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Inhibitory Effect of Phosphate Buffer on Denaturation of Fish Muscle Actomyosin by Freezing*1

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Abstract

The inhibitory effect of the K_2- and Na_1 phosphate buffer on the freeze-denaturation of actomyosin in Kcl and in the minced salt-added fish muscle was compared with that of the known denaturation-inhibitors (sorbitol and Na-glutamate). 1) When the actomyosin in 0.5 M Kcl was frozen-stored at −6°C, the decrease of its ATPase activity was inhibited by the addition of glutamate and phosphate of not less than 0.05 and 0.1 M, respectively, while the decrease of activity was scarcely inhibited even by the addition of sorbitol in 0.3 M concentration. However, when frozen at −25°C, any kind of the additives retained the activity of actomyosin nearly completely. 2) The combined effect of phosphate and glutamate on inhibiting the denaturation at −6°C was slightly lower than or almost the same as the effect of glutamate alone. 3) The jelly product prepared from the frozen phosphate-added mince was superior in the textural properties, especially in the water holding capacity, to that from the frozen sorbitol- and glutamate-added ones.

The authors[1-3] previously reported that the inorganic salts in the fish muscle fluid was occupied more greatly by phosphate than by chloride, and that the phosphate buffer was noticeably inhibitory to the denaturation of actomyosin in chloride during freezing of the solution. They further inferred that the relative effect of phosphate and chloride on the protein should be taken into account, in order to elucidate the relation of inorganic salts in the frozen fish muscle to their protein denaturation.

The experiment was performed to compare the magnitude of inhibitory effect of the phosphate buffer on the protein denaturation with that of the denaturation-inhibitors known before, sorbitol and Na-glutamate, and to obtain additionally some information on the mode of inhibitory effect of the phosphate.

Materials and Methods

Test with actomyosin solution Carp muscle actomyosin was isolated by the same procedure as described previously[3] and dissolved into 0.5 M Kcl containing K_2- and Na_1 phosphate buffer, Na-glutamate and sorbitol, respectively. After the solution was centrifuged for 15 min at 20,000×g and 0–2°C, its upper layer was submitted to the test. Definite aliquots of the actomyosin solution were frozen for

*1 Significance of Inorganic Phosphates in Fish Muscle, with Reference to Its Protein Denaturation in Freeze-storage-II.

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reasonable periods at −6° and −25°C, respectively. After the frozen solution was thawed to 0°C internal temperature in running water of about 20°C, the solubility and ATPase activity of actomyosin in the thawed solution were determined by the same method as previously described[3].

Test with minced fish muscle Ordinary muscle of the fresh horse mackerel was cut out and minced. The minced muscle was washed with tap water at 0–2°C, and de-watered with a centrifugal dehydrator. To the washed mince, concentrated solutions of the additives to be tested (K_2- and Na_1 phosphate buffer, Na-glutamate and sorbitol) each was added such that the additive was in the final concentration of 3.5%, and it was ground for 10 min. From the one half of the ground muscle, jelly product ("kamaboko") was prepared by a modification of the procedure described by NOGUCHI et al.[4] The other half of the ground muscle was frozen-stored at −25°C for 25 days and subsequently at −10°C for 10 days, and then the frozen muscle was thawed by being left overnight at 0–2°C. From the thawed muscle, the product was prepared in the same manner as with the unfrozen muscle.

The jelly strength and water-holding capacity of the product was determined by the methods developed by OKADA et al.[5] and SHIMIZU et al.[6], respectively. The water-holding capacity was expressed in terms of diffused area (cm^2) of the liquid exuded onto the filter paper from the product under a pressure of 10 kg/cm^2. The folding test for the product was run according to the method of OKADA et al.[5]

Results

Inhibitory effect of the K_2- and Na_1 phosphate buffer on freeze-denaturation of protein as compared with sorbitol and glutamate Figs. 1 and 2 show the changes in the ATPase activity of actomyosin when to its solution in 0.5 M Kcl was added in 0.3 M each of sorbitol, glutamate and phosphate and the solution was frozen-stored at −6° and −25°C. At −6°C, there was a distinctive difference respectively of the effect of the three additives on the enzymatic activity of protein. When glutamate and phosphate were added almost no change of the activity occurred, while when sorbitol was added the activity decreased steeply in the early period of freeze-storage. At −25°C, however, any kind of the additives kept the activity of protein stable. On the other hand, at both −6° and −25°C no the solubility of actomyosin in frozen solution did alter in any kind of the additives.

In Fig. 3 are shown the effect of concentration of added phosphate and glutamate on the retardation of denaturation of the actomyosin in Kcl stored at −6°C. By the addition of phosphate of 0.3 and 0.5 M concentration, the activity of actomyosin was retained completely for the storage periods, while with the concentration of 0.1 M or below the activity decreased markedly. However, when glutamate of 0.1 M or above was added, the activity of protein was kept nearly unchanged.

The combined effect of phosphate and glutamate on the denaturation of actomyosin in frozen Kcl solution is as shown in Fig. 4. In the combined addition of 0.05 M

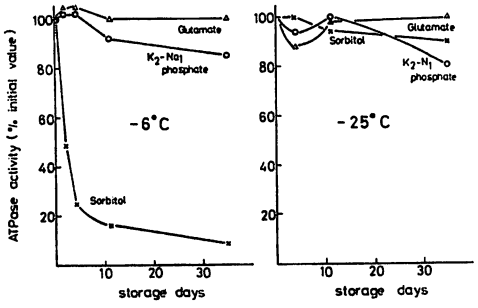


Fig. 1. Effects of Na-glutamate, sorbitol & K_2-Na phosphate on freeze-denaturation of actomyosin in KCl.

Test solution: KCl conc., 0.48M; protein conc., 3.3–3.8 mg/m*l*; additives conc., 0.3M; initial activity, 0.24–0.26 μmole Pi/mg/min.

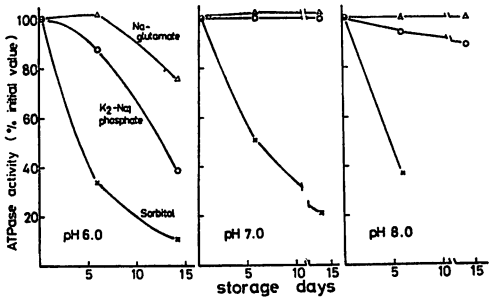


Fig. 2. Effects of three additives on freeze-denaturation of actomyosin in KCl at different pH values.

Test solution: protein conc., 3.8–5.1 mg/m*l*; additives conc., 0.3M; KCl conc., 0.48M; initial activity, 0.28–0.32 μmole Pi/mg/min. Storage temp., −6°C.

phosphate and 0.05 M glutamate, the effect of inhibiting the denaturation was considerably lower than that in the addition of 0.05 M glutamate alone, though it was higher than that in the case of 0.05 M phosphate alone. But the effect of the combined use of 0.05 M glutamate and 0.1 M phosphate resulted in almost the same effect as in the addition of 0.05 M glutamate alone. These results were considerably different from what expected.

Effect of phosphate, glutamate and sorbitol mixed to the washed and

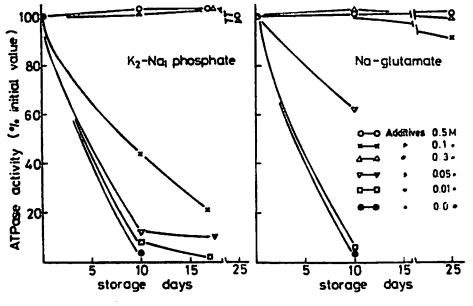


Fig. 3. Effect of concentration of added phosphate and glutamate on freeze-denaturation of actomyosin in KCl.

Test solution: KCl conc., 0.48M; protein conc., 6.3–6.8 mg/m*l*; initial activity, 0.23–0.25 μmole Pi/mg/min. Storage temp.: −6°C.

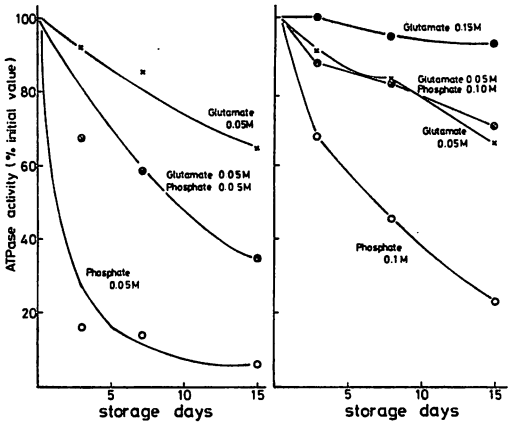
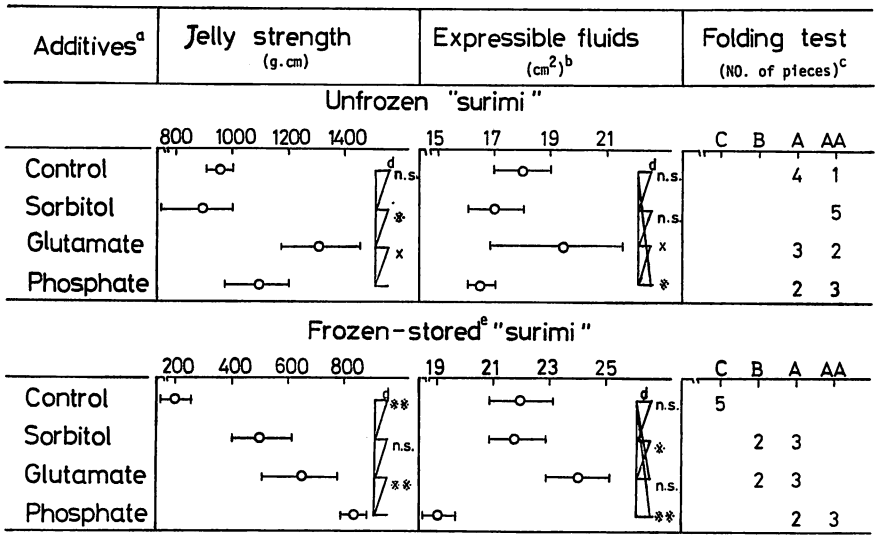


Fig. 4. Combined effect of additives on freeze-denaturation of actomyosin in KCl.

Test solution: KCl conc., 0.48M; protein conc., 6.0–6.3 mg/m*l*; initial activity, 0.32–0.34 μmole Pi/mg/min. Storage temp.: −6°C.

minced fish meat ("surimi") on the textural quality of jelly product ("kamaboko") The results of tests of the quality of products prepared from the unfrozen and frozen-stored horse mackerel "surimi" to which phosphate, glutamate and sorbitol respectively was added, are as shown in Fig. 5. In the case of unfrozen "surimi", the jelly strength of product was higher in the glutamate-added "surimi" than in other two "surimi", the water-holding capacity was higher in the phosphate-added one, and the folding test gave the better result in the sorbitol-added one.

However, in the case of frozen "surimi', the texture characteristics were all higher in the product made from phosphate-added "surimi", followed by the glutamate-added, sorbitol-added and additive-free (control) "surimi" in decreasing order. On the other hand, the product made of phosphate-added material was more briny, as would have naturally been anticipated, than other products.



a: added in 3.5%, b: area of fluid diffused onto filter paper. c: number of pieces of each grade assigned to 5 test pieces. d: difference test (※※ $p<.01$, ※ $p<.05$, × $p<.10$, n.s. no significance). e: 25 days at −25°C & 10 days at −10°C

Fig. 5. Quality of jelly products made from unfrozen and frozen-stored washed and minced horse-mackerel meat "Surimi".

Discussion

The results in Figs. 1 and 2 demonstrate that the phosphate buffer is more effective than sorbitol for inhibiting the denaturation of actomyosin in Kcl by freeze-storage, though its effectiveness is slightly lower than that of glutamate. The fact indicating the remarkably inhibitory effect of added glutamate coincides with the results obtained by some researchers[4,7,8] from the experiment at −20°C. However, the fact that inhibitory effect of sorbitol is much small at −6°C does not always agree with the

finding of OGUNI et al.[9], where the ATPase activity of actomyosin in Kcl stored at −8°C did scarcely alter, while its viscosity decreased appreciably. The inconsistency seems to have been resulted from the difference of concentration of protein in the test solution used.

The results in Fig. 3 also reveal that the inhibitory effect on the freeze-denaturation of protein in 0.5 M Kcl appeared in the addition of glutamate of 0.05 M or more and in the phosphate of 0.1 M or more. The concentration of added glutamate available to inhibiting the denaturation was higher than that found by NOGUCHI et al.[4] The discrepancy is probably due to the difference of the storage temperature of the test solution in both the experiments.

The resutls as shown in Fig. 4 imply that the combined use of phosphate and glutamate did not exert not only synergistically but also additionally inhibitory effect on the denaturation of protein. The fact suggests that both salts differ from each another in the mode of stabilizing effect on the protein in Kcl. The suggestion may be strengthened by the fact that jelly product from the frozen-stored phosphate-added mince was higher in the textural properties examined, especially in the water-holding capacity it was markedly high compared with the products made of the other materials, although the product made of the unfrozen phosphate-added mince was rather inferior in the above properties to other products (Fig. 5).

Thus, it may be concluded that the phosphate buffer is nearly as effective as glutlmate in inhibiting the denaturation of actomyosin by freeze-storage both in Kcl and in minced fish muscle, but that its effect is probably produced in a mode different from that in the case of glutamate.

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