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Lowering Rate of Freshness and Relative Thermo-stabilities of Actomyosin-ATPase of Muscles of Some Fish from Amami Island Sea

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Abstract

To determine the freshness qualities of fish caught from the fishing grounds of Amami Island, the fish K-values at 0, 10, 20 and 30°C, and also the thermo-stabilities of the actomyosin-ATPase at 20, 25, 30, 35 and 40°C were monitored.

The rate constant (K_f) of the decrease in the remaining K-values was highest in Yumekasago > Sokohobo > Mahata > Sokodara > Ohmonhata > Ohhime at low temperatures. On the other hand, the first order rate constant (K_D) of the inactivation of actomyosin-ATPase was highest in Sokohobo at 30°C. The actomyosin of Mahata and Ohhime appeared to be more stable than Sokohobo, Yumekasago, Sokodara and Ohmonhata.

Compared with frigid water fish at 30°C, the actomyosin of the above sub-tropical fish was apparently more unstable.

As a key to handling and storage of fish, one fundamental information that must be known is the inherent capacity of the fish to maintain its freshness at varying temperatures. Some fish are very sensitive to temperature effect but others have been noted to deteriorate less even on prolong storage. This property can be regarded as inherent with the fish species[1,2] and perhaps may vary with the place of catch, being of different ambient temperatures. For measurement of freshness of fish, the degradation of ATP (Adenosine 5'-triphosphate) related compounds (K-value) coupled with the characteristic actomyosin-ATPase (AM-ATPase) activity are usually monitored. These information on freshness quality of most common fish such as mackerel, cod and trout have already been made known, but for others which may have far economic importance like the fish from sub-tropical sub-deep sea, they are yet to be reported. In this regard, this study dealt on the fish coming from the fishing grounds of Amami Island, a sub-tropical sea north of Ryukyu Island.

Materials and Methods

Sample preparation

Fresh frozen fish from the fishing grounds of Amami Island namely Ohmonhata (*Epinephelus areolatus*), Ohhime (*Pristipomoides filamentosus*), Mahata (*Epinephelus*

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spetemfasciatus), Yumekasago (*Helicolemus hilgendorfi*), Sokodara (*Coryphaenoides sp.*), Sokohobo (*Peterygotrigle hemisticta*) and Tsumaritsunozame (*Squalus megalops*) were placed in −70°C freezer and withdrawn on sampling. Anterior dorsal muscles were ground and used for determination of AM-ATPase activities at 20, 25, and 30°C. Ground whole fish muscles which were kept at 0, 10, 20 and 30°C were used for determination of K-values.

Chemical analyses

A. K-value[3] Muscle perchloric acid extract was chromatographed through Dowex 1×4 (Cl^-, 50–100 mesh) with dilute HCl and NaCl, and the fractionated nucleotides were read at 250 nm. The K-value was calculated by SAITO's formula[4].

B. AM-ATPase activity The AM solution was prepared using the method of TAKASHI et al.[5]. The solution was incubated at 20, 25 and 30°C and then reacted with ATP substrate at 25°C for 5 minutes. The liberated phosphorus was measured by FISKE-SABBROW's method[6].

Results and Discussion

Lowering of freshness

The freshness of the fish sample muscle apparently decreased with time and the logarithm of the decrease, log (100-K), gave characteristically straight lines in all the fish sample muscles. This suggests that the decomposition of ATP and its derivatives followed a first order reaction, and as such the deterioration rate constants (K_f) were calculated accordingly and are shown in Table 1.

Table 1. Rate constants of the decrease in the remaining K-value in the fish sample muscles at various storage temperatures.

Species	Rate constants, $K_f \times 10^{-3}(hr^{-1})$			
	0	5	15	25 (°C)
Ohhime	0.53	3.78	(154.83)	
Ohmonhata	1.74	2.50	(4.92)	(9.27)
Sokodara	2.15	2.97	4.78	34.27
Mahata	2.73	6.13	(29.38)	(117.22)
Sokohobo	9.14	14.62	57.39	206.13
Yumekasago	13.77	30.87	54.56	70.76

( ): Estimated value

Among the 6 species, Yumekasago showed the fastest deterioration rate (highest K_f), followed by Sokohobo, Mahata, Sokodara, Ohmonhata and Ohhime at low temperatures. It may be concluded then that Yumekasago and Sokohobo are fast-

deteriorating at low keeping temperatures. In fact, they are even faster than known fast-deteriorating Saba (mackerel) and Hanafuedai[2] (*Tropidinus ameonus*) from Ryukyu fishing grounds.

Thermo-stabilities of the AM-ATPase activities

Apparently in Fig. 1, the logarithm of the remaining specific AM-Ca^{2+}-ATPase activities of the samples decreased with incubation period at 20, 25 and 30°C (at 35 and 40°C in the case of shark muscles), and the decrease was greater at higher temperatures. The plots of log remaining specific AM-Ca^{2+}-ATPase activities with incubation time were linear with correlation coefficient ranging from 0.9 to 1.0 indicating almost perfect linearity and first-order nature of the reaction. Accordingly, the inactivation rate constants (KD) were calculated. The first-order rate constants of the inactivation of the AM-ATPase activities evidently increased with temperature (Table 2). The order of their thermo-stabilities at 30°C was as follows: Mahata> Ohhime>Ohmonhata>Sokodara>Yumekasago>Sokohobo.

The thermo-stabilities of the shark muscle AM-Ca^{2+}-ATPase activities however were higher than any of the samples at 30°C.

Effect of temperature on the inactivation of AM-Ca^{2+}-ATPase in the dorsal muscles of samples

The rates of inactivation of AM-Ca^{2+}-ATPase on incubation for definite periods

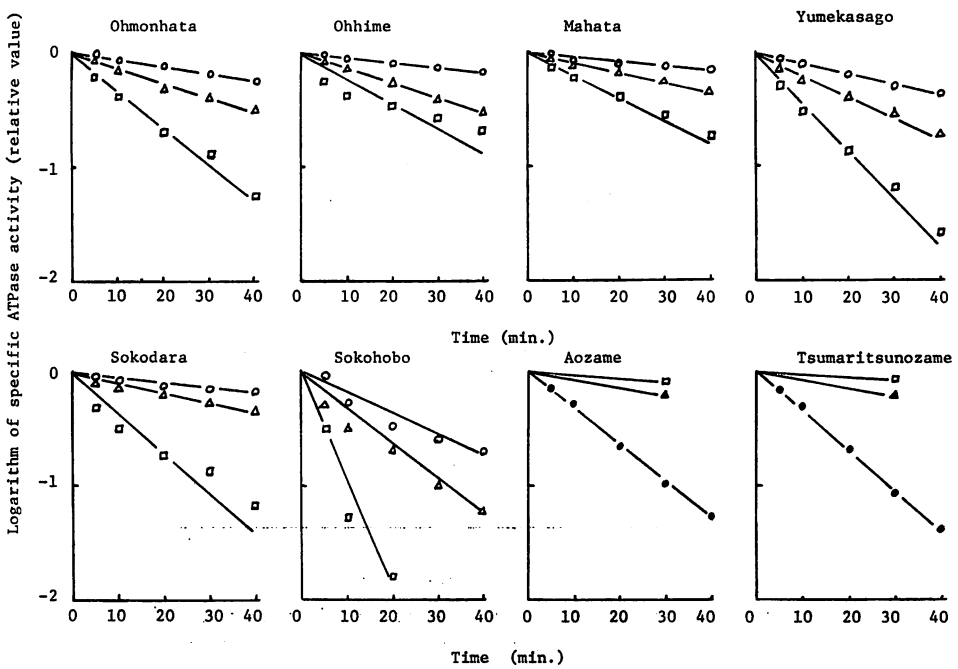

Fig. 1. Logarithm of activities of actomyosin Ca^{2+}-ATPase from the dorsal muscles as a function of time.

○: 20°C, △: 25°C, □: 30°C, ▲: 35°C, ●: 40°C

Table 2. Rate constants of the inactivation of actomyosin Ca^{2+}-ATPase from the dorsal muscle of samples in 0.6 M KCl at various temperatures.

Species	Rate constants (K_D) ($\times 10^{-4}$/sec)				
	20	25	30	35	40 (°C)
Ohmonhata	2.4	5.8	13.7	—	—
Ohhime	1.9	5.2	11.5	—	—
Mahata	1.7	3.6	8.3	—	—
Yumekasago	3.9	8.8	17.9	—	—
Sokodara	2.5	4.0	16.3	—	—
Sokohobo	2.0	10.1	27.0	—	—
Aozame	—	—	1.0	2.1	11.7
Tsumaritsunozame	—	—	1.1	2.5	12.8

as affected by temperature were measured and their curves are shown in Fig. 2. The temperature at which approximately fifty percent of the ATPase activity was lost within 30 minutes varied among AM of the various fish species. Shark muscles gave high stability even at about 30°C, while Mahata gave at about 27.8°C, Sokodara about 25.6°C, Ohhime about 23.0°C, Ohmonhata about 22.6°C, and Yumekasago about 20.2°C.

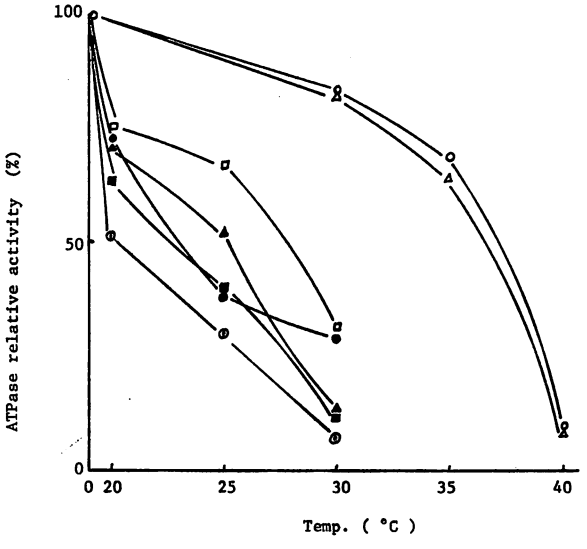

Fig. 2. Effect of temperature on inactivation of actomyosin Ca^{2+}-ATPase from the dorsal muscle of samples (pH 6.8 for 30 min).

○: Aozame, △: Tsumaritsunozame, □: Mahata,
●: Ohhime, ▲: Sokodara, ■: Ohmonhata,
◉: Yumekasago

Many investigators[7,8,9] have reported that the thermo-stabilities of the AM or myofibrils-ATPase prepared from dorsal muscles of epipelagic and mesopelagic and of various fish species living in the frigid water zone were very unstable. Comparing the rate constants and the temperatures of fifty percent reduction in the activity with representative frigid water fish like Atka mackerel and Alaska pollack, the sub-tropical fish have generally lower values. In other words, the AM of the muscles of Sokohobo, Yumekasago and Sokodara can be said to be more unstable than those of frigid water fish. While it has been considered that the thermo-stability of AM-ATPase in the fish muscle is adapted to the fish environmental temperature[7,10], the finding of this experiment showed otherwise.

For confirmation, it will be necessary to check whether the thermo-stability of AM-ATPase of the muscles of the sub-tropical fish samples are more unstable than those of frigid water fish.

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