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Influence of Temperature on the Stability of *M. rosenbergii* and *P. monodon* Muscle Protein under Various Storage Conditions

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Abstract: The influence of temperature on the changes in Ca^{2+}-ATPase activity and solubility of *M. rosenbergii* and *P. monodon* muscle myofibrils were studied in a wide range of temperature from 20 to 55°C for 30 min. Both ATPase activity and solubility almost remain unchanged up to 25°C while both ATPase activity and solubility decreased with the raise of temperature. The decreasing of ATPase activity and solubility after 25°C clearly indicates the influence of temperature on the denaturation of *M. rosenbergii* muscle myofibrils. The influence of temperature on the inactivation rate of Ca^{2+}-ATPase at 30 and 35°C on myofibrillar proteins of *M. rosenbergii* and *P. monodon* were investigated at various pH values. The inactivation rate of *M. rosenbergii* was low at pH 7.8 to 8.5 where the rate was quite high both in acidic and alkaline pH region irrespective of incubation temperature. The Kd value at 35°C was markedly higher than at 30°C throughout all the pH ranges. Similar studies were also conducted on *P. monodon* muscle myofibrils. The results obtained from *P. monodon* muscle myofibrils were more or less similar to that of Kd value obtained from *M. rosenbergii* muscle myofibrils where the myofibrils were found more stable at neutral pH ranges form 7.1 to 8.8. However, with the progress of acidic and alkaline pH value the Kd value gradually increased. The result also shows that higher temperature of 35°C accelerated the Kd value in myofibrils compared to that of incubated at 30°C throughout the pH ranges used.. Studies were also conducted to evaluate the effect of temperature on the coagulation time of sarcoplasmic protein of *M. rosenbergii* and *P. monodon.* At 40°C coagulation of *M. rosenbergii* sarcoplasmic protein was started in 8 min and coagulation time decreased gradually with the increase of temperature and at 60°C the coagulation of sarcoplasmic protein was started in 3 min. On the other hand, at 40°C coagulation of *P. monodon* sarcoplasmic protein started in 5 min and the coagulation time decreased with the raise of incubation temperature and it was found that at 60°C, coagulation was started within 1.7 min. The results obtained from present studies also shows that sarcoplasmic protein of *P. monodon* denature more quickly than that of *M. rosenbergii* sarcoplasmic protein at higher temperature.

Key words: Temperature, stability, myofibrillar protein, *P. monodon*, *M. rosenbergii*

INTRODUCTION

In tropical countries like Bangladesh, the environmental temperature generally rises up to 40°C, where fish and shellfish are generally transported for marketing from remote place to urban cities without adequate preservation in ice through various means of communication such as boat, roads and trains. Since the temperature plays vital role in the denaturation of muscle proteins of fish, therefore, it seems interest to see the influence of temperature on the stability of their muscle proteins by measuring ATPase activity and solubility. Since ATPase activity is not all together a satisfactory criterion for muscle protein denaturation, some workers have looked other evidence of denaturation[1,2]. The solubility has been adopted for that purpose and it is often assumed that solubility and ATPase activity represent the same state of myofibrillar denaturation[3].

Since myofibrillar protein is the major constituents of the muscle protein composition, it seems important to investigate the inactivation rate constant value (Kd) of myofibrillar protein of *M. rosenbergii* and *P. monodon.* Although considerable information are available on the thermostability and inactivation rate constant (Kd) of myofibrillar protein.

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However, this study reports the influence of temperature on the stability of *M. rosenbergii* and *P. monodon* muscle protein under various storage conditions.

MATERIALS AND METHODS

Materials: Giant freshwater prawn (*Macrobrachium rosenbergii*) and marine tiger shrimp (*Panaeus monodon*) 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[22]. 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: To assay the ATPase activity and solubility the myofibrils were pipetted (5 mg mL^{-1}) in different test tubes and incubated at 20, 25, 30, 35, 40, 45, 50 and 55°C for 30 min. Then the tubes containing the myofibrils were taken out at due time intervals and kept in ice. For Ca^{2+}-ATPase activity the reaction mixture was prepared in a final volume of 10 mL in each tube containing 25 mM Tris-maleate (pH 7.0), 5 mM $CaCl_2$, 0.1M KCl and 1 mM ATP. Then the Ca^{2+}-ATPase activities were measured at 25°C as described before. For solubility the protein concentration of each tubes was determined by Biuret method[4].

Assay of inactivation rate constant (Kd): The myofibril suspension contained 5 mg mL^{-1} myofibril, 20 mM Tris-maleate or glycine-KOH buffer ranging from 5.5 to 10.0 of respective pH values and 0.1 M KCl were pipetted into different test tubes and incubated for 30 min at 30°C for one series and the other series at 35°C and the tubes were transferred into ice at due time intervals. The reaction mixture was prepared in a final volume of 10 mL in each tube with 25 mM Tris, 5 mM $CaCl_2$, 0.1 M KCl and 1 mM ATP. Then the Ca^{2+}-ATPase activity were measured at 25°C as described before. The inactivation rate constant were determined according to the method of Yasui *et al.*[5].

The first order rate constant (Kd) for inactivation (denaturation) of Ca^{2+}- ATPase was calculated from the following formula:

$$Kd = 1/t(InC_o - InC_t)$$

Where, In C_o and InC_t are the natural logarithms of the ATPase activity before and after adequate incubation time (t).

Preparation of sarcoplasmic protein and assay of coagulation time: Fresh ice stored shrimp and prawn were used for the study to prepare sarcoplasmic protein. After removing the shell, twenty grams of muscle was fractionated by a procedure described by Hashimoto *et al.*[6]. All the operations were performed at 3-4°C as quantitatively as possible. After fractionation, about 10 mL of sarcoplasmic protein was taken in the transparent test tube and heated in the controlled water bath and coagulation time was measured from the first appearance of coagulation at subsequent temperature.

RESULTS AND DISCUSSION

The influence of temperature on the changes in Ca^{2+}-ATPase activity and solubility of *M. rosenbergii* and *P. monodon* muscle myofibrils were studied in a wide range of temperature from 20°C to 55°C for 30 min. As shown in Fig. 1 both ATPase activity and solubility of *M. rosenbergii* muscle myofibrils almost remain unchanged up to 25°C while both ATPase activity and solubility decreased with the raise of temperature. The highest ATPase activity of 0.518 μ mol Pi/min mg was found at 25°C which declined to about 0.011 μ mol Pi/min mg at 55°C. Similarly, the highest solubility of 82% was found to decline to about 33% from 25 to 55°C. The decreasing of ATPase activity and solubility after 25°C clearly indicates the influence of temperature on the denaturation of *M. rosenbergii* muscle myofibrils. The result also shows that the trend in changes in ATPase activity and solubility in a higher temperature is more or less similar where ATPase activity was found more sensitive than the measurement of solubility.

Similar studies were also conducted to evaluate the effect of different temperature on the ATPase activity and solubility of *P. monodon* muscle myofibrils after incubation at a wide range of temperature from 25 to 55°C (Fig. 2). There was little or no changes in both ATPase activity and solubility after incubation at temperature up to 25°C for 30 min. But with the raise of temperature both ATPase activity and solubility declined rapidly though the ATPase activity was also found more sensitive

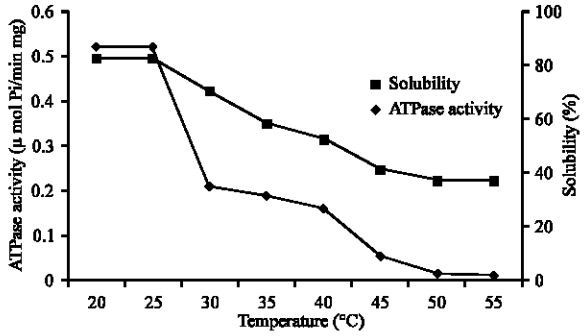

Fig. 1: Influence of temperature on the ATPase activity and solubility of *M. rosenbergii* muscle muofibrils

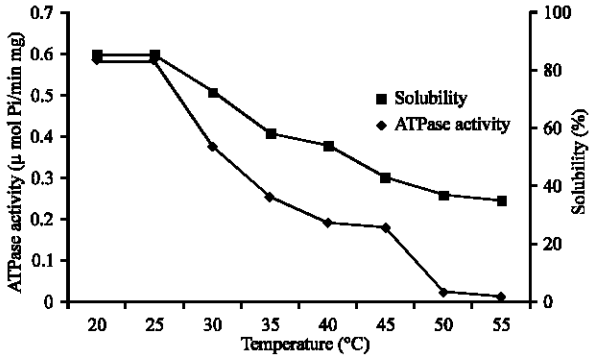

Fig. 2: Influence of temperature on the ATPase activity and solubility of *P. Monodon* muscle muofibrils

than solubility at higher temperature. The result clearly indicates that the higher temperature above 25°C has a definite denaturing effect on the myofibrillar proteins.

Studies were conducted on the changes in Ca^{2+}-ATPase activity and solubility of *M. rosenbergii* and *P. monodon* muscle myofibrils at various temperature. There was little or no change of both ATPase activity and solubility upto 25°C but they were declined with the further raise of temperature incase of both the species. ATPase activity declined more rapidly than that of solubility which indicate that ATPase activity is more sensitive than solubility to assess the myofibrillar protein quality. The results of the present study indicate that the higher atmospheric tropical temperature above 25°C may accelerate the degradation of myofibrillar proteins of fish and shellfish while kept for a longer period without using ice. Recent studies on delayed icing for *M. rosenbergii* and *P. monodon* indicates that longer exposure at higher temperature drastically reduce their shelf life[7-9]. Incase of *P. monodon* the sample stored in ice immediately after catch were organoleptically acceptable condition for 10 days while delayed icing of 4, 8 and 12 h shorten the shelf-life to 7, 6 and 5 days, respectively[10].

Incase of *M. rosenbergii* the samples kept in ice immediately after catch remain acceptable condition for 7 days whereas, delaying of 4, 8 and 12 h shortening the shelf life to 3, 2 and 1 days, respectively[8]. According to Reilly[11] storage life of *P. monodon* drastically reduced approximately one day for every hour delay in icing. However, the results obtained from present study clearly indicate that the temperature above 25°C not only spoils the fish quickly but also accelerated the degradation of myofibrils of *M. rosenbergii* and *P. monodon*. Considerable information are also available on the influence of higher temperature on the denaturation of myofibril by measuring ATPase activity and solubility[12-15]. The present study also indicates that both ATPase activity and extractability of myofibrillar protein are effective methods for evaluating the denaturation of myofibrils under various storage conditions.

The influence of temperature on the inactivation rate of Ca^{2+}- ATPase at 30 and 35°C on myofibrillar proteins of *M. rosenbergii* and *P. monodon* were investigated at various pH values to find out whether the Ca^{+2}-ATPase activity was really related with the stability of muscle myofibrils. As shown in Fig. 3 the inactivation rate of *M. rosenbergii* was low at pH ranges from 7.8 to 8.5 with the value ranges from 3.2×10^{-4} s^{-1} to 3.9×10^{-4} s^{-1} where the rate was quite high both in acidic and alkaline pH region irrespective of incubation temperature. The Kd value at 35°C was markedly higher than that of 30°C throughout all the pH ranges. The higher Kd value both in acidic and alkaline pH clearly indicates the effect of low and high pH value on the denaturation of *M. rosenbergii* muscle myofibrils.

Similar studies were also conducted on *P. monodon* muscle myofibrils which are shown in Fig. 4. As shown in Fig. the results obtained from *P. monodon* muscle myofibrils were more or less similar to that of Kd value obtained from *M. rosenbergii* muscle myofibrils where the myofibrils were found more stable at neutral pH ranges form 7.1 to 8.8 with the inactivation rate constant 2.8×10^{-4} s^{-1} to 3.31×10^{-4} s^{-1}. With the progress of acidic and alkaline pH the Kd value gradually increased. The result also shows that higher temperature of 35°C accelerated the Kd value in myofibrils compared to that of incubated at 30°C throughout the pH ranges used. However, the results also clearly indicate that the myofibrils of *P. monodon* are more stable compared to that of *M. rosenbergii* muscle myofibrils irrespective of wide ranges of pH values. The influence of pH on the inactivation rate constant (Kd) value of *M. rosenbergii* and *P. monodon* were investigated at 30° and 35°C under various pH values.

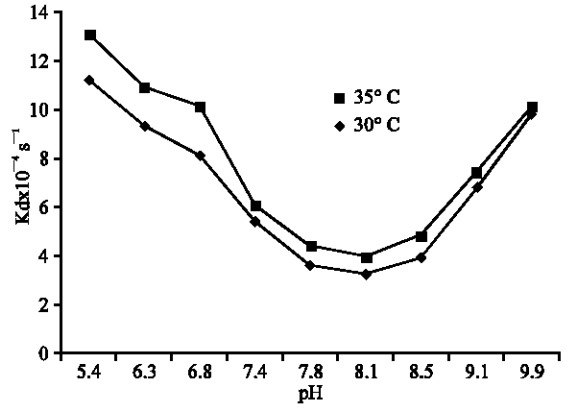

Fig. 3: Influence of temperature on the inactivation rate constant (Kd) of *M. rosenbergii* muscle myofibrils at various pH values

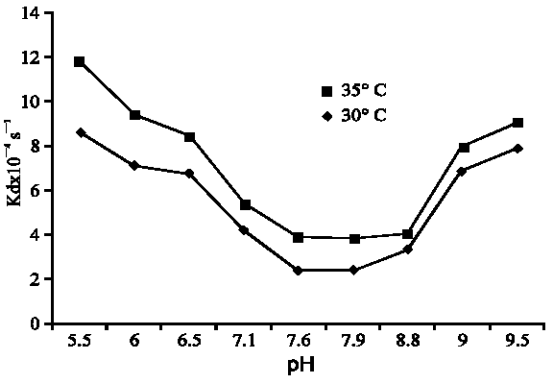

Fig. 4: Influence of temperature on the inactivation rate constant (Kd) of *P. monodon* muscle myfibrils at various pH values

The denaturation rate constant is high in acidic and alkaline pH values in both the species. This is an agreement with the results obtained previously where both ATPase activity and solubility were higher in neutral pH region but the activity drastically reduced both in acidic and alkaline pH region. A similar inactivation curve was reported for sardine ordinary muscle myofibrils[16,17] and yellow tail ordinary and dark muscle myosins[18]. In this study, the remaining Ca^{2+}-ATPase activity of both *M. rosenbergii* and *P. monodon* myofibrils after storage at various pH was found positively proportional to their stability. In present study, higher inactivation rate was observed at 35°C compare to that of 30°C in a wide pH ranges which clearly indicates the influence of higher temperature on the denaturation rate of myofibrillar proteins.

The denaturation rate constant of *M. rosenbergii* muscle myofibrils was higher than that of *P. monodon*

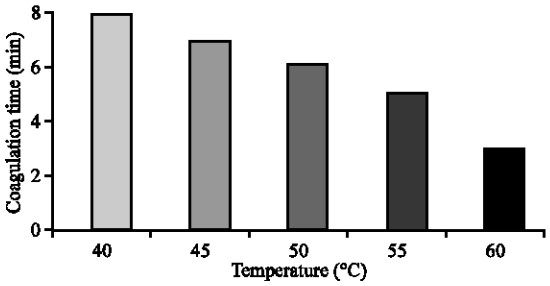

Fig. 5: Influence of temperature on the coagulation time of *M. rosenbergii* sarcoplasmic protein

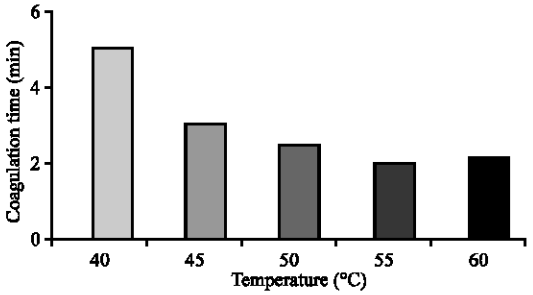

Fig. 6: Influence of temperature on the coagulation time of *P. monodon* sarcoplasmic protein

under similar storage conditions. The result is also correlated with those reported that the delayed icing of *M. rosenbergii* and *P. monodon* for certain period drastically reduce the shelf- life. In this studies delayed icing to 4, 8 and 12 h shorten the shelf-life of *P. monodon* to 7, 6 and 5 days, respectively[10] where incase of *M. rosenbergii*, delaying 4, 8 and 12 h shorten the shelf-life to 3, 2 and 1 days, respectively[8]. The study also clearly indicates that higher temperature may contribute higher driploss together with degradation of myofibrillar proteins.

Studies were also conducted to evaluate the effect of temperature on the coagulation time of sarcoplasmic protein of *M. rosenbergii* and *P. monodon*. Figure 5 shows the coagulation time of sarcoplasmic protein of *M. rosenbergii* at different temperature. It was found that at 40°C coagulation was started in 8 min and coagulation time decreased gradually with the increase of temperature and at 60°C the coagulation of sarcoplasmic protein was started in 3 min.

Similar studies were also conducted with the sarcoplasmic protein of *P. monodon* at different temperature (Fig. 6). As shown in Fig. 6 at 40°C coagulation of sarcoplasmic protein started in 5 min and the coagulation time decreased with the raise of incubation temperature and it was found that at 60°C,

coagulation started at 1.7 min. The results obtained from present studies also shows that sarcoplasmic protein of *P. monodon* denature more quickly than that of *M. rosenbergii* sarcoplasmic protein at higher temperature. The results obtained from the present study is a clear indication that sarcoplasmic protein of *P. monodon* denatured even at 40°C within a relatively short time. Studies were also conducted on the coagulation time of sarcoplasmic protein of *M. rosenbergii* and *P. monodon* at various temperature. The results indicate that the coagulation time of sarcoplasmic protein of *P. monodon* is much higher than that of *M. rosenbergii*. The phenomena is not clearly understood and more studies are needed to evaluate more precisely on this aspect. Available information suggests that coagulation time of sarcoplasmic protein varies from species to species.

Most sarcoplasmic proteins of pelagic fishes are coagulated when heated in water above 50°C, e.g. 90°C for 10 min. Only 65-75% of the sarcoplasmic protein from demersal fishes is heat coagulated[19]. The low molecular mass (12 kDa) parvalbumins remain soluble when heated at 70°C or above[20]. It was also reported that proteins from deep-sea fishes are less heat stable than those from warmer water[21].

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