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Protein Content Test of Tilapia Fish Extract (Oreochromis Mossambicus) before and after Freeze Dry Using Biuret Method

Walin[1], Hardono Susanto[2], Bagoes Widjanarko[3], Nurjazuli Nurjazuli[4]

[1]Doctoral Student, Faculty of Public Health, [2]Professor, Faculty of Medicine, [3,4]Associate Professor, Faculty of Public Health, Diponegoro University, Semarang, Indonesia.

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Abstract

Objective: To determine the difference in protein content of tilapia extract before and after drying process.

Materials and Methods: This study was designed with a true experimental design. The research sample was tilapia fish extract with 3 replications in each sample. The measurement of protein content was carried out on samples before and after drying. The drying process of tilapia extract was carried out for 48 hours using a freeze dryer. Furthermore, the dried samples were tested for protein content using the biuret method supported by the UV-Vis Spectrophotometer instrument. The testing stages include making the standard solution, determining the maximum wavelength, determining the operating time, and making a standard curve.

Results: Showed that the protein content of tilapia extract before and after drying was 2.23% and 27.4%, respectively. The results of statistical analysis showed that there was a significant difference between the protein content of tilapia fish before and after drying.

Conclusion: There was an increase in protein content of tilapia fish extract after drying by 25.17%.

Keywords: Protein; Tilapia Fish; Extract; Freeze Dry; Biuret.

Introduction

Tilapia fish (Oreochromis mossambicus) is one of the freshwater fishery commodities. Tilapia fish easily live and breed in various conditions such as water with high salinity and low salinity/saltiness content. There are several nutritional contents that is very beneficial for human health, one of them is protein which help the process of growth and development of organs, membranes and cells. The protein content in tilapia fish is higher when compared to other fish, which is around 18.7%.[1] The high nutritional value contained in tilapia fish encourages people to process it into various kinds of food products.[2]

Tilapia fish can also be used as a supplement with various processing techniques such as steaming, boiling, roasting and frying. Fish meat that is processed with high heating temperatures and for a long time can cause protein denaturation.[3] This problem can be overcome by making the mozambique tilapia fish in extract form and dried using a freeze dryer which is safer against the risk of degradation of compounds in the extract. Freeze dryer is the best drying process

Corresponding Author: Walin, Doctoral Student, Faculty of Public Health, Diponegoro University, Semarang, Indonesia.

Email: walinpwt@gmail.com

for thermolabile products because it can reduce the nutritional degradation of a product. Freeze dryers are widely applied to the food industry, pharmaceutical industry, and health product industry.[4]

Based on the research of Asrullah et al. (2012), it is known that the decrease in content and changes in protein digestibility depend on the treatment method, so it is necessary to measure protein content before and after freeze dry using the biuret method. Determination of protein content of tilapia fish extract can be done using the biuret test supported by a UV-Vis spectrophotometer instrument.[5] The biuret test was chosen because it is simpler and has less color deviation occurs when compared to other methods. The biuret method is based on measuring the absorption of purple light that comes from the reaction between protein and biuret reagent.[6] This study was conducted to determine the protein content of tilapia fish extract (*Oreochromis mossambicus*) before and after the drying process freeze dryer using the biuret method.

Materials and Methods

This study was designed with a true experimental design.

Tools and materials. The tools used in this study are hydraulic press (modification), centrifuge, freeze dryer, UV spectrophotometer, measuring cup, measuring flask, test tube, beaker glass, analytical scale, water bath, napkin, gas stove, pan, volume pipette, spatula, plastic container, horn spoon, mortar, stamper, watch glass, and stirring rod. The materials used in this study are tilapia fish (*Oreochromis mossambicus*), aquadest, biuret reagent, Bovine Serum Albumin (BSA) solution.

Sample preparation. Tilapia fish with 300 grams weight is cleaned form its scales and then the contents of its stomach are removed and washed thoroughly with running water. The fins and head are cut and the body of the fish is sliced. The fish is steamed in steamer pan for 50 minutes, keeping the heat from getting too high so it doesn't boil (about 90-95°C) and the water doesn't get into the fish team. Put the fish in a high-pressure squeezer machine while it is still hot. The juice is filtered and packaged immediately to avoid contamination in a 200 ml capasity thick plastic then sealed it with electric sealer. After packing is completed, check that there are no leaks in the packing. After all the steps are done, stored the product in the freezer with temperature below 10°C.

Freeze Dry. The water phase of tilapia fish extract was put into a tube with an equalized volume then the tube was put into the freeze dryer. Drying is processed automatically, the freeze dry state must be ensured that the power is in the "off" position, all buttons and valves are in the "off" and "close" position. After the "power" cable is connected to an electric current source, pressed the power button to "on" position (for lexol circulation) ± 24 hours before the operation.[7]

Protein Content Test Using Biuret Method

Preparation of Solutions. A total of 0.05 grams of Bovine Serum Albumin (BSA) was dissolved with distilled water in a 10 ml volumetric flask to the mark, so that a standard solution with a concentration of 5000 ppm was obtained.

Determination of Maximum Wavelength. 0.4 ml of standard solution was added with distilled water up to 4 ml then added with 6 ml of biuret reagent, then analyzed by UV spectrophotometry at a wavelength of 400-700 nm. The results of this study indicate that the maximum wavelength is 592.8 nm at an absorbance of 0.2385.

Determination of Operating Time. 0.4 ml of standard solution was added with distilled water up to 4 ml, then 6 ml of biuret reagent was added, then analyzed by UV spectrophotometry for5-45 minutes at the maximum wavelength obtained in the previous stage. Operating time is determined by measuring the absorbance at the maximum wavelength from the data that has been generated at the determination of the maximum wavelength of 592.8 nm. The best operating time result in this study was in minutes 35 because in this time a stable product compound began to form, with an absorbance value of 0.2318.

Standard Curve Creation. Standard curve is made to determine the equation of linear regression. This is done by preparing 12 test tubes. The first tube is filled with blank solution (solvent). In the second tube and so on, it is filled with the composition according to the table below and the absorbance is measured using a spectrophotometer with a maximum wavelength then made it into a curve to obtain a linear equation.

Table 1: Composition of standard curve tube

BSA standard solution (ml)	H_2O (ml)	Biuret Reagent (ml)	Standard Consentration (ppm)
0.0	4	6	0
0.1	3.9	6	125
0.2	3.8	6	250
0.4	3.6	6	500
0.6	3.4	6	750
0.8	3.2	6	1000
1.0	3	6	1250
1.2	2.8	6	1500
1.4	2.6	6	1750
1.6	2.4	6	2000
1.8	2.2	6	2250
2.0	2	6	2500

Based on the concentration data of the standard solution with the absorbance of the solution, the results obtained from the linear equation y = 0.00008x + 0.0964 with a value of R2 = 0.9984. The equation shows that b is positive, which means that the higher the concentration, the higher the absorbance value. The value of the correlation coefficient (R2) in this study was in the range between 0.80 to 0.99 which was classified as a very strong correlation.[8]

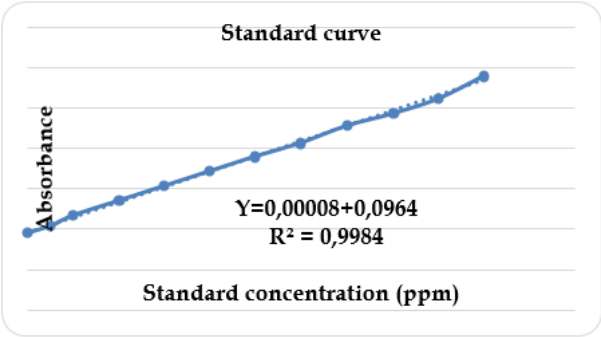

Figure 1: Standard curve

Results

Protein content tests were carried out on a liquid tilapia fish extract (before freeze dry) and solid tilapia fish extract (after freeze dry). The protein content test was replicated 3 times on each sample using the UV spectrophotometer. The volume of each liquid samples were 5 ml. From Table 2 it is known that the average protein content of liquid tilapia fish extract (before freeze dry) is 2.230% and the average protein content of solid fish extract (after freeze dry) is 27.400%.

Table 2: Results of Protein Content Analysis of Tilapia Fish Extract

Sample (replication)	Protein content (%)		Statistical test results paired t-test (p-value)
	Before drying	After drying	
1	2,165	27,900	0,0001
2	2,215	26,750	
3	2,310	27,550	
Average	2,230	27,400	

The results of the statistical paired t-test showed the p-value = 0.0001 < (0.05). This means that there is a significant difference between the average level of protein of liquid tilapia fish extract (before freeze dry) and solid (after freeze dry). The difference in the average value of protein content before and after freeze dry is 25.17%. A positive difference value indicates that there is an increase in the average protein content of tilapia fish extract after freeze dry.

Discussion

The results were significantly difference between the average level of protein of liquid tilapia fish *extract* (before *freeze dry*) and solid (after *freeze dry*). The difference in the average value of protein content before and after *freeze dry* is 25.17%. A positive difference value indicates that there is an increase in the average protein content of tilapia fish *extract* after *freeze dry*. Supported by the research of Saputro et al. (2019) which stated that there was a significant difference between the protein content of snakehead fish *extract* before and after drying. This study is also in accordance with previous research by Andana (2018) which stated that there was an increase in protein content of processed fish products (surimi) after the drying process from 9.69% to 44.87%.[9,10]

The drying process of fish extract can reduce the percentage of water content which can increase the percentage of protein content. This is supported by Ahmed et al. (2010) who stated that reduced water content resulted in high ash, protein and fat content. In the study of Alhanannasir et al. (2017), water absorption by freeze dry proven to be higher and shorter than the oven method. The amount of water absorbed by oven method is 4.09% for 60 minutes, while the amount of water absorbed by freeze dry can reach 45.29% in just 20 minutes. The research of Setyowati et al. (2017) also stated that the absorption of water with the freeze dry method is the best when compared to the cabinet and oven drying methods.[11–13]The drier the material, the higher the protein content contained in it.[14]

In this study, the drying temperature using freeze dry reached until -100°C. The use of freeze dry method or drying at the temperature lower than the ambient temperature can prevent product damage caused by oxidation or chemical modification.[15] Supported by the research of Shen, et al., (2021) which stated that the protein content of freeze-dry was proven to be higher because it was less denatured during processing and showed better functional properties than protein which was dried by spray-drying and vacuum-drying.[16]

In contrast to the research, Fathonit et al. (2019) also stated that there was a decrease in protein content of cork fish extract after freeze dry up to 22.489%. The decrease in protein content can also be caused by the ineffective freeze dry process, so the frozen final product loses its nutritional composition and will most likely lose its taste.[17,18] Smida et al. (2014) also stated that drying rate has a deleterious effect on protein content.[19] Limitations of the research is : 1) Drying tilapia fish extract using the freeze dry method requires a long process and time, and 2) Not all laboratorium have freeze dry kit.

Conclusion

The research results showthat an increase in protein level of tilapia fish extract after drying from 2.23% to 27.4%. The results of statistical analysis concluded that there was a significant difference of protein content between before and after drying with freeze-dry.

What is already known on this topic?

A significant difference of protein content between before and after drying with freeze-dry.The difference in the average value of protein content before and after *freeze dry* is 25.17%.

What doesthis study add?

This research has confirmed that drying with freeze-dryincrease in protein level of tilapia fish extract after drying from 2.23% to 27.4%.

Potential conflicts of interest: None.

Ethical clearance

The present study was approved by the Health Research Ethics Committee of the POLTEKKES Ministry of Health in Semarang No.469/EA/KEPK/2021.

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