BIOCHEMICAL AND NUTRITIONAL STUDIES ON EAST PAKISTAN FISH

Part XII—Investigation on the Solubility of True Protein of Fish and Prawn in Fresh and decomposed Conditions and after Freezing Storage for One Year

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Ten varieties of fish and one variety of *crustacea* (prawn) were investigated with regard to the changes in the quantity of the total soluble nitrogen and soluble protein nitrogen due to decomposition and due to storage at -15°C. for a period of one year. The results show that actual protein nitrogen extracted by the solvents (KCl of ionic strength 0.05M) was much lower than that simply deduced from the soluble nitrogen fraction and this is because of the presence of non-protein nitrogen.

the In case of both fish and prawn there is not much effect in the extraction of soluble total nitrogen and soluble protein nitrogen due to decomposition. Due to frozen storage, there is decrease of soluble fraction to 70 percent whereas in case of prawn the soluble fraction remains unchanged. Higher decrease of soluble fraction in the case of marine teleosts to 25 per cent in contrast to 70 percent in the case of fresh water teleosts as noted here, and solubility of the same fraction in the case of prawn when those are stored in freezing condition for a considerable period, has been discussed in the light of difference in the osmosis of the cell membrance and the participation of the bone in causing the change of the salt concentration of the extracellulur fluid.

Introduction

In the previous communication from these Laboratories[1,2] reports were submitted about the distribution of protein and non-protein nitrogenous constituents in the muscles of the different varieties of fresh-water fish (teleosts) of this region and their relation with the condition of the fish and their age. The present report submits the results of investigation about the changes in the distribution of soluble sarcoplasmic and myofibrillo protein and insoluble protein (stroma) of some fresh-water fish of this region in their fresh, decomposed and under frozen storage condition over a year.

Extensive work about the distribution of the above protein and their sub-fractions in the different species of toleosts and elasmobranch fish in various regions has been carried out in different laboratories and has been reviewed by Hamolir, [3] Dyer Dingle [4] and recently by Connell. [5] From all these reports new information has been gathered which has helped us to understand to a certain extent the relationship between the protein denaturation, their texture and loss of water-binding capacity as judged by the solubility of the protein in water and in salt solution of different ionic strengths under condition of freezing storage at various temperature-ranges for different periods. But no information is yet available in the literature about the above aspect of denaturation of proteins of fish of this region when these are subjected to long storage under freezing condition and dehydrated to fish flour. The necessity for an extensive investigation on this aspect is now keenly felt in view of the prospects of fish preservation industries in this country by canning, dehydration to fish flour or by freezing technique where the maintenance of texture of fish tissue accompanied by high nutritional qualities is of primary importance to be judged by objective tests.

Experimental

In all the previous work [6–8] the amount of soluble protein was deduced by the estimation of the nitrogen of the fractions extracted by water and salts of different ionic strength and by multiplication of the values with the factor 6.25. But since these fractions contain NPN to the extent of 9 to 18 percent of the total nitrogen in the case of teleosts and more than 30% in case of elasmobranch fish as has beeen observed by De *at al.*,[2] Shewn *et al.*[9] and others,[10] quantities of soluble protein as reported in the literatures do not therefore, represent the true value but only the crude values of the soluble proteins. In order to makea real assessment of the soluble protein of the fish under various conditions of storage, Connell's method[11] with some modifications was adopted. The soluble-protein fraction obtained by this method was also used for the determination of ATPase activity of fish muscle protein as affected by decomposition and freezing-storage, an account of which will be submitted in a subsequent communication.

The fish were collected early in the morning in fresh condition from the market and were divided into three batches. One batch was kept as fresh, another batch was allowed to decompose by storing at the laboratory temperature of 26-30°C. and the third batch was stored in the ice chamber of the refrigerator at -15°C. for over a year.

Fishes of the first batch were sliced before the onset of rigor since it was reported by Nikhita and Kiuko[12] and Love[13] that proteins of the pre-rigor frozen herring and cod are less denatured than that of post-rigor ones. In the case of air-breathing species no such precaution was necessary as these were in the living condition before being sliced. In all cases muscular tissue was collected from different parts of the body so as to make a homogenous distribution of myotomes and myocommata. 10 g. of the replicate sample were then coarsely minced with phosphate buffer of ionic strength, 0.05M and pH 7.5. Washing was removed by centrifugation. The process was repeated twice with the above solution. This was then followed by repeated stirring and extraction with a mixture containing 50 ml. of 0.9M KCl+phosphate buffer of ionic strength 0.1 and pH 7.5, 150 ml. of 0.45 M KCl+10 ml. 0.2M sodium pyrophosphate and 0.5 ml. of 0.2M $Mg.Cl_2$. After each stirring the supernatant fluid was separated by centrifugation. All the above operations were carried out at 0°C.

The above washings of lower and higher ionic strength solutions were combined together and made to volume (200 cc.) with the second solvent. An liquot was subjected to analysis for total nitrogen and another portion (10 ml.) was deproteinised by T.C.A. The NPN of the TCA extract was determined by taking an aliquot of the extract after making up the volume to 25 ml.

The insoluble residue (stroma), after the extraction of the soluble proteins, was analysed for total nitrogen. All the nitrogen estimation were carried out by microKjeldahl technique as described in the previous communications.[2]

In a similar manner replicate sample of decomposed fish and frozen fish were similarly extracted. The frozen sample was previously thawed by keeping under the tap water the temperature of which was high enough to carry on the thawing. After thawing the fish was sliced and the tissue was extracted according to the same procedure as described above.

Results

Fish.—The detailed analytical value of the total N (TN), non-protein N (NPN), total soluble N (TSN), proteins nitrogen of the soluble fraction (PN) and the insoluble N of different varieties of fish (teleosts) in their fresh, decomposed and freezing storage condition are shown in Table 1. The above values on an average expressed as percent of TN or TSN are shown in Table 2A. Values of prawn are shown separately in Table 2B.

The results of Table 1 show a great variation in the amount of TSN extracted in different fish under fresh, spoiled and freezed storage condition.

The results of Table 2 (a) show that the average values of TN of fresh, decomposed and freeze-stored fish are almost of the same order of 2.89-2.90 g./100 g. fish tissue. The values of TSN are of the order of 2.60 to 2.64 g. per 100 g. for both fresh and decomposed fish and constitute 90% of TN. Freezing storage for one year caused decrease of the TSN to 2.02 g./100 g. fish tissue and this constitutes about 70% of TN.

The values of TSN as shown under column 2 do not actually represent the nitrogen of the different proteins extracted but include some non-protein nitrogen (NPN) which on average represents 0.32 g. per 100 g. fish tissue and constitutes 11.0 percent of TN or 12.3 percent of the TSN. On decomposition this value wasfound to increase to 14.8 percent of the TN or 16.3 percent of TSN. Freezing, though, causes decrease of the TSN, but effects an increase of the NPN to the level constituting 13.1 percent of TN or 18.8 percent of TSN. Deduction of NPN from the TSN values causes the decrease of the SPN values to 78.6, 76.2 and 56.8 percent of TN or 88.0, 83.7 and 81.1 percent of TSN respectively for fresh, decomposed and freezestored fish.

The insoluble nitrogen representing stroma etc. and comprising of collagen, elastin etc. ranges from 5.92 to 10.09 percent or 8.76 percent of the TN on an average in the case of fresh fish, slight decrease in the case of the decomposed fish and increase to the level of 31.6 percent of TN in the case of freezingstored fish.

Prawn.—The results with respect to prawn as presented in Table 2 B show some increase of TN values during decomposition and under frozen storage and this is perhaps due to dehydration. The average value of TN of fresh prawn is higher than that of fresh fish as shown in Table 2A and this is due to high NPN content of the prawn to the extent of 24.9 percent of TN. Due to this high NPN the actual soluble protein N (SPN) in prawn approaches the same order as that of fish *i.e.* 2.28 g./100 g.

Decomposition na causes increase of NPN to a larger extent as compared to that in fish and for this reason the actual PN of the soluble fraction decreases considerably although the TSN does not show much change.

Insoluble nitrogen fraction, *i.e.*, stroma etc. was 3.15 percent of TN in the case of fresh prawn and this was not affected by decomposition as in the

TABLE 1.—SHOWING THE DISTRIBUTION OF TOTAL NITROGEN (TN), TOTAL SOLUBLE NITROGEN (TSN), NON-PROTEIN NITROGEN (NPN) OF THE SOLUBLE FRACTION AND INSOLUBLE NITROGEN IN DIFFERENT SPECIES OF FRESH WATER FISH (TELEOSTS) IN FRESH, DECOMPOSED AND UNDER FREEZE STORAGE CONDITION.

Name of Fish		Total N (TN) content g./100 g. fish			Total soluble N (TSN) content g./100 g. fish.			NPN in the soluble N Fraction g./100 g. fish			Protein-N(PN) in soluble N extract TSN-NPN g./100 g. fish.			Insoluble N. g./ 100 g. fish.			Insoluble N as percent of TN		
Local	Zoological	Fresh	Decom-posed	Freeze-stored	Fresh	Decom-posed	Freeze-stored	Fresh	Decom-posed	Freeze-stored	Fresh	Decom-posed	Freeze-stored	Fresh	Decom-posed	Freeze-stored	Fresh	Decom-posed	Freeze-stored
Rohi	Labeo rohita	2.96	3.0	3.0	2.69	2.80	2.14	0.22	0.42	0.32	2.47	2.38	1.82	0.186	0.12	0.85	5.94	4.00	28.33
Mirka	Cirrhina mrigala	2.70	2.78	2.8	2.50	2.63	2.01	0.29	0.37	0.35	2.21	2.26	1.66	0.21	0.17	0.80	7.77	6.11	28.57
Kali-Baos	Labeo calbasu	2.80	2.82	2.87	2.52	2.60	1.86	0.28	0.46	0.32	2.24	2.14	1.54	0.27	0.28	1.0	9.64	8.15	34.84
Kai	Anobes testudimius	3.10	3.12	3.10	2.80	2.91	1.76	0.37	0.41	0.40	2.43	2.50	1.36	0.25	0.20	1.35	8.06	6.57	43.54
Shingi	Heteroepnestes fossilis	3.0	3.0	3.0	2.64	2.65	1.75	0.37	0.47	0.42	2.27	2.23	1.33	0.30	0.31	1.31	10.00	10.33	43.66
Baila	Glossogobius giuris	2.86	2.86	2.71	2.55	2.50	1.95	0.33	0.40	0.36	2.22	2.10	1.59	0.27	0.29	0.77	9.44	10.13	28.41
Lata	Ophicephalus punctuatus	2.78	2.79	2.62	2.44	2.50	2.13	0.37	0.45	0.39	2.07	2.05	1.74	0.26	0.30	0.56	9.35	10.78	21.37
Puti	Barbus puntius	2.78	2.78	2.78	2.50	2.47	2.41	0.32	0.46	0.40	2.18	2.01	2.01	0.28	0.30	0.64	10.07	10.76	23.02
Shoul	Ophicephalus striatus	2.82	2.82	2.81	2.55	2.54	2.04	0.35	0.43	0.39	2.20	2.11	1.65	0.27	0.28	0.77	9.57	9.92	27.40
Hilsa	Clupea ilisa	3.1	3.1	3.25	2.84	2.84	2.15	0.28	0.49	0.43	2.56	2.35	1.72	0.25	0.28	1.08	8.06	9.03	33.23

*The nitrogen of the insoluble fraction was determined directly and not calculated from the difference in the values of total nitrogen and soluble nitrogen.

TABLE 2 A.—SHOWING THE AVERAGE VALUES OF TOTAL NITROGEN AND SOLUBLE NITROGEN, NON-PROTEIN NITROGEN, PROTEIN NITROGEN AND INSOLUBLE NITROGEN EXPRESSED AS PERCENTAGE OF TOTAL NITROGEN.

Condition of fish	Total Nitrogen (T.N)	Total soluble N (TSN)		Soluble N. P.N.			Soluble protein N (PN)			Insoluble N		
		Total	As p.c. of total N	Total	As p. c. of total N.	As p. c. of soluble N.	Total	As p. c. of total N.	As p. c. of soluble N.	Total per 100 g.	As p. c. of total N.	As p. c. of soluble PN.
	1	2	3	4	5	6	7	8	9	10	11	12
Fresh ..	2.90	2.60	90	0.32	11.0	12.3	2.28	78.6	88.0	0.254	8.76	9.75
Decomposed..	2.90	2.64	90	0.43	14.8	16.3	2.21	76.2	83.7	0.249	8.60	9.42
Freezed stored	2.89	2.02	70	0.38	13.1	18.8	1.64	56.8	81.1	0.913	31.6	45.4
TABLE 2 (b).—(THE VALUES WITH RESPECT TO PRAWN)												
Fresh ..	3.17	3.07	96.8	0.79	24.9	25.7	2.28	71.9	74.3	0.10	3.15	3.25
Decomposed..	3.30	3.16	95.8	1.16	35.1	36.7	2.00	60.6	63.3	0.13	3.4	4.1
Freezed Stored	3.38	3.20	95.7	0.84	25.0	25.0	2.36	69.8	70.4	0.17	5.0	5.3

case of fish. But under freezing storage condition the prawns, unlike fish, did not show any change iu the soluble and insoluble fraction indicating the ineffectiveness of freezing storage in case of prawn to conversion of the soluble protein to its insoluble stage.

Discussion

The following are the main features of the results of the present investigation.

1. The amount of actual protein-N is much less than the values reported by other workers due to the presence of NPN fraction.

2. There was no effect on the solubility of the protein due to decomposition at ordinary room temperature. Some decrease in the protein solubility in the case of fish and none in the case of prawn was noted due to their freezing storage.

These may be discussed in the perspectives of the findings reported from other laboratories.[3,4,5] In the previous investigations by Ironside and Love[14–16] and other workers reviewed elsewhere[4–6] particular attention was given to the protein fraction extracted by different solvents. The determination of the NPN fraction along with the actual protein fraction as carried out in these laboratories has thrown new light on the understanding of the mechanism of spoilage or denaturation of the proteins due to decomposition or freezing storage. This is evident from the results of Table 2 which shows that decomposition by autolysis is associated with increase of NPN without any change in the content of the soluble-N whereas freezing storage is associated with the decrease of the total soluble-N. This information leads us to visualise that the decrease in the soluble fraction of nitrogen due to freeze-storage is not the result of more formation of NPN constituents.

Insoluble nitrogen fraction as determined in the different fresh-water fish of this region shows a higher value ranging from 5 to 10 percent of the total nitrogen in contrast to lower range of 3 to 8 percent as noted in case of marine fish—cod. This difference of the insoluble nitrogen fraction, mainly constituting the stroma, may be explained on the basis of the physico-chemical behaviour of the cell structures of the fish of sweet and marine water origin as hypothesised by Baldwin.[17]

In the case of marine teleosts blood containing 1 percent of the salt is separated from the surrounding water having 3 percent salt by the semipermeable membrane of the gill and mucous. This creates an osmotic force away from the fish tissue producing a tendency for dehydration of the cell and in order to counteract this, the marine teleosts have to acquire more capacity for hydration or for more water-binding for which additional structural myofibrillo proteins have to be synthesised in the cells as a result of which the percent of stroma is decreased. In the case of the fresh-water teleost the position is the reverse for much comparatively less structural protein but more stroma have to be maintained as the cells are not thrust by osmosis to create a condition of dessication. It is also strikingly noted in the present investigation that storage at-15°C for over a year caused decrease of the soluble nitrogenous fraction to 70 percent of the TN in contrast to 25% as noted in the case of cod stored under same freezing temperature only for a few weeks. Such higher

stability of the structural proteins of the fresh water teleosts of this region in comparison to those of marine fish is also perhaps due to the different physico chemical behaviour of the cell structure of these two groups of fish while stored at ordinary or freezing temperature. The differential change in the pH of the cell structures [18] of the above two groups of fish causing varied concentration of salt in the extra cellular fluid by the process of dissolution of their bones may also be one of the factors for the above difference in the solubility of the protein of fish while stored at -15°C. This possibility is sustantiated from the existence of correlation between the protein denaturation and the ash content of expressed fluid of the fish as noted by Bank[19] and Love.[20] Although the increase in the ash content is not due to increase of Na and K as later reported by Love[21] yet the possibility of the involvement of Ca and Mg. in the increase of ash content of expressed fluid cannot be overruled.

It is not improbable that the salt concentrate of the tissue fluid is further concentrated by formation of ice crystal causing denaturation of protein and decrease in water-binding capacity as viewed by Dyer and Dingle.[22]

The importance of fish bones in causing protein denaturation as discussed above is further supported from the findings carried out with the prawn where the freeze-storage did not cause any change in the protein solubility. The cellular salt concentration in this case probably did not change because of the absence of bone in the muscle structures.

It is so evident from the foregoing discussion that the freshwater teleost and prawn impose too different problems with regard to the denaturation of their proteins during freezed storage, and this requiresa thorough investigation to evaluate the differential mechanism with their marine counterpart. It may be mentioned that there are many factors which cause fish protein denaturation and their insolubility due to freezing. The possibility of the involvement of osmosis of the cell membrance and participation of small bones in increasing the salt concentration of the tissue fluid cannot be overruled.

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