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Extraction Of Albumin Of Snakehead Fish (*Channa Striatus*) In Producing The Fish Protein Concentrate (FPC)

Muhammad Asfar, Abu Bakar Tawali, Nurlailah Abdullah, Meta Mahendradatta

Abstract: This study aimed to investigate the optimal extraction method in extracting the albumin of snakehead fish and to know the nutrient content of fish protein concentrate so it can be consumed as functional food. The extraction of snakehead fish albumin was conducted by using water, HCl 0,1M, and ethanol 50% solvent with and without heating at temperature of 50-60°C for 10 minutes. The drying was conducted by mechanic dryer at temperature of 60-70°C. The parameters of this research were albumin, fat, total protein, yield, and dry basis water concentration. The result showed that the fish protein concentrate of snakehead fish with highest albumin (20,80%) and lowest fat (1,78%) was produced by the treatment of HCl 0,1M solvent by heating at temperature of 50-60°C for 10 minutes. Whereas the fish protein concentrate of snakehead fish with highest yield (6,41%) and total protein content (76,13%) was produced by the treatment of water solvent without heating. The optimal extraction method in extracting the fish protein concentrate of snakehead fish was the extraction by using HCl 0,1M solvent with heating at temperature of 50-60°C for 10 minutes.

Key words: Albumin, Extraction, Fish protein concentrate, Snakehead fish.

1 INTRODUCTION

In Indonesia, the snakehead fish is known as haruan, kutuk, kanjilo or bale bolong fish and classified in family of channidae or ophiocephalidae with scientific name *Channa striatus* (Pillay and Kutty, 2005) or in several references is called with scientific name of *Ophiocephalus striatus* (Bijaksana, 2004). Snakehead fish is one of the biological resources with high economic value due to its high content of albumin (Asfar, et al., 2007; Tawali, et al., 2012; Mustafa, et al., 2012; Sulistiyati, 2010). Albumin is a kind of globular protein dissolving in water, salt solvent and diluted acid (Winarno, 2004). This fact indicate that albumin protein of snakehead fish can be extracted to be a concentrate of fish protein. Fish protein concentrate is one of product result of fishery processing such as fish where the protein is a special component compared to other component in fish body. Fish protein concentrate is sometimes abbreviated with FPC. Fish protein concentrate is almost similar with fish powder, nevertheless the making of fish powder is not objected for human consumption but for livestock wool (Windsor, 2001). Fish protein concentrate is one way in providing the fish for human consumption with protein as the special component. The discovering of albumin protein in snakehead fish which beneficial for health promote this fish to have a high functional potency. The albumin extraction of snakehead fish for producing the albumin protein concentrate is expected to be the alternative of cheaper albumin source for clinical use. Based on this information, this study was conducted to investigate the method in processing snakehead fish (*Channa striatus*) into fish protein concentrate with high albumin.

2. MATERIALS AND METHODS

2.1. Equipment and Materials

Materials used in this study were snakehead fish (*Channa striatus*) obtained from Bili-bili watershed in South Sulawesi, Indonesia, hexane solvent, aquades, and clean water. Material for protein analyzing (Lowry method) were Na₂CO₃, NaOH, CuSO₄·5H₂O, NaK-tartrat, Bovinalbumin Folin-Ciocalteu Phenolreagenz, aluminium foil, roll tissue, plastic clip, plain plastic, and labeling paper. Materials for Kjehdahl method were concentrated sulfuric acid, oxide hydrargyrum, sulfuric potassium, hydroxide-sodium thiosulphate solution, saturated boric acid solution, chloride acid solution, and diethyl ether solvent.

2.2 Research Procedures

Extraction of albumin protein. Snakehead fish was weeded (remove the scales, gills, and stomach content) then was washed until there was no more blood and mucus, cut to be small pieces and remove the bones. Then it was smoothed using blender by adding solvent with ratio 1:1 (100ml solvent : 100g fish). Treatments in this research were as follows: A1B1= water solvent without heating; A1B2 = water solvent with heating at temperature 50-60°C for 10 minutes; A2B1 = HCl 0.1M solvent without heating; A2B2 = HCl 0.1M solvent with heating at temperature 50-60°C for 10 minutes; A3B1= ethanol 50% solvent without heating; A3B2 = ethanol 50% solvent with heating at temperature 50-60°C for 10 minutes. Sample from each treatments was filtered to separate liquid and dregs. Liquid was separated with its oil by adding 200ml of hexane solvent then shaken for 30 minutes. After forming two phases, the oil was separated by funnel. Extract liquid was dried in oven at temperature of 60-70°C. The dry extract was measured analyzed.

2.3 Samples Analyzing

Dry albumin protein extract was analyzed for water, albumin protein, total protein, total fat, and yield content.

- 1. Water content** (Apriyantono, et al., 1989). Smoothed materials was weighed as many as 3 grams then was put into aluminum foil that has been weighed.

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Materials was dried in oven at temperature of 100-105°C for 3-5 hours, then chilled in desiccators and weighed. Materials then was dried again in oven for 30 minutes, chilled in desiccators and weighed. This trial was repeated until the weight was constant. Calculation for water content used this formula:

$$\text{WaterContent} = \frac{(\text{Beginning_weight}) - (\text{ending_weight})}{(\text{ending_weight})} \times 100\%$$

2. **Albumin Protein Content** (Apriyantono, et al., 1989). Protein content was analyzed using Lowry Method as follow:

Reagent. 1) Natrium carbonate 2 gram in 500 ml NaOH 0,1 mol/L solvent. 2) Copper Sulfur 0,5 gram in 100 ml Na-K tartarat 1% solvent (made only before analysis). 3) Mixture of 50 ml reagent (1) with 1 ml reagent (2) (made only before analysis, stabil only for 1 day). 4) Reagent Folin Ciocalteau (fenol reagent), usually was available commercially, solution with aquades 1:1 before used. 5) Standard protein solution : 0,02 mg/ml Bovine serum albumin (BSA).

Standard Curve. A serie of concentration : 0 (blank), 0,1, 0,2, 0,3, 0,4, 0,6, 0,7, 0,8 and 1 ml standard protein was put into test tube. That water was added until the total volume of each was 4 ml. In each test tube 5 ml reagent (3) was added, mixed evenly and left for 10-15 minutes at room temperature. A 0,5 ml solvent (4) was added, mix evenly immediately after adding, then it was left for about 30 minutes until blue was formed coloration. Absorption was measured at 650 nm. The standard curve was made.

Sample Preparation. Sample must be in liquid state by adding water. The Solid phase was filtered after centrifugation. Attention should be given to dilution factor.

Determining of Samples. 0,1–1 ml sample was pipetted and put into test tube, then treated in the same way to standard determination.

3. **Protein Content** (Kjeldahl-Mikro Method). Approximately 0.5 gram of sample was weighed carefully, then added into kjedahl flask 100 ml. After that, an approximately 1 gram mixture of selenium and 10 ml concentrated H₂SO₄ (technical) was added. Khjedhal flask with its content was shaken until all the sample was wetted by H₂SO₄. Then it was destructed in acid cupboard until clear. The solution was left cool poured in to volumetric flask 100ml and rinsed by aquades, then added aquades until sign. An erlemeyer consisted of 10 ml H₃BO₃ 2 % + 4 drops of mixture indicator solution in erlemeyer 100 ml was prepared. 5 ml NaOH 30 % and 100 ml aquades were pipetted, then distilled until the container was filled about 50 ml. The end of distiller was rinsed use aquades then the container and its content was titrated using HCl or H₂SO₄ 0,0222 N solution until the solution changed into light red and did not disappear for 30 minutes.

Calculation:

$$\text{Protein_Content}(\%) = \frac{V \times N \times 0.014 \times 6.25 \times P}{\text{sample(gram)}} \times 100\%$$

V = Sample titration volume

N = Solution normality of HCl or H₂SO₄ 0,0222 N

P = Dilution factor = 100/5

4. **Fat Content** (Soxhlet method). Volumetric flask with suitable volume with soxhlet extraction tool was dried in oven, chilled in desiccator and weighed. Sample in powder state was weighed 5 g on lead filter, with suitable size, and closed by free-fat wool. As an alternative, the sample can be covered using filter paper. Lead or paper filter was put in soxhlet extraction, then the condensor was set on top and fat flask in down. Dietyl eter solvent was caunted and put into fat flask as needed, according to the size of soxhlet used. Reflux for minimum 5 hours was conducted until the fall solvent back to the clear fat flask. The solvent was destilated in fat flask, and collected then the fat flask containing fat of extraction was heated in oven at temperature of 105° C. After drying until constant weight and chilled in desiccator, the flask and its fat were weighed. The fat weight can be calculated as below:

$$\text{Fat}(\%) = \frac{\text{FatWeight(g)}}{\text{SampleWeight(g)}} \times 100\%$$

5. **Yield.** To calculate the yield of albumin protein concentrate, the formulation used was as follow :

$$\text{Yield}(\%) = \frac{\text{Result_Material_Weight(g)}}{\text{Raw_Material_Weight(g)}} \times 100\%$$

6. **Data Analysis.** Data analysis was conducted using data processing method of quantitative descriptive and Complete Randomized Design with four fold replication.

3 RESULTS AND DISCUSSION

Albumin Protein

Mean of concentrate albumin protein range from 13.83 % to 20.80%. Result of analysis of variance showed the interaction between the treatments significantly different at $\alpha = 0.05$.

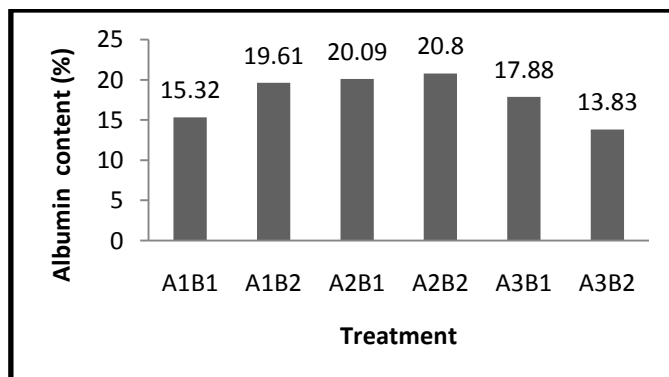


Figure 1. Histogram of mean of albumin content of snakehead fish protein concentrate

Analysis of albumin content as shown in figure 1 showed that albumin protein content was lowest among ethanol 50% solvent with heating (A3B2) (13.83%), and the highest among HCl 0.1M solvent with heating at temperature 50-60°C for 10 minutes (A2B2) (20.80%). It was found that dissolved protein (albumin) was soluble among all solvent. This fact agreed with Winarno (2002), that albumin is a globular protein, spherical protein dissolve in water, salt solvent and aqueous acid, and also easier change under temperature influence, salt concentration, and acid solvent. It was also found that dissolved protein (albumin) could dissolve in ethanol 50% solvent although with lower dissolved protein content.

Total Protein

Mean of concentrate total protein ranged from 64.12 % to 76.13 %. Result of analysis of variance showed that the interaction between treatments was not significantly different at $\alpha=0.05$.

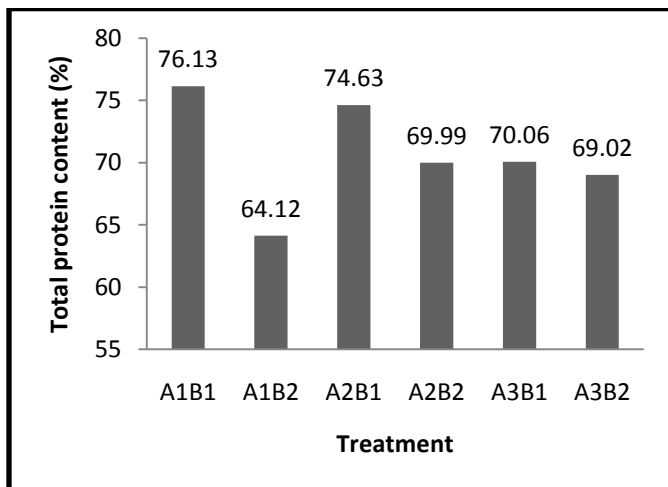


Figure 2. Histogram of mean of protein content of snakehead fish protein concentrate

Result of total protein content analysis in figure 2 showed that the highest content of total protein was by treatment water solvent without heating (76.13%), whereas the lowest was by treatment water solvent by heating at temperature of 50-60°C for 10 minutes (64.12%). Based on this protein content of concentrate, the result generally met the criteria as fish protein concentrate because according to Ruiter (1995), the specific criteria of fish protein concentrate for quality I has protein content of 80%, the quality II has protein content of 75% and quality III has protein content 55%. This was agreed with Windsor (2001) opinion that generally fish protein concentrate product contained of 65% protein and high quality of fish protein concentrate contained 80% of protein.

Fat

Mean of fat content in concentrate ranged from 1.71 % to 4.76 %. Result of analysis of variance showed that the interaction between treatments significantly different at $\alpha=0.05$.

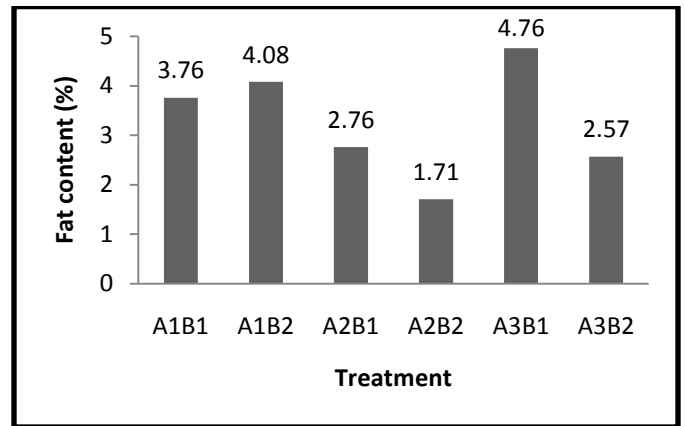


Figure 3. Histogram of mean of fat content of snakehead fish protein concentrate.

Analysis of fat content of protein concentrate as shown in figure 3 showed that the highest content of fat content was by treatment with ethanol solvent without heating (4.76%), whereas the lowest was by treatment with HCl 0.1M solvent with heating for 10 minutes (1.71%). The lower fat content the better quality of fish protein concentrate, because its correlation with fat oxidation could cause rancidity in concentrate. According to classification of fish protein concentrate type by Windsor (2001), the fish protein concentrate can be classified into 3 type: 1) type A: tasteless and odorless powder with total content of fat 0.75%, 2) type B: powder without specific border for odor and taste, but has the fish taste with total fat content 3%, 3) type C: fish powder produced at hygiene condition.

Water Content

Mean of water content of fish protein concentrate ranged from 8.49% to 21.53%. Analysis of variance showed the interaction between treatments was significantly different at $\alpha=0.05$.

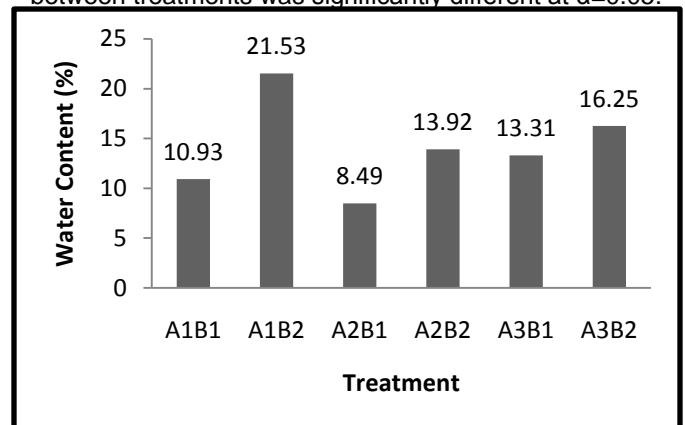


Figure 4. Histogram of mean of water content of snakehead fish protein concentrate.

Water content analysis of fish protein concentrate as shown in figure 4 showed that the highest water content was by the treatment of water solvent by heating at temperature of 50-60°C for 10 minutes (21.53%), and the lowest was by treatment of HCl 0.1M without heating (8.49%). The lower water content the better quality of concentrate, this is due to the activity of microbe. Quality criteria for fish protein concentrate by Ruiter (1995) was as follow : quality I

contained maximum 10% of water content in fish protein concentrate, while quality II and III contained maximum 12%. Based on the water content, treatment of water solvent without heating only which met the criteria for quality II and III, and HCl 0.1M solvent without heating which met the criteria for quality I of fish protein concentrate.

Yield

Mean of concentrate yield obtained in this study ranged from 2.74% to 6.41%. Analysis of variance showed the interaction between treatments was not significantly different at $\alpha=0.05$.

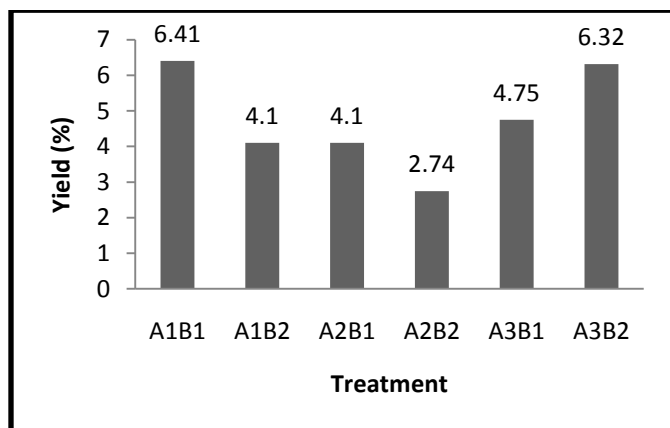


Figure 5. Histogram of mean of yield content of snakehead fish protein concentrate.

Yield of snakehead fish concentrate as shown in figure 5 showed that the highest yield was by the treatment of water solvent without heating (6.408%), while the lowest was by the treatment of HCl 0.1M by heating at temperature 50-60°C (2.74%). The higher yield of concentrate the better result for economic factor of a method.

4 CONCLUSIONS

Snakehead fish albumin protein concentrate containing highest albumin (20.80%) with lowest fat content (1.78%) was in the treatment of HCl 0.1M by heating at temperature of 50-60°C for 10 minutes, whereas the concentrate with highest yield (6.41%) and raw protein content (76.13%) was in the treatment of water solvent without heating. The optimal extraction method in producing snakehead fish protein concentrate was the albumin protein extracted using HCl 0.1M by heating at temperature 50-60°C for 10 minutes.

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