KnE Life Sciences

ISSN 2413-0877 Volume 1 (2015)
The 1st International Symposium on Aquatic Product Processing 2013

STUDY OF COLLOIDAL CHITIN HYDROLYSIS TO PRODUCE THE N ACETYL GLUCOSAMINE FROM SHRIMP SHELL WASTE USING HYDROCHLORIC ACID AND NITRIC ACID

Sri Wahyuni

Food Technology Dept. Halu Oleo University, Indonesia
e-mail: sriwahyuni_aan@yahoo.com

ABSTRACT

Shrimp processing industries produces shrimp waste by 30-75% is wasted without being processed causing environmental pollution. The purpose of this study examines efforts to produce monomers of chitin hydrolysis of colloidal chitin compound derived from shrimp shells are chemically (hydrochloric acid and nitric acid) to produce N acetyl glucosamine bioactive compounds that have health benefits. The method used in this study is the optimization of chemical hydrolysis conditions using a solution of HCl and HNO_3. On determining the optimum time to sample variation colloidal chitin 0,5%, 1% and 2%, the sample is heated with time variation 1,3,6, 9,12, and 24 hours at a temperature of 60 °C and the concentration of acid was 4 M. In the determination of the optimum temperature, each sample was heated at a temperature variation of 40, 60, and 80 °C at the optimum time (9 hours) and acid concentration of 4 M. In the determination of the optimum concentration of acid, each sample was heated at a temperature and the optimum reaction time. Production results showed the highest compound N acetyl glucosamine as 623.3 ppm, using hydrochloric acid hydrolysis reaches optimum temperature 40° C, the optimum reaction time 9 hours, the optimum concentration of 4 M hydrochloric acid, the concentration of 2% colloidal chitin. Production of N acetyl glucosamine supreme as 625.83 ppm, using nitric acid hydrolysis reaches optimum temperature 60° C, the optimum reaction time 9 hours, the optimum concentration of 4M nitric acid, and the concentration of 1% colloidal chitin. Therefore we can conclude the results of 1% colloidal chitin hydrolysis using nitric acid more efficiently produce N acetyl glucosamine than hydrochloric acid, due to the temperature, reaction time, and acid concentration are not the same ability to hydrolyze colloidal chitin. Differences in the results of hydrolysis reached 11,59 ppm.

Key words : Chitin, N acetyl glucosamine, acid hydrolysis, optimum reaction

INTRODUCTION

Chitin waste in Indonesia in 2002 have produced about 112 208 tons (Anonim 2004), with the increasing number of industrial activities in this fishery shrimp processing, either frozen or other products, indicating increasing amount of chitin waste produced. But until now it seems the fishing industry has not looked chitin waste processing business so far not been utilized properly and efficiently, even mostly just a waste material which also pollute the environment, such phenomena commonly found in fish processing industry that does not treat waste that is processed shrimp shells. Shrimp processing business generated from shrimp waste by 30% - 75% is wasted without being processed even cause pollution. The amount is very large for the size of the industrial waste (Marganof 2003).

A total of 50-60% of shrimp waste, can produce 25% chitin than 32% by weight of the dry waste (Muzzarelli, 1985). Approximately 35% of dried shrimp shells contain chitin. chitin of shrimp can be produced about 80% chitosan (No and Meyer 1997). Chitosan in the world

Selection and peer-review under responsibility of the 1st ISAPPROSH 2013
DOI : http://dx.doi.org/10.18502/kls.v1i0.89

market price is around U.S. $ 7.5/10g for chitosan with good standards. Currently, 90% of the world market dominated by Japanese chitosan with production of more than 100 million tons each year (Tsigos *et al.* 2000). Indonesia with potential sea wider than the Japanese have the opportunity to take part of the world market chitosan.

D-glucosamine and N-acetyl-glucosamine is widely used for the treatment of various diseases such as osteoarthritis, gastritis, food allergies, diverticulitis, inflammatory bowel disease and is used as a prebiotic. In developed countries such as Japan, N-acetyl-glucosamine has been applied in the food industry / beverages (Aiba, 2009). In addition, these compounds can also be applied to cosmetics since shown to have anti-aging activity, repair skin damage due to UV rays and reduce hyperpigmentation in skin cells. Until now, there are no side effects from the use of N acetylglucosamine as medicine (Anonymous, 2010). When compared with the D-glucosamine, N-acetyl-glucosamine is preferred because it is more stable and sweetened so more acceptable if applied through the oral (Widhyastuti 2010).

To produce compound N-acetyl-glucosamine can be done by acid hydrolysis and enzymatic hydrolysis. The results of Wulandari research (2009), reported the production of N-acetyl-glucosamine (GlcNAc) through a semi-solid fermentation process by chitinase enzymes from *Aspergillus rugulosus* 501. The results were obtained optimal GlcNAc 2.228 mg / mL (2.228 ppm) in media with initial pH 4 and source of urea nitrogen on day 10. Bohlman *et al.,* (2004) also reported the production of N-acetyl-glucosamine is chemically by acid hydrolysis of chitin rough at suitable reaction conditions including approximately 15-36% HCl and temperature of 40-80 ° C. In this procedure up to 6.42 g / L (6420 ppm) GlcNAc can be generated for 1 h. The use of hydrochloric acid is able to cut the chitin polymer into units lower.

Enzymatic hydrolysis is also frequently used in the hydrolysis of chitin produced monomer because the process can be controlled precisely. However, enzymatic hydrolysis requires very expensive and difficult to obtain in sufficient quantities for industrial applications.

Therefore, to be examined efforts produce chitin monomer of colloidal chitin hydrolysis compounds using chemical means (hydrochloric acid and nitric acid). Optimum conditions in the hydrolysis process need to be studied for effectiveness and efficiency in obtaining the expected product. Therefore, identification of conditions such as temperature, reaction time and concentration of the acid hydrolysis products of chitin be expected, important to note that the optimum characteristics obtained from the hydrolysis process.

MATERIALS AND METHODS

1. Materials

Shrimp shell waste, HCl, NaOH, HNO_3, Schales reagent (Na_2CO_3 and K_3 [Fe $(CN)_6$], and N-acetyl-glucosamine anhydrous (Sigma) as standard.

2. Procedures

2.1 Sample preparation

Shrimp shell waste materials cleaned from impurities by using water. Samples of shrimp shell that have been cleaned and dried in an oven at 60°C for 1 x 24 hours. The dried shrimp shell and then crushed with a particle size of 70 mesh.

2.2 Chitin Powder production From Shrimp Shell Waste (No *et al.* 1989)

Shrimp shells that have been crushed then performed the removal of minerals (demineralization) with the addition of 1 L of 1.5 M hydrochloric acid in 100 grams of shrimp shell powder (10:1), and stir for 24 hours 6000 rpm. Solid remains washed back to a neutral pH. After that is done the process of removal of protein with the addition of NaOH solution 3.5% ratio of 10:1, and then heated at 60°C for 2 hours. After it cooled, filtered, and then washed with water until neutral pH, and then dried. Solid residue obtained is chitin.

2.3 Preparation of Colloidal Chitin (Arnold and Solomon, 1986)

To produce 25 g ± 20 g colloidal chitin chitin required. chitin is dissolved in 400 ml of concentrated HCl using an ice jacket because these reactions generate heat. After the solution was kept for 1 night in cold temperatures. The next day 200 ml of cold water was added into the mixture, then filtered with glass wool. Results matched filtration pH to 7.0 with NaOH 12 M. After the solution was centrifuged, and then retrieved sediment.

2.4 Standard curves N Acetyl Glucosamine

N acetyl glucosamine standard solution with a concentration of 1000 ppm was 8 times dilution in order to obtain a working solution with a concentration: 100, 200, 300, 400, 500, 600, 700, and 800 ppm. Into a test tube 5 ml sample included 200 µL demineralized water plus 800 µL, and 1000 µL reagent Schales. Blank consisting of 1000 µL demineralized water plus 1000 µL Schales reagent. Once it is done reading the absorbance at 442 nm wavelength maximum (Ueda and Arai, 1992).

2.5 Reaction Kinetics of Colloidal Chitin Hydrolysis

Chemical hydrolysis conditions using a solution of HCl and HNO_3. On determining the optimum time to sample variation colloidal chitin 0,5, 1 and 2%, respectively when the sample is heated with a variation of 1,3,6, 9,12, and 24 hours at a temperature of 60°C and the concentration of acid used was 4 M. Later in the determination of the optimum temperature, each sample was heated at a temperature variation of 40, 60, and 80 °C at the optimum time and acid concentration of 4 M. In the determination of the optimum concentration of acid, each sample was heated to the optimum temperature and optimum over time.

RESULT AND DISCUSSION

1. Determination of Optimum Reaction Time

Time optimization is done to obtain the condition of optimum production of N-acetyl glucosamine from chitin hydrolysis with time variation 1,3,6,9,12 and 24 hours at a temperature of 60°C with acid concentration of 4M. Time optimization is intended to hydrolysis of chitin into N-acetyl glucosamine using hydrochloric acid (HCl) and nitric acid (HNO_3) through heating process. Time optimization results for colloidal chitin concentration of 0.5, 1 and 2% can be seen in Figure 1and 2.

Figures 1 and 2 show the hydrolysis of colloidal chitin 1% using HCl and HNO_3, both appear to give a higher yield than colloidal chitin 0.5 and 2% at the reaction time of 9 hours and a temperature of 60°C. By hydrolysis using HCl produce N acetyl glucosamine as much as 600.08 ppm and 628.9 ppm using HNO_3.

Samples of colloidal chitin 0.5, 1, and 2% to an increase in all levels of N-acetyl glucosamine (ppm) starting from the hydrolysis time 1 hour to 24 hours, and the results of the highest hydrolysis occurs at the time of 9 hours with levels of N-acetyl glucosamine is 620 ppm. On the graph also shows after 9 hours hydrolysis time, decreased levels of N-acetyl glucosamine, which is the hydrolysis time of 12 hours and 24 hours. Production of N-acetyl glucosamine in reaction time 9 hours to different concentrations of colloidal chitin, respectively the difference reached 37.6 ppm to 0.5% colloidal chitin; 28.1 ppm to 1% colloidal chitin, and 2.71 ppm to 2% colloidal chitin.

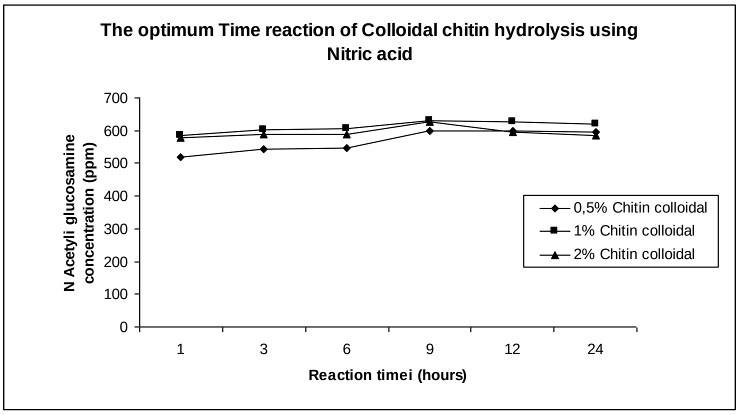

Figure 1. The optimum time of hydrolysis of colloidal chitin using Nitric acid (HNO_3)

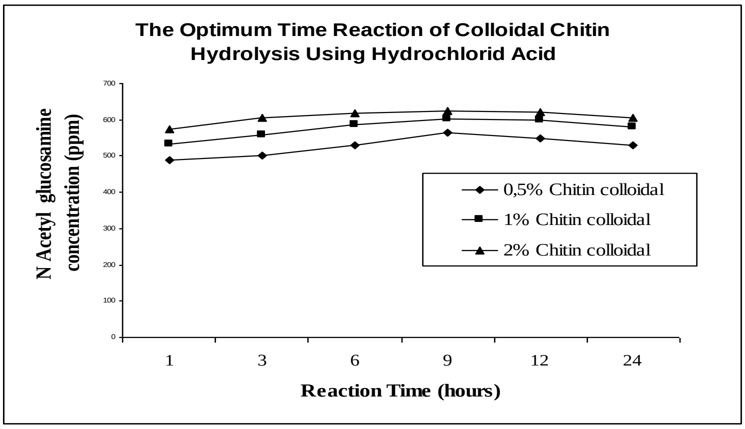

Figure 2. The optimum time of hydrolysis of colloidal chitin using Hydroclorid acid (HCl)

2. Determination of Optimum Reaction Temperature

Optimization is conducted by varying the temperature of temperature ranging from 40, 60, and 80^{0}C for 9 hours of reaction, the results of the optimization time. shown in Figure 3 and 4 .

Figures 3 and 4 shows the hydrolysis reaction with different concentrations of colloidal chitin using HCl and HNO_3 showed the highest production of N-acetyl glucosamine occurs at a temperature of 60° C, with the results of the hydrolysis of N acetyl glucosamine is higher using nitric acid than hydrochloric acid. Thus the temperature of 60 ° C is the optimum temperature production of N-acetyl glucosamine. It appears the hydrolysis reaction using colloidal chitin concentration of 1% is higher produce N-acetyl glucosamine than colloidal chitin 0,5 and 2%.

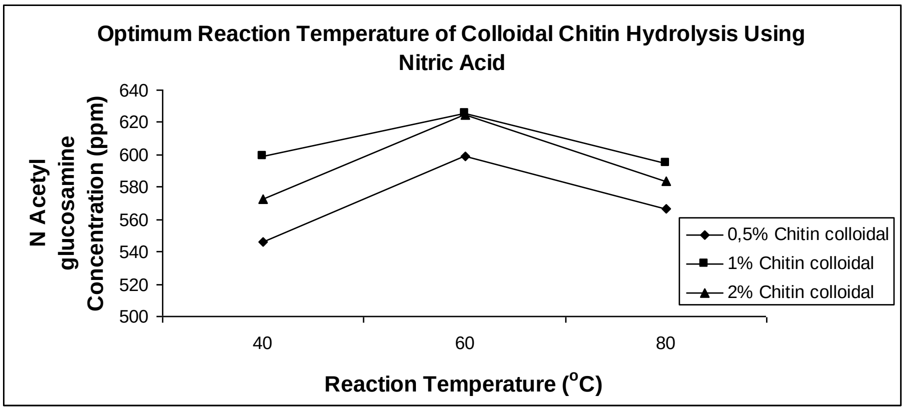

Figure 3. The optimum temperature of the hydrolysis reaction with different concentrations of colloidal chitin using HNO_3

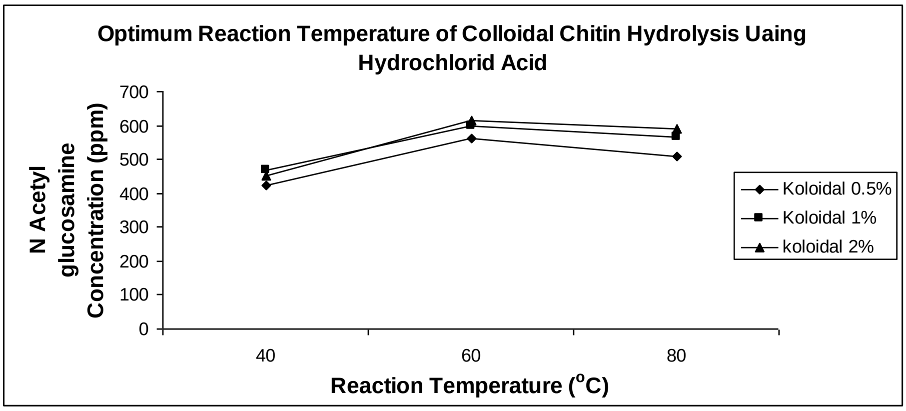

Figure 4. The optimum temperature of the hydrolysis reaction with different concentrations of colloidal chitin using HCl

HNO_3 ability higher than HCl in situations similar reaction conditions to produce compound N-acetyl glucosamine from colloidal chitin with various concentrations. Each of these differences reached 36.8 ppm to 0.5% colloidal chitin; 28.03 ppm for 1% colloidal chitin, and 11.59 ppm to 2% colloidal chitin.

3. Determination of Optimum Concentration of Acid

Chitin is soluble in concentrated acids can be degraded to the monomer. In this study, optimization of the concentration of hydrochloric acid and nitric acid are meant to see the effect of the concentration of acid used for hydrolysis of colloidal chitin. Acid hydrolysis process is done by variation of the concentration of acid used was 4 M, 8 M, and 12 M. Colloidal chitin hydrolysis results for 0.5, 1, and 2% can be seen in Figure 5 and 6.

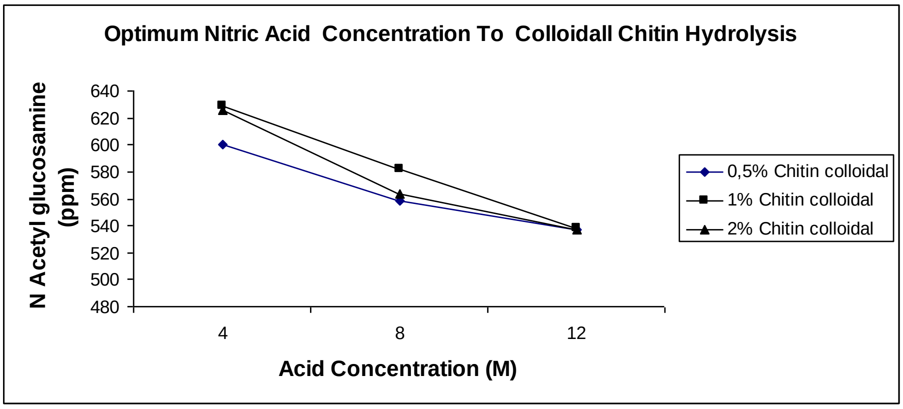

Figure 5. The optimum HNO_3 concentration on colloidal chitin hydrolysis

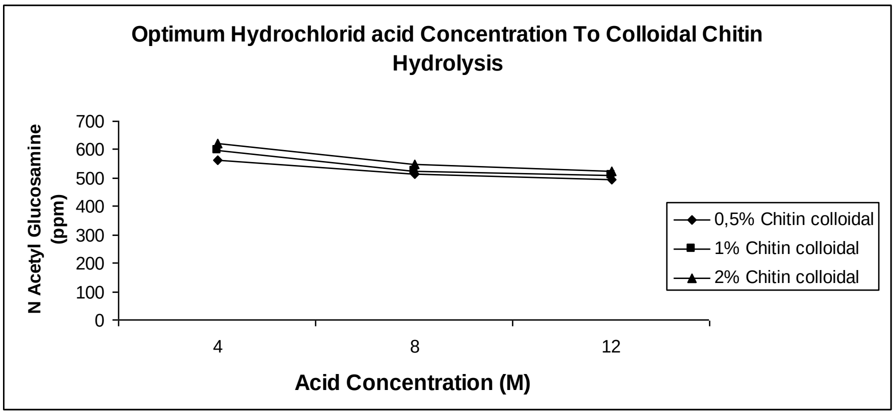

Figure 6. The optimum concentration of HCl hydrolysis of colloidal chitin

Figures 5 and 6 shows the hydrolysis reaction with different concentrations of colloidal chitin using HCl and HNO_3 shows the the highest production of N-acetyl glucosamine at a temperature of 60 ° C for 9 hours contained in the acid concentration of 4 M, with the result of the hydrolysis of N acetyl glucosamine more high by using nitric acid than hydrochloric acid. Thus the concentration of hydrochloric acid and nitric acid 4M is optimum hydrolysis colloidal chitin on the production of N-acetyl glucosamine. It appears the hydrolysis reaction using colloidal chitin concentration of 1% more produce N-acetyl glucosamine compared with colloidal chitin 0.5 and 2%.

Production of N-acetyl glucosamine from colloidal chitin hydrolysis reaction with various concentrations, providing information HNO_3 ability to produce compound N-acetyl glucosamine HCl is higher compared with the situation of the same reaction conditions. Each of these differences reached 38.2 ppm to 0.5% colloidal chitin; 29.9 ppm to 1% colloidal chitin, and 2.47 ppm for 2% colloidal chitin.

Hydrolysis results colloidal chitin 0,5, 1, and 2% increases with time until the optimum reaction time. Hydrolysis results shows the that the optimum at the same time is 9 hours obtained levels of N-acetyl-glucosamine at the highest concentration of 2% colloidal chitin. The longer the reaction time, the results obtained by hydrolysis will increase in size because of the sample in contact with acids longer. But the increase is not directly proportional to the results of hydrolysis with the addition of the time because it was too long can cause the deacetylation reaction.

Research conducted by Bolman (2004) hydrolyze chitin in the rough, also obtaining optimum conditions of hydrochloric acid in the range of 4-12 M. the maximum concentration of 10 M HCl in the hydrolysis of chitin into N-acetyl-glucosamine. Another researcher, Dolgopyatova *et al.*, (2013) to obtain the maximum concentration at 7.8 M HCl with levels of N-acetyl-glucosamine produced by 75.5% from the hydrolysis of chitin by acid ratio 1:20. Liu *et al.*, (2013) reported that Microbial fermentation by filamentous fungi or recombinant *Escherichia coli*, as an alternative method for the production of GlcN and GlcNAc, is attracting increasing attention because it is an environmentally friendly process. Although the microbial production of GlcN and GlcNAc is hampered by low yield and high production cost.

Hydrolysis results colloidal chitin 0.5%, 1%, and 2% at a temperature of 40⁰C to 60⁰C increased concentration of N-acetyl-glucosamine. The reaction will be running faster if the reaction temperature is raised. This is because the movements of molecules becomes faster with increasing the reaction temperature. However, the optimum reaction temperature graphics shows the decreased levels of N-acetyl-glucosamine at a temperature of 80⁰C. According to Chen *et al.*, (2010) uses the temperature is too high in chitin degradation process can lead to the destruction of N-acetyl-glucosamine. This opinion is supported by Defaye *et al.*, (1989) and Hasegawa *et al.*, (1993) which states that the higher the temperature rises, the more so that the levels of soluble N acetyl glucosamine be reduced.

CONCLUSION

Production of N acetyl glucosamine hydrolysis using hydrochloric acid and nitric acid reaches optimum temperature 60°C, the optimum reaction time 9 hours, 4 M optimum acid concentration, and the concentration of 1% colloidal chitin. Nitric acid hydrolysis yield is higher than hydrochloric acid at the same acid concentration and reaction time.

Based on the results obtained, it can be suggested for the manufacture of N acetyl glucosamine from shrimp shell waste that has been converted into colloidal chitin compounds, which nitric acid more efficient use than hydrochloric acid.

Acknowledgments

Prime Research Fund Higher Education in 2013 from the Director General of the Republic of Indonesia higher education that has funded this research

REFERENCES

Anonim. 2004. Statistik Perikanan Tangkap 2002. Direktorat jenderal Perikanan. Jakarta.

Anonim. 2010. (http://www.bio-medicine.org/biology-news

Aiba S. 2009. *Chemical And Enzymatic Modification Of Chitin And Chitosan Towards Functional Materials*. [Laporan Penelitian]. Ibaraki: Environmentally Degradable Polymer Research Group, Institute for Biological Resources and Functions-AIST

Arnold LD, Solomon NA. 1986. Manual of Industrial Microbiology and Biotechnology. American Society for Microbiology, Washington.

Bohlman. J.A. Schisler. D.O. Hwang, K.O. Hennling. J.P.; Trinkle, J.R.; Anderson, T.B. Steinke, J.D. Vanderhoff, A. 2004. *N-Acetyl-D-Glucosamine and Process for Producing N-Acetyl-D-glucosamine*. US Patent NO. 6693188B2

Chen JK, Shen CH, and Liu CL. 2010. N-Acetylglucosamine: Production and Applications. *Mar. Drugs,* 8: 2493-2516.

Defaye J, Gadelle A, Pedersen C. 1989. Chitin and Chitosan Oligosaccharides. *Di dalam*: G. Skjak-Braek, T. Anthonsen, dan P.A. Sandford (eds.). Chitin and Chitosan. Elsevier Applied Science. London

Dolgopyatova, V.Yu. Novikov, I.N. Konovalova, N.M. Putintsev, 2013. Mechanism of acid hydrolysis of N-acetyl-D-glucosamine. *Russian Journal of Applied Chemistry* 86(7): 986-991

Hasegawa M, Isogi A, Onabe F. 1993. Preparation of low molecular weight chitosan using phosphoric acid. *Carbohydr. Pol.* 20:279-283.

Liu L, Liu Y. Shin HD, Chen R, Li J, Chen J. 2013. Microbial production of glucosamine and *N*-acetylglucosamine: advances and perspectives Applied Microbiology and Biotechnology Volume 97(14) : 6149-6158

Marganof. 2003. Potensi Limbah Udang sebagai Penyerap Logam Berat (Timbal, Kadmium dan tembaga) di Perairan. http://rudict.topcities.com [28 Maret 2010].

Muzzarelli RAA. 1985. Chitin, Dalam GO. Aspinal (Ed.) *The Polysaccharides*. Academic Press Inc. New York. 3: 417–450.

No, H. K., Meyer, S. P., and Lee, S. P. 1989. Isolation and Characterization of Chitin Crawfish Shell waste. *Journal angricultural and food Chemistry*. 37: 493-575.

No HK, SP Meyers. 1997. Preparation of chitin and chitosan. Di dalam RAA Muzzarelli dan MG Peter (ed). Chitin Handbook. European Chitin Soc., Grottamar.

Tsigos I, A. Martinou, Kafetzopoulos, V. Bouriotis. 2000. Chitin deacetylases: *New versatile tools in biotechnology. TIBTECH Rev,* 18: 305-312.

Ueda M, Arai M. 1992 Purification and some properties of chitinases from *Aeromonas* sp. No 10S-24. *Biosci. Biotech. Biochem.* 56:460-464.

Ilankovan, P., S. Hein, C.H. Ng, T.S. Trung and W.F. Stevens, 2006. Production of N-acetyl chitobiose from various chitin substrates using commercial enzymes. *Carbohydr. Polym.* 63: 245-250.

Widhyastuti N. 2010. *Purifikasi N-Asetil-0-Glukosamina' Hasil Sintesa Secara Enzimatis Untuk Bahan Obat Dan Pangan Fungsional*. Laporan Akhir Program Insentif Peneliti Dan Perekayasa Lipi. Pusat Penelitian Biologi.

Wulandari F. 2009. *Optimasi Produksi N-Asetilglukosamina Dari Kitin Melalui Fermentasi Oleh Aspergillus Rugulosus 501*. [Skripsi] Institut Pertanian Bogor. Bogor.