

Production and characteristics of fish protein hydrolysate from parrotfish (*Chlorurus sordidus*) head

Asep A. Prihanto^{1,2}, Rahmi Nurdiani^{1,2} and Annas D. Bagus²

¹ Department of Fishery Product Technology, Faculty of Fisheries and Marine Science, Brawijaya University, Malang, East Java, Indonesia

² BIO-SEAFOOD Research Unit, Brawijaya University, Malang, East Java, Indonesia

ABSTRACT

Background: Fish byproducts are commonly recognized as low-value resources. In order to increase the value, fish byproducts need to be converted into new products with high functionality such as fish protein hydrolysate (FPH). In this study, FPH manufactured from parrotfish (*Chlorurus sordidus*) heads using different pH, time and sample ratio was investigated.

Methods: Hydrolysis reactions were conducted under different pHs (5, 7, and 9) and over different durations (12 and 24 h). Control treatment (without pH adjustment (pH 6.4)) and 0 h hydrolysis duration were applied. Hydrolysates were characterized with respect to proximate composition, amino acid profile, and molecular weight distribution. The antioxidant activity of the hydrolysate was also observed.

Results: The pH and duration of hydrolysis significantly affected ($p < 0.05$) the characteristics of FPH. The highest yield of hydrolysate ($49.04 \pm 0.90\%$), with a degree of hydrolysis of $30.65 \pm 1.82\%$, was obtained at pH 9 after 24 h incubation. In addition, the FPH had high antioxidant activity ($58.20 \pm 0.55\%$), with a high level of essential amino acids. Results suggested that FPH produced using endogenous enzymes represents a promising additive for food and industrial applications.

Subjects Biotechnology, Food Science and Technology, Aquatic and Marine Chemistry

Keywords Endogenous enzyme, Parrotfish, Fish-by product, Fish protein hydrolysate, Antioxidant

INTRODUCTION

Parrotfish (*Chlorurus sordidus*) are one of the most important fish commodities in Indonesia (Adrim, 2010). Parrotfish have unique and exceptional arrangements of teeth and body shape (Chen, 2002). In 2014, parrotfish fishing production increased by 18.8% (76 tons) compared to all reef fish fisheries in the Asian region (Food & Agriculture Organization (FAO), 2015). In Indonesia, a total of 1.8 ton parrotfish production was recorded for 2019 (Ministry of Marine Affairs & Fisheries, 2019). An increase in the number of catch means an increase in the amount of byproduct processing, as processing requires the removal of bones, skin, head, scales, and viscera. Out of all the other body parts, the head accounts for approximately 19% of the total fish processing-byproducts from fillet processing (Anil, 2017). Several parts of by product such as scales and bone was applied as gelatin (Herpandi, Huda & Adzitey, 2011). In contrast, fish head was still underutilized.

Submitted 4 October 2019
Accepted 26 November 2019
Published 20 December 2019

Corresponding author

Asep A. Prihanto,
asep_awa@ub.ac.id

Academic editor
Rogerio Sotelo-Mundo

Additional Information and
Declarations can be found on
page 12

DOI 10.7717/peerj.8297

© Copyright
2019 Prihanto et al.

Distributed under
Creative Commons CC-BY 4.0

OPEN ACCESS

Fish byproducts, commonly recognized as low-value resources, can be further developed into products with high economic value if handled and processed appropriately (Hapsari & Welasi, 2013). In general, fish byproducts contain many elements, such as nitrogen, phosphorus, potassium, and others, which are the constituents of proteins and fats (Lepongbulan, Tiwow & Diah 2017). Thus, the protein fraction of byproducts can be utilized for the production of fish protein hydrolysate (FPH) with desirable functionality. In addition, FPH has reported to exhibit bioactive properties, such as antihypertensive, antioxidant (Yang et al., 2011), antithrombotic (Qiao et al., 2018), anticancer, and immunomodulatory activities (Kim & Mendis, 2006).

Fish protein hydrolysate can be manufactured from the decomposition of fish proteins into simple peptides (2–20 amino acids) through hydrolysis by adding enzymes, acids, or bases (Nurilmala, Nurhayati & Roskananda, 2018). The characteristics and quality of FPH are highly influenced by several factors, including the type of proteases or chemicals used, temperature, pH, and duration of hydrolysis (Nazeer & Kulandai, 2012).

In previous studies, FPH was developed using various fisheries byproducts, including cod head waste (Himonides, Taylor & Morris, 2011), catfish (Nurilmala, Nurhayati & Roskananda, 2018), tuna (Bougatef et al., 2012; Herpandi, Huda & Adzitey, 2011), Sardinella (Jeevitha, Priya & Khora, 2014), and tilapia (Srikanya et al., 2017). Nevertheless, the production of FPH from parrotfish byproducts remains limited. This study aimed to determine the characteristics of protein hydrolysates from parrotfish (*C. sordidus*) heads, extracted at different pHs and hydrolysis duration periods.

MATERIALS AND METHODS

Materials

All materials used in this experiment were of analytical grade and were purchased from Merck (Darmstadt, Germany, USA). Parrotfish (*C. sordidus*) heads with the average weight of 250 ± 18 gr were obtained from a local fish processing plant (PT. Alam, Surabaya, Indonesia). The heads were transported to the laboratory using a storage box maintained at 4 °C.

Preparation of fish protein hydrolysate

Preliminary experiments on the optimum water: substrate ratios were conducted to obtain the highest yield and antioxidant activity of hydrolysate. Fish heads were crushed in Philips-Food Processor, model HR7627, 650 W, capacity 2.1 L. Briefly, 20 g of minced fish head was mixed with dH₂O in ratios of 1:0, 1:1, 1:2, and 1:3 (w/v). Hydrolysis for 18 h was conducted using an orbital shaker at 150 rpm at temperature of 30 ± 2 °C. Next, the mixture was centrifuged at 3,000 rpm for 30 min. Each layer formed after centrifugation was separated and weighed. The liquid protein layer was also analyzed for antioxidant activity. The data were obtained by triplicate analysis.

The effect of pH and duration of hydrolysis on the characteristics of FPH was investigated as per a modified method of that previously described (Sabtecha, Jayapriya & Tamilselvi, 2014; Nurdiani et al., 2016). Minced parrotfish head (20 g) was mixed with dH₂O (1:2 w/v). The pH of the mixture was adjusted to 5, 7, and 9, and the hydrolysis

was conducted for 12 and 24 h. Samples without pH adjustment served as the control (pH 6.4). A similar procedure as the preliminary experiments was carried out to obtain hydrolysate.

Process optimization

An optimum process was obtained by analyzing the data using response surface methodology (RSM). An overlaid contour plot was applied to select the best hydrolysis conditions for FPH. Minitab version 18 was used for all statistical analysis.

Yield

The yield of protein hydrolysate products is defined as the percentage of the number of hydrolysate products produced against the raw materials used before hydrolysis. Yield is calculated as per the following formula:

$$\text{Yield} = \frac{A}{B} \times 100\%$$

where A = final weight of hydrolysate (after centrifugation) (g), and B = initial weight of the sample after mixing (before incubation) (g).

Antioxidant assay (DPPH radical scavenging activity)

The antioxidant activity of FPH was examined according to a modified protocol described by [Donkor et al. \(2012\)](#). As much as 100 μL of liquid protein was added to 3,900 μL 0.075 mM 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) in 95% methanol; the mixture was kept in the dark for 1 h. The absorbance value of the solution was measured at a wavelength of 517 nm using an ultraviolet-visible spectrophotometer. Antioxidant activity was calculated using the following equation:

$$\% \text{ antioxidant activity} = \left[\frac{\text{blank absorbance} - \text{sample absorbance}}{\text{blank absorbance}} \right] \times 100\%$$

Proximate analysis

Protein, fat, water content, and ash analyses were performed according to the method described by [AOAC \(2005\)](#). Protein was analyzed following the Kjeldahl method, and fat was analyzed using the Soxhlet method. Ash was determined by heating the samples in a furnace at 550 $^{\circ}\text{C}$ for 8–12 h.

Degree of hydrolysis

A slightly modified method of that described by [Hoyle & Merritt \(1994\)](#) was employed for the DH analysis. Liquid FPH (two mL) was combined with Trichloroacetic acid 20% (v/v); the aliquot was left for 30 min prior to centrifugation (5,000 rpm, 30 min). The supernatant was decanted and analyzed for nitrogen content following the Kjeldahl method ([AOAC, 2005](#)). DH was calculated using the following formula:

$$\text{Degree of Hydrolysis (DH)} = \frac{\text{TCA} - \text{soluble nitrogen}}{\text{Total nitrogen in sample}} \times 100\%$$

Molecular weight analysis (SDS–PAGE)

Fish protein hydrolysate molecular weight was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE), based on the Laemmli method (Laemmli, 1970). SDS-PAGE analysis utilized a 12% separating gel and 4% stacking gel. Mixed samples and loading buffers, as much as 30 μ L, were run at 20 mA and 100 V for 3 h. The gel was then stained with staining solution Coomassie Brilliant Blue (CBB) R-250 1 g, methanol 450 mL, glacial acetic acid 100 mL, and distilled water 450 mL. The stained gel was subsequently de-stained using the same solution without CBB R-250.

Free amino acid analysis

Fish protein hydrolysate free amino acid profiles were determined according to a slightly modified method of that described by Boogers *et al.* (2008). Ultra-High Performance Liquid Chromatography (UPLC), using an Acquity system (Waters), was utilized for free amino acid analysis. Sample (0.50 mL) was pipetted into a 100 mL volumetric flask, and 2.0 mL of alpha amino butyric acid 10 mM internal standard solution was added. The solution was diluted to the limit mark with 0.1 N HCl, before being homogenized. Next, the solution was filtered through a 0.22 μ m membrane filter. Ten microliters of the solution was added to 70 μ L of AccQ-Fluor Borate. After that, up to 20 μ L fluorine reagent A was added, before being vortexed, and allowed to stand for 1 min. One microliter of sample solution was injected into the UPLC system (ACCQ-Tag Ultra C18, fluid rate system of 0.7 mL per minute, the column temperature was maintained at 55 °C, and a photodiode array detector at a wavelength of 260 nm.

Statistical analysis

All data and RSM optimizations were analyzed by using Minitab 18 Statistical software (Minitab Pty Ltd., Sydney, NSW, Australia). Except data for optimization, all data obtained were subjected to one-way analysis of variance, followed by post-hoc test (Tukey analysis). Data are presented as the mean from three independent experiment \pm SD of the results.

RESULTS

Proximate composition of parrotfish heads

The proximate composition of minced parrotfish (*C. sordidus*) heads is listed in Table 1. The protein content, at $20.37 \pm 2.33\%$, was higher than salmon and Mackarel head. The fat content (3.92%) was slightly higher than Mackarel fish 3.70%, and far lower than salmon (17.4%). The water content ($71.68 \pm 2.33\%$) was higher than that of salmon (65.9%) but lower Mackarel fish (65.9%).

Fish protein hydrolysate from parrotfish heads

Five layers were formed after centrifugation. The first layer was oil/fat, followed by light lipoprotein, soluble protein, fine particles, and coarse particles layers (Fig. 1A). Soluble protein layers were carefully separated and collected (Fig. 1B). The yield and antioxidant

Table 1 Proximate composition of minced Parrotfish, Salmon, and Nile.

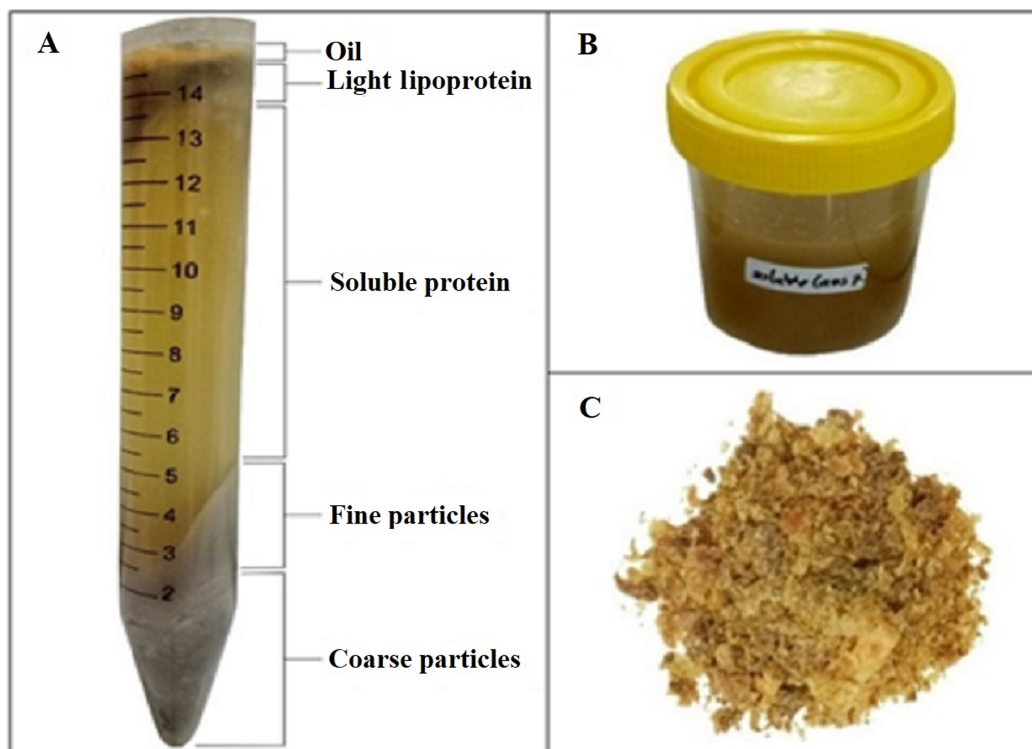
Parameter	Parrotfish*	Salmon**	Nile***
Carbohydrate (%)	0.52 ± 0.13	–	37.78
Protein (%)	20.37 ± 2.33	11.90	29.80
Fat (%)	3.92 ± 0.38	17.40	3.10
Water (%)	71.68 ± 1.87	65.90	5.70
Ash (%)	4.19 ± 0.66	4.30	21.80

Notes:

* This study.

** Wu et al. (2011).

*** Kefas et al. (2014).

**Figure 1** FPH from parrotfish head. (A) Formed layers after centrifugation. (B) Collected soluble protein layer. (C) Dried FPH. [Full-size !\[\]\(302f6ef9ec4729e9f737ac0cd0297b05_img.jpg\) DOI: 10.7717/peerj.8297/fig-1](https://doi.org/10.7717/peerj.8297/fig-1)

activity of liquid/soluble protein were measured. The soluble protein layer was also spray-dried (Fig. 1C).

Effect of substrate: water ratio on the yield and antioxidant activity of soluble protein

The ratio of minced head: dH₂O significantly affected ($p < 0.05$) the yield and antioxidant activity of the FPH produced, as seen in Fig. 2. Among the four ratios (1:0, 1:1, 1:2, and 1:3), the highest yield and antioxidant activity were obtained from the ratio of 1:2 (w/v), with values of 42.70 ± 0.70 and $51.50 \pm 0.90\%$, respectively. The ratio of 1:0 generated the lowest yield and antioxidant activity.

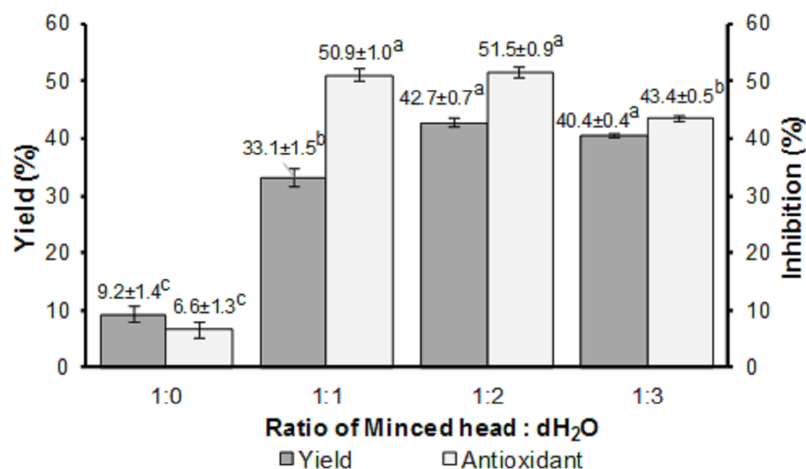


Figure 2 Yield and antioxidant activity of FPH.

Full-size DOI: 10.7717/peerj.8297/fig-2

Effect of pH and hydrolysis duration on FPH characteristics

The characteristics of FPH from parrotfish head hydrolyzed at various pH and time durations are shown in [Table 2](#).

Yield of FPH

Yields of FPH ranged from $4.96 \pm 0.72\%$ to $49.0 \pm 0.9\%$. The highest yield ($49.0 \pm 0.9\%$) was obtained at pH 9 after 24 h of hydrolysis. The lowest yield ($4.96 \pm 0.72\%$) was obtained at pH 7 and 0 h of hydrolysis. The result suggested that pH, duration of hydrolysis, and its interaction significantly affected the yield ($p < 0.05$).

Antioxidant activity

The highest antioxidant activity ($58.20 \pm 0.55\%$) was obtained after 24 h hydrolysis at pH 9. FPH showed the lowest antioxidant activity ($5.69 \pm 4.57\%$) at pH 9 and 0 h of hydrolysis. Both pH and duration of hydrolysis significantly affected the antioxidant activity ($p < 0.05$).

Proximate composition

pH and hydrolysis time significantly affected ($p < 0.05$) all proximate parameters. The highest protein content ($69.15 \pm 1.11\%$) was obtained at pH 9, with 24 h of hydrolysis time. The fat content of parrotfish head FPH ranged from $0.68 \pm 0.13\%$ to $5.882.99\%$; the highest fat content was obtained at pH 5 with 0 h of hydrolysis time and the lowest fat content was obtained at pH 9 with 24 h of hydrolysis time.

The ash content of the FPH of parrotfish head ranged from $4.55 \pm 0.35\%$ to $8.60 \pm 0.78\%$; the highest ash content was obtained at pH 9 with a 12 h hydrolysis time, while the lowest was observed in the control treatment (pH 6.4) with a 12 h hydrolysis time ($4.60 \pm 0.35\%$). ANOVA analysis revealed that different pH treatments resulted in significantly different results ($p < 0.05$). The water content of the parrotfish FPH ranged from $7.25 \pm 1.06\%$ to $9.01 \pm 0.71\%$; the highest water content was obtained at pH 9 with a 12 h hydrolysis time ($9.01 \pm 0.71\%$), while the lowest water content was obtained at pH 7 with a 24 h hydrolysis time ($7.25 \pm 1.06\%$).

Table 2 Characteristics of FPH from parrotfish heads with different pH and hydrolysis duration.

Parameter	5			7			9					
	Control*	12	24	0**	12	24	0**	12	24			
Yield	5.78 ± 0.85 ^a	39.15 ± 0.87 ^b	47.48 ± 1.29 ^{cd}	5.50 ± 2.03 ^a	37.73 ± 0.92 ^b	45.4 ± 1.17 ^c	4.96 ± 0.72 ^a	36.36 ± 1.03 ^b	48.37 ± 0.63 ^{cd}	6.58 ± 2.13 ^a	40.28 ± 0.63 ^{bc}	49.04 ± 0.90 ^e
Antioxidant	6.22 ± 2.28 ^a	43.79 ± 1.13 ^b	54.58 ± 1.31 ^d	6.53 ± 0.67 ^a	44.5 ± 1.5 ^b	49.24 ± 1.35 ^c	5.89 ± 1.47 ^a	43.34 ± 0.62 ^b	56.31 ± 0.78 ^c	5.69 ± 4.57 ^a	48.85 ± 1.57 ^c	58.20 ± 0.55 ^f
DH	0.28 ± 0.17 ^a	21.46 ± 1.71 ^c	28.09 ± 1.75 ^e	0.59 ± 0.12 ^a	22.47 ± 0.73 ^{cd}	24.77 ± 1.69 ^{cd}	0.44 ± 0.05 ^a	19.76 ± 0.75 ^b	29.60 ± 1.65 ^e	0.26 ± 0.11 ^a	24.04 ± 1.36 ^{cd}	30.65 ± 1.82 ^{ef}
Protein	51.81 ± 2.45 ^{bc}	48.98 ± 2.45 ^b	63.16 ± 1.11 ^{de}	49.3 ± 2.89 ^b	50.72 ± 0.89 ^{abc}	59.69 ± 0.89 ^d	49.3 ± 2.00 ^b	44.89 ± 1.56 ^a	64.26 ± 0.89 ^e	48.98 ± 2.48 ^b	55.13 ± 1.78 ^c	69.15 ± 1.11 ^f
Fat	5.72 ± 1.01	1.2 ± 0.14 ^a	1 ± 0.28 ^a	5.88 ± 2.99	1.35 ± 0.35 ^a	1.02 ± 0.23 ^a	5.49 ± 0.70	1.25 ± 0.5 ^a	0.89 ± 0.25 ^a	5.52 ± 2.12	0.97 ± 0.47 ^a	0.68 ± 0.13 ^a
Ash	7.00 ± 2.83 ^b	4.55 ± 0.35 ^a	4.85 ± 0.35 ^a	6.5 ± 0.71 ^{ab}	6.8 ± 0.69 ^{ab}	7.04 ± 1.06 ^b	8.00 ± 1.41 ^{bc}	5.05 ± 0.64 ^a	5.5 ± 0.7 ^a	7.00 ± 1.25 ^b	8.56 ± 0.78 ^c	8 ± 0.17 ^c
Water	8.38 ± 0.74 ^{ab}	8.39 ± 0.74 ^{ab}	7.82 ± 0.55 ^a	8.64 ± 0.98 ^{ab}	8.63 ± 0.99 ^{ab}	8.24 ± 1.06 ^{ab}	8.41 ± 0.67 ^{ab}	8.41 ± 0.67 ^{ab}	7.25 ± 1.06 ^a	9.00 ± 0.71 ^b	9.01 ± 0.71 ^b	7.85 ± 1.2 ^a

Notes:

* Control was done without pH adjustment (pH 6.4).

** Control time for hydrolysis.

Means in the same row with different superscripts (a-f) are significantly different ($p < 0.05$).

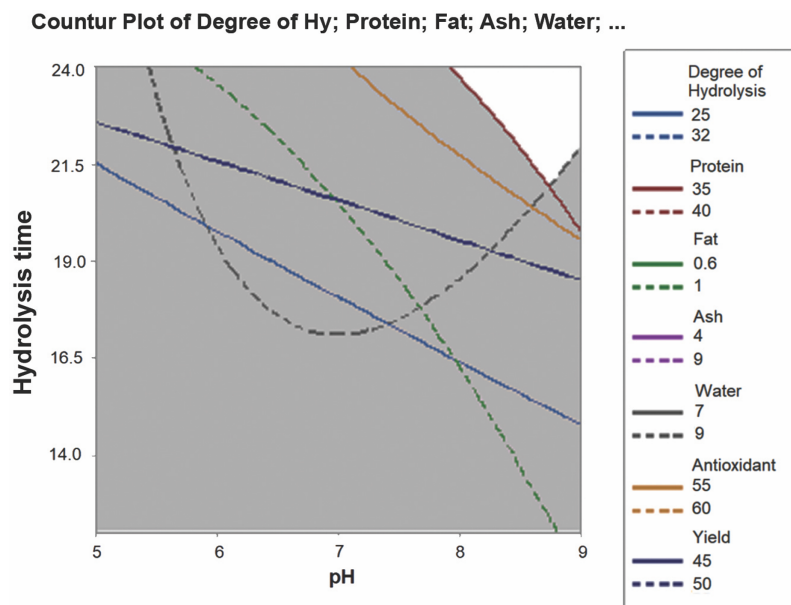


Figure 3 Overlaid contour plot for optimum FPH.

Full-size DOI: 10.7717/peerj.8297/fig-3

Degree of hydrolysis

The essential properties of FPH rely on the DH of the process. A high DH can be used as an indicator of effective hydrolysis. The result of DH analysis ranged from $0.26 \pm 0.11\%$ to $30.65 \pm 1.82\%$. The highest DH was observed at pH 9 after 24 h hydrolysis, while the lowest DH was obtained at pH 7 after 2 h hydrolysis. Both pH and duration of hydrolysis significantly affected ($p < 0.05$) DH.

Optimum conditions for preparation of FPH

The optimum conditions for parrotfish FPH production were analyzed using the RSM, based on the yield, antioxidant activity, protein, fat, water, ash, and DH of the FPH. The overlaid contour plot as a result of RSM analysis is shown in Fig. 3.

Based on Fig. 3, it was apparent that pH 8–9 and 21.5–24 h of hydrolysis were considered the optimum conditions for producing FPH. As the longer hydrolysis time gave better FPH characteristics, pH 9 and 24 h hydrolysis were considered the optimum conditions for generating FPH with the best characteristics from the head byproduct of parrotfish.

SDS–PAGE analysis

SDS–PAGE analysis was carried out to observe the molecular weight range of the FPH obtained under optimum conditions (pH 9; 24 h hydrolysis). The result showed that the molecular weight of FPH ranged from 18.05 kDa to 75.89 kDa (Fig. 4).

Amino acid composition

The amino acid composition of the FPH from parrotfish heads extracted at pH 9 with 24 h hydrolysis was compared to the FPH from tuna heads (Bougatef et al., 2012) and commercial FPH (International Quality Ingredients (IQI), 2005) (Table 3).

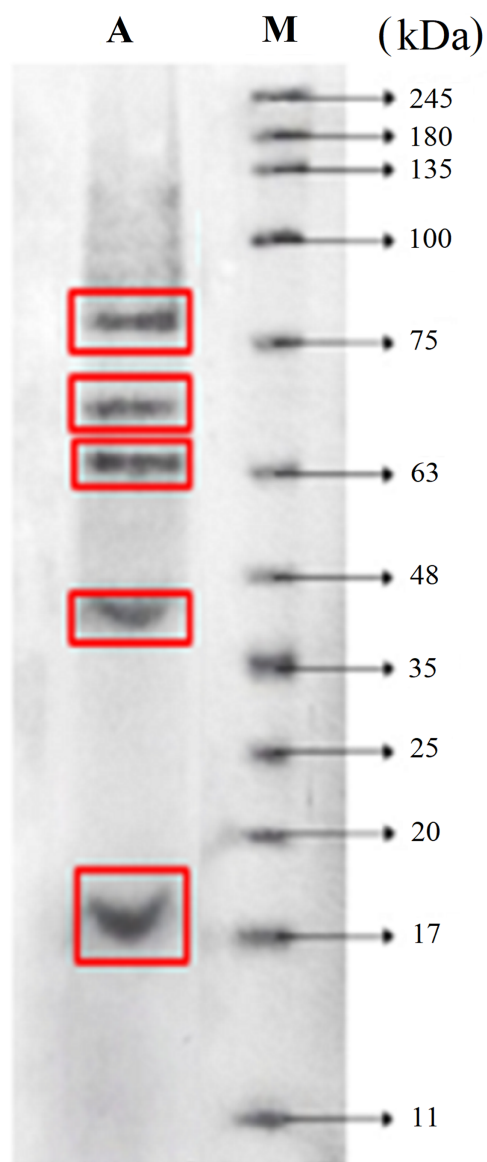


Figure 4 Molecular weight distribution of parrotfish FPH. (A) Sample (pH 9 and 24 h). (M) Molecular weight of protein standard. [Full-size !\[\]\(fcc3264021d438d9732560e78099f674_img.jpg\) DOI: 10.7717/peerj.8297/fig-4](https://doi.org/10.7717/peerj.8297/fig-4)

Parrotfish FPH consists of essential amino acids (histidine, threonine, valine, isoleucine, leucine, phenylalanine, and lysine) and non-essential amino acids (aspartic acid, glutamic acid, serine, arginine, glycine, alanine, tyrosine, and proline).

DISCUSSION

The yield of FPH from parrotfish heads is higher than that obtained from Yellow-Spotted Trivaly fish heads (71.77%) (*Tawfik, 2009*), tuna fish heads (9.85%) (*Parvathy et al., 2018*), Grouper fish head (71.20) (*Tawfik, 2009*). The antioxidant activity of parrotfish heads was much lower than that derived from Catla fish heads (77.92%)

Table 3 Comparison of amino acid composition of several FPH.

No.	Amino acids	FPH from parrotfish head (%)	FPH from tuna (%)	Commercial FPH (%)
1.	L-Ser	1.81	5.18	4.90
2.	L-Glu	14.43	11.20	14.00
3.	L-Phe	5.53	06.18	3.70
4.	L-Ile	4.34	4.83	4.00
5.	L-Val	5.38	7.49	4.90
6.	L-Ala	7.41	2.88	7.30
7.	L-Arg	6.12	11.53	6.80
8.	L-Gly	7.63	3.32	11.00
9.	L-Lys	8.3	10.23	7.50
10.	L-Asp	11.06	9.91	9.50
11.	L-Leu	8.48	6.48	6.50
12.	L-Pro	5.64	3.62	–
13.	L-Tyr	4.22	5.44	2.90
14.	L-Thr	6.80	2.17	4.40
15.	L-His	2.85	9.52	2.60
Total Essential Amino Acid		41.69	46.90	42.70
Total Hydrophobic Amino Acid (HAA)		41.00	36.92	29.30

(*Elavarasan, Kumar & Shamasundar, 2014*). Heating can be applied to increase the yield, because it allows water unbound to materials to dissipate (*Khan et al., 2017*). Furthermore, the longer the hydrolysis time, the higher the yield. *Kim (2013)* stated that the FPH yield increases as a function of time of hydrolysis until it reaches a stationary phase. The highest yield obtained in this study ($49.00 \pm 0.9\%$) was lower than that of tuna (60.73%) (*Ramakrishnan et al., 2013*) and codfish (75%) (*Himonides, Taylor & Morris, 2011*).

Compared to FPH from the heads of catfish and mackerel, the antioxidant activity of FPH from parrotfish heads was still lower (*Le Vo et al., 2016; Ediriweera, Aruppala & Abeyrathne, 2019*). The size of peptides and the composition of free amino acids affect the antioxidant activity of FPH. The longer the hydrolysis time, the more abundant free amino acids become. Hydrophobic amino acids such as Pro, Leu, Ala, Trp, and Phe will increase antioxidant activity. In addition, Tyr, Met, His, and Lys are able to act as antioxidants (*Le Vo et al., 2016*).

The protein content of parrotfish head FPH was higher than that obtained from catfish heads (39.03%) (*Utomo, Suryaningrum & Harianto, 2014*), tuna heads (28.39%) (*Ramakrishnan et al., 2013*), but still lower than commercial FPH (73–75%) (*International Quality Ingredients (IQI), 2005*). According to *Nurdiani et al. (2016)*, the protein content of FPH can be influenced by the amount of water dehydrated from the material. (*Windsor, 2001*) has categorized FPC/FPH into three types; type A (protein content is more than 80%), type B (protein content is less than 80%), and type C (low quality). Based on its protein content, FPH from parrotfish heads could be classified as a type B hydrolysate.

The fat content of parrotfish head FPH was lower than the FPH from croaker fish head waste ($5.1 \pm 4.0\%$) (*Amorim et al., 2016*) and commercial FPH (19–22%) (*International Quality Ingredients (IQI), 2005*). The low-fat content of parrotfish head FPH was due to the low-fat content in fish head raw materials (3.92%). According to *Bechtel (2003)*, the fat content in hydrolysate products is influenced by the characteristics of the raw materials used and the process of separating fat after hydrolysis. The fat was separated mechanically during the centrifugation process.

The ash levels were higher than that of cod head waste FPH (1%) (*Himonides, Taylor & Morris, 2011*), but still met commercial FPH standards (4–7%) (*International Quality Ingredients (IQI), 2005*). The ash content in FPH tends to increase with an increasing amount of buffer (HCl and NaOH) added. According to *Salamah, Nurhayati & Widadi (2011)*, high ash content in FPH was a result of the addition of alkali compounds, such as NaOH, or acid compounds, such as HCl, in the process of protein hydrolysis. Mixing acid and alkali compounds in the protein hydrolysate solution will cause the formation of salt compounds, which increases the ash content in protein hydrolysates. The water content of parrotfish head FPH was higher than that of cod head FPH (5%) (*Himonides, Taylor & Morris, 2011*) and commercial FPH standards (3–5%) (*International Quality Ingredients (IQI), 2005*).

The optimization result indicated that the best FPH would be produced from pH 9 and a 24 h hydrolysis time. pH 9 has previously been recorded as the best pH for hydrolyzing fish byproducts (*Singh & Benjakul, 2018*). One parameter that should be considered during this optimization process is the low protein content; the protein content was below commercial FPH (*International Quality Ingredients (IQI), 2005*). This result was also corroborated by the DH result. *Norma et al. (2005)* and *Hau et al. (2018)*, reported that a longer incubation time increased the DH.

The DH of parrotfish hydrolysate was higher than that of Nile fish heads (14.3%) (*Srikanya et al., 2017*) and kurisi byproducts (15%) (*Gajanan, Elavarasan & Shamasundar, 2016*). This is possibly due to the high level of endogenous parrotfish head proteases. For the first two hours, the DH was similar from the result from *Herpandi et al. (2012)* and *Herpandi et al. (2013)*, which use commercial enzymes for the hydrolysis. However, our result was lower in the 3rd h of hydrolysis. It is clear that the enzyme plays an important role in the DH. Furthermore, the physical structure and protein molecules, which exist in the sample, were affecting the DH (*Kanu et al., 2009*).

The DH affects protein molecular weight and amino acids. FPH from Nile fish had a wider range of molecular weight (14.4–116 kDa) (*Tejpal et al., 2017*) than that obtained in this study. The dominance of small peptides will increase the potency of the FPH as a bioactive substance.

The total essential amino acids of FPH from parrotfish heads (41.69%) approached the commercial standard of FPH (42.70%) (*International Quality Ingredients (IQI), 2005*), but was still lower than FPH from tuna heads (46.90%) (*Bougatef et al., 2012*). According to *Chobert, Bertrand-Harb & Nicolas (1988)*, the content of essential amino acids indicates the potential of hydrolysates to serve as a useful source of nutrition. The difference in

amino acid composition between hydrolysates depends on differences in enzyme specificity and hydrolysis conditions.

The total hydrophobic amino acid content of parrotfish FPH (41%) was higher than the FPH from tuna heads (36.92%) (*Bougatef et al., 2012*) and commercial FPH (29.30%) (*International Quality Ingredients (IQI), 2005*). The amino acid composition can also affect the functional properties of FPH, such as the nature of the antioxidant activity. According to *Zainol et al. (2003)*, hydrophobic amino acids (alanine, leucine, and proline) have been shown to have free radical quenching activities. Hydrophobic aromatic amino acids (tyrosine and phenylalanine) can also function as antioxidants by donating electrons.

Analysis of amino acids can determine the quality of FPH manufactured, specifically from the ratio of amino acids contained in these proteins (*Nurilmala, Nurhayati & Roskananda, 2018*). According to *Annisa, Darmanto & Amalia, 2012*, the amino acids in each fish species vary depending on internal and external factors. Internal factors include fish species, sex, age, and the reproduction phase of the fish, while external factors are typically environmental.

CONCLUSIONS

Characteristics of FPH from the heads of parrotfish (*C. sordidus*) were affected by the ratio of minced fish head: dH₂O, pH, and duration of hydrolysis. The yield, antioxidant activity, protein content, ash content, and DH of the FPH were dependent on pH and time of hydrolysis. The optimum conditions for the production of FPH from parrotfish heads include a minced head: dH₂O ratio of 1:2 (w/v), at pH 9, with a 24 h hydrolysis time. The process generated an essential amino acid profile of 41.69%. To the best of our knowledge, this is the first report on the added value of *C. sordidus* heads.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This work was supported by the Ministry of Research, Technology and Higher Education, the Republic of Indonesia (No. 167/SP2H/LT/DRPM/2019), and the Brawijaya University for Basic Research on Higher Education (No. 330.32/UN10.C10/PN/2019). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:

Ministry of Research, Technology and Higher Education, the Republic of Indonesia:
167/SP2H/LT/DRPM/2019.

Brawijaya University for Basic Research on Higher Education: 330.32/UN10.C10/PN/2019.

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Asep A. Prihanto conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Rahmi Nurdiani conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the paper, approved the final draft.
- Annas D. Bagus performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, approved the final draft.

Data Availability

The following information was supplied regarding data availability:

The raw data is available in the [Supplemental File](#) and at figshare: Prihanto, Asep Awaludin; Nurdiani, Rahmi; Bagus, Annas Dwi (2019): Raw Data: Production and characteristics of fish protein hydrolysate from parrotfish (*Chlorurus sordidus*) head. figshare. DOI [10.6084/m9.figshare.10264805.v2](https://doi.org/10.6084/m9.figshare.10264805.v2).

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.8297#supplemental-information>.

REFERENCES

- Adrim M.** 2010. Biological aspect of Parrotfish (Scaridae). *Oseana* **33**(1):41–50 [in Indonesian].
- Amorim RG, Oliveira FC, Deschamps C, Pessatti ML.** 2016. Protein hydrolysate waste of whitemouth croaker (*Micropogonias furnieri*) as a way of adding value to fish and reducing the environmental liabilities of the fishing industry. *Latin American Journal of Aquatic Research* **44**(5):967–974 DOI [10.3856/vol44-issue5-fulltext-9](https://doi.org/10.3856/vol44-issue5-fulltext-9).
- Anil KA.** 2017. *Food processing by-products and their utilization*. New York: John Wiley & Sons.
- Annisa S, Darmanto YS, Amalia U.** 2012. The effect of various fish species on fish protein hydrolysate with the addition of Papain Enzyme. *Indonesian Journal of Fisheries Science and Technology* **13**(1):24–30 DOI [10.14710/ijfst.13.1.24-30](https://doi.org/10.14710/ijfst.13.1.24-30).
- AOAC.** 2005. *Official methods of analysis*. Washington D.C.: Association of Official Analytical Chemists.
- Bechtel PJ.** 2003. Properties of different fish processing by-products from Pollock, Cod and Salmon. *Journal of Food Processing and Preservation* **27**(2):101–116 DOI [10.1111/j.1745-4549.2003.tb00505.x](https://doi.org/10.1111/j.1745-4549.2003.tb00505.x).
- Boogers I, Plugge W, Stokkermans YQ, Duchateau ALL.** 2008. Ultra-performance liquid chromatographic analysis of amino acids in protein hydrolysates using an automated pre-column derivatisation method. *Journal of Chromatography A* **1189**(1–2):406–409 DOI [10.1016/j.chroma.2007.11.052](https://doi.org/10.1016/j.chroma.2007.11.052).
- Bougatef A, Balti R, Haddar A, Jellouli K, Souissi N, Nasri M.** 2012. Protein hydrolysates from bluefin tuna (*Thunnus thynnus*) heads as influenced by the extent of enzymatic hydrolysis. *Biotechnology and Bioprocess Engineering* **17**(4):841–852 DOI [10.1007/s12257-012-0053-y](https://doi.org/10.1007/s12257-012-0053-y).
- Chen LS.** 2002. Post-settlement diet shift of *Chlorurus sordidus* and *Scarus schlegeli* (Pisces: Scaridae). *Zoological Studies* **41**(1):47–58.

- Chobert JM, Bertrand-Harb C, Nicolas MG. 1988.** Solubility and emulsifying properties of caseins and whey proteins modified enzymatically by trypsin. *Journal of Agricultural and Food Chemistry* **36**(5):883–892 DOI [10.1021/jf00083a002](https://doi.org/10.1021/jf00083a002).
- Donkor ON, Stojanovska L, Ginn P, Ashton J, Vasiljevic T. 2012.** Germinated grains: sources of bioactive compounds. *Food Chemistry* **135**(3):950–959 DOI [10.1016/j.foodchem.2012.05.058](https://doi.org/10.1016/j.foodchem.2012.05.058).
- Ediriweera TK, Aruppala ALYH, Abeyrathne EDNS. 2019.** Analysis of bioactive properties of fish protein hydrolysates from *Scomber japonicus* fin wastes. *Journal of Technology and Value Addition* **1**(1):31–45.
- Elavarasan K, Kumar VN, Shamasundar BA. 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):207–214 DOI [10.1111/jfpp.12081](https://doi.org/10.1111/jfpp.12081).
- Food and Agriculture Organization (FAO). 2015.** Fishery and aquaculture country profiles barbados. Available at <http://www.fao.org/fishery/jacp/BRB/en> (accessed 26 January 2019).
- Gajanan PG, Elavarasan K, Shamasundar BA. 2016.** Bioactive and functional properties of protein hydrolysates from fish frame processing waste using plant proteases. *Environmental Science and Pollution Research* **23**(24):1–11 DOI [10.1007/s11356-016-7618-9](https://doi.org/10.1007/s11356-016-7618-9).
- Hapsari N, Welasi T. 2013.** Utilization of fish by product as biofertilizer. *Jurnal Teknik Lingkungan* **2**(1):1–6.
- Hau EH, Mohd ZZ, Zuraidah N, Shaharudin NA, Zainol MK. 2018.** Physicochemical properties of powdered protein hydrolysate from yellowstripe scad (*Selaroides leptolepis*) fish. *International Food Research Journal* **25**(6):2553–2559.
- Herpandi H, Huda N, Adzitey F. 2011.** Fish bone and scale as a potential source for halal gelatin. *Journal of Fisheries and Aquatic Science* **6**(4):379–389 DOI [10.3923/jfas.2011.379.389](https://doi.org/10.3923/jfas.2011.379.389).
- Herpandi H, Huda N, Rosma A, Nadiyah WAW. 2013.** Optimizing the enzymatic hydrolysis of skipjack tuna (*Katsuwonus pelamis*) dark flesh using Alcalase[®] enzyme: a response surface approach. *Journal of Fisheries and Aquatic Science* **8**(4):494–505 DOI [10.3923/jfas.2013.494.505](https://doi.org/10.3923/jfas.2013.494.505).
- Herpandi H, Huda N, Rosma A, Wan Nadiyah W. 2012.** Degree of hydrolysis and free tryptophan content of skipjack tuna (*Katsuwonus pelamis*) protein hydrolysates produced with different type of industrial proteases. *International Food Research Journal* **19**(3):863–867.
- Himonides AT, Taylor AKD, Morris AJ. 2011.** Enzymatic hydrolysis of fish frames using pilot plant scale systems. *Food and Nutrition Sciences* **2**(6):586–593 DOI [10.4236/fns.2011.26082](https://doi.org/10.4236/fns.2011.26082).
- Hoyle NT, Merritt JH. 1994.** Quality of fish-protein hydrolysates from herring (*Clupea harengus*). *Journal of Food Science* **59**(1):76–79 DOI [10.1111/j.1365-2621.1994.tb06901.x](https://doi.org/10.1111/j.1365-2621.1994.tb06901.x).
- International Quality Ingredients (IQI). 2005.** Fish protein hydrolysate. Available at <http://www.eyequye.nl> (accessed 26 January 2019).
- Jeevitha K, Priya M, Khora SS. 2014.** Antioxidant activity of fish protein hydrolysates from *Sardinella longiceps*. *International Journal of Drug Development and Research* **6**(4):137–145.
- Kanu PJ, Kanu JB, Sandy EH, Kande JBA, Mornya PMP, Huiming Z. 2009.** Optimization of enzymatic hydrolysis of defatted sesame flour by different proteases and their effect on the functional properties of the resulting protein hydrolysate. *American Journal of Food Technology* **4**(6):226–240 DOI [10.3923/ajft.2009.226.240](https://doi.org/10.3923/ajft.2009.226.240).
- Kefas M, Michael KG, Abubakar KA, Edward A, Wahide JA. 2014.** Proximate and mineral contents of flesh and body parts of *Oreochromis niloticus* and *Synodontis clarias* in Mubi, Nigeria. *Global Journal of Biology, Agriculture and Health Sciences* **3**(3):116–121.

- Khan MIH, Wellard RM, Nagy SA, Joardder MUH, Karim MA. 2017.** Experimental investigation of bound and free water transport process during drying of hygroscopic food material. *International Journal of Thermal Sciences* **117**:266–273 DOI [10.1016/j.ijthermalsci.2017.04.006](https://doi.org/10.1016/j.ijthermalsci.2017.04.006).
- Kim SK. 2013.** *Marine proteins and peptides: biological activities and applications*. New York: John Wiley & Sons.
- Kim SK, Mendis E. 2006.** Bioactive compounds from marine processing byproducts: a review. *Food Research International* **39**(4):383–393 DOI [10.1016/j.foodres.2005.10.010](https://doi.org/10.1016/j.foodres.2005.10.010).
- Laemmli UK. 1970.** Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* **227**(5259):680–685 DOI [10.1038/227680a0](https://doi.org/10.1038/227680a0).
- Lepongbulan W, Tiwow VMA, Diah AWM. 2017.** Nutrient analysis of organic liquid fertilizer from waste of mujair fish (*Oreochromis mosambicus*) from Lindu lake using local microorganism (MOL) of banana weevil variation. *Jurnal akademik kimia* **6**(2):92–97.
- Le Vo TD, Nguyen TTH, Phan DV, Nguyen HDM, Tran HQ. 2016.** Investigation of antioxidant activity of the hydrolysate derived from Tra catfish byproducts using Alcalase 2.4 L FG for application as a natural antioxidant ingredient. *Journal Science and Technology Development* **19**(6):109–121 DOI [10.32508/stdj.v19i3.577](https://doi.org/10.32508/stdj.v19i3.577).
- Ministry of Marine Affair and Fisheries. 2019.** Production and price of fish in Indonesia. Available at http://pipp.djpt.kkp.go.id/profil_pelabuhan/1304/produksi_harga (accessed 24 October 2019).
- Nazeer RA, Kulandai KA. 2012.** Evaluation of antioxidant activity of muscle and skin protein hydrolysates from giant kingfish, *Caranx ignobilis* (Forsskål 1775). *International Journal of Food Science and Technology* **47**(2):274–281 DOI [10.1111/j.1365-2621.2011.02836.x](https://doi.org/10.1111/j.1365-2621.2011.02836.x).
- Norma I, Jamilah B, Saari N, Yakakob BCM. 2005.** Optimization of hydrolysis condition for the production of treadfin bream (*Nemipterus japonicus*) hydrolysate by Alcalase. *Journal of Muscle Foods* **16**(2):87–102 DOI [10.1111/j.1745-4573.2005.07404.x](https://doi.org/10.1111/j.1745-4573.2005.07404.x).
- Nurdiani R, Dissanayake M, Street WE, Singh TK, Donkor ON, Vasiljevic T. 2016.** In vitro study of selected physiological and physicochemical properties of fish protein hydrolysates from 4 Australian fish species. *International Food Research Journal* **23**(5):2042–2053.
- Nurilmala M, Nurhayati T, Roskananda R. 2018.** By product of catfish fillet Industry for protein hydrolysate. *Jurnal Pengolahan Hasil Perikanan Indonesia* **21**(2):287–294 DOI [10.17844/jphpi.v21i2.23083](https://doi.org/10.17844/jphpi.v21i2.23083).
- Parvathy U, Nizam KM, Zynudheen AA, Ninan G, Panda SK, Ravishankar CN. 2018.** Characterization of fish protein hydrolysate from red meat of *Euthynnus affinis* and its application as an antioxidant in iced sardine. *Journal of Scientific and Industrial Research* **77**:111–119.
- Qiao M, Tu M, Wang Z, Mao F, Chen H, Qin L, Du M. 2018.** Identification and antithrombotic activity of peptides from blue mussel (*Mytilus edulis*) protein. *International Journal of Molecular Sciences* **19**(1):138 DOI [10.3390/ijms19010138](https://doi.org/10.3390/ijms19010138).
- Ramakrishnan VV. 2013.** Extraction of proteins from mackerel fish processing waste using alcalase enzyme. *Journal of Bioprocessing & Biotechniques* **3**(2):1–9 DOI [10.4172/2155-9821.1000130](https://doi.org/10.4172/2155-9821.1000130).
- Sabtecha B, Jayapriya J, Tamilselvi A. 2014.** Extraction and characterization of proteolytic enzymes from fish visceral waste: potential applications as destainer and dehairing agent. *International Journal of ChemTech Research* **6**(10):4504–4510.
- Salamah E, Nurhayati T, Widadi IR. 2011.** Production and characterization of protein hydrolysates from African catfish (*Clarias gariepinus*) using papain. *Jurnal Pengolahan Hasil Perikanan Indonesia* **15**(1):9–16 DOI [10.17844/jphpi.v15i1.5328](https://doi.org/10.17844/jphpi.v15i1.5328).

- Singh A, Benjakul S. 2018.** Proteolysis and its control using protease inhibitors in fish and fish products: a review. *Comprehensive Reviews in Food Science and Food Safety* **17(2)**:496–509 DOI [10.1111/1541-4337.12337](https://doi.org/10.1111/1541-4337.12337).
- Srikanya A, Dhanapal K, Sravani K, Madhavi K, Kumar GP. 2017.** A study on optimization of fish protein hydrolysate preparation by enzymatic hydrolysis from tilapia fish waste mince. *International Journal of Current Microbiology and Applied Sciences* **6(12)**:3220–3229 DOI [10.20546/ijcmas.2017.612.375](https://doi.org/10.20546/ijcmas.2017.612.375).
- Tawfik MS. 2009.** Proximate composition and fatty acids profiles in most common available fish species in Saudi market. *Asian Journal of Clinical Nutrition* **1(1)**:50–57 DOI [10.3923/ajcn.2009.50.57](https://doi.org/10.3923/ajcn.2009.50.57).
- Tejpal CS, Lekshmi RGK, Asha KK, Anandan R, Chatterjee NS. 2017.** Antioxidant, functional properties and amino acid composition of pepsin-derived protein hydrolysates from whole tilapia waste as influenced by pre-processing ice storage. *Journal of Food Science and Technology* **1(1)**:11–29 DOI [10.1007/s13197-017-2897-9](https://doi.org/10.1007/s13197-017-2897-9).
- Utomo BSB, Suryaningrum TD, Harianto HR. 2014.** Optimization of enzymatic hydrolysis of protein hydrolysate processing from waste of catfish filet production. *Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology* **9(3)**:107–114 DOI [10.15578/squalen.v9i3.79](https://doi.org/10.15578/squalen.v9i3.79).
- Windsor ML. 2001.** Fish protein concentrate. Torrey advisory note nr. 39. FAO fisheries and aquaculture department, Rome. Available at <http://www.fao.org/wairdocs/tan/x5917E/x5917e01.htm> (accessed 2 November 2019).
- Wu TH, Nigg JD, Stine JJ, Bechtel PJ. 2011.** Nutritional and chemical composition of by-product fractions produced from wet reduction of individual red salmon (*Oncorhynchus nerka*) heads and viscera. *Journal of Aquatic Food Product Technology* **20(2)**:183–195 DOI [10.1080/10498850.2011.557524](https://doi.org/10.1080/10498850.2011.557524).
- Yang P, Ke H, Hong P, Zeng S, Cao W. 2011.** Antioxidant activity of bigeye tuna (*Thunnus obesus*) head protein hydrolysate prepared with Alcalase. *International Journal of Food Science & Technology* **46(12)**:2460–2466 DOI [10.1111/j.1365-2621.2011.02768.x](https://doi.org/10.1111/j.1365-2621.2011.02768.x).
- Zainol MK, Abd-Hamid A, Yusof S, Muse R. 2003.** Antioxidative activity and total phenolic compounds of leaf, root and petiole of four accessions of *Centella asiatica* (L.) Urban. *Food Chemistry* **81(4)**:575–581 DOI [10.1016/S0308-8146\(02\)00498-3](https://doi.org/10.1016/S0308-8146(02)00498-3).