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Changes in Protein during Drying of Milk Fish (*Chanos chanos*) at 60°C

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Milk fish collected from brackishwater culture ponds was dressed and dried at 60°C for 24 h and biochemical changes taking place during drying were studied. Alpha amino nitrogen (AAN) showed a slight increase but total free amino acids (FFA) showed significant reduction during 24 h drying. The variation in individual free amino acid was found to be different for each amino acid. Salt soluble nitrogen and SH group decreased considerably as a result of drying. Electrophoretic study showed a decrease in the number of high molecular weight and low molecular weight protein fractions, but intermediate molecular weight proteins did not show considerable change.

Key words: Biochemical changes, fish drying, milk fish, *Chanos chanos*

Considerable quantities of dried fish are consumed in developing countries like India. Natural drying by exposure to sun and wind is widespread and is possibly the first method used for preserving seafoods. This is still applied to a large extent to preserve fish and squid (Sikorski *et al.*, 1995). Conventional air drying at relatively higher temperature is detrimental to fish muscle, because, as a result of evaporation, more moisture and salts are diffused to the surface resulting in increase in salt concentration and changes in pH which affect solubility and water binding properties (Zyas, 1997). Extensive work on biochemical and nutritional changes during heating at higher temperature (100°C and above) has been done by many workers (Sano *et al.*, 1994; Synowiecki & Shahidi, 1991) and only a few reports are available on biochemical and nutritional changes during dehydration (Mao & Sterling, 1970; Raghunath *et al.*, 1995)

Heat treatments bring about complex changes in the muscle proteins. Reduction in solubility due to protein aggregation, mainly by formation of disulfide bonds, isopeptide bonds, etc., is the major consequence of these changes. Different fractions of muscle get denatured at different temperatures, starting from 60°C for myosin to 81°C for actin (Poulter *et al.*, 1985) This study was undertaken to evaluate the biochemical changes during drying of farmed milk fish at 60°C.

Materials and Methods

The study was carried out using *Chanos chanos* collected live from the culture ponds near Cochin. The fish was iced immediately and transported to the laboratory and drying was carried on the same day. The total length of the fish ranged from 37 to 42 cm and weight from 420 to 430 g. The fish were eviscerated and split open from dorsal side and washed thoroughly to remove blood and visceral parts. They were kept skin side down on perforated aluminum trays and placed in oven preset at 60°C. The temperature was maintained constant throughout 24 h of drying. The samples were drawn at intervals of 0, 2, 4 , 6 ,8 ,12 and 24 h of drying. Skin was removed and meat ground as finely as possible. The samples were then stored in sealed polythene cover at-18°C until analyses were carried out.

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Moisture and total nitrogen were determined according to AOAC (1975). Dyer's buffer extract of the samples was prepared by homogenizing 3 g of accurately weighed sample in 60 ml of pre-cooled Dyer's buffer (5% NaCl buffered with 0.02M $NaHCO_3$) and centrifuging the homogenate (Dyer *et al.*, 1950). Centrifugation was at 10,000 rpm for 20 min at 0°C. The supernatant was used for determination of salt soluble protein (Gornell *et al.*, 1949) and salt soluble nitrogen (SSN) (Dyer *et al.*,1950). The Dyer's extract was diluted to 5mg/ml for electrophoresis, which was done according to Laemmli (1970). Sulfhydril group (SH) was estimated as described by Sedlak & Lindsay (1968). Trichloro acetic acid (TCA) extract was prepared by grinding 10 g of accurately weighed sample with 10% TCA and quantitatively filtering through Whatman filter no.1. The volume was made up to 100 ml. The TCA extract was used to measure α-amino nitrogen (AAN) (EBC, 1975), non-protein nitrogen (NPN) (AOAC, 1975), and total free amino acids (FFA) (Ishida *et al.*, 1981). Total amino acids were estimated by hydrolysis of about 0.5 mg of the fish muscle for 24 h at 110°C in vacuum sealed tubes. The sample was filtered and flash evaporated to remove the acid and then the pH was adjusted to around 2.5. Separation and estimation of amino acids were done by HPLC (Ishida *et al.*, 1981). Tryptophan was determined by colorimetry after alkali hydrolysis (Sastry & Tummuru,1985). All the determinations were carried out in triplicates except amino acids and moisture for which only duplicates were done.

Result and Discussion

Table 1 gives the changes in moisture, SSN, NPN, AAN, Total FAA, and SH group during drying of *C. chanos* at 60°C. The moisture content came down to 25.8% from 73.6% in 24 h (Table1). The values for NPN and AAN decreased slightly by 2 h of drying and then increased up to the completion of drying. The reduction in NPN and AAN by 2 h may be due to the loss of some of NPN and AAN in the water that oozed out and drained during the initial stages. Some of the soluble components also might have been lost in the exudate water. Protein breakdown on further heating might have caused the increase in NPN and AAN.

The SH group content in the fish muscle decreased during drying at 60°C, retaining only about 23% of the initial level. Raghunath *et al.* (1995) studied the effect of drying of fish at 50, 60, and 70°C on proteins and found that the SH groups registered a regular and sharp decrease with drying except at 50°C where initially an increase was observed. The initial increase found in this case was attributed to the unfolding of protein chains exposing the buried SH groups, which subsequently became oxidized. It was reported that temperatures higher than 50°C are required for oxidative transformation of SH groups to S-S bonds (Opstvedt *et al.*, 1984). Loss in cystiene also can cause reduction in SH groups.

Table 1. Changes in Moisture, SSN, NPN, AAN, Total FAA and SH group during drying of *Chanos chanos* at 60°C

Parameters	Drying time						
	0 h	2 h	4 h	6 h	8 h	12 h	24 h
Moisture (%)	73.55	70.44	66.26	62.71	54.99	48.04	25.82
NPN ($mg.g^{-1}$)*	21.1±0.09	16.2±0.12	17.94±0.39	18.75±0.32	19.3±0.10	21.24±1.27	22.5±0.91
AAN ($mg.g^{-1}$)*	4.0±0.007	3.4±0.01	3.5±0.011	3.61±0.07	3.96±0.003	3.82±0.002	4.3±0.008
Total FAA ($mg.g^{-1}$)*	46.7	36.43	39.97	43.15	36.47	39.39	24.11
SSN ($mg.g^{-1}$)*	115±10.35	90.7±9.5	75.6±3.78	61.58±2.6	46.4±1.35	44.8±3.58	42.3±0.01
SH group (μ mole.g^{-1})*	76.1±1.52	61.3±0.35	53.7±1.14	44.6±0.71	37.6±3.56	20.2±1.01	16.8±1.34

*On dry weight basis

The heat treatment studies with *Tilapia* and *Siganus* muscles (Poulter *et al.*, 1985) showed that the total extractable protein remained at its initial level until a temperature of 35-40°C was reached, while cod muscle protein began to lose its extractability at temperatures between 25 and 30°C. It was reported that aggregation of cod myosin molecule increased with temperature (35-55°C) and was considerably higher than those for herring at all temperatures (Gill *et al.*, 1992). Salt enhanced aggregation of cod myosin on heating above 50°C. But thermal aggregation of herring myosin showed little dependence on salt concentration. Aggregation affects protein solubility. Thus, the change in protein solubility varies from species to species. Solubility of protein in solvents like water 0.6M NaCl, 1.5M urea, 8M urea and 1% SDS decreased as drying progressed at 50, 60 and 70°C (Raghunath *et al.*, 1995). From the lower solubilisation in water, 0.6 M NaCl and 1.5 M urea it would appear that hydrogen and ionic bonding did not contribute as much towards denaturation by drying, as do hydrophobic bonds, which appeared to play a major role. Formation of covalent disulphide linkages and reaction of ε-NH_2 groups of lysine with lipid oxidation products or amide groups (Bjarnason & Carpenter, 1970) could be implicated in these denaturation processes.

Reduction in the salt solubility of protein of *C. chanos* at 60°C proceeded at a faster rate till 8 h of drying and afterwards it was very negligible. But SH groups decreased at a regular rate till the end of drying. The loss of all SH groups may not be due to oxidation of SH to S-S bonds. Destruction of cysteine also leads to loss of reactive SH groups.

Though an increase in AAN was observed, the total free amino acid content decreased (Table 1). This may be due to the breakdown of protein into intermediate weight polypeptides and smaller peptides. The N terminal of these would have answered the ninhydrin reaction for AAN. But since the filtrate was not hydrolysed before the estimation of free amino acids, the amino acids of the peptides were not estimated. Hence the free amino acids registered a decrease in value.

Table 2 shows the changes in the major free amino acids and Table 3 gives the changes in essential amino acids during drying of *C. chanos* at 60°C As seen from Table 2, the major free amino acids did not show a regular pattern in their changes during drying at 60°C. However, the values of taurine, glutamic acid and lysine were less than the initial value after 24h drying while those of glycine registered an increase and that of alanine did not change. These variations may be attributed mainly to the interactions of amino acids with the other constituents, mainly lipid oxidation products during drying, which results in a reduction in the amino acids content. At the same time some free amino acids may be formed by hydrolysis of peptides and proteins which increases the amino acid content.

Table 2. Changes in the major free amino acids during drying of *Chanos chanos* at 60°C

Amino acid ($mg.g^{-1}$)*	Drying time						
	0 h	2 h	4 h	6 h	8 h	12 h	24 h
Tau	6.09	6.06	5.08	5.45	6.92	8.41	5.21
Glu	0.78	0.91	1.06	0.95	1.33	1.06	0.64
Gly	1.52	1.81	1.50	1.16	1.80	2.97	1.94
Ala	0.57	0.68	0.65	0.65	0.78	0.90	0.55
His	33.47	22.17	26.84	30.73	20.31	21.1	12.22
Lys	1.13	0.95	1.00	0.88	1.05	0.67	0.52

*On dry weight basis

Among essential amino acids, maximum loss was observed in the case of cysteine (35%) followed by lysine (28.4%) and methionine (27.2%) (Table 3). In the present study, about 4% of tryptophan was lost durng drying. Other essential amino acids showed a loss of around 20%. Experiments have shown that a reaction between amide groups and the ε-amino group of lysine in protein can occur at practical drying temperatures. Reaction of the ε-amino group of lysine with degradation products of cysteine is also considered to be partially responsible for the lysine binding in heated protein (Bjarnson & Carpenter, 1970). Methionine, histidine and cysteine are the amino acids most labile to damage by heat and peroxidised lipids (Neilsen *et al.*, 1985).

Dehydration resulted in considerable loss of moisture which could lead to protein denaturation. These changes caused hardening of muscle and significant reduction in extractability. Lipid oxidation products also react with proteins forming stable bonds, which render the protein insoluble. Synowiecki & Shahidi (1991) found a large decrease in solubility of seal meat protein and an increase in their degree of coagulation at temperature ranges from 50 to 75°C. There was 22.07% reduction in SH group as a result of oxidation of SH groups to S-S bonds. Hamm & Hofmann (1965) observed that heating meat to 70°C increased the availability of SH groups as a result of unfolding of protein molecules. In the present study this was not noticed even though the dehydration temperature was 60°C. This may be due to the longer duration of exposure to 60°C so that some of the SH groups would have been oxidized to S-S bonds.

Table 3. Changes in the essential amino acids during the drying of *Chanos chanos* at 60°C

Amino acid* ($mg.g^{-1}$)	Initial	After 24 h drying	% Loss
Thr	37.9	29.68	21.7
Cys	16.93	11.01	35.9
Met	20.76	15.12	27.2
Val	41.62	32.48	21.9
Ile	37.52	28.86	23.1
Leu	59.6	46.04	22.8
Tyr + Phe	57.3	42.0	26.7
His	69.37	55.1	20.6
Lys	54.13	38.76	28.4
Trp	11.68	11.2	4

* Dry weight basis

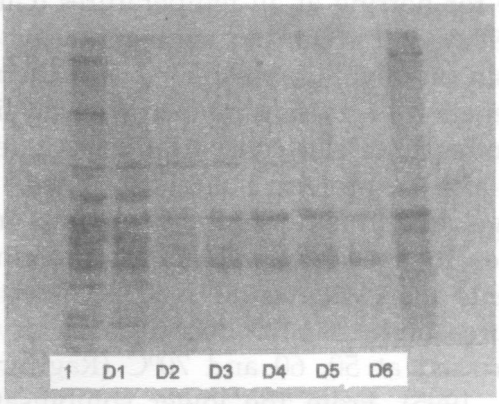

Fig. 1. Electrophoretogram (SDS PAGE) of samples dried at 60°C

Fig. 1 presents the electrophoretogram of protein during drying of *C. chanos* at 60°C. With the increase in drying time, protein bands disappeared gradually. In general, higher molecular weight protein fractions were found to be more sensitive and these disappeared earlier during drying than the lower molecular weight protein bands. Only the medium molecular weight protein bands remained after 4 h of drying which also disappeared slowly. But two of the bands were seen in the 24h dried sample also. It is likely that stainability by coomassie brilliant blue was lost upon denaturation by drying. Poor staining of protein extracted with 8M urea from fish meal has been recorded earlier (Moodie & Eva, 1974). This may also contribute to the non-visibility of the protein bands.

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