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Research Article

Effect of Extraction Time on Chemical, Physical and Organoleptic Characteristics of South Asian Applesnail (*Pila ampullacea*) Protein Concentrate

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Abstract:
This research aims to investigate the effect of protein extraction time using 90% ethanol on the protein concentrate product's physical, chemical, and organoleptic characteristics. The findings of this study could promote the use of protein concentrate developed from SAA. The study was conducted from April to June 2024, utilizing SAA collected from rice fields in the PALI (Penukal Abab Lematang Ilir) Regency, South Sumatra Province, Indonesia. A completely randomized design was employed, using different extraction times of proteins from SAA with 90% ethanol. Four extraction time levels were tested: 0 hours (P0 or control), 24 hours (P1), 28 hours (P2), and 32 hours (P3), each with three repetitions. Data were analyzed using ANOVA, followed by a post-hoc test employing Duncan's multiple range test, utilizing SPSS version 20.0. Results have shown that the optimal protein extraction time was P2 (28 hours), yielding a protein concentration of 51.6% on a dry basis. Additionally, protein profile analysis conducted via SDS-PAGE revealed the presence of histone proteins and an antibacterial protein derived from snail mucus in SAA. Treatment P2 exhibited five distinct protein bands with molecular weights of 17 kDa, 20 kDa, 25 kDa, 35 kDa, and over 48 kDa respectively. On the other hand, treatments P1 and P3 displayed only three protein bands that are 20 kDa, 35 kDa, and over 48 kDa. Results from the organoleptic analysis indicated that protein isolates with suitable attributes in terms of color and aroma were obtained from treatment P1 (24-hour extraction). This study has potential applications in the food industry.

Keywords: Chemical Characteristics, Organoleptic Characteristics, Physical Characteristics, Protein Concentrate, South Asian applesnail (*Pila ampullacea*)

1. INTRODUCTION

South Asian applesnails (SAA) (*Pila ampullacea*) are primary pests of rice plants. Despite their appealing taste and high nutritional content, their consumption remains limited due to a chewy texture and fishy aroma.

SAA are mollusks commonly consumed in various regions of Asia, including the Philippines, Singapore, and the Indonesian islands of Kalimantan, Sumatera, and Java. They are typically found in rice fields, ditches, lakes, ponds, and wetlands, and can survive the dry season. SAA exhibit a diverse diet, primarily feeding on organic matter in their habitat, with a preference for vegetables and plant material (Delvita et al., 2015). They pose significant threats to rice crops in Southeast Asia and Indonesia (K. Saputra et al., 2018).

Penukal Abab Lematang Ilir (PALI) is an autonomous region located in South Sumatra Province of Indonesia. The local economy of the province is predominantly agricultural, with crops ranging from rubber and oil palm to rice. The extensive rice fields in PALI Regency serve as a habitat for the SAA population.

Research conducted by Oktasari (2014) revealed that SAA contain 15% protein, 2.4% fat and 24% ash. Additionally, SAA are rich in essential minerals, particularly calcium, thus underscoring their potential as nutritious food.

Protein extraction is the process of isolating proteins from biological samples usually with the aid of

solvents. Aquatic protein extraction can employ various polar solvents, including phosphate buffers (Nasyiruddin, Noman, et al., 2019; Nasyiruddin, Mahdi, et al., 2019; Nasyiruddin et al., 2021) and ethanol (Rieuwpassa & Cahyono, 2019; Rieuwpassa et al., 2021). Ethanol is a colorless, flammable liquid with a distinctive odor that can easily evaporate and mix with water. Its ability to penetrate cell walls facilitates cell dispersion and accelerates the extraction of bioactive compounds (Laili, 2021). Despite its application in extraction from other sources, there is limited data on use of ethanol as a solvent for extracting protein isolates from SSA. Against this background, therefore it was the aim of this study to determine the potential of extracting SAA protein by using a 90% ethanol solution. This study is expected to provide useful insights into the economic value of SAA, their utility in food, feed and pharmaceutical industries.

2. MATERIAL AND METHOD

The study was conducted from April to June 2024 across three locations. It involved several key processes: the preparation of SAA meat, the production of SAA meat flour, the extraction of SAA protein concentrate (SPC), the determination of proteins and organoleptic properties in particular colour and aroma of the SPC product. The study was conducted at the Fisheries Product Technology Workshop Laboratory within the Faculty of Fisheries and Marine Sciences at the University of PGRI Palembang.

Proximate analysis including assessments of protein, fat, water content and color measurements (*L**, *C**, *H**, *a**, *b** values) were conducted in Chemistry and Microbiology Laboratory at Sriwijaya University. Protein profiling was performed using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) in Laboratory of Purification and Molecular Biology, Faculty of Technobiology, University of Surabaya.

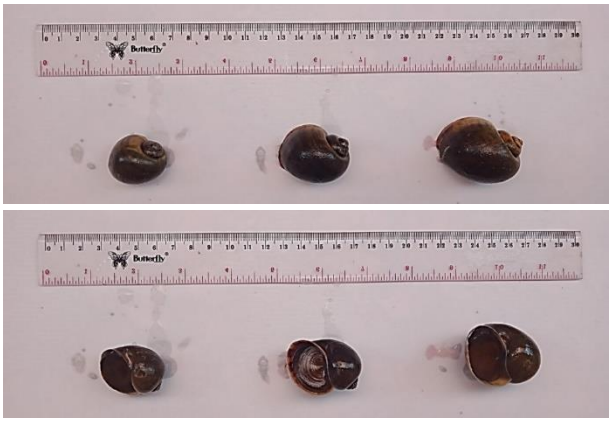

Figure 1. South Asian applesnail (*Pila ampullacea*)

The primary material used in this research was South Asian applesnail (*Pila ampullacea*), with individual weights ranging from 10 to 30 g. These specimens were collected from rice fields in PALI (Penukal Abab Lematang Ilir) Regency, South Sumatra Province, Indonesia.

Chemicals employed included ethanol (95%, technical grade, Onemed, Cikarang, Indonesia), selenium (99.0-100.5%, SeO2 in HN), and boric acid (99.5-100.5%), were all sourced from Merck (Darmstadt, Germany). Additional chemicals included SDS (Promega, Switzerland), tris base (Himedia, India), and acrylamide (Sigma Aldrich, India). High-purity and analytical-grade chemicals and reagents were utilized throughout this study.

Equipment used in the study included a blender (HR 2115, Philips, Netherlands), an oven for drying the protein concentrate (EO-35ST, Sharp, Indonesia), a colorimeter (NH310, Master Supply, Indonesia), Kjeldahl flasks (50 mL, Iwaki, Indonesia), a Soxhlet extractor (500 mL, Iwaki, Indonesia), a Mini Protean Tetra Cell (Bio-Rad Laboratories, Inc., Berkeley, California, USA), and an analytical balance (0.0001 g, Fujitsu).

2.1 Preparation of South Asian applesnail (SAA) Meat

Preparation of SAA meat was conducted following the methods of Laili (2021) and Rieuwpassa & Cahyono (2019), with modifications. The SAA were washed multiple times until the mucus on their outer surface was completely removed. The SAA were then immersed in boiling water set at 100℃ for 15 minutes. The SAA were separated into shell, meat and innards. The meat was washed again with tap water. Finally, the SAA meat was cut into small pieces (1-5 g).

Figure 2. South Asian applesnail/SAA (*Pila ampullacea*) Meat

2.2 Processing South Asian applesnail (SAA) Meat into SAA Meat Flour

Preparation of SAA meat flour was performed by using the methods of Laili (2021) and Rieuwpassa & Cahyono (2019), with modifications. The SAA meat, previously cut into pieces, was dried in an oven at 70°C for 6 hours. After drying, the SAA meat was ground using a blender (food blender, medium speed for around 5 min to produce SAA meat flour, which was designated as the control (P0).

2.3 Processing South Asian applesnail (SAA) Meat into SAA Protein Concentrate Flour (SPC)

Preparation of SAA meat concentrate flour (SPC) was done following the methods of Laili (2021) and Rieuwpassa & Cahyono (2019), with modifications. A total of 200 g of small pieces of SAA meat was placed into a glass jar and mixed with 300 mL of 90% ethanol, maintaining a ratio of 2:3 (weight of meat to volume of ethanol). The jar was sealed and allowed to stand for 24 hours (P1), 28 hours (P2), and 32 hours (P3), with stirring every 4 hours. During the extraction process, the solvent transitioned from clear to yellowish. The mixture was then filtered using calico cloth to separate the solid and liquid components. The resulting solid, identified as SAA concentrate (SPC), was dried in an oven at 70°C for 6 hours. The dried SPC was subsequently ground using a blender to produce SPC flour, which served as the research samples. The SPC flour was packaged in tightly sealed plastic containers and stored in a dry location before use.

Figure 3. South Asian applesnail (*Pila ampullacea*) Meat Flour (P0) and Protein Concentrate Flour (P1, P2, P3)

2.4 Proximate Analysis

Proximate analysis included moisture content, protein, fat, carbohydrate and ash content. The tests were conducted according to the SNI 01-2891-1992 method (BSN, 1992).

2.4.1 Protein Content Analysis

The sample was carefully weighed to 0.51 g and then placed into a 100 ml Kjeldahl flask. Subsequently, 2 g of a selenium mixture was added (the mixture consisted of 2.5 g of SeO_2 powder, 100 g of K_2SO_4, 30 g of $CuSO_4 \cdot 5H_2O$, and 25 ml of concentrated H_2SO_4). The mixture was then heated on an electric heater until boiling, and the solution turned clear and slightly greenish (approximately 2 hours). The solution was allowed to cool and then diluted by transferring it to a 100 ml volumetric flask, which was filled to the mark with distilled water. Next, 5 ml of the solution was pipetted and placed into a distillation apparatus, followed by the addition of 5 ml of 30% sodium hydroxide (NaOH) solution (prepared by dissolving 150 g of NaOH in 350 ml of water and storing it in a rubber-stoppered bottle) and a few drops of phenolphthalein (PP) indicator (prepared by mixing 10 ml of bromocresol green solution (0.1% in 95% alcohol) and 2 ml of methyl red solution (0.1% in 95% alcohol)). The solution was distilled for approximately 10 minutes, with 10 ml of a 2% boric acid (H_3BO_3) solution used as the receiver (prepared by dissolving 10 g of H_3BO_3 in 500 ml of distilled water, and after cooling, transferred to a glass-stoppered bottle). The boric acid solution was then mixed with 5 ml of the indicator solution. The condenser was rinsed with distilled water, and the tip was titrated with 0.01 N HCl solution. A blank determination was also performed.

Protein content was calculated according to the formula:

$$\textbf{Protein Content}\ (\%) = \frac{(V1 - V2) \times N \times 0.014 \times fk \times fp}{W} \times 100\%$$

Where :

W = The weight of the sample
V1 = Volume of 0.01 N HCl used for titration of the sample
V2 = Volume of HCl used for titration of the blank
N = Normality of HCl
fk = Protein conversion factor (6.25 for food ingredients)
fp = Dilution factor

2.4.2 Fat Content Analysis

The sample was carefully weighed to 1-2 g and placed into a paper sleeve lined with cotton. The paper sleeve containing the sample was then sealed with dry cotton and dried in an oven at a temperature of 80°C for one hour. Afterward, it was transferred into a Soxhlet apparatus that was connected to a fat flask containing a boiling stone, which had been dried and its weight previously determined. The sample was extracted with hexane for 6 hours. The extract was then distilled

with hexane and the fat extract was dried in a drying oven at 105°C. After cooling, the extract was weighed. The drying process was repeated until a constant weight was obtained.

Fat content was calculated according to the formula:

$$\text{Fat Content (\%)} = \frac{W - W1}{W2} \times 100\%$$

Where :

W = The weight of the sample, in grams
W1 = The weight of the fat flask before extraction, in grams

W2 = The weight of the fat flask after extraction, in grams

2.4.3 Moisture Content Analysis

The sample weighing 1-2 g was carefully measured into a pre-weighed, sealed weighing bottle. For liquid samples, the weighing bottle was equipped with a stirrer and either quartz sand or folded filter paper. The sample was dried in an oven at 105°C for 3 hours. It was then cooled in a desiccator. The sample was weighed, and the procedure was repeated until a constant weight was obtained.

Moisture content was calculated according to the formula:

$$\text{Water Content (\%)} = \frac{W}{W1} \times 100\%$$

Where :
W = The weight of the sample before drying, in grams
W1 = The weight loss after drying, in grams

2.4.4 Ash Content Analysis

A 2-3g sample was carefully weighed into a pre-weighed porcelain (or platinum) dish. For liquid samples, the sample was evaporated over a water bath until dry. It was then ashed over a flame and subsequently ignited in a muffle furnace at 550°C until complete combustion occurred (occasionally, the furnace door was slightly opened to allow oxygen to enter). The sample was cooled in a desiccator and weighed until a constant weight was obtained.

Ash content was calculated using the formula:

$$\text{Ash Content (\%)} = \frac{W1 - W2}{W} \times 100\%$$

Where :
W = The weight of the sample before ashing, in grams
W1 = The weight of the sample and dish after ashing, in grams
W2 = The weight of the empty dish, in grams

2.5 Protein Profile Analysis

Analysis of the protein profile was conducted using SDS-PAGE, following the method described by Laemmli (1970) and Fadhila & Darmawati (2017).

A 50 mg sample powder was mixed with 500 µL of PBS (Phosphate-Buffered Saline/PBS) prepared by dissolving 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4, and 0.24 g of KH_2PO_4 in 800 ml of distilled H_2O. The pH was adjusted to 7.4 using HCl. Distilled water was added to bring the total volume to 1 liter. The solution was dispensed into aliquots and sterilized by autoclaving for 20 minutes at 15 lb/sq.in. on the liquid cycle. The solution was stored at room temperature. The mixture was briefly vortexed then sonicated for 3.5 minutes. The solution was centrifuged at 10,000 rpm for 15 minutes at 4 ℃. A 15 µL aliquot of the supernatant was taken and mixed with 15 µL of 2x sample buffer (1 M Tris-HCl pH 6.8 0.6 ml, 50% glycerol 5 ml, 10% SDS 2 ml, 0.5 ml of 2-mercaptoethanol, 1% bromophenol blue 1 ml, and 0.9 ml of aquabides). The solution was vortexed, spun down, and then incubated in a water bath at 100 ℃ for 5 minutes.

The preparation of the separating and stacking gel was carried out as follows. The gel plates were arranged properly and checked to ensure that no wells were leaking. The separating gel (12.5%) was prepared by mixing 3.125 ml of acrylamide stock and 2.75 ml of 1 M Tris pH 8.8 in a 50 ml beaker. The beaker was then covered and gently shaken. A 1.505 ml of aquabidest, 75 µL of 10% SDS, 75 µL of 10% APS, and 6.25 µL of TEMED (tetra methyl ethylene diamine) were added sequentially, in a beaker, covered and the beaker was gently shaken after each addition. The solution was immediately poured into the gel casting plate, ensuring that no air bubbles formed. Aquabidest was slowly added on top of the gel solution in the plate to prevent the gel surface from becoming uneven. The gel was allowed to polymerize for 30 minutes (indicated by the formation of a transparent line between the water and the formed gel), after which the water overlaying the separating gel was discarded. After the gel had polymerized, the stacking gel was prepared following the same procedure as above, with the following composition: 0.45 ml of 30% acrylamide-bis, 0.38 ml of 1 M Tris pH 6.8, 2.11 ml of aquabidest, 30 µL of 10% SDS, 5 µL of TEMED, and 30 µL of 10% APS.

The running of the sample on the gel wells was carried out as follows. The plate containing the gel was placed into the electrophoresis chamber, and running buffer was poured until the top and bottom of the gel were submerged. A running buffer with pH 8.3, prepared by dissolving 1.52 g of Tris, 7.2 g of

glycine, 0.5 g of SDS, and adding aquabidest to a final volume of 500 ml, was used. Then, 10-20 µL of the sample (with protein content between 1 µg and a maximum of 20-40 µg) was carefully loaded into the bottom of the gel wells using a micropipette. Running was initiated by connecting the electrophoresis apparatus to the power supply. The electrophoresis was carried out at 20 mA for 40-50 minutes until the tracking dye reached a distance of 0.5 cm from the bottom of the gel. After completion, the gel was stained with a staining solution (1 gram of Coomassie Blue R-250, 450 ml of methanol, 450 ml of aquabidest, and 100 ml of glacial acetic acid) for 15 minutes with gentle shaking. The gel was then washed in a destaining solution consisting of 100 ml of methanol, 100 ml of glacial acetic acid, and 800 ml of aquabidest for 30 minutes with gentle shaking. The gel was pressed and dried for 48 hours in a dark room. To determine the molecular weight of the protein, the Rf (retention factor) value was calculated and plotted on a logarithmic graph against the Rf values of protein markers with known molecular weights.

2.6 Color Test

The color test was conducted using a standardized colorimeter following the method described by Hendra Permana Aditya, Herpandi, Susi Lestari (2016). The sample was then placed under the colorimeter lens, and the reflectance values for *L**, *C**, *H**, *a** and *b** were recorded from the measuring device.

2.7 Organoleptic Test (Aroma)

Organoleptic test was conducted to determine differences in sensory quality among several similar products based on the aroma of SPC samples, following the method described by Asriani et al. (2019). The 20 semi-trained panelists were engaged in testing a triplicate of the samples. Prior to the scoring test, panelists filled out a standardized form, assigning scores as follows: 5 for "Very strong aroma," 4 for "Strong aroma," 3 for "Weak aroma," 2 for "Very weak aroma," and 1 for "No aroma."

2.8 Research Design

The study employed a completely randomized design with a single treatment, namely the extraction time of South Asian applesnails (SAA) using a 90% ethanol solution. Four treatment levels were assessed, namely 0 hours (P0 or control), 24 hours (P1), 28 hours (P2), and 32 hours (P3), with each level replicated three times.

2.9. Data Analysis

The data obtained were expressed as means. A one-way analysis of variance (ANOVA) was conducted to determine statistical differences among the treatment groups. Significant differences between means were identified using Duncan's multiple range test at p value < 0.05. Statistical analyses were performed using IBM SPSS Statistics 20.0 (IBM SPSS Software, USA).

3. RESULT AND DISCUSSION

3.1 Proximate Composition of SAA Meat

Proximate composition included the percentage of five basic nutritional components, namely moisture, protein, fat, ash, and carbohydrate content. Proximate analysis revealed that the moisture, ash, fat, and protein contents were 65.92%, 3.15%, 0.31%, and 32.76%, respectively, with carbohydrate content (calculated by difference) at 1.26%. The results indicate that SAA meat is high in protein content (32.76%), low in fat (0.31%), and contains a modest amount of carbohydrates (1.26%), making it suitable for consumption. The moisture content, which influences the freshness and shelf life of food ingredients, was found to be 65.92% in fresh SAA meat. Additionally, the ash content in fresh SAA meat was measured at 3.15%.

Previous studies have shown that the nutritional content of SAA meat competes with other aquatic commodities. The protein content of SAA was found to be higher, while its fat content was lower compared to other snails (*Archachatina marginata* and *Archatina fulica*) and fishes (*Catla catla* and *Labeo rohita*). Additionally, the ash content was higher in comparison to those snails but lower than in fishes (Ngobidi et al., 2024; Mahboob et al., 2019).

3.2 Protein, Fat and Water Content in SAA Meat Flour and SPC

3.2.1 Protein Content

The results of the protein content analysis for SAA meat flour (control P0) and SAA protein concentrate (SPC) flour (samples P1, P2, and P3) are presented in Figure 4 below.

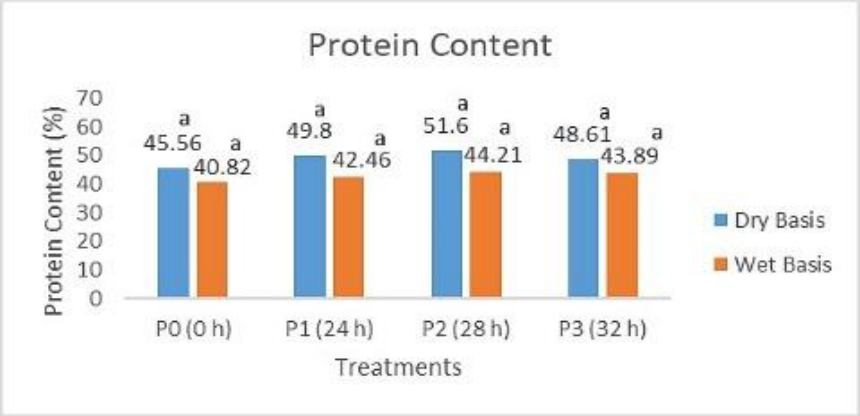

Figure 4. Effect of extraction time on the protein content of South Asian applesnail meat flour (P0) and protein concentrate flour (P1, P2, P3). Duncan's multiple range test ($p < 0.05$, $n = 3$)

Protein is an essential nutrient for the body, serving not only as a source of energy but also as a builder and regulator of bodily functions. It can act as a backup energy source when the body's energy requirements are not met by carbohydrates and fats. Additionally, protein is crucial for forming new tissues and maintaining existing ones in the human body (Apriansyah et al., 2021).

The analysis of protein content in SPC flour revealed that the average protein content on a dry basis varied with extraction time. Specifically, treatment P3 (32 hours) exhibited the lowest protein content, while treatment P2 (28 hours) showed the highest. On the other hand, results as shown in Figure 4 indicated no significant differences between samples, based on both dry and wet basis data. This is in line with the findings of Setyarini et al. (2024), which concluded that the number of extractions, which correlates with a longer extraction time, did not have a significant effect on the protein content of catfish (*Clarias gariepinus*). Previous research by Nkansah et al. (2021) showed that the values of dry meat protein contents in three snail species, namely *Achatina achatina*, *Achatina fulica*, and *Archachatina marginata*, were higher than SAA meat flour and SPC content.

3.2.2 Fat Content

Fat is a class of lipids characterized by its insolubility in water and solubility in organic solvents. It serves as a highly effective source of energy, surpassing both protein and carbohydrates in energy yield (Pargiyanti, 2019).

In terms of dry basis fat content, the analysis of SPC flour revealed that varying extraction times influenced fat levels. Specifically, treatment P1 (24 hours) exhibited the lowest fat content, whereas treatment P3 (32 hours) showed the highest fat content. The protein extraction process using alcohol was found to increase protein content and decrease fat content (Setyarini et al., 2024). The higher fat content in the P3 treatment was correlated with a lower protein content compared to the other treatments. The observed low fat content is indicative of successful protein concentrate preparation. Factors contributing to this outcome include the type of raw material, extraction method, drying technique, and extraction duration. The values of SAA and SPC fat content was lower than the dry meat fat content of other snail species, namely *Achatina achatina*, and *Archachatina marginata* (5.06%, and 4.37%, respectively) (Nkansah et al., 2021).

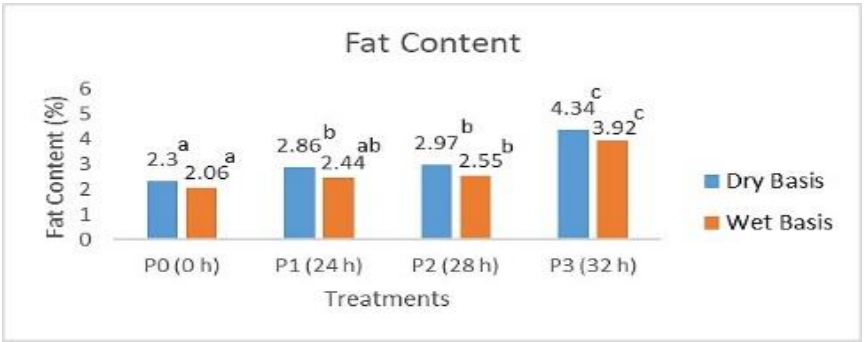

Figure 5. Effect of extraction time on the fat content of South Asian applesnail meat flour (P0) and protein concentrate flour (P1, P2, P3). Different letters (a - c) indicate significant differences identified by Duncan's multiple range test ($p < 0.05$, $n = 3$). Comparisons were made within the same data presentation (either dry basis or wet basis).

3.2.3 Moisture Content

Moisture content reflects water content which can exist as either an intracellular or extracellular component. Moisture content significantly influences the acceptability, freshness, taste, and overall quality of food ingredients (Daud et al., 2019).

The analysis of moisture content in SPC flour extracted over different time intervals revealed that the sample extracted for 24 hours contained 14.71% moisture, while the sample extracted for 28 hours showed a slightly lower water content of 14.38%. The sample extracted for 32 hours had an even lower water content of 9.69%. The decrease in moisture content of the protein concentrate, which is correlated to an increasing number of extractions and longer extraction time, is consistent with previous studies by Setyarini et al. (2024). The ethanol solution used in the extraction process may facilitate water evaporation, and the reduction in water content can also be attributed to the prolonged extraction duration (Laili, 2021).

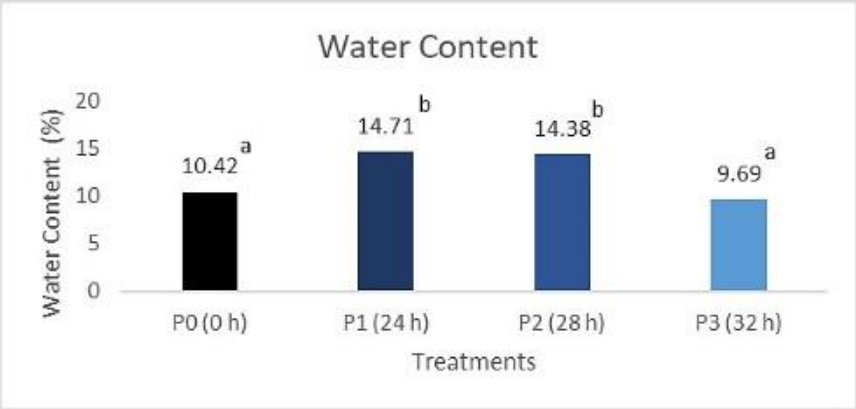

Figure 6. Effect of extraction time on the moisture content of South Asian applesnail meat flour (P0) and protein concentrate flour (P1, P2, P3). Different letters (a - b) indicate significant differences identified by Duncan's multiple range test ($p < 0.05$, $n = 3$).

3.3 Protein Profile by SDS-PAGE

Protein profile characterization was conducted using SDS-PAGE was conducted to determine protein types based on their molecular weight and monitor protein purification processes.

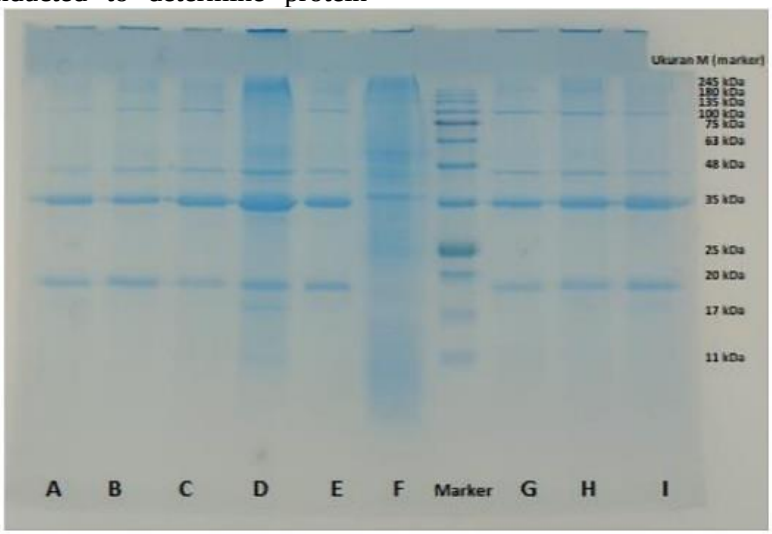

Figure 7. SDS-PAGE test results of South Asian applesnail meat flour (control P0) and SAA protein concentrate (SPC) flour (samples P1, P2, P3). Sample designations: A, B, C correspond to P1; D, E, F correspond to P2; G, H, I correspond to P3.

Differences in protein profiles within the SPC flour are evident from the distinct bands observed in the electrophoresis results, along with variations in molecular weight. The SDS-PAGE analysis revealed five protein bands with molecular weights of 11 kDa, 17 kDa, 20 kDa, 25 kDa, 35 kDa, and 48 kDa. The most abundant band were 35 kDa. The proteins within the range of 11-37 kDa were identified as histone proteins (Joshi et al., 2012). Additionally, the 48 kDa band is attributed to a protein derived from snail

mucus, which serves an antibacterial function (Velkova et al., 2024).

Histone proteins are a type of protein found in the nuclei of eukaryotic cells, where they associate with DNA to form nucleosome structures. There are five main types of histone protein subunits: H1, H2A, H2B, H3, and H4. Each of these subunits is characterized by a high content of cationic (positively charged) and hydrophobic amino acids. Histone proteins have demonstrated antibacterial properties against *Staphylococcus aureus* and *Escherichia coli*, suggesting their potential as functional food ingredients (Viruly et al., 2019).

According to Nurilmala & Ochiai (2016) and Nurilmala et al. (2017), several factors influence molecular weight during extraction, including extraction temperature, extraction time, and the concentration of the solvent used. Prolonged extraction times can lead to the cleavage of peptide bonds, resulting in amino acids with lower molecular weights. Therefore, it is crucial to optimize extraction conditions, including time, temperature, and solvent concentration, to achieve the desired protein profile.

3.4 Color Test

Color analysis was conducted using a colorimeter, with the following parameters measured: *L* (Lightness), *C* (Chroma), *H* (Hue), *a* (Redness), and *b* (Yellowness) (Rohmah et al., 2022).

3.4.1 *L** Value

Color correction for brightness ranges from 0 (representing the darkest color, black) to 100 (representing the lightest color, white) (R. Saputra et al., 2017).

The average Lightness value (*L*) in the SPC flour, as shown in Figure 8, indicates that the lowest Lightness value is observed in the P3 treatment (extraction time of 32 hours), while the highest value is found in the P1 treatment (extraction time of 24 hours). This trend suggests that Lightness in SAA flour decreases with increasing extraction time. Duncan's multiple range test revealed significant differences in the *L* (Lightness) values among the different extraction times in the SPC flour.

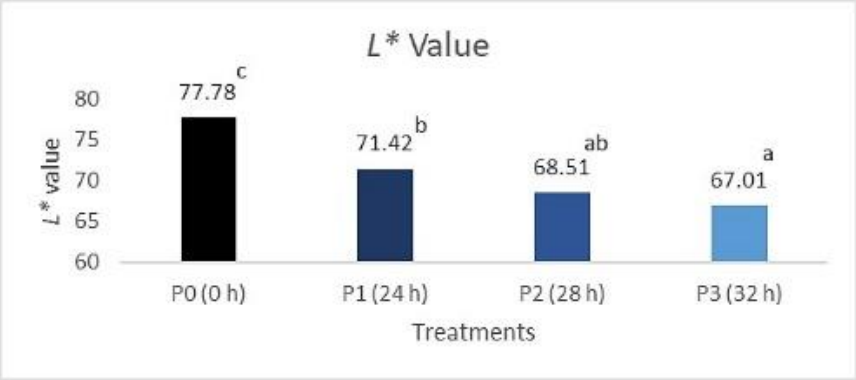

Figure 8. Effect of extraction time on the *L** value of South Asian applesnails meat flour (P0) and protein concentrate flour (P1, P2, P3). Different letters (a - c) indicate significant differences identified by Duncan's multiple range test ($p < 0.05$, $n = 3$).

Treatment P3 shows a significant difference compared to treatments P2 and P1. The results indicate that as the extraction time increases, the Lightness value of the SPC flour decreases, resulting in a darker color. This suggests that extraction time has a notable effect on the brightness of the SPC flour (Sari et al., 2017). The whiteness level indicates the ability of a material to reflect light that strikes it. The higher the whiteness level, the whiter the color of the produced flour. The whiteness level is inversely related to the fat content in the fish protein concentrate; if the fat content is high then the whiteness level will be lower (Setyarini et al., 2024). This can explain why the P3 treatment, which had the highest fat content, exhibited the lowest lightness value compared to the other treatments.

3.4.2 *C** Value

C (Chroma) indicates the level of color sharpness, determining the intensity of a product's color, whether it appears shiny or dull. The chroma value is expressed as a percentage, ranging from 0% for the dullest color to 100% for the sharpest or shiniest color (R. Saputra et al., 2017). In the analysis of SPC flour, the results show that the lowest Chroma value is found in treatment P3 with a soaking time of 32 hours, while the highest value is in treatment P1 with a soaking time of 24 hours.

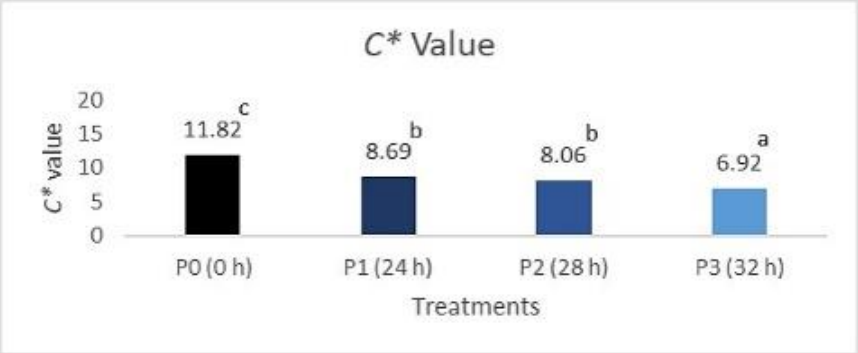

Figure 9. Effect of extraction time on the C* value of South Asian applesnails meat flour (P0) and protein concentrate flour (P1, P2, P3). Different letters (a - c) indicate significant differences identified by Duncan's multiple range test ($p < 0.05$, $n = 3$).

The chroma of the SPC flour decreases with increasing soaking time, likely due to color changes that occur during the extraction process, which can lead to a duller appearance (Sari et al., 2017). A higher chroma value corresponds to a greater intensity of color brightness (Aditya et al., 2016).

3.4.3 *H** Value

The dominant color value of a material or object is expressed in degrees of hue, as noted by (R. Saputra et al., 2017). This hue value indicates the predominant wavelength of light reflected from the material, determining its color characteristics. The range of colors produced can include red, yellow, green, blue, and purple.

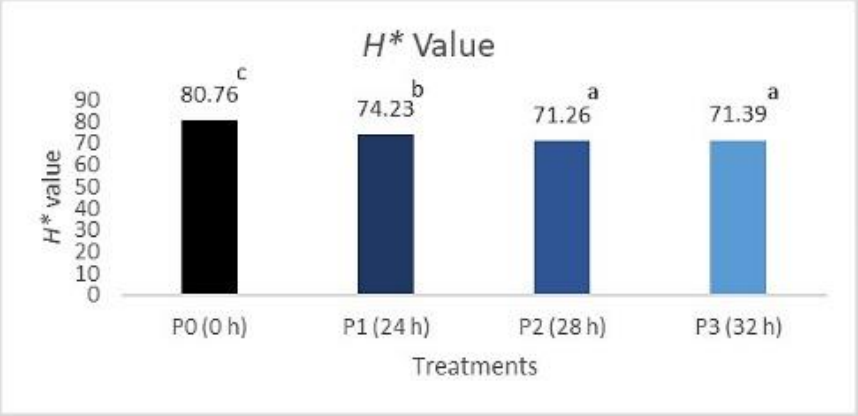

Figure 10. Effect of extraction time on the H* value of South Asian applesnail meat flour (P0) and protein concentrate flour (P1, P2, P3). Different letters (a - c) indicate significant differences identified by Duncan's multiple range test ($p < 0.05$, $n = 3$).

Based on the results of the hue (*H*) color test analysis of the SPC flour, the average hue value for treatment P2 (28 hours) was found to be the lowest, while treatment P1 (24 hours) exhibited the highest hue value.

The hue of the SPC flour tends to decrease with longer extraction times. This reduction in hue suggests a shift in color characteristics, with the resulting SPC flour displaying a yellow hue within the H° angle range of 0° to 90°, involved red, orange, and yellow tones (Sari et al., 2017).

3.4.4 *a** Value

According to Fathinatullabibah et al. (2014), the positive *a** value indicates a redder product, while the negative *a** value corresponds to a greener product.

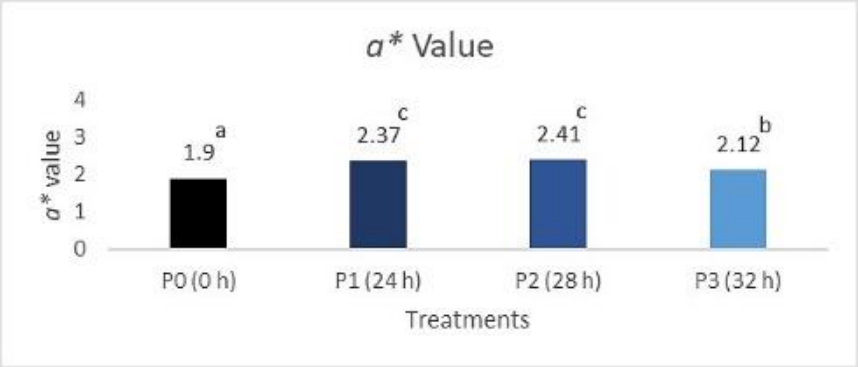

Figure 11. Effect of extraction time on the a* value of South Asian applesnail meat flour (P0) and protein concentrate flour (P1, P2, P3). Different letters (a - c) indicate significant differences identified by Duncan's multiple range test ($p < 0.05$, $n = 3$).

Based on the analysis of the *a** (Redness) color test for the SPC flour, it was observed that the average *a** value for treatment P3 (32 hours) was the lowest, while treatment P1 (24 hours) exhibited the highest value. The redness of the SPC flour tends to decrease with longer extraction times. This reduction in redness is attributed to color changes in the SPC flour, shifting towards greener tones as extraction time increases (Sari et al., 2017).

3.4.5 *b** Value

According to Fathinatullabibah et al. (2014), an increasing *b** value indicates a more intense yellow hue in the product, whereas a decreasing *b** value suggests a shift towards a bluer hue.

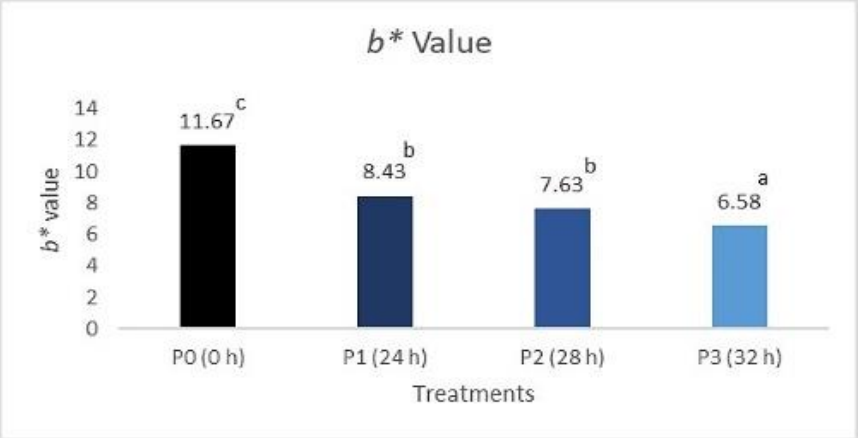

Figure 12. Effect of extraction time on the b* value of South Asian apple snail meat flour (P0) and protein concentrate flours (P1, P2, P3). Different letters (a-c) indicate significant differences determined by Duncan's multiple range test ($p < 0.05$, $n = 3$).

The results of the *b** (yellowness) color test analysis of SPC flour indicate that varying extraction times significantly affect the average *b** values. Specifically, treatment P3 (32 hours) exhibits the lowest *b** value, while treatment P1 (24 hours) shows the highest value. This trend suggests that yellowness in SPC flour decreases with longer extraction times, indicating that prolonged extraction may lead to a reduction in the yellowness value of SPC flour (Wibawanti & Rinawidiastuti, 2018).

3.5 Organoleptic Test (Aroma)

In the food industry, aroma testing is crucial as it provides a rapid assessment of consumer acceptance of a product. Aroma, as an olfactory stimulus, significantly influences the overall acceptance of food products. Notably, even when food appears visually appealing, deviations in aroma can diminish product acceptability (Tarwendah, 2017).

The results of the organoleptic test indicate that the level of panelist acceptance for the aroma of SPC flour showed the highest average total score for treatment P1 (24-hour soaking), while treatment P3 (32-hour soaking) received the lowest score.

Although individual preferences may vary, there is a noticeable decrease in average score or increase in acceptance related to differences in aroma assessment (Chandra et al., 2021). The organoleptic scale values and preference ranges are illustrated in the preference

criteria for the three samples. The distinctive aroma of SPC flour is attributed to the 24-hour extraction time, which is influenced by the strong characteristic aroma of SAA and the use of a 90% ethanol solvent (Amanah et al., 2015). Furthermore, the Duncan analysis revealed that results for P1 (24-hour soaking) are significantly higher than P3 (32-Hour soaking) confirming that treatment time had an effect on aroma. This is consistent with the study by Setyarini et al. (2024), which showed that an increasing number of extractions, related to a longer extraction time of catfish protein (*Clarias gariepinus*) resulting into a weaker fish aroma.

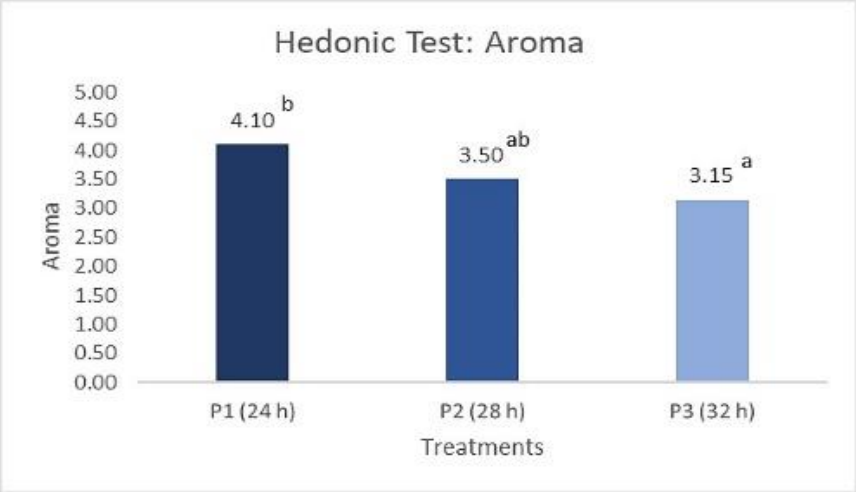

Figure 13. Effect of extraction time on the hedonic test results for the aroma of South Asian apple snail meat flour (P0) and protein concentrate flours (P1, P2, P3). Assigning scores of aroma: 5 = Very strong aroma, 4 = Strong aroma, 3 = Weak aroma, 2 = Very weak aroma, and 1 = No aroma. Different letters (a-b) indicate significant differences identified by Duncan's multiple range test ($p < 0.05$, $n = 3$).

4. CONCLUSION

Based on the results of this research, it can be concluded that the optimal treatment for protein content was the P2 treatment (SAA extraction for 28 hours), which yielded a protein content of 51.6% (dry basis). Additionally, protein profile analysis using SDS-PAGE revealed that P2 exhibited five protein bands with molecular weights of 17 kDa, 20 kDa, 25 kDa, 35 kDa, and greater than 48 kDa, which corresponds to histone proteins and an antibacterial protein derived from snail mucus. In contrast, the other treatments displayed only three protein bands.

However, the results of color and organoleptic aroma analysis indicated that optimum treatment for color and aroma was the P1 treatment (SAA extraction for 24 hours).

This research proposes that further studies can optimize protein extraction by extending the extraction time or using other solvents. Additionally, a more detailed analysis of the amino acid composition and the characteristics of the extracted protein, such as its antioxidant potential, is required.

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