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To the Graduate Council:

I am submitting herewith a thesis written by Abdur Rasheed Khan entitled "Shrimp quality response to chemical treatments." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

Melvin R. Johnston, Major Professor

We have read this thesis and recommend its acceptance:

Ivon E. McCarty, David L. Coffey

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

March 2, 1971

To the Graduate Council:

I am submitting herewith a thesis written by Abdur Rasheed Khan entitled "Shrimp Quality Response to Chemical Treatments." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science with a major in Food Technology.

Melvin R. Johnson
Major Professor

We have read this thesis and recommend its acceptance:

Ivan E. McCaig

David L. Coffey

Accepted for the Council:

Hilton A. Smith
Vice Chancellor for
Graduate Studies and Research

SHRIMP QUALITY RESPONSE TO CHEMICAL TREATMENTS

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

Abdur Rasheed Khan

March 1971

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Thanks are due to Professor Thomas Puroff, Mr. Ray Fliescher, Miss Elizabeth Payne, and Miss Ruth Hill for their assistance.

Deep appreciation is due to my parents for their ever present encouragement and support of my educational efforts.

CRANES CREST

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ABSTRACT

This study was undertaken to determine the penetration trend of tetra sodium pyrophosphate, phosphoric acid, and sodium chloride into raw shrimp, and their effect on water-holding capacity, texture, and color of raw and cooked shrimp.

Shrimp were treated with 5 percent tetra sodium pyrophosphate, 5 percent phosphoric acid, and 5 percent sodium chloride for five, 10, and 15 minutes. The penetration was determined by measuring the amount of phosphorus and chlorine at three levels (outer, intermediary, and center) of raw shrimp. Water-holding capacity was determined by measuring the area of the pressed juice of shrimp samples on a Harco-Hydraulic Press. Allo-Kramer Shear Press, with recorder attached, was used to determine the texture of the samples. The changes in the color of shrimp were recorded by color-eye.

Under the experimental conditions reported in this study, several results were indicated. The concentration of tetra sodium pyrophosphate, phosphoric acid, and sodium chloride was highest in the outer layer and decreased toward the center. When compared with the control, both tetra sodium pyrophosphate, and sodium chloride improved the texture and water-holding capacity of shrimp; however, tetra sodium pyrophosphate, had a greater effect than sodium chloride. Phosphoric acid had an inverse effect. Treatments did not differ markedly in their effect on the color of raw shrimp; however, tetra sodium pyrophosphate, differs

markedly from the other treatments in its effect on the whiteness of cooked shrimp.

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GRAVES CREST

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CHAPTER I

INTRODUCTION

Shrimp are among the most popular fishery food products in the world. The domestic shrimp industry has expanded rapidly, in many countries, within the past 20 years. In the United States the yearly catch at present is 317 million pounds. The United States imported 220.1 million pounds of shrimp and exported 5.7 million pounds of canned shrimp in 1969 (70).[1]

Shrimp are frozen as soon as they are caught because of the bacteriological and other quality problems. But when the frozen shrimp are thawed and cooked they become tough in texture. Loss of moisture and development of rancidity are important changes taking place in cooked frozen shrimp (43). When fish products are kept in freezer storage, they show an increase in toughness and dryness. This might be due to actual loss of moisture because of inadequate packaging or to protein denaturation resulting in shrinking of the protein structure (52; 62).

According to Garnatz et al. (20) when shrimp are soaked in sodium pyrophosphate or other alkaline salts before cooking, the cooking losses are decreased, also the shrimp are more tender. Jones (35) reported that sodium acid pyrophosphate improved moisture retention in canned

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[1]The numbers in parentheses represent similarly numbered references in the bibliography.

king crab meat as compared with the regular commercial addition of salt and citric acid.

However, very little work has been done in this field and Moorjani and Dani (51) stressed the necessity of improving the water-retention property of shrimp so that texture could be improved.

The purpose of this study was to determine the depth of penetration of tetra sodium pyrophosphate, sodium chloride and phosphoric acid into the tissue and their effect on the water-holding capacity (WHC), texture and color of raw and cooked shrimp.

CRANES CREST

CHAPTER II

LITERATURE REVIEW

I. WATER-HOLDING CAPACITY

Water-holding capacity is one of the most important features of meat quality. It is closely related to taste, tenderness, and color. Also, it effects the quality of meat during processing operations such as transport, storage, aging, grinding, freezing, thawing, heating, salting, drying and curing (26).

Muscle proteins are responsible for the binding of water in meat (27). Sponsler et al. (64) reported that the reaction groups responsible for binding of water are of two types. One type consists of the polar groups of the side chains of protein, such as the carboxyl-, amino, hydroxyl-, and sulfhydryl- groups. The other group includes the undissociated carbonyl- and imido- groups of the peptide bonds, in which the binding of water is due to the dipolar character of water. Water being a dipole, since the negative charge of oxygen and positive charge of hydrogen do not coincide, acts as a molecular magnet. This molecular magnet is attracted by all the groups in the protein and are bound by hydrogen bonds. Olcott and Fraenkel-Conrat (54) showed that the activity of proteins in binding water could be diminished by blocking polar groups with certain reagents; thus proving that the polar groups are responsible for the bindings of water.

Also, they showed that the carboxyl groups play a less important role in hydration than do amino groups. According to Pauling (56) in the binding of water by proteins one polar group binds one water molecule at first. Since polar groups vary in their affinity for water, the water attaches first to the most active groups and then to the less active.

In muscle tissue, different kinds of water binding may exist with apparently no sharp demarcation between tightly bound water and loosely bound water (23; 27). Hamm (27) showed that only 4 to 5 percent of the total water content in a muscle is bound firmly to the proteins and has a lower freezing point, a lower vapor-pressure, and a lower dissolving power than normal water. He named this bound water the "true hydration water." According to Haurowitz (29) most of the water in the muscle is "free." It freezes at the same temperature as normal water, has the same solvent power and exhibits no indication of being tightly bound to the protein molecules. Though it is free it is mechanically immobilized by the network of the cellular protein membranes and protein filaments, probably even by cross linkages and electrostatic forces between the peptide chains.

Kuprianoff (42) reported that scientists have not agreed upon a definition of "bound water" because there is no standard method for its determination. This term is generally assumed to indicate water that is very closely united with other compounds in the system and differs from the remainder or "free water." According to Meryman (49), "current usage in cryobiology loosely defines bound water is

that which does not freeze." Mazur (48) stated that the unfrozen water in cells is water bound to cellular solids.

Hamm (27) concluded that the tightly bound water is hardly influenced by changes in the structure and charges of the proteins. Thus the changes in water-holding capacity of meat are mainly caused by the changes in "free water" which in turn is influenced by the changes of protein charges and protein structure. He defined water-holding capacity as "the ability of meat to hold fast to its own or added water during application of any force (pressing, heating, grinding, etc.)." It could be represented in two ways; in terms of the amount of loose water related to the total content of moisture in muscle, or in terms of the amount of bound water related to muscle or muscle protein.

II. FACTORS AFFECTING WATER-HOLDING CAPACITY

Heating affects the quality of cooked meat and meat products because of the change in the hydration of muscle proteins (5; 74). Juice is released by the meat during heating, and the amount depends on the temperature and this loss influences the juiciness and texture of meat (3; 26). The WHC of meat decreases as the temperature increases in the range of 0 to 25°C (71). Hamm and Deatherage (28) pointed out that the difference in hydration between 30°C and 50°C could be determined by the disappearance of acidic groups in the muscle proteins. They explained that the decrease of negative protein charges, at pH values lower than the isoelectric point, produces a decrease of the electrostatic repulsion between the peptide chains, causing a tighter

network of protein structure and a lower WHC. But at pH values greater than the isoelectric point; a decrease of negative protein charges will break salt cross linkages, resulting in a losing of protein structure, and as a result, the WHC is increased. Change of hydration is delayed significantly in the range of 50 to 55°C due to the decrease of acidic groups (28; 74). The pH of meat is changed by heating and is dependent on the initial pH of tissue. This change is caused by a decrease in acidic groups in muscle proteins (57). Within the range of pH 3 to pH 8, the pH of meat has a marked influence on WHC and not only before but after heating. Meat has its lowest WHC and maximum water loss when heated at its isoelectric point (28). Meats cooked at higher or lower pH values than the isoelectric point, exhibit higher WHC (5).

Grau et al. (24) reported that WHC is dependent on pH value and showed that hydration was minimum around pH 5. Hollwede and Weber (32) showed that small changes in pH may cause relatively great changes of WHC. Hamm (27) reported that the bound water related to muscle was highest at pH 10 and lowest at pH 5.

Magnesium, calcium and zinc, which are present in meat naturally, have an important influence on WHC (27). Partial extraction of calcium by ethylene diamine-tetra-acetate and of magnesium by polyphosphate increases the swelling capacity of muscle tissue (9). Bound calcium and bound magnesium decrease the hydration of muscle (26).

Muscle fibers affect the WHC. The thicker the muscle fibers, the lower WHC they have. Meat having high intramuscular fat content has greater WHC than meat of low fat content (55). Meat containing

large amounts of connective tissue also exhibits high WHC. Not only is the quality of connective tissue important but also the quantity. The fine connective tissue of young animals has better WHC than that of older animals (27).

III. SODIUM CHLORIDE, TETRA SODIUM PYROPHOSPHATE, AND PHOSPHORIC ACID

Sodium chloride and phosphates improve WHC in proteinacious foods. The concentrations of anions and cations absorbed from salt and alkaline polyphosphate solutions influence water retention in meat (27).

Cann and Phelps (12) explained that on the acidic side of the isoelectric point of muscle, the anions are bound, thereby decreasing the electrostatic repulsion between positively charged groups of the protein molecule. This results in tightening of the protein structure with a corresponding decrease in WHC. The stronger the anions are bound, the greater the effect of dehydration will be. On the basic side of the isoelectric point, the binding of anions has the opposite effect due to the presence of more negatively charged groups in the protein, which form salt bridges with the opposing positive charges.

Klotz (37) noted that ions are bound to proteins by strong electrostatic attraction, and not by adsorption forces. The first bound ions are held very strongly but the binding strength decreases for additional ions progressively because of electrostatic repulsion by the first bound ions. He related binding of ions by proteins to their composition.

The smaller the number of hydroxy amino acid residues in the protein molecule, the greater the concentration of anions bound.

The effect of sodium chloride on WHC of meat is due mainly to the chlorine ion (53). Hydration of muscle increases with increasing concentration of sodium chloride, reaches a maximum, and then decreases, finally falling below the original WHC (39). Rapid loss of WHC after slaughter could be prevented by salting the ground or cut meat during the first hours after death (58).

Hamm (27) reported that the influence of salt on the WHC of large pieces of meat depends on the rate at which the salt penetrates the meat. Callow (11) showed that the amount of swelling of muscular tissue depends on the manner in which immersion is done. Much greater gain could be achieved by gradually increasing the strength of the salt solution rather than immersing the tissue immediately in the final solution. Also he said that no more salt penetrates the tissue after a certain period of time although equilibration by no means exist. This might be due to the "closed" structure in the exterior layers of the muscle due to increase in swelling due to salt and that further diffusion of salt is therefore inhibited.

Empey and Howard (18) reported that treating meat with sodium chloride before freezing or before thawing reduced drip losses. Meat with added sodium chloride releases bound anions and cations on heating to 100°C, but the anions are still preferentially retained (59). When ground meat with sodium chloride is heated the pH of meat increased but

not that of the released fluid. This indicates that the sodium ions are preferentially retained by the meat proteins (74).

Sherman (60) reported that pyrophosphate and polyphosphate increased WHC in fresh pork. He explained that the increase in WHC was primarily due to the solubilization of proteins particularly (acto) myosin. Mahon (52) and Boyd and Southcott (8) found that fish fillets treated with sodium tripolyphosphate prior to freezing had less drip and better flavor when thawed and cooked. Spinelli et al. (62) reported that the drip in fish fillets and steaks dipped in sodium tripolyphosphate was decreased. The drip was effectively retarded when the concentration of sodium tripolyphosphate in the dipping solution exceeded 5 percent.

Jones (35) reported that the use of sodium acid pyrophosphate in treating king crab improved retention of moisture. Barnett et al. (4), showed that shrinkage was reduced when Halibut and Blackcod were treated with sodium tripolyphosphate before smoking.

Scheurer (59) treated haddock fillets with 12.1 percent sodium tripolyphosphate solution for 10 sec. to 10 min. He found that the concentration of sodium tripolyphosphate was highest at the surface increased with an increase in dip time but the concentration at the center remained about the same.

Spinelli et al. (62) reported that when fish fillets were dipped in sodium tripolyphosphate solution of different concentrations for different periods, the uptake of sodium tripolyphosphate was fairly uniform.

A review of literature indicates that orthophosphoric acid (H_3PO_4) has not been used to improve the WHC of any proteinacious food products.

The increased use of phosphoric acid is due not only to its relatively low cost but to the fact that salts of phosphoric acid have certain recognized medicinal value. Moreover, compounds of phosphorus play an important role in human nutrition. It is argued that acidity furnished in the form of orthophosphoric acid is certainly not deleterious but probably beneficial to health (71).

IV. TEXTURE

The word "texture" is derived from the latin verb "to weave" and was probably first applied in the English language with reference to fabrics (47).

A study of consumer awareness revealed that texture is the most often mentioned of the three food quality attributes namely flavor, appearance, and texture (67). Texture is the second most important food attribute, with appearance being first (47).

Webster's dictionary defines texture as "the disposition or manner of union of the particles of a body or substance." A committee of the Institute of Food Technologists (1959) defined texture as "those properties apprehended by the eyes and the skin and muscles of the mouth" (47). Kramer (40) defined texture "as those sensations perceived by the sense of feel only and as that part of rheology dealing with the deformation of matter by forces greater than gravity."

Methods of evaluation of textural characteristics of raw or manufactured food materials are divided into (1) subjective or sensory evaluation and (2) objective or instrumental measurements. In the sensory evaluation of texture, some physiological and psychological aspects as well as mechanical processes are involved, in the objective or instrumental measurements, the mechanical properties of the material are the only criteria for evaluation of the texture (47).

According to Szczesniak (67) the concept of texture refers to several properties related to the structural elements of the food, detectable with the physiological senses. It thus includes tenderness, juiciness, and all the other properties which effect the manner in which the meat "eats and feels in the mouth."

According to Hill (30) the sensation of toughness (lack of tenderness) could be classified into three components: "(a) the ease with which the teeth penetrate into the meat when chewing begins; (b) the friability or ease with which meat breaks into small fragments in the mouth; and (c) the quality of residue which remains after chewing."

Cover and her co-workers (14) differentiated beef tenderness into six components: "softness as judged by the sense of feel to the tongue and cheek; softness to tooth pressure as sensed by exerted muscular force; ease of fragmentation during cutting or breaking the muscle fibres across the grain; mealiness as judged by the quality of tiny, dry and hard meat fragments adhering to the inner surfaces of the mouth; apparent adhesion between muscle fibres; and, finally, the amount and apparent hardness of connective tissue.

Tenderness is closely related to the WHC. The increasing tenderness of meat during aging is accompanied by an increasing WHC. Tenderness or texture of meat is not only a matter of muscle hydration but it depends on some other factors like amount of connective tissue, and muscle characteristics etc. Muscle containing much connective tissues are less tender than those containing less connective tissues (65). Hiner et al. (31) found significant correlation between the muscle characteristics and tenderness. Muscles with small fiber diameter were tender.

According to John et al. (34) evaluation of texture in fresh, frozen and canned fishery products is important in two fields: (1) quality control; and (2) preservation research. In Quality control of fishery products, there is need for standards that reflect consumer preferences and act as guide lines for inspection. In preservation research it is helpful in evaluating the effect of variables in packaging and processing.

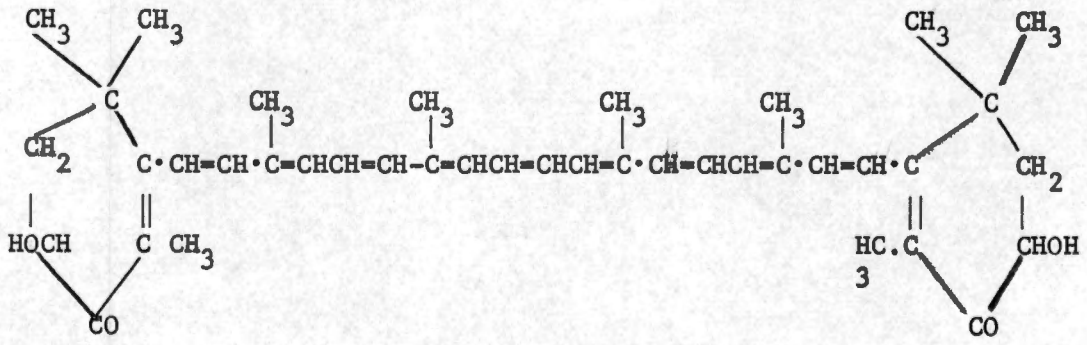
Increasing toughness and dryness of fish products in freezer storage may be due to actual loss of moisture because of inadequate packaging or to protein denaturation resulting in shrinking of the protein structure and freeing of bound water from the tissue (52; 61).

The WHC and texture of fish could be improved by treating them with phosphates and sodium chloride (8; 46).

V. COLOR

The reddish pink color in shrimp is due to a carotenoid pigment called "astaxanthin." In experiments with fresh prawns esterified astaxanthin from the hypodermis and free astaxanthin from cayapace, attached to protein were isolated. Early workers named astaxanthin as astacin. But it is clear now that astacin does not occur naturally and that reports of its presence are due to its formation by oxidation during the manipulative process (22). De Nicola (17) in his studies of carotenoids of a starfish (Ophidiaster ophidianus) identified a reddish-pink fraction (the major fraction) as astaxanthin and an orange-red fraction as astacin. Astacin was assumed to be an artifact produced during the extraction process.

Kuhn and Sorensen (38), on the basis of the formula of astacene established by Karrer and co-workers (36), assigned the structure of 3:3-dihydroxy-4:4diketo-β carotene to astaxanthin, which is as follows:



The red pigment in stored shrimp gradually fades to an almost colorless state (19). Studies on frozen pink salmon showed that the

normal pink to red color would fade and turn yellowish (16). Rancidity in salmon is accompanied by the bleaching of the red astacin pigments. This bleaching is similar to the oxidation of β-carotene (68). According to summer (66) when fat oxidizes with formation of peroxides, associated carotene is simultaneously oxidised and bleached. Hence there is a possibility that the bleaching of astaxanthin, the carotenoid pigment of shrimp, is related to the oxidation of the fat present.

Lusk et al. (45) reported that heating breaks the bonding between protein and astacin, oxidation product of astaxanthin, to release the red pigment and results in a sudden change of color of lobster, and to a lesser extent of shrimp. Also they showed that temperature of storage and oxygen concentration are the most important factors determining the rate of loss of the pigment in freeze dried shrimp and salmon.

Czerpak and Czeczuga (15) studied the pigments in the shrimp crangon crangon and reported the presence of β-carotenes, cantaxanthin, astaxanthin, free and esterified lutein, zeaxanthin, and a form of xanthophyll in it.

Krinsky (41) reported that keto-carotenoids, canthaxanthin and echinenone are present in the brine shrimp Artimia salina.

CHAPTER III

MATERIALS AND METHODS

I. SOURCE AND PREPARATION OF SHRIMP

Green shrimp were supplied by the Travisco Meat and Seafood Company, Knoxville, Tennessee. They were first thawed for 24 hours at 34°F and then in running cold tap water for five minutes. They were graded for size uniformity by visual selection of 10 shrimps per 100 gm and were used in the experiment.

The graded shrimp were divided into four main lots, each of which was then again divided into three sublots. In the first lot, sublot one was treated with 5 percent tetra sodium pyrophosphate ($Na_4P_2O_7 \cdot 10H_2O$ - molarity = 0.188) five minutes, sublot two for 10 minutes, and sublot three for 15 minutes, in a 1:2 ratio. The other three lots were divided and treated in the same manner, using 5 percent phosphoric acid (Molarity = 0.510) with the second lot and 5 percent sodium chloride (molarity = 0.854) with the third lot, and the fourth lot was run as a control by treating the shrimp with water.

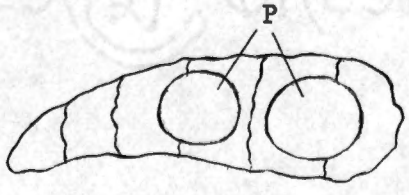
The shrimp were then stored at -10°F, until analysis could be performed, to determine the penetration of tetra sodium pyrophosphate, phosphoric acid, and sodium chloride into raw shrimp, and their effect on the water-holding capacity, texture, and color, in raw and cooked shrimp.

II. EXPERIMENTAL DESIGN

Determination of Penetration of Tetra Sodium Pyrophosphate, Phosphoric Acid, and Sodium Chloride

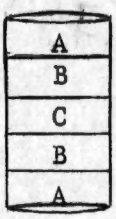
To determine the penetration of tetra sodium pyrophosphate and phosphoric acid, the amount of phosphorus in the shrimp was measured at three depths--the outer, intermediary, and center. Phosphorus content was also determined in the control at three levels. The penetration of sodium chloride was determined by measuring the chlorine content of the shrimp at the same three positions.

Sample Preparation. The shrimp samples were prepared following the procedure of Scheuer (58). Core samples of the frozen shrimp were taken with two cork borers (of 1 and 1.2 cm inner diameters, depending on the width of the shrimp). The cores were sectioned with a razor blade into sections of approximately one third thickness of the cores as outer, intermediary, and center sections. Each of the three types of sections were kept separate. Then, each type was further cut into small pieces and mixed well for greater uniformity and used in determination of phosphorus and sodium chloride.



Top View of Shrimp

P = Core samples taken.



A = Outer sections. B = Intermediary sections. C = Center section

Reagents. Perchloric acid--60 percent.

Ammonium molybdate solution--dissolved 5 gm of ammonium molybdate in 100 ml H_2O, kept overnight, and filtered into a dark bottle.

1,2,4--Aminonaphtholsulfonic acid (ANSA), recrystallized--dissolved 0.125g ANSA in 44 ml 15 percent sodium-bi-sulphite ($NaHSO_3$). Added dropwise a 20 percent solution of sodium sulphite until the solution cleared, then filtered into a dark bottle.

Potassium dihydrogen phosphate (KH_2PO_4)

Primary standard--dissolved 0.439 gm KH_2PO_4 (reagent grade) in H_2O and diluted to one litre.

Secondary standard--diluted 10 ml of the primary standard to 200 ml with H_2O. One ml of secondary standard contains 5 m mg phosphorus.

Silver nitrate solution--0.1N standardized against 0.1N Nacl solution.

Ammonium thiocyanate solution--0.1N standardized against 0.1N $AgNO_3$ solution.

Ferric indicator--saturated solution of $FeNH_4(SO_4)_2 \cdot 12H_2O$ in H_2O.

Preparation of Standard Curve. Two, 4, 6, 8, and 10 ml of the secondary standard were pipetted into 25 ml volumetric flasks. Then 2.5 ml $HClO_4$ and 0.8 ml of the ANSA solution were added to each flask. After 30 sec. 2 ml $(NH_4)_2MoO_4$ were added and the mixture diluted to volume with H_2O, mixed, and let stand for 15 minutes for color development. The absorbance of optical density was determined using a Bausch and Lomb Spectronic 20 colorimeter at 730 m μ against a blank.

The standard curve was plotted from the values obtained, the ml of the secondary solution used being on the 'x' axis and optical density on the 'y' axis. From the curve, a value of 1.0 m μ optical density, a constant, was calculated which equals 104.5 m mg of phosphorus since one ml of the secondary solution equals 5 m mg of phosphorus.

Phosphorus content was determined following the procedure of Gersten (21). A 2 gm sample was digested by adding 30 ml HNO_3 and 10 ml sulfuric acid. Near completion of digestion, a few drops of HNO_3 were added, until the solution became colorless. The solution was cooled, diluted to 400 ml, mixed, and let stand for two hours. Ten ml of the mixture was then pipetted into a 25 ml volumetric flask. Then 2.5 ml of perchloric acid, 0.8 ml of the ANSA solution, and 2 ml of ammonium molybdate solution (color developing reagents) were added. The mixture was brought to volume with the addition of H_2O, and the solution was left standing for 15 minutes for color development. The optical density was determined by the Bausch and Lomb Spectronic 20 colorimeter at 730 mμ, using red filter. The optical density was converted into

m mg of phosphorus by multiplying each value by 104.5, the constant obtained from the standard curve.

The AOAC method for fish and other marine products (1) was used to determine the sodium chloride content which is as follows: 25 ml of 0.1 N silver nitrate solution was added to a 10 gm shrimp sample, which was dipped for five minutes, to precipitate all the chlorine as silver chloride. Then, 20 ml of nitric acid was added and boiled gently on a hot plate for 15 minutes until all solid materials, except silver chloride were dissolved. The mixture was cooled and 50 ml of water and 5 ml of the indicator, saturated ferric-ammonium-sulfate, were added. This solution was titrated with 0.1 N ammonium-thiocyanate solution until a permanent light brown color appeared. The amount (ml) of 0.1 N ammonium-thiocyanate used was subtracted from the amount (ml) of 0.1 N silver nitrate solution added. The quantity of Cl expressed as sodium chloride in 10 gm of the sample was calculated (each ml of 0.1 N silver nitrate is equal to 0.058 percent sodium chloride).

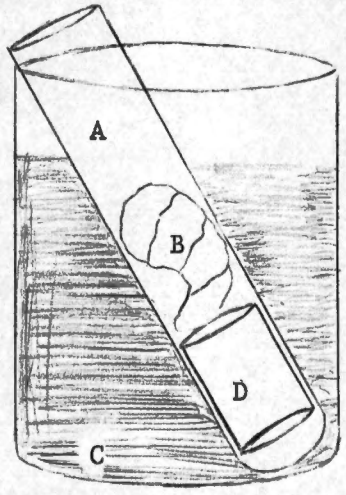
The same procedure was used for samples which had been treated for 10 and 15 minutes, but the amount of 0.1 N silver nitrate used was 30 ml, instead of 25 ml.

The experiment was replicated three times, with two observations being made of each replication.

Determination of Water-Holding Capacity, Texture, and Color

Preparation and Method of Cooking of the Samples. Treated frozen shrimp samples were placed in polythelene bags (one sample consisting of one shrimp in each bag) and thawed by holding

them under cold running tap water for 15 minutes. Following the procedure of Asselbergs and Whitaker (2), samples were placed in glass tubes (1.3 x 6 in.) and cooked for 10 minutes in boiling water. During cooking, the shrimp rested on vertical 1 in. length of tygon tubing (3/4 in. outer diameter), placed inside the glass tubes, so that the juice released did not come in contact with the shrimp.

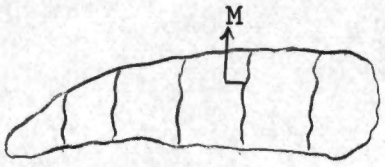


A = Test tube. B = Shrimp. C = Boiling water. D = Tygon tube

Procedure. The water-holding capacity of shrimp was determined by a method similar to that used by Rogers et al. (57). Samples of raw and cooked shrimp, cut horizontally from the first segment of each shrimp and each weighing 500 mg, were taken. Each sample was then placed in the center of a 15 cm diameter, Whatman No. 42 ashless, filter paper. A unit, consisting of two pieces of filter paper and two samples, one raw and one cooked, alternately stacked between three 6 x 6 in. plexiglass plates, was pressed on a Harco-hydraulic press at 305.5

PSig[1] for 60 seconds. Following pressing, flesh and exuded moisture rings on the filter paper were outlined with pencil and the areas measured with a Keuffel and Esser compensating polar planimeter. The area of the juice, assumed to be free water area, was obtained by substracting the flesh area from the flesh plus juice area.

An Allo-Kramer Shearpress model SP-12, with recorder attached, was used to determine the texture of the shrimp. A 100 pound proving ring was used, and the recorder was set at 10 percent range. The 10 percent range position provided a span of 10 pounds to the full scale of the recorder. The raw shrimp were cut at the joint of the first and second segments, and the cooked shrimp at the joint of the second and third segments (segments M and L). The single blade shear cell and 18 second thrust was used. The texture, force of shear, was recorded in the form of curves on the graph paper. The areas of the curves were calculated in square centimeters. The greater the area, the greater the shear force required.



Cooked Shrimp

M = Site of cutting.

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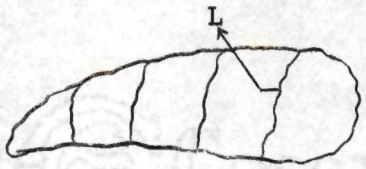
[1] Unit pressure = $\frac{\text{total force}}{\text{unit area}} = \frac{11{,}000 \text{ lb.}}{36 \text{ sq. in.}}$



Raw Shrimp

L = Site of cutting.

Color-eye (Model D-1, Instrument Development Laboratory, Division of Kollmorgan Company, Attleboro, Massachusetts) was used to determine the color of the samples. Before making any measurement, a reference standard (Illuminant "C"--barium sulphate) was placed over the standard reference port. Then raw and cooked samples, consisting of one shrimp each, were placed, one at a time, over the sample port. The selector switch was set on "Hi" and color-eye values χ, x, y, and z were read directly from the microdial. From these values, the color coordinates were calculated.

III. STATISTICAL ANALYSIS

The ANOVAR program (6) adapted for the IBM Model 360 Computer at The University Computer Center was used to perform the analysis of variance calculations. The data of water-holding capacity, texture, and color of raw and cooked shrimp were analyzed. The data was analyzed as a factorial design (13).

Duncan's New Multiple Range Test was used to determine significant differences at the 0.05 level among means.

CHAPTER IV

RESULTS AND DISCUSSION

I. PENETRATION MEASUREMENT

The results presented in Tables 1 and 2 show that the concentration of tetra sodium pyrophosphate, phosphoric acid, and sodium chloride was highest at the surface and decreased toward the center. Increase in the length of time increased the concentration at all levels. However, the degree of increase in concentration, with increase in the length of time, was higher at the surface or in the outer level than at the other two levels. This could be explained by the Fick's law of diffusion, according to which the rate of diffusion of a substance depends not only on the concentration difference but also on the area across which the substance is diffusing and the distance between two concentrations. The smaller the distance, the greater the number of molecules crossing it per unit time (33).

II. WATER-HOLDING CAPACITY

There was a significant difference (Table 3) among the treatments in their effect on the water-holding capacity of raw shrimp. Tetra sodium pyrophosphate and sodium chloride improved the water-holding capacity of shrimp, when compared with the control, where as phosphoric acid had an inverse effect (Table 4). Shrimp treated with tetra sodium pyrophosphate had the highest and those treated with phosphoric acid

TABLE 1

EFFECT OF TIME ON PENETRATION OF TETRA SODIUM PYROPHOSPHATE, AND PHOSPHORIC ACID (EXPRESSED AS M MG OF PHOSPHORUS/2.0 GM)

	Outer Section			Intermediary Section			Center Section		
Treatments	5 min.	10 min.	15 min.	5 min.	10 min.	15 min.	5 min.	10 min.	15 min.
Control	14.68	14.63	14.63	14.63	14.66	14.66	14.68	14.63	14.63
Tetra Sodium Pyrophosphate	27.83	35.57	39.78	22.71	25.46	29.94	20.69	23.86	27.38
Phosphoric Acid	32.57	37.62	40.55	22.64	23.86	28.34	19.12	22.94	25.01

TABLE 2

EFFECT OF TIME ON PENETRATION OF SODIUM CHLORIDE IN SHRIMP
(EXPRESSED AS PERCENT OF THE 10 GM SAMPLE)

	Outer Section			Intermediary Section			Center Section		
Treatments	5 min.	10 min.	15 min.	5 min.	10 min.	15 min.	5 min.	10 min.	15 min.
Control	0.029	0.030	0.029	0.026	0.029	0.029	0.029	0.027	0.030
Sodium Chloride	0.935	1.112	1.143	0.367	0.501	0.543	0.208	0.311	0.402

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TABLE 3

ANALYSIS OF VARIANCE FOR THE EFFECT OF TREATMENTS ON WATER-HOLDING CAPACITY OF SHRIMP

Source	D.F.	M.S.	
		Raw	Cooked
Treatments	3	267.1838**	161.0328**
Time	2	2.0969	0.5586
Treatments x Time	6	18.9362**	11.8593**
Replication	2	2.1011	1.7353
Error	22	1.8063	0.6835

*Significant at 0.05 level.

**Significant at 0.01 level.

TABLE 4

EFFECT OF TREATMENTS ON WATER-HOLDING-CAPACITY* OF SHRIMP

Treatments	Raw	Cooked
Control	25.767b	33.900q
Tetra Sodium Pyrophosphate	19.078d	28.444s
Phosphoric Acid	31.678a	38.189p
Sodium Chloride	21.933c	30.689r

*Water-holding capacity correlated with free moisture area expresses as sq. cm. within each column, means followed by the same letter are not significantly different at 0.05 level of probability.

had the lowest water-holding capacity. This is due to the action of phosphoric acid, which, being a strong acid, would have lowered the pH of shrimp to an acidic pH. On the acidic side of the isoelectric point of muscle, the electrostatic repulsion between positively charged protein groups is reduced by the binding of anions, which results in a tightening of protein structure and a decrease in water-holding capacity. The pH of shrimp treated with tetra sodium pyrophosphate, and sodium chloride would have become basic. On the basic side of the isoelectric point, more negatively charged groups are present in the protein, which form salt bridges with the positive groups. Therefore, the binding of anions has the opposite effect in the basic muscle structure, and consequently an increase of water-holding capacity (27). Exposure time to treatments beyond five minutes did not show significant effect on water holding capacity.

The increased effectiveness of tetra sodium pyrophosphate, over sodium chloride may be explained by the fact that tetra sodium pyrophosphate is a polyvalent anion and polyvalent anions strongly influence muscle hydration.

There was a significant difference among the treatments in their effect on the water-holding capacity of cooked shrimp (Table 3). Tetra sodium pyrophosphate and sodium chloride improved the water-holding capacity, whereas phosphoric acid had an inverse effect (Table 4). Water-holding capacity was higher with cooked shrimp treated with tetra sodium pyrophosphate, and sodium chloride, because these salts are known to retain their effect to a certain extent, even after cooking (27).

III. TEXTURE

Treatments differ significantly (Table 5) in their effect on texture of raw and cooked shrimp. Comparison of the control with other treatments indicates that tetra sodium pyrophosphate, and sodium chloride improved the texture (Table 6) of raw and cooked shrimp where as phosphoric acid had an inverse effect.

This could be explained by the favorable effect of tetra sodium pyrophosphate, and sodium chloride on water-holding capacity of shrimp which as a result, improved its texture.

IV. COLOR

The results of color calculations are presented in Tables 7 through 11. The analysis of variance on CIE color coordinate 'x' in raw and cooked shrimp (Tables 7 and 9) indicates a significant difference among the treatments. Phosphoric acid has the highest (Table 8) and control, the lowest 'x' value in raw shrimp. But in cooked shrimp (Table 10), tetra sodium pyrophosphate, and H_3PO_4 have the highest 'x' value and sodium chloride and control, the lowest. Treatments did not differ significantly on the color coordinate 'y'. Time had no significant effect on either the 'x' or 'y' coordinates in raw shrimp, but had a significant effect on coordinate 'x' in cooked shrimp (Table 9). Treatments also differed significantly in their effect on lightness index, whiteness index, and saturation index in both raw and cooked shrimp. But they did not differ significantly in the values of Hunter 'A', 'B', and hue angle. Interpretation of the color by CIE color system (Table 11) shows no marked change among the treatments in their

TABLE 5

ANALYSIS OF VARIANCE FOR THE EFFECT OF TREATMENTS ON TEXTURE OF SHRIMP

Source	D.F.	M.S. Raw	M.S. Cooked
Treatment	3	33.7538**	10.1914**
Time	2	0.0827	0.0309
Treatment x time	6	1.9762**	0.6570**
Replication	2	0.1042	0.0227
Error	22	0.3945	0.0493

*Significant at 0.05 level.

**Significant at 0.01 level.

TABLE 6

EFFECT OF TREATMENTS ON TEXTURE* OF SHRIMP

Treatments	Raw	Cooked
Control	7.160q	3.574b
Tetra Sodium Pyrophosphate	5.338s	2.632d
Phosphoric Acid	9.781p	5.060a
Sodium Chloride	6.123r	3.024c

*Texture correlated to the force of shear calculated from the work curve as sq. cm. within each column, means followed by the same letter are not significantly different at the 0.05 level of probability.

TABLE 7

ANALYSIS OF VARIANCE FOR THE EFFECT OF TREATMENTS ON THE COLOR OF RAW SHRIMP

Source	D.F.	M.S.				
		CIE Color Coordinates		Lightness Index	Whiteness Index	Saturation Index
		x	y			
Treatments	3	0.0006**	0.0002	2.2304**	3035.4322*	33.8043*
Time	2	0.0000	0.0002	0.1409*	262.5060**	5.3349
Treatments x Time	6	0.0002	0.0002	0.1001	237.3317**	14.3572
Replication	2	0.0002	0.0002	0.0544	57.9419	96.4670**
Error	22	0.0001	0.0002	0.0404	42.1194	7.8962

*Significant at 0.05 level.

**Significant at 0.01 level.

TABLE 8

EFFECT OF TREATMENTS ON THE COLOR OF RAW SHRIMP

Treatment	CIE Color Coordinate X	Lightness Index	Whiteness Index	Saturation Index
Control	0.324c*	5.901k	105.024y	4.482n
Tetra Sodium Pyrophosphate	0.338ab	5.329L	84.351z	7.678m
Phosphoric Acid	0.342a	6.479i	125.351x	8.714m
Sodium Chloride	0.330bc	6.234j	119.123x	5.527n

*Within each column, means followed by the same letter are not significantly different at the 0.05 level of probability.

TABLE 9

ANALYSIS OF VARIANCE FOR THE EFFECT OF TREATMENTS ON THE COLOR OF COOKED SHRIMP

Source	D.F.	CIE Color Coordinates x	CIE Color Coordinates y	Lightness Index	Whiteness Index	Saturation Index
Treatments	3	0.0004**	0.0005	2.0339**	2659.3301**	33.1756*
Time	2	0.0003*	0.0004	0.0460	67.3271	21.9097
Treatments x time	6	0.0000	0.0003	0.3397**	324.4378**	2.3028
Replication	2	0.0000	0.0005	0.0359	54.6927	4.9699
Error	22	0.0001	0.0003	0.0378	28.3424	8.1509

*Significant at 0.05 level.

**Significant at 0.01 level.

TABLE 10

EFFECT OF TREATMENTS ON THE COLOR OF COOKED SHRIMP

Treatment	CIE Color Coordinates χ	Lightness Index	Whiteness Index	Saturation Index
Control	0.348b*	6.316r	119.909k	13.927m
Tetra Sodium Pyrophosphate	0.361a	6.339r	116.298k	9.90n
Phospheric Acid	0.353a	7.266p	150.8841	11.14mn
Sodium Chloride	0.346b	6.987q	143.798j	9.81n

*Within each column, means followed by the same letter are not significantly different at the 0.05 level of probability.

TABLE 11

EFFECT OF TREATMENTS ON THE COLOR OF RAW AND COOKED SHRIMP

Treatments	Chromaticity Coordinates x	y	Dominant Wavelength (nm)	Luminocity	Purity (percent)
		Raw			
Control	0.324	0.332	580	34.87	10
Tetra Sodium Pyrophosphate	0.338	0.337	592	28.44	7
Phosphoric Acid	0.342	0.335	581	42.05	11.3
Sodium Chloride	0.330	0.327	587	38.93	7.8
		Cooked			
Control	0.348	0.346	601	31.09	20
Tetra Sodium Pyrophosphate	0.361	0.333	581	40.90	32
Phosphoric Acid	0.353	0.350	582	52.83	21
Sodium Chloride	0.346	0.340	581	48.92	16

effect on the color of raw shrimp; however, tetra sodium pyrophosphate, differed markedly from the other treatments in its effect on the color of cooked shrimp. It has the highest purity value and sodium chloride, the lowest.

CHAPTER V

SUMMARY

This study was conducted to determine the depth of penetration of tetra sodium pyrophosphate, phosphoric acid, and sodium chloride into raw shrimp and the effect of these treatments on water-holding capacity, texture, and color of raw and cooked shrimp. From this experiment, the following conclusions are drawn:

1. The concentration of tetra sodium pyrophosphate, phosphoric acid, and sodium chloride was highest in the outer layer and decreased toward the center.
2. The amount of phosphorus increased with an increase in time exposure.
3. Both tetra sodium pyrophosphate and sodium chloride improved the water-holding capacity of shrimp; however, tetra sodium pyrophosphate had the greater effect. Phosphoric acid decreased the water-holding capacity.
4. Time exposure to treatments beyond five minutes did not show a significant effect on water-holding capacity.
5. Tetra sodium pyrophosphate and sodium chloride had a favorable effect on texture; the tenderness increased with the increase in time of treatment, but phosphoric acid had an inverse effect on texture.

6. Treatments did not show marked effect on the color of raw shrimp; however, tetra sodium pyrophosphate differed markedly from other treatments in its effect on the whiteness of cooked shrimp.

Finally, it could be concluded that tetra sodium pyrophosphate was superior to sodium chloride on the water-holding capacity, texture, and color of shrimp. Phosphoric acid was shown to be inferior to the other treatments in all respects.

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APPENDIX

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TABLE A-1

DETERMINATION OF PHOSPHORUS CONTENT IN SHRIMP IN M MG

Treatment	Replication	Outer		Intermediary		Center	
				5 minutes			
Control	1	14.63	14.63	14.63	14.63	14.63	14.63
	2	14.63	14.86	14.63	14.63	14.63	14.73
	3	14.73	14.63	14.63	14.63	14.73	14.63
Tetra Sodium-Pyrophosphate	1	29.26	29.17	21.95	20.90	19.86	18.81
	2	28.26	26.33	24.04	24.45	23.62	21.53
	3	29.54	28.42	22.99	21.95	21.95	20.17
Phosphoric Acid	1	31.35	32.40	20.90	21.95	19.44	19.02
	2	33.44	29.78	24.14	22.99	19.33	20.38
	3	33.44	32.81	20.90	25.08	17.77	18.81
				10 minutes			
Control	1	14.63	14.63	14.63	14.73	14.63	14.63
	2	14.63	14.63	14.63	14.63	14.63	14.63
	3	14.63	14.63	14.63	14.63	14.63	14.63
Tetra Sodium-Pyrophosphate	1	34.49	35.02	25.08	24.56	24.04	24.66
	2	37.62	35.63	25.39	25.08	22.99	22.99
	3	35.35	35.32	26.75	26.13	24.04	24.45

TABLE A-1 (continued)

Treatment	Replication	Outer		Intermediary		Center	
				10 minutes			
Phosphoric Acid	1	34.62	38.14	22.99	22.99	21.95	21.95
	2	38.67	37.67	24.04	24.56	24.04	22.47
	3	37.10	36.68	25.29	24.04	23.10	24.24
				15 minutes			
Control	1	14.63	14.63	14.73	14.63	14.63	14.63
	2	14.63	14.63	14.63	14.63	14.63	14.63
	3	14.63	14.63	14.63	14.63	14.63	14.63
Tetra Sodium-Pyrophosphate	1	39.71	38.67	30.31	29.78	27.17	28.22
	2	40.96	39.81	29.26	29.36	25.08	28.01
	3	40.23	39.50	31.35	29.78	28.22	27.59
Phosphoric Acid	1	40.76	41.80	28.22	28.74	25.08	26.13
	2	39.19	40.76	28.22	27.38	24.04	26.60
	3	39.71	40.96	30.31	29.26	24.66	24.45

TABLE A-2

DETERMINATION OF SODIUM CHLORIDE IN SHRIMP (EXPRESSED AS PERCENTAGE OF 10 GM SAMPLE)

Treatment	Replication	Outer		Intermediary		Center	
				5 minutes			
Control	1	0.029	0.029	0.023	0.029	0.029	0.029
	2	0.029	0.029	0.029	0.029	0.029	0.029
	3	0.029	0.029	0.029	0.029	0.029	0.029
Sodium Chloride	1	0.930	0.905	0.360	0.336	0.232	0.230
	2	0.986	0.997	0.411	0.360	0.157	0.180
	3	0.928	0.893	0.348	0.389	0.244	0.244
				10 minutes			
Control	1	0.035	0.029	0.029	0.029	0.023	0.023
	2	0.029	0.029	0.029	0.029	0.029	0.029
	3	0.029	0.029	0.029	0.029	0.029	0.029
Sodium Chloride	1	1.127	1.095	0.447	0.458	0.319	0.313
	2	1.124	1.143	0.574	0.563	0.290	0.221
	3	1.073	1.114	0.441	0.528	0.337	0.389
				15 minutes			
Control	1	0.029	0.029	0.029	0.029	0.035	0.029
	2	0.029	0.029	0.029	0.029	0.029	0.029
	3	0.029	0.029	0.029	0.029	0.029	0.029
Sodium Chloride	1	1.201	1.189	0.545	0.541	0.382	0.371
	2	1.134	1.154	0.629	0.534	0.516	0.383
	3	1.207	1.183	0.522	0.499	0.406	0.360

TABLE A-3

EFFECT OF TREATMENTS ON THE WATER-HOLDING CAPACITY AND TEXTURE OF SHRIMP (EXPRESSED IN SQ. CM.)

	Replication	5 min.	10 min.	15 min.	5 min.	10 min.	15 min.
Water-holding capacity			Control			Tetra sodium-Pyrophosphate	
Raw	1	25.90	25.50	25.60	20.36	18.62	16.32
	2	26.30	26.20	26.10	22.15	19,80	18.24
	3	25.40	25.10	25.80	21.40	18.26	16.80
Cooked	1	33.40	33.72	33.60	30,12	29.26	26.94
	2	33.80	33.30	33,92	29.46	28.38	27.24
	3	34.10	34.20	34.52	31.44	27.84	25.76
Texture							
Raw	1	7.10	7.22	7.16	6,00	4,82	4.78
	2	7.30	7.00	7.18	5.56	5.50	5.00
	3	7.14	7.10	7.24	6.74	5.02	4.62
Cooked	1	3.60	3.61	3.51	3.20	2,40	2.36
	2	3.50	3.63	3,56	3,00	2.50	2.08
	3	3.54	3.58	3.58	28.30	2.82	2.50

TABLE A-3 (continued)

	Replication	5 min.	10 min.	15 min.	5 min.	10 min.	15 min.
Water-holding capacity		Phosphoric acid			Sodium Chloride		
Raw	1	28.50	32.00	35.50	24.62	22.30	19.00
	2	27.41	29.30	32.20	25.41	20.59	17.60
	3	30.00	34.20	36.00	23.35	23.21	21.50
Cooked	1	35.40	38.60	39.40	32.10	30.10	29.42
	2	34.80	36.90	41.12	31.60	29.54	30.00
	3	36.80	39.10	41.62	33.16	32.00	28.41
Texture							
Raw	1	8.78	10.20	11.10	6.80	6.41	6.00
	2	9.90	8.32	10.30	6.59	6.12	5.42
	3	7.43	10.90	11.10	7.00	5.72	5.10
Cooked	1	4.32	5.12	5.60	3.30	3.00	2.90
	2	4.62	5.41	5.45	3.02	2.84	2.64
	3	3.96	4.88	6.16	3.22	3.22	3.02

TABLE A-4

EFFECT OF TREATMENTS ON THE COLOR OF SHRIMP

Replication		Color eye values	5 min.	10 min.	15 min.	5 min.	10 min.	15 min.
				Control			Tetra sodium-pyrophosphate	
Raw	1	x	38.26	34.32	38.14	26.91	33.02	30.43
		y	37.50	36.40	36.23	26.66	32.08	29.70
		z	31,79	30.16	32.32	24,13	26.15	25.28
		χ	30.68	29.45	29.41	23.74	25.34	24.71
	2	x	33.87	37.13	35.31	29.78	36,19	34.53
		y	33.16	32.84	33.72	29.32	28.43	27.56
		z	28.78	29.16	30.54	20.31	23,40	23.26
		χ	28.31	26.32	30.16	19.40	22.15	22.03
	3	x	36.34	35.41	34.72	24,54	29.92	32.41
		y	34.24	34.18	35.31	23.63	30.32	28.31
		z	30.62	30.86	31.41	22.31	21.54	22.15
		χ	32.23	31.54	31,30	21.30	20.83	21.62
Cooked	1	x	43.80	45,60	38.36	49.53	45.43	46.73
		y	40.94	42.80	35.10	37.50	38,10	42,56
		z	28.90	31.60	24,84	31.64	32.42	31.43
		χ	28.15	30.93	24.56	30.80	30.60	28.95
	2	x	47.32	53.41	35.34	47.24	48.19	48.32
		y	44.43	47.94	31.31	43.40	40.62	40.17
		z	24.16	34.15	30.06	29,60	30.53	33.61
		χ	23.85	33.31	29.34	28.50	31.43	31.36
	3	x	44.14	46,91	43.17	48.43	46.23	49.19
		y	39.15	45.11	36.34	40.31	39.23	37.32
		z	30.16	32.31	34.76	30,20	30.32	30.14
		χ	23.85	31.83	33.42	30,84	29.36	30.56

TABLE A-4 (continued)

Replication		Color eye values	5 min.	10 min.	15 min.	5 min.	10 min.	15 min.
			Phosphoric Acid			Sodium chloride		
Raw	1	x	47.91	41.13	47.30	37.62	37.33	39.22
		y	45.60	38.27	44.74	37.00	36.90	38.78
		z	36.13	28.56	36.92	31.04	32.18	36.27
		X	35.68	28.11	36.71	30.24	31.97	35.63
	2	x	53.54	45.12	53.93	44.36	46.31	44.31
		y	40.31	41.83	47.45	39.01	40.41	42.31
		z	39.01	25.13	43.14	30.06	39.13	37.53
		X	38.63	24.16	42.82	29.43	38.35	37.04
	3	x	43.26	39.63	44.93	35.53	42.12	47.14
		y	41.51	36.12	42.62	31.36	41.15	43.52
		z	37.32	31.32	34.32	28.42	37.31	40.31
		X	36.72	30.81	34.01	28.03	36.86	39.17
Cooked	1	x	58.74	56.28	58.13	50.35	54.90	51.24
		y	54.09	51.09	53.81	46.68	50.65	51.11
		z	38.74	34.20	40.27	31.40	34.74	39.58
		X	38.30	33.78	39.87	30.38	33.95	39.08
	2	x	64.52	52.43	63.43	47.54	58.45	56.17
		y	57.16	47.31	56.73	43.14	53.31	50.41
		z	35.17	37.41	41.15	33.19	42.32	44.15
		X	34.84	37.01	40.06	32.84	41.73	43.29
	3	x	61.31	57.24	55.34	52.41	51.36	60.14
		y	52.19	51.53	51.63	40.46	38.54	56.04
		z	40.34	34.84	39.53	38.54	39.44	41.54
		X	39.73	34.03	38.93	38.06	38.66	40.67

VITA

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