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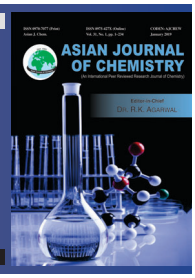

Optimization of Degree of Deacetylation of Chitosan Snail Shells (*Pilla ampulaceae*)

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Snails fields (*Pilla ampulaceae*) have a hard shell and are known to be one of the sources of biomaterial called chitin. Chitosan is a natural biopolymer that can be obtained from chitin deacetylation. The objective of this study was to obtain the optimum conditions of chitin deacetylation in highest degree deacetylation of chitosan. This study used random complete method that contains various of NaOH concentration (30, 40, 50, 60 and 70 %), temperature of deacetylation (60, 90, 120, 150 and 180 °C), time of deacetylation (1, 2, 3, 4 and 5 h) and ratio between NaOH and chitin (1:4, 1:6, 1:8, 1:10 and 1:12 b/v) with three repetitions. The deacetylation degrees of chitosan were determined by FTIR. The results show the optimum condition of deacetylation degrees of NaOH concentration, temperature, time and ratio of NaOH and chitin are 60 %, 150 °C, 4 h and 1:10, respectively. This produces the highest deacetylation degree of chitosan rate of 83.23 %.

Keywords: Chitin, Chitosan, Degree of deacetylation, Shell of snails.

INTRODUCTION

Indonesian territorial waters are rich in abundant resources. One of them is the diversity of Mollusca. According to Chapman [1] mollusca generally lives in water but some lives on land. Some mollusca have important value because their shells can be used for a variety of decorations of high economic value. Some are used as a source of food ingredients for which meat can be consumed. Snail field (*Pila ampullace*) is one group of mollusca that is often considered as a pest on rice plants. This snail is widespread in freshwater areas in Southeast Asia. In Indonesia this snail is found mostly in rice fields. Snail field has a hard shell and is known as one of the sources of biomaterials called chitin. Chitin is a polysaccharide that is non-toxic and is biodegradable. Furthermore, deacetylation can be carried out to produce chitosan.

Chitosan is a polycationic polymer which is the product of deacetation of chitin which is a long chain glucosamine polymer (2-amino-2-deoxy-D-glucose, m.f. $[C_6H_{11}NO_4]_n$. Today the use of chitosan in various industries is very large, including in the pharmaceutical, biochemical, biotechnology, preservation, cosmetics industries and is used to complex heavy metal ions found in surface water and industrial waste. Referring to the benefits of highly chitosan advantages, so that the snail field shell is very potential as one of the sources of chitosan [2].

In general the quality of chitosan used is influenced by the degree of deacetylation [3-5]. For technical quality, the deacetylation degree is around 85 %, for the quality of the deacetylated degree needed around 90 %, while for parmasetis quality the degree of deacetylation is around 95 % [6]. The degree of deacetylation of chitosan is influenced by various factors, such as high NaOH concentrations, which can break the bonds between the acetyl group with nitrogen at chitin. Temperature influences the degree of deacetylation, where the higher the deacetylation temperature of chitin, the greater the rate of breakdown of the acetamide bond. Meanwhile, the long deacetylation time will result in the termination of more acetamide bonds. Furthermore, the effect of ratio of NaOH to chitin will increase the number of amine groups formed from the interaction of chitin and solvents and particle size [7].

Although either acids or alkalis can be used to deacetylate chitin, the fact that glycosidic bonds are more susceptible to acid which would destroy the chain, therefore alkali deacetylation process is used more frequently [8]. Tsiah and Chen [9]

studied the effect of reaction time and temperature during heterogenous alkali deacetylation on degree of deacetylation and molecular weight of resulting chitosan. They found that the degree of deacetylations of the resulting chitosan that were obtained from 140 ºC were higher than those reacted at 99 ºC. Also, the degree of deacetylation of chitosans increased fast at the beginning of reaction process then slowed over time. The reaction rate and rate constant of the deacetylation reaction decreased with increasing degree of deacetylation of reactant.

This study was conducted to determine how the effect of NaOH concentration, deacetylation temperature, deacetylation time and NaOH ratio with chitin, on the determination of the degree of deacetylation of the chitosan of the snails field.

EXPERIMENTAL

The basic ingredients used were snails shell obtained from Bo'e village, Poso Regency, Sulawesi Tengah, Indonesia and other required chemicals including NaOH p.a, HCl 1 M. p.a, aquadest, universal pH paper, pH meter and Whatman filter paper.

The equipment used analytic balance, oven, magnetic stirrer, vacuum pump, FTIR (Shimadzu Type 21), hot plate stirrer, reflux tool, 60 mesh sieve, thermometer, petri dish, porcelain saucer, spatula and glassware commonly used in chemical laboratories.

This study used a completely randomized design (CRD) consisting of independent variables in the form of NaOH concentration, deacetylation time and ratio of NaOH solution to chitin snails shell in each variable consists of five levels with three repetitions. The fixed variable used is the degree of deacetylation.

Preparation of snail field shell samples: First, the snail shell used as the test sample was removed from the shell. Secondly, the removed contents of the shell were washed with water until clean. Thirdly, the contents of the clean shell were dried in the sun. Finally, the clean and dry shell was reduced in size with pestle mortar then sieved with a 60 mesh sieve.

Isolation of chitin: The process of making chitin consists of two stages, namely deproteination and demineralization [7]. As much as 100 g of snail field samples in the form of flour are put into a reflux flask containing 4 % of NaOH w/v. The comparison of the number of samples to the volume of NaOH used is 1:10 w/v. The mixture is stirred with a magnetic stirrer for 2 h at ± 65 °C. This protein-free of shells of snails is then washed with distilled water, neutralized by using HCl. Dried in an oven at 60 °C until dry and then weighed.

Demineralization: The demineralization process is done according the reported method [10], where 50 g of snail field shell reacted with 750 mL of 1 M solution of HCl (comparison of the amount of shell powder to the volume of HCl used is 1: 15 w/v). The mixture was stirred with a magnetic stirrer for 3 h at room temperature. Snail shells were then separated by filtering using a vacuum suction, washed with water to neutral pH and dried in an oven at 60 °C until dry and then weighed.

Chitosan making

Variation of concentration: Chitin is reacted with NaOH solution in a percentage of 30 to 70 % with an increase of 10 % with a ratio of the amount of chitin to NaOH solution of 1:8 (w/v). The mixture was stirred with a magnetic stirrer with an agitation speed of 250 rpm while being heated at 120 °C for 3 h, then filtered with vacuum filter. Meanwhile, the sediment was washed with distilled water to neutral pH. Chitosan was dried in an oven at 60 °C for 12 h [11].

Time variation: Chitin is reacted with NaOH solution with the best concentration obtained in the previous treatment with a ratio of the amount of chitin to 1:8 (w/v) NaOH solution. The mixture was stirred with a magnetic stirrer with agitation speed of 250 rpm while being heated at the best temperature obtained from the previous treatment with a variation of 1, 2, 3, 4 and 5 h. The mixture was then filtered with vacuum filter. The precipitate was washed with distilled water to neutral pH. Then, chitosan was dried in the oven at 60 °C for 12 h.

Determination of degrees of deacetylation: The degrees of deacetylation can be determined by the infrared spectrum obtained by comparing the absorbance of C=O amide in the wave number region of (1650-1500) cm^{-1} (A_{1655}) with respect to O-H absorbance in the wave number area of (3500-3200) cm^{-1} (A_{3450}) with an absorbance value of 1.33 in the perfect deacetylation process. This is based on determining the degrees of deacetylation using the baseline method as proposed by Domszy and Robert [12] using the following formula:

$$\text{Degrees of deacetylation (\%)} = 1 - \left(\frac{A_{1655}}{A_{3450}} \times \frac{1}{1.33} \right) \times 100 \quad (1)$$

RESULTS AND DISCUSSION

The results show the effect of NaOH concentration on the degree of deacetylation of chitin and the effect of deacetylation time and ratio of NaOH solution to chitin shells of snails in order to produce high levels of degrees of deacetylation. The fixed parameters observed are degrees of deacetylation. The effect of variations in NaOH concentration on chitin degrees of deacetylation is shown in Fig. 1.

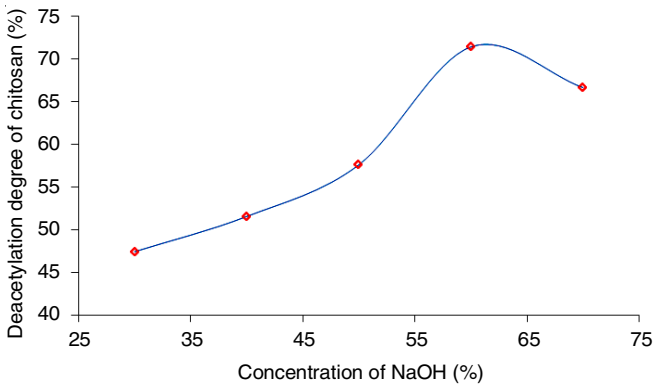

Fig. 1. Concentration of NaOH with chitosan deacetylation degree

The concentration of NaOH affects the value of chitosan degrees of deacetylation. This result is in line with the results of research conducted by Hargono and Djaeni [13]. The use of high NaOH concentrations can break the bonds between carbon in the acetyl group (-$COCH_3$). The reaction will form a negative charged amine group (NH_2). The concentration is influential on chitosan degrees of deacetylation. The results of variance analysis and further tests showed that the best

NaOH concentration at a concentration of 60 % with a degrees of deacetylation of 71.39 %.

Degrees of deacetylation based on the effect of deacetylation time: The degrees of deacetylation of shells of snails chitosan obtained at time variations shown in Fig. 2.

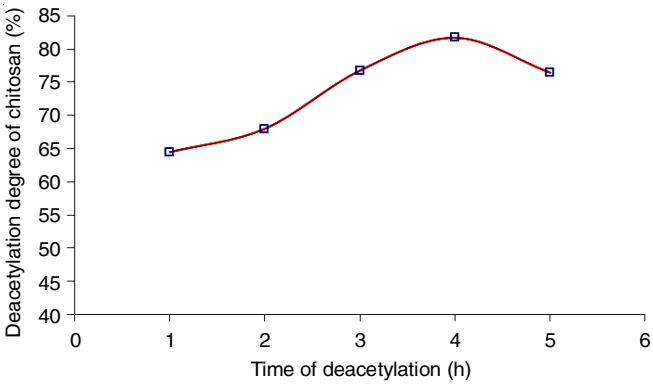

Fig. 2. Process of deacetylation with the percentage of chitosan deacetylation rate

The deacetylation time of chitin greatly influences the value of chitosan deacetylation. The longer the reaction time, the greater the impact intensity in a reaction. The driving factor for the increase in the degree of deacetylation of chitosan is the change in morphology of the chitin chain so that the acetamide group allows hydrolysis along with the length of time deacetylation [14,15]. The long deacetylation time will result in the termination of more acetamide bonds which affect the value of degrees of deacetylation of chitosan [16]. The results of the analysis of variance and the results of the analysis of advanced tests show that the best time is at 4 h with a degrees of deacety-lation of 81.70 %.

Degree of deacetylation based on effect of NaOH solution ratio on chitin: It can be seen in Fig. 3, the effect of the ratio between NaOH solution and chitin on the degrees of deacetylation of shells of snails.

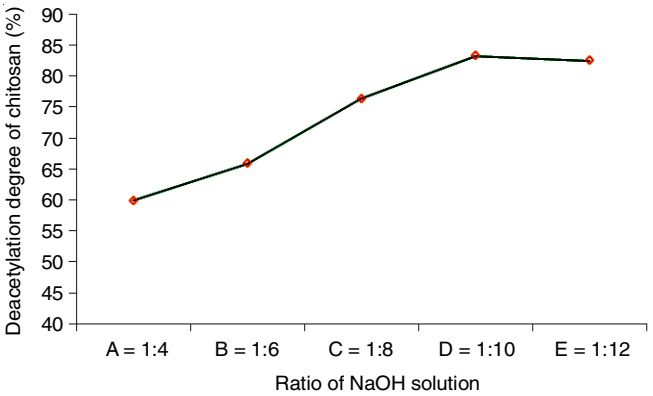

Fig. 3. Ratio of NaOH solution to chitin

These results proved that the number of amino groups formed from the interaction of chitin and solvents will increase [7]. This is also supported by the results of Alexander *et al.* [18]. They concluded that the amount of solvent affects the extent of solid contact with the solvent so that more acetamide groups (-$NHCOCH_3$) are released from chitin. The optimum condition was achieved at a ratio of 1:10 with a deacetylation degree of 83.23 %. Meanwhile, at a ratio of 1:12 the degree of deacetylation decreases due to NaOH which is saturated with chitin to break the bonds of acetamide.

Spectra of FTIR of shells of snails: The degree of deacetylation shows a reduction in the acetyl group from chitin to an amino group in chitosan (Fig. 4). The degree of deacetylation can be determined from the FTIR spectrum by applying four treatments, namely the effect of NaOH concentration, the effect of deacetylation time, deacetylation temperature and the ratio of NaOH to chitin. The base line method proposed by Domszy and Roberts [12] used to record the highest peak, measure the base band selected and to determine the highest deacetylation degree.

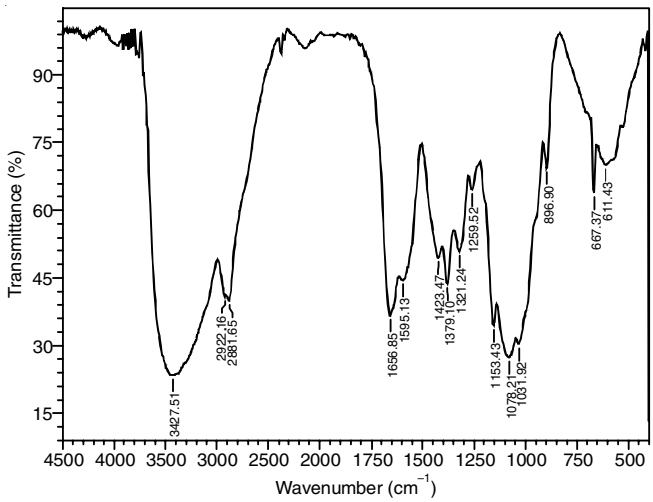

Fig. 4. Fourier transformation infrared spectra of chitosan snail shell

Fig. 4 showed the chitosan spectra of fields shells of snails that shows the absorption band at wave number of 3346.50 cm^{-1} and stretching OH and NH functional groups. The absorption band at wave number of 2991.59 cm^{-1} shows CH_2 stretching functional group, while the absorption band at wave number 1654.92 cm^{-1} shows the presence of a group C=O amide. In the wave number absorption band 1012.63 cm^{-1} belongs to the functional group C-O-C.

Conclusion

The optimum condition of the deacetylation of chitin from rice conch shells was 60 % NaOH concentration, deacetylation temperature 150 °C, deacetylation time 4 h and the ratio of NaOH solution to chitin 1:10 obtained 83.23 %.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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