BIODIVERSITAS
Volume 24, Number 7, July 2023
Pages: 4027-4033

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240740

SDS-PAGE protein profile of albumin extracted by steaming from four marine and three brackish-water fishes

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Manuscript received: 14 June 2023. Revision accepted: 22 July 2023.

Abstract. *Fatma N, Metusalach, Taslim NA, Nurilmala M. 2023. SDS-PAGE protein profile of albumin extracted by steaming from four marine and three brackish-water fishes. Biodiversitas 24: 4027-4033.* Snakehead fish is a type of fish that has been known as the best source of fish albumin. However, the increasing demand for and exploitation of snakehead fish albumin is not accompanied by the availability of sustainable snakehead fish stocks. Snakehead fish stocks in nature continue to experience depletion and cultivation efforts that have been carried out so far have not yielded satisfactory results. For this reason, it is necessary to find alternative sources of fish albumin. The purpose of this study was to determine the molecular weight of the albumin proteins extracted by water bath steaming from four marine fishes (Indian scad *Decapterus russelli*, short-bodied mackerel *Rastrelliger brachysoma*, goldband fusilier *Pterocaesio chrysozona*, Japanese threadfin bream *Nemipterus japonicus*) and three brackish-water fishes (white-lipped eel catfish *Paraplotosus albilabris*, barramundi *Lates calcarifer*, milkfish *Chanos chanos*). The method used was Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) electrophoresis. Each sample was steamed in a water bath at 50°C for 30 minutes to obtain the crude extract's optimal yield and albumin content. The molecular weights of each protein band were analyzed using the Photocapt application, visualized and tabulated, and analyzed descriptively. Indian scad had six protein bands with three major (48 kD, 37 kD, 32 kD) and three minor bands (66 kD, 25 kD, 15 kD). Shortbodied mackerel had one major protein band (26 kD). Goldband fusilier had one major (33 kD) and four minor bands (50 kD, 69 kD, 57 kD, 26 kD). Japanese threadfin bream had one major (62 kD) and three minor bands (171 kD, 72 kD, 26 kD). White-lipped eel catfish had two major (41 kD, 34 kD and two minor bands (70 kD, 26 kD). Barramundi had one major (26 kD) and two minor bands (171 kD, 87 kD). Milkfish had one major (41 kD) and four minor bands (333 kD, 135 kD, 31 kD, 25 kD). It is hoped that this research will provide information on which fish have the potential as a source of fish albumin in terms of protein composition which resembles snakehead fish so that the properties provided are as good as albumin from snakehead fish.

Keywords: Albumin extract, marine/brackish-water fishes, protein profile, SDS-PAGE

INTRODUCTION

People in several countries have long believed that fish protein, especially striped snakehead (*Channa striata*) extract, has therapeutic properties beneficial to human health and wellbeing (Haniffa et al. 2014). In several areas of Sulawesi, Indonesia, snakeheads have been used in traditional medicine. For example, in South Sulawesi, snakeheads are often prepared for women to eat after giving birth, as it is thought that consuming this fish will speed up the women's recovery and enhance breast milk production. Recent research has shown that snakefish consumption can promote the healing of perineal wounds in postpartum mothers (Purwanti et al. 2019).

Albumin is a protein normally present in plasma at levels ranging from 3.5 to 5 g dl^{-1}, around 55% to 60% of total serum protein, and also be found in interstitial and lymph fluids in concentrations up to 40-60% of the plasma level (Hülshoff et al. 2013; Soedjanaatmadja et al. 2021; Belinskaia et al. 2021). Albumin plays several important roles, including forming new cells, accelerating wound healing, and maintaining blood osmotic pressure (Belinskaia et al. 2021). Low albumin levels in blood serum can impair the binding and transport of endogenous and exogenous compounds, including drugs, throughout the body. Medical professionals have administered intravenous infusions of Human Serum Albumin (HSA), extracted from blood serum to substitute for low patient albumin levels, but this treatment is expensive (Soedjanaatmadja et al. 2021), and the high cost has made this treatment inaccessible to many people in Indonesia (Tungadi 2020).

Snakehead extract has also been shown to benefit patients with low albumin levels associated with the symptoms or complications of diseases such as tuberculosis (Fadhilah and Sari 2021), malnutrition and nephrotic syndrome (Herumuryawan and Hardaningsih 2017; Gilda and Muryawan 2017; Muryawan et al. 2019; Pratiwi 2021), liver disease (Yulianda 2020), human immunodeficiency virus (HIV), possibly also diabetes, gastritis, and ulcers (Yulizal et al. 2020; Hadisaputro and Sunarjo 2021; Saraswati

et al. 2022). It has been suggested that snakehead extract can increase postoperative serum albumin because it contains active antioxidant compounds that react with and suppresses the production of free radicals. The increased catch of snakeheads for albumin has raised concerns about the sustainability of the fishery (Ndobe et al. 2014, 2019; Rahman and Awal 2016). Therefore, there is a need to find other albumin sources, including from other species of fish with albumin properties similar to those of snakehead albumin.

The fish's nutritional composition, particularly albumin content, can vary between species, individuals, and populations (Firlianty et al. 2013; Chasanah et al. 2015). Potential influencing factors include sex, sexual maturity and age, the reproductive cycle and spawning season, other seasonal factors, and geographical location. However, there is some evidence that environmental conditions may influence more than population genetic traits (Khasani and Astuti 2019). The protein content in fish is strongly influenced by the water and fat content, and albumin content can vary between the fish flesh and filtrate extracts (Firlianty et al. 2013). Therefore, Identifying the characteristics of albumin protein from other fish species is a necessary first step in exploring albumin sources besides snakeheads.

The albumin protein profile can be identified from the molecular weight of the proteins in fish albumin using the Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS PAGE) method. SDS-PAGE is based on the electrophoresis' ability to separate biological molecules, especially proteins, into distinct bands without affecting the structure of the biopolymers present. The electrophoresis is also very sensitive to small differences in charge and molecular weight (Hemes 1998). This study aimed to determine the protein profiles (based on molecular weight) of albumin extracted from marine and brackish-water fishes, compare these profiles with the striped snakeheads, and investigate the similarities. Hopefully this research will provide information on whether these potential sources of fish albumin have a protein composition resembling that of the striped snakehead so that the therapeutic properties might be comparable to those of striped snakehead albumin.

MATERIALS AND METHODS

Materials and equipment

The materials used in this study consisted of specimens from seven fish species known to have relatively high total protein and albumin content (Fatma et al. 2020). These comprised four marine fishes (Indian scad *Decapterus russelli*, short--bodied mackerel *Rastrelliger brachysoma*, goldband fusilier *Pterocaesio chrysozona*, and Japanese threadfin bream *Nemipterus japonicus*) and three brackish water fishes (white-lipped eel catfish *Paraplotosus albilabris*, barramundi *Lates calcarifer*, and milkfish *Chanos chanos*). Marine fish samples were obtained from the Paotere Fish Landing Base, Makassar City, South Sulawesi Province, Indonesia. Also, the brackish-water fish samples were obtained from the Hasanuddin University (Unhas) Education Ponds in Bojo District, Barru Regency, South Sulawesi Province.

The selected sea fish were taken from Makassar's Paotere fish landing base (PPI Paotere), and brackish water fish were obtained from fish farming from Barru district. The fish samples were put into a styrofoam box and then given enough bulk (fine) ice to prevent damage during transportation and then taken to the laboratory. The fish that have been obtained are weighed per head to determine the weight of each fish, then cleaned, gutted, then filleted. The filleted fish meat is separated from the skin and then mashed with a blender. As much as 100 g of blended fish meat was weighed and put into a measuring flask (beaker glass), 400 mL of distilled water was added and then homogenized using a homogenizer. Furthermore, heating with a water bath at 50°C for 30 minutes. The fish extract was then filtered using filter paper. The filtered fish extract was then measured for volume and put into a glass bottle and then stored in the refrigerator until analysis was carried out.

Equipment used for electrophoretic analysis of the albumin protein extracted from the samples included a vertical electrophoresis unit (Mini-PROTEAN® Tetra Cell), a PowerPac™ power supply, a heat block/water bath, micropipette sets, and a shaker. Materials used for the extraction and electrophoresis included micropipette tips, 1.5 mL microtubes, gloves, ammonium persulfate (APS) 10%, distilled water (ddH_2O), Laemmli sample buffer, 2-mercaptoethanol, bis-acrylamide solution, sodium dodecyl sulfate (SDS), tris-glycine-SDS running buffer, Tris-HCL, Coomassie brilliant blue staining solution, Coomassie de-staining solution, tetramethylethylenediamine (TEMED), protein samples, protein markers, and polyvinylidene difluoride (PVDF) membranes.

Procedures

Sample extraction

The samples that have been obtained are then prepared and weighed. The sample is then weeded by washing and separating from the skin and bones so that only the meat remains. The sample meat that has been obtained is then pureed using a blender. Each type of fish was then weighed 50 g and added with distilled water (1:4 ratio) and homogenized with a homogenizer and then heated using a water bath at 50°C for 30 minutes. After that the sample was filtered with a filter cloth. The filter results are then put into glass bottles and stored in the freezer to maintain their quality before proceeding to the analysis stage.

Molecular weight with SDS-PAGE

A protein profile describes the protein content in the sample by separating the protein molecules based on their molecular weight using the Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) method. The principle of SDS-PAGE is to determine the molecular weight of protein compounds using a vertical electrophoresis device to separate the compounds present due to their differential response to the presence of an electric current (Kaimudin 2020). Ionic detergent (SDS) is used to form

protein complexes that are negatively charged so that the protein moves in the positive direction.

Each filtrate sample (volume 500 µL) was buffered by adding a buffer solution at a ratio of 1:1. This buffer solution was a mix of Laemmli buffer solution and β-mercaptoethanol (ratio 950:50). The buffered protein extract was then heated in a water bath at 85°C for 5 minutes.

The SDS-PAGE gel was then prepared. Furthermore, to print the gel, the spacer, and short plates were inserted into the gel mold, taking care to avoid leakage. A 12% separating gel mix was prepared as follows: 6 mL of 30% acrylamide/Bis solution was placed in a 50 mL beaker to which 3.75 mL of 1.5M tris pH 8.8 was added and mixed by placing on the shaker at a low speed. Further ingredients were then added, placing the mix on the shaker after each addition: 5.03 mL distilled water was added first, followed by 150 µL of 10% SDS (sodium dodecyl sulfate), then 75 µL 10% ammonium persulfate (APS) and finally 7.5 µL of tetramethylethylenediamine (TEMED). The mix was immediately poured into the mold and left to set for ±30 minutes.

The next step was to make a 4% stacking gel solution, again placing the mix on the shaker after adding each ingredient. Firstly, 0.99 mL of 30% Bis-acrylamide was placed in a tube, and 1.89 mL of 0.5M Tris (pH 6.8) was added, followed by 4.5 mL distilled water, then 75 µL of 10% sodium dodecyl sulfate (SDS), 40 µL of 10% APS, and finally 7.5 µL of TEMED. The mixture was immediately poured over the separating gel, and the comb was placed in the mold to create wells for the samples. The gel was then left to solidify, ready for the SDS-PAGE process.

Furthermore, the gel was placed in the mini protein tetra cell chamber to run the SDS-PAGE. A running buffer was added, and the comb was removed. A molecular weight ladder (marker) was loaded into one well, and aliquots of each protein extract sample were loaded into their designated wells using a pipette. The mini protean tetra cell chamber was closed after loading the protein marker and protein samples. The running voltage and time were 100-120 V and 60-90 minutes. The SDS-PAGE results (protein bands) were made visibly by staining the gel with Coomasie brilliant blue staining solution and then rinsing it with Coomasie de-staining solution. The bands could then be observed directly without using an imaging system, and the molecular weight of each band was inferred by comparing its position with that of the standard protein bands in the molecular ladder (marker).

Data analysis

The molecular weight (BM) data were obtained using Photocapt software. The results were presented visually and tabulated. The data were analyzed descriptively.

RESULTS AND DISCUSSION

Protein profiles

The pattern of protein bands (bands) obtained from the extracts of four marine and three brackish-water fish species using the SDS-PAGE electrophoresis method clearly shows visible differences between these species (Figure 1). In particular, the protein band of short-bodied mackerel (F2) was considerably thicker than the other fish species in this study. The thickness of the protein band produced through electrophoresis indicates the protein concentration; protein bands can be categorized as main or major bands and faint or minor bands (Alberts et al. 2015). The thickness and intensity of a band are related to the number of migrating molecules, and bands with a higher ionic charge will migrate further than bands with lower ionic charges (Sinlae et al. 2015). The main bands are thicker and have a greater color intensity, indicating that the proteins forming these bands are in higher concentrations than those forming the fainter (minor) bands (Alberts et al. 2015). Meanwhile, changes in the protein pattern of SDS-PAGE electropherograms indicate changes in the protein composition, and thinning or loss of protein bands indicates a change in the nature of the protein (Ilminingtyas et al. 2000).

The electropherogram of albumin extract from the seven fish species in this study (Figure 1) shows the presence of thick and thin (major and minor) protein bands, indicating the presence of several proteins in different concentrations. These results were then analyzed using the Photocapt application to determine the molecular weight of the proteins represented by each band. The results of the Photocapt application analysis can be seen in Figure 2 and Table 1.

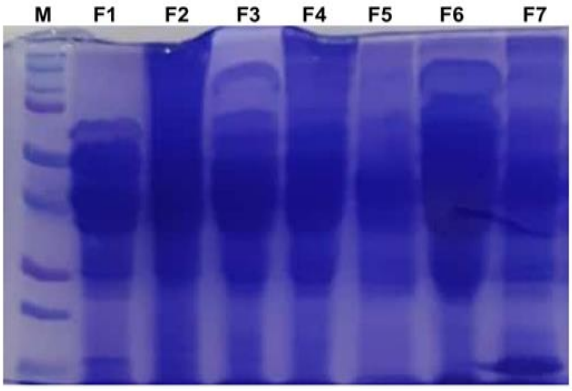

Figure 1. Electropherogram of the molecular weight of four seawater fish and three types of brackish water fish. Legend: M: Marker; F1: Indian scad; F2: short-bodied mackerel; F3: goldband fusilier; F4: Japanese threadfin bream; F5: white-lipped eel catfish; F6: barramundi; F7: milkfish

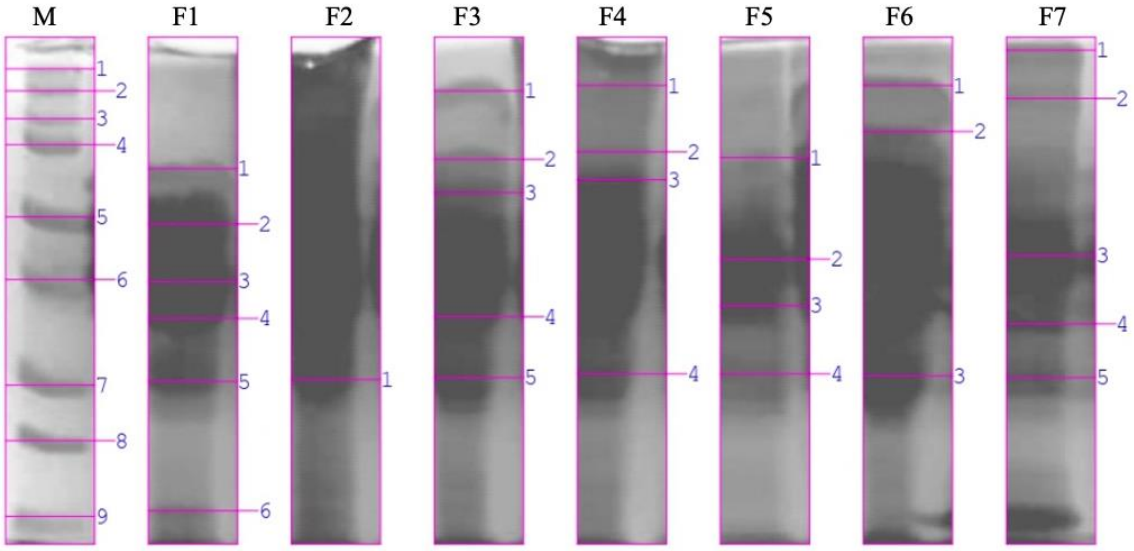

Figure 2. Photocapt analysis of the electropherogram of albumin extract from seven fish species. Left to right: standard molecular weight ladder. M: Marker, F1: Indian scad, F2: short-bodied mackerel, F3: goldband fusilier, F4: Japanese threadfin bream, F5: white-lipped eel catfish, F6: barramundi, F7: milkfish

Table 1. Molecular weight (kD) from the Photocapt analysis of the electropherogram of albumin extract from seven fish species. The bold font indicates major bands

Marker	*C.striata*	Indian scad *D. russelli*	Short-bodied mackerel *R. brachysoma*	Goldband fusilier *P. chrysozona*	Japanese threadfin bream *N. japonicus*	White-lipped eel catfish *P. albilabris*	Barramundi *L. calcarifer*	Milkfish *C. chanos*
		F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**
250	**250**	65.9	**25.6**	150.0	170.7	70.1	170.7	333.3
150	150	**48.3**		69.4	72.2	**40.8**	86.7	135.3
100	75	**36.8**		57.4	**61.9**	**33.9**	**26.0**	**41.5**
75	50	**32.4**		**32.6**	26.1	26.1		31.7
50	37	25.4		25.8				25.8
37	**25**	15.3						
25	20							
20	**10**							
15								
10								

The band formed in each sample identified the type of protein by comparing the position of the sample band with the band on the marker/standard route whose molecular weight is known. Markers are protein standards that have 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.

Protein bands are divided into two, namely major bands and minor bands. The major band has a higher protein concentration than the other bands, this is indicated by the thickness of the band and the greater intensity of the color. Conversely, the minor band has a lower protein concentration than the major band with a smaller band thickness and color intensity (Widowati and Wijaya 1997).

Based on Figures 1 and 2 and Table 1, the number of bands varied from one to six, with molecular weights of the separated proteins varying from 15 to 333 kD. All seven fish species had a band with a molecular weight of around 25-26 kD; the major bands of all species were between 25 and 62 kD, but each fish species had a different albumin extract protein profile. A marine fish, the Indian scad (F1), had the most (six) protein bands (three major and three minor bands) from around 15 to 66 kD, while the shortbodied mackerel (F2), also a marine fish, had the least with just one major protein band at a relatively low molecular weight around 26 kD. The goldband fusilier (F3) had five protein bands (one major and four minor bands) from around 26 to 150 kD. Japanese threadfin bream (F4) had four protein bands (one major and three minor bands) from around 26 to 171 kD. White-lipped eel catfish (F5) also had four protein bands (two major and two minor bands) ranging from 26 to 70 kD. Barramundi (F6) had three protein bands (one major and two minor bands) from 26 to 171 kD. Milkfish was the brackish-water fish with the most (Five) bands (one major and four minor bands) ranging from 26 to 333 kD.

In addition to differences between species, differences in geographic origin also affect the protein profile and the bands formed (Mabrur et al. 2018). The methods used for albumin extraction can also influence the bands, as heat is needed to obtain a high dissolved protein content, but proteins become denatured above certain temperatures. In general, albumin is relatively stable, but the higher the cooking temperature and the longer the cooking time, the greater the protein structure changes (Yu et al. 2017). It is reported that steaming at a temperature of 60°C can yield albumin isolates with high dissolved protein content, while albumin extracted at 40°C contained high concentrations of a complex mix of dissolved proteins, with a large number of clear and thick protein bands detected through electrophoresis (Nugroho 2013). Plasma albumin denaturation begins at a heating temperature of 69.1±0.3°C and peaks at 78±0.2°C.

Meanwhile, other research indicates that heating above 60°C can damage the albumin and lead to low yields (Raoufinia et al. 2016). Heating BSA (Biovine Serum albumin) and alpha-lactalbumin to 78°C for 15 minutes reduced the number of bands detected, while snakehead albumin solubility is reduced to around 81% at 70°C and greatly reduced at 90°C. Such protein properties changes resulted in fewer and thinner SDS-PAGE protein bands. In this study, before electrophoresis, the albumin was extracted by heating the fish in a water bath at 50°C for 30 minutes; while it seems unlikely that denaturation sufficient to reduce the number of bands would have occurred at this temperature, therefore, it possible that some protein components may not have been extracted.

During electrolysis, protein molecules with the same charge and size will accumulate in adjacent zones or bands so that multiple bands indicate that the sample is composed of several proteins or a protein complex. The molecular weights of standard protein compounds used in the molecular ladder (marker) are shown in Table 2. Few of these correspond to the molecular weights of the extracts obtained from the seven fish species in this study. However, the protein in fish meat is generally in the form of bioactive peptides, fragments of an original protein with no biological activity. Bioactive peptide compounds that can work actively and positively affect human health and the digestive tract usually have a low molecular weight below 100 kD and consist of 3 to 10 amino acids. This description is consonant with the results in Table 1.

Starting with the lightest compound in Table 2, aprotinin (10 kD) is an antifibrinolytic molecule that inhibits trypsin and related proteolytic enzymes, commonly used to reduce bleeding and needed for blood transfusions during surgery (Hoffman et al. 2018). It can reduce the risk of organ damage in patients with hypotension (low blood pressure) due to significant blood loss, maintain platelet function, and be an anti-inflammatory drug. However, bands consonant with this protein were not detected in any samples by the Photocapt analysis.

Lysozyme (15 kD) is an enzymatic protein or bacteriolytic hydrolase enzyme known as muramidase or N- acetylmuramyl hydrolase (Dekina et al. 2015). Lysozyme is important in inhibiting the growth and invasion of infectious pathogens in the skin, mucus, serum, and other fish organs (Song et al. 2021). Fish lysozyme is active against Gram+ and Gram- bacteria by damaging bacterial cell walls and is used as a preservative in the food industry (Mei et al. 2019; Song et al. 2021). A band consonant with lysozyme was detected in just one species, the Indian scad *D. russelli*. Trypsin inhibitors (20 kD) are compounds that can bind tightly and inhibit the function of the trypsin enzyme to digest protein. Trypsin inhibitors have anticarcinogenic (anti-cancer) abilities and can lower blood sugar levels (hypoglycemic). However, bands consonant with this protein were not detected in any samples by the Photocapt analysis.

Myoglobin (25 kD) is an oxygen-binding heme protein with a high iron (Fe) content commonly found in the skeleton and heart muscle of vertebrates and gives such tissue its red color, including the so-called red meat of some fishes (Hart and Reynolds 2004; Lall and Kaushik 2021). Myoglobin maintains the supply and diffusion of oxygen, especially to the mitochondria, and strongly influences metabolic processes in the body (Hoffman et al. 2018). In addition, myoglobin can bind glucose and amino acids and carry them through the cell membrane into the cells (Gao et al. 2022). All seven fish species studied had bands consonant with this protein, including the sole thick band of the short-bodied mackerel *R. brachysoma* and the major band of the barramundi *L. calcarifer*.

Carbonic anhydrase (37 kD) is an important enzyme in stabilizing carbon dioxide concentration, converting carbon dioxide to carbonate, and vice versa (Boone et al. 2013). In fish, this enzyme plays a role in maintaining the acid-base balance in the fish body despite fluctuations in its environment (Schreck et al. 2016). Carbonic anhydrase is used in blood transfusions in the event of trauma or major surgery, combined with catalase and superoxide as a substitute for free stroma of hemoglobin to treat acidosis, a condition of increased CO_2 levels in the body during transfusion processes which, if not treated immediately can lead to coma and even death (Boone et al. 2013). While a band clearly consonant with carbonic anhydrase was detected in just one species, the Indian scad *D. russelli*, two other species had bands with molecular weights quite close to that of carbonic anhydrase and which might indicate the presence of this protein (Figure 2 and Table 1). These were the white-lipped eel catfish *P. albilabris* and the milkfish *C. chanos*.

Table 2. Molecular weights of standard proteins in the Nacalai Tesque standard molecular weight marker ladder

Molecular weight (kD)	Protein	Molecular weight (kD)	Protein
250	Myosin	37	Carbonic anhydrase
150	β-galactosidase	25	Myoglobin
100	Bovine serum albumin	20	Trypsin Inhibitor
75	Glutamate dehydrogenase	15	Lysozyme
50	Ovalbumin	10	Aprotinin

Source: Nacalai Tesque (2017)

Ovalbumin (50kD) is a carrier of small molecules in cells and may have antioxidant and anti-mutagenic properties. It can also accelerate the formation of immunoglobulin E and trigger allergic reactions and is often used in the medical field in allergen testing. A recent study did not find ovalbumin in the striped snakehead *C. striata*, but it was found in at least four other fishes: three-spot gourami *Trichogaster trichopterus*, African catfish *Clarias gariepinus*, Nile tilapia (*Oreochromis niloticus*), and common carp *Cyprinus carpio* (Nurfaidah et al. 2020).

Glutamate dehydrogenase (75 kD) is an enzyme that catalyzes the (reversible) oxidative deamination of L-glutamate to α-ketoglutarate and NH_3, is important in the incorporation of ammonia into the central amino acid metabolic pathway, and both catabolic and anabolic reactions (Plaitakis et al. 2017). In the medicinal, glutamate dehydrogenase can be used to evaluate liver function (Schomaker et al. 2020). Three fishes had minor bands very close to the Glutamate dehydrogenase band and similar molecular weights from the Photocapt analysis. The goldband fusilier *P. chrysozona*, Japanese threadfin bream *N. japonicus*, and the white-lipped eel catfish *P. albilabris*. Meanwhile, none of the seven fishes had a band matching the bovine serum albumin (100 kD) band, although, in Figure 2, a minor band of the barramundi *L. calcarifer* (87 kD) appears quite close.

The protein β-galactosidase (150 kD) is an enzyme commonly known as lactase. Excessive lactose in the intestine due to a lack of β-galactosidase causes tissue dehydration, low calcium absorption and cramps, abdominal pain, and diarrhea. Furthermore, β-galactosidase hydrolyzes lactose to glucose and galactose monomers and important in overcoming lactose intolerance symptoms. One fish, the goldband fusilier *P. chrysozona*, had a minor band that was exactly matched. Furthermore, two fishes in this study had minor bands very close to the band for β-galactosidase, the Japanese threadfin bream *N. japonicus* and the barramundi *L. calcarifer*. In addition, the milkfish *C. chanos* also a band quite close to the reference lactase band (Figure 2).

Myosin (250 kD) is the most abundant of the myofibril protein group, which comprises the largest protein fraction in animal skeletal muscle (Ojima 2019), including fish meat. Myosin is functioning to convert chemical energy in the form of ATP into mechanical energy, thereby producing force and motion. Widely used as a gelling and coagulation agent in the surimi industry (Tolano-Villaverde et al. 2016), myosin is present in the cytoplasm it is important in forming fibroblasts and hence in wound healing (Darby et al. 2014). None of the fishes in this study had bands close to the myosin band, although the milkfish *C. chanos* had a band with a molecular weight higher than any protein in the marker ladder (333 kD). As a larger molecule, it is possible that myosin may not have been dissolved or could have been broken down during the extraction process.

Snakehead albumin is complex and contains at least 17 proteins (phenylalanine, isoleucine, leucine, methionine, valine, threonine, lysine, histidine, aspartic acid, glutamic acid, alanine, proline, arginine, serine, glycine, cysteine, and tyrosine) with up to 28 bands from 24 to 191 kD (Mabrur et al. 2018). However, study using the same methods found 7 bands for *C. striata*, while six other freshwater fishes had four to eight bands (Nurfaidah et al. 2020). Based on the results, although the albumin extract from all seven fish studied contained valuable proteins, none had the complexity or nearly approached the full range of proteins in striped snakehead (*C. striata*) albumin. However, the variation between species indicates that some combination of albumin extracts from several fishes might be more effective than a single species for healthcare applications.

In conclusion, the SDS-PAGE analysis showed that the steamed albumin extract profiles of the seven fish species in this study differed considerably. Of the four marine species, the Indian scad (F1) had six protein bands consisting of three major bands of 48 kD, 36 kD, and 32 kD and three minor bands of 65 kD, 25 kD, and 15 kD; the short-bodied mackerel (F2) had one major protein band (25 kD); the goldband fusilier (F3) had five protein bands (a major band of 32 kD and minor bands at 150 kD, 69 kD, 57 kD and 25 kD); while the Japanese threadfin bream (F4) had four protein bands (one major band at 61 kD and minor bands at 170 kD, 72 kD and 26 kD. Concerning the three brackish-water fishes, the white-lipped eel catfish (F5) has four protein bands consisting of two major bands of 40 kD and 33 kD and two minor bands of 70 kD and 26 kD. Barramundi (F6) has three protein bands: one major band of 25 kD and two minor bands of 170 kD and 86 kD. Milkfish has five protein bands consisting of one major band of 41 kD and four minor bands, namely 333 kD, 135 kD, 31 kD, and 25 kD. Based on the results of molecular weight analysis of the seven samples of seawater fish and brackish water fish, it is known that the protein content in fish albumin has various types and different levels. This is influenced by various factors, both internal and external factors. Such as the type of fish, size, sex, food, environment and many other factors. Thus the seven samples analyzed have potential as a source of albumin.

ACKNOWLEDGEMENTS

The authors are grateful to the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia for funding to support this research. We also thank the laboratory technicians of the Fish Product Technology Laboratory, Faculty of Marine Sciences and Fisheries, and the Integrated Biotechnology Laboratory, Faculty of Animal Science at Hasanuddin University, for their assistance with sample analysis.

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