The Examination of The Quality of Chitosan from Bamboo Shell Waste with Variations of NaOH Concentration in the Deacetylation Process

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ABSTRACT

The bamboo shells that have been unused will be thrown away. Bamboo shell waste is still not utilized or processed optimally, better processing and utilization are needed so that the waste does not pollute the surrounding environment. Bamboo shells can be used as chitosan. The main organic material found in the mollusca animal group contains organic material that forms chitin and chitosan compounds. This study aims to determine the quality of chitosan from bamboo shell waste with variations in NaOH 60%, 65%, 70%, 75%. Analysis of research data using simple regression test. The results of quality inspection of bamboo shell chitosan showed that yield and degree of deacetylation obtained significance of $p = 0.03$ and 0.01 ($p<0.05$) so NaOH effect on yield quality and degree of deacetylation, water content and ash content are significant $p = 0.21$ and 0.20 ($p>0.05$) so NaOH has no effect on the quality of water content and ash content. The conclusion of this study is chitosan from bamboo shells has the quality standards of SNI 7949 in 2013. Suggestions for other researchers are to add other variables and parameters, while for industry use chitosan as an absorbent for heavy metals, cosmetics, food preservatives.

Keywords: Bamboo shellfish, quality of chitosan, NaOH concentration, daecetylation process

INTRODUCTION

The high number of shellfish for consumption by the general public will also be directly proportional to the amount of shellfish waste produced. If the waste is disposed of continuously without proper treatment, the environmental balance will be disturbed due to the accumulated waste. *Solen* sp. often found on the coast of Madura in Pamekasan district, East coast of Surabaya, Cirebonan Kejawan beach and in the waters of Tanjung Solok Jambi. Bamboo shells burrow vertically into the sediment and will come to the surface at low tide. Has a long flat shape like a bamboo for an adult's finger. Its unique shape resembles a razor blade, so it is also called a knife shell, razor clam or also a jack knife(Muhammad et al, 2017). Bamboo shell waste is still not utilized or processed optimally, so that better processing and utilization is needed so that the waste does not pollute the surrounding environment. One of the uses of bamboo shell waste that can be done is to make the shell as a material for making chitosan. Chitosan is a polysaccharide consisting of D-glucosamine and N-acetyl-D'glucosamine linked through - (1,4) bonds.

Chitosan has excellent biological properties including biocompatible, biodegradable, non-toxic, and antioxidant(Huang et al., 2020). Chitosan is derived from chitin compounds produced from the deacetylation process(Alawiyah and Taufikul, 2016). The transformation of chitin into chitosan is called the deacetylation step, namely by adding a base at a high concentration. The reaction produced in the deacetylation process using alkali at high temperatures will cause the release of the acetyl group (-COCH3) from the chitin molecule and form a free amine group (–NH2) which means that the chitosan molecule has been formed (Wulandari, et al 2020). The benefits of chitosan, especially in the field of environmental health, are also quite broad, such as: coagulant, flocculant, waste adsorbent, food product preservative, especially fishery.

The benefits of chitosan, especially in the field of environmental health, are also quite broad, such as: coagulant, flocculant, waste adsorbent, food product preservative, especially fishery. Utilization of waste bamboo shellfish (*Solen* sp.) compared to other types of shellfish, such as blood clams, green mussels, and scallops as chitosan is still very little, so it needs to always be developed. This study aims to determine the content of chitosan made from waste bamboo shells using a variation of 60% NaOH concentration. 65%, 70%

and 75% in the deacetylation process in order to get the best deacetylation degree (DD) results, which is at least 75% according to SNI 7949 of 2013, this is because the degree of deacetylation (DD) is a very important chemical characteristic in determining the content and the quality of the chitosan itself(Azhar et al., 2010).

METHODS

The research that will be conducted is experimental research or(experimental research experimental research). The type of research used in this study is a quasi(quasi-experimental design-experimental design). The research design used in this study is the One Shot Case Study, where the subject will be given treatment and then the results of the treatment are observed. The object of the research is the content and characteristics of chitosan, including: yield, moisture content, ash content, and the degree of deacetylation to determine the level of chitosan produced based on different concentrations of NaOH in the deacetylation process. Each variation of NaOH 60%, 65%, 70% and 75% chitosan that will be studied has 6 (six) repetitions without control. Data collection techniques by means of literature study, observation, and laboratory examination. Data analysis used a simple regression test on the SPSS application.

The first stage is demineralization, then deproteinization stage to produce chitin. Demineralization was carried out by giving 100 ml of 1N NaOH solution in 25 grams of the sample of bamboo shell powder. The powder was heated in a magnetic stirrer for 30 minutes at a temperature of 65^0C, rinsed with distilled water and dried in an oven at 100^0C for 1 x 24 hours.

The deproteinization stage was carried out after the powder was dry. This stage was carried out by giving 100 ml of HCL solution, heated in a magnetic stirrer for 30 minutes at a temperature of 75^0C. The powder was rinsed with aquased and dried in an oven at 100^0C for 1 x 24 hours. The chitin of the bamboo shells was formed and the chitin yield was calculated.

The transformation of chitin into chitosan was carried out through a deacetylation step using 60%, 65%, 70%, and 75% NaOH solutions. Chitin powder was heated with a magnetic stirrer for 1 hour at a temperature of 100^0C. The powder was rinsed with distilled water and dried in an oven at 100^0C for 1 x 24 hours. Chitosan was then examined for yield, moisture content, ash content, FTIR spectro, and determination of the degree of deacetylation(Laila and Niken, 2012).

RESULTS

Chitin and Chitosan Yield of Bamboo Shells

Table 1. Chitin weight (gr)

No.	Result	No.	Result	No.	Result	No.	Result
1.	19,683	**7.**	20,129	**13.**	19,614	**19.**	20,312
2.	17,054	**8.**	20,312	**14.**	19,461	**20.**	22,127
3.	19,278	**9.**	19,421	**15.**	20,325	**21.**	21,079
4.	18,731	**10.**	19,269	**16.**	20,211	**22.**	20,414
5.	21,476	**11.**	20,224	**17.**	22,312	**23.**	18,618
6.	20,379	**12.**	21,379	**18.**	20,182	**24.**	20,312

The table above is the result of shrinkage of bamboo shell powder (chitin) after going through demineralization and deproteinization resistance. The initial powder sample is 25gr. The following is the result of measuring the chitin yield with the formula :

$$\text{Yield} = \frac{chitin\ weight}{initial\ weight\ sample} \times 100\%$$

Table 2. Chitin weight yield (gr)

No.	Result	No.	Result	No.	Result	No.	Result
1.	78,732	**7.**	80,516	**13.**	81,248	**19.**	78,456
2.	68,216	**8.**	88,508	**14.**	88,508	**20.**	77,844
3.	77,112	**9.**	77,684	**15.**	84,316	**21.**	81,3
4.	74,924	**10.**	77,076	**16.**	81,656	**22.**	80,844
5.	85,904	**11.**	80,896	**17.**	75,471	**23.**	89,248
6.	81,526	**12.**	85,516	**18.**	81,248	**24.**	80,728
Total average							**80.72%**

The variation of NaOH here has not been applied, so the calculation of the average value is carried out thoroughly. The results of the transformation of chitin into chitosan after going through the deacetylation stage with various concentrations of NaOH 60%, 65%, 70%, and 75% were then calculated using the same formula as the previous chitin yield calculation to determine the chitosan yield.

Table 3. Chitosan weight yield (%)

Concentrtion of NaOH	Weight yield chitosn bmboo shell						average
	1	**2**	**3**	**4**	**5**	**6**	
60%	78,708	67,876	76,348	71,072	85,868	81,444	76.886
65%	74,856	75,844	76,024	73,664	79,976	83,588	77.325
70%	75,056	72,704	70,596	69,784	79,456	79,488	74.514
75%	67,896	75,564	67,984	78,912	71,872	78,996	73.537

Based on the results in the table above, the highest average yield of bamboo shell chitosan was obtained by 77.325% with a variation of 65% NaOH concentration, while the lowest average was 73.537% using a 75% NaOH concentration variation.

Bamboo Shell Chitosan Water Content

The water content examination was carried out using the method Gravimetric/oven. The sample of chitosan used for measuring the water content is 1 gram.

Table 4. Chitosan water content

Concentrtion of NaOH	Chitosan water content						average
	1	**2**	**3**	**4**	**5**	**6**	
60%	1.6 %	1.2 %	0,4 %	0,5 %	0,6 %	0,8 %	0,85 %
65%	1,4 %	1,2 %	0,9 %	0,7 %	0,6 %	0,5 %	0,88 %
70%	0,6 %	1,2 %	0,7 %	0,6 %	0,7 %	0,7 %	0,75 %
75%	0,6 %	0,7 %	0,6 %	0,6 %	0,7 %	1,4%	0,76 %

Based on the table above, it can be seen that the highest average water content in chitosan is 65% variation, which is 0.88% and the lowest average is 70% variation of 0.75%. It is known that each sample does not exceed the quality standard of water content in chitosan according to SNI 7949 of 2013 which is a maximum of 12%.

Bamboo Shell Chitosan Ash Concent

Examination of ash content using method Drying Ash. The chitosan samples used for measuring the water content were 4 grams each.

Table 5. Chitosan ash content

Concentrtion of NaOH	Chitosan ash content						average
	1	2	3	4	5	6	
60%	0,175%	0,15%	0,15%	0,525%	0,175%	0,4%	0,26,%
65%	1,075%	0,575%	0,225%	0,15%	0,4%	0,175%	0,43%
70%	1,95%	1,275%	0,175%	0,925%	0,1%	0,225%	0,77%
75%	1,4%	0,925%	0,35%	0,3%	2,025%	0,25%	0,87%

Based on the measurement results above, it can be seen that the highest average is in the 75% NaOH concentration variation of 0.87% while the lowest average is in the 60% NaOH concentration of 0.26%. Of the 24 samples, none exceeded the quality standard of ash content in chitosan in accordance with SNI 7949 of 2013 which is a maximum of 5%.

FTIR Spectrophotometry of Bamboo Shell Chitosan

Spectrophotometry FTIR was used to determine the characteristics of the functional groups contained in chitosan. The wavelength used is 4000-400cm^{-1}. The following is one of the results of FTIR spectrophotometer on chitosan:

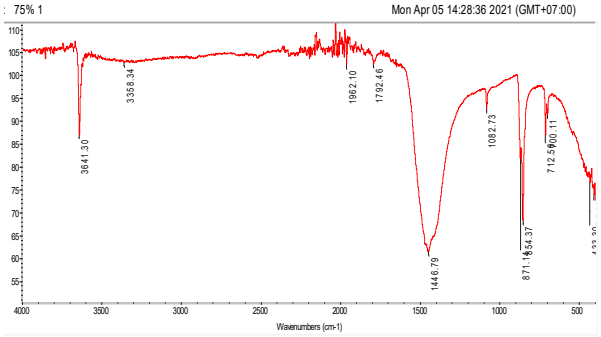

Figure 1. Chitosan FTIR spectrum

Table 6. FTIR adsorbtion characteristic of chitosan

Type of vibration	Wavenumber (cm^{-1})
Streching vibrtion OH	3450,3340
Streching vibrtion NH	3400
Asymmetric streching vibrtion CH	2926
Symmetric streching vibrtion CH	2864
Streching vibrtion C = O	1650
Bending vibration NH	1596
Streching vibrtion CN	1200 – 1020
Asymmetric bending vibrtion CH	1418
Symmetrical bending vibrtion CH	1377
Asymmetric streching vibrtion C – O	1082
Symmetric streching vibrtion C – O	1033
Streching vibrtion β – 1,4 – glikosidik	850

bamboo shell chitosan deacetylation degree

The calculation or determination of the degree of deacetylation in this study uses the method *Baseline* or the equation formula with the formula:

$$\%DD = 100 - \left[\frac{A1655}{A3450} \times \frac{100}{1,33}\right] \%$$

Description :

A = $\log \frac{Po}{P}$

Po = The distance between the baseline and the tangent, the highest peak at wavelengths 1655^{cm-1} and 3450 $^{cm-1}$

P = The distance between the baseline and the lowest valley at wavelengths 1655cm-1 and 3450 cm-1

1,33 = Determination of the perfect degree of deacetylation

A1655 = Absorbance of amide/acetamide groups

A3450 = Absorbance of hydroxyl groups

The following is the result of measuring the degree of deacetylation of chitosan from bamboo shells based on the results of the FTIR spectro:

Table 7. deacetylation degree of chitosan

Concretation of NaOH	Deacetylstion degree				Average
	1	2	3	4	
60%	96,84%	96,67%	96,59%	96,76%	96,71%
65%	97,87%	97,33%	97,47%	97,45%	97,53%
70%	98,47%	97,74%	97,79%	97,84%	97.88%
75%	98,64%	98,94%	98,71%	98,86%	98,78%

Based on the table above, it can be seen that the highest average value of the degree of deacetylation is 98.78% at 75% NaOH concentration variation, while the lowest average is 96.71% at 60% NaOH concentration variation. The highest deacetylation degree value from all variations and replications was 98.94% at 75% NaOH concentration variation, then the lowest deacetylation degree value was 96.59% at 60% NaOH concentration variation. The results of all concentration variations show that there is no value of the degree of deacetylation below the quality standard of SNI 7949 in 2013. The value of the degree of deacetylation according to the regulation is 75%.

Characteristics of Bamboo Shell Chitosan

Table 8. Characteristics of Bamboo Shell Chitosan

Parameter	chitosan SNI 7949 : 2013	Chitosan bamboo shell

			NaOH 60%	NaOH 65%	NaOH 70%	NaOH 75%
Praticle shape		Flakes to powder	Powder	Powder	Powder	Powder
color		Light brown to white	Brownish white	Brownish white	Brownish white	Brownish white
physical		**Requirement**				
-	**Foreign object**	Negative	-	-	-	-
chemical		**requirement**				
-	**Deacetylation degree**	Min 75%	96,48 %	97,33 %	97, 79%	98,86 %
	Test type	**requirement**				
-	**pH**	7-8	7	7	7	7
-	**Ash content**	Maks 5%	0,175%	0,575%	0,35%	0,925%
-	**Water content**	Maks 12%	1,6 %	1.2 %	0,7 %	0,6 %

Based on the comparison table above, it can be seen that the bamboo shell chitosan in this study has met the requirements in accordance with SNI 7949 of 2013

DISCUSSION

Bamboo Shell Chitosan Yield

The yield of chitosan in each concentration variation was different. The higher the concentration of NaOH given, the lower the yield value obtained. The results of different yields can be influenced by several factors, including: temperature and time of stirring in the deacetylation process, the concentration of alkali used, as well as the size and thickness of the raw materials used. Alkali concentration and high temperature can cause a decrease in polymer and yield value(Mursida et al., 2018). In the deacetylation process, alkali can bind more acetyl groups. The yield produced by the variation of the 75% NaOH concentration is indeed lower than the 60% NaOH concentration, but the quality of the chitosan obtained is getting better and purer, because many acetyl groups in chitosan are released from chitin. High temperatures can also affect the resulting yield value. The concentration of NaOH and high stirring and heating temperatures in the deacetylation process can accelerate the release of excess acetyl chitin chains, so that finer chitosan particles dissolve in NaOH and can cause the chitosan mass to decrease(Anggun et al., 2018).

Bamboo Shell Chitosan Water Content

The average ash content produced based on the variation of NaOH concentration was 0.85%, 0.88%, 0.75%, and 0.76%, respectively. The lower the water content produced, the better the quality of chitosan obtained. A good drying process will produce a low (good) moisture content. The size of the water content value is fully influenced by the drying method, drying time, means of drying, the amount of chitosan being dried, and the drying area used so that the concentration of NaOH in chitosan has no effect on the high or low water content value obtained (Loede Muhammad et al ., 2018).

Bamboo Shell Chitosan Ash Content

Factors that can affect the quality of the ash content in chitosan are the duration of stirring and the consistency of stirring, as well as washing to a neutral pH during the demineralization process. Ash content is used to remove mineral residues during the demineralization process. If the ash content obtained is still high, then the demineralization carried out has not been effective and perfect. Washing the pH to neutral can minimize the occurrence of acids that are entangled and diffuse with free amino acids and protein residues that can cause damage during the drying process (Pinta Purbowati, 2016). The demineralization process in this study was carried out using a stove *magnetic,* stirrer so that the stirring and the heat that was delivered were stable or homogeneous. Stable stirring process causes HCL to bind minerals effectively. The low ash content indicates that the quality and purity of the processed chitosan is good.

FTIR Spectrophotometry of Bamboo Shells

The absorption pattern shows the presence of OH (hydroxyl) and NH (amine) functional groups, which means that the two groups indicate that the acetyl group (COCH3) has been lost. Another characteristic of chitosan lies in the amide group at wave number 1655 – 1310 cm^{-1} and the hydroxyl group at wavenumber 3550 – 3300 cm^{-1}, then there is also a -1,4-glycosidic bond. The results of the FTIR spectrum of bamboo shell chitosan show the location of the amide group and hydroxyl group at a predetermined wave number, and there is a -1,4-glycosidic bond so that bamboo shell chitosan has been successfully formed(Sari, 2013).

Bamboo Shell Deacetylation Degree Value

The deacetylation process is the stage of releasing the acetyl group from the chitin molecular chain, so that if the value of the degree of deacetylation is high, the purer the quality of the chitosan produced(Fatmawati, 2019). Factors that affect the degree of deacetylation are the concentration of NaOH, stirring time, and stirring temperature. In this study, the concentration of NaOH in the deacetylation process used was stratified, namely 60%, 65%, 70%, and 75%. The higher the concentration of NaOH given, the higher the degree of deacetylation produced will also be.The addition of high NaOH causes the hydroxyl group for the hydrolysis process (transformation of chitin to chitosan) to be high, so that the acetyl group can be eliminated properly and the formation of a high amine group is also better(Bahri, 2015).

The higher the degree of deacetylation, the better the quality and purity of the chitosan. The data on the degree of deacetylation in table 7 shows the highest degree of deacetylation at 98.95% in the 75% NaOH concentration variation, and the lowest at 96.84% at the 60% NaOH concentration variation, so that the NaOH concentration affects the value of the chitosan deacetylation degree. Chitosan with a deacetylation degree value of about 90% can be applied for food quality and pharmaceuticals, as long as the moisture content, ash content and other standard requirements have been determined(Century Siregar, 2016).

Characteristics of Bamboo Shell Chitosan

The characteristics of chitosan in this study refer to SNI 7949 of 2013 which has several parameters, including: chitosan powder form, water content, ash content, pH, color, and degree of deacetylation. The results of the research on the quality of bamboo shell chitosan showed that all of them had met the quality standards. The form of chitosan in this study was a fine powder. The texture of the powder of the raw material is smooth and evenly distributed, and can provide good effectiveness during the chitosan manufacturing process, so that it can absorb evenly and well. The pH of chitosan shells is 7 (neutral). pH that is too acidic or too alkaline will affect the demineralization process which can cause high ash content values, so rinsing with distilled water must really be carried out until neutral. In this study, the color of the chitosan produced was brownish white. The color of chitosan can vary depending on the raw materials used. Chitosan with a degree of deacetylation of about 90% can be applied for food quality and pharmaceuticals, as long as it takes into account the moisture content, ash content and other predetermined standard requirements.

CONCLUSION

Examination of the quality of bamboo shell chitosan using the reference of SNI 7949 in 2013. The results of this study were in accordance with the provisions of quality standards, both the NaOH concentrations of 60%, 65%, 70%, and 75%. The chemical quality of bamboo shell chitosan from each concentration of NaOH has met the quality standard requirements. The results of the analysis showed that the concentration of NaOH had a significant effect on the yield quality and degree of deacetylation, while the water content and ash content had no significant effect.

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