JIPK. Volume 15 No 2. November 2023
Sinta 1 (Decree No: 158/E/KPT/2021)
e-ISSN:2528-0759; p-ISSN:2085-5842
Available online at https://e-journal.unair.ac.id/JIPK

JIPK
(JURNAL ILMIAH PERIKANAN DAN KELAUTAN)
Scientific Journal of Fisheries and Marine

Research Article

Antioxidant Activity and Potential Bioactive Peptides from Skin Protein Hydrolysate of Yellowfin Tuna (*Thunnus albacares*)

Sitti Hardiyanti Rachman, Joko Santoso*, and Sugeng Heri Suseno

Department of Aquatic Product Technology, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor. Indonesia

OPEN ACCESS

ARTICLE INFO

Received: December 16, 2022
Accepted: March 27, 2023
Published: April 12, 2023
Available online: August 15, 2023

*) Corresponding author:
E-mail: jsantoso@apps.ipb.ac.id

Keywords:
Antioxidant
Bioactive Peptides
Defatting
Enzymatic Hydrolysis
Tuna Skin Hydrolysate

Abstract

The tuna fillet industry produces abundant skin by-products with high protein, which has the potential as a raw material for fish protein hydrolysate (FPH) for a source of bioactive peptides. Exploration of bioactive peptides from fish skin is generally from hydrolyzed gelatin and collagen. The study aimed to produce FPH directly from tuna skin as an antioxidant and identify potential bioactive peptides. The research began by defatting using multiple concentrations of $NaHCO_3$ and immersion times. The defatted fish skin was produced as FPH by enzymatic hydrolysis method using different papain enzyme concentrations and hydrolysis times. The selected treatments were assessed for antioxidant activity and bioactive peptides. The results showed that the defatting process using a 0.50% $NaHCO_3$ for 30 minutes generated the highest reducing fat content in value was 80.53%. Using papain enzyme gave a significant effect on the DH of FPH, with value was 29.72-67.64%. Therefore, FPH obtained from different concentrations for 4 hours was chosen to characterize the antioxidant activity and bioactive peptide. Hydrolysis using 5% enzyme papain showed the highest antioxidant activity of DPPH, ABTS, and reducing power with values of 0.965 mg/mL, 0.495 mg/mL, and 0.415 absorbances, respectively. FPH possesses a molecular weight of 10.15-48.50 kDa. Functional groups detected were amides A, B, I, II, and III. Glycine, proline, and arginine became amino acids dominant of FPH. Based on the diversity of biopeptide compounds, several biological function candidates were detected, namely antioxidants, ACE inhibitors, and antifibrinolytics which have the potential to be used as nutraceutical products.

Cite this as: Rachman, S. H., Santoso, J., & Suseno. S. H. (2023). Antioxidant Activity and Potential Bioactive Peptides from Skin Protein Hydrolysate of Yellowfin Tuna (Thunnus albacares). *Jurnal Ilmiah Perikanan dan Kelautan*, 15(2): 248-263. http://doi.org/10.20473/jipk.v15i2.41625

1. Introduction

The tuna industry is the most complex and dynamic industry; dominating the export of fishery products in Indonesia. The main export products for tuna are divided into four categories: canned tuna of 82,234 tons, frozen fillet of 58,871 tons, frozen tuna of 24,261 tons, and fresh tuna of 3,067 tons of a total export volume of 168,433 tons with a value of US$ 713,9 million, generally originating from yellowfin tuna (KKP, 2020). The high total export volume of processed products will undoubtedly increase the volume of by-products. The tuna fillet industry has the highest export volume after canning products, which can be utilized because of the characteristics of fresh by-products compared to canned by-products that have undergone a heating process (Irianto and Akbarsyah, 2007; Gamarro *et al*., 2013). Several studies have shown that the most effective protein content in yellowfin tuna by-products is found in the skin 27.12-36.09% (Karunarathna and Attygalle, 2010; Nurilmala *et al*., 2019), in dark meat, 20.22-26.92% (Karunarathna and Attygalle, 2010; Sánchez-Zapata *et al*., 2011) and the minor protein content in the swim bladder is 17.52-19.17% (Hadinoto and Idrus, 2018; Hadinoto *et al*., 2019). The high protein content in tuna skin shows the potential to be utilized as a raw material for fish protein hydrolysate (FPH) products which can be value-added products.

Enzymatic hydrolysis is the best way to hydrolyze proteins with minimum losing the nutritional value of raw materials, the easiest to control in producing, and can improve the functional and biological properties of raw materials (Huang *et al*., 2016; Schmidt *et al*., 2020). The fat content in raw materials will affect the hydrolysis, requiring a defatting process to get the best FPH. In order to reduce the fat content is generally done by chemical and mechanical processes (Hua *et al*., 2020). FPH is used as an alternative protein source in commercial products (Lopera *et al*., 2018) and is also used as a source of additional nutrition (Kiewiet *et al*., 2018; Asmak *et al*., 2020), flavor enhancer (Vijaykrishnaraj *et al*., 2016; Witono *et al*., 2019) and to improve product characteristics (Jeewanthi *et al*., 2015). FPH is proven as a bioactive component source in the form of bioactive peptides released during hydrolysis. These bioactive peptides have positive effects on health due to biological activities, so they have the potential to be used as functional and nutraceutical food products (Li-Chan, 2015).

Bioactive peptides have very diverse functional properties and is determined by the constituent amino acid sequence (Baehaki *et al*., 2020). Several studies report that FPH has functional properties as glucoregulatory and anti-inflammatory (Henaux *et al*., 2021), antihypertensive (Naik *et al*., 2021), anticancer (Yaghoubzadeh *et al*., 2020), antibacterial (Baco *et al*., 2022), antidiabetic (Rothwell *et al*., 2021), and antioxidants (Qiu *et al*., 2019; Baehaki *et al*., 2020; Agustin *et al*., 2021). Research related to the exploration of bioactive peptides from fish skin by-products is generally in the form of hydrolyzed protein products in gelatin products (Gonz *et al*., 2022) and collagen products (Prastyo *et al*., 2020; Tkaczewska *et al*., 2020). Exploration of bioactive peptides by direct hydrolysis fish skin is still limited so this research is essential to evaluate the potential of bioactive peptides in tuna skin FPH. Thus, this study has several objectives, first to get the best treatment in the defatting process. Second, to evaluate the effect of enzyme concentration and time on the degree of hydrolysis (DH) attribute as well as to assess the antioxidant activity and to identify bioactive peptides.

2. Materials and Methods

2.1 Material

The main material in this study was tuna skin obtained from PT. Bumi Menara Internusa, Makassar, Indonesia. The material used for the defatting process was $NaHCO_3$ powder (Merck, Germany). The protein hydrolysate production was performed by the enzyme papain (EC3.4.22.2) commercial (Himedia, India) activity 1,000 units/mg. The chemicals used for analysis were 2,2-Diphenyl-1-Picrylhydrazyl (DPPH; Sigma-Aldrich, St. Louis, MO, USA), 2,2-Azinobis 3-ethyl benzothiazoline 6-sulphonic acid (ABTS; Sigma- Aldrich, St. Louis, MO, USA), ethanol (Merck, Germany), phosphate buffer (Sigma-Aldrich, St. Louis, MO, USA), $K_3Fe(CN)_6$ (Merck, Germany) Trichloroacetic acid (TCA; Merck, Germany) and $FeCl_3$ (Merck, Germany).

2.2 Tuna Skin Preparation and Defatting

Skin obtained from a by-product of the tuna industry was thawed and washed to remove dirt that sticks to the surface. Then it was cut into 2x2 cm^2 pieces and crushed, then analyzed for proximate composition (AOAC, 2005). The mashed skin was then subjected to a defatting process according to Ramadhan *et al*. (2014), which was modified by immersion in $NaHCO_3$ solution with a concentration of 0%, 0.25%, 0.5%, and 0.75% (w/v) for 30, 60, and 90 minutes, respectively, using a 1:4 ratio of sample and $NaHCO_3$ solution at 4°C with periodic stirring. A fat content test was performed (AOAC, 2005) to determine the best $NaHCO_3$ concentration and immersion time.

2.3 Production of Protein Hydrolysate

Hydrolysate production based on Mutaminah *et al.* (2018) was modified with different concentrations of papain enzymes 4, 5, and 6% (v/v) at four, five, and six hours. The ratio of sample and distilled water 1:2 (w/v), pH 7 hydrolysis with a water bath shaker (BS-21, Jeio Tech, Korea) at 55°C, speed 100xg. The incubation process is complete followed by the inactivation process used, water bath (DWB-18, B-One, China) at 85°C and centrifuged (Sorval Lynx 6000, Thermo Scientific, Germany) at 10,000xg temperature 4°C, 20 minutes. Then the supernatant was collected and stored in a dark bottle at -15°C. The selected treatment was determined by testing the degree of hydrolysis (DH). The filtrate was mixed with 10% TCA with a ratio of 1:1 (v/v) and centrifuged at 7800xg for 15 minutes) (Hoyle and Merritt, 1994). The nitrogen content of the supernatant was measured using the Kjeldahl method (AOAC, 2005). This formula can calculate the degree of hydrolysis (%):

$$\text{Degree of hydrolysis (\%)} = \frac{\text{TCA dissolved nitrogen 10 \%}}{\text{Sample total nitrogen}} \times 100\% \text{..Eq 1}$$

2.4 Evaluation of Antioxidant Activity

The filtrate obtained from hydrolysis was dried using a freeze dryer (Missouri 64132, Labconco, USA) at -50°C, 0.5 Mbar pressure, and analyzed for antioxidant activity of DPPH, ABTS, and reducing power.

2.4.1 DPPH radical scavenging activity

The 1.5 mL sample solution was added to 1.5 mL of 0.15 mM DPPH in 95% ethanol incubated in the dark for 30 minutes and the absorbance was measured at a wavelength of 517 nm (Wu *et al.*, 2003). Percent DPPH inhibition is calculated using the following formula:

$$\text{DPPH inhibition (\%)} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100 \quad \text{... Eq 2}$$

The sample used was dissolved with various concentrations to obtain the IC_{50} value representing the concentration of the sample solution required to reduce DPPH free radicals by 50%. The linear regression equation (y = a + bx) obtained from the inhibition value at each concentration is used to find the IC_{50} value.

2.4.2 ABTS radical scavenging activity

The ABTS radical scavenging activity was performed according to the method by Stämpfli *et al.* (2007) with slight modifications. ABTS stock solution was prepared by reacting 7 mM ABTS solution and 2.45 mM potassium persulfate in 1:1 ratio in a dark room for 16 hours at room temperature. The solution was then added with ethanol to obtain an absorbance of ±0.785 at a wavelength of 750 nm. A total of 1 mL of ABTS solution was added to 1 mL of the hydrolyzed sample. It was left for 10 minutes at room temperature. Absorbance was measured at a wavelength of 750 nm. The percentage of ABTS radical inhibition was calculated based on the formula:

$$\text{ABTS inhibition (\%)} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \text{x100... Eq 3}$$

The sample used was dissolved with various concentrations to obtain the IC_{50} value representing the concentration of the sample solution required to reduce ABTS free radicals by 50%. The linear regression equation (y = a + bx) obtained from the inhibition value at each concentration is used to find the IC_{50} value.

2.4.3 Reducing power

Evaluation of reducing power was carried out using 1 mL sample (5 mg/mL) added to 2 mL of 0.2 M phosphate buffer (pH 6.6) and $K_3Fe(CN)_6$ and then incubated for 20 minutes at 50°C. After incubation, the sample was added with 1 mL of 10% TCA and centrifuged at 3500xg for 10 minutes. The 1 mL supernatant was added with 1 mL distilled water and 200 µL $FeCl_3$. After 10 minutes of incubation, the absorbance was measured at a wavelength of 700 nm (Oyaizu, 1988).

2.5 Molecular Weight Analysis

The molecular weight of the samples was determined using the SDS-Polyacrylamide Gel electrophoresis method based on Laemmli (1970) with a concentration of 12% acrylamide gel as separator gel and 5% acrylamide gel as collector gel. The sample tested was tuna skin protein hydrolysate; 20 µL of the sample was mixed with 10 µL of sample buffer containing 2-mercaptoethanol, then heated at 85°C for five minutes, and then put into the well of the gel. Ten µL of the marker was included in the gel well. The gel was mounted in the electrophoresis apparatus by pouring the electrophoretic buffer into the chamber. The electrophoresis process lasted for 180 minutes at 180 volts and 50 mA in the electrophoresis apparatus, after which the gel was removed from the glass plate. The gel was soaked in Coomassie blue gel stain solution for two hours, followed by distaining until protein bands were obtained.

2.6 Functional Group Analysis

Functional group analysis was carried out using the *Fourier-transform infrared spectroscopy* (FTIR) principle based on Qiu *et al.* (2019) using an infrared spectrophotometer (Bruker Tensor 37, Ettlingen, Germany). A 2 mg sample was mixed with 100 mg KBr and pulverized. Then the sample was measured using the FTIR instrument at a wavelength of 500 - 4000 cm^{-1}. FTIR instruments will detect absorption peaks at certain wave numbers. The wave numbers detected as peaks are identified based on the specific absorption region range of the protein functional groups.

2.7 Amino Acid Composition Analysis

The type of amino acid was characterized to determine the amino acid type in the sample. The instrumentation used was Ultra Performance Liquid Chromatography (UPLC) ACCQ-Tag Ultra C18 column, temperature 49°C, and mobile phase gradient composition system. The standard amino acids used in this analysis consist of 15 types of amino acids. A total of 100 mg of sample was weighed and put into a 20 mL headspace vial. Hydrolysis was carried out with an HCl solution. Then hydrolysate was removed and transferred into a 50 mL measuring flask, added distilled water the tera mark, and homogenized. The test solution was filtered with a 0.2 µm syringe filter to collect the filtrate. A total of 1 µL was included in the UPLC instrument (SIG, 2021). Calculation of amino acid levels in the sample using the ratio of the analyte area to the internal standard, with the following formula:

$$\text{Standard or sample ratio} = \frac{\text{The area of the amino acid analyte}}{\text{AABA standard internal area}} \quad \ldots \text{Eq 4}$$

$$\text{Amino acid content} = \frac{\frac{\text{Sample ratio}}{\text{Standard ratio}} \times \frac{C_{std}}{1000000} \times MW \times Va \times DF}{W_{spl} \text{ or } V_{spl}} \quad \ldots \text{Eq 5}$$

where:

MW = Amino acid molecular weight (g/mol)

C_{std} = Concentration of standard amino acid solutions (pmol/µL)

Va = The final volume of the test solution (µL)

DF = Dilution factor

W_{Spl} = Test portion weight (g)

V_{Spl} = Volume of test portion (mL)

2.8 Diversity of bioactive peptides

The best protein hydrolysate was identified as a bioactive peptide using LC-MS, referring to the method of Suetsuna *et al.* (2000). A 5 µL hydrolyzed tuna skin was injected into the LC-ESI-QTOF instrument. LC-MS analysis was performed using a UPLC-MS equipped with a binary pump. The LC was connected to a QTOF mass spectrometer with an ESI ionization source. The MS used was a Xeco G2-S TOF system with a positive ionization mode. The ESI parameters included a capillary temperature of 120°C and a 500 L/hour fogging gas with a voltage source of 3 kV. The full scan mode from m/z 100-1500 was carried out with a source temperature of 110°C. The UPLC column used was Acquity UPLC HSS C18 1.8 µM×2.1×150 mm. The eluent was H_2O + 0.1% formic acid (A) and 0.1% formic acid + acetonitrile (B). The eluent was set at a 0.3 mL/min flow rate. The elution system was run isocratic at 0-1 minute with a ratio of 95:5, 0-5 minutes with a linear gradient elution of solvent A from 95% to 5%, 6-7 minutes isocratic elution with a ratio of 0:100, 6-7 minutes with a gradient elution linear solvent A from 0% to 100%, minutes 7.5-9 isocratic elution ratio 95:5, minutes 7.5-9 linear gradient elution solvent A from 95% to 5%. The resulting chromatogram data were converted to NetCDF format to make it easier to process data with Mzmine. The results obtained were in the form of mass array data from peptide hydrolysate chromatograms which included three variables, namely m/z, retention time, and peak intensity. Identification of the metabolite compounds contained in the samples was carried out by matching the m/z of the resulting chromatogram with the accurate mass database for the peptide compounds contained in tuna skin hydrolysate.

2.9 Data Analysis

The research design at the stages of defatting and protein hydrolysis used a Completely Randomized Design Factorial (CRDF), while for the determination of antioxidant activity a Completely Randomized Design (CRD) was used. Each result of the analysis of variance was significantly different; further testing was carried out with the Tukey test using Minitab software.

3. Results and Discussion

3.1 Results

3.1.1 The proximate composition of tuna skin

Yellowfin tuna skin as raw material for FPH is obtained from tuna fillet processing companies with an average fish weight of 15–70 kg/head. Several studies reported the proximate composition of scombroid fish skin (Table 1). Characteristics of raw materials can be determined by analyzing the proximate composition especially protein content, is of concern in this research.

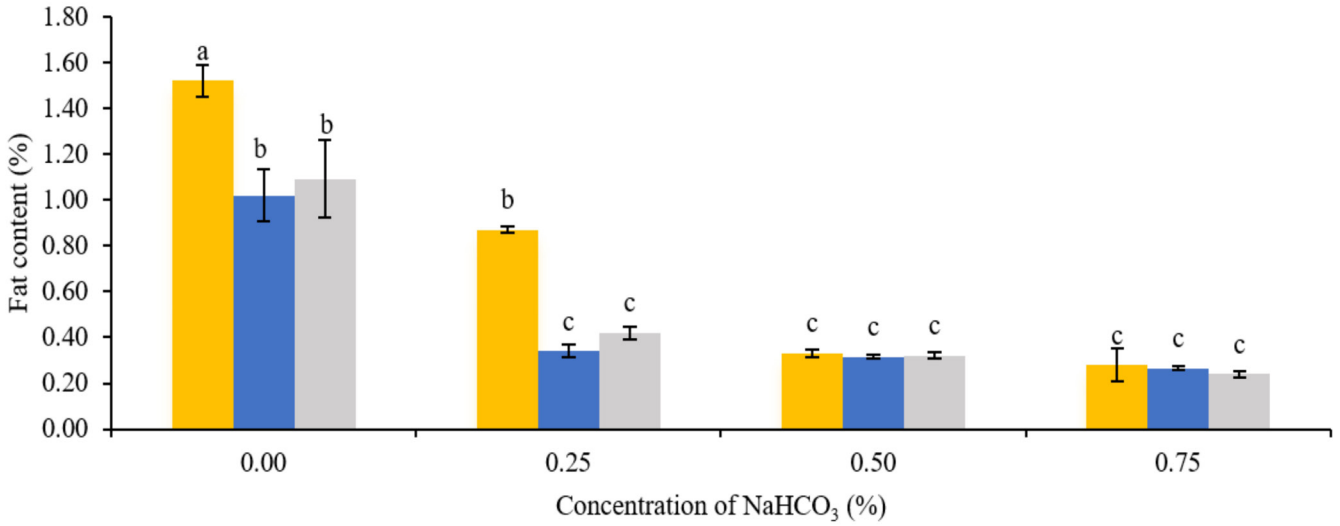


Figure 1. Fat content with defatting treatment in the concentrations of $NaHCO_3$ and immersion time at 30 ( ); 60 ( ); 90 ( ) minutes. Different superscripts show significant differences ($p<0.05$).

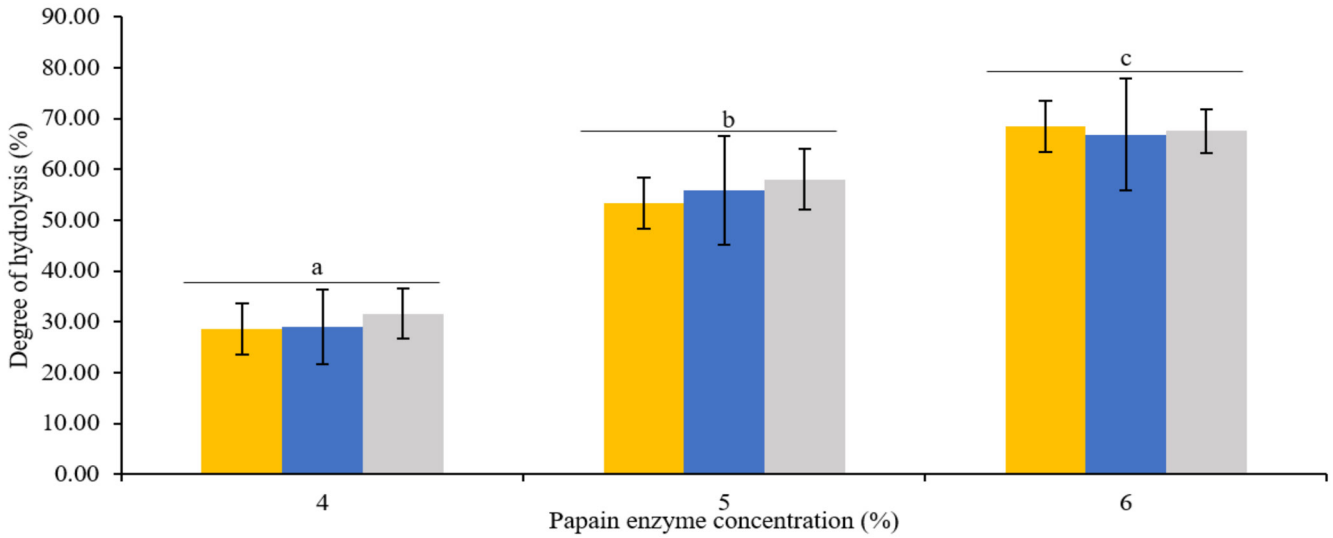


Figure 2. The degree of hydrolysis in the treatment of differences in the concentration of the enzyme papain and the duration of hydrolysis at 4 ( ); 5 ( ); 6 ( ) hours. Different superscripts show significant differences ($p <0.05$).

Table 1. The proximate composition of scombroid fish skin

Composition	Yellowfin tuna (*Thunnus albacares*)		Mackerel tuna[1] (*Euthynnus affinis*)		Skipjack[2] (*Katsuwonus pelamis*)		Yellowfin tuna[3] (*Thunnus albacares*)	
	(ww) %	(dw) %	(ww) %	(dw) %	(ww) %	(dw) %	(ww) %	(dw) %
Moisture	69.18 ± 0.06	-	71.66	-	59.6	-	57.42	-
Protein	26.77 ± 1.08	81.5	24.63	87.43	28.31	70.07	37.45	87.95
Fat	1.69 ± 0.29	5.2	2.72	9.65	7.45	18.44	3.8	8.92
Ash	0.20 ± 0.05	0.59	0.17	0.6	1.33	3.29	0.49	1.15

Description: [1]Komala *et al.* (2015), [2]Karunarathna and Attygalle (2010), [3]Nurjanah *et al.* (2021)
(ww=wet weight; dw=dry weight). Data represented as means ± SD (n=3)

3.1.2 Decreased fat content of tuna skin

The defatting method in this study was carried out by immersing the samples in various concentrations of $NaHCO_3$, low temperature (4°C), and immersion time (Figure 1). The analysis of variance showed that the concentration of $NaHCO_3$, immersion time and their interactions significantly affected the decrease in the lipid content of the tuna skin ($p<0.05$). The alkaline salt immersion treatment results were proven to remove 45-85% fat in the tuna skin.

Table 2. Antioxidant activity at different enzyme concentrations

Enzyme concentration (%)	DPPH IC_{50} (mg/mL)	ABTS IC_{50} (mg/mL)	*Reducing power* (Absorbance 700 nm)
4	1.277± 0.016[b]	0.647± 0.016[b]	0.415± 0.007[a]
5	0.964± 0.002[a]	0.495± 0.002[a]	0.415± 0.001[a]
6	0.963± 0.001[a]	0.491± 0.001[a]	0.416± 0.004[a]

Data represented as means ± SD (n=3). Different superscripts in the same column show significant differences ($p < 0.05$)

Table 3. Characteristics of tuna skin hydrolysate functional groups

Amide	Absorption area (cm^{-1})	Absorption peak (cm^{-1})	Explanation
Amide A	3400 – 3440[a]	3430	N-H *stretching*
Amide B	2922 – 2940[b]	2934	C-H *asymmetrical stretching*
Amide I	1625 – 1690[c]	1650	C=O *stretching*, C-N *stretching*
Amide II	1500 – 1600[d]	1542	N-H *bending*, C-N *stretching*, C=H *stretching*
Amide III	1229 – 1301[e]	1244	C-N *stretching*, N-H *bending*

Description: [a]Kaewdang *et al.* (2014); [b]Devita *et al.* (2021); [c]Kaewdang *et al.* (2014); [d]Rasli and Sarbon (2019); and [e]Kong and Yu (2007)

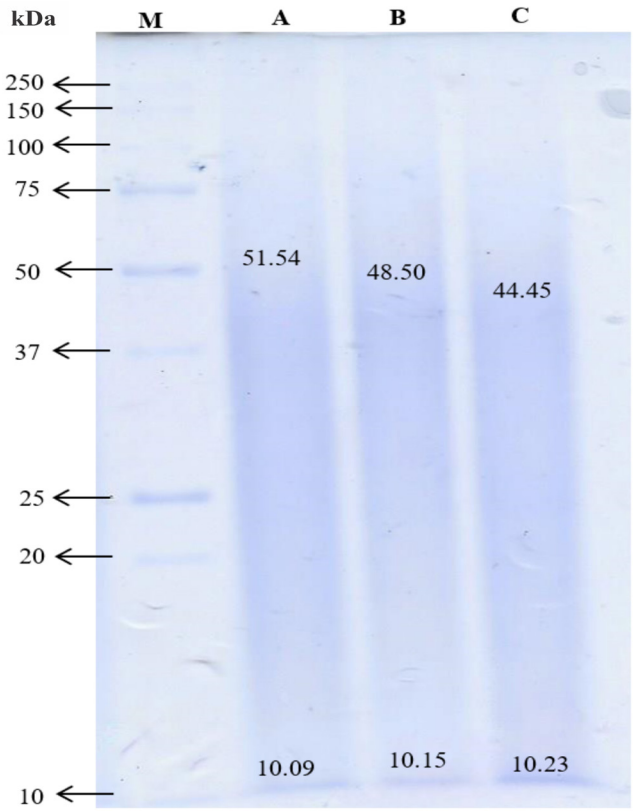


Figure 3. Molecular weight of marker (M), tuna skin hydrolysate with papain enzyme concentration 4% (A); 5% (B) and 6% (C)

3.1.3 Degree of hydrolysis

The level of enzymatic hydrolysis of proteins was measured by the degree of hydrolysis (DH) (Figure 2). The DH value in the hydrolysis process was affected by the addition of different concentration of papain enzyme ($p<0.05$), while time of hydrolysis and interaction had no significant effect ($p>0.05$).

3.1.4 Antioxidant activity

The antioxidant activity was evaluated using three simple spectrophotometric methods (DPPH, ABTS, and reducing power) (Table 2). Analysis of variance showed a significant effect ($p<0.05$) with the addition of different concentration of papain enzyme on the value of antioxidant activity in the DPPH and ABTS methods. In the reducing power method, different concentration of papain enzyme did not have a significant effect ($p>0.05$).

3.1.5 Molecular weight profile

The molecular weight profile of tuna skin hydrolysate was determined using the SDS-PAGE (Figure 3). The results obtained were that the MW value tended

to decrease with increasing the concentration of the papain enzyme in the hydrolysate in line with the increase in the DH value and the antioxidant activity obtained.

3.1.6 Function group of tuna skin hydrolysate

The polypeptide and protein units generally provided nine characteristic absorption peaks: amides A, B, and I–VII. The functional group spectrum analysis resulted from tuna skin hydrolysate samples using 5% enzyme concentration treatment for four hours (Table 3). The tuna skin hydrolysate functional groups showed absorption peaks in the amide A, B, I, II, and III absorption areas (Figure 4).

3.1.7 Amino acid composition of tuna skin hydrolysate

The amino acid composition was essential in determining the bioactivity of protein products. Amino acids of tuna skin, tuna skin hydrolysate, and collagen hydrolysate showed differences in total amino acids (Table 4). Tuna skin hydrolysate with the highest antioxidant activity had the highest amino acid composition in hydrophobic amino acids.

3.1.8 Bioactive peptide diversity

LC-MS is an integrated proteomics system for analyzing a component in a material. The results of LC-MS analysis related to peptides, molecular weight, and biological activity of tuna skin hydrolysate (Table 5). Based on various molecular and peptide formulas in tuna skin hydrolysate, various bioactive potentials are obtained as antibacterial, ACE inhibitor, antioxidant and antifibrinolytic.

3.2 Discussion

Fish are classified into two categories based on their protein and fat content. Two categories of fish, namely high protein with, moderate fat and high protein with, low fat. Yellowfin tuna is a type of fish with high protein content and low fat (Bahurmiz, 2019). The protein and fat content of the yellow fin tuna skin produced were 26.77% and 1.69%, respectively. The value of this protein content was lower than of mackerel tuna skin (Komala *et al*., 2015), and higher than skipjack tuna skin (Karunarathna and Attygalle, 2010d), and yellowfin tuna reported by Nurjanah *et al*. (2021). Differences in proximate composition can be influenced by species, habitat, body size, environmental conditions, type of feed, and preparation of ingredients (Songchotikunpan *et al*., 2008; Porto *et al*., 2016). The protein content of yellowfin tuna skin obtained in this study is relatively high, making it possible to use it as raw material for FPH.

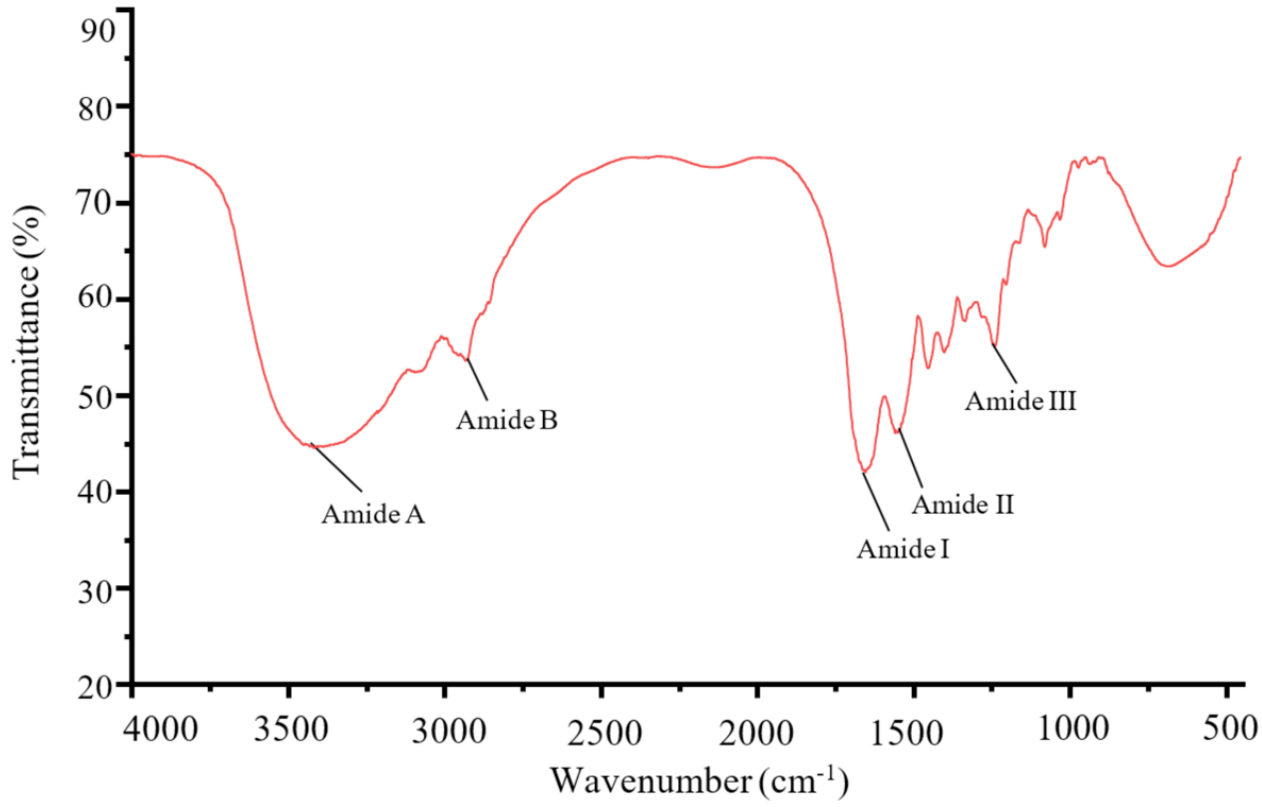


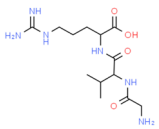
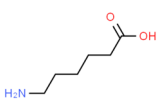
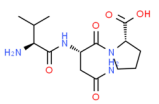
Figure 4. FPH infrared spectrum of tuna skin using 5% papain enzyme concentration. Amide spectrum: A, B, I, II, and III

Table 4. Amino acid composition of tuna skin, tuna skin hydrolysate and collagen hydrolysate

Types of amino acids	Skin[1]	Tuna skin hydrolysate	Collagen hydrolysate[2]
Lysine[a]	2.3	3.06	4.04
Valine[ab]	1.4	2.37	2.3
Isoleucine[ab]	1.06	1.23	1.13
Leucine[ab]	1.97	2.63	2.94
Histidine[ac]	0.86	0.82	-
Threonine[ac]	1.51	3.76	3.44
Phenylalanine[ab]	1.23	2.11	2.63
Glycine[b]	6.18	21.92	23.86
Alanine[b]	3.66	8.46	8.79
Arginine[c]	3.24	8.94	10.56
Aspartate[c]	2.82	4.22	4.66
Glutamate[c]	5.18	8.41	8.42
Proline[b]	-	9.76	11.82
Serine[c]	1.13	3.91	4.13
Tyrosine	0.59	0.49	0.97
AA Essential	10.33	15.98	16.48
AA Hydrophobic	11.84	48.48	44.68
AA Hydrophilic	14.74	30.06	31.21
Total amino acids	33.13	82.09	89.69

Description: [1]Suseno (2015) and [2] Prastyo (2021); [a]essential amino acids, [b] hydrophobic amino acids, and [c] hydrophilic amino acids

Table 5. Bioactive peptide diversity in tuna skin protein hydrolysate

Peptide Confirmed[1]	Molecular formula[2] $[M+H]^-$	Molecular weight[1] (Da)	Structure Molecule[1]	Biological activity of peptide[1]
Gly-Val-Arg	$C_{13}H_{26}N_6O_4$	330.383		Inhibitor ACE
Aminocaproic	$C_6H_{13}NO_2$	131.173		Antifibrinolytic
Val-Asn-Pro	$C_{14}H_{24}N_4O_5$	328.364		Antioxidant

Description: [1]Chemspider Database 2022; [2] MassLynMassLynx

The chemical composition of tuna skin shows a low-fat content, but the presence of this fat can inhibit the effectiveness of the hydrolysis process and the results obtained. Several chemical and mechanical methods have been developed for degreasing (Hua *et al*., 2020). The application of the alkaline salt immersion method in this study intends to avoid economic, environmental, and food safety problems. The immersion treatment in $NaHCO_3$ at a low temperature of 4°C was effective in reducing the fat content, which can reduce the fat content of 85% in tuna skin before the defatting process. This condition is caused by the alkalinity of $NaHCO_3$, which can remove fat and open the matrix bond structure in the skin so that fat and other compounds can be quickly released (Cho *et al*., 2005; Hemung, 2013). Immersion with 0.5% $NaHCO_3$ (w/v) and soaking time of 30 minutes resulted in 0.33% fat content with a reduction percentage of 80.53% from before the defatting process, which was the selected treatment for the next stage based on the efficient use of time in production. The decrease in fat content shows higher results when compared to research by Sae-leaw *et al.* (2016) on seabass skin gelatin with 30% isopropanol, decreased fat 50.32% and in Xu *et al.* (2017) on skin of southern catfish (*Silurus meridionalis* Chen) with 10% $NaHCO_3$, the fat reduction was 69.37%. Lower results when compared with reported by Ramadhan *et al.* (2014) using catfish fillet with 0.75% $NaHCO_3$, soaking time 10 minutes, which reduced fat 89.64%. Nilsuwan *et al.* (2021) on fat in collagen hydrolysate with a disk stack centrifugal separator (DSCS) two cycles and the addition of a solution 30% isopropanol which decreased fat 100%. Using different immersion media, tools, and raw materials in defatting, produces different fat reduction values. According to Hierro *et al.* (2021), low-fat insect extracts produce high antioxidant activity, so the low-fat content in the defatting process is also expected to produce high antioxidants from hydrolyzed tuna skin.

The DH value reflects changes in peptide bonds during enzymatic hydrolysis and is an essential parameter for measuring the enzymatic process of proteins (Shu *et al*., 2018). Based on the results obtained, it was found that hydrolysis of tuna skin with papain enzymes 4-5% for 4-6 hours, DH 29.72–67.64%. These results indicate a higher DH when compared of reported by Prastyo *et al.* (2020) on tilapia collagen hydrolysis with papain enzyme for 15 minutes, DH 12.21-34.01% by Nurilmala *et al.* (2020) on skin collagen of yellowfin tuna with alcalase enzyme for three hours, DH 52.71%. However, these results are lower when compared to research reported by Karnila *et al.* (2020) on yellow pike conger (*Congresox talbon*) 0.15% papain enzyme for four hours, DH 99.06%. Several factors that affect the DH value are the enzyme concentration, hydrolysis time, and the type of protease used. Significantly different DH values can affect the molecular weight and amino acid composition, affecting the resulting peptides' antioxidant activity (Alahmad *et al*., 2022). The multiple comparison follow-up test results showed that an increase in hydrolysis time above four hours did not show a significant increase in DH value. This condition is likely related to a decrease in the level of enzyme activation due to the autohydrolysis process at high temperatures for a long time (Fonseca *et al*., 2016). Therefore, only treatments with enzyme concentrations of 4, 5, and 6% (v/v) and hydrolysis time of four hours were evaluated for their antioxidant activity.

The evaluation results showed that the antioxidant activity was higher in the ABTS method compared to the DPPH. Similar results were also reported by Kusumaningtyas *et al.* (2019) and Nurilmala *et al.* (2020) on the hydrolysis of collagen from fish skin. The ABTS method tends to detect hydrophobic and hydrophilic compounds, while the DPPH method is suitable for hydrophobic compounds. This finding can be attributed to the raw material properties of tuna skin reported by Suseno (2015), which consists of 15.50% hydrophobic and 14.74% hydrophilic amino acids of the total detected amino acids. The differences of amino acids in the hydrolysate result in varying antioxidant activity. The type of proteases and different raw materials exhibit peptides with different amino acid lengths and compositions that hydrolyze proteins. The amino acid composition influences the hydrophobicity and charge of the peptide, which determines its bioactivity (Venuste *et al*., 2013). The absorbance value of the reducing power method showed a value of 0.415-0.416 at a sample concentration of 1 mg/mL. Samples with higher absorbance values show more ability to donate electrons to free radical elements. This research results showed an absorbance higher than that reported by Arlina *et al.* (2019) on eel myofibril hydrolysis at a sample concentration of 3 mg/mL with an absorbance of 0.305 nm and lower than that reported by Chen *et al.* (2014) on the protein hydrolysate sarcoplasm of tilapia at a sample concentration of 3 mg/mL with an absorbance of 0.629 nm. These results indicate that differences in raw materials and sample concentrations affect the samples' absorbance values and antioxidant activity. The antioxidant activity value of tuna skin hydrolysate was included in the very weak antioxidant category (>0.20 mg/mL). From the results obtained it was concluded that the concentration of hydrolysate used from tuna skin needs to be increased to obtain strong antioxidant activity.

The selected treatment from the antioxidant

test on tuna skin hydrolysate with an enzyme concentration of 5% (v/v) showed an MW value in the range of 10.15-48.50 kDa. The results obtained showed a higher MW value than that reported by Nurilmala *et al.* (2020) on collagen hydrolysate where the MW was in the range of 2.94-11.93 kDa and is lower than that reported by Mahmoodani *et al.* (2014) on the hydrolysate of catfish (*Pangasius sutchi*) skin gelatin, where the MW ranged from 45-66 kDa. The observed differences in MW from several hydrolysates were caused by the distribution of MW in different peptide of raw materials, the type of enzymes used Fonseca *et al.* (2016) and hydrolysis conditions. A lower MW value indicates a higher antioxidant activity value. Generally, bioactive peptides with high antioxidant activity have a molecular size of <10 kDa (Daliri *et al.*, 2017).

The functional groups of tuna skin hydrolysate showed absorption peaks in the amide A, B, I, II and III absorption areas which showed similar results to those reported by Prastyo (2021) on tilapia skin collagen hydrolysate and Nurilmala *et al.* (2019) on tuna skin collagen hydrolysate. This is associated with the same type of raw material in the hydrolysate used. The absorption peaks obtained indicated that the tuna skin hydrolysate product had similar functional group characteristics to the collagen hydrolysate product. It is known that tuna skin hydrolysate shows a secondary protein structure in the form of α-helix with amide I vibration at the absorption peak of 1651 cm^{-1} (Table 3). The secondary structure of protein can vary depending on the type of material used, for example tilapia skin collagen hydrolysate reported by Prastyo (2021) shows an absorption peak at 1645 cm^{-1} secondary protein structure is random coil while in tilapia skin collagen reported by Bi *et al.* (2019) at the absorption peak of 1628 cm^{-1} the secondary protein structure is *β-sheet*.

The dominant types of amino acids in tuna skin hydrolysate are glycine, proline and arginine which are not different from the amino acid content reported by Prastyo (2021) in tilapia skin collagen hydrolysate by Blanco *et al.* (2017) in tuna skin collagen hydrolysate and collagen of mackerel (*Scomberomorus guttatus*) by Fatemi *et al.* (2021). Tuna skin hydrolysate contains the amino acids glycine, proline, and arginine, 21.92%, 9.76%, and 8.94%, respectively. These amino acids show similar results to collagen hydrolysate products, in which these amino acids are also reported to have a role in biological activity. The tuna skin hydrolysate obtained had the highest amino acid composition among hydrophobic amino acids. According to Chi *et al.* (2015), hydrophobic amino acids can prevent the oxidation of macromolecules by donating protons to reactive radicals. The arrangement of aromatic amino acid side chains (tyrosine, phenylalanine, and threonine) in hydrolysate also has radical scavenging and metal-chelating activities (Mutaminah *et al.*, 2018). The presence of these aromatic amino acid affects the evaluation of the antioxidant activity obtained previously.

Some of the peptides in the skin hydrolysate identified are multifunctional and exhibit bioactivity other than as antioxidant. The detected presence of Val, Met, Tyr, Leu, Phe, Lys, His, and Arg have been shown to contribute greatly to the potency of antioxidant peptides (Pezeshk *et al.*, 2019). In addition, the hydrophobic nature of peptides can enhance their interaction with lipid targets or the entry of peptides into target organs through hydrophobic composition, which is desirable to achieve antioxidant effects (Wiriyaphan *et al.*, 2012). Jiang *et al.* (2021) reported that the peptide component Val-Asn-Pro in baijiu drink showed potential antioxidant activity and was in line with the peptide component identified in tuna skin hydrolysate. This proves that tuna skin with an enzymatic hydrolysis process can be utilized as an antioxidant source in food and nutraceutical products, which was also supported by testing the antioxidant activity carried out in the previous stages. Findings by Miguel *et al.* (2006) showed that the presence of proline amino acid at the C-terminal end and hydrophobic amino acid residues at the N-terminal end (alanine, valine, leucine) had an impact on the good ACE ability of ovalbumin. The high percentage of hydrophobic amino acids in tuna skin hydrolysate is believed to increase ACE inhibitors. Manoharan *et al.* (2017) reported that the peptide components Gly-Val-Arg (GVR) from oyster mushroom extract (*Pleurotus pulmonarius*) produces antihypertensive effects that are competitive by analysis of ACE inhibitors in vitro and in vivo. The peptide components Gly-Val-Arg (GVR) was also detected in tuna skin hydrolysate with an integrated proteomic system. Identification was continued by matching the m/z of the resulting chromatogram with the accurate mass from the ChemSpider database. Confirmed peptides have the potential to be developed as nutraceutical products to reduce hypertension. Aminocaproic acid is a derivative and analogue of the amino acid lysine detected in tuna skin hydrolysate samples. Estcourt *et al.* (2016) reported the presence of aminocaproic acid as an antifibrinolytic for the prevention of bleeding in patients with hematological disorders.

4. Conclusion

Pretreatment of tuna skin with $NaHCO_3$ at a low temperature of 4°C effectively reduces fat content

by 80.53%. Tuna skin hydrolysate which was hydrolyzed for four hours with 5% (v/v) enzyme concentration had the best average percentage degree of hydrolysis and the best average IC_{50} value. The most effective antioxidant method for testing tuna skin hydrolysate is the ABTS method. FTIR results showed absorption peaks of amides A, B, I, II and III. The dominant amino acids are glycine, proline, and arginine. The detected potential bioactive peptides are ACE inhibitor, antioxidant and antifibrinolytic. This research should be continued with the fractionation and purification processes on protein hydrolysates to obtain higher antioxidant activity and specific bioactive peptides as antioxidants.

Acknowledgment

The author would like to thank PT. Bumi Menara Internusa Makassar for providing material support and special handlers for research raw materials.

Authors' Contributions

All authors have contributed to the final manuscript. The contributions of each author are as follows, SHR; developed conceptual ideas and manuscript, prepared, ran, and controlled the course of the research, as well as collected and analyzed data. JS and SHS; developed conceptual ideas, monitored, and controlled the course of the research and critically revising articles. All authors discussed the results and contributed to the final manuscript.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding Information

This research was supported by Raw Material Characteristics of Aquatic Products Laboratory, Molecular Biology of Aquatic Products Laboratory in the Aquatic Product Technology Department Faculty of Fisheries and Marine Sciences, Bogor Agricultural University, and also received raw material support from PT. Bumi Menara Internusa Makasaar.

References

Association of Analytical Chemist Publisher (AOAC). (2005). Official methods of analysis of the association of official analysis chemist. Arlington Virginia (US): The Association of Official Analytical Chemist, Inc.

Agustin, V., Husni, A., & Putra, M.M.P. (2021). Antioxidant activity of protein hydrolysate from snakehead fish (*Channa striata*) viscera obtained by enzymatic process. IOP Conf Series: *Earth and Environmental Science*, 919(012046):1-7.

Alahmad, K., Xia, W., Jiang, Q., & Xu, Y. (2022). Effect of the degree of hydrolysis on nutritional, functional, and morphological characteristics of protein hydrolysate produced from bighead carp (*Hypophthalmichthys nobilis*) using ficin enzyme. *Foods*, 11(9):1-17.

Arlina, H., Santoso, J., Desniar. (2019). Aktivitas antioksidan hidrolisat protein miofibril belut (*Synbranchus bengalensis*) yang dihidrolisis dengan enzim papain. *Jurnal Teknologi Industri Pertanian*, 29(3):247-259.

Asmak, N., Kusmiyati-Tjahjono, D. K., Chasanah, E., Fawzya, Y. N., Martosuyono, P., Nuryanto, & Afifah, D.N. (2020). The effect of fish protein hydrolysate (FPH) substitution complementary feeding formula on the albumin levels of Sprague Dawley rat. *Food Research*, 4(Suppl. 3):18-23.

Baco, N., Oslan, S. N. H., Shapawi, R., Mohhtar, R. A. M., Noordin, W. N. M., & Huda, N. (2022). Antibacterial activity of functional bioactive peptides derived from fish protein hydrolysate. IOP Conference Series: *Earth and Environmental Science*, 967(012019):1-12.

Baehaki, A., Widiastuti, I., Nainggolan, C., & Gofar, N. (2020). Antioxidant activities of snakehead (*Channa striata*) fish skin: Peptides hydrolysis using protease TP2 isolate from swamp plant silage. *Slovak Journal of Food Sciences*, 14:379-384.

Bahurmiz M. (2019). Proximate and fatty acid composition of three tuna species from *Hadhramout* coast of the Arabian Sea, Yemen. *Hadhramout University Journal of Natural & Applied Sciences*, 16(1):63-71.

Bi, C., Li, X., Xin, Q., Han, W., Shi, C., Guo, R., Shi, W., Qiao, R., Wang, X., & Zhong, J. (2019). Effect of extraction methods on the preparation of electrospun/electrosprayed microstructures of *Tilapia* skin collagen. *Journal of Bioscience and Bioengineering*, 128(2):234-240.

Blanco, M., Vázquez, J. A., Pérez-Martín, R. I., & Sotelo, C. G. (2017). Hydrolysates of fish skin collagen: An opportunity for valorizing fish industry by-products. *Marine Drugs*, 15(5)1-15.

Chen, X. X., Hu, X., Li, L. H., Yang, X. Q., Wu, Y. Y., Lin, W. L., Zhao, Y. Q., Ma, H. X., & Wei, Y. (2014). Antioxidant properties of *Tilapia* component protein hydrolysates and the membrane ultrafiltration fractions. *Advanced Materials Research*, 1812-1817.

Chi, C. F., Wang, B., Hu, F. Y., Wang, Y. M., Zhang, B., Deng, S. G., & Wu, C. W. (2015). Purification and identification of three novel antioxidant peptides from protein hydrolysate of bluefin leatherjacket (*Navodon septentrionalis*) skin. *Food Research International*, 73:124-129.

Cho, S. M., Gu, Y. S., & Kim, S. B. (2005). Extracting optimization and physical properties of yellowfin tuna (*Thunnus albacares*) skin gelatin compared to mammalian gelatins. *Food Hydrocolloids*, 19(2):221-229.

Daliri, E. B. M., Oh, D. H., & Lee, B. H. (2017). Bioactive peptides. *Foods*, 6(5):1-21.

Devita, L., Nurilmala, M., Lioe, H. N., & Suhartono, M. T. (2021). Chemical and antioxidant characteristics of skin-derived collagen obtained by acid-enzymatic hydrolysis of bigeye tuna (*Thunnus obesus*). *Marine Drugs*, 19(4):1-19.

Estcourt, L. J., Desborough, M., Brunskill, S. J., Doree, C., Hopewell, S., Murphy, M. F., & Stanworth, S. J. (2016). Antifibrinolytics (lysine analogues) for the prevention of bleeding in people with haematological disorders. *Cochrane Database of Systematic Reviews*, (3):1-53.

Fatemi, M. J., Garahgheshlagh, S. N., Ghadimi, T., Jamili, S., Nourani, M. R., Sharifi, A. M., Saberi, M., Amini, N., Sarmadi, V. H., & Yazdi-Amirkhiz, S. Y. (2021). Investigating the impact of collagen-chitosan derived from *Scomberomorus guttatus* and shrimp skin on second-degree burn in rats model. *Regenerative Therapy*, 18:12-20.

Fonseca, R. A. S., Silva, C. M., Fernandes, G. R., & Prentice, C. (2016). Enzymatic hydrolysis of cobia (*Rachycentron canadum*) meat and wastes using different microbial enzymes. *International Food Research Journal*, 23(1):152-160.

Gamarro, E., Orawattanamateekul, W., Sentina, J., & Gopal, S. (2013). By-products of tuna processing. Rome: Food and Agriculture Organization of the United Nations.

Gonz, D. J., Hadidi, M., Varcheh, M., Jelyani, A. Z., Moreno, A., & Lorenzo, J. M. (2022). Bioactive peptide fractions from collagen hydrolysate of common carp fish by-product: antioxidant and functional properties. *Antioxidants Article* 2022, 11(3):1-11.

Hadinoto, S., & Idrus, S. (2018). Proporsi dan kadar proksimat bagian tubuh ikan tuna ekor kuning (*Thunnus albacares*) dari perairan maluku. *Majalah Biam*, 14(2):51-57.

Hadinoto, S., Kolanus, J. P. M., & Idrus, S. (2019). Characterization of swim bladder and its collagen of yellowfin tuna (*Thunnus* sp.) produced from acetic acid extraction. *Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan*, 14(2):129-140.

Hemung, B. (2013). Properties of *Tilapia* bone powder and its calcium bioavailability based on transglutaminase assay. *International Journal of Bioscience, Biochemistry and Bioinformatics*, 3(4):2-5.

Henaux, L., Pereira, K. D., Thibodeau, J., Pilon, G., Gill, T., Marette, A., & Bazinet, L. (2021). Glucoregulatory and anti-inflammatory activities of peptide fractions separated by electrodialysis with ultrafiltration membranes from salmon protein hydrolysate and identification of four novel glucoregulatory peptides. *Membranes*, 11(7):1-16.

Hierro, J. N. del, Cantero-bahillo, E., Fornari, T., & Martin, D. (2021). Effect of defatting and extraction

solvent on the antioxidant and pancreatic lipase inhibitory activities of extracts from *Hermetia illucens* and *Tenebrio molitor*. *Insects*, 12(9):1-17.

Hoyle, N. T., & Merritt, J. H. (1994). Quality of fish protein hydrolysates from herring (*Clupea harengus*). *Journal of Food Science*, 59(1):76-79.

Hua, K. C., Feng, J. T., Yang, X. G., Wang, F., Zhang, H., Yang, L., Zhang, H. R., Xu, M. Y., Li, J. K., Qiao, R. Q., Lun, D. X., & Hu, Y. C. (2020). Assessment of the defatting efficacy of mechanical and chemical treatment for allograft cancellous bone and its effects on biomechanics properties of bone. *Orthopaedic Surgery*, 12(1):617-630.

Huang, C. Y., Kuo, J. M., Wu, S. J., & Tsai, H. T. (2016). Isolation and characterization of fish scale collagen from tilapia (*Oreochromis* sp.) by a novel extrusion-hydro-extraction process. *Food Chemistry*, 190:997-1006.

Irianto, H. E., & Akbarsyah, T. M. I. (2007). Pengalengan ikan tuna komersial. *Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology*, 2(2):43-50.

Jeewanthi, R. K. C., Lee, N. K., & Paik, H. D. (2015). Improved functional characteristics of whey protein hydrolysates in food industry. *Food Science of Animal Resources*, 35(3):350-359.

Jiang, Y., Yin, Z., Zhao, J., Sun, J., Zhao, D., Zeng, X. an, Li, H., Huang, M., & Wu, J. (2021). Antioxidant mechanism exploration of the tripeptide Val-Asn-Pro generated from *Jiuzao* and its potential application in baijiu. *Food and Chemical Toxicology*, 155:112402.

Kaewdang, O., Benjakul, S., Kaewmanee, T., & Kishimura, H. (2014). Characteristics of collagens from the swim bladders of yellowfin tuna (*Thunnus albacares*). *Food Chemistry*, 155:264-270.

Karnila, R., Dewita, Ilham, D., & Sidauruk, S. W. (2020). Utilization of papain enzymes in the production of protein hydrolysates of yellow pike conger (*Congresox talabon*). *AACL Bioflux*, 13(3):1285-1291.

Karunarathna, K., & Attygalle, M. V. E. (2010). Nutritional evaluation in five species of tuna. *Vidyodaya Journal Science*, 15(1&2):7-16.

Kiewiet, M. B. G., Dekkers, R., Ulfman, L. H., Groeneveld, A., De Vos, P., & Faas, M. M. (2018). Immunomodulating protein aggregates in soy and whey hydrolysates and their resistance to digestion in an *in vitro* infant gastrointestinal model: New insights in the mechanism of immunomodulatory hydrolysates. *Food and Function*, 9(1):604-613.

Kementrian Kelautan dan Perikanan (KKP). (2020). Peluang usaha dan investasi tuna. Jakarta (ID): Kementrian Kelautan dan Perikanan.

Komala, A. H., Suptijah, P., & Nurhayati, T. (2015). Ekstraksi dan karakterisasi kolagen dari kulit ikan tongkol (*Euthynnus affinis*). Bogor: Institut Pertanian Bogor.

Kong, J., & Yu, S. (2007). Fourier transform infrared spectroscopic analysis of protein secondary structures. *Acta Biochimica et Biophysica Sinica*, 39(8):549-559.

Kusumaningtyas, E., Nurilmala, M., & Sibarani, D. (2019). Antioxidant and antifungal activities of collagen hydrolysates from skin of milkfish (*Chanos chanos*) hydrolyzed using various *Bacillus proteases*. IOP Conference Series: *Earth and Environmental Science*, 278(012040):1-7.

Laemmli. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 228:726-734.

Li-Chan, E. C. Y. (2015). Bioactive peptides and protein hydrolysates: Research trends and challenges for application as nutraceuticals and functional food ingredients. *Current Opinion in Food Science*, 1(1):28-37.

Lopera, L. M. S., Sepúlveda, C. T. R., Vásquez, M. P., Figueroa, O. A. M., & Zapata, J. E. M. (2018). By-products of aquaculture processes: Development and prospective uses. Review. *Vitae*, 25(3):128-140.

Mahmoodani, F., Ghassem, M., Babji, A. S., Yusop, S. M., & Khosrokhavar, R. (2014). ACE inhibitory activity of pangasius catfish (*Pangasius sutchi*) skin and bone gelatin hydrolysate. *Journal of Food Science and Technology*, 51(9):1847-1856.

Manoharan, S., Shuib, A. S., Abdullah, N., Mohamad, S. Bin, & Aminudin, N. (2017). Characterisation of novel angiotensin-I-converting enzyme inhibitory tripeptide, Gly-Val-Arg derived from mycelium of *Pleurotus pulmonarius*. *Process Biochemistry*, 62(07):215-222.

Miguel, M., Aleixandre, M. A., Ramos, M., & López-Fandiño, R. (2006). Effect of simulated gastrointestinal digestion on the antihypertensive properties of ACE-inhibitory peptides derived from ovalbumin. *Journal of Agricultural and Food Chemistry*, 54(3):726-731.

Mutaminah, D., Ibrahim, B., & Trilaksani, W. (2018). Aktivitas antioksidan hidrolisat protein mata ikan tuna (*Thunnus* sp.) dengan hidrolisis enzimatik. Bogor: Institut Pertanian Bogor.

Naik, A. S., Whitaker, R. D., Albrektsen, S., Solstad, R. G., Thoresen, L., & Hayes, M. (2021). Mesopelagic fish protein hydrolysates and extracts: a source of novel anti-hypertensive and anti-diabetic peptides. *Frontiers in Marine Science*, 8(09):1-9.

Nilsuwan, K., Chantakun, K., Chotphruethipong, L., Benjakul, S. (2021). Development of hydrolysis and defatting processes for production of lowered fishy odor hydrolyzed collagen from fatty skin of sockeye salmon (*Oncorhynchus nerka*). *Foods*, 10(10):1-16.

Nurilmala, M., Hizbullah, H. H., Karnia, E., Kusumaningtyas, E., & Ochiai, Y. (2020). Characterization and antioxidant activity of collagen, gelatin, and the derived peptides from yellowfin tuna (*Thunnus albacares*) skin. *Marine Drugs*, 18(2):1-12.

Nurilmala, M., Pertiwi, R. M., Nurhayati, T., Fauzi, S., Batubara, I., & Ochiai, Y. (2019). Characterization of collagen and its hydrolysate from yellowfin tuna *Thunnus albacares* skin and their potencies as antioxidant and antiglycation agents. *Fisheries Science*, 85(3):591-599.

Nurjanah, Baharuddin, T. I., & Nurhayati, T. (2021). Ekstraksi kolagen kulit ikan tuna sirip kuni (*Thunnus albacares*) menggunakan enzim pepsin dan papain. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 24(2):174-187.

Oyaizu, M. (1988). Antioxidative activities of browning products of glucosamine fractionated by organic solvent and thin-layer chromatography. *Nippon Shokuhin Kogyo Gakkaishi*, 35:771-775.

Prastyo, D.T. (2021). Aktivitas antioksidan dan inhibitor enzim pengubah angiotensin (ACE) hidrolisat kolagen kulit ikan nila (*Oreochromis niloticus*). Bogor: Institut Pertanian Bogor.

Prastyo, D. T., Trilaksani, W., & Nurjanah. (2020). Aktivitas antioksidan hidrolisat klagen kulit ikan nila (*Oreochromis niloticus*). *Jurnal Pengolahan Hasil Perikanan Indonesia*, 23(3):423-433.

Pezeshk, S., Ojagh, S. M., Rezaei, M., & Shabanpour, B. (2019). Fractionation of protein hydrolysates of fish waste using membrane ultrafiltration: investigation of antibacterial and antioxidant activities. *Probiotics and Antimicrobial Proteins*, 11(3):1015-1022.

Porto, H. L. R., de Castro, A. C. L., Filho, V. E. M., & Rádis-Baptista, G. (2016). Evaluation of the chemical composition of fish species captured in the lower stretch of Itapecuru river, Maranhão, Brazil. *International Journal of Advances in Agricultural and Environmental Engineering*, 3(1):181-186.

Qiu, Y. T., Wang, Y. M., Yang, X. R., Zhao, Y. Q., Chi, C. F., & Wang, B. (2019). Gelatin and antioxidant peptides from gelatin hydrolysate of skipjack tuna (*Katsuwonus pelamis*) scales: Preparation, identification and activity evaluation. *Marine Drugs*, 17(10):1-16.

Ramadhan, W., Santoso, J., & Trilaksani, W. (2014). Pengaruh defatting, frekuensi pencucian dan jenis dryoprotectant terhadap mutu tepung surimi ikan lele kering beku. *Jurnal Teknologi dan Industri Pangan*, 25(1):47-56.

Rasli, H. I., & Sarbon, N. M. (2019). Preparation and physicochemical characterization of fish skin gelatine hydrolysate from shortfin scad (*Decapterus macrosoma*). *International Food Research Journal*, 26(1):287-294.

Rothwell, P. A. H., Khatib, N., Sharkey, S., Lafferty, R. A., Gite, S., Whooley, J., O'harte, F. P. M., & Fitzgerald, R. J. (2021). Physicochemical, nutritional and in vitro antidiabetic characterisation of blue whiting (*Micromesistius poutassou*) protein hydrolysates. *Marine Drugs*, 19(7)1-16.

Sae-leaw, T., Benjakul, S., & O'Brien, N. M. (2016). Effects of defatting and tannic acid incorporation during extraction on properties and fishy odour of gelatin from seabass skin. *LWT – Food Science and Technology*, 65:661-667.

Sánchez-Zapata, E., Amensour, M., Oliver, R., Fuentes-Zaragoza, E., Fernández-López, J., Sendra, E., Sayas, E., & Pérez-alvarez, J. A. (2011). Quality characteristics of dark muscle from yellowfin tuna (*Thunnus albacares*) to its potential application in the food industry. *Food and Nutrition Sciences*, 2011(January):22-30.

Schmidt, M. M., Da Fontoura, A. M., Vidal, A. R., Dornelles, R. C. P., Kubota, E. H., Mello, R. de O., Cansian, R. L., Demiate, I. M., & De Oliveira, C. S. (2020). Characterization of hydrolysates of collagen from mechanically separated chicken meat residue. *Food Science and Technology*, 40(Suppl. 1):355-362.

Shu, G., Huang, J., Bao, C., Meng, J., Chen, H., & Cao, J. (2018). Effect of different proteases on the degree of hydrolysis and angiotensin I-converting enzyme-inhibitory activity in goat and cow milk. *Biomolecules*, 8(4)1-8.

Suetsuna, K., Ukeda, H., & Ochi, H. (2000). Isolation and characterization of free radical scavenging activities peptides derived from casein. *Journal of Nutritional Biochemistry*, 11(3):128-131.

Saraswati Indo Genetech Laboratory. (SIG). (2021). Metode uji asam amino (UPLC). Bogor: Saraswati Indo Genetech Laboratory.

Songchotikunpan, P., Tattiyakul, J., & Supaphol, P. (2008). Extraction and electrospinning of gelatin from fish skin. *International Journal of Biological Macromolecules*, 42(3):247-255.

Stämpfli, R., Brühwiler, P., Mourad, S., Verdejo, R., & Shaffer, M. (2007). Development and characterisation of carbon nanotube-reinforced polyurethane foams. *Original Contribution*, 26(2007):51.

Suseno, H. S. (2015). Proximate, fatty acid, and mineral composition of tuna (*Thunnus* sp.) by-product from West Sumatra Province, Indonesia. *Pakistan Journal of Nutrition*, 14(1):62-66.

Tkaczewska, J., Borawska-Dziadkiewicz, J., Kulawik, P., Duda, I., Morawska, M., & Mickowska, B. (2020). The effects of hydrolysis condition on the antioxidant activity of protein hydrolysate from *Cyprinus carpio* skin gelatin. *Food Science and Technology*, 117:108616.

Venuste, M., Zhang, X., Shoemaker, C. F., Karangwa, E., Abbas, S., & Kamdem, P. E. (2013). Influence of enzymatic hydrolysis and enzyme type on the nutritional and antioxidant properties of pumpkin meal hydrolysates. *Food and Function*, 4(5):811-820.

Vijaykrishnaraj, M., Roopa, B. S., & Prabhasankar, P. (2016). Preparation of gluten free bread enriched with green mussel (*Perna canaliculus*) protein hydrolysates and characterization of peptides responsible for mussel flavour. *Food Chemistry*, 211:715-725.

Wiriyaphan, C., Chitsomboon, B., & Yongsawadigul, J. (2012). Antioxidant activity of protein hydrolysates derived from threadfin bream surimi byproducts. *Food Chemistry*, 132(1):104-111.

Witono, Y., Fauziah, R. R., Windrati, W. S., Taruna, I., Azkiyah, L., & Wijayanti, R. P. (2019). Formulation of flavor enhancer from common barb (*Rasbora jacobsoni)* protein hydrolysate. *AIP Conference Proceedings*, 2199(1):1-11.

Wu, H. C., Chen, H. M., & Shiau, C. Y. (2003). Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). *Food Research In-*

ternational, 36(9–10):949-957.

Xu, S., Yang, H., Shen, L., & Li, G. (2017). Purity and yield of collagen extracted from southern catfish (*Silurus meridionalis* Chen) skin through improved pretreatment methods. *International Journal of Food Properties*, 20(sup1):S141-S153.

Yaghoubzadeh, Z., Peyravii Ghadikolaii, F., Kaboosi, H., Safari, R., & Fattahi, E. (2020). Antioxidant activity and anticancer effect of bioactive peptides from rainbow trout (*Oncorhynchus mykiss*) skin hydrolysate. *International Journal of Peptide Research and Therapeutics*, 26(1):625-632.