Antioxidant Properties of Tilapia Skin Collagen Peptides

Nuttapong Jantaratch, Wanchai Worawattanamateekul and Jirapa Hinsui*

Department of Fishery Products, Faculty of Fisheries, Kasetsart University, Bangkok 10900

Received: 21 May 2022/ Revised: 10 June 2022/ Accepted: 14 June 2022

Abstract

Tilapia processing wastes (skin, bone and scale) can be utilized to reduce waste and environmental problems. Tilapia skin mostly contain of collagen and suitable for collagen hydrolysate extraction. The objective of this research was to study relationship between molecular weight and amino acids on antioxidant properties of tilapia skin collagen peptides. Tilapia skin collagen peptides was isolated by ultrafiltration (1, 5 and 10 kDa). Soluble proteins, antioxidants activities and amino acids composition were analyzed. The extracted collagen hydrolysate had high antioxidant activity of ABTS and FRAP. There are 3 group of ultrafiltration peptide fractions by molecular weight: <1kDa, 1-5kDa and >10kDa. The <1kDa peptide fraction had the highest antioxidant activity (ABTS and FRAP). Molecular weight of peptide fraction influenced on antioxidant properties more than amino acid composition. Tilapia skin collagen hydrolysate, was fishery processing by-product, contributed to antioxidant activity and useful in the development of dietary supplement.

Keywords: ABTS, antioxidants peptides, fish skin, tuna pepsin, ultrafiltration

Corresponding: jirapa_h@hotmail.com

Introduction

Tilapia (*Oreochromis niloticus*) is a freshwater fish that has been of economic importance since 1965. In 2020, Thailand exported 185.5 tons of frozen tilapia fillets and 0.5 tons of chilled tilapia fillets [1]. The processing of fillets produces large amounts of waste, including the head (33%), bones (16%), intestines (8%), and scales and skin (7%). These wastes are often disposed of in landfills, causing environmental problems, including ground water pollution and toxic gas emissions [2]. The waste can be used to make animal feed, but the resulting product has low value [3]. Tilapia skin contains high amounts of collagen and can be used as raw material to produce collagen [4], gelatin [5] and collagen hydrolysate [6].

Collagen hydrolysate can be extracted from tilapia skin using thermal hydrolysis or enzymatic proteolysis. Enzymatic proteolysis takes less time than thermal hydrolysis and produces hydrolysate with a higher antioxidant activity and smaller peptides than from thermal hydrolysis [8]. Enzymatic extraction is a potential method for extracting collagen hydrolysate. To maximize the collagen yield and improve extraction efficiency, several enzymes are used under optimal conditions to increase the solubilization of collagen in the acidic medium [7]. Commercial proteases and protease from microorganism (*Bacillus licheniformis* and *Vibrio* sp.) were used for extracted collagen hydrolysate from porcine [9], common carp fish [10], jumbo squid [11] and salmon skin [12, 13], resulting in high antioxidant activity. Tuna pepsin was used to produce fish protein hydrolysate with high levels of antioxidant activity [14]. Tuna protease can be used to extract collagen hydrolysate from tilapia skin. This collagen hydrolysate is as effective as commercial porcine pepsin [15]. The biologically active peptide properties, antimicrobial and antioxidant properties, depend on the type of amino acids and the molecular size of the peptide chain [16, 17]. Collagen hydrolysates, consist of various molecular size of peptide chain, were separated by ultrafiltration. Therefore, separation of the hydrolysate by ultrafiltration can be used to increase the biologically active peptide properties. Small peptide fraction of tilapia skin [18], yellowfin tuna skin [19], salmon skin [12], squid [20], and fish by-products [13] collagen hydrolysate produced the highest antioxidant activity. Tilapia collagen hydrolysate consists of amino acid in peptide chain that may affect to antioxidant properties. The purpose of the current research was to study the relationship between molecular weight and amino acids on the antioxidant properties of tilapia skin collagen peptides.

Materials and Methods

Reagents and raw material preparation

Skins from tilapia (*Oreochromis niloticus*) were obtained from fillet processing waste from Mankit Mankhong Co., Ltd., Samut Prakan province, Thailand. Stomachs from tuna (*Katsuwonus pelamis*) were provided by Thai Union Group PCL, Samut Sakhon, Thailand. All samples were packed in polyester bags and kept on ice in an insulated box to maintain the temperature below 10 °C and transported to the Department of Fishery Products, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand within 2 hr, where they were stored at -20 °C until further experimentation.

Preparation of tilapia skin

The tilapia skin was pre-treated by removing blood, meat, mucus, lipids, non-collagen substances, and fishy odor according to Tohmadlae, Worawattanamateekul, & Hinsui [21]. The tilapia skin sample was progressively soaked in 1.5% sodium chloride solution for 1 hr, 0.2% sodium hydroxide solution for 2 hr, 0.2% sulfuric acid solution for 2 hr, and then 1% citric acid solution for 2 hr (all at a skin-to-solution ratio of 1:4). After chemical pre-treatment, the tilapia skin sample was rinsed with water until the pH was neutral (pH 7) and then mixed in a blender for 2 min.

Extraction of crude enzymes from tuna stomachs

The tuna stomach sample was homogenized for tuna protease extraction according to Tohmadlae et al. [15]. The sample was blended with a sodium phosphate buffer solution at pH 7 at a ratio of 1:3 (w/v), stirred for 3 hr at 4 °C, and then centrifuged at 10,000×g for 30 min at 4 °C. The supernatant was collected and activated with 2 M hydrochloric acid at a ratio of 1:1 (v/v) at 4 °C for 30 min. The protein concentration of the crude enzyme was analyzed according to Lowry et al. [22] and Peterson [23] using bovine serum albumin (BSA) as a standard. Enzyme activity was measured according to Nalinanon et al. [24] and Tohmadlae et al. [15] using hemoglobin as a substrate. The absorbance of the resulting solution was measured at 750 nm using a UV- 1700 spectrophotometer (Shimadzu, Japan). The blank was prepared in the same manner, except that distilled water was used instead of the sample. A standard curve was prepared using tyrosine in the range of 0-1 μM. Pepsin activity was then calculated using the formula:

$$\text{Enzyme activity (unit/ml)} = ([\text{Protein}]_{\text{Sample}} - [\text{Protein}]_{\text{Control}})/(\text{Time of the reaction (min)} \times \text{Volume of enzyme solution (ml)}) \quad (1)$$

Specific activity was then calculated using the following formula:

$$\text{Specific enzyme activity (unit/mg protein)} = \text{enzyme activity (unit/ml)} / \text{soluble protein of enzyme (mg/ml)} \quad (2)$$

Methods

1.Tilapia skin collagen hydrolysate extraction

Tilapia skin collagen hydrolysate was extracted using crude enzyme solution (1:10 (w/v)) [15] at 50 °C for five durations (1, 2, 3, 4 and 5 hr), and then enzyme activity was stopped by heating at 100 °C for 15 min. The mixture was centrifuged at 10,000×g at 4 °C for 30 min. The supernatant was collected and adjusted to pH 7.0, before the water was evaporated to produce collagen hydrolysate (with the same protein concentration as before adjusting the pH) for fractionation and analysis of chemical properties.

2.Fractionation of tilapia skin collagen hydrolysate using ultrafiltration

Collagen hydrolysate was fractionated into peptides based on molecular weight, following a method modified from Charoenphun et al. [25].

Amicon® Stirred Cells were used, along with 10, 5 and 1 kDa molecular weight cut-off (MWCO) membranes and nitrogen gas pressure was set at 50–75 psi. Peptides of different sizes were collected and analyzed for their chemical properties.

3.Chemical properties analysis

3.1. Soluble protein content

The soluble protein content (mg/ml) was analyzed according to Lowry et al. [22] and Peterson [23] using bovine serum albumin (BSA) as a standard.

3.2. Determination of degree of hydrolysis (DH)

The total nitrogen content of the tuna protein hydrolysate was analyzed using the Kjeldahl method [26]. DH of tilapia skin collagen hydrolysate was determined by TCA-solubility index as describe by Rutherfurd [27] according to the method of Tohmadlae et al. [15] and calculated using the formula:

DH (%) = Total nitrogen in tilapia skin collagen hydrolysate × 100 / Total nitrogen in tilapia skin (3)

3.3. Antioxidant activities

Antioxidant activity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (ABTS) and ferric reducing antioxidant power (FRAP) assays following methods modified from Sukkwei et al. [28], Aleman et al. [29], Ketnawa and Liceaga [30], and Wu et al. [31], respectively, with trolox as a positive control. A standard curve was prepared using trolox according to Tohmadlae et al. [15] in the range of 10-50, 25.71-60 and 0-850 μM, respectively. Metal chelating assay was modified from Boyer and McCleary [32], using Ethylene diamine tetraacetic acid (EDTA) as a positive control. A standard curve was prepared using EDTA according to Tohmadlae et al. [15] in the range of 0-150 μM. The blank used was distilled water instead of the sample.

3.4. Determination of molecular weight distribution

Collagen hydrolysate samples were determined for their molecular weight distribution using high-performance liquid chromatography (HPLC) (Agilent 1260 Infinity, USA) equipped with an ultraviolet detector, auto sampler, and vacuum degasser. The samples were loaded onto an Advance Bio SEC column (300 × 4.6 mm, 2.7 μm, Agilent Technologies, Wilmington, DE, USA) and eluted with 150 mM sodium phosphate buffer (pH 7.0) at a flow rate of 0.350 mL/min. The injection (Agilent 1260 Infinity auto sampler, USA) volume was 2 μl and the detection (Agilent 1260 Infinity II Variable Wavelength Detector, USA) wavelength was set at 220 nm. Ovalbumin (44 kDa), approtinin (6.5 kDa), and neurotensin (1.6 kDa) (Advance Bio SEC 130A Protein Standard 4.0 mg, lyophilized 1.5 mL vial) were used as the molecular weight markers.

3.5. Amino acid composition

Collagen hydrolysate and peptides in different size ranges were hydrolyzed in 6M HCl at 110 °C for 24 hr and then analyzed using HPLC (Agilent 1260 Infinity, USA). The samples were loaded onto an Agilent Poroshell column (HPH C18,

4.6 × 100 mm, 2.7 μm, Agilent Technologies, Wilmington, DE, USA) and eluted with the mobile phase A containing 10 mM Na_2HPO_4, 10 mM $Na_2B_4O_7$, and 5 mM NaN_3, with a final pH of 8.2 and the mobile phase B containing acetonitrile: methanol: water (45:45:10, v:v:v) at a flow rate of 1.5 ml/min. The injection (Agilent 1260 Infinity autosampler, USA) volume was 1 μl and the detection (Agilent 1260 Infinity II Variable Wavelength Detector, USA) wavelengths were set at 230 nm and 450 nm. Determination of amino acids followed AOAC [33].

4.Statistical Analysis

A completely randomized design (CRD) was used with three replications. Data were presented as mean ± standard deviation (SD) values. Means among treatments were compared based on Duncan's multiple range test and analysis of variance (ANOVA) was tested at a statistical significance of 95%.

Results and Discussion

The tuna protease activity and specific enzyme activity were 0.0475±0.0002 units/ml and 0.0010±0.0000 units/mg protein, respectively. Even though both these values were lower than for skipjack protease activity of 3.42±1.008 units/ml and specific enzyme activity of 0.0983±0.61 units/mg protein [15], the extraction time patterns were similar. Table 1 shows that the DH of tilapia skin collagen hydrolysate was not significantly different for extraction times in the range 1–5 hr; thus, this extraction time range was suitable for producing tilapia skin collagen hydrolysate. The collagen hydrolysate solutions were not gelatinous when refrigerated at 4 °C, in contrast to the control (collagen hydrolysate at 0 hr), which was gelatinous at 4 °C.

Table 1 Antioxidant activities of tilapia skin collagen hydrolysate using 5 extraction durations.

Extraction time (hr)	DH (%)	DPPH (μmol TE/mg protein)	ABTS (μmol TE/mg protein)	FRAP (μmol TE/mg protein)	Metal Chelating (mM EDTA/mg protein)
1	72.23 ±4.42^{ns}	2,171.69 ±118.26^{a}	5,215.43 ±698.12^{c}	758.54 ±46.64^{a}	44.10 ±0.49^{a}
2	72.23 ±2.21^{ns}	1,944.97 ±287.86^{ab}	5,676.96 ±407.21^{bc}	702.83 ±27.12^{a}	45.03 ±0.88^{a}
3	73.50 ±3.38^{ns}	2,076.96 ±209.25^{a}	6,751.81 ±293.66^{a}	703.24 ±16.89^{a}	45.17 ±0.77^{a}
4	72.23 ±5.58^{ns}	2,105.58 ±179.60^{a}	4,285.51 ±567.31^{d}	610.80 ±44.45^{b}	44.13 ±1.41^{a}
5	72.87 ±2.92^{ns}	1,763.69 ±61.66^{b}	6,199.71 ±461.78^{ab}	533.13 ±13.43^{c}	39.56 ±1.12^{b}

* Values presented as mean ± SD from triplicate determinations. Different lowercase superscript letters (a–d) in same column indicate significant ($p<0.05$) differences between means; ns indicates no significant ($p>0.05$) difference in column.

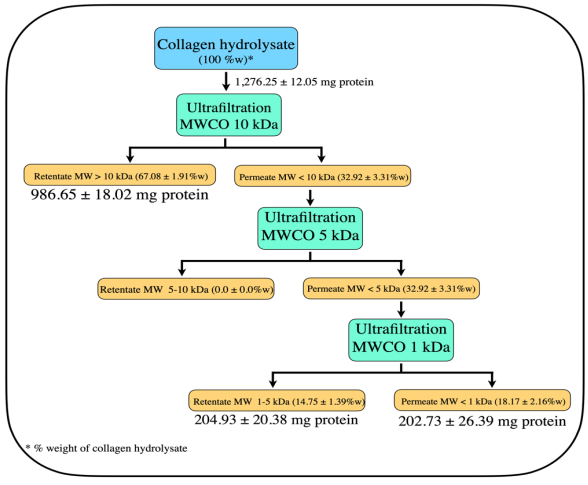

Figure 1 Fractionation ratio of tilapia skin collagen hydrolysate using ultrafiltration.

Table 1 presents data on the collagen hydrolysate extracted at 1–5 hr. The levels for metal chelating, FRAP, and DPPH scavenging capacity for 1–3 hr in the extracted collagen hydrolysate were not significantly different. The hydrolysate after 3 hr extraction having the highest ABTS activity (6,751.81 ± 293.66 µmol TE/mg protein), which was similar to the values for tilapia skin collagen hydrolysate in another study [15], for jumbo squid collagen hydrolysate [19] and for white jellyfish collagen hydrolysate [34]. Based on the current study results, the extraction time of 3 hr for tilapia skin collagen hydrolysate was considered optimal.

Fractionated peptides were isolated from collagen hydrolysate after 3 hr extraction and the resulting ratios are shown in Figure 1. The yield of peptides > 10 kDa represented 67.08% of the total, peptides 1–5 kDa were 14.75%, and peptides < 1 kDa were 18.17%. Soluble protein contents were 986.65 ± 18.02 mg/ml for > 10kDa, 204.93 ± 20.38 mg/ml for 1–5 kDa and 202.73 ± 26.39 mg/ml for < 1 kDa. The <1 kDa fraction had the highest levels of antioxidant capacity (ABTS, FRAP, metal chelating, and DPPH), similar to yellowfin skin collagen hydrolysate [20], tilapia skin [18] and fish by-products collagen hydrolysate [13]. The antioxidant capacity of the fractionated peptides decreased compared with the crude collagen hydrolysate, similar to porcine peptide fractions [9]. This may have been due to a decrease in the protein concentration [10, 13].

Extracted tilapia skin collagen hydrolysate contained glutamic acid (449.07 mg/100g), glycine (425.50 mg/100g), and proline (282.29 mg/100g) as the main amino acids. As well as hydrophobic amino acids (955.70 mg/100g) and hydrophilic amino acids (267.68 mg/100g), as shown in Table 3. ABTS radicals can react with hydrophilic and hydrophobic compounds, while the DPPH assay is only suitable for hydrophobic compounds [20, 35]. Tilapia skin collagen hydrolysate contains hydrophilic and hydrophobic amino acids, resulting in high ABTS antioxidant activity. Hydrophilic and hydrophobic amino acids play an important role in antioxidant activity as hydrogen donors [12, 36-39]. Tilapia skin collagen hydrolysate contains acidic amino acids (aspartic and glutamic) and basic amino acids (histidine, lysine, and arginine) that play important roles in metal chelating ability [40].

Table 2 Antioxidant activities of peptide fractions from tilapia skin collagen hydrolysate.

Peptide size	DPPH (μmol TE/mg protein)	ABTS (μmol TE/mg protein)	FRAP (μmol TE/mg protein)	Metal Chelating (mM EDTA/mg protein)
<1 kDa	10.92 ± 0.84 [a]	3,178.22 ± 11.85 [a]	564.61 ± 17.87 [a]	151.48 ± 1.58 [a]
1-5 kDa	8.28 ± 0.22 [b]	2,830.58 ± 58.09 [b]	454.88 ± 6.54 [b]	119.00 ± 3.23 [c]
>10 kDa	8.14 ± 0.45 [b]	2,639.57 ± 23.46 [c]	466.62 ± 3.69 [b]	134.13 ± 1.51 [b]

* Values presented as mean ± SD from triplicate determinations. Different lowercase superscript letters (a–d) in same column indicate significant ($p<0.05$) differences between means.

From the current study on the relationship between molecular weight and amino acids on the antioxidant properties of tilapia skin collagen hydrolysate, it was found that the molecular weight of the peptide fraction influenced on the antioxidant properties more than the amino acid composition. The antioxidant capacity of the small peptide fraction (<1kDa) increased. Peptides with a low concentration of hydrophilic and hydrophobic amino acids that contribute to antioxidant capacity. While longer peptides with a high amino acid content that cannot unfold and expose their amino acid sequences to exert their bioactivity [20]. Indicating that the antioxidant properties depended on the molecular weight.

Table 3 Amino acid composition of tilapia skin collagen hydrolysate and fraction peptides.

amino acid profiles (mg/100g)	collagen hydrolysate	Peptide fractions		
		>10 kDa	1-5 kDa	<1 kDa
Alanine**	267.68	170.62	39.51	48.64
Aspartic acid*	261.72	165.29	37.25	-
Glutamic acid*	449.07	287.25	66.58	81.60
Glycine	425.50	293.90	67.42	77.31
Leucine**	177.57	108.37	24.39	32.26
Lysine*	221.17	137.71	32.23	40.19
Proline**	282.29	163.47	39.73	51.29
Valine**	131.46	75.01	17.07	23.89
Total	2216.46	1401.63	324.19	355.18

Table 3 (cont.)

amino acid profiles (mg/100g)	collagen hydrolysate	Peptide fractions		
		>10 kDa	1-5 kDa	<1 kDa
Hydrophilic amino acids	267.68	170.62	39.51	48.64
Hydrophobic amino acids	955.70	614.99	141.11	173.65

* Hydrophilic amino acids

** Hydrophobic amino acids

Conclusions

Tilapia skin collagen hydrolysate had high levels of ABTS and FRAP antioxidant activity. The peptide fraction <1 kDa derived from this collagen hydrolysate had the highest antioxidant activity, mainly in terms of the ABTS antioxidant activity. Thus, tilapia skin collagen hydrolysate could be considered an excellent source of antioxidants, making it possible to utilize this waste more effectively and to add value to tilapia and tuna resources. The further studies are needed to isolate small peptide (<1 kDa) to apply for food, dietary supplement and pharmaceuticals.

References

1. Nurit K. Situation of tilapia products and products in 2020. Available from: https://www.fisheries.go.th/strategy/fisheconomic/Monthly% 20report/tilapia/สถานการณ์ปลานิล%20Q4%2063.pdf.
2. Sarker S. By-products of fish-oil refinery as potential substrates for biogas production in Norway: A preliminary study. Results in Engineering 2020;6:100137.
3. Goldhor S, Regenstein J. Handbook of Waste Management and Co-Product Recovery in Food Processing. Cornell University: Woodhead Publishing; 2007.
4. Bi C, Li X, Xin Q, Han W, Shi C, Guo R, et al. Effect of extraction methods on the preparation of electrospun/electrosprayed microstructures of tilapia skin collagen. J Biosci Bioeng 2019;128 (2):234-40.
5. Santos JP, Esquerdo VM, Moura CM, Pinto LAA. Crosslinking agents effect on gelatins from carp and tilapia skins and in their biopolymeric films. Colloids Surf A: Physicochem Eng Asp 2018;539: 184-91.
6. Lin S, Hu X, Li L, Yang X, Chen S, Wu Y, et al. Preparation, purification and identification of iron-chelating peptides derived from tilapia (Oreochromis niloticus) skin collagen and characterization of the peptide-iron complexes. LWT 2021;149:111796.
7. Ahmed M, Verma AK, Patel R. Collagen extraction and recent biological activities of collagen peptides derived from sea-food waste: A review. Sustain Chem Pharm 2020;18:100315.
8. Wang W, Li Z, Liu J, Wang Y, Liu S, Sun M. Comparison between thermal hydrolysis and enzymatic proteolysis processes for the preparation of tilapia skin. Czech J Food Sci 2013;31:1-4.
9. Hong G-P, Min S-G, Jo Y-J. Anti-Oxidative and Anti-Aging Activities of Porcine By-Product Collagen Hydrolysates Produced by Commercial Proteases: Effect of Hydrolysis and Ultrafiltration. Molecules 2019;24(6):1104.

10. González-Serrano DJ, Hadidi M, Varcheh M, Jelyani AZ, Moreno A, Lorenzo JM. Bioactive Peptide Fractions from Collagen Hydrolysate of Common Carp Fish Byproduct: Antioxidant and Functional Properties. Antioxidants 2022;11(3)509.
11. Cuevas-Acuña DA, Robles-Sanchez RM, Torres-Arreola W, Marquez-Rios E, Ezquerra-Brauer J-M. Collagen from jumbo squid fin: extracting conditions and influence of the protease system on collagen hydrolysate antioxidant activity. CYTA J Food 2016;14(2):193-9.
12. Wu R, Wu C, Liu D, Yang X, Huang J, Zhang J, et al. Antioxidant and anti-freezing peptides from salmon collagen hydrolysate prepared by bacterial extracellular protease. Food Chem 2018;248:346-52.
13. Zamorano-Apodaca JC, García-Sifuentes CO, Carvajal-Millán E, Vallejo-Galland B, Scheuren-Acevedo SM, Lugo-Sánchez ME. Biological and functional properties of peptide fractions obtained from collagen hydrolysate derived from mixed by-products of different fish species. Food Chem. 2020;331:127350.
14. Taheri A, Bakhshizadeh G A. Antioxidant and ACE Inhibitory Activities of Kawakawa (Euthynnus affinis) Protein Hydrolysate Produced by Skipjack Tuna Pepsin. J Aquat Food Prod Technol. 2020;29(2):148-66.
15. Tohmadlae P, Worawattanamateekul W, Hinsui J. Characterization of Acidic Tuna Protease and Its Application for Extraction of Tilapia Collagen Hydrolysate. Chiang Mai Univ J Nat Sci 2020;19(2):206-21.
16. Korhonen H, Pihlanto A. Bioactive peptides: Production and functionality. Int Dairy J 2006;16(9):945-60.
17. Chew LY, Toh GT, Ismail A. Enzymes in Food Biotechnology. Selangor Darul Ehsan: Academic Press; 2019.
18. Liang Q, Wang L, He Y, Wang Z, Xu J, Ma H. Hydrolysis kinetics and antioxidant activity of collagen under simulated gastrointestinal digestion. J Funct Foods 2014;11:493-9.
19. Nurilmala M, Hizbullah HH, Karnia E, Kusumaningtyas E, Ochiai Y. Characterization and Antioxidant Activity of Collagen, Gelatin, and the Derived Peptides from Yellowfin Tuna (Thunnus albacares) Skin. Mar Drugs 2020;18(2):98.
20. Suarez-Jimenez GM, Burgos-Hernandez A, Torres-Arreola W, Lopez-Saiz CM, Contreras CAV, Ezquerra-Brauer JM. Bioactive peptides from collagen hydrolysates from squid (Dosidicus gigas) by-products fractionated by ultrafiltration. Int J Food Sci Technol 2019; 54(4):1054-61.
21. Tohmadlae P, Worawattanamateekul W, Hinsui J. Tilapia Gelatin : Elimination of Fishy Odor. RMUTSV Res J 2019;11(3):402-11.
22. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193(1):265-75.
23. Peterson GL. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal Biochem 1977;83 (2):346-56.

24. Nalinanon S, Benjakul S, Visessanguan W, Kishimura H. Partitioning of protease from stomach of albacore tuna (Thunnus alalunga) by aqueous two-phase systems. Process Biochem 2009;44:471-6.
25. Charoenphun N, Cheirsilp B, Sirinupong N, Youravong W. Calcium-binding peptides derived from tilapia (Oreochromis niloticus) protein hydrolysate. Eur Food Res Technol 2013;236(1):57-63.
26. Nielsen SS. Food analysis laboratory manual. 2nd ed. West Lafayette: Springer; 2010.
27. Rutherfurd SM. Methodology for Determining Degree of Hydrolysis of Proteins in Hydrolysates: A Review. J AOAC Int 2010;93(5):1515-22.
28. Sukkwai S, Kijroongrojana K, Benjakul S. Extraction of gelatin from bigeye snapper (Priacanthus tayenus) skin for gelatin hydrolysate production. Int Food Res J 2011;18(3):1129-34.
29. Alemán Pérez A, Gimenez B, Montero P, Gomez-Guillen M. Antioxidant activity of several marine skin gelatins. LWT 2011;44(2):407-13.
30. Ketnawa S, Liceaga AM. Effect of Microwave Treatments on Antioxidant Activity and Antigenicity of Fish Frame Protein Hydrolysates. Food Bioproc Tech 2017;10(3):582-91.
31. Wu H-C, Chen H-M, Shiau C-Y. Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (Scomber austriasicus). Food Res Int 2003;36(9):949-57.
32. Boyer RF, J. McCleary C. Superoxide ion as a primary reductant in ascorbate-mediated ferretin iron release. Free Radic Biol Med 1987;3(6):389-95.
33. Szkudzińska K, Smutniak I, Rubaj J, Korol W, Bielecka G. Method validation for determination of amino acids in feed by UPLC. Accred Qual Assur 2017;22:247–252.
34. Sahaworarak R, Worawattanamateekul W, Hinsui J. Extraction and Characterization of Collagen from White Jellyfish (Lobonema smithii). RMUTSV Res J 2016;8(1):112-124.
35. Dash P, Ghosh G. Amino acid composition, antioxidant and functional properties of protein hydrolysates from Cucurbitaceae seeds. J Food Sci Technol 2017;54(13):4162-72.
36. Levine RL, Mosoni L, Berlett BS, Stadtman ER. Methionine residues as endogenous antioxidants in proteins. Proc Natl Acad Sci U.S.A. 1996;93(26):15036-40.
37. Ao J, Li B. Amino acid composition and antioxidant activities of hydrolysates and peptide fractions from porcine collagen. Food Sci Technol Int. 2012;18(5):425-34.
38. Chen H-M, Muramoto K, Yamauchi F, Nokihara K. Antioxidant Activity of Designed Peptides Based on the Antioxidative Peptide Isolated from Digests of a Soybean Protein. J Agric Food Chem 1996;44(9):2619-23.
39. Weng W, Tang L, Wang B, Chen J, Su W, Osako K, et al. Antioxidant properties of fractions isolated from blue shark (Prionace glauca) skin gelatin hydrolysates. J Funct Foods 2014;11:342-51.
40. Saiga A, Tanabe S, Nishimura T. Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. J Agric Food Chem 2003;51(12):3661-7.