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Fish protein concentrate from freshwater shark (*Wallago attu*)

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Abstract. The possibility of utilisation of a low priced catfish known as freshwater shark (*Wallago attu*) for the production of cheap and widely acceptable form of protein concentrate (FPC) has been studied. The fish could be converted into a product having good dispersible and better functional qualities of protein by solvent-extraction and other process techniques. Conventional processing was found to be improved by washing the fish in brine and initial steaming operation. Both steaming and use of brine during blending gave good appearance of the product. Slight hydrolysis with enzyme pepsin produced better foaming and required dispersion. The treatment with acetic acid resulted in a good swelling effect of the FPC.

Keywords. Homogeneous flesh; isopropyl alcohol; acetification; protein isolates; proteolytic enzymes; dispersion.

1. Introduction

Freshwater shark (*Wallago attu*) a catfish is available in large quantities all over India. Although, it is good to eat, it is not liked by some people and does not fetch better price in the market. The fish can be transported to other areas but involves problems of transportation and preservation. Processing the fish for extracting protein concentrate (FPC) may be more economic if the method of extraction improves the functional quality of the final product. Anon (1967), Cobb and Hyder (1972) and Spinelli *et al* (1972) reported that the product does not attain its desirable properties and needs further studies for the improvement of its dispersibility, swelling, foaming and rehydration capacity. Chemical improvement of these properties was made by Hermansson *et al* (1972), Groninger (1973) and Koury and Spinelli (1975); whereas Spinelli *et al* (1973) and Groninger and Miller (1975) have tried to modify the product both by chemical and biological means.

The purpose of this study is to develop a suitable process for the utilisation of *W. attu* and to examine the effect of proteolytic enzymes on the product. An attempt to evaluate the cost estimates is also made.

2. Maerials and methods

Fish protein concentrate (FPC) was prepared from *W. attu*, which was procured from the catches of Gudha reservoir (Bundi District). The fish was kept in the deep freeze until use. Pepsin and papain were obtained from Pfizer Chemicals Division, New York, USA. Folin-Ciocalteu's phenol reagent, from E Merck, Germany, was used. Reagent grade chemicals and solvents were used.

For preparation of the fish samples, outer slimes were washed with 0·5% salt solution. After removal of the bones and skin, the flesh was blended either by the addition of 2·5% sodium chloride solution or steamed under pressure for 10 min. Moisture, fat, protein and ash in fresh fish and finished products were determined (AOAC 1965). Enzyme activity was measured by taking 5 mg enzymes in 5 ml 1% substrates and allowing the reaction under specified conditions of time, temperature and pH. The enzyme was quickly inactivated by heating at 80° C for 15 min. Protein in supernatant liquid was determined (Lowry *et al* 1951) and the enzyme units were calculated. After blending, the pH of fish samples was adjusted to its isoelectric range of about 4 and the protein was extracted with isopropyl alcohol.

Partial hydrolysis of protein concentrate was done by pepsin and papain using enzymes in 0·01, 0·005 and 0·001% at a temperature of 30° C for a specified period. Buffers with pH 2·0 (citrate) and 6·5 (borate) were used for pepsin and papain respectively. The enzyme action was terminated in the same way as was done for enzyme assay. Proteolysis was measured by determining protein in the supernatant liquid (Lowry *et al* 1951). After inactivation of enzyme and cooling, pH of the product was adjusted to 7·0. The extracted and hydrolysed protein isolates were dried at controlled temperature of 85–90° C.

2.1. *Process steps*

The process steps for obtaining the finished product involved the following flow charts :

Raw fish → (treated with 0·5% sod. chloride sol.) → washed fish → (steaming for 10 min) → homogeneous flesh → (pH 4·0, treatment with isopropyl alcohol at 65°C for 20 min; 4 vols. three times followed by similar treatments with water) → deboned and deskinned communited fish → (blending) → defatted fish → (acetification; pepsin treatment, pH 2·0) → partially hydrolysed protein isolates → (pH adjusted to 7·0, drying and grinding) → finished product

2.2. *Dispersion, foaming and acetification*

For determining the dispersibility, 1 g of the prepared product in 100 ml of water was mixed in a blender for 7–8 min and immediately transferred to a 250 ml graduated cylinder. The dispersion in each run was noted by measuring the maximum settling.

Foaming of both hydrolysed and untreated protein concentrates was tested. For foam preparation, 1 g of prepared and treated material was whipped in a waring blender for a specified period using 100 ml of water. Initial foam volume was determined by matching the level with that of the calibration already done on the blender glass.

For determining the stability of foam, the whipped material was quickly transferred to a graduated cylinder after noting the initial volume. The stability of foam at the room temperature was measured by noting the reduction in volume at regular intervals of time. The treatment of FPC was made with acetic acid in the ratio of 1 : 10 (wt./vol) for 20 min at room temperature.

3. Results and discussion

The brine solution at the rate of 0·5% helped clearing the extraneous slimy material. Bones and skin could easily be detached and separated by steaming the fish under low pressure for 10 min. Blending with salt solution softened the rigidity of fish muscles, while steaming before blending or adding brine during blending improved the colour of the final product. Both steaming and salting might have inactivated biochemical changes in the flesh.

For deodorisation of protein concentrate, isopropyl alcohol was found to be the most efficient solvent. Petroleum ether also extracted lipids and absorbed flavour, but the product was not suitable. Carbon tetrachloride did not extract oil completely but made the flesh into a sticky mass. The results show that for an ideal extraction, the ratio of fish flesh to iso-propyl alcohol at 65° C for a period of 20 min was 1 : 4 (wt/vol). The same procedure when replicated at least 4 times resulted in almost complete removal of lipids. The extracted protein followed by water treatments 4 times under the same conditions of time and temperature made the product free from solvent and flavour.

The average percentage composition of the flesh of fish and the FPC is recorded in table 1.

Table 1. Per cent composition of fish and FPC.

	Fresh fish (wet-weight basis)	FPC
Moisture	78·0	3·70
Fat	5·0	traces
Protein	17·0	88·8
Ash	1·1	5·0

Under the same conditions of time, temperature and enzyme-protein ratio for digestion, it was observed that the pepsin hydrolysed more protein than that by papain. Smoothness in texture of the product increased with increase in the percentage of hydrolysis. The dispersion was more (about 80%) higher percentages of hydrolysis. The untreated product had very little dispersion. The acetic acid could improve in the swelling of the product. The acidified product treated with enzyme gave good dispersion. However, whipping is necessary for a complete dispersion. As the whipping time increased, the product became more viscous and opaque. The dispersion was observed to be highest within 10 min of whipping.

Table 2 presents the relationship between the degree of hydrolysis of protein and foam stability. Higher amount of foam is observed with greater degree of hydrolysis which also favours better foam stability. The untreated product does not produce any foam. The foam quantity increases with increase in whipping time and is maximum in 10–12 min. The maximum loss in foam occurs within 10 min of whipping which is stable after 30 min. The increase in protein concentration gave more foam, but became constant beyond 5% concentration of protein. However, the quantity of foam increased in the acidified product treated with enzyme.

3.1. *Cost estimate*

The cost estimate of extracting 1 kg protein concentrate by treating 10 kg of whole fish was made and these are given in table 3.

The protein extract from the *W. attu* appears to be promising as a food ingredient for a number of reasons. The cost of the product is reasonable compared to that of other similar proteins. Although there is a high flesh ratio, the *W. attu* do not fetch a better price. However, the process cost indicates that the fish could be made into a proteinous food at cheaper rates. The returns of this processed food may further add to the national economy.

From the viewpoint of its both quality and price, it is observed that the protein isolate may find its use in different food systems as one of the ingredients. Groninger and Miller (1975) attempted the suitability of its application in foods,

Table 2. Foam formation of FPC and its stability.

Treatment	Per cent hydrolysis	Initial foam volume (ml)	Foam reduction from initial volume (ml) after		
			10 m	30 m	1 hr
Pepsin	5·0	34	11	13	15
	8·0	38	8	10	11
	19·0	55	6	9	10
Papain	2·5	16	12	14	15
	5·0	30	11	12	13
	5·3	36	10	12	14

Table 3. Cost estimates for extraction of protein concentrate from fish.

(1) Cost of raw fish—10 kg @ Rs. 3/kg	Rs. 30·00
(2) *Chemicals*	
(i) iso-propyl alcohol, 4 litres @ Rs. 11·55 litres (24 × 4 litres = 96 litres for one preparation, loss during recovery by distillation 4·1% = 4 litres)	Rs. 46·20
(ii) pepsin, 0·01% (0·1 g @ Rs. 80/25 g)	Re. 0·32
(iii) other chemicals (lumpsum)	Rs. 21·00
Cost of overhead charges (labour, depreciation, utilities, contigencies, etc., 20% of material cost)	Rs. 19·50
Total	Rs. 117·02

e.g., frozen dessert, souffle and dessert toppings, etc. Study is also in progress in our laboratory for its possible use as a protein-fortifying agent in some formulated cereal foods.

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