Effect of Time/Temperature Treatment Parameters on Depolymerization of Chitosan

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ABSTRACT: Depolymerization of the biopolymer chitosan by an autoclaving process at 121°C and 15 psi was investigated using various treatments. Acetic acid was found to be the most effective solvent in decreasing chitosan viscosity among the six organic acids tested. The rate of viscosity decrease increased with increasing chitosan concentration. The viscosity of 1% chitosan in 1% acetic acid decreased rapidly to 91% of the initial viscosity following the initial 15 min of autoclaving. This decreased gradually to 93% and 94% in 30 and 60 min, respectively, without being adversely affected by the chitosan solution volume. The degree of deacetylation was comparable before and after autoclaving for 60 min. Chitosan at three molecular weights (M_r = 1597, 1110, and 789 kDa) decreased in molecular weight by 46%–51% in the 15-min treatment, 55%–60% in the 30-min treatment, and 60%–62% in the 60-min treatment. The addition of 0.1%–1.0% (v/v) concentrations of hydrogen peroxide to the chitosan solution autoclaved for 15 min decreased viscosity by 94%–98% and molecular weight by 69%–83%. This process is a simple, timesaving, homogeneous depolymerization procedure, and it is possible to prepare partially hydrolyzed chitosan with specified molecular weights by regulating the time of treatment. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 87: 1890–1894, 2003

Key words: depolymerization; viscosity; molecular weight; chitosan; autoclaving

INTRODUCTION

Chitosan is a natural, nontoxic biopolymer derived by deacetylation of chitin, a major component of the shells of crustaceans such as crab, shrimp, and crawfish. During the past several years chitosan has received considerable attention for its commercial applications in biomedical, food, and chemical industries.[3]

Chitosan is now widely produced commercially from crab and shrimp waste shells with a molecular weight (MW) usually in the range of 10^5–10^6 and viscosity reaching or exceeding 1500 cP.[4–6] Recently, chitosan and its oligomers have attracted considerable attention because of antimicrobial,[7–9] antitumor,[10] hypocholesterolemic, and other biological activities.[5] Uchida et al.[9] found in their antimicrobial activity studies that chitosan hydrolyzate, which is slightly hydrolyzed with chitosanase, was more active as an antibacterial agent than was native chitosan and chitosan oligomers. Cho et al.[11] reported that the antibacterial activity of chitosan against *E. coli* and *Bacillus* sp. increased with viscosity decreasing from 1000 to 10 cP.

Low-molecular-weight chitosan can be obtained by chemical or enzymic hydrolysis. In the latter, papain,[12] lipase,[13] pepsin,[14] lysozyme,[15,16] and cellulase[17] are applied to depolymerize chitosan as an alternative to using chitosanase itself.[5] Although enzymatic treatment provides chitosan oligomers with relatively high yields, it is not effective in preparing products with molecular weights greater than a chito-heptamer.[18] In chemical methods, the hydrolysis of chitosan using hydrochloric acid (HCl),[19,20] nitrous acid,[21–23] phosphoric acid,[24] and hydrogen peroxide[5] have been attempted. However, the use of HCl leads either to complete depolymerization or to large polydispersity. Deaminated products of the 2,5-anhydromannose type are formed when using nitrous acid.[12] Hydrolysis of chitosan with phosphoric acid at room temperature usually requires lengthy treatment times of 1–6 weeks. Currently, there is no available information on the preparation of low-molecular-weight chitosan by use of a selective heat/pressure process.

The present article reports on research on the development of a relatively simple and inexpensive procedure for the depolymerization of chitosan using standard autoclaving techniques.

EXPERIMENTAL

Materials

The chitosan (M_r = 1110 kDa) used throughout this research was obtained from Kitto Life (Seoul, Korea).

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In the comparative studies of chitosan of different molecular weights, chitosan with molecular weights of 1597 (Kitto Life) and 789 kDa (Keumho Chemical Co., Seoul, Korea) also were used. All chitosan was in powder form prepared from crab shells and was placed in plastic bottles and stored at ambient temperature during experiments. Hydrogen peroxide (Duksan Pharmaceutical Co., Seoul, Korea), used as an oxidizing agent, was a first-grade reagent.

Preparation of chitosan solutions

The chitosan solution was prepared in 1% acetic acid at a 1% concentration on a dry basis, except for the studies on the effects of various concentrations of organic acids (1%, 3%, and 5% concentrations of acetic, lactic, formic, ascorbic, succinic, and malic acids) and on the effects of chitosan concentrations (0.5%, 1.0%, and 1.5%). All solutions were freshly prepared before autoclaving treatments.

Autoclaving

Each 50-mL screw-capped test tube used received 30 mL of chitosan solution and was treated for 15–60 min at 121°C and 15 psi. The autoclave was preheated to 100°C before introduction of the samples, requiring about 11 min to reach 121°C. Immediately following autoclaving, the test tubes containing the chitosan solution were rapidly cooled in running tap water. For evaluation of the effect of repeated treatment times, 1% chitosan solution was autoclaved at 121°C and 15 psi for 15 min and then cooled under running tap water. This process was repeated up to four times. In studies on the effect of chitosan solution volumes, 50, 100, and 150 mL of 1% such solution were placed in 250-mL screw-capped brown-glass bottles.

Determination of viscosity

The viscosity of the chitosan solutions before and after autoclaving was determined with a Brookfield viscometer, model LVDV-II+ (Brookfield Engineering Labs, Stoughton, MA). Measurements were made using a small sample adapter on solutions (8 mL) at 23 ± 0.3°C, with values reported in centipoise (cP) units. The percentage of viscosity decrease was calculated as follows: viscosity decrease (%) = $(V_i - V_t)/V_i \times 100$, where V_i is the initial viscosity and V_t is the viscosity after t, time of autoclaving.

Determination of recovery yield, degree of deacetylation, and molecular weight

Chitosan solution (30 or 150 mL) autoclaved for a given time at 121°C was added to 2% NaOH solution (60 or 300 mL) and the precipitate filtered using a filter paper (Whatman No. 4). The residue was washed thoroughly with distilled water, freeze-dried, and ground in a mortar for analyses of the degree of deacetylation and molecular weight. The recovery yield (%) of chitosan was calculated as: (g of chitosan recovered/g of original chitosan) × 100.

The degree of deacetylation was determined according to a colloid titration method[25] using N/400 potassium polyvinyl sulfate (PVSK; f = 1.006; Wako Pure Chemical Ind., Osaka, Japan). Viscosity-average molecular weight was established with an automated solution viscometer (Relative Viscometer, Model Y501, USA) using a 0.1*M* acetic acid–0.2*M* NaCl solvent. The percentage of molecular weight decrease was calculated as follows: M_r decrease (%) = $(M_ri - M_rt)/M_ri \times 100$, where M_ri is the initial molecular weight and M_rt is the molecular weight after t, time of autoclaving.

Statistical analysis

All experiments were carried out in triplicate, except for duplicate determinations of degree of deacetylation and molecular weight. Average values or means ± standard deviations are reported. Means of the main effects were separated by Duncan's multiple-range test using the SPSS (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL) software package.

RESULTS AND DISCUSSION

Effect of different chitosan solvents

The possible effects of using different organic acids as chitosan solvent on decreased chitosan viscosity after heat/pressure treatment were compared. Solutions of 1% chitosan were prepared in 1%, 3%, and 5% organic acids and then treated for 15 min at 121°C and 15 psi. As seen in Table I, acetic acid was more effective in decreasing viscosity (%) than were the five other organic acids, particularly at the lower concentration. The greatest decrease in viscosity (92%) was obtained with 1% acetic acid. Among the six organic acids tested, ascorbic, succinic, and malic acids required concentrations greater than 1% or 3% to completely dissolve the chitosan. Based on these results, all subsequent experiments were conducted using 1% acetic acid as the chitosan solvent.

Acetic acid is a commonly used organic acid for solubilizing chitosan[26,27] and promotes acidic hydrolysis more effectively than do malic, lactic, and citric acids.[12] Lactic acid was reported to be less effective in decreasing chitosan viscosity than were citric, tartaric, and propionic acids under reflux reaction.[28] Such a trend also was observed in the present study, although the organic acids tested were somewhat different from those used in previous studies.

TABLE I
Effect of Concentration of Various Organic Acids as Chitosan Solvents on Chitosan Viscosity Decrease by Autoclaving for 15 min at 121°C and 15 psi

Organic acid	Concentration of organic acid (%)		
	1	3	5
Acetic	92[1cC]	90[bE]	88[aF]
Lactic	84[cB]	75[bB]	72[aB]
Formic	82[abA]	84[bC]	81[aD]
Ascorbic	ND[2]	60[aA]	60[aA]
Succinic	ND	87[aD]	87[aE]
Malic	ND	ND	78[C]

[1] Average viscosity decrease (%) of three replicates: [a–c]Different superscripts within a row indicate significant differences ($p < 0.05$). [A–F]Different superscripts within a column indicate significant differences ($p < 0.05$). Thirty milliliters of a 1% chitosan (1110 kDa) solution was placed in each 50-mL screw-capped test tube and autoclaved.

[2] Not determined because of incomplete dissolution of chitosan.

Effect of chitosan concentration

The effect of various chitosan concentrations on chitosan viscosity before and after autoclaving (15 min at 121°C and 15 psi) is given in Table II. The results show a greater viscosity decrease with increasing chitosan concentration. The viscosity of the 0.5%, 1.0%, and 1.5% chitosan solutions decreased by 85%, 92%, and 94%, respectively. An increase in chitosan concentration to 1.5% yielded a highly viscous solution requiring a considerable dissolution time in 1% acetic acid. Therefore, a concentration of 1% was considered appropriate for chitosan depolymerization.

Effect of autoclaving time

The viscosity values for the 1% chitosan solution autoclaved at 121°C and 15 psi for 15, 30, and 60 min are shown in Table III. The viscosity decreased rapidly to 91% of the initial value after the first 15 min of autoclaving and then decreased gradually to 93% and 94% of the initial value in 30 and 60 min, respectively.

TABLE II
Effect of Chitosan Concentration on Chitosan Viscosity Before and After Autoclaving for 15 min at 121°C and 15 psi

Chitosan concentration (%)	Viscosity[a] (cP)		Viscosity decrease (%)
	Before autoclaving	After autoclaving	
0.5	55 ± 1	8 ± 0	85
1.0	258 ± 2	20 ± 2	92
1.5	891 ± 2	52 ± 3	94

[a] Mean ± standard deviation of three replicates. Thirty milliliters of a chitosan (1110 kDa) solution (0.5%–1.5% in 1% acetic acid w/v) was put in each 50-mL screw-capped test tube and autoclaved.

TABLE III
Effect of Autoclaving Times at 121°C and 15 psi on Chitosan Viscosity

Autoclaving time (min)	Viscosity[a] (cP)	Viscosity decrease (%)
0	227 ± 1	0
15	21 ± 1	91
30	17 ± 1	93
60	13 ± 1	94

[a] Mean ± standard deviation of three replicates. Thirty milliliters of a chitosan (1110 kDa) solution (1% in 1% acetic acid w/v) was put in each 50-mL screw-capped test tube and autoclaved.

Autoclaving beyond 1 h did not produce any additional decrease in viscosity (94% in 2 h). Similar to our results, Choi et al.,[28] who studied depolymerization of chitosan in 3% tartaric acid under conditions of 120°C/4.5 atm, found that a reaction longer than 1 h did not further decrease viscosity.

Table IV shows the effects of repeated 15-min autoclaving treatments on chitosan viscosity. The first 15-min treatment resulted in a 90% viscosity decrease; the second, 93%; and the third and the fourth, 94%. These results demonstrated no differences in viscosity decrease between repeated (Table IV) and continuous (Table III) autoclaving treatments at comparable reaction times. Therefore, a continuous process was considered more advantageous because of the savings of energy and time in any projected commercial process.

Effect of chitosan solution volume

All experiments whose results are shown in Tables I–IV were conducted with 30 mL of chitosan solution in 50-mL screw-capped test tubes in order to obtain optimal conditions. To determine differences in autoclaving efficiency with solution volume, 50, 100, and 150 mL of chitosan solution were placed in 250-mL screw-capped brown-glass bottles and treated for 15, 30, and 60 min at 121°C and 15 psi (Table V). The results reveal no noticeable difference in the percent-

TABLE IV
Effect of Repeated Autoclaving of 15 min at 121°C and 15 psi on Chitosan Viscosity

Repeated time	Viscosity[a] (cP)		Viscosity decrease (%)
	Before autoclaving	After autoclaving	
15 min × 1	238 ± 7	23 ± 1	90
15 min × 2		17 ± 3	93
15 min × 3		14 ± 3	94
15 min × 4		15 ± 2	94

[a] Mean ± standard deviation of three replicates. Thirty milliliters of a chitosan (1110 kDa) solution (1% in 1% acetic acid w/v) was put in each 50-mL screw-capped test tube and autoclaved.

age of viscosity decrease among the three chitosan solution volumes at comparable autoclaving times. This indicates that depolymerization of chitosan can be effectively achieved by autoclaving without being adversely affected by the volume of the chitosan solution used.

Muzzarelli et al.[12] documented that a decrease in viscosity of a chitosan solution of as much as 94% could be obtained in 1 h with free papain at pH 3.2 and 50°C. In the present study a comparable decrease in viscosity was obtained within 1 h.

The degree of deacetylation of chitosan after autoclaving for 60 min was determined and compared with that of the initial chitosan before treatment. The results revealed no significant differences in degree of deacetylation between the depolymerized chitosan (98.2%) and the initial chitosan (98.9%). A similar result was observed by Choi et al.[28] using HCl or acetic acid for chitosan hydrolysis.

Based on the aforementioned results, the present method appears to be more effective than enzymatic treatment with papain for depolymerizing chitosan without affecting its degree of deacetylation.

Depolymerization of chitosan of different molecular weights

Changes in the molecular weight of chitosan (M_r = 1110 kDa) with autoclaving time were determined and compared with two chitosan samples of different molecular weights (Table VI). The molecular weights of the three chitosans decreased by 46%–51% in 15 min, 55%–60% in 30 min, and 60%–62% in 60 min. Relatively narrow ranges of decrease in molecular weight (%) with autoclaving time indicate that the molecular weight of chitosan can be manipulated by regulating treatment times.

TABLE V
Effect of Chitosan Solution Volume and Autoclaving Time at 121°C and 15 psi on Chitosan Viscosity and Degree of Deacetylation

Chitosan volume (mL)	Autoclaving time (min)	Viscosity[a] (cP)	Viscosity decrease (%)	DD[b] (%)
50	0	265 ± 3	0	
	15	29 ± 1	89	
	30	18 ± 1	93	
	60	11 ± 1	95	
100	0	265 ± 3	0	
	15	26 ± 1	90	
	30	18 ± 1	93	
	60	12 ± 0	95	
150	0	268 ± 1	0	98.9[a]
	15	27 ± 1	90	
	30	19 ± 1	93	
	60	15 ± 1	94	98.2[a]

[a] Mean ± standard deviation of triplicate determinations. I Chitosan (1110 kDa) solution (1% in 1% acetic acid (w/v) was put in 300-mL screw-capped brown glass bottles and autoclaved.

[b] DD = degree of deacetylation. Values are average of duplicate determinations. Means with same superscripts within a column indicate no significant differences ($P > 0.05$).

TABLE VI
Effect of Autoclaving Time at 121°C and 15 psi on Molecular Weight of Three Chitosans of Different Molecular Weights

MW (kDa)	Autoclaving[a] time (min)	MW[b] (kDa)	MW decrease (%)
1110	15	588	47
	30	505	55
	60	419	62
1597	15	868	46
	30	638	60
	60	600	62
789	15	390	51
	30	323	59
	60	313	60

[a] Thirty milliliters of chitosan solution (1% in 1% acetic acid w/v) was put in each 50-mL screw-capped test tube and autoclaved.

[b] Average of duplicate determinations.

Effect of oxidizing agent

In an effort to further depolymerize chitosan, hydrogen peroxide as an oxidizing agent was added to the chitosan solution at concentrations of 0.1%–1.0% and autoclaved for 15 min. The results (Table VII) show that the percentages of viscosity and MW decrease increased with increasing hydrogen peroxide concentrations. The addition of hydrogen peroxide at a 0.1% concentration decreased the viscosity by 94% and the molecular weight by 69%. This percentage of viscosity decrease corresponds to that (94%) obtained after autoclaving for 60 min without hydrogen peroxide. However, the percentage of molecular weight decrease was higher than that (62%) obtained after 60 min autoclaving without hydrogen peroxide. Further increase in hydrogen peroxide concentration to 1.0% resulted in a molecular weight decrease by 83%.

Recovery of chitosan by precipitation with dilute NaOH from the autoclaved chitosan solutions without and with hydrogen peroxide yielded 91% and 94%–98%, respectively. Incomplete recovery may be a result of incomplete precipitation, loss during filtration, or depolymerization to water-soluble chitosan.

CONCLUSION

This investigation has demonstrated the effectiveness of an autoclaving process (at 121°C and 15 psi) for depolymerization of chitosan. Acetic acid was the

TABLE VII
Effect of Hydrogen Peroxide Concentration on Viscosity, Molecular Weight, and Recovery Yield of Chitosan after Autoclaving for 15 min at 121°C and 15 psi

Concentration (%)	Viscosity[a] (cP)	Viscosity decrease (%)	MW[b] (kDa)	MW decrease (%)	Recovery yield (%)
Before autoclaving	224 ± 4		1110		
After autoclaving					
0	21 ± 1	91	588	47	91
0.1	13 ± 1	94	339	69	94
0.25	9 ± 0	96	290	74	94
0.5	7 ± 1	97	285	74	97
1.0	5 ± 0	98	194	83	98

[a] Mean ± standard deviation of three replicates. Thirty milliliters of chitosan (1110 kDa) solution (1% in 1% acetic acid w/v) containing various concentrations of hydrogen peroxide was put into each 50-mL screw-capped test tube and autoclaved.
[b] Average of duplicate determinations.

most effective chitosan solvent in decreasing viscosity. The viscosity of 1% chitosan in 1% acetic acid decreased by 91%, 93%, and 94% from the initial level in 15, 30, and 60 min of autoclaving, respectively. The degree of deacetylation was comparable before and after autoclaving for 60 min. The molecular weight of the chitosan decreased by 46%–51% in 15 min, 55%–60% in 30 min, and 60%–62% in 60 min autoclaving. The addition of hydrogen peroxide at 0.1%–1.0% concentrations decreased the molecular weight by 69%–83% after 15 min of autoclaving. A significant decrease in chitosan viscosity and molecular weight by a combination of heat and pressure indicates that this process can be utilized for depolymerization of chitosan, an effect usually achieved by chemical or enzymatic hydrolysis. This temperature/pressure process is a homogeneous depolymerization procedure and is relatively simple to do for an industrial-scale operation, as it uses less reaction time than chemical and enzymatic processes. Importantly, it is possible to prepare partially hydrolyzed chitosan of specified molecular weights by regulating the reaction time. Because this process is a batch system, there is a need for close monitoring of product quality in the ultimate development of a continuous processing system.

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