Characteristics of Fish Protein Hydrolysate from Yellowstripe Scad (*Selaroides leptolepis)* Produced by a Local Microbial Protease

Yusro Nuri Fawzya[1*], *Safira M.* Nursatya[2], *Rini* Susilowati[1], *Ekowati* Chasanah[1]

[1] Research Centre for Marine and Fisheries Product Processing and Biotechnology
Jalan K.S.Tubun, Petamburan VI, Jakarta
[2] Universitas Jenderal Soedirman, Purwokerto

Abstract. Fish protein hydrolysate (FPH) containing small protein or peptides and amino acids has a great attention related to the provision of high protein foods to overcome the problem of malnutrition. This research was purposed to prepare FPH from yellowstripe scad (*Selaroides leptolepis)* by using a local microbial protease from *Bacillus subtilis* BII-1. Hydrolysis process was done in a laboratory scale (500 g minced fish) at 55ºC for 6 h. The liquid hydrolysate was then spray dried using whey protein and maltodextrin at a concentration of 20 and 30% for each filler. The treatment of whey protein powder produced FPHs with higher protein content (31.71-33.97% db) and slightly yellowish in color compared to maltodextrin (11.88-16.66% db). Their foaming capacity and stability were 20-100% and 15% in 5-10 min, respectively. However, FPHs prepared with maltodextrin had no foaming capacity. The hydrolysates from both treatments had low water and oil absorption with the value less than 3 mL/g hydrolysate. A trial on scaling up production using 30 kg fish, showed that optimization or adjustment should be carried out due to the high amount and high protein content of the residual products.

1 Introduction

The supply of nutritious food, particularly rich in protein is required by most of the world, including Indonesia, which currently deals with the problem of stunting. Children suffering from stunting reached 37.2% in 2013, higher than that at the year of 2010 (36.8%) [1]. Many factors involved in this problem including the low income, poor of sanitation and hygiene, lack of education, and provision of nutritious food.

Fish as source of high quality animal protein is available abundantly at affordable prices. However, it has not proportionately distributed in its role to achieve nutritional adequacy for Indonesian people. In addition, fish are very perishable and vary in their chemical composition which present specific problems in their processing.

*Corresponding Author: nurifawzya@gmail.com

Fish protein hydrolysate (FPH) is a hydrolyzed fish protein which is more easily digested and absorbed than native protein due to its simple form as peptides and amino acids. The product can be processed by the enzymatic as well as chemical methods. By hydrolyzing, the fish protein is broken down into peptides and amino acids with smaller molecular weight. Fish protein hydrolysate has a wide application in accordance with its functional properties including as protein supplement and fortificant, stabilizer, flavor enhancer, and milk replacers [2]. Many researches on FPHs production have been published which mostly done by enzymatic method. Commonly, the commercial enzymes were used, including papain and alcalase. Enzymatic FPHs production was derived from various fish or fish by-products, such as sardines (*Sardinella lemuru*) [3], 'lele dumbo' (*Clarias gariepinus)* [4], fish by-products [5], *Clarias batrachus*, a freshwater catfish [6], and tilapia fish waste mince [7]. Local proteases used in FPHs processing were found in a limited number of papers, such as biduri protease [8], local papain [9] [10] and microbial protease produced from *Bacillus* sp. isolated from marine environment [11] [12].

Previous study on preparation of protein hydrolysate from yellow stripe scad using a local *Bacillus subtilis* BII-1 protease showed that the optimum hydrolysis time was 6 h. The total activity of enzyme used was 500U for 75 mL fish slurry [11]. The resulting liquid FPH needs to be converted into a powder form through drying process, to extend the shelf life and make easy in distribution. Freeze drying is considered to be the best method because this process allows us to maintain the high protein of FPH. However, this method is quite expensive to apply on a larger scale. Other drying method used to prepare powdered FPH at an industrial scale is spray drying [13], which is lower in cost, but lower in protein content due to the addition of filler before spray dried. Protein hydrolysate from yellowstripes cads contained protein in the range of 39.94 – 51.76% when it was dried by ultrasonic spray drier; and 50.90 – 60. 23% when was dried by a freeze drier [14]. Whereas, the protein content of peptones powder from *Lutjanus* sp. spray dried with maltodextrin as the filler, was 20-38% [15]. Maltodextrin and arabic gum were also used as the carrier agent in pink perch protein hydrolysate for reducing its bitter taste and to characterize the protein hydrolysates [16]. Maltodextrin is a polysaccharide which is the most frequently used as encapsulant in spray drying of food due to its effectivity in protecting food from oxidation, besides its other advantages properties such as high solubility and rapid dispersion [17, 18]. In this research we used maltodextrin, compared to whey protein powder in spray-drying FPH from yellowstripe scad, and evaluate their effect on the protein content and properties of FPH powder produced.

Production of FPH in laboratory scale is the basis of production process before scaling up process at pilot plant and industrial scale. Pilot plant scale production is intended to get products which are identical (if possible) on a larger scale than the predetermined production scale. In this work, an initial trial of scaling up FPH production was studied to obtain mass balance information and evaluate the changes or differences of the FPH quality during scaling up.

2 Materials and methods

2.1 Materials

Fish used for a laboratory scale study was fresh Yellostripe scad (*Selaroides leptolepis*) obtained from Muara Angke, Jakarta with the average size of 14.36 ± 0.77 cm in length, 4.15 ± 0.16 cm in width and 34.18 ± 4.14 g in weight. The fish had moisture content of 81.28±0.61 %, ash content of 0.49±0.02 %, protein content of 13.83±2.35 % and fat content of 0.75%. Meanwhile, frozen fish was used for the scaling up trial. The fish was bought from the auction

place (TPI) in Tegal, Central Java, packed in a Styrofoam box to keep cold, and brought to the laboratory in Jakarta. The fish was then eviscerated and filleted in a cold chain system by using pieces of ice, and kept in a cold storage until processed into Fish Protein Hydrolysate (FPH). Local microbial protease was prepared from *Bacillus subtilis* BII-1, a collection isolate of RCMFPB laboratory isolated from hot spring at Banyuwedang, Bali [19].

2.2 Preparation of enzyme

Protease enzyme was produced from *Bacillus subtilis* BII-1 following the method of Fawzya's trial [11]. Fresh isolate was firstly inoculated in Minimal Synthetic Medium (MSM) containing 0.1% K_2HPO_4; 0.1% NaCl; 0.7% $(NH_4)_2SO_4$; 0.05% yeast extract; 0.01% $MgSO_4$; 0.6% technical grade skim milk, then incubated at 37ºC 125 rpm for 18 h. Crude enzyme was separated as supernatant from centrifugation the culture at 8,000 rpm, 4ºC for 20 min. The enzyme activity was assayed based on Zilda's method [19].

2.3 Preparation of fish protein hydrolysate (in a laboratory scale)

Fish protein hidrolysate was prepared according to Fawzya et al. [11]. Fish fillet was chopped and blended with water (ratio 1:2 w/v). Approximately 1.5L of fish slurry from 500 g minced fish with 1L of water was heated until the temperature of 55°C achieved, and then added with 33 mL of 300 U/mL *Bacillus* protease. The mixture was stirred for 6 h at 55ºC for hydrolysis process. After 6 h hydrolysis, the reaction was stopped by increasing the temperature to 90°C, kept for 20 min. The hydrolysate was separated by centrifugation (8000 x g) for 10 minutes. Hydrolysate powder was prepared by spray drying using 2 types of fillers, i.e. a commercial whey protein powder (coded as PC) and maltodextrin (coded as MD) with the concentration of each filler was 20 and 30%.

2.4 Preparation of fish protein hydrolysate in a larger scale (± 30 kg fish)

Similar with the preparation of FPH in a laboratory scale (500 g minced fish or approx. 1.0 kg fish), the equipment used was slightly different with the lab-scale experiment. Hydrolysis process was done in a stainless steel tank equipped with temperature control and an agitation with a capacity of 100 kg of fish slurry. Whereas, hydrolysate was separated by using gradual filtration, starting from filter with size of 300 mesh, 600 mesh, microfiltration (5 µm) and ultrafiltration (0.1 µm). The liquid hydrolysate was partially sampled for freeze drying and the rest was spray dried which was preceded by addition of maltodextrin and whey protein powder with the concentration of 20% for each filler and homogenization at 16,000 rpm for 10 min. Spray drying was done at inlet temperature of 160ºC, outlet temperature of 90ºC and aspirator of 90%. Mass balance at each processing step was observed as well as moisture and protein content.

2.5 Analysis

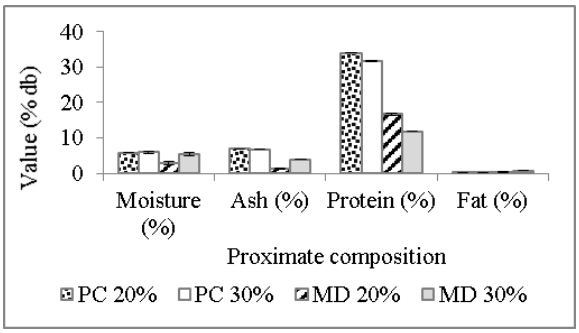
The resulting FPHs were analyzed for their nutritional content and physical properties. Proximate composition were determined according to SNI. Moisture content was determined by SNI 2354.2:2015 [20], ash content by SNI2354.1:2010 [21], protein by modification of SNI 01-2354.4-2006, using Kjeltec [22], fat content by SNI 2354-3-2006 [23]. The analysis of physical properties of FPHs included color by using the Hunterlab colorimeter (ColorFlex EZ) with the parameters of L* from black (0) to white (100), a* from green (1) to red (+), and b* from blue (-) to yellow (+) [24], foam capacity and stability [25], water

absorption [26], and oil absorption [27 with modification]. Mass balance was observed in the preparation of FPH at the pilot scale. Research was done in 2 replicates, except scaling up production which was done with no replication. The data were presented as an average values, and were analyzed by descriptive method.

3 Results and discussion

3.1 Chemical composition

Yellowstripe scad (*Selaroides leptolepis)* used in this study was categorized as low fat fish because the fat was less than 5% [28, 29]. When processing into FPHs powder, their protein content were affected by filler used. Whey protein powder coded as PC produced higher protein content of FPH compared to maltodextrin. It is due to the protein content of filler, which PC has protein content much higher than maltodextrin (20.36 vs 0.42%). Before drying process, the liquid hydrolysate contained of 90.85% (db) protein, meanwhile the protein content of FPHs powder were at the range of 11.88±0.08 to 33.97% (db) (Figure 1). This result was lower compared to yellowstripe scad protein hydrolysate reported previously [14]. They found that the hydrolysate powder produced by hydrolysis of yellowstripe scad using alcalase and ultrasonic spray dried contained protein varied from 40.06 to 51.76%, meanwhile those freeze dried had the protein content ranged from 52.86 to 60.23%. Many studies reported that fish protein hydrolysates contained much higher protein content, ranged from about 60 to 90% [30]. This is mainly because the FPHs were commonly produced in liquid form and the presented data are in % dry base. Besides, they were prepared in a powder form by freeze drying method with no addition of other substances, for example liquid FPH from milkfish (*Chanos chanos* Forsk) [31] and freeze dried protein hydrolysate from Pacific whiting muscle [32].



Note : PC : a commercial whey protein powder
MD : maltodextrin

Fig. 1. Proximate composition of fish protein hydrolysate powder from yellowstripe scad

The research produced a comparable result with FPH powder from silver catfish frame which spray dried using 5% maltodextrin and consisted of protein in the range of 32.9–35.6% [34]. Meanwhile, spray dried black Tilapia hydrolysates with 10% maltodextrin contained 37.7% [35]. A high protein hydrolysate powder which was dried using a spray drier was reported on hydrolyzed red meat of *Euthynnus affinis* [30]. The protein content of FPH was much higher than our result (~90% vs ~30%). It might be due to the less ratio of fish to water (1 : 1) used in the process [30] resulted in more concentrated liquid FPH that no need addition

of filler to spray dry it. The addition of filler in our research was aimed at reducing the stickiness and wall deposition in spray-drying [35].

3.2 Physical properties

3.2.1 Color

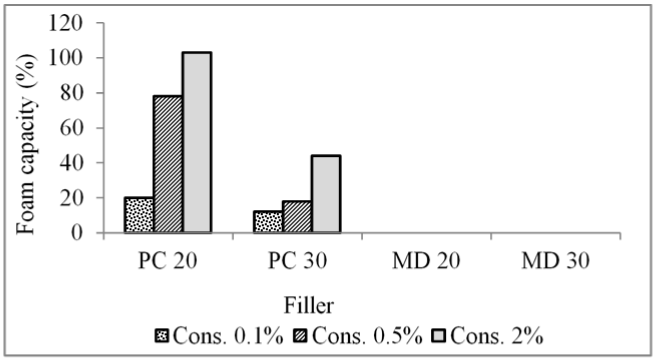
Color is one of the factors affecting in overall product's acceptance. It may be influenced by raw materials used, as well as hydrolysis and drying condition. The color measurement of the FPH showed that FPH produced by using PC filler exhibited a light yellowish color compared to the use of MD filler which had the higher value of L* and lower value of b* (Table 1). It might be due to the higher protein content of FPH-PC that was more sensitive to heat during the drying process for Maillard reaction.

Table 1. Hunter lab color parameter values of yellowstripe scad protein hydrolysate (FPH)

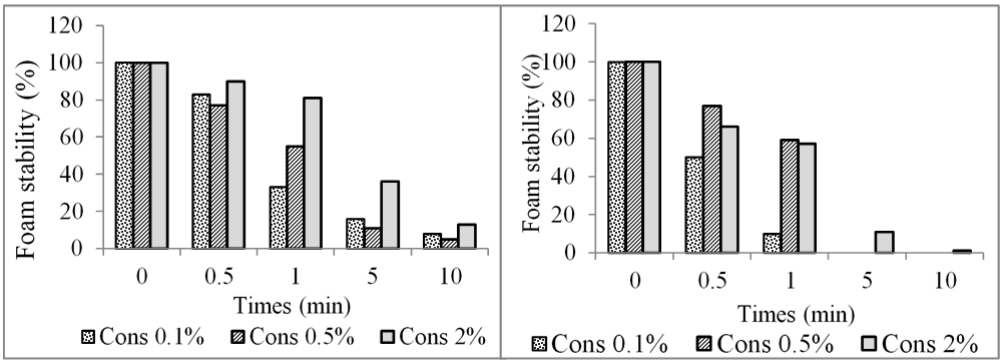
Treatments	L*	a*	b*
PC 20	94.97	-0.41	13.09
PC 30	95.49	-0.82	11.36
MD 20	96.70	-0.18	5.70
MD 30	96.27	-0.33	5.26

3.2.2 Foaming properties

Foaming capacity and stability of FPHs from yellowstripe scads were presented in Figure 2. It is shown that treatment MD did not produce foam at all, so there is no stability data either. Meanwhile, foam capacity from treatment PC increased in line with the increased of FPH concentration. This FPH with the concentration of 2% had the foam ability of 40 – 100%. Similar result was shown by protein hydrolysate from fish by product. At the concentration of 3%, the foam expansion of FPH reached 60-80%, meanwhile 0.1% FPH had foam capacity of 40 to 50% [36]. On the contrary, higher concentration of filler decrease foam capacity of FPH but increase their stability which can be seen from PC 30 vs PC 20. Compared to other FPH with higher protein content, such as freeze-dried FPH (protein of 70-80%) the foam stability of FPH produced by this research was much lower (remained 15% in 5-10 min vs remained 15% in 60 min). Another factor that may affect the foaming properties is degree of hydrolysis. Foam capacity and stability generally increase by the increase in degree of hydrolysis (DH), however excessive hydrolysis can have contrary effects on the foaming properties. Protein hydrolysis at the initial process (DH up to 12-15%) increased the lower molecular weight of protein, improve the solubility and hydrophobicity as well as the molecular flexibility, which then forming a more stable foam. Further hydrolysis with DH more than 15% led small peptides to increase the incorporation of air into the dispersion than larger peptides, thereby reducing foam capacity and stability [37, 38].



A



B C

Fig. 2. Foaming properties of FPH from yellowstripe scad : foam capacity (A), foam stability of FPH using 20% PC (B) and foam stability of FPH using 30% PC (C)

3.2.3 Other physical properties

Other physical properties observed were water and oil absorption (Table 2). It is shown that oil absorption of the FPH from yellowstripe scad was relatively low, ranged from 1.3 to 2.1 mL/g. This oil absorption capacity was similar with that from rainbow trout viscera protein hydrolysate (2.8 – 3.1 mL/g hydrolysate) [24]. The oil absorption is affected by size and nature of peptides [39]. Hydrophobic peptides with large molecule size have good ability in oil absorption. They found that oil absorption of protein hydrolysate from fresh water mussel *Lamellidens marginalis* varied from 12-35 mL/g FPH. Similar results were obtained in water absorption, where FPH from yellowstripe scads showed low absorption of water (0.1 – 0.3 mL/g). Previous report found that protein hydrolysate from rainbow trout viscera had water holding capacity 5.1 mL/g hydrolysate [24].

Table 2. Oil and water absorption of FPH from yellowstripe scad

Physical properties	PC 20	PC 30	MD 20	MD 30
Oil absorption (mL/g)	1.9	2.1	2.1	1.3
Water absorption (mL/g)	0.3	0.1	0.3	0.23

3.3 A trial on scaling up production of FPH

In order to scaling up production of FPH, a trial has been conducted by using a stainless steel hydrolysis tank with the capacity of 100 L slurry and an ultrafiltration membrane to separate the hydrolysate. Filtration using a 300 and 600 mesh filter bag initiated the separation step of hydrolysate, followed by filtration using micro and ultrafiltration. The first filtering step produced the largest amount of by-product, reached 7.5% composed of 1.3% frame and scales, and 6.2% meat fibers. Whereas, micro and ultrafiltration leaving soft fish pulp or paste about 2.5% (Table 3). The abundance in amount of meat fibers residue was found which may be caused by the non-optimal hydrolysis process of fish protein at upscale condition. The upscale process requires adjustment or optimization to reduce the by-products, such as prolong the hydrolysis time, reducing the ratio of fish meat: water or increasing the concentration of enzymes.

Table 3. Mass balance of FPH processing from yellowstripe scad at production scale of 30 kg fish

Step	**Product**	**Yield** (% based on 30 kg fish)
Fish	Fillet 15 kg	50
Minced meat	13.5 kg	45
Water	27 L	
Enzyme (300 U/mL)	900 mL	
Liquid FPH	37 L	
Residue 300 mesh	400 g	1.3
Residue 600 mesh	1.850 g	6.2
Residue microfiltration	450 g	1.5
Residue ultrafiltration	300 g	1.0
Liquid FPH (ultrafiltration)	31.5 L	
Filler (20%)	6.3 kg	
HPI padat (spray dried filtration)	5.9 kg	19.7
Total residues	3.000 g	10

Observation on moisture and protein content of products from each step are presented on Table 4. Both liquid and freeze dried FPHs contained high protein reached above 90% (db), meanwhile the protein of spray dried FPHs were much lower, ranged from 14.09 to 32.44% (db). This was because of the addition of filler up to 20%. Maltodextrin is a polysaccharide produced from starch that is used as a food additive. Based on the nature, liquid hydrolysate produced industrially concentrated from 25 to 50-60% solid before drying [40]. Concentration of the liquid FPH reduces the amount of filler used, and increase the protein content of FPH powder. High protein by-products of FPH processing were also observed on dried residue of 600 mesh filtration, with the protein content of 80.83% (db) similar with the centrifugation's residue of 80.70% (db). This remaining protein content in these residues may

be used as raw material to obtain higher value-added products. The residue of sardinella protein hydrolysate preparation had high content of glutamic acid which potential to be used as flavor enhancer [12].

Table 4. Moisture and protein content of each step products of FPH processing

Sample	Lab scale		Upscale	
	Moisture (% wb)	**Protein (% db)**	**Moisture (% wb)**	**Protein (% db)**
Liquid FPH	97.27	90.85	93.33	91.45
Freeze dried FPH	-	-	8.56	92.47
Spray dried FPH MD 20%	2.8	16.66	2.87	14.09
Spray dried FPH PC 20%	5.76	33.97	3.49	32.44
Centrifugation's residue	74.25	80.70	-	-
Dried Residue 300 mesh (vacuum oven)	-	-	4.57	76.40
Dried Residue 600 mesh (vacuum oven)	-	-	5.06	80.83

It was also investigated that the resulting FPH had the whiteness value of 87.3% and did not have any bitter taste. Small hydrophobic peptides were the main contributor to the bitter taste [41]. In addition, the type of proteases had the most significant impact on the properties of FPH. The *Bacillus subtilis* protease used in this research has similar properties to alcalase, including its optimum temperature and pH [42]. Alcalase is widely used for FPH preparation because it produced FPH with relatively high degree of hydrolysis in relative short time [2].

4 Conslucion

Hydrolysis of yellowstripe scads protein by using a local *Bacillus subtilis* BII-1 protease produced protein hydrolysate which had no bitterness. Spray drying using whey protein powder produced FPHs with higher protein content than that using maltodextrin. The use of whey protein powder as filler gave better quality of hydrolysate powder. However, process improvement needs to be done in order to increase the protein content or other properties of hydrolysates, and minimize the residual amount.

5 Aknowledgement

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