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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

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Abstract

The effects of different freeze–thaw cycles (0, 1, 3 and 5) on the physicochemical properties and microstructures of black tiger shrimp (*Penaeus monodon*) and white shrimp (*Penaeus vannamei*) muscle were investigated. White shrimp had greater exudate loss and higher α-glucosidase (AG), as well as β-*N*-acetyl-glucosaminidase (NAG) activities, than did black tiger shrimp, especially when the number of freeze–thaw cycles increased ($P < 0.05$). The decreases in Ca^{2+}-ATPase activity, sulfhydryl group content and protein solubility with concomitant increases in disulfide bond formation and surface hydrophobicity were more pronounced in white shrimp muscle, than in black tiger shrimp muscle, particularly after five cycles of freeze–thawing ($P < 0.05$). The shear force of both shrimps was decreased after five freeze–thaw cycles ($P < 0.05$). The microstructure study revealed that the muscle fibers were less attached, with the loss of Z-disks, after subjection to five freeze–thaw cycles. Therefore, the freeze–thawing process caused denaturation of proteins, cell disruption, as well as structural damage of muscle in both shrimps. White shrimp generally underwent physicochemical changes induced by the freeze–thawing process to a greater extent than did black tiger shrimp.
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Keywords: Black tiger shrimp; White shrimp; Freeze–thawing; Denaturation; Muscle protein; Microstructure

1. Introduction

Black tiger shrimp (*Penaeus monodon*) and white shrimp (*Penaeus vannamei*) are important commercial species in Thailand, with a catch volume over 1000 tons per year (Suphamongkhon, 2002). Both shrimps generally have high market value and have become the essential income generator of the country. During transportation, storage, retail display, and consumption, spoilage of shrimp, caused by microorganisms generally occurs. To prevent such a deterioration, freezing technology has been successfully applied. Frozen storage is an important preservation method for seafood. Despite microbial spoilage being effectively terminated, quality deterioration, especially in texture, flavour and colour, still takes place during frozen storage (Benjakul, Visessanguan, Thongkaew, & Tanaka, 2003; Shenouda, 1980). The freezing process induces muscle tissue changes by the formation and accretion of ice crystals, dehydration, and increases in solute concentration (Shenouda, 1980). Freezing and thawing disrupt muscle cells, resulting in the release of enzymes from mitochondria into the sarcoplasm (Hamm, 1979). Thawed meat tends to display higher shear force than does nonfrozen meat (Hale & Waters, 1981). Such increase in toughness during frozen storage of seafood is

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attributed to myosin denaturation, as well as aggregation of myofibrillar proteins (Sikorski, Olley, & Kostuch, 1976). Denaturation and aggregation of muscle proteins are associated with the formation of disulfide bond formation (Jiang, Wang, & Chen, 1989). The freeze–thaw process can also promote lipid oxidation, which may affect the texture of crustacean muscle (Srinivasan, Xiong, Blanchard, & Tidwell, 1997). Deterioration of muscle proteins during frozen storage depends on many factors, including species, storage temperature, time and enzymatic degradation (Ang & Hultin, 1989; Badii & Howell, 2001; Hsieh & Regenstein, 1989). Those alterations determine the quality of frozen seafoods, especially in terms of protein functionality. The degree of denaturation of myofibrillar proteins varies, depending on species (Fukuda, Kakehata, & Arai, 1981; Seo, Endo, Fujimoto, Moku, & Kawaguchi, 1997). The freeze–thawing process may influence the quality of shrimps differently, depending on species. Nevertheless, no basic information regarding the effect of the freeze–thawing process on the physicochemical properties and microstructures of black tiger shrimp or white shrimp cultured in Thailand has been reported. Our objective was to elucidate the changes in the properties and quality of black tiger shrimp and white shrimp, as influenced by multiple freeze–thawing.

2. Materials and methods

2.1. Chemicals

Ammonium molybdate, 5-5′-dithio-bis (2-nitrobenzoic acid) (DTNB), adenosine 5′-triphosphate (ATP), ρ-nitrophenyl-α-glucopyranoside, ρ-nitrophenyl-*N*-acetyl-β-D-glucose amide,1-anilinonaphthalene-8-sulphonic acid (ANS) and glutaraldehyde were procured from Sigma (St. Louis, MO, USA). Potassium chloride (KCl) was obtained from Ajax Finechem (Wellington, Auckland, New Zealand). Sodium dihydrogen phosphate and absolute ethanol were purchased from Merck (Darmstadt, Germany).

2.2. Sample preparation

Black tiger shrimp (*P. monodon*) and white shrimp (*P. vannamei*), of the size of 60 shrimps/kg, were obtained from the farms in Songkhla and Suratthani provinces, respectively. The shrimps were placed in ice with an ice/shrimp ratio of 2:1 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai, Songkhla within approximately 1 and 4 h, respectively. Upon arrival, the shrimps were washed with clean water and deheaded. The shells were then peeled off. The shrimps were packaged in polyethylene bags and frozen at −20 °C using an air-blast freezer for 48 h. The frozen shrimps were thawed, using running water (27 °C) until the core temperature reached 0–2 °C. The freeze–thawing was performed for 0, 1, 3 and 5 cycles. The shrimps were then subjected to analyses.

2.3. Determination of exudate loss

The samples (25 g) were chopped into small pieces, followed by centrifuging at 10,000*g* for 60 min at 4 °C, using a Sorvall RC 26 Plus refrigerated centrifuge (Sorvall, Norwalk, CT, USA). The exudate formed was collected using a Pasteur pipette and the volume obtained was measured. The exudate was brought to 25 ml with distilled water before enzyme assay. The protein content in the exudate was determined by the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951).

2.4. α-Glucosidase (AG) and β-N-acetyl-glucosaminidase (NAG) activity assays

The AG (E.C. 3.2.1.20) and NAG (E.C. 3.2.1.30) activities were assayed according to the method of Benjakul and Bauer (2000) with a slight modification. For AG activity assay, the activity was measured spectrophotometrically using ρ-nitrophenyl-α-glucopyranoside as a substrate. The reaction mixture contained 0.3 ml of 0.1 M Na citrate buffer (pH 4.0), 0.2 ml of 1.0 M NaCl, and 1 ml of diluted shrimp muscle exudate. The reaction mixture was pre-incubated at 37 °C for 10 min. The reaction was initiated by adding 1 ml of 4.2 mM ρ-nitrophenyl-α-glucopyranoside. After 60 min, the reaction was terminated by adding 1 ml of 0.3 M KOH. The absorbance was measured at 405 nm. The blank was performed using distilled water instead of shrimp muscle exudate. A negative control was carried out by adding the stopping reagent prior to the addition of substrate. NAG activity was determined using ρ-nitrophenyl-*N*-acetyl-β-D-glucose amide as a substrate. The reaction mixture consisted of 0.3 ml of 0.1 M Na-citrate buffer (pH 4.5), 0.2 ml of 0.6 M KCl and 0.2 ml of diluted shrimp muscle exudate. The reaction was initiated by adding 0.2 ml of ρ-nitrophenyl-*N*-acetyl-β-D-glucose amide and incubated at 37 °C for 10 min. The reaction was stopped by adding 1 ml of 0.3 M KOH. The absorbance was measured at 405 nm. The amount of ρ-nitrophenol released was monitored at 405 nm and calculated using a molar extinction coefficient of 19,500 $M^{-1}\,cm^{-1}$. One unit of enzyme was defined as the activity which released 1 nmol of ρ-nitrophenol per min.

2.5. Determination of Ca^{2+}-ATPase activity

ATPase activity was determined by the method of Benjakul, Seymour, Morrissey, and An (1997). Natural actomysin (NAM), prepared as described by Benjakul et al. (1997), was diluted to 3–5 mg/ml with 0.6 M KCl, pH 7.0. Diluted NAM solution (0.5 ml) was added to 0.3 ml of 0.5 M Tris–maleate, pH 7.0. The mixture was treated with 0.5 ml of 10 mM $CaCl_2$ and 3.45 ml of distilled water. To initiate the reaction, 0.25 ml of 20 mM ATP were added. The reaction was conducted for 10 min at 25 °C and terminated by adding 2.5 ml of chilled 15% (w/v) trichloroacetic acid. The reaction mixture was centrifuged

at 3500*g* for 5 min and the inorganic phosphate liberated in the supernatant was measured by the method of Fiske and Subbarow (1925). Specific activity was expressed as μmol inorganic phosphate released/mg protein/min. A blank solution was prepared by adding chilled trichloroacetic acid prior to addition of ATP.

2.6. Determination of total sulfhydryl content

Total sulfhydryl content was determined, using 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB), according to the method of Ellman (1959), as modified by Benjakul et al. (1997). To 1 ml of NAM solution (0.4%), 9 ml of 0.2 M Tris–HCl buffer, pH 6.8, containing 8 M urea, 2% SDS and 10 mM EDTA, were added. To 4 ml of the mixture, 0.4 ml of 0.1% DTNB, in 0.2 M Tris–HCl (pH 8.0), was added and the mixture was incubated at 40 °C for 25 min. The absorbance at 412 nm was measured using a UV-16001 spectrophotometer (Shimadzu, Kyoto, Japan). A blank was conducted by replacing the sample with 0.6 M KCl. Sulfhydryl content was calculated, using the extinction coefficient of 13,900 M^{-1} cm^{-1}.

2.7. Determination of disulfide bond content

Disulfide bond content in NAM was determined by using 2-nitro-5-thiosulfobenzoate (NTSB) assay, according to the method of Thannhauser, Konishi, and Scheraga (1987). To 0.5 ml of NAM sample (1 mg/ml), 3.0 ml of freshly prepared NTSB assay solution, pH. 9.5 were added. The mixture was incubated in the dark at room temperature (25–27 °C) for 25 min. Absorbance at 412 nm was measured. Disulfide bond content was calculated, using the extinction coefficient of 13,900 M^{-1} cm^{-1}.

2.8. Determination of surface hydrophobicity

Surface hydrophobicity was determined, as described by Benjakul et al. (1997), using 1-anilinonaphthalene-8-sulphonic acid (ANS) as a probe. NAM dissolved in 10 mM phosphate buffer, pH 6.0, containing 0.6 M NaCl, was diluted to 0.1, 0.2, 0.3, and 0.5% (w/v) protein, using the same buffer. The diluted protein solution (2 ml) was treated with 10 μl of 8 mM ANS in 0.1 M phosphate buffer, pH 7.0. The fluorescence intensity of ANS-conjugates was measured using a FP-750 spectrofluorometer (Shimadzu, Kyoto, Japan) at an excitation wavelength of 374 nm and an emission wavelength of 485 nm. The initial slope of the plot of fluorescence intensity versus NAM concentration was referred to as SoANS.

2.9. Determination of protein solubility

Protein solubility was determined by the method of Benjakul and Bauer (2000). To 1 g sample, 20 ml of 0.6 M KCl were added and the mixture was homogenised for 1 min at a speed of 12,000 rpm, using an IKA homogeniser (Salangor, Malaysia). The homogenate was stirred at 4 °C for 4 h, followed by centrifuging at 8500*g* for 30 min at 4 °C. To 10 ml of supernatant, cold 50% (w/v) trichloroacetic acid was added, to obtain a final concentration of 10%. The precipitate was washed with 10% trichloroacetic acid and solubilised in 0.5 M NaOH. The sample was also directly solubilised by 0.5 M NaOH and used for total protein determination. Protein content was determined by the Biuret method (Robinson & Hodgen, 1940) and expressed as the percentage of the total protein in the sample.

2.10. Determination of shear force

Shrimp meats, raw and 5 cycle-freeze–thawed, were examined for shear force, using a TA-XT2i texture analyser (Stable Micro Systems, Surrey, England), equipped with a Warner-Bratzler shear apparatus (Brauer, Leyva, Alvarado, & Sandez, 2003). The operating parameters consisted of a cross head speed of 10 mm/s and a 25 kg load cell. The shear force, perpendicular to the axis of muscle fibres, was measured in six replicates for each sample. The peak of the shear force profile was regarded as the shear force value.

2.11. Determination of microstructure by scanning electron microscopy (SEM)

Raw and five cycle-freeze–thawed shrimps of both species were subjected to SEM analysis, as described by Jones and Mandigo (1982). Shrimp meat (middle part) 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 deionised water for 15 min and washing was twice repeated. The samples were then dehydrated with a serial concentration of 50–100% ethanol, for 15 min each. All specimens were coated with 100% gold (Sputter coater SPI-Module, PA, USA). The microstructure was visualized using a scanning electron microscopy (JEOL, JSM-5800 LV, Tokyo, Japan).

2.12. Statistical analysis

Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's multiple range test (DMRT) (Steel & Torrie, 1980). Statistical analyses were performed using the Statistical Package for Social Science (SPSS 11.0 for windows, SPSS Inc., Chicago, IL).

3. Results and discussion

3.1. Effect of freeze–thawing on exudate loss, AG and NAG activities

Exudate losses of black tiger shrimp and white shrimp, subjected to multiple freeze–thaw cycles, are presented in

Table 1
Exudate loss (%) of black tiger shrimp and white shrimp meats subjected to different freeze–thaw cycles

Freeze–thaw cycles	Shrimp species	
	Black tiger shrimp	White shrimp
0	0.69 ± 0.10aB	0.78 ± 0.02aB
1	0.83 ± 0.04aB	0.87 ± 0.09aB
3	0.92 ± 0.06aB	1.06 ± 0.13aB
5	1.39 ± 0.39bA	2.09 ± 0.37aA

Values are given as means ± SD from triplicate determinations. Different letters in the same row indicate significant differences ($P < 0.05$). Different capital letters in the same column indicate significant differences ($P < 0.05$).

Table 1. Higher amounts of exudate were observed when the freeze–thaw cycles increased ($P < 0.05$). With the freeze–thawing lower than three cycles, no differences in exudate volumes were noticeable between the species ($P > 0.05$). Nevertheless, the volume of exudate of white shrimp was greater, than that of black tiger shrimp when subjected to five cycles of freeze–thawing ($P < 0.05$). The increase in exudate volume indicates loss of water-holding capacity of the muscle. Therefore, repeated freeze–thawing showed detrimental effects on the shrimp muscle. Repeated melting and reformation of ice crystals caused damage to cell membranes and organelles. Freeze–thawing was also shown to increase the cooking loss of shrimp muscle (Srinivasan et al., 1997). The drip loss of muscle can lead to less acceptability, due to the loss of tasteful constituents, e.g. some amino acids or nucleotides.

AG and NAG activities in the exudate increased when the freeze–thaw cycles increased, as shown in Fig. 1a and b, respectively. At the same freeze–thaw cycle, AG and NAG activities of white shrimp were higher than those found in black tiger shrimp ($P < 0.05$). The differences in activities indicated differences in stability of shrimp tissues during the freeze–thawing process, as well as differences in enzyme activity in the organelles between the shrimp species. The formation and acceleration of ice crystals, dehydration, and the increase in solute, result in changes in muscle tissues (Shenouda, 1980). The cell damage of muscle was mainly attributed to ice crystal growth, as well as to the increased salt concentration in the unfrozen phase. Therefore, freezing can disrupt muscle cells, resulting in release of mitochondrial and lysosomal enzymes into the sarcoplasm (Hamm, 1979). AG and NAG have been used as markers of the freezing and thawing process of fish muscle (Benjakul & Bauer, 2000; Rehbein, 1979; Shimomura, Takahashi, Morishita, & Ueno, 1987). From the results, white shrimp muscle tissues were more susceptible to damage induced by freeze–thawing than were the tissues of black tiger shrimp. Thus, the quality of black tiger shrimp might be retained during frozen storage and freeze–thawing to a higher degree, than white shrimp.

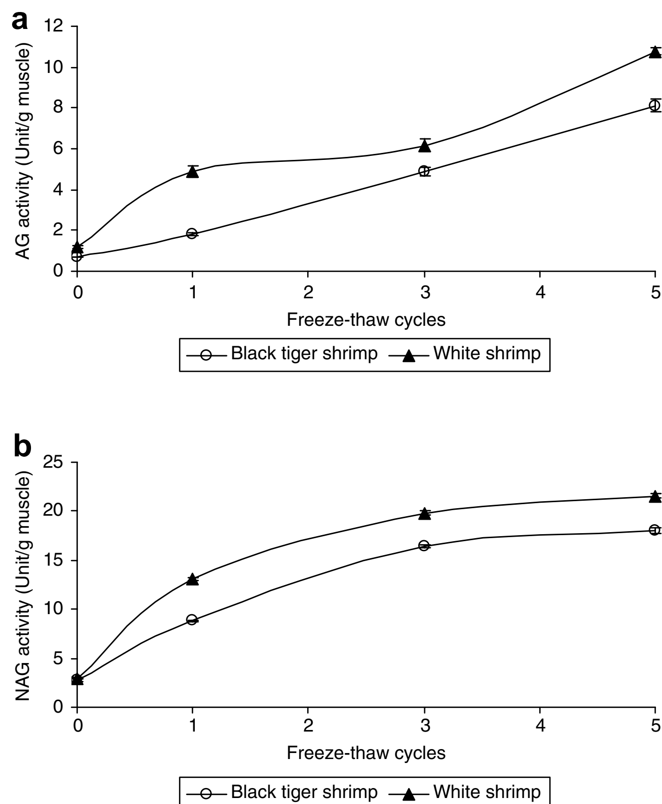

Fig. 1. Changes in α-glucosidase (a) and β-*N*-acetyl-glucosaminidase (b) activities of black tiger shrimp and white shrimp subjected to different freeze–thaw cycles. Bars represent the standard deviation from triplicate determinations.

3.2. Effect of freeze–thawing on Ca^{2+}-ATPase activity

Ca^{2+}-ATPase activities of NAM extracted from black tiger shrimp and white shrimp muscles subjected to different freeze–thaw cycles are depicted in Fig. 2. Decreases in Ca^{2+}-ATPase activity were noticeable after one cycle of freeze–thawing ($P < 0.05$). Thereafter, no marked changes were found with increasing freeze–thaw cycles up to five cycles ($P > 0.05$). It was found that the activities in black

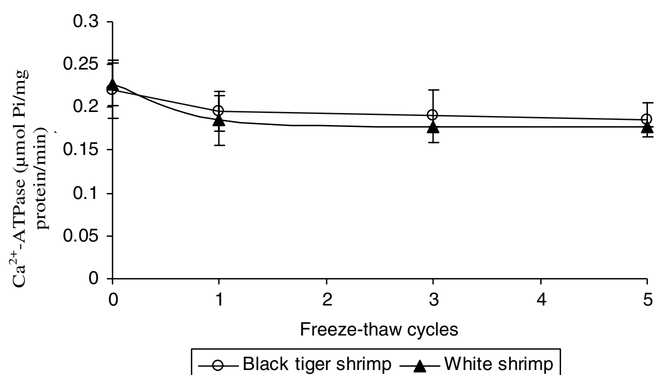

Fig. 2. Changes in Ca^{2+}-ATPase activity of natural actomyosin extracted from black tiger shrimp and white shrimp subjected to different freeze–thaw cycles. Bars represent the standard deviation from triplicate determinations.

tiger shrimp and white shrimp decreased by 16.4% and 22.0%, respectively, after five cycles of freeze–thawing. The decrease in Ca^{2+}-ATPase activity was possibly due to the conformational changes of the myosin globular head as well as the aggregation of this portion (Okada, Inoue, & Akiba, 1986). Rearrangement of proteins, via protein–protein interaction induced by the freeze–thawing process, might contribute to the loss in ATPase activity (Benjakul & Bauer, 2000). From the results, no differences in Ca^{2+}-ATPase activity were found between the species, regardless of freeze–thawing cycles. Thus, it was most likely that the stabilities of muscle proteins, especially myosin, toward the freeze–thawing process of both species, were similar. The result suggested that muscle protein, mainly myosin, underwent denaturation to a greater extent with increasing freeze–thaw cycles.

3.3. Effect of freeze–thawing on sulfhydryl and disulfide bond contents

Sulfhydryl (SH) group content of extracted NAM from both shrimps decreased with increasing freeze–thaw cycles ($P < 0.05$) (Fig. 3a). The SH group content of black tiger shrimp and white shrimp NAM decreased to 2.88 and 2.46 mol/10^5 g protein, respectively, after five freeze–thaw cycles. From this result, the decrease in SH group content was in agreement with the increase in disulfide bond content (Fig. 3b). The accelerated denaturation of myosin molecules, especially the conformational changes, in which the reactive sulfhydryl groups were exposed to oxidation, might result in increased disulfide bond formation. Cysteine is perhaps the most susceptible amino acid residue and it is usually one of the first to be oxidised (Thanonkaew, Benjakul, & Visessanguan, 2006). Another sulphur-containing amino acid, methionine, is also readily oxidised to methionine sulfoxide derivative (Vogt, 1995). The amino acids with reactive side chains (sulfhydryl, thioether, amino group, imidazole ring and indole ring) are particularly susceptible to oxidation initiated by oxidising lipids and their products (Gardner, 1979). Oxidised myofibrils showed substantial changes in sulfhydryls and disulfide bonds (Xiong, 2000). From the results, white shrimp tended to show greater decrease in sulfhydryl group content with a greater disulfide bond content, than black tiger shrimp, particularly after five cycles of freeze–thawing. Thus, white shrimp was possibly more prone to sulfhydryl oxidation than was black tiger shrimp. Benjakul et al. (2003) reported that the difference in sulfhydryl content among species during frozen storage might be due to differences in susceptibility to sulfhydryl oxidation of myofibrillar proteins.

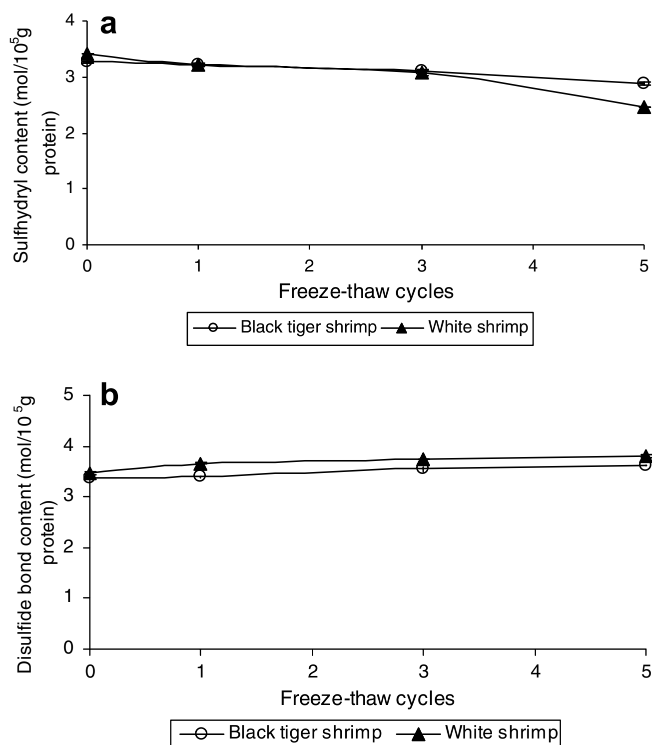

Fig. 3. Changes in sulfhydryl group content: (a) and disulfide bond content; (b) of natural actomyosin extracted from black tiger shrimp and white shrimp subjected to different freeze–thaw cycles. Bars represent the standard deviation from triplicate determinations.

3.4. Effect of freeze–thawing on surface hydrophobicity

The changes in surface hydrophobicity (SoANS) in extracted NAM from both shrimps, as influenced by freeze–thaw cycles, are shown in Fig. 4. In general, surface hydrophobicity of NAM from black tiger shrimp and white shrimp increased when the freeze–thaw cycles were greater than three cycles. After five cycles, surface hydrophobicity of NAM from both species increased by 21.9% and 37.9%, respectively. The polar (hydrophilic) residues are generally exposed to water, while the nonpolar (hydrophobic) groups or moieties are generally localised in the molecule. An increase in SoANS, regardless

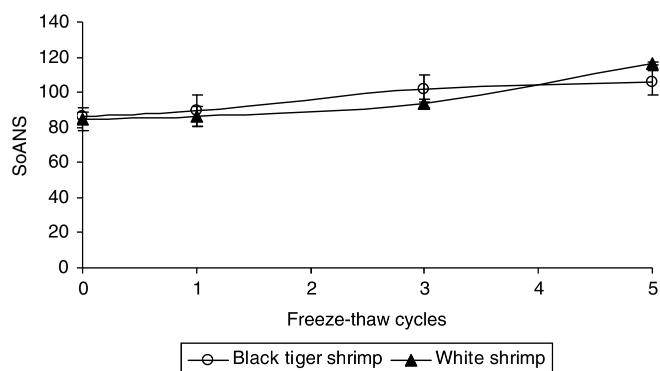

Fig. 4. Changes in surface hydrophobicity of natural actomyosin extracted from black tiger shrimp and white shrimp subjected to different freeze–thaw cycles. Bars represent the standard deviation from triplicate determinations (SoANS is initial slope of fluorescence intensity against NAM concentration).

of the cause, is assumed to result from structural alterations, which, in some cases, would mean an irreversible denaturation (Nakai & Li-Chan, 1988). After five cycles of freeze–thawing, SoANS of white shrimp was greater than that of black tiger shrimp, suggesting that the former was more prone to the conformational changes induced by repeated freeze–thawing.

3.5. Effect of freeze–thawing on solubility

Protein solubility (in 0.6 M KCl) of black tiger shrimp and white shrimp muscle subjected to multiple freeze–thaw cycles is depicted in Fig. 5. Protein solubility of both shrimps slightly decreased when the freeze–thaw cycles increased ($P < 0.05$). White shrimp had a greater decrease in protein solubility than had black tiger shrimp, particularly after five freeze–thaw cycles ($P < 0.05$). The decrease in solubility was in accordance with the increase in surface hydrophobicity (Fig. 4). The loss in salt-soluble protein suggested that protein denaturation was induced by the freeze–thawing process. The decrease in solubility was most likely associated with the formation of disulfide bonds (Fig. 3). The decrease in solubility of protein has been used as a marker of oxidative deterioration of muscle protein (Decker, Xiong, Calvert, Crum, & Blanchard, 1993; Srinivasan & Hultin, 1997; Xiong & Decker, 1995). Thermodynamically, a decrease in protein solubility is the result of a shift from a balance of protein intermolecular interaction and protein–water interaction, resulting in a situation where protein intermolecular interaction is strengthened, while protein water interaction is weakened (Vojdani, 1996). As a result of loss of ordered tertiary structure, the cross-linkages are formed among proteins, as evidenced by the decrease in solubility. Free radical attack is also a major cause of decreased protein solubility (Decker et al., 1993). The decrease in solubility was also associated with the increase in exudate of both species with increasing freeze–thaw cycles (Table 1).

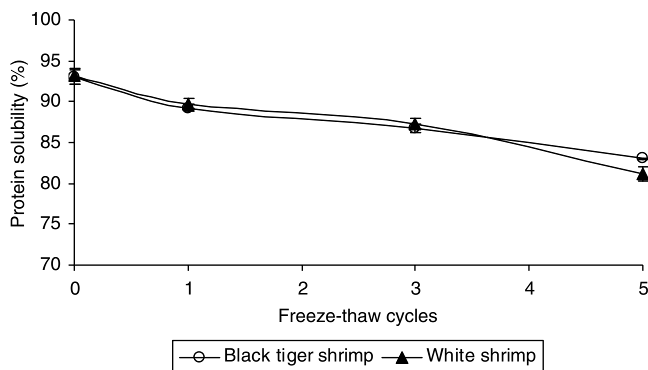

Fig. 5. Changes in protein solubility of black tiger shrimp and white shrimp subjected to different freeze–thaw cycles. Bars represent the standard deviation from triplicate determinations.

3.6. Effect of freeze–thawing on shear force

Shear force of black tiger shrimp and white shrimp, before and after subjecting to five freeze–thaw cycles, is presented in Table 2. Decrease in shear force of both shrimps occurred after freeze–thawing ($P < 0.05$). The shear force value of white shrimp was lower, than that of black tiger shrimp, regardless of freeze–thawing ($P < 0.05$). The decrease in shear force suggested loss in integrity of muscle fibres, leading to the weakening of muscle. Repeated melting and reformation of ice crystals caused damage of cell membranes, organelles, as well as muscle structure. Shear force correlated either with the diameter (width) of the muscle portion sheared or with the weight of the shrimp (Srinivasan et al., 1997). Size of muscle or bundles had a major effect on shrimp meat tenderness (Srinivasan et al., 1997). Due to the same size of shrimp used, the differences in shear force might be caused by the different compositions, particularly in collagen content (Sriket, Benjakul, Visessanguan, & Kijroongrojana, 2006). Differences in microstructure, particularly in the arrangement of muscle fibres between species, are also possible. Therefore, freeze–thawing showed a detrimental effect on shrimp texture, as manifested by the lower shear force.

3.7. Effect of freeze–thawing on microstructure

Microstructures of black tiger shrimp and white shrimp subjected to five freeze–thaw cycles, in comparison with fresh shrimp, are shown in Fig. 6a and b. For the longitudinal sections, shrinkage of fibres was noticeable, as evidenced by the gaping formed (Fig. 6a). Also, losses of Z-disks were observed after freeze–thawing. However, the destruction of Z-disks was more pronounced in white shrimp after freeze–thawing. It was reported that the freeze–thawing process caused shrinkage and drip loss of muscle fibers (Hale & Waters, 1981). Cross-linking of myosin heavy chain, through disulfide and nondisulfide covalent bonds during frozen storage, contributed to the formation of high-molecular-weight polymers and aggregates (Ragnarsson & Regenstein, 1989). This might be associated with the shrinkage of muscle fibres. For the transverse section, muscle bundles were more separated when subjected to five

Table 2
Shear force (N) of black tiger shrimp and white shrimp meats before and after subjecting to five freeze–thaw cycles

Shrimp species	Freeze–thaw cycle	
	0	5
Black tiger shrimp	26.42 ± 1.03aA	20.56 ± 0.56bA
White shrimp	22.26 ± 0.35aB	16.52 ± 0.43bB

Values are given as means ± SD from triplicate determinations. Different letters in the same row indicate significant differences ($P < 0.05$). Different capital letters in the same column indicate significant differences ($P < 0.05$).

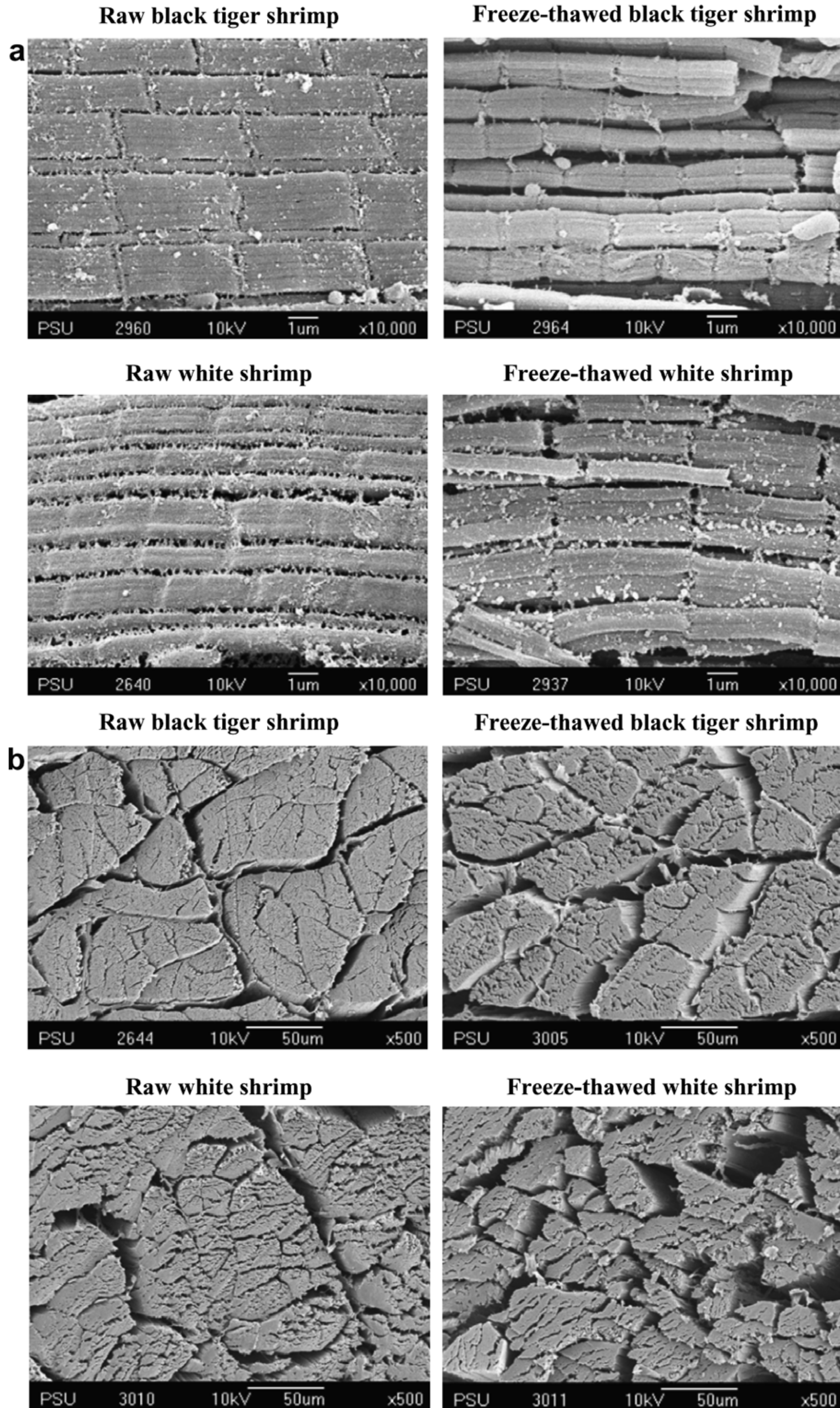

Fig. 6. Microstructure of black tiger shrimp and white shrimp before and after subjecting to five freeze–thaw cycles; longitudinal section (a) and transverse section (b).

freeze–thaw cycles (Fig. 6b). Protein denaturation and disruption of endomysium, induced by freeze–thawing, possibly resulted in a less compact structure. The looser structure and disruption of muscle fibres were coincidental with the lower shear force value of both shrimps. The disrupted structure, together with the denaturation of myofibrillar protein induced by freeze–thawing, was most likely associated with the lowered water-holding capacity of muscle, as shown by the higher drip loss (Table 1).

4. Conclusion

Changes in physicochemical properties of protein induced by freeze–thawing were somehow different

between white shrimp and black tiger shrimp and the degree of changes was more pronounced with increasing freeze–thawing cycles. Freeze–thawing caused protein denaturation, tissue disruption and damage to muscle fibres. Disulfide bond formation and hydrophobic interaction were different between species and might be associated with the loss in protein integrity of both species. Such differences might govern the varying quality losses between the species, particularly as affected by freeze–thawing process.

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