Applied Acoustics xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Applied Acoustics

journal homepage: www.elsevier.com/locate/apacoust

Ultrasound-assisted conversion of alpha-chitin into chitosan

Willian Garcia Birolli, Jorge Augusto de Moura Delezuk, Sérgio Paulo Campana-Filho *

Instituto de Química de São Carlos/Universidade de São Paulo, São Carlos, Brazil

ARTICLE INFO

Article history:
Received 31 January 2015
Received in revised form 24 September 2015
Accepted 1 October 2015
Available online xxxx

Keywords:
Chitin
Chitosan
Ultrasound-assisted deacetylation

ABSTRACT

This paper discusses the production of chitosan by applying high intensity ultrasound irradiation to alpha-chitin suspended in 40% aqueous sodium hydroxide. The average degree of acetylation (DA) of chitosan was determined by ^{1}H NMR spectroscopy and titrimetry while its viscosity average molecular weight (Mv) was calculated from the intrinsic viscosity as determined by capillary viscometry. The results show that fully acid-soluble chitosans (DA < 32%; 100,000 g/mol ⩽ Mv ⩽ 200,000 g/mol) are produced at very high yield (>95%) by applying non-isothermal ultrasound-assisted *N*-deacetylation process to alpha-chitin suspension (44 mg/mL). It is also shown that such a process is more efficient than thermochemical *N*-deacetylation, even being carried out at a lower temperature due to the effects of high intensity ultrasound irradiation.

1. Introduction

Chitin and chitosan are linear polysaccharides composed by 2-acetamide-2-deoxy-D-glucopyranoside (GlcNAc) and 2-amine-2-deoxy-D-glucopyranoside (GlcN) units linked by β-(1–4) glycosidic bonds however, the GlcNAc units being largely predominant (>80%) in chitin while chitosan is richer in GlcN units (>60%). Such different contents of GlcNAC and GlcN units, expressed by the average degree of acetylation (DA), strongly affects the arrangement of the polymeric chains in the solid state as well as the physical–chemical properties and biological activities of chitin and chitosan [1–4]. Actually, the main properties and activities of chitin and chitosan are strongly affected by their crystallinity degree, molecular weight, DA, and also by the source of production and method used to extract chitin, as well as by the processing conditions used to convert chitin into chitosan [5,6].

Chitin exists as two main allomorphs, namely α-chitin and β-chitin, which are arranged according to orthorhombic and monoclinic cells, respectively, the former exhibiting a more compact structure. Indeed, the packaging structure of α-chitin is strongly stabilized by numerous hydrogen bonds along the three unit cell axis due to antiparallel arrangement of its chains inside the polymer sheets while the parallel arrangement observed in β-chitin precludes the establishment of intra-sheet hydrogen bonds [7]. Although both allomorphs of chitin are insoluble in aqueous and common organic solvents, β-chitin displays higher reactivity, swelling and solubility as compared to α-chitin [8], but as this latter is more widely spread in the biomass, mainly as a major component of the crustaceans shells, it is preferentially used in industries and in research laboratories. In contrast, chitosan exhibits lower crystallinity as compared to both chitin allomorphs, better reactivity and improved solubility due to the presence of numerous GlcN units, in which protonated amino groups in diluted acidic media turns the polymer soluble in aqueous media.

Chitosan occurs as a component of the cell wall of some fungi and yeast but it is mainly produced, in industries as well as in research laboratories, by carrying out the *N*-deacetylation of chitin by enzymatic [9] and chemical [10,11] processes, the latter being the most frequently used. The chemical conversion of chitin into chitosan is preferentially carried out by treating chitin with aqueous sodium hydroxide, a process presenting the following drawbacks: (i) the need for executing several consecutive reactions to attain a low average degree of acetylation, (ii) the poor reproducibility of most heterogeneous processes, which causes the variation of the main characteristics and properties of chitosan, and (iii) the occurrence of severe depolymerization due the harsh reaction conditions with respect to the high concentration of alkali (>40% w/w), long reaction time (>60 min) and high reaction temperature (80–130 °C) [12–14].

The ultrasound-assisted deacetylation (USAD) was recently reported as an efficient process to produce chitosan while avoiding severe depolymerization by treating the alkaline suspension of β-chitin with high intensity ultrasound irradiation [15]. Thus, it was shown that although carried out at lower temperature (50–80 °C) and for shorter time (10–60 min) as compared to the conventional chemical processes used to carry out the

* Corresponding author at: IQSC/USP, Avenida Trabalhador são-carlense 400, 13566-590 São Carlos, SP, Brazil. Tel.: +55 1633739929.
E-mail address: scampana@iqsc.usp.br (S.P. Campana-Filho).

http://dx.doi.org/10.1016/j.apacoust.2015.10.002
0003-682X/

N-deacetylation of chitin, the USAD process efficiently promoted the conversion of β-chitin into chitosan of variable degree of acetylation (6% < DA < 17%), high weight average molecular weight (600,000 g/mol < Mw < 1,080,000 g/mol) and low dispersity (Đ < 1.6), according to the ultrasound irradiation amplitude and processing temperature.

The present study focuses on the application of high intensity ultrasound irradiation to convert α-chitin into chitosan and it initially aims to compare the efficiency of such a conversion to that attained when the conventional thermochemical *N*-deacetylation is applied to α-chitin. The reaction efficiency is evaluated by determining the relative amount of water soluble and insoluble products and its characteristics, namely average degree of acetylation (DA) and viscosity average molecular weight (Mv), which are determined by ^{1}H NMR and capillary viscometry, respectively. Additionally, X-ray diffraction is used to evaluate the changes in the solid state arrangement due to the occurrence of ultrasound-assisted *N*-deacetylation of α-chitin.

2. Experimental

2.1. Extraction of α-chitin

Alpha-chitin was extracted from the cephalothoraxes of *Macrobachium rosenbergii* by applying to this biomass the treatments aiming its demineralization, deproteinization and depigmentation, as described in the literature [16]. The resulting α-chitin (Cht) was grinded, sieved and the fraction with particles size in the range 0.125–0.250 mm was used in the experiments aiming the ultrasound-assisted conversion of α-chitin into chitosan.

2.2. Ultrasound-assisted N-deacetylation

The high intensity ultrasound irradiation was applied to α-chitin (440 mg) suspended in 10 mL of 40% (w/w) sodium hydroxide aqueous solution. Thus, the chitin suspension (44 mg/mL) was poured in a double-walled cylindrical glass reactor (internal diameter ≈ 3.5 cm) and then it was submitted to ultrasound irradiation by using a Branson Sonifier Model 450 (ν = 20 kHz) coupled to a 0.5 in (1.3 cm) stepped probe whose position inside the reactor was fixed in all experiments, as described in the literature [15]. The ultrasonic device was adjusted for intermittent irradiation, the ultrasound irradiation was fixed at high amplitude (75 –95%) and the reaction temperature was monitored as a function of the irradiation time.

The reaction was interrupted at the desired time by pouring the reaction medium into crushed ice followed by the addition of 0.1 M HCl up to pH ≈ 8.5; the solid was filtered, thoroughly washed with 80% ethanol/deionized water and dried in air-circulating oven at 30 °C during 48 h.

The dried solid was suspended in 0.05 M HCl and the suspension was kept at room temperature and constant stirring for 24 h to promote the dissolution of chitosan. The insoluble fraction, corresponding to α-chitin insufficiently deacetylated (Cht_Ac), was filtered and thoroughly washed with deionized water while chitosan (Chs) was isolated from the supernatant by addition of aqueous sodium hydroxide up to pH ≈ 8.5, followed by filtration and extensive washing with deionized water. Both fractions, Cht_Ac and Chs, were dried in air-circulating oven at 30 °C during 48 h.

2.3. Average degree of acetylation (DA)

2.3.1. ^{1}H NMR spectroscopy

According to their solubility, the samples were dissolved in 20% DCl/D_2O (α-chitin) or in 1% HCl/D_2O (chitosan).

The procedure of Varum et al. [17] was adapted to acquire the ^{1}H NMR spectrum of α-chitin, as described in the literature [10,18]. Thus, α-chitin (10 mg) was dispersed in 1 mL of 20% DCl/D_2O (w/w), kept under constant magnetic stirring at 70 °C overnight to promote its depolymerization *via* acid hydrolysis and then an aliquot of the clear slightly yellow solution of depolymerized α-chitin was used to acquire its ^{1}H NMR spectrum.

To acquire the ^{1}H NMR spectrum of chitosan, the sample (10 mg) was dissolved in 1 mL of 1% HCl/D_2O (pD = 3–4) upon constant magnetic stirring at room temperature for 24 h to result in a clear solution.

The average degrees of acetylation (DA) of α-chitin and chitosan samples were determined from the corresponding ^{1}H NMR spectra according to Hirai et al. [19] by taking into account the intensity of the signals due to methyl hydrogens from acetamido groups (≈2.0 ppm) and due to H2–H6 (4.1–3.4 ppm). The ^{1}H NMR spectra were acquired at 80 °C by using a Bruker AVANCE III spectrometer (υ = 400 MHz), 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS) being added to the solutions of α-chitin and USAD chitosans as an external reference. Also, the composite pulse sequence "ZGCPPR" was used to suppress the signals of hydrogens from water, resulting in improved Signal-to-Noise (S/R) ratio for the resonance of hydrogens from beta-chitin and chitosans.

2.3.2. Titrimetry

Owing to its solubility in moderately acidic media, the DA of chitosan was also determined by titration. Thus, chitosan (0.1 g) was dissolved in 110 mL of 0.05 M HCl, an aliquot (50 mL) of the chitosan solution was poured into a glass cell thermostated at 25.0 ± 0.1 °C and 0.1 M sodium hydroxide solution was added dropwise by using a Titronic Universal (Schott-Geräte) while the solution conductivity was measured by using a Handylab LF1 conductivimeter (Schott-Geräte). The DA of chitosan was determined from the titration curve as described in the literature [20].

2.4. Viscosity average molecular weight (Mv)

The intrinsic viscosities of α-chitin and chitosan samples were determined by dissolving the polymers in *N,N*-dimethylacetamide/5% LiCl (w/w) and 0.3 mol L^{-1} acetic acid/0.2 mol L^{-1} sodium acetate buffer (pH = 4.5), respectively. The polymer solutions were initially filtered through 0.80 μm and 0.45 μm mixed cellulose esters membrane (WSCWP/Millipore) and polymer concentrations were sufficiently low to result in $1.2 < \eta_{rel} < 2.0$. The AVS-360 viscometer and the AVS-20 automatic burette, both from Schott-Geräte, were used for the viscosity measurements. The glass capillary (ϕ = 0.53 mm) containing 15 mL of the polymer solution was immersed in a water bath maintained at 25 °C ± 0.01 °C and previously programmed volumes of the solvent, *i.e. N,N*-dimethylacetamide/5% LiCl (w/w) in the case of α-chitin and 0.3 mol L^{-1} acetic acid/0.2 mol L^{-1} sodium acetate buffer in the case of chitosan, were sequentially added to provide the desired dilution. The intrinsic viscosity was determined from the extrapolation of the curves η_{sp}/C *versus* C to infinite dilution.

The values of viscosity average molecular weight (Mv) of α-chitin and chitosan samples were determined from the corresponding intrinsic viscosities by using the Mark–Houwink–Sakurada parameters (K' and α) according to the polymer nature, solvent and temperature. Thus, different values of K' and α were used in the case of chitosan samples according to their DA [21], while $K' = 2.4 \times 10^{-4}$ L g^{-1} and $\alpha = 0.69$ were used to determine the Mv of α-chitin [22].

Please cite this article in press as: Birolli WG et al. Ultrasound-assisted conversion of alpha-chitin into chitosan. Appl Acoust (2015), http://dx.doi.org/10.1016/j.apacoust.2015.10.002