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Dissolution of mechanically milled chitin in high temperature water

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ABSTRACT

Chitin is high in crystallinity in its natural form and does not dissolve into high temperature water (HTW), which often leads to decomposition reactions such as hydrolysis, deacetylation and dehydration when hydrothermally processed. In this work, we investigated the reactions of mechanically milled chitin in HTW. Mechanical milling pretreatment combined with HTW treatment improved the liquefaction of chitin giving a maximum water soluble fraction of 80%, where the untreated chitin was 55%. The reaction mechanism of the milled and raw chitin in HTW was shown to be different. For milled chitin, the dissolution of chitin occurred during the heating period to supercritical water conditions (400 °C) at short reaction times (1 min). Extended reaction time (10 min) led to decomposition products and aromatic char formation. For raw chitin, the dissolution of chitin in HTW did not occur, due to its high crystallinity, so that liquefaction proceeded via decomposition reactions.

1. Introduction

Chitin, the (1–4)-2-acetamido-2-deoxy-β-D-glucan, is industrially produced from marine resources (Muzzarelli, 2012; Muzzarelli et al., 2012). High crystallinity of chitin is often observed due to extensive intra- and inter-molecular hydrogen bonding (Draget, Smidsrod, & Skjak-Braek, 2002; Pearson, Marchessault, & Liang, 1960). Chitin and chitosan activate the macrophages, and are mucoadhesive, antimicrobial, biodegradable and non-toxic; therefore they are widely used for the repair of wounded human tissues (Muzzarelli, 2009), drug delivery and cancer diagnosis (Jayakumar, Menon, Manzoor, Nair, & Tamura, 2010; Jayakumar, Prabaharan, Kumar, Nair, & Tamura, 2011). Future applications are suggested; however, processing chitin is difficult, due to its high crystallinity and low solubility to solvents (Pearson et al., 1960; Takai et al., 1992). Solvents such as dimethylacetamide with LiCl (Brine & Austin, 1975; Tsioptsias & Panayiotou, 2008; Weng & Wang, 2001), methanol saturated calcium chloride dehydrate (Tamura, Nagahama, & Tokura, 2006), methanesulfonic acid (Nishi, Noguchi, Tokura, & Shiota, 1979), hexafluoroisopropanol and hexafluoroacetone sesquihydrate (Capozza RC. Solution of poly-(N-acetyl-D-glucosamine), US Patent 3; Carpozza RC. Spinning and shaping poly-(N-acetyl-D-glucosamine), US Patent 3; Louvier-Hernandez, Luna-Barcenas, Thakur, & Gupta, 2005; Salinas-Hernandez et al., 2009) have been reported to dissolve chitin. However these solvents are toxic and the utilization is not preferable for medical applications.

High temperature water (HTW) is an environmental friendly solvent. Solvent properties such as dielectric constant, viscosity, and dissociation constant can be varied by changing the temperature and pressure of the HTW (Akiya & Savage, 2002; Savage, 1999). Therefore, HTW can take place of organic solvents and acid–base catalysts. Natural polymers such as cellulose and hemicellulose depolymerize and dissolve into HTW due to the high concentration of hydronium and hydroxyl ions compared to water at room temperature (Sasaki, Fang, Fukushima, Adschiri, & Arai, 2000). Reports of treating chitin with HTW have been reported (Quitain, Sato, Daimon, & Fujie, 2001; Sakanishi, Ikeyama, Sakaki, Shibata, & Miki, 1999; Sato, 2004). In HTW, the liquefaction of chitin is suppressed due to its high crystallinity and high temperatures over 300 °C are required (Sakanishi et al., 1999; Yoshida, Ehara, & Saka, 2004). Amorphous chitin, prepared by a concentrated base and acid, showed that the hydrolysis reaction occurred in a much higher rate compared to the original chitin in HTW at 180 °C (Sato, 2004). These results suggest that the reaction mechanism of chitin in HTW is dependent to the degree of crystallinity of the chitin.

The crystallinity of biopolymers can be reduced by mechanically milling (Takeda et al., 2009; Craeyveld, Delcour, & Courtin,

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2008; Van Craeyveld et al., 2009; Venkataraman & Narayanan, 1998). This technique does not require any organic nor inorganic chemical. Mechanically milling has been shown to reduce the crystallinity of polymers such as cellulose (Sasaki et al., 2000) and chitin (Nakagawa et al., 2011). Osada et al. (2012) and Osada, Miura, et al., (2013) reported that a combination of milling and HTW pretreatment enhanced the enzymic decomposition from 5% (untreated chitin) up to 93%. In that work, it was reported that the increased enzymic degradation rate was due to the increased hydrophilicity of the pretreated chitin. However, to our knowledge, the dissolution of milled chitin in high temperature water has not yet been reported. The objective of this work is to investigate the reactions of raw and milled chitin in high temperature water and elucidate the reactions mechanisms.

2. Experimental

2.1. Materials

Chitin was obtained from Sigma–Aldrich and was used as received. Water was purified with a water distillation apparatus (Yamato Co., model WG-220) having a conductivity of 5.5 μs m^{-1}. Formic acid (>99%, Wako chemicals), 5-hydroxymethylfurfural (5-HMF) (99%>, Sigma–Aldrich), acetic acid (99.7%>, Wako chemicals), glycolic acid (>98%, Tokyo Chemical Industry Co., Ltd., Saitama, Japan), and dihydroxyacetone dimer (96%, Tokyo Chemical Industry Co., Ltd., Saitama, Japan) were used as standards for the HPLC analysis.

Milling of chitin was conducted in the interest of understanding the effect of polymer structure especially crystallinity on the dissolution phenomena of chitin. Chitin samples were grinded using a planetary ball mill (Pulverisette-7, Fritsch). A brief procedure is as follows. First, chitin (1.0 g) samples and 24 grinding balls (10 mm diameter) were loaded into a pair of grinding bowls (45 cm^3) and sealed. Both the grinding balls and grinding bowls were made of zirconium oxide. A pair of the loaded and sealed grinding bowls was attached to a revolution disk that was rotated at 700 rpm for 15 min followed by a 15 min rest period and this was repeated for 7 intervals. After the grinding, the milled chitin was removed from the pots for characterization and hydrothermal treatment experiments. The proximate and ultimate analysis of the raw chitin and milled chitin is shown in Table S1.

2.2. Characterization of the raw chitin and milled chitin

Characterization of the raw and the milled chitin samples were conducted. A simplified scheme of the mechanical pretreatment and the characterization preformed on the samples are shown in Fig. S1. Morphologies of the samples were observed by a scanning electron microscope (SEM) (JOEL JSM-6390, JOEL, Japan). Prior to the observation by the SEM, samples were pretreated by carbon deposition to eliminate charging and improve contrast of the SEM images. X-ray diffraction analysis (RU-200A, RIGAKU, Japan) was conducted on the samples to evaluate the crystallinity using a method reported in previous literature (Minke & Blackwell, 1978; Schiffman, Stulga, & Schauer, 2009). The mean particle diameter of the raw and milled chitin samples was measured by laser diffraction analysis (MT3300EX, NIKKISO, Japan). Chemical structures of the samples were investigated by Fourier transform FT-IR analysis (FT/IR-230, JASCO, Japan). Specific surface area of the samples was analyzed by volumetric gas adsorption (BELSORP-mini, BEL Japan), where the data reported are a result of the averages and standard deviations of two separate measurements. The solubility of the raw and milled chitin in water at room temperature was evaluated by the following procedure. First, raw or milled samples (0.1 g) were mixed in distilled water (3.0 g) in a flask at 25 °C for 2 h. After this treatment, the mixture was filtered to obtain a solid and solution. The obtained solid was dried at 60 °C for 24 h under vacuum. The solubility of the samples was evaluated by the net weight between the initial sample and solid obtained after the drying. The obtained solution was analyzed by MALDI-TOFMS to understand the molecular weight of the dissolved compounds. Also, to understand the chemical structure of the dissolved compounds in the solution, this solution was freeze-dried and the obtained solid was analyzed by FTIR.

2.3. Procedure

2.3.1. Hydrothermal treatment of chitin

The hydrothermal treatment of chitin was conducted on both raw and milled chitin samples. The simplified scheme of the experimental procedure and analysis is shown in Fig. S2. First, chitin samples (0.1 g) and distilled water (3.0 g) were loaded into a stainless steel (SUS316) tube bomb reactor with an inner volume of 6.0 cm^3. Then the contents of the reactor were purged with Argon atmosphere for removal of oxygen and then sealed. The reaction was initiated by immersion of the reactor into a fluidized sand bath controlled at the reaction temperature. The reaction temperatures examined were 220 and 400 °C. After the required reaction time, the reactor was quickly quenched by transferring the reactor from the sand bath into a water bath at room temperature. The reaction time was defined as the time when the reactor was first placed into the fluidized sand bath until the reactor was quenched in the water bath. The time required for the reactor to reach the reaction temperature (400 °C) was 4 min, while the time for cooling to below 100 °C was 30 s. The reaction times examined in this work were 1, 10 and 20 min. After the reactor was cooled, the contents of the reactor was washed out with distilled water (60 g) and filtered with a 10 μm membrane filter. The obtained solid was dried in a vacuum oven at 60 °C for 24 h and weighed in a weighing vial after cooling to room temperature in a dry desiccator under N_2 atmosphere. This solid was defined as the product solid. The solid recovery (SR) of chitin after hydrothermal treatment was evaluated by the following equation:

$$\text{Solid recovery}\,(\%) = \left[1 - \frac{W_{\text{product solid}}\,(\text{g})}{W_{\text{chitin}}\,(\text{g})}\right] \times 100 \qquad (1)$$

where $W_{\text{product solid}}$ and W_{chitin} are the weight of the product solid and the weight of the loaded chitin sample of the experiment.

The total carbon concentration of the liquid product was analyzed by a TOC (Shimadzu, model TOC-5000A) and the water soluble fraction (WS) of chitin was evaluated by the following equation.

$$\text{Water soluble fraction of chitin}\,(\%) = \frac{W_{\text{C\_liquid}}\,(\text{g})}{W_{\text{C\_chitin}}\,(\text{g})} \qquad (2)$$

where $W_{\text{C\_liquid}}$ and $W_{\text{C\_chitin}}$ are the amount of carbon in the liquid evaluated from the TOC analysis and the amount of carbon calculated from the elemental analysis respectively.

The liquid product was stored at room temperature for 24 h. After storing the liquid product for 24 h, solid precipitation was observed. The solid precipitate was collected by decantation and dried at 60 °C under vacuum. This solid was defined as the solid precipitate. SEM observation and FTIR analysis were done on the obtained solid precipitate.

Identification and quantification of the compounds obtained in the liquid product were evaluated by high performance liquid chromatography (HPLC). The HPLC system was equipped with a RI detector (RI SE-61, SHODEX), two RSpak KC-811 columns (Showa Denko) and H_3PO_4 aqueous solution (5 mM) was used as mobile phase at a flow rate of 1.0 mL/min at 80 °C. The products