FOODSCITECH
Food Science and Technology Journal

Relative Mobility (Rf) Analysis of Albumin Isolates from Snakehead Fish (*Ophiocephalus striatus*) Extracted at Different Temperatures and Times

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ABSTRACT

Snakehead fish was a fish that has a high albumin content. Snakehead fish was used in the health sector known as medicinal freshwater fish, to accelerate the process of healing wounds after surgery and childbirth. The purpose of this study was to analyze the relative mobility (Rf) of the snakehead fish (Ophiocephalus striatus) albumin isolates by steaming at different temperatures and times. The research method for analyze the relative mobility (Rf) of the snakehead fish (Ophiocephalus striatus) albumin isolates by using gel filtration column chromatography, was detected by the SDS-PAGE electrophoresis method. Electrophoresis was carried out at a constant voltage of 125 v/ slab. Gel staining was carried out with Commasie Brilliant Blue R-250 in methanol-acetic acid-water. A standard protein mixture consisting of: myosin (200.0 kDa), β-galactosidase (116.3 kDa), phosphorylase B (97.4 kDa), bovine serum albumin (66, 2 kDa), ovalbumin (45.0 kDa), carbonic anhydrose (31.0 kDa), trypsin inhibitor (21.5 kDa), lysozyme (14.4 kDa) and aprotinim (6.5 kDa). The data analysis of this research was descriptive analysis to see the photos of the electrophoresis results. Meanwhile, for the analysis, the measurement of albumin isolation by gel filtration Sephadex G-75 was carried out with a divided plot design. The results showed that SDS-PAGE electrophoresis with the most complex amount of protein was albumin isolate, the effect of the steaming temperature was 40 ^{o}C for 30 minutes, located in 5 ml of the 1st fraction, 5 ml of the 2nd fraction and 5 ml of the 3rd fraction, with values of relative mobility (Rf) 0.0833 to 0.6944.

Key words*: snakehead fish; albumin isolate, relative mobility*

INTRODUCTION

Albumin was the highest plasma protein in about 60% and has various functions that are very important for health, namely the formation of new cell tissue, accelerating the recovery of damaged body tissue and maintaining fluid balance in blood vessels with fluid in the interstitial cavity within the limits normal limit, albumin levels in the blood 3.5-5 g/dl (Fulks et al., 2010). One way to meet the needs of albumin in the body is by administering human serum albumin (HSA) (Kusumaningrum et al., 2014; Firlianty et al., 2014).

Snakehead fish was a fish that has a high albumin content. Snakehead fish was used in the health sector known as medicinal freshwater fish, to accelerate the process of healing wounds after surgery and childbirth (Rahayu et al., 2016). Research in the nutrition installation and the surgical section of the Dr. Saiful Anwar Malang Hospital on postoperative patients with low albumin levels (1.8 g/dl). With the treatment of 2 kg of cooked snakehead fish per day, it has increased the patient's blood albumin levels to normal (3.5-5.5 g/dl) (Putri & Agustina, 2017). Snakehead fish has very beneficial biomedical benefits, such as anti-inflammatory, anti-microorganisms, anti-pain nociception and anti-cancer properties (He et al., 2017). Susilowati et al. (2015) in their research states that snakehead fish extract was a prospective alternative as raw material for nutraceutical products. Prastari et al. (2017) reported that snakehead fish protein hydrolyzate has antihyperglycemic potential. Other benefits of snakehead fish albumin, including maintaining intravascular oncotics (colloid

osmotics), facilitate the movement of body fluids and facilitate the transfer of substances (Fulks et al., 2010). Several types of amino acids contained in snakehead fish include arginine (3.55%), valine (7.58%), isoleucine (5.36%), aspartic acid (16.09%), tyrosine (1. 99%), alanine (15.62%), and tyrosine (2.68%) (Firlianty et al., 2014). Other research results related to the albumin content of snakehead fish were the interaction between various treatment factors with the higher temperature range of 40-90 ^{o}C, and steaming time ranging from 25-35 minutes. The highest albumin yield of snakehead fish extract was 2.459 g/100g, by steaming temperature of 60 ^{o}C for 25-35 minutes (Nugroho, 2013).

Romadhoni et al. (2016) stated that albumin quality was influenced by the method used. The use of temperature treatment and steaming time are alternatives to obtain crude albumin extract of snakehead fish. Many methods have been used to extract albumin, including steaming, vacuum drying and freeze drying as well as the use of various solvents (Asfar et al., 2014).

Based on the data from the above research, it was necessary to carry out an analysis of the relative mobility (Rf) of snakehead fish albumin (Ophiocephalus striatus) with using gel filtration column chromatography, was detected by the SDS-PAGE electrophoresis method, to test the value of the relative mobility (Rf) of snakehead fish albumin with serum albumin, where the application of snakehead fish albumin can replace serum albumin in an effort to help maintain and improve nutritional value and human health.

METHODS

Material

The materials used in this research were snakehead fish (*Ophiocephalus striatus*) from Karangkates-Malang reservoir and aquadest. The test materials for albumin levels in the bromine cresol green method were succinate buffer (7 mmol/ l pH 4.2), bromine cresol green 0.15 mmol/l, brij 35 and aquadest can succinate (0.01 M; pH 4.2), while for the albumin content after the gel filtration column was purified by UV testing, the ingredients included standard BSA 0.5 g/l phosphate buffer 0.1 M pH 7.1 and aquabidest. Materials used to purify the crude albumin extract consisted of 1 g of sephadex G-75, phosphate buffer (0.1 M pH 7.1), glasswool and 0.2% sodium azid were purchased from Sigma-Aldrich. Electrophoresis analysis materials include: 12.5% separator gel (acrylamide 30% 4.126 ml, 1.5 M tris pH 8.8 2.5 ml, 10% SDS 100 µl, TEMED 20 µl, 10% ammonium persulfate 25 µl), gel stacker 4% (acrylamide 30% 1.03 ml, 0.5 m tris pH 6.8, H2O 2,650 ml, 10% SDS 50µl, 10% ammonium persulfate 15µl), running buffer (glycine 14.4 g, tris base 1.0 g, ad aquabidest 100 ml), reducing sample buffer (RBS) (H_2O (aquabidest) 3 ml, 0.5 M tris pH 6.8 1 ml, glycerol 10% 1.6 ml, SDS 10% 1, 6 ml, 0.4 ml mercaptoetanol and 0.4 ml bromophenol blue), staining (comassie brilliant blue 0.1 g, 40 ml absolute methanol, 10 ml acetic acid, and 100 ml ad aquabidest), destainning (methanol 20 ml, acetic acid 10 ml and ad aquabidest 100 ml) were purchased from Sigma-Aldrich. All chemicals used were analytical grade.

Tool

The equipment used includes: knives, scissors, waterbath, thermocouple, 100 ^{o}C thermometer, scale, measuring cup, filter cloth, plastic and hydraulic press. Equipment for analyzing albumin levels includes: 1 cm diameter cuvette, Shimadzu UV-100-02 spectrophotometer and SMA autoanalyzer spectrophotometer. Gel filtration column measuring 2.5 x 60 cm, with sephadex G-75 for the purification of crude albumin extract. Characteristics of the molecular weight of albumin with the following equipment: 1

ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
DOI: http://dx.doi.org/10.25139/fst.v4i1.3799

electrophoretic unit of the Bio-Rad brand apparatus, refrigerator, sample plate, scapel, tweezers, deep freezer, ruler, pipette, knife and digital balance. Equipment for gel preparation includes: measuring cup, erlenmeyer, digital balance, pipette, heater, gloves, glass, plastic, gel plate and vacuum pump. Coloring tools include: cutting tools, glass, mica, ballast, pipette, digital balance, gel plate, incubator. Bufer making tools: measuring cup, pipette and erlenmeyer.

The research method for analysis of relatife mobility (Rf) of albumin isolates was detected by the SDS-PAGE electrophoresis method, 4% acrylamide stacking gel separation, 12% separating gel, 1 mg/ml albumin isolate dissolved in 0.0625 M Tris-HCl buffer pH 6.8 which contains SDS (2% w/v), glicerol (7% w/v), urea (8 M) and 2-mercaptoethanol (5% w/v). Electrophoresis was carried out at a constant voltage of 125 v/slab. Gel staining was carried out with Commasie Brilliant Blue R-250 (0.1% w/v) in methanol-acetic acid-water (10:7.5:82.5% v/v/v). A standard protein mixture (Novex Mark 12, San Diego, CA) consisting of: myosin (200.0 kDa), β-galactosidase (116.3 kDa), phosphorylase B (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45.0 kDa), carbonic anhydrose (31.0 kDa), trypsin inhibitor (21.5 kDa), lysozyme (14.4 kDa) and aprotinim (6.5 kDa). The data analysis of this research was descriptive analysis to see the photos of the electrophoresis results. Meanwhile, for the analysis, the measurement of albumin isolation by gel filtration Sephadex G-75 was carried out with a divided plot design (RPB).

RESULT AND DISCUSSIONS

The results of the electrophoretic analysis of the determination of the relative mobility (Rf) of the snakehead fish albumin isolates presented in Table 1. explained that the albumin isolates were treated with 80 ^{o}C waterbath steaming temperature (meat temperature 55 ^{o}C), and continued with the fractionation of the sephadex G-75 gel filtration column to extract 5 ml of the 2nd fraction after electrophoresis produces six protein bands. 1st band Rf value 0.0938; Rf 2nd band 0.1563; Rf 3rd band 0.1719; Rf 4th band 0.2500; Rf 5th band 0.3042; no Rf values in the 6th, 7th and 9th bands; the Rf value of the 8th band was 0.7813. Taking 5 ml of the 3rd fraction after electrophoresis resulted in 5 protein bands. Missing 1st band Rf value; Rf 2nd band 0.1406; Rf 3rd band 0.1875; Rf 4th band 0.2344; Rf 5th band 0.3041; no RF values in the 6th, 7th and 9th bands; the Rf value of the 8th band was 0.7813. Taking 5 ml of the 4th fraction after electrophoresis resulted in 4 protein bands. Missing 1st band Rf value; Rf 2nd band 0.1406; Rf 3rd band 0.1719; the Rf value of the 4th band is missing; Rf 5th band 0.3125; no RF values in the 6th, 7th and 9th bands; the Rf value of the 8th band was 0.7813.

Nugroho (2013), the results of the relevant research on the molecular weight (MW) characteristics of snakehead fish albumin isolation explained that taking 5 ml of the 2nd fraction after electrophoresis resulted in six protein bands, consisting of two major bands, namely bands 5 and 8 with a molecular weight of 66.3 kD and 11.2 kD, and four minor bands, namely bands 1, 2, 3 and 4 with a molecular weight of 145, 3 kD, 115.1 kD, 108.5 kD and 81.1 kD; for 5 ml of the 3rd fraction after electrophoresis it produces five protein bands, which consist of two major bands, namely bands 5 and 8 with molecular weights of 66.3 kD and 11.2 kD, and three minor bands namely bands 2, 3, and 4 with molecular weights of about 121.9 kD, 102.4 kD and 85.9 kD, respectively; where as for 5 ml of the 4th fraction after electrophoresis it produced four protein bands, which consisted of two major bands, namely bands 5 and 8 with molecular weights of 66.2 kD and 11.2 kD, and two minor bands, namely bands 2 and 3 with weight 121.9 kD and 108.5 kD molecules.

FOODSCITECH, Vol. 4 No. 1 July 2021
ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
DOI: http://dx.doi.org/10.25139/fst.v4i1.3799

Nugroho (2013), the results of the relevant research on the molecular weight characteristics of snakehead fish albumin isolation, explained that SDS-PAGE electrophoresis was treated with 80 ^{o}C waterbath steaming temperature (meat temperature 55 ^{o}C) in the 2nd to 4th fractions, this can be seen in rows B, C, and D (Figure 1A). In the 2nd to 4th fractions, the thickness of the protein bands was almost the same, it was assumed that at 5 ml of the 2nd fraction, 5 ml of the 3rd fraction and 5 ml of the 4th fraction have similarities in terms of the amount of albumin content that dissolves. The albumin isolate resulted from the fractionation of the gel filtration column with relatively the same albumin content when taking 5 ml of the 2nd fraction to 5 ml of the 4th fraction.

Asikin and Kusumaningrum (2018), describing the results of egg white ovalbumin fractionation, the highest average concentration of ovalbumin (%) was found in fractions II and III while fractions I, IV and V tended to decrease ovalbumin concentrations. Protein molecules with the same charge and size would accumulate in adjacent zones or bands. It was further explained that more bands indicate that the sample is composed of complex proteins.

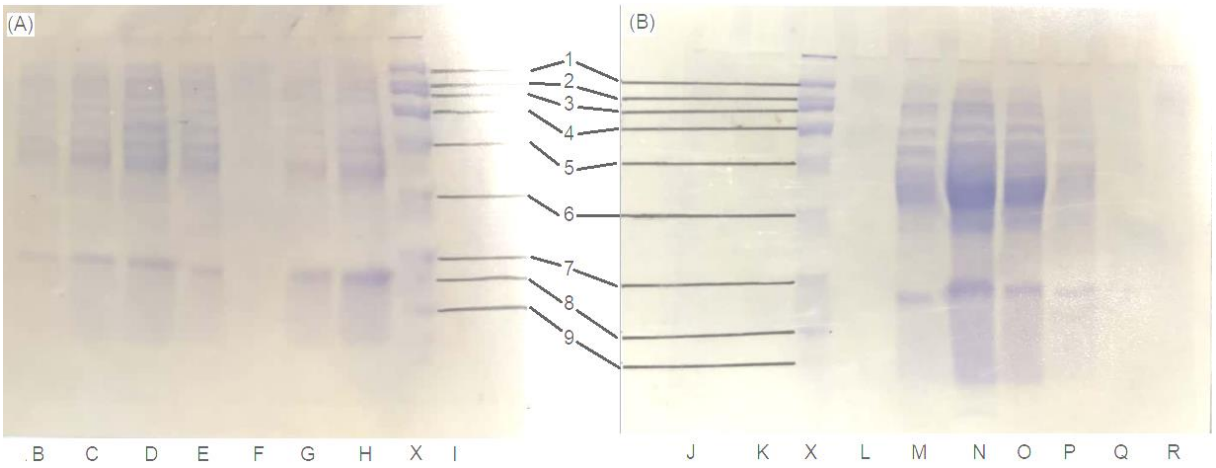

Figure 1. SDS-PAGE Electrophoresis of albumin isolates after fractionation (Nugroho, 2013)
Noted: X= protein signature kits: myosin (1), β-galactosidase (2), phosporylase B (3), bovine serum albumin (4), ovalbumin (5), carbonic anhydrase (6), trypsin inhibitot (7), lysozyme (8), aprotinin (9); B=5 ml albumin isolate of 2nd fraction of WT/MT (80/55 ^{o}C); C=5 ml albumin isolate of 3rd fraction of WT/MT (80/55 ^{o}C); 5 ml albumin isolate of 4th fraction of WT/MT (80/55 ^{o}C); E=5 ml albumin isolate of 1st fraction of WT/MT (60/45 ^{o}C); F=5 ml albumin isolate of 2nd fraction of WT/MT (60/45 ^{o}C); 5 ml albumin isolate of 3rd fraction of WT/MT (60/45 ^{o}C); 5 ml albumin isolate of 4th fraction of WT/MT (60/45 ^{o}C); I=5 ml albumin isolate of 1st fraction of WT/MT (90/66 ^{o}C); J=5 ml albumin isolate of 2nd fraction of WT/MT (90/ 66 ^{o}C); K=5 ml albumin isolate of 3rd fraction of WT/ MT (90/ 66 ^{o}C); L=5 ml albumin isolate of 4th fraction of WT/ MT (90/ 66 ^{o}C); M=5 ml albumin isolate of 1st fraction of WT/ MT (40/36 ^{o}C); N=5 ml albumin isolate of 2nd fraction of WT/ MT (40/36 ^{o}C); O=5 ml albumin isolate of 3rd fraction of WT/ MT (40/36 ^{o}C); P=5 ml albumin isolate of 4th fraction of WT/ MT (40/36 ^{o}C); Q=5 ml albumin isolate of 2nd fraction of WT/ MT (90/66 ^{o}C). WT=waterbath temperature; MT=meat temperature

The results of the electrophoretic analysis of the determination of the relative mobility (Rf) of the snakehead fish albumin isolates presented in Table 1, explained that the albumin isolates were treated with 60 ^{o}C waterbath steaming temperature (meat temperature 45 ^{o}C), and continued fractionation of the sephadex G-75 gel filtration column when taking 5 ml of the 1st fraction, resulting in six protein bands. 1st band Rf value 0.0938; Rf 2nd band 0.1563; Rf 3rd band 0.1875; Rf 4th band 0.2344; Rf 5th band 0.3040; no Rf values in the 6th, 7th and 9th bands; the Rf value of the 8th band was 0.7813. Taking 5 ml of the 2nd fraction, produces five protein bands. 1st band Rf value 0.0780; Rf 2nd band 0.1406; Rf 3rd band

0.1875; Rf 4th band 0.2344; The Rf of the 5th band was 0.3040; while for bands 6, 7, 8 and 9 did not produce the value of Rf. Taking 5 ml of the 3rd fraction, produced 6 protein bands, with each Rf value as follows: 1st band Rf value 0.0780; Rf 2nd band 0.1250; Rf 3rd band 0.1719; Rf 4th band 0.2344; Rf 5th band 0.3042; no RF values in the 6th, 7th and 9th bands; the Rf value of the 8th band was 0.7344. Taking 5 ml of the 4th fraction, produced 6 protein bands, with each Rf value as follows: 1st band Rf value 0.0780; Rf 2nd band 0.1406; Rf 3rd band 0.1719; Rf 4th band 0.2500; Rf 5th band 0.3041; no Rf values in the 6th, 7th and 9th bands; the Rf value of the 8th band was 0.7188.

Nugroho (2013), the results of the relevant research on the molecular weight characteristics of snakehead fish albumin isolation explained, taking 5 ml of the 1st fraction, resulted in six protein bands consisting of of the two major bands, namely band 5 and band 8 with molecular weights of 66.3 kD and 11.2 kD, and four minor bands, namely fractions 1, 2, 3 and 4 with molecular weights of about 145.3 kD, 115.1 kD, 102.4 kD and 85.9 kD; for 5 ml of the 2nd fraction after electrophoresis it produces six protein bands consisting of one major band, namely band 8 with a molecular weight of 11.2 kD, and five minor bands, namely bands 1, 2, 3, 4 and 5 with molecular weights respectively 154.2 kD, 121.9 kD, 102.4 kD, 85.9 kD and 66.3 kD; for 5 ml of the 3rd fraction after electrophoresis produced six protein bands, consisting of two major bands, namely bands 5 and 8 with molecular weights of 66.2 kD and 13.3 kD, and four minor bands namely 1, 2, 3 and 4 with molecular weights respectively 154.2 kD, 129.3 kD, 108.5 kD and 85.9 kD; while 5 ml of the 4th fraction after electrophoresis produced six protein bands consisting of two major bands, namely bands 5 and 8 with molecular weights of 66.3 kD and 14.1 kD, and four minor bands namely 1, 2, 3 and 4 with molecular weights respectively 154.2 kD, 121.9 kD, 108.5 kD and 81.1 kD.

Table 1. Relative mobility (Rf) and estimated molecular weight of albumin isolates due to different steaming temperatures for 30 minutes

NO	(80 : 55) ^{o}C 5/2		(80 : 55) ^{o}C 5/3		(80 : 55) ^{o}C 5/4		(60 : 45) ^{o}C 5/1		(60 : 45) ^{o}C 5/2		(60 : 45) ^{o}C 5/3		(60 : 45) ^{o}C 5/4	
Band	Rf	MW kD	Rf	MW kD	Rf	MW kD	Rf	MW kD	Rf	MW kD	Rf	MW kD	Rf	MW kD
1	0.093	145.3	-	-	-	-	0.093	145.3	0.078	154.2	0.078	154.2	0.078	154.2
2	0.156	115.3	0.140	121.9	0.140	121.9	0.156	115.1	0.140	121.9	0.125	129.3	0.140	121.9
3	0.171	108.5	0.187	102.4	0.171	108.5	0.187	102.4	0.187	102.4	0.171	108.5	0.171	108.5
4	0.250	81.1	0.234	85.9	-	-	0.234	85.9	0.234	85.9	0.234	85.9	0.250	81.1
5	0.304	66.3	0.304	66.3	0.312	64.2	0.304	66.3	0.304	66.3	0.304	66.2	0.304	66.3
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	0.781	11.2	0.781	11.2	0.781	11.2	0.781	11.2		11.2	0.734	13.3	0.719	14.1
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Note: (80/55) ^{o}C = WT/MT; (60/45) ^{o}C = WT/MT; (90/66) ^{o}C = WT/MT; (WT = waterbath temperature; MT= meat temperature); 5/1= 5 ml 1st fraction from the sephadex G-75 gel filtration column; 5/2 = 5 ml 2nd fraction from the sephadex G-75 gel filtration column; 5/3 = 5 ml 3rd fraction from the sephadex G-75 gel filtration column; 5/4 = 5 ml 4th fraction from the sephadex G-75 gel filtration column.

Nugroho (2013), the results of the relevant research on the molecular weight characteristics of snakehead fish albumin isolation, explained that SDS-PAGE electrophoresis was treated with 60 ^{o}C waterbath steaming temperature (meat temperature 45 ^{o}C), the amount of dissolved protein content for each fraction of the Sephadex G-75 gel column was still high. This was evidenced by the number of protein bands in the four fractions that are almost the same (lines E, F, G, H Figure 1A). Seeing this, it was suspected that the albumin isolate from water bath temperature steaming was 60 ^{o}C, the albumin

FOODSCITECH, Vol. 4 No. 1 July 2021
ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
DOI: http://dx.doi.org/10.25139/fst.v4i1.3799

plasma had not yet occurred. The highest albumin content occurred in 5 ml of the 1st fraction, 5 ml of the 3rd fraction and 5 ml of the 4th fraction, this can be seen from the condition of the protein band thickness which was almost the same in the three bands of fractions (rows E, G and H Figure 1A), and the thickness lies in the protein band 5 with a molecular weight of 66.2-66.3 kD. Suardi et al. (2020) stated the results of research that the initiation of plasma albumin denaturation was at a heating temperature of 69.1 ± 0.3 ^{o}C, while the peak of plasma denaturation occurred at a heating temperature of 78 ± 0.2 ^{o}C.

The results of the electrophoretic analysis of the determination of the relative mobility (Rf) of the snakehead fish albumin isolates were presented in Table 2. which explains that the albumin isolates were treated with 90 ^{o}C waterbath steaming temperature (66 ^{o}C meat temperature), and continued fractionation of the sephadex G-75 gel filtration column when taking 5 ml of the 1th fraction does not get the value of relative mobility (Rf). When taking 5 ml of the 2nd fraction, the relative mobility (Rf) value was not obtained. Likewise, for taking 5 ml of the 3rd and 4th fractions at the same heating temperature after electrophoresis there was no value for relative mobility (Rf).

Nugroho (2013) results of relevant research on the characteristics of the molecular weight of snakehead fish albumin isolation, explaining that SDS-PAGE electrophoresis was treated with 90 ^{o}C waterbath steaming temperature (meat temperature 66 ^{o}C), and continued with fractionation of the sephadex G-75 gel phytration column to extract 5 ml of the 1st fraction, no protein bands were obtained. The results of electrophoresis (Figure 1A) of albumin isolates were treated with a temperature measurement of 90 ^{o}C (meat temperature of 60 ^{o}C), and continued with fractionation of the sephadex G-75 gel filtration column for 5 ml of the 2nd fraction, no protein bands were found. Likewise, taking 5 ml of the 3rd and 4th fractions at the same heating temperature after dielectrophoresis did not produce protein bands. The results of the electrophoresis showed that the albumin isolate was treated with a temperature of 90 ^{o}C water bath (meat temperature 66 ^{o}C), the amount of content and quality of the dissolved protein in the albumin isolate was low, presumably at 90 ^{o}C waterbath measurement temperature the protein had undergone denaturation. The four fractions of the sephadex G-75 gel filtration column, after electrophoresis, none of the protein bands were detected (row I Figure 1, rows J, K, L Figure 1B).

Table 2. Relative mobility (Rf) and estimated molecular weight of albumin isolates due to different steaming temperatures for 30 minutes

No	(90:66) ^{o}C 5/2		(90:66) ^{o}C 5/3		(90:66) ^{o}C 5/4		(40:36) ^{o}C 5/1		(40:36) ^{o}C 5/2		(40:36) ^{o}C 5/3		(40:36) ^{o}C 5/4	
Band	Rf	MW kD	Rf	MW kD	Rf	MW kD	Rf	MW kD	Rf	MW kD	Rf	MW kD	Rf	MW kD
1	-	-	-	-	-	-	0.083	133	0.083	133	0.083	133	-	-
2	-	-	-	-	-	-	0.138	108.8	0.125	114.4	0.125	114.4	-	-
3	-	-	-	-	-	-	0.152	103.5	0.152	103.5	0.138	108.8	0.152	103.5
4	-	-	-	-	-	-	0.208	84.7	0.208	84.7	0.208	84.7	0.208	84.7
5	-	-	-	-	-	-	0.276	66.2	0.276	66.2	0.276	66.2	0.277	66.5
6	-	-	-	-	-	-	0.430	37.9	0.416	39.9	0.402	41.9	0.416	39.9
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	0.694	14.6	0.694	14.6	0.694	14.6	0.694	14.6
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Noted: (90/66) ^{o}C = WT/MT; (40/36) ^{o}C = WT/MT; (WT= waterbath temperature; MT= meat temperature); 5/1 = 5 ml 1st fraction from the sephadex G-75 gel filtration column; 5/2 = 5 ml 2nd fraction from the sephadex G-75 gel filtration column; 5/3 = 5 ml 3rd fraction from the sephadex G-75 gel filtration column; 5/4 = 5 ml 4th fraction from the sephadex G-75 gel filtration column.

ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
DOI: http://dx.doi.org/10.25139/fst.v4i1.3799

Fitriyani and Deviarni (2018) explained in their research, that when BSA and alpha-lactalbumin were heated at 78 ^{o}C for 15 minutes, it would reduce the number of zones detected. The heating more than 90 ^{o}C albumin began to reach the maximum gel. Furthermore, it was explained that at a temperature of 70 ^{o}C the solubility of albumin was around 81% so that the steaming temperature of 90 ^{o}C would reduce the solubility of albumin in the snakehead fish albumin isolate.

The results of the electrophoretic analysis of the determination of the relative mobility (Rf) of the snakehead fish albumin isolates presented in Table 2. explained that the albumin isolates were treated at 40 ^{o}C waterbath steaming temperature (meat temperature 36 ^{o}C), and continued with fractionation of the sephadex G-75 gel filtration column when taking 5 ml of the 1st fraction produce seven protein bands. Rf value for the 1st band 0.0833; Rf for the 2nd band 0.1389; Rf 3rd band 0.1528; Rf 4th band 0.2083; Rf 5th band 0.2763; Rf 6th band 0.4306; no Rf values in the bands 7 and 9; the Rf value of the 8th band was 0.6944. In taking 5 ml of the 2nd fraction, it produces seven protein bands. Rf value for 1st band 0.0833; 2nd band Rf 0.1250; Rf 3rd band 0.1528; Rf 4th band 0.2083; Rf 5th band 0.2762; Rf 6th band 0.4167; no RF values in the bands 7 and 9; the Rf value of the 8th band was 0.6944. In taking 5 ml of the 3rd fraction, it produces seven protein bands. Rf value for 1st band 0.0833; 2nd band Rf 0.1250; Rf 3rd band 0.1389; Rf 4th band 0.2083; Rf 5th band 0.2763; Rf 6th band 0.4028; no Rf values in the bands 7 and 9; the Rf value of the 8th band was 0.6944. When taking 5 ml of the 4th fraction, it produces 5 protein bands. There were no Rf values for the 1st and 2nd bands; Rf 3rd band 0.1528; Rf 4th band 0.2083; Rf 5th band 0.2778; Rf 6th band 0.4167; no RF values in the bands 7 and 9; the Rf value of the 8th band was 0.6944.

Nugroho (2013), the results of the relevant research on the molecular weight characteristics of snakehead fish albumin isolation explained that taking 5 ml of the 1st fraction after electrophoresis produced seven protein bands, consisting of two major bands, namely bands 5 and 8 with molecular weights of 66.2 kD and 14.6 kD, and five minor bands, namely 1, 2, 3, 4 and 6 with their respective molecular weights. 133 kD, 108.8 kD, 103.5 kD, 84.7 kD and 37.9 kD, respectively; for 5 ml of the 2nd fraction, after electrophoresis, there were seven protein bands, consisting of two major bands, namely bands 5 and 8 with molecular weights of 66.2 kD and 14.6 kD, and five minor bands namely 1, 2, 3, 4 and 6 with molecular weights of 133 kD, 114.4 kD, 103.5 kD, 84.7 kD and 39.9 kD, respectively; for 5 ml of the 3rd fraction, after electrophoresis, there were seven protein bands, consisting of two major bands, namely bands 5 and 8 with molecular weights of 66.2 kD and 14.6 kD, and five minor bands namely 1, 2, 3, 4 and 6 with molecular weights, respectively, 133 kD, 114.4 kD, 108.8 kD, 84.7 kD and 41.9 kD; whereas for 5 ml of the 4th fraction after electrophoresis, five protein bands were obtained, consisting of one major band, namely the 8 band with a molecular weight of 14.6 kD, and four minor bands namely 3, 4, 5 and 6 with BM respectively. 103.5 kD, 84.7 kD, 66.5 kD and 39.9 kD.

Nugroho (2013) in the relevant research results on the characteristics of the molecular weight of snakehead fish albumin isolation, explained that SDS-PAGE electrophoresis was treated with 40 ^{o}C waterbath steaming temperature (meat temperature 36 ^{o}C), the type and content of protein dissolved in the albumin isolate were still complex and high. That each uptake fraction from the sephadex G-75 gel filtration column, after electrophoresis produced a more constant number of protein bands. This can be seen from (M, N, O and P Figure 1B, Tables 1 and 2), the four fractions have the same number of protein bands, namely seven bands. It was suspected that the four fractions have almost the same protein content, while

for the thickness of the ribbon there is similarity for taking 5 ml of the 1st fraction to 5 ml of the 3rd fraction, each of which has two major bands. It was suspected that these three initial fractions had almost the same molecular weight charge of the albumin. Alviodinasyari et al. (2019) reported the results of their research, that the major band had a thickness and color intensity greater than other bands, so the conclusion was that the major band is a protein band that has a higher concentration compared to other bands (minor bands). The heating BSA (bovine serum albumin) 40 ^{o}C to 70 ^{o}C, the number of relatively complex zones was obtained (7 bands) and two of them were thick with soluble protein ranging from 6.5-3.5 mg/ml, but at heating 80 ^{o}C above the number of zones reduced drastically (1 band) and thin with soluble protein ranging from 1-1.5 mg/ml.

The results of electrophoresis (Figure 1B) albumin isolates were treated with 90 ^{o}C waterbath steaming temperature (66 ^{o}C meat temperature), which was a conversion from the previous 90 ^{o}C waterbath steaming temperature treatment (rows I, J, K and L Figure 1B), taking 5 ml fractions 2 and 3 did not produce protein bands. The electrophoresis results showed that at the waterbath steaming temperature of 90 ^{o}C (meat temperature 66 ^{o}C), which was the conversion result of the previous 90 ^{o}C waterbath steaming temperature, it also had the same tendency for missing and undetectable protein bands (Q and R rows Figure 1B). It was suspected that the protein solubility of the albumin isolate was damaged due to heat denaturation, and a change in the characteristics of the albumin isolate, namely the formation of a gel. Kong et al. (2007) states that the heating process in fish meat causes changes in the texture of fish meat, such as damage to myofibrillar or collagen proteins, protein mass formation, and drastic changes in protein formation, the greater the damaged protein until it reaches a constant level so that the results were low enough.

Changes in the protein pattern of SDS-PAGE results indicate that there were changes that occur in proteins, thinning and loss of protein bands indicate changes in the properties of these proteins. Albumin was a water-soluble protein. However, protein content has decreased at temperatures above 40 ^{o}C, for example at temperatures of 50-70 ^{o}C. Albumin was composed of a single polypeptide chain which has a molecular weight of around 66.4 kDa and is composed of 585 amino acids (Alviodinasyari *et al*., 2019).

CONCLUSION

Relatife mobility of albumin isolate from snakehead fish (*Ophiocephalus striatus)* the result of SDS-PAGE electrophoresis with values of mobility relative of 0.0833 to 0.6944 and the most complex amount of protein were found at the steaming temperature was 40 ^{o}C for 30 minutes, located in 5 ml of the 1st fraction, 5 ml of the 2nd fraction and 5 ml of the 3rd fraction. The albumin isolates from snakehead fish (*Ophiocephalus striatus*) is promising to replace serum albumin in an effort to help maintain and improve nutritional value and human health.

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