



International Journal of Agriculture Extension and Social Development

ISSN Print: 2618-0723
ISSN Online: 2618-0731
NAAS Rating (2026): 5.04
IJAESD 2026; SP-9(10): 11-23
www.extensionjournal.com
Received: 21-08-2026
Accepted: 23-09-2026
Published: 03-10-2026

Ajay Eknath Sonavane
Assistant Fisheries
Development Officer,
Department of Fisheries,
Government of Maharashtra,
Maharashtra, India

Vinay Maruti Hatte
Assistant Fisheries
Development Officer,
Department of Fisheries,
Government of Maharashtra,
Maharashtra, India

Production, characterization and bioactivity evaluation of protein hydrolysates from Indian mackerel (*Rastrelliger Kanagurta*) using commercial proteases

Ajay Eknath Sonavane¹, Vinay Maruti Hatte²

DOI: <https://www.doi.org/10.33545/26180723.2026.v9.i10Sa.4359>

ABSTRACT

The present study was undertaken to investigate the extraction and characterization of bioactive compounds from Indian mackerel (*Rastrelliger kanagurta*), with particular emphasis on fish protein hydrolysate (FPH). Fish protein hydrolysate was prepared using different concentrations of the enzyme Alcalase (0.5%, 1.0%, and 2.0%) at different hydrolysis periods (1, 2, and 3 h). The results revealed that the yield of FPH increased with an increase in both enzyme concentration and hydrolysis time. At 0.5% Alcalase, the hydrolysate yield increased from 8.42% at 1 h to 13.72% at 3 h, whereas at 1.0% and 2.0% Alcalase, the yield increased from 8.93% to 15.91% and from 9.12% to 18.36%, respectively. The proximate composition of FPH showed variations with enzyme concentration and hydrolysis time. An increase in enzyme concentration from 0.5% to 2.0% resulted in a decrease in protein and moisture contents, while fat and ash contents increased. The degree of hydrolysis (DH) also increased progressively with increasing enzyme concentration and hydrolysis time. At 0.5% Alcalase, the DH increased from 8.75% to 13.76%, while at 1.0% Alcalase it increased from 11.14% to 14.76%. The highest DH of 23.12% was recorded with 2.0% Alcalase after 3 h of hydrolysis. PAGE analysis of the FPH revealed protein bands with molecular weights ranging from approximately 50 to 180 kDa. Furthermore, in vitro antioxidant and antimicrobial assays demonstrated positive bioactivities of the prepared FPH. The findings indicate that Indian mackerel can serve as a valuable source of functional protein hydrolysates with potential applications in nutraceutical, food, pharmaceutical, and other value-added products.

Keywords: Indian Mackerel, *Rastrelliger kanagurta*, Fish Protein Hydrolysate, Alcalase, Degree of Hydrolysis, Antioxidant Activity, Antimicrobial Activity

Introduction

Indian mackerel (*Rastrelliger kanagurta*) is an economically important pelagic marine fish widely distributed throughout the Indo-Pacific region and forms a major component of the marine fisheries of India. The species is one of the most abundant members of the genus *Rastrelliger* occurring in Indian waters and supports an important commercial fishery along both the west and east coasts. India contributes a substantial proportion of the global Indian mackerel production, with the west coast accounting for the major share of the national landings (Ayyappan, 2011; CMFRI, 2017) [1, 2]. Indian mackerel is consumed fresh and traded mainly in frozen form with limited quantities being traded as direct landings.

In recent years, increasing attention has been directed towards the utilization of marine fish as sources of value-added bioactive compounds. Fish proteins are not only nutritionally valuable but also serve as precursors of biologically active peptides. Enzymatic hydrolysis of fish proteins can release peptides with specific molecular characteristics and biological activities that may differ considerably from those of the parent proteins. Depending on the source, enzyme used, degree of hydrolysis, and processing conditions, fish protein hydrolysates may exhibit antioxidant, antimicrobial, antihypertensive, immunomodulatory, antidiabetic, and other physiological activities (Harnedy *et al.*, 2012) [3].

Proteins from marine sources show promise as functional ingredients in foods because they possess numerous important and unique properties such as film and foaming capacity, gel forming ability and antimicrobial activity. Bioactive peptides can be naturally occurring biomolecules, produced by microbial fermentation or generated with a variety of different enzymes, such as commercially available enzymes or for instance gastro intestinal enzymes. Bioactive peptides are specific protein fragments, and in addition act as amino acids and nitrogen sources; they have numerous potential physiological functions within the body (Harnedy *et al.*, 2012) [3]. Marine fish oils contain great amount of omega-3 fatty acids, whereas seaweeds and shellfish such as crustaceans have potent antioxidants including carotenoids and phenolic compounds (Rasmussen *et al.*, 2007) [4].

Bioactive peptides derived from marine proteins have attracted considerable interest because of their potential applications in functional foods, nutraceuticals, pharmaceuticals, and health-promoting products. Previous investigations have demonstrated that enzymatically derived fish protein hydrolysates can possess

Corresponding Author:

Vinay Maruti Hatte
Assistant Fisheries
Development Officer,
Department of Fisheries,
Government of
Maharashtra, Maharashtra,
India

significant antioxidant activity, which may be associated with the presence of specific amino acid sequences and peptides capable of scavenging free radicals or inhibiting oxidative reactions (Je *et al.*, 2008) [5]. Fish-derived peptides have also been reported to exhibit antimicrobial activity against selected microorganisms (Liu *et al.*, 2008) [6], while other studies have demonstrated immunomodulatory, anti-cancer, angiotensin-I-converting enzyme (ACE)-inhibitory, and antidiabetic properties (Picot *et al.*, 2006; Yang *et al.*, 2009; Li-Chan *et al.*, 2012) [8, 9, 7].

Therefore, the present study was undertaken to prepare and characterize fish protein hydrolysate from Indian mackerel (*Rastrelliger kanagurta*) using Alcalase at different enzyme concentrations and hydrolysis periods. The study specifically evaluated hydrolysate yield, degree of hydrolysis, proximate composition, protein profile by PAGE, and in vitro antioxidant and antimicrobial activities of the resulting hydrolysates. The findings may provide useful information for the development of value-added functional ingredients and nutraceutical products from Indian mackerel.

Materials and Methods

The Indian mackerel (*Rastrelliger kanagurta*) in the iced condition was procured from, Mirkarwada, Ratnagiri fish landing center, Maharashtra, India. Fish was stored in chilled box and transported to the laboratory and kept at -20 °C prior to further study.

Enzymatic extraction of protein hydrolysate from whole fish

Whole fish minced without adding water
50 g minced fish was weighted and heated in water bath at 90° C for 10 min to inactivate endogenous enzymes
Cooled and 50 mL potassium phosphate buffer (1M and pH 8.0) was added to the mince in the ratio of 1:1
Mixture placed in a water bath at 55° C and occasionally stirred for 30 min
Alcalase was added to the mixture in the concentration of 0.5, 1.0, 2 % (w/v)
Incubation was carried out for 60, 120 and 180 min. at room temperature
The mixture was heated at 90°C for 5 min to inactivate the enzyme
Cooled for 15 min
Centrifuged at 4100 rpm for 40 min
Four layers formed after centrifugation
Decanting fat layer and collect protein hydrolysate (middle layer)
Drying in vacuum oven dryer at 55°C
Packed in air tight container and stored for further study at room temperature

Protein and peptide pattern of FPH

Tricine SDS-polyacrylamide gel electrophoresis (SDS-PAGE), which is based on Tricine-Tris buffer system, was performed essentially as described by Schagger (2006) [12]. Hydrolysates were prepared as 2 mg/mL (w/v, in protein content) in distilled water at 60 °C and diluted to a final concentration of 1 mg/mL with sample buffer containing β-mercaptoethanol (Hi-media, Mumbai) then heated to 85 °C for 10 min. Gels were prepared according to Laemmli (1970) [13] in 4 % stacking gel and 15 % resolving gel. SDS-PAGE gels were loaded with 10 µL of protein hydrolysates and molecular weight markers (low molecular weight peptide marker).

The extraction of Fish protein hydrolysate was done according to the method of Ramkrishnan *et al.* (2013) [10], with certain modifications from the whole *Rastrelliger kanagurta*. The alcalase enzyme was used at three concentrations (0.5, 1.0, and 2%) and incubated at different time intervals (60, 120, 180 min.). Detailed procedure described in below table and flow chart.

Table 1. Different FPH with different enzyme concentration and reaction time

Sample No.	Reaction condition for hydrolysis at 55°C	
	Enzyme Concentration (%)	Reaction time (min.)
1	0.5	60
2	0.5	120
3	0.5	180
4	1	60
5	1	120
6	1	180
7	2	60
8	2	120
9	2	180

Yield of fish protein hydrolysate

The yield of FPH was determined by using following formula,

$$\text{Yield} = \frac{\text{Dry weight of FPH (g)}}{\text{Wet weight of raw material (g)}} \times 100$$

Characterisation of FPH

Degree of hydrolysis

The degree of hydrolysis (DH) is defined as the ratio of the number of peptide bonds broken with respect to the total number of bonds per unit weight and was determined following the method of Tang *et al.* (2009) [11]. This method is based on the fact that the α-amino groups released by hydrolysis react with OPA and dithiothreitol (DTT) to form a compound that absorbs strongly at 340 nm.

The degree of hydrolysis (DH) of fish samples was determined by the ratio of trichloroacetic acid (TCA) soluble nitrogen to total nitrogen in the sample. Hydrolysis was determined by triplicate batch experiments in which 50-g portions of frozen fish were thawed and prepared by the different fat extraction methods. To each portion of 50g fish mince were added equal volumes of water and the mixtures were adjusted to optimum conditions for enzyme activity and hydrolyzed. Aliquots (50 mL) were removed at intervals of 0, 15, 30, 45, and 60 min and mixed with 50 mL 20% TCA to create 10% TCA-soluble and TCA-insoluble fractions. The mixtures were centrifuged at 10,000 rpm and the supernatants analysed for nitrogen by the macro-Kjeldahl method (AOAC, 1980). The degree of hydrolysis (DH) was calculated as:

$$\text{DH} = \frac{10\% \text{TCA-soluble N in sample}}{\text{Total N in sample}} \times 100$$

The analysis was run in an Atto Dual Mini-slab Size Electrophoresis System AE-6450 (Atto Corporation, Tokyo, Japan) at a constant current of 25 mA/gel. Gels were then silver stained according to Sorensen *et al.* (2002) [14]. The gels were fixed in 40 % methanol, 10 % acetic acid and 50 % water for 20 min, washed 3 times for 10 min in 30 % ethanol, followed by incubation for 1 min in 1.25 mM sodium thiosulfate. After washing 3 times in water, gels were stained for 20 min in 0.2 % silver nitrate, 0.02 % formaldehyde and 0.03 mM sodium thiosulfate and then washed briefly in water. The gel was developed until the bands appeared (5 - 10 min) in 280 mM sodium carbonate and 0.05 % formaldehyde and finally with 5 % acetic acid.

The low molecular weight peptide marker contained a mixture of peptides: myoglobin (16,950 Da), myoglobin I + II (14,440 Da), myoglobin I + III (10,600 Da), myoglobin I (8,160 Da), myoglobin II (6,210 Da) and myoglobin III (2,510 Da).

Results

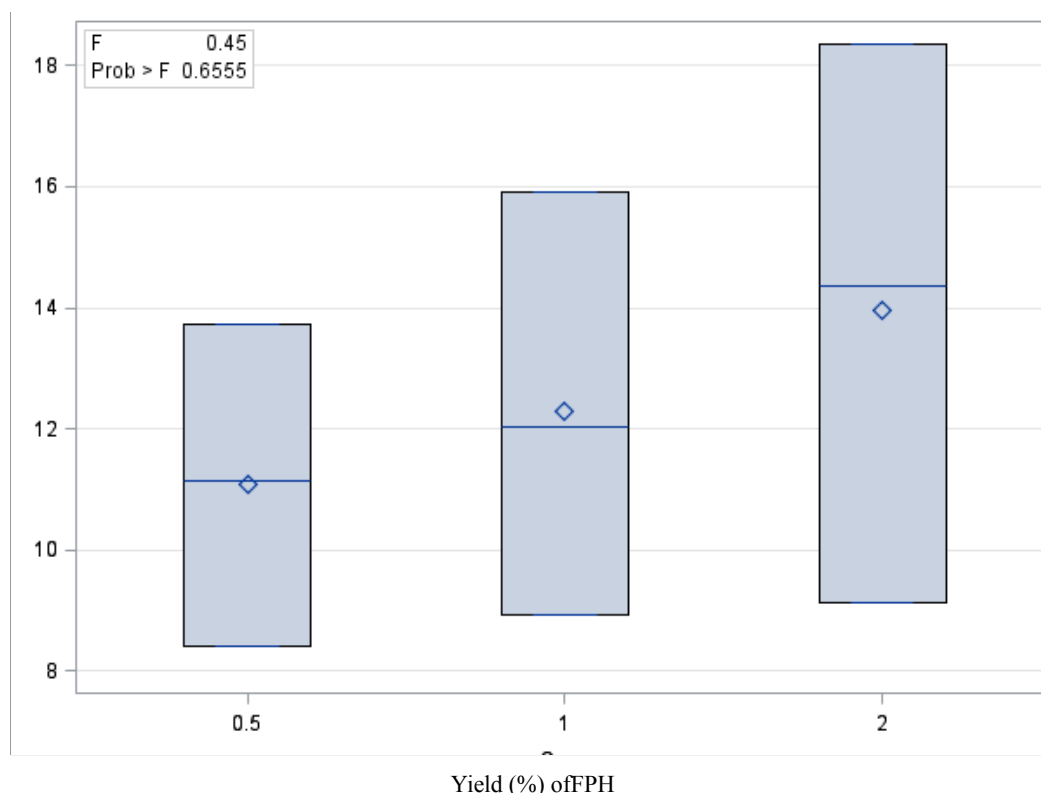
Preparation of fish protein hydrolysates

Fish protein hydrolysate was prepared using alcalase at three different concentrations and hydrolysis time. FPH extracted from mackerel muscle hydrolysed with enzyme for 1, 2 & 3 hr. It was observed that as the hydrolysis progressed, the turbidity decreased and the solution become clear.

The yield of FPH of mackerel prepared using various concentrations at duration of 180 min. was recorded to be higher. The result indicate

that when 0.5 % alcalase used for extraction of FPH at different hydrolysis period i.e. 60, 120 and 180 minutes the hydrolysate yield was observed as 8.42 ± 0.95 , 11.14 ± 1.03 and 13.72 ± 1.36 % respectively. When 1 % alcalase was used for extraction of FPH for 60, 120 and 180 minutes, then hydrolysate yield was recorded as $08.93 \pm 0.65\%$, $12.04 \pm 1.30\%$ and 15.91 ± 2.40 % respectively. But, when 2 % alcalase was used for different hydrolysis period i.e. 60, 120 and 180 minutes, then FPH yield was recorded as 9.12 ± 1.15 , 14.36 ± 1.75 and $18.36 \pm 2.70\%$ respectively as shown in (Table 2 and Figure 1).

There was statistically significant difference ($\alpha = 0.05$) the effect of concentrations on the yield (%) of fish protein hydrolysate. Values are mean $\pm$ standard deviation of triplicate



Concentration (%) of alcalase

Figure 1. Distribution of yield of FPH using different alcalase concentrations and duration hydrolysis

Proximate composition of FPH prepared using different concentration and duration hydrolysis using alcalase enzyme

The proximate composition of fish protein hydrolysate i.e. crude protein, fat, moisture and ash content in fish protein hydrolysate was recorded after hydrolysis with different concentrations of alcalase en-

zyme and hydrolysis time. At 0.5 % concentration of enzyme the result indicates that the protein and moisture values were decreasing while fat and ash values were increasing with hydrolysis time. At 1 % concentration of enzyme the result indicates that the protein and moisture values were decreasing while fat and ash values were increasing with hydrolysis time. At 2 % concentration of enzyme the result indicates that the protein and moisture values were decreasing while fat and ash values were increasing with hydrolysis time is shown in Table-2 and Figure- 2-4.

Table 2. Yield of fish protein hydrolysate obtained using different alcalase concentrations for different hydrolysis time

Sr. No.	Concentration of alcalase (% and w/v)	Hydrolysis duration (minutes)	Hydrolysate yield (%)
1	0.5	60	08.42 ± 0.95
		120	11.14 ± 1.03
		180	13.72 ± 1.36
2	1	60	08.93 ± 0.65
		120	12.04 ± 1.30
		180	15.91 ± 2.40

(continued on next page)

3 2

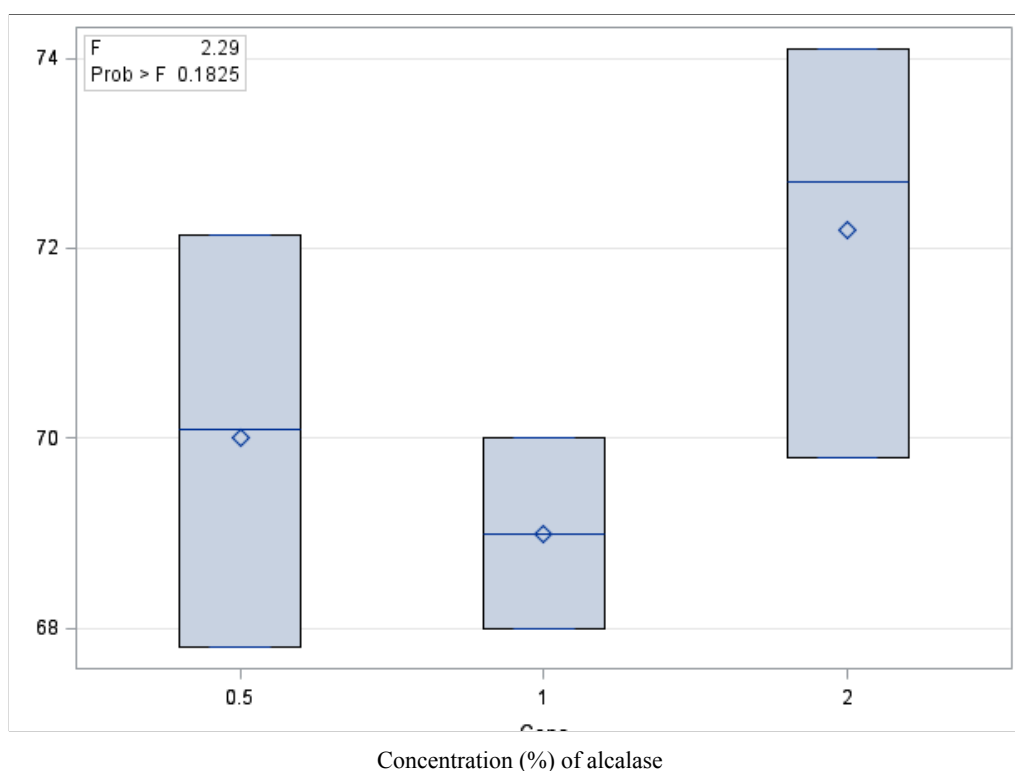
60 9.12 ± 1.15
 120 14.36 ± 1.75
 180 18.36 ± 2.70

There was a significant difference ($\alpha = 0.05$) between the enzyme concentrations on the proximate composition (%) of FPH.

Table 3. Proximate composition of FPH prepared using different concentration and time using alcalase enzyme

Sr. No.	Conc. of enzyme (%)	Hydrolysis time (minutes)	Protein (%)	Fat (%)	Moisture (%)	Ash (%)
1	0.5	60	72.14 ± 1.5	3.25 ± 0.7	5.6 ± 1.2	18.1 ± 1.5
2	0.5	120	70.09 ± 0.9	4.7 ± 0.65	5.3 ± 1.0	20.7 ± 1.5
3	0.5	180	67.8 ± 1.3	5.8 ± 0.53	4.9 ± 0.7	23.1 ± 2.0
4	1.0	60	70.0 ± 1.65	4.0 ± 0.4	6.1 ± 1.2	19.7 ± 1.2
5	1.0	120	68.0 ± 0.9	4.5 ± 0.95	5.8 ± 0.85	20.1 ± 1.0
6	1.0	180	69.0 ± 1.2	4.9 ± 1.05	6.01 ± 0.4	20.6 ± 2.1
7	2.0	60	74.1 ± 2.2	2.4 ± 0.35	5.4 ± 1.35	18.6 ± 0.7
8	2.0	120	72.7 ± 1.7	3.1 ± 0.70	5.06 ± 0.9	19.4 ± 1.2
9	2.0	180	69.8 ± 0.9	4.41 ± 0.9	5.2 ± 0.65	21.7 ± 1.9

Values are given as mean ± standard deviation of triplicate



Protein (%)

Fat (%)

Figure 2. Protein level of FPH prepared by using different concentration and hydrolysis time of alcalase

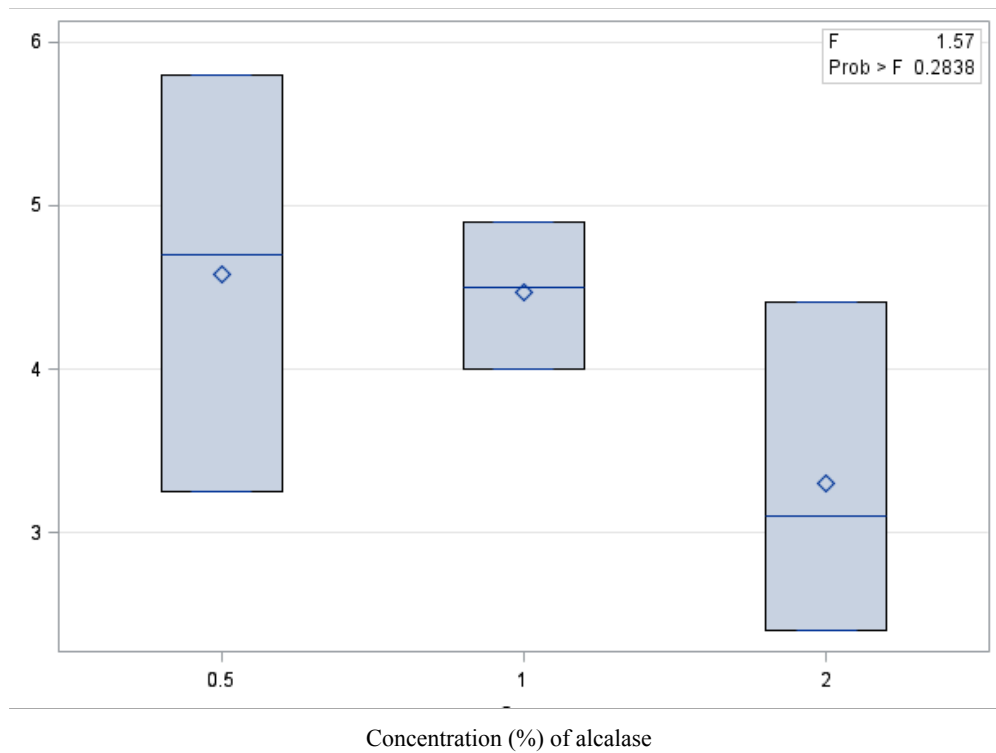


Figure 3. Fat level of FPH prepared by using different concentration and hydrolysis time of alcalase

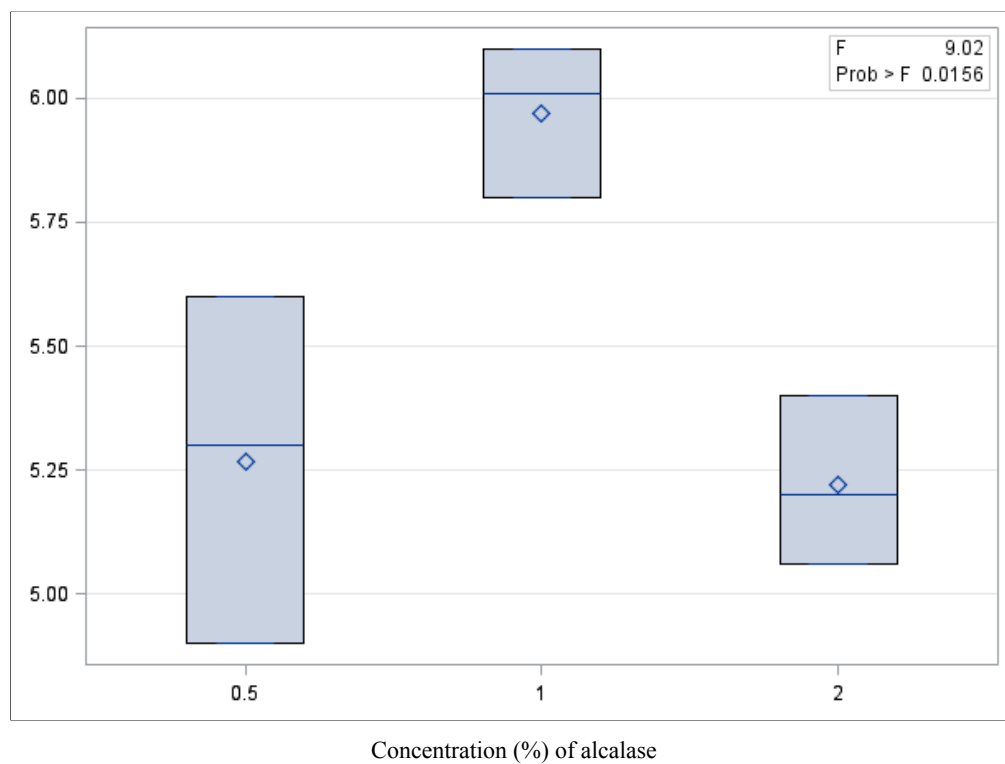
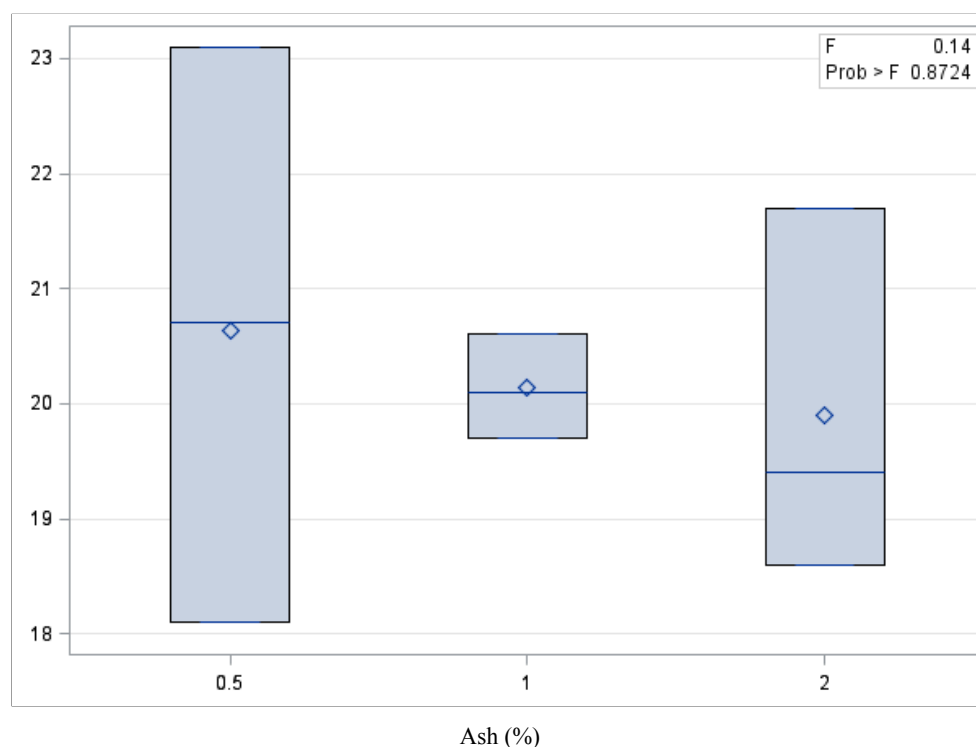


Figure 4. Moisture level of FPH prepared by using different concentration and hydrolysis timing of alcalase

Moisture (%)



Concentration (%) of alcalase

Figure 5. Ash content of FPH prepared by using different concentration and hydrolysis time of alcalase

Degree of hydrolysis

The degree of hydrolysis was determined as percentage of protein nitrogen in the hydrolysate with respect to the total nitrogen content of original nitrogen sample. The protein degradation extract using alcalase enzyme was measured by analysing the degree of hydrolysis of mackerel was found to be maximum at the concentration of 2 % as 23.12 ± 2.15 %.

The degree of hydrolysis of mackerel FPH was maximum at 180 min. of hydrolysis time with of various enzyme concentrations. The results indicate that use of 0.5 % alcalase for extraction of FPH at different

hydrolysis time i.e. 60, 120 and 180 minutes then degree of hydrolysis was recorded as 08.75 ± 0.35 , 10.08 ± 0.50 and 13.76 ± 1.00 respectively. When 1 % alcalase was used for extraction of FPH at different hydrolysis time the degree of hydrolysis was recorded as 11.14 ± 0.50 , 13.13 ± 0.85 and 14.76 ± 1.2 respectively. Similarly, when 2 % alcalase was used for extraction of FPH at different hydrolysis time the then degree of hydrolysis was recorded as 18.34 ± 0.90 , 21.14 ± 1.45 and 23.12 ± 2.15 respectively (Table 4 and Figure 5). There was a statistically significant difference ($\alpha = 0.05$) in the effect of concentrations on the degree of hydrolysis (%).

Values are given as mean $\pm$ standard deviation of triplicate

Figure 6. Degree of hydrolysis of FPH using different alcalase concentrations and hydrolysis time

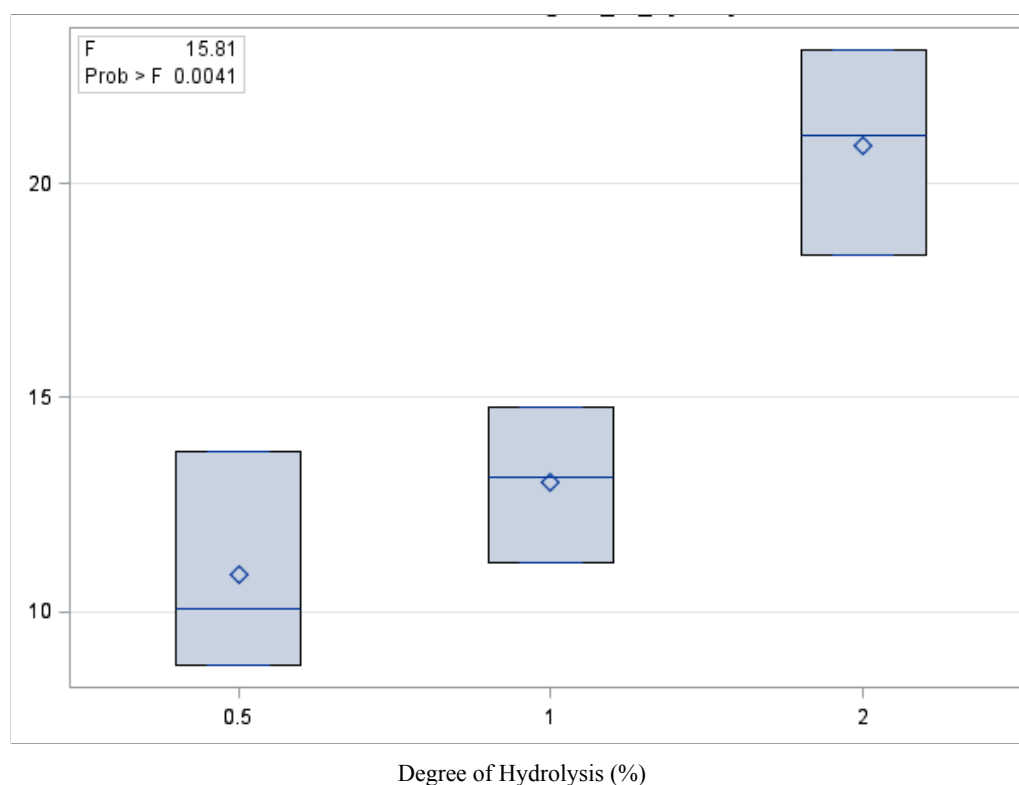


Table 4. Degree of hydrolysis of FPH using different alcalase concentrations and hydrolysis time

Sr. No.	Conc. of enzyme (%)	Hydrolysis time (minutes)	Degree of Hydrolysis (%)
1	0.5	60	08.75 ± 0.35
2	0.5	120	10.08 ± 0.50
3	0.5	180	13.76 ± 1.00
4	1.0	60	11.14 ± 0.50
5	1.0	120	13.13 ± 0.85
6	1.0	180	14.76 ± 1.2
7	2.0	60	18.34 ± 0.90
8	2.0	120	21.14 ± 1.45
9	2.0	180	23.12 ± 2.15

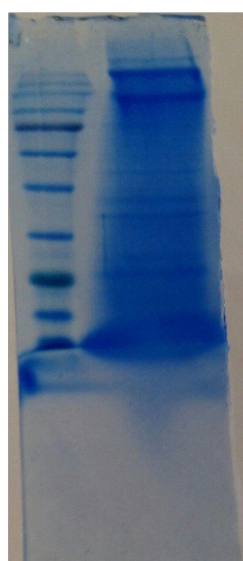
Protein and peptide pattern of FPH

Results were recorded better for 2% alcalase at 180 minutes hydrolysis time as compared to 0.5 % and 1% enzyme concentrations at 60 and 120 minutes hydrolysis time used for extraction of hydrolysate on the basis of hydrolysate yield and degree of hydrolysis. Therefore, this hydrolysate sample was used for further studies of antioxidant activity and effect of FPH on microbial growth.

The molecular weight pattern of peptides of FPH (2% alcalase at 180 minutes hydrolysis time) against the molecular weight markers each shown in figure-7. The protein pattern of FPH was analysed by 10% resolving gel and 5% stacking gel. Molecular weight pattern from mackerel FPH showed various bands with molecular weight in between 50 to 180kDa in FPH pattern.

FPH Lane

Protein marker lane



25

53
100
180
11
kDa

Figure 7. SDS- PAGE patterns of FPH of mackerel

Lane 1 marker standard protein, carbonicunhydus (30 kDa), Ovalbum (45 kDa), Serum albumin (66 kDa), Phosphorylase – b (96 kDa), β- galactosidase (120 kDa), myosin (200 kDa), lane 2 is FPH.

Antioxidant activity of FPH

Fish protein hydrolysate of 2% alcalase at 180 minutes hydrolysis time was produced using enzymes that can be determined for the antioxidant activity. The antioxidant activity of FPH of mackerel was estimated by DPPH assay and reducing power. Reducing power of hydrolysate was determined for the concentration of 1 mg/ml to 5 mg/ml and concentration of 5 mg/ml showed maximum reducing power. Reducing power of fish protein hydrolysate is shown in Table-5 and Figure-8. Result of DPPH radical scavenging ability was determined of the FPH at 1 mg/ml to 5 mg/ml concentration. The scavenging activity was recorded maximum at concentration of 5 mg/ml. The scavenging effect increased with the increasing concentration of hydrolysate.

Table 5. Antioxidant activity of FPH

Sr. No.	Concentrations (mg/ml)	Reducing power (%)	DPPH free radical Scavenging activity (%)
1	1	0.35	34.12
2	2	0.60	39.80
3	3	0.90	52.15
4	4	1.2	63.35

(continued on next page)

5

5

1.4

70.14

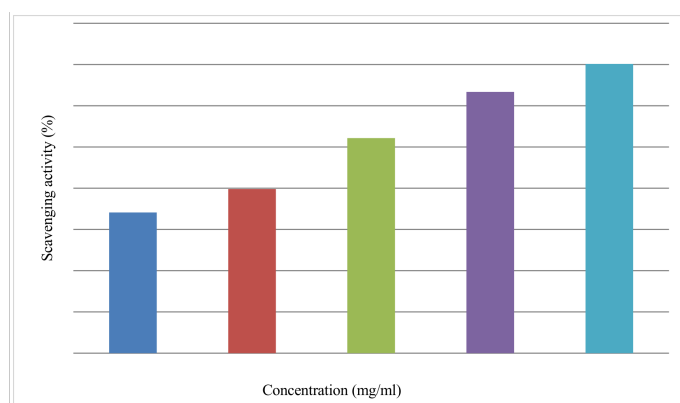


Figure 8. Reducing power of FPH

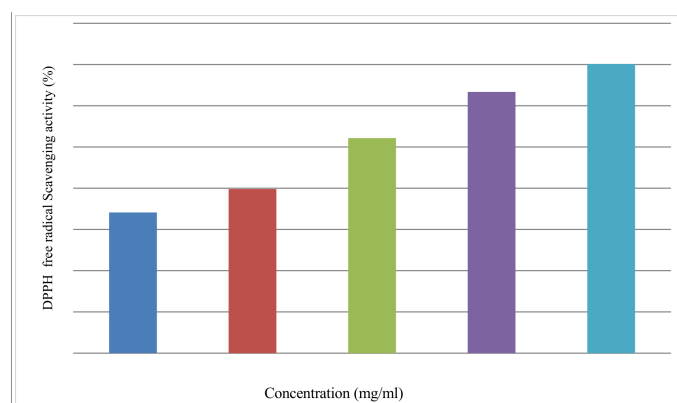
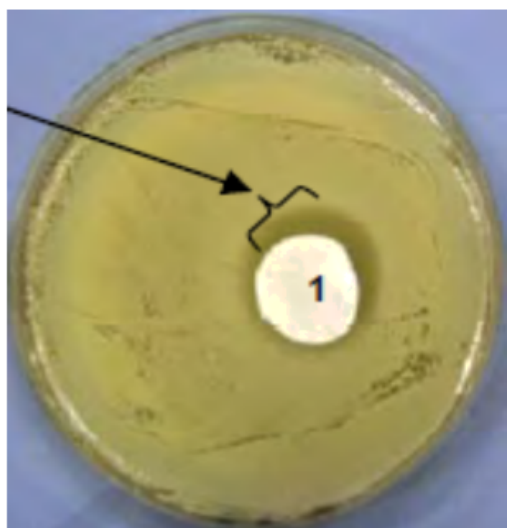


Figure 9. DPPH free radical scavenging power of FPH

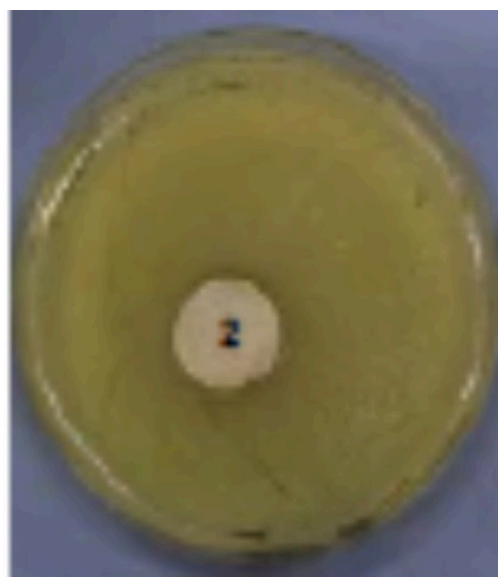
Effect of FPH on microbial growth

Fish hydrolysate had not affected antimicrobial activity against Gram negative bacteria (*Pseudomonas spp.* and *Escherichia coli*). When tested against Gram positive bacteria, slightly inhibited *Staphylococcus aureus* (Figure-10).

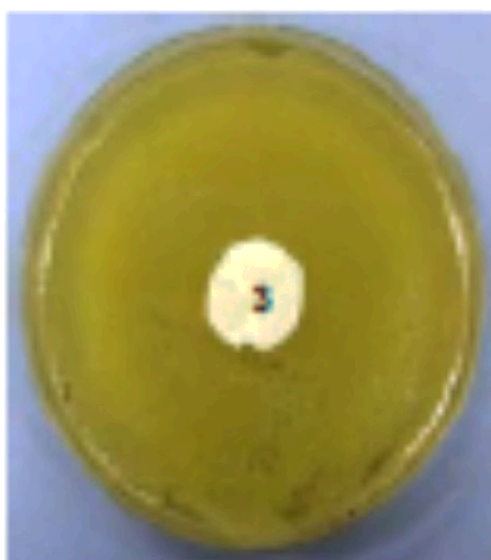
Zone of inhibition



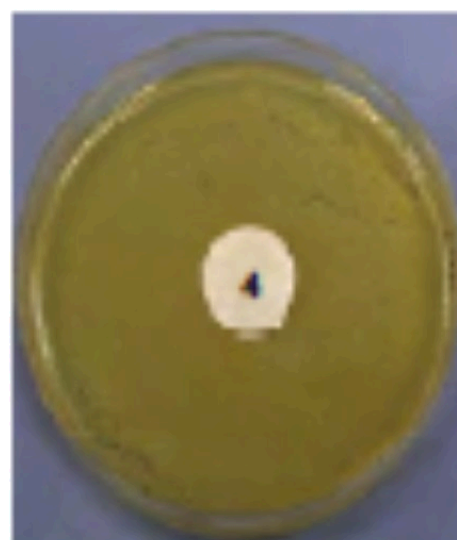
A



B

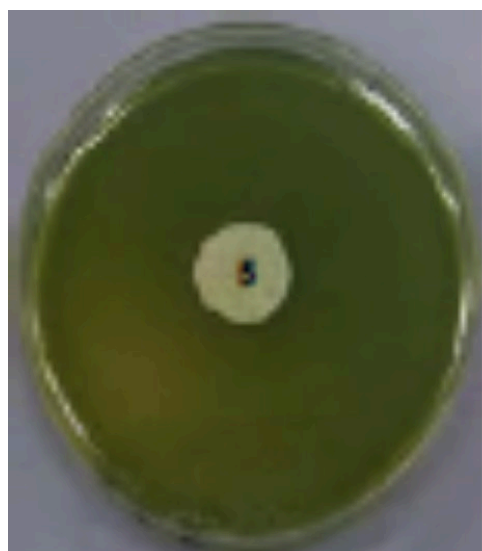


C

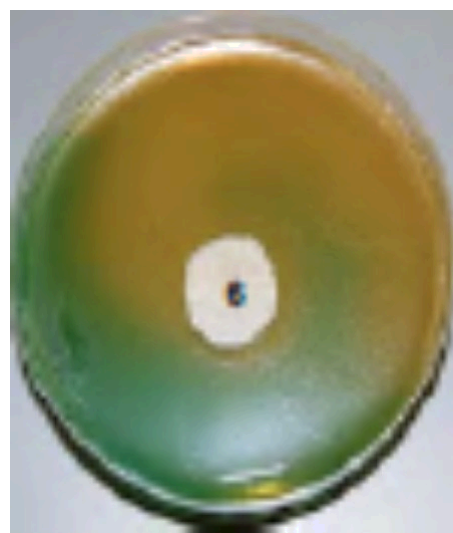


D

(continued on next page)



E



F

Figure 10. Zone of inhibition obtained in *anti*-bacterial disc diffusion assay. Discs 1, 3 & 5 were soaked in hydrolysates from mackerel muscle for 24 h and discs 2, 4 & 6 were soaked in distilled water (control). A & B assay against *S. aureus*, C & D assay against *E. coli* and E & F assay against *Pseudomonas spp.*

Discussion

Preparation of fish protein hydrolysates and yield

It is known that huge amount of protein rich materials from seafood processing plants are discarded without any attempts on recovery of protein. At the same time fish processors are no longer allowed to discard their offal directly into the ocean, resulting in a very high cost of refining the material before it is discarded. To meet the need of the seafood processing industry, an alternative to discarding this offal should be developed. Recovery and alteration of the fish muscle proteins and using these as functional ingredients in food is a very exciting and promising alternative. By applying enzyme technology for protein recovery in fish processing, it may be possible to produce a broad spectrum of food ingredients or industrial products for a wide range of applications. The present study involved fish protein hydrolysate (FPH) preparation from mackerel (*R. kanagurta*) using commercial enzyme alcalase. The hydrolysis condition was maintained at pH 7.5, temperature 55 °C for different hydrolysis durations and enzyme concentrations. The yield of fish protein hydrolysate (FPH-A) from croaker (*Otolithus ruber*) was 8%. Typical yield of FPH have been reported between 10-15% based on fresh fish wet weight basis (Hale, 1972 ;Quagila *et al.*, 1990) [15, 16]. Higher yield obtained in present study when concentration of alcalase enzyme was 2 % and hydrolysis time 180 minutes was 18.36 % and lower yield reported when 0.5 % alcalase and 60 minutes time of hydrolysis was 8.42. The present study revealed that yield of FPH was depends on time of hydrolysis and alcalase concentration. Higher yields were obtained with higher concentration of enzyme and hydrolysis time. Slizyte *et al.*, (2015), reported that yield of fish protein hydrolysate from cod using various enzyme i.e. trypsin, papain and bromelain etc. similar tendency was observed. Hoyle *et al.* (1994) [17] obtained total yield of herring hydrolysate between 2.9 to 4.8% with papain at 23.84 - 43.14% DH and 3.6 to 5.5% concentration with alkalase at 26.8 - 4.7% DH. Red hake hydrolysate had typical yield between 13.5 to 15.2% (Imm *et al.*, 1999) [18], however Sripathy *et al.* (1964) obtained about 8% total yield from *Barbus dubius* hydrolysate using papain.

Proximate composition of FPH prepared using different concentration and time using alcalase enzyme

Table-3 showed the protein, fat, moisture and ash content of fish protein hydrolysate prepared using different time period of hydrolysis i.e. 60 minutes and 0.5 % concentration from mackerel was 72.14%, 3.25 %, 5.6 % and 18.1 % respectively. In present study, as per increasing hydrolysis time and concentration of enzyme, protein and moisture decreases and fat and ash increases. Same results were reported in 1 %, 2 % concentrations of enzyme and 120, 180 minutes hydrolysis time. The protein content reported for fish protein hydrolysate is due to solubilization of proteins during hydrolysis and removal of insoluble solid matter by centrifugation Chalamaiah *et al.* (2010) [19]. Similar trend was reported in the sardine hydrolysates Souissi *et al.* (2007) [20] as well as pacific whiting muscle hydrolysate Pacheco-Aguilar *et al.* (2008) [21]. The amount of protein content in spray dryer sardine hydrolysate and freeze dried pacific whiting muscles hydrolysate were within the range of 72- 75 % and 85-88 % respectively. The low fat content of fish protein hydrolysate is due to the removal of lipids and insoluble protein fractions by centrifugation (Chalamaiah *et al.*, 2010) [19]. The low moisture content of protein hydrolysate is related to the type of sample and the effect of freeze drying. During the process, the sample loses most of its moisture (Bueno-Solano *et al.*, 2009) [22]. The low ash content of sample might due to the minimal addition of alkali required for pH adjustment and its control during the hydrolytic process.

Kristinsson *et al.* (2000) [23] and Motamedzadegan *et al.* (2010) [24] reported that, the reduction in lipid content in FPH in the present may be due to the exclusion in centrifugation and mostly due to lipid oxidation. However, the elimination of lipid can improve the stability and quality FPH products. Sawant (2008) [25], reported that moisture, protein, fat and ash values for fish protein hydrolysate (FPH-A) were 3.15%, 72.25%, 2.60% and 5.05% respectively. Crude protein content of FPH decreased as DH increased. The crude protein content of FPH- A (39.87% DH) was 72.25% while FPH-E (95.99% DH) has 59.25% protein. The moisture content ranged between 3.10% (FPH-A) and 3.65% (FPH-E). Fat content of FPH-A was 2.60% and FPH-E contained 4.37%. Ash content was in range of 5.05% (FPH-A) to 5.38% (FPH-E).

Benjakul *et al.* (1997) [26] prepared hydrolysate from pacific whiting solid waste which had moisture, protein, fat and ash content at the levels of 2.77%, 82.24%, 3.94% and 13.82% respectively. Warriar *et al.* (1996) [27] analyzed Dhoma hydrolysate for proximate composition i.e. moisture, protein, fat and ash obtained values at 3.54%, 90%, 5.00% and 3.20% respectively. Red hake hydrolysate contained 1.87% moisture, 77.18% protein, 6.10% lipid and 13.61% ash (Imm *et al.*, 1999) [18]. Herring hydrolysate had 3.98% moisture, 77.18%

protein, 0.77% lipid and 13.7% ash (Liceaga-Gesualdo *et al.*, 1999; Warriar *et al.*, 1996) [28, 27] reported 90% protein in FPH prepared from *Dhoma*.

Degree of hydrolysis of fish protein hydrolysate

The degree of hydrolysis was determined as percentage of protein nitrogen in the hydrolysate with respect to the total nitrogen content in the muscle. In present study, DH was estimated by adding different enzyme concentrations and carried out hydrolysis for different durations. With 0.5% enzyme concentration at for 60 min, 120 min, 180 min, the DH obtained was 8.75 %, 10.08 % and 13.26 % respectively, with 1 % enzyme concentration at reaction time for 60 min, 120 min, 180 min, the DH obtained was 11.14 %, 13.13 % and 14.76 % respectively and with 2 % enzyme concentration at reaction time for 60 min, 120 min, 180 min, the DH obtained was 18.34 %, 21.14 % and 23.12 % respectively was showed in Table -4 and Figure-6.

Enzymatic hydrolysis of fish protein generates a mixture of free amino acids, di-, tri-, and oligopeptides, increase the number of polar groups and the solubility of hydrolysate, and therefore modifies functional characteristics of protein, improving their functional properties. The chain length of peptides or breaking of linkage is dependent on extent of hydrolysis, concentration of enzyme and type of protein to be hydrolysed.

Wisuthipha, *et al.* (2016) studied, the effect of temperature and enzyme concentration on DH of fish protein hydrolysate produced by enzymes. For both papain and alcalase, the only factor that significantly affected DH was the concentrations of the enzyme. The result showed that DH increased as the enzyme concentration increased, while changes in temperature and reaction time had no significant effect on DH and the effect of acid concentration and temperature on DH of fish protein hydrolysate hydrolysed by acid. The result showed that temperature was the only factor that affected with an increase in temperature resulting in increased DH. The highest value for DH was in the range of 40-50 % when the temperature was 120°C, while an increase in acid concentration made no change in DH of fish protein hydrolysate of mackerel (*Decapterus maruadsi*).

Sheriff *et al.* (2014) [29] observed that DH was 19.86% for 1:100 enzyme substrate ratio, 21.74 % for 1.5:100 and 22.99% for 2:100. The rapid phase of proteolytic cleavage was observed in the first 60 minutes and a steady increase was recorded up to 180 minutes. Upon further hydrolysis, the rate of hydrolysis was nearly unchanged. Higher of proteolytic cleavage was 60 minutes of *R. Kanagurta*. This rapid increase in the hydrolytic process resulted in the proportional increase in DH during this period.

Martinez *et al.* (2005) [30] carried out hydrolysis of golden carp at maximum DH which was 43.6% with flavourzyme at pH 5.9 and temperature 53 °C. They observed that as pH and temperature increased DH decreased. Benjakul *et al.* (1997) [26] studied the effect of concentration of alcalase and neutrase on DH in pacific solid waste. They found that as enzyme concentration increased DH increased; however the progressive rate of DH and nitrogen recovery (NR) reduced with increase in enzyme concentration. They obtained 10% and 35% DH for neutrase and alcalase respectively for 10 AU/ kg enzyme concentration and it increased near to 15% to 55% for 110 AU/kg. They chose alcalase at concentration of 20 AU/kg for 1 h reaction which gave about 60-65% DH. In harp seal hydrolysate, the DH was influenced by both time and temperature of hydrolysis as well as type and concentration of enzyme employed. The DH after 3 h period at 30 AU/kg of alcalase gave 21.42% DH; at same condition neutrase did not show DH more than 16.19%. FPH with high DH possessed slight bitter taste, therefore DH of about 19.5% was recommended for preparation of fish protein hydrolysate to contain product with bland taste (Shahidi *et al.*, 1994; Imm *et al.*, 1999) [31, 18] studied the effect of endogenous protease, water/fish ratio on DH and change in DH according to type of enzyme. They observed that frame mince of red hake contained higher endogenous protease activity than headed and gutted mince, although its endogenous protease activity was not enough to liquefy the sample alone. Therefore, they added flavoen-

zyme and savorase to increase the yield. They did not observe much difference in DH according to water/fish ratio. In comparison of flavoenzyme and savorase, savorase yielded higher DH than flavourzyme.

Jeevitha *et al.* (2014) [32] reported that, the protein degradation extend using proteolytic enzymes was measured by analyzing the degree of hydrolysis. The degree of hydrolysis and yield of protein hydrolysate of *Sardinella longiceps* was found to be maximum at the concentration of 2 (v/w) as 25.12 ± 0.87%, 23.35 ± 0.45% respectively.

Herring hydrolysate made by using alcalase at pH 8 and 50 °C for 1 h yielded FPH with 36% DH (Liceaga-Gesualdo *et al.*, 1999; Hoyle *et al.*, 1994) [28, 17] prepared hydrolysate using raw herring, herring press cake and ethanol-extracted herring using alcalase and neutrase. They obtained higher DH with alcalase ranging from 26.8% in herring press cake to 44.7% in raw herring, while values ranged from 23.84% to 43.14% with papain. They observed that raw herring had 23% to 40% higher recovery of soluble nitrogen than ethanol extracted herring or herring press cake on hydrolysis with alcalase. Similarly, recoveries with papain were higher by 30% - 40%.

DH estimates the change of peptide content in a hydrolytic reaction. It is generally used as a proteolysis monitoring parameter and an important factor highly related with the hydrolytic process yield Adler-Nissen (1986) [33]. DH actually measures the extent of protein degradation by the respective enzymes in different proportions with that of the recovered protein hydrolysate. Higher the DH, more number of peptides would be produced in the solution. The increased production of peptides during hydrolytic reaction will result in the increased solubility of the protein and will increase the possibility of recovered protein to be used as food grade protein additive or replacer in various food preparations. Moreover, the cleavage of recovered protein would yield peptides of different sizes that are proven to be bioactive and antioxidant in nature Sampath *et al.* (2011) [34]. Many investigators have isolated peptides with antioxidant properties from the protein hydrolysates prepared from various parts of different fish species Najafian *et al.* (2012) [35]. Nalinanon *et al.* (2011) [36] has isolated an antioxidant peptide from the protein hydrolysate prepared with the muscle of ornate threadfin bream by skipjack tuna pepsin with 20% DH.

Sheriff *et al.* (2014) [29], measured values for DH at the end of 6 h were, 24.7% and 18.1% for pepsin and papain, respectively. The higher level of breakdown 14.3% for pepsin and 11.8% for papain was achieved during the first hour of digestion. After that, hydrolytic reaction proceeded gradually and steadied toward the end. This typical pattern of hydrolytic curve was obtained in many investigations by different investigators earlier (Guerard *et al.*, 2002; Sathivel *et al.*, 2003 and Kong *et al.*, 2007) [37, 38].

Difference in DH may be due to difference in hydrolysis procedures or conditions maintained for hydrolysis, such as enzyme concentration, hydrolysis time, pH and temperature.

Protein and peptide pattern of FPH

The SDS-PAGE was used to study the molecular weight distribution of hydrolysate after hydrolysis. Result showed that high molecular weight proteins in intact hydrolysate were degraded in to smaller peptides. Figure-7 shows the molecular weight pattern of peptides of FPH against the molecular weight markers. The protein pattern of FPH was analysed by 10% resolving gel and 5% stacking gel. Molecular weight pattern from Mackerel skin showed various bands, these bands with molecular weight in between 75 to 180kDa in FPH pattern.

Results were obtained better by using 2% alcalase and 180 minutes hydrolysis time as compared to 0.5 % and 1% enzyme concentrations and 60 and 120 minutes hydrolysis time used for extraction of hydrolysate on the basis of hydrolysate yield and degree of hydrolysis. SDS-PAGE was mainly used for the detection of low molecular peptides the result showed no distinct low molecular weight peptides other than some faint smearing that appeared in the 200 – 17 kDa region.

The molecular pattern of the samples has been notified through SDS-PAGE, and the results show that the samples have low molecular weight. The banding pattern in the lane second indicates that the samples possess low molecular weight protein as the bands coincide with bands of the marker. This indicates the molecular weight of the samples lie in the range of 50-180 kDa due to the extent of protein hydrolysis was supported by the disappearance of large proteins in the electrophoretic profile of the protein hydrolysates. The molecular weight peptides in the protein hydrolysates indicating the efficacy of these protein hydrolysates as a source of bioactive peptides (Bhaskar *et al.*, 2008) [39].

Galla *et al.* (2012) [40] studied, roe protein concentrates that contained protein bands between 205 and 30 kDa. A thick protein fraction at 103 kDa was observed in the soluble and insoluble portions of Alaska pollock roe (Bechtel *et al.*, 2007) [41]. Four abundant protein bands with molecule weight between 40 and 100 kDa were seen in spray dried catfish roe protein powder (Sathivel *et al.*, 2003) [38]. A similar SDS-PAGE profile with lower molecular weight protein (10 kDa) was observed in meriga roe hydrolysate (Chala-maiah *et al.*, 2010) [19].

Antioxidant activity of fish protein hydrolysate

Protein hydrolysate are produced using enzymes have different sized peptides that can determine the antioxidant activity. The antioxidant activity of FPH of *R. kanagurta* was estimated by DPPH assay and reducing power. In the present study detail result had shown in Table-5 and Figure 8-9. Antioxidant power of the compound is determined by their capability to reduce $\text{Fe}^{(3+)}$ to $\text{Fe}^{(2+)}$, in present study, reducing power of hydrolysate was determined for the different concentrations, at the concentrations of 5% mg/ml, it has shown maximum reducing power. DPPH is a stable free radical that shows maximum absorbance at 517 nm. DPPH assay works based on the reduction of DPPH in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H^+ by the reaction (Shimada *et al.*, 1997) [42]. Figure-8 shows the results of the DPPH radical scavenging ability of the fish hydrolysates at different concentration. The scavenging effect increased with the increasing concentration of hydrolysates was observed, this results indicates a dose dependent activity. Protein hydrolysate with the highest DH values produced with alcalase has shown higher scavenging activity of 70.14 % at 5 mg/ml. Similar result was reported for scavenging activity by Jeevitha *et al.* (2014) [32]. Sheriff *et al.* (2014) [29] reported that, radical scavenging ability of pepsin and papain derived protein hydrolysate of Indian mackerel and it was found to be a maximum of 46% and 36%, respectively. The radical scavenging ability increased with an increase in the concentration of hydrolysate. These values were well above in case of pepsin and equal for papain to that of synthetic antioxidant BHT in comparison. Similar findings were reported in earlier study in frog skin (Qian *et al.*, 2008) [43] and *Exocoetus volitans* muscle (Shabeena *et al.*, 2010) [44]. The peptide fraction exhibited higher scavenging activity $P < 0.05$ over papain fraction, which was also observed in the backbones of big eye tuna (*Thunnus obesus*) muscle by Je *et al.*, (2008) [5].

Wu *et al.* (2003) [45] reported that the scavenging effect for the hydrolysate increased slightly in the first 5 h, and remained unchanged during the elongated period of hydrolysis, the reducing power of both hydrolysis by autolysis and with protease N increase gradually with the increasing hydrolysis time.

Slizyte *et al.* (2016) [46], reported that the highest DPPH ability was shown in FPH obtained using a mixture of bromelain + papain (BrP) and trypsin FPH is the lowest DPP scavenging ability were made using seabzyme and protex 6L. the effect of both enzyme and hydrolysis time of hydrolysis of the FPH was also obtained in other studies (Bougatf *et al.*, 2010) [47].

In present study, result of DPPH radical scavenging ability of the FPH and the scavenging effect increased with the increasing concentration of hydrolysate was observed.

Effect of FPH on microbial growth

In present study the fish protein hydrolysate have an antibacterial activity, which may inhibit the growth of gram positive. Lehner *et al.* (2005) [48] similar results reported in their experiment of FPH on microbial growth. They reported no fish protein hydrolysate had antimicrobial activity against gram negative bacteria (*Pseudomonas spp.* and *E. coli*). When tested against gram positive bacteria, hydrolysate obtained from mackerel after hydrolysis with alcalase, slightly inhibited *Staphylococcus aureus*. It is known that gram negative bacteria are more resistant to antibacterial activity than gram positive bacteria due to the difference in the structure of their cell wall. Aspmo *et al.* (2005) [49] reported that, no fish protein hydrolysate had antimicrobial activity against gram negative bacteria (*Pseudomonas spp.* and *E. coli*). When tested against gram positive bacteria, hydrolysate obtained from mackerel after hydrolysis with alcalase, slightly inhibited *Staphylococcus aureus*.

References

1. Ayyappan S. Handbook and fisheries and aquaculture. New Delhi: Indian council of Agricultural Research; 2011.
2. CMFRI. Annual Report- 2017. Kochi, India: Central Marine Fisheries Research Institute; 2017.
3. Harnedy A, Fitzgerald RJ. Bioactive peptides from marine processing waste and shellfish: a review. Journal of Functional Foods. 2012;4:6-24.
4. Rasmussen R, Morrissey MT. Marine Biotechnology for Production of Food Ingredients. Advances in Food and Nutrition Research. 2007;52:237-292.
5. Je JY, Qian ZI, Lee SH, Byun HG, Kim SK. Purification and antioxidant properties of bigeye tuna (*Thunnus obesus*) dark muscle peptide on free radical-mediated oxidative systems. Journal of Medicinal Food. 2008;11(4):629-637.
6. Liu Z, Dong S, Xu J, Zeng M, Song H, Zhao Y. Production of cysteine-rich antimicrobial peptide by digestion of oyster (*Crassostrea gigas*) with alcalase and bromelin. Food Control. 2008;19:231-235.
7. Li-Chan ECY, Hunag SL, Jao CL, Ho KP, Hsu KC. Peptides derived from Atlantic salmon skin gelatin as dipeptidyl-peptidase IV inhibitors. Journal of Agricultural and Food Chemistry. 2012;60:973-978.
8. Picot L, Bordenave S, Didelot S, Fruitier-Arnaudin I, Sannier F, Thorkelsson G, Bergé JP, Guérard F, Chabaud A, Piot JM. Antiproliferative activity of fish protein hydrolysates on human breast cancer cell lines. Process Biochemistry. 2006;41(5):1217-1222.
9. Yang R, Zhang Z, Pei X, Han X, Wang J, Wang L, Long Z, Shen X, Li Y. Immunomodulatory effects of marine oligopeptide preparation from Chum Salmon (*Oncorhynchus keta*) in mice. Food Chemistry. 2009;113:464-470.
10. Ramkrishnan VV, Ghaly AE, Brooks MS, Budge SM. Extraction of oil from Mackerel fish processing waste using Alcalase Enzyme. Enzyme Engineering. 2013;2:115.
11. Tang CH, Peng J, Zhen DW, Chen Z. Physicochemical and antioxidant properties of buckwheat (*Fagopyrum esculentum* Moench) protein hydrolysates. Food Chemistry. 2009;115:672-678.
12. Schagger H. Tricine-SDS-PAGE. Nature Protocols. 2006;1:16-22.
13. Laemmli UK. cleavage of structural proteins during the assembly of the head of Bacteriophage T4. Nature. 1970;227:680-685.
14. Sorensen BK, Hojrup P, Ostergard E, Jorgensen CS, Enghild J, Ryder LR, Houen G. Silver staining of proteins on electro blotting membranes and intensification of silver staining of proteins separated by polyacrylamide gel electrophoresis. Analytical Biochemistry. 2002;304:33-41.

15. Hale MB. Making fish protein concentrates by enzymatic hydrolysis. A status report on research and some processes and products studied by NMFS. Seattle, WA: NOAA Technical Report NMF SSRF-657; 1972.
16. Quagila G, Orban E. Influence of enzymic hydrolysis on structure and emulsifying properties of sardine (*Sardina pilchardus*) protein hydrolysate. *Journal of Food Science*. 1990;55(6):1571-1573.
17. Hoyle NT, Merritt JH. Quality of fish protein hydrolysate from herring (*Clupea harengus*). *Journal of Food Science*. 1994;59:76-79.
18. Imm JY, Lee CM. Production of seafood flavor from red hake (*Urophycis*) by enzymatic hydrolysis. *J. Agric. Food Chem.* 1999;47:2360-2366.
19. Chalamaiah M, Narsing Rao G, Rao DG, Jyothirmayi T. Protein hydrolysates from Meriga (*Cirrhinus mrigala*) egg and evaluation of their functional properties. *Food Chemistry*. 2010;120:652-657.
20. Souissi N, Bougatef A, Triki-Ellouz Y, Nasri M. Biochemical and functional properties of *Sardinella* (*Sardinella aurita*) by-product hydrolysates. *Food Technology and Biotechnology*. 2007;45:187-194.
21. Pacheco-Aguilar R, Mazorra-Manzano MA, Ramirez-Suarez JC. Functional properties of fish protein hydrolysates from Pacific whiting (*Merluccius productus*) muscle produced by a commercial protease. *Food Chemistry*. 2008;109(4):782-789.
22. Bueno-Solano C, Lopez-Cervantes J, Campas-Baypoli ON, Lauterio-Garcia R, Adan-Bante NP, Sanchez-Machado DI. Chemical and biological characteristics of protein hydrolysates from fermented shrimp by-products. *Food Chemistry*. 2009;112:671-675.
23. Kristinsson HG, Rasco BA. Fish protein hydrolysate: production, biochemical and functional properties. *Critical Review on Food Science and Nutrition*. 2000;40(1):43-81.
24. Motamedzadegan A, Davarniam B, Asadi G, Abedian A, Ovissipour M. Optimization of enzymatic hydrolysis of yellow fin tuna, *Thunnus albacares viscera* using neutrase. *International Aquatic Research*. 2010;2(3):173-81.
25. Sawant DV. Preparation of enzymic fish protein hydrolysate from Croaker (*Otolithus ruber*). M.F.Sc thesis submitted to Dr. Balasaheb Sawant Kokan Krishi Vidhyapeeth, Dapoli, Maharashtra, India. 2008.
26. Benjakul S, Morrissey MT. Protein hydrolysates from Pacific whiting solid wastes. *Journal of Agricultural and Food Chemistry*. 1997;45(9):3423-3430.
27. Warriar SB, Ghadi SV, Ninjoor V. Functional and nutritional properties of fish protein hydrolysate from *Dhoma* (*Johnius dussumieri*). *Fishery Technology*. 1996;3(2):101-106.
28. Liceaga-Gesualdo AM, Li-Chan ECY. Functional properties of hydrolysates from herring (*Clupea harengus*). *Journal of Food Science*. 1999;64:1000-1004.
29. Sheriff SA, Balasubramanian S, Baranitharan R, Ponmurugan P. Synthesis and in vitro antioxidant functions of protein hydrolysate from backbones of *Rastrelliger kanagurta* by proteolytic enzymes. *Saudi Journal of Biological Sciences*. 2014;21:19-26.
30. Martinez I, Bathen T, Standal IB, Halvosen J, Aursand M, Gribbestad IS, Axleson DE. Bioactive compounds in Cod (*Gadus morhua*) products and suitability of ¹H NMR metabolite profiling for classification of the products using multivariate data analyses. *Journal of Agricultural and Food Chemistry*. 2005;53:6889-6895.
31. Shahidi F, Synowiecki J, Balejko J. Proteolytic hydrolysis of muscle protein of harp seal (*Phocagroen landica*). *Journal of Agricultural Food Chemistry*. 1994;42:2634-2638.
32. Jeevitha K, MohanaPriya K, Khora SS. Antioxidant activity of Fish Protein Hydrolysates from *Sardinella longiceps*. *International Journal of Drug Development and Research*. 2014;6(4):137-145.
33. Adler-Nissen J. Enzymatic hydrolysis of food protein. New York: Elsevier, Applied Science Publishers; 1986.
34. Sampath KNS, Nazeer RA, Jaiganesh R. Purification and biochemical characterization of antioxidant peptide from horse mackerel (*Magalaspis cordyla*) viscera protein. *Peptides*. 2011;32:1496-1501.
35. Najafian L, Babji AS. A review of fish derived antioxidant and antimicrobial peptides: Their production, assessment, and applications. *Peptides*. 2012;33:178-185.
36. Nalinanon S, Benjakul S, Kishimura H, Shahidi F. Functionalities and antioxidant properties of protein hydrolysates from the muscle of ornate threadfin bream treated with pepsin from skipjack tuna. *Food Chem.* 2011;124:1354-1362.
37. Guerard F, Guimas L, Binet A. Production of tuna waste hydrolysates by a commercial neutral protease preparation. *Journal of Molecular Catalysis B: Enzymatic*. 2002;19-20:489-498.
38. Sathivel S, Bechtel PJ, Babbitt J, Smiley S, Crapo C, Reppond KD. Biochemical and functional properties of herring (*Clupea harengus*) by-product hydrolysates. *Journal of Food Science*. 2003;68:2196-2200.
39. Bhaskar N, Benila T, Radha C, Lalitha RG. Optimization of enzymatic hydrolysis of visceral waste proteins of catla (*Catla catla*) for preparing protein hydrolysate using a commercial protease. *Bioresource Technology*. 2008;99:335-343.
40. Galla NR, Pamidighantam PR, Akula S, Karakala B. Functional properties and in vitro antioxidant activity of roe protein hydrolysates of *Channa striatus* and *Labeo rohita*. *Food chemistry*. 2012;135(3):1479-1484.
41. Bechtel PJ, Chantarachoti ACM, Sathivel S. Characterization of protein fractions from immature Alaska Walleye Pollock (*Theragra chalcogramma*) roe. *Journal of Food Science*. 2007;72(5):S338-S343.
42. Shimada KK, Fujikawa K, Yahara Nakamura T. Antioxidative properties of xanthan on the antioxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry*. 1997;40:945-948.
43. Qian ZJ, Jung WK, Kim SK. Free radical scavenging activity of a novel antioxidative peptide purified from hydrolysate of bull frog skin *Rana catesbeiana* Shaw. *Bioresources Technology*. 2008;99:1690-1698.
44. Shabeena YN, Nazeer RA. Antioxidant activity of hydrolysates and peptide fractions of *Nemipterus japonicus* and *Exocoetus volitans* muscle. *Journal of Aquatic Food Product Technology*. 2010;19:182-192.
45. Wu HC, Chen HM, Shiau CY. Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomberus triasicus*). *Food Research International*. 2003;36(9):949-957.
46. Rasa Slizyte, Katariina R, Revilija M, Peter E, Kathrine F, Turid R. Bioactivities of fish protein hydrolysates from defatted salmon backbones. *Biotechnology Reports*. 2016;11:99-109.
47. Bougatef N, Nedjar-Arroume L, Manni R, Ravallec A, Barkia D, Guillochon M, Nasri. Purification and identification of novel antioxidant peptides from enzymatic hydrolysates of sardine (*Sardinella aurita*) by-products proteins. *Food Chemistry*. 2010;118:559-565.
48. Lehner A, Loy A, Behr T, Gaenge H, Ludwig W, Wagner M, Schleifer KH. Oligonucleotide microarray for identification of *Enterococcus* species. *FEMS Microbiology Letters*. 2005;246:133-142.
49. Aspino SI, Horn SJ, Eijssink VGH. Hydrolysates from Atlantic cod (*Gadus morhua* L.) viscera as components of microbial growth media. *Process Biochemistry*. 2005;40:3714-3722.

50. Bordenave S, Fruitier I, Ballandier I, Sannier F, Gildberg A, Batista I, Piot M. HPLC preparation of fish waste hydrolysate fractions. Effect on guinea pig ileum and ACE activity. *Preparative Biochemistry and Biotechnology*. 2002;32(1):62-77.
51. Kong X, Zhou H, Qian H. Enzymatic hydrolysis of wheat gluten by proteases and properties of the resulting hydrolysates. *Food Chemistry*. 2007;102:759–763.
52. Lehner A, Riedel K, Eberl L, Breeuwer P, Diep B, Stephan R. Biofilm formation, extracellular polysaccharide production, and cell-to-cell signaling in various *Enterobacter sakazakii* strains: aspects promoting environmental persistence. *Journal of Food Protection*. 2005;68(11):2287-2294.
53. Sheriff SA, Balasubramanian S, Baranitharan R, Ponmurugan P. Production and antioxidant properties of protein hydrolysate from *Rastrelliger kanagurta* (Indian mackerel). *Pakistan Journal of Pharmaceutical Sciences*. 2014;27(5):1295-1302.

How to cite this article: Sonavane AE, Hatte VM. Production, characterization and bioactivity evaluation of protein hydrolysates from Indian mackerel (*Rastrelliger Kanagurta*) using commercial proteases. *International Journal of Agriculture Extension and Social Development*. 2026;SP-9(10):11-23. doi: <https://www.doi.org/10.33545/26180723.2026.v9.i10Sa.4359>