Physicochemical Changes in Prawns (*Machrobrachium rosenbergii*) Subjected to Multiple Freeze-Thaw Cycles

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ABSTRACT

Fresh prawns were subjected to five freeze-thaw cycles (−29°C ⇄ 22°C) and associated physicochemical changes in muscle were determined. Shear force to break prawn tails was dependent on prawn size, more on weight (r = 0.75-0.81) than on diameter (r = 0.56-0.66). No change (P > 0.05) in shear force was observed in raw prawns after five freeze-thaw cycles. However, shear force of cooked prawns decreased after three freeze-thaw cycles which coincided with accelerated lipid oxidation in raw prawns. Electrophoretic analysis revealed gradual decreases in myosin, actin, and most other myofibrillar proteins with each successive freeze-thaw cycle. Enthalpy of protein denaturation decreased from 16.6 J/g (fresh) to 13.5 J/g after one freeze-thaw cycle with minor changes thereafter.

Key Words: prawns, freeze thaw, protein stability, quality

INTRODUCTION

FROZEN STORAGE is an important preservation method for seafood but quality deteriorations (e.g., texture, flavor, and color) in muscle tissues occur during frozen storage and have been extensively reviewed (Connell, 1964; Matsumoto, 1979; Shenouda, 1980). The extent of quality loss is dependent upon many factors, including storage temperature, rate of freezing and thawing, temperature fluctuations, and freeze-thaw abuse during storage. Repeated freeze-thaw is especially common at retail and in the home or restaurant (Giddings and Hill, 1978), but any related changes in protein and muscle texture have not been fully characterized.

The freezing process induces muscle tissue changes by the formation and accretion of ice crystals, dehydration, and increases in solute (Shenouda, 1980). Freezing and thawing disrupt muscle cells causing the release of enzymes from mitochondria into the sarcoplasm (Hamm, 1979). Fiber shrinkage and exudation of fluid (drip loss) are deleterious changes in muscle tissue following freezing and thawing. Thawed meat tends to be higher in shear force than nonfrozen meat (Hale and Waters, 1981). Such increase in toughness during frozen storage of shrimp and other seafood is attributed to myosin denaturation, as well as cross-linking and aggregation of myofibrillar proteins (Sikorski et al., 1976). Freeze-thaw processes can also promote lipid oxidation which may affect texture of crustacean muscle.

The principal forces maintaining the native structure of most proteins are hydrophobic and the principal destabilizing forces appear to be entropic (Dill, 1990). Hydrogen-bonding, dipole-dipole interactions, electrostatic interactions, and disulfide linkages also are important to the stability of proteins. The difference between native and denatured states of many proteins is about 10–20 Kcal/mole (Creighton, 1994). Destabilizing forces on proteins increase with changes such as temperature abuse (e.g., cyclic freezing and thawing), and eventually result in protein unfolding. During multiple freeze-thaw cycles, recrystallization of water or exudate may increase protein denaturation and fiber shrinkage by mechanical damage. Such temperature fluctuations/abuse during storage, transportation, retail display, and consumption may deteriorate the quality of prawns. Although there is much published information on the effects of frozen storage on quality of food products, reports of the effects of repeated freeze-thaw cycles on quality of prawns are few. Our objective was to determine the stability of freshly harvested prawns (*Machrobrachium rosenbergii*) compared to those subjected to multiple freeze-thaw cycles and assess any associated physicochemical deterioration in raw and cooked prawns.

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MATERIALS & METHODS

Materials

Juvenile prawns (0.5g live mean weight) were grown in 0.02-ha ponds for 117 days to a mean live weight of 31.5g at Kentucky State University Aquaculture Research Center, Frankfort. Harvested prawns were held live in 3,000L flow-through tanks until processed. Randomly selected prawns (200) from a pool of 1700 were deheaded and immediately cooled on ice. The tails with shells intact were individually frozen in a still freezer at −29°C for 2h before packaging.

Freezing and thawing

Individually frozen prawns were loosely packaged in a moisture-impermeable polyethylene bag (Cryovac, Duncan, SC) and stored at −29°C for 4 days. The frozen prawns were thawed in a stainless steel pan with running tap water (22 ± 2°C) for 30 min simulating handling practices during food services (e.g., restaurants). The flow rate of water was maintained low to avoid agitation. The temperature at the center of thawed prawns, measured with a thermocouple, was 20 ± 2°C. Randomly selected prawns (20 from 200) were immediately placed on ice for analysis. The remaining 180 thawed prawns were again individually frozen in a still freezer for 2h at −29°C and stored in a polyethylene bag at the same temperature for another 4 days as described. The freeze-thaw procedure, i.e., 4 days frozen at −29°C followed by thawing in tap water for 30 min, was repeated for five cycles.

Cooking

Half the thawed prawns (10 of 20) were cooked in boiling water for 2 min and placed immediately on ice for further analysis. The other half were analyzed raw. Cooking loss (%) was expressed as the weight loss (g, after cooking) divided by the original weight (g, before cooking) of prawns then multiplying by 100.

Preparation of samples for analysis

Shells of cooked and uncooked deheaded prawns were removed. The width of the first major muscle from the anterior of the tail (head end) was measured with a vernier caliper, and the weight of each shell-less prawn was recorded. The individual tails (10/treatment) were then subjected to textural analysis (shear test). After textural analysis, three randomly selected prawns from each freeze-thaw cycle were homogenized in a Mini Chopper (Black and Decker, Inc., Shelton, CT) for 30 sec. The minced muscle was analyzed immediately for physicochemical properties.

Textural analysis

Shear forces required to rupture the first intact major muscle from anterior of the tail were measured using a Model 4301 Instron Universal Testing Instrument (Instron Corp., Canton, MA) with a Warner-Bratzler

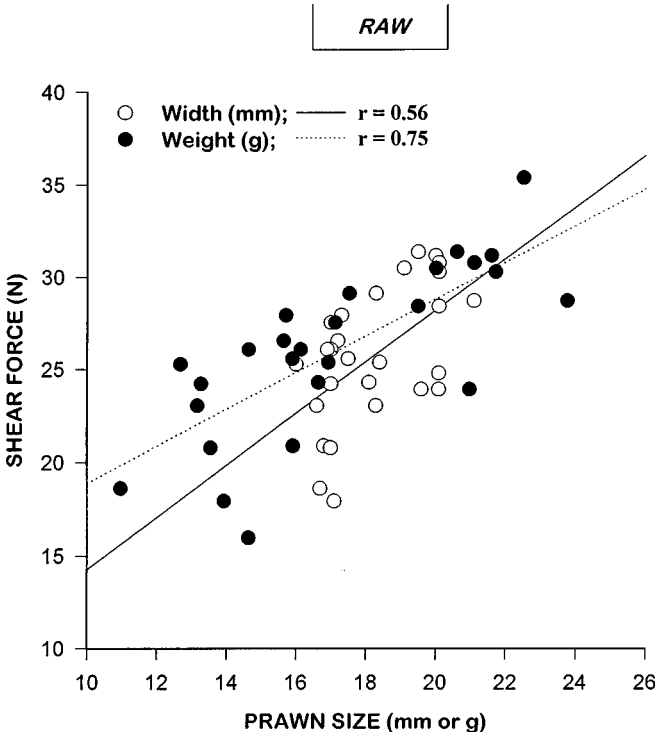

Fig. 1—Correlation of raw prawn size (width or weight) with shear force.

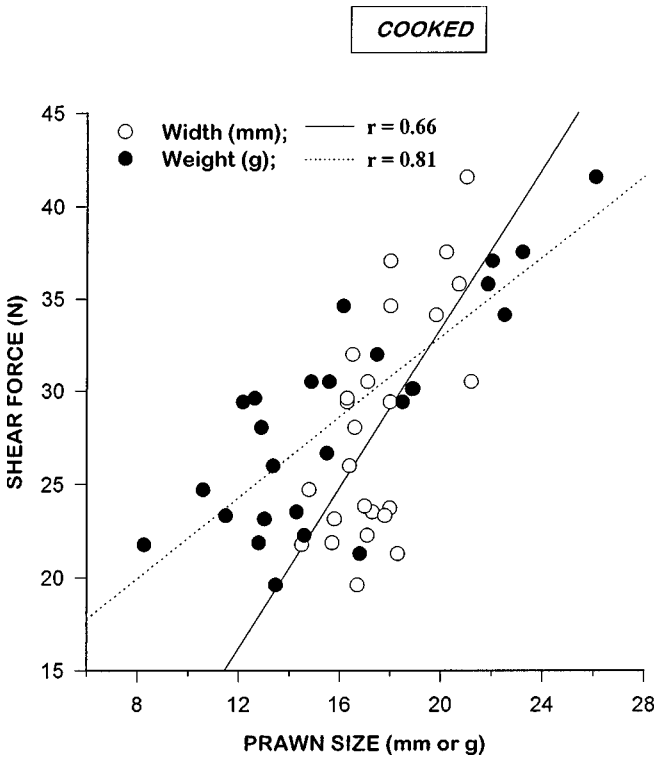

Fig. 2—Correlation of cooked prawn size (width or weight) with shear force.

shearing device attached to the load cell (100 kg capacity). The tail was placed in a transverse position to the blade so that the blade would cut the tail across the muscle fibers. The cross-head speed of the Instron was 20 mm/min. The first major peak (usually the highest, which represented the maximal shear force required to cut the muscle sample) was recorded. Shear force values for individual prawns were normalized based on the diameter of the first intact muscle from the anterior of the tail (N/mm) and on the weight of the prawns (N/g) to determine any correlations with prawn size.

Thiobarbituric acid-reactive substances (TBARS)

The TBARS in raw prawn muscle tissues were determined using the colorimetric method described by Witte et al. (1970).

Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was done by the method of Laemmli (1970) using an SE 250 Mighty Small II slab gel electrophoresis unit (Hoefer Scientific Instruments, San Francisco, CA). The stacking gel and the resolving gel contained 3% and 10% acrylamide. Samples for electrophoresis were prepared by homogenizing 1g minced muscle in 100 mL cold (~5°C) distilled deionized water with a Polytron (Brinkman Instruments, Inc., Westbury, NY) for 30 sec. The homogenate was diluted 1:1 in the sample buffer containing 4% SDS, 0.125 M Tris (pH 6.8), 20% glycerol, and 10% β-mercaptoethanol.

Differential scanning calorimetry (DSC)

Thermal denaturation of proteins was measured in a Model 2920 modulated DSC machine (TA Instruments, New Castle, DE). The instrument was calibrated for temperature and baseline using indium as standard. Accurately weighed samples (14–17 mg) were placed in hermetically sealed polymer-coated aluminum pans (TA Instruments, New Castle, DE). An empty, sealed pan was used as reference. A heating rate of 10°C/min was used to thermally scan samples from 10 to 100°C. The total enthalpy change (ΔH) associated with protein denaturation was estimated by measuring the area under the DSC transition curve with a straight baseline constructed from the start to the end of the endotherm. Temperature at maximum heat flow (T_{max}), i.e., temperature at peak of the endotherm, was recorded. The onset melting temperature (T_m) was determined by constructing a tangent to the leading edge of the transition and determining the temperature at the point of intersection with the baseline using Universal Analysis Ver 1.2 N (TA Instruments, New Castle, DE).

Statistical analysis

Data were analyzed using the General Linear Models procedure of the Statistix 3.5 software package (Analytical Software, Inc., St. Paul, MN) for microcomputers. Analysis of variance (AOV) was done to determine the significance of main effects of freezing and thawing. Significant ($P \leq 0.05$) differences between means were identified using Least Significant Difference procedures (Snedecor and Cochran, 1989).

RESULTS & DISCUSSION

SHEAR FORCE correlated either with the diameter (width) of the muscle portion sheared or with the weight of the prawn (Fig. 1 and 2). The correlation coefficient (r) of shear force with width of raw prawns was 0.56 while that of shear force with weight was 0.75. Measurement of width of raw prawns with a vernier caliper was somewhat difficult due to softness of raw tissue. After cooking, correlation coefficients of both width and weight with shear force increased (Fig. 2). Measurement of width of cooked prawns with the caliper was facilitated by firmness of cooked tissue resulting in a more accurate width determination. This might explain the higher correlation value with width for cooked samples. The correlation of shear force with weight (r = 0.86) for cooked prawns was higher than with width (r = 0.66). Therefore, all further shear force measurements were done on prawns of known weights (rather than widths). The positive correlation of prawn size with shear force indicated that the size of muscle or bundles had a major effect on meat tenderness. Since all prawns were of the same age, the size variation might be a reflection of a complex population structure involving different morphotypes (Tidwell et al., 1994). Therefore, it is important to measure the shear forces on individual prawns of known weights and normalize values for meaningful comparisons between treatments.

Tissue toughening is common to many low-fat fish species stored at sub-freezing temperatures and is an important factor for reduced acceptability. The composition of prawns (moisture: 77.5%; protein: 20.5%; fat: <0.1%) (Tidwell et al., 1993) is somewhat similar to white-fleshed, less-fatty fish. Thus, prawn muscle was expected to toughen in texture due to frozen storage. Hale and Waters (1981) reported toughening of raw muscle from frozen freshwater shrimp, and they attributed this to fiber shrinkage and drip loss. They also found, however, that muscle tissue of the same prawns after cooking was softer than that of non-frozen controls. The softening effect was attributed more to the prawns being boiled with an intact head than to changes due to frozen storage. Our results showed that shear force of raw prawn tissue was not affected ($P > 0.05$) by freeze-thaw treatment, but shear force of cooked muscle decreased by ~21% after 3 cycles with no further changes ($P > 0.05$) thereafter (Fig. 3). Kim et al. (1986) also reported that increasing the number of freeze-thaw cycles reduced the strength and deformation (measured by torsion test) of fish surimi gels and suggested that certain domains of proteins may be particularly labile to freeze-thaw abuses.

Note that textural changes not observed in raw prawns after exposure to multiple freeze-thaw cycles occurred in cooked prawns. Repeated melting (i.e., thawing) and reformation of ice crystals (i.e., freezing) in multiple freeze-thaw situations is clearly detrimental to muscle tissues by causing mechanical damage to cell membranes and organelles. Hamm (1979) demonstrated the release of enzymes from muscle cell mitochondria into the sarcoplasm upon freezing and thawing. The losses of muscle cell integrity and other associated changes due to multiple freeze-thaw cycles were apparently nondetectable in raw prawns by the Instron shear testing. Cooking may have increased any changes in the freeze-thaw abused prawn muscle tissue, including cell membrane constituents and susceptible structural proteins such as myosin. The internal temperature of the prawn muscle after cooking was 73 ± 6°C at which the majority of muscle endogenous proteases would have been inactivated. Note, however, that the likelihood still existed that some heat stable proteases might be activated during cooking and they could have affected tissue softening. Cooking yield as determined by weight loss after cooking was affected by freeze-thaw cycle abuse (Fig. 4). Weight loss of cooked prawns was 11.7% (fresh) while the loss increased to 15.2–17.8% for freeze-thaw abused prawns.

In order to explain textural changes in temperature-abused prawns, physicochemical analyses were performed on raw and cooked muscle. Since lipid oxidation was implicated in textural deterioration in many fish species (Shenouda, 1980), it was monitored in each freeze-thaw cycle. The TBARS of raw prawn samples increased gradually during the first three freeze-thaw cycles and then rapidly after four cycles (Fig. 5). The accelerated lipid oxidation after three freeze-thaw cycles could be due to the release of oxidative enzymes and prooxidants from various cellular organelles, and additionally, to the depletion of antioxidant defense systems in the prawn muscle. Rehbein and Orlick (1990) showed that strong lipid oxidation slightly influenced texture of frozen stored fish mince. This was confirmed by our observation that although lipid oxidation occurred in raw prawns after multiple freeze-thaw cycles, there were little changes in shear forces of raw prawns. The rapid increase in TBARS of raw prawns after three freeze-thaw cycles however coincided with a decrease in shear force of cooked prawns. Any relationship between lipid oxidation and tissue changes in prawns could not be established based on our results.

Electrophoretic analysis revealed gradual decreases in the intensity of myosin heavy chain, actin, and most other myofibrillar protein bands with each successive freeze-thaw cycle. Two protein bands (tropomyosin and myosin light chain 1), tentatively identified by reported relative mobilities (Porzio and Pearson, 1977), appeared to be unaffected by freeze-thaw treatments (Fig. 6). Huidobro and Tejada (1995) showed that tropomyosin in whiting and mackerel muscle remained stable even after 1-yr storage at −18°C but significant reductions in myosin heavy chain and actin occurred. Cross-linking of myosin heavy chain through disulfide and nondisulfide covalent bonds contributed to the formation of high-molecular-weight polymers and aggregates (Ragnarsson and Regenstein, 1989) which might explain the loss of myosin heavy chain and actin upon freezing and thawing. However, the gradual loss of myofibrillar proteins due to freeze-thaw cycles did not fully account for the observed decrease in textural quality of cooked prawns after three cycles. We could speculate that the effect of protein insolubilization might be cu-

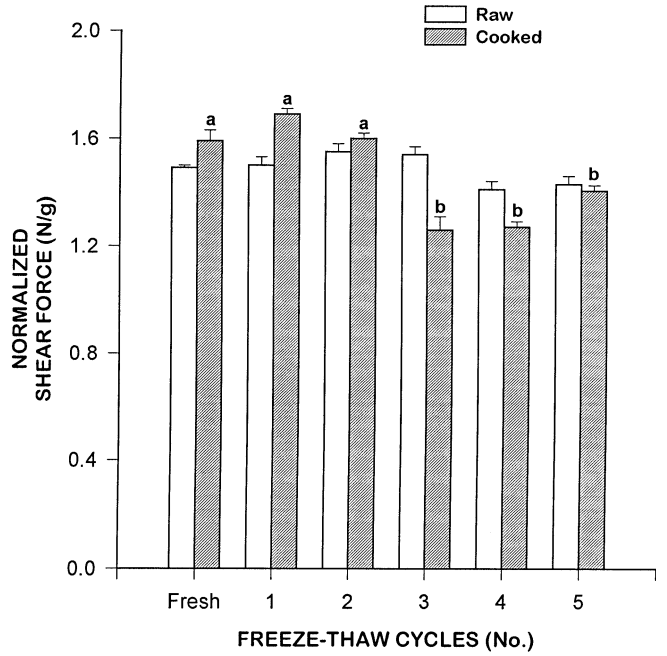

Fig. 3—Effect of freeze-thaw cycles on shear force of prawn muscle. No shear force differences ($P \geq 0.05$) were found between raw prawns with different freeze-thaw cycles; for cooked prawns, shear forces with different letters differed significantly ($P \leq 0.05$).

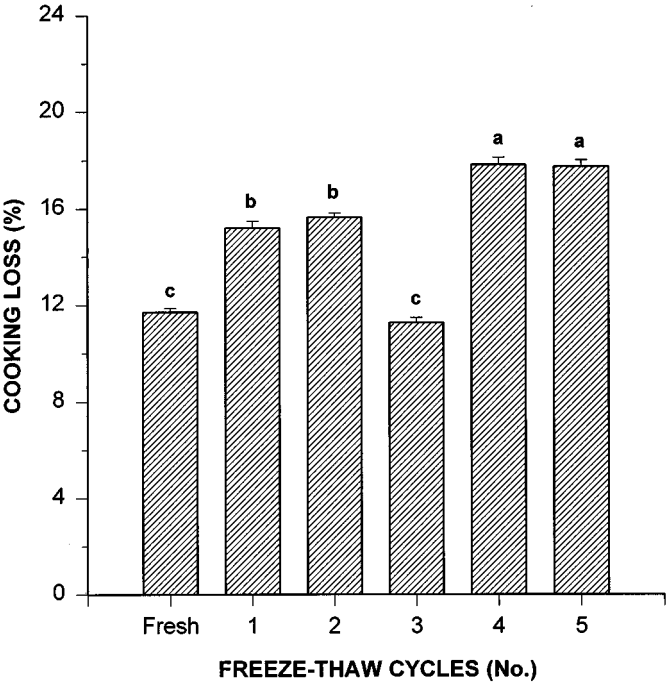

Fig. 4—Effect of freeze-thaw cycles on cooking loss in prawns. Cooking losses with different letters differed significantly ($P \leq 0.05$).

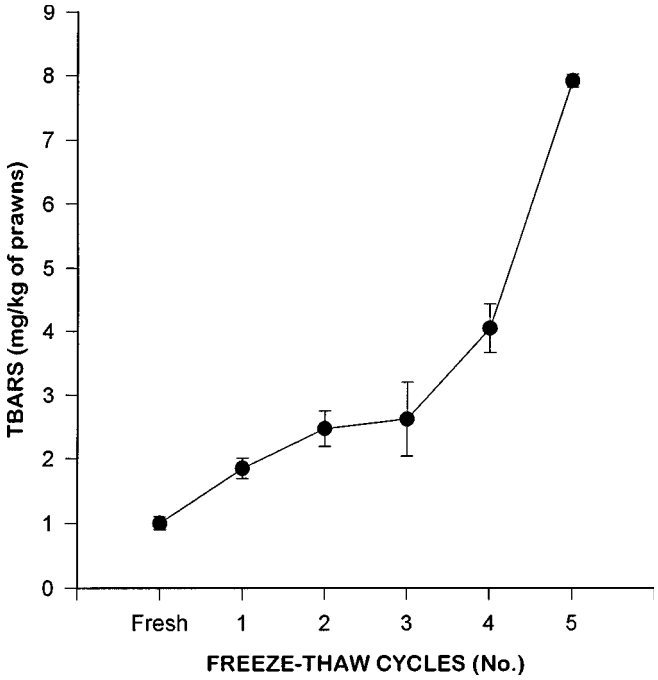

Fig. 5—Formation of thiobarbituric acid-reactive substances in raw prawn tails subjected to five freeze-thaw cycles.

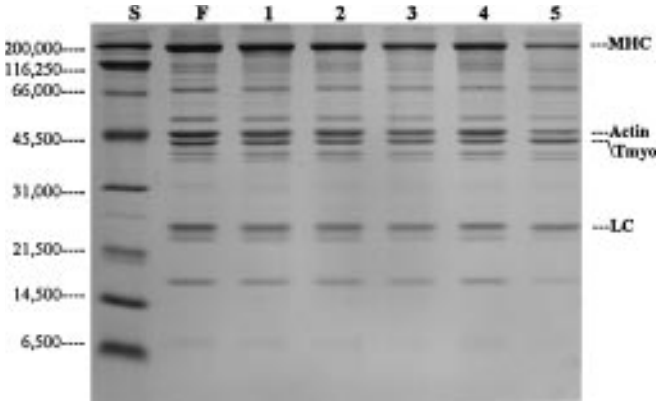

Fig. 6—Electrophoretic patterns of prawn tails subjected to five freeze-thaw cycles. Lane S contained standard proteins with molecular weights indicated on the left margin. Lane F: fresh prawn; lanes 1 to 5: number of freeze-thaw cycles. MHC: myosin heavy chain; Tmyo: tropomyosin; LC: myosin light chain 1.

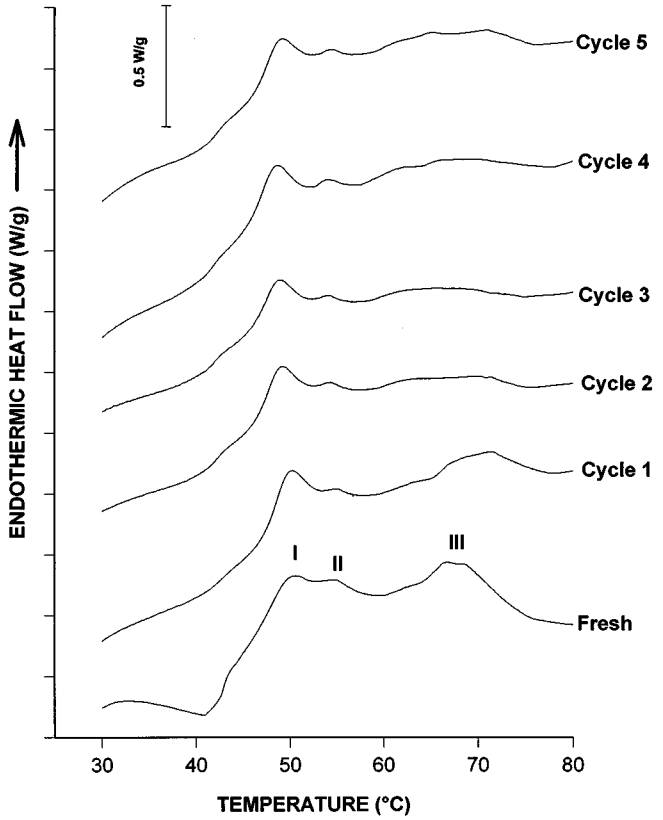

Fig. 7—DSC thermograms of prawn tail muscle subjected to five freeze thaw cycles. Fresh: prawn muscle less than 6h postmortem. Cycles 1 to 5: number of freeze-thaw cycles to which prawn muscles were subjected.

mulative, i.e., only after it reached a certain point did textural changes in muscle tissue become detectable.

Differential scanning calorimetric analysis produced multiple transition peaks for fresh prawn whole muscle presumably corresponding to myosin (I) and actin (III) denaturation (Stabursvik and Martens, 1980) (Fig. 7). Peak II could be attributed to myosin, sarcoplasmic proteins, and connective tissue. The height of peak I for fresh prawns was quite low compared to those of freeze-thaw treated prawns. The small size of peak I for fresh prawn (<6h postmortem) could be attributed to an exothermic heat flow (~40°C) which has been widely observed in muscle tissue shortly after death. Wright et al. (1977) attributed the exothermic heat flow at temperatures lower than the myosin denaturation temperature (commonly observed in pre-rigor muscle) to the muscle contraction process. Park and Lanier (1989) hypothesized the exothermic heat flow as rapid ATP hydrolysis induced with rising temperature. This exothermic process was conspicuously absent in prawns subjected to freeze-thaw cycles. Freezing and thawing treatments resulted in changes in muscle thermograms. The peak corresponding to actin denaturation (III) (67.7°C) broadened after freezing and thawing treatments, and the maximum temperature (T_{max}) associated with this transition became difficult to measure. The peak broadening might be indicative of partial destablization of actin or reduced cooperative nature of the major segments of the protein. The onset temperature for myosin denaturation (T_m) decreased from 45.3°C (fresh) to 43.5°C after four cycles, and to 44.3°C after five cycles (Table 1). A concomitant decrease in T_{max} for myosin from 50.5°C (fresh) to 48.9°C (five cycles) occurred. The total enthalpic change during denaturation of whole muscle tissue decreased from 16.6 J/g for fresh muscle to 11.4 J/g after five freeze-thaw cycles, with more than half the decrease resulting from the first freeze-thaw cycle. Hastings et al. (1985) reported an initial reduction in myosin enthalpy of cod muscle after 2 wk storage at −10°C with no further changes in myosin enthalpy during subsequent storage. The initial reduction in enthalpy was ascribed to partial denaturation of myosin due to freezing that occurred in the initial stages of frozen storage.

Overall, from the physicochemical and textural analyses softening of cooked prawn tissue appeared to be related to the loss of muscle cell integrity. Disruption of muscle cells could be caused by mechanical damage associated with repetitive ice crystal formation during multiple freeze-thaw cycles. The involvement of endogenous proteases was also possible. Many endogenous proteases are considered important in postmortem degradation of muscle proteins (Asghar and Bhatti, 1987; Jiang et al., 1990). Among them is calpain which has been isolated from crustacean muscle (Mykles and Skinner, 1982). The activity of proteases was possibly low in frozen muscle tissue. However, the thawing and cooking processes may stimulate proteases which could cause considerable textural changes in cooked prawns. As there was no strong evidence of protein hydrolysis in raw, thawed muscle when analyzed by electrophoresis, we speculated that the

Table 1—Differential scanning calorimetric analysis of prawns subjected to freeze-thaw cycles

Freeze-thaw cycles (No.)	T_m[a] (°C)	T_{max}[b] (°C)	ΔH[c] (J/g)
Fresh	45.33	50.51	16.6
1	45.31	49.64	13.5
2	44.56	49.32	11.4
3	44.16	49.53	11.6
4	43.50	48.55	12.9
5	44.32	48.86	11.4

[a] T_m (°C): Onset melting temperature for peak I (Fig. 7).
[b] T_{max} (°C): Temperature at maximum heat flow corresponding to peak I (Fig. 7).
[c] ΔH (J/g): Total enthalpy change in denaturation of the whole prawn muscle.
Data represent an average of six replicates of DSC thermograms. Variability of temperature in thermograms was less than $\pm$ 0.5°C.

activation of proteases, if any, occurred only during cooking. Factors which caused the two opposing effects, i.e., tissue-softening (e.g., due to proteases and ice crystal formation) and tissue-toughening (e.g., due to protein denaturation and cross-linking), may have both been active. Because of their counter-action, individual effects on textural changes in cooked prawns during the first three freeze-thaw cycles were diminished. After the fourth cycle of freezing and thawing, the tissue-softening effect apparently became prevalent.

CONCLUSIONS

FRESH WATER PRAWNS (*Machrobrachium rosenbergii*) remained stable against temperature abuses up to three freeze-thaw cycles. However, additional freeze-thaw cycles were detrimental to overall physicochemical and textural quality. A rapid increase in lipid oxidation in raw prawns was accompanied by softening of cooked prawns as evidenced by decreases in shear force. Thus, freeze-thaw cycles should be kept to less than three to ensure high eating quality.

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