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EXTRACTION OF SNAKEHEAD FISH [*Ophiocephalus striatus* (Bloch, 1793)] INTO FISH PROTEIN CONCENTRATE AS ALBUMIN SOURCE USING VARIOUS SOLVENT

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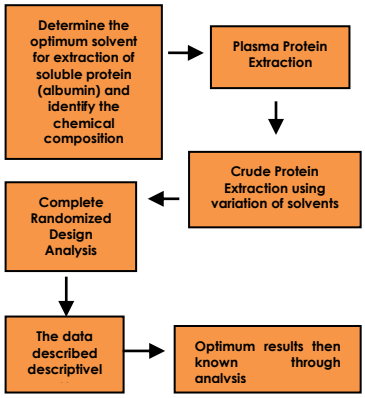
Graphical abstract



Abstract

Study aimed to determine the optimum solvent for extraction of soluble protein (albumin) and identify the chemical composition of snakehead fish protein concentrate. The method was experimental while the treatments were the variation of solvents: distilled water, HCl 0.1 M, and NaCl 0.9 %. Soluble protein (albumin) and yield parameters analyzed by using completely randomized design (CRD) which consist three treatments and four replications, the other parameters were described descriptively. The result showed that the highest soluble protein (albumin) (7.65 %) was produced by HCl 0.1 M solvent with 2.55 % yield, 10.76 % dry basis moisture content, 63.78 % total protein content, and 2.54 % fat content.

Keywords: Extraction, fish protein concentrate, snakehead fish [*Ophiocephalus striatus* (Bloch, 1793)], soluble protein, solvent

1.0 INTRODUCTION

Albumin is a protein which is soluble in water and could be coagulated by heat. This compound can be found in blood serum and eggwhite. In human plasma, albumin is the majority protein (4.5 g dL^{-1}) which is about 60 % of total plasma [1]. Along with the increasing number of hospitals that utilized snakehead fish as a source of albumin for hipoalbumin and wound healing, the albumin products have a specific target market. Traditionally albumin is obtained by steaming the fish until the cloudy white color filtrate is acquired. The volume that can be generated is 65 mL per 100 g of material. Levels of albumin filtrate ranged from 0.46 g to 1.50 g per 100 mL [2].

Albumin extraction of snakehead fish is expected to be the alternative of cheaper albumin source for clinical use. The use of a suitable solvent can increase the amount of albumin that can be extracted. Albumin is a globular protein which soluble in water, salt and acid solvents [3]. This fact indicates that albumin protein of snakehead fish can be extracted to be a concentrate of fish protein using among solvents. Fish protein concentrate is a product that is produced by removing fat and water, thus, containing higher protein concentration [4].

Based on the information, this study was conducted to determine the solvent which could optimize the extraction of soluble protein (albumin). The other objective is to identify the chemical composition of snakehead fish protein concentrate.

2.0 MATERIAL AND METHOD

2.1 Equipment and Materials

Equipment used in this study were knife, cutting board, digital scales, blender and grinder, cabinet oven, glasswares, separating funnel, water bath, Buchner funnel, Ultraviolet-visible spectrophotometer, cuvette, and 50 mL ependorf tubes. Materials used in this study were snakehead fish [*Ophiocephalus striatus* (Bloch, 1793)] from Gedebage Fish Market, Bandung, West Java, hexane solvent, distilled water, and clean water. Material for protein analyzing (Lowry method) were Na_2CO_3, NaOH, $CuSO_4.5H_2O$, 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 Procedures

Snakehead fish was weighed then cleaned/ weeded (discarded scales, entrails, gills, fins, head and skin). Fish were washed (no blood) then drained and separated between the meats with bones, cut into small pieces. All the fish meats that has been cut combined into one. The Meats were put in a blender and solvent was added with a ratio of 1:1 (100 mL solvent : 100 g of fish meat).

The treatments in this study are:
A: Fish meat was added distilled water and heated at a temperature of (50 ± 10) °C for 15 min.
B: Fish meat was added 0.1 M HCl and heated at the temperature (50 ± 10) °C for 15 min.
C: The fish meat is added 0.9 % NaCl and heated at temperature (50 ± 10) °C for 15 min.

Sample from each treatment was filtered to separate liquid and dregs. Liquid was separated with its oil by adding hexane solvent with ratio 1: ¼ (liquid extract: hexanes solvent), then shaken till no gas. After forming two phases, the oil was separated by funnel. The filtrate is dried using an oven with a temperature of 50 °C to 55 °C for approximately 12 h and grinded till smooth. The dry extracted was analyzed.

2.3 Sample Analysis

2.3.1 Soluble Protein (Albumin)

Soluble protein content was analyzed using Lowry method as follows [5]. Initially, four reagents were prepared, i.e.:
(i) Natrium carbonate 2 g in 500 mL NaOH 0.1 mol L^{-1}.
(i) Copper Sulfur 0.5 g in 100 mL Na-K tartarat 1 % (w/v)
(ii) Mixture of 50 mL of reagent A and 1 mL of reagent B (stable for 1 d).
(iii) Reagent Folin Ciocalteau or fenol reagent (usually available commercially, dillute with distilled water 1:1 v/v before used)
(iv) Standard protein solution, i.e. 0.02 mg mL^{-1} Bovine serum albumin (BSA).

For standard curve preparation, a series of standard protein, i.e. 0 mL, 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL, 0.6 mL, 0.7 mL, 0.8 mL and 1 mL was placed into test tube. Distilled water was added until the total volume of each solution was 4 mL. In each test tube, 5 mL of reagent C was added, mixed evenly and left for 10 min to 15 min at room temperature. Next, 0.5 mL of reagent D was added, mixed evenly immediately after addition, then it was left for about 30 min until blue color was formed. Absorption was measured at 650 nm.

Sample was added with distilled water to form liquid state. The solid phase was filtered after centrifugation. Dilution factor should be taken into consideration for sample determination. Subsequently, 0.1 mL to 1 mL of sample was placed into test tube, then treated in the same way as standard protein determination.

2.3.2 Yield

To determine the yield of snakehead fish protein concentrate, the formulation is as the following:

$$\% \text{ Yield} = \frac{\text{Dry Extract (g)}}{\text{Fish Meats (g)}} \times 100\ \% \quad (1)$$

2.3.3 Total Protein Content

Total protein content was analyzed using Kjeldahl-Micro Method [6]. Approximately 0.5 g of sample was weighed carefully the placed into 100 mL Kjedahl flask. After that, approximately 1 g mixture of selenium and 10 mL of concentrated H_2SO_4 was added. The flask was shaken until all the sample was wetted by H_2SO_4. Then it was destructed in acid cupboard until clear. The solution was diluted with distilled water until 100 mL. An erlenmeyer consisted of 10 mL H_3BO_3 2 % and four drops of mixture of indicator solution was prepared. Into the mixture, 5 mL of NaOH 30 % and 100 mL of distilled water were added, then distillation was performed until the container was filled for approximately 50 mL. The end

of distiller was rinsed with distilled water then the container and its content was titrated using 0.0222 N HCl or H_2SO_4 until the solution became light red and did not disappear for 30 min. The calculation of total protein content is as follows:

$$\% \text{ Total Protein Content} = \frac{V \times N \times 0.014 \times 6.25 \times P}{\text{Sample (g)}} \times 100\ \% \quad (2)$$

in which
V = sample titration volume
N = solution normality of HCl or H_2SO_4 (0.0222 N)
P = dillution factor = 100/5

2.3.4 Fat Content

Volumetric flask with suitable volume with soxhlet extraction tool was dried in oven, chilled in desiccator and weighed. Then, 5 g of sample in solid state (powder) was placed 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 condenser was set on top and fat flask at bottom. Diethyl ether was inserted into fat flask. Reflux was performed for minimum 5 h was until the fall solvent came back to the clear fat flask. The solvent was distillated in fat flask and collected, then the fat flask containing fat extract was heated in oven at 105 °C. After drying until constant weight and chilled in desiccator, the flask and its fat were weighed. The fat weight can be calculated using formulation below:

$$\% \text{ Fat} = \frac{\text{Fat Weight (g)}}{\text{Material Weight (g)}} \times 100\ \% \quad (3)$$

2.3.5 Dry Basis Moisture Content

Prior to analysis, 3 g of grinded sample was placed into aluminum foil that has been weighed. Sample was dried in oven at 100 °C to 105 °C for 3 h to 5 h, then chilled in desiccators and weighed. Sample was dried again in oven for 30 min, chilled in desiccators and weighed. The experiment was repeated until the weight was constant. Value of water content was calculated using following equation:

$$\% \text{ Dry Basis Moisture} = \frac{\text{Beginning Weight} - \text{Ending Weight (g)}}{\text{Ending Weight (g)}} \times 100\ \% \quad (4)$$

2.4 Data Analysis

Data analysis was conducted using Complete Randomized Design consisting of three treatments with four fold replication using analysis of variance following by duncan test at α= 0.05.

3.0 RESULTS AND DISCUSSION

3.1 Soluble Protein (Albumin)

Mean of soluble protein (albumin) content of snakehead fish protein concentrate range from 5.83 % to 7.65 %. Result of analysis of variance showed significantly different between treatments at α = 0.05. Result of each treatment can be seen in Table 1.

Table 1 Mean of soluble protein (albumin) of snakehead fish protein concentrate

Treatments	% soluble protein (albumin)
A	6.51 [a]
B	7.65 [b]
C	5.83 [a]

Note: Numbers which followed by the same letters are not significantly different at α= 0.05.

Table1 shows that (albumin) or dissolved protein in the snakehead fish could be soluble amongst all of solvent. Albumins are soluble in water, able to dilute acid solutions and saline solution. Albumin is a globular protein which is soluble in water, in acidic or alkaline solution also in ethanol [7]. Solubility of albumin in water indicates that the albumin is polar, to dissolved a compound that was polar, the polar solvent must be used, in this case distilled water, 0.1 M of HCl and 0.9 % of NaCl were the polar solvent [8].

In general, increased level of soluble protein after treatment was due to hydrolysis of the soluble protein in the fish. Hydrolysis of proteins is the process of the breakup or breakdown of peptide bonds of proteins into simpler molecules with solvents assistance. Hydrolysis of peptide bonds will cause some changes in the protein, which increases the solubility due to the increase in the ion content of the amine ($NH3^+$) ions and carboxyl (COO^-), thus, results in lower molecular weight proteins or polypeptides as well as unraveling the structure of globular proteins [9].

From the Table 1 it was found that treatment B have the highest soluble protein content because the addition of HCl 0.1 M acidic solvent would increase the solubility of proteins because the positive ions from the acid induced positively charged protein. The addition of 0.1 M HCl solvent acidic results in acid solvent which affects the solubility of the protein. Distilled water has a pH of 7, 0.9 % NaCl solvent has a pH between 4.5 to 7.0 and 0.1 M HCl solvent has pH below 2 [10]. The isoelectric point of albumin ranged from 4.6 to 4.9 [11]. Each amino acid has a different isoelectric point. Isoelectric point was the moment when the amino acids are in the form of amphoteric (zwitter ion) or double–charged. Zwitter ion form is volatile because it is influenced by the circumstances or the pH of the environment. At isoelectric point, protein solubility decreases and reaches the lowest number, then the protein will precipitate and agglomerate, when the

isoelectric point is the number of cations and anions that are formed as much [12]. In addition, protein solubility will increase if the acidity of the protein solution is getting away from the isoelectric point [13].

One of the amino acids which is contained in snakehead fish is tyrosine. Based on the properties, tyrosine is slightly soluble in water and alcohol, but is more soluble in a dilute acid solvent and alkali hydroxide solvent because tyrosine has the R groups and tends to release H^+ ions which also slightly ionized at neutral pH [14]. Tyrosine tends to be more soluble in acid, thus, it is likely that 0.1 M HCl could dissolve more tyrosine amongst the other solvents which have a correlation on the results of the analysis.

3.2 Yield

Mean of yield obtained in this study ranged from 2.55 % to 4.37 %. Analysis of variance showed significantly different between treatments at a = 0.05. Result of each treatment can be seen in Table 2.

Table 2 Mean of yield content of snakehead fish protein concentrate

Treatments	% yield
A	4.37[b]
B	2.55[a]
C	3.26[a]

Note: Numbers which followed by the same letters are not significantly different at α= 0.05.

From Table 2, it was found that treatment A had the highest yield due to the amount of moisture in the fish protein concentrate that affected the yield. Snake head fish protein concentrate which was made using distilled water solvent had high water content among the other concentrated produced from other solvents, thereby affecting the mass of the product. The amount of yield of the products can be known from the moisture content of the product. The higher the moisture content of a product, the higher the yield produced, because high water levels resulted in increased product mass [15]. While the lower water content would cause a reduction in material weight, because the water in the material was the main component that affects the weight of the material. If water was removed, the material would be lighter so it would affect the yield of the final product [16].

The high yield was generated by using distilled solvent and this signifies that distilled water can dissolve chemicals compared to other solvents. Due to the polarity, water can interact and dissolve many compounds chemical judging from the highest yield produced from distilled solvent [17].

The yield of fish protein concentrate from snakehead fish had a relatively low yield. The low yield of fish protein concentrate cork is also caused by the drying effect. Besides for preservation, drying also aims to reduce the volume and weight of the product [18]. Therefore, the moisture content could decrease 60 % to 70 % producing materials in a low yield value.

3.3 Proximate Analysis

Proximate analysis results from treatment A were 58.77 % protein content, 5.13 % fat content and 13.51 % dry basis moisture content. Treatment B were 63.78 % total protein content, 2.54 % fat content and 10.76 % dry basis moisture content. Treatment C were 64.01 % protein content, 2.23 % fat content and 9.01 % dry basis moisture content. Result of each treatment can be seen in Table 3.

Table 3 Proximate analysis of snakehead fish protein concentrate

Treatments	protein content (%)	fat content (%)	dry basis moisture content (%)
A	58.77	5.13	13.51
B	63.78	2.54	10.76
C	64.01	2.23	9.01

Snakehead fish protein concentrate which was made using 0.9 % NaCl solvent had the lowest dry basis moisture content due to the salts which can bind with water, leading to the withdrawal of water from food, thus the water activity in food would decrease and inhibit the growth of microorganisms. Salt inhibits bacteriological and enzymatic activity caused by the action of the osmotic salt solution to food because food acts as a semi-permeable membrane, thus lowering the water content [19].

Good quality requirements of fish protein concentrate were at least 67.5 % of total protein content, maximum 0.75 % of fat, does not smell fishy and has good color. The highest protein content was obtained at treatment using 0.9 % NaCl solvent, which was 64.01 %. The result was higher than previous study with a total value of 62.9 % protein [20]. The percentage of protein of some fish protein concentrate showed a high protein content. In addition, the content of proteins that vary in some protein concentrate is influenced by several factors, including the type of fish, methods of extraction, also types of solvent and drying [21].

High or low value of protein content can also be influenced by the amount of moisture loss (dehydration) of the material. Protein content will be even greater if the amount of water loss increases. The protein content depends on the amount of ingredients added and is largely influenced by moisture content [22]. Moreover, protein content of the fish is affected by moisture, where there is an inverse relationship between protein and water content, in which the higher the protein content increases as the water content decreases [23].

The protein content of food will be higher than the raw material if other components (non-protein) have been removed because the protein in food has affinity complex with other components such as carbohydrate, fats, minerals and water. However, the process of removal of non-protein components causes the protein to be free, thus, it is more easily to measure current and the analysis becomes accurate. There is an increase in the protein content of fish protein concentrate cork from all treatment variations compared to the raw material. Fresh snakehead fish had a protein content of 21.98 %, but once processed into fish protein concentrate using a variation of the solvent, content of protein increased by 58.77 % to 64.01 % [24, 25].

Fat content of snakehead fish protein concentrate ranged from 2.23 % to 5.13 %. This suggests that fish protein concentrate had a low fat content. Low fat content is an indicator of the quality of good fish protein concentrate, because the high fat content also affected the process of rancidity in product [26]. Rancidity occurs when components of taste and smell of volatile formed as a result of oxidative damage of unsaturated fats and oils. These components caused the smell and taste of unwanted fat and oil and products containing fats and oils [23].

Low level of fat in the snakehead fish protein concentrate is also influenced by the process of meat cutting which can help releasing fat and water because it can increase the surface area in contact with hot material upon heating. Downsizing will facilitate the penetration of moisture and hot air in the cells containing fat. The smaller the surface area formed as a result of cuts, the greater the contact material with the heat, causing the composition of fatty damaged impact on the levels of fat [27].

The use of hexane solvent in this study also affected the low fat content of the product. All the filtrate was extracted using a solvent variation of distilled water, HCl 0.1 M, NaCl 0.9 %. Fat was partially separated using hexane. The non–polar *n*–hexane will dissolve non–polar fatty acid; thus, affect the fat content of a product which was treated with fat separation. As well as principles like-dissolved-like that says that polar material is only soluble in polar solvents, as well as non–polar material is only soluble in non- polar solvents, in this case the fat in the filtrate will be dissolved in hexane, because both are non–polar. Hexane is a good enough to bind most of the fat contained in food and volatile material [28].

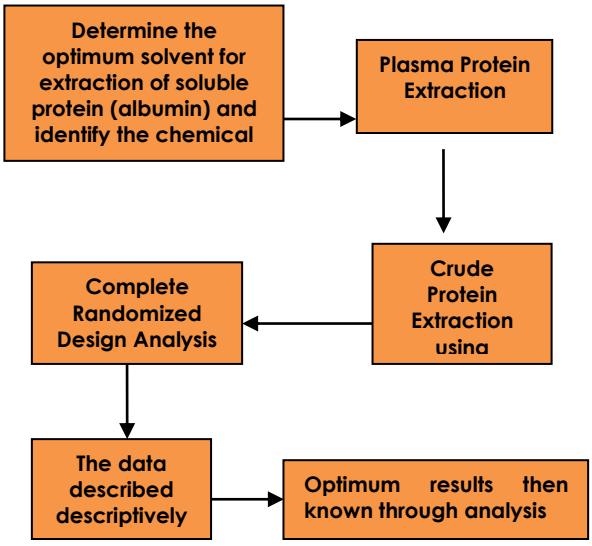


Figure 1 Block diagram of the processes of the system

From Table 1 it can be concluded that treatments B and C match the criteria of B type in fish protein concentrate, because generally fish protein concentrate products contain 60 % to 80 % of total protein, maximum 10 % of dry basis moisture content and a powder without specific border for odor and taste, but has the fish taste with total fat content 3 % [23], while treatment A met the criteria of C type. C type fish protein concentrate was produced at hygiene condition and it contained maximum 12 % of dry moisture content and slightly lower than 60 % total protein content [26]. The summarize of procedures in this research can be found in Figure 1.

4.0 CONCLUSION

The result showed that the fish protein concentrate with highest soluble protein (albumin) (7.65 %) was produced by the treatment of HCl 0.1 M solvent with 2.55 % yield, 10.76 % dry basis moisture content, 63.78 % total protein content and 2.54 % the fat content.

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