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Original Article

Isolation and Characterization of Snakehead Fish Meal Extract with Fresh, Boiled, and Steamed Treatments and Its Potential for Health Drinks and Immunomodulators

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ABSTRACT

Albumin is one of the proteins that currently have an important role for the organism's body. This study aims to determine the characteristics of albumin with several treatments which will later be applied to cendol drinks. The treatments for extraction and albumin flour were fresh, boiled, and steamed, which were tested for extract yield, amino acids, and molecular identification using SDS-Page. The results showed that the steam treatment of the extract produced the highest yield of 0.42g/dL. The highest amino acid was in albumin flour with boiled treatment, namely each essential amino acid, namely leucine and non-essential amino acid, namely leucine. Identification of albumin molecular weight showed that albumin was found only in fresh and flour conditions when fresh. Other results indicate the possibility of processing factors affecting the presence of albumin which is easily soluble in water. albumin isolation from snakehead fish can potentially be developed into immunomodulatory health products

Key words: Albumin, Extract, Immunomodulator, Food product, Sds page.

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INTRODUCTION

Albumin is one of the sarcoplasmic proteins that has an important role for the organism's body with the unique characteristic that it does not contain carbohydrates.[1] Albumin can be found in the human body about 55-60% of the total plasma protein.[2] Albumin can also be found in fish with different levels of each fish, such as: in seawater and brackish water fish consisting of yellowtail fish (Pterocaesio chrysozona) by 4.87%; nineg fish (Paraplotosus albilabris) by 5.45%; flying fish (Decapterus ruselli) by 6.87%; white snapper (Lates calcarifer) by 8.19%; kurisi fish (Nemipterus japonicus) by 10.41%; and freshwater fish such as snakehead fish (Channa striata) by 13.44%.[3]

Albumin extraction from snakehead fish has been widely carried out by testing its potential, especially in the health sector, such as a good source of albumin for hypoalbumin sufferers (low albumin,[4] accelerating the healing process of post-operative wounds and childbirth,[5] anti-inflammatories, anti-pain, anti-inflammatory, anti-inflammatory, and anti-inflammatory. cancer,[6] and as an immunomodulator.[7,8] The role of snakehead fish albumin as an immunomodulator can be used to increase immunity or body resistance in preventing the transmission of the COVID-19 virus. The use of albumin as an immunomodulator can be applied to food products that can be consumed by all the community, not limited to age or economy.

Albumin has a role in COVID19, albumin levels will decrease in inflammatory conditions or in severe COVID19 patients.[9,10] Hypoalbumin (low albumin) is associated with mortality in various clinical conditions as critically ill patients.[11] Hypoalbumin was also detected in COVID19 patients who did not survive, so decreased albumin levels were associated with increased mortality, with the mean albumin level being 2.86±0.5 g/dL.[12,13] Normal albumin levels are 3.8 – 5.0 g/dL.[14] Albumin can be used as a therapeutic, stabilizer, and drug delivery agent (antiviral drug) that effectively targets extracellular and intracellular viral components in the therapy of patients infected with the SARS-CoV-2 virus (COVID19).[15]

Several albumin isolation techniques that have been carried out include: isolation of albumin from snakehead fish can be done through the ultrafiltration method. The results showed that the extraction of surimi washing water with an ultrafiltration membrane resulted in albumin type of Human Serum Albumin.[16,17] Extraction of snakehead fish albumin can be done chemically by using HCl solvent and NaCl solvent.[18,19] Isolation of albumin from snakehead fish has also been carried out by boiling using water in a ratio of 1:4 at a temperature of 100°C for 2 hours with an albumin content of 3.53 g for every 100 g of snakehead fish fillet.[20]

Isolation of albumin from snakehead fish meat by steaming for 10 minutes at a temperature of 100°C with the final product in the form of serum albumin was proven to have potential as an immunomodulator by detecting the presence of Immunoglobulin G. Immunoglobulin G (IgG) contained in serum albumin acts as an antibody and anti-inflammatory. Snakehead fish serum albumin was identified as having characteristics similar to Human Serum Albumin.[8] Human serum albumin (HSA) acts as an immunomodulator. The albumin can help patients with chronic liver disease in reducing inflammation caused by bacteria.[21]

Research on snakehead fish extraction and application has been carried out by the authors since 2015 and has been published in reputed national

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and international journals. In 2015, the authors found an increase in protein content of 22.69% in ice cream fortified with snakehead fish meal,[22] an increase in protein content of 23.61% in mochi fortified with snakehead fish protein concentrate (KPI),[23] and the addition of KPI cork on biscuits was 22.23%.[24] In 2016, the author has found the best formulation of mixing catfish meat and snakehead fish meat in making surimi.[25] In 2017, the authors found that donuts fortified with KPI cork were acceptable to consumers with an average consumer preference level of more than 90%,[26] and in 2020, the authors also made cendol fortified with catfish bone meal which was preferred. by consumers and has a calcium value of 0.86%.[27] KPI cork has also been fortified with Chlorella sp. in cookies with an increase in protein content of 21.65% and fiber content of 3.68%.[28] In addition to fish flour and protein concentrate, the authors have also investigated snakehead fish protein isolate using different pH methods, namely acidic pH and alkaline pH with the characteristics of snakehead fish protein isolate at pH 4 (acidic) having a protein content of 84.20% while at pH 11 (alkaline).) has a protein content of 83.45%.[29] Utilization for food products with cork isolation with different methods needs to be done. Therefore, this study aims to determine the characteristics and the best source of albumin to be applied to food products.

MATERIALS AND METHODS

Materials and tools

The main ingredient used in this study was fresh snakehead fish obtained from Bengkuang Lake, Kab. Kampar and distilled water, and other chemicals used for analysis. The equipment used includes a modified steamer, blender, filter, digital scale, porcelain dish, measuring cup, thermometer, and oven. The instruments used for the analysis are AAS (Atomic Absorption Spectrophotometer), UV-VIS spectrophotometry, and others.

Procedure research

Characterization of snakehead fish albumin and determination of the best albumin source

The cork fish obtained from the traditional market of Pekanbaru city was categorized as fresh. Samples were distributed to the laboratory using a cool box. The initial stage, cork fish was carried out by organoleptic testing to ensure that the fish to be processed to the next stage was included in the fresh category and the morphometric characteristics of the fish were carried out. Samples were prepared to separate the fish flesh from the bones, scales, fins, head, and offal, then calculated the body proportions obtained, proximate analysis, analysis of amino acid content, and minerals. The second stage, isolation of snakehead fish albumin from different extraction methods and characterization of the snakehead fish albumin obtained. The method used in albumin isolation is the method of steaming and boiling method.[8]

Albumin isolation

Isolation of snakehead fish albumin[30] Snakehead fish meat was chopped and weighed as much as 100 g. The water is preheated until it reaches a temperature of 100°C. The meat is put into steaming and boiling, then meat extract, meat extract by filtering, and fish meat are obtained.

Albumin filtration[8]

Filtration and separation of meat extract by filtering and meat flour Isolation of the best albumin extract with the highest albumin content in four treatments, filtered using 100 mesh nylon mesh to obtain meat extract while the best albumin meat was weighed and dried using an oven on Filtration and separated meat extract by filtering and meat flour Isolated the best albumin extract with the highest albumin content in four treatments, filtered using 100 mesh nylon mesh to obtain meat extract while the best albumin meat was weighed and dried using an oven.

PROCEDURE ANALYSIS

Amino acids

Referring to,[31] it consists of four stages. The first step is making protein hydrolysate. Samples were weighed as much as 3 mg and crushed. The crushed sample was hydrolysed using 1 mL of 6 N HCl which was then heated in an oven at 110°C for 24 hours. The second stage is drying. The sample which had been hydrolysed at room temperature was transferred to a 50 mL evaporator flask, rinsed with 2 mL of 0.01 N HCl and the rinse liquid was added to the evaporator flask. This process is repeated up to 2-3 times. The sample was then dried using a rotary evaporator for 15-30 minutes. The dry sample was added with 5 mL of 0.01 N HCl and then filtered with milipore filter paper. The third stage is derivatization. The derivatization solution was added as much as 30 L to the drying product. The derivatization solution was prepared from potassium borate buffer solution with a 1:1 sample and then mixed with Orthophthalaldehyde (OPA) solution in a 5:1 ratio with the sample. Then the mixture was filtered using Whatman filter paper. The fourth step is injection and amino acid analysis. The mixture was injected into the HPLC as much as 5 L. Then wait until the separation of all the amino acids is complete and the time required is about 25 minutes. The calculation of the concentration of amino acids present in the material is carried out by making standard chromatograms using ready-to-use amino acids that undergo the same treatment as the sample.

Molecular weight analysis

Referring to the Sodium Dodesyl Sulfate-Polyacrilamide Gel Electrophoresis (SDS-PAGE) method refers to[32] using a gelatin sample with 3% stacking gel and 12.5% separating gel. The making of separating gel was carried out for ±40 minutes, while the manufacture of stacking gel was carried out for approximately two hours. The analysis procedure was as follows: 5 L sample was put into polyacrylamide gel; electrophoresis was run constantly at 10 mA and 125 volts for three hours; SDS-PAGE detection was carried out by removing the electrophoretic gel from the mold then the gel was stained with coomasie brilliant blue; the gel is removed and soaked for one hour in the dye solution. The destaining process is carried out until the protein bands can be seen clearly. The resulting gel was analyzed by molecular weight of gelatin using Photocapt software.

Data analysis

Data processing is carried out using an statistic descriptive with MS Excel tools.

RESULT AND DISCUSSION

Albumin extract

Protein Content of Cork Fish Meat, Meat Extract, and Meat Extract with Filtering. Albumin is a plasma protein that is abundant in the human body and is the largest protein fraction in snakehead fish extract, which is about 64.61% of the total protein. The normal range of albumin in the body is 3.5-5.5 g/dL. The results of the analysis of albumin content are presented in Table 2. Table 2 shows that the albumin extract during 10 minutes of steaming contained a higher albumin content of 3.76±0.15 g/100 mL than the extracts of the other four treatments. Serum albumin content is relatively high, namely 4.6 ± 0.15 g/100 mL. Albumin is a protein that is very susceptible to the influence of temperature. Excessive heating process can cause denaturation of albumin protein. Differences in albumin content of extracts are influenced by temperature and steaming time. Based on the analysis of variance at a confidence level of 0.05, it showed that

Table 1: Albumin content of fresh, steamed, and boiled snakehead fish.

Treatment		Albumine(g/dL)
Fresh	Extract	0.32±0.07
	Powder	0.02±0.07
Steaming	Extract	0.42±0.09
	Powder	0.04±0.014
Boiling	Extract	0.09±0.014
	Powder	0.01±0.0

Table 2: Amino Acids Albumin of snakehead fish treated fresh, steamed, and boiled.

	Fresh		Steaming		Boiling	
	Extract	Powder	Extract	Powder	Extract	Powder
As. Aspartate	0.0363	3.442	0.0567	4.152	0.0331	3.565
As. Glutamate	0.0521	6.015	0.0823	6.417	0.0463	6.924
Serine	0.0064	1.335	0.0128	1.469	0.0070	1.515
Glisine	0.0149	1.483	0.0190	1.598	0.0102	1.602
Histidine	0.0031	1.592	0.0068	1.476	0.0028	1.492
Arginine	0.0093	1.266	0.0109	1.637	0.0087	1.559
Treonine	0	1.102	0	1.181	0	1.229
Alanine	0.0077	0.966	0.0110	1.093	0.0082	1.262
Proline	0.0196	1.812	0.0280	2.283	0.0156	2.486
Tirosine	0.0048	1.543	0.0155	2.069	0.0052	2.168
Valine	0	1.277	0	1.286	0	1.350
Metionine	0.0125	1.809	0.0135	2.193	0.0112	2.075
Sisteine	0.0097	1.377	0.0109	1.389	0.0059	1.442
Isoleusine	0.0078	1.678	0.0170	1.809	0.0080	1.755
Leusine	0.0144	2.630	0.0310	3.196	0.0121	3.255
Phenilalanine	0.0063	1.456	0.0080	1.591	0.0054	1.654
Lisine	0.0185	1.887	0.0219	2.654	0.0165	3.011

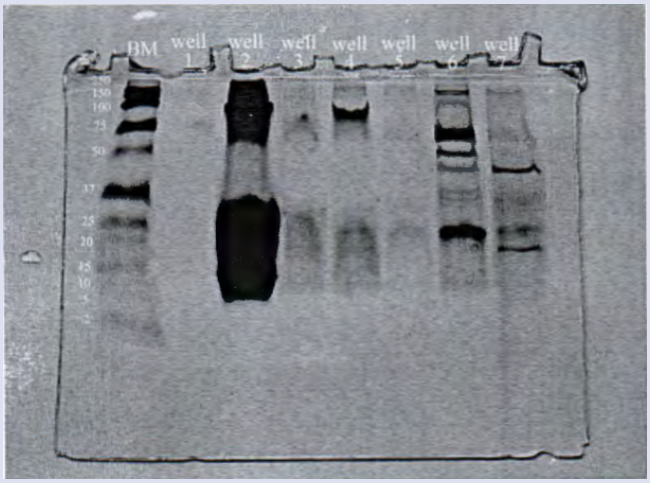

Figure 1:

WELL 2 :	Extract (steaming)
WELL 3 :	Powder (steaming)
WELL 4 :	Extract (boiling)
WELL 5 :	Powder (boiling)
WELL 6 :	Extract (fresh)
WELL 7 :	Powder (Fresh)

the length of steaming treatment had a significant effect on albumin levels. Processing factors greatly affect the albumin content. The results of table 1 show that the steam treatment resulted in higher albumin extract yields, both extracts and flour. According to[33] reported that the separation of albumin and other proteins can be done by heat shock method at a temperature of 60-100°C.[34] explained that heating creates a porous structure, because protein bonds will separate from each other and form aggregates, thus facilitating the separation of liquids during pressing.

Amino acid

Amino acids have a very close role in the synthesis of albumin in tissues. The amino acid composition of meat and flour extracts can be seen in table 2. The extracts and flour contain 17 amino acids. The highest non-essential amino acid was found in all treatments, namely glutamic acid, while the essential amino acid leucine was higher in all treatments. Albumin is rich in the amino acids lysine, arginine, glutamic acid, and aspartic acid arranged in -helic series with 17 sulfide bridges.[35] Amino acids found in meat and flour extracts are needed by the body to synthesize body tissues and energy reserves.

Identification of albumin proteins based on molecular weight

The protein analysis of meat extract by qualitative screening was carried out using the SDS-PAGE method. The results of the sds page for albumin can be seen in Figure 1.

Figure 1 shows that albumin can be detected in fresh snakehead fish extract with a molecular weight of 47.78-57.86 kDa. In addition, it was also seen in albumin flour with a molecular weight of 57.86 kDa. This is in line with the research.[8] 2022 which states that albumin is found in snakehead fish meat at a molecular weight of 40-57 kDa. Steamed and boiled treatment showed that albumin was not detected. The heating factor is thought to cause albumin to obey because albumin is a sarcoplasmic protein that is easily soluble in water, so that when the temperature is high, albumin is easily dissolved. In addition, according to,[36] the presence of albumin in fish is influenced by internal and external factors, such as salinity levels, body shape, and food.

CONCLUSION

The conclusion of this study was that the steam treatment of the extract produced the highest yield of 0.42g/dL. The highest amino acid was in albumin flour with boiled treatment, namely each essential amino acid, namely leucine and non-essential amino acid, namely leucine. Identification of albumin molecular weight showed that albumin was found only in fresh and flour conditions when fresh. Other results indicate the possibility of processing factors affecting the presence of albumin which is easily soluble in water. albumin isolation from snakehead fish can potentially be developed into immunomodulatory health products

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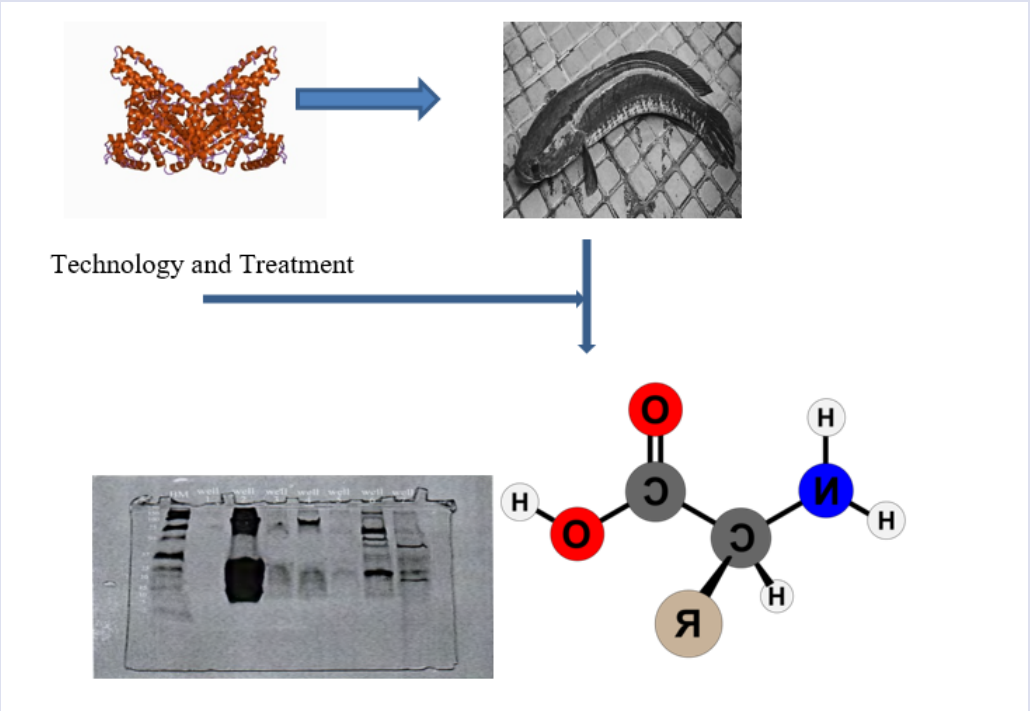
GRAPHICAL ABSTRACT



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