High Pressure Processing Of Sous-vide Lobster (Homarus Americanus) Tails

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HIGH PRESSURE PROCESSING OF SOUS-VIDE

LOBSTER (Homarus americanus) TAILS

By

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HIGH PRESSURE PROCESSING OF SOUS-VIDE

LOBSTER (*Homarus americanus*) TAILS

By Sami A. Humaid

Dissertation Advisor: Dr. Denise I. Skonberg


Sous-vide and high pressure processing (HPP) are promising techniques in the development of high-quality seafood products. Lobsters are high-value seafood products that are highly susceptible to being overcooked using conventional methods, producing a tough and rubbery texture. Lobsters are usually sold either live or frozen due to their high perishability. The application of sous-vide cooking may provide evenly cooked lobster products with a succulent and juicy texture, while HPP may increase the shelf-life of lobster products without the use of additives. The objectives of this research were to: 1) evaluate the impact of three different sous-vide cooking conditions on physicochemical properties and consumer acceptability of lobster tails, 2) evaluate the effects of HPP application on physicochemical properties of vacuum-packaged raw and subsequently sous-vide cooked lobster tails, and on consumer acceptability of the sous-vide cooked lobster tails, 3) determine the effects of HPP on the refrigerated shelf-life of vacuum-packaged raw and subsequently sous-vide cooked lobster tails.

In the first study, hand-shucked lobster tails were vacuum-packaged in boilable bags and sous-vide cooked to internal temperatures of 55, 60, and 65 °C for equivalent times values (208,
45, and 10 minutes, respectively) aimed to control the target foodborne pathogen, *Listeria monocytogenes*. Results revealed that sous-vide cooked lobster tails at all parameters were more tender than those conventionally cooked in boiling water. In addition, no significant differences were observed in lobster qualities among the sous-vide cooking parameters. In support of the physicochemical results for sous-vide cooked tails, hedonic testing confirmed that there were no significant differences in consumer acceptability response to the sous-vide cooking parameters. Therefore, the 65 °C for 10 minutes treatment was chosen for subsequent studies because it represents the most convenient cooking treatment.

In the second study, hand-shucked raw lobster tails were high pressure processed at two moderate processing pressures (150 or 350 MPa) and two processing times (5 or 10 min), then half were subsequently sous-vide cooked at 65 °C to achieve a core temperature of 65 °C/10 minutes.

Hardness of raw tails decreased in the 150 MPa/10 min samples, while the shear force to cut raw and sous-vide cooked samples increased in response to 350 MPa for 5 or 10 minutes. Although HPP induced significant textural changes, consumer acceptability of the HPP pretreated sous-vide cooked lobster tails was not influenced.

The third study investigated the effects of 150 MPa or 350 MPa for 10 minutes on microbial, sensory, and physicochemical qualities of raw and subsequently sous-vide cooked (65 °C) lobster tails during 28 days of storage at 2 °C. Higher pressure (350 MPa) samples maintained acceptable quality throughout 28 days storage compared to the control and 150 MPa treatment, although a considerable histamine content was observed in raw lobsters which reached the hazard limit after 14 days in the 350 MPa treatment. Furthermore, HPP
pretreatment did not contribute to additional shelf-life extension of the sous-vide cooked lobster tails.

The results of these studies have important implications for the lobster industry and for consumers of value-added lobster products. These results suggest that HPP has the potential to increase the commercial availability of refrigerated raw lobster tails and to be applied in combination with sous-vide cooking to produce high-quality and consumer-acceptable ready-to-eat lobster products.
DEDICATION

I dedicate this manuscript with sincerest gratitude to my wife, Tayba Humaid.
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CHAPTER 1
INTRODUCTION

Many seafood consumers around the world are attracted to fish and shellfish because of their unique texture and taste profiles, along with health benefits. In the modern world, as time available for cooking at home is limited, there is a growing consumer demand for fresh, easy-to-prepare, and ready-to-eat food products that are high in quality, safe, and minimally processed. These demands have led to increased utilization of new food processing technologies such as high pressure processing (HPP) and sous-vide (SV) cooking. In the current study, soft-shell American lobster has been used as a model to study the potential impacts of HPP and SV applications on high-value seafood products.

1.1 Lobsters

Lobsters are aquatic arthropods of the class crustacea. They live in all oceans, dwelling in crevices or in burrows under rocks (Mente, 2008). Globally, the four main commercial species (Figure 1.1) include American lobster (*Homarus americanus*), European lobster (*Homarus gammarus*), rock lobster (*Jasus* spp.), and spiny lobster (*Panulirus* spp.) (Pereira & Josupeit, 2017).

The American lobster is economically more important than other lobster species. American lobster landings, in the U.S. and Canada combined, represent more than half of all lobster landings worldwide (Pereira & Josupeit, 2017). The National Marine Fisheries Service NMFS (2017) reported that American lobster landings in the U.S. reached 158.6 million pounds valued at $666.7 million in 2016. Maine and Massachusetts produced more than 94% of the total landings in the U.S. (NMFS, 2017). According to the Maine Department of Marine Resources
(DMR, 2019), in 2018, American lobster landings generated 119.6 million pounds valued at around $484.5 million, which was an increase of nearly 8 million pounds over 2017 (Figure 1.2). Increased lobster landings have stimulated the development and sales of value-added lobster products in seafood markets (Massachusetts DMF, 2018).

![Figure 1.1. World production of the four main commercial lobster species. Source: Nguyen et al. (2017)](image1)

![Figure 1.2. American lobster landings in the State of Maine from 1950 to 2018. Source: Maine Department of Marine Resources.](image2)
From a nutritional perspective, American lobster is one of the most nutritious and healthful aquatic animal foods. They are a good source of high-quality protein and contain less saturated fat, fewer calories, and less cholesterol than red meat. According to the USDA FoodData Central (FDC, 2019), a 100 gram (~3/4 cup) serving of cooked lobster (boiled or steamed) contains around 19 grams of protein, 90 calories, and 145 milligrams of cholesterol. In addition, lobsters are rich in vitamins and minerals such as vitamin B12 (0.85 µg), selenium (72.6 µg), copper (1.2 mg), and phosphorous (129 mg).

The segmented body of the American lobster consists of two major body parts, a cephalothorax (head and thorax) and an abdomen (tail). A lobster has ten legs: eight are walking legs and the first two legs near the mouth have developed into claws. These claws include a big shell-crusher claw and a smaller, serrated claw, both of which are used in the feeding process. Lobsters are benthic crustaceans and live in the cold bottom waters in the North Atlantic from Virginia up through the Canadian Maritimes (Billings, 2014). They are abundant in the coastal waters of New England, particularly in Maine (NMFS, 2017). American lobsters grow slowly in cold waters (Straus, 1991) and they grow by molting or shedding their shells (Billings, 2014). It takes about 20 – 25 molts over 5 – 7 years for a lobster to reach sexual maturity (Hughes et al., 1972). After molting, lobsters are soft until their new shells become hard (Pereira & Josupeit, 2017). Lobsters are harvested year-round in Maine. However, soft-shell or new shell lobsters (newly molted) are usually harvested from late summer to early fall (Billings, 2014), while hard-shell lobsters are harvested in late spring, from May to June, and in fall between October and late November. In Maine, the minimum size lobster weighs approximately one pound, while the maximum size lobster weighs between 3 – 4 pounds. According to the Maine DMR, the legal
minimum and maximum lobster carapace lengths are 3¼ and 5 inches, respectively. Lobster carapace lengths are measured from the extreme rear of the eye socket to the beginning of the tail by a special double-sided gauge.

Hard-shelled lobsters have more meat in proportion to total body weight than soft-shelled lobsters and their meat has a firm texture, while soft-shell lobster meat is softer and tends to contain more water (Nenes & Manville, 2016). After molting, new shell lobsters take up water to enlarge the new shell before it hardens (Bayer et al., 1999). This rehydration causes blood dilution, consequently the serum protein (hemolymph protein) levels significantly decrease directly after the molt, from their high pre-molt levels (Barlow & Ridgway, 1969). Serum protein is used as an indicator of quality and physiological condition in American lobsters (Leavitt & Bayer, 1977; Wang & McGaw, 2014). Typically, a healthy hard-shell lobster has a high serum protein level, greater than a Brix level of 8, while a soft-shell lobster is weak and has a lower blood protein level (Wang & McGaw, 2014). Lobster dealers commonly ship lobsters with a Brix level of 10 or higher, however some may use a Brix level of 8 depending on their standards.

Lobsters are usually caught by baited traps made of wire, wood, or plastic; the bait is mostly herring (Grabowski et al., 2010). After harvesting, lobsters are stored live in crates and typically sold live to retailers and restaurants where they are stored in tanks or floatation pools to keep the lobsters alive until sold live (graded and priced according to weight) or cooked (Marsh, 2012). Live lobsters are typically cooked by boiling, steaming, grilling, or baking. The color of live lobster varies from brownish rust to bright blue to greenish brown. The shell of cooked lobsters turns bright red due to the release of astaxanthin resulting from the thermal denaturation of crustacyanin, a protein which suppresses the red hue of the astaxanthin and gives live lobster
shell a blue color (Belitz et al., 2009). Cooking conditions for American lobster can vary depending on the type of the shell (Alfiero et al., 2013). Soft-shell lobsters are cooked slightly less than hard-shell lobsters due to their softer texture. Hard-shell lobsters with sizes ranging from 1 to 2 ½ pounds are cooked from 15 to 28 min, while for soft-shell lobsters cooking times range from 9 to 20 min (Table 1.1). Cooking lobster for longer than the recommended times usually makes the meat rubbery and too tough (Keller et al., 2008). Lobster meat is mild and sweet in flavor, and the texture is firmer in the tail than in the claws.

<table>
<thead>
<tr>
<th>Size of lobsters (pounds)</th>
<th>Cooking times (min)</th>
<th>Hard-shell lobsters</th>
<th>Soft-shell lobsters</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 1 ¼</td>
<td>15 – 17</td>
<td></td>
<td>9 – 11</td>
</tr>
<tr>
<td>1 ¼ to 1 ½</td>
<td>18 – 20</td>
<td></td>
<td>12 – 15</td>
</tr>
<tr>
<td>1 ½ to 2</td>
<td>22 – 25</td>
<td></td>
<td>15 – 18</td>
</tr>
<tr>
<td>2 to 2 ½</td>
<td>25 – 28</td>
<td></td>
<td>18 – 20</td>
</tr>
</tbody>
</table>

*Table 1.1. Cooking (boiling or steaming) times of different sizes of hard-shell and soft-shell American lobster a*

*Lobsters are fragile animals, and there is considerable potential for physical damage and mortality when handling a live lobster, which may result in a significant loss of product and incurred costs. The distribution, handling, and storage of live lobsters require a substantial cost compared to processed lobster products. Therefore, Massachusetts Division of Marine Fisheries (2018) recommends processing whole live lobsters into value added lobster products such as frozen or raw shell-on lobster products.*

*Raw and cooked lobsters are typically processed by freezing. Freezing extends the shelf-life of lobster products, consequently providing more access to a wider range of seafood markets*
because of easy shipping with reduced icing and labor costs (Billings, 2014). Lobster products that are available in the seafood markets include frozen whole shell-on (raw, blanched, or cooked) lobster including frozen claws and knuckles (C/K) (raw or cooked), tails shell-on (raw or cooked), and frozen deshelled whole and parts lobster meat (raw or cooked) (Marsh, 2012).

Lobsters are received live and banded in crates, then transported via refrigerated trucks to the processing facilities. Lobsters are inspected to ensure that they are alive and active by checking leg movements and whether the tail is curled when the lobster is lifted. Live lobsters are conveyed by a conveyor belt to a weight grader which grades them by individual weight. They are graded according to shell hardness and by weight (1.25 lb, 1.5 lb, 1.75 lb, etc.) (Marsh, 2012). Live lobsters are butchered into parts including tails, claws and knuckles, bodies, and legs. Separation of lobster parts is conducted by hand. Graded lobsters (whole or parts) are cooked either in batch or with a continuous (steam or boil) cooker. All cooking techniques must receive a thermal process review to locate cold spots and establish appropriate cooking parameters to achieve a 6-log reduction process for *Listeria monocytogenes* based on table A-3 in the FDA Fish and Fisheries Seafood Hazard Guide. Cookers are equipped with a thermocouple data logger to monitor cooking process temperature (chamber temperatures or water temperature) and belt speed to monitor cooking time (if it is a continuous process). Post cooking, lobster products are moved to a water chiller (cooler operating range: 32 °F/0 °C to 36 °F/2.2 °C) using potable water so that every part of the lobster product is cooled to below 40 °F (4.4 °C). After the product has been adequately cooled, whole lobster or parts are moved to a picking area. This step includes extracting meat from the tails, claws and knuckles by hand. Legs are fed into a leg roller machine. Bodies are fed into deboning machines that mince and
separate the meat from the shell. The minced meat may be partially dewatered to remove excess water by either pressing or centrifugation, then is immediately packaged and frozen. Prior to packaging, lobster meat is inspected to ensure that small pieces of shell from butchering and meat extraction are removed from the final product. All packaged lobster products are blast frozen, cryogenically frozen with liquid nitrogen ($N_2$) or liquid carbon dioxide ($CO_2$), or by other flash-freeze methods, at an ambient temperature of -31 °F (-35 °C) or below until solid. The freezing process typically takes less than 30 min. The advantages of value-added frozen lobster products include stabilizing the boat price and flow of lobsters to the markets as well as the market price consumers pay for the product (Work et al., 1997). However, there are critical quality disadvantages due to the freezing process such as toughening of meat and development of off-flavors during storage (Calder et al., 2006). In addition, freezing live lobsters can make it difficult for the consumer to extract the meat easily from the shell (Work et al., 1997). In the lobster processing industry, a blanching treatment is commonly applied to lobster before freezing to facilitate separating the thawed meat from the shell. However, the blanching conditions should be sufficient only to cook the meat next to the shell and not the meat below the surface. Getchell and Highlands (1957) reported that heat treatment for 70 sec in a 91 °C 2 % salt brine could help to extract the meat easily from the shell, while keeping the meat as raw as possible. After the blanching treatment, lobsters are chilled in cold running water or in ice water (1:1, v/v) for approximately 15 min. After cooling, the lobsters are blast or cryogenically frozen and packaged individually in a moisture-vapor-resistant wrapper, such as plastic film, to prevent dehydration and oxidation.
Lobster meat is attached to the shell continuously across the entire body of the crustacean by a continuous series of intracuticle fibers (Figure 1.3) (Jabbour et al., 2011). These intracuticle fibers extend from the surface of the muscle tissue to the outer surface of the shell via pore canals in the shell (Tabilo-Munizaga et al., 2016). This continuous attachment renders it difficult to shuck the shell by hand. To facilitate meat extraction, several processing methods have been developed to weaken the connective tissue attaching the shell to the meat, such as immersing the crustacean in a solution of protease enzymes and freezing the crustacean followed by vacuum aspiration (Jabbour et al., 2009; Jabbour et al., 2011).

Figure 1.3. The meat of lobster is attached to the shell continuously across the body of the animal.

However, these methods have not been widely adopted by the seafood industry due to their inconsistent results. Thermal treatments are commonly used to facilitate shucking lobsters. Heat can break the tissues connecting the meat to the shell (Jabbour et al., 2009) thereby facilitating shell removal. However, there are limitations to this method. Lobster meat underneath the shell becomes cooked, consequently affecting the physicochemical properties of
the meat, such as texture, color, and heat-sensitive nutrients. In addition, after thermal treatment, removing the meat from the shell by hand is time-consuming and laborious. Moreover, mechanical methods can damage the meat, causing it to be minced or flaked, thus limiting its potential applications (Tabilo-Munizaga et al., 2016).

More recently, some lobster processors have upgraded their processing operations with high pressure processing (HPP) technology, a non-thermal system. This non-thermal process efficiently separates lobster meat from the shell by denaturing the connective tissues holding the shell to the meat (Jabbour et al., 2009; Jabbour et al., 2011). Consequently, the shucked lobster meat is completely raw and intact without physical damage to its texture, while maintaining its natural flavor and nutritional properties. The HPP conditions for shucking lobster range from 172.4 to 689.4 MPa for short exposure times of 15 to 180 seconds (sec) at temperatures between 10 °C and 30 °C (Jabbour et al., 2011). HPP represents an opportunity for lobster processors to create new markets for value-added lobster products that are enjoyed by consumers, particularly since HPP products retain their fresh-like sensory characteristics.

1.2. High pressure processing

High pressure processing (HPP) is a novel emerging technology applied in the food industry to improve shelf-life, safety, and quality properties of food products (James & James, 2014). HPP, which is also known as ultra-high pressure (UHP) or high hydrostatic pressure (HHP), is a non-thermal food processing technology applied such that the food is subjected to high hydrostatic pressures (100 – 900 MPa), at a variety of holding times (5 – 30 min) and temperatures (5 – 90 °C), depending on the product and desired effects. The pressure generated is transmitted by liquid medium, usually water, which is assumed to treat all parts of the food
uniformly (Campus, 2010). When a liquid medium is compressed, its temperature will increase due to adiabatic heating (heat of compression). For water and nonfatty products, adiabatic heat is approximately 3 °C per 100 MPa, while fats have larger adiabatic heat (up to 10 °C per 100 MPa) because of the higher compressibility of fat compared to water (Ting et al., 2002). This temperature rise caused by the pressurization of the liquid medium (adiabatic heating) may be removed by (partial) cooling of the pipeline.

HPP of foods was first investigated as early as 1894 (Hite, 1899), by Bert Hite at the agricultural experiment station at West Virginia University. Hite demonstrated that shelf-life of milk (Hite, 1899), and vegetables and fruits (Hite et al., 1914) could be extended by pressure treatment. HPP was extensively explored in the 1970s. For example, many studies investigated the effect of HPP on tenderness of pre-rigor sheep (Macfarlane, 1973) and beef (Bouton et al., 1977) muscles. The application of pressure showed improvement in tenderness due to activation of proteolytic enzymes in the sheep and beef muscle. In the early 1980s, HPP applications grew rapidly as an alternative preservation method to conventional thermal processing methods, that could improve quality and safety of food products (Yang & Powers, 2016). In the early 1990s, HPP jams and jellies were introduced in the Japanese market (Suzuki, 2002), followed by the introduction of HPP guacamole in U.S. markets (Palou et al., 2000). Currently, HPP is effectively implemented in the food industry on meats, seafoods, beverages, dairy products, fruits, and vegetables to meet consumer demands for minimally processed, safe, and high-quality food products. Many commercial food companies have adopted HPP such as Butterball, Ocean Choice International, Sofina, Freybe, Viau Food, Maple Leaf, Golden Valley and Casa Italia, Well Juicery, and Impress Juice (Hyperbaric commercial customers, 2020). Greenhead Lobster, Shucks Maine Lobster, and Ready Seafood are among the U.S. lobster processors currently using
HPP technology, and many Canadian processors have been applying HPP to lobsters for over 20 years. In addition, many research studies have been conducted to understand the mechanism and impacts of HPP on food products.

There are many advantages to using HPP on foods. It promotes retention of flavors, pigments, and nutritional content of foods due to its negligible effects on covalent bonds, however HPP can disrupt noncovalent interactions resulting in structural changes in proteins (Bolumar et al., 2016) which can lead to tender or tough meat, depending on the muscle type (e.g. shank, shoulder, loin), rigor stage, the pressure level, the temperature of the liquid medium, and the duration of the HPP (Sikes & Warner, 2016).

1.2.1. Effects of high pressure processing on muscle proteins

Changes in protein structures during processing significantly alter protein functionality and result in changes in food quality and sensory properties (Messens et al., 1997). Proteins are macromolecules made up of amino acid polypeptide chains. Proteins make up the major structure of muscle foods (Tornberg, 2005) and have four types of structure: primary, secondary, tertiary, and quaternary. Primary structure refers to amino acids linked covalently by peptide bonds to form polypeptide chains. Secondary structure is folding of the polypeptide chains into regular structures. The most common types of secondary structures are the alpha helix and the beta pleated sheet. These structures are held in shape by hydrogen bonds which form between the carbonyl oxygen of one amino acid and the amino hydrogen of another amino acid (C=O and NH). Tertiary structure is the three-dimensional (3D) shape of a protein. The 3D shape is stabilized due to side chain interactions (hydrophobic interactions and Van Der Waals interactions) and bonds (hydrogen bonds, ionic bonds, and covalent bonds). Finally, quaternary
structure consists of two or more polypeptide chains. These chains are held together by noncovalent interactions like hydrogen bonds, ionic bonds, and hydrophobic interactions (Damodaran, 2008). Protein structures are not stable and can be subjected to conformational changes at the secondary, tertiary, and quaternary structural levels, while the primary structure is not affected by denaturation (Damodaran, 2008). Conformational changes of proteins are usually called denaturation (Tornberg, 2005).

The effects of HPP on protein structures result in changes such as denaturation, dissociation, aggregation, and gelation (Gross et al., 2014). Changes in protein structure under pressure are governed by Le Chatelier’s principle. The principle indicates that a chemical system in an equilibrium state will shift to a new equilibrium to minimize the effects of an external factor (Yang & Powers, 2016). HPP changes protein structures primarily by rupturing or forming non-covalent electrostatic and hydrophobic interactions and hydrogen bonds (Sikes and Warner, 2016), and the impact of HPP on protein denaturation primarily influences tertiary and quaternary structure (Yang and Powers, 2016). The effects on non-covalent bonds by HPP causes the protein chains to partially or fully unfold with minimal impact on small molecules associated with desirable food quality attributes such as flavor, color, and nutrients (Yang & Powers, 2016).

Protein quaternary structures unfold at pressures lower than 150 MPa (Balny & Masson, 1993), while pressures above 200 MPa can affect the tertiary structure, and pressures ranging from 300 to 700 MPa can induce changes in the secondary structure (Lullien-Pellerin & Balny, 2002). In addition, pressures less than 200 MPa reversibly denature proteins, while proteins are often irreversibly denatured at pressures higher than 300 MPa (Balny and Masson, 1993; Smeller
et al., 2002). Generally, HPP does not result in the formation or breakage of covalent bonds of a protein, however it can form new disulfide bonds, thereby stabilizing the denatured proteins or producing protein aggregation (Yang & Powers, 2016). In addition, changes in protein structures can lead to decreased protein solubility, which was suggested to be caused by pressure-induced denaturation followed by aggregation due to intermolecular disulfide bonds or hydrophobic interactions (Gross et al., 2014). However, protein denaturation depends on the structure of individual proteins, as large numbers of disulfide bonds in a native protein can help the protein to resist HPP denaturation (Yang & Powers, 2016).

Fish and shellfish muscle proteins, like those of all other muscle foods, can be classified based on their solubility at different salt concentrations into sarcoplasmic, myofibrillar, and connective tissue or stromal proteins which account for 30 – 34%, 50 – 55%, and 10 – 15% of the total muscle proteins, respectively (Tornberg, 2005; Strasburg et al., 2008). These muscle proteins play major roles in meat qualities.

Sarcoplasmic proteins are water-soluble (soluble in low ionic strength buffer) and mostly consist of water-soluble enzymes involved in the biochemical processes of muscle tissues such as glycolytic enzymes, lysosomal enzymes (e.g. cathepsin), and pigments, such as hemoglobin/myoglobin. Sarcoplasmic proteases, particularly calpains and cathepsins, are important and responsible for post-mortem muscle softening (Chéret et al., 2005; Teixeira et al., 2013). The effects of HPP on the sarcoplasmic proteins are confounding. For example, studies using differential scanning calorimetry (DSC) and protein electrophoresis (SDS-PAGE) indicated that in salmon (Oncorhynchus kisutch) muscle pressurized at 135, 170, and 200 MPa for 30 seconds, sarcoplasmic protein content significantly decreased as pressure increased (Ortea
et al., 2010). In contrast, in cod (Gadus morhua) muscle treated at levels of 100 to 800 MPa, sarcoplasmic proteins at 200 MPa had a similar profile compared to the untreated sample, but at 300 MPa, most of the sarcoplasmic proteins were denatured. However, some sarcoplasmic protein fractions were resistant to pressure-denaturation and increased in concentration at 400 MPa and higher (Angsupanich & Ledward, 1998). In addition, when cod and mackerel muscle were processed with high pressure, certain sarcoplasmic proteins covalently linked together and were consequently resistant to extraction with sodium dodecyl sulfate (SDS) (Ohshima et al., 1992).

Structural changes in sarcoplasmic proteins induced by HPP can influence meat quality characteristics. Marcos et al. (2010) reported that HPP at pressure levels above 200 MPa induced denaturation of sarcoplasmic proteins which significantly correlated with modifications of color and water holding capacity (WHC) in beef. In carp, Sequeira-Munoz et al. (2006) suggested that changes in the color values of muscle samples were a consequence of coagulation of sarcoplasmic and myofibrillar proteins induced by HPP. In shrimp muscle, lightness significantly increased immediately after pressurization (at 100, 270, and 435 MPa for 5 min at 25 °C) and the bleaching effect was higher at high pressures. These color changes in shrimp after HPP were suggested to be linked to the denaturation of the myofibrillar and sarcoplasmic proteins (Kaur et al., 2013). In addition, shrimp texture experienced an increase in springiness at 270 and 435 MPa, which led to increases in gumminess and hardness which could be attributed to unfolding of actin and sarcoplasmic protein and formation of hydrogen bonded networks (Angsupanich & Ledward, 1998). Sarcoplasmic proteins are less stable than myofibrillar
proteins. Myofibrillar proteins are directly associated with meat texture due in part to their state of contraction (Damodaran, 2008).

Myofibrillar proteins are salt-soluble (soluble in high ionic strength buffer) and include contractile proteins such as myosin (thick filaments) and actin (thin filaments), regulatory proteins such as tropomyosin and troponin, and other structural proteins, such as titin, nebulin, α-actinin, and β-actinin (Damodaran, 2008; Hopkins, 2014). Myosin and actin alone account for around 65 – 70 % of total myofibrillar proteins (Strasburg et al., 2008). Approximately 80 % of water in the muscle is held between the actin and myosin filaments (Baldwin, 2012).

The muscle of American lobster consists mainly of myosin and actin (Govind, 1995). These filaments are grouped into bundles of various diameters to form a muscle myofibril. Each myofibril is organized longitudinally as a chain of contractile units called sarcomeres. Sarcomeres consist of Z lines and dark (A) and light (I) bands. The sarcomere is bound by adjacent Z lines. The I bands consist of only actin while the A bands consist of overlapping actin and myosin filaments that also contain the H band which consists of only thick myosin filaments. In the middle of the H band is a dark region parallel to the Z line called the M line where the thick filaments attach to each other. The distance between adjacent Z lines represents the sarcomere length (Strasburg et al., 2008). Sarcomere length varies depending on contraction status or stretch force applied to the fiber and ranges from 2 to 20 µm (Govind, 1995). Every sarcomere of every myofibril is surrounded by tubules forming the sarcoplasmic reticulum (Figure 1.4). The myofibrils congregate and form a muscle fiber that also contains granular sarcoplasm, mitochondria, and nuclei, along with a connective tissue sheath (Strasburg et al., 2008).
The mechanism for muscle contraction was explained by Huxley and Hanson (1954): when the muscle fiber contracts the Z lines all move toward each other, sliding the actin filaments over myosin filaments and consequently the I band disappears and the sarcomere length is shortened (Figure 1.5).

Actin and myosin filaments are not shortened during contraction, but they move over each other. In contrast, Z lines separation causes stretching when the thin and thick filaments move away from each other. Disruption of the Z line is one of the primary processes in the development of tenderness in poultry and in beef (Strasburg et al., 2008). Muscle contractions within the sarcomere are regulated by two other components of the actin filament: tropomyosin and troponin. They are proteins which block the myosin-binding sites to prevent actin from binding to myosin. The interactions between the actin and myosin filaments significantly affect the textural properties of meat. Muscle toughness is caused by the formation of actomyosin cross-bridges that result from the overlap of myosin and actin filaments that causes shortening of the sarcomere (Lawrie, 2006).
The application of pressure is known to influence myofibrillar proteins (Bolumar et al., 2016; Sikes & Warner, 2016). For example, in raw blue crab meat (*Callinectes sapidus*) pressurized at 100, 300 and 600 MPa (10 °C/5 min), myosin and actin denaturation increased with increasing pressure level (Martínez et al., 2017).

![Diagram of muscle contraction](image)

**Figure 1.5. Illustration of the sliding filament mechanism of muscle contraction.**
*Source: Tortora and Derrickson, (2006).*

DSC and SDS-PAGE analyses showed that the increase in the pressure level resulted in a decrease in denaturation enthalpy and increased denaturation of both proteins (myosin and actin). In addition, results from Fourier transform infrared spectroscopy (FTIR) indicated a reduction in α-helix and an increase in β-turn structures as a result of denaturation induced by HPP (Martínez et al., 2017). In cod muscle, myosin was denatured at 100 – 200 MPa and actin at 300 MPa (Angsupanich & Ledward, 1998). In addition, a significant decrease in the enthalpy of myosin was observed after HPP treatment at 200 MPa in cod and mackerel (*Scomber scombrus*), while in salmon, a significant decrease in the enthalpy of myosin and actin was induced at 500 MPa.
(Christensen et al., 2017). In addition, myosin and actin denaturation resulted in a lighter appearance.

HPP affects myofibrillar protein solubility. Decreased solubility of myofibrillar protein as pressure level increased was observed in pork (Grossi et al., 2016) and beef (Marcos & Mullen, 2014). Grossi et al. (2016) reported that the decreased protein solubility in raw pork muscle on increasing pressure above 200 MPa was a result of the pressure impact on the individual myofibrillar proteins. Myosin and actin lose their native solubility at pressures above 400 MPa, while α-actinin and troponin-T are less affected by pressure. The decrease in protein solubility is attributed to formation of larger insoluble protein aggregates linked by disulfide bonds that cannot be extracted. In addition, exposing hydrophobic groups as a result of protein unfolding due to HPP may promote protein aggregation through hydrophobic interactions, resulting in a reduction in solubility (Olsen & Orlien, 2016). However, in pork meat, it was observed that the concentration of the solubilized protein in the pressurized myofibrils was increased with increasing pressure level up to 200 MPa (Iwasaki et al., 2006). The solubilized pork muscle proteins did not form large aggregates at 200 MPa, therefore the amount of myosin in the supernatant increased up to 200 MPa. Although most studies on the effects of HPP on muscle proteins have been conducted on myofibrillar proteins (Yang & Powers, 2016), the effects of HPP on connective tissue have also been studied (Ichinoseki et al., 2006).

Connective tissue or stromal proteins, composed mostly of collagen (90 %), are insoluble in both low and high ionic strength buffers (Strasburg et al., 2008). Fish and shellfish generally contain a lesser amount of collagen and higher myofibrillar protein levels than terrestrial animals (Tornberg, 2005). Collagen content and crosslinking have been linked to the toughness of muscle
food (Sikes and Warner, 2016). HPP induces minimal or no effects on collagen (Macfarlane et al., 1981; Suzuki et al., 1993). For example, in beef, no significant changes were observed in the collagen among the control and pressurized muscles at 100, 150, 200 and 300 MPa for 5 min at about 2 °C (Suzuki et al., 1993). Therefore, it was thought that since collagen was not affected by HPP, changes in the myofibrillar protein structures were responsible for the textural modification induced by HPP (Suzuki et al., 1993).

### 1.2.2. Effects of high pressure processing on muscle texture

Texture is one of the most critical quality attributes impacting consumer acceptability of seafood products. Shear force and hardness parameters are frequently investigated to assess the influences of HPP on texture profiles of seafoods. The hardness value is the peak force, expressed in Newtons (N), that occurs during the first compression of texture profile analysis (TPA) (Texture Technologies, 2020), while shear force measures the maximum force (N) as a function of blade movement and the force required to shear through the meat samples perpendicular to the longitudinal positioning of the muscle fibers (Destefanis et al., 2008). Many studies have reported that high pressure significantly changes the texture profile of fish and shellfish products (Chevalier et al., 2000; Chéret et al., 2005; Jantakoson et al., 2012; Kaur et al., 2013; Jiranuntakul et al., 2018). For example, shear force values of raw Norway lobster (*Nephrops norvegicus*) pressurized at 200 MPa for 30 min significantly increased compared to the unpressurized samples (Chevalier et al., 2000). Moreover, hardness of raw black tiger shrimp muscle significantly increased with increasing pressure levels (Jantakoson et al., 2012; Kaur et al., 2016; Kaur & Rao, 2018). Myofibrillar protein denaturation and aggregation were thought to be responsible for the effects of HPP on texture changes, as myosin denaturation induced by HPP
resulted in forming structures that contained hydrogen bonds and were also stabilized by disulfide bonds. These disulfide bonds led to tougher shrimp muscle (Jantakoson et al., 2012). In contrast, many authors reported a decrease in hardness of fish muscle induced by HPP (Chéret et al., 2005; Teixeira et al., 2013). Pressures below 300 MPa can decrease hardness, possibly due an increase in proteolytic activity. Chéret et al. (2005) reported that pressures below 300 MPa increased the activity of proteases that hydrolyze muscle structural proteins, resulting in softening of the texture of seabass. In addition, the increase in enzymatic activity was due to damage of the lysosomal membrane by HPP, consequently releasing proteases with access to myofibrillar proteins (Teixeira et al., 2013). The decrease in proteolytic activity at pressures above 300 MPa was likely due to structural modification of the enzymes. Overall, unfolding of myofibrillar and sarcoplasmic proteins and formation of new hydrogen-bonded networks lead to increase hardness in seafoods (Angsupanich & Ledward, 1998).

1.2.3. Effects of high pressure processing on muscle color

Color is one of the most important sensory properties of seafood products, as it plays a significant role in product acceptability. HPP effect on the color of raw seafood muscles has been well studied by many authors (Matser et al., 2000; Yagiz et al., 2007; Yagiz et al., 2009; Picouet et al., 2011; Bindu et al., 2013; Kaur et al., 2013; Hughes et al., 2016). The main reason for this interest is because HPP has the potential to bleach the color of fish muscle resulting in a cooked appearance (Matser et al., 2000). The color of fish muscle is generally measured with the L* (lightness), a* (redness), b* (yellowness) parameters. Fish muscle can obtain a cooked appearance due to HPP application depending on the pressure intensity. Ashie and Simpson (1996) reported that the lightness of bluefish and sheephead generally increased with increasing
pressure above 200 MPa. In addition, Matser et al. (2000) reported that pressurization higher than 100 MPa for 5 min at 0 °C resulted in a cooked appearance of pollock (*Pollachius virens*), mackerel (*Scomber scombrus*), tuna (*Thunnus thynnus*), cod, salmon trout (*Salmon trutta*), carp (*Cyprinus carpio*), plaice (*Pleuronectus platessa*) and anglerfish (*Lophius piscatorius*), while octopus (*Octopus vulgaris*) muscle experienced a cooked appearance at pressures higher than 400 MPa. The increase in lightness can be accounted for by increased light scattering due to protein denaturation in muscle (Campus, 2010). The lightness of cod and salmon increased at 200 and 500 MPa pressurization for 2 min (Christensen et al., 2017). In shellfish, $L^*$ value of prawns increased significantly with increasing pressure levels (100, 270, 435 and 600 MPa) (Bindu et al., 2013). Similar results were observed in the muscle of black tiger shrimp (Kaur and Rao, 2018). The authors reported that the shrimp muscle became whiter and more opaque with increasing pressure levels of 100, 270, and 435 MPa for 5 min. In addition, after pressure treatment, $a^*$ values increased and $b^*$ values were increased. The increase in lightness as a result of pressure treatment has been associated with unfolding of carotenoprotein (Truong et al., 2015) and lipid oxidation due to degradation of carotenoid pigments of shrimp such as astaxanthin (Cruz-Romero et al., 2004). In carp, Sequeira-Munoz et al. (2006) suggested that pressure-induced coagulation of sarcoplasmic and myofibrillar proteins was responsible for the changes in the color values of the samples.

### 1.2.4. Effects of high-pressure processing on microbial shelf-life

The rapid deterioration of quality of refrigerated seafoods occurs mainly as a consequence of microbial activity (Cruz-Romero et al., 2008). Microbial inactivation is one of the significant applications for HPP to improve the shelf-life of refrigerated seafood products.
HPP has been shown to be effective in extending the microbial shelf-life of many different fish and shellfish products such as salmon, cod and mackerel (Rode et al., 2016), oyster (Cruz-Romero et al., 2008), abalone (Hughes et al., 2016), and shrimp (Ginson et al., 2012; Kaur et al., 2017).

The pressure levels most often used in commercial applications in the food industry range from 100 to 600 MPa which is sufficient to extend microbial shelf-life (Hugas et al., 2002). For example, HPP can inactivate microorganisms and extend the shelf-life of oysters. Cruz-Romero et al. (2008) reported a reduction in the total viable counts (TVC), anaerobic plate counts (APC) and counts of H$_2$S-producing bacteria after pressurization at 260, 400 and 600 MPa for 5 min in oyster (Crassostrea gigas) stored at 2 °C for 31 days. In prawns (Fenneropenaeus indicus), Ginson et al. (2012) reported that non-pressurized prawns and prawns pressurized at 100 MPa reached the microbial rejection limit (>7 log CFU/g) before day 7 of storage at 2±1 °C, whereas samples pressurized at 270 and 435 MPa reached the limit before the 21st and 28th days of storage, while samples pressurized at 600 MPa were still acceptable after 28 days of storage due to the destruction of bacteria. In addition, a significant reduction was observed in total Enterbacteriaceae due to increased pressure levels.

HPP can inactivate microorganisms and delay microbial growth due to a breakdown in the bacterial cell membranes, changes in the permeability of the cell wall, and denaturation of proteins including enzymes (Chong et al., 1983; Campus, 2010). However, the types and strains of microorganism affect the microbial inactivation by HPP. For example, Gram-positive bacteria, such as lactic acid bacteria (LAB) are generally more resistant to physical stresses caused by pressure treatment compared to Gram-negative bacteria, such as Campylobacter (Simonin et al.,
2012). In addition, spores are extremely HPP resistant compared to vegetative cells (Smelt, 1998). Spores of non-proteolytic *Clostridium botulinum* tend to be extremely resistant to inactivation at pressures approaching 600 MPa (Lenz et al. 2015). Moreover, the inactivation by HPP also depends on the microbial growth phase. Bacteria in growth phase are more sensitive than bacteria in stationary phase. Mackey et al. (1995) reported that HPP at 400 MPa for 10 min caused a 7.0 log reduction in the viable counts of *L. monocytogenes* in growth phase, while the stationary phase cells were reduced by only 1.3 log. Non-proteolytic *C. botulinum* and *L. monocytogenes* are the pathogens of interest for sous-vide cooked products.

In addition to the improvement in shelf-life of raw seafoods, HPP has the potential to pasteurize and improve the shelf-life stability of cooked seafood products, particularly those that are prepared by cooking methods using lower temperatures, such as the sous-vide method (Picouet et al., 2011; Espinosa et al., 2015). For example, HPP effectively decreased total bacterial count and *Enterobacteriaceae* of sous-vide cooked salmon products, extending refrigerated shelf-life while maintaining their desired sensory qualities (Picouet et al., 2011).

### 1.3. Sous-vide cooking

Sous-vide (SV) is increasingly used to fulfill consumer demands for high quality, safe, and minimally processed ready-to-eat meals (Ayub & Ahmad, 2019), and its products are widely available in global markets (Kilibarda et al., 2018). In contrast to conventional cooking methods, sous-vide is controlled, low-temperature cooking of vacuum-sealed foods in a water bath or under steam (Baldwin, 2012). Sous-vide provides consistent, repeatable, and perfectly cooked foods every time. Sous-vide processed products are immediately chilled after cooking and kept below 3.3 °C to prevent *Clostridium botulinum* growth and toxin formation (FDA, 2020). They
are typically cooked at low temperatures for a longer period (depending on the type of the food) than conventional cooking methods (Keller et al., 2008). Sous-vide processing offers evenly cooked food products which helps to preserve their sensory characteristics and nutritional value. Sous-vide is French for "under vacuum". Historically, in the late 1960s, sous-vide cooking was used to extend the shelf-life of foods (Ayub & Ahmad, 2019). Professional chefs have been using sous-vide cooking since the 1970s, however it did not become widely known until the 2000s. Currently, sous-vide cooking has significantly increased in use in restaurants and homes (Baldwin, 2012). Sous-vide is applicable to a wide variety of foods including fish, meat, fruits and vegetables (Kilibarda et al., 2018). Sous-vide cooking temperatures in the range of 50 °C – 70 °C are applied to seafoods and meats and maintained for several hours or even days, while for vegetables, sous-vide cooking temperatures range from 90 to 100 °C (Kilibarda et al., 2018).

The advantages of sous-vide cooking are well described by many authors (Keller et al., 2008; Baldwin, 2012; Aguilera, 2018; Kilibarda et al., 2018; Ayub & Ahmad, 2019). Sous-vide cooking results in uniform and efficient heat transfer from water to the food. At relatively low temperatures, juiciness and tenderness of meat are improved (Aguilera, 2018), and nutrients are better preserved, especially heat-sensitive nutrients such as vitamins. Additionally, vacuum packaging limits food contact with the air, preventing off-flavors from oxidation, maintaining food flavor by reducing evaporative losses of flavor volatiles and moisture during cooking, and eliminating the risk of recontamination during storage.

Sous-vide cooking can enhance microbiological safety and quality of foods, thus extending their shelf-life. In seafood, sous-vide cooking was an effective method to ensure the safety and extend the shelf-life of trout (González-Fandos et al., 2004) and salmon (González-
Fandos et al., 2005). For example, González-Fandos et al. (2005) reported that neither aerobic nor anaerobic sporeforming bacteria were detected in salmon sous-vide processed at 90 °C for 15 min and stored at 2 °C for 45 days, while the product maintained its sensory characteristics including texture and appearance. However, the sous-vide cooking parameters were not validated to ensure pathogen inactivation. Singh et al. (2016) reported that sous-vide cooking extended the refrigerated shelf-life of seerfish steaks for up to 65 days based on microbial, sensory, and biochemical parameters, such as trimethylamine (TMA), total volatile base nitrogen (TVBN), and thiobarbituric acid reactive substances (TBARS). The precisely controlled temperatures of sous-vide cooking allow seafoods to be thoroughly cooked, resulting in excellent flavor and texture attributes. For example, according to chefs, lobster meat sous-vide cooked at 50 °C for 12 min better retained the natural flavor and texture attributes of the lobster compared to the conventional cooking method (Rodgers & Young, 2008). In addition, pork ham sous-vide cooked at 61 °C for 45 min had a tenderer texture compared to hams cooked conventionally at 100 °C for 45 min and sous-vide cooked at 71 °C for 45 min (Jeong et al., 2018). In general, sous-vide cooked products are heated at relatively mild temperatures. However, during cooking, structural changes in muscle proteins induced by the heating process are highly associated with changes in meat qualities, particularly texture.

1.3.1. Effects of the temperatures of sous-vide on muscle proteins

During cooking, structural changes in sarcoplasmic, myofibrillar, and connective tissue proteins occur at different temperatures (Tornberg, 2005). At 40 to 60 °C, the sarcoplasmic proteins start to aggregate and gel (Baldwin, 2012). At sous-vide cooking temperatures, most of the sarcoplasmic proteases remain active and contribute to improving muscle tenderness. For
example, Cathepsin B and L remained active for up to 24 hours of cooking at 55 °C, however calpains were inactivated within 10 min (Ertbjerg et al., 2012). Calpains appear to be more important than cathepsins in the tenderizing process. Calpain causes destruction of the Z line and leads to its disappearance. This disruption of the Z line is associated with increased tenderness of meat (Strasburg et al., 2008).

Myofibrillar proteins, including myosin and actin, shrink during heating resulting in the contraction and shrinkage of the muscle fibers (Baldwin, 2012). When muscle is subjected to heat ranging from 35 to 40 °C, the muscle fibers start to shrink and the shrinkage increases at temperatures up to 80 °C. Between 40 and 60 °C, the muscle fibers shrink transversely, consequently widening the gap between fibers (Palka & Daun, 1999). As temperature further increases (above 60 – 65 °C), the muscle fibers shrink longitudinally and cause substantial loss in the water held between the myosin and actin filaments. The extent of this contraction increases with increasing temperature, and temperatures approaching 80 °C cause myofibrillar toughening due to shrinking of myofibrils and associated loss of water (Tornberg, 2005). In addition, the formation of disulfide bonds in actomyosin leads to increased muscle toughness at temperatures between 70 and 90 °C (Warriss, 2010).

Connective tissues (collagen) start to denature and shrink at temperatures around 60 °C (Martens et al., 1982; Tornberg, 2005). If the collagen fibers are not stabilized by heat-resistant intermolecular bonds, shrinking (on further heating) mostly will destroy the triple helix structure of collagen leading to the formation of water-soluble random-coiled gelatin, and decreasing the adhesion between muscle fibers (Baldwin, 2012).
Myofibrillar proteins and collagen in seafood are more sensitive and have lower thermal stability than those from land animal muscles (Tahergorabi et al., 2011). Structural and thermal properties of muscle proteins have been mostly investigated using DSC (Tornberg, 2005), and DSC has been used to relate the denaturation of individual muscle proteins to the textural changes in meat caused by cooking (Martens et al., 1982; Findlay et al., 1986). The three major endothermic transitions seen in beef muscle, attributed to myosin (between 54 and 58 °C), collagen and sarcoplasmic proteins (between 65 and 67 °C), and actin (between 80 and 83 °C), have been associated with specific changes in beef texture (Wright et al., 1977; Findlay et al., 1986; Tornberg, 2005; Ishiwatari, et al., 2013). Due to its delicate texture, fish muscle requires milder cooking temperatures of between 35 to 50 °C to solubilize the muscle collagen (Espinosa et al., 2015). Ogawa et al. (1993) studied the thermal stability of myosin in different fish species over the range of 20 – 80 °C. The authors reported that fish myosin is thermally unstable compared to the myosin of mammals. Moreover, they found that myosin stability considerably differs among fish species and tends to have more than one denaturation peak. In addition, a high correlation between the denaturation enthalpy ($\Delta H$) and a decrease in $\alpha$-helicity ($\Delta h$) was observed. The DSC analysis of skipjack tuna muscle proteins showed that the first peak corresponds to myosin denaturation at 52 °C, the second peak corresponds to collagen at 59 °C, and the third peak corresponds to actin at 68 °C. Upon reaching their thermal denaturation temperatures, myosin and collagen caused changes in qualities of the cooked skipjack tuna muscle including moisture loss and toughness (Bell et al., 2001). Schubring (2008) studied the effects of heat (ranging from 30 to 70 °C) on DSC pattern, color, texture, and water holding capacity (WHC) of rainbow trout muscles. DSC curves revealed only a small peak for myosin at 40 °C, however at 50 °C, the myosin peak completely disappeared. The actin peak also
disappeared in the samples at 70 °C. The author also found that the color, texture, and WHC of trout muscle were strongly related to heat treatment. For example, the lightness of trout muscle increased when the temperature increased, the muscle firmness increased in the range of 40 – 70 °C, and WHC decreased at temperatures ranging from 30 to 60 °C. In tuna muscle (*Thunnus maccoyii*), myosin was denatured at temperatures between 35 – 51°C, while denaturation temperatures for actin ranged from 58 to 76 °C (Llave et al., 2018). For shellfish, the enthalpy of denaturation decreased significantly with increasing core temperature in shrimp cooked at temperatures ranging from 30 to 80 °C (Schubring, 2009). Myosin of shrimp muscle was fully denatured at 35 °C, as indicated by the complete disappearance of the myosin peak. Peaks of sarcoplasmic and stroma proteins were almost invisible at 50 °C, and the actin was fully denatured at 70 °C. In addition, a temperature of 80 °C caused a significant loss of shrimp muscle tenderness.

**1.3.2. Effects of sous-vide temperatures on muscle texture**

Fish and shellfish have an inherently delicate texture. Therefore, temperature control is needed when cooking seafoods to ensure optimal texture, particularly since muscle proteins and interactions between them are the basis for the mouthfeel of cooked muscle. Texture is one of the main quality characteristics of fish muscle. Tenderness and juiciness are important texture attributes. Muscle juiciness is highly affected by WHC (Hughes et al., 2014) and WHC is significantly influenced by the structural changes occurring in muscle proteins during cooking (Tornberg, 2005; Baldwin, 2012). Most of the tissue water (80 %) is located in the spaces between the myosin and actin filaments (Tornberg, 2005). Thus, shrinking or swelling of these filaments can significantly influence WHC of the muscle. WHC decreases are typically
associated with lower sensory juiciness (Hughes et al., 2014). In trout, shrinkage of myofibrillar proteins was found to be less at 65 °C than at higher temperatures (75 or 85 °C), which reduced water loss (Oz & Seyyar, 2016). Muscle tenderness is mainly attributed to the conversion of collagen to gelatin during normal cooking of fish muscle (Strasburg et al., 2008). It has been reported that meat toughness is due to actomyosin toughness and background toughness (Marsh & Leet, 1966; Findlay & Stanley, 1984; Ueno et al., 1999). Actomyosin toughness is due to myofibrillar protein denaturation whereas the background toughness is attributed to the connective tissue and other stromal proteins (Ueno et al., 1999). However, in fish muscle, myofibrillar proteins seem to contribute significantly more to the toughening process than connective tissue since fish muscle contains a relatively high concentration of myofibrillar proteins (70 – 90 %) and a low concentration of connective tissue (3-10 %) compared to the myofibrillar protein (39 – 68 %) and connective tissue protein (16 – 28 %) content in muscle of land animals (Haard, 1992). Llave et al. (2018) studied the effects of thermal protein denaturation on quality characteristics, such as texture and color, of tuna (Thunnus maccoyii) using sous-vide cooking at temperatures of 50, 60, and 70 °C for 30 min. The authors found that texture values (breaking strength) significantly decreased at 70 °C compared to the other two treatments. These lower breaking strength values at 70 °C were thought to be due to the high degree of actin denaturation. In general, during sous-vide cooking in the range of 50 to 65 °C, muscle tenderness increases most likely due to: 1) the solubilization of the connective tissue resulting in tenderization of sous-vide cooked products (García-Segovia et al., 2007), 2) the change from a viscoelastic to an elastic material and the aggregation and gelation of sarcoplasmic proteins (Baldwin, 2012; Tornberg, 2005), 3) the hydrolysis of myofibrillar proteins by the endogenous proteolytic enzymes (Bouton & Harris, 1981; Erthjørg et al., 2012),
and 4) water retention in the muscle structure at these low temperatures (Hughes et al., 2014). To achieve optimum textural attributes for sous-vide products, cooking temperature should be set at a value high enough for collagen solubilization and low enough for massive myofibrillar shrinkage (Ruiz et al., 2013). For example, heating at a temperature between 55 and 65 °C can contribute to solubilizing collagen resulting in gelatin formation and leading to the tenderness of cooked meat. Meanwhile, these temperatures minimize myofibrillar toughening due to shrinking of the myofibrils when cooked at temperatures above 65 °C. However, microbiological inactivation should be considered when selecting appropriate sous-vide cooking temperatures.

1.3.3. Effects of sous-vide temperatures on microbial shelf-life

In general, the shelf-life of sous-vide cooked products depends on both the temperature-time treatment and the storage temperature and can range from 6 to 42 days (Schellekens, 1996; González-Fandos, 2005; Diaz et al., 2009). Sous-vide cooked products can be classified into three groups depending on the thermal treatment applied: 1) lightly processed, 2) pasteurized, short shelf-life chilled food, and 3) long shelf-life chilled food products (Stringers & Metris, 2018). The lightly processed group includes foods that receive a heat treatment that is not enough to ensure inactivation of pathogens. Therefore, if pathogens are present in raw seafood, they may survive after cooking. The pasteurized, short shelf-life chilled food group includes products that receive a heat treatment that can destroy all vegetative pathogens to an acceptable level. FDA (2020) requires that a 6-log reduction of L. monocytogenes should be achieved since these bacteria are the most heat resistant vegetative pathogen. For example, if the food were pasteurized for Salmonella species instead of Listeria then the growth of Listeria would limit shelf-life to less than 7 days at storage temperatures ranging from -0.4 to 5 °C (FDA, 2020;
Baldwin, 2012). The long shelf-life chilled food products group receives a heat treatment that can achieve a 6-log reduction of non-proteolytic *C. botulinum*. Strains of *C. botulinum* can be classified into two groups (FDA, 2020): the proteolytic (do break down proteins) strains including *C. botulinum* type A and some of types B and F and the nonproteolytic (do not break down proteins) strains including *C. botulinum* type E and some of types B and F. Non-proteolytic *C. botulinum* type E is often associated with food products from aquatic environments (Hauschild & Dodds, 1993), and is the primary pathogen of concern for sous-vide products since it can grow in the absence of oxygen at chilled temperature. Seafoods are very heat sensitive products. When applying the high thermal treatment (90 °C for 10 min or equivalent based on FDA recommendations) necessary to reach a 6-log reduction of non-proteolytic *C. botulinum*, seafood products can experience unacceptable changes in quality properties, such as texture, flavor, appearance, and nutritional value (González-Fandos et al., 2005; Baldwin, 2012). Therefore, lower heat treatments are preferable, however in that case additional hurdles should be incorporated (González-Fandos et al., 2005). In addition, the FDA (2020) requires two hurdles to control non-proteolytic *C. botulinum* in seafood products. Storing sous-vide cooked products at temperatures below 3.3 °C can be a potentially effective hurdle for non-proteolytic *C. botulinum* type E (FDA, 2020), however if temperature abuse occurs then bacterial growth and toxin formation can result (Peck et al., 2011). Therefore, where refrigeration is the sole barrier to prevent toxin formation, the FDA (2020) requires adequate temperature control and a thermal history for any hermetically sealed, reduced oxygen package containing seafood. Time-temperature indicators (TTI) are currently being used to monitor thermal histories of seafood products (Endoza et al., 2004). TTIs must be applied to each of the smallest package units (the units of packaging that will not be distributed any further; usually the
Sous-vide cooking can retain the microbiological quality and effectively extend the shelf-life of seafoods at storage temperature below 3 °C. Nayti (2000) studied the shelf-life and microbiological quality of different types of food products including beef, lamb, chicken, and fish that were sous-vide cooked at 80 °C (to achieve an internal product temperature of 70 °C for 2 min) then stored at 3°C for five weeks. The author reported that all sous-vide cooked products showed negligible microbial growth by the end of the fourth week and were organoleptically acceptable throughout the storage period. In shrimp, sous-vide cooking at 90 °C for 4 min extended the shelf-life of products for up to 28 days during storage at 1 – 2 °C (Mohan et al., 2016). The microbial counts did not exceed the upper acceptability limit (7 log CFU/g) for freshness throughout the 28-day storage period, while air-packed and vacuum-packed raw shrimps exceeded the freshness limit on day 11 and 18 respectively, indicating the effectiveness of the heat process employed. In the same study, an approximately 3 log reduction in total mesophilic count was observed for sous-vide cooked shrimp. In finfish, sous-vide cooking effectively extended the shelf-life of salmon products. Aerobic and anaerobic spore-forming bacteria were inhibited in salmon slices sous-vide cooked at 90 °C for 15 min and stored at 2 °C for up to 45 days (González-Fandos et al., 2005). Additionally, *Staphylococcus aureus, Bacillus cereus, Clostridium perfringens,* and *Listeria monocytogenes* were not found in any of the salmon slices. Similar results for inhibition of aerobic and anaerobic spores were previously reported in rainbow trout sous-vide cooked at 90 °C for 3.3 min (González-Fandos et al., 2004). Although the authors in both salmon and trout studies did not detect any pathogens in their
samples, none of the products had been inoculated with pathogens. Therefore, the studies did not actually show pathogen inactivation based on the sous-vide cooking parameters applied.
1.4. Research needs

The American lobster is a highly valued seafood product with delicate meat and a very short shelf-life. Sous-vide cooking and HPP are promising techniques for the development of high-quality, refrigeration-stable, and convenient-to-use seafood products without the use of preservatives and other additives. Sous-vide cooking is reported to provide evenly cooked seafood products with a succulent and juicy texture compared to conventional cooking methods (Ayub & Ahmad, 2019). The application of HPP may help support the expansion and convenient distribution of prepackaged lobsters for subsequent sous-vide cooking to retailers, restaurants, or home cooks without the substantial concern for burden of live shipment and subsequently short shelf-life. In addition, the application of HPP has the potential to meet the increasing global demand for raw and minimally processed seafood products, including lobster, in a wide variety of popular dishes such as sushi, leading to the creation of new markets for American lobster products. Moreover, sous-vide ready-to-eat products processed following inadequate thermal processes may allow microbial growth consequently limiting their refrigerated shelf-life. The combination of HPP pretreatment and sous-vide has the potential to increase the refrigerated shelf-life and safety of novel ready-to-eat seafood products. The impacts of HPP on raw or subsequently sous-vide cooked lobsters have not been reported. Therefore, research is needed to understand and assess the impacts of HPP on the physiochemical, microbial, and sensory qualities of refrigerated, raw and subsequently sous-vide cooked lobsters.

1.5. Objectives

The overall objective of this research was to evaluate the use of HPP and sous-vide cooking for the development of high-quality, convenient-to-use, and refrigeration stable lobster
products. The specific objectives were to: 1) evaluate the impact of three different sous-vide cooking conditions on physicochemical properties and consumer acceptability of lobster tails, 2) evaluate the effects of HPP application on physicochemical properties of vacuum-packaged raw and of subsequently sous-vide cooked lobster tails, and on the consumer acceptability of the subsequently sous-vide cooked lobster tails, and 3) determine the effects of HPP on the refrigerated shelf-life of vacuum-packaged raw and subsequently sous-vide cooked lobster tails.
CHAPTER 2

EFFECTS OF SOUS-VIDE COOKING ON PHYSICOCHEMICAL PROPERTIES AND CONSUMER ACCEPTABILITY OF LOBSTER (Homarus americanus) TAILS

2.1. Introduction

Globally, seafood consumers are increasingly seeking high quality, safe, and minimally processed products with characteristics approaching those of fresh products (James & James, 2014), and improving the sensory quality of prepared seafoods to meet consumer expectations is a major challenge for gourmet chefs (Keller et al., 2008). Seafoods have delicate muscle structure and are prone to overcooking when using conventional cooking methods, often resulting in tough, dry products. The quality of cooked muscle foods, such as fish and shellfish, is determined by their texture, color, water retention, and flavor (Ishiwatari et al., 2013). American lobsters are expensive menu items in restaurants worldwide. These high-value, nutritious crustaceans are more sensitive to thermal treatment than red meat, and appropriate control of cooking temperatures can help to obtain high quality lobster products (Ayub & Ahmed, 2019). Sous-vide (SV) processing, the temperature-controlled cooking of vacuum packaged raw foods, represents a growing trend in the food services industry due to the superior quality products prepared (Baldwin, 2012). During sous-vide cooking, the vacuum-packaged food is heated to the exact optimal temperature using a water bath or steam convection oven (Schellekens, 1996; Baldwin, 2012; Llave et al., 2017). Numerous articles have reported the benefits of the sous-vide method on muscle foods, particularly those with delicate texture such as seafood (Keller et al., 2008; Baldwin, 2012; Kilibarda et al., 2018; Ayub & Ahmed, 2019). According to the authors, sous-vide cooking results in evenly cooked and extremely tender and
juicy products, as compared to conventional cooking methods. According to Rodgers and Young (2008), sous-vide cooked lobster meat better retained natural texture characteristics than those lobsters conventionally cooked. Tenderness and juiciness of meat are highly correlated and are important textural attributes of fish and shellfish (Ayub & Ahmed, 2019), valuable to both chefs and gourmet consumers alike.

Changes in texture characteristics are associated with changes in muscle proteins (Ayub & Ahmad, 2019). The thermal denaturation of muscle proteins including myosin, sarcoplasmic, collagen, and actin typically occur during cooking of meat (Tornberg, 2005; Baldwin, 2012). Myosin and actin are the major components of myofibrillar protein (Ishiwatari et al., 2013) and cooking meat to temperatures approaching 80 °C causes toughening of myofibrillar proteins due to shrinking of the myofibrils and associated loss of water (Tornberg, 2005). Temperatures of between 50 – 75°C are reported to be usually applied to sous-vide cooked seafood and meat (Kilibarda et al., 2018), however heating to above 65 °C can result in tougher meat (Tornberg, 2005; Baldwin, 2012). In addition, 54.4 °C is the lowest temperature recommended for sous-vide cooking to control all non-spore forming pathogens (Baldwin, 2012; FDA, 2020).

In the food service industry, various time-temperature combinations for sous-vide cooked seafood are applied, however the impacts of these processing parameters on physicochemical and sensory qualities of seafood have not been reported. In this study, three different cooking temperatures of 55, 60, and 65 °C against equivalent time values (208, 45, and 10 min, respectively) were selected (see section 2.2.) to control the target food pathogen, *Listeria monocytogenes*, with the assumption that these sous-vide parameters would correspond with a minimal impact on lobster qualities. Two experiments were conducted to evaluate the potential
influences of sous-vide cooking on physicochemical properties and consumer acceptability of lobster products. The first experiment was designed to determine the effects of different sous-vide cooking parameters on selected meat quality attributes (moisture content, weight loss, water holding capacity (WHC), shear force, and instrumental color) and thermal stability (DSC pattern) of lobster muscle proteins. The objective of the second experiment was to evaluate the impacts of sous-vide cooking parameters on consumer acceptability of lobster tails, to inform the selection of the "best" sous-vide cooking parameters for use in subsequent objectives in this research.

2.2. Materials and Methods

To ensure safety of cooked products, the FDA requires time/temperature combination adequate for achieving a 6-log reduction of *L. monocytogenes* In this study, time and temperature combinations for the sous-vide cooking treatments were calculated based on the Fish and Fishery Products Hazards and Controls Guidance (FDA, 2020). Table A-3 in the guide provides 23 combinations corresponding to the length of time at a specific internal product temperature (\(z = 7.5 \, ^\circ\text{C}\)) needed to accomplish a six-logarithm reduction in the number of *L. monocytogenes*. A linear regression was built using data points provided in table A-3 to calculate the slope, y-intercept and the square of the correlation coefficient (\(R^2\)) or the coefficient of determination to measure how well the regression equation fits the data (Figure 2.1). The equation generated was \(y = 5 \times 10^9 e^{-0.0309x}\) with \(R^2 = 0.9984\) for the relationship between temperature in degrees Celsius (x) and time in minutes (y). The calculated equivalent time values were 208 and 45 min for a core product temperature of 55 and 60 °C, respectively. The core product temperature of 65°C had a corresponding time value of 9.3 min necessary to achieve a 6-log reduction of *L. monocytogenes*, but a time value of 10 min was used for this study as a conservative value.
2.2.1. Experimental design

Two experiments were conducted to assess the physicochemical quality and consumer acceptance of sous-vide cooked lobster tails. For the consumer acceptance experiment, one hundred approximately 4 to 5-ounce, fresh raw soft-shell lobster (*Homarus americanus*) tails were purchased from Maine Fair Trade Lobster (Prospect Harbor, ME, USA) in October 2016, while two hundred tails were purchased in June 2019 for the physicochemical quality experiment. Tails were hand-shucked, vacuum packed, and sous-vide cooked to achieve a core temperature of 55 °C for 208 min, 60 °C for 45 min, or 65 °C for 10 min (Figure 2.2). Sous-vide cooked samples were compared to tails boiled in-shell for 10 min. All cooked samples were evaluated for moisture content, weight loss, water holding capacity (WHC), shear force and color (L*, a*, b*), salt soluble protein (SSP) content, differential scanning calorimetry (DSC patterns), and sarcomere length. In addition, quality properties of vacuum-packed, shucked raw tails were
assessed. Each of the five treatments was prepared in triplicate. Sensory evaluation was conducted to determine consumer acceptability of sous-vide cooked treatments only.
Figure 2.2 Process flow.

1. Receiving lobster tails
2. Blanching
   - 90-92 °C hot water for 20 s, placed in ice-water for at least 2 min
3. Shucking (by hand)
4. Vacuum packaging
5. Packing in ice
   - -55°C/208min
   - 60°C/45min
   - 65°C/10min
6. Boiling (for 10 min)
7. No cooking “raw”
8. SV Cooking
   - 55°C/208min
   - 60°C/45min
   - 65°C/10min
9. Storing overnight (at 2 °C)
10. Analyses
   - Moisture content
   - Weigh loss
   - Water holding capacity (WHC)
   - Salt soluble protein content
   - Shear force
   - Color (L*,a*,b*)
   - DSC
   - Sarcomere length
   - Consumer acceptability (only SV treatments)
2.2.2. Sample preparation

Fresh raw lobster tails were hand-shucked after 20 sec immersion in 90 to 92 °C tap water to facilitate shell removal. Based on a preliminary experiment, this process facilitated shell removal without apparent physical change to the tail meat which still appeared translucent and raw. Also, Getchell and Highlands (1957) reported that heat treatment for 70 sec in a 91 °C 2 % salt brine could help to extract the meat easily from the shell, while keeping the meat in as raw a state as possible. Blanched tails were hand-shucked using kitchen shears and dissecting scissors (Figure 2.3). Tails were cut directly down both sides of the shell without damaging the meat, then peeling back the middle bottom shell section. After shucking, the weight and thickness of forty lobster tails were measured by using a digital scale and digital caliper, and ranged from 61 to 83g, and from 12.45 – 19.38 mm, respectively.

Figure 2.3. Shucked lobster tail

Shucked tails were packed under 99 % vacuum (Model UV550, Wichita, KS, USA) in 3.2 mil plastic bags (3.3 cm³/100 in² oxygen transmission rate, 80 micron, 100 °C tolerance; UltraSource, Kansas, MO, USA) (Figure 2.4). The bags were labelled with the following codes: Raw, SV 55, SV 60, and SV 65. There were six bags per treatment (2 bags per treatment
replicate) and each bag contained five tails (n=30 per treatment). Thirty in-shell tails were used for the boiling treatment. An additional six tails per treatment were prepared to be used for recording sample core temperature throughout the cooking processes. All sample bags containing tails were completely covered by ice in 70-quart coolers (Coleman, USA) and stored in a walk-in refrigerator at 2 °C until subjected to further processing on the next day.

![Vacuum packaged lobster tails ready for sous-vide cooking.](image)

**Figure 2.4. Vacuum packaged lobster tails ready for sous-vide cooking.**

### 2.2.3. Sous-vide cooking

Sample bags containing shucked lobster tails were randomly cooked in a polycarbonate processing vessel (5-gal Storplus™; Carlisle, OK) using an immersion circulator (Sous-vide™ Professional Creative, PolyScience, Niles,IL) with a temperature control proficiency of ± 0.05 °C. The water bath was set to 55, 60, or 65 °C. Warm tap water (46 °C) was poured into the processing vessel until the maximum water level mark of the circulator unit was reached (approximately, 16 L volume). Once the circulator was switched on, the pump started to circulate the water. The direction of the water flow was tested by squeezing a few drops of food coloring into the water bath. The flow was observed to be horizontal. To check heat stability and uniformity in the water bath, after the desired temperature was reached, the temperature values
recorded at various locations in the water bath on the data logger were compared to the
temperature values shown on the circulator display panel. This step was repeated three times.
Once the water bath reached the set point temperature, two sample bags per treatment replicate
were positioned on a wire rack that was placed in the processing vessel ensuring that the sample
bags were completely submerged in the water bath. The processing vessel was covered with a
plastic serving tray to reduce water loss by evaporation (Figure 2.5).

Figure 2.5. Cooking lobster tails sous-vide.

To monitor core temperatures of the samples, two K-type thermocouple probes (Omega,
Stamford, CT) were inserted into the center of the thickest part of two tails that were individually
vacuum packed (Figure 2.6). Closed-cell foam tape (~2 x 3 x 1 cm) (ThermoWorks, Salt Lake
City, UT) was used to maintain the seal and vacuum of each thermocouple bag during cooking.
Thermocouple probes were attached to a data logger thermometer (RDXL4SD, Omega,
Stamford, CT) and temperatures were recorded every 30 secs throughout the entire cooking
process. To ensure accuracy of the temperature values, K-type thermocouple probes were
calibrated before each experiment using boiled water and ice water (2:1, v/v). The temperature
was within +/- 0.5 °C of 100 °C for boiling and 0 °C for ice water slush, with the probe
contacting neither the sides nor the bottom of the containers. Once the core lobster temperatures reached 55, 60, or 65 °C, they were processed for 208, 45, 10 min, respectively. After cooking, bags were promptly immersed in an ice:water slush (2:1 v/v) to bring the meat core temperature to ≤ 2.7 °C. Rapid cooling to less than 4 °C within 90 min is recommended to control microbial growth (Tansey and Gormley 2005). Cooked sample bags were packed in ice in 70-quart coolers (Coleman, USA) and stored in the walk-in refrigerator at 2 °C until subjected to analyses.

![Thermocouple probe inserted into the center of the thickest part of the tail for monitoring sous-vide cooking temperatures.](image)

**Figure 2.6. Thermocouple probe inserted into the center of the thickest part of the tail for monitoring sous-vide cooking temperatures.**

### 2.2.4. Boiling processing

Lobster tails were conventionally cooked according to a modified method of Dagbjartsson & Solberg (1973). In-shell lobster tails were cooked by boiling in 30 quarts of 1.5% salt solution (one tablespoon kosher salt per quart water) in a steam-jacketed kettle located in the Matthew Highlands Pilot Plant (Orono, ME). After water was brought to a rolling boil, five tails (at a time) were placed inside a boil basket, then submerged in the boiling water for 10 min total thus bringing the core temperature to 80-85 °C (Dagbjartsson & Solberg, 1973). These temperatures are reported to achieve a six-log reduction of *L. monocytogenes* (FDA, 2020). After cooking, tails were immediately cooled in an ice bath for approximately 30 min.
Boiled shell-on tails were packed on ice in coolers and stored in a walk-in refrigerator at 2 °C until subjected to analyses. The boiled tails were shucked prior to analyzing.

2.2.5. Weight loss

Weight loss was quantified as the liquid released after processing from fresh or cooked meat (known as “purge”). One bag per treatment replicate, containing five tails, was drained to remove any excess liquids, and then used for the weight loss determination. The percentage of cumulative weight loss resulting from the liquid of five lobster tails released after processing was calculated as:

\[
\% \text{ Weight loss} = \frac{\text{initial sample wt (g)} - \text{processed sample wt (g)}}{\text{initial sample wt (g)}} \times 100
\]

2.2.6. Moisture content

Lobster meat was ground and homogenized using a food processor (Oster FPSTMC3321-015-NP2, Sunbeam Products, USA) for 15 secs, stirred and then ground for an additional 15 secs. Moisture content (%) was determined gravimetrically by drying samples (5 g) of ground lobster overnight in a 105 °C oven (Fisher Isotemp, Barrington, IL) (AOAC, 2005). Each of three treatment replicates (n=3) was evaluated in duplicate samples, and values were averaged.

2.2.7. Water holding capacity (WHC)

Water holding capacity (WHC) is the ability of meat to retain its own water even though external pressures, such as gravity or heating are applied to it (Huff-Lonergan & Lonergan, 2005). WHC of meat samples was determined in duplicate per treatment replicate (n=3) according to a modified method of Jiang et al. (1985). Lobster tails (n=3) were cut into cubes weighing 2 g, wrapped in two pieces of pre-weighed Whatman #1 filter paper, placed in 50 mL
test tubes, and then spun at 1000 x g for 15 min in a bench top centrifuge (model 5430, Eppendorf, Hamburg, Germany). Following centrifugation, the filter paper was re-weighed, and the difference in weight was recorded. WHC was calculated as the percent of water retained by the meat with respect to water present in meat prior to centrifugation using the following equation:

\[
\frac{\text{[\% moisture x sample wt. (g)]} - \left\{ \text{[final paper wt.(g)]} - \text{[initial paper wt. (g)]} \right\} \times 100}{\text{[\% moisture x sample wt.(g)]}}
\]

2.2.8. Salt soluble protein content

Salt soluble protein (SSP) content was obtained by using 5 g ground tail meat (sample preparation explained in section 2.2.7.) homogenized with 95 mL of cold 5 % NaCl solution for 60 secs using a Waring blender. The homogenate was centrifuged at 12,000 x g for 20 min at 2 °C in a bench top centrifuge (model 5430, Eppendorf, Hamburg, Germany) according to a modified method of Work et al. (1997). The collected supernatant was then used for determining soluble protein content according to the method of Lowry et al. (1951). Solution A (2 % anhydrous Na₂CO₃ in 0.4 % NaOH), Solution B (1 % cupric sulfate 5H₂O), Solution C (2.7 % sodium potassium tartrate), Solution D (100 mL solution A + 1mL solution B + 1 mL solution C), Solution E (diluted 1:1 v/v Folin-Ciocalteu phenol reagent), and a series of bovine serum albumin standards were used for calculating protein concentrations from measured absorbance at 700 nm using a UV/Vis spectrophotometer (DU 530, Beckman Coulter, Fullerton, CA). Results were expressed as mg salt soluble protein/g tail meat. Each determination was carried out in duplicate.
2.2.9. Color

A colorimeter (LabScan XE, Hunter Labs, Reston, VA, USA) was used to measure differences in color among treatments. Twenty mm long portions (n=10) per treatment replicate were cut from the central region of each lobster tail. Each sample was measured in a 50 x 9 mm polystyrene petri dish (BD Falcon Steril, VMR, Corning, USA) (Figure 2.7.A). The colorimeter was standardized using white and black tiles for a port size of 30.5 mm, area view of 12.7 mm, and an illumination of 10° (D65, Hunter Labs, Reston, VA). The Hunter L*, a*, b* values of the ventral side of the tail were recorded as the average of three (initial and rotated 120° twice) readings per sample by the colorimeter software (Universal, version 4.10, 2001, Hunter Labs, Reston, VA).

2.2.10. Texture

Following color analyses, texture measurements were performed using a calibrated texture analyzer platform (TA-XTi2, Texture Technologies Inc., Scarsdale, NY, USA) with a 5 kg load cell. Lobster portions (n=10 per treatment replicate), 2 cm in length were sheared perpendicularly to muscle fibers (Figure 2.7.B). Each portion was positioned with the red colored dorsal surface of the sample facing upward so the Warner-Bratzler blade (TA-42 knife blade with 45° chisel end) cut through the muscle fibers. The texture analyzer was configured to a 90 % depth, a 2 mm/s test speed, and a trigger force of 0.049 Newtons (N). The maximum peak force (N) required to shear through the sample was recorded as shear force and averaged by the texture analysis software (Exponent 32, version 5.0.6.0 2010, Texture Technologies Inc., Scarsdale, NY).
2.2.11. Differential scanning calorimetry (DSC)

Thermal denaturation temperatures and enthalpies of the lobster muscle proteins were analyzed by DSC. Tail meat (5-15 mg) from the core central region of the lobster tail muscle was accurately weighed into aluminum pans and sealed hermetically (T0 pans and lids, TA instruments, New Castle, DE). An empty pan was used as the reference. Samples were heated and scanned from 5 to 105 °C at a rising rate of 2 °C/min using a differential scanning calorimeter (DSC Q2000, TA instruments, New Castle, DE). Onset temperature (T_onset), peak temperature (T_max), and enthalpy (ΔH) were estimated using the Universal Analysis 2000 software (v5.5.24, TA instruments, New Castle, DE). T_onset represents the initiation of protein unfolding. The protein denaturation temperature (T_max) was estimated from the peak temperature of the thermal transition. The enthalpy (ΔH) of protein denaturation was measured as area under the curve, expressed as (J/g). Samples were analyzed in triplicate per treatment.

2.2.12. Sarcomere length (SL)

Histological preparation for muscle tissues was performed according to Prophet et al. (1992). Approximately 0.5 cm thickness of longitudinal section tissue was removed from the
core central region of the lobster tail. Tissues were fixed with 10 % buffered formalin fixative to prevent tissue destruction. Fixed tissues were processed in separate runs on an automated tissue processor (TP1020, Leica-microsystems, Wetzlar, Germany). Samples were embedded in a paraffin bath and sectioned into 5-micron thick sections which were placed onto microscope slides and stained with hematoxylin and with eosin. Sarcomere length (SL) was measured according to a modified method of Williams et al. (1986). A drop of immersion oil was placed on top of the coverslip and the slide was placed under a microscope (LABOMED Lx400, Labo America Inc, CA, US) at 1000X magnification. The images were captured by a digital camera (5.0 MP, iVu 3100, LABOMED, Labo America Inc, CA, US) connected to microscope using software (PixelPro V2.8, LABOMED, Labo America Inc, CA, US). SL measurements were performed using an image analysis software package (Digimizer, version 5.4, MedCalc Software bvba, Ostend, Belgium) to determine the length of ten measurements of a five-sarcomere unit from five random areas on each slide (Figure 2.8) and average SL was expressed in micrometer (µm).

Figure 2.8. Light microscope picture (1000x) showing the five sarcomeres across measured as one unit.
2.2.13. Sensory evaluation

A total of one-hundred untrained participants, over the age of 18, who were interested in consuming lobsters were recruited via email and flyer notice (Appendix A) to assess acceptability of sous-vide cooked lobster tails. Panelists who were allergic to seafood or were not interested in consuming lobsters were requested to not participate in this study. Participants were asked to refrain from eating, drinking (except water), or smoking for a minimum of one-hour prior the test. Samples were served at room temperature (21 °C) with melted salted butter. A 9-point hedonic scale method (Peryam & Pilgrim, 1957) was conducted to measure the consumer acceptability of the sous-vide cooked (SV55, SV60, and SV65) samples. The questionnaire was distributed on paper ballots (Appendix B).

Samples were sous-vide cooked as explained in section 2.2.4. For sample preparation for consumer acceptance testing, bags of each sous-vide treatment were removed from the ice coolers. The initial core temperatures of the samples were below 2.7 °C. Core temperature was checked by inserting the thermocouple probes through the holes in the foam-tape attached to thermocouple bags. After the tails were removed from the bags, each lobster tail was cut into three portions. The weights of the portions ranged from 15 g to 20 g. These portions of lobster tails were large enough (over two bites) to be evaluated for mouthfeel. Portion sizes were determined based on preliminary estimates, as three people were asked in advance to assess the size of lobster tail portions.

Tail portions of each of the three sous-vide treatments (SV55, SV60, and SV65) with their juices were placed into 2-oz ceramic bowls and held at room temperature for approximately 30 min until serving to participants. Participants were seated individually in booths with fluorescent lighting at the Sensory Evaluation Center at the University of Maine. The three
products were labelled with 3-digit random codes and were served on a tray with a plastic fork and knife, a napkin, a cup of spring water, and melted salted butter (Figure 2.9). The butter was served because people commonly consume lobster with butter. Participants were instructed to evaluate samples, taking a sip of water (to cleanse their palate) after testing each sample, and to rate the acceptability of specific sensory attributes of the samples. A 9-point hedonic scale (from 1=“Dislike Extremely” to 9=“Like Extremely,” with 5=“Neither Like Nor Dislike”) was used to assess acceptability of texture, flavor, color, aroma, and overall liking of samples. Participants were asked to answer a set of questions relating to demographic characteristics, purchasing frequency, and their attitudes toward consuming lobsters. A section for comments was also provided on the questionnaire. Participants were pre-scheduled to show up at thirty min intervals between 11:00 am and 4:00 pm. There were ten participants per testing interval. Participants were requested to read an informed consent form (Appendix C) before taking the test. Responses were collected anonymously. At the end of the test, participants were compensated with $5 cash for their participation. The consumer acceptability study was approved (application number, 2016-10-10; approval date, October 18th, 2016) by the University of Maine Institutional Review Board (IRB) for the Protection of Human Subjects.

Figure 2.9. Sous-vide cooked lobster tail ready for consumer acceptance test.
2.2.14. Statistical analysis

Data were analyzed using SPSS 26 (IBM, Armonk, NY, USA) at a significance level of $P<0.05$. Outliers were identified by SPSS based on the rule of $3 \times$ interquartile range (IQR). The Shapiro-Wilk test was used to assess normality and Levene’s equality of variances test was used to assess homogeneity. One-way analysis of variance (ANOVA) was used to assess all one-level (treatment) effects. Separation of treatment means was accomplished using Tukey’s honest significant difference (HSD) post hoc test. Pearson’s correlation was performed to evaluate correlations among variables.

2.3. Results and Discussion

2.3.1. Time-temperature profiles during sous-vide cooking

The internal temperature profiles of lobster tails during sous-vide cooking and cooling processes are shown in Figure 2.10. During the sous-vide cooking process, the water temperature dropped by about 4.0 °C after chilled sample bags were placed into the water bath. However, the water temperature recovered to the desired level of 55, 60, and 65 °C within approximately 5 min. The maximum come-up time to reach the desired core temperatures of 55, 60, and 65 °C ± 0.5 °C were approximately 27, 26, and 35 min, respectively. After sous-vide cooking, the time required to cool down the lobster tails to ≤ 2.7 °C were 39, 32, and 31 min for 55, 60, and 65 °C, respectively.
Figure 2.10. Representative graph of core temperature profile of lobster tails during cooking at (A) 55°C/208min, (B) 60°C/45min, and (C) 65°C/10min followed by cooling to below 2.7 °C.
2.3.2. Moisture content

The mean moisture content of the raw tails was 82.7 ± 0.7 %. This moisture content value was approximately 4 % to 6 % higher than moisture content of raw American lobsters reported by Dagbjartsson & Solberg (1973) and Calder et al. (2006). No significant differences were observed among the cooking treatments. Mean moisture contents for cooked tails were 80.3 ± 0.3 % for boiled, 80.7 ± 0.2 % for SV 55, 80.8 ± 0.4 % for SV 60, and 80.7 ± 0.5 % for SV 65 (Figure 2.11). Similarly, there were no significant differences found among mean moisture content of salmon slices that were sous-vide cooked at 65 °C for 5 min, 90 °C for 10 min, and 90 °C for 15 min.

After thermal treatment, the moisture content of cooked lobster tails was lower by approximately 2 % compared to the raw tails (Figure 2.11). Similar moisture content reduction after cooking (approximately 2 %) was reported by Dagbjartsson & Solberg (1973). The authors found that the moisture content of lobster tails boiled for 10 min in 2.5 % sodium chloride (NaCl) brine solution was 76.9 %, while moisture content was 78.8 % in raw lobster tails. They also found that thermal treatment reduced the ability of the proteins to retain water.

Sous-vide cooking is known to decrease meat exposure to dehydration compared to conventional cooking methods due to low cooking temperature, uniform heating, and vacuum packaging (Baldwin, 2012; Ayub & Ahmad, 2019). However, our results revealed no significant differences in moisture content between boiled and sous-vide cooked treatments. Our results were in contrast to Jeong et al. (2018), who reported that moisture contents were significantly higher in pork samples sous-vide cooked at 61 °C (for 46 or 90 min) or at 71 °C (for 46 min).
compared to the air-packaged controls cooked for 45 min in boiling water. This conflict among studies could be because lobsters were directly immersed and boiled in a salt solution.

**Figure 2.11. Moisture content of lobster tails by treatment.**

![Moisture content of lobster tails by treatment](image)

Each bar represents the mean value ± standard deviation (n=3). Treatments not sharing an uppercase letter are significantly different ($P < 0.05$) based on one-way ANOVA followed by Tukey’s HSD post hoc test.

* Moisture content of the raw sample was not statistically compared to cooked treatments.

Dilute salt solutions can cause muscle proteins to absorb and retain water while they are cooked (Offer & Trinick, 1983). In addition, salt compounds, such as sodium tripolyphosphate (STPP) have been reported to exhibit a cryoprotective effect in lobsters by increasing moisture retention and increasing the ability of lobster protein to reabsorb liquid when thawed (Calder et al., 2006; English et al., 2019). Reduced cooking time could be another reason for moisture retention in lobster compared to pork as tails were boiled for approximately 10 min while pork was boiled for 45 min. In false abalone (*Volutharpa ampullacea perryi*) heated at 95-100 °C, significant decreases in moisture content were observed with an increase in heating time (5, 10, 15, 30, and 60 min) (He et al., 2018). Generally, moisture content decreases during cooking due
to denaturation of muscle proteins that occurs during heating, consequently muscle fibers shrink and cause water loss. The extent of muscle shrinkage increases with temperature (Tornberg, 2005; Baldwin, 2012). However, boiling in salted water resulted in similar moisture retention in lobster muscle compared to those samples sous-vide cooked. Moisture retention has a great economic implication as processed seafoods are often sold by weight. Moreover, moisture retention is important to meat texture as higher moisture content can improve tenderness and juiciness of the muscle.

2.3.3. Weight loss

Raw lobster tails had an initial weight loss after packaging of 1.5 ± 0.3 % which was likely due to the 99% vacuum applied during packaging (Figure 2.12). All cooked lobster tails experienced a loss of weight (15-20 %) compared to raw tails due to cooking induced shrinkage of muscle proteins and consequent water loss (Keller et al., 2008; Baldwin, 2012). The weight loss for the 65 °C/10 min treatment (18.9 ± 0.5 %) was significantly higher than for the boiled (15.7 ± 1.9 %), SV 55 (15.6 ± 0.7 %), and SV 60 (15.1 ± 1.0 %) treatments. Weight losses in sous-vide processed meats generally increase as the processing temperature increase. For example, pork samples sous-vide cooked at 71 °C showed significantly higher weight loss than those cooked at 61 °C (Jeong et al., 2018). In addition, beef sous-vide cooked at 60 °C had significantly lower weight loss than samples heated at 70 and 80 °C (Garcia-Segovia et al., 2007). Moreover, cook loss increased with increasing temperature in fresh cod heated at different temperatures in the range from 40 to 100 °C for 10 min (Skipnes et al., 2007). Between 40 and 60 °C the cook losses among cod samples were not significantly different but cook losses were significantly higher at 75 °C than at 60 °C. Above 60 – 65 °C the muscle fibers shrink
longitudinally and cause significant water loss, increasing almost linearly with temperature up to 80 °C (McGee, 2004; Tornberg, 2005; Baldwin, 2012). In addition, endomysium and primarily perimysium collagens shrink intensely at 60 – 70 °C. This shrinkage of the perimysium and endomysium forces water out and increases toughness (Foegeding et al., 1996).

Figure 2.12. Weight loss of lobster tails by treatment.

Each bar represents the mean value ± standard deviation (n=3). Treatments not sharing an uppercase letter are significantly different \((P < 0.05)\) based on one-way ANOVA followed by Tukey’s HSD post hoc test

* Weight loss of the raw sample was not statistically compared to cooked treatments.

Although increased cooking temperatures increase weight loss (Baldwin 2012), the conventional cooked (boiling at 100 °C) lobster tails did not have substantial weight loss compared to sous-vide cooking treatments. Our results contrasted those of Jeong et al. (2018) who reported that pork boiled at 100 °C experienced a significant weight loss compared to pork sous-vide cooked at 61 or 71 °C. However, lobster tails were boiled in salted water and the presence of the salt may have minimized the weight loss. The interactions of salt and muscle proteins can result in a greater water holding capacity in the muscle tissues, which then absorb
water from the brine (Offer & Trinick, 1983; McGee, 2004). Sodium triphosphate-treated lobsters had a significantly lower cook loss compared to controls (Calder et al., 2006). Water is not the only component released during cooking: other water-soluble components, such as sarcoplasmic proteins, solubilized collagen and vitamins, can also be released (Foegeding et al., 1996). Extreme liquid losses in cooked meat can critically impact meat quality, particularly tenderness and juiciness of muscle texture (McGee, 2004; Baldwin 2012).

2.3.4. Water holding capacity (WHC)

WHC of cooked tails ranged from 64.5 to 67.0 % and did not significantly differ between the control (boiled) and sous-vide cooked treatments (Figure 2.13). The initial WHC (raw tails) was 91.7 ± 0.4 %, approximately 29 % higher than the WHC of raw lobster tails (62.6 %) that was previously reported by Dagbjartsson & Solberg (1973). WHC of minced raw cod muscle was significantly lower than the whole cut sample likely due to severe changes in the muscle structure caused by the mincing process (Skipnes et al., 2007). Most of the water in the muscle is held in the spaces between the thick and thin filaments (Tornberg, 2005). This water is free to migrate throughout the muscle structure (Skipnes et al., 2007) and any changes in this spacing will affect the ability of myofibrils to hold water (Tornberg, 2005).

The reduction of WHC has also been reported to be temperature dependent (Schubring, 2008). After cooking, the WHC of the cooked tails in all cooking treatments decreased by approximately 27 % compared to raw tails. This reduction in WHC was due to thermal treatment as cooking induces structural changes due to shrinkage of both the filaments (thick and thin) which decreases the water holding capacity of the muscle (McGee, 2004; Baldwin, 2012).
Figure 2.13. Water holding capacity of lobster tails by treatment.

Each bar represents the mean value ± standard deviation (n=3). Treatments not sharing an uppercase letter are significantly different ($P < 0.05$) based on one-way ANOVA followed by Tukey’s HSD post hoc test.

* WHC of the raw sample was not statistically compared to cooked treatments.

Although different cooking treatments were applied to the lobster tails, no significant differences in WHC were detected among the treatments. Blikra et al. (2019) measured WHC of cod after cooking at 0-100 °C for 10 min. The authors found that from 25 to 40 °C, the WHC decreased until reaching a minimum of 67.0 % (WHC of raw fish was 81.3 %), while there were no significant changes in WHC observed from 40 to 90 °C. The authors proposed that the water holding capacity reached a plateau after the proteins were denatured. In rainbow trout muscle, the largest decrease in water-binding capacity during heating in the range of 30-70 °C was found at 30-50 °C and thus in the range in which the coagulation of myofibrillar proteins occurs (Schubring, 2008). In addition, within 2 to 30 min (depending on temperature) WHC decreased very rapidly and reached minimum values. This minimum WHC is in general reached faster at high temperatures (above 70 °C) compared to lower temperatures (Skipnes et al., 2011).
Boiling in a dilute salt solution may promote swelling of the fibers and enhance the ability of muscle to take up additional water (Offer & Trinick, 1983). Sodium chloride gives muscle the ability to take up additional water. A concentration of approximately 2-6% NaCl is used in the manufacture of meat products (Offer & Trinick, 1983). Gains in water are economically important since meat is sold by weight, and water uptake enhances juiciness and tenderness of meat.

2.3.5. Salt soluble protein content

The SSP content for raw tails was 85.6 ± 2.9 mg salt soluble protein/ g meat. The control SSP content (12.0 ± 0.3) was significantly lower by approximately 8% than the sous-vide cooked treatments which ranged from 19.9 to 21.4 (Figure 2.14). No significant differences were observed among the sous-vide cooked treatments. The results of the current study indicate that lobster myofibrillar proteins may be denatured or aggregated to form insoluble proteins more readily in the boiled than the sous-vide cooked samples, thereby decreasing their solubility.

In finfish including carp and threadfin bream (*Nemipterus bleekeri*), solubility of actomyosin started to gradually decrease at temperatures above 40 °C and reached a minimum at 80 °C (Sano et al., 1994; Yongsawatdigul & Park, 2003). The decrease in SSP content in carp and threadfin bream muscles was attributed to heat-induced protein conformational changes. During heating, conformational changes in proteins occur due to denaturation and aggregation which result from changes in protein structures (secondary, tertiary, and quaternary structure) (Nguyen et al., 2011). Conformational changes in proteins resulted in losses of hydrophilic surfaces and increased hydrophobic groups, leading to a decrease in SSP (Yongsawatdigul &
Park, 2003; Odoli et al., 2019). Yongsawatdigul and Park (2003) reported that the conformation of threadfin bream actomyosin began to unfold and expose the nonpolar amino acids at temperatures above 30 °C in a polar environment.

**Figure 2.14. Salt soluble protein content of lobster tails by treatment.**

![Figure 2.14](image)

Each bar represents the mean value ± standard deviation (n=3). Treatments not sharing an uppercase letter are significantly different (P < 0.05) based on one-way ANOVA followed by Tukey’s HSD post hoc test.

*Salt soluble protein content of the raw sample was not statistically compared to cooked treatments.*

In addition, although the aggregation of proteins causes them to be less soluble, it has also been assumed to make the muscle texture tough due to crosslinking of the myofibrillar proteins (Work et al., 1997). However, Calder et al. (2006) reported that although control lobster tails were lower in SSP levels than sodium tripolyphosphate (STP)-treated samples, sensory panelists did not observe any significant differences in texture among the treatments at month 2 of storage at -15 °C.
2.3.6. Texture

The average shear force value was 26.2 ± 1.1 (N) for raw lobster tails (Figure 2.15). For cooked tails, the average shear force value was significantly \((P < 0.05)\) higher for the boiled control (45.5 ± 3.4 N) than the sous-vide cooked treatments (38.5 – 39.5 N) indicating that sous-vide cooked lobsters were more tender than the control. In pork ham, a higher shear force value was reported for the control (100 °C for 45 min at 0 % vacuum) compared to the sous-vide cooked samples (at 61 °C or 71 °C for 44 min or 90 min at 96.58 % or 98.81 % vacuum).

Moreover, a significant loss of tenderness was observed in northern shrimp \((Pandalus borealis)\) when heated to a core temperature of 80 °C (Schubring, 2009). Tougher beef muscle at temperatures approaching 80 °C was attributed to the elastic modulus increases which required larger tensile stress to extend fractures (Tornberg, 2005; Baldwin, 2012). Muscle proteins shrink and decrease the distance between the crosslinks that form between the myosin and actin filaments and cause significant water loss (Baldwin, 2012). Muscle shrinkage and water loss result in increasing the elastic modulus, which results in tough meat. The elastic modulus drastically increases when actin starts to denature, while myosin denaturation has a small impact on the elastic modulus of meat (Ishiwatari et al., 2013). In addition, Jantakoson et al. (2012) boiled Black tiger shrimp \((Penaeus monodon Fabricius)\) at 100 °C for 2 min and reported that heat induced denaturation and aggregation of shrimp muscle proteins led to the shrinkage of both the thick and thin filaments and collagen. They proposed that the exposure of hydrophobic domains of myofibrillar protein allowed new intra- and interprotein interactions, consequently leading to more dense protein structure. In current study, correlations showed shear force had a strong negative correlation \((P<0.01)\) with SSP content, with a value of \(r = -0.72\). This correlation indicates that lower SSP content can serve as an indication of tougher lobster texture.
Figure 2.15. Shear force values of lobster tails by treatment.

Each bar represents the mean value ± standard deviation (n=3). Treatments not sharing an uppercase letter are significantly different ($P < 0.05$) based on one-way ANOVA followed by Tukey’s HSD post hoc test.

* Shear force of the raw sample was not statistically compared to cooked treatments.

Results indicated no significant differences in shear force values among the sous-vide cooked treatments (55 °C/208 min, 60 °C/45 min, and 65 °C/10 min). In contrast, Schubring (2009) reported that shear force values of deep-water pink shrimp (*Parapenaeus longirostris*) decreased significantly when cooked to core temperatures of 50 to 70 °C, indicating increased tenderness. Shrimp muscle proteins including myosin and actin heated to 70 °C were completely denatured, according to DSC measurements. In addition, tuna (*Thunnus maccoyii*) sous-vide cooked to a core temperature of 50, 60, and 70 °C experienced a lower breaking-strength ratio (which is an indicator of stress at which a specimen fails via fracture) at 70 °C than at 50 °C and 60 °C (LIave et al., 2017). These lower breaking-strength ratios at 70 °C were attributed to the complete denaturation of actin. Our results indicated that better preservation of lobster texture was obtained by sous-vide cooking than the conventional cooking method.
2.3.7. Color

There were no significant differences in L* (lightness) and a* (redness) among all cooking treatments (Table 2.1). Similarly, the whiteness of cod muscle after heating at 70, 80, or 90 °C was not significantly different among treatments (Skipnes et al., 2011). In control lobster tails, b* values (14.03±0.11) were significantly higher than in sous-vide cooked samples (9.26-10.43). Turner and Larick (1996) reported that chicken breasts sous-vide cooked to 77 °C were less yellow than those processed to 94 °C.

Table 2.1. Instrumental color (L*, a*, b*) values of lobster tails by treatment.

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Color</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L*</td>
<td>a*</td>
</tr>
<tr>
<td>Raw</td>
<td>43.59±1.36*</td>
<td>1.50±0.038*</td>
</tr>
<tr>
<td>Boiled</td>
<td>68.17±0.75a</td>
<td>4.96±0.70a</td>
</tr>
<tr>
<td>SV 55</td>
<td>67.15±2.32a</td>
<td>7.34±1.84a</td>
</tr>
<tr>
<td>SV 60</td>
<td>68.57±1.44a</td>
<td>5.69±0.57a</td>
</tr>
<tr>
<td>SV 65</td>
<td>68.62±0.29a</td>
<td>6.86±1.23a</td>
</tr>
</tbody>
</table>

Each bar represents the mean value ± standard deviation (n=3). Treatments not sharing a letter are significantly different (P < 0.05) within columns based on one-way ANOVA followed by Tukey’s HSD post hoc test. * Raw samples were not statistically compared to cooked treatments.

Higher b* values in control lobster tails can be explained by a higher rate in the non-enzymatic (Maillard) browning reaction between muscle proteins and reducing sugars at higher temperatures (Koomyart et al., 2017; Ayub & Ahmad, 2019). Interestingly, b* values for lobster tails showed a strong positive correlation (P < 0.01) with shear force and a strong negative correlation (P < 0.01) with SSP content with values of r = 0.73 and r = -0.94, respectively. This correlation indicates that higher b* values (more yellow) can serve as an indication of tougher
lobster texture. Calder et al. (2006) found a negative relationship between $b$ values and consumer acceptability ratings for exterior lobster meat color with values of $r = -0.75$, indicating that the yellower the lobster meat, the lower its consumer acceptability. Taken together, these correlations indicate that consumers may not like the color of tougher lobster meat, which would be interesting to clarify in future research evaluating the acceptability of boiled and sous-vide cooked lobsters.

After thermal treatment, lightness and redness of raw tails increased by approximately 64% and 25%, respectively. The increase in lightness of cooked tails may have been due to the effect of denaturation and aggregation of myofibrillar proteins associated with increased light scattering from the surface (Robb et al., 2000). Llave et al. (2017) reported that myosin denaturation was mainly responsible for changes in color and appearance of sous-vide cooked tuna, which starts to denature at temperatures around 35 °C. Regarding increased redness in tails after cooking, similar results were observed in black tiger shrimp (Jantakoson et al., 2012) and Pacific krill (*Euphausia pacifica*) (Koomyart et al., 2017) as the redness of cooked shrimp and krill increased compared to raw samples. The increase in redness was attributed to the release of astaxanthin from heat-denatured carotenoproteins (Lorenz, 1998). Astaxanthin is a red carotenoid pigment which interacts non-covalently with various proteins (Weesie et al., 1995), consequently crustaceans range in color from green, yellow, and blue to brownish. Astaxanthin content is higher in shell than in muscle of crustaceans (Venugopal & Gopakumar, 2017). In addition, Koomyart et al. (2017) reported that changes in the $a^*$ values correlated well with the astaxanthin content in krill.
Meat color is a critical visual factor that affects consumer perception of product quality and strongly impacts their purchasing decisions. Our results indicate that sous-vide cooking may promote better color attributes of lobsters than conventional cooking methods which can result in a less appealing color in lobster products.

2.3.8. DSC

The DSC thermograms of raw lobster tail showed two endothermic transitions (peaks) (Figure 2.16). For the first and second peak, $T_{onset}$ were $37.06 \pm 0.12$ and $55.06 \pm 0.49 \degree C$, $T_{max}$ were $40.17 \pm 0.08 \degree C$ and $58.18 \pm 0.19 \degree C$, and enthalpy ($\Delta H$) were $0.32 \pm 0.09$ J/g and $0.15 \pm 0.02$ J/g, respectively. Similarly, Sriket et al. (2007) found two endothermic peaks in black tiger shrimp and white shrimp ($Penaeus vannamei$). The authors reported that the two peaks corresponded to myosin (peak I) and actin (peak II). However, the $T_{max}$ of myosin and actin in both shrimp species were higher by approximately 10 \degree C than those in our samples. In addition, enthalpies of denaturation for myosin and actin in lobster muscle were lower than those of shrimp (1.40-1.46J/g for myosin; 0.66-0.67J/g for actin). The lower denaturation temperatures and enthalpies of lobster proteins indicate that myosin and actin are less stable and require lower temperatures and energies to unfold than those in shrimps. The lower stability of lobster myofibrillar proteins could be attributed to a correlation between the thermostability of muscle proteins and the environmental temperature in which the species live. For example, Poulter et al. (1985) reported that the denaturation temperature of myosin from warm-water species may be almost 20 \degree C higher than myosin from cold-water species. Ogawa et al. (1993) reported that thermal denaturation represented by the endothermic peaks of fish myosin comes mainly from breaking $\alpha$-helical structures.
In a different study, DSC thermograms showed three peaks in raw deep-water pink shrimp at 33.4, 50.4, and 57.9 °C (Schubring, 2009). The authors assumed these peaks corresponded to myosin (peak I), sarcoplasmic and stromal proteins (peak II), and actin (peak III). Compared to our results, \( T_{\text{max}} \) of myosin from deep-water pink shrimp were lower by approximately 7 °C, while the required \( \Delta H \) (1.14J/g) to denature myosin was higher by 0.82 J/g. However, the \( T_{\text{max}} \) (57.9 °C) and \( \Delta H \) (1.14J/g) for actin of deep-water pink shrimp were similar to those of the lobster muscle samples in the present study.

![Graph of DSC thermograms of lobsters by treatment.](image)

**Figure 2.16.** Representative graph of DSC thermograms of lobsters by treatment. Samples were heated and scanned from 5 to 105 °C at a rising rate of 2 °C/min.

After cooking, both peaks (myosin and actin) disappeared in all treatments as displayed by flat, straight lines in the DSC curves, indicating that myosin and actin were completely denatured. Surprisingly, actin completely denatured in sous-vide cooked tails at 55 °C, below its \( T_{\text{max}} \) as determined in the raw muscle (58.18±0.19 °C). The actin denaturation at 55 °C could be attributed to the long cooking time (208 min). Proteins can be denatured by temperatures below
their maximum thermal denaturation temperatures, if the samples are maintained at these temperatures for longer time (Vilgis, 2015).

During cooking, heat denatures the muscle proteins and consequently meat becomes more edible with acceptable texture (Pathare & Roskilly, 2016). However, excessive heating can result in undesirable meat qualities, such as a lack of tenderness or juiciness. Controlling the thermal denaturation of muscle proteins is crucial to reach the desired qualities, particularly for texture (Vilgis, 2015; Llave et al., 2017), because texture is a very important parameter for seafood products (Espinosa et al., 2015). Our results indicated that myosin and actin from lobster muscle were completely denatured by all cooking treatments. However, the higher SSP content and lower shear force values observed in sous-vide cooked samples compared to boiled samples indicate that the sous-vide cooking conditions used in this study can produce high quality lobster products.

2.3.9. Sarcomere length (SL)

Sarcomere length (SL) was significantly different among the treatments (Figure 2.17). Average SL of raw tails (5.25±0.11µm) was significantly shorter than SV 55 (6.92±0.89 µm), SV 65 (7.67±0.51 µm), and boiled (8.21±0.61 µm) treatments. However, no significant differences were observed between raw and SV 60 (5.31±0.63 µm) treatments, which could be due to muscle fibers shrinking longitudinally at temperatures above 60 °C, while shrinking transversely between 40 and 60 °C (Tornberg, 2005). Sarcomere length in American lobster muscle was reported to vary depending on contraction status or stretch force applied to the fiber and ranged from 2 to 20 µm (Govind, 1995). Our results indicated that at higher cooking
temperatures (boiling and SV 65) the sarcomeres were significantly expanded. These results were in contrast to Hearne et al. (1978) who reported that increased heating of bovine semitendinosus muscle fibers to internal temperatures of 40, 50, 60 and 70 °C resulted in a decrease of sarcomere length. However, Bendall and Restall (1983) reported no change in sarcomere length when muscle fibers were heated in aqueous medium to final temperatures of 40 to 90 °C.

**Figure 2.17. Sarcomere length of lobsters by treatment.**

![Sarcomere length of lobsters by treatment](image)

Each bar represents the mean value ± standard deviation (n=5). Treatments not sharing an uppercase letter are significantly different ($P < 0.05$) based on one-way ANOVA followed by Tukey’s HSD post hoc test.

The increase in sarcomere length in lobster muscle at higher temperatures could be attributed to increased water absorption by proteins. Leander et al. (1980) reported that after cooking bovine muscles to an internal temperature of 63 °C swelling of perimysial connective tissue occurred, and that increasing the internal temperature to 68 °C resulted in more swelling in the A band. At 70 to 90 °C swelling of the perimysium seems to occur (Tornberg, 2005). In
addition, myofibrils can swell to more than twice their original volume when immersed in 0.8 M salt solutions (Offer & Trinick, 1983).

In general, there is a negative correlation between sarcomere length and meat toughness in raw and cooked muscle of land animals (Strasburg et al., 2008; Starkey et al., 2016). In contrast, our results showed a strong positive correlation ($P < 0.05$) between sarcomere length and shear force, with an $r = 0.55$ indicating that the longer sarcomeres in cooked samples could be related to tougher muscle, consequently influencing the eating quality of cooked lobster meat.

2.3.10. Sensory evaluation

Among all participants ($n=100$), 70 % were female and 30 % were male (Table 2.2). Since the sensory evaluation was conducted on the University of Maine campus, most participants (56%) were younger age (18-24 years old) and 45% of participants reported that they consume lobsters 1-2 times a year. Younger age and lower income have been associated with lower seafood (fish + shellfish) consumption in the U.S. (Jahnas et al., 2014). The gender of participants did not affect ($P > 0.05$) the overall ratings for all sous-vide cooked products as females and males gave overall liking scores between 6.7 and 7.1. In addition, there were no significant differences among the age categories (18-24, 25-34, 35-44, 45-54, 55-64, >65) for overall liking (6.0-8.1) of all sous-vide cooked products. Eighty-seven percent of the participants reported that they typically consume boiled rather than baked, fried, and grilled lobsters. Seventy-one percent of participants indicated that flavor is the most important sensory characteristic of lobster tails, followed by texture (25 %).
Table 2.2. Demographics, frequency, and attitudes toward consuming lobsters of sensory participants.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Female</th>
<th>70%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>30%</td>
</tr>
<tr>
<td></td>
<td>Rather Not Say</td>
<td>0%</td>
</tr>
<tr>
<td>Age</td>
<td>18-24</td>
<td>56%</td>
</tr>
<tr>
<td></td>
<td>25-34</td>
<td>19%</td>
</tr>
<tr>
<td></td>
<td>35-44</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td>45-54</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td>55-64</td>
<td>6%</td>
</tr>
<tr>
<td></td>
<td>&gt;65</td>
<td>3%</td>
</tr>
<tr>
<td>Consumption frequency</td>
<td>1-2 times a week</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>1-2 times a month</td>
<td>12%</td>
</tr>
<tr>
<td></td>
<td>Every 3-4 months</td>
<td>38%</td>
</tr>
<tr>
<td></td>
<td>1-2 times a year</td>
<td>45%</td>
</tr>
<tr>
<td></td>
<td>Less than once a year</td>
<td>5%</td>
</tr>
<tr>
<td>Most common preparation method</td>
<td>Boiled</td>
<td>87%</td>
</tr>
<tr>
<td></td>
<td>Baked</td>
<td>9%</td>
</tr>
<tr>
<td></td>
<td>Fried</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td>Grilled</td>
<td>7%</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>9%</td>
</tr>
<tr>
<td>Most important sensory characteristic</td>
<td>Color</td>
<td>1%</td>
</tr>
<tr>
<td></td>
<td>Aroma</td>
<td>3%</td>
</tr>
<tr>
<td></td>
<td>Texture</td>
<td>25%</td>
</tr>
<tr>
<td></td>
<td>Flavor</td>
<td>71%</td>
</tr>
</tbody>
</table>

The mean liking scores for the five sensory attributes (color, aroma, texture, flavor, and overall liking) of sous-vide cooked lobsters ranged from 5.9 to 7.1 on the 9-point hedonic scale (Table 2.3). No significant differences ($P > 0.05$) were found in consumer acceptability of color, aroma, texture, flavor, and overall liking among the sous-vide cooking treatments, indicating consumers liked all sous-vide cooked treatments the same. In addition, 38 to 44 of the participants rated overall acceptability of all three sous-vide treatments as $\geq 8$, while 2 to 7 of the participants gave the samples scores of $\leq 2$. However no significant differences were found in the distribution of overall acceptability scores that any sample received.
Although no significant differences were found in overall acceptability, SV 65 and SV 60 each received an average score of 7.0 ± 1.5 (like moderately), while SV 55 (6.8 ± 1.5) samples were rated slightly lower. In another study, sous-vide cooking at 65 °C showed better sensory results than sous-vide cooking at higher temperatures. For example, in salmon, sensory characteristics (taste and appearance) of salmon slices sous-vide cooked at 65 °C for 5 min received higher scores than those sous-vide cooked at 90 °C for 10 or 15 min (González-Fandos et al., 2005). Each characteristic of cooked salmon was scored on a point scale from 1 (poor quality) to 7 (high quality). However, in the current study, the temperature differences among treatments did not impact consumer acceptability scores.

Table 2.3. Mean scores for consumer acceptance of sous-vide cooked tails on a 9-point hedonic scale.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Sous-vide treatments*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>6.8±1.7</td>
<td>6.8±1.6</td>
</tr>
<tr>
<td>Aroma</td>
<td>6.3±1.7</td>
<td>5.9±1.7</td>
</tr>
<tr>
<td>Texture</td>
<td>6.4±1.9</td>
<td>6.8±1.8</td>
</tr>
<tr>
<td>Flavor</td>
<td>7.0±1.6</td>
<td>7.1±1.5</td>
</tr>
<tr>
<td>Overall</td>
<td>6.8±1.5</td>
<td>7.0±1.5</td>
</tr>
</tbody>
</table>

Each value is the mean ± standard deviation (n = 100). There were no statistically significant differences among treatments (P > 0.05). 1= Dislike Extremely and 9= Like Extremely.

* SV55, sous-vide cooking at 55°C/208 min; SV60, sous-vide cooking at 60°C/45min; SV65, sous-vide cooking at 65°C/10 min.

Although the majority of participants (71%) said flavor is the most important sensory characteristic of lobster tails, most of their comments were about texture (Appendix D). There were 71 comments for all treatments. For SV 55 there were 20 comments; only in four of them participants said they liked the texture, while the other 16 comments mentioned that participants did not appreciate the texture of SV 55 samples. For SV 60, there were 16 positive and 10
negative comments about texture. For SV 65, 15 out of 25 comments praised the texture of the samples, while the other 10 comments expressed dissatisfaction with the texture. For flavor, out of 71 total comments, only two described the flavor as “sweet” and “mild” for SV 65 and SV 60, respectively. Comments from panelists who consume lobsters at least every 2-3 months were further investigated since it is important to understand the opinions of actual lobster consumers in real life (Figure 2.18). These panelists characterized the texture of the SV 60 and SV 65 treatments as "best," although some also thought the texture of SV 60 was mushy, while SV 55 was frequently described as mushy. These comments indicated that the SV 60 and SV 65 treatments were preferred by the panelists who consume lobsters more frequently.

**Figure 2.18.** Word clouds of texture descriptors of sous-vide cooked products by panelists who consume lobsters at least every 2-3 months. SV55, sous-vide cooking at 55°C/208min; SV60, sous-vide cooking at 60°C/45min; SV65, sous-vide cooking at 65°C/10 min.

Correlations among sensory attribute scores revealed that overall acceptability had strong, significant \((P < 0.01)\) positive correlations with hedonic scores for flavor \((r = 0.84)\) and texture \((r = 0.83)\) and significant \((P < 0.01)\) moderate correlations with aroma \((r = 0.45)\) and color acceptability scores \((r = 0.45)\). These correlations indicate that texture and flavor attributes of the lobster strongly influenced overall acceptability of the samples, more so than aroma and color attributes. In addition, consumer acceptability ratings for texture had a strong, significant
negative correlation ($r=-0.98$) with $b^*$ values. These correlations indicate that higher $b^*$ values (indicating a stronger yellow color) corresponded with lower consumer acceptability ratings of texture which potentially can affect overall acceptability ratings, particularly since panelists rated their overall liking based on the flavor and texture attributes of the lobster.

2.4. Conclusions

Sous-vide cooking treatments improved the physicochemical properties of lobster tails compared to the boiling treatment. Lobster tails sous-vide cooked at all three time/temperature conditions were more tender than those conventionally cooked by boiling. The more tender texture was not attributed to changes in moisture content or WHC of the lobster muscle, which were not significantly different among cooking treatments. Differences in texture between boiled and sous-vide cooked samples were likely due to differences in the level of protein denaturation and aggregation as evidenced by the negative correlation between salt soluble protein content and shear force. Additionally, differences in shear force values were significantly correlated with sarcomere length and $b^*$ value of lobster muscle. $L^*$ and $a^*$ values of lobster muscle were not significantly different among treatments, while $b^*$ values were significantly higher in the boiled than sous-vide cooked tails. Lobster muscle protein denatured at temperatures below 60 °C as evidenced by DSC measurements that revealed two peaks at approximately at 40 and 58 °C for myosin and actin, respectively. Although the 65 °C/10 min treatment significantly increased sarcomere length and weight loss of the samples compared to the 60 °C/45 min and 55 °C/208 min treatments, there were no significant differences in shear force or color attributes among sous-vide cooked treatments, which could be due to the thermal denaturation of myosin (mainly responsible for color changes) and actin (mainly responsible for texture changes) in all sous-vide treatments.
The sous-vide cooking parameters resulted in no significant differences in consumer acceptability for the tested sensory attributes among treatments. The mean overall liking score for the 65 °C/10 min and 60 °C/45 min treatments was 7 on a 9-point hedonic scale, whereas a score of ≥ 7 is usually associated with a highly acceptable sensory quality (Everitt, 2009). The slightly higher acceptability of the 65 °C/10 min and 60 °C/45 min treatments was supported by consumer comments that revealed both treatments provided better texture than the 55 °C/208 min treatment. Physicochemical and consumer acceptance studies have not been previously reported for any sous-vide shellfish products and have important implications for the upscale food service industry for selecting sous-vide processing treatment conditions to prepare high quality lobster products. Lobsters are susceptible to being overcooked using conventional methods and these data confirm that sous-vide cooking can produce more tender lobster meat. Since there were no significant differences among sous-vide cooking treatments based on consumer acceptability, the 65 °C/10 min treatment was selected for the subsequent studies despite its higher weight loss because it is the most convenient of the time-temperature treatments tested, based on time and energy used.
CHAPTER 3

PHYSICOCHEMICAL PROPERTIES AND CONSUMER ACCEPTANCE OF HIGH-PRESSURE PROCESSED, SOUS-VIDE COOKED LOBSTER TAILS

Most of the content of this chapter was published in the Journal of Food Science, 84(12): 3454-3462, 2019

3.1. Introduction

There is a growing consumer demand for fresh, high-quality, minimally processed, and refrigeration-stable meals. These demands have led to increased utilization of new processing technologies (James & James, 2014). Sous-vide (SV) and high-pressure processing (HPP) are among the most promising emerging technologies in the food industry, including retail and food service, and their products are widely available in global markets (Rastogi, 2013; Kilibarda et al., 2018). Sous-vide is the controlled cooking of vacuum-sealed foods in a water bath or under steam (Baldwin, 2012). After heating, sous-vide cooked products are immediately chilled and kept below 3.3 °C to prevent Clostridium botulinum growth and toxin formation (FDA, 2020). In addition, the use of a time-temperature indicator (TTI) is required particularly where refrigeration is the sole barrier to prevent toxin formation.

Sous-vide products are typically cooked at relatively low temperatures for a longer period of time compared with conventional cooking methods (Keller et al., 2008), which helps to preserve their sensory characteristics and nutritional value. Fish and shellfish products particularly benefit from the sous-vide process, which reduces overcooking and results in a more tender and juicy texture (Baldwin, 2012). Textural changes in muscle foods as a result of thermal processing are a consequence of major changes in muscle protein structure. Cooking meat to
temperatures approaching 80 °C causes myofibrillar toughening due to shrinking of the myofibrils and associated loss of water (Tornberg, 2005). However, in comparison to red meat, collagen and myofibrillar proteins in seafood are more sensitive to thermal treatment (Tahergorabi et al., 2011), therefore precise temperature control is critical during cooking of these products.

HPP has been adopted by the food industry due to its capacity to control food spoilage, enhance food safety, and extend shelf-life while maintaining the quality of fresh, additive-free foods (James & James, 2014). In commercial settings, HPP is commonly applied to prepackaged food using select pressures (400 to 600 MPa), holding times (10 to 30 min), and temperatures (5 to 90 °C), depending on the product and desired effects. HPP promotes retention of flavors, pigments, and nutritional content of processed foods due to its negligible effects on covalent bonds; however, HPP can disrupt noncovalent (electrostatic and hydrophobic) interactions, resulting in structural changes in proteins which can negatively impact texture (Bolumar et al., 2016). Several studies have evaluated the effects of high-pressure treatment on various quality characteristics of raw fish and shellfish products (Chéret et al., 2005; Jantakoson et al., 2012; Kaur et al., 2013; Jiranuntakul et al., 2018). Texture and color attributes of products post processing are frequently examined due to their strong impact on consumer acceptability and marketability. In raw shrimp, pressurization at 435 MPa for 5 min at 25 °C increased toughness compared to the untreated samples and led to color changes observable to the human eye (Kaur et al., 2013). Similar results were observed in prawns pressurized at 400 MPa for 10 min at 7 °C (López-Caballero et al., 2000). Overall, the reported effects of HPP on texture and color
attributes are dependent on pressure, duration, temperature, and the type of the product (Campus, 2010).

Lobsters are one of the most valuable crustaceans consumed worldwide (NMFS, 2017). They are prized for their healthful nutrient content and unique texture and flavor profiles. However, lobsters are prone to being overcooked when boiled or steamed, which can yield tough and rubbery products (McGee, 2004). Professional chefs have adopted sous-vide cooking of lobsters because it ensures perfectly and evenly cooked lobsters every time (Keller et al., 2008). Combining HPP with sous-vide cooking techniques may facilitate the development of safe, refrigeration stable lobster products with minimal effects on consumer acceptability and physicochemical qualities, particularly texture and color attributes. In finfish, application of HPP after sous-vide cooking had conflicting effects on texture and color characteristics of seabream and salmon fillets (Picouet et al., 2011; Espinosa et al., 2015). However, to the best of our knowledge, no previous studies have reported on the impacts of either HPP or sous-vide on the quality attributes of lobsters. Therefore, two experiments were conducted that focused on quality characteristics of high-pressure processed and subsequently sous-vide cooked lobster tails. The objective of the first experiment was to evaluate the impacts of two moderate processing pressures (150 or 350 MPa) and two processing times (5 or 10 min) on selected physicochemical properties of raw and subsequently sous-vide cooked lobster tails. In the second experiment, we evaluated whether these physicochemical effects influence consumer acceptability of the sous-vide cooked lobster tails. Moderate HPP conditions were employed to limit any negative impacts on raw and sous-vide cooked lobster quality.
3.2. Materials and Methods

3.2.1. Experimental design

Fresh, shucked raw American lobster (*Homarus americanus*) tails were vacuum packaged and treatments were processed in triplicate based on a full $2 \times 2$ multifactorial design evaluating moderate pressures (150 and 350 MPa) and HPP duration (5 and 10 min). Subsequently, half the samples were sous-vide cooked at 65 °C (Figure 3.1). The samples were evaluated post processing for treatment effects on instrumental color and texture, moisture content, weight loss, water holding capacity (WHC), and salt soluble protein content. HPP lobster tails were compared to appropriate non-HPP-treated raw and sous-vide cooked controls. Based on the results of the physicochemical analyses, two HPP treatments were selected for subsequent consumer acceptability testing in comparison to the non-HPP control.

3.2.2. Sample preparation

Three hundred and seventy, 4 to 5-ounce deveined fresh raw soft-shell lobster tails were purchased from a local seafood supplier (Maine Fair Trade Lobster, Prospect Harbor, ME, USA) in July 2017. The tails were hand-shucked after 20 secs immersion in hot water (90 to 92 °C) to facilitate shell removal. Shucked tails were packed (four tails per bag) in 3.2 mil (0.001 inch) plastic bags (3.3 cm$^3$/100 in$^2$ oxygen transmission rate, 80 micron, 100 °C tolerance; UltraSource, Kansas, MO, USA) under 99 % vacuum (Model UV550, Wichita, KS, USA). Bags were completely submerged in ice (held below 3.3 °C) until subjected to HPP.
Figure 3.1. Process Flow

1. Receiving lobster tails
2. Blanching
3. Shucking (by hand)
4. Vacuum packaging
5. Packing in ice
6. HPP (150 MPa or 350 MPa for 5 or 10 min)
   - Raw
   - Sous vide cooking (65 °C for 10 min, core temperature)
7. Physicochemical Analyses

- 90-92 °C hot water for 20 s, placed in ice-water for at least 2 min
3.2.3. HPP and sous-vide cooking

Sample bags containing lobster tails were randomly pressurized at 150 or 350 MPa for a holding time of 5 or 10 min using a 55 L unit (Hiperbaric, Miami, FL, USA) (Figure 3.2). Water maintained at 4 °C was used as the pressure-transmitting medium. Subsequently, half of the HPP-processed tails were sous-vide cooked at 65 °C for approximately 37 min in a polycarbonate processing vessel (5-gal StorplusTM; Carlisle, OK, USA) using an immersion circulator (Sous-videTM Professional Creative, PolyScience, Niles, IL, USA) to achieve a sample core temperature of 65 °C for 10 min. This cooking process was previously validated to ensure a 6-log reduction of *L. monocytogenes* using the FDA Fish and Fisheries Products Hazards and Controls Guide, Appendix 4: Bacterial Pathogen Growth and Inactivation, Table A-3 (FDA, 2020). Cooking temperature was monitored using K-type thermocouple probes (Omega, Stamford, CT, USA) attached to a data logger thermometer (RDXL4SD, Omega) and recorded every 30 sec. Approximately 2 x 3 x 1 cm of closed cell foam tape (ThermoWorks, Salt Lake City, UT, USA) was used to maintain the seal and vacuum of the bags during cooking. After cooking, bags were promptly immersed in an ice: water slush (2:1 v/v) to bring the meat core temperature to ≤ 2.7 °C within 25 min. Samples were packed in ice and stored in a refrigerator at ≤ 2 °C until subjected to physicochemical analysis.
3.2.4. Weight loss

The percentage of cumulative weight loss resulting from the liquid of four lobster tails released after HPP and/or after sous-vide processing was calculated as:

\[
\% \text{ Weight loss} = \frac{\text{initial sample wt (g)} - \text{processed sample wt (g)}}{\text{initial sample wt (g)}} \times 100
\]

3.2.5. Moisture content

Moisture content (%) was determined gravimetrically by drying samples (5 g) of ground tail meat (sample preparation explained in section 2.2.7.) overnight in a 105 °C oven (Fisher Isotemp, Barrington, IL, USA) (AOAC, 2005). Each of three treatment replicates (n = 3) was evaluated in duplicate samples, and values were averaged.

3.2.6. Water holding capacity (WHC)

The WHC of meat samples was determined in duplicate per treatment replicate (n = 3) according to a modified method of Jiang et al. (1985). Lobster tail was cut into cubes (2 g), wrapped in two pieces of preweighed Whatman #1 filter paper, placed in 50 mL test tubes, and then spun at 1,000 \( \times \) g for 15 min in a bench top centrifuge (model 5430, Eppendorf, Hamburg,
Germany). Following centrifugation, the filter paper was reweighed, and the difference in weight was recorded. WHC was calculated as the percent of water retained by the meat with respect to water present in meat prior to centrifugation using the following equation:

\[
\frac{\text{[% moisture x sample wt. (g)]} - \{\text{[final paper wt.(g)]} - \text{[initial paper wt. (g)]}\}}{\text{[% moisture x sample wt.(g)]}} \times 100
\]

3.2.7. Salt soluble protein (SSP) content

Lobster muscle was ground as explained in section 2.2.7. A 5 g subsample was blended with 195 mL of cold 5 % NaCl solution for 60 secs in a Waring blender, and the homogenate was centrifuged at 12,000 × g for 20 min at 2 °C. The supernatant was collected, and soluble protein content was determined according to the method of Lowry et al. (1951). Solution A (2 % anhydrous Na₂CO₃ in 0.4 % NaOH), Solution B (1 % cupric sulfate 5H₂O), Solution C (2.7 % sodium potassium tartrate), Solution D (100 mL solution A + 1mL solution B + 1 mL solution C), Solution E (diluted 1:1 v/v Folin-Ciocalteu phenol reagent). Results were expressed as mg salt soluble protein/g tail meat. Bovine serum albumin was used as a standard and absorbance was evaluated at a wavelength of 700 nm using a UV/Vis spectrophotometer (DU 530, Beckman Coulter, Fullerton, CA, USA).

3.2.8. Texture

Texture measurements were performed using a calibrated texture analyzer platform (TA-XTi2, Texture Technologies Inc., Scarsdale, NY, USA) with a 5-kg load cell. Two different texture analyses were conducted, texture profile analysis (TPA) and the Warner–Bratzler shear test. With the red colored dorsal surface of the sample facing upward, lobster portions (20 mm in length) were compressed or sheared perpendicular to muscle fibers. For TPA, analyses (n = 10) were performed using a TA-25, 50.8 mm diameter aluminum cylinder at a 2 mm/s test speed.
Two 75% compressions were applied with a 5 s gap between compressions. Force (Newtons, N), area (N*s), and time (s) were recorded by the texture analysis software (Exponent 32, version 5.0, Texture Technologies Inc.) to calculate the TPA parameters hardness, springiness, chewiness, and resilience. Hardness was expressed in Newtons (N) and the other TPA parameters are unitless. For shear force analyses, samples (n = 10) were placed so the blade (TA-42 knife blade with 45° chisel end) cut across the muscle fibers. The blade was applied to a 90% depth using a 2 mm/s test speed and force (N) was recorded.

3.2.9. Color

A colorimeter (LabScan XE, Hunter Labs, Reston, VA, USA) was used to measure differences in color among treatments. Twenty mm in length lobster portions (n=10 per treatment replicate) were cut from the central region of each lobster tail. The colorimeter was standardized using white and black tiles for a port size of 30.5 mm, area view of 12.7 mm, and an illumination of 10° (D65, Hunter Labs). The Hunter L*, a*, and b* values of the ventral side of the tail were recorded as the average of three (initial and rotated 120° twice) readings per sample by the colorimeter software (Universal, version 4.10, 2001, Hunter Labs).

3.2.10. Sensory evaluation

Sensory evaluation was conducted to determine the effects of HPP on consumer acceptability of sous-vide cooked lobster tails. Approximately 120 lobster tails (obtained from Maine Fair Trade Lobster in October 2018) were pressurized at 150 or 350 MPa for 10 min as described previously using a 100 L HPP unit (Avure Technologies, Erlanger, KY, USA) (Figure 3.3). Subsequently, HPP-processed tails and the non-HPP control were sous-vide cooked using 65 °C for 10 min treatment. One hundred sensory panelists (older than 18 years) who enjoy
consuming lobsters were recruited via email and flyer notice to assess the acceptability of sous-vide cooked lobster tails. Each tail was cut into three pieces and then warmed to between 35 and 45 °C in a covered aluminum dish placed on a water bath at 50 °C for 30 min. Panelists were seated in booths with fluorescent lighting at the Sensory Evaluation Center at the University of Maine. The three products were labeled with 3-digit random codes and were served with a ceramic ramekin containing melted salted butter. Panelists were instructed to evaluate the samples, take a sip of water after testing each sample, and rate the acceptability of specific sensory attributes of the samples. A 9-point hedonic scale (from 1 = “Dislike Extremely” to 9 = “Like Extremely,” with 5 = “Neither Like Nor Dislike”) was used to assess the acceptability of texture, flavor, color, aroma, and overall liking of samples, and a 5-point Just-About-Right (JAR) scale was used to examine specific texture attributes (tenderness and juiciness) (Appendix E). Penalty analysis was performed for scores that were not JAR.

Participants were asked to answer a set of questions relating to demographic characteristics, purchasing frequency, and their attitudes toward consuming lobsters. In addition, they were asked to best describe the texture of each sample using only one of the following words: tender, chewy, tough, mushy, soft, firm, juicy, dry.
The test process was executed and assessed using SIMS 2000 (Sensory Computer Systems, Morristown, NJ, USA) software. The consumer acceptability study was approved (application number, 2018-09-16; approval date, October 11th, 2018) by the University of Maine Institutional Review Board (IRB) for the Protection of Human Subjects.

### 3.2.11. Statistical analysis

Data were analyzed using SPSS 25 (IBM, Armonk, NY, USA) at a significance level of $P < 0.05$. Outliers were identified by SPSS based on the rule of $3 \times$ interquartile range (IQR). One-way analysis of variance (ANOVA) was used to assess all one-level (treatment) effects. Separation of treatment means was accomplished using Tukey’s honest significant difference (HSD) post hoc test. Multiway ANOVA was used to assess overall effects of processing pressures and processing times. Adjustment for multiple comparisons was conducted by the Bonferroni method. A chi square test with Bonferroni was used to compare the differences among frequencies. Pearson’s correlation was performed to evaluate correlations among variables.

### 3.3. Results and Discussion

#### 3.3.1. Weight loss

HPP treatments did not significantly affect weight loss, WHC, or moisture content of raw or sous-vide cooked lobsters compared to the controls (Figure 3.4). Weight loss of raw meat ranged from 2.5 to 4.0 % and from 16.9 to 21.2 % for sous-vide cooked lobsters. Similarly, Jantakoson et al. (2012) found that pressurization at different levels (200, 400, 600, and 800 MPa for 20 min at 28°C) had no effect on weight loss of raw black tiger shrimp. Compared to the raw lobster samples, sous-vide cooking increased weight loss by about six-fold. Sous-vide-induced
denaturation of the muscle protein apparently caused shrinkage of the muscle fibers that resulted in extruding the water held between the myosin and actin filaments of the myofibrils (Baldwin, 2012). Water loss has considerable economic implications for processed lobsters since they are sold by weight, and retention of water is equally important for optimal texture of muscle because of its substantial impact on meat tenderness and juiciness (Hughes et al., 2014).

**Figure 3.4. Weight loss of raw and sous-vide cooked lobster tails.**

![Graph showing weight loss of raw and sous-vide cooked lobster tails](image)

Each value is the mean ± standard deviation (n = 3). Treatments not sharing a letter are significantly different (P < 0.05) based on one-way ANOVA followed by Tukey’s HSD post hoc test.

### 3.3.2. Moisture content

HPP treatments did not significantly affect moisture content of raw or sous-vide cooked lobsters compared to the controls (Figure 3.5). Moisture content of raw (80.6 to 82.0 %) and sous-vide cooked lobsters (79.2 to 79.9 %) did not significantly change due to HPP. These findings are in agreement with Lakshmanan et al., (2007) who reported that neither pressure (100, 150, or 200 MPa) nor processing time (10 or 20 min) influenced moisture content in fresh
salmon. However, moisture content of oysters increased with increasing pressures up to 800 MPa (Cruz-Romero et al., 2004), likely due to increased water absorption by protein.

**Figure 3.5. Moisture content of raw and sous-vide cooked lobster tails.**

![Graph showing moisture content of raw and sous-vide cooked lobster tails.](image)

Each value is the mean ± standard deviation (n = 3). Treatments not sharing a letter are significantly different (P < 0.05) based on one-way ANOVA followed by Tukey’s HSD post hoc test.

### 3.3.3. Water holding capacity (WHC)

HPP treatments did not significantly affect WHC of raw or sous-vide cooked lobsters compared to the controls (Figure 3.6). Average WHC of HPP lobsters was 88.1 % for raw and 77.9 % for sous-vide cooked treatments. Effects of HPP observed were not significant among raw or sous-vide cooked treatments. In raw finfish, WHC decreased with increasing pressure (Chéret et al., 2005; Jiranuntakul et al., 2018). Although muscle proteins can denature due to pressure resulting in decreasing WHC, the effects of HPP on the WHC of muscle foods are confounding (Chauhan, 2019). For example, skipjack tuna loin processed at 150 MPa for 3 min had a higher (P < 0.05) WHC compared to the control (Jiranuntakul et al., 2018). In contrast,
Atlantic salmon subjected to the same pressure (regardless of processing time) exhibited lower (P < 0.05) WHC compared to the control (Lakshmanan et al., 2007).

Figure 3.6. WHC of raw and sous-vide cooked lobster tails.

![Graph showing WHC of raw and sous-vide cooked lobster tails with statistics and letters indicating significance.]

Each value is the mean ± standard deviation (n = 3). Treatments not sharing a letter are significantly different (P < 0.05) based on one-way ANOVA followed by Tukey’s HSD post hoc test.

3.3.4. Salt soluble protein (SSP) content

Higher pressure (350 MPa) significantly (P < 0.05) decreased salt soluble protein (SSP) content in raw tails by about 25% compared to the control and 150 MPa samples, regardless of processing time (Figure 3.7). However, no significant effects of pressure on SSP were observed among the sous-vide cooked treatments. Grossi et al. (2016) reported that the decreased protein solubility in raw pork muscle on increasing pressure above 200 MPa was a result of the pressure impact on the individual myofibrillar proteins. They concluded that myosin and actin lose their native solubility at pressures above 400 MPa, while α-actinin and troponin-T are less affected by pressure. Lobster muscle proteins experienced a decrease in solubility at pressures less than 400 MPa, suggesting that lobster myofibrillar proteins are less stable than pork muscle proteins to
pressurization. Denaturation of crustacean myofibrillar proteins in response to a mild 100 MPa (5 min) pressurization was reported by Martínez et al. (2017) in Blue crab, as evidenced by significant \( P < 0.05 \) changes in \( \alpha \)-helix and \( \beta \)-sheet composition. Moreover, differential scanning calorimetry (DSC) results confirmed a decrease in enthalpy and denaturation temperature in myosin and a complete disappearance of the actin peak at 300 MPa, suggesting that in Blue crab, actin is more sensitive than myosin to pressure.

**Figure 3.7. Salt soluble protein content of raw and sous-vide cooked lobster tails.**

Each value is the mean ± standard deviation \( (n = 3) \). Treatments not sharing a letter are significantly different \( (P < 0.05) \) based on one-way ANOVA followed by Tukey’s HSD post hoc test.

Shellfish myofibrillar proteins are also more sensitive to thermal treatment than their mammalian counterparts (Tahergorabi et al., 2011), and differences in thermal stability have even been reported among lobster species, with the American lobster showing lower myofibrillar thermal stability than the Japanese spiny lobster, presumably related to the temperatures of their marine habitats (Shimada et al., 2000).
3.3.5. Texture

HPP significantly ($P < 0.05$) affected the texture of raw and sous-vide cooked lobsters (Figure 3.8 and 3.9). As the pressure level increased from 150 to 350 MPa, the hardness and shear force values increased ($P < 0.05$) in raw and sous-vide cooked lobsters. Similarly, compression force and shear force of raw black tiger shrimp muscle pressurized at 200, 400, 600, and 800 MPa significantly ($P < 0.05$) increased with increasing pressure (Jantakoson et al., 2012).

**Figure 3.8. Hardness of raw and sous-vide cooked lobster tails**

![Graph showing hardness of raw and sous-vide cooked lobster tails](image)

Each value is the mean ± standard deviation ($n = 3$). Treatments not sharing a letter are significantly different ($P < 0.05$) based on one-way ANOVA followed by Tukey’s HSD post hoc test.

However, shrimp cooked at 100 °C for 2 min had higher ($P < 0.05$) shear force than the pressurized and unpressurized raw control shrimps. In addition, shear force values of raw Norway lobster pressurized at 200 MPa for 30 min significantly ($P<0.05$) increased (1.3 times) compared to the control samples (Chevalier et al., 2000). HPP-induced modification of texture can be explained by myofibrillar protein denaturation and aggregation (Yagiz et al., 2009).
addition, it was suggested that the increase in hardness of pressurized fish muscles was due to the formation of new hydrogen bonded networks (Angsupanich & Ledward, 1998). Moreover, the increase in hardness following HPP might be due to unfolding of the myofibrillar proteins, which may coincide with compression of myosin into the Z-line of the muscle (Macfarlane, 1985; Edwards, 1995; Zamri et al., 2006). All other TPA parameters (springiness, chewiness, and resilience) of raw and sous-vide cooked lobsters were not significantly influenced by HPP (Table 3.1).

### Table 3.1. Springiness, chewiness, and resilience of raw and sous-vide cooked lobster tails.

<table>
<thead>
<tr>
<th></th>
<th>Springiness</th>
<th>Chewiness</th>
<th>Resilience</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw</td>
<td>SV</td>
<td>Raw</td>
</tr>
<tr>
<td>Control</td>
<td>0.6±0.0a</td>
<td>0.5±0.0a</td>
<td>4.9±0.6a</td>
</tr>
<tr>
<td>HPP150/5</td>
<td>0.7±0.0a</td>
<td>0.6±0.0a</td>
<td>4.7±0.7a</td>
</tr>
<tr>
<td>HPP150/10</td>
<td>0.7±0.1a</td>
<td>0.6±0.0a</td>
<td>3.9±1.4a</td>
</tr>
<tr>
<td>HPP350/5</td>
<td>0.5±0.0a</td>
<td>0.6±0.0a</td>
<td>5.5±0.2a</td>
</tr>
<tr>
<td>HPP350/10</td>
<td>0.5±0.1a</td>
<td>0.6±0.0a</td>
<td>4.0±0.3a</td>
</tr>
</tbody>
</table>

Each value is the mean ± standard deviation (n = 3). Values not sharing a lowercase letter are significantly different within columns, analyzed by ANOVA (Tukey’s HSD post-hoc).

For raw lobsters, the longer processing time (10 min) significantly (P < 0.05) decreased the hardness by approximately 18 % compared to the 5 min processed samples. The same trend was observed in shear force values of raw lobsters; however, differences between 5 and 10 min were not statistically significant. Although the higher-pressure treatment significantly (P < 0.05) increased the hardness by approximately 22 % compared to 150 MPa, the hardness significantly (P < 0.05) decreased by approximately 37 % in the raw lobsters pressurized at 150 MPa for 10 min compared to the control. The decrease in hardness in the 150 MPa for 10 min treatment
could be due to an increase in proteolytic activity. Pressures below 300 MPa increased the activity of proteases that hydrolyze muscle structural proteins, resulting in softening the texture of seabass (Chéret et al., 2005).

**Figure 3.9. Shear force of raw and sous-vide cooked lobster tails.**

![Shear force graph]

Each value is the mean ± standard deviation (n = 3). Treatments not sharing a letter are significantly different (P < 0.05) based on one-way ANOVA followed by Tukey’s HSD post hoc test.

In addition, an increase in processing duration from 0 to 30 min at 250 MPa increased cathepsin B activity by approximately threefold, consequently promoting the softening of seabass muscle tissues (Teixeira et al., 2013). The authors explained that the increase in enzymatic activity was due to damage of the lysosomal membrane by HPP, consequently releasing proteases with access to myofibrillar proteins. The decrease in proteolytic activity at pressures above 300 MPa was likely due to structural modification of the enzymes. The texture results of raw lobsters have useful implications for the food service industry due to the increasing global demand for raw seafood products, including lobster, in a wide variety of popular dishes, including sushi, sashimi, crudo, and poke (Robertson, 2018).
For sous-vide cooked lobsters, processing pressures and processing times significantly 
($P<0.05$) affected the shear force values. Lobster pressurized at 350 MPa and then sous-vide 
cooked experienced the highest ($P < 0.05$) increase in shear force values by approximately 50 % 
and 46 % compared to the control and 150 MPa treatment, respectively. Shear force values at 
10 min processing time were significantly ($P < 0.05$) higher by approximately 8 % compared to 
5 min. Similar results were found by Jung et al. (2000) in beef *Biceps femoris* and *Longissimus 
dorsi* muscles pressurized at 520 MPa for 260 s at 10 °C and then cooked at 65 °C for 1 hr. The 
authors reported a significant ($P < 0.05$) increase in shear force values in both muscles when 
compared to the non-HPP cooked controls. Macfarlane et al. (1981) attributed the increase in 
shear force values of pressurized beef (150 MPa for 3 hr. at 0 °C) to the occurrence of a pressure-
induced denaturation and coalescence of the myosin filaments for samples heat treated at 25 and 
50 °C. Our textural results suggest that HPP at 350 MPa for 10 min followed by thermal 
treatment induced significant modification of myofibrillar proteins. Yu et al. (2016) reported that 
pressures above 200 MPa for 20 min at 60 °C caused loss of actin and myosin structure in beef 
muscle, while collagen remained relatively intact. Moreover, hardness and shear force 
significantly negatively correlated ($P < 0.01$) with SSP content, with values of $r = -0.80$ and 
$r = -0.64$, respectively. These correlations indicate that lower SSP content can serve as an 
indicator of tougher texture in lobster tails. However, the tougher texture caused by HPP may be 
preferred by some consumers. For example, Espinosa et al. (2015) found that seabream samples 
sous-vide cooked at 65 °C for 46.3 min and then pressurized at 600 MPa for 5 min at 5 °C 
experienced tougher texture compared to the control and 300 MPa treatment, however the 
samples pressurized at 600 MPa were rated the most acceptable by panelists. Therefore, although 
sous-vide cooked tails pressurized at 350 MPa were instrumentally tougher ($P < 0.05$) compared
to the control and 150 MPa treatments, the texture may be acceptable or even preferred by some consumers.

In this research, both the texture profile analysis (TPA) and Warner–Bratzler shear methods were used since there are no standard established methods to evaluate the texture of lobster meat. Our texture results indicated that shear force evaluation was more responsive to textural changes and showed less variability than TPA in both raw and cooked tails. Therefore, the Warner–Bratzler shear method was used in subsequent research for evaluating lobster texture.

3.3.6. Color

The appearance of lobster tails was clearly modified by HPP treatment (Figure 3.10). The raw meat was translucent and yellowish white. After 150 MPa treatment, the raw meat did not appear different in comparison to the control. However, differences were visually apparent at 350 MPa compared to the control and 150 MPa treatment. Lobsters subjected to 350 MPa treatment became more opaque and whiter. Similar observations were found in raw shrimps processed at 270 and 435 MPa (Kaur et al., 2013), and at 300 and 450 MPa in salmon fillets (Yagiz et al., 2009). For the sous-vide cooked lobsters, samples previously pressurized at 350 MPa appeared whiter compared to the control and 150 MPa treatment. Similarly, lightness of sous-vide cooked salmon meat increased with increasing pressure up to 400 MPa (Picouet et al., 2011).

In support of our visual observations, instrumental color analysis confirmed that L*, a*, and b* values were significantly \( P < 0.05 \) affected by HPP. Lightness (L*), red-green (a*), and yellow-blue (b*) values were significantly \( P < 0.05 \) different among treatments (Table 3.2).
Changes in raw and sous-vide cooked meat color depended on the pressure level and processing time. L* values of raw and sous-vide cooked lobsters pressurized at 350 MPa were significantly ($P < 0.05$) higher compared to the control and 150 MPa treatment. Raw tails processed for 10 min showed higher ($P < 0.05$) L* values and lower ($P < 0.05$) a* and b* values than those processed for 5 min. Lobsters processed at higher pressure (350 MPa) and subsequently sous-vide cooked had significant ($P < 0.05$) increases in L* values compared to sous-vide cooked control and 150 MPa treatments, and no significant differences were observed in a* and b* values among sous-vide cooked samples. These results indicate that raw lobsters became whiter with higher processing pressure and longer processing time, while the sous-vide cooked lobsters were impacted by higher processing pressure alone. The changes in L* values
noted here are in agreement with reports on prawn (Bindu et al., 2013) and black tiger shrimp (Kaur et al., 2013). Bindu et al. (2013) reported that lightness (L* value) of prawns increased ($P < 0.05$) with increasing pressure levels (100, 270, 435, and 600 MPa).

### Table 3.2. Instrumental color (L*, a*, b*) values for raw and sous-vide cooked tails.

<table>
<thead>
<tr>
<th>Product</th>
<th>Sample Code</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L*</td>
</tr>
<tr>
<td>Raw</td>
<td>Control</td>
<td>49.56±2.32c</td>
</tr>
<tr>
<td>150/5</td>
<td>48.13±5.13c</td>
<td>0.66±0.21ab</td>
</tr>
<tr>
<td>150/10</td>
<td>57.93±1.53b</td>
<td>-1.53±0.31c</td>
</tr>
<tr>
<td>350/5</td>
<td>59.10±0.95a</td>
<td>1.93±0.71a</td>
</tr>
<tr>
<td>350/10</td>
<td>59.90±0.60a</td>
<td>-1.68±0.65c</td>
</tr>
<tr>
<td>SV Cooked</td>
<td>Control</td>
<td>69.43±0.96b</td>
</tr>
<tr>
<td>150/5</td>
<td>69.73±1.02b</td>
<td>2.83±0.12a</td>
</tr>
<tr>
<td>150/10</td>
<td>69.90±0.26b</td>
<td>2.40±0.56a</td>
</tr>
<tr>
<td>350/5</td>
<td>72.10±0.78a</td>
<td>1.97±0.38a</td>
</tr>
<tr>
<td>350/10</td>
<td>72.93±0.76a</td>
<td>1.53±0.50a</td>
</tr>
</tbody>
</table>

Each value represents a mean ± standard deviation (n=3). Treatments not sharing a letter are significantly different ($P < 0.05$) within columns, within product form, based on one-way ANOVA followed by Tukey’s HSD post hoc test.

The increase in L* values as a result of HPP has been associated with unfolding of carotenoprotein (Truong et al., 2015) and degradation of carotenoid pigments, such as astaxanthin (Cruz-Romero et al., 2004). Increases in L* values of carp fillets were attributed to pressure-induced coagulation of sarcoplasmic and myofibrillar proteins (Sequeira-Munoz et al., 2006). Color of cooked muscle can impact consumer acceptability of lobster products (Calder et al., 2006).

### 3.3.7. Sensory evaluation

A mean liking score of $\geq 7$ on a 9-point hedonic scale is usually associated with a highly acceptable sensory quality (Everitt, 2009). The mean acceptability scores for five sensory
attributes (aroma, color, texture, flavor, and overall liking) of the sous-vide cooked lobsters ranged from 6.5 to 7.3 on the 9-point hedonic scale (Table 3.3). Consumer acceptance of the control and HPP samples did not significantly differ. The mean hedonic scores clarified that the changes in the instrumental texture and color due to HPP did not significantly impact sensory acceptability of the sous-vide cooked lobsters.

Table 3.3. Mean scores for consumer acceptance of sous-vide cooked tails on a 9-point hedonic scale.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Control</th>
<th>150/10</th>
<th>350/10</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aroma</td>
<td>6.7±1.6</td>
<td>6.7±1.4</td>
<td>6.6±1.7</td>
<td>0.89</td>
</tr>
<tr>
<td>Appearance</td>
<td>7.0±1.6</td>
<td>6.9±1.7</td>
<td>7.1±1.7</td>
<td>0.72</td>
</tr>
<tr>
<td>Texture</td>
<td>6.5±1.9</td>
<td>6.5±1.8</td>
<td>6.9±1.8</td>
<td>0.33</td>
</tr>
<tr>
<td>Flavor</td>
<td>6.9±1.5</td>
<td>6.9±1.6</td>
<td>7.3±1.5</td>
<td>0.15</td>
</tr>
<tr>
<td>Overall</td>
<td>6.8±1.8</td>
<td>6.8±1.7</td>
<td>7.0±1.6</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Each value is the mean ± standard deviation (n = 98). There were no statistically significant differences among treatments (P > 0.05). 1= Dislike Extremely, 9= Like Extremely.

Overall acceptability scores had strong, significant (P < 0.01) positive correlations with flavor (r = 0.86) and texture scores (r = 0.87) and significant (P < 0.01) moderate correlation with aroma (r = 0.48) and color scores (r = 0.45). These correlations indicate that panelists rated their overall liking based on the flavor and texture attributes of the lobster more than on aroma and color. Moreover, prior to tasting the samples, 79 % and 20 % of the panelists reported that they considered flavor and texture, respectively, to be the most important sensory attributes of lobsters. Interestingly, overall acceptability scores were significantly (P < 0.05) influenced by reported consumption frequency, with the 43 % of panelists who said that they consume lobsters every 2 to 3 months rating the overall acceptability higher than the 48 % of panelists who reported consuming lobsters 1 to 2 times per year. The most frequent consumption group (every 2 to 3 months) rated overall acceptability for all lobster products an average of 7.1, while the least frequent consumption group (1 to 2 times per year) gave an average overall acceptability
The apparent influence of consumption frequency on overall acceptability has implications for selection of panelists in lobster sensory evaluation studies. Although participants were selected based on age (> 18 years), lack of seafood allergies, and their liking for consuming lobsters, future studies should consider including a minimum consumption frequency as part of the selection criteria. Based on a check-all-that-apply question, lower prices and more availability of lobster products had the potential to motivate 91 % and 32 % of the panelists, respectively, to consume lobster more often. Panelists who consume lobsters at least every 2 to 3 months, and whose opinions are likely more representative of actual lobster consumers in the U.S. market as compared to panelists who consume lobster 2 or fewer times per year, predominantly described the texture of the three products as “tender” (Figure 3.11). However, the samples pressurized at 150 or 350 MPa were described as “juicy” more than the control. All panelists’ comments about the samples are listed in appendix F.

Figure 3.11. Word clouds of texture descriptors of sous-vide cooked products provided by panelists who consume lobsters at least every 2-3 months.

In the present study, JAR analysis focused on the specific sensory texture attributes of tenderness and juiciness, and whether consumers considered them to be ideal. Tenderness and moistness (juiciness) were found to be positively associated with overall liking scores of American lobster meat after 6 months of frozen storage (English et al., 2019).
be considered ideal, at least 70% of the responses should be “Just About Right” (Rothman & Parker, 2009). A large majority (approximately 75%) of panelists judged all three lobster products to have just the right degree of juiciness (Figure 3.12.A). This ideal juicy texture was likely due in part to the low water vapor permeability of the sous-vide packaging materials, which generally reduces moisture loss during cooking (Baldwin, 2012). As previously noted, there was negligible loss in moisture content or WHC in sous-vide cooked HPP lobsters compared to the control. However, pressurization at 300 or 600 MPa for 5 min significantly ($P < 0.05$) decreased the juiciness of previously sous-vide ready-to-eat seabream as evaluated by panelists using an unstructured 10 cm scale (Espinosa et al., 2015). In contrast to the results for juiciness, only approximately 50% of panelists perceived tenderness of all treatments as “JAR” (Figure 3.12.B), indicating that tenderness was not considered to be ideal.

Figure 3.12. Just-About-Right (JAR) categorical scores (n=98 consumers) for (A) Juiciness and (B) Tenderness for control, HPP 150/10, and HPP 350/10 lobster tails.
Significantly ($P < 0.05$) more panelists (approximately 29 %) rated control lobster tails as too tender compared to approximately 13 % for the 350 MPa samples. In contrast, the 350 MPa samples were significantly ($P < 0.05$) rated as too chewy by approximately 35 % of panelists compared to the control samples (approximately 16 %).

The chewiness of the HPP samples may have been due to a stabilizing effect of pressure on the hydrogen bonds of the collagen structure (Sikes et al., 2010) that remained relatively intact at low cooking temperatures (Yu et al., 2016), while in the control sample the low temperature sous-vide cooking dissolved and denatured the collagen into gelatin, rendering the meat more tender, as well as softer and mushier (Baldwin, 2012).

Penalty analyses of the control, 150 MPa, and 350 MPa products were performed to determine whether respondents’ ratings for tenderness which were not JAR (less than 70 % of responses were JAR) were associated with a mean drop in hedonic ratings of texture (Figure 3.13). Control samples received concerning penalties for “too chewy.” 150 MPa samples received concerning penalties for “too tender,” and a slightly concerning penalty for “too chewy,” while 350 MPa samples received slightly concerning penalties for too tender. 350 MPa had less concerning penalties for each texture attributes compared to the control and 150 MPa treatment. These results reflected the texture hedonic scores where 350 MPa was rated slightly higher (6.9 ± 1.8) compared to the control (6.5 ± 1.9) and 150MPa (6.5 ± 1.8), however these slight differences in texture hedonic scores among the three products were not statistically different.
Figure 3.13. Mean drops (penalties) in texture liking on a 9-point hedonic scale (n=100 consumers) from penalty analysis corresponding to the scale ends for each JAR texture attribute of firmness and tenderness for control, 150 MPa, and 350 MPa products. Mean drops of 1.5 -1.9 are concerning, drops of 1- 1.49 are slightly concerning, and 0- 0.99 are very slightly concerning (Rothman & Parker, 2009; Varela & Ares, 2012).

Finally, although the higher pressure (350 MPa) increased the L* and shear force values of sous-vide cooked lobster, overall consumer acceptability was not affected by these changes. Approximately 84 % of the panelists reported that the 350 MPa samples met their expectations compared to the control (approximately 76 %) and 150 MPa (approximately 75 %) treatments; these values were not statistically different among treatments. Overall, the sensory results indicate that pressurization of lobster tails at 150 or 350 MPa for 10 min followed by sous-vide cooking (65 °C for 10 min) resulted in a juicy product with a desirable texture.

3.4. Conclusions

Application of moderate pressures (150 and 350 MPa) significantly influenced the texture and color of lobster tails; however, processing time (5 or 10 min) showed less effect. Observed differences in texture were not a result of changes in moisture content or WHC of the muscle,
which were not significantly different among treatments. Although pressurization at 350 MPa significantly increased L* values and shear force, and decreased salt soluble protein content, these HPP-induced changes did not influence the overall acceptability of the sous-vide cooked lobster tails as evaluated by a consumer panel. The effects of HPP on the texture, color, and consumer acceptability of lobster tails have not previously been reported, and these findings have important implications for the seafood industry and for consumers of value-added lobster products. Additionally, although HPP has been reported to promote weight loss in some seafood products, the moderate pressurization applied in this study did not significantly impact weight loss of the processed lobster tails. This finding is important for a product that generates approximately $550 million in annual landings in New England alone (NMFS, 2017). Sous-vide is becoming increasingly popular and is a convenient and reliable method to produce perfectly cooked, juicy lobsters in the home or in a food service environment. The application of HPP to vacuum-packaged lobsters for subsequent sous-vide cooking may help promote the development of novel refrigeration-stable, raw or ready-to-eat products; however, shelf-life evaluation and pathogenic bacteria validation are warranted to clarify the impacts of HPP on product quality and stability during storage.
CHAPTER 4

REFRIGERATED SHELF-LIFE EVALUATION OF HIGH PRESSURE PROCESSED,
SOUS-VIDE COOKED LOBSTER

Most of the content of this chapter was accepted for publication in the journal of High Pressure Research

4.1. Introduction

Lobsters are one of the highest value fisheries worldwide (Pereira & Josupeit, 2017), and their consumption has been increasing steadily over the past decade (Massachusetts DMF, 2018). They are well known for their high nutritional value, sweet distinctive flavor, and firm texture. Unfortunately, lobsters are highly susceptible to rapid spoilage post-harvest, resulting in a very short refrigerated shelf-life ranging from 3 to 10 days depending on the type of lobster (Boziaris et al., 2011; Gornik et al., 2013; Arulkumar et al., 2019). Consequently, lobsters are usually sold either live or frozen (Billings, 2014). However, the distribution, handling, and storage of live lobsters require a substantial cost (Massachusetts DMF, 2018). Moreover, cooking live lobsters can be an intimidating task for those who prefer easy-to-prepare seafood products. Freezing, although it offers convenience and significantly extends the shelf-life of lobster products, can come at a cost to optimal texture and flavor qualities (Calder et al., 2006; English et al., 2019). As a result, seafood processors are increasingly interested in developing new varieties of processed lobster products (Massachusetts DMF, 2018).

Novel processing technologies have been developed which may help ensure the safe and convenient distribution of prime quality seafood products to retailers, restaurants, or consumers (James & James, 2014). High pressure processing (HPP) is an emerging technique that offers the potential to meet the expectations for high-quality, convenient-to-use, and refrigeration-stable
seafood products. HPP, a cold pasteurization method, may extend the refrigerated shelf-life of vacuum-packed, ready-to-prepare lobsters without the use of synthetic additives. HPP has been shown to be effective in extending the shelf-life of different seafoods such as shrimp (Bindu et al., 2013; Kaur et al., 2013; Kaur et al., 2017), abalone (Hughes et al., 2016), salmon, cod and mackerel (Rode & Hovda, 2016). The advantages associated with HPP include the inactivation of endogenous enzymes and food spoilage microorganisms, along with minimal effects on the sensory and nutritional attributes of the product (Campus, 2010).

Processing pressures above 400 MPa for processing times of 5–20 min can extend the shelf-life of raw seafoods compared to non-HPP controls. For example, refrigerated shelf-life of raw shrimp was extended to 15 days when processed at 435 MPa for 5 min compared to a 5-day shelf-life in unprocessed controls, but pressurization also significantly increased the toughness and lightness of the muscle (Kaur et al., 2013). Higher processing pressures (435 and 600 MPa) induced significant changes in biochemical parameters, such as total volatile base nitrogen (TVBN) and pH, and physical parameters, including instrumental color and texture of prawns (Bindu et al., 2013). In contrast, pressurization of raw abalone meat at 300 MPa for 10 min significantly increased its refrigerated shelf-life without causing toughening or undesirable color changes (Hughes et al., 2016).

Recent work conducted in our laboratory (chapter 3) showed that HPP has the potential to be applied in combination with sous-vide cooking to produce consumer-acceptable, value-added lobster products (Humaid et al., 2019). Sous-vide products are cooked in a hot water bath under controlled mild temperatures for a specific time in heat-stable, vacuum-packed bags (Aguilera, 2018). The precisely controlled temperatures during sous-vide cooking result in tender and moist seafood texture (Keller et al., 2008; Baldwin, 2012). Humaid et al. (2019) reported that HPP at
150 or 350 MPa for 10 min induced significant changes in physicochemical properties of raw lobster tails, particularly texture and color, without influencing consumer acceptance of the subsequently sous-vide cooked (at 65 °C) product. The application of HPP to prepackaged lobsters for subsequent sous-vide cooking may help promote the development of novel refrigeration-stable, raw or ready-to-eat products. However, the effects of HPP pretreatment on the refrigerated shelf-life of raw or of sous-vide cooked lobsters have not been reported. Shelf-life evaluation is needed to clarify the effects of HPP on product quality and stability throughout refrigerated storage. Therefore, our specific objective was to investigate the impacts of two different mid-level processing pressures (150 MPa or 350 MPa) for 10 min on the refrigerated shelf-life of vacuum-packaged raw and subsequently sous-vide cooked lobster tails. These processing pressures were selected to limit any undesired impacts on the physicochemical and sensory qualities of the lobsters.

4.2. Materials and Methods

4.2.1. Experimental design

Fresh, shucked raw lobster (*Homarus americanus*) tails were vacuum packaged and processed at 150 MPa or 350 MPa for 10 min at 4 °C (Figure 4.1). Subsequently, half the samples were sous-vide cooked to achieve a core temperature of 65 °C for 10 min. The samples were evaluated immediately post processing and then every 7 days for up to 28 days storage at 2 °C for total bacterial count (TBC), lactic acid bacteria (LAB), total volatile basic nitrogen (TVB-N), biogenic amines (BA), pH, sensory quality (aroma), shear-force, and color (L*, a*, b*). HPP lobster tails were compared to appropriate non-HPP treated raw and sous-vide cooked controls. Each treatment was processed in triplicate.
Figure 4.1. Process flow

- Receiving lobster tails
- Blanching
  - 90-92 °C hot water for 20 s, placed in ice-water for at least 2 min
- Shucking (by hand)
- Vacuum packaging
- Packing in ice
- HPP
  - 150 or 350 MPa for 10 min
  - 65 °C for 10 min (core temperature)
- Raw
- Sous vide cooking
  - 65 °C for 10 min (core temperature)
- Storing (at 2°C)
- Quality Analyses
  - Initially and every 7 days for up to 28 days
  - Initially and at end of shelf-life
  - Total bacterial count (TBC)
  - Lactic acid bacteria (LAB)
  - Total volatile base nitrogen (TVBN)
  - Biogenic amines (BAs)
  - pH
  - Sensory quality (aroma)
  - Shear-force and color (L*,a*,b*)
4.2.2. Sample preparation and high-pressure processing (HPP)

Six hundred, 4 to 5-ounce deveined fresh raw soft-shell lobster tails were sourced from a local seafood supplier (Maine Fair Trade Lobster, Prospect Harbor, ME, USA) in June 2018. To facilitate shell removal, lobster tails were dipped in hot water (90-92 °C) for a brief period (20 sec) and then immediately placed in ice-water to bring their temperature to ≤ 2 °C. Tails (n=6) were packed in 3.2 mil plastic bags (3.3 cm³/100 in² oxygen transmission rate, 80 micron, 100 °C tolerance; UltraSource, Kansas, MO, USA) under 99 % vacuum (Model UV550, Wichita, KS, USA), then packed in ice until subjected to HPP. Prepackaged lobster tails were randomly pressurized in a 55 L unit (Hiperbaric, Miami, FL, USA) at 150 MPa or 350 MPa for a holding time of 10 min. The chamber water temperature was maintained at 4 °C to achieve hydrostatic pressure throughout processing.

4.2.3. Sous-vide (SV) cooking

Subsequently, half of the HPP processed tails were sous-vide cooked for approximately 40 min in a polycarbonate processing vessel (5-gal Storplus™; Carlisle, OK) using an immersion circulator (Sous-vide™ Professional Creative, PolyScience, Niles, IL) to achieve a sample core temperature of 65 °C for 10 min. Cooking temperature was monitored using K-type thermocouple probes (Omega, Stamford, CT) attached to a data logger thermometer (RDXL4SD, Omega, Stamford, CT) and recorded every 30 secs. A strip (~ 2 x 3 x 1 cm) of closed-cell foam tape (ThermoWorks, Salt Lake City, UT) was used to maintain the seal and vacuum of the bags during cooking. After cooking, bags were promptly immersed in an ice: water slush (2:1 v/v) to bring the tail core temperature to below 2.7 °C within 25-30 min. Samples were thoroughly packed in ice and stored in a refrigerator at 2 °C until subjected to quality analyses.
4.2.4. Microbiological analyses

For microbial analyses, tails were individually packaged to avoid any potential cross-contamination from other tails. On each testing day, one lobster tail per treatment replicate, in its package, was thoroughly mashed by hand (Figure 4.2). A 25 g subsample was aseptically placed in a filtered stomacher bag with sterile 0.1 % bactopeptone (BD Diagnostics, Sparks, MD, USA) (1:10 w/w ratio). The bags were mechanically mixed for 2 min using a BagMixer 400 (Model P, SpiralBiotech, Advanced Instruments, Norwood, MA, USA). Three serial dilutions (e.g. 1:10, 1:100, and 1:1000) were prepared at each time point, increasing the dilution when necessary.

Figure 4.2. Lobster tail mashed for microbiological analyses

Aliquots of 0.1 mL of each dilution were spread onto tryptic soy agar (TSA) and DeMann Rogosa Sharpe agar (MRS) (Alpha Biosciences, Baltimore, MD) in 100 mm x 15 mm plastic Petri-dishes to quantify TBC and LAB, respectively. Petri-dishes were incubated for 48 h at 35 °C and at 30 °C for TBC and LAB, respectively. Post incubation, Petri-dishes having between 25-250 counts were recorded and then multiplied by the appropriate dilution factor to give the microbial count of the original sample expressed as colony forming units per gram (CFU/g). All treatment-replicates were plated in duplicate and the counts were averaged. Averaged bacterial counts were log-transformed for graphical presentation. In cases where no colonies were detected, the results were reported as < 2 log CFU/g for statistical analysis (Clontz, 2009).
4.2.5. Total volatile basic nitrogen

The TVBN content of the samples was determined in triplicate per treatment (n=3) according to a modified method of Botta et al. (1984). Lobster muscle (15 g) was homogenized 1:2 (w/v) with 30 mL of 7.5 % trichloroacetic acid (TCA) for 30 sec in a Waring blender, and then the homogenate was centrifuged (Beckman Model J2-21, USA) for 20 min at 2,000 x g. The supernatant (15 mL) was added to a micro-Kjeldahl distillation unit (Rapid distillation unit, Labconco, Kansas City, MO) along with 4 mL of 10% sodium hydroxide solution. Samples were distilled into 15 mL of 4 % boric acid solution containing 8 drops of indicator (0.2 % methyl red and 0.2 % methylene blue, 2:1 in ethanol) to a final volume of approximately 40 mL, then the distillate was titrated until a constant purple color was obtained using 0.05 N hydrochloric acid (HCl). The amount of TVBN (mg/100g) extracted from each sample was calculated as follows: 

\[
\text{[(volume (mL) HCl used for titrating sample – volume (mL) HCl used for titrating sample blank) x normality of HCl) x molecular weight of N] x [(volume (mL) of extraction solution/ volume (mL) of extract added to the distilling unit) x (100/original weight (g) of sample).}
\]

4.2.6. Sensory quality (aroma)

Ten panelists who self-identified as lobster consumers rated the quality of the samples throughout storage based on their odor characteristics using a 6-inch (15.2 cm) horizontal line scale (Meilgaard et al., 2015). Lobster tails were cut into 40 mm wide cross sections which were served blind in coded plastic disposable cups with lids. Prior to participating in this study, participants were screened using an odor recognition test to ensure that they had adequate sensory acuity and could detect specific odor compounds (Appendix G). A series of ten odor substances was presented blind, then the trainees were asked to identify the odor of each sample.
Trainees who could correctly identify at least seven of the odor substances were trained for approximately 60 min to become familiar with the quality attributes of lobster tails based on their odor characteristics and to develop descriptors for aroma. Two groups of lobster tails, raw and cooked, were presented to the trainees. Each group had three lobster tail samples representing a certain level of quality: high quality (very fresh, recently butchered), medium quality (butchered, then packed in ice for 10 days), and poor quality (butchered, then thermally abused to accelerate spoilage). Trainees were also instructed how to rate the odor of the lobster tails during the shelf-life study using the 6-inch horizontal line scale. Furthermore, a group discussion was conducted in order to develop descriptors for the 6-inch line scale.

Odor descriptors for high quality and poor quality were “fresh, ocean-like” and “putrid,” respectively, for raw samples, and “very fresh, lobster-smell” and “sour,” respectively, for sous-vide cooked samples. Panelists took 3 short sniffs, then marked the line scale (0 = high quality, 15 = poor quality) on a paper ballot to represent their perception of sample odor (Appendix H). Panelists waited 30 seconds before evaluating the next sample. Panelists were also asked whether or not they would consume the sample product. Samples were considered unacceptable when more than 50% of the consumers rejected the product (Boziaris et al., 2011; Østli et al., 2013). Participants were requested to read an informed consent form (Appendix C) before taking the test (Appendix I). The sensory study was approved (application number, 2018-06-01; approval date, June 13th, 2018) by the University of Maine Institutional Review Board (IRB) for the Protection of Human Subjects.
4.2.7. pH analyses

Lobster muscle was ground using a food processor (Oster FPSTMC3321-015-NP2, Sunbeam Products, USA) for 30 secs. Ten grams of ground tails were homogenized with 90 mL of deionized water in a Waring blender (Eberbach Corporation, Ann Arbor, MI) for 1 min. The pH of the tail muscle homogenate was measured using an Orion™ Model 320 PerpHecT LogR meter (Thermo Scientific™, Waltham, MA) at room temperature. Two subsamples were analyzed per treatment replicate and readings were averaged.

4.2.8. Biogenic amines

Lobster muscle was ground using a food processor (Oster FPSTMC3321-015-NP2, Sunbeam Products, USA) for 30 secs. Five grams of ground tail muscle was homogenized 1:4 (w/v) with 20 mL of 6 % TCA. The homogenate was centrifuged at 12000 x g for 10 min at 4 °C then filtered through Whatman #1 filter paper and brought to 50 mL with distilled water (Özogul et al., 2006). The supernatant and analytical standards were derivatized using an AccQ-Fluor Reagent Kit purchased from Waters Corporation (Milford, MA, USA). An analytical standard cocktail was prepared using histamine (ICN Biomedicals Inc., Aurora, OH), putrescine (Acros Organics, Fairlawn, NJ, USA), and cadaverine (Sigma–Aldrich, St. Louis, MO, USA). Biogenic amine concentrations in the standard curve prepared from this cocktail ranged from 1 to 100 µg/mL.

Ten µL aliquots of the standard and samples were mixed with 70 µL of AccQFluor borate buffer then added to 1 mL Target Micro-Sert tubes (Thermo Scientific™, Waltham, MA) and vortexed briefly. Twenty µL of reconstituted AccQFluor Reagent was added to the tube and then the sample tube was incubated in a sand-filled heating block at 55 °C for 10 min. Ten µL
aliquots of samples and standards were injected on the HPLC (1100/1200, Agilent Technologies, Santa Clara, CA, USA). A gradient mobile phase consisting of Eluent A (AccQ-Tag Eluent A Buffer, Waters Corporation), Eluant B (acetonitrile), and Eluent C (water) was modified to maximize separation and resolution of the target biogenic amines. The flow rate was set to 1.5 mL/min and an AccQ-Tag C18 column (3.9 x 150mm, Waters Corp) was used to separate the amines at ambient temperature. The fluorescence detector excitation was set at 250 nm and the emission was 410 nm. ChemStation software (Agilent Technologies, Santa Clara, CA, USA) was used to calculate peak areas. Biogenic amines in the samples were reported as mg/100 g meat.

4.2.9. Texture analyses

Texture measurements were performed using a calibrated texture analyzer platform (TA-XTi2, Texture Technologies Inc., Scarsdale, NY, USA) with a 5000 g load cell. Twenty mm long portions (n=10 per treatment replicate) were cut from the central region of each lobster tail. Each portion was positioned with the red colored dorsal surface of the sample facing up so the Warner-Bratzler blade (TA-42 knife blade with 45° chisel end) cut perpendicularly through the muscle fibers. The texture analyzer was configured to a 90 % depth, a 2 mm/s test speed, and a trigger force of 0.049 Newtons (N). The maximum peak force (N) required to shear through the sample was recorded as shear force and averaged by the texture analysis software (Exponent 32, version 5.0.6.0 2010, Texture Technologies Inc., Scarsdale, NY).

4.2.10. Color analyses

A colorimeter (LabScan XE, Hunter Labs, Reston, VA, USA) was used to measure differences in color among treatments. The colorimeter was standardized using white and black tiles for a port size of 30.5 mm, area view of 12.7 mm, and an illumination of 10° (D65, Hunter
Labs, Reston, VA). The Hunter L*, a*, b* values of the ventral side of 20 mm long lobster tail portions (n=10 per treatment replicate) were recorded as the average of three (initial and rotated 120° twice) readings per sample by the colorimeter software (Universal, version 4.10, 2001, Hunter Labs, Reston, VA).

4.2.11. Statistical analysis

Data were statistically analyzed by repeated-measures ANOVA at a 95% confidence interval (P < 0.05) using SPSS 26 (IBM, Armonk, NY, USA). Outliers were identified by SPSS based on the rule of 3 x interquartile range (IQR). Adjustment for multiple comparisons was conducted by the Fisher's Least Significant Difference (LSD). One-way ANOVA was performed to detect statistical differences for all one-level (treatment) analyses. Tukey’s honest significant difference (HSD) was used for post-hoc separation of means. Significant differences between the means of two sets of observations were analyzed by t-test. Pearson’s Correlation was used to evaluate correlations among dependent variables.

4.3. Results and Discussion

4.3.1. Microbiological analyses

LAB is a significant spoilage indicator for vacuum-packed seafood products (Gram & Huss, 1996), and LAB counts of raw lobster tails increased significantly (P < 0.05) over time for the control and both HPP treatments (150 and 350 MPa). On days 7, 14, and 21, significantly lower (P < 0.05) average counts were observed in the 350 MPa treatment (1.95-3.92 log CFU/g) compared to the control (3.30-4.00 log CFU/g) and 150 MPa treatment (3.64-5.67 log CFU/g). Surprisingly, by day 28, LAB counts for the 350 MPa raw samples exceeded 6 log CFU/g, significantly (P < 0.05) higher than the control and 150 MPa treatments which ranged from
4.25-5.09 log CFU/g, indicating that stressed or sub-lethally injured bacteria had recovered and were able to grow rapidly during extended storage (Campus, 2010). Moreover, the lack or the absence of competitive microbiota (due to their sensitivity to pressure) could be another reason for the higher LAB counts of the 350 MPa treatment, after recovery. For example, Gram-negative bacteria such as Campylobacter are generally more pressure-sensitive than Gram-positive bacteria such as LAB. Moreover, Campylobacter were observed to not recover after high pressure inactivation (Simonin et al., 2012). LAB were consistently observed to recover to high values during chilled storage (4 °C) in cooked ham and marinated beef loin samples after a 600 MPa treatment (Garriga et al., 2004; Jofré et al., 2009; Vercammen et al., 2011). The TBC (ranged from 1.95 to 6.56 log CFU/g) and LAB counts (ranged from 1.95 to 6.67 log CFU/g) for all raw treatments were nearly identical and did not significantly ($P < 0.05$) differ throughout the storage (Figure 4.3.A; B).

For sous-vide cooked samples, LAB and TBC counts remained below 2 log CFU/g throughout the study for all treatments indicating that HPP pretreatment did not contribute to additional shelf-life extension of the cooked lobster tails. Similar results were observed in seabream sous-vide cooked at 65 °C for 46.3 min and then pressurized at 300 or 600 MPa for 5 min at 5 °C (Espinosa et al., 2015). The authors reported that LAB counts were below the detection limit (< 1 log CFU/g) in all treatments for up to 62 days of refrigerated storage at 2 °C. Moreover, no significant differences were observed in TBC counts (ranged from 2.36 to 2.84 log CFU/g) between the unpressurized (control) and pressurized seabream samples throughout the shelf-life study. These results from the present study indicate that sous-vide cooking at 65 °C can preserving microbial quality and extending shelf-life of lobster products without the application of HPP.
LAB growth is the main limiting factor in the refrigerated shelf-life of vacuum-packed products due to its contribution to spoilage and quality losses including sour off-flavors, unpleasant off-odors, gas production, discoloration, slime production, and pH reduction (Ramesh, 2007), which constitute the main qualitative criteria for meat rejection by consumers (Nychas et al., 2008). TBC can be disregarded or used interchangeably with LAB counts to determine shelf-life in future studies (Table 5.1) of vacuum-packed lobster products, as TBC in
this type of product includes facultative anaerobes which can spoil vacuum-packed food products.

4.3.2. Total volatile basic nitrogen (TVBN)

TVBN represents the sum of trimethylamine (TMA), dimethylamine (DMA), ammonia, and other basic nitrogen compounds (Altissimi et al., 2018). Compared to fish, crustacea contain a high content of non-protein nitrogen (NPN) compounds, including trimethylamine oxide (TMAO), which may be converted to TVBN compounds by endogenous enzymes and microbial activity resulting in unacceptable off-odors and off-flavors (Gram & Huss, 1996; Boziaris et al., 2011). TVBN values for raw lobster tails increased significantly ($P < 0.05$) during storage for the control and 150 MPa treatment (Figure 4.4.A). The control and 150 MPa treatment had significantly ($P < 0.05$) higher TVBN values than the 350 MPa treatment. Throughout 28 days of storage, TVBN values for raw tails pressurized at 350 MPa were below the upper limits for fresh seafood (35-40 mg N/100g meat) (Özogul et al., 2008). In contrast, TVBN values for the control and 150 MPa treatment were ~five times higher compared to 350 MPa. The effect of pressurization on decreasing TVBN values were also observed by Kaur et al. (2013), who reported that the higher processing pressure of 435 MPa significantly decreased the development of TVBN (27.7 mg N/100g meat) in shrimp muscle for up to 20 days of storage at 2 °C compared to the untreated control (92.4 mg N/100g meat), 100 MPa (45.7 mg N/100g meat) and 270 MPa (39.8 mg N/100g meat) treatments. TVBN reduction was also found in raw prawns pressurized at 100, 270, 435, and 600 MPa (Bindu et al., 2013). Furthermore, the shelf-life of Norway lobster stored at 0 °C was limited to 4 days due to the high levels of TVBN products (Boziaris et al., 2011).
For sous-vide cooked tails, although TVBN levels increased significantly over time for all treatments (Figure 4.4.B), values remained below 35 mg/100g throughout 28 days storage, confirming that sous-vide cooking inhibited the microbial activity responsible for producing these volatile compounds, as indicated by the microbial reduction in the sous-vide cooked lobster tails.

Figure 4.4. Total volatile base nitrogen concentrations of (A) raw and (B) sous-vide cooked lobster tails during refrigerated (2 °C) storage. Each value is the mean ± standard deviation (n = 3).
In the present study, TVBN values were significantly \((P < 0.01)\) and positively correlated with LAB counts \((r= 0.71)\), indicating that the lower TVBN values observed in the raw lobster at 350 MPa and sous-vide cooked tails correspond with lower LAB counts. However, when LAB counts of raw tails pressurized at 350 MPa increased sharply after day 14 and reached over 6 log CFU/g on day 28, TVBN values remained below 35 mg N/100g meat. The lower TVBN values in the 350 MPa samples despite increasing LAB counts could be attributed to various species of bacteria present in the spoilage flora unable to convert TMAO to TMA (Sotelo & Rehbein, 2000; Summers et al., 2016). Moreover, LAB produced lactate when grown in the presence of TMAO but did not produce large amounts of TMA (Hoyles et al., 2018). Bacteria responsible for the majority of TMA found in spoiled seafood, such as \textit{Enterobacteriaceae} and Pseudomonads are generally pressure sensitive. Diez et al. (2008) reported that the application of HPP treatments of 300, 500, and 600 MPa for 10 min at 15 °C in blood sausage reduced enterobacteria and pseudomonads counts to levels below the detection limit (< 1 and 2 log CFU/g, respectively) throughout 28 days of chilled storage (2 °C). Furthermore, the delayed development of TVBN may be also attributed to the effect of higher pressure (350 MPa) on trimethylamine oxide demethylase (TMAOase). TMAOase converts TMAO to DMA and formaldehyde (Gill & Paulson, 1982), which are responsible for unpleasant fishy odors during refrigerated storage (Gou et al., 2010). Although TMAOase is heat stable and can still be active after heating at 80 °C for 30 min (Kimura et al., 2003), Gou et al. (2010) reported a significant decrease in TMAOase activity in squid pressurized at 300 MPa for 5 min at 20 °C.

### 4.3.3. Sensory quality (aroma)

Aroma plays a significant role in consumer acceptability of seafood products. Odor scores of the control and pressurized raw tails stored at 2 °C increased over time for all the
treatments and ranged from 1.7 to 12.1. The mean initial odor score for control raw samples was higher (5.4) compared to the 150 MPa (2.2) and 350 MPa (2.3) treatments. However, strong putrid off-odors developed rapidly in the controls and 150 MPa samples, consequently aroma scores increased rapidly and both products were rejected by 90 % and 60 % of respondents, respectively, by day 7 (Figure 4.5). Aroma scores for the 350 MPa samples remained at ~ 7 throughout the study, with 70 % of respondents rating the 350 MPa samples as still acceptable after 28 days of refrigerated storage. Erkan et al. (2010) used a 9-point descriptive scale to assess odor characteristics of raw red mullet pressurized at 220-330 MPa for 5 to 10 min then stored at 4 °C, with scores below 4 indicating spoiled fish. The authors observed that the odor scores of all pressurized samples were above 4 for up to 11 days compared to 9 days for the control. The development of off-odors in seafoods can be partially attributed to the TMA resulting from endogenous enzymatic reactions and bacterial degradation of TMAO (Gram & Huss, 1996; Campos et al., 2005; Erkan et al., 2010; Østli et al., 2013). In the present study, panelist rejection of raw lobster tails had a significant ($P < 0.01$) strong positive correlation ($r=0.94$) with TVBN values indicating that volatile nitrogen compounds were likely a limiting factor to sensory acceptability of raw lobster tails, with sensory rejection calculated as occurring at a TVBN level of 63 mg N/100g meat. For sous-vide cooked tails, the aroma scores increased significantly over time for all the treatments (from 4.8 to 8.1), and no differences were observed among the treatments. By day 28, only 20% of the respondents had rejected any of the sous-vide cooked samples. Sous-vide conditions that combine low cooking temperatures and long cooking times with vacuum packaging may delay the development of undesirable off-odors during storage (Díaz et al., 2009). The lower TVBN levels in sous-vide cooked tails compared to raw tails at 350 MPa also contributed to the extension of aroma acceptability, as TVBN, particularly TMA is
responsible for fishy off-odors during storage (Gram & Huss, 1996; Gou et al., 2010). However, sous-vide cooked cod developed putrid off odors after 3 weeks of storage at 5 °C due to growth of spore-forming gram-positive bacteria (Embarek, 1994). Overall, these results indicate that the higher pressure treatment (350 MPa) preserved a fresher aroma in raw tails for up to 28 days, while for sous-vide cooked lobster tails, the thermal treatment was responsible for decreasing off-odors development in refrigerated samples longer without a synergistic beneficial effect of HPP pretreatment.

![Graph showing sensory rejection of raw lobster tails during refrigerated storage](image)

**Figure 4.5. Sensory rejection (aroma) of raw lobster tails during refrigerated (2 °C) storage (n=10 panelists).**

* Control and 150 MPa samples were not evaluated after day 7.

4.3.4. pH

HPP significantly affected pH values of raw lobster tails (Table 4.1). The initial pH values of raw lobsters pressurized at 350 MPa (6.35±0.01) were significantly higher than those of the control (6.15±0.06) and 150 MPa (5.97±0.04) treatments. The increase in pH value may be
due to conformational changes induced by higher pressure (350 MPa) treatment associated with the unfolding of some protein fractions consequently exposing basic amino acids (Angsupanich & Ledward, 1998; Kaur et al., 2013). Over time, pH values of raw tails increased significantly for all the treatments. The increase in pH values is similar to results reported by Bindu et al. (2013) who observed a significant increase in pH values (ranging from 6.75 to 7.26) of prawns high pressure processed at 100, 270, 435 and 600 MPa for 5 min and stored at 2°C for 30 days. The increase in pH may be attributed to bacterial spoilage resulting in the formation of basic volatile nitrogen compounds such as TMA, DMA, ammonia, and other alkaline substances (Lopez-Galvez et al., 1995; Gou et al., 2010; Briones-Labarca et al., 2012), supported by the significant ($P < 0.01$) strong positive correlation ($r=0.90$) between pH values and TVBN content of lobster tails.

**Table 4.1. pH values of raw and sous-vide cooked lobster tails during refrigerated (2 °C) storage.**

<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th>150 MPa</th>
<th>350 MPa</th>
<th>Control</th>
<th>150 MPa</th>
<th>350 MPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.15±0.06Cc</td>
<td>5.97±0.04Bc</td>
<td>6.35±0.01Ab</td>
<td>6.46±0.13Aa</td>
<td>6.33±0.10Aa</td>
<td>6.44±0.03Ab</td>
</tr>
<tr>
<td>7</td>
<td>7.15±0.05Ab</td>
<td>6.87±0.08Bb</td>
<td>6.53±0.04Ca</td>
<td>6.41±0.11Ba</td>
<td>6.33±0.08Ba</td>
<td>6.63±0.01Aa</td>
</tr>
<tr>
<td>14</td>
<td>7.44±0.11Aa</td>
<td>7.28±0.14Aa</td>
<td>6.49±0.04Bab</td>
<td>6.41±0.19Aa</td>
<td>6.23±0.12Aa</td>
<td>6.47±0.04Ab</td>
</tr>
<tr>
<td>21</td>
<td>7.62±0.13Aa</td>
<td>7.22±0.03Ba</td>
<td>6.51±0.10Cab</td>
<td>6.54±0.01Aa</td>
<td>6.47±0.12Aa</td>
<td>6.49±0.06Ab</td>
</tr>
<tr>
<td>28</td>
<td>7.16±0.15Ab</td>
<td>7.26±0.06Aa</td>
<td>6.64±0.08Ba</td>
<td>6.37±0.8ABa</td>
<td>6.29±0.09Ba</td>
<td>6.49±0.04Ab</td>
</tr>
</tbody>
</table>

Each value is the mean ± standard deviation (n =3). Values not sharing an uppercase letter are significantly ($P < 0.05$) different within rows. Values not sharing a lowercase letter are significantly ($P < 0.05$) different within columns, analyzed by ANOVA (Tukey’s HSD post hoc test).

Throughout storage, the mean pH values of raw samples ($\bar{x} = 6.51$) for the higher-pressure treatment (350 MPa) were significantly lower compared to the control ($\bar{x} = 7.10$) and 150 MPa treatment ($\bar{x} = 6.92$), apparently due to the inhibition of TVBN development as previously discussed. Similarly, Briones-Labarca et al. (2012) reported that HPP treatments of 500 MPa and
550 MPa significantly decreased pH values of red abalone muscle throughout storage at 4 °C in comparison to raw controls. For sous-vide cooked lobster tails, pH values were stable (6.33-6.44) throughout storage for all samples, and no differences were observed among the control and HPP treatments, likely due to the inactivation of LAB by the thermal treatment.

### 4.3.5. Biogenic amines

Biogenic amines are formed as a consequence of microbial decarboxylation of free amino acids (Altissimi et al., 2018; Arulkumar et al., 2019). Although many biogenic amines have been found in fish, only putrescine, cadaverine, and histamine are significant in terms of quality determination (Briones-Labarca et al., 2012). In raw lobster tails, putrescine and cadaverine levels increased significantly ($P < 0.05$) over time for all treatments (Table 4.2).

#### Table 4.2. Putrescine and cadaverine content (mg/100 g muscle) of raw and sous-vide cooked lobster tails during refrigerated (2 °C) storage.

<table>
<thead>
<tr>
<th>Raw</th>
<th>Putrescine</th>
<th>Cadaverine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td>Control</td>
<td>150 MPa</td>
</tr>
<tr>
<td>0</td>
<td>3.8±0.7Ab</td>
<td>3.8±0.1Ac</td>
</tr>
<tr>
<td>7</td>
<td>41.1±11.5Aa</td>
<td>24.7±14.8ABab</td>
</tr>
<tr>
<td>14</td>
<td>47.9±10.7Aa</td>
<td>41.7±5.8Aab</td>
</tr>
<tr>
<td>21</td>
<td>39.6±25.6Aab</td>
<td>21.4±4.7ABbc</td>
</tr>
<tr>
<td>28</td>
<td>40.4±8.5Aab</td>
<td>44.4±3.1Aa</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SV Days</th>
<th>Control</th>
<th>150 MPa</th>
<th>350 MPa</th>
<th>Control</th>
<th>150 MPa</th>
<th>350 MPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.8±1.4Aa</td>
<td>1.3±0.5Ab</td>
<td>2.1±0.3Aa</td>
<td>4.2±0.9Aa</td>
<td>2.3±2.4Aa</td>
<td>2.1±0.4Aa</td>
</tr>
<tr>
<td>7</td>
<td>2.7±0.8Aa</td>
<td>2.2±0.8Aab</td>
<td>1.4±0.3Aa</td>
<td>7.6±2.5Aa</td>
<td>1.6±1.5Ba</td>
<td>1.0±0.3Ba</td>
</tr>
<tr>
<td>14</td>
<td>4.8±2.8Aa</td>
<td>3.7±0.9Aa</td>
<td>0.9±1.2Aa</td>
<td>7.5±1.6Aa</td>
<td>7.3±6.4Aa</td>
<td>3.9±3.4Aa</td>
</tr>
<tr>
<td>21</td>
<td>1.6±0.2Aa</td>
<td>2.0±0.9Aab</td>
<td>0.8±0.3Aa</td>
<td>11.1±5.9Aa</td>
<td>2.8±2.9Aa</td>
<td>0.9±0.3Ba</td>
</tr>
<tr>
<td>28</td>
<td>2.4±0.4Aa</td>
<td>1.8±0.5Aab</td>
<td>0.8±0.7Aa</td>
<td>12.9±7.1Aa</td>
<td>6.7±6.2Aa</td>
<td>2.7±1.8Aa</td>
</tr>
</tbody>
</table>

Each value is the mean ± standard deviation (n =3). Values not sharing an uppercase letter are significantly ($P < 0.05$) different within rows, analyzed by ANOVA (Tukey’s HSD post hoc test). Values not sharing a lowercase letter are significantly ($P < 0.05$) different within columns, analyzed by ANOVA (Tukey’s HSD post hoc test).

Throughout storage, overall average putrescine levels for the 350 MPa tails (3.2±1.4 mg/100g) were significantly lower than the average putrescine levels for control...
(34.6±1.4 mg/100g) and 150 MPa (27.2±1.4 mg/100g) treatment. Although cadaverine production in raw lobster samples was somewhat lower than putrescine, cadaverine showed similar trends as putrescine for all treatments, as cadaverine levels for the 350 MPa tails (2.7±1.5 mg/100g) were significantly lower compared to the control (28.6±1.5 mg/100g) and the 150 MPa (21.6±1.5 mg/100g) treatment.

Similarly, putrescine and cadaverine levels of white prawn continued to increase at a rapid rate throughout storage at 4 °C for 8 days, ranging from < 0.1 mg/100g at day 0 to 12.5 and 23.4 mg/100g, respectively, at day 8 (Zhao et al., 2007). In the present study, putrescine content had a significant ($P < 0.01$) and strong correlation (r=0.86) with cadaverine indicating that either putrescine or cadaverine can serve as an indicator for decomposition in a vacuum-packaged lobster tails under refrigeration.

Histamine levels in illness-causing fish are generally above 20 mg histamine/100g meat (200 ppm) and often above 50 mg histamine/100g meat (500 ppm) (FDA, 2020). However, due to the high variability in histamine levels between fish and within an individual fish, 5 mg histamine/100g meat (50 ppm) has been set by the U.S. FDA as a guidance level in the edible portion of fish (FDA, 2020). While other biogenic amines can potentiate histamine toxicity, histamine is the only biogenic amine with regulatory limits set by the European Commission (2005), up to a maximum of 20 mg histamine/100g meat (200 ppm) in fresh fish. Histamine levels in raw lobster tails increased significantly over time for all the treatments (Figure 4.6), and ranged from 0-195 mg/100g, 4.8-134.0 mg/100g, and 3.6-188.0 mg/100g for the control, 150 MPa, and 350 MPa treatments, respectively. Histamine levels for the 350 MPa treatment were below the health hazard limit (50 mg/100g) reported by the FDA (2020) for the first 14 days. Conversely, at day 14, the histamine levels were ~195 and ~135 mg/100g for the control and
150 MPa treatment, respectively. These results are in contrast to previous studies that reported negligible histamine contents in fresh and spoiled crustaceans (Mietz & Karmas, 1978; Prester, 2011).

![Histogram showing histamine content of raw lobster tails during refrigerated (2 °C) storage.](image)

**Figure 4.6. Histamine content of raw lobster tails during refrigerated (2 °C) storage. Each value is the mean ± standard deviation (n = 3).**

For example, the histamine level was 5.6 mg/100g in the muscle of mud spiny lobster (*Panulirus polyphagus*) after 15 days storage on ice (Arulkumar et al., 2019). However, a more significant histamine level (39.4 mg/100g) was observed in fermented shrimp sauces (Tsai et al., 2006). Histamine development in fermented seafoods can be due to LAB activity, as LAB are considered the main biogenic amine producers in fermented foods (Barbieri et al., 2019). The high level of histamine observed in the lobster tail muscle may be related to the histidine and histamine content of lobster bait. Atlantic herring (*Clupea harengus*), the most popular bait in lobster traps throughout northeastern U.S. (Grabowski et al., 2010), naturally contain considerable quantities of free histidine in their muscle which can be converted to histamine as a result of temperature abuse (FDA, 2020). Therefore, the presence of histamine in the bait can be significant, likely contributing to the histamine content in lobster tissues after consuming the
bait. In rats, as the levels of dietary histidine and histamine increased, their concentrations also increased in all the tissues measured (Lee et al., 1981).

Histidine is converted to histamine by certain gram-negative bacteria that are able to produce histidine-decarboxylase such as *Morganella morganii*. In the present study, histamine content significantly (*P* < 0.01) correlated (*r*=0.67) with LAB counts. LAB growth was maintained below 2 log CFU/g for up to 14 days, however histamine levels increased ~11 fold by day 14. This increase in histamine levels on day 14 could be attributed to the lag phase of recovered bacteria. After the lag phase, the recovered bacteria adjust to the new conditions and start producing enzymes required to degrade the surrounding substrate thereby preparing them for exponential growth (Rolfe et al., 2011). A number of the histamine-forming bacteria are facultative anaerobes that can grow in reduced oxygen environments (FDA, 2020). Moreover, once histidine decarboxylases are produced, their activity continues at refrigerated temperature to produce histamine in fish even if the bacteria are inactivated (FDA, 2020). For sous-vide cooked tails, putrescine, cadaverine, and histamine did not significantly change over time in all treatments (Table 4.2; Table 4.3). Overall, putrescine, cadaverine, and histamine levels were below 3 mg/100g, 9 mg/100g, and 3 mg/100g, respectively, due to reduced bacterial growth in the cooked samples.

**Table 4.3. Histamine content (mg/100 g meat) of sous-vide cooked lobster tails during refrigerated (2 °C) storage.**

<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th>150 MPa</th>
<th>350 MPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.1±0.2</td>
<td>2.4±2.1</td>
<td>4.9±2.1</td>
</tr>
<tr>
<td>7</td>
<td>3.8±1.7</td>
<td>4.2±1.3</td>
<td>3.1±0.2</td>
</tr>
<tr>
<td>14</td>
<td>2.3±0.53</td>
<td>3.7±2.4</td>
<td>1.4±1.2</td>
</tr>
<tr>
<td>21</td>
<td>2.2±1.4</td>
<td>2.7±0.5</td>
<td>2.5±1.2</td>
</tr>
<tr>
<td>28</td>
<td>3.6±0.7</td>
<td>3.2±0.9</td>
<td>2.9±0.1</td>
</tr>
</tbody>
</table>

Each value is the mean ± standard deviation (*n* =3). There were no statistically significant differences among treatments (*P* > 0.05).
4.3.6. Texture

Shear force values for raw and sous-vide cooked tails did not significantly change throughout storage for all three treatments despite the microbial and biochemical changes that occurred, particularly in raw tails. For raw lobsters, pressurization at 150 MPa significantly decreased shear force values by ~ 8 N compared to the control and 350 MPa treatment (Table 4.4), and no significant differences were observed between the control and 350 MPa treatment. These results support findings from our previous study on HPP lobster tails (Humaid et al., 2019), in which pressurization at 150 MPa for 10 min significantly decreased hardness of lobster tails compared to controls. In seabass, processing pressures below 300 MPa were found to increase enzymatic activity due to the rupture of the lysosomal membrane, consequently releasing proteases with access to muscle proteins. In contrast, processing pressures above 300 MPa decreased the proteolytic activity due to structural modification of the enzymes (Chéret et al., 2005; Teixeira et al., 2013). For the cooked lobsters, shear force values were not significantly different among all treatments, suggesting that the thermal treatment (65 °C) associated with sous-vide cooking inactivated endogenous proteases such as calpains and cathepsins responsible for softening the muscle (Porter et al., 1995). However, our previous study on lobster tails (Humaid et al., 2019) showed that 350 MPa for 10 min significantly increased the shear force in sous-vide cooked lobsters. The difference in shear force results between the present study and the previous study may be due to different environmental conditions for pre-harvest lobsters that influenced their muscle structure (Spees et al., 2002). Location of harvest, time of year, and physiological condition are all reported to impact the sensory attributes of aquatic species (Botta et al., 1986).
Table 4.4. Shear force (N) values of high pressure processed raw and sous-vide cooked tails stored during refrigerated (2 °C) storage

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Raw Initial</th>
<th>Raw Final</th>
<th>SV-cooked Initial</th>
<th>SV-cooked Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.76±4.19Aa</td>
<td>22.43±0.96Aa</td>
<td>48.97±8.80Aa</td>
<td>45.17±2.47Aa</td>
</tr>
<tr>
<td>150 MPa</td>
<td>16.70±1.27Ba</td>
<td>16.76±1.02Ba</td>
<td>47.10±10.07Aa</td>
<td>51.07±4.97Aa</td>
</tr>
<tr>
<td>350 MPa</td>
<td>25.65±3.14Aa</td>
<td>24.98±3.62Aa</td>
<td>46.39±5.55Aa</td>
<td>47.72±3.63Aa</td>
</tr>
</tbody>
</table>

a Each value is the mean ± standard deviation (n = 3). Values not sharing an uppercase letter are significantly (P < 0.05) different within columns, analyzed by ANOVA (Tukey's HSD post hoc test). Values not sharing a lowercase letter are significantly (P < 0.05) different within rows for each group (raw and SV-cooked), analyzed by t-test.
b At day 7 for the control and 150 MPa treatments, and at day 28 for the 350 MPa treatment.

In the present study, the textural results of sous-vide cooked lobster tails were comparable to those of Angsupanich et al. (1999) who reported no significant differences in hardness between cooked cod muscle and cod muscle pressurized at 400 MPa prior to cooking. Similarly, the firmness of salmon loins sous-vide cooked at 50 °C was not significantly different compared to samples sous-vide cooked and then pressurized at 210, 310, or 400 MPa (Picouet et al., 2011).

4.3.7. Color

Storage did not affect the color (L*, a*, b*) of raw lobster tails in any of the treatments, however HPP induced color changes immediately after processing. Higher processing pressure (350 MPa) significantly increased the L* and a* values and decreased b* values of raw samples compared to the control and 150 MPa treatment (Table 4.5), and gave the 350 MPa pressurized tails an opaque and cooked appearance. The increase in lightness of tail muscle after pressurization can be attributed to unfolding of carotenoprotein (Truong et al., 2015) and the degradation of carotenoid pigments such as astaxanthin (Cruz-Romero et al., 2004; Bindu et al., 2013). However, carotenoids in crustaceans vary depending on molt stage along with reproductive cycle, food source, and habitat (Menasveta et al., 1993). The color bleaching effect
of HPP on fish and shellfish products is well documented (Bindu et al., 2013; Kaur et al., 2013; Kaur & Rao, 2018), with numerous reports of muscle lightness increasing with increasing pressure levels. However, changes in a* and b* values of HPP lobster tails were contrary to the results found in shrimp (Bindu et al., 2013; Kaur et al., 2013), which had higher b* values and lower a* values with increasing pressure intensity. For sous vide cooked samples, no significant differences were observed in L* values between initial and final assessment for all the treatments.

Table 4.5. Instrumental color (L*, a*, b*) values of raw and sous vide cooked tails during refrigerated (2 °C) storage.

<table>
<thead>
<tr>
<th></th>
<th>Raw</th>
<th>SV-cooked</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>L*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>43.62±0.09&lt;sup&gt;Ca&lt;/sup&gt;</td>
<td>45.23±1.7&lt;sup&gt;1Ba&lt;/sup&gt;</td>
</tr>
<tr>
<td>150 MPa</td>
<td>47.73±0.13&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>46.15±0.55&lt;sup&gt;Ca&lt;/sup&gt;</td>
</tr>
<tr>
<td>350 MPa</td>
<td>65.15±1.31&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>66.42±1.29&lt;sup&gt;Aa&lt;/sup&gt;</td>
</tr>
<tr>
<td>a*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.98±0.38&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>3.07±0.76&lt;sup&gt;Aa&lt;/sup&gt;</td>
</tr>
<tr>
<td>150 MPa</td>
<td>2.96±0.58&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>3.50±0.46&lt;sup&gt;Ca&lt;/sup&gt;</td>
</tr>
<tr>
<td>350 MPa</td>
<td>4.50±0.19&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>4.44±0.77&lt;sup&gt;Ba&lt;/sup&gt;</td>
</tr>
<tr>
<td>b*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.35±0.32&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>2.14±1.76&lt;sup&gt;Ca&lt;/sup&gt;</td>
</tr>
<tr>
<td>150 MPa</td>
<td>-0.55±0.09&lt;sup&gt;Ca&lt;/sup&gt;</td>
<td>-1.15±0.70&lt;sup&gt;Ba&lt;/sup&gt;</td>
</tr>
<tr>
<td>350 MPa</td>
<td>-0.23±1.43&lt;sup&gt;ABAa&lt;/sup&gt;</td>
<td>-1.48±1.04&lt;sup&gt;Ba&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Each value is the mean ± standard deviation (n = 3). Values not sharing an uppercase letter are significantly (P < 0.05) different within columns, analyzed by ANOVA (Tukey’s HSD post hoc test). Values not sharing a lowercase letter are significantly (P < 0.05) different within rows for each group (raw and SV-cooked), analyzed by t-test. <sup>b</sup> At day 7 for the control and 150 MPa treatments, and at day 28 for 350 MPa treatment.

Similarly, redness and yellowness did not significantly change over time for all the treatments, ranging from 7.99 to 12.88 and from 6.46 to 11.26, respectively. However, pressurization affected color of cooked samples, as yellowness-value of 350 MPa sous vide cooked samples were significantly lower than the control and 150 MPa treatment. In addition, lobsters pressurized at 350 or 150 MPa and subsequently sous vide cooked had significantly
higher L* values compared to non-HPP sous vide cooked control samples. An increase in L* values in sous vide cooked seafood due to HPP was also observed by Picouet et al. (2011) in salmon loins, however in their study pressure was applied after sous vide cooking. The color changes observed in raw and sous vide cooked lobster in response to HPP have important implications for the seafood industry, particularly for restaurants and other food services operations.

Color is a significant factor in consumer perception of meat quality and in their purchasing decisions (Jung et al., 2003). The cooked appearance of pressurized raw tails may be more appealing to those who consume raw or minimally processed seafood products such as ceviche, in which citric acid denatures muscle proteins, giving it an opaque and cooked appearance. In addition, for sous vide cooked lobster tails, higher pressure (350 MPa) promoted a potentially more desirable appearance, as the less yellow the meat color, the greater the acceptability of lobster meat. Calder et al. (2006) found a negative relationship ($r=-0.75$) between yellowness and consumer acceptability ratings for exterior color of lobster meat and a negative relationship ($r=-0.70$) between hue (red, yellow, green, blue) and consumer acceptability ratings for overall acceptability after six months of frozen storage.

### 4.4. Conclusions

This study provides information relevant for the application of HPP technology to lobster products. Results indicate that HPP at 350 MPa for 10 min can be utilized as an effective tool to extend the refrigerated shelf-life of vacuum-packed raw lobsters. However, HPP pretreatment did not contribute to additional shelf-life extension of the sous-vide cooked lobster tails. Based on microbial, TVBN, aroma, and biogenic amines results, raw lobsters processed at 350 MPa maintained acceptable quality throughout 28 days storage. Texture and color were not
significantly affected by storage but HPP significantly affected texture and color of raw tails. However, previous research showed that these changes in texture and color did not affect consumer acceptance of the lobster tails after sous-vide cooking. These findings have important implications for the lobster processing industry. HPP at 350 MPa can increase the commercial availability of refrigerated lobster tails and promote the development of diverse lobster products that are more convenient than live lobsters and having better quality than frozen products. Surprisingly, a considerable histamine content was observed in raw lobsters that reached the hazard limit after 14 days, however, according to the FDA (2020) histamine is not considered a hazard likely to be present in lobster products. Further studies monitoring histamine-forming bacteria in vacuum packaged lobsters are warranted (Table 5.1), particularly since a large number of histamine-forming bacteria are facultative anaerobes and can grow in a reduced oxygen environment. In addition, a future HPP validation study is necessary to clarify the impacts of 350 MPa for 10 min on product safety during refrigerated storage.
CHAPTER 5

OVERALL CONCLUSIONS

These studies show that high pressure processing (HPP) and sous-vide (SV) cooking are promising techniques in the development of lobster products. HPP can help food service operations or home cooks to receive fresh, additive-free, ready to sous-vide lobster products with extended refrigerated shelf-life for convenient sous-vide cooking. Sous-vide also offers a greatly extended shelf-life of minimally processed, high quality, ready to be served foods. This unique method of cooking provides perfectly cooked foods with a tender and juicy texture, which are crucial attributes for consumer satisfaction. HPP has the potential to be combined with sous-vide cooking to produce consumer-acceptable, value-added lobster products. However, there are some disadvantages associated with sous-vide and HPP techniques. For example, sous-vide requires more time to cook foods compared to conventional cooking methods. In addition, drip loss post sous-vide cooking may result in packages that are unappealing to consumers. Moreover, improper pasteurization of sous-vide cooked products is a potential safety risk. For HPP, the price of equipment along with operating costs including labor, maintenance, and energy are major barriers that have limited the widespread commercialization of this technology. Moreover, although HPP can inactivate bacterial spores at pressures above 600 MPa, some spores require a combination of higher pressure levels and heat to be inactivated. These extreme conditions required to inactivate spores by HPP can cause undesirable sensory changes, such as producing tough texture and cooked appearance in raw foods.

Although this research focused solely on lobster quality, all sous-vide time-temperature combinations were established based on safety to achieve a 6-log reduction of \textit{L. monocytogenes}. 

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because this species is the most heat resistant vegetative pathogen likely to occur in sous-vide cooked products. Non-proteolytic _C. botulinum_ type E, an anaerobic spore-forming bacteria is another pathogen of concern in sous-vide cooked products. However, based on literature, applying thermal treatment necessary to destroy _C. botulinum_ spores can produce unacceptable changes in the qualities of cooked lobster products. Therefore, immediately bringing the sous vide cooked products to temperatures below 3 °C was used as a hurdle to prevent growth and toxin formation of non-proteolytic _C. botulinum_ type E.

In the first study, the physicochemical properties and consumer acceptance of lobster tails sous-vide cooked under different time-temperature combinations were evaluated. The impacts of these sous-vide processing parameters on lobster qualities and consumer acceptability have not previously been reported. Sous-vide cooked lobsters were more tender and less yellow than those conventionally cooked in boiling water. The tougher meat induced by boiling may be attributed to increased denaturation and aggregation of muscle proteins as partially evidenced by correlations between the lower solubility of myofibrillar proteins and tougher texture. Myosin and actin of lobster muscle were completely denatured when cooked at 55 °C although the thermal denaturation temperature of actin was 58 °C based on DSC measurements. The long cooking time for the 55 °C treatment, a total of 231 min in a 55 °C water bath, likely contributed to the denaturation of actin at temperatures below its thermal denaturation temperature. Since actin denaturation was reported to be the main contributor to textural changes in seafood, cooking at 55 °C or below for shorter time has the potential to provide better texture attributes. However, these conditions would come at a cost to the microbial safety of sous-vide cooked
products. According to the FDA, 54.4 °C is the lowest temperature recommended for sous-vide cooking to control all non-spore forming pathogens such as *L. monocytogenes*.

The acceptability of sous-vide cooked lobster tails under different time-temperature combinations was evaluated by a consumer panel to establish the best sous-vide cooking parameters for the use in the subsequent studies. However, a direct sensory comparison between sous-vide cooked and boiled samples, and correlation of instrumental color and shear force results with texture and color attributes as determined by a consumer panel, would offer further insight. Consumer acceptability testing revealed that all sous vide cooking treatments were equally acceptable despite the physicochemical results which indicated that cooking at 65°C/10min significantly increased weight loss compared to the other two sous vide cooking treatments (55°C/208 min and 60°C/45min). In addition, correlations showed that the potential toughness associated with the expansion of sarcomeres in sous vide cooked lobsters at 65 °C did not influence consumer acceptability. Based on convenience during cooking, the 65 °C/10 min treatment was selected for further testing.

In the second study, we evaluated the impacts of HPP application on physicochemical properties of vacuum-packaged raw and sous-vide cooked tails, and subsequently determined consumer acceptability of the HPP pretreated sous-vide cooked (65 °C/10min) lobster tails. Although moderate HPP conditions were chosen to limit any negative impacts on lobster qualities, these moderate HPP pretreatment conditions significantly altered the physicochemical qualities of raw, more than of sous vide cooked lobster meat. Regardless of processing time, 150 MPa induced significant softening in the raw tails, while 350 MPa did not affect their texture. This decrease in hardness in the 150 MPa treatment may have been due to an increase in
proteolytic activity. Therefore, evaluating the impacts of HPP on proteolytic activity including calpains and cathepsins in lobster muscle is recommended for future studies (Table 5.1) since these enzymes are known to affect the texture of meat.

**Table 5.1. Proposed future research directions.**

- The impacts of HPP on proteolytic activity including calpains and cathepsins in lobster muscle.
- Evaluating the effects of HPP on the enthalpy and denaturation temperature of lobster muscle proteins using DSC.
- Determining the effects of HPP on hard-shell lobster meat.
- Monitoring histamine-forming bacteria in vacuum packaged lobsters.
- HPP validation study to clarify the impacts of 350 MPa for 10 min on product safety during refrigerated storage.

Both processing pressures (150 and 350 MPa) had bleaching effects on the color of raw muscle but 350 MPa produced a lighter color than the 150 MPa treatments. The cooked appearance induced by HPP in lobster muscle might be unacceptable to some consumers who prefer a translucent appearance in fresh raw seafood products. Sensory evaluation by a consumer panel would be an excellent approach to investigate consumer acceptance of high pressure processed raw lobsters. For HPP pretreated cooked lobsters, consumer acceptability evaluation showed that the changes in texture and color induced by HPP did not influence the consumer acceptability. Furthermore, the sensory results indicated that both pressures (150 and 350 MPa) along with the control resulted in an ideal juicy product. The effects of HPP on the texture, color, and consumer acceptability of cooked lobster tails have not previously been reported.

For both consumer acceptability studies, the rating scores (7 or below) of overall acceptability on the 9-point hedonic scale were disappointing, particularly since lobsters were cooked sous-vide, which is reported to produce exceptional texture and flavor attributes. These
lower than expected overall liking scores could be attributed to the panelists’ consumption frequency of lobster products. Results indicated that overall acceptability scores were influenced by reported consumption frequency. The most frequent consumption group (every 2 to 3 months) rated overall acceptability for all lobster products an average of 7.1, while the least frequent consumption group (1 to 2 times per year) gave an average overall acceptability rating of 6.6. In addition, some panelists did not appear to enjoy consuming lobster. To avoid these limitations and obtain better results in future consumer acceptability studies, we recommend limiting participation to consumers who consume lobster more frequently (at least every 2-3 months), as a part of the selection criteria. In addition, cluster analysis and correlations between sensory attributes and overall liking scores within different consumer clusters will help to better clarify and identify subgroups of respondents based on their degree of similarity (or differences) in certain demographic characteristics such as gender, age, and income.

The present study showed that myofibrillar proteins in raw lobsters experienced a decrease in solubility at 350 MPa indicating unfolding of muscle proteins. Evaluating the effects of HPP on the enthalpy and denaturation temperature of lobster muscle proteins using DSC is recommended for future studies (Table 5.1). Studying the thermal stability of muscle proteins in pressurized lobsters is important for selecting HPP conditions to manufacture high-quality lobster products, particularly since myosin is reported to be very sensitive to pressure and is responsible for the cooked appearance of muscle when denatured.

The sensory qualities of lobster meat, including texture and color, naturally vary depending on several factors such as molting stage and diet. For example, hard-shell lobster meat is firmer, while soft-shell meat is softer and tends to have a brighter red color. The effects of
HPP on soft-shell lobsters have been covered in this research. However, evaluating the effects of HPP on hard-shell lobster meat is recommended for future studies (Table 5.1), to better understand how lobster qualities respond to HPP based on their physiological state (soft-shell vs hard-shell). Measuring the concentration of serum protein using a refractometer should be considered as part of the sample selection criteria. For example, lobsters having a brix (°Bx) level of 8 or higher should be selected. Using serum protein level as a part of sample selection criteria can minimize any potential natural variations and provide more consistent lobster samples, possibly resulting in data with less variability.

The third study was conducted to determine the effects of HPP on refrigerated shelf-life of raw and subsequently sous-vide cooked lobster tails. The shelf-life study provided new information on the applications of HPP on lobster products. Based on microbial, biochemical, physical, and sensory results, 350 MPa for 10 min can be utilized as an effective tool to extend the refrigerated shelf-life of vacuum-packed, raw lobster tails. Raw tails pressurized at 350 MPa maintained lactic acid bacteria and total bacterial count below 2 log CFU/g throughout 14 days of refrigerated storage. Total volatile base nitrogen and biogenic amines were reduced and based on odor characteristics, 70% of sensory respondents rated the 350 MPa samples as still acceptable on day 28. Based on these results, HPP can increase the commercial availability of refrigerated raw lobsters and reach new markets with convenient and high quality products. For safety purposes, time temperature indicators (TTI) should be applied on vacuum packages to ensure product temperatures below 3.3 °C to prevent *C. botulinum* growth and toxin formation particularly when refrigeration is the sole barrier to prevent toxin formation (FDA, 2020).
However, histamine levels in raw tails pressurized at 350 MPa reached the hazard level (50 mg/100g) after 14 days of refrigerated storage. This unexpected histamine development could limit the refrigerated shelf-life of vacuum-packaged raw tails. Therefore, further studies are recommended to evaluate the presence and growth of histamine-forming bacteria in refrigerated, vacuum-packaged raw lobsters (Table 5.1). In addition, since development of histamine in seafoods mainly results from temperature abuse, monitoring time/temperature exposure during lobster processing is highly recommended. Moreover, a pathogenic bacteria validation study including *L. monocytogenes* and *C. botulinum* is warranted to clarify the impacts of 350 MPa for 10 min on product safety during refrigerated storage (Table 5.1).

In conclusion, this research provided valuable information about the potential to combine HPP and sous vide processing in the development of convenient and high quality seafood products. The combination of high pressure processing and sous vide cooking techniques has the potential ensure the production and distribution of minimally processed, preservative-free, value-added lobster products for food service and retail markets.
REFERENCES


Food and Drug Administration. (2020). *FDA fish and fishery products hazard and control guidance document.* FDA.


Are you interested in trying sous-vide cooked lobsters?

If you are at least 18 years old and like eating lobsters, please help University of Maine researchers evaluate sous-vide cooked lobsters. Sous-vide is a technique to cook vacuum packaged food at low temperatures to retain their quality.

Testing will take about 20 minutes, and you will be paid $5 for completing the survey of how much you like 3 samples of lobsters.

Testing will be held on: October 2016

Please call 917-215-4507 or sami.humaid@maine.edu to schedule an appointment for this study, or for more information.

Testing will occur from 11:00 am to 4:00 pm

If you don’t like lobsters, have never eaten seafood before or have allergies to seafood, please do not participate.
APPENDIX B: CONSUMER ACCEPTABILITY OF SOUS-VIDE COOKED LOBSTER QUESTIONNAIRE

Thank you for taking the time to participate in our research. Please evaluate the samples in the order that they are served to you from left to right and take a sip of water before tasting each sample. Please make sure that the sample code on the sample and on the computer screen match. You may use the butter provided, if you wish.

Please indicate your gender.
  o Male
  o Female
  o Rather Not Say

Please indicate your age.

Approximately how often do you consume lobsters?
  o 1-2 times a week
  o 1-2 times a month
  o Every 2-3 months
  o 1-2 times a year
  o Less than once a year

How are your lobsters typically prepared?
  o Boiled
  o Baked
  o Fried
  o Grilled
  o Other

Which sensory characteristic of lobster is most important to you?
  o Flavor
  o Texture
  o Color
  o Aroma
  o Other: _______

How much do you like the aroma of this sample?
  o Dislike Extremely
  o Dislike Very Much
  o Dislike Moderately
  o Dislike Slightly
  o Neither Like nor Dislike
  o Like Slightly
  o Like Moderately
  o Like Very Much
  o Like Extremely
How much do you like the color of this sample?
-o Dislike Extremely
-o Dislike Very Much
-o Dislike Moderately
-o Dislike Slightly
-o Neither Like nor Dislike
-o Like Slightly
-o Like Moderately
-o Like Very Much
-o Like Extremely

How much do you like the texture of this sample?
-o Dislike Extremely
-o Dislike Very Much
-o Dislike Moderately
-o Dislike Slightly
-o Neither Like nor Dislike
-o Like Slightly
-o Like Moderately
-o Like Very Much
-o Like Extremely

How much do you like the flavor of this sample?
-o Dislike Extremely
-o Dislike Very Much
-o Dislike Moderately
-o Dislike Slightly
-o Neither Like nor Dislike
-o Like Slightly
-o Like Moderately
-o Like Very Much
-o Like Extremely

How much do you like the sample overall?
-o Dislike Extremely
-o Dislike Very Much
-o Dislike Moderately
-o Dislike Slightly
-o Neither Like nor Dislike
-o Like Slightly
-o Like Moderately
-o Like Very Much
-o Like Extremely
Is there anything else you would like to tell us about this sample? If you refer to other samples in this test, please use their three-digit code.
APPENDIX C: CONSENT FORM FOR CONSUMER ACCEPTABILITY OF SOUS-VIDE COOKED LOBSTER

Dear Seafood Consumer,

You are invited to take part in a research project titled “High Pressure Processing of Sous-vide Seafood Products” by Sami Humaid, in the School of Food and Agriculture at the University of Maine. The purpose of the research is to learn about consumer acceptability of sous-vide cooked lobsters. Sous-vide refers to the low-temperature, long-time controlled cooking of vacuum-packaged foods in a hot water bath. You must be at least 18 years old to take part in this project.

What Will You Be Asked to Do?
If you choose to take part in this study, you will be asked to answer a few questions about yourself. Then you will be served three samples of lobster tails with warm butter on the side. For each sample, you will be asked to rate how much you like its odor, color, texture and taste.

Risks
If you have never eaten or do not like lobsters, or have an allergy to seafood or dairy, please do not participate. The risks involved in taking part in this study are small, and are not expected to be more than those occurring in normal eating. The test may take about 20 minutes to complete.

Benefits
• You may enjoy eating the lobsters.
• Your opinions will help a University of Maine graduate student in completing his research project.

Compensation
Upon completion of today’s test, you will receive $5. No compensation will be provided if you decide not to complete the test.

Confidentiality
Your name will not be on any files that contain your answers to our questions. Data will be kept in the Consumer Testing Center’s locked office. Your name or other identifying information will not be reported in any publications. All data will be destroyed within two years or after the research is published, whichever comes first.

Voluntary
Taking part in this study is voluntary. If you choose to take part in this study, you may stop at any time, but you will not receive any compensation.

Contact Information
If you have any questions about this study, please contact me at sami.humaid@maine.edu or by phone at (917) 215-4507. If you have any questions about your rights as a research participant, please contact Gayle Jones, Assistant to the University of Maine’s Protection of Human Subjects Review Board, at 581-1498 (or e-mail Gayle.Jones@umit.maine.edu).
## APPENDIX D: SUMMARY COMMENTS REPORT FOR SOUS-VIDE COOKED LOBSTER TAILS

Sample coded 185: Sous-vide cooked 55°C/208min  
Sample coded 560: Sous-vide cooked 60°C/45min  
Sample coded 749: Sous-vide cooked 65°C/10min

<table>
<thead>
<tr>
<th>Sample</th>
<th>Comments</th>
</tr>
</thead>
</table>
| **SV55** | Meat is too soft.  
| | A little bit tough.  
| | Least desirable texture.  
| | Texture was much better than 560.  
| | Very mushy.  
| | Texture was very mealy and unappealing.  
| | Best texture.  
| | Best texture.  
| | Soft and chewy texture.  
| | I did not find the texture as pleasurable as 560.  
| | Texture was a bit hard.  
| | Mushy.  
| | It is not springy - it is more mushy + sticky which is less appealing.  
| | Best texture.  
| | It was sticky.  
| | Texture was too chewy.  
| | Too mushy.  
| | Too mushy.  
| | Chewy.  |

| **SV60** | Most firm.  
| | Much looser than 185.  
| | Best texture.  
| | Soft texture.  
| | Texture was good.  
| | Best texture.  
| | Mushy.  
| | Part is mushy and part is very firm.  
| | Fall a part better than 749.  
| | Not as chewy as 749.  
| | Easier to chew.  
| | Texture too smoother compared to 749.  
| | Undercooked.  
<p>| | Texture better than 749.  |</p>
<table>
<thead>
<tr>
<th>Text</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mushy</td>
<td>Texture was much springier/crunchier than 185.</td>
</tr>
<tr>
<td>Favorite one.</td>
<td>Texture extremely perfect.</td>
</tr>
<tr>
<td>Mild flavor.</td>
<td>Soft texture.</td>
</tr>
<tr>
<td>Good texture.</td>
<td>Good sample.</td>
</tr>
<tr>
<td>Very tender.</td>
<td>Good texture.</td>
</tr>
<tr>
<td>Tough texture.</td>
<td>Similar to 185.</td>
</tr>
<tr>
<td>SV65</td>
<td>Too chewy.</td>
</tr>
<tr>
<td>Meat is the less toughness.</td>
<td>Perfect texture.</td>
</tr>
<tr>
<td>Texture was much better than 560.</td>
<td>Texture was good.</td>
</tr>
<tr>
<td>The most preferable.</td>
<td>A little chewy but this is happens with regular lobster.</td>
</tr>
<tr>
<td>Texture is ok.</td>
<td>Mushy.</td>
</tr>
<tr>
<td>Texture not good.</td>
<td>Too chewy.</td>
</tr>
<tr>
<td>I liked it because it was easier to chew.</td>
<td>More chewy than 185.</td>
</tr>
<tr>
<td>Tougher texture.</td>
<td>Texture create a good impression (fresh).</td>
</tr>
<tr>
<td>Crunchy.</td>
<td>Sweetest flavor.</td>
</tr>
<tr>
<td>Texture is better than 560 not too chewy.</td>
<td>Favorite one.</td>
</tr>
<tr>
<td>Good texture.</td>
<td>Texture was perfect.</td>
</tr>
<tr>
<td>Good texture.</td>
<td>Strange chewiness.</td>
</tr>
<tr>
<td>Great texture.</td>
<td>Unusual.</td>
</tr>
</tbody>
</table>
APPENDIX E: QUESTIONNAIRE OF CONSUMER ACCEPTABILITY OF HIGH PRESSURE PROCESSED, SOUS-VIDE COOKED LOBSTER

Thank you for participating. Please answer some questions about yourself, then evaluate all three samples, in order from left to right. Make sure that the sample code on the sample and on the computer screen match.

Please indicate your gender.
- Male
- Female
- Rather Not Say

Please indicate your age.

Approximately how often do you consume lobsters?
- 1-2 times a week
- 1-2 times a month
- Every 2-3 months
- 1-2 times a year
- Less than once a year

How are your lobsters typically prepared?
- Baked
- Fried
- Grilled
- Boiled/Steamed
- Other

What would make you consume lobsters more often? (Select all that apply)
- Lower price
- More availability
- Longer shelf life
- Sustainably grown
- Minimally processed
- Sold fresh
- Sold in ready to eat dishes
Which sensory characteristic of lobsters is most important to you?

- Flavor
- Texture
- Color
- Aroma
- Other: _______

How much do you like the appearance of this sample?

- Dislike Extremely
- Dislike Very Much
- Dislike Moderately
- Dislike Slightly
- Neither Like nor Dislike
- Like Slightly
- Like Moderately
- Like Very Much
- Like Extremely

How much do you like the aroma of this sample?

- Dislike Extremely
- Dislike Very Much
- Dislike Moderately
- Dislike Slightly
- Neither Like nor Dislike
- Like Slightly
- Like Moderately
- Like Very Much
- Like Extremely

Please take a bite and evaluate the texture questions below.

How much do you like the texture of this sample?

- Dislike Extremely
- Dislike Very Much
- Dislike Moderately
- Dislike Slightly
- Neither Like nor Dislike
- Like Slightly
- Like Moderately
- Like Very Much
- Like Extremely

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How would you rate the texture of this sample? (Part 1)
- Much too tender
- Somewhat too tender
- Just about right
- Somewhat too chewy
- Much too chewy

How would you rate the texture of this sample? (Part 2)
- Much too juicy
- Somewhat too juicy
- Just about right
- Somewhat too dry
- Much too dry

Which one word best describes the texture of this sample? (choose one)
- Tender
- Chewy
- Tough
- Mushy
- Soft
- Firm
- Juicy
- Dry

Please take another bite and evaluate the flavor and overall liking.

How much do you like the flavor of this sample?
- Dislike Extremely
- Dislike Very Much
- Dislike Moderately
- Dislike Slightly
- Neither Like nor Dislike
- Like Slightly
- Like Moderately
- Like Very Much
- Like Extremely
How much do you like the sample overall?
- Dislike Extremely
- Dislike Very Much
- Dislike Moderately
- Dislike Slightly
- Neither Like nor Dislike
- Like Slightly
- Like Moderately
- Like Very Much
- Like Extremely

Did this sample meet your expectations for lobster tail? Please let us know anything else you would like to say about this sample in the comment box below. When referring to other samples please use the 3-digit sample codes.

- Yes
- No

Is there anything else you would like to tell us about this sample? If you refer to other samples in this test, please use their three-digit code.
Sample coded 759: Non-HPP cooked (control).
Sample coded 529: Cooked pretreated at 150MPa for 10 min.
Sample coded 823: Cooked pretreated at 350MPa for 10 min.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>This is the way I am used to lobster tasting. Fresh with just the right amount of chewiness and succulent!</td>
</tr>
<tr>
<td></td>
<td>759 parts of this sample have excellent texture while others are very soft and mushy, this is weird because I’m getting both in each bite.</td>
</tr>
<tr>
<td></td>
<td>If I compare sample to sample I would prefer 529 over 759 but I enjoyed both overall.</td>
</tr>
<tr>
<td></td>
<td>This was actually probably the best lobster tail I’ve tried. Usually I find lobster tail to be a little bit tough but this was very tender and juicy. The color was nice (a nice deep red) and there wasn`t any mushiness about the sample. The flavor was buttery and sweet.</td>
</tr>
<tr>
<td></td>
<td>For sample 759 it was surprisingly soft, but still rich. It`s flavor was better than sample 823, but it is still missing something from the iconic lobster taste. Sample 759 also went down very well and I enjoyed how easy it was to eat.</td>
</tr>
<tr>
<td></td>
<td>I think that this sample (759), although it was less flavorful and had a stranger aroma than the other two, was the perfect texture between not firm enough and not being chewy, and had a very good moisture content.</td>
</tr>
<tr>
<td></td>
<td>It retains or gains tenderness once cooked, something I am unable to achieve when cooked at home or eating out. I am accustomed to tails being too chewy, or rubbery, but these have none of those qualities. Very good samples.</td>
</tr>
<tr>
<td></td>
<td>This one is the second favorite among all the three samples. I enjoyed the sample 759 the most and the 529, I didn’t find too much discrepancy between the samples on taste but the texture was a little bit more firm but not chewy on 759.</td>
</tr>
<tr>
<td></td>
<td>Sample 759, had a fishy flavor and odor. Not so much like lobster. There were tough fibers that normally are not present in lobster meat. When trying to pull apart the meat with a knife and fork there was not a clean tear. The fibers prevented the easy flaky or pull apart texture of lobster.</td>
</tr>
<tr>
<td></td>
<td>This was very tender and good tasting...</td>
</tr>
<tr>
<td></td>
<td>The appearance, texture and flavor were right on with this sample. Extremely real done!!!!</td>
</tr>
<tr>
<td></td>
<td>#759 was not a texture that I enjoyed, nor did I enjoy the flavor. I did eat the whole piece to make sure I was being fair in my review.</td>
</tr>
<tr>
<td></td>
<td>The color is right, but its far too soft and mushy to be acceptable.</td>
</tr>
<tr>
<td></td>
<td>It was very tender, maybe slightly mushy, but good. I enjoyed the flavor.</td>
</tr>
</tbody>
</table>
|          | 759- With no butter, slight but unpleasant aftertaste. Not fishy, but more chemical-like (same with 823 and 529). With butter, not really noticeable. By far the best of 759- With no butter, slight but unpleasant aftertaste. Not fishy, but more chemical-like (same with 823 and 529). With butter, not really noticeable. By far the best of 759- With no butter, slight but unpleasant aftertaste. Not fishy, but more chemical-like (same with 823 and 529). With butter, not really noticeable. By far the best of 759- With no butter, slight but unpleasant aftertaste. Not fishy, but more chemical-like (same with 823 and 529). With butter, not really noticeable. By far the best of
the three samples in all categories.

This lobster was very good but it was just a bit softer than I like my lobster.

759 had a soft texture that was not appealing it also did not have as good of a taste as 823.

The best sample overall.

Rough and almost crunchy.

This sample, 759, was juicier than the other two samples. While they all had some differences, each of them have tasted delicious, and I would be more than satisfied with each of them.

Just about rights were better than previous 823 this one 759 was more tender

As with Sample 529, Sample 759 first appears dry, tough. Taste passable but uninteresting, does not show through the butter.

Better flavor, worse texture.

It fell apart on my fork and basically disintegrated. #759

To me, this seemed the most `normal` sample--more or less what I expect from a lobster tail.

759 had a bit less lobster flavor.

759 was a little on the mushy side for me.

Nice firm texture, good flavor

This sample (759) was my favorite! It had the best texture. The most like what I would expect of a lobster tail!

I liked this sample very much. The texture did seem slightly grainy when it first touched my tongue, but when I bit into it the sample met my expectations of lobster.

Just a little too soft for my liking. Coloration of sample was a little off-putting and the fishy smell seemed much stronger compared to other samples. 823 had the least fishy smell to me.

Seems to mushy, overall good taste, but much too gooey and soft.

Sample 759: the color is too dark compare with usual. This sample is way too soft compare to other two sample, however, the skin is chewy. It kind of feel wired while eating the meat.

759’s initial bite was very tough. Once it was in my mouth it was much easier to eat. 759’s smell was very fishy. 759’s temperature was just right.

I think I associated greater color depth with greater firmness.

This sample didn’t appeal for me at first because it looked dry. But when I took a bite the flavor and texture is actually amazing.

759-Odd sweet undertone. Not bad for a processed product, but definitely didn’t taste fresh.

This was very good. The flavor was nice and sweet.

Best flavor.

Sample 759 was my least favorite of the three. It tasted off. I am trying to think, but it didn’t taste like 823 or 529. It tasted more sandy and fishy. The taste was strong
and even butter did not take the harshness of it. I tried all three samples both a bite without butter and a bite with. Overall, 529 is my favorite followed by 823 and 759.

<table>
<thead>
<tr>
<th>Sample 529 was my favorite followed by 823 and 759.</th>
</tr>
</thead>
<tbody>
<tr>
<td>The first bite, which was the edge of the sample, was very mushy. The texture is okay but the sample tends to fall apart without chewing.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>759 sample was favorable and had the right consistency that I am use to. It also looked like a regular lobster. It wasn’t too chewy or tough, just right.</th>
</tr>
</thead>
<tbody>
<tr>
<td>759 had a very good texture and flavor. Aroma was good, but had a slight after smell, but not bad, just different.</td>
</tr>
<tr>
<td>This is the perfect tenderness. Love it!</td>
</tr>
<tr>
<td>This was delicious, and better than the lobster I usually consume.</td>
</tr>
<tr>
<td>Not as tender as the other two samples (823 and 529)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>759 tasted like a traditional lobster tail, some bits were tough but overall it was tender, which was good.</th>
</tr>
</thead>
<tbody>
<tr>
<td>The texture of this sample was the softest. The flavor was also enjoyable. Thank you!</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>529 This one was my least favorite for taste. It tasted more like dirt than the sea. However, the texture was better than 759. 823 is my favorite.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 529 tasted like I expect a lobster tail to taste and was slightly softer than what I`m used to but this did not detract from the taste.</td>
</tr>
<tr>
<td>Based on the look of this sample, it wasn’t as appealing to me. The color was very bright pink/orange and didn’t have that rich red color that I associate with lobster tail.</td>
</tr>
<tr>
<td>There was a bit of softness on the outside of the tail, but then when I bit through it had a snap to it and was very juicy. I liked this sample less than 823 and 759 just because I found this look and texture a little less appealing. It was very juicy but a little too much so I think. The taste was still good, but had a bit of a more bitter taste to it compared to 759 (which was very sweet).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample 529 was tough and chewy. It felt like it was an inconvenience to eat, and was very difficult to chew due to its chewy nature. The taste of sample 529 was</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>150MPa There was a fishy flavor and was too soft for my liking. Did not have a <code>freshness.</code></th>
</tr>
</thead>
<tbody>
<tr>
<td>529 This one was my least favorite for taste. It tasted more like dirt than the sea. However, the texture was better than 759. 823 is my favorite.</td>
</tr>
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</tr>
</tbody>
</table>

| Sample 529 was tough and chewy. It felt like it was an inconvenience to eat, and was very difficult to chew due to its chewy nature. The taste of sample 529 was |

170
also very bland and uninteresting. One last thing was that the color looked a little off. When I look for a piece of lobster I want to see the red flesh and white meat; in the case of sample 529, the flesh was red but the meat was pink.

This sample was perfectly moist and tasted excellent, and I’m not sure if the softness was only somewhat off-putting to me because I’m not used to lobster this tender but it was overall very good.

Too soft, and a little bland in flavor

I don’t like seafood, but if the flavor is good, I will try it. But the lobster is not the one.

I really enjoyed the soft texture of the lobster meat. I usually find lobster tails to be rather dense and chewy but this was the opposite. It almost melts in your mouth and doesn’t require much chewing to break it down.

Sample 529, reminds me of lobster that was cooked at an outdoor restaurant during the summer in Maine surrounded by many tourists. Typically, the lobster will be cooked under high pressure within a short amount of cook time to fulfill all orders.

Sample 529 - it is a little bit juice for me.

It is a little too soft...I like the flavor but much of it was too soft.

I would eat any of the lobsters and be satisfied with them. They were very good texture and flavor wise.

Very very tasty!!!! Good appearance and very flavorful!

#529 was hard to cut into pieces and I did not care for the flavor at all. This was the first piece that I dipped in butter and still did not like the taste. Yuck

The color of 529 is off, but I think it would generally meet my expectation

529: Looked mushy, a little yellowish/off color. Too mushy in mouth. Undesirable aftertaste. Not sure how to explain, tangy/bitter, but not good.

This lobster was also very good but still a little too soft for me - it seemed a little firmer than sample # 759 though.

529 was more chewy than 823 it had good flavor which was similar to 823

About average. Tastes and feels almost identical to 529.

This sample, 529, was a bit more chewy than 823, but had more flavor, in my opinion.

Sample 823 had a great texture and was juicy, which made the lobster tail have such a savory flavor and even with the butter.

Tender but texture seemed a bit oddly 'consistent'

I think the texture of this sample was a little bit too tough and a little bit dryer than what I would expect from a lobster tail.

Sample first appears dry, stale. Surprised to find it is soft and juicier than anticipated.

Flavor lacks fresh salty character.

Great sample, but practically no aroma. #529

Taste is fine, but it's a little mushy.

The color was a little lighter than sample 759 and it was a little mushier but still good.
<table>
<thead>
<tr>
<th>Text</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Texture too soft, flavor is not as pleasant as the other two samples (823 and 759)</td>
<td>I found the taste a bit brinier than the other samples. I did not like the brown on the outside of the sample. I found this sample more difficult to cut than the other samples.</td>
</tr>
<tr>
<td>529 good texture, just a little chewy but I think 823 had the same level of chewiness. Hard to distinguish if a sample is more chewy than another due to lobster being a hard to digest food in general.</td>
<td>Just a bit too firm. This seemed very close in comparison to sample 823, but is a bit more firm. Personally, I like the firmness, but I think sample 823 beats this one.</td>
</tr>
<tr>
<td>Sample 529: the meat fall apart after my first bite, usually only unfresh seafood do that after it cook. Other than that, this sample tastes pretty good. The color is slightly too dark compare to what I usually eat.</td>
<td>529's appearance much more pleasing than 759's. The color was more vibrant but not as much as 823's. 529 was chewier than 759 and tougher as well.</td>
</tr>
<tr>
<td>529 was great as well, great flavor, great texture, and looked great as well. Not too stringing, soft, gummy, etc.</td>
<td>This seemed softer than what I'm used to but it was fine.</td>
</tr>
<tr>
<td>Hands-down, sample 823 was the best as far as texture and flavor. I liked sample 529 the least; it had an unpleasant bitterness to it and left an aftertaste as well. I found the texture to be its strong point.</td>
<td>Most tender of all, very nice to eat flavor not pronounced but probably best of the three I'm surprised and curious about butter being provided. How can flavor and moistness be properly evaluated if the sample is taken with butter?? (I did not use any. And I don't normally, when having lobster out or at home. I like to taste my FOOD not stuff added to it!)</td>
</tr>
<tr>
<td>529 met my expectations for texture, but fell a bit short in taste (was not very sweet)</td>
<td>Very similar to sample 823, but I liked this sample better. One thing I forgot to mention is 823 was more difficult to cut. It was much firmer. For this sample, I had to trouble cutting it in half.</td>
</tr>
</tbody>
</table>
Has a slightly mealy texture in some spots, flavor was right on.
Everything was great, except for the flavor. I was hoping that it would be a little sweeter, and there seemed to be a slight aftertaste that I was not expecting.
slightly softer than 759 and 823, but still a great texture and flavor. Not a bad level of softness.

Very tender and juicy.

529- the skin part was a little too chewy and the taste was slightly too strong
not as juicy as sample 823.

529 had a tough red top piece that looked appealing but was hard to chew.
I enjoyed this sample a lot more than 823. It was much more tender to eat and I like the appearance a bit more too.

Little tough.

It had good texture and taste it also looks the most appealing compared to the others.

**350MPa**

This sample had beautiful flavor, texture was slightly chewy.

823 I like this sample a lot. I wish the lobster flavor and smell were slightly stronger

This sample was the least flavorful but I still enjoyed eating it.

Compared to sample 759, this sample was lighter in color (it didn’t have that deep red look to it). I liked the flavor of this one a lot but it was less sweet than 759. It was tender overall, but there was a bit of softness on the outside that made it a bit less enjoyable to me.

The biggest enjoyment of lobster for me is the taste, and in sample 823 the taste was pretty lack luster and bland. On the other hand I did like the firm texture of the lobster tail.

I think that this sample, 823, was less appealing in texture and moisture content than 529, however the flavor was more intense and appealing.

good balance of flavor and tenderness, not an overwhelming sea smell. Not as preferable as sample 759, but an overall good sample

Better for me than 529.

Sample 823, had the right flavor and texture. The color was a mild red which was inviting unlike samples 759 and 529, which were dark red more so associated with the color of dry blood.

Sample 823 - it is so tasty and perfect texture for eating. Sample 759 - it is a little bit soft than I wish. Sample 529- it is chewy. Overall, I like the 823 most. Thanks

but a little to chewy. the flavor was not as good as 759 but close.

This sample was overall very good, just a little mushy but not enough to not make me eat it.

#823 had good flavor but was a bit too soft for my liking. I prefer a bit more firm lobster texture. Was still yummy and I wouldn’t throw it away lol

Does not have any smell, but not a huge factor for me

823-Too mushy, (but it was extremely tough to cut in the little cup). In the mouth it was not tough. Still seems to be a little aftertaste.

The lobster was very good in appearance, aroma, moistness & flavor but it was the softest sample of the test and actually was mushy - I like my lobster much firmer.
823 was perfect for taste and texture.
Better than 529.
Seems about average.
Sample 823 was my favorite because it had the right texture and it was savory. Very pleased with the overall sample.
Seemed like a normal boiled lobster tail eating experience
This sample was much too tough.
This sample was very chewy but the flavor was pretty good.
Sample 823 still appears dry, does not look as tough at first glance as Samples 529 and 759 did. Texture is much more familiar and meets expectations of a lobster tail. Flavor still lacking but not off-putting.
A little soft, but really great texture.
This sample was the best of all of them. It was a little chewy which is good and it wasn’t as fishy as the others were slightly bland and soft.
823 - this was the best sample if the three. Texture, taste and color were the best.
Sample 823 is softer than lobster tails that I’ve had before. I liked the flavor though.
Sample 823 was borderline between soft and mushy.
Inconsistent texture - some parts were mushy, other parts were about perfect.
I prefer the flavor of this sample to sample 759. It tasted a bit sweeter. I did not find the dark spots visually appealing. The red outer part of the sample had an odd texture on my tongue, it felt a bit sandpapery.
823 good flavor and texture wasn’t too bad but a little soft for me traditional liking
What I expect from lobster, firm and a bit juicy. Texture a bit dry on outside but flavor and texture are quite outstanding.
823 had a vibrant red color.
I think this is tough compared to what I usually eat.
Sample 823: this sample is too hard to bite into for the first bite, then it’s too chewy. However, the color of sample 823 looks yummy. (I forgot to mention that I didn’t smell anything/ slightly smell fishy for sample 529).
823 has a vibrant color but its surface looks dry. 823 had the least fishy smell of the 3 samples. 823’s initial bite was tough but then chewy after that. 823 was not as juicy as 759.
Again, an odd sweet flavor. Color was a bit too vibrant. Not bad for a frozen or processed product.
Very very good - a little firm, but not unpleasantly so.
This one (823) had more of a fishy taste than sample 529. While the flavor was stronger, I much prefer the first sample. This sample had a stronger fishy flavor and was more chewy. It wasn’t terribly chewy, but it was more than I am used to.
was good
It’s too juicy and soft.
823 had a darker color on the top red part, but still looked good enough to eat. The texture was right on and the flavor was great, a little on the lighter side on the taste,
but still great.

Again a little on the soft side and lacking a lot of aroma and flavor

The flavor is good, just not as strong as I expected. The softness isn’t a deal-breaker, I just like my lobster meat slightly firmer.

Detected no aroma at all slightly chewy but not unpleasantly so sweet compared with 759 (not at all bitter)

Although 823 met my expectations, it was a bit too chewy/tough for my liking (I’d go back to the restaurant but I wouldn’t rave about it :) )

This sample smells fishier than the others. But overall taste was good.

This one threw me off. It looked questionable..no offence. I’m not sure what you did to it, lol. But it was actually the best tasting sample out of all three; I have no critiques on texture, flavor or otherwise aside from how it looks!

The texture of this sample (823) had a lot more depth than the texture of sample 529.

This sample did not have much of an aroma compared to 529 and 759, but the taste was sweeter than the other two.

Slightly more juicy than 759. Just as flavorful and great texture

This sample does not have quite the same aroma as the other two even though they all look the same. The scent does not scream ‘lobster’ the way the other two do. Also the flavor is not quite as rich. Still quite good. It is hard to go wrong with lobster.

I liked it, but I usually expect a better consistency from Lobster when I buy it.

823 -tastes like fresh steamed.

The taste stays with you. The red color stains the fork a little.

Sample 823 had a weird top piece that was hard and fibrous, that was the only thing that made it look and feel weird.

This sample was quite tough and chewy to eat. The appearance was nice but it seemed a little over cooked once eaten.

This one did meet my expectations for a lobster tail but not the best one I have had. It was a little chewy and the taste was not as strong (or good) as 823.
APPENDIX G: SCREENING AND TRAINING PANELISTS FOR ODOR CHARACTERISTICS OF LOBSTER TAILS

Introduction

The objective of this shelf-life study was to determine whether or not HPP treatments of 150MPa/10min and 350MPa/10min would extend the refrigerated shelf-life of raw and subsequently sous-vide cooked lobster tails at 65ºC for 10min. In this objective, the planned shelf-life parameters were microbial, biochemical, and physicochemical analyses. However, sensory evaluation was added to provide insight about the practical significance of the laboratory analyses of the refrigerated lobster tails. Panel member screening and training were required to ensure that any sensory results are meaningful. Sensory evaluation as well as the microbial, biochemical and physicochemical analyses were conducted once per week for five weeks for raw tails and for six weeks for cooked tails. Panelists did NOT consume the samples. Evaluation was based primarily on the odor of the lobster samples. It took approximately 10 mins to complete the evaluation each test day. Screening and training were according to a modified method of Learson & Ronsivalli (1969).

Screening and Training

Ten potential panel members were recruited via word of mouth and/or emails to participate on the sensory panel for the shelf-life study. The potential panelists were screened using an odor recognition test and then trained to detect differences in odors between high quality, medium quality, and poor-quality lobster tails. Participants were also educated about how to rate the odor of the lobster tails during the shelf-life study using the 15cm horizontal line scale. Furthermore, a group discussion was conducted in order to develop descriptors for the 15cm line scale.

Odor recognition test

In this test, potential panelists were screened to ensure they had adequate sensory acuity and that they could detect certain odor compounds. A series of ten odor substances were presented blind, then participants were asked to identify the odor of each sample. Samples were prepared as follows: 2 cotton balls were placed in a 2oz plastic cup, then 3 drops of a specific food flavor extract (McCormick & Co., Inc. Hunt Valley, MD, U.S.A.) were added to the cup. These odors included coconut, orange, almond, vinegar, banana, coffee, garlic, lemon, mint, and vanilla. Cups were capped and held at room temperature for 2 hours before the screening process. Participants were asked to partially open the lid and take 3 short sniffs of the sample, through the nose, then to identify the odor. Samples were presented on a tray, and each tray had 10 cups total (Figure 1). Participants were instructed to wait 30 seconds before continuing to the next sample. They were graded according to number of correct answers. Criteria for panelists
selection were that they should recognize (correctly answer) at least 7 of the odor substances. The odor recognition test took approximately 15 mins to complete.

![Figure E.1 Odor recognition test samples.](image)

**Training for the odor characteristics of lobster tails**

Panelists that passed the odor recognition screening were trained to detect the freshness of lobster tails based on their odor characteristics. Panelists were asked to smell and describe the odor of raw and cooked samples (40 mm portions) having different levels of quality (high, medium, and poor quality) so that they would become familiar with the freshness and spoilage odor characteristics of lobsters.

Throughout the training, two groups of lobster tails, raw and cooked, were presented to the trainees. Each group of tails had three samples representing a certain level of quality; high quality (very fresh, recently butchered), medium quality (butchered, then packed in ice for 10 days), and poor quality (butchered, then thermally abused to accelerate spoilage). Samples were presented at room temperature in capped 2oz plastic cups (Figure 2).

![Figure E2 High quality (HQ), medium quality (MQ), and poor quality (PQ) of raw (R) and cooked (C) lobster samples](image)

Trainees were asked to smell the six lobster samples by partially opening the lid and taking 3 short sniffs of each sample, through the nose, starting with raw samples followed by cooked.
They were instructed to wait 30 seconds before continuing to the next sample within each group and to take a five min pause before switching from raw to cooked samples. Characteristic odors of each sample were described by the trainees in a group discussion. Throughout the group discussion, trainees’ comments were used to develop descriptors to anchor the endpoints of the 15 cm line scales.

Trainees were tested to rank the quality level of raw and cooked samples based on the odor of each sample: 1 = high quality, 2 = middle quality, and 3 = poor quality. Samples were presented blind with 3-digit codes (Figure 3). Trainees were also asked about their lobster consumption frequency. Trainees who were able to correctly identify the high or poor quality lobster tails were selected to participate in the sensory shelf-life study.

![Figure E.3 Coded raw and cooked lobster samples](image)

**Results**

For the odor recognition test, four trainees out of the ten correctly recognized all of the ten different odor samples. Three trainees recognized nine odors, and three trainees were able to recognize eight odors. Thus, all ten potential panelists met the minimum sensory acuity to move forward to the training.

After a group discussion for approximately 60 min, all participants agreed on the following descriptions of odor (Table 1).

Table E.1 Odor descriptor for raw and cooked lobsters at different levels of quality.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Perceived odor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw, High Quality</td>
<td>Fresh, “ocean like”</td>
</tr>
<tr>
<td>Raw, Medium Quality</td>
<td>Intense fishy/lobster smell “acceptable”</td>
</tr>
<tr>
<td>Raw, Poor Quality</td>
<td>Putrid</td>
</tr>
<tr>
<td>Cooked, High Quality</td>
<td>Very fresh, “lobster smell”</td>
</tr>
<tr>
<td>Cooked, Medium Quality</td>
<td>Fresh lobster smell, “sweet”</td>
</tr>
<tr>
<td>Cooked, Poor Quality</td>
<td>Sour</td>
</tr>
</tbody>
</table>

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For the subsequent ranking test, nine trainees correctly ranked all three quality (high, medium, and poor) levels of raw samples, and one trainee correctly ranked only the poor quality sample. For cooked samples, six trainees correctly ranked all of three quality levels, three trainees correctly ranked only the poor quality sample, and one trainee correctly ranked only the high quality sample.

**Conclusion**

All ten trainees successfully passed the odor recognition test and sample ranking test. All trainees who participated in this screening and training session were selected to participate in sensory evaluation for shelf-life study. Trainee odor perception comments were used to develop descriptors for the 15cm horizontal line scale for raw and cooked lobster.
APPENDIX H: FORMS FOR EVALUATING ODOR CHARACTERISTICS OF RAW AND SOUS-VIDE COOKED LOBSTER TAILS

A. Raw Lobster Tails Sensory Evaluation Form

Thank you for participating. Please rate the lobster tail samples using the line scales provided. Partially open the lid and take 3 short sniffs of the sample, through the nose; then close the lid and evaluate aroma. Please wait 30 seconds before continuing to the next sample. Mark an X on the line scale which represents how you perceive the odor.

Panelist name: Date: Sample code:

Please rate the aroma of the sample

Fresh ———— Intense fishy/lobster smell ———— “Putrid”

“Ocean like” ———— “Acceptable”

Would you consume this product?

☐ Yes. ☐ No.

Please try to explain your choices


B. Cooked Lobster Tails Sensory Evaluation Form

Thank you for participating. Please rate the lobster tail samples using the line scales provided. Partially open the lid and **take 3 short sniffs** of the sample, through the nose; then close the lid and evaluate aroma. Please wait **30 seconds** before continuing to the next sample. Mark an X on the line scale which represents how you perceive the odor.

**Panelist name:**  
**Date:**  
**Sample code:**

Please rate the **aroma** of the sample

- Very fresh
- “Lobster smell”
- Fresh lobster smell
- “Sweet”
- Sour

Would you consume this product?

☐ Yes. ☐ No.

Please try to explain your choices

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APPENDIX I: CONSENT FORM FOR SHELF LIFE EVALUATION OF HIGH PRESSURE PROCESSING (HPP), SOUS-VIDE LOBSTER (*Homarus americanus*) TAILS UNDER REFRIGERATED STORAGE

Dear Seafood Consumer,

You are invited to take part in a research project titled “Shelf Life Evaluation of High Pressure Processed (HPP), Sous-vide Lobster (*Homarus americanus*) Tails Under Refrigerated Storage” by Sami Humaid and Denise Skonberg, in the School of Food and Agriculture at the University of Maine. The purpose of the research is to learn about shelf-life of processed refrigerated lobster tails. Sous-vide refers to the low-temperature, long-time, controlled cooking of vacuum-packaged foods in a hot water bath. HPP is a non-thermal pasteurization technique by which products are introduced into a vessel and exposed to high pressure. HPP prior to sous-vide cooking offers the potential to increase the safety and refrigerated shelf life of sous-vide products without the use of additional heat or food additives. You must be at least 18 years old to take part in this project. Although panelists will not be consuming the lobster tails, If you do not like lobsters, have never eaten seafood before or have allergies to seafood, please do not participate.

What Will You Be Asked to Do?

If you choose to take part in this study, you will be participating in all sensory evaluation sessions. The evaluation will be conducted each week for five weeks total. You will be presented six coded samples of raw and cooked lobster tails. Then, you will be asked to evaluate the quality of the samples by evaluating the aroma and meat color. In this sensory test, you will NOT consume the products. Each testing session may take up to 10 minutes to complete.

Risks

There are no risks to you from participating except the loss of your time and inconvenience.

Benefits

There are no direct benefits to you. The overall potential benefit of this research is that sous vide and HPP processed seafoods will be able to provide high quality, minimally processed products to the consumer. Benefits should outweigh any risks.

Compensation

Upon completion of all five test sessions, you will receive a $15 Walmart gift card. No compensation will be provided if you decide not to complete all five tests.

Confidentiality

Paper ballots will be used for data collection. Your name will not be on any files that contain your answers to our questions and answers will be kept confidential. Data will be kept in the
Sensory Testing Center’s locked office. Your name or other identifying information will not be reported in any publications. All data will be destroyed within two years i.e. June 2020 or after the research is published, whichever comes first.

Voluntary

Taking part in this study is voluntary. If you choose to take part in this study, you may stop at any time, but you will not receive any compensation.

Contact Information

If you have any questions about this study, please contact me at sami.humaid@maine.edu or by phone at (917) 215-4507. You may also reach the faculty advisor of this study at denise.skonberg@maine.edu If you have any questions about your rights as a research participant, please contact the Office of Research Compliance, University of Maine, 207/581-1498 or 207/581-2657 (or e-mail umric@maine.edu).
BIOGRAPHY OF THE AUTHOR

Sami Humaid was born in Mukalla-Hadhramout, Yemen on 13\textsuperscript{th} June, 1984. He graduated from high school in Yemen in 2003. He attended Hadhramout University and graduated in 2007 with a Bachelor of Science in Food and Fish Technology. Sami moved to the Kingdom of Saudi Arabia in 2011 to purse his Master of Science in Marine Biology and received the degree in 2014. After completing his Ph.D., Sami will be working in Hadhramout University in Yemen. Sami is a candidate for the Doctor of Philosophy degree in Food and Nutrition Sciences from the University of Maine in August 2020.