Effects of High Pressure and Sous-Vide Processing On Quality of Atlantic Sea Scallops (Placopecten Magellanicus)

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EFFECTS OF HIGH PRESSURE AND SOUS-VIDE PROCESSING ON QUALITY OF
ATLANTIC SEA SCALLOPS (*Placopecten magellanicus*)

By

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A DISSERTATION
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Sous-vide is the cooking of vacuum-sealed foods at precise temperatures in a water bath. Vacuum packaging and cooking at lower temperatures offer multiple benefits to maintain the nutritive and sensory value of foods in comparison to traditional cooking methods. Sea scallops are high-value products that have a very short refrigerated shelf-life (<7 days). High pressure processing (HPP) may facilitate the development of convenient-to-use, high quality, refrigerated scallop products for sous-vide applications to be sold in retail and foodservice facilities. The objectives of this research were to evaluate the effects of HPP and subsequent sous-vide cooking on the 1) physicochemical and sensory attributes, 2) refrigerated shelf-life, and 3) protein structural modifications of sea scallops.

Sous-vide cooking scallops at 55 °C for 208 min, 60 °C for 45 min, or 65 °C for 10 min revealed that consumer acceptability did not differ significantly (p<0.05) in response to the cooking parameters. Therefore, the 65 °C for 10 min sous-vide treatment was chosen for
subsequent studies. In study 2, scallops were processed at moderate pressures (150-350 MPa) and times (5-10 min), and subsequently sous-vide cooked (65 °C/10 min), with longer
pressurization treatments resulting in tougher scallops. Consumer acceptability testing of sous-vide cooked, HPP scallops revealed that despite the textural differences caused by HPP, the “overall liking” scores on a 9-point hedonic scale did not differ among treatments. For study 3, the shelf-life of HPP and sous-vide cooked scallops in ice was evaluated for 42 days to assess changes in microbiological, biochemical and physical qualities. HPP (350MPa) extended the iced shelf-life of raw scallops from 8 days (control) to 28 days, with minimal effects of pressurization time (5-10 min) on physicochemical quality attributes. In the final study, thermo-analytical and biochemical methods were used to evaluate the physical changes in scallop muscle in response to HPP.

The results of these studies present valuable information for diversifying the availability of seafood products using sous-vide processing. HPP could be applied to sea scallops prior to sous-vide cooking to extend their iced shelf-life and maintain their quality during distribution and storage.
DEDICATION

I dedicate this manuscript with all my love to my closest confidante, Surbhi Khanna.
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CHAPTER 1
INTRODUCTION

Growing consumer awareness and attraction toward minimally processed, clean-labeled foods is on the rise (Sloan, 2017). In response to such consumer demands, the food industry is charged to innovate food products using milder preservation techniques that eliminate the use of chemical additives while extending foods’ shelf-life. Shellfish, typically sold at a premium, are valued for their taste and texture as well as high protein content. Conventional cooking and preservation methods such as cooking at high temperatures, canning or freezing may negatively impact shellfish quality due to increased drip loss, freezing injury or development of rancid off-flavors. Sous-vide (SV) is the temperature-controlled cooking of vacuum-packaged foods in a water bath or steam which preserves much of the flavor and nutrient profile of foods while providing consistent reproducibility. To make an economically sustainable supply chain of refrigerated SV seafood products for food service and retail, it is important to have shelf-life that can accommodate distribution without compromising quality. High pressure processing (HPP) is a non-thermal processing technique that has shown promising results in shelf-life extension of selected seafood products, while maintaining favorable nutritional and sensorial qualities. The combination of HPP and SV techniques has the potential to develop high quality, convenient-to-use, refrigeration stable seafood products for multiple channels including food service and retail. Sea scallops are high-value shellfish that are already commonly prepared using SV in restaurants, making them an excellent choice for testing the combined effects of HPP and SV.
1.1 Sea Scallop Industry

Scallops are economically important, marine bivalve mollusks that belong to the *Pectinidae* family. There are several scallop species that are harvested globally but the Atlantic sea scallop (*Placopecten magellanicus*) is one of the most commercially relevant species in the United States and Canada (Fig 1.1). Sea scallops may be wild harvested or farm-raised, with the wild catches ranging from Maine to North Carolina in the U.S. (NOAA, 2019). The Atlantic sea scallop is the world’s largest and most valuable wild scallop fishery and is regulated through the National Shellfish Sanitation Program in the U.S (NOAA, 2019). This fishery is considered one of the best managed fisheries in the world, and the New England Fishery Management Council (NEFMC) and the National Marine Fisheries Service (NMFS) jointly manage the scallop fishery in federal waters (NOAA, 2019). Although Massachusetts and New Jersey remain the top states in wild scallop harvests, the scallop fishery contributes over $9 million in annual landing value to Maine with around 800,000 pounds harvested in Maine waters (NOAA, 2019).
Once harvested, sea scallops are typically cleaned in sea water and hand- or mechanically-shucked to obtain their adductor muscle while the viscera and roe are removed (DuPaul et al., 1990; Downey et al., 2012). Post cleaning, the adductor meat may receive additional treatment with ice and sea water slush, 2.5 % sodium tripolyphosphate or 1 % sodium chloride, which increases their moisture content, after which they are referred to as “wet scallops.” On the other hand, untreated scallops are marketed as “dry scallops,” which are more expensive than the “wet scallops.” The U.S Food and Drug Administration (FDA) has established a standard of identity for scallops based on the moisture content of the adductor meat, with the label “scallops” reserved for adductor meat having an average moisture content below 80 % (Fisher, 2000). The average moisture content of untreated scallops is ~79 % (Naidu & Botta, 1978). Scallops
containing 80-83.9 % moisture content must be labeled as “X % water added scallop product,” but the value of X cannot exceed 25 % whereas any scallop with more than 84 % of moisture content is considered adulterated (Fisher, 2000).

Shucked adductor muscle has two parts; 1) larger, cross-striated muscle; and 2) smaller catch or smooth muscle attached to the larger portion of the meat (Fig 1.2). The smaller portion may remain attached to the cross-striated muscle, but more often is lost during handling and processing (Fisher, 2000; Chantler, 2016). The adductor meat is sold fresh or frozen in the market. Scallop meat is classified into different size grades by number of meats per pound; less than 10 scallops per pound are U10 (>45 g/piece), followed by 10/20 with 10-20 meats per pound (25-45 g/piece), 20/30 (15-23 g/piece), 30/40 (11-15 g/piece) and 40+ (Hart & Rago 2006; NOAA 2019). During distribution and sale, fresh scallops are stored at chilled temperatures to maintain their initial quality. Chilled shellfish shelf-life ranges from 6-10 days (Venugopal & Gopikumar, 2017), with a shelf-life of 6 days reported by Bremmer and Statham (1983) for untreated sea scallops held at 4 °C in ice.

![Fig 1.2. Photograph of Atlantic sea scallop adductor muscle in shell with all the other organs removed. Striated muscles are represented as S whereas catch muscle is represented as C. Modified from Scallop Adductor Muscle: Structure and Function (Chantler, 2016).](image)
Consumers pay a premium to enjoy juicy and succulent scallop adductor meat. Sea scallops are typically off-white in color but may also have an orange or yellowish tinge, depending on their diet. Although there is no difference in their safety or nutritional quality, consumers often reject colored scallops due to lack of familiarity (Bourne & Bligh, 1965; Fisher, 2000; NOAA, 2019). In general, scallops are a rich source of protein (~17 g/100 g), and are low in lipids (<1 g/100 g) and carbohydrates (< 3 g/100 g) (Naidu & Botta, 1978; USDA, 2017). Moreover, they are also a good source of minerals such as zinc and magnesium, as well as vitamin A (King et al., 1990; Venugopal & Gopikumar, 2017). They have a mildly sweet and briny taste, and are cooked using various methods including grilling, steaming, searing and sautéing with different sauces. Today, freshly prepared or frozen scallop dishes such as bacon wrapped, breaded and smoked scallops can be found at the seafood counter of supermarkets and grocery stores. Typically, fresh, wild caught, dry sea scallops may range from $14.99 to $19.99 per pound, depending on their size (Hannaford, 2019). Several seafood companies retail frozen sea scallops for ~$24.99/lb with attractive packaging and claims including “no preservative,” “chemical free,” “sustainably sourced,” and “wild caught” (BJ’s, 2019). More recently, Lund’s Fisheries launched ready-to-cook frozen scallops in two exciting flavors; butter and roasted garlic, and white wine and herb, retailing at $29/lb (QVC, 2019). These scallop products are being marketed as an easy gourmet restaurant experience at home.

1.2 Muscle Proteins

Protein makes up the majority of the muscle tissue (~20 %, wet weight basis (wwb)), after water (~75%, wwb), giving the muscle food its structure (Tornberg, 2005). Proteins are amino acid chains, where individual amino acids are attached to each other by peptide bonds. Muscle proteins are divided into three classes based on their solubility: 1) sarcoplasmic, 2)
myofibrillar, and 3) stromal (Ochiai & Chow, 2000). Myofibrillar (salt soluble) proteins make up
the major portion (50-55%) of the muscle protein, followed by sarcoplastic (water soluble)
proteins (30-34%) and then stroma (insoluble in water or salt solution) proteins or connective
tissue proteins (10-15%) (Tornberg, 2005). The two major components of myofibrillar proteins
are myosin (~550 kDa) and actin (~42 kDa); other myofilbrillar proteins include regulatory
proteins such as tropomyosin, paramyosin, actinin, and scaffold proteins such as titin, desmin
and nebulin (Tornberg, 2005). Sarcoplastic proteins are relatively low molecular weight
globular proteins including proteolytic enzymes and myoglobin and are found in the cytoplasm
of a muscle cell, whereas collagen is the major component of connective tissue that strengthens
the muscle structure (Tornberg, 2005).

Scallop adductor meat is composed of cross-striated sarcomeres containing thick and thin
filaments (Findlay & Stanley, 1984; Chantler, 2016). Actin and tropomyosin are present in the
thin filaments whereas myosin and paramyosin are parts of the thick filament, and the
actomyosin along with other regulatory proteins, ATP and calcium ions are responsible for
muscle movements in scallops, such as rapidly opening and closing the shell for propulsion
through water (Findlay & Stanley, 1984; Chantler 2016). In contrast to vertebrate muscles,
paramyosin can be found in invertebrates, which may play a role in the thermal properties of
their myofibrillar proteins (Ehara et al., 2004).

1.2.1 Effects of Thermal Processing on Muscle Proteins

The stability or modifications of muscle proteins in response to thermal processing has
important implications for their properties and those of the resulting food product. Heating
induces significant structural changes to muscle proteins, affecting their native conformations.
To better understand the effects of thermal processing on proteins, it is important to discuss their
structure. Proteins, in general, can be divided into four levels of structures: 1) primary, 2) secondary, 3) tertiary, and 4) quaternary. The primary structure of protein is a sequence of amino acids held together with peptide bonds. The secondary structure forms α-helices and β-sheets within the polypeptide chain, stabilized by hydrogen bonds or intra-molecular bonding. The tertiary structure is a three-dimensional conformation of the protein molecule, and is formed by non-covalent and covalent (disulfide) bonds. Finally, the quaternary structure is a combination of two or more protein subunits that serves a biochemical, physiological or structural role, such as myosin or hemoglobin (Damodaran, 2017).

Denaturation of muscle proteins has been widely studied through differential scanning calorimetry (DSC), depicting key proteins denaturing at different temperatures. Myosin is very thermally-labile and typically denatures between 54-58 °C, followed by connective tissue denaturing between 53-63 °C and sarcoplasmic proteins between 65-67 °C (Tornberg, 2005). As the temperature rises to 80 °C, collagen starts to solubilize and finally, actin denatures between 80-83 °C (Tornberg, 2005). Compared to land animals, fish and shellfish contain a higher proportion of myofibrillar proteins and lower collagen content, contributing largely to their softer texture. Fish muscle collagen solubilizes with mild heating (35-50 °C) (Espinosa et al., 2015), and overheating may cause the meat to toughen rendering it inedible. For example, yesso scallops (*Patinopecten yessoensis*) cooked at 45 °C exhibited less moisture loss, hardness and protein degradation compared to scallops cooked at 65 °C (Dong et al., 2018). According to Findlay & Stanley (1984), muscle fibers of sea scallops are damaged above 65 °C resulting in tough meat mostly due to protein denaturation. Moreover, high temperature (>65 °C) cooking also leads to increased protein oxidation, denaturation and precipitation (Zhang et al., 2013; Dong et al., 2017, Dong et al., 2018).
Myofibrillar proteins hold up to 80% of muscle water content between the thick and thin filaments, and their denaturation may directly affect the water holding capacity of the meat as well as the overall moisture content (Tornberg, 2005). Meat tenderness and juiciness are two of the most important sensory attributes of eating quality, with tough beef deemed unacceptable by consumers (Wood et al., 1999). As heating temperatures during thermal processing increase, a decrease in water holding capacity and an increase in cook loss may be observed, resulting in tougher and less juicy seafood meat (Hughes et al., 2014; Dong et al., 2018). Conventional pasteurization methods used in the industry require fish and shellfish to meet the FDA’s criteria for safe processing of seafood (FDA, 2011). However, these temperatures are typically high (>80 °C) and may be harmful to the intrinsic characteristics of tender shellfish muscle. For example, cooking black tiger shrimp at 100 °C for merely 2 minutes produced tough meat with over 25% water loss (Jantakoson et al., 2012). Stryker et al. (2018) reported 50% shrinkage in size and profuse cook loss in shrimp cooked using commercial pasteurization methods, contributing to tough and rubbery shrimp.

Consumers consider meat tenderness and juiciness important sensory quality attributes (Pearce et al., 2011). Sea scallops have inherently tender and succulent texture, which is highly susceptible to toughening depending on the heating parameters employed while cooking (Findlay & Stanley, 1984). Increased toughness in cooked (25-80 °C) sea scallops was reported as temperature increased, likely due to myofibrillar protein denaturation (Findlay & Stanley, 1984). Moreover, yesso scallops boiled (98 °C) and steamed (95 °C) for 15 min had 50% and 64% loss in extractive nitrogen, respectively (Abe & Miyashita, 2007). Extractive nitrogen is the total amount of amino acids, water-soluble peptides, and nucleotides, that contribute to the taste of the meat and overall quality (Abe & Miyashita, 2007). The same authors also reported uneven heat
transfer, and rapid surface water loss and protein denaturation when the scallops were cooked using superheated steam (150 °C and 200 °C), but only a 14 % loss in extractive nitrogen.

These studies demonstrate the need to develop and optimize cooking techniques for sea scallops that preserve their favorable sensory attributes and quality. Effects of SV cooking on quality of scallops have been scarcely reported, with no work on sea scallops. Sous-vide has the potential to further diversify the sea scallop market, while delivering perfection in flavor and texture in the final preparations. However, comprehensive research is required to assess the quality changes due to SV in sea scallops.

1.3 Sous-vide

Sous-vide is a term for foods cooked under vacuum in a water-bath or steam, and it literally translates as “under-vacuum” from French. This cooking technique was first developed by a French chef, George Palus, then garnered the attention of several gourmet chefs. At present, sous-vide cooking has traveled across countries and has become a part of the mainstream fine-dining restaurant experience. More recently, the availability of reasonably priced and convenient-to-use table top sous-vide machines has made it possible for home cooks to explore this cooking method as well (Ramsden, 2013). In a nutshell, SV cooking comprises vacuum-packaging raw or partially cooked food in heat-stable plastic pouches, immersing the pouches in a water-bath for a precise time-temperature combination, and typically consuming immediately. Less frequently cooked product is rapidly chilled, and then stored under refrigeration to inhibit the growth of pathogens (FDA, 2013).

The acceptance and exploration of SV cooking is widely due to the benefits of this technique over conventional, thermal cooking methods such as searing, boiling and grilling. For example, vacuum-packaging of the raw foods allows uniform heat distribution resulting in
evenly cooked product as well as increased moisture and nutrient retention as the volatile compounds cannot escape during cooking (Schellekens, 1996; Church and Parsons, 2000). Sous-vide also minimizes off-flavors due to lipid oxidation, positively contributing to the flavor profile of the food (Church and Parsons, 2000; Baldwin, 2012). Once vacuum-packed, foods are typically cooked at precise, low temperatures allowing better control and reproducibility of doneness (Baldwin, 2012). The sous-vide method typically uses temperature below 100 °C, with muscle foods cooked below 70 °C and vegetables cooked around 95 °C (Schellekens, 1996; Sampels, 2015). The time-temperature combinations used during sous-vide target the elimination of vegetative bacteria, and the rapid chilling process post-cooking ensures enhanced safety of the food during refrigerated or frozen storage (Baldwin, 2012). In addition, vacuum-packaging minimizes the risk of cross-contamination during storage or distribution (Baldwin, 2012).

As the popularity of SV grows amongst consumers, several food companies are experimenting with innovative ways to sell SV cooked products at the food service and retail level. Moreover, Roascio–Albistur & Gámbaro (2018) reported that consumers perceived a ready-to-heat SV dish as “premium” quality that offers convenience, and would like the packaging to highlight some of the benefits of SV including “absence of preservatives.” Wayne Farms sells ready-to-heat SV cooked grilled chicken across 19 states in the U.S. (Nelson, 2019). Cuisine Solutions has been offering a variety of sous-vide products ranging from meat and seafood dishes to pasta and vegetables in the U.S. since 1971 (Cuisine Solutions, 2019). More recently, Starbucks diversified their menu by incorporating “velvety textured”, sous-vide cooked egg whites as a high-protein, healthy breakfast option (Starbucks, 2019). Several companies offer sous-vide product development consultation for food service and retail, citing the technology as a balance between quality and convenience.
1.3.1 Sous-vide of Muscle Foods

Texture, one of the most important quality attributes of muscle foods, has been of keen interest to researchers with respect to sous-vide. In general, the lower cooking temperature used during SV compared to traditional cooking methods yields juicier and more tender meat (Baldwin 2012; Rinaldi et al., 2014). Beef SV cooked at 75 °C for 36 h exhibited lower hardness values and higher vitamin B12 retention compared to beef SV cooked at 100 °C for 2 h, and to traditionally boiled beef (100 °C for 2 h, no vacuum packaging) (Rinaldi et al., 2014). In another study, SV cooked beef evidenced increased cook loss and decreased shear force values as the temperature was increased from 50 to 60 °C, but no significant effects of cook time (90 to 360 min) were observed (Vaudagna et al., 2002). Moreover, the same authors reported a shelf-life of 21 days for the beef samples stored at 1 °C for all the SV treatments, citing decreased sensory quality beyond 21 days.

The low temperature/long time combination used during SV cooking has the potential to uniformly cook and protect the organoleptic profiles of seafood products (Schellekens, 1996; González-Fandos et al., 2005; Sampels, 2015). Loss of color and protein precipitation in SV processed (90 °C for 10 min) salmon slices were major contributors to lower scores for sensory appearance by trained panelists, in contrast to samples SV cooked at 65 °C for 10 min (González-Fandos et al., 2005). The lower temperature treatment received higher scores for taste as well, but had a 21-day shelf-life at 2 °C versus 45-days for the higher temperature treatment (González-Fandos et al., 2005). Similarly, SV processing (85 °C for 10 min) of mussels better preserved their quality and resulted in a shelf-life of 21 days, compared to traditionally steamed mussels with a 14-day refrigerated shelf-life (Bongiorno et al., 2019). These studies clearly show
the advantages of low temperature SV cooking in retaining seafood quality and extending its iced shelf-life.

Vacuum-packaged, SV cooked seafood would be well-suited for ready-to-eat/heat dishes, providing ease to consumers interested in consuming seafood in different forms (Bongiorno et al., 2019). However, the reduced oxygen packaging and use of low temperatures during SV pose some safety challenges for these products. Pathogens of concern for SV cooked products stored at chilled temperatures include non-proteolytic, type B and F, and proteolytic type E *Clostridium botulinum* and *Listeria monocytogenes* (Peck, 2005; Bolton 2015). The FDA requires stringent time and temperature combinations as critical controlling factors to eliminate *C. botulinum* and *L. monocytogenes* growth during refrigerated storage (FDA 2013).

Neurotoxins produced by *Clostridium botulinum* are the most toxic substances known to humans (Peck et al., 2010). There are several strains of *C. botulinum* (*C. bot*) but type E and non-proteolytic types B and F are associated with seafood and seafood products (Bolton, 2015). *C. botulinum* is a spore forming anaerobic pathogen, and reduced oxygen packaging may foster its growth even at refrigerated temperatures as low as 3.3 °C, with limited competition from aerobic microorganisms (Peck & Stringer, 2005; Bolton, 2015). Although *C. bot* spores do not produce neurotoxins, these spores have the ability to germinate into vegetative cells under favorable conditions and produce harmful toxins in the process. Temperature abuse during cooking or refrigerated storage of fish may facilitate production of *C. bot* type E neurotoxins (Peck et al., 2010), hence this pathogen remains a safety concern in refrigerated SV foods (Peck & Stringer, 2005; Bolton, 2015). In particular, reduced oxygen packed raw products that do not have any added hurdles to control *C. bot* toxin formation have to maintain supply chain at refrigerated temperatures below 3.3 °C to ensure safety (FDA, 2011).
Listeria monocytogenes is a non-spore forming, facultative anaerobic pathogen that can potentially survive and grow at refrigerated temperatures, making it a major concern for foods prepared using SV (Doyle et al., 2001). L. monocytogenes can be harbored in processing environments, and improper handling during processing can contaminate foods, rendering them unfit for human consumption. Ready-to-eat refrigerated seafood products at the retail level and several seafood processing facilities have tested positive for L. monocytogenes, which has a zero tolerance limit in the U.S. (Zarei et al., 2012; Leong et al., 2015).

A systematic design for processing and refrigerated storage of SV cooked seafood is essential to mitigate risk of pathogenic contamination. Clean and sanitized processing facilities following Hazard Analysis Critical Control Points (HACCP) and stringent temperature control during processing and storage (<3 °C) can minimize the risk of L. monocytogenes and C. bot.

The FDA requires the thermal history for any vacuum-packaged, reduced oxygen packaging seafood products, and requires the use of time-temperature indicators (TTIs) throughout processing and distribution.

1.4 High Pressure Processing

High pressure processing (HPP) is a novel food processing technique by which packaged foods are subjected to isostatic pressure (100 – 800 MPa or 14,504 – 116,060 psi) imparted using a liquid (typically water or glycerol) for a specific duration. In contrast to thermal processes, pressure applied during HPP disperses through the food uniformly and quasi-instantaneously, regardless of its geometry and size, without causing physical damage to the food in most cases. However, HPP needs flexible packaging to withstand the high pressures during processing. Depending on the HPP parameters used, bacterial cell wall destruction, protein structure modification, enzyme inactivation and pigment alteration may occur to varying degrees due to
changes at the cellular level. Since pressure is used as the primary energy source to process foods during HPP, it potentially eliminates the need for additional heat or chemical additives. However, heat or other processing techniques also may be applied to achieve certain quality attributes or enhance shelf-life and safety of the food.

Key benefits of HP processing of foods include refrigerated shelf-life extension, reduction of microbial load, enzyme inactivation, and retention of favorable sensorial characteristics and nutritional value (Murchie et al., 2005; Teixeira et al., 2013; de Oliveira et al., 2017). HPP is based on Le Chatelier’s principle, whereby a change in the existing equilibrium within a system must be compensated by a change to achieve a new equilibrium. During HPP, the increase in the pressure within the system is counterbalanced by a decrease in volume to achieve equilibrium. Hence, HPP favors volume reduction reactions within the food system, with the subsequent depressurization bringing the food back to its original volume. This pressure change is the primary cause of bacterial cell wall destruction, resulting in shelf-life extension and pasteurization effects observed in a variety of foods (Hughes et al., 2016; de Oliveira et al., 2017; Suemitsu & Cristianni, 2019). Moreover, hydrogen bonds are stabilized due to increased pressure whereas electrostatic and hydrophobic interactions are destabilized, targeting cell membrane fluidity and thus contributing to reduced cellular functions in bacterial populations. Unlike thermal processes, HPP does not affect the covalent bonds, thus the primary structures of proteins (amino acids linked by dipeptide bonds) remain intact. Protein denaturation due to HPP has been aimed to inactivate enzymes, tenderize meat, and shuck crustaceans and mollusks (Cruz-Romero et al., 2007; Teixeira et al., 2013; Yi et al., 2013). In a typical high pressure system, a rise in temperature of the food occurs due to adiabatic heating which means as the
pressure changes, the volume of the food changes to achieve equilibrium but is not enough to compensate for the pressure effect, and hence the temperature rises (de Oliveira et al., 2017).

The food industry is poised to respond to the ever-increasing consumer demand for high-quality, minimally-processed, preservative-free food products. HPP utilizes pressures ranging from 100 MPa to 800 MPa to process solid and liquid food products. HPP, also known as cold-pasteurization, has already been widely accepted in the beverage industry for refrigerated shelf-life extension, owing to its ability to retain better color, flavor and nutritive profile of juices compared to thermal processing (Oey et al., 2008). Other HP-treated commercial food products include salad dressings, dips, cheese, and ready-to-eat meat and seafood products.

Proven success in shelf-life extension of HP-treated food products has led to increasing commercialization of this technology. The ability of high pressures to successfully reduce pathogenic microbial activity to safe levels while maintaining fresh-like appearance and nutritive profile, and sensory appeal has resulted in keen focus on HPP from the food industry. Microbial sensitivity to pressure conditions is dependent on the type, form, species, strain, shape and growth phase of the cells (Hayman et al., 2007; Kaur et al., 2016). For example, effective destruction of Gram-negative bacteria, such as *Escherichia* spp., *Vibrio vulnificus*, *Salmonella* spp., has been observed at a pressure level of 300 MPa whereas a pressure of 600 MPa was needed to destroy Gram-positive bacteria including *Listeria monocytogenes*, *Clostridium* spp., *bacillus* spp., and *Staphylococcus* spp. (Kural & Chen, 2008; Porto-Fett et al., 2010). Additionally, bacterial cell growth and phase also affect their resistance toward HPP, with cells in growth phase less resistant to pressure, in comparison to stationary phase (McClements et al., 2001). Moreover, differences in microbial inactivation are also a function of the food product under investigation. For example, Yagiz et al. (2007) reported a 6-log reduction of initial
microbial counts in rainbow trout (fresh water fish) versus a 4-log reduction in mahi mahi (salt water fish) processed at 300 MPa for 15 min.

1.4.1 HPP of Seafood

Keen interest in application of high pressures on seafood has existed for some time, however wide commercialization demands more committed research and investments in this food category. Generally speaking, seafood is highly perishable due to its high moisture content and higher levels of polyunsaturated fatty acids (PUFAs) compared to terrestrial animals. High consumer awareness of seafood nutritional value and interest in minimally-processed foods has spurred the need to develop more high-quality seafood products for the market. Consumers enjoy specific textural and sensorial attributes associated with seafood, and HPP may offer minimal impact on the desired texture and sensorial properties of seafood while extending its short shelf-life.

The delicate texture of seafood muscle and its susceptibility to PUFA oxidation support the use of moderate pressure processing parameters to maintain favorable quality attributes (Angsupanich & Ledward, 1998; Kaur et al., 2016). Changes in seafood muscle appearance due to the application of ultra-high pressures have been a well-documented limitation of HPP. Kaur et al. (2016) reported harder texture and cooked appearance of black tiger shrimp processed at 500 and 600 MPa, and recommended 300 MPa for 3-9 min or 400 MPa for 3 min to achieve physicochemical (texture, color and lipid oxidation) and microbial qualities closer to unprocessed samples. Tilapia fillets processed at 400 MPa for 3 min were preserved for 7 days under refrigeration, however the sensory panel visually preferred samples processed at 200 MPa for 3 min due to the change in appearance at higher pressure (Suemitsu & Cristianini, 2019). Similarly, Yagiz et al. (2007) revealed that lower pressure treatment (150 MPa) did not affect
texture but recommended pressurization level of 350 MPa and 450 MPa for rainbow trout and mahi mahi, respectively, based on the change in appearance, lipid oxidation and reduction in microbial load over time. Previous research emphasizes the crucial need to optimize HPP parameters to balance shelf-life extension and maintenance of seafood quality and consumer acceptance.

Efficacy of HPP to shuck shellfish compared to traditional physical methods, while enhancing their safety and shelf-life, has also promoted the expansion of this processing technique in the seafood industry. Oysters processed at 207 to 310 MPa for 0, 1 and 2 min demonstrated a significantly higher percent of adductor muscle release, and reduced aerobic plate counts (<6-log CFU/g) until day 20 of storage (<4 °C), compared to the unprocessed control which reached 6-log CFU/g by day 9 (He et al., 2002). Moreover, Yi et al. (2013) demonstrated no significant changes in physical attributes during 150 days of frozen storage of previously HPP-shucked scallop meat, but reported reduced microbial counts and increased meat yield in HPP-treated scallops compared to manually shucked scallops. Similarly, abalones processed at 300 MPa for 10 min experienced refrigerated shelf-life extension up to 25 days (Hughes et al., 2016), whereas pressures of 500-550 MPa resulted in 35-days shelf-life (Briones et al., 2010; Briones-Labarca et al., 2012) compared to the unprocessed controls. These studies demonstrate the efficacy of HPP to date in achieving prolonged shelf-life of highly perishable and expensive seafoods. However, the effect of HPP conditions on refrigerated shelf-life of scallops has not been reported yet.
1.4.2 Effects of HPP on Seafood Proteins

In general, high pressure induces changes in protein secondary, tertiary, and quaternary structures, affecting different bonds of the native protein structure. Protein denaturation, i.e. conformational changes in the native protein structure, occurs to varying degrees during pressurization, depending on the pressurization conditions applied and the specific food product (Campus 2010, Bolumar et al., 2016). Some non-covalent bonds (electrostatic and hydrophobic interactions) are disrupted by pressure whereas hydrogen bonds are stabilized in response to HPP. Moreover, covalent bonds are unaffected by pressure due to their high dissociation energy requirement compared to other bonds. Quaternary structure of proteins is affected at pressures above 100 MPa, followed by tertiary structure at 200 MPa, while pressures of 300-700 MPa are required to affect the secondary structure of a protein (Lullein-Pellerin & Balny, 2002) which is more stable. The primary structure of the protein, bound by peptide (covalent) bonds, remains unaffected by pressure (de Oliveira et al., 2017).

HPP modifies the native protein structure of seafood muscle, which directly impacts its texture to varying degrees, depending on the parameters applied while processing. Myosin is considered the most pressure-sensitive protein, with significant reduction in Digital Scanning Calorimetry (DSC) peaks observed at pressures >100 MPa in cod (100 MPa, 20 min, ambient temp) (Angsupanich & Ledward, 1998), turbot (140-200 MPa, 15–30 min, 4 °C) (Chevalier et al., 2001), black tiger shrimp (200–800 MPa, 20 min, 28 °C) (Jantakoson et al., 2012), silver carp (300 – 500 MPa, 10 min, 20 °C) (Qiu et al., 2014) and red swamp crayfish (200 – 500 MPa, 5 min, 25 °C) (Shao et al., 2018). As pressure levels increase above 300 MPa, actin and sarcoplasmic protein DSC peaks begin to disappear, with the possible formation of new aggregates observed in some seafood species (Angsupanich & Ledward, 1998; Jantakoson et al.,
Several studies have further confirmed the structural protein changes observed through DSC using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). These studies clearly indicate that the effects of HPP are related to the specific parameters and species used (Schubring, 2005).

Sarcoplasmic proteins consist of an array of enzymes, including proteolytic cathepsins and calpains that are responsible for post mortem degradation of proteins. Increased enzyme activity may lead to desirable or undesirable changes in muscle texture including tenderization, tissue softening, or gaping, affecting seafood quality and shelf-life (Godiksen et al., 2009; Teixeira et al., 2013). Moderate pressure conditions (~100-200 MPa) increased proteolytic activity in cod, sea bass and cold-smoked salmon, whereas pressures >200 MPa, especially >400 MPa, reportedly decreased enzymatic activity (Angsupanich & Ledward, 1998; Lakshamanan et al., 2005; Teixeira et al., 2013). Proteolytic activity of cod muscle increased at 200 MPa but decreased at 400-800 MPa (Angsupanich & Ledward, 1998). Lakshamanan et al. (2005) demonstrated that a pressure level of 300 MPa for 20 min successfully reduced activities of cathepsin B+L-like and calpains compared to unprocessed controls and 100-200 MPa for 20 min in cold smoked salmon, with no reactivation of these enzymes for up to 12 days of refrigerated storage. In sea bass, varying pressurization level (0.1-400 MPa) and holding time (0-30 min) combinations significantly modified activities of several enzymes, with highest reductions in activities of acid phosphatase, cathepsin D and calpains observed at 400 MPa (Teixeira et al., 2013). Pressurization ruptures lysosomes resulting in release of enzymes at lower pressures; at ~200 MPa enzyme inactivation begins, with pressures ~400 MPa dominating enzyme inactivation (Homma et al., 1994; Lakshamanan et al., 2005; Teixeira et al., 2013).
Changes in myofibrillar and sarcoplasmic proteins have important implications for seafood quality and shelf-life (Yagiz et al., 2007; Teixeira et al., 2013; de Oliveria et al., 2017). Muscle texture is directly affected by modifications in myofibrillar proteins as it alters their capacity to hold water as well as reducing the space between muscle fibers resulting in tougher, chewier and less juicy meat (Angsupanich & Ledward 1998; Campus, 2010; Jantakoson et al., 2012). Enzymatic activity may degrade seafood proteins post-mortem, during processing and storage, affecting textural quality and limiting the shelf-life (Lakshamanan et al., 2005; Teixeira et al., 2013), making it crucial to evaluate the effects of HPP on myofibrillar and sarcoplasmic proteins of scallops.

1.5 Research Needs

Consumers seek and pay a premium for delicious, tender and succulent sea scallops. The controlled low temperature cooking of such delicate meat under vacuum has the potential to evenly cook the meat while mitigating the negative impacts of high temperature cooking. Sea scallops already pre-portioned and vacuum-packaged, ready to be cooked via SV may offer commercial kitchens and consumers convenience, quality and consistent results. However, the short refrigerated shelf-life of sea scallops (<7 days) poses a challenge to build a successful supply chain of value-added scallop products. Application of HPP pre-treatment may extend the refrigerated shelf-life of vacuum-packaged sea scallops while maintaining their physicochemical and sensory qualities. There are no scientific reports on the effects of HPP on quality and shelf-life of sea scallops. Moreover, there have been no previous studies assessing the effects of HPP on the quality attributes of subsequently SV cooked seafood products.
1.6 Objectives

This research was carried out to promote the development of SV seafood products using HPP as a pre-treatment. The overall objective of this project was to evaluate the effects of moderate HPP parameters on raw and subsequently SV cooked scallops in order to develop high-quality, convenient-to-use, refrigerated scallop products. The specific objectives were to: 1) assess consumer acceptability of sea scallops SV cooked using different parameters (55 ºC/208 min, 60 ºC/45 min, 65 ºC/10 min), 2) evaluate the effects of HPP (150/350 MPa for 5/10 min) on physicochemical and sensory quality of raw and subsequently cooked scallops, 3) determine shelf-life of HPP (350 MPa for 5/10 min) raw and then sous-vide cooked (65 ºC/10 min) scallops under refrigeration, and 4) investigate effects of HPP (150/350 MPa for 5/10 min) on myofibrillar and sarcoplasmic proteins of scallop muscle.
CHAPTER 2
EFFECTS OF SOUS-VIDE COOKING PARAMETERS ON SENSORY EVALUATION OF SEA SCALLOPS (Placopecten magellanicus)

2.1 Introduction

Sous-vide (SV) processing is the temperature-controlled cooking of vacuum packaged raw foods in a water bath or steam (Baldwin, 2012). This cooking technique has been associated with producing consistent and high quality muscle foods (Baldwin, 2012). Cooking food after vacuum packaging or sealing offers several benefits including inhibition of oxidative deterioration, reduction in moisture loss, retention of volatile flavors, reduction in growth of aerobic bacteria, efficient energy transfer and uniform heating (Church & Parsons, 2000; Baldwin 2012). Moreover, the controlled temperature during cooking allows more control of doneness and texture of the resulting food product. At present, SV cooking methods are employed at fine-dining restaurants around the world as well as at home. Moreover, with the increasing popularity and acceptance of SV, food companies have begun selling SV cooked dishes at the retail level as well (Cuisine Solutions, 2019; Nelson, 2019; Starbucks, 2019).

Sea scallops are protein-rich, low calorie shellfish popular for their tender texture and mild, sweet flavor. Fresh scallops are soft and tender, and the delicate quality of the muscle meat may benefit from low cooking temperatures. Schellekens (1996) also recommended low SV cooking temperatures for seafoods in order to retain their intrinsic qualities. Moreover, cooking sea scallops at temperatures above 65 °C has resulted in tough meat due to excessive protein denaturation (Findlay & Stanley, 1984). However, it is crucial to choose SV cooking parameters that ensure safety of the resulting product. The FDA provides a series of time and temperature combinations considered safe for SV cooking based on the length of time required to accomplish
a six logarithmic reduction in the number of *L. monocytogenes* at a specific internal product temperature as suggested by the Fish and Fisheries Products Hazards and Controls Guidance (FDA, 2013).

Although SV dishes are popular among innovative chefs and cooks, there are no reports to date discussing the effects of SV parameters on consumer acceptability of seafood, and in particular, of sea scallops. This work aimed to evaluate the effects of three SV cooking conditions on consumer acceptability of sea scallops. A second objective of this study was to select one of the three treatments for subsequent SV studies based on consumer acceptability. The three time–temperature treatments (55 °C for 208 min (SV55), 60 °C for 45 (SV60), and 65 °C for 10 min (SV 65)) chosen for the sensory evaluation were based on ensuring safety of the SV cooked scallops while retaining maximum sensory quality and appeal as well.

### 2.2 Materials and Methods

#### 2.2.1 Sous-vide cooking

Fresh, dry sea scallops (size 10-12) sourced from Seatrade International Company Inc. (Topsfield, MA, USA) were vacuum packed into 3.3 mil plastic bags (3.3 cm$^3$/100 in$^2$ oxygen transmission rate, 80 micron, 100 °C tolerance; Ultrasource, Kansas, MO, USA), with six scallops per bag and three bags per treatment. Samples were SV cooked using an immersion circulator (Sous-vide™ Professional Creative, PolyScience, Niles, IL) to bring the core temperature to 55, 60 or 65 °C and held at that temperature for the stipulated duration depending on the treatment. The time and temperature combinations were determined by extrapolating the 23 combinations provided by the FDA (2013) corresponding to the duration at a specific internal product temperature required to accomplish six logarithmic reduction in the number of *L. monocytogenes*. Using these data points, a linear regression was created to calculate the slope, y-
intercept and the square of the correlation coefficient ($R^2$) to measure how well the regression equation fit the data. The equation generated was $y = 5 \times 10^9 e^{-0.309x}$ with $R^2 = 0.9984$ for the relationship between temperature in degree Celsius ($x$) and time in minutes ($y$). Cooked samples were immediately cooled in an ice-water slush for approximately 30 min until the core temperature reached $<2.7 \, ^\circ C$. Chilled samples were stored on ice at $<3.3 \, ^\circ C$ overnight and sensory analysis was performed the next day.

2.2.2 Sensory analysis

Sensory analysis was conducted to determine the consumer acceptability of the SV cooked samples. Panelists ($n=95$), who enjoyed consuming seafood products, were recruited to rate the color, aroma, texture, flavor and overall acceptability of the samples on a nine-point hedonic scale ($1 =$ dislike extremely, $5 =$ neither like nor dislike, $9 =$ like extremely). Scallop samples were removed from the vacuum bag $\sim$30-40 min prior to serving to bring them closer to room temperature ($\sim 22 \, ^\circ C$) and served in ceramic bowls with unsalted, melted butter in randomized and balanced order. The test was designed and executed using SIMS 2000 (Sensory Computer Systems, Morristown, NJ). Consumer testing was conducted at the University of Maine Sensory Evaluation Center with approval from the University of Maine Institutional Review Board prior to conducting the test. Each panelist was requested to read the consent form prior to the test (Appendix A). The questionnaire (Appendix B) was identical for each subject, and consisted of sample evaluation and a series of demographic and scallop consumption pattern questions. A comment box was also made available for the panelists to leave any comments, which were categorized and counted to understand panelists’ impressions of the samples. The panelists were recruited via flyers and email lists (Appendix C) and were compensated with $5 in cash upon completion of the test.
2.2.3 Statistical analysis

Sensory evaluation data were extracted from SIMS 2000 and statistical analysis was performed using SPSS version 22 (IBM, New York, USA) at a significance level of $p<0.05$. One-way ANOVA and correlations were performed on hedonic scores. Microsoft Excel (Microsoft corporation, Redmond, CA, USA) was used to calculate means and standard errors.

2.3 Results and Discussion

Sensory analysis was conducted with 95 panelists in total, of whom 48% were male and 51% were female, with 78% of the panelists aged 18-35 years old (Table 2.1). This study was conducted on a university campus, contributing to the low the average age of the panelists, which was 27 years old. Sixty-eight percent of panelists reported that they typically consumed scallops at a restaurant, suggesting that SV cooked scallops could be introduced to more consumers by incorporating them as a dish in restaurants (Table 2.2). Almost half of the panelists consumed scallops two or fewer times a year, which was not surprising since younger, college aged panelists might find them too expensive or not easily accessible. As the age group increases, the percentage of people who consume seafood at least twice a week also increases significantly in the U.S. (Terry et al., 2018). When asked which sensory characteristic of a sea scallop was most important them, 63% responded “flavor,” followed by 34% response for “texture,” clearly indicating the importance of flavor and texture as compared to aroma, color or any other characteristics.
Table 2.1. Age and gender of sensory panelists

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number of panelists</th>
<th>Percent of panelists (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>46</td>
<td>48</td>
</tr>
<tr>
<td>Female</td>
<td>48</td>
<td>51</td>
</tr>
<tr>
<td>Rather not say</td>
<td>1</td>
<td>0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>Number of panelists</th>
<th>Percent of panelists (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-23</td>
<td>45</td>
<td>47</td>
</tr>
<tr>
<td>24-29</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>30-35</td>
<td>10</td>
<td>11</td>
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<td>36-41</td>
<td>3</td>
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<td>42-47</td>
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<td>4</td>
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<td>48-53</td>
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<td>6</td>
</tr>
<tr>
<td>54-59</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>60+</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 2.2. Place and frequency of scallop consumption, and important attribute of scallops among sensory panelists

<table>
<thead>
<tr>
<th>Question</th>
<th>Response</th>
<th>Percent of panelists (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Where do you usually consume scallops?</td>
<td>At restaurant</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>At home</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>How often do you consume scallops?</td>
<td>2 or fewer times a year</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>Every 2-3 months</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>1-8 times a month</td>
<td>16</td>
</tr>
<tr>
<td>What sensory characteristic of scallop is the most important to you?</td>
<td>Flavor</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>Texture</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Color</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Aroma</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>0</td>
</tr>
</tbody>
</table>

There were no significant differences ($p>0.05$) among the three treatments for any of the sensory attributes tested, suggesting consumers liked scallops cooked using different time – temperature parameters equally (Table 2.3). In contrast, trout SV cooked at 90 °C for 5 and 15 min for was rated higher for appearance, smell and acceptability compared to trout SV cooked at 70 °C for 10 min (González-Fandos et al., 2004). The authors further explained that the higher cooking temperature may have contributed in maintaining favorable characteristics during
refrigerated storage by limiting bacterial growth (González-Fandos et al., 2004). A score of 7 or higher on a 9-point hedonic score is associated with highly acceptable sensory quality (Everitt, 2009). Despite no significant differences in overall acceptability, SV65 received an average score of 7.1 ± 0.9 or ‘like moderately,’ whereas SV55 (6.8 ± 1.9) and SV60 (6.9 ± 1.5) were rated slightly slower.

Table 2.3. Consumer acceptability scores on a 9-point hedonic scale for SV cooked scallops

<table>
<thead>
<tr>
<th>Attribute</th>
<th>SV55</th>
<th>SV60</th>
<th>SV65</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aroma</td>
<td>6.7 ± 1.6</td>
<td>6.7 ± 1.1</td>
<td>6.7 ± 1.3</td>
<td>0.93</td>
</tr>
<tr>
<td>Color</td>
<td>6.3 ± 1.7</td>
<td>6.3 ± 1.6</td>
<td>6.5 ± 1.2</td>
<td>0.51</td>
</tr>
<tr>
<td>Texture</td>
<td>6.4 ± 2.3</td>
<td>6.4 ± 1.8</td>
<td>6.8 ± 1.6</td>
<td>0.26</td>
</tr>
<tr>
<td>Flavor</td>
<td>6.8 ± 2.0</td>
<td>6.8 ± 1.7</td>
<td>7.1 ± 1.2</td>
<td>0.35</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>6.8 ± 1.9</td>
<td>6.9 ± 1.5</td>
<td>7.1 ± 0.9</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Above values represent mean (n=95) scores on a 9-point hedonic scale where 1=dislike extremely and 9=like extremely.

Out of 42 comments received for SV65, there were 13 comments appreciating the texture of the scallop and some pointing out that they liked the texture of SV65 best out of the three samples. On the other hand, 9 panelists mentioned they did not like the texture of SV65, with the most common comment on the sample being “chewy”. For both SV55 and SV60, there were more negative (6-10) comments than positive (4-5) related to texture, with most of the panelists mentioning that the sample was “too soft” or “undercooked” for their liking. With respect to flavor, 9 out of 11 comments characterized the flavor of SV65 as favorable whereas SV55 received 5 negative comments out of a total of 9. These results suggest that the texture and flavor of SV65 appealed more to the panelists in comparison to SV55 and SV60. There were numerous comments about not liking that the scallops were served at room temperature, which likely decreased the acceptability scores across all treatments.
Correlations among sensory attribute scores revealed significant \((p<0.05)\), strong, positive correlations of overall acceptability with texture \((r=0.770)\) and flavor \((r=0.864)\) scores, indicating that texture and flavor of the scallop samples strongly affected overall liking scores of the samples. However, when overall acceptability scores were sorted based on “most important sensory characteristic of scallops,” no significant differences were found among treatments (Fig 2.1). Interestingly, for all three treatments, overall acceptability scores were above 7, which corresponded to “like moderately” for consumers who picked flavor and texture as the most important characteristic of scallops.

Fig 2.1. Overall acceptability scores based on panelists’ response to most important sensory characteristic of scallops.
Reported consumption frequency had no significant effects on flavor, texture and overall acceptability scores of SV cooked scallops (Fig 2.2). However, approximately 75% of the panelists rated overall acceptability and flavor of SV65 scallops ≥ 7 compared to ≤ 65% for SV55 and SV60 samples (Fig 2.3). These results corroborate the average scores for the sensory attributes.
2.4 Conclusions

Consumer acceptability results demonstrated that SV cooking temperature and holding time did not affect the ratings for any of the sensory attributes tested in this study. However, samples cooked at 65 °C for 10 min scored marginally higher on the 9-point hedonic scale for flavor, texture and overall acceptability, regardless of consumption patterns. A strong, positive correlation of overall acceptability scores with flavor and texture scores indicated that these two attributes are key when consumers evaluate sea scallops. Consumer comments provided a deeper insight on their perceptions of SV cooked scallop texture, with samples cooked at 65 °C for 10 min receiving the most positive comments. Additionally, one of the important lessons from the consumer comments was that the sea scallops should not be served at room temperature. Based on the fact that the higher temperature-short time treatment offers convenience during cooking and is potentially more cost and energy efficient than other treatments, 65 °C for 10 min was
chosen for the subsequent studies. Moreover, gentle heating or warming the scallop samples to a specific temperature right before serving may aid in better consumer evaluation and experience.
CHAPTER 3

MODERATE HIGH PRESSURE PROCESSING CONDITIONS ALTER
PHYSICOCHEMICAL AND SENSORY QUALITIES OF RAW AND
SUBSEQUENTLY SOUS-VIDE COOKED SEA SCALLOPS

3.1 Introduction

Sous-vide (SV) cooking refers to the precision thermal processing of vacuum-packaged foods in a temperature-controlled water bath. In recent years, SV cooked muscle foods have found a lasting place in fine dining and fast casual restaurants, and with the current availability of reasonably priced, restaurant-quality SV equipment, adventurous home cooks are adopting this innovative cooking technique as well (Ramsden, 2013; McHugh, 2017). Uniform cooking along with reduced moisture loss due to vacuum packaging offer multiple advantages including better flavor retention and textural properties, minimal loss of water-soluble compounds, and reduced lipid oxidation during SV cooking (Baldwin, 2012; Sampels, 2015). For muscle foods, low-temperature, slow SV cooking results in evenly cooked, tender products, particularly important for seafood, which is prone to being overcooked. In restaurants, center-of-plate proteins are typically vacuum-packaged on-site, SV cooked at low temperatures, then subsequently finished and plated just prior to serving. However, individual proteins already portioned, vacuum-packaged, distributed under refrigeration, and ready to be SV cooked, can offer convenience to commercial kitchens and the added benefit of less potential cross-contamination during meal preparation. To make this a viable process, adequate shelf-life for refrigerated distribution is essential.

High pressure processing (HPP), recognized for its ability to reduce pathogenic and spoilage microorganisms while maintaining fresh-like appearance and nutritive value of foods,
has already been shown to extend the shelf-life of a variety of foods (Murchie et al., 2005; Campus, 2010). Bacterial cell lysis occurs due to pressurization and depressurization, often yielding an extended shelf-life of the food product (Campus, 2010). A non-thermal processing technique, HPP dispatches pressure uniformly and instantaneously in foods packaged under vacuum, and reduces the use of chemical preservatives. Moreover, texture and flavor components are better preserved post-HPP in comparison to thermal processing (Murchie et al., 2005; Campus, 2010; de Oliveira et al., 2017). HPP could serve as a potential pre-treatment for vacuum-packaged seafood intended for SV, depending upon its effects on the quality attributes of the food.

Use of HPP to shuck shellfish is now a well-accepted practice in the seafood industry, owing to higher quality and better meat yield when compared to traditional physical shucking methods (Campus et al, 2010). Pressures of 100-800 MPa have also been studied to reduce the microbial load to improve shelf-life and microbial safety of various mollusks (Hughes et al., 2016; de Oliveira et al., 2017, Bonfim et al., 2019). Yi et al. (2013) reported harder texture in bay scallops shucked at 300 MPa, but not when shucked at lower pressures. HPP of already-shucked mollusks was shown to affect appearance and texture, depending on the pressure and holding time applied. Cruz-Romero et al. (2008) reported an increase in L* value and shear force values in oyster meats that were processed in-shell at 260, 400 and 600 MPa for 5 min. In contrast, hardness in already shucked scallop adductor meat decreased after HPP at 200 and 400 MPa for 10 min (Pérez-Won et al., 2005). Moreover, application of high pressures, especially >400 MPa, in tender seafood products can negatively affect protein structure, resulting in diminished protein-hydration properties and altered texture (Murchie et al., 2005; Jantakoson et al., 2012; Martínez et al., 2017), which may negatively impact quality.
HPP may prove to be a powerful tool to extend the shelf-life of vacuum-packaged scallops intended for SV cooking in commercial kitchens or by home chefs. However, it is crucial to use pressure parameters yielding minimal negative impacts on product quality while delivering prolonged shelf-life. For example, Sun et al. (2017) reported that high pressure treatment of raw beef steaks improved their safety, while achieving greater than 5-log microbial reduction (aerobic plate counts and *Escherichia coli* counts) at 600 MPa for 10 min. However, after SV cooking, the texture of the steaks changed significantly compared to the non-HPP control. To our knowledge, studies evaluating the effects of high pressure pre-treatment of seafood, and in particular scallops, that are subsequently SV cooked, have not been reported. Furthermore, to develop a successful foundation for HPP-treated, SV cooked seafood products, it is important to understand the effects of these processing techniques on their sensory qualities. Several reviews have addressed the pressing need for sensory studies on HPP-treated seafood (Murchie et al. 2005; de Oliveira et al. 2017; Bonfim et al. 2018), emphasizing the importance of consumer acceptability of these products. Atlantic sea scallops (*Placopecten magellanicus*) are economically important, high-value molluskan shellfish enjoyed for their tender, juicy and succulent adductor meat, making them an appropriate model product for testing the effects of combined HPP and SV processing.

The present work was conducted to gain a comprehensive insight on the physicochemical and sensorial qualities of HPP and subsequently SV cooked scallops. The specific objectives of this study were to evaluate the effects of moderate HPP conditions (150/350 MPa for 5/10 min) on the physicochemical qualities of raw and subsequently SV cooked scallops, and to determine their acceptability by a consumer sensory panel. Moderate pressures of 150 and 350 MPa were
selected to minimize damage to the physical integrity and characteristic texture of sea scallops while potentially providing prolonged shelf-life under refrigeration.

3.2. Materials and Methods

Two separate experiments were conducted to evaluate the effects of HPP and SV cooking on physicochemical and sensory qualities of scallops. In the first experiment, physicochemical quality attributes of HPP raw and SV cooked scallops were tested. In the second experiment, consumer acceptability testing of the HPP and subsequently SV cooked scallops was conducted using untrained panelists.

3.2.1 Physicochemical Study

Fresh dry, raw scallops (size 10-12) were vacuum packed and then subjected to moderate pressures (150 and 350 MPa) for 5 or 10 min, for a total of 5 treatments, including the non-HPP control. Subsequently, half of the samples were SV cooked (65 ºC for 10 min) and half remained uncooked (Fig 3.1). Treatments were HPP and SV processed in triplicate batches, with 24 scallops per replicate. Samples were stored in ice at <3.3 ºC and analyzed within 2 days of processing.
**3.2.1.1 Sample Preparation and High Pressure Processing**

Dry, shucked scallops (size 10-12) were sourced from Seatrade International Company Inc. (Topsfield, MA, USA) and vacuum packed in 3.3 mil plastic bags (3.3 cm³/100 in² oxygen transmission rate, 80 micron, 100 °C tolerance; Ultrasource, Kansas, MO, USA), six scallops per bag. Samples were high pressure processed in a 55 L HPP unit (Hiperbaric, Miami, FL, USA). Water was used to achieve hydrostatic pressure and was maintained at 5 °C throughout processing. Once processed, plastic bags containing scallops were packed in ice in coolers at < 3.3 °C until sous-vide processed or analyzed.

**3.2.1.2 Sous-vide Processing**

Samples were SV cooked using an immersion circulator (Sous-vide™ Professional Creative, PolyScience, Niles, IL) to bring the scallop core temperature to 65 °C for 10 min. Sample core temperature was monitored throughout cooking and cooling by inserting K-type...
thermocouples attached to a data logger at the core of the scallop (RDXL4SD, Omega, Standford, CT, USA). Cooked samples were immediately cooled until the core temperature reached <2.7 °C in an ice-water slush, within 30 min. Chilled samples were packed in ice in a cooler at <3.3 °C overnight.

3.2.1.3 Physicochemical Analyses

3.2.1.3.1 Moisture Content

Moisture content was determined gravimetrically in triplicate, following AOAC method (950.46) by drying 5 g of ground scallops overnight in a convection oven at 105 °C (AOAC, 2005). The difference in initial and final weight was used to calculate the moisture content in g/100 g.

3.2.1.3.2 Weight Loss

Weight loss due to HPP and SV processing was measured by weighing scallops (n=6/treatment-replicate) pre- and post-processing. Percent water loss was calculated as follows:

\[
Weight\ loss\ (%) = \frac{(W_1 - W_2)}{W_1} \times 100
\]

where \(W_1\) and \(W_2\) are the pre- and post-processing weight of 6 scallops, respectively.

3.2.1.3.3 Water Holding Capacity

Water holding capacity (WHC) was determined by cutting scallops (n=3) into cubes weighing 2 ± 0.05 g and wrapping them in two pieces of pre-weighed filter paper. Wrapped samples were centrifuged at 1,000 x g for 15 min. After removing the samples from the filter paper, the filter papers were reweighed. WHC (g/100g) was calculated as follows,

\[
\left(\frac{MC}{100} \times \text{sample weight (g)}\right) \div (F_2 - F_1)
\]

\[
\times 100
\]
where MC is moisture content of scallops (g/100 g), and F₁ and F₂ are initial and final weight (g) of the filter papers, respectively.

3.2.1.3.4 Salt Soluble Protein

Salt soluble protein (SSP) was extracted from the scallops by following the method of Work et al. (1997). Briefly, scallop meat was ground and homogenized using a food processor (Oster FPSTMC3321-015-NP2, Sunbeam Products, USA) for 15 s, stirred and then ground for an additional 15 s. A 5 g subsample was blended with 95 mL of 5 % NaCl solution for 60 s in a Waring blender and the homogenate was centrifuged (Beckman Model J2-21, USA) at 30,074 x g for 20 min at 4 °C. Protein analysis was performed on the supernatant as described by Lowry et al. (1951), with bovine serum albumin as the standard. Sample absorbance was read at 700 nm, and SSP was reported as mg/g sample.

3.2.1.3.5 Instrumental Color

Color analyses of scallops (n=10/treatment-replicate) were performed using a colorimeter (LabScan XE, Hunter Labs, Reston, VA, USA). The colorimeter was standardized using black and white tiles, and the L*, a* and b* measurements were recorded using colorimeter software (Universal, version 4.10, 2001, Hunter Labs). The surface color of each scallop sample was evaluated three times by rotating 120° from the previous reading, and the three readings were averaged.

3.2.1.3.6 Texture Analyses

Instrumental texture analysis was performed using two methods: texture profile analysis (TPA) and shear analysis. Scallop samples were prepared for each method by coring the center of the scallops using an apple corer (2.3 cm diameter) to ensure uniformity. The coring remnants were ground and used for moisture and salt soluble protein analyses.
3.2.1.3.6.1 TPA

Scallop cores (n=10/treatment-replicate) were placed vertically on the flat platform of a texture analyzer (TA-XTi2, Texture Technologies Inc., Scarsdale, NY). A 2-inch cylindrical probe was used to compress samples by 70%, with 2 mm/sec pre, test and post-test speed and a gap of 2 s between two cycles. Hardness (resistance to compression, Newton (N)), chewiness (resistance to elasticity, unitless), springiness (the ability of the meat to spring back after compression, unitless) and resilience (the immediate springiness of the meat as the probe is withdrawn between “bites,” unitless) (Bourne 2002) were recorded by the texture analyzer software (Exponent 32, version 5.0, 6.0, 2010, Texture Technologies Inc., Scarsdale, NY).

3.2.1.3.6.2 Shear Force

Scallop cores (n=10) were placed on a texture analyzer (TA-XTi2, Texture Technologies Inc., Scarsdale, NY) platform, perpendicular to a knife blade with chisel end (TA 42). A 90% strain was applied to shear the muscle fibers with pre, test and post-test speeds of 2, 1, and 2 mm/sec, respectively. Firmness (N) and toughness (N.sec) were recorded by averaging 10 values for each treatment-replicate using texture analyzer software (Exponent 32, version 5.0, 6.0, 2010, Texture Technologies Inc., Scarsdale, NY).

3.2.1.3.7 Statistical Analysis

Data analysis was performed using SPSS version 22 (IBM, New York, USA) at a significance level of \( p<0.05 \). Extreme outliers were removed based on the 3 * interquartile range. One-way and two-way ANOVA were performed to analyze the differences among treatments and to study the effects of independent treatment variables, respectively. Tukey’s HSD test was used to determine differences among treatment means.
3.2.2 Sensory Analysis

Sensory analysis was conducted to determine consumer acceptability of sous-vide cooked samples. Panelists (n=99) rated appearance, aroma, color, texture, flavor and overall acceptability of samples on a nine-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely). Moreover, panelists also rated specific textural attributes on a five-point “just-about-right” (JAR) scale (Table 3.1). Scallop samples were removed from the vacuum bags and reheated in aluminum pans placed on a water bath at 50 °C for at least 30 min to a maximum of 90 min prior to serving. Scallops were served with melted salted butter in a randomized and balanced order. The test was conducted at the University of Maine Sensory Evaluation Center, and was designed and executed using SIMS 2000 (Sensory Computer Systems, Morristown, NJ). Approval from the University of Maine Institutional Review Board (IRB) was granted to prior conducting the test. The panelists were recruited through email lists and were compensated $5 in cash, upon completion of the study.

<table>
<thead>
<tr>
<th>Textural attribute</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firm - Soft</td>
<td>Much too firm</td>
<td>Somewhat too firm</td>
<td>Just about right</td>
<td>Somewhat too soft</td>
<td>Much too soft</td>
</tr>
<tr>
<td>Chewy - Tender</td>
<td>Much too chewy</td>
<td>Somewhat too chewy</td>
<td>Just about right</td>
<td>Somewhat too tender</td>
<td>Much too tender</td>
</tr>
<tr>
<td>Dry – Juicy</td>
<td>Much too dry</td>
<td>Somewhat too dry</td>
<td>Just about right</td>
<td>Somewhat too juicy</td>
<td>Much too juicy</td>
</tr>
</tbody>
</table>

Table 3.1. Textural attributes assessed using five-point JAR scale

3.2.2.1 Treatments and Processing

The three scallop treatments tested were 350 MPa/5 min, 350 MPa/10 min, and the non-HPP control. Shucked scallops (size 12-14) were purchased (Seatrade International, Bedford, MA, USA) in October 2018. Scallops were washed in fresh water to remove any sand or grit, and pat dried using paper towels. Samples were subjected to pressure at 350 MPa for either 5 or 10
min using a 100 L unit (Avure Technologies, Erlanger, KY) as described previously, and stored on ice until cooking. The treatments were SV cooked by bringing the internal temperature of the scallops to 65 °C for 10 min. The samples were cooled immediately to <2.7 °C and packed in ice at <3.3 °C overnight.

3.2.2.2 Statistical Analysis

Statistical analysis of the sensory data was performed using SPSS version 22 (IBM, New York, USA) at a significance level of $p<0.05$. One-way ANOVA was performed on hedonic and JAR scores and Tukey’s HSD was conducted to determine differences among treatment means. A chi-square test was used to determine differences in frequency distributions of JAR scores whereas Bonferroni’s test was performed for post hoc for the frequency distributions.

3.3 Results and Discussion

3.3.1 Moisture Content, Weight Loss and Water Holding Capacity

Moisture content of the raw scallops was not significantly ($p>0.05$) affected by the HPP parameters tested in this study (Table 3.2). Overall, the moisture content of the raw scallops ranged from 74.9 – 77.3 g/100g, slightly lower than previously reported values for raw sea scallops (Naidu and Botta, 1978). Similarly, Pérez-Won et al. (2005) reported no significant change in moisture content of bay scallops treated at 200 MPa and 400 MPa for 10 min compared to the unprocessed control. SV cooking following HPP did not significantly change the moisture content of the pressurized samples compared to the control. Moreover, when compared to the raw scallops, the moisture content of the cooked scallops was not significantly different. In contrast, salmon slices SV cooked at temperatures of 65 °C or 90 °C for 5-15 min experienced significant reduction in moisture content compared to the raw samples (González-Fandos et al., 2005).
Table 3.2. Moisture content, weight loss, and water holding capacity of raw and sous-vide cooked scallops.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Moisture Content (g/100g)</th>
<th>Weight Loss (%)</th>
<th>Water Holding Capacity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>75.1 ± 0.1</td>
<td>3.5 ± 0.7</td>
<td>81.8 ± 2.9a</td>
</tr>
<tr>
<td>150/5</td>
<td>77.3 ± 0.1</td>
<td>2.3 ± 1.1</td>
<td>83.8 ± 3.8a</td>
</tr>
<tr>
<td>150/10</td>
<td>77.1 ± 0.1</td>
<td>1.9 ± 0.8</td>
<td>78.9 ± 3.4a</td>
</tr>
<tr>
<td>350/5</td>
<td>74.9 ± 0.0</td>
<td>1.7 ± 1.2</td>
<td>76.0 ± 5.2a</td>
</tr>
<tr>
<td>350/10</td>
<td>75.8 ± 0.1</td>
<td>3.0 ± 0.9</td>
<td>73.5 ± 3.7a</td>
</tr>
</tbody>
</table>

| Cooked    |                           |                 |                           |
| C         | 75.4 ± 0.0                | 7.1 ± 1.8       | 68.0 ± 2.3                |
| 150/5     | 75.4 ± 0.1                | 10.7 ± 0.5      | 68.8 ± 5.3                |
| 150/10    | 74.9 ± 0.0                | 13.1 ± 3.1      | 70.9 ± 5.4                |
| 350/5     | 76.4 ± 0.2                | 11.7 ± 4.1      | 64.7 ± 9.0                |
| 350/10    | 74.7 ± 0.0                | 15.1 ± 3.3      | 63.9 ± 6.2                |

Each value represents mean ± standard deviation (n=3). No letters indicate no significant differences among treatments by one-way ANOVA.

Weight loss due to vacuum packaging and HPP remained below 3.5 % for raw scallops. HPP treated samples had lower weight loss compared to the control, with 350/5 experiencing half the weight loss of the raw control; although the treatment differences were not statistically significant. Reduced weight loss of scallops due to HPP would have important implications with regard to physicochemical quality and economic value. Similar to the observed results in scallops, black tiger shrimp experienced less than 5 % weight loss, with no significant differences among samples HPP treated at 200, 400, 600, and 800 MPa for 20 min, at 28 ºC (Jantakoson et al., 2012). Extreme weight loss due to thermal or non-thermal processing has previously been reported to result in tough, chewy or hard texture of cooked meat (Botinestean et
SV cooking clearly increased weight loss compared to the raw scallops, with values ranging from 7.1-15.1 %. Pressurization appeared to increase weight loss of cooked scallops, however the treatments were not significantly different than the control. Sea scallops heated at atmospheric conditions to internal temperatures of 50-80 ºC experienced a 20-45 % weight loss (Findlay & Stanley, 1984), considerably higher than the weight loss of vacuum-packed scallops in the current study. Llave et al. (2018) reported slightly lower weight loss values, 5-9 % in SV cooked (65 ºC) scallops that were not HPP processed. Protein denaturation and aggregation due to thermal processing may lead to shrinkage, and often cause cellular liquid loss as proteins lose their water binding capacity.

No significant effect of time or pressure was observed on water holding capacity of raw, HPP-treated scallops, however, the 350 MPa/10 min treatment (most severe treatment tested) appeared to reduce WHC compared to the other treatments. The WHC of 350/10 scallops was 73.5 % compared to the control, which was 81.8 %. This reduced WHC seen in 350/10 samples could be attributed to protein denaturation along with muscle fiber compression previously reported in HPP-treated cold smoked salmon and scallop adductor meat (Pérez-Won et al., 2005; Gudbjornsdottir et al., 2010; de Oliveira et al., 2017). Aubourg et al. (2013) reported that pressure level, but not holding time, affected the WHC of raw Atlantic mackerel whereas the effect of HPP on cooked muscle was minimal. In the current study, the WHC of pressure treated, SV cooked scallops dropped to 63.9-70.9 %. and no significant effects of pressure level or holding time were observed. Reduced WHC in thermally treated samples is expected because of changes in the native protein structure and muscle fiber aggregation.
3.3.2 Salt Soluble Protein

HPP increased salt soluble protein content of raw scallops in comparison to the control (Fig 3.2), however, no significant effects of pressure or time were found. Pressures below 400 MPa have been shown to activate proteases in beef muscle (Ohmori et al., 1991) and to denature quaternary and tertiary protein structures, which may have led to the increased solubility of proteins observed in the raw, HPP processed scallops. Once SV cooked, the soluble protein content of all the treatments dropped to similar levels, 11.8-14.4 mg/g (Appendix D). However, higher pressure significantly lowered the SSP content in the cooked samples when compared to 150 MPa. The salt soluble protein fraction in muscle consists primarily of myofibrillar proteins, with myosin and actin comprising ~70% of the myofibrillar protein content. Pressures greater than 100 MPa significantly reduce the myosin peak in DSC thermographs, whereas pressures >200 MPa led to complete disappearance of the myosin peak, and a reduction of actin and sarcoplasmic protein peaks of raw seafood meat (Angsupanich et al., 1999; Chevalier et al., 2001; Jantakoson et al., 2012). These reports may explain the higher loss of SSP in the 350 MPa treatments post SV cooking. The combination of cooking and higher pressure (350 MPa) used in this study led to higher protein denaturation and hence, reduced SSP content in scallop meat.
Fig 3.2. Salt soluble protein content of raw scallops. Each value represents a mean ± standard deviation (n=3). Values not sharing a letter are significantly (p<0.05) different, analyzed by one-way ANOVA followed by Tukey’s HSD post hoc test.

3.3.3 Instrumental Color

Increased pressure magnitude, but not holding time, significantly (p<0.05) increased L* values of HPP-treated raw scallops, indicating a bleaching effect due to the 350 MPa treatment whereas a* and b* values were not affected by pressure or holding time (Table 3.3). L* values increased significantly for pressurized samples in comparison to the control. The 350 MPa treatments exhibited a slightly cooked appearance, due to their increased L* values and increased opacity. Numerous studies have reported lighter color in shellfish muscle post HPP processing (Yi et al, 2013; Hughes et al., 2015). In a previous study, the L* values for scallop adductor meat increased significantly compared to the control at 400 MPa but not 200 MPa for 5 and 10 min treatments (Pérez-Won et al., 2005). Although in red muscle a lighter color is considered a defect and is largely due to oxidation of the heme in myoglobin (Campus, 2010; de Oliveira et al.,
darker scallop meat was deemed unacceptable by a trained sensory panel (Weiqing et al., 2011) suggesting that lighter colored scallop meat is more desirable to consumers. In white muscle, the bleached appearance caused by high pressures is likely due to the partial denaturation of myofibrillar and sarcoplasmic proteins, with decreased hydration status and increased oxidation of proteins and lipids as additional contributors (Chevalier et al., 2001; Cruz-Romero et al., 2007; de Oliveira et al., 2017). Contrary to previous reports (Pérez-Won et al., 2005; Cruz-Romero et al., 2007), a* values of HPP scallops significantly increased compared to the unprocessed control whereas b* values of HPP scallops were not affected by pressure or time, or different than the control. SV cooking masked the differences in L* and a* values among raw treatments caused by HPP, and no significant differences were found in L*, a*, and b* values among HPP treated, SV cooked scallops.

Table 3.3. Instrumental color of raw and SV cooked scallops

<table>
<thead>
<tr>
<th>Treatment</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>68.2 ± 0.7a</td>
<td>1.5 ± 0.2a</td>
<td>12.6 ± 0.5</td>
</tr>
<tr>
<td>150/5</td>
<td>70.1 ± 1.2b</td>
<td>2.4 ± 0.2b</td>
<td>12.8 ± 0.3</td>
</tr>
<tr>
<td>150/10</td>
<td>71.1 ± 1.0b</td>
<td>2.4 ± 0.3b</td>
<td>13.1 ± 0.7</td>
</tr>
<tr>
<td>350/5</td>
<td>74.6 ± 0.5b</td>
<td>2.5 ± 0.2b</td>
<td>13.2 ± 0.3</td>
</tr>
<tr>
<td>350/10</td>
<td>74.6 ± 1.5b</td>
<td>2.5 ± 0.7b</td>
<td>12.5 ± 0.8</td>
</tr>
<tr>
<td>Cooked</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>72.1 ± 0.8</td>
<td>1.1 ± 0.6</td>
<td>13.0 ± 1.2</td>
</tr>
<tr>
<td>150/5</td>
<td>72.5 ± 1.4</td>
<td>1.7 ± 1.0</td>
<td>13.5 ± 0.8</td>
</tr>
<tr>
<td>150/10</td>
<td>72.7 ± 1.1</td>
<td>1.5 ± 0.4</td>
<td>13.2 ± 0.8</td>
</tr>
<tr>
<td>350/5</td>
<td>74.9 ± 0.9</td>
<td>1.2 ± 0.2</td>
<td>12.8 ± 0.7</td>
</tr>
<tr>
<td>350/10</td>
<td>72.9 ± 0.7</td>
<td>1.7 ± 0.3</td>
<td>13.6 ± 3.0</td>
</tr>
</tbody>
</table>

Each value represents mean ± standard deviation (n=3). Values not sharing a letter are significantly (p<0.05) different, analyzed by one-way ANOVA followed by Tukey’s HSD post hoc test. No letters indicate no significant differences among treatments by one-way ANOVA.
3.3.4 Instrumental Texture

3.3.4.1 Texture Profile Analysis

HPP did not alter the hardness of raw scallops compared to the control, with no significant differences among the treatments (Table 3.4). These results suggest that even the 350/10 treatment could be applied to sea scallops without affecting the texture of raw samples. In a prior study, neither pressure magnitude nor duration affected compression force (N) of HPP-treated tilapia fillets (Suemitsu & Cristianini, 2019). However, HPP at 200 and 400 MPa for 10 min was shown to soften bay scallop muscle tissue due to changes in connective tissue structure (Pérez-Won et al., 2005). The differences in results from the current study and Pérez-Won et al. (2005) could be attributed to higher HPP temperature (22 °C) and smaller size of bay scallops (~3 g) in the latter study.

Table 3.4. TPA attributes of raw and SV cooked scallops

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hardness</th>
<th>Springiness</th>
<th>Chewiness</th>
<th>Resilience</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>11.6 ± 0.9</td>
<td>0.4 ± 0.1a</td>
<td>0.7 ± 0.1</td>
<td>5.8 ± 1.6</td>
</tr>
<tr>
<td>150/5</td>
<td>14.7 ± 1.3</td>
<td>0.3 ± 0.0b</td>
<td>1.2 ± 0.5</td>
<td>8.8 ± 2.2</td>
</tr>
<tr>
<td>150/10</td>
<td>13.3 ± 1.2</td>
<td>0.4 ± 0.0b</td>
<td>1.1 ± 0.1</td>
<td>8.1 ± 2.1</td>
</tr>
<tr>
<td>350/5</td>
<td>14.4 ± 1.5</td>
<td>0.4 ± 0.1b</td>
<td>1.0 ± 0.2</td>
<td>10.6 ± 5.3</td>
</tr>
<tr>
<td>350/10</td>
<td>13.5 ± 1.5</td>
<td>0.3 ± 0.1b</td>
<td>0.9 ± 0.2</td>
<td>10.7 ± 3.0</td>
</tr>
</tbody>
</table>

Each value represents mean ± standard deviation (n=3). Values not sharing a letter are significantly (p<0.05) different, analyzed by one-way ANOVA followed by Tukey’s HSD post hoc test. No letters indicate no significant differences among treatments by one-way ANOVA.

In contrast to the uncooked samples, the SV cooked samples demonstrated treatment differences in hardness. The hardness values in the 350/10 treatment were significantly higher than the control and 150/5 treatment, likely due to the combined effect of pressure, time and SV cooking (Fig. 3.3 (a)). Moreover, both of the 5 min treatments resulted in lower hardness values than 10 min treatments whereas pressure had no effects on hardness of cooked samples. These
results suggest that the duration of pressurization rather than magnitude of pressure caused textural changes in subsequently SV cooked scallop meat. Llave et al. (2018) reported that SV cooking of scallops (65 °C) led to considerable protein denaturation, with myofibrillar proteins (myosin and actin) denaturing at around 40 °C and 80 °C, respectively, causing reduced spaces between muscle fibers. Dong et al. (2017) reported that vacuum-packaged scallops (Patiniopecten yessoensis) cooked at 55 °C for 2 h – 18 h experienced an increase in hardness and shear force values compared to the raw control as the cooking time increased due to denaturation of proteins in scallops. The authors cited loss of cellular liquid (cook loss), due to protein denaturation and subsequent inability of proteins to hold water as one of the primary reasons for the textural changes, and further confirmed the contribution of protein degradation due to increased proteolytic activity during cooking in changing meat quality during the cooking process as well (Dong et al., 2017). Denaturation of myofibrillar proteins resulting in water loss in scallop meat has been previously correlated to increased hardness values (Findlay & Stanley, 1984), as evidenced in the current study. Weight loss had a significant \((p=0.002)\), moderately strong positive correlation \((0.724)\) with hardness of cooked samples, indicating an increase in hardness in the SV cooked samples as the weight loss increased. Additionally, salt soluble protein content was significantly \((p=0.042)\), negatively correlated \((-0.530)\) with hardness.
Fig. 3.3 (a). Texture profile analysis hardness values for HPP, sous-vide cooked scallops. Each value represents a mean ± standard deviation (n=3). Values not sharing a letter are significantly ($p<0.05$) different, analyzed by one-way ANOVA followed by Tukey’s HSD post hoc test.

Processing parameters used in this study did not significantly ($p<0.05$) affect chewiness, resilience or springiness (also known as elasticity) of raw samples (Table 3.4). In contrast, Pérez-Won et al. (2005) reported that chewiness of raw HPP scallop meat (0.387) processed at 200 MPa for 10 min decreased by half compared to the control (0.753), whereas the resilience increased as the pressure level increased, and springiness was not significantly affected by HPP at 400 MPa or 200 MPa for 10 min (Pérez-Won et al., 2005). However, chewiness of pressurized, cooked sea scallops in the current study was significantly ($p<0.05$) affected by HPP, with 150/10 and 350/10 treatments resulting in higher values compared to the control (Fig. 3.3 (b)). Moreover, SV cooked scallops pressurized for 10 min were significantly chewier than the 5 min treatments, following the same trend as TPA hardness values. Resilience and springiness of the SV cooked scallops were significantly higher in the 150 MPa treatments compared to the
control for SV cooked scallops. Overall, the TPA results indicate that 10 min of pressurization significantly affected scallop texture post cooking, making meat harder and chewier than that from the 5 min treatments. The effects of HPP on scallop texture were evident once SV cooked, as observed by differences among treatments in TPA parameters only in cooked samples.

Fig. 3.3 (b). TPA chewiness, resilience and springiness values for HPP, sous-vide cooked scallops. Each value represents a mean ± standard deviation (n=3). Values not sharing a letter are significantly (p<0.05) different, analyzed by one-way ANOVA followed by Tukey’s HSD post hoc test.

3.3.4.2 Shear Force

For uncooked, pressurized scallops, no significant differences in firmness and toughness (Appendix E) were detected during shearing in comparison to the control. However, in SV cooked scallops, pressurization time, but not pressurization level, significantly affected firmness and toughness, with the 10 min treatment resulting in firmer and tougher SV cooked scallops, following a similar trend as the hardness values (Fig. 3.4). In contrast, both, elevated pressure (600 MPa) and longer pressurization time, resulted in tougher SV beef steak with increased shear force values compared to lower (450 MPa) and shorter HPP treatment (Sun et al., 2017). Beef muscle tissue comprises a higher proportion of connective tissue than scallops, which in addition
to higher pressure levels used in the beef study may have contributed to the differences in shear force results in response to pressure level.

Fig 3.4. Shear firmness values of raw and sous-vide cooked scallops. Each value represents the mean ± s.d. (n=3). Within independent factors, values not sharing a letter are significantly (p<0.05) different analyzed by two-way ANOVA.

Protein denaturation due to increased pressurization magnitude and duration has been reported to cause hardening, firming and toughening in seafood meat (de Oliveira et al., 2017). This was further supported by differential scanning calorimetry (DSC) studies showing reduction in or disappearance of myosin, actin and various sarcoplasmic protein peaks post HPP in species including cod, tilapia, herring, ocean perch, and salmon (Angsupanich & Ledward, 1998; Hsu & Ko 2001; Schubring et al, 2003; Schubring 2005; de Oliveira et al., 2017). In addition, the compaction of muscle fibers due to protein interactions and their aggregation in response to HPP was reported to contribute to texture changes in cod (Angsupanich et al., 1999) and black tiger shrimp (Jantakoson et al., 2012). Martínez et al., (2017) confirmed conformational changes in
pressurized (100-600 MPa) crabmeat proteins by demonstrating significant increases in β-sheet and decreases in α-helix content compared to an unprocessed control, however there were no significant differences in protein confirmation among pressure levels. β-sheets are important components of aggregated proteins as they stack uniformly when compared to α-helices. In the present study, increased hardness and chewiness of HPP-treated, SV cooked scallops indicate that the combination of these processes altered their texture even though HPP alone did not result in significant differences in texture.

3.3.4.3 TPA Compared to Shear Testing Method

Compression and shear tests are commonly used to analyze seafood texture with varying probes and parameters, demanding cautious comparisons among studies (de Oliveira et al., 2017). TPA is a double compression test mimicking biting behavior during chewing whereas the Kramer Shear test reports the force and work required to shear the muscle tissue with a blade (Texture Technologies, 2015). In this study, texture was evaluated using both the TPA and Kramer Shear methods because there are no standard established methods to assess scallop meat texture. Consequently, results from the two methods were compared for responsiveness to textural changes and variability of the data. Overall, TPA was more responsive to textural changes in scallops with lower overall variability in data compared to the shear testing method. The TPA testing parameters used identified changes in hardness whereas the shear test did not demonstrate differences in firmness of HPP treated uncooked or cooked scallops compared to the control. Based on these results, the TPA method was a more effective tool for evaluating scallop texture.
3.3.5 Sensory Evaluation

Based on the results from the physicochemical analysis, the 350/5 and 350/10 treatments were selected for consumer acceptability study since HPP holding time, and not pressure level, was found to significantly affect the texture of scallop meat. Thus, it was important to evaluate whether holding time also affected consumer acceptability of the scallops. The 350 MPa pressure level was chosen because the higher pressure is likely more effective in reducing the microbial load and extending shelf-life of vacuum-packaged scallops, making the 350 MPa a more viable option for the industry. Sensory quality was evaluated using 9-point hedonic scale to understand acceptability of sensory attributes as well as the 5-point “just-about-right” (JAR) scale to determine the appropriateness of the level of specific textural attributes (Lawless and Heymann, 2013).

Table 3.5. Mean scores for consumer acceptability of sensory attributes on 9-point hedonic scale for SV cooked scallops.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Control</th>
<th>350/5</th>
<th>350/10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>6.37 ± 1.7</td>
<td>6.35 ± 1.6</td>
<td>6.20 ± 1.7</td>
</tr>
<tr>
<td>Aroma</td>
<td>6.55 ± 1.5</td>
<td>6.41 ± 1.7</td>
<td>6.54 ± 1.5</td>
</tr>
<tr>
<td>Texture</td>
<td>6.67 ± 1.8</td>
<td>6.57 ± 1.8</td>
<td>6.57 ± 1.6</td>
</tr>
<tr>
<td>Flavor</td>
<td>6.96 ± 1.4</td>
<td>6.80 ± 1.6</td>
<td>7.04 ± 1.4</td>
</tr>
<tr>
<td>Overall Quality</td>
<td>6.88 ± 1.5</td>
<td>6.69 ± 1.7</td>
<td>6.74 ± 1.6</td>
</tr>
</tbody>
</table>

Each value represents mean ± standard deviation (n=99). There were no significant differences among treatments by one-way ANOVA.

Consumer acceptability of the control and HPP samples did not differ significantly, with all three treatments receiving similar scores for sensory attributes based on the 9-point hedonic scale (Table 3.5). It is noteworthy, however, that a higher proportion of panelists (65.7 %) rated the overall quality of the control and 350/10 samples as ≥ 7 compared to 350/5 (61.6 %) samples. On a 9-point hedonic scale, a score of 7 or higher is typically considered highly acceptable.
sensory quality (Everitt, 2009). Based on the 9-point acceptability scores, 350 MPa for 5 and 10 min may be applied to scallops intended for SV cooking without negatively impacting their overall acceptability. Interestingly, results from the “just-about-right” questions indicated significant differences in firmness, tenderness, and juiciness between HPP treated and control samples (Table 3.6). A higher proportion of panelists rated the firmness of HPP treated samples as “JAR,” compared to the control scallops. For chewy/tender and dry/juicy attributes, the proportion of panelists rating treatments as “JAR” dropped from control to 350/5, and then 350/10. Although TPA hardness values were higher for the 350/10 SV cooked scallops and significant differences in texture among treatments were observed on the JAR questions, the consumer acceptability scores for texture on the 9-point scale were not affected. Similarly, despite significant differences in instrumental hardness values, HPP (200 MPa/3min or 350MPa/0min) bay scallops boiled for 90 s in vacuum bags demonstrated no differences in consumer acceptability (9-point hedonic scale) of flavor, color, chewiness and comprehensive impression of the treatments and cooked control (Yi et al., 2013). Sensory evaluation does not always corroborate with instrumental analysis, making consumer or trained panel evaluations crucial (de Oliveira et al., 2017), as the consumers are the end users of the product. Moreover, it is also important to recruit panelists from the target audience for the intended product, since panel composition can significantly affect hedonic results.
Table 3.6. Mean scores for consumer acceptability of sensory attributes on 5-point Just-About-Right (JAR) scale for SV cooked scallops and percent respondents who selected JAR for specific attributes.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Mean scores</th>
<th>Respondents selecting JAR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>350/5</td>
</tr>
<tr>
<td>Firm-Soft</td>
<td>3.43 ± 0.64a</td>
<td>2.81 ± 0.76b</td>
</tr>
<tr>
<td>Chewy-Tender</td>
<td>3.27 ± 0.71a</td>
<td>2.85 ± 0.71b</td>
</tr>
<tr>
<td>Dry-Juicy</td>
<td>3.09 ± 0.57a</td>
<td>2.85 ± 0.65b</td>
</tr>
</tbody>
</table>

Each value represents mean ± standard deviation (n=99). Different letters indicate significant differences (p<0.001) among treatments by one-way ANOVA.

Consumers were asked to select the word that they thought best described the scallop sample to help clarify the effects of pressurization on overall scallop meat sensory quality. A significantly higher percentage of people chose “chewy” (21-25 %) and “firm” (17-26 %) to describe HPP treated samples compared to the control (5 %), while a higher percentage of people characterized the control sample as “mushy” (Fig 3.5). Moreover, more panelists described the control and 350/5 samples as “soft” compared to 350/10, indicating that the 350/10 samples were perceived as firmer. However, when panelists were asked if the samples met their expectation of a scallop, no significant differences were found among treatments, with 82.8 %, 79.8 %, and 78.8 % of the panelists saying yes for the control, 350/5, and 350/10 samples, respectively.

Seafood texture is an important attribute to consumers, and over-processing may affect its flavor or texture negatively (Botta 1991, Kim 2014). These results indicate that significant differences in consumer ratings of texture on the JAR scale were not critically associated with overall liking of processed scallops, and that all of the treatments equally met consumer expectations. Moreover, a pressure of 350 MPa for 10 min, which may have higher potential to extend refrigerated shelf-life, could be applied to scallops without compromising consumer acceptance.
Fig 3.5. Spider map depicting average percent of panelists (n=99) selecting response as best word to describe the scallop samples.

3.4 Conclusions

HPP at 350 MPa for 10 min significantly modified instrumentally-measured textural attributes of SV cooked scallops, with increased hardness and chewiness compared to the control. However, despite the changes in physicochemical qualities and significant texture differences among treatments in firmness, chewiness and juiciness based on the JAR scale, overall consumer acceptability on a 9-point hedonic scale was not affected by the 5 and 10 min exposure times at a moderate pressure of 350 MPa. Pressurization resulted in increased L* values of the raw scallops, however, subsequent SV cooking masked the effects of HPP on color. HPP likely led to modified native protein structures as observed by increased salt soluble protein levels in pressurized scallops. These results indicate that the higher pressure, longer time treatment (350/10) may serve as an effective way to potentially increase refrigerated shelf-life of vacuum-packaged scallops, without affecting the palatability of SV cooked scallops. However, a
systematic refrigerated (<3.3 °C) shelf-life evaluation of HPP scallops is warranted to assess quality changes over time and to determine viability of the process to develop SV scallop products.
CHAPTER 4

QUALITY EVALUATION OF HIGH PRESSURE PROCESSED AND SUBSEQUENTLY
SOUS-VIDE COOKED SEA SCALLOPS DURING ICED STORAGE

4.1 Introduction

Sea scallops are highly perishable with a shelf-life of less than a week at chilled temperatures (Bremner & Statham, 1983). Freezing scallop meat has the potential to maintain biochemical quality for longer compared to chilled storage (Vidode Mattio et al., 2001; Goya et al., 2012). However, freezing and frozen storage may cause increased protein denaturation, negatively affecting texture and other physical parameters of seafood meat (Strasburg et al., 2008; Aubourg et al., 2013). Frozen scallops are often chemically treated with polyphosphates to maintain their moisture content during frozen storage (Manthey-Karl et al., 2015). However, the recent consumer interest in “chemical-free” clean label products has put such processing practices under scrutiny (Manthey-Karl et al., 2015). Moreover, thawing is a time intensive step, posing a challenge for chefs and at-home consumers.

Iced storage of sea scallops intended for sous-vide (SV) cooking can contribute to the expansion of high quality, value-added seafood products in the market but may require additional processing to achieve an adequate shelf-life for ample time to process, distribute and sell the products. Additionally, SV cooked sea scallops that are sold under refrigeration temperatures are a great way to deliver protein-dense seafood to consumers. High pressure processing (HPP) has been successful in refrigerated shelf-life extension of selected seafoods including salmon, cod, mackerel, tilapia, abalone and oysters without the need for additives (Campus, 2010; Rode & Hovda, 2016; Hughes et al., 2016; Suemitsu & Cristianini, 2019), and hence makes an excellent contender as a way to maintain quality of vacuum-packaged sea scallops during iced storage.
However, there is no comprehensive research assessing the effects of HPP on quality of vacuum-packaged raw or cooked sea scallops during iced storage.

Immediate effects of HPP conditions on physicochemical and sensory qualities of vacuum-packaged raw and SV processed scallops were presented in Chapter 3. Although 350 MPa for 10 min caused textural changes in SV cooked scallops, overall acceptability of these samples did not differ significantly from the unprocessed samples. Higher pressure treatment has the potential to reduce initial microbial load, and minimize spoilage in seafood during refrigerated storage compared to lower pressures (Rode & Hovda, 2016; Suemitsu & Cristianini, 2019). Based on these studies and the results from Chapter 3, the higher pressure (350 MPa) was chosen for the shelf-life evaluation. The objectives of this study were to assess the effects of pressurization (350 MPa) for 5 and 10 min on quality of raw and SV cooked sea scallops over 42 days of iced storage.

4.2 Materials and Methods

Quality analyses were conducted pre- and post-sous-vide cooking (65 °C for 10 min) on sea scallops processed at 350 MPa for 5 and 10 min, along with appropriate controls. Samples were packed in ice and stored in a refrigerator for 42 days. Microbiological analyses were conducted on day 1 after processing and then every 7 days (seven total analyses), whereas physicochemical analyses were conducted on day 1 and then biweekly (4 total analyses).

4.2.1 Sample Preparation and High Pressure Processing

Fresh, dry sea scallops (size 10-12) were sourced from Seatrade International Company Inc. (Topsfield, MA, USA) and were divided into equal groups according to the experimental design. Six scallops were vacuum packed (99%) for physicochemical analyses whereas one scallop was vacuum packed separately for microbiological analyses per bag in 3.3 mil plastic
bags (3.3 cm$^3$/100 in$^2$ oxygen transmission rate, 80 micron, 100 °C tolerance; Ultrasource, Kansas, MO, USA). On each testing day, 2 bags containing 12 scallops were used in total per treatment-replicate for physicochemical analyses. Samples were high pressure processed in a 55 L HPP unit (Hiperbaric, Miami, FL, USA). Water was used to achieve hydrostatic pressure and was maintained at 5 °C throughout processing. Once processed, plastic bags containing scallops were packed in ice in coolers, and then stored in walk-in refrigerator at temperatures between 2-3.3 °C until sous-vide processed or analyzed.

4.2.2 Sous-vide Processing

Samples were SV cooked using an immersion circulator (Sous-vide™ Professional Creative, PolyScience, Niles, IL) to bring the scallop core temperature to 65 °C for 10 min. Sample core temperature (in the cold spot of the bag) was monitored throughout cooking and cooling by inserting K-type thermocouples attached to a data logger with a foam tape to prevent leakage (RDXL4SD, Omega, Standford, CT, USA). Cooked samples were immediately cooled in ice-water slush (approximately 30 min) until the core temperature reached <2.7 °C. Chilled samples were packed in ice in a cooler and were placed in a walk-in refrigerator overnight.

4.2.3 Microbiological Analyses

Each testing day, scallop samples were manually mashed in their individual vacuum bags and a 25 g subsample was placed in a sterile stomacher bag with filter and sterile 0.1 % bactopeptone (BD Diagnostics, Sparks, MD) (1:10 w/v). The samples were mechanically mixed for 2 min using a BAGMixer 400 (Model P, SpiralBiotech, Advanced Instruments, Norwood, MA) and serial dilutions were prepared in 0.1 % bactopeptone. One mL aliquots of the serial dilutions were plated onto DeMann Rogosa Sharpe agar (MRS) (Alpha Biosciences, Baltimore, MD) and tryptic soy agar (TSA) to enumerate lactic acid bacteria (LAB) and total aerobic
microflora (APC), respectively. The MRS and TSA plates were incubated for 48 h at 30 °C and 37 °C, respectively. After incubation, plates with 30-300 colonies were counted and duplicate values were averaged. The dilutions were increased as necessary. All treatment-replicates were plated in duplicate and the counts were averaged and reported as Log colony forming units (CFU)/g.

4.2.4 Physicochemical Analyses

4.2.4.1 Total Volatile Base Nitrogen (TVBN)

Fifteen grams of ground scallops were homogenized with 30 mL 7.5% trichloroacetic acid (TCA) in a Waring blender (Eberbach Corporation, Ann Arbor, MI) for 30 s. The homogenate was centrifuged (Beckman Model J2-21, USA) for 20 min at 2,000 x g. The supernatant was stored at -20 °C until further analyses. Fifteen mL of thawed supernatant was added to a micro-Kjeldahl distillation unit (Rapid distillation unit, Labconco, Kansas City, MO). The blank was made with 20 mL TCA and 6 mL distilled water, and indicator dye was prepared by mixing 0.2% methyl red and 0.2% methylene blue in 2:1 in ethanol. Four mL of 10% sodium hydroxide solution were slowly added to the supernatant in the receiving flask. Samples were distilled into 15 mL of 4% boric acid solution containing 8 drops of indicator to a final volume of approximately 40 mL. The distillate was then titrated using 0.05 N hydrochloric acid until a constant purple color was obtained. The amount of TVBN (mg/100g of wet sample) was calculated as follows:

\[
\text{TVBN (mg/100g)} = \left(\frac{\text{volume (mL) HCl required for titrating sample} - \text{volume (mL) HCl used for titrating blank}}{\text{HCl normality}}\right) \times \text{molecular weight of N} \times \left(\frac{\text{volume of supernatant (mL)}}{\text{volume of supernatant used for distillation (mL)}}\right) \times \left(\frac{100}{\text{original weight (g) of sample}}\right)
\]
4.2.4.2 Biogenic Amines

Ground scallop (5 g) was homogenized in a Waring blender (Eberbach Corporation, Ann Arbor, MI) with 20 mL of 6% TCA for 30 s. The homogenate was centrifuged at 12,000 x g for 10 min at 4 °C and then filtered through Whatman #1 paper and brought to 50 mL with distilled water (Özogul et al., 2006). The supernatant was frozen at -20 °C until further analysis. Samples were thawed at 4 °C overnight prior to derivatizing using a AccQ•Tag ultra derivatization kit purchased from Waters Corporation (Milford, MA, USA). A standard cocktail was prepared using putrescine, cadaverine, histamine, agmatine and tyramine. A 10 μL aliquot of standard was added to the sample tube followed by 70 μL of AccQFluor borate buffer and then vortexed briefly. Twenty μL of reconstituted AccQFluor Reagent was added to the tube and then the sample tube was incubated in a heating block at 55 °C for 10 min. Using a Pasteur pipette, the sample tube contents were transferred to an autosampler vial limited volume insert.

Five μL aliquots of samples and standards were randomly injected on the HPLC (1100/1200, Agilent Technologies, Santa Clara, CA, USA). The mobile phase was Eluent provided in the kit (Waters Corporation, Milford, Massachusetts, USA). A Nucleosil C18 column was used to elute amines at a flow rate of 1.5 mL/min at ambient temperature. The fluorescence detector was set at 250 nm and ChemStation software (Agilent Technologies, Santa Clara, CA, USA) was used to calculate peak areas. Biogenic amines were reported as mg/100g.

4.2.4.3 Weight Loss

Weight loss due to HPP and SV processing during storage was measured by weighing scallops (n=6 per treatment-replicate) pre-processing and then on days 1, 14, 28 and 42. Percent (%) weight loss during storage was calculated by subtracting the weight (g) of six processed...
scallops from the weight of six unprocessed scallops, dividing by the weight of six unprocessed scallops, then multiplying by 100.

4.2.4.4 Texture Profile Analysis (TPA)

Following color analyses, whole scallops (n=10 per treatment-replicate), were cored using an apple corer to one inch in diameter and were placed vertically on the flat platform of a texture analyzer so that the muscle fibers were perpendicular to the platform (TA-XTi2, Texture Technologies Inc., Scarsdale, NY). A 2-inch cylindrical probe was used to compress samples by 70%, with 2 mm/sec pre, test and post-test speeds and a gap of 2 s between two cycles. Hardness (resistance to compression, Newton (N)), chewiness (resistance to elasticity, unitless), springiness (the ability of the meat to spring back after compression, unitless) and resilience (the immediate springiness of the meat as the probe is withdrawn between “bites,” unitless) (Bourne 2002) were recorded by the texture analyzer software (Exponent 32, version 5.0, 6.0, 2010, Texture Technologies Inc., Scarsdale, NY).

4.2.4.5 Instrumental Color

Color analyses were performed on whole scallops (n=10 per treatment-replicate) using a colorimeter (LabScan XE, Hunter Labs, Reston, VA, USA). On each testing day, the colorimeter was standardized using black and white tiles. Using a port size of 30.5 mm, area view of 25.4 mm, and illumination of 10° (D65, Hunter Labs, Reston, VA). The L*, a* and b* measurements were recorded using colorimeter software (Universal, version 4.10, 2001, Hunter Labs). The surface color of each scallop sample was evaluated three times by rotating 120° from the previous reading, and the three readings were averaged.
4.2.5. Statistical analysis

Data were analyzed using SPSS version 22 (IBM, New York, USA) for repeated-measures ANOVA (RMANOVA) at a significance level of $p<0.05$ to determine differences in treatments over time. One-way ANOVA was used at a significance level of $p<0.05$ to measure the differences among treatments on each testing day. Separation of treatment means was achieved by using Tukey’s honest significant difference (HSD) post hoc test.

4.3 Results and Discussion

4.3.1 Microbiological Quality

Lactic acid bacteria counts (LAB) increased significantly ($p<0.05$) over time for all the raw treatments, however at different rates for different treatments (Fig 4.1(a)). Although the aerobic plate counts also increased significantly over time for all the treatments, there were no differences among the three treatments overall (Appendix F). For raw seafood, the upper acceptable APC microbial limit is typically considered to be $10^6$ colony forming units (CFU)/g, with counts of $10^8$ CFU/g or higher resulting in sensory rejection (Gram & Huss, 1996). Increased microbial counts contribute to increased organoleptic changes caused by specific spoilage organisms, contributing to the loss of quality in seafood (Gram & Huss, 1996). By day 7, LAB counts for the raw control were significantly higher compared to the 5 and 10 min treatments, suggesting that the HPP delayed LAB growth. Moreover, by day 28, the control and 5 min treatment exceeded the upper microbial (APC) limit, whereas the 10 min treatment remained below the limit until day 35. These results suggest that a pressure of 350 MPa for 10 min extended the microbial shelf-life of raw sea scallops by 7 days under refrigeration, compared to scallops treated at 350 MPa for 5 min and the unprocessed control. In this study, the 10 min treatment resulted in lower LAB counts and longer microbial shelf-life compared to the 5 min
treatment at 350 MPa. On the contrary, Hughes et al. (2016) reported a microbial shelf-life of 35 days at 300 MPa, irrespective of 5 or 10 min pressure holding time, for raw abalone meat, in comparison to a 7-day microbial shelf-life of the unprocessed control under refrigeration.

In SV cooked scallops, LAB counts increased for each of the treatments over time (Fig 4.1(b)). By day 28, the LAB values for SV control samples had reached >7 log CFU/g and >6 log CFU for 350/5 treatment while the samples processed at 350 MPa for 10 mins remained below 3 log CFU/g. These results clearly indicate that pressurization at 350 MPa for 10 min successfully maintained lower LAB values than the other treatments for longer during iced storage. Bongiorno et al., (2018) reported a shorter, 21-day shelf-life for SV cooked (85 °C for 10 min in core) mussels stored at 3 °C. In comparison, in the current study, a 28-day shelf-life was observed for HPP-treated (350/10) SV cooked scallops even though the scallops were cooked at a much lower temperature (65 °C), demonstrating HPP’s potential in extending shelf-life of SV cooked scallops.
Fig 4.1 (a). Lactic acid bacteria counts of raw scallops over 42 days of iced storage. (b) Lactic acid bacteria counts of SV cooked scallops over 42 days of iced storage. Each value represents grouped mean ± standard deviation (n=3).
4.3.2 Total Volatile Base Nitrogen

TVBN values increased significantly ($p<0.05$) over time for all the raw treatments, but HPP significantly lowered the TVBN values compared to the control (Fig 4.2 (a)). TVBN measures the amount of volatile nitrogenous compounds including trimethylamine, ammonia and methylmercaptan, which may be produced by the bacteria present in the sample from nitrogen (Gram and Huss 1996). It has been used to track spoilage in seafood, and concentrations of 25-35 mg nitrogen (N)/100g are typically considered the upper limits for freshness in raw seafood (Hassoum & Karoui, 2017). For this study, samples with TVBN values >30 mg N/100g were considered to have lost substantial freshness and to be unacceptable for human consumption (Fatima & Qadri, 1985; Shamshad et al., 1990). The initial TVBN values for all the treatments were extremely low and could not be detected, and were reported as 0 mg N/100g. However, by day 14, the control samples exhibited a sharp increase in the TVBN values reaching over 83 mg N/100g whereas the HPP treated raw samples remained below the 30 mg N/100g upper limit. Similarly, TVBN values of unprocessed Pacific lion-paw scallops reached 30.7 mg N/100g) (Pacheco-Aguilar et al., 2008) while TVBN values for catarina scallops were reported to be ~21 mg N/100g muscle, both stored at 0 ºC for 15 days (Ocaño-Higuera et al., 2006), much lower than values observed for control sea scallops in this study potentially due to differences in species. On day 28, 350/5 samples exceeded the upper limit for TVBN with 33.5 mg N/100g whereas the 350/10 samples continued to be below the upper limit with 26.2 mg N/100g. In comparison to the less than 14-day shelf-life of the unprocessed scallops, HPP at 350 MPa for 10 min more than doubled the shelf-life of raw scallops, resulting in a shelf-life of 28 days in ice based on the TBVN analysis, which is in accordance with microbial evaluation.
Fig 4.2 (a). Total volatile base nitrogen concentrations of raw scallops over 42 days of iced storage. Each value represents mean ± standard deviation (n=3). Fig 4.2 (b). Total volatile base nitrogen concentrations of SV cooked scallops over 42 days of iced storage. Each value represents mean ± standard deviation (n=3).
For pressurized and SV processed scallops, TVBN values increased significantly with time, however, unlike the raw scallops, no treatment differences were observed in SV cooked scallops (Fig 4.2 (b)). The initial TVBN values were below the detection limit, however by day 14 the average TVBN values ranged between 18.9-23.2 mg N/100g. The control and the 350/5 treatment for the cooked samples reached the 30 mg N/100g limit for TVBN by day 42 whereas the 350/10 samples remained at ~21 mg N/100g. In contrast, TVBN values (13.9 mg N/100g) of in-shell mussels SV cooked at 85 ºC for 10 min and stored at 3 ºC remained constant for over 50 days (Bongiorno et al., 2018). Compared to the raw and SV-cooked controls, the HPP treated samples at 350 MPa for 10 min maintained acceptable TVBN values until day 42 under iced storage. Although the specific upper limit for TVBN is for raw seafood, it is a useful measure of microbial deterioration in cooked seafood as well.

4.3.3 Biogenic Amines

Biogenic amines are naturally occurring compounds at low levels in fresh seafood, but their concentration typically increases with increased spoilage over time due to microbial decomposition in post-mortem muscle tissue (Hassoun & Karoui, 2017). Biogenic amines increased significantly ($p<0.05$) during iced storage of raw samples, except for agmatine and tyramine (Appendix G1). Putrescine and cadaverine concentrations were highest among the tested biogenic amines in the raw scallops, with the control raw scallops having significantly higher levels compared to the HPP treated scallops (Fig 4.3 & 4.4). Similarly, Hughes et al. (2016) detected high levels of putrescine and cadaverine in unprocessed and HPP treated abalone at 100 MPa over 35 days of iced storage, but not in samples treated at 300 MPa. In this study, higher levels of putrescine and cadaverine were observed for 350/5 treatment in
Fig 4.3. Putrescine concentrations of raw scallops during iced storage. Each value represents mean ± standard deviation (n=3).

Fig 4.4. Cadaverine concentrations of raw scallops during iced storage. Each value represents mean ± standard deviation (n=3).
comparison to 350/10 treatments, although the differences were not significant. These biogenic amines results corroborate with microbial and TVBA data, with higher LAB counts and TVBN values found in the 350/5 treatment compared to 350/10 (Fig 4.1(a) & 4.2(a)). In contrast to the raw scallops, SV cooking tremendously prevented increases in all biogenic amine levels during storage (Appendix G2).

Histamine is a biogenic amine of particular concern due to its toxicological effects in humans, if consumed in high quantities (Hassoun & Karoui, 2017). The Fish and Fishery Products Hazards and Controls Guidance stipulates an upper limit for histamine as 50 mg/100g in seafood (FDA, 2011). Histamine concentrations of the raw samples increased significantly during iced storage, with the 350/10 treatment resulting in significantly lower values over time compared to the control (Fig 4.5). By the end of the study, the histamine level in the control samples was ~4 times higher than the 350/10 treatment. However, histamine concentrations remained below the 50 mg/100g threshold throughout storage for all the raw samples.

![Histamine concentrations of raw scallops during iced storage. Each value represents mean ± standard deviation (n=3).](image)

Fig 4.5 Histamine concentrations of raw scallops during iced storage. Each value represents mean ± standard deviation (n=3).
During refrigerated storage, proteins and peptides are broken down into free amino acids, which can be converted into biogenic amines due to microbial activity. Putrescine is produced from ornithine, a derivative of arginine, whereas cadaverine is produced from lysine. Mackie et al. (1997) reported arginine to be the most dominant amino acid in the scallop (*Pecten maximus*) adductor muscle stored in ice over 10 days, followed by lysine, potentially explaining the high levels of putrescine and cadaverine found in this study in the raw samples. However, agmatine, observed in much lower quantities in this study (Appendix G1), is derived directly from arginine. It is possible that a considerable amount of arginine was converted to ornithine, resulting in higher levels of putrescine compared to agmatine. Mackie et al. (1997) also found that free histidine present in the cooked scallops increased during iced storage, however, they did not detect any histamine during the course of the 10-day shelf-life.

Biogenic amines proved to be a useful indicator of quality during iced storage in this study, particularly for the raw samples. The maximum upper limit for total biogenic amine content in any food product is recommended to be 75-90 mg/100g (Ladero et al., 2010). By day 42, the total biogenic amine content of all treatments was below 75 mg/100g. However, the unprocessed raw treatment resulted in highest (65.9 mg/100g) the total biogenic amine content followed by the 350/5 treatment (11.9 mg/100g) and the 350/10 treatment (6.3 mg/100g). Based on these results, the 350/10 treatment could be applied to vacuum-packed scallops to keep biogenic amine levels to a minimum during iced storage.

**4.3.4 Weight Loss**

A significant \( p<0.05 \) increase in weight loss was observed for all the raw treatments during storage, but HPP for 5 and 10 min more than doubled the weight loss compared to the control (Fig 4.6 (a)). As reported in the physicochemical study (Chapter 3), there were no
Immediate effects of HPP on weight loss of raw scallops on day 1 and the values were <3.5%, similar to current results. However, by day 28, weight loss in raw HPP samples (~12%) was about 3 times higher compared to the non-HPP control (~5%). Reduced water holding capacity during iced storage of unprocessed lion-paw scallops was reported by Pacheco-Aguilar et al. (2008), who specified break down and aggregation of myofibrillar proteins as the possible major contributor to the increased water loss. Moreover, pressurization level of >200 MPa in seafood often causes compression of myofibrillar proteins (Pérez-Won et al., 2005; Yagiz et al., 2007; Gudbjornsdottir et al., 2010), which hold ~80% of water in the muscle tissue, possibly contributing to the increased water loss during storage in HPP-treated scallops.

Weight loss also increased significantly over time for the treatments of SV-cooked scallops, and the 350/5 treatment resulted in significantly higher weight loss compared to the cooked control and 350/10 treatment (Fig 4.6 (b)). By day 28, 18-23% weight loss was observed in SV cooked treatments, with no differences among treatments. Overall, similar to results in the physicochemical study (Chapter 3), cooking increased weight loss compared to the raw samples, as thermal treatment of seafood muscle promotes muscle coagulation resulting in increased water loss and toughening of the muscle tissue (Jantakoson et al., 2012; Botinestean et al., 2016).
Fig 4.6 (a) Weight loss of raw scallops over 42 days of iced storage. (b) Weight loss of SV cooked scallops over 42 days of iced storage. Each value represents grouped mean ± standard deviation (n=12). Values not sharing a letter are significantly different analyzed by RM-ANOVA, followed by Tukey’s HSD post hoc test.
4.3.5 TPA Hardness

Scallop texture is of great importance to its sensory quality, further confirmed by consumer responses in Chapter 2, in which panelists chose flavor and texture to be the most important attributes for sea scallops. Hence, it is crucial to evaluate textural changes in scallops during storage post processing. Hardness values of raw scallops did not change significantly over time for any of the treatments, but HPP significantly ($p<0.05$) increased the hardness of raw scallops (Fig 4.7 (a)). However, the initial values for hardness (15-21 N) did not differ significantly among the raw treatments, which corroborates the results found in the physicochemical study (Chapter 3), which evaluated immediate effects of HPP processing on raw scallops. Harder texture in seafood muscle due to pressures $>300$ MPa has been previously reported in shrimp (Kaur et al., 2016), rainbow trout and mahi mahi (Yagiz et al., 2007). The increase in hardness observed in HPP treated scallops, in comparison to the control could potentially be due to structural changes induced in the native proteins of scallops during HPP. Myofibrillar proteins, in particular myosin, begin to denature at pressures $>100$ MPa, reducing their ability to hold water in muscle resulting in loss of cellular liquid and tougher meat (Angsupanich & Ledward, 1998; Qiu et al., 2014; de Oliveira et al., 2017). Similarly, in this study, HPP at 350 MPa for 5 and 10 min significantly increased weight loss and hardness of raw scallops.
Fig 4.7 (a). Average hardness of raw scallops during iced storage. Each value represents grouped mean ± standard deviation (n=12). Values not sharing a letter are significantly different analyzed by RM-ANOVA, followed by Tukey’s HSD post hoc test.

(b) Hardness of SV cooked scallops over 42 days of iced storage. Each value represents mean ± standard deviation (n=3).
In contrast to the raw samples, hardness of SV cooked scallops increased significantly over time, but there were no significant differences among treatments (Fig 4.7 (b)). These results demonstrate again that SV cooking masks the textural changes due to HPP in raw scallops, suggesting that HPP can be applied as a pre-treatment to SV cooked sea scallops without harming their texture. Thermal processing of sea scallops cooked (25-80 ºC) at atmospheric conditions has been reported to increase toughness with increase in temperature (Findlay & Stanley, 1984), however there have been no reports on textural changes in SV cooked sea scallops during iced storage.

4.3.6 Instrumental Color

L* values significantly ($p<0.05$) changed over time for all the raw treatments, but no differences were found in the a* and b* values for any of the raw scallops over time (Table 4.1). However, HPP for 5 and 10 mins significantly increased the L* and a* values whereas HPP for 10 mins significantly decreased the b* values in comparison to the 5 min treatment based on two-way ANOVA. An increase in L* value, i.e. making the appearance lighter, is a common effect of HPP in seafood muscle including scallops (Pérez-Won et al., 2005; Yi et al, 2013; Hughes et al., 2015). In this study, L* values decreased over time for HPP treatments whereas a decrease and then slight significant increase toward the end of the study was observed for the control samples. HPP treated scallops became less light during iced storage, however, their L* values at the end of storage were still significantly higher than those of the control scallops. Increasing L* values indicate whitening of scallops, which may be desirable to the consumers (Weiqing et al., 2011).
Table 4.1. Instrumental color of raw and SV cooked scallops during iced storage.

<table>
<thead>
<tr>
<th>Days</th>
<th>Raw</th>
<th>Cooked</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>350/5</td>
</tr>
<tr>
<td>1</td>
<td>70.0±0.5bA</td>
<td>76.6±1.8bB</td>
</tr>
<tr>
<td>14</td>
<td>67.0±0.2aA</td>
<td>72.4±0.7aB</td>
</tr>
<tr>
<td>28</td>
<td>67.0±1.0aA</td>
<td>74.0±0.7abB</td>
</tr>
<tr>
<td>42</td>
<td>68.8±0.3bA</td>
<td>73.3±1.6abB</td>
</tr>
</tbody>
</table>

Each value is the mean ± standard deviation (n=3). Values not sharing a lowercase letter are significantly different within columns and values not sharing an uppercase letter are significantly different within rows for product form (raw, cooked), analyzed by ANOVA, followed by Tukey’s HSD post hoc test.

The color (L*, a* and b*) of SV-cooked scallops did not change significantly over time, but similar to the raw samples, L* values of HPP samples were higher than the control (Table 4.1). Similarly, Yi et al. (2013) reported higher L* values of HPP treated (200 and 350 MPa), cooked bay scallops (100 °C for 90 s) compared to the control. HPP often results in whitening of raw fish muscle, resulting in a cooked appearance (Chevalier et al., 2001; Yagiz et al., 2007; de Oliveira 2017), contributing to the higher L* values observed in the HPP treated, SV cooked scallops. Although increased whiteness is considered a defect in red muscle, HPP enhances the inherent off-white color of the scallops, potentially making them more appealing to the
consumers. There were no significant differences among treatments in the a* and b* values of the cooked samples.

4.4 Conclusions

Microbial and chemical quality evaluations clearly indicated that high pressure processing at 350 MPa for 10 min significantly extended the shelf-life of raw and sous-vide cooked sea scallops compared to the 350 MPa for 5 min treatment and control during iced storage. However, physical parameters of raw scallops were affected by HPP, with weight loss exhibiting a sharp increase in the HP treated scallops versus the unprocessed control. Interestingly, scallops processed at 350 MPa for 10 min and then SV cooked had similar weight loss and hardness as the SV cooked control. Based on these results and on industry guidelines, scallops HP treated at 350 MPa for 10 min had a 28-day and 35-day shelf-life during iced storage for raw and sous-vide cooked scallops, respectively. As reported in Chapter 2, SV cooked scallops pre-treated at 350 MPa for 10 mins received similar consumer acceptability scores compared to the control. Together, these studies confirm that HPP at 350 MPa for 10 min can successfully maintain the quality of raw and sous-vide cooked scallops for longer during iced storage compared to the other treatments, benefiting the development of sous-vide cooked scallop market and providing consumers with high-quality products.
CHAPTER 5
EFFECTS OF MODERATE PRESSURES ON MYOFIBRILLAR AND SARCOPLASMIC PROTEINS OF SEA SCALLOPS

*(Placopecten magellanicus)*

5.1 Introduction

High pressure processing (HPP) is an emerging, non-thermal technology and the application of HPP to high value seafood products continues to grow in the food industry. HPP has been applied to seafood products for shucking, inactivation of microorganisms and enzymes, and shelf-life extension, with minimal impacts on flavor, appearance and nutritional quality of foods (Yi et al., 2013; Teixeira et al., 2014; Hughes et al., 2016; de Oliveira et al., 2017; Martínez et al., 2017). However, several studies have confirmed conformational changes in seafood proteins due to HPP, reporting changes in the resulting meat texture (Yagiz et al., 2007; Kaur et al., 2016; Martínez et al., 2017).

Myofibrillar proteins, which comprise 75-95% of muscle proteins, hold up to 80% of the water in the muscle tissue and contribute substantially to the meat texture. HPP-induced changes in myofibrillar protein structure have been reported in several seafood species including rainbow trout (Günlü et al., 2014), silver carp (Qiu et al., 2014), red swamp crayfish (Shao et al., 2018), blue crab (Martinez et al., 2017) and black tiger shrimp (Kaur et al., 2016). Sarcoplasmic proteins present in muscle tissue include many proteolytic enzymes that hydrolyze proteins post-mortem, causing tissue softening and consequently contributing to quality loss and limited shelf-life (Lakshamanan et al., 2005; Teixeira et al., 2013). Moderate pressurization (100-200 MPa) of seafood muscle has been shown to increase proteolytic enzyme activity, while higher pressures (>400 MPa) decreased enzymatic activity in Atlantic cod, cold-smoked salmon and sea bass.
myofibrillar and sarcoplasmic proteins play a significant role in textural quality of meat post-processing and during storage. Limited reports on effects of HPP on shucking of mollusks have discussed changes in protein and texture post processing (Cruz-Romero et al., 2007; Gupta et al., 2015), but focused more on physical changes including color and texture. To the best of our knowledge, there have been no studies reporting effects of HPP on myofibrillar and sarcoplasmic proteins of sea scallops.

HPP has already shown promising results in shelf-life extension of selected seafoods including mollusks such as oysters and abalone (Campus, 2010; Hughes et al., 2016). Sea scallops are an economically important fishery in the U.S., and are particularly enjoyed by consumers for their tender and succulent adductor muscle. Scallops are rich in protein and low in calories, with low lipid and carbohydrate content (Naidu & Botta, 1978), with a shelf-life of <7 days for fresh scallops under refrigeration. HPP successfully extended the shelf life of raw and sous-vide (SV) cooked scallops in comparison to the non-HPP controls (Chapter 4). However, pressurization modified scallop texture of raw scallops, resulting in harder scallops with higher weight loss during iced storage (Chapter 4). Moreover, the physicochemical study (Chapter 3) found that HP-processing at 350 MPa for 10 min resulted in significantly harder SV cooked scallops. Further investigation on structural changes at the protein level in response to HPP will provide deeper insight on the textural changes observed in HP-treated scallops using instrumental techniques in the previous studies. The objectives of this study were to evaluate the effects of moderate pressures and holding times on thermal and biochemical properties of myofibrillar and sarcoplasmic proteins of sea scallops.
5.2 Materials & Methods

5.2.1 Experimental Design

This study had a 2x2 factorial design. Treatments included 150 and 350 MPa pressurization for 5 and 10 min, with an unprocessed control, for a total of five treatments. Already shucked dry sea scallops (size 10-12) were purchased from Seatrade International Inc. (Bristol, Maine, USA), and six scallops were vacuum-packed (99% vacuum) in individual 3.3 mil boil-in-bags (3.3 cm³/100 in² oxygen transmission rate, 80 micron, 100 °C tolerance; Ultrasource, Kansas City, MO, USA) per treatment-replicate. Triplicate batches of samples were high pressure processed in a 55 L HPP unit (Hiperbaric, Miami, FL, USA) at 150 or 350 MPa for 5 or 10 min. Water was used to achieve hydrostatic pressure and was maintained at 5 °C throughout processing.

5.2.2 Preparation of Sarcoplasmic and Myofibrillar Protein Extracts

The protein fractions were extracted following the method of Lv et al. (2018) with some modifications. Three scallops per treatment-replicate were ground for 15 s in a chopper (Model FPRVMC3002, Rival, Boca Raton, FL). Ground scallop meat (10 g) was homogenized with 90 mL of cold Tris-maleate buffer (20 mM, in 0.05 M KCl, pH 7) for 30 s in a Waring blender (Eberbach Corporation, Ann Arbor, MI). The homogenate was centrifuged (Beckman Model J2-21, USA) at 15,000 xg for 10 min at 4 °C, and the resulting supernatant was collected as the sarcoplasmic protein extract. The pellet was suspended and homogenized with high ionic strength Tris-maleate buffer (20 mM in 0.6 M KCl, pH 7) for 15 s and then extracted for 1 h at 4 °C on an orbital shaker. The homogenate was centrifuged again at 10,000 xg for 10 min at 4 °C, and the supernatant was collected as the myofibrillar protein extract.
5.2.3 Soluble Protein Content

Protein content of the extracts was determined as described by Lowry et al. (1951), using bovine serum albumin as the standard. Water was used as a blank. Sample absorbance was measured spectrophotometrically (Beckman Du 530, Brea, CA) at 700 nm and the soluble protein concentration was expressed as mg of protein/g of meat.

5.2.4 SDS-PAGE

SDS-PAGE was performed according to Laemmli (1970). Sarcoplasmic and myofibrillar extracts were adjusted with extraction buffers to 660 µg/mL and 500 µg/mL protein concentration, respectively. Diluted extracts were mixed with Laemmli buffer (1:1, v/v), vortexed and heated at 95 °C for 5 min in a water bath. Samples were then centrifuged at 10,000 xg for 5 min. Subsequently, 20 µL aliquots of sample supernatants and 10 µL of a molecular weight reference ladder (10-250 kDa) (Precision Plus Protein™, Dual Color Standards, Bio-Rad, Hercules, CA) were loaded into individual wells of a 10% polyacrylamide gel (Bio-Rad, Hercules, CA). Gels were run at 80 V for 20 min and then at 120 V for 40 min in a Mini-PROTEAN 3 Cell (Bio-Rad, Hercules, CA), then fixed with a solution containing 50 % methanol and 7 % glacial acetic acid for 15 min, following a wash step with ultrapure water where water was replaced every 15 min for 45 minutes. Gels were stained overnight using GelCode Blue Stain Reagent (Thermo Scientific, Waltham, MA) and then destained by rinsing with ultrapure water several times for 1-2 hrs. Images of the gels were captured using a digital single-lens camera (Canon EOS 550D, Tokyo, Japan).
5.2.5 Digital Scanning Calorimetry (DSC)

Thermal characteristics of scallop meat for all the treatments were analyzed by DSC. Scallop meat (7-12 mg) from the core of the adductor 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 from 5 to 105 °C at a rising rate of 2 °C/min in a differential scanning calorimeter (DSC Q2000, TA instruments, New Castle, DE). Thermograms, peak temperatures and change in enthalpy (J/g), measured as area under the curve, were extracted using Universal Analysis 2000 software (v5.5.24, TA instruments, New Castle, DE).

5.2.6 Ca$^{2+}$ATPase Activity

The Ca$^{2+}$ATPase activity of myofibrillar extracts was determined according to Lv et al. (2018), with slight modifications. Briefly, a reaction solution containing 0.25 mL of 0.5 M Tris-maleate buffer (pH 7.0), 0.5 mL of 1 M KCl, 0.25 mL of 0.1 M CaCl$_2$ and 1.75 mL distilled water was incubated in a water bath at 25 °C for 10 min. A 2 mL aliquot of myofibrillar extract along with 0.25 mL of adenosine triphosphate (ATP) solution (20 mM, pH 7.0) was then added to the reaction solution and vortexed briefly. The resulting solution was incubated in a water bath at 25 °C for 10 min. The reaction was stopped by addition of 2.5 mL of 15 % trichloroacetic acid (TCA) solution, and the mixture was centrifuged at 3000 xg for 10 min. One mL of supernatant was mixed with 3 mL of 0.66 % ammonium molybdate solution in 0.75 M sulfuric acid to make a phosphomolybdate complex. A 0.5 mL aliquot of freshly prepared 10 % FeSO$_4$ in 0.15 M sulfuric acid was then added to the complex. After an incubation of 2 min at room temperature, the absorbance was measured spectrophotometrically (Beckman Du 530, Brea, CA) at 700 nm to
determine the Ca$^{2+}$ATPase activity based on liberation of inorganic phosphate. A standard using 0.02 M NaH$_2$PO$_4$ solution was prepared and the results were expressed as µmol/min/mg protein.

5.2.7 Cathepsin D Activity

Cathepsin D activity of crude enzyme extracts of scallop meat was determined as described by Texeira et al. (2013) and Ge et al. (2016) with slight modifications. Ground scallop meat (10 g) was homogenized in a Waring blender with distilled water (40 mL) (1:4, w/v) for 45 s, and the homogenate was shaken on an orbital shaker at 4 °C for 30 min. Samples were centrifuged (Beckman Model J2-21, USA) at 15,000 xg for 20 min, and the supernatants were frozen until further analysis. To measure the cathepsin D activity, the crude enzyme extract (300 µL) was mixed with substrate solution (450 µL) (2 % denatured hemoglobin from bovine blood in 0.1 M citrate buffer, pH 3.1) and incubated at 37 °C for 2 h. The reaction was stopped with 450 µL of 15% TCA, vortexed for 10 s, and centrifuged at 20,000 xg for 5 min. For the sample blanks, the TCA solution was added immediately after the addition of enzyme extract. Absorbance of the supernatants was measured at 280 nm spectrophotometrically (Beckman Du 530, Brea, CA) and cathepsin D activity was expressed as AU/hour/mg protein.

5.2.8 Statistical Analysis

Data were analyzed using SPSS version 22 (IBM, New York, USA) for analysis of variance (ANOVA) at a significance level of $p<0.05$. Separation of treatment means was achieved by using Tukey’s honest significant difference (HSD) post hoc test.
5.3 Results & Discussion

5.3.1 Protein Solubility

Contrasting effects of HPP were observed in the protein concentrations of sarcoplasmic and myofibrillar extracts of sea scallops. Protein content of the sarcoplasmic extracts increased significantly ($p<0.05$) with higher pressure level but decreased significantly in myofibrillar extracts whereas pressurization time had no effect on protein solubility (Table 5.1). In contrast, sarcoplasmic proteins in finfish including salmon, sea bass and hake muscle showed decreased solubility post pressurization (100-600 MPa) (Ortea et al., 2010; Teixeira et al., 2013; Villamonte et al., 2016). However, several studies have reported increases in proteolytic enzyme activity due to lysosomal rupture in seafood muscle as a result of moderate pressures (<400 MPa) (Angsupanich & Ledward, 1998; Gou et al., 2010; Teixiera et al., 2013), which may explain the increase in sarcoplasmic protein concentration observed in this study, as enzymes belong to sarcoplasmic proteins. In general, myofibrillar proteins are less resistant to pressure treatment in comparison to sarcoplasmic proteins (Jantakoson et al., 2012; de Oliveira et al., 2017). Several authors have reported pressure induced denaturation in myofibrillar proteins (myosin and actin) of seafood (Zhang et al., 2015; de Oliveira et al., 2017), leading to decreased protein solubility. Variable degree of myosin and actin denaturation has been reported with increase in pressure level from 100 to 600 MPa in crustaceans including blue crab (Martínez et al., 2017) and red swap crayfish (Shao et al., 2018). In the current study, myofibrillar protein solubility decreased in the 150 and 350 MPa treatments, dropping by ~57 and 86 % compared to the control, respectively, suggesting that the myofibrillar proteins in sea scallops were at least partially modified due to HPP. Conformational changes in sarcoplasmic and myofibrillar proteins have been previously shown to modify muscle texture in seafood, and potentially
explain textural changes in sea scallops observed due to HPP discussed in previous chapters (Chapter 3 & 4).

Table 5.1. Protein concentration of sarcoplasmic and myofibrillar extracts of sea scallops.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sarcoplasmic extract</th>
<th>Myofibrillar extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>13.0 ± 1.7a</td>
<td>61.0 ±14.6c</td>
</tr>
<tr>
<td>150/5</td>
<td>17.9 ± 1.2b</td>
<td>26.0 ± 3.8b</td>
</tr>
<tr>
<td>150/10</td>
<td>18.6 ± 0.5b</td>
<td>26.2 ± 1.0b</td>
</tr>
<tr>
<td>350/5</td>
<td>22.6 ± 1.1c</td>
<td>8.3 ± 0.8ab</td>
</tr>
<tr>
<td>350/10</td>
<td>21.7 ± 0.5c</td>
<td>7.7 ± 1.4a</td>
</tr>
</tbody>
</table>

Each value represents mean ± standard deviation (n=3). Values not sharing a letter within columns are significantly different (p<0.05) by ANOVA, followed by Tukey’s HSD test.

5.3.2 SDS-PAGE

SDS-PAGE showed the impact of HPP on sarcoplasmic and myofibrillar proteins, revealing different protein profiles between the control and HPP treated scallops (Fig 5.1(a) & (b)). More sarcoplasmic proteins were extracted as pressure level increased (Table 5.1), as observed by the increasing intensity of protein bands at around 40 kDa, with increasing pressure, as compared to the control (Fig 5.1(a)). Similarly, higher intensities of certain bands of sarcoplasmic protein extracts from HPP treated sea bass and squid, including bands at ~40 kDa, were reported by Teixeira et al. (2013) and Gou et al. (2010), respectively. However, the intensity of the band observed in the control scallop sample around 100 kDa dropped in the 150 MPa treatments and completely disappeared in the 350 MPa treatments. These results indicate that HPP likely either increased proteolytic activity or decreased quaternary structure of sarcoplasmic proteins, which exhibited lower molecular weight profiles.

The myofibrillar protein extract showed distinct bands around 240, 100, 45 and 37 kDa, representing myosin heavy chain (MHC), paramyosin, actin, and tropomyosin, respectively (Shao et al., 2018) (Fig 5.1(b)). In comparison to the control, the MHC band lost intensity as the
pressure level increased, while the intensity of the lower molecular weight band at ~37 KDa increased for 150 MPa scallops. Similar results were observed in crab meat proteins and rainbow trout subjected to HPP, whereby an increase in pressure decreased MHC band intensity and increased intensity of lower molecular weight bands (Günlü et al. 2014; Martínez et al., 2017). These results suggest that HPP promoted loss of native quaternary structure of MHC, consequently increasing lower molecular weight proteins. Protein bands at ~45 kDa, generally associated with actin, were observed in all the treatments and the control. However, the band intensity decreased greatly in the 350 MPa treatments, in comparison to the control. These SDS-PAGE results clearly demonstrate the effects of HPP on myosin and actin, the two major myofibrillar proteins, and confirm that pressure level modified the native structure of scallop myofibrillar proteins and likely contributed to toughening of HPP treated scallops.

![Fig 5.1. SDS-PAGE of sarcoplasmic (a) and myofibrillar (b) protein extracts. STD=MW ladder C: control; 150/5: HPP at 150 MPa for 5 min, 150/10: HPP at 150 MPa for 10 min; 350/5: HPP at 350 MPa for 10 min; 350/10: HPP at 350 MPa for 10 min.](image)
5.3.3 Differential Scanning Calorimetry

Thermal characteristics of sea scallop meat were examined by DSC and the representative thermograms depict endothermic peaks for the different treatments and control (Fig 5.2). In these samples, the endothermic peaks indicate energy absorption for protein unfolding during denaturation. Two transition peaks were observed at around 45 °C and 71 °C in unprocessed sea scallop meat, representing myosin and actin denaturation, respectively. These temperatures for myosin and actin denaturation are similar to several other reports on seafood muscle thermal properties including cod, turbot and crab meat (Angsupanich & Ledward, 1998; Chevalier et al., 2001; Martínez et al., 2017). The heat flow for 150 Ma samples was below the control and the other treatments (Fig 5.2). Pressurization significantly (p<0.05) decreased change in enthalpy compared to the control, indicative of partial denaturation of scallop proteins due to HPP (Table 5.2). Moreover, differences in myosin and actin peak depths for different treatments can be clearly seen in the thermogram, indicating that pressurization affected myosin and actin conformation of scallop meat. Myosin peaks reduced in height in the 150 and 350 MPa treatments, suggesting partial denaturation of scallop myosin due to pressurization. Interestingly,
no peaks were registered for actin in the 350 MPa treatments, indicating that actin was fully denatured at 350 but not at 150 MPa (Table 5.2). Although actin is generally considered more resistant to pressure (de Oliveira et al., 2017), crab meat HPP treated at 300 also showed no endothermic peaks for actin via DSC (Martínez et al., 2017). These results suggest that shellfish actin, in particular scallop actin, may be more sensitive to pressures above 300 MPa as compared to myosin.

Table 5.2. Effects of HPP parameters on thermal characteristics of sea scallops

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No of peaks</th>
<th>T_{p1} (ºC)</th>
<th>T_{p2} (ºC)</th>
<th>ΔH_T (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>2</td>
<td>44.5 ± 0.5b</td>
<td>69.4 ± 2.0a</td>
<td>0.81 ± 0.20b</td>
</tr>
<tr>
<td>150/5</td>
<td>2</td>
<td>39.3 ± 0.2a</td>
<td>71.4 ± 0.8a</td>
<td>0.35 ± 0.18a</td>
</tr>
<tr>
<td>150/10</td>
<td>2</td>
<td>40.0 ± 2.4ab</td>
<td>71.8 ± 0.2a</td>
<td>0.32 ± 0.10a</td>
</tr>
<tr>
<td>350/5</td>
<td>1</td>
<td>42.5 ± 0.8b</td>
<td>-</td>
<td>0.16 ± 0.03a</td>
</tr>
<tr>
<td>350/10</td>
<td>1</td>
<td>42.9 ± 0.5b</td>
<td>-</td>
<td>0.10 ± 0.01a</td>
</tr>
</tbody>
</table>

T_{p1}, T_{p2}, and ΔH_T indicate temperature of first peak, temperature of second peak and total denaturation enthalpy of sea scallops, respectively. Each value represents mean ± standard deviation (n=3). Values not sharing a letter within column are significantly different (p<0.05) by ANOVA, followed by Tukey’s HSD test.
5.3.4 Ca\textsuperscript{2+} ATPase Activity

HPP significantly ($p<0.05$) decreased the Ca\textsuperscript{2+} ATPase activity of the scallop myofibrillar extracts, with the 350 MPa treatments reducing the activity by around 60% in comparison to the control (Fig 5.3). Moreover, 350 MPa treatments also significantly reduced the Ca\textsuperscript{2+} ATPase activity compared to the 150 MPa treatments. Similarly, decreased Ca\textsuperscript{2+} ATPase activity was observed as pressure level increased from 100 to 200 MPa in silver pomfret during pressure assisted thawing, with an ~81% decrease at 200 MPa (Cui et al., 2019). Moreover, Ko et al. (2003) also reported a sharp decline in Ca\textsuperscript{2+} ATPase activity of tilapia treated at >100 MPa, with only 21% activity remaining for samples treated at 200 MPa for 10 min. Myosin Ca\textsuperscript{2+} ATPase activity is considered a good index to evaluate protein denaturation in muscle foods, with the loss of activity indicating denaturation of the myosin head (Ochiai & Chow, 2000; Cao et al., 2015). The loss of Ca\textsuperscript{2+} ATPase activity observed further confirms the decrease in soluble myofibrillar content in response to HPP (Table 5.1). The reduced Ca\textsuperscript{2+} ATPase in HPP treated scallops shows that HPP generated conformational changes in the myosin head, with the 350 MPa treatments exhibiting more pronounced effects compared to the 150 MPa treatments.
5.3.5 Cathepsin D Activity

Cathepsin D activity decreased significantly \((p<0.05)\) for all the HPP treatments compared to the control (Fig 5.4). However, no effects of pressure and time were observed among the treatments. Similarly, Teixeira et al. (2013) reported decrease in cathepsin D activity of sea bass at 100 and 400 MPa but a slight increase at 250 MPa. The authors further explained that although at low pressure (100 MPa) cathepsin D activity could be inactivated, the higher cathepsin D activity observed in sea bass at 250 MPa may be due to lysosomal rupture and subsequent release of enzymes. However, at 400 MPa, inactivation of cathepsin D predominated due to the effects of high pressure in seabass (Teixeira et al., 2013). Cathepsins are a group of proteolytic enzymes that hydrolyze proteins in muscle foods post-mortem and thus contribute to muscle softening (Godiksen et al., 2009; Teixeira et al., 2013). Cathepsin D activity is of particular importance in post-mortem changes of muscle foods as it is known to tenderize meat.
over time, but ultimately causes extensive proteolysis due to the absence of a specific inhibitor in the fish muscle (Chéret et al., 2005; Buckow et al., 2010; Teixeira et al., 2013). Moderate pressures (100-200 MPa) have been shown to increase proteolytic activity of endogenous enzymes in fish muscle (Angsupanich & Ledward, 1998; Lakshamanan et al., 2005). Cathepsin D activity decreased due to pressure treatment in this study, possibly limiting muscle softening of scallops post HPP treatment and contributing to harder texture of HPP treated scallops observed in previous studies (Chapter 3 & 4).

Fig 5.4. Cathepsin D activity of scallop sarcoplasmic extract by treatment. Each value represents a mean ± standard deviation (n=3). Values not sharing a letter are significantly (p<0.05) different, analyzed by one-way ANOVA followed by Tukey’s HSD post hoc test.

5.4 Conclusions

The HPP parameters (pressure level and holding time) used in this study changed the thermal and biochemical properties of sarcoplasmic and myofibrillar proteins of sea scallops. The increase in sarcoplasmic and decrease in myofibrillar proteins extracted indicate that the pressurization had different effects native protein structures. DSC, SDS-PAGE and Ca²⁺-ATPase analyses further confirmed the graded denaturation and change in molecular weights of myosin
and actin in response to pressure level. Cathepsin D activity of the sarcoplasmic extracts decreased significantly for all the HPP treatments, indicating that pressure inactivated cathepsin D in sea scallop meat. Myofibrillar proteins are key to muscle texture, and we hypothesized that changes in their conformation due to HPP affected the sea scallop texture as observed in Chapter 3 and 4. On the other hand, proteolytic sarcoplasmic proteins can cause softening of seafood muscle post-mortem. The changes in sarcoplasmic and myofibrillar proteins in response to HPP observed in this study help explain some of the textural changes observed in sous-vide cooked HPP scallops (Chapter 3) and in raw HPP scallops during iced storage (Chapter 4), including increased toughening and weight loss. This study provided a deeper understanding of structural changes in myofibrillar and sarcoplasmic proteins due to moderate pressures and holding times, and corroborated the physical changes observed in sea scallop texture in previous studies.
CHAPTER 6

OVERALL CONCLUSIONS

This series of studies confirms that high pressure processing (HPP) is an effective tool in the development of refrigeration-stable sous-vide processed sea scallops for the seafood products market. Sous-vide offers a unique method of cooking vacuum-packaged foods in a water bath delivering uniform heating during processing. This cooking method offers better control over texture and doneness, which are crucial attributes of muscle foods. Already vacuum-packaged and HPP treated raw scallops may make an excellent product for retail, restaurant or other commercial food preparation facilities, potentially delivering extended iced shelf-life and ease for on-site sous-vide cooking. Moreover, using HPP as a pre-treatment for sous-vide cooked scallops could promote the development of convenient ready-to-eat seafood products that only need to be heated prior to eating.

The first study showed that moderate pressures (150-350 MPa) and holding times (5-10 min) did not have a significant impact on physicochemical qualities of raw scallops, except for salt soluble protein and color. L* values of pressurized raw scallops increased in comparison to the control, which may prove to be a desirable effect for consumers seeking scallops that are whiter in appearance. However, the effects of pressurizing scallops at 350 MPa for 10 mins were subsequently observed in the sous-vide cooked scallops, which had a harder and chewier texture compared to the control. Despite the textural differences revealed by instrumental analysis, consumer ratings for overall acceptability of 350MPa/10 min scallops did not differ from the control or 350MPa/5 min sous-vide cooked scallops. Consequently, a pressure of 350 MPa for 10 min can be applied to scallops prior to sous-vide processes, with minimal impact on their “eating” quality. It is important to note that the panelists used for the consumer testing enjoyed
consuming seafood, but may not have been the ideal target audience for premium scallop products, since many were relatively low income college students. In future sensory evaluations, thorough screening of panelists may provide more meaningful data. In this study, the objective of the consumer testing was to determine the differences in acceptability scores among treatments, hence the sous-vide cooked scallops were only reheated prior to serving. However, the overall scores would likely have been higher if the scallops had been flash-seared to develop a crust, making the end product closer to how consumers typically consume scallops.

Pressurization at 350 MPa for 10 min successfully extended the iced shelf-life of raw and subsequently sous-vide cooked scallops to 28 and 35 days, respectively. The significant extension of an otherwise <7-day shelf-life of fresh scallops in ice due to HPP could prove advantageous to seafood processing facilities, by increasing the distribution and sale window for sea scallops. However, pressurization increased the weight loss during iced storage compared to the control, negatively affecting the sea scallop quality. This negative effect may prove to be detrimental to the sales and revenue as scallops are sold by weight and their moisture content is an important parameter for their standard of identity. Hence, it is crucial to assess the economic benefits of extended shelf life versus increased weight loss due to HPP. On the other hand, processing at 350 MPa for 10 min caused minimal impact on quality of sous-vide cooked scallops during iced storage, indicating that cooking masked the previously observed effects of HPP in the raw samples. In this study, more rapid deterioration was observed in the raw treatments compared to the cooked scallops. In future research, it may be more valuable to assess quality changes, in particular microbial and biochemical changes, in raw treatments weekly rather than biweekly. Microbial and physicochemical evaluations provided substantial information on quality changes during storage, but sensory assessment by a trained panel is
recommended in future studies to further confirm results obtained from laboratory based techniques and to more clearly define high quality shelf life. In this study scallops were packed in ice and placed in a refrigerator to maintain quality; however the use of time-temperature indicators would have provided real-time information about any temperature fluctuations during storage. Moreover, a shelf life study mimicking the refrigerated storage temperatures commonly used in grocery stores could provide useful information for HPP, sous-vide scallops intended for retail.

The effects of HPP on sea scallop proteins have not been reported previously and studies evaluating effects of HPP on molluscan proteins were limited to myofibrillar proteins, with scarce attention to sarcoplasmic proteins. Sea scallops are high in protein, which also contributes to their juicy and tender texture. Given the significant impacts of HPP on sea scallop texture, an investigation of modifications in native protein structures in response to HPP conditions was warranted. HPP at moderate pressure modified the native myofibrillar and sarcoplasmic structures in raw scallops, corroborating the physical changes we observed in previous studies. Changes in soluble protein content due to HPP were further confirmed by changes in intensities of various protein bands as observed via SDS-PAGE of sarcoplasmic and myofibrillar proteins. DSC thermograms showed the disappearance of the actin peak in the 350 MPa samples but not in the 150 MPa treatments, indicating that HPP impacted myofibrillar proteins differently in response to pressure level but not pressurization time. Evaluating the changes in muscle fibers in response to HPP using scanning electron microscopy (SEM), which was not performed in this study, is recommended to observe visual changes at the cellular level. SEM could visually show how muscle cell structure is modified due to HPP, and further explain the physical changes observed in Chapter 3 and 4.
HPP is a novel preservation technique, and the moderate pressure parameters used in this study delivered compelling results by increasing whitening, and extending the iced shelf-life of raw sea scallops. A comparative consumer acceptability test of scallop appearance between unprocessed and HP-treated scallops would confirm if HPP improves consumer perception of raw scallop appearance. The texture of HP-treated and subsequently sous-vide cooked scallops was altered, but consumer ratings of overall acceptability were not impacted by this change. HPP at the pressures and times evaluated could prove to be an effective way to develop scallop products for sous-vide applications, bolstering the refrigerated ready-to-eat sous-vide seafood market. However, a systematic evaluation of economic and logistical feasibility is imperative to make the combination of HPP and sous-vide viable. HPP is an energy and financially intensive technology currently, and a resource assessment of combining HPP and sous-vide would be helpful in comparing benefits versus costs of these technologies.

Stringent time and temperature controls were followed to ensure safety of the scallops in these studies, however, research on the microbial safety of vacuum-packaged sea scallops is recommended to validate the combination of HPP and sous-vide cooking in pathogens of concern in seafood products, including *Listeria monocytogenes* and *Clostridium botulinum*. In conclusion, the current work provided foundational information on the development of refrigerated sous-vide scallop products, with HPP proving to be an effective method to process vacuum-packaged scallops for shelf-life extension. Combining HPP with sous-vide may be suitable for diversification of value-added scallop products, contributing to increased profits for the seafood industry.
REFERENCES


APPENDIX A: CONSUMER ACCEPTABILITY OF SOUS-VIDE COOKED SCALLOPS
CONSENT FORM

Dear Seafood Consumer,
You are invited to take part in a research project titled “High Pressure Processing of Sous-vide Seafood Products” by Dhriti Nayyar and Denise Skonberg, 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 scallops. 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. If you have never eaten or do not like scallops, or have an allergy to seafood or dairy, please do not participate.

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 scallops 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. The test may take about 20 minutes to complete.

Risks
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 scallops.
• This study will help in developing convenient-to-use, safe and high quality scallops for American consumers using new cooking methods.

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. All data will be destroyed by December 2018 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 dhriti.nayyar@maine.edu or by phone at (315) 447-3914. 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).
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 on the tray, 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.
- Male
- Female
- Rather Not Say

Please indicate your age on your last birthday.
Where do you usually consume scallops?
- At a restaurant
- At home
- Other

Approximately how often do you consume scallops?
- 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 scallops typically prepared?
- Baked
- Fried
- Grilled
- Other

Which sensory characteristic of scallops is most important to you?
- Flavor
- Texture
- Color
- Aroma
- Other: ________
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

How much do you like the color 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 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

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 this sample overall?

- Dislike Extremely
- Dislike Very Much
- Dislike Moderately
- Dislike Slightly
- Neither Like nor Dislike
- Like Slightly
- Like Moderately
- Like Very Much
- 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: CONSUMER ACCEPTABILITY OF SOUS-VIDE COOKED SCALLOPS
RECRUITMENT NOTICE

Are you interested in trying sous-vide cooked scallops?

If you are at least 18 years old and like eating scallops, please help University of Maine researchers evaluate sous-vide cooked scallops. 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 scallops.

Testing will be held on: October 2016

Please call 315-447-3914 or dhriti.nayyar@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 scallops, have never eaten scallops before or have allergies to seafood, please do not participate.
Fig D. Salt soluble protein content of SV cooked scallops. Each value represents a mean ± standard deviation (n=3). No letters indicate values are not significantly (p<0.05) different, analyzed by one-way ANOVA.
**APPENDIX E: SHEAR FORCE VALUES OF RAW AND SV COOKED SCALLOPS**

Table E. Shear force firmness and toughness

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Firmness (N)</th>
<th>Toughness (N.sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Raw</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2.9 ± 0.1</td>
<td>34.4 ± 0.9</td>
</tr>
<tr>
<td>150/5</td>
<td>2.9 ± 0.3</td>
<td>35.6 ± 1.9</td>
</tr>
<tr>
<td>150/10</td>
<td>2.8 ± 0.3</td>
<td>33.4 ± 2.2</td>
</tr>
<tr>
<td>350/5</td>
<td>3.0 ± 0.2</td>
<td>35.4 ± 3.7</td>
</tr>
<tr>
<td>350/10</td>
<td>3.1 ± 0.2</td>
<td>36.5 ± 3.1</td>
</tr>
<tr>
<td><strong>Cooked</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>11.8 ± 0.8</td>
<td>154.0 ± 13.9</td>
</tr>
<tr>
<td>150/5</td>
<td>12.8 ± 1.4</td>
<td>160.4 ± 24.6</td>
</tr>
<tr>
<td>150/10</td>
<td>17.5 ± 1.1</td>
<td>230.3 ± 40.0</td>
</tr>
<tr>
<td>350/5</td>
<td>13.4 ± 0.9</td>
<td>178.3 ± 12.3</td>
</tr>
<tr>
<td>350/10</td>
<td>15.5 ± 0.7</td>
<td>200.5 ± 12.7</td>
</tr>
</tbody>
</table>

Each value represents mean ± standard deviation (n=3). No letters indicate no significant differences among treatments by one-way ANOVA.
APPENDIX F: AEROBIC PLATE COUNTS OF RAW AND SV COOKED SCALLOPS DURING REFRIGERATED STORAGE

**Fig F 1.** Aerobic plate counts of raw scallops over 42 days of iced storage. Each value represents grouped mean ± standard deviation (n=3).

**Fig F 2.** Aerobic plate counts of SV cooked scallops over 42 days of iced storage. Each value represents grouped mean ± standard deviation (n=3).
APPENDIX G. BIOGENIC AMINES

Table G 1. Agmatine and tyramine concentrations of raw scallops during iced storage

<table>
<thead>
<tr>
<th>Days</th>
<th>C</th>
<th>350/5</th>
<th>350/10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.4 ± 0.3</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>28</td>
<td>3.0 ± 0.8</td>
<td>2.8 ± 0.6</td>
<td>0.9 ± 0.0</td>
</tr>
<tr>
<td>42</td>
<td>4.6 ± 7.5</td>
<td>n.d</td>
<td>n.d</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Days</th>
<th>C</th>
<th>350/5</th>
<th>350/10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5 ± 0.0</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>28</td>
<td>0.9 ± 0.7</td>
<td>1.1 ± 0.3</td>
<td>n.d</td>
</tr>
<tr>
<td>42</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.2</td>
<td>0.9 ± 0.4</td>
</tr>
</tbody>
</table>

Each value is the mean ± standard deviation (n=3).
### Table G 2. Biogenic amines concentrations of SV cooked scallops during iced storage

<table>
<thead>
<tr>
<th></th>
<th>Putrescine (mg/100g)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td></td>
<td>350/5</td>
<td>350/10</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.6 ± 0.5</td>
<td>0.2 ± 0.4</td>
<td>0.2 ± 0.3</td>
</tr>
<tr>
<td>28</td>
<td>1.0 ± 0.2</td>
<td>0.9 ± 0.0</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>42</td>
<td>n.d</td>
<td>n.d</td>
<td>0.6 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Cadaverine (mg/100g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days</td>
<td></td>
<td>350/5</td>
<td>350/10</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3.6 ± 1.2</td>
<td>1.3 ± 1.1</td>
<td>2.0 ± 0.6</td>
</tr>
<tr>
<td>28</td>
<td>3.2 ± 1.0</td>
<td>3.3 ± 0.9</td>
<td>4.3 ± 0.7</td>
</tr>
<tr>
<td>42</td>
<td>5.8 ± 1.4</td>
<td>3.2 ± 1.0</td>
<td>1.9 ± 0.0</td>
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<td></td>
<td>Histamine (mg/100g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days</td>
<td></td>
<td>350/5</td>
<td>350/10</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td></td>
<td></td>
</tr>
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<td>1</td>
<td>0.7 ± 0.9</td>
<td>n.d</td>
<td>0.8 ± 0.0</td>
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<tr>
<td>28</td>
<td>1.1 ± 0.3</td>
<td>1.2 ± 0.0</td>
<td>2.1 ± 0.8</td>
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<td>42</td>
<td>3.3 ± 0.0</td>
<td>3.0 ± 0.0</td>
<td>n.d</td>
</tr>
<tr>
<td></td>
<td>Agmatine (mg/100g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days</td>
<td></td>
<td>350/5</td>
<td>350/10</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>n.d</td>
<td>n.d</td>
<td>4.0 ± 2.3</td>
</tr>
<tr>
<td>28</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>42</td>
<td>n.d</td>
<td>0.8 ± 0.3</td>
<td>n.d</td>
</tr>
<tr>
<td></td>
<td>Tyramine (mg/100g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days</td>
<td></td>
<td>350/5</td>
<td>350/10</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>n.d</td>
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</tr>
<tr>
<td>28</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>42</td>
<td>0.7 ± 0.0</td>
<td>n.d</td>
<td>n.d</td>
</tr>
</tbody>
</table>

Each value is the mean ± standard deviation (n=3).
BIography of the author

Dhriti Nayyar was born in Mumbai, India on 27th September, 1990. She graduated from High School in Mumbai in 2006. She attended North Caroline State University and graduated in 2012 with a Bachelor of Science in Biological Sciences and minoring in Genetics. Dhriti moved to Maine in 2014 to pursue her Master of Science in Food Science and Human Nutrition and received the degree in 2016. After completing her PhD, Dhriti will be working in the food industry to develop new food products. Dhriti is a candidate for the Doctor of Philosophy degree in Food and Nutrition Sciences from the University of Maine in December 2019.