CHAPTER VI

DENATURATION OF THE MUSCLE PROTEIN OF SOME FRESHWATER TILAEOSTS DURING STORAGE AT -4°C

INTRODUCTION

The loss in the biological activity of tissue may be produced by the denaturation of protein. To quote Love (1966): "Protein denaturation is defined as an alteration in the conformation of polypeptide chain to produce a more disordered structure".

The earlier findings (Pennington, 1908; Smith, 1913; Perlweig and Gies, 1913), based on the theory that denatured protein could be quantitatively expressed as percentage of coagulable nitrogen and/or water soluble nitrogen, failed to produce expected results when the period of storage of the tissue was extended. Later, a few investigators (Nortswarp and Heen, 1938; Banks, 1955a,b) tried to assess protein denaturation by the quantitation of exuding fluid (drip) after thawing, but the fact could not offer a wide acceptance due to the unreliability of the amount of drip exuded.

Reay (1933), while working with haddock, observed
that denatured protein is not water soluble but could be
dissolved in dilute salt solution. This criterion which
was used in all subsequent studies formed one of the bases
to understand the internal or external causes of protein
denaturation occurring in the stored tissue. Albeit, there
had been some apparent refinements in the protein solubility
determinations (Dyer et al., 1950), Love (1958, 1959, 1960)
and Love and Mackay (1962) evolved the technique of 'cell
fragility' for measuring protein denaturation, limiting
the applicability of salt solubility method, where the
nutritional state of the fish, which might cause consider-
able variations, was almost neglected.

Disatisfaction with the precision of the different
methods, however, led various workers to attempt to measure
the denatured protein in other ways. Seagran (1966) and
Ueda et al. (1962), while assuming that the shape and size
of the protein are greatly affected by the phenomenon of
denaturation, extracted actomyosin and attempted to corre-
late the viscosity with the degree of denaturation, though
they failed to produce any generalization. Hotani (1955)
measured the internal friction during storage by an appara-
tus capable of oscillating with a specific amplitude/parti-
cular friction. But this technique could not show its
sensitivity when the internal friction increased to a
particular point. Since certain proteins act as enzymes, the angle of enzyme activation was also taken for assessing the denatured protein (Partmann, 1954; Connell, 1960). Husaini and Alam (1955), Connell (1960) and Golovkin and Pershina (1962) made use of the sulphhydril groups, while Cornell and Howgate (1964) used the shape of the hydrogen ion titration curves in a suspension of cod myofibril, but the two techniques could not be used as possible indices to judge the rate of protein denaturation during extended period of storage.

Of considerable technological importance is the fact that even under refrigerated condition, fish muscle protein gets denatured and this lead to some textural changes and toughness which, in turn, appears to reduce the consumers appeal of the product. Though a considerable body of literature exists on the subject (Dyer et al., 1950, 1957, 1964; Dyer, 1951; Heen, 1954; Luijpen, 1957; Ironside and Love, 1958; Connell, 1962, 1969; Love, 1962; Jamberga, 1964; Dykov, 1971), little progress has been made to understand the possible mechanisms involved in the process of denaturation. The theory that the production of the free fatty acids in the frozen fish may be accompanied by an insolubilization of protein (Dyer and Fraser, 1959; Olley and Lovorn, 1960) could not secure general acceptance. However, when Olley
At al. (1962) extended this theory to many other fish species; they found that though cod, halibut and lemon sole produced very similar amounts of free fatty acids on cold storage at -14°C, yet they showed completely dissimilar rates of protein denaturation as measured by the solubility in 5% sodium chloride solution. Thereafter, Hansen and Olley (1965) produced evidence to indicate that neutral lipid protects the protein from free fatty acid denaturation.

With few notable exceptions, much of the work on fish protein denaturation has been conducted at the laboratories of Torry Research Station, Aberdeen, Scotland and Technological Stations of the Fisheries Research Board of Canada. A historical background and comprehensive account of the process of denaturation in fish has been given by Love (1966), and Dyer and Mingle (1961).

An account of the protein fractions affected by the process of denaturation has been given by Connell (1966). Connell (1968) has also summarized the possible causes of protein denaturation in fish. The work of Dyer (1951) has indicated the rate of protein denaturation with the taste panel of fish, but Luijpen (1957) discarded the rationale and pointed that these two phenomena did not always go together. Despite the variability in the methods to
measure the rate of denaturation, Love (1958) has discussed the various factors which might affect the rate of protein denaturation in fish.

However, the most satisfactory method evolved for the assessment of the rate of protein denaturation in fish during storage at low temperature appeared to be the solubility of protein in neutral salt solution (5% NaCl).

The following account is based on the observed changes in the rate of protein denaturation, as measured by the solubility in 5% neutral sodium chloride solution, of three freshwater teleosts, namely, Ophicephalus punctatus Blech, Clarias batrachus (Linn.) and Heteromesaspis fossilis (Blech) during 16 days of storage at -4°C.

MATERIALS AND METHODS

The method of storage, tissue sampling and processing were the same as described in the earlier section (see page 51). The techniques of extraction and estimation of protein denaturation have been described under 'Procedure and Methodology'.

Fig. 20. Changes in the solubility of muscle protein of *C. punctatus* during storage at -4°C.
Fig. 21. Changes in the solubility of muscle protein of *C. batrachus* during storage at -4°C.
RESULTS AND DISCUSSION

The values of the percentage of soluble protein in the muscle of the three species during 16 days of storage at -4°C have been given in the Tables 22 - 24, and plotted in Figs. 26 - 22.

In the three species examined, storage at -4°C were characterized by a steady decline in the percentages of soluble protein. In the fresh condition, the protein solubility figures varied little in the three teleosts (Tables 22-24). On a comparative basis, however, the solubility was higher (8.018%) in C. punctatus, while in C. baya and H. fasciata, the solubility value were 7.337% and 7.299%, respectively. The latter two species, being systematically close to each other, showed very close values. Also, the percentage of fall in the muscle protein solubility of C. baya and H. fasciata during frozen storage were almost similar (about 33%) as compared to the percentage of decline (about 30%) in C. punctatus.

The postmortem loss observed in the solubility of the muscle protein of the three freshwater species during storage at low temperature (-4°C) could be the result of some such factors as dehydration, increase in the salt
concentration—as more water is frozen out, a change in
the water holding capacity of protein and/or increased
oxidation of lipid.

The results have clearly indicated both to the
thermal effect as well as to the effect of the length of
storage on the phenomenon of protein denaturation in
fish.

It has earlier been shown that the extractibility and
solubility of both sarcoplasmic and myofibrillar proteins
of pig, ox and rabbit muscle are markedly altered by post-
mortem conditions of pH and temperature (Bendall and
Wismer-Federsen, 1962; Sayre and Briskey, 1963; Scopes and
Alterations of a similar nature related to the postmortem
denaturation may occur in fish muscle.

It appears to be generally accepted that denaturation
of fish muscle protein, soluble at low ionic strength, does
not occur, or is very slight during cold storage, unless it
is accompanied by dehydration (Dyer and Mingie, 1961).
Dyer (1951) has indicated that improper wrapping of the fish
leads to the dehydration resulting in the denaturation of
protein in the surface layer. On the other hand, as tempe-
rature is reduced, more water is frozen out and concentration
of salt increases (Birdeeey, 1929; Reay, 1933, 1934; Finn, 1934; Dyer et al., 1957; Love and Haroldson, 1958). Furthermore, the increase in the salt concentration brings the protein molecules into closer contacts, causing considerable damage to them (Reay, 1933; Snow, 1930; Tokunaga and Nakamura, 1961). The shrinkage of the cells as a result of ice dehydration (Maryman, 1968) may also cause damage to the muscle protein of fish. Some of the changes, such as water holding capacity of the protein, are reduced by low temperature. This change in the hydration characteristic of the protein has been attributed to the formation of more hydrophobic surface on protein micelles produced by the absorption of fatty acids (Segalman, 1958).

The oxidation of lipid (Gunstone and Hilditch, 1946; Toyama, 1952) is considered another important factor causing denaturation of fish protein (Nishimoto, 1962; Ota and Nishimoto, 1963). The increased oxidation of fat in the presence of free fatty acids is also known to cause an inextractibility of fish protein (Lovern, 1962).

The liberation of free fatty acids from phospholipid by the action of the enzyme phospholipase (Olley and Lovern, 1960; Jonas and Tomlinson, 1962) may possibly be due to the activation of lysome, containing this enzyme, by the
concentrated salt produced by freezing. An increase in the tissue enzyme activity during freezing and thawing has been demonstrated by Hokin and Hokin (1963). It has more often been demonstrated that the unsaturated fatty acids, especially oxidizing free fatty acids, actually combine with protein to form highly insoluble lipid protein complexes (Desai and Tappel, 1963; Andre, et al., 1965; Embal and Tappel, 1966; Embal, 1971). The neutral lipids and fats have also been indicated to inhibit the denaturation of fish muscle protein (Dyer and Mortram, 1966; Simidu, et al., 1958; Hanson and Olley, 1963). Two different mechanisms are known to be involved in this phenomenon, firstly, the relation between proteins and unsaturated fatty acids or oxidation products and, secondly, the relation between protein and formaldehyde, the latter being a breakdown product of fat oxidation (Castell, 1971; Castell et al., 1973). The formaldehyde can combine with any one of a number of functional groups found in proteins, producing, among other things, highly insoluble polymers.

The rate of protein denaturation, particularly in the early period of storage, may probably be related to the amount of neutral lipids and fats.

The proper understanding of the process of denaturation can be had from the assumption that actomyosin fraction
of the myofibrillar protein gets soluble in salt solution. It has been shown that myosin - the principal myofibrillar protein, making about 50% of total protein, in the muscle is most sensitive to freezing and frozen storage. During frozen storage in cod, the myosin is reported to be inextractible in solvent, like the neutral 0.5 - 1.0 M salt, which is a native muscle extract (Cornell, 1962; King, 1966). On the other hand, the actin, the other principal myofibrillar protein, remains usually undamaged, as does the sarcoplasmic group of proteins (Cornell, 1960). The change in the extractibility of myosin appears to be the result of denaturation reaction as ATPase of this protein diminishes concurrently with the diminution of extractibility (Cornell, 1960; Savant and Magar, 1961). In addition, the usual form of enzymically inactive myosin is reported to appear in the extracts of frozen stored cod (Mackie, 1966) which has some of the properties expected from a denatured intermediate form of the protein. This denatured form of myosin appears to interact with native actin in the muscle in some way because extractibility of a complex of actin and myosin (actomyosin) diminishes in such a way that ultimately no more actin remains extractable in salt solution.
SUMMARY

The degree of denaturation of the muscle protein, as measured by the solubility in 5% neutral sodium chloride solution, was studied in three important teleosts, *O. munkiana* Moehl, *C. batrachus* (Linn.) and *H. fossilis* (Bloch) during storage for 16 days at -4°C. In all the three species, a decline in the percentage of soluble protein occurred with the period of storage at -4°C. The rate of fall was, however, found to vary with species, being less in the murrel than in the cat-fishes. The possible cause and significance of protein denaturation in fish muscle have been discussed in the light of the earlier informations on this phenomenon.