Fishery Technology 53 (2016) : 115 - 124

Extraction of Protein from Yellowfin Tuna (*Thunnus albacares*) Waste by Enzymatic Hydrolysis and its Characterization

U. Parvathy[1]*, A. A. Zynudheen[2], S. K. Panda[2], A. Jeyakumari[1] and R. Anandan[2]
[1] *Mumbai Research Centre of ICAR-CIFT, Vashi, Navi Mumbai - 400 703, India*
[2] *ICAR-Central Institute of Fisheries Technology, P. O. Matsyapuri, Cochin - 682 029, India*

Abstract

The focus of this investigation was to utilize the protein present in tuna waste by enzyme hydrolysis and characterization of protein hydrolysate (TPH). Yellowfin tuna waste was used to prepare the hydrolysate using 0.5% (w/w) bromelain enzyme. It was characterized for nutritional, functional and antioxidant properties. The protein content in the derived protein hydrolysate was 95.3 ± 3.1%. The foaming capacity as well as foam stability was maximum at pH 6.0 and the property decreased when pH deviated from neutral. Emulsifying properties *viz.,* emulsifying activity index as well as emulsion stability index was least at 4.0 pH. DPPH radical scavenging activity of TPH revealed an IC_{50} value of 4.06 mg ml^{-1}.

Keywords: Tuna waste, fish protein hydrolysate, bromelain, functional properties, antioxidant property

Introduction

Processing and value addition of fish products results in the generation of large quantities of discards which are commonly recognized as low-value resources with negligible market value. In addition, inapt disposal is a major cause of environmental pollution. Traditionally, these wastes have been used for feeding animal. Research efforts for effective utilization of these protein rich discards are often attempted and one such approach is to convert them into hydrolysates which have a wide range of applications (Faid et al., 1997; Kurbanoglu & Algur, 2002). Enzymatic hydrolysis has been used due to the desirable end product attained. They allow the quantification of asparagine and glutamine and other sensitive residues, which are normally destroyed by acid and alkali hydrolysis, and do not cause any racemization during digestion. A variety of enzymes have been widely used to improve the functionality of proteins to meet specific needs (Panyam & Kilara, 1996). Kim & Wijesekara (2010) reported that enzymatic hydrolysis of fish proteins with appropriate enzymes such as alcalase, pronase, collagenase, pepsin, papain, protamex, bromelain, chymotrypsin and trypsin allows for the generation of bioactive peptides made up of specific length of amino acids. Hydrolysates generally contain small fragments of peptides making them readily available sources of amino acids that have various physiological functions. Hydrolysates possess good functional properties and hence find application as additives in food (Quaglia & Orban, 1990; Kristinsson & Rasco, 2000). They exhibit an excellent solubility at high degree of hydrolysis which is a constructive attribute for many food applications (Gbogouri et al., 2004; Shahidi et al., 1995). Other functional properties include water holding capacity, oil absorption capacity, emulsifying and foaming properties (Gbogouri et al., 2004; Kristinsson & Rasco, 2000). Protein hydrolysates also contain bioactive peptides which exhibit potential antioxidant properties. Levels and compositions of free amino acids and peptides were reported to determine the antioxidant activities of protein hydrolysates (Wu et al., 2003). However optimization of the hydrolytic conditions is to be done to obtain peptides of predefined range of activities as different properties require different hydrolytic conditions. Present work was carried out to explore nutritional,

Received 10 December 2015; Revised 30 January 2016; Accepted 15 February 2016

* *E-mail: p.pillai2012@gmail.com*

functional and antioxidant properties of protein hydrolysate developed from the waste of yellow fin tuna (*Thunnus albacares*) using bromelain enzyme digestion.

Materials and Methods

Fresh yellow fin tuna fish (*Thunnus albacares*) was procured from the local fish landing center at Cochin, India and brought to the laboratory in iced condition. Tuna waste *viz.,* skin, gills, viscera, fins and head were collected manually for preparing protein hydrolysate. Bromelain enzyme from pineapple stem (Sigma-Aldrich) was used for hydrolyzing the tuna waste protein. All other chemicals used for the study were of analytical grade.

Tuna waste was minced thoroughly using an electric grinder. The mince was transferred to a beaker and cooked in equal quantity of water for 30 min at 100ºC to completely inactivate the endogenous enzymes present in the waste. After cooking, the temperature was lowered and maintained at 55ºC and pH 6.5 (optimum temperature and pH of the enzyme respectively) and hydrolysis reaction was initiated by the addition of bromelain @ 0.5% (w/w) for 45 min. Bromelain, a natural enzyme from pineapple stem, is an endoprotease which has been used mainly for meat tenderization (Kolle et al., 2004). An enzyme substrate ratio of 0.5% (w/w) was used for hydrolysis, as it is an optimized ratio for good functional and bioactive properties (Tanuja et al. 2012). Aliquots were drawn at regular intervals (15, 30 and 45 min) to determine the nitrogen recovery during hydrolysis. On completion of hydrolysis, the solution was immediately heated to 90ºC for 20 min to arrest the hydrolytic process. The solution obtained was then course filtered followed by centrifugation at 8000 g at 10ºC for 20 min and the supernatant collected was dried using spray drier (Basic Technology Private Limited, Kolkata, India) to get hydrolysate powder which was further stored in an air tight plastic container.

Proximate composition of tuna waste and tuna protein hydrolysate (TPH) was estimated as per AOAC (2012). Amino acid analysis was accomplished by HPLC (high-performance liquid chromatography) (Hitachi, Japan) by following the methodology proposed by Ishida et al. (1981). The instrument was equipped with Shimadzu FL 6A fluorescence detector (FL- Detector 2485) and the oven temperature was maintained at 60°C. For mineral profiling, samples were digested under specific conditions (Table 1) for 40 min in a microwave assisted extraction system, Milestone START D (Milistone Srl., Italy), set with easy CONTROL software and HPR 1000/10S high pressure segmented rotor. The digested samples were diluted with ultra-pure water in known volume prior to elemental profiling. Inductivity Coupled Plasma–Optical Emission Spectrometer (iCAP 6300 Duo, Thermo fisher Scientific, Cambridge, England) with dual configuration (axial and radial) and iTEVA (version 2.8.0.97) operational software was used for elemental analysis. The experimental conditions used in the determination of the above elements are shown in Table 2. ICP multi-element standard solution (CertiPUR, Merck, Mumbai, India) was used for the preparation of calibration solutions. Yttrium was used as internal standard.

Nitrogen recovery (NR) was used as an index of nitrogen solubilization to describe the hydrolysis yield. The nitrogen recovery at different times (15, 30, 45 min) of hydrolysis were carried out. For this, aliquots were drawn and the soluble fraction was separated from the insoluble fraction by centrifuging (Heraeus multifuge X1R centrifuge, Thermofisher Scientific, Germany) at 22,000 × g for 10 min. The total nitrogen in the soluble fraction and the total

Table 1. Microwave digestion conditions in Milestone START D[a]

Sample	1.0 g
Nitric acid (HNO_3) (TraceMetal™ Grade, Fisher Scientific)	8.0 ml
Hydrogen peroxide (H_2O_2) (30-32%, Optima, Fisher Scientific).	2.0 ml
Pressure	400 psi (max.)
Power	1200 W
Temperature[b]	Step I: Ramp to 150ºC over 30 minutes Step II: Hold at 150ºC for 10 minutes Step III: Allow to cool to room temperature over 1 hour

[a] microwave condition and digestion procedures were adapted from Milestone Cookbook Digestion Rev. 03_04

[b] Temperature and pressure sensors were used to monitor digestion conditions and to prevent over-pressurization of vessels

Table 2. Experimental conditions for elemental analysis using ICP-OES

RF power	1150 Watt
Optics temperature	38°C
Camera temperature	-44°C
Nebulizer	MiraMist, Cyclonic Chamber
Main Argon flow rate	15 L min^{-1}
Auxiliary Argon flow rate	0.5 L min^{-1}
Nebulizer Gas Flow	0.5 L min^{-1}
Maximum integration time	30 sec

nitrogen in the original substrate were determined using the Kjeldahl method (AOAC, 2012). NR was calculated according to Diniz & Martin (1997a) using the following equation:

$$\text{NR } (\%) = \frac{\text{Total nitrogen in supernatant x 100}}{\text{Total nitrogen in the substrate}}$$

Yield of fish protein hydrolysate was calculated from the ratio of the amount of spray-dried hydrolysate powder to the amount of initial raw material (tuna waste) used for hydrolysis.

$$\text{Yield } \% = \frac{\text{Weight of the spray dried hydrolysate x 100}}{\text{Weight of tuna waste used for hydrolysis}}$$

Colour of the hydrolysate powder was evaluated using Hunter Lab colorimeter (MiniScan XE Plus Hunter Associates Lab inc., Reston, Virginia, USA) to produce numeric results indicative of the color of the sample by measuring L* (the degree of lightness: black (0) to white (100)), a* (degree of redness(+)/greenness (-)) and b* (the degree of yellowness (+) or blueness (-)).

Oil absorption capacity (OAC) was determined according to the modified method of Shahidi et al. (1995). About 1.0 gm hydrolysate sample was taken in a pre-weighed centrifuge tube and thoroughly mixed with 5 ml sunflower oil. Further it was centrifuged (Heraeus multifuge X1R centrifuge, Thermofisher Scientific, Germany) at 3000 g for 30 min (25°C). The supernatant was drained off at 45°C angle immediately and the centrifuge tube weighed again.

$$\text{FAC (g oil g}^{-1}\text{ sample)} = \frac{W_3 - W_2}{W_1}$$

W_1 = Wt of dry sample alone

W_2 = Wt of tube + dry sample

W_3 = Wt of tube + Sediment

Foaming capacity and stability of fish protein hydrolysate were determined according to the modified method of Sze-Tao & Sathe (2000). Protein solution (0.5%) was prepared and the pH was adjusted to 2, 4, 6, 8 and 10. This protein solution was whipped for 2 min at a speed of 11000 rpm using a homogenizer (IKA® Ultra-turrax T18™ basic, Germany) and poured into a 100 ml graduated cylinder. The total sample volume was taken at the zero minutes for foam capacity. The foaming capacity was calculated according to the following equation:

$$\text{FC } \% = \frac{V_2 - V_1 \text{ x } 100}{V_1}$$

where V_2 is the volume after whipping (ml) and V_1 is the volume before whipping (ml). The whipped sample was allowed to stand at room temperature for 15 min and the volume of whipped sample was then recorded. Foam stability was calculated as follows:

$$\text{FS } \% = \frac{V_2 - V_1 \text{ x } 100}{V_1}$$

where V_2 is the volume after standing (ml) and V_1 is the volume before whipping (ml).

Emulsifying properties were determined according to the method of Pearce and Kinsella (1978). Vegetable oil (10 ml) and protein solution (30 ml, 1%) were mixed and homogenized using a homogenizer (IKA® T18™ basic, Germany) at a speed of 20 000 rpm for 1 min. An aliquot of the emulsion (50 μl) was pipetted from the bottom of the container at 0 and 10 min after homogenization and mixed with 5 ml of 0.1% sodium dodecyl sulphate (SDS) solution. The absorbance of the diluted solution was measured at 500 nm using a spectrophotometer (Spectronic Genesys 5, USA). The absorbance was measured immediately (A_0) and 10 min (A_{10}) after emulsion formation was used to calculate the emulsifying activity index (EAI) and the emulsion stability index (ESI) as follows:

$$\text{EAI (m g}^{-2}) = \frac{2 \text{ x } 2.303 \text{ x } A^0}{0.25 \text{ x Wt of protein}}$$

$$ESI\ (min) = \frac{A10 \times \Delta t}{\Delta A}$$

Δt =Time

$\Delta A = A_0 - A_{10}$

DPPH (2, 2-Diphenyl-1-picryhydrazyl) radical-scavenging activity was measured, using the method described by Yen & Wu (1999) with slight modifications. Hydrolysate was dissolved in distilled water to obtain different concentrations (1- 8 mg protein ml^{-1}). To 2 ml of sample solutions, 2 ml of 0.06 mM DPPH was added and mixed vigorously. After incubating for 30 min, the absorbance of the resulting solution was measured at 517 nm using a spectrophotometer (Spectronic Genesys 5, USA). The control was conducted in the same manner, except that distilled water was used instead of sample. DPPH radical scavenging activity was calculated according to the following equation:

DPPH radical-scavenging activity =

$$\frac{1-\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The antioxidant activity of the protein sample was expressed as IC_{50}, which is defined as the concentration of hydrolysate solution (mg ml^{-1}) required to scavenge 50% of DPPH radicals. IC_{50} values were estimated by a non-linear regression. A lower IC_{50} value indicates higher antioxidant activity.

Total phenolic content (TPC) was determined spectrophotometrically using gallic acid as a standard, according to the method described by Singleton & Rossi (1965) with modifications. About 0.5 ml of the protein sample was mixed with 2.5 ml of diluted Folin-Ciocalteu's reagent in water (1:9). The mixture was allowed to react for 5 min and 2.5 ml of a sodium carbonate solution (7.5% w/v) was added to the sample. The tubes were then allowed to stand at room temperature for 30 min and the absorbance was measured at 765 nm using a spectrophotometer (Spectronic Genesys 5, USA). The concentration of polyphenols in samples was derived from gallic acid standard curve.

All analysis were done in triplicate and results were expressed as mean ± standard deviation.

Results and Discussion

Proximate compositions of tuna waste and tuna protein hydrolysate were determined (Table 3). Motamedzadegan et al. (2010) reported that yellow fin tuna viscera contains 21.5±0.5% protein, 5.08±1.53% fat, 69.66±2.32% moisture and 4.46±1.21% ash similar to the observations made in the study. Foh et al. (2011a) reported a protein content of 97.57±0.12 %, 1.22±0.02% moisture, 0.67±0.04% lipid and 2.25±0.13% ash respectively in tilapia fish protein hydrolysate. An increase in protein content was observed on account of drying, which reduced the moisture content in TPH. Solubilisation of protein during hydrolysis, removal of insoluble undigested non-protein substances as well as the partial removal of lipid after hydrolysis results in high protein content in hydrolysates (Benjakul & Morrissey, 1997). Low moisture content was related to the type of sample and to the higher temperatures employed during the process of evaporation and spray drying. Several studies reported fat content for various fish protein hydrolysates below 5% (Bhaskar et al., 2008; Ovissipour et al., 2009) which was on account of the removal of lipids along with insoluble protein fractions by centrifugation. Ash content of fish protein hydrolysates reported in many studies ranged between 0.45% to 27% of total composition (Chalamaiah et al., 2010; Ovissipour et al., 2009; Thiansilakul et al., 2007).

Protein hydrolysates exhibit many advantages as nutraceuticals or functional foods because of their amino acid profile (Santos et al., 2011). The variation in amino acid composition of different fish protein hydrolysates mainly depends on several factors such as raw material, enzyme source, and hydrolysis conditions (Klompong et al., 2009). TPH was found to be rich in glutamic acid and aspartic acid (Table 4). Fair levels of leucine, arginine, proline and glycine were found whereas cysteine was very

Table 3. Proximate Composition of Tuna Waste and Tuna Protein Hydrolysate

	Tuna waste	Tuna Protein Hydrolysate
Moisture	72.77 ± 0.45	3.03 ± 0.04
Protein	18.94 ± 0.94	95.3 ± 3.1
Fat	6.03 ± 0.24	2.28 ± 0.64
Ash	3.58 ± 0.14	0.54 ± 0.04

low. Iminoacids are abundantly present in connective tissue and skin that contains collagen (Taheri et al., 2011). The higher levels of glycine, and proline in TPH might be on account of the higher amounts of connective tissue in the skin which was also used as raw material for the protein hydrolysate preparation. The amino acid profile indicated a high essential amino acid/non-essential amino acid ratio of 1.05 thus finding application as a dietary protein supplement. Iwasaki & Harada (1985) reported that fish and shellfish contain high essential amino acid/ non-essential amino acid ratio. Wasswa et al. (2007) reported that grass carp skin protein hydrolysates contain rich quantities of glycine, alanine, glutamine and proline. Bhaskar et al. (2008) reported that glutamine was the most prominent amino acid in catla protein hydrolysate. Arginine and glycine were also found in higher levels. Tryptophan and cystine (0.23%) were the limiting amino acids.

Table 4. Amino acid composition of Tuna protein hydrolysate

Aminoacid composition	Percentage of total aminoacids
Essential Amino acids (EAA)	
Arginine	7.73
Histidine	4.07
Isoleucine	4.16
Leucine	9.58
Phenyl alanine	4.79
Threonine	4.27
Valine	5.83
Methionine	2.98
Lysine	3.87
Tyrosine	3.51
Non Essential Amino acids (NEAA)	
Alanine	5.46
Aspartic acid	9.88
Glycine	6.15
Glutamic acid	16.41
Proline	6.50
Serine	3.63
Cysteine	0.22
EAA/NEAA	1.05

Minerals in diet are known to play a variety of bodily functions and are also important for metabolic processes. Mineral profiling of TPH indicated higher levels of Na, K, P, Ca and Mg (Table 5). Similarly TPH was found to be a good source of selenium and zinc. Selenium has been known to counter arsenic toxicity in a variety of animal models (Gailer, 2009). Thiansilakul et al. (2007) reported higher levels of Na, K, Ca and Mg in freeze-dried round scad protein hydrolysate. Similar results were also reported by Sathivel et al. (2003) in herring and herring byproduct hydrolysates with an abundance in minerals like K, Mg, P, Na, S and Ca. Foh et al. (2011a) observed higher levels of Na and K in Tilapia protein hydrolysate. The toxic heavy metals like Ba, Cd, Hg were within the acceptable limits in the sample. TPH is a dry powder having low moisture content of about 3.0 ±0.04 % and hence it didn't exceed the acceptable limit of 0.3 ppm for cadmium and 1.0 ppm for mercury, on wet weight basis as per current FSSAI regulations.

Table 5. Mineral profile of Tuna Protein Hydrolysate

Element	ppm
Aluminium	17.29±2.05
Barium	0.45±0.02
Calcium	910.40±9.75
Cadmium	1.16±0.5
Chromium	0.93±0.64
Copper	4.37±0.07
Iron	59.98±0.57
Gallium	0.63±4.73
Potassium	7335.49±25.77
Magnesium	502.29±4.99
Manganese	0.53±0.18
Sodium	10090.65±19.52
Nickel	1.01±0.49
Selenium	21.50±3.79
Strontium	7.71±0.06
Tellurium	1.05±4.22
Zinc	24.45±0.19
Phosphorous	5673.00±38.59
Arsenic	3.38±0.14
Mercury	0.12±0

Nitrogen recovery was observed to increase with hydrolysis time ranging from 39.66±0.86 at 15 min to 45.95±4.79 % at 45 min (Table 6). Liaset et al. (2002) reported an increase in nitrogen recovery with hydrolysis time. The protein recoveries in the enzymatic hydrolysis of different fish processing waste with alcalase has been reported to be between 60% and 70% (Shahidi et al., 1995; Benjakul & Morrisey, 1997; Liaset et al., 2000). Increased umamizyme enzyme/substrate ratio resulted in higher nitrogen recovery of 57% after a 4 h reaction in tuna waste hydrolysate (Guerard et al., 2002).

Yield of the TPH powder was 3.9% (Table 6). Typical yields of fish protein hydrolysates have been reported to be 10–15%, based on fresh fish substrate (Hale, 1972; Quaglia & Orban, 1990). The lower yields obtained in this study was probably due to the fact that only the soluble fraction was spray–dried and due to significant loss during spray drying operation.

Colour of fish protein hydrolysate depends on the composition of the raw material, the hydrolysis conditions and the drying method adopted. Analysis of colour using colourimeter was indicated by an L*(lightness), a*(greenness), b* (yellowness) value (Table 6). Foh et al. (2011a) reported that FPH from tilapia were light yellow in colour and exhibited an L*, a*, b* value of 92.45±2.51, 0.45±0.18 and 10.72±3.19 respectively.

Oil absorption capacity (OAC) is an important functional characteristic of food ingredients and it correlates with surface hydrophobicity (Kristinsson & Rasco, 2000). OAC of TPH was observed to be 1.42±0.03 g oil g^{-1} protein. An OAC of 3 g oil g^{-1} protein was observed in blue whiting fish processing co-product hydrolysate (Geirsdottir et al. 2011) while 3.38 ml oil g^{-1} powder was the OAC of tilapia fish processing co-product hydrolysate (Foh et al., 2011b). Foh et al. (2010) reported an OAC of 2.27±0.06 ml g^{-1} for 2.4 L alcalase hydrolysed tilapia protein.

Table 6. Characteristics of Tuna Protein Hydrolysate

Characteristics	Values
Nitrogen Recovery @	
15 min	39.66±0.86%
30 min	41.4±1.05%
45 min	45.95±4.79%
Yield	3.9%
Colour	
L*	86.86±0.47
a*	-0.1±0.06
b*	14.1±0.41
DPPH radical scavenging activity(2 mg ml^{-1})	71.39±0.41%
Total Phenolics	31.85±2.31 mg GAE g^{-1}

Foaming properties at different pH levels *viz.,* 2, 4, 6, 8 and 10 indicated that both foaming capacity as well as foam stability at 15 min were maximum at pH 6.0 and the property decreased when pH deviated from 6.0 (Fig. 1). The foaming capacity of yellow stripe trevally hydrolysate using alcalase reached a maximum at pH 6 with a slight decrease at alkaline pH (Klompong et al., 2007). The lowest foaming properties of proteins also coincided with the lowest solubilities at their isoelectric pH (Pearson, 1983). Taheri et al. (2013) also observed

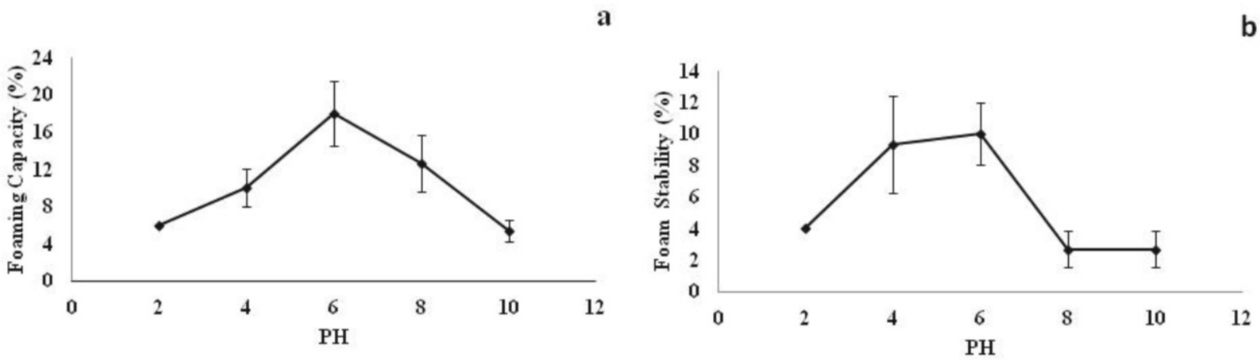

Fig. 1. Foaming capacity (a) and foam stability (b) of TPH as influenced by pHs. Bars represent standard deviations from triplicate determinations.

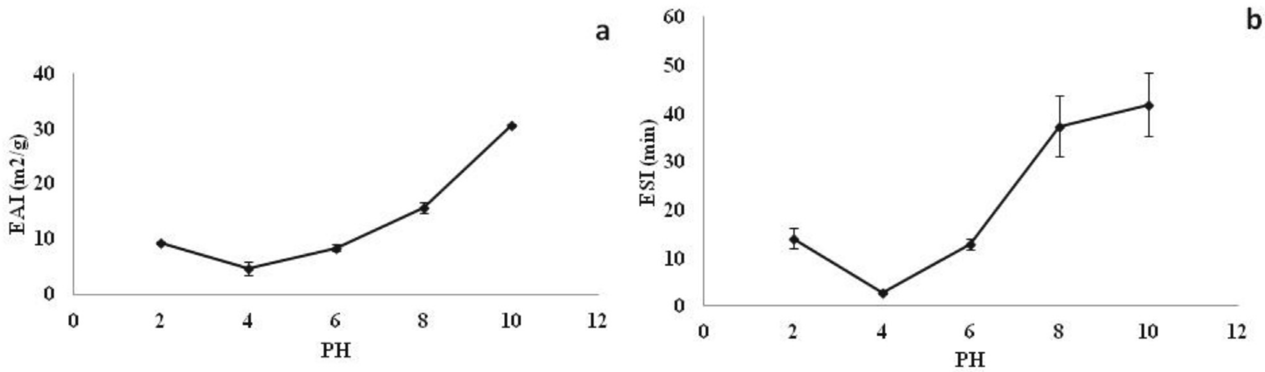

Fig. 2. Emulsifying activity index (EAI) (a) and emulsion stability index (ESI) (b) of TPH as influenced by pHs. Bars represent standard deviations from triplicate determinations.

similar results in rainbow trout visceral protein hydrolysate where in the foaming properties were maximum at pH 6.0 and were minimum at pH 4.0. Foaming capacity observed in shark hydrolysate exhibited about 50% increase in volume (Diniz & Martin, 1997b).

With a limited degree of hydrolysis, the hydrolysates have exceptional emulsifying activity and stability (Kristinsson & Rasco, 2000). Hydrolysates with a higher DH had poorer EAI and ESI due to their small peptide size as small peptides migrate rapidly and adsorb at the interface, but show less efficiency in decreasing the interface tension due to their inability to unfold and reorient at the interface like large peptides to stabilize emulsions (Gbogouri et al., 2004; Rahali et al., 2000). Environmental pH also affects emulsifying properties by changing the solubility and surface hydrophobicity of proteins, as well as the charge of the protective layer surrounding the lipid globules (Taheri et al., 2013). In the TPH samples, emulsifying properties *viz.,* EAI and ESI were least at pH 4.0 and they increased as pH moved away from 4.0 (Fig. 2). Klompong et al. (2007) reported that when considering the effect of pH on EAI and ESI, the lowest EAI and ESI were found at pH 4, with coincidental decrease in solubility. Taheri et al. (2013) also reported a maximum and minimum EAI at pH 10 and 4 for rainbow trout visceral hydrolysate.

Antioxidants are substances capable of delaying, retarding or preventing oxidation processes (Shuler, 1990). In order to prevent lipid peroxidation in food products, many synthetic antioxidants have been used (Kim & Wijesekara, 2010). But recently more interest is generated towards finding antioxidants from natural sources that have little or no side effects (Mendis et al., 2004). Protein hydrolysates (peptides) are potential antioxidants than free amino acids, due to their chemical composition and physical properties (Elias et al., 2008). DPPH radical scavenging activity of 2mg ml^{-1} of TPH solution was evaluated (Table 6) and an IC $_{50}$ value of 4.06 mg ml^{-1} was observed for the sample. Bougatef et al. (2009) reported an IC_{50} value of 1.2±0.014 mg ml^{-1} for smooth hound muscle protein treated with crude enzyme extract. Foh et al. (2010) reported a DPPH scavenging of 86.67±1.15% at 5 mg ml^{-1} for alcalase, 70.20±1.06% for flavourzyme and 82±1.73% for neutrase hydrolysed tilapia protein.

Recently, the ability of phenolic substances including flavonoids and phenolic acid to act as antioxidants has been extensively investigated (Shahidi, 2000; 2008). The total phenol content in TPH was observed to be 31.85±2.31 mg GAE g^{-1} sample. Arunrat et al. (2011) observed a total phenolic content of 0.916±0.053 mg GAE ml^{-1} sample in 1.5 times diluted protein hydrolysate prepared by conventional fermentation of fish frame, shrimp cephalothorax and NaCl.

Intelligent utilization of fishery waste from fish processing industry is the need of the hour. The protein present in these wastes can be better utilized and recovered in the form of hydrolysates by the application of enzymes. In the present study, the derived protein hydrolysate gave a protein content of 95.3±3.1% with a balanced profile of amino acids. However they exhibited low functional properties. Foaming properties *viz.,* foaming capacity and foam stability was maximum at pH 6.0. Similarly emulsifying properties *viz.,* emulsifying activity index as well as emulsion stability index was least at pH of 4.0. Present study revealed strong anti-oxidant activity with DPPH radical scavenging activity of TPH revealing an IC_{50} value of 4.06 mg ml^{-1}. Hence the derived protein hydrolysate finds better application as an anti-oxidant in food systems. Further optimization of the hydrolytic conditions is essential to obtain fish protein hydrolysates having peptides with desirable properties for their specific end application.

Acknowledgements

The authors wish to thank the Director, ICAR- Central Institute of Fisheries Technology, Cochin, for providing the support and facilities to carry out this work.

References

AOAC (2012) Official Methods of Analysis, 19th edn., Association of Official Analytical Chemists, Washington DC

Arunrat, K., Siripongvutikorn, S. and Thongraung, C. (2011) total phenolic content and antioxidative activity in seasoning protein hydrolysate as affected by pasteurization and storage. J. Food Sci. Eng. 1: 252-262

Benjakul, S. and Morrissey, M. (1997) Protein hydrolysates from Pacific whiting solid wastes. J. Agri. Food Chem. 45: 3423-3430

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 Technol. 99: 335-343

Bougatef, A., Hajji, M., Balti, R., Lassoued, I., Triki-Ellouz, Y. and Nasri, M. (2009) Antioxidant and free radical-scavenging activities of smooth hound (*Mustelus mustelus*) muscle protein hydrolysates obtained by gastrointestinal proteases. Food Chem. 114(4): 1198-1205

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

Diniz, F. M. and Martin, A. M. (1997a) Optimisation of nitrogen recovery in the enzymatic hydrolysis of dogfish (*Squalusacanthias*) protein. Composition of the hydrolysates. Int J. Food Sci. Nutr. 48: 191-200

Diniz, F. M. and Martin, A. M. (1997b) Effects of the extent of enzymatic hydrolysis on functional properties of shark protein hydrolysate. Lebensm. Wiss. Technol. 30(3): 266-272

Elias, R. J., Kellerby, S. S. and Decker, E. A. (2008) Antioxidant activity of proteins and peptides. Crit. Rev. Food Sci. 48: 430-441

Faid, M., Zouiten, A., Elmarrakchi, A. and Achkari-Begdouri, A. (1997) Biotransformation of fish waste into stable feed ingredient. Food Chem. 60(1): 13-18

Foh, M. B. K., Amadou, I., Foh, B. M., Kamara, M. T. and Wenshui, X. (2010) Functionality and antioxidant properties of tilapia (*Oreochromis niloticus*) as influenced by the degree of hydrolysis. Int. J. Mol. Sci. 11: 1851-1869

Foh, M. B. K., Kamara, M. T., Amadou, I., Foh, B. M. and Wenshui, X. (2011a) Chemical and Physicochemical properties of Tilapia (*Oreochromis niloticus*) fish protein hydrolysate and concentrate. Int. J. Bio. Chem. 5(1): 21-36

Foh, M. B. K., Wenshui, X., Amadou, I. and Jiang, Q. (2011b) Influence of pH shift on functional properties of protein isolated of Tilapia (*Oreochromis niloticus*) muscles and of soy protein isolate. Food Bioprocess Tech. 5: 1-9

Gailer, J. (2009) Chronic toxicity of As (III) in mammals: the role of (Gs)(2) AsSe(–) Biochimie. 91(10): 1268-1272

Gbogouri, G. A., Linder, M., Fanni, J. and Parmentier, M. (2004) Influence of hydrolysis degree on the functional properties of salmon byproduct hydrolysates. J. Food Sci. 69: 615-622

Geirsdottir, M. S., Sigurgisladottir, S., Hamaguchi, P. Y., Thorkelsson, G., Johannsson, R., Kristinsson, H. G. and Kristjansson, M. M. (2011) Enzymatic hydrolysis of Blue whiting (*Micromesistiuspoutassou*); functional and bioactive properties. J. Food Sci. 76(1): C14-C20

Guerard, F., Guimas, L. and Binet, A. (2002) Production of tuna waste hydrolysates by a commercial neutral protease preparation. J. Mol. Catal. B-Enzym. 19-20: 489-498

Hale, M. B. (1972) Making fish protein hydrolysate by enzymatic hydrolysis, A status report on research and some processes and products studied by NMFS, NOAA Technical Report, NMFS SSRF - 657, Seattle, WA

Ishida, Y., Fujita, T. and Arai, K. (1981) New detection and separation method for amino acid by high performance liquid chromatography. J. Chromatogr. 204: 143-148

Iwasaki, M. and Harada, R. (1985) Proximate and amino acid composition of the roe and muscle of selected marine species. J. Food Sci. 50: 1585-1587

Kim, S. and Wijesekara, I. (2010) Development and biological activities of marine derived bioactive peptides: A review. J. Funct. Foods 2: 1-9

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

Klompong, V., Benjakul, S., Yachai, M., Visessanguan, W., Shahidi, F. and Hayes, K. D. (2009) Amino acid composition and antioxidative peptides from protein hydrolysates of yellow stripe trevally (*Selaroidesleptolepis*). J. Food Sci. 74: C126-C133

Kolle, B. K., McKenna, D. R. and Savell, J. W. (2004) Methods to increase tenderness of individual muscles from been rounds when cooked with dry or moist heat. Meat Sci. 68(1): 145-154

Kristinsson, H. G. and Rasco, B. A. (2000) Fish protein hydrolysates: Production, biochemical and functional properties. Crit. Rev. Food Sci. 40: 43-81

Kurbanoglu, E. B. and Algur, O. F. (2002) The influence of ram horn hydrolyzate on the crop yield of the mushroom *Agaricus bisporus*. Sci. Hort. 94: 351-357

Liaset, B., Lied, E. and Espe, M. (2000) Enzymatic hydrolysis of by-products from the fish-filleting industry, chemical characterization and nutritional evaluation. J. Sci. Food Agr. 80: 581-589

Liaset, B., Nortvedt, R., Lied, E. and Espe, M. (2002) Studies on the nitrogen recovery in enzymatic hydrolysis of Atlantic salmon (*Salmosalar, L.*) frames by Protamex protease. Process Biochem. 37: 1263-1269

Motamedzadegan, A., Davarniam, B., Asadi, G., Abedian, A. and Ovissipour, M. (2010) Optimization of enzymatic hydrolysis of yellowfin tuna *Thunnusalbacares* viscera using Neutrase. Int. Aquat. Res. 2: 173-181

Mendis, E., Rajapakse, N., and Kim, S. (2004) Antioxidant properties of a radical scavenging peptide purified from enzymatically prepared fish skin gelatin hydrolysate. J. Agri. Food Chem. 53(3): 581-587

Ovissipour, M., Abedian K. A., Motamedzadegan, A., Rasco, B., Safari, R. and Shahiri, H. (2009) The effect of enzymatic hydrolysis time and temperature on the properties of protein hydrolysates from Persian sturgeon (*Acipenser persicus*) viscera. Food Chem. 115(1): 238-242

Panyam, D and Kilara, A. (1996) Enhancing the functionality of food proteins by enzymatic modification. Trends Food Sci. Technol. 7: 120-125

Pearce, K. N. and Kinsella, J. E. (1978) Emulsifying properties of proteins: Evaluation of a turbidimetric technique. J. Agri. Food Chem. 26: 716-723

Pearson, A. M. (1983) Soy proteins. In: Developments in food proteins-2, pp 67-108 (Hudson, B. J. F., Ed), Applied Science Publishers, England

Quaglia, G. B. and Orban, E. (1990) Influence of enzymatic hydrolysis on structure and emulsifying properties of sardine (*Sardina pilchardus*) protein hydrolysate. J. Food Sci. 55: 1571-1573

Rahali, V., Chobert, J. M., Haertle, T., and Gueguen, J. (2000) Emulsification of chemical and enzymatic hydrolysates of b-lactoglobulin: characterization of the peptides adsorbed at the interface. Nahrung 44: 89-95

Santos, S. A., Martins, V. G., Salas-Mellado, M. and Prentice, C. (2011) Evaluation of functional properties in protein hydrolysates from blue wingSearobin (*Prionotus punctatus*) obtained with different microbial enzymes. Food Bioprocess Technol. 4: 1399-1406

Sathivel, S., Bechtel, P., Babbitt, J., Smiley, S., Crapro, C. and Reppond, K. (2003) Biochemical and functional properties of herring (Clupeaharengus) byproduct hydrolysates. J. Food Sci. 68: 2196-2200

Shahidi, F., Hang, X. Q. and Synowiecki, J. (1995) Production and characteristics of protein hydrolysates from capelin (*Mallotus villosus*). Food Chem. 53: 285–293

Shahidi, F. (2000) Antioxidant factors in plant foods and selected oilseeds. Biofactors 13: 179-185

Shahidi, F. (2008) Nutraceuticals and functional foods: Whole versus processed foods. Trends Food Sci. Technol. 20: 376-387

Shuler P. (1990) Natural antioxidants exploited commercially. In: Food antioxidants, pp 65-98 (Hudson, B., Ed), Elsevier Applied Science, New York

Singleton, V.L. and Rossi, J.A. (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16: 144-158

Sze-Tao, K. W. C. and Sathe, S. K. (2000) Functional properties and in vitro digestibility of almond (*Prunusdulcis L.*) protein isolate. Food Chem. 69: 153-160

Taheri, A., AbedianKenari, A., Motamedzadegan, A. and Habibi-Rezaei, M. (2011) Poultry By-Products Enzymatic Hydrolysis: Optimization by response Surface Methodology Using Alcalase®2.4L.Int. J. Food Eng. 7(5): 1-17

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. Iran J. Fish. Sci. 12(1): 154-169

Tanuja, S., Viji, P., Zynudheen, A. A. and Joshy, C. G. (2012) Composition, functional properties and antioxidative activity of hydrolysates prepared from the frame meat of Striped catfish (*Pangasianodon hypophthalmus*) Egypt. J. Biol. 14: 27-35

Thiansilakul, Y., Benjakul, S. and Shahidi, F. (2007) Compositions, functional properties and anti-oxidative activity of protein hydrolysates prepared from round scad (*Decapterus maruadsi*). Food Chem. 103(4): 1385-1394

Wasswa, J., Tang, J., Gu, X. and Yuan, X. (2007). Influence of the extent of enzymatic hydrolysis on the functional properties of protein hydrolysate from grass carp(*Ctenopharyngodon idella*) skin. Food Chem. 104: 1698-1704

Wu, H., Chen, H., and Shiau, C. (2003) Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). Food Res. Int. 36: 949-957

Yen, G. and Wu, J. (1999) Antioxidant and radical scavenging properties of extract from *Ganoderma tsugae*. Food Chem. 65: 375-379