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Functional Properties and Proximate Analysis of Fish Waste Protein Hydrolysate Processed using Enzymes

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Abstract

A huge production of fish and their processing waste give rise to by-products comprises up to 70% depending on the species, size and processing method. The waste includes visceral parts, head, frames, bones, skin and cut-offs are rich source of protein with high functional properties. It is generally discarded which is a wastage of nutrient source and leading to environmental issues. Therefore, it was aimed to utilized the by-products for maximum recovery of nutrients by enzyme hydrolysis method for the preparation of protein hydrolysate with the used of papain and pepsin for digestion following different hydrolysis conditions. With the hydrolysis of papain enzyme (1 to 6%), the protein content of finfish waste protein hydrolysate ranges from 19.17% ± 0.06 to 73.14% ± 0.08 and that of shellfish waste protein hydrolysate prepared with 5,10 and 15% papain enzyme showed 26.73% ± 0.04 to 40.4% ± 0.5 which is comparatively low. Whereas the highest protein content was observed in 1% pepsin enzyme treated finfish waste protein hydrolysate with 80.55% ± 0.07. Besides, the hydrolysates were composed of 6.91% ± 0.05 to 10.46% ± 0.05 (moisture content), 0.6% ± 0.01 to 2.4% ± 0.01 (ash content) and 0.02% ± 0.005 to 0.09% ± 0.005 (fat content). The hydrolysates were highly soluble ranges from 72.73% ± 0.05 to 93.83% ± 0.1 which indicates development of small size hydrophilic with highly solvated polypeptide particles. A reduced phenomena of foaming capacity and stability were observed in shellfish waste protein hydrolysate in contrast with finfish waste protein hydrolysate.

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Similar pattern was also resulted in emulsifying stability index. Whereas the emulsifying activity index was in the range of 6.15 ± 0.03 to 9.85 ± 0.07 m^2/g. The water holding capacity of finfish and shellfish waste protein hydrolysate ranges from 3.4 to 4.23 gm/gm hydrolysate and 1.53 to 1.63 gm/gm hydrolysate respectively which is resulted by the difference in molecular weight of the peptide. The hydrolysates extracted from different sample with different enzyme and concentration at varying conditions were more or less similar ranges from 3.7 to 4.1 gm/gm protein hydrolysate (oil holding capacity). Hence, high protein content with good functional properties of the protein hydrolysate prepared with the utilization of fish waste is a positive impact on the attempt made to recover nutrient by enzymatic hydrolysis.

Introduction

The total fisheries production of the world is 177.8 million tonnes. Out of this, 157.4 and 20.8 million tonnes are of human consumption and non-food uses respectively. There is an expansion of fisheries processing sector which results to the increase in by-products up to 70% of processed fish based on the species, size as well as processing method. Fish waste comprises of approximately 9 to 12% head portion, 12 to 18% visceral part, 1 to 3% skin, 9 to 15% bone or frame and around 5% scales.[1] Similarly, a huge sum of fish catch roughly 60% is rejected during processing which comprises of visceral parts, frame, fins, skin and head portion without any recovery.[2] The fish by-product is a potential material which can used as an ingredient in value added products to enhance the nutritional properties with stable shelf-life and moreover utilization of by-products reduced environmental impact and economic loss.[1] The wastes can be utilized in the preparation of hydrolysate and incorporate in fish meal for animal feeds. Fish protein hydrolysate being rich in protein have the potential for pharmaceutical and nutritional applications.[3,4,5] The importance of protein hydrolysate extracted from marine by-products has increased the popularity due to the high protein content.[6] The protein hydrolysis involves disintegration of protein with a protein-digesting enzyme.[7] It has mentioned that fish waste contained approximately 58% protein, 19% fat and minerals, 22% monosaturated fatty acids along with oleic acid and palmitic acid.[8] Therefore, timely assortment and treating the by-products are critical step for advance processing. For fish protein hydrolysate production using fish waste, enzymatic method is found to be safe, fast and easily controlled process for human consumption. This method enhanced protein quality in the raw material, their functional properties and peptides.[9,10] Fish protein hydrolysate can be obtained by the process of putrefaction of fish protein into simpler peptides by enzymatic hydrolysis, or by hydrolytic reaction with the incorporation of acids or bases.[11] Several factors comprise of temperature, pH, time of hydrolysis and proteases or chemical used are highly responsible for the quality and characteristics of the fish protein hydrolysate.[12]

With the utilization of salmon frame, fish protein hydrolysate has been developed with the application of enzymatic process using alcalase and papain enzyme at different percentage ranges from 1%-3%.[13] Fish protein hydrolysate has also prepared from frozen eel fish with the process of hydrolyzation using papain enzyme.[14] The experiment with Chinese sturgeon muscle using papain enzyme at variable percentage ranging from 0.5% - 5%, maintaining the pH 5.5 -7 for different enzyme percentage by applying 25 mM sodium phosphate buffer and the final hydrolysate product obtained by the processed of lyophilisation method.[15] Grass turtle (*Chinemys reevesii*) with different ratio of muscle and liquid (distilled water), different percentage of enzyme, varying pH using sodium phosphate buffer and maintaining different time and temperature for the enzymatic hydrolysis process with papain enzyme was followed for the preparation of protein hydrolysate.[16]

Therefore, the study aimed to utilized finfish and shellfish processing waste material for maximum protein recovery with the preparation of protein hydrolysate by enzymatic method with papain and pepsin enzyme hydrolysis and to compare the difference in proximate composition and functional properties of the protein hydrolysate extracted from finfish and shellfish waste. The evaluation was

conducted to identify the highest protein content in the different hydrolysate with a good functional property to meet the recommended daily protein requirement.

Materials & Methods

Sample comprises of finfish and shellfish wastes were collected from Mirkarwada fish landing centre, Ratnagiri, Maharashtra. The samples were collected in cleaned plastic pouch and transported to the laboratory of Department of Fish processing Technology and Microbiology, College of Fisheries, Ratnagiri, Maharashtra, India in chilled condition (iced). Further, the samples were washed with tap water, segregated the finfish and shellfish waste. Then packed in plastic bag and stored in deep freezer at -18°C for the experiment.

Table 1: The factors levels maintained to acquire the ideal enzymatic reaction of fish waste using Papain enzyme

Factors	Signs	Factor levels					
		1	2	3	4	5	6
Fish waste: Distilled water (W/V)	S: L	1:1	1:2	1:3	1:4	1:5	1:6
Enzyme (%)	E: S	1	2	3	4	5	6
pH (pH)	pH	5.5	6	6.5	7	7	7
Temperature (^{0}C)	T	40	45	50	55	60	65
Time (hour)	t	2	3	4	5	6	7

Preparation of Protein Hydrolysate from Fish Waste using Papain Enzyme

Enzymatic reaction of fish waste with papain enzyme with following different factors levels (Table: 1) to acquire the ideal enzymatic reaction condition. Protein hydrolysates were prepared following the protocol with the modification in drying process.16 The 25×10-3M sodium phosphate buffer was added to maintain a constant pH throughout the process of hydrolysis. The substrate was subjected at 90°C for in water bath (Bio Techno Lab, Mumbai, India) for 20 min for enzyme deactivation. Then, immediately shift the digested mixture in ice bath and proceeded by centrifugation for 20 min, 10000g at 4°C. The supernatant was transferred in a steel tray and dried in cabinet dryer (Fourtech, Techno industries, 1000 rpm, 230 volt, India) for 6 h at 55°C whereas exceeding the time and temperature resulted browning reaction. The final hydrolysate powder was stored at -20°C for further analysis.

Development of Fish Waste Protein Hydrolysate using Pepsin Enzyme

Fish waste protein hydrolysate was also developed with the addition of 1% pepsin enzyme in the substrate with the modification in drying process.17 Fish waste and distilled water was homogenated by mixing in the ratio of 1:2. With the inclusion of 2M HCl, the homogenated substrate was calibrated at pH 2.5 prior to the addition of enzyme and kept at 37°C for 5 min to attain temperature equilibrium. The process of hydrolysis took place for 3 h at 37°C and terminated the process of digestion by incubating the mixture at water bath (Bio Techno Lab, Mumbai, India) in boiling condition for 15 min. Then, reduced the temperature of the mixture at ambient temperature and pH was adjusted at 7 with 2 M NaOH. With the use of a muslin cloth, the mixture was filtered and centrifuged (Hettich Zentrifugen, D-78532, Germany) the filtrate at 10,000 rpm for 15 min. The supernatant was poured in a steel tray and dried in cabinet dryer (Fourtech, Techno industries, 1000 rpm, 230 volt, India) at 55°C for 6 h as optimum condition as exceeding the time and temperature resulted browning reaction. The hydrolysate obtained was packed airtight and stored at -20°C for analysis.

Development of Shellfish Waste Protein Hydrolysate using Papain Enzyme

Shellfish waste protein hydrolysate preparation was conducted with the addition of drying process.18 The shellfish waste was crush using mortar and pestle and blend with distilled water at 1:1 ratio (weight/volume) and allowed to homogenize for about 2 min. Then, adjusted the pH at 7.0 (optimal

pH of papain enzyme) using 1M NaOH solution and 1M HCl solution. Hydrolysates were prepared with three different enzyme concentrations (5%, 10% and 15%). The mixtures were incubated at 60°C at water bath (Bio Techno Lab, Mumbai, India) for 24 h. Later, the mixture was subjected at 85°C for 15 min for the termination of enzymatic activity. The product was centrifuged (Hettich Zentrifugen, D-78532, Germany) at 10,000g for 15 min and collected the supernatant and dried using cabinet dryer (Fourtech, Techno industries, 1000 rpm, 230 volt, India) at 55°C for 6 h to prevent browning resulted with the increase in time and temperature and stored the hydrolysate in -20°C. As the result of the papain hydrolysis with shellfish waste, the protein content in the hydrolysate was low. Therefore, shellfish waste was not subjected to pepsin enzyme hydrolysis.

Analysis of Protein Hydrolysates

Proximate Analysis

The proximate analysis includes determination of protein, fat, moisture and ash content of protein hydrolysates was evaluated following the standard protocol.[19]

Functional Properties

The protein solubility, emulsifying properties, foaming capacity, water and oil holding capacity of the fish waste and shell fish waste protein hydrolysates were analyzed.

Protein Solubility

The protein solubility of hydrolysate was evaluated with slight modification.[16] The hydrolysate sample (200mg) was dissolved in 20ml water (deionized), adjusted the pH of the solution with 0.1M HCl or 0.1M NaOH in the range of 2 to 10. The solution was then kept for 30 min at 30°C in water bath (Bio Techno Lab, Mumbai, India). By Kjeldahl (KelPlus Classic DX, Pelican, India) method, the protein content in supernatant was detected.[2] Therefore, protein solubility was determined by using the formula:

Protein solubility (%) = (Protein content in supernatant)/(Total protein content in sample)×100

Emulsifying Properties

According to guidelines given, the emulsifying activity index (EAI) and emulsifying stability index (ESI) was estimated with minor changes.[20] 30ml protein hydrolysate solution (1%) mixed with 10ml vegetable oil was homogenised in a homogeniser (Remi motor, RPM 8000) at 800rpm for 1 min. Pipetted 50µl aliquot of emulsion after homogenization at 0 and 10 min from the bottom of the container and mixed with 5ml 0.1% sodium dodecyl sulphate (SDS) solution. Using spectrophotometer (UV Probe, Ver 2.43, Shimadzu) at 500nm, the absorbance of the solution was measured immediately after emulsion formation at 0 min (A0) and 10 min (A10) which is mixed with 5ml 0.1% sodium dodecyl sulphate (SDS) solution. The following formula was applied to determined EAI and ESI:

Emulsifying activity index (EAI) = (2 × 2.303 × A0)/(0.25 ×weight of protein)

Emulsifying stability index (ESI) = (A10 × Δt)/ΔA

Where: Δt = time; $\Delta A = A_0 - A_{10}$

Foaming Properties

Foaming capacity and stability of fish and shellfish waste protein hydrolysate was evaluated as given in the protocol.[20] Whipped 20ml 0.5% sample solution using homogeniser (Remi motor, RPM 8000) at a speed of 800rpm at ambient temperature for 2 min to incorporate air. Immediately transferred the whipped sample in 25ml measuring cylinder and recorded the volume. The foaming capacity (FC) was determined by the formula given below:

Foaming capacity % (FC) = (V2-V1)/V1 ×100

Where: V_2 = volume after whipping (ml)
V_1 = volume before whipping (ml)

Rest the whipped sample for 3 min at ambient temperature and recorded the volume. The foam stability was calculated by the given formula:

Foaming stability % (FS) = (V2-V1)/V1 ×100

Where: V_2 = volume after rest (ml)
V_1 = volume before whipping (ml)

Water and Oil Holding Capacity

Water and oil holding capacity was studied according to the protocol given by the author with slight modification.[15] Dissolved 0.5g hydrolysate in 10ml distilled water or 10ml soyabean oil using centrifuged tube and dispersed the mixture with the used of

vortex mixer (Cyclo mixer, Eppendorf, India Ltd.) for 1 min. The mixture of protein hydrolysate and water and oil with protein hydrolysate in the centrifuged tube were allowed to settle at 25°C for 7h and 20 min respectively and centrifuged (Hettich Zentrifugen, D-78532, Germany) (5000g) for 25 min at 4°C. To obtained the water holding capacity, filtered the supernatant using filter paper (Whatman No. 1) and recorded the difference in weight of the sample before dissolving in water and oil and after filtration of the sample recovered in the filter paper. The result was expressed as gm/gm protein hydrolysate.

Results

Sample code of fish waste protein hydrolysate and hydrolysis condition is described in table 2.

Table 2: Sample code and their description

Sr. No.	Raw material	Enzyme used	Enzyme (%)	Solid to Liquid ratio	Temperature and Time	pH	Sample code assigned
1	Fish waste	Papain	1	1:1	40°C, 2 h	5.5	FPH 1
2			2	1:2	45°C, 3 h	6	FPH 2
3			3	1:3	50°C, 4 h	6.5	FPH 3
4			4	1:4	55°C, 5 h	7	FPH 4
5			5	1:5	60°C, 6 h	7	FPH 5
6			6	1:6	65°C, 7 h	7	FPH 6
7	Shellfish waste		5	1:1	60°C, 24 h	7	SFPH 7
8			10	1:1	60°C, 24 h	7	SFPH 8
9			15	1:1	60°C, 24 h	7	SFPH 9
10	Fish waste	Pepsin	1	1:2	37°C, 3 h	2.5	FPH 10

Table 3: Proximate Composition of papain and pepsin enzyme treated fish waste protein hydrolysate

Sample code	Protein (%)	Ash (%)	Fat (%)	Moisture (%)
FPH 1	73.14 ± 0.02 ^{i}	0.6 ± 0.01 ^{a}	0.02 ± 0.005 ^{a}	8.61 ± 0.03 ^{d}
FPH 2	58.25 ± 0.07 ^{h}	2.24 ± 0.02 ^{h}	0.04 ± 0.005 ^{b}	10.46 ± 0.05 ^{j}
FPH 3	46.81 ± 0.05 ^{g}	1.93 ± 0.04 ^{g}	0.04 ± 0.005 ^{b}	10.14 ± 0.10 i
FPH 4	35.3 ± 0.13 ^{e}	1.55 ± 0.03 ^{d}	0.07 ± 0.005 ^{c}	9.85 ± 0.08 ^{h}
FPH 5	23.99 ± 0.07 ^{b}	1.36 ± 0.02 ^{b}	0.08 ± 0.001 ^{c}	9.67 ± 0.06 ^{g}
FPH 6	19.17 ± 0.02 ^{a}	1.44 ± 0.05 ^{c}	0.09 ± 0.005 ^{d}	9.16 ± 0.06 ^{e}
SFPH 7	40.4 ± 0.04 ^{f}	1.84 ± 0.08 ^{f}	0.03 ± 0.005 ^{a}	7.49 ± 0.10 ^{c}
SFPH 8	33.85 ± 0.15 ^{d}	1.85 ± 0.04 ^{f}	0.02 ± 0.005 ^{a}	7.29 ± 0.06 ^{b}
SFPH 9	26.73 ± 0.20 ^{c}	1.73 ± 0.05 ^{e}	0.04 ± 0.002 ^{b}	6.91 ± 0.05 ^{a}
FPH 10	80.55 ± 0.02 ^{j}	2.4 ± 0.01 ^{i}	0.05 ± 0.005 ^{b}	9.36 ± 0.05 ^{f}

Mean ± Standard Deviation. Values within a group column followed by the same superscript letter are significantly different from each other ($P < 0.05$), according to Tukey's test.

Proximate Analysis of Fish Waste Protein Hydrolysates

The proximate composition of fish waste protein hydrolysates is given in table 3.

Proximate composition of fish protein hydrolysate prepared from fish and shellfish waste using papain and pepsin enzyme at different percentage were done. The values of protein, fat, ash and moisture present in the samples are given in Table 3. The protein content of papain enzyme (1 to 6%) treated fish waste protein hydrolysate (FPH 1 to FPH 6) ranges from 19.17% ± 0.02 – 73.14% ± 0.02 and shellfish waste protein hydrolysate (SFPH 7 to SFPH

9) prepared with 5, 10 and 15% papain enzyme showed 26.73% ± 0.20 - 40.4% ± 0.5 protein content. Among FPH 1 to FPH 6, the highest protein content was recorded in FPH 1 (73.14%) with 1% enzyme treatment and lowest was found in FPH 6 (19.17%) and out of shellfish waste protein hydrolysate, SFPH 7 showed highest protein content 40.4% with 5% enzyme treatment and lowest protein (26.73%) was recorded in SFPH 9 by 15% enzyme treatment. Whereas the highest protein content was observed in 1% pepsin enzyme treated (FPH 10) fish waste protein hydrolysate with 80.55% ± 0.02. The range of moisture content was 6.91% ± 0.05 - 10.46% ± 0.05, ash content 0.6% ± 0.01 - 2.4% ± 0.01 and fat content 0.02% ± 0.005 - 0.09% ± 0.005 were observed in the hydrolysates treated with different enzymes at varying percentage with fish and shellfish waste. Moisture content in all FPH samples were found below 10%. Also, in all FPH treatments fat content were showed in the lowest level which was help to avoid oxidation of fat. It was noticed that higher protein content was observed in FPH treated with lower percentage of enzyme.

Fish waste consist of head, tail, skin, gut, fins and frames. Approximately 45% comprises of 24-34% bones, 20-25% head and 4-5% skin are unutilized in the whole fish filleting procedure.[21] Moreover, the by-products are rich in protein and amino acids, collagen, gelatin, oil and enzymes.[22] Therefore, the present study utilized the fish waste for extraction of fish protein hydrolysate by enzymatic method. The nature of the hydrolysate was hygroscopic which is supported by the statement given in the documentation.[13] Additionally, it was also mentioned that hydrolysate possess a high water holding capacity leading to hygroscopic nature.[23]

Several researchers reported different protein percentage obtained from varying raw materials by different enzymatic hydrolysis process. A protein content of 48%,[24] 73.35% to 76.63%,[16] 19.6%,[25] 84.81%,[18] 13.1 to 14.2%[26] and 79.20% to 82.01%.[13] In the present study, the protein content ranges from 19.17% to 80.55%. the highest protein content was obtained from fish waste hydrolysis with 1% pepsin enzyme. The difference in raw material, enzyme and its percentage, temperature, pH and ratio of raw material and water in the substrate are the factors for the diverse protein content in the FPH. The solubilization of protein and removal of lipids and undigested non-protein particles after hydrolysis results in high protein content.[27]

The fat content was recorded a range of 0.02 to 0.09%. In the FPH prepared from sardine solid waste, 2.1-9.2% of fat content was observed,[26] in salmon frame protein hydrolysate 6.03% – 6.34% fat content[13] and 2.39 to 3.4% fat was present in shrimp hydrolysate.[18] The low fat content in the study may be due to the variation in raw material and filtration technique in which fat are eliminated. Moreover, during hydrolysis, cell membranes of muscle likely gather together and formation of insoluble vesicles takes place losing the lipid membrane.[28] The amount of lipid content affects the oxidation stability. Hence, the protein hydrolysate acquired in the present experiment are more stable.[29]

Ash content of 9.81% - 11.09%13, 6.58%[15] and 9.07%[17] were recorded. In the current study, ash content of 0.6% to 2.4% was found. The variation may be due to the composition of raw material which is supported by the statement that the mineral content is attributed by bones, scales and head portion of the fish.[30]

Moisture is an essential unit that holds all the other components in the biological substance. The moisture content was found to be 6.91% to 10.46%. a more or less similar results were also reported 5.06%[31] and 5.04%.[17]

Functional Properties of Fish Protein Hydrolysate (Fph) Prepared Using Fish and Shellfish Waste Using Papain and Pepsin Enzyme

Functional properties of the hydrolysates are depicted in table 4 and table 5. The foaming properties of FPH 1 to FPH 6 ranges from 17.43% ± 0.02 to 19.89% ± 0.05 (capacity) and 10.79% ± 0.07 to 11.91% ± 0.02 (stability), SFPH 7 to SFPH 9 in the range of 7.34% ± 0.04 to 7.64% ± 0.01 and 4.75% ± 0.09 to 4.91% ± 0.03 respectively while FPH 10 showed 20.27% ± 0.06 and 12.08% ± 0.01 foaming capacity and stability respectively. Among the treatment, the highest foaming capacity and stable hydrolysate was FPH treated with 1% pepsin. The value of emulsifying activity index (EAI) and emulsifying stability index (ESI) for FPH 1 to FPH 6 were found in the range of 6.15 m^2/g ± 0.03 to 9.85 m^2/g ± 0.07 and 44.33 min ± 0.5 to 49.66 min ± 0.5 respectively, SFPH 7 to SFPH 9 showed the value

in the range of 6.43 m^2/g ± 0.04 to 6.51 ± 0.02 and 17.66 min ± 0.5 to 19.66 min ± 0.5 respectively. The EAI and ESI of FPH 10 was recorded 6.54 m^2/g ± 0.05 and 44 min ± 0.08 respectively. The statistical analysis includes ANOVA and Tukey's test was performed to observed the significant difference among protein hydrolysates. It was found that FPH 1 to FPH 10 were significant to each other ($p < 0.05$) with respect to foaming and emulsifying properties.

Table 4: Functional properties (Foaming capacity, foaming stability, emulsifying properties) of hydrolysate treated with papain and pepsin enzyme

Sample code	Foaming (%)		Emulsifying properties	
	Capacity	Stability	EAI (m2/g)	ESI (min.)
FPH 1	19.89 ± 0.05 ^{d}	10.79 ± 0.07 ^{c}	6.15 ± 0.03 ^{a}	49.66 ± 0.5 ^{i}
FPH 2	17.7 ± 0.01 ^{bc}	11.89 ± 0.07 ^{g}	6.81 ± 0.02 ^{e}	48.66 ± 1.1 ^{h}
FPH 3	17.43 ± 0.02 ^{b}	11.91 ± 0.02 ^{h}	9.85 ± 0.07 ^{f}	45 ± 0.03 ^{g}
FPH 4	17.88 ± 0.04 ^{bc}	11.36 ± 0.09 ^{d}	6.43 ± 0.04 ^{c}	44.33 ± 0.5 ^{e}
FPH 5	18.05 ± 0.02 ^{c}	11.68 ± 0.06 ^{f}	6.33 ± 0.08 ^{b}	44.53 ± 0.5 ^{e}
FPH 6	18.2 ± 0.03 ^{c}	11.57 ± 0.02 ^{e}	6.31 ± 0.1 ^{b}	44.66 ± 0.5 ^{f}
SFPH 7	7.64 ± 0.01 ^{a}	4.85 ± 0.01 ^{b}	6.51 ± 0.02 ^{d}	19.66 ± 0.5 ^{c}
SFPH 8	7.38 ± 0.03 ^{a}	4.91 ± 0.03 ^{b}	6.50 ± 0.04 ^{d}	18.33 ± 0.5 ^{b}
SFPH 9	7.34 ± 0.04 ^{a}	4.75 ± 0.09 ^{a}	6.43 ± 0.04 ^{c}	17.66 ± 0.5 ^{a}
FPH 10	20.27± 0.06 ^{e}	12.08 ± 0.01 ^{h}	6.54 ± 0.05 ^{d}	44 ± 0.08 ^{d}

Results in the table represent the mean of triplicate measurements.
Mean ± standard deviation. Values within a group column followed by the same superscript letter are significantly different from each other ($P < 0.05$), according to Tukey's test.

Foaming Properties

In the experiment, foaming capacity and stability was recorded 19.89% and 10.79% respectively with 1% papain enzyme and 20.27% and 12.08% respectively with 1% pepsin enzyme treated fish waste hydrolysate. A similar result of 20.3% and 10.67% foaming capacity and stability respectively with 0.5% papain enzyme hydrolysis of red meat of *Euthynnus affinis* was reported.[20] A variable result on foaming properties were reported. Foaming capacity ranges from 9.96% to 69.72% with the application of alcalase enzyme32, 18.75 - 60% foaming capacity and 4.68 - 28.12% foaming stability[14] and 100% foaming capacity was also reported.[16]

The factors for the difference in foaming properties may be due to the different raw material, enzyme and protein concentration which is justified by the statement that the foam is more stable with higher protein concentration in the hydrolysate.[33] Foam stability is intensified by adjustable protein domains that strengthen the cohesiveness of the aqueous phase, film and amount of protein.[34] More the protein concentration, higher the rate of diffusion.[35] At isoelectric point, foam stability reduces. Therefore, change in pH is advantageous for the insensitivity of protein hydrolysate.[36]

Emulsifying Properties

The EAI and ESI ranges from 6.15 to 9.85 m^2/g and 17.66 to 49.66 min respectively. A similar result of 6.8 m^2/g EAI and 40 min ESI in red meat (*Euthynnus affinis*) protein hydrolysate treated with 0.5% papain enzyme was also reported.[20]

A few statements on the emulsifying properties were given by some authors. The protein hydrolysates carry both hydrophilic and hydrophobic charged group which is surface-active materials that promotes oil-in-water emulsion.[37] At low protein concentration, protein absorption in diffusion-controlled at the interface of non-miscible liquids (oil-water) and at the presence of high amount of protein, accumulation of protein in aqueous phase leading to the reduction in EAI and ESI of protein hydrolysate. There is more

protein-protein interaction at low concentration at the interface.[33]

Water Holding Capacity (Whc) and Oil Holding Capacity (Ohc)

The WHC and OHC was in the range of 1.53 to 4.23 g and 3.71 to 4.10 g respectively. With the used of papain enzyme, the maximum WHC was recorded at pH 5.5 and lowest at pH 7 whereas both the maximum and minimum OHC was recorded at pH 7. A more or less similar finding has been who reported the oil holding capacity at 2.4 - 3.6 ml oil/g FPH[38] and 1.93 gm/gm FPH and 2.59 gm/gm FPH WHC and OHC respectively.[15] WHC and OHC ranges from 2.87-4.38 gm/gm protein hydrolysate and 2.33-3.66 gm/gm protein hydrolysate were also found.[16] The difference in the WHC is possibly due to dissimilarity in the molecular weight of peptides. The low molecular weight peptides can hold more water than larger peptides.[23] Besides, the thickness of peptides, enzymes and specificity of substrate also determine the OHC of hydrolysate.[39]

Table 5 :Functional properties (water holding capacity [WHC], oil holding capacity [OHC] and solubility) of hydrolysates

Sample code	WHC (g)	OHC (g)	Solubility (%)
FPH 1	4.23 ± 0.01 ^{g}	3.73 ± 0.02 ^{a}	88.53 ± 0.12 ^{d}
FPH 2	3.46 ± 0.02 ^{cd}	3.96 ± 0.03 ^{c}	93.83 ± 0.11 ^{f}
FPH 3	3.53 ± 0.05 ^{e}	4.03 ± 0.01^{d}	92.53 ± 0.10 ^{e}
FPH 4	3.40 ± 0.03 ^{c}	3.76 ± 0.05 ^{b}	87.61 ± 0.02 ^{bc}
FPH 5	3.45 ± 0.01 ^{d}	3.71 ± 0.04 ^{a}	87.32 ± 0.21 ^{bc}
FPH 6	3.46 ± 0.04 ^{d}	3.76 ± 0.01 ^{b}	88.13 ± 0.11 ^{c}
SFPH 7	1.63 ± 0.05 ^{b}	4.03 ± 0.05 ^{a}	85.23 ± 0.10 ^{bc}
SFPH 8	1.53 ± 0.01 ^{a}	4.10 ± 0.01 ^{e}	85.93 ± 0.09 ^{bc}
SFPH 9	1.56 ± 0.02 ^{a}	4.02 ± 0.02 ^{d}	84.73 ± 0.12 ^{b}
FPH 10	3.86 ± 0.03 ^{f}	4.06 ± 0.01 ^{de}	92.73 ± 0.12 ^{a}

Results in the table represent the mean of triplicate measurements.
Mean ± standard deviation. Values within a group column followed by the same superscript letter are significantly different from each other ($P < 0.05$), according to Tukey's test.

Solubility

Solubility stands the prime functional and physicochemical properties of hydrolysate.[40] The solubility of protein hydrolysates was in the range of 72.73% - 93.83%. The dissemination of protein in solvent is the main cause for the solubility and high solubility indicates development of small size hydrophilic and solvated polypeptide particles.[41] Good solubility promotes emulsification properties, foam formation and gelling capacity.[42] The high protein solubility of the hydrolysate shows a good potential in food formulation to enhance appearance and smooth texture of the product.[43] Enzymatic reaction of the protein slowly splits the protein into smaller peptide units. Therefore, solubility of hydrolysate increases over an extensive range of pH which is a suitable quality for application in food industries. The reason for a better solubility may be a result of the increase in hydrophobicity due to ionizable hydrophillic amino and carboxyl groups of the amino acids.[44] A solubility of 93.83% was observed at pH 6. Similarly, a researcher was also found solubility of 95.56% at the same pH.[16]

Conclusion

A huge amount of fish waste has been generated from seafood industries and fish market which remains unutilized and discarded in water bodies that leads to pollution and pathogenic infections. On the other hand, many reports claimed that fish waste can be a good source of nutrients mainly protein. Hence several attempts have been made to isolate the protein for the utilization as functional components in food industries, cosmetics and nutraceuticals companies.

The highest protein and ash content was obtained with 1% pepsin enzyme treated fish waste protein hydrolysate along with lower fat and moisture content. Additionally, the foaming capacity and stability and WHC was also found highest whereas moderately high emulsifying properties and OHC in 1% pepsin treated hydrolysate was observed. The solubility was more than 90%. Research findings in the study suggested that protein extraction using fish waste was found successful with enzyme treatment and recovered a high amount of protein with good physicochemical and functional properties. Moreover, pepsin enzyme shows more effective than papain enzyme in the production of finfish waste hydrolysate.

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Conflicts of interest

All authors mentioned no conflicts of interest in this research.

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