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# CHARACTERIZATION OF CHITOSAN FROM WHITE SNAPPER (*LATES CALCARIFER*) SCALES USING CHEMICAL METHODS

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## ABSTRACT

White snapper (*Lates calcarifer*) scales can be used as raw material for chitin extraction, and then modified into chitosan. Chitosan is a deacetylation product of chitin either through chemical or enzymatic reactions. The chemical reaction process can be carried out through several stages, namely deproteination, demineralization, depigmentation and deacetylation. Chitosan has many uses such as anti-microbial, water purifier, anti-oxidant, and fat absorbent. This study aims to determine the characterization of chitosan from white snapper scales and the yields of chitin and chitosan extracted from white snapper scales. Extraction of chitin from white snapper scales was carried out by deproteination, demineralization and depigmentation stages. Then the transformation of chitin into chitosan from fish scale waste was carried out through a deacetylation process. The chitosan produced was then tested for the characteristics of chitosan which included determining the degree of deacetylation, moisture content, ash content, color, odor, and texture. The results obtained are presented in tabular form where the chitosan characterization of white snapper scales has a deacetylation degree of 72.80%; 8.36% moisture; and 0.48% ash, has a texture that is not too smooth in the form of flakes and is creamy white in color as well as odorless. While the yield of chitin was 20.10% and the yield of chitosan was 10.72%. Chitin extraction from white snapper scales at each stage obtained a protein of 30.41%; 37.70% mineral; and 11.78% pigment. Based on this research, it can be seen that chitosan from the scales waste of white snapper (*L. calcarifer*) can be a product of high economic value because it can be used in various fields.

**KEYWORDS:** Chitosan, white snapper scales, antimicrobial.

## INTRODUCTION

Indonesia has a large sea area of ± 3,446,488 km$^2$ with potential natural wealth, including biological creatures as a result of fisheries. So that it has very high potential in the fisheries sector.[1] According to the Ministry of Maritime Affairs and Fisheries in 2019, the sustainable potential of Indonesia's marine fish resources is estimated at 12.54 million tonnes per year which are spread across Indonesian territorial waters and the waters of the Indonesian Exclusive Economic Zone (ZEEI). Based on statistical data from the Ministry of Maritime Affairs and Fisheries (KKP) 2020, Maluku produces 18,759.9 tons of snapper.

The large consumption of white snapper by the community and the existence of culinary restaurants whose main menu is fish, resulting in waste, both liquid waste and solid waste. Liquid waste is usually in the form of blood, mucus, and fat. While organic solid waste is mostly in the form of heads, gills, stomach contents, bones, fins, skin and scales. Lack of management of these wastes can cause environmental pollution, such as the appearance of unpleasant odors and disturbing aesthetics and comfort, as well as decreasing water quality which can pollute the environment and disrupt the health of people in the surrounding area.[2]

White snapper fish scales can be used to produce valuable products such as chitosan. According to[3] fish scales can be used as raw material for the extraction of chitin and then modified into chitosan. Chitin is a natural biomaterial belonging to the second most abundant structural polysaccharide after cellulose. Chitosan is a deacetylation product of chitin either through chemical or enzymatic reactions. This compound can be found in shrimp shells, fish scales, crabs, clams, insects, annelids and some fungal and algae cell walls.[4]

Processing fish scales into chitosan using chemical methods can be carried out through several processes,

namely deproteination, demineralization, depigmentation and deacetylation. Chitosan can be applied in various fields such as in agriculture, chitosan is usually used in natural seed treatments and plant growth enhancing agents, and as an environmentally friendly biopesticidal substance that increases the ability of plants to defend themselves against fungal infections. In the environmental field as an adsorbent for heavy metals, such as lead (Pb), iron (Fe), nickel (Ni) which are found in polluted water. In the food sector it can be used as a substitute for formalin because it has antimicrobial activity. In the medical field, especially as a biopolymer which is usually combined with bone and tooth replacement materials.[2]

The application of chitosan in various fields is largely determined by the quality characteristics which include the degree of deacetylation, moisture content, ash content, color, odor and texture. For this reason, this research is very important so that the utilization of waste, especially fish scales, into products of high economic value can be carried out optimally.

## MATERIAL AND METHODS

### Materials and equipment

The materials used in this study were White snapper (*Lates calcarifer*) scales, hydrochloric acid (HCl), sodium hydroxide (NaOH), Proclin, distilled water, filter paper, litmus paper. The tools used in this study were blenders, analytical balances, magnetic stirrers, ovens, furnaces, desiccators, vacuum pumps, hotplate UV-Vis spectrophotometers, and several glassware, such as erlenmeyer, beakers, measuring cups, pipettes, stir bars, measuring flasks, petridisk, porcelain dish, and stir bar.

### Extraction of chitin from fish scales

Extraction of chitin from white snapper (*Lates calcarifer*) scales was carried out using the Hong method[2] in the following way: (i) prepare 100 g of white snapper scales which have been washed with water until clean, then dried under sunlight. Scales that have been clean and smoothed using a blender; (ii) The next step is deproteination, namely into a 2000ml erlemeyer containing fish scales powder, 3.5% NaOH solution was added with a ratio of 10:1 (v/w), then heated while stirring with a magnetic stirrer for 2 hours at a temperature of 65$^0$C. After the sample has cooled, it was then filtered and neutralized with distilled water until the pH was neutral. The solid obtained was dried in an oven at 60$^0$C until dry, then weighed; (iii) Demineralization, namely deproteinated fish scales powder added with 1N HCl solution with a ratio of 15:1 (v/w) in a 2000ml Erlenmeyer and refluxed at 40$^0$C for 30 minutes, then cooled. After cooling, it was filtered and the solid was neutralized with distilled water until the pH was neutral, then dried in an oven at 60$^0$C, and weighed; and (iv) Depigmentation, namely Proclin solution added to the demineralized powder in a ratio of 10:1 (v/w) in a 1000ml glass beaker. Reflux was carried out for 1 hour at 40$^0$C, then the solid was filtered and neutralized with distilled water until the pH was neutral. The neutralized solid was dried in an oven at 80$^0$C until the weight remains constant, then weighed.

The yield of chitin was calculated based on the ratio between the weight of chitin and the weight of the raw material using the following formula equation:

$$\text{Chitin yield} = \frac{\text{Chitin weight (g)} \times 100\%}{\text{Weight of fish scales (g)}}$$

### Transformation of chitin into chitosan from fish scales

The production of chitosan from chitin on fish scales was carried out through a chitin deacetylation process using the Knorr method[2], namely by adding 60% NaOH with a ratio of 20:1 (v/w) and refluxing it at 100-140$^0$C for one hour. After chilling, it was filtered and the solid obtained was neutralized with distilled water until the pH was neutral. The solid was then dried in an oven at 80$^0$C for 24 hours, after which it was weighed and the chitosan was ready to be analyzed. Chitosan was identified using a UV-visible spectrophotometer at a wavelength of 201nm.

The yield of chitosan is the ratio between the weight of chitosan (g) and the weight of chitin (g) multiplied by 100%. The weight of chitosan (g) is the weight of chitin after undergoing the deacetylation process, while the weight of chitin (g) is the final weight of barramundi scale powder after undergoing deproteination, demineralization and depigmentation processes.

### Characterization of chitosan

The characterization of chitosan includes determining the degree of deacetylation, moisture content, ash content, color, odor and texture with the following description: 4 mg of chitosan that has been produced was dissolved in 50 ml of 0.1 M HCl solution and the absorbance was recorded with a genesis spectrophotometer 10 UV-Vis at a wave length of 201nm. The degree of acetylation (DA) of deacetylated chitosan and the degree of deacetylation (DD) were determined according to the method.[5] Observation of color, smell, and texture of chitosan extracted from white snapper fish scales was observed visually, by looking at the appearance of its color and texture, while the smell was inhaled.

## RESULTS AND DISCUSSION

### Extraction of chitin from scales of white snapper (*L. calcarifer*)

The deproteination stage was carried out to remove the protein content contained in the white snapper scales by using a strong base, namely sodium hydroxide (NaOH). From 100g of white snapper fish scales have lost protein by 30.41%. More detail is presented ain Table1.

This is in accordance with what was reported by[6] that the protein content in marine fish scales is 30-40%, so that the protein content contained in the white snapper fish scales has been hydrolyzed quite optimally. In this

deproteination reaction, a few bubbles were formed on the surface of the solution in the Erlenmeyer and the solution became slightly thickened.

**Table 1: Protein, mineral and pigment content in white snapper (*L. calcarifer*) scales.**

| Nutrient (%) | White snapper scale |
|---|---|
| Protein | 30.41 |
| Ash | 37.70 |
| Pigment | 11.78 |

The demineralization stage is carried out to remove the minerals contained in fish scales using HCl. At this stage, a residue of 31.88 g was obtained and a mineral loss of 37.70%. According to[6] that the mineral content in marine fish scales is 30-50%, so that the mineral content contained in the scales of this white snapper fish has been hydrolyzed quite optimally.

The depigmentation stage is to remove the color (pigment) contained in the fish scales sample. This process is carried out using proclin because proclin contains sodium hypochlorite (NaClO). At this depigmentation stage, a residue of 20.10 g was obtained so that the dye was lost by 11.78%. At this stage, it has produced chitin. The stage of the transformation of chitin into chitosan involves only one step, namely the deacetylation stage which transforms chitin into chitosan.

**Yield of chitin and chitosan from white snapper (L. calcarifer) scales**

The percentage of chitin and chitosan yields extracted from white snapper (*L. calcarifer*) scales can be seen in Table 2. Meanwhile, the appearance of chitin and chitosan from white snapper (*L. calcarifer*) scales can be seen in Figure 1.

**Table 2: Percentage of chitin and chitosan yields extracted from barramundi (*L. calcarifer*) scales.**

| Variable | Percentages (%) |
|---|---|
| Chitin yield | 20.10 |
| Chitosan yield | 10.72 |

The yield of chitin extracted from the scales of white snapper (*L. calcarifer*) in this study was 20.10% (Table 2). This is in accordance with what was reported by[6], that the chitin content in marine fish scales is 20-30%. The presence of high chitin content in fish scales indicates that fish scales have high economic value because they have the potential to be used as chitosan which is very useful in various fields, while the yield of chitosan was 10.72%. There have been several previous studies regarding the yield of fish scales such as the results of the study of[7] stating that the yield of chitosan produced from parrot fish scales was 3.33%. Bangngalino and Akbar[8] stated that the chitosan obtained from milkfish scales has a yield of 12.5%. When compared with several previous studies, regarding the yield of chitin and chitosan yield from fish scales, various results were obtained. This is caused by the type of fish scale samples used, and also by temperature and time.

For the visualization of chitosan, it can be seen in Fig. 1b, showing that this chitosan has a texture that is not too smooth, flake-shaped and cream-white in color, and has no odor.


**Fig 1: Photos of chitin (A) and chitosan (B) extracted from the scales of white snapper (*L. calcarifer*).**

**Characterization of chitosan from white snapper (*L.calcarifer*) scales**

Characterization of chitosan from barramundi scales including the degree of deacetylation (DD), moisture content, ash content, color, and texture, is presented in Table 3. The degree of deacetylation (DD) test of the scales of white snapper (*L. calcarifer*) was 72.80%, this result met the established standard of more than 70%.

Several previous studies regarding the degree of deacetylation contained in chitosan extracted from fish scales, including research conducted by[9] which resulted in the degree of deacetylation of red snapper scales, namely 73.40%. Joris et al.[7] obtained a degree of deacetylation of parrotfish scales of 90.23%, and Bangngalino and Akbar[8] reported a degree of deacetylation of milkfish scales of 81.56%.

**Table 3: Characteristics of chitosan produced from white snapper (*L.calcarifer*) scale waste.**

| Variables | White snapper | Standart (Lab Protan, Dewoo) |
|---|---|---|
| Degree of deacetylation | ≥70% | 72.80% |
| Water content | ≤10% | 8.36% |
| Ash content | ≤2% | 0.48% |
| Color | White/yellow | White-cream |
| Smell | No smell | No smell |
| Texture | powder | flakes |

The DD obtained from previous studies varied widely, due to the fish scale samples used which were sourced from different types of fish, the amount of alkaline solution, time, and reaction temperature used during the deacetylation process. The water content obtained has met the specified quality standard, which is equal to 8.36%. This is caused by the drying process which takes quite a long time, and the amount of chitosan that is dried is small, and uses a large container. In accordance with what was stated by[10] that the water content in chitosan is affected by the drying process, drying time, the amount of chitosan to be dried, and the surface area where the chitosan is dried.

The ash content in this study is 0.48%. These results indicate that the chitosan obtained met the chitosan quality standard, which is < 2%, which means that the remaining mineral content is very small. The lower the ash content contained in chitosan, the higher the quality and purity of chitosan. The chitosan produced from this study is creamy white, flake-shaped, and odorless. The color produced from this study was caused during the demineralization and deproteination processes, there was still organic matter that had not completely disappeared. So get a color that is not in accordance with the standards set, which has a white color. The resulting color is also affected by the depigmentation process.

## CONCLUSION

Based on this study, it can be concluded that chitosan extracted from white snapper (*L. calcarifer*) scales has a degree of deacetylation of 72.80%, moisture content of 8.36%, ash content of 0.48%, creamy white color, flaky texture, and odorless. While the yield of chitin extracted from barramundi scales was 20.10% and the yield of chitosan from barramundi scales was 10.72%.

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