OBSERVATIONS ON CHANGES IN THE MAJOR PROTEIN NITROGEN FRACTION OF PRAWNS AND SARDINES DURING ICE STORAGE

K. DEVADASAN AND M. RAJENDRANATHAN NAIR
Central Institute of Fisheries Technology, Ernakulam, Cochin-11

Changes in the major protein nitrogen fractions viz; sarcoplasmic, myofibrillar and stroma have been studied in two species of prawns and in oil sardine held in ice storage. Myofibrillar proteins were observed to get denatured at a rapid rate as determined by salt extractability method. The sarcoplasmic proteins were not denatured to any considerable extent. With sardine however, the extraction of myofibrillar proteins was inhibited rather in the uniced condition itself presumably owing to the presence of free fatty acids.

INTRODUCTION

The effect of storage at various temperatures on fish muscle proteins has been a subject matter of several investigations. Seagran (1958) followed the method of differential solubility for fractionation of proteins of king crab. Working with certain fresh water fishes, Moorjani *et al* (1962) and Baliga *et al* (1962) have followed the change in major protein fractions during storage in ice. Govindan (1962) studied the changes in total nitrogen and water soluble nitrogen in ice-stored Indian prawns. More recently postmortem changes of horse mackerel proteins stored in ice have been investigated by Japanese workers (Maruyama and Suzuki (1968), However a systematic study of the changes in protein fractions of fish during chill storage has not been attempted in India and the present study forms part of an exhaustive investigation on such changes occurring in important commercial fishes and shell fishes of the Indian waters.

MATERIALS AND METHODS

Marine prawns *(M. dobsoni* and *P. indicus)* and sardines *(S. longiceps)* procured in prime condition were used for the study. They were beheaded, washed clean of adhering dirt and slime, packed in thin guage polythene bags and stored in ice. Preferential solubility technique of Seagran *loc cit* modified according to Paul (1966) was employed for the extraction and determination of the different protein fractions.

Buffers used: (a) Sarcoplasmic proteins: KCl–Borate buffer μ = 0.05, pH = 7.5 at 0°C.

(b) Myofibrillar proteins ; $KCl - NaH_2PO_4 - Na_2HPO_4$ buffer μ = 0.6 pH = 7.5 at 0°C.

(c) Denatured proteins:– 0.1 N NaOH at room temp.

Extraction procedure:–

Muscle freed from bones, skin and shell was minced well in a high speed mixer. 10g of the homogenised meat was weighed into a steel centrifuge tube. About 35 ml of KCl-Borate buffer were added to it, mixed well with a glass rod and kept over-night at 0°C. The suspension was centrifuged at 0°C next day at 5000 r. p. m. for 30 minutes and the supernatant transfered to a 100 ml standard flask kept at 0°C. The residue was extracted twice with the same buffer at 0°C, using 25 ml portions and the supernatant decanted into the standard flask. The volume was made up with the buffer and the protein nitrogen content determined in the aliquot according to A. O. A. C. (1960). The residue after extraction of the sarcoplasmic nitrogen was extracted with KCl-Phosphate buffer in the manner described above. Myofibrillar protein nitrogen content was determined in an aliquot of the made up solution. The residue after this extraction was similarly extracted with 0.1 N NaOH at room temperature to give the inextractable denatured protein. The residual stroma protein was estimated by direct digestion with sulphuric acid.

Results and discussion

The results obtained on different protein nitrogen fractions during ice storage of prawns *P. indicus* and *M. dobsoni* and sardines *(S. Longiceps)* are represented in tables I-III. The values are expressed as percentage of total protein

Table I. Changes in protein nitrogen fractions of prawn P. *indicus* held in ice.

No. of days in ice	Sarcoplasmic protein-nitrogen	Myofibrillar protein-nitrogen	Denatured protein-nitrogen	Stroma protein-nitrogen
	(Results expressed as % total protein nitrogen)			
0	28.95	27.31	37.19	3.77
2	27.38	27.06	37.20	3.77
5	24.86	22.29	45.86	4.70
7	25.46	16.67	49.12	5.57
10	23.48	11.89	59.94	5.57
12	23.35	7.23	62.15	5.82

Table II Changes in protein nitrogen fractions of prawn *M. dobsoni* held in ice.

No. of days in ice	Sarcoplasmic protein-nitrogen	Myofibrillar protein-nitrogen	Denatured protein-nitrogen	Stroma protein-nitrogen
	(Results expressed as % total protein nitrogen)			
0	29.36	25 01	35.92	4.91
2	29.29	19.43	39.91	5.04
4	— —	18.01	41.91	5.22
6	27.77	16.98	49.27	5.81
9	27.77	16.90	52 01	5.93
11	25.98	13.63	55.34	5.93
13	25.20	10.27	57.90	6.11

Table III Changes in protein nitrogen fractions of sardines *S longiceps.*

Nor of days in ice	Sarcoplasmic protein-nitrogen	Myofibrillar protein-nitrogen	Denatured protein-nitrogen	Stroma protein-nitrogen
	(Results expressed as % total protein nitrogen)			
0	26.33	8.77	55.60	6 84
2	23.71	7.86	59 15	7.03
4	23.15	6 66	61 24	7.15
6	22.35	6.32	62 86	6 98
8	22.24	5.96	64 91	7.08
10	21.80	5.47	65 47	7.24
13	20.39	5.43	66.48	7.39

nitrogen. It has been found that the sarcoplasmic protein nitrogen decreased only by 3–6% during about 2 weeks of storage in ice in the case of both sardine and the two species of prawns studied. Myofibrillar protein nitrogen on the other hand showed a decrease of 15-20% during the same period in prawns which tends to indicate that this protein gets denatured with storage time. The dccrease of myofibrillar protein nitrogen is accompanied by the increase of denatured protein to the extent of 20–25% during the same period. There is only slight variation in the amounts of stroma protein during ice storage. These observations are in agreement with the findings of Dyer (1950) and Sawant and Magar (1961) that it is the actomyosin fraction of the muscle which becomes insoluble during chill storage. Matsumoto *et al* (1967) working with post rigor muscle of cod found that larger part of the protein precipitated from extracts on standing corresponded to myosin B.

In the case of oil sardine the yield of myofibrillar protein by the above method was very low even with the fresh raw material. Although the reasons for this extremely low yield of myofibrillar proteins are not definitely known it is possible that appreciable amounts of free fatty acids present in this muscle might have rendered the protein insoluble during extraction. The insolubilizing effect of free fatty acids on fish protein has been extensively investigated by several workers (King *et al*, 1962, Anderson *et al*, 1963). It has also been shown that development of free fatty acids parallels protein insolubility in frozen fish (Dyer and Frazer 1959, Olley and Lovern 1960, Bligh 1961 and Olley *et al* 1962). The differences in the behaviour shown by the muscle protein of sardine and prawns during extraction were also apparent in their action toward added C_{18} unsaturated fatty acids. The results of the investigations on this subject will be communicated in a subsequent publication.

ACKNOWLEDGEMENT

The authors are grateful to Dr. A. N. Bose, former Director and Dr. V. K. Pillai, Fishery Scientist (Processing) of this Institute for their keen interest in this work.

REFERENCES

A. O. A. C. 1960, *Official Methods of Analysis* (Association of Official Agricultural Chemists) 9th Edn.

Anderson M. L., F. J. King and M. A. Steinberg. 1963, *J. Fd. Sci.* **28** (3), 286.

Baliga B. R., M. N. Moorjani and N. L. Lahiry 1962, *Food Technol.*, **16** (2), 84.

Bligh, E. G. 1961, *J. Fish. Res. Bd. Canada*, **18**, (1), 143.

Dyer, W. J. 1950, *Ibid*, **7** (4), 585.

Dyer, W. J. and Frazer D. I. 1959, *Ibid* **16** (1). 43.

Govindan, T. K. 1962, *Indian J. Fish*, **9**, B; 7.

King F. J. M. L. Anderson and M. A. Steinberg 1962, *J. Food Sci;* **27** (4), 363.

Maruyama Y. and T. Suzuki 1968, *Bull Jap. Soc. Sci Fish;* **34** (5), 415.

Matsumoto J. J., W. J. Dyer, J. R. Dingle and D. G. Ellis 1967, *J. Fish. Res. Bd. Canada.* **24** (4), 873.

Moorjani, M. N. Baliga B. R. Vijayaranga and N. L. Lahiri 1962 *Food Technol;* **16** (2) 80.

Olley, J., and J. A. Lovern 1960, *J. Sci. Fd. Agric;* **11**, (8), 644.

Olley, J., R. Pierie and Helen Watson 1962, *Ibid*, **13** (6), 501.

Paul, Pauline C. 1966 *J. Agric. Fd. Chem* **14** (5), 490.

Savant P. L. and N. G. Magar 1961, *J. Fd. Sci.* **26** (2), 253.

Seagran H. L. 1658, *Comm. Fish. Rev;* **20** (11), 15.

—