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Physico-chemical, amino acid composition, functional and antioxidant properties of roe protein concentrates obtained from *Channa striatus* and *Lates calcarifer*

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ABSTRACT

Roe protein concentrates prepared from *Channa striatus* (CRPC) and *Lates calcarifer* (LRPC) were investigated for physico-chemical characteristics, amino acid composition, functional properties and antioxidant activity. Channa and Lates roes yielded 20.7% and 22.5% of protein concentrates possessing 90.2% and 82.5% protein, respectively. Major differences were not observed in each of the amino acids except leucine in CRPC and LRPC. The solubility of protein was 3.93–54.6% and 1.6–55.5% over a pH range of 2–12 in CRPC and LRPC, respectively. Water absorption, oil absorption, foam capacity, stability and emulsifying capacity were found to be higher in CRPC than in LRPC. Antioxidant activity determined by the radical scavenging activity and ferric reducing power was higher in CRPC. SDS–PAGE of both roe protein concentrates showed protein bands of 170, 95 and 55 kDa. Moisture sorption isotherms of protein concentrates indicated their hygroscopic nature.

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1. Introduction

Proteins possessing functional characteristics and antioxidant activity have immense importance in the food processing industry. During processing of fish, a variety of fish by-products such as scales, head, viscera and roes are released. By-products utilisation will improve the economic aspects of processing industry and further their nutritional beneficiation through valuable essential amino acids and fatty acid components. Majority of fisheries by-products are presently utilised to produce fish oil, fishmeal, fertilizers, pet food and fish feed (Dong & Bechtel, 2010). Marine byproducts were reported to be good sources of nutraceuticals and functional food ingredients (Barrow & Shahidi, 2007).

Literature on chemical and functional characteristics of fish and protein utilisation from its by-products is available, however data on characterisation of the roe protein concentrates is limited. Balaswamy, Prabhakara Rao, Narsing Rao, Rao, and Jyothirmayi (2009) studied functional properties of roes from fresh water fish species such as catla, carp, rohu and murrel, and their application in bakery and traditional foods. They reported that roes contain protein in the range of 16.6–28.2%. Sathivel, Yin, Bechtel, and King (2009) prepared roe protein powder (67% protein) from catfish with four major bands between molecular weights of 40 and 100 kDa.

Balaswamy, Jyothirmayi, and Rao (2007) prepared protein concentrate (78% protein) from the roes of rohu (*Labeo rohita*) and examined its sorption behaviour. The studies revealed that the protein concentrate was non-hygroscopic in nature and stable in polyethylene pouches at room temperature.

Antioxidant activity of fish protein derivatives such as amino acids and peptides are reported in the literature. Antioxidant activities of peptides from Alaska Pollack were reported (Je, Park, & Kim, 2005; Kim et al., 2001). However, all amino acids have been shown to have antioxidant activity in some systems, which probably reflect the antioxidant nature of the amino group (Taylor & Richardson, 1980). The use of a protein or a hydrolysate for the improvement of the antioxidative activity in functional foods might be a more practical approach than the use of individual amino acids, because proteins and hydrolysates have some desired functional properties. The antioxidative effect of peptides derived from the enzymatic hydrolysates of capelin proteins was described by Amarowicz and Shahidi (1997).

India produces 6.57 MMT of fish annually out of which 55% is from fresh water (FAO, 2008). Fish roes constitute about 25–30% of the weight of fish during the spawning seasons. Fish roe is one of the important by-products of fish processing industry, which possesses good quantity of essential amino acids and fatty acids (Bechtel, Chantarachoti, Oliveira & Sathivel, 2007). In India, the roes of fresh water or marine sources are the most underutilised fish by-products, which have considerable scope for value-addition to produce food and feed. Roes are highly perishable with short shelf-life and hence, the roes should be processed

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immediately or converted into value added foods to enhance their shelf-life. Waste generated from fish processing results in odour problems, reduction in dissolved in oxygen in water bodies and turbidity (Clarke, 1997). By-product utilisation will help to reduce the by-product wastage and environmental pollution. Though literature on antioxidant activity of fish protein concentrates are abundant, antioxidant activity of roe protein concentrates is not reported earlier. *Channa striatus* is a fresh water fish, known for its specific taste, soft texture and higher lipid content. The fish is locally called murrel fish and sold live in Indian markets at premium prices. In India *C. striatus* is available in large quantities through out the year (58,365 tonnes) either from natural resources or as cultured species. The fishlings of this species is also used in administering traditional medicine for patients suffering from pulmonary diseases in Andhra Pradesh, India. *Lates calcarifer* is a medium sized marine fish some times observed in estuarine waters near the sea. The fish is popular with people living near the coastal regions and central plains and 70,162 tonnes were produced during 2006–07 (Anonymous, 2007). Literature on roes of these fishes is very limited and hence, the present investigation was undertaken to evaluate physico-chemical, amino acid compositions, functional and antioxidant properties of roe protein concentrates prepared from *C. striatus* and *L. calcarifer*. Studies were also conducted to determine sorption characteristics of roe protein concentrates in turn to ascertain their storage behaviour and packaging requirements.

2. Materials and methods

2.1. Materials

Fresh fish roes (750 and 640 g) were collected from live fishes viz., Channa (10 Nos.) and Lates (7 Nos.) at a fish market in Hyderabad, India. Chemicals and solvents used in the study were of analytical grade and were procured from Sd Fine-Chem Ltd. (Mumbai, India). Standard pre-stained protein molecular marker ranging from 10 to 170 kDa was procured from M/s. Peqlab Ltd., Salisbury, UK.

2.2. Preparation of roe protein concentrates (RPCs)

Fresh roes from *Channa* and *Lates* were separated manually from skins and blood vessels. The roes were homogenised using a high speed mixer (Sumeet, Nasik, India) and dried at 45 ± 2 °C for 10 h in a cabinet tray dryer (Chemida, Mumbai, India). The dried roes were powdered and defatted using isopropanol maintaining a solid to solvent ratio of 1:3 (w/v) at room temperature (RT) 28 ± 2 °C for 2 h with occasional stirring (Sikorski & Naczk, 1981). The solvent was decanted and the extraction was repeated for three times for maximum removal of lipid. The residue was dried in a vacuum dryer at 45 ± 2 °C for a period of 8 h. Defatted and dried roes were ground to powder using a mixer and passed through 180 μ mesh to obtain roe protein concentrate of *C. striatus* (CRPC) and *L. calcarifer* (LRPC). The roe protein concentrates were prepared in duplicate, packed in metallized polyester polyethylene (MPE) laminate pouches and kept at 4 °C.

2.3. Chemical composition, colour units and bulk density

Roe protein concentrates were analysed for physico-chemical characteristics using standard methods (Ranganna, 1986). Colour measurement of roe protein concentrates was conducted using a Lovibond Tintometer (Model F, Salisbury, UK). The bulk density of fish roe protein concentrates was measured by noting the volume occupied by 20 g RPC in a 100 ml graduated cylinder.

2.4. Amino acid composition

The amino acid content of CRPC and LRPC was determined by hydrolysing the protein (5 mg) in 6 M HCl for 24 h at 110 ± 2 °C. In an air oven (Dalal, Mumbai, India). The contents were made up to 25 ml and an aliquot of 20 μl was injected into an automatic amino acid analyser (Biochrom 30, England). Amino acids were detected after post-column derivatization with Ninhydrin reagent. Eluates were spectrophotometrically monitored at 570 nm and the concentrations of the unknown samples were determined by comparing with peak areas of standards (Agilent amino acid standard kit, Palo Alto, CA, USA). Cysteine and methionine contents in CRPC and LRPC were determined after converting them into cysteic acid and methionine sulphone respectively (Moore, 1963).

2.5. Water absorption capacity

The water absorption capacity (WAC) of RPCs was measured following the method of Shahidi, Han, and Synowiecki (1995) with a minor modification. One gram RPC was taken into a 50 ml centrifuge tube and 10 ml water was added. The mixture was thoroughly vortexed for 10 min at 26 °C and centrifuged at 1431g for 20 min at room temperature. The water absorbed by the sample was determined from the difference in weights and expressed as grams of water absorbed per 100 g RPC.

2.6. Fat absorption capacity

The fat absorption capacity (FAC) of RPCs was measured according to the method of Shahidi et al. (1995) with a minor modification using one gram RPC in 10 ml sunflower oil. The fat absorbed by the sample was determined and expressed as grams of fat absorbed per 100 g RPC.

2.7. Emulsification capacity (EC)

Emulsification capacity was determined by following a reported method (Gagne & Adambounou, 1994) with minor modification. The method involves dispersion of 1 g RPC in 25 ml of distilled water, stirring and gradual addition of sunflower oil until separation of oil layer was observed. EC is expressed as millilitres of oil emulsified per gram of RPC.

2.8. Foam measurements

Foam capacity (FC) and foam stability (FS) of roe protein concentrates were measured by a reported method (Lawhon, Carter, & Matil, 1972). One gram of RPC was dispersed in 100 ml distilled water. The contents were thoroughly stirred and the volume of foam generated was recorded after 1 min and reported as foam capacity. Volume of foam recorded after time intervals of 15, 30, 45, 60, 75 and 90 min was expressed as percentage foam stability at respective time intervals.

2.9. Protein solubility

Protein solubility of roe protein concentrates were determined according to the method reported by Klompong, Benjakul, Kantachota, and Shahidi (2007) by dispersing 1 g of protein concentrate in 40 ml distilled water and adjusting to pH values of 2, 4, 6, 8, 10 and 12 with 0.5 M HCl or NaOH. The mixture was stirred at room temperature (28 ± 2 °C) for 30 min and centrifuged at 1431g for 20 min. Protein content in the supernatant was determined by Biuret method (Sadasivam & Manickam, 1997). Percentage protein solubility was calculated on the basis of protein content present in RPC.

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