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MINERAL CONTENT PROFILE OF CARAPACE FLOUR, CHITIN, AND CHITOSAN SLIPPER LOBSTER (*Thenus orientalis*)

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ABSTRACT

Slipper lobster live on the bottom of sandy and muddy waters at a depth of 10-200 m and are found throughout Indonesian waters. The carapace is the most significant part of its body, of which approximately 50% is a source of waste, but it can be utilized as functional food in the form of chitin and chitosan. One of the determinations of chitin and chitosan quality depends on the demineralization process. The potential of this shrimp has not been fully utilized due to the lack of information on mineral content after the demineralization process. This study aims to obtain information on flour, chitin, and chitosan mineral content. This research used a descriptive method. The parameters observed were yield, water, ash, and mineral content (K, Mg, Ca, Na, Fe, and P). The results showed that slipper lobster weighed about 80-240 g with a length of 15-25 cm with the yield and mineral content of flour, chitin and chitosan of slipper lobster carapace as follows: yield 29.04%, 41.02%, 33.42%, water 2.36%, 3.96%, 3.25%, ash 52.89%, 8.27%, 3.16%, potassium 55.73 mg/L, 9.09 mg/L, 4.55 mg/L, magnesium 37.23 mg/L, 5.59 mg/L, 5.95 mg/L, calcium 766.87 mg/L, 137.62 mg/L, 126.91 mg/L, sodium 146.62 mg/L, 17.82 mg/L, 10.73 mg/L, iron 12.82 mg/L, 1.89 mg/L, 1.72 mg/L, phosphorus 0.17 mg/L, 0.06 mg/L, 0.05 mg/L.

Keywords: Minerals, Chitin, Chitosan, Slipper lobster

1. INTRODUCTION

Slipper lobster (*Thenus orientalis*) live on the bottom of sandy and muddy waters at a depth of 10-200 m and are often found throughout Indonesian waters, such as in the waters of Sumatra, Indian Ocean, Makassar Strait, Java Sea, East Kalimantan, and Kupang[1,2]. Slipper lobster is also often known as Flathead lobster (English), Cigale raquette (French), and Cigarra chata (Spanish). The difference in names depends on various regions. This shrimp has a characteristic fan-shaped head. The shrimp has a dark brown and pale fan-shaped tail, and the skeleton of the head is very thick, flattened, and covered with large and small spines with a body length of generally 8-10 cm, sometimes reaching 25 cm[3,4]. The proportion of meat and carapace of this shrimp is quite large. Unfortunately, the carapace has not been utilized, so it becomes a waste source with the proportion of carapace, which is ± 50% of the shrimp's body weight[5].

Slipper lobster carapace contains 17.50% chitin with 65% deacetylation degree[6]. Chitin can be applied to various industrial fields, such as chromatography, paper, textile, nutrition, agriculture, and pharmacy. In addition, shrimp carapace chitin has been reported to be converted into chitosan, which is used as raw material for food, cosmetics, antibacterial, and other pharmaceutical products[7]. Chitin and chitosan can be used as various functional food products due to the nature of natural polysaccharide biopolymers with many amine groups (NH_2) that are ionized in solution to neutralize target compounds. Chitosan is a derivative of chitin, and the

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process of forming chitin and chitosan must go through an isolation process.

Chitin and chitosan can bind to other polysaccharides such as cellulose, glucan, mannan, and polygalactosamine, making isolation more difficult[8], so it must release mineral, protein, and acetyl groups to increase its function. Chitin isolation generally consists of three main processes: mineral removal by immersion in HCl solution, protein removal by immersion in NaOH solution enzymatically using protease enzymes or microorganisms, and pigment removal (decolorization) using absolute ethanol or acetone[9].

This shrimp carapace contains higher minerals than its meat. It is mainly formed by Ca, K, Na, Mg, Fe, and P[10,11], so it becomes a significant concern in the mineral removal stage. Research Tobing et al.[12], optimum demineralization conditions can be obtained by extracting 1 N HCl, NaOH, and H_2O_2 for 30 minutes and 1 to 2 hours at room temperature with a solution ratio of 1:10 (b/v). This condition can reduce the ash content of chitin to 38.02-70.70%. The difference between high and low ash content is directly proportional to the presence and amount of mineral content in the chitin and chitosan isolation process.

The lower the mineral content of chitin and chitosan, the more effective its use. This means that the amine group is more reactive to other molecules due to the detachment of the mineral chain. Based on that, information about the mineral content of fan shrimp chitin and chitosan after the demineralization process is still limited, so it is necessary to carry out mineral analysis to determine the mineral content that is still dominantly bound to the chitin and chitosan structure. Therefore, researchers are interested in researching the mineral content profile of chitin and chitosan of slipper lobster carapace. This study aimed to determine the description, proportion, yield, and chemical composition of slipper lobster carapace minerals.

2. RESEARCH METHOD

Time and Place

This study was conducted from April to September 2023. Samples were processed and analyzed at the Fishery Products Chemistry Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Riau.

Method

This study used a descriptive method. Parameters observed in mineral analysis include ash, potassium, magnesium, calcium, sodium, iron, and phosphorus.

Procedures

Carapace Raw Material Preparation

The shrimp were separated into carapace, meat, and entrails. The carapace was washed using running water and brushed to remove any remaining meat attached to the carapace. Then, the carapace was dried in an oven for five days at a temperature of 40°C. Next, it was pulverized by grinding (grinder), and chemical parameters were measured, including moisture, ash, minerals K, Mg, Ca, Na, P, and Fe[13].

Extraction of Chitin and Chitosan

Chitosan extraction refers to the procedure of Suptijah[14], namely the initial process of chitin preparation, including demineralization, deproteination, and drying. Then, chitosan is obtained from chitin deacetylation. The smooth slipper lobster carapace was demineralized with 1N HCl solution (ratio of shell and HCl solution 1:7 (b/v)) for 1 hour at ±90°C. The results of the demineralization process were then precipitated to separate the solids and liquids by letting them stand. The solids obtained were washed three times with distilled water to neutralize the pH close to pH 7. The solids were then deproteinated with 3N NaOH solution (ratio of solids and NaOH solution 1:10 (w/v) for 1 hour at ±90°C. The results of the deproteination process were then precipitated to separate the solids and liquids by allowing the solids to separate from the liquid. The solids obtained were washed

repeatedly with tap water to neutralize the pH to close to pH 7. The solids were dried in an oven at 60°C for 6 hours. The chitin formed was analyzed for moisture, ash, nitrogen, and degree of deacetylation.

Chitosan was obtained by deacetylating chitin using 30% NaOH solution (ratio of chitin and NaOH solution 1:2 (b/v)) for 1 hour at ±100°C. The results of the deacetylation process were then precipitated to separate the solids and liquids by allowing the solids to separate from the liquid. The solids obtained were washed repeatedly with tap water to neutralize the pH to close to pH 7. The solids were dried in an oven at 60°C for 6 hours. The chitosan formed was analyzed for yield, moisture content, ash, minerals (K, Mg, Ca, Na), and Fe[13].

Research Parameters

Yield Analysis

The yield of slipper lobster carapace chitosan, according to Karnila et al.[15]. The yield is calculated by comparing the material's final weight and the raw material's initial weight. The yield results are represented in the form of percent (%). The calculation of yield can be done using the formula:

$$Y = \frac{W_t}{W_0} \times 100\%$$

Description:
Y = Yield (%)
W0 = Initial weight of raw material (g)
Wt = Final product weight (g)

Water Content Analysis

Moisture content analysis was carried out according to the AOAC[13] procedure, and the porcelain cup was dried in an oven at 105°C for 2 hours. Then, it was placed into a desiccator for ±15 minutes and weighed, then the sample (crushed) was weighed 3-4 g into a porcelain cup and placed in an oven at 102-105°C for 5-6 hours. After that, the sample was put into a desiccator and cooled for 30 minutes. Then, it is weighed (repeat this procedure until a fixed weight is obtained). Calculation of moisture content can be done using the formula:

$$\% \text{ moisture} = \frac{B-C}{B-A} \times 100\%$$

Description:
A = Empty weighing porcelain cup (g)
B = Weighing porcelain cup filled with sample (g)
C = Weighing porcelain cup with dried sample (g)

Ash Content Analysis

Ash content analysis was carried out according to the procedure of AOAC[13]. The porcelain cup was cleaned and dried in an oven at 105°C for 2 hours. Then, the sample was put in a desiccator for 30 minutes, and the sample (crushed) was weighed 4-5 g in a porcelain cup. After that, it was burned in a furnace at 550°C until it reached complete ignition. Next, the cup was placed in a desiccator for 30 minutes and weighed. Calculation of ash content can be done using the formula:

$$\% \text{ ash} = \frac{C-A}{B-A} \times 100\%$$

Description:
A = Weight of empty cup (g)
B = Weight of cup with sample (g)
C = Weight of the cup with the annealed sample (g)

Phosphorus Content Analysis

Total phosphorus was analyzed using a spectrophotometer according to the procedure of AOAC[13]. Samples were weighed 1-2 g into a 200 mL Erlenmeyer, added 5 mL of HNO_3, and allowed to stand for 1 hour at room temperature in an acid chamber, then heated on a hot plate at 60°C for 4-6 hours. After that, it was allowed to stand for 12 hours in a closed state. Next, 0.4 mL of H_2SO_4 was added to the solution and heated again on a hot plate at 80°C until the solution was more concentrated (±1 hour), and 2-3 drops of $HClO_4$:HNO_3 (2:1) were added. Heating is continued until a color changes from brown to light yellow (±1 hour). After a color change, heating is continued for 10-15 minutes. Then, the

sample was cooled, and 2 mL of distilled water and 0.6 mL of HCl were added. The sample was reheated for ± 15 minutes and then put into a 100 mL measuring flask. If there is a precipitate, it is filtered with glass wool. Samples were pipetted as much as 0.5 mL into a test tube, distilled water added up to 3 mL and 2 mL of lanthan solution ($LaCl_3.7H_2O$), and homogenized. Then, the sample was measured using a spectrophotometer with a wavelength of 660 nm. Calculation of phosphorus content using the formula:

$$\% \text{ phosphorus} = \frac{\text{P in solution x } f_p}{W} \text{ x } 100\%$$

Description:
fp = Dilution factor
W = Sample weight (g)

Analysis of Calcium, Potassium, Iron, Sodium and Magnesium Levels

Iron (Fe), sodium (Na), magnesium (Mg), and metal absorption values were analyzed using the Atomic Absorption Spectrophotometer (AAS) method. A total of 2 g of sample was put into 150 mL Erlenmeyer, then 5 mL of 65% nitric acid, which aims to dissolve the inorganic content heated on a hot plate and cooled. After cooling, 2 mL of perchloric acid was added to the Erlenmeyer, which seeks to evaporate the organic content in the sample and then heat it on a hot plate and cool it. The solution was diluted with distilled water to 100 mL in a measuring flask, and then the solution was filtered with Whatman filter paper until a clear solution was obtained. The standard solution, blank, and sample have flowed into the Atomic Absorption Spectrophotometer (AAS) brand Perkin Elmer Analyst 100 with the wavelength of each mineral type, then the absorbance or peak height of the standard, blank, and sample at the appropriate wavelength and parameters for each mineral with a spectrophotometer. Calcium content was measured at a wavelength of 422.7 nm, sodium at a wavelength of 589.00 nm, potassium at a wavelength of 766.49 nm, and iron at a wavelength of 248.3 nm.

3. RESULT AND DISCUSSION

Description of Slipper Lobster Flour, Chitin, and Chitosan

Slipper lobster carapace flour produces a white-brown color, while chitin extraction is obtained from the demineralization and deproteination process, and then the deacetylation process (CH_3CO^-) of chitin produces chitosan. More details can be seen in Figure 1.

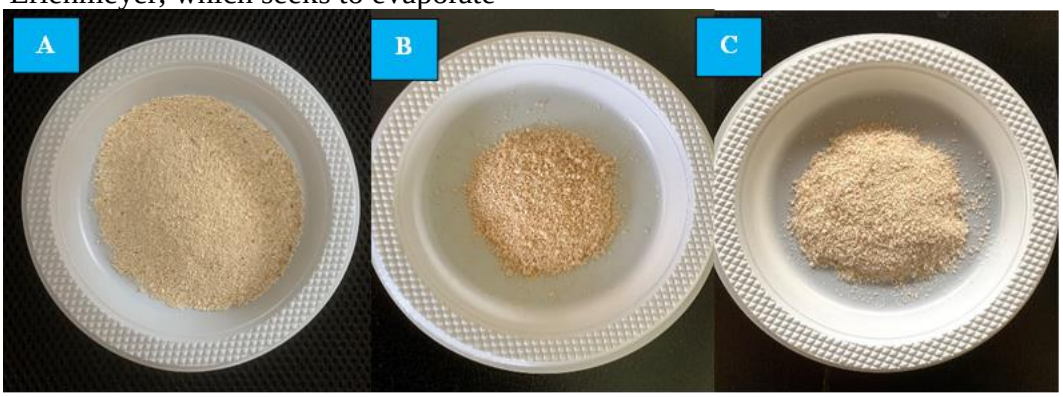

Figure 1. A. Flour; B. chitin; C. slipper lobster carapace chitosan

The color of slipper lobster carapace flour is slightly faded with the initial color of fresh slipper lobster carapace due to the carapace washing and drying process. The flour has a crystalline texture and some fine and coarse grains with a distinctive lobster aroma. While chitin produces a characteristic light brownish-white color with a crystalline texture and fine grains, chitosan produces a slightly yellowish-white color with a fine powder texture, uniform and odorless. This is because soaking in HCl acid and NaOH base results in a condensation reaction by hydrolyzing the N-

acetylglucosamine monomer, thus changing the characteristics of chitin and chitosan to be smoother, whiter, and odorless typical of shrimp[16]. The purity characteristics of chitin and chitosan are close to SNI standards, namely light brown to white color[17].

Yield of Carapace Flour, Chitin, Chitosan

Based on Table 1, the average yield of flour produced was 29.04%. According to Karnila[18], economically, the higher the yield value, the more profitable. The oven drying process obtains a more controlled material moisture content than drying by drying[19]. The yield of chitin was 41.03%, which was due to the concentration of 1N HCL acid (demineralization) and 3N NaOH base (deproteinization) that could release mineral, protein, and polysaccharide bonds reaching 38.02-70.70%[12]. The resulting chitin yield is higher than sea cucumber chitin 39.08%[20], slipper lobster chitin 17.50%[6]. The difference in chitin yield also depends on the type of material, the amount of mineral, and protein content that is hydrolyzed from the length of acid and intense base immersion.

Table 1. Flour, chitin, and chitosan yields of slipper lobster carapace

Repeat	Flour (%)	Chitin (%)	Chitosan (%)
1	28.98	38.33	30.33
2	29.04	42.24	34.09
3	29.09	42.48	35.84
Average	29.04 ± 0.06	41.02 ± 2.33	33.42 ± 2.81

Meanwhile, the average value of chitosan yield was 33.42%. The low chitosan yield is due to the high concentration of NaOH base of 30-50% which can reduce the chitosan yield by 14-50%[21,22,23]. The concentrated NaOH solution can break the CH_3CO group (acetyl bond), thus increasing the degree of deacetylation and decreasing the molecular weight. The resulting chitosan yield was higher than crab chitosan 20.64%[24], rama-rama lobster chitosan 27.51%[25], Vanname shrimp chitosan 19.5%[26], and tiger shrimp chitosan 14%[23].

Mineral Chemical Content of Flour, Chitin, Carapace Chitosan

Based on Table 2, flour, chitin, and chitosan moisture content were 2.36%bb, 3.96%bb, and 3.25%bb, respectively. The difference in moisture content in these materials is thought to be the effect of the level of dryness of the sample during preparation with the oven heating process. The heating process is one of the energies that can break the molecular bonds of water (H_2O) bound to other molecules such as polar compounds (hydrophilic) on N, O, F atoms, and hydroxyl groups (OH^-) found in carbohydrates and proteins, and non-polar compounds (hydrophobic) with many macromolecular atoms and carboxylic groups ($COOH^-$) found in fats, as well as ionic bonds on anion cation group.

Then, chitosan's water content is lower than chitin's due to the release of chitin water molecules using a concentrated NaOH solution to produce chitosan. NaOH molecules dissociate into Na^+ and OH^- ions so that OH^- interacts with H and O bonds in the water molecules of the material, which can affect physical properties, chemical content, and boiling and freezing points[27]. In addition, NaOH solution exposed to air can react with CO_2 to form sodium carbonate (CO_2), which can reduce the strength of the solution by binding to chitosan water molecules[28]. The chitin and chitosan characteristics of slipper lobster have met the standard of a maximum of 12%[17198], although higher than the water content of Homarus americanus lobster of 2.9%[29].

The ash content of slipper lobster carapace flour was higher at 52.89 %bk, compared to slipper lobster chitin ash at 8.27% and chitosan at 3.16%. The high ash

content of flour indicates the amount of minerals and lime substances formed in the lobster carapace. In contrast, the ash and chitin content decreased dramatically due to the release of minerals from using HCl acid and decantation.

Table 2. Chemical content of flour, chitin, and chitosan of slipper lobster carapace

No	Content	Flour	Chitin	Chitosan
1.	Moisture	2.36 ± 0.16	3.96 ± 0.04	3.25 ± 0.20
2.	Ash (dw)	52.89 ± 3.79	8.27 ± 0.64	3.16 ± 0.71
3.	Potassium (mg/L)	55.73 ± 7.44	9.09 ± 0.04	4.55 ± 15.90
4.	Magnesium (mg/L)	37.23 ± 0.39	5.59 ± 0.04	5.95 ± 1.05
5.	Calcium (mg/L)	766.87 ± 11.11	137.62 ± 0.05	126.91 ± 22.80
6.	Sodium (mg/L)	146.62 ± 10.27	17.82 ± 0.07	10.73 ± 3.22
7.	Iron (mg/L)	12.82 ± 2.61	1.89 ± 0.08	1.72 ± 0.48
8.	Phosphorus (mg/L)	0.17 ± 0.13	0.06 ± 0.00	0.05 ± 0.01

The chitin and chitosan minerals of slipper lobster are mainly in the form of calcium, namely chitin 137.62 mg/L and chitosan 126.91 mg/L (Tabel 2), due to the high calcium content of slipper lobster carapace flour of 766.87 mg/L (Table 2). The decrease in calcium levels is due to the acid-base reaction process that forms inorganic salts, namely calcium carbonate ($CaCO_3$) and calcium phosphate (Ca_3PO4), which can dissolve water[30,31]. This situation is consistent with the low phosphorus content maximizing water solubility with calcium phosphate (Ca_3PO_4) salts.

Bates[32] added that the release of phosphorus due to reaction with HCl acid forms phosphorus pentachloride (PCl_5), and NaOH base forms sodium phosphate (Na_3PO_4), which is dissolved with H_2O molecules. Then, iron (Fe) is an active element, namely bivalent (II) or Fe^{2+} and bivalent (III) or Fe^{3+}, which is physically soluble in acids. HCl and NaOH solutions will adsorb through ion exchange to form $FeCl_2$ and $Fe(OH)_2$, whose bonds are easily separated by demineralization, deproteination, and deacetylation processes[33].

Potassium and sodium levels in flour and chitin to chitosan experienced a significant decrease in value, reaching ±50%. This situation is due to HCl acid-forming salts, namely KCl and NaCl and NaOH base forming KOH and 2NaOH. These compounds are the most hygroscopic, so they can reduce the melting point of metals when extracted by acids[34]. Meanwhile, the magnesium content in chitin also decreased by 5.59 mg/L from the high magnesium content of slipper lobster carapace flour of 37.23 mg/L (Table 2) due to HCl immersion forming $MgCl_2$ which can reduce the reactivity of the material through the release of magnesium bands, in contrast to the magnesium content in chitosan which is difficult to release due to NaOH immersion forming Mg $(OH)_2$ which is difficult to dissolve in water and increases the reactivity of magnesium (fly ash) in the extractor[35].

4. CONCLUSION

Slipper lobsters have an average weight of 80-240 g and a length of 15-24 cm. The yield and mineral content of the flour, chitin, and chitosan of slipper lobster carapace are as follows: yield 29.04%, 41.02%, 33.42%, water 2.36%, 3.96%, 3.25%, ash 52.89%, 8.27%, 3.16%, potassium 55.73 mg/L, 9.09 mg/L, 4.55 mg/L, magnesium 37.23 mg/L, 5.59 mg/L, 5.95 mg/L, calcium 766.87 mg/L, 137.62 mg/L, 126.91 mg/L, sodium 146.62 mg/L, 17.82 mg/L, 10.73 mg/L, iron 12.82 mg/L, 1.89 mg/L, 1.72 mg/L, phosphorus 0.17 mg/L, 0.06 mg/L, 0.05 mg/L.

Based on this research, the author suggests that further research is needed on the value of the degree of deacetylation and

the structure of the glycosidic chain in chitin and chitosan to see the detached acetyl chain and the correctness of the chemical structure of chitin and chitosan.

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