Studies on the Preparation of Functional Fish Protein Concentrate from *Nemipterus japonicus* by Enzymatic Method

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The study was carried out to develop a suitable method for the preparation of functional fish protein concentrate (FFPC) from the underutilized fish, Japanese threadfin bream (*Nemipterus japonicus*) and to evaluate their functional properties, sensory and chemical characteristics. The FFPCs were prepared employing proteolytic enzymes to partially hydrolyse proteins and then dried in vacuum as well as in a spray drier. The FFPCs obtained by these processes exhibited better functional and sensory properties when compared to conventionally prepared fish protein concentrate.

Development of fish protein concentrate(FPC) represents the first concerted effort to increase the use and the value of underutilized fish by converting it into a more readily acceptable form. However, FPC produced by these technologies was deficient in some of the functional properties and their cost of production was high. Recognising this problem several investigations have aimed at improving the functional properties of FPC by rationally modifying the parameters of extraction and by employing enzymes to partially hydrolyse proteins (Sripathi, 1975; Sikorski, 1981).

The present investigation has been directed towards studying processing techniques that could form the basis for the preparation of a functional fish protein concentrate (FFPC) using enzymes and to evaluate their functional and other properties. Another objective was to develop a commercially feasible processing method for production of FFPC from low value fish, which can be used as a food ingredient, emulsifier, fortifier for bakery products and beverages and milk replacer in animal ration.

Materials and Methods

Fresh threadfin bream (*Nemipterus japonicus*) were obtained from the fish landing centre, Mangalore and were washed and iced immediately for the preparation of FFPC. The fish was dressed, washed thoroughly and the meat was separated using a meat picking machine. The minced fish was washed in chilled water 4-5 times and after separating free water by a manual screw press, minced in a meat mincer. It was then mixed with 0.5% sucrose and 0.1% sodium tripolyphosphate in a silent cutter for 15 min, maintaining a low temperature. Part of it was mixed with 0.5% papain for one hour at room temperature, then dried in a vacuum drier (25" Hg) at 65°C for 16 h, powdered to fine texture and packed in bottles. Another part was treated with 0.25% papain and a third part with 0.5% papain for one hour at 55°C. They were then heated to 80-90°C for 5 min, the slurry filtered through a nylon filter cloth and dried in a spray drier (Niro atomiser, Denmark) where the inlet temperature was maintained at 200-250°C and outlet at 90-110°C with a compressor pressure of 5kg/cm^2.

Proximate composition of raw material, mixed meat and FFPC were determined according to AOAC (1975) and the values were expressed on wet weight basis. The sugar content of the product was analysed according to the method of Munson-Walker

(AOAC, 1975), while the inorganic phosphorus was analysed as per the method of Fiske & Subbarow (1972).

Functional properties such as protein solubility (Warrier & Ninjoor, 1981) in different solvents such as distilled water, hot distilled water (80°C), 5% NaCl, 0.2 N NaOH and 1.0 N NaOH solutions, emulsifying capacity (Sosulski, 1962), fat absorption capacity (Sosulski *et al.*, 1976), bulk density (Thompson & Cho, 1984) wettability and relative viscosity (Spinelli *et al.*, 1973) were also analysed.

The analysis for sensory characteristics such as colour, flavour, odour and taste was carried out by experienced panelists.

Results and Discussion

The proximate composition of the raw meat, mixed meat and different types of FFPC is shown in Table 1. The mixed meat had slightly higher moisture content and lower fat content than raw meat. These were due to washing of the picked meat. The higher values of ash content in mixed meat might be due to the added phosphate, while the reduction in protein content was presumably due to loss of soluble protein during washing. The proximate composition of the final product almost correlated with the results obtained by Hiroshiniki *et al* . (1982).

The present investigation showed higher solubility for enzyme treated products, when compared to the products prepared without enzyme treatment (Mahesh, 1986). This may be due to either the enzyme treatment which altered the properties of fish protein or the prevention of protein denaturation by added sugar. The development of such enzyme processes have been favoured because the end product properties, such as solubility, make it more suitable for certain applications than the solvent extracted FPCs (Sripathi, 1975).

The emulsifying capacities of the FFPCs prepared in this investigation (Table 2) compare fairly well with that of active fish protein powder prepared by Hiroshiniki *et al.* (1983) but were much higher than those of oven dried and vacuum dried FFPCs prepared without enzyme treatment (Mahesh, 1986). The products prepared by Spinelli *et al.* (1973) had good emulsifying capacity.

The water holding capacities of the different types of FFPC (Table 2) were found to be considerably less than those reported

Table 1. *Proximate composition of raw meat, mixed meat and FFPC prepared under different processing methods*

Processing methods	Processing stage	Parameters Moisture %	Protein %	Fat %	Ash %	Sugar %	Inorganic phosphorus, %
Enzyme treated	Raw meat	77.9	18.3	2.0	1.4	-	-
and vacuum dried	Mixed meat	78.9	12.2	1.4	2.2	-	
FFPC	Dried FFPC	5.8	66.3	2.8	1.8	22.9	0.3
Enzyme treated	Raw meat	77.8	18.4	2.0	1.4	-	-
(0.25%)and spray	Mixed meat	79.5	12.5	1.3	1.8	-	-
dried FFPC	Dried FFPC	3.2	65.9	2.6	2.2	22.6	0.3-
Enzyme treated	Raw meat	77.8	18.4	2.0	1.4	-	-
(0.5%) and spray	Mixed meat	79.6	12.4	1.3	1.9	-	-
dried FFPC	Dried FFPC	3.4	65.5	2.7	2.4	22.7	0.3

Table 2. *Functional properties of FFPC prepared under different processing conditions*

Sl. No.	Functional properties	Enzyme treated and vacuum dried FFPC	Enzyme treated (0.25%) & spray dried FFPC	Enzyme treated (0.5%) & spray dried FFPC
I	Protein solubility (%) in			
	i) Distilled water	47.76	62.50	60.27
	ii) Hot distilled water (80°C)	51.86	65.15	63.90
	iii) 5% NaCL	77.61	82.57	81.20
	iv) 0.2 N NaOH	100.00	100.00	100.00
	v) 1.0 N NaOH	Gel	Gel	Gel
II	Emulsifying Capacity (ml oil/g FFPC)	120.00	140.00	138.00
III	Water holding capacity, (%)	232.50	252.40	278.20
IV	Fat absorption capacity(ml/100 g FFPC)	193.30	213.40	206.60
V	Bulk density (g/ml)	0.80	0.43	0.45
VI	Wettability	Excellent	Excellent	Excellent
VII	Relative Viscosity (in centipoises) at different concentration of FFPC (%)			
	i) 0.5	114.00	116.00	116.00
	ii) 1.0	116.00	118.00	118.00
	iii) 1.5	118.00	121.00	121.00
	iv) 2.0	120.00	123.00	123.00
	v) 2.5	123.00	125.00	125.00
	vi) 3.0	125.00	127.00	127.00

for modified fish proteins (Miller & Groninger, 1976; Suzuki, 1981). The fat absorption capacity of different products were in the range of 193.2 to 213.4 ml/100 g (Table 2), which were found to be better than those obtained for FFPCs prepared without enzyme treatment (Mahesh, 1986). The bulk densities of spray dried products were found to be much lower than that of vacuum dried products(Table 2). Unlike the solvent extracted FPC (Dubrow et al., 1973), all the three types of FFPC prepared in the present study were wettable and the wettability could be judged as excellent (Table 2).

Viscosity changes are used to evaluate the thickening power of proteins, a property of practical interest in fluid foods such as soups, beverages and batters. The relative viscosity of all types of FFPC prepared under different conditions has been found to increase with the increased concentrations of FFPC (Table 2). However, high values going upto 5000 centipoises at 2% concentration have been reported for succinylated fish protein by Spinelli *et al* (1973).

In the present study, the colour of FFPC dried under vacuum was light brown, while that of spray dried products were creamy white (Table 3). The colour of the concentrated fish protein products depends on the species of fish, the parts of their body used as raw material and the parameters of extraction and drying.

Most of the published literature on the sensory quality of concentrated fish protein products are concerned with the presence of odour and flavour compounds and the influence of the quality and quantity of residual lipids on odour reversion during

Table 3. *Sensory quality characteristics of FFPC prepared under different processing conditions*

Sensory characteristics	Enzyme treated and vacuum dried FFPC	Enzyme treated (0.25%) and spray dried FFPC	Enzyme treated (0.5%) and spray dried FFPC
Colour	Light brown	Cream white	Cream white
Flavour	Mild sweety flavour	Mild sweety flavour	Mild sweety flavour
Odour	No fishy odour	No fishy odour	No fishy odour
Taste	Sweetish and slightly bitter after taste	Sweetish and slightly bitter after taste	Sweetish and slightly bitter after taste

storage (Sikorski, 1981). All the three types of FFPCs prepared in the present investigation had mild sweety flavour and there was no fishy odour (Table 3) immediately after their preparation. Whether odour reversion takes place during storage cannot be predicted and needs further study.

The tastes of the products prepared in the present study were sweetish(Table 3) due to the sugar added during processing and had slightly bitter after-taste. The bitterness may be due to peptides formed during the partial hydrolysis of the proteins before vacuum drying or spray drying.

The yield of different FFPCs varied with the process selected. It was around 13 to 15% of picked meat and around 5% of the raw material. Similar results have been reported by Setty *et al.* (1977) for partially hydrolysed and deodourised fish flour and Mahesh (1986) for FFPC.

The products of the present study, at the best, may be used for partial replacement of fish meat in the preparation of fish sausages. Also these may be used for developing the products like milk replacers and for fortification of various types of snacks, bread and biscuits. Compared to other conventional FPCs and solvent extracted FPCs, these products score over them, since the undesirable solvent residues in the final product is eliminated, apart from making the process much simpler.

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