Cryoprotective Effects of Chemicals on Proteins of Fish Muscle

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Introduction

Since freezing appeared at the beginning of this century as a new technique of storing fish, very many works have been published with regard to the technical improvement of freezing in order to improve the quality of frozen fish. Studies were also carried out to clarify the cause of deterioration of meat during frozen storage.[1,2]

Results of studies on the factors affecting the quality of frozen fish may be summarized as follows:-

1. Species of fish,
2. Prefreeze freshness of fish meat,
3. Freezing speed,
4. Condition of frozen storage:
   - Temperature
     — Depth
   - Stability (including repeat of freezing)
   - Contact with air
     — Glazing,
     — Package,
5. Thawing speed, and
6. Cooking method.

In contemporary fisheries the practice of freezing seems to satisfy much of these factors, so that frozen fish is sold in fairly good quality. However, there is still left a definite gap in meat quality between the fresh fish and the frozen fish. Particularly in some fish like the Gadoidae, the deteriorative effect of freezing is serious, and specially so in the decrease of the functional properties for processing the meat. Namely, the fish meat becomes hardly processable into fish gels like *kamaboko* and fish balls. Such damage was attributed mainly to the denaturation of proteins caused by freezing and storage.

The above gap was overcome by a novel technique called *"reito surimi"* (frozen mince) or rather popularly "surimi". The technique was proposed in 1959 by a group of fish technologists in the Prefectural Government of Hokkaido, Northern Japan. The group was headed by K. Nishiya.[3] This technique was able to significantly reduced the denaturation of protein and the processed meat could be used for producing *kamaboko* even after frozen storage for more than a few years. It must be pointed out that this technique was used successfully to freeze meat of gadoid fish which have been regarded the most susceptible to freezing denaturation. This finding has resulted in the full use of the previously underutilised resources of Alaska pollack in Northern Pacific.

As is well known now, surimi processing consists of two elements; one, removal of water soluble matters by washing, and the other, protection of proteins against freeze denaturation by addition of cryoprotectants.[4] During washing, inorganic ions, low molecular organic matters and water-soluble proteins are removed. The cryoprotectants employed in the original surimi of Nishiya are sucrose (5-10%), sorbitol (0-5%) and polyphosphates (0.3%).

Search for Other Cryoprotectants

In our laboratory, we have carried out a series of experiments to search for other potential cryoprotectants, resulting in the finding of several effective ones other than those used in the original prescriptions.[2,5-14] In the present paper our contributions will be briefly reviewed; suggestions for further studies on fish freezing and surimi production and production of novel processed foods will also be discussed.

Our experiments were mainly based on *in vitro* freezing tests of isolated fish proteins.[5] The cryoprotectants were added to the protein solution prior to freezing. After frozen storage for a definite period the content of the test tube is thawed and submitted to analyses and measurements of various items. This

experimental approach allowed us to acquire quicker answers with higher precision.

After screening more than 150 kinds of compounds we found several substances which demonstrated very good cryoprotective effects. Representative cryoprotective compounds found effective on fish actomyosin are:

Amino acids	with marked effect	Na-glutamate, Na-aspartate, lysine-HCl, cysteine, cysteate
	with moderate or little effect	alanine, serine, threonine, β-alanine, γ-butyrate
Dicarboxylic acids	with marked effect	malonate, glutarate
	with moderate or little effect	adipate, pimelate, maleate
Hydroxy carboxylic acids	with marked effect	glycolate, glycerate, lactate, gluconate, malate, tartrate, citrate
Polyalcohols	with marked effect	ethylene glycol, propylene glycol, glycerol, sorbitol
	with moderate or little effect	1,4 butanediol
Sugars	with marked effect	arabinose, glucose, mannose, galactose, fructose, sucrose, lactose, melizitose, stachiose

Each compound was added in a neutral form, viz acidic or basic ones were neutralized before use. Among the compounds listed above some of them like glucose, galactose, fructose, lactose and sucrose had been already reported by Nishiya's group.

With those compounds found very effective, application tests for *kamaboko* processing were also conducted, turning out similarly successful results.

Behaviour of Carp Actomyosin During Frozen Storage

Now, how the cryoprotective effects were proved will be described. For the additive cryoprotectant, sodium glutamate was most frequently used because it was outstandingly effective among the compounds found effective.

Carp actomyosin was freeze stored either in the presence or absence of 0.1-0.3 M sodium glutamate. Without sodium glutamate, actomyosin showed that it was denatured in every aspect tested, namely solubility, viscosity, ATPase activity, sedimentation profiles, etc. In the election microscopic profiles of the freeze denatured actomyosin the fine structures of the native actomyosin were lost and the filaments became aggregated with each other. When sodium glutamate was added prior to freezing those changes were definitely prevented.[5-18]

As far as there is aggregation of protein molecules taking place, there must be some kind of intermolecular bonding having been formed during frozen storage. This was proved by a differential redissolving experiment on actomyosin and myosin. The bonding was attributed to hydrogen bonds, ionic bonds, S-S bonds and nonpolar bonds.[19]

Behaviour of Myosin, Actin and Other Constituent Proteins During Frozen Storage

Similar experiments were conducted on each constituent protein of fish muscle, namely myosin, myosin subunits, actin, tropomyosin and lactate dehydrogenase. The last was studied as a representative of water soluble proteins, the so called myogens.

Decrease of solubility and viscosity during frozen storage was found in myosin and actin. Fall of biochemical properties such as ATPase activity of myosin, G-F transforming capacity of actin and enzymic activity of lactate dehydrogenase, was proved to occur in frozen storage. However, these changes, due to frozen storage, were depressed markedly when sodium glutamate was added to the system before freezing.[4,20,21]

Similar experiments were conducted on

subunits of myosin, namely H-meromyosin (HMM), L-meromyosin (LMM), S-1 and rod fragments, confirming denaturation taking place in each subunit, as well as its prevention by added sodium glutamate. It was to be noticed that the freeze denaturation occurred not only in the α-helical structure (tropomyosin, LMM and rod fraqment) but also in the nonhelical, random structures (actin, lactate dehydrogenase, HMM and S-1 fragment).

These results suggested that there was not only inter-molecular aggregation of whole protein molecules but also transconformation of each molecule, namely change of intramolecular structures.

To confirm these, experiments were conducted to study the changes in the intramolecular confirmation by use of differntial spectrometry, and measurements of natural fluorescence, probe fluorescence by naphtalene sulfonic acid (ANS) and circular dichrosism. Thus, it was confirmed that the transconformation occurs both in α-helical structure and in randomly coiled structure. This finding was to rewrite the conventional model of freeze denaturation of fish proteins[2] where the intermolecular aggregation of protein molecules not accompanying any transconformation is the major and sole change which occurs in the case of freeze denaturation.[14,21] It was remarkable that these conformational changes were also definitely or considerably depressed under the presence of sodium glutamate.

Mechanism of Freeze Denaturation and of Effects of Cryoprotectants

Based on the whole results of the screening tests for the cryoprotective effects of various compounds, all the tested compounds were lined up according to mutual similarity of the molecular structure.

Such study led us to assume the following regarding the structure of a cryoprotective compound.[2,5,13,14*]

1. The molecule must bear one essential functional group as well as more than one supplementary functional group as listed below:

- Essential functional groups

  Organic compounds — COOH or — OH

  Polyphosphates — PO_3H_2

- Supplementary functional groups — COOH, — NH_2, — SO_3H, OH and/or — SH

  Polyphosphates — PO_3H_2

2. Suitable distance and suitable configuration between the functional groups on a molecule.

3. Moderate molecular size.

When the cryoprotectant molecules are brought to the neighbourhood of a protein molecule, the protectant molecules are associated with the protein molecule via bonding between the functional groups, one on the protein, and the other on the cryoprotectant. The bonds are either of hydrogen bonds, ionic bonds, or S-S bonds. It results in a state where the protein molecule is coated with the cryoprotectant molecules. Because each cryoprotectant has more than two functional groups and since it keeps more than one functional groups free, these free groups must work either to hinder association of the protein molecules by electrostatic repulsion (the case with ionic groups) or to hydrate themselves as to bring about increased hydration of the protein as a whole (case with ionic groups and other hydrophilic groups like — OH). These must result in reduced associative force between the protein molecules.

In the case of the globular proteins, the increased hydration should resist against the removal of water on the occasion of freezing and must retain some portion of water in the neighbourhood of protein molecule. This must prevent the rupture of the intramolecular nonpolar bonds which are contributing to stabilization of the folded (randomly coiled) structure of the globular proteins.

The cryoprotective effect of the polyhydroxylic compounds such as mono- and oligosaccharides and polyalcohols, is interpreted as working on a principle distinct from above. Namely, these componds must function by interfering with the freezing-out of the ice crystals from the system. This view has its experimental bases in the facts, one, that the amount of freezing water is much less in a sugar-added meat system than a non-sugar system,[23] and the other, that the election microscopic profiles of carp actomyosin are very different between the glucose added system and the glutamate added system,[14,15]

* Mainly based on Noguchi's proposal[13] with partial amendments.

though the freeze denaturation is well depressed in both the cases.

The details of the facts and the reasonings will be published elsewhere.

Conclusion

Although the current surimi industry mainly relies on the principle of the original technique and its prescriptions of the additive cryoprotectants, namely sucrose, sorbitol and phosphates, there are good ground for developing other cryoprotectants to replace the popular ones. Introduction of a new cryoprotectant might enable us to broaden the variety of material fish, to bring about gel products of novel types of flavour, taste and texture, and to create new kinds of foods attractive for local consumers. This might stimulate the birth of an even more prosperous food industry related with surimi.

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1. Fennema, O.R., Powerie, W.D. and Marth, E.H. eds., 1973. Low temperature preservation of foods and living matter. Marcel Dekker Inc., New York, New York: 577.
2. Matsumoto, J.J., 1979. Denaturation of fish muscle proteins during frozen storage. In Fennema, O., ed., Proteins at Low Temperatures. Advances in Chemistry Series 180. American Chemical Society, Washington DC: 205-224.
3. Nishiya, K., Takeda, F. and Tamoto, O., Tanaka, O., Fukumi, T., Kitabayashi, T. and Aizawa, S., 1961, Monthly Rept. Hokkaido Municipal Fisheries Experimental Station, 18: 122.
4. Matsumoto, J.J., 1978. Minced fish technology and its potential for developing countries. In Indo-Pacific Fishery Commission Proceedings, Section III, IPFC Secretariat, FAO Regional Office of Asia and Far East, Bangkok: 267-271.
5. Matsumoto, J.J. and Noguchi, S. 1971. Control of the freezing-denaturation of fish muscle proteins by chemical substances. Proceedings of the XIIIth International Congress of Refrigeration Washington DC, Vol. 3: 237-241.
6. Noguchi, S. and Matsumoto, J.J., 1970. Studies on the control of the denaturation of the fish muscle proteins during the frozen storage-I. Preventive effect of Na-glutamate. Bulletin of the Japanese Society of Scientific Fisheries, 36: 1078-1087.
7. Noguchi, S. and Matsumoto, J.J., 1971. Studies on the control of the denaturation of the fish muscle proteins during frozen storage-II. Preventive effect of amino acids and related compounds. Bulleting of the Japanese Society of Scientific Fisheries, 37: 1115-1122.
8. Noguchi, S. and Matsumoto, J.J. 1975. Studies of the control of denaturation of the fish muscle proteins during frozen storage-III. Preventive effect of some amino acids, peptides, acetylamino acids and sulfur compounds. Bulletin of the Japanese Society of Fisheries, 41: 243-249.
9. Noguchi, S. and Matsumoto, J.J., 1975. Studies on the control of the denaturation of the fish muscle proteins during frozen storage-IV. Preventive effect of carboxylic acids. Bulletin of the Japanese Society of Scientific Fisheries, 41: 329-335.
10. Noguchi, S., Shinoda, E. and Matsumoto, J.J., 1975. Studies on the control of denaturation of fish muscle proteins during frozen storage — V. Technological application of cryoprotective substances on the frozen minced fish meat. Bulletin of the Japanese Society of Scientific Fisheries, 41: 779-786.
11. Noguchi, S., Oosawa, K. and Matsumoto, J.J. 1976. Studies on the control of denaturation of fish muscle protens during frozen storage-VI. Preventive effect of carbohydrates. Bulletin of the Japanese Society of Scientific Fisheries, 42: 77-82.
12. Ohnishi, M., Tsuchiya, T. and Matsumoto, J.J. 1978. Electron microscopic study of the cryoprotective effect of amino acids on freeze denaturation of carp actomyosin. Bulletin of the Japanese Society of Scientific Fisheries, 44: 755-762.
13. Noguchi, S., 1974. The control of denaturation of fish muscle protein during frozen storage. Doctoral Thesis, Sophia University.
14. Matsumoto, J.J., 1980. Chemical deterioration of proteins during frozen storage. In Whitaker, J.R. and Fujimaki M. eds., Chemical Deterioration of Proteins. ACS Symposium Series 123, American Chemical Society, Washington DC: 94-124.
15. Tsuchiya, T., Tsuchiya, Y., Nonomura, Y. and Matsumoto, J.J., 1975. Prevention of freeze denaturation of carp actomyosin by sodium glutamate. Journal of Biochemistry, 77: 853-862.
16. Oguni, M., Kubo, T. and Matsumoto, J.J., 1975. Studies on the denaturation of fish muscle proteins — I. Physicochemical and electron microscopic studies of freeze denatured carp actomysoin. Bulletin of the Japanese Society of Scientific Fisheries, 41: 1113-1123.
17. Ohnishi, M., Tsuchiya, T. and Matsumoto, J.J. 1978. Kinetic study on the denaturation mechanism of carp actomyosin during frozen storage. Bulletin of the Japanese Society of Scientific Fisheries, 44: 27-37.
18. Iguchi, S.M.M., Tsuchiya, T. and Matsumoto, J.J., 1981. Studies on the freeze denaturation of squid actomyosin. Bulletin of the Japanese Society of Scientific Fisheries, 47: 1499-1506.
19. Tsuchiya, Y., Tsuchiya, T. and Matsumoto, J.J., 1980. The nature of cross bridges constituting aggregates of frozen stored carp myosin and actomyosin. In Connell, J.J. et al. eds., Advances in Fish Science and Technology. Fishing News Books Ltds., Farnham, Surrey, England: 434-438.
20. Akahane, T., Tsuchiya, T. and Matsumoto, J.J., 1981. Freeze denaturation of carp myosin and its prevention by sodium glutamate. Cryobiology, 18: 426-435.
21. Akahane, T., 1982. Freeze Denaturation of Fish Muscle Proteins. Doctoral Thesis, Sophia University: 195.
22. Tamiya, T., Okahashi, N., Sakuma, R., Aoyama, T., Akahane, T. and Matsumoto, J.J., 1985. Freeze denaturation of enzymes and its prevention with additives. Cryobiology, 22: 446-456.
23. Love, R.M., Private communication.