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Characterization of Physicochemical Properties of Chitosan Coconut Crabs Shells (*Birgus latro*)

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***Abstract*—The coconut crab, or in the language of North Maluku called coconut crab, is one of the biological resources with high economic value. People consume coconut crab dishes that have a flavor similar to lobster. The utilization of coconut crab shells has not been done. At the same time, this study aims to isolate and characterize chitosan coconut crab shells by deacetylation method using different concentrations of NaOH and different heating times. The results of the best chitosan synthesis with a variation of 15% NaOH concentration, 120 minutes of heating included the characterization of FTIR spectrum DD values (89.15%) so that there was an increase in crystallinity in each peak spectrum of 3448 cm^{-1} area that binds hydrogen bonds (O-H). Likewise, for molecular weight (368 kDa), the morphology of chitosan CC is shown using SEM, which shows the similarity of the structure of the microfibrils structure. In comparison, the XRD pattern shows distinctive characteristics of peak crystallinity. Likewise, the quality of water content, ash, protein, fat, color of chitosan CC is under the standards of Protan Biopolymer. Chitosan CC micrograph has the same shape of microfibril structure due to the heating process. While the average crystal peak is 19.2-29.4 ^{O}, the best treatment recommended in this study is the deacetylation process using a 10% NaOH concentration with a heating time of 120 minutes. Thus chitosan, which has the best characterization value, is used as a polymer that can be used in the food sector.**

***Keywords*— Chitosan; coconut crab; SEM; XRD; North Maluku.**

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I. INTRODUCTION

The coconut crab (*Birgus latro*) is one of the biological resources that have high economic value. Coconut crabs are one of the decapod groups that spend a lot of time on land. Coconut crabs in North Maluku people call walnut crabs to have different names in each region. Even the efforts of the mass cultivation of coconut crabs in several islands in the North Maluku region such as in the villages of Posi, Patani, Gebe in Central and South Halmahera Districts, Tobololo Village in Ternate, and Samada Island in Pulau Taliabu District [1]. Moreover, a preliminary study was carried out breeding coconut crabs in North Maluku as an initial step in the cultivation of coconut crabs [2]. The utilization of coconut crab shells has not been utilized properly. Unlike mangrove crab, shrimp shells, and crab shells, lobster shells have been widely studied as sources of chitin and chitosan [3].

The application of chitosan is one of the polysaccharides used as a coating on medicines and foodstuffs, especially in the manufacture of the film [4]. Chitosan is the most studied polysaccharide as a coating by giving an overview of the characteristics of the chitosan layer [5]. Conventionally, chitosan is found in many types of crustaceans, especially in shrimp shells developed into nanomaterials sourced from alternative biotechnology products tailored to the industry's needs [6]. Furthermore, chitosan nanocomposites were developed as drug delivery [7].

The source of polysaccharides from crustaceans as biomaterials other than cellulose is in the form of chitosan [8]. Even chitosan is a compound produced by deacetylation from chitin by determining the value of the degree of deacetylation (DD), which varies according to the source of the raw material and its treatment method [9,10]. Furthermore, the chitosan preparation process to get the characteristics of chitosan is the main parameter is the value of the degree of deacetylation and

molecular weight determine the quality of chitosan [11]. Therefore, chitin isolation from mangrove crab shells has been carried out through the deproteination and demineralization process and the transformation of chitin into chitosan by the deacetylation process. In comparison, chitosan is a compound of the result of chitin deacetylation by determining the degree of deacetylation (DD), which varies according to the source of the raw material and its treatment method. The chitosan quality requirements obtained from crustacean waste have a DD value of 70% and some even reach 100%; several studies report that the source of chitosan from research results with DD values has good quality standards [12]. The increase in the value of the degree of deacetylation is produced through NaOH concentration, reaction temperature, and reaction time [13].

The protein content removal method uses deproteinization by dissolving crab shell powder into NaOH solution while heated [14]. Furthermore, remove the mineral content by demineralization method into the HCl solution, then proceed with removing the acetyl group on chitin using NaOH. Chitosan composition consists of 2-amino-2-deoxy-β-D-glucose [15].

In this study, how to prepare coconut crab shells before and after heating which has the best physical and chemical properties, and how to isolate and characterize chitosan coconut crab shells as chitosan coconut crabs (chitosan CC) products. In comparison, the purpose of this study is to prepare the waste of coconut crab shells before and after heating with the best physical and chemical properties and to isolate and characterize chitosan coconut crab shells as chitosan products based on the value of the degree of deacetylation using different NaOH concentrations and heating times.

II. Material and Method

A. Materials

The materials used in this study were coconut crab shells obtained from 2 locations in North Maluku (traditional markets of Patani District and Restaurants in Ternate City), NaOH 1 M, HCl 2 M, aquadest, and proximate analysis chemicals. The tools used in this study are a set of ball-mill, oven, Ohaus analytical balance, pH meter, stopwatch, cabinet drying, thermometer, 200 mesh sieve. Analyzer in the form of Fourier-transform infrared spectroscopy (FT-IR) (Nicolet ™ iS ™ 5 TFS Inc.), crystallinity test with X-ray Diffraction (X-RD) (Rigaku Corp; Japan), micrograph analysis with Scanning Electron Microscopy (SEM)) (Shimadzu, SS 550), Metal content analysis namely X-Ray Fluorescence (XRF) (Philips Magix Pro XRF), Color reader (Minolta Camera Co., Ltd), as well as proximate analysis tools.

B. Purification of Chitosan Coconut Crab

Chitin isolation process was carried out starting from handling raw material of coconut crab shells to become powder, then continued with demineralization stages with 3.5% NaOH, deproteination stage with 1.5 M HCl, and deacetylation with NaOH [16]. Furthermore, the transformation of chitin into chitosan is carried out by the deacetylation method. A total of 4 g chitin was deacetylated with sodium hydroxide (1 M NaOH): 1, 3, 5 (% w/v) in the reflux flask. Furthermore, heat using a magnetic heating stirrer at 105 ^{0}C for (60 and 120 minutes) while stirring with a magnetic stirrer. After cooling, the solid is filtered off, and the residue left behind is washed with aquadest. Then the solids obtained were in the form of chitosan and dried in an oven at 60 ^{0}C for 24 hours [14], [17].

C. Physicochemical Properties of Chitosan

1) Yield: The yield of powder and chitosan coconut crab shell is calculated based on the ratio between the weight of the powder and the weight of chitosan with the weight of the coconut crab shell waste or the weight of chitin before deacetylation into chitosan, using the following equation:

$$Rendemen\ (\%) = \frac{\text{Powder weight / Weight of chitosan (g)}}{\text{The weight of a coconut crab shell (g)}} x\ 100 \qquad (1)$$

2) Chemical composition: Determination of water content (AOAC 930.15) and ash (AOAC 942.05) in coconut crab powder and chitosan according to standard methods [18]. The fat content using dry samples, namely powder and chitosan, was determined gravimetrically after the Soxhlet method was extracted using hexane. Moreover, the powder and chitosan protein content was determined by extracting the sample with 10 (% w/v) NaOH for 20 minutes at 120 ^{0}C. The filtration process is carried out at the supernatant and diluted with distilled water to 100 ml. According to the Kjeldahl procedure, this extract was used for protein determination (total nitrogen content × 6.25)—carbohydrates (by difference) [19].

3) Viscosity: Chitosan viscosity was measured using the Ubbelohde dilution viscometer. Chitosan solution was made in various concentrations in 0.1 M acetic acid solvent and 0.25 M sodium acetate. Each sample was placed in a 10 ml viscometer. The sample is pulled up to the flask at the top of the viscometer slowly. The time taken for the sample to flow between the two boundaries flanking the flask is recorded. As blanks, 0.1 M acetic acid solvent and 0.25 M sodium acetate were used, and the viscosity was determined in the same way [20]. Specific viscosity is calculated in the following way:

$$\eta = \frac{T - t0}{t0} \qquad (2)$$

η_{cp}= Specific viscosity (seconds)
T = the time required for the flow of sample solution (seconds)
t_0 = the time needed for the flow of the solvent solution (seconds)

4) Solubility: The calculation of the solubility value of coconut crab chitosan was determined according to [21]. Where 0.1 g chitosan was put into a centrifuge tube, then dissolved in 10 ml of 1% acetic acid for 30 minutes at 25 ^{0}C and stirred continuously. Then the solution obtained is heated for 10 minutes. Furthermore, the suspension is cooled using normal (27 ^{0}C), followed by 6000 rpm centrifugation for 15 minutes (MPW-350 R centrifuge), obtained a supernatant. The pellets are dissolved in distilled water (25 ml) and centrifuged at 8000 rpm. The supernatant is released, and the insoluble pellet is dried at 50 ^{0}C, ± 24 hours. Calculation of chitosan solubility value is based on the equation below:

$$\text{Solubility}\ (\%) = \frac{W1-W2}{W1-W0} x\ 100 \qquad (3)$$

Note: where W1 and W2 are the initial weight of the tube + chitosan and the final weight of the tube + chitosan, and W0 is the initial mass of the tube [20].

5) Antioxidant Activity: Testing the antioxidant activity of coconut crab powder and chitosan using the DPPH method according to the method [22]. In short, 1 mL of each sample was mixed with 3 mL of DPPH (3 M) methanol solution. The reaction mixture is vortexed, and 30 minutes incubated. The measured absorbance value of the solution at a wavelength of 517 nm. A standard solution in the form of ascorbic acid is used [10]. The percentage of DPPH inhibition the calculation is based on the equation below.

$$\text{Antioxidant Activity (\%)} = \frac{Abs\ \text{sampel}}{\text{Abs control}}\ x\ 100 \qquad (4)$$

6) Color: The color measurements of powder and chitosan coconut crab shells were measured with CR 310 Minolta Chroma Meter (Minolta Camera Co., Ltd) with color values (L*, a*, b*). Powder and chitosan are placed on a standard white plate (calibration plate), and the Hunter Lab color scale is used to measure color [24]. Each sample is measured at four different reading positions.

7) Molecular Weight (BM) Chitosan: Chitosan molecular weight is one parameter that can be used as a quality standard because its molecular weight low is suitable for use as an antibacterial, antioxidant, and anti-tumor agent. Chitosan having medium weight molecular has higher anti-cholesterol activity compared to high molecular weight Chitosan [24]. Intrinsic viscosity [η] of the chitosan made was determined by dissolving the chitosan 0.5 M acetic acid 0.5 M sodium acetate buffer, and the viscosity measurement was carried out at 25 ^{0}C. The average weight viscosity of chitosan (Mv) was determined using the Mark-Houwink equation:

$$[\eta] = K[MV]a \qquad (5)$$

D. Characterization of Coconut Crab Chitosan

1) Fourier-transform infrared spectroscopy Chitosan: Fourier Transform Infra-Red (FT-IR) spectrum of chitosan produced using the deacetylation method using a modified method [25]. Characterization of samples using FT-IR using KBr pellets in the scanning range of 400-4000 cm^{-1} (FTIR-8400S, Shimadzu). Before obtaining the spectrum, the KBr pellet was prepared (1 mg chitosan with 100 mg KBr) and stabilized under relative humidity control. The measurement waveforms obtained are compared with standard chitosan spectrum wave numbers [24].

$$\text{DD\%} = 100 - \left[\left(\frac{A1655}{A3450}\right) x\ \frac{100}{1.33}\right] \qquad (6)$$

2) Scanning Electron Microscopy Chitosan: Testing of coconut crab powder and chitosan using Scanning Electron Microscope (SEM) (Hitachi High Technologies) has a magnification range of 1000-5000 times and an acceleration voltage of 20 kV. The average particle diameter was calculated by SEM image analysis using Meter © software size, version 1.1, with the differentiation threshold set according to the image scale [23].

3) X-Ray Diffraction Chitosan: Characterization of chitosan samples using XRD spectra with X-ray diffraction (X-RD) techniques using a diffractometer (XRD-7000, Shimadzu,) using radiation (k = 40 kV, 30 mA). The results of X-ray diffractometers can show high vertical goniometer results and can accept up to a maximum value of up to 400 mm (w) × 5500 mm (d) × 400 mm (h). Measurements were taken with a scan range of 5–80 at a scanning speed of 50 d − *I*. To determine the crystallinity index (Cr*I*) you can use the following equation:

$$\text{CrI (\%)} = \frac{[I110 - Iam]}{I110}\ x\ 100 \qquad (7)$$

Where I_{110} is the maximum intensity at 2θ ≈20^o. *I*am is the amorphous diffraction intensity at 2θ≈10^o [26].

E. Statistical Analysis

Data were evaluated using a one-way analysis of variance (ANOVA), and significant differences were analyzed by DMRT test (P <0.05).

III. Result and Discussion

A. Coconut Crab Chitosan Preparation

Purification of coconut crab chitin using acidic and alkaline compounds produces pure chitin which is 76.48 - 78.78%. The results of pure CC chitosan powder obtained from chitin purification by deacetylation method using different NaOH concentration treatments with different heating times obtained values of degrees of deacetylation (DD) in the range 81.72-89.15%. Therefore, DD chitosan CC value with a concentration of 15% NaOH and a heating time of 120 minutes has a higher DD value reaching 89.15%. Conversely, a short heating time of 60 minutes with 5% NaOH concentration has a fairly low DD value of 81.72%. This shows that the heating process will cause many components to be degraded by NaOH. However, the different heating durations and NaOH concentrations did not significantly influence the chitosan DD value (P <0.05) and meet the chitosan standard with a DD value> 70%. Likewise, the heating of 120 minutes causes an increase in the long enough reaction so that the value of DD chitosan in crab shell waste has a DD value of 93.3% [14].

B. Fourier-transform infrared spectroscopy Chitosan

Figure 1 shows the FTIR spectrum there is a diffraction pattern that appears new peaks at other angles in the deacetylation process using different NaOH concentrations and heating times. This shows that each spectrum shows a diffraction angle which is a group of chitosan coconut crabs. So it can be concluded that the results of the demineralization, deproteinization and deacetylation stages can reduce the protein groups found in the coconut crab shells. Based on the results of calculations from the FTIR graph, the value of DD chitosan CC for all treatments> 80%, and the highest DD value in the treatment of 120 minutes heating with 15% NaOH concentration. Besides that, the characteristics of chitosan lobster shells have FTIR spectrum at the peak of the area of 1590 cm^{-1} due to N-H bending from the secondary amide II band -CONH- [19].

In Figure 4.A and B, the FTIR spectrum describes the spectral characteristics of the chitosan CC polymer particles from the method of deacetylation of chitin to chitosan. FTIR chitosan CC spectra as shown in Figure 5 shows that chitosan CC has a relatively high deacetylation rate (89% DD). In the

spectrum of CC chitosan, strong peaks and widths in the area of 3448 cm^{-1} are associated with vibrations that bind to hydrogen bonds (O-H). In addition, there is overlap in the same peak region in type 2 amides and primary amine groups from above the N-H region. On the other hand, the asim peak stretching area (C-O-C) at the peak of 1082 cm^{-1} and CH at the peak of 1418 cm^{-1} belongs to the C-N vibrational strain at its peak. The release of acetyl groups from chitosan causes positively charged chitosan, which can bind negatively charged compounds such as proteins and polysaccharide anions to form neutral ions [28].

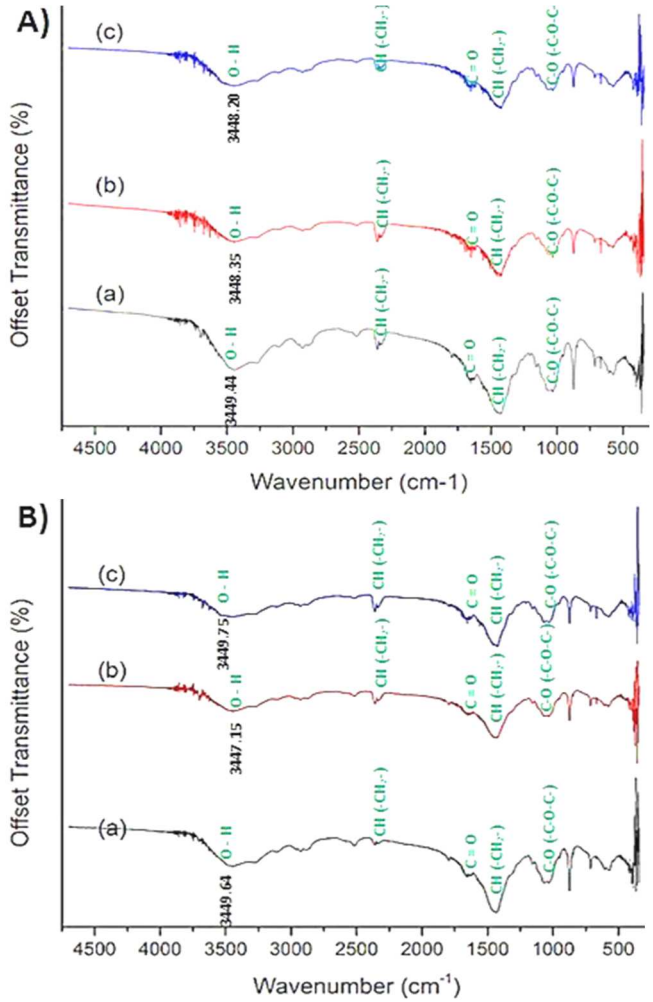

Fig. 1 FT-IR Spectra Chitosan Coconut Crab (CsCC). A). Deacetylation Long heating 60 minutes. B). Deacetylation Old Heating 120 minutes. a) 5% NaOH, b) 10% NaOH, c) 15% NaOH

C. *Degree of Deacetylation*

In Table 1, in general, the DD value of CC chitosan produced between 81.72-89.15%, this can be seen from the increasing value of the concentration of NaOH and the heating temperature during the deacetylation process. NaOH concentration is very influential in the stages of deacetylation. The higher the concentration of NaOH 15% and the heating time of 120 minutes will increase the DD value. The relatively high concentration of NaOH during deproteinization will also facilitate the termination of the acetyl group during the deacetylation process of chitin CC. Likewise, the demineralization process can reduce the acetyl group in the coconut crab shell. Therefore the release of acetyl groups in the production of chitosan will cause the content of positively charged chitosan to increase negatively charged compounds, such as polysaccharide anions and proteins that form neutral ions. Likewise, the optimum deacetylation rate will be obtained if the NaOH concentration used is 50 (% w/v), resulting in better deacetylation if deacetylation will be carried out at low temperatures [29].

The value of the degree of deacetylation (DD) in chitosan CC is the percentage of the acetyl group that must be removed from chitin CC. Table 3 shows that the higher concentration of NaOH and the use of heating time will result in a higher DD, i.e. at a concentration of 15% NaOH with a heating time of 120 minutes, it will produce a DD value of 89.15%. This shows that a high DD value shows high chitosan content, and the acetyl group contained in chitosan is getting lower. Therefore, the acetyl group in CC chitosan has a strong interaction between hydrogen and ionic bonds. Likewise, the content of chitosan CC obtained through the deacetylation process is influenced by several factors, including process temperature and NaOH concentration. DD value of chitosan crab shell with 120 minutes heating treatment can increase the effective reaction time by a value of 93.3%, compared to DD values with heating 60 and 90 minutes [14].

NaOH solution with a high concentration (15% w / v) has a role to break the bonds between carboxyl groups with nitrogen atoms from chitin which has a thick and long crystalline structure, Besides, the functional groups in the form of amino groups (NH_3^+) influence substitution of acetyl chitin groups in the solution will be more active so that the process of deacetylation of chitin to chitosan cc will be more perfect. On the other hand, the chitosan quality standard of various crustacean shells with a perfect deacetylation process has been set by Protan Biopolymer, which is ≥70% [29]. Likewise, the DD value depends on the chitin source and the morphology of the initial raw material consisting of a hydrogen-bonding network [12]. The difficulty of producing chitosan with a high degree of deacetylation is suspected because chitin is naturally shaped crystalline containing chitin polymer chains with a very high density. Each other is bound by very strong hydrogen bonds, thus preventing NaOH from reaching its specific group [28]. Therefore, to obtain chitosan, it is sometimes carried out repeatedly or stratified deacetylation with the consequences of depolymerization, which affects the intrinsic viscosity and molecular weight (BM) of chitosan produced.

D. Molecular Weight

The average molecular weight (Mw) of chitosan CC in the treatment of NaOH concentration and heating time is in the range 280-368 kDa, and there is a decrease in Mw along with the heating time (120 minutes) with high NaOH concentrations (15%) (Table 1). Conversely an increase in Mw at a heating time of 60 minutes with a 5% NaOH concentration. However, chitosan with Mw medium value (30 kDa) turned out to have advantages in the form of anti-cholesterol activity which was quite high compared to chitosan which had high Mw (250 kDa) [30]. While chitosan production from shrimp shell waste can produce Mw chitosan an average of 4.2 x 103 with deacetylation stages using 60% (w/v) NaOH solution and carried out enzymatically using

chitin enzymes resulting from ammonium sulfate precipitation.

E. Yield

The chitosan CC powder yield in this research is shown in Figure 3. Chitosan CC was obtained from the deacetylation results of chitin CC, namely removing the acetyl group. The use of sodium hydroxide (NaOH) in this deacetylation process uses concentrations of 5, 10, 15 (% w / v) with a heating time of 60 and 120 minutes at a temperature of 80 ^{0}C. Therefore, the CC chitosan yield values presented in Table 1 show an 80.63–84.22% average yield. The increase in the yield of the lowest NaOH concentration (5%) with a heating time of 60 and 120 minutes showed the lowest yield. The Mw chitosan CC value influences this. The use of high NaOH concentrations will result in the depolymerization of chitosan molecular chains, which will ultimately cause a decrease in Mw chitosan CC. Conversely, an increase in NaOH concentration and the use of temperature can affect the decrease in the value of chitosan CC. Likewise, the yield and molecular weight (Mw) have a close relationship [31]. Meanwhile, chitosan polymers consist of 2-amino-β-D-glucose obtained from chitin processing using a concentrated base solution [32].

F. Chitosan CC Quality

This study showed that the quality of chitosan CC obtained under the standard quality of chitosan, in general, can be seen in Table 2. The levels of chitosan CC with different NaOH concentrations and heating times during the deacetylation process showed an average of 9.18 - 10.12%. The test results (P> 0.05) did not show a significant difference between treatments. The water content of chitosan CC has a low water content because the drying time factor influences it, the surface area of the dryer uses a drying cabinet for 6-8 hours at 60 ^{0}C, and the number of chitosan CC with a size of 200 mesh as the main process of success in decreasing water content. The structure of chitosan CC is also degraded by the demineralization and deproteinization process using HCl and NaOH solution, which is a strong base compound that causes a lot of protein and mineral content loss and causes chitosan to become lighter, making it easier to dry the CC chitosan material.

Likewise, according to its quality, chitosan water content is set at ≤ 10% [30]. Likewise, the low water content can influence and reduce the level of damage to chitosan polymers, especially in conditions of high humidity, which will lead to microorganism contaminants, especially fungi, and a tendency that chitosan can draw moisture from the environment due to hygroscopic chitosan properties due to its ability to form groups chitosan amen in binding water molecules.

Chitosan CC ash content is a parameter to determine the mineral yields produced through the demineralization process in coconut crab shells using strong base compounds. Low ash content indicates mineral chitosan CC content is also low. Chitosan CC ash content has an average value of 1.28–2.18% (Table 4), from the test results (P> 0.05) did not show a significant difference between treatments. This shows that the lower the ash content, the higher the purity level of chitosan CC from deacetylation. The decrease in mineral content is influenced by a continuous stirring process (agitation) so that it remains homogeneous during heating for 60 and 120 minutes and causes heat to be evenly distributed using HCl solvents. Besides, the decrease in ash content occurs during washing, where minerals will be released from the materials which bind with solvents that can be wasted with washing water and produce neutral pH. Meanwhile, the quality of chitosan ash content determined by Protan Biopolymer is <2%. Likewise, mineral residues from the deacetylation of chitin lobster to chitosan are very low at 0.99% [33].

Protein level or CC chitosan nitrogen level is a parameter in determining the success of deproteinization stages in chitin CC, which is indicated by a decrease in the total nitrogen content of an average of 6.56–8.51% (Table 2), from the test results (P> 0.05) shows no difference which was signed between the treatments of NaOH concentration and heating time. Although it still does not meet the nitrogen content value standard based on chitosan quality standards according to Protan Biopolymer with a maximum value of 5%. Therefore, the decrease in levels of nitrogen chitosan CC can be affected when deproteinization uses too high a concentration of NaOH and an imperfect washing process, so it is unable to remove water-soluble proteins. Meanwhile, the protein residue from the deacetylation of chitin lobster to chitosan is very low at 1.6% [33].

Chitosan CC fat content has an average value of 6.56 - 8.31% (Table 2), from the test results (P> 0.05) did not show a significant difference between the treatment of NaOH concentration and the heating time. Carbohydrate levels of CC chitosan are done by calculation of difference where chitosan CC carbohydrate levels have an average value of 77.99–80.31% (Table 2), the test results (P> 0.05) do not show a significant difference between the treatment of NaOH concentration and heating time. Carbohydrate content is influenced by other quality components such as water content, ash, fat, and protein, and it shows that a carbohydrate component is a group of polysaccharides, and among them, there are chitosan results from the deacetylation process.

TABLE I
DEACETYLATION COCONUT CRAB CHITOSAN

Heating Time (minutes)	NaOH concentration (%)	Yield (%)	DD (%)	*M*w (kDa)
60	5	84.22 ±1.13C	81.72 ± 0.7A	368 ± 5.6C
	10	83.18 ±0.45B	85.7 8± 1.5B	353 ± 8.3B
	15	80.69 ±0.72A	88.90 ± 0.1C	321 ± 5.6A
120	5	84.70 ±0.90C	83.62 ± 0.8A	309 ± 9.9B
	10	82.32 ±0.83B	86.93 ± 0.5B	288 ± 8.4A
	15	80.63 ±0.50A	89.15 ± 0.2C	280 ± 8.4A

Values followed by different letters indicate that they are significantly different from each other (α = 0.05).

TABLE II
PROXIMATE TEST RESULTS FOR CHITOSAN DEACETYLATION RESULTS USING NAOH CONCENTRATION AND LENGTH OF HEATING TIME

Heating Time (minutes)	NaOH concentration (%)	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrates (%)
	5	9.46 ±1.4B	1.46 ±0.01A	2.07 ±0.13A	6.69 ±0.02B	80.31 ±1.2A
60	10	10.30 ±0.7A	1.80 ±0.01A	1.56 ±0.65B	7.25 ±0.38A	79.08 ±0.3B
	15	9.78 ±0.6B	1.28 ±0.07A	1.24 ±0.41B	7.65 ±0.82A	80.05 ±1.0A
	5	10.03 ±0.7A	1.53 ±0.02B	1.99 ±0.40A	6.56 ±0.57C	79.89 ±1.6A
120	10	9.18 ±0.5B	2.18 ±0.48A	1.02 ±0.09A	7.75 ±0.18B	79.86 ±0.7A
	15	10.12 ±0.1A	2.02 ±0.13A	0.66 ±0.53B	8.31 ±0.02A	77.99 ±0.8B

Values followed by different letters indicate that they are significantly different from each other (= 0.05).

TABLE III
VISCOSITY, SOLUBILITY, AND ANTIOXIDANTS OF CHITOSAN CC

Heating Time (minutes)	NaOH concentration (%)	Viscosity (cp)	Solubility (%)	Antioxidant (%)
	5	74.05 ±1.48A	0.81 ±0.06A	26.27 ±0.18C
60	10	74.35 ±0.64A	0.83 ±0.02A	28.95 ±0.28B
	15	73.35 ±1.20B	0.90 ±0.02A	29.99 ±0.18A
	5	70.90 ±0.71A	0.82 ±0.04A	29.72 ±0.95C
120	10	69.84 ±0.71B	0.89 ±0.02A	33.53 ±0.97B
	15	67.90 ±0.85C	0.95 ±0.06A	35.66 ±0.18A

Values followed by different letters indicate that they are significantly different from each other (= 0.05)

TABLE IV
CHITOSAN CC COLOR RESULTS OF DEACETYLATION USING NAOH CONCENTRATION AND LENGTH OF HEATING TIME

Heating Time (minutes)	NaOH concentration (%)	Hunter scale		
		L^*	a^*	b^*
	5	75.73 ±0.40B	7.08 ±0.22B	8.32 ±0.35A
60	10	79.93 ±0.29A	6.90 ±1.10C	7.90 ±0.50B
	15	79.55 ±1.71A	8.08 ±0.21A	7.60 ±1.22B
	5	79.50 ±0.68C	7.28 ±0.09B	7.67 ±0.31A
120	10	80.35 ±0.15B	8.15 ±0.14A	7.85 ±1.51A
	15	81.08 ±0.16A	8.19 ±0.38A	7.04 ±0.45A

Values followed by different letters indicate that they are significantly different from each other (= 0.05)

G. The viscosity of Chitosan CC

Table 3 shows the viscosity of chitosan CC with an average value of 67 - 74 cp. The test results (P> 0.05) did not show a significant difference between the treatment of NaOH concentration and heating time. This shows that the intrinsic viscosity value has the ability of the polymer to increase the viscosity of the solution. The viscosity value of chitosan CC is related to the degree of polymerization, and molecular weight (Mw) will increase, thereby affecting the increase in viscosity of chitosan CC. Therefore, the linear relationship between log value is intrinsic viscosity and log value of the molecular weight for chitosan solution with the same degree of deacetylation. Likewise, the use of high temperature (140 ^{0}C) for 4 hours at the stage of deacetylation of chitin crabs will cause a breakdown of the chitosan molecular chain polymer bonds and cause an increase in viscosity and decrease in molecular weight [12]. Therefore, the viscosity, particle size, and morphology of chitin deacetylation to chitosan can affect the molar mass with the factors of high-temperature use, NaOH concentration, reaction time, and preparation of the material before becoming chitin [34].

H. The Solubility of Chitosan CC

Based on Table 2, the average solubility of CC chitosan is 0.81 - 0.95%. The test results (P> 0.05) did not significantly differ between the NaOH concentration treatments and the heating time. The increase in solubility is directly proportional to the increase in deacetylation of chitin CC, especially in the acetyl group, will disappear and leave an amine group. The amine group has H^+ ions, making it easy for chitosan CC to interact with water through hydrogen bonds. Likewise, the use of NaOH solution and varying heating temperatures can affect the solubility of chitosan CC. Therefore, chitosan has soluble properties only in dilute acid, acetic acid, citric acid, and formic acid. While chitosan, which can be sea in water, has been substituted between chitosan and other ingredients. However, chitosan in an acidic atmosphere where free amine groups will be protonated to form a cationic amino group (NH_3^+). Likewise in chitosan cation groups will react with anionic polymers and form electrolyte complex compounds [35].

I. Antioxidant Chitosan CC

The antioxidant properties of chitosan CC are related to the activity of DPPH free radicals on chitosan CC, which has an average value of 26.27-35.66% (Table 2). The test results (P> 0.05) did not show a significant difference between the NaOH concentration treatments and the heating time. The heating process will make the coconut crab shell, and chitin CC resulting from deproteinization will turn reddish. This is presumably because the pigment content in coconut crab skin contains astaxanthin pigment which is integrated with protein. Like pigments from crab shells and shrimp shells containing astaxanthin, pigments include canthaxanthin, lutein, zeaxanthin, β-carotene, Heinonen phoenicoxanthin, and astaxanthin [27].

The antioxidant activity showed DPPH free radical activity from the results of demineralization and deproteinization of chitin and deacetylation of chitosan, respectively 4.71% to 21.25% and 11.45% to 32.78% [36]. Likewise, free radicals

in chitosan are carried out by a cleansing mechanism so that hydrogen ions from ammonium ions (NH_3^+) form stable molecules [37]. The results of testing the antioxidant properties of chitosan shell chitosan deacetylation results with heating 60, 90, and 120 minutes are influenced by the ability to capture free radicals in Fe ions, which can be used as a source of antioxidants, especially in food supplements and pharmaceutical industry development [38].

J. Chitosan Color CC

The color of chitosan CC is the result of the purification of deacetylation from chitin CC. The results showed that CC chitosan produced from the deacetylation process with different NaOH concentrations and heating times showed a reddish-white color. Table 4 shows that the color of chitosan CC using a color reader shows the level of Brightness (L*) chitosan CC has an average value of 75.73–81.08, while the redness value (a*) chitosan CC has an average value of 6.90–8.19, and a yellowish value (b*) Chitosan CC has an average of 7.04–8.32. In general, chitosan crab deacetylation results using alkali treatment and C60, C90, and C120 heating times produce cream to brown colors and show different brightness levels and brightness index values [14]. Likewise, the measurement of chroma value on the color of chitosan lobster shell is more influenced by the raw material of the product itself and is also influenced by the extraction results from the component of water content activity with an average value of L* = 98.12, b* = 5.28, a* = 3.35 [19].

K. Morphology Chitosan CC

The morphology of CC chitosan is demonstrated using SEM. The result of the deacetylation of chitosan using NaOH concentration and heating time showed that the crystal structure showed a different shape. The morphology of CC chitosan (Figure 2A) shows that the use of 5% NaOH concentration with a heating time of 60 minutes shows a spherical micrograph, whereas the concentrations of NaOH 10 and 15% are thin sheets. Likewise, the NaOH concentration and the heating time of 120 minutes (Figure 2B) have the same micrograph. Chitosan CC at N-deacetylation 60 and 120 minutes generally showed the same microfibrils, except at 5% NaOH concentrations, which were still around. Likewise, chitosan crabs with heating 60, 90, and 120 minutes showed the same microfibrils of their structure [14]. Hence, the particle size and morphology of chitosan deacetylation result into chitosan is very influential on the molar mass in the presence of high-temperature usage factors, NaOH concentration, reaction time and preparation of the material before becoming chitin [34].

L. X-Ray Diffraction Chitosan CC

The results of the XRD pattern show that chitosan CC has an average crystal peak characteristic of 2θ = 19.2 - 29.4^O (Figures 3 A and B). A sharper peak in both treatments is shown at 2θ = 29.4 ^{O}. However, different NaOH concentrations and heating times did not affect the crystal reflection during N-deacetylation of chitin CC that formed eight crystal peaks, with one highest crystal peaks at each treatment. Likewise, the results of XRD pattern research showed the characteristics of different crystal peaks, namely chitosan shrimp (19.2 ^{O}), lobster (19.3 ^{O}), crab (18.8^O). Likewise, chitosan extracted from crab shell chitin has higher crystallinity when associated with molecular interactions and produces the highest peak of 9.36 ^{O} with a percentage of crystallinity of 35.2% [12].

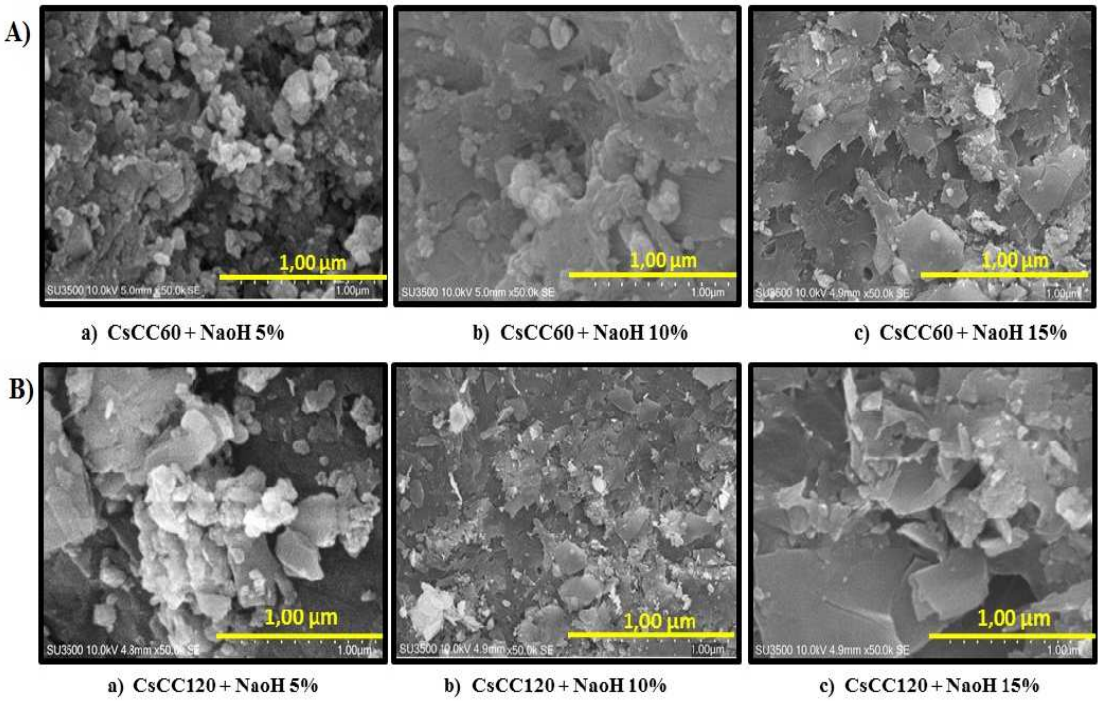

Fig. 2 Morphology of CC Chitosan. A). Deacetylation Long heating 60 minutes. B). Deacetylation Old Heating 120 minutes. a) 5% NaOH, b) 10% NaOH, c) 15% NaOH

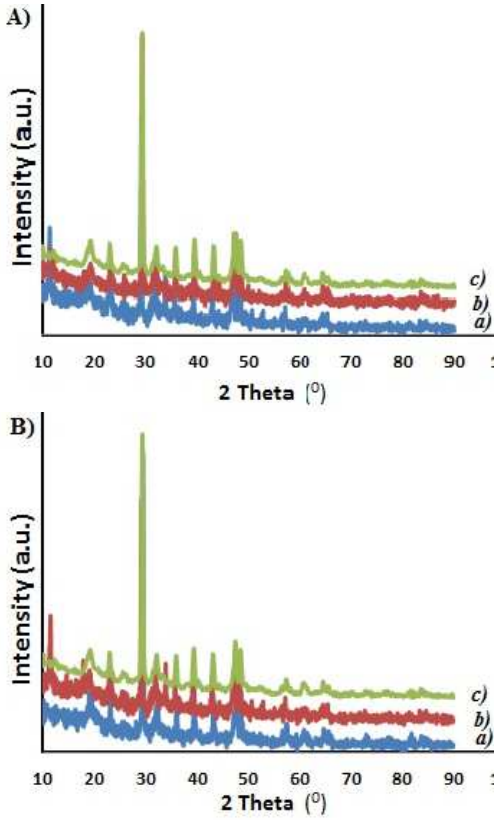

Fig. 3 XRD Chitosan CC Spectra. A). Deacetylation Long heating 60 minutes. B). Deacetylation Old Heating 120 minutes. a) 5% NaOH, b) 10% NaOH, c) 15% NaOH

IV. Conclusions

Coconut crab shell (CC) is a waste material that is one of the important biopolymers. The chitin CC deacetylation process using different NaOH concentrations and heating times produced chitosan CC with the highest DD value (89.15%) and low molecular weight of 280 kDa at 15% NaOH concentration with a 120-minute heating time. Likewise, the quality of water content, ash, protein, fat, color of chitosan CC is under the standards of Protan Biopolymer. Chitosan CC micrograph has the same shape as microfibril structure due to the heating process. While the average crystal peak is 19.2-29.4 ^{O}, the best treatment recommended in this study is the deacetylation process using a 10% NaOH concentration with a heating time of 120 minutes.

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