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# The Use of Polyphosphates to Maintain Yield and Quality of Whole Cooked, Cryogenically Frozen Lobster (*Homarus americanus*) and the Use of Sorbitol and Tocopherol to Maintain Quality of Whole Cooked, Cryogenically Frozen Crab (*Cancer irroratus*)

Beth Louise Calder

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**THE USE OF POLYPHOSPHATES TO MAINTAIN YIELD AND  
QUALITY OF WHOLE COOKED, CRYOGENICALLY FROZEN  
LOBSTER (*HOMARUS AMERICANUS*) AND THE USE OF SORBITOL  
AND TOCOPHEROL TO MAINTAIN QUALITY OF WHOLE COOKED,  
CRYOGENICALLY FROZEN CRAB (*CANCER IRRORATUS*)**

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A.S. University of Maine at Farmington, 1990

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A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Doctor of Philosophy

(in Food and Nutrition Sciences)

The Graduate School

The University of Maine

May, 2003

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By Beth Louise Calder

Thesis Advisor: Dr. Alfred Bushway

An Abstract of the Thesis Presented  
in Partial Fulfillment of the Requirements for the  
Degree of Doctor of Philosophy  
(in Food and Nutrition Sciences)  
May, 2003

The lobster fishery is valuable to Maine seafood processors, a commodity worth over \$160 million dollars in 2001. Jonah and Rock crab are underutilized Maine seafood species, which are incidentally caught with lobster. Maine seafood processors could process this non-essential catch, and lobster into high quality frozen seafood products to increase national and international markets, which may have a significant economic impact.

Cryogenic freezing can retain high quality texture and flavor attributes in seafood. Freezing can still cause cellular damage, however minimal. Sodium tripolyphosphate (STP) and sorbitol are noted

for their cryoprotectant effects, and tocopherol has been found to have antioxidant effects to help prevent off-flavor and odor formation during storage.

This study was designed to utilize a patent developed at the University of Maine to improve seafood quality by injecting food additives to enhance shelf-life of frozen seafood. Two objectives were investigated to determine:

- 1) effects of sodium tripolyphosphate to maintain quality and increase yield of whole cooked, cryogenically frozen lobster
- 2) effects of sorbitol and tocopherol to maintain quality of whole cooked, cryogenically frozen Rock crab.

STP concentrations of 0.1 and 0.3% prepared in 0.9% saline solution were injected into lobster. Controls were injected with 0.9% saline solution. Crabs were injected in their crusher claw joints with either 1g sorbitol/2.5g tocopherol or 2g sorbitol/2.5g tocopherol. Control crabs were not treated. Both crab and lobsters were stored at -15°C until further chemical, physical, and sensory analyses were conducted on reheated samples. Lobsters were analyzed at storage months 0, 2, 4, and 6, while crab samples were analyzed at months 0, 3, 6, 9, and 12.

Lobster results indicate that polyphosphates added at low concentrations may extend shelf-life of cryogenically frozen lobsters, decrease lipid oxidation, maintain texture, color, and flavor attributes, decrease drip loss and increase yield (at 0.3% STP).

Crab results indicate that injecting crabs with 1g sorbitol/tocopherol may be beneficial in maintaining the shelf-life of whole cooked, cryogenically frozen crab, as indicated by sensory ratings.

Significance of this research indicates that Maine processors could economically profit from this injection technology, while meeting future demand for lobster, and creating new markets for Maine crab species.

## **ACKNOWLEDGMENTS**

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## **INTRODUCTION:**

### **Lobster Industry:**

The lobster industry is a valuable fishery to Maine seafood businesses. Preliminary data compiled from the Maine Department of Marine Resources (DMR) and the National Marine Fisheries Service (NMFS) for 2001 Maine Commercial Landings by Value has determined the total value of Maine's seafood commodities to be worth approximately \$300,375,307 dollars (DMR and NMFS, 2001). Maine lobster contributed 51% (\$153,191,406) of this total value. Lobster landings have increased steadily since the early 1990's reaching a record high of approximately 50 million pounds of lobster, close to a value of over \$160 million dollars as determined by preliminary data published by DMR (2001). Maine is the major producing State of *Homarus americanus* lobster species in the United States, which produced more than two-thirds of the U.S. American lobster catch in 2000 (Seafood Business, 2002). FAO (2000) reports that Canada and the United States are both leading lobster producers, each sharing 25% of the world's lobster production. The U.S. accounts for 36% of the world's live export of fresh and chilled lobster while Canada is the leading frozen lobster producer, representing 25% of the world's output. The U.S. import of Canadian frozen lobster products has increased from 3,000 to 7,500 metric tons since 1995 (Seafood Business, 2002). There is an obvious rising demand for frozen lobster. These facts reveal an

opportunity for Maine seafood processors to increase frozen lobster production to compete with Canada's frozen market. Frozen lobster products could have a significant economic impact for Maine seafood processors in the future.

### **Crab Industry:**

Jonah (*Cancer magister*) and Rock (*Cancer irroratus*) crab are underutilized seafood species in Maine. They are often incidentally caught in lobster traps and contribute to the crab meat picking industry in Maine. The two crab species can be over a pound in weight and most of the crab meat is contained in the crusher claws.

Preliminary data compiled from the Maine DMR and NMFS for 2001 has determined Maine's crab to be worth approximately 1% of the total value of Maine's commercial landings, approximately \$3 million dollars (DMR and NMFS, 2001). However, crab landings have steadily increased since 1999 from 1.5 to over 6 million pounds, with a value of \$3.5 million dollars (DMR, 2001). Seafood processors could process this non-essential catch into a high quality, whole cooked frozen product, which could open a new market for Rock and Jonah crab in Maine. Processing a whole frozen crab product would decrease byproduct shell waste for seafood processors and also increase the market value of Maine crab.

### **Freezing Lobster and Crabs:**

Research that supports extending the shelf-life of a whole cooked, frozen lobster product to compete with Canada's frozen brine "popsicle pack" lobster would be in the best interest of seafood processors in Maine. Providing a high quality, frozen Maine seafood product would open many national and international markets for both Maine lobster and crabs.

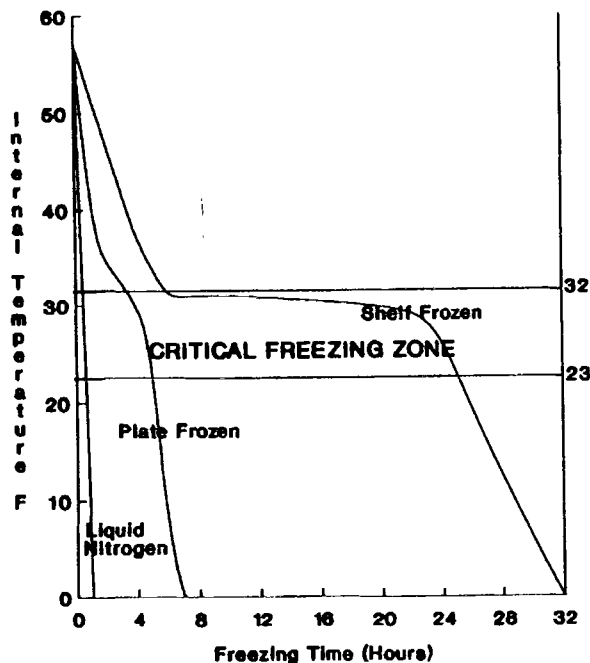
Preserving whole lobster by freezing to aid Maine's seafood industry is not a novel idea. In the 1950's, researchers investigated ways to freeze Maine lobster to better meet demands and cover seasonal availability for the lobster industry (Getchell and Highlands, 1957). A frozen lobster product would be more accessible to a wider range of markets because of ease in shipping with reduced icing and labor costs.

However, investigators have encountered problems with freezing seafood in the past, such as toughening of meat, development of off-flavors in storage, and difficulty separating meat from the shell after thawing and cooking (Getchell and Highlands, 1957). Dagbjartsson (1970) investigated the alterations in heated lobster muscle during low temperature storage and reported that precooked lobster meat suffers textural changes in the form of toughening during frozen storage. The changes could be detected organoleptically from a trained panel and objectively by Warner-Bratzler shear measurements. He determined that the rate of textural change at -12°C was greater than for lobsters stored at -27°C. The rate of toughening, measured as increase in Warner-Bratzler

shear force, was twice as great at -12°C than at -27°C. Seasonal variations in lobster texture were also detected in fresh lobster. Lobsters were found to be more tender during the warmer months of the year (May-November). Cooking the lobster muscle pre and post-rigor, did not result in differences of shear force measurement, but lobster cooked during rigor had a tougher texture.

Prior to the arrival of cryogenic freezing, frozen meat products suffered major losses of flavor, nutrients, texture, and moisture resulting in excessive drip upon thawing (Henry et al., 1995). Indicating the importance of reducing seafood internal temperature through the critical freezing zone (32-23° F/0-5° C) as rapidly as possible (Licciardello, 1990). This temperature region is where most cellular freezing damage occurs. The faster seafood passes through the critical freezing zone, the less likely that large ice crystals will form, which can puncture tissues causing cellular damage.

**Figure 1.1-Critical Freezing Time of Three Types of Freezing**



(Licciardello, 1990)

Figure 1.1 illustrates three common freezing types used in the seafood industry. The shelf-frozen freezing technique has a slow freezing rate, and depending on the thickness of the seafood product, can pass slowly (19 hours) through the critical freezing zone. Seafood is placed onto trays in a freezer room with no air circulation. The plate freezing process has a quicker freezing rate. The seafood can pass through the critical zone in less than 2 hours. This type of freezing circulates refrigerant between two plates placed next to the seafood product. Mechanical air-blast freezing provides another means for quick freezing as well, by circulating cold air over the product. Liquid nitrogen utilizes an ultra-rapid freezing rate, and cryogenic freezing is an example of an ultra-rapid freezing technique. Cryogenic freezing involves spraying or submerging the product in liquid

nitrogen (b.p.  $-195^{\circ}\text{C}$ ) or liquid carbon dioxide (b.p.  $-78^{\circ}\text{C}$ ) resulting in extremely fast freezing. The seafood product passes through the critical zone in less than two hours, and depending on the thickness of the product, potentially in only a matter of minutes. Due to the fact that the seafood product passes so quickly through the critical freezing zone, the quality of the frozen product closely resembles that of the fresh product (Licciardello, 1990).

Research supports the use of cryogenic freezing to retain high quality texture and flavor attributes in seafood. Work et al. (1997) investigated the effect of cryogenic freezing on the quality of soft and hard shell lobsters. They found that fast freezing of soft shell lobsters maintained good quality attributes during frozen storage for up to 9 months. None of the treatments evaluated were found to have any fishy off-flavors over the storage period. The researchers attributed the superior textural qualities of both hard and soft shell samples to the rapid rate of freezing. Henry et al. (1995) determined that cryogenically frozen hand-picked blue crab meat with cryoprotectants added, maintained acceptable texture and chemical qualities after approximately 8 months of frozen storage at  $-29^{\circ}\text{C}$ , compared to crab that had been pasteurized or frozen without cryoprotectants.

Freezing, as a method of food preservation, may cause cell damage by affecting the mechanical structure of membranes and causing proteins to denature due to ice crystal formation (Sanz et al., 1999).



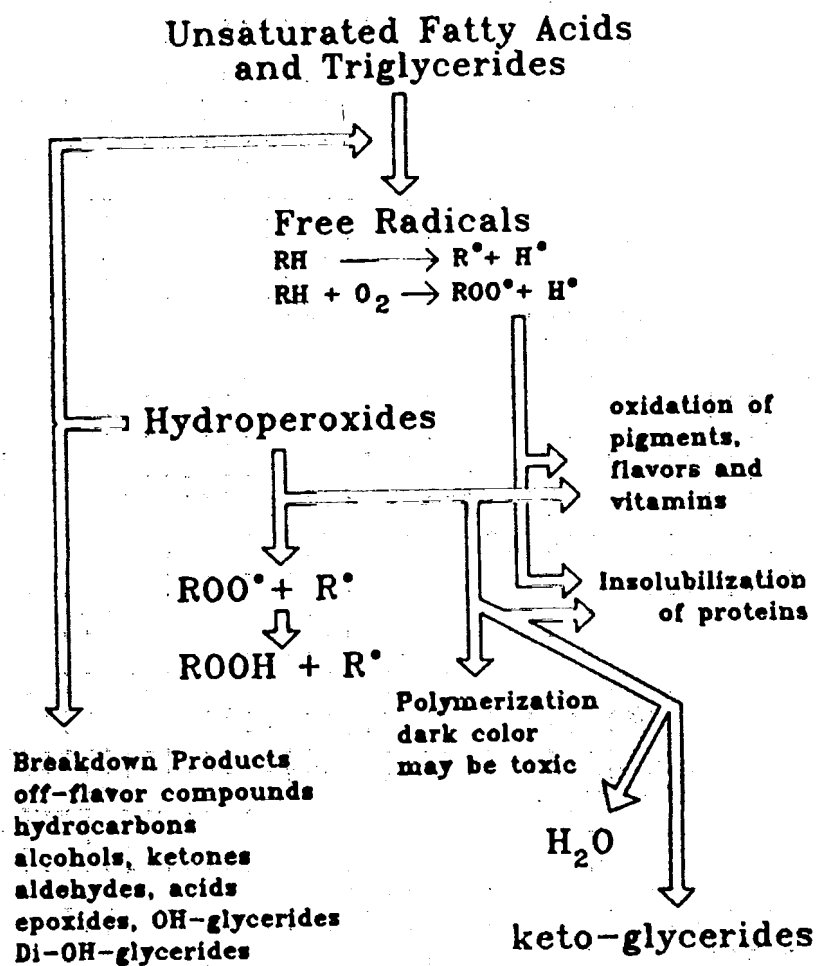
Muscle fiber dehydration increases the ionic strength in the cell, causing the proteins to denature. When proteins are damaged, the product will exude fluid upon thawing and the texture will toughen (Licciardello, 1990). Even though cryogenic freezing techniques will decrease moisture loss during freezing more than using conventional methods, some ice crystals can form due to the nature of water transition from a liquid to solid state and some cellular damage is inevitable, however minimal.

#### **Cryoprotectants and Antioxidants:**

Krivchenia and Fennema (1988) found that cryoprotectants such as sorbitol, and sodium tripolyphosphate reduce the rate of deteriorative changes in fish and fishery products during frozen storage. Sodium tripolyphosphate (STP) has been shown to reduce drip loss and increase tenderness, and the addition of sorbitol to surimi has been shown to maintain protein functionality during long periods of frozen storage (Krivchenia and Fennema, 1988)

Fish are more prone to lipid oxidation than other meat products because of the high degree of unsaturation in fish lipids and also the presence of metals in seafood (Ramanathan and Das, 1992). Lipid oxidation is defined as the oxidative deterioration of unsaturated fatty acids or triglycerides in either fresh or frozen seafood. It occurs via a chain reaction mechanism initiated by free radicals either enzymatically or nonenzymatically.

**Figure 1.2-Lipid Oxidation Pathway**



(Khayat and Schwall, 1983)

The proposed mechanism of lipid oxidation (autoxidation) occurs in three steps (Khayat and Schwall, 1983):

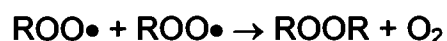
1) Initiation:  $\text{RH (unsaturated lipid)} \rightarrow \text{R}\bullet + \text{H}\bullet$  (free radicals)



2) Propagation:  $\text{R}\bullet + \text{O}_2 \rightarrow \text{ROO}\bullet$



3) Termination:  $\text{ROO}\bullet + \text{R}\bullet \rightarrow \text{ROOR}$



(Non-radical breakdown products)

The mechanism for lipid oxidation is via free radical attack, and the initial substrate for the reaction is unsaturated lipids (Khayat and Schwall, 1983).

Once the reaction has been initiated, oxidation is propagated by the removal of hydrogen atoms at  $\alpha$  positions to fatty acid double bonds, producing free radical species (Nawar, 1996). Oxygen addition then occurs, resulting in the production of peroxy radicals, and these radicals in turn remove hydrogen from  $\alpha$  positions of other unsaturated fat molecules. This reaction yields hydroperoxides and new free radicals, which react with oxygen, and the sequence of reactions are repeated (Nawar, 1996). The formed hydroperoxides that are converted to free radicals, in turn can accelerate the rate of lipid oxidation (Khayat and Schwall, 1983). Hydroperoxides are not stable products, and enter into numerous complex reactions resulting in a variety of compounds such as hydrocarbons,

alcohols, ketones, aldehydes, acids, epoxides, and keto-glycerides, which can produce off-flavor compounds.

The lipid oxidation process can cause the qualitative deterioration of muscle foods producing off-flavors and odors (Ramanathan and Das, 1992). The use of various antioxidants in controlling lipid oxidation in fish systems has been reported including tocopherol, which acts as free radical terminator (Khalil and Mansour, 1998).

Research indicates that tocopherol, sorbitol, and sodium tripolyphosphate could hold potential antioxidant and cryoprotective effects for frozen seafood products, which would minimize potential cryogenic freezing damage and possibly maintain quality attributes over frozen storage time. These three food additives will be further discussed in greater detail.

#### **Sodium Tripolyphosphate:**

Phosphates are refined from calcium phosphate, a naturally occurring substance. Food grade phosphates have many functional uses in seafood products, including the retention of moisture and flavor, prevention of lipid oxidation by metal chelation, shelf-life extension and cryoprotection (Lampila, 1992). Seafood proteins are unique since their myofibrillar proteins can readily denature and possibly lose 80% of their water binding capacity within 5 days at normal refrigerated temperatures. Beef, in comparison, takes an excess of 45 days at temperatures  $>20^{\circ}\text{C}$

(Lampila, 1992). Lengthened phosphate chains, such as STP, are associated with greater polyelectrolyte properties. Phosphate chains have a greater tendency to attach to positive sites on protein molecules, which improves protein solubility and enhances binding of water (Lampila, 1992). Phosphates are applied by either dipping or spraying onto the surface of seafood, or injecting the phosphate solution into the product. Uniform and consistent application of phosphates to seafood results in retention of natural juices, preventing fluid losses during shipment and prior to sale, and also in retention of flavor and moisture (Lampila, 1992).

Sodium chloride is sometimes used in conjunction with sodium tripolyphosphate in seafood processing. Rippen et al. (1996) added six processing treatments of water, phosphate, and sodium chloride to sea scallops. The scallops were evaluated during iced storage for moisture content, aerobic plate count, pH, drip and cook loss, and sensory freshness attributes. The 10% STP prepared in 1% sodium chloride dip produced the least drip and cook loss, and lower plate counts compared to freshwater washed scallops.

Meat and poultry researchers have investigated sodium tripolyphosphate and sodium chloride treatments as well, demonstrating the versatility of phosphates in meat applications. Froning and Sackett (1985) injected turkey breast muscle with solutions of phosphate blends along with sodium chloride. The results indicated that sensory properties, such as binding, flavor, and juiciness were significantly improved by the

presence of phosphates and sodium chloride. Moiseev and Cornforth (1997) investigated sodium hydroxide and sodium tripolyphosphate effects on the binding strength, cook yield, and sensory characteristics of restructured beef rolls. They revealed that rolls with STP had significantly higher bind strength and cooked yield than sodium hydroxide treated or control rolls. STP treated rolls generally had higher sensory scores than sodium hydroxide rolls or controls for cohesiveness and overall acceptability as well.

Woyewoda and Bligh (1986) have studied the effects of STP alone added to seafood products. They dipped Atlantic cod fillets in tripolyphosphate solutions for 45 seconds, froze the fillets, and then stored them at either -12°C or -30°C for up to 26 weeks. The phosphate treated cod showed decreased drip loss and resulted in a higher protein content of both cooked and raw product under abusive conditions of frozen storage. After 26 weeks of storage at -30°C, all phosphate treated fillets were the most tender in texture and were highly accepted by sensory panelists. Krivchenia and Fennema (1988) treated whitefish fillets with STP, monosodium glutamate (msg), or a high pH buffer by high-pressure injection, then froze the fillets and stored them at -12°C. The samples treated with STP and high pH buffer demonstrated better textural qualities than the control (untreated samples). The STP and high pH buffer samples had lower drip loss and less firmness than the control. Sensory analysis at 18 weeks of storage revealed that STP treated samples stored

at -12°C were significantly ( $p \leq 0.05$ ) preferred over the control at the same storage temperature. Regenstein and Stamm (1979) researched the water holding capacity of pre and post-rigor chicken, trout, and lobster with the presence of polyphosphates and the influence of added divalent cations, which included magnesium and calcium. Phosphates caused a large increase in water holding capacity of pre-rigor lobster muscle and some increases in post-rigor muscle. However, divalent cations had a depressing effect on water-holding capacity values when added to lobster muscle whether phosphates were present in the sample or not. The effects of sodium tripolyphosphate on frozen canned lobster meat were investigated by Sims et al. (1975). They were able to demonstrate that the addition of STP was able to minimize thaw drip from canned frozen lobster meat. They determined the optimum level of STP to be 0.25%, which was sufficient to retain original fill-in weight, and at the same time, ensure a good quality product. They also studied the uptake of STP in lobster meat and found that half of the added STP was present in the lobster meat within one hour of canning and that the maximum effect of the STP additive could be obtained even in a product that was frozen immediately after canning. Crapo and Crawford (1991) studied the influence of polyphosphate soak and cooking procedures on the quality and yield of Dungeness crab. The researchers soaked raw sections of crab meat in STP solutions and cooked them in either water or steam. They found that optimum meat yield, quality, and frozen storage stability

was obtained by soaking the crab meat in a 10% STP solution >60, but <120 minutes and then cooking in steam for approximately 8 minutes. The results indicated that moisture and proteins retained the STP and cooking in steam was believed responsible for improving meat yield, quality and storage stability. Nielsen and Piggott (1994) investigated the affect of polyphosphates on gel strength of low-grade heat-set surimi. The researchers determined that gel strength was increased in commercial surimi by the addition of phosphate blends, even in thermally abused surimi prepared from Alaskan pollock mince. The results indicated that lower grades of surimi would probably be able to be used to produce an acceptable surimi product with blended phosphates. van Wyk et al. (1984) investigated the effect of chemical treatments, including STP, on the yield and quality of rock lobster leg meat. Cooked, pre-dipped legs with STP treatment produced one of the best yields with no significant dehydration observed after 4 weeks of frozen storage at -40°C, and leg meat was also easily extracted. As far as organoleptic scores, STP treated rock lobster leg meat scores were rated higher for texture attributes than all other chemical treatments and control leg meat.

The maximum permitted legal level of sodium tripolyphosphate is 0.5% in processed meat and poultry (Lampila, 1992). At excess concentrations, a soapy after-taste can develop, which contraindicates the use of phosphates over the legal limit. An initial study investigating the effects of injecting American lobsters with STP, completed by the



University of Maine Food Science and Human Nutrition Department, determined that injecting lobsters with sodium tripolyphosphate solutions at 0.5% prepared in a 0.9% sodium chloride solution might be lethal to lobsters. After injection, some lobsters dropped claws, indicating that future STP lobster studies should evaluate STP solutions at less than 0.5%. This phenomenon was not seen in lobsters injected at STP concentrations below this level (Calder et. al, 2000).

### **Sorbitol:**

Polyhydric alcohols, such as sorbitol, are carbohydrate derivatives. Sorbitol is water-soluble and exhibits moderate viscosity in high concentrations in water (Lindsay, 1996). It is produced by the hydrogenation of glucose, and is 60% as sweet as sugar. Sorbitol can function as a cryoprotectant, crystallization inhibitor, freeze point depressant, and shelf-life extender (SPI Polyols, 2002). The most important function of sorbitol is thought to be the ability to deter myosin denaturation during frozen storage along with subsequent textural changes that occur from freezing damage. Some researchers have found that sorbitol enhanced the softness of thermally-induced gels by controlling the cross-linking reactions of myosin heavy chains that take place during the gel setting period of surimi production as mentioned by Konno et al. (1997). Salt solubilization of myosin before heating is required to produce a firm, elastic gel in surimi. Konno et al. (1997)

suggested that sorbitol promoted the solubilization of myofibrillar proteins or reduced the required sodium chloride concentration for solubilization as revealed from the results of their study.

There are numerous studies that justify sorbitol's cryoprotective effects in food systems during frozen storage. Yoon et al. (1991) investigated the changes in textural and microstructural properties of washed and unwashed frozen fish mince as affected by the addition of non-fish proteins and 6% sorbitol. They determined that all non-fish proteins and sorbitol stabilized myofibrillar structure by reducing freeze-induced contraction of myofibrils. Sensory panelists rated the fish mince treated with sorbitol the highest for overall acceptability when the mince was formed in a fish patty and steam cooked after intentional freeze thaw abuse. Contrary to the non-fish proteins, sorbitol dissolved into the extracellular fluid and was available to interact with myofibrillar proteins that could possibly increase the hydration of protein molecules for stabilization during frozen storage. Yoon et al. (1991) mentioned that other researchers have hypothesized that certain solutes, such as polyols, promote a preferential hydration of proteins with non-freezable water, which forms the basis of cryoprotection. Sultanbawa and Li-Chan (1998) studied the effects of cryoprotectant blends for their ability to stabilize ling cod surimi during frozen storage at -18°C for 4 months. The results showed that a gel could not be formed from ling cod surimi that had been frozen for 4 months without cryoprotectants. The researchers suggested

that ling cod surimi is similar to other types of surimi in that it is susceptible to freezing damage and requires cryoprotectants for extended storage. A commercial mix of 4% sucrose and 4% sorbitol was effective in ensuring good gel formation from ling cod after frozen storage for 4 months.

Medina and Garrote (2001) investigated the cryoprotectant effects of two mixtures (sucrose-sorbitol and maltodextrin-sorbitol) with regard to frozen storage and functional quality of surimi gels made from surubí. The researchers found surubí flesh was suitable for surimi production. They determined that even with the addition of cryoprotectants, the surimi gel strength was decreased by 32% compared to fresh surimi gel. Sucrose-sorbitol treated surubí surimi was more suitable than maltodextrin-sorbitol treated surimi because of the superior gel strength discovered in frozen gels treated with sucrose-sorbitol stored for 45 and 90 days.

Researchers have also investigated the cryoprotective effects of sorbitol added with sodium tripolyphosphate in seafood and other meat systems during frozen storage. Chang and Regenstein (1997) studied the textural changes and functional properties of cod mince proteins stored at -14°C as affected by added kidney tissue and cryoprotectants. They were studying mixtures of sucrose and sorbitol along with polydextrose or phosphates to improve texture and water retention properties of cod mince with added kidney tissue during frozen storage. Kidney tissue, if not totally removed, may be incorporated into the mince during deboning. The quality of the fish mince may be lowered and protein functional properties

may deteriorate faster due to increased dimethylamine and formaldehyde formation during frozen storage due to the increased enzyme (trimethylamine oxide demethylase) activity introduced from the kidney tissue. The expressible moisture (EM) of the sucrose-sorbitol treatment increased by 15% and increased by 24% with the addition of phosphates added to fish mince over the 12 weeks of frozen storage. However, the combination of sucrose-sorbitol and phosphates only increased EM by 12% over the same amount of frozen storage time. Their results indicate that phosphates and sucrose-sorbitol could increase water retention during frozen storage, suggesting that water uptake ability and cook loss was affected more by phosphate treatment, while protein solubility was affected more by sucrose/sorbitol treatment. Sucrose-sorbitol treatments helped to inhibit dimethylamine production at 2, 4, and 8 weeks of frozen storage, with sucrose-sorbitol in combination with phosphate treated samples showing greater inhibition at 8 weeks than sucrose/sorbitol alone. Sucrose-sorbitol in combination with phosphates had lower hardness ratings for texture in fish mince after frozen storage. The results demonstrated that phosphates exhibited beneficial effects on water retention properties, while phosphates and sucrose-sorbitol maintained good expressible moisture, water retention properties, reduced cook loss, as well as textural qualities in modified mince. Henry et al. (1995) compared the storage stability of blue crab meat either pasteurized and stored at 1.1°C or treated with cryoprotectants or water and cryogenically

frozen and held at -29°C to determine changes in physical and chemical properties after 8 months of refrigerated or frozen storage. Control samples were untreated and stored at -65°C. They found that the addition of sucrose-sorbitol and sodium tripolyphosphate to crab meat improved the frozen storage stability. This treatment had increased water holding capacity and lower TBAR values than the control. The researchers concluded that addition of cryoprotectants to cryogenically frozen crab meat enhanced the quality of crab meat compared with either pasteurization or freezing without cryoprotectant and phosphates when stored at -29°C. Ryu et al. (1994) researched the optimal level of cryoprotectants in reducing protein denaturation in pollock surimi. The cryoprotective effects of sucrose, sorbitol, sodium tripolyphosphate, and sodium pyrophosphate were assessed at -25°C for 16 weeks. The most effective treatments were achieved from using sucrose-sorbitol (1:1, w/w) at 8% with 0.2% sodium tripolyphosphate and pyrophosphate (1:1, w/w) in surimi, as measured by salt extractable protein and drip loss. Protein digestibility was not significantly affected by polyphosphate addition to pollock surimi. Yu et al. (1994) studied the cryoprotective effects of sucrose, sorbitol and phosphates on surimi as well. Results determined that 5% sucrose, 5% sorbitol, and 0.3% STP were the most effective levels of cryoprotectants during 16 weeks of frozen storage at -30°C. The phosphate treated samples had a higher water holding capacity and had higher salt soluble protein levels, indicating less protein denaturation

during frozen storage. The phosphates, in concentrations used in this study, were more effective in reducing protein denaturation compared to sucrose and sorbitol. The researchers concluded that phosphates may prevent the denaturation of actomyosin by binding to active sites of the proteins in the surimi, preventing them from irreversibly binding to each other. The researchers also discussed that sucrose seemed to have a better antifreeze protection than sorbitol during frozen storage.

#### **Tocopherol:**

Tocopherol (vitamin E) is a lipid-soluble antioxidant vitamin, which acts primarily by scavenging peroxy radicals as mentioned by Faustman et al. (1999). Among natural antioxidants, tocopherol has been found to offer a protective role against the adverse effects of reactive oxygen and other free radicals, which are the initiators of the oxidation process of polyunsaturated membrane phospholipids, critical proteins, or both, as noted by Gatta et al. (2000). Free radicals, hydroxyl radicals, and other oxygenated radicals are involved in these oxidative processes. However, the oxidation chain may be broken by the addition of tocopherol, which in trace amounts, will intercept radicals before they react with unsaturated fats, as discussed by Gatellier et al. (2000). Muscle-based foods containing higher concentrations of  $\alpha$ -tocopherol (at maximum antioxidant effect threshold concentrations) demonstrate higher stability of lipids than muscle-based foods at lower concentrations (Faustman et al., 1999).

Researchers have determined that increased tocopherol tissue levels have been shown to retard the onset of rancidity in frozen turkey meat and to depress TBA values from rising in storage as mentioned by Sklan et al. (1983). Oxidation of lipids is a particular concern in frozen seafood and in refrigerated products, and researchers have noted the presence of oxidative products (lipid hydroperoxides) already evident after a few days of cold storage (Watanabe et al., 1996). Furthermore, these chemical processes are promoted by the presence of highly unsaturated fatty acids, which are well represented in fish, although their level may vary according to diet composition, as mentioned by Gatta et al. (2000). Increasing the degree of polyunsaturation accelerates oxidative processes, leading to deterioration in meat flavor, meat color, and nutritional composition seen in pig and poultry meat, as noted by Gatellier et al. (2000). Tocopherols are considered more “consumer friendly” being deemed a “natural” antioxidant versus synthetic antioxidants such as BHA and BHT, which have been recently questioned about their potential carcinogenic effects especially in Japan, as discussed by Takashi et al. (1983).

Tocopherol's function is important for quality preservation in cooked seafood and meat products during cold and frozen storage, and studies have shifted towards  $\alpha$ -tocopherol research because it is the main tocopherol isomer in the human body. Higgins et al. (1998) investigated the effects of dietary supplementation of  $\alpha$ -tocopheryl acetate and post mortem addition of  $\alpha$ -tocopherol on lipid and color stability in cooked

turkey breast patties. They found that  $\alpha$ -tocopherol, added post-mortem, significantly protected patties against lipid oxidation compared to the control. However, endogenous tissue levels at 5.9 $\mu$ g/g of  $\alpha$ -tocopherol, were the most stable against lipid oxidation and had higher Hunter 'a' values. Even though exogenous  $\alpha$ -tocopherol concentrations were higher than endogenous  $\alpha$ -tocopherol concentrations in meat throughout the 9-day refrigerated (4°C) study. Whang et al. (1986) researched the effect of  $\alpha$ -tocopherol at different concentrations (0, 100, 200ppm) on inhibiting lipid oxidation of cooked or uncooked pork during aerobic storage at 4°C and -20°C. They found that  $\alpha$ -tocopherol slowed the rate of oxidation in cooked ground pork stored at both storage temperatures and uncooked samples refrigerated up to 12 days. Khalil and Mansour (1998) investigated the effects of antioxidants on lipid oxidation of common carp fillets stored at 5°C for 16 days. They determined that mixed function tocopherols (35-38%) combined with citric acid or ascorbyl palmitate were less effective antioxidants than others tested (rosemary extract, TBHQ, propyl gallate, BHT) to control lipid oxidation in carp. However, control fish had higher TBARS values than all fish samples treated with antioxidants. They revealed that the best antioxidant dip to depress TBARS values in carp fillets was a treatment of propyl gallate, TBHQ, and citric acid.

Researchers have also studied the effects of feeding  $\alpha$ -tocopherol to fish to see if antioxidants added to feed may later decrease lipid



oxidation during storage due to increased  $\alpha$ -tocopherol tissue concentrations. Jensen et al. (1998) studied the effect of dietary levels of fat,  $\alpha$ -tocopherol, and astaxanthin on color and lipid oxidation of rainbow trout during frozen storage and chilled storage of smoked trout. They found that during frozen storage, astaxanthin protected against lipid oxidation and against degradation of  $\alpha$ -tocopherol. High concentrations of  $\alpha$ -tocopherol improved the stability of fillet lipids against oxidation in the chilled, smoked product only. Gatta et al. (2000) investigated the influence of different levels of dietary vitamin E on sea bass flesh quality. They determined that samples with higher vitamin E concentrations were more resistant to forced lipid oxidation for 12 days at 1°C. They found that higher tocopherol levels in muscle fillets correlated to lower TBARS values, indicating that high levels of  $\alpha$ -tocopherol may have the potential to improve seafood quality if fed to fish prior to processing.

Other researchers have investigated the effects of blending sodium tripolyphosphate with tocopherol to examine their combined antioxidant effects. Miles et al. (1986) researched the antioxidant effects of STP and  $\alpha$ -tocopherol, on lipid oxidation of restructured pork. They determined that sodium tripolyphosphate acted as an effective antioxidant, as revealed by lower TBARS values, and  $\alpha$ -tocopherol seemed less effective in lowering TBARS values. The researchers hypothesized that  $\alpha$ -tocopherol may be a less effective antioxidant than  $\delta$ -tocopherol, and that sodium tripolyphosphate, as determined by their study, was more effective than  $\alpha$ -

tocopherol. Srinivasan et al. (1996) studied protein and lipid oxidation inhibition by using various wash media added with antioxidants during the preparation of beef heart surimi-like material. They found that sodium tripolyphosphate at 0.2% was an effective water-soluble antioxidant. They also found propyl gallate (0.02%) and  $\alpha$ -tocopherol (0.2%) to be effective lipid-soluble antioxidants when added to the washing solution. These antioxidants lowered both TBARS and conjugated diene levels during storage. Vara-Ubol and Bowers (2002) studied the effect of  $\alpha$ -tocopherol and STP in combination, as well as their solitary effects on hexanal values and sensory attributes of refrigerated cooked ground turkey or pork with or without sodium chloride added. The 0.03%  $\alpha$ -tocopherol concentration alone was found not to be the most effective antioxidant in stored cooked meats. A combination of 0.03%  $\alpha$ -tocopherol with 0.3% STP was very effective and nearly as efficient as 0.5% STP alone in preserving desirable meat notes and inhibiting hexanal development and oxidative changes that may cause stale aroma and flavor. However, a slick mouthfeel and metallic aftertaste were determined to be more intense for turkey with 0.5% STP added than STP added with an antioxidant combination. Besides an antioxidant effect, STP helped to maintain juiciness of both cooked meats during storage.

**Preliminary Dye Studies:**

Researchers at the University of Maine Food Science and Human Nutrition Department conceptually designed a method to add food additives, such as cryoprotectants, to seafood to maintain quality attributes over frozen storage. Initial dye studies were conducted at the University of Maine Food Science and Human Nutrition Department to implement this concept. Live North American lobster (*Homarus americanus*) and Rock crab (*Cancer irroratus*) were injected with 5mL of blue food coloring mixed in distilled water. The lobsters were injected in the ventral side of the lobster tail below the carapace with a B-D 5mL syringe and 20G (3.81cm) sized needle (Becton Dickson & Co., Rutherford, NJ). Rock crabs were injected in the flexible joints of both crusher claws. Dye was delivered throughout both crustacean systems within minutes. Crustaceans were cooked after injection and meat was evaluated. Blue dye was detected throughout the lobster and crab meat including the crusher claws. Preliminary dye injection studies on lobster led to the development of a patented method to inject food additives into seafood to potentially maintain frozen storage quality.

**Patent:**

Bayer et al. (1999) patented a method to improve frozen seafood quality by enhancing flavor and shelf-life so that seafood can be stored under commercial conditions. This method involves the injection of food

additives into the flesh or circulatory system of live seafood prior to cooking and freezing. A food additive injection apparatus can be used such as a pump (manual, gas, or fluid) that forces additives in a solution reservoir through tubing and a hollow needle into the flesh to be injected. These injected substances can be cryoprotectants or antioxidants, which circulate throughout the tissues, allowing for a uniform distribution throughout the flesh. The seafood is then cooked, frozen and stored for later use.

#### **Preliminary Injection Study:**

A preliminary injection study was completed at the University of Maine Food Science and Human Nutrition Department (Work et al., 1998) during the patent application process to further implement this method in a research study to determine the method's effectiveness. The objectives of the study were to examine the effect of sucrose as a cryoprotectant and an antioxidant to maintain and possibly enhance the flavor and texture attributes of cooked, cryogenically frozen whole lobsters stored under commercial storage conditions over 15 months. Lobsters were obtained from Icebrand Foods, Portland, Me., and 60 lobsters per treatment were used. Injection treatments were as follows:

1g sucrose + 2.5g antioxidant/lobster

2g sucrose + 2.5g antioxidant/lobster

3g sucrose + 2.5g antioxidant/lobster

Untreated = Control

The antioxidant was a mixed function, water dispersible tocopherol (Henkel Corporation; LaGrange, IL). After the initial experiment evaluation, lobsters were evaluated for sensory, chemical, and physical characteristics at three month intervals during frozen storage.

The researchers found that sucrose concentration had a significant effect on panelist perception of sweetness. Overall, control and the lower sucrose concentration samples were rated higher for flavor at each evaluation period. The samples injected with higher sucrose concentrations were rated lower for flavor. For control samples, TBARS values increased over the first three months of frozen storage and then decreased through twelve months of frozen storage, and then spiked higher at 15 months. TBARS values for 1g sucrose+antioxidant remained relatively constant with no significant differences. However, 2g and 3 g sucrose+antioxidant treated lobster had significant decreases in TBARS levels after an initial rise in TBARS levels at month 3. These changes were similar to those reported by other researchers in which TBARS are further metabolized to other compounds resulting in lower TBARS values over time. Initially, there was a dose response to the level of sucrose added to lobster prior to freezing.

Salt soluble protein levels decreased over frozen storage time until 15 months when the values increased dramatically. Mechanically measured shear values indicated an increase in toughening for all treatments up to 6 months of frozen storage and then values fell back to initial levels after 15 months of storage. These changes may have been related to interactions (cross-linking) of the muscle proteins over storage.

The researchers concluded that the addition of sucrose and antioxidant had a positive effect on lobster quality over 6 months of frozen storage. The results were not as clear following longer frozen storage periods. They further stated that two areas of interest in future studies would be to further examine the use of sorbitol as a replacement for sucrose as a cryoprotectant, and the use of tripolyphosphate to increase cook yield.

#### **Initial Phosphate Injection Study:**

An initial phosphate injection study with lobsters was conducted at the University of Maine (Calder-Bussell et al., 2000). These findings were never published due to the extreme variability in lobster size provided by Icebrand Foods, Inc. Lobster weight ranges were 1½ to 2+ lbs. Due to the unexpected difference in lobster size, injection amounts had to be estimated during injection to accommodate larger lobster weights so that tripolyphosphate residues were equally distributed within treatments. The purpose of this study was to further research the recommendations of

Work et al. (1998). The objective was to determine the effect of sodium tripolyphosphate on the quality of fully cooked lobster during 6 months of frozen storage. The lobster injection treatments were as follows:

Control = 0.9% sodium chloride

0.1% STP in 0.9% sodium chloride

0.3% STP in 0.9% sodium chloride

0.5% STP in 0.9% sodium chloride

One-hundred and sixty lobsters were donated by and processed at Icebrand Foods, Inc., Portland, ME. Lobsters were injected utilizing the patented method of injecting additives into live lobster tails. Lobsters were cooked in a steam cooker for 9 minutes and then cooled in an ice bath for 15 minutes. The lobsters were then cryogenically frozen with liquid nitrogen in a 30-foot cryogenic freezing tunnel. Lobsters were placed into commercial cardboard cartons and held under commercial frozen storage conditions (-20°C) at Icebrand Foods, Inc. until evaluation. Chemical analyses were performed at frozen storage months 0, 1, 4, and 6. Sensory affective tests to determine consumer acceptance tests were performed at storage months 4 and 6.

The researchers determined significant ( $p \leq 0.05$ ) differences in salt soluble protein levels at month 6. The 0.5% STP treated lobsters had a significantly ( $p \leq 0.05$ ) higher salt soluble protein concentration than the other treatments, with the control having the lowest salt soluble protein level at month 6. Sensory results revealed significant ( $p \leq 0.05$ ) differences

among panelists' ratings at month 4, but no statistically significant ( $p \geq 0.05$ ) differences were detected at month 6. At month 4, panelists gave 0.5% STP treated lobster tails significantly ( $p \leq 0.05$ ) higher ratings than the control or 0.1% STP treated tails for meat color. The panelists also rated 0.1% STP treated tails significantly ( $p \leq 0.05$ ) lower for texture than all other treatments. The 0.1% STP treated tails were also rated significantly ( $p \leq 0.05$ ) lower in overall acceptability than 0.3 and 0.5% STP injected tails. No statistical differences were determined for flavor attributes.

The researchers concluded that polyphosphates injected into lobster may extend the shelf-life of cryogenically frozen lobsters over a frozen storage period of 6 months.

#### **Justification of Current Seafood Injection Study:**

Preliminary studies at the University of Maine and research supporting the use of sodium tripolyphosphate, sorbitol, and tocopherol indicate the effectiveness of these cryoprotectants and antioxidants in maintaining the quality of frozen seafood products over storage time, and support the justification for the purpose of this current seafood injection study. The current research project could possibly justify the implementation of a commercial injection system of food additives to seafood products to increase yield and to maintain quality over frozen storage time. Maine seafood processors could potentially benefit from this new injection technology and increase economic gains, while also



meeting the rising demand for frozen lobster products and creating new markets for Maine crab species.

## **LOBSTER MATERIALS AND METHODS:**

### **Objective:**

The objective of this study was to determine the effect of sodium tripolyphosphate (STP) on the quality and yield of fully cooked lobster over a frozen storage period of six months.

### **Experimental Design:**

As the result of a preliminary experiment, STP concentration was set below 0.5% because this level seemed lethal to lobsters as claws were observed dropping away from the lobster body after injection. Therefore, two STP levels were selected at 0.1 and 0.3% along with control lobsters, which were injected with 0.9% saline solution.

### **Processing:**

Fifty-eight lobsters per treatment were tagged and weighed at D.B. Rice Fisheries, Bunkers Harbor, Maine prior to processing at Cranberry Point Seafood Processing Facility, Gouldsboro, Maine. Lobster weights ranged from 1  $\frac{1}{8}$  to 1  $\frac{1}{2}$  lbs. STP (sodium tripolyphosphate, FMC; Philadelphia, PA) solutions of 0.1% and 0.3% were prepared in 0.9% saline solution and were injected into live lobsters. The injection site of the lobster was located on the ventral side of the tail near the carapace to utilize the patented injection method of Bayer et al. (1999). Five mL of

solution were injected into each lobster tail and STP concentrations were calculated prior to injection in order to deliver either 0.1 or 0.3% STP into the tail. Controls were injected with 0.9% saline solution. Lobsters were cooked for 16 minutes in a continuous steam cooker at 100°C (CTX Steam Cooker, model# VSWDM3546, Laitram Machinery; New Orleans, LA). The lobsters were then immediately cooled in an ice bath for approximately 15 to 20 minutes to reduce overall temperature for optimization of cryogenic freezing. Lobsters were re-weighed after cooling and then cryogenically frozen using a 30-foot freezing tunnel equipped with liquid nitrogen at temperatures between temperatures of -55 to -57°C (Modular Tunnel Freezer, model# KFT36-10M, BOC Gases; Murray Hill, NJ). Approximately 15 minutes were required for lobsters to exit the tunnel. Lobsters were then placed into plastic storage totes equipped with covers and transported to the University of Maine Department of Food Science and Human Nutrition and held at -15°C frozen storage until evaluation.

#### **Chemical Analyses:**

Chemical analyses were performed on lobster samples at months 0, 2, 4, and 6. Sample preparation consisted of thawing lobsters at refrigerated temperatures (4°C) for 24 hours. Lobsters were reheated by steaming in a colander over boiling water for approximately 20 minutes. Tail meat samples were removed from shell by hand, lightly rinsed, and

homogenized per treatment using a hand-powered food processor (Culinare RocketChef, Best Direct; Pacoima, CA) to avoid adding heat to samples, which possibly could accelerate oxidation of lobster meat. Claw meat samples were removed from shell, homogenized per treatment, and analyzed separately.

#### Thiobarbituric Acid Reactive Substances:

Lobster tail and claw samples were analyzed according to the method described by Tarladgis et al. (1960) and Rhee and Watts (1966). Four g of each sample were placed in 50 mL disposable polypropylene conical tip centrifuge tubes 30x115 mm (Falcon BlueMax, Becton Dickson Labware; Franklin Lakes, NJ) in 12 mL of cold buffer (50mM PO<sub>4</sub> (Sodium phosphate, monobasic monohydrate (product# BP329-500) and dibasic anhydrous (product# S374-500), Fisher Scientific; Pittsburgh, PA), 0.1% EDTA (product# E-4884, Sigma Chemical Co.; St. Louis, MO), 0.1% n-propyl gallate (product# P-3130, Sigma Chemical Co.; St. Louis, MO)) and vortexed (Vortex-Genie, model# K-550-G; Bohemia, NY) vigorously for 10 seconds. The samples were then homogenized on a Polytron (Kinematica; Switzerland) set at level 5 for 1-2 minutes. Four mL of 30% trichloroacetic acid (product# A322-100, Fisher Scientific; Pittsburgh, PA) were added to samples followed by vortexing for 15 seconds. The slurry was filtered through a Whatman #1, 15 cm P-8 fluted filter paper (product# 9-790-14E, Fisher Scientific; Pittsburgh, PA) and collected in screw cap glass test tubes. Four mL of 20 mM 2-thiobarbituric acid (product # T-

5500, Sigma Chemical Co.; St. Louis, MO) solution was added to each test tube, the tubes were capped, and vortexed briefly. Test tubes were placed in a boiling water bath for 20 minutes. The test tubes were then immediately placed into an ice bath to inhibit further reaction. Cooled filtrates with TBA were transferred to polystyrene disposable cuvettes 4.5 mL capacity with 10 mm light path (Fisher Scientific; Pittsburgh, PA) and the sample absorbance was read at 530 nm in a DU-64 Spectrophotometer (Beckman Instruments, INC., CA). Each treatment was analyzed in duplicate.

#### Salt Soluble Protein:

Claw and tail lobster samples were analyzed for salt soluble protein levels using the method of Lowry et al. (1951). Ten g of meat was weighed per treatment in 250 mL polypropylene copolymer Nalgene centrifuge bottles (Nalge Nunc International, Fisher Scientific; Pittsburgh, PA) and homogenized for 1 minute with 90 mL of 5% NaCl (product# SX 0420-1, EM Science; Gibbstown, NJ) solution for 1-2 minutes using a Polytron. The homogenate was then centrifuged for 20 minutes at 12,000 rpm (48,000 g) on a Sorvall RC-5B Refrigerated Superspeed Centrifuge (Sorvall Instruments, Dupont Co. Biomedical Div.; Newton, CT). The supernatant was collected as the salt soluble fraction. Fifty  $\mu$ L of supernatant was pipetted into glass screw cap test tubes and then the volume was brought up to 0.5 mL with distilled water. Five mL of solution D (100 mL of Solution A: 20 g  $\text{Na}_2\text{CO}_3$  (anhydrous, Sigma Chemical Co.;

St. Louis, MO), 4 g NaOH pellets (product# SX 0593-3, EM Science; Gibbstown, NJ), distilled water to 1 L + 1 mL of Solution B: 1% cupric sulfate (product# C-493, Fisher Scientific; Pittsburgh, PA) + 1 mL of Solution C: 2.7% sodium potassium tartrate (product# S-2377, Sigma Chemical Co.; St. Louis, MO) were added to all test tubes including the bovine serum albumin standard (product# A-7030, Sigma; St. Louis, MO). A series of dilutions of bovine serum albumin solution (1 mg/mL), which ranged from 10, 20, 40, 80, 100, to 200 µl were created to produce a standard curve, which was used to estimate the salt soluble protein concentration of the lobster tail and claw samples. Sample tubes were then capped and vortexed briefly, and held at room temperature for 10 minutes. Then 0.5 mL of 1 N phenol reagent (product# P-3653, Sigma Chemical Co.; St. Louis, MO) was added to all test tubes, the tubes were capped, vortexed briefly, and held at room temperature for 20 minutes. Samples were transferred to disposable cuvettes (4.5 mL capacity with 10 mm light path) (Fisher Scientific; Pittsburgh, PA.) and the sample absorbance was read at 530 nm in a DU-64 Spectrophotometer (Beckman Instruments, INC., CA). Each treatment was analyzed in duplicate.

#### Moisture:

Five g tail and claw samples were placed into pre-weighed scintillation vials (Wheaton Scientific; Milville, NJ) per treatment, which were analyzed in duplicate. Samples placed in vials were dried for 24 hours in a vacuum oven (National Appliance Co.; Portland, OR) set at

70°C. The samples were then re-weighed and percent moisture was calculated using the following formula:  $((\text{vial wt.} + \text{wet sample wt.}) - (\text{vial} + \text{dry sample wt.})) / (\text{sample wt.}) \times 100$ .

Ash:

Dried samples were placed into a Thermolyne muffle oven (model F-A1730; Dubque, IA) set at 550°C for 6 hours. Ashed samples were then re-weighed and the percent ash was calculated using the following formula:

$$(((\text{vial wt.} + \text{ash wt.}) - (\text{vial wt.})) / (\text{sample wt.})) \times 100.$$

Minerals:

Ashed claw and tail lobster samples were dissolved in 1 mL of hydrochloric acid (EM Science; Gibbstown, NJ) and 1 mL of nitric acid (EM Science; Gibbstown, NJ). Ten mL of distilled water was added to the samples and followed by vortexing. Samples were then transferred to 100 mL volumetric flasks and diluted to that volume using distilled water.

Lobster samples were analyzed for sodium, phosphorus, calcium, iron, potassium, magnesium, and zinc content using an Inductively Coupled Argon Emission Spectrophotometer (Jarrell Ash, 975 Atom Co.; Franklin, MA) by the Analytical Lab located in Deering Hall at the University of Maine. Mineral concentrations were then calculated using the following formula:

$$(\text{mineral ppm} \times \text{dilution factor } (\mu\text{g})) / \text{wt. of wet sample (g)}.$$

## **Physical Analyses:**

### **Texture Analysis:**

Lobster tail meat firmness was analyzed using a custom-designed, metal shear cell machined with a beveled edge for an Instron (model# 4466 with a Series IX Automated Materials Testing System 7.5 – Instron, Inc.; Canton, MA) in order to mimic the human bite. The lobster tail samples for Instron testing were prepared exactly the same as sensory samples, reheated and removed from shell. The tails were then cut in half as duplicates per lobster, and the length and width of each section was measured using a Mitutoyo Digimatic caliper (model # CD-6"BS, Mitutoyo Corporation, Japan). The scale load on the Instron was 0.5 kN, crosshead speed was 500 mm/min with a platen separation of 50.1 mm, and sample rate (pts/sec) was 20.0. The compression test was to determine the shear force needed to penetrate through the lobster tail meat. Maximum displacement (peak load) was measured in mm/cm, and force (load) was measured in kN per pound. The peak load or breaking point of the sample was calculated in kN/mm.

### **Colorimetric Analysis:**

Colorimetric analyses were performed on tail muscle at the same time as sensory analysis at months 2 and 6. Color was measured using a Hunter LabScan II (Hunter Associates Laboratory; Reston, VA). An optical aperture of 6 cm was used. The instrument was standardized with a white tile (Illuminant D65 10 degree observer, CIE # 15E-1.3.1 1971)



and black tile (no company specification) before samples were analyzed. Samples of tail meat were placed in transparent plastic dishes. L (lightness), a (redness), and b (yellowness) values were recorded, and the sample dish was rotated 1/3 of a turn for a total of three turns. These lobster samples were analyzed in duplicate. Chroma and hue values were then calculated. Chroma indicates the intensity of color and was calculated by:  $(a^2 + b^2)^{1/2}$ . Hue angle is an indication of red versus yellow color and was calculated by taking the arctan of (b/a).

### **Sensory Analysis:**

Sensory evaluation was performed using an affective test to determine the consumer acceptability of the frozen lobster product upon re-heating at months 2 and 6. The College of Natural Sciences, Forestry, and Agriculture Human Subjects' Protection Committee previously approved the procedures of this sensory study. The lobsters were removed from frozen storage and placed at refrigerated temperatures (4°C) for 24 hours before evaluation. Lobsters were steamed for approximately 20 minutes. Tail meat was removed from the shell, lightly rinsed, dried, weighed. The tail meat was cut vertically in half, and then cut into 6 horizontal proportionate sections. Each panelist received one tail section per treatment, which was presented on small white, plastic plates. The samples were labeled with three-digit randomized codes and presented in random order. Butter (sweet cream unsalted butter,

Hannaford; Scarborough, ME) was provided for panelists to mimic typical lobster consumption in the home. The butter was melted in a saucepan set at low heat and served in 2 oz. opaque cups (Solo; Urbana, IL) upon request. Thirty panelists (over the age of 18) were recruited for this study from the University of Maine community by either verbal or written announcement. Volunteers were allowed to participate after signing an informed consent form (copy of informed consent form Appendix A), which stated that they must not have any food allergies in order to participate in this research study. Panelists were seated in booths with fluorescent lighting located at the Consumer Testing Center, in Holmes Hall at the University of Maine. Sensory panelists were asked to sit in front of a computer, which gave specific instructions to guide panelists through the sensory test. They were asked to provide information such as their age, gender, and how often do they eat boiled lobster. Panelists were provided a cup of water and were asked to take a sip of water to cleanse their palate before tasting the next sample. Sensory panelists evaluated sample attributes of exterior and interior meat color, flavor, texture, and overall acceptability using a 9-point hedonic scale (1=dislike extremely to 9=like extremely) (copy of sensory ballot in Appendix A). Data from the computerized sensory ballots were collected by SIMS 2000 program for Windows. Volunteers received incentives after participating in the study.

### **Cook Loss and Yield:**

Lobsters were thawed, steamed and weighed prior to sensory, chemical or physical analyses following the same procedure as mentioned previously in the sensory analysis method. Cook loss and yield were calculated to see if STP treatment would have any effect on retaining moisture in the product. Percentage of cook loss was calculated as:

$$\frac{\text{initial wt. (g)} - \text{cooked wt. (g)}}{\text{initial wt. (g)}} \times 100 \text{ (Young et al., 1987).}$$

Percentage of steamed lobster tail meat yield was calculated as:

$$\frac{\text{lobster tail meat wt. without shell (g)}}{\text{whole steamed lobster wt. (g)}} \times 100$$

### **Analytical Studies:**

High performance liquid chromatography methods were developed to detect STP, sucrose, and tocopherol, and injected into lobster tissue in separate lobster studies.

#### **Quantification Studies of Sodium Tripolyphosphate in Lobster:**

Methods were developed to attempt quantification of sodium tripolyphosphate (STP) injected into lobster using High Performance Liquid Chromatography (HPLC) and Capillary Electrophoresis procedures. However, lobster has a high natural phosphate content, which masks the injected STP. Polyphosphates also rapidly break down to orthophosphate increasing the difficulty of detection. Further STP detection studies were

discouraged due to the cost of necessary equipment, and also the lack of facilities to radioactively tag phosphate for detection.

#### Quantification Study of Sucrose in Lobster:

The objective of this study was to determine if sucrose injected into lobster tissue could be detected by High Performance Liquid Chromatography (HPLC).

The procedure for this study began after lobsters were injected with 1 or 2 g sucrose as described in the preliminary sucrose injection study. Control lobsters were not injected. Lobsters injected with 2 g sucrose were preferred for this HPLC study due to the higher sucrose concentration for detection purposes. Lobsters were injected, frozen, and then reheated before extraction. Duplicate 10 g samples of lobster tail tissue were homogenized using a Polytron (Kinematica; Switzerland) in 30 mL 60% ethanol (Fisher Scientific; Pittsburgh, PA) at 60°C in 100mL glass beakers. (This procedure also worked using ethanol at room temperature.) Samples were centrifuged for 5 minutes using a Sorvall RC-5B Refrigerated Superspeed Centrifuge (Sorvall Instruments, Dupont Co. Biomedical Div.; Newton, CT). Supernatants were filtered using a B-D 5 mL syringe (Becton Dickson & Co., Rutherford, NJ) and 0.45 µm Acrodisc syringe filter (product # 4184, Pall Corporation; Ann Arbor, MI). Sucrose (Sigma Chemical Co.; St. Louis, MO) standard (1 mg/ml) was prepared in HPLC grade water (Fisher Scientific; Pittsburgh, PA). The initial mobile phase was 75:25 acetonitrile (Fisher Scientific; Pittsburgh,

PA) to HPLC grade water, and was later changed to 80:20 acetonitrile to water for cleaner baseline resolution. HPLC was a Waters Associates liquid chromatograph model ALC/GPC 244 (Milford, MA), equipped with a 6,000A pump, a U6K injector, and a differential refractometer. The detector signal was recorded on a Houston Instruments (Austin, TX) dual pen recorder. HPLC settings were a flow rate of 2.0 ml/min attenuation was set at 4, and a chart speed of 0.5. A Phenomenex Primesphere 5 $\mu$ , NH<sub>2</sub>, 4.6 x mm column was used with a 25  $\mu$ m injection loop.

An additional enzyme study was conducted to justify the effectiveness of the HPLC method in detecting sucrose previously injected into lobster tissue. Invertase was used in this study, which is an enzyme that hydrolyzes sucrose to glucose and fructose.

Lobster tail samples were prepared according to the method above. Control lobster tail extraction in 60% ETOH was considered the control sample. Sucrose injected lobster supernatant to be treated with enzyme was then evaporated using a roto-evaporator (Rotavapor R-3000; Buchi Switzerland) at 55°C at a speed of 85 to evaporate ethanol, to concentrate the sample, prevent enzyme denaturation, and as a preliminary cleanup step. A 3 mL sucrose (1 mg/mL) solution prepared in citrate/phosphate buffer at pH 4.6 was added with 1 mL of invertase enzyme (Sigma Chemical Co; St. Louis, MO). This buffer was used because invertase functions at an optimum enzymatic activity at a pH of 4.6. The 3:1 sucrose/buffer/enzyme solution was added to the evaporated lobster

sample residue and incubated for 30 minutes at 25°C in a water bath. The lobster sample in sucrose/buffer/enzyme solution was then diluted 1:1 with 60% ETOH.

#### Quantification Study of Tocopherol in Lobster:

The objective of this study was to determine if mixed function tocopherol injected into lobster could be detected using High Performance Liquid Chromatography (HPLC) in both tail and lobster claw meat.

The procedure included using a mobile phase of 95:5 HPLC grade methanol and water. Lobster tissue samples (10 g) were homogenized using a Polytron (Kinematica; Switzerland) in 20 mL of mobile phase, and then centrifuged using a Sorvall RC-5B Refrigerated Superspeed Centrifuge (Sorvall Instruments, Dupont Co. Biomedical Div.; Newton, CT). Supernatant and standards were filtered using a B-D 5 mL syringe (Becton Dickson & Co., Rutherford, NJ) and 0.45 µm Acrodisc syringe filter (product # 4184, Pall Corporation; Ann Arbor, MI). Standard was a mixed function tocopherol (Covi-ox® T-30P, Cognis Corporation; LaGrange, IL) that had been previously injected into lobster tails. Standard was prepared in mobile phase at a concentration of 1mg/ml. Samples and standard were injected into a LC-FLD system – HP 1100 series (Hewlett Packard; Burlington, MA) flow rate of 4ml/min., using a Prodigy 5µ ODS-2, 4.6 x 250mm (Phenomenex, Inc.; Torrance, CA), along with ultraviolet (UV) and fluorescence detection (FLD; 6-11) Ex=292, Em=324.

**Data Analysis:**

Chemical and physical data were analyzed using Systat 9.0 (Systat Software Inc., 1998). One-way analysis of variance (ANOVA) was used to detect statistical differences ( $p \leq 0.05$ ) among sodium tripolyphosphate and control treatments at a specific frozen storage month. Significant differences ( $p \leq 0.05$ ) between means were analyzed using the Tukey HSD post hoc test. A multi-way ANOVA was used to evaluate differences of the potential effects of treatment over frozen storage time, and the Tukey HSD Multiple Comparisons were used to determine significant differences ( $p \leq 0.05$ ) of treatment means across time. Lobster tail and claw data were independently studied during statistical analyses.

Sensory data on lobster tails only were analyzed using SAS 6.12 (SAS Institute Inc., 1989) ( $p \leq 0.05$ ) with Duncan's long range multiple post-hoc test.

Pearson correlation matrix analyses were performed on chemical, physical, and sensory data using Systat 9.0 (Systat Software Inc., 1998).

## **CRAB MATERIALS AND METHODS:**

### **Objective:**

The objective of this study was to determine the effect of sorbitol and tocopherol on the quality of fully cooked rock crab (*Cancer irroratus*) over a frozen storage period of twelve months.

### **Experimental Design:**

As the result of a preliminary experiment conducted at the University of Maine using sucrose and tocopherol in lobster, sorbitol residues were established at 1g sorbitol (product # BP439-500, Fisher Scientific; Pittsburgh, PA) and 2.5 g tocopherol and 2 g sorbitol and 2.5 g tocopherol (Henkel Corporation) per crab along with a control, which was not injected.

### **Processing:**

One hundred and twenty-five rock crabs per treatment were tagged and separated in plastic totes at D.B. Rice Fisheries, Bunkers Harbor, Maine prior to processing. Crabs were processed at Cranberry Point Seafood Processing Facility in Gouldsboro, Maine. Crab weight was approximately 1 lb. Crabs were injected with either 1 g sorbitol and 2.5 g tocopherol or 2 g sorbitol and 2.5 g tocopherol solution. Treated crabs were injected with 2.5 mL of solution into each pincher joint to utilize the



patented injection method of Bayer et al. (1999). After injection, crabs were cooked for 16 minutes in a continuous steam cooker at 100°C (CTX Steam Cooker, model# VSWDM3546, Laitram Machinery; New Orleans, LA). Crabs were then immediately cooled in an ice bath for 15-20 minutes to reduce overall temperature for optimal cryogenic freezing conditions. Crabs were cryogenically frozen with a 30-foot freezing tunnel equipped with liquid nitrogen between temperatures of -55 to -57°C (Modular Tunnel Freezer, model# KFT36-10M, BOC Gases; Murray Hill, NJ). Crabs were then placed into Styrofoam storage totes equipped with covers and transported back to the University of Maine Department of Food Science and Human Nutrition and held at -15°C frozen storage until evaluation.

#### **Chemical Analyses:**

Chemical analyses were performed on crab samples at months 0, 3, 6, 9, and 12. Sample preparation consisted of thawing crabs at refrigerated temperatures (4° C) for 24 hours. Crabs were reheated by steaming in a colander over boiling water for approximately 15 minutes. Claw and body meat were removed from shell by hand and homogenized using a hand-powered food processor (Culinare RocketChef, Best Direct; Pacoima, CA) to avoid adding heat to samples, which possibly could accelerate oxidation of crab meat.

#### **Thiobarbituric Acid Reactive Substances:**

Method used to analyze crab meat for TBARS was the same as previously mentioned in lobster material and methods.

#### **Salt Soluble Protein:**

Method used to analyze crab meat for salt soluble protein was the same as previously mentioned in lobster material and methods.

#### **Moisture, Ash, and Minerals:**

Methods and calculations used to analyze crab meat for ash, moisture, and mineral content were the same as previously mentioned in lobster material and methods.

#### **pH:**

pH was analyzed as a method of monitoring quality attributes in crab meat over time. Approximately 25 g of crab meat per treatment was placed into a 50 mL glass beaker. A glass, flat surface electrode probe (Beckman 32pH meter, Beckman Instruments, Inc.; Fullerton, CA) was inserted approximately 24.4 mm into the crabmeat and the pH level was digitally read. Each treatment was analyzed in duplicate.

#### **Colorimetric Analysis:**

Method used to analyze crab meat for color differences was the same as previously mentioned in lobster material and methods.

**Sensory Analysis:**

Sensory analyses were performed at month 3 and 9 using a home use test in order to test consumer acceptance of crab samples as a potentially new food product to utilize Maine rock crab. The home use test was selected to allow panelists the convenience of preparing and consuming the samples at home, while providing a more typical consumption environment for this product. Thirty panelists over the age of 18 were recruited from the local area by either written or verbal announcement. Volunteers were allowed to participate after signing an informed consent form (see Appendix A), which stated they did not have any food allergies. Volunteers were delivered a whole frozen crab sample and sensory ballot (see Appendix A) per week for three weeks per sensory testing period to reduce sample bias or confusion. Each panelist received a different randomized sequence of crab samples at each sensory testing time. They were also initially given written handouts with reheating directions (see Appendix A) for consistency in crab meat sample preparation. Panelists were asked to evaluate sample attributes of flavor, texture, and overall acceptability using a 9-point hedonic scale (1=dislike extremely to 9=like extremely). Panelists were asked to complete a post-study questionnaire (Appendix A) to determine ease of preparation of samples, expected cost of such a product if bought at a store, and comments and opinions of the product. Panelists received an incentive after successfully completing both sensory testing months in the study.

**Data Analysis:**

Chemical, colorimetric, and sensory data were analyzed using Systat 9.0 (Systat Software Inc., 1998). One-way analysis of variance (ANOVA) was used to detect statistical differences ( $p \leq 0.05$ ) among sorbitol/tocopherol and control crab treatments at a specific frozen storage month. Significant differences ( $p \leq 0.05$ ) between means were analyzed using the Tukey HSD post hoc test. A multi-way ANOVA was used to evaluate differences of the potential effects of treatment over frozen storage time, and the Tukey HSD Multiple Comparisons were used to determine significant differences ( $p \leq 0.05$ ) of treatment means across time.

Pearson correlation matrix analyses were performed on chemical and sensory data using Systat 9.0 (Systat Software Inc., 1998).

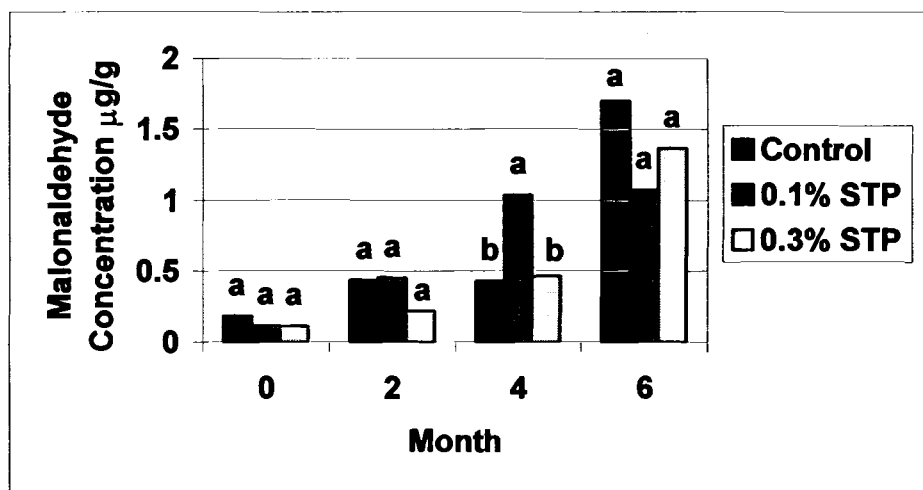
## LOBSTER RESULTS:

### Chemical Analyses:

#### Thiobarbituric Acid Reactive Substances:

One-way ANOVA results reveal a significant ( $p \leq 0.05$ ) difference in TBARS levels at month 4 in lobster tail meat. The 0.1% STP treated tails were significantly higher in TBARS than control and 0.3% STP tails. At month 6, control tail TBARS levels were higher than 0.1 and 0.3% tails, however the difference was not significant. TBARS levels in lobster tail meat indicate an increased malonaldehyde concentration over frozen storage time as illustrated by Figure 2.1. Lobster tail malonaldehyde concentrations remained low over 6 months of frozen storage, and did not surpass 2  $\mu\text{g/g}$ .

**Figure 2.1- Lobster Tail TBARS Means Over Frozen Storage Time\***



\*Significance  $p \leq 0.05$

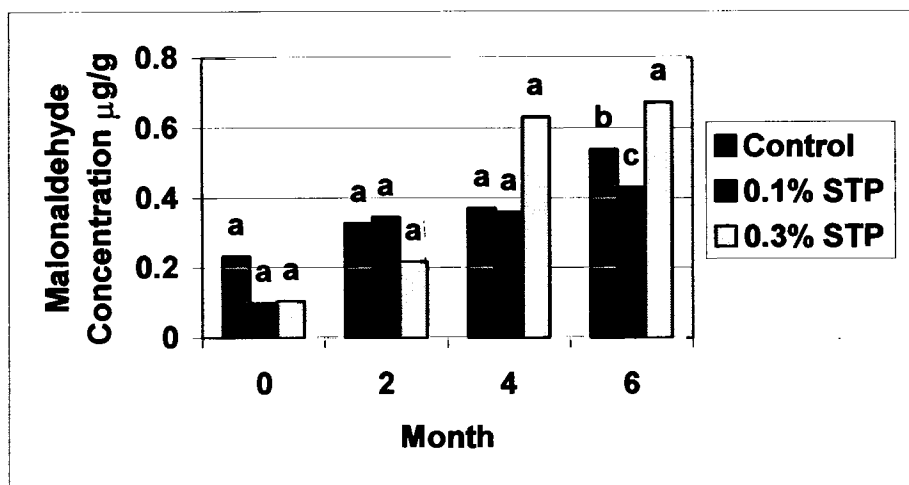
Means are an average of duplicate samples.

Means within each evaluation period sharing a common letter are not significantly different.

Multi-way ANOVA results detect significant effects of time and treatment/month interactions on TBARS levels in lobster tail meat. TBARS values for all three treatments significantly ( $p \leq 0.05$ ) increased over storage time.

One-way ANOVA results on TBARS levels in lobster claws show an increased malonaldehyde concentration over storage time as well (Figure 2.2). At month 6, significant ( $p \leq 0.05$ ) differences were detected between all three treatments. The 0.3% STP treatment was significantly higher than 0.1% STP and control claw means. The 0.1% STP treated claws were significantly ( $p \leq 0.05$ ) lower in malonaldehyde concentration than both control and 0.3% STP treated claw meat. Claw meat malonaldehyde concentrations remained low as well, and did not exceed  $0.7 \mu\text{g/g}$ .

**Figure 2.2- Lobster Claw TBARS Means Over Frozen Storage Time\***



\*Significance  $p \leq 0.05$

Means are an average of duplicate samples.

Means within each evaluation period sharing a common letter are not significantly different.

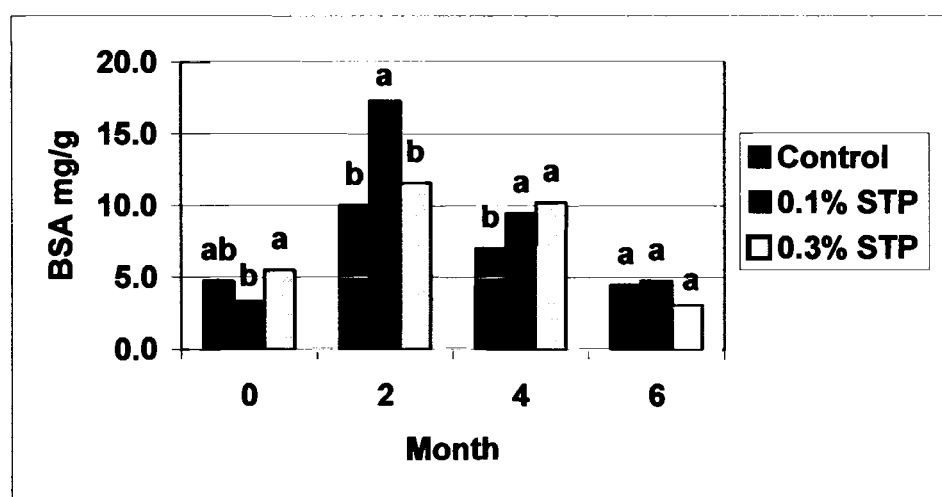
Multi-way ANOVA results reveal significant time and treatment effects and significant effects of treatment/month interactions on TBARS levels in lobster claw meat. TBARS values in lobster claw meat significantly increased ( $p \leq 0.05$ ) over frozen storage time across all treatments.

#### Salt Soluble Protein:

One-way ANOVA of Lowry results in lobster tail meat distinguish significant ( $p \leq 0.05$ ) differences among treatments in salt soluble protein levels at months 0, 2, and 4. At month 0, 0.1% STP treated tail salt soluble protein content was significantly lower than 0.3% STP treated lobster tails. At month 2, 0.1% STP tail levels were significantly ( $p \leq 0.05$ ) higher than both control and 0.3% STP treated lobster tail levels. At month 4, the control salt soluble protein level was significantly lower than

0.3 and 0.1% STP tail levels. At month 6, 0.3% STP tail salt soluble protein concentration was slightly lower, but not significantly different than control and 0.1% STP treated lobster tail meat. Salt soluble protein concentrations were variable across frozen storage time as illustrated by Figure 2.3.

**Figure 2.3-Salt Soluble Protein Means of Lobster Tail Meat Over Frozen Storage Time\***



\*Significance  $p \leq 0.05$

Means are an average of duplicate samples.

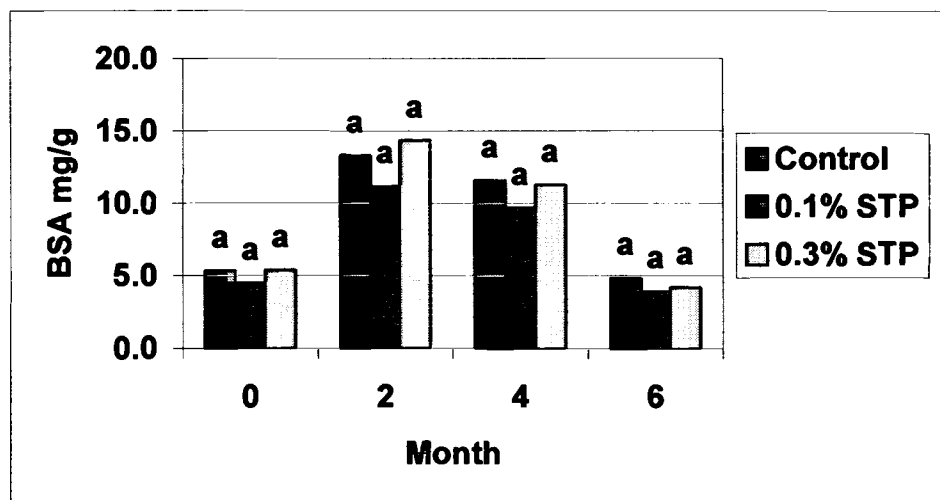
Means within each evaluation period sharing a common letter are not significantly different.

Results from multi-way ANOVA indicate that there were significant effects of treatment, month, and treatment/month interactions on salt soluble protein levels in lobster tail meat. Lowry values were significantly ( $p \leq 0.05$ ) higher at month 2 for the control than storage months 0, 4, and 6. Treated lobster tails with STP had significantly ( $p \leq 0.05$ ) higher Lowry values at month 2 and 4 than storage months 0 and 6.



Lobster claw salt soluble protein levels were variable as well, demonstrating no particular trend as shown by Figure 2.4. Control and 0.3% STP treated lobster claw meat consistently had higher salt soluble protein levels than 0.1% STP claw meat. However, differences across treatments were not found to be significant.

**Figure 2.4-Salt Soluble Protein Means of Lobster Claw Meat Over Frozen Storage Time\***



\*Significance  $p \leq 0.05$

Means are an average of duplicate samples.

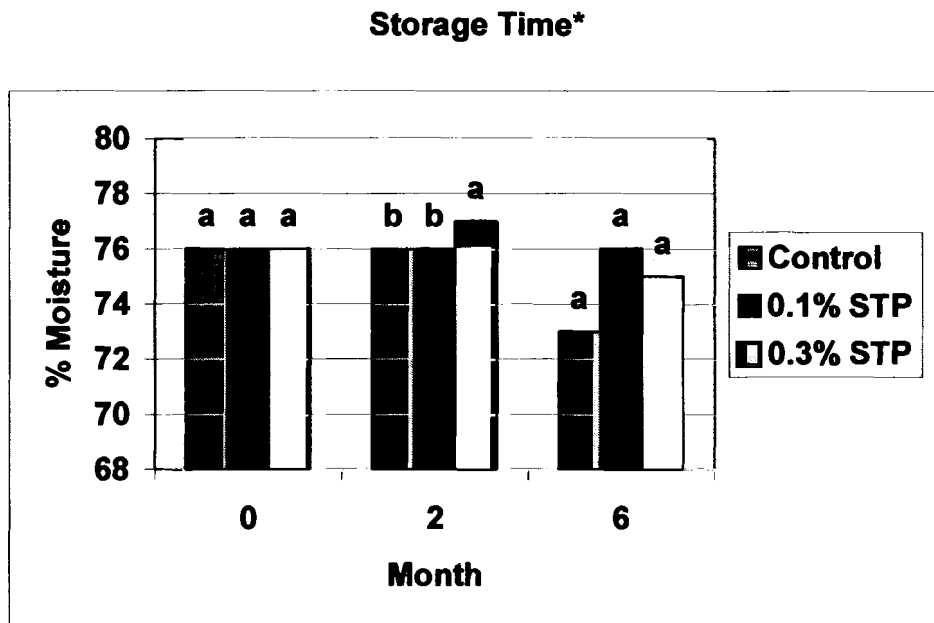
Means within each evaluation period sharing a common letter are not significantly different.

Multi-way ANOVA results reveal significant effects of treatment and time, but no significant treatment/month interactions on salt soluble protein levels in claw meat. All three treatments had significantly ( $p \leq 0.05$ ) higher Lowry values at months 2 and 4 than at months 0 and 6.

### Moisture:

According to one-way ANOVA results, moisture content of lobster tail meat varied significantly ( $p \leq 0.05$ ) among treatments at month 2. The 0.3% STP treated lobster tail meat had a significantly higher moisture level than the other two treatments. Moisture content seemed to decrease at month 6 for control and 0.3% STP treated lobster tails. Treated tails with STP were higher in moisture content at month 6 than control. Moisture data at month 4 was eliminated due to experimental error. Lobster tail moisture levels can be seen in Figure 2.5.

**Figure 2.5-Mean Percentage of Moisture in Lobster Tails Over Frozen**



\*Significance  $p \leq 0.05$

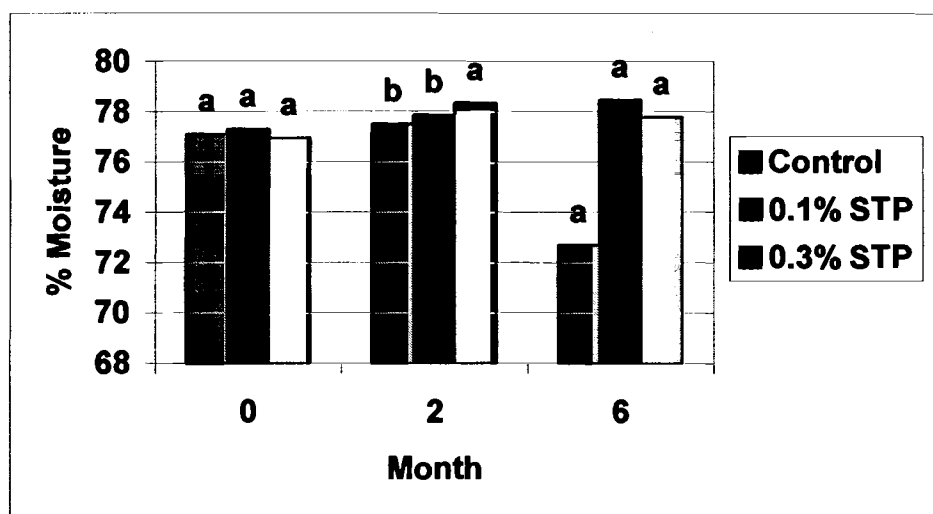
Means are an average of duplicate samples.

Means within each evaluation period sharing a common letter are not significantly different.

Moisture multi-way ANOVA results indicate no significant effects of treatment, time or treatment/month interactions on moisture levels in lobster tail meat.

Claw moisture content varied over frozen storage time in a similar manner to lobster tail meat as can be seen in Figure 2.6. One-way ANOVA results show claw meat moisture differences were significant ( $p \leq 0.05$ ) at months 2 and 6. At month 2, 0.3% STP treated claw meat was slightly higher than control and 0.1% STP treated lobster claws. At month 6, control claw meat was lower in moisture levels than the STP treated lobster claws. Month 4 data was not reported due to experimental error.

**Figure 2.6-Mean Percentage of Moisture in Lobster Claws Over Frozen Storage Time\***



\*Significance  $p \leq 0.05$

Means are an average of duplicate samples.

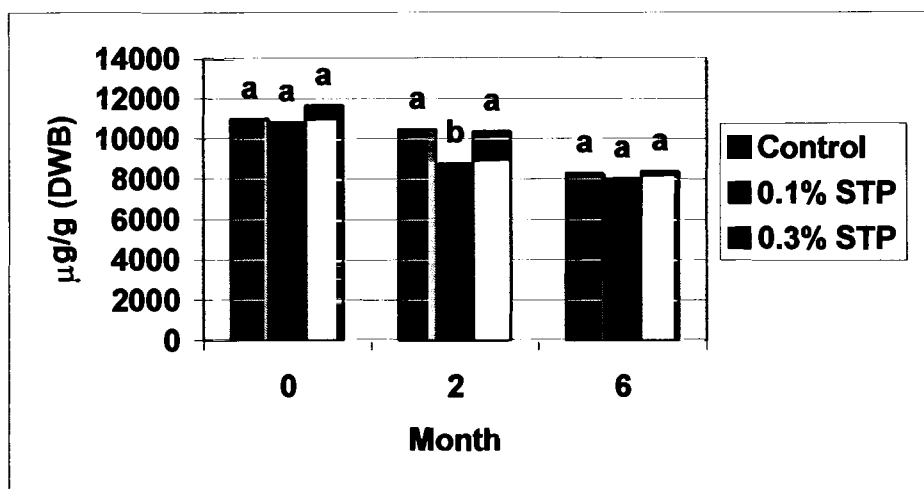
Means within each evaluation period sharing a common letter are not significantly different.

Moisture multi-way ANOVA results indicate no significant effects of treatment, time or treatment/month interactions on moisture levels in lobster claw meat.

**Minerals:**

Phosphorus levels (reported on a dry weight basis) were significantly ( $p \leq 0.05$ ) different among treatments at month 2 in lobster tails, according to one-way ANOVA results. At month 2, mean phosphorus concentrations in 0.1% STP lobsters were significantly lower than in control or 0.3% STP tails. There seemed to be a trend of decreasing phosphorus levels over frozen storage time as illustrated in Figure 2.7. Moisture data at month 4 was eliminated due to experimental error.

**Figure 2.7- Mean Phosphorus Concentrations in Lobster Tails Over Frozen Storage Time\***



\*Significance  $p \leq 0.05$

Means are an average of duplicate samples.

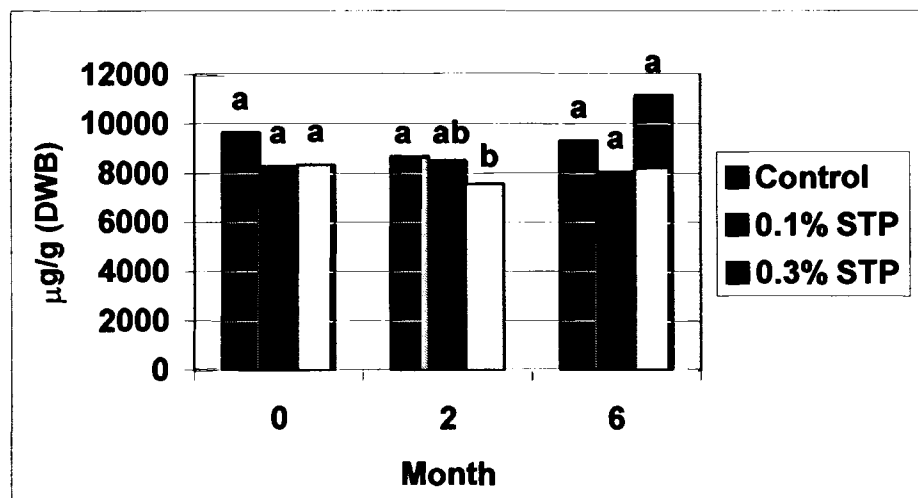
Means within each evaluation period sharing a common letter are not significantly different.

Multi-way ANOVA results indicate significant treatment and time effects, but no significant treatment/month interactions on phosphorus levels in lobster tail meat. Phosphorus concentrations were significantly ( $p \leq 0.05$ ) lower at month 6 than month 0 across all treatments. Month 2 phosphorus levels were significantly ( $p \leq 0.05$ ) higher than month 6 for control and 0.1% STP treated lobster tails.

Claw meat one-way ANOVA results reveal significant ( $p \leq 0.05$ ) differences in phosphorus levels. At month 2, control claw meat was significantly ( $p \leq 0.05$ ) higher in phosphorus concentration than 0.3% STP treated claws. Phosphorus levels decreased over frozen storage time for most treatments until month 6. There was a noticeable increase in phosphorus content across treatments at month 6 as shown in Figure 2.8.

Worth noting, phosphorus levels in claw meat were in a similar concentration range to phosphorus levels in tail meat. Month 4 data was not reported due to experimental error.

**Figure 2.8-Mean Phosphorus Concentrations in Lobster Claws Over Frozen Storage Time\***



\*Significance  $p \leq 0.05$

Means are an average of duplicate samples.

Means within each evaluation period sharing a common letter are not significantly different.

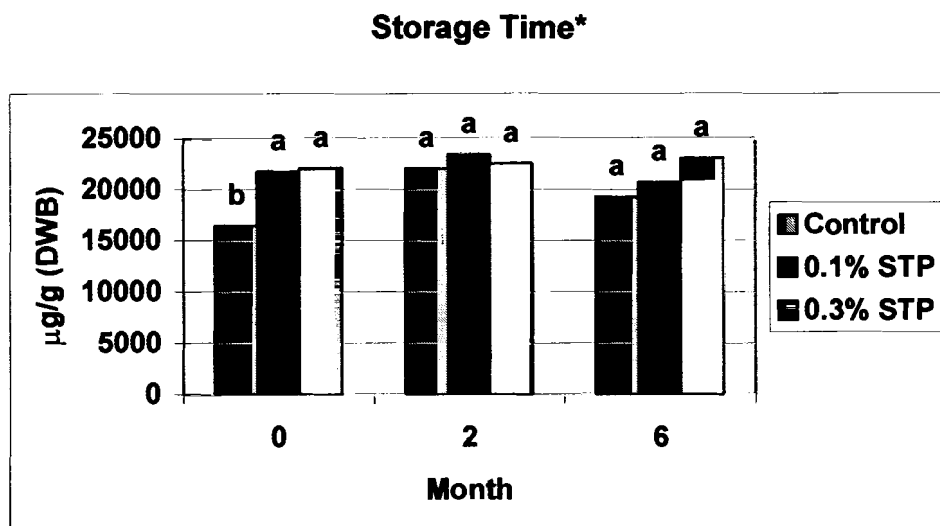
According to multi-way ANOVA results, there were no significant effects of treatment, time or treatment/month interactions on phosphorus levels in lobster claw meat.

Sodium content in lobster tail meat varied significantly ( $p \leq 0.05$ ) at month 0, as determined by one-way ANOVA sodium results. Control tails were significantly lower in sodium than 0.1 and 0.3% STP treated tails.

Sodium concentrations in lobster tail meat can be seen in Figure 2.9.

Month 4 data was eliminated due to experimental error.

**Figure 2.9-Mean Sodium Concentrations in Lobster Tails Over Frozen**



\*Significance  $p \leq 0.05$

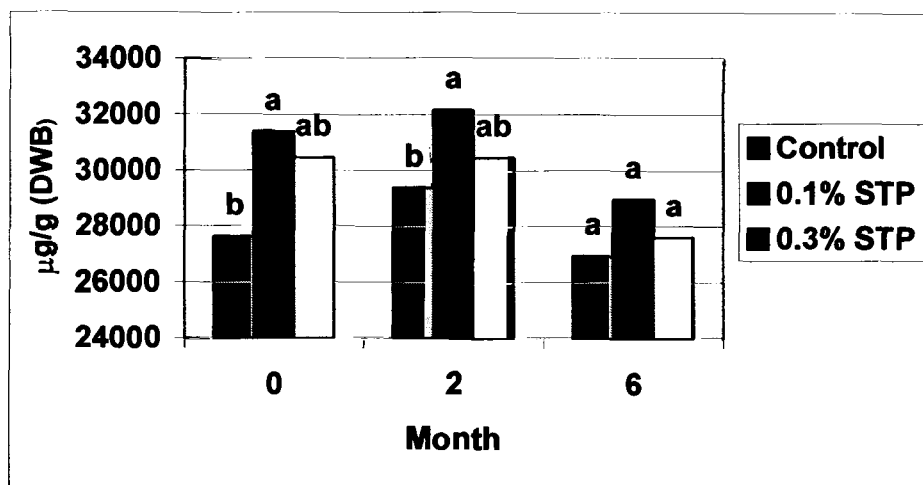
Means are an average of duplicate samples.

Means within each evaluation period sharing a common letter are not significantly different.

Multi-way ANOVA results indicate no significant effects of treatment, time or treatment/month interactions on sodium levels in lobster tail meat.

One-way ANOVA results indicate that claw meat sodium levels were significantly ( $p \leq 0.05$ ) different. At months 0 and 2, 0.1% STP treated lobster claws were significantly higher in sodium than control claw meat. Sodium levels were variable over frozen storage time in a similar fashion to tail meat levels as demonstrated by Figure 2.10. Month 4 data was not reported due to experimental error.

**Figure 2.10-Mean Sodium Concentrations in Lobster Claws Over Frozen Storage Time\***



\*Significance  $p \leq 0.05$

Means are an average of duplicate samples.

Means within each evaluation period sharing a common letter are not significantly different.

As determined by multi-way ANOVA results, no significant effects of treatment, time or treatment/month interactions were detected in sodium levels in lobster claw meat.

No significant differences were detected in calcium levels of lobster tail and claw meat, as indicated by one-way ANOVA results for both samples. Calcium levels were variable across storage time, and did not illustrate any particular trends as noted in Table 2.1 and Table 2.2. Month 4 data was eliminated due to experimental error.



**Table 2.1-Mean Calcium Concentrations in Lobster Tails on DWB  
(µg/g) Over Frozen Storage Time\***

<b>Sample:</b>	<b>Month 0</b>	<b>Month 2</b>	<b>Month 6</b>
<b>Control</b>	4590.5±1384.1 <sup>a</sup>	6403.8±344.6 <sup>a</sup>	2960.9±824.7 <sup>a</sup>
<b>0.1%STP</b>	6357.7±916.3 <sup>a</sup>	4605.7±571.9 <sup>a</sup>	3185.4±77.4 <sup>a</sup>
<b>0.3%STP</b>	6483.9±1272.9 <sup>a</sup>	5967.00±581.9 <sup>a</sup>	4835.2±3358.5 <sup>a</sup>

\*Significance  $p \leq 0.05$

Means are an average of duplicate samples ± standard deviation.

Means within each evaluation column sharing a common letter are not significantly different.

**Table 2.2-Mean Calcium Concentrations in Lobster Claws on DWB  
(µg/g) Over Frozen Storage Time\***

<b>Sample:</b>	<b>Month 0</b>	<b>Month 2</b>	<b>Month 6</b>
<b>Control</b>	8526.7±1647.2 <sup>a</sup>	9685.9±469.2 <sup>a</sup>	8580.0±1085.4 <sup>a</sup>
<b>0.1%STP</b>	8234.3±2889.3 <sup>a</sup>	9733.9±414.6 <sup>a</sup>	9010.3±150.2 <sup>a</sup>
<b>0.3%STP</b>	6627.7±247.1 <sup>a</sup>	8321.5±543.4 <sup>a</sup>	6710.2±2673.5 <sup>a</sup>

\*Significance  $p \leq 0.05$

Means are an average of duplicate samples ± standard deviation.

Means within each evaluation column sharing a common letter are not significantly different.

However, there were significant effects of time on calcium levels in lobster tail meat, according to multi-way ANOVA results. In lobster tails, calcium levels were found to be significantly ( $p \leq 0.05$ ) lower in calcium at month 6 than at month 0. According to multi-way ANOVA results, calcium

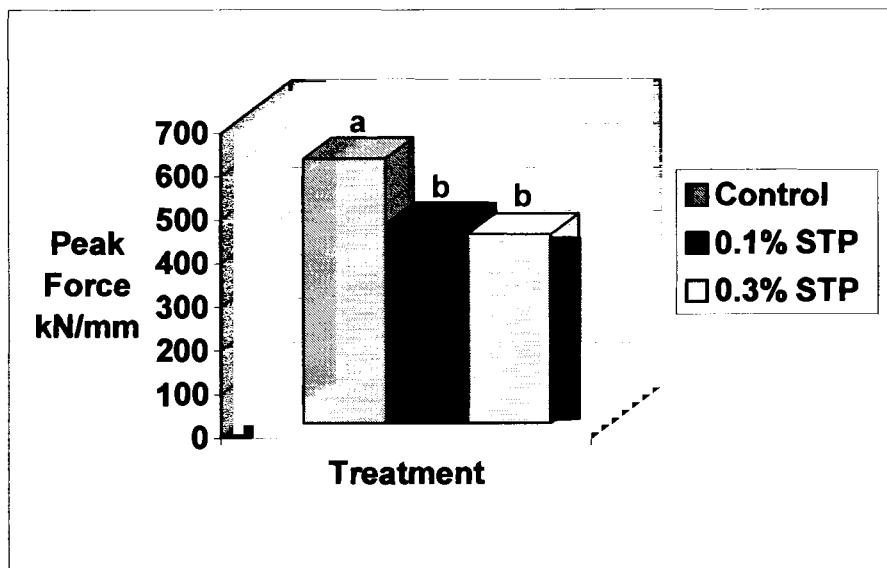
levels were not significantly different in claw meat across treatments over time.

### **Physical Analyses:**

#### **Texture Analysis:**

One-way ANOVA results reveal significant ( $p \leq 0.05$ ) differences among treatments in peak force at month 0 (Figure 2.11). Control had a significantly higher maximum displacement mean than 0.1 and 0.3% STP treated tails. No significant differences were determined at frozen storage months 2, 4, and 6. Unequal sample sizes were a problem for texture analyses due to “mushy” tail meat seen across treatments. Disintegrated lobster tails were rendered unfeasible for Instron testing. However, the STP treatments had more intact tails for Instron testing over frozen storage time as illustrated by Figures B.1-B.4 in Appendix B, which include individual Instron lobster tail data graphed separately by month.

**Figure 2.11-Instron Means of Lobster Tail Results at Month 0\***



\*Significance  $p \leq 0.05$

Means are an average of 14 tails for control, 18 tails for 0.1% STP, and 18 tails for 0.3% STP.

Means within each evaluation period sharing a common letter are not significantly different.

#### Colorimetric Analysis:

No significant Hunter color differences were detected at month 2.

The 0.1% STP treated lobster tails had a much higher “a” and hue value than the other two treatments as noted in Table 2.3.

**Table 2.3-Hunter Means of Lobster Tail Results at Month 2\***

<b>Sample:</b>	<b>L</b>	<b>a</b>	<b>b</b>	<b>Chroma</b>	<b>Hue</b>
<b>Control</b>	58.67±3.77 <sup>a</sup>	-0.34±0.61 <sup>a</sup>	7.16±1.34 <sup>a</sup>	7.17±1.36 <sup>a</sup>	2.30±122.85 <sup>a</sup>
<b>0.1%STP</b>	50.53±8.93 <sup>a</sup>	3.76±4.14 <sup>a</sup>	5.52±0.21 <sup>a</sup>	7.09±2.35 <sup>a</sup>	60.74±28.95 <sup>a</sup>
<b>0.3%STP</b>	53.94±5.18 <sup>a</sup>	-0.69±0.33 <sup>a</sup>	5.84±2.19 <sup>a</sup>	5.90±2.13 <sup>a</sup>	-82.17±6.04 <sup>a</sup>

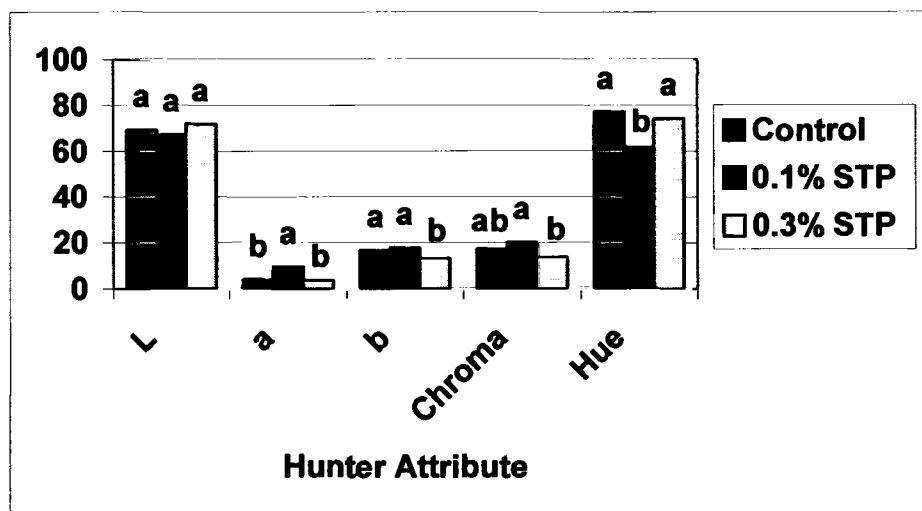
\*Significance  $p \leq 0.05$

Means are an average of duplicate samples  $\pm$  standard deviation.

Means within each evaluation column sharing a common letter are not significantly different

However, one-way ANOVA of colorimetric results reveal significant ( $p \leq 0.05$ ) differences among treatments at month 6. Lobster tails treated with 0.3% STP had a significantly lower b (measure of yellowness - blueness) value than 0.1% STP treated lobster tails. The 0.3% STP treated tails also had a significantly lower chroma (measure of intensity of saturation of a particular hue) value than 0.1% STP and control treatments. Tails treated with 0.1% STP were found to have a lower hue (attribute by which we distinguish primary colors) value than control and 0.3% STP tails; yet 0.1% STP treated tails had a significantly higher "a" (measure of greenness-redness) value than control and 0.3% STP lobster tails. Color analysis results for month 6 can be seen in Figure 2.12.

**Figure 2.12- Hunter Means of Lobster Tail Results at Month 6\***



\*Significance  $p \leq 0.05$

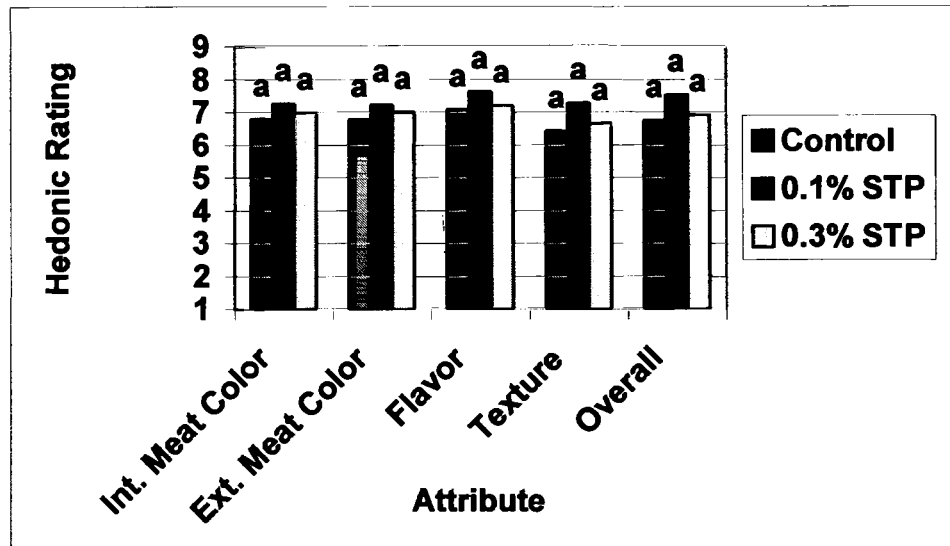
Means are an average of duplicate samples.

Means within each evaluation period sharing a common letter are not significantly different.

### Sensory Analysis:

No statistically significant differences were detected across treatments at month 2. However, STP treated lobster tails were rated slightly higher by panelists for all attributes including interior/exterior meat color, flavor, texture and overall acceptability as illustrated by Figure 2.13.

**Figure 2.13-Mean Sensory Ratings of Lobster Tails at Month 2\***



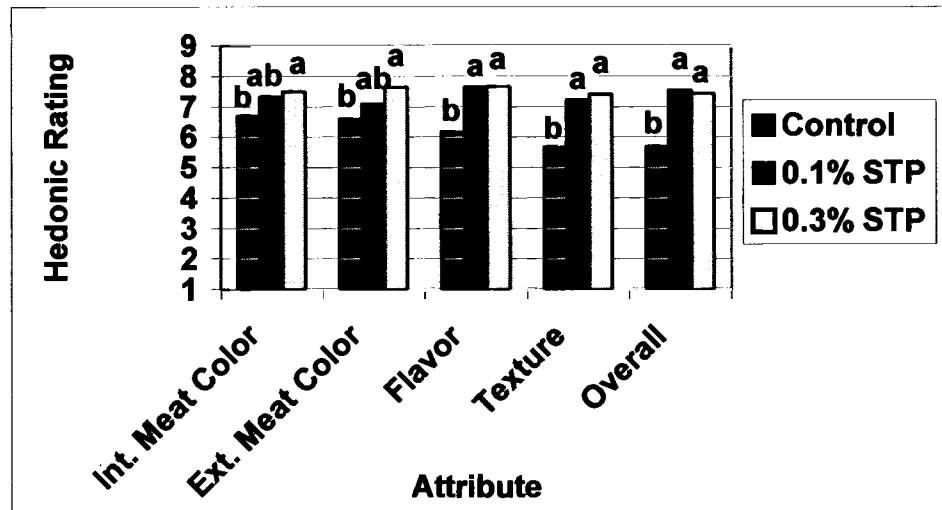
\*Significance  $p \leq 0.05$

Each value is a mean score of 43 panelists.

Means within each evaluation period sharing a common letter are not significantly different.

Significant differences were revealed at month 6 amongst panelist's ratings. The 0.3% STP treated lobster tails were rated significantly ( $p \leq 0.05$ ) higher than control tails for interior and exterior meat color. Panelists also rated 0.1 and 0.3% STP injected tails significantly ( $p \leq 0.01$ ) higher in flavor and texture, and significantly ( $p \leq 0.001$ ) higher in overall acceptability than control tails (Figure 2.14). Multi-way ANOVA sensory results for lobster tails indicate no significant effects of time, treatment, or time/treatment interactions on panelists' ratings.

**Figure 2.14- Mean Sensory Ratings of Lobster Tails at Month 6\***



\*Significance  $p \leq 0.05$  for interior and exterior meat color,  $p \leq 0.01$  for flavor and texture, and  $p \leq 0.001$  for overall acceptability attributes. Each value is a mean score of 30 panelists.

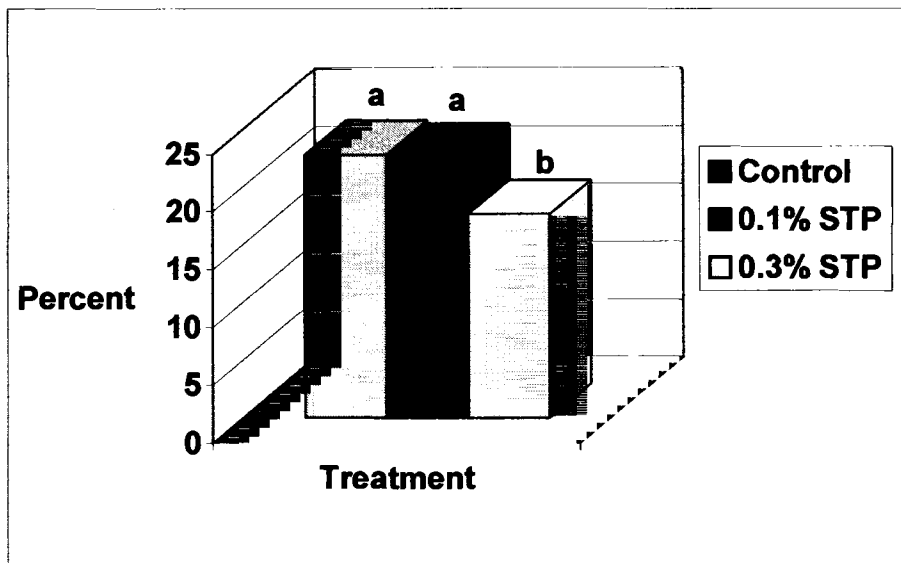
Means within each evaluation period sharing a common letter are not significantly different.

### **Cook Loss and Yield:**

Overall cook loss results determined significant ( $p \leq 0.05$ )

differences between 0.3% STP treated lobsters and 0.1% STP and control lobsters. The 0.3% STP treated lobsters had a 5% lower cook loss than the other two treatments as illustrated by Figure 2.15.

**Figure 2.15-Mean Percentage of Lobster Tail Meat Cook Loss\***



\*Significance  $p \leq 0.05$

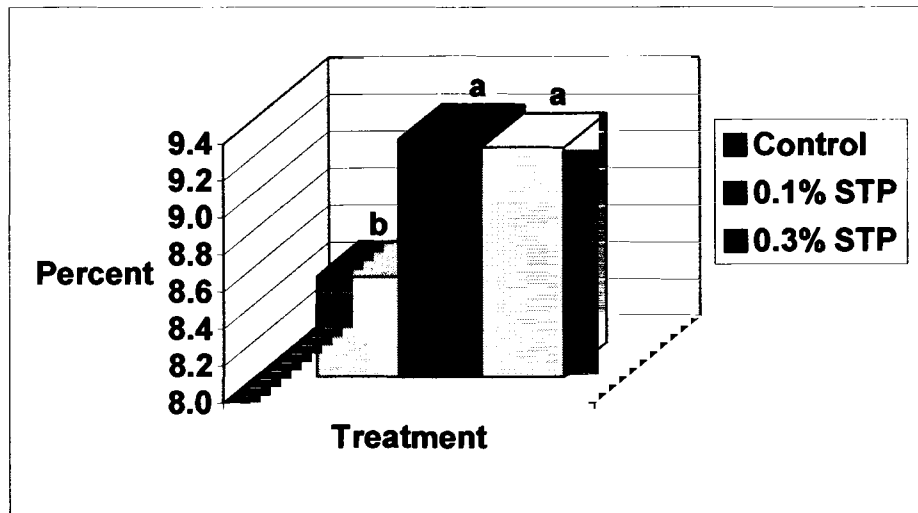
Each value is a mean of 57 lobster tails taken from random periods of frozen storage. Means within each evaluation period sharing a common letter are not significantly different.

The 0.3% and 0.1% STP treated lobster tails were significantly ( $p \leq 0.05$ ) higher in the percentage of steamed meat yield, based on whole steamed lobster weight, than control lobster tails. STP treated tails had 0.7-0.8% higher meat yield than control as shown in Figure 2.16.



**Figure 2.16- Mean Percentage of Steamed Lobster Tail Meat**

**Yield\***

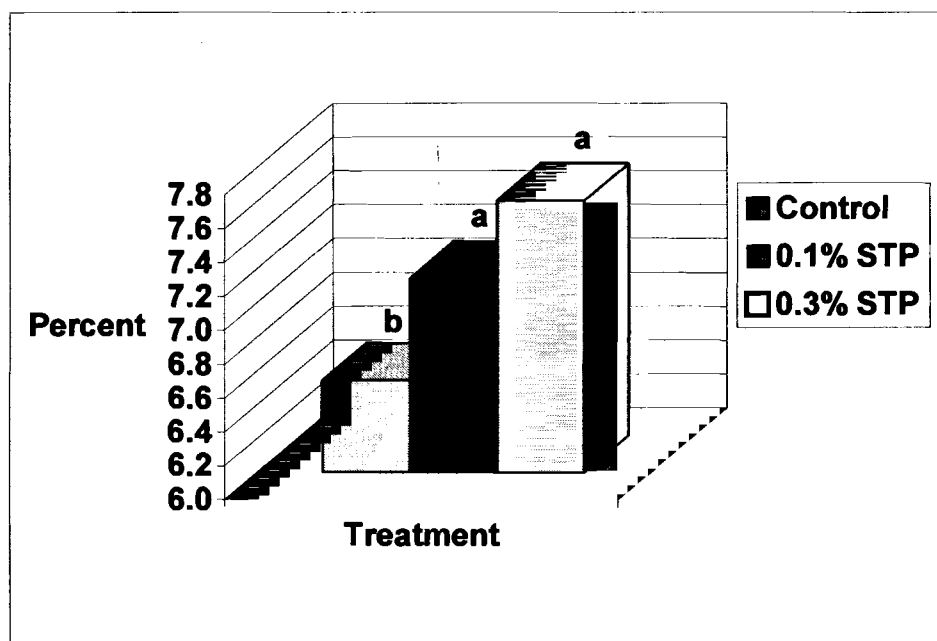


\*Significance  $p \leq 0.05$

Each value is a mean of 57 lobster tails taken from random periods of frozen storage. Means within each evaluation period sharing a common letter are not significantly different.

The percentages of lobster tail yield based on initial lobster weight are shown in Figure 2.17. The STP treated lobster tails were significantly ( $p \leq 0.05$ ) higher in the percentage of tail meat yield based on initial weight compared to control tails. The STP yields were 0.6 to 1.1% higher than control yields.

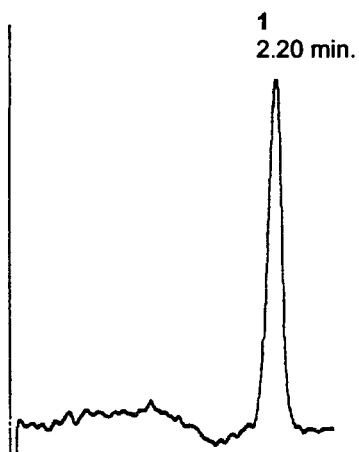
**Figure 2.17- Mean Percentage of Lobster Tail Meat Yield Based on Initial Lobster Weight\***



## Analytical Studies:

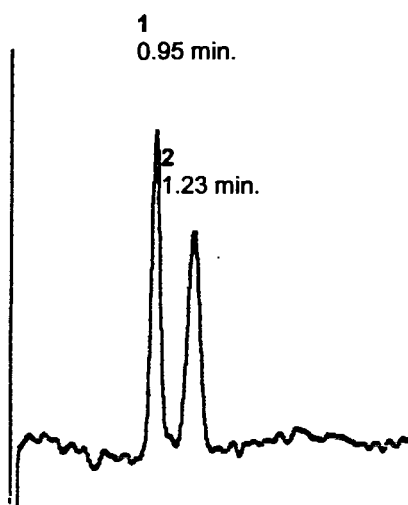
### Quantification Study of Sucrose in Lobster:

**Figure 2.18-Chromatogram of Sucrose-Buffer Standard**



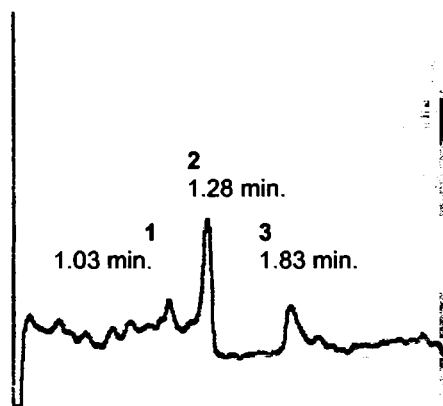
1) Sucrose

**Figure 2.19-Chromatogram of Sucrose/Buffer and Enzyme Standard**



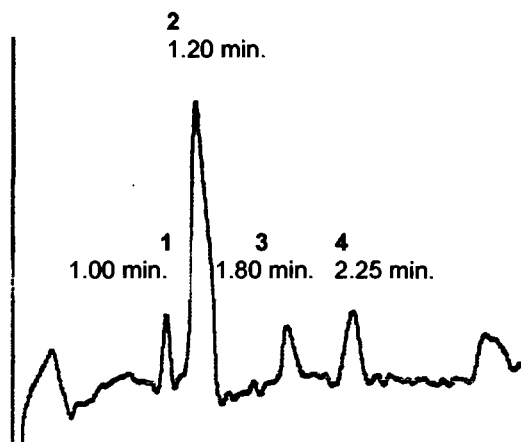
1) Fructose, 2) Glucose

**Figure 2.20-Chromatogram of Control Lobster (No Sucrose Injection) with Enzyme**



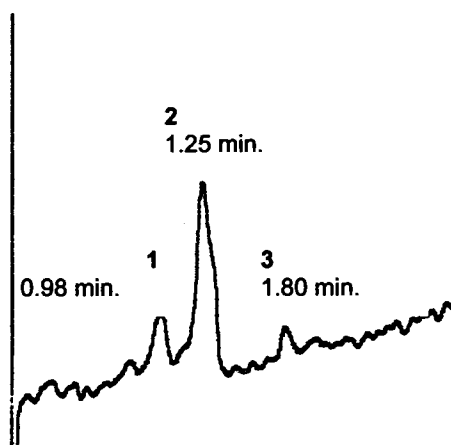
1, 2, 3) Unknown peaks

**Figure 2.21-Chromatogram of Sucrose Injected Lobster without Enzyme**



1, 2, 3) Unknown peaks 4) Sucrose

**Figure 2.22-Chromatogram of Sucrose Injected Lobster with Enzyme**

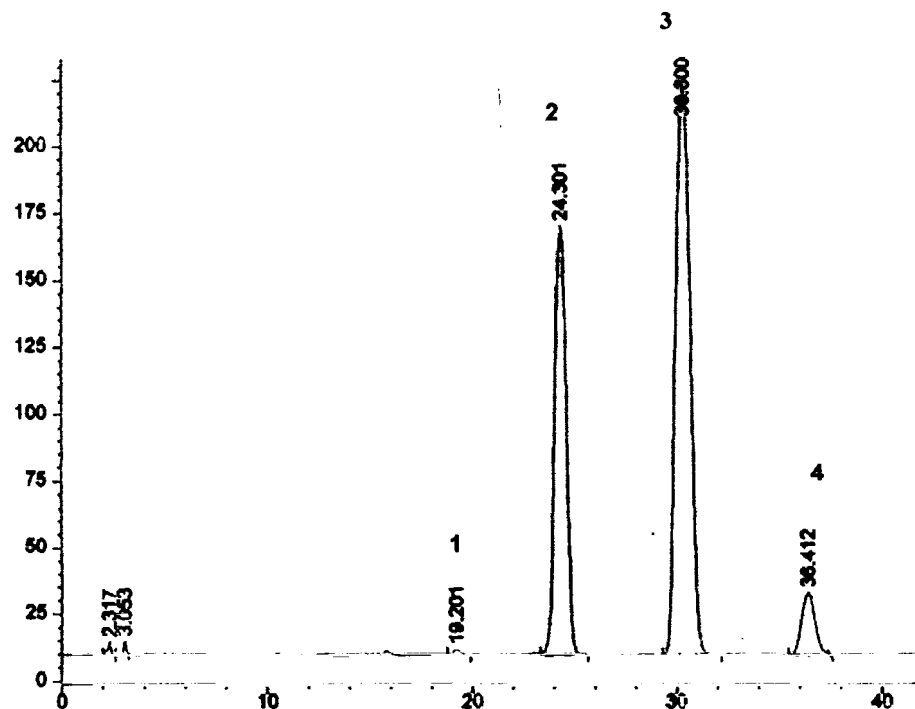


**1) Fructose, 2) Glucose, 3) Unknown peak**

Chromatogram results illustrate that invertase breakdown of sucrose to glucose and fructose can be detected by HPLC as shown by Figures 2.19 and 2.22. Due to baseline noise caused by interferences in lobster tissue, quantification of sucrose and breakdown products could not be obtained by this method. Figure 2.20 shows two unknown peaks found in control lobster detected near the same area as fructose and glucose. These unknown peaks would more likely be interfering fat or amino acids. Figure 2.21 shows that injected sucrose can be detected in lobster tissue, and with subsequent enzyme addition, sucrose is hydrolyzed by invertase to fructose and glucose as shown in Figure 2.22, resulting in the disappearance of the sucrose peak.

Quantification Study of Tocopherol in Lobster:

**Figure 2.23-Chromatogram of Mixed Function Tocopherol Standard**



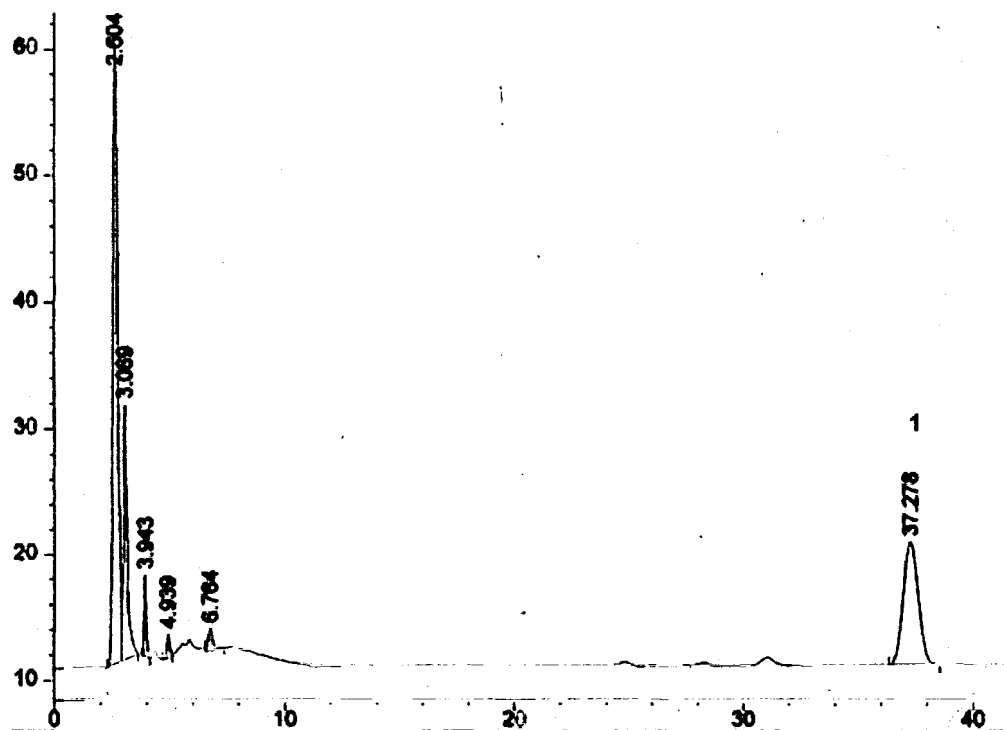
1) d- $\delta$ -tocopherol

2) d- $\gamma$ -tocopherol

3) d- $\beta$ -tocopherol

4) d- $\alpha$ -tocopherol

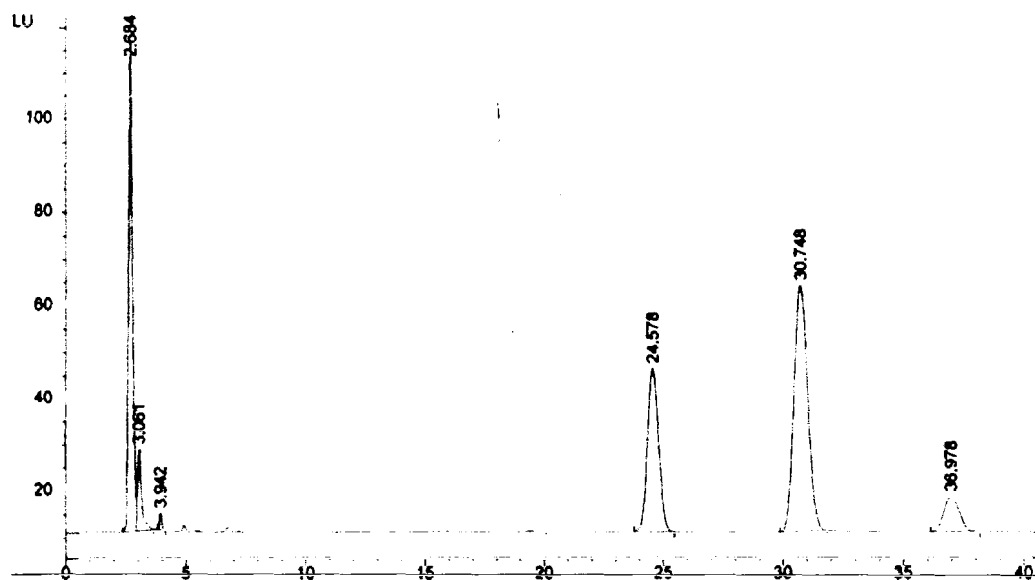
**Figure 2.24-Chromatogram of Control Lobster (No tocopherol injected)**



1) Unknown peak

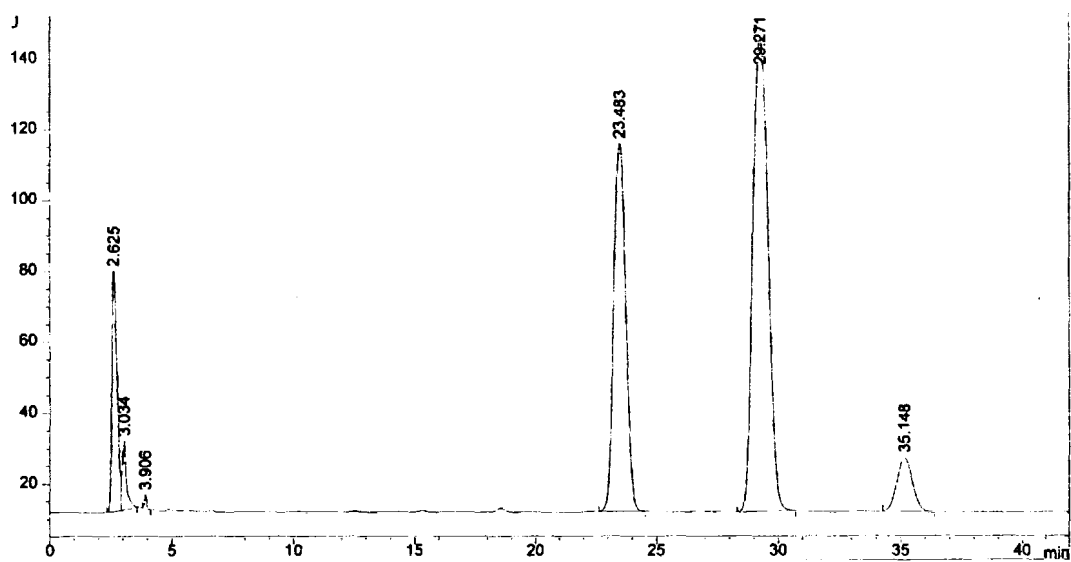
**Figure 2.25-Chromatogram of Lobster with Mixed Function**

**Tocopherol Added**



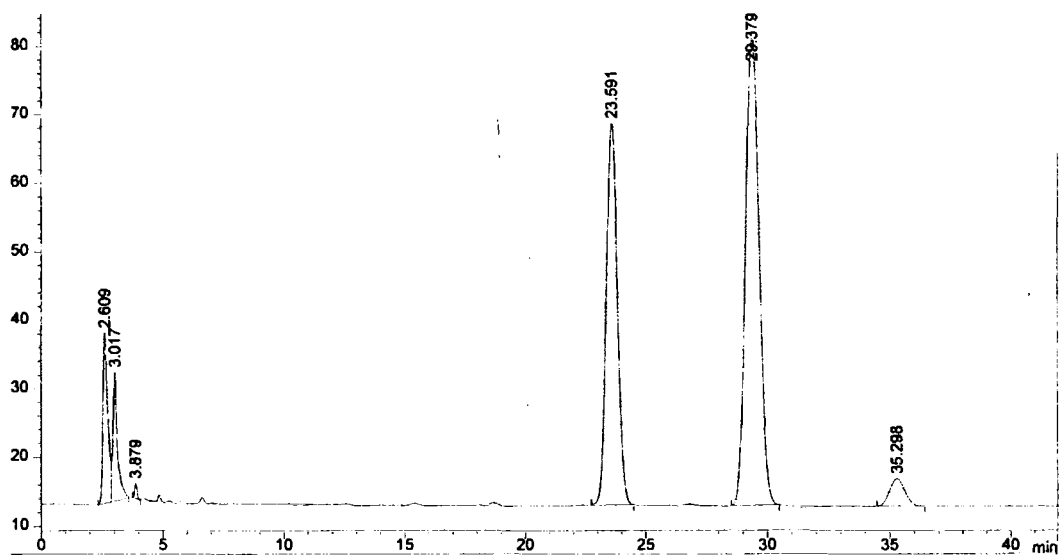
**Figure 2.26-Chromatogram of Lobster Tail Meat Injected with**

**Tocopherol**



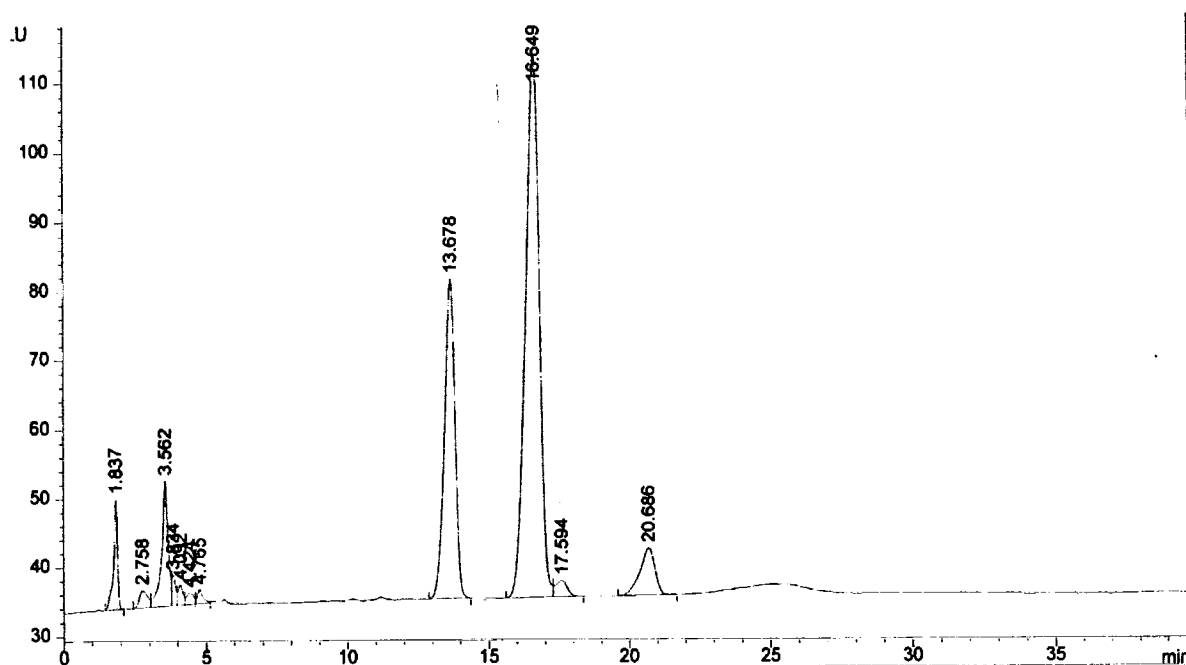


**Figure 2.27-Chromatogram of Lobster Claw Meat Injected with  
Tocopherol**



**Figure 2.28-Chromatogram of Further Separation of Lobster Tail Meat**

**Added with Tocopherol**



Covi-ox® T-30P contains a mixture of 4 tocopherols. The approximate percentages are 14% of d- $\alpha$ -tocopherol, 2% of d- $\beta$ -tocopherol, 60% of d- $\gamma$ -tocopherol, and 24% of d- $\delta$ -tocopherol, and these tocopherols were separated by this HPLC method as shown by Figure 2.23. These peaks were later identified by standards. Lobster meat was initially mixed with the tocopherol standard and a chromatogram of this mixture is shown in Figure 2.25. As illustrated by Figures 2.26 and 2.27,  $\beta$ -tocopherol and  $\gamma$ -tocopherol appear to be combined in one peak, similar to results of Huo et al. (1999). Figures 2.26 and 2.27 show only three tocopherol peaks. Changing columns for better peak separation with

lobster samples were successful as demonstrated by Figure 2.28, illustrating that  $\beta$ -tocopherol and  $\gamma$ -tocopherol are combined as one peak. When tocopherol standards were analyzed,  $\beta$ -tocopherol and  $\gamma$ -tocopherol were separated only a minute apart.  $\beta$ -tocopherol and  $\gamma$ -tocopherols appear to be slower to separate when added to or injected into lobster tissue indicating that possible interferences are occurring from within the lobster tissue matrix, possibly due to protein interferences.

## **CRAB RESULTS:**

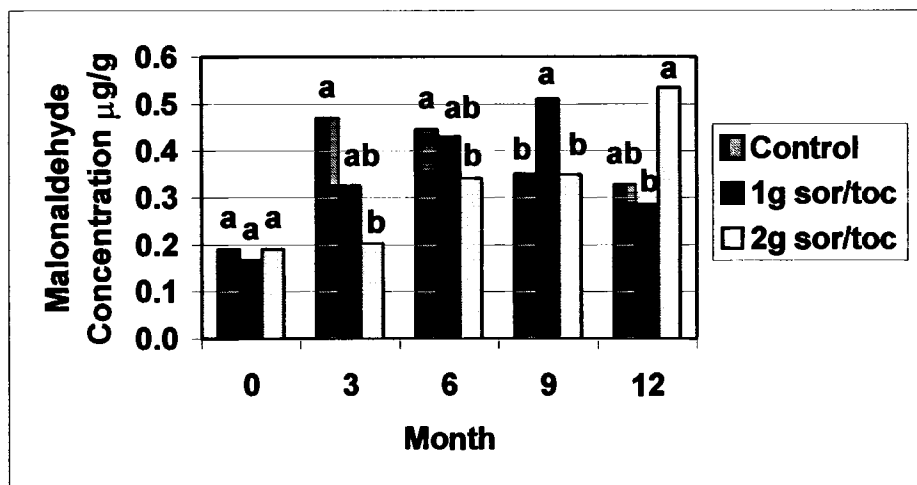
### **Chemical Analyses:**

#### **Thiobarbituric Acid Reactive Substances:**

One-way ANOVA results reveal that 2g sorbitol/tocopherol treated crabs were significantly ( $p \leq 0.05$ ) lower in malonaldehyde concentration than the control samples at months 3 and 6. At month 9, 1g sorbitol/tocopherol treated crabs had significantly ( $p \leq 0.05$ ) higher TBARS values than control and 2g sorbitol/tocopherol treated crab meat. At month 12, 2g sorbitol/tocopherol crab meat samples were significantly ( $p \leq 0.05$ ) higher in malonaldehyde concentration than 1g sorbitol/tocopherol treated crab. Multi-way ANOVA results indicate significant time and treatment/month interaction effects on TBARS levels in crab meat. The 1g sorbitol/tocopherol treated crab TBARS level was significantly ( $p \leq 0.05$ ) lower at month 12 than at months 6 and 9. The 2g sorbitol/tocopherol treated crab TBARS level was significantly ( $p \leq 0.05$ ) higher at month 12 than at months 0 and 3. The control crab TBARS level was significantly ( $p \leq 0.05$ ) lower at month 0 than at month 3.

A trend of increased malonaldehyde concentration over time can be seen in Figure 3.1. However, control crab meat increased up to month 3, and then decreased for the latter three months of frozen storage.

**Figure 3.1-Crab TBARS Means Over Frozen Storage Time\***



\*Significance  $p \leq 0.05$

Means are an average of duplicate samples.

Means within each evaluation period sharing a common letter are not significantly different.

1g sor/toc=1g sorbitol with mixed function tocopherol

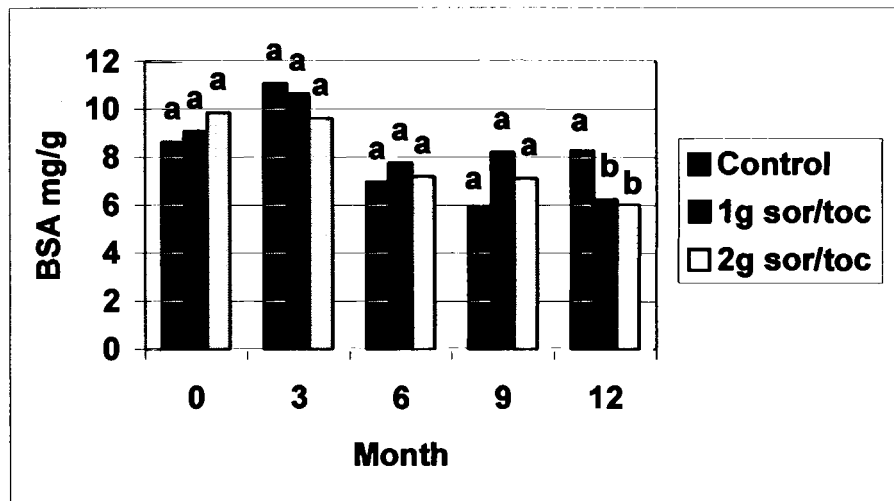
2g sor/toc=2g sorbitol with mixed function tocopherol

### Salt Soluble Protein:

Salt soluble protein results reveal significant ( $p < 0.05$ ) differences among treatments at month 12, as determined by one-way ANOVA analyses. Control crabs were significantly higher in salt soluble protein content than both treated crabs. Salt soluble protein levels were variable over time. However, 2g sorbitol/tocopherol crab meat decreased progressively in salt soluble protein content over storage time as illustrated by Figure 3.2. According to multi-way ANOVA results, there were significant time and treatment/month interaction effects on salt soluble protein levels in crab meat. The control Lowry value at month 0 was found to be significantly ( $p \leq 0.05$ ) higher than at months 6, 9, and 12. The 1g sorbitol/tocopherol treated crab Lowry value at month 0 was determined to be significantly ( $p \leq 0.05$ ) higher than at month 12, and the

salt soluble protein level at month 3 was also significantly ( $p \leq 0.05$ ) higher than months 6 and 12. The 2g sorbitol/tocopherol treated crab meat was significantly ( $p \leq 0.05$ ) higher in salt soluble protein levels at month 0 than months 6, 9, and 12, and also higher at month 3 than months 9 and 12.

**Figure 3.2-Mean Salt Soluble Protein Levels in Crab Over Frozen Storage Time\***



\*Significance  $p \leq 0.05$

Means are an average of duplicate samples.

Means within each evaluation period sharing a common letter are not significantly different.

1g sor/toc=1g sorbitol with mixed function tocopherol

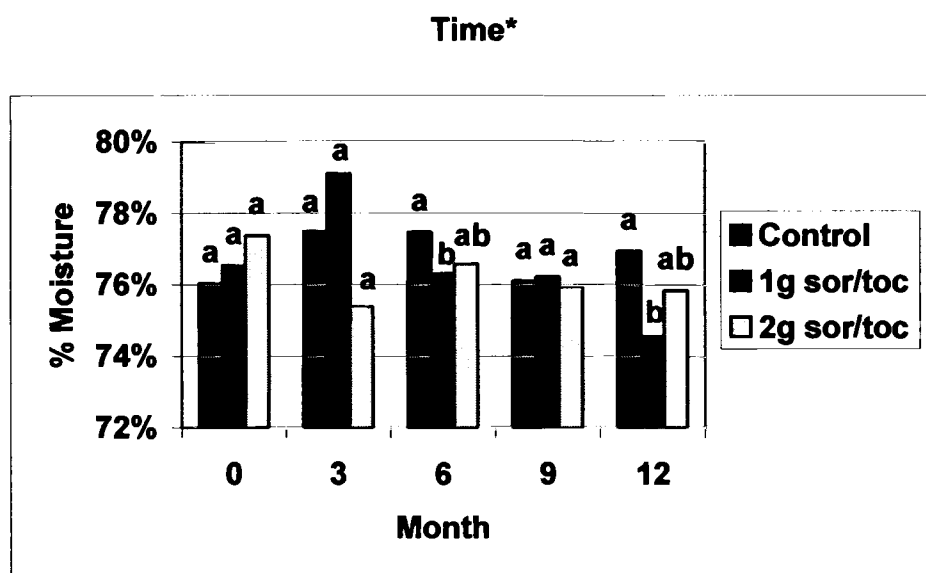
2g sor/toc=2g sorbitol with mixed function tocopherol

### Moisture:

Significant ( $p \leq 0.05$ ) differences in moisture content in crab meat were detected at months 6 and 12, according to one-way ANOVA results. At months 6 and 12, control had a significantly higher moisture percentage than 1g sorbitol/tocopherol treated crabs. Multi-way ANOVA results indicate no significant effects of treatment, time, or treatment/month

interactions on moisture levels in crab meat. The variability of moisture content over time in crab meat is demonstrated in Figure 3.3, which indicates no set moisture trends across storage months.

**Figure 3.3-Mean Percentage of Moisture in Crab Over Frozen Storage**



\*Significance  $p \leq 0.05$

Means are an average of duplicate samples.

Means within each evaluation period sharing a common letter are not significantly different.

1g sor/toc=1g sorbitol with mixed function tocopherol

2g sor/toc=2g sorbitol with mixed function tocopherol

### Minerals:

One-way ANOVA results indicate that phosphorus levels were not significantly ( $p \leq 0.05$ ) different across crab meat treatments. Multi-way ANOVA results reveal significant effects of time on phosphorus content in crab meat. The control crab was found to be significantly ( $p \leq 0.05$ ) higher in phosphorus at month 9 than at month 0. Phosphorus levels remained relatively stable over frozen storage time as shown by Table 3.1.

**Table 3.1-Crab Phosphorus Means on DWB (µg/g) Over Frozen Storage Time\***

	Month 0	Month 3	Month 6	Month 9	Month12
<b>Sample:</b>					
<b>Control</b>	7001.8±814.4 <sup>a</sup>	7963.6±801.9 <sup>a</sup>	7742.9±420.8 <sup>a</sup>	9399.0±403.2 <sup>a</sup>	7804.03±339.3 <sup>a</sup>
<b>1g sor/toc</b>	7098.0±171.5 <sup>a</sup>	7724.5±1253.1 <sup>a</sup>	8338.6±327.6 <sup>a</sup>	8133.5±361.3 <sup>a</sup>	7743.6±85.4 <sup>a</sup>
<b>2g sor/toc</b>	7888.1±476.3 <sup>a</sup>	7795.8±1194.8 <sup>a</sup>	8044.8±353.2 <sup>a</sup>	8431.8±25.2 <sup>a</sup>	7899.8±177.0 <sup>a</sup>

\*Significance  $p \leq 0.05$

Means are an average of duplicate samples.

Means within each evaluation column sharing a common letter are not significantly different.

1g sor/toc=1g sorbitol with mixed function tocopherol

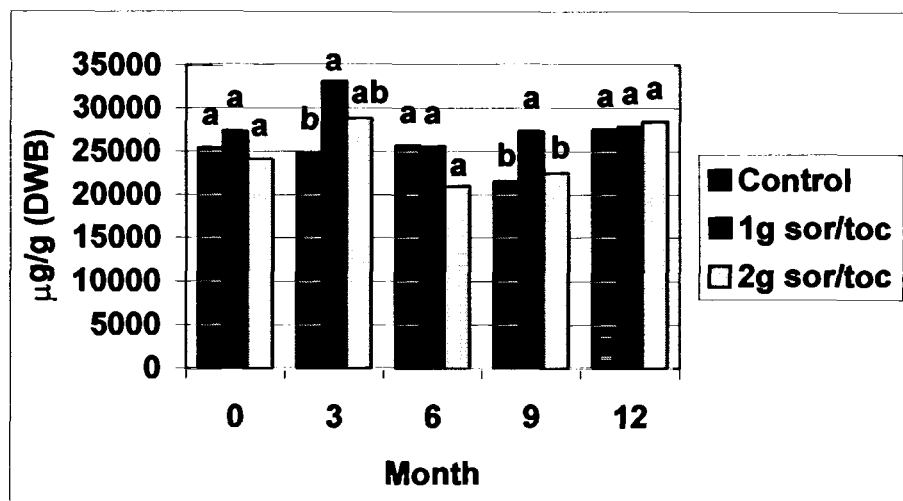
2g sor/toc=2g sorbitol with mixed function tocopherol



Sodium content was significantly ( $p \leq 0.05$ ) different among treatments at months 3 and 9, as determined by one-way ANOVA analyses. The 1g sorbitol/tocopherol treated crabs were significantly ( $p \leq 0.05$ ) higher in sodium than the control at month 3 and significantly higher than the control and 2g sorbitol/tocopherol at month 9. Multi-way ANOVA analysis indicate significant treatment, time, and treatment/month interaction effects on sodium content in crab meat. Control crab meat sodium level at month 0 was significantly ( $p \leq 0.05$ ) higher than month 9. Month 3 sodium level for 1g sorbitol/tocopherol treated crabs was significantly ( $p \leq 0.05$ ) higher than at all other storage months. The 2g sorbitol/tocopherol treated crab sodium level was also significantly ( $p \leq 0.05$ ) higher at month 3 than months 0, 6, and 9, and month 12 was significantly higher in sodium than at months 6 and 9. Sodium levels in crab meat were variable over time as illustrated by Figure 3.4.

**Figure 3.4-Mean Sodium Concentrations in Crab Over Frozen**

**Storage Time\***



\*Significance  $p \leq 0.05$

Means are an average of duplicate samples.

Means within each evaluation period sharing a common letter are not significantly different.

1g sor/toc=1g sorbitol with mixed function tocopherol

2g sor/toc=2g sorbitol with mixed function tocopherol

One-way ANOVA results for calcium content in crab meat indicated no significant ( $p \leq 0.05$ ) differences across treatments. However, multi-way ANOVA results revealed significant effects of time on calcium content in crab meat. The calcium level at month 9 for the control was significantly ( $p \leq 0.05$ ) higher than all other storage months for control samples and was higher than sorbitol/tocopherol treated crab across all storage months.

Calcium levels were relatively stable over storage time except at month 9. Calcium levels seemed to spike across treatments at this month especially for control and 1g sorbitol/tocopherol treated crab meat as noted in Table 3.2

**Table 3.2-Crab Calcium Means on DWB ( $\mu\text{g/g}$ ) Over Frozen Storage Time \***

	Month 0	Month 3	Month 6	Month 9	Month 12
<b>Sample:</b>					
<b>Control</b>	4059.4 $\pm$ 876.4 <sup>a</sup>	4222.6 $\pm$ 204.7 <sup>a</sup>	5906.6 $\pm$ 345.9 <sup>a</sup>	18271.5 $\pm$ 9251.1 <sup>a</sup>	6153.9 $\pm$ 2175.7 <sup>a</sup>
<b>1g sor/toc</b>	4174.5 $\pm$ 45.7 <sup>a</sup>	6202.7 $\pm$ 1883.1 <sup>a</sup>	6279.5 $\pm$ 1079.6 <sup>a</sup>	12015.5 $\pm$ 1718.2 <sup>a</sup>	3486.8 $\pm$ 111.7 <sup>a</sup>
<b>2g sor/toc</b>	5527.9 $\pm$ 577.5 <sup>a</sup>	6030.2 $\pm$ 3180.4 <sup>a</sup>	5165.1 $\pm$ 56.9 <sup>a</sup>	6691.2 $\pm$ 413.6 <sup>a</sup>	4589.8 $\pm$ 199.1 <sup>a</sup>

\*Significance  $p \leq 0.05$

Means are an average of duplicate samples.

Means within each evaluation column sharing a common letter are not significantly different.

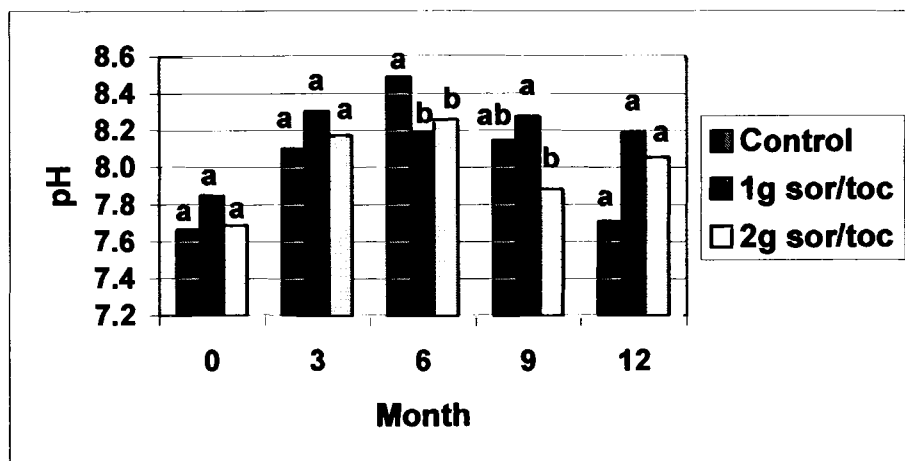
1g sor/toc=1g sorbitol with mixed function tocopherol

2g sor/toc=2g sorbitol with mixed function tocopherol

pH:

pH results are shown in Figure 3.7. Significant ( $p \leq 0.05$ ) differences were detected at months 6 and 9, as determined by one-way ANOVA analyses. Control crab pH levels were significantly higher than treated crabs at month 6. At month 9, 1g sorbitol/tocopherol crab meat was significantly higher in pH than 2g sorbitol/tocopherol treated crabs. There were significant effects of month, treatment, and treatment/month interactions on pH in crab meat, according to multi-way ANOVA analysis. The control crab meat pH levels were significantly ( $p \leq 0.05$ ) higher at months 6 and 9 than at months 0 and 12. The 1g sorbitol/tocopherol treated crab meat was significantly ( $p \leq 0.05$ ) higher in pH levels at months 3 and 9 than month 0, and the 2g sorbitol/tocopherol treated crab was significantly ( $p \leq 0.05$ ) higher at months 3 and 6 than at month 0.

**Figure 3.5- Crab pH Means Over Frozen Storage Time\***



\*Significance  $p \leq 0.05$

Means are an average of duplicate samples.

Means within each evaluation period sharing a common letter are not significantly different.

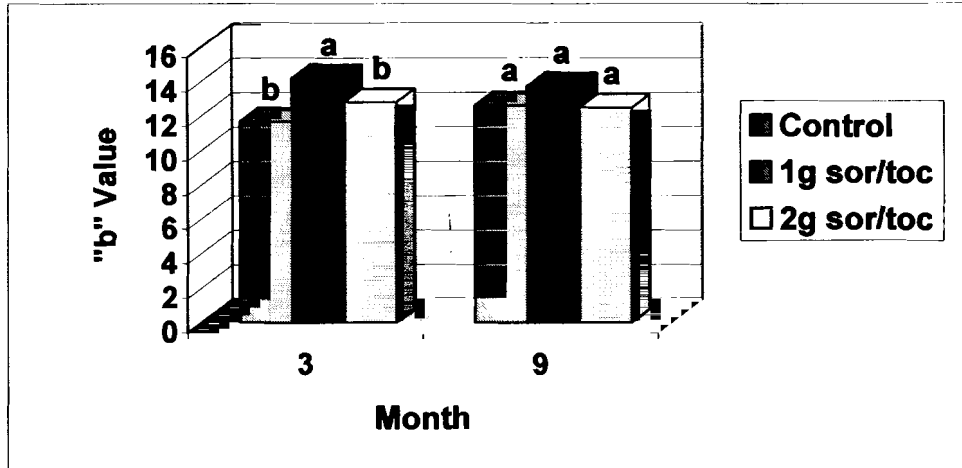
1g sor/toc=1g sorbitol with mixed function tocopherol

2g sor/toc=2g sorbitol with mixed function tocopherol

### Colorimetric Analysis:

Significant ( $p \leq 0.05$ ) differences were revealed for Hunter b and calculated chroma values, according to one-way ANOVA results. The 1g sorbitol/tocopherol treated crabs had a significantly higher b and chroma values than control and 2g sorbitol/tocopherol treated crabs at month 3, but no significant differences were detected at month 9 as shown in Figures 3.5 and 3.6. Tables 3.3 and 3.4 give complete Hunter results of crab meat at months 3 and 9.

**Figure 3.6- Crab Hunter "b" Value Means \***



\*Significance  $p \leq 0.05$

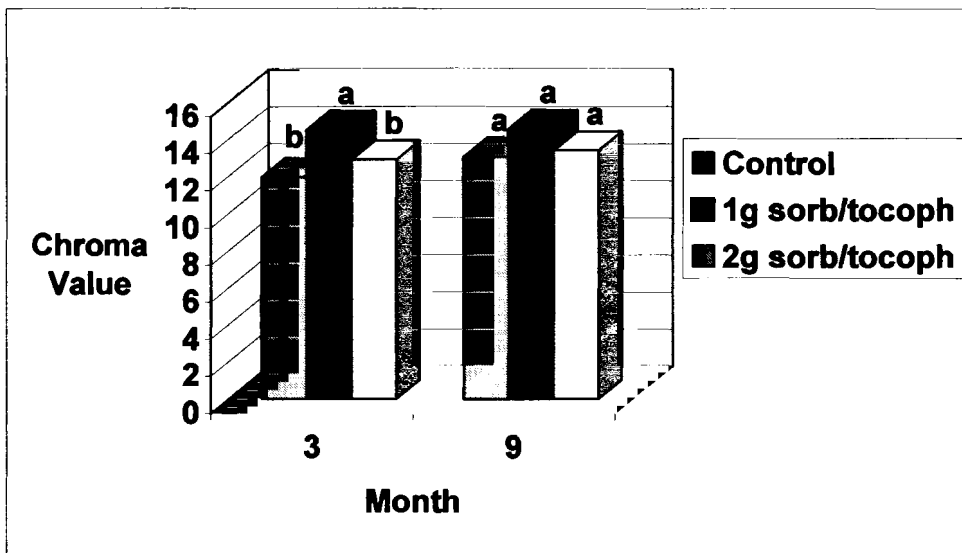
Means are an average of duplicate samples.

Means within each evaluation period sharing a common letter are not significantly different.

1g sor/toc=1g sorbitol with mixed function tocopherol

2g sor/toc=2g sorbitol with mixed function tocopherol

**Figure 3.7-Crab Hunter Chroma Means\***



\*Significance  $p \leq 0.05$

Means are an average of duplicate samples.

Means within each evaluation period sharing a common letter are not significantly different.

1g sor/toc=1g sorbitol with mixed function tocopherol

2g sor/toc=2g sorbitol with mixed function tocopherol

**Table 3.3-Crab Hunter Means at Month 3\***

<b>Sample:</b>	<b>L value</b>	<b>a value</b>	<b>b value</b>	<b>Chroma</b>	<b>Hue</b>
<b>Control</b>	64.8±0.8 <sup>a</sup>	2.5±0.9 <sup>a</sup>	11.7±0.8 <sup>b</sup>	12.0±0.7 <sup>b</sup>	77.9±4.5 <sup>a</sup>
<b>1g sor/toc</b>	63.8±3.0 <sup>a</sup>	3.1±0.9 <sup>a</sup>	14.2±0.3 <sup>a</sup>	14.5±0.1 <sup>a</sup>	77.8±3.6 <sup>a</sup>
<b>2g sor/toc</b>	68.0±0.9 <sup>a</sup>	1.8±0.6 <sup>a</sup>	12.8±0.3 <sup>b</sup>	12.9±0.3 <sup>b</sup>	82.2±2.9 <sup>a</sup>

\*Significance  $p \leq 0.05$

Means are an average of duplicate samples.

Means within each evaluation column sharing a common letter are not significantly different.

1g sor/toc=1g sorbitol with mixed function tocopherol

2g sor/toc=2g sorbitol with mixed function tocopherol

**Table 3.4-Crab Hunter Means at Month 9\***

<b>Sample:</b>	<b>L value</b>	<b>a value</b>	<b>b value</b>	<b>Chroma</b>	<b>Hue</b>
<b>Control</b>	66.4±2.8 <sup>a</sup>	3.4±0.1 <sup>a</sup>	12.6±1.1 <sup>a</sup>	13.1±1.1 <sup>a</sup>	74.7±1.1 <sup>a</sup>
<b>1g sor/toc</b>	64.4±2.4 <sup>a</sup>	4.9±1.0 <sup>a</sup>	13.7±1.0 <sup>a</sup>	14.6±0.9 <sup>a</sup>	70.5±4.3 <sup>a</sup>
<b>2g sor/toc</b>	66.8±2.9 <sup>a</sup>	4.9±0.5 <sup>a</sup>	12.5±0.7 <sup>a</sup>	13.4±0.8 <sup>a</sup>	68.7±1.4 <sup>a</sup>

\*Significance  $p \leq 0.05$

Means are an average of duplicate samples.

Means within each evaluation column sharing a common letter are not significantly different.

1g sor/toc=1g sorbitol with mixed function tocopherol

2g sor/toc=2g sorbitol with mixed function tocopherol

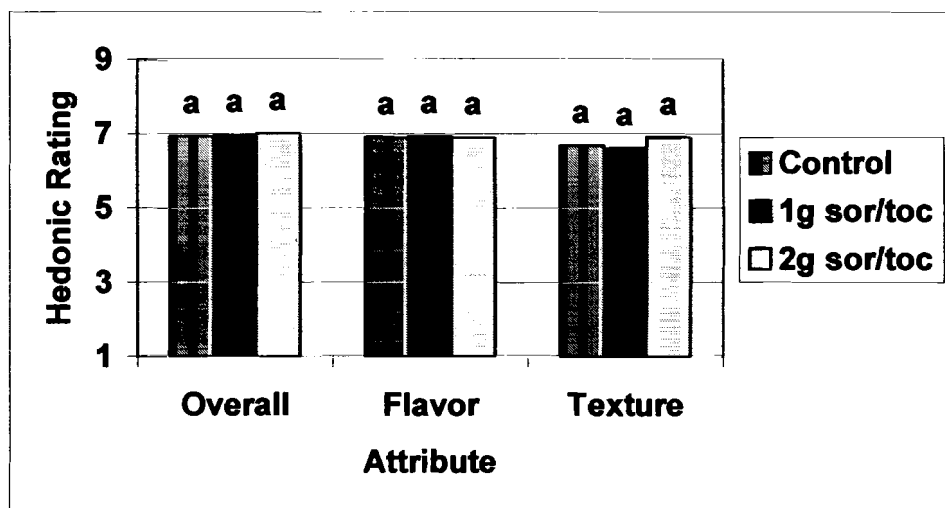
### **Sensory Analysis:**

One-way ANOVA results indicate no significant differences at month 3. Sensory panelists gave treatments almost the same rating for overall acceptability and flavor, but the 2g sorbitol/tocopherol treated crab had a slightly higher rating for texture than the other two treatments.

Sensory results for month 3 can be seen in Figure 3.8.

At month 9, control and 1g sorbitol/tocopherol treated crab meat were rated significantly ( $p \leq 0.05$ ) higher for overall acceptability and flavor than 2g sorbitol/tocopherol treated crabs. The 1g sorbitol/tocopherol treated crabs had the highest ratings for all attributes. No significant differences were detected for texture. Sensory results for month 9 are shown in Figure 3.9. Multi-way ANOVA sensory results for month 3 and 9 indicate significant ( $p \leq 0.05$ ) differences for overall acceptability. Overall acceptability was rated significantly ( $p \leq 0.05$ ) higher across treatments and months than 2g sorbitol/tocopherol treated crab meat at month 9.

**Figure 3.8-Mean Sensory Ratings of Crab at Month 3\***



\*Significance  $p \leq 0.05$

Each value is a mean score of 30 panelists.

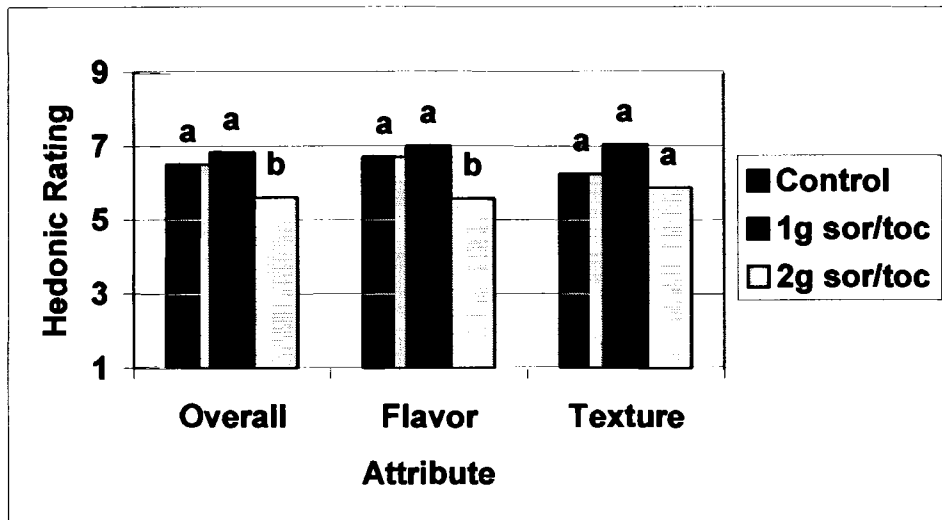
Means within each evaluation period sharing a common letter are not significantly different.

1g sor/toc=1g sorbitol with mixed function tocopherol

2g sor/toc=2g sorbitol with mixed function tocopherol



**Figure 3.9-Mean Sensory Ratings of Crab at Month 9\***



\*Significance  $p \leq 0.05$

Each value is a mean score of 30 panelists.

Means within each evaluation period sharing a common letter are not significantly different.

1g sor/toc=1g sorbitol with mixed function tocopherol

2g sor/toc=2g sorbitol with mixed function tocopherol

## **DISCUSSION:**

### **Thiobarbituric Acid Reactive Substances:**

The thiobarbituric acid reactive substances analysis (TBARS) measures a secondary product of lipid oxidation, malonaldehyde (Pike, 1998). Malonaldehyde reacts with TBA to yield a colored compound that is measured spectrophotometrically. The TBARS test correlates better with sensory evaluation of rancidity than does peroxide values, but like peroxides, it is a measure of a transient product of oxidation (i.e., malonaldehyde can react with other compounds) (Pike, 1998). The TBARS test, with minor modifications, is frequently used to measure lipid oxidation, especially in meat products (Pike, 1998). Quantification of hexanal, one of the major secondary products formed during the oxidation of linoleic acid, and other aldehydes, has also been utilized to determine the extent of lipid oxidation in meat systems (Lai et al., 1995). A gas chromatography method was developed at the University of Maine Food Science and Human Nutrition Department in an effort to measure hexanal in oxidized lobster meat, but results revealed that hexanal levels were too low to be quantified in lobster. Therefore, hexanal values were not analyzed in this study.

TBARS results indicate a trend of increasing lipid oxidation over frozen storage with a higher amount of malonaldehyde detected at month 6 seen across treatments for lobster claw, tail, and crabmeat. Higher TBARS values indicate an increased probability of off-flavor and odor

formation. However, after month 6, crab malonaldehyde concentrations decreased for some treatments indicating possible interferences. Lai et al. (1995) mentioned that the interaction of malonaldehyde with amino acids and the presence of other non-lipid TBA-reactive substances in biological samples might interfere with quantitation of oxidation products in the TBARS test. However, malonaldehyde concentrations never surpassed  $2\mu\text{g/g}$ , indicating that sensory panelists would probably not be able to detect any off flavors or odors at these concentrations. At month 6, TBARS results correspond well to sensory results. Pearson correlation matrix results indicate a strong negative relationship between TBARS values and panelists' ratings for overall acceptability ( $r = -0.90$ ) and flavor ( $r = -0.87$ ) of lobster tails at month 6. STP treated lobster tails had lower TBARS values than control, and sensory panelists rated STP treated tails significantly ( $p \leq 0.01$ ) higher for flavor and significantly ( $p \leq 0.001$ ) higher for overall acceptability than control lobster tails. TBARS results reveal that 0.1% STP treated lobster claw meat was significantly ( $p \leq 0.05$ ) lower in malonaldehyde concentration at month 6, and also lower in lobster tail meat than the other two treatments. Crab TBARS results indicate that sorbitol-tocopherol treatment was effective in preventing TBARS values to increase up to 6 months of frozen storage, and Pearson correlation matrix results indicate a strong negative relationship between panelists' ratings of overall acceptability and TBARS values ( $r = -0.99$ ) at month 3.

**Salt Soluble Protein:**

The Lowry method is a sensitive test to estimate salt soluble protein concentration by using a Folin-Ciocalteu phenol reagent, which is reduced by tyrosine and tryptophan residues in the protein of the sample. As the result of the reaction, a bluish color develops and is measured spectrophotometrically (Chang, 1998). Decreases in salt soluble protein concentrations usually indicate an increase in the toughening of texture due to an increase in cross-linking of myofibrillar protein (Chang and Regenstein, 1997, Konno et al., 1997).

Lowry results indicate that salt soluble protein concentrations in lobster tail and claw meat were variable over frozen storage time across treatments. However, sensory panelists did not indicate any significant differences among treatments in regards to texture at month 2 for lobster tails. Pearson correlation matrix results indicate a strong positive relationship between Lowry values and panelists' ratings for texture ( $r=0.99$ ) and overall acceptability ( $r=1.00$ ) at month 2. At month 6, sensory panelists rated STP treated lobster tails higher for more desirable texture even though no significant differences were noted for salt soluble protein concentrations across treatments.

Salt soluble protein concentrations decreased in crab over frozen storage time with control having a significantly ( $p \leq 0.05$ ) higher Lowry value than sorbitol-tocopherol treated crab at month 12. Pearson correlation matrix results reveal a strong negative relationship between

texture ( $r = -0.88$ ) and overall acceptability ( $r = -0.97$ ) at month 3. However, sensory panelists did not rate the texture of the crab meat significantly different across treatments.

Salt soluble protein and sensory results do not correspond well for lobster and crab meat. Variable concentrations in salt soluble protein levels over time have been detected in other lobster studies conducted at the University of Maine (Calder-Bussell et al., 2000, Work et al., 1998). Possibly the salt soluble protein variation could be due to protein stability changes over frozen storage as ice crystal aggregates change in conformation affecting the protein structure during frozen storage due to the cycling nature of the freezer. Researchers have noted that in lean fish species, quality loss often involves alteration of proteins accompanied by loss of water holding capacity and toughening of the muscle during prolonged frozen storage at  $-20^{\circ}\text{C}$  and above, as discussed by Krivchenia and Fennema (1988). Uncooked, frozen fish may undergo extensive textural changes during frozen storage because of the high sensitivity of fish proteins to denaturing factors such as increased salt concentrations, pH changes, and oxidized lipid products which may interact with proteins during frozen storage (Foegeding et al., 1996). Foegeding et al. (1996) found that myofibrillar proteins can be stabilized during freezing and frozen storage by the infusion of sugars or sugar alcohols, and salt soluble protein levels did seem to be more stabilized in the crab meat samples

injected with sorbitol-tocopherol over time compared to lobster claw and tail samples injected with STP.

### **Moisture:**

Moisture levels were measured to determine the protective effects of injected cryoprotectants (STP and sorbitol) on countering the harsh effects of freezing that slowly dehydrate muscle fibers over frozen storage time, increase the incidence of protein denaturation, and increase drip and subsequent moisture loss (Krivchenia and Fennema, 1988). Sodium tripolyphosphate injected into lobster seemed to maintain moisture levels in lobster meat over 6 months of frozen storage. Control lobster claw meat appeared to lose more moisture over time and STP treatments significantly ( $p \leq 0.05$ ) increased moisture levels in lobster claw and tail meat compared to control samples at month 6. Researchers have noted quality changes in frozen Jonah crabs appear much earlier in leg than in body meat, and studies with frozen lobster showed that claw tips deteriorate first, exhibiting yellowing and rancidity as mentioned by Rebach et al. (1990). Yellowing of lobster claw meat was also observed in our study after 4 months of frozen storage, which could explain the decrease in moisture levels in control lobster claws after month 2. Pearson correlation matrix results for lobster suggest a strong positive relationship between moisture levels and panelists' ratings of flavor ( $r=0.92$ ), texture ( $r=0.89$ ), and overall acceptability ( $r=0.94$ ) at month 6.

Sorbitol-tocopherol treated crab seemed to maintain moisture levels over 12 months of frozen storage. However, the 1g sorbitol/tocopherol treated crab appeared to have the widest range in moisture levels across frozen storage time and fluctuated by approximately 4%. Control crab moisture levels were also well maintained. Control crab had a significantly ( $p \leq 0.05$ ) higher moisture level at frozen storage months 6 and 12 than the 1g sorbitol/tocopherol treated crab meat. Pearson correlation matrix results for crab indicate a moderately strong positive relationship between moisture and panelists' ratings for flavor ( $r=0.83$ ) at month 3. At month 6, Pearson correlation matrix results suggest a strong positive relationship between moisture levels and sensory panelists' ratings at month 6 for flavor ( $r=0.99$ ), texture ( $r=0.92$ ), and overall acceptability ( $r=0.99$ ).

The moisture results indicate that the cryogenic freezing process was adequate in maintaining moisture quality in whole crab products over frozen storage time. Lobster meat appears to be more delicate and seemed to benefit from the added cryoprotectant effects of sodium tripolyphosphate over frozen storage time.

#### **Minerals:**

Mineral levels were monitored over frozen storage time to detect any significant differences due to the injection of cryoprotectants into the crustaceans' system. Mineral concentration varied across treatments over frozen storage. Crab calcium levels were elevated across treatments at

month 9, which could be an error from the Analytical Lab in Deering Hall. Sodium and phosphorus levels were slightly higher in STP treated lobster than controls in lobster claw and tail meat (except for phosphorus levels in lobster claw meat), as expected due to the initial injection of sodium tripolyphosphate. Control lobster tail and claws were significantly ( $p \leq 0.05$ ) lower in sodium content than STP treated lobster meat. The variability of mineral concentrations were not likely due to release of minerals from thaw drip loss because moisture levels remained constant, lowering slightly at month 6. Mineral extra- and intracellular content could possibly change due to cross-linking of myofibrillar proteins, tying up minerals in altered protein states during frozen storage. The amount of minerals lost on steaming may vary depending on the ratios of sodium and phosphorus in the crustaceans' muscle.

Treating lobster and crab with cryoprotectants may change electrolyte balances in lobster and crab tissue, however, lobster and crabs were not adversely affected after injection of cryoprotectants at these concentrations. Crabs and lobsters were steamed within 5-10 minutes after injection, but the cryoprotectants were not found to be lethal to these crustaceans.

### **Texture Analysis:**

Peak load measurements were performed on duplicate pieces of each lobster tail to determine shear force. This mechanical method is an



objective test of lobster tail texture differences over storage time. Aside from consumer acceptability, viscoelastic properties of food products are considered important in determining textural changes. Seafood researchers have performed Instron testing (with minor modifications) to determine shear force changes over storage time (Henry et al., 1995.) In our study significant ( $p \leq 0.05$ ) differences were detected at month 0 only. Control tail meat had a significantly higher peak force than STP injected lobster tails, indicating that control tails had a tougher or more chewy texture at month 0 than treated tails. Pearson correlation matrix results indicate a moderately strong negative relationship between Instron values and panelists' ratings for texture ( $r = -0.83$ ) and overall acceptability ( $r = -0.82$ ) at month 2. As mentioned previously, unequal sample sizes were an unexpected problem, rendering some lobster tails unfeasible for Instron testing. Random samples of disintegrated lobster tail meat inside the shell upon reheating were observed across treatments. This problem has been noted in previous cryogenic frozen lobster studies at the University of Maine, but it is not known if "mushy" tails are caused by the cryogenic freezing process, due to time since molt, or by unknown structural muscle changes, which may take place during frozen storage. Mizuta et al. (2001) discovered that partial disintegration of pericellular connective tissue was observed in soft shell crab muscle, while muscle fibers in the hard crab muscle connected well to each other through the endomysium, indicating the muscle structure was well maintained and

orderly. These histological characteristics of the soft crab muscle, suggest structural weakening of muscle fibers or connective tissue due to a physiological phenomenon related to molting, such as the uptake of seawater. The same physiological phenomena could occur in lobster muscle during their molting process as well. Mushy lobster tail meat has also been reported with fresh cooked soft shell lobsters from personal communications with the Maine Lobster Promotion Board with the Food Science and Human Nutrition Department at the University of Maine. It is unlikely to be related to biochemical or enzymatic reactions because of the cooking process, which would denature and inactivate enzymes. Worth mentioning, STP treated lobster tails had more intact tail meat over frozen storage time that was suitable for Instron testing, indicating that STP treatment may decrease the incidence of “mushy” lobster tail meat in cryogenically frozen, whole cooked lobsters. (See Instron individual lobster tail data in Figures B.1-B.4 in Appendix B.)

#### **Cook Loss and Yield of Lobster:**

Initial, steamed whole, and lobster tail meat (removed from tail shell) weights were taken to determine if sodium tripolyphosphate injection had any influence on decreasing cook loss and increasing tail yield. Results indicate that injecting 0.3% STP into lobster significantly decreased cook loss by 5%, and STP treatments significantly ( $p \leq 0.05$ ) increased tail meat yields based on steamed and initial weights. These

findings agree with the results of Froning and Sackett (1985). They injected turkey breast muscle with salt and various types of phosphates. The presence of salt and phosphates significantly reduced expressible moisture and cooking losses, but did not significantly affect shear values. Crapo and Crawford (1991) had similar results from their study. They soaked Dungeness crab in polyphosphate solutions and determined that moisture and proteins, retained by polyphosphate treatment after steam cooking, were believed responsible for improving meat yield, quality, and storage stability.

### **Sensory Analyses:**

Sensory affective tests to determine consumer acceptability of lobster samples were not found to be significantly different in lobster tails at month 2. However, panelists rated the STP treated lobster tails higher in overall acceptability, flavor, texture, and interior/exterior meat color than control lobster tails. At month 6, treatment differences were determined to be significant. Sensory results at month 6 reveal that sensory panelists rated both 0.1 and 0.3% STP injected lobster tails significantly ( $p \leq 0.01$ ) higher for flavor and texture, and significantly ( $p \leq 0.001$ ) higher in overall acceptability than control samples. The 0.3% STP treated tails were also rated significantly ( $p \leq 0.05$ ) higher than control for interior and exterior meat color. These results indicate that over frozen storage time, injection of STP maintained quality sensory attributes in lobster.

Crab sensory results reveal no significant ( $p \leq 0.05$ ) differences in panelists' ratings of crab samples at month 3. However, panelists rated 2g sorbitol/tocopherol treated crab slightly higher for texture than control and 1g sorbitol/tocopherol treated crab. At month 9, significant ( $p \leq 0.05$ ) differences were revealed across treatments. Sensory panelists rated control and 1g sorbitol/tocopherol injected crabs higher for overall acceptability and flavor than 2g sorbitol/tocopherol injected crabs, rating the 1g sorbitol/tocopherol treated crabs slightly higher for both attributes over the control. Sensory panelists rated 1g sorbitol/tocopherol higher for texture than the other two treatments, however the differences were not significant.

Panelists detected ammonia notes across crab treatments while cracking open claws at both sensory testing periods. However, panelists mentioned that it did not seem to permeate or affect the flavor of the meat. The ammonia odor was detected more often at month 9 sensory testing in crab treated with 2g sorbitol/tocopherol than the control or 1g sorbitol/tocopherol treated crab. Rebach et al. (1990) reported similar findings in their study. Sensory judges were asked to note the presence of ammonia odor in whole, frozen Jonah crabs at storage weeks 2, 20, 30, 40, and 50 after reheating and removing meat from the crab. Negative comments and ammonia odor were detected at 30 weeks of frozen storage or approximately 7 ½ months, which is similar to the results found in this study.

Twenty-eight sensory panelists out of 30 completed a post-test questionnaire and selected descriptors that best suited their opinion of the crab product. The ratings of their overall opinion were excellent (3 panelists), good (13 panelists), fair (11 panelists), or poor (1 panelist). Eighteen of the panelists noted that they would either definitely, probably, or maybe buy the crab product if it was offered at the grocery store. Fifteen panelists wrote that they would purchase the crab if the price range were from \$ 2.00 to \$ 7.00/crab. Fifteen panelists noted that the crabs were very easy to prepare, but 7 panelists mentioned that it was tedious to pick a small amount of meat from one crab. Three panelists noted that larger crabs were better. The cooked, frozen crab product may be best marketed in a package of 6 to 10 crabs as a “party pack” for social gatherings.

#### **pH:**

pH was measured in crab meat samples to determine any changes in pH over storage time. Foegeding et al. (1996) explained that the texture of cooked fish is closely related to postmortem pH of the flesh, the lower the ultimate pH, the tougher the texture. pH apparently exerts its effect on the texture of cooked fish muscle by influencing the contractile elements, since cooking disrupts fish collagen. Results show variability in pH over frozen storage time in crab meat. Muscle tissue pH often will be reduced postmortem by ATP hydrolysis (Foegeding et al., 1996), however,

ATP is quickly hydrolyzed in crab post-rigor. pH levels in crab meat appeared to rise until month 6 and then began to decrease, possibly due to a chemical phenomenon in the crab product over frozen storage time. Chitin is a nitrogenous polysaccharide, which functions as a structural carbohydrate in crab shell. An unknown chemical reaction may have taken place within the crab shell deacetylating chitin and releasing nitrogen compounds, such as amines, that may have increased pH in crab meat, and volatilized during the reheating process. No significant pH or sensory differences were detected at month 3. However, significant ( $p \leq 0.05$ ) pH and sensory differences were detected at month 9. The 1g sorbitol/tocopherol treated crab had a significantly ( $p \leq 0.05$ ) higher pH than 2g sorbitol/tocopherol treated crab. Sensory panelists rated 1g sorbitol/tocopherol treated crab higher in texture than 2g sorbitol/tocopherol treated, indicating that 1g sorbitol/tocopherol injected into crab may maintain a higher pH and more favorable texture over longer frozen storage periods. Pearson correlation matrix results indicate a strong positive relationship between pH levels and panelists' ratings for flavor ( $r=0.93$ ) at month 3. At month 9, Pearson correlation matrix results suggest a strong positive relationship between pH levels and sensory panelists' ratings for flavor ( $r=0.99$ ), texture ( $r=0.92$ ), and overall acceptability ( $r=0.99$ ). pH results indicate that pH levels in crab may have affected panelists' ratings for flavor of crab meat over time. The control and 1g sorbitol/tocopherol treated crab had similar pH levels at months 3

and 9. However, the 2g sorbitol/tocopherol treated crab pH level decreased from month 6 to month 9. Sensory panelists detected more ammonia notes in the 2g sorbitol/tocopherol treated crab indicating that the non-protein nitrogen may have been more volatile during the steaming process at month 9 than the other treatments. Non-protein nitrogen, such as IMP (phosphorylated inosinic acid), may have been in higher concentrations at elevated pH levels and possibly contributed to a more acceptable crab flavor in the control and 1g sorbitol/tocopherol treated crab meat.

#### **Colorimetric Analysis:**

Hunter color analyses were performed during sensory testing as an objective measure of possible color changes to compare with sensory panelists' color ratings of lobster tail meat. No significant differences were detected at month 2. Pearson correlation matrix results indicate a strong negative relationship between "L" values and sensory panelists' ratings for interior meat color ( $r = -0.98$ ), exterior meat color ( $r = -0.99$ ), and overall acceptability ( $r = -0.93$ ) at month 2. A moderate to strong, negative relationship between "b" values and sensory panelists' ratings for overall acceptability ( $r = -0.81$ ) and exterior meat color ( $r = -0.95$ ) were also suggested at month 2. Strong negative relationships were indicated between "a" values and sensory panelists' ratings for interior meat color ( $r = -0.90$ ) and overall acceptability ( $r = -0.95$ ), while a moderate positive

association between “a” values and sensory panelists’ ratings for exterior meat color ( $r=0.81$ ) were noted at month 2. At month 6, 0.1% STP treated lobster were determined to be significantly ( $p\leq 0.05$ ) redder and also significantly ( $p\leq 0.05$ ) lower in hue than the other two treatments. The 0.3% STP treated lobster tails also had more blueness in color and less color saturation than the other the two treatments. These significant ( $p\leq 0.05$ ) differences in color seemed acceptable and not noticeable to panelists. They rated interior and exterior meat color higher than control. Pearson correlation matrix results indicate a moderate negative relationship between “b” values and sensory panelists’ ratings for exterior meat color ( $r= -0.75$ ) and between hue angle and panelists’ ratings for overall acceptability ( $r= -0.70$ ) at month 6.

Meat color differences were measured in crab meat as well, even though sensory panelists were not asked to rate crab meat color on their sensory ballots. The only significant ( $p\leq 0.05$ ) differences were detected at month 3, 1g sorbitol/tocopherol treated crab had significantly ( $p\leq 0.05$ ) higher color saturation and significantly ( $p\leq 0.05$ ) higher yellow notes compared to the other two treatments, indicating that treatment of 1g sorbitol/tocopherol injected into crab may yellow the crabmeat. However, sensory panelists did not note any color differences under comments and rated 1g sorbitol/tocopherol treated crab highest in overall acceptability.



### **Analytical Studies:**

Chromatogram results indicate that invertase breakdown of sucrose to glucose and fructose and mixed function tocopherols previously injected into lobster can be detected by the HPLC methods used in this study. Control lobster appears to have a natural form of tocopherol, possibly a form of  $\alpha$ -tocopherol as illustrated by Figure 2.24. Lobster contains 1.47 mg ATE ( $\alpha$ -tocopherol equivalence) of vitamin E, according to USDA nutrition database information (USDA, 1999).

The importance of Figures 2.26 and 2.27 illustrate that tocopherols, when injected into lobster tails, are indeed circulating within the lobster circulatory system as previously theorized by preliminary dye studies conducted at the University of Maine that were mentioned previously. Tocopherols are detected in lobster tail, as well as claw meat using this HPLC method. However, tocopherol recovery, quantification, and reproducibility studies have not been completed, but would be interesting future work. Identifying the natural tocopherol in lobster would be another future avenue for research, as there seems to be no published information on vitamin E in lobster at the present.

## **CONCLUSIONS:**

Lobster results indicate that polyphosphates added at low concentrations may extend the shelf-life of cryogenically frozen lobsters. STP injection of lobster, prior to processing, may decrease lipid oxidation over frozen storage time, increase yield, and extend more desirable texture, color, and flavor attributes.

The results of the crab study indicate that injecting crabs with sorbitol and tocopherol especially at the 1g sorbitol level may be beneficial in maintaining the shelf-life of whole cooked cryogenically frozen crabs. Our results indicate that the 2g sorbitol/tocopherol treatment would not be beneficial for crabs held at longer periods (9 months) of frozen storage. Sensory panelists rated crabs highly across all treatments, especially for 1g sorbitol/2.5g tocopherol treated crabs, indicating that whole cooked, cryogenically frozen crabs have potential to be a marketable product. However, many panelists indicated that picking the crab meat from the shell is tedious work. Future studies could investigate ways to market this product or find other product forms for Rock and Jonah Maine crab that might be more attractive and easy to prepare for consumers.

The significance of this research indicates that seafood processors can increase profitability from utilizing this injection technology. For example, small increases in the percentage of yield for lobster, which is often sold by weight, can contribute to large economic gains if the price of lobster remains consistent. This study also indicates that cryogenic

freezing lobster and crabs along with added cryoprotectants maintains a high quality product over storage time allowing for more seafood product versatility and availability, reducing seasonal fluctuations.

Further research studies could investigate other cryoprotectant combinations such as sorbitol combined with sodium tripolyphosphate injections or using mixed function tocopherols with sodium tripolyphosphate in seafood products. The disintegrated lobster meat phenomenon would be worth researching as well to determine if the cause is due to shell molting biochemical changes in the lobster muscle and subsequent sea water uptake or due to muscle structural changes from the cryogenic freezing process.

Injection technology could also be studied further to enhance delivery of cryoprotectants in crustacean systems, and feasibility studies could be performed to justify commercializing this technology. Currently research is being conducted at the University of Maine on determining the effectiveness of a needleless system that injects food additive solutions by using a high- pressure delivery from syringe into lobster.

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## **APPENDIX A:**

### **INFORMED CONSENT FORMS AND SENSORY BALLOTS**

#### **Informed Consent-Lobster Study 2000-2001**

I authorize Beth Calder to include me in the research study The Use of Polyphosphates to Extend the Shelf life of Cryogenically Frozen Fully Cooked Lobsters. I have been asked to participate in a research project designed to evaluate the flavor, color, texture, and overall acceptability of frozen lobster.

I understand that I can participate in this research study if I am over the age of 18. I also understand that there will be three sensory evaluation periods and that I will be able to participate in all three.

I understand that if I am a subject, I will evaluate the lobster samples that will be presented to me in this research project. I understand that this lobster product contains phosphates. I understand that I will be excluded from this study if I have an allergy to seafood or phosphates, or if I am not a regular consumer of lobster (at least once a year annually).

I understand that the study described above does not involve any more risk than occurs in the course of everyday living.  
I understand that Beth Calder (#581-1635) or Dr. Mary Ellen Camire (#581- 1627) will answer any questions I may have at any time concerning details of the procedures performed as part of this study.

I understand that I have the right to refuse to participate in, or to withdraw from, this research at any time without penalty or loss of benefits to which I am entitled.

I understand that no information, which identifies me, will be released without my separate consent except as specifically required by law.

I understand that the University of Maine at Orono will not provide any medical treatment reasonably necessary for any injury or illness, which I suffer as a direct result of my participation in this research project. The University does not provide any other form of compensation for injury.

Subject's signature \_\_\_\_\_

Date: \_\_\_\_\_

Witness \_\_\_\_\_

## Lobster Sensory Ballot

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

AGE: \_\_\_\_\_

GENDER: \_\_\_\_\_

How frequently do you have boiled lobster? \_\_\_1x/yr. \_\_\_2-4 x/yr. \_\_\_>4x/yr.

Please drink some water provided before tasting samples. Using the scale below, assign a whole number that best describes your opinion of the meat color, flavor, texture, and overall acceptability of each sample. Evaluate the samples from left to right and rinse with the water provided between each sample.

1	2	3	4	5	6	7	8	9
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

### SAMPLE

**OVERALL  
ACCEPTABILITY**

\_\_\_\_\_

**MEAT COLOR:**

**INTERIOR**

\_\_\_\_\_

**EXTERIOR**

\_\_\_\_\_

**FLAVOR**

\_\_\_\_\_

Please comment on why you assigned the scores you did.

## **Informed Consent-Frozen Crab Study 2000-2001**

I agree to participate in the research study 'The Use of Sorbitol and Tocopherol to Extend the Shelf-life of Cryogenically Frozen Fully Cooked Crab'.

I understand that my participation in the study requires that:

- I do not have an allergy to shellfish or seafood.
- I purchase whole crabs or crabmeat at least twice a year.
- I will steam (reheat) a cooked, frozen crab in my home according to directions provided and write my opinions about the crab on the questionnaire after I have eaten the crab.
- I will cook, eat, and evaluate a crab sample once per week for three consecutive weeks.

This study has no more risks than those encountered in everyday living. The benefits to me include free crabs and a \$10.00 certificate to the Olive Garden.

I understand that I can withdraw from the study at any time, and that I can contact Beth Calder at #581-1635 or Dr. Mary Ellen Camire at #581-1627 if I have any questions. The information that I provide will be kept confidential.

\_\_\_\_\_Date

\_\_\_\_\_Signature

\_\_\_\_\_Witness

## **Crab Reheating Directions**

### **Important!**

Please keep the frozen crab in the freezer until you are ready to reheat them. Do not thaw them before preparing them – they are meant to be reheated from the frozen product stage.

### **Please Follow These Reheating Directions:**

Pour 3 cups of water in a 2 quart saucepan and cover with a lid. Bring to a boil, then place the crab in a steamer in the saucepan over the boiling water. Replace the lid back on the saucepan to cover the crab and steam for approximately 15 minutes. Allow the crab to cool to touch and open claws and legs. Please read and complete enclosed crabmeat evaluation sheet. Thank you!

**Panelist Code** \_\_\_\_\_

### **Crabmeat Sensory Ballot**

Please drink some water before tasting the sample. Using the scale below, assign a whole number that best describes your opinion of the meat flavor, texture, and overall acceptability of each sample.

1	2	3	4	5	6	7	8	9
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

**OVERALL  
ACCEPTABILITY**

\_\_\_\_\_

**FLAVOR**

\_\_\_\_\_

**TEXTURE**

\_\_\_\_\_

Please comment on why you assigned the scores you did and please tell us anything that you feel is important about this product, either in its preparation or consumption.

## **Post Questionnaire Ballot**

### **Frozen Crab Study, 2000**

1. Overall, what was your impression of this crab product?

<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Excellent	Good	Fair	Poor	Terrible

2. Would you buy frozen crab at the store?

- ☐ Definitely would buy
- ☐ Probably would buy
- ☐ Maybe/Maybe not
- ☐ Probably would not buy
- ☐ Definitely would not buy

3. What price would you be willing to pay for this  
crab product at the store? \$\_\_\_\_\_

**4. How easy was it to prepare this product?**

- ☐ **Very easy**
- ☐ **Somewhat easy**
- ☐ **Just right**
- ☐ **Somewhat hard**
- ☐ **Very hard**

**5. Did you eat this product:**

- ☐ **with butter**
  - ☐ **with mayonnaise**
  - ☐ **as crabcakes**
  - ☐ **other:**
- 

**Any additional comments:**

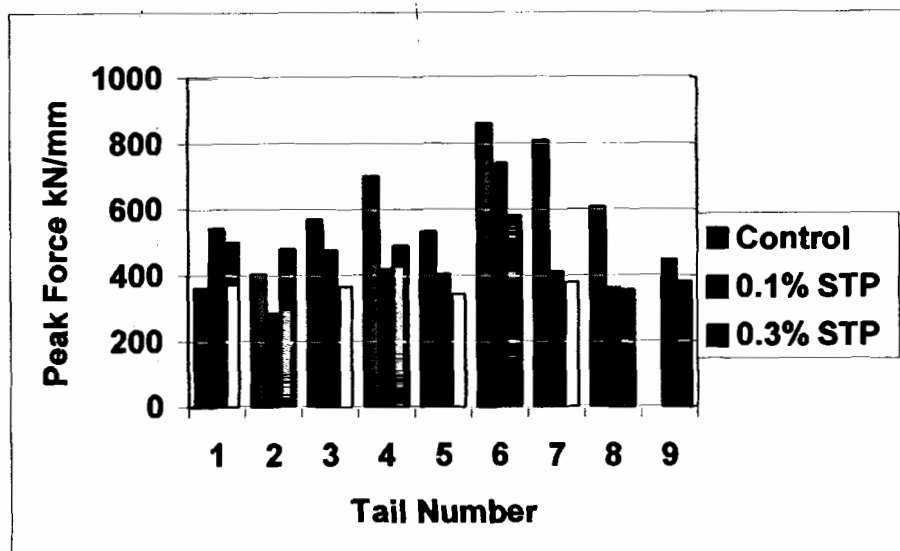
**(We value your opinions!)**

The principal researchers of this project want to thank you again for your participation. We couldn't have completed this study successfully without help from our volunteers.

## APPENDIX B:

### GRAPHS OF INSTRON INDIVIDUAL LOBSTER TAIL DATA

**Figure B.1- Instron Individual Lobster Tail Data at Month 0\***



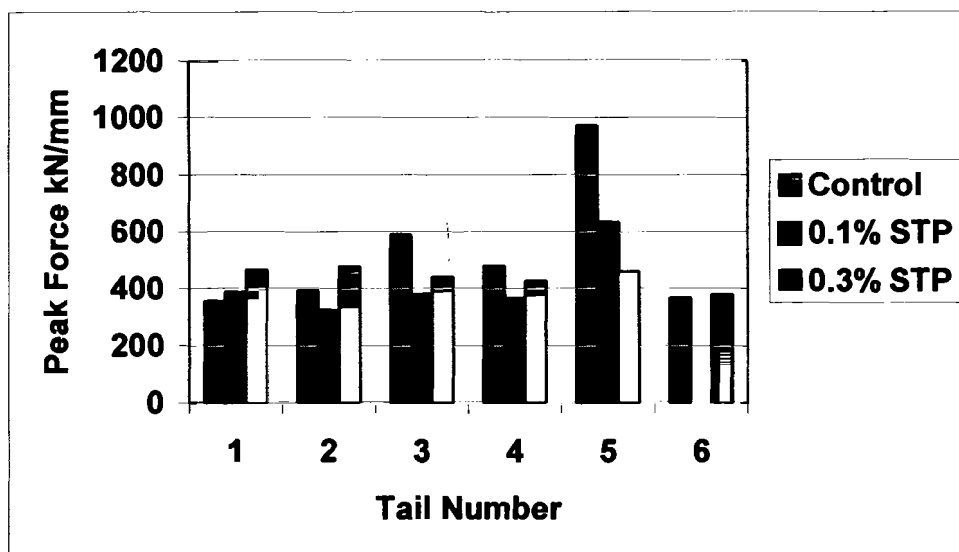
\*Significance  $p \leq 0.05$

Control<sup>a</sup>, 0.1% STP<sup>b</sup>, and 0.3% STP<sup>b</sup>

Means within each evaluation period sharing a common letter are not significantly different.

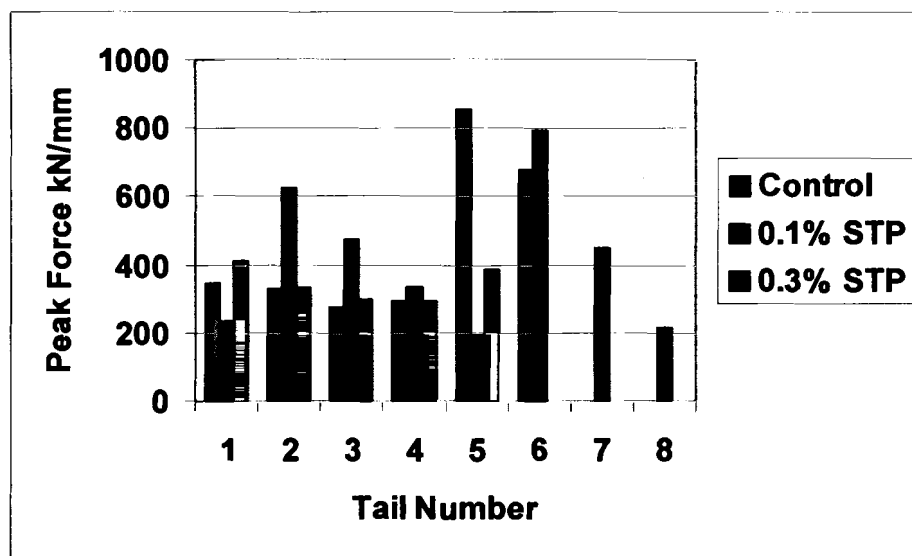


**Figure B.2-Instron Individual Lobster Tail Data at Month 2\***



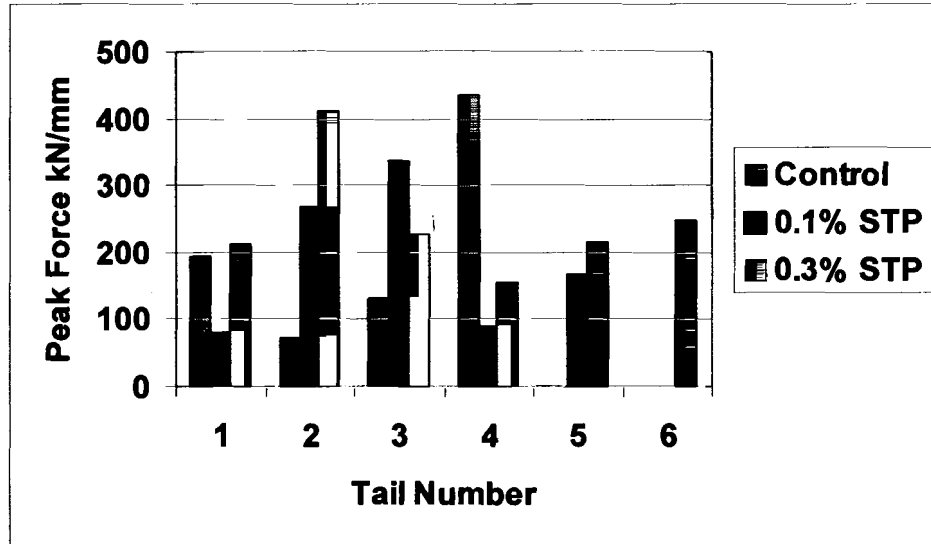
\*No significance

**Figure B.3- Instron Individual Lobster Tail Data at Month 4\***



\*No significance

**Figure B.4- Instron Individual Lobster Tail Data at Month 6\***



\*No significance

## **BIOGRAPHY OF THE AUTHOR**

Beth Louise Calder was born in Skowhegan, Maine on December 3, 1969. She was raised in Skowhegan, Maine and graduated from Skowhegan Area High School in 1988. She attended the University of Maine at Farmington and graduated in 1990 with an Associate's degree in Dietetics Technology. She returned to college at the University of Maine and graduated in 1997 with a Bachelor's degree in Food Science and Human Nutrition. She further pursued her studies at the University of Maine in the spring of 1998.

Beth is a candidate for the Doctor of Philosophy degree in Food and Nutrition Sciences from The University of Maine in May, 2003.