Analysis of molecular weight albumin concentrate on various types of freshwater fish using SDS-page electrophoresis method

Nurfaidah¹, Metusalach², Meta Mahendradatta³ and Sukarno⁴

¹Doctoral Program in Fisheries Science, Faculty of Maritime and Fisheries, Hasanuddin University
²Lecturer of Fisheries Product Technology Faculty of Marine Sciences and Fisheries, Hasanuddin University
³Lecturer in Food Science and Technology Faculty of Agriculture, Hasanuddin University
⁴Lecturer in Food Science, Faculty of Agricultural Technology, Bogor Agricultural University

Email: nurfaidahanwah@gmail.com

Abstract. This study aims to determine the distribution of molecular weight distribution in the extraction results of freshwater fish albumin that will be compared with Snakehead fish to see the similarity of distribution. The samples used were 7 types of fish obtained from the waters around Makassar. Fish albumin concentrates were analyzed for molecular weights using the SDS-PAGE electrophoresis method. Running SDS-PAGE is done at 100-120 V, for 60-90. The gel reading is done using a solution coomassie blue. The results showed that Three spot gourami fish had the most protein bands, which were 8 bands. Snakehead fish has 7 protein bands. Catfish, Tilapia, Pangas Catfish, and Common carp fish have 6 protein bands. Eels have the least protein bands, which are 4 bands. Judging from the protein composition in each type of fish, Three spot gourami fish have a complete protein and are similar to Snakehead fish.

1. Introduction
Snakehead fish is a type of fish that has been known as the best source of fish albumin. However, increasing demand and exploitation albumin catfish is not accompanied by the availability of fish stocks sustainable Snakehead. Snakehead fish stocks in the natural world continue to experience depletion, and cultivation efforts that have been carried out have not provided satisfactory results. For that, it is necessary to look for alternative sources of fish albumin. The protein content of the albumin concentrate of each type of fish has a different composition [1]. The composition of the constituent proteins will affect the efficacy of the fish albumin concentrate produced. This difference is influenced by factors of habitat, food, species, age, sex, and many other internal and external factors. The protein composition of albumin can be analyzed based on observations of its molecular weight.

Determination of protein molecular weight can be done by the SDS-PAGE electrophoresis method [2]. Wibowo confirmed that through electrophoresis, it could be known the type of protein in a material [3]. This determination is based on the nature of the protein's ionic charge and also carries an
electric charge. Each protein molecule has mobilization varying the electric field, the rate of mobilization is directly proportional to the magnitude of the charge [1,4]. The SDS-PAGE electrophoresis method will separate the protein polypeptide chain by adding SDS detergent and heating, which will damage the three-dimensional structure of the protein. After the disulfide protein bond is broken, it will be reduced to a sulfidihydril group. SDS will form complexes with proteins [5].

This study aims to determine the composition of the protein through the distribution of molecular weight distribution in the extraction results of freshwater fish albumin, which will be compared with Snakehead fish to see the similarity of distribution. It is hoped that this research will provide information on what fish have the potential as a source of fish albumin but has a protein composition that resembles Snakehead fish so that the properties given are as good as fish albumin from Snakehead fish.

2. Research methods

2.1. Tools and materials

Vertical Electrophoresis (Mini-PROTEAN® Tetra Cell), PowerPac™ Power Supply, Heat Block/ Water Bath, Micropipet Set + Tips, Microtube 1.5 ml, Gloves, Shaker, APS 10%, ddH2O/equates, Laemmli sample buffer, β-mercaptoethanol, Acrylamide / Bus, Tris-Glycine- SDS Running Buffer, SDS, Tris-HCL, Brilliant Blue Staining Solution, Coomasie brilliant blue, TEMED, Protein Samples, Protein Markers, PVDF Membranes.

2.2. Molecular Weight Analysis

2.2.1. Sample Assessment

Create a sample buffer by mixing another sample buffer that has been added β mercaptoethanol (50 µl β-mercaptoethanol + 950 µl sample buffer). A 500 µL buffer sample is mixed with a 500 µL sample (1: 1 ratio). Next, the protein that has been added to the sample buffer is heated in an 85 °C water bath for 5 minutes.

2.2.2. Making SDS-PAGE Gel

Prepare tools that will be used to print gels such as spacer plates, short plates, and gel casting systems. Assemble a tool for making gels, by inserting spacers and short plates into the casting gel evenly to avoid leakage. First made solution gel separator (separating gel) 12% with the composition as follows: 6 mL of stock 30% acrylamide / Bis in tube/bottle of 50 mL, add 3.75 mL of 1.5 M Tris pH 8.8, shaker slowly. Add aquabidest 5, 0.3 mL, shaker slowly returning. Add 150 uL of 10% SDS (sodium dodecyl sulfate), shaker back. Add 75 µL of 10% APS (Ammonium persulfate). Add Themed 7.5 µL, shaker and pour into the mold, wait up to 30 minutes.

The next is to make a solution for the stacking gel was 4 %, with the following composition: 0.99 mL of 30% Akrilamide / bis is inserted in the tube add 1.89 mL of 0.5 M Tris pH 6.8, shaker slowly. Add aquabidest 4.5 mL, shaker slowly returning. Add 75 µL of 10% SDS (sodium dodecyl sulfate), shaker back. Add 40 µL 10% APS (Ammonium persulfate) and add Themed 7.5 µL, the shaker returns, immediately pouring it over the separating gel. Install the comb, and wait for the gel to solidify. After the gel has frozen, the gel is ready to be used for the SDS-PAGE process.

2.2.3. Running SDS-PAGE

Gel inserted into the chamber contained in the mini protean tetra cell, add a running buffer, and then com opens. Protein samples and markers put in well / well. After the protein marker and sample protein are inserted, close the chamber mini protean tetra cell. Running gel at 100-120 V, for 60-90 minutes. The verification of SDS-PAGE results can be done by the coloring process with Coomassie brilliant blue. After the coloring process, the next step is destaining the gel. Visualization can be done directly without using an imaging system.
3. Results And Discussion

The pattern of protein bands in various types of freshwater fish using the SDS-PAGE electrophoresis method can be seen in the following figure:

![Figure 1. Freshwater fish concentrate protein albumin pattern](image)

Bands formed in each sample are identified by their protein type by comparing the position of the sample band with the tape in the marker / standard path that has known molecular weight. A marker is a protein standard that has a mixture of molecules of different sizes. Sample particles that have the same molecular weight will accumulate at one point and form a band in the same gel lane as the marker.

The composition of the SDS-PAGE marker protein can be seen in table 1:

| Molecular Weight (kD) | Type of Protein                  |
|----------------------|----------------------------------|
| 250                  | Miosin                           |
| 150                  | B-galactosidase                  |
| 100                  | *Bovine serum albumin*           |
| 75                   | Glutamate dehydrogenase          |
| 50                   | Ovalbumin                        |
| 37                   | Carbonic anhydrase                |
| 25                   | Myoglobin                        |
| 20                   | Trypsin Inhibitor                |
| 15                   | Lysozyme                         |
| 10                   | Aprotinin                         |

Table 1. SDS-PAGE Marker protein composition[6]
The results of the analysis of serum albumin molecular weight can be seen in Table 2.

**Table 2.** Analysis of protein bands of freshwater fish concentrate albumin

| Molecular Weight (kD) | Fish Type |
|-----------------------|-----------|
|                       | Trichogaster trichopterus | Mohopterus albus | Clarias Gariepinus | Oreochromis niloticus | Pangasius Pangasius | Cypinus carpio | Channa striata |
| 250                   | √          | -                | √                   | -                 | -                 | √               | √            |
| 150                   | √          | -                | -                   | -                 | -                 | -               | -            |
| 100                   | -          | √                | √                   | √                 | √                 | -               | -            |
| 75                    | √          | -                | -                   | -                 | -                 | -               | √            |
| 50                    | √          | -                | -                   | √                 | √                 | √               | -            |
| 37                    | √          | -                | √                   | √                 | √                 | √               | √            |
| 25                    | √          | √                | √                   | √                 | √                 | √               | √            |
| 20                    | √          | √                | √                   | √                 | √                 | √               | √            |
| 15                    | -          | -                | -                   | -                 | -                 | -               | √            |
| 10                    | √          | √                | √                   | √                 | √                 | √               | √            |

Source: Primary Data, 2019

In Table 2, it can be seen that the three spot gourami fish albumin concentrate has the most protein bands, which are eight bands, and the albumin concentrate has the least protein bands, namely four bands. Catfish, Tilapia, Pangas Catfish, and Common carp fish concentrates have six protein bands, while Snakehead albumin concentrate has seven protein bands. In this study, the protein bands formed in each fish were compared with the protein bands in Snakehead fish, which are known as the best albumin sources.

Protein bands are divided into two, namely major and minor bands. The major band has a higher protein concentration than the other bands; this is characterized by the thickness of the band and greater color intensity. In contrast, minor bands have lower protein concentrations compared to major bands with smaller band thicknesses and color intensities [7].

### 3.1. Analysis of Protein Ribbons in Snakehead Fish Albumin Concentrates

Based on Figure 01, the Snakehead fish albumin concentrate has three major bands, namely 10 kD, 25 kD, and 250 kD, and four minor bands, namely 15 kD, 20 kD, 37 kD, and 75 kD. Proteins with the highest concentration of albumin concentrate catfish are Aprotinin, Myoglobin, and Myosin, while the protein concentration is small at concentrations of albumin catfish is Lysozyme, Trypsin Inhibitor, Carbonic anhydrase, and glutamate dehydrogenase.

Aprotinin is also known as a pancreatic bovine trypsin inhibitor [8]. The main effect given by aprotinin is slowing down the process of fibrinolysis, a process that leads to the breakdown of blood clots. Aprotinin is used as a drug to reduce bleeding and the need for blood transfusion during surgery, besides aprotinin is also able to reduce the risk of organ damage in patients with hypotension (low blood pressure) due to significant loss of large amounts of blood. In addition to maintaining platelet function, aprotinin is also used as an anti-inflammatory drug [9].

Myoglobin is a protein *heme-binding* oxygen which is generally present in the skeleton and muscles heart vertebrates that make this network is red. Myoglobin serves to maintain the availability of oxygen supply and diffusion, which increases the rate of oxygen transport in muscle cells [10-13]. The function of myoglobin as oxygen transport is closely related to the amount of iron mineral content (Fe) in myoglobin. It is this iron mineral that acts as an oxygen carrier for the body's oxidative function, which will determine the metabolic processes in the body. Also, myoglobin serves to bind and carry glucose and amino acids from the cell membrane into the cell.

Myosin is included in the myofibril protein group. Myosin covers 50-58% of the total myofibril fraction, where myofibril is the largest protein fraction in fish meat [14]. Myosin functions to convert chemical energy in the form of ATP into mechanical energy, thus producing force and movement.
Myosin is widely used as a gel-forming and coagulation process in the fish meat (surimi) luminescence industry. While wound healing, myosin is found in all parts of the cytoplasm and is a constituent part of fibrous, which is the main agent in the process of wound healing.

Lysozyme is a bacteriolytic enzyme which is a hydrolase enzyme and is known as muramidase or N-acetylmuramoylhydrolase [15]. Lysozyme is a protein enzyme that acts as an inhibitor of bacterial P no fish; lysozyme role is important to inhibit the growth and invasion of infected pathogenic are contained in the mucus of the skin, serum and organs other fish [16]. Fish lysozyme has activity against pathogenic fish bacteria, including gram-positive and gram-negative bacteria by damaging bacterial cell dinging. Lysozyme also used as a preservative in the food industry [17].

Trypsin inhibitors are compounds that can bind tightly and inhibit the function of the enzyme trypsin to digest proteins. Trypsin inhibitors have carcinogenic (anti-cancer) abilities and can reduce blood sugar levels (hypoglycemia) [18-19].

Protein anhydrase protein is an enzyme that plays an important role as a stabilizer of carbon dioxide concentration. The main function of carbonic anhydrase is to convert carbon dioxide to carbonate or vice versa. In fish, this enzyme plays a role in maintaining the balance of acid and base in the body of the fish and its environment [20]. Carbonic anhydrase is used in blood transfusions in the event of trauma or major surgery. Carbonic anhydrase is combined with catalase and superoxide as a substitute for free hemoglobin stroma to treat acidosis, which is a condition of increasing levels of CO 2 in the body during the transfusion process and if not treated immediately can lead to coma and even death [21]. Glutamate dehydrogenase is an enzyme that causes α-ketoglutarate reductive amination to glutamate for the incorporation of ammonia into a central amino acid metabolic pathway [22-23]. In the medical world, glutamate dehydrogenase is used to evaluate liver function.

3.2. Comparison of Protein Tape Patterns for Snakehead Fish and Other Fresh Fish
Concentrated fish albumin three spot gourami has two major bands, namely ten kD and 25 kD and six minor bands of 20 kD, 37 kD, 50 kD, 75 kD, 150 kD, and 250 kD. The highest concentrations of protein in three spot gourami fish are Aprotinin and Myoglobin. While the protein concentrations of more small fish are Trypsin inhibitor Three spot gourami, Carbonic anhydrase, Ovalbumin, Glutamate dehydrogenase, B-galactosidase, and Myosin.

Eel albumin concentrate has one major band, which is 25 kD and three minor bands, which are 10 kD, 20 kD, and 100 kD. The highest concentration of protein in eels is Myoglobin. While proteins with smaller concentrations in eels are Aprotinin, Trypsin inhibitors, and Bovine serum albumin.

Catfish albumin concentrates have two major bands, namely ten kD and 37 kD and 4 minor bands, namely 20 kD, 25 kD, 100 kD, and 250 kD. The highest concentrations of protein in catfish are Aprotinin and Carbonic anhydrase. While proteins with smaller concentrations in catfish are Trypsin inhibitors, Myoglobin, Bovine serum albumin, and Myosin.

Tilapia and catfish albumin concentrates have the same protein banding pattern, having 1 major band of 10 kD and five minor bands of 20 kD, 25 kD, 37 kD, 50 kD, and 100 kD. The highest concentration of protein in tilapia and catfish is Aprotinin. While proteins with smaller concentrations in tilapia and catfish are Trypsin inhibitors, Myoglobin, Carbonic anhydrase, Ovalbumin, and Bovine serum albumin.

Common carp fish albumin concentrate has one major band, which is ten kD and five minor bands, which are 20 kD, 25 kD, 37 kD, and 50 kD and 250 kD. The highest concentration of protein in carp is Aprotinin. While proteins with smaller concentrations in common carp fish are Trypsin inhibitors, Myoglobin, Carbonic anhydrase, Ovalbumin, and Myosin.

Based on the analysis of protein band patterns from the albumin concentrate of each type of freshwater fish compared to Snakehead fish, there are three types of protein found in all types of fish, namely Aprotinin, Trypsin inhibitors, and Carbonic anhydrase. These three types of protein can be used as a protein in fish albumin. There are three types of protein that are not present in Snakehead fish albumin concentrate but in other types of fish, namely Ovalbumin, Bovine serum albumin, and B-galactosidase.
Ovalbumin is often used in the health field in allergen tests. Ovalbumin also uses to neutralize victims of metal poisoning [24]. Ovalbumin is a protein that can trigger allergic reactions. Ovalbumin acts as a carrier protein, which makes mastocyte cells more sensitive, and when exposed to other allergens causes the formation of immunoglobulin E (IgE) to be faster and cause allergies [25-26]. There are 4 types of fish that contain Ovalbumin protein, namely three spot gourami fish, tilapia, catfish, and common carp fish.

Bovine serum albumin (BSA) is often used as a nutrient in cell culture and microbes. BSA is generally used to determine the number of other proteins, by comparing the number of known BSAs and the amount of protein to be analyzed. This is because of the stable and minimal nature of BSA influences many biochemical reactions.

Protein B-Galactosidase is an enzyme commonly known as lactase. This enzyme hydrolyzes lactose as its natural substrate into glucose and galactose monomers. B-Galactosidase plays an important role in overcoming intolerant lactose symptoms. Excessive lactose in the intestine due to a lack of B-Galactosidase can cause the tissue to become dehydrated, low absorption of calcium and cramps, abdominal pain, and diarrhea [27].

4. Conclusion

Based on the results of the analysis of molecular weights in the albumin concentrates of various types of freshwater fish, it is known that under three spot gourami fish have more protein bands than other fishes as many as eight bands namely two major bands 10 kD and 25 kD and six minor bands namely 20 kD, 37 kD, 50 kD, 75 kD, 150 kD, and 250 kD. Meanwhile, who has the least protein bands are eel fish, where all the protein present in eel fish has the potential to be used as a source of fish albumin.

Acknowledgments

The author, thanks, Prof. Dr. Ir. Metusaelch, M.Sc, Prof. Dr. Ir. Meta Mahendradatta and Dr. Ir. Sukarno M.Sc, who has contributed thought and direction to the author, Head of the Department of Chemistry, Faculty of Marine Sciences and Fisheries, Hasanuddin University, Makassar who has provided facilities to conduct research as well as to Kemenristek-Dikti who have funded this research.

References

[1] Nair Viswanathan P G and Suseela Mathew 2000 Biochemical composition of fish and shell fish CIFIT Advisory series Central Institute of Fisheries Technology Cochin
[2] Wilson K, Walker J 2000 Principles and Techniques of practical Biochemistry Fifth Edition United Kingdom: Cambridge University Press
[3] Wibowo M S 2010 Elektroforesis Sekolah Farmasi Institut Teknologi Bandung
[4] Ferguson A 1974 Biochemical Systematics and Evolution, Glassgow (First edition). Blackie and Son limited ix: 194
[5] Hermes B D 1998 Gel Electrophoresis of proteins Oxford university press. New York
[6] Nacalai 2017 www.nacalai.com
[7] Widowati S dan Wijaya S K 1997 Isolasi dan Karakterisasi Globulin 7S dan 11S dari Sepuluh Varietas Kedelai Indonesia Dalam Budianto, S ; Zakaria, F ; Hariyadi dan Satiyowiharjo, B (ed). Prosiding Seminar Nasional Teknologi Pangan Denpasar
[8] Edelstein C L and Faubel S 2011 Biomarkers in Acute Kidney Injury. Biomarkers of Kidney Disease 177–232
[9] Jaffer I H, Reding M T, Key N S, and Weitz J I 2018 Hematologic Problems in the Surgical Patient. Hematology 2304–2312 e4
[10] Salathe E P and Chen C 1993 The role of myoglobin in retarding oxygen depletion in skeletal muscle. Math. Biosci 116 1-20
[11] Gödecke A Fligel U, Zanger K, Ding Z, Hirchenhain J, Deeding U K M. and Schrader J 1999 Disruption of myoglobin in mice induces multiple compensatory mechanisms. Proc. Nat Acad. Sci. USA 96 10495-10500
[12] Wittenberg J B and Wittenberg B A 2003 Myoglobin function reassessed. J. Exp. Biol. 206 2011-2020
[13] Kanatous S B and Garry D J 2006 Gene deletional strategies reveal novel physiological roles for myoglobin in striated muscle. Respir. Physiol. Neurobiol 151, 151-158
[14] Irianto H E, Giyatmi S 2006 Teknologi Pengolahan Hasil Perikanan Penerbit Universitas Terbuka Jakarta
[15] Dekina SS, Romanovska II, Ovsepyan AM, Bodyu MG, Toptikov VA 2015 Isolation and purification the hen egg white. Biotechnol Acta 8:41-47
[16] Hikima J, Hirono I, Aoki T 2003 The lysozyme gene in fish In: Shimizu N, Aoki T, Hirono I, Takashima F. (eds) Aquatic Genomics. Springer, Tokyo. https://doi.org/10.1007/978-4-431-65938-9_27
[17] Hiidenhovi J 2007 Ovomucin. In: Huopalahti R, Lopez-Fandino R, Anton M, Schade R, editors. Bioact Egg Compd. Berlin (Germany): Springer p 61-68
[18] Losso J N 2002 Preventing degenerative diseases by anti-angiogenic functional foods. Food Tech 56 (6): 78-88
[19] Zuheid Noor dan Rheizy Fitriana 2002 Penjajagan kacang merah sebagai komponen makanan fungsional bagi penderita diabetes (Lidm). Proceedings of the PATPI National seminar, Malang
[20] Lucas AYH 2010 Analisis Tingkat Konsumsi Oksigen Ikan Nila Gift (Oreochromis sp.) Pasca Terekpos Logam Berat Cu pada Berbagai pH. Media Exacta 10 (2): 1 – 11
[21] Boone CD, Habibazadegan A, Gill S, dan McKenna R 2013 Carbonic Anhydrases and Their Biotechnological Applications. Biomolecules 2013 3: 553 – 562
[22] Horton Robert 2006 Principle of Biochemistry
[23] McKee 2004 Biochemistry: The Molecular Basis Of Life , 3rd edition. Mc Graw Hill.New York
[24] Huntington JA, Stein PE 2001 Structure and Properties of ovalbumin. J of Chrom Phy B 756: 189-198
[25] Harlow E, Lane M 1998 Antibodies: A Laboratory Manual. New York: Cold Spring Harbor
[26] Winter WE, Hardt NS, Fuhrman S 2000 Immunoglobulin E: Importance in parasitic infections and hypersensitivity
[27] Panesar PS, J F Kennedy, C J Knill and M Kosseva 2010 Production of L(+) lactic acid using Lactobacillus casei from whey. Braz Arch Biol Technol 53 (1): 219-226