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Gelation of Previously Cooked Jonah Crab (Cancer borealis) Minced Meat in New Food Product Development

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GELATION OF PREVIOUSLY COOKED JONAH CRAB (*CANCER BOREALIS*) MINCED MEAT IN NEW FOOD PRODUCT DEVELOPMENT

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Thesis Advisor: Dr. Denise Skonberg

An abstract of the Thesis Presented
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Gelation of meat products plays an important role in utilization of by-products. Low-value muscle tissue that may go to waste can be reformed into new products that consumers find acceptable and want to purchase. In the crab industry, the need for by-product utilization is great. Crab processing typically involves cooking whole crabs and picking the claw meat. A lower grade minced meat can then be extracted mechanically from the walking legs and carapace of the crab. Traditional protein chemistry indicates that only raw muscle proteins can form gels. However, by using a modified surimi processing technique, previously cooked Jonah crab (*Cancer borealis*) meat was used to create protein gels. The effects of cryoprotectants, sodium chloride, protein additives, and carbohydrate additives in the crab meat gels were investigated. Preliminary work to determine the gelation mechanism of the cooked proteins was conducted. The crab mince was then used in new food product development as a primary ingredient and evaluated by consumers. This research is the first reported to show that cooked crustacean proteins can form gels upon further treatment. Not only can previously

cooked crab meat form gels, but through the use of additives it can be used in new food product development as a primary ingredient, thereby better utilizing the resource.

DEDICATION

I dedicate this manuscript to my loving parents who always pushed me to do my best and reach for the stars, without whose love and support I would not be where I am today.

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CHAPTER 1. INTRODUCTION

Jonah Crab

Harvesting

Crabs are an important economic part of the United States fishery industry. There are thousands of species worldwide but the marine species are most important to the U.S. industry. Commercially important species include: 1) blue crab (*Callinectes sapidus*), 2) Dungess crab (*Cancer magister*), 3) King crab (several species in the family *Paralithodes*), 4) stone crab (*Menippi mercenaria*), 5) Jonah crab (*Cancer borealis*), 6) red or golden crabs (*Geryon quinquedens* or *G. fenneri*) and 7) snow or tanner crabs (*Chionoecetes bairdi*, *C. opilio*, or *C. tanneri*) (Osterling 2000). Only a few of these species live in the coastal waters of Maine: Atlantic rock crab (*Cancer irroratus*), deep-sea red crab (*Geryon quinquedens*), and Jonah crab (*Cancer borealis*).

Jonah crab is typically caught as a by-catch of the lobster industry. Residing in waters 40 + m deep, Jonah crabs are caught live in lobster pots. The U.S. and Maine fishery industry has seen a significant increase in Jonah crab landings over the past five years (Table 1). In 2000, 2.6 million pounds of Jonah crab were landed in the U.S., 260,000 pounds of which were caught in Maine. Five years later, the landings almost tripled with 7.2 million pounds landed in the U.S., 2.7 million of which was in Maine. Financially, this increase in landings resulted in a jump in value from $280,000 in 2000 to $1.1 million in 2005. Jonah crab is only harvested in the waters of the Atlantic oceans, with five states reporting landings since 1985 (Table 2). Maine ranks 2nd in landings only to Massachusetts, landing approximately 1 million fewer pounds of Jonah crab in 2005 (NMFS 2005).

Table 1: Jonah crab catch and value from 1985 to 2005 in million of pounds and millions of dollars

	Maine		New England		U.S.	
Year	lbs.	$	lbs.	$	lbs.	$
1985	-	-	0.5	0.2	0.6	0.2
1990	0.007	0.003	1.3	0.4	1.3	0.5
1995	0.4	0.09	1.8	1.1	1.9	1.1
2000	0.3	0.3	2.6	1.5	2.6	1.6
2005	2.7	1.1	7.1	3.5	7.2	3.5

(NMFS 2005)

Table 2: Jonah crab landing statistics ranked by state with millions of pounds landed per year and value in millions of dollars in 2005

Rank	State	Pounds (millions)	$ (millions)
1	Massachusetts	3.7	2.0
2	Maine	2.7	1.1
3	Rhode Island	0.8	0.4
4	New Jersey	0.003	0.003
5	New York	0.001	0.0005

(NMFS 2005)

Processing and Waste Production

Jonah crab is typically steamed or boiled live and then the meat is picked by hand in small businesses and home operations. Larger businesses also process Jonah crab. One local processor receives crabs live in totes, eviscerates by use of a hook to remove the top shell, followed by thermal processing. Batches of crabs (approximately 2500 pounds) are boiled for 14 min then cooled in an ice slurry. Claws are removed and used either as cocktail claws for buffets or appetizers or the claw meat is picked for use as

lump meat or salad meat. The meat in the larger companies is picked one of two ways, either by hand or using high pressure water to extract the meat. Water extracted meat has less crab flavor due to loss of flavor components in the wash water. Residual crab meat, from the walking legs and carapace, is then extracted using a drum deboning machine. Mince is packaged in 10-pound polyethylene bags and frozen.

The average meat yield from hand-picked crab is between 10% and 12% (Ward 1990). Several mechanical methods have been developed to increase meat yields while decreasing the labor involved in the meat picking process. Methods such as vacuuming the meat out of the shell, shaking the meat out of the shell by vibration, removing the meat from the shell by centrifugal force, squeezing the meat out with rollers, and crushing the shell with a hammer mill to allow the meat to float to the top at which time the meat is harvested have all been researched (Ward 1990). Unfortunately, consistent high-quality results have not been obtained using any of these methods. In fact, most processors still rely on human labor to remove the meat from the shell.

Annual crab landings in the U.S. are over 300 million pounds (NMFS 2002) which generates an estimated 270 million pounds of processing waste per year. In New Brunswick, Canada, over 75% of the annual crab catch is discarded as solid waste (Jaswal 1990). It is estimated that the global annual production of shell waste from crab, lobster, krill, shrimp, clams, and oysters combined is 1.4 million metric tons (3.2 billion pounds) (Shahidi and Synowiecki 1991). The cost of waste removal from processing plants is increasing due to labor and fuel costs, therefore new methods of extracting usable material from the crabs have been developed. The shells of crustaceans are largely insoluble and resistant to biodegradation due to the putrification of shells leading

to the inactivation of enzymes needed for degradation (Shahidi and Synowiecki 1991; Healy and others 1994). The main portion of meat used from crab, specifically Jonah crab, is the claw meat, leaving residual meat in the walking legs and body. In the past, this residual meat was disposed of with the shell. Now, some processors are mechanically separating this edible tissue from the shell as mince, increasing meat yields by 40% (CIFT 2007). This cooked mince can then be further utilized in a variety of products, thereby maximizing processors' efficiency, resources, and profits while decreasing the waste stream. The development of new value-added products incorporating by-products can reduce waste, lower overhead costs, and help sustain and promote the aquaculture and seafood industries (Meyers 1994).

Composition of Crab Meat

The composition of crab meat is variable depending on factors such as sex, animal size, season and cooking method (Lee and others 1993). The nutritional composition of Jonah crab meat can be seen in Table 3. The major component of the crab meat is water, followed by protein at 16.2%. Interestingly, Jonah crab has a higher carbohydrate content than most other species of crab, averaging 3% of the meat composition. For instance, Queen crab (*Chionoecetes opilio*) has a carbohydrate content of 0.075% in the leg and claw meat and red crab (*Geryon quinquedens*) has only 1.5% carbohydrate. This higher percentage of carbohydrate is responsible for the sweet flavor often associated with Jonah crab meat (Lauer and others 1974). Though low in total fat content, approximately 38% of the fat in crab is poly-unsaturated omega-3 fatty acids (Krzynowek and others 1982). The mineral found in the meat in the greatest quantity was potassium, though this content may vary depending on the processing method.

Table 3: Composition of Jonah crab edible meat

Component	%
Moisture	78.2
Crude fat	1.9
Protein	16.2
Ash	1.5
Carbohydrate	3.1

Fat Content	% of Total Fat
Saturated fat	18.3
Mono-unsaturated fat	25.9
Poly-unsaturated fat	45.7
20:5 ω 3	27.9
22:6 ω 3	9.7

Mineral Content	g/100 g Ash
Na^{+}	18.8
K^{+}	19.0
Ca^{2+}	6.5
Mg^{2+}	2.2
PO_4^{2-}	2.7

(Lauer and others 1974; Krzynowek and others 1982)

Minced meat tends to have a higher mineral content due to small shell fragments in the mince (Gates and Parker 1992; Lee and others 1993). The amino acid composition is integral to the function of the muscle in both physiological functions and food systems. Though the essential amino acid composition of Jonah crab has yet to be determined, the composition of snow crab (*Chionoecetes bairdi*) is provided in Table 4. Arginine is the most prevalent amino acid in snow crab meat whereas tryptophan is the limiting amino acid. Approximately 15% of the crab meat contains polar essential amino acids whereas 22% are nonpolar. The nonpolar portions may therefore be the driving force behind the secondary structure of these myofibrillar proteins.

Most of the research to date on Jonah crab has focused on the physiological function of crab muscle proteins. The muscle fiber structure and histology are most often researched with a focus on the composition of myofibrillar proteins and myosin structure of various crab species, including Jonah crab (Mykles and Skinner 1982; Maeda 1983; Rathmayer and Erxleben 1983; Tse and others 1983; Mizuta and others 2001). Studies thus far have not addressed the functionality of cooked crab proteins.

Table 4: Essential amino acid composition of snow crab as % of protein removed from the crab offals by alkaline (2% aqueous KOH) extraction

Amino Acid	% of Protein
Arginine	6.66
Histidine	3.58
Isoleucine	2.67
Leucine	5.14
Lysine	2.51
Methionine	1.93
Phenylalanine	5.98
Threonine	4.74
Tryptophan	0.78
Valine	7.07

(Shahidi and Synowiecki 1991)

Muscle Proteins

General Characteristics

There are three basic classes of proteins in muscle: 1) sarcoplasmic, 2) myofibrillar, and 3) stromal. Sarcoplasmic proteins are the proteins dissolved in the cytoplasm inside the muscle cell which are mainly involved in regulation of cell and energy homeostasis. Proteins included within this group include myoglobin and glycolytic enzymes. Myofibrillar proteins, making up 50% to 60% of the muscle proteins, are the proteins that comprise the muscle fibers and are responsible for the contraction and relaxation of the muscle. Myofibrillar proteins can be dissolved in concentrated (0.3 M NaCl) salt solutions. The major myofibrillar proteins will be discussed in detail later. The final group, stromal proteins, is the fibrous connective tissues that strengthen and protect muscles. Stromal proteins contribute to the toughness of meat. An example of a stromal protein is collagen, which is the most abundant stromal protein in muscle (Byrem and Strausburg 2000).

When investigating the functionality of muscle proteins, myofibrillar proteins are the most often discussed. The type, percentage, approximate molecular weight and function of each major type of myofibrillar protein are presented in Table 5. The myofibrillar proteins are further broken down into thin and thick filaments. Thin filaments consist of four proteins: actin, tropomyosin, troponin and nebulin. The thick filaments are composed primarily of myosin, though titin is also often associated with the thick filaments (Byrem and Strausburg 2000).

Table 5: Protein composition of muscle including % of myofibrillar proteins, function, and molecular weight

Protein Type	%	Function	Molecular Weight (kDa)
Contractile			
Myosin	45	Muscle contraction in association with actin	500
Actin	20	Muscle contracts in association with myosin	42
Regulatory			
Troponin	5		
Troponin C	1.3	Binds calcium	
Troponin I	1.5	Inhibits actin/myosin interaction	
Troponin T	2.2	Binds troponin	
Tropomyosin	5	Binds actin and troponin	74
Cytoskeletal			
Titin	10	Possible scaffold for sarcomere organization; myofibril elasticity	3000
Nebulin	4	Regulates titin filament assembly and length	800

(Byrem and Strausburg 2000)

Myosin is the most abundant protein in muscle making up 43% of the myofibril. Myosin is a large molecule (~ 500 kDa) consisting of six polypeptide chains: 2 heavy chains (~ 205 kDa) and 4 light chains (18 to 30 kDa). At the amino end of the myosin molecule, the protein exhibits a globular structure referred to as the myosin head (Figure 1). From the head, the polypeptide chain extends into a long α-helical tail. The α-helical tails of two myosin heavy chains intertwine to form a helical coiled-coil. During muscle contraction, myosin acts as an enzyme that splits ATP, liberating chemical energy. The active site of the ATPase activity resides in the myosin head region. The myosin heads then form crossbridges with actin molecules resulting in a muscle contraction (Byrem and Strausburg 2000).

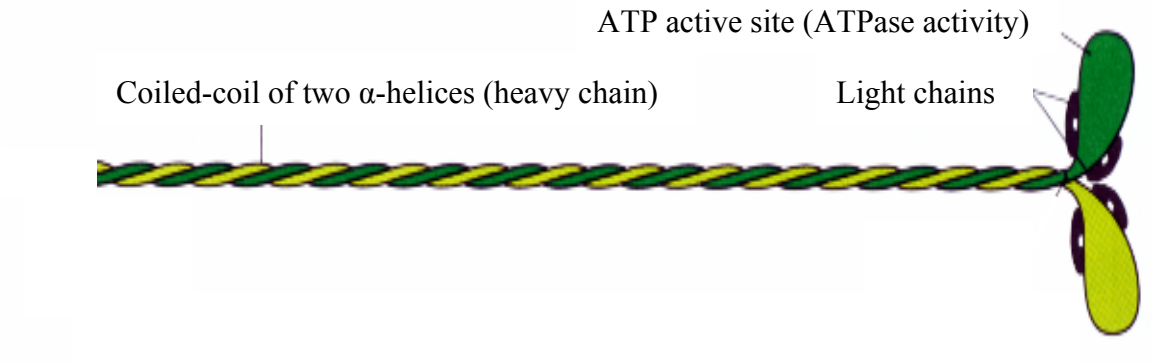

Figure 1: Diagram of the thick filament, myosin, showing the two heavy polypeptide heavy chains and four polypeptide light chains
(MITACS 2007)

To begin to understand how protein molecules interact and function, the levels of structure within the protein molecule must be further discussed. There are four main levels of structure within a protein molecule (Figure 2). The first, or primary structure, is the sequence of amino acids in the polypeptide chain. Within this polypeptide chain, hydrogen bonding occurs between amino acids, forming the secondary structure. The two main components of secondary structure are α-helices and β-sheets. The α-helix is found in fibrous proteins, such as myosin and collagen, and results from the winding of the polypeptide chain around a long axis with the functional groups protruding outward from the center. The α-helix is held together by hydrogen bonds between the amino acid groups. The second, β-sheets, form when the polypeptide chains extend into sheet-like structures with the functional groups projecting outward in an alternating pattern from the plane of the sheet. The β-sheets are formed by the inter-chain hydrogen bonding of a carbonyl group of one polypeptide bonding with an amide group of another polypeptide chain. The third level of structure is tertiary structure, which is the three-dimensional conformation of the protein molecule. Tertiary structure forms from a combination of non-covalent and covalent bonding between amino acid residues. This structure is the most thermodynamically favorable and requires the least amount of energy to maintain this form. The final structure is quaternary structure. Quaternary structure is the higher-ordered organization of two or more proteins into a complex protein aggregate that serves a particular physiological, biochemical, or structure role, such as hemoglobin (Rasco and Zhong 2000).

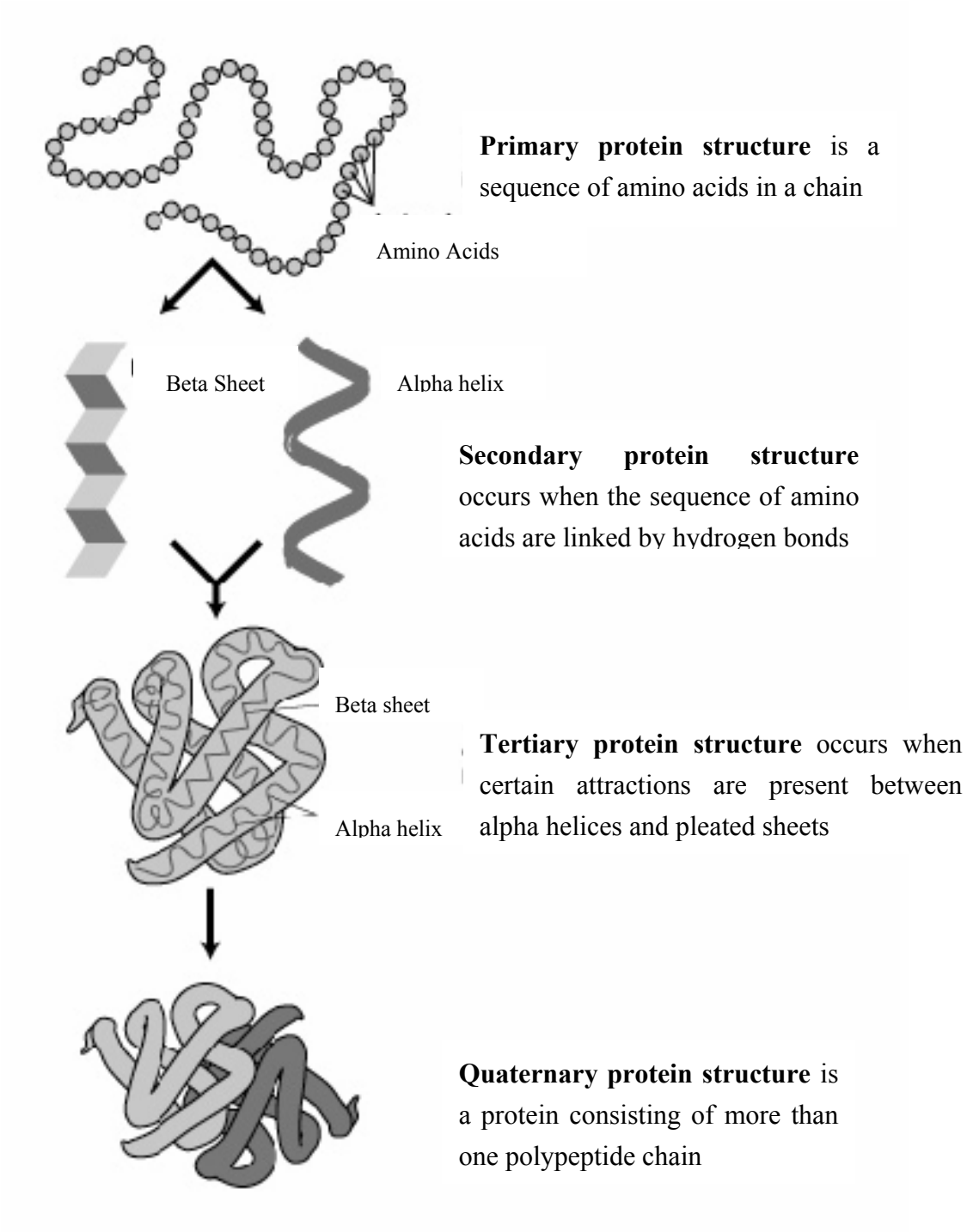

Figure 2: The four levels of protein structure
(Seidman and Mowery 2006)

Functional Properties

Proteins exhibit many functional properties that are important to foods. These properties include: solubility, gelation, water binding, emulsification, and foaming. The functional properties of the proteins depend on the characteristics of the specific protein. For example, a peptide should have a minimum length of 20 amino acid residues to possess good emulsifying and foaming properties (Lee and others 1987). Herring and arrowtooth flounder can be used to create functional protein powders that could be used as emulsifying and gelling agents (Sathivel and others 2004). Fish protein isolated from cod muscle is effective in stabilizing oil-in-water emulsions (Petursson and others 2004). This research focuses on the gelation and water binding properties of myofibrillar proteins, which will be discussed further.

Gelation is the process by which proteins form a continuous three-dimensional solid-like cross-linked network that has an embedded aqueous solvent. Protein gels can either be reversible or irreversible depending on the type of proteins used in gel formation. Most gels made from myofibrillar proteins are irreversible gels. Irreversible gels are formed by heat denaturation, or partial unfolding of the protein, followed by aggregation of the unfolded molecules to form a cross-linked matrix. Protein gels are commonly formed by hydrophobic interactions, hydrogen bonds, or electrostatic interactions. Covalent bonds, such as disulfide linkages, may also play a part in the formation of some gels. If the forces between protein molecules are weak, the proteins will not aggregate upon heating. On the other hand, if the forces are too strong, the proteins may become highly cross-linked which results in the proteins no longer being able to bind water. Figure 3 demonstrates gelation of proteins. First the solvent diffuses

into the protein molecules. As the molecules unfold due to application of heat, aggregation begins to occur. The proteins then begin to aggregate and a cross-linked protein matrix is formed. The solvent, typically water, is trapped within the matrix and a gel is formed (Smith and Culbertson 2000). The gel properties of a muscle protein gel are most dependent on the myosin molecule (Iwata and others 1977).

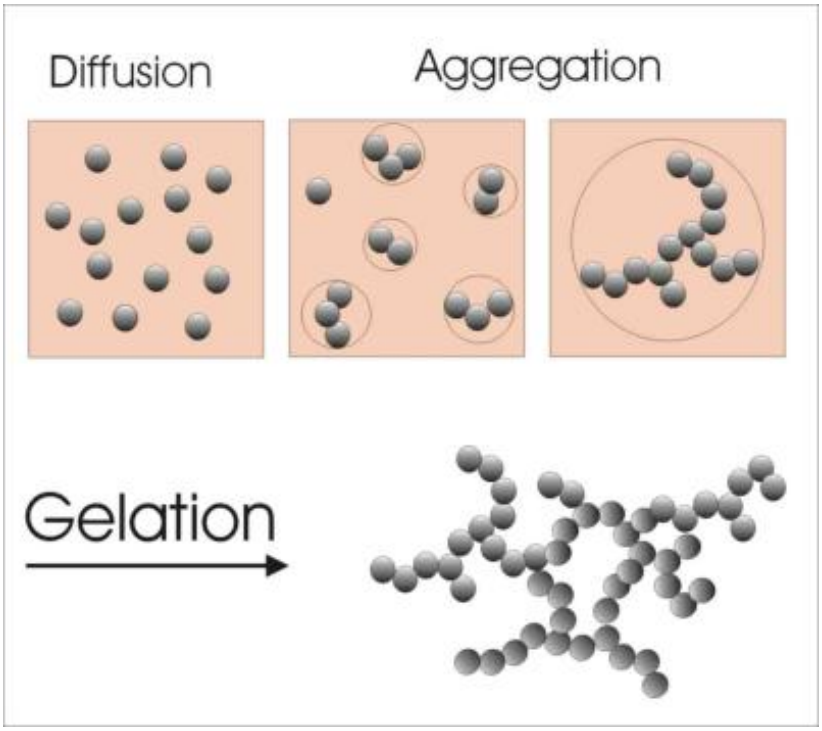

Figure 3: Gelation mechanism of proteins
(Bissig and others 2004)

Gelation of myofibrillar proteins is a physicochemical process by which protein-protein interactions occur to form a three-dimensional viscoelastic matrix, which is capable of entrapping large amounts of water (Xiong 2000). It is widely believed that high salt conditions are required for solubility of myofibrillar proteins and proper gelation, however recent reports indicate that these proteins may be highly functional at very low ionic strengths, even though they are insoluble (Chang and others 2001; Kristinsson and Hultin 2003c). Gelation properties of washed, minced fish muscle are a widely-studied topic, as more researchers are investigating the potential of incorporating numerous underutilized fish species into processed surimi-based products. Amount and type of salt, mince washing method, heating process, pH, and fish species are a few of the many factors that influence gelation properties of raw minced fish muscle (Venugopal and others 1995; Dagher and others 2000; Luo and others 2001; Kristinsson and Hultin 2003b, 2003a).

The water binding ability of proteins is a crucial property in food proteins due to the large quantity of water within food products. Water associates with the surface of proteins by dipole-dipole interactions forming a single layer of water on the surface called the monolayer. This water accounts for approximately 0.3 g water per g of protein and does not have the same properties as free water due to the tight bond with the protein molecule. When evaluating other functional characteristics of proteins, such as gel formation, the water-holding capacity is often tested. The water measured in these tests is the free water in the gel, not the monolayer water. One of the factors affecting gel formation is the level of salt, typically, sodium chloride added to the meat. As the salt concentration increases the intermolecular forces between the muscle fibers weaken.

This weakening allows for more water to bind to the proteins, creating less protein-protein interactions and more water-protein interactions (Smith and Culbertson 2000).

Protein Denaturation

Protein denaturation is defined as any change in a protein's native structure. This change typically involves a significant shift in the molecular shape, including changes or loss of tertiary and/or secondary structure (Rasco and Zhong 2000). Any change in environmental conditions can lead to denaturation. Proteins can partially or fully unfold due to very low or high pH, high salt concentrations, presence of organic solvents, or changes in temperature. Most thermal processes in the food industry cause protein denaturation which can lead to desirable products, such as protein gels (Smith and Culbertson 2000).

The effects of thermal processing on loss of protein functionality in muscle foods have not been well described. Cooking muscle causes denaturation, and denatured proteins are assumed to have limited functionality. Denatured proteins lose their native conformation or ordered structure, unfolding from their natural configurations into other reversible or irreversible forms. Denaturation often causes myofibrillar proteins to lose some functional properties, including loss of solubility and water-holding capacity, due in part to the exposure of hydrophobic amino acid residues and sulfhydryl groups upon unfolding, and the subsequent aggregation of proteins by hydrophobic interactions and the formation of disulfide bonds. However, the onset and extent of denaturation of myofibrillar proteins during heating can be influenced by several factors including protein purification, presence of salt, extent of processing, and fish species (Park and Lanier 1989; Esturk and others 2004). Sano and others (1994) researched the effects of

heating on structural and functional changes in carp actomyosin. They reported that the soluble protein decreased by 25% after heat treatment to 80 °C. However, unfolding appeared reversible, since after cooling protein solubility increased to 42%. With regard to crab muscle proteins, the effects of thermal processing on the extent of denaturation and on subsequent protein functionality have not been reported.

Surimi Production

Processing

Surimi is defined as a wet concentrate of proteins of fish muscle that is mechanically deboned, water-washed fish flesh. A flow diagram of the surimi process resulting in the production of a surimi-based product is shown in Figure 4. Fish is first gutted and meat is separated from the bone creating minced fish. The mince is then washed, strained and dewatered resulting in what is called raw surimi. The raw surimi is then mixed with cryoprotectants and frozen in blocks. To make a surimi-based product, the frozen surimi is ground with salt and ingredients and heat processed, resulting in the final product.

The production of surimi involves washing of raw fish flesh to remove fat and water soluble substances, such as sarcoplasmic proteins, pigments, amines, and vitamins and also to concentrate the myofibrillar proteins to enhance gelation (Mendes and Nunes 1992; Lin and others 1995). The number of washing cycles and ratio of water to mince needed varies with fish species, initial condition of the fish, and the type of operation, either continuous or batch processing (Lee 1984). In general, a five minute agitation in each of two washing cycles using a 3:1 water to mince ratio has been considered adequate for raw fish surimi production (Lee 1986).

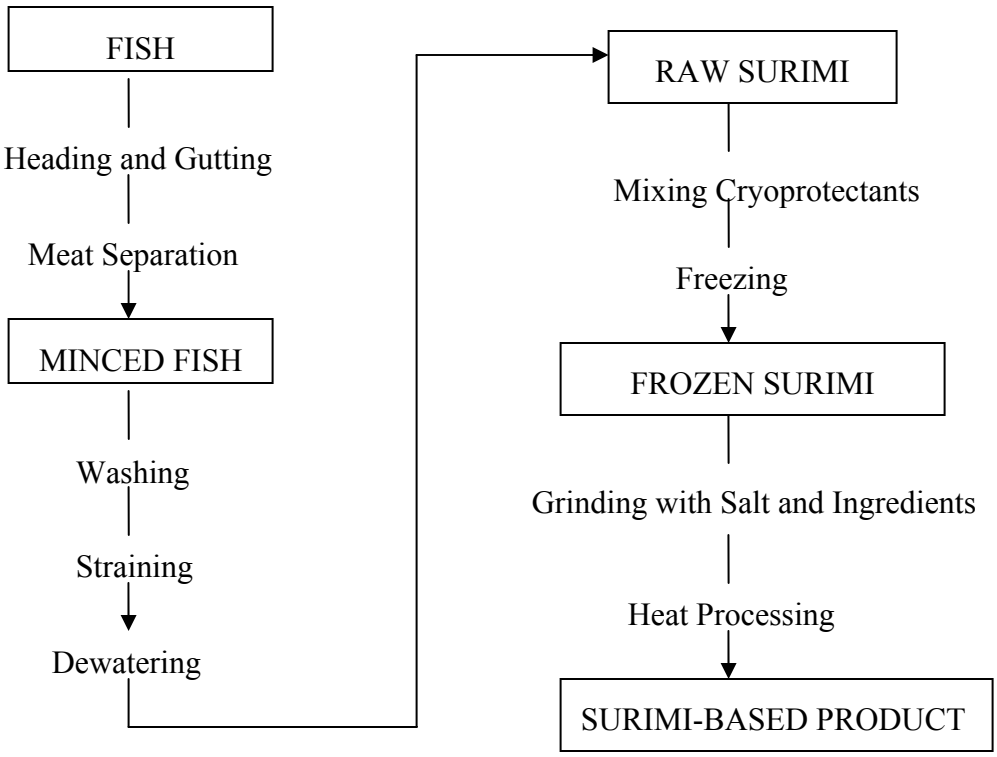

Figure 4: Surimi process resulting in a surimi-based product

(Okada 1992)

Pacheco-Aguilar and others (1989) investigated the effect of washing in Pacific whiting surimi production. Removal of lipids was not efficiently achieved using a single wash with a 3:1 ratio (water to mince) but resulted in high solids and protein recoveries which are often lost in multiple exchange washing procedures. During the washing and dewatering process, approximately 40% to 50% of fish solids are lost (Adu and others 1983; Pacheco-Aguilar and others 1989; Yang and Froning 1992). When electrophoretically analyzing wash water, low molecular weight protein bands (29 to 45 kDa) and an insignificant amount of myosin heavy chains present suggested that the first

washing removed mainly sarcoplasmic proteins and did not impart notable losses of myosin. Subsequent washing indicated a greater loss of myofibrillar proteins (Lin and others 1995). The benefit of single exchange washing was the high solids and protein recoveries. Processes that employ multiple exchange washing procedures lose many of the recoverable solids from the wash water during the first exchange which is enhanced by the dewatering mechanism between washings. For instance, loss from screening was more substantial than loss from pressing (Pacheco-Aguilar and others 1989). Therefore, reducing the number of washing cycles is important in overall recovery of solids and maximization of yield.

When a fish meat gel is formed by heating surimi, two processes occur: unfolding of the myosin and formation of the gel network. The thermostability of myosin is correlated with the environmental temperature at which the organism lives (Nakaya and others 1995; Kakinuma and others 1998; Kakinuma and others 2000). Differential scanning calorimetry thermograms were different for rabbit, Alaska pollock, and carp myosin. Each type of myosin showed three major endothermic peaks. For the species tested, Alaska pollock lives in the coldest environment and rabbit lives in the warmest. The endothermic peaks correlated to the environment with Alaska pollock myosin being the least thermostable and the rabbit being the most. Gel formation is considered to be associated with changes in myosin structure which means that the unfolding of the coiled-coil structure of α-helices in myosin rod results in exposure of hydrophobic amino acids which are normally buried between the two α-helices facing each other in the intact myosin molecule (Fukushima and others 2003). There are two main types of surimi that correspond with different cooking methods. The first, *suwari*, is produced by setting the

gel at a low temperature (5 °C to 45 °C). The second, *kamaboko*, is created by setting at a low temperature followed by a cooking treatment (≥ 80 °C). The cooking time varies with species. Gel strength of sardine *kamaboko* gels was much higher than the corresponding *suwari* gels. The higher gel strength was due to the protein-protein interactions that were established during setting and strengthened during cooking (Alvarez and Tejada 1997).

To guarantee the safety of surimi foods, the U.S. Department of Commerce has guidelines for the Packed Under Federal Inspection label and FDA's HACCP for vacuum-packaged surimi seafood. The guidelines indicate that products must be pasteurized at 85 °C (internal temperature) for 20 min. For a package with 2 layers of surimi crab sticks in 90 °C water, it takes 60 minutes to reach the internal temperature of 85 °C (Jaczynski and Park 2003). There is no standard pasteurization method used by the industry, therefore different manufacturers use different conditions (Park 1994).

Underutilized fish species and by-products of fish processing can be used in the production of new products to gain a greater value from the original resource. Mackerel (*Scomber australasicus*) and golden threadfin-bream (*Nemipterus virgatus*) surimi gels have been investigated as an alternative use of those species (Jiang and others 2000; Hsieh and others 2002). The concept of creating value-added products is not limited only to fish, but includes shellfish by-products as well. Yoshida and others (2003) investigated the use of scallop (*Patinopecten yessoensis*) striated adductor muscle in the production of a kamaboko-like gel and found that weak gels could be created from the salted scallop adductor paste.

Effect of Additives on Surimi Processing

The quality of surimi is affected by sodium chloride content, cooking temperature and time, the species of fish, and any additives to the surimi. In traditional surimi processing, sodium chloride is added to raw muscle mince to aid in gel formation. The sodium chloride initiates the conversion of surimi into a *kamaboko* style gel by forming an actomyosin sol from the actin and myosin present in the meat (Hall and Ahmad 1997). Typically, between 2% and 3% sodium chloride has been added to aid in gel formation. High concentrations of sodium chloride are thought to be required to solubilize myofibrillar proteins that are required for the formation of fish gels (Suzuki 1981; Lee 1984; Shimizu 1985; Lee 1986). However, Lin and others (1995) showed that significant amounts of myofibrillar proteins are solubilized in very low (near zero) ionic strength solutions. In raw cod muscle, the solubility of proteins at near zero ionic strength was greater than the solubility at high ionic strengths (Stefansson and Hultin 1994). Solubilization of muscle proteins occurs in two steps: depolymerization of thick filaments (myosin) followed by dissociation of actin from myosin (Parsons and Knight 1990). Therefore, the salt concentration once thought to be required in gelation of myofibrillar proteins, may in fact be lower than traditional processes suggest.

The salt content of a solution can affect the solubility of the proteins present. Low salt concentrations allow the proteins to be surrounded by salt ions of opposite charge which decreases the electrostatic free energy leading to increased activity of the solvent resulting in greater solubility of proteins (Debye and Huckel 1923). This increased solubility is referred to as salting-in of proteins. The salting-in phenomenon, which may occur in this situation, is attributed to nonspecific electrostatic interaction

between charged proteins and the ionic environment which leads to solubilization (von-Hippe and Schleich 1969). As the salt concentration in a solution increases, the salt ions compete with the proteins for binding sites on the water molecules. This results in greater protein-protein interactions and fewer protein-solvent interactions leading to the precipitation of proteins. Salting-out occurs at higher salt concentrations and is attributed to the loss of a stable hydrophilic surface, causing exposed hydrophobic areas of the proteins to interact, aggregate, and precipitate.

Another type of additive in surimi products are cryoprotectants. Addition of sucrose, sorbitol, and phosphates has been shown to bind water surrounding the protein molecules thereby preventing denaturation of the protein during frozen storage. During freezing, proteins in fish begin to aggregate leading to tougher textures and lower water-holding capacity of the proteins (Sikorski and others 1976; Nilsson and Ekstrand 1995). The addition of cryoprotectants prevents cell disruption and ice crystal formation within the meat caused by frozen storage (Franks 1985; Privalov and others 1986). Sucrose and sorbitol are often used due to availability and low cost, though less sweet alternatives, such as trehalose, are being investigated (Lee 1984; Jittinandana and others 2005).

Other types of additives, such as hydrocolloids, proteins, and carbohydrates can be added to surimi to improve the textural characteristics. Additives function by filling the interstitial spaces of the myofibrillar protein network but do not seem to interact with myosin and have the tendency to weaken gels (Burgarella and others 1985a; Burgarella and others 1985b). The added ingredients exert their effects on overall texture, appearance, and other properties due to effects on water mobility or just by their presence in the gel which serves to reinforce the structure formed by the surimi gel matrix (Lee

and others 1992; Park 2000). This reinforcement can lead to either a strengthening or weakening of the gel matrix. Though the mechanism by which both protein and carbohydrate additives interact with myofibrillar proteins is not clearly defined, these additives can change the textural attributes of surimi gels. Additives such as hydrolyzed beef plasma protein, egg white, soy protein, wheat gluten, whey protein, potato starch, corn starch, *iota*-carrageenan, *kappa*-carrageenan, and polysaccharide gums have been researched extensively (Burgarella and others 1985a; Burgarella and others 1985b; Chang-Lee and others 1990; Chung and Lee 1990; Chen and Trout 1991; Chung and Lee 1991; Lee and others 1993; Shand and others 1993; Yoo and Lee 1993; Park 1994; Yoon and others 1997; Luo and others 2004a, b; Murphy and others 2005). Incorporation of these additives can be used to change the textural characteristics of the surimi gels based on concentration and additive type.

The textural properties of surimi can also be enhanced through the addition of transglutaminase. Transglutaminase is an enzyme of the sarcoplasmic fraction of fish muscle that catalyzes the cross-linking of proteins through the formation of non-disulfide covalent bonds between glutamine and lysine residues (Lanier 2000). In surimi production, there are two types of transglutaminase that are used: endogenous and microbial. Endogenous transglutaminase is calcium-dependent whereas microbial transglutaminase will form bonds without calcium. In traditional surimi processing, the endogenous transglutaminase is washed away or inactivated by processing (Perez-Mateos and others 2004). Striped mullet (*Mugil cephalus*) and lizardfish (*Saurida* spp.) are species that have low economic value and have benefited from the use of transglutaminase, resulting in higher quality surimi gels, thereby increasing their value

(Ramirez and others 2000; Yongsawatdigul and Piyadhammaviboon 2005).

Quality Attributes of Surimi

The Technical Subcommittee of the Surimi Committee, National Fisheries Institute, was formed in 1986 to set standard testing methods for the assessment of raw surimi composition and functional properties. They established the "benchmark" methods to evaluate the composition and functional properties of raw surimi in the United States. No grades of raw surimi were established because the grade would be based on the criteria for only one consumer group. Therefore standardized tests were implemented to evaluate the quality and each buyer could purchase raw surimi based on these characteristics. The compositional tests most often performed are protein content, moisture content, fat content, and visual contaminants. Measurement of pH is also considered compositional but is mainly used as an indirect measure of functionality (Lanier 1992).

Direct functionality tests are also performed. Raw surimi is cooked using five different time and temperature combinations to provide a range of functional data. The gel-forming properties are determined by torsional deformation, or twisting, of a dumbbell shaped sample. Puncture tests, in which a plunger is lowered at a constant rate into a sample of the cooked surimi gel, can also be used to determine the gel strength, though the puncture measurements do not directly correlate with torsion tests. Color is another important characteristic. Generally, surimi will have L* (or lightness) values well above 50, and low (near zero) a* (redness) and b* (yellowness) values (Lanier 1992). Though not an official method of analysis, the amount of water retained within the surimi gel network is also often measured to determine quality. The more water the

gel retains, the higher the quality of the gel.

Cooking time and temperature play an important role in the quality of surimi gels that are obtained. In sardine surimi gels, setting at 25 °C for 30 min is too short of a time for the gel network to become stable enough to hold water (Alvarez and Tejada 1997). The yield strength determines the quality of the surmi that is formed. Yield strength of sardine surimi *kamaboko* gels was much higher than that of corresponding *suwari* gels. The higher yield strength was due to protein-protein bonds that were established during setting and strengthened during cooking (Alvarez and Tejada 1997). The strength is dependent on the bonds that are formed. At cooking temperatures, mainly intermolecular disulfide bonds and extensive intermolecular hydrophobic interactions are formed (Seki and others 1990; Niwa and others 1992). The cooking of *suwari* gels to form *kamaboko* gels causes further aggregation of the proteins due to these disulfide bridges and hydrophobic interactions (Sano and others 1988; Niwa and others 1992; Careche and others 1995). The denaturation of proteins during the setting process results in a finer gel structure according to Hermansson (1978).

The effect of storage on surimi quality was studied using hoki. Hoki were caught and dressed, then stored on ice to be processed into surimi at an on-land processing facility. Fish were stored in ice from 4 to 21 days and gels were formed at 60 °C and 90 °C and washed mince was compared to unwashed mince. The strength and puncture deformation of the washed mince was greater than that of the unwashed mince. In the later stages of the experiment, the gels made from unwashed mince gelled at 60 °C underwent textural deterioration indicated by the decline in puncture deformation. The study indicates that surimi made from fish immediately after rigor, as would happen in a

factory ship production, results in a better quality product (Macdonald and others 1990).

The breaking force and deformation of mackerel surimi gels can be enhanced with the addition of microbial transglutaminase. Samples with 0.5 unit/g microbial transglutaminase had about 2.5 fold the breaking force and 1.5 fold the deformation of the control. When the microbial transglutaminase was added at levels higher than 0.5 unit/g, there was a significant decrease in deformation and breaking force. The addition of microbial transglutaminase showed a decrease in myosin heavy chain intensity in the surimi (Hsieh and others 2002). Transglutaminase can also improve the break force, deformation, and gel strength of golden threadfin-bream surimi gels. Gels with 0.3 unit/g mince had 2-fold the gel strength of that of the control (Jiang and others 2000). The effect of microbial transglutaminase on the gel-forming ability of Alaska pollock surimi was similar to that of golden threadfin-bream with the highest breaking force obtained with the addition of 0.2 unit/g microbial transglutaminase (Jiang and others 2000). The amount of microbial transglutaminase needed is highly dependent on the fish species as well as freshness, protein quality and harvesting season (Jiang and others 2000).

Lee and Park (1998) found that adding 0.2% calcium chloride to cold water fish species surimi increased the shear stress properties of the surimi. An acceptable maximum stress can be obtained without the addition of calcium, but the addition of calcium to surimi created a gel with better mechanical properties, determined by the higher values of shear stress and strain. Setting of gels is a phenomenon dependent on both protein denaturation/aggregation and enzymatic activity of the endogenous transglutaminase with both processes occurring during setting (Ramirez and others 2003).

Use of Surimi in Food Products

Different flavors and additives can be added to surimi to produce different products. Analog products, such as roe analog, crab analog, and scallop analog can be created from the bland surimi by the addition of flavors and colors. The analogs are made either by extrusion into either sheets for fibrous products or ropes for a more homogenous product, such as analog crab legs. Molding is also sometimes used in the production of shrimp and lobster analogs. Kamaboko, a Japanese favorite, is a homogenous fish product. A block of surimi paste is formed on top of a triangular wooden board and then steamed to create the kamoboko (Wu 1992).

Restructured fish products are created to maintain the native characteristics, flavor, texture, etc., of the fish. In surimi production, the meat is washed prior to gelling, whereas with restructured products the meat is not washed prior to setting and cooling (Ramirez and others 2000; Ramirez and others 2002; Tellez-Luis and others 2002). In the creation of restructured products, the most widely used technique is to solubilize and extract myofibrillar proteins with salt to obtain sticky exudates, which in turn bind meat pieces. Transglutaminase is used as the binding agent in restructured meat products without salt or cooking (Kuraishi and others 1997).

Crustacean Product Development

Most crab product development research has focused on optimizing the shelf-life of crustacean or crab meat. Canning of crab meat greatly extends its shelf-life. By developing processing schedules for canned meat, less meat goes to waste due to spoilage. Ghazala and others (1996) developed a processing schedule for the pasteurization of rock crab meat to better utilize the resource. Another method that has

been investigated is the use of irradiation to decrease microbial loads in hand picked meat, thereby extending the shelf-life. Chen and others (1996) found that minimal changes occurred in volatile flavor compounds and consumer acceptability of crab meat irradiated with 1 kGy. Though not a pasteurization process, the treatment did extend the shelf-life of the crab by an additional 3 days.

Blue crab minced meat has been studied to determine usability in further processed crab products. Blue crab mince is used as an extender in devilled crab, seafood stuffings, soups, and chowders. The dark color and high microbial values are a limitation in the marketability of the minces. Gates and Parker (1992) investigated ways to improve the characteristics of minced meat from picking plant by-products. They found that an additional 22% of the original uncooked crab could be recovered after picking using a commercial deboning machine to remove additional meat. Meat from different locations on the crab body had different properties, for example, minced leg meat was rated as wetter and having a stronger ammonia odor than minced white meat. Claw meat was also more fibrous than minced mixed meat. From a microbial perspective, pre-pasteurization levels ranged from 10^5 to 10^7 CFU/g. Pasteurization of mixed crab mince was conducted at both 80.6 °C and 83.3 °C. Both temperatures effectively reduced microbial levels in the mince but the lower heat treatment resulted in less bluing of the mince. Acid-phosphate buffer was added to try to improve color. Samples treated with buffer had improved color upon pasteurization compared to samples without added buffer. Lee and others (1993) further investigated using minced meat from blue crab in the production of crab cakes. Minced meat was used in combination with claw meat and binders such as soy protein concentrate and surimi. A feasible crab cake product utilizing minced blue

crab meat was formed with similar textural characteristics as a crab cake made solely with lump meat.

Other crab product development, specifically using Jonah crab, has focused on using meat as a secondary ingredient. Extruded crab snacks, similar to corn puffed snacks, were developed as a means of reducing processing wastes and improving value (Obatolu and others 2005). The snacks were coated using a cheese powder, though sensory testing was not reported. Jonah crab mince was also used in an extruded pasta product (Gillman 2001). Pasta containing 10% to 20% crab mince was developed and received consumer acceptability scores of "like slightly" to "like moderately". Most of the work conducted with Jonah crab has utilized the mince as a secondary ingredient. More product development research is needed to develop a commercially viable product that contains Jonah crab mince as a primary ingredient.

Objectives

The overall goal of this research was to explore the functional properties of cooked Jonah crab meat mince and to utilize the mince as a primary ingredient in new food product development. A series of studies were conducted over three years.

The specific objectives of the studies were to:

1. Determine the optimal processing parameters for gelation of previously cooked Jonah crab meat mince, including the effects of sodium chloride, cooking temperature, cryoprotectants, and protein and carbohydrate additives.

2. Conduct preliminary research investigating the effects of thermal processing on the denaturation of crab proteins.

3. Develop a consumer acceptable new food product using cooked Jonah crab meat mince as the primary ingredient.

CHAPTER 2. THERMAL GELATION OF PREVIOUSLY COOKED MINCED MEAT FROM JONAH CRAB (*CANCER BOREALIS*)

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Chapter Abstract

Crab processing typically involves picking meat from whole cooked product. Remaining meat can be recovered as mince and potentially processed into value-added products. This study focused on gelation of commercially processed crab mince. Objectives of the research were to determine the effects of cryoprotectants, freezing and various heat treatments on gel formation of washed mince from previously cooked crab. Previously frozen minced meat from thermally processed Jonah crab was washed to remove soluble components. Four different treatments were applied to the washed mince: 1) freezing with cryoprotectants, 2) freezing with no cryoprotectants, 3) no freezing with cryoprotectants, and 4) no freezing with no cryoprotectants. Unwashed mince was used as a control treatment. Sodium chloride (2.5%) was mixed into the mince prior to stuffing into sausage casings and heating at 35 °C/30 min, 90 °C/30 min, or 35 °C/30 min followed by 90 °C/30 min. Gels were tested for proximate composition, color, water-holding capacity, and gel strength. All mince samples formed gels except for the unwashed control. Gels with no cryoprotectants had 10% to 20% greater water-holding capacity, lower L* values, and greater gel strength than those with cryoprotectants. Freezing of washed mince resulted in lower water-holding capacity of gels and higher a* values. A two-stage heating treatment resulted in gels with the greatest gel strength, whereas gels cooked at 35 °C had the greatest distance to fracture. Results indicate that

protein gels can be formed using previously cooked crab meat, which may be useful in the development of value-added products.

Introduction

Jonah crab (*Cancer borealis*) is a by-catch of the lobster industry with 578 metric tons landed in Maine and 1456 metric tons landed in the United States in 2003 (NMFS 2003). Gaining a market in the culinary world, Johan crab is seen on many buffets across the United States and consumer demand for Jonah crab claws has increased due to its mild flavor and relatively low cost (Redmayne 2002). Though the claws have become more popular, much of the remaining crab meat is wasted after removal of the claws. A mechanical deboner can be used to produce low-value crab mince which is added to bisques, soups, stuffings, and crab cakes (Portland Shellfish, Portland, ME, U.S.A.). However, newer higher value uses for the crab mince are needed to increase profitability and total utilization of the resource.

Underutilized fish species and byproducts of fish processing can be used in the production of new products to gain a greater value from the original resource. Mackerel (*Scomber australasicus*) and golden threadfin-bream (*Nemipterus virgatus*) surimi gels have been investigated as an alternative use of those species (Jiang and others 2000; Hsieh and others 2002). Fish mince from species that produce a poor quality surimi gel can be enhanced through the use of transglutaminase, an enzyme that aids in gel formation. Striped mullet (*Mugil cephalus*) and lizardfish (*Saurida* spp.) are species that have low economic value and have benefited from the use of transglutaminase, resulting in higher quality surimi gels, thereby increasing their value (Ramirez and others 2000, 2003; Yongsawatdigul and Piyadhammaviboon 2005). The concept of creating value-

added products is not limited only to fish, but includes shellfish byproducts as well. Yoshida and others (2003) investigated the use of scallop (*Patinopecten yessoensis*) striated adductor muscle in the production of a kamaboko-like gel and found that weak gels could be created from the salted scallop adductor paste.

Studies investigating the mechanical extraction of blue crab (*Callinectes sapidus*) byproducts reported an additional 22% of the crab could be recovered as mince meat (Gates and Parker 1992). However, the dark color and high microbial values limited the marketability of the mince. Pasteurization of the minced meat and addition of citric acid-phosphate buffer improved appearance and controlled microbial levels. In another study, minced meat from undersized blue crab claw was combined with picked claw meat, 10% pollock surimi (*Theragra chalcogramma*), and 30% to 50% soy protein concentrate to produce a crab cake acceptable to consumers (Lee and others 1993). Additional research that focuses on the use of crab mince as the primary component of value-added products is needed to increase utilization of this resource.

When muscle proteins are cooked, their physicochemical and biochemical properties change due to denaturation, causing a significant loss of functionality. Studies thus far have not addressed the thermal gelation properties of cooked crab proteins. However, unexpected findings in our lab indicated that previously cooked and frozen crab meat could apparently form protein gels. The purpose of this study was to confirm that cooked, frozen, and presumably extensively denatured crab mince could form protein gels under controlled conditions. Our ultimate objective is to utilize the cooked crab mince in the development of protein-gel based value-added products such as sausages or nuggets that consist of over 85% minced crab meat. Since surimi is the closest

comparison to minced crab meat, despite having very different characteristics, we tested the effects of typical surimi processing parameters on the attributes of the crab protein gels. The specific objectives of this study were to determine the effects of cryoprotectants, freezing, and various heat processes on the gel formation of washed crab mince produced from cooked Jonah crab meat.

Materials and Methods

Frozen, commercially processed Jonah crab meat mince processed in the following manner was obtained from Portland Shellfish (Portland, Maine, U.S.A.). Crabs had been eviscerated, boiled for 14 minutes without the top shell and cooled. Cooked crabs were kept on ice until mince was separated later the same day. Claws were removed and the remaining meat was separated using a mechanical deboning machine. The mince consisted of meat from the body and walking legs. Ten pounds of mince was packaged in polyethylene bags and frozen.

Surimi Production

A 10-lb block of frozen Jonah crab meat mince was thawed at 4 °C for 48 h. The mince was washed in 200 g batches a total of three times to remove water-soluble proteins, minerals, and small peptides using a ratio of 4:1, water to mince. The first wash consisted of a slurry of mince in water that was mechanically stirred for 15 min and filtered through a mesh fabric screen. The final 2 washes were completed in a Waring blender for 1 minute and dewatered by centrifugation at 15,000 rpm for 10 min. The first 2 washes were with cold deionized water and the final wash was with a cold 0.3% NaCl solution.

Treatment

Approximately 1 kg of washed crab mince was used per treatment. Four different treatments were applied to the washed mince: 1) freezing with cryoprotectants (FC), 2) freezing with no cryoprotectants (FNC), 3) no freezing with cryoprotectants (NFC), and 4) no freezing with no cryoprotectants (NFNC) (Table 6). Unwashed mince was used as a control treatment (C). The cryoprotectant mixture used was sucrose (4%), sorbitol (4%), and sodium tripolyphosphates (0.3%). Upon addition of cryoprotectants to the washed mince and mixing in a kitchen stand mixer for 5 min, samples were vacuum packaged before receiving the designated freezing treatment. The freezing treatment consisted of storage at -20 °C for 1 wk. Samples that were not frozen were kept at 4 °C for 24 h. After the appropriate storage, each batch was stuffed into 30 mm dia cellulose casing of 150 mm length for a total of 9 sausages per treatment. Three sausages from each of the 5 mince treatments were then cooked using one of the 3 heat processes: 35 °C for 30 min; 90 °C for 30 min; and 35 °C for 30 min followed by 90 °C for 30 min. Immediately after cooking the sausages were placed in ice water for 1 h to cool. The cellulose casings were removed, sausages were cut into 30 mm tall cylinders, and cylinders were stored in food-grade plastic bags at 4 °C for no longer than 24 h.

Table 6: Sample abbreviations used to designate each of 15 cryoprotectant and freezing treatments used in the experimental design

Freezing and Cryoprotectant Treatment	Heat Process		
	35°C	**90°C**	**35/90°C**
Control	C35	C90	C35/90
No freezing with cryoprotectants	NFC35	NFC90	NFC35/90
No freezing with no cryoprotectants	NFNC35	NFNC90	NFNC35/90
Freezing with cryoprotectants	FC35	FC90	FC35/90
Freezing with no cryoprotectants	FNC35	FNC90	FNC35/90

Proximate Analysis

Fat, protein, ash, and moisture content were measured in all samples. Nitrogen content was analyzed using the Elementar Rapid N III nitrogen analyzer (Elementar Americas, Inc., Mount Laurel, N.J., U.S.A.). Total fat was determined using the SafTest (SafTest, Inc., Tempe, Ariz., U.S.A.) percent fat kit. Ash and moisture were both analyzed using AOAC methods (AOAC 2005). Moisture and protein analysis were performed in triplicate; ash and fat analysis were run in duplicate.

Water-Holding Capacity

The water-holding capacity of the gels was determined using the method described by Rouseel and Cheftel (1990) and modified by Alvarez and others (1992). Surimi-like gels were cut into approximately 3 mm cubes and 2 g of sample were centrifuged for 15 min at 1000 x *g*. Exudate was collected on Whatman filter paper. Samples were analyzed in triplicate and water-holding capacity was expressed as

percentage of water retained with respect to the water present in the gel prior to centrifugation.

Gel Strength

A penetration test was performed on 30 x 30 mm cylinders of each gel using a cylindrical stainless steel ball probe of 5 mm dia with a speed of 60 mm per min into 1 end of the sample. The probe was attached to a 25 N load cell connected to TA-XT2i texture analyzer (Technology Technologies, Scarsdale, N.Y., U.S.A.). Both the force (g) required for fracture and the distance (cm) to fracture were recorded. Nine measurements were taken per treatment.

Color

Ground sample from each of the 3 sausages per treatment were analyzed using the LabScan XE Hunter Lab colorimeter (Hunter Associates Laboratory, Reston, Va., U.S.A.). Each sausage was analyzed 3 times resulting in a total of 9 measurements for each treatment. Average L*, a*, and b* values were determined from the 9 measurements per treatment.

Statistical Analysis

The experimental design used for the study was an incomplete block design. A control of unwashed mince was subjected to the 3 heat processes (30 °C, 90 °C, and 35 °C followed by 90 °C), but not to the 2 levels of freezing (freezing and no freezing) or 2 levels of cryoprotectants (addition and no addition). Washed mince was subjected to all 3 treatment variables: heat process, freezing, and cryoprotectant. All possible treatment combinations were used resulting in an additional 12 treatments. There were a total of 15

treatments used in the design.

Analysis for water-holding capacity, force to fracture, and distance to fracture was conducted on the 12 washed samples only due to insufficient gel formation in the control. A 2 x 2 x 3 factorial design was used for this analysis. Factors used were 2 levels of cryoprotectants, 2 levels of freezing, and 3 heat processes. Color (L*, a*, and b*) values were evaluated on all 15 samples using an incomplete block design. Statistical analyses were performed using SAS (9.1) using least square means with Tukey mean separation. Significance was established at $p < 0.05$.

Results and Discussion

Average yield of washed mince obtained at the end of 3 washes was 49% of the starting material on a wet weight basis. Forty replicates of 200 g samples were used to calculate the average yield. A scale-up study using larger batch sizes would be beneficial to help determine the commercial value of this process.

Proximate analysis characterized the crab mince gels as having between 0.1% to 0.2% ash, <1% total fat, 11.2% to 18.2% protein, and 77.0% to 81.1% moisture content. Protein content of the gels varied with addition of cryoprotectants. Samples with no cryoprotectants had average protein contents of 15.0% to 18.2% whereas those with cryoprotectants had average protein contents of 11.2% to 12.8%. The control samples had higher average moisture contents at 84% than the treatment gels.

All treatments, excluding the unwashed control, formed gels from the previously cooked crab mince (Figure 5). Gels from washed mince were firm and had a hotdog-like consistency. The control did not form a gel under any of the 3 cooking procedures used. Therefore, only the 12 gels from the washed mince treatments could be analyzed for

water-holding capacity and texture.

Figure 5: Gelation of samples with the cryoprotectant and freezing treatments

Control sample gel formation, as shown in the top 3 samples, as compared to washed sample gels without cryoprotectants and no freezing (NCNF), bottom samples. Pictured on top from left to right: control heated at 35 °C for 30 min, control heated at 90 °C for 30 min, and control heated at 35 °C for 30 min followed by 90 °C for 30 min. Pictured on bottom from left to right: NCNF heated at 35 °C for 30 min, NCNF heated at 90 °C for 30 min, NCNF heated at 35 °C for 30 min followed by 90 °C for 30 min.

The water-holding capacity of the gels was significantly ($p < 0.05$) affected by both the freezing and cryoprotectant treatment. Samples that were not frozen (NFC, NFNC) had significantly ($p < 0.05$) higher water-holding capacity based on multi-way analysis of variance with an overall average of 63.3% water retained compared to those that were frozen (FC, FNC), which only retained an average of 56.6% of the water in the gel (Figure 6). The frozen samples had a lower water-holding capacity due to the denaturation of proteins during freezing, which would allow less water to be retained during processing (Jittinandana and others 2005). Samples with no cryoprotectants (FNC, NFNC) added had significantly ($p < 0.05$) higher water-holding capacity with an average 68.5% of the water being retained within the gel (Figure 6). Samples with cryoprotectants (FC, NFC) retained only an average of 51.3% of the water in the gel (Figure 6). The addition of cryoprotectants to washed mince is well known to reduce the rate of protein denaturation during frozen storage (Jittinandana and others 2005). In washed crab mince, adding cryoprotectants may have reduced the protein denaturation during freezing; however the extent of the denaturation was not measured. The crab mince was already denatured due to the commercial cooking process. The lower water holding capacity observed in the cryoprotectant treatments may have been due to the cryoprotectants in the crab mince interfering with the formation of a protein network between less denatured proteins thereby preventing a strong protein gel from forming (Yoon and others 1991). The cooking temperature had no impact on the water holding capacity of the gels. Lin and others (1995) found that Pacific whiting surimi gels had a water-holding capacity of 60% to 65%, which was comparable to the average water-holding capacity of the gels from the non-frozen crab mince treatments.

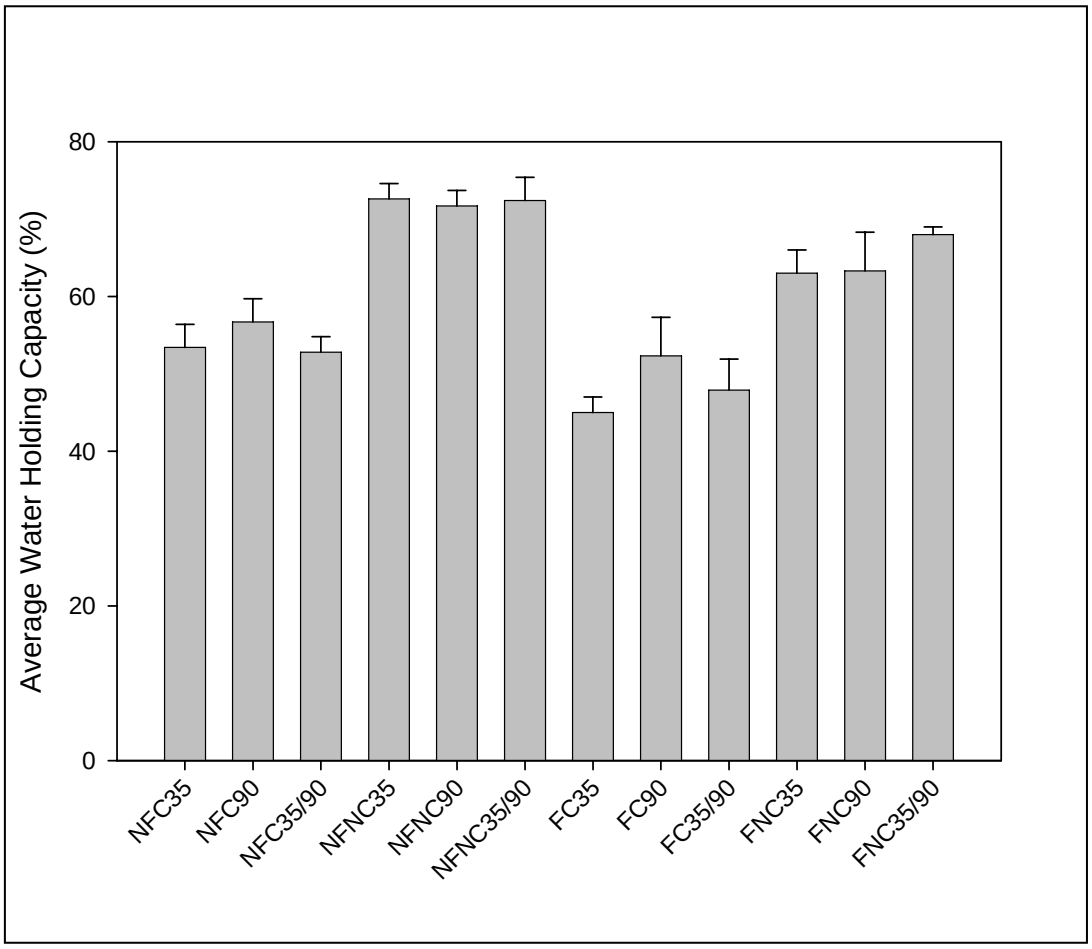

Figure 6: Water-holding capacity (percent water retained in gel) of crab mince gels from cryoprotectant and freezing treatment

Values shown are averages of three measurements with standard deviations. Sample codes: NFC35, NFC90, and NFC35/90 (non frozen washed mince with cryoprotectants heated at 35°C, 90°C, and both 35°C and 90°C, respectively); NFNC35, NFNC90, and NFNC35/90 (non frozen washed mince with no cryoprotectants heated at 35°C, 90°C, and both 35°C and 90°C, respectively); FC35, FC90, and FC35/90 (frozen washed mince with cryoprotectants heated at 35°C, 90°C, and both 35°C and 90°C, respectively); FNC35, FNC90, and FNC35/90 (frozen washed mince with no cryoprotectants heated at 35°C, 90°C, and both 35°C and 90°C, respectively).

Alvarez and Tejada (1997), on the other hand, produced sardine surimi suwari gels with water-holding capacities of 75% to 80% dependent on setting time and temperature. Although the water-holding capacities obtained for the crab mince gels were lower than those for sardine surimi suwari gels, they were surprisingly higher than expected given that the crab mince had already been cooked and frozen.

Both the cryoprotectant and cooking process had a significant ($p < 0.05$) effect on the force to fracture of the gels, but only the cooking process had an effect on the distance to fracture of the gels (Figure 7 and 8). Samples with no added cryoprotectants (FNC and NFNC) had significantly ($p < 0.05$) higher gel strength with an average value of 194.6 g compared to that of samples with cryoprotectants (FC and NFC) at 30.8 g (Figure 7). The addition of cryoprotectants did not result in a significant ($p > 0.05$) difference in distance to fracture. Cooking treatment significantly ($p < 0.05$) impacted both gel properties. A 2-stage heating process (35 °C for 30 min followed by 90 °C for 30 min) had the significantly ($p < 0.05$) highest average mean force to fracture of 160.3 g followed by the 90 °C for 30 minutes with an average force of 118.9 g and the 35 °C for 30 min treatment having the lowest mean force to fracture at 72.6 g (Figure 7). Samples cooked at 35 °C had the greatest average distance to fracture at 0.79 cm that was significantly ($p < 0.05$) higher than both the other cooking treatments with averages of 0.61 cm to 0.67 cm (Figure 8). The application of a freezing treatment (FC, NFC) did not significantly ($p > 0.05$) impact gel strength.

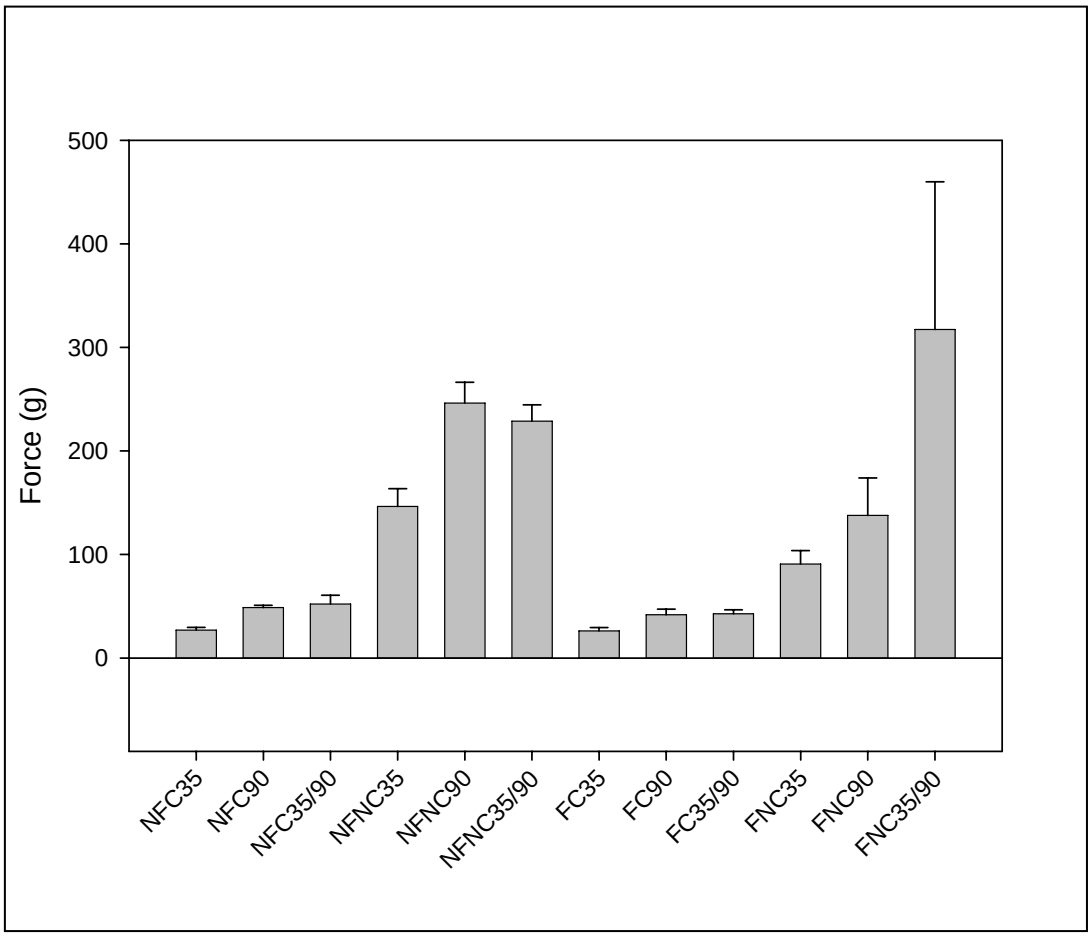

Figure 7: Force to fracture (g) of crab mince gels treated with cryoprotectants and freezing treatment

Values shown are averages of nine measurements with standard deviations. Sample codes: NFC35, NFC90, and NFC35/90 (non frozen washed mince with cryoprotectants heated at 35°C, 90°C, and both 35°C and 90°C, respectively); NFNC35, NFNC90, and NFNC35/90 (non frozen washed mince with no cryoprotectants heated at 35°C, 90°C, and both 35°C and 90°C, respectively); FC35, FC90, and FC35/90 (frozen washed mince with cryoprotectants heated at 35°C, 90°C, and both 35°C and 90°C, respectively); FNC35, FNC90, and FNC35/90 (frozen washed mince with no cryoprotectants heated at 35°C, 90°C, and both 35°C and 90°C, respectively).

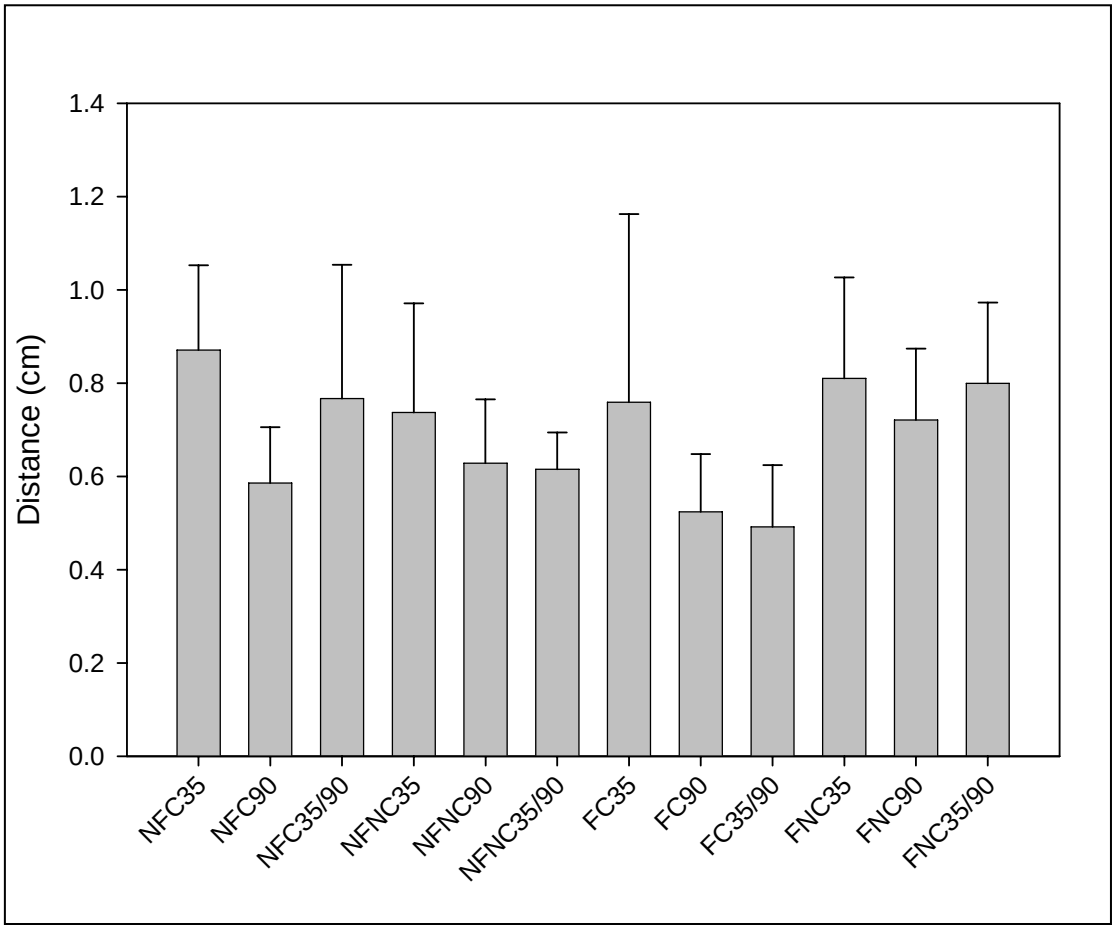

Figure 8: Distance to fracture (cm) of crab mince gels treated with cryoprotectants and freezing treatment

Values shown are averages of nine measurements with standard deviations. Sample codes: NFC35, NFC90, and NFC35/90 (non frozen washed mince with cryoprotectants heated at 35°C, 90°C, and both 35°C and 90°C, respectively); NFNC35, NFNC90, and NFNC35/90 (non frozen washed mince with no cryoprotectants heated at 35°C, 90°C, and both 35°C and 90°C, respectively); FC35, FC90, and FC35/90 (frozen washed mince with cryoprotectants heated at 35°C, 90°C, and both 35°C and 90°C, respectively); FNC35, FNC90, and FNC35/90 (frozen washed mince with no cryoprotectants heated at 35°C, 90°C, and both 35°C and 90°C, respectively).

The gelation properties of muscle proteins are highly dependent on the species. For example, Jiang and others (2000) found pollock surimi gels required approximately 900 g of force to fracture at a distance of 0.16 cm. Yongsawatdigul and Park (2004) found that rockfish surimi gels required only 340 g of force to fracture at a distance of 0.13 cm. Atlantic croaker surimi gels were found to require even less at 75 g of force to fracture at a distance of 0.06 cm (Perez-Mateos and others 2004). The washed crab mince gels exhibited gel strength between the rockfish surimi and Atlantic croaker surimi. The crab mince gels were similar to the Atlantic croaker gels in terms of force to fracture, though overall the distance to fracture was greater in the crab mince than in other species.

Color analysis (Table 7) indicated that freezing, addition of cryoprotectants, and heating process all impacted L* values. Unwashed mince (control) treatments were significantly ($p < 0.05$) more white than both frozen and non-frozen samples, as well as those with and without cryoprotectants. Non-frozen samples were more white than frozen samples as were those with cryoprotectants compared to those without. Samples that received the 2-stage heat process were significantly ($p < 0.05$) more white than both of the other heat processes. The higher L* values for the unwashed control mince treatments were unexpected because with traditional surimi, L* values increase with number of washings. Yongsawatdigul and Park (2004) found that rockfish surimi had an L* value of 81.22 which was higher than what was obtained for the washed crab mince gels, which ranged from 62.40 to 69.76. Mexican flounder surimi gel had an L* value of 82.45, which was also higher than that found in the washed crab mince gels (Tellez-Luis and others 2004). In traditional surimi processing, washing removes water soluble

colorants, thereby increasing L* values. In this case, L* decreased with washing.

Table 7: Hunter color values (L*, a*, b*) of crab mince gels from cryoprotectant and freezing treatments

Sample Code	L*	a*	b*
C35	70.92 ± 0.56	2.24 ± 0.08	13.48 ± 0.07
C90	70.34 ± 0.10	3.76 ± 0.29	13.38 ± 0.07
C35/90	70.37 ± 0.61	1.83 ± 0.26	12.14 ± 0.14
NFC35	69.47 ± 0.03	8.56 ± 0.04	16.33 ± 0.07
NFC90	69.76 ± 0.22	8.05 ± 0.09	16.29 ± 0.11
NFC35/90	69.66 ± 0.52	7.73 ± 0.07	16.03 ± 0.02
NFNC35	66.69 ± 0.02	8.92 ± 0.02	16.84 ± 0.06
NFNC90	65.38 ± 0.20	7.67 ± 0.06	16.59 ± 0.99
NFNC35/90	66.21 ± 0.28	8.08 ± 0.03	16.32 ± 0.20
FC35	66.48 ± 0.17	7.94 ± 0.06	16.14 ± 0.04
FC90	67.34 ± 0.23	7.02 ± 0.05	16.16 ± 0.09
FC35/90	67.79 ± 0.36	6.95 ± 0.09	15.74 ± 0.15
FNC35	62.40 ± 0.07	7.94 ± 0.09	15.95 ± 0.19
FNC90	64.17 ± 0.04	7.50 ± 0.02	16.64 ± 0.28
FNC35/90	66.02 ± 0.32	8.00 ± 0.12	18.19 ± 0.59

Values shown are the average of three measurements ± the standard deviation. Sample codes: NFC35, NFC90, and NFC35/90 (non frozen washed mince with cryoprotectants heated at 35°C, 90°C, and both 35°C and 90°C, respectively); NFNC35, NFNC90, and NFNC35/90 (non frozen washed mince with no cryoprotectants heated at 35°C, 90°C, and both 35°C and 90°C, respectively); FC35, FC90, and FC35/90 (frozen washed mince with cryoprotectants heated at 35°C, 90°C, and both 35°C and 90°C, respectively); FNC35, FNC90, and FNC35/90 (frozen washed mince with no cryoprotectants heated at 35°C, 90°C, and both 35°C and 90°C, respectively).

All factors had a significant ($p < 0.05$) effect on a* values. Non-frozen samples (NFC, NFNC) were significantly ($p < 0.05$) more red than frozen (FC, FNC) samples and the control. Samples with no cryoprotectants (FNC, NFNC) were significantly ($p < 0.05$) more red than those with cryoprotectants (FC, NFC) or the control. Again, the higher a* values in the washed samples was surprising because washing was expected to reduce the colorants in the mince. In this case it increased a* values. The unwashed control had average a* values ranging from 1.83 to 3.76 while samples with no cryoprotectants and no freezing had average values ranging from 7.67 to 8.92. The only difference between the control and the other treated samples was the wash step intended to remove soluble compounds that might interfere with gel formation. Heat process also significantly ($p < 0.05$) impacted a* values. The samples cooked with the 2-stage heat process were less red than the other heat processes.

The b* values were less affected by the various treatments. Heat processing did not significantly ($p > 0.05$) affect the b* values of the samples. Freezing and no freezing treatments were not significantly ($p > 0.05$) different from one another but were significantly ($p < 0.05$) more yellow than the control. The samples with cryoprotectants were significantly ($p < 0.05$) different than those without cryoprotectants. Samples with no cryoprotectants had the highest b* values, with the control having the lowest b* values.

Since a traditional surimi process was used to treat the previously cooked mince, color was measured. In raw fish surimi, a high L* value and low a* and b* values are indicative of high-quality surimi; therefore washed cooked crab mince was compared to traditional raw fish surimi. Raw fish becomes more white with washing, whereas the

opposite was true for the previously cooked Jonah crab mince. This study showed that both a* and b* increased in the mince that was washed. In contrast to fish, crabs and other crustaceans contain the blue colored oxygen-carrying pigment hemocyanin as well as blue and red colored carotenoproteins, which may affect the color of the mince (Garate and others 1984; Garcia-Carreno and others 1999; Terwilliger and others 2005). Endogenous enzymes and chemical reactions such as Maillard browning and oxidative discoloration may also influence the color of the crab meat during processing (Boon 1975). Though color may not be important for further manufacturing of gels, it is interesting to note that the previously cooked mince did not respond similarly to raw fish when washed.

Conclusion

We believe this is the 1st study to demonstrate gel formation in previously cooked muscle proteins. Further studies are underway to investigate the mechanism involved in gelation of these cooked crab proteins. Washing the mince aided in gel formation and the washed, cooked proteins displayed gelling characteristics with water-holding capacity comparable to Pacific whiting surimi. The gel strength was weaker than traditional Pollock surimi, but stronger than Atlantic croaker surimi gels. The addition of cryoprotectants did not aid in improved gel characteristics and in most cases reduced the quality as measured by both water-holding capacity and gel strength. Freezing of the washed mince resulted in lower water-holding capacity but did not affect the force to fracture of the gels. The use of a 2-stage heating process produced a crab mince gel with the highest strength and whitest color. Based on these results, washed crab mince from previously cooked Jonah crab meat can produce gels with water-holding capacity and gel

strength similar to those of surimi from some raw fish.

The gelation properties of the previously cooked crab mince suggest that it can be used as a major component in protein gel-based products. Investigations are currently under way to evaluate the effects of carbohydrate and protein additives on the textural attributes of the crab mince gels. Ultimately, the cooked crab mince will be utilized in the formation of value-added products such as sausages, restructured meats, or nuggets.

Acknowledgements

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CHAPTER 3. GELATION PROPERTIES OF PREVIOUSLY COOKED MINCED MEAT FROM JONAH CRAB (*CANCER BOREALIS*) AS AFFECTED BY WASHING TREATMENT AND SALT CONCENTRATION

Chapter Abstract

Jonah crab processing involves boiling live product followed by removal of a portion of the edible tissue resulting in large quantities of by-products. In this study two factors, washing treatment (dewatered only, one wash, and three washes) and sodium chloride (NaCl) concentration (0%, 2%, and 4%), were investigated to determine the impact of each on the gelation properties of cooked Jonah crab mince. Gels were tested for color, water holding capacity (WHC), gel strength, and electrophoretic profile. Washing treatment and NaCl concentration affected the color of gels. Four percent NaCl increased L* and a* values, and b* values decreased with increased washing. Washed samples exhibited significantly higher WHC, retaining 14% more water than dewatered samples. The 4% NaCl treatment decreased the WHC by 13% compared to lower NaCl levels. Multiple washing steps increased the force to gel deformation, doubling the force required for dewatered samples. Based on these results, cooked Jonah crab meat treated with three washes and 0% NaCl resulted in the strongest gels with the best water holding capacity.

Introduction

Jonah crab (*Cancer borealis*) is a by-catch of the lobster industry with 578 metric tons of crab landed in Maine and 1456 metric tons landed in the United States in 2003 (NMFS 2003). Jonah crab claws have seen an increase in consumer demand due to their

mild flavor and relatively low cost (Redmayne 2002). Though the claws have become more popular, much of the remaining crab meat is wasted after removal of the claws. A mechanical deboner can be used to produce low-value cooked crab mince from the walking legs and body that is added to bisques, soups, stuffings, and crab cakes (Portland Shellfish, Portland, ME, U.S.A.). However, newer higher value uses for the crab mince are needed to increase profitability and total utilization of the resource.

Underutilized fish species and by-products of fish processing can be used in the development of new products to gain a greater value from the original resource. Processing these meat byproducts into surimi is one way of increasing utilization of the resource. Earlier research showed that previously cooked minced crab meat could be used in the formation of surimi-like gels (Baxter and Skonberg 2006). In traditional surimi processing, sodium chloride is added to raw muscle mince to aid in gel formation. The sodium chloride initiates the conversion of surimi into a *kamaboko* style gel by forming an actomyosin sol from the actin and myosin present (Hall and Ahmad 1997). Typically, between 2% to 3% sodium chloride has been added to aid in gel formation. However, Lin and others (1995) showed that significant amounts of myofibrillar proteins in fish are solubilized in very low (near zero) ionic strength solutions. In raw cod muscle, the solubility of proteins in near zero ionic strength solutions was greater than the solubility at high ionic strengths (Stefansson and Hultin 1994). Solubilization of muscle proteins occurs in two steps: depolymerization of thick filaments followed by dissociation of actin from myosin (Parsons and Knight 1990). The salt content of a solution can affect the solubility of the proteins present. Low salt concentrations allow the proteins to be surrounded by salt ions of opposite charge which decreases the

electrostatic free energy leading to increased activity of the solvent resulting in greater solubility of proteins (Debye and Huckel 1923). This increased solubility is referred to as salting-in. The salting-in phenomenon is attributed to nonspecific electrostatic interactions between charged proteins and the ionic environment which leads to solubilization (von-Hippe and Schleich 1969). As the salt concentration in a solution is increased, the salt ions compete with the proteins for binding sites on the water molecules. This results in greater protein-protein interactions and fewer protein-solvent interactions leading to the precipitation of proteins. Salting-out occurs at high salt concentrations and is attributed to the loss of a stable hydrophilic surface, causing exposed hydrophobic areas of the proteins to interact, aggregate, and precipitate. Both salting-in and salting-out are affected by ion type, with calcium ions promoting salting in and sulfate ions promoting salting-out. In the case of cod, all of the proteins in the cod muscle were soluble when the ionic strength was reduced below 0.025 (Stefansson and Hultin 1994). The contradictory research, showing that proteins are most soluble at both high and low ionic strengths, suggest that traditionally used levels of sodium chloride may be higher than what is needed for gelation of muscle proteins.

Processing of raw fish into surimi requires several washing steps. The washing step not only removes fat, sarcoplasmic proteins, and other undesirable substances such as blood, pigments and odorous compounds, but also concentrates the myofibrillar proteins to enhance gelation (Mendes and Nunes 1992). The number of washing cycles and ratio of water to mince needed varies with fish species, initial condition of the fish, and the type of operation, either continuous or batch processing (Lee 1984). In general, a five minute agitation in each of two washing cycles using a 3:1 (water to mince) ratio has

been considered adequate for raw fish surimi production (Lee 1986). Pacheco-Aguilar and others (1989) investigated the effect of washing in Pacific whiting surimi production. Removal of lipids was not efficiently achieved using a single wash with a 3:1 ratio (water to mince) but resulted in high solids and protein recoveries which are often low in multiple exchange washing procedures. Up to 38% of the solids were lost in the third wash cycle (Lin and Park 1996). Reducing the number of washing cycles is important in overall recovery of solids and maximization of yield as well as decreasing the wash water volume.

Previous studies by other researchers have focused on gelation of raw fish muscle. The purpose of this study was to investigate the impact of washing and sodium chloride addition on the gelation of cooked muscle proteins. The denaturation of the cooked crab minced meat was expected to increase the number of washing cycles and the amount of sodium chloride needed for gelation. The objectives of this study were to determine the effects of washing treatment (dewatered only, one wash, and three washes) and sodium chloride level (0%, 2%, and 4%) on the gel formation of previously cooked Jonah crab meat mince.

Materials and Methods

Frozen, commercially available Jonah crab meat mince processed in the following manner was obtained from Portland Shellfish (Portland, Maine, U.S.A.): crabs had been eviscerated, boiled for 14 minutes without the top portion of the carapace, and cooled. Cooked crabs were kept on ice until mince was separated later the same day. Claws were removed and the remaining meat was separated using a mechanical deboning machine. The mince consisted of meat from the body and walking legs. Ten pounds of mince

were packaged in polyethylene bags and frozen.

Mince Processing

A 10-pound block of frozen Jonah crab meat mince was thawed at 4 °C for 48 h. The thawed mince was subjected to one of three wash procedures: 1) dewatered only, 2) dewatering followed by one wash, or 3) dewatering followed by three washes. Due to the high water content of the thawed mince it was dewatered via centrifugation at 15,000 rpm for 10 minutes. Dewatered mince was then further washed in 200 g batches in a Waring blender for 1 minute using a ratio of 4:1 (water to mince). A cold (4 °C) 0.3% sodium chloride solution was used for the one wash treatment. For the three wash treatment, two washes of cold deionized water were followed by a third wash using a cold 0.3% sodium chloride solution. Mince was dewatered via centrifugation at 15,000 rpm for 10 minutes after each wash. The supernatant from each wash was collected and stored at 4 °C for further analysis. Once washed and dewatered, approximately 500 g of the mince was mixed with 0%, 2%, or 4% sodium chloride in a stand mixer (Kitchenaid, St. Joseph, MI, U.S.A.) with paddle attachment for five minutes. The moisture of the mince was adjusted to 80%. Mince was then stuffed into 30 mm cellulose casings (Nojax E-Z Peel; Viskase, Kentland, IN, U.S.A.) using a hand operated sausage stuffer (The Sausage Stuffer, Buffalo, NY, U.S.A.) to form approximately 3 sausages per treatment. The mince was cooked at 35 °C for 30 minutes followed by 90 °C for 30 minutes and then cooled on ice for one hour. The cellulose casings were removed, gels were cut into 30-mm tall cylinders, and the cylinders were stored in food-grade plastic bags at 4 °C for no more than 24 h before testing.

Proximate Analysis

Gels from each treatment were pooled and ground to create one homogenous sample per treatment for proximate analysis. Fat, protein, ash, and moisture content were measured in all samples. Nitrogen content was analyzed using the Elementar Rapid N III nitrogen analyzer (Elementar Americas, Inc., Mount Laurel, N.J., U.S.A.) to determine total protein content. Total fat, ash, and moisture were analyzed using AOAC methods, methods # 922.96, 938.08, and 950.46, respectively (AOAC 2005). Moisture, ash, and protein analysis were performed in duplicate, and fat analyses were performed in triplicate.

Water-Holding Capacity

The water-holding capacity of the crab mince gels was determined using the method described by Rouseel and Cheftel (1990) and modified by Alvarez and others (1992). The gels in each treatment were pooled and cut into approximately 3-mm cubes. Approximately 2 g of sample was centrifuged for 15 min at 1000 x *g* and the exudate was collected on Whatman No. 1 filter paper. Samples were analyzed in triplicate and water-holding capacity was expressed as percentage of water retained with respect to the water present in the gel prior to centrifugation.

Gel Strength

A penetration test was performed on 30 x 30 mm cylinders of each gel using a cylindrical stainless steel ball probe of 5 mm dia with a speed 60 mm per min into 1 end of the sample. The probe was attached to a 25 N load cell connected to TA-XT2i texture analyzer (Texture Technologies, Scardale, N.Y., U.S.A.). Both the breaking force (g)

and deformation (cm) were recorded. Nine measurements were taken per treatment.

Color

Ground sample was pooled from each of the 3 gels per treatment and analyzed using the LabScan XE Hunter Lab colorimeter (Hunter Associates Laboratory, Reston, Va., U.S.A.). Three sub-samples from each treatment were analyzed 3 times, resulting in a total of 9 measurements for each treatment. Average L*, a*, and b* values were determined from the 9 measurements per treatment.

Protein Molecular Weight Identification

The molecular weight of proteins in each gel sample and wash water supernatant was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Samples were solubilized in a SDS-urea solution containing 2% SDS, 8 M urea, 50 mM Tris-HCl (pH 8.0), and 2% 2-mercaptoethanol heated at 100 °C for 2 minutes and incubated overnight at 25 °C (Seki and others 1990; Ni and others 1998; Park and others 2003; Yoshida and others 2003). SDS-PAGE was performed by the Laemmli (1970) method using a 10% separating and 4% stacking polyacrylamide gel (Sathivel and others 2003; Esturk and others 2004). Proteins were separated using a Bio-Rad Mini-PROTEAN III Cell (Bio-Rad Laboratories, Inc., Hercules, CA, U.S.A.). The Precision Plus Protein Duel Color Standard (Bio-Rad Laboratories, Inc., Hercules, CA, U.S.A.) was used for comparison. Separated proteins were stained with 0.125% Coomassie brilliant blue R-250 (Mallinckrodt Baker, Phillipsburg, NJ) and destained in a solution containing 10% methanol and 10% acetic acid. The total protein concentration in the wash water supernatant was determined using the method by Lowry and others (1951).

All analyses were conducted in duplicate.

Statistical Analysis

A 3 x 3 factorial randomized complete block design was used for statistical analysis. Three wash treatments (dewatered only, dewatered followed by one wash, and dewatered followed by three washes) and three sodium chloride levels (0%, 2%, and 4%) were used resulting in 9 total treatments. Multi-way analysis of variance of water-holding capacity, breaking force, deformation, L*, a*, and b* values, and proximate composition was conducted on all nine treatments. Statistical analyses were performed using SAS (9.1) using least square means with Tukey mean separation. Significance was established at $p < 0.05$.

Results and Discussion

Proximate Analysis

Proximate analysis characterized the different crab mince gels as having less than 1% fat, 5.2 to 6.7% ash, 13.5 to 16.9% protein, and 79.6 to 81.0% moisture (Table 8). Based on multi-way analysis of variance, the crude fat content was significantly greater ($p < 0.05$) in the control (dewatered only) with an average of 1.0% fat than the other wash treatments at 0.9% fat for one wash and 0.7% fat for three washes. The addition of salt did not affect the fat content of the samples. The ash content was not significantly ($p > 0.05$) affected by either wash treatment or addition of sodium chloride. The moisture content of the gels was significantly ($p < 0.05$) higher in gels with 4% sodium chloride with 80.7% average moisture. Gels with 2% sodium chloride averaged 80.1% moisture and gels with no sodium chloride averaged 80.0% moisture. The protein content of the

gels was not significantly ($p < 0.05$) affected by wash treatment but was affected by addition of sodium chloride. Sample with no sodium chloride had a greater percentage of protein (16.6%) than samples with 2% sodium chloride (15.4%) or those with 4% sodium chloride (13.9%). This difference was due to the increase in non-protein solids which lowered the overall percentage of protein in the sample.

Table 8: Proximate analysis of cooked crab mince gels treated with varying wash treatments and sodium chloride levels

Sample	% Crude Fat	% Ash	% Protein	% Moisture
Control 0% NaCl	1.1 ± 0.2	6.7 ± 0.2	16.7 ± 0.3	79.6 ± 0.7
Control 2% NaCl	1.0 ± 0.1	6.9 ± 0.1	14.9 ± 0.3	80.6 ± 0.4
Control 4% NaCl	0.9 ± 0.1	6.6 ± 0.2	13.6 ± 0.3	81.0 ± 0.3
One wash 0% NaCl	0.9 ± 0.1	6.5 ± 0.0	16.3 ± 0.9	80.5 ± 0.6
One wash 2% NaCl	0.8 ± 0.1	6.5 ± 0.1	15.6 ± 0.6	79.9 ± 0.7
One wash 3% NaCl	0.8 ± 0.1	6.6 ± 0.2	14.2 ± 0.4	80.6 ± 0.6
Three washes 0% NaCl	0.6 ± 0.0	6.6 ± 0.1	16.8 ± 0.2	79.9 ± 0.4
Three washes 2% NaCl	0.8 ± 0.2	6.5 ± 0.1	15.7 ± 0.7	79.9 ± 0.9
Three washes 4% NaCl	0.7 ± 0.5	6.6 ± 0.1	13.9 ± 1.0	80.6 ± 1.1

Values shown are averages of four measurements with standard deviations.

Water-Holding Capacity

The water holding capacity of the gels was significantly ($p < 0.05$) affected by both the washing treatment and sodium chloride levels (Figure 9). Multi-way analysis of variance of gels from mince that was washed, either once or three times, had significantly ($p < 0.0.5$) higher water-holding capacity than gels from the control (dewatered only).. Previous research conducted by Baxter and Skonberg (2006) showed that gels from washed crab mince and 2.5% sodium chloride produced gels with 68.5% water-holding capacity. The washed gels from this study were similar in water-holding capacity (70.9%), whereas gels from control mince (dewatered only) retained on average only 60.3% of the original water. The water-holding capacity for these gels is also comparable to that of surimi from raw Pacific whiting at 60-65% retained water (Lin and others 1995). The washed crab mince gels did not retain as much water as sardine surimi suwari gels, which retained 75-80% water (Alvarez and Tejada 1997). Karthikeyan and others (2006) found that 10% more water was retained in the gel after three washes than with no washing in surimi made from raw threadfin bream (*Nemipterus japonicus*). Differences in water-holding capacity are dependent on the stability of the protein network. Washing of the crab mince removed components, such as fat, that may interfere with the stability of this protein network, therefore increased washing resulted in gels with a higher water-holding capacity.

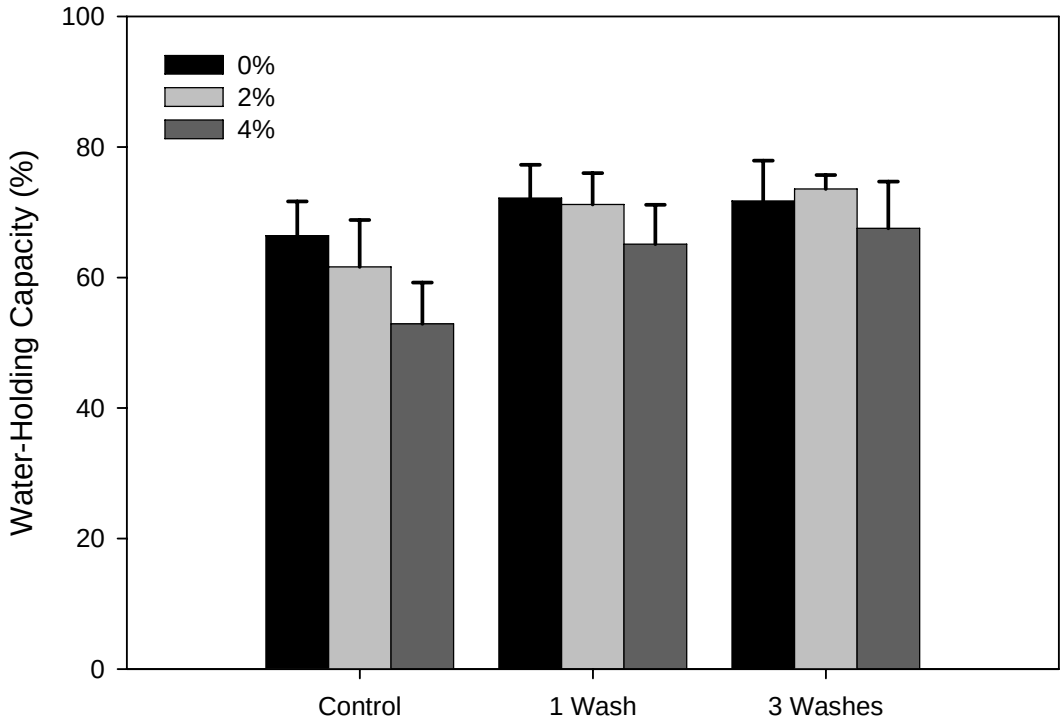

Figure 9: Water-holding capacity (percent water retained) of crab mince gels as affected by sodium chloride level and number of washes.

Values shown are averages of three measurements with standard deviations.

Sodium chloride level also affected the water-holding capacity of the crab mince gels. Multi-way analysis of variance showed that the addition of 4% sodium chloride significantly ($p < 0.05$) decreased the water holding capacity of gels compared to the two other salt treatments (Figure 9). Addition of sodium chloride above the isoelectric point of the proteins in a meat system caused swelling of the proteins which led to a greater water-holding capacity (Honikel 1989). In raw fish surimi, the water-holding capacity of the fish does increase with increased sodium chloride concentration. Tellez-Luis and others (2002) found that the water retained by raw silver carp (*Hypophthalmichthys molitrix*) surimi increased as the sodium chloride concentration increased from 0 to 20 g kg^{-1}. The same held true in restructured hams made from raw silver carp. The water-holding capacity of the hams was greater with 2% sodium chloride than with 0% by almost 3-fold (Ramirez and others 2002). The trend observed in raw fish is opposite of that seen in the washed, cooked, crab mince gels. Gels with 0% added sodium chloride retained an average of 70.1% of the water within the gel, whereas gels with 4% sodium chloride retained only 61.9% water. In raw fish, the increased water-holding capacity is related to the isoelectric point of the fish proteins. Though not investigated in this study, the isoelectric point of the cooked crab proteins may be different from that of raw fish, resulting in the unexpected relationship between salt concentration and water-holding capacity.

Breaking Force and Deformation

Breaking force (g of force) was affected by both the number of washes and amount of added sodium chloride in the gels. The force significantly ($p<0.05$) increased with increasing number of wash treatments. Gels from mince washed three times

required on average 175.5 g of force, gels from mince washed once required 117.4 g, and gels from dewatered mince required on average 83.1 g of force (Figure 10). Previous work showed that gels made from washed crab mince and 2.5% sodium chloride had an average breaking force ranging from 160.3 g to 194.6 g (Baxter and Skonberg 2006). The breaking force of the washed crab mince gels in this study have similar values to gels from previous work. Washing concentrates the myofibrillar proteins which may result in a stronger breaking force due to a stronger protein network (Chen and others 1997); this correlates well with the results from the washed cooked crab mince gels. Lee (1986) found that the breaking force of surimi made from raw fish increased with up to two washings, at which point the breaking force leveled off.

The breaking force (g of force) significantly ($p < 0.05$) decreased with increasing sodium chloride concentration (Figure 10). Gels without additional sodium chloride were the firmest (175.9 g) whereas gels with 4% sodium chloride were the softest (72.9 g).. This may be due, in part, to the decreased protein concentration due to the addition of salt. These results are unique when compared to gels made from raw fish muscle. Kubota and others (2006) found that walleye pollock (*Theragra chalcogranmma*) surimi breaking force increased with increasing salt concentration, with a maximum breaking force at 3% sodium chloride. The same was found in another study investigating walleye pollock. Akahane and Shimizu (1989) found that the breaking force of walleye pollock surimi increased when up to 3% sodium chloride was added. In the crab mince gels, deformation was affected only by the number of washes and not the sodium chloride content. Gels made from dewatered mince exhibited significantly ($p < 0.05$) greater deformation than either of the washed sample gels (Figure 10).

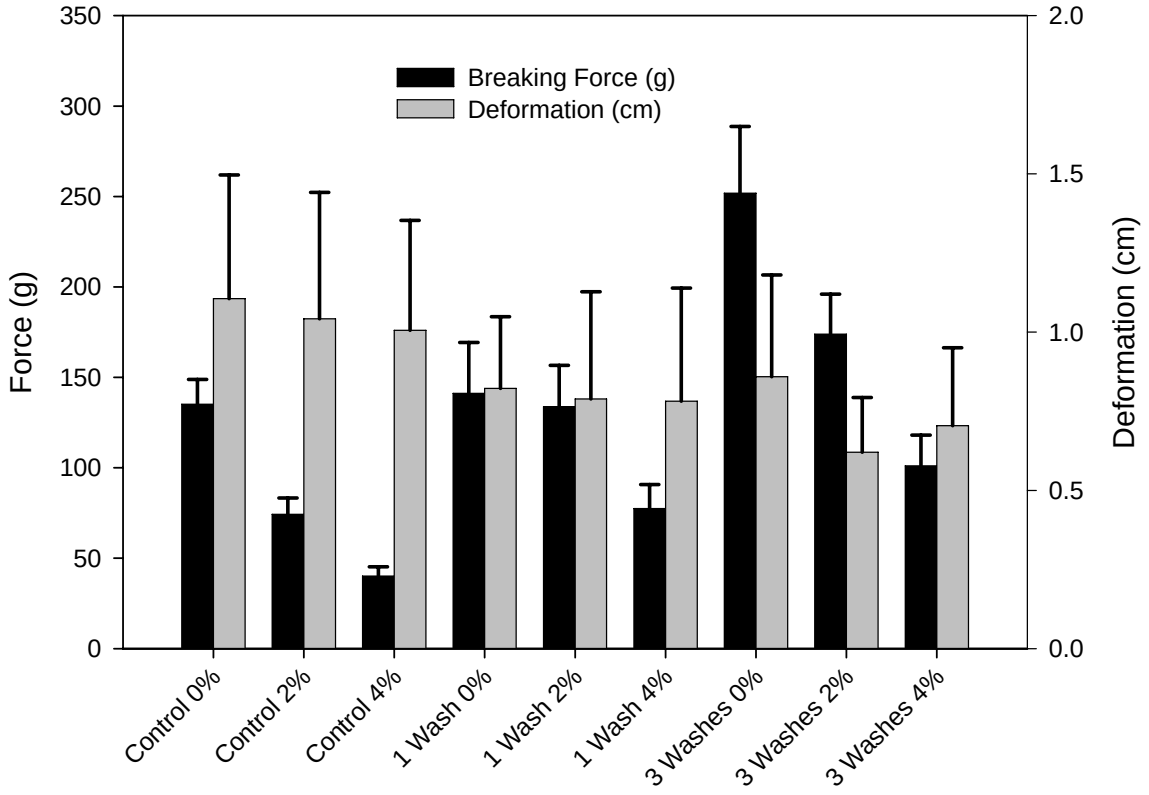

Figure 10: Breaking force (g) and deformation (cm) of crab mince gels as affected by sodium chloride level and number of washings.

Values shown are averages of 9 measurements with standard deviations.

Color

The L* values were not significantly affected by the number of washings but increased significantly ($p < 0.05$) with the addition of sodium chloride. The lack of change in L* values in response to increased washing was surprising, given that washing usually increases L values in raw surimi. Gels with 4% sodium chloride had average L* values of 62.4 whereas those with no or 2% sodium chloride had average L* values of 61.2 (Figure 11).

A* values (redness) were significantly ($p < 0.05$) affected by both the number of washing cycles and the addition of sodium chloride. Dewatered mince gels were more red (13.9) than those washed once (13.3) and those washed three times (10.8) (Figure 11). In the previous work done with cooked crab mince, a* values increased with washing, which is contradictory to the current results. Washing reduced the redness whereas the addition of sodium chloride increased the redness. Gels with 4% sodium chloride were significantly more red (12.9) than those with no sodium chloride (12.4).

Although yellowness (b* value) was not affected by addition of sodium chloride, it was significantly affected by the number of washings. Gels made from mince washed three times were significantly ($p < 0.05$) less yellow than those washed once or only dewatered. Average b* values for gels made from mince washed once or from dewatered mince ranged from 21.1-21.9, whereas average b* values for gels from mince washed three times was 19.1 (Figure 11).

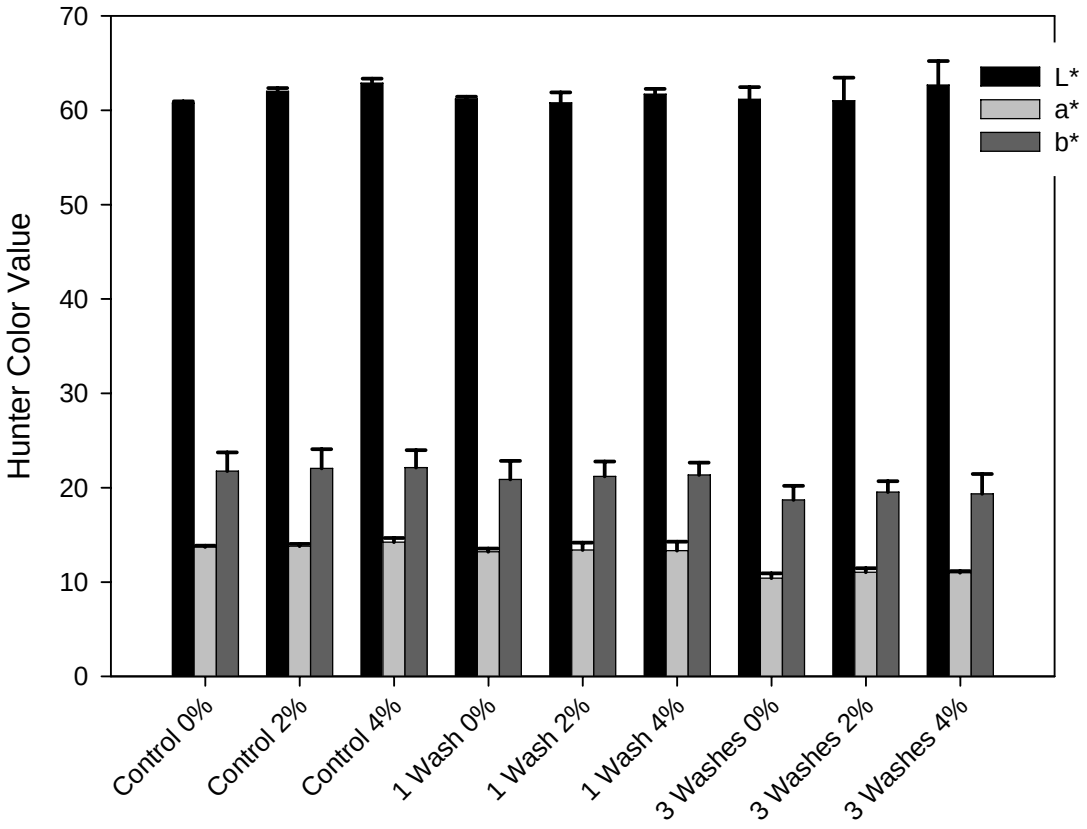

Figure 11: Hunter color values (L*, a*, b*) of crab mince gels as affected by sodium chloride level and number of washes.

Values shown are the average of three measurements with standard deviations.

Muscle tissue color is dependent on season, age of crabs, and dietary composition. In surimi, color is an important quality indicator (Lanier 1992). The lighter the washed muscle, the higher the quality of surimi. In washed crab mince, color may not be as important. Crabs and other crustaceans contain the blue colored oxygen-carrying pigment hemocyanin as well as blue and red colored carotenoproteins, which may affect the color of the mince (Garate and others 1984; Garcia-Carreno and others 1999; Terwilliger and others 2005). Browning may also occur during the cooking process that may influence the color of the crab meat during processing (Boon 1975). The color of the washed crab mince may not be as important of a quality indicator as it is in surimi processing. In fact, for new food product development, a more intense color of the meat may enhance the product properties.

Protein Molecular Weight

Proteins in the crab mince, gels, and wash water were analyzed using SDS-PAGE. SDS-PAGE gels (Figure 12) indicated that the crab mince gels consisted of proteins in the 190 kDa range, 75-100 kDa range, and 36-45 kDa range. The wash water contained a protein band at 37 kDa, identified as likely one strand of the tropomyosin molecule, with some small proteins in the 18-20 kDa ranges. The band at 190 kDa was probably the myosin heavy chain (Thorarinsdottir and others 2002). As the major protein involved in meat gelation, the presence of myosin heavy chain in the gels was expected. The banding in the 36 to 45 kD range was believed to contain both the actin and tropomyosin. The actin, which is also integral to gelation, was expected in all of the gels. The band identified as likely tropomyosin, 37 kDa, was found in both the gels and wash supernatant. French (1986) found that the stiffness of the Alaska pollock surimi gels was

inversely correlated to tropomyosin content. The relationship between tropomyosin content and breaking force was also seen in the crab mince gels. The breaking force of the gels increased with increasing number of wash cycles, resulting in less tropomyosin within the gel. The protein bands in the 18 to 20 kDa range in the gels and wash supernatant were correlated with myosin light chain and troponin (Thorarinsdottir and others 2002).

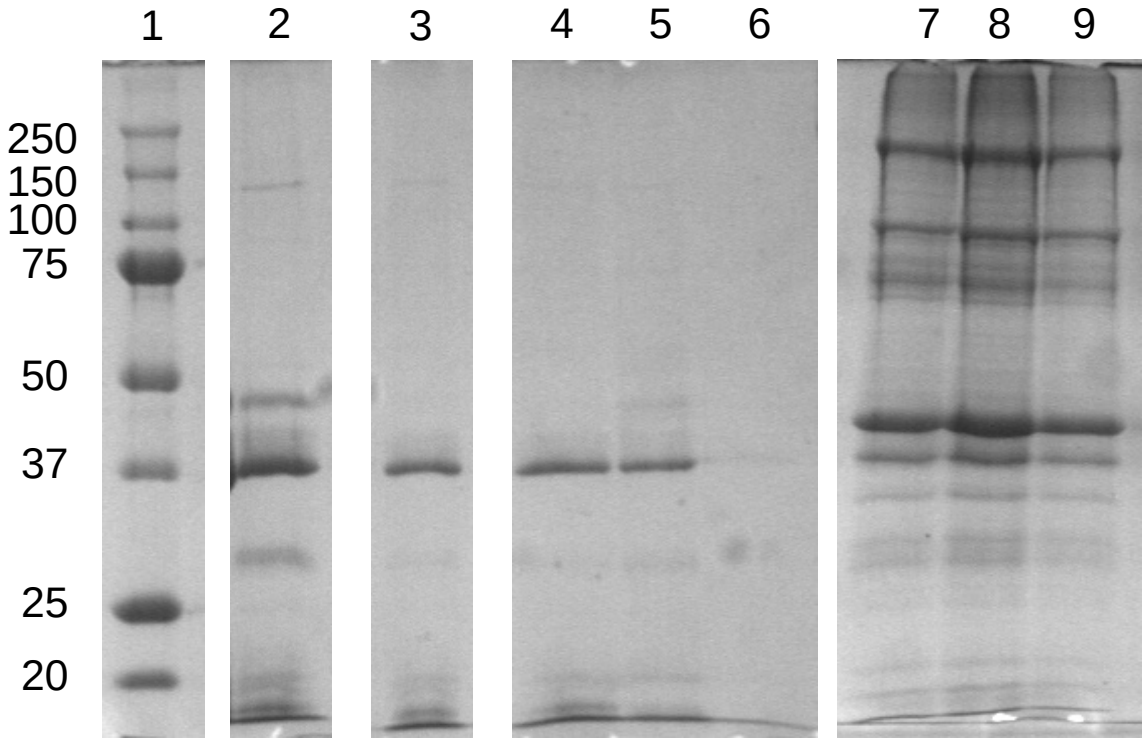

Figure 12: SDS-PAGE analysis of wash water and gel from previously cooked Jonah crab meat.

Lane 1 is the molecular weight standard. Lane 2 is the supernatant from the dewatering step. Lane 3 is the supernatant recovered from one wash. Lanes 4-6 are the supernatants recovered from each of the three washes. Lanes 7-9 are the gels from mince washed three times with 0, 2, and 4% sodium chloride added, respectively.

Interestingly, the major protein component removed in the wash water was tropomyosin, as such, it is suggested that tropomyosin may be the main protein component interfering with gelation of cooked crab proteins. Some smaller muscle components, such as myosin light chain and troponin, were also removed during washing, though at smaller concentrations. The first two washing cycles removed mostly tropomyosin, troponin, and myosin light chain. The myosin was most likely present in the wash water due to the freezing treatment, though further research is necessary to determine the cause. Future research will focus on identifying the specific proteins removed and their function in the crab protein gels.

Conclusion

Protein gels were formed using previously cooked proteins from Jonah crab (*Cancer borealis*) minced meat. A three wash treatment resulted in the gels with the best gel strength and water holding capacity. Given that little protein was removed in the third washing cycle, it was determined that two wash cycles will be adequate for future gelation studies. The improved characteristics due to washing are likely due to the removal of tropomyosin, troponin, and myosin light chain in the first two washes that may interfere with protein-protein interactions involved in gel formation. The washed gels had similar water-holding capacity and breaking force similar to that of other cooked crab mince gels. Increased sodium chloride concentration decreased the breaking force contrary to gelation results common for raw fish proteins. The addition of sodium chloride was detrimental to all physical gel characteristics and is not recommended for gelation of cooked proteins. With regard to color, the whiteness values were not affected by washing, whereas the redness and yellowness values decreased. Based on these

results, washed crab mince from previously cooked Jonah crab meat can produce gels with water-holding capacity and gel strength similar to those of surimi from some raw fish but without the addition of sodium chloride.

The gelation properties of the previously cooked crab mince suggest that it can be used as a major component in protein gel-based products. The ability of cooked Jonah crab minced meat to form gels upon washing will enable future product development endeavors to develop a value-added product with crab mince as the primary ingredient.

CHAPTER 4. CARBOHYDRATE AND PROTEIN ADDITIVES IN GELS FROM PREVIOUSLY COOKED JONAH CRAB (*CANCER BOREALIS*) MINCED MEAT

Chapter Abstract

Fully cooked crab meat mince, a crab processing by-product, is currently under investigation for its use as a primary ingredient in value-added food products. The gelation properties of muscle proteins are crucial in the formulation of nuggets, sausages, and other protein gel-based products. Previous research conducted in our lab showed that previously cooked, washed Jonah crab mince formed gels upon further heat treatment. The current study focused on the use of protein and carbohydrate additives to modify the water-holding capacity and textural attributes of crab mince gels. The cooked Jonah crab *(Cancer borealis)* mince was washed and one of two additive groups was applied to the mince: carbohydrate additives (*iota*-carrageenan, *kappa*-carrageenan, and potato starch), or protein additives (soy protein isolate, whey protein isolate, and dried egg white). Carbohydrate additives were used at levels of 0.5%, 1%, and 2% and protein additives were used at the 5%, 10%, and 15% level. A control of washed mince with no additive was used for comparison. Gels were tested for water-holding capacity, breaking force, deformation, hardness, springiness, cohesiveness, and gumminess. Addition of *kappa*-carrageenan resulted in gels with the highest breaking force whereas gels with *iota*-carrageenan and soy protein isolate exhibited the lowest breaking force. The addition of 5% or 10% whey protein isolate severely disrupted gel formation. Texture profile analysis indicated that the dried egg white and *kappa*-carrageenan treatments exhibited the highest levels of hardness and springiness. Results indicate that both protein and carbohydrate additives can be used to alter textural characteristics of gels from previously

cooked Jonah crab but do not improve water-holding capacity.

Introduction

Jonah crab (*Cancer borealis*), a by-catch of the lobster industry, is increasing in consumer demand due to its mild flavor and relatively low cost (Redmayne 2002). The processing of the crab results in a quantity of waste, including residual meat in the walking legs and body. This meat can be extracted as a low-value mince through use of a mechanical deboner. Landings of Jonah crab doubled in Maine from 2003 to 2004, intensifying the need to find higher value uses for the crab mince thereby increasing the profitability and total utilization of the resource.

Various additives have been shown to change the textural properties of raw fish surimi gels. The protein-water, protein-protein, and protein-lipid-water interactions are important to the formation of a stable gel network (Regenstein 1984). Additives function by filling the interstitial spaces of the myofibrillar protein network but do not seem to interact with myosin and have a tendency to weaken gels (Burgarella and others 1985a; Burgarella and others 1985b). The added ingredients exert their effects on the overall texture, appearance, and other properties by altering water mobility or simply by their presence in the gel, which serves to reinforce the structure formed by the surimi gel matrix (Lee and others 1992; Park 2000). This reinforcement can lead to either a strengthening or weakening of the gel matrix. Though the mechanism by which both protein and carbohydrate additives interact with myofibrillar proteins function is not clearly defined, these additives can change the textural attributes of raw fish surimi gels.

The use of protein additives in changing the textural characteristics of surimi has been well researched. Hydrolyzed beef plasma protein, egg white, soy protein, wheat

gluten, and whey protein have been researched extensively (Burgarella and others 1985a; Burgarella and others 1985b; Chang-Lee and others 1990; Chung and Lee 1990; Chen and Trout 1991; Chung and Lee 1991; Lee and others 1993; Park 1994; Luo and others 2004a; Luo and others 2004b; Murphy and others 2005). The effects of the different protein additives are dependent on fish species and additive concentration. Pollock surimi became tougher with the addition of hydrolyzed beef plasma protein, dried egg white, and frozen egg white; whereas soy protein isolate, wheat gluten, whey protein concentrate and whey protein isolate made pollock gels more brittle (Park 1994). Chung and Lee (1990; 1991) found that the addition of whey protein concentrate and egg white detrimentally affected the gel strength of pollock surimi gels. The researchers attributed gel weakening to a reduced myofibrillar protein concentration due to addition of test ingredients and interference of these non-fish proteins in myofibrillar gelation, specifically cross-linking. Chang-Lee and others (1990) found that the addition of 3% to 5% dried egg white produced the hardest whiting gels but whey protein isolate and soy protein isolate did not change the textural characteristics. Murphy and others (2005) found contradictory results in whiting surimi gels. The addition of whey protein isolate and egg white decreased the hardness of gels and egg white decreased the water-holding capacity of the whiting gels. Soy protein was used successfully to create a harder texture in restructured beef steaks and improve the texture of crab cakes from blue crab minced meat by-products (Chen and Trout 1991; Lee and others 1993). Though the effects of various protein additives on gel texture are not consistent they can clearly be used to modify the properties of surimi and restructured meat gels.

Carbohydrate additives have also been used to alter the textural attributes of

surimi gels. Potato starch, corn starch, *iota*-carrageenan, *kappa*-carrageenan and polysaccharide gums have been investigated for use in surimi (Shand and others 1993; Yoo and Lee 1993; Yoon and others 1997; Murphy and others 2005). Addition of 4% unmodified potato starch produced stronger whiting gels than a control without additives (Murphy and others 2005). Corn and potato starch added to pollock and whiting surimi gels improved the textural properties through reinforcing the protein gel network (Yoo and Lee 1993; Yoon and others 1997). In another study, the addition of potato starch increased the water-holding capacity in whiting surimi gels (Murphy and others 2005). Although both are carrageenans, *iota*- and *kappa*-carrageenan function differently in food gels. The addition of *kappa*-carrageenan improved yields and maintained product texture in restructured beef rolls, whereas *iota*-carrageenan resulted in loss of yield and structure (Shand and others 1993). As seen with protein additives the effects of carbohydrates on gel texture are not predictable, however carbohydrate additives can be used to alter the textural characteristics of muscle protein gels.

Research conducted in our lab has shown that gels can be formed from previously cooked, washed Jonah crab meat without additives (Baxter and Skonberg 2006). Further investigation into how to alter the textural characteristics of the gels is needed so that this resource can be better utilized. One way of altering gel texture is through the use of protein and carbohydrate additives. The objectives of this study were to determine the effects of commonly used protein and carbohydrate additives on the textural attributes of gels from previously cooked Jonah crab mince.

Materials and Methods

Frozen, commercially available Jonah crab meat mince processed in the following manner was obtained from Portland Shellfish (Portland, Maine, U.S.A.): crabs had been eviscerated, boiled for 14 min without the top portion of the carapace, and cooled. Cooked crabs were kept on ice until mince was separated later the same day. Claws were removed and the remaining meat was separated using a mechanical deboning machine. The mince consisted of meat from the body and walking legs. Ten pounds of mince were packaged in polyethylene bags and frozen.

Mince Processing and Treatment

A 10-pound block of frozen Jonah crab meat mince was thawed at 4 °C for 48 h. Due to the high water content in the dewatered mince, the meat was dewatered via centrifugation at 15,000 rpm for 10 minutes. The mince was washed in 200 g batches with a 4:1 (water to mince) ratio twice prior to treatment, once with a wash of cold deionized water and once with a cold solution of 0.3% sodium chloride. Washes were completed in a Waring blender for 1 min and dewatered by centrifugation at 15,000 rpm for 10 min.

Approximately 750 g of washed crab mince were used per treatment. Three carbohydrate and three protein additives were used in this study. The protein additives, soy protein isolate (Ardex ® F Dispersible; ADM, Decatur, IL, U.S.A.; 94% protein), whey protein (Glanbia Nutritionals, Monroe, WI, U.S.A.; > 90% protein), and dry egg white (Deb El, Elizabeth, NJ, U.S.A.; 81% protein), were used at levels of 5%, 10%, and 15%. The carbohydrate additives, iota- (GENUVISCO carrageenan type MB-11F; CP Kelco, Atlanta, GA, U.S.A.) and kappa-carrageenan (GENU Texturizer Type MB-150F;

CP Kelco, Atlanta, GA, U.S.A.) and potato starch (Novation 6600; National Starch, Bridgewater, NJ, U.S.A.) were used at 0.5, 1, and 2%. All of the additives were added at levels commonly used in studies with raw fish surimi. A control of washed mince with no additive was used. Mince, water to adjust the moisture content to 80%, and assigned additive were mixed together in a Kitchenaid mixer with a paddle attachment for 5 min before being stuffed into 30-mm dia cellulose casings and cooked in a water bath using a two-stage heat treatment: 35 °C for 30 min followed by 90 °C for 30 min. Approximately six sausages per treatment were immediately cooled, the casings removed, and then stored at 4 °C in re-sealable plastic bags (Ziploc sandwich; S.C. Johnson & Son, Racine, WI, U.S.A.) until further testing was completed. The texture and water holding capacity of the gels were measured within 24 h. The entire experiment was duplicated.

Texture Profile Analysis

Gel samples (30 mm high by 30 mm diameter) were subjected to a double compression test by a cylindrical plunger (dia 50 mm) to 60% of the original height at a speed of 50 mm min^{-1} using a 25 N load cell connected to a TA-XT2i texture analyzer (Texture Technologies) (Chang-Lee and others 1990; Changlee and others 1990; Alvarez and Tejada 1997; Tellez-Luis and others 2004). Samples were stored at room temperature for no longer than 24 h in re-sealable plastic bags to prevent moisture loss prior to testing. Hardness (N), springiness (mm), cohesiveness (dimensionless), and gumminess (N) were calculated according to Bourne (1968). A total of nine replicates were used per treatment.

Water-Holding Capacity

The water holding capacity of the crab mince gels was determined using the method described by Roussel and Cheftel (1990) as modified by Alvarez and others (1992). Gels from each treatment were pooled and cut into approximately 3-mm cubes and 2 g of sample were centrifuged for 15 min at 1000 x *g*. Exudate was collected on Whatman filter paper. Samples were analyzed in triplicate and water-holding capacity was expressed as percentage of water retained with respect to the water present in the gel prior to centrifugation.

Puncture Test

A penetration test was performed on 30 x 30 mm cylinders of each gel using a cylindrical stainless steel ball probe of 5-mm dia with a speed of 60 mm per min into 1 end of the sample. The probe was attached to a 25 N load cell connected to a TA-XT2i texture analyzer (Texture Technologies, Scarsdale, N.Y., U.S.A.). Both the breaking force (g) and deformation (cm) were recorded. Nine measurements were taken per treatment.

Moisture and Protein Analysis

Sausages from each treatment were pooled and ground creating one uniform sample per treatment for proximate analysis. Protein and moisture content were measured in all samples. Nitrogen content was analyzed using the Elementar Rapid N III nitrogen analyzer (Elementar Americas, Inc., Mount Laurel, N.J., U.S.A.) to determine total protein content. Moisture was analyzed using AOAC methods using method # 950.46 (AOAC 2005). Both moisture and protein were analyzed in duplicate.

Statistical Analysis

Statistical analysis was performed on each group of additives separately (protein additives and carbohydrate additives). Each group had 9 treatments (3 additives at 3 levels each) plus a control. An incomplete block design was used as the experimental design. Analysis of water holding capacity, breaking force, deformation, hardness, springiness, cohesiveness, gumminess, moisture, and protein was conducted on all treatments and the control. Multi-way analysis of variance was performed using SAS (9.1) with least square means and Tukey mean separation to determine the effect of additive type (dried egg white, soy protein isolate, whey protein isolate; or *kappa*-carrageenan, *iota*-carrageenan, and potato starch) and additive concentration (5%, 10%, 15%; or 0.5%, 1%, 2%). Significance was established at $p < 0.05$.

Results and Discussion

Moisture and Protein Analysis

Proximate analysis characterized the different crab mince gels as having between 78.2% to 82.3% moisture and 14.9% to 20.5% protein on a wet weight basis (Table 9). Moisture analyzed by additive type showed that there were significant ($p < 0.05$) differences among protein additive treatments. The egg white additive gels had on average more moisture (81.5%) than soy protein isolate gels (80.9%) which was significantly higher than the moisture content of the control gels (80.4%) and the whey protein isolate gels (80.2%). The level of additives and the addition of carbohydrate additives did not affect the moisture content of the gels.

Table 9: Protein and moisture analysis for crab mince gels with carbohydrate and protein additives

CARBOHYDRATE ADDITIVES		
Sample	**% Protein**	**% Moisture**
Control	18.0 ± 0.1	78.6 ± 0.3
0.5% *kappa*-carrageenan	17.2 ± 0.1	78.9 ± 0.1
1% *kappa*-carrageenan	16.9 ± 0.3	78.6 ± 0.3
2% *kappa*-carrageenan	15.5 ± 0.6	79.6 ± 0.6
0.5% *iota*-carrageenan	17.3 ± 1.0	78.6 ± 0.5
1% *iota*-carrageenan	16.9 ± 0.1	78.9 ± 0.3
2% *iota*-carrageenan	16.0 ± 0.1	79.2 ± 0.2
0.5% potato starch	16.7 ± 0.7	79.6 ± 1.1
1% potato starch	16.9 ± 0.5	79.1 ± 0.5
2% potato starch	16.2 ± 0.0	78.9 ± 0.1
PROTEIN ADDITIVES		
Control	17.7 ± 1.4	80.4 ± 0.3
5% dried egg white	15.8 ± 1.1	81.5 ± 0.7
10% dried egg white	14.0 ± 4.1	81.4 ± 0.2
15% dried egg white	16.8 ± 1.0	81.6 ± 0.2
5% soy protein isolate	17.2 ± 0.1	80.9 ± 0.2
10% soy protein isolate	17.6 ± 0.3	80.8 ± 0.3
15% soy protein isolate	18.3 ± 0.2	81.0 ± 0.3
5% whey protein isolate	17.0 ± 0.4	80.9 ± 0.3
10% whey protein isolate	17.8 ± 0.3	80.2 ± 0.7
15% whey protein isolate	18.7 ± 1.1	79.6 ± 0.3

Each value is the average ± standard deviation of four replicates per treatment.

The protein content of the gels was significantly ($p < 0.05$) affected by the level of carbohydrate additive but not by level of protein additive. Samples with 0% carbohydrate additive had significantly more protein, averaging 18.0% protein, than those with 1% or 0.5% additive. Those with 2% carbohydrate additive had the least protein, averaging 15.9%. The difference in total protein content was due to the addition of a non-protein additive which reduced the overall percent protein in a sample.

All treatments formed gels except for those treated with 5% whey protein isolate. Gels with 10% whey protein isolate only formed in the second replication and were not strong enough to test for water-holding capacity, though texture profile analysis could be conducted.

Water-Holding Capacity

The water-holding capacity was not significantly affected by the addition of the carbohydrate additives (Figure 13) but was affected by both the type and level of the protein additive (Figure 14) based on multi-way analysis of variance. None of the gels with additives were significantly ($p < 0.05$) different from the control, however, the soy protein isolate gels had a greater water-holding capacity, averaging 82.6% water retained, than the other two protein additive gels. The 10% additive treatment resulted in the lowest water-holding capacity for the protein additives, averaging 72.0% water retained. Interestingly, even at the lowest water-holding capacity, all of the gels, including the control, had greater water-holding capacity than gels from previous work with washed crab except for the whey protein isolate averaging ~60% water retained.

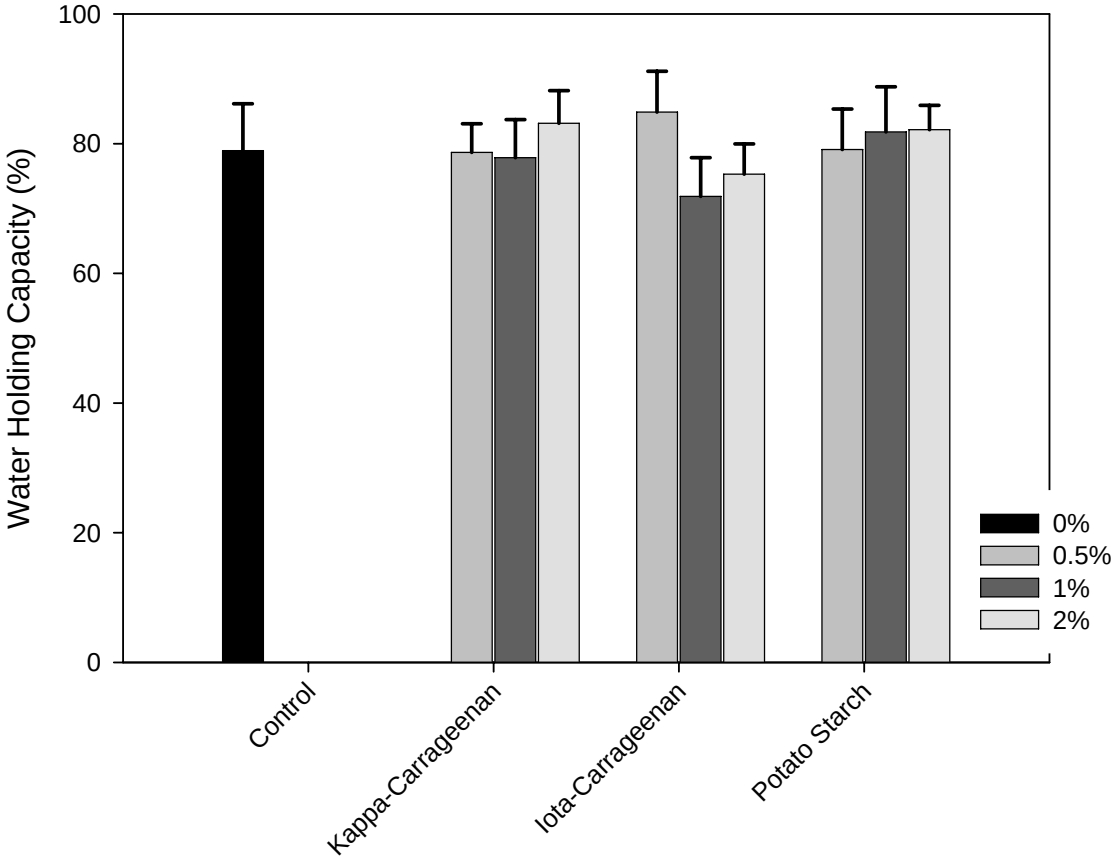

Figure 13: Water-holding capacity of washed crab mince gels with carbohydrate additives.

Each value is the average ± standard deviation of three replicates per treatment.

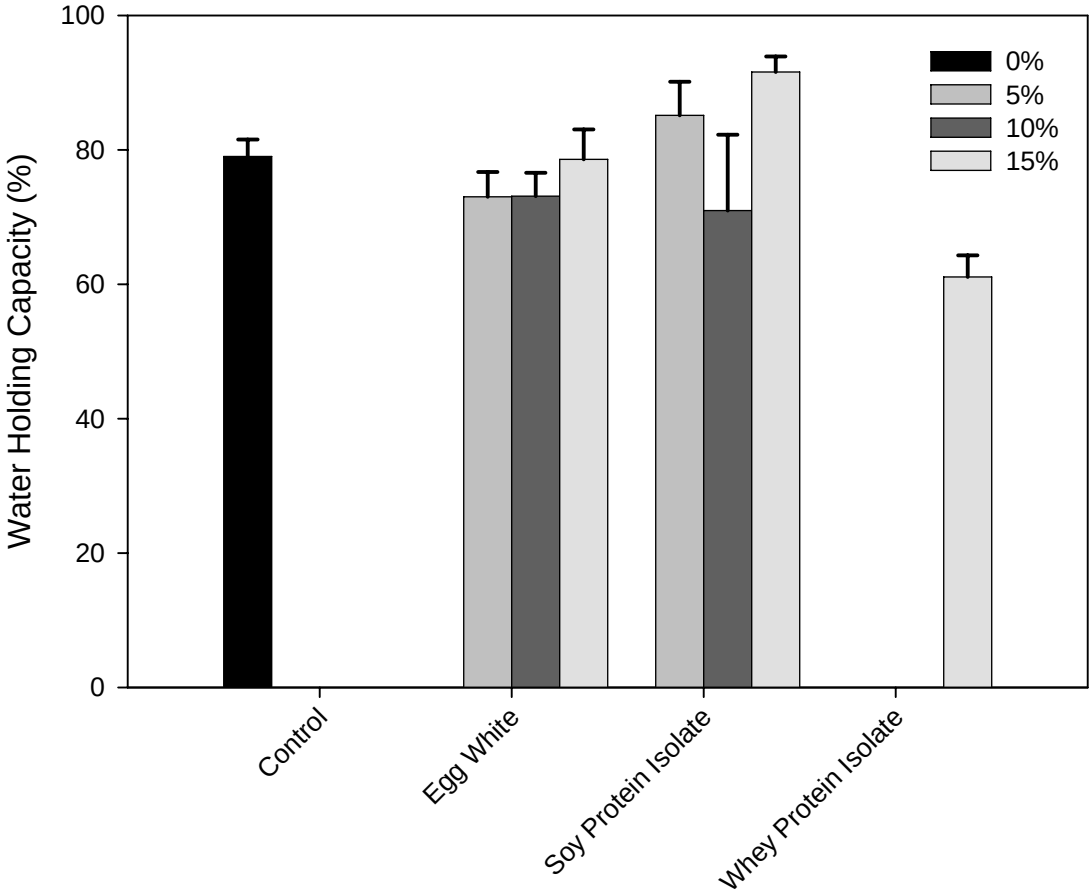

Figure 14: Water-holding capacity of washed crab mince gels with protein additives.

Each value is the average ± standard deviation of three replicates per treatment.

** 5% and 10% whey protein isolate treatments are not included due to the lack of gel formation needed for the methodology.

Baxter and Skonberg (2006) found that washed, cooked crab mince with 2.5% sodium chloride had an average water-holding capacity of 68.5% which is less than that of gels in the current study. In Pacific whiting, the addition of dried egg white and whey protein isolate resulted in water-holding capacities ranging from 72% to 79% (Murphy and others 2005). Crab gels with additives were comparable to the Pacific whiting surimi gels. The increase in water-holding capacity compared to previous reports is likely due to the increased water-binding ability of the additives thereby increasing the water-holding capacity of the gels.

Breaking Force and Deformation

The breaking force of gels from both additive groups was significantly affected by additive type and level. The addition of soy protein isolate significantly ($p < 0.05$) decreased the strength of the gels though the addition of whey protein isolate and dried egg white significantly ($p < 0.05$) increased the gel strength as compared to the control. Addition of soy protein isolate resulted in the softest gels (80.51 g) compared to all other protein additives or the control (Figure 15). None of the additive levels were different than the control; of the additive levels, gels with 5% protein resulted in the softest gels (83.95 g). Previous work conducted on washed crab mince showed that the gels had an average breaking force of 160.3 g to 194.6 g (Baxter and Skonberg 2006) which was similar to the control in the current work. Within carbohydrate additives the addition of *kappa*-carrageenan produced the strongest gels (451.59 g) whereas the *iota*-carrageenan produced the softest gels (107.26 g) (Figure 16). *Kappa*-carrageenan was the only carbohydrate additive that increased the breaking force to greater than the control.

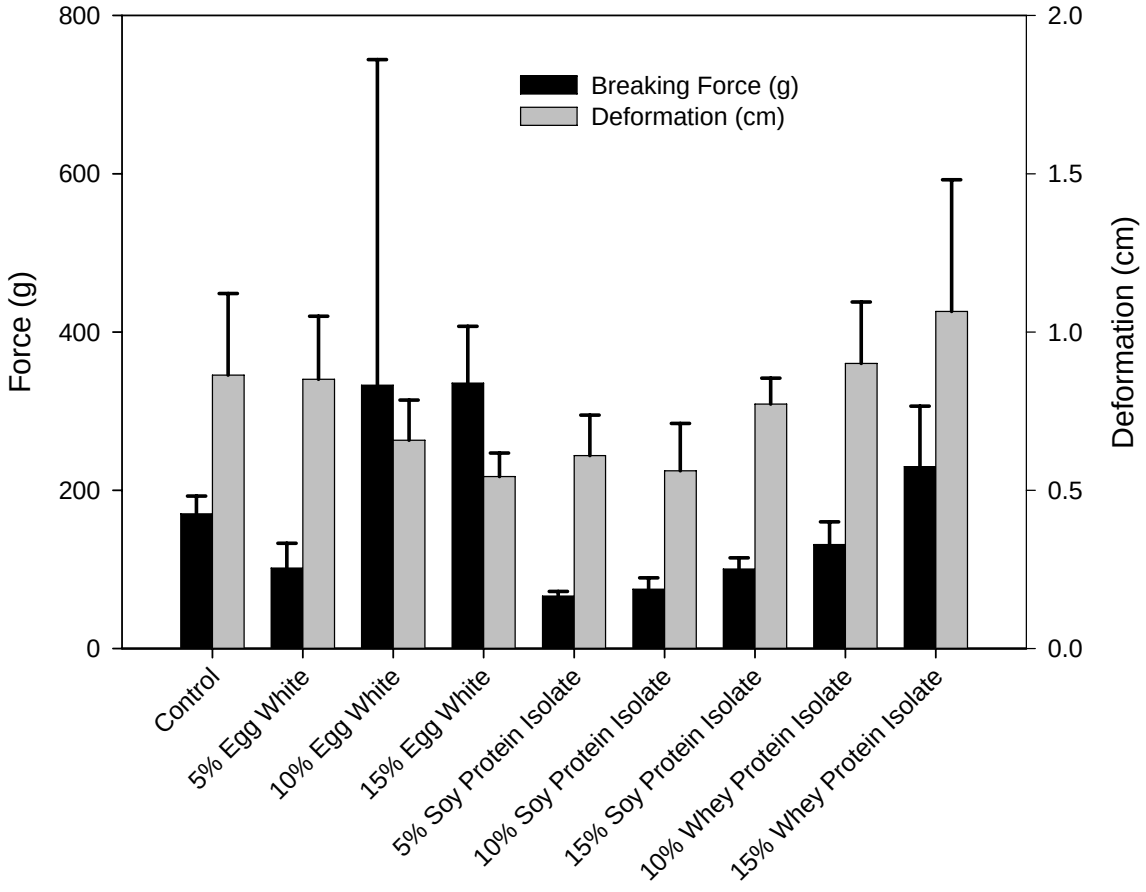

Figure 15: Breaking force (g) and deformation (cm) of crab mince gels with protein additives.

Each value is the average ± standard deviation of three replicates per treatment.

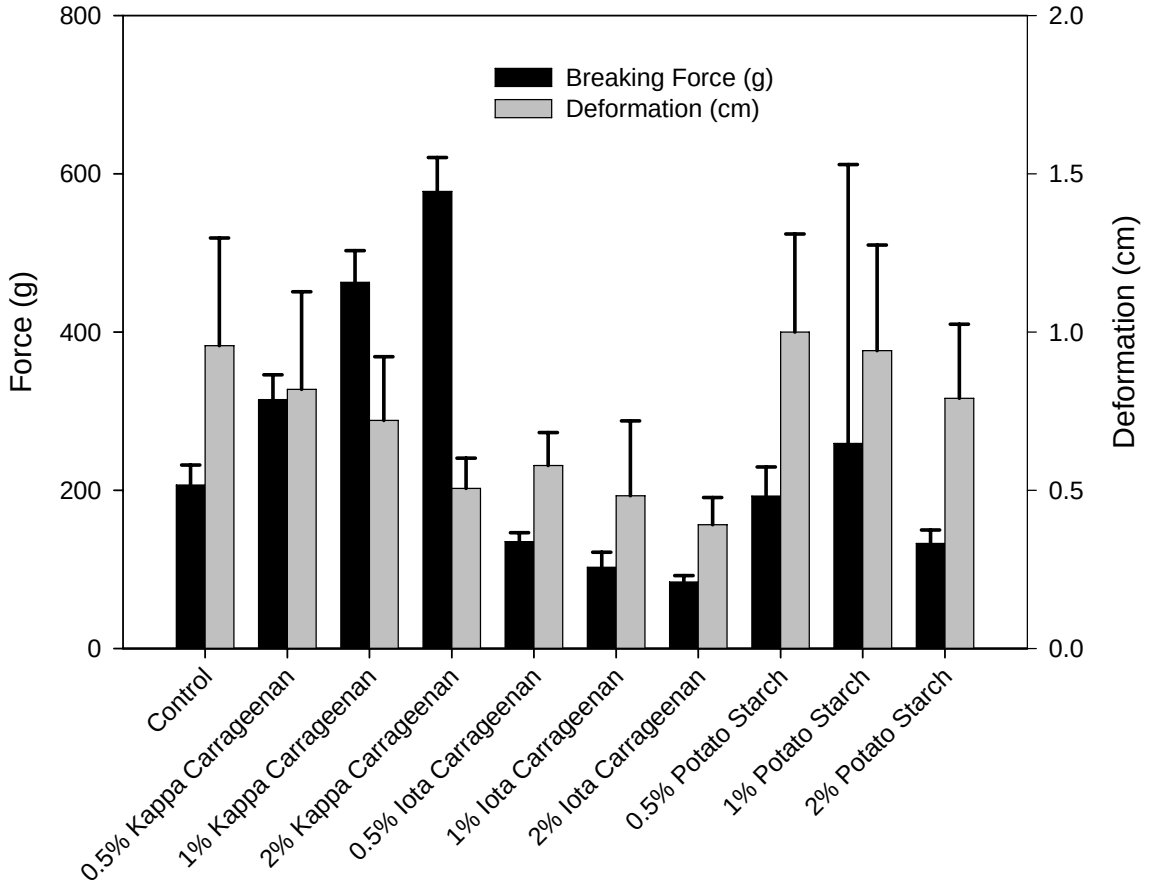

Figure 16: Breaking force (g) and deformation (cm) of crab mince gels with carbohydrate additives.

Values shown are the average of nine measurements with standard deviations.

The addition of 1% carbohydrate additive resulted in the strongest gels (278.13 g) which were significantly stronger than the control (206.52 g).

In pollock surimi gels, the addition of 10% soy protein isolate resulted in a breaking force of 798.20 g (Luo and others 2004a). The gel strength of pollock is considerably greater with soy protein isolate than in the crab gels likely due to the difference in gelation of raw proteins and cooked proteins. Raw muscle proteins form a more stable network due to the stronger protein-protein interactions. It appears that the soy and whey protein additives interfered with the gelation of the cooked crab proteins likely due to stronger protein-protein interactions between the additives than with the crab proteins. These interactions result in a less structured and weaker protein network. On the other hand, the *kappa*-carrageenan and potato starch additives worked with the crab protein network. These carbohydrate additives served to reinforce the protein gel matrix thereby enhancing the gel properties.

The deformation (cm) of the gels was also affected by both type and level of both protein and carbohydrate additives based on multi-way analysis of variance. Addition of dried egg white and soy protein isolate decreased the deformation whereas addition of whey protein isolate resulted in gels similar to the control. Addition of *kappa*- and *iota*-carrageenan decreased the deformation in the carbohydrate additive gels. Increasing the level of carbohydrate additive also decreased the deformation of the gels. Increasing the level of protein additive also decreased the deformation of the gels. Whey protein isolate and the control both had a greater deformation distance (1.01 cm and 0.88 cm, respectively) than the dry egg white and soy protein isolate (Figure 15). The control, at 0% additive, had a greater deformation distance than all protein additive levels, except

15% (0.88 cm). In the carbohydrate additive group, the control and potato starch had the greatest deformation distance (0.96 cm and 0.91 cm, respectively) (Figure 16). *Kappa*-carrageenan followed with an average distance of 0.68 cm and *iota*-carrageenan resulted in the shortest distance (0.48 cm). The control, at 0% additive, had the greatest deformation distance (0.96 cm). The 0.5% and 1% carbohydrate additive deformation distance was smaller than the control but greater than the 2% carbohydrate deformation distance. Previous work on cooked crab gels showed deformation distances ranging from 0.61 to 0.67 cm (Baxter and Skonberg 2006). The control in this study had a greater deformation distance than in the previous work. Only the addition of whey improved the deformation distance out of all additives and levels. At 1.01 cm, the deformation was still below that of pollock surimi, at 1.73 cm (Luo and others 2004a). The difference is believed to be due to the greater elasticity in gels made from raw muscle compared to those made from cooked muscle proteins.

Texture Profile Analysis

Texture profile analysis is a quantitative method for determining the sensory attributes of a food product. Seven different attributes (hardness, fracturability, cohesiveness, springiness, chewiness, gumminess, and resilience) can be calculated based on a double compression instrumental analysis (Figure 17). Each attribute relates directly to a sensory characteristic as defined by Szczesniak (1963). Hardness is defined as the force necessary to attain a given deformation. Springiness (or elasticity) is defined as the rate at which a deformed material goes back to its undeformed condition after the deforming force is removed. Cohesiveness is defined as the strength of the internal bonds making up the body of the product. Finally, gumminess is defined as the energy

required to disintegrate a semi-solid food product to a state ready for swallowing. Gumminess is related to the hardness and cohesiveness of the product.

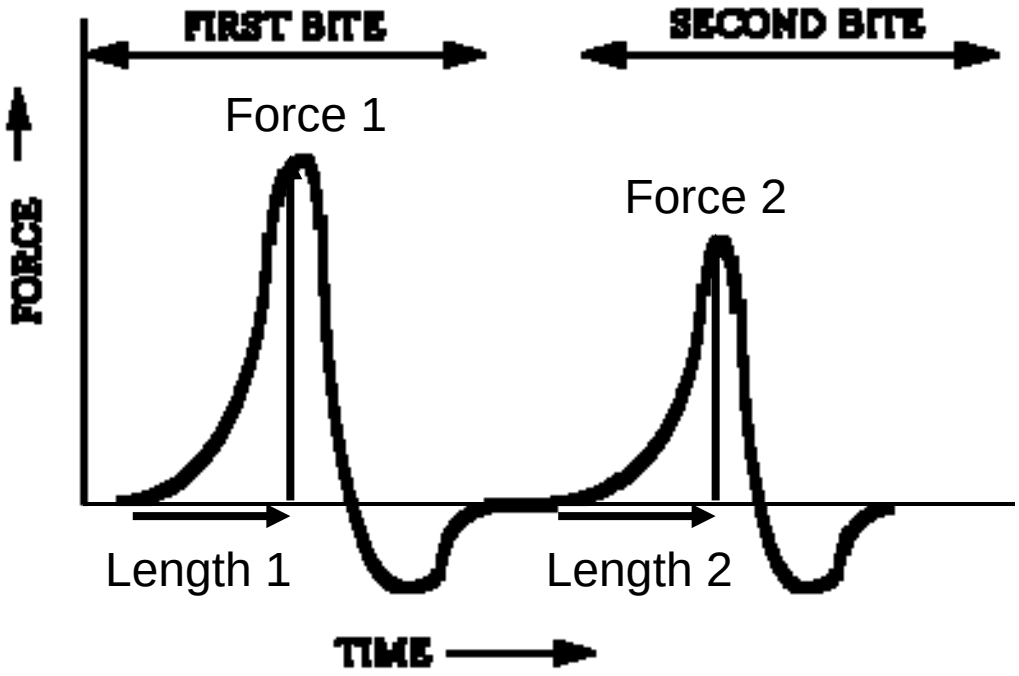

Figure 17: Texture profile analysis curve detailing important parameters for calculating attributes
(Texture Technologies 2007)

Texture profile analysis evaluated four measures of texture: hardness, springiness, cohesiveness, and gumminess. The effect of additive type, in both carbohydrate and protein additive categories, and level of additive significantly ($p < 0.05$) affected the hardness of the gels based on multi-way analysis of variance. The addition of egg white protein resulted in significantly the hardest gels of all the additives, averaging 41.0 N (Table 10). The soy protein isolate, control, and whey protein isolate were all statistically similar to one another and softer than the egg white protein by almost half (17.4 N, 15.3 N, and 14.6 N, respectively). When evaluating the effects of protein additive levels, a 15% protein additive level resulted in significantly ($p < 0.05$) harder gels than all other samples (41.5 N), followed by the 10% level (21.3 N) and 0% additive (15.3 N). Addition of 5% protein resulted in gels that were the softest. Addition of *kappa*-carrageenan resulted in the strongest carbohydrate additive gels (52.2 N) (Table 11). The potato starch gels were statistically the same as the control gels (17.6 N) and were softer than the *kappa*-carrageenan gels, but harder than the *iota*-carrageenan gels. The *iota*-carrageenan gels were softest (14.4 N). The 2% carbohydrate additive level resulted in the hardest gels (34.8 N). The 1% additive level was next hardest (26.4 N) followed by the 0.5% additive level (22.9 N). The 0% additive level resulted in the softest gels (17.6 N).

Addition of 4% whey protein isolate to whiting surimi gels resulted in a gel with a hardness of 3.24 N whereas addition of 4% egg white resulted in a greater force at 4.73 N (Murphy and others 2005). In restructured beef products, the addition of whey protein isolate resulted in gels with a hardness of 9.17 N and gels with additional soy protein isolate had a hardness of 12.09 N (Chen and Trout 1991).

Table 10: Texture profile analysis of washed crab mince gels with protein additives.

Additive	Hardness (N)	Springiness (mm)	Cohesiveness	Gumminess (N)
Control*	15.31 ± 1.88	0.54 ± 0.05	0.23 ± 0.02	3.59 ± 0.60
5% Egg White*	8.80 ± 4.11	0.63 ± 0.14	0.43 ± 0.10	3.85 ± 2.09
10% Egg White	33.97 ± 7.07	0.81 ± 0.06	0.35 ± 0.06	12.18 ± 4.46
15% Egg White	78.32 ± 12.41	0.79 ± 0.03	0.34 ± 0.07	27.35 ± 8.05
5% Soy Protein Isolate	9.19 ± 1.00	0.26 ± 0.02	0.19 ± 0.01	1.75 ± 0.23
10% Soy Protein Isolate*	14.33 ± 2.46	0.64 ± 0.06	0.32 ± 0.03	4.57 ± 0.93
15% Soy Protein Isolate	28.64 ± 2.92	0.89 ± 0.02	0.69 ± 0.05	19.89 ± 2.73
5% Whey Protein Isolate	nd	nd	nd	nd
10% Whey Protein Isolate**	8.93 ± 1.73	0.39 ± 0.06	0.23 ± 0.03	2.05 ± 0.57
15% Whey Protein Isolate	17.47 ± 4.75	0.58 ± 0.05	0.43 ± 0.13	7.71 ± 3.70

Values shown are the average of 18 measurements with standard deviations.
nd = not determined
* Average and standard deviation of 17 measurements.
** Average and standard deviation of 9 measurements.

Table 11: Texture profile analysis of washed crab mince gels with carbohydrate additives.

Additive	Hardness (N)	Springiness (mm)	Cohesiveness	Gumminess (N)
Control	17.58 ± 2.04	0.62 ± 0.03	0.24 ± 0.02	4.23 ± 0.76
0.5% *kappa* – Carrageenan*	31.80 ± 3.25	0.61 ± 0.06	0.22 ± 0.02	6.91 ± 1.24
1% *kappa* – Carrageenan*	49.73 ± 3.75	0.67 ± 0.05	0.23 ± 0.02	11.67 ± 1.94
2% *kappa* – Carrageenan*	75.05 ± 13.92	0.68 ± 0.06	0.23 ± 0.04	18.04 ± 6.15
0.5% *iota*-Carrageenan	17.93 ± 4.65	0.31 ± 0.04	0.19 ± 0.02	3.37 ± 0.91
1% *iota*-Carrageenan	11.96 ± 3.13	0.31 ± 0.08	0.17 ± 0.02	2.06 ± 0.58
2% *iota*-Carrageenan	10.88 ± 2.93	0.29 ± 0.05	0.16 ± 0.02	1.79 ± 0.55
0.5% Potato Starch	17.66 ± 2.14	0.58 ± 0.05	0.24 ± 0.02	4.25 ± 0.74
1% Potato Starch	15.92 ± 1.49	0.53 ± 0.05	0.22 ± 0.02	3.54 ± 0.56
2% Potato Starch	16.61 ± 1.80	0.44 ± 0.13	0.22 ± 0.01	3.71 ± 0.51

Values shown are the average of 18 measurements with standard deviations.

* Average and standard deviation of 17 measurements.

The crab gels with additives were all harder than both whiting and restructured beef products. These values are higher than those cited above most likely due to the higher concentrations of protein additives used in this cooked crab mince study. In whiting, addition of 4% potato starch resulted in a hardness of 5.92 N (Murphy and others 2005). Restructured beef rolls were harder with the addition of 1% *kappa*-carrageenan at 175 N whereas the addition of 1% *iota*-carrageenan resulted in a hardness of 150 N (Shand and others 1993). Overall, only the addition of dry egg white and *kappa*-carrageenan increased the hardness of the gels whereas *iota*-carrageenan decreased the hardness. All of the other additives maintained gel hardness similar to the control. The addition of dry egg white and *kappa*-carrageenan produced gels harder than whiting gels with additives but did not exceed the hardness of restructured beef rolls with carbohydrate additives.

Regression analysis showed a positive relationship between hardness and breaking force data though the R^2 values were low (carbohydrate gels $R^2 = 0.58$; protein gels $R^2 = 0.14$). The regression between hardness and breaking force was significant ($p < 0.01$) for both the carbohydrate and protein additive gels. The low R^2 values were likely due to the large variation among treatments within each additive category. However, given the highly significant relationship between the two variables, both hardness and breaking force analysis are not necessary in future work. It is recommended that hardness measurements be conducted in future products due to the large amount of information gained in using texture profile analysis.

The springiness of the crab mince gels was affected in both additive groups by type and level. The egg white protein gel was the springiest, averaging 0.75 mm, followed by the soy protein isolate gels, averaging 0.60 mm (Table 10). The control gels

and whey protein isolate gels were statistically the same, averaging 0.54 mm and 0.52 mm, respectively. Gels with 15% additive were the springiest, averaging 0.75 mm, followed by 10% additive, averaging 0.66 mm, and 0% additive, averaging 0.54 mm. Gels with 5% protein additive were the least springy, averaging 0.44 mm. Putting this in perspective, whiting surimi gels with the addition of whey protein had a springiness value of 0.68 mm. The addition of soy protein isolate to whiting increased the springiness to 0.74 mm (Chen and Trout 1991). Though the additives did not increase the springiness to the levels that Chen and colleagues (1991) found, the addition of dry egg white resulted in springiness values comparable to that of whiting with soy protein isolate. Within the carbohydrate additive group, gels with 0% additive were the springiest, averaging 0.62 mm, followed by all of the carbohydrate additive levels which were statistically equal. Within the carbohydrate additives, the *kappa*-carrageenan resulted in the springiest gels, averaging 0.66 mm, followed by the control, averaging 0.62 mm, and potato starch averaging 0.52 mm (Table 11). The addition of *iota*-carrageenan resulted in the least springy gels, averaging 0.31 mm.

The protein and carbohydrate additives affected cohesiveness of the crab mince differently. The protein additives resulted in significantly more cohesive gels than the control. At the 15% protein additive level, gels had average cohesive values of 0.49, which was more cohesive than the 10% or 5% treatments, which were statistically equal. The control treatment was the least cohesive, averaging 0.23. In whiting surimi gels with 4% whey protein isolate or 4% dry egg white was, cohesiveness was 0.33 and 0.31, respectively (Murphy and others 2005). Addition of whey protein isolate in the crab gels resulted in cohesiveness of 0.36 and the addition of egg white resulted in cohesiveness of

0.37. The higher cohesiveness values observed in the crab gels was likely due to the higher levels of additives used in this study. Soy protein isolate was added to restructured beef steaks and cohesiveness was measured at 0.46 (Chen and Trout 1991) which is larger than the average cohesiveness in the crab gels at 0.40. Chang-Lee and others (1990) reported much lower cohesiveness when evaluating whiting surimi gels with added whey protein, soy protein isolate, and dry egg white. In that study, cohesiveness values for all of the treated gels ranged from 0.16 to 0.20, half the cohesiveness measured in the crab mince gels. The addition of protein additives increased the cohesiveness of the gels though all additives aided the gel network to the same degree. The level of carbohydrate additive did not significantly affect cohesiveness, however gels were affected by the type of additive. The *iota*-carrageenan gels were the least cohesive of all the carbohydrate treatments, averaging 0.17. Overall the cohesive values for the carbohydrate additive gels were less than that of the protein additives, though the controls in both cases were similar. Addition of potato starch in crab mince gels resulted in cohesive values of 0.23 which was less than that found with the addition of 2% potato starch in whiting, resulting in a cohesive value of 0.35 (Murphy and others 2005). The cohesive values for the protein additives are believed to be higher due to greater protein-protein interactions allowing the network, though it was not necessarily structured, to stay together when a force was applied.

Gumminess of gels was affected by type of additive, in both the protein and carbohydrate groups, and level of additive. Addition of *kappa*-carrageenan resulted in the gummiest of carbohydrate gels, averaging 12.2 N. The control and potato starch gels, which were statistically equal, followed, averaging 4.2 N and 3.8 N, respectively. The

addition of *iota*-carrageenan resulted in the least gummy gels, averaging 2.5 N. The 2% carbohydrate additive level was the gummiest, averaging 8.0 N. The 1% additive level was significantly gummier than the 0% level, averaging 5.9 N. The 0.5% additive level was statistically equal to both the 1% and 0% levels, averaging 4.9 N and 4.2 N, respectively. Addition of dry egg white, soy protein isolate, and *kappa*-carrageenan increased the gumminess of crab mince gels. The addition of potato starch and whey protein isolate did not change the gumminess compared to the control while the addition of *iota*-carrageenan reduced the gumminess of the gels. The highest additive levels (15% for protein and 2% for carbohydrate) produced the gummiest gels whereas the lower levels (5% for protein and 0.5% for carbohydrate) did not change the gumminess from the control. Chen and others (1991) found that the addition of whey protein to restructured beef resulted in a gumminess value of 3.95 mm whereas the addition of soy protein isolate increased the gumminess to 5.66 mm.

The addition of egg white protein resulted in a gummier gel (14.7 N) than other protein additives. Soy protein isolate gels (8.8 N) were gummier than whey protein isolate gels or control gels. Whey protein isolate gels and control gels were statistically equal, averaging 5.8 N and 3.6 N, respectively. Addition of 15% protein additive resulted in the gummiest gels, averaging 18.3 N, followed by 10% additive, averaging 7.2 N. The 5% and 0% additive levels were statistically equal averaging 3.6 N and 2.8 N, respectively. The level of gumminess is relative to the type of product produced therefore the data cannot be used to determine quality but can be used in the development of a new food product.

Conclusion

The use of additives can aid in altering the texture of crab muscle protein gels. Addition of dried egg white and soy protein isolate increased the hardness and springiness of the crab mince gels. However, whey protein isolate caused inhibition of gelation when added at the 5% and 10% level. Addition of *kappa*-carrageenan resulted in the strongest carbohydrate additive gels whereas potato starch and *iota*-carrageenan did not perform as well. None of the additives improved the water-holding capacity of the crab mince gels in comparison to the control gels. However, texture profile analysis indicated that hardness, springiness, cohesiveness, and gumminess could be significantly modified by the inclusion of protein and carbohydrate additives. Based on these findings, functional additives can be used to change the textural characteristics of gels from previously cooked crab mince in the creation of value-added food products, and ultimately result in better utilization of the Jonah crab resource.

CHAPTER 5. EFFECT OF THERMAL PROCESSING ON DENATURATION OF JONAH CRAB (*CANCER BOREALIS*) MEAT COOKED IN VERSUS OUT OF THE SHELL

Chapter Abstract

Crustaceans are unique in that their meat is typically cooked within the shell before it is extracted. However, the effect of cooking the meat within the shell on the myofibrillar proteins of the crustaceans has not been reported. The objective of this study was to investigate the effect of cooking Jonah crab (*Cancer borealis*) meat in the shell versus out of the shell to determine if there are any differences in ATPase activity, total sulfhydryl groups, undenatured myosin and extent of protein denaturation. Experimentation was conducted in two stages. The first investigated the effect of cooking the meat in the shell. Four freshly eviscerated raw crabs were assigned to each of five heat processing treatments: 1) raw, 2) 62.8 °C, 3) 75.5 °C, 4) 82.2 °C, and 5) 100 °C. The second stage investigated the effect of cooking the meat out of the shell. Raw meat was extracted from 6 crabs and divided equally into four heat treatments. Extracted meat and meat in the shell were cooked in a water bath under controlled heat to eliminate cold spots. There were no significant differences in myosin ATPase activity or total sulfhydryl groups between the two cooking methods. Retention of ATPase activity suggests that the myosin head was not fully denatured under either of the two cooking methods at any of the tested temperatures. Myosin was not extracted from any of the treatments in either cooking method. Differential scanning calorimetry (DSC) analysis showed that proteins were not completely denatured when heat was applied, and cooking

the meat in versus out of the shell resulted in fewer endothermic peaks. Results indicate that the crab proteins were not fully denatured at cooking temperatures, either in or out of the shell. More research is needed to determine the cause of this unique phenomenon.

Introduction

Cooked Jonah crab (*Cancer borealis*) minced meat is a by-product of the crab processing industry. Little research has been conducted on the gelation properties of cooked muscle proteins because proteins lose functionality as they are denatured. Previous work conducted in our lab has shown that cooked crab meat can form gels by using a modified surimi process (Baxter and Skonberg 2006). Our previous research on the gelation of cooked crab proteins has focused on optimizing the processing parameters of the mince. However, the mechanism by which the cooked proteins form gels is still unknown. Traditional protein chemistry has established that during the heat setting of gels, proteins unfold and interact with nearby proteins forming the gel matrix. If the proteins are completely denatured, extensive aggregation occurs and the gel matrix is not formed. More research is needed to determine how these previously cooked crab proteins form a gel network.

The use of differential scanning calorimetry (DSC) can provide information about thermal denaturation of materials by measuring changes in enthalpy. Fully denatured proteins show no enthalpy changes with increased temperature, while native proteins show endothermic or exothermic activity. The first stage of gel formation is the unfolding of proteins which then interact to form a gel matrix. This protein unfolding can be seen as endothermic peaks using DSC. A benefit to using DSC for studying proteins is that the samples do not have to be solubilized, therefore the actual meat system can be

investigated in its natural state. Parsons and Patterson (1986) used DSC to track protein denaturation and temperature abuse in beef. As proteins became denatured, endothermic peaks decreased in size or were absent in the thermograms, indicating temperature abuse. DSC has been utilized to monitor the structural stability of actin and myosin in sausage processing (Quinn and others 1980). The monitoring of the structural changes occurring in the myofibrillar proteins helped to determine the mechanism by which the sausages formed. Research has shown that myosin is the protein most responsible for the heat-induced gelation of meat (Yasui and others 1980; Samejima and others 1981). Myosin stability is closely associated with the temperature in which an organism lives, for instance, the colder the environment, the less heat stable the myosin (Hashimoto and others 1982). Through the use of DSC, the denaturation of myosin can be monitored to help in understanding the mechanism of gelation of proteins from various sources.

Sulfhydryl groups and disulfide bonds are integral in maintaining the structure and functional properties of myofibrillar proteins. As these proteins are heated, disulfide cross-linking due to oxidation of the sulfhydryl groups may occur (Hamm and Hofmann 1965; Opstvedt and others 1984; Synowiecki and Sikorski 1988). As oxidation and the formation of disulfide linkages occur, the total amount of sulfhydryl groups decrease in the meat (Srinivasan and Hultin 1997). In ovalbumin, Broersen and others (2006) found that aggregation may be driven by physical interactions and the disulfide bonds in the ovalbumin are important to the physical gel or aggregate characteristics, such as gel strength. The same may be true in gels from cooked crab proteins.

The thermal denaturation of myosin should be monitored since it is the protein most responsible for heat-induced gelation of meat (Yasui and others 1980; Samejima

and others 1981). Changes in the Ca^{2+} ATPase activity of the myosin head can be monitored as a measure of myosin denaturation. As myosin unfolds, Ca^{2+} ATPase activity of the myosin head decreases (Ko and others 2003). Yamamoto and others (2002) found that after 12 hr incubation at 30 °C, 42% of Ca^{2+} ATPase activity was retained in carp myofibrils. At 40 °C, the reduction in Ca^{2+} ATPase activity was more notable decreasing to 42% activity in only 10 min. Given that the cooking process for Jonah crabs occurs at a much higher temperature (100 °C) but for a relatively short time (approximate 14 min) we hypothesize that the short time might lead to the retention of some Ca^{2+}ATPase activity.

The extent of denaturation of previously cooked crab mince has not been reported. Crustaceans are unique in that when thermally processed, the meat is cooked within the shell, forming a closed vessel. Environmental factors within the shell may affect the rate of protein denaturation of the meat, such as pressure build-up within the shell. The objective of the current study was to determine if cooking crab meat in the shell impacts the thermal denaturation of the proteins.

Materials and Methods

Freshly eviscerated, raw Jonah crab was obtained from Portland Shellfish (Portland, Maine, U.S.A.). Crabs were transported to the University of Maine on ice. The crabs were assigned to two experimental groups. Group 1 crabs were cooked with the meat still in the shell. In Group 2, raw meat was extracted from the crab shells and pooled prior to cooking. The meat was then divided into five heat processing treatments: 1) no heat, 2) cook to 62.8 °C, 3) cook to 75.5 °C, 4) cook to 82.2 °C, and 5) cook to 100 °C. Crabs and extracted meat were cooked in a controlled heat water bath (Julabo Model

SW22; Blackforest, Germany) to ensure uniform cooking and eliminate temperature gradients within the meat. Commercially, crabs are boiled until the meat reaches a set internal temperature. The temperatures chosen for this study include one value (62.8 °C) below the normal internal temperature (~ 75.5 °C) and two (82.2 °C and 100 °C) above the commercial processing temperature. For meat cooked in the shell, three thermocouples were placed in each crab (in the claw, walking leg, and body) to insure uniform heating. One thermocouple was used to monitor meat cooked out of the shell. Meat outside of the shell was cooked in a plastic laboratory sample bag (NASCO Whirlpak, Fort Atkinson, WI, U.S.A.) at a controlled temperature. All samples reached the appropriate temperature within 15 to 30 minutes. After cooking, crab meat cooked in the shell was picked and pooled, creating one uniform sample for further testing.

Differential Scanning Calorimetry

The thermal denaturation of the muscle from all 9 treatments was evaluated using differential scanning calorimetry (DSC). Muscle samples (15 to 20 mg) were hermetically sealed in aluminum pans and scanned at 10 °C per min from 5 °C to 95 °C (Mettler Toledo, Columbus, OH, U.S.A.). An empty pan was used as a reference. To determine the effect of cooking the meat out of the shell, raw muscle was heated in the instrument to the assigned temperature and then a scan from 5 °C to 95 °C was performed. Peak temperatures and change in enthalpy (J/g), measured as the area under the curve, were estimated from the thermograms (Park 1994; Benjakul and others 2004; Yongsawatdigul and Park 2004). To insure accuracy, calibration was performed using indium prior to analyses. Six replicates were analyzed for each treatment.

Ca^{2+} ATPase Activity

Denaturation of the crab meat was also monitored by measuring the change in myosin Ca^{2+} ATPase activity (Carvajal and others 1999). The ATPase activity was measured according to the method of Perry (1955) as modified by Ang and Hultin (1989). The myosin ATPase in the sample was used to liberate inorganic phosphate which was measured spectrophotometrically. An incubation medium containing 1.3 mL of 0.2 M glycine buffer (pH 9.1), 0.2 mL of 0.1 M $CaCl_2$, 0.3 mL of 0.05 M ATP was warmed to 25 °C at which time 0.2 mL of crab mince solution (0.45 mg crab mince/mL in 0.5 M KCl) was added. The mixture was incubated for 5 min at 25 °C. The reaction was stopped by the rapid addition of 1 mL of 15% trichloroacetic acid. After centrifugation (10 min at 10,000 rpm), an aliquot of 0.5 mL of the supernatant was mixed with 3.2 mL of 12% trichloroacetic acid and incubated at 25 °C for 10 min. An aliquot of 3 mL of this mixture was then combined with 2 mL of a 5% ferrous sulphate – 1% ammonium molybdate reagent. The absorbance of the reaction mixture was measured at 363 nm in a polycarbonate cuvette. A blank without the addition of the crab mince homogenate was used and a standard curve was created using potassium phosphate. The activity was measured as the concentration of inorganic phosphate released per g of mince.

Total Sulfhydryl Content

Protein molecules contain sulfhydryl groups may form disulfide bridges. The total sulfhydryl content (T-SH) was determined by reacting the groups with 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) as described by Simplicio and others (1991). A 0.5 g sample of crab mince was homogenized in 25 mL of cold (5-7 °C) deionized water using

a polytron (Kinematica; Neward, NJ, U.S.A.) for 1 min. An aliquot of the homogenate was dissolved in a urea-SDS solution containing 8 M urea, 2 M thiourea, 3% SDS, and 0.05 M Tris (pH 8.0) at a ratio of 1:1 homogenate to urea-SDS solution. To 1 mL of the sample in urea-SDS solution, 2.0 mL of 0.1 M phosphate buffer (pH 7.4), and 0.5 mL of 10 mM DTNB (in 0.1 M phosphate buffer, pH 7.4) were added. The absorbance of the solution was read at 412 nm after a 5 min reaction time. A blank with DTNB in the absence of proteins was used. The concentration of sulfhydryls was estimated using a molar extinction coefficient of 11 400 M^{-1} cm^{-1}.

Myosin Extraction

Native myosin was extracted from each of the treated samples. As native myosin becomes denatured, less should be extracted from a muscle sample. Myosin was extracted using the method of Connell (1958) as modified by Ang and Hultin (1989). Solutions used in the analysis were KCl-phosphate buffer (ionic strength 0.46) prepared by dissolving 17.9 mmol KH_2PO_4, 82.1 mmol Na_2HPO_4, and 200 mmol KCl in 1 L deionized water and KCl-phosphate buffer (ionic strength 0.66) prepared by dissolving 17.9 mmol KH_2PO_4, 82.1 mmol Na_2HPO_4 and 400 mmol KCl in deionized water. The mince was homogenized (Kinematica; Newark, NJ, U.S.A.) with 3 volumes (w/v) of cold KCl-phosphate buffer (ionic strength 0.46) for 1 min. The suspension was then centrifuged at 3000 x *g* for 15 min. The supernatant was diluted by slow addition of 6 volumes cold deionized water. Precipitation was allowed to proceed overnight at 4 °C. The precipitate was collected by centrifuging at 3000 x *g* for 15 min. The precipitated myosin was then dissolved in 5 volumes of cold KCl-phosphate buffer (ionic strength 0.66). After stirring for 5 min, the myosin solution was filtered using Whatman No. 1

qualitative filter paper. The protein concentration of the myosin solution was measured using the method described by Lowry and others (1951). The concentration was diluted to 0.2% protein using KCl-phosphate buffer (ionic strength 0.66). Solutions were then analyzed for extracted myosin using SDS-PAGE gels as described by Laemmli (1970).

Statistical Analysis

An incomplete randomized block design was used. A total of nine treatments were analyzed. Multi-way analysis of variance was conducted on total sulfhydryl concentration and inorganic phosphate concentration using SAS (9.1) with least square means and Tukey mean separation to evaluate the effects of cook method (in shell and out of shell) and cook temperature (62.8 °C, 75.5 °C, 82.2 °C, 100 °C). Significance was established at $p < 0.05$.

Results and Discussion

Differential Scanning Calorimetry

Differential scanning calorimetry showed that some of the heat treatments did not fully denature all the proteins within a muscle sample at temperatures below the specific cooking temperature (Tables 12 and 13). For example, when the meat was cooked in the shell to 82.2 °C, three of the six samples subsequently exhibited an endothermic peak in the 76.8 to 78.4 °C temperature range. A representative thermogram of raw crab meat is shown in Figure 18. Not all sample replicates exhibited exactly the same peak patterns. The variability in endothermic peak patterns within treatments may have been due to potential gender, age, or size differences in the commercially obtained crabs.

Table 12: Exothermic and endothermic peaks from DSC analysis for crab meat cooked in the shell.

Peak Temperature Range	Treatment Temperature									
	Raw Crab		62.8 °C		75.5 °C		82.2 °C		100 °C	
	#**	ΔH (J/g)	#	ΔH (J/g)	#	ΔH (J/g)	#	ΔH (J/g)	#	ΔH (J/g)
19.3-21.2°C*	1	0.30	2	0.13-0.26	1	0.37	3	0.13-0.36	2	0.13-0.25
40.1-46.5 °C	6	0.31-0.42	1	0.22	0	-	0	-	0	-
50.7-63.1 °C	0	-	0	-	0	-	0	-	1	0.26
71.1-75.0 °C	4	0.16-0.32	0	-	0	-	0	-	0	-
76.8-78.4°C	0	-	1	0.09	0	-	3	0.02-0.19	0	-
83.2-88.8°C	0	-	0	-	1	0.08	0	-	0	-
91.6-97.7°C	2	0.15-2.34	0	-	1	2.27	0	-	0	-

* All peaks in this range were exothermic.

** Number of peaks in the indicated temperature range out of a total of 6 replications.

Table 13: Exothermic and endothermic peaks from DSC analysis for crab meat cooked out of the shell.

Peak Temperature Range	Treatment Temperature							
	62.8 °C		75.5 °C		82.2 °C		100 °C	
	#**	ΔH (J/g)	#	ΔH (J/g)	#	ΔH (J/g)	#	ΔH (J/g)
19.3-21.2°C*	0	-	0	-	0	-	0	-
40.1-46.5 °C	0	-	1	0.57	0	-	0	-
50.7-63.1 °C	1	1.54	1	1.04	0	-	0	-
71.1-75.0 °C	1	0.86	1	0.19	1	0.04	0	-
76.8-78.4°C	1	0.38	1	27.65	0	-	0	-
83.2-88.8°C	0	-	1	37.39	3	0.24-42.25	0	-
91.6-97.7°C	3	0.32-1.76	2	1.2-4.46	0	-	0	-

* All peaks in this range were exothermic.

** Number of peaks in the indicated temperature range out of a total of 6 replications.

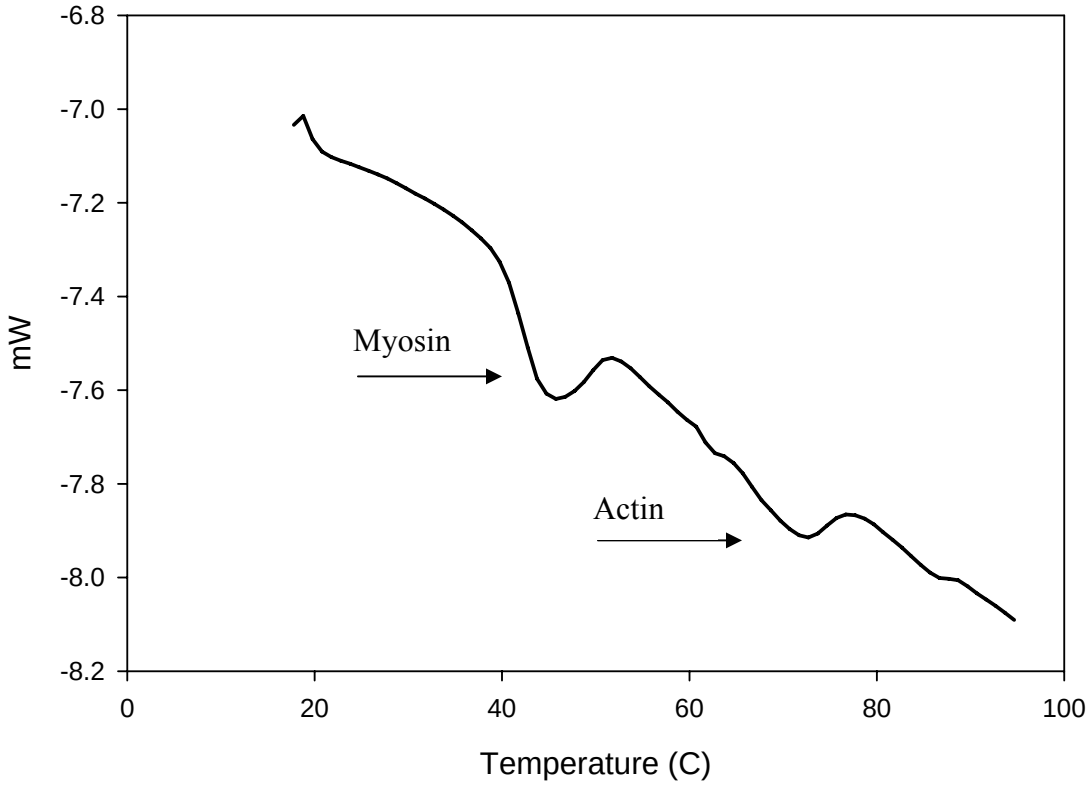

Figure 18: Representative DSC thermogram of raw crab mince

Surprisingly, meat cooked outside the shell exhibited more endothermic peaks (a total of 17 peaks for the 6 samples) than the meat cooked inside the shell (8 peaks). Another interesting difference between the two cooking methods is the appearance of an exothermic peak in the 19.3 to 21.2 °C range that only appeared in the meat cooked in the shell. Meat cooked out of the shell did not exhibit this peak.

An exothermic peak indicates that the material is releasing energy at this temperature, unlike protein denaturation which requires energy to unfold. As of yet, the reason for this peak has not been identified. Another item of interest is the high

temperature (83.2 to 88.8 °C) peak that is exhibited in both the meat cooked in the shell and out of the shell. Myosin is denatured at approximately 40 to 45 °C. The denaturation temperature of actin is often associated with the 70 to 75 °C range but when isolated is much higher, in the 80 to 85°C range (Wright and others 1977). The raw crab did not exhibit all peaks collectively observed among all samples, likely due to not all proteins being present in a heterogeneous 10 to 15 mg sample. The high temperature peak in the crab muscle may represent denaturation of actin, although the protein was not isolated. The peaks in the 70 to 80 °C range may also have been actin, or the protein titin. Pospiech and others (2002) showed that porcine titin, a stromal protein that aids in myofibril elasticity, denatures in the 74 to 78 °C range. Titin is also an important stromal protein in crabs to stabilize thick filaments in crab muscle tissue (Akimoto and Sugi 1999). The peaks in the 70 to 80 °C range may have been the stromal proteins of the crab muscle denaturing, however further testing is required to confirm this hypothesis. Whatever the identity of the muscle proteins, it is clear from the DSC data that cooking to a temperature of 75.5 °C does not fully denature all crab muscle proteins.

Crabs contain trimethyl amine oxide (TMAO), which has the primary function of being an osmoregulator in the crab muscle tissue (Hayashi 1976). The amount of TMAO in marine animals varies depending on the depth at which the species lives. Research has shown that TMAO *in vitro* can offset the pressure inhibition of the polymerization of actin and the enzyme-substrate binding for two enzymes (Yancey and Siebenaller 1999; Yancey and others 2002). TMAO, which is present in other species such as snow crab (Hayashi and others 1981) may be helping to prevent the complete denaturation of the proteins in the crab meat.

Ca^{2+}ATPase Activity

All crab muscle samples retained some ATPase activity after thermal processing regardless of the cook method used, indicating that the protein was not completely denatured. The Ca^{2+} ATPase activity, measured as the concentration of released inorganic phosphate, did not significantly differ between meat cooked in or out of the shell (Figure 19).

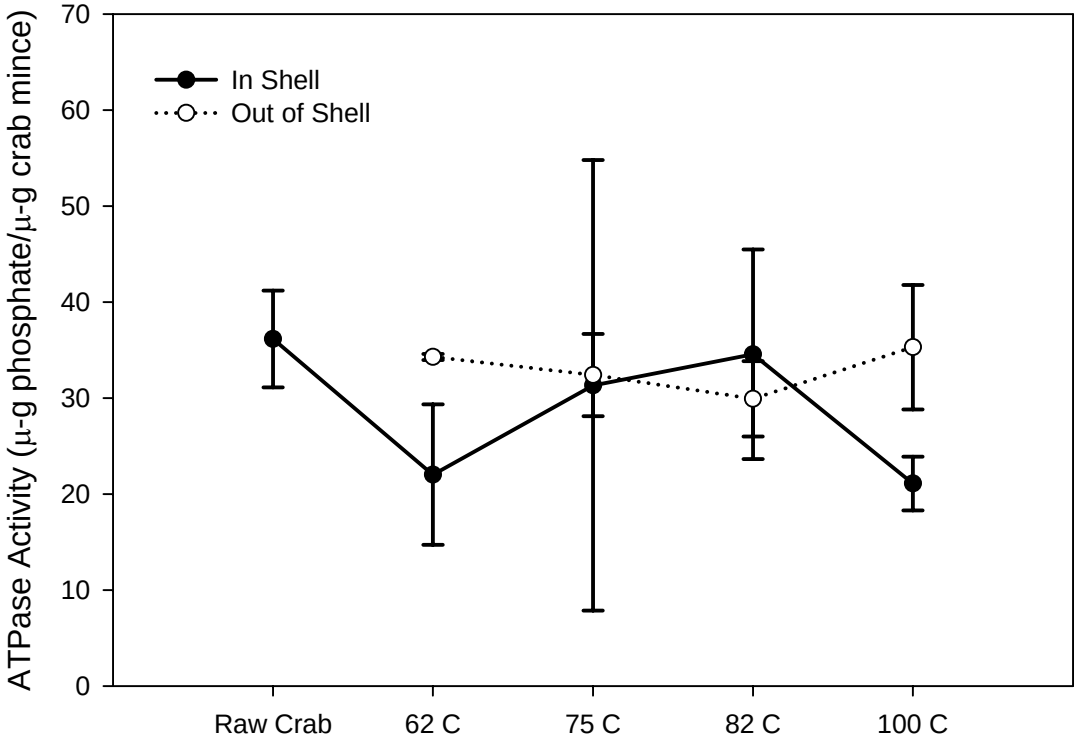

Figure 19: ATPase activity of crab meat cooked in and out of the shell.

Values shown are the average of three measurements with standard deviations.

However, the ATPase activity of the crab meat cooked in the shell had a larger standard deviation than that cooked out of the shell. The variations may be attributed to environmental conditions within the crab shell upon cooking. Preliminary results (Appendix A) showed a consistent 40% decrease in ATPase activity of crab meat when boiled to varying internal temperatures. The current study results do not show a consistent downward trend in ATPase activity in response to increased cook temperature, most likely due to the elimination of temperature gradients within the meat. When boiled, the meat closest to the shell gets a greater heat treatment than the meat in the center of the claw, which leads to temperature gradients within the meat. The crab meat was boiled for less than 3 minutes in the preliminary study whereas it took 15 to 30 min to cook the crab meat (both in shell and out) to the assigned temperature under controlled heating conditions. Further investigation is needed to determine the thermostability of the crab muscle proteins and how the cooking method (boiling or controlled heat) impacts their denaturation.

Myosin ATPase concentration can be directly correlated with the gel strength of the corresponding thermal gel. For example, the greater the ATPase activity, the greater the gel strength (Katoh and others 1979). A correlation also exists between the ability of actomyosin to form a cohesive gel and the level of ATPase activity (Haard 1992). Though the level of ATPase is important, this level varies with species. Myosin from cold water species tends to be less heat stable than that from warm water species (Hashimoto and others 1982). Jonah crab, a cold water species, should therefore have myosin that is not very heat stable. The results obtained from this study show that the ATPase activity of the muscle did not consistently decrease with increasing temperature

which contradicts the myosin stability research previously reported for other species.

Total Sulfhydryl Content

Although the total sulfhydryl content of the meat cooked outside the shell was consistently higher for each temperature tested, the differences were not significant between heating methods (Figure 20). The lack of significant change between raw and cooked meat indicates that the sulfhydryl groups did not become oxidized or form additional disulfide linkages during any of the heat treatments. The formation of disulfide bonds is important in determining the aggregate rheological properties (Broersen and others 2006). As seen in ATPase data, the standard deviation for the total sulfhydryl groups content were greater for the meat cooked in the shell versus out of the shell. In this case a correlation cannot be made between the sulfhydryl content and properties of the meat gel. To further understand the unfolding of the protein molecules, the reactive sulfhydryl content should also be determined.

Myosin Extraction

No native myosin, myosin that had not been denatured, was extracted from any of the crab meat treatments (Figure 21). The method used in this study should have extracted any myosin in the treatments that had not unfolded due to thermal processing. Myosin heavy chain, (M_w ~ 205 kDa) is the main protein responsible for gelation of meats (Yasui and others 1980; Samejima and others 1981).

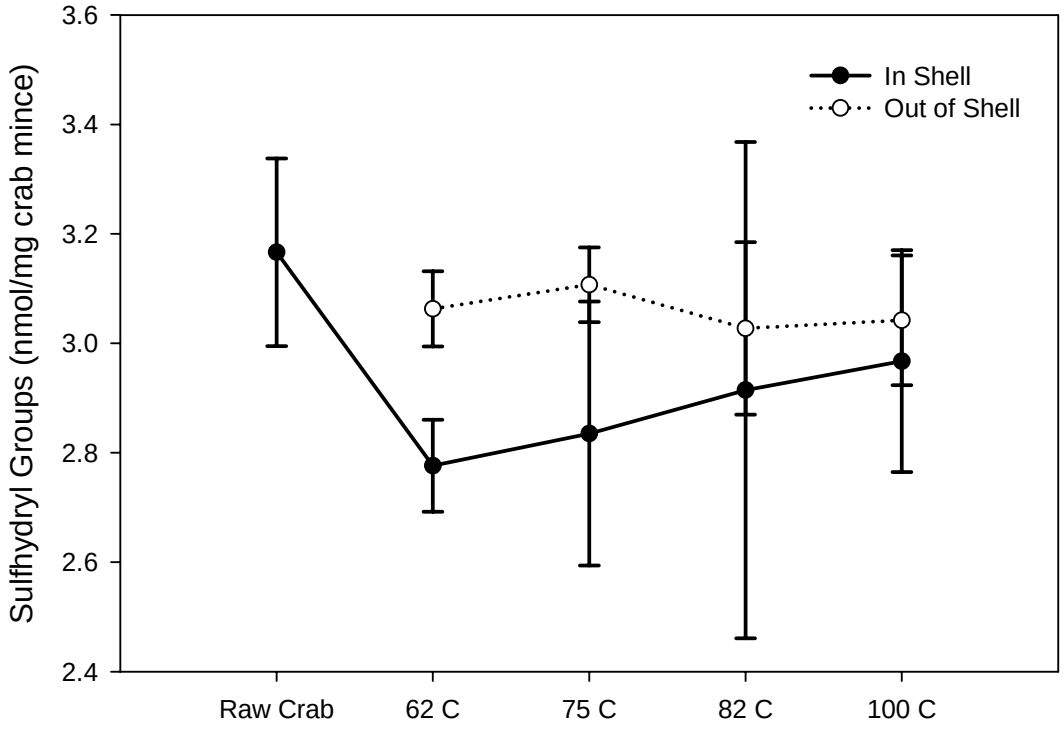

Figure 20: Total concentration of sulfhydryl groups in crab meat cooked in and out of the shell

Values shown are the average of three measurements with standard deviations.

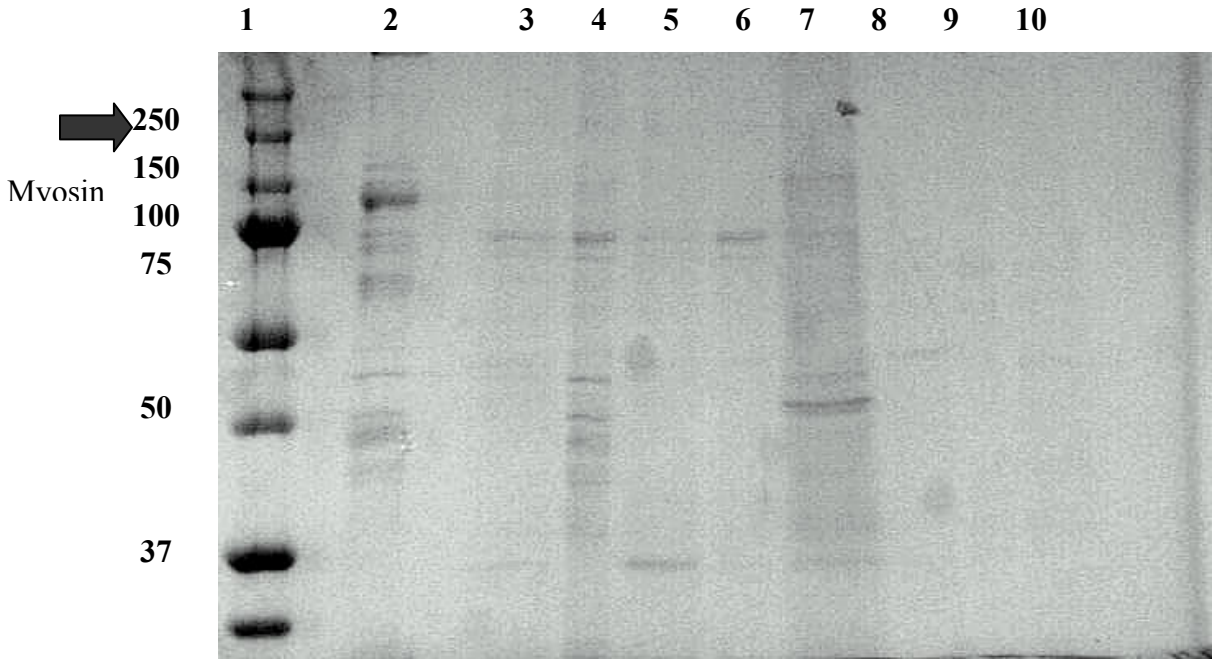

Figure 21: SDS-PAGE analysis of muscle extracts from each crab treatment.

Lane 1 is the molecular weight standard. Lane 2 is the extract from raw crab. Lanes 3 and 4 are extracts from meat cooked to 62.8 °C, in and out of shell, respectively. Lanes 5 and 6 are extracts from meat cooked to 75.5 °C, in and out of shell, respectively. Lanes 7 and 8 are extracts from meat cooked to 82.2 °C, in and out of shell, respectively. Lanes 9 and 10 are extracts from meat cooked to 100 °C, in and out of shell, respectively.

Interestingly the ATPase activity of the muscle did not change with heat treatment, but no native myosin was extracted. It may indicate that ATPase activity was not from myosin but from thermostable ATPases present in the cell and organelle membranes. The method used in this study was developed for extraction of cod myosin, therefore modification may be needed to extract myosin from cooked crab meat. Many lower molecular weight bands (< 205 kDa) appeared in the SDS-PAGE gels which may indicate enzymatic break down of the myosin molecule during extraction. This was

especially true in the raw sample which should have contained the largest quantity of myosin. Raw crab meat contains active proteolytic enzymes that may have degraded myosin molecules during the extraction process.

Conclusion

Jonah crab muscle proteins did not become fully denatured as a result of thermal processing as indicated by the presences of ATPase activity and endothermic peaks. There was no measurable effect of cooking the meat in versus out of the shell on the protein structure of Jonah crab meat. The number of sulfhydryl bonds remained constant indicating a lack of oxidation of sulfhydryl groups and disulfide bond formation. Interestingly, ATPase activity was preserved but no native myosin was extracted from any of the crab meat. Differential scanning calorimetry analysis indicated that though some protein denaturation did occur, not all of the proteins below the treatment temperature were denatured, in or out of the shell. Based on these results it is concluded that cooking the meat in the shell does not significantly affect protein denaturation compared to cooking outside the shell but does increase the variability of the protein denaturation measurements possibly due to increased pressure within the shell during cooking or the presence of compounds (such as osmoregulators) that help stabilize the proteins in the shell.

CHAPTER 6. DEVELOPMENT OF A CRAB APPETIZER FROM JONAH CRAB (*CANCER BOREALIS*) MINCED MEAT

Chapter Abstract

In crustacean processing, large quantities of edible by-products are produced annually. These by-products are often used in low-value products or are used as animal feed. Our objectives were to use edible minced meat from Jonah crab (*Cancer borealis*) processing by-products to create a crab appetizer. Consumer focus groups were conducted to determine preferences for crab flavor, cooking methods, and new product concepts. Three varieties of crab appetizers were developed: Curry, Jalapeno cheese, and Italian. Sixty-six consumers evaluated each using a 9-point hedonic scale to evaluate appearance, flavor, crab flavor, texture, and overall acceptability. The Italian variety was the most acceptable receiving scores of "like somewhat" to "like moderately". The least acceptable variety was the Jalapeno cheese receiving overall acceptability scores of "neither like nor dislike" to "like somewhat". These findings show that minced meat by-products from Jonah crab can be used to create a consumer acceptable crab appetizer product.

Introduction

Annual crab landings in the U.S. are over 300 million pounds (NMFS 2002), which generates an estimated 270 million pounds of processing waste per year. Average meat yields of picked crab meat range from 10% to 12% of the live crab weight (Ward 1990). Mechanical methods of removing meat from the shell have been explored to increase the yield, however consist high-quality results have not yet been obtained. The

meat from the processing discards, walking legs and bodies, can be mechanically separated to further extract residual meat as mince, thereby increasing the meat yield by 40% (CIFT 2007). Frozen, minced Jonah crab meat extracted in such a way can then be used as a filler and flavor enhancer in bisques, soups, stuffing, dips, and crab cakes for the food service industry. Blue crab mince, used as an extender in devilled crab, seafood stuffings, soups, and chowders, has a dark color and high microbial values which limit the marketability of the mince (Gates and Parker 1992). Lee and others (1993) investigated the use of this blue crab mince with additional binders and claw meat in the production of crab cakes. Further product development with Jonah crab mince is needed to fully utilize the resource.

One of the major problems with seafood product development is the concern about seafood safety. In May 2005, approximately 60% of consumers said that they were extremely concerned about mercury in seafood (Sloan 2006a). Consumers also stated that *trans*-fats were a concern in both restaurant and retail products with 41% "very concerned" about *trans*-fat levels (Hedlund 2007). However, even with the concerns, seafood consumption is at an all time high and is expected to continue growing through 2020 (Sloan 2007).

Sales of seafood have been increasing, with crustacean sales making up 41% of the total. Crab sales alone account for 22% of the crustacean market (Sloan 2007). Different age groups in the population seem to want different types of products from the food industry. Empty-nesters, who are entertaining more at home, want more gourmet main dishes, appetizers and desserts (Sloan 2006a) whereas 18 to 44 year olds are looking for spicier foods with a foreign flavor (Sloan 2005). With over 85% of

Americans consuming seafood, 45% of whom consume it on a weekly basis, new products are needed to meet consumer demands (Hedlund 2006).

Fish and seafood products are most dominant in the restaurant industry as appetizers. Crab cakes rank 7th on the list of most popular appetizer items with calamari close behind in 8th position (Sloan 2006c). Young diners are looking for more courses in a meal, specifically for appetizer options (Sloan 2006b) whereas older diners are looking to "buy up" to more gourmet and adventuresome products (Sloan 2006a). When speaking in terms of "fancy" foods, lobster and crab are always at the top of the list (Sloan 2005). In fact, crab cakes have started to appear on more menus across the country within the past few years (Ramseyer 2006). The increasing popularity of crab cakes in restaurants has been largely due to the availability of low-cost imported crab meat (Ramseyer 2005). Crab meat consumption has increased markedly over the past 10 years. In 1994, the average consumption of crab meat per capita was 0.3 pounds, which doubled to 0.6 pounds per capita in 2004 (Anon. 2006b).

Not only has consumer preference for crab changed, but the types of flavors consumers enjoy have also begun changing. Italian, Mexican, and Chinese cuisine rank at the top of consumer preferences (Sloan 2006c). Companies are now coming out with new product lines with more exotic flavors. Products such as *Curry and Cashew Chicken* (Sable and Rosenfeld), *Calamari Rings and Tentacles* (Tampa Maid), *Jumbo Crab Bites* (Po-Boy), *Phillip's Crab Slammers* (Phillip's Food, Inc.), *Lobster Bites* (Long John Silver's), and *Crab Spread* (Beck's) are all new value-added products developed to meet the demand for new flavors and textures (Sloan 2005; Anon. 2006a).

With the demand increasing not only for seafood products, but more specifically

crab products, product development research is needed to formulate minced crab meat into new value-added products. Consumer focus groups can be used to gain a better understanding of consumer preferences for flavors and cooking methods of crab which can aid in the development of new products. The purpose of this study was to determine consumer preferences regarding reformed crab products and to use these to create a consumer acceptable product that utilizes Jonah crab meat mince as a primary ingredient.

Materials and Methods

Focus Groups

The test protocol for the focus groups was approved by the University of Maine Committee for the Protection of Human Subjects. Twelve participants were recruited from the University of Maine community to participate in one of two consumer focus groups. A flyer was sent out to the general University community via email. Participants had to be at least 18 years of age and consume fish at least once per month. During the 2 hr sessions, participants were asked questions about shopping for seafood products, eating preferences for seafood products, crab preferences, and ideas for new crab product development. Both sessions were video taped and audio recorded. Transcripts of each session were made from the video and audio recordings and summarized (Appendix B). Trends in consumer responses were determined based on the responses provided most often from the panelists.

Product Development

Based on consumer feedback and analysis of market trends, we chose to develop a crab appetizer product which was a cross between a traditional crab cake and a nugget-

style product. Frozen, commercially processed Jonah crab meat mince was obtained from Portland Shellfish (Portland, Maine, U.S.A.). Raw crabs were eviscerated, boiled for 14 minutes, and cooled. Claw meat was removed and the remaining crab sections were used in the production of the mince. Meat from the walking legs and carapace was removed using a mechanical deboner, packaged in 10-pound polyethylene bags and frozen.

Frozen mince was thawed at 4 °C for 48 hr before processing. Three varieties of crab appetizers were formulated: Curry, Jalapeno cheddar, and Italian. Dry egg white was used to increase the hardness and springiness of the crab mince gels. The formulations of the three appetizers are summarized in Table 14. Appetizer cores were made by combining the listed ingredients (except for the batter, breading and crab mince) for each variety in a food processor and chopping finely. Ingredients were then mixed with the mince for 5 min in a Kitchenaid mixer with a paddle attachment. The mince mixtures were then stuffed into cellulose sausage casings and cooked in water using a two-stage heat treatment: 35 °C for 30 minutes followed by 90 °C for 30 minutes. Gels were cooled on ice for 30 minutes and stored at 4 °C overnight. Gels were then sliced into 1.27 cm thick slices, battered (Onion Ring Batter, Concord Foods, Brockton, MA, U.S.A.), and breaded (Plain Bread Crumbs, 4C, Brooklyn, NY, U.S.A.) before deep frying in an industrial deep fat fryer (Hotpoint, General Electric Company, Fairfield, CT, U.S.A.) at 182 °C (360 °F) for 1 minute. Appetizers were then drained and placed in holding pans in a steam table covered loosely with foil for consumer testing. Appetizers were cooked to an internal temperature of at least 73.9 °C (165 °F) and held for no longer than 20 min.

Table 14: Formulation for each of the three crab appetizer varieties.

		% of Formulation		
Core Ingredients	**Source**	**Italian**	**Jalapeno**	**Curry**
Jonah crab minced meat	Portland Shellfish, Portland, ME, U.S.A.	70.6	84.2	77.5
Dry egg white	Deb El, Elizabeth, NJ, U.S.A.	4.0	4.0	4.0
Sun dried tomato	Bella Sun Luci, Mooney Farms USA, Chico, CA, U.S.A.	3.5		
Green pepper	Hannaford Bros. Co., Scarborough, ME, U.S.A.	5.7		
Onion	Hannaford Bros. Co.	6.4		
Red pepper flakes	McCormick & Company, Inc., Sparks, MD, U.S.A.	0.2		
Italian seasoning	Gourmet Garden Botanical Food Company, Queensland, Australia	1.4		
Tomato paste	Hunts, Conagra Foods, Omaha, NE, U.S.A.	3.5		
Chopped garlic	Spice World, Orlando, FL, U.S.A.	3.9		
Salt	Hannaford Bros. Co.	0.9		1.0
Raisins	Hannaford Bros. Co.			8.1
Coconut flakes	Hannaford Bros. Co.			4.8
Honey	Hannaford Bros. Co.			3.9
Curry powder	McCormick & Company, Inc.			0.7
Hot Jalapeno pepper slices	Vlasic, Cherry Hill, NJ, U.S.A.		3.0	
Mild banana pepper slices	Mt. Olive, Mount Olive, NC, U.S.A.		3.8	
Shredded mild cheddar cheese	Kraft Foods, Northfield, IL, U.S.A.		4.6	
Cayenne pepper	McCormick & Company, Inc.		0.2	
Cheddar cheese powder	Kraft Foods, Northfield, IL, U.S.A.		0.2	

Sensory Evaluation

Consumer acceptance testing was conducted on the three crab appetizer varieties in the University of Maine Consumer Testing Center. The test protocol for consumer acceptability testing was approved by the University of Maine Committee for the Protection of Human Subjects. Subjects were recruited via flyers and email postings. All subjects were required to be at least 18 years of age, not have allergies to shellfish, dairy, wheat, or soy, and consume crab at least twice per year. A total of 66 subjects were recruited for the testing. SIMS 2000 software (version 3.3, Sensory Computer Systems, Morristown, N.J., U.S.A.) was used to generate the questionnaire and to collect and analyze data. Each subject was seated in a booth with partitions and evaluation rooms were climate controlled with positive-pressure air flow to prevent odors from the preparation area to bias judgments. A combination of fluorescent and incandescent lighting was used.

Subjects were first asked demographic questions including: age, gender, how often they consume crab products, which crab products they had eaten in the past 6 months, and where they most often purchase crab products. Subjects were then asked to rate the overall acceptability, appearance, overall flavor, crab flavor, and texture of each of the crab appetizers (Appendix C). Subjects were also asked if they would be willing to purchase the product, what was the most they would be willing to pay at a grocery store for 6 servings (3 pieces per serving) of the product, and if the product were labeled as containing no *trans*-fat and had a total fat content of less then 10 g per serving how it would influence their choice to buy the product.

Each of the three crab appetizer varieties was assigned a random, 3-digit code.

Subjects were served samples in a randomized order determined by the SIMS software. A 9-point hedonic scale (1 = dislike extremely, 9 = like extremely) (Peryam and Pilgrim 1957) was used to assess the degree of liking for the product attributes. Samples were served on paper plates with a single fork and a cup of spring water. Each sample was presented individually.

After acceptance testing, those 12 subjects who had participated in the focus groups were asked to fill out a secondary survey. These subjects were asked how the crab appetizers met their overall expectations, how the crab appetizer flavor met their expectations, how the crab appetizer texture met their expectations, and whether they would be willing to purchase the crab appetizer product.

Proximate Analysis

Five appetizers from each variety were pooled and ground to determine moisture, crude fat, ash and total protein content. Moisture, crude fat, and ash content were analyzed using AOAC methods using methods # 950.46, 922.06, and 938.08, respectively (AOAC 2005). Total protein was determined by measuring the nitrogen content using the Elementar Rapid N III nitrogen analyzer (Elementar Americas, Inc., Mount Laurel, N.J., U.S.A.). All analyses were performed in triplicate.

Statistical Analysis

Proximate analyses data were analyzed using SAS (9.1) with least square means and Tukey mean separation. Sensory data were analyzed by SAS (version 8e, SAS Inst. Inc., Cary, N.C., U.S.A.), and Tukey's mean separation. Significance was established at $p < 0.05$.

Results and Discussion

Focus Group

Each focus group was asked general questions about shopping for seafood products, such as the types of products they buy and where they typically purchase seafood. Combined results from both groups showed that all of the subjects preferred to buy fresh fish and to only use frozen-at-sea fish in recipes such as chowders. If forced to purchase frozen seafood, then flash frozen was preferred. All of the subjects bought fish at either the grocery store or fish market, mainly due to ease and location. The choice of fish products mainly depended upon cost, appearance and odor of the product.

In evaluating the attributes consumers look for in seafood products, each group was asked about flavors, textures, and types of products they preferred to eat. The most important attribute to the group members was freshness of the fish. They preferred fish that was flaky, not chewy. Most preferred their fish with butter or white wine sauces, with some herbs such as dill. When asked about formed seafood products, such as fish sticks and nuggets, most of the panelists said they did not consume fried fish often and preferred baked or broiled fish. The only formed seafood eaten by either group was imitation crab legs (surimi) due to its low cost.

The groups were each asked about preferences in eating crab, such as deterrents in eating crab, texture preferences, and flavors associated with crab. The most commonly consumed crab product, except for crab lump meat, were crab cakes. The greatest deterrent in eating crab was the cost so it is often used only for special occasions. The texture of crab was expected to be similar to that of chopped egg white, slightly flaky, and not pasty. Few flavors were used in crab preparations, but the most common were

mayonnaise, butter, garlic, and mild herbs.

The discussion ended with a brain-storming session in which the groups expressed opinions on some new restructured crab products and suggested new ideas. The concept of crab nuggets was not liked by either group and was considered a product for children. The idea of crab sausages was liked by several members in each group, as long as the sausages had synthetic casings. Suggested flavors for the sausages included spicy, Italian, and fennel seed. The concept of a crab cake with little to no breading in the meat was suggested as a crab burger or patty. Subjects preferred a firm texture for this product that would be plump and work well on a bun. Several crab dips were also suggested, such as Cajun, dairy-based, avocado, crab salsa, and spinach with parmesan. Subjects also liked the idea of a smoky crab paté that could be frozen or have a shelf life of up to 2 weeks. A Mexican burrito or tamale with cilantro, green salsa, and spicy sauce was also suggested by the subjects. The product would be frozen and served as microwavable product.

Comments from the focus group fit into reported consumer trends. Younger consumers are looking for more ethnic flavor with a greater kick (Sloan 2005). The consumer focus group confirmed this trend by suggesting ethnic food products, such as burritos. The spicy trend was also suggested by the focus group through suggestions of Cajun dips and spicy sausages. The overall U.S. consumer trends matched closely with those of the focus groups conducted.

Sensory Evaluation

Sixty-six people completed the consumer acceptance testing of the three varieties of crab appetizers. Demographic information is provided in Table 15. Approximately

61% of the subjects were women and the age of panelists ranged from 20 to 56+ with an average age of 33. Approximately 98.5% of the subjects had consumed crab products within the past year, with 61% consuming crab 1 to 2 times a year and 1.5% consuming crab more than twice per week. When asked about the crab products eaten in the past 6 months, 56% said they had eaten canned or fresh crab meat, 44% had eaten crab cakes and 29% had consumed crab claws. 55% of the subjects most often purchased crab products in a supermarket/grocery store whereas only 42% most often purchased crab at restaurants (Table 16).

Table 15: Subject demographic information from consumer acceptability testing.

Gender	**Number**	**% of Total**
Female	40	61
Male	26	39
Age		
18-25	21	32
26-35	11	17
36-45	10	15
46-55	12	18
56+	12	18

Table 16: Crab consumption data on 66 subjects participating in consumer acceptability testing of 3 varieties of crab appetizer.

Question	Response	% of Subjects
How often do you consume crab products?	Never	1.5
	1-2 times per year	60.6
	1-2 times per month	34.9
	1-2 times per week	1.5
	More than 2 times per week	1.5
Which crab products have you eaten in the past 6 months? Mark all that apply.	I have not eaten crab in the past 6 months	9.1
	Whole crab	9.1
	Crab claw (e.g. cocktail claws)	28.8
	Canned or fresh crab meat	56.1
	Crab cakes	43.9
	None of these	16.7
Where do you most often purchase crab products?	Supermarket/grocery store	54.6
	Seafood market	19.7
	Restaurant	42.4
	Roadside seafood vendor	3.0
	Do not purchase crab myself.	7.6

In testing consumer acceptability of product attributes, the Italian appetizer received the highest scores compared to the Curry and Jalapeno varieties. The Italian and Jalapeno varieties received the greatest appearance score at 7.2 with the Curry variety close behind at 6.9 (Table 17), consumers ranking all three at "like moderately". The three varieties varied significantly ($p < 0.05$) in flavor acceptability with the Italian variety being the most acceptable (6.9) to consumers ("like moderately"). Subjects thought that the Jalapeno variety had the lowest flavor acceptability (5.9) ranking just below "like slightly". Consumers liked the crab flavor of the Italian variety the most. It received average crab flavor scores of 6.6, which was significantly higher than crab flavor scores for the Jalapeno (5.6) or Curry (5.6) varieties. Based on the comments received, consumers were expecting more crab flavor in all the varieties, even the Italian variety, which ranked the highest in crab flavor. The acceptability scores for texture were the same for all varieties and ranked between "like slightly" and "like moderately", though interestingly the comments received stated that one of the varieties, typically different between different subjects, had a softer and "mushier" texture than the other two. Of the three varieties, the Italian variety had the best overall acceptability score at 6.8. The Italian was statistically the same as the Curry (6.2) but more acceptable than the Jalapeno variety (5.9). Analysis for the top two score levels (8 = like very much; 9 = like extremely) showed that 38% of the subjects ranked the overall acceptability of the Italian variety as an 8 or 9, versus 32% and 23% for the Curry variety and the Jalapeno variety, respectively.

Table 17: Acceptability scores for three varieties of crab appetizers.

Variety	Appearance	Flavor	Crab Flavor	Texture	Overall
Italian	7.2 a	6.9 a	6.6 a	6.6 a	6.8 a
Jalapeno	7.2 a	5.9 b	5.6 b	6.5 a	5.9 b
Curry	6.9 a	6.5 ab	5.7 b	6.5 a	6.2 ab

Values shown are the average of 66 measurements. Statistical differences with significance at $p < 0.05$ are indicated by the small letters in each column. Varieties with the same letter are not statistically different.

After trying the three varieties of crab appetizers, subjects were asked whether they would be willing to purchase the product (Table 18). The Italian variety was most likely to be purchased with 64% of the subjects reporting that they would purchase the product. The Jalapeno variety was the least likely to be purchased with only 42% of the subjects stating that they would purchase the product. In a retail market, 65% of the consumers were willing to pay at least $4.00 for 18 Italian crab appetizers, whereas only 56% were willing to pay at least $4.00 per 18 Curry or Jalapeno appetizers. Based on focus group input, consumers were concerned with the amount of fat and *trans*-fat a product contained. In response to the question "If this product were labeled as containing no *trans*-fat and had a total fat content of less than 10 g per serving, would you be more likely to buy the product, less likely to buy the product, or would it not affect your choice?" between 56% to 61% of the subjects said that they would be more likely to purchase the product. The differences between varieties are likely due to the consumers' liking of the product. For example, if the consumer did not like the product, even a label that states it has no *trans*-fat and less than 10 g of fat per serving may not influence their choice to purchase it.

Table 18: Purchase intent of 66 subjects who participated in consumer acceptability of 3 varieties of crab appetizer.

		% of Subjects		
Question	**Response**	**Italian**	**Jalapeno**	**Curry**
Would you be willing to purchase this product?	Yes	63.6	42.4	51.5
	No	36.4	57.6	48.5
What is the most you would be willing to pay at a grocery store for 6 servings (3 pieces per serving) of the product you just sampled?	Less than $4	36.4	45.5	47.0
	Between $4 and $5	43.9	30.3	24.2
	Between $5 and $6	15.2	18.2	22.7
	Between $6 and $7	4.5	7.6	9.1
	More than $7	1.5	0	0
If this product were labeled as containing no *trans*-fats and had a total fat content of less than 10 g per serving, how would this influence your choice to buy the product? `	I would be more likely to purchase the product.	59.1	60.6	56.1
	I would be less likely to purchase the product.	6.1	0	1.5
	It would not affect my choice.	34.8	39.4	42.4

The focus group follow-up survey was completed by 6 of the 12 subjects. Half of the subjects agreed that the appetizer met their overall expectations, with one subject responding that two of the three varieties met their expectations but the third (Jalapeno variety) did not. Two-thirds of the subjects reported that the flavor met their expectations, with one subject stating that it was variety dependent. The most common comment was that the spices used masked the "crab" flavor and that if they had not been told they would not have known it was crab meat. The same was reiterated in the comments from the 66 subjects conducting acceptability testing. Future product development is needed to determine the appropriate level of spice that would still retain the crab flavor of the appetizers. Overall the crab appetizer texture met the expectations of 5 of the 6 focus group subjects. The one panelist who did not think the texture met their expectations stated that the texture of one of the samples (unknown) was too mushy though the others were fine. Of the focus group participants, 4 of the 6 would be willing to purchase at least one variety of the appetizers. The other two subjects stated that they might purchase on a special occasion but only in a restaurant.

Previous product development research has shown that seafood ingredients can be used in the development of value-added products. Lee and others (1993) incorporated blue crab meat mince into a crab cake. Though sensory analysis was not performed, a feasible formulation combined the mince with claw meat, soy protein concentrate, and surimi. Fish burgers have also been developed (Kasapis and others 2004). The fish burgers, incorporating vegetables, fruits, corn starch and other spices and preservatives received overall consumer acceptability scores between 5.0 and 5.5 (5 = neither like nor dislike). In comparison, the crab appetizers were more acceptable to consumers.

In general, the scores for the appetizer product indicate that all of the appetizers were acceptable. To improve these scores the incorporation of spices into the mix needs to be further researched. Other variations, such as a plain crab appetizer with no spices, or an appetizer with Cajun or Mexican flavors should be formulated to test consumer acceptance. Though two focus groups were conducted, additional groups outside of the University setting may be required to gain a better understanding of the "typical" seafood consumer.

Proximate Analysis

The proximate composition of the three crab appetizer varieties varied due to differences in formulation (Table 19). The moisture content was significantly ($p < 0.05$) different in all of the samples; the Jalapeno variety had the greatest moisture content at 57.5% and the Curry variety had the least at 53.3%. Both the Italian and Curry varieties had statistically the same fat content at 10.3% and 10.2%, respectively, whereas the Jalapeno variety had significantly less fat at 9.3%. Protein content also changed with variety with the Jalapeno variety having the greatest protein content at 31.1% and the Italian and Curry varieties with significantly less at 23.6% and 23.3%, respectively. The difference in protein was due to the variation in the ingredients. For example, the Jalapeno variety had the greatest percentage of crab mince (84.2%) with additional protein in other ingredients such as the cheddar cheese. The Curry variety (77.5% crab) and Italian variety (70.6% crab) had less mince and fewer ingredients that contributed to the overall protein content of the products.

Table 19: Proximate analysis of three crab appetizer varieties.

Variety	Moisture (%)	Fat (%)	Ash (%)	Protein (%)
Italian	56.2 b	10.3 a	2.3 a	23.6 b
Jalapeno	57.5 a	9.3 b	2.0 a	31.1 a
Curry	53.3 c	10.2 a	2.1 a	23.3 b

Each value shown is the average of three measurements. Statistical differences with significance at $p < 0.05$ are indicated by the small letters in each column. Varieties with the same letter are not statistically different.

Conclusions

The development of new value-added food products to increase the utilization of seafood resources can be accomplished through monitoring consumer trends and compiling feedback from the consumers. All varieties of the crab appetizer, Italian, Curry, and Jalapeno, were acceptable to consumers with overall acceptability scores of 6.8, 6.2 and 5.9, respectively. The Italian variety received the highest scores for every attribute tested. Consumers were more likely to purchase the product if labeled as containing less than 10 g of fat per serving and no *trans*-fats. Shelf-life studies are needed to optimize the formulations and determine the effect of long-term storage on consumer acceptability. The findings of this study suggest new products from crustacean by-products, such as Jonah crab meat mince, can be developed that are acceptable to consumers and meet the changing demands of the marketplace.

CHAPTER 7. CONCLUSIONS

Jonah crab can be more fully utilized through the development of technologies and processes that utilize the functional characteristics of the mince. This is the first reported research to show that cooked crab proteins can form gels upon washing and further heat treatment. The addition of traditionally used cryoprotectants (4% sorbitol, 4% sucrose, and 0.3% tripolyphosphate) inhibited gelation of the cooked proteins. Further freezing of the crab meat induced freeze denaturation that was also detrimental to gelation. A two stage cooking process, setting at 35 °C for 30 min followed by cooking at 90 °C for 30 min, produced the strongest gels. Gelation was best achieved through the use of at least two washing cycles with no additional sodium chloride, unlike traditional surimi processing that requires 2.5% sodium chloride for maximal gelation of the raw fish proteins. More research is needed to determine the gelation mechanism of the proteins. One area of investigation should determine if the high shear washing method is breaking down protein aggregates thereby allowing for the formation of a more homogenous gel matrix.

Addition of protein and carbohydrate additives altered the crab meat gel characteristics. Crab mince with the addition of 15% dried egg white and 4% *kappa*-carrageenan produced the hardest gels. The addition of 5% and 10% whey protein isolate was detrimental to gel formation and interfered with the protein matrix. Depending on the textural characteristics desired, soy protein isolate, dried egg white, *iota*-carrageenan, *kappa*-carrageenan, and potato starch can all be used to alter the characteristics of the crab gels. An investigation of more thermal-stable additives is needed as well as use of additives in unwashed mince. To maximize product development endeavors, more

information is needed regarding which additives function best in the unwashed mince and if the additives are thermally stable in newly developed products.

As the first reported gelation of cooked crab proteins, the mechanism behind gelation of these proteins is unknown. Preliminary research investigated the denaturation of crab proteins during cooking under controlled heating conditions and how the shell impacted denaturation. When cooked both in and out of the shell, the proteins were not fully denatured. There was greater variability in the physical characteristics of the proteins cooked within the shell. For example, the Ca^{2+} ATPase activity was not significantly different in the meat cooked in and out of the shell, but more variability in the levels existed in the meat cooked in the shell. The presence of osmoregulators may be contributing to the thermal stability of the proteins, though more research is needed. Future research should include measuring the amounts of osmoregulators, such as trimethyl amine oxide, and determining if an increase in this compound relates to more thermal stable crab proteins.

The Jonah crab mince was successfully used in new food product development. Three varieties of a crab appetizer, a cross between a crab cake and a nugget, were developed and consumer tested. All of the varieties were found acceptable by consumers, though improvements can still be made. Investigations to determine appropriate scale-up processes and long-term storage stabilities should be conducted.

Other interesting projects that should be conducted based on this research would further investigate the protein structure of all crustaceans. We have found that gelation can occur in Jonah crab muscle proteins, but we do not know if the functional property of gelation extends to other crustaceans, such as blue crab, crayfish, and lobster. Gelation

studies utilizing meat from these species may help to decrease the processing waste for multiple crustacean industries. Another potential project involves the full evaluation of crab muscle structure. Investigation of the amino acid composition, myosin and actin content, and secondary and tertiary structure may provide clues into the mechanism by which these proteins can form stable gel networks.

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APPENDIX A. THERMAL PROPERTIES OF COOKED JONAH CRAB (*CANCER BOREALIS*) MEAT: A DIFFERENTIAL SCANNING CALORIMETRY STUDY

Objective

The extent of denaturation of previously cooked crab mince has not been reported. Crustaceans are unique in that when thermally processed, the meat is cooked within the shell, forming a closed vessel. Environmental factors within the shell, such as pressure build-up, may affect the rate of protein denaturation. The objective of this preliminary study was to establish methodology for further research and to determine if cooking temperature affects the extent of protein denaturation in crab meat.

Materials and Methods

Freshly eviscerated, raw Jonah crab was obtained from Portland Shellfish (Portland, Maine, U.S.A.). Crabs were transported to the University of Maine on ice. Upon arriving, four crabs were used for each of six heat treatments: 1) no heat, 2) cook to 62.8 °C, 3) cook to 75.5 °C, 4) cook to 82.2 °C, 5) cook to 93.3 °C, and 6) boiled for 14 min. The temperatures were chosen to include a temperature (62.8 °C) below the normal internal cooking temperature while still reducing pathogen loads, and to include temperatures above the commercial processing temperature (82.2 °C and 93.3 °C). The final treatment, boiled for 14 min, was used to simulate the commercial process which boils a large batch of crabs for 14 min. Each set of four crabs was boiled in a 6 L cooking pot until the internal temperature of the claw meat reached the appropriate treatment temperature. Thermocouples were placed in one crab per batch which

measured the temperature in the claw, one walking leg, and within carapace. Once cooked, the crabs were cooled on ice until meat was removed. Meat from the walking legs and carapace of the four crabs per treatment was pooled to insure one uniform sample per treatment. The four raw crabs were kept on ice until meat was extracted and pooled together. The entire experiment was duplicated.

Differential Scanning Calorimetry

The thermal denaturation of muscle from of all treatments was evaluated using differential scanning calorimetry (DSC). Muscle samples (15 to 20 mg) were hermetically sealed in aluminum pans and scanned at 10 °C per min from 5 °C to 95 °C. An empty pan was used as a reference. Peak maximum temperatures and change in enthalpy (J/g), measured as the area under the curve, were estimated from the thermograms (Park 1994; Benjakul and others 2004; Yongsawatdigul and Park 2004). To insure accuracy, calibration was performed using indium prior to analysis. Four replicates were analyzed for each treatment.

Ca^{2+} ATPase Activity

Denaturation of the crab meat was also monitored by measuring the change in myosin Ca^{2+} ATPase activity (Carvajal and others 1999). The ATPase activity was measured according to the method of Perry (1955) as modified by Ang and Hultin (1989). The myosin ATPase in the sample was used to liberate inorganic phosphate which was measured spectrophotometrically. An incubation medium containing 1.3 mL of 0.2 M glycine buffer (pH 9.1), 0.2 mL of 0.1 M $CaCl_2$, 0.3 mL of 0.05 M ATP was warmed to 25 °C at which time 0.2 mL of crab mince solution (0.45 mg crab mince/mL

in 0.5 M KCl) was added. The mixture was incubated for 5 min at 25 °C. The reaction was stopped by the rapid addition of 1 mL of 15% trichloroacetic acid. After centrifugation (10 min at 10,000 rpm), an aliquot of 0.5 mL of the supernatant was mixed with 3.2 mL of 12% trichloroacetic acid and incubated at 25 °C for 10 min. An aliquot of 3 mL of this mixture was then combined with 2 mL of a 5% ferrous sulphate – 1% ammonium molybdate reagent. The absorbance of the reaction mixture was measured at 363 nm in a polycarbonate cuvette. Analysis was performed in triplicate and reported as the percent ATPase activity of each sample relative to the ATPase activity of raw crab.

Sulfhydryl Content

The sulfhydryl (SH) groups exposed on the surface of the protein molecule were measured as the reactive SH group (R-SH) (Riddles and others 1979). A crab homogenate of 0.45 mg crab mince/mL cold water was prepared. A 2.75 mL aliquot of crab homogenate was mixed with 50 μL Ellman's reagent (10 mM 5,5'-dithiobis(2 nitrobenzoic acid)) and held at 5 °C for 1 hr. The R-SH content was measured spectrophotometrically using an absorbance of 420 nm and the concentration was calculated using a molar extinction coefficient of 13 600 mol/cm. The total SH (T-SH) content was determined using a modified method of Choi and Park (2002). A mixture of 0.25 mL crab mince homogenate (0.45 mg crab mince/mL), 2.5 mL of 8 M urea, 2% SDS and 10 mM EDTA in 0.2 M Tris-HCl buffer (pH 7.0) and 50 μL Ellman's reagent was incubated at 40 °C for 15 min. The T-SH concentration was measured at absorbance 420 nm (Ellman 1959; Kim and others 2003; Ko and others 2003). Analyses were performed in triplicate for both R-SH and T-SH.

Statistical Analysis

A complete randomized block design was used. A total of six treatments were analyzed. Statistical analyses were performed using SAS (9.1) with least square means and Tukey mean separation. Significance was established at $p < 0.05$.

Results and Discussion

Cooking Method

The cooking method employed in this study used boiling water to cook the crab meat in the shell. One of the biggest disadvantages to using boiling water is the creation of heat gradients within the shell. This results in the meat closest to the shell having a higher temperature than the meat in the center of the shell. The heat gradient within the meat could have also created cold spots in which the meat did not reach the appropriate temperature, potentially leading to inaccurate results for all of the tests. The cooking time required to reach the treatment was short. All of the treatments, except for the crab boiled for 14 min, reached the assigned internal temperature within 4 min of boiling. Though boiling is the cooking method used by commercial producers, in this type of study boiling was not ideal for producing uniform meat temperatures. For future studies, it is suggested that crabs be cooked in a constant temperature water bath until all of the meat reaches the same internal temperature.

Differential Scanning Calorimetry

The results indicated that the crab muscle proteins were not completely denatured during the various heat treatments. All of the treatments, including the raw haddock, exhibited an exothermic peak in the 15 to 24 °C range (Table A. 1). Seven other temperature ranges exhibited peaks in at least one of the treatments. Seven of the eight replicates of raw crab exhibited a peak between 44.2 to 46.2 °C. This peak is associated with the denaturation of myosin. Interestingly even when the crab meat was cooked to a specific internal temperature, peaks still appear in that same temperature range. For example, it was anticipated that the crab cooked to 82.2 °C would not exhibit peaks below 82.2 °C. However, in this case, 3 peaks appeared in this sample below the cook temperature meaning that the proteins were not fully denatured during the process or the proteins were being reversibly denatured. Some peaks occurred below the cook temperature in all of the samples. Interestingly, with increased cook temperature, the number of low temperature peaks began to decline indicating that the proteins become more denatured with higher internal temperatures. One of the more intriguing peak temperature ranges was 87.5 to 90.8 °C. Up to 4 peaks (82.2 °C) appeared in this temperature range for the crab samples, though the raw haddock had no peaks in this range. Connective tissue may add additional peaks to the denaturation pattern (Martens and Vold 1976; Starbursvik and Martens 1980). The enthalpy (ΔH in J/g) of the peaks varied widely even within treatment. A decrease in enthalpy would indicate an increase in the extent of denaturation as seen in frozen mackerel (Saeed and Howell 2004).

Table A. 1: Exothermic and endothermic peaks from DSC analysis for crab meat boiled in shell.

Peak Temperature Range	Treatment Temperature: Raw Crab		62.8 °C		75.5 °C		82.2 °C		93.3 °C		100 °C for 14 min		Raw Haddock	
	#**	ΔH (J/g)	#	ΔH (J/g)	#	ΔH (J/g)	#	ΔH (J/g)	#	ΔH (J/g)	#	ΔH (J/g)	#	ΔH (J/g)
15.0-24.0 °C*	4	0.06-0.40	5	0.06-0.23	3	0.10-0.15	7	0.09-0.24	5	0.03-0.26	7	0.12-0.31	7	0.10-0.35
44.2-46.2 °C	7	0.23-0.57	0	-	0	-	0	-	0	-	0	-	8	0.22-0.55
52.3-54.5 °C	0	-	1	0.01	0	-	0	-	0	-	0	-	3	0.08-0.12
64.5-69.0 °C	1	0.29	0	-	2	0.11-0.39	0	-	0	-	0	-	0	-
70.2-73.7°C	6	0.11-0.27	0	-	0	-	3	0.02-0.19	0	-	0	-	0	-
75.2-79.5°C	2	0.08-0.32	1	0.54	0	-	0	-	2	0.01-0.14	1	0.30	8	0.07-0.44
81.7-84.5°C	1	0.13	1	0.13	1	0.14	1	0.02	0	-	1	0.32	0	-
87.5-90.8°C	0	0	2	0.19-0.43	0	-	4	0.05-0.44	1	0.42	0	-	0	-

* All peaks in this range were exothermic.

** Number of peaks in the indicated temperature range out of a total of 8 replications.

The partial denaturation of the proteins could be due to one of several factors. The potential presence of cold spots in the cooked meat is the most likely reason. The cold spots would result in proteins that were not denatured to the same extent as the adequately processed proteins therefore leading to "false" peaks in the thermograms. This problem can be remedied by using a controlled heat source. The second reason for partial denatuaration could be due to cooking the meat in the shell. The shell itself may be impacting the extent of denaturation of the proteins, either through insulating the proteins from the full heat treatment or by causing pressure which would limit the unfolding process. To test the effect of the shell on heating and the extent of protein denaturation, crab meat must be cooked outside of the shell to see if differences in the thermograms, ATPase activity and sulfhydryl concentration exist.

Ca^{2+} ATPase Activity

The raw crab had significantly more ATPase activity than the rest of the heat treated samples. All of the heat treated samples had statistically the same amount, approximately 40% that of raw crab, of ATPase activity as seen in Figure A. 1. The concentration of inorganic phosphate released was not quantified therefore these results cannot be directly compared to other research. It is recommended that future studies directly quantify the ATPase content in the cooked meat.

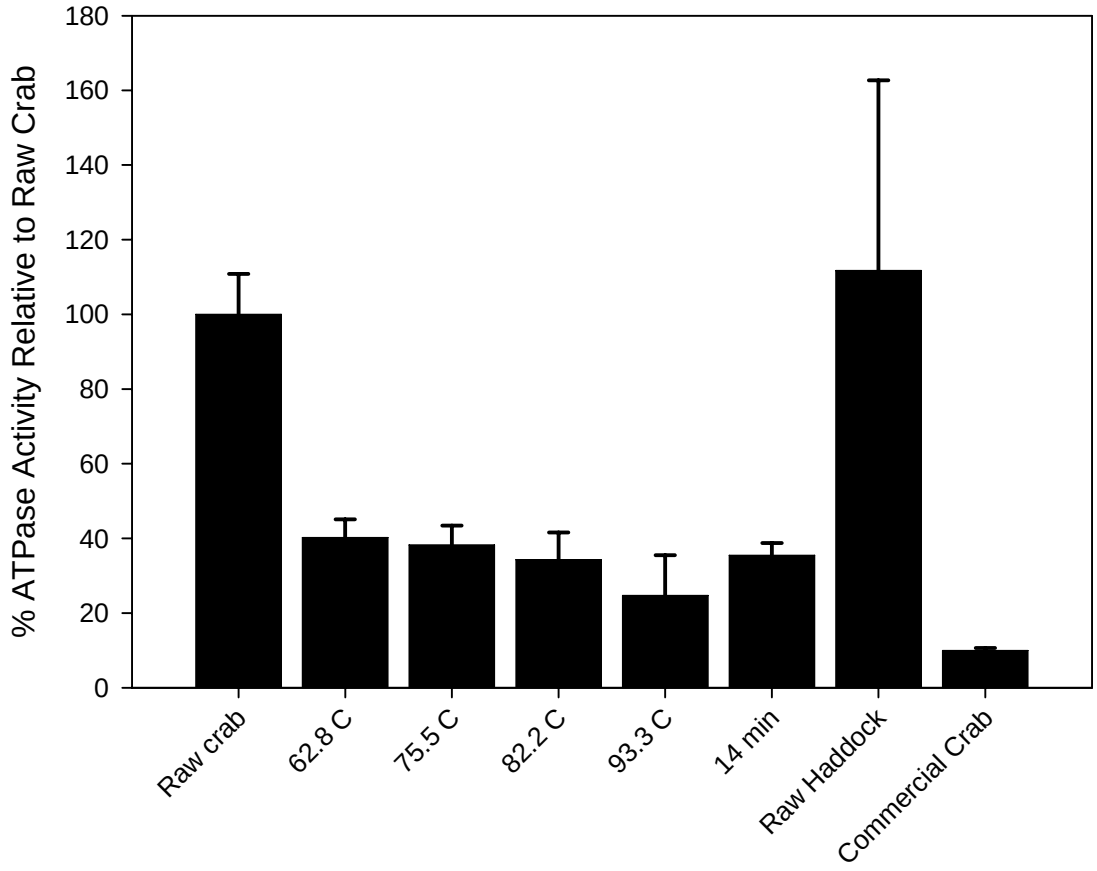

Figure A. 1: ATPase activity of crab boiled in the shell relative to the activity of raw crab

Values shown are the average of three measurements with standard deviations.

Sulfhydryl Content

There were no significant differences in the concentration of total sulfhydryls in all of the samples (Figure A. 2). There was a large standard deviation in the samples which may indicate a problem in the methodology. The total concentration of sulfhydryl groups may change due to oxidation of the sulfhydryl groups or formation of disulfide linkages which are not measured in this assay. What was expected to change is the number of reactive sulfhydryl groups. The raw crab had more reactive sulfhydryl groups than all of the heat treated samples. Once heated to 62.8 °C, the amount of reactive sulfhydryl groups did not change with increased heat. This means that as the meat was heated, the reactive sulfhydryl groups were lost in the initial stages of heating, before the meat reached a safe temperature. The loss of reactive sulfhydryls groups was unexpected. As a protein unfolds more sulfhydryl groups should be exposed to the surface thereby increasing the concentration of reactive sulfhydryls. The reason for the decrease in this study is not known. It is very likely that sulfhydryl groups increase with moderate heating, but as you increase temperature more protein aggregates are formed, blocking the reaction of the sulfhydryl with the Ellman's reagent. In order to overcome this, the sulfhydryl groups would need to be lightly heated for measurement. One possible explanation is that the method used was not appropriate for denatured proteins. Little research exists on characterizing cooked proteins and therefore some method modifications may be needed for future studies.

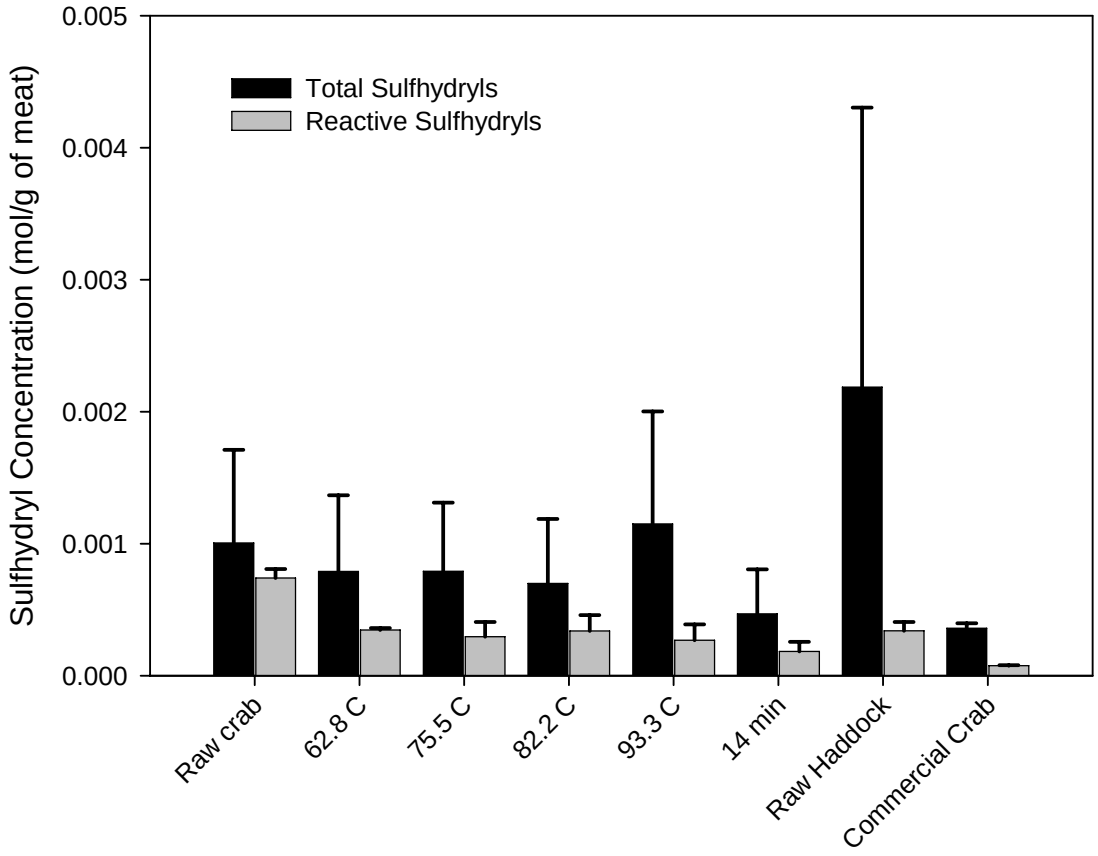

Figure A. 2: Total and reactive sulfhydryl content of crab meat boiled in the shell

Values shown are the average of three measurements with standard deviations.

Conclusion

Previous research has shown the cooked Jonah crab minced meat can form gels but the mechanism is not yet known. To be able to begin to understand the mechanism of gelation the proteins involved in the formation of the gel need to be better understood. This study was the preliminary research used to gain a better understanding of the crab proteins. Results indicated that when boiled, crab proteins are not fully denatured and the extent of denaturation can be measured using DSC. Myosin was one of the myofibrillar proteins not fully denatured based on the presence of ATPase activity in meat boiled for 14 min. The sulfhydryl data from this study is inconclusive. There were problems with using this method for cooked proteins that make the results unreliable. Many recommendations can be made for future research. By quantitatively measuring the concentration of inorganic phosphate released by ATPase, smaller differences may be seen between the cooked samples. Also a more controlled heating method should be employed to reduce cold spots, thereby creating a more uniformly heated meat sample. Results from this study indicate that even with extensively cooking, crab proteins may not be fully denatured; functional characteristics such as ability to form a gel may be retained which may help determine the mechanism for gelation of these proteins.

APPENDIX B. FOCUS GROUP SYNOPSIS

Introduction and Group Orientation (10 minutes)

Thank you for participating in this focus group today. I will be leading you in a discussion about shopping for seafood products. I want you to know that there are no right or wrong answers. We expect to receive a wide range of opinions and are eager to hear everyone's ideas and thoughts.

Tonight we are interested in your seafood shopping and eating experiences, the different concerns you may have while you shop and the type of information you use when shopping for seafood products.

(Read and discuss the informed consent)

General Discussion on Shopping for Seafood Products (20 minutes)

Imagine you are planning a dinner and you want to incorporate seafood into your plans. As you are walking through the grocery store, what things are you looking for in a product?

What types of products are you looking for?

Do you prefer fresh or frozen products?

- *Salmon (West coast) for the taste – but not farmed because it is too fatty, it's mushier*
- *Texture is important*
- *Already thought about recipe before going to the grocery store*
- *Seafood is spontaneous when it is on sale*
- *Straight for the scallops, only buy if they are the giant, fresh not frozen due to freezer burn*

- *Texture is important, flavor of giant scallops is better*
- *No price considerations always buy flounder, then haddock or salmon*
- *How fresh it looks*
- *Price*
- *Pre-cooked shrimp for an appetizer from the case, not frozen*
- *Canned tuna*
- *Frozen haddock fillets for fish and shrimp*
- *Won't buy if too expensive, will search for another store*
- *Love shrimp, pre-peeled, buy frozen*
- *Rely on suggestions from "fish guy"*
- *Know what fish you want to buy but not how to prepare it*
- *Grocery stores*
- *What's on special, salmon, tilapia, haddock*
    - *If at fish market – crab and other shell fish*
- *Panelist said they eat fish about 5 times a week*
    - *This panelist was a fish expert, freshness and smell*
- *Fish cooks fast*
- *Don't always want to eat fish on the day you buy it.*
- *Availability at the store and the store must be near by*

Is the type of preparation required something you consider? What cooking methods do you prefer (baked, broiled, fried, etc)? How important is the preparation method to your decision to purchase?

Does convenience play a role in choosing seafood products?

- *Growing up only knew frozen but later on I wouldn't eat it so when I moved here I won't buy it anymore*
- *Stay away from prepared due to sodium content and fat*
- *Tend not to buy frozen fish if I have a choice*
- *Day old in frozen tubs for paella*
- *Fresh seasoned fish in the grocery store that you cook in home*
- *Nothing takes more than 20 min, consider seafood a convenience food*
- *Too heavily seasoned and tastes strange*
- *Never think to buy prepared except for the shrimp rings*
- *Crustaceans must be live when you buy them*
- *Don't buy lobster live to cook with because I can't boil alive*
- *Eat lobster in a restaurant, frozen crab legs are OK but never frozen lobster tail*
- *Frozen crab meat and frozen lobster meat for a stuffing*
- *Uncooked clams from the wharf to steam at home*
- *Mostly buy lobsters cooked at the store or at a restaurant*
- *Pollock they sell as crab – Imitation crab meat will purchase*
- *Fresh preferred by all*
- *Used to buy the frozen fried fish but not anymore*
- *Flash frozen fish preferred*
- *Frozen at sea haddock for chowder*
- *Frozen shrimp (cooked or uncooked)*

Eating Preferences for Seafood Products (15 minutes)

What is the most important attribute that you consider when eating seafood products (flavor, texture, preparation, etc)?

- *Flavor*
- *Texture – white fish like haddock by poach or broil*
- *Freshness but it relates to flavor*
- *Salmon was too soft but can't be too chewy (not steak or chicken chewiness); firm but not tough; Alaskan salmon is perfect*
- *Filet mignon has perfect texture*
- *Swordfish is good*
- *Grilled, medium (not rare or well done) color must be uniform*
- *Scallops seared on each side for ~ 1 min*
- *Salmon steak, grilled or broiled*
- *Scallops, fried*
- *White fish, baked*
- *Freshness*
- *No ammonia smell*
- *How they display it in the stores (displayed on ice)*
- *Not chewy- want flaky*

What types of flavors do you look for in seafood products (spicy, citrus, etc)?

- *Lemon*
- *Garlic*
- *Don't overpower seafood taste*

- *Don't like creative sauces*
- *Citrus*
- *Chutney*
- *Cajun seasoning on scallops (spicy not hot)*
- *Subtle herbs; cilantro, thyme, dill*
- *Italian seasoning and breading*
- *Dill sour cream sauce*
- *Dijon mustard*
- *Seasoned bread crumbs in crab cakes*
- *Poach in white wine for tartness*
- *Butter*
- *White Wine*
- *White fish- shallots, tomato*
- *Shrimp-herbs*
- *Herb- Dill*
- *McCormick*

How does texture affect your choice to eat seafood products? What type of texture do you prefer in products? Are there products currently on the market that have the same type of textural attributes (fish sticks, firm vs soft fillets etc)?

- *flakes off*
- *Scallop texture*
- *Filet Mignon*

- *No chewyness*
- *Mahi Mahi*
- *Fresh caught red snapper*
- *Oysters- like texture and taste*
- *Baked haddock*

How do you feel about formed seafood products (fish sticks, fish nuggets, surimi products etc)?

- *Fish sticks and nuggets are for kids*
- *Fake crab is OK; makes a good salad or with alfredo*
- *High in sodium*
- *Don't like that its made from lots of different fish*
- *The "hotdog" of fish*
- *Probably eaten more by lower income people*
- *Don't like fried fish*
- *Imitation crab meat*
    - *Cheap- different than tuna*
    - *Artificial but not bad*
- *Salmon sausage*
- *Do not like the fish burgers*

Crab Preferences (15 minutes)

I want you to continue to think about your preferences but we are going to narrow the field down to talk just about crab. I want you to think about when and why you purchase crab and what it is about crab that you like.

How often do you eat crab meat? What are the occasions on which you eat crab?

- *I don't think about crab meat*
- *Avacodo with light mayo and crab meat*
- *I don't know what to do with it besides mix with mayo and put on a roll*
- *King crab in the shell for special occasions*
- *I like the taste but I don't know what to do with it*
- *I like it if it isn't too strong – too strong a crab taste*
- *Blue crab is a little strong*
- *Not many recipes*
- *Fry it – egg, bread and fry*
- *Don't eat much crab meat*
- *Like fresh crab meat better than lobster*
- *Crab cakes*
- *Tube (not shelf stable) crab- stuffing, pasta salad*
    - *Very easy*
    - *Not too expensive*

If a low cost option were available, would you purchase it? What information about the product would be useful when deciding to purchase it?

- *Only if it was prepared by the seafood people in the fresh case*
- *Salt content has to be low; no MSG*
- *All natural product*
- *Have recipes in the store*
- *Never go to the prepared food section first*
- *Would only like it if it was pure crab not other things (crab cake ok)*
- *Frozen may be ok*

What type of texture do you look for in a crab product?

- *Like something you can bite in to*
- *Not too chewy but not mushy*
- *Like shredded chicken*

What flavors do you associate with crab products (spicy, citrus, etc)?

- *Garlic*
- *Spice*
- *Crab boil the heat is OK*
- *I don't know, I don't know what to do with it*
- *Wouldn't want citrus*
- *A little bit of bite would be good*
- *Mayo or alfredo sauce or cream sauce*
- *Mayo because that's all I've had*
- *No cheese*

- *Crab dips*
- *Onion*
- *Flaky*
- *I want to be able to determine it was crab not extruded fish*
- *Firmness- not pasty*
    - *Firm- chopped up egg white*
- *Not canned dog food*
- *Turkey meat (ground turkey)*
- *Some mayo*
- *Butter*
- *Cakes - herbs*

New Seafood Products (45 minutes)

The purpose of the group today is to brainstorm new product concepts for crab meat. Don't be afraid to think outside the normal bounds of food products currently on the market. We are interested to know what types of new products you would like to see in your local grocery store.

***NOTE: Use the five concepts as a starting point for discussion. If participants bring up ideas, go with those. All 5 concepts do not have to be discussed.*

What new type of crab product would you purchase if it were on the market? What would it look like? What flavors would it have? What would the texture be?

Crab dog

Crab paste to add flavor

- *Want it to be shelf stable to have on hand*
- *Like wasabi*
- *Don't want it to be concentrated*
- *Artificially distilling and adding back flavor is bad*

Paté for crackers

- *Many would try it to put it in something*
- *Original, garlic and herb, Cajun – would rather have plain, garlic*
- *Need someone offering you a free sample in order to notice*
- *Texture may be an issue – must be spreadable not chunky*
- *Reasonable color – flesh tone; not a putty color; a little white; pinkish but not too pink; more orangish*
- *Must taste good*
- *Fat content, sugar (high fructose corn sugar), farmed or wild, know that it's an added value product (more likely to buy); lower cost*
- *Don't like the word by-product*
- *Don't like preservatives*
- *Want it to stay fresh about a week after purchase*

Pet food

Chicken fries made out of crab

- *Cheese may be nice*
- *Appetizer in a restaurant*
- *Like calamari*

- *Kick in the sauce, mild on their own*
- *Beer batter flour*
- *Fish and chips*
- *Want the breading to stick*
- *Bite size*
- *Chicken nugget would be good, not hotdog*
- *Somewhat flaky so that you know its crab*
- *Stringy, fibrous*

Nuggets are appetizers

Part of a main dish

Incorporate crab meat into a cracker

- *Crab flavor in the cracker*

Japanese whitish color cracker

Jerky

- *Salt*
- *Dried crab*

Crab bacon

- *Sodium*
- *Smoked*

Mini quiches for appetizers

Ice cream with seaweed

OK to incorporate crab meat into non meat products

Crab loaf- ok though the texture in loafs is bad

Crispy coating is good

Crab patty would be ok

- *Crab cakes*
- *If it wasn't too processed*
- *Taste would be interesting*
- *Low in sodium*
- *Alternative to a burger*
- *Like the idea of no breading*
- *Pre-formed patties*
- *Enough consistency to hold together but not as firm as a chicken patty*
- *See a little bit of stringiness*
- *Onion*
- *Vegetables mixed in*
- *No peppers*
- *Cilantro*
- *Something fresh*
- *Plain or garlic*

Crab whiz – cheese whiz but made out of crab

Sausages are too greasy; too processed

- *Prefer synthetic casing*
- *Too high in sodium*
- *Red flakes for distinctive crab color*
- *Want plain crab; with hot peppers*

- *Would not eat a sausage on a hot dog roll*
- *Too much sausage to eat alone; mostly because they are fatty*

Make a crab hotdog that tastes like a hot dog (like turkey dog)

Crab cubes – bad

- *Like shredded cheese but made of crab*

Want mince as is, not touched at all, not processed

Sausage

- *Casing - synthetic*
- *Spicy*
- *Italian one*
- *Fennel seed*

Crab Patties (similar to a chicken patty, not a crab cake)

- *Plump – not too much breading*
- *Mound of meat-bread crumbs mix in*
- *Firm with bun*

Crab Loaf

- *would try it*
- *maybe make it*

Crab Cubes for Salad

- *No cubed , shredded*

Crab cream sauce

- *Homogenized*
- *Some chunks*

Crab BALLS

Dim Sum

- *Crab dumpling*
- *Some texture but not too much*
- *Frozen*
- *Don't care if smooth or chunky*
- *If it took more than one bite, chunks*
- *If it is one bite- homogenous*
- *Dipping sauce*

Crab Jerky

Crab and fruit

- *fruit bar*

Crab Dip

- *Cajun*
- *Dairy based – low fat sour cream or yogurt (any flavored)*
- *Or Avocado- smooth but chunks of crab meat or without*
- *Spinach in crab, parmesan dip (frozen)*

Crab salsa

Spicy crab rice

- *crab sausage or shredded*
- *veggies*
- *Frozen*

Crab Block or cubes

Crab tube (like Jimmy Dean)

Crab lasagna

- *ground into paste*
- *CRAB flavor*
- *Cream or tomato*

Crab paste

- *to add flavor*

Crab pasta

- *FRESH*

Paté

- *Preferred plain Crab*
- *Smoky*
- *Maybe frozen*
- *Fresh – two weeks*
- *Color – Muted brown*

Mexican

- *burrito*
    - *homogenous but some "tooth"*
    - *frozen*
    - *Spices*
    - *Green salsa spice*
    - *cilantro*
    - *Topping sauce*

- *Tamale*
    - *Homogenous*

Closing (5 minutes)

We want to thank you for participating in our study today. We have a small gift of our appreciation for you before you leave.

APPENDIX C. CRAB APPETIZER SENSORY EVALUATION SCORECARD

Crab Appetizer Evaluation

Please answer the following questions about yourself. The information will remain confidential.

Please indicate your age by sliding the red bar to the number that corresponds to your age on your last birthday.

+----------+

18 90

Please tell us your gender.

( ) Female
( ) Male

How often do you consume crab products?
( ) Never
( ) 1-2 times a year
( ) 1-2 times a month
( ) 1-2 times a week
( ) More than two times a week

Which crab products have you eaten in the past 6 months? (mark all that apply)
( ) I have not consumed crab in the past 6 months
( ) Whole crab
( ) Crab claws (e.g. cocktail claws)
( ) Canned or fresh crab meat
( ) Crab cakes
( ) None of these products

Where do you most often purchase crab products?

( ) Supermarket/grocery store

( ) Seafood market

( ) Restaurant

( ) Roadside seafood vendor

( ) Do not purchase crab myself

Crab Appetizer Hedonic Evaluation

Using the scale below, assign a whole number that best describes your opinion of the crab appetizer's overall acceptability, appearance, overall flavor, crab flavor, and texture.

1	2	3	4	5	6	7	8	9
Dislike Extremely	**Dislike Very Much**	**Dislike Moderately**	**Dislike Slightly**	**Neither Like Nor Dislike**	**Like Slightly**	**Like Moderately**	**Like Very Much**	**Like Extremely**

Overall acceptability _____

Appearance _____

Overall flavor _____

Crab flavor _____

Texture _____

Would you purchase this product? YES NO

What is the most you would be willing to pay at a grocery store for 6 servings (2 pieces per serving) of the product you just sampled?

( ) Less than $4.00
( ) Between $4.00 and $5.00
( ) Between $ 5.00 and $6.00
( ) Between $6.00 and $7.00
( ) More than $7.00

If this product were labeled as containing no trans fat and had a total fat content of less than 10g per serving, how would this influence your choice to buy the product?

( ) I would be more likely to buy the product.

( ) I would be less likely to buy the product.

( ) It would not affect my choice.

Please give your opinion of this product.

Thank you for your opinions. Please don't forget to collect your gift before leaving.

BIOGRAPHY OF THE AUTHOR

Shari R. Baxter was born in Defiance, Ohio in 1980. She was raised in Hickory, North Carolina and graduated from St. Stephens High School in 1998. She attended North Carolina State University where she received her Bachelor's Degree in Food Science in 2002. Shari continued her education at the University of Tennessee where she received her Master's degree in Food Science and Technology in 2004. She then relocated to Bangor, Maine and continued her work in the Department of Food Science and Human Nutrition at the University of Maine.

Shari is a candidate for the Doctor of Philosophy Degree in Food and Nutritional Sciences from The University of Maine in May, 2007.