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Effect of Cryoprotectants on Suppression of Protein Structure Deterioration Induced by Freeze-thaw Cycle in Pacific White Shrimp

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ABSTRACT

This study aimed to elucidate the changes in Pacific white shrimp (*Litopenaeus vannamei*) myofibrillar protein as influenced by multiple freeze-thaw cycles as well as the stabilization effects of sucrose and trisodium citrate on shrimp myofibrils. Shrimp myofibrils in 0.1 M NaCl, 20 mM Tris-HCl (pH 7.5) were mixed individually with sucrose and citrate at concentrations of 0.05 M and were evaluated for Ca^{2+}-ATPase activity, salt solubility, total and reactive sulfhydryl, and surface hydrophobicity during three freeze-thaw cycles. Sucrose and citrate had strong cryoprotective effects against freeze denaturation by retaining higher Ca^{2+}-ATPase activity and salt-soluble myosin and actin, by slowing the reduction of reactive sulfhydryl (SH) and by exposing less hydrophobic groups at the surface of the protein compared with the no-additive sample. Results indicated that both cryoprotectants had suppressive effect against protein denaturation and helped stabilize white shrimp myofibrillar protein during the freeze-thaw process. This study suggests that sucrose and citrate stabilized the protein structure by retarding the unfolding of protein; thus, the native protein could be protected during frozen storage.

KEYWORDS

Ca^{2+}-ATPase activity; salt solubility; sucrose; citrate; freeze denaturation

Introduction

Currently, Pacific white shrimp (*Litopenaeus vannamei*) is the main shrimp species cultured in Thailand, and it has high value in commercial markets, especially as a frozen product (Sriket et al., 2007). Freezing is considered the best method for preserving shrimp for the highest quality; however, quality can still be affected during frozen storage, especially by the denaturation of protein (Benjakul et al., 2003; Boonsumrej et al., 2007; Sriket et al., 2007). Storage at low temperature can lead to aggregation of myofibrillar proteins, which are the most abundant protein of the shrimp myofibril (McCormick, 1994; Rupnow, 1992). Aggregation and/or alteration of protein structure may cause physicochemical changes and directly affect shrimp quality. Several cryoprotectants have been used to reduce alterations in fish myofibrils due to denaturation and aggregation during freezing and storage (Herrera and Mackie, 2004; Jiang et al., 1987; MacDonald and Lanier, 1994; Ooizumi et al., 1984). Sugars and sugar alcohols are used commercially as cryoprotectants in surimi (MacDonald and Lanier, 1994). Other compounds, such as sodium salts of carboxylic acids including lactate and trisodium citrate, have been found to stabilize fish proteins (Kuwahara and Konno, 2010; Ooizumi et al., 1984), while amino acids had protective effect on mackerel actomyosin denaturation during

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frozen storage (Jiang et al., 1987). Researchers (Jaczynski et al., 2006) have proposed that compounds exhibiting cryoprotective effects have to possess one essential group of the various functional groups, such as -COOH, -OH or $-OP_3H_2$, and more than one supplementary group of the type -COOH, -OH, NH_2, SH, $-SO_3H$, or $-OP_3H_2$. Furthermore, the functional groups must be suitably spaced and properly oriented about each other, and the molecule must be comparatively small. Previous reports (Jiang et al., 1987; Kuwahara and Konno, 2010; Ooizumi et al., 1984) also indicated that the cryoprotective capacity may be related to the number of such functional groups in the structure of the compounds. The number of hydroxyl groups in sugar that are available for hydrogen bonding with proteins may lead to a different degree of decreased aggregation or denaturation (Ooizumi et al., 1984). Also, the effectiveness of salt containing carboxylic groups to stabilize protein is proportional to the number of carboxyl groups in their structure (Kuwahara and Konno, 2010).

Most of the studies of cryoprotectants have focused on fish meat, especially for application in surimi. Meanwhile, many of the studies on shrimp quality during frozen storage have focused on shrimp meat rather than the shrimp myofibrillar protein. No basic information regarding the effect of the freeze-thaw process on shrimp myofibrils has been reported. Our objectives were to elucidate the changes of shrimp myofibrillar protein as influenced by multiple freeze-thaw cycles as well as the stabilization effects of sucrose and citrate on shrimp myofibrils.

Materials and methods

Materials and chemicals

Fresh shrimp, with the size of 60 shrimps/kg, were used for myofibril preparation. The reagent-grade additives included D-sucrose from Merck KGaA (Darmstadt, Germany) and D-sorbitol and citrate from Ajax Manufacturing Co. (Auckland, New Zealand). Bovine serum albumin, 2-mercaptoethanol, 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB) and N, N, N, N-tetramethyl ethylene diamine were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, US). Acrylamide and bis-acrylamide were purchased from Vivantis Technologies Sdn. Bhd. (Selangor Darul Ehsan, Malaysia). Adenosine 5′-triphosphate disodium salt was obtained from Oriental Yeast Co., Ltd. (Tokyo, Japan). Ammonium molybdate, ammonium persulfate, bromophenol blue (BPB) sodium salt, calcium chloride, maleic acid, perchloric acid, sodium dodecyl sulfate, and urea were purchased from Ajax Manufacturing Co. (Auckland, New Zealand). Potassium chloride was purchased from Merck KGaA (Darmstadt, Germany). Standard unstained protein marker (2–212 kDa) was produced by New England Biolabs Ltd. (Hertfordshire, UK).

Preparation of shrimp myofibril

Shrimp were deheaded, peeled, and deveined on a chilled surface. The connective tissue and pigment on the exterior surface were removed, and shrimp meat was finely chopped. Shrimp myofibrils were prepared according to the method of Jantakoson et al. (2013) with slight modifications. Firstly, chopped shrimp meat was washed with 10 volumes of chilled buffer 0.1 M NaCl, 20 mM Tris-HCl (pH 7.5) into a centrifuge tube, and stirred while standing for 15 min in ice-water bath. Then, the washed meat was centrifuged at 3,000 RPM (equivalent to 1,045 g) for 5 min at 4°C using a Hettich Universal 32 R refrigerated centrifuge equipped with a 1620A fixed angle rotor with radius of 104 mm for 85 mL tubes (Ø 38 × 106 mm) (Andreas Hettich GmbH and Co. KG, Tuttlingen, Germany). The washing and centrifugation were conducted twice. Next, the washed meat was homogenized in the solution for 30 s using a Polytron PT 10–35 homogenizer equipped with PTA 10S dispersing generator (Ø10 mm) (Kinematica AG, Lucerne, Switzerland) at 15,000 RPM, followed by a 30 s rest interval for a total of 4 times. Then, 5 µL of 1-octanol was added to dissolve the foam of protein. The solution was discarded from the homogenate after washing by centrifuging at 3,000 RPM for 5 min at 4°C. The homogenate was

twice washed with the same buffer and centrifuged. The pellets were then collected and suspended in 0.1 M NaCl, 20 mM Tris-HCl (pH 7.5). Finally, the suspension was filtered through two layers of cotton gauze and used as myofibril suspension stock. Protein concentration was determined using the biuret method (Gornall et al., 1949) using bovine serum albumin as a standard.

Preparation of treated samples

Shrimp myofibrils in 0.1 M NaCl, 20 mM Tris-HCl (pH 7.5) were mixed with sorbitol, sucrose, and citrate individually at the concentration of 0.05 M with a final concentration of 2.5 mg/mL protein for 20 min. Due to the sticky characteristic of myofibrils, specified volumes of myofibril suspensions were transferred into test tubes for individual analysis in order to obtain homogeneity of samples, especially after freeze-thaw (FT) cycles in which aggregation might be expected. Treated myofibril suspensions at volumes of 0.2 mL (for Ca^{2+}-ATPase activity assay), 0.72 mL (for salt solubility determination), 2 mL (for SH group content determination), and 1 mL (for surface hydrophobicity) in test tubes were kept in a Sanyo MDF-1155 freezer (Sanyo Electric Biomedical Co., Ltd., Tokyo, Japan) at −20 °C. The samples were taken out after 4 days of frozen storage and then thawed in an Eyela NCB-2600 water bath (Tokyo Rikakikai Co., Ltd., Tokyo, Japan) at 25 °C (until thawed) and refrozen, then repeated for a total of three FT cycles. At 0, 1, 2, and 3 cycles, treated myofibrils were selected to measure remaining Ca^{2+}-ATPase activity, salt solubility, total and reactive SH group content, and surface hydrophobicity.

Determination of ca^{2+}-atpase activity

Ca^{2+}-ATPase activity of myofibrils in each FT cycle was assayed in a buffer containing the reaction mixture of 0.5 M KCl, 25 mM Tris-maleate (pH 7.0), 1 mM ATP, and 5 mM $CaCl_2$ with a total volume of 2 mL. In the measurement of the Ca^{2+}-ATPase activity of myofibril with citrate, 15 mM $CaCl_2$ was used instead of 5 mM $CaCl_2$ in the reaction mixture (Kuwahara and Konno, 2010). The reaction was conducted at 25 °C for 20 min in an Eyela NCB-2600 water bath (Tokyo Rikakikai Co., Ltd., Tokyo, Japan) with myofibril concentration at about 0.25 mg/mL and was terminated by addition of 1 mL chilled 15% (w/v) perchloric acid. A blank was performed without incubation at 25°C. The inorganic phosphate liberated in the supernatant was measured, and specific activity was expressed as μmole inorganic phosphate (Pi) released/mg protein/min.

Determination of salt solubility

The salt solubility of myofibrils were determined using the method of Jantakoson et al. (2013). At each FT cycle, a series of three tubes of myofibril were taken from the freezer and placed in an Eyela NCB-2600 water bath (Tokyo Rikakikai Co., Ltd., Tokyo, Japan) at 25°C until thawed. The myofibril samples (2.5 mg/mL) in 0.1 M NaCl, 20 mM Tris-HCl (pH 7.5) were then adjusted to a final concentration of 2 mg/mL soluble protein in 0.5 M NaCl, 20 mM Tris-HCl (pH 7.5) and placed in an ice-water bath for 30 min before being measured separately. The first sample was kept as whole myofibrils without centrifugation. The second was centrifuged without ATP addition, while the third was treated with 2 mM Mg-ATP and left for 5 min to dissociate myosin from actin before centrifugation at 20,000 *g* for 20 min at 4°C using a Tomy Suprema 21 refrigerated centrifuge (Tomy Digital Biology Co., Ltd., Tokyo). Myosin and actin content in the supernatant were referred to as soluble myosin and soluble actin, respectively (Jantakoson et al., 2013). The dissolved myofibrils in the supernatant and whole myofibrils were assayed with an Atto AE-6500 Compact-PAGE apparatus (Atto Corporation, Tokyo, Japan), and the quantities were estimated by measuring the staining intensity of myosin heavy chain (MHC) band and actin band appearing on sodium dodecyl

sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970) with Image J software (version 1.41, NIH-Informer Technologies, Inc., Bethesda, MD, US).

Determination of total and reactive SH group content

Total SH group content of myofibrils at each FT cycle was determined using DTNB according to Ellman (1959) and Sompongse et al. (1996a) with a slight modification. To a 2 mL myofibril suspension (2.5 mg/mL) was added 18 mL of 8 M urea, 2% (w/v) SDS, and 10 mM EDTA in 0.2 M Tris-HCl buffer (pH 6.8). A 3 mL aliquot of the myofibril mixture was mixed with 0.3 mL of 0.1% (w/v) DTNB. The reaction mixture was incubated at 40 °C for 25 min using an Eyela NCB-2600 water bath (Tokyo Rikakikai Co., Ltd., Tokyo, Japan), and absorbance of the mixture was measured on a Shimadzu UV-700 spectrophotometer (Shimadzu Co., Kyoto, Japan) at 412 nm. Reactive or surface SH group content of myofibrils was determined according to the method of Sompongse et al. (1996b) with a slight modification in the same manner as the total SH group content except that the solvent used was urea-SDS free solution (pH 6.8) containing 0.6 M NaCl and reaction with DTNB was carried out at 15 °C for 1 h using a Eyela NCB-2600 water bath (Tokyo Rikakikai Co., Ltd., Tokyo, Japan). Total and reactive SH group content were calculated from the absorbance using a molar extinction coefficient of 13,600 M^{-1} cm^{-1} for 2-nitro-5-thiobenzoic acid at this wavelength and was expressed as mol/10^5 g protein.

Determination of surface hydrophobicity

Hydrophobicity of myofibrils at each FT cycle was determined using BPB sodium salt for electrophoresis as described by Chelh et al. (2006) with a modification. A 1 mL myofibril suspension (2.5 mg/mL) was mixed with 200 µL of 1 mg/mL BPB (in distilled water). The addition of the weak acid BPB slightly decreased the pH of the solution from 6 to 5.8. A control, without myofibrils, consisted of the addition of 200 µL of 1 mg/mL BPB (in distilled water) to 1 mL of 0.1 M NaCl, 20 mM Tris-HCl (pH 7.5). Samples and control were kept at room temperature for 10 min and then centrifuged at 2,000 *g* for 15 min using a Hettich Universal 32 R refrigerated centrifuge (Andreas Hettich GmbH and Co. KG, Tuttlingen, Germany). Absorbance of the supernatant (diluted 1/10) was measured at 595 nm and compared to a Tris-buffer blank. The amount of bound BPB was calculated as follows:

$$\text{BPB bound } (\mu g) = 200\ \mu g \times \frac{\text{Abs595 Control} - \text{Abs595 Sample}}{\text{Abs595 Control}},$$

where Abs_{595} is the absorbance at 595 nm.

Determination of microstructure of pacific white shrimp muscle

Fresh shrimp with the size of 60 shrimps/kg were kept in an ice bath (1:2 shrimp: ice) for 72–96 h in a refrigerator at 4 ± 1°C prior to study. The deveined, peeled, headless shrimp were treated by soaking in 6% (w/v) sucrose or 5% (w/v) citrate, with a shrimp: solution ratio of 1:2 (w/v) for 6 h at 4 ± 1°C. Samples with and without additives were packaged in zip lock bags, and kept frozen at −60° C in a Sanyo MDF-1155 freezer (Sanyo Electric Co., Ltd., Osaka, Japan) for 2 h, then stored at −20°C until thawing. The frozen shrimp were thawed with running water (22–25°C) for 20 min until the core temperature reached 0–2°C.

For microstructure observation, the thawing of samples was done every day for 0 and 3 cycles. The structure of fresh and thawed shrimps was determined by microscopy. Samples were prepared from the 3rd abdominal segment into cylindrical frozen section compounds. The embedded samples were sliced with a Leica CM1850 Cryostat Microtome (Leica Biosystems Nussloch GmbH, Nussloch, Germany) at −20°C into 5 µm thick sections, and the sliced specimens were stained with 1% Eosin Y

(Hagiwara et al., 2003). The longitudinal sections were placed under an Olympus BX51 microscope (Olympus Co., Tokyo, Japan) connected to an Olympus DP27 digital camera (Olympus Co., Tokyo, Japan) and then visualized with 200x magnification. Light micrographs were scanned using DP2-BSW software (version 2.1, Olympus Co., Ltd., Tokyo, Japan).

Statistical analysis

All tests were conducted with three replicates, and values were expressed as the mean ± standard deviation. Statistical analyses were performed using SPSS for Windows (version 11.5, SPSS Inc., Chicago, IL, US). Differences between means were evaluated by univariate analysis of variance (ANOVA) and compared by Duncan's new multiple range test (DMRT) at the 5% significance level ($P < 0.05$).

Results and discussion

Effects of cryoprotectants on ca^{2+}-atpase activity of shrimp myofibrils during the FT cycle

The remaining Ca^{2+}-ATPase activity of myofibrils mixed with food additives during 3 FT cycles was evaluated and expressed as μmole Pi/mg protein/min as shown in Figure 1. Ca^{2+}-ATPase activity has been shown to be an effective indicator of myofibrillar protein denaturation at low temperature storage (Park and Lin, 2005). The freeze-thaw process used in this study was aimed to induce severe denaturation. A sharp rate of decrease in Ca^{2+}-ATPase activity was observed for the control samples (no additives) at the first FT cycle and decreased to less than 10% after 3 FT cycles. This indicated the denaturation of protein, which might result from conformational changes of myosin heads as well as the aggregation of this myosin head portion (Chantarasuwan et al., 2011; Godiksen et al., 2003). Since the Ca^{2+}-ATPase activity can be used as a primary indicator of the integrity of myosin molecules, the loss of Ca^{2+}-ATPase activity was possibly due to the tertiary structural changes of myosin heads as well as the aggregation of protein caused by ice crystals and the increase in ionic strength of the system (Benjakul et al., 1997; Okada et al., 1986; Sriket et al., 2007). The formation of

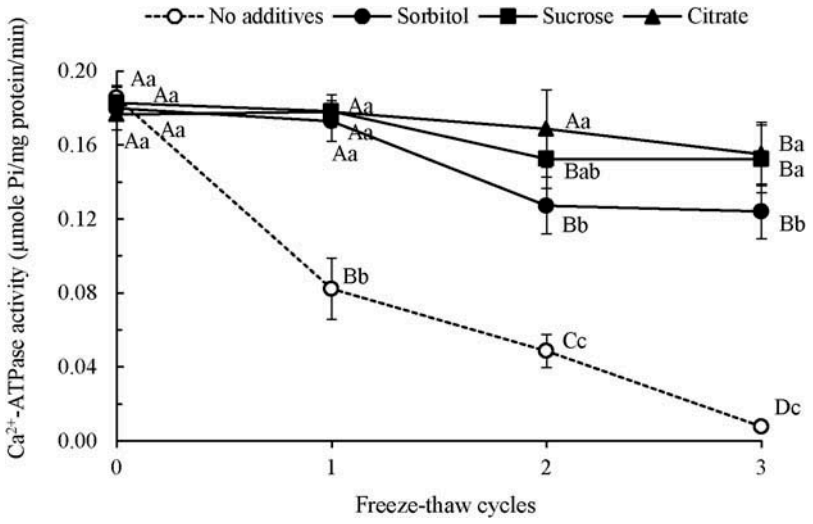

Figure 1. Effect of cryoprotectants on Ca2+-ATPase activity of shrimp myofibrils during the freeze-thaw (FT) cycles. Values are means of triplicate determinations ± standard deviation. Different uppercase letters (A-D) indicate significant differences between FT cycles for the same additive, and different lowercase letters (a-c) indicate significant differences between additives at the same FT cycle ($P < 0.05$).

ice crystals from either intracellular or extracellular water can result in mechanical damage caused by irregular ice crystals and by disrupting cell activities (Xiong, 1997). As crystallization proceeds, extracellular solutes become more concentrated, and in an attempt to balance the chemical potential, intracellular moisture flows outward, leading to dehydration and an increase in the ionic strength of the cell. Water drawn from the interior of the cell will freeze onto the existing extracellular ice crystals, causing them to grow, thus distorting and damaging the membrane and protein. This can cause structural changes in myofibrillar proteins, subsequently causing protein denaturation and aggregation (Love, 1968; Roy et al., 2012). The rearrangement of protein due to protein-protein interactions has also been presumed to contribute to the loss in activity (Benjakul and Bauer, 2000). Therefore, the decrease in the Ca^{2+}-ATPase activity of myofibrils without additives during the multiple freeze-thaw process indicated that myosin underwent denaturation.

Similarly, it was found previously that the Ca^{2+}-ATPase activity of triggerfish surimi protein decreased with an increase of the freezing-thawing from 0 to 3 cycles (Ai-Mei et al., 2004); this was also found for common carp surimi (Guo et al., 2014; Kong et al., 2013). A similar decrease was shown in natural actomyosin extracted from white shrimp muscle when subjected to five cycles of freezing-thawing (Sriket et al., 2007). Conversely, the activity of Ca^{2+}-ATPase was maintained when treated with each of the food additives studied. Sucrose and citrate seem to have better cryoprotective effects on shrimp myofibrillar protein than the sorbitol, as sucrose and citrate samples retained high Ca^{2+}-ATPase activity (90–95%), while almost 80% activity was observed for the sorbitol sample at the three FT cycles (Figure 1). Different cryoprotective effects of sugars may be attributed to the number of hydroxyl groups available for hydrogen bonding with proteins, leading to differing degrees of reduced aggregation or denaturation (Ooizumi et al., 1984). Sucrose contains eight hydroxyl groups, while sorbitol has six hydroxyl groups. Apart from the number of hydroxyl groups, we also suggest that the different suppressive effects on myofibrils may be due to the molecular weight and structure. The results were consistent with many previous studies on the freeze denaturation suppression of some sugars and polyols on frozen fish actomyosin (Herrera and Mackie, 2004; Noguchi et al., 1976) and carp myofibrils (Hayashi et al., 2007). Hironaka et al. (1976) reported that the protective effect of sugar did not depend on the direct interaction between protein and sugar but by preventing the salting out effect due to increased salt solvation.

Citrate also stabilized the shrimp protein by maintaining approximately 95% Ca^{2+}-ATPase activity (Figure 1) after three FT cycles. This result is consistent with a previous report that even though citrate is a trivalent carboxylic acid with high ionic strength, it effectively suppressed myosin denaturation in carp, both in heating and at low temperature conditions (Kuwahara and Konno, 2010).

Our result suggested that the cryoprotective effect of sucrose and citrate was beneficial in protecting shrimp myofibrils, especially on the myosin head region.

Effects of cryoprotectants on structure and properties of shrimp myofibrils during the FT cycle

Salt solubility

Salt solubility of white shrimp myofibrils was determined by protein patterns in the soluble fraction in both the absence and presence of 2 mM Mg-ATP using SDS-PAGE electrophoresis (Jantakoson et al., 2013). Each sample produced a set of three lanes appearing on SDS-PAGE gel in the following order: sample not centrifuged, representing total protein (MF); centrifuged sample without Mg-ATP (S); and centrifuged sample with Mg-ATP (S+ATP). Myosin heavy chain (MHC) and actin are two major protein components that were found in the band patterns (Figure 2). The addition of Mg-ATP to the myofibrils was to detach myosin from F-actin without changing the yield of myosin and actin. If the salt-soluble fraction contained inactivated myosin and actin, the protein would not be recovered by Mg-ATP addition (Jantakoson et al., 2013). Thus, the salt-soluble MHC and actin in the presence of Mg-ATP of treated myofibrils were compared with a freshly prepared myofibril sample using SDS-PAGE with the aim of identifying the state of myosin and actin in each treatment.

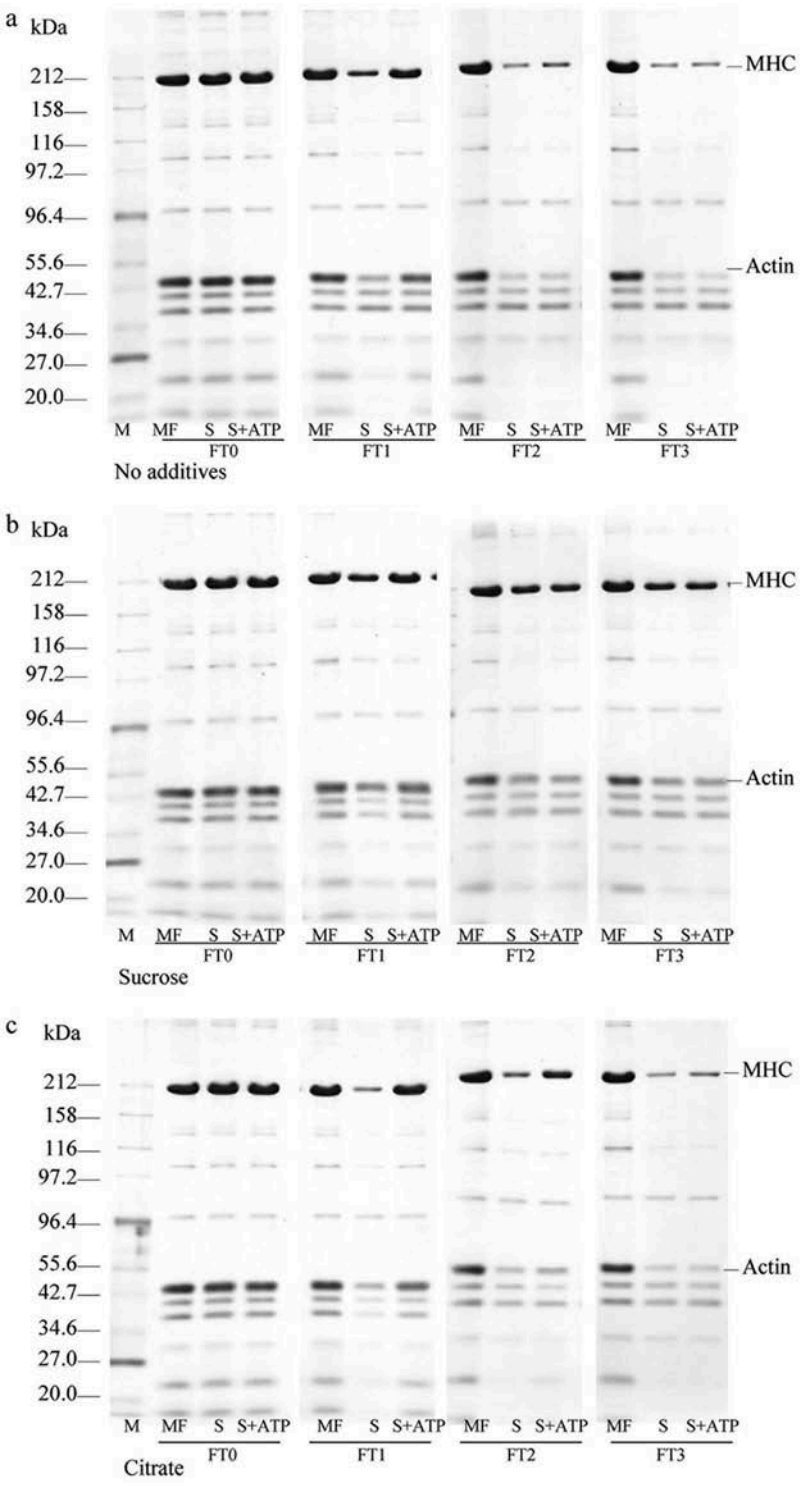

Figure 2. The effect of cryoprotectants on salt solubility of shrimp myofibrils (a) without additives, (b) with sucrose and (c) with citrate during freeze-thaw (FT) cycles. The first lane is standard proteins (M) and the samples were measured by dissolving the myofibril (MF) in 0.5 M NaCl, 20 mM Tris-HCl (pH 7.5) in the absence (S) and presence (S+ATP) of 2 mM Mg-ATP.

In order to demonstrate the percentage of salt solubility loss of myofibrils during the FT cycles, the ratio of band intensity of myosin and actin in the soluble fraction in the presence of Mg-ATP to those of myofibril fraction shown on SDS-PAGE (S+ATP lane to MF lane) was calculated and plotted against FT cycle (Figure 3). For all sets of fresh samples (without freezing-thawing), almost 100% of both soluble myosin and actin were recovered, whether in the absence or presence of Mg-ATP, indicating the native state of proteins (Figure 2). After one FT cycle, salt-soluble myosin and actin decreased noticeably more in fresh MF than in other samples (Figure 3). A sharp drop in myosin and actin was observed after two FT cycles in all samples, especially those without additives, which were further reduced to approximately 12% and 14%, respectively, after three FT cycles (Figure 3). When denaturation occurs, the hydrophobic amino acid side chains are occluded in the protein molecules and are exposed to the surface. In the attempt to maintain the lowest free energy, nonpolar groups tend to interact with one another to form protein aggregates, thus decreasing water binding and solubility (Xiong, 1997). Therefore, the aggregation of partly unfolded protein molecules results in the loss of protein solubility (Benjakul et al., 2003).

Addition of sucrose resulted in retention of up to 60% of soluble myosin and approximately 45% of soluble actin after the second and third FT cycles. Similarly, adding citrate caused retention of both myosin and actin at levels as high as with sucrose after the second FT cycle. However, this dropped to roughly 20% for both myosin and actin after the third FT cycle. A decrease in protein solubility is a main indicator of the freeze denaturation of protein and may be related to conformational changes in the peptide chain and a different arrangement from the native structure in which the formation of hydrogen or hydrophobic bonds as well as disulfide bonds and ionic interactions during frozen storage are involved (Benjakul and Bauer, 2000; Jiang et al., 1988; Riebroy et al., 2009; Tironi et al., 2007; Zhou et al., 2006). Among the additives studied, sucrose exhibited the highest protection against denaturation according to the band intensity.

Previous research pointed out that as sugar attaches via its OH-group to the side chain of a protein molecule, hydration of the protein is increased. Thus, each protein molecule gets covered by hydrated cryoprotectant molecules resulting in increased hydration (Noguchi et al., 1976). In addition, Kong et al. (2013) also found that the addition of sucrose/sorbitol mixture significantly inhibited carbonyl formation and lipid oxidation and thus reduced protein oxidation and structure

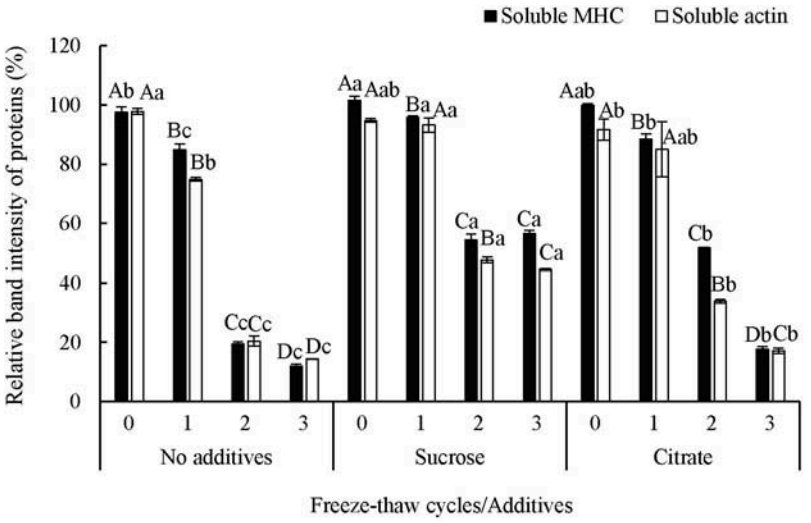

Figure 3. The effect of cryoprotectants on salt-soluble protein content of shrimp myofibrils during freeze-thaw (FT) cycles. Values are means of triplicate determinations ± standard deviation. Different uppercase letters (A-D) indicate significant differences between FT cycles for the same additive, and different lowercase letters (a-c) indicate significant differences between additives at the same FT cycle ($P < 0.05$).

deterioration in common carp surimi. On the other hand, salts affect proteins in frozen fish, probably by depressing the freezing point of tissue fluids, dehydration, altering interfacial tension, and by ionic interaction of charged groups of the side chains. The characteristic capacity of ions to hydrate and interfere with water structures makes various salts at different concentrations exert specific influences on the conformation of proteins. Arakawa et al. (1990) investigated the correlation between protein solubility and the preferential interactions of proteins with magnesium chloride and found that the proteins were preferentially hydrated at low salt concentrations. Chantarasuwan et al. (2011) studied the effects of sodium carbonate and bicarbonate on shrimp protein and concluded that the effect was due to the net surface charge at different pH, and was most likely governed by the different unfolding or exposure of charged amino acids, in which protonation or deprotonation could take place at different degrees. Thus, distinct negative net charge on the surface of the protein might enhance the dissociation of actomyosin complex, leading to the increased solubility of proteins. MacDonald et al. (1996) reported that sodium lactate is an effective stabilizer of actomyosin and that its effectiveness is strongly correlated with its ability to increase the solution surface tension. Based on these reports, it may be that sodium citrate can exert indirect action on freezing changes in shrimp protein. The ability of a salt to stabilize proteins in solution is a balance between its ability to increase the solution surface tension and its binding to anionic sites and dipolar peptide bonds of the protein. Increasing surface tension tends to stabilize intramolecular hydrophobic interactions, while binding of ions may be stabilizing or destabilizing depending on the specific interactions between the protein and the ions (Arakawa et al., 1990; MacDonald et al., 1996). The addition of carboxylic acids to a solution presents a potential complication due to their charged state, because electrostatic interactions are possible with the charged sites on proteins (MacDonald et al., 1996).

Total and reactive SH content

The total and reactive SH content of shrimp myofibrils are shown in Figure 4 (a and b), respectively. The initial total SH content of fresh myofibril samples without additive, with citrate, and with sucrose were 9.80, 9.61, and 9.44 mol/10^5 g protein, respectively (Figure 4a). Total SH content of myofibrils without additive was reduced to 40% of the original value after the first FT cycle, followed by a slight decrease in SH content after the second cycle, and was 30% or less after the third FT cycle. The reduction of total SH content was considered to be due to the formation of disulfide (SS) bonds through the oxidation of SH groups or disulfide interchanges (Hayakawa and Nakai, 1985; Xia et al., 2009).

In this study, the decrease in total SH content of shrimp myofibrils after the first FT cycle was retarded by the addition of cryoprotectants, i.e. sucrose and citrate at concentrations of 0.05 M, maintaining 50–70% of the original total SH content throughout the three FT cycles ($P < 0.05$). The total SH content of both additive-treated samples gradually decreased during the freeze-thaw process, although citrate samples retained higher SH content than sucrose samples ($P < 0.05$). The results agree with Kong et al. (2013) who noted that the addition of cryoprotectants could serve as a protective barrier against oxidation initiators. Thus this result suggested that addition of sucrose or citrate was able to reduce the decrease in SH content and indicated the protection against denaturation.

The reactive or surface SH content was slightly lower than the total SH content in all samples, and both values decreased during the FT cycles in a similar trend (Figure 4a and 4b). The total and reactive SH content of samples without an additive were found to decrease when subjected to the freeze-thaw process, indicating an increase in disulfide bond formation due to protein denaturation (Jiang et al., 1988). The accelerated denaturation of myosin molecules, in which the reactive SH groups are exposed to oxidation, might result in increasing disulfide bond formation (Sriket et al., 2007). During FT cycles, the total and reactive SH of samples treated with sucrose and citrate decreased more slowly than that of samples without additives ($P < 0.05$). The reduction of reactive SH was coincident with the decrease in Ca^{2+}-ATPase activity as mentioned earlier (Figure 1). Again, these results suggest that conformational change occurred in the myosin head region. The finding

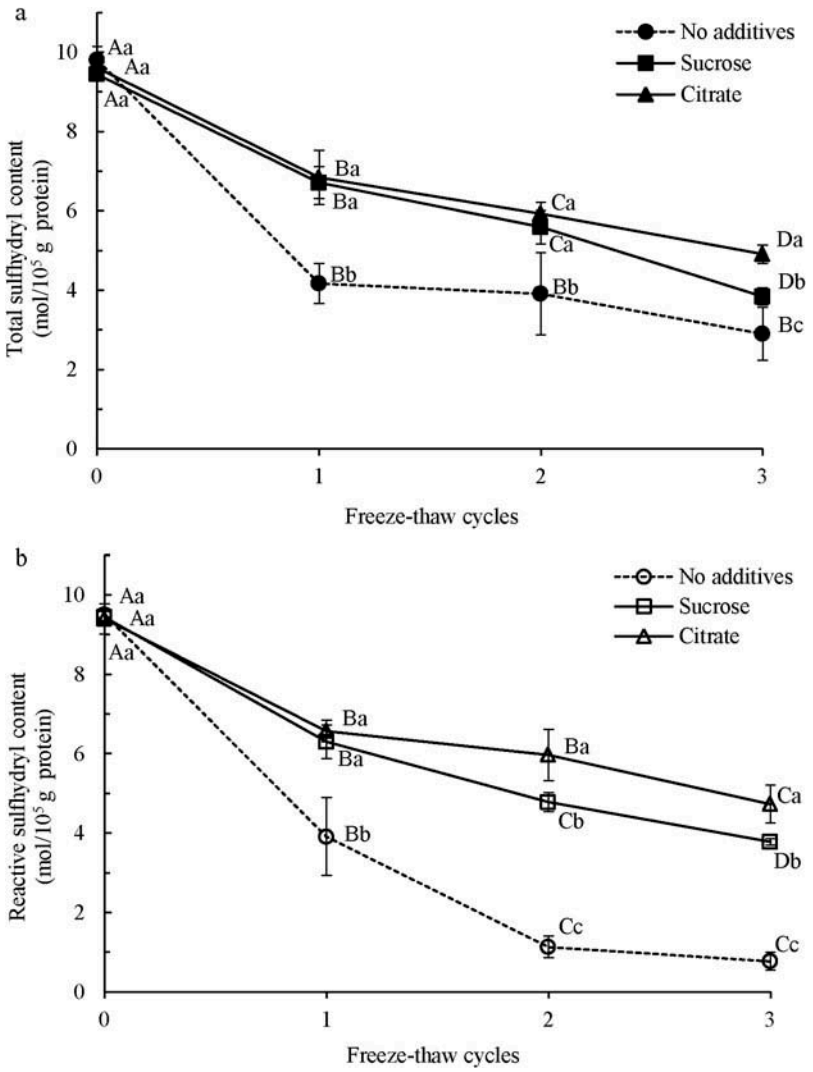

Figure 4. The effect of cryoprotectants on (a) total and (b) reactive SH group content of shrimp myofibrils during freeze-thaw (FT) cycles. Values are means of triplicate determinations ± standard deviation. Different uppercase letters (A-D) indicate significant differences between FT cycles for the same additive, and different lowercase letters (a-c) indicate significant differences between additives at the same FT cycle ($P < 0.05$).

was supported by Wang et al. (2014), who reported that the reactive SH content of grass carp myofibrillar proteins decreased sharply throughout a period of frozen storage, especially after 10 days. Similarly, common carp myofibrils were found to have a sharp decrease in reactive SH content after five FT cycles (Guo et al., 2014; Kong et al., 2013).

The addition of sucrose can retard the oxidation of sulfhydryl groups as shown by the decrease in polymerization of MHC through SS bonding of SH in myosin (Sompongse et al., 1996b) to form disulfide bonds or disulfide interchange (Benjakul and Bauer, 2000). Lee and Timasheff (1981) found that the apparent activation energy of the unfolding of proteins was increased by the addition of sucrose. Furthermore, protein-solvent interaction studies indicated that sucrose was preferentially excluded from the protein domain, thereby increasing the free energy of the system. Thermodynamically, this leads to protein stabilization since the unfolded state of the protein is accompanied by an increase in protein-solvent surface contact area, and hence unfolding becomes

thermodynamically less favorable in the presence of sucrose. The strengthening of intramolecular hydrophobic interactions and the maintenance of organization of water have been postulated to be the major effects of cryoprotection on protein (Kong et al., 2013). For citrate salt, its stabilizing effect may possibly be correlated with its ability to increase the solution surface tension and the preferential interaction of salts with the proteins.

Surface hydrophobicity

The surface hydrophobicity of shrimp myofibrils was measured using BPB bound method, which is based on the interaction of the hydrophobic chromophore bromophenol blue with myofibrillar protein and the separation of free and bound BPB by centrifugation (Chelh et al., 2006). Fixation of BPB to shrimp myofibrils in this study is shown in Figure 5.

The amount of BPB in sample without additive was only 10 μg prior to the FT process, markedly increased to 30 μg after the first FT cycle ($P < 0.05$), and then was rather constant after the second and third FT cycles ($P \geq 0.05$). In native myofibrillar proteins, hydrophobic residues, especially nonpolar amino acids are buried inside the natural folded structure. Protein can undergo unfolding, which increases with temperature abuse, e.g. cyclic freezing and thawing (Srinivasan et al., 1997; Riebroy et al., 2009). High hydrophobicity indicates the unfolding of protein and the exposure of non-polar amino acids that become available for formation of hydrophobic interaction and leads to protein aggregation (Benjakul and Bauer, 2000; Benjakul et al., 2003; Riebroy et al., 2009).

When appropriate cryoprotectants are added, protein denaturation can be prevented to some extent (Zhou et al., 2006). However, the addition of sucrose and citrate in this study increased the initial amount of bound BPB of the myofibrils ($P < 0.05$) before freezing, especially for the citrate, which was raised to 20 μg. No clear reasons were provided based on the available data, but this might be due to the effect of additives on thermodynamic balances of protein or protein conformation changes resulting in partially unfolded but not denatured protein. Even though the initial values in samples with additives were higher, the increase in surface hydrophobicity of these samples was lower than the samples without additive after being subjected to the FT process.

The increase in surface hydrophobicity in the sucrose-treated sample was retarded during FT cycles. This result might be explained by the hypothesis of Matsumoto and Noguchi (1992) that

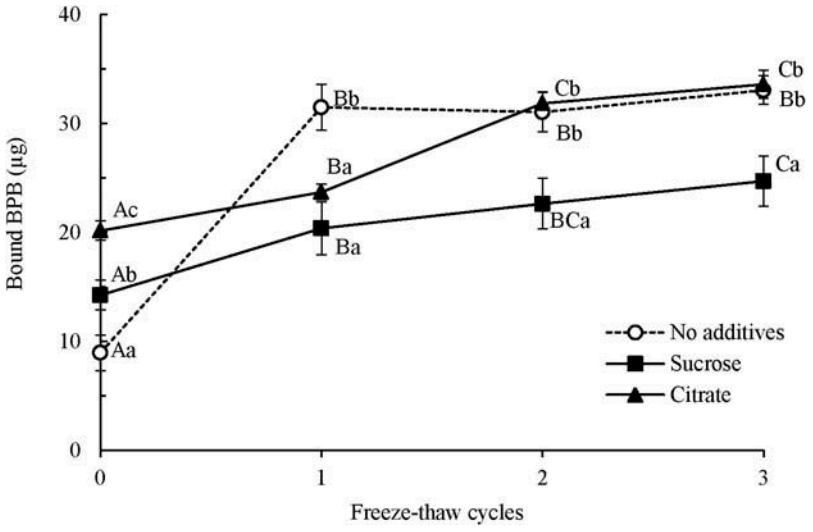

Figure 5. The effect of cryoprotectants on surface hydrophobicity of shrimp myofibrils during freeze-thaw (FT) cycles. Values are means of triplicate determinations ± standard deviation. Different uppercase letters (A-C) indicate significant differences between FT cycles for the same additive, and different lowercase letters (a-c) indicate significant differences between additives at the same FT cycle ($P < 0.05$).

during freezing, water molecules migrate from the protein surface to the surface of ice crystals and are replaced by the cryoprotectants. The interactions between proteins and the cryoprotectants prevent the crosslinking with adjacent proteins and decrease protein aggregation. The sucrose-treated sample had the lowest surface hydrophobicity, indicating less unfolding (Figure 5). It was demonstrated that sucrose could prevent the exposure of hydrophobic clusters of protein. Hydrogen bonding between hydroxyl groups of saccharides and proteins has been proposed as the mechanism of cryoprotection (Sych et al., 1990; Wang et al., 2014).

For the citrate-treated sample, surface hydrophobicity gradually increased during the first two cycles and reached a value similar to the sample without additives. A hypothetical mechanism for the cryoprotective effect of citrate on protein is that citrate interacts and bonds with the protein molecule via functional groups on the surface. Water molecules are hydrated onto the other remaining functional groups of the cryoprotectant, thus each protein is covered with the hydrated cryoprotectant molecules. In the case of sodium citrate, which possesses three anionic carboxylic groups, the protein molecules then were covered by anionic charges. These anionic surface charges result in a repulsive force between protein molecules and an increased hydration of the protein-cryoprotectant conjugates via other anionic groups on the cryoprotectant molecules. Thus, in our study, citrate could have protected the protein from unfolding during frozen storage as the surrounding water was displaced to form ice crystals. However, although citrate retarded the increase in surface hydrophobicity to some extent, the effect was lower than that of sucrose.

Effects of cryoprotectants on microstructure of shrimp muscle along the FT cycle

The effect of sucrose and citrate on the microstructure of unfrozen shrimp and shrimp after three freeze-thaw cycles was observed from light micrographs as shown in Figure 6. The muscle structure of raw shrimp without treatment (Figure 6a) had well-organized structure of fibers. After being soaked with 6% sucrose or 5% citrate solution (Figure 6b and c), the muscle contained slightly larger spaces between fibers, possibly where water had been held. As the hydroxyl groups of sugars can interact with water molecules, adding sucrose may contribute to retardation of ice crystallization (Uedaira et al., 1990). The citrate-treated sample (Figure 6c) seemed to have larger voids distributed throughout the muscle. The structure of sodium citrate contains three carboxyl groups, which has a major effect on ionic strength and could extract myosin from myofibrillar structures in meat. However, citrate does not remove the links between actin and myosin but contributes only to the swelling of the muscle fiber structure and not to protein solubilization (Feiner, 2006).

After freezing-thawing for three cycles, the muscle without additives (Figure 6d) became denser, with many larger voids representing gaping and indicating a damaged structure. This result is similar to an earlier study on microstructure of white shrimp subjected to five FT cycles. In longitudinal sections of the tissue, shrinkage of fibers was evidenced by the gaping formed (Sriket et al., 2007). In another study, increased extracellular space was observed between the fibers of freeze-thawed salmon fillets (Sigurgisladottir et al., 2000). The increase in the intra-myofibrillar protein spaces has been associated with the effect of the freezing process, and this change became more evident with the increased number of FT cycles (Ramirez Guerra et al., 2012; Sriket et al., 2007). In this study, microstructure of samples treated with additives became increasingly fine and with a continuous pattern (Figure 6e and 6f). Using sucrose enhanced the uniformity in structure (Figure 6e), resembling the microstructure of fresh shrimp (Figure 6a). The structure of the citrate-treated sample (Figure 6f) after three FT cycles was not different from the structure before freezing (Figure 6c). The microstructure results also support the earlier suggestion that sucrose and citrate can enhance the stability of muscle protein.

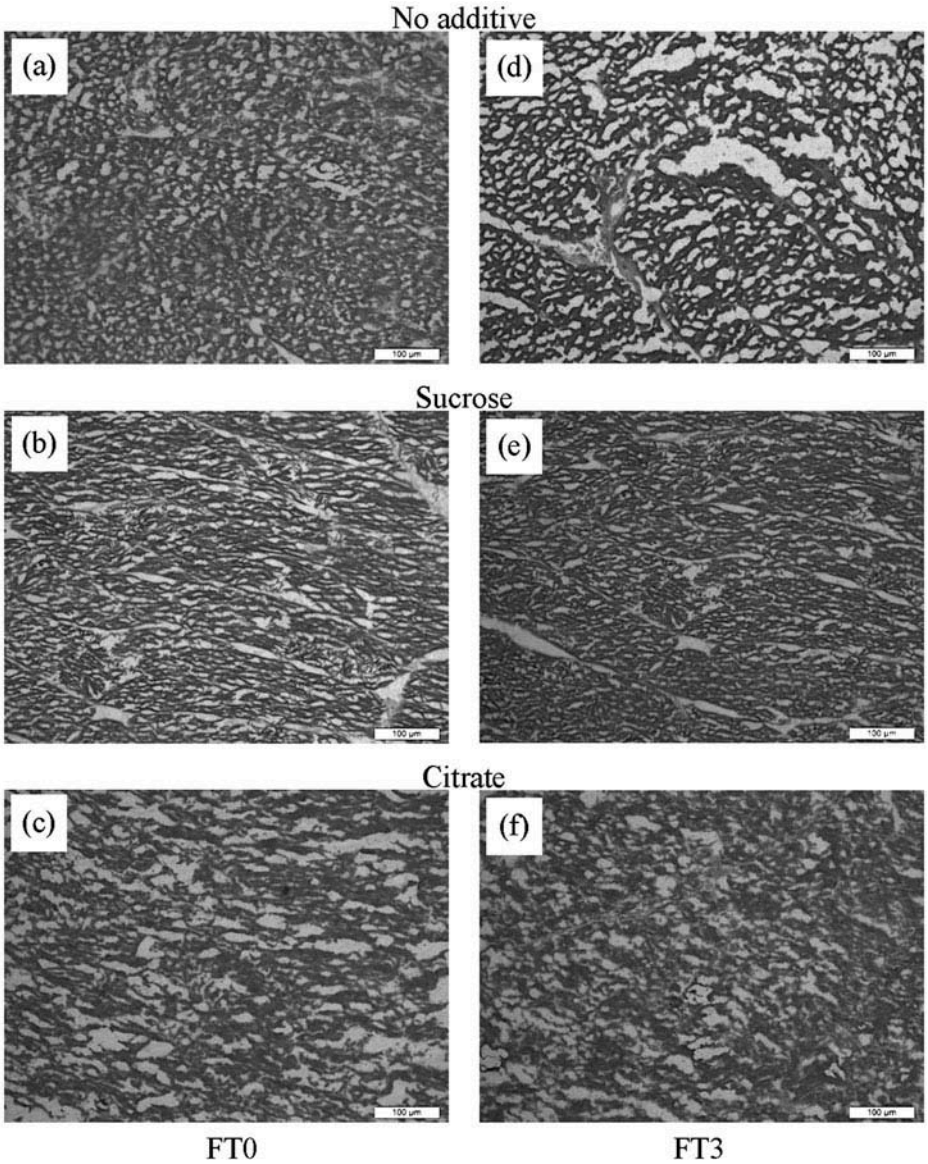

Figure 6. Light micrographs of longitudinal section of the 3rd abdominal segment of fresh (a-c) and freeze-thawed (d-f) shrimp muscle without treatment and treatment with solutions of 6% sucrose and 5% citrate, respectively before (FT0) and after (FT3) freeze-thaw process for three cycles (magnification 200x).

Conclusion

This investigation has shown that Pacific white shrimp myofibrillar protein rapidly denatured during the freeze-thaw process. Sucrose and citrate had the highest stabilizing effect on shrimp myofibrillar proteins by retarding freeze denaturation as indicated by preventing loss in Ca^{2+}-ATPase activity and salt soluble myosin and actin. The structural changes accompanying the denaturation of the myofibril were shown by the reduction of total and reactive SH and by the increase in the surface hydrophobicity. Results demonstrated that both additives were able to retard the unfolding of protein; thus, the native protein could be protected during the freeze-thaw process.

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References

Ai-Mei, Z., Benjakul, S., and Qing Xiao, Z. 2004. Effect of the freeze-thaw cycle on the protein denaturation of *Starry triggerfish* (*Abalistes stellaris*) surimi. J. South China Univ. Technol. (Natl. Sci. Ed.). 32(7): 93–96.

Arakawa, T., Bhat, R., and Timasheff, S. N. 1990. Preferential interactions determine protein solubility in three-component solutions: the $MgCl_2$ system. Biochem. 29: 1914–1923. doi:10.1021/bi00459a036

Benjakul, S., and Bauer, F. 2000. Physicochemical and enzymatic changes of cod muscle proteins subjected to different freeze-thaw cycles. J. Sci. Food Agric. 80: 1143–1150. doi:10.1002/(ISSN)1097-0010

Benjakul, S., Seymour, T., Morrissey, M. T., and An, H. 1997. Physicochemical changes in Pacific whiting muscle proteins during iced storage. J. Food Sci. 62(4): 729–733. doi:10.1111/j.1365-2621.1997.tb15445.x

Benjakul, S., Visessanguan, W., Thongkaew, C., and Tanaka, M. 2003. Comparative study on physicochemical changes of muscle proteins from some tropical fish during frozen storage. Food Res. Int. 36: 787–795. doi:10.1016/S0963-9969(03)00073-5

Boonsumrej, S., Chaiwanichsiri, S., Tantratian, S., Suzuki, T., and Takai, R. 2007. Effects of freezing and thawing on the quality changes of tiger shrimp (*Penaeus monodon*) frozen by air-blast and cryogenic freezing. J. Food Eng. 80: 292–299. doi:10.1016/j.jfoodeng.2006.04.059

Chantarasuwan, C., Benjakul, S., and Visessanguan, W. 2011. The effect of sodium carbonate and sodium bicarbonate on yield and characteristics of Pacific white shrimp (*Litopenaeus vannamei*). Food Sci. Technol. Int. 17: 403–414. doi:10.1177/1082013211398802

Chelh, I., Gatellier, P., and Sante-Lhoutellier, V. 2006. Technical note: A simplified procedure for myofibril hydrophobicity determination. Meat Sci. 74: 681–683. doi:10.1016/j.meatsci.2006.05.019

Ellman, G. L. 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 82: 70–77. doi:10.1016/0003-9861(59)90090-6

Feiner, G. 2006. Meat Products Handbook. Abington Hall, Abington Cambridge: Wood Head Publishing Limited.

Godiksen, H., Hyldig, G., and Jessen, F. 2003. Sarcoplasmic reticulum Ca^{2+}-ATPase and cytochrome oxidase as indicators of frozen storage in cod (*Gadus morhua*). J. Food Sci. 68(8): 2579–2585. doi:10.1111/j.1365-2621.2003.tb07064.x

Gornall, G., Bardawill, C., and David, M. 1949. Determination of serum protein by means of the biuret reaction. J. Biol. Chem. 177: 751–766.

Guo, Y., Kong, B., Xiufang, X., Tao, Y., and Qian, L. 2014. Changes in physicochemical and protein structural properties of common carp (*Cyprinus carpio*) muscle subjected to different freeze–thaw cycles. J. Aquat. Food Prod. Technol. 23: 579–590. doi:10.1080/10498850.2012.741663

Hagiwara, T., Hayashi, R., Suzuki, T., and Takai, R. 2003. Fractal analysis of ice crystals in frozen fish meat. Japan J. Food Eng.. 4(1): 11–17.

Hayakawa, S., and Nakai, S. 1985. Contribution of hydrophobicity, net charge and sulfhydryl groups to thermal properties of ovalbumin. Can. Inst. Food Sci. Technol. J. 18: 290–295. doi:10.1016/S0315-5463(85)71960-8

Hayashi, K., Azuma, Y., Koseki, S., and Konno, K. 2007. Different effects of ionic and non-ionic compounds on the freeze denaturation of myofibrils and myosin subfragment-1. Fish. Sci. 73: 178–183. doi:10.1111/j.1444-2906.2007.01316.x

Herrera, J. R., and Mackie, I. M. 2004. Cryoprotection of frozen-stored actomyosin of farmed rainbow trout (*Oncorhynchus mykiss*) by some sugars and polyols. Food Chem. 84: 91–97. doi:10.1016/S0308-8146(03)00178-X

Hironaka, Y., Hayashi, S., and Ooshiro, Z. 1976. Interaction between protein and sugar during frozen storage. Mem. Fac. Fish. Kagoshima Univ. 25(1): 101–105.

Jaczynski, J., Hunt, A., and Park, J. W. 2006. Safety and quality of frozen fish, shellfish, and related products. In: Handbook of Frozen Food Processing and Packaging. Sun, D. W. (Ed.) Boca Raton: CRC. pp. 360–364.

Jantakoson, T., Thavaroj, W., and Konno, K. 2013. Myosin and actin denaturation in frozen stored kuruma prawn *Marsupenaeus japonicas* myofibrils. Fish. Sci. 79: 341–347. doi:10.1007/s12562-012-0589-y

Jiang, S. T., Hwang, B. S., and Chen, C. S. 1988. Effect of storage temperatures on the formation of disulfides and denaturation of milkfish actomyosin (*Chanos chanos*). J. Food Sci. 53: 1333–1335. doi:10.1111/j.1365-2621.1988.tb09270.x

Jiang, S. T., Tsao, C. Y., and Lee, T. C. 1987. Effect of free amino acids on the denaturation of mackerel myofibrillar proteins in vitro during frozen storage at −20 °C. J. Agric. Food Chem. 35: 28–33. doi:10.1021/jf00073a007

Kong, B., Guo, Y., Xia, X., Liu, Q., Li, Y., and Chen, H. 2013. Cryoprotectants reduce protein oxidation and structure deterioration induced by freeze-thaw cycles in Common carp (*Cyprinus carpio*) surimi. Food Biophys. 8: 104–111. doi:10.1007/s11483-012-9281-0

Kuwahara, K., and Konno, K. 2010. Suppression of thermal denaturation of myosin and salt-induced denaturation of actin by sodium citrate in carp (*Cyprinus carpio*). Food Chem. 122: 997–1002. doi:10.1016/j.foodchem.2010.03.056

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of head of bacteriophage T4. Nature. 227: 680–685. doi:10.1038/227680a0

Lee, J. C., and Timasheff, S. N. 1981. The stabilization of proteins by sucrose. J. Biol. Chem. 256: 7193–7201.

Love, R. M. 1968. Ice formation in frozen muscle. In: Low Temperature Biology of Foodstuffs. Hawthorn, J., and Rolfe, E. J. (Eds.) Oxford, U.K.: Pergamon Press Ltd. pp. 105–124.

MacDonald, G. A., and Lanier, T. C. 1994. Actomyosin stabilization to freeze-thaw and heat denaturation by lactate salts. J. Food Sci. 59(1): 101–105. doi:10.1111/j.1365-2621.1994.tb06907.x

MacDonald, G. A., Lanier, T. C., Swaisgood, H. E., and Hamann, D. D. 1996. Mechanism for stabilization of fish actomyosin by sodium lactate. J. Agric. Food Chem. 44: 106–112. doi:10.1021/jf940698y

Matsumoto, J. J., and Noguchi, S. F. 1992. Cryostabilization of proteins in surimi. In: Surimi Technology. Lanier, T. C., and Lee, C. M. (Eds.) New York: Marcel Dekker, Inc. pp. 357–388.

McCormick, R. J. 1994. Structure and properties of tissue. In: Muscle Foods: Meat, Poultry and Seafood Technology. Kinsman, D. M., Kotula, A. W., and Breidenstein, B. C. (Eds.) Hall, New York: Chapman. pp. 25–62.

Noguchi, S., Oosawa, K., and Matsumoto, J. J. 1976. Studies on the control of denaturation of fish muscle proteins during frozen storage. VI. Preventive effect of carbohydrates. Bull. Jap. Soc. Sci. Fish. 42: 77–82. doi:10.2331/suisan.42.77

Okada, T., Inoue, N., and Akiba, M. 1986. Electron microscopic observation and biochemical properties of carp myosin B during frozen storage. Bull. Jap. Soc. Sci. Fish. 52: 345–353. doi:10.2331/suisan.52.345

Ooizumi, T., Nara, Y., and Arai, K. 1984. Protective effect of carboxylic acids, sorbitol and Na-glutamate on heat denaturation of Chub mackerel myofibrils. Nippon Suisan Gakkaishi. 50: 875–882. doi:10.2331/suisan.50.875

Park, J. W., and Lin, T. M. J. 2005. Surimi: manufacturing and evaluation. In: Surimi and Surimi Seafood. 2nd. Park, J. W. (Ed.) Boca Raton: Taylor and Francis Group. pp. 33–106.

Ramirez Guerra, H. E., Garcia-Sifuentes, C. O., Pacheco-Aguilar, R., Ramirez-Suarez, J. C., Lugo-Sanchez, M. E., and Scheuren-Acevedo, S. M. 2012. The influence of ante-mortem hypoxia on the physicochemical stability of myofibrillar proteins in the muscle tissue of white shrimp (*Litopenaeus vannamei*) exposed to multiple freeze–thaw cycles. Eur. Food Res. Technol. 235: 37–45. doi:10.1007/s00217-012-1702-2

Riebroy, S., Benjakul, S., Visessanguan, W., Erikson, U., and Rustad, T. 2009. Acid-induced gelation of natural actomyosin from Atlantic cod (*Gadus morhua*) and burbot (*Lota lota*). Food Hydrocoll. 23: 26–39. doi:10.1016/j.foodhyd.2007.11.010

Roy, B. C., Ando, M., Itoh, T., and Tsukamasa, Y. 2012. Structural and ultrastructural changes of full-cycle cultured Pacific bluefin tuna (*Thunnus orientalis*) muscle slices during chilled storage. J. Sci. Food Agric. 92: 1755–1764. doi:10.1002/jsfa.v92.8

Rupnow, J. H. 1992. Proteins: biochemistry and applications. In: Encyclopedia of Food Science and Technology. Hui, Y. H. (Ed.) USA: John Wiley & Sons Inc. pp. 2182–2191.

Sigurgisladottir, S., Ingvarsdottir, H., Torrissen, O. J., Cardinal, M., and Hafsteinsson, H. 2000. Effects of freezing/thawing on the microstructure and the texture of smoked Atlantic salmon (*Salmo salar*). Food Res. Int. 33: 857–865. doi:10.1016/S0963-9969(00)00105-8

Sompongse, W., Itoh, Y., and Obatake, A. 1996a. Effect of cryoprotectants and a reducing reagent on the stability of actomyosin during ice storage. Fish. Sci. 62(1): 73–79. doi:10.2331/fishsci.62.73

Sompongse, W., Itoh, Y., and Obatake, A. 1996b. Role of SH_a in the polymerization of myosin heavy chain during ice storage of carp actomyosin. Fish. Sci. 62(1): 110–113. doi:10.2331/fishsci.62.110

Sriket, P., Benjakul, S., Visessanguan, W., and Kijroongrojana, K. 2007. Comparative studies on the effect of the freeze–thawing process on the physicochemical properties and microstructures of black tiger shrimp (*Penaeus monodon*) and white shrimp (*Penaeus vannamei*) muscle. Food Chem. 104: 113–121. doi:10.1016/j.foodchem.2006.11.004

Srinivasan, S., Xiong, Y. L., and Blanchard, S. 1997. Effect of freezing and thawing methods and storage time on thermal properties of freshwater prawns (*Macrobrachium rosebergii*). J. Sci. Food Agric. 75: 37–44. doi:10.1111/j.1365-2621.1997.tb04381.x

Sych, J., Lacriox, C., Asambounou, L. T., and Castaigne, F. 1990. Cryoprotective effects of some materials on cod-surimi proteins during frozen storage. J. Food Sci. 55: 1222–1227. doi:10.1111/j.1365-2621.1990.tb03902.x

Tironi, V. A., Tomas, M. C., and Anon, M. C. 2007. Lipid and protein deterioration during the chilled storage of minced sea salmon (*Pseudopercis semifasciata*). J. Sci. Food Agri. 87: 2239–2246. doi:10.1002/jsfa.2949

Uedaira, H., Ishimura, M., Tsuda, S., and Uedaira, H. 1990. Hydration of oligosaccharides. Bull. Chem. Soc. Jpn. 63 (12): 3376–3379. doi:10.1246/bcsj.63.3376

Wang, L., Xiong, G., Peng, Y., Wu, W., Li, X., Wang, J., Qiao, Y., Liao, L., and Ding, A. 2014. The cryoprotective effect of different konjac glucomannan (KGM) hydrolysates on the glass carp (*Ctenopharyngodon idella*) myofibrillar during frozen storage. Food Bioprocess Technol. 7: 3398–3406. doi:10.1007/s11947-014-1345-3

Xia, X., Kong, B., Liu, Q., and Liu, J. 2009. Physicochemical change and protein oxidation in porcine longissimus dorsi as influenced by different freeze-thaw cycles. Meat Sci. 83: 239–245. doi:10.1016/j.meatsci.2009.05.003

Xiong, Y. L. 1997. Structure-function relationships of muscle proteins. In: Food Proteins and Their Applications. Damodaran, S., and Paref, A. (Eds.) New York: Marcel Dekker. pp. 341–392.

Zhou, A., Benjakul, S., Pan, K., Gong, J., and Liu, X. 2006. Cryoprotective effects of trehalose and sodium lactate on tilapia (*Sarotherodon nilotica*) surimi during frozen storage. Food Chem. 96: 96–103. doi:10.1016/j.foodchem.2005.02.013