Changes of Myofibrillar Proteins and Texture in Freshwater Prawn, *Macrobrachium rosenbergii*, During Iced Storage

H. W. KYE, W. K. NIP and J. H. MOY

ABSTRACT—Changes in myofibrillar proteins and texture of freshwater prawn, Macrobrachium rosenbergii, *during 14-day iced storage were studied. Degradation of myofibrillar proteins with 113,000, 103,000, and 80,000 daltons and an increase of 25,000 and 31,000 dalton protein subunits were observed during iced storage of the prawns. Significant changes in texture of the ice-stored and cooked prawn muscle were demonstrated. Relationship of myofibrillar protein degradation and textural changes are discussed.*

Introduction

Shelf life of ice-chilled freshwater prawn, *Macrobrachium rosenbergii* has been reported to be 3-4 days with development of mushiness at the proximal end (Sidwell, 1977; Nip et al., 1985a). Microstructural changes as related to development of mushiness has been demonstrated (Nip and Moy, 1988). Proteolysis of muscle proteins by proteolytic and/or collagenolytic enzymes has been postulated as the main mechanism which contributes to postmortem mushiness in freshwater prawn (Rowland et al., 1982; Baranowski et al., 1984; Nip et al., 1985b; Premaratne et al., 1986). However, the nature of this protein degradation is not fully understood. Reports on changes of prawn/shrimp protein components have been very limited. Wong (1982) studied the microstructural changes in muscles in penaid shrimp during iced storage and demonstrated myofibrillar breakdown, especially Z-lines and sarcoplasmic reticular degradation.

Objective/instrumental methods have been used successfully in assessing the textural quality in shrimp and prawn (Ma et al., 1983; Soo and Sander, 1977; Ahmed et al., 1972; Angel et al., 1985; Tillman and Finne, 1983; Waters and Hale, 1981; Nip and Moy, 1981). Nip et al. (1985b) reported that the texture, as measured by the PEP Texture Tester[1] (PEP Co., Houston, TX), showed significant softening with ice chilling time and was related to the mushiness problem in *M. rosenbergii*.

Relationship on structural protein degradation and textural changes in prawn or shrimp has not been reported. The purpose of this study was to investigate the degradation of myofibrillar proteins and the textural changes in freshwater prawn, *M. rosenbergii*, and their relationship during iced storage.

Materials and Methods

Muscle Samples

Freshly harvested live prawns averaging 78 g in weight and 200 mm in length (tip of rostrum to tip of telson) were obtained from a local (Honolulu, Hawaii) market and held in a tank with running tap water overnight at ambient temperature (20°-23°C). The prawns were killed by placing them in an ice slurry. Upon death, whole prawns were stored in slush ice in an insulated container at 0°C. After 0, 1, 3, 5, 7, 10, and 14 days of storage, the abdomen (tail) muscle was separated from the shell and head and then sectioned. After a brief washing with distilled water, the first proximal segment (section in the vicinity of the stomach) and/or the third proximal segment (the third section away from the stomach) from three prawns were pooled and subject to protein extraction.

Protein Extraction

The first and third segments from the prawns stored for 0, 1, 3, 5, 7, and 14 days were subject to myofibrillar isolation according to the procedure of Olson et al. (1977). Myofibril suspensions were then brought to 50 percent glycerol (w/w) and placed in the freezer at −20°C for 2-14 days. At the end of the iced storage period, the myofibril suspensions were analyzed within 2-3 days.

Protein Determination

The protein concentrations of all samples were determined according to the Bio-Rad Protein Assay (Bio-Rad Lab., Richmond, Calif.) with bovine gamma globulin as the standard.

Protein Electrophoresis

Polyacrylamide gel electrophoresis was performed in the presence of sodium dodecyl sulfate (SDS) according to the procedures of Porzio and Pearson (1977) except that a Bio-Rad Protean slab gel was used rather than a rod gel. The marker dye was 0.005 percent bromophenol blue.

A 26 μg protein sample extracted from

[1]Mention of trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

The authors are with the Department of Food Science and Human Nutrition, University of Hawaii at Manoa, 1920 Edmondson Road, Honolulu, HI 96822.

the prawn muscles stored for 0, 1, 3, 7, and 14 days at 0°C was applied to each sample well. A Bio-Rad silver staining kit (Bio-Rad Lab., Richmond, Calif.) was used to stain the slab gels.

Textural Changes

The prawn samples obtained from the same source (killed and stored in similar manner as described above for 0, 1, 3, 5, 7, and 14 days at 0°C) were cooked in boiling water for 5 minutes. After the heads and shells were removed, the first and third segments were further sectioned into halves parallel with the muscle fibers. Another set of prawn samples was treated as described above except for the heat treatment.

The PEP Texture Tester equipped with a standard multiple-blade shearing cell was used to measure forces required to shear individual samples. Each sample was placed on the platform so that the cut face was made contact with the stationary shearing cell and the muscle fibers were perpendicular to the moving cell. Data were recorded as the force-distance curve and total integrated work (force-distance) required to compress, shear, and push the sample through (a combination of adhesive and cohesive forces is encountered as the sample is pushed through the stationary cell). There were 10-24 replicates for each treatment.

Statistical analyses (i.e., the analysis of variance, correlation coefficients, and regression analysis) were conducted on the textural measurement data to determine the significance of textural changes in *M. rosenbergii* during cold storage.

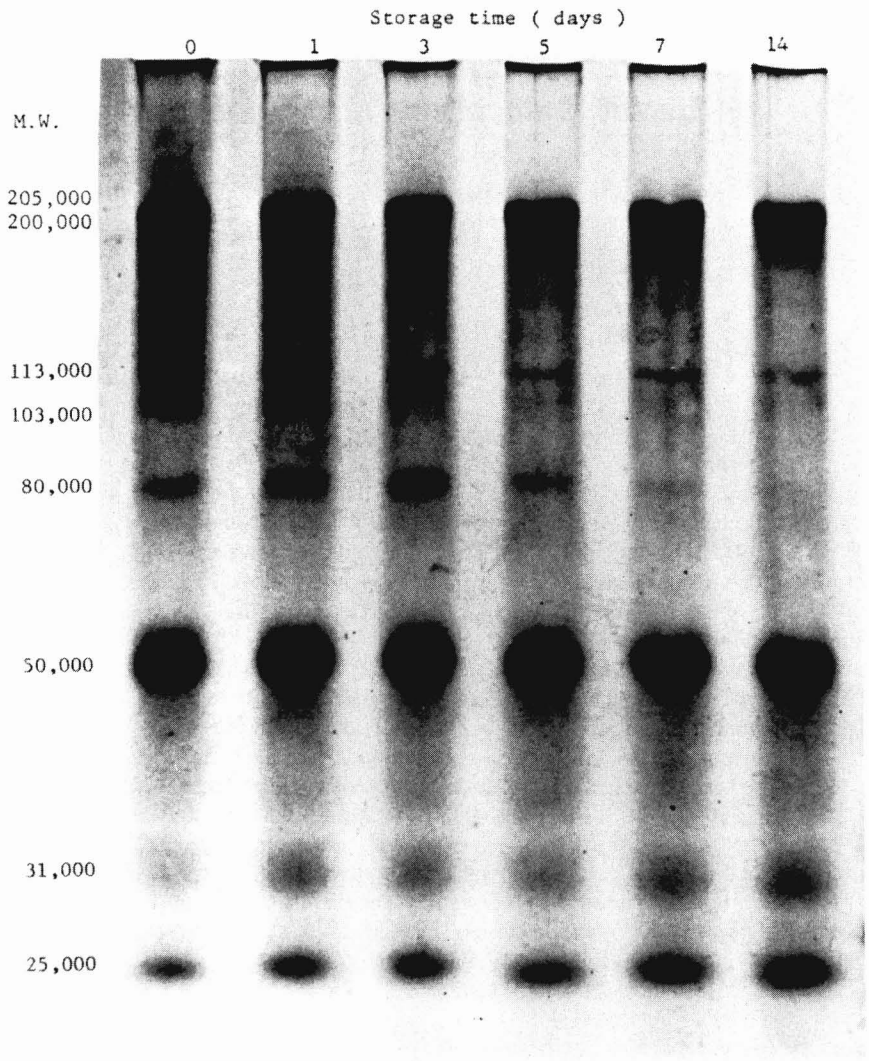

Figure 1.—The effects of iced storage on the electrophoretic patterns of myofibrillar proteins extracted from the first segment of the freshwater prawn.

Results and Discussion

SDS-PAGE of Myofibrillar Proteins

The results of SDS-PAGE of myofibrillar proteins from the first segment of prawn stored for various periods at 0°C are shown in Figure 1. An identical pattern was obtained for the third segment.

Extensive protein degradation occurred during 14 days of iced storage (Fig. 1): The decrease in intensity of the 113,000 and 80,000 dalton subunits during the 14-day iced storage and a complete dissolution of α-actinin (103,000 daltons) after 3 days of iced storage (Fig. 1).

Myosin heavy chains have been known to be very stable myofibrillar components. Degradation of this component has been reported only under specific or controlled conditions, such as at an acidic pH to investigate lysosomal proteolytic reaction (Dutson, 1982), at a high temperature to study the nature of major contractile proteins (Betchel and Parrish, 1983) or from a specific animal such as the squid (Stanley and Hultin, 1984). The gradual disappearance of these heavy chain subunits implies the existence of a highly-active enzyme system in the freshwater prawn during iced storage.

The protein α-actinin and its postmortem changes are also known to be barely detectable. Studies have shown that α-actinin is released from the Z-line as a result of partial degradation of my-

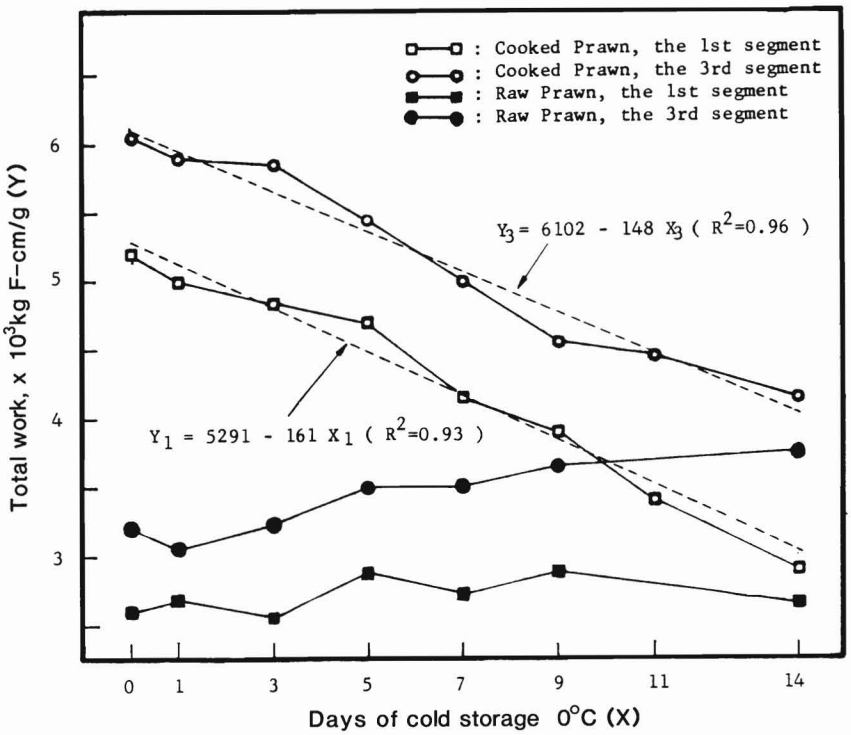

Figure 2.—The effects of iced storage and subsequent heat treatment on the texture (firmness or softness expressed as the total work required for shearing a given sample in a PEP Texture Tester) of different segments of the freshwater prawn tail muscle. Each point represents a mean of 10-24 replicates.

ofibril caused by a calcium-activated enzyme (Dayton et al., 1976, Nagainis and Wolfe, 1982). This phenomenon has been considered as one of the most important events that cause myofibril fragmentation. Observations on the dissolution of α-actinin are quite limited: A very slight decrease during the postmortem storage of bovine muscle (Goll et al., 1977) or the occurrence of dissolution at 37°C (Betchel and Parrish, 1983).

In this study, the disappearance of the α-actinin band after 3 days of cold storage is a significant observation and is in agreement with the microstructural changes in ice-chilled prawn (Nip and Moy, 1988). This postmortem change in the freshwater prawn might be useful in determining its storage history when it is iced.

An increase in intensity of bands with 25,000 and 31,000 daltons during iced storage was clearly demonstrated (Fig. 1). The increase in these lower molecular weight proteins corresponds to the decrease in the higher molecular weight proteins.

From this experiment, it is apparent that extensive myofibrillar protein degradation occurs during the first few days of iced storage.

Texture Measurements

The results of texture measurement are shown in Figure 2, demonstrating significant differences ($P \leq 0.01$) between the cooked segments and among the days of iced storage.

Maximum firmness of cooked prawn tissues can be obtained right after death (Fig. 2). Both the raw and cooked samples of the first segment are softer than those of the third segment (Fig. 2). The rate of textural deterioration in the cooked prawn tissues is higher in the first segment than the third segment by about 9 percent (Fig. 2). The cooked prawn tissues lose an average of 11 percent of their original firmness during the first 4 days of iced storage, as measured by the PEP Texture Tester (Fig. 2). This is considerably less than that reported by Waters and Hale (1981).

The textural change is clearly related to the myofibrillar protein degradation reported in the previous sections. The decrease in work (force-distance) values during the first 3 days (Fig. 2) coincides with the complete dissolution of the α-actinin (103,000 daltons) (Fig. 1). The change of texture deterioration after 7 days also coincides with the complete disappearance of the 80,000-dalton band.

The results of statistical analyses show significant correlations between the differences of cooked muscle texture and the duration of iced storage: $r = -0.96$ and $r = -0.98$ for the first and third segments, respectively.

These results strongly imply the diminishing effect of the degraded proteins, which contribute to mechanical strength, and in part in a consequence of the proteolytic reaction during iced storage.

Acknowledgment

This research was supported in part by the United States-Israel (Binational) Agricultural Research & Development Fund (BARD) US-421-81 and the Hawaii Institute of Tropical Agriculture and Human Resources, University of Hawaii at Manoa. This article is Journal Series No. 3332, the Hawaii Institute of Tropical Agriculture and Human Resources.

Literature Cited

Ahmed, E. M., J. A. Koburger, and V. T. Mendenhall. 1972. Factors affecting texture of cocktail shrimp. J. Texture Stud. 3:186-193.

Angel, S., Z. G. Weinberg, B. J. Juven, and P. Lindner. 1985. Quality changes in the freshwater prawn, *Macrobrachium rosenbergii* during the storage in ice. J. Food Technol. 20:553-560.

Baranowski, E. S., W. K. Nip, and J. H. Moy. 1984. Partial characterization of a crude enzyme extract from the freshwater prawn, *Macrobrachium rosenbergii*. J. Food Sci. 49:1494-1495, 1505.

Betchel, P. J., and F. C. Parrish, Jr. 1983. Ef-

fects of postmortem storage and temperature or muscle protein degradation: Analysis by SDS-Gel electrophoresis. J. Food Sci. 48:294-295, 297.

Dayton, W. R., W. J. Reville, D. E. Goll, and M. H. Stromer. 1976. A Ca^{2+}-activated protease possibly involved in myofibrillar protein turnover. Partial characterization of the purified enzyme. Biochemistry 15:2159-2167.

Dutson, T. R. 1982. Meat proteolysis. *In* P. F. Fox and J. Z. Cindon (editors), Food proteins, p. 333-336. Appl. Sci. Publ., Lond.

Goll, D. E., M. M. Robson, and M. H. Stromer. 1977. Muscle protein. *In* J. R. Whitaker and S. R. Tannenbaum (editors), Food proteins, p. 121-174. Avi Publ. Co. Westport, Conn.

Ma, L. Y., J. C. Deng, E. M. Ahmed, and J. P. Adams. 1983. Canned shrimp texture as a functional of its heat history. J. Food Sci. 48:360-363.

Nagainis, P., and F. H. Wolfe, 1982. Calcium activated neutral protease hydrolyzes z-disc actin. J. Food Sci. 47:1358-1364.

Nip, W. K., and J. H. Moy. 1988. Microstructural changes of ice-chilled and cooked freshwater prawn, *Macrobrachium rosenbergii*. J. Food Sci. 53:319-322.

Nip, W. K., J. H. Moy, and Y. Y. Tzang. 1985a. Effect of purging in quality changes of ice-chilled freshwater prawn, *Macrobrachium rosenbergii*. J. Food Technol. 20:9-15.

Nip, W. K., C. Y. Lan, and J. H. Moy. 1985b. Partial characterization of a collagenolytic enzyme fraction from the hepatopancreas of the freshwater prawn, *Macrobrachium rosenbergii*. J. Food Sci. 50:1187-1188.

Nip, W. K., and J. H. Moy. 1981. Effect of thawing and subsequent chilling on the quality of prawn, *Macrobrachium rosenbergii*. J. Food Process. Preserv. 5:207-213.

Olson, D. G., F. G. Parrish Jr., W. R. Dayton, and D. E. Goll. 1977. Effect of postmortem storage and calcium-activated factor on the myofibrillar proteins of bovine skeletal muscle. J. Food Sci. 42:117-124.

Porzio, M. A., and A. M. Pearson. 1977. Improved resolution of myofibrillar proteins with sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Biochem. Biophys. Acta 490:27-34.

Premaratne, R. J., W. K. Nip, and J. H. Moy, 1986. Characterization of proteolytic and collagenolytic psychrotrophic bacteria of ice-stored freshwater prawn, *Macrobrachium rosenbergii*. Mar. Fish. Rev. 48(2):44-47.

Rowland, R., G. Finne, and R. Tillman. 1982. A morphological study of muscle proteolysis in the tails of *Macrobrachium rosenbergii*. *In* R. Nickelson II (compiler), Proc. 7th Annu. Trop. Subtrop. Fish. Technol. Conf. Am., p. 105-112. Tex. A&M Univ. Publ. TAMU-SG-82-110.

Sidwell, V. D. 1977. The status of prawn processing. *In* J. A. Hanson and H. L. Goodwin (editors), Shrimp and prawn farming in western hemisphere, p. 292-297. John Wiley & Sons, Inc., N.Y.

Soo, H. M., and E. H. Sander. 1977. Prediction of sensory response to textural parameters of breaded shrimp shape using Instron texture profile analysis. J. Food Sci. 42:163-167.

Stanley, D. W., and H. O. Hultin. 1984. Proteolytic activity in North American squid and its relationship to quality. Can. Inst. Food Sci. Technol. 17:163-167.

Tillman, R., and G. Finne. 1983. Texture analysis of tailmeat for *Macrobrachium rosenbergii*. *In* R. Nickelson II (compiler), Proc. 8th Annu. Trop. Subtrop. Fish. Conf. Am., p. 260-269. Tex. A&M Univ. Publ. TAMU-SG-83-112.

Waters, M. E., and M. B. Hale. 1981. Quality changes during iced storage of whole freshwater prawns (*Macrobrachium rosenbergii*). *In* R. Nickelson II (compiler), Proc. 6th Annu. Trop. Subtrop. Fish. Technol. Conf. Am., p. 116-127. Tex. A&M Univ. Publ. TAMU-SG-82-101.

Wong, T. M. 1982. Ultrastructural-biochemical changes and sensory corrections in shrimp during cold storage. Ph.D. Dissert. La. State Univ., Baton Rouge, 193 p.