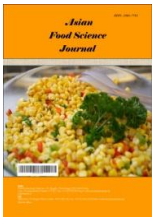

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Chemical Composition, Amino Acid Profile and Angiotensin Converting Enzyme (ACE) Inhibitory Activities of Skipjack (*Katsuwonus pelamis*) Roe Hydrolyzate

Max Robinson Wenno[1*], Johanna Louretha Thenu[2] and Martha Loana Wattimena[1]

[1]Department of Fisheries Processing Technology, Fishery and Marine Science Faculty, Pattimura University, Ambon, Indonesia.
[2]Fishery Training and Extantion Center of Ambon, Indonesia.

Authors' contributions

This work was carried out in collaboration among all authors. Author MRW designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JLT and MLW managed the analyses of the study. Author MRW managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Roe has a high protein content and a number of amino acids. The process of removing fat and hydrolyzate with enzymes leads to the breaking of the bonds, so that complex proteins are converted into short chain proteins or peptides and free amino acids. The peptide can act as bioactive and has an effect as antihypertensive, antibacterial, antioxidant and so on. This research was aimed at utilizing processed roes to make hydrolyzate which had previously viewed the chemical composition both fresh and defatted, and to determine the protein profile of the roes from hydrolyzate. The research data were analyzed descriptively, and the average value and standard deviation were calculated. The results showed that skipjack roes have a fairly complete chemical composition, such as Proximate (protein, fat, moisture, ash, and carbohydrates), with values, respectively 19,19%, 0,67%, 76,32%, 2,51% and 1,31%. It was also found that the dominant amino

**Corresponding author: Email: maxwenno@yahoo.com, wennomaxrobinson@gmail.com;*

acid composition of defatted skipjack mature roes is lysine, glutamate and leusine with values, respectively 12.65, 11.20 and 7.72 g/100 g protein and have activity as an angiotensin converting enzyme inhibitory. The ACE inhibitory activity of Skipjack roe hydrolysates of crude papain enzyme from immature and mature value, respectively 36.62% and 38.82%, while pure papain enzyme from immature and mature value respectively 42.63% and 47.54%. The protein profile of the immature roe hydrolyzate range from 10.88 to 125,80 kDA, while the mature roe hydrolysates range from 10.08 to 125,30 kDa.

Keywords: Skipjack roe; defatted; angiotensin converting enzyme inhibitory; bioactive peptides; fish protein hydrolyzate.

1. INTRODUCTION

Hypertension or high blood pressure is one of the chronic diseases and is a major factor causing several diseases such as stroke, coronary heart disease, kidney dysfunction and myocardial infarction. It is estimated that 20% of the world's population have hypertension and is a cardiovascular disease that mostly occurs in developing countries (Li et al., 2012). Referring to WHO, the definition of hypertension is a systolic blood pressure flow of 140 mmHg or more or a diastolic blood pressure flow of 90 mmHg or more. Hypertension is the number 3 cause of death after stroke and tuberculosis, which reaches 6.7% of the population of deaths at all ages in Indonesia [1]. An angiotensin I (ACE) converting enzyme is a glycoprotein peptidyldipeptide hydrolase and belongs to the class of zinc proteases which requires zinc and chloride to be active. ACE plays a role in the body in the process of regulating blood pressure. This type of peptide basically catalyzes the reaction of angiotensin I to angiotensin II, by breaking down the histidyl-leucine dipeptide from the C-tip of angiotensin I to produce angiotensin II and hypuric acid [2]. If the hydrolysis of angiotensin I is excessive, the blood pressure will increase [3]. Synthesis of ACE inhibitors, such as captopril, enalapril, alacepril, or lisinopril are some of the most commonly used drugs to treat hypertension [4] It has been reported that ACE synthesis inhibitors have side effects ,such as effects on the inflammatory response, swelling of the gums, dry cough, abnormal changes in taste and swelling under the skin [5].

A concept that marine organisms are a new source of substance as an alternative to medicine is currently being increasingly studied. The biological and chemical characteristics of marine organisms are unlimited new bioactive sources. Marine organisms and their by-products contain a number of very high numbers of nutritional components such as minerals, fatty acids, amino acids, polysaccharides and other compounds with unique characteristics. The by-product of the processing of fishery products such as heads, skins, scales, bones, viscera, and roes is a substance that has a variety of high added values. So, it needs to be exploited further and requires serious attention because it can be used as an excellent source of pharmaceutical and neutraceutical ingredients, with a specific effect in curing various diseases. More than 91 million tonnes of fish and non-fish are produced each year. From the capture and cultivation process, it is estimated that the total waste produced is around 25% [6,7]. Fisheries waste management is a major problem and needs serious attention because it is related to environmental pollution. Until now, various applications for the utilization of marine waste products have been carried out, such as animal feed, biodiesel / biogas, dietary products and food packaging (chitosan), cosmetics (collagen), natural pigments, and enzymes. Recent research is more aimed at obtaining various bioactive components such as peptides from fish bones, skin, and internal organs including roes produced. By-product of the fishing industry is a source of nutrition and functional food ingredients [8,9]. By-products such as of roe have a high protein content and several of amino acids. Some of the research on the chemical composition of roe include alaska pollock (*Theragra chalcogramma*) and cod (Gadus morhua) with a moisture of 67.4-80.0%, protein 16.0-25.8%, fat 0.3-5.2 % and ash 1.7-2.3% [10]. In addition, it was found that from skipjack tuna, bonito, moisture was 72.17-73.03%, protein 18.16-20.15%, fat 3, 39-5.68% and ash 1.79-2.10% and amino acids, namely glutamic acid 12.8-12.65 g / 100g protein, aspartic acid 8.27-8.85 g / 100g protein, leucine 8 , 28-8.64 g / 100g protein and lysine 8,24-8,30 g / 100g protein [11]. Fish roe has a high protein content and some of amino acids. Some research showed the composition of roe consisted of glutamine (12.65%), leucine (8.44%) and lysine (8.30%).

The protein content of skipjack and tuna roe were 20.15% and 18.44%, respectively. The dominant amino acids in tuna consists of histidine, leucine and protein with successive values of 10.30, 10.80, and 11.70 g / 100 g protein, while the dominant non-essential amino acids are glutamate, proline, and serine with values respectively 5.89, 3.79, and 3.23 g / 100 g protein [11,12].

Protein hydrolyzate is the enzymatic breakdown of proteins into smaller peptides. Generally, hydrolyzed proteins are small fragments of peptides containing 2-20 amino acids. Biotechnology currently used to improve the nutrition and physiological importance of peptides is the enzymatic hydrolysis of fish proteins that produce protein and bioactive hydrolyzates from fish species of low economic value and are underutilized. Some proteolytic enzymes that are commonly used to hydrolyze fish protein are Alkalase, papain, pepsin, trypsin, α-chemotrypsin, pancreatin, Flavourzyme, Pronase, Neutrase, Protamex, bromelain, cryotin-F, proteaseN, protease A, Oricentase, thermolysin, and Valid [13–17]. The hydrolysis reaction of fish protein using proteolytic enzymes under controlled conditions of temperature, pH and hydrolysis time can produce the final product in the form of the quality fish protein hydrolyzate. Fish protein hydrolyzate is obtained through enzymatic techniques from a variety of processed fish protein waste and has various applications in the pharmaceutical, cosmetic and animal nutrition fields [9,13]. Free amino acids and short chain peptides resulting from roe protein hydrolyzate can provide many advantages as nutraceuticals or functional foods due to the composition of amino acids they contain. Various studies on ACE inhibitors in Indonesia are still limited and are produced from a number of terrestrial plants. ACE inhibitors from marine products are still very rarely studied. Several studies abroad have produced ACE inhibitors of marine organisms including: pepsin hydrolyzate red meat tuna, skin and skeletal bone base for pollack, salmon skin and alkalase hydrolyzed skipjack roe [4,11,18,19]. This research is intended to utilize the byproducts of fishery products in the form of skipjack roe that have not been utilized properly. Skipjack is one type of large pelagic fish that has high economic value. Apart from being an export commodity, local fish for consumption, smoked fish processing and freezing are also getting higher. With this high enough demand, of course, it also produces high waste and side products. Therefore, the utilization of byproducts from skipjack fish to produce roe protein hydrolyzate which can be isolated by ACE Inhibitory peptides, which have the potential as antihypertensive drugs, is a reason why this study was conducted.

2. MATERIALS AND METHODS

The main ingredient used in this study was skipjack roe (*Katsuwonus pelamis*) obtained from fish sellers at the Mardika Market of Ambon City. Crude papain enzyme (Paya brand) and pure papain enzyme (Sigma Singapore) are also used as well as some of chemicals for proximate analysis and chemical composition of fresh roe and roe protein hydrolyzate. Equipments used for the process of making protein hydrolyzate are analytical scales, centrifuges, vacuum drying, refrigerators, homogenizers, electric ovens, High Performance Liquid Chromatography / HPLC (Waters), and a spectrophotometer.

2.1 Research Stages

This research consisted of 2 steps. The first stage was the testing of proximate and amino acid profiles of fresh and defatted skipjack roes and the second step was the testing of Angiotensin converting Enzyme inhibitors of the skipjack roes. Fresh skipjack roes collected from fresh fish sellers in the Mardika Market of Ambon City and put in the refrigerator. Some samples were subjected to proximate analysis (protein, fat, water, ash and carbohydrates) and some were defatted and hydrolyzed. The process of making roe protein hydrolyzate (the modified [20] method, is as follows: roes are washed thoroughly using ice water. Roes that have been clean are homogenized, then isopropyl alcohol (IPA) is added with a ratio of 1:3, with the aim of removing fat. The process of removing fat was carried out for 8 hours. The defatted were then filtered and dried in a dry cabinet for 4-5 hours. Dried defatted roes were then added with distilled water with a ratio of 1: 10 and the pH value of the mixture was adjusted to the optimum pH of the enzyme namely pH 7.0 with the addition of 1 M NaOH solution and 1 M HCl solution. Then, enzymes (crude papain brand Paya and pure papain enzymes from Sigma Singapore) were added to the defatted mixture and distilled water. The hydrolyzate was carried out at 55^{o}C using a water bath shaker for 1.5 hours for crude papain enzymes and 6 hours for pure enzymes. After the hydrolysis process was complete, the enzyme was inactivated at 80^{o}C

for 20 minutes. Samples were centrifuged at 5000 rpm for 20 minutes, at 4°C to separate the dissolved fraction (supernatant) and the non-dissolved fraction (pellet). The supernatant was dried using vacuum drying.

2.2 Analysis Procedure

Chemical composition analysis procedures (moisture, ash, protein and fat, amino acid profile used the AOAC method [21], and carbohydrate (by-different). The ACE inhibitor activity assay according to the Cushman and Cheung method [22] with slight modification. Abaout 50 mL 0f sample olution and 50 mL solution ACE (≥ 2.0 units per mg protein, from Sigma-Aldrich Chemie) were pre-incubated for 32 °C for 10 min. The mixture was then incubated for 30 min at the same temperature with the addition of 50 mL of substrat (Hip-His-Leu 8 mM in buffer 50 mM HEPES from Sigma-Aldrich Chemie containing NaCl 300 mM at pH 8.3. The reaction was ferminated by the addition of 1 M HCl (200 mL). The Solution was extracted wit the addition of 1.5 mL ethyl acetate and centrifuged (4000 x g) for 15 min.After that, 1 mL of the supernatant was transferred to another tes tube and was evaporated until dry (63°C, for 45 min). Once dried, it was dissolved in 1 mL of distilled water and the absorbance was determined at a wavelenght of 228 nM using a UV-Vis spectrophotometer (Thermo spectronic).

2.3 Data Analysis

The research data were analyzed descriptively, where all data were displayed in Tables and Figures and the average value and standard deviation were calculated.

3. RESULTS AND DISCUSSION

3.1 Yield of Defatted Skipjack Roe

Yield is a part that can be utilized. During the defatted process, the yield of skipjack roes has decreased due to fat removal, filtering and drying processes. Data on defatted yield of skipjack roes can be seen in Table 1.

Table 1. Yield of *Defatted* Skipjack Roe

Defatted Roe	Yield (%)
Mature	10.85 ± 1.63
Immature	14.57 ±1.04

n = 3

Referring to Table 1, it can be seen that the mature skipjack roes has a higher yield than immature roes. This is influenced by roe size and the total amount of protein contained. Roes that are mature have a larger size than immature roes or immature gonads.

3.2 Chemical Composition

3.2.1 Chemical composition of skipjack roe

Proximate values (protein, fat, moisture, ash, and carbohydrates) of fresh skipjack roes can be seen in Table 2.

Protein content and fat of mature gonad roe are higher than those of pre-cooked gonads, both fresh roe (wet weight) and dry weight. Fish roes with a high level of maturity has high levels of protein and fat. Roes have different protein, moisture, fat, ash and carbohydrate content, depending on several factors such as the kind and size of the fish, the level of maturity of the roe, the habitat of the fish, the season and several other factors. Some research results show that roes of several species have a moisture content above 50%. When roes mature, the ratio between water or fat content generally increases [10]. Variations in roe chemical composition generally depend on biological factors, such as fish species, roe maturity, food, season, catching area and processing conditions [23]. Furthermore, the amount and composition of fat is strongly influenced by species and habitat [24].

Table 2. Proximate of Skipjack Roe

Composition	Content			
	% Wet Weight		% Dry Weight	
	Immature	Mature	Immature	Mature
Protein	19.19±0.18	20.99±0,12	62.29±0.03	69.77±0.06
Fat	0.67±0.02	1.23±0,28	7.01±0.04	10.64±0.58
Moisture	76.32±0.42	75.85±0,98	13.19±0.43	10.94±0.02
Ash	2.51±0.01	1.24±0,35	10.64±0.17	6.53±0.02
Carbohidrate (*by different*)	1.31	0.69	6.87	2.10

n = 3

Table 3. Proximate of *Defatted Roe*

Composition	Content (% Dry Weight)	
	Immature	**Mature**
Protein	70.45 ± 0.39	72.78 ± 1.02
Fat	6.39 ± 0.03	10.39 ± 0.40
Moisture	12.97 ± 0.18	10.69 ± 0.02
Ash	6.99 ± 0.18	4.01 ± 0.06
Carbohidrate (*by different*)	3.19	2.12

n = 3

Table 4. Amino Acids Profile of *Defatted* Roe

Amino Acids (g/100 g protein)	Immature	*Mature*
Valine*	2.45	5.01
Threonine*	2.92	4.46
Lysin*	5.01	12.65
Serine	2.81	4.65
Isoleusine*	1.85	4.20
Alanine	2.83	5.77
Histidine*	1.44	2.09
Phenilalanine*	2.34	3.10
Glutamate	5.81	11.20
Tirosine	2.06	2.44
Proline	2.18	4.84
Arginine*	8.70	5.00
Glysine	2.96	3.39
Leusine*	4.01	7.72
Aspartate	3.65	7.39
Metionine*	0.83	1.49
Sistine	0.16	0.20
Total	**52.02**	**85.60**

**Essential amino acids*

3.2.2 Chemical composition of defatted skipjack roe

Proximate of defatted skipjack roe can be seen in Table.

Table 3 shows that the protein content of skipjack roe has improved when compared to the protein content of fresh roe (Table 3). When the fat content of the roe decreases due to the defatted process, the protein will increase. When the moisture has declined, the non-water nutrient component will rise. Some research results show that defatted roes from several fish species have a moisture content above 50%, while hydrolyzed roes generally have a low moisture content because it has undergone a drying process. The average defatted protein of roes ranges from 16-20%, whereas in roes the results of hydrolyzate have a significant grow. Levels of fat, ash and carbohydrates from defatted and hydrolyzed roes are generally quite low.

3.2.3 Amino acids profile

The amino acid composition of defatted skipjack roes can be seen in Table 4.

It can be seen on table that the total amino acids of defatted mature roes were higher compared to defatted fish roes before mature. Some essential amino acids in the mature roe of skipjack fish such as lysine, glutamic, leucine and aspartic acid are more dominant than other essential and non essential amino acids, whereas defatted roes before mature contain essential amino acids such as lysine, arginine and leucine which are more dominant than other amino acids. The dominant amino acid composition of skipjack roes is histidine, leucine and proline with values respectively 10.80, 10.30 and 11.70 g / 100g protein, while the dominant nonessential amino acids were glutamic acid, proline and serine with the values respectively 5.89, 3.79g and 3.23 g / 100 g protein [11]. The difference between the

composition of fresh fish roe amino acids and fat is caused by different habitats, food and seasons [12]. Fish roes can be used as an alternative source of amino acids with high nutritional value.

3.2.4 Angiotensin converting enzyme inhibitory activities from hydrolyzate roe

The activities of ACE hydrolyzate inhibitors of papain enzymes in skipjack fish roes can be seen in Table 5.

Referring to Table 5, ACE inhibitor activity of skipjack roe hydrolyzate of crude papain enzyme treatment was lower compared to pure papain enzyme treatment, but it did not show a significant difference in activity, both in immature and mature. ACE inhibitor activity is influenced by enzyme and substrate activity. The higher activity of the enzymes used was thought to be the higher activity of ACE inhibitors. The enzyme functions to cut the polypeptide chain into short chain peptides which have activity as ACE inhibitors. Literature studies explain that there is a strong relationship regarding the size and structure of the amino acid peptide with the activity of ACE inhibitors. Peptides that have bioactive activity are usually small peptides with a molecular weight range of <5 kDa or composed of 2-5 amino acid residues [25]. Potential ACE inhibitor peptides are generally in the form of short sequences composed of 2-12 amino acids [26]. The large number of peptide bonds break during hydrolysis producing a number of oligopeptides, short chain peptides and free amino acids. Literature studies show that peptides derived from food proteins are bioactive compounds whose activity is still latent when in their original protein. The process of hydrolysis or digestion process causes the peptide to be separated from its original protein and if consumed, it can provide physiological functions to the human body, one of which is antihypertensive activity that can be demonstrated by the activity of ACE inhibitors [27].

Table 5. The activities of ACE hydrolyzate inhibitors of papain enzymes in skipjack fish roes

Treatment	ACE Inhibitory Activities (%)	
	Immature	Mature
Crude Papain Enzyme	36.63	38.82
Pure Papain Enzyme	42.63	47.54

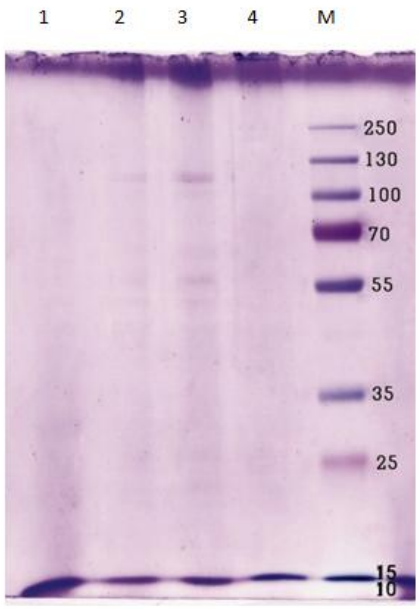

Fig. 1. Roe Hydrolyzate Protein Profile with SDS-PAGE (1,2: Hydrolyzate from immature roe; 3,4: Hydrolyzate from mature roe; M: Marker)

Table 6. Molecule Weight of Hydrolyzate Defatted Roe

Sample	Molecule Weight (kDa)
Hydrolyzate from immature roe	125.80; 59.11; 24.28; 11.50; 10.88
Hydrolyzate from mature roe	125.30; 67.11; 11,87; 11.44; 11.08

3.2.5 Protein profile with SDS-PAGE (Sodium dodecyl sulfate polyacrylamide Gel electrophoresis)

Protein profile samples of skipjack roe defatted and skipjack roe hydrolyzate from immature and mature roes can be seen in Fig. 1 and Table 6.

Table 6 shows that the protein profile of immature roe hydrolyzate range from 10.88 to 125,80 kDA, while the mature roe hydrolyzate range from 10.08 to 125,30 kDA. These proteins are short chain proteins or peptides that have activity as angiotensin converting enzyme inhibitors. [28].

4. CONCLUTION

From the results of the research, it can be concluded that the skipjack roes have a fairly complete chemical composition, especially the protein content and its amino acid constituents. It was found that a number of essential and non-essential amino acids are dominant in skipjack roes, and have activity as an inhibitor of angiotensin converting enzymes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ministry of Health Rupublic of Indonesia; 2009. Available:http://www.depkes.go.id/index.php/berita/press-release/810-hipertensi-penyebab-kematian-nomor-tiga.html.
2. Goretta L, Ottaviani JI, Keen CLFCG. Inhibition of Angiotensin Converting Enzyme (ACE) Activity By Flavan-3-Ols and Procyanidin FEBS Lett. 555; 2003.
3. Shalaby SM, Zakora M, Otte J. Performance of Two Commonly Used Angiotensin-Converting Enzyme Inhibition Assays Using FA-PGG and HHL as Substrates J. Dairy Res. 2006;73:178–86.
4. Je JY, Park PJ, Kwon JY, KSK. A Novel Angiotensin I Converting Enzyme Inhibitory Peptide from Alaska Pollack (Theragra chalcogramma) Frame Protein Hydrolysate J. Agric. Food Chem. 2004;52:7842–5.
5. Wijesekara I, Kim SK. Angiotensin-I-Converting Enzyme (ACE) Inhibitors from Marine Resources: Prospects in the Pharmaceutical Industry. Mar. Drugs. 2010;8:1080–93.
6. Eurostat. Fishery Statistic Data; 2006. Available:hhtp//ec.europa.eu/eurostat, last accessed
7. Rustad T, Storrø I, Slizyte R. Possibilities for the Utilisation of Marine by-Products Int. J. Food Sci. Technol. 2011;46:2001–14.
8. Barrow C, Shahidi F. Marine Nutraceuticals & Functional Foods (CRC Press, Taylor & Francis, Boca Raton, FL. USA); 2007.
9. Dong FD, Bechtel P. New Fish Feeds Made from Fish by Products; 2010.
10. Bledsoe GE, Bledsoe CD, Rasco B. Caviars and Fish Roe Products Crit. Rev. Food Sci. Nutr. 2003;43:317–56.
11. Intarasirisawat R, Benjakul S, Visessanguan W. Chemical Compositions of the Roes from Skipjack, Tongol and Bonito. Food Chem. 2011;124:1328–34.
12. Ziaeian H, Moini S, dan Jamili S. Consequences of Frozen Storage for Amino Acids and Unsaturated Fatty Acids of Tuna (Thunnus tonggol) Roe. J. Fish. Aquat. Sci. 2008;3:410–5.
13. Je JY, Lee KH, Lee MH, Ahn CB. Antioxidant and Antihypertensive Protein Hydrolysates Produced from Tuna Liver by Enzymatic Hydrolysis. Food Res. Int. 2009;42:1266–72.
14. Ngo DH, Qian ZJ, Ryu BM, Park JW, Kim SK. Invitro Antioxidant Activity of a Peptide Isolated from Nile Tilapia (Oreochromis niloticus) Scale Gelatin in Free Radical-Mediated Oxidative Systems. J. Funct. Foods. 2010;2:107–17.
15. Raghavan S, Kristinsson HG. Antioxidative Efficacy of Alkali-Treated Tilapia Protein Hydrolysates: A Comparative Study of Five Enzymes. J. Agric. Food Chem. 2008;56:1434–41.
16. Ren J, Zhao M, Shi J, Wang J, Jiang Y, Cui C, Kakuda Y, Xue SJ. Purification and

Identification of Antioxidant Peptides from Grass Carp Muscle Hydrolysates by Consecutive Chromatography and Electrospray Ionization-Mass Spectrometry. Food Chem. 2008;108:727–36.
17. Samaranayaka AGP, Li-Chan ECY. Autolysis-Assisted Production of Fish Protein Hydrolysates with Antioxidant Properties from Pacific Hake (Merluccius productus). Food Chem. 2008;107:768–76.
18. Qian ZJ, Jung WK, Lee SH, Byun HG, Kim SK. Antihypertensive Effect of an Angiotensin I-Converting Enzyme Inhibitory Peptide from Bullfrog (Rana catesbeiana Shaw) Muscle Protein in Spontaneously Hypertensive Rats. Process Biochem. 2007;42:1443–8.
19. Gu RZ, Li CY, Liu WY, Yi WX, Cai MY. Angiotensin I-Converting Enzyme Inhibitory Activity of Low-Molecular-Weight Peptides from Atlantic Salmon (Salmo salar L.) Skin. Food Res. Int. 2011;44:1536–40.
20. Nurhayati T, Salamah EHT. Hydrolyzed Protein Characteristics of Caranx leptolepis which is Processed Enzymatic. "BuletinTeknologi Hasil. Perairan". 2007;10:23–34.
21. Association of Official Analitical Chemist [AOAC]. Official Methods of analysis. 17th edition. Maryland; 2005.
22. Cushman DW, Cheung HW. Spectrophotometric Assay and Properties of The Angiotensin Converting Enzyme of The Rabbit Lung. Biochem Pharmacol J. 1971;20:1637–48.
23. Al-Sayed Mahmoud K, Linder M, Fanni J, Parmentier M. Characterisation of the Lipid Fractions Obtained by Proteolytic and Chemical Extractions from Rainbow Trout (Oncorhynchus mykiss) Roe Process Biochem. 2008;43:376–83.
24. Sahena F, Zaidul ISM, Jinap S, Saari N, Jahurul HA, Abbas KA, Norulaini NA. PUFAs in Fish: Extraction, Fractionation, Importance in Health Compr. Rev. Food Sci. Food Saf. 2009;8:59–74.
25. Geirsdóttir M. Isolation, Purification and Investigation of Peptides from Fish Proteins with Blood Pressure Decreasing Properties; 2009.
26. Norris R, FitzGerald RJ. Antihypertensive Peptides from Food Protein (INTECH). 2013;46–70.
27. Ryan JT, Ross RP, Bolton D, Fitzgerald GF, Stanton C. Bioactive Peptides from Muscle Sources: Meat and fish. Nutrients. 2011;3:765–91.
28. Wenno MR, Suprayitno E, Aulanni'Am A, Hardoko. The Physicochemical Characteristics and Angiotensin Converting Enzyme (ACE) Inhibitory Activity of Skipjack Tuna (*Katsuwonus pelamis*) "Bakasang". J. Teknol. 2016;78:119–24.

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