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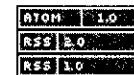
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Comparison of the Chitosan Degradation through Hydrothermal and Sonication-Hydrothermal Processes

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Abstract

Chitosan is a natural substance that has many applications in the fields of pharmacy and medicine. Because it has high molecular weight and does not dissolved easily in a neutral pH solution, then there is an effort to depolymerize chitosan into low molecular weight chitosan and oligomers. Nowadays, one of the methods used to degrade biomass is hydrothermal. Hydrothermal is one method of biomass degradation and polymers that is quite effective and environmentally friendly. Because chitosan has strong hydrogen bonds in addition to high molecular weight, it is necessary to treat chitosan by sonication before subjected to hydrothermal process. This study will compare degrading chitosan by only hydrothermal process and also sonication-hydrothermal processes. The hydrothermal of chitosan was carried out using a stainless steel tube reactor of 4-mL capacity at 200 °C for 4 s under pressures of 25 MPa for both of hydrothermal systems. For sonication-hydrothermal processes, chitosan was treated with sonication at 40 °C for 30 and 120 min before subjected to hydrothermal process. After hydrothermal, chitosan was characterized by viscosimetry and HPLC to determine molecular weight and also the dissolved product. Based on the product yield of the process, the sonication (40 °C, 120 mins, 1 %)-hydrothermal process (200 °C, 4 mins) was the best process on this study and gave lactose yield as much as 90 %.

Keywords: degradation, chitosan, hydrothermal, sonication-hydrothermal, oligomers

1. Introduction

Chitosan, (1→4)-2-acetamido-2-deoxy-β-D-glucan (GlcNAc) and 2-amino-2-deoxy-β-D-glucan (GlcN), is a natural copolymer produced by deacetylation of chitin isolated from crustacean shells. Chitosan and its oligomers have interesting properties such as non toxicity, biocompatibility, controllable biodegradability, and non antigenicity (Lo'pez & Bodmeier, 1997, p. 215-225). These properties cause chitosan as a potential biopolymer for various areas of applications such as biotechnology, pharmaceuticals, wastewater treatments, cosmetics, agricultures, food sciences, and textiles (Li, et. al., 1997). Chitosan has a high molecular weight and solubility restriction in water or neutral pH solution, which limits its applications. For medicine or pharmacy applications, the degree of deacetylation must be > 80 % and the degree of polymerization should be low, because low molecular weight chitosan (LMWC) and chito oligomers (COS) are responsible for chitosan biological activity (Folkman & Pospieszny, 2001; Suzuki et.al, 1986). Muzzarelli *et. Al.* (Muzzarelli, Tomasetti & Iari, 1994, pp. 110-114) studied that chitosan in the range of 1,000 – 10,000 Da is most potential for a number of medical and biotechnological applications. In addition, low molecular weights of chitosan and chitosan oligomers have high solubility in water. Thus the efficient processes for hydrolyzing chitosan, without changing its chemical structure, are of great interest. Like other biopolymer, chitosan is susceptible to a variety of degradation techniques, including chemical hydrolysis (Varum, Ottoy & Smidsrod, 2001, pp. 89-98; Rege & Block, 1999, pp. 235-245), oxidative degradation (Allan & Peyron, 1995, pp. 257-272; Shao, Yang & Zhong, 2003, p. 295) and enzymatic methods (Xia W, Liu P, Liu J, 2008, pp. 6751-6762).

Hydrothermal reaction has been interesting because of the fascinating characteristics of water as reaction medium at elevated temperatures and pressures (Savage, 1999, pp. 603-607). The degradation process by hydrothermal reaction proceeds through a variety of steps including hydrolysis, oxidation and gasification (Jomaa et. al., 2003, pp. 647-653). Hydrothermal decomposition also acts on the large organic molecules

reducing them into smaller fragments, some of which were dissolved in water. Recently, hydrothermal was used to degrade biopolymers such as cellulose, starch, protein, chitin, etc. to produce many valuable products.

Chitosan has strong intra and intermolecular hydrogen bondings. It also has high molecular weight. It will not easy to be hydrolyzed further without any treatments to produce weaker its hydrogen bonding and lower molecular weight. Sonication is particularly effective process in splitting up aggregates, and reducing the size of particles. Besides degrading, sonication also gives changing on physical and structural properties of chitosan. The changes on physical and structural properties of chitosan might make chitosan easy to be hydrolyzed on hydrothermal process (Chen, Chang, Shyur, 1997, pp. 287-294). In this study, the different concentration effects on chitosan molecular size and dissolved product after hydrothermal process were explored with the absence and presence of sonication process. The observation emphasized on the intrinsic viscosity changes and yield product of processes.

2. Method

2.1 Materials

The main material used was medical grade chitosan, which was purchased from Biotech Surindo (Cirebon, Indonesia). Chitosan as initial material was used directly without any treatment. The degree of deacetylation (DD) and the viscosity-average molecular weight (M_v) of chitosan are 80 % and 3,783 kDa, respectively. The solvent for chitosan solution was acetic acid (Merck, Darmstadt, Germany). Ultra High Purity N₂ gas was supplied by Tri Gases (Surabaya, Indonesia).

2.2 Methods and Analysis

2.2.1 Hydrothermal

The hydrothermal experiment was conducted in a batch-type tube reactor. The experimental unit consisted of a pressure vessel (reactor), electrical heater, pressure gauge, thermocouple, and temperature controller. The reactor was made of stainless steel super duplex type tubing (ϕ 6.35 mm \times 350 mm, V = 5 cm³) with one sealing screw-caps. The reactor was loaded with 4 mL of chitosan solution with sonication treatment and set up in the apparatus. The reactor was pressurized with N₂ gas and then was added by compressing with hydraulic system up to 230 bars. Electrical heater was heated up to 200 °C and the reactor was introduced for 4 minutes. The reaction time is defined as the duration that the reactor was kept in the electrical heater. After 4 mins, the reactor was pulled out from an electrical heater and quickly quenched in a water bath at room temperature. After cooling, the reactor was opened and then liquid was collected to be analysed.

2.2.2 Sonication-Hydrothermal

Chitosan was sonicated as Savitri et.al conducted in 200 mL of 0.5 and 1 % (v/v) of acetic acid solution. The experiments were conducted in a 400 mL Pyrex glass as a reactor for 30 and 120 mins at 40 °C. Sonication was performed using the high-intensity ultrasonic processor VCX 500, Sonics and Materials Inc., USA (500 W, 20 kHz, 50 % amplitude) equipped with a titanium alloy probe transducer. The converter was made of piezoelectric lead zirconate titanate crystals. The tip of the probe was 1.3 cm in diameter and was immersed in the solution up to 1 cm above the bottom of the reactor. The reactor was immersed in a water bath equipped with a circulating water thermostat, model 9102 (PolyScience, USA), to maintain the process temperature (Savitri et.al, 2014, pp. 244-252). After sonication, 4 mL of sonicated sample was subjected to hydrothermal as previous procedure (hydrothermal).

2.2.3 Measurement of Intrinsic Viscosity

Intrinsic viscosity of each sample solution was measured by an Ubbelohde viscometer (Type Nr 285400526, Schott Gerate, Germany) at 25 °C. Intrinsic viscosity could be calculated based on the ASTM (American Society for Testing and Materials, 2001). Viscosity of dissolved chitosan in acetic acid was calculated as the reduction viscosity (η_{red}) or as inherent viscosity (η_{inh}).

Reduction viscosity was calculated as:

$$\eta_{red} = (\eta/\eta_0 - 1)/c \quad (1)$$

Inherent viscosity was calculated as:

$$\eta_{inh} = \ln(\eta_{sp})/c \quad (2)$$

and Intrinsic viscosity was calculated as :

$$\eta_{ins} = \lim_{c \rightarrow 0} \eta_{red} = \lim_{c \rightarrow 0} \eta_{inh} \quad (3)$$

2.2.4 Analysis of the Oligosaccharides by High Performance Liquid Chromatography and Yield Determination

Water soluble chitosan was analyzed for identification and quantitative determination of chitoooligosaccharides. The analysis used high performance liquid chromatography (HPLC) on Zorbax NH₂ column. HPLC was performed with Agilent 1100 (Agilent Technologies, Inc, USA) instrument equipped with RI 1100 Refractive Index detector. The sample was eluted with 75 : 25 (v/v) mixture of CH₃CN/H₂O as the mobile phase, at a flow rate of 1 ml/min. Glucosamine (GlcN), N-acetyl Glucosamine (GlcNAc), chitosan dimer, hexoses (Sigma Aldrich Pte Ltd, Singapore) were used as authentic standard. The composition of the degradation product was calculated from peak areas in the HPLC profile using the standard curve obtained from pure standard solution.

The percentage yield of each hydrothermal product (i) was calculated as follow:

$$\% \text{ Yield} = \frac{\text{mg of each hydrothermal product (i)}}{\text{mg of initial chitosan in 4 mL}} \times 100 \% \quad (4)$$

where i was each compound which produced by hydrothermal process such as glucosamine, lactose, and N,N diacetyl chitobiose.

3. Results

3.1 The Intrinsic Viscosity of Chitosan after Processes

Table 1 shows the intrinsic viscosity of chitosan after hydrothermal process and also sonication-hydrothermal processes at various acetic acid concentrations. The results described that the intrinsic viscosity of chitosan after combination sonication-hydrothermal processes at various acetic acid concentrations were lower than that after only hydrothermal process. They were almost half of the intrinsic viscosity of chitosan after hydrothermal process on the range of 0.3 – 1 % v/v acetic acid. The change on intrinsic viscosity referred to the alteration on molecular weight of chitosan after processes. The comparison of intrinsic viscosity would describe the effect of processes with different treatments (the absence and presence of sonication). The study compared the hydrothermal and sonication-hydrothermal process and also observed the effect of acetic acid concentration during hydrothermal process and the duration of sonication treatment. The intrinsic viscosity changes of chitosan solution after hydrothermal only and sonication-hydrothermal process were shown in Table 1. Table 1 also shows the intrinsic viscosity on various acetic acid concentrations. The concentration of acetic acid solution during the hydrothermal process and sonication-hydrothermal processes influences the reduction of the intrinsic viscosity of chitosan. The intrinsic viscosities decreased for the more dilute solutions than for the concentrated solutions. Table 1 shows that the higher the acetic acid concentration, the higher the intrinsic viscosity of the solution.

Table 1. The intrinsic viscosity of chitosan of hydrothermal only and sonication-hydrothermal processes

Concentration, % v/v	η intrinsic		
	Hydrothermal	Sonication (40 °C 30 min) –Hydrothermal	Sonication (40 °C 120 min) –Hydrothermal
0.3	4.61	2.92	0.11
0.5	5.95	3.1	0.86
0.6	12.28	5.45	5.92
1	21.04	4.09	9.47

3.2 The Yield of Dissolved Chitosan after Processes

The chemical changes of chitosan could be followed by the degraded and dissolved product. Degraded and dissolved products were analyzed by high performance liquid chromatography. The product yield of hydrothermal and sonication- hydrothermal processes can be described in Figure 1-3.

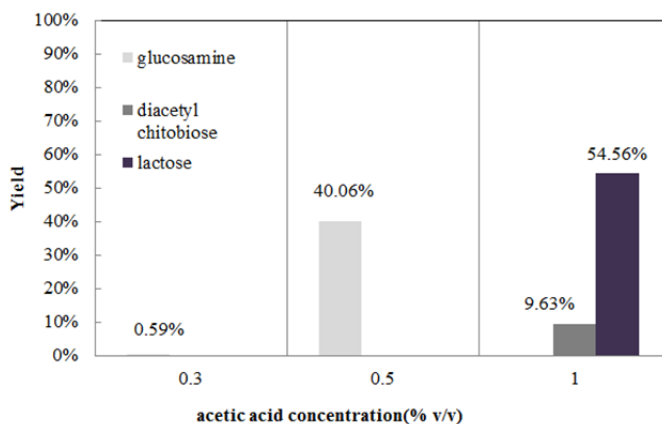


Figure 1. The yield product of hydrothermal process (200 °C, 4 min)

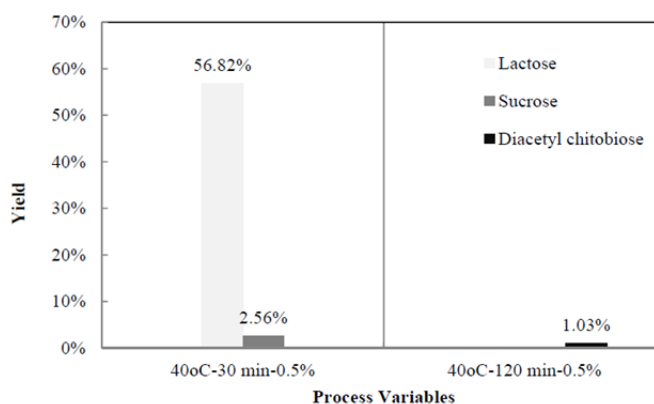


Figure 2. The yield product of combination process sonication (40 oC-0,5 % v/v-30 and 120 min) and hydrothermal (200 oC, 4 mins)

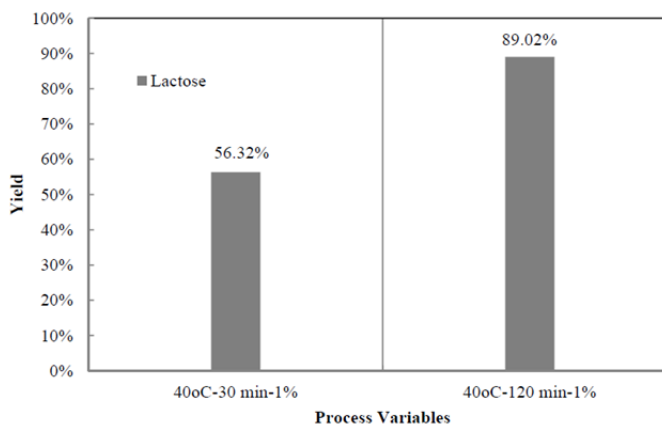


Figure 3. The yield product of combination process sonication (40 oC-1 % v/v-30 and 120 min) and hydrothermal (200 oC, 4 min)

4. Dissucions

4.1 The Intrinsic Viscosity of Chitosan after Processes

The degradation process by hydrothermal reaction was proceeded through a variety of mechanisms including hydrolysis, oxidation and gasification (Jomaa et. al., 2003, pp. 647-653). Hydrolysis refers to breaking up of the organic substances into smaller organic fragments in water. Hydrothermal decomposition also acts on the large organic molecules reducing them into smaller fragments, some of which were dissolved in water. Because of reducing the size of molecules, the hydrothermal will give changing on intrinsic viscosity of chitosan.

The change in intrinsic viscosity suggested that sonication was an effective method to reduce the molecular size of chitosan. Sonication creates cavitation that is responsible for chitosan depolymerization by temporarily dispersing the aggregates, breaking the weakest chemical linkage in chitosan and accelerating mass transport. When the cavities collapse, they produce high energy in the form of a shock wave, shear deformation, local high pressures and temperatures, and elongation flow, which induce the disintegration of the polymer (Suslick, 1989, pp. 80-86; Masselin et al, 2001, pp. 213-220; Grönroos et al, 2001, pp. 259-264; Nguyen, Liang, Kausch, 1997, pp. 3783-3793). Peter Riesz also showed that the heat from a cavity implosion decomposes water (H_2O) into extremely reactive hydrogen atoms (H^\bullet) and hydroxyl radicals (HO^\bullet). During the quick cooling phase, hydrogen atoms and radicals reunite to form hydrogen peroxide (H_2O_2) and molecular hydrogen (H_2). Hawkins and Davies also studied that the amino groups on the C-2 of chitosan facilitated a site-specific fragmentation of the glycosidic linkage during β -cleavage (Fang et al, 1999, pp. 423-432; Kawakishi, Kito & Kito, 1977, pp. 951-957). The hydroperoxide anion is very reactive and easily decomposes into a highly reactive hydroxyl radical (HO^\bullet) which is a very powerful oxidant. The main chemical action of HO^\bullet with a chitosan is shown to be hydrogen abstraction. This radical reacts with chitosan very quickly, and the reaction occurs by HO^\bullet pulling off a hydrogen atom and recombining with it to form water. Water is responsible for the hydrolysis reaction of chitosan. After reducing molecular size of chitosan during sonication, the chitosan also further depolymerised through hydrothermal. The hydrothermal condition supports the chitosan hydrolysis process on acid condition. On hydrothermal condition, the ion products of water increase and the dielectric constant of water also reduces. The conditions had advantages on chitosan hydrolysis.

The concentration of acetic acid also gave effect on the intrinsic viscosity. This indicates that the concentration of the acetic acid solution used changes the chitosan solubility. Some systems (system with sonication pre-treatment for all concentration and without sonication on 1 % v/v) provide clear solutions; a number of systems had solid remained after the hydrothermal process (even in the small amount). This indicates that the amount of chitosan dissolved in the acetic acid solution was different and depended on the solvent concentration. Lower-concentration acetic acid dissolved smaller amounts of chitosan and produced lower concentrations of chitosan during the homogenous phase. In hydrothermal system, hydrolysis was easier occurred in soluble system so that the intrinsic viscosity reduced. But, for partly soluble system, ion hydrogen produced from ionization of water / acetic acid tended to attach NH_2 group of chitosan molecules that has not been dissolved and produced NH_3^+ that made molecules dissolved in the system and not to break the glycosidic linkage. The charged amino group (NH_3^+) at the neighboring C2 atom of chitosan at low pH values seems to increase water exchange in the region of the O3 atom destabilizing the $HO_{3(n)} \cdots O_{5(n+1)}$ hydrogen bond (Eduardo et al, 2008, pp. 2141-2149). Destabilization of hydrogen bond make chitosan easy to be dissolved in the solution. The Table describes that hydrothermal for longer time gave lower intrinsic viscosity of chitosan solution except for system with acetic acid concentration 1 % v/v.

4.2 The Yield of Dissolved Chitosan after Processes

The hydrothermal process in different acetic acid concentration gave different products (Figure 1). On 0.3 % acetic acid, hydrothermal of chitosan gave small amount of glucosamine (0.59 %) and the major product was unknown substances (99.41 %). The yield of glucosamine increased on the system hydrothermal with 0.5 % acetic acid. Otherwise, on 1 % acetic acid produced diacetyl chitobiose (9.63 %) and lactose (54.56 %) and the rest were unknown substances. The difference product might relate to the acetic acid concentration used. It gave effect on the solubility of chitosan in the system as previous explanation. On low acetic acid concentration, only small amount of chitosan can be dissolved initially and for the same operation condition 200 °C (it mean that the energy gave to the system was the same), the energy entering the system will be used to degrade further a small amount of chitosan dissolved to produce unknown substances. On higher concentration (0.5 % acetic acid), the amount of chitosan dissolve initially was increase and the energy was used to degrade chitosan into oligomer followed by degrading into glucosamine. On this system, the glucosamine was increased and the unknown substance was decreased compare to 0.3 % acetic acid system. For 1 % acetic acid system, the amount of chitosan dissolved was high and the energy used to degrade chitosan on higher concentration and the product dominated by dimer (lactose and diacetyl chitobiose). The availability of lactose also suggested that the OH^- ions in the hydrothermal system attack side group of chitosan dimers and replaced it to form lactose.

The combination of sonication and hydrothermal process in acetic acid concentration 0.5% v/v gave products such as diacetyl chitobiose, lactose, sucrose and some degraded products of glucose and glucosamine (unknown substances) as describes in Figure 2. From the figure 2, chitosan dimers dominated the product when sonication was conducted for 30 min. After sonication both on 0.5 % and 1 % acetic acid, all system were homogenous, and all chitosan was dissolved completely. In this case ion products on the system were responsible on degrading

chitosan and produced chitosan dimers. It was similar to the system hydrothermal only when dissolved on 1 % acetic acid. But, for 120 min sonication, chitosan dimers were degraded further into monomer and unknown substances (98.97 %). It was appeared that the product was chitosan dimers (diacetyl chitobiose) in small amount.

Otherwise, the combination of sonication and hydrothermal process in acetic acid concentration 1% v/v gave majority products lactose, and some degraded products of glucose and glucosamine (unknown substances) as describes in Figure 3. On both cases, all system were homogenous initially even before sonication. In this system, ion products on the system were also responsible on degrading chitosan and produced chitosan dimers. According to the results show that ion hydrogen in hydrothermal system did not attack glycosidic linkage of chitosan only, but also attacked groups (NH₂ and NHAc) that was attached in C-2 and exchanged with -OH group. This phenomenon due to NH₂ groups that dissolved in acidic environment was weaker base than hydroxide ions (OH⁻). Thus, -NH₂ groups were easily attacked and replaced by-OH groups, and the products were lactose, in addition to degraded product as unknown substances. It occurred for both the system 30 and 120 min.

The rate constants for the exo-site glucosidic bonds were slightly greater than those for the endo-site bonds. The activation energy for the cleavage of the exo-site bond was smaller than that for the cleavage of the endo-site bond (Grönroon et al, 2001, pp. 259-264). If the products after sonication were introduced in the hydrothermal system, dimer products were hydrolyzed to produce monomer products and then further degraded to form 5 HMF. In this study, degraded product of glucose and glucosamine could not be quantified by HPLC because of the non availability of standard. But, it has already analyzed qualitatively by liquid chromatography – mass Spectrophotometer (LC-MS). The products was analyzed as 5-hydroxyl methyl furfural (5- HMF) which has molecular weight 125 Da.

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