Bull. Fac. Fish. Hokkaido Univ.
30(4), 282–287. 1979.

Interaction between Lipid and Protein during Frozen Storage

I. Effect of oil dipping on rainbow trout muscle during frozen storage*

Sei-ichi ANDOU,** Kōzō TAKAMA*** and Kōichi ZAMA***

Abstract

The effect of oil dipping on rainbow trout muscle stored at −20°C for 37 days was studied mainly by microscopic observation of frozen muscle section.

Lots of very small ice crystals were formed in the muscle dipped in olive oil, whereas with undipped muscle only large ice crystals were formed. Muscle bundles of the sample dipped in olive oil were fine and elongated at close intervals, whereas they were thick and short at large intervals in the undipped muscle. In the undipped muscle about 17.6% salt soluble protein was damaged during frozen storage compared with the muscle dipped in olive oil.

Relationships among lipid, water and protein during frozen storage are also discussed.

Introduction

It is well known that fish proteins (salt soluble proteins) become insoluble when fish are frozen and stored. The degree of protein denaturation is influenced by several factors such as freezing rate, method of freezing, temperature of frozen storage, state of rigor at time of freezing and especially the concentration of solutes in the liquid phase of frozen fish.

It is also a well known fact that the size of ice crystals is related to the extent of protein denaturation. In general, slow freezing results in the formation of large extracellular ice crystals with cell rupture, whereas quick freezing results in the formation of small evenly distributed ice crystals and the absence of any ruptured cells.[1)]

Previous study in this laboratory[2)] has found that fish dipped in olive oil have a small degree of protein denaturation as compared with that not dipped in olive oil during frozen storage. Also, fish dipped in olive oil have a higher available lysine content and digestibility in vitro by pepsin and trypsin.

This paper shows the effectiveness of oil dipping treatment on rainbow trout muscle during frozen storage based on microscopic observation of frozen muscle

* Contribution No. 11 from Nanae Fish-Culture Experimental Station, Faculty of Fisheries, Hokkaido University
(北海道大学水産学部七飯養魚実習施設業績 No. 11)
** Course of Nutritional Science, Hakodate Junior College
(函館短期大学食物栄養学科)
*** Laboratory of Food Chemistry I, Faculty of Fisheries, Hokkaido University
(北海道大学水産学部食品化学第一講座)

section and the determination of extractability of salt soluble protein content. Also, the relationships among lipid, water and protein during frozen storage are discussed.

Materials and Methods

Rainbow trout (*Salmo gairdneri irideus* GIBBONS), about 26 cm in length and 300 g in weight was obtained from Nanae Fish-Culture Experimental Station, Hokkaido University, filleted and pieces of dorsal lumps of almost the same size (about $1.3\times1.3\times4.0$ cm) were taken from bilateral parts along lateral lines. Immediately, one piece from the side dorsal lump was coated with corn starch, while another piece from the other side was first dipped in olive oil for a short time and then coated with corn starch. Both samples were packed in polyethylene bags and stored at –20°C for 37 days.

Frozen stored muscles were cut in thickness of 30 μ in frozen state and sections were fixed with iodine. The ice crystal marks and muscle bundle forms were observed microscopically.

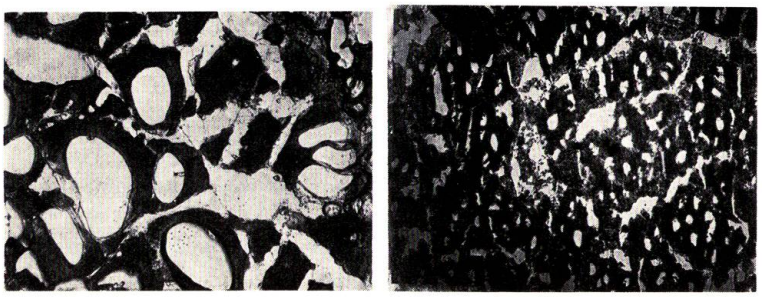
Also, salt soluble protein was extracted from both muscles according to the procedure shown in Fig. 1 and salt soluble protein content was determined using biuret method[3].

Coarsely minced muscle of rainbow trout (1 g)
|—washed with 20 ml of phosphate buffer (pH 7.5, μ=0.05)
|—centrifuged at 5,000×g for 15 min
Residue
|—extracted with 25 ml of 0.8 M KCl-phosphate buffer (pH 7.0, μ=0.85)
|—centrifuged at 5,000×g for 20 min
Supernatant (salt soluble protein)

Fig. 1. Procedure of the preparation of rainbow trout salt soluble protein.

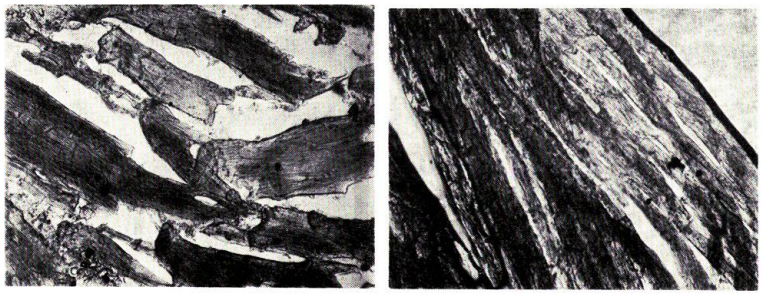
Results

Fig. 2 and Fig. 3 show microscopic photographs of cross and vertical sections of frozen muscle of both undipped and dipped rainbow trout in olive oil, respectively.



2-A. 2-B.

Fig. 2-A. Cross section of undipped frozen rainbow trout muscle (–20°C, 37 days). (×130)
Fig. 2-B. Cross section of frozen rainbow trout muscle dipped in olive oil (–20°C, 37 days). (× 130)



3-A. 3-B.

Fig. 3-A. Vertical section of undipped frozen rainbow trout muscle (–20°C, 37 days). (× 130)

Fig. 3-B. Vertical section of frozen rainbow trout muscle dipped in olive oil (–20°C, 37 days). (× 130)

A and B show microscopic photographs of undipped and dipped muscle in olive oil, respectively.

As shown in Fig. 2, there are some large sized air-bladders in the undipped muscle (A), whereas there are lots of small sized air-bladders in the muscle dipped in olive oil (B). As these air-bladders are considered ice crystal marks formed during freezing, it is shown that in the muscle dipped in olive oil lots of very small sized ice crystals have been formed during freezing. This differs from the undipped muscle. Furthermore, as Figs. 3-A, -B show, illustrating vertical sections of these muscles, the muscle bundles are thick and short with large intervals in the undipped muscle, whereas they are fine and long with close intervals in the muscle dipped in olive oil. These sections, as well as the cross sections, show that there are remarkable differences between undipped and dipped muscle in olive oil.

Also, as shown in Table 1, salt soluble protein content extracted from undipped and olive oil dipped muscle was 16.4% and 19.9%, respectively, and in the undipped muscle about 17.6% salt soluble protein was damaged during frozen storage as compared with the muscle dipped in olive oil.

Table 1. *Content of salt soluble protein extracted from rainbow trout muscle undipped and dipped in olive oil.*

Content of salt soluble protein	
Undipped	Dipped in olive oil
16.4% (82.4)	19.9% (100)

The values in parenthesis are relative to rainbow trout muscle dipped in olive oil.

Discussion

It is well known that salt soluble protein, which is the main constituent of fish muscle, is remarkably unstable and is easily denatured during frozen storage. Many studies associated with interaction between salt soluble protein and the other

components are reported. Interaction between actomyosin and sugars, amino acids and their derivatives is discussed from the viewpoint of preventing denaturation of fish muscle protein during frozen storage.[4)–12)]

Concerning interaction of lipid with protein, studies have shown that insolubility of actomyosin during frozen storage is promoted by free fatty acids produced by hydrolysis of lipid, low aliphatic aldehydes, and fatty acids produced from oxidized lipid.[13)14)]

However, the present facts concerning the prevention of denaturation of fatty fish muscle protein during frozen storage are reported[15)–17)]. As stated above, according to determinations of digestibility in vitro by pepsin and trypsin and of available lysine content, it was found that fish dipped in olive oil had a smaller degree of protein denaturation during frozen storage than fish undipped in olive oil. Moreover, the results of this study showed that muscle dipped in olive oil was closer to a normal state than undipped muscle, so it was considered that fish protein insolubility caused during frozen storage in muscle dipped in olive oil was slighter than in undipped muscle.

Thus, it is considered that the effect of lipid on protein during frozen storage differs from the case stated above.

Akiba[18)] determined total water, free water and bound water in the raw fresh meat of various kinds of fish. On the basis of these determined values, the relationship between bound water and total water is shown in Fig. 4. Note a negative reciprocity in the relationship. Moreover, it is shown in Fig. 5 that there is negative reciprocity in the relationship between total water and total lipid.[19)] As stated above, it was estimated that fish muscle possessing high lipid content has a large quantity of bound water.

Thus, judging from the relationship between lipid and protein, and between bound water and lipid, it was considered that there is an existing close relationships among these three — lipid, protein and water.

Further study of interaction between myofibrils and highly unsaturated fatty acid methylester, and lecithin is needed to elucidate the basic knowledge for preventing denaturation of fatty fish muscle protein during frozen storage.

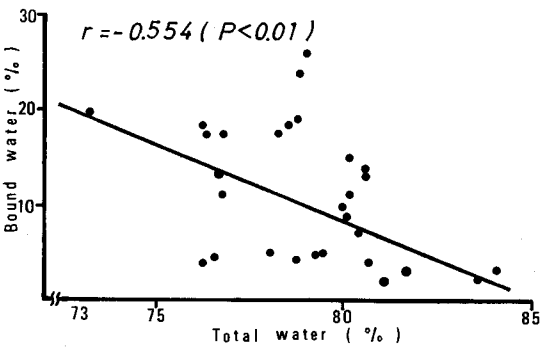


Fig. 4. Relationship between total water and bound water in the raw fresh meat of various kinds of fish.

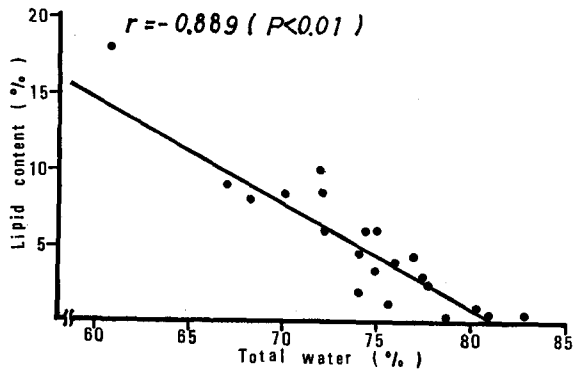


Fig. 5. Relationship between total water and lipid content in the raw fresh meat of various kinds of fish.

Acknowledgements

The authors wish to express their thanks to Dr. T. Kubo, Nanae Fish-Culture Experimental Station, Faculty of Fisheries, Hokkaido University, for providing the rainbow trout sample and to Mr. N. Kimura, Laboratory of Marine Food Technology, Faculty of Fisheries, Hokkaido University, for his kind preparation of frozen muscle section.

References

1) Kreuzer, R. (1965). *The technology of fish utilization.* 280p. Fishing News (Books) Ltd., London.
2) Takama, K., Andou, S., Zama, K., Nakamura, S. and Akatsuka, S. (1978). The quality of frozen sand lance treated with a water-dispersible tocopherol mixture. *Bull. Fac. Fish. Hokkaido Univ.* **29**, 56–64.
3) Gornall, A.G., Bardawill, C.J. and David, M.M. (1949). Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **177**, 751–766.
4) Arai, K., Takahashi, H. and Saito, T. (1970). Studies on muscular proteins of fish-III. Inhibition by sorbitol and sucrose on the denaturation of carp actomyosin during frozen storage. *Bull. Japan. Soc. Sci. Fish.* **36**, 232–236. (In Japanese with English abstract).
5) Noguchi, S. and Matsumoto, J.J. (1970). Studies on the control of denaturation of the fish muscle proteins during the frozen storage-I. Preventive effect of Na-glutamate. *ibid.* **36**, 1078–1087.
6) 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. *ibid.* **37**, 1115–1122.
7) Arai, K. and Takashi, R. (1973). Studies on muscular proteins of fish-XI. Effect of freezing on denaturation of actomysoin ATPase from carp muscle. *ibid.* **39**, 533–541. (In Japanese with English abstract).
8) Noguchi, S. and Matsumoto, J.J. (1975). Studies on the control of the denaturation of fish muscle proteins during frozen storage-III. Preventive effect of some amino acids, peptides, acetylamino acids and sulfur compounds. *ibid.* **41**, 243–249.
9) Noguchi, S. and Matsumoto, J.J. (1975). Studies on the control of denaturation of

fish muscle proteins during frozen storage-IV. Preventive effect of carboxylic acids. *ibid.* **41**, 329–335.

10) Noguchi, S., Oosawa, K. and Matsumoto, J.J. (1976). Studies on the control of denaturation of fish muscle proteins during frozen storage-VI. Preventive effect of carbohydrates. *ibid.* **42**, 77–82.
11) Ooshiro, Z., Hironaka, Y. and Hayashi, S. (1976). Preventive effect of sugars on denaturation of fish protein during frozen storage. *Mem. Fac. Fish., Kagoshima Univ.* **25**, 91–99.
12) Hironaka, Y., Hayashi, S. and Ooshiro, Z. (1976). Interaction between protein and sugar during frozen storage. *ibid.* **25**, 101–105.
13) Anderson, M.L. and Ravesi, E.M. (1968). Relation between protein extractability and free fatty acid production in cod muscle aged in ice. *J. Fish. Res. Bd. Canada* **25**, 2059–2069.
14) Takama, K., Zama, K. and Igarashi, H. (1972). Changes in the flesh lipids of fish during frozen storage. Part-III. Relation between rancidity in fish flesh and protein extractability. *Bull. Japan. Soc. Sci. Fish.* **38**, 607–612. (In Japanese with English abstract).
15) Dyer, W.J. (1951). Protein denaturation in frozen and stored fish. *Food Research* **16**, 522–527.
16) Dyer, W.J., Morton, M.L., Fraser, D.I. and Bligh, E.G. (1956). Storage of frozen rosefish fillets. *J. Fish. Res. Bd. Canada* **13**, 569–579.
17) Okada, M., Yokozeki, G. and Kinumaki, T. (1974). Gyoniku neriseihin, Riron to ōyō. 431p. Kōseishakōseikaku, Tokyo. (In Japanese).
18) Akiba, M. (1952). Studies on bound water in fish meat muscle (III). *Bull. Fac. Fish. Hokkaido Univ.* **2**, 239–261.
19) Nonaka, J., Hashimoto, Y., Takahashi, T. and Suyama, M. (1976). Suisan syokuhingaku. 298p. Kōseishakōseikaku, Tokyo. (In Japanese).