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Effects of supercritical water and mechanochemical grinding treatments on physicochemical properties of chitin

Mitsumasa Osada*, Chika Miura, Yuko S. Nakagawa, Mikio Kaihara, Mitsuru Nikaido, Kazuhide Totani

Department of Chemical Engineering, Ichinoseki National College of Technology, Ichinoseki, Iwate, 021-8511, Japan

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ABSTRACT

This study examined the effects of a combined pretreatment with supercritical water and mechanochemical grinding with a ball mill on the physicochemical properties of chitin and its enzymatic degradation. Following pretreatment with a combination of supercritical water and grinding, chitin had a lower mean molecular weight, a lower crystallinity index, a lower crystallite size, greater *d*-spacing, weaker hydrogen bonds, and the amide group was more exposed compared with untreated chitin. These properties increased the hydrophilicity of the chitin and enhanced its enzymatic degradation. The *N*,*N*′-diacetylchitobiose $(GlcNAc)_2$ yield after enzymatic degradation of chitin following pretreatment with supercritical water (400 °C, 1 min) and grinding (800 rpm, 10 min) was 93%, compared with 5% without any treatment, 37% with supercritical water pretreatment alone (400 °C, 1 min), and 60% with grinding alone (800 rpm, 30 min).

1. Introduction

N,*N*′-diacetylchitobiose, $(GlcNAc)_2$, is a dimer of *N*-acetylglucosamine (GlcNAc). GlcNAc, derived from crustacean chitin (α-chitin), is a versatile functional compound used in skin moisturizers, analgesics for joint pain, and antitumoral and antimicrobial agents (Liang, Chen, Yen, & Wang, 2007; Liu et al., 2011; Muzzarelli, 2011; Muzzarelli et al., 2012; Suzuki et al., 1986; Wang et al., 2008). $(GlcNAc)_2$ has superior physiological activities compared with GlcNAc and it is an attractive building block for the production of oligomers (Usui, Matsui, & Isobe, 1990). Chitin oligomers have elicitor activities in plants and are implicated in the activation of immune responses, the control of intentional inflammation and the stimulation of bifidobacteria growth (Hirano, 2004). The production of $(GlcNAc)_2$ and GlcNAc from crab shells involves numerous steps that require strongly acidic conditions because of the crystallinity and insolubility of α-chitin. An enzymatic process that depolymerizes chitin completely would be environmentally favorable since it would avoid the use of deleterious substances and the generation of large amounts of wastewater. However, α-chitin is insoluble in water under ambient conditions because of its hydrophobicity, high crystallinity, and strong hydrogen bonds. These properties make the enzymatic depolymerization of α-chitin difficult. In light of this, pretreatments that reduce the hydrophobicity of chitin, by loosening the crystal structure and weakening the hydrogen bonds, are important.

Previously, we reported that sub- and supercritical water (Tc = 374.3 °C, Pc = 22.1 MPa) pretreatment improves the enzymatic degradation of chitin and the $(GlcNAc)_2$ yield after enzymatic degradation with an optimum supercritical water pretreatment at 400 °C for 1.0 min was 37%, compared to 5% without pretreatment (Osada et al., 2012). We also reported that mechanochemical grinding pretreatment of chitin using a ball mill enhanced enzymatic degradation (Nakagawa et al., 2011). Although each pretreatment with supercritical water and mechanochemical grinding was effective for enzymatic degradation, detailed physicochemical properties of chitin after these pretreatments were not investigated. In addition, combined pretreatment with supercritical water and mechanochemical grinding of chitin was not conducted. The aim of this study was to investigate the effect of the combined pretreatment with supercritical water and mechanochemical grinding on physicochemical properties of chitin and its enzymatic degradation.

2. Materials and methods

2.1. Materials and enzymes

The sources of chitin and enzymes have been reported previously (Osada et al., 2012).

* Corresponding author. Tel.: +81 191 24 4767; fax: +81 191 24 2146.
E-mail address: osada@ichinoseki.ac.jp (M. Osada).

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2.2. Supercritical water pretreatment

The method used for the pretreatment of chitin with supercritical water was reported previously (Osada et al., 2012). The treatment conditions used in this study were 400 °C for 1.0 min.

2.3. Mechanochemical grinding pretreatment

The grinding equipment was a converge mill produced by Makabe Giken (internal capacity, 1000 cm^3). The details of the mill structure were reported previously (Nakagawa et al., 2011). Two grams of the chitin and 704 g of chromium steel balls (5 mm dia.) were ground at 800 rpm for 10–30 min.

2.4. Average particle size

The average particle size (median size D_{50}) was determined using a particle size distributor (Nikkiso, HRA [X-100]). Methanol was used to disperse the samples.

2.5. Surface area

The Brunauer–Emmett–Teller (BET) surface areas of untreated and pretreated chitins were determined using a nitrogen adsorption method (Bel Japan, Belsorp-2).

2.6. Molecular weight distribution

The molecular weight distribution of chitin was measured using a gel permeation chromatography (GPC) system (Shimadzu, LC-10Avp), which was equipped with a refractive index detector, a GPC column (Tosoh, TSK gel G5000H_{HR}), and a guard column (Tosoh, TSK guard column HHR-H). Pullulan standards (Shodex STANDARD P-82) of 708, 340, 200, 107, 47.1, 21.1, 9.6, and 5.9 kDa were used. A total of 1% of the sample was dissolved in 5% LiCl/DMAC with continuous stirring for two days. Prior to measurement, the samples were filtered through a 0.45 μm nylon filter. The flow rate of the mobile phase was 0.5 mL min^{-1}. The temperatures of the columns were set at 30 °C and the detector was 30 °C. A calibration curve was plotted for the elution time versus the absolute molecular weight of the standards. The relative mean molecular weight of the chitin samples was estimated from the standard curve.

2.7. X-ray diffraction (XRD)

The method used to calculate the crystallinity index of the (1 1 0) lattice (at $2\theta = 20$), the *d*-spacings of the (1 1 0) and (0 2 0) (at $2\theta = 9$) lattices, and the crystallite size of the (1 1 0) lattice from the XRD data were identical to those described previously (Osada et al., 2012).

2.8. Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra of chitin were measured using a Nicolet iS10 spectrometer (Thermo Fisher Scientific Inc.).

2.9. Near-infrared (NIR) spectroscopy

The NIR spectra of chitin were measured using a PlaScan-W spectrometer (OPT Research Inc.).

2.10. Thermogravimetric and differential thermal analysis (TG-DTA)

Thermal analysis of chitin was conducted in a nitrogen atmosphere using a TG-DTA instrument (Rigaku, Thermo plus EVO TG-8120). The temperature program was set to produce a temperature range of 30–600 °C at a rate of 20 °C min^{-1}.

2.11. Enzymatic degradation of chitin

The conditions used for the enzymatic degradation of chitin were reported previously (Osada et al., 2012).

2.12. High-performance liquid chromatography (HPLC)

The HPLC system and the method used to calculate the yields of $(GlcNAc)_2$ and GlcNAc have been reported previously (Osada et al., 2012).

3. Results

3.1. Average particle size

Table 1 shows the effect of various pretreatments on the average particle diameter, as well as mean molecular weight, crystallinity index, *d*-spacing of the (1 1 0) and (0 2 0) lattices, the crystallite size, and the $(GlcNAc)_2$ yield after enzymatic degradation for 48 h.

The average particle size after supercritical water pretreatment was approximately 3000 μm (run 2), which was the same as that of the untreated chitin flakes (run 1). After grinding both with and without supercritical water pretreatment, the particle size decreased significantly to 13–24 μm (runs 3–8). The particle size after combined pretreatment with supercritical water and grinding for 10 min was 18 μm (run 3), which was slightly larger than that observed when grinding was performed for 30 min (run 4). When grinding was performed alone for 10 min or 30 min, the particle size decreased with increasing grinding time (runs 5 and 6). After combined pretreatment of grinding for 10 min or 30 min followed by supercritical water treatment, the particle size was 24 μm in both cases (runs 7 and 8), which was almost the same size as when only grinding was performed (runs 5 and 6). When grinding was performed after supercritical water treatment (runs 3 and 4), the particle size was smaller than that observed with grinding alone (runs 5 and 6). These results indicate that supercritical water pretreatment made the chitin flakes fragile.

3.2. Surface area

The BET surface areas during runs 2–8 were from 22–36 $m^2\ g^{-1}$, which were similar to that of untreated chitin flakes (33 $m^2\ g^{-1}$). These results indicate that the supercritical water and grinding pretreatment did not affect the surface area, although the particle diameters after grinding (runs 3–8) were lower than those before grinding (runs 1 and 2) as shown in Table 1.

3.3. Mean molecular weight

The untreated chitin flake was not dissolved completely in the LiCl/DMAC solvent and we measured the mean molecular weight of only soluble part of chitin. Therefore, the real mean molecular weight of untreated chitin would be higher than 760 kDa. On the other hand, the chitin samples of runs 2–8 were dissolved completely in the LiCl/DMAC solvent. The mean molecular weight decreased to 209 kDa following supercritical water pretreatment (run 2). Following combined pretreatment with supercritical water and grinding for 10 and 30 min (runs 3 and 4), the mean molecular weight decreased significantly. The mean molecular weight was also reduced after grinding alone for either 10 or 30 min (runs 5 and 6), but the reduction was greater when both treatments were performed (runs 3 and 4). This result indicates that the grinding pretreatment reduced the mean molecular weight by making the