EFFECT OF C_{18} UNSATURATED FATTY ACIDS ON THE EXTRACTABILITY OF FISH MUSCLE PROTEINS

It has been suggested that actomyosin insolubilization in fish held in frozen storage is due to free fatty acid (FFA) accumulation in the muscle as a result of lipid hydrolysis. (Dyer and Fraser, 1959; Olley and Lovern, 1960; Olley *et al*, 1962; Boyd *et al*, 1967) Kind *et al* (1962) and Anderson *et al* (1963, 1964) have further shown that FFA and their salts if added to the extractant could cause inextractability of fish proteins. Fatty acids are known to be denaturants, their effectiveness increasing with length of C-chain. Species differences in the reactivity of fish muscle proteins towards fatty acid salts in muscle homogenate has already been demonstrated (Anderson and Steinberg, 1964). In the present investigation the authors have attempted to study some of the above factors which govern the extractability of proteins from teleost and crustacean muscle under the influence of certain added C_{18}- unsaturated fatty acids.

10g of homogenised muscle of teleost fatty fish, *Sardinella longiceps* or crustacean, prawn, *P. indicus* were blended for 1 minute with 200 ml of chilled extractant (5% NaCl in 0.02 M $NaHCO_3$) in waring blender using a baffle technique. Blending was repeated for another minute and measured quantities of fatty acid added to the extract and again blended for a minute. The extract was centrifuged and protein nitrogen determined in the supernate by the micro kjeldahl method. All the preparations were conducted in a cold room at 5-7°C. In a different series, the actomyosin protein fraction of the muscle was prepared by the method of King *et al* (1962) by washing off the sarcoplasmic proteins by low ionic strength buffer. To 40 ml of the extract containing actomyosin were added the fatty acid in calculated amounts by a capillary jet tube, mixed and stored in cold room (5-7°C) and protein nitrogen determined at intervals of days.

Table I summarises the effect of added linoleic acid on the extractability of muscle proteins of *S. longiceps*. The fatty acid added to the extractant, as is shown, causes inextractability of fish muscle progressively with increase in concentration of the acid. The soluble protein N content decreases regularly with added linoleic acid, a level of 0.25 ml having been found to be sufficient to insolubilize essentially all myofibrillar proteins in the extract. In the case of prawns (Table II) the soluble protein nitrogen shows a small but consistent increase with lower amounts of fatty acid added to the extractant, followed by a regular loss of solubility with increasing amounts of the acid. Both oleic and linoleic acids gave similar trend of results with prawns, the effect being more marked with linoleic acid.

TABLE I EFFECT OF ADDED LINOLEIC ACID ON EXTRACTABILITY OF MUSCLE PROTEINS OF *S. longiceps*.

S. No.	Volume of linoleic acid added to 200 ml extractant (ml)	Salt soluble protein nitrogen (as % of total protein N)
1	0 00	47 10
2	0.05	45.04
3	0 10	40.12
4	0.15	29 88
5	0.25	25.77
6	0.30	25.31

It has been shown by Bull and Bruse (1967) that the rate of denaturation by fatty acids follows first order kinetics in respect to protein but order in respect to fatty acids is higher and that it would be necessary to bind about 10 mols of fatty acid per mole of protein before denatura-

TABLE II EFFECT OF ADDED OLEIC ACID AND LINOLEIC ACID ON EXTRACTABILITY OF MUSCLE PROTEINS OF PRAWN *P. indicus*

Acid	Volume of acid added to 200ml of extractant (ml)	Salt soluble protein N (as % of total protein N) Series I	Series II
Oleic acid	0.00	81.87	75.77
	0.10	84.13	77.62
	0.20	80.88	75.39
	0.30	51.49	52.85
Linoleic acid	0.00	78.86	75.41
	0.20	84.01	81.32
	0.40	63.40	61.75
	0.60	36.63	40.88

TABLE III EFFECT OF ADDED LINOLEIC ACID ON INSOLUBILIZATION OF FISH ACTOMYOSIN (from *S. longiceps*) HELD AT 5–7°C

S. No.	Amount of linoleic acid added (mg/g of soluble protein N)	Soluble protein Nitrogen concentration (mg/ml of actomyosin prepn.) at 5–7° C 24 hrs.	120 hrs.
1	0.000	0.409	0.349
2	0.088	0.296	0.279
3	1.978	0.245	0.191
4	3.054	—	0.175
5	5.005	0.232	0.141
6	10.380	0.134	0.107

tion begins. It is possible that in the case of prawn, the fatty acid added in smaller levels are utilized for emulsification of sarcoplasmic proteins (occurring to an extent of 30% in the muscle) which are rendered extractable, sufficient FFA being not available originally due to its poor lipid content. The C_{18} acids are not dissociated completely in buffered salt solutions during blending (Ralston, 1948) and they interact with sarcoplasmic proteins of the muscle without resulting in insolubilization (Putnam, 1948.) Increased insolubility on addition of larger amounts of the acid may be due to the contractile protein–FFA interaction, after saturation of binding sites of sarcoplasmic proteins, resulting in the formation of cross linked net work with muscle fibre which becomes resistant to fragmention and solubilization by the extractant. On the other hand in the case of *S. longiceps* which has a higher fat content and low sarcoplasmic protein content (about 20%) the critical FFA level would have been already available for reaction with sarcoplasmic proteins by lipid hydrolysis so that the system showed regular trend of insolubilisation with increasing addition of linoleic acid. This is further corroborated by the relatively lower values of soluble protein nitrogen obtained during extrrction from fresh fish in the absence of added FFA.

The fish actomyosin held in solution has also been found to be increasingly insolubilized by the addition of linoleic acid depending upon the concentration of the fatty acid and length of storage at low temperatures (Table III).

On comparing the values obtained after 24 hours and 120 hours of standing at low temperature, it may be seen that the loss of solubility of actomyosin is enhanced with prolonged contact by the fatty acids. Although the actual mechanisms responsible for the observed phenomenon are not fully understood, the important factor involved might be the loss of water holding capacity of the protein during storage and the change in its hydration characteristics (Seagran, 1958) brought about by the formation of a more hydrophobic surface on the protein miscelles due to adsorption of fatty acids.

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