FUNCTIONAL PROPERTIES OF ROE PROTEIN HYDROLYSATES FROM CATLA CATLA

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ABSTRACT

Fish roe is a by-product from a majority of fish species were not utilised due to its ignorance of its nutritional quality and and vulnerability towards deterioration. Roe protein hydrolysates (RPHs) were prepared using defatted roe protein concentrates (RPCs) of Catla catla by applying 1% alcalase at optimal conditions of pH 8.5-9.0 and temperature 50-55 °C. RPCs containing 81.6-86.7% protein, yielded 13-38.2% RPHs with a protein content of 37.1-62.4% depending up on the buffer/alkali used. The degree of hydrolysis varied from 22 to 68.5% after 90 min at 50-55 °C. Protein solubilities of RPHs were observed to be 70.5-99% over a wide pH range (2-12). Functional properties viz., oil absorption capacity, emulsifying capacity and foaming capacity were found to be protein content dependent on the respective RPHs. SDS-PAGE profile showed the presence of simple peptides known to be responsible for desired functional properties.

KEYWORDS

Catla catla, fish roe, alcalase, roe protein hydrolysates, functional properties, SDS-PAGE.

INTRODUCTION

Value addition to by-products is one of the important aspects needs to be attended in food processing industry. Fish roe is an imperative underutilised by-product having essential amino acids and fatty acids. The preparation of caviars and fish roe products from marine fish species, its chemical composition and food safety issues were reviewed [1]. Few products like fish egg protein concentrates from Labeo rohita [2] and protein powders from Alaska Pollock by-products [3] were prepared with good functional properties. The fish waste can be converted into fish protein hydrolysates (FPH} using various enzymes and chemical treatments for better utilisation [4]. The literature on production of FPHs by means of acid, base, endogenous enzymes and bacterial proteases, biochemical and functional properties of hydrolysates and their possible applications in food systems were reviewed earlier [5]. Among commercially available enzymes many workers preferred alcalase, neutrase, papain [6], fungal protease [7]. Alcalase is an alkaline protease enzyme produced from Bacillus licheniformis has been mostly favoured for fish protein hydrolysis [8,5,6]. Alcalase was also used as hydrolytic enzyme in preparation of protein hydrolysates from defatted echinoderm by-products [9], shrimp [10] and tuna liver [11]. The protein hydrolysates were reported to be possess potent antioxidant activity. Protein hydrolysates were suggested for use as emulsifying agents in various food systems [12]. Fortification cereals with protein hydrolysate prepared from Chilean hake (Merluccius gayi) at 2 to 10% increased in both quantity and quality of dietary protein [13]. The importance of protein hydrolysates in human nutrition was discussed in detail by Clemente [14].

Annually world wide fish processing industry discards were estimated to nearly 25% of the total production [15]. Unlike seafood processing, fresh water fish processing is an un-organised sector in Asian countries. Catla catla is a major carp in terms of production among the freshwater fish species in India. During spawning seasons, roes were produced to the extent of one third of the total body weight of the fish. In India, fish roes are mostly an underutilised food by-product. By-products are very prone to microbial spoilage, enzymatic changes and oxidation if the handling is not satisfactory. Most of the literature available is pertaining to marine fish species. The present study was aimed to prepare protein hydrolysates from catla roes and to evaluate functional properties for determining their suitability in food systems.

MATERIALS AND METHODS

Fresh catla roes were obtained from a local fish market, Hyderabad, India immediately after fish dressing and brought to laboratory, stored at 4 °C for not more than 2 h before experimental work. Alcalase enzyme was procured from Novo Laboratories, Denmark. The chemicals and solvents used in the present study were of analytical grade and procured from Sd Fine Chemicals (Mumbai, India). Protein markers for electrophoresis were procured from Sigma Chemicals Co., St. Louis, USA.

Preparation of roe protein concentrates (RPCs) and roe protein hydrolysates (RPHs)

Roes were separated from blood vessels, skeins and homogenized using high speed mixer (Sumeet, Nasik, India). One portion was defatted with isopropanol and dried at 48±2 °C for 8 h in a cabinet tray dryer (Chemida, Mumbai, India) to obtain RPC-1. The other portion of homogenate was dried at 48±2 °C for 8 h and defatted with isopropanol to obtain RPC-2. Isopropanol was recovered by distillation to obtain lipid portion. RPCs were ground to powder using a high speed mixer to pass through a 180 μ mesh, packed in polyethylene

pouches and kept at 4 °C until use. RPHs were prepared from RPCs according to a method of Hoyle & Merritt [16] with a minor modification. RPCs (10 g each) were suspended in 200 ml Tris-HCl or NaOH at pH 9.0. The mixtures were pre-incubated at 50-55 °C for 10 min. The protein hydrolysis reaction was initiated by the addition of the alcalase 1% (v/w) based on protein content in the RPC and allowed for stirring for 90 min at pH 8.5-9.0 and temperature between 50-55 °C. The enzyme activity was terminated by heating the contents at 85 °C for 20 min, cooled and centrifuged at 4500 × g for 30 min at 4 °C. The supernatant containing hydrolyzed and soluble portion was vacuum dried at 40 °C, sealed in polypropylene bags and stored at 4 °C. Average yields of RPHs were calculated by determining the protein content of vacuum dried products with respect to total protein in RPC samples.

Protein content, colour readings and sensory analysis

Protein contents in fresh fish roe, RPCs and RPHs were determined using standard methods [17]. Tintometer colour readings of dried fish roe and RPCs were measured using Lovibond Tintometer (Model F, Salisbury, UK). RPHs were evaluated for sensory parameters viz., taste, flavour and overall acceptability with a panel of 10 semi-trained judges using a 9-point Hedonic scale (1 = dislike extremely, 5 = neither like nor dislike and 9 = like extremely) [18].

Determination of degree of hydrolysis (DH)

Degree of hydrolysis was carried out as a function of time maintaining the optimum conditions (50-55 °C, pH 8.5-9.0) as per the method described in the literature [16]. At the end of each hydrolysis time of 0, 15, 30, 45, 60, 75 and 90 min, an aliquot (20 ml) was taken, mixed with 20 ml of 20% trichloroacetic acid (TCA) and centrifuged at 4500 × g for 30 min at 4 °C. The supernatant was analyzed for nitrogen by the micro-Kjeldahl method [17]. Degree of hydrolysis (%) was calculated as:

$$DH\ (\%) = \frac{10\%TCA - so\text{lub}le\ Nitrogen\ in\ sample}{Total\ Nitrogen\ in\ sample} \times 100$$

Functional properties

The fat absorption capacity (FAC) of RPHs was measured according to a reported method with slight modification [6]. One gram RPH was taken into a 50 ml centrifuge tube and 10 ml sunflower oil was added. The mixture was thoroughly vortexed for 10 min at 25 °C and centrifuged at 4500 × g for 30 min at 25 °C. The fat absorbed by the sample was determined from the weight difference and expressed as grams oil absorbed per gram sample. Emulsification capacity (EC) was determined by a method described [19]. One gram protein hydrolysate and 24.5 ml of distilled water were blended for 30 seconds in a mixer (Kenstar make, Mumbai, India) at low speed. After complete dispersion, refined sunflower oil was added from a burette with blending until phase separation was observed. EC is expressed as ml of oil emulsified per gram of protein hydrolysate. Foam capacity (FC) and foam stability (FS) of RPHs were measured by the following a reported method [20]. One gram RPH was dispersed in 100 ml distilled water and stirred for 10 min using mechanical stirrer (Eltek Motor type 1, Mumbai, India). The contents along with resulting foam were poured into a 250 ml measuring cylinder and the volume of the foam was recorded after 1 min. Foam volume was recorded after 10, 15 and 30 min and expressed as % foam stability.

Determination of protein solubility

Protein solubility of RPHs was determined following a reported method [21] by dispersing

200 mg of RPHs in 20 ml distilled water and adjusting to pH values of 2 to 12 with 0.5 N HCl or 0.5 N NaOH. The mixture was stirred at room temperature (25±2 °C) for 30 min and centrifuged at 4500 × g for 30 min at 4 °C. Protein content in the supernatant was determined using the Biuret method [22] and % protein solubility was calculated.

SDS-PAGE

Gradient sodium dodecylsulfate - polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the method of Laemmli [23] using a 4% stacking gel and 8-15% separating gel. Protein hydrolysate samples (1 µg/µl) were prepared in 60mM Tris-HCl buffer (pH 6.8), 25% glycerol, 2% SDS, 10% β-mercaptoethanol and 0.1% bromophenol blue. The samples were heated in a boiling water bath for 5 min, and loaded (30 µl) on the SDS - polyacrylamide gel and electrophoresis was performed at constant current. Gels were stained in a staining solution containing 0.1% Coomassie brilliant blue R-250, 30% methanol and 20% acetic acid, and de-staining was carried out using a solution containing methanol, glacial acetic acid and water (3:2:5). A broad range pre-stained protein marker from Bangalore Genei, Bangalore, India was used for the determination of molecular weight in the range 14.4 to 116.0 kDa.

Statistical analysis

All analytical determinations were carried out in triplicate and mean values with standard deviation (SD) are presented. Average yields, protein content and protein solubility of RPHs were analysed statistically by ANOVA using SPSS 15.0 to ascertain whether differences were significant at $p<0.05$.

RESULTS AND DISCUSSION

Quality of roe protein concentrates (RPCs) and colour readings

The results showed fresh fish roes containing 26.5% protein (Table 1). Earlier studies [24] revealed that catla roes contained more protein (28.2%) than other fish species studies. The colour of fresh roes (on dry basis) was brighter with red and yellow units to the extent of 2.6 and 4.6 respectively due to the presence of fat. Fresh roes yielded 30% RPC-1 and 31.8% RPC-2 with 86.7% and 81.6% protein respectively.

Sample	Protein (%)	Colour readings		
		Red	Yellow	Blue
Fresh roes	26.5 ± 0.20	2.6[a]	4.6[a]	1.0[a]
RPC-1	86.7 ± 0.31	1.6	2.8	0.9
RPC-2	81.6 ± 0.35	1.6	3.6	0.9
[a] Values are on dry basis				

Table 1. Protein content and Lovibond Tintometer colour readings in fish roe preparations.

Both RPCs showed lower red value of 1.6, but slightly higher yellow values (3.6) in RPC-2 than that of RPC-1 (2.8). The protein content and colour values of RPC-2 are similar to that of RPC prepared from rohu [2]. A spray dried roe protein powder prepared from catfish containing 18.3% fat and 67% protein was light yellow in colour [25].

Effect of processing on preparation of RPHs and sensory analysis

The flow chart for the preparation of RPHs was presented in Fig. 1. Moisture contents in RPHs were in the range of 5.6 to 6.8%. The average yield and protein content in each RPH varied significantly. Average yield of vacuum-dried protein hydrolysates were in the range of 13-38.2% (Table 2) and the protein contents in RPHs were in the range of 37.1 – 62.4%. The yields of RPHs were more in presence of Tris-HCl and significantly decreased in presence of NaOH. Fig. 2 also confirmed the lower yields of RPHs in the presence of NaOH. Alkali assisted RPHs contained higher amounts of proteins which may be due to solubilisation of native proteins instead of hydrolysates.

Catla fish roe

↓

Homogenization

Defatting and drying → FEPC-1   Drying and defatting → FEPC-2

Recovery of isopropanol

Adjusting pH to 9.0 by adding Tris HCl or NaOH and maintaining temperature between 50-55 °C

Addition of alcalase 1% (v/w)

Enzymatic hydrolysis for 90 min

85-90°C, 15-20 min

Enzyme inactivation

4500 × g, for 20 min

Centrifugation

Supernatant

Vacuum drying

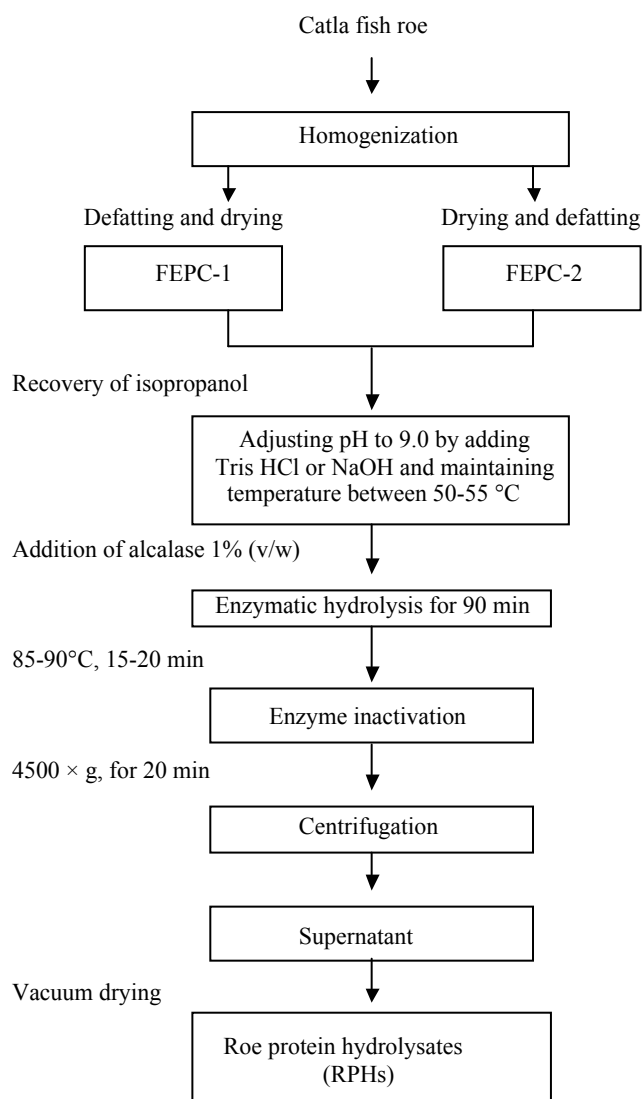Roe protein hydrolysates (RPHs)

Figure 1. Flow chart for the preparation of roe protein hydrolysates (RPHs)

In contrast, RPCs yielded higher amounts of RPHs in the presence of Tris-HCl due to higher DH and exhibited higher protein solubility at various pH levels. (Table 3). It was reported that 79% of protein hydrolysates were yielded (on fat free basis) from visceral waste of Catla when a commercial protease was used at 1.25% (v/w) under optimized conditions of 55 °C for 165 min [26]. Earlier, protein hydrolysates from Cirrhinus mrigala roes were prepared using alcalase at 62% hydrolysis which showed higher protein content (85%) and good functional properties [27]. All RPHs scored good (≥7) during sensory analysis in terms

of taste and overall acceptability and were devoid of off-flavours.

Parameter	RPHs from RPC-1		RPHs from RPC-2	
	Tris-HCl	NaOH	Tris-HCl	NaOH
Moisture (%)	5.6 ± 0.05	6.1 ± 0.12	6.3 ± 0.26	6.8 ± 0.12
Yield (%)	33.6 ± 0.5^a	15.7 ± 0.40^b	38.2 ± 1.40^c	13.0 ± 0.35^d
Protein (%)	44.0 ± 0.50^a	48.6 ± 0.87^b	62.4 ± 0.69^c	37.1 ± 0.61^d
Fat absorption capacity (%)	15.0 ± 0.50	18.0 ± 1.00	30.0 ± 1.00	32.0 ± 1.50
Emulsifying capacity (ml/g)	5.0 ± 0.40	5.6 ± 0.20	6.9 ± 0.10	6.5 ± 0.20
Foam capacity (%)	70.0 ± 0.20	220.0 ± 3.00	94.0 ± 1.00	110.0 ± 2.00
Foam stability (%), after 10 min	15.0 ± 0.02	140.0 ± 1.50	22.0 ± 0.50	80.0 ± 0.50
Foam stability (%), after 15 min	15.0 ± 1.00	20.0 ± 0.50	22.0 ± 0.50	60.0 ± 0.50
Foam stability (%), after 30 min	15.0 ± 1.00	20.0 ± 0.50	22.0 ± 0.50	60.0 ± 0.50

Mean values with different letters in the same rows are statistically different at $P < 0.05$

Table 2. Yield and functional properties of RPHs

Degree of hydrolysis (DH)

The effect of time on degree of hydrolysis of RPC by 1% alcalase in both NaOH and Tris-HCl buffer is presented in Fig. 2. There was a steady increase in degree of hydrolysis (DH) with increased time intervals for both RPCs in the presence of Tris-HCl. The DH after 90 min was 60.8% and 68.5% for RPC-1 and RPC-2, respectively. The present study showed lower DH from 4 to 22% was observed in the presence of NaOH.
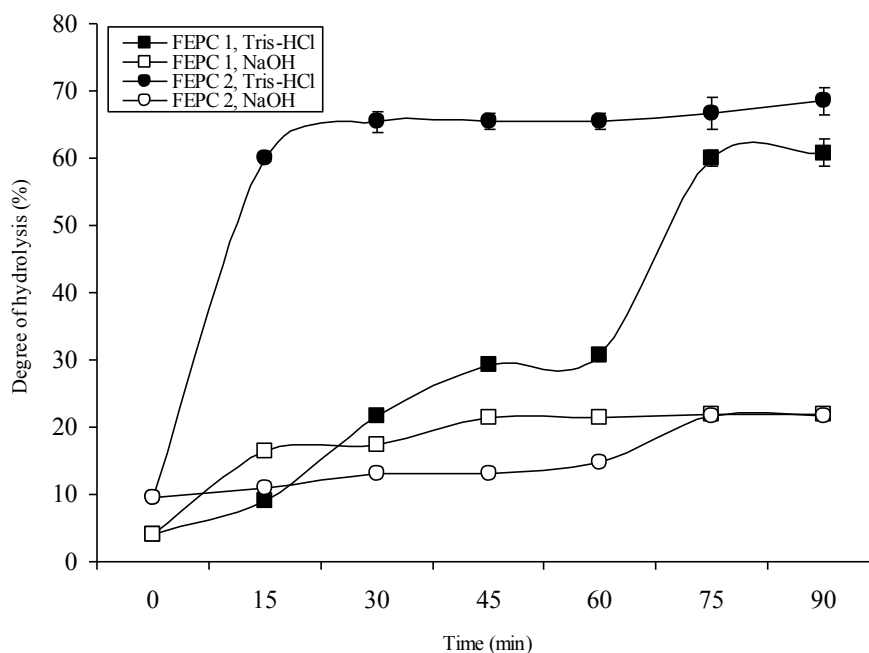

Figure 2. Time Vs % Degree of hydrolysis of roe proteins. Each point represents the mean values of triplicate determination. Vertical bars represent standard deviations

Functional properties of RPHs

Functional properties such as fat absorption capacity (FAC), emulsifying capacity (EC) and foam properties have been reported in Table 2. FAC and EC values for RPHs were much

lower compared to reported values of 3.7 -7.3 ml oil / g protein and 11.1 to 20.6 ml of oil / 200 mg of protein respectively for herring byproduct hydrolysates [28]. The foam capacities of RPHs were in the range between 70 to 220%. Foaming capacity for capelin protein hydrolysates was as high as 90% [6]. Fish protein hydrolysate from raw herring (Clupea harengus) prepared at 36% hydrolysis showed an adequate (142%) foam expansion [29]. In the present study, RPHs prepared in alkali showed higher foam capacity and lower foam stability, which will be of immense help in bakery industry.

Protein solubility of RPHs

Table 3 showed the protein solubility of various RPHs. Protein hydrolysates prepared from both RPCs in Tris-HCl buffer were completely soluble at various pH ranges. Alkali assisted RPHs were soluble to the extent of minimum 70.5%. Increase in solubility might be a result of reduction in molecular weight during enzymatic hydrolysis of proteins. RPHs prepared from both RPC-1 and RPC-2 in presence of NaOH has shown differential solubility. Solubility of RPHs from RPC-2 showed significant increase (85.6-99%) compare to that of RPHs from RPC-1 (70.5-83.6%) at respective pH. The results indicated that the method of processing, which includes the drying followed by defatting of roes is a critical step to obtain highly soluble FPHs in the presence of alkali. Higher solubility of RPHs help in production of health drinks, beverage mixes and protein supplements.

pH	RPH 1, Tris-HCl	RPH 1, NaOH	RPH 2, Tris-HCl	RPH 2, NaOH
2	98.8 ± 0.25	70.5 ± 0.61[a]	96.2 ± 0.30	85.7 ± 0.83[b]
3	99.0 ± 0.25	74.5 ± 0.60[a]	99.1 ± 0.40	90.3 ± 0.35[b]
4	98.2 ± 1.00	70.5 ± 0.76[a]	96.1 ± 0.15	92.5 ± 0.45[b]
5	99.0 ± 0.88	71.8 ± 0.10[a]	99.0 ± 0.57	89.7 ± 0.78[b]
6	98.6 ± 0.45	75.4 ± 0.60[a]	98.0 ± 0.23	89.2 ± 1.10[b]
7	98.0 ± 0.20	74.1 ± 0.46[a]	97.2 ± 1.41	90.7 ± 0.56[b]
8	98.6 ± 0.45	77.2 ± 0.59[a]	98.1 ± 0.34	91.1 ± 0.88[b]
9	98.2 ± 0.62	74.7 ± 0.29[a]	98.7 ± 0.22	92.8 ± 0.76[b]
10	98.8 ± 0.90	78.2 ± 0.05[a]	98.2 ± 1.00	93.4 ± 0.44[b]
11	99.0 ± 0.62	78.5 ± 0.20[a]	98.6 ± 0.80	98.2 ± 0.80[b]
12	98.6 ± 1.00	83.5 ± 0.55[a]	96.1 ± 0.46	98.4 ± 0.40[b]

Mean values with different letters in the same rows are statistically different at P < 0.05

Table 3. Protein solubility of RPHs at different pH levels

SDS-PAGE

SDS-PAGE electrophoresis (Fig. 3) shows that the RPCs had smaller and intermediate molecular weight proteins. The banding pattern for RPC indicated the presence of four abundant proteins with molecular weights between 25 and 116 kDa. Major bands were observed between 45 to 116 kDa. The presence of four abundant proteins with molecular weights between 40 and 100 kDa in spray dried catfish roe protein powder was also observed [25]. A major protein band with molecular weight of 103 kDa was observed in protein powders prepared from immature Alaska Pollock roe [30].
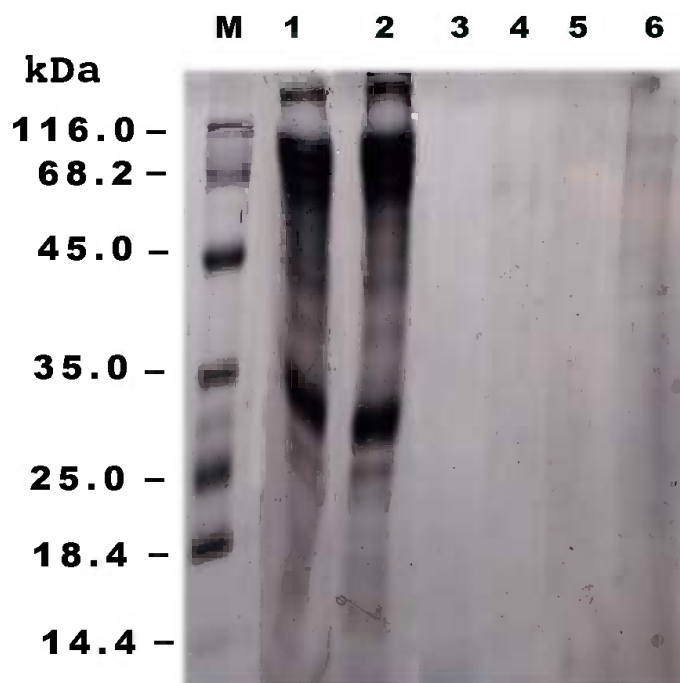
Figure 3. SDS-PAGE pattern of RPCs and RPHs of Catla. M: broad range molecular weights of standard protein markers; 1: RPC-1; 2: RPC-2; 3: RPH-1, Tris-HCl; 4: RPH-1, NaOH; 5: RPH-2, Tris-HCl; 6: RPH-2, NaOH

The disappearance of protein bands in RPHs is the indication of uncontrolled hydrolysis resulted in higher yield of amino acids/smaller peptides. Small bands were observed in RPH prepared from RPC-2 with NaOH might be due to lower degree of hydrolysis and presence of soluble native proteins. These results suggested that hydrolysis yielded a high proportion of shorter peptides below 14.4 kDa.

ACKNOWLEDGEMENTS

REFERENCES

1. G.E. Bledsoe, C.D. Bledsoe, B. Rasco. Caviars and fish roe products. Crit. Rev. Food Sci. Nutri. 43, 317-356 (2003).
2. K. Balaswamy, T. Jyothirmayi, D.G. Rao. Chemical composition and some functional properties of fish egg (roes) protein concentrate of rohu (*Labeo rohita*). J. Food Sci. Technol. 44, 293-296 (2007).
3. S. Sathivel, P.J. Bechtel. Properties of soluble protein powders from Alaska Pollock (*Theragra chalcogramma*). Int. J. Food Sci. Technol. 41, 520-529 (2006).
4. S. I. Aspmo, S.J. Horn, V.G.H. Eijsink,. Enzymatic hydrolysis of Atlantic cod (*Gadus morhua* L.) viscera. Process Biochem. 40, 1957–1966 (2005).
5. H.G. Kristinsson, B.A. Rasco. Fish protein hydrolysates: production, biochemical, and functional properties. Crit. Rev. Food Sci. Nutri. 40, 43-81 (2000).
6. F. Shahidi, X.Q. Han, J. Synowiecki. Production and characteristics of protein hydrolysates from capelin (*Mallotus villosus*). Food Chem. 53, 285-293 (1995).
7. N. Bhaskar, V.K. Modi, K. Govindaraju, C. Radha, R.G. Lalitha. Utilization of meat industry by products: Protein hydrolysate from sheep visceral mass. Bioresource Technol. 98, 388-394 (2007).

8. N. Bhaskar, T. Benila, C. Radha, R.G. Lalitha. Optimization of enzymatic hydrolysis of visceral waste proteins of catla (*Catla catla*) for preparing protein hydrolysate using a commercial protease. Bioresource Technol. 99, 335–343 (2008).

9. J. Mamelona, R. Saint-Louis, E. Pelletier. Nutritional composition and antioxidant properties of protein hydrolysates prepared from echinoderm byproducts. Int. J. Food Sci. Technol. 45: 147–154 (2010).

10. W. Cao, C. Zhang, P. Hong, H. Ji. Optimising the free radical scavenging activity of shrimp protein hydrolysate produced with alcalase using response surface methodology. Int. J. Food Sci. Technol. 44, 1602-1608 (2009).

11. C.B. Ahn, K.H. Lee, J.Y. Je. Enzymatic production of bioactive protein hydrolysates from tuna liver: effects of enzymes and molecular weight on bioactivity. Int. J. Food Sci. Technol. 45, 562-568 (2010).

12. C.S. Badal, H. Kiyoshi. Debittering of protein hydrolysates. Biotech. Adv. 19, 355-370 (2001).

13. E. Yanez, D. Ballester, F. Monckeberg, W. Heimlich, M. Rutman. Enzymatic fish protein hydrolyzate: Chemical composition, nutritive value and use as a supplement to cereal protein. J. Food Sci. 41, 1289 – 1292 (1976).

14. A. Clemente. Enzymatic protein hydrolysates in human nutrition. Trends Food Sci. Technol. 11, 254-262 (2000).

15. T. Rustad. Utilizationof marine by-products. EJEAFChe. 2, 458-463 (2003).

16. N.T. Hoyle, J.H. Merritt. Quality of fish protein hydrolysates from herring (*Clupea harengus*). J. Food Sci. 59, 76-79 (1994).

17. AOAC. Official Methods of Analysis (15th ed.), Association of Official Analytical Chemists, Washington, D.C. (1995).

18. D.R. Peryam, F.J. Pilgrims. Hedonic scale method of measuring food preferences. Food Technol. 11(suppl), 9–14 (1957).

19. N. Gagne, L.T. Adambounou. Physico-chemical and functional properties of roes from autumn spawning herring (*Clupea herengus herengus* L.). Food Res. Int. 27, 405-408 (1994).

20. J.F. Lawhon, C.M. Carter, K.F. Matil. A comparative study of the whipping potential of an extract from several oil seed flours. Cereal Sci. Today 17, 240-244, 246, 294 (1972).

21. V. Klompong. S. Benjakul, D. Kantachota, F. Shahidi. Antioxidative activity and functional properties of protein hydrolysate of yellow stripe trevally (*Selaroides leptolepis*) as influenced by the degree of hydrolysis and enzyme type. Food Chem. 102, 1317-1327 (2007).

22. T.E. Weichselbaum. An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. Am. J. Clin. Pathol. 16 (Tech. Suppl.), 40 (1946).

23. U.K. Laemmli. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature 227, 680-685 (1970).

24. K. Balaswamy, P.G. Prabhakara Rao, G. Narsing Rao, D.G. Rao, T. Jyothirmayi. Physico-chemical composition and functional properties of roes from some fresh water fish species and thei application in some foods. EJEAFChe. 8, 806-812 (2009).

25. A. Sathivel, H. Yin, P.J. Bechtel, J.M. King. Physical and nutritional properties of catfish roe spray dried protein powder and its application in an emulsion system. J. Food Eng. 95, 76–81 (2009).

26. N. Bhaskar, N.S. Mahendrakar. Protein hydrolysate from visceral waste proteins of catla (*Catla catla*): Optimization of hydrolysis conditions for a commercial neutral protease. Bioresource Technol. 99, 4105–4111 (2008).

27. M. Chalamaiah, G. Narsing Rao, D.G. Rao, T. Jyothirmayi. Protein hydrolysates from meriga (*Cirrhinus mrigala*) egg and evaluation of their functional properties. Food Chem. 120, 652-657 (2010).

28. S. Sathivel, P.J. Bechtel, J. Babbitt, S. Smiley, C. Crapo, K.D. Reppond, W. Prinyawiwatkul. Biochemical and functional properties of herring (*Clupea harengus*) byproduct hydrolysates. J. Food Sci. 68, 2196-2200 (2003).

29. A.M. Liceaga-Gesualdo, E.C.Y. Li-Chan. Functional properties of fish protein hydrolysate from herring (*Clupea harengus*). J. Food Sci. 64, 1000-1004 (1999).

30. P.J. Bechtel, J. Chantarachoti, A.C.M. Olivera, S. Sathivel. Characterization of protein fractions from immature Alaska Walleye Pollock (*Theraga chalcogramma*) roe. J. Food Sci. 72, 338-343 (2007).