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Original Research Article

Identification of Protein Types and Molecular Weight of Mangrove Snail (*Telescopium Telesco*pium) Isolate at Different pH

Rahman Karnila[1]*, Andarini Diharmi[1], Harifa Syah Putra[1], Dian Iriani[1], Bustari Hasan[1], Muthia Elvia Avissa[1], Melania Syahada[1]

[1]*Faculty of Fisheries and Marine Science, Riau University, Kampus Bina Widya Soebrantas, Pekanbaru 28293, Indonesia*

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ABSTRACT

Mangrove snail (*Telescopium Telescopium*) is a gastropods species with low economic value serving as an alternative source of animal protein in the form of isolate. Therefore, this research aimed to evaluate the effect of different pH values in the yield, proximate composition, soluble protein content, and molecular weight of mangrove snail isolate. An experimental method was used with completely randomized design (CRD) consisting of three pH treatment levels (4.5; 5.5; 6.5) and three replications. Furthermore, data were subjected to descriptive statistics and analysis of variance (ANOVA). The stages included sample preparation, grinding of mangrove snail meat, and production of protein isolates at different pH levels. The results showed that mangrove snail flour, moisture content, ash content, and protein content had a yield of 25.52%, 5.27%, 4.58%, and 42,88%, respectively. The highest protein isolate yield (5,06%) and soluble protein contenct (1.90 mg/mL) were obtained at pH 4.5. molecular weight profiles varied by 4-191, 4-44, and 4-54 kDa at pH 6.5, 5.5, and 4.5, respectively. Protein identification reported the presence of actin, α-tropomyosin, myosin light chain, lysozyme, and peptides, suggesting potential applications in health food products such as protein supplements.

Keywords: Protein isolate, *Telescopium telescopium*, Soluble protein, Moleculer weight

Introduction

Mangrove snails (*Telescopium telescopium*) is a group of gastropods widely distributed in mangrove forest areas, typically found on muddy substrates. These gastropods are in abundance in ponds adjacent to mangrove forests. In addition, mangrove snails are also found in rivers near the poud areas.[1 2] snail is often attached to the roots and trunks of mangrove trees, which have a conical shell shape, long, slender, flat, and tightly spiraled. The utilization of mangrove conch is common among fishing communities, consumed as a protein source alternative to chicken and beef. Nutritional content is relatively high, containing 12.16% protein and 0.38% fat. Mangrove snail also possesseas bioactive compounds (alkaloids, steroids, flavonoids) that have potential anticancer, antitumor, antioxidant and liver cell repair activities in rats. Snail slime functions as an adhesive, emollient, moisturizer, and lubricant suitable for application in medical and cosmetic products. [4] furthermore, snail contains a veriety of substances, including protein, peptides, hyaluronic acid, and allantoin, such as 1.56% histidine and 1.20% glutamic acid. These results show the potential of mangrove snail in different biological actions, including anti-tyrosinase, antioxidant, and antitumor effect. [3,5-6]

*Corresponding author. Email: rahman.karnila@lecturer.unri.ac.id
Tel: 081318406022

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Protein isolates (Bradford method) found *in A. fulica, C. bistrialis, P. globose, P. virens, B. dissimilis, B. pulchella,* and *M. tuberculata* produced protein molecular weights of 20, 30, 45, 66, 97 kDa for ecaluating biologival activities such anti-inflammatory, antiviral, anticancer, and antioxidant protperties. [6] For example, at pH 5.5-7.5, 3.5-5.1, and greater than 7.5 or below 3.0, β-Lg forms an octamer, monomers, and a proportion of β-turn, respectively. [7-8] at pH 4 and 7, structural changes occur in almond protein to increase viscosity, and particulate gel-related networks are formed, respectively. In addition, *Phaeodactylum tricornutum* isolates obtained at different pH levels (3,5,7,9, and 11) showed varying molecular weight, namely below 5 kDa (51.49%), between 5 and 100 kDa (2%), and above 100 kDa (46.51%). [9] this is because of the electrostatic forces between protein molecules at pH 5, which are the primary cause of aggregation of form protein aggregates. [10] furthemore, increasing Ph (2,5,8,11) in the preparation of soy protein isolates showed higher fat absoption, gelation capacity, gel hardness, cohesiveness and chewiness. Pea isolates reported the highest and lowest hydrophobicity at pH 4 and 9 while maintaining very compact β-sheet and α-helix secondary structures with proportions exceeding 75%.[11] in this context, pH significantly affects the solubility, hydrophobicity, emulsification, foam formation, volatile compounds, and gelation properties of proteins, [12] remaining a major challenge for mangrove snail protein isolates as fuctional foods. Limited data have evaluated the functional properties of mangrove conch proteins. Therefore, this research aimed to examine the impact of pH combinations on yield, soluble protein contenct, and molecular weight (Sodium Dodecyl Sulphate (SDS)-polyacrylamide Gel (PAGE) to assess protein purity and damage, as well as to determine isolectric point.[13]

A detailed molecular weight profile and protein identification of mangrove snail protein isolates was provided across different pH levels while optimizing conditions for maximum protein isolation yield. Additionally, the research shows pH-dependent degradation and identifies functional proteins and bioactive peptides with potensial applications in health products. Mangrove snail is positioned as a valuable alternative protein source and offer a replicable methodology for isolation from other marine gastropods, supporting the sustainable utilization of mangrove resources.

Materials and Methods

Preparation of phytochemicals and target protein
Materials and methods
The main material in this research was a medium-sized mangrove snail obrained from the forest of Bengkalis Regency, Sungai Pakning village, Bukit Batu District, Riau (Figure 1). Furthermore, the chemical materials are HCL 6N, distilled water, Bovine Serum Albumin (BSA), Bradford reagent, bis Acrylamide, ammonium persulphate (APS), Tetra Methyl Diamine, this HCL (Merck), phosphate-buffered saline (PBS), buffer, detergent SDS, methanol, acetic acid, formaldehyde. The tools used are knives, cutting boards, basins, scales, grinders, sieves (60 mesh), ovens (Memmert-Germany), dropper pipettes, pH meters)HM-205), centrifuge (Eppendorf 5424 R), SDS-PAGE, Incubator (binder), measuring cylinder (Pyrex), beaker (Pyrex), Erlenmeyer flask (Pyrex), hot plate (Thermo Scientific), porcelain cup (Oem), filter paper (Hellma), desiccator (Duran), and UV-Vis spectrophotometer (T70).,

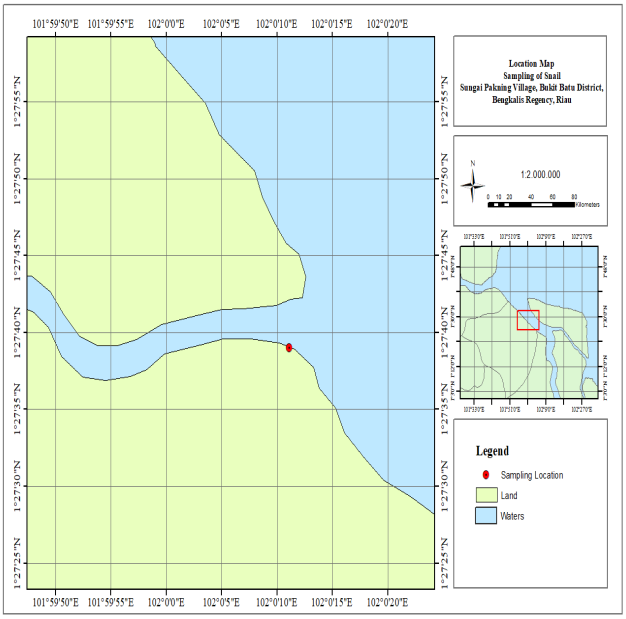

Figure 1: Geographical location of mangrove snail

Experimental design
Mangrove snail collected from the forest of Sungai Pakning Village was cleaned using running water before separating the shells, meat, and innards. The meat was dried in the sun, processed by grinding into flour, and sifted to homogenize the particle size. Before making protein isolates, the flour was analyzed according to proximate analysis methods,[14] including protein, moisture, and ash contents.
Mangrove snail flour (50 g) was weighed and suspended in purified water in a proportion of 1:15 (w/v). subsequently, a 35% (w/v) sodium hydroxide (NaOH) solution was gradually added dropwise using a dropper to adjust pH of the mixture to 11 while stirring continuously. The mixture was heated at 40°C for 30 minutes and stirrer using a magnetic stirrer before centrifuging to obtain the supernatant. The superntatant was subjected to acidic pH treatments (4.5, 5.5, and 6.5) and heated at 40°C for 30 minutes of continuous stirring using a magnetic stirrer. Additionally, centrifugation was conducted at 1000 rpm for 15 minutes at 40°C to obtain protein isolates for each treatment.[15]

Biochemical test
Molecular weight analysis was performed using the SDS-PAGE method, as described in the study by. [16] First, the gels were prepared using a 12% and 3% separating and stacking gel. A 12% acrylamide solution was poured into a glass plate before adding butanol to prevent drying and allowed to polymerize. The butanol was discarded using Whatman filter paper and a 3% acrylamide solution was poured over the hardened separating gel. A comb was inserted and left for a few minutes until the gel hardened. The gel and plate were mounted on an aelctrophoresis apparatus, and a running buffer was poured. Samples weighing 20 mg were dissolved in 1X phosphate-buffered saline (PBS), while 20 µL was blended with a buffer in a 1:1 ratio and heated for 3-5 minutes. The sample was heated and put into the wells of the gel soaked in a running buffer. The electrophoresis apparatus was closed and run at 80 volts. Completion of the run was carried out when the blue dye in Laemmli buffer migrated to the bottom of the gel. The machine was then turned off, and the gel was stained with Coomassie Brilliant Blue while shaking on a shaker for 30 minutes. Furthermore, the stained gel was soaked in 150 mL of acetic acid, followed by a destaining solution while shaking for 30 minutes. The gel was wahed with acetic acid and shaken on a shaker. The electrophoresis results appeared as bands, from which Rf (retardation factor) of each band can be calculated.

Data Analysis
The experiment was performed in triplicate, with results presented as mean ± standard deviation, and experimental data were analyzed using analysis of variance (ANOVA). Significant differences among treatments ($P<0.001$) were determined using Duncan Multiple Range Test (DMRT) with the Statistical for Social Science (SPSS) software, version 26.

Results and Discussion

Identification body components of mangrove snail
Mangrove conch used in this research had a sehll length of 8.27 cm but varied in size due to geographical differences.[17] in addition, variations in the length of mangrove snail can be caused by food availability, competition, and pollution, which act as disturbances and pressures affecting growth. The body components of mangrove sanil consist of the shell, meat, and viscera. The percentage of the body composition is 70.56% shell, 12.78% meat, and 16.67% viscera. The high percentage of shell in mangrove conc is due to increased lime content in the shell, leading to hidh yield.[18] the meat of mangrove snail has two different colors, where the legs or abdominal muscles are whiter, while the head is blackish-green. The weight can be influenced by environmental conditions, food availability, and energy expenditure.
The yield was 25.52% and the variation between high and low values was attributed to the drying process of the flour. Factors such as low drying temperature, drying duration, grinding, and sieving affect the yield.[19-20]

Chemical composition of mangrove snail flour
The analysis of the chemical composition of mangrove snail is shown in Table 1. Mangrove conch flour contains 5.27% moisture, 4.58% ash, and 42.88% protein. In this research, moisture content of mangrove conch flour is higher than *Archachatina marginata* 4.75%.[21] The variation in ash content is attributed to differences in the ability to absrob and excrete minerals. Protein content of mangrove snail flour is higher than general and *Brotia costula* snail flours, ranging from 14% to 19%[22] and 13.83%, respectively.[23] however, the content is slightly lower than gold snail flour at 46.2%.[24]

Table 1: Chemical composition of mangrove snail flour (*Telescopium Telescopium)*

Parameters	Percentage (%)
Water (*)	5.27 ± 0.08
Ash (**)	4.58 ± 0.08
Protein (**)	42.88 ± 0.13

*wet basis
**dry basis

Yield of mangrove snail isolate protein
The results showed that the yield value was influenced by isoelectric pH, where protein precipitates and produces the largest amount of isolate. This is evidenced by the change in yield at pH 4.5, which decreased from 5.06% to 2.64% following an increase in pH to 6.5 (a

more alkaline condition). Previous studies [25] shown that hydrolysate yield can decrease at higher pH levels due to suboptimal enzyme activity in peptide chain hydrolysis In this context, the concentration of soluble protein in the solution is at the minimum, leading to precipitation or coagulation. In addition, differences in yield values may be attributed to variations in the raw materials used. [26,27] the yield is influenced by several factors obtained during protein isolation process, such as meat sources (species, freshness, as well as the use of fillets or whole raw materials, water ratio, fat content, separation methods, and pH values applied during protein solubilization and precipitation.

Table 2: Yield of mangrove snail isolate at different pH

Treatment	Yield (%)
pH 4.5	5.06 ± 0.06^{C}
pH 5.5	3.25 ± 0.03^{B}
pH 6.5	2.64 ± 0.12^{A}

Data in the same column marked with different manuscripts show significant differences ($p<0.001$).

Dissolved protein of mangrove snail isolate

Measurement of soluble protein at acidic pH is conducted to determine the quality of remaining protein soluble at the minimun pH as presented in Figure 2. In this research, protein solubility increased with higher pH treatments. The highest concentration was found at pH 6.5 (2.18 mg/mL), followed by pH 5.5 (2.01 mg/mL) and pH 4.5 (1.90 mg/mL) ($p<0.01$). The higher solubility at pH 6.5 [28] showed that protein isolates increase solubility by 65.13% compared to more acidic pH levels. This condi-tion is also due to the greater number of negatively charged ions at pH levels distant from isoelectric point.[29,30] however, the increased level of pH will also affect to decrease in viscosity.[31] in this contet, the solubility of protein can be used to enhance food protein effectiveness, diet, and digestibility.[31,32]

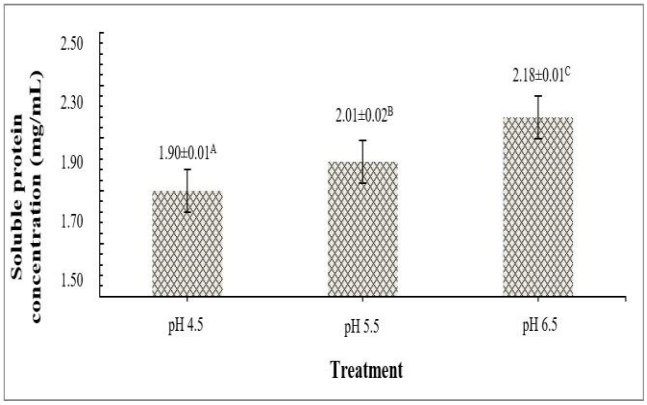

Figure 2: Soluble protein concentration value of protein isolate pH

Molecular weight and type of mangrove snail protein

Molecular weight testing on mangrove snail protein isolates was conducted using SDS - PAGE method with a 7.5%-17.5% gradient separating and 4% stacking gel, stained with Coomassie Brilliant Blue. Electrophoresis was performed at 80 volts for 125 minutes, using protein markers with molecular weight between 2 and 250 kDa. The obtained molecular weight data were used to assess to impact of pH on the profiles of three protein isolate samples subjected to different pH treatments. Molecular weight patterns of protein isolates under various acidic pH conditions are presented in Figure 3.The results obtained from the gel were analyzed by calculating Rf using the marker. Rf value was determined by calculating the ratio of the distance moved by protein band to the tracking dye. Rf values and the logarithm of molecular weight (MW) were plotted in a regression formula, leading to the linear equation y=-1.754x+5.3198. This linear equation is used to obtain molecular weight, where y is the logarithm and x represents Rf value of the sample, allowing the calculation of the weight. The calculations obtained on each molecular weight isolate with different pH treatments have different results. In Figure 2, pH 6.5 detected 15 bands with protein molecular weights ranging from 4, 6, 8, 14, 19, 25, 41, 54, 83, 96, 137, 147, 169, 181, and 195 kDa, pH 5.5 has 4 bands with protein molecular weights of 4, 6, 17, and 44 kDa, and at pH 4.5 there are 5 bands with weights of 4, 6, 13, 31, and 54 kDa.

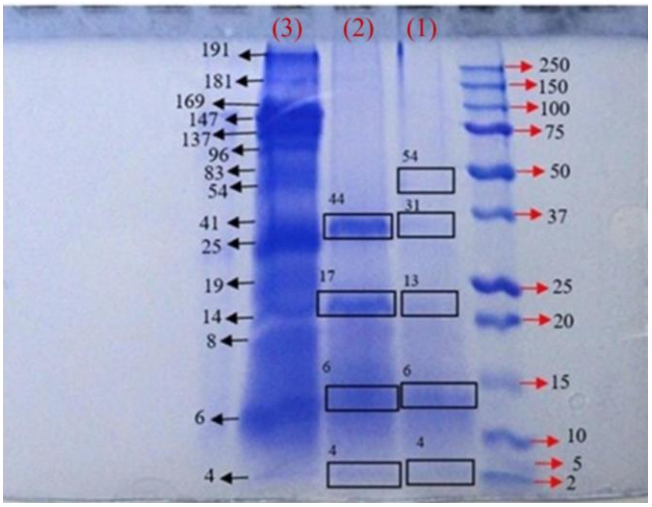

Figure 3: Weight profile of mangrove snail protein isolate with pH 4.5 (1); pH 5.5 (2); pH 6.5 (3); and marker (M)

Molecular weight results of mangrove snail protein isolate at different pH levels show that each treatment affect the number of protein bands. The results show that the number of protein bands decreases at pH 5.5. protein bands, which initially numbered 15 at pH 6.5, decreased by 11 bands at pH 5.5. Meanwhile, at Ph 4.5, a band with molecular weight of 54 kDa was detected, which was not observed at pH 5.5. in previous research [33], proteins vulnerable to deterioration at isoelectric point led to increased degradation at low pH. Protein degradation can be observed by the reduction or disappearance of some protein bands during pH treatment. This obervation is also under the results of [34] where acidic pH will make protein bands with large molecular weight disappear. According to previous research[35], protein isolate subjected to acid treatment showed degradation and increased protease sensitivity since pH shift process enhances digestibility. In addition, protein identification corresponding to molecular weights observed in the three pH treatments waas carried out as presented in Table 3.

Table 3: Protein identification of different pH mangrove snail protein isolates

Research Result				
Protein Molecular Weight (kDa)			Identical Protein Types	References
pH 6.5	pH 5.5	pH 4.5		
181,191	-	-	Myosin heavy chain	30,33
137,147,169	-	-	Heavy meromyosin	30,32
83,96	-	-	Paamyosin	32
54	-	54	Desmin	31,32
41	44	-	Actin	30
-	-	31	α Tropomyosin	28,32
19,25	17	-	Myosin light chain	30,33
14	-	13	Lysozyme	34,35
4,6,8	4.6	4.6	Peptide	36,37

(-) not detected

Based on Table 3, most proteins detected in muscle fibers are myofibrillar.[36] myofibrillar proteins are categorized as salt-soluble and constitute the main compnent, accounting for 70% of the total protein in fish muscle. In this research, molecular weights at pH 4.5 and pH 5.5 were below 100 kDa.37 peptides with molecular weights below 100 kDa, consisting of 3-10 amino acids, have positive on health and digestion.

Conclusion

In conclusion, mangrove snail protein isolate at acidic pH had a significant effect on yield and soluble protein, where pH 4.5 being the optimal treatment, producing a yield of 5.06% and soluble protein concentration of 1.90 mg/mL. Molecular weight at pH 4.5 (4 - 54 kDa), pH 5.5 (4 - 44 kDa), and pH 6.5 (4 - 191 kDa) showed evidence of protein degradation in isolates. The detected proteins included actin, α-tropomyosin, light chain myosin, lysozyme, and peptide with potential benefits for human health and digestion.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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