

CHARACTERIZATION BY FT-IR OF CHITOSAN OLIGOMERS PRODUCED BY ENZYMATIC SEQUENTIAL ACTION

E.M. DEL AGUILA¹, L. P. GOMES¹, C.I.R. OLIVEIRA², M.C. SILVA², C.T. ANDRADE², J.T. SILVA¹, V.M.F.PASCHOALIN¹

¹ Instituto de Quimica. Univ. Federal do Rio de Janeiro. UFRJ. Brazil, e-mail: emda@iq.ufrj.br.

² Instituto de Macromoléculas Professora Eloisa Mano. Univ. Federal do Rio de Janeiro. Brazil.

Chitosan is a linear polymer formed by β -1,4-linked residues of 2-acetamido-2-deoxy-D-glucose (N-acetyl-D-glucosamine) and 2-amino-2-deoxy-D-glucose (D-glucosamine), usually obtained from deacetylation of chitin. Chitosan and its derivatives have found extensive applications in biological areas. Several studies have shown that the molecular weight and acetylation degree (DA) of the polysaccharide affect its properties, with many special functions appearing when its molecular weight and DA decline to some extent. The development of viable processes for the controlled hydrolysis of chitosan is attracting growing interest because the emergence of new biomedical and food applications for chitooligosaccharides. In this context, enzymatic are preferable to chemical processes, since the hydrolysis course and product distribution are subject to easier control.

We report the characterization of chitooligosaccharides prepared from chitin using chitinase from *Vitis vinifera* L. cv. Red Globe and recombinant chitin deacetylase from *S. cerevisiae* [1]. The enzymatic preparation of chitooligosaccharides has been described previously [2].

Fourier Transform Infrared (FT-IR) spectra were recorded in a Perkin-Elmer spectrometer, model 1720, with a resolution of 2 cm⁻¹ and accumulation of 20 scans. FTIR was used to estimate degrees of acetylation (DA) from the ratio between transmittance at 1320 cm⁻¹ and 1420 cm⁻¹ (characteristic and reference bands, respectively) (Table 1) and the regression curve $A_{1320}/A_{1420} = 0.3822 + 0.03133 \times DA$ [3]. The amide II band at 1550 cm⁻¹ (attributed to the C-N bond stretching and to C-N-H bonds bending) is characteristic of secondary amides. Crystalline shrimp chitin treated with chitinase in pH 3.0 and pH 6.0 prior to deacetylation with chitin deacetylase (Cda2p) showed chitooligosaccharides with DA of 29.3 and 32.5%, respectively (Table 1 and Fig. 1).

Table 1. FT-IR transmittance at 1320 cm⁻¹ and 1420 cm⁻¹ and DA of chitin and chitosan samples

Sample	A1320	A1420	A1320/A1420	DA
Crystalline shrimp chitin (1a)*	0.124	0.035	3.52	100.0%
Chitin + CHase pH 3.0 + CD (1b)	0.017	0.013	1.30	29.3%
Chitin + CHase pH 6.0 + CD (1c)	0.021	0.015	1.40	32.5%
Chitosan (2c)	0.023	0.046	0.50	3.8%
Chitosan (2b)	0.123	0.054	2.27	60.2%
Chitosan + CHase pH 6.0 + CD (2a)	0.012	0.038	0.32	≅ 0.0%

CHase - chitinase; **CD** - chitin deacetylase. The number in parentheses corresponds to FT-IR curves in figure 1 and 2. One milligram of crystalline shrimp chitin was submitted to deacetylation with purified chitin deacetylase, with or without previous treatment by chitinase. Purified chitin deacetylase was used in each assay, which was performed in a volume of 0.15 μ L of 25mM Tris-HCl buffer at pH 8.0. After 1h and 2h at 50°C, the reaction was stopped by heating at 100°C prior to amine determination.

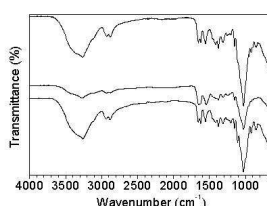
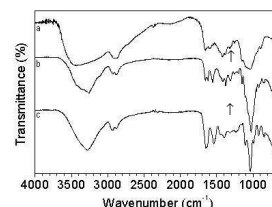


Fig. 1. FT-IR spectra of crystalline shrimp chitin and chitosan products. (a) pure chitin, (b) chitosan treated with chitinase (pH 3) and chitin deacetylase; (c) chitosan treated with chitinase (pH 6) and chitin deacetylase.

Chitosan obtained by alkaline hydrolysis with DA of 60.2% and Mw 759.217 was subjected to the sequential enzymatic treatment producing chitosan polymers completely deacetylated (Table 1 and fig. 2).

Fig. 2. FT-IR spectra of chitosans.

(a) chitosan from chitinase (pH 6) and CD reaction using chitosan with chitin deacetylase of 60.2% as substrate; (b) chemically produced chitosan with DA of 60.2%; (c) control chitosan with DA of 3.8%.



We showed that the sequential enzymatic treatment of crystalline shrimp chitin with chitinase and chitin deacetylase can produce chitosan with DA of 30%, whereas chitosan with DA of 60.2% resultant from alkaline hydrolysis produced chitooligosaccharides completely deacetylated. Optimization of the chitinase and chitin deacetylase reaction conditions seems to be necessary in order to produce chitosan with the required DA and molecular weight.

ACKNOWLEDGEMENTS

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