Wintari Taurina et al /J. Pharm. Sci. & Res. Vol. 13(10), 2021, 630-634

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Determination of Total Protein Levels in Snakehead Fish (*Channa striata*) Water Phase Extract Before and After Freeze Drying using Biuret Method

Wintari Taurina*, Mohamad Andrie
Department of Pharmacy, Faculty of Medicine
Tanjungpura University, Pontianak, Indonesia

Jl. Prof. Dr. H. Hadari Nawawi,
Pontianak, West Kalimantan, Indonesia
wintari.taurina@pharm.untan.ac.id

Abstract

Snakehead fish (*Channa striata*) is a freshwater fish that has a high protein content which is needed for the formation of new cell tissue such as wound healing due to surgery. Extract contains amino acids and fatty acids that are important in the synthesis of collagen fibers, especially glycine, during the wound healing process. Snakehead fish albumin is very good for people with hypoalbumin and postoperative wound healing and burns. *Freeze dry* is done so that there is no remaining moisture content so that the sample can be more stable in storage. The purpose of this test was to determine the albumin content and water phase protein content of snakehead fish (*Channa striata*) extract before and after *freeze dry*. The aqueous phase sample of snakehead fish extract was tested for albumin content by heating method and analyzed for protein content using visible spectrophotometry based on the absorption of purple light from the protein reacting with Biuret reagent. The results of the analysis of albumin content in the aqueous phase of snakehead fish extract were analyzed qualitatively using the heating method. The results of the analysis showed that the albumin content in the water phase of snakehead fish extract and the total protein content of the snakehead fish water phase extract before *freeze dry* was 21775 ppm while after freeze dry was smaller, namely 16876 ppm where the difference in levels decreased The total protein in the aqueous phase of snakehead fishextract before and after *freeze dry* was 22.489%.

Keywords: albumin, biuret method, freeze dry, protein content, water phase of snakehead fishextract.

INTRODUCTION

Snakehead fish is one of the endemic swamp fish species whose presence is decreasing.[1] Snakehead fish (*Channa striata*) is a swamp fish that has not been widely cultivated. This fish is a type of freshwater carnivorous fish that inhabits the Southeast Asian region. Snakehead fish albumin is very good for people with hypoalbumin (low albumin), and postoperative wound healing and burns. Albumin is used in controlling the nutritional status of acute and chronic sick patients.[1] The nutritional content of snakehead fish consists of high protein content, especially albumin and essential amino acids, fats, especially essential fatty acids, and minerals, especially zinc/zinc (Zn) which are needed for cell development and the formation of new cell tissues such as wounds and wound healing due to surgery. [2,3]

Wound healing is a natural phenomenon of the body that is able to overcome the damage to the tissue itself, but the healing rate is relatively slow and the probability of being infected with microbes is high. This causes a high demand for nutrients to accelerate the wound healing process. Snakehead fish extract derived from natural ingredients has been proven to contain nutrients that can be used as a safer and more effective alternative for wound healing. The results showed that the topical preparation of snakehead fish extract gel had the effectiveness of wound closure at a concentration of 5%.[4]

The role of albumin protein for clinical purposes is increasingly important, especially for hospitalized patients who experience hypoalbuminemia (low plasma albumin levels, below 3.5 g/dl), the process of recovering the patient's plasma volume, and the healing process of burns or patients who have recently been operated. [5] The decrease in protein content depends on the way of treatment. [6] Preparation of powder preparations can be done using the *Freeze dryer* process so that it is expected to be accepted by everyone and is more stable in storage. [7] It is necessary to measure protein levels before and after *freeze dry* using the biuret method, so it is necessary to analyze the albumin content seen by the presence of lumps when heated.

Measurement of protein content before and after *freeze dry* using the biuret method is based on the measurement of the purple light absorption of the protein that reacts with the biuret reagent.

[8] This study was conducted to determine the albumin content and total protein content in the aqueous phase of snakehead fish (*Channa striata*) extract before and after *freeze dry*.

Figure 1. Snakehead Fish

METHOD

Among the tools used in this research are the hydraulic press (modifier), the centrifuge (PLC series), freeze dryers (Labconco model 7948030 Freezone-Stoppering Tray Dryers Made in US), the spectrometer uv (shimadzu 2450), evaporating dish, the measuring cylinder 500 ml (Pyrex), the measuring cylinder 20 ml (Pyrex), the beaker glass (Pyrex), the analytical balance (precise type xb 4200c), the water bath (Memmert®), the high-resolution camera, the towel, stove, steaming pot, volumetric Pipette (Pyrex), spatula, plastic container, mortar and pestle, watch glass, stirrer bar.

The main ingredient used is the water extracts of the snakehead fish (*Channa striata*) , aquabidest, biuret reagent (Merck), albumin serum bovin solution (BSA) (Merck).

Procedure

Preparation of Sample. The snakehead fish was cleaned, removed at the head and contents of the abdomen, and removed from the scales, where the meat was weighed. The snakehead fish meat was steamed in a pan for minutes on a gas stove with a temperature of 70°C, and then it was wrapped in flannel and stuffed into a hydraulic press. Next, a resurgent removal was made to take the extract of a snakehead fish. The extract of the snakehead fish that had been obtained was contained, then fed into the test-tubes and was sealed with clean packs and aluminum foils, and then the extract was sentrifuged for 60 minutes at 6000 rpm after that the snakehead fish extract was separated from the oil phase, water phase, and impuruties (sediment) used a dropper and stored in dark glass bottle covered with aluminium foil and clean packs. The top of layer as oil phase and the bottom layer as the water extract phase. Then, taken the water phase and continued in the freezer dryer process.

Albumin Content Analysis Used the Heating Method

Albumin Content Analysis. The research sample was the water phase of snakehead fish (*Channa striata*) extract carried out by heated at a temperature of 90°C for 30 minutes used a water bath.

Determination of Rate Used Biuret Method

Preparation of Main Solution. Weighed 0.05 grams Bovin Serrum Albumin (BSA), asdissolved with distilled water in the 10 ml volumetric flask to the mark, so that main solution was obtained of 5000 PPM concentrations. [9]

Determination of Maximum wavelength. A standard solution of 0.4 ml was added aquadest up to 4 ml and then reagent biuret 6 ml, analyzed by UV-Spectrophotometry on a wavelength of 450-700 nm. [10]

Determination of Operating time. A standard solution of 0.4 ml was added aquadest up to 4 ml, then reagent biuret 6 ml, and then analyzed by UV-Spectrophotometry at a maximum of 20,30,40 minutes.[10]

Making of a Standard Curve. Making a standard curve to determine the linear regression equation, was prepared with six test-tubes, and the first one was filled with blanko solution. In other tube was filled with composition according to the table below and measured the absorption was used a maximum wavelength spectrophotometer and was made curve so that it had linear equations (Anggraini, et al., 2015).

Table 1. Composition of Standard Curve Tube

Main Solution (ml)	Distillwater	Biuret Reagent (ml)	BSA concentration (ppm)
0	Ad 10 ml	6	0
2	Ad 10 ml	6	1000
2,4	Ad 10 ml	6	1200
2,8	Ad 10 ml	6	1400
3,2	Ad 10 ml	6	1600
3,6	Ad 10 ml	6	1800

Determination of Sample Rate. The research sample was a water phase snakehead fish (*Channa striata*) extract, which was made up of an unused sample at freeze dried which has been diluted by 2.8 ml add 10 ml of aquadest, and then add a 6 ml dose of biuret solution, before freeze dried was tried triplo, further stored in a volumetric flask at room temperature for a time operating time, so that the solution was reacted, until it formed a perfect purple color and then read absorbance in wavelength length. [10]

RESULT AND DISCUSSION

The raw material for this research was a snakehead fish with a weight of 1,730 kilograms, a length of 25-40 cm, which was about 3-5 months old. The sample was obtained and collected from fish traders located in the Flamboyan market in Pontianak, West Kalimantan. Sampling was done by purposive sampling (samples taken with certain considerations). The raw material selected with these criteria aimed to obtain maximum protein content, where snakehead fish which had a length, weight, and age that was more than the specified criteria had the same protein content, but had a higher fat content.

Steaming was carried out for ± 30 minutes at a temperature of 65-70˚C and tightly closed with a steaming pan to prevent evaporation and contamination from the outside. The steaming process at the temperature of 65-70˚C aimed to break down the cells of the snakehead fish meat so that during the pressing process, the nutrients contained in the snakehead fish meat could come out optimally because when the orientation with temperatures below 65-70ºC was done, the result of the extract of snakehead fish was not optimal which was characterized by the extract still contained blood. The snakehead fish that had passed steaming was then wrapped using a flannel cloth or napkin and pressed with a hydraulic press. The working principle of this tool is to give pressure to the material that is placed on the bottom side of the tool, then the top and bottom sides are close together when pressure is applied by hydraulics that are attached to the bottom side of the tool, so that it can separate the liquid phase from thesample or material used.

The snakehead fish extract that had been accommodated and put in a test tube was tightly closed with plastic wrap to prevent damages to the sample. The snakehead fish extract was centrifuged for 60 minutes at a speed of 6000

rpm, this aimed to separate the water phase and the oil phase from the extract so that the water phase of the snakehead fish extract could be obtained. The extract that had passed the centrifuge appeared to have 3 layers, namely the oil phase, the water phase, and the impurities. The oil layer was clear and bright yellow and was at the top. The water layer was pale yellow and was in the middle. The layer of impurities was brown or blackish brown and was at the bottom.

Analysis of albumin content in the aqueous phase of snakehead fish extract by heating at 70°C for 30 minutes resulted clumping or coagulation which indicated the presence of albumin in the sample. Snakehead fish (Channa striata) has a high protein content, including albumin and essential amino acids, fats especially essential fatty acids, and minerals especially zinc (Zn) which are needed for cell development and the formation of new cell tissues such as due to wounds and postoperative wound healing. [2,3] The characterization of the water phase of snakehead fish extract was that it had a distinctive odor, it was slightly thick and cloudy. The water phase of snakehead fish extract had a weakness that was easy to decay and not durable. Storage in the cooler or freezer could only last for 12 hours. After 12 hours, the water phase of the snakehead fish extract would be damaged which was characterized by anunpleasant odor.

Freeze drying used a freeze dryer. The freezing process used the vacuum freezing method. This method is based on the rate of evaporation of moisture from the surface and inside the product based on pressures below the triple point. The temperature of the product begins to decrease when the water begins to evaporate thereby eliminating the evaporation of heat in the product due to the pressure drop. The water phase of snakehead fish (*Channa striata*) extract used was 350 mL. After the freeze dryer process was carried out, the extract of snakehead fish water phase was 20.60 g.

Determination of total protein content of snakehead fish extract was carried out using a UV-Visible spectrophotometer. Some steps were carried out before the determination of the protein content. First, the determination of the operating time. The operational time was the time required for a compound to react with other compounds to form a stable product compound. The best operating time result in this study was 40 minutes, because a stable product compound began to form at this temperature, with an absorbance value of 0.1902. The second step was the determination of the maximum wavelength. The wavelength used was the wavelength that had the maximum absorbance. From the results of this study, the maximum wavelength obtained was 607.00 nm at an absorbance of 0.19024. This result was different from previous research which stated that the wavelength that had the maximum absorbance was 558.00. [10]The difference in the determination of this wavelength could be influenced by several factors including the place of research, the laboratory used, UV-Visible spectrophotometry equipment, and the person conducting the research. Then next step was making a standard curve. The results of the determination of the standard curve obtained a linear equation, that was y = 0.0001 x + 0.1258 with a correlation coefficient (r) = 0.9950. This result was still in the linearity range. The value of the correlation coefficient (r) = 0.9950 had a very strong correlation value because according to Sarwono in 2006, the correlation value between 0.75 - 0.99 is included in the classification of very strong correlation. The relationship between correlation and linearity was the correlation indicated the strength of the linear relationship in the two variables and the variables seen in the determination of the standard curve were the concentration level and the resulting absorbance value.[11]

Table 2. Yield of Water Phase and Oil Phase of Snakehead Fish Extract (*ChannaStriata*) .

Raw material	Treatment	Results	Yield of fishmeat	Yield of extract	Observation
Clean snakehead meat 1,730 kg	Snakeheadextract	420 mL	24, 2774 % v/b	-	Typical fishy smell of fish, frothy cloudy yellow color
	Water phase of snakehead extract	350 mL	20, 2312 % v/b	83,333% v/v	Typocal fishy smellof fish, pale yellowcolor
	Oil phase of snakeheadextract	70 mL	4, 0462 % v/b	16,666% v/v	Fat smell, darkyellow color

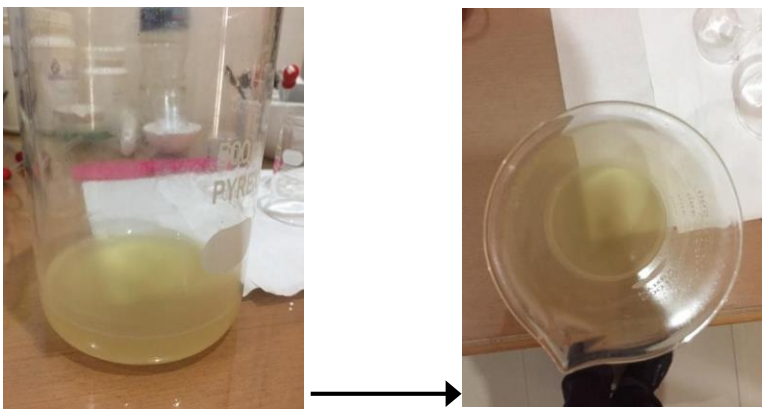

Figure 2. Results of Identification of Snakehead Water Phase Extract

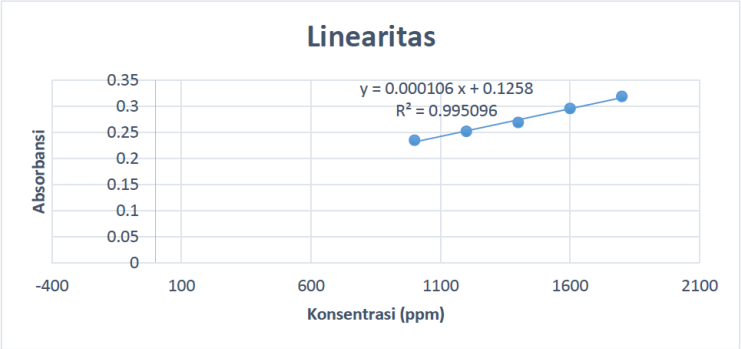

Figure 2. Linearity of Standard Curve

Table 3. Results of Calculation of Sample Content before *Freeze dryer*

Sample(ml	Dilution Volume(ml)	Diluent Factor	Abs	Sample Content (ppm)	Real Content(ppm)	Average (ppm)
2,8	10	3,571	0.7957	6319.811	22568	
2,8	10	3,571	0.7272	5673.584	20260	21775
2,8	10	3,571	0.7936	6300	22497	

Table 4. Results of Calculation of Sample Content after *Freeze dryer*

Sample(ml)	DilutionVolume (ml)	DiluentFactor	Abs	SampleContent (ppm)	Real Content ppm)	Average(ppm)
2,8	10	3,571	0,6558	5000	17855	
2,8	10	3,571	0,6178	4641.509	16574	16876
2,8	10	3,571	0,6067	4536.792	16200	

Table 5. Statistical test result

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1 sebelum – sesudah	4898.67	1315.36	759.426	1631.12	8166.21	6.450	2	.023

Determination of total protein content of snakehead fish extract aimed to determine the comparison and changes in total protein content of snakehead fish extract water phase before and after the freeze dryer process. The results obtained that the concentration of sample levels contained in the extract of snakehead fish water phase before the freeze dryer process was 21775 ppm or 21.775 grams and after the freeze dryer process was 16876 ppm or 16.876 grams. The results obtained were shrinkage by 22.498%. Comparison of protein levels of several types of fish was snakehead fish contains 25.2 g, Belida fish contains 16.5 g, Carp contains 16g, Mujahir fish contains 18.7 g, Catfish Pencok fish contains 7.8 g, and Seluan fish contains 10 g. [1]

The decrease in the total protein content of snakehead fish extract could be caused by the length of processing and storage before the freeze dry process was carried out. The protein value during storage at room temperature decreased due to degradation because of enzyme activity. Protein degradation was from molecules complexes into simple molecules such as amino acids, ammonia, and other nitrogen elements. These compounds were generally volatile so that there was a reduction in the total nitrogen (N) value measured in the measurement of protein content. Testing the paired sample T test resulted the P value was 0.023. From the results that had been known, the data had a significant difference because the P value <0.05.

CONCLUSION

The results of analysis of albumin content in water phase of snakehead (*Channa Striata*) extract showed clumping which indicated the presence of albumin. The total protein content of the water phase of snakehead extract (*Channa Striata*) before freeze dryer was 21775 ppm or 21.775 grams and the total protein content of snakehead extract after freeze dryer was 16876 ppm or 16,876 grams with a difference of 22.498% decrease in content.

Acknowledgement

Thank you to University of Tanjungpura who has provided financial assistance for this research.

REFERENCES

1. Tanjung, M., Nursal., Karimah S. Type of Helminth Parasite in Snakehead fish (*Channa striata*) from Seuneubok Cina, Indra Makmur, Aceh Timur, Indonesia. *International Journal of*

Ecophysiology. 2019; 1(1): 47-55.
2. Alviodinasyari R, Pribadi ES, Soejoedono. Kadar Protein Terlarut dalam Albumin Ikan Gabus (Channa striata dan Channa micropeltes) Asal Bogor. Jurnal Veteriner. 2019
3. Mustafa, A., Widodo, A.M., Kristianto,Y.. Albumin And Zinc Content Of Snakehead Fish (*Channa striata*) Extract And Its Role In Health. IEESE *International Journal of Science and Technology (IJSTE).* 2012; 1(2): 1-8.
4. Utomo, D., Wahyuni, R., Wiyono, R. Pemanfaatan Ikan Gabus (*Ophiocephalus striatus*) Menjadi Bakso Dalam Rangka Perbaikan Gizi Masyarakat Dan Upaya Meningkatkan Nilai Ekonomisnya. Pasuruan: Fakultas Pertanian Universitas Yudharta Pasuruan . 2003.
5. Andrie, M., Sihombing, D. Efektivitas Sediaan Salep yang Mengandung Ekstrak Ikan Gabus (Channa striata) pada Proses Penyembuhan Luka Akut Stadium II Terbuka pada Tikus Jantan Galur Wista. *Pharm Sci Res*. 2017; 4(2): 88-101
6. Hidayanti. (2006). Pengaruh pemberian konsentrat ikan gabus pada pasien pasca bedah di RSU. DR. Wahidin Sidurohusodo Makassar (Tesis, Program Pasca Sarjana UNHAS, Makassar).
7. Asrullah, M., Mathar,A.H., Citrakesumasari. Denaturasi dan Daya Cerna Protein pada Proses Pengolahan Lawa Bale (Makanan Tradisional Sulawesi Selatan). *Media Gizi Masyarakat Indonesia*. 2012; 1(2): 84-90.
8. Yuniarti, D.W., Sulistiyati, T.D., Suprayitno, E. Pengaruh Suhu Pengeringan Vakum Terhadap Kualitas Serbuk Albumin Ikan Gabus *(Ophiocephalus Striatus)*. *THPi Student Journal.* 2013; 01(01).
9. Jubaidah, S., Nurhasnawati, H., Wijaya, H. Penetapan Kadar Protein Tempe Jagung (*Zea Mays* L.) Dengan Kombinasi Kedelai (*Glycine Max* (L.) Merill) Secara Spektrofotometri Sinar Tampak. *Jurnal Ilmiah Manuntung.*2016; 2(1): 111-119.
10. Anggraini,A. Yunianta. Pengaruh Suhu dan Lama Hidrolisis Enzim Papain Terhadap Sifat Kimia, Fisik dan Organoleptik Sari Edamame. *Jurnal Pangan dan Agroindustri.* 2015:3(3).
11. Sari,F.A. Handayani,S. Rahmi,N. Pengaruh Penetapan Kadar Albumin Dalam Ikan Gabus (*Channa striata*) Kukus Dengan Metode Spektrofotometri Visibel. *CERATA Journal of Pharmacy Science.* 2013.
12. Jonathan, Sarwono,. Metode Penelitian kuantitatif dan Kualitatif. Yogyakarta: Graha ilmu.2006.