Effect of Lipids on Insolubilization of Protein in Frozen Fish Muscle during Storage*

Fuyuo OHTA and Jun-ichi NISHIMOTO

Abstract

Insolubilization of salt-soluble protein of the fish during the freezing storage was slightly affected by fatty acid esters mixed into the flesh or by fish body oil, but it was accelerated by fatty acid. The extent of acceleration was enlarged with the increase of mixed quantity, that by lower fatty acids being greater than that by the higher ones; and it was affected by storage temperature, while on the other hand, addition of antioxidant was inhibitory against insolubilization.

Addition of glycerine, within certain range of concentration, was preventive against insolubilization of protein; on the other hand, glycerine was stimulative for lipase activity of fish muscle juice in the frozen state, but inhibitory at the moderate temperature.

The results mentioned above indicate that the prevention of lipids in frozen fish against oxidation and decomposition is indirectly effective in reducing the denaturation of protein.

The largest deterioration of frozen stored fish is denaturation of muscle protein. The extent of denaturation is divergent among defferent kinds of fishes, the cause of which is not distinctly known yet. DYER et al. (1956, 1959)[1) 2)] mentioned lipid contents and contents of fatty acid in the flesh as the cause of this difference, while OLLEY et al. (1961)[3)] disagreed with the view claiming its modification. On the other hand, SHIMIZU et al. (1957)[4)] reported that the stability of fish protein may have a relation with the softness of muscle, and CONNEL (1961)[5)] made clear that the stability of salt-soluble protein was essentially different among different kinds of fishes. The authors inferred that the extent of insolubilization in the case of stationary fishes was larger in general than of migratory ones, and that it was influenced by the content of free fatty acid and the oxidation degree of lipid in the flesh.[6)]

Therefore, in the present experiment, mixing test was performed to examine the influences of deterioration products of lipid conceived to be most significant among coexisting substances in the flesh, excluding the point of essential difference of protein.

Materials and Methods

Preparation of samples

Fresh flesh of mackerel obtained commercially was chiefly used in the experiment. Each of the test materials mentioned below was mixed in the mince of normal muscle and every 10 g of the mixture was made in blocks of fixed size and

* Accepted for presentation at the 11th International Congress of Refrigeration, Munich, August 1963.

stored −10° and −20°C cold room. At certain intervals during storage, frozen flesh taken out of a cold room was submitted to analysis after thawing at 0°C for 2.5 hr. All of the preparative operation was carried out in 5°–10°C cold room and each operation for mixing was made for a fixed time. For part of experiment, saline extract of flesh obtained with 15 volumes of 0.6 M KCl of pH 7.5 was used and to the extract was added KCl solution of the test materials.

Test materials

Free fatty acid (oleic, linoleic caprylic, and caproic acids were obtained from Tokyo Kasei Co. and purified as needed); fatty acid ester (methyl-oleate was prepared by usual method); fish oil (tuna, cachucho and horse-mackerel oils were separated from minced muscle by water-cooking method); Oxidized oil and fatty acid (tuna oil and oleic acid taken in a petri dish were arbitrarily autoxidized at 30°C); alcohol (chemical pure, glycerine, propylene glycol, ethanol and methanol); antioxidant (technical BHT and BHA).

Analysis

In the case of block, salt-soluble protein wes measured by the Biuret method after extracting with 0.6 M KCl of pH 7.5. The precipitation obtained by diluting the extract 8 times with cold water and standing at 0°C overnight was measured as actomyosin.

In the case of saline extract, it was slightly mixed after thawing and centrifuged after standing for 20 minutes and the upper layer was analyzed for protein.

Measurement of lipase activity of muscle juice

Muscle juice was separated from the minced mackerel muscle by centrifugation and was used as a crude enzyme solution. An aliquot of juice was added to the mixture of aqueous solution of triacetin and phosphate buffer solution (0.075 M, pH 7.2). The reaction mixtures obtained was kept at 37° and −10°C. Fatty acid liberated was titrated with alcoholic potassium hydroxide solution.

Results and Discussion

Effect of free fatty acid upon insolubilization of protein

The results of the case where oleic, linoleic, caprylic and caproic acids were mixed in mackerel flesh is as shown in Figs. 1 and 2. It is surely seen that the existence of free acid facilitated insolubilization of protein, and that the extent of facilitation was enlarged with the increase of mixed quantity, those by caprylic and caproic acids being greater than that by oleic acid; and it was greater at −10° than at −20°C.

This is conceived presumably, as Mabrouk (1961)[7] testified with linoleic acid, due to the lowering of pH value of the flesh, though it may be very slight, when the fatty acid was dispersed in the water. In the experiment, the pH value of flesh was observed lowered, though very slight. These facilitating effects of free acid can be obviously seen in Fig. 3 where compared results of oleic acid with

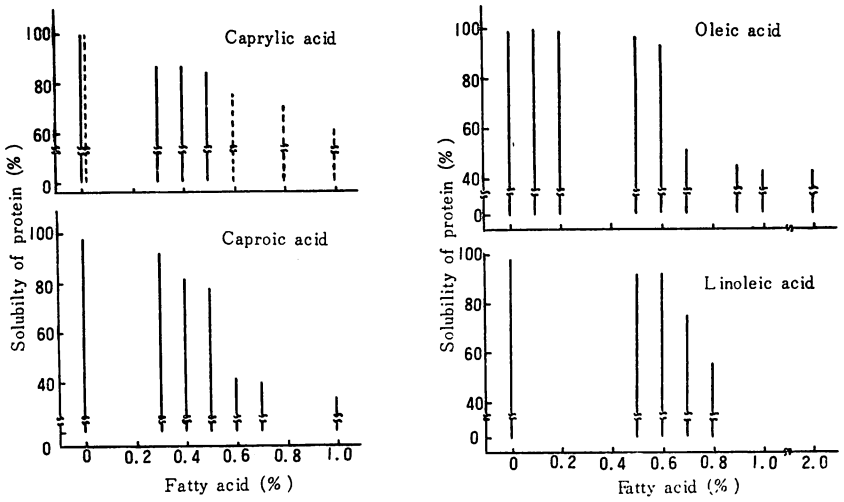

Fig. 1. Effect of added fatty acids on salt-soluble protein of mackerel muscle stored at 0°C for a day.

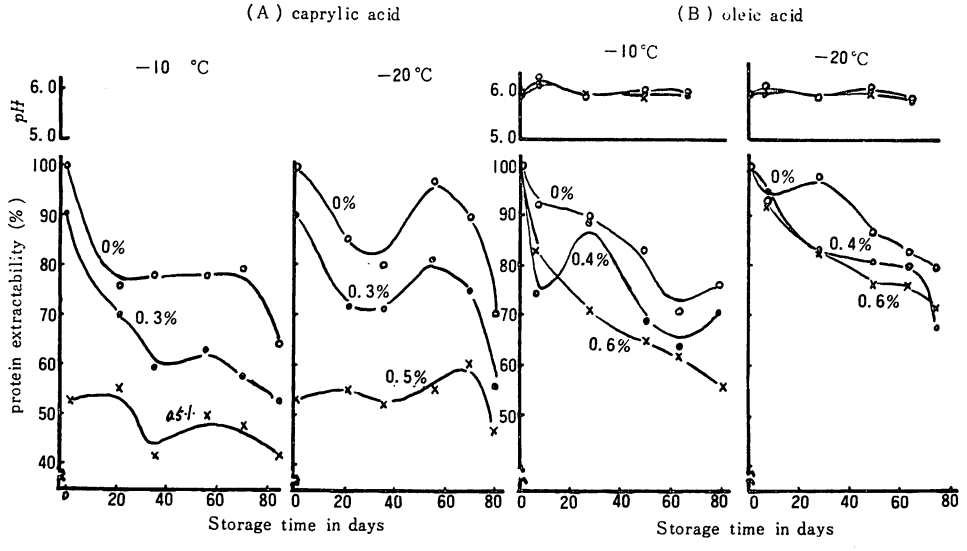

Fig. 2. Influence of added caprylic and oleic acids on extractability of salt-soluble protein in minced mackerel flesh during frozen storage.

methyl-oleate are illustrated, and in the case of fish oil containing almost no free acid, as shown in Fig. 4, its effect was found only slight.

Therefore, formation of free fatty acid by hydrolysis of lipid in the muscle appears to be one significant factor affecting protein denaturation. This gives experimental support to Dyer's hypothesis mentioned above.

Effect of oxidized fat and fatty acid upon insolubilization of protein

The effect when autoxidized fish oil and oleic acid were mixed are as shown

in Fig. 5. The percentage of extracted protein was smaller in either case of them when they were more autoxidized. These results show plainly the facilitation of insolubilization of protein by oxidized substances. This also may be seen from Fig. 6 where the effect of antioxidants mixed in the flesh by 0.01% is shown. Addition of antioxidants was inhibitory against the oxidation of lipid and simultaneously, though slightly, against insolubilization of protein. These facts show that oxidation of lipid may be one of factors affecting protein denaturation.

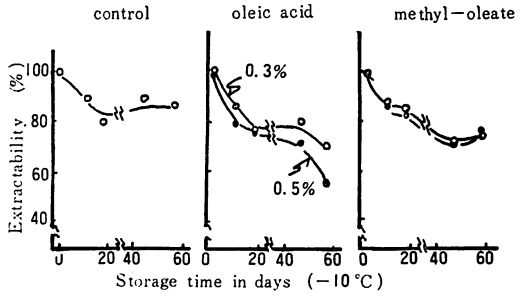

Fig. 3. Influence of added methyl-oleate and oleic acid on extractability of protein in frozen mackerel muscle.

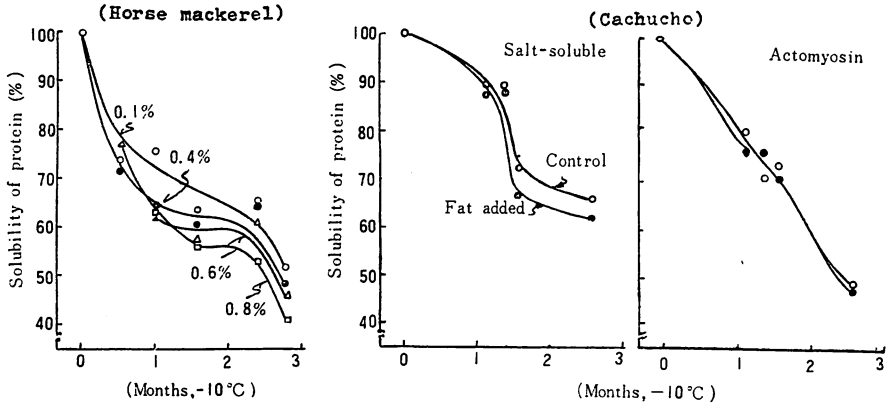

Fig. 4. Effect of addition of oil separated from fish muscle on solubility of its protein during frozen storage.

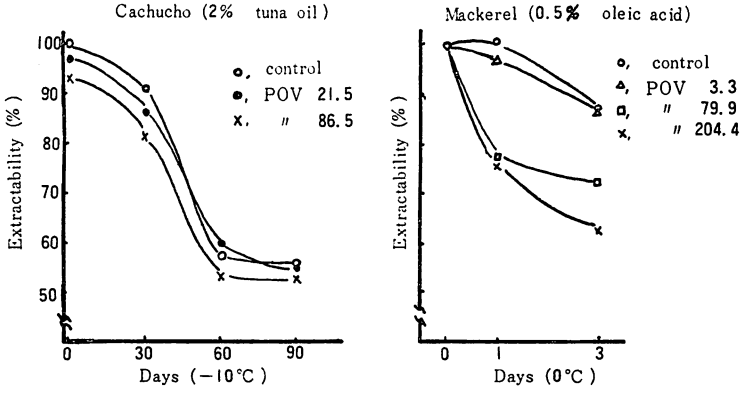

Fig. 5. Influence of addition of oxidized fish oil and fatty acid on protein extractability.

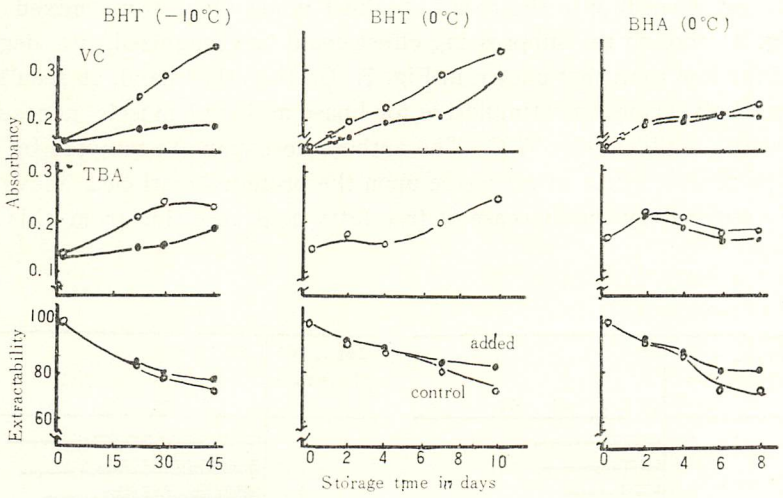

Fig. 6. Influence of added antioxidants on extractability of protein in mackerel muscle during frozen storage.

Effect of alcohols upon insolubilization of protein

The relation of protein insolubilization in the KCl extract with added alcohols is shown in Fig. 7. Any kinds of alcohols, within certain range of concentration, were proved to have surely suppressed insolubilization, which is conceived to be due to great hydrophilic property of alcohols and to its property of lowering the freezing point of the solution.

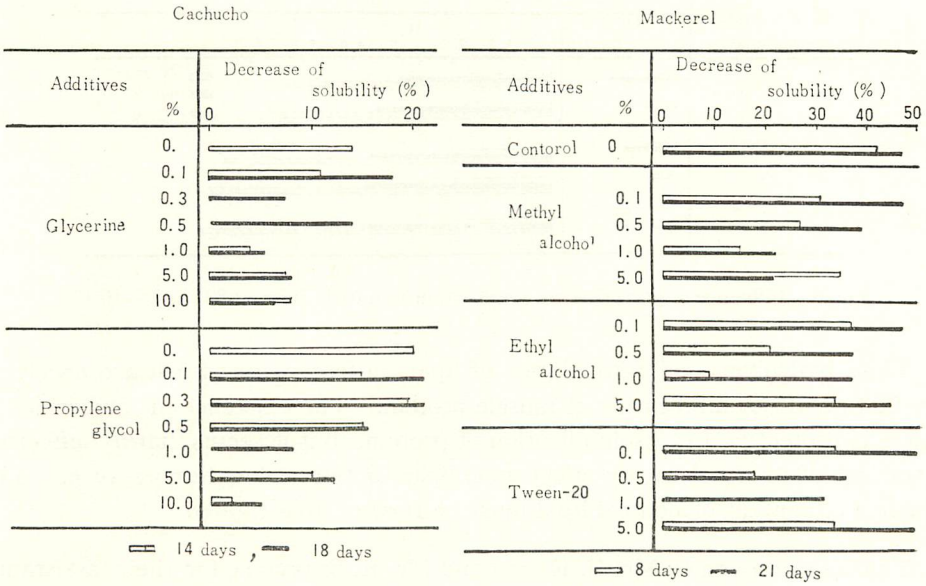

Fig. 7. Effect of added alcohols on solubility of protein in saline extracts of fish mucsle stored at −10°C.

However, according to the result obtained when they were mixed into the flesh (Fig. 8), though the suppressing effect could be recognized, its degree was observed far low than that shown in Fig. 7. On the other hand, as seen in Fig. 9, existence of glycerine was stimulative for lipase action of muscle juice stored at −10°C, while inhibitory at 37°C. The authors hereupon are tempted to conceive that the protective effect of glycerine upon the protein mentioned above will be indirectly reduced by the increase of free fatty acid resulting from this facilitating action.

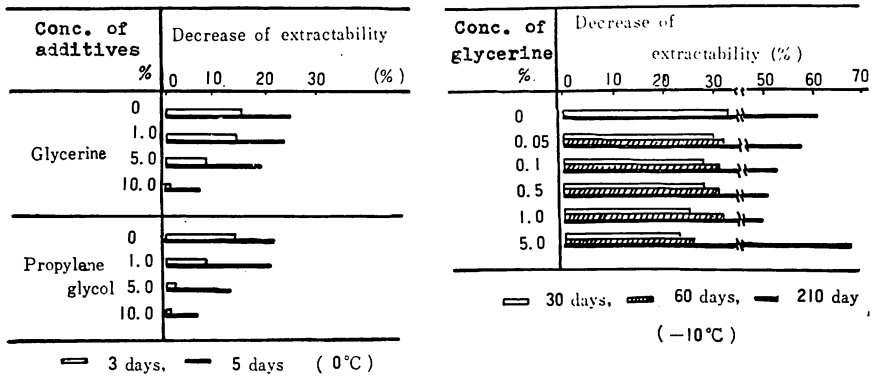

Fig. 8. Effect of added glycerine on extractability of salt-soluble protein in minced mackerel muscle during storage.

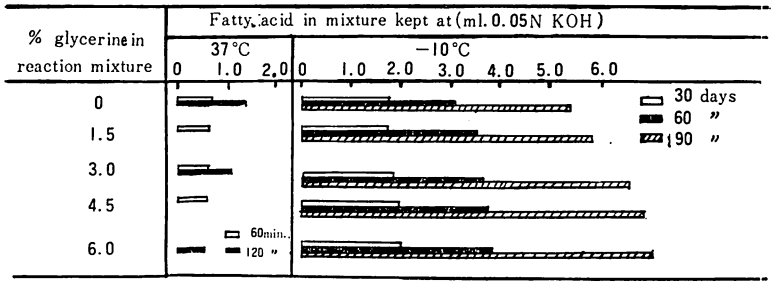

Fig. 9. Influence of glycerine on lipase action of muscle juice at 37° and −10°C.

Thus, hydrolysis and autoxidation of lipids in frozen stored fish are acceleratory factors of insolubilization of muscle protein. The existence of glycerine in muscle is protective for insolubilization of protein, but it seems hardly effective on account of its acceleratory effect upon lipase action at low temperature. The influence of content of neutral lipid must be further investigated.

Thanks are given to Mr. H. Kondo and Mr. K. Shinmura for their assistance in the experimental work.

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