Characteristics of Chitooligosaccharides Hydrolysate from Crab Shell (Portunus Pelagicus) Waste by Chitosanase Hydrolysis

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ABSTRACT

Enzymatic hydrolysis of chitooligosaccharides can be carried out using the chitosanase enzyme. Chitosanase enzyme is a glycosyl hydrolase enzyme that catalyzes the hydrolysis of β-1,4 glycosidic bonds of chitosan to produce low molecular weight chitooligosaccharides. The crab shell (Portunus pelagicus) has the potential to be used as chitooligosaccharides because the crab shell contains 20-30% chitin. This study aimed to determine the effect of chitosanase enzyme concentration and duration of hydrolysis on the characteristics of chitooligosaccharides (COS) from crab shells. This research was conducted using a factorial completely randomized design (CRD) method with two factors and two replications. The factor I was enzyme concentration (0.5%, 1%, and 1.5%), factor II was hydrolysis time (3 hours, 4 hours and 5 hours). The data were obtained using ANOVA if there was a significant difference, followed by Duncan’s Test (DMRT). The best results were obtained in the A2B2 treatment, enzyme concentration (1.0%) and hydrolysis time (4 hours) with a chemical composition of 84.96% yield, 4.83 KDa molecular weight, 86.87% degree of deacetylation and FTIR test results indicated the availability of a functional group, the OH group was obtained at a wavelength of 3624.25 cm⁻¹ and the NH group is at a wavelength of 3404.36 cm⁻¹.

Keywords: chitooligosaccharides, chitosanase, crab shell, hydrolysis.

INTRODUCTION

Chitin is a polymer that is insoluble in water and weak acids, while chitosan is chitin in the form of partially deacetylated (more than 50%) or 100% free of acetyl, which has better properties than chitin in solubility and functional properties. Chitosan is soluble in weak acids and has a wider application than chitin because it has more active groups. However, because it is only soluble in weak acids, the application of chitosan also has limitations in industry. Chemical modification of chitosan with the addition of a hydroxyl group (hydrophilic) chemically so that it becomes carboxyl methyl chitosan which is water-soluble, is a strategy in utilizing chitosan (Yusro Nuri Fawzya et al., 2009).

According to several researchers studying on chitooligosaccharides (COS) that compounds derived from chitosan resulted in the chitin deacetylation process, which is a complex compound of the glycoprotein group that has β-1,4 glucosamine bonds (Agnes Sri Harti, 2011); and the chitosan (1, 4) glycosidic bonds chain cutting several techniques such as chemical degradation, enzymatic, and mechanical treatmen (Singh, 2016). Enzymatic hydrolysis is specific, is controlled, is produced chitosan oligomers with higher polymer degrees and less glucosamine which are environmentally friendly (Sarni et al., 2016). In the opinion of other researchers the chitosanase enzyme is a glycosyl hydrolase that catalyzes the hydrolysis of β-1, 4 glycosidic bonds of chitosan to produce glucosamine and low molecular weight COS (Heggset, 2012) and (de Assis et al., 2010).

Chitooligosaccharides (COS) are compounds derived from chitosan resulted from the deacetylation of chitin with complex compounds belonging to the glycoprotein group that has 1, 4 glucosamine bonds (Listiyana, 2018). In addition, COS has chains of 20 or less and is water-
soluble chitosan (Dewi & Fawzya, 2006). COS has a unique polycationic nature that it has polycationic which can protect proteins and suppress the growth rate of pathogenic bacteria so that it has the potential as an 'alternative antibiotic' which has safer value without causing residue (A S Harti, 2007).

According Peng et al. (2013), the characteristic of COS derived from shrimp shells obtaining a yield of 14.56 g / 100 g of weight of shrimp shells. COS showed high water solubility as much as 0.97 g /100 g water. Then for the molecular weight obtained by 13 KDa and the degree of COS deacetylation of 54.83%. Research by Yusro Nuri Fawzya et al., (2009) showed that hydrolyzed chitooligosaccharides using cellulase enzymes (as chitosanase) at concentrations of 10, 13, and 15 U/g with hydrolysis time of 1, 2 and 3 hours produced COS with a viscosity of 2.15 Cps and an average molecular weight of 323.76 Da.

Sánchez et al. (2017) tested on the COS molecular weight of shrimp hydrolyzed using a commercial chitosanase enzyme with a concentration of 1% with a hydrolysis time of 4 hours. The results showed that the molecular weight was 5.6 KDa and the degree of deacetylation was 11%.

Several studies on testing of the characterization of chitoologosalidalita from crab waste are still very limited. Therefore, this study aimed to determine the characterization of chitoologosalidalita from crab waste by treating the concentration of chitosanas enzyme and the duration of hydrolysis of crab shells on the resulting chitooligosaccharides.

METHODS

Material and Tools

The materials used were crab shells obtained from the PT. Kelola Mina Laut and the chitosanase enzyme used GF- Chitosanase 51570-20-8. The materials for the analysis of the characteristics of chito oligosaccharides of crab shell were 2% CH₃COOH, 4% NaOH, 60% NaOH, 1.25 N HCl, and distilled water. The tools used in this research included tools for processing and analysis. Tools for processing included drying cabinet 100 mesh sieve, baking sheet, and disk mill. Tools for analysis included analytical balance, kiln, volumetric flask, oven, volumetric pipettemicropipee, and FTIR (Fourier Transform Infra Red) to determine the COS functional group (Muyonga et al., 2004).

Research design

This method was developed using a completely randomized design (CRD) with two-factor factorial pattern, involving 0.5% chitosanase enzyme concentrations; 1.0%; 1.5%, and hydrolysis time of 3 hours; 4 hours, 5 hours so that 9 treatment combinations were obtained, each of which was repeated twice. The data were analyzed by analysis of variance (ANOVA). If there was a significant difference between the treatments, the Duncan test (DMRT) is continued at the 95% level. The results obtained are then analyzed to determine the effectiveness index to get the best product. Determination of the optimum formula was based on performances of the chemical using an effectiveness index (DeGarmo et al., 1997).

Production of Chitooligosaccharides

Crab shells are selected by choosing materials that match the standard. Then the crab shells were weighed. Next, the crab shells were washed and drained an oven at 100°C for 2 hours. The dried shells were crushed in a blender for 2 minutes until a smooth texture was formed then 100 mesh sieving was carried out, the next process was chitin extraction.
**Demineralization Process**
A total of 100 g of crab shell powder was put into 2 L glass beaker, added 1.5 L of 1.5 M HCl solution in a ratio of 1:15 (w/v). Those ingredients were heated on a hotplate at 70°C for about 4 hours and stirred it with a magnetic stirrer. Then, It was filtered. To obtain insoluble precipitate then washed with distilled water until the supernatant was neutral. It was dried in the oven for 24 hours at 70°C.

**Deproteination Process**
A total of 23 g of demineralized powder was then added with 230 ml of 3.5% NaOH solution in a ratio of 1:10 (w/v). The mixture was heated at 70°C for 4 hours while stirred with a magnetic stirrer then filtered and the precipitate was washed with distilled water until the supernatant was neutral. Dry in the oven for 24 hours at 70°C, and then carry out the process of taking chitosan

**Deacetylation Process**
As many as 17 g of deproteinized powder was added with 170 ml 50% NaOH solution in a ratio of 1:10 (w/v). The mixture was heated at 100°C for 4 hours while stirred with magnetic stirrer. It was filtered and the precipitate was washed until the filtrate was neutral. Dried in the oven for 24 hours at 70°C. The resulted chitosan was then characterized by FT-IR.

**Chitoooligosaccharide Manufacturing Process**
As many as 5 g of chitosan was dissolved in 500 ml of 1% acetic acid and stirred for 10 minutes and the pH was adjusted at 7 ± 0.5. Chitosanase enzyme concentrations of 0.5%, 1%, and 1.5% were added to the chitosan solution and hydrolyzed at 60°C for 3, 4, and 5 hours. The enzyme was inactivated at 100°C for 5 minutes. The results were centrifuged for 15 minutes at 9000 rpm to obtain a supernatant containing chitooligosaccharides. The results of the hydrolysis were stored in a refrigerator then identified and other tests were carried out.

**Chitoooligosaccharide Analysis**
Yield is one of the important parameters in determination of the final weight of a material after the production process. The percentage (%) by weight of dissolved was calculated by the formula:

\[
Yield (\%) = \frac{\text{Weight COS (g)}}{\text{Chitosan Sample Weight (g)}} \times 100
\]

The degree of deacetylation was analyzed using an IR spectrophotometer. The sample was made into pellets with 1% KBr, then scanned in the frequency range between 4000 cm\(^{-1}\) – 400 cm\(^{-1}\). The degree of deacetylation of chitosan can be determined by the "Base Line" method. The calculation of the degree of deacetylation (DD) was by comparing the absorbance value at a wavenumber of 1655 cm\(^{-1}\) (amide absorption band) with a wavenumber of 2450 cm\(^{-1}\) (hydroxyl band absorption). The ratio of absorbance at 1655 cm\(^{-1}\) with absorbance at 3450 cm\(^{-1}\) was doubled one by one with the standard N-diacetyl chitosan (1,33). The measurement of the degree of deacetylation can be calculated using the formula:

\[
N - \text{deasetyl} (\%) = \left[1 - \left(\frac{A_{1655}}{A_{3450}} \times \frac{1}{1.33}\right)\right]
\]

The molecular weight of COS was determined from the calculation of the intrinsic viscosity value, then calculated using the Mark-Khun Houwing equation (Wang et al., 2010). The
calculations have been adjusted to the provisions of Mark-Khun Houwing for chitosan polymers. Calculation of the molecular weight of chitosan is presented in the following equation:

\[ M = \frac{\text{antilog} ([\eta] - \log k)}{\alpha} \]

**Determination of the Best Treatment**

The best treatment was determined based on the effective index method (DeGarmo et al., 2003). This method was based on the following procedure: variables were sorted by priority and contribute to the results. It should be given a burden value to each variable (BV) according to its contribution with a relative number of 0-1. These weights vary depend on the importance of each variable which results were obtained as a result of the treatment. Normal weight (BN) was determined from each variable by dividing the variable weight (BV) by the sum of all weighted values. Devided the analyzed variables into two groups, namely: 1. Group A, consisted of variables in which the larger the average the better the value (desirable for processed products). 2. Group B consisted of variables that were getting worse (unwanted). The effectiveness value (NE) of the variable was determined, using the formula: treatment value - the worst value and the best value - the worst value, for the variable with a larger average is better, that the lowest value is the worst value and the highest value is the best. Besides that, for variables with smaller values, the better, the highest value being the worst value and the lowest value being the best. Calculate the yield value (NH) of each variable obtained by multiplying the normal weight (BN) with the effectiveness value (NE). So that the lowest value is the worst value and the highest value is the best. On the other hand, for variables with smaller values, the better, the highest value being the worst value and the lowest value being the best. Calculate the yield value (NH) of each variable obtained by multiplying the normal weight (BN) with the effectiveness value (NE). The amount of the result values of all variables then be calculated. The best combination was selected from the treatment combination that has the highest yield value (NH)

\[ N \text{ Effectiveness} = \frac{\text{treatment value} - \text{the worst value}}{\text{the best value} - \text{the worst value}} \]

Result value = NE x weight

**RESULTS AND DISCUSSION**

**Enzyme Activity of Chitosanase**

Table 1. The results of the analysis of enzyme activity of chitosanase

<table>
<thead>
<tr>
<th></th>
<th>Analysis Results</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosanase Activity (Unit/g)</td>
<td>38,000</td>
<td>36,212 U/g a)</td>
</tr>
</tbody>
</table>

a) (Alain Gohi et al., 2016)

The results of testing the activity of the chitosanase enzyme can be seen in Table 1. According to Uhlig (1998), the increasing of enzyme activities was proportional to the increasing of
temperature until reaching expected optimum temperature. This is because the kinetic energy of enzyme molecules increases before reaching the optimum. In addition, microbial chitosanase produced relatively higher COS yields, compared to enzymes from other sources (Kim et al., 2007). The higher decrease in chitosan viscosity (up to 99.5%) at 16 hours of hydrolysis use a similar enzyme (activity 36.212 U/g) on a larger scale (10-15 times capacity) (Alain Gohi et al., 2016). Hydrolysis of chitosan (DD 74.6%) using pepsin reduced the viscosity by 86% after 1 hours (Yusro Nuri Fawzya et al., 2019).

### Chemical Properties of Chitosan

<table>
<thead>
<tr>
<th>Component</th>
<th>Analysis Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield (%)</td>
<td>5.17 ± 0.41</td>
</tr>
<tr>
<td>Deacetylation degree (%)</td>
<td>75.60 ± 0.07</td>
</tr>
<tr>
<td>Molecular weight (Da)</td>
<td>178.41 x 10³ ± 0.01</td>
</tr>
</tbody>
</table>

Based on Table 2, the yield of chitosan obtained in this study was 5.17%. This result was different from the result of the research by (Renuka et al., 2019) by 4.4%. This was probably due to differences in temperature and heating time during the demineralization process, differences in temperature and duration of the deacetylation process, as well as the length of the drying process of chitosan. In the deproteination process, were dissolved in 1.5 M hydrochloric acid solution. After that, were heated at different temperatures, namely to crab shell, in this study at a temperature of 70°C for 4 hours, while in the study of (Renuka et al., 2019) using shrimp shell material at a demineralization temperature of 36°C for 30 minutes. The difference in temperature and duration of the demineralization process can affect the difference in yield results. Maryati et al., (2016) stated that the yield decrease with temperature treatment and the length of the demineralization process increases. During the demineralization process, calcium compounds react with hydrochloric acid which is soluble in water. Crustaceae shells contain minerals calcium carbonate (CaCO₃) and calcium phosphate (Ca₃(PO₄)₂). These minerals can be removed from the matrix using HCl solution (Aisyah, 2012).

Based on the data shown in Table 2, deacetylation degree of crab shell chitosan was 75.6 ± 0.07%, while the degree of deacetylation of shrimp shell in (Renuka et al., 2019) was 76.43 ± 0.91% This indicates that the amine content contained in the crab shell lower than shrimp shells. The deacetylation process in this study used a temperature of 100°C with a processing time of 4 hours, while in the study of (Renuka et al., 2019) used a temperature of 900°C with a processing time of 3 hours. In other opinion of researcher stated that the higher of heating temperature and the longer oh the reaction time, causing the higher the degree of deacetylation (Omali et al., 2015).

This difference could be caused by the initial difference in the value of the degree of deacetylation of chitin before the formation of chitosan. In this study, the degree of deacetylation of chitin in crab shells was 62.71 ± 0.31%, while in the study of Renuka et al. (2019) the degree of deacetylation of chitin was 69.21 ± 0.72%.

The next parameter, namely the molecular weight of chitosan obtained in this study was shown in Table 4. The molecular weight of crab chitosan was 178.41 ± 0.35 kDa which is different from the research by (Renuka et al., 2019), the molecular weight of shrimp shell chitosan was 110.64 ± 1.65 kDa. This result was not in accordance with the research of (Sularsih, 2013) that the value of the molecular weight increases in line with the increase in temperature and deacetylation time. The deacetylation process that uses high temperatures can cause a polymer to depolymerization and in turning also cause the breakdown of polymer molecular chains,
thereby reducing viscosity and molecular weight (Bastaman, 1989 in Junaid et al., 2019). This difference could be caused by differences in the raw materials used, this study used crab raw materials while the Renuka et al (2019) study used shrimp shells. According to Sularsih (2013) the molecular weight of chitosan can be grouped into three types, namely low, medium and high molecular weight. Chitosan with low molecular weight has a molecular weight of 150 kDa, medium molecular weight chitosan is 600 kDa and has a molecular weight of 1250 KDa.

### Chemical Properties of Chitooligosaccharide

Table 3. The average value of yield, molecular weight, deacetylation degree content of chitooligosaccharide

<table>
<thead>
<tr>
<th>Enzyme Concentration</th>
<th>Hydrolysis Time</th>
<th>Yield (%)</th>
<th>Molecular Weight (kDa)</th>
<th>Deacetylation Degree (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 Hour</td>
<td>4 Hour</td>
<td>5 Hour</td>
<td></td>
</tr>
<tr>
<td>0.5%</td>
<td>79.85±0.34^a</td>
<td>82.11±0.26^b</td>
<td>83.82±0.04^b</td>
<td>5.34±0.02^a</td>
</tr>
<tr>
<td>1.0%</td>
<td>83.89±0.03^c</td>
<td>84.96±0.33^c</td>
<td>85.61±0.84^c</td>
<td>4.90±0.08^b</td>
</tr>
<tr>
<td>1.5%</td>
<td>85.63±0.13^c</td>
<td>86.45±0.39^c</td>
<td>87.05±0.44^d</td>
<td>4.69±0.01^b</td>
</tr>
</tbody>
</table>

*Note: Different notations show a significant difference (p≤0.05).*

Based on the Table 3, the yield content of the chito oligosaccharide ranged from 79.85% to 87.05%. Based on the analysis of variance, it can be seen that there was a significant interaction (p≤0.05). The higher the enzyme concentration and the duration of the hydrolysis time, the higher the yield of chitooligosaccharides.

Chitosanase enzyme with a certain concentration can break the acetyl group chain, causing the yield value to increase. According to (Siagian, 2002) the yield increase with temperature treatment, and the length of the process of neutralization increases. During the demineralization process, calcium compounds react with hydrochloric acid which is soluble in the water. Crustacea shells contain minerals calcium carbonate (CaCO₃) and calcium phosphate (Ca₃(PO₄)₂). These minerals can be removed from the matrix using HCl solution.

The longer the hydrolysis time, the higher the yield, which indicates the hydrolysis process was more effective. According to Rokhati et al. (2017) which states that the efficiency and effectiveness of the raw material extraction process in the manufacture of chitosan can be seen from the yield value produced. The greater the yield produced, the more efficient the treatment applied.

Based on the analysis of variance (Table 3), there was a significant interaction (p≥0.05). The higher concentration of the chitosanase enzyme and the duration of hydrolysis, the lower the molecular weight. This was because of the breakdown of chitosan and the duration of hydrolysis. Then, the 1,4 beta glycosidic bonds were cut more and more this causes the viscosity to decrease. So that the molecular weight also decreases. This is in the statement of (Heggset, 2012) and (de Assis et al., 2010), the chitosanase enzyme is a glycosyl hydrolase that catalyzes the hydrolysis of 1,4 glycosidic bonds of chitosan to produce glucosamine and produce low molecular weight COS.

Based on Table 3, the degree of deacetylation ranged from 86.24% to 88.64%. Based on the analysis of variance, it can be seen that there was a significant interaction (p≥0.05). The higher
the concentration of chitosanase enzyme and the duration of hydrolysis, the degree of deacetylation increases. This was due to the high amine content in crab shell chitosan, which increases the degree of deacetylation. From the results obtained, the higher the enzyme concentration, the higher the degree of deacetylation in chitooligosaccharides, this was because the percentage of the acetyl group in chitosan is eliminated by the chitosanase enzyme, resulting in chitooligosaccharides containing amines, as well as the duration of hydrolysis. The longer the hydrolysis time, the process of forming chitooligosaccharides will occur in the process of breaking the double bond. In this process, the OH group enters the NHCOCH3 group, and then the elimination of the CH3COO group results in an amine, namely chito oligosaccharides. According to (Sartika et al., 1996) statement in (Agusnar, 2007), in determining the degree of deacetylation, the amide and hydroxyl absorption bands play an important role. The higher the amine content contained in chitosan, the higher the degree of deacetylation produced. Based on the analysis of chitosan, it was known that the degree of deacetylation of gold snail shell chitosan was 77.38%, this result is higher than the research of (Panggalo et al., 2016), namely the deacetylation degree of chitosan of mangrove snail shells 64%.

**Determination of the Best Treatment**

Determination of the best treatment that can be seen in Table 4 was carried out by scoring method. The data needed for decision analysis was the aspect of quantity: yield, molecular weight, and degree of deacetylation. Determination was done by giving a score on each average of the results of the response analysis and giving a rank for each sample that has the lowest to the highest score. The sample with the highest score was the sample with the highest rating and was automatically the product of choice. The selected sample was analyzed by FTIR.

<table>
<thead>
<tr>
<th>No</th>
<th>Treatment</th>
<th>Yield (%)</th>
<th>Molecular Weight (kDa)</th>
<th>Deacetylation Degree (%)</th>
<th>Total Score</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td>Score</td>
<td>Average</td>
<td>Score</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>a1b1</td>
<td>79.85</td>
<td>5</td>
<td>5.34</td>
<td>1</td>
<td>86.24</td>
</tr>
<tr>
<td>2</td>
<td>a1b2</td>
<td>82.11</td>
<td>4</td>
<td>5.04</td>
<td>1</td>
<td>86.25</td>
</tr>
<tr>
<td>3</td>
<td>a1b3</td>
<td>83.82</td>
<td>3</td>
<td>4.97</td>
<td>3</td>
<td>86.39</td>
</tr>
<tr>
<td>4</td>
<td>a2b1</td>
<td>83.89</td>
<td>3</td>
<td>4.90</td>
<td>3</td>
<td>86.50</td>
</tr>
<tr>
<td>5</td>
<td>a2b2</td>
<td>84.96</td>
<td>3</td>
<td>4.83</td>
<td>4</td>
<td>86.87</td>
</tr>
<tr>
<td>6</td>
<td>a2b3</td>
<td>85.61</td>
<td>2</td>
<td>4.77</td>
<td>4</td>
<td>87.78</td>
</tr>
<tr>
<td>7</td>
<td>a3b1</td>
<td>85.63</td>
<td>2</td>
<td>4.69</td>
<td>4</td>
<td>88.03</td>
</tr>
<tr>
<td>8</td>
<td>a3b2</td>
<td>86.45</td>
<td>2</td>
<td>4.56</td>
<td>5</td>
<td>88.42</td>
</tr>
<tr>
<td>9</td>
<td>a3b3</td>
<td>87.05</td>
<td>1</td>
<td>4.41</td>
<td>5</td>
<td>88.64</td>
</tr>
</tbody>
</table>

A1 = enzyme concentration 0.5%, A2 = enzyme concentration 1%, A3 = enzyme concentration 1.5%, B1 = hydrolysis time 3 hours, B2 = hydrolysis time 4 hours, B3 = hydrolysis time 5 hours

Based on the Table 4, the results of the analysis carried out, COS with enzyme concentration treatment (1.0%) and hydrolysis time (4 hours) was the product with the best treatment with chemical composition (A2B2), namely yield of 84.96%, molecular weight 4.83 kDa, degree of deacetylation 86.87%. This alternative then be continued with functional group of COS analysis.
The Functional Group of COS Analysis

Based on the determination of the selected product through the scoring test, it was known that the A2B2 sample, namely the treatment of enzyme concentration (1.0%) and hydrolysis time (4 hours) becomes the selected product to be tested for the functional group.

FTIR test results show the spectrum of chitooligosaccharides at a wavelength of 400-3700 cm\(^{-1}\) can be seen in Figure 1.

![Figure 1. FTIR functional group of chitooligosakarida](image)

Based on Figure 1, chitooligosaccharides have a typical FTIR spectrum absorption band on the OH- and NH functional groups of amines. The results of the OH- functional group from the FTIR absorption spectrum in this study showed a peak wavelength of 3624.25 cm\(^{-1}\). There is not much difference from the results of S, namely the OH- group has a wavenumber of 3423.41 cm\(^{-1}\). According to Matheis (Zulaikah et al., 2014) that the absorption of OH a wavelength of 3650-3100 cm\(^{-1}\). For a wavelength of 1633.71 cm\(^{-1}\) in this study, it showed the presence of amide I and amide II groups. This amide group was also found in Sarni et al. (2016) on chitooligosaccharides from tiger prawns at wavelengths of 1658 cm\(^{-1}\), 1625 cm\(^{-1}\), and 1554 cm\(^{-1}\). The NH stretching functional group in this study shows the wavenumber at the peak of 1271.09 cm\(^{-1}\); 1157.29 cm\(^{-1}\) and 1078.21 cm\(^{-1}\). Ridho et al. (2017) states that the wavelength of NH stretching is in the range of 1180-1360 cm\(^{-1}\). The stretching vibration of the –COC- group appears at the absorption wavelength of 1091.75 which indicates the presence of β-1,4 glycosidic bonds that have not been hydrolyzed. According to Thadathil & Velappan (2014), the enzymechitosanase cuts β-(1,4) glycosidic bonds from the interior of the chitosan structure and produce chitosan oligomers with chain lengths of 2-10. These results were not much different from the results of Rokhati et al.(2017) that wavelength –COC- is 1072.3 cm\(^{-1}\).

CONCLUSION

The results of statistical analysis of chitooligosaccharides from crab shells showed that, there was an interaction between enzyme concentration which was significant (p≤0.05) in the treatment and duration of hydrolysis on yield parameters, molecular weight, and degree of deacetylation. Based on the results of decision analysis, the best treatment of chitooligosaccharides from crab shells that can be accepted by consumers was the treatment of enzyme concentration (1%) and hydrolysis time of 4 hours with a yield of 84.96%, molecular weight 4.83 kDa, degree of deacetylation 86.87%.
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REFERENCES


