Physico-Chemical, Functional and Antioxidant Properties of Roe Protein Concentrates from *Cyprinus carpio* and *Epinephelus tauvina*

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**ARTICLE INFO**
- Received 12 December 2012
- Revised form 30 January 2013
- Accepted 31 January 2013
- Available online 12 May 2013

**ABSTRACT**
Roe protein concentrates prepared from *Cyprinus carpio* (CRPC) and *Epinephelus tauvina* (ERPC) were investigated for physico-chemical characteristics, functional properties and antioxidant activity. *Cyprinus* and *Epinephelus* roes yielded 19.5% and 21.5% of protein concentrates possessing 70.71% and 85.9% protein, respectively. Moisture sorption isotherms of roe protein concentrates indicated the non-hygroscopic nature of CRPC with initial moisture content (IMC) of 8%, which equilibrated at 63% RH and hygroscopic nature of ERPC with IMC of 4.9%, which equilibrated at 42% RH. Water absorption capacity, oil absorption capacity, foam capacity and lower foam stability and emulsifying capacity were found in ERPC than in CRPC respectively. Maximum solubility of protein was 17.79% and 16.89% at pH 12, observed in CRPC and ERPC respectively. Higher buffer capacity was observed in both roe protein concentrates in alkali medium. Antioxidant activity determined by the DPPH radical scavenging activity was higher, and ferric reducing power was observed to be lower in ERPC.

**Keywords**: Roe protein concentrates, *Cyprinus carpio*, *Epinephelus tauvina*, physico-chemical properties, Equilibrium moisture content, Antioxidant properties

1. **Introduction**
Although most protein concentrates and isolates have been traditionally prepared from oilseeds, animal and fish proteins have also been used in the food industry because of their functional characteristics. India produces 6.57 MMT (Million metric tons) of fish annually, out of which 55% is from freshwater (FAO, 2008). Fish processing yields a wide variety of fish by-products such as scales, head, skin, fat, viscera and roes in large quantities. Most of these by-products are discarded as waste, without processing them into value-added products, either for industrial applications or for human consumption. In addition, indiscriminate disposal of fish by-products is a serious cause of environmental pollution. Among fish by-products, roes are highly perishable in nature, nutritious material rich in essential minerals, amino acids and fatty acids. Majority of fisheries by-products are presently utilized to produce fish oil, fishmeal, fertilizers, pet food and fish feed (Bechtel, Chantarachoti and Sathivel, 2007; Dong and Bechtel, 2010). By-products utilization will improve the economic aspects of processing industry, and further their nutritional beneficiation through valuable essential minerals, amino acids and fatty acid components. Particularly, by-products with high protein content, suitable functional characteristics and specially, antioxidant activity has immense importance in the food processing industry. Marine by-products were reported to be good sources of nutraceuticals and functional food ingredients (Barrow and Shahidi, 2007).

Among these by-products, roes are rich source of essential minerals, amino acids and fatty acids. Fish roes constitute about 25-30% of the weight of fish during the...
spawning seasons (Balaswamy et al., 2007; Narsing Rao et al., 2012). In India, the roes of fresh water or marine sources are the most underutilized 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 immediately or converted into value added foods to enhance their shelf-life. Waste generated from fish processing resulted in odor problems, reduction in oxygen in water bodies and turbidity (Clark, 1997).

Literature on chemical and functional characteristics of fish and protein utilization from its by-products is available, however data on chemical characterization and antioxidant activity of the roe protein concentrates are limited (Taylor and Richardson, 1980; Amarowicz and Shahidi, 1997; Kim et al., 2001; Je et al., 2005; Balaswamy et al., 2007; Balaswamy et al., 2009; Sathivel et al., 2009; Narsing Rao et al., 2012).

India is the third largest producer of fish, where production increased from 4.16 MMT in 1991-1992 to 7.85 MMT in 2009-2010 (Anonymous, 2011). Fresh water fish species Cyprinus carpio was worldwide with the annual availability of 3.5 MMT in 2012 (FAO, 2012). This fish produces an average of 200000-300000 roes/kg body weight. Epinephelus tauvina releases an average of 50,428 eggs/kg body weight (Mathew, 2010).

Literature on roes of these fishes is very limited, and hence, the present investigation was undertaken to evaluate physico-chemical, sorption, functional characteristics and antioxidant activity of roe protein concentrates prepared from Cyprinus carpio and Epinephelus tauvina.

2. Materials and methods

2.1. Materials

Fresh fish roes Cyprinus carpio (1.8 kg) and Epinephelus tauvina (10 kg) were collected from live fishes at a fish market in Hyderabad and Visakhapatnam, India. Chemicals and solvents used in the study were of analytical and laboratory grade and were procured from Sd Fine-Chem Ltd. (Mumbai, India).

2.2. Preparation of roe protein concentrates (RPCs)

Cyprinus and Epinephelus roes were individually separated from blood vessels, skins and homogenized using a high speed mixer (M/s. Sumeet, Nasik, India) and dried at 45 ± 2 °C for 10 h in a cabinet tray dryer (Chemida, Mumbai, India). The dried roes were subjected to lipid removal using isopropanol (IPA) at room temperature (RT) of 28 ± 2 °C. The defatted roes were dried, ground, and passed through a 240μ mesh to obtained roe protein concentrates. They were packed in metallized polyester polyethylene (MPE) laminate pouches and stored at 4 °C. The isopropanol extracts were pooled, dried by passed through sodium sulphate bed, filtered and concentrated on Rota vapor. The crude (lipid) weight was noted, calculated the total lipid content and expressed as percent of total lipid for 100g dried roes.

2.3. Physico-chemical composition

Roe protein concentrates were analysed for physical properties such as colour units measured using Lovibond Tintometer (Model F, Salisbury, UK). The bulk density of CRPC and ERPC was measured by noting the volume occupied by 20 g sample in a 100 ml graduate cylinder.

Chemical composition of CRPC and ERPC was carried out using standard methods (Ranganna, 1986). Protein content was estimated by using standard micro-Kjeldahl method, and the protein conversion factor used is 6.25 (Pellett and Young, 1980). The percentage of nitrogen and protein was calculated using following equations.

\[
\% \text{ Protein} = \frac{\% \text{ Nitrogen} \times 6.25}{100}
\]

The percent carbohydrate content, by difference, was calculated by difference using the following equation:

\[
\% \text{ Carbohydrate} = [100 - (\text{Moisture} + \text{Total Ash} + \text{Crude protein} + \text{Crude fiber})]
\]

The energy value was calculated using following expression and reported as kcal/100g roe protein concentrate.

\[
\text{Energy (kcal/100g)} = 4 \times (\% \text{ Protein} + \% \text{ Carbohydrate})
\]

2.4. Mineral content

Minerals such as calcium, iron and phosphorous were determined in CRPC and ERPC from the ash, which was prepared and dissolved in 6 M hydrochloric acid and made up to 100 ml. Calcium content was estimated by titrimetric method, and iron content was estimated using UV-Visible spectrophotometer (Shimadzu, UV-160A model) at 480 nm (AOAC, 1995). Phosphorous was analyzed by Ranganna (1986) method. The blue colour developed was read at 650 nm in UV-Visible spectrophotometer (Shimadzu, UV-160A model) and expressed as phosphorus mg/100g roe protein concentrate.

2.5. Equilibrium moisture content-relative humidity (EMC-RH) studies

The sorption behavior of CRPC and ERPCs was determined by exposing the samples (5 g) to different relative humidity (RH) ranging from 10% to 100% using appropriate concentrations of sulphuric acid in glass desiccators at room temperature (RT) of 28 ± 2°C. The samples were observed visually for change in colour, lump formation and mold growth during the study. The samples were weighed at regular intervals till they attained constant weight. Moisture sorption isotherms were plotted (Ranganna, 1986) for the equilibrium moisture content (EMC) and the corresponding
equilibrium relative humidity (ERH). The dry solids and EMC were calculated using following formulas.

\[
\text{Dry solids of the sample (A)} = \frac{B \times 100 - C}{100}
\]

Weight of experiment sample (B) initial moisture content (IMC) of sample (C)

\[
\text{Equilibrium Moisture Content (EMC), } % = \frac{D - A}{D} \times 100
\]

Weight of sample after equilibration (D)

2.6. Protein solubility studies

The protein solubility of CRPC and ERPC was estimated by the method of Klompong et al., (2007). The extractions were carried out by dispersing 1 g (CRPC or ERPC) in 40 ml distilled water and the pH of the mixture was adjusted to 2, 4, 6, 8, 10 and 12 with 0.5 M hydrochloric acid (HCl) or 0.5 M sodium hydroxide (NaOH). The mixture was stirred for 30 min and centrifuged at 4500 x g for 30 min at RT. Protein content in the supernatant was determined using the Biuret method (Sadasivam and Manickam, 1997). Protein solubility was calculated as follows:

\[
\text{Protein solubility (\%)} = \left( \frac{\text{Protein content in supernatant}}{\text{Total protein content in sample}} \right) \times 100
\]

2.7. Buffer capacity

The buffer capacity of CRPC and ERPC was estimated by the method of Narsing Rao and Govardhana Rao (2010). A set of two 1 g samples each was dispersed in 40 ml distilled water independently. To one portion was added a measured quantity of 0.1 M NaOH and to the other was added with 0.1 M HCl. As the quantity addition of acid or alkali increased, there was change in pH, and were noted. The amount of alkali and acid added was plotted against pH, and the buffer capacity in each range was expressed as the mean value of mM of NaOH or HCl per gram of roe protein concentrate required to bring about a change in pH by 1 unit.

2.8. Functional properties

2.8.1. Water absorption capacity

The water absorption capacity (WAC) and fat absorption capacity (FAC) for CRPC and ERPC were determined by the method of Shahidi et al., (1995). A 1 g sample was suspended in 10 ml of distilled water, vortexed for 2 min and then centrifuged at 4500 x g for 30 min. The free water was decanted and the water absorbed by the sample was expressed as grams of water absorbed 100 g of roe protein concentrate. Similarly, the fat absorption capacity (FAC) was determined by dispersing in 10 g of refined sunflower oil, and repeated the above operation. The fat absorption capacity was expressed as grams of fat absorbed per 100 g of CRPC and ERPC.

2.8.2. Emulsification capacity (EC)

Emulsification capacity was determined by following reported method (Gagne and Adambou, 1994) with minor modification. The method involves dispersion of 1 g roe protein concentrates (CRPC or ERPC) in 25 ml of distilled water, stirring and gradual addition (0.5-1 ml portions) of sunflower oil until separation of oil layer was observed. EC is expressed as milliliters of oil emulsified per gram of roe protein concentrate. EC was also determined for a standard Bovine serum albumin (BSA) for comparison.

2.8.3. Foam measurements

Foam capacity (FC) and foam stability (FS) of CRPC and ERPC were measured by a reported method (Lawhon et al., 1972). One gram of sample was dispersed in 100 ml distilled water. The contents were 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 10, 20, 30 and 60 min was expressed as percentage FS at respective time intervals. FC was also determined for a standard Bovine serum albumin (BSA) for comparison.

2.9. Antioxidant activity

2.9.1. DPPH radical scavenging activity

Radical scavenging activity of CRPC and ERPCs was measured using DPPH (2,2-diphenyl-1-picrylhydrazyl) (Nanjo et al., 1996). Varying quantities of CRPC and ERPCs such as 2, 4, 6, 8 and 10 mg were dispersed in 1 ml water. Methanolic solution, (4 ml) of DPPH (0.004%) was added, and the contents were vortexed for 1 min and incubated at RT for 30 min and the absorbance was measured at 517 nm. The radical scavenging activity of CRPC and ERPCs percent inhibition (\%) were compared with the activity of butylated hydroxytolulene (BHT). The percentage inhibition was calculated using following expression

\[
\% I = \frac{\text{Absorbance of control} - \text{Absorbance of CRPC or ERPC}}{\text{Absorbance of Control (without sample)}} \times 100
\]

2.9.2. Iron reducing power

The iron reducing power of the CRPC and ERPCs were measured according to the reported method (Yildirim et al., 2001) with minor modification. Roe protein concentrates such as 2, 4, 6, 8 and 10 mg were dispersed in a mixture of water (1 ml) and phosphate buffer (2.5 ml) in different test tubes. Potassium ferricyanide (2.5 ml) solution 1% was added and the contents of test tubes were incubated for 20 min at 50 °C. After incubation, 2.5 ml of 10% tri-chloroacetic acid was added to each test tube and centrifuged at 1073 x g for 10 min. Aliquot (2.5 ml) was mixed with 2.5 ml of water and 0.5 ml of 0.1% ferric chloride and the absorbance was read at 700 nm. Iron reducing power of CRPC and ERPCs were compared with BHT.
2.10. Statistical analysis

Physico-chemical composition, sorption isotherm, functional and antioxidant properties of CRPC and ERPC were carried out in triplicate, and mean values with standard deviation (SD) were computed by using MS Excel, 2007.

3. Results and Discussion

3.1. Physico-chemical and Mineral content of CRPC and ERPC

Unit operations involved in the production of roe protein concentrates and total lipids were presented in Fig. 1. *Cyprinus carpio* and *Epinephelus tauvina* fresh roes yielded 19.50 and 21.50% of CRPC and ERPCs with possessing 70.71 and 85.98% protein respectively. The isopropanol extract yielded 13.65 and 20.22% total lipid of *Cyprinus carpio* and *Epinephelus tauvina* roes respectively. Fig. 2 was depicting the photographs of CRPC and ERPC. A Marginal difference was found in bulk density, total ash and fiber contents. The tintometer colour 3.4, 2.1 (red) and 10.0 (yellow) units found for CRPC and ERPCs (Table 1). Higher red colour units were observed in CRPC, which may be due to the presence of bound carotenoids. Marginal difference in calcium was found in both CRPC and ERPCs, and higher iron (1.32mg/100g) in CRPC and higher phosphorous (1172mg/100g) was observed in ERPC. Balaswamy et al. (2007) also reported 78% protein and red colour units 2.1 for rohu roe protein concentrates and a protein content of 67% with light yellow colour of catfish roe protein powder was also reported by Sathivel et al. (2009).
3.2. Experimental sorption isotherms

Experimental sorption isotherms of CRPC and ERPC indicated that CRPC was more stable than ERPC at RT (Fig. 3). Initial moisture contents (IMC) and critical moisture contents (CMC) of 8 and 9.52% which equilibrated at 63 and 68% RHs, respectively. Similarly, IMC and CMC of ERPCs were 4.9% and 11.50%, which equilibrated at 42% and 70% RHs respectively. Moisture sorption isotherm of *Channa striatus* and *Lates calcarifer* roe protein concentrates are reported to have IMCs 5.78 and 5.36%, which equilibrated at 40 and 38%. They concluded that both roe protein concentrates are hygroscopic in nature (Narsing Rao et al., 2012). The present study indicated that CRPC was non-hygroscopic and ERPC was found to be hygroscopic in nature and PE and MPE pouches with high moisture barrier properties for storage at RT are suitable for CRPC and ERPC respectively. Fish protein concentrate was stable below 84% RH at 35 °C reported by Balaswamy et al. (2007). In our earlier study on seed protein concentrates from Sterculia and bael the critical moisture content were 9.2 %, 8.76%, which equilibrated at 72% and 68% RH respectively (Narsing Rao & Rao, 2010; Narsing Rao et al., 2011).

3.3. Protein solubility

Solubility is one of the most important functional properties of proteins (Kristinsson and Rasco, 2000). Good solubility of proteins is required for many functional applications, especially for making emulsions and foams. The protein solubility of CRPC and ERPC in the pH range of 2-12 is shown in Fig. 4. Both roe protein concentrates were soluble minimum over a wide pH range with not more than 17% solubility. The lower solubility at pH 4 might be attributed to the difference in the isoelectric points of the peptides. Similar trend in protein solubility was also reported in fish protein concentrate (Sikorski and Naczk 1981; Balaswamy et al., 2009).

3.4. Buffer capacities

Buffer capacities of CRPC and ERPC are presented in Fig. 5. High buffer capacity values in acid and alkaline pH were found in ERPC than that of CRPC. The pH of dispersions in distilled water for CRPC and ERPC was found to be respectively at RT. Higher buffer capacity was observed in both roe protein concentrates in alkali.
than in acid, which indicating larger volumes of alkali required for changing the pH for preparation of protein isolates and hydrolysates. Preparation of protein isolates at pH 12 from CRPC and ERPC required 1.2 and 1.6 mM of NaOH/g. *S. urens* seed protein concentrate, 0.44 mM (NaOH/g) was required to change the pH from 6.83 to 10 (Narsing Rao and Rao, 2010). These studies can be utilized during preparation of protein isolates or hydrolysates in bulk at industry.

The emulsification capacity data generated for BSA were comparable to that of rohu roe protein concentrate (Balaswamy et al., 2007). Their ability of emulsify water dispersion had good emulsifying agents. Their ability of emulsify water-oil dispersion had good industrial applications. Hence we reported here the emulsification capacity of CRPC and ERPC, which was noted to be 6.5 and 5.5 ml/g, respectively. The results were comparable to that of rohu roe protein concentrate (Balaswamy et al., 2007). Similar emulsifying capacity (7.1 ml/g) of *Cirrhinus mrigala* roe protein concentrate was reported (Chalamaiah et al., 2011). Emulsification capacity (12 ml/g) was reported in lates roe protein concentrate (Narsing Rao et al., 2012). The emulsification capacity data generated for BSA were also presented in Table 2 for comparison. EC is a very important property if the protein concentrate is to be used in products such as salad dressings.

The foam capacities of CRPC and ERPC were found to be 26 and 56 ml with stability of 17 and 7 ml over a period of 60 min observation. This property generally depends on protein content with hydrophilic nature of peptides. These characteristics are desirable in bakery and traditional food formulations. The poor protein solubility might be one of the reasons of reduction of values in functional characteristics. Hence, further studies can be taken up to improve the protein solubility and functional characteristic of roe protein concentrates for use as functional food ingredients.

### 3.5. Functional properties of roe protein concentrate

WAC, FAC, EC, FC and FS are higher values found for ERPC than CRPC (Table 2). Both the roe protein concentrates absorbed more water than oil, which may be due to the higher protein content with hydrophilic peptides, which may be binding water molecules. Roe proteins were good emulsifying agents. Their ability of emulsify water-oil dispersion had good functional properties and antioxidant activity of roe protein concentrates

DPPH radical scavenging activity

Data on DPPH radical scavenging activity was presented in Table 3. DPPH radical scavenging activity was higher in ERPC than in the CRPC and its activity increased with increase in concentration of the roe protein concentrate. Radical inhibition of 29.43% was observed when 2 mg concentration of ERPC was used and 50.66% inhibition with 10 mg. Similarly for CRPC, the values were 12.04 and 28.54% for 2 and 10 mg, respectively. Under identical conditions, percent inhibition of 87 and 90% was noticed with BHT at 0.2 and 0.3 mg, respectively. The activity of protein depends on solubility of protein, nature of peptides and free amino acids present in the sample (Jun et al., 2004).

### Table 3. Antioxidant activity of roe protein concentrates

<table>
<thead>
<tr>
<th>Weight of roe protein concentrate (mg)</th>
<th>CRPC</th>
<th>ERPC</th>
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</thead>
<tbody>
<tr>
<td><strong>DPPH (Inhibition, %)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>12.04 ± 1.05</td>
<td>12.04 ± 1.05</td>
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<tr>
<td>4</td>
<td>18.56 ± 1.06</td>
<td>18.56 ± 1.06</td>
</tr>
<tr>
<td>6</td>
<td>23.07 ± 1.05</td>
<td>23.07 ± 1.05</td>
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<tr>
<td>8</td>
<td>25.55 ± 1.05</td>
<td>25.55 ± 1.05</td>
</tr>
<tr>
<td>10</td>
<td>28.54 ± 1.16</td>
<td>28.54 ± 1.16</td>
</tr>
<tr>
<td><strong>Ferric reducing power (OD)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.370 ± 0.015</td>
<td>0.370 ± 0.015</td>
</tr>
<tr>
<td>4</td>
<td>0.520 ± 0.008</td>
<td>0.520 ± 0.008</td>
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<tr>
<td>6</td>
<td>0.764 ± 0.004</td>
<td>0.764 ± 0.004</td>
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<tr>
<td>8</td>
<td>0.806 ± 0.004</td>
<td>0.806 ± 0.004</td>
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<tr>
<td>10</td>
<td>0.910 ± 0.006</td>
<td>0.910 ± 0.006</td>
</tr>
<tr>
<td><strong>DPPH (Inhibition, %)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>29.43 ± 1.27</td>
<td>29.43 ± 1.27</td>
</tr>
<tr>
<td>4</td>
<td>34.61 ± 0.77</td>
<td>34.61 ± 0.77</td>
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<tr>
<td>6</td>
<td>37.62 ± 1.45</td>
<td>37.62 ± 1.45</td>
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<tr>
<td>8</td>
<td>42.80 ± 1.75</td>
<td>42.80 ± 1.75</td>
</tr>
<tr>
<td>10</td>
<td>50.66 ± 0.70</td>
<td>50.66 ± 0.70</td>
</tr>
<tr>
<td><strong>Ferric reducing power (OD)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.302 ± 0.004</td>
<td>0.302 ± 0.004</td>
</tr>
<tr>
<td>4</td>
<td>0.358 ± 0.002</td>
<td>0.358 ± 0.002</td>
</tr>
<tr>
<td>6</td>
<td>0.313 ± 0.019</td>
<td>0.313 ± 0.019</td>
</tr>
<tr>
<td>8</td>
<td>0.302 ± 0.004</td>
<td>0.302 ± 0.004</td>
</tr>
<tr>
<td>10</td>
<td>0.313 ± 0.019</td>
<td>0.313 ± 0.019</td>
</tr>
</tbody>
</table>

*Values are average of triplicate analysis with ± SD

CRPC: *Cyprinus carpio* roe protein concentrate
ERPC: *Epinephelus tauvina* roe protein concentrate
BASA: Bovine serum albumin
The results revealed that ERPC possessed higher radical scavenging activity than CRPC, which could be due to reaction of free radicals to form stable products in preventing oxidative degeneration and also may be rich in smaller peptides and free amino acids. It was reported that peptides prepared from catfish protein isolate possessing higher molecular weight proteins exhibited greater radical scavenging activity (Theodore et al., 2008). The higher antioxidant activity of Mackerel (Scomber australius) hydrolysates, due to the presence of <1000 Da peptides was reported by Hui-Chun et al. (2003).

3.7. Reducing power

The iron reducing power of CRPC and ERPC was presented in Table 3. Higher reducing power was found with CRPC than that of ERPC (Table 3). Optical density (OD) of 0.379 and 0.302 ODs of CRPC and ERPC for 2 mg (w/v) respectively, and the ODs 0.910 and 0.516 ODs of CRPC and ERPC were noticed for 10 mg respectively. This activity was compared with reported synthetic BHT, which was 0.42/0.05 mg (w/v) (Narsing Rao et al., 2012). The higher electron donating power of CRPC leads to the higher reducing power. The higher iron reducing power of Mackerel (Scomber australius) hydrolysates was due to the presence of lower peptides reported by Hui-Chun et al. (2003).

4. Conclusion

The unit operations involved in the production of CRPC and ERPCs from fish roe is a simple and easy process. The results showed that considerable variation was observed between physico-chemical parameters, moisture sorption isotherms, functional characteristics, protein solubility and antioxidant activity of roe protein concentrates from Cyprinus carpio and Epinephelus tawitna. The roe protein concentrates were found to be rich in protein, minerals like phosphorous, suitable functional characteristics and antioxidant activity for supplementing in bakery, traditional food formulations and functional ingredient for specialty foods. Fish roe, a by-product in fish industry, can be utilized as a low cost source of protein for value addition. The sorption isotherms are recommended these roe protein concentrates are packed in moisture barrier packaging. The roe protein concentrates were rich in protein but solubility of protein was very poor, hence, these studies further encouraging to investigation on protein solubility, amino acid, lipid and SDS profile.

Acknowledgements

The author thanks the Director, CFTRI, Mysore and CFTRI-RC staff for their support and encouragement to carry out the work.

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