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Utilizing Near-infrared Spectroscopy to Detect Sodium Tripolyphosphate in Treated Penaeus aztecus and setiferus Shrimp

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UTILIZING NEAR-INFRARED SPECTROSCOPY TO DETECT SODIUM TRIPOLYPHOSPHATE IN TREATED *PENAEUS AZTECUS* AND *SETIFERUS* SHRIMP

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Abstract

Applying sodium tripolyphosphate to shrimp may cause excess water absorption and has become a major concern to phosphate producers, the consumer and regulatory agencies, such as, the United States Food and Drug Administration. The objective of this study was to examine Near Infrared (NIR) spectroscopy as a nondestructive and rapid method of detecting shrimp treated with sodium tripolyphosphate solutions of varying concentrations and treatment times. Wild caught *Penaeus setiferus* and *aztecus* Louisiana gulf coast shrimp were submerged in distilled water, 2.5% sodium tripolyphosphate (STPP), and 5% sodium tripolyphosphate solutions for 30, 60, 120, 240, 480, 960, 1920 minute time intervals. The total moisture was determined and correlated with the water peaks at 5330 and 7180 cm^{-1} on the resulting NIR spectra. Mineral analyses performed by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) for total phosphorus was intended for comparison with the baseline spectra at 5241 cm^{-1} for the spectra-structure of P-OH; however, there was not an identifiable trend for phosphorus. Partial least square calibration methods were applied to the spectral analyses to develop prediction models based on the changes in moisture content associated with the sodium tripolyphosphate shrimp treatments. As long as the immersion time is known, the concentration of the sodium tripolyphosphate solution can be determined using the moisture content of the treated shrimp samples. The low standard errors of prediction and validation coupled with recent advances in chemometrics have rendered NIR spectroscopy a viable option for the detection of sodium tripolyphosphate in treated shrimp.

Chapter I

Introduction

Consumers became increasingly aware of the relationship between health and food in the 1980's and early 1990's. The popular diet trend was geared towards the reduction of fat intake and required a lean protein source (Freedman et al., 2001). Shrimp is a source of lean protein; therefore, the demand for shrimp has increased. The increase in shrimp consumption coincides with an increase in imports (National Marine Fisheries Services, 1982). The domestic fisheries had to compete with foreign aquaculture; in 2009, the U.S. imported 1.2 billion pounds of fresh and frozen shrimp worth 3.75 billion dollars (National Oceanic and Atmospheric Administration, 2010). The increase in imports have driven down the annual revenue of Louisiana shrimpers by approximately 40% in the last decade (Finn, 2009). The storage and distribution time increased, so the potential for muscle degradation and water loss became a major issue.

Shrimp are comprised mainly of water with a moisture content that ranges from 75.6 to 76.8 % for *Penaeus aztecus* and 76.2 to 81.4% for *Penaeus setiferus* (as cited by Sidwell et al., 1981). The sensory and organoleptic attributes of shrimp are most influenced by the moisture content. Therefore, most commercial shrimp producers focus on water retention during storage, processing, and distribution. One of the more common techniques utilized in the seafood industry is the application of condensed phosphates to help retain the initial moisture content.

An article in the Wall Street Journal highlighted the Louisiana shrimpers' discontent with the industry's overuse of sodium tripolyphosphate (Opdyke, 2009). An unnamed, local shrimp processor provided some samples to the seafood laboratory at Louisiana State University for evaluation and testing. The effects of shrimp treated with excess amounts of sodium

tripolyphosphate were readily apparent. The shrimp had a slippery texture, soapy appearance, and a moisture content of 89%. A sensory analysis of the cooked concluded the shrimp had a rubbery texture and off-flavors (metallic and bitter).

Section 402 (b)(4) of the Food, Drug, and Cosmetic Act states that if any substance has been added thereto or mixed or packed therewith so as to increase its bulk or weight, or reduce its quality or strength, or make it appear better or of greater value then it is adulterated. The FDA currently utilizes sensory analysis to detect adulteration in seafood. Shrimp treated with high levels of sodium tripolyphosphate have bitter metallic taste and a soapy appearance. Trained sensory analysts are able to detect the any off flavors, odors, and texture that may be present. Sensory analysis is time intensive. The sensory analyst and methods must be reliable, and have a record that can withstand the scrutiny of the legal system (FDA, 2010). The current method of detection does not satisfy industry needs for rapid assessment of quality parameters; therefore, the objective of this study was to utilize Near Infrared Spectroscopy to detect shrimp treated with sodium tripolyphosphate.

Chapter II

Literature Review

Penaeus aztecus and *setiferus*

Taxonomy and Species Description

The shrimp species *Penaeus setiferus* was first classified by Linnaeus in 1767. The species taxonomy for *Penaeus setiferus* and *aztecus* is as follows: kingdom, Animalia; phylum, Arthropoda; class, Malacostraca; order, Decapoda; family, Penaidae; and genus, Penaeus. Some of the more common names listed for *Penaeus setiferus* are white, gray, lake, and blue-tailed shrimp. *Penaeus aztecus*, brown shrimp, has been classified as a grooved and burrowing species (Muncy, 1984).

The two primary segments of the *P.setiferus and P. aztecus* body are the cephalothorax and pleon segment. The cephalothorax is comprised of the head, thorax, carapace, rostrum, stalked eyes, antennas, antennules, peropods, maxillipeds, and mandibles. The pleon segment includes tergum (upper abdomen), pleuron (lower abdomen), pleopods, uropods, and telson.

The carpace, dorsal section of the shell, has a medial carina, which is continuous with the anterior rostrum. The ridge stretches posteriorly two-thirds the length of the carapace. The rostrum is slim, drawn out, and curves slightly distally upwards between 5 and 11 sharp teeth located along the dorsal surface and two teeth are positioned on the ventral edge of the rostrum. The pleuron of the abdomen is carinate with the sixth segment narrowly grooved on both sides. The telson has a sharp tip and a medial length-wise groove. The body color of *P. setiferus* is blue and white with black speckles and pick sides. The wide spacing of the chromatophores

lends to the lighter body color of *Penaeus aztecus*. The tail uropodos are green along the edges, and the pleopods are marked with dark red (Williams, 1984).

P. aztecus are known to have antennae that extend beyond the length of the body. *Penaeus setiferus* commonly have very long antennae, which can grow to 2.5 to 3 times the body length. Williams (1955) described the carpace of *P. aztecus* as having a rounded and grooved medial carina on the anterior surface. The rostrum had an upward curvature with five to ten upper edge teeth. The walking legs were chelated and uropods of the distal segments are rounded. The telson was anteriorally grooved had a sharp tip. Some species have been reported red and green in appearance; however *P. aztecus* have chromatophores, which give the shrimp a brown to olive-green color.

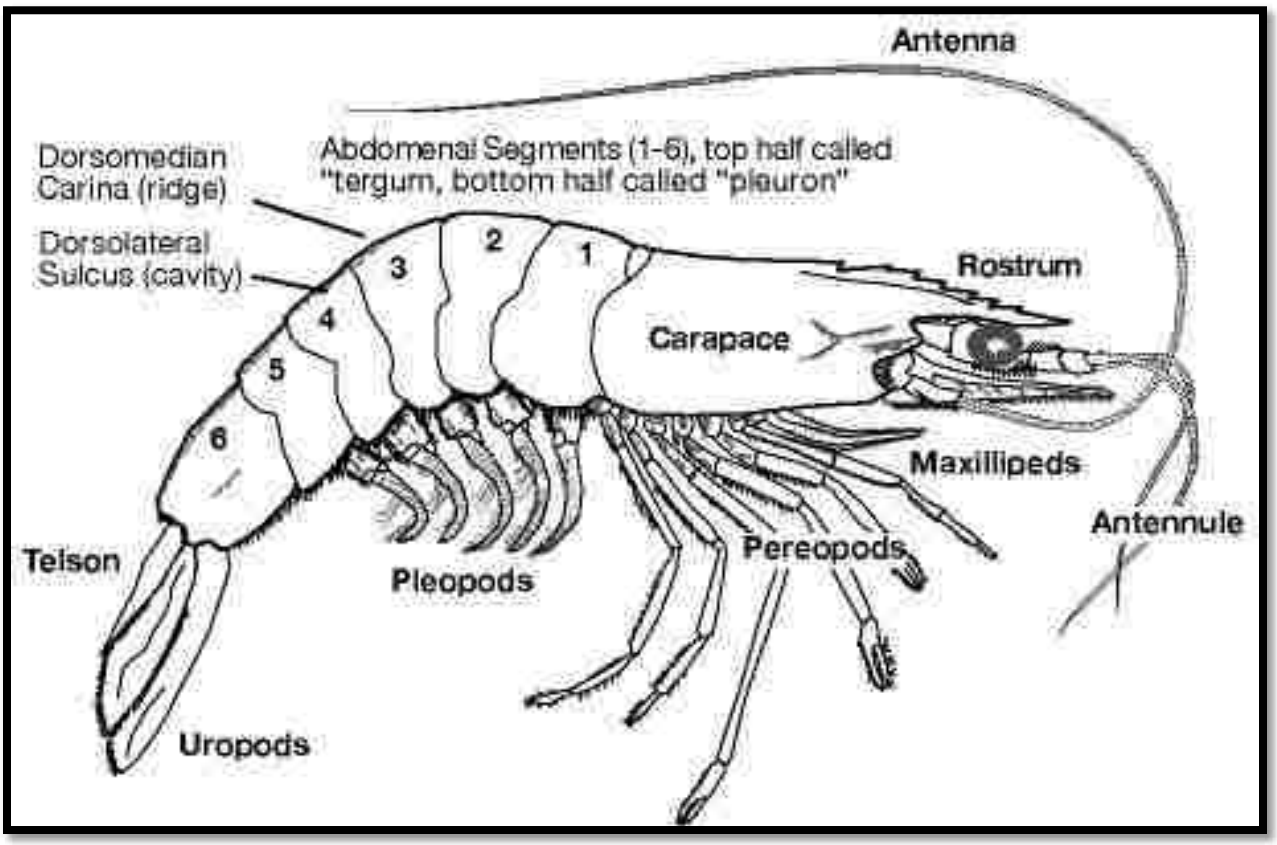

Figure 1 - A diagram of shrimp in the Penaeid family (sci.tamucc.edu, 2010).

Habitat

The habitat of *Penaeus setiferus* has been identified as estuaries and the inner littoral zone along the east coast from New York to Florida and the Gulf of Mexico. The most abundant populations of *Penaeus setiferus* in the Gulf of Mexico have been pinpointed in the brackish wetlands and shallow coastal areas. *Penaeus setiferus* burrow in the shallow, muddy substrata, but have been located at depths of 80 meters (Williams, 1984).

P. setiferus and *P. aztecus* have been classified as congeners; therefore, their habits are similar. Brown shrimp inhabit the estuaries and littoral zones along coasts from the intertidal zone to a depth of 110 m. *P. aztecus* prefer the muddy bottom areas at depths between 27 – 55 m. It is atypical of the brown shrimp to dwell at depths exceeding 165 m (Williams 1984).

Lifespan

Klima et al. (1982) determined the lifespan of white shrimp ranges from 27 months to four years, but most do not survive longer than a year. Female *P. setiferus* have reached a length of 200 mm, and their male counter can achieve a length of 182 mm (Williams, 1984). White shrimp have been classified as a shallow burrowing species (Anderson, 1966). Williams (1965) reported the spawning phase of *P. setiferus* occurred from March to September. Linder and Cook (1970) concluded fertilization takes place in the water column. The fertilized eggs hatched within 10 to 12 hours into planktonic nauplii larvae. Perez-Farfante (1969) categorized the ten day larval period into 5 naupliar, 3 protozoeal, 3 mysis, and 2 postlarval stages. The postlarval white shrimp were classified as juvenile with the appearance of 4 – 10 upper rostral teeth and 1 to 3 lower rostral teeth (Perez-Farfante, 1969). Muncy (1984) ascertained juvenile white shrimp

inhabited the estuaries during the fall and winter months, but their growth was impeded until the following spring.

William (1955) established male *P. aztecus* can grow to 195 mm in length, while the female brown shrimp can reach 236 mm. Wilson (1969) determined the growth of the male *P. aztecus* decreased with age. Larson et al. (1989) reported brown shrimp have an extended offshore spawning period at depths greater than 18 meters. The eggs of *P. aztecus* hatched in 24 hours into larve that undergo 5 naupliar, 3 protozoeal, and 3 mysis phases. After approximately 11 to 17 days of postlarval metamorphosis the brown shrimp entered the juvenile phase (Cook and Murphy, 1969). Copeland and Truitt (1966) reported *P. aztecus* postlarvae inhabited estuaries on flood tides and migrated to shallow, low-salinity waters. Williams (1955) determined the growth of brown shrimp is increased during the summer months as they migrate toward the more saline waters of the offshore regions.

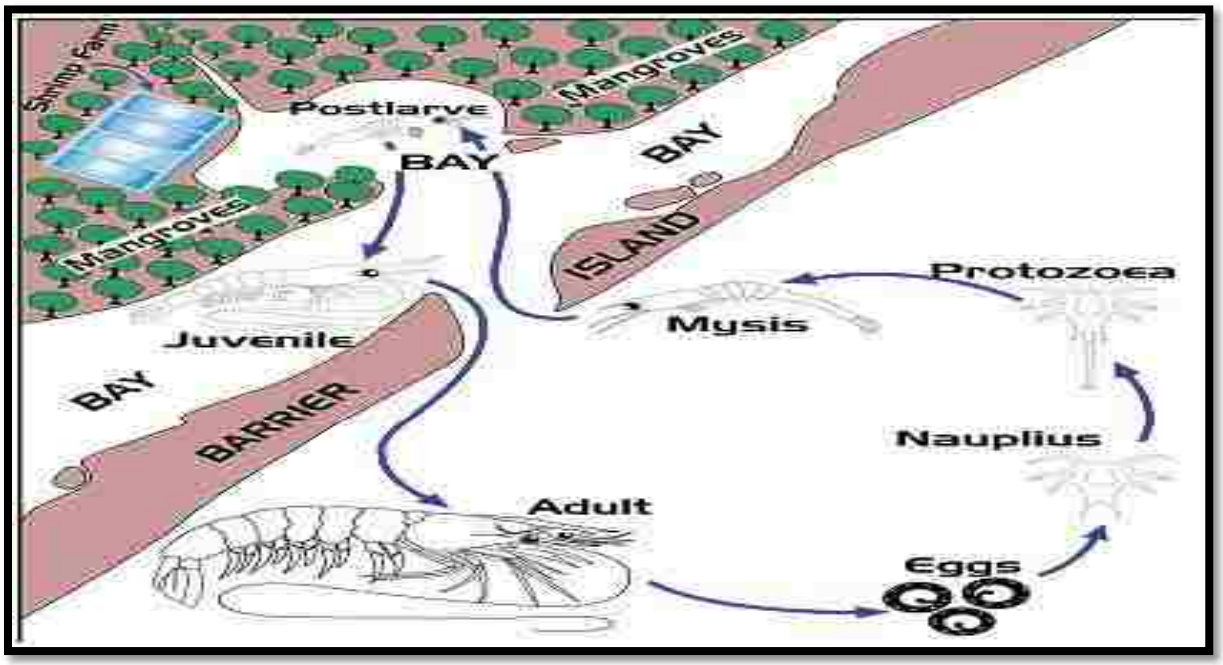

Figure 2 - A map of the Penaeid habit in the Louisiana Gulf Coast (Ozello Shrimper, 2010).

Louisiana Shrimp Industry Processing History

The shrimp industry in Louisiana began in 1867 with the opening of the first canning factory. Initially, consumers only purchased fresh shrimp; however, the introduction of canned shrimp shifted the focus of the Louisiana shrimp industry from local domestic to international markets. At the end of the Civil War, canning and drying plants were under construction and the Louisiana shrimp industry was increasing in scale. The market expansion of the early 1900's led to an increase in the shrimp production which was estimated to be six million pounds annually and valued at three hundred thousand dollars. All of the streams drained into the Barataria Bay of the Gulf of Mexico in southeastern Louisiana. The fishermen set up shrimping stations all along the Barataria Bay from October to April to capitalize on the vast concentration of shrimp (Becnel, 1962).

Seines comprised of a one-half inch wire mesh lined with lead on the lower edge and upper cork lines were employed to capture the shrimp. The size of the seines increased and was virtually impossible to handle with a small crew, so the shrimpers established seining companies to address the issue. The demand for shrimp was still greater than the supply, so the Louisiana shrimp industry initiated the use of the trawls, fishing nets, in 1915. The trawls were large fishing nets attached to the boat's stern that captured shrimp, as they were raked along the ocean floor. Shrimp production increased because trawling extended the industry from seasonal to a year-round operation, expanded the shrimping grounds, and reduced manual labor. The industry continued to flourish over the next two decades with the introduction of gasoline operated shrimping boats.

The two forms of processed shrimp were dried and canned, until the 1930's. When spoilage problems became an issue on board the boats, the shrimpers were supplied with ice, water, fuel, and supplies by ice boats, but this was an expensive operation. In order to reduce spoilage and reduce the shipping rate, the processors began de-heading the shrimp in 1934. The shrimp head comprises 35 to 40 percent of the total body mass; therefore, removal reduced the amount of storage space required. The fishermen noticed that head-on shrimp spoiled faster than their de-headed counterparts. Over 80 percent of the spoilage bacteria are located in the heads of shrimp, which coincides with the observations of the fishermen (Perkins, 1995). A new domestic market was formed, and increased demand led to soaring profits. Headless shrimp were packed in ice and refrigerated transport permitted distribution across the United States (Anonymous, 1937).

In 1938, canning was the most prevalent form of processed shrimp; however, the value was on the decline and freezing was emerging as a method of preservation. After 1944, the industry shifted from canning to freezing. By 1946, headless shrimp had more value for the Louisiana shrimp industry than canned, dried, or frozen. In the mid 1950's, the demands of the frozen shrimp led to the development of peelers, deveiners, and graders in shrimp processing plants. After 1954, market decline and an increase in imports triggered the foundation of shrimp associations to protect the Louisiana shrimp industry. In the years that followed, the Louisiana shrimp industry is still thriving, but new issues have arisen that threaten the domestic market (Becnel, 1962).

Modern Day Louisiana Shrimp Processing

Shrimp are held in cold storage (4 °C) during harvesting, processing, and distribution. The edible portion of shrimp is comprised mainly of water and protein, which are extremely important to the organoleptic attributes of shellfish. Peeling machines used by shrimp processors use water and pressure to remove the heads and shells. The yield is low ranging from 45 to 50% of the initial shrimp weight. The mechanical peeling can damage the tissue and cause leaching of moisture. The peeled shrimp are then packed in five pound boxes and blast frozen.

During blast freezing, rapid circulation of air is forced around a product to result in moderately fast freezing. The goal of rapid freezing is to produce small ice crystals and minimize the movement of water from the muscle cells. Temperatures should be quickly reduced to between −2 and −7 °C (28 and 20 °F), which is the optimum range for maximum ice crystal formation in the cells of the flesh. This temperature range represents the zone of maximum ice crystal formation in the cells of the flesh. Rapid freezing results in small crystals, minimum dislocation of water, normal appearance of cells in the frozen state and superior quality. During slow freezing large ice crystals form, which rupture the cell membranes and allow water to migrate out of the cells resulting is a product of lower quality (Jeremiah, 1996). When slow-frozen flesh is thawed, the ruptured cells release water (thaw-drip) and many compounds that provide certain flavor characteristics. A major concern with cold storage is drip loss, which can range from 10% in peeled and deveined to 3% in cooked shrimp (Demann and Melnychyn, 1971).

Some of the factors that affect the quality of a product after freezing are microbial growth, storage time, and temperature fluctuations. Freezing does not sterilize foods or destroy

the organisms that cause spoilage but instead slows growth rate by restricting water availability. Over time the water sublimates from the surface of the product into the surrounding atmosphere, ice crystals form on the surface resulting in freezer burn. Most consumers are aware of the color, texture, and weight deficiencies associated with freezer burn in products. The undesirable changes that a frozen product undergoes during storage can be precluded with the selection and application of a combination proper freezing technique and food ingredients, such as, cryoprotectants (Jeremiah, 1996). Temperature fluctuations cause some melting and subsequent re-freezing. The ice crystals that are formed during the re-freezing process are larger and can potentially damage the cells resulting in a decline in the quality (Archer et al.,1998).

Degradation of Shrimp Muscle during Frozen Storage

The freeze-thaw process disrupts cells, denatures proteins, and results in damage to the muscle structure of shrimp (Sriket et al., 2007). When the muscle cells are opened, mitochondrial enzymes are discharged into the sarcoplasm (Hamm, 1979). The volume of thaw exudates was correlated with the decrease in the water-holding capacity of the shrimp muscle, which affected the structure of shrimp muscle. The sulfhydryl content of the shrimp decreased with the increase of freeze-thaw sequences and formation of disulfide bonds. The conformational changes and denaturation of myosin led to the boost in disulfide bonds formed as the result of oxidation of sulfhydryl groups. The hydrophobic groups were normally bound within the molecule are released due to irreversible denaturation; therefore, the surface hydrophocity increased during freezing and thawing (Nakai and Li-Chan, 1988). Protein solubility decreased during the freeze-thaw process in agreement with the increased surface hydrophobicity. An analysis of the microstructure of the shrimp subjected to freeze-thaw cycles depicted shrinkage of muscle fibers, loss of Z-disks, and separation of muscle bundles due to

protein denaturation and disruption of the endomysium (Sriket et al., 2007). Srinivasan et al. (1997) established a link between the freeze-thaw process and cook loss. Kye and others (1988) published data that related myofibrillar protein degradation to textural changes in fresh water prawn, *Macrobrachium rosenbergii*.

Sodium Tripolyphosphate

History of Condensed Phosphates

Berzelius (1816) ignited phosphoric acid and produced sodium pyrophosphate capable of coagulating a solution of albumen. In 1828, Clark expounded upon the work of Berzelius and generated sodium pyrophosphate; however, with the addition of silver nitrate the color of the product changed from yellow to white. Graham (1833) classified all phosphates into orthophosphates, pyrophosphates, and metaphosphate groups. In 1845, Fleitmann and Henneberg prepared polyphosphoric acids by extracting water from phosphoric acid. Kroll (1912) was the first scientist to research the ultraphosphate region, which lies between the pure phosphorus pentoxide and metaphosphate. The generic titles, "condensed" and "molecularly dehydrated", were assigned to pyro-, meta-, and poly- phosphates comprised of more orthophosphoric acid than water. Due to a lack in analytical methods, it was virtually impossible to distinguish between condensed phosphates until the introduction of quantum mechanics in 1925 (Van Wazer, 1950).

Phosphate Structure

Van Wazer et al. (1955) defined phosphates as compounds comprised of phosphorus anions bordered by four oxygen atoms with a tetrahedron structure. Van Wazer (1958) divided the structure of phosphate into five groups. The "branching point" was described as the PO_4

group where three oxygen atoms are shared with the adjacent phosphate groups. The "middle group" was defined as the PO_4 group with two shared oxygen atoms and one negative charge or ester bond. The "end group" was depicted as the phosphate group with one shared oxygen and two negative charges or ester linkages. The ortho- or monophosphate group was characterized as the PO_4 group with three negative charges or ester bonds. The final phosphate building block was a "four-way branching point" with either an anionic or neutral structure. The combination of end groups, middle groups, and branching points yielded the structure of sodium tripolyphosphate.

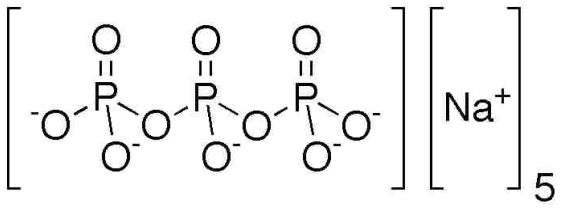

Figure 3 - The structure of sodium tripolyphosphate.

Sodium Tripolyphosphate Preparation

In 1895, Schwartz prepared pure crystalline pentasodium tripolyphosphate by melting the combination of tetrasodium pyrophosphate and sodium metaphosphate. The solution was slowly cooled to form the crystalline salt. Sodium tripolyphosphate is a white crystalline salt with the chemical formula $Na_5P_3O_{10}$. Partridge et al. (1941) identified two anhydrous crystalline forms of sodium tripolyphosphate. The first form, Phase I, was described as a high temperature variety, and the second, Phase II, was designated a low temperature form. Approximately 5 moles of Na_2O and three moles of P_2O_5 were the starting materials used to prepare both forms STPP.

Some manufacturers have been known to add an excess of Na_2O to prevent turbidity in the final product. The second form of STPP was prepared by heating an orthophosphate mixture to a final temperature between 350-400 ˚C. When the orthophosphate mixture was heated to a final temperature ranging from 450 to 615 ˚C and cooled, Form II transformed into Form I (Van Wazer, 1958). Richard (2007) reported sodium tripolyphosphate may contain a maximum of 15% mixture of ortho-, meta-, and pyrophosphates.

Properties of Sodium Tripolyphosphate

Powdered, granular, and food were the established grades for sodium tripolyphosphate (Richard, Sr., 2007). Lampila and Godber (2001) reported sodium tripolyphosphate has a pH value of 9.8 and solubility of 15 g in 100 grams of water at 20 °C. The hygroscopic nature of sodium tripolyphosphate was noted in the Chemical Abstract Service (CAS) Database (2010) as well as the melting point of 622 °C and molecular weight of 367.86 g/mole. Sodium tripolyphosphate was deemed incompatible with strong oxidizing agents and strong acids and slightly corrosive in the presence of copper, steel, aluminum and zinc (CAS, 2010). Sodium tripolyphosphate was classified as moderately toxic when orally ingested (3900 mg/kg, rat), subcutaneously applied (4640 mg/kg, rabbit), and intraperitoneally injected; however, sodium tripolyphosphate is poisonous when introduced intravenously. When sodium tripolyphosphate is heated to a temperature of decomposition (> 622 °C), it emits toxic PO_x and Na_2O fumes (CAS, 2010).

Sodium Tripolyphosphate Laws and Regulations

There were several notices and proposed regulations published in the Federal Register on July 26, 1973, which resulted in the launching of an evaluation into the safety of sodium

tripolyphosphate in agreement with title 21 of the Code of Federal Regulations (CFR) 170.35 to affirm the generally recognized as safe, GRAS, status (FDA, 2000). The chemical toxicity, occupational hazards, metabolism, reaction products, degradation products, carcinogenicity, dose response, reproductive effects, histology, embryology, behavioral effects, detection, and processing of sodium tripolyphosphate were evaluated by the Select Committee on GRAS Status [(SCOGS), (FDA, 1975)] . The Food and Drug Administration proposed affirmation of the generally recognized as safe (GRAS) status of sodium tripolyphosphate as a multiple purpose food ingredient in 21 CFR182.1810 (FDA, 2010). Sodium tripolyphosphate was presumed approved for use as a sequestrant with good manufacturing practices in 21 CFR 182.681(FDA, 2010). In 1959, the Federal Register approved sodium tripolyphosphate as GRAS for use as nutrient and dietary supplements. The FDA (1961) published a regulation (26 FR 5224) in the Federal Register that granted sodium tripolyphosphate as a GRAS status for substances that migrate from food to cotton in packaging materials. Sodium tripolyphosphate was approved for use as a diluent in Citrus Red aqueous solutions (21 CFR 74.302), a starch modifier (21 CFR 172.892), a boiler water additive (21 CFR 173.310), and a meat preparation agent (9 CFR 318.7) (eCFR, 2010). Part 182 of the CFR was amended as a result of the investigation into the GRAS status of sodium tripolyphosphate in accordance with the Federal Food, Drug, and Cosmetic Act (sections 201(s), 409, and 701(a)); however, the amendments were never affirmed. Sodium tripolyphosphate was removed from the list of substances that migrate from food into cotton in 21 CFR 182.70 and 182.90 (eCFR, 2010). Part 184 was amended to include the following stipulations on the use of sodium tripolyphosphate: the ingredient must meet the standards of the Food Chemical Codex and used in accordance with good manufacturing practices in 21 CFR184.1(b)(1) (eCFR, 2010). The amendment also proposed the maximum level cannot

exceed 0.5% of the fish products (§170.3(n)(13)), 0.7% for gelatin and puddings (§170.3(n)(22)), 0.5% for seasonings and flavorings (§170.3(n)), 0.6% for meat products (§170.3(n)(29)), and 0.5% for poultry products (§170.3(n)(34)). Sodium tripolyphosphate can be used as an antioxidant (§170.3(o)(3)), flavor enhancer (§170.3(11)), curing agent (§170.3(o)(5)), humectant (§170.3(o)(16)), pH control agent (§170.3(o)(23)), stabilizer and thickener (§170.3(o)(26)), and texturizer (§170.3(o)(32)) (FR 44, 1979). In meat food products, which will be cooked or frozen after processing, it can be used alone or in combination with sodium metaphosphate and sodium polyphosphate at a level not to exceed 0.5% of total product (Smith and Hong, 2003).

Patent History of Sodium Tripolyphosphate in the Seafood Industry

There are a vast number of patents regarding the use of polyphosphates and seafood. The following patents are highlighted because of the intricate role they played in the development of sodium tripolyphosphate dipping solutions used in the prevention of drip loss and retention of the natural organoleptic quality of shrimp. In 1946, an innovative technique designed to improve the appearance, palatability, and moisture retention during the preparation and processing of "humanly consumable cooked shrimp" was patented. The overall goal was to improve the cook yields from around 50 to 67% by dipping raw shrimp into an aqueous solution of 2% dibasic sodium phosphate (DSP) by weight for two hours before boiling in a brine solution or 0.5 to 2.5% aqueous solution by weight of alkaline salt comprised of alkali metal and ammonium dibasic (DAP) and tribasic phosphate (TSP), metaphosphate, pyrophosphate, tartrate, carbonate, and hydroxide just long enough for the shrimp proteins to exchange ionic bases with the alkali metal salt (Garnatz et al., 1949). Subsequently, Ekkehard and McFee (1949) created a treatment to combat the formation of struvite, transparent magnesium ammonium phosphate crystals, during canning. To suppress the formation of struvite crystals water soluble glassy phosphates

were added to the canned fish and shellfish as a 0.25 to 1.5% by weight solution based on the total moisture content.

Meyer (1956) invented a polymeric phosphate treatment for fish to improve the taste, stability, digestibility, and color. The patent protected the use of compounds of polymeric phosphoric acid, water-soluble alkali-metal and ammonium salts of the pyro-, meta-, and other phosphoric acids, for example tripolyphoshphoric acid. Kurrol's, alkaline earth, and the heavy metal salts of the polymeric phosphoric acid were also patent protected. The research concluded that the phosphates were effective when used either alone or in conjunction with one of the salts of the palatable acids (Meyer, 1956). Albright and Wilson, Ltd. (1961) patented a fish treatment comprised of an aqueous solution of sodium or potassium salts of polyphosphoric acid. This treatment was able to reduce the drip loss for frozen fish. The sodium or potassium salts of polyphosphoric acid also helped to retain moisture during cooking when the fish were treated with 10 to 15% solutions of the polyphosphates.

In 1962, Mahon patented a method for the preservation of fish to inhibit the loss of moisture, soluble proteins, minerals, and vitamins during thawing and cooking. Haddock fillets were dipped in a solution consisting of sodium and potassium salts of polyphosphates with a molar ratio of H_2O to P_2O_5 ranging from 1:1 to 2:1. Falci and Scott (1979) devised a method to preserve the natural color and texture of whole, peeled, and deveined shrimp by soaking in an aqueous solution of at least one phosphate salt in the presence of calcium and/or a magnesium salt, an application which was subsequently abandoned. Stone (1981) conceived an invention designed to store fresh shrimp with approximately 1 to 2% by weight sodium tripolyphosphate treated crushed ice to reduce moisture and nutrient loss.

Penetration Mechanism and Distribution Gradients of Sodium Tripolyphosphate

Love and Abel (1966) were the first to detect the formation of a film on the surface of fillets treated with sodium tripolyphosphate. Nikkila et al. (1967) used a tracer technique to detect changes in fish proteins stored in saline solutions and their inhibition by phosphates. The researchers reported the tracer migrated into the fillets quickly; however, after the first day transfer from the solution to the fillets began to slow-down. The remainder of the tracer in solution took up to five days to migrate into the fillets. The migration of the phosphate was independent of the sodium chloride in solution. Scheurer (1968) designed a study to ascertain the penetration gradients of sodium nitrite and sodium tripolyphosphate in haddock fillets. The experiment employed a radioactive tracer to measure the absorption and distribution of the salts. The concentration of sodium tripolyphosphate was greatest at the surface. The concentration at the surface increased with the length of dip time, but the concentration at the center of the fillets remained low. The total concentration of sodium tripolyphosphate of Haddock fillets dipped in a 12% (wt. %) solution for 10 seconds and 10 minutes were 0.21% and 0.28% respectively. Kang and Park (1975) demonstrated that besides chain length, phosphate binding was directly dependent on phosphate concentration. The researchers dipped Alaska Pollock fillets in five or ten percent aqueous solutions of STPP/SPG (1:1, w/w) for one or five minutes, and regardless of immersion time, 101 mg phosphate per gram muscle, measured as P_2O_5, was absorbed by fillets dipped in 10% phosphate solutions.

Tenhet et al. (1981) experimented with P^{32} labeled sodium tripolyphosphate to determine the effects of dip time and concentration on the penetration and distribution gradients of STPP in peeled and deveined shrimp muscle. The concentration of sodium tripolyphosphate at the surface of the shrimp was proportional to the dip time. A surface-to-center phosphate

penetration gradient was formed when the concentration of the dipping solution was low regardless of treatment time. However, the dipping solution with the highest concentration of sodium tripolyphosphate (10%) penetrated evenly throughout the shrimp muscle. Unal et al. (2004) designed an experiment to explore the experimental theory, fundamentals and mathematical evaluation of phosphate diffusion in meat. Beef samples were dipped in sodium tripolyphosphate solutions with concentrations that varied from 0% to 6% (weight/volume) for 90 minutes. The treated meat samples and dipping solutions were analyzed with a modified spectrophotometric ammonium molybdate method. The data reported a counter-current diffusion of orthophosphates and sodium tripolyphosphate in the dipping solutions and beef samples. The water-soluble proteins and sodium tripolyphosphate interacted to form a film. The results proved the diffusion of phosphates can be measured by evaluating the changes in phosphate concentration to establish a diffusion coefficient. Initially, the orthophosphates from the beef samples diffused into solution, but after the barrier was formed the orthophosphate diffusion decreased. The phosphate concentration of the beef increased because of the sodium tripolyphosphate into the meat samples. The study by Unal et al. (2006) was extended and *Longismus dorsi* beef muscle was dipped in 0 (control), 2%, 4%, and 6% (weight/volume) sodium tripolyphosphate solutions for 30 minutes at different temperatures ranging from 18 to 36±2 °C to determine the effect of temperature on phosphate diffusion. The phosphate concentration was derived using a modified spectrophotometric method for both the beef muscle and sodium tripolyphosphate solutions because of the naturally occuring orthophosphates in the beef, which resulted in a counter-current diffusion of phosphates between the meat and dipping solution. The orthophosphates diffused from the meat samples to the solution faster than sodium tripolyphosphate into the muscle until a barrier film of proteins formed at the surface.

Temperature did affect the diffusion rate of sodium tripolyphosphate into the meat. At low temperatures the barrier film formed at a slower rate allowing more penetration of STPP into the muscle, but the barrier films formed rapidly at high temperatures. The researchers were successful in defining the fundamentals of diffusion for meat samples dipped in sodium tripolyphosphate.

Water-binding Capacity of Phosphates

Tanikawa et al.(1963) determined that a combination of trisodium polyphosphate and sodium tripolyphosphate diminished the thaw drip loss, decreased the concentration of deoxyribonucleic acid phosphorus in the exudates, and enhanced the ability of the flesh protein to reabsorb liquids for treated cod fillets. A study performed by Mahon and Schneider (1964) reported that Haddock fillets dipped in polyphosphate and NaCl solutions of varying concentrations did not diminish thaw drip until the pH was alkaline. Dover sole, Pacific cod, halibut, red snapper, and Chinook salmon were treated with sodium tripolyphosphate before freezing and a decrease in drip loss was observed (Boyd and Southcott, 1965). An investigation into enhancing the quality of frozen fish treated with sodium tripolyphosphate determined a reduction in drip thaw loss and improved water holding. The study also concluded water retention and time of dip and solution concentration were directly proportional. The researchers observed the samples with superior water retention had longer dip time intervals and dipping solutions of higher sodium tripolyphosphate concentrations (Sutton and Ogilvie, 1968). The study was extended to quantify the effects of sodium tripolyphosphate dipping solutions on fish muscle during storage. Sodium and phosphate ions were absorbed by the cod muscle and were retained during freezing, storage and thawing. When diluted polyphosphate dipping solutions were utilized, the phosphorus diffused from the cod muscle into the solution (Sutton and Ogilvie,

1968). Halibut, silver salmon, and black cod fillets dipped in sodium tripolyphosphate and sodium chloride solutions before hot smoking had a higher yield than untreated samples. The investigators noted the synergistic effects of salt and sodium tripolyphosphate on water retention (Barnett et al., 1969). The main sites of water holding in shrimp muscle were the myofibrillar proteins. The sodium tripolyphosphate molecule interacted with the positively charged groups of the shrimp protein, and the remainder of the phosphate molecule bound water molecules. The addition of sodium tripolyphosphate caused the shrimp protein's myofilamental lattices to stretch. The distention was the result of an increase in the charge repulsions of the myofilaments and removal of transverse myofibrillar proteins. The expansion allowed for physical entrapment of water and an increase in the water holding capacity of treated meat (Xiong, 2005).

Sodium Tripolyphosphate Degradation

Van Wazer and Holst (1950) stated," In any environment in which reactions involving degradation of condensed phosphates are possible, it is to be expected that assemblies in which three of the four oxygens of PO_4 tetrahedra will be exceedingly unstable and will degrade more rapidly as compared to those in which one or two oxygen atoms are shared. Such an environment is found for example, in aqueous solutions." In 1956, the Antibranching Rule founded on X-ray and titration results was established, which characterized the instability of PO_4 branching points.

Shen and Dyroff (1966) evaluated the degradation of sodium tripolyphosphate in solution in the presence of ions. The degradation followed first order kinetics and the reactions were defined for dilute sodium tripolyphosphate solutions in Equations 1-6:

(1) $P_3O_{10}^{-5} + H_2O \rightarrow HP_2O_7^{-3} + HPO_4^{-2}$

(2) $P_3O_{10}^{-5} + H_2O \rightarrow P_3O_{10}^{-5} \cdot H_2O$ (rate controlling)

(3) $P_3O_{10}^{-5} \cdot H_2O \rightarrow (HP_2O_7^{-3}) + HPO_4^{-2}$

(4) $(HP_2O_7^{-3}) \rightarrow HP_2O_7^{-3}$

(5) $(HP_2O_7^{-3}) + H_2O \rightarrow HPO_4^{-2} + HPO_4^{-2}$

(6) $2(P_3O_{10}^{-5} \cdot H_2O) \rightarrow H_2O + 2\ HP_2O_7^{-3} + P_2O_7^{-4}$

The molar ratio of pyro- to orthophosphate was faintly less than one; however, the ratio increased with the phosphate concentration. This was an improvement upon the one to one ratio of ortho- to pyrophosphate for hydrolyzed sodium tripolyphosphate established by Bell (1947). Pyrophosphate degraded to orthophosphate at a rate less than 2% in 10,000 minutes at 70 ˚C and a pH 11 with 35% solids. An activated complex of sodium tripolyphosphate was formed to break the P-OP bond in the anion (Equation 2).

Analytical Methods of Sodium Tripolyphosphate Detection

In 1987, Sturno employed ion chromatography to detect tripolyphosphate and the orthophosphate and pyrophosphate residuals in treated shrimp. The method was able to detect 35% of the sodium tripolyphosphate in the shrimp after eight weeks of frozen storage. Ravelo et al. (1991) used percent moisture to percent protein ratios to identify sodium tripolyphosphate treated shrimp based upon the natural phosphate content of 39 to 397 mg/100g of shrimp defined by Sidwell (1981) and Sullivan and Otwell (1992). The study reported the percent moisture to percent protein ratio was directly related to phosphate treatment, but the concentration of the sodium tripolyphosphate treatment solution could not be quantified. The ratio of percent moisture to phosphorus and sodium tripolyphosphate treatment was inversely related.

Near-infrared Spectroscopy

Description

Near-infrared (NIR) spectroscopy measures the combination and overtone bands that are related to absorption frequencies between 12000 – 4000 cm^{-1}. The combination and overtone bands correspond to the frequencies of vibrations between the bonds of the atoms making up the material under evaluation. These bands are primarily due to stretches of O-H, C-H, and N-H bonds. Every compound is comprised of a unique combination of atoms, which results in a distinctive near-infrared spectrum. Therefore, near-infrared spectroscopy can be used for identification in a qualitative analysis. The size of the peaks in the spectrum can be correlated with the concentration in a quantitative analysis (Workman and Weyer, 2008). Near-Infrared spectroscopy has been identified as a pattern recognition technology. NIR spectroscopy can rapidly recognize spectral patterns for chemical and physical composition of a sample and store the data in a library (Kradjel, 1991).

Overtones and Combination Bands

Overtones were defined as the replications of the Mid Infrared absorption bands. The 1^{st} and 2^{nd} overtones were mathematically derived by dividing the fundamental frequency by a factor of either 2 or 3 respectively. The overtones had a reduced intensity when compared with their fundamental bands. The relative intensity of the 1^{st} and 2^{nd} overtones was, respectively, 1 and 2 orders of magnitude beneath their fundamental absorbance band counter-parts. The overtone bands of water are intense and absorb in the 1400-1500 nm and 1900-2000 nm regions of the spectrum (Workman and Weyer, 2008). The changes associated with stretching and bending vibrations of fundamental absorbances were designated combination bands. Kradjel

(1991) determined molecular symmetry greatly influences the individuality of combination bands.

NIR Data Pretreatments

Data pretreatments counteract the deviations from a linear relationship between spectral signals and analyte concentrations, which can be the result of light scatter, interference, or molecular interactions. The most frequently used data pretreatments are normalization, derivatives (1^{st} and 2^{nd}), multiplicative scatter correction (MSC), Kubelka-Munk (KM), and absorbance. The most commonly used pretreatment combinations are normalization and 1^{st} derivative; MSC and 1^{st} derivative; and subtract DC and 1^{st} derivative (Workman and Weyer, 2008).

Kubelka-Munk

The most commonly referred to theory to rationalize isotropic light scattering is Kubelka-Munk, which was based on the absorption (K) and scattering (S) coefficients.

Kubelka-Munk utilized the following formula:

$K/S= (1-R_\infty)^2/(2- R_\infty)$,

where the reflectance term defined as R_∞ to correlate the K and S with the absolute reflectance of an infinitely thick layer. The near-infrared spectrophotometer detector logarithmically amplified the signal. The data was in the form log R/R', where R is the reference reflectance and R' is the sample reflectance. The reference does not change; therefore, R is a constant. The log of the inverse sample reflectance (1/R') must be calibrated against the results of a proximal analysis of samples utilizing approved methods before performing the NIR analysis (Osborne, 1981).

Initially, the Kubelka-Munk theory was used to evaluate paint films, but the application potential was infinite. Law and Thachuk (1977) validated the Kubelka-Munk theory in an experiment that measured the moisture content of wheat by NIR diffuse reflectance spectrophotometry. The Kubelka-Munk values were derived from 20 spectral wavelengths between 1.12 and 2.49 μ. The particle size was smaller than the total thickness and uniformly distributed. Geladi et al. (1985), evaluated linearization and scatter-correction for Near-Infrared reflectance spectra of meat. The partial least squares regression multivariate calibration method was utilized to predict fat in meat. The light scatter distortions were linearized and corrected using KM transformations and the inverse in combination with multiplicative scatter correction. The scattering coefficient of Kubelka-Munk is related to the size of the sample particles (Davies and Grant, 1987).

Partial Least Squares Regression

Partial least squares (PLS) regression has been applied as a prediction tool for models with multiple colinear factors. The PLS method was originated by Herman Wold in the late 1960's for econometrics studies (Geladi & Kowalski, 1986). The PLS method was first used to analyze chemical compounds in the late nineteen seventies (Geladi & Kowalski, 1986). Partial least squares regression has been characterized as an extension of principal component and canonical variables (Dijkstra, 1983). PLS is an extension of principal component and canonical variables (Dijkstra, 1983).

Blanco et al. established the primary sources of error in near infrared quantitative calibration modeling are non-linearity due to variations in sample particle size or the relationship between absorbance-concentration (Blanco et al., 1999). Trygg reported PLS regression models the relationship between the predictors, X, and responses, Y (Trygg & Wold, 1998). The X and

Y scores were selected to establish the strong relationship amongst the pairs altering the response toward accurate predictions (Geladi & Kowalski, 1986). Rambla et al. (1997) used PLS-NIR to derive the total sugar, glucose, fructose, and sucrose in fruit juice. The study concluded PLS applied to the first order derivative can accurately and precisely measure the total sugar concentration in samples where the primary carbohydrates are glucose, fructose, and sucrose. Dupuy et al. (1992) determined PLS regression has no limitations on the number of selected wavelengths that can be used for calibration.

Advantages of NIR

The advantages of NIR include but are not limited to non-destructive, rapid analysis in line to alter formulation during production, and ease of operation. NIR spectroscopy requires little to no sample preparation and has the ability to analyze a sample through glass and packaging materials; therefore, costs and analysis time are decreased. The estimated time to obtain a spectrum from a routine sample varies from one to ten minutes depending on the type of instrument and the resolution required. This is considerably less time than the antiquated techniques used for analysis like the previously mention evaluation of percent moisture to percent protein ratios to determine the sodium tripolyphosphate concentration of treated shrimp. NIR spectrometers are mechanically simpler than traditional instruments; thus, there is minimal chance of user error. NIR spectroscopy can determine the physical and chemical composition of a material and generate complete spectral patterns in seconds, which are archived in a database. Near Infrared analysis is also utilized as a pattern recognition tool. NIR spectroscopy offers a practical alternative to time-consuming wet-chemical methods and liquid chromatographic techniques. The use of toxic, corrosive, or expensive chemicals is eliminated; thus, reducing the costs associated with acquisition and disposal. Large or multi-component samples can be

analyzed, which also decreases cost. NIR spectroscopy is a green science, which is extremely important to the emerging environmental conscious population.

Disadvantages of NIR

The primary disadvantages of NIR technology are expensive equipment, calibration, and data analysis. The majority of the disadvantages associated with NIR spectroscopy have been overcome by recent advances in modern technology. For example, the initial cost of the equipment can be negated with the analysis of large or multi-component samples, which decreases cost. The NIR equipment must be calibrated before each use, but it is not a time intensive process. The accuracy and precision of the results obtained from NIR are directly to laboratory procedures. For a routine analysis under favorable conditions, the accuracy of NIR spectroscopy is greater than ± 5%. Modern software algorithms and statistical treatments (chemometrics) have virtually eliminated the problems with data analysis and rendered NIR spectroscopy an excellent tool for qualitative and quantitative analysis. The accuracy and precision of the results obtained from NIR are directly related to laboratory procedures. The method of data analysis must be carefully selected, and the NIR results should be compared with wet chemistry on a regular basis. Another disadvantage of NIR technology has a sensitivity limit of approximately 0.1%; therefore, the detection of minor constituents is restricted (Iwamoto and Kawano, 1992).

Applications of NIR in Assessing Food Quality

Near infrared spectroscopy is a reliable, rapid, and non-destructive assessment tool capable of evaluating food quality. The first application of NIR for food analysis was conducted by Norris (1964) to measure the moisture in grain. Near infrared spectroscopy has been applied

in the beverage, dairy, meat industries. Paradkar et al. (2002) utilized NIR to classify adulterants in maple syrup. Partial least squares (PLS) and principal component regression (PCR) were applied to the quantitative analysis, which resulted in a correlation coefficient greater than 0.93. Near-infrared spectroscopy was used to detect adulteration in hamburger meat in a study conducted by Ding and Xu (2000). The adulterants, 5−25% mutton, pork, skim milk powder, and wheat flour, were detected with accuracy up to 92.7%, as the adulteration levels increased the accuracy improved. Laporte and Paquin (1999) determined the fat, crude protein, true protein, and casein contents of cow milks by near-infrared transmission spectroscopy. Free fatty acids and moisture in fish oils were analyzed (Cozzolinoa and et al., 2005). A study completed by Pedro and Ferreira (2005) was able to generate spectral calibration models for tomato products for chemical composition regarding solids and carotenids. The models are being used by Unilever Brazil to evaluate the quality parameters of tomato products.

Future Applications of NIR for Food Analysis

In the future of food analysis coupling of NIR technology with HPLC or gas chromatography may negate the problems associated with low level detection. Also incorporating NIR equipment into the production line was possible because of its non-destructive, continuous analytical and chemical-free attributes (Hoyer, 1997). NIR used as an in-line processing tool could detect the inconsistencies in ingredients (Brodersen and Bremner, 2001). Narratil et al. (2004) explored NIR combined with an electronic nose for on-line monitoring of yogurt and Filmjölk fermentations for pH and titratable acidity. The PLS calibration model yielded a correlation value of 0.99, which far exceeded industry standards. Experimentation with non-contact transflectance NIR with multi spectral imaging to determine the moisture content of dried and salted coal fish (bacalao) produced better results than

reflectance NIR. The correlation value for the PLS calibration model was approximately 0.92, and this was primarily due to the deeper light penetration of the transflectance NIR spectroscopy (Wold et al., 2006). Recent technological advances with fiber optic probes increased the sensitivity of monitor and process controls for remote on/in-line NIR spectrometers (Huang et al., 2008).

Justification

Shrimp are comprised mainly of water and protein, and in order to maintain nutritive quality and palatability during preservation the secondary focus is water retention. The use of excessive amounts of sodium tripolyphosphate and water cause an increase in water weight of the treated shrimp. Essentially, the consumer is purchasing water at the price of seafood. Shrimp are a commodity that is sold by the pound. Whether it is intentional or unintentional adulteration of seafood products has led to an increased demand for rapid assessment technology. The current method of detection practiced by the FDA, sensory analysis, is antiquated and impractical. Seafood is extremely perishable and by the time the analysis is complete the shrimp has either it has already been sold or discarded. Near-infrared spectroscopy has previously been established as a rapid non-destructive assessment tool used in the food industry. The attributes of cooked and conditioned shrimp had been measured using NIR reflectance spectroscopy successfully by Brodersen et al. (2001). This led to the decision to evaluate the use of Near-infrared Spectroscopy to detect shrimp treated with excessive amounts of water stabilized by phosphates.

Chapter III

Materials and Methods

Penaeus setiferus and *aztecus*

Penaeus setiferus and *aztecus* were obtained from Annamarie Seafood, LLC. (Dulac, LA). The shrimp were wild-caught in Louisiana Gulf coast coastal waters and not treated with any additives, preservatives, or humectants prior to freezing. The shrimp were individually quick frozen (IQF) within one-hour of capture onboard the vessel using a plate freezer (-40° F). The frozen shrimp were then dipped in potable water to form an encasing thin layer of ice; this process is most commonly referred to as glazing. The glazed shrimp were then loosely layered in a plastic lined carton, shatter packed.

Sample Preparation

The samples were defrosted in a sealed plastic bag (Poly-America, Grand Prairie, Texas) submerged in ice water and held at 4 ℃ for approximately three hours. The test shrimp were washed, the shells and heads were removed and discarded, and the tails were separated. The shrimp were also deveined to reduce the risk of interference during the ICP-AES mineral analysis. The samples were placed into a one gallon plastic zip lock bag (Great Value, Bentonville, AR) and held on ice until the experimental treatments were applied.

Phosphate Application

Once the sample preparation was complete, glass sampling jars were washed with Liquinox™ (Alconox Inc., NY, USA) solution to avoid any phosphate residues and dried in a bench top oven. The glass jars were removed from the oven and given ample time to cool. Once

the jars returned to room temperature they were labeled, and three jars were designated for each treatment. The jars were placed on ice, while two shrimp (approximately 60 grams) were placed into the sampling jars. The solutions of 5 g/100 mL food grade sodium tripolyphosphate (Prayon SA, Engis Belgium), 2.5 g/100 mL sodium tripolyphosphate, 100 mL of deionized water, 1 g NaCl and 1 g STPP blend/100 mL, 2 g NaCl and 2 g STPP blend/100 mL of tap water, or in potable water (control group) were poured into the sampling jars. The solutions were made with potable water to replicate the current industry practices. The shrimp remained immersed in solution for the allotted time interval of 30, 60, 90, 120, 240, 480, 960, 1920 minutes at approximately 4 °C. Subsequently, the samples were drained for 30 seconds on USS number 5 (wire mesh) screen and returned to the sampling jars to be held on ice for further testing. The immersion times were selected in part because they represented possible immersion times that may be practiced by shrimp processors. Approximately three grams of shrimp from each sample was set aside for moisture analysis and subsequent phosphorus content determination.

Moisture Analysis

The total moisture was determined according to the protocol outlined in the AOAC 39.1.02 part B (AOAC, 1995). The procedure was altered by increasing the sample size to 3.5±0.5 grams, and utilizing porcelain crucibles rather than aluminum dishes. The crucibles were acid washed with a 10% nitric acid (Coroco Chemical, Fairless Hills, PA) solution and dried in an oven at 150 °C for four hours before being placed in a dessicator. After the crucibles had cooled to room temperature for approximately two hours, they were labeled and weighed. Each individual whole shrimp sample was manually cut into small pieces and the equivalent of three grams was added to each crucible. The crucibles and comminuted shrimp samples were weighed and placed into the preheated oven at 100-102 °C for 16-18 hours. The crucibles were

removed from the oven, placed in dessicator, cooled, equilibrated to ambient temperature, and re-weighed. The wet-basis moisture content (M_c) was determined by the weight difference after oven drying using the following formula:

$$M_c = \left[\left(\frac{M_i - M_f}{M_i}\right)\right] \times 100,$$

where M_i is the initial weight and M_f is the dried weight of the shrimp samples.

Phosphorus Analysis

The mineral content was determined for the phosphorus concentrations. The AOAC official methods 938.08 and 969.23 were used to complete the dry ashing procedure. The dried samples in crucibles were charred on an electric hot plate under a fume hood until the presence of smoke was no longer detectable and crucibles returned to the dessicator. Once the crucibles had reached ambient temperature, the samples were placed in a cold muffle furnace and brought up to temperature of 525 °C. The samples were held at 525 °C overnight until the charred shrimp turned into white ash. The samples were carefully removed from the furnace and placed into a dessicator and allowed to cool. After the samples had cooled, they were weighed for the total ash.

The nitric acid solution was prepared by diluting 10 mL HNO_3 into 100 mL of deionized water. Nine milliliter aliquots of the nitric acid were added into each crucible of dried ash and a glass stirring rod was used dissolve the ash into solution. The sample solutions were filtered through 0.20 micron syringe filter (Mallinkrodt, Phillipsburg, NJ), and an addition milliliter of HNO_3 solution was used to rinse the filter and bring the samples up to volume of 10 mL The solutions were transferred to acid washed with HNO_3 glass vials and capped with Teflon lined

caps. A blank solution was also prepared using 10 mL of the dilute nitric acid solution. The ash samples were analyzed using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES).

ICP-AES, an emission spectrophotometric technique, measured the energy that the excited electrons emitted at a given wavelength as they returned to ground state. ICP-AES assigned a single wavelength to each element. Every element emits multiple wavelengths; therefore, the intensity of the energy emitted at the chosen wavelength was proportional to the concentration of the element in the sample. The proportion of the phosphorus was calculated on a dry weight basis and quantified by deriving the wavelengths emitted and its intensity.

Near Infrared Analysis

The remaining portions of shrimp not utilized in the moisture and mineral analysis were blended into a paste using a Waring Blender (Model 51BL31, Turrington, CT) at the highest setting for 30 seconds. The blender jars were held on ice while the NIR was activated and calibrated. The homogenized shrimp samples were transferred from the blending jar to the NIR glass sample dishes.

The NIR spectra were generated with a Büchi NIRLab N-200 spectrophotometer (Flawil, Switzerland). The near-infrared spectrophotometer measured the absorbance versus the wavenumber in the region from 12,500 to 4000 cm^{-1}. A wavenumber (cm^{-1}) has been defined as an inverse of the wavelength, the distance the light traveled. The wavenumbers of significance were the O-H stretching band of water in the region of 6240-7100 cm^{-1} and the P-OH, phosphate functional group, located at 5241 cm^{-1} (Workman and Weyer, 2008). The reflectance spectra for each time interval and treatment were collected over a three month period. Two hundred and ten

samples were analyzed. Three reflectance spectra were obtained for each sample and mean centered prior to data analysis.

NIR Data Analysis

The spectra were then exported to the NirCal® 4.21 (Büchi, Flawil, Switzerland) chemometric software to mathematically transform the spectral data utilizing a regression analysis. The NIR data was pre-processed to transform the multivariate signals to fit Beer's Law defined as follows:

$$A = \varepsilon bc,$$

where A represented the absorbance, ε signified the molar absorptivity constant, b denoted the path length, and c denoted the concentration, in an attempt to linearly correlate the absorbance with concentration. The NirCal® 4.21 software employs principal component, multiple linear and partial least squares regression.

Partial Least Squares Regression

The NirCal® 4.21 chemometric software wizard selected the partial least square regression (PLS) to build the linear predictive model for the sodium tripolyphosphate concentration based on the spectrum. The spectrum consisted of approximately 1,500 different frequencies which are the factors. The responses were the six different concentrations of the sodium tripolyphosphate dipping solutions. The PLS regression modeled the relationship between the factors, X, and responses, Y (Trygg et al., 1998). The spectra were divided into calibration and validation sets for the partial least squares regression analysis. Approximately, two-thirds of the spectra were randomly assigned to the calibration set, and the remaining one-

third was designated the validation set by the NirCal® 4.21 Wizard. The PLS regression mathematical algorithm was applied to the calibration wavelengths from 3999.67-10001.1 cm^{-1}. Three calibration properties used to aid in the sodium tripolyphosphate concentration determination were the moisture content, phosphorus concentration, and concentration of the sodium tripolyphosphate dipping solutions. The validation spectra were used to cross-validate the calibration spectra with the wet analytical laboratory values. In an attempt to surmount the obstacles associated with the chemical and physical properties of the samples, the mean centering and Kubelka-Munk pre-treatments were applied to the spectra.

Statistics

A three-way factorial analysis of variance (ANOVA) was conducted to compare the absorbances for each treatment for the following wavenumbers: 5237.75, 5241.61, 5245.46, 7177.8, 7181.66, and 7185.51 cm^{-1}. The wavenumbers selected had the greatest deviations from the control. The three factors were defined as concentration of the dipping solution, wavenumber, and dip time. There were three two factor interactions: concentration and dip time, concentration and wavenumber, and dip time and wavenumber. The final interaction analyzed was a combination of all three factors, concentration, dip time, and wavenumber.

Chapter IV

Results and Discussion

Moisture Analysis

The moisture content for all of the samples increased over time with the exception of the control. The sodium tripolyphosphate treatments improved the water retention of the shrimp, which was evident in the increasing moisture contents over time in Figure 6. The 2.5% STPP dipping solution provided the best results for moisture retention, which coincides with the literature. Garnatz and others (1946) previously reported that treating shrimp prior to freezing produced the maximum stability and water retention.

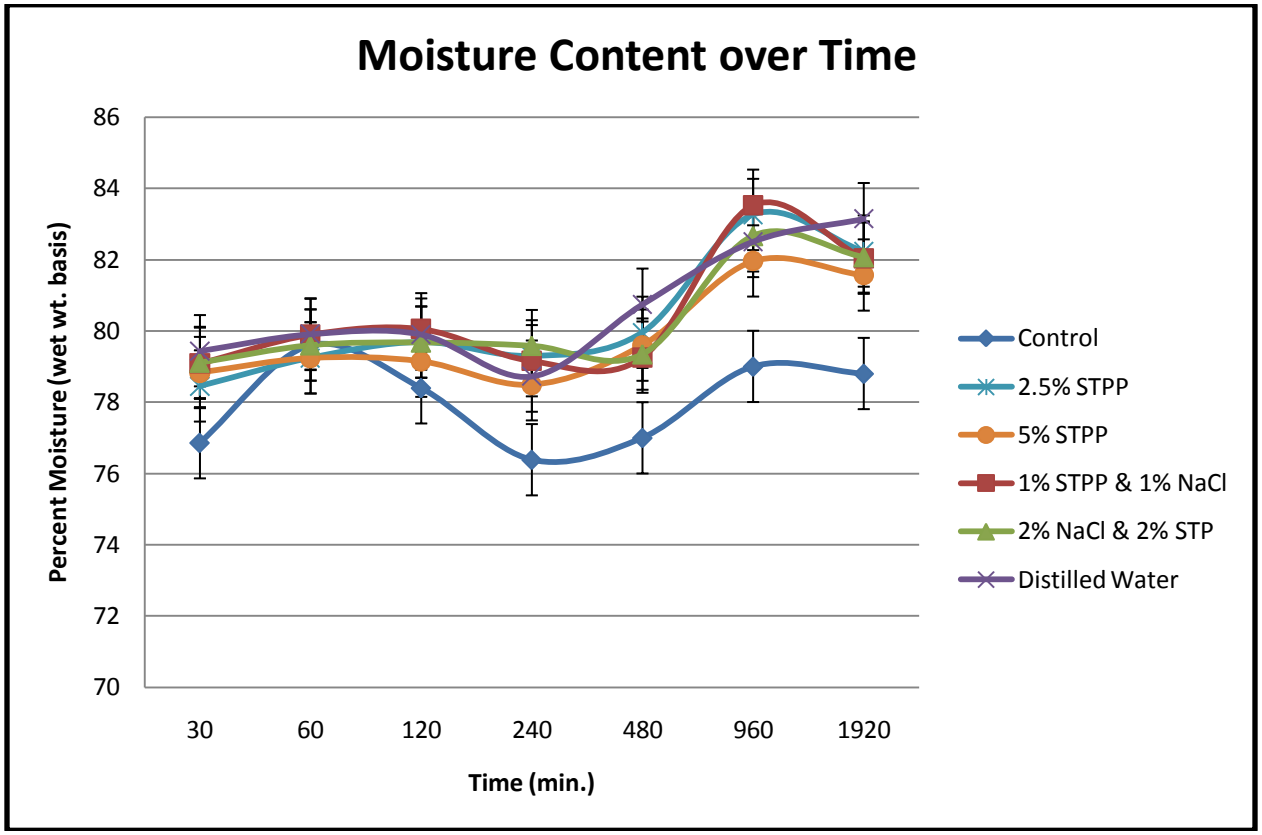

Figure 4 - A graphical representation of the changes in moisture content over time for all of the treatments.

The moisture contents of the 1% and 2% sodium chloride and STPP combination solutions imitated the results of the 2.5% and 5% sodium tripolyphosphate treatments. The synergistic effect of the salt and sodium tripolyphosphate was previously noted in a study performed by Mahon and Schneider (1964). The moisture content was affected by the ions from the sodium chloride. The sodium tripolyphosphate and sodium chloride bind the charged groups on the protein surfaces and caused the muscle proteins to separate from one another. The increased distance between the proteins within the muscle fiber increased the number of water binding sites (Richardson, 1987). The distention was the result of an increase in the charge repulsions. The expansion allowed for physical entrapment of water and an increase in the water holding capacity of treated meat (Xiong, 2005).

The initial decline in the moisture content at the 240 minute mark in Figure 4 could be attributed to the degradation of the shrimp muscle proteins. During the intial stages of the postmortem shrimp degradation, the myofibrillar proteins released the bound water. Baranowski et al. (1984) reported proteolytic and/or colagenolytic enymes were the cause of postmortem degradation of shrimp. A study conducted by Kye et al. (1988) reported the existence of highly active enzymes during ice storage. The results of the SDS-PAGE analysis of the myofibrillar proteins in Figure 5 showed the intensity of the smaller bands (25,00 and 31,000 daltons) increased, while the heavy chain units (205,000 and 200,000 daltons) decreased during one day of ice storage. This was evidence of the enzymatic digestion of shrimp proteins into peptide fragments and amino acids.

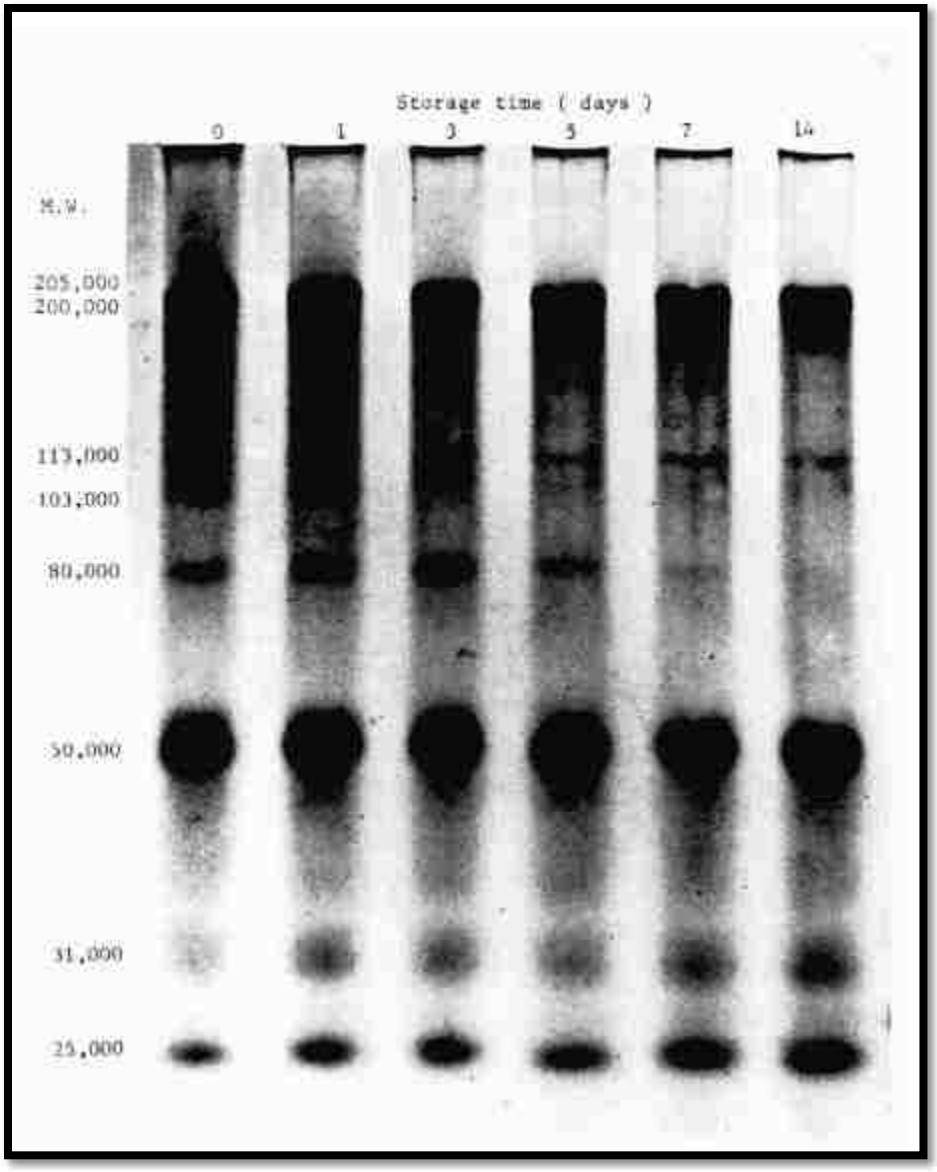

Figure 5 - The results of SDS-PAGE analysis of the shrimp myofibrillar proteins during 14 days of ice storage (Kye et al., 1998).

Phosphorus Analysis

The phosphorus content increased proportionally along with the concentration amongst the sodium tripolyphosphate dipping solutions in Figure 6. The phosphorus content for the control decreased over time. Shen and Dyroff (1966) proved the rate of degradation of STPP increased exponentially in the presence of sodium ions. This phenomenon was evident in the

decrease in detectable phosphorus for the combination 1% sodium chloride and 1% sodium tripolyphosphate solutions in Figure 6.

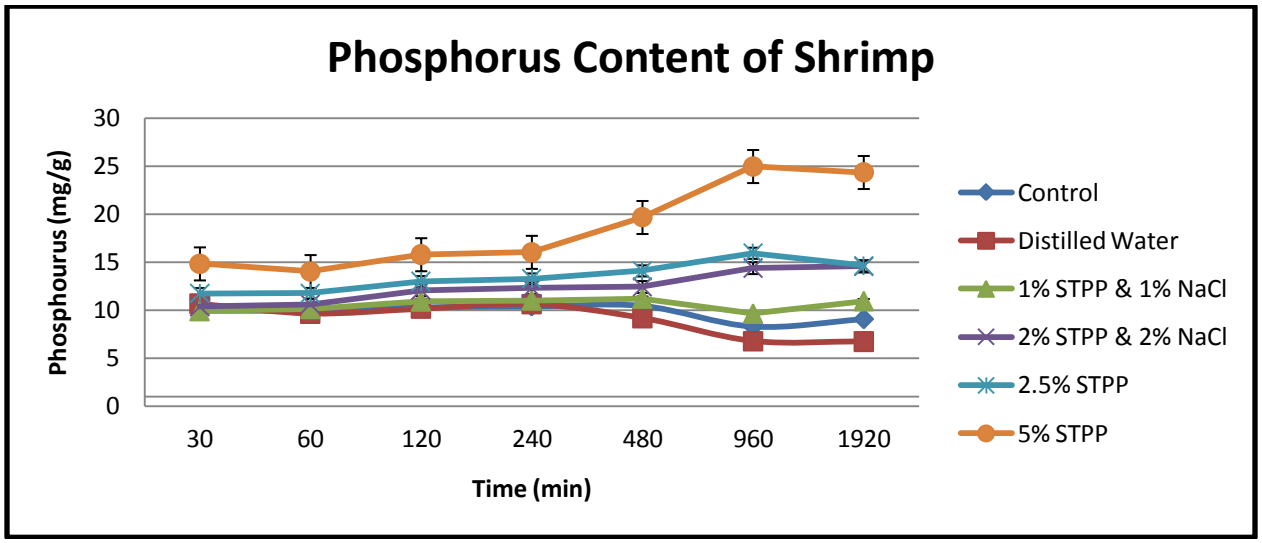

Figure 6 - The results of the ICP-AES mineral analysis for phosphorus content of the shrimp over time.

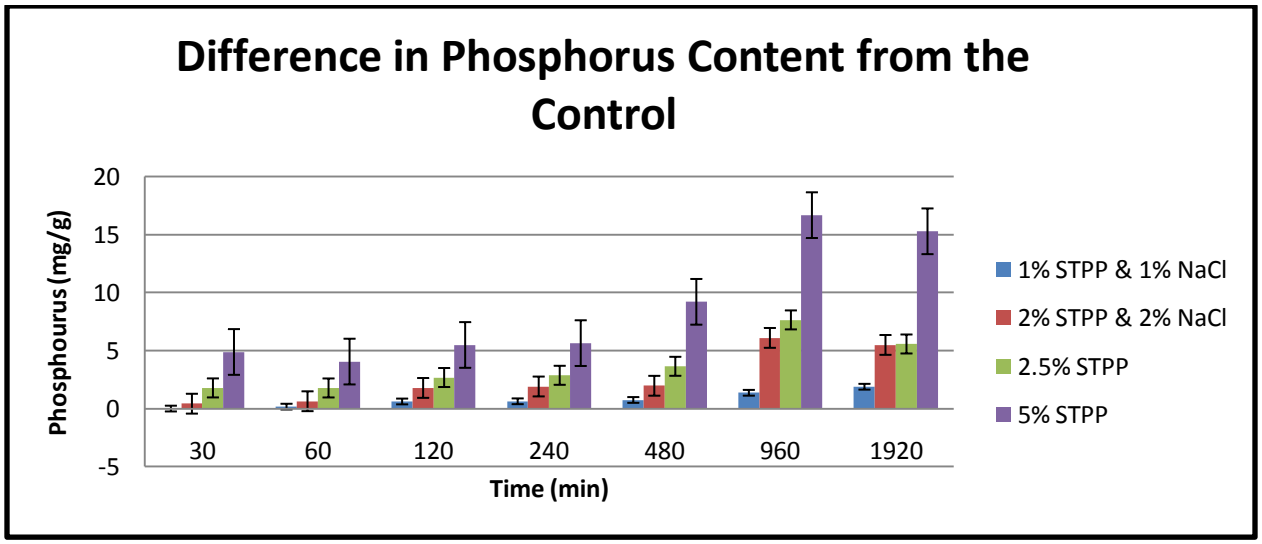

Figure 7 - The difference in the phosphorus content per gram of shrimp from the control.

The temperature was held constant at 4 °C to avoid the pitfalls associated with changes in temperature affecting diffusion in the shrimp flesh. Unal et al. (2004) determined that the counter-current diffusion of phosphates was temperature dependent. Utilizing the modified spectrophotometric ammonium molybdate method (AOAC, 1995), the phosphate levels were determined in terms of orthophosphate for beef (*Longismus dorsi* muscle) samples in a study performed (Unal et al., 2006). It was extremely difficult to make a distinction between the naturally occurring orthophosphate in meat from the polyphosphates that were absorbed by the flesh and transformed into orthophosphate. Unal et al. (2006) noticed an initial drop in the phosphate concentration and determined that is most likely due to the naturally occurring orthophosphates of the meat. The study concluded the high levels of orthophosphates in the meat would diffuse into the solution before being reabsorbed. This coincides with the drop in the phosphorus concentration at the 60 minute time interval. The naturally occurring orthophosphates of the shrimp diffused into the dipping solution, which had a lower solute concentration.

NIR Spectroscopy

The spectral analysis was based on the group frequencies, spectra-structure correlation, and the difference spectra methods. Initially, the project was focused on developing a regression equation to predict the sample sodium tripolyphosphate concentration of the treated shrimp regardless of the dip time. The NirCal® 4.21 chemometric software was employed to generate a regression equation for the spectra of all the treatment times and solutions, which were incorporated into one project. The NirCal® calibration wizard was unable to compute a regression equation with the ability to distinguish between the different sodium tripolyphosphate concentrations of the shrimp over all dip times. The calibration properties, moisture content,

phosphorus concentration, and the STPP treatment solution concentration, were used in every combination in an attempt to produce a regression equation with an acceptable quality value. The errors in the regression calibration arose from the similarity in the spectra for the shrimp dipped in different solutions. For example, the shrimp dipped in 1% NaCl and 1% STPP solutions behaved similarly to the control samples. This phenomenon was evident for several of the dipping solutions.

The NirCal® software was unable to separate the spectra based on the STPP concentration regardless of dip time. The spectra were divided according to the following seven dip times; 30, 60, 120, 240, 480, 960, and 1920 minutes. The trials were divided according to dip time rather than the concentration to avoid the previous errors that occurred in the regression calculations attributed to the similarities in the spectra for the treated shrimp. The spectra for shrimp dipped in the 2.5% STPP solution for 60 minutes mimicked the behavior of the shrimp immersed in the 5% STPP solution for 30 minutes. The NirCal calibration wizard was applied to the spectra separated according to the dip times. The resulting spectra in Figures 7-14 were analyzed for the stretching of the hydroxyl groups of water located in the region from 6240 cm^{-1} to 7100 cm^{-1} and the phosphate functional group at 5241 cm^{-1}, which were established by Workman and Weyer (2008).

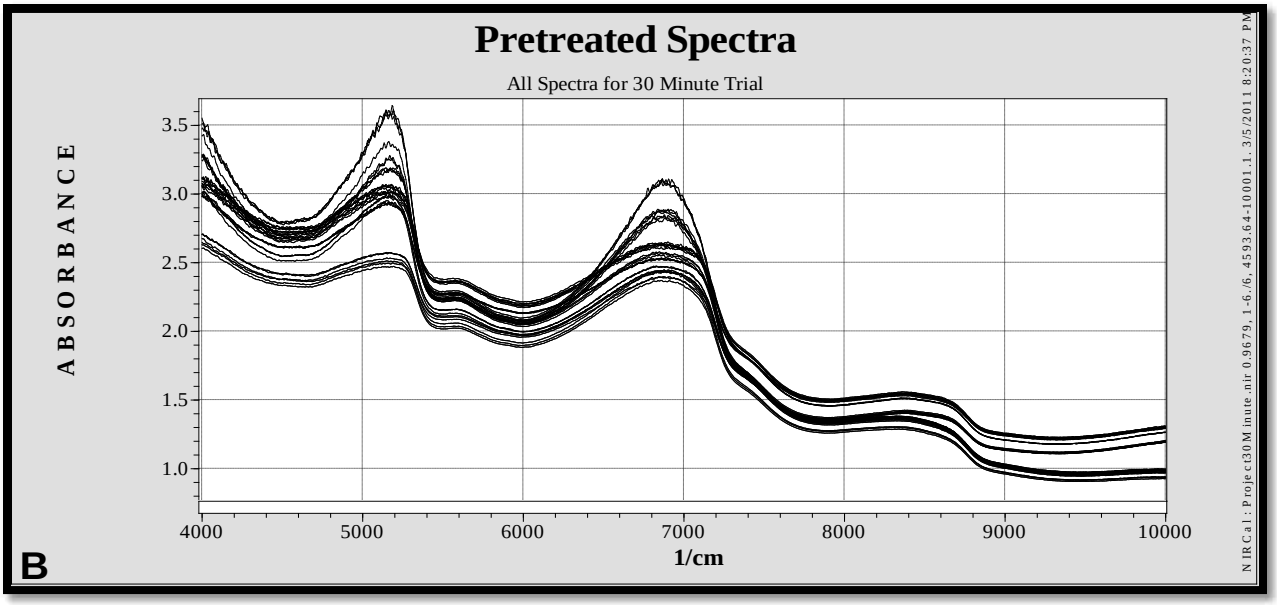

Figure 8 – The results of the Near Infrared spectral analysis of the 30 minute treatment.

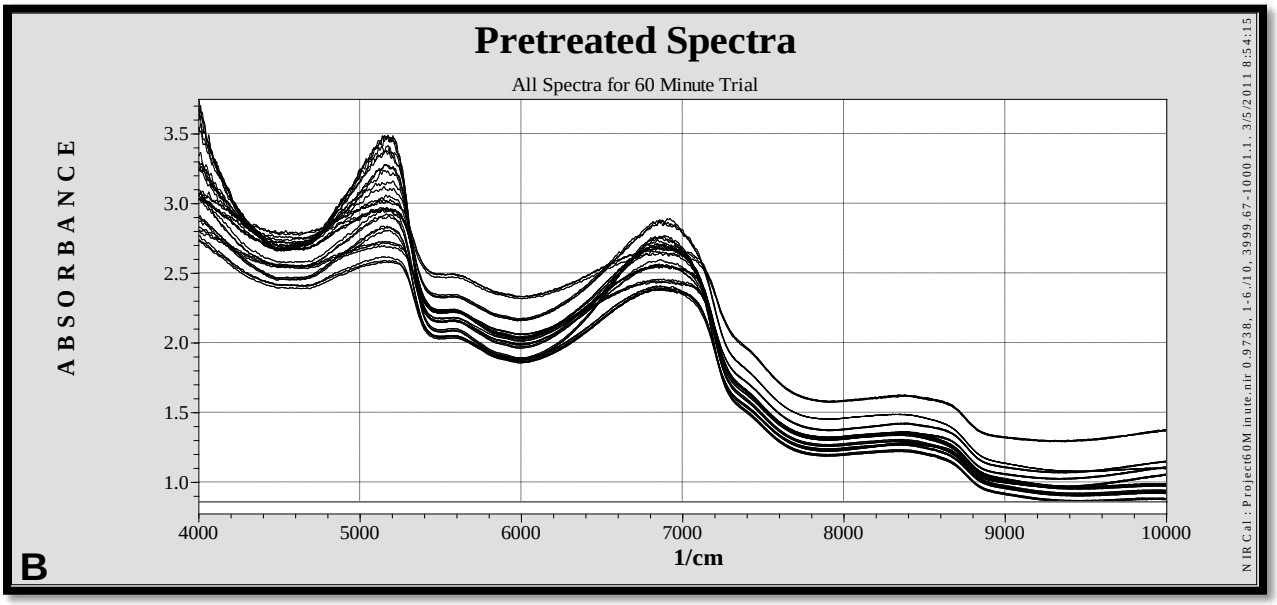

Figure 9 - The results of the Near Infrared spectral analysis of the 60 minute treatment.

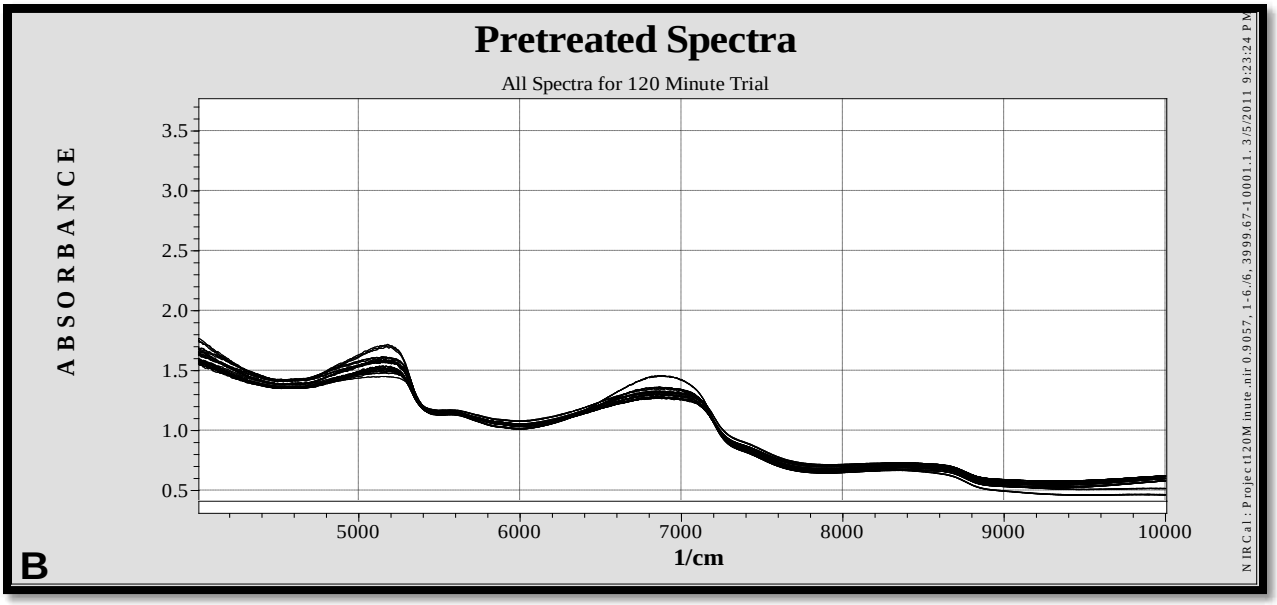

Figure 10 - The results of the Near Infrared spectral analysis of the 120 minute treatment.

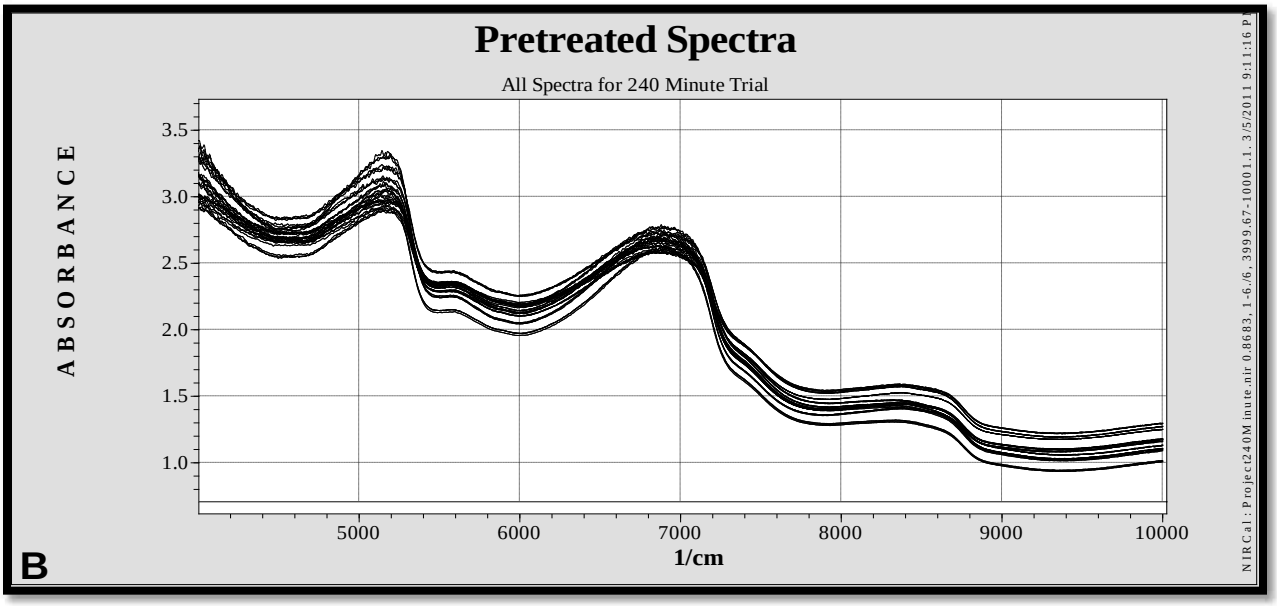

Figure 11 - The results of the Near Infrared spectral analysis of the 240 minute treatment.

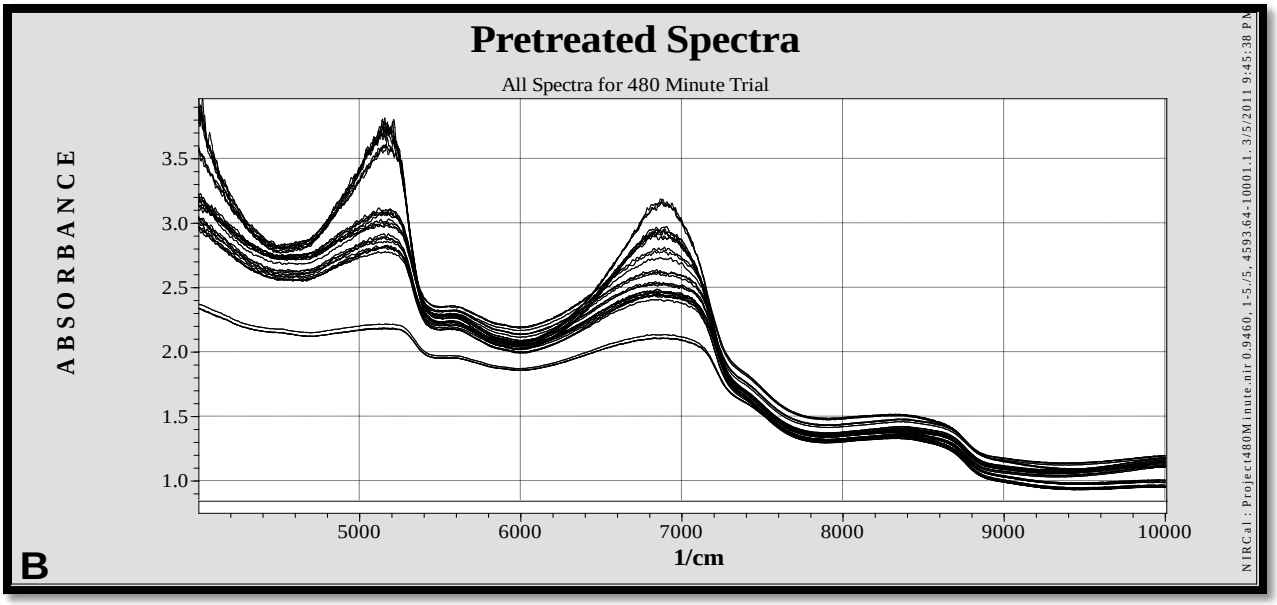

Figure 12 - The results of the Near Infrared spectral analysis of the 480 minute treatment.

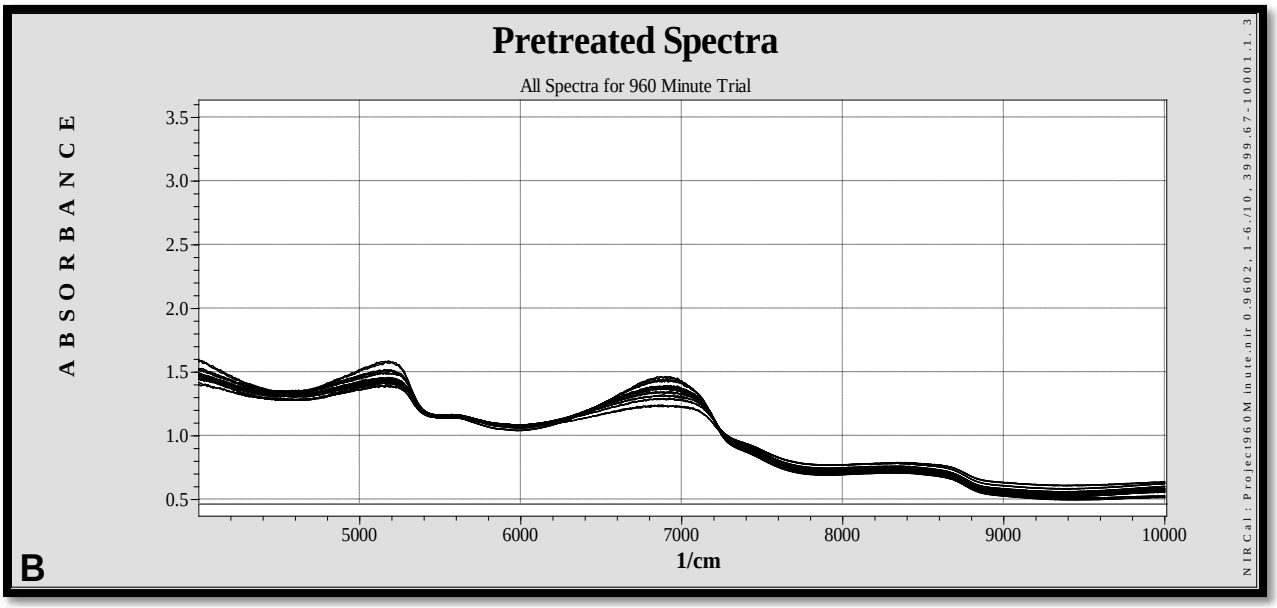

Figure 13 - The results of the Near Infrared spectral analysis of the 960 minute treatment.

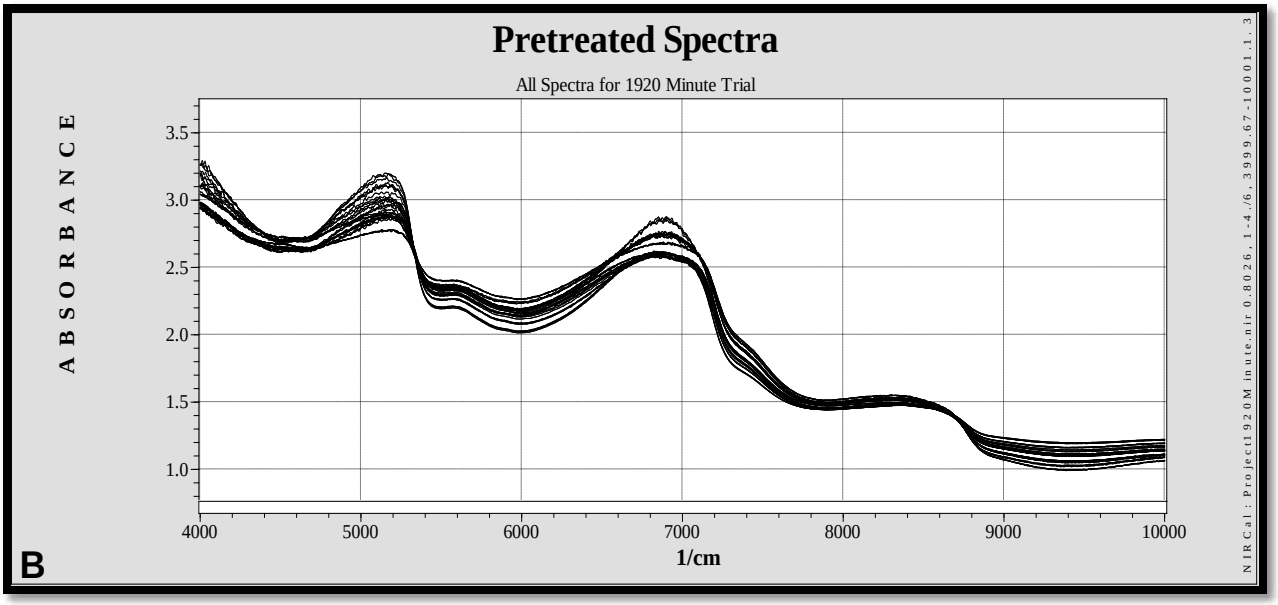

Figure 14 - The results of the Near Infrared spectral analysis of the 1920 minute treatment.

The decline in the absorbances depicted in Figure 9 for the 120 minute trial and the 960 minute trial in Figure 12 were most likely attributed to temperature differences. The samples were measured at room temperature during the summer months. The average high temperature from June to August in Louisiana is 90±2 °F, and the average low was 70±1 °F. A study performed by Uddin et al. (2006) documented that fish thermally processed at higher temperatures had lower absorbances.

The variations of the overlapping spectral bands caused light scattering, random noise or non-linearity. For a thick sample like shrimp paste where the particles were closely packed, isotropic scattering distribution was expected; however light diffusion still occurred (Kortum, 1969).

A predictive model for the quantitative analysis was built, and statistical analyses were performed by the PLS regression. Osborne et al. (1981) established a sub-set of two to three readings are sufficient for a regression analysis. For the PLS regression analysis, approximately

one-third (9/30) of the spectra were selected at random for the validation set, and the remaining two-thirds were designated the calibration set. The partial least squares regressions generated linear predictive models for quantification of the sodium tripolyphosphate from the results of the near-infrared spectrophotometric analysis in Figures 14-20. The projects had three calibration properties; moisture content, sodium tripolyphosphate concentration, and the phosphorus content. Utilizing all three calibration properties the NirCal® calibration wizard generated unacceptable Q-values of less than 0.2 for all the projects. All combinations of the calibration properties were tried to produce regression equations with acceptable Q-values. The poor performance of the phosphorus calibration property was attributed to the errors in the ICP-AES analysis. The results were not consistent for the mineral analysis replications and seemingly varied by operator.

The focus of the project was shifted to the evaluation of the moisture content of the treated shrimp. As previously indicated, Ravelo et al. (1991) used percent moisture to percent protein ratios to identify sodium tripolyphosphate treated shrimp based upon the natural phosphate content of 39 to 397 mg/100g of shrimp defined by Sidwell (1981) and Sullivan and Otwell (1992). The study reported the percent moisture to percent protein ratio increased as the phosphate treatment intensified, but the concentration of the sodium tripolyphosphate treatment solution could not be quantified.

Calibration Curve

The original property/ predicted property (2D) graphs in Figures 15-21 provided an interpretation of the calibration curve generated by the NirCal® calibration wizard. The graphs

depict the reproducibility of multiple measurements for identical samples and the errors in the calibration and validation sets.

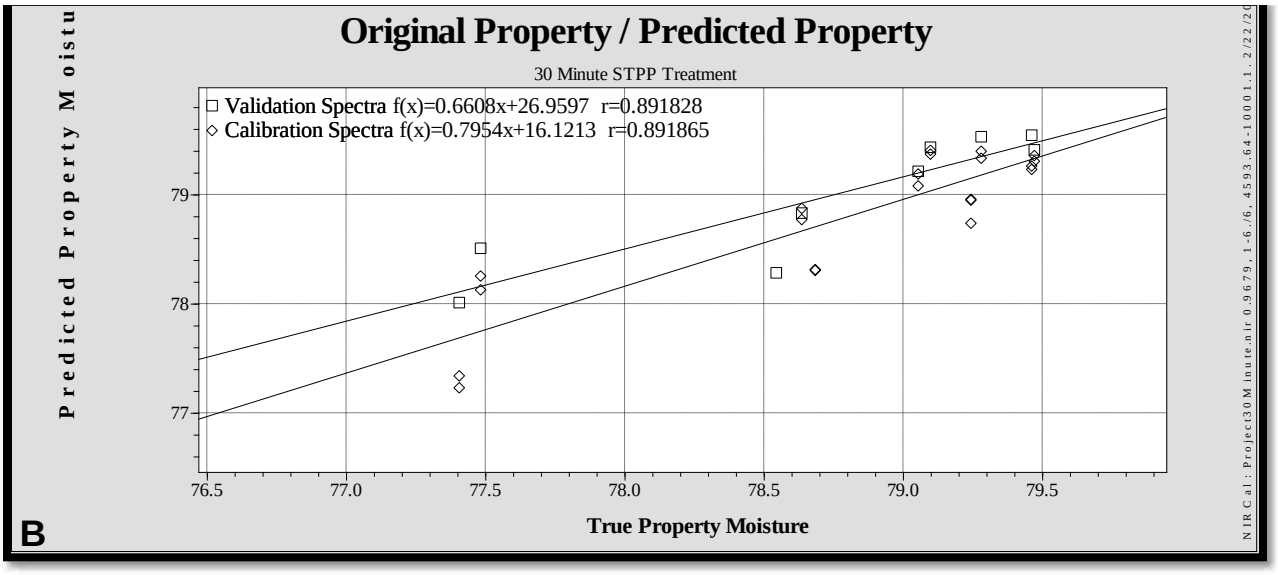

Figure 15 – A plot of the original property against the predicted the predicted property for the partial least squares regression for the 30 minute treatment.

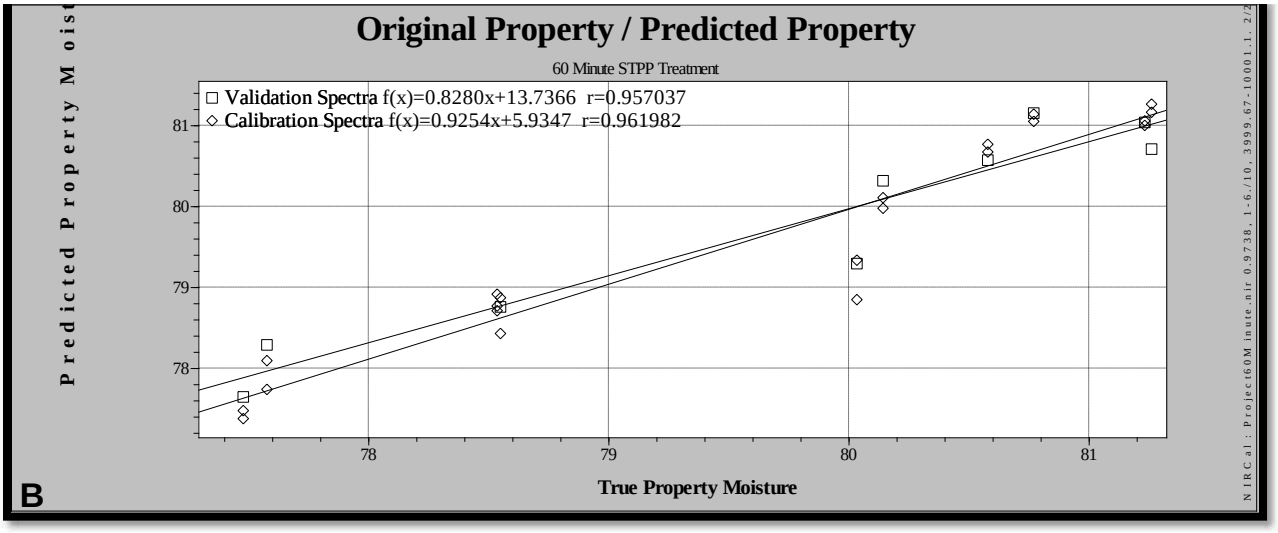

Figure 16 – A plot of the original property against the predicted the predicted property for the partial least squares regression for the 60 minute treatment.

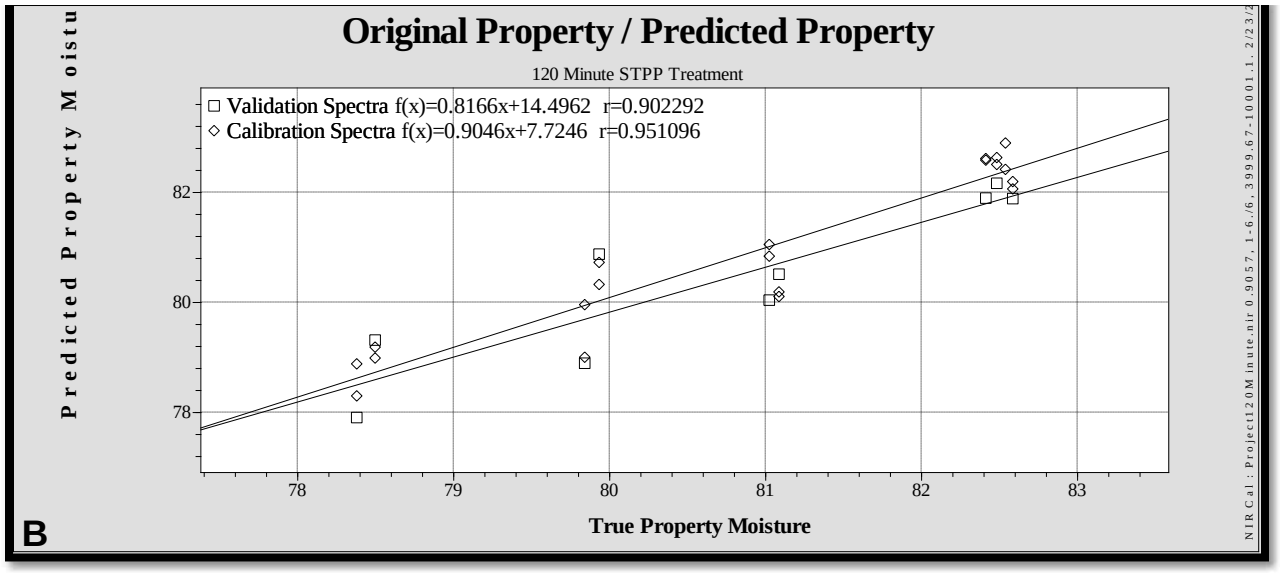

Figure 17 – A plot of the original property against the predicted the predicted property for the partial least squares regression for the 120 minute treatment.

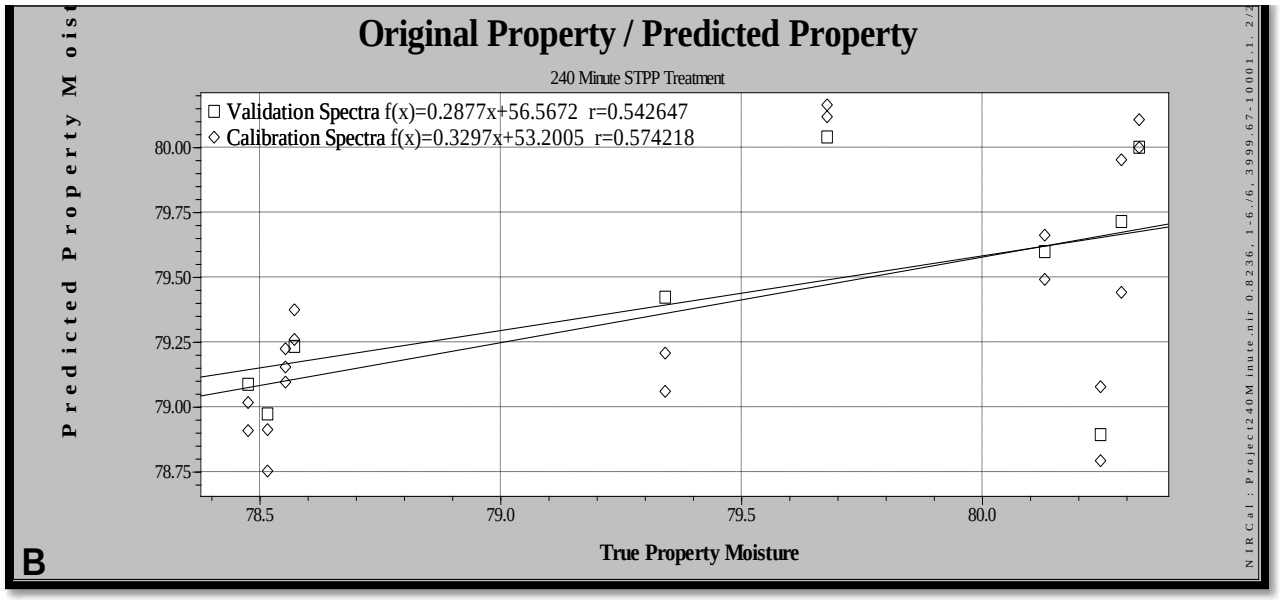

Figure 18 – A plot of the original property against the predicted the predicted property for the partial least squares regression for the 240 minute treatment.

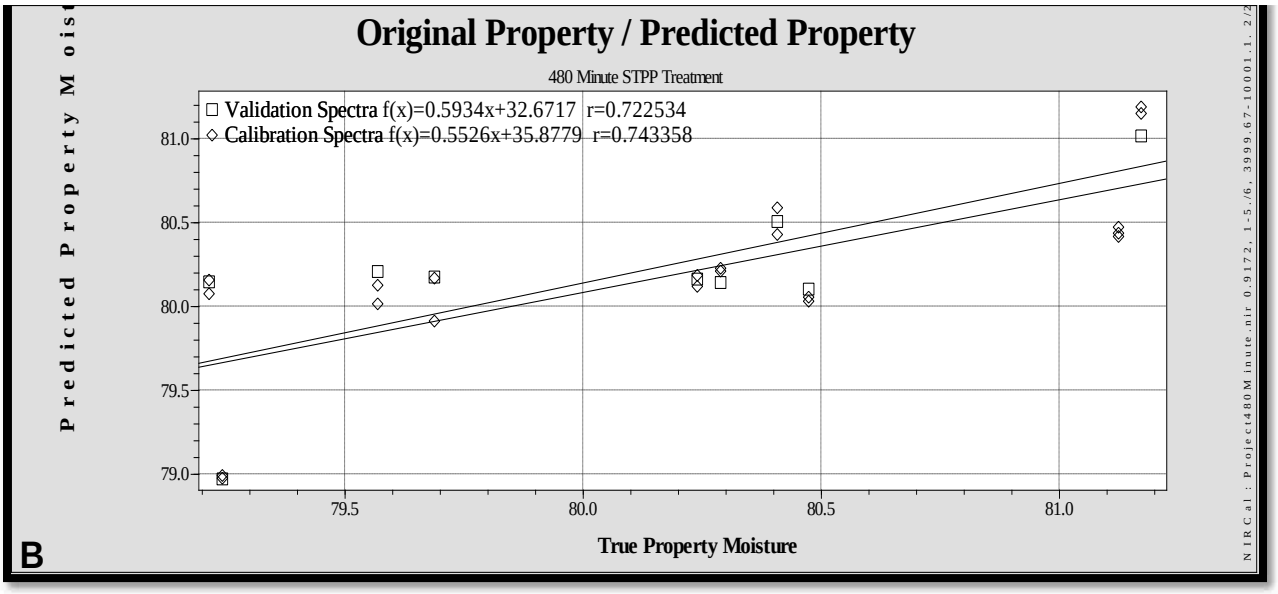

Figure 19 – A plot of the original property against the predicted the predicted property for the partial least squares regression for the 480 minute treatment.

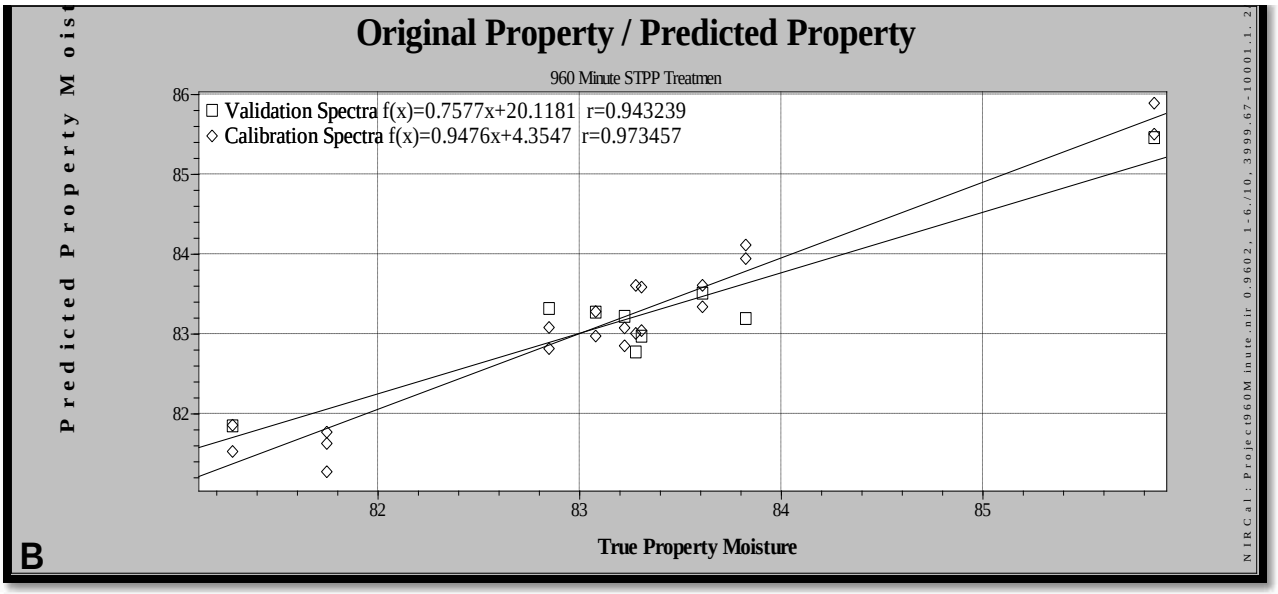

Figure 20 – A plot of the original property against the predicted the predicted property for the partial least squares regression for the 960 minute treatment.

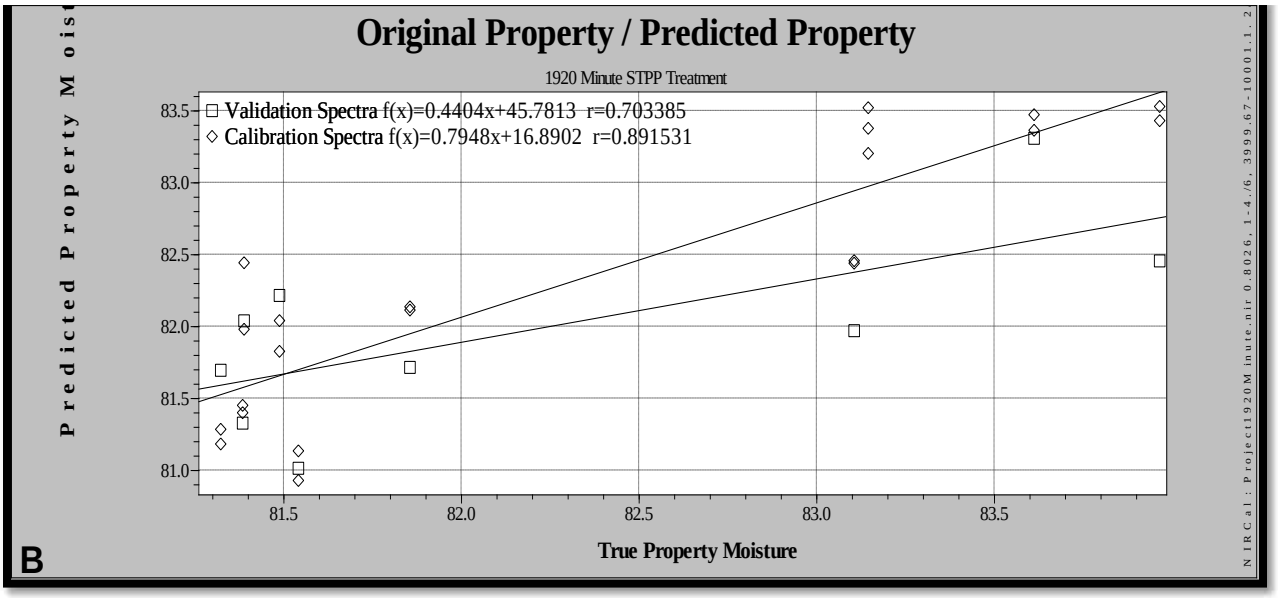

Figure 21 – A plot of the original property against the predicted the predicted property for the partial least squares regression for the 1920 minute treatment.

The quality of the PLS calibrations were evaluated with the following statistical parameters listed in Table1: regression coefficient (r), predicted residual sum of squares (PRESS), standard error of the estimate/calibration (SEE), and standard error of prediction (SEP). The true moisture plotted against the predicted moisture yielded the regression equation and coefficient.

Regression Coefficient

The regression coefficient (r) designated the strength and direction of the linear relationship between the predicted and true values. When the value of the correlation coefficient is close to 1, a strong positive linear relationship exists between the factors. A strong negative linear relationship exists, when the correlation coefficient close to -1. The correlation coefficient is near zero, when there is an absence of correlation. A strong correlation coefficient is greater than 0.8. A correlation coefficient with a value less than 0.5 is described as weak (Stockburger,

1996). The correlation coefficients for all of the treatment times were greater than 0.8; therefore, all of the correlation coefficients had a strong positive linear relationship.

Quality Value

The NirCal® 4.21 calibration wizard was employed to compute all of the possible combinations of wavenumber ranges, calibration algorithms, and data pretreatments to garner the optimal calibration (Bossart et al., 2002). The different combinations are assigned a quality value ranging from zero to one. A calibration with a quality value greater than or equal to 0.95 is considered very good; 0.9 is good; 0.75 is medium; and 0.5 is acceptable, but not accurate. The highest quality value was used to select the optimal calibration model. The quality of a calibration is measure of the robustness, sensitivity, and selectivity of the model. The quality value often referred to as the Q-value was the most important statistical measure because it is a comparison of the number of spectra, standard error of the calibration, standard error of prediction, predicted residual sum of squares, and BIAS (Büchi, 2002). All of the Q-values listed in Table 1 exceeded the minimal requirement of acceptability (≥ 0.5). The Q-values for the 30, 60, and 960 minute treatments were very good (≥ 0.95). The quality values for the 120 and 480 minute were ≥ 0.90 and were considered good. The calibrations for the 240 and 1920 minute treatments were of medium quality.

Table 1 - A summary of the pretreatment, multivariate calibration methods, and statistical parameters for the calibration and validation sets used during the development of the predictive model for the sodium tripolyphosphate concentration of treated shrimp.

Treatment Time (min)	Pre-treatment	Multivariate Calibration	PLS Primary factors	Q-value	V-Set Correlation Coefficient (r)	C-Set Correlation Coefficient (r)	C-Set SEE	V-Set SEP	C-Set BIAS	V-Set BIAS
30	KM	PLS	6	0.9679	0.8918	0.8919	0.3289	0.3762	8.12E-15	-0.2630
60	KM	PLS	10	0.9738	0.9570	0.962	0.3825	0.4533	6.09E-15	-0.0210
120	KM	PLS	6	0.9057	0.9023	0.9511	0.4967	0.7065	1.35E-15	0.3019
240	KM	PLS	6	0.8236	0.5426	0.5742	0.6531	0.6767	-5.41E-15	0.0661
480	KM	PLS	6	0.9172	0.7225	0.7434	0.4682	0.4532	-1.76E-14	-0.1280
960	KM	PLS	10	0.9602	0.9432	0.9735	0.2761	0.4259	4.06E-15	0.0784
1920	KM	PLS	6	0.8026	0.7034	0.8915	0.459	0.7618	-1.22E-14	0.2116

SEE and SEP

The standard error of the estimate/calibration (SEE) compared different calibrations from the same data set, and the standard error of prediction (SEP) measured the calibration potential. The SEE and SEP had values in close proximity to one another. The corrected SEP denoted the optimal calibration performance. The BIAS represented the average difference between the measured and predicted values (Williams and Norris, 2001). The following formula was used to derive the SEE:

$$\text{SEE} = \frac{1}{N} \times \sqrt{\sum (x_n - y_n - BIAS)^2} \ .$$

The standard error of prediction was calculated using the following formula:

$$\text{SEP} = \frac{1}{N} \times \sqrt{\sum (x_n - y_n - BIAS)^2} \ .$$

The BIAS value was calculated from the NIR reflectance predictions of data of the validation sample as follows:

$$\text{BIAS} = \frac{1}{N} \times \sqrt{\sum (x_n - y_n)} \ .$$

PRESS

The predicted residual sum of squares (PRESS) was the most important measure in the selection of primary factors for the calibration. The primary factors were used to reconstruct the spectra and determine the residual values. The model was designed for prediction; therefore, the PRESS was used as a statistical tool to establish the number of required factors. In order to

optimize the NIR calibration, the number of factors used had the minimum PRESS, which is evident in Figures 21-27.

The optimum number of factors to be included in the calibration model was determined by the computed PRESS for each cross-validated model. Cross-validation was required to develop a prediction model with the appropriate number of factors (Geladi and Kowalski, 1986). The cross-validation method employed was designed to eliminate one sample at a time, while the PLS regression calibrated the remaining spectra. The concentration of the excluded sample was predicted, and the cross-validation was completed.

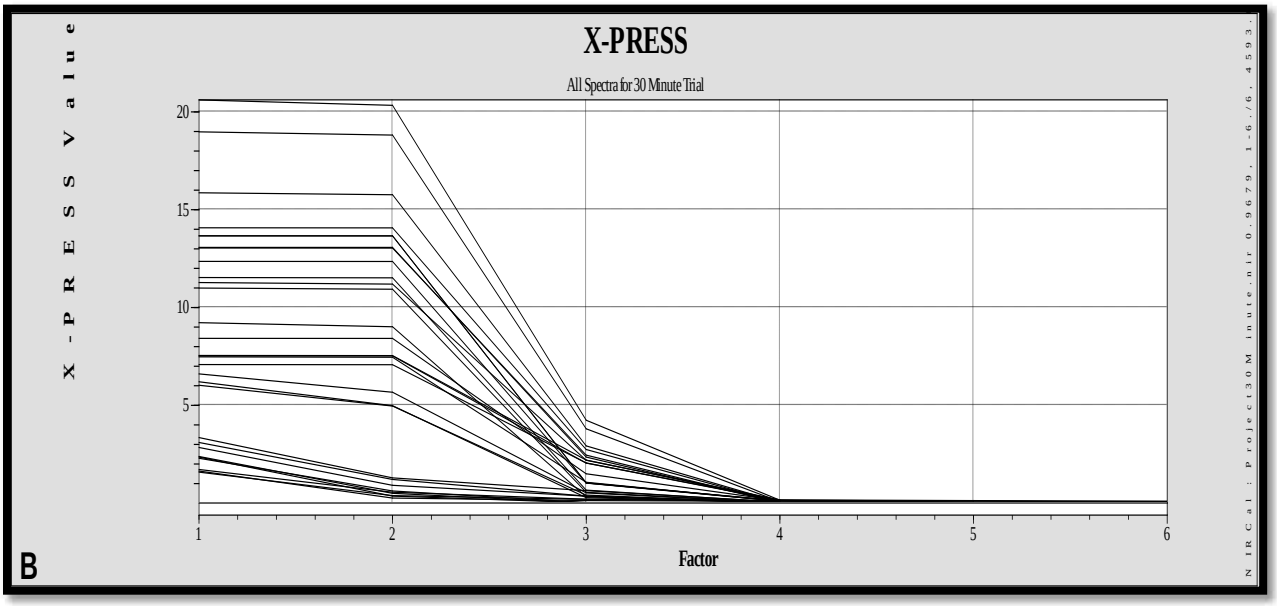

Figure 22 – The predicted residual sum of squares (X-PRESS) for the 30 minute trial.

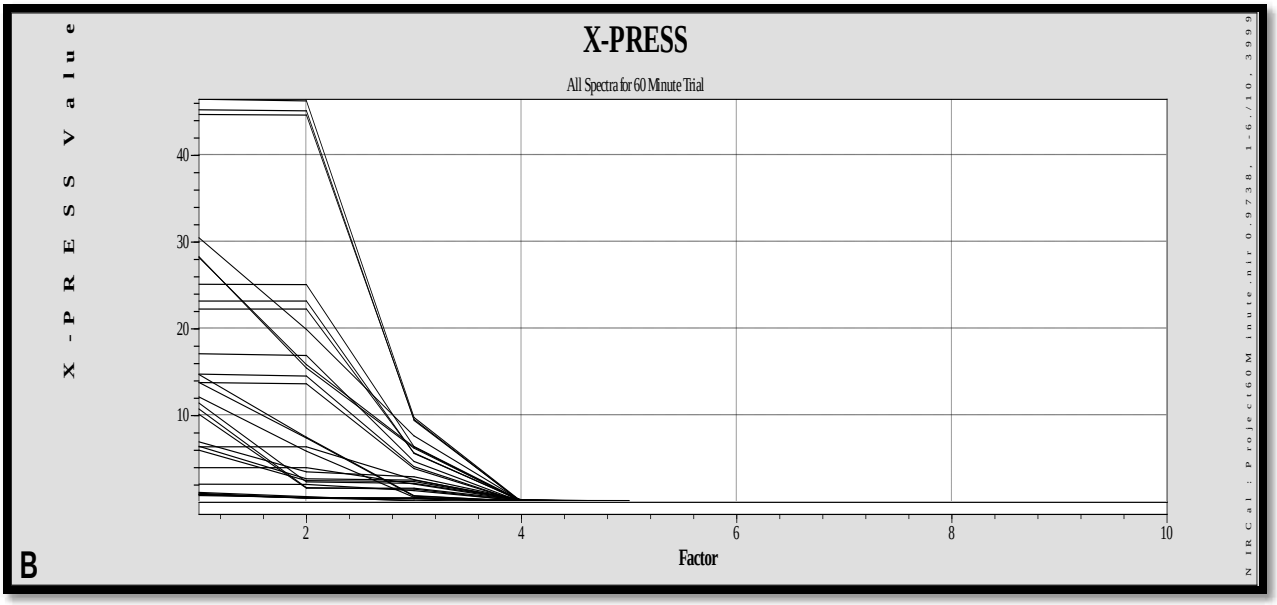

Figure 23 – The predicted residual sum of squares (X-PRESS) for the 60 minute trial.

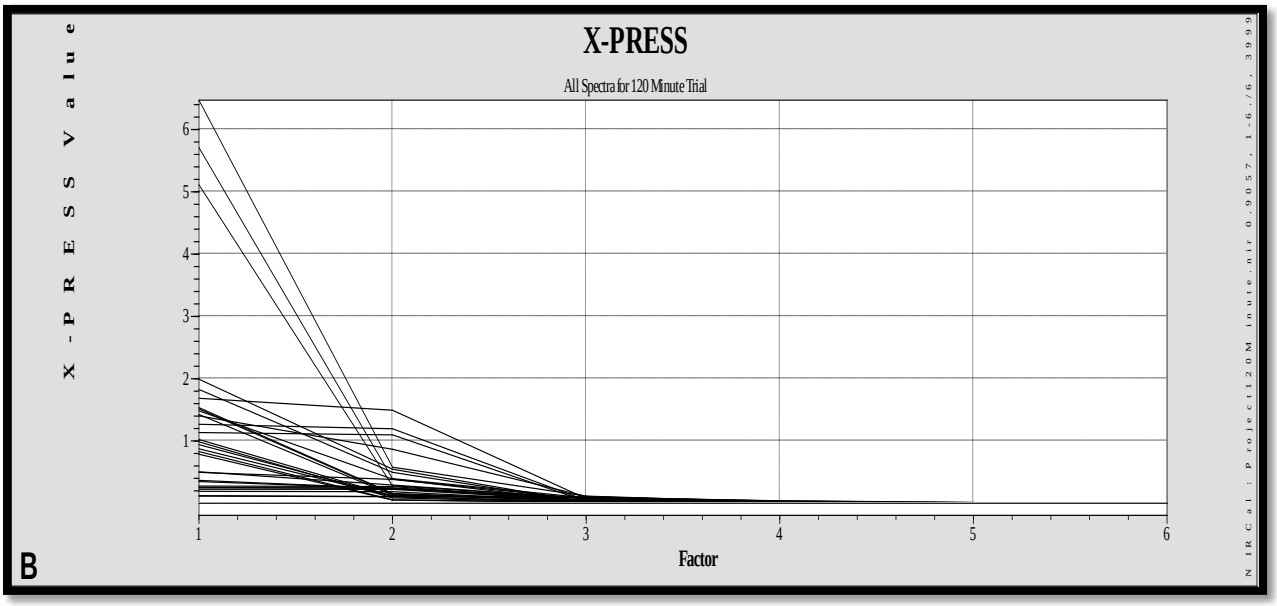

Figure 24 – The predicted residual sum of squares (X-PRESS) for the 120 minute trial.

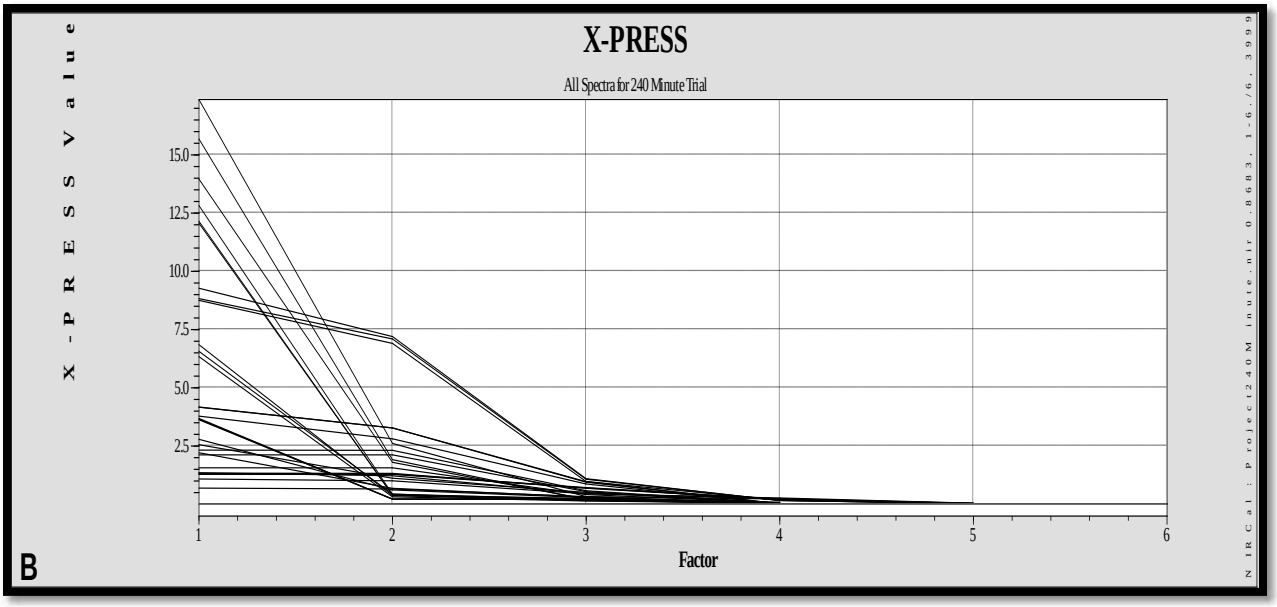

Figure 25 – The predicted residual sum of squares (X-PRESS) for the 240 minute trial.

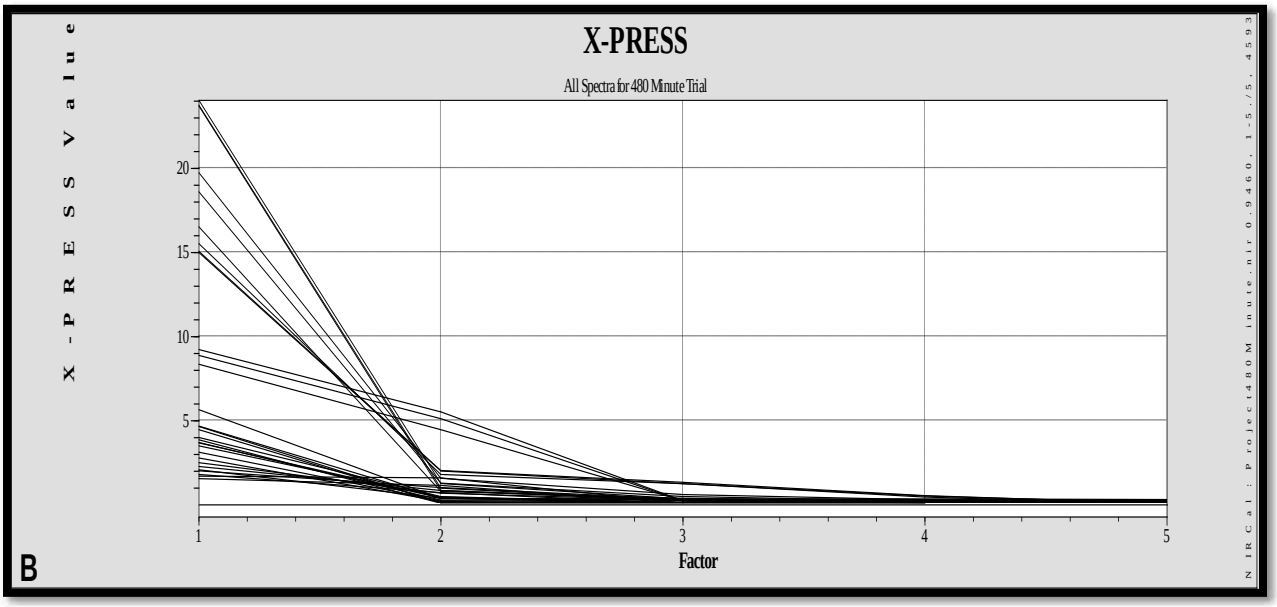

Figure 26 – The predicted residual sum of squares (X-PRESS) for the 480 minute trial.

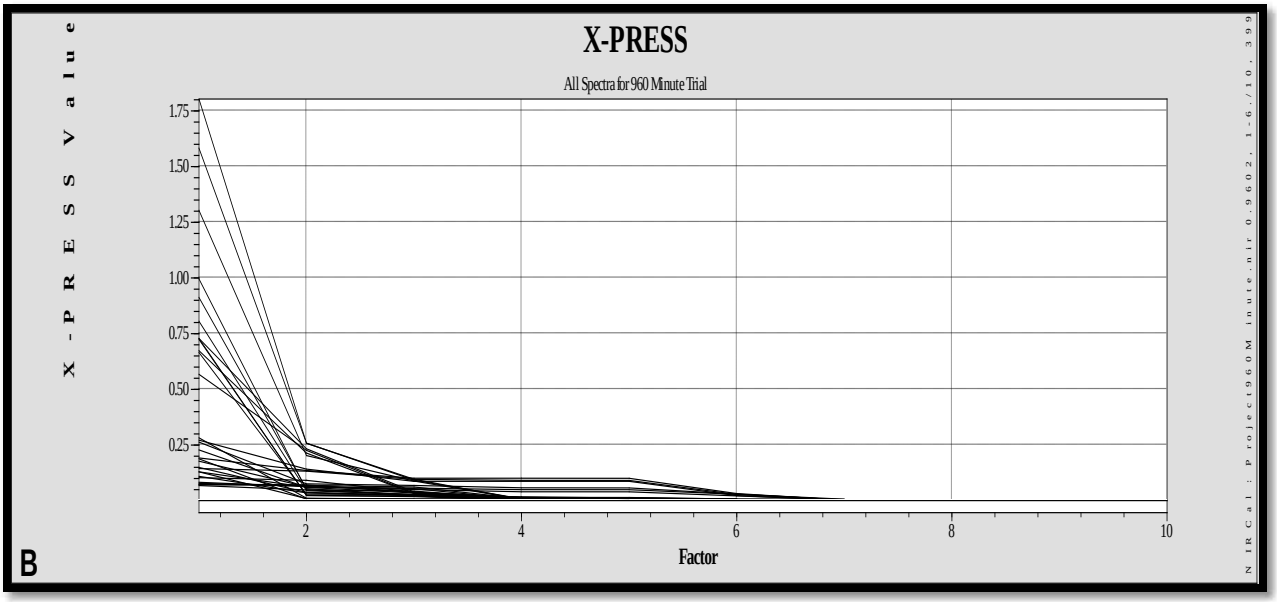

Figure 27 – The predicted residual sum of squares (X-PRESS) for the 960 minute trial.

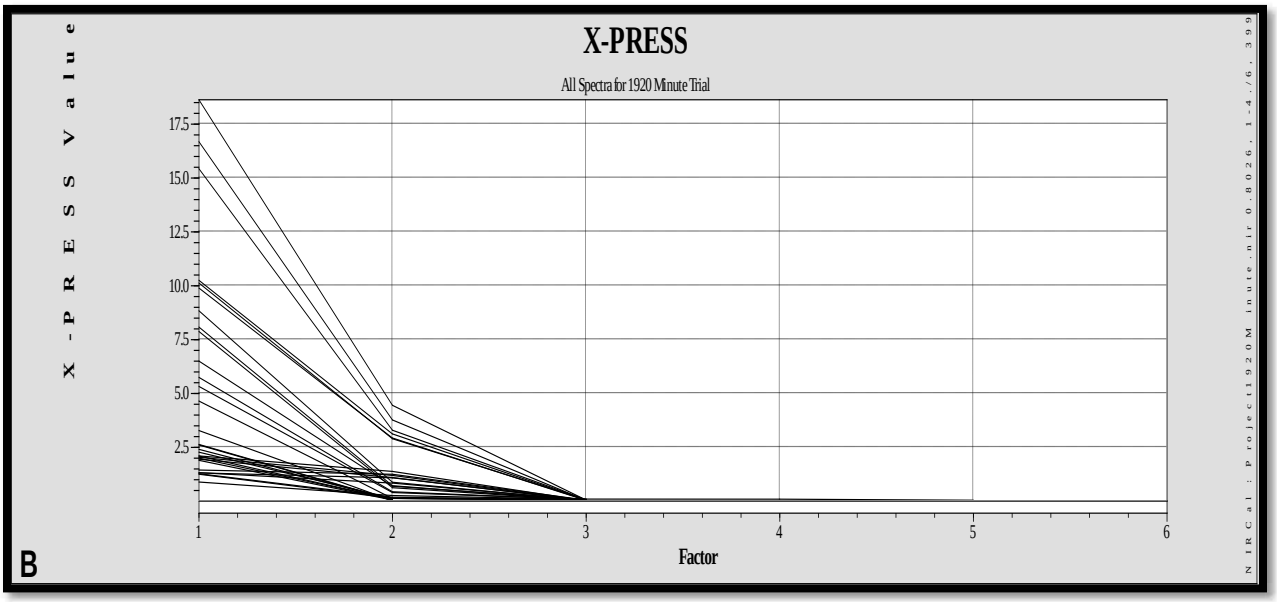

Figure 28 – The predicted residual sum of squares (X-PRESS) for the 1920 minute trial.

PLS Factors

The PLS factors accounted for the variations in response and were a function of all the input factors. Some of the smaller components that characterize the noise were excluded to avoid collinearity. The PLS factors in Figures 28-34 depict the regions from 4585.92-5330.32 and 6363.98-7208.65 cm^{-1} for the 30 minute trial, 4003.5-5353.46 and 6495-7293.51 cm^{-1} for the 60 minute trial, 4003.52-5334.17 and 6541.4-7227.94 cm^{-1} for the 120 minute trial, 4007.38-5326.46 and 6495.12-7200.94 cm^{-1} for the 240 minute trial, 4589.78-5372.74 and 6579.97-7208.65 cm^{-1} for the 480 minute trial, 4003.52-5649.6 and 6248-7301.22 cm^{-1} for the 960 minute trial, and 4007.38-5365.03 and 6360.12-7278.08 cm^{-1} for the 1920 minute trial of the spectra that made positive contributions to the regression equations. The aforementioned regions coincided with the established O-H stretching band of water in the region of 6240-7100 cm^{-1} and the P-OH, phosphate functional group, located at 5241 cm^{-1}.

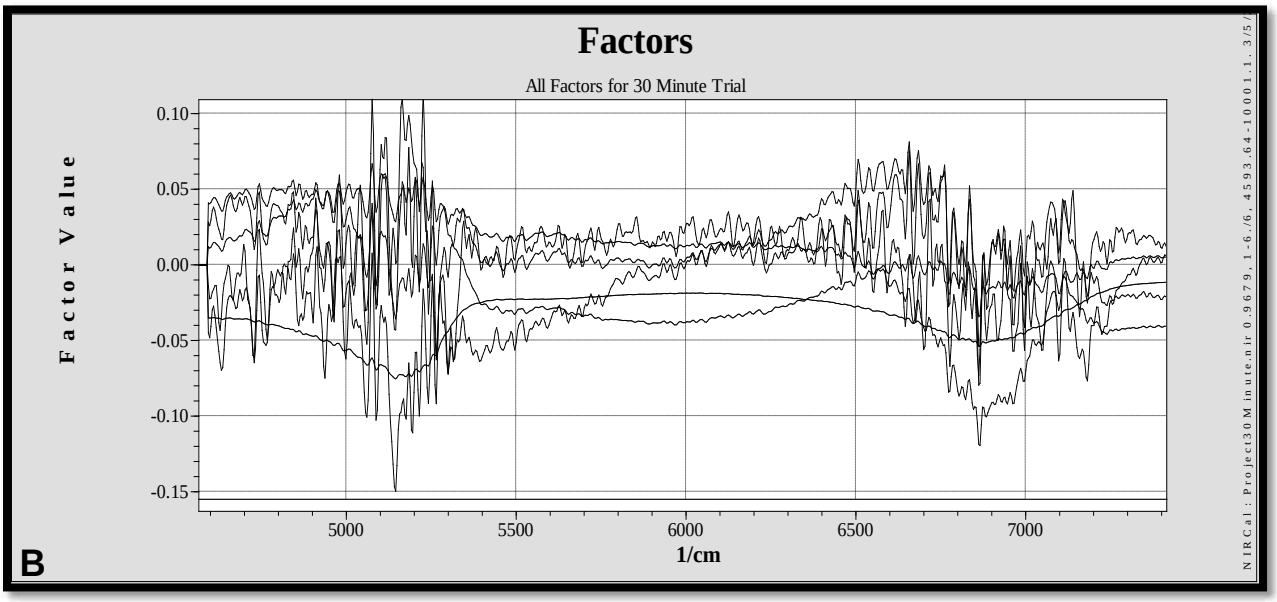

Figure 29 – The factors for the 30 minute treatment trial.

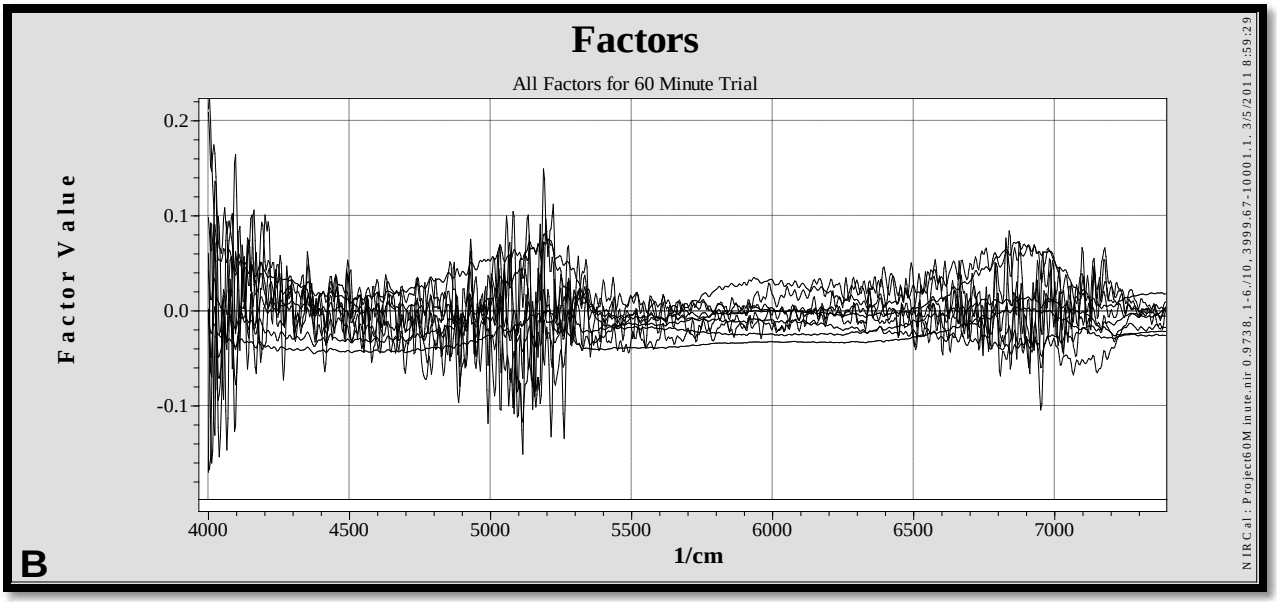

Figure 30 – The factors for the 60 minute treatment trial.

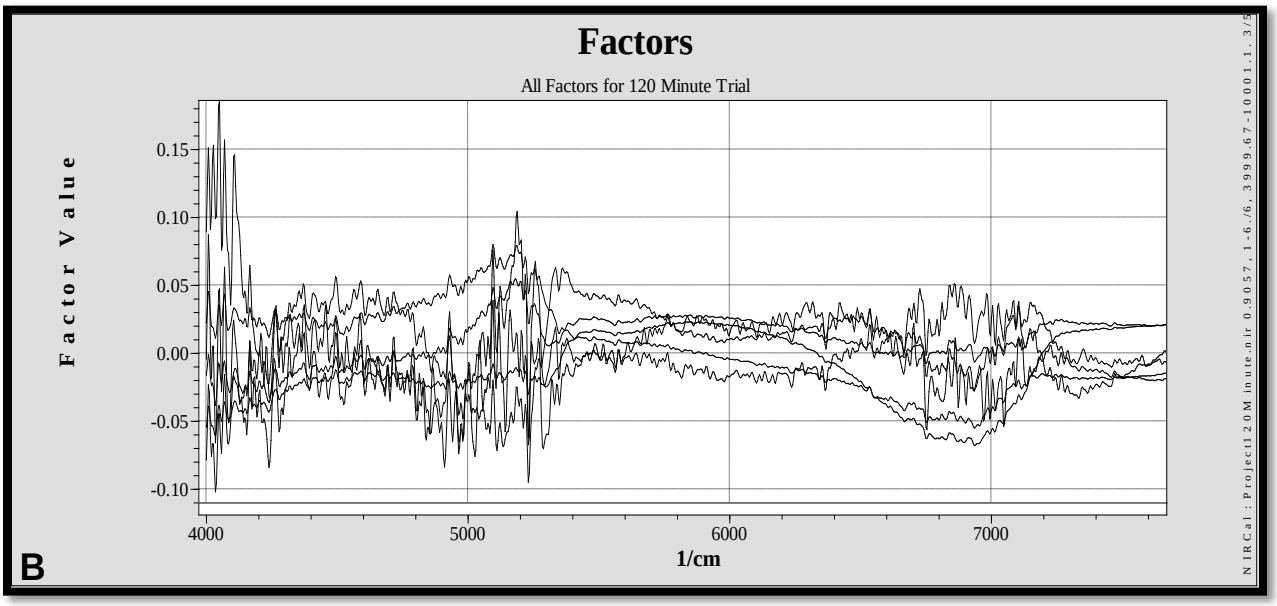

Figure 31 – The factors for the 120 minute treatment trial.

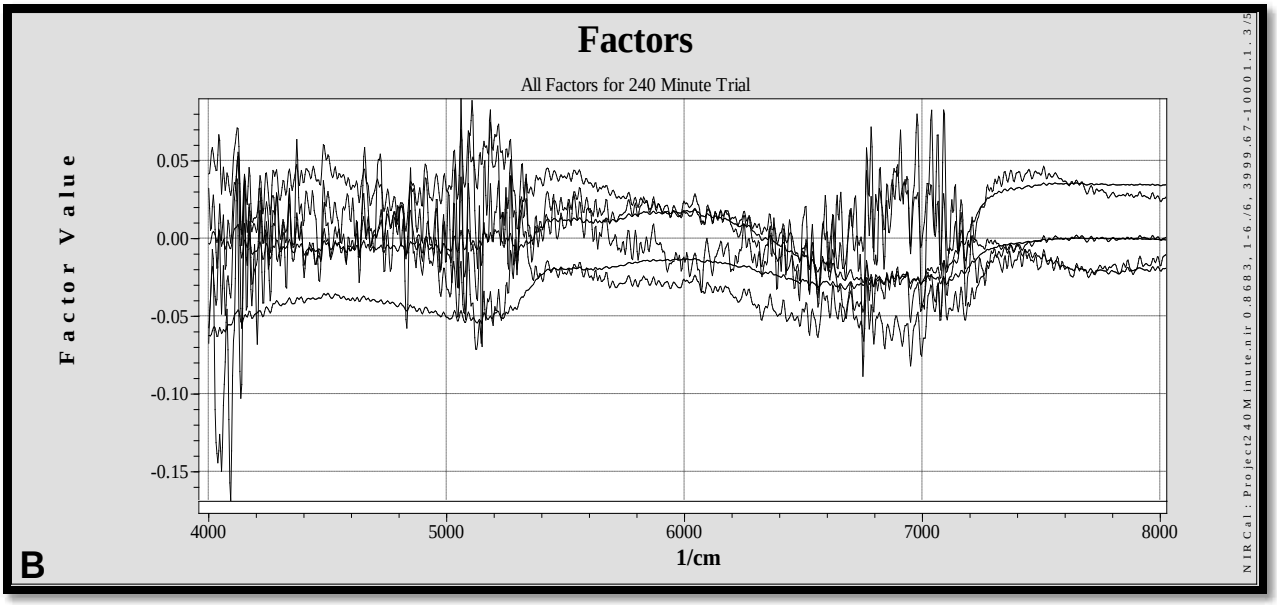

Figure 32 – The factors for the 240 minute treatment trial.

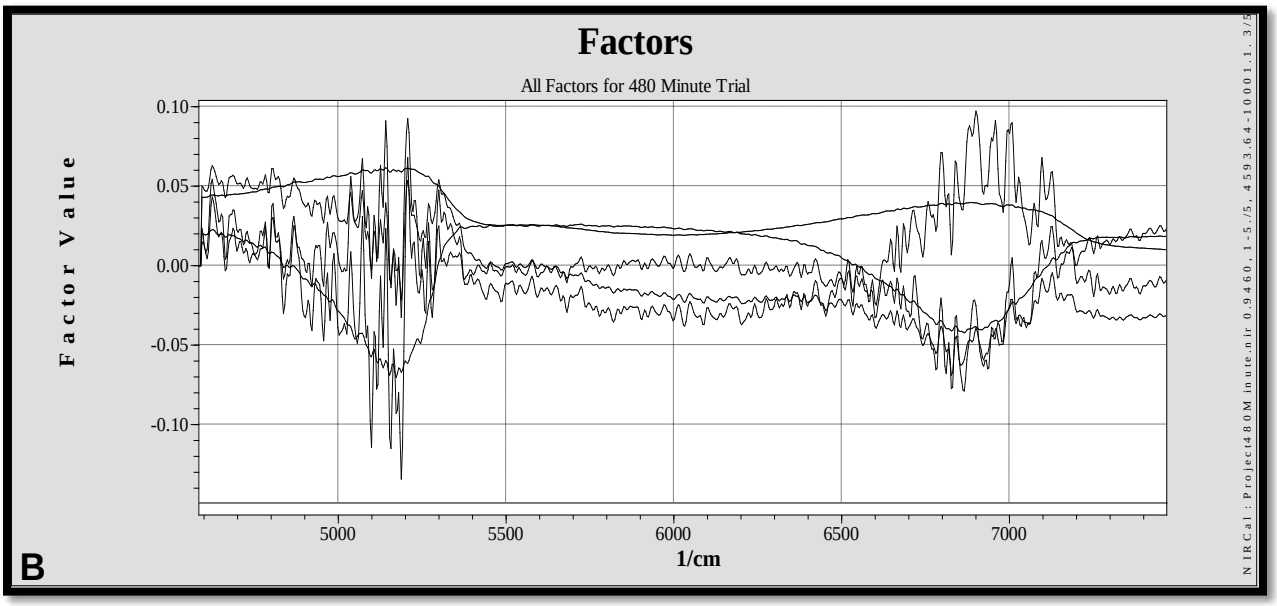

Figure 33 – The factors for the 480 minute treatment trial.

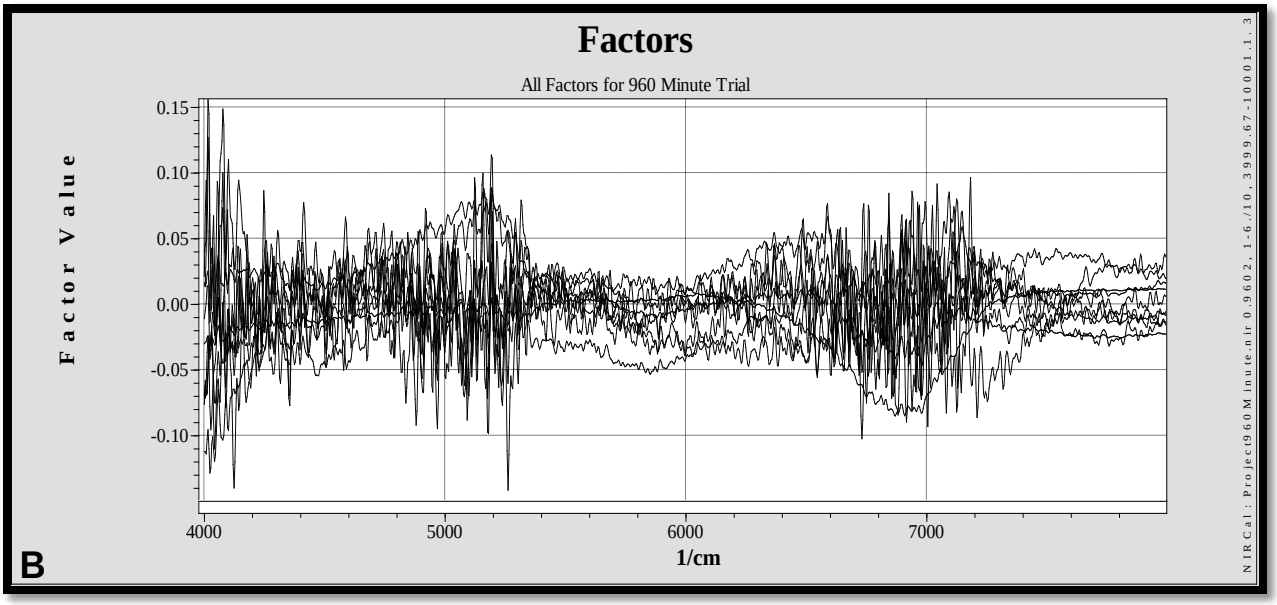

Figure 34 – The factors for the 960 minute treatment trial.

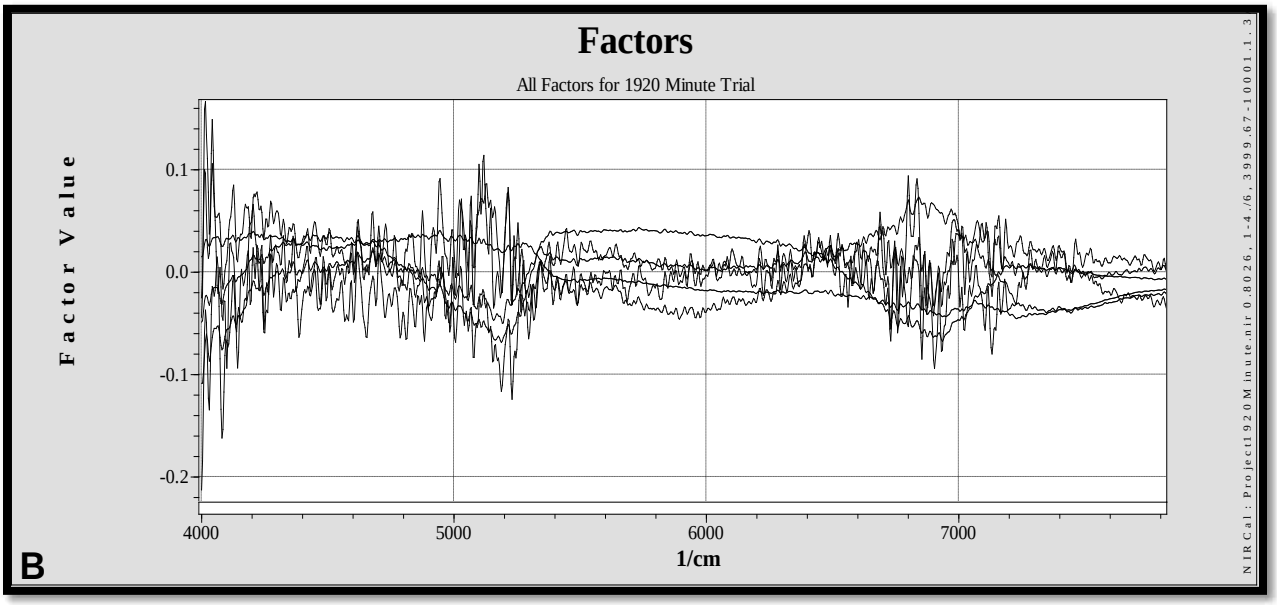

Figure 35 – The factors for the 1920 minute treatment trial.

Statistical Analysis

Table 2 – Results of the 3 way factorial analysis of variance for NIR absorbance.

Source	Type III Sum of Squares	df	Mean Square	F	Significance
Corrected Model	38.597	251	0.154	53.590	0.000
Intercept	1731.374	1	1731.374	107980.460	0.000
Wavenumber	34.252	5	6.850	427.241	0.000
Time	0.272	6	0.045	0.263	0.954
Concentration	1.364	6	0.227	1.318	0.251
Wavenumber * Time	0.595	30	0.020	1.237	0.196
Wavenumber * Concentration	0.170	24	0.007	0.408	0.994
Time * Concentration	1.831	36	0.051	0.295	1.000
Wavenumber * Time * Concentration	1.110	150	0.007	0.005	<0.001
Error	0.000	0	.		
Total	1769.971	252			
Corrected Total	38.597	251			

Dependent Variable: Absorbance

The level of acceptability, α, was set to 0.05. The results of the three-way factorial analysis of variance listed in Table 2 indicate the overall model is statistically significant (F=53.590, p=0.000). The wavenumber was also statistically significant (F=427.241, p=0.000). The results of the three-way factorial ANOVA concluded that the combination interaction of wavenumber, dip time, and solution concentration was statistically significant (F=0.005, p<0.001). The two factor interactions between concentration, time, and wavenumber were not statistically significant (p>0.05).

Chapter V

Conclusions

Workman and Weyer (2008) established the location of the phosphate functional group having a spectra-structure of P-OH at 1908 nm (5241 cm^{-1}). The elevated water content of the shrimp was a constraint for chemical parameter NIR analysis causing light scattering and interference. Due to the interference of the water bands there was a great deal of noise; therefore, it was virtually impossible to analyze the phosphate functional group. Bechman and Jorgensen (1998) also experienced this problem when analyzing whole cod, and concluded the sample temperature affected the accuracy. The experimental analysis was adapted to combine the changes in the moisture and phosphorus content. Unfortunately, the chemometric software was unable to process both the moisture and phosphorus content as calibration properties. The application of the phosphorus content as a calibration property was unable to identify sodium tripolyphosphate treated shrimp because of small variances in the levels of phosphorus residuals (Ravelo et al., 1991). Utilizing the moisture content as the sole partial least squares calibration property yielded the most acceptable results.

The experiment utilized an older model of the NIR, as well as the chemometric software. Some of the problems that arose during the chemometric analyses have been eliminated in the more recent generations of chemometric software. Shrimp are sensitive to time and temperature; therefore, the analysis is extremely complex. One of more other persistent issues arose from the internal power source of the older model NIR. The internal energy source of the Büchi N-200 spectrophotometer generated heat, which may have influenced the sample temperature; thus, interfering with the NIR analysis. The new NIR models utilize an eternal power source, which

would alleviate the temperature variations. The application of a repeatability file would off-set the temperature variations and improve the calibration. A study performed by Tillman and Paul (1998) illustrated the benefits of repeatability files with 50% or greater improvements in the performance values for NIR calibrations with moisture variations.

These experiments should be repeated with the most recent software and equipment to improve upon the results. NIR reflectance should not be completely rule out as a technique capable of evaluating shrimp treated with sodium tripolyphosphate. There were some clear distinctions among the various treatment times and concentrations. As long as the dip time was known, the PLS regression equations predicted the concentration of the sodium tripolyphosphate. It is highly improbable that a shrimp processor will divulge reliable information for the dip time of the sodium tripolyphosphate treatment.

In the future, fiber optic probes combined with non-contact transflectance near infrared imaging spectroscopy may be able to detect the changes in phosphorus content of sodium tripolyphosphate treated shrimp. An alternative way to solve this problem efficiently is to combine different detection techniques with NIR spectroscopy, such as X-ray fluorescence spectroscopy, and UV light. Perhaps freeze drying to remove the water and reduce the noise will provide more reliable results and a better calibration model can be built. Davies et al. (1998) have demonstrated the possible optimization of NIR spectroscopy with a sample database for chemical and NIR readings, so unknown samples can be analyzed. The preliminary results of the near infrared analysis were promising, but require further exploration to determine if this is an applicable procedure for sodium tripolyphosphate detection in treated shrimp.

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Appendix: Additional Statistics Tables

Table 3 – Descriptive Statistics based on 30 Minute Dip Time.

Time			Statistic	Std. Error
30.00	Mean		2.6408	.06541
	95% Confidence Interval for Mean	Lower Bound	2.5080	
		Upper Bound	2.7735	
	5% Trimmed Mean		2.6397	
	Median		2.8443	
	Variance		.154	
	Std. Deviation		.39245	
	Minimum		2.12	
	Maximum		3.17	
	Range		1.05	
	Interquartile Range		.77	
	Skewness		-.238	.393
	Kurtosis		-1.827	.768

Table 4 – Descriptive Statistics based on 60 Minute Dip Time.

Time			Statistic	Std. Error
60.00	Mean		2.6149	.06818
	95% Confidence Interval for Mean	Lower Bound	2.4765	
		Upper Bound	2.7533	
	5% Trimmed Mean		2.6121	
	Median		2.5813	
	Variance		.167	
	Std. Deviation		.40909	
	Minimum		2.15	
	Maximum		3.14	
	Range		.99	
	Interquartile Range		.79	
	Skewness		.059	.393
	Kurtosis		-1.973	.768

Table 5 – Descriptive Statistics based on 120 Minute Dip Time.

Time			Statistic	Std. Error
120.00	Mean		2.5431	.06570
	95% Confidence Interval for Mean	Lower Bound	2.4097	
		Upper Bound	2.6764	
	5% Trimmed Mean		2.5455	
	Median		2.5419	
	Variance		.155	
	Std. Deviation		.39418	
	Minimum		2.07	
	Maximum		2.97	
	Range		.90	
	Interquartile Range		.79	
	Skewness		-.003	.393
	Kurtosis		-2.064	.768

Table 6 – Descriptive Statistics based on 30 Minute Dip Time.

Time			Statistic	Std. Error
240.00	Mean		2.6660	.06593
	95% Confidence Interval for Mean	Lower Bound	2.5322	
		Upper Bound	2.7999	
	5% Trimmed Mean		2.6682	
	Median		2.6723	
	Variance		.156	
	Std. Deviation		.39558	
	Minimum		2.20	
	Maximum		3.09	
	Range		.89	
	Interquartile Range		.77	
	Skewness		-.013	.393
	Kurtosis		-2.086	.768

Table 7 – Descriptive Statistics based on 480 Minute Dip Time.

Time			Statistic	Std. Error
480.00	Mean		2.6626	.07936
	95% Confidence Interval for Mean	Lower Bound	2.5014	
		Upper Bound	2.8237	
	5% Trimmed Mean		2.6564	
	Median		2.5029	
	Variance		.227	
	Std. Deviation		.47618	
	Minimum		2.13	
	Maximum		3.31	
	Range		1.18	
	Interquartile Range		.93	
	Skewness		.185	.393
	Kurtosis		-1.887	.768

Table 8 – Descriptive Statistics based on 960 Minute Dip Time.

Time			Statistic	Std. Error
960.00	Mean		2.6196	.05088
	95% Confidence Interval for Mean	Lower Bound	2.5163	
		Upper Bound	2.7229	
	5% Trimmed Mean		2.6198	
	Median		2.6534	
	Variance		.093	
	Std. Deviation		.30528	
	Minimum		2.25	
	Maximum		2.98	
	Range		.74	
	Interquartile Range		.59	
	Skewness		-.017	.393
	Kurtosis		-1.994	.768

Table 9 – Descriptive Statistics based on 1920 Minute Dip Time.

Time			Statistic	Std. Error
1920.00	Mean		2.6013	.06206
	95% Confidence Interval for Mean	Lower Bound	2.4753	
		Upper Bound	2.7273	
	5% Trimmed Mean		2.5927	
	Median		2.5484	
	Variance		.139	
	Std. Deviation		.37237	
	Minimum		2.16	
	Maximum		3.19	
	Range		1.02	
	Interquartile Range		.64	
	Skewness		.302	.393
	Kurtosis		-1.469	.768

Table 10 – Descriptive Statistics based on Wavenumber (5237.75 $^{cm-1}$).

Wavenumber			Statistic	Std. Error
5237.75	Mean		2.9956	.02057
	95% Confidence Interval for Mean	Lower Bound	2.9540	
		Upper Bound	3.0371	
	5% Trimmed Mean		2.9930	
	Median		2.9837	
	Variance		.018	
	Std. Deviation		.13333	
	Minimum		2.74	
	Maximum		3.31	
	Range		.57	
	Interquartile Range		.20	
	Skewness		.333	.365
	Kurtosis		-.281	.717

Table 11 – Descriptive Statistics based on Wavenumber (5241.61^{cm-1}).

Wavenumber			**Statistic**	**Std. Error**
5241.61	Mean		2.9902	.02053
	95% Confidence Interval for Mean	Lower Bound	2.9487	
		Upper Bound	3.0316	
	5% Trimmed Mean		2.9876	
	Median		2.9801	
	Variance		.018	
	Std. Deviation		.13306	
	Minimum		2.73	
	Maximum		3.30	
	Range		.57	
	Interquartile Range		.20	
	Skewness		.335	.365
	Kurtosis		-.274	.717

Table 12– Descriptive Statistics based on Wavenumber (5245.46 $^{cm-1}$).

Wavenumber			**Statistic**	**Std. Error**
5245.46	Mean		2.9834	.02040
	95% Confidence Interval for Mean	Lower Bound	2.9421	
		Upper Bound	3.0246	
	5% Trimmed Mean		2.9806	
	Median		2.9737	
	Variance		.017	
	Std. Deviation		.13222	
	Minimum		2.73	
	Maximum		3.29	
	Range		.56	
	Interquartile Range		.19	
	Skewness		.342	.365
	Kurtosis		-.247	.717

Table 13 – Descriptive Statistics based on Wavenumber (7177.80^{cm-1}).

Wavenumber			**Statistic**	**Std. Error**
7177.80	Mean		2.2697	.02023
	95% Confidence Interval for Mean	Lower Bound	2.2289	
		Upper Bound	2.3106	
	5% Trimmed Mean		2.2551	
	Median		2.2464	
	Variance		.017	
	Std. Deviation		.13108	
	Minimum		2.10	
	Maximum		2.97	
	Range		.87	
	Interquartile Range		.10	
	Skewness		3.787	.365
	Kurtosis		19.641	.717

Table 14 – Descriptive Statistics based on Wavenumber (7181.66^{cm-1}).

Wavenumber			**Statistic**	**Std. Error**
7181.66	Mean		2.2526	.02050
	95% Confidence Interval for Mean	Lower Bound	2.2112	
		Upper Bound	2.2940	
	5% Trimmed Mean		2.2376	
	Median		2.2276	
	Variance		.018	
	Std. Deviation		.13285	
	Minimum		2.08	
	Maximum		2.96	
	Range		.88	
	Interquartile Range		.11	
	Skewness		3.827	.365
	Kurtosis		19.910	.717

Table 15 – Descriptive Statistics based on Wavenumber (7185.51^{cm-1}).

Wavenumber			Statistic	Std. Error
7185.51	Mean		2.2356	.02081
	95% Confidence Interval for Mean	Lower Bound	2.1936	
		Upper Bound	2.2776	
	5% Trimmed Mean		2.2201	
	Median		2.2088	
	Variance		.018	
	Std. Deviation		.13485	
	Minimum		2.07	
	Maximum		2.96	
	Range		.89	
	Interquartile Range		.11	
	Skewness		3.882	.365
	Kurtosis		20.291	.717

Table 16 – Descriptive Statistics based on Concentration of Dipping Solution (1% STPP & 1% NaCl).

Concentration			Statistic	Std. Error
1% STPP & 1% NaCl	Mean		2.6179	.06620
	95% Confidence Interval for Mean	Lower Bound	2.4842	
		Upper Bound	2.7516	
	5% Trimmed Mean		2.6083	
	Median		2.5749	
	Variance		.184	
	Std. Deviation		.42900	
	Minimum		2.10	
	Maximum		3.31	
	Range		1.21	
	Interquartile Range		.77	
	Skewness		.142	.365
	Kurtosis		-1.778	.717

Table 17 – Descriptive Statistics based on Concentration of Dipping Solution (2.5% STPP).

Concentration			Statistic	Std. Error
2.5% STPP	Mean		2.5964	.05514
	95% Confidence Interval for Mean	Lower Bound	2.4850	
		Upper Bound	2.7077	
	5% Trimmed Mean		2.5902	
	Median		2.5434	
	Variance		.128	
	Std. Deviation		.35736	
	Minimum		2.13	
	Maximum		3.17	
	Range		1.05	
	Interquartile Range		.61	
	Skewness		.142	.365
	Kurtosis		-1.725	.717

Table 18 – Descriptive Statistics based on Concentration of Dipping Solution (2% STPP & 2% NaCl).

Concentration			Statistic	Std. Error
2% STPP & 2% NaCl	Mean		2.5790	.06245
	95% Confidence Interval for Mean	Lower Bound	2.4529	
		Upper Bound	2.7052	
	5% Trimmed Mean		2.5683	
	Median		2.6197	
	Variance		.164	
	Std. Deviation		.40471	
	Minimum		2.07	
	Maximum		3.28	
	Range		1.21	
	Interquartile Range		.69	
	Skewness		.181	.365
	Kurtosis		-1.548	.717

Table 19 – Descriptive Statistics based on Concentration of Dipping Solution (5% STPP).

Concentration			Statistic	Std. Error
5% STPP	Mean		2.5904	.05914
	95% Confidence Interval for Mean	Lower Bound	2.4710	
		Upper Bound	2.7098	
	5% Trimmed Mean		2.5852	
	Median		2.5567	
	Variance		.147	
	Std. Deviation		.38329	
	Minimum		2.15	
	Maximum		3.12	
	Range		.98	
	Interquartile Range		.75	
	Skewness		.115	.365
	Kurtosis		-1.848	.717

Table 20 – Descriptive Statistics based on Concentration of Dipping Solution (control – 0%).

Concentration			Statistic	Std. Error
control	Mean		2.6458	.06155
	95% Confidence Interval for Mean	Lower Bound	2.5214	
		Upper Bound	2.7701	
	5% Trimmed Mean		2.6423	
	Median		2.6222	
	Variance		.159	
	Std. Deviation		.39890	
	Minimum		2.16	
	Maximum		3.19	
	Range		1.03	
	Interquartile Range		.76	
	Skewness		.066	.365
	Kurtosis		-1.912	.717

Table 21 – Descriptive Statistics based on Concentration of Dipping Solution (water - 0%).

Concentration			Statistic	Std. Error
water	Mean		2.6975	.05972
	95% Confidence Interval for Mean	Lower Bound	2.5769	
		Upper Bound	2.8181	
	5% Trimmed Mean		2.6998	
	Median		2.9177	
	Variance		.150	
	Std. Deviation		.38705	
	Minimum		2.16	
	Maximum		3.19	
	Range		1.03	
	Interquartile Range		.72	
	Skewness		-.216	.365
	Kurtosis		-1.833	.717

Table 22 - The Results of the LSMEANS Statistical Analysis for 30 minute treatment.

Wavenumber	Dip Time	Dipping Solution Concentration	abs LSMEAN
5237.75	30	1% STPP & 1% NaCl	2.954367
5237.75	30	2% STPP & 2% NaCl	2.823944
5237.75	30	2.5% STPP	3.173589
5237.75	30	5% STPP	2.875611
5237.75	30	control	2.9949
5237.75	30	DI Water	2.9764
5241.61	30	1% STPP & 1% NaCl	2.9431
5241.61	30	2% STPP & 2% NaCl	2.816311
5241.61	30	2.5% STPP	3.1656
5241.61	30	5% STPP	2.870689
5241.61	30	control	2.989867
5241.61	30	DI Water	2.976267
5245.46	30	1% STPP & 1% NaCl	2.932067
5245.46	30	2% STPP & 2% NaCl	2.808211
5245.46	30	2.5% STPP	3.154156
5245.46	30	5% STPP	2.864556
5245.46	30	control	2.986
5245.46	30	DI Water	2.972667
7177.8	30	1% STPP & 1% NaCl	2.176111
7177.8	30	2% STPP & 2% NaCl	2.157433
7177.8	30	2.5% STPP	2.278044
7177.8	30	5% STPP	2.204278
7177.8	30	control	2.23925
7177.8	30	DI Water	2.966867
7181.66	30	1% STPP & 1% NaCl	2.158933
7181.66	30	2% STPP & 2% NaCl	2.140144
7181.66	30	2.5% STPP	2.258789
7181.66	30	5% STPP	2.186756
7181.66	30	control	2.221883
7181.66	30	DI Water	2.9613
7185.51	30	1% STPP & 1% NaCl	2.143089
7185.51	30	2% STPP & 2% NaCl	2.123444
7185.51	30	2.5% STPP	2.239222
7185.51	30	5% STPP	2.170022
7185.51	30	control	2.205317
7185.51	30	DI Water	2.958067

Table 23 - The Results of the LSMEANS Statistical Analysis for 60 minute treatment.

Wavenumber	Dip Time	Dipping Solution Concentration	abs LSMEAN
5237.75	60	1% STPP & 1% NaCl	3.135389
5237.75	60	2% STPP & 2% NaCl	2.844678
5237.75	60	2.5% STPP	2.988889
5237.75	60	5% STPP	3.123522
5237.75	60	control	3.003689
5237.75	60	DI Water	3.003689
5241.61	60	1% STPP & 1% NaCl	3.1269
5241.61	60	2% STPP & 2% NaCl	2.839989
5241.61	60	2.5% STPP	2.985178
5241.61	60	5% STPP	3.118578
5241.61	60	control	3.000489
5241.61	60	DI Water	3.000489
5245.46	60	1% STPP & 1% NaCl	3.114122
5245.46	60	2% STPP & 2% NaCl	2.832478
5245.46	60	2.5% STPP	2.978278
5245.46	60	5% STPP	3.1105
5245.46	60	control	2.994289
5245.46	60	DI Water	2.994289
7177.8	60	1% STPP & 1% NaCl	2.228322
7177.8	60	2% STPP & 2% NaCl	2.1849
7177.8	60	2.5% STPP	2.3301
7177.8	60	5% STPP	2.186589
7177.8	60	control	2.246356
7177.8	60	DI Water	2.246356
7181.66	60	1% STPP & 1% NaCl	2.209511
7181.66	60	2% STPP & 2% NaCl	2.168433
7181.66	60	2.5% STPP	2.313856
7181.66	60	5% STPP	2.1668
7181.66	60	control	2.227589
7181.66	60	DI Water	2.227589
7185.51	60	1% STPP & 1% NaCl	2.190322
7185.51	60	2% STPP & 2% NaCl	2.151878
7185.51	60	2.5% STPP	2.298278
7185.51	60	5% STPP	2.146589
7185.51	60	control	2.208756
7185.51	60	DI Water	2.208756

Table 24 - The Results of the LSMEANS Statistical Analysis for 120 minute treatment.

Wavenumber	Dip Time	Dipping Solution Concentration	abs LSMEAN
5237.75	120	1% STPP & 1% NaCl	2.965889
5237.75	120	2% STPP & 2% NaCl	2.875989
5237.75	120	2.5% STPP	2.878133
5237.75	120	5% STPP	2.964229
5237.75	120	control	2.967156
5237.75	120	DI Water	2.967156
5241.61	120	1% STPP & 1% NaCl	2.9587
5241.61	120	2% STPP & 2% NaCl	2.868122
5241.61	120	2.5% STPP	2.873611
5241.61	120	5% STPP	2.95775
5241.61	120	control	2.960267
5241.61	120	DI Water	2.960267
5245.46	120	1% STPP & 1% NaCl	2.9498
5245.46	120	2% STPP & 2% NaCl	2.860333
5245.46	120	2.5% STPP	2.866256
5245.46	120	5% STPP	2.949706
5245.46	120	control	2.951833
5245.46	120	DI Water	2.951833
7177.8	120	1% STPP & 1% NaCl	2.132189
7177.8	120	2% STPP & 2% NaCl	2.101333
7177.8	120	2.5% STPP	2.2234
7177.8	120	5% STPP	2.199311
7177.8	120	control	2.194744
7177.8	120	DI Water	2.194744
7181.66	120	1% STPP & 1% NaCl	2.114056
7181.66	120	2% STPP & 2% NaCl	2.084311
7181.66	120	2.5% STPP	2.206978
7181.66	120	5% STPP	2.180189
7181.66	120	control	2.177489
7181.66	120	DI Water	2.177489
7185.51	120	1% STPP & 1% NaCl	2.096856
7185.51	120	2% STPP & 2% NaCl	2.068144
7185.51	120	2.5% STPP	2.190389
7185.51	120	5% STPP	2.160889
7185.51	120	control	2.160322
7185.51	120	DI Water	2.160322

Table 25 - The Results of the LSMEANS Statistical Analysis for 240 minute treatment.

Wavenumber	Dip Time	Dipping Solution Concentration	abs LSMEAN
5237.75	240	1% STPP & 1% NaCl	3.050767
5237.75	240	2% STPP & 2% NaCl	3.091522
5237.75	240	2.5% STPP	3.019367
5237.75	240	5% STPP	3.078011
5237.75	240	control	3.063044
5237.75	240	DI Water	3.063044
5241.61	240	1% STPP & 1% NaCl	3.046378
5241.61	240	2% STPP & 2% NaCl	3.083167
5241.61	240	2.5% STPP	3.014278
5241.61	240	5% STPP	3.071433
5241.61	240	control	3.057178
5241.61	240	DI Water	3.057178
5245.46	240	1% STPP & 1% NaCl	3.039878
5245.46	240	2% STPP & 2% NaCl	3.076311
5245.46	240	2.5% STPP	3.007444
5245.46	240	5% STPP	3.061544
5245.46	240	control	3.051056
5245.46	240	DI Water	3.051056
7177.8	240	1% STPP & 1% NaCl	2.301244
7177.8	240	2% STPP & 2% NaCl	2.238089
7177.8	240	2.5% STPP	2.307111
7177.8	240	5% STPP	2.247311
7177.8	240	control	2.3371
7177.8	240	DI Water	2.3371
7181.66	240	1% STPP & 1% NaCl	2.284067
7181.66	240	2% STPP & 2% NaCl	2.219056
7181.66	240	2.5% STPP	2.2923
7181.66	240	5% STPP	2.229611
7181.66	240	control	2.319389
7181.66	240	DI Water	2.319389
7185.51	240	1% STPP & 1% NaCl	2.267178
7185.51	240	2% STPP & 2% NaCl	2.200811
7185.51	240	2.5% STPP	2.277733
7185.51	240	5% STPP	2.211267
7185.51	240	control	2.302589
7185.51	240	DI Water	2.302589

Table 26 - The Results of the LSMEANS Statistical Analysis for 480 minute treatment.

Wavenumber	Dip Time	Dipping Solution Concentration	abs LSMEAN
5237.75	480	1% STPP & 1% NaCl	3.308756
5237.75	480	2% STPP & 2% NaCl	3.274778
5237.75	480	2.5% STPP	2.7575
5237.75	480	5% STPP	3.054078
5237.75	480	control	3.161389
5237.75	480	DI Water	3.161389
5241.61	480	1% STPP & 1% NaCl	3.298656
5241.61	480	2% STPP & 2% NaCl	3.275478
5241.61	480	2.5% STPP	2.752411
5241.61	480	5% STPP	3.047667
5241.61	480	control	3.154433
5241.61	480	DI Water	3.154433
5245.46	480	1% STPP & 1% NaCl	3.288511
5245.46	480	2% STPP & 2% NaCl	3.272922
5245.46	480	2.5% STPP	2.745767
5245.46	480	5% STPP	3.0427
5245.46	480	control	3.144256
5245.46	480	DI Water	3.144256
7177.8	480	1% STPP & 1% NaCl	2.237622
7177.8	480	2% STPP & 2% NaCl	2.219789
7177.8	480	2.5% STPP	2.156722
7177.8	480	5% STPP	2.248511
7177.8	480	control	2.260078
7177.8	480	DI Water	2.260078
7181.66	480	1% STPP & 1% NaCl	2.217056
7181.66	480	2% STPP & 2% NaCl	2.198567
7181.66	480	2.5% STPP	2.1409
7181.66	480	5% STPP	2.2306
7181.66	480	control	2.241722
7181.66	480	DI Water	2.241722
7185.51	480	1% STPP & 1% NaCl	2.196644
7185.51	480	2% STPP & 2% NaCl	2.177244
7185.51	480	2.5% STPP	2.125111
7185.51	480	5% STPP	2.212967
7185.51	480	control	2.223622
7185.51	480	DI Water	2.223622

Table 27 - The Results of the LSMEANS Statistical Analysis for 960 minute treatment.

Wavenumber	Dip Time	Dipping Solution Concentration	abs LSMEAN
5237.75	960	1% STPP & 1% NaCl	2.982489
5237.75	960	2% STPP & 2% NaCl	2.9849
5237.75	960	2.5% STPP	2.890378
5237.75	960	5% STPP	2.898632
5237.75	960	control	2.883522
5237.75	960	DI Water	2.883522
5241.61	960	1% STPP & 1% NaCl	2.979611
5241.61	960	2% STPP & 2% NaCl	2.980644
5241.61	960	2.5% STPP	2.886089
5241.61	960	5% STPP	2.894762
5241.61	960	control	2.879078
5241.61	960	DI Water	2.879078
5245.46	960	1% STPP & 1% NaCl	2.973956
5245.46	960	2% STPP & 2% NaCl	2.973444
5245.46	960	2.5% STPP	2.880011
5245.46	960	5% STPP	2.891425
5245.46	960	control	2.8756
5245.46	960	DI Water	2.8756
7177.8	960	1% STPP & 1% NaCl	2.279056
7177.8	960	2% STPP & 2% NaCl	2.4312
7177.8	960	2.5% STPP	2.340956
7177.8	960	5% STPP	2.385456
7177.8	960	control	2.297667
7177.8	960	DI Water	2.297667
7181.66	960	1% STPP & 1% NaCl	2.261456
7181.66	960	2% STPP & 2% NaCl	2.416967
7181.66	960	2.5% STPP	2.324089
7181.66	960	5% STPP	2.371922
7181.66	960	control	2.282189
7181.66	960	DI Water	2.282189
7185.51	960	1% STPP & 1% NaCl	2.245156
7185.51	960	2% STPP & 2% NaCl	2.402122
7185.51	960	2.5% STPP	2.307656
7185.51	960	5% STPP	2.357411
7185.51	960	control	2.265489
7185.51	960	DI Water	2.265489

Table 28 - The Results of the LSMEANS Statistical Analysis for 1920 minute treatment.

Wavenumber	Dip Time	Dipping Solution Concentration	abs LSMEAN
5237.75	1920	1% STPP & 1% NaCl	2.857233
5237.75	1920	2% STPP & 2% NaCl	2.8406
5237.75	1920	2.5% STPP	2.884311
5237.75	1920	5% STPP	2.735811
5237.75	1920	control	3.185489
5237.75	1920	DI Water	3.185489
5241.61	1920	1% STPP & 1% NaCl	2.853022
5241.61	1920	2% STPP & 2% NaCl	2.837044
5241.61	1920	2.5% STPP	2.879089
5241.61	1920	5% STPP	2.7314
5241.61	1920	control	3.181511
5241.61	1920	DI Water	3.181511
5245.46	1920	1% STPP & 1% NaCl	2.848556
5245.46	1920	2% STPP & 2% NaCl	2.8322
5245.46	1920	2.5% STPP	2.874678
5245.46	1920	5% STPP	2.727844
5245.46	1920	control	3.175189
5245.46	1920	DI Water	3.175189
7177.8	1920	1% STPP & 1% NaCl	2.2191
7177.8	1920	2% STPP & 2% NaCl	2.197411
7177.8	1920	2.5% STPP	2.276844
7177.8	1920	5% STPP	2.224656
7177.8	1920	control	2.368867
7177.8	1920	DI Water	2.368867
7181.66	1920	1% STPP & 1% NaCl	2.202067
7181.66	1920	2% STPP & 2% NaCl	2.1814
7181.66	1920	2.5% STPP	2.260422
7181.66	1920	5% STPP	2.210144
7181.66	1920	control	2.35
7181.66	1920	DI Water	2.35
7185.51	1920	1% STPP & 1% NaCl	2.184867
7185.51	1920	2% STPP & 2% NaCl	2.164256
7185.51	1920	2.5% STPP	2.242956
7185.51	1920	5% STPP	2.195333
7185.51	1920	control	2.331056
7185.51	1920	DI Water	2.331056

Table 29 – Analysis of variance for moisture content.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
time	6	216.1877873	36.0312979	14.85	<.0001
treatment	5	99.1315373	19.8263075	8.17	<.0001
time*treatment	30	38.0722794	1.2690760	0.52	0.9762

Table 30 – Least-squares means for moisture content influenced by treatment and time.

Treatment	Time							Average	s.d.
	30	60	120	240	480	960	1920		
Control	76.86	79.6	78.40	76.38	76.99	79.03	78.80	78.01	1.08
Deionized Water	79.44	79.91	79.91	78.73	80.75	82.51	83.15	80.63	1.41
1% NaCl & 1% STP	79.09	79.90	80.05	79.16	79.26	83.52	82.04	80.43	1.47
2% NaCl & 2% STP	79.11	79.60	79.68	79.58	79.35	82.66	82.07	80.29	1.25
2.5% STP	78.45	79.24	79.69	79.30	79.96	83.26	82.23	80.30	1.52
5% STP	78.83	79.24	79.15	78.49	79.59	81.96	81.56	79.83	1.18

Table 31 – Analysis of variance for phosphorus content.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
time	6	96.001728	16.000288	6.70	<.0001
treatment	5	1257.340532	251.468106	105.30	<.0001
time*treatment	30	438.449330	14.614978	6.12	<.0001

Table 32 – Least-squares means for phosphorus content influenced by treatment and time.

Treatment	Time							Average	s.d.
	30	60	120	240	480	960	1920		
Control	9.95	9.97	10.30	10.38	10.45	8.29	9.06	9.77	0.74
Deionized Water	10.65	9.64	10.14	10.63	9.17	6.76	6.71	9.10	1.57
1% NaCl & 1% STP	9.92	10.14	10.91	11.01	11.20	9.65	10.94	10.54	0.57
2% NaCl & 2% STP	10.36	10.61	12.08	12.29	12.42	14.38	14.55	12.38	1.51
2.5% STP	11.73	11.75	12.98	13.25	14.10	15.94	14.63	13.48	1.42
5% STP	14.83	14.03	15.79	16.03	19.66	24.97	24.35	18.52	4.22

Vita

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