PART I

GENERAL

FISH MUSCLE STRUCTURAL PROTEINS

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Processing of fish and fishery products requires basic information on microbial degradation reactions in addition to ante-mortem and post-mortem alterations in muscle proteins. Based on the differences in their physico-chemical properties the proteins are broadly categorised as sarcoplasmic and fibrillar proteins. The sarcoplasmic proteins, forming approximately 15-20% of the total proteins depending on the fish species, are generally soluble in water or buffers of low ionic strength. To this class of proteins belong enzymes of glycolytic pathway (Tarr, 1966; Nagayama, 1966; Siebert and Schmitt, 1965; Gumbmann and Tappel, 1962; Gould 1965; Martin and Tarr, 1961.) and autolytic reactions (Siebert, 1958; Siebert and Bottke, 1963; Bird *et al.* 1969; Warrier *et al.* 1972A; Warrier *et al.* 1972 B). Most of these are low molecular weight proteins normally in the range not exceeding 40,000 to 70,000. The fibrillar proteins consisting 60-80% of the total proteins are soluble only in salt solutions of high ionic strength and have molecular weight in the range of $4x10^5$ to $6x10^5$. This article aims to review particularly some of the physico-chemical properties of the proteins which have distinguished themselves as 'structural' or 'textural' proteins. As denaturation in these proteins caused by a variety of factors manifests itself in terms of changes in the quality attributes of the product, relevent mechanisms have been particularly highlighted.

Textural changes in frozen and dehydrated fishery products

Preservation of fish by freezing and dehydration is based on the retardation of biochemical and microbial reactions and as such are commonly employed. However, the freeze induced physico-chemical changes in the colloidal structure of fish protein pose many technological problems such as the exudation of drip from thawed fish and toughness of muscle on prolonged storage which result in economic loss and reduced acceptability. In dehydrated fishery products, much is desired in respect of retention of original texture, colour and flavour on rehydration.

Drip loss

Drip is defined as the exudate of tissue fluids that flow free from fish muscle during thawing of frozen fish or

muscle (Heen and Karsti 1965). Drip leaches along with it soluble proteins, vitamins, minerals and confers an undesirable appearance on thawed fish (Mahon and Schneider, 1964). Drip was regarded occurring as a result of cell damage caused by freezing. Love (1955) examined the DNA content from the expressible fluid of thawed fish which were frozen at different rates. He suggested that the appearance of DNA in the expressible fluid was indicative of rupture of cell membrane. Seagran (1958) reported that the sarcoplasmic proteins were not associated with the release of the drip. According to Seagran (1958,1959) the cell damage alone cannot account for the release of drip, but it is also related to the capacity of muscle proteins to imbibe free liquid.

Structure of muscle proteins

Fibrillar proteins play an important role in contributing textural quality to the flesh food. Our knowledge of muscle proteins is mainly derived from the work on rabbit muscle proteins. The chief contractile protein has been identified as actomyosin which is composed of actin and myosin. The properties and structure of these proteins and their sub-units have been extensively studied in rabbit muscle (Scifter and Gallop, 1966). Striated muscle consists of two sets of filaments one containing myosin and the other containing actin. The A bands (A for anisotropic) contain filaments of 100°A diameter spaced about 45°A apart. These bands correspond exactly to the length of myosin filaments and each filament spanning an A band contains 200-400 myosin molecules. A second array of filaments extends on either side of the Z line through the I bands (I for isotropic) terminating at the edges of the H zones. These thinner filaments contain actin.

Properties of myofibrillar proteins

It is well recognized that the textural qualities associated with muscle such as fibrousness, plasticito and gel-forming ability are controlled by the myofibrillar proteins. The deleterious changes in texture occuring in fish muscle are attributed to the denaturation reactions involving this group of proteins (Connell, 1964).

Solubility characteristics

The salt soluble myofibrillar proteins extracted using an extractant of ionic strength 0.6 - 0.9 are generally found to be turbid, exhibit birefringence and have a high non-neutonian viscosity due to the presence of particles of high molecular weight (Dyer and Dingle, 1961). When ATP was added to these extracts, there was a sudden fall in viscosity, followed by a slow recovery to approximately original value. When diluted to ionic strength of 0.2 or less, these proteins form precipitates which can be redissolved completely only at an ionic strength of 0.60. The amount of precipitated protein was found to be about 75 per cent of the total protein. These studies suggested that fish muscle contain large proportions of actomyosin, a protein composed of actin and myosin. Another type of protein called tropomyosin is also associated with myofibrillar proteins (Hamoir, 1951). The physical and biochemical properties of these molecules have been reviewed by Scifter and Gallop, (1966) and Szent-Gyorgyi (1960).

Actomyosin

Investigations carried out by Ueda *et al.*, (1964, 1968) on the behaviour of the purified actomyosin revealed that there was no species differences in intrinsic viscosity value, electrophoretic mobility, salting-in and salting-out range etc. while there existed a difference in the temperature of denaturation as well as the velocity of denaturation from species to species.

Taguchi and Tkeda (1968) have reported the presence of lecithin in fish muscle actomyosin. Myosin content dissociable from the actomyosin in presence of ATP varied from 43-56 per cent of actomyosin. According to Connell and Howgate (1959), the ratio of myosin to actin in cod actomyosin lies between 2 and 4. Actomyosin having a high level of lecithin was found to have a high myosin content (Taguchi and Tkeda, 1968).

Myosin

Isolation of myosin from fish species such as cod, carp and mullet has been reported by Connell (1962) and Hamoir *et al.* (1960). Further, Connell (1958) has determined the sedimentation and diffusion coefficient, intrinsic viscosity and molecular weight of cod myosin (Table I). Based on the amino acid composition, Connell (1964) suggested that in cod muscle myosin contributes 40% of the total proteins. Fish myosin was observed to be more unstable in comparison to myosin from other muscle (Connell, 1964). Among fish myosins, cod myosin exhibited maximum unstability. Cod (Connell, 1961) and carp (Stainier Lambrecht, 1962) myosin are readily split by trypsin into meromyosins, but due to their unstability further characterization was found to be difficult.

Actin

Even though the isolation of fish actin has been attained by Connell (1954), Connell and Howgate (1959) and Dingle (1959) from cod muscle, comparatively little is known about fish actin. The depolymerised or G-actin obtained could be polymerised upon addition of salts to a viscous solution of F-actin. Studies have also been carried out on the molecular shape and size of cod actin. Some of the properties of cod actin are summarised in Table 1. According to Connell and Howgate (1959), the amount of actin present in cod actomyosin is to the extent of 15-20 per cent. In 0.6 M potassium iodide cod G-actin appears to exist as a dimer, though its exact molecular dimensions are uncertain (Connell, 1954).

Tropomyosin

A third type of protein associated with the myofibrillar proteins is tropomyosin which is present to the extent of 3 per cent in cod muscle (Connell, 1964). Hamoir (1959) studied the solubility, electrophoretic and ultracentrifugal patterns of tropomyosin. Tropomyosin extracted from cod and haddock muscle appear as well defined and monodisperse components on electrophoresis (Dyer and Dingle, 1961). Once extracted, tropomyosin remained soluble at pH 6 although under these conditions the viscosity was found to increase considerably.

Contractile proteins account for as much as 65-75% of the total proteins and water holding capacity resides mainly in these proteins. According to Hamm (1960) about 65% of the total water holding capacity of beef muscle can be accounted for by structural proteins while water soluble proteins account for only 5%. The remaining 30% is due to water soluble non-proteins. However, Hamm (1960) attributes the effect of the latter to their interaction with structural proteins.

Status of Water

The water molecule being dipolar is attracted by polar groups in the proteins. According to Sponsler *et al.* (1940), the hydrophilic groups responsible for fast binding of water are of two types: the polar groups of the side chains of proteins such as carboxyl, amino, hydroxyl and sulphydryl groups and the undissoci-

TABLE I

Properties of fish myosin and actin

Properties	Myosin	Actin
Molecular weight	5,30000	1,30000
Intrinsic viscosity	1.80	—
Sedimentation coefficient (S)	6.45	3.3
Diffusion coefficient (cm^2 sec^{-1})	1.10×10^{-7}	2.3×10^{-7}

Cited from Connel (1958 B)

ated carbonyl and imido groups of the peptide bonds. Since the water molecules combined with protein polar groups also possess unshared electron pairs, they may combine with more water to form aggregates attached to polar groups.

Concepts of water holding by muscle proteins

Hamm (1960) has suggested that only 4-5% of the total water of muscle is tightly bound to the muscle proteins and is not influenced by changes in the structure and charges of proteins. Most of the remaining water is termed as free water and is retained within the protein structure. The free water has a tendency to pass into a state of loose water which can be forced out by application of low pressure. In contrast with the tightly bound water, the free water is influenced by changes and spatial structure of muscle proteins. The term 'water holding capacity' (WHC) is used to express the ability of meat or fish to hold water during the application of force like pressing, centrifugation etc. Water liberated by the application of force is termed as 'loose' water and the water retained by the tissues as 'bound' water. Suitable modifications of centrifuge or press method have been developed for the measurement of WHC (Wierbicki and Deatherage, 1958; Wierbicki *et al.* 1957; Urbin *et al.* 1962).

Influence of pH

Grau *et al.* (1953) first showed that muscle has minimum WHC around pH 5. On either side of the isoelectric point, the muscle exhibits higher WHC. According to Kuntzel (1944) this may be due to a repulsion between protein groups of the same charge, positive or negative, and the space between the peptide chains is probably enlarged which allows more water to penetrate.

Post-mortem changes

Hamm (1960) has observed that the loss in WHC of muscle post-mortem is due to the degradation of ATP which may further release the bound Ca^{++} ions. The subsequent degradation products of ATP have a low binding capacity for calcium. The liberated Ca^{++} ions are then bound by the negatively charged groups of proteins. This leads to tightening of muscle structure and a loss in WHC. Hamm (1960) has further shown that addition of ATP to post-mortem muscle increases its WHC and attributes it to the complex binding of Ca^{++} ions.

The decrease in pH during rigor also accounts for the loss in WHC. It is estimated (Hamm, 1960) that 2/3 drop in WHC of beef muscle is due to breakdown of ATP and 1/3 to the decrease in pH.

Under certain conditions, when ATP is not degraded, ATP induces relaxation of muscle. Weber and Portzehl (1952) call this property as the plasticizer effect. This effect is produced by the inorganic polyphosphates also. According to Hamm

TABLE II

Effect of pre-dip treatment on sodium tripolyphosphate solution on Bombay duck fillets.

Treatment	Storage period	Dip treatment	%Drip loss	Total Nitrogen* (mg./100g. tissue)	Ribose* (mg./100g. tissue)	%Extractable proteins in 5% NaCl, pH 7.5
Unirradiated	10-12°C, 3 days	Nil	14.0	19.5	1.8	57.0
		Dip treatment	4.0	12.0	1.0	71.0
Irradiated, 0.5 Mrad	10-12°C, 3 days	Nil	22.0	29.5	15.8	75.0
		Dip treatment	12.5	14.0	3.6	75.0
Irradiated, 3.0 Mrad	10-12°C, 3 days	Nil	27.5	31.0	30.0	35.0
		Dip treatment	16.0	17.0	6.0	36.0
Frozen at - 20°C	-20°C, 3 days	Nil	20.0	24.5	20.0	44.0
		Dip treatment	4.5	10.0	4.0	72.0

Fresh Bombay duck fillets were given dip-treatment in 10% sodium tripolyphosphate solution for 15 min. and the fillets were sealed in polythene bags and given the above treatments and stored for 3 days.

*This refers to the amount of total nitrogen and ribose present in the drip released from 100g. fish subjected to dip treatment.

(1960), the hydrating effect is identical with the plasticizing effect. The exact mechanism of this effect is not known, but is related to the dissociation of actomyosin into actin and myosin (Hamm, 1960).

Bendall (1954) in his studies on meat suggested that the effect of pyrophosphate in improving the WHC is probably due to its ability to split the link between the two components actin and myosin. To do this, the specific links (I) or (II) are required (Fig. 1). Both these types of links are found in the compound ATP.

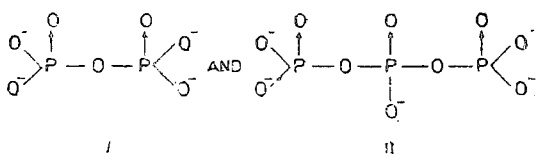

Fig. 1
Specific links required for splitting actin and myosin.
Cited from Bendall (1954)

Influence of sodium chloride

The effect of sodium chloride in increasing the WHC of meat has been observed by many workers (Hamm 1960; wierbicki, 1957; Bendall 1954). The nature of binding of salt ions to proteins is mainly an electrostatic one due to the attraction of salt ions by positively or negatively charged groups of proteins (Sehellmann, 1953). Also, the binding strength of ions to proteins varies (Hamm, 1960). The effect of sodium chloride in increassing the WHC is principally attributed to the effect of Cl^- ions, since with sodium acetate no increase in WHC of muscle was observed (Hamm, 1960). In the basic range of isoelectric point (I.P) of the muscle proteins, the Cl^- ions bring about greater repulsion between the peptide chain leading to increase in WHC. In the acidic range of I. P., Cl ions decrease the repulsion. This may explain the tightening of protein structure and lower WHC.

Influence of salts of weak acids

Hamm (1960) studied the influence of several sodium salts of weak acids on the WHC of muscle homogenate. He attributes the increased WHC of muscle by polyphosphates to the elimination of alkaline earth metals in muscle by precipitation or the formation of undissociated complexes.

The effect of sodium pyrophosphate and sodium tripolyphosphate has been recognised by many workers in increasing the water holding capacity of flesh foods. However, there is no full agreement about the mode of action. Bendall (1954) attributes the beneficial effects of these phosphates to the splitting of actomyosin into actin and myosin. According to Sherman (1961) the ability of these phosphates in improving the WHC does not reside in their ability to complex Ca^{++} and Mg^{++} ions. Hollendoorn (1962) holds similar views.

According to Popp and Muhlbrecht (1958) the polyhosphates do not increase the water absorption capacity of meat, but merely restore it to the original. Fukazawa *et al.* (1961) correlated the influence of the phosphates in improving the WHC to their effect in promoting the extraction of proteins from the fibrils. Yasui *et al.* (1964) extensively studied the extractability of proteins from myofibrils in the presence of pyrophosphate, tripolyphosphate and hexametaphosphate with or without sodium chloride, magnesium chloride and calcium chloride. They report

that the affinity of sodium pyrophosphate and tripolyphosphate was greatly improved in the presence of high salt concentration and divalent cations. Hexametaphosphate, however, was bound directly with salt free myosin B (actomyosin) and the binding was inhibited by the presence of high salt concentrations and divalent cations. Specific interaction of polyphosphates with myosin B was further investigated by viscosity changes, ultracentrifugal and enzymatic methods (Yasui *et al.* 1964). Sodium pyrophosphate and tripolyphosphate seemed to show specific reaction with myosin B, similar to ATP (Yasui *et al.*, 1964). According to Yasui *et al.* (1964) the factor which influenced the improvement of the water binding of meat in sausage may be the dissociation of actomyosin by pyrophosphate while tripolyphosphate becomes effective only after its decomposition to pyrophosphate by tripolyphosphatase in meat.

Technological significance of WHC

The concepts of muscle hydration have now been extensively applied to improve the quality of frozen fish in terms of reduced drip and cooking loss and textural improvement. MacCallum *et al.* (1964) reported that the drip loss of frozen cod pretreated with 10% sodium tripolyphosphate solution was greatly reduced in the initial period of frozen storage and up to 27 weeks of storage, the treated fillets showed better textural quality than the untreated. Boyd and Southcott (1965) reported that treatment with sodium tripolyphosphate of Dover sole, Pacific cod, halibut and red snapper prior to freezing was found effective in reducing drip loss. Also, sodium chloride was found effective in reducing drip and cooking lossses (Boyd and Southcott, 1965). It was also observed that less shear force was required to shear the fibres of tripolyphosphate treated fillets than the control samples which exhibited tough quality. Mahon and Schneider (1941) examined the effect of pre-dip treatments in sodium tripolyphosphate, sodium hexametaphosphate, citrate and carbonate solutions in reducing the drip of a variety of fish. They observed that the tripolyphosphate treated fish fillets retained more of their fresh caught composition after frozen storage. Migita and Otake (1960) observed that ATP added to the muscle of exhausted cod that died during struggling changes the chemical properties of muscle proteins to that of muscle of fish killed instantly and polyphosphate had a like effect. Tanikawa *et al.* (1963) reported that the polyphosphate treatment helped to maintain good quality of frozen cod. Kumta & Gore (1970) have observed that the drip loss in Bombay duck during frozen storage or irradiation can be minimised by pre-dip treatment in sodium tripolyphosphate or sodium chloride. Further, the dip treatment reduced the loss of ribose and total nitrogen and increased the extractability of proteins in 5% sodium chloride (Table II). Antioxidant properties

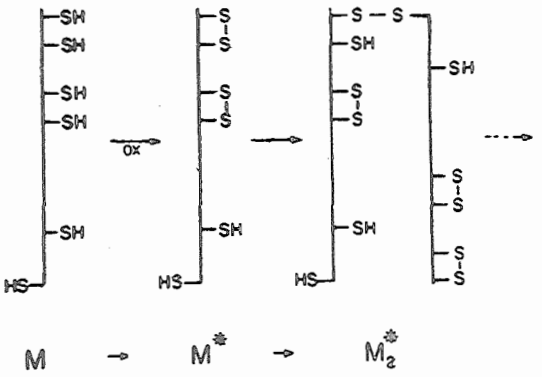

Fig. 2

Schematic representation of the oxidation and the consequent sulphydryl - disulphide exchange reactions of myosin.

Cited from Buttkus (1970).

of sodium tripolyphosphate in meat products have been reported (Timms and Watts, 1958; Zisper and Watts, 1961; Ramsey and Watts, 1963; Thomson, 1964: Zisper, Tai -wan Kwon and Watts, 1964), but in frozen chinook salmon (Boyd and Southcott, 1965) and cod (MacCallum *et al.* 1964) pretreated with sodium tripolyphosphate liquid oxidation was not retarded.

Denaturation of proteins

The most important biochemical change associated with proteins is denaturation. Numerous definitions have been proposed for this phenomenon to arrive at an understanding of the complicated mechanisms (Joly, 1965). In general, denaturation may be defined as any modification of the secondary, tertiary or quarternary structure of the protein molecule. This structural modification may bring about definite change in chemical, physical or biological properties of the protein. Some of the molecular parameters usually adopted to measure the extent of denaturation are solubility, sedimentation or diffusion constants, molecular weight, electrophoretic mobility, absorption coefficient, intrinsic viscosity and specific rotation.

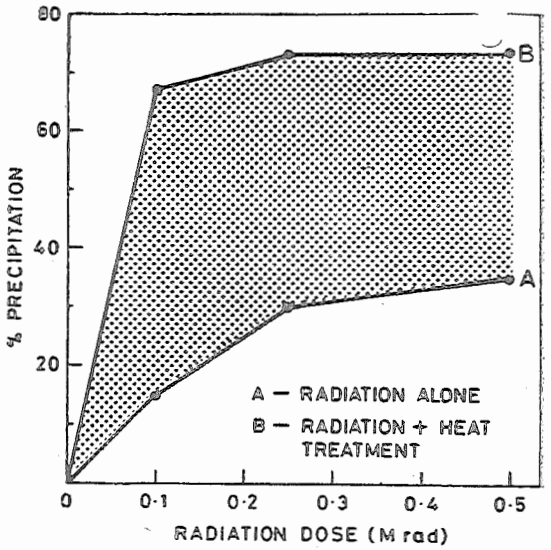

Fig. 3

Synergestic effect of radiation and heat treatment on the precipitation of filbrillar proteins of Bombay duck muscle.

(Bombay duck muscle proteins extracted in 0.01 M phosphate buffer containing 5% sodium chloride was subjected to radiation, heat and radiation heat combination treatment. While heat treatment alone bring about precipitation to the extent of 6% post irradiation (0.25 Mrad) heat treatment (60°C for 10 min.) enhanced the precipitation of proteins to 73%.)

Machanism of denaturation

Various machanisms have been suggested by Tanford (1968),Linderstrom-Lang (1952), Putnam (1953), Kauzmann(1959), Joly (1965) and Connell (1960 A) to explain the phenomenon of denaturation. Generally, it is considered to be a two-step process involving the opening of the peptide bonds followed by the splitting or combination of the molecules. Some of the denaturation effects occurring in fish muscle proteins due to heat, radiation or during frozen storage are reviewed below.

Heat denaturation

Connell (1957) has made an attempt to elucidate some of the changes in the fish muscle proteins during dehydration and storage in the dried state. He observed that in the dried fish the protein gel system of the fresh fish is much disorganized, and the fillets were tougher and drier in texture than fresh fish after reconstitution. Moreover, the solubility of vacuum-contact dried cod protein in 0.5M potassium chloride was reduced from 90 to 16 per cent. From these studies, he speculated that the main

TABLE III

Comparison of the extent of precipitation and solubility of fish muscle proteins by radiation, heat and pH treatments.

Method of precipitation	Protein Precipitated %	% solubility in 0.01 M phosphate buffer containing 5% sodium chloride
Isoelectric precipitation (pH 4.0)	96.7	12.0
Heat, 60°C, 10 min.	2.0	—
Radiation, 250 Krad + Heat, 60°C, 10 min.	73.0	60.0

The solubility of the protein precipitate was tested by suspending the proteins in 5% sodium chloride for 15 min. with frequent shaking. The suspension was centrifuged and the protein in the supernatent was measured.

structural protein complex of the muscle actomyosin, had been denatured on drying. Sawant and Magar (1961) have shown that denaturation of proteins of Bombay duck during drying was not only restricted to the actomyosin fraction but it was also extended to the sarcoplasmic protein fraction. Further, the solubility behaviour of the dried product in urea and in urea containing thioglycolate solutions suggested the formation of hydrogen-bond and disulphide cross-linkages during drying. Kishimoto *et al.* (1956) have also concluded from their studies on air dried cuttle fish flesh that the protein net work of the water swollen material is partly crosslinked by hydrogen bonds which could be broken by the action of urea solutions.

Frozen storage and alterations in fish muscle proteins

During frozen storage, the rate of protein denaturation was found to depend on various factors such as storage temperature, state of rigor at the time of freezing (Love, 1962A), freezing rate (Love 1962 A, B) and time required for thawing (Dyer and Dingle, 1961). Amount of extractable proteins is often taken as a criterion of denaturation. It has been reported that during frozen storage the extractability of myofibrillar group of proteins is decreased whereas the extractability of sarcoplasmic proteins is unaffected (Connell, 1964; Dyer and Dingle, 1961; Sawant and Magar 1961). The loss in characteristic texture due to decrease in water holding capacity of myofibrillar proteins brings about drip loss in some varieties of fish such as cod and Bombay duck (Idler *et al.* 1965; Kumta and Gore 1970). Warrier *et al.* (1972A) have observed increased activity of hydrolytic enzymes in drip obtained from Bombay duck muscle following irradiation or freezing. Also treatment of muscle with sodium tripolyphosphate reduced the levels of hydrolytic enzymes released into the drip. Seagran (1958) by his studies on rock fish found similarity between the proteins of drip and sarcoplasmic proteins. Pre-dip treatments in sodium chloride or sodium

tripolyphosphate were found to reduce the drip loss and maintain good quality of a number of species of fish during frozen storage (Malon and Schneider, 1964; Boyd and Southcott, 1965; Tanikawa, Akiba and Shitamosi, 1963; Kumta & Gore 1970).

Connell (1964) observed that the extractability of cod myosin was reduced during storage at-14°C though at a slower rate than that of actomyosin whereas the extractable actin remained unchanged. Sawant and Magar (1961) and Connell (1960 B) noticed decline in ATPase activity. Decrease in the intrinsic viscosity of myofibrillar proteins of eight species of fish was observed by Ueda *et al.* (1962).

Salt denaturation theory

It is presumed that the protein is damaged as a consequence of continued exposure to concentrated solutes in the frozen-stored muscle. Evidence for the salt denaturation theory stems from the observations of Duerr and Dyer (1952) on the behaviour of proteins upon continuous immersion of fish muscle in concentrated brine at 0°C. They observed a rapid decrease in water holding capacity and inextractability of myofibrillar protein fraction when the average salt content reached about 10per cent. These finding were further supported by the observations of Simidu and Hibiki (1952), Nikkila and Linko (1954) and Simidu and Simidu (1957) in many species of fish. Studies by Snow (1950) in model systems on fish actomyosin have shown that actomyosin is rendered insoluble by freezing the solutions below the eutectic point of the solutes or by freezing the suspension. According to Connell (1959) cod myosin solutions can be frozen and thawed repeatedly without damage but quickly form aggregates on frozen storage.

Lipid hydrolysis theory

Dyer (1951) suggested that the lipid constituents of fish muscle may have an important influence on the stability of proteins. This suggestion was later proved by Dyer and Fraser (1959) and Olley and Lovern (1960) by obtaining a good correlation between protein extractability and rise in the amount of free fatty acids in cod muscle. King *et al.* (1962), Anderson *et al.* (1963) and Anderson and Steinberg (1964) have shown that *in vitro* addition of long chain fatty acids would result in decrease in protein extractability. Anderson *et al.* (1965) have observed that interaction of proteins with fatty acids resulting in insolubilization of the protein is optimal at an ionic strength of 0.5, which should exist in the cellular fluid of cod muscle as a result of freezing at-1.5°C. Olley *et al.* (1962) have provided evidence to show that phospholipases are active under frozen storage.

Aggregation of myosin

Connell (1958 A, B) observed that monodisperse cod myosin has a pronounced tendency to aggregate when neutral solutions are kept at 0°C. The aggregation is characterised by the appearance of at least one and sometimes two discrete components which sediment faster than myosin as evidenced in the ultracentrifuge picture. Lowey and Holtzer (1959) by their light scattering and sedimentation experiments and Connell (1958 A) from his sedimentation results on cod myosin suggested that the main aggregation mechanism is a side-by-side process. According to Connell (1960 A) the aggregation reaction involves two steps : a first order step which does not affect the sedimentation coefficient followed by a second or higher order step. The two steps of this kinetic scheme can be rationalized in terms of a small con-

Fig. 4
Methods for the preparation of fish protein concentrate

- Precipitation of proteins
  1. Isoelectric pH
  2. Combination of radiation and heat
- Other methods
  1. Chemical methods solvent treatment for extraction of lipids.
  2. Biological methods Hydrolysis of proteins by proteolitic microorganisms.
  3. Physical methods High voltage for seperation of components of fish tissue.

figurational alteration in the myosin monomer (M) proceeding the step-wise aggregation on the altered molecule (M_D) as follows.

$$M \longrightarrow M_D$$

$$M_D + M_D \longrightarrow 2\,M_D$$

$$2\,M_D + M_D \longrightarrow 3\,M_D \text{ etc.}$$

Buttkus (1970) on the basis of his comparative studies on the aggregation of rabbit and trout myosins in frozen solutions suggested a mechanism which involves disulphide-sulphydryl exchange reactions between activated myosin molecule and aggregates. This is on the basis of the observed behaviour of the sulphydryl groups in the monomeric myosin molecule and the effect of reducing conditions on the high molecular weight aggregates. The proposed mechanism for the polymerization is as follows.

According to Buttkus (1970) about ten of the very reactive sulphydryl groups, diagrammatically represented in the top portion of the myosin molecule (M) are first oxidised (Fig. 2). The molecule M* with the intramolecular disulphide bonds is in an activated state being susceptible to sulphydryl - disulphide interchange reactions with its own kind to form M_2^* or with molecules being already multiples of M* having a molecular weight of M*.

$$M \longrightarrow M^*$$

$$2\,M^* \longrightarrow M_2^*$$

$$M^*_2 + M^* \longrightarrow M^*_3$$

$$M^*_{(x-1)} + M^* \longrightarrow M^*_x$$

Also M and M* could probably form M – M* which would then oxidise to M_2^*.

Further Buttkus (1970) has shown that while native myosin will unfold and dissociate into randomly coiled peptide

chains in 8 M urea solution, the aggregated polymeric myosin, which is insoluble in salt solutions, redissolved in 6 M guadine hydrochloride (pH 8.0 - 10.5) only after the addition of reducing agent sodium borohydride. From these results he has suggested the involvement of S – S as well as noncovalent hydrogen, hydrophobic and possibly ionic bonds in the formation of polymeric myosin. Extensive studies carried out by Buttkus (1974) on the denaturation of proteins have shown that these denaturation mechanisms are of a more general nature and are also responsible in the hardening, firming or gelling of the protein structures of egg white, kamaboko and meats from fish and mamals during cooking.

Physico-chemical alterations of fibrillar proteins of fish muscle caused by heat, radiation or isoelectric pH are used to define optimum conditions for the preparation of fish protein isolates. Meinke *et al.* (1972) have studied the solubility characteristics of fish muscle proteins of carp, mullet and golden croaker. They have shown that 70% of the proteins of carp and golden croaker can be brought in solution at pH 3 and pH 10-11. By adjusting the pH to 5.5-6.0, 50% of the protein was obtained as a curd. When precipitation of fibrillar proteins isolated from Bombay duck by heat treatment at temperatures varying from 50-100°C was studied it was observed that these treatments did not bring about significant precipitation(Warrier *et al.*1973). However, when fibrillar proteins were subjected to optimum radiation doses in the range of 0.10-0.25 Mrad followed by heat treatment at 60°C for 10min. maximum precipitation to the extent of 67 - 73% was obtained ; radiation treatment at these doses alone caused only 15-30% precipitation (Fig.3). These results thus point out the synergestic effect of radiation and heat on the precipitation of proteins. This is probably the first instance of employing gama radiation for enhancing aggregation reaction in protein by mild heat treatment - an observation with potential for sealing up as a new process. Warrier *et al.* (1974) have shown that proteins from the muscle homogenates of Bombay duck could be precipitated by adjusting the pH of the homogenate to isoelectric point. Among the pH values examined pH 4 was most effective and at this pH about 96% of the proteins could be precipitated.

Although there are several methods for isolation of fish protein concentrates based on solvent extraction procedures (Stillings, 1969; Levin, 1959; Hevia *et al.* 1971; Dreosti, 1962; Sen *et al.* 1966) as well as microbiological and physical methods (Roels, 1969; Burkholder *et al.* 1968;Knobel, 1967) (Fig. 4) several problems such as persistence of residual solvent, reversion of flavour, loss in functional properties and probably less acceptance by the food industry have retarded the demand for FPC. Fish protein precipitate obtained by combination of radiation and heat treatment shows minimum alterations in functional properties as evidenced from its high solubility and dispersibility (Table 3). Researches on the properties of structural proteins of trash fish varieties may enlarge the scope for their effective utilization as a source of protein.

References

Anderson, M. L., F. J. King and M. A. Steinberg. 1963. *J. Food Sci.* 28:286

Anderson, M. L. and M. A. Steinberg. 1964. *J. Food Sci.* 29 : 327.

Anderson, M. L., M. A. Steinberg and F. J. King. 1965. In 'The technology of fish utilization', Ed. Kreuzer, R., Fishing News(Books)Ltd., London,105.

Bendall, J. R. 1954. *J. Sci. Fd Agric.*, **5**: 468.

Bird, J. W.C., T.Berg, A.Milanesi and W.T. Stauber, 1969. *Comp. Biochem. Physiol.* **30**: 457.

Boyd, J. W. and B. A. Southcott. 1965. *J. Fish. Res. Bd. Can.*, **22**: 53.

Burkholder, L., P. R. Burkholder, A. Chu, N. Kostyk and O. A. Roels. 1968. *Food Technol.*, **22**: 1278.

Buttkus, H. 1970. *J. Food Sci.*, **35** : 558.

Buttkus, H. 1974. *J. Food Sci.*, **39** : 484.

Connell, J. J. 1954. *Biochem. J.*, **58**: 360.

Connell, J. J. 1957. *J. Sci. Fd Agric.*, **8**:527.

Connell, J. J. 1958. *B. Biochem. J.*, **70**: 81.

Connell, J. J. 1958, *A. Biochem. J.*, 69: 5.

Connell, J. J. 1959. *Nature* (Lond).,**183**:664.

Connell, J. J. 1960. A. *Biochem. J.*, **75**:530.

Connell, J. J. 1960. B. *J. Sci. Fd Agric.*, **11**: 245.

Connell, J. J. 1961. *Biochem. J.*, **80**: 503.

Connell, J. J. 1962. *J. Sci. Fd Agric.*, **13**: 607.

Connell, J. J. 1964. In 'Proteins and their reactions' Eds., H. W. Schultz and A. F. Anglemier, AVI Publishing Co. Inc. Connecticut, p. 255.

Connell, J. J. and P. F. Howgate. 1959. *Biochem. J.*, **71**: 83.

Dingle, J. R. 1959. *J. Fish. Res. Bd Can.*, **16**: 243.

Dreosti, G. M. 1962. In 'Fish in Nutrition' Eds. E. Heen and R. Kreuzer, Fishing News Books, London, England, 425.

Duerr, J. D. and W. J. Dyer. 1952. *J. Fish. Res. Bd Can.*, **8**: 325.

Dyer, W. J. 1951. *Food Research.*, **16**: 522.

Dyer W. J. and J. R. Dingle. 1961. In 'Fish as Food'. Ed., G. Borgstrom. Academic Press, New York, p. 275.

Dyer, W. J, and D. I. Fraser., 1959. *J. Fish. Res. Bd can.*, **16**: 43.

Fukazawa, T., Y. Hashimoto and T. Yasui 1961. *J. Food. Sci.*, **26**: 550.

Gould, E. 1965. In 'The technology of fish utilization' Ed. R. Kreuzer, Fishing News (Books) Ltd. London, p. 126.

Grau, R., R. Hamm and A. Baumann. 1953. *Biochem. Z.* **325**: 1.

Gumbmann, M. and A. L. Tappel. 1962. *Archs Biochem. Biophys.*, **98**: 262.

Hamm, R. 1960. Advances in Food Research. **10**: 355.

Hamior, G. 1951. *Biochem.* J., **50**: 140.

Hamior, G. 1951. *Biochem. J.*, **48**: 146.

Hamior, G., H. A. Mekenzie. and M. B. Smith. 1960. *Biophys. Acta.*, **40**: 141.

Heen, E. and O. Karsti, 1965. In 'Fish as Food' Ed., G. Borgstrom. Academic Press, New York, Vol. IV, p. 355.

Hevia, P., F. Aeevedo and S. Kaiser, 1971. *J. Food Sci.*, **36** : 708.

Hollendoorn, E. W. 1962. *Food Technol.*, **16** : 119.

Idler, D. R., W. A. MacCallum, D. Chalker and J. T. Lauder, 1965. In 'The Technology of Fish Utilization', Ed., R. Kreuzer, Fishing News (Books) Ltd., London, p. 99.

Joly, N. 1965. 'A Physico-chemical approach to the denaturation of Proteins', Academic Press, New York, p. 2.

Kauzmann, W. 1959. In 'Advances in Protein Chemistry' Eds., C. B. Anfinsen Jr., M. L. Anson, K. Bailey and J. T. Edsall, Academic Press, New York, vol. XIV, p. 1.

King, F. J., M. L. Anderson, and M. A. Steinberg, 1962. *J. Food Sci.*, **27**: 363.

Kishimoto, A., K. Mitsuhashi and H. Fujita. 1956 *Bull. Jap. Soc. Scient. Fish.*, 22; 289.

Knobl Jr, G. M. 1967. *Food Technol.*, **21**; 1108.

Kumta, U. S. and M. S. Gore. 1970. *J. Food Sci.*, **36**: 456.

Kuntzel, A. 1944. In 'Handbuch Der Lederfabrikation, vol. I, p. 511.

Levin, E., 1959. *Food Technol.*, **13**: 132.

Linderstrom-Lang, K. U. 1952. In 'Proteins and Enzymes', Lane Medical Lectures, Stanford University Press, California, p. 53.

Love, R. M. 1962. *J. Food Sci*, **27**: 544.

Love, R. M. 1962. *J. Sci. Fd Agric.*, **13**:269.

Love, R. M. 1955. *J. Sci. Fd Agric.*, **6**:30.

Lowey, S. and A. Holtzer. 1959. *J. Am. Chem. Soc.*, **81**: 1378.

MacCallum, W. A., D. A. Chalker, and J. T. Lander. 1964. *J. Fish. Res. Bd Can.*, **21**: 1397.

Mahon, H. J. and C. G. Schneider. 1964. *Food Technol.*, **18** : 1941.

Martin, G. B. and H. L. A. Tarr. 1961. *Can. J. Biochem. Physiol.*, **39**: 297.

Meinke, W. W., M. A. Rahman, and K. F. Mattil, 1972. *J. Food. Sci.*, **37**: 195.

Migita, M. and S. Otake. 1960. *Bull. Jap. Soc. Scient. Fish.*, **24**: 821.

Nagayama, F. 1966. *Bull. Jap. Soc. Scient. Fish.*, **32**: 118.

Nikkila, O. E. and R. R. Linko, 1954. *Food Research.*, **19** : 200.

Olley, J. and J. A. Lovern, 1960. *J. Sci. Fd Agric.*, **11**: 644.

Olley, J., R. Pirie, and H. Watson, 1962. *J. Sci. Fd Agric.*, **13**: 501.

Popp, H. and F. N. Muhlbrecht. 1958. *Fleischwirtschaft*, **10**: 399.

Putnam, F. W. 1953. In 'Proteins', vol. I, Eds. H. Newrath, and K. Bailey, Academic Press, New York, vol. I, p. 807.

Ramsey, M. B. and B.M. Watts. 1963. *Food Technol.*, **17**: 1056.

Roels, O. A. 1969. *Nutrition Revs.*, **27**: 35.

Sawant, P. L. and N. G. Magar. 1961. *J. Sci. Fd Agric.*, **12** : 298.

Sawant, P. L. and N. G. Magar. 1961. *J. Food Sci*, **26**: 253.

Schellmann, J. A. 1953. *J. Phys. Chem.*, **57**: 472.

Scifter, S. and P. M. Gallop. 1966. In 'The Proteins' Ed., H. Newrath, Academic press, New York, Vol. IV, p. 372.

Seagran, H. L. 1958. *Fd Res.*, **23**:143.

Seagran, H. L. 1959. *Fd Res.*, **24**:681.

Sen, D. P., T. S. S. Rao and N. L. Lahiry 1966. *J. Food Sci.*, **31**: 344.

Sherman, P. 1961. *Food. Technol.*, **15**: 79.

Siebert, G. 1958. *Experientia*, **14**: 65.

Siebert, G. and I. Bottke. 1963. *Arch. Fish wiss*; **14**: 57.

Siebert, G. and A. Schmitt. 1965. In 'The technology of fish utilization' Ed., R. Kreuzer, Fishing News (Books) Ltd. London, p. 47.

Simidu, W. aud S. Hibiki. 1952. *Bull. Jap. Soc. Scient. Fish.*, **17** : 301.

Simidu, U. and W. Simidu. 1957. *Bull. Jap. Soc. Scient. Fish.* **23** : 442.

Snow J. M. 1950. *J. Fish. Res. Bd Can.* **7** : 599.

Sponsler, O. L., J. D. Bath and J. W. Ellis. 1940. *J. Phys. Chem.* **44**: 996.

Stainier-Lambrecht, A. 1962. *Archs. Int. Physiol. Biochim.*, **70** : 682.

Stillings, B. R., 1967. Beureau of Commercial Fisheries Laboratory, College Park, Maryland, Activities Report, **19** : 109.

Szent-Gyorgyi, A. G. 1960. In 'The Structure and function of muscle', Ed., G. H. Bourne. Academic Press, New York, Vol. II, p. 1.

Taguchi, T. and S. Tkeda. 1968. *Bull. Jap. Soc. Scient. Fish.*, **34** : 411.

Taguchi, T. and S. Tkeda. 1968, *Bull. Jap. Soc. Scient. Fish.*, **34** : 339.

Tanford, C. 1968. In 'Advances in Pro-

tein Chemistry', Vol. XXIII, Eds., C. B. Anfinsen. Jr., M. L. Anson, J. T. Edsall, and F. M. Richards, Academic Press, New York, p. 121.

Tanikawa, E., M. Akiba and A. Shitamori. 1963. *Food Technol.*, 17: 1425.

Tarr, H. L. A. 1966. *J. Food Sci.*, 31 : 846.

Thomson, J. E. 1964. *Food Technol.*, 18 : 1805.

Timms, M. J. and B. M. Watts. 1958. *Food Technol*, 12 : 240.

Ueda, T., Y. Shimizu and W. Simidu. 1962. *Bull. Jap. Soc. Scient. Fish.*, 28 : 1010.

Ueda, T., Y. Shimizu and W. Simidu. 1964. *Bull. Jap. Soc. Scient. Fish.*, 30 : 353.

Ueda T., Y. Shimizu and W. Simidu. 1968. *Bull. Jap. Soc. Scient. Fish.*, 34 : 357.

Urbin, M. C., D. A. Zessin and G. D. Wilson. 1962. *J. Anim. Sci.*, 21 : 9

Warrier, S.B.K., V. Ninjoor, M.G. Hirlekar, P. L. Sawant and U. S. Kumta. 1972A. *Indian. J. Biochem. Biophys.*, 9:207.

Warrier, S. B. K., V. Ninjoor, P. L. Sawant, M. G. Hirlekar, and U. S. Kumta. 1972B. *Ind. J. Biochem. Biophys.*, 9: 278.

Warrier, S. B. K., V. Ninjoor, P. L. Sawant and U. S. Kumta. 1973. *J. Food Technol.*, 8 : 283.

Warrier, S. B. K., S. N. Doke, M. S. Gore, P. Harikumar and U. S. Kumta. 1974. *Labensmittel-Wirsenschaft and Technologie* (Communicated).

Weber, H. H. and H. Portzehl. 1952. Advances in Protein Chemistry. 7:162.

Wierbicki, E. and F. E. Deatherage. 1958. *J. Agric. Fd Chem*, 6 : 387.

Wierbicki, E., L. E. Kunkle and F. E. Deatherage. 1957. *Food Technol.*, 11 : 69.

Yasui, T., T. Fukazawa, K. Takahashi, M. Sakamishi and Y. Hashimoto. 1964. *J. Agric. Fd Chem.*, 12 : 399.

Yasui, T., M. Sakanishi and Y. Hashimoto. 1964. *J. Agric. Fd Chem.*, 12:392.

Zisper, M. W., Tai-Wan Kwon. and B. M. Watts. 1964. *J. Agric. Fd Chem.*, 12 : 105.

Zisper, M. W. and B. M. Watts. 1961. *Food Technol.*, 15 : 318.