



ORIGINAL ARTICLE

OPEN ACCESS

Effect of different concentration of Pepsin Enzyme on extraction of Fish Protein Hydrolysate from Malabar Sole Fish(*Cynoglossus macrostomus*)

O. R. Bhingarde¹, J. M. Koli², A. E. Sonavane⁴, S. B. Patange², P.E. Shingare³, P.P. Relekar¹ and V.B. Mulye²

¹ Post Graduate Institute of Post Harvest Management, Killa - Roha, Raigad

² College of Fisheries, Shirgaon, Ratnagiri, Maharashtra

³ Taraporewala Marine Biological Research Station, Mumbai

⁴ College of Fishery Science, Jabalpur (NDVSU, Jabalpur) Madhya Pradesh

Corresponding author: jmkoli@gmail.com

ABSTRACT

*Fish protein hydrolysate (FPH) is a healthy and highly nutritive product produced hygienically from whole fish or fish waste in which, protein and other nutrients are more concentrated than fresh fishes. In the present study an attempt was made to study the functional properties and other parameters of FPH derived from Malabar sole fish (*Cynoglossus macrostomus*) so as to know the quality. The important findings are summarized as: Proximate composition of Malabar sole fish was observed to be moisture 75.13%, crude protein 15.73%, fat 2.2% and ash 2.43%. Proximate composition of FPH from Malabar sole fish (*Cynoglossus macrostomus*) using different pepsin concentrations (1, 1.5, 2, 2.5 and 3%) were observed moisture content as 7.40, 7.48, 7.06, 7.52 and 7.61% respectively; crude protein content as 63.50, 66.00, 73.00, 65.00 and 62.50% respectively; fat content as 1.64, 1.70, 1.25, 1.79 and 2.01% respectively; ash content as 2.25, 2.49, 3.65, 2.90 and 2.70% respectively. Functional properties of FPH were observed: solubility as 83.66, 84.83, 92.43, 89.46 and 90.23% respectively and was higher in 2% pepsin concentration. Emulsifying capacity, emulsifying stability, foaming capacity, foaming stability and water holding capacity showed higher range in the same. Parameters of FPH were observed pH in the range of 6.46-7.20; isoionic point as pH 5, 5, 7, 8 and 8 respectively. For enzymatic hydrolysis, among different concentrations of pepsin(1, 1.5, 2, 2.5 and 3%) 2% is more suitable resulting 22.63% degree of hydrolysis at 180min. Microbiological analysis (TPC) of FPH were observed in the range of 0.42×10^2 - 0.62×10^2 respectively. The observations of present study suggest that 2% pepsin was an ideal choice for better quality of FPH.*

Keywords: *Cynoglossus macrostomus, FPH, Pepsin Enzyme*

Received 09.04.2018

Revised 21.06.2018

Accepted 31.07. 2018

INTRODUCTION

Protein Hydrolysate are defined as proteins that are chemically or enzymatically broken down into free amino acids and/or peptides, which can present a large range of molecular weight depending on the greater or lesser degree of hydrolysis [19]. Nowadays, fish protein hydrolysate with good nutritional composition, amino acid profile, and antioxidant activities has gained great attention of food scientists. Protein Hydrolysate is used as readily available sources of protein for humans and animals due to their good functional properties.

Large amount of fish by-product are currently disposed or used for low-value products. There is a large potential for reducing the amount of by-product and to utilize a larger amount of the by-product for value added product for human consumption [11]. Every year, huge quantities of fishery wastes and by-products are generated by fish processing industries. Either these marine wastes are underutilized to produce low market value products such as fish meal, fish oil, fertilizers or simply dumped leading to environmental issues [26]. The development of fish protein hydrolysates and derived peptides as functional food ingredients have been relatively a recent technology gaining popularity due to the array of potential bioactive properties associated with them, including antioxidant, antihypertensive, immunomodulatory, neuroactive, antimicrobial, and mineral or hormone regulating abilities [4]. Unlike

Protein of vegetable origin, fish proteins are very unstable and much more sensitive to temperature, pH, salts and contact with polar solvents. This sensitivity may result in a substantial loss in functional properties of fish protein when prepared as dry protein concentrates or isolates for use in food ingredients [35].

There are a number of different proteolytic enzymes that can be used for the production of hydrolysates [36]. Enzymatic proteolysis and solubilization of proteins from various sources has been studied extensively and described by several different authors over the last 60 years [6]. Addition of proteolytic enzymes could make a hydrolytic process more controllable. Alcalase an alkaline bacterial protease produced from *Bacillus licheniformis* has been proven to be one of the best enzymes used in the preparation of fish protein hydrolysate [15]. Flavourzyme is a fungal protease/peptidase complex produced by submerged fermentation of a selected strain of *Aspergillus oryzae* which has not been genetically modified and is used for the hydrolysis of proteins under neutral or slightly acidic conditions. Flavourzyme has been used to produce a protein hydrolysate with acceptable functional properties [19].

In general, fish protein hydrolysates have improved physicochemical properties, such as oil binding capacity and emulsifying capacity, compared with intact fish protein. These improved properties enable fish protein hydrolysates to be used as functional food ingredients in many food products, such as meat products and spread-texture food. Protein hydrolysis has shown continuous development over time, but in a general context, this process is still in the early stages of discovering peptides and individual amino acid combinations to produce desired effects for different applications. The fish protein hydrolysates thus produced is widely used as nutritional supplements, functional ingredients, and flavor enhancers in food, beverage and pharmaceutical industries [16].

Annually large amount of marine fish are caught to use as a raw material in sea food industries leading to approximately 100,000 tons per year of fish by product are obtained from sea food process [25] including a lot of small fish that do not match the quality criteria and cannot be used in industrial process. These types of fish waste are usually be either discarded from fishery and aquaculture or sold as low valued products. These low valued fish contain valuable protein and essential amino acids. Therefore, hydrolysis of fish protein would be a proper strategy for economic gain under the consideration of fish processing waste into high value products with the improvement in both quality and quantity.

Malabar Sole fish (*Cynoglossus macrostomus*) belong to the family Cynoglossidae. The catch of sole fish in India during the year 2015 was 41,535 tons [10]. Malabar sole fish (*Cynoglossus macrostomus*), which is a low value fish, is the predominant fish among all the species of flatfishes landed along the west coast of India, in spite of paucity of targeted fisheries for the species. With the increase in targeted fisheries for shrimps, this species is also being heavily fished. [30]. Sole fish have been not that economically used fresh flesh. It is not used for culinary purpose on large scale and considered as waste. Good waste management practice leading to additional economic benefit. Therefore, the main aims of this research work are to determine the potential of Sole fish for the production of Fish Protein Hydrolysate and evaluating functional and biochemical properties.

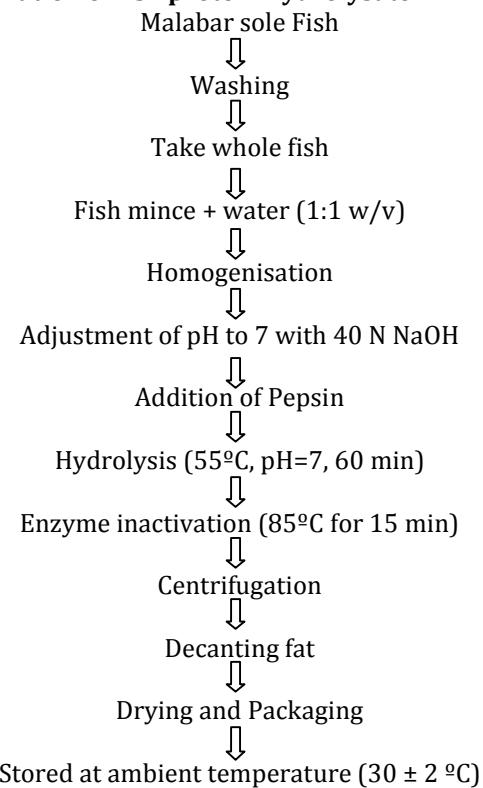
MATERIAL AND METHODS

Raw material

Malabar Sole Fish (*Cynoglossus macrostomus*) locally known as Lepa was collected from Ratnagiri fish market. The fish was washed and stored at - 20°C until further use.

Preparation of fish mince for Fish Protein Hydrolysate

The whole fish was washed in fresh water and kept in iced condition during processing. It was minced using a mixer. Fish mince was divided into 5 batches. All the batches include 200 gm fish mince and water in 1:1 proportion. Hydrolysate was carried out by adding Pepsin enzyme 1%, 1.5%, 2%, 2.5% and 3% respectively. Enzyme inactivation was done by increasing temperature to 85 °C for 15 min. For decantation of fat, the mixture was centrifuged and final supernatant was dried. The procedure of preparation of fish protein hydrolysate is mentioned in Flow 1.

Flow chart 1 Method for preparation of fish protein hydrolysate

Proximate composition: Proximate composition viz, Protein, Fat, Moisture, Ash were estimated according to AOAC [5].

pH of fish protein hydrolysate

About (5g) sample was ground with 45ml distilled water and filtered using a filter paper. The pH of filtrate was recorded using a PH meter [5].

Determination of degree of hydrolysis

The DH was determined by the Trinitrobenzene sulphonic acid (TNBS) technique described by Adler-Nissen [3] with slight modifications: A mixture of 10 ml of hydrolysate and 10 ml of 24% TCA and centrifuged at 12,100 × g for 5 min. From the supernatant, 0.2mL was mixed with 2mL of 0.2 M Sodium borate buffer (pH 9.2) and 1.0mL of 2.0 N NaH₂PO₄ containing 18mM Na₂SO₃ was added. The absorbance was read at 420 nm, using a spectrophotometer.

$$DH (\%) = \frac{h}{h_{tot}} \times 100$$

Where,

h = percent ratio of peptide bond broken *h_{tot}*= total no of bond per unit weight (8.6 meq/g)

Emulsifying capacity and stability

The method of Yasumatsu *et al.*, [38] with a slight modification was used to determine emulsifying capacity and stability. Emulsions were prepared with 1 g of each sample, 50 ml of cold distilled water (4° C) and 50 ml of sunflower oil. The samples were dispersed with a homogenizer/blender. Each blended samples was equally into 50 ml centrifuge tubes. One centrifuge tube was directly centrifuge at 4000 × g for 10 min while the other was centrifuged under the same conditions after heating in a water bath at 80° C for 30 min and cooling to room temperature (25° C). The height of emulsified layer, as a percentage of the total height of material in the unheated tubes, was used to calculate the emulsifying capacity and stability, using following formulae:

$$\text{Emulsifying Capacity} = \frac{\text{Height of emulsion layer}}{\text{Height of whole layer}} \times 100$$

$$\text{Emulsifying stability} = \frac{\text{Height of emulsion layer after heating}}{\text{Height of whole layer}} \times 100$$

Water holding capacity

Water holding capacity (WHC) was determined using the centrifugation method [11]. Duplicate samples (0.5 g) hydrolysate were dissolved in 20 ml of water in centrifuge tubes and dispersed, with a vortex mixer for 30s. The dispersion was allowed to stand at room temperature for 6 h, and then centrifuge at $2800 \times g$ for 30 min. The supernatant was filtered with Whatman No.1 filter paper and the volume recovered was measured. The difference between the initial volume of distilled water added to the protein sample and the volume of the supernatant was determined, and the result were reported as ml of water absorbed per gram of hydrolysate sample.

Determination of Colour

Colour measurement was made by using a Hunter Lab Scan XE colorimeter (Hunter Association Laboratory, Inc., VA, USA). The tristimulus L*a*b* measurement mode was used as it relates to the human eye response to colour. The L* variable represents lightness (L*=0 for black, L*=100 for white), the a* scale represents the red/green (+a* intensity of red and -a* intensity of green) and the b* scale represents the yellow/blue (+b* intensity of yellow and -b* intensity in blue). The samples were filled into clear Petri dish and readings were taken. Clarity was determined by measuring transmittance (%T) at 620 nm in spectrophotometer (Thermospectronic, Cambridge, U. K)

Determination of isoionic point (pI)

Isoionic point of FPH was determined according to the method described by Zhang et al., [44]. The pI was determined by measuring the transparency of 2 % (w/v) FPH solution with different pH values at 660 nm spectrophotometer (Thermospectronic, Instrument). The pH value at which the solution has the lowest transparency was the pI value of the FPH.

Determination of Foaming Capacity and Stability

The method of Miller and Goninger [21] was used to determine foaming properties. The FPH powder (1gm) was added to 100 ml of distilled water and homogenized for 1min. The mixture was carefully transferred into a 250 ml calibrated beaker for volume measurement. The foam was calculated as the volume of mixture after blending compared to the original volume. The foaming stability was the ratio of the foam capacity after 30 min divided by the original foam capacity.

Microbiological Analysis

Samples were analyzed for total plate count (TPC) as per the USFDA [36].

Statistical Method

The data was analysed using appropriate statistical methods [32]. Using ANOVA techniques significant difference between the treatments was determined. The significance of difference between means of treatments was further subjected to SNK test.

RESULTS AND DISCUSSION

The fish processing industry produces more than 60% by-products as waste, which includes head, skin, trimmings, fins, frames, viscera and roes, and only 40% fish products for human consumption. Several proteolytic enzymes are most commonly used to hydrolyse the fish proteins for the production of fish protein hydrolysates. With the advent of these enzymatic techniques, several fish protein hydrolysates are produced from various protein rich fish by-product wastes. In many countries, traditional and commercial preparations of fish protein hydrolysates are currently used as health nutraceutical food. The present study involved fish protein hydrolysate (FPH) preparation from Malabar Sole fish (*Cynoglossus macrostomus*) using Pepsin enzyme. Various functional and biochemical properties were studied and discussed in this chapter.

Proximate composition of Malabar Sole fish:

The composition of fish can generally be summarized as moisture 65-80%, protein 15-20%, fat 5-20% and ash 0.5-2%. The composition varies considerably depending on size, weight, water, temperature, state of spawning and feeding habits. Protein and ash content do not register much difference. Lipid content shows remarkable variation [14]. In present study the proximate composition of Malabar Sole fish were carried out i.e. moisture, protein, fat and ash as 75.13, 15.73, 2.2 and 2.43% respectively (Table 1).

Table 1. Proximate composition of Malabar Sole Fish (*Cynoglossus macrostomus*)

Malabar Sole Fish	Proximate Composition (%)
Moisture	75.13
Protein	15.73
Fat	2.2
Ash	2.43

Proximate composition of Fish Protein Hydrolysate extracted from Malabar Sole fish (*Cynoglossus macrostomus*) by using different Pepsin enzyme concentrations:

Moisture content

In present study, the moisture content of FPH extracted from Malabar sole fish by using 5 different Pepsin enzyme concentrations (1%, 1.5%, 2%, 2.5% and 3%) were found to be 7.40, 7.48, 7.06, 7.52 and 7.61% respectively (Table 2). The moisture content was significantly ($p<0.05$) different in 2% as compared to other concentrations. Yin et al. [39] reported that the moisture content of *Ictalurus punctatus* skin protein hydrolysate was 6.75% and similarly Sathivel et al. [28] derived FPH from *Oncorhynchus nerka* head using enzymes such as Alcalase, Flavourzyme 500L, Palatase 2000L, protex 6L, GC 106 and Neutrase and it resulted as 5.9%, 5.0%, 6.1%, 6.2%, 5.6% and 5.7% respectively. Most of the studies demonstrated that FPH from various fish proteins contain moisture below 10% [8].

Protein content

In present study, the protein content of FPH extracted from Malabar sole fish by using 5 different Pepsin enzyme concentrations (1%, 1.5%, 2%, 2.5% and 3%) were found to be 63.50, 66, 73, 65 and 62.50% respectively (Table 2). The protein content was significantly ($p<0.05$) higher in 2% as compared to other concentrations. High protein content of fish protein hydrolysates demonstrates its potential use as protein supplements for human nutrition.

The high protein content reported for fish protein hydrolysates is due to solubilisation of proteins during hydrolysis and removal of insoluble solid matter by centrifugation. [9]. Sawant et al. [30] also reported similar results of protein of FPH-A, FPH-B FPH-C FPH-D FPH-E as 72.25, 68.12, 64.81, 64.31 and 59.25% respectively. Shahidi et al. [31] reported that the protein content of *Mallotus villosus* protein hydrolysate using Alcalase, papain and Neutrase enzyme was 72.4%, 78.3% and 71.2% respectively and similarly Souissi et al. [33] using *Sardinella aurita* by-product protein hydrolysate using FPH1, FPH2, FPH3 using Alcalase enzyme with 3 different Degree of Hydrolysis percentage he found protein content 75.01%, 72.99% and 73.05% respectively. Sathivel et al. [28] derived *Oncorhynchus nerka* head protein hydrolysate using Alcalase, Flavourzyme 500L, Palatase 2000L, protex 6L, GC 106 and Neutrase enzymes and reported slightly low protein (%) i.e. 63.3%, 62.8%, 62.3%, 63.6%, 64.8% and 64.8% respectively.

Fat content

In present study, the fat content of FPH extracted from Malabar sole fish by using 5 different Pepsin enzyme concentrations (1%, 1.5%, 2%, 2.5% and 3%) were found to be 1.64, 1.70, 1.25, 1.79 and 2.01% respectively (Table 2). The fat content was significantly ($p<0.05$) different in 2% as compared to other concentrations. Abdul-Hamid et al. [2] reported that the fat content of *Oreochromis mossambicus* protein hydrolysates of Type-A and Type-B was 2.80% and 2.56% respectively and similarly Nilsang et al. [23] using Tuna by-product protein hydrolysate he found fat content 2.37% respectively. Sawant et al. [29] reported slightly higher results of fat of FPH-A, FPH-B FPH-C FPH-D FPH-E as 2.60, 4.20, 4.23, 4.33 and 4.37% respectively. Bhaskar et al. [8] derived *Catla catla* visceral protein hydrolysates and reported 1.94% fat content.

Ash content

In present study, the ash content of FPH extracted from Malabar sole fish by using 5 different Pepsin enzyme concentrations (1%, 1.5%, 2%, 2.5% and 3%) were found to be 2.25, 2.49, 3.65, 2.90 and 2.70% respectively (Table 2). The ash content was significantly ($p<0.05$) higher in 2% as compared to other concentrations. Foh et al. [13] reported that the fat content of *Oreochromis niloticus* meat hydrolysates of Fresh minced meat Hydrolysate (FMMH) as 2.25% and of hot water dip hydrolysate (HWDH) was slightly different and valued as 9.85% and similarly Souissi et al. [32] reported using *Sardinella aurita* by-product protein hydrolysates using FPH1, FPH2, FPH3 he found fat content 14.81, 13.06 and 12.10% respectively. Yin et al. [39] derived *Ictalurus punctatus* skin protein hydrolysate by Catfish skin soluble hydrolysates (CSSH) and Catfish skin insoluble hydrolysates (CSISH) and reported 3.07% and 1.89% fat content.

Table. 2. Proximate composition of Fish Protein Hydrolysate from Malabar Sole Fish (*Cynoglossus macrostomus*) by using different Pepsin concentrations

Proximate Composition	Pepsin Concentration in FPH				
	1%	1.5%	2%	2.5%	3%
Moisture	7.40 ± 0.05	7.48 ± 0.02	7.06 ± 0.17	7.52 ± 0.02	7.61 ± 0.05
Protein	63.50 ± 1.45	66.00 ± 0.88	73.00 ± 0.88	65.00 ± 1.73	62.50 ± 1.45
Fat	1.64 ± 0.03	1.70 ± 0.11	1.25 ± 0.02	1.79 ± 0.09	2.01 ± 0.05
Ash	2.25 ± 0.05	2.49 ± 0.02	3.65 ± 0.05	2.90 ± 0.17	2.70 ± 0.13

Functional properties of Fish Protein Hydrolysate extracted from Malabar Sole Fish (*Cynoglossus macrostomus*) extracted by using different Pepsin enzyme concentrations

Solubility

Solubility is one of the most important physicochemical and functional properties of protein hydrolysates [17]. Solubility of hydrolyzed protein in a broad pH range is one of the most desirable physicochemical and functional properties from which derived the rest of the functionalities (emulsifying and foaming) in a food system [26]. In present study solubility of FPH extracted from Malabar Sole fish (*Cynoglossus macrostomus*) by using different Pepsin enzyme concentrations (1, 1.5, 2, 2.5 and 3%) were found to be 83.66%, 84.83%, 92.43%, 89.46% and 90.23% respectively (Table 3). The solubility was significantly ($p<0.05$) higher at 2% enzyme concentration. Taheri et al. [33] reported that the solubility of poultry by-product protein hydrolysates (PPH) and rainbow trout protein hydrolysate prepared by alcalase enzyme had a maximum solubility of 96% and the least solubility was at 4pH and 5pH respectively. Sathivel et al. [27] reported a slightly different solubility of different herring hydrolysates, 85.1% of whole herring hydrolysate (WHH), 78.6% of herring body hydrolysate (HBH), 84.9% of herring head hydrolysate (HHH) and 56% of herring gonad hydrolysate (HGH).

Emulsifying capacity

Most processed foods contain oil which exists as an emulsion together with other constituents. The most frequent emulsion is an oil-water emulsion [23] in the form of spread-texture food such as vinaigrette, mayonnaise and hollandaise sauce. In present study emulsifying capacity of FPH extracted from Malabar Sole fish by using different Pepsin enzyme concentrations (1, 1.5, 2, 2.5 and 3%) were found to be 63.83%, 63.1%, 69.93%, 67.06% and 66.53% respectively (Table 3). The emulsifying capacity was significantly ($p<0.05$) higher at 2% enzyme concentrations. Kristinnson and Rasco, [18] reported that the Emulsifying capacity of Atlantic Salmon muscle protein hydrolysates prepared by using various Alkaline protease i.e alcalase, Flavourzyme, Corolase PN-L, Corolase 7089 and resulted as 192.51, 191.91, 222.14 and 234.79 in the unit of mL of oil/200g of protein. Foh et. al. [12] reported a slight change in Emulsifying capacity of tilapia fish muscle as 85.32%.

Emulsifying stability

In present study Emulsifying stability of FPH extracted from Malabar Sole fish by using different Pepsin enzyme concentrations (1, 1.5, 2, 2.5 and 3%) were found to be 59.90%, 59.20%, 64.83%, 61.13% and 61.16% respectively (Table 3). The emulsifying stability was significantly ($p<0.05$) higher at 2% enzyme concentrations. Kristinnson and Rasco [18] reported that the Emulsifying stability of Atlantic Salmon muscle protein hydrolysates prepared by using various Alkaline protease i.e alcalase, Flavourzyme, Corolase PN-L, Corolase 7089 and resulted as 70.3%, 67.3%, 68.0%, 70.2% and 69.7% respectively at 5% DH and 61.0%, 55.7%, 61.5%, 61.1% and 67.2% respectively at 10% DH. Sathivel et al. [33] reported Emulsifying stability using herring by-product hydrolysates, 48.6% of whole herring hydrolysate (WHH), 51.8% of herring body hydrolysate (HBH), 53.3% of herring head hydrolysate and 54.2% of herring gonad hydrolysate (HGH).

Foaming capacity

In present study foaming capacity of FPH extracted from Malabar Sole fish by using different Pepsin enzyme concentrations (1, 1.5, 2, 2.5 and 3%) were found to be 114.26%, 112.2%, 124.4%, 116.9% and 119.93% respectively. The foaming capacity was significantly ($p<0.05$) higher at 2% enzyme concentrations. Elavarasan et al. [11] reported that the Foaming capacity of fresh water carp protein hydrolysates prepared by using different enzymes i.e. alcalase, bromelain, Flavourzyme, Protamex resulted in the range of 75%-130%. Taheri et al. [33] reported that the foaming capacity of poultry by-product protein hydrolysates (PPH) and rainbow trout protein hydrolysate prepared by alcalase enzyme had foaming capacity between 100%-250% in FPH and in range of 50%-100% in PPH, it showed a slight difference. Foh et al. [12] reported that the foaming capacity of Tilapia fish protein hydrolysate of Fresh minced meat hydrolysate (FMMH) 125.50% and Hot water dip hydrolysate (HWDH) 124.50%.

Foaming stability

In present study foaming stability of FPH extracted from Malabar Sole fish by using different Pepsin enzyme concentrations (1, 1.5, 2, 2.5 and 3%) were found to be 114.26%, 112.2%, 124.4%, 116.9% and 119.93% respectively (Table 3). The foaming stability was significantly ($p<0.05$) higher at 2% enzyme concentrations. Elavarasan et al. [11] reported a slight difference in the Foaming stability of fresh water carp protein hydrolysates prepared by using different enzymes i.e. alcalase, bromelain, Flavourzyme, Protamex resulted in the range of 25%-95%. Taheri et al. [27] also reported the slight change foaming capacity of poultry by-product protein hydrolysates (PPH) and rainbow trout protein hydrolysate prepared by alcalase enzyme had foaming capacity between 55%-100% in FPH and in range of 50%-95% in PPH, it showed a slight difference. Foh et al. [12] reported that the foaming stability of Tilapia fish protein hydrolysate of Fresh minced meat hydrolysate (FMMH) 38.2% and Hot water dip hydrolysate

(HWDH) 37.25%. All the above differences are due to the difference in the pH of the product as optimum pH gives the best results.

Water Holding Capacity

In present study water holding capacity of FPH extracted from Malabar Sole fish by using different Pepsin enzyme concentrations (1, 1.5, 2, 2.5 and 3%) were found to be 2.99%, 3.06%, 3.75%, 3.41% and 3.44% respectively (Table 3). The water holding capacity was significantly ($p<0.05$) higher at 2% enzyme concentration. Kristinnson & Rasco, [18] reported slight different and low water holding capacity of Atlantic Salmon muscle protein hydrolysates prepared by using various Alkaline protease i.e alcalase, Flavourzyme, Corolase PN-L, Corolase 7089 and resulted as 0.96%, 1.79%, 2.13%, 2.74% and 2.29% respectively at 5% DH and 0.92%, 1.92%, 2.09%, 2.62% and 2.94% respectively at 10% DH. Taheri et al. [27] reported that the water holding capacity of poultry by-product protein hydrolysates (PPH) and rainbow trout protein hydrolysate prepared by alcalase enzyme had water holding capacity 5.1% in FPH and 2.8% in PPH. Foh et al. [12] reported that the water holding capacity of Tilapia fish protein hydrolysate of Fresh minced meat hydrolysate (FMMH) 2.10% and Hot water dip hydrolysate (HWDH) 1.77%.

Table. 3. Functional properties of FPH extracted from Malabar Sole Fish (*Cynoglossus macrostomus*) extracted by using different Pepsin enzyme concentrations

Functional Properties	Pepsin Concentrations				
	1%	1.5%	2%	2.5%	3%
Solubility (%)	83.66 ± 0.83	84.83 ± 0.23	92.43 ± 0.21	89.46 ± 0.44	90.23 ± 1.06
Emulsifying Capacity (%)	63.83 ± 0.78	63.1 ± 0.68	69.93 ± 0.72	67.06 ± 0.21	66.53 ± 0.34
Emulsifying Stability (%)	59.90 ± 1.27	59.20 ± 0.23	64.83 ± 1.04	61.13 ± 0.17	61.16 ± 0.14
Foaming Capacity (%)	114.26 ± 2.09	112.2 ± 0.7	124.4 ± 2.80	116.9 ± 1.56	119.93 ± 1.16
Foaming Stability (%)	81.00 ± 0.41	86.76 ± 1.19	92.03 ± 0.17	85.10 ± 0.32	87.56 ± 1.50
WHD (ml/g)	2.99 ± 0.05	3.06 ± 0.04	3.75 ± 0.11	3.41 ± 0.11	3.44 ± 0.01

pH

In present study pH of FPH extracted from Malabar Sole fish by using different Pepsin enzyme concentrations (1, 1.5, 2, 2.5 and 3%) were found to be 6.46, 6.53, 6.96, 7.20 and 6.66 respectively (Table 4). Kristinnson & Rasco, [18] reported that the pH for reaction conditions was controlled at 7.5pH for Atlantic salmon muscle protein hydrolysates prepared by using various alkaline proteases. Foh et al. [12] reported that the optimum pH for Tilapia fish protein hydrolysate of Fresh minced meat hydrolysate using Alcalase enzyme is 8 pH. Elavarasan et al. [11] studied that the optimum hydrolysis pH for fresh water carp protein hydrolysates prepared by using different enzymes i.e. alcalase, bromelain, Flavourzyme and Protamex is 9, 6, 6 and 6 pH respectively.

Isoionic Point

In present study Isoionic point of FPH extracted from Malabar Sole fish by using different Pepsin enzyme concentrations were found to be 5pH, 5pH, 7pH, 8pH and 8pH respectively (Table 4). Limited study of this parameter was carried out in Fish protein hydrolysate. This parameter was done to understand the zero charge of protein at different pH values.

Table.4. pH and Isoionic point of FPH extracted from Malabar Sole Fish (*Cynoglossus macrostomus*) extracted by using different Pepsin enzyme concentrations

Parameters	Pepsin Concentrations				
	1%	1.5%	2%	2.5%	3%
pH	6.46 ± 0.14	6.53 ± 0.20	6.96 ± 0.06	7.20 ± 0.11	6.66 ± 0.08
Isoionic Point	5 pH	5 pH	7 pH	8 pH	8 pH

Degree of Hydrolysis

In present study Degree of Hydrolysis of FPH extracted from Malabar Sole fish by using different Pepsin enzyme concentrations (1, 1.5, 2, 2.5 and 3%) and different time intervals (60, 120 and 180min) were found to be 15.80, 17.43, 18.36; 16.50, 18.30, 18.53%; 17.30, 20.36, 22.63%; 16.26, 19.13, 20.13% and 15.93, 18.80, 19.83% respectively (Table 5). Foh et al. [12] reported that for Tilapia fish protein hydrolysate of Fresh minced meat hydrolysate using Alcalase enzyme DH of 23.03% and 23.53% at 80

min, 23.40 and 25.43% at 120 min for fresh minced meat hydrolysate (FMMH) and hot water dip hydrolysate (HWDH) respectively. Abdulazeez *et al.* [1] reported that the DH increases with increase in incubation time. The DH was observed to be 22.2% for enzyme substrate ratio 1:100, 23.6% for 2:100 and 24.7% for 4:100. Sheriff *et al.* (2014) reported DH of protein hydrolysate derived by pepsin and papain enzyme from backbone of Indian mackerel and he derived 14.3, 15.8, 17.3 for pepsin enzyme substrate ratio of 1:100, 2:100, 4:100 respectively for 60 min; 18.4, 19.6, 20.9 for 120min and 21.3, 21.7 and 22.2 for 180 min respectively.

Table 5. Degree of Hydrolysis with respect to time of FPH extracted from Malabar Sole Fish (*Cynoglossus macrostomus*) extracted by using different Pepsin enzyme concentrations

Degree of Hydrolysis	Pepsin Concentrations				
Time	1 %	1.5 %	2 %	2.5 %	3 %
60 min	15.80 %	16.50 %	17.30 %	16.26 %	15.93 %
120 min	17.43 %	18.03 %	20.36 %	19.13 %	18.80 %
180 min	18.36 %	18.53 %	22.63 %	20.13 %	19.83 %

Colour (L* a* b*) value of FPH extracted from Malabar sole fish

In present study, the colour value of FPH extracted from Malabar sole fish by using different Pepsin enzyme concentrations (1, 1.5, 2, 2.5 and 3%) were found to be (L* value 62.73, 64.3, 68.56, 67.5 and 63.5) (a* value 8.13, 7.86, 6.05, 7.84 and 7.84) (b* value 16.18, 17.18, 18.6, 17.92 and 16.84) (Table 6). The moisture content was different in 2% as compared to other concentrations. There was difference in the results obtained in various hydrolysates due to drying factors. Taheri *et al.*, [33] reported that the colour value of Rainbow trout viscera protein hydrolysate (FPH) was (L* 68.9 a* -3.73 b* 18.4) and of poultry by-products protein hydrolysate (PPH) was (L* 78.8 a* -4.71 b* 11.1). Sathivel *et al.* [27] derived colour value of herring by-product hydrolysates (L* 89.4 a* 3.3 and b* 8.0) of whole herring hydrolysate (WHH), (L* 84.3 a* 2.8 and b* 13.4) of herring body hydrolysate (HBH), (L* 79.3 a* 4.2 and b* 14.0) of herring head hydrolysate (HHH) and (L* 74.6 a* 3.1 and b* 18.0) of herring gonad hydrolysate.

Table 6 Colour of FPH extracted from Malabar Sole Fish (*Cynoglossus macrostomus*) extracted by using different Pepsin enzyme concentrations

Colour	Pepsin enzyme Concentrations (%)				
	1%	1.5%	2%	2.5%	3%
Lightness(L*)	62.13 ± 0.49	64.3 ± 0.28	68.56 ± 0.44	67.5 ± 0.15	63.5 ± 0.30
Redness(a*)	8.13 ± 0.15	7.86 ± 0.24	6.05 ± 0.14	7.84 ± 0.16	7.45 ± 0.16
Yellowness(b*)	16.18 ± 0.17	17.18 ± 0.19	18.6 ± 0.43	17.92 ± 0.48	16.84 ± 0.29

Total Plate Count (TPC):

In present study the total plate count (TPC) of FPH sample was found to be in the range of 0.51×10^2 to 0.62×10^2 cfu/g are considered as the TPC limit of acceptable. No major changes are seen in range of TPC of FPH due to no storage study period. Jeyasanta *et al.* [16] has reported the results of changes in total plate count (TPC) of edible fish powder during the storage period at fresh, 1st, 2nd, 3rd, 4th and 5th as 2.0×10^2 , 2.0×10^2 , 1.4×10^2 , 1.2×10^2 , 1.0×10^2 and 1.0×10^2 cfu/g respectively.

Table 7. Microbiological analysis of FPH extracted from Malabar Sole Fish (*Cynoglossus macrostomus*) extracted by using different Pepsin enzyme concentrations

Microbiological characteristic	Pepsin enzyme concentrations (%)				
	1%	1.5%	2%	2.5%	3%
TPC (cfu/g)	0.51×10^2 (1.70)	0.53×10^2 (1.72)	0.42×10^2 (1.62)	0.62×10^2 (1.79)	0.59×10^2 (1.77)

CONCLUSION

It can be concluded that Malabar sole fish is suitable for preparation of fish protein hydrolysate using pepsin enzyme. Higher protein concentration is observed in the FPH with good biochemical and functional properties with low range of Total Plate Count (TPC). Pepsin was found to be efficient enzyme for production of FPH. The optimal conditions were determined to be 2%, of Pepsin enzyme at 55°C , pH 7 for 60 min. The FPH could also be considered as alternatives for other protein ingredient sources that are being used in the food industry. The future research on FPH is very bright since the FPH market is expanding enormously all over the world.

REFERENCES

1. Abdulazeez, S. S., Ramamoorthy, B., and Ponnusamy, P. (2013). Proximate analysis and production of protein hydrolysate from king fish of Arabian Gulf Coast-Saudi Arabia. International Journal of Pharmacy and Biological Sciences, 3(1), 138-144.
2. Abdul-Hamid, A., Bakar, J., and Bee, G. H. (2002). Nutritional quality of spray dried protein hydrolysate from Black Tilapia (*Oreochromis mossambicus*). Food chemistry, 78(1), 69-74.
3. Adler-Nissen, J. (1979). Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. Journal of agricultural and food chemistry, 27(6), 1256-1262.
4. Alasalvar, C., Shahidi, F., and Quantick, P. (2002). Food and health applications of marine nutraceuticals: a review. In Seafoods—Quality, Technology and Nutraceutical Applications (pp. 175-204). Springer Berlin Heidelberg.
5. AOAC, (2005). Official methods of analysis of the Association of Official Analytical Chemists International, 18th edition, In: Association of Official Analytical Chemists, Washington (D. C.).
6. Aspmo, S. I., Horn, S. J., and Eijssink, V. G. (2005). Enzymatic hydrolysis of Atlantic cod (*Gadus morhua*) viscera. Process Biochemistry, 40(5), 1957-1966.
7. Bhaskar, N., Benila, T., Radha, C., and Lalitha, R. G. (2008). Optimization of enzymatic hydrolysis of visceral waste proteins of Catla (*Catla catla*) for preparing protein hydrolysate using a commercial protease. Bioresource Technology, 99(2), 335-343.
8. Chalamaiyah, M., Hemalatha, R., and Jyothirmayi, T. (2012). Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: a review. Food Chemistry, 135(4), 3020-3038.
9. CMFRI.2015.Annual Report-2015.Central Marine Fisheries Research Institute, Kochi, India.
10. Diniz, F. M., and Martin, A. M. (1997). Effects of the extent of enzymatic hydrolysis on functional properties of shark protein hydrolysate. LWT-Food Science and Technology, 30(3), 266-272.
11. Elavarasan, K., Naveen Kumar, V., and Shamasundar, B. A. (2014). Antioxidant and functional properties of fish protein hydrolysates from fresh water carp (*Catla catla*) as influenced by the nature of enzyme. Journal of Food Processing and Preservation, 38(3), 1207-1214.
12. Foh, M. B. K., Kamara, M. T., Amadou, I., Foh, B. M., and Wenshui, X. (2011). Chemical and physicochemical properties of tilapia (*Oreochromis niloticus*) fish protein hydrolysate and concentrate. International journal of Biological chemistry, 5(1), 21-36.
13. Gopakumar, K. (1997). Biochemical composition of Indian food fish. Central Institute of Fisheries Technology, cochin, pp. 1-5.
14. Hoyle, N. T., and Merritt, J. O. H. N. (1994). Quality of fish protein hydrolysates from herring (*Clupea harengus*). Journal of food Science, 59(1), 76-79.
15. Je, J. Y., Qian, Z. J., Lee, S. H., Byun, H. G., and Kim, S. K. (2008). Purification and antioxidant properties of bigeye tuna (*Thunnus obesus*) dark muscle peptide on free radical-mediated oxidative systems. Journal of medicinal food, 11(4), 629-637.
16. Jeyasanta, K. I., Aiyamperumal, V., and Patterson, J. (2013). Utilization of trash fishes as edible fish powder and its quality characteristics and consumer acceptance. World Journal of Dairy and Food Sciences, 8(1).
17. Kinsella, J. E., and Melachouris, N. (1976). Functional properties of proteins in foods: a survey. Critical Reviews in Food Science and Nutrition, 7(3), 219-280.
18. Kristinsson, H. G., and Rasco, B. A. (2000). Biochemical and functional properties of Atlantic salmon (*Salmo salar*) muscle proteins hydrolyzed with various alkaline proteases. Journal of Agricultural and Food Chemistry, 48(3), 657- 666.
19. Kristinsson, H. G., and Rasco, B. A. (2000). Fish protein hydrolysates: production, biochemical, and functional properties. Critical reviews in food science and nutrition, 40(1), 43-81.
20. Miller, R. and Goninger, H.S. (1976). Functional properties of enzyme modified acylated fish protein derivatives. J. Food Sci. 41 (2): 268-272.
21. Neitzel, J. J. (2010). Enzyme catalysis: the serine proteases. Nature Education, 3(9), 21.
22. Nilsang, S., Lertsiri, S., Suphantharika, M., and Assavanig, A. (2005). Optimization of enzymatic hydrolysis of fish soluble concentrate by commercial proteases. Journal of food Engineering, 70(4), 571-578.
23. Panyam, D., and Kilara, A. (1996). Enhancing the functionality of food proteins by enzymatic modification. Trends in food science and technology, 7(4), 120- 125.
24. Peter, D., and Clive, H. (2006). An overview of the Australian Seafood Industry.[Online].Available: <http://aaa ccpit.org/Category7/mAttachment/2006/Dec/13/asset000070002007202file1.pdf>.
25. Quaglia, G. B., and Orban, E. (1987). Enzymic solubilisation of proteins of sardine (*Sardina pilchardus*) by commercial proteases. Journal of the Science of Food and Agriculture, 38(3), 263-269.
26. Raymundo, A., Empis, J., and Sousa, I. (2000). Effect of pH and NaCl on rheological and textural properties of lupin protein emulsions. Special Publication-Royal Society of Chemistry, 251, 350-365.
27. Sathivel, S., Bechtel, P. J., Babbitt, J., Smiley, S., Crapo, C., Repond, K. D., and Prinyawiwatkul, W. (2003). Biochemical and functional properties of herring (*Clupea harengus*) byproduct hydrolysates. Journal of Food Science, 68(7), 2196-2200.
28. Sawant, D. (2008). Preparation of enzymatic fish protein hydrolysate from croaker (*Otolithus ruber*). Submitted M.F.Sc thesis to Dr. B. S. K. K. V. Dapoli.
29. Sawant, R. R., Metar, S. Y., Nirmale, V. H., Koli, J. M., Vishwasrao, V. V., and Satam, S. B. (2016). Elicitation and characterization of skin gelatin of Malabar sole fish at different extraction temperatures. Animal Science, 10(4).

30. Shahidi, F., Han, X. Q., and Synowiecki, J. (1995). Production and characteristics of protein hydrolysates from capelin (*Mallotus villosus*). Food chemistry, 53(3), 285-293.
31. Snedecor, G. W., and Cochran, W. G. (1967). Statistical methods 6th edition. The Iowa State University.
32. Souissi, N., Bougatef, A., Triki-Ellouz, Y., and Nasri, M. (2007). Biochemical and functional properties of *Sardinella* (*Sardinella aurita*) by-product hydrolysates. Food Technology and Biotechnology, 45(2), 187.
33. Taheri, A., Anvar, S. A. A., Ahari, H., and Fogliano, V. (2013). Comparison the functional properties of protein Hydrolysates from poultry byproducts and rainbow trout(*Onchorhynchus mykiss*) viscera. Iranian Journal of Fisheries Sciences, 12(1), 154-169.
34. Tannenbaum, S. R., Stillings, B. R., and Scrimshaw, N. S. (Eds.). (1974). The economics, marketing, and technology of fish protein concentrate. Cambridge, Massachusetts: MIT Press.
35. US Food and Drug Administration. (2001). FDA guidance for industry: bioanalytical method validation. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research: Rockville, MD.
36. Venugopal, V. (1994). Production of fish protein hydrolyzates by microorganisms. In Fisheries Processing (pp. 223-243). Springer US.
37. Yasumatsu, K., Sawada, K., Moritaka, S., Misaki, M., Toda, J., Wada, T., and Ishii, K. (1972). Whipping and emulsifying properties of soybean products. Agricultural and Biological Chemistry, 36(5), 719-727.
38. Yin, H., Pu, J., Wan, Y., Xiang, B., Bechtel, P. J., and Sathivel, S. (2010). Rheological and functional properties of catfish skin protein hydrolysates. Journal of food science, 75(1), E11-E17.
39. Zhang, F., Xu, S., and Wang, Z. (2010). Pre-treatment optimization and properties of gelatin from freshwater fish scales. Food and Bioproducts Processing, 89(3), 185-193.

CITATION OF THIS ARTICLE

O. R. Bhingarde, J. M. Koli, A. E. Sonavane, S. B. Patange, P.E. Shingare, P.P. Relekar and V.B. Mulye. Effect of different concentration of Pepsin Enzyme on extraction of Fish Protein Hydrolysate from Malabar Sole Fish(*Cynoglossus macrostomus*). Bull. Env. Pharmacol. Life Sci., Vol 7 [9] August 2018: 94-103