Studies on Muscle of Aquatic Animals—XXXVII. The Influence of Frozen Storage on Muscle Protein of Lizard-Fish (*Saurida tumbil*)

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Previous workers^{1),2)} have reported that the fish muscle proteins, above all the actomyosin fraction (so-called "myosin" or "globulin fraction") is denaturated during cold storage and loses its solubility in neutral salt solution, though in general, the denaturation proceeds the more slowly the lower the temperature.

The extreme dislike to frozen fish held by some fishery processors such as *Kamaboko* (steamed fish cake) makers suggests that denaturation of muscle protein may occur very rapidly in some sorts of fishes. This work was undertaken to reveal the deterioration of muscle proteins during the cold storage with the muscle cut from lizard-fish (*Saurida tumbil*) which is known as a fish very liable to post-mortem change.

Method

As criterions for changes on muscle protein, the amount of free SH group and acid-soluble phosphates, pH value, stickiness of muscle brei and solubility in NaCl solution were taken.

SH group : The amount of free SH group in muscle was determined by a method similar to that described by MORI³⁾.

Phosphates : The determination of acid-soluble phosphates was carried out according to the directions of NOGUCHI⁴⁾. Total P organic P and Δ^7 P were determined in trichloroacetic acid extracts of fish skeletal muscle.

Stickiness : The muscle brei used for measurement of stickiness was prepared as follows. A mixture of 100g. of sliced fish skeletal muscle, 300 ml. of cold water, and 1.6 g. of NaCl was treated for 3 min. by Warling Blender (Homogenizer, Nihon Keiki Co. Ltd.). Then, 100 ml. of 2.4 M NaCl was added under stirring so that 0.6 M NaCl solution was obtained, which was filtered later with cotton cloth. Stickiness of the brei was measured by rotational viscometer (Type B viscometer, Tokyo Keiki Co. Ltd.) at 2° to 3° C. after 5 hours standing under chilling.

Solubility : Extraction with NaCl solution was used to determine the solubility of the muscle protein. If muscle was homogenized directly with salt solution, there occurred froth formation inducing protein denaturation, resulting in a low recovery of soluble protein. To avoid this surface denaturation, a mixture of about equal quantities of muscle and water was homogenized, and then NaCl solution was added to the muscle brei and stirred. In this way, more than 90 per cent of the muscle protein of fresh fish could always be

Fig. 1. Relation between time of extraction and the amount of soluble protein nitrogen extracted from lizard-fish muscle.

20 min. at 3500 r.p.m. and protein nitrogen was determined for the extract by the method using the biuret reaction⁷). The curves II show the results obtained for the storage at -14° to -18° C. for 48 days which set in 2 days after catch.

From figure 1 it is seen that it requires considerable time to extract soluble protein with salt solution of a lower concentration, and that this is emphasized by frozen storage. It may possibly be necessary to extract with a stronger salt solution for more than 12 hours to determine the really extractable proteins. In view of this investigation, however, the problem of the extracting time is rather complicated. For emphasizing the change in solubility during frozen storage, the shorter time extraction seems to be suitable. In the following experiments, a period of 2 or 3 hours was taken arbitrarily as the extracting time.

Experimental

(A) Change in lizard-fish muscle stored at -3° to -10° C.

Table 1. Acid-soluble phosphates and free SH group of frozen lizard-fish muscle stored at -3° to -10° C.

Samples frozen 2 days after being caught					Samples frozen 11 days after being caught				
Days of storage	SH (m. M.)	P. (μ M.)			Days of storage	SH (m. M.)	P. (μ M.)		
		T. P.	O. P.	Δ 7P.			T. P.	O. P.	Δ 7P.
0*	0.054	7,700	1,400	100	0*	0.054	7,700	1,400	100
1	0.065	7,910	1,360	10	2*	0.055	7,600	1,910	40
3	0.065	7,262	1,450	20	5*	0.051	7,080	1,210	30
6	0.071	7,220	1,320	0	9*	0.049	7,400	1,050	0
10	0.245	7,320	1,340	0	11	0.056	6,500	1,020	20
19	0.057	7,960	1,410	10	14	0.100			
27	0.054				19	0.057	6,580	1,020	0
44	0.040	7,120	1,180	50	27	0.059			
					44	0.044			

* unfrozen samples T.P. Total phosphate ; O.P. Organic phosphate ;
 Δ 7P. The amount of P corresponding to the sum of $2/3$ ATP-P and $1/2$ ADP-P.

A lizard-fish weighing about 1,800 g. had elapsed about 20 hours after catch was divided into two fillets. One of them was stored at 1° to 3° C., and the other was frozen in the ice box of refrigerator at -3° to -10° C. and stored there. At the same time, some lizard-fish weighing 315 g. on the average, were stored 10 days at 1° to 3° C. and then frozen and stored at -3° to -10° C. At intervals, samples were taken out for determination of solubility, free SH group, acid soluble phosphates, and pH value. In the determination of solubility, muscle was extracted with 0.6 M NaCl solution 20 times the volume of the muscle for 3 hours.

Results obtained are shown in Table 1 and Figure 2. From Figure 2 it is clear that the soluble protein nitrogen decreases rapidly during frozen storage. As for the solubility of the sample frozen from the start, the first decrease was followed by a transient increase, but the solubility dropped finally. It is known that the water soluble muscle protein is not affected by cold storage, and in lizard-fish the amount of water soluble protein nitrogen was about 730 mg. N per 100 g. muscle on the average. So, about 64 per cent of actomyosin fraction might well be inferred to have been turned insoluble in 44 days storage at -4° to -10° C. On the contrary, solubility increased in the storage at 1° to 3° C. It seems to be caused by the rise in pH value. After 5 days storage, the amount of total soluble nitrogen reached 94.0 per cent of total muscle nitrogen.

The pH value fell slightly in the first stage, then rose gradually. The amount of free SH group showed no remarkable change. The amount of phosphates did not show any definite tendency of variation. ATP had been lost already in the first experiment, apparently owing to the lapse of considerable time before the first analysis.

(B) Changes in lizard-fish muscle stored at -14° to -17° C.

Lizard-fish caught the day before were divided into two groups, one lot was stored at 1° to 3° C., and the other at -14° to -19° C. At intervals, pH value, free SH group stickiness and solubility was estimated as above. Soluble protein was extracted with 0.5 M and 1.5 M NaCl solution 20 times the volume of the muscle for 3 hours. The results were expressed as the percentage of soluble protein nitrogen for total nitrogen.

The results are given in Table 2 and Figure 3. The decrease of solubility is seen, but it is slower than the decrease given in Figure 2 which is concerned with the sample stored at a higher temperature. The protein nitrogen soluble in 0.6 M NaCl fell to about 35 per cent of original amount in 47 days storage. More protein was extracted with 1.6M

Fig. 2. pH value and soluble protein nitrogen of frozen lizard-fish muscle stored at -3° to -10° C. Open symbols show the samples frozen 2 days after being caught; solid symbols show the samples frozen 11 days after being caught (unbroken lines show unfrozen samples).

Table 2. Stickiness of muscle mush made from lizard-fish stored at -14° to -17°C. and 1° to 3°C. , respectively. (Poise)

Unfrozen sample (1° to 3°C.)					Frozen sample (-14° to -17°C.)				
Days of storage	Rate of rotation of rotor* (r.p.m.)				Days of storage	Rate of rotation of rotor* (r.p.m.)			
	2	4	10	20		2	4	10	20
0	435	255	125	73					
2	359	217	107	64	2	389	236	111	66
9	314	184	946	57	9	236	141	718	43
20	369	225	114	69	20	355	214	99	58
					35	192	122	65	42
					47	412	235	108	62

* Rate of rotation corresponds to the share rate.

Fig. 3. pH value, free SH group and soluble protein nitrogen of lizard-fish muscle stored at -14° to -17°C. and 1° to 3°C.
— 1° to 3°C. -14° to -17°C.

Fig. 4. Effect of NaCl salting on the solubility of lizard-fish muscle.

NaCl than with 0.6 M NaCl, though the tendency of the extractability was similar in both cases.

Apparent viscosity of the mush showed no remarkable change in spite of the decrease in protein solubility. In preliminary experiments, the viscosity was suggested to be almost directly proportional to the logarithm of the protein concentration of mush. The pH values showed the similar tendency to those given in Figure 2, though the change was not very clear. The amount of free SH group did not change remarkably.

(C) Effect of NaCl on denaturation of muscle protein

SIMUDU & HIBIKI⁷⁾ suggested, with ground yellowtail muscle that condensation of salts in muscle plasma caused by freezing might accelerate denaturation of muscle protein.

An experiment was carried out to reveal the relation between salt concentration in muscle and degree of the protein denaturation. Muscle of lizard-fish weighing 1460 g. was divided into fillets each weighing 10 g., and they were added respectively with various

amounts of NaCl and stored at 1° to 3° C. At intervals the samples were taken out and extracted with NaCl solutions respectively 20 times their volumes, which were adjusted of concentration so as to give 1 M as the final concentration of the extracting solution.

The values of soluble protein nitrogen are shown in Figure 4, which shows that salt soluble protein decreases rapidly when muscle was stored with NaCl, and that the higher the salt concentration, the more remarkable the decrease.

Discussion

Change in solubility was greater than changes in other indexes such as free SH group or stickiness. Considered from the absence of the increase in free SH groups, the change in the earlier stage of frozen storage may not seem to be real denaturation of muscle protein.

NOGUCHI⁴⁾ suggested that the contraction-like phenomenon is caused in fish muscle by freezing and thawing. In this investigation ATP in muscle had been lost at the first experiment, though fluctuation of organic P and fall in pH value were seen. It seems likely that the contraction of muscle brings about the difficulty in the extraction of muscle protein with salt solution especially with a dilute salt solution.

It seems clear that the elevation of salt concentration in muscle plasma caused by freezing may hasten the change of muscle during the frozen storage. The reasons for this acceleration are not as yet clear. It may be possible that the rise of salt concentration affects the enzyme equilibration in muscle so as to promote the muscle denaturation, or advance the "suwari" phenomenon, causing the loss of solubility of protein as it is seen in the salted fresh flesh or on standing a ground fresh fish muscle containing about 3 per cent NaCl.

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