http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 8 (7): 982-988, 2005
ISSN 1028-8880

Influence of pH on the ATPase Activity and Solubility of *Macrobrachium rosenbergii* and *Penaeus monodon* Muscle Myofibrillar Protein

[1]Md. Kamal, [1]Md. Moshiur Rahman, [2]Mohammad Abu Jafor Bapary, [3]Lubna Yasmin and [3]Md. Nurullah
[1]Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[2]Sheikh Fajilatunnesa Mujib Fisheries College, Jamalpur, Bangladesh
[3]Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh

Abstract: The influence of pH on the changes in myofibrillar remaining ATPase activities of *M. rosenbergii* and *P. monodon* was studied after storage at -20°C for 2 days, 0°C for 2 days and 35°C for 30 min. In all the three storage conditions, ATPase activities for both samples were lower in acidic and alkaline pH regions and the activity remains relatively high of 0.403 μ mol Pi/min mg at pH 8.1. The influence of pH on the remaining Mg^{2+}-ATPase activity and Ca-sensitivity of *M. rosenbergii* and *P. monodon* were evaluated after storage at 0°C for 2 days and -20°C for 2 days. Mg^{2+}-ATPase activities both in presence and absence of Ca^{2+} remain high at neutral pH compared to those of acidic and alkaline region. Ca-sensitivity were also very high (about 38 to 50% at pH ranges from 7.4 to 8.3) in neutral pH region where the sensitivity declined sharply both in acidic and alkaline pH region. The influence of pH on the changes in solubility of *M. rosenbergii* muscle myofibrils during ice and frozen storage was investigated. The highest solubility of 83 to 84% was obtained from myofibrils in the range of pH 7.4 to pH 7.9 after storage for 2 days in ice and -20°C for 2 days in frozen storage. The solubility decreased gradually both in acidic and alkaline pH regions. In the case of *P. monodon,* the maximum solubility of 84% were obtained at pH 7.9 after 2 days of storage at 0°C. Similarly the maximum solubility of 79% were obtained at same pH of 7.8 in myofibrils after storage at -20°C for 2 days. The solubility of *P. monodon* myofibrils was quite high in a wide range of pH from 6.8 to 8.3 and the solubility decreased gradually outside of these pH ranges. Comparatively higher solubility of 84% in a wide range of pH in myofibrils stored at 0°C compared to that of frozen storage also indicates the combined effect of pH and frozen storage on the denaturation of muscle myofibrils.

Key words: pH, ATPase activity, solubility, muscle protein, *M. rosenbergii*, *P. monodon*

INTRODUCTION

Considerable information is available on the denaturation of fish muscle myofibrillar proteins during various storage conditions. In recent years, the influence of pH on the denaturation of muscle protein during various storage conditions has received a great attention. The correlation between pH and organoleptic scores suggests that pH can be used as a quite reliable index of quality. Above the pH value of 7.5, sweet flavour characteristic has completely disappeared[1]. The pH value of 7.8 was reported to be critical level suggesting that spoilage has commenced[2]. Available reports also suggest that low pH has denaturing effect on myofibrillar proteins.[3] During postmortem changes muscle pH generally decreases with onset of rigor mortis but it increases with the relaxation of rigor period. In the present study, it is of interest to see the influence of pH on the protein quality of prawn and shrimp during storage at various pH values. The idea is that changes in muscle protein occurring in postmortem muscle should also occur *in vitro*. Myofibrils contain regulatory protein in addition to actin and myosin and moreover possesses structural arrangement which closely resemble to the contractile apparatus as exists *in vivo*. For this reason, isolated myofibrils, as a unit should give information regarding the postmortem changes.

However, this study reports the influence of pH on the ATPase activity and solubility of *M. rosenbergii* and *P. monodon* muscle myofibrillar protein during various storage conditions.

MATERIALS AND METHODS

Materials: Giant freshwater prawn (*Macrobrachium rosenbergii*) and marine tiger shrimp (*Panaeus monodon*)

Corresponding Author: Dr. Md. Kamal, Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

were used for the study. Live freshwater giant prawns were collected from two commercial farms located in Trishal and Goripur Upazillas of the Mymensingh district. The prawns were harvested using seine net. The tiger shrimps were obtained in lots from farms of Paikegacha Upazilla of Khulna district in live condition. The samples were transported to the Laboratory, Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh in ice condition in an insulated box.

Preparation of myofibrils: Myofibrils were prepared from the *M. rosenbergii* and *P. monodon* muscles separately according to Perry and Grey[4]. Well-washed myofibrils were suspended in 39 mM borate buffer (pH 7.1) containing 0.1 M KCl and 0.1 mM DTT at a concentration of 10-15 mg mL^{-1}.

Storage condition of myofibrils and Ca^{+2}-ATPase assay: The myofibrillar suspension (5 mg mL^{-1}) for storage experiment were prepared in a series of test tubes with a volume of 1 mL containing 0.1 M KCl plus 10 mM Tris-maleate (pH 5.5-8.0) or glycine-KOH (pH 8.5-10.0) as shown in Fig. 1. The tubes containing samples thus prepared were stored at 0°C for 2 days, -20°C for 2 days and 35°C for 30 min. After storage all the tubes containing samples were taken out and kept in ice and the reaction mixture was prepared in each tube which contained 40 mM Tris-maleate (pH 7.0), 5 mM $CaCl_2$, 0.1 M KCl and 1 mM ATP in a final volume of 10 mL. The pH of the reaction mixture was around 7.0 during ATPase assay. All activities were measured at 25°C. The mixture was continuously stirred and 2 mL aliquot was taken after 1, 2, 4 and 6 min and was added to 1 mL of 10% TCA to stop the reaction. The mixture was centrifuged at 900×g for 5 min and the supernatant obtained was analyzed for inorganic phosphate (Pi) by the method of Fiske and SubbaRow[5].

Storage condition of myofibrils and Mg^{2+}-ATPase assay: In order to quantify the Mg^{2+}-ATPase activity and Ca-sensitivity, myofibrillar suspension was prepared according to the method described for Ca^{2+}-ATPase assay. Myofibril suspension thus prepared were stored at 0°C for 2 days, -20°C for 2 days and 35°C for 30 min (Fig. 2). After storage, the Mg^{2+}-ATPase activities were assayed at 25°C as reported earlier in 40 mM Tris-maleate (pH 7.0) containing 5 mM $MgCl_2$, 50 m M KCl, 1 mM ATP and 5 mg mL^{-1} of myofibrils in the presence of either 0.25 mM $CaCl_2$ or 1 mM ethylene glycol bis (b-aminoethyl ether) N, N, N′, N′-tetra acetic acid (EGTA) in a final volume of 10 mL. The pH of the reaction mixture was around 7.0 during ATPase assay. The inorganic phosphate (Pi) liberated was analyzed by the method of Fiske and SubbaRow[5].

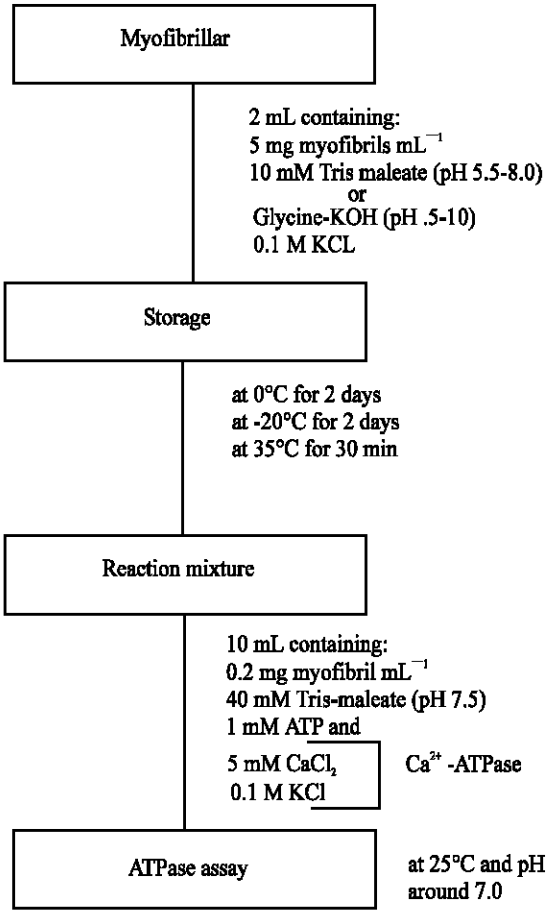

Fig. 1: Storage condition of myofibrils and Ca^{2+}-ATPpase assay

Ca^{2+}-sensitivity was calculated from Mg^{2+}-ATPase activity in the presence of Ca^{2+} relative to that in the presence of EGTA as follows:

Storage condition of myofibrils and solubility test: For solubility test, 2 mL of myofibrillar suspensions (5 mg mL^{-1}) containing 0.1 M KCl plus 10 mM Tris-maleate (pH 5.5-8.0) or glycine -KOH (pH 8.5-10.0) was stored essentially under the same conditions as above. Myofibrillar suspensions thus prepared were stored either at 0°C or at -20°C for 2 days (Fig. 3). After storage, each tube was taken out, cooled at 4°C and homogenized with equal volume of 0.6M KCl plus 0.03 M Tris-HCl (pH 7.5) and the homogenate was centrifuged in a refrigerated centrifuge machine for 30 min at 900×g and protein concentration in the supernatant was determined by the biuret method[6].

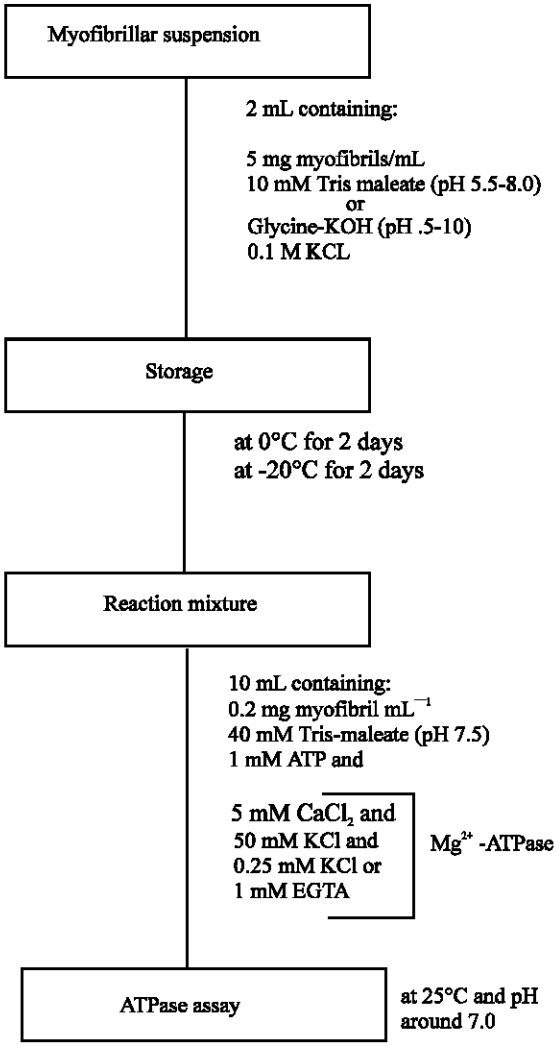

Fig. 2: Storage condition of myofibrils and Mg^{2+}-ATPpase assay

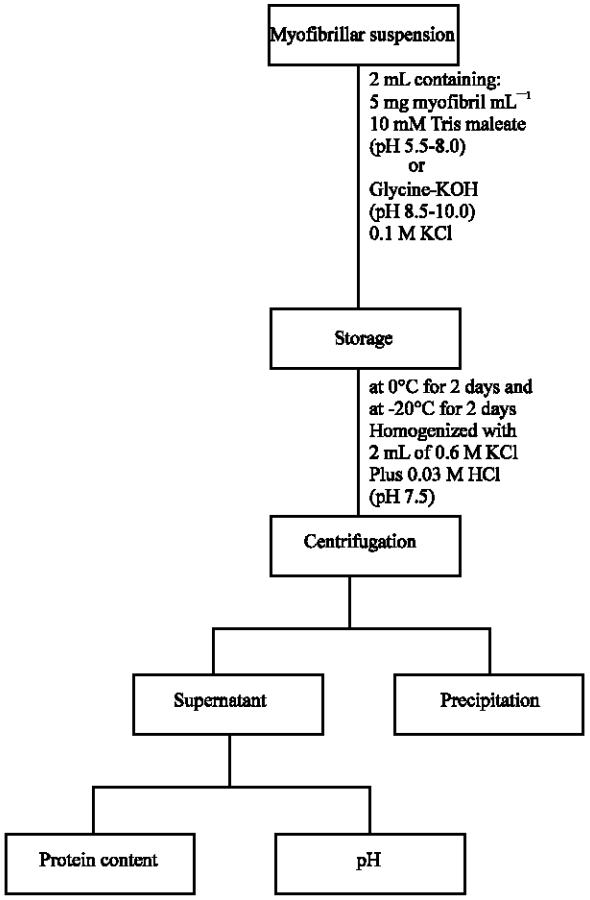

Fig.3: Storage condition of myofibrils and solubility determination

RESULTS AND DISCUSSION

The influence of pH on the changes in myofibrillar Ca^{2+}-ATPase activities of *M. rosenbergii* and *P. monodon* was studied under various storage conditions. Figure 4 shows the influence of a wide range of pH on the remaining Ca^{2+}-ATPase activity of *M. rosenbergii* muscle myofibrils after storage at -20°C for 2 days, 0°C for 2 days and 35°C for 30 min. In all the three storage conditions, ATPase activities were lower in acidic and alkaline pH regions and the activity remains relatively high of 0.403 μ mol Pi/min mg at pH 8.1. The overall ATPase activity was high in myofibrils stored at 0°C followed by -20 and 35°C, respectively. The poor activity in myofibrils incubated at 35°C for 30 min clearly indicates the denaturation of myofibrillar proteins at that temperature. On the other hand, slightly poor activity in myofibrils stored at -20°C compared to that of 0°C also indicates the freeze denaturation of myofibrillar proteins.

Studies were also conducted on the influence of pH on the remaining Ca^{2+}-ATPase activity of *P. monodon* muscle myofibrils after storage at -20°C for 2 days, 0°C for 2 days and 35°C for 30 min (Fig. 5). The pattern of ATPase activity was more or less similar to that of *M. rosenbergii* where the ATPase activities were low at acidic and alkaline pH region. The highest ATPase activity of 0.484 μ mol Pi/min mg was found at pH 7.5 at 30°C. The result shows that ATPase activities of myofibrils incubated at 0°C for 2 days were also high compared to myofibrils stored at -20 and 35°C. Relatively poor activities during frozen storage at -20 and at 35°C which indicates the denaturation of myofibrillar proteins during storage periods.

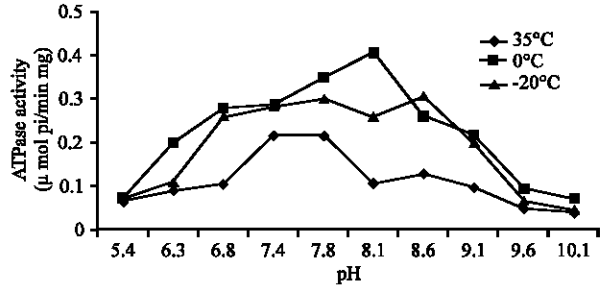

Fig. 4: Influence of pH on the Ca^{2+} -ATPase activity of *M. rosenbergi* muscle myofibrils after storage at -20°C for 2 days, 0°C for 2 days and 35°C for 30 min

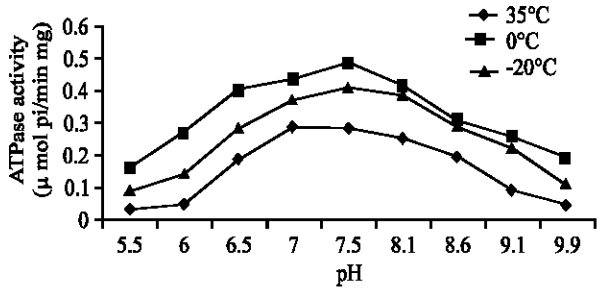

Fig. 5: Influence of pH on the Ca^{2+} -ATPase activity of *P. monodon* muscle myofibrils after storage at -20°C for 2 days, 0°C for 2 days and 35°C for 30 min

The influence of pH on the remaining Mg^{2+}-ATPase activity and Ca- sensitivity of *M. rosenbergii* muscle myofibrils was evaluated after storage at 0°C for two days (Fig. 6). Mg^{2+}-ATPase activities both in presence and absence of Ca^{2+} remain high at neutral pH compared to those of acidic and alkaline region. Ca-sensitivity were also very high (about 38 to 50% at pH ranges from 7.4 to 8.3) in neutral pH region where the sensitivity declined sharply both in acidic and alkaline pH region. The poor Ca-sensitivity indicates the influence of acidic and alkaline pH on the denaturation of myofibrillar protein.

The influence of pH on the Mg^{2+}-ATPase activity and Ca-sensitivity of *M. rosenbergii* muscle myofibrils after storage at -20°C for 2 days were also studied (Fig. 7). Although the overall activity during frozen storage both in presence and absence of Ca^{2+} was low compared to that of myofibrils stored at 0°C for 2 days, the pattern of ATPase activity changes in a wide range of pH values were more or less similar to that of ice (0°C) storage. The maximum activities were obtained in neutral pH region (0.313 μ mol Pi/min mg at pH 7.8 in presence of Ca^{2+} and 0.179 μ mol Pi/min mg at pH 7.4 in absence of

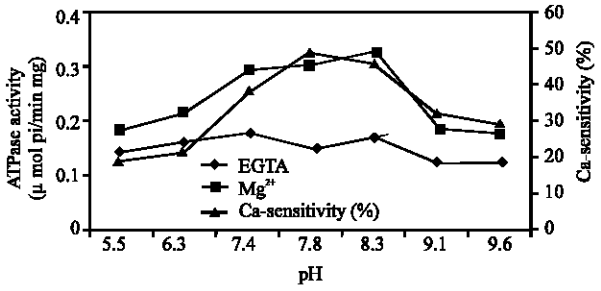

Fig. 6: Influence of pH on the Mg^{2+} -ATPase activity and ca-sensitivity of *M. rosenbergi* muscle myofibrils after storage at 0°C for 2 days

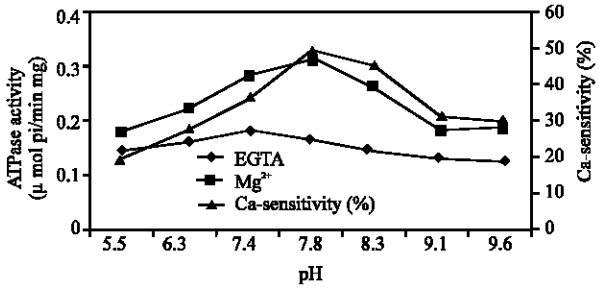

Fig. 7: Influence of pH on the Mg^{2+} -ATPase activity and ca-sensitivity of *M. rosenbergi* muscle myofibrils after storage at -20°C for 2 days

Ca^{2+}) where the activity sharply declined both in acidic and alkaline pH region. Similarly Ca-sensitivity was also very poor in acidic and alkaline pH region.

Studies were also conducted to evaluate the influence of pH on the changes in Mg^{2+}- ATPase activity and Ca-sensitivity of *P. monodon* muscle myofibrils after storage at 0°C for 2 days (Fig. 8). The maximum Mg^{2+}-ATPase activity of 0.568 μ mol Pi/min mg was found at pH 7.5 in presence of Ca^{2+}. The activity declined gradually both in acidic and alkaline pH region. On the other hand, Mg^{2+}-ATPase activity in absence of Ca^{2+} were also high in neutral pH region.

Maximum Ca^{2+}-sensitivity were also observed at pH 7.1 (about 55%) and the sensitivity declined gradually both in acidic and alkaline region. The loss of Ca^{2+}-sensitivity and poor ATPase activities both in acidic and alkaline pH region clearly indicates the influence of pH on the denaturation of myofibrillar proteins.

Figure 9 shows the influence of pH on the Mg^{2+}-ATPase activity and Ca-sensitivity of *P. monodon* muscle myofibrillar protein after storage at -20°C for 2 days. The pattern of changes in ATPase activity were almost similar to that of the myofibrils stored at 0°C for 2 days both in presence and absence of Ca^{2+}. In presence of Ca^{2+} the maximum Mg^{2+}-ATPase activity of

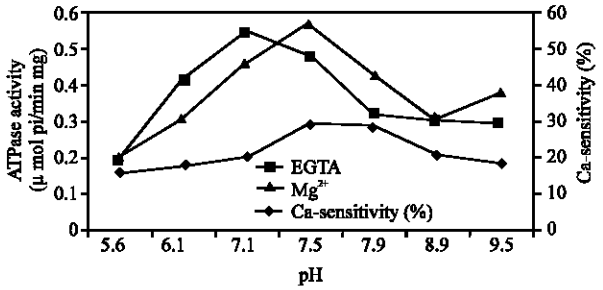

Fig. 8: Influence of pH on the Mg^{2+} -ATPase activity and ca-sensitivity of *P. monodon* muscle myofibrils after storage at 0°C for 2 days

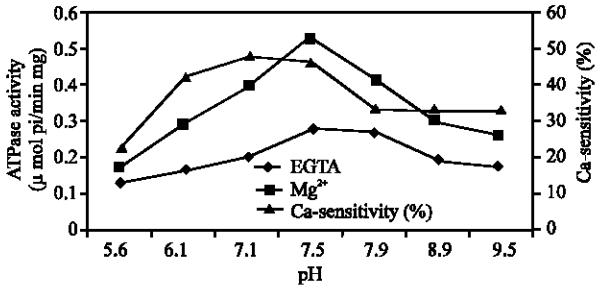

Fig. 9: Influence of pH on the Mg^{2+} -ATPase activity and ca-sensitivity of *P. monodon* muscle myofibrils after storage at -20°C for 2 days

0.55 μ mol Pi/min mg was observed at pH 7.5 which declined gradually both in acidic and alkaline pH region. Similar results were also obtained in absence of Ca^{2+} where maximum activity of 0.285 μ mol Pi/min mg were found at same pH 7.5. The Ca-sensitivity was also high in a wide range of pH from 6.0 to 7.5.

In the present study the influence of pH on the remaining ATPase activities of *M. rosenbergii* and *P. monodon* muscle myofibrils in a wide range of pH under various storage conditions was evaluated. The results indicate that the lower activity in acidic and alkaline pH region is due to denaturing effect of low and alkaline pH. In post-mortem muscle, the pH above 7.2 in shrimp and prawn has been considered as the upper limit of quality for acceptable conditions. The present study indicates that the shrimp/prawn at pH above 7.2 not only spoils due to formation of non-protein nitrogenous substances but also degrade the myofibrillar proteins due to function of pH. The present study reveals that changes in pH in muscle protein particularly in acidic and alkaline pH have denaturing effect on myofibrillar protein of shrimp and prawn. In this study although the pattern of ATPase activity changes are more or less similar in *M. rosenbergii* and *P. monodon* muscle myofibrils, even though the myofibrils of *P. monodon* was found more stable than that of *M. rosenbergii* in wide pH ranges by measuring ATPase activity. Watabe *et al.*[7] reported that ordinary muscle myofibrils showed the remaining activity maxima at pH 7.0 and 8.6 when stored at 0°C for two days. The activity sharply decreased below pH 6.5. The dark muscle myofibrils of sardine showed the remaining Ca^{2+}-ATPase activity maximum at pH 6.7 when stored at 35°C for 30 min and at pH 6.4 when stored at 0°C for 2 days. In this connection Seki *et al.*[8] found that sardine ordinary myofibrils lost about half Ca^{2+}-ATPase activity when ice stored at pH 6.0 for 10 days, whereas, the activity hardly decreased when ice stored at pH 7.0. When incubated at 35°C for 30 min the ordinary myofibrils gave a pH activity relationship similar to that at 0°C with the maximum at around pH 7.0 and the other at pH 8.6. The overall remaining activity of the myofibrils stored at 35°C was considerably lower than that of 0°C which is similar to the present study.

Kamal *et al.*[3] reported on Mg^{2+}-ATPase activity and Ca^{2+}-sensitivity of sardine muscle myofibrils and mentioned that storage at 0°C for 2 days give rise to two activity maxima both in the presence and absence of Ca^{2+}, one at pH around 7.5 and another at pH 9.5 and the highest Ca^{2+}-sensitivity at pH 7.5 regardless of muscle type. After 2 days of storage the activity was extremely low at pH between 5.5 and 7.5 for both myofibrils when myofibrils were incubated at 35°C for 30 min Mg^{2+}-ATPase activities in the presence and absence of Ca^{2+} were both increased with the maximum values at pH 7.0 for ordinary muscle and at pH 7.5 for dark muscle myofibrils. Ca^{2+}-sensitivity of ordinary muscle myofibrils almost disappeared while that of dark muscle to some extent remained, especially in alkaline pH ranges. It was also reported that Mg^{2+}-ATPase activities of sardine ordinary muscle were comparatively stable in the acidic pH region. Similar results were reported for Seabass by measuring Mg^{2+}-ATPase activity by Katoh *et al.*[9] Studies conducted elsewhere also indicated a significant correlation between muscle pH and Mg^{2+}-ATPase activity of myofibrillar proteins.[3,10,11] Yasmin *et al.*[12] also studied the influence of pH on the myofibrillar Mg^{2+}-ATPase activity from Carps. The pH activity curve of the Catla myofibrils in the presence of Ca^{2+} showed two peaks, at around pH 7.0 and around pH 8.0. The activity in the absence of Ca^{2+} showed the two similar maxima at pH 7.0 and 8.0. The activities below pH 6.5 and above 8.0 were decreased markedly which was also similar to the result of present study. The maximum Ca^{2+}-sensitivity (55%) was observed at pH 6.5 while the sensitivity immediately after preparation of myofibrils at pH 7.0 was 65-70%. The highest Ca^{2+}-sensitivity of 52% was also observed in case of rohu at around pH 7.0.

In frozen storage also there was a decrease in Mg^{2+}-ATPase activity in both *M. rosenbergii* and *P. monodon* myofibrils below pH 6.0 and above 8.5 which again revealed a direct function of pH. Fukuda *et al.*[13] observed a rapid denaturation of mackerel ordinary myofibrillar proteins at around pH 6.0 during storage at -30°C. Fukuda *et al.*[14] also reported that sardine meat block lost about half of the initial myofibrillar Ca^{2+}-ATPase activity after 8 months of storage at -20°C. After frozen storage ATPase activities in all assay systems were slightly lower than that of myofibrils stored in ice for 2 days. This phenomena may be associated with side-to-side aggregation of myosin molecules as observed in electron microscope by Buttkus[15] on rabbit myosin during frozen storage. These structural changes of myosin would result in a decrease affinity to actin as observed by Onishi *et al.*[16] with carp ordinary muscle actomyosin during frozen storage. Chemical modification studies confirmed that one myosin molecules contains two reactive cysteine residues termed SH_1 and SH_2 groups at or near the active site of myosin[17]. Blocking of SH_1 results in an activation of Ca^{2+}- ATPase with concomitant inactivation for EDTA-ATPase. It was found that myosin preferentially blocked at SH_1 or SH_2 exhibits a high level of Ca^{2+}-ATPase activity but no EDTA-ATPase activity, indicating that both sides are essential to EDTA-ATPase activity, whereas either site alone is sufficient for the expression of Ca^{2+}-ATPase activity. It is therefore likely that the side-to-side aggregation of myosin molecules might block either SH group and result in decreasing EDTA-ATPase activity in acidic and neutral pH regions.

Figure 10 shows the influence of pH on the changes in solubility of *M. rosenbergii* muscle myofibrils during ice (0°C) and frozen (-20°C) storage. The highest solubility of 83 to 84% was obtained from myofibrils in the range of pH 7.4 to 7.9 after storage for 2 days in ice. The solubility decreased gradually both in acidic and alkaline pH regions. Similar results were also obtained from myofibrils when stored at -20°C for 2 days in a wide range of pH values, where maximum solubility of 80% was obtained at pH 7.4. The poor solubility in both storage conditions at acidic and alkaline pH region clearly indicates the influence of acidic and alkaline pH on the denaturation of myofibrillar proteins. Studies were also conducted to evaluate the influence of pH on the solubility of *P. monodon* muscle myofibrils after ice storage at 0 and frozen storage at-20°C for 2 days (Fig. 11).

The maximum solubility of 84% were obtained at pH 7.8 after 2 days of storage at 0°C . Similarly the maximum solubility of 79% were obtained at same pH of 7.8 in myofibrils after storage at -20°C for 2 days.

The results indicate that the solubility of *P. monodon* myofibrils were quite high in a wide range of pH from 6.8 to 8.3 and the solubility decreased gradually

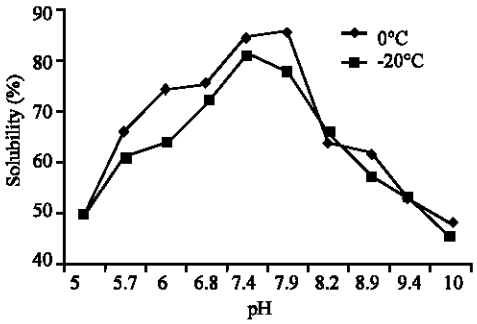

Fig. 10: Influence of pH on solubility of *M. rosenbergii* muscle myofibrils after storage at 0°C for 2 days and frozen storage -20°C for 2 days

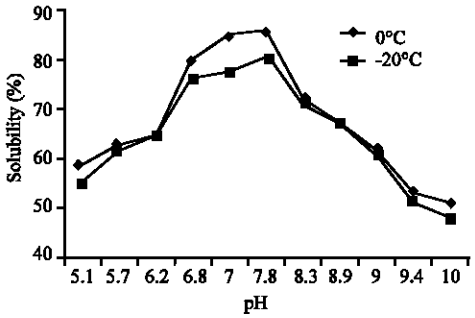

Fig. 11: Influence of pH on solubility of *M. rosenbergii* muscle myofibrils after storage at 0°C for 2 days and frozen storage -20°C for 2 days

outside of these pH ranges. Comparatively higher solubility of 84% in a wide range of pH in myofibrils stored at 0°C compared to that of frozen storage also indicates the combined effect of pH and frozen storage on the denaturation of muscle myofibrils.

Studies were also conducted to evaluate the influence of pH on the solubility of *M. rosenbergii* and *P. monodon* muscle protein under various storage conditions. The higher solubility was obtained at neutral pH region while the lower solubility was observed both in acidic and alkaline pH region indicating the denaturing effect of acidic and alkaline pH on the muscle myofibrils of both the species. Studies conducted elsewhere also represent the similar results indicating the denaturation effect of acidic and alkaline pH. Kamal *et al.*[3] reported the maximum solubility of 84% with ordinary muscle myofibrils at pH 7.0 while 95% at pH around 7.5 with dark muscle myofibrils after storage for 2 days. Again both the sardine ordinary and dark muscle myofibrils showed the maximum solubility at pH 7.7 with 79 and 83% , respectively after 2 days of frozen storage at-20°C and when incubated at 35°C for 30 min, ordinary muscle myofibrils decreased in solubility below 40% irrespective of pH below 6.0 and 10.0 Yasmin *et al.*[12] studied the solubility of both catla and rohu muscle myofibrils after

2 days of ice storage at various pH ranging from 5.0 to 10.0. The highest solubility around 90% was obtained from both catla and rohu ordinary muscle myofibrils at pH 7.0. The solubility of both fish muscle myofibrils were comparatively high at pH ranging from pH 6.5 to 8.5 indicate the denaturation effect of acidity and alkalinity but they are tended to decrease outside this pH ranges was in agreement with the present study. Noguchi and Matsumo[18] reported that frozen storage denaraturation of the carp actomyosin as revealed in the changes of solubility; as well as ATPase activity appear similar to those obtained by Connell[19] on the extractable proteins from the frozen stored cod muscle except for the Connell's data of the ATPase activity for the storage at -20°C where no fall was found. Yoshikawa *et al.*[20] reported that the solubility decreased after 3 weeks at - 4 and -11°C.

Comparison of the solubility results to those obtained by measuring ATPase activities indicate that both solubility and ATPase activity were sensitive to follow denaturation. The marked decrease in ATPase activity and solubility are reported to be insolubilization of myofibrillar proteins and it can be concluded that denaturation of myofibrillar proteins appears prior to the formation of insoluble aggregates[21].

REFERENCES

1. Rahman, M., L. Yasmin, M. Kamal, M. Kamruzzaman, M.N. Islam and Y. Ochiai, 2001b. Effect of delayed icing on the quality changes in freshwater prawn Macrobrachium rosenbergii during subsequent storage. Bull. Fac. Edu. Ibaraki Univ. Natl. Sci., 50: 51-57.
2. Chung, C.Y. and J.L. Lain, 1979. Studies on the decomposition of frozen shrimp II. Deterioration during iced and refrigerated storage. Natl. Sci. Council Monthly, 7: 1136.
3. Kamal, M., S. Watabe and K. Hashimoto, 1990. Stability of sardine myofibrils during storage at various pH values. Nippon Suisan Gakkashi, 56: 83-90.
4. Perry, S.V. and T.C. Grey, 1956. A study on the effect of substrate concentration and certain relaxing factors on the magnesium activated myofibrillar adenosine triphosphatase. Biochem. J., 64: 184-192.
5. Fiske, C.H. and Y. SubbaRow, 1925. The colometric determination of phosphorus. J. Biol. Chem., 66: 375-400.
6. Gornall, A.G., C.J. Bardawill and M.M. David, 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., 177: 751-766.
7. Watabe, S., H. Ushio, M. Iwamoto, H. Yamanaka and K. Hashimoto, 1989. Temperature dependency of rigor-mortis of fish muscle: Myofibrillar Mg^{+2}-ATPase activity and Ca^{2+}-ATPase by sarcoplasmic reticulum. J. Food Sci., 54: 1107-1110.
8. Seki, N., Y. Oogane and T. Watanabe, 1980. Changes in ATPase activity and other properties of sardine myofibrillar proteins during ice storage. Nippon Suisan Gakkaishi, 46: 607-615.
9. Katoh, N., H. Uchiyama, S. Tsukamoto and K. Arai, 1977. A biochemical study on fish myofibrillar ATPase. Bull. Japan. Soc. Sci. Fish., 43: 857-867.
10. Hashimoto, A. and K. Arai, 1978. The effects of pH and temperature on the stability of myofibrillar Ca^{2+}-ATPase from some fishes. Nippon Suisan Gakkaishi, 44: 1389-1393.
11. Watabe, S., M. Kamal and K. Hashimoto, 1989. Changes in Ca^{2+}-ATPase activity of sardine ordinary and dark muscle myofibrils during storage at various pH values. Nippon Suisan Gakkasihi, 55: 703-707.
12. Yasmin, L., K. Ahmed, M. Kamal and Y. Ochiai, 2000b. Changes in ATPase activity and solubility of myofibrillar proteins from Indian major carps during ice storage. Bull. Fac. Edu. Ibaraki Univ. Natl. Sci., 49: 131-137.
13. Fukuda, Y., Z. Tarakita and K. Arai, 1984. Effect of chub mackerel on the freeze-denaturation of myofibrillar proteins. Nippon Suisan Gakkaishi, 50: 845-852
14. Fukuda, Y., Z. Tarakita, M. Kawamura, K. Kakehata and K. Arai, 1982. Denaturation of myofibrillar protein in chub mackerel during freezing and storage. Nippon Suisan Gakkaishi, 46: 1627-1632.
15. Buttkus, H., 1970. Accelerated denaturation of myosin in frozen solution. J. Food Sci., 35: 558-562.
16. Ohnishi, M., T. Tsuchiya and J.J. Matsumoto, 1978. Kinetic study on the denaturation mechanism of carp actomyosin during frozen storage. Nippon Suisan Gakkaishi, 44: 27-37.
17. Sekine, T. and W.W. Kielley, 1964. The enzymic properties of N-ethylmaleimide modified myosin. Biochem. Biophys. Acta, 81: 336-345.
18. Noguchi, S. and J J. Matsumoto, 1970. Studies on the denaturation of fish muscle proteins during the frozen storage-I. Preventive effect of Na-glutamate. Nippon Suisan Gakkaishi, 36: 1078-1087.
19. Connell, J.J., 1960. Changes in the adenosine triphosphatase activity and sulphydryl groups of cod flesh during frozen storage. J. Sci. Food Agric., 13: 607-617.
20. Yoshikawa, K., N. Inoue, Y. Kawai and H. Shinano, 1995. Changes of the solubility and ATPase activity of carp myofibrils during frozen storage at different temperatures. Fish. Sci., 65.
21. Wagner, J.R. and M.C. Anon, 1985. Effect of freezing rate on the denaturation of myofibrillar proteins. J. Food Technol., 20: 735-744.