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Influence of Microwave Irradiation on Extraction of Chitosan from Shrimp Shell Waste

Dwi Titik[1,2,*)], Heru Susanto[1,2)], and Nur Rokhati[1,2)]

[1)]Chemical Engineering Department, Engineering Faculty, Diponegoro University
Jl. Prof. Soedarto SH Tembalang Semarang, Telp./Fax. (024)7460058/(024)76480675
[2)]Membrane Research Center (MeR-C), Center of Research and Services- Diponegoro University
Jl. Prof. Soedarto SH Tembalang Semarang, Telp./Fax.: (024)7460057/(024)7465879

[*)]Coresponding author: dwi.titik07@gmail.com

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Abstract

Chitosan is natural polysaccharides which is nontoxic, biodegradable, and biocompatible and have many advantages in various kinds of fields including health, food, agriculture, and industry. Chitosan usually take long time to extract by conventional method for deacetylation process of chitin. Raw material for chitosan can be found in shrimp shell waste. Chitosan manufactures usually need high temperatures and chemicals in large quantities and it takes much time and consumes a lot of energy where will give bad effect to the environment. Recently microwave irradiation as nonconventional energy sources is widely used in chemical reactions. To reduce the impact of environmental pollution due to excessive use of chemical treatment, the objective of this work is processing chitosan under microwave irradiation. Expected production of chitosan with the same mass requires fewer chemicals than conventional heating. In particular, the study will examine the effect of making the chitosan and adding chemicals, reaction time and operating temperature and degree of deacetylation in chitosan with conventional heating methods that the results will be compared using a microwave. In this research will be developed to the design and fabrication of prototype scale extractor for manufacturing chitosan from shrimp shell waste after optimum results obtained from the research laboratory scale. From the research we can conclude that microwave will speed up reaction time. FTIR also showed functional group of chitosan formed from microwave irradiation have same results.

Keywords: *chitosan; microwave; shrimp shells*

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INTRODUCTION

Chitosan is a thin, odorless, white-colored, natural copolymer comprising a monomer (2-deoxy-2-acetylamin-2-glucose) and (2-deoxy-2 aminoglycose) related β (1,4) (Shahidi and Abuzaytoun, 2005). Chitosan produced from chitin by removing the acetyl group (CH_3-CO) so that the molecule is soluble in acid solution. In application, chitosan has advantages compared with other biopolymers. The existence of a free electron pair of amine groups located in position C-2 makes chitosan has a characteristic as a cation and a strong nucleophile. This causes chitosan easily react with anionic compounds through ionic bonds or hydrogen bonds (Muzzarelli, 1973; Furusaki *et al.*, 1996).

Several methods of chitosan synthesis from shrimp waste have been reported in various references such as portion of NaOH solution while heated at high temperature and enzymatic deacetylation (Percot *et al.*, 2003). Commercial chitosan produced by extracted NaOH solution (40-50% w/v) at high temperature and pressure (Bristow, 2013). In the process requires several hours of operation time to produce chitosan with high purity (Sahu *et al.*, 2009). In previous studies, the required process required large amounts of chemicals that could damage the environment due to the waste generated, and also the required energy is relatively high as it takes hours of heating (Samar *et al.*, 2013). To overcome this problem, we need to develop other technologies and one advanced technologies is microwave instead of conventional method for heating.

Microwave technology has been widely used for various applications for food and chemical industry. Also, another function as a source of energy to heat as well as dry a material, and catalyze chemical reactions in the manufacture of industrial and agricultural materials (Liu *et al.*, 2005). Microwave application provides many advantages including: startup time and relatively short heating, energy efficiency and process costs, easy and precise process control, selective heating, improved end product quality and can improve dry material quality (Sumnu, 2001). Microwaves are often used as external sources to help speed up the occurrence of a chemical reaction (microwave assisted reactions).

Microwave irradiation as a nonconventional energy source is widely used in chemical reactions. Microwave irradiation can transfer energy directly and quickly into the biomass substrate and catalyst, thereby increasing the efficiency of the reaction (Tsubaki and Azuma, 2013). In addition, the use of microwave irradiation can reduce chemicals in the process of making chitosan (Sahu *et al.*, 2009). By using microwave, temperatures will rise rapidly due to low conductivity so that the time required to heat shrimp shell waste become more faster (Hossan., 2010).

Isolation of shrimp shell waste to formed chitin and chitosan still using conventional heating that requires a large energy to require high production costs. Based on this problem, the objective of this work is to compare the sustainability of the innovation of microwave irradiation to replace conventional heating so chitosan processing from shrimp shell waste will take low energy and high efficiency.

MATERIALS AND METHOD

Materials

Shrimp shell waste was obtained from fish market in Tambak Lorok, Semarang. Sodium Hydroxide (NaOH), Acetic Acid (CH_3COOH) and HCl from Merck (Hohenbrunn, Germany). Commercial chitosan as reference material $(C_6H_{11}NO_4)n$, purchased from Biotech Surendo, Cirebon, Indonesia. The aquadest is self-produced in the MeR-C laboratory (Membrane Research Center).

Method

Stage for chitosan production can be done with 3 processes: deproteination (protein separation), demineralization (mineral separation) and deacetylation (separation of acetyl groups). Shrimp shell washed to dry and constant weight then grinding and sieving to 250 μm size and formed shrimp shell powder (SSP).

Deproteination process is dissolving SSP into NaOH solution (ratio: 1 g to 4 mL). It then heated and constantly stirred. In principle the process of deproteination is to separate the protein bonds from the shrimp shell and formed Na-proteinate.

The next process is demineralization to remove the mineral calcium carbonate ($CaCO_3$) contained in the SSP. The product of the deproteination process is pouring into a beaker glass with a volume variable ratio of 1: 5, plus a HCl solution of variation (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 N), in 250 ml. The mixture was stirred with constant stirring for 30 minutes at various temperature (30, 40, 50, 60, 70, 80°C) to forming chitin.

To produce chitosan, the next process is deacetylation (remove acetyl group) where already done by Kurita (2001), by dissolving chitin into 50% NaOH solution (ratio 1:20) at 90^{o}C. the chitosan formed then washed with aquadest to a neutral pH and dried to a constant weight.

To analyze the results of research using several methods including: protein content calculated by kjedahl method. Ash content anlysis by furnaced at 700^{o}C for 6 hours. The degrees of deacetylation (DD) in chitosan were calculated by FTIR analysis. The equation obtained at the absorption intensity at the wavelength of 1655 cm^{-1} (amide group) with wavelength 3450 cm^{-1} (OH group) as the internal standard. DD is then calculated by the Baxter equation below (Biskup *et al.*, 2012).

$$DD\ \% = 100 - [(A1655/A3450) \times 115]$$

RESULTS AND DISCUSSION

Deproteinization and Demineralization by Conventional Heating

The overall process for deproteination shrimp chitin is given in Figure 1. Various variable has applied to control chitin characteristics in protein content. Figure 1 shows that the higher NaOH concentration, then protein content in SSP (shrimp shell powder) getting decreases, it indicating the amount of protein released from SSP is getting bigger. The higher the NaOH concentration, then the space between the particles closer and allowing the collisions occured and formed Na-proteinat more frequently and also reaction faster. After 4% NaOH concentration, the protein content is relatively constant. This same statement was found in previous studies, where the best levels of NaOH for deproteination process was 4% (Khanafari *et al.*, 2008).

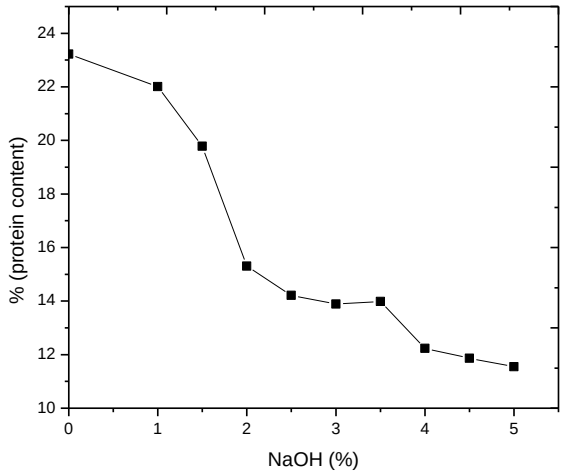

Figure 1. The effect of NaOH concentration on protein content during deproteination.

Figure 2 shows that the higher temperature then the protein content contained in the chitin is getting lower. When the temperature rises up, will lead to more and more degraded reaction that occur and protein became dissolve into the water. The optimum temperature was obtained at 80ºC and yield 11.66% protein content. This is influenced by the speed of motion of particles where the higher the speed, then the collision between particles is also higher (El Knidri *et al.*, 2016). The Archenius equation states that the higher the temperature the activation energy of a reaction will decrease, thus increasing the rate of reaction.

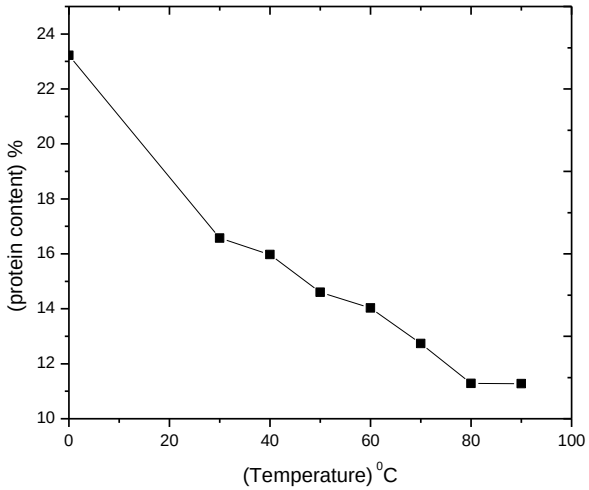

Figure 2. The effect of deproteination temperature on protein content.

The longer contact time between SSP and NaOH solution is causing the degraded protein to formed Na-proteinate and more water-soluble (Figure 3). From this process obtained at 2 hours and produce protein levels 12.76%. In the process of deproteinisation, possibly in addition to the degradation of protein also occurs amine group degradation of chitin compounds.

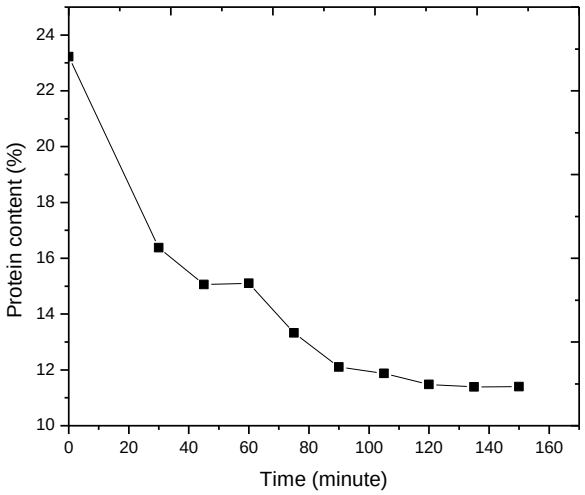

Figure 3. The effect of deproteination time on protein content

Figure 4 shows that the higher HCl concentration, then mineral content in SSP (shrimp shell powder) getting decreases Demineralization is the step chitin extraction which aims to eliminate inorganic compounds in shrimp waste. According to Oduor-Odote *et al.* (2005), that crustacean skin, generally containing 30-50% minerals based on dry weight, with the most minerals being $CaCO_3$. In addition, there are also $Ca_3(PO_4)_2$ with levels of 8-10% of total inorganic materials (Rinaudo, 2008).

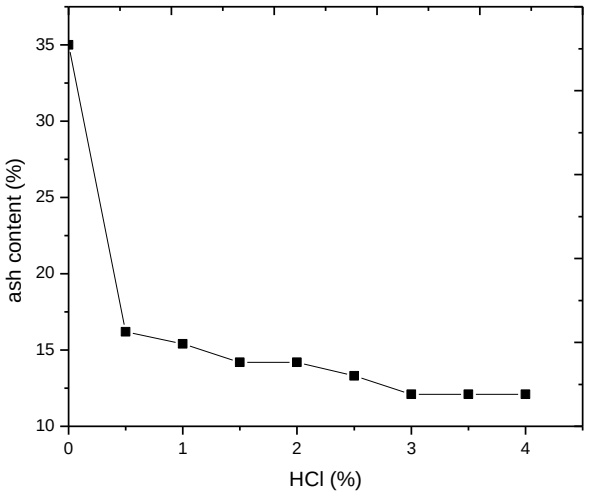

Figure 4. The effect of HCl concentration on ash content during demineralization.

Figure 5 shows the higher temperature during reaction of demineralization process, the less the ash content of the chitin product. Operating temperature is also very influential during the demineralization process (Nouri *et al.*, 2015). The greater the concentration of HCl, the inorganic compounds that react to form salt chloride dissolved in water more and more so ash content decreases. The reaction between $CaCO_3$ and HCl leads to the formation of CO_2 gas which is characterized by the presence of air bubbles at

the time of addition of HCl solution into the sample. This shows that there has been a process of separation of minerals in shrimp waste (Percot *et al.*, 2003). The demineralization reaction in the acid solvent is as follows:

$$Ca_3(PO_4)_{2(s)} + 6HCl_{(aq)} \rightarrow 3CaCl_{2(aq)} + 2H_3PO_{4(aq)}$$
$$CaCO_{3(s)} + 2HCl_{(aq)} \rightarrow CaCl_{2(aq)} + H_2O_{(l)} + CO_{2(g)}$$

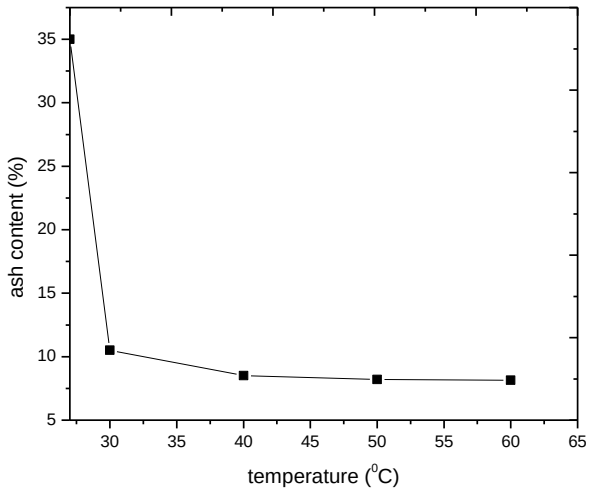

Figure 5. The effect of demineralization temperature on ash content

Figure 6 shows that the longer reaction time, the minerals released from the shrimp shell are getting bigger. The concentration of HCl, time and temperature have been reported in different studies. Most of these, however agree in that the removal should be carried out at very long time to prevent polymer degradation (Goycoolea *et al.*, 2000). From demineralization process optimization it was found that optimum condition was obtained at 3.5% HCl, reaction time 1 hour, and operating temperature 50°C.

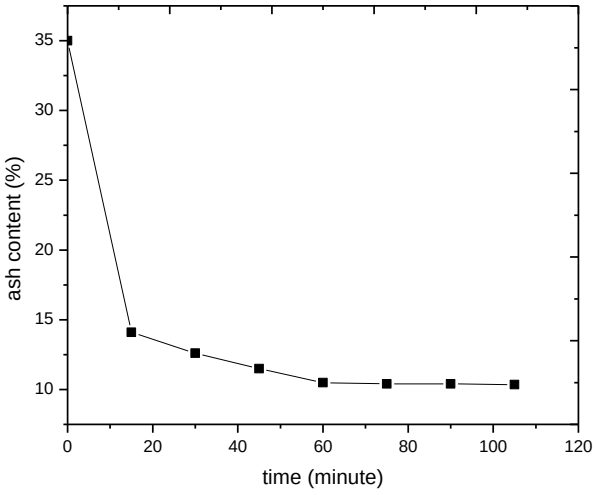

Figure 6. The effect of demineralization time on ash content.

Chitin Isolation (Deproteination and Demineralization) under Microwave Irradiation

Figure 7 shows that in the 5 minute of the reaction the protein content had dropped from 24.64% to about 13% on 400 watt operation process. Power on the microwave is not very influential because the results of deproteination almost the same on various power variables.

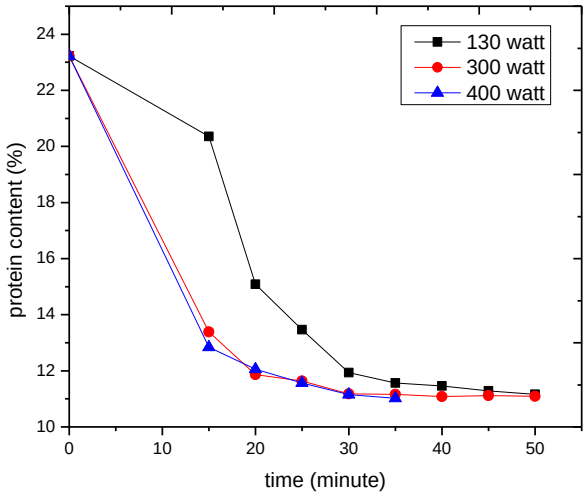

Figure 7. The effect of deproteination time on various power (watt) to protein content.

The microwave energy can be absorbed rapidly by polar bonds and can decrease the activation energy so that it will increase the reaction activity on the polar bond such as the glycoside bond (C - O - C) (Li, 2012).

Microwave irradiation is known to heat materials more efficiently compared to conventional method. Based on previous research, chemical reactions process will faster by microwave irradiation (Hirao *et al.*, 2009). It is not only improved the reaction time but also the power of microwave will effect on protein content. The more we scale up the power of microwave, it will also speed up the reaction time.

Table 1 show that the demineralization reaction by microwave heating is much more effective than regular heating. This is because every molecule that interacts with the microwave will generate heat in the solution so that the heating process occurs evenly. In ionic conduction, the liquid dielectric material interacting with the microwave results in charge movement as a result of the interaction between ions with microwave electric field oscillations. Based on previous researched the movement of ions against the resistance of other ions in the liquid produces heat which will cause an increase in temperature around each ion (Mello *et al.*, 2014).

Tabel 1. The effect of microwave power and reaction time on ash content of chitin during demineralization

No	Power (watt)	Time	Ash content (%)
1	130	5	10,63
2	130	10	5,44
3	300	5	11,82
4	300	10	4,44

Characterization of Chitin by FTIR analysis

The degree deacetylation process of shrimp shells can be simply followed by the measurement by FTIR spectrum. FTIR spectrum results from conventional conventional chitosan, chitosan with microwave heating and chitosan resulting from commercial industry are shown in Figure 8. The spectrum of chitosan samples contained a peak at a wavelength of 3452 cm^{-1} associated with the -NH and -OH groups, the peak of 2882 cm^{-1} is the stretching of the CH-aliphatic bands fused with OH. Characteristics of C=O (carbonyl) lies at the wavelength of 1660 cm^{-1}. The peak of 1427 cm^{-1} corresponds to the symmetric deformation of the CH group and at a wavelength of 1566 corresponding to the NH deformation of amide II. The vibration of the band at 1087 cm^{-1} shows the vibration of COC in the chitosan ring and produces many peaks caused by the presence of hydroxides of chitosan containing a single C=O bond.

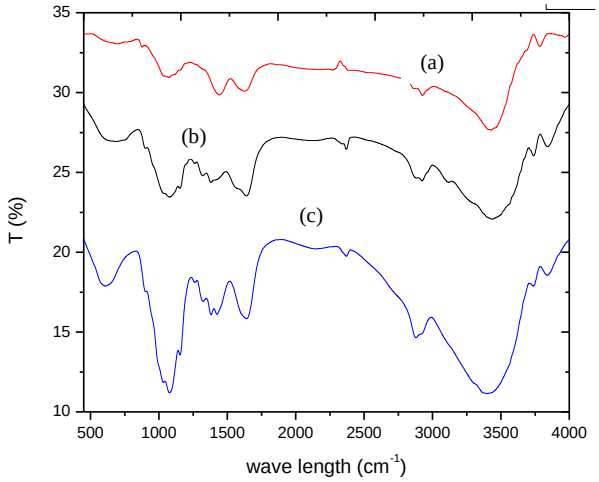

Figure 8. FTIR spectra of chitosan by conventional heating (a), microwave irradiation (b) and commercial chitosan(c)

CONCLUSION

The results showed that the demineralization condition of shrimp leather waste was achieved at the concentration of HCl 3,5 N solution with the weight ratio of shrimp shell waste and HCl solution of 1: 5 (w/v), at a temperature of 500^{o}C during 1 hour heating. In those conditions, the ash content was 8.06%. Ash content decreases to 5.4% if the demineralization reaction is carried out under microwave irradiation with 130 watts for 10 minutes. The optimum condition of the deproteination process was achieved by heating at a temperature of 700^{o}C for 2 hours, a 4% NaOH concentration with shrimp shrimp waste ratio: a NaOH solution of 1:5 (w/v). In this condition obtained nitrogen levels of 1.882% (11.763% protein content). If the deproteination reaction was performed under microwave irradiation with 130 watts of power for 15 minutes the nitrogen content obtained was 1.833% (11.461% protein content).

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