Relative Mobility

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Relative Mobility (Rf) and Molecular Weight Identification of Fish Albumin Isolate (*Ophiocephalus striatus*)

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ABSTRACT

The purpose of this study was to determine the relative mobility (Rf) and to identify the molecular weight of the albumin from steaming the snakehead fish (Ophiocephalus striatus). The research method for detecting the molecular weight of albumin, using gel filtration column chromatography, 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. The results showed that the SDS-PAGE electrophoresis with the most complex amount of protein was albumin isolate which was influenced by the steaming temperature of 40 °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 protein band consists of 2 major bands and 5 minor bands with the relative mobility (Rf) 0.6944 – 0.1250 and molecular weight 14.6-133 kD.

Key words: *snakehead fish; (*Ophiocephalus striatus*); albumin isolate, molecular weight*

Abstrak

Tujuan penelitian ini adalah menentukan mobilitas relatif (Rf) dan identifikasi berat molekul isolasi albumin pengukusan Ikan Gabus (Ophiocephalus striatus). Metode penelitian pendeteksian berat molekul albumin, dengan menggunakan kromatografi kolom filtrasi gel, dideteksi dengan metode elektroforesis SDS-PAGE, separasi 4% gel penumpuk (acrylamide stacking gel), gel pemisah (separating gel) 12%, isolat albumin 1 mg/ml dilarutkan dalam 0,0625 M Tris-HCl bufer pH 6,8 yang berisi SDS (2% w/v), glicerol (7% w/v), urea (8 M) dan 2-mercaptoethanol (5% w/v). Elektroforesis dijalankan dengan voltase konstan 125 v/slab. Pewarnaan gel dilakukan dengan Commasie Brilliant Blue R-250 (0,1% w/v) dalam methanol-asam asetat-air (10:7,5:82,5% v/v/v). Campuran standar protein (Novex Mark 12, San Diego, CA) yang terdiri dari: myosin (200,0 kDa), β-galaktosidase (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) dan aprotinim (6,5 kDa). Analisa data penelitian ini adalah analisa deskriptif untuk melihat foto hasil elektroforesis. Sementara untuk hasil analisa pengukuran isolasi albumin secara filtrasi gel sephadex G-75 dilakukan dengan Rancangan Petak terbagi (RPB). Hasil penelitian menunjukkan bahwa Elektroforesis SDS-PAGE dengan jumlah protein paling komplek adalah isolat albumin pengaruh suhu pengukusan 40^{O}C selama 30 menit, terletak pada 5 ml fraksi ke-1, 5 ml fraksi ke-2 dan 5 ml fraksi ke-3. Pita protein terdiri dari 2 pita mayor dan 5 pita minor dengan berat molekul 14,6-133 kD.

Kata kunci *: ikan gabus (Ophiocephalus striatus), isolat albumin, berat molekul*

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) (Suprayitno, 2003). 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, 2012).

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).

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 °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 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 detecting the molecular weight of albumin, using gel filtration column chromatography, 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 electrophoresis results of the characteristic molecular weight characteristics of the fish albumin isolate were presented in Figures 1 and Tables 1 and 2. It explains that the albumin isolates were treated with 80 °C waterbath steaming temperature (meat temperature 55 °C), and followed by fractionation of the sephadex G-75 gel filtration column to extract 5 ml fractions, second after dielectrophoresis produced 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; whereas 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.

The electrophoresis results showed that of the 3 fractions of the sephadex G-75 gel filtration column after electrophoresis produced protein bands on SDS polyacrylamide electrophoregrams in the 2nd to 4th fractions, this can be seen in rows B, C, and D (Figure 1). 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.

Kato et al. (1981), 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. Sutiman *et al.* (1996) explained the results of their research that 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.

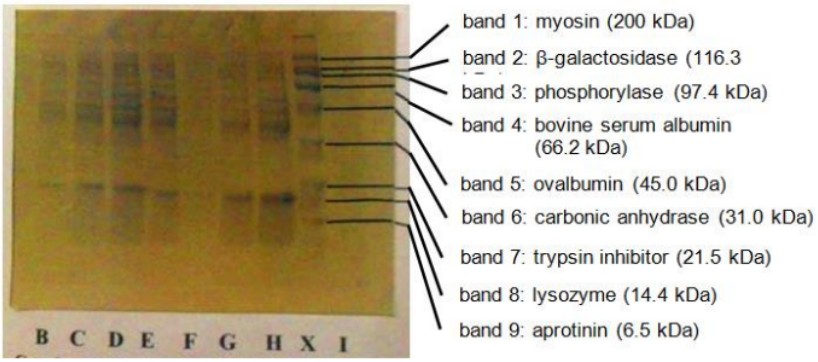

Figure 1. SDS-PAGE Electrophoresis of Albumin Isolates after Fractionation

Note:
X : protein signature kits: myosin, β-galactosidase, phosporylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, trypsin inhibitot, lysozyme and aprotinin
B : 5 ml albumin isolate of 2nd fraction of WT/MT (80/55 °C)
C : 5 ml albumin isolate of 3rd fraction of WT/MT (80/55 °C)
D : 5 ml albumin isolate of 4th fraction of WT/MT (80/55 °C)
E : 5 ml albumin isolate of 1st fraction of WT/MT (60/45 °C)
F : 5 ml albumin isolate of 2nd fraction of WT/MT (60/45 °C)
G : 5 ml albumin isolate of 3rd fraction of WT/MT (60/45 °C)
H : 5 ml albumin isolate of 4th fraction of WT/MT (60/45 °C)
I : 5 ml albumin isolate of 1st fraction of WT/MT (90/66 °C)
WT = waterbath temperature; MT= meat temperature

The results of electrophoresis were in Figure 1 and Tables 1 and 2. Albumin isolates were treated with 60 °C waterbath steaming temperature (meat temperature 45 °C), then fractionation of the sephadex G-75 gel filtration column, 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) °C 5/2		(80 : 55) °C 5/3		(80 : 55) °C 5/4		(60 : 45) °C 5/1		(60 : 45) °C 5/2		(60 : 45) °C 5/3		(60 : 45) °C 5/4	
Band to-	Rf	BM kD	Rf	BM kD	Rf	BM kD	Rf	BM kD	Rf	BM kD	Rf	BM kD	Rf	BM 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	0.781	11.2	0.734	13.3	0.719	14.1
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Note: (80/55) °C = WT/MT; (60/45) °C = WT/MT; (90/66) °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.

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

NO	(90/ 66)°C 5/2		(90/66) °C 5/3		(90/ 66)°C 5/4		(40/36) °C 5/1		(40/36) °C 5/2		(40/36) °C 5/3		(40/36) °C 5/4	
Band to-	Rf	BM kD	Rf	BM kD	Rf	BM kD	Rf	BM kD	Rf	BM kD	Rf	BM kD	Rf	BM 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) °C = WT/MT; (40/36) °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.

Based on the electrophoresis results, it was shown that the albumin isolate treatment temperature 60 °C waterbath steaming (meat temperature 45 °C), the amount of dissolved protein content for each fraction of the Sephadex G-75 gel column was still high. This is evidenced by the number of protein bands in the four fractions that are almost the same (lines E, F, G and H in Figure 1., Tables 1 and 2.). Seeing this, it was suspected that the albumin isolate from water bath temperature steaming was 60 °C, the albumin 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 in Figure 1., Tables 1 and 2.), and the thickness lies in the protein band 5 with a molecular weight of 66.2-66.3 kD. Raeker and Johnson (1995),stated the results of his research that the initiation of plasma albumin denaturation was at a heating temperature of 69.1 ± 0.3 °C, while the peak of plasma denaturation occurred at a heating temperature of 78 ± 0.2 °C.

Electrophoresis results (Figure 1., Tables 1 and 2.) albumin isolates were treated with a temperature measurement of 90 °C waterbath (meat temperature 66 °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 1) of albumin isolates were treated with a temperature measurement of 90 °C (meat temperature of 60 °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 °C water bath (meat temperature 66 °C), the amount of content and quality of the dissolved protein in the albumin isolate was low, presumably at 90 °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 (see row I Figure 1., rows J, K and L Figure 2., Tables 1 and 2.).

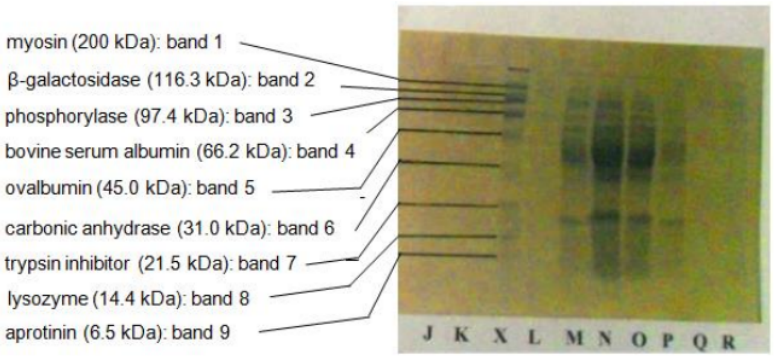

Figure 2. SDS-PAGE Electrophoresis of albumin isolates after fractionation

Note:

X	:	protein signature kits: myosin, β-galactosidase, phosporylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, trypsin inhibitot, lysozyme and aprotinin
J	:	5 ml albumin isolate of 2nd fraction of WT/MT (90/ 66 °C)
K	:	5 ml albumin isolate of 3rd fraction of WT/ MT (90/ 66 °C)
L	:	5 ml albumin isolate of 4th fraction of WT/ MT (90/ 66 °C)
M	:	5 ml albumin isolate of 1st fraction of WT/ MT (40/36 °C)
N	:	5 ml albumin isolate of 2nd fraction of WT/ MT (40/36 °C)
O	:	5 ml albumin isolate of 3rd fraction of WT/ MT (40/36 °C)
P	:	5 ml albumin isolate of 4th fraction of WT/ MT (40/36 °C)
Q	:	5 ml albumin isolate of 2nd fraction of WT/ MT (90/66 °C)

WT = waterbath temperature; MT= meat temperature

Weissler et al. (1981) explained in their research, that when BSA and alpha-lactalbumin were heated at 78 °C for 15 minutes, it would reduce the number of zones detected. Foegeding, et al. (1986) also presented the results of their research, that on heating more than 90 °C albumin began to reach the maximum gel. Furthermore, it was explained that at a temperature of 70 °C the solubility of albumin was around 81% so that the steaming temperature of 90 °C would reduce the solubility of albumin in the snakehead fish albumin isolate.

The electrophoresis results of the characteristics of the molecular weight of the snakehead fish albumin isolate (Figure 2.Tables 1 and 2.), at 40 °C waterbath steaming temperature treatment (36 °C meat temperature), and continued fractionation of the sephadex G-75 gel filtration column, to extract 5 ml fraction 1, obtained 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.

The electrophoresis results indicated that the albumin isolates were treated with a waterbath steaming temperature of 40 °C (meat temperature 36 °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 (lines M, N, O and P Figure 2. 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. Widowati and Wijayati (1997) 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). Foegeding *et al.* (1986) showed the results of their research, on heating BSA (bovine serum albumin) 40 °C to 70 °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 °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 2., Tables 1 and 2.) albumin isolates were treated with 90 °C waterbath steaming temperature (66 °C meat temperature), which was a conversion from the previous 90 °C waterbath steaming temperature treatment (see rows I, J, K and L Figure 2. Tables 1 and 2.), taking 5 ml fractions 2 and 3 did not produce protein bands. The electrophoresis results showed that at the waterbath steaming temperature of 90°C (meat temperature 66 °C), which was the conversion result of the previous 90 °C waterbath steaming temperature, it also had the same tendency for missing and undetectable protein bands (Q and R rows Figure 2., Table 1 and 2.). 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 (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 °C, for example at temperatures of 50-70 °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

Treatment of 40 °C steaming temperature for 30 minutes, taking 5 ml of the 2nd fraction, resulted in the highest albumin content of albumin isolates at 1.77 mg/g. SDS-PAGE electrophoresis with the most complex amount of protein was albumin isolate with the effect of steaming temperature of 40 °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 protein band consists of 2 major bands and

5 minor bands with the relative mobility (Rf) 0.6944 – 0.1250 and molecular weight 14.6-133 kD.

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