DOI: https://doi.org/10.37905/jfpj.v4i2.15254

Jambura Fish Processing Journal
Vol. 4 No. 2 Tahun 2022
P-ISSN: 2655-3465 E-ISSN: 2720-8826

ANALYSIS OF PROTEIN AND BIOACTIVITY OF NIKE FISH (*Awaous melanocephalus*) EXTRACT AS ANTIOXIDANT

Yayurulia Hadju[1], Yuszda K. Salimi[1*], Nurhayati Bialangi[1], Netty Ino Ischak[1]
[1]Department of Chemistry, Universitas Negeri Gorontalo
Jl.Jenderal Sudirman No.06, Kota Gorontalo 96128, Gorontalo, Indonesia

*Korespondensi: yuszda.salimi@ung.ac.id
(Diterima 28-06-2022; Direvisi 20-07-2022; Dipublikasi 30-07-2022)

ABSTRACT

This study aims to determine the protein content and activity of nike fish (*Awaous melanocephalus*) extract as an antioxidant. Nike fish (amphidromous goby larva) is an endemic fish of Gorontalo which is consumed as food and contains minerals, fats, carbohydrates, amino acids, and high protein. Nike fish extract uses a buffer with several components such as water, Tris HCl 0.1 M pH 8.3, NaCl 2 M, CaCl 0.01 M, 2-Mercaptoethanol 1%, and Triton X-100 0.5%. Based on qualitative analysis, Nike fish protein extract was positive through biuret, ninhydrin, xantoproteate, and Pb sulfide tests. Judging from the quantitative analysis, nike fish extract using the biuret method using UV VIS and TVB-N spectrophotometers obtained 47.60% and 0.098%, respectively. The antioxidant activity of nike fish extract using the DPPH method (1,1-diphenyl-2-pycryhydrazyl) was 20.29 mg AEAC/g (Ascorbic acid Equivalent Antioxidant Capacity). IC_{50} (Inhibitory Concentration) is 520 ppm at low levels but has potential as an antioxidant.

Keywords: Antioxidant; IC50; Nike Fish; Protein; TVBN.

INTRODUCTION

Fish is one of the potential animal protein foods that contain bioactive peptides . Fish contains bioactive compounds such as omega-3 polyunsaturated fatty acids, protein hydrolysates, polypeptides, peptides, amino acids, vitamins, and minerals. Fish has a higher absorption of protein than other animal products. The protein content in fish consists of muscle protein which consists of about 70% structural protein (myofibril protein), myofibril protein contains about 32-38% myosin, 13-17% actin, 7% actomyosin, 30% water-soluble protein sarcoplasmic protein, and 6% stromal protein (Natsir, 2018).

Bioactive peptides usually consist of amino acids. Bioactive activity depends on the composition of amino acids as a constituent of protein. Proteins containing amino acids are very beneficial for health, especially as antioxidants because of their high quality with high digestibility (Susanto & Fahmi, 2012). Antioxidant bioactive peptides derived from foodstuffs can be developed as supplements or functional

foods. Can be used to help overcome the problem of inhibiting the oxidation of other molecules that generate free radicals that can trigger normal cell destruction reactions, proteins, and fats. The group content of a peptide compound can react with free radicals (Rinto *et al*., 2019). One of the fish that is used as a source of protein is Nike fish which contains 16.89% protein. Nike fish is one of the endemic fish species in the Gorontalo area with a cycle of emergence in large numbers in certain locations with a small size (2 -4 cm) it contains a source of animal protein. This protein is composed of essential amino acids and peptides which are important food substances for the body (Liputo *et al*., 2013). Yusuf (2011) conducted a study on the nutritional characterization of Nike fish. From the results of the study, it was found that Nike fish had 16.89% protein, 0.30% carbohydrates, 0.76% fat, and high magnesium and calcium minerals. Meanwhile, Kadir *et al*., (2007) analyzed the protein contained in Nike fish and obtained crude protein levels. The results showed differences in protein levels in Nike fish from the first day (2.7315%) and the last day of appearance (4.083%). This research was continued with the paper chromatography method to identify amino acids. The identification results indicate the presence of essential amino acids, namely Leucine, Isoleucine, Methionine, and Threonine in Nike fish.

Several treatments in fish processing cause loss of material in the form of sarcoplasmic protein and low molecular weight such as volatile amine levels from fish. Based on the description above, the researchers aimed to analyze the protein content and bioactivity of nike fish extract as an antioxidant.

RESEARCH METHOD

Material and Equipment

The materials used in this study were Nike fish as samples, Tris HCl 0.1M pH 8.3, NaCl 2M CaCl 0.01 M, 2-mercaptoethanol 1%, and Triton X-100 0.5%, distilled water, BSA (Bovine Serum Albumin), DPPH , biuret reagent, ethanol, H_2SO_4 , $Pb(NO_3)_2$, NaOH, HNO_3, Ninhydrin reagent, $CuSO_4$. K_2CO_3, HCl, TCA, and H_3BO_2. The tools used in this study include centrifuge, beaker, pipette, measuring cup, measuring flask, mortar, pestle, spatula, test tube, test tube rack, stand and clamp, conway dish, burette, erlenmeyer, analytical balance, oven, and a spectrophotometer. UV-Vis.

Research Method

Sample Preparation

Nike fish samples were taken from the sea waters of the city of Gorontalo. Fresh nickel is cleaned with clean water and drained. The samples were ground using a mortar and pestle and extracted using

buffer (Aquades, Tris HCl 0.1 M pH 8.3, NaCl 2M, CaCl 0.01 M, 2-Mercaptoethanol 1%, and Triton X-100 0.5%) with ratio of 1:10 then filtered to get the supernatant. The supernatant obtained was centrifuged at 7000 rpm for 30 minutes. The supernatant was then pipetted for further analysis.

Protein Qualitative Analysis

Qualitative analysis of protein is a test method to determine the presence of protein and amino acids in nike fish extract. These tests include the biuret test, ninhydrin test, xanthoprotein test, and Pb-sulfide test.

Protein Quantitative Analysis

The protein content of Nike fish extract was analyzed by the biuret method using a UV-Vis spectrophotometer. The extract was reacted with biuret reagent and incubated for 15 minutes. After measuring the absorbance at the maximum wavelength, the absorbance results are entered into the standard curve linear regression equation. The standard used is Bovine Serum Albumin (BSA) with concentrations of 2000 ppm, 4000 ppm, 6000 ppm, 8000 ppm, and 10000 ppm.

Protein content was obtained using the following formula: (concentration/sample weight) x 100%

Total Volatile Base (TVB) Analysis

The sample was weighed as much as 0.27 grams and added 10 mL of 7% TCA. is 1 mL of boric acid is inserted into the chamber in the Conway dish while the sample is inserted outside the Conway dish and then 1 ml of saturated K_2CO_3 is added. The same treatment was carried out on blanks with 5% TCA solution. Then incubated at 55℃ for 30 minutes. After incubation, both the sample and the blank were titrated with 0.02 N HCl until the color changed to pink as in the blank. The results of the titration are recorded and included in the calculation:

$$TVB = ((V\ sample - V\ blank) \times N\ HCl \times 14.007)/(sample\ weight)) \times 100\%$$

This test was carried out based on SNI 01-2354.8-2009 by introducing boric acid into the chamber in the Conway dish, while the supernatant and saturated potassium carbonate were added to the outside of the Conway dish. At that time there was decomposition of the meat extract which released the base which was evaporated by potassium carbonate and would be absorbed by boric acid. Boric acid is titrated with dilute (0.02 N) hydrochloric acid. This process is intended to remove volatile bases bound to Boric Acid. The same was done on the blank using 5% TCA. Fish is declared rotten if it has a VB content of >30 mg N/100 grams (Suwetja, 2013).

Antioxidant Activity Test

Testing of bioactivity as an antioxidant was carried out using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method to measure absorbance using a UV-Vis spectrophotometer. Standard solutions of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm were made, each taken as much as 2 mL, added 2 mL of DPPH, and incubated for 30 minutes. Then the absorbance was measured at a wavelength of 517 nm. A total of 0.00197 grams of DPPH was dissolved in 100 mL of ethanol, then the absorbance was measured at a wavelength of 400-600 nm.

Measurement of absorbance and % inhibition (IC_{50}) of the sample

Extract samples were made with concentrations of 200 ppm, 400 ppm, 600 ppm, 800 ppm, and 1000 ppm. 2 mL of each extract was taken and reacted with 2 mL of DPPH. The absorbance of the extract was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm to determine the percent inhibition of free radicals. The absorbance data was obtained from each concentration of each extract and the % inhibition value was calculated using the equation. The resulting linear equation is used to obtain the IC50 value. This value is the concentration obtained when the % inhibition is 50 from the equation y=ax + b. When % inhibition = 50, then to calculate the IC50 value the equation becomes: 50 = ax +b.

Data Analysis

The data were analyzed descriptively through tabulation of images and calculations through excell of standard curves and compared with secondary data.

RESULTS AND DISCUSSION

Nike Fish Extraction

The sample used in this study was fresh nike fish taken by fishermen in the Pohe sea. Extraction aims to break down cells so that the proteins contained in the cells can be dissolved. Triton X-100 is a kind of detergent that chemically damages cell membranes and causes chromosomal damage through the process of cell lysis by stretching the plasma so that with little physical friction the cells will split. Extraction with 2-Mercaptoethanol was carried out because it can act as a biological antioxidant that provides water solubility. Changes in pH decrease can affect the amount of protein to be extracted. The result of homogenization of the sample and the solvent in the form of a cloudy solution was then separated between the protein and its residue by centrifugation method. After being separated by centrifugation the extraction result in the form of residue (pellet) will be discarded and the filtrate (supernatant) will be analyzed further.

Figure 1. Nike Fish Extract

Protein Qualitative Analysis

The results of the qualitative protein test showed that the nike fish extract was positive for the biuret and ninhydrin tests with a purple color change. The xanthoprotein test was indicated by the presence of yellow lumps after the addition of NaOH and a positive Pb-sulfide test was indicated by the presence of lumps with a slightly black color. The results of the qualitative analysis are presented in Table 1.

Table 1. Results of Qualitative Analysis of Nike Fish Extract Protein

Protein Test	Results	Picture
Biuret Test	The color changes to purple (+)	
Ninhydrin Test	The color changes to purple (+)	
Test Xantoprotein	There is a change in color to purple (+)	
Test Xantoprotein	The color changes to purple (+)	

Positive results on the protein qualitative test indicate that nike fish extract contains protein and amino acids. Biuret test was carried out to identify the presence of peptides which were marked by a purple

color change. The results showed the presence of peptide bonds to form peptides in nike fish extract. Meanwhile, the ninhydrin test was used to determine the free amino acids in the form of isoleucine and leucine contained in the extract. The xanthoprotein test aims to determine amino acids containing aromatic groups with a yellow color change after heating. The results obtained are suspected to contain Nike fish extract containing acidic amino acids such as phenylalanine, tyrosine, and tryptophan. Pbsulfide test was carried out to identify the presence of sulfur in the amino acid cysteine. Based on the test results on nike fish extract containing sulfur which is characterized by the occurrence of precipitation by Pb metal which binds sulfur to form PbS salt.

Protein Quantitative Analysis

Quantitative analysis of protein was carried out to determine protein content by spectroscopic techniques using UV-Vis spectrophotometer. This technique is done by calculating the protein content based on the ability of the protein to absorb light in the UV-Visible region based on Biuret reagent. Determination of protein content by the biuret method was carried out on a sample solution and a standard solution of 2% BSA at a wavelength of 569 nm with an absorbance of 0.243. As a standard solution, pure albumin was used, namely Bovine Serum Albumin (BSA) with various concentrations of 2000 ppm, 4000 ppm, 6000 ppm, 8000 ppm, and 10000 ppm.

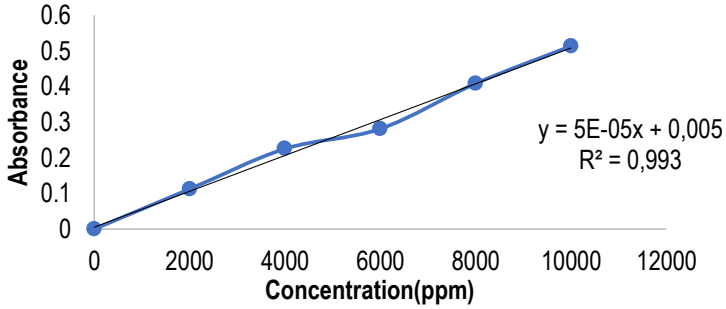

Figure 2. The BSA standard curve

From the results of the data obtained a curve (figure 2) between the absorbance of protein solution and its concentration. The regression value obtained is 0.993 which is close to 1 so that the value obtained is a good relationship between concentration and absorbance and follows Lambert Beer's Law. Based on the results of laboratory analysis, the protein content in 5 grams of sample was 2.38 grams so that the protein content was 47.60%. In contrast to the results of Yusuf's research (2011) which reported that the

protein content was 16.89%. This technique is performed by calculating protein levels based on the protein's ability to absorb light in the UV-Visible area based on biuret reagents. This method is based on the principle of substances containing two or more peptide bonds can form complex compounds of purple color with Cu salts in alkaline solutions. Determination of protein levels by the biuret method was carried out on Nike extract and a standard solution of 2% BSA at a long wave of 569 nm with an absorbance of 0.243. The advantage of using this method is the absence of interference from compounds that absorb at lower wavelengths. Protein quality is determined by the type and proportion of amino acids it contains. Complete protein or high quality protein is protein that contains all types of essential amino acids in amounts and proportions according to the body's needs. The results of Yusuf's research (2011) show that Nike fish contains essential amino acids in the form of arginine, histidine, isoleucine, valine, lysine, methionine, phenylalanine, threonine, and leucine.

Total Volatile Base (TVB) Analysis

TVB levels are used to measure the freshness of fish and as a limit for consumption. The quality of fish freshness can be known based on the amount of TVB levels, the higher the TVB levels, the lower the quality of a fish. Fish is declared rotten if it has a VB level > 30 mg Nitrogen/100 grams (Suwetja, 2013). The principle of TVB analysis is that volatile base compounds are evaporated from previously crushed samples, then these compounds are bound with boric acid and titrated with HCl (Wally *et al*., 2015). From the results of the study, the levels of TVB (mg N) were 0.098%. Fish is declared rotten if it has a VB content of >30 mg N/100 grams. So it can be concluded that the freshness level of Nike fish is in very fresh condition. So it can be concluded that the freshness level of Nike fish is in very fresh condition.

Antioxidant Activity Test

The method used in testing the antioxidant activity of the DPPH radical absorption method. DPPH acts as a model of free radicals that will bind to antioxidant compounds. This study uses ascorbic acid (Vitamin C) as a standard antioxidant for the manufacture of standard curves so that the unit of measurement is expressed as AEAC (Ascorbic Acid Equivalent Antioxidant Capacity). Vitamin C was chosen as the standard because Vitamin C is a natural antioxidant that has high antioxidant activity so its absorbance is easy to observe. In addition, Vitamin C also has a free hydroxy group that acts as a free radical and if it has a polyhydroxy group it will increase antioxidant activity so that it can be used as a comparison of natural antioxidants contained in the sample extract. The results of making standard curves are shown in Figure 3.

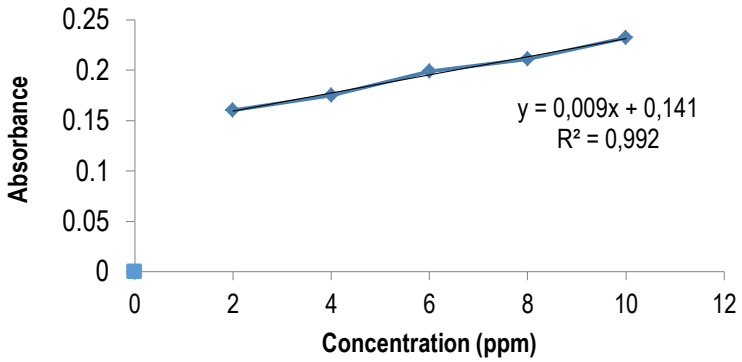

Figure 3. Ascorbic acid curve

Based on the figure above, a linear regression equation was obtained to determine the antioxidant activity (mg AEAC/g) of the sample. Next, the antioxidant activity of the sample extract was calculated which was expressed in AEAC. The antioxidant activity value obtained in the sample extract was 20.298 g/g or 20.29 mg AEAC/g where each gram of the extract was equivalent to 20.29 mg of vitamin C. The antioxidant activity test was carried out using a UV-Vis spectrophotometer. This quantitative test was conducted to determine the residual absorbance of DPPH after the extract was added. If a compound has activity as an antioxidant, there will be a decrease in the absorbance value of DPPH at a wavelength of 517 nm. From the obtained DPPH absorbance value, it can be determined the percentage value of DPPH radical inhibition (% inhibition). From the value of % inhibition can be determined the value of IC_{50} (inhibitory concentration).

The IC_{50} value is a number that indicates the concentration of the extract (ppm) which can inhibit the oxidation process by 50%. The smaller the IC_{50} value, the higher the antioxidant activity. The IC_{50} value was obtained from the linear regression equation while the antioxidant activity index value was determined by comparing the concentration of DPPH used in the test (ppm) with the IC_{50} value obtained (ppm) from the sample extract. The inhibition of DPPH free radicals is due to its ability to donate hydrogen. When DPPH free radicals meet proton donor substances such as antioxidants, radicals are captured and their absorbance value will decrease (decrease). The absorbance value obtained can then be calculated as the percentage value of free radical inhibition of DPPH, the greater the inhibition percentage, the higher the antioxidant activity. The IC_{50} value of the sample extract is 520 ppm, which means that 520 ppm of the sample extract is required to inhibit 50% of free radicals. The IC_{50} value in this extract is in the range of 100-1000 ppm when viewed from the level of antioxidant power, including at a very weak level but still has potential as an antioxidant. Bordbar *et al*., (2013) revealed that the size of the peptide and its solubility,

composition of amino acids, strands, and some free amino acids are the keys that determine the scavenging capacity of DPPH radicals.

According to Kedare and Singh (2011), weak antioxidant levels are influenced by DPPH radicals which are sensitive to compounds containing Lewis bases and the absorbance of DPPH in ethanol will decrease when exposed to light. This factor is another trigger for weak antioxidant activity. In addition, nike fish extract is a crude extract that is very influential on weak antioxidant activity, so that other compounds such as salt, minerals, and other nutrients that do not have a synergistic effect on antioxidant activity can inhibit the work of antioxidant compounds.

CONCLUSION

The nike fish extract was qualitatively positive for the biuret test, ninhydrin test, xanthoprotein test, and Pb-Sulfide test. Nike fish extract was analyzed quantitatively by the biuret method using a UV-Vis spectrophotometer, 47.60% content and 0.98% TVB-N content. The antioxidant activity of nike fish extract using the DPPH method (1.1-diphenyl-2-picrylhydrazyl) obtained a low antioxidant activity value of 20.29 mg AEAC/g and an inhibitory concentration (IC_{50}) of 520 ppm.

REFERENCES

Hanani, E., Munim, A., & Sekarini, R. (2005). Identification of Antioxidant Compounds In Callyspongia Sp Sponge From Thousand Islands. *Pharmaceutical Science Magazine, 2*(3), 127–133. https://scholarhub.ui.ac.id/mik/vol2/iss3/2.

Kedare, S. B., & Singh, R. P. (2011). Genesis and Development of DPPH Method of Antioxidant Assay. *Journal of Food, Scince, and Technology, 48*(4), 412-422. https://pubmed.ncbi.nlm.nih.gov/23572765/.

Liputo, S. A., Berhimpon, S., & Fatimah, F. (2013). Nutritional Value Analysis And Amino Acid Components And Fatty Acids From Nike Fish Nuggets (Awaous Melanocephalus) with the Addition of Tempeh. *Jurnal Chemistry. 6*(1). https://doi.org/10.35799/cp.6.1.2013.2070

Kadir, N. A., Bialangi, N., & Ischak, N. I. (2007). Protein Analysis of Nike Fish from Gorontalo. *Jurnal Entropi*, *2*(2).

Natsir, N. A. (2018). Analysis of Total Protein Content of Red Snapper and Duck Grouper Fish. *Biosel: Biology Science and Education, 7*(1), 49. http://dx.doi.org/10.33477/bs.v7i1.392.

Rinto, R., Baehaki, A., & Subarka, H. (2019). Study Of Antioxidant Aktivity, Anticolestrol And Antihypertence Of Extract Rusip. *Jurnal FishtecH, 8*(1), 18–27.

Susanto, E., & Fahmi, A. S. (2012). Functional Compounds From Fish: Their Application In Food. *Journal of Food Technology Applications, 1*(4), 95–102. http://www.jatp.ift.or.id/index.php/jatp/article/view/78.

Suwetja, I K. (2013). Fish Freshness Quality Index. Published by Bayumedia Publishing. Malang.

Wally, E., Mentang, F., & Montolalu, R. I. (2015). Chemical Quality Study of Skipjack Tuna (Katsuwonus pelamis L.) Smoke (Fufu) During Storage Room Temperature And Cold Temperature. *Fishery Products Technology Media, 4*(2), 7–12.

Yusuf, N. (2011). Nutritional characterization and estimation of the shelf life of nike fish savory chips (Awaous melanocephalus). Thesis. Bogor: Graduate School, Bogor Agricultural University.