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Preparation of Glucosamine by Acid Hydrolysis of Chitin Under Microwave Irradiation

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Abstract. Glucosamine or 2-amino-2-deoxy-D-glucose is a natural monosaccharide. Glucosamine has been used extensively in the fields of nutrition, pharmacy, and cosmetics. Usually, glucosamine is obtained by complete hydrolysis of chitin using strong mineral acid at high temperature. Although acid hydrolysis has several disadvantages as caramel formation and low selectivity, this method is still interesting because it is simple and easy. Recently, microwave irradiation has been widely used in organic chemical reactions because of its ability to reduce both energy consumption and reaction time. In this study, preparation of glucosamine by acid hydrolysis of chitin using irradiation microwave was compared to conventional heating. The result showed that using conventional heating with hydrochloric acid 8 N, 95ºCof temperature process, ratio of chitin and HCl in 1:4 (w/v), and reaction time of 120 minutes yielded 58.8%. Whilst chitin hydrolysis using microwave irradiation with the power of 400 watts and for 25 minutes of reaction time yielded 67.1%. FTIR analysis showed that the glucosamine product from chitin hydrolysis using both conventional heating and microwave irradiation have similar structure with standard glucosamine. Glucosamine from Merck with the purity of 99% was used as the standard.

INTRODUCTION

Glucosamine (2-Amino-2-deoxy-D-glucose) is an amino monosaccharide derived mainly from chitin, a compound found in the shell of marine invertebrates. Glucosamine is easily metabolized in all parts of the human body in various quantities in some tissues [1]. Glucosamine can stimulate the production of hyaluronic acid. Hyaluronic acid plays role in skin regeneration, wound healing acceleration, and wrinkles reduction [2,3,4]. Because of its cationic character and excellent safe profile, glucosamine is widely used as drug delivery [5]. Glucosamine has therapeutic activity in osteoarthritis, analgesic (pain reliever), anti-inflammatory, and antipyretic (fever) or commonly called Nonsteroidal anti-inflammatory drugs (NSAIDs) [6].

Generally, glucosamine can be obtained by complete hydrolysis reaction from chitin using concentrated mineral acid at high temperature under conventional heating [7]. Despite low selectivity, this method is simple, easy, and more cost-effective compared to the enzymatic hydrolysis. In recent years, the microwave irradiation as a non-conventional energy source has become useful energy sources in various chemical processes. This technology has been widely used as an instrument for the synthesis of organic and inorganic chemicals, chemical industrial processes, biosciences, and

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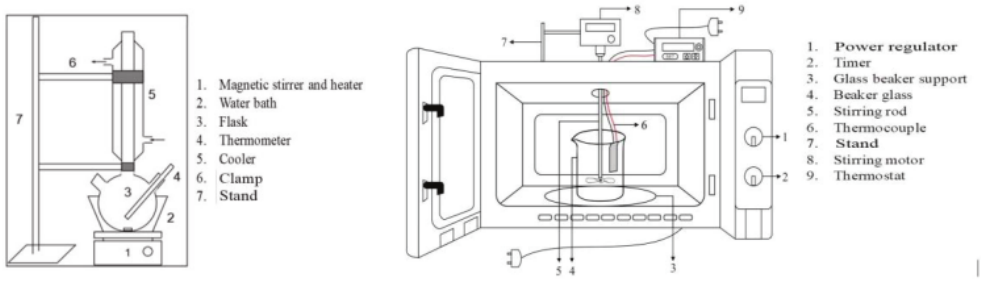
environmental management. The advantages of irradiation are the faster reaction rate, higher product yield, and lower energy consumption.

The objectives of this research are to study the effect of hydrochloric acid concentration and the ratio of chitin-hydrochloric acid on glucosamine yield using conventional heating, the effect of microwave irradiation on the reaction rate and glucosamine yield, and the characteristics of glucosamine products obtained using FTIR analysis.

EXPERIMENTAL DETAILS

Materials and Equipment

Raw Chitin was obtained from PT. Biotech Surindo, Cirebon, Indonesia. Hydrochloric acid (HCl), sodium carbonate (Na_2CO_3), ethanol, and standard glucosamine (Glucosamine-HCl) were purchased from Merck. Potassium ferricyanide purchased from Sigma-Aldrich. Distilled water was prepared from Mer-C Laboratory, Diponegoro University, Indonesia. The main equipment used were conventional heating hydrolysis equipment (Fig. 1.a) and modified microwave irradiation (Fig. 1.b).



(a) (b)

FIGURE 1. Equipment of chitin hydrolysis: (a) Conventional heating and (b) Microwave irradiation

Methods

Hydrolysis

Chitin was dissolved in hydrochloric acid (HCl) solution at concentration varied from 2 to10 N. The ratio of chitin-HCl varied from 1:4 to 1:12 (w/v). The chitin hydrolysis was conducted using conventional hydrolysis equipment at 95°C for 120 minutes. Afterwards, the samples were filtered.

The chitin hydrolysis using microwave irradiation was carried out at the power of 400 watts and reaction time from 5 to 30 minutes. The HCl concentration and the chitin-HCl ratio used were based on the optimum conditions of chitin hydrolysis using conventional hydrolysis equipment. After the hydrolysis process, the samples were filtered.

The filtrate of the sample was then evaporated using rotary evaporator for more concentrated product. Subsequently, ethanol was added to the concentrated solution to remove the remaining acid. Glucosamine crystals were separated from the filtrate using centrifuge and dried in a vacuum oven at 40°C. The crystalline produced was subjected to reducing sugar analysis then characterized using FTIR analysis.

Analysis of Reducing Sugars

Reducing sugars from hydrolyzed chitin was measured according to the method which was developed by Roncal. A mixture containing 1.5 ml of sample and 2 ml of Imoto reagent (0.50 g of potassium ferricyanide was mixed to 100

ml of 0.5 M sodium carbonate solution) were placed in covered glass bottle, then heated in 100°C water for about 15 minutes followed by cooling to room temperature. Afterwards, the mixture solution was filtered to remove precipitation. The absorbance of the mixture solution measured at 420 nm. Reducing sugars was determined using a standard curve of standard glucosamine [7]

The TRS (total reducing sugars) yield was calculated as follows:

$$TRS\ yield = \frac{(reducing\ sugar\ concentration\ (\frac{mg}{1.5\,ml})x\ sample\ volume\ (ml))}{(1000\frac{mg}{gr}\ x\ initial\ weight\ (g))} x\ 100\% \quad (1)$$

Characterization of Glucosamine

Chemical structure of the glucosamine crystalline produced was characterized with KBr pellets in the scanning range of 400–4,000 cm^{-1} using instrument FTIR-8400S, Shimadzu. KBr pellets were prepared (1mg of chitosan with 100 mg of KBr) and stabilized under controlled relative humidity before acquiring the spectrum.

RESULTS AND DISCUSSION

Chitin Hydrolysis Using Conventional Heating

The reaction of chitin hydrolysis is degradation of the chitin polymer chain. The reaction involves cleavage of the glycoside bonds between one monomer and the others via reaction with water. Each cleavage of the glycoside bonds generates the new reducing sugar. In this research, the complete hydrolysis of chitin was aimed to produce glucosamine using hydrochloric acid.

The following steps are the cleavage mechanism of glycoside bonds: (i) protonation of oxygen on glycoside bonds, (ii) addition of water molecules to the reducing sugar, (iii) decomposition of the protonated glycoside bonds, (iv) cleavage of the macromolecular chains into two shorter chains. HCl acted as a catalyst in the hydrolysis reaction [8].

The effect of HCl concentration at the temperature of 95°C and reaction time of 120 minutes on glucosamine yield is showed at Fig. 2. Increasing HCl concentration up to 8N increased the yield of glucosamine product. However, at higher HCl concentration higher than 8 N, the yield of glucosamine was relatively constant.

Fig. 3 shows the effect of chitin-HCl ratio on glucosamine yield. The reaction was carried out at HCl concentration of 8 N and temperature of 95ºCfor 120 minutes using conventional heating. The results showed that increasing ratio of chitin-HCl increased the glucosamine yield. Nevertheless, the yield was relatively constant at chitin-HCl ratio higher than 1:12.

Increasing amount of HCl compound in the solution reduced the pH of the mixture solution. At pH lower than four, the functional group of amines in chitin would be fully protonated, thus the molecular bonds in chitin were became unstable. Higher HCl concentrations did not provide a significant increase in the protonation of amine groups [9]. Protonated amines would lead chitin to dissolve in HCl solution so that the contact and reaction between chitin and HCl could go optimally.

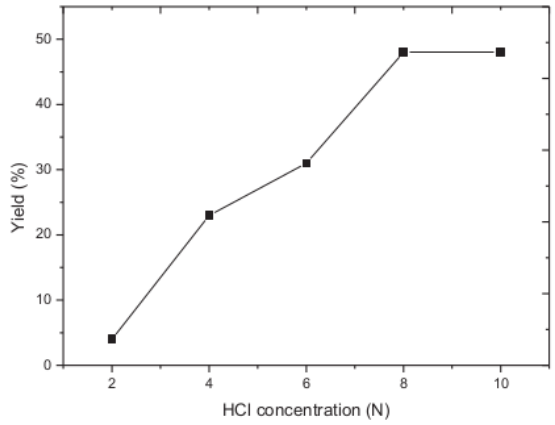


FIGURE2. The effect of HCl concentration on glucosamine yield (temperature = 95°C; reaction time = 120 min)

The constant reaction rate on chitin hydrolysis using acid was not only affected by hydrogen ion concentration but also affected by the number of water molecules. Increasing acid concentration caused the number of water molecules as the main hydrolysis agent to decrease. This affected the decrease of reaction rate [10].

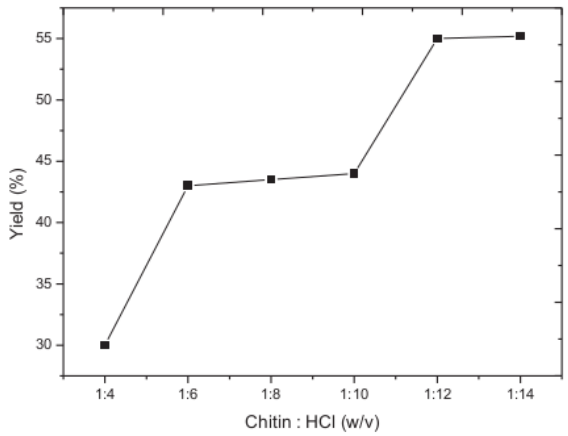


FIGURE3. The effect of chitin-HCl ratio (w/v) on glucosamine yield (HCl concentration = 8 N; temperature = 95°C; reaction time = 120 min)

Based on this discussion, the chitin hydrolysis using conventional heating obtained the optimum HCl concentrations of 8 N and the optimum ratio of chitin:HCl at 1:12 (w/v). This optimum variable was then used to carry out hydrolysis of chitin using microwave irradiation.

Chitin Hydrolysis Using Microwave Irradiation

Effect of hydrolysis reaction time using microwave irradiation on glucosamine yield is shown in Fig. 4. It shows that longer reaction time increased the glucosamine yield. After the reaction time of 25 minutes, the glucosamine yield tended to be constant.

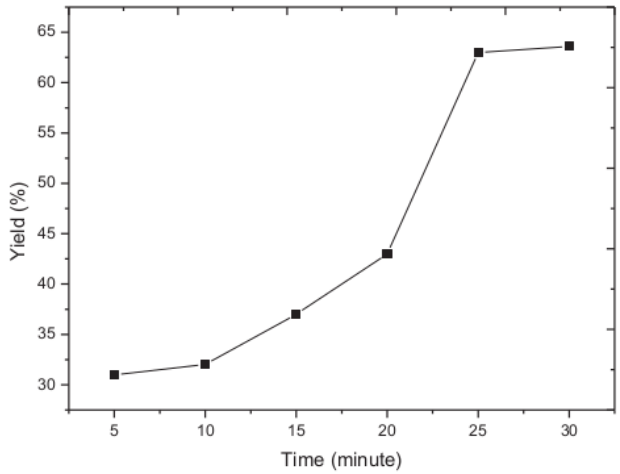


FIGURE4. The effect of irradiation time on glucosamine yield (HCl concentration = 8 N, chitin-HCl ratio= 1:12(w/v))

Longer microwave irradiation time caused more energy to be transmitted into the chitin solution during the hydrolysis process. This energy generated vibration in the chitin molecules which was triggered by the oscillating movement of microwaves (microwave irradiation), the vibration was causing friction between the molecules of chitin which generated heat. Hence, the temperature of the solution increased and the bonding of chitin monomers loosed. These results are similar to the previous publication by Wasikiewicz and Yeates [11] and Ha et al. [12]. The more monomers bonding appeared from the chitin structure, the more glucosamine would be produced, this resulted a higher yield. At the hydrolysis time of more than 25minutes, the yield remained constant. longer irradiation time tended to break and decompose the formed glucosamine. These results are similar to the previous publication by Sibi et al. [13].

The rate of chitin hydrolysis using microwave irradiation (25 minutes; yield = 67.1%) was significantly higher compared to using conventional heating (120 minutes; yield = 58.8%). Microwave irradiation could shorten the reaction time and save energy requirements for hydrolysis process. Microwave irradiation could stretch glycoside bonds in the chitin chain. Therefore, the glycoside bonds became easier to split during the hydrolysis process [12,14]. The split of the chain caused shorter chain to form glucosamine [8].

Characterization of Glucosamine Product

Characterization of glucosamine product was performed with FTIR spectra. Fig. 5 shows the FTIR spectra of glucosamine under the conventional heating, glucosamine under microwave irradiation and standard glucosamine. This showed that products of hydrolysis using both conventional heating and microwave irradiation did not contain acetyl groups. This was indicated by the absence of a carboxyl (C = O) group at a wavelength of 1700 cm^{-1} on the FTIR spectrum.

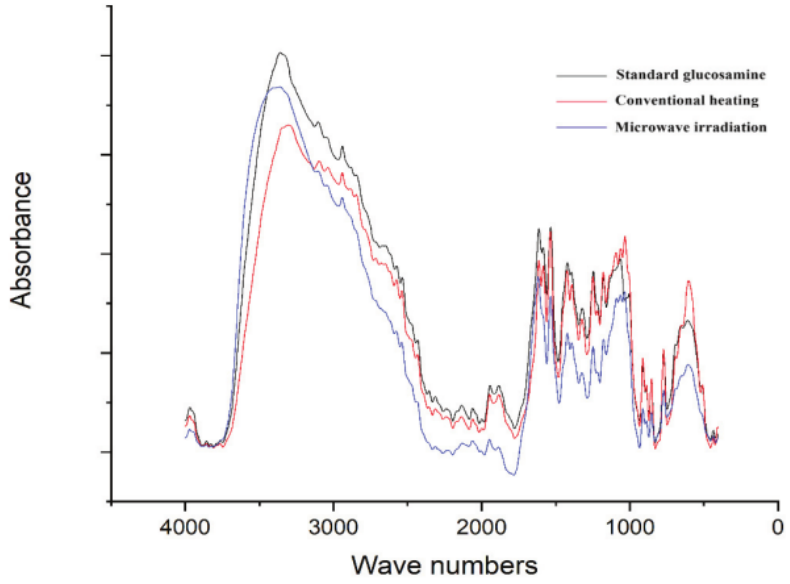


FIGURE5. FTIR spectra of glucosamine product

In chitin hydrolysis with acid catalyst, occurred not only depolymerization process but also deacetylation process. Therefore, the nitro-acetyl group in chitin polymer turns into an amine group [15]. The FTIR spectrum results from the main functional groups of glucosamine are shown in Table 1. The spectra of the products of chitin hydrolysis using conventional heating and microwave irradiation did not show significant difference with the standard glucosamine (from Merck).

TABLE 1. The functional group of glucosamine product

Functional group	**Wave numbers**		
	Glucosamine product using microwave irradiation	Glucosamine product using conventional heating	Standard glucosamine
Stretching O-H	3359.95	3302.08	3359.95
Bending N-H	1618.23 1535.29	1614.37 1537.10	1614.37 1535.29
Stretching C-N	1321.49	1325.05	1321.19

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CONCLUSION

It was found that the increase of HCl concentration until 8 N and chitin-HCl ratio to 1:12 could increase the glucosamine yield. Microwave irradiation was found as an alternative method to increase the reaction rate and the glucosamine yield. Hydrolysis process by microwave irradiation using the same HCl concentration and chitin-HCl

ratio (w/v) accelerated the reaction time from 120 minutes to 25 minutes, resulted higher glucosamine yield (67.1%) than the conventional heating (58.8%). The FTIR spectra showed that glucosamine produced using the conventional heater and microwave irradiation were both similar to the standard glucosamine in terms of the functional group.

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