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Effect of Swamp Eel (*Monopterus albus*) Plasma on The Quality of Tilapia (*Oreochromis mossambicus*) Surimi

(Kesan Plasma Belut Paya (*Monopterus albus*) terhadap Kualiti Surimi Tilapia (*Oreochromis mossambicus*))

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ABSTRACT

The effects of crude plasma and ethanol-extracted of swamp eel plasma at different concentrations (0, 0.25, 0.5, 0.75, 1, 1.5, 2 mg/g) as inhibitor protease in the quality of tilapia (*Oreochromis mossambicus*) surimi were investigated. The parameters analyzed were gel strength, expressible moisture, whiteness, microstructure, protein solubility, hydrolysis pattern of surimi by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, percent inhibition, and zymogram. The results showed that ethanol-extracted plasma could maintain gel strength, expressible moisture, microstructure, protein solubility, and percent inhibition in lysis of tilapia surimi from protease activity that caused formation of modori structure better than crude plasma. The whiteness decreased as the concentration of plasma used increased. SDS-PAGE for hydrolysis pattern analysis showed that the effectiveness of crude plasma as a protease inhibitor decreased as the concentration increased. There was an increase in the inhibition of protease in the ethanol-extracted plasma at a concentration of 1.5 mg/g. It suggested that the crude plasma contained an internal protease enzyme that might interfere with the reduced myosin intensity.

Keywords: Crude plasma; modori; plasma ethanol fraction; swamp eel

ABSTRAK

Kesan plasma mentah belut paya dan diekstrak etanol pada kepekatan berbeza (0, 0.25, 0.5, 0.75, 1, 1.5, 2 mg/g) sebagai perencat protease dalam kualiti surimi tilapia (*Oreochromis mossambicus*) telah dikaji. Parameter yang dianalisis ialah kekuatan gel, lembapan boleh nyata, keputihan, struktur mikro, keterlarutan protein, corak hidrolisis surimi oleh elektroforesis gel natrium dodesil sulfat-poliakrilamida, perencatan peratus dan zimogram. Keputusan menunjukkan bahawa plasma yang diekstrak etanol boleh mengekalkan kekuatan gel, kelembapan yang boleh diungkapkan, struktur mikro, keterlarutan protein dan peratus perencatan dalam lisis surimi tilapia daripada aktiviti protease yang menyebabkan pembentukan struktur modori lebih baik daripada plasma mentah. Keputihan berkurangan apabila kepekatan plasma yang digunakan meningkat. SDS-PAGE untuk analisis pola hidrolisis menunjukkan bahawa keberkesanan plasma mentah sebagai perencat protease menurun apabila kepekatan meningkat. Terdapat peningkatan dalam perencatan protease dalam plasma yang diekstrak etanol pada kepekatan 1.5 mg/g. Ia mencadangkan bahawa plasma mentah mengandungi enzim protease dalaman yang mungkin mengganggu keamatan miosin yang dikurangkan.

Kata kunci: Belut paya; modori; plasma mentah; serpihan etanol plasma

INTRODUCTION

Surimi is an intermediate product used to make gel-based processed food products, including kamaboko, chikuwa, sausage, fish cake, and imitation crab. Surimi is made from minced fish that has undergone a process of washing (leaching), pressing (dewatering), mixing with cryoprotectants, and freezing (Park et al. 2013). Surimi is generally made from marine fish, such as pollock,

pacific whiting, hoki, southern blue whiting, northern blue whiting, and some tropical fish (threadfin bream, croaker, lizard fish, pike-conger). The utilization of tropical fish as raw material for surimi had increased by more than 60% due to increasing demand (Jaziri et al. 2021). In addition, freshwater fish could also be used as an alternative raw material for surimi. Crabsticks are made from silver carp surimi, a freshwater fish in China. Several studies had been conducted to examine the characteristics of surimi produced from freshwater fish (tilapia, catfish, carp). Studies showed that these freshwater fishes could produce good gel strength (Park et al. 2013).

Surimi must have specific characteristics as an essential ingredient in manufacturing gel-based products. Gel strength and colour/whiteness are the primary quality attributes for cooked surimi gel (Park 2005). The value of gel strength is influenced by the raw materials used and is also influenced by the cooking process.

With the relatively high protein content of fish as raw material for surimi, the presence of internal protease enzymes cannot be avoided. Quality deterioration is very easy to occur in fish and its processed products; one of the causes is a protease enzyme. This enzyme causes the autolysis of fishery products. Cathepsin L is one of the protease enzymes found in fish and is classified as cysteine protease. The enzyme could be an obstacle for the surimi industry because it could induce the modori phenomenon or gel softening. It happens during slow heating at a temperature of 55 °C, which is part of the surimi process in making various derivative products.

One way to inhibit the activity of protease enzymes is to use protease inhibitors. Studies on protease inhibitors used for surimi had been carried out. Some sources of protease inhibitors used were salmon plasma (Fowler & Park 2015a), rainbow trout plasma (Li, Lin & Kim 2008), and commercial protease inhibitors (dried egg whites, beef plasma, potato starch) (Weerasinghe, Morrissey & An 1996). Our on-going study also reported the use of crude plasma from swamp eel (*Monopterus albus*), which could inhibit cysteine proteases (papain) better than serine proteases (trypsin) (Nopianti et al. 2019). Initially, the surimi industry used cow plasma as a protease inhibitor, but its use was discontinued due to the outbreak of mad cow disease. The surimi industry then used dried egg whites as a protease inhibitor, but its inhibitory activity was less effective than that of the plasma. It was because dried egg whites were more effective in inhibiting serine proteases, while cathepsin L, which caused gel softening, was a cysteine protease (Fowler & Park 2015a). In addition, plasma contains a protease inhibitor, namely alpha-2-macroglobulin (Baret 1981), which was known to inhibit cysteine protease.

This study used crude plasma and ethanol-extracted plasma from swamp eel as protease inhibitors. The objectives were to find alternative sources of protease inhibitors from freshwater fish and examine the ability of crude plasma and ethanol-extracted plasma to maintain the quality of surimi tilapia (*Oreochromis mossambicus*) from protease activity that causes the modori phenomenon.

Materials and Methods

Materials

Live swamp eel (*Monopterus albus*) with the size 10-15/kg were obtained from wild catches in the Karawang area of West Java, Indonesia. The swamp eels were transported by bus at night to minimize contact with the sun. Tilapia fish were purchased from the Caringin traditional market in the Margajaya village, West Bogor sub-district, Bogor City, West Java, Indonesia. Tris base, 2-mercaptoethanol, and sodium dodecyl sulfate were purchased from Sigma Aldrich (USA). Urea and Triton X-100 were obtained from Merck-Millipore (USA); meanwhile, Trichloroacetic acid was purchased from Merck-Supelco (Germany). A broad range protein standard was purchased from Thermo Fisher Scientific (USA).

Methods

Preparation of crude plasma and ethanol-extracted plasma

Blood was directly collected from bleeding swamp eel and added with 10% EDTA (Ethylenediaminetetraacetic acid) to avoid coagulation with the ratio of 100:1. Blood was centrifugated at 1811 × g for 25 min to collect plasma. The plasma was centrifuged at 1811 × g for 25 min, then kept at -20 °C before used. Ethanol-extracted plasma was collected by fractionating crude plasma with 40% ethanol pH 5.5 following Cohn et al. (1946). Crude plasma was dissolved with distilled water at a ratio of 1:1 to get the protein concentration to be 30-35 mg/mL. Plasma was then mixed with 40% cold ethanol at a ratio of 2:1 (Denizli 2011). The precipitation process was carried out for 1.5 h at a temperature of ± 4 °C, then followed by a centrifugation process at 1811 × g for 10 min. The pellets obtained were dried with nitrogen gas for ± 30 min or until the weight was constant. The resulting dry pellet was then dissolved with 200 µL of 20 mM Tris-HCl pH 8.0. The resulting ethanol-extracted plasma was then stored at -20 °C before use for analysis.

MODORI GEL PREPARATION

Surimi was made from tilapia fish with the stages of the manufacturing process, namely gutting and heading, filleting, grinding, washing two times (leaching), and dewatering (straining). Surimi 150 g and 2% (w/w) salt (Park 2000) were mixed using a chopper. Swamp eel crude plasma and ethanol-extracted plasma were added to surimi with final concentrations of 0.25, 0.50, 0.75, 1.00, 1.50, and 2.00 mg/g. The water content of surimi was conditioned at ± 78%. Surimi and protease inhibitors were stirred for 5 min, and the temperature was kept below 10 °C. The surimi paste was then put into a polyvinylidene casing with a diameter of 30 mm. The surimi paste was twice heated, first at 55 °C for 1 h, then at 90 °C for 20 min, to produce the modori gel. Modori is softening of the surimi gel due to activity of cathelpsin L enzyme which occur during the surimi making at 55 °C. After completing the heating process, all surimi gels were cooled with ice water for 30 min and stored overnight at 4 °C before being analyzed.

GEL STRENGTH

The texture analysis of the surimi gel was carried out using a Texture Analyzer. The modori gel was conditioned at room temperature for 2 h, then cut into 20 mm lengths before being analyzed. Gel strength was measured with a spherical probe, which had a diameter of 0.25 inches, a distance of 11 mm, a force of 5 g, and a 25 kg load cell. The gel strength was calculated according to the following formulation (Park 2005):

$$\text{Gel strength} = \text{force (g)} \times \text{distance (mm)}$$

DETERMINATION OF EXPRESSIBLE DRIP

Expressible drip measurement followed the method of Park (2005). The surimi gel was cut with a thickness of 5 mm, weighed (X), then placed between 2 pieces of Whatman paper number 1. A standard weight of 5 kg was placed on the top of the sample for 2 min, and then the modori gel was weighed again (Y). Expressible drip was calculated according to the following formulation:

$$\text{Expressible drip (\%)} = 100 \times ((X-Y)/X))$$

DETERMINATION OF PROTEIN SOLUBILITY

Protein solubility was determined following the method of Robinson and Hudgens (1940). One gram of surimi gel sample was dissolved in 10 mL 20 mM Tris-HCl (pH 8.0) containing 1% SDS, 2% -mercaptoethanol, and 8 M urea. The sample was homogenized for 1 min, then boiled for 2 min, and then stirred with a magnetic stirrer for 4 h at room temperature. The sample was then centrifuged at 1811 × g for 45 min, and 10 mL of supernatant was added with 10 mL of cold 20% TCA and stored at 4 °C for 18 h. The following process centrifuged the sample at 1811 × g for 30 min, and the resulting precipitate was washed with 10% TCA and immediately dissolved in 0.5 M NaOH. Protein concentration was measured using the Bradford method (1976). Protein solubility was expressed as a percent of total protein.

WHITENESS ANALYSIS

Modori gel with a diameter of 30 mm and a length of 25 mm was measured for whiteness using a Chromameter CR-400 (Minolta, Japan). The values of L*, a*, and b* were measured. Whiteness values were calculated according to the formula below (Park 2005):

$$\text{Whiteness} = L^*-3b^*$$

SCANNING ELECTRON MICROSCOPY (SEM)

The microstructure of the modori gel can be observed using SEM. Sample preparation followed the method of Nopianti et al. (2012). Modori gel was cut into 0.5 × 0.5 cm. The samples were frozen using liquid nitrogen and stored at -20 °C before being freeze-dried for 26 h. The dried samples were then coated with gold and then analyzed for the microstructure of the modori gel using a Scanning Electron Microscope JSM-IT200 (Jeol Ltd.) with a magnification of 1000x.

PROTEIN PROFILE

The effect of crude and ethanol-extracted plasma in inhibiting hydrolysis of surimi protein during the heat at 55 °C could be reflected in its protein profile. Sample preparation followed the method of Benjakul and Visessanguan (2000) and Rawdkuen et al. (2007). As much as 3 g of surimi was added with swamp eel crude plasma and ethanol-extracted plasma (0, 0.25, 0.5, 0.75, 1, 1.5, 2) (w/w), then homogenized. The mixture was incubated in a waterbath at 55 °C for 60 min. For SDS-PAGE analysis, 27 mL of 5 % SDS solution was added, then heated for 1 h at 85 °C in waterbath. The sample solution was then centrifuged at 1811 × g for 20 min. The supernatant obtained was then examined for the pattern of myofibril. SDS-PAGE was conducted using Laemmli's method (1970). A 12% separating gel and 4% stacking gel were used. Beta mercaptoethanol at 5% was added

to the buffer, and a ratio of 1:1 of this buffer and protein sample solution was mixed, then heated at 90 °C for 5 min to denature the proteins.

The sample was loaded with 20 µL for each well and contained 20 µg protein. After the running process, gels were immersed in deionized water for 5 min and then stained with a coomassie blue stain for 30 min. The gel was soaked with a destaining solution until protein bands appeared clearly. The molecular weight of protein samples was compared to a protein standard.

PROTEASE INHIBITION

Protease inhibition of surimi from degradation was carried out by adding crude plasma and ethanol-extracted plasma to 3 grams of surimi with different concentrations (0, 0.25, 0.5, 0.75, 1, 1.5, 2) (w/w), then homogenized by placing in a container filled with ice to maintain a temperature of 0-4 °C. The samples were then incubated at 55 °C for 2 h. The reaction was stopped by adding 27 mL of cold 5% TCA. The mixture was then incubated at 4 °C for 15 min and centrifuged at 1811 × g for 25 min. The peptides dissolved in TCA were measured according to Lowry et al. (1951). Tyrosine was used as standard and expressed as mmol of tyrosine released. The calculation of percent inhibition follows the formula below:

$$\% \text{ Inhibition} = \frac{(TC-TC_b) - (TS-TS_b)\times 100}{TC-TC_b}$$

ZYMOGRAM ANALYSIS

The zymogram analysis was aimed to prove the presence of proteases in the crude and ethanol-extracted of swamp eel. The substrate used was casein 0.75% in 1 M NaOH. The sample was calculated to obtain a protein concentration of 40-50 µg, dilution of the sample using Phosphate Buffered Saline (PBS) pH 7.2. The amount of protein as much as 40-50 µg was the amount of protein in the total volume of the sample to be injected, which was 20 µL after being mixed with the buffer sample. The sample was mixed with buffer sample with a ratio of 1:1, left the mixture at room temperature for 5-10 min before being injected.

Gel electrophoresis consisting of 12% separating gel and 4% stacking gel was installed into the electrophoresis apparatus. The electrophoresis chamber was filled with running buffer, then injected 20 µL of sample and standard protein into each gel well. Run the sample at 50 V for 2 h 30 min or until the sample reaches the bottom of the gel. The next step was the renaturation process. The gel was transferred to a container which was then soaked with 100 mL Triton X-100 2.5%. The gel was incubated for 1 h at room temperature while being shaken using a shaker.

The next process was gel development. Triton X-100 2.5% was discarded, then the gel was rinsed using distilled water. The gel was then soaked with 50-100 mL of phosphate buffer pH 8 or until submerged, incubated for 15 min at room temperature while shaking. The phosphate buffer was removed, the gel was soaked again with phosphate buffer pH 8 and then incubated overnight (18 h) at 37 °C.

The final stage was the process of staining and destaining gel. The phosphate buffer pH 8 was removed, then, the gel was stained using coomassie brilliant blue (CBB) until it was submerged. The gel was incubated for 1 h at room temperature by shaking. Then the CBB was removed, the gel was soaked with destaining solution until the clear band was clearly visible. The visible clear band was then compared with a protein standard to determine its molecular weight.

Results and Discussion

GEL STRENGTH OF TILAPIA (*Oreochromis mossambicus*) MODORI GEL

Modori gel without adding crude and ethanol-extracted plasma showed the lowest gel strength. Li, Lin and Kim (2008) also reported that Alaska Pollock modori gel produced lower gel strength without the addition of trout plasma.

In Figure 1, the presence of protease inhibitors (crude and ethanol-extracted plasma) increased the gel strength value of the surimi gel. Mishra (2022) showed that the excellent quality surimi has a gel strength above 1000 g.cm. Surimi gel mixed with ethanol-extracted plasma resulted in better gel strength than the crude plasma. It indicates that the ethanol-extracted plasma could preserve tilapia surimi from gel softening compared to crude plasma. In addition, as the concentration of the inhibitor in the plasma used increased, the gel strength produced was also increased. Fowler and Park (2015b) reported that the gel strength of pacific whiting modori gel increased with increasing plasma concentrations of salmon at 0.5 g/100 g - 1 g/100 g. Li, Lin and Kim (2008) stated that the leading role in the formation of surimi gel was the presence of alpha-2-macroglobulin in the plasma so that surimi was avoided from hydrolysis due to the activity of internal cathepsin L, which belongs to the sulfhydryl protease.

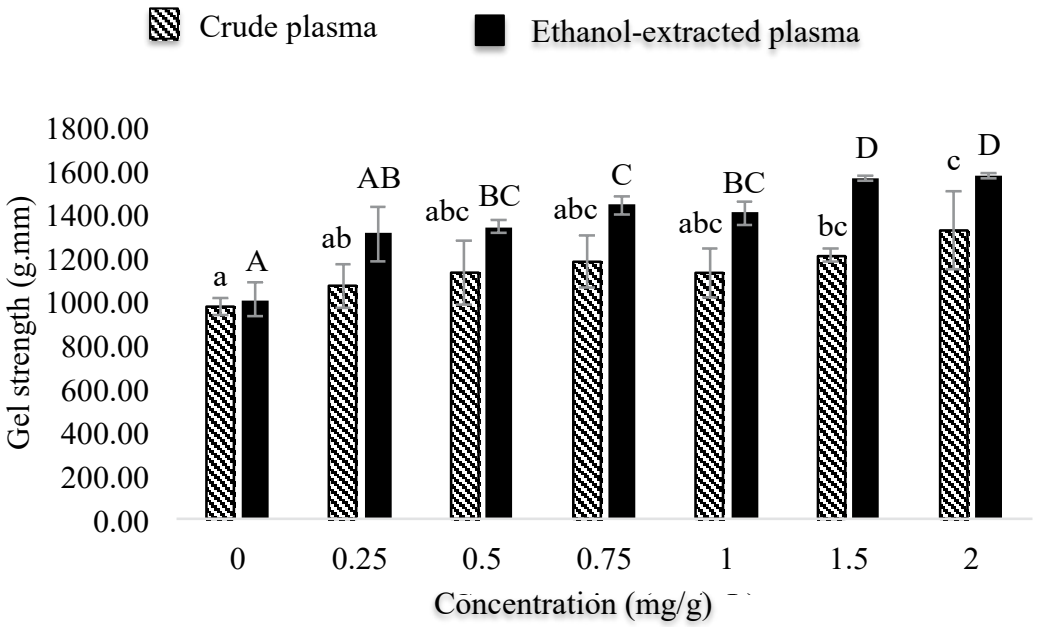

FIGURE 1. The average gel strength of tilapia modori gel on different concentrations of plasma (different letters indicate significant differences in Duncan's test ($P<0.5$)). Bars represent standard deviation of 3 replications

EXPRESSIBLE MOISTURE OF TILAPIA (*Oreochromis mossambicus*) MODORI GEL

Expressible moisture indicates the amount of liquid released from the surimi gel due to the applied pressure or load. Pressure is one method for analyzing expressible moisture, and the other is the centrifugation technique (Shand 2012).

Modori gel without the addition of swamp eel crude plasma and ethanol-extracted plasma showed a higher expressible moisture. Li, Lin and Kim (2008b) reported that Alaska Pollock modori gel produced lower expressible moisture without the addition of trout plasma. Kaewudom et al. (2013) showed that the structurally damaged protein network causes a loss of water holding capacity so that the expressible moisture increases. Damage to the protein structure in the surimi gel was caused by the internal protease enzyme activity, which was termed autolysis (Payne 2019). Table 1 showed that protease inhibitors in the crude plasma and ethanol-extracted plasma reduced the expressible moisture of modori gel. Modori gel mixed with ethanol-extracted plasma produced better expressible moisture than that of the crude plasma. In addition, based on the concentration of the inhibitor used, the expressible moisture produced generally decreased as the concentration of the inhibitor used increased. It indicates that the increasing concentration of inhibitors used could protect the protein structure of tilapia surimi from degradation caused by internal protease enzymes, and the most effective was using ethanol-extracted plasma.

WHITENESS OF TILAPIA (*Oreochromis mossambicus*) MODORI GEL

Changes in the whiteness of modori gel made from tilapia surimi with the addition of crude plasma and ethanol-extracted plasma at different concentrations were shown in Table 2. The result showed that modori gel without adding crude plasma and ethanol-extracted plasma has a higher whiteness than other concentrations. The whiteness of modori gel decreased as the concentrations of crude and ethanol-extracted plasma increased. The lowest whiteness was produced by gel modori with the addition of crude plasma at a concentration of 2.0 mg/g. The decrease in the whiteness of the modori gel was due to hemolysis that occurred in the red blood cells of the plasma. Hemolysis is a common problem for plasma sample preparation. The change in plasma colour from transparent orange to a more intense colour was due to the release of hemoglobin (Fowler & Park 2015a; Li, Lin & Kim 2008).

TABLE 1. The average expressible moisture of tilapia modori gel on different concentrations of inhibitors

Concentration (mg/g)	Expressible moisture (%)	
	Crude plasma	Ethanol-extracted plasma
0	12.91 ± 1.53[a]	9.7 ± 0.76[a]
0.25	7.91 ± 0.83[b]	7.19 ± 0.35[b]
0.5	6.79 ± 0.31[bcd]	6.89 ± 0.41[b]
0.75	6.06 ± 0.21[cd]	6.86 ± 0.76[b]
1	7.20 ± 0.34[bcd]	6.95 ± 0.26[b]
1.5	7.49 ± 0.86[bc]	6.58 ± 0.03[b]
2	5.99 ± 0.33[d]	5.11 ± 0.21[c]

*Values with different superscripts was different significantly (P<0.05)

TABLE 2. The average whiteness of tilapia modori gel on different concentrations of inhibitors

Concentration (mg/g)	Whiteness	
	Crude plasma	Ethanol-extracted plasma
0	68.21 ± 1.10[a]	63.26 ± 1.26[a]
0.25	67.43 ± 0.22[a]	63.15 ± 0.17[a]
0.5	66.63 ± 3.71[a]	63.14 ± 0.17[a]
0.75	66.69 ± 0.31[a]	63.51 ± 0.40[a]
1	66.12 ± 0.33[a]	61.66 ± 0.27[b]
1.5	61.01 ± 0.53[b]	61.56 ± 0.18[b]
2	56.77 ± 0.83[c]	60.76 ± 0.26[b]

*Values with different superscripts was different significantly (P<0.05)

MICROSTRUCTURE OF TILAPIA (*Oreochromis mossambicus*) SURIMI GEL

Changes in the microstructure of the modori gel after the addition of swamp eel crude plasma and ethanol-extracted plasma was evaluated using a scanning electron microscope. It aimed to prove protein degradation in modori gel without the addition of inhibitor (plasma) caused by protease enzymes, especially cathepsin L, and inhibition of protein degradation by crude plasma and ethanol-extracted plasma. In Figure 2, it was shown that the modori gel without adding of a protease inhibitor had relatively large voids/pores. The voids were reduced by adding of high concentration of plasma. Similar conditions in the modori gel made from Pacific whiting surimi mixed with salmon plasma also had many cavities. The structure was less compact than the modori gel mixed with salmon plasma (Fowler & Park 2015). Results from this study, the modori gel tilapia mixed with 1.5 mg/g

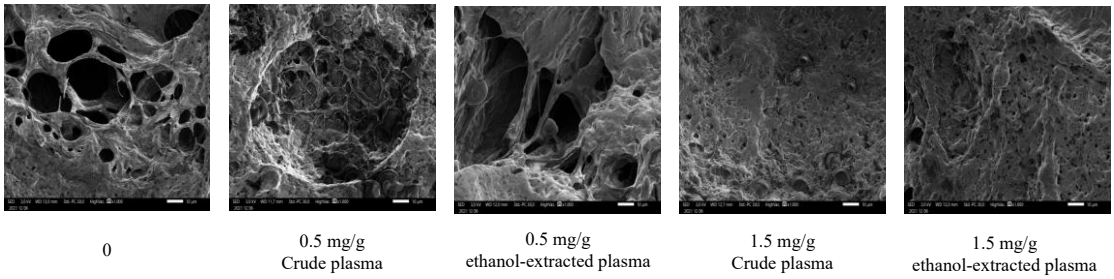

FIGURE 2. Modori gel microstructure with the addition of crude and ethanol-extracted plasma of swamp eel with different concentrations (0, 0.5, 1.5 mg/g)

crude plasma and ethanol-extracted plasma from the swamp eel produced more compact and homogeneous gel network. Hu et al. (2012) showed that all proteins were aggregated to form a gel matrix during the gel formation process, which was then bound by water by the gel matrix to form a compact and more homogeneous gel network. It indicates that swamp eel plasma could protect tilapia surimi from protein degradation during modori gel formation.

PROTEIN SOLUBILITY OF TILAPIA (*Oreochromis mossambicus*) MODORI GEL

Modori gel without addition of swamp eel crude and ethanol-extracted plasma showed the highest protein solubility (Figure 3). The modori gel made from Alaska pollock surimi also showed the same trend. The highest protein solubility was obtained in the Alaska pollock modori gel without the addition of trout plasma (Li, Lin & Kim 2008). The presence of inhibitors, namely crude plasma and plasma ethanol fraction, reduced protein solubility. The modori gel, previously added with plasma ethanol fraction, produced the lowest protein solubility. It indicated that the plasma ethanol fraction could maintain the protein structure of surimi from degradation due to the activity of internal Cathepsin L.

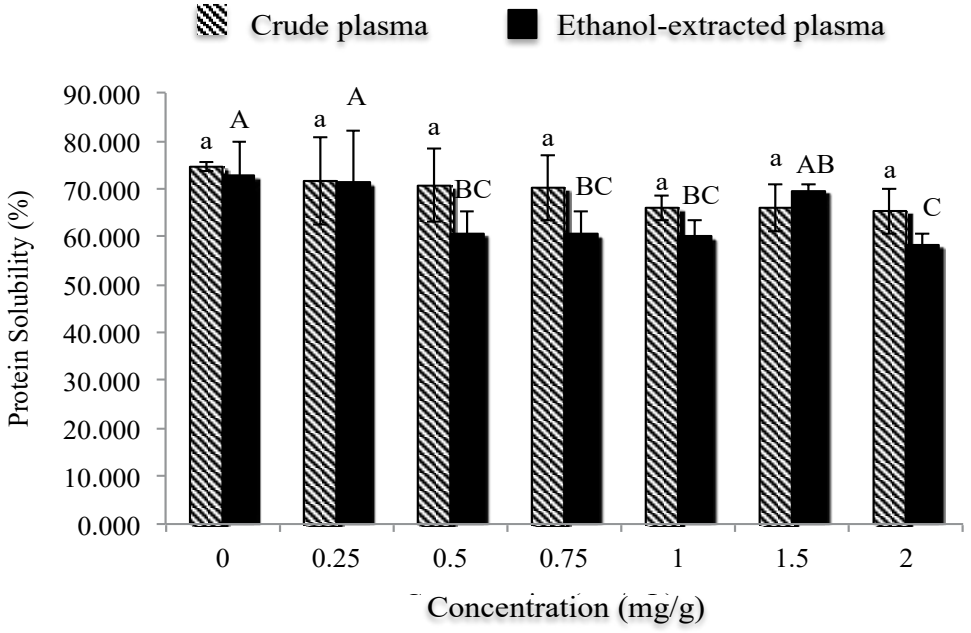

FIGURE 3. The average protein solubility of tilapia modori gel on different concentrations of plasma (different letters indicate significant differences in Duncan's test ($P<0.5$)). Bars represent standard deviation of 3 replications

HYDROLYSIS PATTERN OF TILAPIA (*Oreochromis mossambicus*) SURIMI WITH THE ADDITION OF INHIBITOR PROTEASE

The protein profile could reflect the effect of the addition of inhibitors (plasma) in inhibiting surimi hydrolysis, especially myosin protein. Figure 4 showed the protein profile of surimi added with crude plasma (a) and ethanol-extracted plasma (b). Surimi without the addition of crude and ethanol-extracted plasma and only protected with ice without being followed by a heating process at a temperature of 55 °C generally had the highest myosin protein intensity compared to other treatments. The results of image J showed that there was a decrease in the intensity of myosin in the sample added with crude plasma and ethanol-extracted plasma. A drastic decrease in myosin intensity was found in the sample added with crude plasma at a concentration of 2.0 mg/g by 55.13% compared to the control. Meanwhile, in the sample added with ethanol-extracted plasma, the highest decrease in myosin intensity was only 18.62%, even an increase in myosin intensity with the addition of ethanol-extracted plasma at a concentration of 1.50 mg/g by 10.48%. Fowler and Park (2015a) reported that Pacific whiting surimi added with plasma increased the myosin intensity (thickening of myosin protein bands) with increasing plasma concentrations of salmon used. The increase in myosin intensity indicated that there was an increase in the inhibition of hydolysis caused by the protease activity present in surimi. Unlike myosin, the actin in tilapia surimi mixed with swamp eel crude plasma, and ethanol-extracted plasma was more stable and not affected by protease activity. The same results

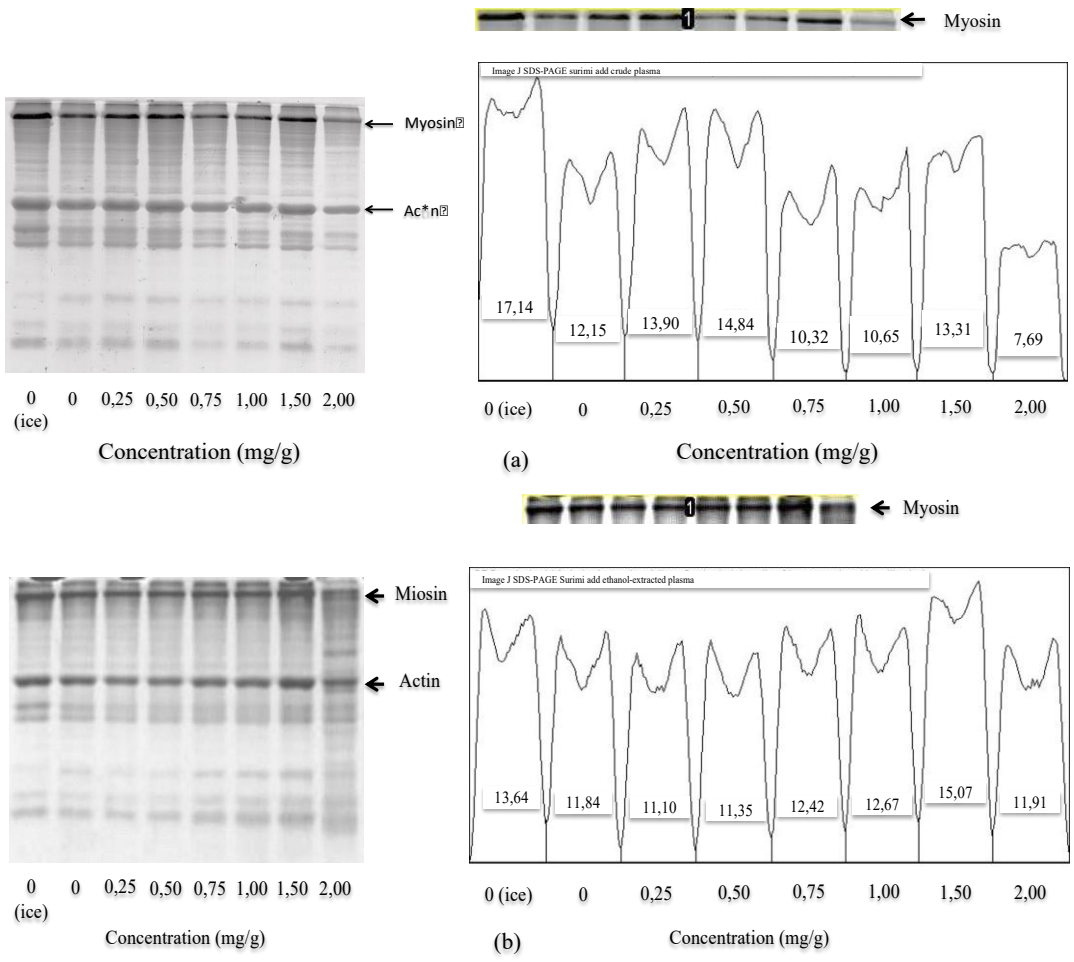

FIGURE 4. Hydrolysis pattern of surimi with the addition of crude plasma (a), ethanol-extracted plasma (b) swamp eel, heated at 55 °C with different concentrations (mg/g), and intensity/area of myosin using image J

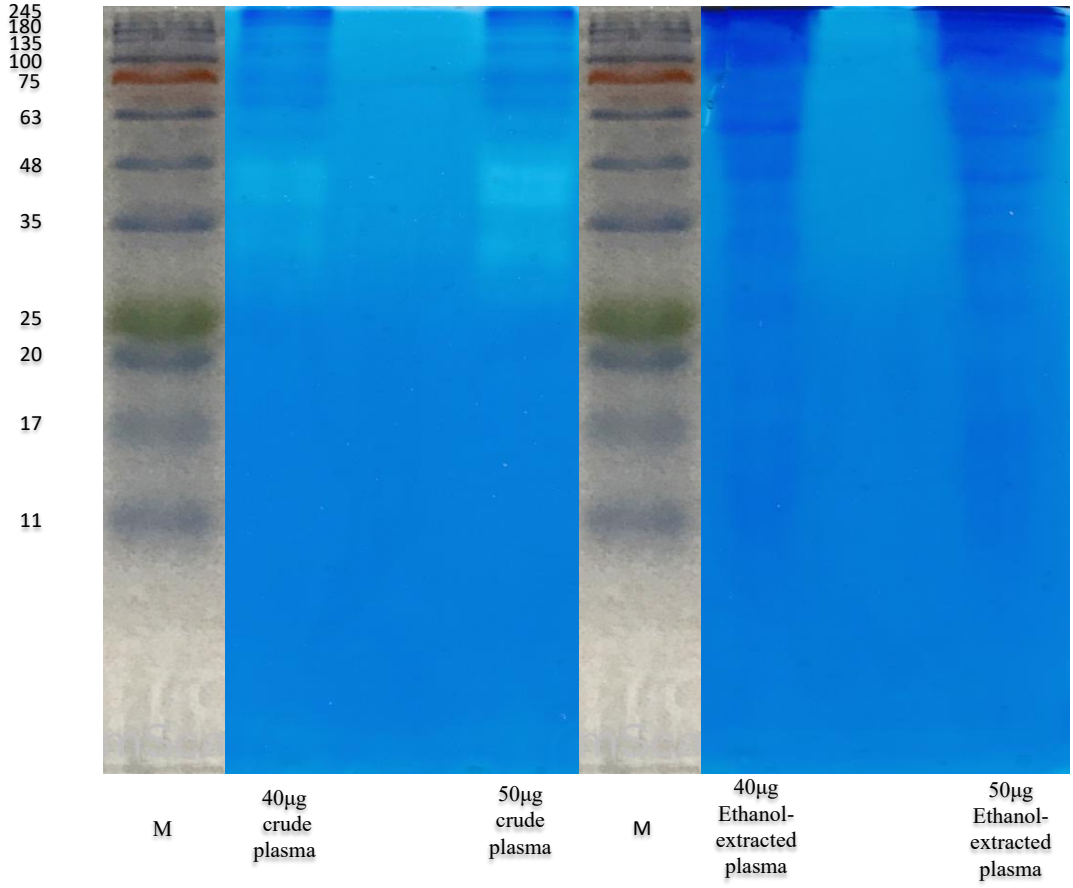

FIGURE 5. Zymogram of swamp eel crude and ethanol-extracted plasma with casein 0.75% as a substrate

were also found in Pacific whiting fish surimi mixed with to salmon plasma, that the actin was not affected by protease activity. Based on previous research, myosin protein was the most affected by the activity of Cathepsin L that attributed the decreasing of myosin. It was probably related to the presence of internal protease. During the washing process in the manufacture of surimi, Cathepsin L was not wasted because it was firmly bound to myofibril proteins. This was because myosin acts as a substrate for myofibril-bound proteases (Fowler & Park 2015a; Li, Lin & Kim 2008). This study analyzed the possibility of protease in tilapia surimi by zymogram analysis (Figure 5).

PERCENT INHIBITION TO INHIBIT PROTEASE ACTIVITY IN TILAPIA (*Oreochromis mossambicus*) SURIMI

The use of protease inhibitors is one way to inhibit or minimize the occurrence of hydrolysis. The inhibition of tilapia surimi from protease activity is shown in Table 3. Crude and ethanol-extracted plasma added to the tilapia surimi could protect surimi from protease activity. Based on the type of plasma, the ethanol-extracted plasma resulted in a better inhibition on hydrolysis of tilapia surimi compared to the crude plasma. There was a positive correlation between the increased inhibitor concentration and the inhibition value of hydrolysis surimi. The highest inhibition was 58.97% in surimi treated with ethanol-extracted plasma. The data was lower when compared to the report by others, where the percent inhibition of hydrolysis on surimi reached 90%, with a plasma concentration of 0.25% to 2% used (Fowler & Park 2015a), which was higher than applied in this experiment.

TABLE 3. The average percent inhibition to inhibit hydrolysis of surimi at different concentrations of inhibitors

Concentration (mg/g)	Percent inhibition	
	Crude plasma	Ethanol-extracted plasma
0.25	16.15 ± 0.00^{a}	17.16 ± 4.90^{a}
0.5	21.49 ± 2.64^{ab}	27.01 ± 3.61^{b}
1	21.74 ± 3.54^{ab}	37.41 ± 2.59^{cd}
1.5	26.19 ± 2.86^{b}	40.28 ± 2.10^{d}
2	48.04 ± 4.80^{c}	58.97 ± 3.49^{e}

*Values with different superscripts was different significantly ($P<0.05$)

ZYMOGRAM ANALYSIS OF CRUDE PLASMA AND ETHANOL-EXTRACTED PLASMA

In this study, the zymogram was conducted to analyze the presence of protease enzymes in swamp eel plasma, which might be responsible for reducing the myosin intensity. Jiang et al. (2019) proved the presence of Cathepsin L activity in yellowtail fish's blood components, including white blood cells, red blood cells, and plasma. The zymogram profile of swamp eel crude and ethanol-extracted plasma was shown in Figure 5. From the image, it could be seen that two clear bands indicated the presence of proteases in crude plasma. It was correlated with the decrease in myosin intensity in the protein profile of tilapia fish surimi upon increasing the concentration of crude plasma. In addition, there was also a decrease in the value of gel strength at the highest concentration of crude plasma (Figure 1). The resulting clear band was visible when the protein concentration of the sample used increased.

Meanwhile, in the ethanol-extracted plasma samples, no clear band was observed in the zymogram analysis, which indicated the ethanol-extracted plasma could remove the internal protease. Further purification might be necessary to ensure a better result in applying the plasma to produce good quality surimi.

CONCLUSION

The ethanol-extracted swamp eel plasma could maintain gel strength, expressible moisture, microstructure, protein solubility, and percent inhibition to tilapia (*Oreochromis mossambicus*) surimi from protease activity better than crude plasma. There was a decrease in whiteness when the concentration of crude plasma was increased in surimi. The protein profile of hydrolyzed surimi demonstrated a drastic decrease in myosin intensity in the protein profile of surimi mixed with crude plasma. The inhibition activity of the protease enzyme increased when the concentration of ethanol-extracted plasma in surimi was 1.5 mg/g, which was reflected in increasing the myosin intensity by 10.49% compared to the control. Zymogram analysis proved the presence of protease enzymes in crude plasma by resulting in two clear bands. Further purification might be necessary to ensure a better result in applying the plasma to produce good quality surimi.

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