Properties, Translucence, and Microstructure of Pacific White Shrimp Treated with Mixed Phosphates as Affected by Freshness and Deveining

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ABSTRACT: Effects of freshness and deveining on some properties, translucence, and microstructure of Pacific white shrimp (Litopenaeus vannamei) soaked in 2.5% NaCl containing different phosphates were studied. Shrimp soaked in all solutions had increases in weight gain and cooking yield with lowered cooking loss, compared with the control (P < 0.05). However, efficacy of mixed phosphates in quality improvement of ice-stored shrimp was lower than fresh shrimp. Deveining resulted in increased weight gain and yield (P < 0.05). Nevertheless, samples treated with phosphates became more translucent. Shrimp stored in ice for 7 d and treated with mixed phosphates were generally more translucent than fresh counterparts (P < 0.05). Shrimp soaked in 2.5% NaCl containing 0.875% sodium acid pyrophosphate (SAPP) and 2.625% tetrasodium pyrophosphate (TSPP) were generally less translucent and had high weight gain and cooking yield along with low cooking loss. The microstructure study revealed that the muscle fibers were less attached with the loss of Z-disks after being treated with mixed phosphates. Cooked meats of fresh shrimp and ice-stored shrimp had more compact fiber arrangement with the shrinkage of sarcomere compared with raw samples. Disintegration was observed at the M-line in ice-stored shrimp treated with mixed phosphates after cooking, while such a phenomenon was not found in the cooked fresh sample treated with phosphates. T_{max} and enthalpy of both myosin and actin peaks shifted to lower values when shrimp were treated with mixed phosphates (P < 0.05). Those changes were generally more pronounced in ice-stored shrimp. Therefore, freshness and deveining process had an impact on the quality of Pacific white shrimp treated with phosphates.

Keywords: ice storage, microstructure, mixed phosphates, Pacific white shrimp, thermal denaturation

Introduction

Thailand has exported seafood and seafood products to different countries in America, Europe, and Asia. In 2005, Thailand exported 279347 tons of shrimp and shrimp products with a value of 1765 million U.S. dollars. Among the products, frozen shrimp and shrimp products accounted for 57.21% and the remainders are processed seafood products (The Customs Dept. 2006). The seafood industry of Thailand is well known for its long-standing excellent reputation worldwide, owing to its outstanding characteristics of quality, freshness, variety, and taste. To maintain the quality of seafoods, some additives have been widely used. The ability of muscle to absorb added water during processing and retain it after cooking is an important functionality, since moisture content influences meat juiciness, tenderness, and mouthfeel (Ogawa and others 1994).

Phosphates have been widely accepted as potential additives in fish and seafood to improve the functional properties of those products by increasing water retention in fresh fish, reducing thaw loss in frozen fish, modifying texture, yielding better color, and reducing cooking loss (Dziezak 1990; Chang and Regenstein 1997). Young and others (1987) noted that salt in combination with phosphates have a synergistic effect in improving water holding capacity and cooking yield. However, these additives can affect the thermal stability of the protein. Robe and Xiong (1992) reported that the addition of ortho-, pyro-, tripoly-, and hexametaphosphate up to 1% altered transition temperatures of salt-soluble proteins. Phosphates may also be applied to shrimp by soaking or by vacuum tumbling. Treating small peeled and deveined shrimp should be of concern due to their tendency to be overtreated (Henson and Kowalewski 1992). Overtreatment generally results in the formation of a translucent and slimy texture. Therefore, much attention has been paid to minimize the translucence as well as improve the quality of Pacific white shrimp treated with phosphates. However, no information on the uses of mixed phosphates on the properties of shrimp meat as well as the influences of freshness and deveining on quality improvement of shrimp by phosphates has been reported. The purposes of this investigation were to elucidate the changes in properties, thermal behavior, and microstructure of Pacific white shrimp treated with mixed phosphates as influenced by shrimp freshness and deveining process.

Materials and Methods

Chemicals

Tetrasodium pyrophosphate (TSPP), sodium hexametaphosphate (SHMP), and sodium dodecyl sulfate (SDS) were obtained from Ajax Finechem (Wellington, Auckland, New Zealand). Sodium
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triopolyphosphate (STPP), sodium acid pyrophosphate (SAPP), β-mercaptoethanol (βME), acrylamide, N,N,N',N'-tetramethyl-ethylenediamide (TEMED), and bis-acrylamide were procured from Fluka (Buchs, Switzerland). Glutaraldehyde and Coomassie Brilliant Blue R-250 were purchased from Sigma (St. Louis, Mo., U.S.A.). Sodium chloride was obtained from Merck (Darmstadt, Germany).

Sample preparation
Pacific white shrimp (Litopenaeus vannamei) with the size of 60 shrimp per kg were obtained from a farm in Songkhla province, Thailand. Three different batches of shrimp were used for each experiment. Shrimp were kept in ice with a shrimp/ice ratio 1:2 (w/w) and transported to the Dept. of Food Technology, Prince of Songkla University, Thailand, within 1 h. Upon arrival, shrimp were washed with clean water and were separated into 2 groups, (1) fresh samples and (2) samples stored in ice for 7 d. For the 2nd group, samples were kept in a styrene foam box containing crushed ice, with a shrimp/ice ratio of 1:2 (w/w) for 7 d. Molten ice was removed and replaced with an equal amount of ice every 2 d. The boxes containing samples and ice were kept at room temperature (28 to 30 °C), K-values determined by the method of Uchiyama and Kakuda 1984 of fresh and ice-stored shrimp were 0.7% and 38.5%, respectively. Before phosphate treatment, both fresh and ice-stored shrimp were washed with clean water and deheaded; the shells were then peeled off. The shrimp were either deveined or nondeveined.

Effects of different phosphates or mixed phosphates on properties of white shrimp
Fresh and 7 d ice-stored shrimp, both deveined and nondeveined, were soaked in 2.5% NaCl in the absence or in the presence of different phosphates, including (1) 3.5% TSPP (2) 0.875% SAPP and 2.625% STPP; (3) 3.5% STPP; (4) 0.875% SAPP and 2.625% STPP for 2 h at 4 °C. Subsequently, the treated samples were drained at 4 °C for 5 min. Both soaking solutions and resulting shrimp were subjected to analyses. All analyses were conducted in triplicate.

Determination of transluence
Sensory evaluation was carried out by 10 trained panelists. Trainings of 5 sessions (2 h each) were performed using the different references: (1) 7 d ice-stored shrimp soaked in 3.5% TSPP for 10 h at 4 °C; (2) fresh shrimp soaked in 3.5% TSPP for 2 h at 4 °C, and (3) fresh shrimp steamed for 5 min, followed by cooling in iced water for 1 min and used as the references for training with the scale of 1, 3, and 5, respectively. Cooked shrimp were evaluated for transluence using a point structured scale with a value of 1 for very translucent, 3 for moderately translucent, and 5 for turbid or opaque. The opacity score was recorded. The higher values represent the higher opacity or less transluence. All samples were identified by a 3-digit code. Sensory testing was held in a clean, well-lighted, and well-ventilated room.

Determination of weight gain, cooking loss, and cooking yield
Weight gain was determined by weighing the shrimp before and after soaking in the solutions. After soaking, the samples were drained for 5 min at 4 °C. Weight gain was calculated as follows:

\[ \text{Weight gain (\%)} = \frac{(B - A)}{A} \times 100 \]

where \( A \) = initial weight (before soaking) and \( B \) = weight after soaking, followed by draining.

Cooking loss and cooking yield were measured by weighing the shrimp before and after steaming. Shrimp were cooked by steaming for 5 min, immediately cooled in iced water for 1 min, and drained at 4 °C for 5 min. Cooking loss and cooking yield were calculated as follows:

\[ \text{Cooking loss (\%)} = \frac{|(B - C)|}{B} \times 100 \]
\[ \text{Cooking yield (\%)} = \frac{(C/A) \times 100}{B} \]

where \( A \) = initial weight (without soaking and steaming), \( B \) = weight after soaking, followed by draining, and \( C \) = weight after steaming, followed by cooling in iced water.

Chemical analyses

Determination of moisture content. Shrimp were finely chopped prior to analyses. Moisture content was determined according to the method of AOAC (2000). The analyses were carried out in triplicate.

Determination of phosphate content. Sample (10 g) was mixed with 20 mL of 10% trichloroacetic acid (TCA). The mixture was homogenized at a speed of 6500 rpm using an Ultra Turrax homogenizer (IKA Labortechnik, Selangor, Malaysia) for 5 min. The homogenate was filtered using Whatman No. 1 filter paper and the sediment was rinsed with 10 mL of 10% TCA. The filtrate obtained was used for analysis according to the method of Fiske and Subbarow (1925). The analysis was performed in triplicate.

Determination of salt content. Salt content was determined by the method of AOAC (2000). Sample (1 g) was added with 10 mL of 0.1 N AgNO\(_3\) and 10 mL of conc. HNO\(_3\). The mixture was boiled gently on a hot plate until all samples except AgCl\(_2\) were dissolved. The mixture was then cooled using running water. Then 50 mL of distilled water and 5 mL of 5% ferric alum (FeNH\(_4\)(SO\(_4\))\(_2\).12H\(_2\)O) indicator were added. The mixture was titrated with standardized 0.1 N KSCN until the solution became a permanent brownish-red. The analysis was conducted in triplicate. The salt content was then calculated as follows.

\[ \text{Salt (\%)} = 5.8 \times \frac{(V_1 \times N_1) - (V_2 \times N_2)}{W} \]

where \( V_1 \) = volume of AgNO\(_3\) (mL); \( N_1 \) = concentration of AgNO\(_3\) (N); \( V_2 \) = volume of KSCN (mL); \( N_2 \) = concentration of KSCN (N); and \( W \) = weight of sample (g).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)
SDS-polyacrylamide gel electrophoresis was carried out according to the method of Laemmli (1970). Soaking solution (20 mL) was mixed with 10 mL of 10% (w/v) SDS solution. The mixture was then homogenized using a homogenizer (IKA Labortechnik). The homogenate was incubated in a water bath (85 °C) for 1 h to dissolve the proteins, followed by centrifuging to remove undissolved debris. The sample with a protein content of 15 mg, determined by the Biuret method (Robinson and Hodgson 1940) using bovine serum albumin standard, was loaded onto the gel. After separation by SDS-PAGE made of 4% stacking gel and 10% separating gel using 15 mA/plate, proteins were fixed and stained for 3 h in 0.125% Coomassie Brilliant Blue R-250 in 40% methanol and 10% glacial acetic acid. Gels were destained for 15 min with destaining solution I (50% methanol and 7.5% glacial acetic acid) and with the destaining solution II (5% methanol and 7.5% glacial acetic acid) for 3 h.
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Differential scanning colorimetry (DSC)

Thermal transition of proteins of shrimp meat without and with phosphate treatment was measured using the differential scanning calorimetry (DSC) (Perkin-Elmer, Model DSCM, Norwalk, Conn., U.S.A.). The samples (15 to 20 mg wet weight) were placed in the DSC hermetic pans, assuring a good contact between the sample and the pan bottom. An empty hermetic pan was used as a reference. The samples were scanned at 10 °C/min over the range of 20 to 100 °C. \( T_{\text{max}} \) was measured and the denaturation enthalpies (\( \Delta H \)) were estimated by measuring the area under the DSC transition curve. The analysis was run in triplicate.

Scanning electron microscopy (SEM)

Raw and cooked shrimp without and with phosphate treatment were subjected to SEM analysis as described by Jones and Mandigo (1982). Shrimp meat (second segment) was cut into a cube (4 × 4 × 4 mm) with a razor blade. The prepared sample was fixed in 2.5% glutaraldehyde in 0.2 M phosphate buffer, pH 7.2 at room temperature for 2 h. All specimens were washed with deionized water for 15 min and washing was repeated twice. The samples were then dehydrated with a serial concentration of 50% to 100% ethanol for 15 min each. All specimens were coated with 100% gold (Sputter coater SPI-Module, Pa., U.S.A.). The microstructure was visualized using a scanning electron microscopy (JEOL, JSM-5800 LV, Tokyo, Japan).

Statistical analysis

CRD (completely randomized design) was used. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan’s multiple range test (DMRT) (Steel and Torrie 1980). Statistical analyses were performed using SPSS 11.0 for Windows (SPSS Inc., Chicago, Ill., U.S.A.).

Results and Discussion

Effects of phosphates and mixed phosphates on the physical properties of fresh and ice-stored shrimp with and without deveining

Cooked fresh and ice-stored shrimp without phosphate treatment had a similar opacity score, regardless of deveining. After being treated with 2.5% NaCl, all samples became more translucent, as evidenced by the lower values (Figure 1). Translucence markedly increased with the treatment of phosphates, as indicated by the decrease in opacity score. In general, cooked ice-stored shrimp treated with all phosphates or mixed phosphates were more translucent than fresh shrimp soaked in the same mixed phosphate solution. Deveining had no impact on translucence, regardless of shrimp freshness. For the fresh shrimp, the solution containing both TSPP and SAPP yielded the less translucent shrimp when compared with the solution without SAPP (\( P < 0.05 \)). Nevertheless, the translucence of fresh shrimp treated with STPP in combination with SAPP was similar to that of sample treated with STPP (\( P > 0.05 \)). The use of SAPP in combination with TSPP or STPP had no impact on the translucence of ice-stored shrimp (\( P > 0.05 \)). When SAPP was used in combination with TSPP and STPP, the pH of solution (7.0 to 7.2) was lower than that of TSPP (10.0) and STPP (8.6). This might lower the repulsion force associated with very alkaline pH of phosphate solution. As a consequence, solubilization of muscle proteins could be decreased. It was postulated that the solubilized muscle proteins might undergo aggregation to form the ordered network, which most likely yielded the gel-like structure with greater translucence. To reduce the translucence caused by phosphate treatments, SAPP might be used in combination with other phosphates.

Weight gains of shrimp soaked in 2.5% NaCl or 2.5% NaCl in combination with different phosphates are shown in Figure 2. Treatment with TSPP or STPP yielded the fresh deveined shrimp with the highest weight gain (\( P < 0.05 \)). The higher weight gain was observed in whole fresh shrimp treated with TSPP, compared with those treated with STPP (\( P < 0.05 \)). For ice-stored shrimp, no differences in weight gain were found between those treated with TSPP and STPP, irrespective of deveining (\( P > 0.05 \)). In the presence of SAPP, the lower weight gain was generally observed. Xiong and others (2000) reported that pyrophosphate and tripolyphosphate were able to promote protein extraction, leading to the improved hydration properties of chicken muscle. Overall, phosphates influenced the ultrastructure of myofibrils and extraction of their constituents in the order PP > TPP > HMP > P ~ non-phosphate control (Xiong and others 2000). The deveined shrimp possessed the greater weight gain, regardless of freshness or phosphates used. Deveining might allow phosphates to penetrate or

![Figure 1](image-url)
Freshness, deveining affect phosphate-treated Pacific white shrimp.

Adsorb into shrimp muscle easily. This could enhance water absorption in the shrimp muscle. After phosphate treatments, weight gain was lower in shrimp stored in ice for 7 days compared with fresh shrimp (Figure 2A). This indicated that freshness, which is related to muscle integrity, was another factor governing the efficacy of phosphate in increasing the yield of treated shrimp. Moisture content of ice-stored shrimp was higher than that of fresh shrimp (P < 0.05) (Figure 2B). During storage in ice, some ice had melted and shrimp were immersed. As a consequence, the water was taken up into shrimp muscle to a high extent, as indicated by the increased moisture content. Deveining mostly had no impact on moisture content of shrimp meat (P > 0.05). However, ice-stored shrimp with deveining had higher moisture content after treatment with 3.5% TSPP and 2.5% NaCl compared with nondeveined shrimp (P < 0.05). Deveining also resulted in an increase in moisture content of fresh shrimp treated with 3.5% STPP together with 2.5% NaCl (P < 0.05). Deveined shrimp had a larger surface area than did nondeveined shrimp. As a result, phosphates as well as water were able to penetrate more easily into shrimp muscle.

Soaking shrimp, either fresh or ice-stored, in phosphate solutions resulted in increased cooking yield and lowered cooking loss compared with the samples without phosphate treatment (P < 0.05, Figure 3). In general, lower cooking yield was obtained in ice-stored shrimp compared with fresh shrimp (P < 0.05). For the sample treated with 2.5% NaCl, the increase in cooking yield was noticeable with ice-stored shrimp (P < 0.05), but it had no effect on the cooking yield of the fresh shrimp (P > 0.05). A slight increase in cooking yield was found in fresh deveened sample treated with 2.5% NaCl compared with nondeveened fresh shrimp (P < 0.05). With the treatment of phosphate in combination with 2.5% NaCl, cooking yield was much increased for both deveened and nondeveened shrimp, particularly for ice-stored shrimp, compared with samples treated with NaCl alone or without any treatment (P < 0.05). Deveining process slightly increased the cooking yield. This might be associated with the higher phosphates adsorbed in the shrimp muscle. Fresh shrimp without and with 2.5% NaCl treatment had the lower cooking loss than ice-stored shrimp (P < 0.05). However, cooking loss was lower after the treatment of 2.5% NaCl together with phosphates. Deveining generally resulted in lower cooking loss of shrimp. Deveining could allow the phosphate or NaCl to penetrate into the shrimp muscle more easily. The use of SAPP in combination of STPP or TSPP rendered the shrimp with slightly lower cooking yield but higher cooking loss compared with the use of STPP or TSPP alone. The results were coincidental with the lower weight gain when SAPP was used in combination with STPP or TSPP. Shrimp soaked in the mixture of TSPP and SAPP had higher cooking yield but lower cooking loss than those soaked in ice-stored shrimp.
in the mixture of STPP and SAPP solution ($P < 0.05$, Figure 3A and 3B). The lower cooking loss and higher cooking yield of the shrimp treated with phosphates indicated that the shrimp muscle had a higher water holding capacity even after cooking. Water molecules might be bound tightly with phosphate or proteins via ionic interaction. Froning and Sackett (1985) reported that use of salt in combination with phosphates had a synergistic effect on tumbling turkey breast muscle to reduce cooking loss and expressible moisture. Xiong and Kupski (1999) found that salt would produce a synergism with phosphate to dissociate actomyosin in chicken fillets.

**Effects of phosphates and mixed phosphates on the chemical composition of fresh and ice-stored shrimp with and without deveining**

Phosphate content (dry basis) and salt content (dry basis) of fresh shrimp and ice-stored shrimp without and with deveining after treatment with different solutions are shown in Figure 4. For the samples treated with phosphates, it was noted that the higher phosphate content was found in ice-stored shrimp, particularly deveined samples ($P < 0.05$). During iced storage, endogenous and bacterial enzymes might be involved in the degradation of shrimp tissues and structure (Martinez and others 2001). Endogenous proteolytic enzymes, including calpain and lysosomal proteases, are certainly related to changes in myofibrillar proteins or collagen, which cause the loosening of the myofibrillar structure (Etherington 1984; Peterson and others 1988). Phosphate content of deveined ice-stored shrimp was higher than nondeveined counterpart ($P < 0.05$), except for samples treated with TSPP together with SAPP, in which deveining had no effect on phosphate content. For fresh shrimp, deveined shrimp treated with TSPP or STPP in combination with SAPP showed the higher phosphate content than nondeveined counterpart ($P < 0.05$). Deveined shrimp tended to have a slightly higher cooking yield, but lower cooking loss (Figure 3). This might be associated with the greater penetration of phosphate into the meat. However, phosphate contents in shrimp were less than the standard value (5000 ppm) (Official Journal of the European Communities 1995).

After soaking in 2.5% NaCl, either without or with phosphates, the increase in salt content was noticeable in shrimp, regardless of freshness and deveining ($P < 0.05$, Figure 4). In general, NaCl content was higher in ice-stored shrimp compared with the fresh counterpart. The impact of deveining on the salt content varied from sample to sample. NaCl has been used in combination with phosphate in order to obtain the synergistic effect on quality improvement. Shrimp, both fresh and ice stored, soaked in 2.5% NaCl containing mixed phosphates (0.875% SAPP and 2.625% TSPP) had a decrease in cooking loss, an increase in cooking yield, and lower

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**Figure 3** — Cooking yield (A) and cooking loss (B) of fresh and ice-stored Pacific white shrimp without and with deveining after soaking in 2.5% NaCl in the absence and the presence of different phosphates. The different lowercase letters within the same treatment indicate significant difference ($P < 0.05$). The different uppercase letters within the same sample indicated significant difference ($P < 0.05$). Bars represent the standard deviation from 5 determinations. □ non-deveined, 0 day ■ deveined, 0 day □ non-deveined, 7 days □ deveined, 7 days.
translucence. Nevertheless, efficacy of mixed phosphates in quality improvement was lower for ice-stored shrimp.

Effect of phosphates and mixed phosphates on protein patterns of soaking solution of fresh and ice-stored shrimp with and without deveining

Protein patterns of different solutions obtained after soaking with shrimp for 2 h are shown in Figure 5. For fresh shrimp, all solutions contained protein bands with MW of 88 and 77 kDa with similar band intensity. Actin was also found in all solutions. However, band intensity of myosin heavy chain (MHC) in all phosphate solutions was greater than that found in 2.5% NaCl. For deveined fresh shrimp, a slightly larger band of MHC was noticeable. The result suggested that more proteins, particularly MHC, were solubilized and leached out for deveined samples. The increases in MHC band intensity correlated with the increases in weight gain and cooking yield (Figure 2 and 3). For ice-stored shrimp, the protein patterns of different soaking solution were observed, compared with those found in fresh shrimp. Much lower band intensity of MHC and proteins with MW of 88 and 77 kDa was found in all solutions. The decrease in band intensity of those proteins might be associated with degradation of protein during iced storage. Nevertheless, no changes in actin were observed. Actin was reported as the most resistant to degradation caused by either endogenous or microbial proteinase (Benjakul and others 1997). Proteolytic degradation of other cytosolic and cytoskeletal proteins present in the muscle caused by microbial growth and structural disintegration also occurred during ice storage of fish (Priacanthus tayenus and P. macracanthus) (Benjakul and others 2002). No marked differences in protein patterns were obtained between all solutions used for soaking the deveined and nondeveined samples. MHC band intensity was greater in the solutions containing TSPP or STPP, regardless of SAPP addition. TSPP and STPP might facilitate protein extraction and dissociation myofibrillar protein due to the ionic effect and pH alteration. Use of higher NaCl concentrations (0.1 to 1.0 M) increased the extraction of myofibrillar proteins from beef tissue, and the inclusion of 10 mM TSPP to NaCl solutions enhanced the extraction of myofibrillar proteins (Xiong and Kupski 1999). Increased myofibrillar proteins extraction was associated with increased beef myofibril swelling and increased beef muscle WHC (Paterson and others 1988).
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**Effects of mixed phosphates on microstructure of fresh and ice-stored shrimp with and without deveining**

Microstructures of Pacific white shrimp muscle treated with and without mixed phosphates are illustrated in Figure 6 and 7, respectively. Fresh shrimp without phosphate treatment had a well-organized structure of the myofibrils. After 7 d of ice storage, the myofibrils were less attached with the loss of Z-disks. After being soaked in mixed phosphates, myofibrils became larger in size. However, myofibrils were less attached as indicated by gaping. The shrinkage of sarcomere was obvious in cooked shrimp. It was suggested that the heating process caused the shrinkage of muscle of shrimp. Heat processing enhanced the firmness and degree of shrinkage of *Penaeus japonicus* (Mizuta and others 1999). After cooking, both fresh shrimp and ice-stored shrimp had more compact myofibril arrangement with the shrinkage of sarcomere compared with raw samples. For fresh shrimp, similar myofibril arrangement was observed between samples with and without phosphate treatment. Interestingly, disintegration of M-line was clearly observed in ice-stored shrimp treated with phosphates after cooking. During ice-chilling of whole freshwater prawn, the muscle fibers at the anterior-most sections were degraded gradually (Nip and Moy 1988). Degradation of shrimp tissue started from the perimysium, endomysium, the Z line, and the H zones with concurrent degradation of the connective fibers and the sarcoplasm due to action of hepatopancreatic enzymes (Baranowski and others 1984; Nip and others 1985). The postmortem degradation might facilitate the penetration of phosphates into the muscle, in which proteins at M-line might be solubilized or removed by phosphates. Proteins associated with M-line are M-protein, myomesin, and creatine kinase (Pearson and Young 1989). Myomesin in M-line was successfully extracted with the aid of Na-pyrophosphate (Masaki and Takaiti 1972).

For the transverse sections (Figure 7), similar microstructures of white shrimp were found. Dense structure was noticeable in fresh shrimp, while sponge-like structure was found in ice-stored shrimp. Cooked meats had more compact myofibril arrangements, compared with raw samples. Treated shrimp with mixed phosphate had looser structure than those without phosphate treatment. For phosphate treated shrimp, less attachment was found for fresh shrimp. Conversely, increased compact structure was observed in ice-stored shrimp after phosphate treatment. When the proteins underwent thermal denaturation, the water was less imbibed or bound in their structure. The release of water from protein molecules might facilitate the myofibrils to align closely, leading to the more compact structure.

**Effects of mixed phosphates on thermal property of fresh and ice-stored shrimp with and without deveining**

Thermal transitions of muscle proteins of shrimp with and without phosphate treatment using DSC are shown in Table 1. A DSC thermogram of Pacific white shrimp meat revealed 2 major...
endothermic peaks with \( T_{\text{max}} \) of 50.1 and 71.3 °C, corresponding to myosin and actin peaks. Sriket and others (2007) reported that myosin from black tiger shrimp (\( T_{\text{max}} = 51.28 \) °C) and from white shrimp (\( T_{\text{max}} = 50.13 \) °C) had a similar temperature required for denaturation. \( T_{\text{max}} \) of actin of black tiger shrimp and white shrimp was 66.20 and 71.17 °C, respectively. Whole cod muscle showed 2 maximal transitions on a DSC thermogram with \( T_{\text{max}} \) at about 45 and 75 °C (Hastings and others 1985), and whole muscle of fresh hake also showed 2 endothermic transitions with \( T_{\text{max}} \) values of 46 and 75 °C (Beas and others 1990). The differences in \( T_{\text{max}} \) among the fish species seem to be correlated with the habitat temperature of the fish (Akahane and others 1985; Hastings and others 1985; Davies and others 1988).

After 7 d of storage in ice, \( T_{\text{max}} \) of both peaks shifted to the lower values. Additionally, \( \Delta H \) was also decreased. This suggested that both myosin and actin underwent denaturation to some extent during the iced storage. After being treated with mixed phosphate, \( T_{\text{max}} \) of both peaks (myosin and actin) of fresh shrimp shifted to the lower temperature. A lower enthalpy was observed for actin peaks after phosphate treatment. Torigai and Konno (1996) reported a promotive effect of pyrophosphate on the dissociation of actin from myosin. As a result, free actin was easily denatured.
by salt. Wu and others (1985) found that only the 1st peak shifted from 43 to 38 °C after addition of 1% salt and the 2nd peak and 3rd peak shifted to lower temperatures after addition of 2% to 3% salt. The addition of salt (3%) in tilapia or hake surimi caused a decrease in denaturation enthalpy and the shift of $T_{\text{max}}$ to the lower temperature (Beas and others 1991). The lower enthalpy of minci plus salt was probably due to the sensitizing effect of the CI anion upon myofibrillar protein denaturation (Beas and others 1991). 

Robe and Xiong (1992) found that addition of 0.25% triphosphate reduced or eliminated the 1st transition ($T_{\text{max}} = 47 ^\circ C$) and enhanced the 2nd transition ($T_{\text{max}} = 57 ^\circ C$) of salt soluble proteins. The disappearance or reduction of thermal transition caused by the addition of triphosphate probably resulted from a decreased thermal stability in the protein domains (Kijowski and Mack 1988). Triphosphate seemed to be more effective than NaCl in modulating the electrostatic forces in proteins, thereby altering salt soluble proteins aggregation pattern (Robe and Xiong 1992). Therefore, salt and pyrophosphate decreased the heat stability of Pacific white shrimp muscle proteins, leading to the denaturation at lower temperature with less energy input. From this result, it can be inferred that the destabilizing effect of salt and pyrophosphate on the shrimp proteins affects the properties of shrimp proteins after heating or cooking. Similar results were obtained in ice-stored shrimp, in which $T_{\text{max}}$ shifted to the lower after phosphate treatment. However, the increase in $\Delta H$ was noticeable in ice-stored shrimp treated with phosphates. Endogenous proteolytic enzymes, including calpain and lysosomal proteases, might partially degrade MHC, leading to the ease of denaturation, particularly after phosphate treatment.

**Conclusions**

Use of mixed phosphates led to quality improvement of both fresh and ice-stored shrimp. Shrimp, both fresh and ice stored, soaked in 2.5% NaCl containing mixed phosphates (0.875% SAPP and 2.625% TSPP) showed a decrease in cooking loss, an increase in cooking yield, and lower translucence. The efficacy of mixed phosphates in quality improvement was governed by the quality of shrimp. The greater translucence was found in shrimp with lower freshness after phosphate treatment, in which M-line was disappeared after heating. The further investigation on the impact of M-line disintegration on the translucence of shrimp should be conducted.

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