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Vacuum Tumbling for the Incorporation of Phosphates in Gulf Shrimp.

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VACUUM TUMBLING FOR THE INCORPORATION OF PHOSPHATES IN GULF
SHRIMP

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Food Science

By
Matthew Daryl Cael
B.S. Texas Tech University, 2010
August 2012

DEDICATION

I would like to dedicate this thesis to my parents, George and Elizabeth Cael, as well as my fiancée, Brittany. Your unwavering support and guidance have led me to this point in my educational and professional career. You have allowed me to travel and grow in my pursuit of knowledge and excellence in my field, and my deep appreciation for this cannot be expressed.

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ABSTRACT

A processing method for shrimp in the Gulf of Mexico region is to place mechanically peeled shrimp in plastic bags in a retail box and add a mixture of water, sodium chloride, and sodium tripolyphosphate (STPP). The box is then placed in frozen storage. As the shrimp and solution freeze, there is a potential for the shrimp to absorb solution inconsistently. When used properly, phosphates help to retain natural moisture, and protect the product through freezing and thawing. However, excessive absorption of phosphate solution can lead to a loss of functional benefits in the finished product. When thawed, the over-treated product has a glassine appearance, soft texture, and occasional soapy taste. The product resists natural changes during cooking, such as development of pink color and coagulation of protein. Some local processors consider this the industry standard, while others have a desire to produce a higher quality product line.

The objective of this study was to determine the potential of a vacuum tumbling method for application of condensed phosphate solutions to produce a value-added, wild-caught, Louisiana Gulf shrimp product.

Shrimp containing no added phosphates from Louisiana, Honduras, South Carolina, and Texas were obtained, peeled, and deveined. Shrimp, plus solutions containing sodium tripolyphosphate (STPP) and sodium acid pyrophosphate (SAPP), were added to a clear tumbling chamber and tumbled under 22 mm Hg vacuum until no free solution was visible. Treated shrimp were compared for moisture content in raw and cooked products, cook-cool loss, and changes in protein content and microstructure after freeze thaw. Standardization of the uptake data showed that there was a consistent level of uptake using the SAPP blend. Standardized cook-cool data indicated that the STPP treatment had equal cook-cool loss values

compared to the control. The tumbled shrimp also had reduced protein solubilization. The measurement of muscle fiber area showed that it is difficult to determine a relationship between fiber area to level of moisture or uptake in the finished product. This study has shown promising initial results for improvements in value-added shrimp using vacuum tumbling. Scale-up studies should be performed to determine its feasibility on an industrial scale.

CHAPTER 1: INTRODUCTION

In 2008, the state of Louisiana began working with the Intensive Technical Assistance program, which is an extension of the USDA Trade Adjustment Assistance program, to provide educational assistance to fishing families that want to change and improve the way that they conduct business (Haby and others, 2008). A goal of this program is to work toward the creation of a premium quality wild-caught Louisiana shrimp product for retail sale. Shrimp, upon harvest, are subject to natural biochemical deterioration in quality during the distribution and processing steps that affect the overall quality of the end product. The attributes that affect consumer decisions at the time of purchase include, but are not limited to, price, appearance, aroma, and taste. Improvements in handling and processing techniques that result in a product that is of high value and consistent quality through distribution and shelf life will ultimately be of greater value to the consumer than that which is currently available in the marketplace (Haby and others, 2008).

On board processes that have been developed to help protect quality include the prevention of melanosis or “black spot” formation and the rapid reduction in the temperature of the product post-harvest. The development of processing techniques that improve the overall quality and consistency of wild-caught, Louisiana Gulf shrimp products will aid in the creation of premium quality value-added product. The objective of this study was to investigate the potential of a vacuum tumbling method for application of condensed phosphate solutions to produce a value-added, wild-caught, Louisiana Gulf shrimp product.

CHAPTER 2. REVIEW OF RELATED LITERATURE

2.1. History of the Shrimp Industry in the Louisiana Gulf

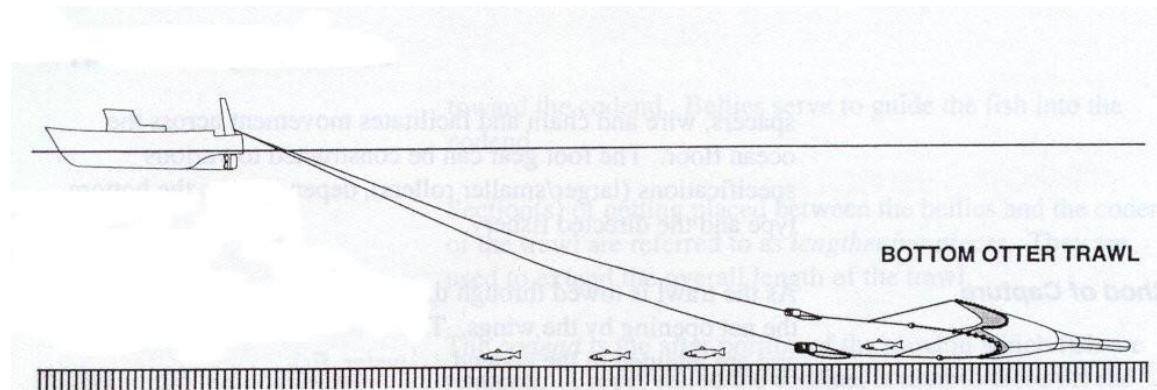
2.1.1. Shrimp Fishing in the Louisiana Gulf

Native Louisianans harvested shrimp for human consumption long before the French immigrated to Louisiana in 1718. A commercial shrimp industry, however, did not begin until 1867 when the process of preserving foodstuffs by canning came to Louisiana (Becnel, 1962). Following the Civil War, the shrimp industry in Louisiana began to grow rapidly. The industry centered on the Barataria Bay area (29.37° N, 89.93° W) near the coast, which spans from as far north as New Orleans and south to the Gulf of Mexico. The early shrimp season was from October to April. Areas fished for shrimp spanned from the Barataria area to Galveston and Matagorda Bay in Texas (Goode, 1889).

Around 1872, the haul seine was put into use in Louisiana, and was considered to be the most important innovation in early shrimp harvesting (Becnel, 1962). The haul seine increased catch and by 1880 was a staple in fishing vessels of the time. By 1915, as the use of engines became standard on boats, the sizes of seines increased. Teams of six to eight shrimpers would cast small nets while maneuvering through the fishing grounds until shrimp were located. When schools of shrimp were identified, the boat would stop and the seine was deployed by rowboat. The seine was then carefully hauled and the shrimp were separated by hand (Becnel, 1962).

The introduction of the trawl (Figure 2.1) to the shrimping industry in the early 1900's revolutionized the shrimp industry (Becnel, 1962). The trawl, which is shaped like a flattened funnel with two sliding downward-facing doors at the opening, drags along the bottom of the water (Becnel, 1962). The trawl increased efficiency because it covered a wider range of fishing

grounds in deeper water, cut manpower from six to eight to two or three men, and yielded a greater production level per harvester (Becnel, 1962). The ability to trawl in deeper water relieved the industry of the reliance on shallow water shrimp. As the trawl gained popularity, the amount of Louisiana catch increased into the 1940's (Table 2.1).



*http://www.ascension.k12.nf.ca/curriculum/social/eastfish/shellfish/shrimp/shrimp_geartype.htm

Figure 2.1. Typical structure of a trawl system.

Table 2.1. Louisiana shrimp harvest for various years (Becnel, 1962)

Year	Shrimp (Thousands of pounds)
1889	7,238
1908	8,851
1927	40,259
1939	100,612
1945	116,904
1951	80,718
1954	83,608

With the introduction of the trawl, it became necessary for the shrimp industry to increase the size and style of boats for shrimping because of the increase in the size of the harvest.

Various styles passed until the introduction of the Florida-type trawler, which had a round bottom, flared bow, and broad square stern (Becnel, 1962). The Florida-type trawler used booms

to haul in nets, with an engine room under the deckhouse, and a fish hold aft. Florida-type boats were 55-80 feet in length, powered by diesel, with cable rigs powered by the main engine. Florida-type trawlers were equipped with steel outriggers and booms situated behind the deckhouse, which would swing outward when hauling in nets (Becnel, 1962). This type of trawler is still used today with improvements of stronger construction materials, a larger flared bow, and better deck arrangements for efficiency. These boats were vastly different than the boats previously used in the industry, which were often flat bottom pirogue-style boats, which were paddled by hand or powered by a small engine (Rudloe and Rudloe, 2009).

Shrimping has changed little since the innovations in the middle 20th century. Use of salt boxes, which are boxes of seawater on board the vessel in which approximately 25% weight to weight (w/w) salt is added, separates shrimp from bycatch. Separated shrimp were often sized by hand aboard the boat and bagged. The invention of onboard freezers and improved cold storage allowed shrimpers to maintain the quality of their product after harvest. This has allowed shrimpers to stay at sea for longer periods of time while maintaining quality product (Rudloe and Rudloe, 2009).

2.1.2. Shrimp Processing in the Louisiana Gulf

The processing of shrimp in Louisiana is believed to have begun in 1867 (Becnel, 1962). G.W. Dunbar opened a floating shrimp cannery at Grand Terre in Barataria Bay as large shrimp landings began to be made in the Gulf of Mexico. Dunbar's business did not begin to prosper until 1875 with the development of a bag lining for cans (Johnson and Linder, 1934; Becnel, 1962). Dunbar's patent application stated that the purpose of this product was to provide an improved method of canning shrimps, prawns, and other shellfish by preventing their discoloration and ensuring the retention of their original freshness and flavor (Emerson and

others, 1881). Dunbar's invention consisted of a textile bag in between the shrimp or other seafood product and the cans' metallic surface (Emerson and others, 1881). During the cooking process, the textile bag held the seafood product to prevent black discoloration, a reaction between the naturally present sulfur in the seafood and the cans' metallic surface caused by the formation of iron sulfide in canned seafood products (Emerson and others, 1881). This innovation sparked a large increase in the number of Louisiana processing facilities. In 1880, Dunbar opened a large canning facility in New Orleans that operated on a seasonal schedule. The plant canned shrimp for five months out of the year during the fall and winter; during the summer it canned fruit (Goode, 1889). The introduction of commercially sterile canned shrimp allowed product to be sold in many sections of the United States, England, and France rather than only locally at the New Orleans markets (Becnel, 1962). The canning industry steadily increased through the early 1900's with a growth of sales from twenty-eight thousand cases in 1897 to one hundred eleven thousand cases in 1918 (Johnson and Lindner, 1934; Becnel, 1962). In the 1870's, Chinese immigrants arrived in Louisiana and set up an extensive shrimp drying operation. Lee Yim, who came to Barataria Bay and established the first drying platform, is considered to be the father of the Louisiana shrimp drying industry (Becnel, 1962). Salted shrimp, dried by the sun on stilted platforms built high above the water, soon became a main item of export from the state to China (Laney, 1938).

Louisiana canned and dried more shrimp annually than it consumed in the fresh state until the introduction of refrigeration and freezing to processing houses (Becnel, 1962). The removal of the head from the shrimp expanded the marketing and distribution of Louisiana wild caught shrimp products around this time. The removal of the head and hepatopancreas from shrimp greatly reduced the spoilage rate of the products by removing enzymes that speed up

spoilage and affect meat quality and reduced the shipping cost by 40% (Becnel, 1962). In 1931, headless shrimp counted for 34% of the South Atlantic and Gulf Coast shrimp marketed and ranked second only to canned shrimp (Becnel, 1962). The new headless product led to large increases in sale to the larger markets in Chicago and New York at this time (Becnel, 1962). The introduction of brine frozen shrimp to the marketplace in the early 1930's also expanded the market for Louisiana product.

In the 1940's, James Lapeyre of Houma, Louisiana developed an idea for a peeling machine that further advanced shrimp processing in the region. Lapeyre's concept was developed after accidentally stepping on shrimp while wearing rubber boots, which forced the muscle from the shell (Lapeyre, 1947). Lapeyre's machine that was patented in 1947 consisted of rollers which gripped shrimp at the protruding edges of the shell as mechanical fingers exerted pressure on the shrimp to remove the shell (Lapeyre, 1947). The introduction of shrimp peelers replaced fifteen to sixty shrimp peeling personnel per plant (Lapeyre, 1947).

The newly developed shrimp peelers were capable of handling small shrimp sizes that further expanded the product availability. In 1951, at the demand of frozen shrimp processors, mechanical methods were developed for the sizing of shrimp (Envoldsen, 1957). This newly developed technology replaced the hand grading method that had been utilized to classify shrimp as jumbo, large, and medium and allowed processors to make more exacting size groupings in a significantly reduced time (Becnel, 1962). By the mid 1950's, the newly mechanized peeling industry in combination with value-added products, such as breaded shrimp, competed with salmon as the top seafood product consumed in the United States (Becnel, 1962).

The modern raw shrimp peeler uses water to fully peel, clean, devein, and grade the shrimp (Laitram, 2011). For the Gulf region, warm water species peelers are used by processors

(Laitram, 2011). These warm water peelers peel between 400-455 kg of shrimp per hour, and have the ability to produce yields within one to two percent of hand peeling processes. The peeler is operated by loading shrimp into the machine which moves them to the top of the process by conveyor belt. The head is then removed by a roller, and the shell is blown off with high pressure water (Laitram, 2011). High pressure water then moves the product down a plane that is lined with sharp metal edges that cut the edge of the shrimp to remove the vein. The product is then graded through sized compartments that allow the product to drop into bins for further processing (Laitram, 2011).

After peeling, five pounds of shrimp are packaged into a plastic bag and a solution containing mixed phosphates, and often NaCl, is poured over the product. The bag is then placed into a box and frozen. The typical process that is used to freeze the product in Louisiana consists of placing the box on a rolling cart into a mechanical freezer at -30°C, until the product is completely frozen. After the product is completely frozen, the product is then moved to a -18 °C freezer for storage until distribution.

Frozen processed shrimp from the 1960's to present has consisted of five basic forms. These forms are frozen headless, frozen peeled and deveined, frozen cooked and peeled, uncooked frozen breaded, and cooked frozen breaded. Improved machinery and processing technology continues to be developed, resulting in continued growth of the shrimp industry (Rudloe and Rudloe, 2009). A key development in the creation of value added products has been the introduction of condensed phosphates into the product, which provide technical improvements such as improved water holding capacity (WHC), a mechanism for the maintenance of natural moisture levels in the product; cryoprotection, a mechanism for

protection of the muscle during freezing; and reduced drip loss upon thawing (Rudloe and Rudloe, 2009).

2.1.3. Similarities and Differences Between Louisiana Gulf and Common Aquaculture Shrimp Species

The two most common species of Louisiana wild-caught Gulf shrimp are white shrimp (*Penaeus setiferus*) or brown shrimp (*Penaeus aztecus*). *P. setiferus* is harvested from the eastern and southern coasts of the United States from New Jersey around to Texas in the Gulf of Mexico where they are especially abundant (Holthius, 1980). These shrimp typically live in two to 90 meters of water in a habitat of mud or peat, and sometimes sand or clay. *P. setiferus* are approximately 175 mm in length, for females, and 200 mm in length, for males, with a maximum carapace, or head, length of 41 mm (Holthius, 1980). *P. setiferus* are the most abundantly caught specie in the Gulf of Mexico, and are typically sold frozen for worldwide distribution (Holthius, 1980).

P. aztecus is the other species of shrimp caught in the Gulf of Mexico region. They are harvested on the Atlantic coast of the United States are abundant in the Gulf of Mexico (Holthius, 1980). They are typically harvested in depths between four and 160 meters of water, with the highest density located between 27 and 54 meters (Holthius, 1980). Their typical habitat is in mud or peat, often with sand, clay, or broken shells (Holthius, 1980).

Aquacultured shrimp species are most commonly of the black tiger (*Penaeus monodon*), and white leg (*Penaeus vannamei*) species. *P. monodon*, originate from the waters of the Indo-West Pacific from Southeast Africa, to Pakistan and Japan (Holthius, 1980). These shrimp typically range in length to 336 mm, with a weight from 60 to 130 grams (Holthius, 1980). They have historically been the predominant farmed species of shrimp, but in recent years have begun

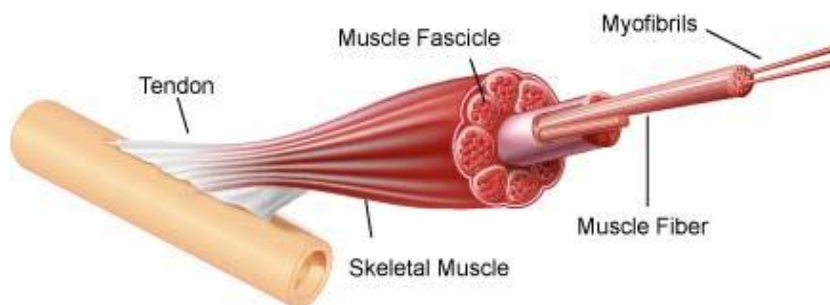
to be surpassed by *P. vannamei*. Within the last three years, *P. monodon* have become an invasive species in the Gulf of Mexico.

P. vannamei originate from the Eastern Pacific region from Mexico to Peru (Holthius, 1980). These shrimp are particularly abundant in the Guatemala and El Salvador regions of Central America (Holthius, 1980). They can grow in very shallow water, which makes them ideal for farming (Holthius, 1980). These shrimp typically range in size to 233mm, with a carapace length of 90 mm.

Haby and others (2003) outlined the influence of foreign aquaculture grown shrimp on the domestically wild-caught shrimp industry. Between the years 1997 and 2001, approximately 80% of shrimp imported into the United States were from farmed sources. Work by Haby and others (2002) investigated the differences in flavor between imported shrimp and domestically wild-caught product. Haby and others (2002) noted that wild caught product contained inherent “built-in” attributes that gives them a superior flavor compared to their imported farmed counterpart. The study stated that the superior flavor profile was thought to be influenced by the increased levels of free amino acids in the muscle that is utilized by the animal to counteract the osmotic gradient that is present in the salty offshore waters as well as high levels of bromophenols in fauna indigenous to the Gulf of Mexico (Haby and others, 2002). Farmed shrimp, on the other hand, are most efficiently raised during the rainy season in ponds that have a salinity equivalent to one-tenth that of sea water (Haby and others, 2002). The natural diet of wild shrimp that is high in protein and natural food are also thought to contribute to the superior flavor of wild shrimp compared to their grain diet based farmed counterpart (Haby and others, 2002).

2.2. Microscopy and Structure

Vacuum tumbling and phosphate solution treatment have been shown to have an effect on the structure of the muscle of products. The effects on the muscle structure have been observed by the use of many different microscopic methods, including light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Lampila (1990) compared the microstructure of red meat, poultry, and fish muscles. It was noted that the muscle structures of beef, lamb, and pork have been determined to be similar by many researchers. The skeletal muscles of these animals tend to vary in shape and size, but generally consist of muscle cells called myofibers (Chiang and others, 2007). Myofibers are encased in a layer of connective tissue known as the endomysium. Many myofibers are held together in bundles by another layer of connective tissue known as the perimysium (Chiang and others, 2007). Bundles are held together to form a muscle by a final layer of connective tissue known as the epimysium (Figure 2.2).



Courtesy: Google Images

Figure 2.2. Muscle Structure

The muscle structure of seafood products differs slightly from their red meat counterparts. The muscles are composed of myotomes that are arranged in concentric circles, which are subdivisions of striated muscle (Suzuki, 1981; Lampila, 1990). These subdivisions consist of one cell or fiber in length (Suzuki, 1981). Microscopy has been used by researchers to

determine the effects of water holding capacity on muscle structure. Hamm (1960) and Lampila (1990) stated that during storage, biochemical changes occur within the protein, resulting in drip loss from the muscle. Jarenback and Liljemark (1975ab) showed that the muscle protein that is responsible for the swelling of muscle fibers is actomyosin, which is present in the salt soluble fraction of the myofilament. Jarenback and Liljemark (1975a) also showed that any crosslinking of the actin and myosin chains could reduce the water holding capacity of muscle because of muscle toughening due to protein crosslinking. Several methods of microscopy have been used to observe the effects of treatments on muscles. Lampila (1990) showed the improved water holding capacity of processed muscle products by the microscopic measurement of fiber diameter. Lampila and Brown (1986) observed the changes in muscle fiber diameter of raw, precooked, and canned skipjack tuna muscle. It was determined that the muscle fiber diameter of raw and precooked or canned product was different ($p < 0.05$.)

2.3. Proteins

Shrimp are composed of two main protein fractions, which are the sarcoplasmic and myofibrillar fractions. The sarcoplasmic protein fraction typically consists of proteins of the sarcoplasm, which are the components of the intracellular fluid (Sikorski, 1990). The sarcoplasmic fraction composes approximately 30% of the total protein content of seafood muscle, but does vary by species (Sikorski, 1990). A major portion of the sarcoplasmic fraction is the albumin group which is composed of more than 100 various proteins over a wide molecular weight range and isoelectric points (Sikorski, 1990). Many of the proteins present in the albumin group of this fraction exhibit enzymatic activity, which upon harvest can contribute to the enzymatic breakdown of muscle over time (Sikorski, 1990). Other sarcoplasmic enzymes that compose the sarcoplasmic fraction include the enzymes of the glycolytic pathway and

hydrolytic enzymes of the lysozymes (Sikorski, 1990). Upon harvest, these enzymes play a role and present challenges for processors in the degradation of muscle quality.

The myofibrillar protein fraction is responsible for a majority of the stiffening of the tissue post-mortem, which is known as rigor mortis (Sikorski, 1990). The myofibrillar fraction is also mainly responsible for the water holding capacity (WHC) of seafood muscle, texture, and protein functionality such as gel formation in value added products (Sikorski, 1990). The main component of the myofibrillar fraction (50 to 58%) is myosin, at 200 kDa (Sikorski, 1990). Myosin presents three main properties that make it an important functional protein in muscle foods as it reacts with ATPase, binds to actin, and it can aggregate with itself to form myosin filaments (Bandman, 1987).

Actin is another myofibrillar protein of importance that composes approximately 15-20% of the fraction (Sikorski, 1990). Actin is present as two main forms, which are the globular (G) and filament (F), and has a combined molecular weight of 42 kDa (Bandman, 1987). G actin is polymerized at physiological salt concentrations to form F actin which creates the mechanical energy required for movement (Bandman, 1987). An important protein for contraction is tropomyosin at 33 kDa, consisting of two filaments that are designated as a and b (Bandman, 1987). Tropomyosin's physiological functional property is its ability to have stoichiometric binding capabilities with actin, and it is believed to be essential in the regulation of contraction by calcium (Bandman, 1987). Troponin, located at 80 kDa, aids in the sensitivity of tropomyosin to calcium ions (Bandman, 1987). It composed of a thin filament and is bound to tropomyosin. Troponin has three separate subunits, troponin C at 18 kDa, troponin I at 20.8 kDa, and troponin T at 38 kDa (Bandman, 1987).

2.4. Electrophoresis

Gel electrophoresis is typically used in food analysis for the separation of proteins, but has the ability to be more widely applied for other purposes (D'Arcy, 2007). Electrophoresis works by the movement of charged molecules in a buffered solution under the effect of an electrical field (D'Arcy, 2007). The degree of migration depends on the pH and the isoelectric point of the protein or other compounds. The migration can also be increased by the charge on the protein and the level of the applied voltage (O'Farrell, 1975). By achieving different migration velocities from different proteins, mixtures can be separated for protein type evaluation (D'Arcy, 2007). Electrophoresis can be performed in many different ways, but one of the most common is the use of polyacrylamide gel electrophoresis, or PAGE (D'Arcy, 2007). PAGE is used primarily for the separation of proteins based on subunit size in the presence of an anionic surfactant, sodium dodecyl sulfate, or SDS (Smith and others, 2003). This procedure involves the solubilization of proteins and their dissociation into subunits (O'Farrell, 1975). The use of reducing agents such as mercaptoethanol helps to reduce the sulfide bonds between and within subunits (D'Arcy, 2007). SDS binds to the proteins producing anions, which are separated by size (D'Arcy, 2007). After electrophoretic treatment, the gels are then stained with Coomassie Brilliant Blue or another stain, such as silver stain. The resulting protein subunit bands allow comparison of the rate of migration to a known protein standard to determine the molecular weight of proteins present in a sample (D'Arcy, 2007)

2.5. Phosphates

2.5.1. Condensed Phosphates

The production of condensed phosphates begins with the creation of merchant grade phosphoric acid by reacting mined phosphate rock with sulfuric acid (Lampila and Godber,

2001). Merchant grade phosphoric acid, which contains impurities, is made into “purified wet” phosphoric acid by using organic solvent extraction methods to remove impurities such as chromium, lead, cadmium, nickel, and iron (Van Wazer, 1958). Lastly, the acid is dearsenified and defluorinated to produce food and pharmaceutical grade phosphates. Phosphates are produced from phosphoric acid by reaction with alkali. The alkali compound can be a number of agents such as NaOH, Na₂CO₃, KOH, K₂CO₃, KCl, CaO, Ca(OH)₂, CaCO₃, or NH₃ (Van Wazer, 1958). Depending on the source of the alkali raw material, impurities can be carried into the phosphate (Lampila and Godber, 2001). It is important to note that food grade alkali sources are used to create food grade phosphates (Lampila and Godber, 2001). Orthophosphates (3 H₂O · P₂O₃ ≈ H₃PO₄), which are the dried product of phosphoric acid adjusted with alkali, serve as the raw materials used to create condensed phosphates (Van Wazer, 1958; Lampila and Godber, 2001). Orthophosphates, such as monosodium phosphate, pH range 3.8 to 4.2, disodium phosphate, pH 6.8 to 7.4, and trisodium phosphate, pH 10.2 to 11.0 (Van Wazer, 1958; Lampila and Godber 2001), are heated to drive off the water present in the molecule to produce condensed phosphates.

The development of condensed phosphates began in the early 1800’s with the creation of orthophosphates (Van Wazer, 1958). The creation of orthophosphates from phosphoric acid was first discovered by Berzelius in 1816 (Van Wazer, 1958). Condensed phosphates were further refined by Clark in 1827 when it was discovered that taking sodium pyrophosphate as prepared by Berzelius and using silver nitrate in the reaction could result in a white precipitate instead of a yellow precipitate (Van Wazer, 1958).

In 1834, Thomas Graham proposed nomenclature to better classify phosphates into the three major groups of orthophosphates, pyrophosphates, and metaphosphates (Van Wazer, 1958).

The classifications made by Graham led to considerable confusion in literature for the nomenclature of condensed phosphates (pyrophosphates, tripolyphosphates, and longer chain species) for the next century (Van Wazer, 1958). Phosphate nomenclature is still rather extensive today, and for the purposes of simplification, phosphates will be explained as described by Lampila and Godber (2001).

Phosphate nomenclature is based on the structural arrangement of the phosphate tetrahedron (Van Wazer, 1958). The simplest form of condensed phosphate is the orthophosphate (OP) containing one tetrahedron. The combination of two orthophosphates creates pyrophosphates (PP), which are also known as diphosphates. Further combination of orthophosphates leads to triphosphates, tetraphosphates, etc. (Van Wazer, 1958). Higher polymers of phosphates are typically sold as mixtures for most poultry and seafood processing (Lampila and Godber, 2001).

Phosphate solubility is a factor that is important when using condensed phosphates in the food industry. For the purposes of this study, sodium tripolyphosphate (STPP) will be discussed. The improvements made in the solubility of STPP have been an important advancement in the use of STPP in industrial settings (Lampila and Godber, 2001). STPP is often thought of as one of slowest phosphates to dissolve in solution; however, increasing the thermodynamic driving force improves dissolution (Lampila and Godber, 2001). STPP has two distinctive exothermic phases of dissolution: a high temperature phase (Phase I) and a low temperature phase (Phase II). Phase I phosphates release more heat during dissolution resulting in a more rapid rate of dissolution as compared to low temperature phase STPP (Van Wazer, 1958). The meat industry favors a high Phase I phosphate blend (90% or greater Phase I) for greater throughput because of the faster rate of dissolution into solution (Lampila and Godber, 2001).

The dissolution of phosphates also creates a need for the understanding of the hydrolysis, or breakdown, of condensed phosphates in solution and usage in food applications (Van Wazer, 1958). To understand the hydrolysis of phosphates in solution, it is important to note that condensed phosphates are thermodynamically unstable over all pH ranges and temperatures (Lampila and Godber, 2001). Several researchers have developed complex calculations to determine the thermodynamic reactions related to the hydrolysis of polyphosphates to pyrophosphates and ultimately orthophosphates when in solution (Saint-Martin and others, 1991; Saint-Martin and others, 1994; Lampila and Godber, 2001). Saint-Martin and others (1994) developed a Monte Carlo simulation that showed evidence to support theories proposed by George and others (1970) that the contributions of hydration energies to the enthalpy of pyrophosphate hydrolysis to orthophosphate is critically important. The research also supported conclusions of DeMeis (1984) that the water activity is relevant to energy output of pyrophosphate hydrolysis to orthophosphate (Saint-Martin and others, 1994). Most importantly, the changes in enthalpy were determined to also be strongly influenced by the hydration layers that are present in the pyrophosphate (Saint-Martin and others, 1994). The hydration layers in the pyrophosphate react with the water in solution. Pyrophosphate as the solute has a loose hydration shell containing the same number of water molecules as each of the orthophosphates molecules of which it is composed (Saint-Martin and others, 1994). Upon hydrolysis, each orthophosphate develops a strongly coordinated shell of water. This development during hydrolysis leads to a gain in enthalpy of the system (Saint-Martin and others, 1994).

The hydrolysis of condensed phosphates in solution occurs in two ways (Lampila and Godber, 2001). The majority of hydrolysis occurs by end group “clipping” in which the terminal phosphate tetrahedron is clipped from the chain (Van Wazer, 1958; Lampila and Godber, 2001).

The other way hydrolysis can occur is by “random cleavage”. Random cleavage in the middle of a chain occurs less often, but it has been shown that it does occur in long chained phosphates (McCullough and others, 1956; Lampila and Godber, 2001). It is believed that the hydrolysis occurs because of an increase of the positive character of the central phosphorus atom that is about to be cleaved from the orthophosphate group (Lampila and Godber, 2001).

The hydrolysis of phosphates in meat products is similar to the process that occurs in solution, but at a much faster rate (Sutton, 1973; Lampila and Godber, 2001). Sutton (1973), Offer and Knight (1988), and Hamm and Neraal (1977) studied polyphosphate stability and hydrolysis in beef muscle as did Molins and others (1985) in pork; Li and others (1993) in turkey; and of particular importance to this study, in shrimp, by Tenhet and others (1981). Hydrolysis of condensed phosphates in muscle is expedited by the presence of enzymes that are naturally present in muscle foods. Kielley (1961) and Kunitz and Robbins (1961) researched the effects of naturally present triphosphatase enzyme activity of myosin on sodium tripolyphosphate (STPP). The studies determined that myosin via triphosphatase activity splits STPP into pyrophosphate (PP) and orthophosphate (OP), however, a different enzyme is responsible for the hydrolysis of PP into OP (Sutton, 1973). Sutton (1973) showed that when 250 mL of a 1 M solution of STPP was applied to muscle samples, the STPP was almost completely hydrolyzed to OP with only a small amount of PP present in 16 hours at both 0 and 25°C.

The hydrolysis of phosphates is important to their functionality in value added products. Hamm and Neraal (1977) suggested that the hydrolysis of longer chained phosphates to PP is probably responsible for the increase of water holding capacity by inducing the dissociation of actomyosin (Hamm, 1960; Hamm, 1971; Li and others, 1993). The effects of condensed phosphates, such as STPP, are also known to contribute to the ionic strength and alkaline pH that

serve to open the muscle structure and through phosphate hydrolysis lead to increased uptake and retention of water (Shults and others, 1972; Shults and Werbicki, 1973; Klose and others, 1978; Froning and Sackett, 1985; Smith and Acton, 2010; Lombard and Lanier, 2011). Whiting (1984) observed that the functionality of batters with SAPP, and its effect on water holding capacity (WHC), is influenced by the pH of a meat batter.

2.5.2. Functional Properties of Phosphate in Muscle Foods

The functional improvements that can be observed in muscle foods due to the treatment of products with mixed solutions of condensed phosphates have been studied by many researchers. Some functional properties that are important to the improvement of quality and consistency in value added products include cryoprotection of muscle during frozen storage, and the chelation of metallic ions, which aid in the inhibition of lipid oxidation, stabilization of color, and flavor, as well as the retention of naturally present moisture by maintaining WHC (Ellinger, 1972; Offer and Trinick, 1983; Sofos, 1986; Dziezak, 1990; Lampila, 1993; Lampila and Schnee, 2000; Godber, 2001; Neto and Nakamura, 2003; Unal and others, 2004; and Goncalves and Ribiero, 2008a)

The use of condensed phosphates to reduce oxidative rancidity in muscle foods has been demonstrated. Regenstein and others (1993) observed the effects of polyphosphate solutions on the oxidative rancidity of samples of minced cod muscle treated for a five minutes. The samples were then stored for 1, 4, 7, 10, and 15 day periods. The samples were tested for oxidative rancidity by thiobarbituric acid reactive substances (TBARS) analysis. The work showed that minced cod had lower TBARS values as concentration of STPP in the dip increased from 0.3 to 0.5, and finally, to 0.7%.

Approximately 80% of the phosphate used in meat processing is sodium tripolyphosphate (Li and others, 1993; Alvarado and McKee, 2007), followed by blends of STPP with SAPP and sodium hexametaphosphate (SHMP). Alkaline phosphates, in this case, primarily STPP, provide increased water binding capacity (Hamm and Neraal, 1977). The effects of phosphates on the WHC in value-added meat products have been shown by many researchers. Ground and cured pork products enhanced by the use of phosphate solutions have shown improvement of yields, tenderness and moisture retention (Shults and Wierbicki, 1973). Whiting (1984) concluded that the addition of 0.25% SAPP to the 1.5% NaCl batter reduced exudate at pH 5.5 to the same extent statistically as treatments with 2.5% NaCl and no added phosphate. Many researchers have observed similar results, suggesting that the use of phosphates can potentially help in the reduction of sodium in products (Offer and Trinick 1983; Lampila 1992; Lombard and Lanier, 2011). Improvements in the water holding capacity of whole pork cuts using vacuum tumbling were also shown by Smith and others (1984), Cannon and others (1993), and Detienne and Wicker (1999). The effects of phosphates, such as STPP, on the WHC of whole and ground chicken meat have been shown by Farr and May (1970), Young and Lyon (1986), and Young and others (1987). Many researchers have also evaluated the effects of phosphate treatment on seafood products. Phosphates are typically applied to seafood by dipping the product into the solution prior to freezing. The effects of this method have been researched by Boyd and Southcott (1965), Sutton (1973), Cormier and Leger (1987), among many others, and have shown improvements in water holding capacity of fish, poultry, and pork products. Tenhet and others (1981), Applewhite and others (1993), Erdogdu and others (2004), and Goncalves and Ribiero (2008a) showed that pickup of solution in seafood products increases as the exposure time of the product to treatment solution is increased.

The influence of phosphates on the WHC is important to seafood processors because of the role water plays in the quality of seafood products. Water, which is the most abundant component in muscle at 70-80% of proximate composition, highly influences the quality and shelf-life of products (Huff-Lonergan and Lonergan, 2005). After harvest, muscle begins to undergo biochemical changes, known as rigor mortis, which directly affect the water holding capacity of muscle. As muscles enter rigor, glycogen is depleted, the pH of muscle drops due to creation of lactic acid, and proteolysis begins to occur as calpain levels rise in the muscle (Huff-Lonergan and Lonergan, 2005). Water is stored within the myofibril, predominately in the I-band of the contractile unit of the muscle, or sarcomere. The process of proteolysis increases the space between the muscle fibers and is believed to promote purge of intracellular water from the muscle (Huff-Lonergan and Lonergan, 2005). The addition of water is not only a technological benefit to processors, but increases the weight of product sold, and affects the consumer's sensory perception of the product (Goncalves and Ribiero, 2008a). Increasing weight of products with added water, however, could potentially result in issues of fraud due to adulteration of products by processors (Lampila, 1992; Otwell, 1992). Excess added water is often lost through thawing, purge, and cooking after purchase and therefore does not benefit the consumer from a quality standpoint. The optimal functional amount of added phosphate is dependent, in part, on the effect of added phosphate on the ability to obtain consistent and stable increases in the water weight (Goncalves and Robiero, 2008a).

This study used phosphate amounts that are suggested in the United States Federal Register (United States Federal Register, 1979) for value added meat and poultry products of 0.5% of the finished weight. Phosphate use is regulated in seafood, but is difficult to enforce, and, because of this, presents the potential for misuse by processors. Lombard and Lanier (2011)

and Rattanasatheirn and others (2007) used 1.6 to 2.5% phosphate solutions in excess for the treatment of products. Lower usage levels provide potential for cost savings to the producer, assurance that they are meeting guidelines, and less likelihood for soapy off flavor created by over-use of phosphates. Many studies have also included the use of sodium chloride to aid in uptake of solution. Rattansatheirn and others (2007) showed levels of uptake in shrimp between 4% and 10% using a static marinade containing 2.5% NaCl. Lombard and Lanier (2011) studied the effects of marinade composition on uptake using NaCl levels of 2 and 6% and 1.6% phosphate in tumbled fish portions. Lombard and Lanier (2011) showed higher levels of uptake than the current study; however, they used 1.6% phosphate solution, and tumbled their product in excess solution. The technique of tumbling in excess solution, as previously discussed, creates more opportunity for solubilization of myofibrillar proteins from the muscle into the sol formed on the tumbling chamber. However, there is also an increase in free water present in the muscle. Lombard and Lanier (2011) showed that a majority of this added water is lost from the muscle upon cooking, resulting in a loss of yield.

2.5.3. Phosphate and Added Water Regulations for Muscle Foods in the United States

The Scientific Committee on GRAS status (SCOGS) first evaluated the phosphate family for affirmation status as direct and human food ingredients as Generally Recognized as Safe (GRAS) (Life Sciences Research Office, 1975). The United States Federal Register of November 20, 1979 (United States Federal Register, 1979) included a proposal that the use of phosphates not exceed 0.5% of the finished product weight in muscle foods. This initial proposal led to six years of research by the United States Food and Drug Administration (FDA) to assess the safety and functionality of phosphates as food additives. After comprehensive studies, the FDA concluded that the use of sodium tripolyphosphate (STPP) and sodium acid pyrophosphate

(SAPP), and many others, when used within the specified limits, had no detrimental effect on human health (United States Federal Register, 1979).

Many researchers have shown that phosphates increase the water holding capacity of muscle. The ability to increase water holding capacity in muscle foods could potentially lead to adulteration (Lampila, 1992; Otwell, 1992). Phosphate and moisture contents in value added muscle foods such as pork, beef, and chicken are regulated by the United States Department of Agriculture Food Safety Inspection Service (FSIS). Title 9 of the United States Code of Federal Regulations (CFR) outlines the regulations by which the meat industry must abide in the production of value added meat products (United States Code of Federal Regulations, 2007). The CFR (United States Code of Federal Regulations, 2007) states that phosphates added to enhanced meats to improve the water holding capacity must not exceed 0.5% of the finished product weight. The USDA Food Safety and Inspection Service (FSIS) states that products that have retained water have to be clearly labeled with the amount of water retained post evisceration or the amount of water or solution added by value added processing (United States Code of Federal Regulations, 2007).

The United States Federal Register of December 18, 1979 outlined the suggested use levels of added phosphate in a treated seafood product to not exceed 0.5% of the total weight of the finished product (United States Federal Register, 1979). This suggested level of added phosphates in seafood is difficult to monitor in final products due to the breakdown of added phosphates in the whole muscle (Sutton, 1973; Otwell, 1992; Kyrznovek, 1995). Gibson and Murray (1973), Sutton (1973), and Reddy and Finne (1986) stated that STPP and SHMP are not very stable when added to seafood and hydrolyze to monophosphates during prolonged frozen storage. Reddy and Finne (1986) showed that 10 mL of a 0.25% STPP and 0.25% SHMP

solution added to 30-40 count tails of shrimp hydrolyzed to monophosphates in 12 days of storage at 5°C and 15 days at 10°C. Otwell (1992) stated that the ability of analytical methods to differentiate between states of hydrolysis in added phosphates is complicated by the tendency of added phosphates to gradually and continuously change states after they are added to the shrimp. Heitkemper and others (1993) proposed new methodology to determine total phosphate in samples of treated shrimp by use of the ion chromatographic method. Heitkemper and other's method was derived from methodology for determining sequestering agents in detergents. The method was determined to sufficiently quantify tripolyphosphate and pyrophosphate in solution; however, orthophosphate quantification is less accurate due to the hydrolysis of the phosphate (Heitkemper and others, 1993). Tripolyphosphate, pyrophosphate, and orthophosphate amounts were able to be obtained from samples of cooked shrimp. The research also showed that phosphate levels remained relatively the same during frozen storage, suggesting that hydrolysis of the condensed phosphates is slowed by frozen storage in the cooked product (Tenhet and others, 1981; Heitkemper and others, 1993). In uncooked samples, it was more difficult to analyze the phosphate levels due to the enzymatic hydrolysis of the compound in the muscle (Heitkemper and others, 1993). Krzynowek (1995) suggested that the use of thin-layer chromatography (TLC) detected polyphosphates applied under current processing methods for up to one year of frozen storage at -18°C. Krzynowek (1995) suggested that after one year, the polyphosphates hydrolyzed to monophosphates and detection was deemed virtually impossible.

The difficulty faced in analytically determining the level of added phosphates in value added seafood products led the FDA to resort to a simplified method of regulating value added products by determining moisture content of the finished product (Otwell, 1992). This method, known as the French HP (Humidité: Protein) method, is used in France to monitor the ratio of

protein to water in scallop muscle (Loreal and Etienne, 1990). The HP method, however, was not realistically enforceable due to the variation in the natural moisture content of various species at different times of the year (Lampila, 1993). Determining regulatory limits in value added seafood products was also complicated due to the fact that the moisture contents of untreated products are variable by nature (Otwell, 1992; Lampila, 1994). Controversy over the use of phosphates in the seafood industry centers on the ability to improve water-holding capacity (Lampila, 1992; Otwell, 1992; Goncalves and Ribiero, 2008ab). Questions arose as to the rate of retention of added water in the muscle. Water could be considered an adulterant in seafood products due to the fact that they are typically sold on a weight basis. Added water that was not stated on the label, and not retained in the cooked muscle, was considered to be misleading to consumers (Goncalves and Ribiero, 2008ab).

Cheng and Regenstein (1997) observed the effects of polyphosphates on water uptake, protein solubility, and protein changes in minced cod that was stored on ice. Cheng and Regenstein's research compared the effects of sodium tripolyphosphate (STPP) and sodium hexametaphosphate (SHMP) at varying levels. It was determined that high molecular weight protein bands at 200kd (myosin), 130kd (calpastatin), and 90kd (actinin) appeared in the extracts of the minced fish treated with SHMP, but were reduced in the STPP treated samples. It was suggested that the bands present at 200kd, 130kd, and 90kd in the SHMP treated meat could potentially be involved in the protein network that held water. The solubilization of these proteins in the STPP samples could have contributed to the observation of higher levels of water uptake in the SHMP treatments as compared to the control and STPP treated samples, as well as enzymatic activity within the muscle. Rattanasatheirn and others (2008) observed the effects of phosphates and mixed phosphates in treatment solutions on the protein pattern of fresh and ice

stored shrimp with and without deveining. Samples were placed in treatment solutions of 2.5% NaCl, 3.5% tetrasodium pyrophosphate (TSPP) + 2.5% NaCl, 0.875% sodium acid pyrophosphate (SAPP) + 2.625% NaCl, 3.5% STPP + 2.5% NaCl, and 0.875% SAPP + 2.625% STPP + 2.5% NaCl for two hours, and stored on ice for 7 days. It was determined that the myosin heavy chain at 200 kDa was present in all 0 day samples of shrimp except the 2.5% NaCl sample. The actin band at 45 kDa was observed in all samples regardless of treatment. After seven days of storage, it was observed that the myosin heavy chain was considerably reduced or completely eliminated in all samples. The actin band, however, still remained in all samples. It was suggested that the myosin heavy chain was solubilized during treatment and leached out over storage. The solubilization of myosin could also be contributed to the activity of muscular proteases, such as alkaline protease, which splits the heavy chain myosin into two fragments of 100 and 80 kDa (Bandman, 1987).

2.6. Freezing

Freezing is the transition from the liquid state to the solid state as a function of decrease in temperature (Fellows, 2009). Seafood muscle is subject to deterioration in frozen storage. One factor is the high levels of polyunsaturated phospholipids that are prone to oxidation during storage (Soliman and Shenouda, 1980). Seafood muscle also undergoes textural damage when kept in frozen storage for long periods of time. Surface dehydration (freezer burn) often leads to irreversible denaturation of proteins in seafood muscle. The denaturation of these proteins can cause loss of water holding capacity of muscle thus affecting the texture of the product for the consumer (Soliman and Shenouda, 1980). The rate of temperature decline at which products are frozen can also cause damage to the muscle due to the formation of ice crystals within the muscle. To limit damage during freezing, muscle should be frozen as quickly as possible after

either harvest or processing. When punctured by large ice crystals, the muscle is unable to retain the natural water present, leading to undesirable changes in texture (Soliman and Shenouda, 1980).

The freezing method most often used during value added processing in Louisiana is air blast freezing. In this method, mechanical freezers use cooled air to remove heat from food products (Fellows, 2009). Air blast style mechanical freezers operate by recirculating air (-30 to -50°C) at a velocity of 1.5 to 6.0 ms⁻¹ over food products (Fellows, 2009). The high velocity of air reduces the thickness of boundary air films surrounding the food and thus increases the surface heat transfer coefficient (Fellows, 2009). When shrimp products are frozen quickly and stored properly, little drip loss occurs from the shrimp muscle, but when frozen slowly, excessive drip loss occurs that seriously affects the quality of thawed products (Goncalves and Ribiero, 2008a). Products that are blast frozen quickly should be kept at low temperatures (< -18°C) to prevent the accretion of large ice crystals during storage (Goncalves and Ribiero, 2008a). Phosphates also can help to protect muscle during frozen storage by working as a cryoprotectant. Muscle treated with phosphate has a decrease in the level of drip loss after freezing and frozen storage (Goncalves and Ribiero, 2008b). The use of phosphate dips increases the water holding capacity of seafood muscle and reduces drip and deterioration of products (Lampila, 1992, 1993; Schnee, 2000; Aitken, 2001; Turan and others, 2003; Goncalves, 2005; Goncalves and Ribiero, 2008b).

The rate of freezing has been shown to severely affect the structure of the muscle in frozen products. Johnston and others (1994) explained that the freezing process for fish muscle occurred in three phases. The initial phase, consisted of a “cooling” step in which the temperature dropped rapidly to just below 0°C. The second phase, referred to as the “thermal arrest” time period, showed that the temperature of the muscle remained fairly stable with a drop

in temperature of only a few degrees Celsius. Johnston and others (1994) explained this observation as the period of time in which a bulk portion of the water is turned to ice. Johnston and others (1994) observed that when approximately 55% of the water in the muscle was frozen, the muscle entered the third phase of muscle freezing in which most of the remaining water froze. Johnston and others (1994) also measured the percentage of water that is frozen in the muscle as a function of temperature to demonstrate phase 2 and 3 of freezing (Table 2.2). Johnston and others (1994) noted that the time that the muscle spent in phase two of freezing was the amount of time in which the majority of ice crystallization occurred, and the longer the time spent in the phase, the more potential there was for increased formation and accretion of ice crystals.

Table 2.2. Proportion of water frozen as a function of temperature in fish muscle.

Temperature (°C)	Water Frozen (%)
0	0
-1	15
-2	55
-3	70
-4	72
-5	75
-6	79
-7	80
-8	81
-9	82
-10	83
-11	84
-12	85
-13	86
-14	87
-15	89

† This data was extrapolated from the graph presented in Johnson and others (1994).

Much research has been done to show the relationship between freezing time and its effects on muscle structure. Jarenback and Liljemark (1975 abc) observed that freezing can cause

shortening of the sarcomere in muscles, which results in a decreased WHC. It has also been demonstrated that large ice crystals can cause muscle cell membranes to rupture during frozen storage, releasing cellular contents (Hamm, 1960; Giddings and Hill, 1979). The damage that occurred during the formation of large ice crystals during freezing also affected the water holding capacity of muscle. Ngapo and others (1999) observed that the drip loss of thawed pork samples that had been frozen at -20°C for one hour and stored at -18°C for 4 weeks had higher drip loss compared to samples that had been frozen at -20°C for 15 hours. The samples that were stored for 15 hours showed less drip loss than the slow frozen samples upon thawing. It was proposed that storage period at -18°C gave time for the water and intracellular salts present in the muscle to migrate out of the cell promoting extracellular crystal growth to disrupt the muscle structure (Ngapo and others, 1999). Lampila and others (1985) also observed the effects in rockfish muscle of ice crystal accretion caused by moving frozen fillets from lower to higher freezer temperatures during storage. As the size of ice crystals increases, there is more potential for muscle damage, potentially resulting in an increase of drip loss from thawed samples.

2.7. Vacuum Tumbling

The addition of phosphate solutions to improve the quality of meat and poultry products began in the 1950's. The addition of these solutions in the beginning stages consisted of dipping or exposing the products to phosphate solutions for extended periods of time by static marination, which is referred to as soaking, to ensure the absorption of solution into the product. Extended holding of product in phosphate solutions has been shown to provide a rise in moisture content (Bendall, 1954; Applewhite and others, 1993). In an effort to provide expedited and more consistent levels of marinade addition to products, injection systems were developed. Moore and others (1968) proposed the development of a marinade injector for muscle foods.

The injector created substantially uniform distribution of marinade throughout the muscle in a faster and more efficient method (Moore and others, 1968). Various versions of needle injectors have been introduced since the 1970's. Fletcher (2004) described an improved version of the injection apparatus as a series or grid of spring-loaded needles that penetrated the muscle and forced marinade into the tissue under pressure. The spring-loaded needles stopped when they came into contact with bones which caused marinade to be injected at various depths within a product (Fletcher, 2004). Injectors were often joined with mechanically operated belts, which created expedited output of product. Lyden (2011) stated that injectors presented various problems in the food production industry because of a separation or undesirable change in physical state of the marinade. Separation of marinade could cause malfunctions to equipment leading to down time and loss of revenue and/or uneven distribution of marinade components into a product. Marinade is often recycled, which can lead to changes in marinade composition and concentration in products (Lyden, 2011).

Marination could also be performed by tumbling a product. The tumbling apparatus for poultry was first patented by Gasbarro (1975). Gasbarro (1975) noted that the injector used at the time did not distribute marinade evenly through the product in the short time periods that were desired by processors. The tumbler, which consisted of a drum with baffles inside, allowed for the impact of products against themselves, resulting in a relatively uniformly marinated product in a reduced time (Gasbarro, 1975). The tumbling apparatus also allowed processors to control the level of marinade that was present in the system from batch to batch, resulting in a more consistent product (Gasbarro, 1975). Much of the early tumbling research of whole muscle product was performed on boneless cured hams. Siegel and others (1978) demonstrated that the binding quality could be improved and cooking losses reduced by the application of tumbling

during processing. Krause and others (1978) suggested that brine distribution within tumbled hams is due to the independent interaction of the tumbling process as well as the use of sodium tripolyphosphate in the marinade mixture. While the effects of tumbling have been demonstrated, it has also been hypothesized that the use of tumbling with the application of vacuum can improve product quality. Gasbarro's patent (1975) suggested that the application of negative pressure on products during tumbling resulted in more efficient distribution of marinade within a product. The wide interest of the application of vacuum in the tumbling process by the meat industry during processing was suggested by Schmidt (1979). Prior to these publications, Rejt and others (1978) determined that vacuum tumbling also decreased cooking loss, improved tenderness, and increased the water holding capacity of hams. Solomon and others (1980ab) further researched the effects of vacuum tumbling on the distribution of marinade in ham muscles. The group determined that the use of vacuum in tumbling marination did increase the absorption of marinade into the muscle. However, while the pick-up of marinade was increased, the distribution of salt in the muscle from the marinade was noticeably different at the three sample depths tested in the experiment (Solomon and others 1980ab). These observations suggested that the vacuum process does not result in equal distribution of marinade throughout the product.

The effects of tumbling on poultry meat have also been researched, as well. Xiong and Kupski (1999) used dye tracing to observe the penetration of marinade solutions during tumble marination. The researchers tested 1.6% and 3.2% alkaline solutions of tetrasodium pyrophosphate (PP), sodium tripolyphosphate (STPP), and sodium hexametaphosphate (SHMP) with and without 8% NaCl. Samples were tumbled in 5, 10, 15, and 30-minute trials. It was determined that the samples absorbed the most marinade during the first five minutes of

processing in the 1.6% solution for the sodium PP, STPP, and SHMP treatments at 196, 171, and 138% pick-up, respectively (Xiong and Kupski, 1999). It was also observed that the levels of pick-up were diminished at phosphate levels of 3.2% when salt was present (Xiong and Kupski, 1999). This diminished pick-up level could have been affected by reduced solubility of phosphate in solutions of high salt content. At high levels of NaCl, the phosphate must compete for available water to solubilize. It is important to note that the salt content used in the work was at 8% in the brine, which might create increased uptake from a functional standpoint, but would most likely be rejected by the consumer because of saltiness. Levels of salt above 5% tend to create more solubilization of myofibrillar protein fractions as well (Thoriarinsdottir and others, 2001). The work by Xiong and Kupski (1999) was further examined by Alvarado and Sams (2004). Alvarado and Sams (2004) noticed that the dye used in the previous experiment was water-soluble and therefore could potentially migrate at a different rate than the ions that are responsible for increasing the tenderization effects of phosphate solutions. Alvarado and Sams (2004) also determined that the application of vacuum during the tumbling process could affect the penetration of marinade into the muscle. Samples of chicken breast were tumbled under a vacuum of 635 mm Hg at 14 rpm with 1 and amount of solution equal to 15% of the mass of the chicken breast,. The solution contained water, 0.54% NaCl, and 0.42% STPP. It was observed that the use of vacuum helped sodium ions present in the solutions reach the center of the muscle. However, it was also observed that the distribution of sodium ions was more concentrated in the outer layers of muscle (Alvarado and Sams, 2004).

Very little research has been conducted to observe the effects of tumbling on whole muscle seafood products due to their delicate muscle structure as compared to meats such as beef, pork, and poultry. Work by Kin and others (2009) and Lombard and Lanier (2011) have

shown that gentle tumbling either alone, or in conjunction with injection, can be an acceptable method for applying marinade to whole muscle seafood products.

The overall benefits of vacuum use in the tumbling process are a debated topic in the scientific literature. Early researchers, such as Rejt and others (1978) and Marriott and others (1984), suggested that the use of vacuum during the tumbling process created equal distribution of marinade throughout a product. Later research by Alvarado and Sams (2004) showed that the ions that are present in marinade solutions, such as sodium, did not necessarily have uniform levels of distribution throughout an entire muscle. Some researchers, such as Smith and Young (2007), suggested that the highly accepted practice of vacuum during the tumbling process did not improve processing or cook losses more than tumbling at ambient or positive pressures. Lombard and Lanier (2011) also suggested that the use of vacuum in the processing step is not necessary after evaluation of a cooked product after processing showed that there was no significant difference in the amount of moisture present in a vacuum and non-vacuum tumble marinated product. Although tumbling has been shown to create improved distribution of marinades containing phosphate in value added protein products, the combination of the mechanical action of the tumbling process mixed with the protein solubilization of phosphates has been shown to affect the integrity of muscle food products. Theno and others (1976) observed disruptions in the muscular tissue of tumbled hams that resulted in higher levels of extracted myofibrillar proteins. Solomon and others (1980) also observed the same phenomenon noting that the tumbling process and disruption of the muscle structure resulted in a decreased breaking strength in tumbled restructured hams. Siegel and others (1978) evaluated the effects of tumbling on the presence of specific skeletal muscle proteins that were present in the exudate from tumbled sectioned and formed ham products. The researchers determined that tumbling in

the presence of phosphate and salt solutions produced higher levels of myosin and actin and a decrease of tropomyosin present in the exudate. Theno and others (1976, 1977) determined the high levels of actin and myosin on the surface of the meat was due to the disruption of myofibrils during the tumbling process. The low levels of tropomyosin in the exudate in the presence of high myofibrillar protein fractions of actin and myosin can be explained by the results of Perry and Corsi (1958), who showed that tropomyosin, was extracted from the myofibril at low levels of ionic strength. It should be noted that the extraction of tropomyosin occurs even in the absence of phosphate and that the application of tumbling in the presence of phosphate acted to aid in the extraction of more insoluble proteins such as actin and myosin (Siegel and others, 1978). Solomon and Schmidt (1980b) observed the effects of vacuum and tumbling time on the extractability and functionality of pre- and post-rigor beef muscle. The research showed that there was a linear relationship between the tumbling time and the amount of crude myofibrillar protein extracted from a sample. It was also shown that there is an increased production of crude myosin. Vacuum was also determined to have a specific effect on the extraction of myosin, as the total protein increased in the vacuum sample extraction.

The objective of this study was to determine the potential of a vacuum tumbling method for application of condensed phosphate solutions to produce a value-added wild-caught Louisiana Gulf shrimp product.

CHAPTER 3. MATERIALS AND METHODS

3.1. Procurement

Frozen samples (LA Frozen) of 16/20 head-on, shell on, untreated (without added phosphate or sodium metabisulfate) Louisiana Gulf Shrimp (*Penaeus setiferus*) were obtained from Anna Marie Shrimp Company of Dulac, Louisiana. Frozen samples were transported to the Department of Food Science at the Louisiana State University Agricultural and Mechanical College (LSU) in Baton Rouge, LA and were stored at -18 °C until further processing. Fresh shrimp (LA Fresh) samples of 21/30 head-on, shell-on, brown shrimp (*Penaeus aztecus*) were obtained from Fourwinds Seafood Co. of Slidell, Louisiana and were immediately transported to the LSU Department of Food Science in Baton Rouge, Louisiana for further processing. Honduran farmed product (Hon), consisting of 21/30 head-on frozen white shrimp (*Litopenaeus vannamei*), was obtained from Grupo Granjas Marinas Empacadora San Lorenzo processing plant in San Lorenzo, Honduras. South Carolina-farmed product (SC), consisting of 16/20 head-off black tiger shrimp (*Penaeus monodon*), was obtained from Ballast Point Seafood (Yemassee, SC). Texas-farmed product (TX) consisting of 16/20 head-off frozen white shrimp (*Litopenaeus vannamei*) was obtained from Harlingen Shrimp in Harlingen, Texas. Samples were shipped frozen to the Louisiana State University Department of Food Science in Baton Rouge, Louisiana and were stored at -18°C until further processing. Food grade high Phase I sodium tripolyphosphate (STPP), and food grade sodium acid pyrophosphate (SAPP) were obtained from Prayon Inc. (Augusta, GA).

3.2. Vacuum Tumbling

LA Fresh shrimp samples were immediately processed upon arrival at the Department of Food Science. Frozen head-on, shell on, and untreated Louisiana Gulf shrimp were placed in a plastic lug with ice and were slack thawed under running water, and drained frequently to

prevent prolonged exposure of the muscle to water until they could be peeled. The head, when necessary, shell, and vein were removed from all shrimp samples by hand. Peeled and deveined (PD) shrimp were then placed in one gallon Ziploc® freezer bags (SC Johnson, Racine, WI) and were kept on ice to prevent desiccation of natural moisture and to prevent protein denaturation by heat until used. Two hundred fifty grams of PD shrimp were then placed in the clear plastic tumbling chamber of a 2270 g capacity Reveo® Mari-Vac Vacuum Tumbler (Eastman Outdoors, Flushing, MI). Solutions of 0.4% (weight /weight (w/w) of the finished product) STPP and STPP/SAPP (70:30) were then prepared according to federal guidelines (Federal Register, 1979), which state that phosphate can be added to value added meat products at 0.5% w/w (maximum). The STPP solution consisted of 15 g water and 0.46 g of sodium tripolyphosphate per 100 g of raw shrimp. The STPP/SAPP solution consisted of 15 g water and 0.46 g phosphate (70% STPP and 30% SAPP) per 100 g of raw shrimp. After preparation, the solutions were held at 4 °C and were used within 24 hours. After preparation of the solutions, the pH was taken using a Milwaukee SMS115 pH meter (Milwaukee Instruments, Rocky Mount, NC). The solutions, which were calculated for a finished level of 0.4% w/w phosphate in the finished product, was determined from preliminary studies performed on a Buchi Rotovapor R114 (Buchi Corporation, New Castle, DE) fitted with a 500 mL Pyrex round bottom boiling flask with thermometer well (Corning Corporation, Tewksbury, MA), to simulate the baffles present in the tumbling chamber. The chilled mixed solution was then added to the tumbler chamber with the PD shrimp. A drop of DOW 1510 Antifoam (Dow Corning Corporation, Midland, MI) was added to the chamber to prevent foaming during tumbling. Samples were then tumbled using the Reveo® Mari-Vac System with 22 mm Hg vacuum at 16 RPM under refrigeration at 4°C until complete uptake of solution into the product could be observed through the clear tumbling chamber. At the

completion of tumbling, samples were rested under vacuum for five minutes. Tumbled samples were weighed to determine solution uptake. Soft water (water containing low levels of phosphate to bind calcium and magnesium) samples were prepared by taking 454 grams of peeled and deveined shrimp and placing them in a one gallon Ziploc® freezer bag (SC Johnson, Racine, WI), and adding 1 L of a solution containing 0.5% sodium tripolyphosphate. Control samples were raw or cooked shrimp with no aqueous treatment applied. Percent moisture was determined by $\% \text{Moisture} = [(\text{Wet Weight} - \text{Dry Weight}) / \text{Wet Weight}] * 100$. The percent uptake of solution into the product was determined by $\% \text{Uptake} = ((\text{treated weight} - \text{initial raw weight}) / \text{initial raw weight}) * 100$. Adjusted percent uptake values were determined by $\text{Adjusted } \% \text{Uptake} = [\text{Actual } \% \text{Moisture} / (\text{Average } \% \text{moisture of controls})] * \text{Actual } \% \text{Uptake}$. Cook-cool loss was determined by using an Oster Model 5711 Food Steamer (Jarden Consumer Solutions, Providence, RI) to steam a 100 gram sample to a constant internal temperature of 62.8 °C (145 °F) which is the suggested cooking temperature for foodservice operations. Steamed samples were cooled for five minutes and then weighed. The cook/cool loss of the samples was then determined by $\% \text{CookCoolLoss} = 100 - [(\text{CookCool weight} / \text{Raw weight}) * 100]$. Adjusted cook-cool loss values were obtained by $\text{Adjusted } \% \text{Cook-Cool} = [\text{Actual } \% \text{moisture} / (\text{Average } \% \text{moisture of controls})] * \text{Actual } \% \text{cook-cool loss}$. The steamed samples and raw samples were then ground using an Oster Osterizer Blender (Jarden Consumer Solutions, Providence, RI). Ground three gram samples were then weighed into 43 mm aluminum weighing dishes (VWR International, Radnor, PA) for moisture analysis (AOAC, 2005). Weighed samples were then placed into a Quincy Lab Model 20 GC Lab Oven (Chicago, IL) at 100°C for 18 to 24 hours. Moisture contents were calculated $\% \text{Moisture} = [(\text{wet weight} - \text{dry weight}) / \text{wet weight}] * 100$.

After obtaining the samples for moisture and cook cool loss analysis, the remaining sample was put in a 2270 g box that is typical to the Louisiana shrimp industry and frozen in a -30°C freezer. The samples were probed using a Hobo model U12 SS temperature logger with a range from -40°C to 125°C (Onset Company, Cape Cod, MA) and were frozen to an internal temperature of -18°C. This data was then analyzed to determine the rate of freezing for the STPP, SAPP blend, and soft water treatments.

3.3. Protein Extraction

Protein extraction was performed according to a modified method of Hashimoto and others (1979) that was used by Chang-Lee and others (1989). The extraction procedures were carried out at 4°C. Three grams of shrimp sample was homogenized using an Oster Osterizer blender (Jarden Consumer Solutions, Providence, RI) with 30 mL of extraction solution for two minutes. The sarcoplasmic shrimp fraction was extracted using a solution of ionic strength (I_s) = 0.05 phosphate buffer at a pH of 7.5 [15.6 mM food grade Na_2HPO_4 , (Prayon Inc. Augusta, GA) and 3.5 mM food grade KH_2PO_4 (Prayon Inc., Augusta, GA)]. The homogenate was centrifuged at 5,500 rpm for 20 minutes at 4°C in a Sorvall RC-5B Refrigerated Centrifuge using a SS-34 Rotor (DJB Labcare Ltd, Buckinghamshire, UK). The supernatant was removed and held at 4°C. This procedure was repeated with the same samples and the supernatants were combined and labeled as the sarcoplasmic protein fraction. The myofibrillar shrimp protein fraction was then extracted from the homogenized shrimp sample using a solution of $I=$ 0.05 phosphate buffer at a pH of 7.5 (0.45 M KCl, 15.6 mM Na_2HPO_4 , and 3.5 mM KH_2PO_4). The samples were centrifuged at 5,500 rpm for 20 minutes at 4°C in a Sorvall RC-5B Refrigerated Centrifuge using a SS-34 Rotor (DJB Labcare Ltd, Buckinghamshire, UK). The supernatant was removed and held at 4°C. This procedure was repeated and the supernatants were combined and labeled as the

myofibrillar protein fraction. Control samples were also extracted with a 2.5% electrophoresis grade SDS (Bio-Rad Laboratories, Hercules, CA) solution to ensure that the myofibrillar and sarcoplasmic fractions achieved extraction. Extracted samples were stored at -80°C until further analysis.

3.4. Electrophoresis

Electrophoresis was performed for separation of protein by molecular weight according to the procedures outlined in the BioRad Ready Gel System Resource Guide (2011). Extracted samples were diluted 5x with their respective pure protein extraction buffer and were analyzed at 655 nm. The 5 μ L of the diluted protein samples, 25 μ L of BioRad D_c Protein Assay Reagent A (BioRad, Laboratories, Hercules, CA), and 200 μ L of using BioRad D_c Protein Assay Reagent B (BioRad, Laboratories, Hercules, CA) were added to a Costar 3590 96 well EIA/RIA plate, and shaken for 15 minutes using a Thermolyne Maxi-Mix III™ Type 65000 at 200 cycles per minute (ThermoFisher Scientific, Walther, MA). The plate was then analyzed using a BioRad Model 680 Microplate Reader (BioRad, Laboratories, Hercules, CA) at 655 nm.

These absorbances were compared to a standard curve that was created using Invitrogen protein standards (Invitrogen, Grand Island, NY) at 0 μ g/mL, 125 μ g/mL, 250 μ g/mL, 500 μ g/mL, 750 μ g/mL, 1000 μ g/mL, 1500 μ g/mL, and 2000 μ g/mL. The average absorbance of the shrimp samples were placed into the equation obtained from the standard curve and samples were diluted to contain 2000 μ g of protein per milliliter of extract. The extracted samples were mixed with BioRad Laemmli Buffer Solution (Bio-Rad Laboratories, Hercules, CA) and 98% electrophoresis grade 2-mercaptoethanol (Sigma Aldrich, St. Louis, MO) at a 2:1 ratio of buffer to protein solution under a fume hood. The sample and buffer mixture was then placed in boiling water at 100°C for three minutes under the fume hood prior to electrophoretic evaluation.

Samples were placed into a 10 well 4-20% gradient Tris-HCl BioRad Ready Gel with a 30 μ L well capacity (Bio-Rad Laboratories, Hercules, CA). Samples were placed into the gel with a BioRad Protein-Plus Pre-Stained 10-250 kDa Marker (Bio-Rad Laboratories, Hercules, CA) in lane one, SDS extraction sample in lane two, and duplicate runs of each sample consisting of control, static marinated, 0.4% STPP with 15% water tumbled, and 0.4% STPP/SAPP (70:30) with 15% water tumbled, respectively, in the remaining lanes. The BioRad 4-20% Tris-HCl gels (Bio-Rad Laboratories, Hercules, CA) were evaluated by electrophoresis using a BioRad Mini PROTEAN Tetra Cell apparatus (Bio-Rad Laboratories, Hercules, CA) filled with diluted BioRad 10x Tris-Glycine-SDS running buffer (Bio-Rad Laboratories, Hercules, CA) at 200V for 35 minutes. After electrophoresis, gels were stained using a solution of 0.5% Coomassie Blue, R-250 (Bio-Rad Laboratories, Hercules, CA), in a 60% solution of pure methanol (Mallenkrodt Chemicals, Philipsburg, NJ), and 40% glacial acetic acid solution for 45 minutes. Samples were then destained with a solution of 60% methanol and 40% acetic acid for 45 minutes. After destaining, the gels were scanned using a Lexmark S305 photocopier (Lexmark International, Lexington KY). Gels were then evaluated for protein type present in the myofibrillar and sarcoplasmic fractions of the samples. Protein amounts were then estimated using the QuantityOne® (Bio-Rad Laboratories, Hercules, CA) software package by analyzing the contrast of the band and comparing results against the protein amounts present in the standard. The protein amounts present in the standard at 37 and 20 kDa were 220 and 150 ng /10 μ L, respectively.

3.3. Microscopy

The fixation of shrimp samples was performed by a modified version of the method proposed by Sabatini (1963). All chemicals used for the fixation step were obtained from Sigma

Aldrich (St. Louis, MO). Shrimp were randomly sampled from each treatment. The third segment of the shrimp tail was removed and taken for fixation. Shrimp samples were cut to small size of approximately 2 mm squares and fixed in 2% glutaraldehyde, 1% formaldehyde in 0.2M cacodylate buffer pH 7.2 for 4 hours, then rinsed 5X in 0.1M cacodylate buffer containing 0.02M glycine over 12 hour period. Samples were post-fixed in 2% osmium tetroxide for two hours, rinsed in water, en bloc stained in 0.5% uranyl acetate in the dark for two hours, rinsed in water 2X, serially dehydrated in ethanol, infiltrated in ethanol: LR White (Sigma Aldrich, St. Louis, MO) resin series for 12 hours, and embedded in LR White (Sigma Aldrich, St. Louis, MO) overnight at 60° C. Sections 1 µm thick were cut on a Sorvall MT-2 microtome (DJB Labcare Ltd, Buckinghamshire, UK), and stained with 0.5% toluidine blue acetate, or TBO, in 2% sodium borate acetate for general tissue staining for light microscope study. The light microscope slides were viewed on an Olympus IX81 (Olympus Corporation, Center Valley, PA) microscope at 600X under oil immersion. Two images for each location and treatment combination, totaling 36 images, were acquired using the Olympus IX81 Camera, and saved for later analysis using the Slidebook software (Intelligent Imaging Innovations, Denver, CO). The images were analyzed using Image J, a free downloadable software program from the United States National Institute of Health (Bethesda, MD), using the free form drawing function and the area of the muscle fiber was calculated using the analyze function (Rasband and Ferreira, 2011). The 70nm ultra-thin sections were cut with the same microtome and mounted on collodion-coated copper grids (Sigma Aldrich, St. Louis, MO), stained with Reynolds lead citrate (Sigma Aldrich, St. Louis, MO), and imaged with a JEOL 100CX TEM (Peabody, MA). Muscle fiber area was measured using the ImageJ software package from the National Institute of Health (Bethesda, MD). A 50 µm scale at 600X was used to calibrate the system for measurements.

3.6. Statistical Analysis

The experiment used a model with three locations of LA Fresh, LA Frozen, and TX; three treatments of control, STPP, STPP/SAPP; and three replications. A soft water treatment was also analyzed for freezing rate and protein levels. The Hon and SC samples were not analyzed statistically due to lack of replication; the data is provided to show the trend. All data was analyzed with the Statistical Analysis System program (SAS, Cary, NC). Moisture, pick up, drip loss, fiber area, and protein amount were evaluated statistically using a generalized linear model, followed by a Tukey's studentized range test at an alpha level of 0.05.

CHAPTER 4: RESULTS AND DISCUSSION:

4.1. Vacuum Tumbling

4.1.1. Uptake of Solution

Samples of Louisiana Wild-Caught Gulf shrimp (LA Frozen and LA Fresh), domestic farm raised product from South Carolina (SC) and Texas (TX), and imported farm raised product from Honduras (Hon) were tumbled at 22 mm Hg with a solution of 15 g water and 0.46 g sodium tripolyphosphate (STPP) or 0.46 g (70% sodium tripolyphosphate and 30% sodium acid pyrophosphate) per 100 g of shrimp. The solution concentration of 0.4% w/w phosphate was determined from preliminary studies that showed that a solution of 0.4% w/w phosphate created similar uptake as a 0.5% w/w phosphate solution at 11.21% , and 11.18%, respectively, after 30 minutes of simulated tumbling. The use of phosphate amounts below the legal limit help to ensure that products are being produced lawfully with regard to phosphate content and could provide potential cost savings for producers. The uptake of solutions by origin and treatment are listed in Table 4.1. Values for Hon and SC are presented to demonstrate the trend of these samples to respond to the treatment, but are not statistically compared because they were not replicated due to procurement difficulties.

The tumbled samples LA Fresh, LA Frozen, and TX samples were similar ($p > 0.05$) for percent uptake of the STPP and SAPP blend at 10.34 ± 0.82 , 11.01 ± 1.16 , 11.31 ± 0.81 , respectively (Table 4.1). The uptake of STPP solution in LA Fresh, LA Frozen, and TX were observed to all be different ($p < 0.05$) at $8.75 \% \pm 3.33\%$, $13.59\% \pm 2.89\%$, $10.25\% \pm 0.15\%$, respectively (Table 4.1).

Table 4.1. Uptake of solution in raw shrimp samples treated with solutions of phosphates and water n=3.

Treatment	Origin				
	LA Fresh	LA Frozen	TX	Hon*	SC*
	Uptake (%) ¹				
STPP ²	8.75 ± 3.33 a	13.59 ± 2.89 c	10.25 ± 0.15 b	11.35	8.34
SAPP ³	10.34 ± 0.82 b	11.01 ± 1.16 b	11.31 ± 0.81 b	11.28	8.91

* Values are present to demonstrate trend of origin, however values could not be statistically compared due lack of replication.

1= % Uptake = ((treated weight – initial raw weight) / initial raw weight)*100

2= 15 g water and 0.46 g sodium tripolyphosphate per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 30 minutes).

3= 15 g water and 0.46 g phosphate (70% sodium tripolyphosphate and 30% sodium acid pyrophosphate) per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 22 minutes).

a-c= Different letters within a column or within a row indicate significant difference of means (p<0.05).

Differences can be noted between the STPP and SAPP blend treatments by sample type. The higher levels of uptake in the SAPP sample can be contributed to pH of the solution and the phosphate composition of the blend. SAPP showed a pH of approximately 4 in a 1% solution as compared to STPP, which had a pH of approximately 10. The addition of SAPP to the blend resulted in a slight drop in pH to around 8.0 resulting in a higher level of uptake, or amount of water taken into the product, in the LA Fresh and TX samples. This is potentially due to the increased amount of pyrophosphates present in the solution, which have been suggested to contribute to an increase in water holding capacity (WHC) (Hamm, 1960), as compared to the STPP solutions. SAPP is not currently used in the phosphate blend that is present in Louisiana processing plants and could present an opportunity for increased throughput and uniformity of the frozen product because of its high level of pyrophosphate and potentially higher level of pick-up. Lombard and Lanier (2011) suggested that phosphate solutions in the pH range of 7 to 8 had the highest levels of pick up in samples from 18-33% in tumbled fish fillets depending on the type of phosphate that was used. However, the levels of uptake observed in the study were

most likely due to the composition of the phosphates used in the solution rather than the pH. In the current study phosphate composition of the STPP and SAPP blend potentially affected the uptake of solution into muscle. As phosphate solutions are applied to muscle samples, they begin to hydrolyze and breakdown from tripolyphosphate to pyrophosphate and finally to orthophosphate (Hamm and Neraal, 1977b). Hamm and Neraal (1977b) suggested that this hydrolysis from polyphosphate to pyrophosphate contributes to higher levels of uptake and retention in muscles by the dissociation of actomyosin. The addition of SAPP in the blend used in the current study causes higher levels of pyrophosphate to be present in the solution at the beginning of treatment, as compared to the STPP, which must be hydrolyzed to the pyrophosphate form. The high initial levels of pyrophosphate affect the actomyosin present in the muscle resulting in a faster uptake of solution.

The LA Frozen and TX samples showed higher levels of solution uptake during tumbling as compared to the LA Fresh samples. The primary freezing process on board the boat helps improve retention of naturally present moisture in the product, and slows biochemical changes in the muscle during storage (Goncalves and Ribiero, 2008b). The researchers also stated that the secondary freezing process, after value-added processing, decreases additional moisture uptake during storage compared to storage on ice.

The effects of vacuum tumbling on uptake have been observed in various muscle foods systems by many researchers. Young and Lyon (1997) suggested that the effects of vacuum tumbling had an influence ($p < 0.05$) on uptake of solutions. However, Smith and Young (2004) observed that tumbling of broiler breast fillet at ambient pressure was not different ($p > 0.05$) compared to those tumbled under vacuum pressure.

In the current trial, shrimp was tumbled at a considerably lower vacuum level (approximately 3 kPa), and therefore cannot be compared to the effects of vacuum (50 kPa) in the Young and Lyon (1997) and Smith and Young (2004) studies. The differences in uptake observed in the LA Fresh product could have been affected by their treatment and storage post-harvest, which could have allowed for biochemical changes in the muscle, or the uptake of water prior to treatment as the control, or pre-treatment, moisture content in these samples was $80.27\% \pm 0.52$, and compared to the STPP samples at $81.55\% \pm 0.24$. The process of tumbling also allows for the uptake of solution prior to freezing, compared to the current static marination method, which occurs during the freezing and storage of the product. Theoretically, the level of water and phosphate present in the finished product can be more accurately controlled through vacuum tumbling. In the current study, however, the variation observed in the amount of solution that was taken up by the muscle was due to the formation of a sol on the tumbling chamber. Further research should be conducted to determine if filling the chamber to a higher level results in less protein loss from the shrimp.

Tumbling with or without vacuum has been conducted using excess solution (Lombard and Lanier 2011; Alvarado and Sams 2004; Krause and others 1978). This study, however, involved a controlled application of water and phosphate addition by batch with a desired amount of uptake and condensed phosphate level in the product. The target uptake for this study was a 15% increase in weight of the product. It was actually observed that approximately 10-12% uptake was obtained across all origins with vacuum tumbling. Table 4.2 shows the differences between the desired uptake and the actual uptake. The remaining solution remained in the chamber as a protein sol. The mechanical action of tumbling extracted proteins that formed a sol on the surface of the tumbling apparatus and the product. The ability of phosphates to

extract myofibrillar protein has been demonstrated by Xiong and others (2000). Xiong and others (2000) treated chicken myofibrils with varying levels of sodium chloride and 10 mM solutions of monophosphate, pyrophosphate, tripolyphosphate, and sodium hexametaphosphate. It was observed that troponin (80 kDa) began to be extracted at 0.3 M or 1.7% NaCl, but that extraction of myosin (200 kDa) and actin (45 kDa) did not occur until 0.5 M or 2.9% NaCl. The phosphate solutions caused varying levels of myofibrillar extraction, and it was determined that the trend for extractability proceeded in the order of pyrophosphate \approx tripolyphosphate > hexametaphosphate > orthophosphate \approx no phosphate control (Xiong and others, 2000).

Table 4.2. Difference in actual percent uptake between desired 15% uptake amount in raw shrimp samples treated with solutions of phosphates and water n=3.

Treatment	Origin				
	LA Fresh	LA Frozen	TX	Hon*	SC*
STPP ²	6.24 \pm 3.32 c	1.41 \pm 2.89 a	4.75 \pm 0.14 b	4.65	6.65
SAPP ³	4.67 \pm 0.81 b	4.42 \pm 0.54 b	3.68 \pm 0.81 b	3.72	6.08

* Values are present to demonstrate trend, however values could not be statistically compared due to lack of replication.

1= Difference = 15 - [(initial raw weight - treated weight) / initial raw weight]*100

2= 15 g water and 0.46 g sodium tripolyphosphate per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 30 minutes).

3= 15 g water and 0.46 g phosphate (70% sodium tripolyphosphate and 30% sodium acid pyrophosphate) per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 22 minutes).

a-c= Different letters within a column or within a row indicate significant difference of means (p<0.05).

Standardization of the raw moisture contents and estimation of the projected uptake in all species of shrimp showed that there were significant differences across the STP treatments in the level of uptake (Table 4.3). The SAPP blend however showed uniform levels of uptake in the LA Frozen, TX and Hon samples. This shows that in this study there was an ability to create a uniform uptake by vacuum tumbling across different species of shrimp using the SAPP blend.

This suggests that there could be potential for vacuum tumbling, but further research should be performed on the method.

Table 4.3. Actual percent uptake, actual percent moisture, and adjusted percent uptake in raw treated and non-treated shrimp in shrimp by origin and treatment with adjusted moisture content of 78.68%.

Sample	Treatment	Actual Uptake (%)	Actual Moisture (%)	Adjusted Uptake at 78.68% Moisture (%)¹
LAFresh	Control		80.27	
LAFresh	STPP ²	8.75 a	81.55	9.07 a
LAFresh	SAPP ³	10.34 b	81.25	10.68 b
LAFrozen	Control		76.07	
LAFrozen	STPP	13.59 c	79.19	13.68 d
LAFrozen	SAPP	11.01 b	78.72	11.02 c
TX	Control		76.27	
TX	STPP	10.25 b	79.02	10.29 b
TX	SAPP	11.31 b	78.63	11.30 c
Hon*	Control		73.78	
Hon*	STPP	11.35	76.45	11.03
Hon*	SAPP	11.28	77.08	11.05
SC*	Control		81.38	
SC*	STPP	8.34	81.30	8.62
SC*	SAPP	8.91	81.48	9.23

* Values are present to demonstrate trend, however values could not be statistically compared due to lack of replication.

1= Adjusted % Uptake= [Actual % Moisture / (Average % moisture of controls)]* Actual % Uptake.

2= 15 g water and 0.46 g sodium tripolyphosphate per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 30 minutes).

3= 15 g water and 0.46 g phosphate (70% sodium tripolyphosphate and 30% sodium acid pyrophosphate) per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 22 minutes).

a-d= Different letters within columns indicate significant difference of means (p<0.05).

4.1.2. Moisture and Cook-Loss

The effects of vacuum tumbling of shrimp with solutions of phosphates on moisture content are shown in Table 4.4. Values for Hon and SC are present to demonstrate the trend of these samples to respond to the treatment, but were not statistically compared because they were not replicated due to procurement difficulties. Samples were not tested for drip loss amounts

following tumbling because most of the industry freezes the product immediately after processing.

The moisture values for the raw control by origin for Hon, LA Fresh, LA Frozen, SC, and TX were $73.78\% \pm 0.20$, $80.27\% \pm 0.52$, $76.07\% \pm 0.48$, $81.38\% \pm 0.37$, $76.27\% \pm 0.49$ respectively. It is difficult to determine the effects of the treatments across all samples because of the varying moisture content of the control in each origin. The LA Frozen STPP and SAPP treated samples were shown to be statistically similar in raw moisture content at 79.19 ± 0.40 , and 78.72 ± 0.54 , respectively. The TX STPP and SAPP treatments were also shown to be statistically similar in raw moisture content at 79.02 ± 0.39 , and 78.63 ± 0.44 , respectively. It was observed that the LA Fresh samples showed the least change in moisture content when treated. The raw moisture content of the STPP and SAPP treated samples in the LA Fresh were shown to be statistically similar at and $81.55\% \pm 0.24$ and 81.25 ± 0.32 , respectively. The LA Fresh samples were stored on ice and were exposed to water via melting ice during storage that could have caused absorption of free water into the muscle prior to processing. The SC samples, which were frozen in water by the processor, demonstrated similar a similar trend as the LA Fresh sample in the STPP and SAPP treated samples at 81.30 ± 0.08 , 81.48 ± 0.49 , respectively. The low level change in moisture content for these two samples could be contributed to the high level of initial moisture present in the control product. The LA Fresh and LA Frozen data suggest that muscle that has been exposed to water prior to treatment could be subsequently limited in its ability to pick-up phosphate solutions during value added processing. The current method for the creation of statically treated value added Gulf shrimp in Louisiana involves the mechanical peeling of shrimp, which uses water to remove the shell and vein, as well as move the product through the peeling machine, exposes the muscle to high levels of water prior to

further processing. Further research should be performed to observe the amount of water that is potentially added by this process, and the effects on the finished statically treated product.

Table 4.4. Moisture content of raw treated and non-treated shrimp n = 3.

Treatment	Origin				
	LA Fresh	LA Frozen	TX	Hon*	SC*
	Moisture (%) ¹				
Control	80.27 ± 0.52 c	76.07 ± 0.48 a	76.27 ± 0.49 a	73.78 ± 0.20	81.38 ± 0.37
STPP ²	81.55 ± 0.24 c	79.19 ± 0.40 b	79.02 ± 0.39 b	76.45 ± 0.25	81.30 ± 0.08
SAPP ³	81.25 ± 0.32 c	78.72 ± 0.54 b	78.63 ± 0.44 b	77.08 ± 0.34	81.48 ± 0.49

* Values are present to demonstrate trend, however values could not be statistically compared due to lack of replication.

1= % Moisture = [(wet weight – dry weight) / wet weight]*100.

2= 15 g water and 0.46 g sodium tripolyphosphate per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 30 minutes).

3= 15 g water and 0.46 g phosphate (70% sodium tripolyphosphate and 30% sodium acid pyrophosphate) per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 22 minutes).

a-c= Different letters within a column or within a row indicate significant difference of means (p<0.05).

The samples were steamed to an internal temperature of 62.8 °C post tumbling to observe the effect of cooking on the moisture content of the treated flesh. Cook-cool loss was calculated for the samples to evaluate the effect of the phosphate treatments on cook yield of the product (Table 4.5). It was observed that there was variation in the cook-cool loss of the control LA Fresh, LA Frozen and Texas samples. The STPP treatment showed the most uniformity for cook-cool loss across samples.

Cook-cool losses in this experiment were lower than the results shown by Rattanasatheirn and others (2008), who studied the effects of freshness and deveining on phosphate treated shrimp. Rattanasatheirn and others (2008) observed cook losses of 15 to 20% in samples of shrimp that were statically treated by soaking in solutions of phosphates and salt ranging from 2.5% NaCl with no phosphates to 2.5% NaCl and 3.5% STPP for 2 hours at 4°C. The increase in

moisture content of these samples ranged from 4 to 8%. Samples evaluated by Rattanasatheirn and others (2008) gained approximately 10 to 12% weight during treatments and had a cook-loss of approximately 16 percent.

Table 4.5. Cook-Cool Loss values for samples of cooked treated and non-treated shrimp n=3.

Treatment	Origin				
	LA Fresh	LA Frozen	TX	Hon*	SC*
	% Cook -Cool Loss ¹				
Control	15.08 ± 0.19 b	8.30 ± 2.40 a	13.64 ± 1.52 b	9.96	15.13
STPP ²	17.38 ± 0.47 b	17.10 ± 6.53 c	16.57 ± 0.24 b	17.71	17.71
SAPP ³	12.74 ± 1.40 a	14.31 ± 3.25 b	21.20 ± 0.99 c	13.73	13.73

* Values are present to demonstrate trend, however values could not be statistically compared.

1= % CookCoolLoss = 100- [(CookCool weight / Raw weight) *100]

2= 15 g water and 0.46 g sodium tripolyphosphate per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 30 minutes).

3= 15 g water and 0.46 g phosphate (70% sodium tripolyphosphate and 30% sodium acid pyrophosphate) per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 22 minutes).

a-c= Different letters within a column or within a row indicate significant difference of means (p<0.05).

Standardization of the raw moisture contents and estimation of the projected cook-cool loss in all species of shrimp showed that there were similarities in the estimated cook-cool loss as compared to the control (Table 4.6). The estimated cook-cool loss was similar (p>0.05) in the STPP treatment. The SAPP treatment had a greater amount of cook-cool loss compared to the control for the LA Fresh but not the TX shrimp.

Table 4.6. Actual percent cook-cool loss, actual percent moisture, and adjusted percent cook-cool loss in cooked treated and non-treated shrimp in shrimp by origin and treatment with adjusted moisture content of 78.68%.

Sample	Treatment	Actual Cook-Cool Loss (%)	Actual Moisture (%)	Adjusted Cook-Cool Loss at 78.68% Moisture (%) ¹
LAFresh	Control	15.08 c	77.16	14.79 d
LAFresh	STPP ³	12.74 b	79.15	12.82 c
LAFresh	SAPP ⁴	17.38 d	80.30	17.74 e
LAFrozen	Control	8.30 a	74.56	7.87 a

(Table cont.)

LAFrozen	STPP	14.31 c	77.40	14.08 c
LAFrozen	SAPP	17.10 d	77.55	16.85 e
TX	Control	13.64 b	74.28	12.88 c
TX	STPP	21.20 e	75.30	20.29 f
TX	SAPP	16.57 d	75.34	15.87 d
Hon*	Control	9.96 a	74.26	9.40
Hon*	STPP	13.73 b	75.53	13.18
Hon*	SAPP	17.71 d	74.61	16.79
SC*	Control	15.13 c	78.50	15.10
SC*	STPP	13.73 b	79.02	13.79
SC*	SAPP	17.71 d	76.10	17.13

* Values are present to demonstrate trend, however values could not be statistically compared due to lack of replication.

1= Adjusted % Cook-Cool = [Actual % moisture/ (Average % moisture of controls)]*
Actual % cook-cool loss.

2= 15 g water and 0.46 g sodium tripolyphosphate per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 30 minutes).

3= 15 g water and 0.46 g phosphate (70% sodium tripolyphosphate and 30% sodium acid pyrophosphate) per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 22 minutes).

a-f= Different letters within columns indicate significant difference of means (p<0.05).

Moisture values for the cooked shrimp samples were obtained (Table 4.7). The moisture contents of the treated product were greater than the control as expected; but more importantly, upon comparison with the values in Table 4.4, the treated samples all showed moisture contents that were similar to moisture content of the raw control product. This suggests that the application of phosphates across all methods contributes to the maintenance of the natural moisture in the product.

Table 4.7. Moisture content of cooked treated and non-treated raw shrimp.

Treatment	Origin				
	LA Fresh	LA Frozen	TX	Hon*	SC*
	Moisture (%) ¹				
Control	77.16 ± 0.85b	74.56 ± 0.03a	74.28 ± 0.27a	74.26 ± 0.24	78.50 ± 0.12
STPP ²	79.15 ± 0.20c	77.40 ± 0.35b	75.30 ± 1.79a	75.53 ± 0.30	79.02 ± 0.16
SAPP ³	80.30 ± 0.31c	77.55 ± 0.22b	75.34 ± 1.15a	74.61 ± 0.06	76.10 ± 0.19

* Values are present to demonstrate trend, however values could not be statistically compared.

1= %Moisture = [(Wet Weight – Dry Weight)/ Wet Weight]*100.

2= 15 g water and 0.46 g sodium tripolyphosphate per 100 g shrimp vacuum tumbled at 22

(Table cont.)

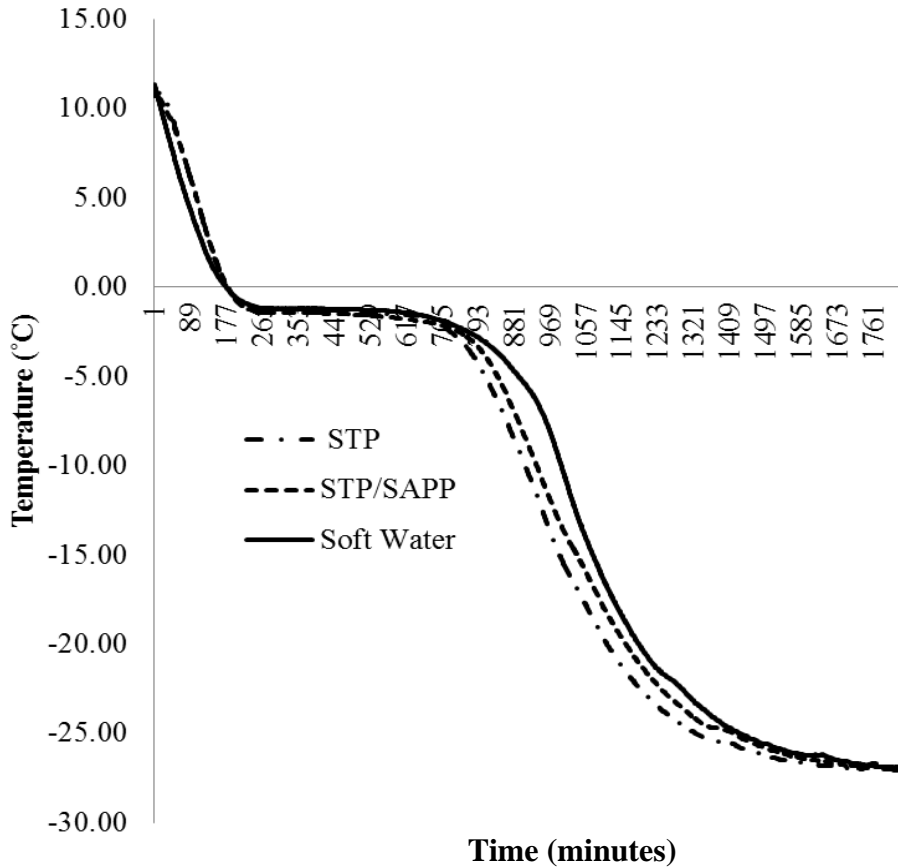
mm Hg until no solution was visible (approximately 30 minutes).

3= 15 g water and 0.46 g phosphate (70% sodium tripolyphosphate and 30% sodium acid pyrophosphate) per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 22 minutes).

a-c= Different letters within a column or within a row indicate significant difference of means ($p < 0.05$).

4.1.3. Freezing

The effects of solution addition by vacuum tumbling on the functional performance of phosphates during post-processing freezing were also observed. Preliminary testing demonstrated that the process of tumbling solution into muscle and freezing showed a faster rate of freezing in the product as compared to the soft water sample (Figure 4.1). Comparing the freezing rate obtained in this trial to the work of Johnston and others (1994), the temperature zone between 0 °C and -8 °C was chosen to observe differences in the freezing rate because it represented the temperature range in which the majority of the water in the muscle would be frozen, therefore this would be the range in which large ice crystal formation could damage the muscle structure. The STPP, SAPP, and soft water samples spent an average of 690, 723, and 787 minutes in the targeted temperature range, respectively. The STPP treatment was determined to be statistically different than the soft water sample ($p < 0.05$), however, the soft water sample was not statistically different than the SAPP blend. Although the SAPP treatments were not statistically different than the soft water treatment, the difference in time that the product was in the selected range was approximately 60 minutes less than the soft water treatment, showing that there was still an improvement in freezing rate. The improvement in the rate of freezing of the tumbled product potentially causes less damage to occur to the muscle structure of the product during freezing. This improvement in freezing rate could also potentially lead to a faster throughput of product for the producer.



1. Samples were frozen at a temperature of -30°C.
 STP= 15 g water and 0.46 g sodium tripolyphosphate per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 30 minutes).
 STP/SAPP= 15 g water and 0.46 g phosphate (70% sodium tripolyphosphate and 30% Sodium acid pyrophosphate) per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 22 minutes).
 Soft Water = 0.5% sodium tripolyphosphate in 1 L of water.
Figure 4.1. Preliminary freezing test of samples of Gulf shrimp treated with phosphate solutions (n=2 five pound boxes)

4.2. Electrophoresis

Samples were evaluated by SDS PAGE electrophoresis to determine if processing by tumbling had an effect on the proteins that were present in the shrimp samples. The myofibrillar and sarcoplasmic fractions of the shrimp samples were extracted after treatment and freezing. The most important band in the myofibrillar fraction, for the purposes of this study, was myosin at 200 kDa because condensed phosphates are expected to solubilize myosin when used in value

added processing (Hamm, 1960). The actomyosin band present at 75 kDa also was sporadically present across all samples in the myofibrillar fraction. The quantification methods used in the experiment were based on comparison of the contrast from the band and the background. For these purposes, two bands that were consistently present across the myofibrillar fraction were chosen for quantification (Table 4.8). The bands selected were at approximately 37-45 kDa and identified as actin in the myofibrillar fraction, and cathepsin D in the sarcoplasmic fraction (Bandman, 1987). The band present at 18-20 kDa was identified as troponin c in the myofibrillar fraction (Bandman, 1987), and sarcoplasmic calcium binding protein in the sarcoplasmic fraction (Shiomi and others, 2008), respectively.

Within the tumbled samples, the SAPP treatment trends to have protein quantities closer to the control than the STPP treatment. The tumbling time for the SAPP treatment required 22 minutes on average for total solution uptake by visual determination as compared to 30 minutes for STPP.

Siegel and others (1978a) suggested that the use of salt and condensed phosphate solutions in the tumbling process contributed to the extraction of myosin and actin from the muscle. Siegel and others (1978a) observed the formation of a protein gel exudate on the surface of the product and within the tumbling chamber. The current study also observed the formation of exudate during tumbling, suggesting that the tumbling process in the presence of phosphate solutions contributed to the disappearance of myosin bands.

STPP and SAPP have been shown to solubilize myosin in muscle tissue, causing an increase in WHC of the muscle (Hamm, 1960). Therefore, the solubilization of this protein was a key in determining the functionality of the condensed phosphates used in the STPP and SAPP treatments. The degree of solubilization for myosin across treatments was unable to be measured

due to an inability to distinguish between the band and the background. The reduction of myofibrillar proteins as a whole was observed across all samples, however, the quantified bands of 37 and 20 kDa showed no significant difference from the control in amount. Comparing these results to the moisture data that was observed, the WHC of the muscle was appears to have not been detrimentally affected by the tumbling treatments. This suggests that the tumbling process caused faster extraction of myofibrillar protein resulting in the uptake of solution in 23 to 30 minutes in the STPP and SAPP samples.

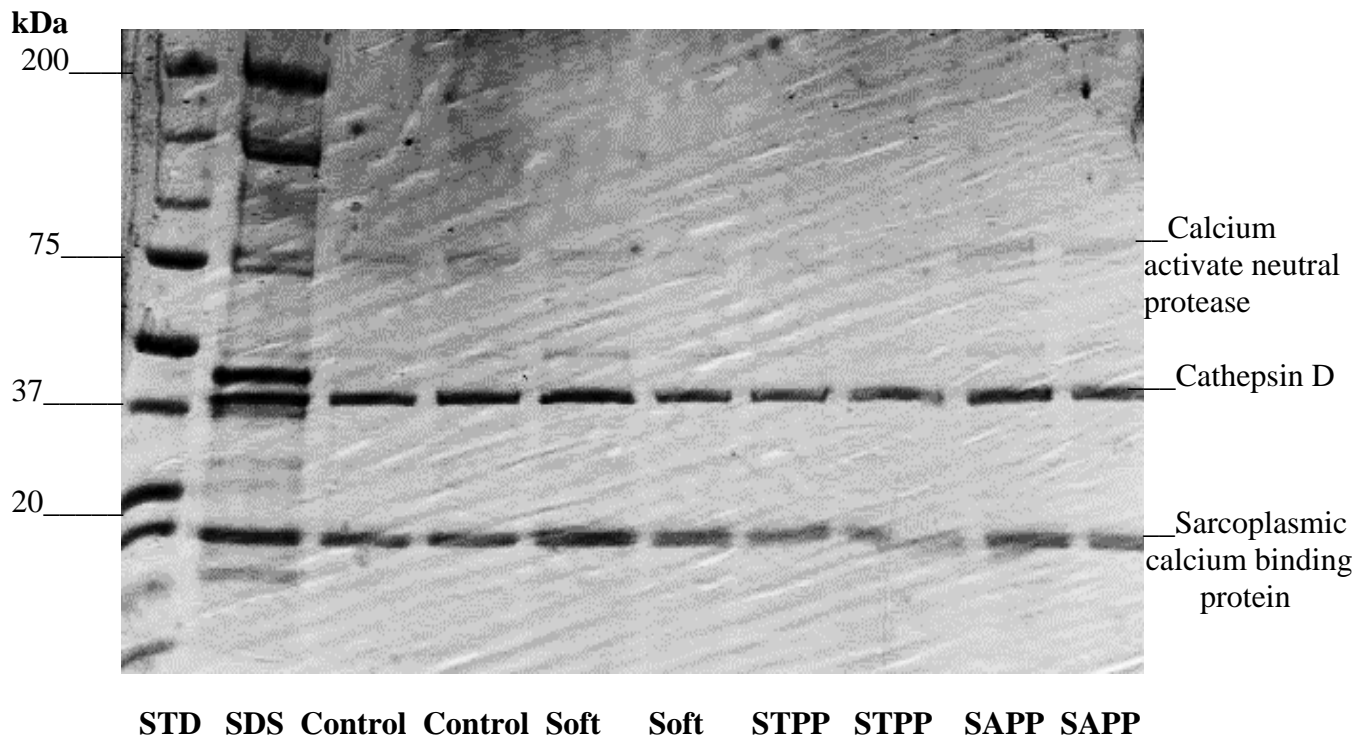


Figure 4.2. Representative SDS PAGE gels of sarcoplasmic protein fractions from treated and non-treated shrimp samples.

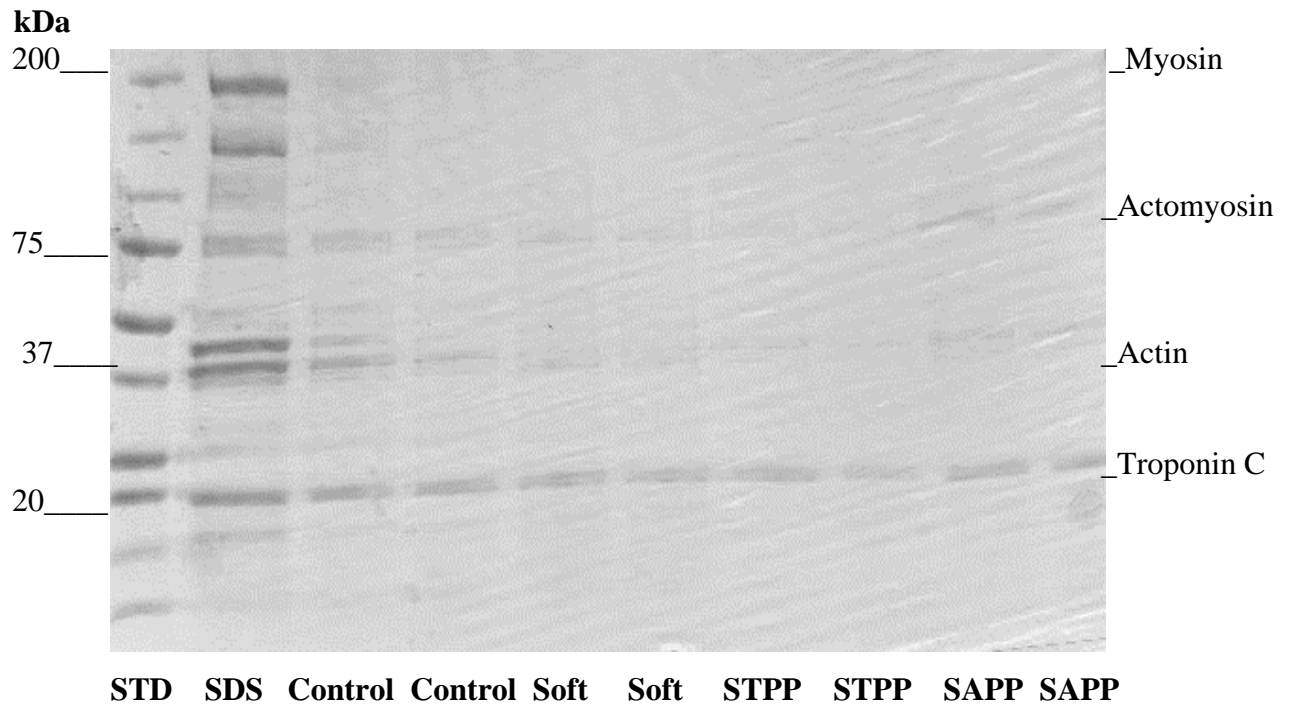


Figure 4.3. Representative SDS PAGE gels of myofibrillar protein fractions from treated and non-treated shrimp samples.

Table 4.8. Protein quantities for sarcoplasmic protein fractions at 20 kDa (n=2).

Treatment	Origin				
	LA Fresh	LA Frozen	TX	Hon*	SC*
	Protein ($\mu\text{g/mL}$)				
Control	3.51 ± 0.20 c	0.97 ± 0.02 a	0.88 ± 0.04 a	0.81 ± 0.36	0.84 ± 0.07
STPP ¹	2.69 ± 0.23 b	0.88 ± 0.04 a	1.10 ± 0.04 a	0.80 ± 0.02	0.83 ± 0.05
SAPP ²	3.05 ± 0.32 c	0.92 ± 0.06 a	1.04 ± 0.05 a	0.94 ± 0.04	0.82 ± 0.01

* Values are present to demonstrate trend, however, values could not be statistically compared due to lack of replication.

1= 15 g water and 0.46 g sodium tripolyphosphate per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 30 minutes).

2= 15 g water and 0.46 g phosphate (70% sodium tripolyphosphate and 30% sodium acid pyrophosphate) per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 22 minutes).

a-c= Different letters within a column or within a row indicate significant difference of means ($p < 0.05$).

Table 4.9. Protein quantities for myofibrillar protein fractions at 20 kDa (n=2).

Treatment	Origin				
	LA Fresh	LA Frozen	TX	Hon*	SC*
	Protein ($\mu\text{g/mL}$)				
Control	1.39 ± 0.11 b	0.91 ± 0.01 a	1.86 ± 0.14 b	1.54 ± 0.21	1.02 ± 0.07
STPP ¹	1.50 ± 0.01 b	0.73 ± 0.01 a	1.39 ± 0.10 b	1.40 ± 0.27	0.99 ± 0.09
SAPP ²	1.11 ± 0.21 a	0.78 ± 0.03 a	1.40 ± 0.13 b	1.08 ± 0.03	0.83 ± 0.16

* Values are present to demonstrate trend, however, values could not be statistically compared due to lack of replication.

1= 15 g water and 0.46 g sodium tripolyphosphate per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 30 minutes).

2= 15 g water and 0.46 g phosphate (70% sodium tripolyphosphate and 30% sodium acid pyrophosphate) per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 22 minutes).

a-b= Different letters within a column or within a row indicate significant difference of means ($p < 0.05$).

Table 4.10. Protein quantities for sarcoplasmic protein fractions at 37 kDa (n=2).

Treatment	Origin				
	LA Fresh	LA Frozen	TX	Hon*	SC*
	Protein ($\mu\text{g}/\text{mL}$)				
Control	4.91 \pm 0.12 e	4.14 \pm 0.06 d	0.86 \pm 0.04 a	1.30 \pm 0.77	2.72 \pm 0.38
STPP ¹	2.97 \pm 0.27 c	3.56 \pm 0.16 d	1.27 \pm 0.14 b	1.56 \pm 0.01	2.67 \pm 0.10
SAPP ²	3.66 \pm 0.22 d	3.71 \pm 0.10 d	1.31 \pm .011 b	1.85 \pm 0.07	2.19 \pm 0.16

* Values are present to demonstrate trend, however, values could not be statistically compared due to lack of replication.

1= 15 g water and 0.46 g sodium tripolyphosphate per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 30 minutes).

2= 15 g water and 0.46 g phosphate (70% sodium tripolyphosphate and 30% sodium acid pyrophosphate) per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 22 minutes).

a-e= Different letters within a column or within a row indicate significant difference of means ($p < 0.05$).

Table 4.11. Protein quantities for myofibrillar protein fractions at 37 kDa (n=2).

Treatment	Origin				
	LA Fresh	LA Frozen	TX	Hon*	SC*
	Protein ($\mu\text{g}/\text{mL}$)				
Control	3.06 \pm 0.10 d	1.00 \pm 0.27 a	2.18 \pm 0.22 c	1.54 \pm 0.24	1.09 \pm 0.07
STPP ¹	1.73 \pm 0.19 b	0.77 \pm 0.002 a	1.68 \pm 0.02 b	1.31 \pm 0.01	0.86 \pm 0.06
SAPP ²	1.56 \pm 0.22 b	0.69 \pm 0.10 a	1.87 \pm 0.14 b	0.73 \pm 0.09	0.73 \pm 0.19

* Values are present to demonstrate trend, however, values could not be statistically compared due to lack of replication.

1= 15 g water and 0.46 g sodium tripolyphosphate per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 30 minutes).

2= 15 g water and 0.46 g phosphate (70% sodium tripolyphosphate and 30% sodium acid pyrophosphate) per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 22 minutes).

a-d= Different letters within a column or within a row indicate significant difference of means ($p < 0.05$).

4.3. Microscopy

Samples were photographed at 600X under light microscopy to determine the effects of processing on the muscle fiber area (Figure 4.2). Samples of muscle fiber area were measured and are shown in Table 4.12. The LA Fresh samples for the STPP and SAPP treatments had different fiber area size at $3.60 \mu\text{m} \pm 0.70$ and $4.91 \mu\text{m} \pm 1.39$, respectively. The LA Frozen samples also had different fiber areas in the STPP and SAPP samples at $3.70 \mu\text{m} \pm 0.95$ and $4.76 \mu\text{m} \pm 1.32$. The TX SAPP sample showed a larger fiber area than the STPP and Control samples. It is important to note that all products in this experiment, with the exception of LA Fresh, were previously frozen and stored for approximately 6 months prior to treatment.

The comparison of the fiber area measurements to the moisture and uptake data show that it was difficult to determine the effects of processing on the change in muscle fiber area. An increase in muscle fiber area could be an indication of the amount of water absorbed into the muscle fiber during treatment. The determination of moisture content in the raw product includes all moisture that is present in the product. This includes water that has been absorbed into the muscle and water that is loosely held between the muscles. The determination of uptake determined the amount of weight change in the treated product as compared to the control, and did not differentiate between water in the muscle fiber and between the muscle fibers. The determination of thaw-drip loss would have given an estimation of the amount of water retained in the muscle. Further research should observe if these values allow for comparison of the effects of tumbling on the ability of processing to affect the muscle fiber area of treated product.

It can, however, be observed that there was a difference in muscle fiber size of samples by origin within the control sample. The Hon and TX samples were of the species *Penaeus vannamei*, and show similarities in muscle fiber area of the control sample. The LA Fresh shrimp

(*Penaeus aztecus*) that were stored on ice post-harvest, and LA Frozen (*Penaeus setiferus*) that were rapidly plate frozen post-harvest show differences in fiber size at $p > 0.05$ as well. Value-added shrimp in the Louisiana Gulf are typically frozen after harvest, thawed, treated with condensed phosphate solution, and are refrozen. This method caused the muscle to undergo multiple freeze thaw cycles before the product would reach the end consumer, which could affect the integrity of the muscle structure. This can be observed in the LA Frozen sample where the STPP treatment resulted in a decrease in muscle fiber size. Comparison of this value shows a difference ($p > 0.05$) from the SAPP and control treatments.

The effects of processing on the muscle structure of seafood have been investigated by Goncalves and Ribiero (2008b), Rattanaseithern and others (2007), and Jarenback and Liljemark (1975a) showed that treatment with mixed condensed phosphate results in less damage to the muscle structure than the control samples after seven days of storage on ice. Other researchers have demonstrated the cryoprotectant capabilities of phosphates in frozen products.

Goncalves and Ribiero (2008b) demonstrated that freezing highly influenced the microscopic structure of the muscle. It was observed that slower freezing rates provided greater opportunity for muscle damage to occur and for muscle fiber area to decrease. Damage to the muscle fiber can potentially lead to losses of intracellular fluids and added water in processed products, which ultimately affect the quality of the finished product. Jarenback and Liljemark (1975a) observed that damage during the freezing process affected the protein amounts present in the muscle structure. Miller and others (1968) observed higher levels of myofibrillar protein loss in samples of frozen beef loin as compared to pre-rigor and post-rigor samples after heating the samples to 70°C and centrifuging the samples at 1000 rpm in a refrigerated centrifuge. The results of Miller and others (1968) were comparable to similar work performed by Wierbicki and

others (1957), who determined that higher levels of myofibrillar protein was in frozen muscle extracted after use of the same method. A primary effect of freezing is the disruption of myosin resulting in the creation of myosin arrowheads. The use of condensed phosphates was shown to result in the creation of less arrowheads suggesting that less damage occurred during storage of treated muscle (Jarenback and Liljemark, 1975a). Less damage in the muscle could potentially result in the retention of more added water after processing.

The current study showed varying results for the measurement of fiber area with regard to the effect of freezing rate on fiber size. The muscle fiber area for the TX and LA Frozen STPP and SAPP treated samples showed a larger fiber area measurement than the soft water treated samples. The SC and Hon samples showed differences in the STPP and SAPP sample fiber sizes, but similar fiber measurements between the STPP and soft water treatments. The LA Fresh samples, which were only frozen after treatment, showed similar fiber area measurements for the STPP and SAPP samples, but the soft water treatment's fiber area was significantly higher. This difference could be due to the uptake of water into the muscle post-harvest during storage on ice prior to processing. The differences observed across all samples and all treatments show that further research should be performed to determine the effects of freezing rate between tumbled and soft water treated shrimp samples on muscle fiber area.

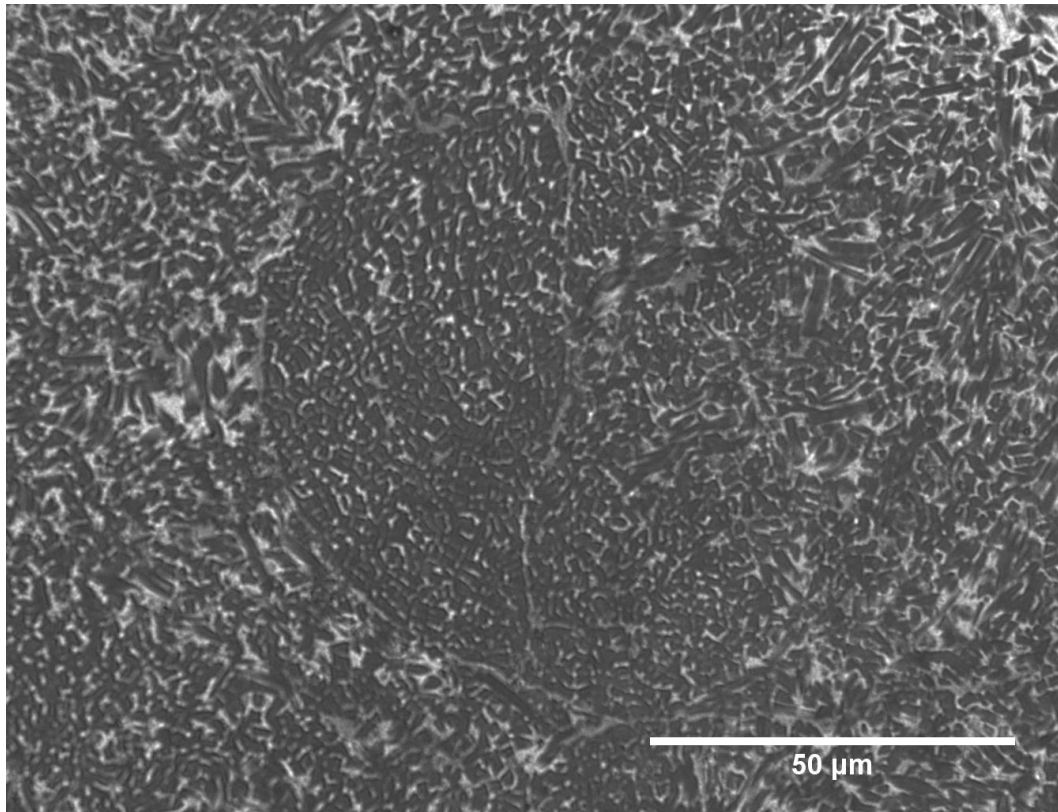


Figure 4.4. Light microscope image of cross section of shrimp sample photographed at 600X magnification.

Table 4.12. Measurements of fiber area* of treated and non-treated shrimp samples at 600X.

Treatment	Origin				
	LA Fresh	LA Frozen	TX	Hon*	SC*
	Fiber Measurements (μm^2)				
Control	3.29 ± 0.70 c	4.49 ± 1.35 d	2.00 ± 0.43 a	1.86 ± 0.41	7.03 ± 1.32
Soft Water ¹	6.31 ± 1.10 e	2.95 ± 0.86 c	1.94 ± 0.32 a	4.01 ± 0.89	5.94 ± 1.23
STPP ²	3.60 ± 0.70 c	3.70 ± 0.95 c	2.04 ± 0.42 a	4.14 ± 0.78	5.68 ± 1.18
SAPP ³	4.91 ± 1.39 d	4.76 ± 1.32 d	2.32 ± 0.42 b	2.79 ± 0.65	2.62 ± 0.54

* Values are present to demonstrate trend, however, values could not be statistically compared due to lack of replication.

1 = Soft Water = 0.5% sodium tripolyphosphate in 1 L of water.

2= 15 g water and 0.46 g sodium tripolyphosphate per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 30 minutes).

3= 15 g water and 0.46 g phosphate (70% sodium tripolyphosphate and 30% Sodium acid pyrophosphate) per 100 g shrimp vacuum tumbled at 22 mm Hg until no solution was visible (approximately 22 minutes).

a-e= Different letters within a column or within a row indicate significant difference of means ($p < 0.05$).

CHAPTER 5: CONCLUSIONS

Data presented in this study suggest that the use of vacuum tumbling of shrimp with known amounts of phosphates and water and subsequent freezing is a feasible method for adding value to shrimp products on a pilot scale. Samples were treated with solutions containing 15 g water and 0.46 g phosphate (STPP, or 70% STPP and 30% SAPP) per 100 g of raw shrimp. Tumbled samples showed a trend of greater or equal levels of solution uptake with the SAPP blend. Standardization of the percent moisture showed that the uptake of solution was not uniform among the shrimp types. Standardized cook-cool data indicated that the STPP and SAPP treatments resulted in moisture levels similar to the natural moisture level of the shrimp. The process of tumbling shrimp and freezing was also demonstrated to have higher losses within the protein fractions, due to adherence of protein to the tumbler chamber, as compared to the control. The measurement of muscle fiber area showed that it is difficult to determine a correlation between fiber area to level of moisture or uptake in the finished product. This may have been due to the inability to distinguish between water absorbed into the muscle fiber and water between muscle fibers. The measurement of fiber area also showed that further research should be performed to investigate the effects of freezing rate on the muscle fiber area between vacuum tumbled shrimp and soft water treated shrimp. This study has shown that the use of vacuum tumbling for the addition of phosphate solutions to shrimp results a consistent level of uptake when the SAPP blend is used. Loss of protein may be a factor due to underfill of the tumbling chamber, and muscle fiber area may be different among species but further research should be performed.

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APPENDIX
ANOVA Tables for Moisture-Raw by Origin

LA Fresh

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	5.34251480	2.67125740	18.57	<.0001
Error	15	2.15829048	0.14388603		
Corrected Total	17	7.50080528			

LA Frozen

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	24.27177358	12.13588679	49.55	<.0001
Error	13	3.18422073	0.24494006		
Corrected Total	15	27.45599431			

TX

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	70.10108376	23.36702792	98.70	<.0001
Error	20	4.73506392	0.23675320		
Corrected Total	23	74.83614768			

ANOVA Tables for Moisture-Cooked by Origin

LA Fresh

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	30.40059369	15.20029684	52.54	<.0001
Error	15	4.33944527	0.28929635		
Corrected Total	17	34.74003896			

LA Frozen

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	13.62656283	6.81328141	93.15	<.0001
Error	7	0.51197926	0.07313989		
Corrected Total	9	14.13854209			

TX

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	4.37047249	2.18523624	1.42	0.2722
Error	15	23.06767529	1.53784502		
Corrected Total	17	27.43814777			

ANOVA tables for Uptake% by Origin

LA Fresh

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	124.4603566	41.4867855	14.10	0.0136
Error	4	11.7663104	2.9415776		
Corrected Total	7	136.2266670			

LA Frozen

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	368.1232656	122.7077552	32.50	<.0001
Error	12	45.3074331	3.7756194		
Corrected Total	15	413.4306987			

TX

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	169.9421153	56.6473718	21.44	0.0063
Error	4	10.5704783	2.6426196		
Corrected Total	7	180.5125936			

ANOVA Tables for Cook Cool Loss by Origin

LA Fresh

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	124.0311531	62.0155765	15.87	0.0254
Error	3	11.7213785	3.9071262		
Corrected Total	5	135.7525316			

LA Frozen

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	367.9810872	183.9905436	50.38	<.0001
Error	11	40.1731427	3.6521039		
Corrected Total	13	408.1542299			

TX

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	156.0482010	78.0241005	342.35	0.0003
Error	3	0.6837301	0.2279100		
Corrected Total	5	156.7319311			

ANOVA Table for Freezing Time

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	9709.00000	4854.50000	10.74	0.0429
Error	3	1356.50000	452.16667		
Corrected Total	5	11065.50000			

ANOVA Table for Myofibrillar Protein Fraction Control Band at 20kDa

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.20151600	0.30037900	18.41	0.0034
Error	5	0.08158293	0.01631659		
Corrected Total	9	1.28309893			

ANOVA Table for Myofibrillar Protein Fraction SAPP Band at 20kDa

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.48718224	0.12179556	7.02	0.0277
Error	5	0.08673030	0.01734606		
Corrected Total	9	0.57391254			

ANOVA Table for Myofibrillar Protein Fraction STPP Band at 20kDa

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.86197637	0.21549409	12.22	0.0086
Error	5	0.08818403	0.01763681		
Corrected Total	9	0.95016040			

ANOVA Table for Sarcoplasmic Protein Fraction Control Band at 20kDa

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	11.14729716	2.78682429	79.29	0.0001
Error	5	0.17574000	0.03514800		
Corrected Total	9	11.32303716			

ANOVA Table for Sarcoplasmic Protein Fraction SAPP Band at 20kDa

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	7.25851577	1.81462894	82.36	<.0001
Error	5	0.11016532	0.02203306		
Corrected Total	9	7.36868109			

ANOVA Table for Sarcoplasmic Protein Fraction STPP Band at 20kDa

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	5.21836232	1.30459058	108.43	<.0001
Error	5	0.06016053	0.01203211		
Corrected Total	9	5.27852285			

ANOVA Table for Myofibrillar Protein Fraction Control Band at 37kDa

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	5.87463305	1.46865826	37.03	0.0007
Error	5	0.19830273	0.03966055		
Corrected Total	9	6.07293578			

ANOVA Table for Myofibrillar Protein Fraction SAPP Band at 37kDa

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2.51300918	0.62825230	25.25	0.0016
Error	5	0.12442267	0.02488453		
Corrected Total	9	2.63743186			

ANOVA Table for Myofibrillar Protein Fraction STPP Band at 37kDa

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.60861615	0.40215404	26.31	0.0015
Error	5	0.07642333	0.01528467		
Corrected Total	9	1.68503947			

ANOVA Table for Sarcoplasmic Protein Fraction Control Band at 37kDa

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	24.55939101	6.13984775	40.27	0.0005
Error	5	0.76241668	0.15248334		
Corrected Total	9	25.32180769			

ANOVA Table for Sarcoplasmic Protein Fraction SAPP Band at 37kDa

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	9.48931413	2.37232853	116.03	<.0001
Error	5	0.10223213	0.02044643		
Corrected Total	9	9.59154627			

ANOVA Table for Sarcoplasmic Protein Fraction STPP Band at 37kDa

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	7.52528376	1.88132094	74.41	0.0001
Error	5	0.12641817	0.02528363		
Corrected Total	9	7.65170193			

ANOVA Table for Myofibrillar Protein Fraction at 20 kDa for Hon

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.33090074	0.11030025	3.82	0.1141
Error	4	0.11546509	0.02886627		
Corrected Total	7	0.44636583			

ANOVA Table for Sarcoplasmic Protein Fraction at 20kDa for Hon

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.05920408	0.01973469	0.51	0.6991
Error	4	0.15616129	0.03904032		
Corrected Total	7	0.21536538			

ANOVA Table for Myofibrillar Protein Fraction at 37kDa for Hon

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.88342965	0.29447655	11.64	0.0191
Error	4	0.10120371	0.02530093		
Corrected Total	7	0.98463336			

ANOVA Table for Sarcoplasmic Protein Fraction at 37kDa for Hon

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.37395328	0.12465109	0.59	0.6547
Error	4	0.84870914	0.21217729		
Corrected Total	7	1.22266242			

ANOVA Table for Myofibrillar Protein Fraction at 20kDa for LA Fresh

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.32644802	0.10881601	3.82	0.1142
Error	4	0.11399226	0.02849807		
Corrected Total	7	0.44044028			

ANOVA Table for Sarcoplasmic Protein Fraction at 20kDa for LA Fresh

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1.77187675	0.59062558	12.00	0.0181
Error	4	0.19686528	0.04921632		
Corrected Total	7	1.96874203			

ANOVA Table for Myofibrillar Protein Fraction at 37kDa for LA Fresh

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	3.41539389	1.13846463	38.67	0.0021
Error	4	0.11776124	0.02944031		
Corrected Total	7	3.53315513			

ANOVA Table for Sarcoplasmic Protein Fraction at 37kDa for LA Fresh

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	9.02662916	3.00887639	83.12	0.0005
Error	4	0.14478856	0.03619714		
Corrected Total	7	9.17141772			

ANOVA Table for Myofibrillar Protein Fraction at 20 kDa for LA Frozen

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.03597482	0.01199161	6.25	0.0544
Error	4	0.00767082	0.00191770		
Corrected Total	7	0.04364564			

ANOVA Table for Sarcoplasmic Protein Fraction at 20kDa for LA Frozen

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.02921302	0.00973767	2.24	0.2255
Error	4	0.01736529	0.00434132		
Corrected Total	7	0.04657831			

ANOVA Table for Myofibrillar Protein Fraction at 37kDa for LA Frozen

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.10401588	0.03467196	1.52	0.3390
Error	4	0.09133290	0.02283323		
Corrected Total	7	0.19534878			

ANOVA Table for Sarcoplasmic Protein Fraction at 37kDa for LA Frozen

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.38249083	0.12749694	4.05	0.1050
Error	4	0.12591717	0.03147929		
Corrected Total	7	0.50840800			

ANOVA Table for Myofibrillar Protein Fraction at 20kDa for NC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.06814395	0.02271465	2.37	0.2120
Error	4	0.03839829	0.00959957		
Corrected Total	7	0.10654224			

ANOVA Table for Sarcoplasmic Protein Fraction at 20kDa for NC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.06945537	0.02315179	12.29	0.0174
Error	4	0.00753344	0.00188336		
Corrected Total	7	0.07698881			

ANOVA Table for Myofibrillar Protein Fraction at 37kDa for NC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.13945529	0.04648510	4.00	0.1070
Error	4	0.04650713	0.01162678		
Corrected Total	7	0.18596242			

ANOVA Table for Sarcoplasmic Protein Fraction at 37kDa for NC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.99520662	0.33173554	7.35	0.0419
Error	4	0.18054869	0.04513717		
Corrected Total	7	1.17575532			

ANOVA Table for Myofibrillar Protein Fraction at 20kDa for TX

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.37348250	0.12449417	9.33	0.0280
Error	4	0.05335534	0.01333884		
Corrected Total	7	0.42683785			

ANOVA Table for Sarcoplasmic Protein Fraction at 20kDa for TX

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.05377941	0.01792647	12.54	0.0168
Error	4	0.00571749	0.00142937		
Corrected Total	7	0.05949690			

ANOVA Table for Myofibrillar Protein Fraction at 37kDa for TX

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.33552893	0.11184298	6.13	0.0561
Error	4	0.07292500	0.01823125		
Corrected Total	7	0.40845393			

ANOVA Table for Sarcoplasmic Protein Fraction at 37kDa for TX

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.33552893	0.11184298	6.13	0.0561
Error	4	0.07292500	0.01823125		
Corrected Total	7	0.40845393			

ANOVA Table for Fiber Area by Origin

Hon

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	699.667495	233.222498	472.21	<.0001
Error	796	393.139150	0.493893		
Corrected Total	799	1092.806645			

LA Fresh

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1143.894211	381.298070	370.04	<.0001
Error	797	821.241819	1.030416		
Corrected Total	800	1965.136030			

LA Frozen

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	401.294711	133.764904	103.00	<.0001
Error	796	1033.746123	1.298676		
Corrected Total	799	1435.040834			

NC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	2149.898540	716.632847	580.94	<.0001
Error	795	980.687703	1.233569		
Corrected Total	798	3130.586242			

TX

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	17.1621258	5.7207086	35.80	<.0001
Error	800	127.8253933	0.1597817		
Corrected Total	803	144.9875191			

ANOVA Tables for Fiber Area by Treatment

Control

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	4307.849295	717.974882	1048.40	<.0001
Error	1391	952.601675	0.684832		
Corrected Total	1397	5260.450969			

SAPP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1268.338568	253.667714	320.97	<.0001
Error	1194	943.629158	0.790309		
Corrected Total	1199	2211.967726			

STPP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1528.882985	305.776597	468.84	<.0001
Error	1197	780.676098	0.652194		
Corrected Total	1202	2309.559083			

Soft Water

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	3188.789494	637.757899	851.83	<.0001
Error	1196	895.436632	0.748693		
Corrected Total	1201	4084.226126			

VITA

Matthew Cael was born in Kansas City, Missouri. He joined the Department of Food Science at Louisiana State University in Baton Rouge, Louisiana. He is a May 2010 graduate of Texas Tech University where he earned his Bachelor of Science in Food Science. He is an active member of the Institute of Food Technologists and the Research Chefs Association. Matthew is also the current President-Elect of the Institute of Food Technologists Student Association, and a member of the Institute of Food Technologists Board of Directors. He completed his Master of Science Degree at Louisiana State University in August of 2012.