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Functional and Nutritional Properties of Fish Protein Hydrolyzate from Dhoma (*Johnius dussumieri*)

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Fish protein hydrolyzate (FPH) was prepared from the minced muscle of an under utilized fish Dhoma (*Johnius dussumieri*), using a plant proteolytic enzyme, papain. The method involves skinning and deboning of fish followed by washing and incubation of the muscle homogenate in presence of papain at 55°C for 2 h. The soluble portion separated after incubation was heated at 95°C for 5 min, cooled and freeze-dried. The final product possessing a creamy white colour contained 90% protein and 5% fat. FPH was soluble to the extent of 70% in distilled water but was completely soluble in 0.2N NaOH. The water absorption capacity was 3.0. Emulsifying capacity was found to decrease with increase in protein concentration. The Wettability of the product was excellent in comparison with defatted casein. Nutritive value of FPH assessed by measuring the growth of *Tetrahymena pyriformis* W could well be compared with that of casein. Incorporation of Sodium tripolyphosphate (NaTPP) and hydrogen peroxide (H_2O_2) during the preparation of FPH yielded a product which retained colour and retarded the development of off-odours during storage for 3 months at room temperature. FPH prepared by the procedure was devoid of viable organisms.

Key words : Protein hydrolyzate, functional properties, nutrition, papain, *Johnius dussumieri*.

During the past few years several attempts have been made for making more effective use of fish proteins from low cost under utilized fishes by developing different protein products for human consumption (Venugopal, 1995). However, many of the products developed so far have not offered much scope as a protein supplement because of the lack of functional properties (Finch, 1970; Holden, 1971; Warrier *et al.*, 1975; Mackie, 1982). Some degree of functionally is essential, if fish proteins are to be incorporated in either conventional or novel foods. In this context among other approaches (Groninger, 1973; Groninger & Miller, 1975; Thankamma *et al.*, 1979), the use of proteolytic enzymes in liquifying the fish and thereby improving the functional properties merit consideration (Mackie, 1982; Venugopal, 1994). Controlled proteolysis may enable us to obtain hydrolyzate having good functional and nutritive properties, in some cases even better than the original (Quaglia & Orban, 1987). The present communication describes a method for the preparation of fish protein hydrolyzate (FPH) from the minced muscle of Dhoma (*Johnius dussumieri*), a low cost fish, using the plant proteolytic enzyme, papain. The product was evaluated in terms of its functional and nutritional properties.

Materials and Methods

Freshly caught Dhoma (*Johnius dussumieri*) were brought to the laboratory in iced condition. They were cleaned, eviscerated, filleted and stored in crushed ice until used. Minced muscle was obtained by passing the fillets through a deboning machine (70% yield). The muscle mince was washed thoroughly with tap water and finally with distilled water. After filtration through two layers of muslin cloth and drying on folds of filter paper, the minced muscle was weighed (75% yield on fillets). A quantity of minced muscle (1.5 kg) was agitated with distilled water (5 litre), 750 mg papain (Sigma, USA, Activity : 1.5-3.5 units mg^{-1} protein) and cysteine (750 mg) and homogenized in a waring blender having a stainless steel jar of 5 litre capacity with an intermittent speed of high and low for 3 min. The protein hydrolysis was carried out at 55°C for 2 h with stirring at low speed. After hydrolysis, the suspension was filtered through muslin cloth and heated at 95°C for 5 min to inactivate the enzyme. After cooling, the enzyme digest was treated with (0.01%) sodium tripolyphosphate (NaTPP), freeze-dried, ground to fine powder and stored in screw capped bottles. In a batch of enzyme digest, H_2O_2 (0.03%) was also added along with NaTPP before freeze drying. The degree of hydrolysis was measured by the ratio, percent of 10% trichloroacetic acid (TCA) soluble nitrogen to total nitrogen.

Nitrogen was determined by Kjeldahl digestion of FPH followed by nesslerization (AOAC, 1965) and the protein calculated (Nx6.25). Protein measurement was also made from suitable aliquots of FPH dissolved in 0.2N NaOH by the procedure of Miller (1959). FPH was refluxed in chloroform for a period of 16 h in soxhlet apparatus. The chloroform extract of FPH was used for the determination of total lipids (AOAC, 1965). Moisture and ash content were measured according to AOAC (1965). The amount of calcium, strontium and potassium was determined by atomic absorption spectrophotometry. The extent of lipid peroxidation during storage was measured in terms of thiobarbituric acid (TBA) value as per Turner *et al.* (1954). The total viable bacterial count (TBC) of FPH was determined from a 10% homogenate in saline by plating on plate count agar.

Solubilization of FPH in different solvents and under varied pH conditions was ascertained. Protein hydrolyzate (1g) was added to 50 ml of distilled water in a 250 ml beaker and agitated continuously for 60 min at room temperature using a magnetic stirrer. The suspension was then centrifuged at 750 x g for 15 min. The protein content of the supernatant as obtained following filtration was determined. The protein content (Nx6.25) of FPH was also estimated by Kjeldahl procedure (AOAC, 1965).

The protein solubility of FPH was tested over a pH range of 2.5-12.5. Five hundred mg of FPH was dispersed in 25 ml of 0.2N NaOH and agitated for 30 min at room temperature using a magnetic stirrer. This treatment dissolved the FPH completely. The pH of the resulting solution was 12.5 which was adjusted to various values by addition of 1 N HCl with continuous stirring. The solution was then centrifuged at 12,000 x g for 10 min. The extent of solubility was determined by measuring the protein content in the supernatant by the procedure of Miller (1959). Emulsifying capacity of FPH was determined by the method described by Dubrow *et al.* (1973) with a few modifications. FPH varying from 10 to 500 mg was separately homogenized in a Sorvall Omnimixer with 20 ml of 5% NaCl containing 0.02M $NaHCO_3$ for 3 min at low speed. *Til* oil (20 ml) was then slowly added to the blender and the mixture was homogenized further for 2 min at low speed. The entire mixture was poured into 3 test tubes in 10 ml portions and the tubes were placed in a boiling water bath for 30 min. After cooling in an ice bath, volume of aqueous and emulsified oil layer was measured. Emulsion capacity was calculated as ml of oil emulsified per mg of protein.

The evaluation of wettability was subjective and based on the extent of wetting when particles were applied to the surface of a water medium. The sample that was wet within 2 min or less was considered as excellent; between 2-5 min good between 5-15 min fair, and any sample that took more than 15 min to become wet was rated poor (Warrier & Ninjoor, 1981).

The method used for the determination of water absorption was essentially that of Smith & Circle (1972). In 20 ml distilled water 1 g of FPH was added gradually to avoid formation of clumps. The tubes were centrifuged at 750 g for 10 min. The supernatant was poured through a funnel containing a small amount of glass wool into a calibrated tube. The volume of the supernatant was substracted from the original 20 ml. The results were reported in terms of ml of water absorbed by 1 g of protein.

The method followed for the evaluation of protein quality with *Tetrahymena pyriformis* W was that of Scott *et al.* (1963). The FPH was extracted three times with diethyl ether at room temperature and once with methanol. The material was finally washed with ether and dried at 60°C for 30 min. The dried material was then ground to fine powder and nitrogen content was determined by the Kjeldahl method (AOAC, 1965). Suspensions of test material were then prepared to give a nitrogen content of 1-6 mg per 4 ml and the pH was adjusted to 8.2. All other stock solutions were prepared as described by Scott *et al.* (1963) and assays were carried out with three replicates for each sample. After incubation for four days the culture was transferred to a ¼ oz screw-capped bottle containing 1 ml preserving fluid. Organisms were counted in a single-cell haemocytometer. The organisms in eight alternate 1 mm squares were counted and the mean number per 1 mm square gave the final population of the test culture in units of 10^4 organisms ml^{-1}. This figure was used to express the nutritive value of fish protein hydrolyzate and was compared with the value obtained with casein.

Results and Discussion

Dhoma muscle contained 15-18% protein, 2-3% fat and 75-80% moisture. The method developed for the preparation of FPH gave a final product having white colour and a faint fishy smell. Initial washing of the muscle mince removes most of the sarcoplasmic proteins (Spinelli *et al.*, 1975) and therefore forms a critical step that helps in reducing the intensity of odour in FPH.

Table 1. Proximate composition of dhoma protein hydrolyzate

Constituents	%
Protein (N x 6.25)	90.00
Ash	3.20
Moisture	3.54
Total lipids	5.00
Calcium	0.080
Strontium	0.010
Potassium	0.450

Values represent the average of three independent estimations

A typical preparation of FPH had a composition of 90% protein, 3.2% ash, 3.5% moisture and 5% lipids (Table 1). The yield of FPH was 66% on basis of solids and 74% on the basis of nitrogen. The elemental analysis profile revealed the presence of calcium, strontium and potassium as the major elements. Similar proximate composition was reported by Mackie (1982) in the case of FPH prepared from a number of fish varieties such as white fish, blue whiting, sprat & mackerel. He observed that the amino acid content, low ash content, high protein content and other factors such as ready dispersibility in water compared favourably with that of casein and suggested that FPH can be used as a substitute for milk protein. No colony forming unit was observed when 10% homogenate in saline was plated on plate count agar.

Despite being slightly deficient in tryptophan, fish protein hydrolysates in general have good nutritional value. The chain length of the peptide generated by the hydrolysis is crucial because the organoleptic and functional properties are generally governed by the length of the peptide. The formation of bitter peptides has been reported to occur during the uncontrolled or prolonged proteolysis of fish muscle (Kilara, 1995). Mohr (1977) has demonstrated a direct relationship between the chain length of the peptides and the time of hydrolysis. In common with all enzymatic hydrolyzates, FPHs have a bitter taste but are comparatively mild compared with those from casein (Clegg & McMillan, 1974). This taste can be reduced by controlling the degree of hydrolysis so that predominantly tasteless peptides of larger molecular weight are produced.

In the present work, papain at a concentration of 0.05% and a digestion time of 2 h at 55°C was found to be ideal for the production of dhoma hydrolyzate. Under ideal conditions 74% of the nitrogen was hydrolyzed by the enzyme. Further increase in the digestion time was observed to increase bitterness in the final product without significant increase in the degree of solubilization. When the enzyme concentration was increased to 0.075% there was no further increase in the solubilization. Bromelain and ficin under similar experimental conditions hydrolyzed the protein to the extent of 65 and 60 per cent, respectively. An enzyme concentration of 0.05% papain was found to be optimum in the case of blue whiting as well as cod FPH (Mackie, 1982). On the basis of feasibility study employing 20 different proteolytic enzymes for the production of fish hydrolyzate, Hale (1969) observed that pancreatin, pepsin and papain were the most suitable enzymes. Sen *et al.* (1962) defined digestion conditions using papain and found that at pH 7.0 maximum solubilization occurred in the first few hours of treatment. They have advocated 40°C instead of 65°C for obtaining longer peptides during digestion. Since plant enzymes possess broad specificity, they are preferred over animal proteases for the production of hydrolyzates of relatively low average molecular weight. Under optimum conditions of digestion the fish muscle homogenate was converted from a viscous mince to a free flowing liquid.

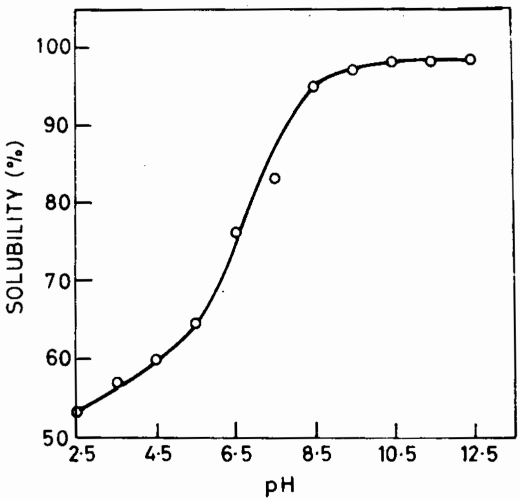

Fig. 1. Influence of pH on the solubilization of FPH

In contrast to Bombay duck fish protein concentrate which was found to be soluble in distilled water to the extent of 3% and 10% in hot distilled water at 60°C (Warrier & Ninjoor, 1981), Dhoma FPH was soluble to the extent of 75% in distilled water. The solubility pattern was further ascertained at different pH ranges varying from 2.5-12.5. The hydrolyzate could be completely solubilized by increasing the pH (Fig. 1). Although many of the protein products including fish protein concentrate are recognized as excellent sources of protein, their use as a food ingredient is limited because of poor functional properties including lack of solubility (Rasekh, 1974). Several approaches have been made in solubilizing the protein concentrate by treatments including alkaline hydrolysis and enzyme digestion (Tannenbaum *et al.*, 1970; Hevia *et al.*, 1976).

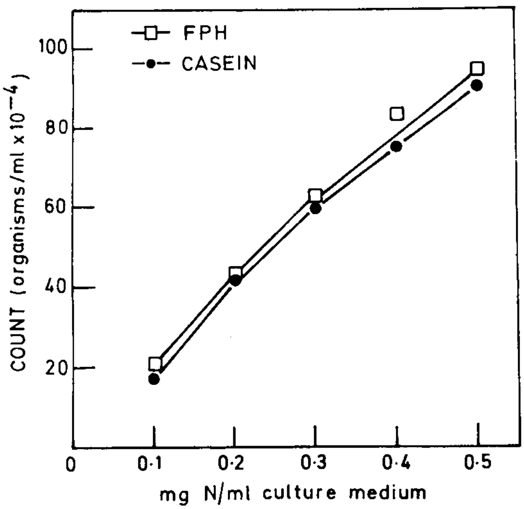

Fig. 2. Growth response of *Tetrahymena pyriformis* W with FPH and casein

When compared with defatted casein the wettability of FPH was found to be excellent. The water absorption ability (ml water g^{-1} protein) was found to be 3.0. Emulsifying capacity of FPH was 3.92 ml oil mg^{-1} protein, but decreased as the concentration of protein in solution increased. Similar results for the oil emulsifying efficiency were reported for different muscle proteins including those of fish protein (Cobb & Hyder, 1972). Nevertheless, it may be indicated that the emulsifying capacity of FPH is comparable with other modified fish proteins (Groninger, 1973; Venugopal *et al.*, 1994). These functional properties are of importance since these are inter related in the production of fabricated foods.

The growth response of *T. pyriformis* W with FPH and casein is shown in Fig. 2. It can be seem from the figure that the growth response of the organism with FPH was similar to casein. A wide range of protein sources has now been assayed with *T. pyriformis* W to determine the nutritive value with the simple criterion of organism count (Scott *et al.*, 1963). Based on the results of *T. pyriforms* W assay Scott *et al.* (1963) graded egg as the best protein followed by casein, soybean and fish protein. The number of organisms per ml of culture medium at 0.3 mg nitrogen ml^{-1} concentration obtained with FPH was comparable with the reported value for casein and soybean. Mackie (1982), from a series of feeding studies, has observed similar nutritive value of FPH and casein.

The FPH prepared with added NaTPP and H_2O_2 maintained white colour during 3 months of storage without vacuum packaging. The preparation was free from undesirable odour. The extent of lipid peroxidation as measured by thiobarbituric acid reacting substances was 38% less in vacuum packed samples than in air packed samples.

It is evident that the FPH prepared from dhoma is an ideal protein possessing good functional and nutritive properties. Dhoma FPH therefore, could serve as a potential protein ingredient in fortifying various conventional and novel foods.

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