International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.12 No.1, pp 112-120, 2019

Preparation of Chitosan with Various Molecular Weight and Its Effect on Depolymerization of Chitosan with Hydrogen Peroxide using Conventional Technique

Matheis F.J.D.P. Tanasale*, Catherina M. Bijang, and Ervina Rumpakwara

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Pattimura University, Ambon, Indonesia

Abstract : Depolymerization of chitosan with hydrogen peroxide (H_2O_2) using conventional techniques has been carried out by varying the molecular weight of chitosan. This study was aimed to determine the optimum conditions of chitosan depolymerization and the effect of molecular weight on the preparation of oligochitosan. Chitosan was produced with different molecular weights in 5% acetic acid with variations of heating time for 2, 12, 24 and 24 h. The molecular weight of chitosan was determined by the viscometry method. Chitosan produced from commercial chitin hadthe molecular weight of $13.30x10^4$ (CC-2), $12.02x10^4$ (CC-12), $7.75x10^4$ (CC-24) and $4.35x10^4$ g / mol (CC-120) for 2, 12, 24, and 120 h of heating time, respectively. The results indicated that the properties of produced chitosan are affected by heating time. The longer the heating time, the lower the molecular weight of chitosan would be. Furthermore, chitosan was depolymerized by varying the concentration of H_2O_2, temperature, and depolymerization time using conventional techniques. The depolymerization process of chitosan with H_2O_2 was influenced by H_2O_2 concentration and heating temperature where the optimum conditions of depolymerization of chitosan using H_2O_2 were obtained for CC-12 when the process was conducted using H_2O_2 concentration of 4%; at 30°C, for 4 h to give the corresponding oligochitosan with the molecular weight of $4.2x10^3$ in 97.57% yield.

Keywords : *chitosan, hydrogen peroxide, molecular weight, oligochitosan.*

I. Introduction

In Indonesia, shrimp is generally exported as frozen product which has been removed from the head, tail and skin. The skin contains protein (25-40%), chitin (15-20%) and calcium carbonate (45-50%)[1]. Chitin consists of monomer of 2-acetamido-2-deoxy-β-D-glucose which is associated to 1,4-glycosidic bond[2]. Chitin (Figure 1) is not soluble in water, which limits its application. By modifying the chemical structure of chitin, the chitin derived products, such as chitosan,whichposses better chemical properties, will be obtained[3].

Matheis F.J.D.P. Tanasale *et al* /International Journal of ChemTech Research, 2019,12(1): 112-120.

DOI= http://dx.doi.org/10.20902/IJCTR.2019.120113

Chitosan (poly-2-amino-2-dioxy-β-D-glucose) can be obtained via partial or complete deacetylation of chitin. The properties of chitosan (Figure 2) depend not only on the changes in structure but also on molecular weight. The applications of chitosan are still not optimal since the length of the chain with high molecular weight leads chitosan to be difficult to dissolve in water[4]. In fact,the solubility is one of important characteristics for chitosan. The increase of solubility will facilitate chitosan to be used more widely in the fields of food, agriculture, pharmacy, and other technical applications. High molecular weight results in chitosan with low solubility in water. This has led to the development of several attempts to cut chitosan bonds to giveshorter oligomers called oligochitosan.

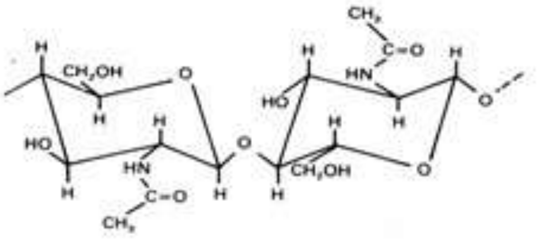

Figure 1. Structure of chitin

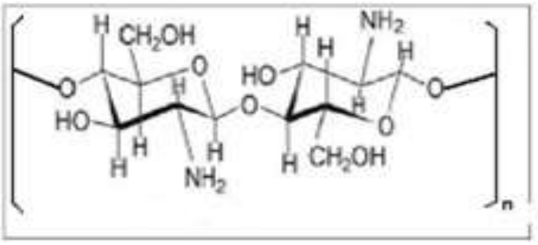

Figure 2. Structure of chitosan

Oligochitosan can be produced from chitosan via the biochemical depolymerization of chitosan using enzymes[5,6]or chemical depolymerization using acids such as HCl, H_2SO_4, and CH_3COOH[7,8,9]. Additionally, the chitosan depolymerization process can be also carried out in the presence of oxidant such as O_3, $NaNO_2$, and H_2O_2[10 - 17].It has been also reported that sodium hypochlorite (NaClO) can be used to produce chitosan with lower molecular weights or oligochitosan[18].Among the reagents aforementioned, hydrogen peroxide is more widely used because it is easy to handle, easy to obtain, and more environmentally friendly[4,13]. In order to effectively and efficiently get oligochitosan, some researchers have combined hydrogen peroxide using microwave irradiation[19,20], gamma ray radiation[21], ultraviolet light[22], ultraviolet-irradiated oxygen[23], heteropoly acid[24,25], and ultrasonic[26].

The reported studiesput more attention on the effect of H_2O_2 concentration, time and temperature of depolymerization without seeing the effect of chitosan properties such as molecular weight and degree of deacetylation. In fact, by understanding the influence of molecular weight and deacetylation degree of chitosan, the use of oligochitosan will be more applicable. In this study, the molecular weight of chitosan, which was converted from commercial chitin, would be varied. The chitosan would be depolymerized into oligochitosan with H_2O_2using conventional techniques. The purposes of this study were to determine the optimum conditions of chitosan depolymerization and the effect of chitosan molecular weight on depolymerized chitosan.

2. Experimental

2.1. Chemical and Instruments

Chemicals used in this study were commercial chitin (Sigma Aldrich), CH_3COOH p.a (E. Merck), NaOH p.a (E. Merck), HCl p.a (E. Merck), H_2O_2 p.a (E. Merck), ethanol p.a (E. Merck), $K_3Fe(CN)_6$ p.a (E.

Merck), Na_2CO_3 p.a (E. Merck), D-glucosaminehydroxide (GAH) (Sigma Aldrich), distilled waterandfilter paper Whatman No. 42.

The instruments used in this study included UV-Vis spectrophotometer(PD-3035) and FTIR spectrophotometer (Prestige 21 Shimadzsu).

2.2. Experimental Procedures

2.2.1. Conversion of chitin into chitosan

The conversion of chitin into chitosan was carried out using No and Meyer method[27]with slight modification. Commercial chitin powder (30 g) was mixed with 300 mL of 50% aqueous NaOH solution. The mixture wasstirred and heated at 100 ^{o}C for 6 h with a ratio of chitin and solvent of 1:10 (m/v). Then, the mixture was filtered and washed with distilled water until the pH was neutral. Residue of chitosan was dried in the oven at 65 ^{o}C for 24 h then weighed. The obtained chitosan was characterized by FTIR spectrophotometer.

2.2.2. Preparation of chitosan with various molecular weights[28]

Chitosan (5 g) was dissolved in 95 mL of 5% acetic acid solution. The mixture was then heated at 50 ^{o}C with variations in heating time of 2, 12, 24 and 120 h. Next, the mixture was cooled and centrifuged at 5000 rpm for 20 min. The supernatant was added with 4 N NaOH until the pH was neutral. The precipitate was filtered and washed with distilled water, then dried in the oven at 50 ^{o}C. The molecular weight of chitosan was determined by the viscometry method and deacetylation degree was determined by UV-Vis spectrophotometry method. Then, the chitosan was characterized by FTIR spectrophotometer.

2.2.3. Determination of molecular weight of chitosan using viscometry method

As much as 0.1 g of various molecular weights of the prepared chitosan was dissolved in 100 mL of 0.01 M HCl solution.The concentration was variedto give the concentration of 0.01%, 0.02%, 0.03%, 0.04% and 0.05 %. Then, 5 mL of each solution was put into the viscometer and the flow time was determined. Chitosan molecular weight was calculated based on Equations 1 and 2 with K and a values for chitosan with 0.01M HCl solvent at 30 °C were 5.48 x 10^{-4} and 0.72, respectively[29].

$$[\eta] = \lim_{c\to 0} \frac{\eta sp}{c} \qquad \ldots(1)$$

$$[\eta] = KM_v^{a} \qquad \ldots(2)$$

Determination of deacetylation degree of chitosan

Determination of deacetylation degree (DD) of chitosan was carried out using Liu *et al.* method[30] with slight modification. As much as 3 mg of chitosan was dissolved in 50 mL of 0.01 M acetic acidsolution. The absorbance of the solution was measured by UV-Vis spectrophotometer at wavelength of 201 nm. The degree of deacetylation is calculated based on Equations 3 and 4.

$$DA = \frac{161,.1.A.V - 0.0218\ M}{3.3615\ M - 42.1.A.V} \qquad \ldots (3)$$

$$DD = (1-DA) \times 100\% \qquad \ldots (4)$$

Preparation of oligochitosan from chitosan using H_2O_2[31]

Into 1.0 g of chitosan was added 20 mL of 2.0% acetic acid solution. The mixture was heated with H_2O_2 concentration of 4, 5 and 6% with the heating time of 2, 4 and 6 h at 30, 40, 50, and 60 ^{o}C. Then, the solution was cooled, neutralized with 10% NaOH solution. The residue was separated by filtration, and the filtrate was added with 2 parts by volume of ethanol. Water-soluble chitosan (oligochitosan) will be obtained after being left overnight. It was dried in the oven at 50 ^{o}C, and was weighed. The yield of oligochitosan was calculated based on Equation 5.

$$\% \text{ yield} = \frac{weight\ of\ water{-}solubled\ chitosan}{weight\ of\ crude\ chitosan}\ x\ 100\% \qquad \ldots (5)$$

Determination of molecular weight of oligochitosan using the end group analysis

Determination of molecular weight of oligochitosan using the end group analysis was performed using Shao *et al* method[19] with slight modification. The coloring reagent was prepared by dissolving 0.5 g of $K_3Fe(CN)_6$ in 1 L of 0.5 M Na_2CO_3 and was then stored in a brown reagent bottle. The standard solution was prepared by dissolving 1 g of D-glucosamine hydrochloride (GAH) in 100 mL of distilled water. Preparation of standard curveswas done by mixing 0.2; 0.4; 0.6; 0.8 and 1 mL of standard GAH solution, respectively with 2 mL of color reagent into the test tube, then the volume was adjusted to 5 mL with distilled water. The test tube was closed, heated in boiling water for 15 min, and cooled to room temperature. Then, the absorbance was measured by UV-Vis spectrophotometer at the wavelength of 420 nm with distilled water as blank. For the oligochitosan sample, 0.1 g of sample was added by 2 mL of the coloring reagent and put into a test tube. The volume was adjusted to 5 mL with distilled water, heated in boiling water for 15 min, cooled and measured by UV-Vis spectrophotometerat wavelength of 420 nm. The average molecular weight ($\bar{M}_n$)was calculated based on Equation 6.

$$\bar{M}_n = \frac{W_1}{W_2} x\ 215.5 \qquad \ldots (6)$$

3. Results and Discussion

3.1. Conversion of chitin into chitosan

First of all, chitin was converted into chitosan via the deacetylation reaction. The deacetylation process was aimed to remove the acetyl groups ($-COCH_3$) by using high concentrated alkaline solutions to generate the amine groups ($-NH_2$). Chitin has a long crystalline structure with strong hydrogen bonds between nitrogen atoms and carboxylic groups in adjacent chains[32].

This deacetylation process can be detected by evaluating the on both chitin and chitosan FTIR spectra at certain characteristic wavenumber. The functional groups which were characteristic for the chitin and chitosan can be seen in the FTIR spectra(Figure 3).

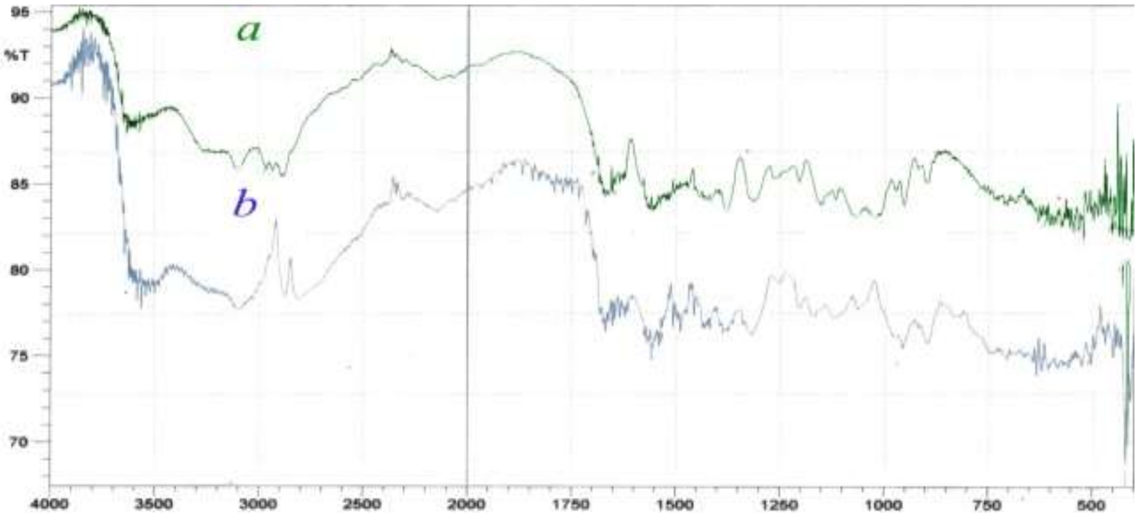

Figure3. FTIR spectra of (a) commercial chitin and (b) chitosan.

Figure 3 showed that most of the absorption bands of chitin were similar with those of chitosan. The band shifts were observed including NH(s) from 3242to 3280-3105, NH(b) from1563-1538to1556and C=O from 1668-1645 to1668-1645cm^{-1}andfrom 1061-1010to1101-1053 cm^{-1}. The shift of some absorption bands indicated that the deacetylation of chitin occurred to give chitosan.

3.2. Preparation of chitosan with various molecular weights

Solubility of chitosan in acetic acid indicated that the deacetylation process had been taken place. The solubility of chitosan was caused by the presence of hydrogen atoms of free aminewhich may facilitate interaction with water through hydrogen bonds. The presence of carboxyl groups in acetic acid will facilitate the solubility of chitosan via the formation of hydrogen bond between carboxyl groups and amine groups of both[33]. Chitosan were prepared by dissolving in 5% of acetic acid solutionwith the variation of heating time of 2, 12, 24 and 120 h, so that chitosan with various molecular weights will be produced. The prepared chitosan were then symbolized by CC-2, CC-12, CC-24, and CC-120 for heating times of2, 12, 24 and 120 h, respectively.

The viscous chitosan solution in acetic acid obtained after heating with various heating time, was then added 4 M of NaOH solution to form a white lump and the solution formed will coagulate[34]. The lump was filtered and neutralized using distilled water, then dried at 50^{o}C. The results showed that heating the chitosan for 2, 12, 24 and 120 h gave the product in 10.56, 16.78, 23.98 and 32.61 g, respectively. The molecular weight of chitosan was calculated using the viscometric method based on Equations 1 and 2 and the degree of deacetylation was determined based on Equations 3 and 4, and symbols were shown in Table 1.

Table 1. Molecular weight and deacetylation degree of chitosan

Term of Prepared Chitosan	Molecular Weight (g/mol)	Deacetylation Degree (%)
CC-2	1.3299 x 10^{5}	79.11
CC-12	1.2021 x 10^{5}	80.77
CC-24	7.7492 x 10^{4}	88.64
CC-120	4.3542 x 10^{4}	95.72

Table 1 showed that the longer the heating time led the increase of the molecular weight and the decrease of the deacetylation degree. The resultswere in accordance with previous studies, where the produced chitosan hadthe molecular weight of 9x10^{6} g/mol with deacetylation degree of 76%[35] and 9x10^{4} g/mol with deacetylation degree of 92%[36]. When compared with previous research by Liu, *et al.*[28], the molecular weight of chitosan preparedwith heating time of 2 - 145 hours ranged between 15.5x10^{4} - 5.5x10^{4} Da and the same deacetylation degree of 80% ± 0.29. The difference was presumably due to the method used for the determination of different molecular weights and deacetylation degrees.

The prepared chitosans were then analyzed using FTIR spectrophotometer (Figure 4) and have absorption bands displayed in Table 2.

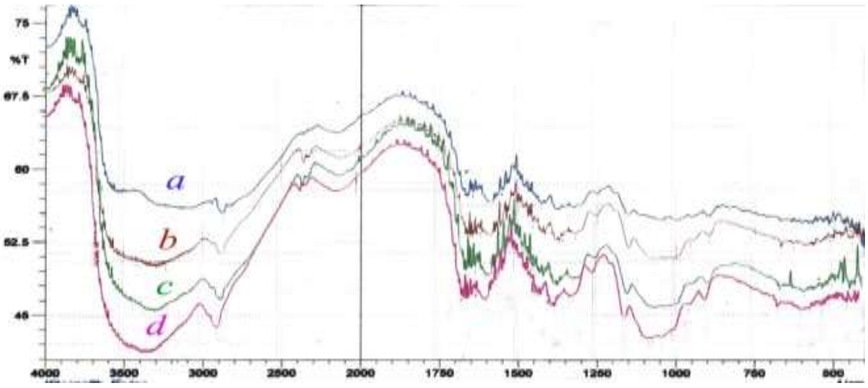

Figure4. FTIR spectra of (a). CC-2, (b). CC-12, (c). CC-24, and (d). CC-120

Table 2. FTIR absorption profile of the prepared chitosan

Term of Prepared Chitosan	Functional Group (wave number, cm^{-1})				
	NH (s)	CH	C=O	NH (b)	C-O
CC-2	3155.54-3116.97	3093.82-2873.94	1658.70-1643.35	1566.20-1552.70	1041.56-1031.92
CC-12	3271.27-3234.62	2881.65	1665.50-1651.07	1556.55 -1537.27	1064.71-1010.70
CC-24	3296.35-3217.27	2881.65	1666.50-1651.07	1556.55-1537.27	1060.85-1012.63
CC-120	3294.42-3194.12	2883.58	1666.50-1651.07	1556.55-1537.27	1072.42-1035.77

Based on Table 2, it can be shown that the FTIR spectra of chitosans with variations in heating time displayed similar pattern. For example,the absorptions of NH (s), C = O, NH (b), and CO were notshifted. While CC-12, CC-24 and CC-120 had the same absorption for NH (b) functional groups, CC-12 and CC-24 displayed the same absorption for for C=O groups.

3.3. Depolymerization of chitosan to produce oligochitosan

The depolymerization of chitosan, which was performed using H_2O_2,generated oligochitosan with a reaction mechanism according to Tian *et al.*[14] which begins with an equilibrium system in Reactions (7) and (8) and a total reaction as in Reaction (9).

$R\text{-}NH_2 + H^+ = R\text{-}NH_3^+$ .... (7)
$H_2O_2 = H^+ + HOO^-$ .... (8)
$R\text{-}NH_2 + H_2O_2 + H^+ = R\text{-}NH_3^+ + HOO^- + H^+$ .... (9)

Anion HOO^- is very unstable and very easily decomposes into highly reactive hydroxyl radicals (Reactions 10 and 11).

$HOO^- \rightarrow OH^- + O\bullet$ .... (10)
$H_2O_2 + HOO^- \rightarrow HO\bullet + O_2^- + H_2O$ .... (11)

Hydroxyl radical(HO•) is strong oxidants. The main chemical action of this radical with a polysaccharide is very fast as shown in Reactions (12) and (13). The hydroxyl radical attracts one H atom and forms water so that it forms a water-soluble oxidation product with a low molecular weight.Depolymerization of chitosan using H_2O_2gave the products with the yields as displayed in Table 3.

Table 3.Optimum conditions of depolymerization of chitosan, weight, yields and average molecular weight ($\bar{M}_n$) of oligochitosan

Term of Prepared Chitosan	Optimum Conditions	Weight Oligochitosan(g)	Yields (%)	$\bar{M}_n$ (g/mol)
CC-2	6%, 30 °C, 4 hours	1.00035	58.31	2404.37
CC-12	4%, 30 °C, 4 hours	1.00040	97.57	4187.31
CC-24	4%, 40 °C, 4hours	1.00045	63.70	3909.13
CC-120	5%, 30 °C, 4 hours	1.00050	50.03	2992.79

Optmization of process conditions was carried out for each preparation time based on the results of the study by taking into account to the concentration, temperature and time during the depolymerization process. Based on the yield of oligochitosan in Table 3, the optimum conditions of chitosan depolymerization process using H_2O_2were obtained when using H_2O_2 concentration of 4% at 30 °C for 4 h for CC-12 which produced oligochitosan with molecular weight of 4.2 x 10^3 g/mol and yield of 97.57%. When compared with research by

Du*et al*[31] which varied H_2O_2 concentration, time, and heating temperature, it indicated that each factor had a significant effect on oligochitosan results. The optimum conditions of chitosan depolymerization can theoretically be determined using Response Surface Methodology (RSM)[19]at H_2O_2concentration of 5.5% at 42.8 ^{o}C for 3.5 h in a yield of 93.5% of oligochitosan product.

3.4. Determination of molecular weight of oligochitosan using the end group analysis

Oligochitosansobtained from depolymerization were characterized for theiraverage molecular weight by the end group analysis method using UV-Vis spectrophotometer. The results can be seen in Table 3.The molecular weight of chitosan significantly decreased after depolymerization with H_2O_2. The molecular weight of chitosan CC-2 had decreased from 1.32986x10^5 to 2404.37 g/mol. Similarly, the molecular weight of chitosan CC-12, CC-24, and CC-120 also decreased. When compared with research conducted by Julianti[26]using 30% H_2O_2, the molecular weight of chitosan decreased from 1.49x10^5 to 3106.705 and 630.957. The difference was probablt due to the use of ultrasonic bath in the degradation process of chitosan.

Based on Table 3, for the optimum conditions of chitosan CC-2 with a high molecular weight of chitosan, the concentration of H_2O_2 used washigher with lower temperature and longer heating time. When compared with the low molecular weight of chitosan, the concentration of H_2O_2 used was small while the time and temperature were higher to break the β-glycosidic bond. Thus, the weight of the oligochitosan molecule produced for each molecular weight of chitosan preparation decreased. The decrease in molecular weight was due to the small size of the polymer chain, so the rate of movement of the transition was fast and the molecular weight became low[37].

4. Conclusion

Based on the results and discussion, it can be concluded that:

1. The optimum conditions of depolymerization of chitosan using H_2O_2 were obtained for CC-12 when the process was conducted using H_2O_2 concentration of 4%; at 30°C, for 4 h to give the corresponding oligochitosan with the molecular weight of 4.2x10^3 in 97.57% yield.
2. The molecular weight of chitosan preparation used for depolymerization has an effect on the molecular weight of oligochitosan, leading to the significant reduction in molecular weight of chitosan.

Acknowledgement

The authors would thank the research institute of Universitas Pattimura Ambon and Ministry of Research, Technology and Higher Education, Republic of Indonesia on the approval and finances of this research based on the letter of agreement of research work *Hibah Bersaing* 2015, number 045/SP2H/PL/DIT.LITABMAS/II/2015.

References

1. Kurniasih M, Kartika D.. Synthesis and characterization of physico-chemistry chitosan *Jurnal Inovasi*., 2011, 5; 42-48 (in Indonesian)
2. Abou-Shoer M. A simple calorimetric method for the evaluation of chitosan. *American Journal of Analytical Chemistry*., 2010, 2; 91-94
3. Manurung M. Potential of khitin / chitosan from shrimp skin as a water purifier biocagulant *Jurnal Kimia*., 2011, 5; 182-188 (in Indonesian)
4. Hien NQ, Phu DV, Duy NN, Lan NTK. Degradation of chitosan in solution by gamma irradiation in the presence of hydrogen peroxide. *Carbohydrate Polymers*., 2012, 87; 935-938
5. Qin C, Li H, Xiao Q, Liu Y, Zhu J, Du Y. Water-solubility of chitosan and its antimicrobial activity. *Carbohydrate Polymers*, 2006, 63; 367 – 374
6. Sarni, Natsir H, Dali S. Chitosan oligomer production from waste tiger shrimp (*Penaeus monodon*) using enzymes chitosanase of bacterial isolates *Klebsiella* sp. *Indonesian Journal of Chemical Research*, 2016, 3; 283 – 289 (in Indonesian)

7. Varum KM, Anthonsen MW, Grasdalen H, Smidsrod O.^{13}C-NMR studies of the acetylation sequences in partially *N*-deacetylated chitins (chitosans). *Carbohydrate Research*, 1991, 217; 19 – 27
8. Kabal`nova NN, Murinov KY, Mullagaliev IR, Krasnogorskaya NN, Shereshovets VV, Monakov VB, Zaikov, GE.. Oxidative destruction of chitosan under the effect of ozone and hydrogen peroxide. *Journal of Applied Polymer Science*, 2001, 81; 875 – 881
9. No HK, Nah JW, Meyers SP. Effect of time/temperature treatment parameters on depolymerization of chitosan. *Journal of Applied Polymer Science*, 2003, 87; 1890 – 1894
10. Allan GG, Peyron M. Molecular weight manipulation of chitosan I: kinetics of depolymerization by nitrous acid. *Carbohydrate Research*, 1995, 277; 257 – 272
11. Tanioka S, Matsui Y, Irie T, Tanigawa T, Tanaka Y, Shibata H, Sawa Y, Kono Y. Oxidative depolymerization of chitosan by hydroxyl radical. *Bioscience, Biotechnology and Biochemistry*, 1996, 60; 2001 – 2004
12. Chang KLB, Tai MC, Xiao L. Kinetics and products of degradation of chitosan by hydrogen peroxide. *Journal of Agricultral and Food Chemistry*, 2001, 49; 4845 – 4851
13. Qin CQ, Du YM, Xiao L. Effect of hydrogen peroxide treatment on the molecular weight and structure of chitosan. *Polymer Degradation and Stability*, 2002, 76; 211 – 218
14. Tian F, Hu K, Zhao B.Study of the depolymerazation behavior of chitosan by hydrogen peroxide. *Carbohydrate Polymers*., 2004, 57; 31-37
15. Mao S, Shui X, Unger F, Simon M, Bi D, Kissel T. The polymerization of chitosan: effects on physicochemical and biological properties. *Inter. J. Pharm.*, 2004, 281; 45 – 54
16. Srijanto B, Paryanto I, Masduki, Purwatiningsih. Effect of the degree of deacetylation of raw materials on chitosan depolymerization.*Akta Kimindo*, 2006, 1, 67 -72 (in Indonesian)
17. Tanasale MFJDP, Telussa I, Sekawael SJ, Kakerissa L. Extraction and Characterization of Chitosan From Windu Shrimp Shell (Penaeus monodon) and Depolymerization Chitosan Process With Hydrogen Peroxide Based On Heating Temperature Variations. *Indonesian Journal of Chemical Research*, 2016, 3; 308-316 (in Indonesian)
18. Zheng X, Yin Y, Jiang W, Xing L, Pu J. Synthesis and characterization of low molecular weight chitosan. *BioResources*, 2015, 10; 2338-2348
19. Shao J, Yang Y, Zhong Q.Studies on preparation of oligoglucosamine by oxidative degradation under microwave irridation. *Polymer Degradation and Stability*, 2003, 82; 395-398
20. Tanasale MFJDP, Bijang CM, Ohorella A.Preparation of chitosan with variations of molecular weight and its effect on depolymerization of chitosan with hydrogen peroxide using microwave technique. 2018. *Abstract poster*. Presented on The 7th International Conferences of Indonesian Chemical Society (ICICS) 2018, Jayapura (Indonesia), September 26 – 27, 2018
21. Kang B, Dai YD, Zhang HQ, Chen D. Synergetic degradation of chitosan with gamma radiation and hydrogen peroxide. *Polymer Degradation and Stability*, 2007, 92; 359 – 362
22. Wang SM, Huang QZ, Wang QS. Study on the synergetic degradation of chitosan with ultraviolet light and hydrogen peroxyde. *Carbohydrate Research*, 2005, 340; 1143 – 1147
23. Yue W, Yao P, Wei Y.Influence of ultraviolet-irradiated oxygen on depolymerization of chitosan. *Polymer Degradation and Stability*, 2009, 94; 851 –858
24. Huang QZ, Wang SM, Huang JF, Zhou LH, Guo YC. Study on the heterogeneous degradation of chitosan with hydrogen peroxide under the catalysis of phosphotungstic. *Carbohydrate Polymers*, 2007, 68; 761 – 765
25. Huang QZ, Zhuo LH, Guo YC. Heterogeneous degradation with H_2O_2 catalysed by phosphotungstate. *Carbohydrate Polymers*, 2008, 72; 500 – 505
26. Julianti S. Preparation of chitosan oligomers through oxidative degradation method with the addition of H_2O_2 and ultrasonic bath and its influence on the viscosity and molecular weight.2012. *Bachelor's Thesis*. Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara (in Indonesian)
27. No HK, Meyers SP. Preparation of chitin and chitosan, *in* R.A.A. Muzzarelli and M.G. Peter (eds), *Chitin Handbook*, European Chitin Society, 1997; 475 – 489
28. Liu N, Chen XG, Park HJ,Meng XH, Yu, LJ.Effect of Mw and concentration of chitosan on antibacterial activity of Escherichia coli. *Carbohydrate Polymers*, 2006, 64; 60-65
29. Cheng RH, Tsaih ML. Effect of temperature on the intrinsic viscosity and conformation of chitosans in dilute HCl solution. *International Journal of Biological Macromolecules*, 1998, 23; 135 – 141

30. Liu D, Wei Y, Yao P, Jiang L. Determination of the degree of acetylation of chitosan by UV spectrophotometry using dual standars. *Carbohydrate Research*, 2006, 341; 782-785
31. Du Y, Zhao Y, Dai S, Yang B. Preparation of water-soluble chitosan from shrimp shell and its antibacterial activity. *Innovative Food Sciences & Emerging. Technologies*, 2008, 10; 103-107
32. Muzzarelli R, Jeaniaux C, Gooday GW (Eds). *Chitin in Nature and Technology*. 1986. Plenum Press, New York
33. Arifin Z. Utilization of indirect sonication technology in the framework of chitosan production.*Konversi*, 2012, 1; 1-6 (in Indonesian)
34. Basuki BR, Sanjaya IGM. Synthesis of chitosan crosslinking with glutaraldehyde and identification of functional groups and degrees of deacetylation.*Jurnal Ilmu Dasar*, 2009, 10; 93-101 (in Indonesia)
35. Kilikily K. Variasi waktu pemanasan pada proses deasetilasi kitin. *Bachelor's Thesis*. 2009. Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Pattimura, Ambon (in Indonesian)
36. Laratmase W. Kinetics of adsorption of Cu (II) metal ions on chitosan isolated from tiger shrimp waste (*Penaeus monodon*). *Bachelor's Thesis*. 2010. Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Pattimura, Ambon (in Indonesian)
37. Dewi NL, Bahri S, Hardi J. The use of various pressures and hydrolysis time on producing glucosamine hydrochloride from chitosan shell snail (*Achatina fucila*). *Kovalen Jurnal Kimia Riset*., 2016., 2(1); 22-32 (in Indonesian)

*****