The Influence of Polyphosphate, Sodium-Chloride and Hydrogen Ion Concentration on Heat Mediated Binding of Crab Meat.

Cheng-nan Lai
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THE INFLUENCE OF POLYPHOSPHATE, SODIUM CHLORIDE AND HYDROGEN ION CONCENTRATION ON HEAT MEDIATED BINDING OF CRAB MEAT

A Dissertation

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

The Department of Food Science

by

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ABSTRACT

The object of this investigation was the recombination of the shredded meat to form lump crab meat. Due to the irregular shape and body structure of the crab, development of suitable cleaning or picking machinery has been difficult. Consequently, at the present time most of the processing is done by hand. Several machines, however, have been developed which crushes the shell and separates the meat from the shell by differences in specific gravity in a brine tank or centrifuge. The problem of this approach is that shredded meat is produced, rather than lump meat, which is the form preferred by consumers.

The effect of three pH's (4.5, 5.5 and 6.5) and four levels (0.45, 1.5, 3.0 and 4.5%) each of sodium acid pyrophosphate, sodium tripolyphosphate and NaCl were studied on heat mediated binding, cooking loss, and moisture retention of crab muscle. The shear strength of crab meat treated by the above reagents were markedly increased as compared to the control samples except at pH 6.5 with high level of polyphosphates. At pH 5.5 samples treated with the various reagents displayed less cooking loss than at other pH's. In general, samples treated with concentration of 0.45% for each of the reagents appeared to have less cooking loss than other treatments with higher concentration of the same reagents at same pH level.

In addition to increase shear strength and reducing cooking loss, there is considerable interest in improving color by adding sodium acid pyrophosphate and sodium tripolyphosphate. The blueing reaction of crab meat was completely inhibited by dipping in
solutions containing above 3.0% sodium acid pyrophosphate or sodium tripolyphosphate.

Solutions of higher phosphate concentrations, though efficient in increasing shear strength and suppressing the blueing reaction, were unsuitable for dip solution due to the production of off flavors attributable to the increased phosphate concentration. Taste panel results indicated that combined solution which contain 1.5% NaCl, 0.45% sodium acid pyrophosphate and 0.63% sodium tripolyphosphate at pH 5.5 were more efficient in obtaining a suitable firmness for the recombined crab meat as well as minimizing objectionable flavors.
INTRODUCTION

The blue crab (Callinectes sapidus Rathbun) is harvested commercially from coastal and estuarine water in all the Atlantic States from New Jersey to the Florida Keys and in all the states bordering the Gulf of Mexico. Prior to 1878, all crabs were sold as hard crabs or as the much highly priced soft crabs. About 1884 marked the first attempt to sell picked crab canned meat. Shortly after this first production of cooked picked crab meat for canning, the commercial production of blue crab increased from about 7.5 million pounds in 1880 to the highest level of 155 million pounds in 1960 (David, 1964). Statistic, have shown a steady decline from 155 million pounds in 1960 to 145 million pounds in 1970 (Wheeland 1970). This decrease in production has been related to soaring labor costs since the early 1960s'. Food processing operations employing a large amount of hand labor have been particular affected by high labor costs.

One of the most laborious parts of the crab industry is the cleaning and picking of crabs. Due to the irregular shape and body structure of the animal, development of suitable cleaning and picking machinery has been difficult. Consequently, at the present time most of the processing is done by hand. Several machines, such as the meat centrifuge (Bird Manufacturing Company, South Walpole, Massachusetts) have been developed which crushes the shell and separates the meat by differences in specific gravity in a brine tank or centrifuge. The problem in this approach is that shredded meat is produced, rather than lump meat which is the form preferred by consumers. Thus, the
object of this investigation was the recombination of the shredded meat to form lump crab meat.

The previous work which has been done on recombination of meat has been primarily with poultry. The common practice with poultry has been to use a mixture of polyphosphate in addition to sodium chloride to minimize cooking losses by water expulsion and to obtain a better structure and consistency in manufactured meat products. The merits of these additions in improving meat products seem well established. (Danish Meat Research Inst. 1956; Giszke, 1958; Kendereski, 1961; Swift and Ellis 1957). Since polyphosphate and sodium chloride have been shown to improve structure and binding ability of poultry meats, it was conceivable that crab meat treated with polyphosphate and sodium chloride may produce meat products with improved structure and consistency.

Many materials such as modified starches are available for improving moisture retention and binding ability of meat products, however, the present study was limited to the effects of various polyphosphates, pH, and sodium chloride on heat-mediated binding of crab meat muscle.
REVIEW OF LITERATURE

The name "polyphosphate" covers a group of compounds having two or more phosphorus atoms joined by an oxygen bridge in a chain structure. The first member of this series, normally referred to as pyrophosphate, have one P-O-P linkage such as sodium acid pyrophosphate. The second in the series of polyphosphates is the triphosphate, a three-phosphorus chain. To minimize the possibility of confusing these phosphates with others having "tri" in the name, the "poly" designation was used. The best known example is sodium tripolyphosphate (Deman and Melnychyn, 1971).

Both sodium acid pyrophosphate and sodium tripolyphosphate are on the GRAS list (Code of Federal Regulation 21:121) and are extensively used through the food industry.

Water-Binding Capacity of Meat as Affected by Phosphates

The polyphosphates (sodium acid pyrophosphate and sodium tri-polyphosphate) are frequently used as additives in many fish products to aid in processing and to improve appearance or eating quality. The main value of polyphosphates in the fish industry have been to reduce or prevent drip from the cut surface of the fish, either during storage or during thawing. Various investigators (Boyd et al. 1965, Mahon 1962 and Spinelli and Dave 1968) have shown that pretreatment of sole fillets with polyphosphate was effective in reducing the amount of weight lost during thawing and cooking. Thaw drip from frozen haddock fillet was also decreased by pretreatment of fresh fish with sodium tripolyphosphate at a concentration of 12.5% in dip solution (Mahon 1962).
Barnett et al. (1969) observed that treatment with sodium tripolyphosphate dissolved in brine effectively reduced the loss of weight in the smoked halibut as compared with the treatment with concentrated brine alone. Use of a short 1-minute dip in the solution containing 7.5% sodium tripolyphosphate and 2.0% sodium chloride reduced the loss of weight from 29.5% to 26.0%.

Spinelli and Dave (1968) observed in sole, perch, cod, and halibut that the loss of drip in fresh (refrigerated) fillets can effectively be minimized by spraying with 7.5 to 10.0% sodium tripolyphosphate solution containing 2.0% salt. Treated fillets such as sole, ocean perch, cod, and halibut steaks lost no more than 0.5 to 1.0% drip during their effective refrigerated shelf life. Drip loss in untreated fish ranged from 3.0 to 6.0% during the same storage period. Tanikawa et al. (1963) also reported that polyphosphates were effective in reducing the amount of drip from frozen cod during thawing.

Mathen (1968) screened various phosphates and their mixtures for their efficiency of preventing drip loss in frozen prawns. The effectiveness of the phosphate decreased in the following order: sodium tripolyphosphate, sodium pyrophosphate, sodium hexametaphosphate, sodium metaphosphate, sodium dihydrogen phosphate; the last two being ineffective. A mixture of 12.0% sodium tripolyphosphate and 8.6% sodium dihydrogen phosphate or 2.0% citric acid in water when used as dip was found to prevent thaw drip losses, and to improve cooking yield.

The addition of certain phosphate compounds to poultry meat increases its water-holding capacity and decreases losses caused by
cooking. The effect of added polyphosphates on water-holding capacity during storage and cooking of poultry meat have been investigated by Bendall (1954), Klose et al. (1962), Schermerhorn et al. (1962), May et al. (1962), Hellendoorn (1962) and Froning (1965).

Klose et al. (1962) reported that moisture uptake during chilling of poultry was appreciably less in the polyphosphate group than in the control, however, cooking shrink was less. The yield of cooked meat as percentage of unchilled, eviscerated weight showed a 4.0% net gain due to polyphosphate, thus suggesting that more of the natural moisture of the meat was retained in the polyphosphate-treated samples. These results were in agreement with Hellendoorn (1962) who reported that the addition of sodium tripolyphosphate did not promote water binding in uncooked beef. He also indicated that the beneficial effect of the sodium tripolyphosphate on the cooked samples increases as the pH increases, and at a pH of about 5.5 and lower there was an inverse effect by adding the phosphate.

Swelling and solubility effects on poultry meat possibly explain the role of the polyphosphate on meat hydration. Morse (1955) reported swelling was produced by phosphate due to binding magnesium and calcium from the meat, thus enabling the meat to increase water uptake. Hamm (1955) indicated that calcium bound to the meat to form bridges between the protein fibers, would play an important part in meat hydration. Displacement of bound calcium by sodium, by means of ion exchange, by complex binding, or by precipitation, would give rise to greater hydration (Grau et al. 1953).

Hamm (1955) attributed polyphosphate effects partly to increasing meat pH and to salt action, and also the importance of chelating
calcium and magnesium was stressed as an important factor affecting the action of the phosphate on meats. Swift and Berman (1959) reported that water retention was inversely proportional to calcium and magnesium ion concentration. On the other hand, Swift and Ellis (1956) observed a slight improvement in the water retention of beef treated with diluted solutions of magnesium chloride.

As seen from the above review there are numerous theories as to the manner in which polyphosphate improve water binding within meat tissue. Whatever the manner of operation, polyphosphates have clearly been shown by the researchers cited above to improve the retention of moisture in protein food during storage or cooking.

**Mechanism of Heat-initiated Binding of Meat**

The mechanism of heat-initiated binding of meat pieces has not been clearly elucidated. Binding is a complex phenomenon involving water holding and/or binding, cell disruption, releasing of intercellular material and the physical and chemical changes in the proteins (connective, sarcoplasmic and contractile proteins) on heating (Schnell et al. 1970).

At present, however, it is thought that, in emulsions at least, binding is caused by salt-soluble proteins. Hashimoto et al. (1959) have reported that the salt soluble protein is important in the binding of emulsified sausages. Hansen (1960), Swift et al. (1961) and Mauer et al. (1969) reported that salt soluble proteins demonstrated better emulsifying power than water soluble proteins. Hansen et al. (1966) were awarded a patent for the production of a poultry product (loaf) in which they claimed that salt soluble proteins have a primary role in binding.
Polyphosphate added to comminuted meat products increased the level of salt soluble protein which results in a finer-textured, more stable emulsion. Upon heating, the salt soluble proteins increased binding of the emulsion by coagulating to form a stable binding structure (Mahon 1961; Bendall 1954; Hellendorn 1962; Yasui et al. 1964).

Swift and Ellis (1957) found that the addition of three combinations of phosphate, 25.0% sodium hexametaphosphate, 10.0% sodium acid pyrophosphate and 65.0% tetrasodium pyrophosphate, markedly increased the relative binding of sausage components. The tensile strength, or cohesiveness, of the bologna containing added phosphate was significantly greater than that of bologna cured only with ordinary curing agents.

Froning (1965, 1966) indicated that the increased pH afforded by polyphosphates probably influenced changes in the properties of proteins and altered the binding properties of the meat. He found in poultry meat that the binding ability of white meat was greater than that observed in dark meat. The texture of the dark meat roll was somewhat more crumbly and possessed a more open structure than the comparable white meat roll. Consequently, the poor binding of dark meat was improved through the use of polyphosphate. May et al. (1962) and Spencer and Smith (1962) reported that the chicken fryer carcass chilled with polyphosphate (sodium tripolyphosphate) treatment resulted in greater tenderness, juiciness and improved binding quality.

Fukazawa (1961) reported an increase in the binding quality of sausage by addition of polyphosphates due to the following facts:
1. In the native state, the effect has been correlated with the action of phosphate in promoting the extraction of protein from intact fibrils. The muscle structural protein is drawn through the sarcolemma of the muscle-cell and such action may be promoted by the use of phosphates.

2. In the denatured state the effect has been correlated with the quantities of "light" components (the upper clear layer obtained by ultracentrifugal separation) dissociated by ultracentrifugation as result of addition of phosphate.

The content of the light component was increased by the addition of pyrophosphate from 11.0% to 30.0% of the total (Nihei and Yuji 1959). The main component of the separate upper clear layer probably has a direct and close relationship to the binding of sausage; the light component consists of myosin A and myosin B (Fukazawa 1961). Hihei and Yuji (1959) suggested that the light component must be myosin A itself or fragments of myosin B, whose molecular characteristics are very similar to those of myosin A.

Maesso et al. (1970) indicated NaCl and Kena (Calgon Corp., formula No. FP-28, sodium tripolyphosphate, tetrasodium pyrophosphate and sodium metaphosphate) were found to enhance binding. When combining NaCl and Kena, there was a significant synergistic effect. The beneficial effect of NaCl may have been due to 1/. an increased solubility of the globular proteins in general and myosin in particular. 2/. increased ionic strength of the medium may have resulted in a structural rearrangement of the meat protein which rendered them more reactive for essential protein binding.
Mahon (1961) reported that addition of increasing amounts of salt to charged protein first reduces the net negative charge to zero, "the first salt induced isoelectric point". If more salt is added to meat at this salt-induced isoelectric point, there is an absorption of more Na\(^+\) ions, which produces a net positive charge on the proteins; this occurrence is generally at a maximum of 4.0 to 5.0\% salt. This protein may then strongly attract the negatively charged tripolyphosphate. Therefore, the presence of NaCl may have been necessary to allow a protein-tripolyphosphate interaction which may cause improved binding.

Schnell (1970) indicated that the meat loaf slices that received no treatment and no beating (control) had a poor bind (score 2.1, binding score 1=worst, 10=best, these scores were measured by Allo-Kramer shear press). Beating for 3 minutes (at low speed 60 RPM) improved the binding considerably (score 6.6). The highest binding scores were obtained with the RNA and Kena treatments which had binding scores of 8.3. Dried meat and NaCl similarly had good binding scores (8.1 and 7.8 respectively). The increase in concentration of Kena greatly increased the binding scores for chicken meat. Increasing the concentration of NaCl increased binding and shear value of the meat. Approximately 2.0\% of NaCl was optimum while 1.5\% Kena gave the best results. Total nucleic acids in the cookout (the fluid expressed on cooking of the loaves) were also affected. There was a slightly increase nucleic acid with Kena as compare to the control, while NaCl showed the highest increase. If the amount of total nucleic acid was taken as an indication of cell disruption, then it would appear that there was more cell disruption with NaCl than Kena. The
Kena effect, therefore, may be merely due to water binding, while NaCl may have an osmotic effect causing cell disruption. The liberation of intercellular material may result in binding with NaCl.

The effect of dried meat on binding appears to be physical, by entrapping the excess moisture. Schnell (1970) reported that ribonucleic acid (RNA) did not markedly decrease the amount of cookout but did improve the before mentioned binding score of poultry meat products. There was no drastic increase in the amount of total nucleic acids indicating that cell disruption was not involved. The effect of RNA may thus be due to its polyionic properties (the interaction of these highly-charged anions with cationic functions of large molecules).
MATERIALS AND METHODS

The aim of the investigation was to study the effects of various concentrations of polyphosphates and sodium chloride at three pH levels on the heat mediated binding of crab meat. Sample preparation and methods of evaluation are described as follows:

Samples:

Twenty pounds of frozen canned crab meat was obtained from a local seafood market in Baton Rouge and kept frozen at -20°F until used. Prior to use, the meat was allowed to thaw for 8 hours in a refrigerator at 40°F. The meat was shredded into fine muscle strands to simulate meat obtained from mechanical picking machinery.

Reagents:

The following reagents were employed during the course of the investigation:

a/. Lemon juice: Fresh lemons were cut in half, pressed, and the collected juice filtered through a No. 1 filter paper in order to obtain a translucent juice.

b/. NaCl solutions: 0.45, 1.5, 3.0, and 4.5% salt solutions were prepared in the following manner:

0.45, 1.5, 3.0, and 4.5 grams respectively of reagent grade NaCl were weighed into a 100 ml volumetric flask and dissolved in 80-85 ml of distilled water. 6.2 ml of lemon juice were added to each flask. The pH was adjusted to 4.5 with 1 N HCl or 1 N NaOH and made up to 100 ml volume. By the same method, solutions of the same concentrations at pH 5.5 and 6.5 were prepared.
c/. Phosphate solutions: sodium acid pyrophosphate and tripolyphosphate solutions of 0.45, 1.5, 3.0, and 4.5% at pH of 4.5, 5.5, and 6.5 were prepared in the same manner as the salt solutions.

d/. Combined solutions: To each of four flasks containing 4.5 g of sodium acid pyrophosphate, 6.3 g of sodium tripolyphosphate, and 6.2 ml of lemon juice, 0.45, 1.5, 3.0, and 4.5 g respectively of reagent grade sodium chloride were added. The mixture was dissolved in 80-85 ml of distilled water, adjusted to pH 4.5 with 1 N HCl or 1 N NaOH and made up to 100 ml volume. Solutions at pH 5.5 and 6.5 were prepared by the same method.

Experimental Solutions:

The samples were immersed in the following experimental solutions, at three pH levels, 4.5, 5.5, and 6.5. The seventeen experimental solutions were as follows:

1/. control, 2/. 0.45% NaCl, 3/. 1.5% NaCl, 4/. 3.0% NaCl
5/. 4.5% NaCl, 6/. 0.45% sodium acid pyrophosphate (S. A. P.)
7/. 1.5% S. A. P., 8/. 3.0% S. A. P., 9/. 4.5% S. A. P.
10/. 0.45% sodium tripolyphosphate (S. T. P.),
11/. 1.5% S. T. P., 12/. 3.0% S. T. P., 13/. 4.5% S. T. P.
14/. 0.45% NaCl + 0.45% S. A. P. + 0.63% S. T. P.,
15/. 1.5% NaCl + 0.45% S. A. P. + 0.63% S. T. P.,
16/. 3.0% NaCl + 0.45% S. A. P. + 0.63% S. T. P.,
17/. 4.5% NaCl + 0.45% S. A. P. + 0.63% S. T. P.

Sample Preparation and Evaluation:

Twenty grams of thawed crab meat was homogenized in a blender at low speed for about 3 minutes to get a more homogenized sample and to improve the appearance of the final products. The samples
were immersed in the previous experimental solutions and kept in a cooler at 4°C for 12 hours.

The meat was separated from the solution by centrifuging twice at 3000 RPM for 15 minutes. These meat loaves formed in the centrifuge tube were weighed and then cooked in an autoclave at 121°C for 30 minutes. After cooking, the meat was separated from the drip and cooled to room temperature for weighing. The percentage of weight lost during cooking was determined from sample weights before and after cooking. These meat loaves were also used to measure shear strength and moisture retention.

Shear strength measurements were made using a Dynamoneter Scale, Chatillon, New York. Meat loaves of 20 mm diameter were utilized for these measurements.

The oven drying method was used for measuring moisture retention. The sample was placed in an oven at 100-110°C for 16 hours. The meat loaves were then cooled in a dessicator to room temperature and weighed. The moisture retention was measured by calculating the difference between the weight of meat loaves before and after drying.

The entire experiment was run in triplicate. The effect of sodium chloride and phosphate on binding, moisture retention, and cooking loss was determined at various pH levels and concentrations of reagent. The combined effect of sodium chloride and phosphates was also investigated.

**Organoleptic Evaluation of Selected Treatments**

After determining the effects of the various treatments on cooking loss, binding ability and moisture retention of the meat loaves, selected treatments which showed desirable properties with
respect to the above were selected for organoleptic evaluation. A panel of five judges evaluated the samples for color, odor, flavor, and overall quality using a six point hedonic scale.
RESULTS AND DISCUSSION

Hydrogen Ion Concentration, Phosphates and Sodium Chloride Effect on Cooking Loss and Moisture Retention

The alterations involving the swelling and liquid-binding power of muscle are largely due to the proteins which are present, especially in view of the fact that there is a pronounced isoelectric zone in which swelling and liquid-binding are least pronounced. A pH of about 4.5 was the approximate isoelectric point of crab protein. At this pH all samples treated with the various salt and phosphate solution retained less moisture after cooking than the control sample (Fig. 1, 2, 3, and 4) except for the treatment using 0.45% NaCl. This treatment resulted in a slight higher level of moisture retention (Table 1 and Fig. 3).

At the isoelectric point there are maximum numbers of inter-molecular salt bridges between the oppositely charged groups of the protein and minimum of electrostatic repulsion between similarly charged group. The net effect is that the phosphates are ineffective in increasing hydration of the tissue at this pH.

In general, there was a trend of moisture retention decrease with increase in level of reagent whether with NaCl or one of the phosphates (Fig. 5, 6, and 7). The addition of NaCl (0.45%) at pH 4.5 however increased the moisture retention of the crab meat loaves. This improvement can be attributed to an increase in electrostatic repulsion between protein charges. This loosening of the micro-structure resulted in increased uptake of immobilized water.
TABLE I
THE EFFECT OF VARIOUS CONCENTRATIONS OF PHOSPHATES, SALT
AND pH ON MOISTURE RETENTION

<table>
<thead>
<tr>
<th>Treatments</th>
<th>pH</th>
<th>MOISTURE RETENTION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.5</td>
<td>5.5</td>
</tr>
<tr>
<td>Control</td>
<td>77.3 ± 1.2</td>
<td>78.4 ± 1.0</td>
</tr>
<tr>
<td>0.45% NaCl</td>
<td>77.6 ± 0.2</td>
<td>77.7 ± 1.0</td>
</tr>
<tr>
<td>1.50% NaCl</td>
<td>77.2 ± 0.1</td>
<td>77.7 ± 0.7</td>
</tr>
<tr>
<td>3.00% NaCl</td>
<td>76.9 ± 0.4</td>
<td>77.3 ± 0.6</td>
</tr>
<tr>
<td>4.50% NaCl</td>
<td>76.1 ± 0.1</td>
<td>76.9 ± 0.4</td>
</tr>
<tr>
<td>0.45% SAP</td>
<td>76.8 ± 0.1</td>
<td>77.1 ± 1.0</td>
</tr>
<tr>
<td>1.50% SAP</td>
<td>76.3 ± 0.1</td>
<td>76.4 ± 1.1</td>
</tr>
<tr>
<td>3.00% SAP</td>
<td>76.7 ± 0.3</td>
<td>75.4 ± 1.0</td>
</tr>
<tr>
<td>4.50% SAP</td>
<td>75.0 ± 0.3</td>
<td>74.6 ± 0.9</td>
</tr>
<tr>
<td>0.45% STP</td>
<td>77.3 ± 0.1</td>
<td>78.3 ± 0.8</td>
</tr>
<tr>
<td>1.50% STP</td>
<td>77.0 ± 0.1</td>
<td>77.3 ± 0.8</td>
</tr>
<tr>
<td>3.00% STP</td>
<td>76.0 ± 0.3</td>
<td>76.7 ± 0.7</td>
</tr>
<tr>
<td>4.50% STP</td>
<td>75.0 ± 0.4</td>
<td>76.3 ± 0.8</td>
</tr>
<tr>
<td>0.45% NaCl+SAP+STP</td>
<td>76.8 ± 0.1</td>
<td>77.7 ± 0.6</td>
</tr>
<tr>
<td>1.50% NaCl+SAP+STP</td>
<td>76.4 ± 0.2</td>
<td>76.7 ± 1.1</td>
</tr>
<tr>
<td>3.00% NaCl+SAP+STP</td>
<td>76.0 ± 0.2</td>
<td>76.5 ± 1.1</td>
</tr>
<tr>
<td>4.50% NaCl+SAP+STP</td>
<td>75.6 ± 0.9</td>
<td>75.9 ± 1.3</td>
</tr>
</tbody>
</table>

\(^a\)Sodium Acid Pyrophosphate  \(^b\)Sodium Tripolyphosphate  \(^c\)0.45% Sodium Acid Pyrophosphate  \(^d\)0.63% Sodium Tripolyphosphate
Fig. 1. The effect of pH at various concentrations of sodium acid pyrophosphate, 0% (○), 0.45% (●), 1.5% (△), 3.0% (▴), and 4.5% (×), on moisture retention.
Fig. 2, The effect of pH at various concentrations of sodium tripolyphosphate, 0% (O), 0.45% (●), 1.5% (Δ), 3.0% (▲) and 4.5% (x), on moisture retention.
Fig. 3, The effect of pH at various concentrations of salt, 0% (○), 0.45% (●), 1.5% (▲), 3.0% (▲), and 4.5% (▲), on moisture retention.
Fig. 4, The effect of pH at various concentrations of combined solutions, 0% (○), 0.45% (●), 1.5% (▲), 3.0% (▲), and 4.5% (x), of salt on moisture retention.
Fig. 5, The effect of various concentrations of sodium acid pyrophosphate (○), sodium tripolyphosphate (Δ), salt (x) and combined solutions (*) on moisture retention at pH 5.5.
Fig. 6. The effect of various concentrations of sodium acid pyrophosphate (o), sodium tripolyphosphate (Δ), salt (x) and combined solution (●), on moisture retention at pH 4.5.
Fig. 7. The effect of various concentrations of sodium acid Pyrophosphate (○), sodium tripolyphosphate (△), salt (×), and combined solution (●) on moisture retention at pH 6.5.
As the pH increases from the isoelectric point (I. P.), the stronger is the electrostatic repulsion between adjacent peptide chains, because of the increasing negative net charge of the proteins. However, the increase in pH accompanied by an increase in electrostatic repulsion did not improve moisture retention over the control for any of the treated samples. In fact, the highest pH level (6.5) showed the least amount of water retention (Table 1).

Cooking losses, however, represented a different picture with regard to the salts and phosphates. The only phosphate treatment which had less cooking loss at pH 4.5 than control sample was sodium tripolyphosphate at the 4.5% level (Table 1 and Fig. 8). At the isoelectric pH (4.5) the sodium tripolyphosphate, sodium acid pyrophosphate and the combination treatments showed considerable greater cooking loss than did the control sample (Table 2 and Fig. 8, 9, and 10). These results were in agreement with Hamm and Grau (1958) who indicated that under I. P. conditions there is a maximum of salt bridges; a cleavage of crosslinkages by bound phosphate ions could hardly increase the hydration of the tissue. This is illustrated in Fig. 11.

at the isoelectric point:
## TABLE II

**THE EFFECT OF VARIOUS CONCENTRATIONS OF PHOSPHATES, SALT AND pH ON COOKING LOSS**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>pH</th>
<th>COOKING LOSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.5</td>
<td>10.6 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>8.4 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>6.5</td>
<td>8.5 ± 0.4</td>
</tr>
<tr>
<td>0.45% NaCl</td>
<td>4.5</td>
<td>9.1 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>6.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>6.5</td>
<td>7.9 ± 0.5</td>
</tr>
<tr>
<td>1.50% NaCl</td>
<td>4.5</td>
<td>9.9 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>7.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>6.5</td>
<td>9.5 ± 0.3</td>
</tr>
<tr>
<td>3.00% NaCl</td>
<td>4.5</td>
<td>10.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>8.8 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>6.5</td>
<td>8.9 ± 0.5</td>
</tr>
<tr>
<td>4.50% NaCl</td>
<td>4.5</td>
<td>9.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>7.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>6.5</td>
<td>8.5 ± 0.4</td>
</tr>
<tr>
<td>0.45% SAPa</td>
<td>4.5</td>
<td>12.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>7.2 ± 0.4</td>
</tr>
<tr>
<td></td>
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<td>9.2 ± 0.2</td>
</tr>
<tr>
<td>1.50% SAP</td>
<td>4.5</td>
<td>10.8 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>8.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>6.5</td>
<td>9.5 ± 0.2</td>
</tr>
<tr>
<td>3.00% SAP</td>
<td>4.5</td>
<td>11.4 ± 0.3</td>
</tr>
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<td>5.5</td>
<td>8.0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>6.5</td>
<td>11.1 ± 0.6</td>
</tr>
<tr>
<td>4.50% SAP</td>
<td>4.5</td>
<td>10.9 ± 0.2</td>
</tr>
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<td>7.4 ± 0.2</td>
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<tr>
<td></td>
<td>6.5</td>
<td>12.4 ± 1.0</td>
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<td>0.45% STPb</td>
<td>4.5</td>
<td>11.0 ± 1.2</td>
</tr>
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<td>5.5</td>
<td>6.8 ± 0.1</td>
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<td>8.7 ± 0.7</td>
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<td>1.50% STP</td>
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<td>12.8 ± 1.4</td>
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<td>7.3 ± 0.3</td>
</tr>
<tr>
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<td>11.1 ± 1.5</td>
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<tr>
<td>3.00% STP</td>
<td>4.5</td>
<td>11.1 ± 1.4</td>
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<td>5.5</td>
<td>8.5 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>6.5</td>
<td>11.8 ± 0.6</td>
</tr>
<tr>
<td>4.50% STP</td>
<td>4.5</td>
<td>9.9 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>8.5 ± 0.2</td>
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<td></td>
<td>6.5</td>
<td>13.9 ± 1.4</td>
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<tr>
<td>0.45% NaCl+SAPc+STPd</td>
<td>4.5</td>
<td>11.0 ± 1.3</td>
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<tr>
<td></td>
<td>5.5</td>
<td>7.9 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>6.5</td>
<td>8.1 ± 0.7</td>
</tr>
<tr>
<td>1.50% NaCl+SAP+STP</td>
<td>4.5</td>
<td>12.1 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>8.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>6.5</td>
<td>10.7 ± 0.3</td>
</tr>
<tr>
<td>3.00% NaCl+SAP+STP</td>
<td>4.5</td>
<td>10.7 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>7.9 ± 0.7</td>
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<td>10.6 ± 0.2</td>
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<tr>
<td>4.50% NaCl+SAP+STP</td>
<td>4.5</td>
<td>11.1 ± 1.2</td>
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<td>5.5</td>
<td>7.5 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>6.5</td>
<td>9.3 ± 0.3</td>
</tr>
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</table>

aSodium Acid Pyrophosphate  
bSodium Tripolyphosphate  
c0.45% Sodium Acid Pyrophosphate  
d0.63% Sodium Tripolyphosphate
Fig. 8. The effect of pH at various concentrations of sodium tripolyphosphate, 0% (o), 0.45 (●), 1.5% (△), 3.0% (▲), and 4.5% (x), on cooking loss.
Fig. 9, The effect of pH at various concentrations of sodium acid pyrophosphate, 0%, (O), 0.45% (•), 1.5% (△), 3.0% (▲), and 4.5% (x), on cooking loss.
Fig. 10. The effect of pH at various concentrations of combined solutions, 0% (o), 0.45% (●), 1.5% (△), 3.0% (▲), and 4.5% (x) of salt on cooking loss.
Fig. 11, The influence of protein net charge on the effect of polyphosphate (PP) anions on swelling and water-holding capacity of meat (from Deman and Melnychyn 1971).

However, all levels of NaCl treated samples at pH had less cooking than the controls (Table 1 and Fig. 13). This was due to the increase in electrostatic repulsion between protein charges caused by the addition of NaCl the latter resulted in loosing the microstructure enabling the meat to hold more water. The effect is illustrated in Fig. 12. Lillie (1921) indicated that very small amount of certain salts may cause a pronounced effect on swelling. Tarr (1942) also reported that sodium chloride prevents cooking loss in muscle by causing the protein to swell and to obtain liquid firmly, not by a surface sealing action.

\[
\begin{align*}
\text{Me} + \text{PP} & \rightarrow \text{MePP} \\
\text{Me} & = \text{Me} - \text{H}_2\text{N}^+ - \text{COOH} \\
\text{PP} & = \text{H}_3\text{N}^+ - \text{COO}^- + 3\text{Na}^+\text{Cl}^- \\
\end{align*}
\]

Fig. 12, The influence of NaCl on loosing the microstructure of meat at pH 4.5.
Fig. 13, The effect of pH at various concentrations of salt, 0% (○), 0.45 (●), 1.5% (△), 3.0% (▲), and 4.5% (×), on cooking loss.
At pH 5.5 the phosphates as well as NaCl tended to reduce cooking loss in the crab meat loaves. This effect is probably due to the fact that there is greater electrostatic repulsion at pH 5.5 which is also facilitated by NaCl and polyphosphate breaking cross-linking bonds between proteins (Fig. 11).

Deman and Melynchn (1971) indicated that at pH 5.5 the binding of the ions of phosphates or sodium chloride by the charged groups of the myofibrillar protein causes a cleavage of salt and hydrogen bonds. The net charge of the protein and the electrostatic repulsion between adjacent peptide chains are increased by binding of polyphosphate anions, particularly between myosin and actin, resulting in additional swelling of the system. If added water is available, more water can be taken up in an immobilized state within the loosened protein network.

Krieley and Martonosi (1968) indicated that the binding of magnesium diphosphate (Mg-PP) by myosin or actomyosin probably result in the formation of a protein-Mg-PP complex. Polyphosphate anions bound in this way increase the negative net charge of the protein, thus causing a loosening of the molecular structure, resulting in a decrease in cooking loss. A single bond between the bivalent cation and protein is broken by binding of the phosphate anion, the other valence of the cation remaining connected with protein:

\[
\text{Protein-Mg}^{+2}\text{-Protein + PP} \rightarrow \text{Protein-Mg-PP + Protein}
\]

In this manner the cross-linking effect of the cation could be eliminated, and the final result would be an increase of water holding capacity or a decrease in cooking loss.
Sodium chloride and sodium tripolyphosphate when used alone at low levels (0.45% and 1.5%) had the least cooking loss of the sample (Fig. 14, 15, and 16). These results are similar with that of Tarr (1942) who reported that in the pH range 4.5 to 6.5 maximal swelling occurred below 1.5% salt, while higher concentration did not cause decrease of cooking loss, presumably due to peptization of the protein.

At pH 6.5 the crab meat loaved showed high cooking losses comparable to the cooking losses at the isoelectric pH 4.5 (Fig. 8,9, 10, and 12). The cause of the high cooking loss is again related to the charge on the protein. At pH 6.5, the electrostatic repulsion exerted by like charges are maximum and there are a minimum amount of salt bridges between protein molecules resulting in a loose microstructure.

Treatments with NaCl and phosphate under these conditions further increase the electrostatic repulsion between protein molecules. This results in a structure which is too loose to hold water resulting in high cooking losses. (Fig. 17).

Moisture retention, as seen from the data (Table 1), was not a good indicator of the changes brought about by the various NaCl and phosphate treatments. This is attributed to the fact that the greatest changes occurred prior to the moisture determination i.e. cooking loss. Moisture determination were based on weight of cooked sample divided by the weight of the dried sample and did not reflect the losses which occurred during cooking. For future investigation of this nature it would be advantageous to determine moisture retention based on the weight of the initial sample prior to cooking or cooking losses alone. This approach would be more reflection of changes occurring due to the various treatments.
Fig. 14, The effect of various concentrations of sodium acid pyrophosphate (○), sodium tripolyphosphate (△), salt (X), and combined solution (●) on cooking loss at pH 5.5.
Fig. 15, The effect of various concentrations of sodium acid pyrophosphate (○), sodium tri-polyphosphate (△), salt (×), and combined solution (●) on cooking loss at pH 5.5.
Fig. 16, The effect of various concentrations of Sodium acid pyrophosphate (O), Sodium tri-polyphosphate (Δ), salt (X) and combined solution (●) on cooking loss at pH 6.5.
Fig. 17, Influence of cross-linking of proteins or swelling of meat on water-holding capacity.

Left: Strong cross-linking causes low water-holding capacity. The bulk of "free" water is not immobilized. Middle: Moderate cross-linking cause a high water-holding capacity. A great part of the "free" water is immobilized within the protein network. Right: Few cross-linking and, therefore, only little water-holding capacity.
Hydrogen Ion Concentration, Phosphate and Sodium Chloride Effect on Shear Strength

The shear strength values for the crab meat loaves are shown in Table 3. The data revealed that pH has a pronounced effect on shearing strength. At pH 5.5 the samples always had lower shearing values than the corresponding treatment (Fig. 18, 19, 20, and 21), however, they had higher moisture retention and less cooking loss than other pH’s. This observation was supported by Froning and Norman (1966) who indicated that a higher moisture: protein ratios are related to lower binding ability.

At concentrations above 1.5%, sodium acid pyrophosphate treated samples had stronger shearing values than either NaCl or sodium tripolyphosphate at pH 4.5 and 5.5 (Fig. 22 and 23). It was also noted that as the level of sodium tripolyphosphate and sodium acid pyrophosphate increase, so does the shearing value (Table 3 and Fig. 22, 23, and 24).

In general, NaCl and the combined solutions showed slight improvements in binding scores compared to control samples, but were not as effective as the phosphate.

Organoleptic Evaluation of Selected Treatments

The following treatment were selected for organoleptic evaluation based on low cooking losses and binding ability: 0.45% sodium tripolyphosphate, 0.45% sodium chloride, 4.5% sodium acid pyrophosphate, and combined solution containing 1.5% NaCl, 0.45% sodium acid pyrophosphate and 0.63% sodium tripolyphosphate. The treatment were maintained at pH 5.5 which exhibited lower cooking losses than other pH levels evaluated. Low cooking losses were considered essential
### TABLE III

THE EFFECT OF VARIOUS CONCENTRATIONS OF PHOSPHATES, SALT AND pH ON SHEAR STRENGTH

<table>
<thead>
<tr>
<th>Treatments</th>
<th>pH 4.5</th>
<th>pH 5.5</th>
<th>pH 6.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.2 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>0.45% NaCl</td>
<td>2.5 ± 0.1</td>
<td>2.3 ± 0.1</td>
<td>2.9 ± 0.1</td>
</tr>
<tr>
<td>1.50% NaCl</td>
<td>2.8 ± 0.1</td>
<td>2.4 ± 0.1</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>3.00% NaCl</td>
<td>2.4 ± 0.1</td>
<td>2.6 ± 0.2</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>4.50% NaCl</td>
<td>2.3 ± 0.1</td>
<td>2.3 ± 0.5</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td>0.45% SAP&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4 ± 0.1</td>
<td>2.2 ± 0.1</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>1.50% SAP</td>
<td>3.2 ± 0.3</td>
<td>2.7 ± 0.1</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>3.00% SAP</td>
<td>3.8 ± 0.1</td>
<td>3.6 ± 0.1</td>
<td>3.2 ± 0.1</td>
</tr>
<tr>
<td>4.50% SAP</td>
<td>4.1 ± 0.1</td>
<td>4.0 ± 0.1</td>
<td>3.0 ± 0.1</td>
</tr>
<tr>
<td>0.45% STP&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.7 ± 0.4</td>
<td>2.4 ± 0.2</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>1.50% STP</td>
<td>2.9 ± 0.3</td>
<td>2.5 ± 0.1</td>
<td>3.0 ± 0.1</td>
</tr>
<tr>
<td>3.00% STP</td>
<td>3.4 ± 0.1</td>
<td>2.7 ± 0.1</td>
<td>2.7 ± 0.1</td>
</tr>
<tr>
<td>4.50% STP</td>
<td>3.6 ± 0.2</td>
<td>3.0 ± 0.1</td>
<td>2.7 ± 0.1</td>
</tr>
<tr>
<td>0.45% NaCl+SAP&lt;sup&gt;c&lt;/sup&gt;+STP&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.3 ± 0.1</td>
<td>2.2 ± 0.3</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td>1.50% NaCl+SAP+STP</td>
<td>2.7 ± 0.2</td>
<td>2.5 ± 0.1</td>
<td>2.9 ± 0.1</td>
</tr>
<tr>
<td>3.00% NaCl+SAP+STP</td>
<td>2.7 ± 0.1</td>
<td>2.2 ± 0.1</td>
<td>3.3 ± 0.1</td>
</tr>
<tr>
<td>4.50% NaCl+SAP+STP</td>
<td>2.9 ± 0.4</td>
<td>2.2 ± 0.1</td>
<td>2.7 ± 0.1</td>
</tr>
</tbody>
</table>

<sup>a</sup>Sodium Acid Pyrophosphate  
<sup>b</sup>Sodium Tripolyphosphate  
<sup>c</sup>0.45% Sodium Acid Pyrophosphate  
<sup>d</sup>0.63% Sodium Tripolyphosphate
Fig. 18. The effect of pH at various concentrations of sodium acid pyrophosphate, 0% (O), 0.45% (•), 1.5% (Δ), 3.0% (▲), and 4.5% (x), on shear strength.
Fig. 19. The effect of pH at various concentrations of sodium tripolyphosphate, 0% (o), 0.45% (●), 1.5% (∆), 3.0% (▲), and 4.5% (x), on shear strength.
Fig. 20, The effect of pH at various concentrations of salt, 0% (o), 0.45% (●), 1.5% (△), 3.0% (▲), and 4.5% (x), on shear strength.
Fig. 21, The effect of pH at various concentrations of combined solutions, 0% (O), 0.45% (D), 1.5% (A), 3.0% (△), and 4.5% (X) of salt, on shear strength.
Fig. 22. The effect of various concentrations of sodium acid pyrophosphate (○), sodium tripolyphosphate (Δ), salt (×), and combined solution (●) on shear strength at pH 4.5.
Fig. 23, The effect of various concentrations of sodium acid Pyrophosphate (○), sodium tripolyphosphate (△), salt (×), and combined solution (●) on shear strength at pH 5.5.
Fig. 24 The effect of various concentration of sodium acid pyrophosphate (O), sodium tripolyphosphate (△), salt (X) and combined solution (●) on shear strength at pH 6.5.
for any type of commercial application due to economics.

Odor of the samples treated with the combined solutions and sodium tripolyphosphate alone were found superior by panel members compared to NaCl and sodium acid pyrophosphate treated samples, however; as can be seen in Table 4 all treatments had lower odor scores than the control's.

Apparently there was little difference in flavor between treated samples and the control. Panel members found that the combined solution resulted in slight improved flavor over the control as well as the other treatments.

Sodium pyrophosphate treated sample had considerable higher scores for color than other treatments or controls. Samples other than sodium acid pyrophosphate treated turned slightly blue during processing. Blueing does not occur in uncooked crab meat. If blueing occurs at all, it is present after cooking rather than before. Fullers and Harris (1940) and Dassow (1963) stated that the blueing in crab meat is due to the interaction of the copper pigment (hemocyanin) in crab blood with ammonia and sulfur compounds formed from the breakdown of the proteins during heat processing. The oxidation of these compounds results in the characteristic blue color in the meat. Sodium acid pyrophosphate is a good sequestering agent and evidently combined with copper to prevent or retard the blueing effect.

The treatment employing the combined solution was judged by the panel members to have the best overall quality. Sodium chloride treated sample were also judged to be slightly superior than the control whereas sodium acid pyrophosphate treatment was judged the poorest by panel members.
<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Combined Solution</th>
<th>bSTP</th>
<th>cNaCl</th>
<th>dSAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odor</td>
<td>4.6 ± 0.2</td>
<td>4.0 ± 0.2</td>
<td>4.0 ± 0.2</td>
<td>3.7 ± 0.3</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td>Flavor</td>
<td>3.7 ± 0.2</td>
<td>4.3 ± 0.2</td>
<td>3.6 ± 0.2</td>
<td>3.7 ± 0.2</td>
<td>3.7 ± 0.4</td>
</tr>
<tr>
<td>Color</td>
<td>3.8 ± 0.3</td>
<td>3.4 ± 0.3</td>
<td>3.5 ± 0.3</td>
<td>3.9 ± 0.2</td>
<td>5.5 ± 0.2</td>
</tr>
<tr>
<td>Overall Quality</td>
<td>3.6 ± 0.2</td>
<td>4.3 ± 0.2</td>
<td>3.5 ± 0.2</td>
<td>3.9 ± 0.3</td>
<td>3.2 ± 0.3</td>
</tr>
</tbody>
</table>

- Excellent: 6
- Fair: 3
- Very Good: 5
- Poor: 2
- Good: 4
- Very Poor: 1

*a1.50% NaCl, 0.45% sodium acid pyrophosphate and 0.63% sodium tripolyphosphate

*b0.45% Sodium tripolyphosphate

*c0.45% NaCl

*d4.50% sodium acid pyrophosphate
SUMMARY AND CONCLUSION

The effect of pH and various levels of NaCl, sodium acid pyrophosphate and sodium tripolyphosphate on cooking loss, moisture retention and binding ability of crab meat were studied.

At the isoelectric point (pH 4.5) the sodium tripolyphosphate, sodium acid pyrophosphate and the combination treatments showed considerable greater cooking loss than control sample, however; all levels of NaCl treated samples had less cooking than the control. At pH 5.5, the phosphate as well as NaCl tended to reduce cooking loss in the crab meat loaves. This effect is due to the greater electrostatic repulsion between protein molecules at pH 5.5 which was also facilitated by NaCl and polyphosphate breaking cross-linking bonds between proteins. At pH 6.5, the structure of meat loaves were too loose in which to hold water resulting in high cooking loss and less moisture retention. Samples at pH 5.5 displayed higher moisture retention and less cooking loss than the other two pH levels. In general, lower cooking losses occurred at the low reagent levels. Cooking loss was a better indicator of the changes brought about by the various NaCl and phosphate treatments than moisture retention.

The shear strength of crab meat treated by sodium acid pyrophosphate, sodium tripolyphosphate and NaCl were markedly increased compared to control sample. Samples at pH 5.5 had lower shearing tests but higher moisture retention than the other two pH levels tested.

According to the panel test, combined solution which contains 1.5% NaCl, 0.45% sodium acid pyrophosphate and 0.63% sodium
tripolyphosphate at pH 5.5 were more efficient in obtaining a good overall quality for recombined crab meat as well as minimizing objectionable flavors.
LITERATURE CITED


Cheng-nan Lai was born on June 26, 1939, at Tainan Hsien, Taiwan. He attended elementary school at Chia-yi, Taiwan and graduated from Taiwan Provincial Chia-yi High School in July, 1958. His undergraduate training was obtained at Chung-Hsing University, Taiwan from which he graduated in July, 1964 with a B. S. degree in Agricultural Chemistry. He came to study at Tuskegee Institute, Alabama for an advanced degree in September 1967. He received the Master of Science degree in Food Science in January 1969, from Tuskegee Institute. In January 1969, he was accepted as a graduate research assistant in the Food Science Department of Louisiana State University. He is currently a candidate for the Degree of Doctor of Philosophy.
Candidate: CHENG-NAN LAI

Major Field: Food Science

Title of Thesis: The Influence of Polyphosphate, Sodium Chloride and Hydrogen Ion Concentration on Heat Mediated Binding of Crab Meat.

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination: November 8, 1972