

Effective Deacetylation of Chitin under Conditions of 15 psi/121 °C

Hong Kyoon No,^{*,†} Young In Cho,[†] Hyeung Rak Kim,[‡] and Samuel P. Meyers[§]

Department of Food Science and Technology, Catholic University of Taegu-Hyosung, Hayang 712-702, South Korea; Faculty of Food Science and Biotechnology, Pukyong National University, Pusan 608-737, South Korea; and Department of Food Science, Louisiana State University, Baton Rouge, Louisiana 70803

Deacetylation of chitin under autoclaving conditions (15 psi/121 °C) was evaluated for the preparation of chitosan under different NaOH concentrations and reaction times. Deacetylation was effectively achieved by treatment of chitin under elevated temperature and pressure with 45% NaOH for 30 min and a solids/solvent ratio of 1:15. Treated chitosan showed similar nitrogen content (7.42%), degree of deacetylation (90.4%), and molecular mass (1560 kDa) but significantly higher viscosity values (2025 cP) compared with those (7.40%, 87.6%, 1304 kDa, and 143 cP, respectively) of a commercial chitosan. Reduction of the solids/solvent ratio from 1:15 to 1:10 did not affect degree of deacetylation, viscosity, and molecular mass of chitosan.

Keywords: Chitin; chitosan; deacetylation; autoclaving

INTRODUCTION

A variety of chemical processes have been developed and proposed for the preparation of chitosan (No and Meyers, 1995). This biopolymer generally is prepared by treating raw chitin with 50% sodium hydroxide solution, usually at 100 °C or higher to effectively remove the acetyl groups (No and Meyers, 1995). During deacetylation, conditions must, in a reasonable time, sufficiently deacetylate the chitin to yield a final chitosan product that is soluble in dilute acetic acid without significant degradation. A limited number of alternative techniques for chitin deacetylation have been proposed. These include application of thermo-mechano-chemical technology (Pelletier et al., 1990), an alkali impregnation technique (Rao et al., 1987), use of water-miscible organic solvents as diluents (Batista and Roberts, 1990), and use of thiophenol to trap oxygen during deacetylation processes (Domard and Rinaudo, 1983).

Although a variety of methods have been developed for chitosan preparation, few attempts have been made to evaluate a combination of feasible elevated pressure and temperature conditions for rapid deacetylation. Earlier, Pelletier et al. (1990) tried thermo-mechano-chemical treatment using a cascade reactor unit operating (90 s at 230 °C) under reduced alkaline conditions of 10% (w/v) NaOH. However, this process required prior mercerization of chitin in 50% NaOH for 24 h at 4 °C.

The present research reports on the development of a relatively simple and inexpensive process for effective deacetylation of chitin.

EXPERIMENTAL PROCEDURES

Materials. Commercial chitin was obtained from Keumho Chemical Co. (Seoul, South Korea). To obtain a uniform size product, the chitin was ground through a Wiley mill (model

4, Thomas Scientific, Swedesboro, NJ), sifted with 20 (0.841 mm) and 40 mesh (0.425 mm) sieves, placed in opaque plastic bottles, and stored at ambient temperature. Chitin of 0.841–0.425 mm particle size was used for deacetylation. Commercial chitosan (Keumho Chemical Co.), prepared according to a conventional method from the same source of chitin as used in this investigation, was used for comparative purposes.

Deacetylation by Autoclaving. Fifteen grams of chitin in a 300-mL autoclavable bottle was deacetylated by pressure of 15 psi for 5–30 min at 121 °C, using 40–50% NaOH and a solids/solvent ratio of 1:10 or 1:15 (w/v). The autoclave was preheated to 100 °C before introduction of the samples, requiring about 12 min to reach 121 °C. The resulting chitosans were washed to neutrality in running tap water, rinsed with distilled water, filtered, and dried at 60 °C for 4 h in a forced-air oven.

Proximate Analyses. Nitrogen was determined in triplicate using an elemental analyzer (EA 1110, CE Instrument, Rodano-Milan, Italy). Moisture content was determined using a halogen moisture analyzer (HG53, Mettler Toledo, Greifensee, Switzerland).

Solubility. Percentage of solubility of chitosan was determined in duplicate at a 0.5% chitosan concentration in 1% acetic acid.

Degree of Deacetylation (DD). The DD of chitosan was determined in duplicate according to a colloid titration method (Kim, 1996) using N/400 potassium polyvinyl sulfate (PVSK; $f = 1.006$; Wako Pure Chemical Industries, Osaka, Japan).

Viscosity. Viscosities of chitosan samples were determined with a Brookfield viscometer, model RVT (Brookfield Engineering Laboratories, Inc., Stoughton, MA). Chitosan solutions (500 mL) were prepared by dissolving chitosan for 4 h in 1% acetic acid at a 1% concentration on a moisture-free basis; the mixture was allowed to stand for 3 h at room temperature to remove air bubbles. Measurements were made in duplicate using a no. 2 spindle at 5 rpm on solutions at 20 °C with values reported in centipoise (cP) units.

Molecular Weight. Weight-average molecular weight (M_w) of chitosan was measured using multiangle laser light scattering (Dawn DSP-F, Wyatt Technology, Santa Barbara, CA) and refractive index (RI; model 7021, Rheodyne, Cotati, CA) detectors operated in a high-performance size exclusion chromatograph following procedures described by You et al. (1999). Operation conditions were as follows: column, TSK G5000PW (7.8 × 300 mm, Tosoh Corp., Tokyo, Japan); RI detector temperature, 35 °C; flow rate, 0.4 mL/min; eluent, 0.2 M

* Author to whom correspondence should be addressed (telephone/fax +82-53-850-3219; e-mail hkno@cuth.cataegu.ac.kr).

[†] Catholic University of Taegu-Hyosung.

[‡] Pukyong National University.

[§] Louisiana State University.

Table 1. Solubility of Chitosans Prepared by Autoclaving at 15 psi/121 °C and a Solids/Solvent Ratio of 1:15

NaOH (%)	time (min)	solubility ^a (%)
50	30	97.4
45	30	95.2
	20	95.6
	10	95.1
	5	48.1
40	30	70.3

^a Solubility was determined in duplicate at a 0.5% chitosan concentration in 1% acetic acid.

ammonium acetate (pH 4.5); solvent, 0.1 M acetic acid + NH₄-OH; dn/dc (change in RI with concentration) of chitosan, 0.162.

Statistical Analysis. All experiments were carried out in duplicate, and average values or means ± standard deviations (SD) are reported. Mean separation and significance were analyzed using the SPSS (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL) software package.

RESULTS AND DISCUSSION

Solubility of Chitosans. The solubility of chitosan in 1% acetic acid is correlated with the effectiveness of the method used for chitin deacetylation. Chitosan, with a DD >60%, usually is dissolved in dilute acetic acid (No and Meyers, 1995). On this basis, the solubility of chitosans deacetylated by autoclaving (15 psi/121 °C and a solids/solvent ratio of 1:15) under different NaOH concentrations and reaction times was determined (Table 1).

The acid-soluble chitosans with >95% solubility in 1% acetic acid at a 0.5% concentration could be obtained by treatment of the original chitin with 45–50% NaOH for 10–30 min. This demonstrates that a combination of elevated pressure and temperature is effective in obtaining acid-soluble chitosan in a relatively shorter reaction time compared with previously reported conventional methods (No and Meyers, 1995).

On the other hand, chitosans treated with 45% NaOH for only 5 min, and with 40% NaOH for 30 min, were not deacetylated sufficiently to be soluble in 1% acetic acid. Insoluble particles were found in both solutions. Bough et al. (1978) noted that the deacetylation rate is largely determined by the extent of swelling of the chitin particles. Thus, a reaction time of 5 min with 45% NaOH may not be enough for chitin particles to be sufficiently swollen. A decrease of the alkali concentration to 40% required increased time of >30 min to obtain a soluble chitosan.

Characteristics of Chitosans. Table 2 shows nitrogen, DD, viscosity, and M_w of the acid-soluble chitosans in Table 1 compared with those of a commercial Keumho chitosan (Keumho Chemical Co.) prepared according to a conventional method from the same source of chitin as used in this study.

The nitrogen content of the chitosans prepared by autoclaving ranged from 7.18 to 7.46%. DD decreased and viscosity increased with a decrease in reaction times from 30 to 20 min with 45% NaOH. Chitosans prepared with 50 and 45% NaOH for 30 min did not show any significant difference in nitrogen content, DD, and viscosity.

Chitosan prepared with 45% NaOH for 30 min by autoclaving showed comparable nitrogen content (7.42%), DD (90.4%), and M_w (1560 kDa) with those (7.40%, 87.6%, and 1304 kDa, respectively) of the Keumho chitosan. However, the former chitosan revealed a notably higher viscosity (2025 cP) than that (143 cP) of the Keumho chitosan. These data clearly indicate that deacetylation was effectively achieved under the autoclaving conditions without significant degradation of the chitin structure compared with the conventional method.

The viscosity of chitosan reported in the literature generally ranges from 60 to 780 cP (Alimuniar and Zainuddin, 1992; Anderson et al., 1978). These ranges of viscosity also were observed by Cho et al. (1998) with five commercially available chitosans. Furthermore, a commercial Keumho chitosan (Keumho Chemical Co.) prepared according to a conventional method from the same source of chitin used in this study showed a viscosity of 143 cP. On the basis of these composite observations, it is apparent that autoclaving is an effective process to yield higher viscosity of chitosan. Earlier, Rao et al. (1987) proposed an alternative method for deacetylation of chitin at low temperature by a novel alkali impregnation technique. However, the prepared chitosan showed a viscosity of only 290 cP.

Effect of Solids/Solvent Ratios. The effect of solids/solvent ratios on DD, viscosity, and average molecular weight of chitosans prepared by autoclaving with 45% NaOH for 30 min was examined in an effort to minimize usage of NaOH. As shown in Table 3, reduction of the solids/solvent ratio from 1:15 to 1:10 did not affect DD, viscosity, and molecular weight of chitosan ($P > 0.05$). Further reduction of the solids/solvent ratio was found to be insufficient to wet the chitin particles. Thus, a solids/solvent ratio of 1:10 was considered to be a minimum ratio to obtain uniformity in reaction without agitation in this autoclaving process.

This investigation has demonstrated the effectiveness of an autoclaving process for deacetylation of chitin in the preparation of chitosan. This process yields a relatively high DD with reduction in usage of NaOH, in addition to providing advantages of increased rates of processing without significant degradation using less reaction time compared with other conventional processes studied. However, because this process is a batch system, the need for regular monitoring of product quality and development of a continuous system should be considered. Finally, pretreatment of chitin by a

Table 2. Comparison of Characteristics^a of Chitosans Prepared by Autoclaving at 15 psi/121 °C and a Solids/Solvent Ratio of 1:15 with a Commercial Chitosan

NaOH (%)	time (min)	N (%)	DD ^b (%)	viscosity ^c (cP)	M_w^d (kDa)
50	30	7.46 ± 0.07 ^b	91.8 ± 2.9 ^d	2230 ± 65 ^{ab}	1560 ± 256 ^a
45	30	7.42 ± 0.01 ^{ab}	90.4 ± 0.1 ^{cd}	2025 ± 21 ^a	
	20	7.44 ± 0.16 ^{ab}	84.3 ± 0.1 ^{ab}	2402 ± 93 ^b	
	10	7.18 ± 0.16 ^a	82.9 ± 0.1 ^a	2470 ± 189 ^b	
commercial chitosan ^e		7.40 ± 0.14 ^{ab}	87.6 ± 0.2 ^{bc}	143 ± 13 ^c	1304 ± 37 ^a

^a Mean ± standard deviation of duplicate (for DD, viscosity, and M_w) and triplicate (for N) determinations, on a dry basis. Means with different superscripts within a column indicate significant differences ($P < 0.05$). ^b DD, degree of deacetylation. ^c Viscosity was measured with 1% chitosan solution in 1% acetic acid using a Brookfield viscometer. ^d M_w , weight-average molecular weight. ^e Commercial chitosan (Keumho Chemical Co.) prepared by a conventional method from the same source of chitin used in this study.

Table 3. Effect of Solids/Solvent Ratio on Characteristics^a of Chitosans Prepared by Autoclaving at 15 psi/121 °C

NaOH (%)	ratio (w/v)	time (min)	DD ^b (%)	viscosity ^c (cP)	M _w ^d (kDa)
45	1:15	30	90.4	2025	1560
	1:10	30	90.7	2050	1466

^a Average of duplicate determinations, on a dry basis. ^b DD, degree of deacetylation. ^c Viscosity was measured with 1% chitosan solution in 1% acetic acid using a Brookfield viscometer. ^d M_w, weight-average molecular weight.

variety of physical methods may improve the penetration of the alkali into the chitin polymer's particles and result in higher deacetylation rates in a shorter time. Therefore, further investigations using pretreatment techniques such as extrusion are under consideration.

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