Available online: journal.ipb.ac.id/index.php/jphpi

ANTIOXIDANT ACTIVITY OF PROTEIN HYDROLYSATE PRODUCED FROM TUNA EYE (*Thunnus* sp.) BY ENZYMATIC HYDROLYSIS

Dewi Mutamimah*, Bustami Ibrahim, Wini Trilaksani
Department of Aquatic Product Technology,
Fisheries and Marine Science Faculty, Bogor Agricultural University
Dramaga IPB Campus, Agatis street, Bogor 16680, Indonesia
Phone (0251) 8622909-8622906, Fax (0251) 8622915
*Correspondence: dewimut91@gmail.com
Diterima: 3 Maret 2018/ Disetujui: 22 Desember 2018

Cara sitasi: Mutamimah D, Ibrahim B, Trilaksani W. 2018. Antioxidant activity of protein hydrolysate produced from tuna eye (*Thunnus* sp.) by enzymatic hydrolysis. *Jurnal Pengolahan Hasil Perikanan Indonesia*. 21(3): 522-531.

Abstract

Tuna (*Thunnus* sp.) by-products from frozen loin and canning industry especially the eye is rich in proteins and in lipids consisting of polyunsaturated fatty acids (PUFA). That requires protective agent (antioxidant) to inhibit the oxidation naturally present and predicted to be protein peptides. Enzymatic hydrolysis of protein is an appropriate method to produce bioactive peptide with such nutraceutical/ pharmaceutical function such as an antioxidant peptide. This study aimed to produce protein hydrolysate having a function as anwith an antioxidant activity from eye of tuna through enzymatic hydrolysis and determining the antioxidant activity by DPPH methods. Protein soluble content of tuna's eye protein hydrolysate (TEPH) ranged from 59.98±0.130 to 94.90±0.002%. The degree of hydrolysis (DH) of TEPH was about 9.10±0.28 to 16.14±0.09%. The highest inhibition of DPPH radical scavenging activity was 93.57±0.05% (at 5 mg/mL) was obtained with a DH of 11.35±0.002% at the concentration 0.1% of papain for 6 hours hydrolysis. The IC50 value of was 1.08±0.008 mg/mL

Keywords: Antioxidant activity, enzymatic hydrolysis, tuna eye protein hydrolysate.

Aktivitas Antioksidan Hidrolisat Protein Mata Ikan Tuna (Thunnus sp.) dengan Hidrolisis Enzimatik

Abstrak

Hasil samping tuna dari bahan baku industri loin beku dan pengalengan terutama pada bagian mata kaya akan protein dan lipid yang terdiri dari *polyunsaturated fatty acids* (PUFA) yang memerlukan perlindungan terhadap oksidasi membutuhkan zat pelindung (antioksidan) untuk menghambat oksidasi secara alami dan diduga yaitu peptida protein. Hidrolisis enzimatik protein merupakan metode yang tepat untuk menghasilkan peptida bioaktif yang memiliki fungsi nutraseutika-farmasi seperti peptida antioksidan. Penelitian ini bertujuan untuk menghasilkan hidrolisat protein sebagai antioksidan dari mata tuna melalui hidrolisis enzimatik dan menentukan aktivitas antioksidan dengan metode DPPH. Kandungan protein terlarut pada hidrolisat protein mata tuna (HPMT) berkisar antara 59,98±0,130 hingga 94,90±0,002%. Derajat hidrolisis (DH) TEPH adalah sekitar 9,10±0,28 hingga 16,14±0,09%. Aktivitas Ppenghambatan tertinggi radikal DPPH (93,57±0,05% pada 5 mg / mL) diperoleh dengan DH 11,35±0,002% pada konsentrasi 0,1% papain selama 6 jam hidrolisis. Nilai IC50 1,08±0,008 mg/mL.

Kata kunci : Aktivitas antioksidan, hidrolisis enzim, hidrolisat protein mata ikan tuna.

INTRODUCTION

Fisheries industry is one of the reliable economic sources in some countries. Indonesia's fisheries industry such as tuna industry generate large amount of waste or by-product (head, skin, fin, frame, tail, and viscera). The abundant by-product of tuna, based on the total catch in 2016 reached 132.691 ton (Ruchimat *et al.* 2017). These by-product rich in nutrition value has not been optimally utilized yet, discarded without any treatment therefore potentially causing pollution to the environment. Tuna eye, a part of tuna head has high economic value, due to the high lipid and protein content. Vijaykumar (2016) obtained PUFA of tuna eye (EPA and DHA) about 7.07% and 36.72% respectively and protein content according to Sandria (2014) was 19.49%. Long chain omega 3 (EPA and DHA) in lipids are protected by antioxidants from oxidation naturally prior to external treatment. One of the antioxidants that work as a protective PUFA predicted to be peptide. A number of studies on protein hydrolysates showed that peptides derived from fish protein are potential sources of antioxidants.

Some antioxidant peptides had already been identified from several by-product sources such as head (Bougatef *et al.* 2012), liver (Young-je *et al.* 2009), backbone (Slizyte *et al.* 2016), dark meat (Saidi *et al.* 2014), frame (Kim *et al.* 2007), skin and viscera (Zhong *et al.* 2011). Yang *et al.* (2011) reported, on the antioxidant activity of tuna bigeye protein hydrolysate at 1.34 mg/mL. Food protein have been known for their nutritional and functional properties related to amino acids content that coincides with the physiological utilization of specific amino acids on digestion and absorption. In addition, functional properties of protein associated with the contribution to the physicochemical and sensory properties of the food (Sila and Bougatef 2016).

It plays single role in escalating human health beyond its nutritional value. Several studies in the past two decades has been reported concerning about protein hydrolysate from various food sources, in addition to its nutritional properties, exhibit different biological functions including antioxidant, (Bougatef *et al.* 2009), antimicrobial (Salampessy *et al.* 2010; Sila *et al.* 2014) anticoagulant (Ren *et al.* 2014), and antihypertensive (Je *et al.* 2009; Furuta *et al.* 2016). Derivative bioactive protein, consist of 2 to 20 of amino acids which inactive in sequence of protein core and it can be released by enzymatic hydrolysis during gastrointestinal digestion in the body or food processing. When liberated in the body, derivative bioactive protein can act as a regulatory compound with the activity as hormone. Furthermore, the specific enzyme used for the hydrolysis is important factor for the production of bioactive compound. Enzyme influences the molecular weight, amino acid content and biological activity of the protein hydrolysate (Van *et al.* 2002). The objectives of this study were to produce TEPH using different concentration of papain and hydrolysis duration times, and to characterize its antioxidant properties.

MATERIALS AND METHODS

Materials and Tools

Raw material for this study was frozen tuna eye (*Thunnus* sp.) obtained from Nizam Zachman Port, Muara Baru Bay, Jakarta. Frozen tuna eye was brought by using coolbox and transported by car for about 2 hours. Papain Merk (30000 USP-U/mg) was purchased from Milipore Co. (Billerica Ma, USA). 2,2-diphenyl-1-picrylhydrazyl (DPPH). Other chemicals and reagents used are commercially available. The tools used include freezer, blender, centrifuge (Beckam), waterbath, pH-meter, spectrophotometer, micro pipette (Pipetman), HPLC (Shimadzu), digital scales (Sartorius), oven (Yamato), and freeze dryer.

Research Methods

Separation of tuna eye protein

Tuna eye was cut into small part and blended by using blender, then centrifuged at 10,000 g, 4^0C for 30 minute to separate the lipid, liquid and precipitant (solid). The precipitant was collected and used in further research stages.

Proximate analysis

Composition of tuna eye precipitant (TEP) was determined by proximate analysis according to AOAC (2005) covering moisture (Gravimetrically), protein (Kjeldahl), lipid (Soxhlet), and ash content.

Production of tuna eye protein hydrolysate (TEPH)

Protein hydrolysate from tuna eye was prepared through enzymatic hydrolysis with papain according to the method Imm and Lee (1999) with slight modification. The concentration of papain used were 0.1% (b/v), 0.15% (b/v), 0.2% (b/v), and 0.25% (b/v) with different hydrolysis duration times for 4; 5; 6 and 7 hours. The hydrolysis was carried out in a glass bottle placed in a water bath with stirred in 100-130 g. Distilled water was added in sample by ratio of TEP and distilled water 1:8 (b/v). The hydrolysis started by adding enzyme with the temperature 55^{o}C combined with different of hydrolysis times, then heated to 85^{o}C for 15 minutes and centrifuged at 6.000 g for 20 minutes, in cold temperature of 4^{o}C. Obtained supernatant was then dried using freeze dryer at -55^{o}C in order to obtain the hydrolysate powder of tuna eye protein and stored in the freezer until characterization an analysis process.

Measurement of degree of hydrolysis (DH)

Degree of hydrolysis was calculated based on the percentage ratio of trichloroacetic acid (TCA) method (Baharuddin *et al.* 2016). The supernatant of protein hydrolysate was added with TCA 10% and incubated for a certain time and followed by precipitation, then centrifuged at 7.500 g for 15 minutes. Obtained supernatant was analysed for the nitrogen content by kjeldahl method (AOAC 2005). Degree of hydrolysis was calculated by the following formula:

$$DH\ (\%) = \frac{\text{Soluble nitrogen TCA 10\%}}{\text{Total nitrogen}} \times 100\%$$

Determination of soluble protein

The soluble protein content of TEPH Tuna's eye protein hydrolysate was performed according to Bintang (2010). Two mL of hydrolysate sample was added 5 mL of Cu-alkali (NaOH 4%, $NaCO_3$ 20%, NaK-tartrate, and 5% $CuSO_4$) and incubated at room temperature for 1 hour. Then added 0.6 mL of folin-ciocalteu, the mixture was kept for 15 minutes at ambient temperature and measured absorbance at a wavelength of 660 nm.

Antioxidant activity 2.2-Diphenil-1-picrylhydrazyl (DPPH)

The DPPH Scavenging activity from TEPH was measured according to Shimada *et al.* (1992) method with slight modifications, 1 mL of sample was mixed with 1 mL of DPPH 0.15 mM solution, vortexed and incubated in a dark room for 30 minutes. The absorbance was measured at wavelength 517 nm using UV-VIS spectrophotometer. Scavenging DPPH activity was calculated by the following equation:

$$\text{Inhibition (\%)} = (A-B)/A \times 100\%$$

Where A is blank absorbance and B is sample absorbance.

Data Analysis

Experimental design was performed referring to Steel and Torrie (1991) method using complete randomized design with factorial pattern: papain concentrations with 4 levels (0.1%; 0.15%; 0.2% and 0.25%) and different hydrolysis times (4; 5; 6 and 7 hours) by twice repetition. Data was processed using Statistical Process for Social Science software (SPSS) version 24. Significant differences ($p<0.05$) between the means value were evaluated by Least Significant Different (LSD) test.

RESULTS AND DISCUSSION

Composition of Tuna Eye

Proximate analysis of tuna eye precipitant was conducted to determine the chemical composition such as moisture, protein, lipid ash of TEP. The results of chemical composition of TEP were shown in Table 1.

Protein content of TEP was 15.06%, indicating that TEP is a good resource of protein for producing protein hydrolysate.

Table 1 Chemical composition of TEP

Chemical composition	TEP (%)
Moisture	78.04 ± 0.042
Protein	15.06 ± 0.028
Lipid	6.11 ± 0.042
Ash	0.62 ± 0.035

Stansby (1982) explained about fish with protein content of 15-20% included into a group of high-protein fish. High protein of fish is one of the requirements in producing protein hydrolysate.

Degree of Hydrolysis

The degree of hydrolysis (DH) is defined as the percent ratio of the number of broken peptide bonds to the total number of peptide bonds on the substrate (hydrolysate) (Bougatef *et al.* 2012). Sila and Bougatef (2016) stated that DH determination is an important parameter for determining the functional and biological properties of protein hydrolysate. DH value of TEPH can be seen in Figure 1.

Value of DH was changed during hydrolysis. ANOVA data showed that hydrolysis period and enzyme concentration gave significant influence on DH value ($p<0.05$). DH of TEPH ranged between 9.1%-16.14%. The higher DH value of hydrolysate, the more low-molecular peptides produced. Several factors that influence DH are enzyme concentration, time of hydrolysis and proteases used. Similar DH was obtained by Saidi *et al.*(2013) about 10.22% hydrolysate from by-products of tuna dark meat and Wasswa *et al.* (2007) revealed 16.11% in grass carp skin. Differences DH obtained from TEPH due to the various in enzyme concentration used and times of hydrolysis (Jamil *et al.* 2016). Beveridge (1996) explained that during the hydrolysis process, papain will cleavage the substrate into product by histidine and cysteine groups in active site of catalytic enzyme. Structural changes that occur among them, a highly reactive cysteine (Cys-25) group bonding to the substrate to produce substrate covalent bonds with tetrahedral enzyme. The histidine group (His-159) is protonated so that binds to the

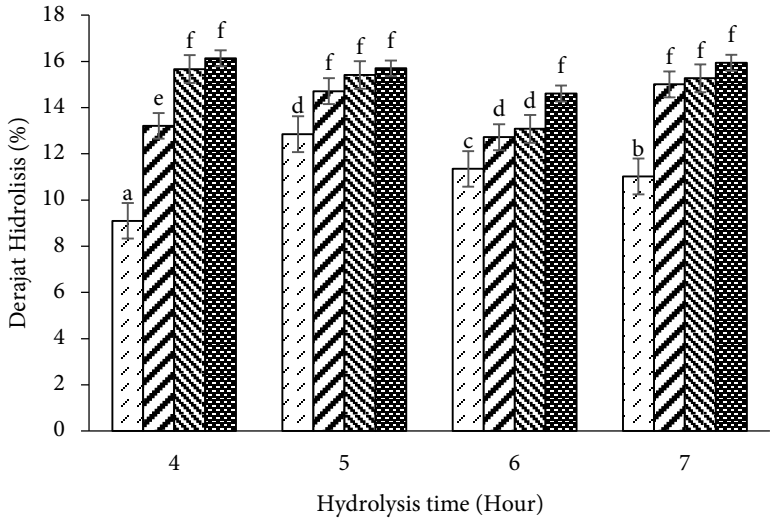

Figure 1 DH of TEPH in various conditions. Different letters indicate significant differences ($p<0.05$). Concentration papain 0.1% (□), 0.15% papain (▨), 2% papain (▩), and 2.5% papain (■).

nitrogen present in the substrate. As a result, the amine group on the substrate diffuses and its position is replaced by water molecules which ultimately hydrolyse the intermediate product thus returning the enzyme to original form and function.

Soluble Protein Content

The protein content of TEPH was performed by calculating the absorbance of hydrolysate on standard absorbance using Bovine Serum Albumin serum (BSA). The soluble protein concentration of TEPH illustrated in Figure 2.

The highest soluble protein concentration 94.90±0.002% ($p<0.05$) was obtained from hydrolysis process for 6 hour with 0.2% papain. Protein is an important component in hydrolysate product. During the Hydrolysis process the insoluble proteins were converted by papain into soluble proteins that will be hydrolysed into smaller component such as peptides and amino acids. Therefore, the total of soluble protein will be increased in optimum condition (Nielsen 1997).

Antioxidant Activity 2.2-Diphenil-1-Picrylhydrazyl (DPPH)

Antioxidants are molecules that can interact with free radicals and form stable compounds that stop oxidation (You *et al.* 2009). DPPH is a stable radical showing the highest absorbance length at 517 nm. When DPPH discovers a hydrogen donor substance such as an antioxidant, the radical is captured. It is accomplished by the change of colour from purple to yellow and the absorbance will decrease. DPPH scavenger activity shows the ability of antioxidant compounds to donate hydrogen or electrons, thereby altering radical substances to be more stable (Binsan *et al.* 2008). Antioxidant activity will increase with increasing concentration used. Tuna eye protein inhibits against DPPH radical was 78.44±0.40% at concentration 10 mg/mL. Elias *et al.* (2008) stated that the ability of proteins and their derivatives to inhibit oxidation of lipids makes it an important component as the antioxidant defence of biological tissue from the food produced. Converting protein structure into short peptide with certain amino acid composition can improve its ability as an antioxidant. The result of antioxidant DPPH radical scavenger of hydrolysate protein from tuna eye presented in Figure 3.

Antioxidant activity changed during hydrolysis process. Hydrolysis period and enzyme concentration gave significant different ($p<0.05$) in ANOVA data results. Figure 3 showed the results of the DPPH radical scavenging ability of TEPH in different condition of treatments. The inhibition activity against DPPH radicals in protein hydrolysates

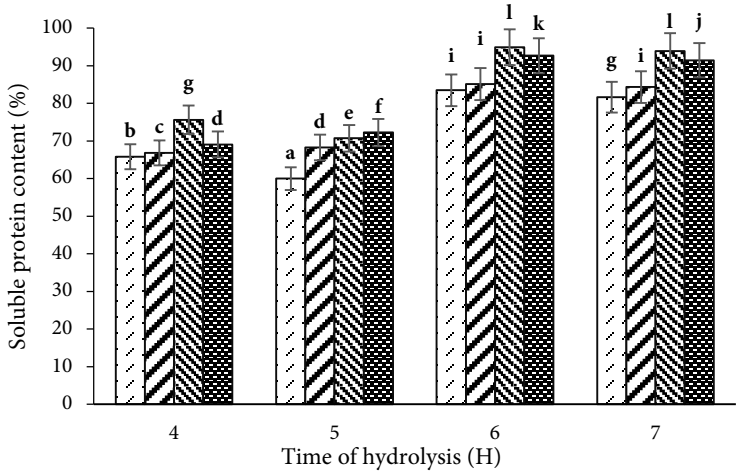

Figure 2 TEPH Soluble protein content. Different letters indicate significant differences ($p<0.05$). Concentration papain 0.1% (□), 0.15% papain (▨), 2% papain (▩), and 2.5% papain (■).

ranged from 78-93%. Compared to the raw material, TEPH had high of antioxidant activity. The hydrolysis process enhances the antioxidant activity. Jamil *et al*. (2016) stated that hydrolysis process had increased the amount hydrophobic amino acids (valine, isoleucine, phenylalanine, methionine) which contributed to the antioxidant activity of protein hydrolysate. The highest activity was obtained from the addition of 0.1% papain with 6 hours hydrolysis about 93.57% with the IC_{50} value 1.08±0.008 mg/mL. IC_{50} value of TEPH is presented in Table 2.

Radical scavenging DPPH can also be expressed with IC_{50} value. The lower of IC_{50} obtained, the highest the activity of antioxidant. The lowest IC_{50} value was obtained in protein hydrolysate with 0.1% enzyme concentration at 6 hours hydrolysis (1.082±0.008 mg / mL) ($p<0.05$) with inhibition percentage of DPPH 93.5% at a concentration of 5 mg/mL. This showed that TEPH with 0.1% concentration for 6 hours probably containing hydrogen donor peptides by quench free radical into stable molecule. High hydrophobic amino acids content has been demonstrated in peptides exhibited high antioxidant activity which is considered in scavenge of free radical.

According to Zou, He, Tang and Xia (2016), hydrophobic amino acids had strong radical scavenging activity in oxidative reaction, especially with enzymatic catalysed reaction due to the present of imidazole ring as important electron donor. Chang *et al.* (2015)

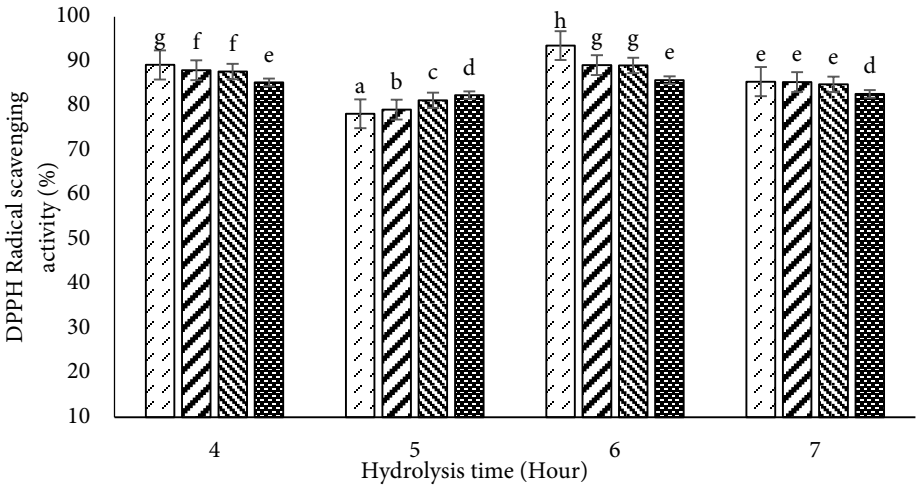

Figure 3 DPPH radical scavenging activity. Different letters indicate significant differences ($p<0.05$). Concentration papain 0.1% (□), 0.15% papain (▨), 2% papain (▩), and 2.5% papain (■).

Table 2 Antioxidant IC_{50} value of TEPH by various condition

Hydrolysis time (Hour)	IC_{50} value of DPPH radical scavenging (mg/mL)			
	Papain 0.1%	Papain 0.15%	Papain 0.2%	Papain 0.25%
4	1.77	1.86	1.89	1.95
5	2.97	2.95	2.93	2.84
6	1.08	1.15	1.49	1.51
7	1.31	1.37	1.39	1.50

reported on the hydrolysate protein of bluefin leatherjacket (*Navodon septentrionalis*) using various enzymes had the highest IC_{50} value of 5.22 mg/mL. Hydrolysate of the head protein of bigeye tuna (*Thunnus obesus*) shows IC_{50} value of 1.34 mg/mL (Yang *et al.* 2011). Zhong *et al.* (2011) reported on protein hydrolysate by-products of grass carp has a radical scavenging inhibition percentage 68.4% at a concentration of 5 mg/mL. These showed that tuna eye protein hydrolysate (*Thunnus* sp.) had better IC_{50} value than protein hydrolysate of bigeye tuna, bluefin leatherjacket, and grass carp IC_{50} value. There were many studies that reported on the potential of bioactive peptides especially from fish protein as antioxidants. However, it cannot be explained in detail about the mechanism of protein as an antioxidant. Many of the antioxidant mechanisms of proteins depend on their amino acid composition (as free-radical scavenging, metal binder, hydro peroxide reduction, or adduction (giving) aldehyde groups). In addition, the antioxidant activity of amino acid residues is limited to the tertiary structure of the polypeptide, so that many amino acids with antioxidant capabilities are enclosed within the protein core that is inaccessible to the pro-oxidant.

Amino Acid Composition

The quality of a food protein can be evaluated by determining the amino acid composition. The amino acid of hydrolysate protein composition has various roles in its physiological activity, which has also been observed for antioxidant activity. The amino acid composition of HPMT is expressed in percent in Table 3.

Amino acid composition obtained from the protein hydrolysate that had the highest antioxidant activity produced in 0.1% concentration of papain for 6 hours hydrolysis. The highest amino acid content was glutamic acid about 19.70/100 ppm. Chang *et al.* (2015) reported similar results in the hydrolysate protein of skipjack dark meat that had the highest glutamic acid content of 127.6 / 1000. Protein that can provide amino acids that almost match human needs, is a high quality protein. Amino acid composition of food

Table 3 Amino acid composition from tuna eye protein hydrolysate

Amino acid composition	TEPH (100 ppm)
Aspartic acid	7.26
Glutamic acid	19.70
Serine	3.06
Glycine	6.27
Histidine	3.50
Arginine	2.90
Threonine	3.83
Proline	6.67
Alanine	2.67
Tyrosine	3.01
Valine	1.80
Methionine	4.50
Cysteine	1.60
Isoleucine	3.01
Leucine	12.36
Phenylalanine	2.80
Lysine	8.17

protein hydrolysate has various roles in its physiological activity, including its activity as an antioxidant. Some amino acids have ability to be antioxidants such as hydrophobic amino acids (alanine, leucine, isoleucine, methionine, proline, tyrosine and valine) and aromatic amino acids (tyrosine, phenylalanine and tryptophan) that have radical scavenging and metal chelating activities (Damodaran 1996).

CONCLUSION

The concentration of the enzyme papain and the duration of hydrolysis significant effect ($p<0.05$) on the antioxidant activity of tuna eye protein hydrolysate. The highest inhibition of DPPH radical scavenging activity (93.5%) obtained at 0.1% concentration of papain for 6 hours of hydrolysis at the concentration 5 mg/mL with an IC_{50} value of 1.082 ± 0.008 mg/mL.

REFERENCES

[AOAC] Association of Official Analytical Chemist. 2005. Official Methods of Analysis (18 Edn). Mayland (USA): Association of Official Analytical Chemist Inc. pp 777-784.

Baharuddin NA, Halim NRA, Sarbon NM. Effect of degree of hydrolysis (DH) on the functional properties angiotensin I-converting enzyme (ACE) inhibitory activity of eel (*Monopterus* sp.) protein hydrolysate. *International Food Research Journal.* 23(4): 1424-1431.

Beveridge AJ. 1996. A theoretical study of the active sites of papain and s195c rat trypsin: implication for the low reactivity of mutant serine proteinases. *Journal Protein Science.* 5: 1355-1365.

Binsan W, Soottawat B, Wonnop V Hideki, K. 2008. Antioxidative activity of mungoong, an extract paste, from the cephalothorax of white shrimp (*Litopenaeus vannamei*). *Journal of Food Chemistry.* 106: 185-193.

Bintang M. 2010. *Biokimia teknik Penelitian.* Jakarta (ID): Erlangga.

Bougatef A, Hajji M, Balti R, Lassoued I, Triki-Ellouz Y, Nasri M. 2009. Antioxidant and free radical-scavenging activities of smooth hound (*Mustelus mustelus*) muscle protein hydrolysates obtained by gastrointestinal proteases. *Food Chemistry*. 114: 1198–1205.

Bougatef A, Balti R, Haddar A, Jellouli K, Souissi N, Nasri M. 2012. Antioxidant and functional properties of protein hydrolysates of bluefin tuna (*Thunnus thynnus*) heads as influenced by the extent of enzymatic hydrolysis. *Biotechnology and Bioprocess Engineering*. 17: 841–852.

Chang F C, Bin W, Yu MW, Bin Z, Shang GD. 2015. Isolation and characterization of three antioxidant peptides from protein hydrolysate of bluefin leatherjacket (*Navodon septentrionalis*) heads. *Journal of Functional Foods.* 12: 1-10.

Damodaran S. 1996. Amino Acids, Peptides and Protein. Food Chemistry. 3rd edition. New York (NYC): Marcel Dekker Inc.

Elias RJ, Kellerby SS, Decker EA. 2008. Antioxidant activity of proteins and peptides. *Critical Reviews in Food Science and Nutrition*. 48: 430–441.

Furuta T, Yoshikatsu M, Hajime Y, Yasunori K, Hideki K. 2016. Angiotensin I converting enzyme inhibitory peptides derived from phycobiliprotein of dulse (*Palmaria palmate). Marine Drugs*. 14(2): 32.

Imm JY, Lee CM. 1999. Production of seafood flavour from red hake (*Urophycis chuss*) by enzymatic hydrolysis. *Journal Agriculture Food Chemistry.* 47: 2360-2366.

Jamil NH, Halim NRA, Sarbon NM. 2016. A response surface approach on hydrolysis condition of eel (*Monopterus* Sp.) protein hydrolysate with antioxidant activity. *International Food Research Journal.* 24(3): 1081-1093.

Je JY, Lee KH, Lee MH, Ahn CB. 2009. Antioxidant and antihypertensive protein hydrolysates produced from tuna liver by enzymatic hydrolysis. *Food Research International.* 42: 1266 1272.

Kim SY, Je JY, Kim SK. 2007. Purification and characterization of antioxidant peptide from hoki (*Johnius belengerii*) frame protein by gastrointestinal digestion. *Journal Nutrition Biochemistry.* 18: 31-38.

Klompong V, Benjakul S, Kantachote D, Shahidi F. 2007. Antioxidative activity and functional properties of protein

hydrolysate of yellow stripe trevally (*Selaroides leptolepis*) as influenced by the degree of hydrolysis and enzyme type. *Food Chemistry*. 102: 1317-1327.

Nielsen PM. 1997. Functionality of Protein Hydrolysate. In: Damodaran S, Paraf A, eds. Food proteins and their applications. 1st edn. New York (NYC): Marcel Dekker Inc. 451 pp.

Ren Y, Wu H, Lai F, Yang M, Li X, Tang Y. 2014. Isolation and identification of a novel anticoagulant peptide from enzymatic hydrolysates of scorpion (*Buthus martensii* Karsch) protein. *Food Research International*. 64: 931–938.

Ruchimat T, Wudianto, Fahmi Z, Setyadji B, Sadiyah L. 2017. Indonesia national report to the scientific committee of the indian ocean tuna commission. *Indian Ocean Tuna Commission*. IOTC-2017-SC20-NR09.

Sandria N, Desmelati Mery S. 2014. A study on an extraction gelatin from tuna eye's (*Thunnus* sp.). *Journal Online Mahasiswa Universitas Riau*. 1(2): 1-11.

Saidi S, Deratani A, Ben AR, Belleville MP. 2013. Fractionation of a tuna dark muscle hydrolysate by a two-step membrane process. *Separation and Purification Technology*. 108: 28–36.

Saidi S, Deratani A, Belleville MP, Ben AR. 2014. Antioxidant properties of peptide fractions from tuna dark muscle protein by-product hydrolysate produced by membrane fractionation process. *Food Research International*. 65: 329–336.

Salampessy J, Phillips M, Seneweera S, Kailasapathy K. 2010. Release of antimicrobial peptides through bromelain hydrolysis of leatherjacket (*Meuchenia* sp.) insoluble proteins. *Food Chemistry*. 120: 556–560.

Shimada K, Fujikawam K, Yaharam K, Nakamura T. (1992). Antioxidative properties of xanthin on autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agriculture and Food Chemistry*. 40: 945-948

Sila A, Malik N, Sayari N, Balti R, Bougatef A. 2014. Chitin and chitosan extracted from shrimp waste using fish proteases aided process: efficiency of chitosan in the treatment of unhairing effluents. *Journal of Polymers and the Environment*. 22: 78–87.

Sila A, Bougatef A. 2016. Antioxidant peptides from marine by-products: isolation, identification and application in food systems. A review**.** *Journal of Functional Foods*. 21: 10-26.

Slizyte R, Rommi K, Mozuraiteyte R, Peter E, Five K, Rustad T. 2016. Bioactivities of fish protein hydrolysates from defatted salmon backbones. *Biotechnology Reports*. 11: 99-109.

Stansby M. E. 1982. Properties of fish oils and their application to handling of fish and to nutritional and industrial use; in Martin RE (ed.). *Chemistry and Biochemistry of Marine Food Products*. Westport Connecticut: The AVI Publishing Company.

Steel RGD, Torrie JH. 1991. *Prinsip dan Prosedur Statistika, Edisi Kedua.* Jakarta (ID): PT. Gramedia.

Van der Ven C, Gruppen H, de Bont DBA, Voragen AGJ. 2002. Optimization of the angiotensin converting enzyme inhibition by whey protein hydrolysates using response surface methodology. *International Dairy Journal*. 12: 813–820.

Vijaykumar R, Sivaraman GK, Mathew S. 2016. Fatty acid profile of yellowfin tuna eye (*Thunnus albacares*) and oil sardine muscle (*Sardinella longiceps*). *Fishery technology.* 53: 151-154.

Wasswa J, Tang J, Gu XH, Yuan XQ. 2007. Influence of the extent of enzymatic hydrolysis on the functional properties of protein hydrolysate from grass carp skin. *Food Chemistry*. 104: 1698-1704.

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 and Technology.* 46: 2460-2466.

You L, Zhao M, Cui C, Zhao H, Yang B. 2009. Effect of degree of hydrolysis on the antioxidant activity of loach *(Misgurnusanguilli caudatus)* protein hydrolysates. *Innovative*

Food Science and Emerging Technology. 10(2): 235–240.

Young-je J, Ka HL, Mi HL. Chang BA. 2009. Antioxidant and antihypertensive protein hydrolysates produced from tuna liver by enzymatic hydrolysis. *Food Research International*. 42: 1266-1272.

Zhong S, Ma C, Lin YC, Luo Y. 2011. Antioxidant properties of peptide fractions rom silver carp (*Hypophthalmichthys molitrix*) processing by-product protein hydrolysates evaluated by electron spin resonance spectrometry. *Food Chemistry*. 126: 1636–1642.