

Microwave-Assisted Hydrolysis of Chitosan from Shrimp Shell Waste for Glucosamine Hydrochloride Production

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Abstract. Chitin is the most widespread renewable natural sources following cellulose as the main source of chitosan. Chitin is isolated from crustacean waste and shrimp shells. Chitosan is derived from chitin through demineralisation, deproteination, decolorisation and deacetylation process using chemicals such as sodium hydroxide, hydrogen chloride and acetone. Glucosamine hydrochloride (GlcN-Cl) can be produced by hydrolysis of chitosan by using hydrogen chloride. During deacetylation and hydrolysis the solution is heated by hotplate or furnace. In this paper we use microwave instead of hotplate for production chitosan and GlcN-Cl. The research investigates effect of microwaves to amount of rendement and their property. The chitosan was characterized its moisture content, solubility, and degree of deacetylation (DDA). Whereas the glucosamine hydrochloride characterized its functional groups using FTIR and crystallization by using X-Ray Diffraction (XRD). The experimental results show that the use of microwave energy on deacetylation of chitosan and hydrolysis processes can decrease time consuming and reactant concentration during production. the DDA value obtained was very high from 70 to 85%. The results also show that microwaves meet chitosan and GlcN-Cl standards.

1. Introduction

Osteoarthritis (OA) is one of the leading causes of disability (disability) in the elderly population in the world. OA is the fifth leading cause of disability in the population of developed countries, and the ninth highest cause in developing countries [1]. The main cause of disability (disability) in Australia and Asia is cancer. After cancer, many disability caused by lower back pain and OA with a prevalence of about 15% of the total cases. The results show in the world 9.6% of men and 18.0% of women aged over 60 have symptoms of OA. 80% of them due to OA causing limitation of movement, and 25% can not perform major activities of daily living [2]. WHO also estimates that in 2020 OA will be one of the causes of physical disability in humans after rheumatoid arthritis, osteoporosis and lower back pain.

The prevalence of OA continues to increase along with the increase in population, age, and risk factors such as obesity [1]. Indonesia is the 4th with the number of elderly people (elderly) the most after China, India and the United States [3]. Approximately 56.7% of patients in the outpatient clinic of the Department of Rheumatology (Cipto Mangunkusumo Hospital) has been diagnosed is one variant OA. Osteoarthritis patients is very haunted by the pain caused by degradation and joint inflammation that occurs [4], so the treatment is focused on reducing pain by administration of nonsteroidal antiinflammatory drug (NSAID) in the long term. However, this drug side effects if used long term. The use of NSAIDs for a long time, especially in older people reportedly turned out to cause a lot of side effects, such as gastrointestinal disorders, impaired liver function,



impaired kidney function and so on [4]. Dietary supplements glucosamine and chondroitin sulfate has been recommended as a safe and effective option for preventing and treating symptoms of OA [5].

Glucosamine is a natural compound found in the human body is composed of glucose and amino acid glutamine, other than that glucosamine is a key element of glycosamino-glycans (GAGs) in the cartilage of cartilage and synovial fluid. The function of glucosamine in the body is to produce synovial fluid that serves as a lubricant to the cartilage, so that the movement of the bones to be good. Shortage of synovial fluid in the body will cause joint disorders, such as stiff joints so that movement will result in OA disease. Giving oral glucosamine can help the production of synovial fluid so as to prevent and cure of disease [5]. Until now, glucosamine has not been produced in Indonesia that are imported from other countries with a fairly high. Research to produce glucosamine have been done by some researchers. Islam et al. (2011) have managed to hydrolyze glucosamine hydrochloride amounted to 63.45% of the chitin shells of shrimp[6]. Afridiana (2011) also managed to extract glucosamine hydrochloride from chitin shells of shrimp through chemical hydrolysis method with a concentration of 45.64%. Hydrolysis is safe for human consumption [5].

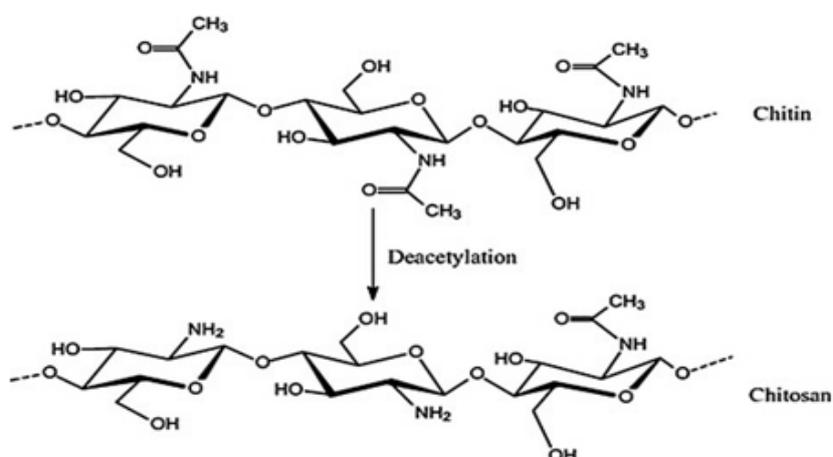


Figure 1. Deacetylation chitin to chitosan

Glucosamine can be obtained from the hydrolysis of chitin. Chitin is an organic material in animal groups molluscs, crustaceans, insects, arthropods and in the cell walls of plants low grade. The content of chitin in the skin of crustaceans such as shrimp shells 20-40%, -35% 15 shell crabs, and squid cartilage 97.20%. Chitin contained in squid cartilage, usually conjugated with a protein. Therefore, in this study glucosamine hydrochloride will produced from shrimp shell waste.

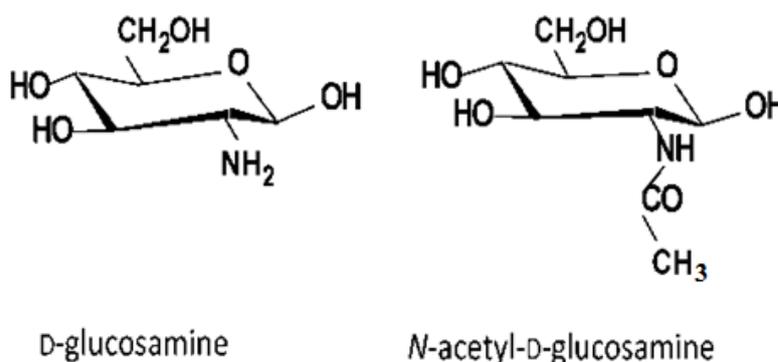


Figure 2. Hydrolysis product of chitosan and chitin

One of main process to produce Glucosamine Hydrochloride is hydrolysis. Conventionally, hydrolysis is performed by soaked in a chemical solution (i.e. hydrochloric acid) with certain concentrations and heated by using hot plate or electric furnace at temperature 80-100 °C. Recently, the

use of microwave energy to heat material or to speed up chemical reaction as a substitute of conventional heating has been reported. Some peculiar results were found such faster densification of ceramics [7-9], improve microstructure and mechanical of ceramics [10-14], drying and chemical reaction [15-17], synthesizing organic materials [18-20]. In this study, we applied this microwave energy on deacetylation and hydrolysis. The experimental results were then compared to the results of conventional ones.

2. Material and Method

The shrimp shells were collected from local fisheries in Kendari, Indonesia. Shrimp shell waste was washed and dried in the sun at room temperature before processing. The composition of the shell parts was determined by Thermogravimetry. The degree of deacetylation (DDA) of the chitin, chitosan, and glucosamine were determined by Fourier Transform Infrared Spectroscopy (FTIR). Crystallinity was determined by X-Ray Diffraction (XRD). All chemicals used in this study was bought from SIGMA-ALDRICH. The procedure of preparation of glucosamine from exoskeleton of shrimp is follow the procedure on Mojarrad and Arifin [21-22].

2.1 Demineralisation

50 grams of shrimp shell dissolved in hydrochloric acid of 1.0 M with a ratio of 1:10 (sample: solvent), then stirred with a stirrer for 1 hour at a temperature of 75°C. After filtered and washed using distilled water until neutral pH, then dried in an oven at 60 °C.

2.2 Deproteination

Deproteination (removal of protein) is done by a solution of NaOH 3.5% with a ratio of 1:10 (sample: solvent), then stirred with a stirrer for 2 hours at a temperature of 65°C. Then the samples were filtered and washed with distilled water until pH neutral and dried at 60°C

2.3 Decolorisation

Decolorisation can be done by solution of 0,315% NaOCl for 5 minute or extracted by acetone for several hours. The resulted then washed with water and dried in oven at 60 °C.

2.4 Deacetylation

Deacetylation performed 50 % sodium hydrokhlorid solution in water, after that continue by heating wich microwave for several minute.

2.5 Hydrolysis

Two gram chitosan and HCl was prepared for glucosamine production. Hydrolysis performed by two methods, microwave and conventional. By using microwave with varying exposure time from 5 to 20 minutes. Conventional hydrolysis was performed by using hot plate at temperature of 90°C for 90 minutes. Samples were soaked in a solution of hydrochloric acid at a ratio 1: 9 (w / v). HCl solution was varied at 20%, 25%, 32% and 37%. The use of microwave in these process are not only in the deacetylation process but also in the hydrolysis of this produced chitosan.

3. Results and Discussion

Main exoskeleton of shrimp components are protein, mineral, and chitin. After characterization, we found the composition as shown in Table 1. to remove the proteins and minerals do deproteinization and demineralization .Deproteinization was done by soaking the samples in NaOH 3,5% solution for 2 hours at temperature 90°C. While demineralization was performed by using HCl 1,0 M at ambient temperature for 1 hour. The use of microwave in the deacetylation of chitin and hydrolysis of chitosan decreasing heating temperature and time significantly.

Table 1. Characterization of shrimp shell

No.	Material	Percentage
1	Protein	29,5 %
2	Mineral	50,75%
3	Chitin	19,75%



Figure 3. Chitin and chitosan produced from exoskeleton of shrimp

Analysis of functional groups to identify functional groups and calculates the degree of deacetylation (DD) was performed by using FTIR (Fourier Transform Infra Red). FTIR spectra of chitosan showed absorption at 3441.87 cm wave number region-1 (O-H stretching), 1658.78 (C = O amide) as shown in Fig. 4 and 5. Absorption at wave number 1658.78 cm-1 (amide peak) appear due to chitosan is yet deasetilation overall. Quality of chitosan can be seen also from the large percent degree of deacetylation (DD). The existence of chitin into chitosan supported by data degree of deacetylation of chitosan which is calculated using the Baseline Method according to Khan *et.al* (2002), is 61.78% [23-24]. According Hargono *et al.* (2008) [25], standard degree of deacetylation of the chitosan from 60 to 100% so that the chitosan found in this experiment meet the standard. The higher DD means the more pure chitosan.

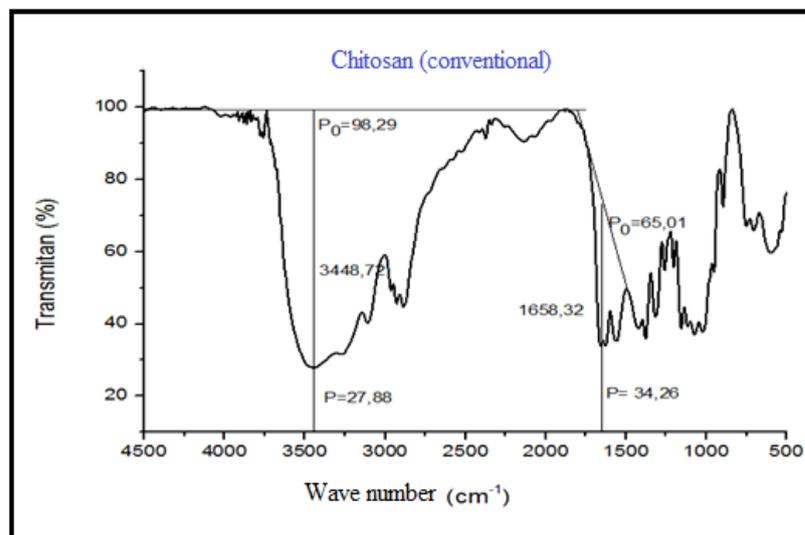


Figure 4. FTIR Spectrum of chitosan produced by hot plate (conventional)

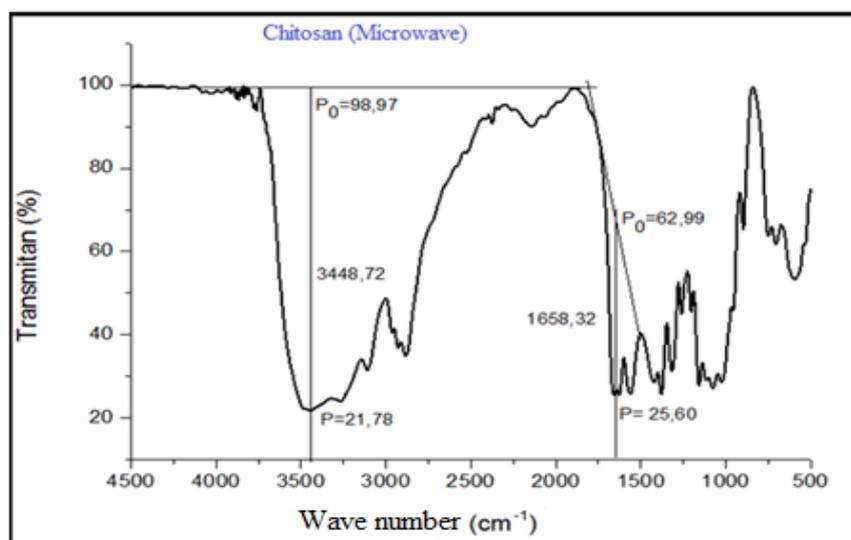


Figure 5. FTIR spectrum of chitosan produced by microwave

Figure 6. show FTIR spectrum of Glucosamine hydrochloride(GlcN-HCl) hydrolysis by using microwave for 15 minutes. The spectrum shows that an absorption at wave number of 3348.42 cm^{-1} as a result of vibration of -OH group and followed by absorption at wave number 3292.49 cm^{-1} derived from stretching vibration of NH amines Fig. 6. Brugnerotto (2001) explained that the monomer GlcN-HCl will show O-H functional group at 3350 cm^{-1} whereas polymer GlcN-HCl will show O-H functional group closer to 3450 cm^{-1} [26]. From the Fig. 6. indicate that the GlcN-HCl found in this experiment is monomer.

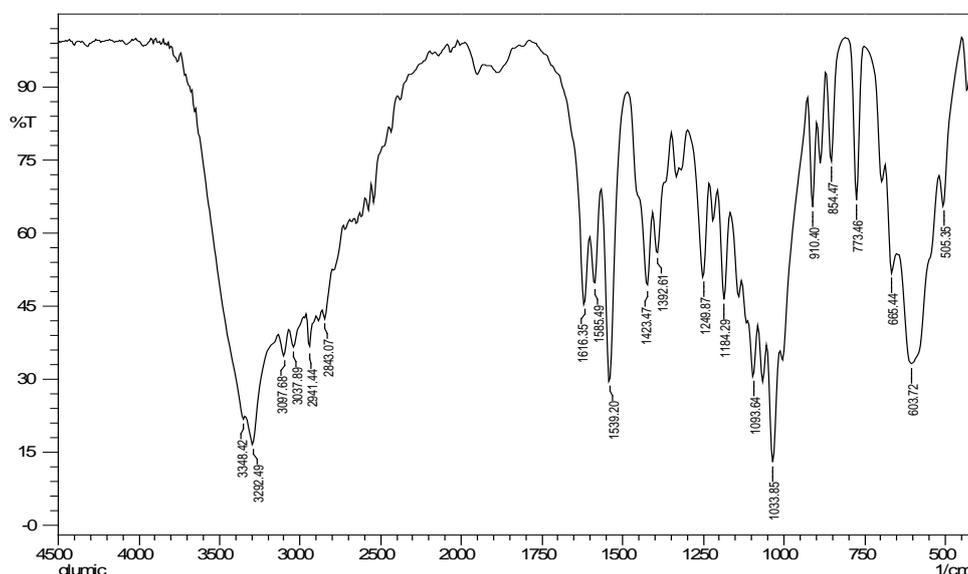


Figure 6. FTIR spectrum of Glucosamine hydrochloride(GlcN-HCl) hydrolysis by using microwave

Figure 6. show FTIR spectrum of Glucosamine hydrochloride(GlcN-HCl) hydrolysis by using by chemical methods with heated by hot plate for 90 minutes. The spectrum shows similarities with the microwave one for 15 minutes. It indicates that microwave accelerate the process of hydrolysis of GlcN-HCl. In addition with shorter time, we can found the almost same yields than that of result in hydrolysis by using conventional method as shown in Figure 8.. It can be seen that the yield obtained in the variation of microwave heating time 15, 11, 7, and 3 minutes give yield of 30.14%, 38.25%, 6.8% and 6.35% respectively. While by using conventional for 90 minutes we found yield of 51.01 %.

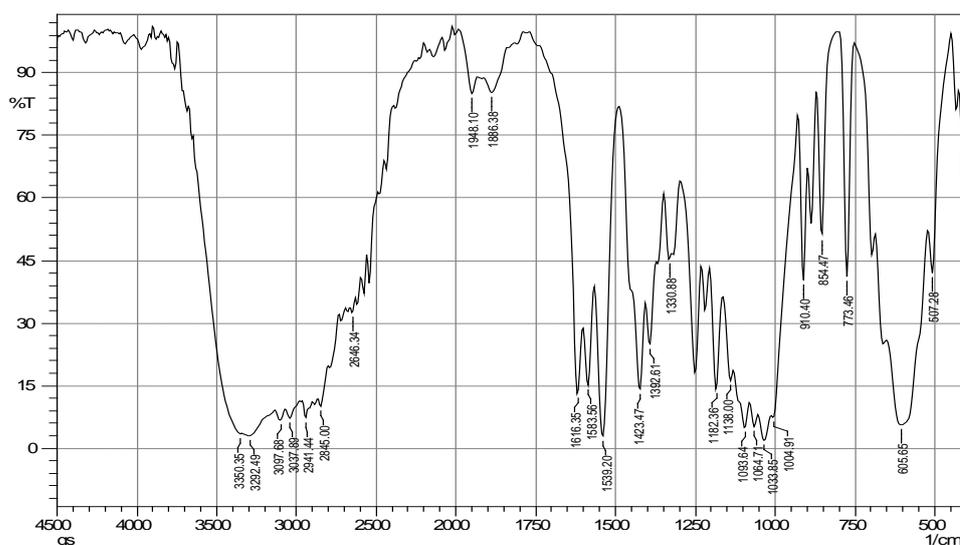


Figure 7. FTIR spectrum of Glucosamine hydrochloride (GlcN-Cl) hydrolysis by using hot plate

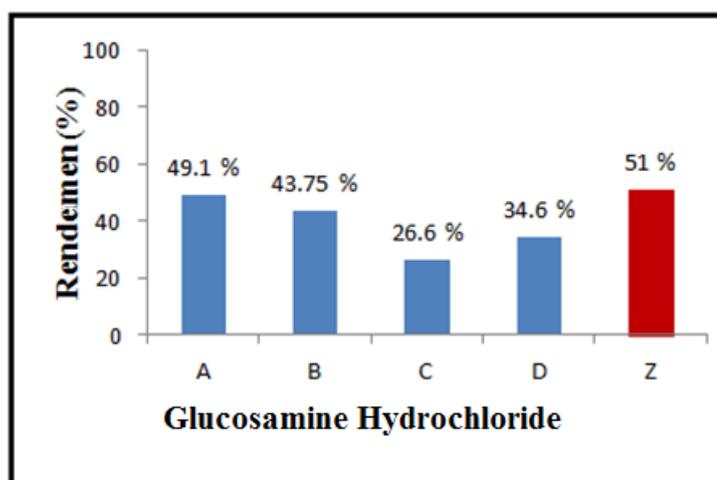


Figure 8. Glucosamine hydrochloride's rendemen from hydrolysis of chitosan (microwave : (A) 3 minutes; (B) 7 minutes; (C) 11 minutes; (D) 15 minutes; and (Z) conventional (hotplate)

4. Conclusion

Application of microwave energy for deacetylation of chitin and hydrolysis of chitosan process for glucosamine hydrochlorid production was successfully performed. Experimental results show that microwave decreased heating temperature and heatingtime significantly. It indicates that microwave accelerates these processes. FTIR results showed that absorption bands of glucosamine hydrochloride meet the standard of GlcN-Cl. These results suggested that microwave is appropriate technology for production of GlcN-Cl.

Acknowledgments

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References

1. S. Tanna, 2014. Osteoarthritis: Opportunities to Address Pharmaceutical Gaps. WHO: Priority Medicines for Europe and the World.
2. __, 2014. World Health Statistics, <http://www.who.int/gho/publications/worldhealthstatistics/>
3. Ernawati, 2012, Pembuatan Glukosamin Hidroklorida (GlcN HCl) dengan Metode Autoklaf, Skripsi, Institut Pertanian Bogor, Bogor.
4. N. Kertia, Danang, 2011. Effect of the combination of curcuminoid and essential oil on the serum ureum and creatinine level of patients with osteoarthritis, *Majalah Farmasi Indonesia*, 22(3), pp. 151 – 157. [in Indonesian]
5. N. Afridiana, 2011. Recovery Glukosamin Hidroklorida dari Cangkang Udang Melalui Hidrolisis Kimiawi sebagai Bahan Sediaan Suplemen Osteoarthritis, Skripsi, Institut Pertanian Bogor. Bogor. [in Indonesian]
6. Islam, M. M., Masum, S. M., Rahman and Shaikh, 2011, Preparation of Glucosamine Hydrochloride from Indigenous Shrimp Processing Waste, *Bangladesh Journal of Scientific and Industrial Research*, **46(3)**, 376-377.
7. W. H. Sutton, Microwave processing of Ceramic Materials, *Microwave Solutions for Ceramic Engineers*, American Ceramic Society, (2005), 35-65.
8. I.N. Sudiana, Ryo Ito, S. Inagaki, K. Kuwayama, K. Sako, S. Mitsudo, Densification of Alumina Ceramics Sintered by Using Sub-millimeter Wave Gyrotron, *J. Infrared, Millimeter, and Terahertz Waves*. 34 (2013), pp. 627-638.
9. M. Zamrun F., I. N. Sudiana, S. Mitsudo, Microwaves Enhanced Sintering Mechanisms in Alumina Ceramic Sintering Experiments, *Contemporary Engineering Sciences*, Vol. 9, 2016, 5, 237 – 247
10. S. Mitsudo, S. Inagaki, I.N. Sudiana, K. Kuwayama, Grain Growth in Millimeter Wave Sintered Alumina Ceramics , *Advanced Materials Research*, Vol.789 (2013), pp. 279-282
11. I.N., Sudiana, S. Mitsudo, K. Sako, S. Inagaki, L. O. Ngkoimani, I. Usman, H. Aripin, The microwave effects on the properties of alumina at high frequencies of microwave sintering, *American Institute of Physics (AIP) Conference Proceeding*, **1719**, 030014 (2016); doi: 10.1063/1.4943709. .
12. I.N. Sudiana, S. Mitsudo, T. Nishiwaki, P. E. Susilowati, L. Lestari, M Zamrun Firihi, H. Aripin, Effect of Microwave Radiation on the Properties of Sintered Oxide Ceramics, *Contemporary Engineering Sciences*, Vol. 8 No. 34, (2015), 1607-1615
13. I. N. Sudiana, S. Mitsudo, M. Z. Firihi, Effect of initial green samples on mechanical properties of alumina ceramic, *Contemporary Engineering Sciences*, Vol. 9, 2016, no. 12, 595-602
14. I. N. Sudiana, S. Mitsudo, M. Z. Firihi, Effect of Initial Green Samples on Mechanical Properties of Alumina Ceramic, *Contemporary Engineering Sciences*, Vol. 9, 2016, no. 12, 595-602.
15. M. Z. Firihi, I.N. Sudiana, 2.45 GHz microwave drying of cocoa bean , *ARPN Journal of Engineering and Applied Sciences* Vol. 12 No. 19, 2016, pp. 11595-11598.
16. Nikolic, S., Mojovic, L., Rakin, M., Pejin, D. dan Savic, D. 2008. A Microwave-Assisted Liquefaction as a Pretreatment for the Bioethanol Production by the Simultaneous Saccharification and Fermentation of Corn meal. *CI and CEO*. Vol 14(4), 231-234.
17. H. Aripin, S. Mitsudo, I. N. Sudiana, N. Jumsiah, I. Rahmatia, B. Sunendar, L. Nurdiwijayanto, S. Mitsudo, S. Sabchevski, Preparation of Porous Ceramic with Controllable Additive and Firing Temperature, *Advanced Materials Research*, Vol. 277 (2011) pp. 151-158.
18. Kumar P, Barrett D.M., Delwiche M.J. dan Stroeve, P. 2009. Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production, *Industrial and Engineering Chemistry Research* Vol. 48 (8), pp 3713–3729
19. I. N. Sudiana, S. Mitsudo, T. Nishiwaki, P. E. Susilowati, L. Lestari, M. Z. Firihi, H. Aripin, Synthesis and Characterization of Microwave on Sintered Silica Xerogel Produced from Rice Husk Ash, *Journal of Physics: Conference Series* Vol. 739 No. 1, (2016) 012059.
20. Y. Wu, Zhang, C., Liu, Y., Fu, Z., Dai, B. dan Yin, D. 2012. Biomass Char Sulfonic Acids (BC-SO₃H)-Catalyzed Hydrolysis of Bamboo under Microwave Irradiation. *Bioresources*. 7(4), 5950-5959.
21. J. S. Mojarrad, Mahboob, N., Valizadeh, H., Ansarin, M., and Bourbour, S., 2007, Preparation of Glucosamine from Exoskeleton of Shrimp and Predicting Production by Response Surface Methodology, *Journal of Agricultural and Chemistry*, **55**, 2245
22. Z. Arifin, dan Dady Irawan., 2015, Microwave-Assisted Deacetylation of Chitin from Shrimp Shells, *Prosiding Seminar Nasional Teknik Kimia “Kejuangan”*, Pengembangan Teknologi Kimia untuk Pengolahan Sumber Daya Alam Indonesia, Yogyakarta [In Indonesian].

23. Khan T.A. Peh K.K, dan Hung S.C.2001, reporting Degree of Deacetylation Value of Chitosan: The Influence Analytical Methods. *J Pharm Pharmaceut Sci.* vol 5. No 3 205-212.
24. H.M.S. Kingston, H.J. Haswell, 1997. *Microwave-Enhanced Chemistry: Fundamental, Sample Preparation, and Applications*, American Chemical Society.
25. A. Hargono, and S. Indro, 2008. Pembuatan Kitosan dari Limbah Cangkang Udang serta Aplikasinya dalam Mereduksi Kolesterol Lemak Kambing, *Reaktor*, 12(1). 53-57.[In Indonesian]
26. J. Brugnerotto ., 2001. An Infrared Investigation Interaction with Chitin and Chitosan Characterization, *Polymer* 42: 3569-3580.