Depolymerization of chitosan from snail (*Pilla ampullacea*) field shell using α-amylase

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**Abstract.** Chitosan is a biopolymer derivative from chitin that has many benefits for applications in various fields. Since chitosan has a large molecular weight, it has constraints to its application. To overcome the disadvantage, it needs hydrolysis of chitosan in order to obtain a lower molecular weight. In this research, the hydrolysis of chitosan from snail field shell (*Pilla ampullacea*) will be operated by the enzymatic process using α-amylase. The chitosan solution is hydrolysis using α-amylase with the varied time of hydrolysis. The results showed that the operating conditions for chitosan hydrolysis using α-amylase enzyme is at 5 hours operating time and concentration of enzyme 0.02 %. The hydrolysis can reduce the molecular weight of chitosan snail field shell from $6.41 \times 10^5$ Da to $1.06 \times 10^4$ Da and the viscosity from 41.7 cPs to 17.3 cPs.

**1. Introduction**

Snail field (*Pilla ampullacea*) is one of the mollusc species that have hard shells and the population is fast breeding. During this time the snail shell is only a waste and a small part is used as animal feed. It is known that hard-shelled animals are one of the biomaterials called chitin, which can be deacetylated to produce chitosan. Chitosan structure is a heteropolysaccharide polymer composed of 2-acetamide-2-deoxy-B-D-glucose (N-acetylglucosamine) monomer and 2-amino-2-deoxy-B-D-glucose (D-glucosamine) [1]. Chitosan constraints in the application is high molecular weight so that the solubility is very low. Therefore, efforts should be made to increase the solubility of chitosan by hydrolyzing chitosan enzymatically. The enzymes used to hydrolyze chitosan can use specific enzymes such as chitosanase and non-specific enzymes such as lipase, cellulase, and protease. Chemically hydrolysis is random, uncontrolled, low efficiency and produces low polymerization (DP) oligomers with more D-glucosamine monomers, but the enzymatic hydrolysis is specific, controlled, it produces a hydrolyzate with a lower molecular weight with high solubility [2, 3].

Commercial chitosan hydrolysis using papain and pronase enzymes was able to decrease the chitosan hydrolysis molecular weight by a 3 hour hydrolysis time from 1,077,919 Da to 395,891 Da, and increase solubility with decreasing molecular weight [4]. Chitosan depolymerization using non-specific enzymes such as α-amylase, cellulase, lipase can be done with regard to hydrolysis factors. Hydrolysis conditions such as the concentration of enzymes used, hydrolysis temperature, enzyme type, and hydrolysis time have an effect on the physicochemical properties of chitosan hydrolysate such as molecular weight, viscosity, solubility, and polymerization degrees [5, 6].
The molecular weight of chitosan hydrolyzate results of hydrolysis effect on its application such as the ability to act as antimicrobial, emulsifier, antitumor, dialysis membrane, water treatment, fertilizer, and fungicide. Besides that, the chloride hydrolysis product by enzymatic method provides advantages such as mild reaction conditions, easy to control reaction, high specificity, high yield, no glucose ring modification, and environmentally friendly [7, 8].

In this research, the chitosan hydrolysis process on the snail's shell using an α-amilase enzyme to obtain chitosan hydrolyzate with lower molecular weight. To determine the effect of variables associated with the hydrolysis process then studied the effect of hydrolysis time on molecular weight and the viscosity of chitosan hydrolyzate produced.

2. Experimental

2.1 Materials

The basic ingredients used in this study were chitosan, the other materials were α-Amylase (20.3 U / mg) enzyme from Sigma Aldrich, glacial acid (CH₃COOH), CH₃COONa (99%), NaOH (99% ), HCL (37%) from Merck, and aquadest. The equipment used is Fourier Transforms Infrared (FTIR-IR Prestige 21 Shimadzu), incubator, Ostwald viscometer, and hotplate.

2.2 Methods

Snail field shell was cleaned and then dried and crushed then sieved using 100 mesh sieve. The chitosan powder is deproteinized with 4% NaOH solution then mineralized with 1 M HCl to obtain chitin. Chitin product then deacetylation using 60% NaOH so that produced chitosan. The chitosan obtained was analyzed by deacetylation degree (DD) with FTIR baseline method. Chitosan obtained with a certain degree of deacetylation was carried out by the hydrolysis process using the α-amilase enzyme. 1 g chitosan was dissolved with 5 mL acetate buffer pH 5.5, then added enzyme α-amilase with the ratio of 1: 0.25 and incubated at 50°C hydrolysis temperature with hydrolysis time according to treatment that is 1 hour, 2 hours, 3 hours, 4 hours, and 5 hours. Hydrolysis was stopped by boiling the solution for 15 minutes. Then 10% NaOH added dropwise to pH 8 and obtained hydrolyzate gel. The gel is filtered and neutralized using aquades, dried for 24 hours at 40°C. Determination of degrees of chitosan deacetylation use equation 1.

\[
DD = 1 - \left[ \frac{A_{1655}}{A_{3450}} \times \frac{1}{1.33} \right] \times 100
\]  

Specific viscosity determination was performed using the Ostwald viscometer tool. Solvent (1% acetic acid solution) is introduced into the viscometer then the flow rate is measured. After that, the sample of chitosan solution which has been in the hydrolysis is inserted into the viscometer and the flow rate is measured. Viscosity value and time of flow rate are measured [9]. The specific viscosity value is determined by equation 2.

\[
\eta_{sp} = \frac{\eta - \eta_0}{\eta_0} = \frac{t - t_0}{t_0}
\]  

Intrinsic viscosity can be determined by measuring the specific viscosity of some conjugations and extrapolating the \( \eta_{sp} / c \) graphs versus c in the concentration equal to zero. Alternative analysis to get the same result using equation 3.

\[
[\eta] = \frac{\eta_{sp}}{c}
\]  

After knowing the intrinsic viscosity, the weight of the chitosan molecule can be calculated using the Mark-Houwink equation as shown in equation 4 [10].
\[ \eta = K[M]^\alpha \]  

where:

- \( \eta_{sp} \) = Specific viscosity (sec)
- \( \eta \) = Viscosity of substance
- \( M \) = molecular weight of g/mol substance
- \( t \) = Sample flow rate (seconds)
- \( t_0 \) = Flow rate of solvent time (sec)
- \( k \) = 3.5 x 10^{-4} ml / g
- \( \alpha \) = 0.76

3. Result and Discussion

3.1 The degree of chitosan deacetylation

The value of degrees of deacetylation of chitosan snail shells was obtained from the calculation results using the FTIR baseline method as shown in figure 1. Deacetylation of chitin into chitosan is carried out by using an alkaline solution and deacetylation time. The intensity of collisions in a reaction increases with the increase of alkali concentration. The driving factor of increasing the degrees of chitosan deacetylation is the morphological change of the chitin chain so that the acetamide group allows hydrolysis along with the length of deacetylation time. A long deacetylation time will result in the breaking of more acetamide bonds thus affecting the degree of deacetylation of chitosan [11].

The degrees of deacetylation are affected by the used alkali concentration, the deacetylation temperature, the deacetylation time, and the ratio between the solvent and the material. The increase of concentration alkali chitosan deacetylation, as well as the increase in temperature and deacetylation time, affect the size of the deacetylation degree.

Based on FTIR spectrum images, snail field shell has a value of deacetylation degree of chitosan equal to 83.23%. The spectrum of chitosan from snail field shell shows absorption bands at wave number 3346.50 cm\(^{-1}\) which shows the functional group of stretching -OH and stretching -NH. The absorption band of 2991.59 cm\(^{-1}\) shows a functional group of stretching CH\(_2\), an absorption band of...
1645.92 cm$^{-1}$ shows the presence of a C=O amide group, the absorption band at 1012.63 cm$^{-1}$ indicating the presence of a C-O-C functional group.

### 3.2 Effect of hydrolysis time on hydrolyzed chitosan molecular weight

The effect of hydrolysis time on the decrease of molecular weight of chitosan hydrolysate chitosan as shown in figure 2. The decrease that produces minimum molecular weight occurs at 5 hours hydrolysis time of 10,642.41 Da. The longer time used to hydrolyze chitosan the more decrease the molecular weight of chitosan hydrolysate product. The decrease in molecular weight shows a change in the degree of chitosan linear chain polymerization. The molecular weight reduction is due to the polymer chain cutting occurring at the center of the chitosan polymer chain. This phenomenon shows that the enzyme α-amylase is an endo-enzyme [12].

![Figure 2. The molecular weight of chitosan hydrolyzate at various hydrolysis times](image)

The role of the α-amylase enzyme in degrading the chlorine hydrolyzate polymer chain by cutting the inner glycosidic bond. This type is endo-action by randomly cutting the glycosidic bond. As a result, the polysaccharides decomposes into smaller forms of random size.

It was deduced from the pattern of molecular weight degradation that dropped significantly in the initial 1 hour of hydrolysis and slowed after 1 hour of hydrolysis time (figure 2). The resulting chitosan hydrolyzate molecular weight is included in the medium molecular weight of chitosan (Medium Molecular Weight of Chitosan) ranging from 30,000 Da – 249,000 Da. From the calculation result of chitooligosaccharide monomer amount of monomer obtained as much as 59 from the amount of monomer beginning 3,574. Further analysis with BNJ test 5% level showed that chitosan hydrolyzate molecular weight significantly influence hydrolysis time 1-5 hours. So it can be concluded that the weight of chitosan hydrolyzed molecules decreased significantly at hydrolysis time of 1-5 hours.

Reduced chitosan molecular weight of the hydrolysis result shows that the enzyme activity of α-amylase is able to hydrolyze the glycosidic bond on the chitosan polymer chain. This is due to the many chains of degraded chitosan resulting in a low molecular weight chitosan depolymerization product. The enzyme’s ability to cuts the polymer chain is also evidenced by the lower viscosity of the chitosan solution of the hydrolysis result. Non-specific enzymes can catalyze two different reactions because they have two functionally active sides. In chitosan, the amount of the N-acetyl group, the N-acetyl group distribution in the linear chain, and the molecular weight or distribution of the long chain will affect the ability of the enzyme in degrading chitosan [12, 13]. Chitosan hydrolyzate that have small molecular weights are reported to have many biological activities such as antimicrobials, anticancer, antioxidants and immunostimulant effects.
3.3 Effect of hydrolysis time on the viscosity of chitosan hydrolyzate

The hydrolysis viscosity value of the snail shells based on hydrolysis time from 1 hour to 5 hours as shown in figure 3. The viscosity of the initial chitosan solution prior to hydrolysis was 41.7 cPs, after which the hydrolysis time lasted for 5 hours the viscosity decrease is 17.3 cPs. A gradual decrease in the viscosity value of chitosan solution shows the ability of the α-amylase enzyme to hydrolyze chromium chain chitosan bonds. The change in the viscosity of the solution reflects the change in the degree of polymerization caused by the polymer chain cutting by the α-amylase enzyme during hydrolysis. Figure 3 also shows that the longer the hydrolysis time the greater the viscosity of the solution, due to the interaction of the enzyme with the substrate capable of breaking the glycosidic bond on the chitosan polymer chain. The more polymer bonds that are cut off have an effect on the decrease in the viscosity of the chitosan solution [10, 13].

![Figure 3. The effect of hydrolysis time on the viscosity of chitosan hydrolyzate](image)

Any termination of the glycosidic bond on the chitosan chain will result in reduced sugar and nonreduction sugar. The formation of reducing sugar shows the activity of the enzyme that cuts the glycosidic bond on the active chitosan. Increased reducing sugar affects the decrease in the viscosity of the solution and the molecular weight of the chitosan hydrolysate of snail field.

4. Conclusion

Chitosan depolymerization using α-amylase can reduce the molecular weight and viscosity of chitosan hydrolyzate of snail field shell. The use of 0.1% α-amylase enzyme concentration was able to reduce the molecular weight from 640,489.55 Da to 17,122.08 Da, while the hydrolysis time for 5 hours was able to decrease the molecular weight to 10,642.41 Da. The viscosity of the chitosan hydrolyzate solution decreased from 41.7 cPs to 17.3 cPs.

Aknowlegement

We would like to thank the Directorate General of Higher Education of the Republic of Indonesia who has provided research funding through DPRM Dikti with contract/number: 281.k/UN28.2/PL/2018.

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