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Protein hydrolysates from meriga (*Cirrhinus mrigala*) egg and evaluation of their functional properties

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ABSTRACT

Protein hydrolysates from underutilised meriga (*Cirrhinus mrigala*) fish egg were prepared by using commercial Alcalase and papain enzymes. The degree of hydrolysis was 62% for Alcalase and 17.1% for papain, after 90 min digestion at 50–55 and 60–65 °C, respectively. The protein content of Alcalase-produced hydrolysate was higher (85%) than that of papain hydrolysate (70%) ($p < 0.05$). Hydrolysis by both enzymes increased protein solubility of fish egg protein hydrolysates to above 72.4% over a wide pH range (2–12). Results showed that the hydrolysates had good fat absorption capacity (0.9 and 1.0 g/g sample), foam capacity (70% and 25%) and emulsifying capacity (4.25 and 5.98 ml/g hydrolysate), respectively for Alcalase and papain protein hydrolysates. Gel filtration chromatograms and SDS–PAGE analysis indicated the distribution of smaller peptides. These results suggested that fish egg protein hydrolysates could be useful in the food industry.

1. Introduction

Large quantities of protein-rich fish processing by-products are discarded as waste, without any attempt to recover the essential nutrients. This would pose serious disposal and pollution problems, especially in developing countries. By developing enzyme technologies for protein recovery and modification, production of valuable food ingredients and industrial products will be possible.

Enzymatic hydrolysis of fish proteins has been employed primarily as an alternative approach for converting underutilised fish biomass, which is commonly used in making feed or even fertiliser into edible protein products. Use of proteolytic enzymes is often an attractive means for improving functional properties of food proteins, without losing their nutritional value. Hydrolysates produced by enzymatic treatment contain well defined peptide profiles and an extensive review exists on the application of enzymatic protein hydrolysates in human nutrition (Clemente, 2000). The hydrolytic process and reaction conditions differ for different substrates and enzymes, which also depend on the properties desired for the hydrolysates.

Several underutilised aquatic protein sources (processing wastes) have been investigated for the production of functional protein hydrolysates; these include *Clupea harengus* (Hoyle & Merritt, 1994; Sathivel et al., 2003), *Selaroides leptolepis* (Klompong, Benjal, Kantachota, & Shahidi, 2007), *Mallotus villosus* (Shahidi, Han, & Synowiecki, 1995), and *Merluccius productus* (Benjakul & Morrissey, 1997). Fish protein hydrolysates have been well studied and reported in terms of their production, biochemical, and functional properties (Kristinsson & Rasco, 2000). Enzymatic hydrolysis of food proteins is an efficient way to recover potential bioactive peptides. Fish protein hydrolysates have been shown to have potential for nutritional or pharmaceutical applications (Wergedahl et al., 2004).

One of the most efficient means of increasing protein solubility as well as improving the functional properties of fish proteins is to subject them to enzymatic hydrolysis. A variety of commercial enzymes have been tested successfully for hydrolysing fish and other food proteins. Proteolytic enzymes from microorganisms and plants are most suitable for preparing fish protein hydrolysates. Alcalase, an alkaline enzyme produced from *Bacillus licheniformis*, and papain from a plant source (*Carica papaya)* have been found to be the best enzymes for the preparation of functional fish protein hydrolysates (FPHs) by many researchers (Benjakul & Morrissey, 1997; Shahidi et al., 1995; Sugiyama, Mukoto, Onzuku, & Oba, 1991).

The meriga carp (*Cirrhinus mrigala*) is an herbivorous freshwater fish and is the most widely farmed species among the major Indian carps; it is also common in Bangladesh and Pakistan. It is an important component of carp polyculture throughout South Asia. The fecundity of meriga ranges from 100,000 to 200,000 eggs/kg body weight (Jhingran, 1982). However, human consumption of these eggs is still limited. Therefore, the production of value-added products, such as protein hydrolysates with good nutritive value as well as good functional properties, can pave the way for complete utilisation of this species. Lot of work has been carried out in the field

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of fish protein products, fish protein hydrolysates and their functional properties (Thiansilakul, Benjakul, & Shahidi, 2007; Wasswa, Tang, Gu, & Yuan, 2007). However, the studies pertaining to meriga egg protein hydrolysates have been scarce. Hence, the present study investigated the preparation of vacuum-dried meriga egg protein hydrolysates using two proteases, namely Alcalase and papain.

The objectives of this investigation were to study the proximate composition, functional properties and molecular weight distribution of fish egg protein hydrolysates prepared by hydrolysing fish eggs of *meriga* (*C. mrigala)* using Alcalase and papain as proteolytic enzymes.

2. Materials and methods

2.1. Materials

Fresh *meriga* carp roes were obtained from a local fish market (Hyderabad, India) immediately after processing and brought to the laboratory, stored at 4 °C for <2 h before experimental work. Roes were separated from blood vessels and skeins, and homogenised using a high speed mixer to give fish egg mince. The egg mass was dried at 48 ± 2 °C for 8 h in a cabinet tray dryer (Chemida, Mumbai), ground to powder using a high speed mixer and sieved to pass through a 180 μm mesh to give fish egg meal. It was packed in polyethylene pouches and kept at 4 °C until used for experimental work. Enzymes used were Alcalase 2.4 L (*B. licheniformis*) from Novo Laboratories, Copenhagen, Denmark, and papain (6000 NF units) a food grade proteolytic preparation isolated from the latex of *C. papaya* (Loba Chemie Limited, Mumbai, India). These enzymes were chosen because of their availability and their apparent suitability for industrial use. The chemicals and solvents used in the present study were of analytical grade and procured from S.D. Fine-Chem Ltd. (Mumbai, India).

2.2. Preparation of fish egg protein hydrolysates (FEPHs)

Fish egg meal (13 g) was suspended in 200 ml of distilled water. The hydrolysis conditions were maintained according to the method of Hoyle and Merritt (1994). The mixture was adjusted to the optimum pH for enzyme activity (8.0–8.5 for Alcalase and 6.0–6.5 for papain). The mixtures were pre-incubated at 50–55 °C or 60–65 °C for 10 min prior to enzymatic hydrolysis using Alcalase or papain, respectively. Enzyme blanks and enzymes inactivated prior to hydrolysis were kept as controls for both Alcalase and papain. The protein hydrolysis reaction was initiated by the addition of the enzyme (Alcalase or papain) at a level of 0.5% (w/w) of the protein content in the fish egg meal. The enzymatic reaction was carried out for 90 min with continuous stirring by maintaining optimum pH and temperature (pH 8.0–8.5, 50–55 °C for Alcalase and pH 6.0–6.5, 60–65 °C for papain) for enzyme activity. The enzyme activity was terminated by keeping the mixture in a water bath at 85 °C for 20 min for Alcalase, or at 90 °C for 30 min for papain. When the mixture was cooled down, it was centrifuged at 4500 *g* for 30 min (4 °C) and the soluble aqueous fraction decanted, vacuum dried, sealed in vacuum bags and stored at 4 °C until further experiments. A flow chart for the production of fish egg protein hydrolysates (FEPHs) is shown in Fig. 1.

2.3. Average yield, proximate composition and colour

Average yields of FEPHs were calculated by determining the protein percentage of vacuum-dried products as a percentage of total fish egg protein when used as control. FEPHs were analysed for moisture, fat, protein and ash using standard procedures (AOAC, 1995). Colour of vacuum-dried FEPHs was determined by using a Lovibond tintometer (Model F, Salisbury, UK).

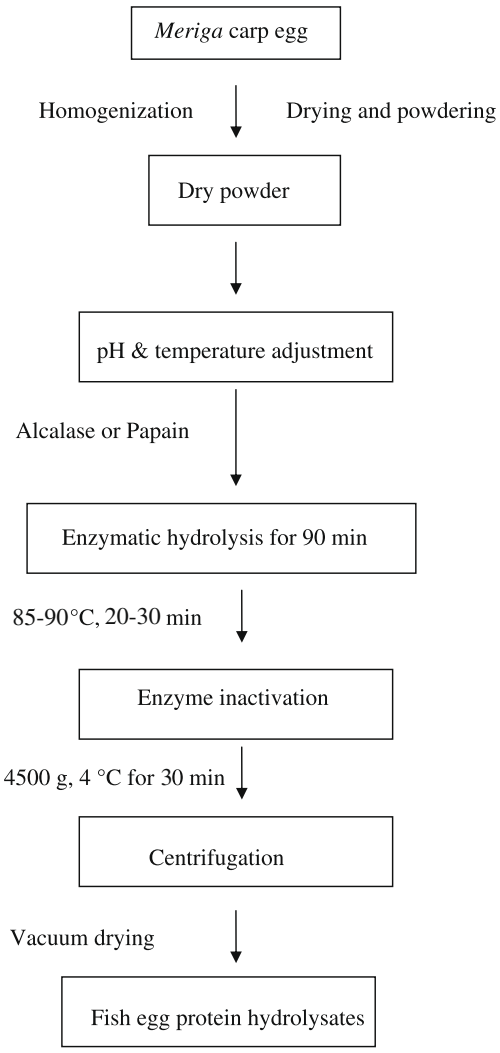

Fig. 1. Flow chart for the production of fish egg protein hydrolysates.

2.4. Degree of hydrolysis (DH)

The effect of hydrolysis time on DH was monitored using the method of Hoyle and Merritt (1994), with slight modification. Water (20 ml) was added to 1 g fish egg meal (protein basis), and the mixture was adjusted to optimum pH (8.0–8.5 for Alcalase and 6.0–6.5 for papain) and temperature (50–55 °C for Alcalase or 60–65 °C for papain). The enzyme was added to the minced protein at 0.5% w/w. At the end of each hydrolysis time of 0, 15, 30, 45, 60, 75 and 90 min, an aliquot (20 ml) was taken and mixed with 20 ml of 20% trichloroacetic acid (TCA) and then centrifuged at 4500*g* for 30 min at 4 °C. The supernatant was decanted and analysed for nitrogen by the micro-Kjeldahl method (AOAC, 1995). The degree of hydrolysis (%) was calculated as:

$$\text{DH } (\%) = \frac{10\%\ \text{TCA-soluble nitrogen in sample}}{\text{total nitrogen in sample}} \times 100$$

2.5. Protein solubility

Protein solubility of FEPHs was determined by the method of Klompong et al. (2007). FEPHs (200 mg) were taken in 20 ml of dis-