

SYNTHESIS AND CHARACTERISTICS OF CHITOSAN FROM WASTE FISH SCALES AND CRAB SHELLS PRODUCED IN KUPANG CITY

Sintesis Dan Karakteristik Kitosan Dari Limbah Sisik Ikan dan Cangkang Kepiting Hasil Buangan di Kota Kupang

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ABSTRACT

Fish scales are the result of fishing activities that are simply thrown away. Fish scales that are thrown away can become waste which has a negative impact on the environment. Crab shells are a problem that needs to be solved. One use of crab shell waste is processing it into chitosan. The aim of this research is to find out how to make chitosan from waste fish scales and crab shells, calculate the yield and water content, and then the method used to make chitosan from waste fish scales and crab shells including the stages of deproteinization, demineralization, and deacetylation. The differences in chitosan synthesis from waste fish scales and crab shells mainly depend on the extraction time, temperature, and concentration of NaOH and differences in the texture of the waste samples used. The results obtained are chitosan from fish scales and crab shells. Chitosan was obtained from waste fish scales (25.33-29.33%) and chitosan from crab shells (50%). From the research carried out it can be concluded that waste fish scales and crab shells can be converted into chitosan, the FT-IR results of chitosan from fish scales, crab shells, and commercial have different results, crab shell chitosan has better FT-IR results than commercial chitosan and fish scales.

Keywords: Chitosan, Crab Shells, Fish Scales, Kupang.

ABSTRAK

Sisik ikan merupakan hasil dari kegiatan perikanan yang dibuang begitu saja. Sisik ikan yang dibuang begitu saja dapat menjadi limbah yang memiliki dampak kurang baik terhadap lingkungan. Cangkang kepiting merupakan masalah yang perlu dicari pemecahannya. Salah satu pemanfaatan limbah cangkang kepiting tersebut adalah pengolahan menjadi kitosan. Tujuan dari penelitian ini adalah untuk mengetahui cara pembuatan kitosan dari limbah sisik ikan dan cangkang kepiting, dihitung rendemen dan kadar air-nya, kemudian metode yang digunakan dalam pembuatan kitosan dari limbah sisik ikan dan cangkang kepiting meliputi tahapan deprotenisasi, demineralisasi, dan deasetilasi. Perbedaan sintesis kitosan dari limbah sisik ikan dan cangkang kepiting terutama pada waktu ekstraksi, suhu dan konsentrasi NaOH

dan perbedaan tekstur sampel limbah yang digunakan. Hasil yang didapatkan berupa kitosan dari sisik ikan dan cangkang kepiting. Kitosan yang didapatkan dari limbah sisik ikan (25,33-29,33%) dan kitosan dari cangkang kepiting (50%). Dari penelitian yang dilakukan dapat disimpulkan bawa limbah sisik ikan dan cangkang kepiting dapat diubah menjadi kitosan, hasil FT-IR kitosan dari sisik ikan, cangkang kepiting dan komersil memiliki hasil yang berbeda, kitosan cangkang kepiting memiliki hasil FT-IR yang lebih bagus dari kitosan komersil dan sisik ikan.

Kata Kunci: Cangkang Kepiting, Kitosan, Kupang, Sisik Ikan.

INTRODUCTION

East Nusa Tenggara (NTT) Province is one of the provinces with quite high fishery resource potential so that it needs to be encouraged to accelerate the development of the marine economy, NTT has a sea area of $\pm 200,000$ km² with a coastline of 5,700 km². The fishery production owned includes those with high economic value such as Tuna, Skipjack, Mackerel, Grouper, Crab, Shrimp, Shellfish, Squid, Lobster, and Pangasius. NTT fishery production from 2017-2021 has increased, explained in Table 1.

Table 1. Fishery Production and Fish Consumption in NTT Province 2017-2021

Year	Fisheries Production (Tons)	Fish Consumption (%)
2017	1.840.355	39,73
2018	4.198.029	42,13
2019	3.116.772	46,26
2020	4.196.688	46,65
2021	4.388.896	47,07

processed data source: NTT Province Ministry of Marine Affairs and Fisheries, 2023

Fish consumption has increased from 2017-2021. High fish consumption produces by-products in the form of fishery waste such as fish scales, fish bones, and crustaceans such as crab shells, lobster shells, and shrimp shells. So far, the utilization of marine resources has only utilized the meat part which is made into processed products such as fish chips, shredded meat, meatballs, nuggets, penpek, and sausages. Waste is a by-product of the processing process or the remaining raw materials with very low quality, and if not managed or utilized properly will cause environmental pollution. Among these wastes, some are further utilized, namely fish scale waste and crab shell waste.

Fish scales are waste that has not been utilized optimally, where so far it has been used more as a source of collagen. By utilizing fish scale waste as a raw material for making chitosan, this has the potential to reduce water, air, and soil pollution. (Indah, 2019).

Crab shells contain the most chitosan compared to other crustaceans, chitosan in crab shells is 71% (Muzzarelli, 1985), which can be used to reduce environmental pollution. The compound contained in crab shells, namely chitin, is the top three of the most abundant polysaccharides found besides cellulose and starch (starch) which will produce chitosan (Sartika, 2016). Chitosan isolated from crab shells can be used as an adsorbent, heavy metal waste and dyes, preservatives, antifungals, cosmetics, pharmaceuticals, flocculants, anticancer, and antibacterial. Chitin is the second largest natural biopolymer found in nature after cellulose. Chitin can be obtained from arthropods, fungi, and yeast, but the most important commercial source comes from crab exoskeletons. The chitin content in shrimp shells ranges from 42% -

57%, while in crab shells it reaches 50% -60%. Chitin is soluble in concentrated mineral acids such as HCl, HNO₃, and H₂SO₄, while chitosan can be obtained through the conversion of chitin obtained by converting chitin (Sartika 2016).

The purpose of this study was to determine the manufacture of chitosan from fish scale waste and crab shells, a comparison of the properties of chitosan from fish scale waste and crab shell waste. And to provide information to the public about the utilization of fishery waste into chitosan.

RESEARCH METHODS

Time and Place

This research was conducted for 4 months starting from July to October 2023. This research was conducted at the Fisheries Laboratory, Biology Laboratory, Muhammadiyah University of Kupang and LPPT UGM Yogyakarta.

Tools and materials

a. Tool

The tools used in this research are glassware (beaker glass, glass measuring , dropper, thermometer , stirrer glass , magnetic stirrer , bar stirrer , oven, scales), blender, and KBr powder

b. Ingredients

The materials used in this study were: fish scales , shell crab , sour chloride (HCl) 1M, NaOH, distilled water , paper filter , paper litmus .

c. Research Variables

In this study, there are two variables used in the chitosan manufacturing process, namely:

- a. Concentration of 5% NaOH solution
- b. Solvent volume 100 ml

Research Procedures

1. Initial Preparation

Fish scales and crab shells are washed until clean, then dried in the sun until completely dry.

2. Stage Making Chitin

a. Sample Collection

The collection of fish scale and crab shell waste is done collectively, which comes from the Liliba fish burning place and the Kupang City Night Market. After the fish scale and crab shell waste is collected, the next stage is to separate the fish scale and crab shell waste.

b. Initial Preparation

Fish scales and crab shells are cleaned with distilled water and dried under the sun until dry. The weight of the raw material of fish scales is 500 grams and crab shells are 500 grams of dry samples.

c. Stage Making Chitin

1) Deproteination

This process is carried out at a temperature of 65°C using a 5% NaOH solution, as much as 100 mL, with a ratio of fish scales to NaOH = 1:10 (grams of scales / mL of NaOH). How to make a NaOH solution at the deproteinization stage by weighing NaOH as much as the specified ratio then mixed with distilled water and inserting fish scale or crab shell samples then stirred for 2 hours and

the crab shell is deproteinized at a temperature of 75-80°C using a 1 M NaOH solution, with a ratio of 1:10 (grams of powder / ml) NaOH is stirred for 60 minutes, then the mixture is filtered using filter paper. The precipitate obtained is then washed with distilled water until it reaches a neutral pH, and then dried.

2) Demineralization

To remove minerals, 100 mL of 0.5N HCl was added with a ratio of fish scales after the deproteinization process using NaOH, which is 1:10 (grams of powder/ml of NaOH). How to make an HCl solution at the demineralization stage is to weigh the fish scales or crab shells from deproteinization then add HCl as much as the ratio (HCl is adjusted to the results of deproteinization). Then put it in a beaker glass, and soaked at 30°C (room temperature) for 30 minutes. Then in the crab shell at this stage the temperature used is 25-30°C using a 2 M solution with a ratio of sample and HCl solution of 1:10 (grams of powder/ml of HCl). The results obtained were filtered using filter paper, then washed with distilled water until they reached a neutral pH. The solid obtained was dried again at room temperature for one day. This process produces a material called chitin.

3) Deacetylation

Chitin from fish scales was put into a beaker glass, added with 50 ml of 10% NaOH solution and heated at 100°C while stirring for 2 hours. The chitin solution was filtered and washed until the pH was neutral and dried at 30°C (room temperature) for one day (Susanti & Purnawati, 2020). Then chitin from crab shells was put into a NaOH solution with a concentration of 20% as much as 50 ml and heated at 90-100°C while stirring for 60 minutes. The results in the form of slurry (suspension), were filtered and then washed with distilled water until the pH was neutral. The results obtained at the deacetylation stage are called chitosan (Sari & Abdiani, 2015).

The resulting chitosan was weighed and then analyzed for water content, yield, and degree of deacetylation. Furthermore, the obtained chitosan was analyzed using FTIR to determine the Degree of Deacetylation (DD) (Susanti & Purwanti, 2019).

Table 2. Chitosan indicators in fish scales and whiting shells.

Research Procedures	Stage	Indicator Achievement
Making Chitin	Deproteinase	Chitin with less content
	Demineralization	Chitin with less mineral content
Making Chitosan	FTIR Spectrum Characteristics	Obtained group function that proves obtained product chitosan

Data analysis

1. Water content

- On Raw Materials

This test was conducted using the gravimetric method, namely by weighing the weight of 10 grams of finely ground fish scales. Furthermore, the drying process was carried out using an oven at a temperature of 105°C for 30 minutes. After that, the fish scales were put into a desiccator for 5 minutes before being weighed again. This process was repeated until a constant weight was obtained.

- On Products Chitosan

Chitosan products were dried using an oven at a temperature of 105°C for 30 minutes. After that, chitosan was put into a desiccator for 5 minutes before being weighed again. This process was repeated until a constant weight was obtained. The water content can be calculated using the following formula:

$$\text{Water content (\%)} = \frac{\text{initial sample weight} - \text{final sample weight}}{\text{Initial sample weight}} \times 100\%$$

2. Rendement Analysis

The chitosan that has been produced is analyzed to determine the rendement. The analysis of chitosan rendement can be calculated using the following formula :

$$\text{Rendement : (\%)} = \frac{A}{B} \times 100\%$$

With: A = Weight of Chitosan dry result water free (grams)

B = Weight of Chitin dry water free (grams)

3. Analysis Degrees Deacetylation

Determination Degrees Deacetylation (DD) by IR spectroscopy was performed use Domszy & Robert's baseline method (Khan *et al.*, 2002) by recording peak highest and measure the selected baseline. The formula for calculating the baseline is:

$$DD = 100 - \left[\frac{A_{1655}}{A_{3450}} \times \frac{100}{1.33} \right]$$

With:

A₁₆₅₅ : Absorbance at the number wave 1655 cm⁻¹ which shows absorption carbonyl from amide .

A₄₃₅₀ : Absorbance number wave 3450 cm⁻¹ which shows absorption hydroxyl and used as an internal standard.

Factor 1.33: Comparative value $\frac{A_{1655}}{A_{3450}}$ for 100% deacetylated chitosan.

RESULTS

Synthesis Chitosan

Synthesis results from waste fish scales and shells crab presented in tables 3 and 4 below:

Table 3. Chitosan Synthesis Results from Waste Fish scales :

Weight in each stage Synthesis (g)	Test	
	1	2
Initial Sample (g)	30 (g)	30 (g)
Deproteinization (g)	20.5 (g)	25.8 (g)
Demineralization (g) (chitin g)	8.8 (g)	9.3 (g)
Deacetylation (g) (chitosan g)	7.6 (g)	8.8 (g)
% rendement	25.33 (g)	29.33%

Source: Data processed 2023

Table 4. Chitosan Synthesis Results from Shell Crab

Weight in each stage Synthesis (g)	Test	
	1	2
Initial Sample (g)	20 (g)	20 (g)
Deproteinization (g)	24.4 (g)	24.7 (g)
Demineralization (g) (chitin g)	11.8 (g)	12.6 (g)
Deacetylation (g) (chitosan g)	10.0 (g)	10.0 (g)
% yield	50%	50%

Source : Data processed 2023

Water content analysis

The chitosan product is weighed and then dried using an oven at a temperature of 110°C for 15 minutes. Then let it stand until the chitosan is completely cool. Then weighed. The water content can be calculated using the following formula:

$$\text{Water Content \%} = \frac{\text{initial sample weight} - \text{final sample weight}}{\text{final sample weight}} \times 100\%$$

Deacetylation Degree Analysis

The results of the study showed that the degree of deacetylation of chitosan using NaOH p.a for fish scale chitosan could not be known because the IR spectrum wave could not be calculated, this is the same as the Degree of Deacetylation for fish scale chitosan using technical NaOH, while in crab shell chitosan using NaOH p.a (DD) 44.5% and in crab shell chitosan using technical NaOH (DD) 53.5%, then in commercial chitosan (DD) 53.5%. DD is calculated manually.

FTIR spectrum

a. FTIR Spectrum of Chitosan

After characterization of chitosan using FTIR spectrophotometer, IR spectrum was obtained for three types of chitosan, namely fish scale chitosan, crab shell chitosan and compared with commercial chitosan. The spectrum results can be seen in the image below.

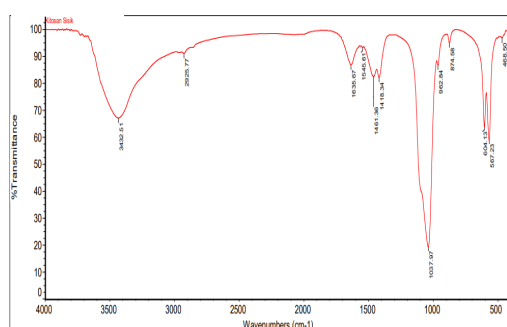


Figure 1. IR spectrum of chitosan Fish Scales

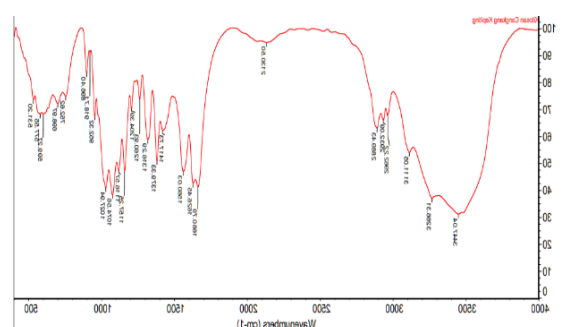


Figure 2. IR Spectrum of Chitosan Shell Crab

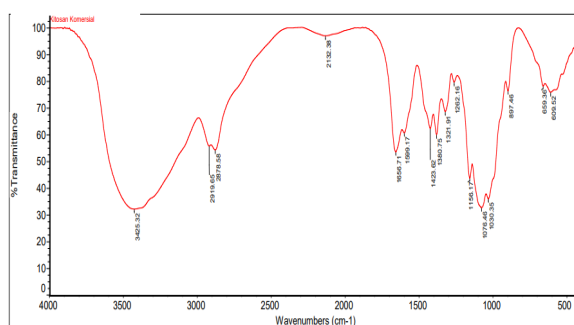


Figure 3. IR spectrum of chitosan Commercial

b. FTIR Spectrum of Technical Chitosan

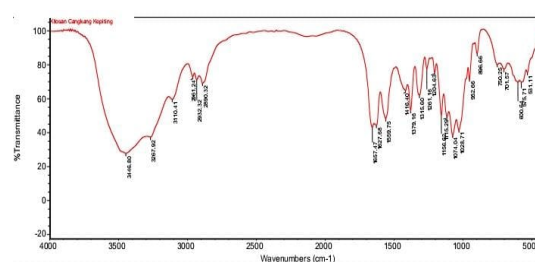
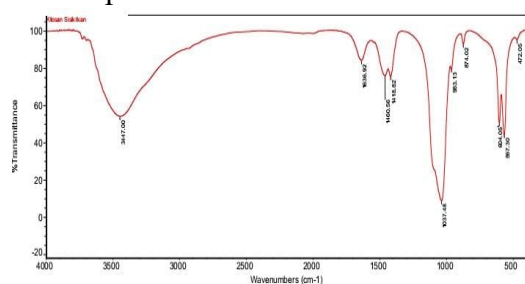


Figure 4. IR spectrum of chitosan Figure 5. IR spectrum of chitosan Shell Crab

DISCUSSION

Synthesis Chitosan

The demineralization stage is the process of removing inorganic salts and mineral content contained in the sample will be removed through the demineralization process. After that, the fish scale and crab shell samples that have gone through the demineralization stage will proceed to the deproteinization stage, which is the process of breaking the protein bonds in chitin. In the deproteinization stage, a reaction occurs to release the protein bound to chitin, where the protein binds to Na^+ from NaOH so that Na-protein is formed, which is marked by the thickening of the solution during the reaction.

The results of the study show that chitosan can be synthesized from fish scale and crab shell waste. The synthesis of chitosan from these two samples generally goes through the same stages, namely deproteinization, demineralization, and deacetylation. The only difference occurs in the length of time, temperature and concentration of NaOH solution at each stage. The difference in length of time and temperature is caused by differences in sample texture. The fish scale waste sample is thinner and lighter, while the crab shell has a thick and hard shell so that at each stage of synthesis there is a difference in time and temperature.

At the stage of deproteinization of fish scale waste, the time is 2 hours while stirring and the temperature is 65°C , at the stage of demineralization, the time used to soak or settle the deprotein results is 30 minutes and the temperature is 30°C and in the deacetylation process, the time is 2 hours while stirring at a temperature of 90°C , the yield results reach 25.33-29.33%. While in crab shells, before carrying out the deproteinization stage, the crab shells are first blended finely because crab shells have a thick and hard texture, after being blended finely, it is continued with the deproteinization stage for 60 minutes while stirring at a temperature of $75-80^\circ\text{C}$, then at the stage of demineralization for 120 minutes while stirring at a temperature of $25-30^\circ\text{C}$ then at the stage of deacetylation for 60 minutes while stirring at a temperature of 95°C , the % yield produced is around 50%.

In line with the opinion of Susanti and Purwati (2020), the longer the extraction process, the longer the NaOH reaction reduces the acetyl group in chitin, so that the yield of chitosan obtained is less, but the quality of chitosan is better. The resulting chitosan can be used as a preservative in fishery products. According to Putra & Rr (2015), the effectiveness of the deproteination process depends on the concentration of NaOH used; the higher the concentration used, the cleavage of intermolecular hydrogen bonds between chitin and protein not only occurs, but it is also suspected that the cleavage of acetyl groups occurs.

Water Content Analysis

The average water content of chitosan from fish scales and crab shells using NaOH p.a. is 4.24% for fish scale chitosan and 64% for crab shell chitosan, then chitosan from fish scales and crab shells using technical NaOH is 11.92% for fish scale chitosan and 66.67% for crab shell chitosan, in addition to chitosan using NaOH p.a. and technical NaOH, there is also commercial chitosan with an average water content of 6.36%.

FTIR spectrum

a. FTIR Spectrum of Chitosan p.a

The IR spectrum image of fish scale chitosan (figure 1) shows that chitosan has a structure consisting of carbon, hydrogen, hydroxyl groups (OH), alkenes (C-H₂), amines (C-N) and alcohols, ethers (C-O). The FTIR spectrum of chitosan from immersion shows the presence of functional groups (O-H) at wave numbers around 3200-3500, namely at number 3432, this is the wave (O-H) in fish scale chitosan. The C-H₂ functional group is shown in the 3500-3100 and 1640-1550 regions, namely at wave 1635, in fish scale chitosan, the functional group is shown in the (C-H) 3000-2850 region, namely at number 1418, in fish scale chitosan, the (C-N) group is shown in the 1350-1000 region, namely at number 1080 in fish scale chitosan, while the (C-O) functional group is shown in the 1300-1000 region at number 1037, in fish scale chitosan (Widyatusi *et al.*, 2021).

The IR spectrum image of crab shell chitosan (figure 2) shows that chitosan has a structure consisting of carbon, hydrogen, hydroxyl groups (OH), alkenes (C-H₂), amines (C-N) and alcohols, ethers (C-O). The FTIR spectrum of chitosan from immersion shows the presence of functional groups (O-H) at wave numbers around 3500-3200, namely at number 3447, this is the wave (O-H) in crab shell chitosan. The C-H₂ functional group is shown in the 3500-3100 and 1640-1550 regions, namely at wave 1626 in crab shell chitosan, the functional group is shown in the (C-H) 3000-2850 region, namely at number 1417, in crab shell chitosan, the (C-N) group is shown in the 1350-1000 region, namely at number 1074 in crab shell chitosan, while the (CO) functional group is shown in the 1300-1000 region at number 1074, in crab shell chitosan.

From the IR spectrum image of commercial chitosan (figure 3) it shows that chitosan has a structure consisting of carbon, hydrogen, hydroxyl groups (OH), alkenes (C-H₂), amines (C-N) and alcohols, ethers (C-O). The FTIR spectrum of chitosan from immersion shows the presence of functional groups (O-H) at wave numbers around 3500-3200, namely at number 3425, this is the wave (O-H) in commercial chitosan. The C-H₂ functional group is shown in the 3500-3100 and 1640-1550 regions, namely at wave 1599 in commercial chitosan, the functional group is shown in the (C-H) region 3000-2850 cm⁻¹, namely at the number 1423 cm⁻¹, in commercial chitosan, the (C-N) group is shown in the 1350-1000 cm⁻¹ region, namely at the number 1076 cm⁻¹, in commercial chitosan, while the (C-O) functional group is shown in the 1300-1000 region at the number 1076, in commercial chitosan.

b. FTIR Spectrum of Technical Chitosan

The IR spectrum image of fish scale chitosan (figure 4) shows that chitosan has a structure consisting of carbon, hydrogen, hydroxyl groups (OH), alkenes (C-H₂), amines (C-N) and alcohols, ethers (C-O). The FTIR spectrum of chitosan from immersion shows the presence of functional groups (O-H) at wave numbers around 3200-3500, namely at number 3447, this is the wave (O-H) in fish scale chitosan. The C-H₂ functional group is shown in the 3500-3100 and 1640-1550 regions, namely at wave 1636, in fish scale chitosan, the functional group is shown in the (C-H) 3000-2850 region, namely at number 1460, in fish scale chitosan, the (C-N) group is shown in the 1350-1000 region, namely at number 1418 in fish scale chitosan, while the (C-O) functional group is shown in the 1300-1000 region at number 1037, in fish scale chitosan (Setiawan *et al.*, 2021).

The IR spectrum image of crab shell chitosan (figure 5) shows that chitosan has a structure consisting of carbon, hydrogen, hydroxyl groups (OH), alkenes (C-H₂), amines (C-N) and alcohols, ethers (C-O). The FTIR spectrum of chitosan from immersion shows the presence of functional groups (O-H) at wave numbers around 3500-3200, namely at number 3446, this is the wave (O-H) in crab shell chitosan. The C-H₂ functional group is shown in the 3500-3100 and 1640-1550 regions, namely at wave 1657, in crab shell chitosan, the functional group is shown in the (C-H) 3000-2850 region, namely at number 1379, in crab shell chitosan, the (C-N) group is shown in the 1350-1000 region, namely at number 1074 in crab shell chitosan, while the (CO) functional group is shown in the 1300-1000 region at number 1074, in crab shell chitosan.

CONCLUSION

Based on the results of the study and analysis of observation data, the following conclusions can be drawn:

1. Chitosan can be synthesized from fish scale and crab shell waste through three stages, namely Deproteinization aims to remove proteins found in fish scales and crab shells. Demineralization aims to remove minerals contained in fish scales and crab shells. While deacetylation aims to remove acetyl groups contained in chitin, thus forming chitosan.
2. Characterization of chitosan using an FT-IR spectrophotometer obtained IR spectra for three types of chitosan, namely fish scale chitosan, crab shell chitosan and commercial chitosan. Crab shell chitosan has better FT-IR results than commercial chitosan and fish scale chitosan.
3. From the research that has been done, it can be concluded that chitosan obtained from fish scale waste is 25.33-35% lower than crab shell chitosan 47-50%.

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