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# FUNCTIONAL PROPERTIES OF ENZYMATICALLY MODIFIED PROTEINS FROM FISH WASTE

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**ABSTRACT:** Fish flour from dried waste consisting of head, tail, fins and entrails was enzymically hydrolysed using various proteases and the hydrolysate was spray dried. The functional properties such as water-fat absorption ratio, foaming and solubility index of the hydrolysates and fish flour revealed that some of the products might find significant uses in the food and/or cosmetics industry. Electrophoretic separation of the proteins from the fish flour and of the hydrolysates indicates that almost all the flour proteins are susceptible to proteolytic cleavage with the exception of one or two. The extent of degree of hydrolysis from 43-70.3% with a simultaneous decrease in unpleasant smell suggests an economical tool for minimizing odour pollution due to fish industry waste deterioration.

**KEY WORDS:** Functional properties - Fish waste flour - Enzyme - Electrophoresis.

## INTRODUCTION

Waste, whether solid, liquid or gaseous in form is an inevitable by product of many industries. For example, the growing cost of red and white meat during the last few decades has increased the consumption of raw and processed fish worldover, with the subsequent accumulation of fish wastes in bulk if this cannot be utilized. Fish processing industry wastes causes atmospheric pollution due to evolution of gases which are the secondary products of deterioration of solids (Willium and Litchfield, 1988). Litchfield (1983) has reviewed the subject in detail. The problem of odour pollution due to fish waste has been suppressed by passing the gaseous discharge from fish waste treatment plant through a moist activated column loaded with aerobic bacteria capable of digesting the compounds responsible for unpleasant smells.

Rossmore (1972) has suggested the biodegradation of solid fish waste through fermentation process to produce oil and protein of good quality. The nutritional value and organoleptic properties of ensiled seafood has also been evaluated after an appropriate fermentation period (Samuels, 1984).

Approximately 70% protein was isolated from fresh ground flounder frames using NaOH-HCl system as the extractant and precipitant respectively (Montecalvo *et al.*, 1984). Isoelectric precipitation at pH 5 was found more effective for protein recovery from alkaline solution containing impurities. The frame portion of solid fish waste has been used as the raw material for obtaining protein isolates which may then be modified enzymically (Montecalvo, 1983).

A milk substitute from solid fish waste (Pastoriza *et al.*, 1985) was prepared by mixing proteolytic hydrolysate (2.6%) with an equal amount of fat and 5.5% lactose in water. A small quantity of the stabilizer Sodium alginate (0.75%), was found suitable for emulsifying the protein.

A simple technique for animal feed production has been patented in Japan, (Sakyo,

1982), which involves $FeCl_3$ precipitation from aquous homogenized fish waste. The solids recovered were washed, dried and sold as additives for feed, specially as chick feed. The acid solubilized waste consisting 73.4% of crude protein, (dry weight basis) promoted chick growth and increased egg production by 130% if only 2.9% of liquified waste was supplemented in the basal diet of chicks (Tetsuo *et al.*, 1955, 56).

Composting sea waste such as fins, crab and shell fish with peat in a room having aereated static windows gave excellent end products with variable nutrient contents reflecting the diversity of the waste material used (Mathur *et al.*, 1988). The Cross Polarization Magic Angle Scanning (CPMAS) NMR were used to measure the degree of completion of the process (Preston *et al.*, 1986).

Recently Revina and co-workers (1990) have reported a methodology for the production of biologically active compounds such as enzymes and phospholipids from fish processing industry waste. The by products of the seafood manufacturing industry have previously been reported as capable of utilization (Takei, 1975).

The solid fish waste residue has long been used as a raw material for the industrial production of gelatin (Mirella, 1953). A fine powder, rich in nuclein and lecithin, from fish waste has found industrial application in manufacturing cosmetic ointments. Albumin dehydration products for use in damp-proofing mortar or adhesive have also been made from marine waste.

The present work deals with the protein isolation from fish waste and an exploration of selected functional properties of protein flour and it's hydrolysate is also studied to evaluate the effect of enzymic degradation of proteins.

## MATERIALS AND METHODS

The sea fish *Pomadasys olivaceum* (Haque *et al.* 1975), locally called "Dhoter" commonly available in coastal region of Pakistan (Fig.1), was freshly obtained from the market. The fish with a length of 51 cm and width of 15 cm weighes around 1.5 Kg. The proteolytic enzymes were obtained from Sigma and the solvents used were of A.R. grade supplied by Merck.

### FISH WASTE FLOUR:

The inedible waste portion consisting of fins, scales, entrails, head and tail was initially sun dried to a moisture content of 25%. The moisture was finally reduced to 10% in a fluidized bed drier. The dried waste was ground in a pin mill to a mesh size of 60 mm producing a fine flour grey in colour.

### ENZYMIC DEGRADATION:

The proteases bromelain, papain, trypsin and proteinase K (fungal origin) were selected for the present study in view of their similar optimum pH. A small amount of each of the enzymes (5 mg) was dissolved in 50 ml of 0.1 M phosphate buffer of pH 7.5 separately. A set of three flasks were taken for each enzymic digestion. Each of the flasks contained 10 g of the fish flour in 100 ml of the same buffer as mentioned above in addition to 5 ml of the enzyme solution. A set of controls of two flasks was also prepared without enzymes for comparison purposes. The flasks were incubated at $40^{\circ}$ C for 20 hours. The hydrolysate was centrifuged at 2000 g for 5 minutes to

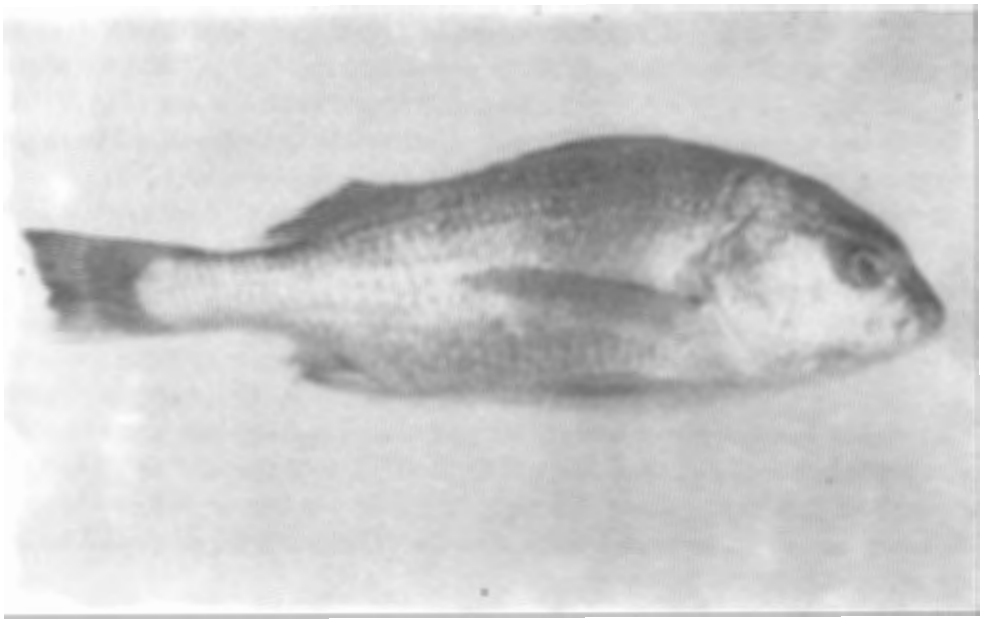


Fig.1. Marine fish *Pomadasys olivaceum*

separate insoluble residues. The supernatents were separately dried to fine powder using a spray drier while the residues were dried in an oven at 80° C to a moisture content of 5%.

**FUNCTIONAL PROPERTIES OF FLOUR PROTEINS:**

The functional properties of the flour proteins and hydrolysate were determined as described by Deeslie and Cheryan (1988).

**Protein Dispersibility Index:** A 10% dispersion of the sample in water was made and its pH was adjusted from 2 to 10 using 0.1 N HCl or 0.1 N NaOH. The sample was blended for 10 minutes and after holding for 15 minutes the solubility was measured in terms of total solids present at each pH in order to determine the optimum pH for solubility.

**Water Absorption (g/g):** Ten grams of each of the samples was dissolved in 100 ml of water with continuous stirring for 30 minutes. It was then centrifuged at 3000 g for 10 minutes and the amount of water retained after centrifugation was measured.

**Fat Absorption:** A sample of 4 g was added to 24 ml corn oil, stirred for 10 minutes and centrifuged in graduated tubes at 1600 g for 10 minutes. The oil layer which separates out was measured and the difference from oil gives the fat absorption by fish waste flour and its hydrolysate.

**Foaming Capacity:** Foaming capacity of each sample was estimated in the following manner. A 6% solution of each sample was prepared by stirring in a blender at 1600 rpm for 5 minutes. It was then pourd into a 100 ml measuring cylinder and total volume was recorded.

**Degree of hydrolysis (DH):** 0.5 g of the protein hydrolysates, obtained by treating with bromelain, papain and proteinase-K were dissolved in the same phosphate buffer to make 10 ml of solution (20% conc. w/v). To each test tube 5 ml of 10% trichloro-

acetic acid (TCA) was added and centrifuged and 3000 g for 10 minutes. The supernatent was further filtered through filter paper and the "$\lambda$max" was measured by spectrophotometer (UV-160 A, Shimadzu), and found to be 256 nm (Fig.2). At this wave length the absorbance of all the hydrolysates was determined. The degree of hydrolysis was calculated according to the method of Adler-Nissen (1976).

**Polyacrylamide gel electrophoresis (PAGE):** The fish waste flour protein and the protein hydrolysate were analysed by Sodium dodecyl sulphate (SDS) - PAGE according to the method of Weber and Osborn (1969) using 10% gels with 3% cross linking. The samples, 40 mg each, were taken in 3.8 ml of sample diluting buffer contained in centrifuge tubes. The mixtures were left overnight to allow maximum extraction of proteins and then centrifuged at 1600 g for ten minutes. A 50 $\mu$l sample of each protein hydrolysate and flour in triplicate with saturated sucrose and 1% bromophenol blue was loaded on top of the gel tubes. The proteins were resolved at 7.0 mA/gel for 2.5 hours. The gels were stained overnight in 0.25% Coomassie brilliant blue R-250 in 10% acetic acid in 1:1 methanol-water mixture. The gels were destained by heating in 7.5% acetic acid in 5% methanol.

## RESULTS AND DISCUSSION

The solid waste remaining after processing of marine fish *P. olivaceum* exceeds 20% of the fresh weight indicating the availability of bulk raw material for commercial utilization. The fish waste flour (FWF) with 5% moisture showed a significant reduction in its unpleasant smell and had an extended shelf life.

**ENZYMIC DEGRADATION:** The FWF, when hydrolysed with the proteolytic enzymes bromelain, proteinase-K, papain and trypsin shows that trypsin is the most effective in cleaving the peptide bonds producing 70.3% of soluble solids while bromelain and proteinase-K equally solublised the FWF. However, papain was next to trypsin in its ability to solublize proteins. The results presented in Fig.3 reveal that the ratio of insoluble to soluble protein was highest in the control (1.59) and decreased gradually from bromelain to trypsin treatment.

**FUNCTIONAL PROPERTIES:** The functional properties of FWF and its proteolytic hydrolysate are compared in Fig.4. The optimum pH for protein dispersibility had a narrow range between 6.9 for FW to 7.6 for FWF while emulsification or fat absorption was similar in all the cases. However, foaming capacity varies with enzymic modification and proteinase-K produced the hydrolysate of the highest foaming capacity of 0.6 compared with untreated waste having a value of 0.2 only. The bromelain treated sample was next to proteinase-K in merit of whipping ability.

The raw fresh waste has the highest water holding capacity of 4 and FWF had the least value of 1.5 for water absorption. The water holding capacity increased from 1.5 with enzymic treatment with bromelain being the most effective with a water abroption value of 3.2.

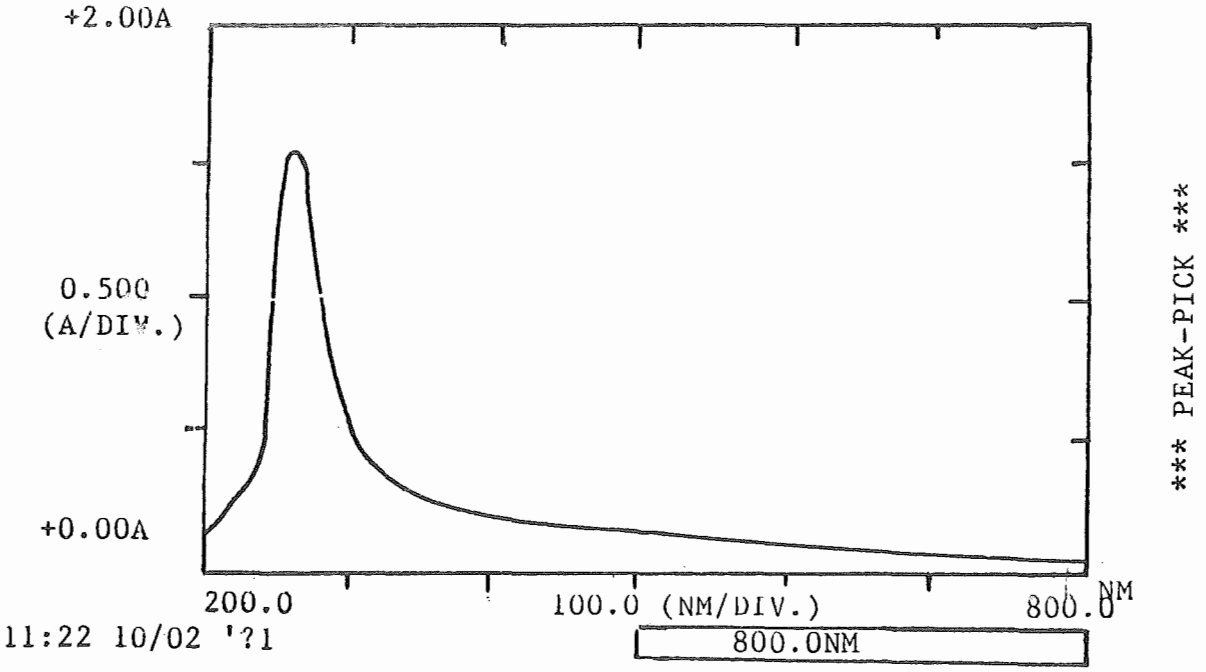



Fig.2. The max value of hydrolysate at 256 ml. NM

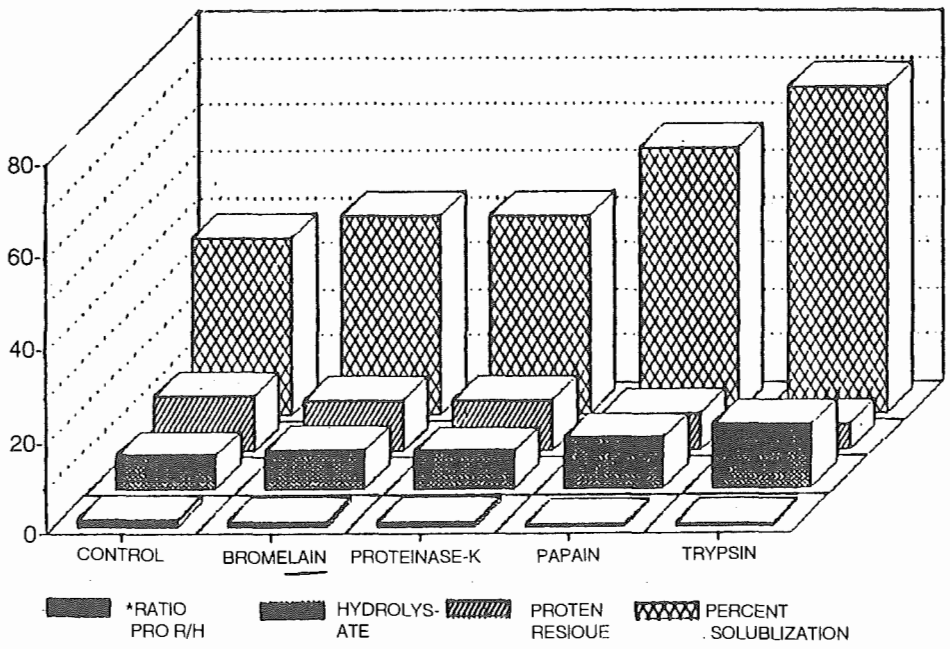



Fig.3. Comparison of effects of proteolytic enzymes on solubility.
(* Ratio of protein residue and hydrolysate).

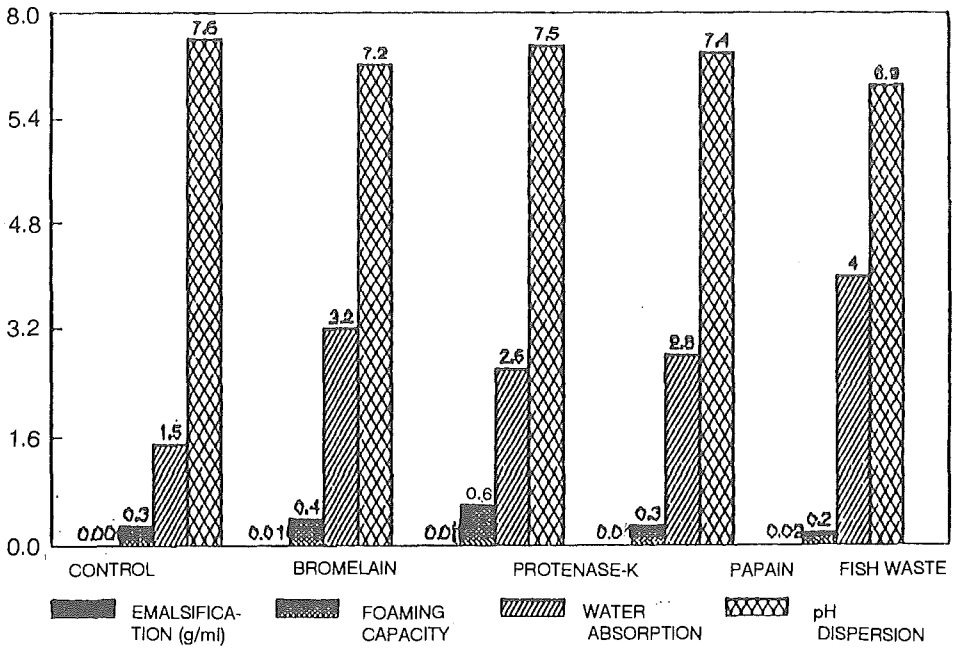



Fig.4. Functional properties of FWF and it's hydrolysates.

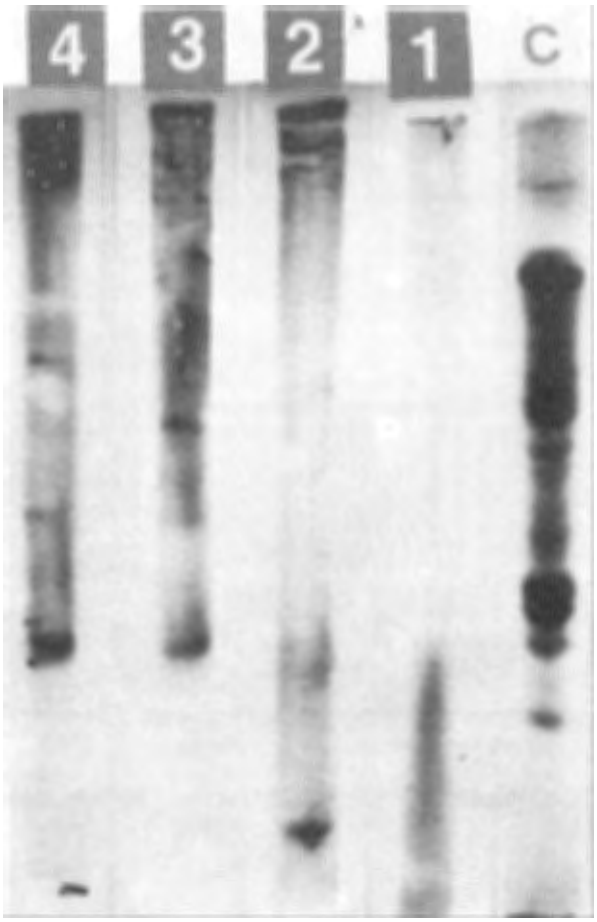



Fig.5. Comparison of electrophoretic mobility. C = untreated FWF, 1,2,3 and 4 = hydrolysates of bromelein, trypsin, proteinase K, and papain respectively.

**DEGREE OF HYDROLYSIS:** The DH for each enzyme treated sample against the control is given in Table 1. Proteinase-K treated sample with a DH value of 92.83 showed the highest level of cleaved peptide bonds.

**Table 1. Degree of hydrolysis (DH) by different proteases.**

| S.NO. | SAMPLE | ABSORBANCE (256 nm) | D.H. (%) |
|---|---|---|---|
| 1. | C. WASTE | 0.098 | - |
| 2. | BROMELAIN | 0.316 | 76.32 |
| 3. | PAPAIN | 0.718 | 87.32 |
| 4. | PROTEINASE-K (FUNGAL) | 1.269 | 92.83 |
| 5. | TRYPSIN | 0.142 | 59.16 |

$$\% \text{ D.H.} = \frac{\text{Absorbance of Sample}}{(\text{Absorbance of Sample} + \text{Absorbance of Control})} \times 100$$

**ELECTROPHORETIC PATTERN:** The electrophoresis of FWF untreated (control) produced atleast 15 protein bands against the hydrolysates of various proteases (Fig.5). The results reveal the effective modification of nearly all the proteins by respective proteases indicating that a mixture of selective proteases may be used to solubilize FWF proteins. The hydrolysates with soluble proteins are less odiferous and posess flat taste and may be used as additives in food or feed for supplementation of amino acids. Similarly, improvement in whipping property by bromelain and proteinase-K projects its use in cosmetic industry for use in shampoo and shaving creams.

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