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FULL LENGTH ARTICLE

The production of fully deacetylated chitosan by compression method

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Abstract Chitosan's activities are significantly affected by degree of deacetylation (DDA), while fully deacetylated chitosan is difficult to produce in a large scale. Therefore, this paper introduces a compression method for preparing 100% deacetylated chitosan with less environmental pollution. The product is characterized by XRD, FT-IR, UV and HPLC. The 100% fully deacetylated chitosan is produced in low-concentration alkali and high-pressure conditions, which only requires 15% alkali solution and 1:10 chitosan powder to NaOH solution ratio under 0.11–0.12 MPa for 120 min. When the alkali concentration varied from 5% to 15%, the chitosan with ultra-high DDA value (up to 95%) is produced.

Introduction

Chitin (CT) as an abundant natural mucopolysaccharide, exists in shrimps, crabs, fungi cell walls and other exoskeleton of insects in crustaceans (Kumar, 2000). Chitosan was obtained from chitin's deacetylation by alkaline hydrolysis or enzymatic method (Morley et al., 2006; Sagheer et al., 2009), whose structure is similar to glycosaminoglycan in the extracellular matrix. The degree of deacetylation (DDA) of chitosan means the content of amino in molecular chain is related to its biological activities directly (Fig. 1) (Tolaimatea et al., 2003). The protonation of amino increases the polyelectrolyte charge, leading to changes in structures, properties and applications.

Chitosan with higher DDA value has more positively charged amine groups when dissolved in acid solution (Alsarra et al., 2002). It is crucial to chitosan's activities, such as antibacterial, lipid-lowering and enhancing immune activities (Dutta et al., 2009; Rinaudo, 2006; Jayakumar et al., 2010, 2011; Xia et al., 2011). In general, the N-deacetylation degree of chitosan between 55% and 70% is called a low degree of deacetylation of chitosan; the 70%–85% is the medium; the 85%–95% is high and the 95%–100% is the ultra-high chitosan. Chitosan with 100% DDA is called full chitosan which is difficult to produce in an industrial field. Moreover, due to good biological activities and chemical modification, the urgent requirements of chitosan materials enlarged its industrial production. Currently, studies on chitin and chitosan mainly concentrated on the refinement of extraction

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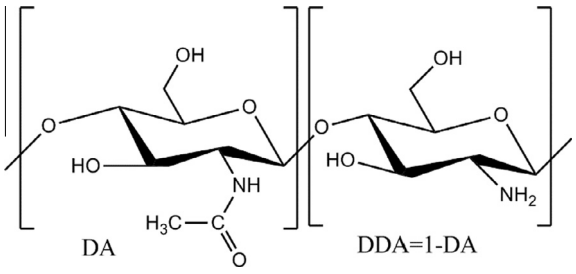

Figure 1 Structure of chitosan.

process and the development of material properties. Therefore, it is necessary to produce chitosan with high DDA value and high Mw, especially can be used in the large-scaled production (Domard, 1987).

A comprehensive knowledge of the Mw and DDA of chitosan is essential for its applications (Yaghobi, 2012). For example, the chitosan hydrogel beads with high Mw and DDA value resulted in a higher loading rate (Alsarra et al., 2002) and the interactions between gelatine and chitosan were stronger (Liu et al., 2012). A higher DDA value also facilitated attachment and proliferation of cells in chitosan coating material (Lieder et al., 2012). Most importantly, all chitosan derivatives derived from 90% deacetylated chitosan revealed excellent cytotoxic activity than those derived from 50% deacetylated chitosan (Je et al., 2006).

According to previous data, traditional method for producing high deacetylated chitosan was adding the chitin to 40% concentrated alkali in 95 °C and stirring for 6–7 h, and then chitosan obtained with approximately 80% DDA value (Jiang, 1997). To improve the efficiency and shorten the reaction time recently, on the one hand, researches raised the temperature to 130 °C, and the DDA value of resultant was up to 94.14% consequently (Ding et al., 2003). On the other hand, some solvents were used to prepare the high DDA chitosan, such as ethanol (Wang and Yu, 1998), *n*-butyl alcohol–sodium hydroxide (Song et al., 2005a,b) and the amyl alcohol (Song et al., 2005a,b) or dimethyl sulfoxide (Ding et al., 2005), and finally 99.7% DDA value was reached. However, the amount of alkali and organic solvents not only increased the economic costs, but also did serious damages on the environment and equipment.

With the development of technology, researches paid more attention to the use of new technology. For example, microwave method can shorten the deacetylated time from 6 h to 10–15 min (Sagheer et al., 2009), but the poor repeatability and high technical requirements limited its use in industry. Tahtat et al found that DDA value increased by 13% by using the 20 kGy gamma ray in 60% concentration alkali in 100 °C (Tahtat et al., 2007). However, the use of gamma ray would cause deleterious effects on researchers (Prasertsung et al., 2013). L. Guillaume created the freeze–pump out–thaw cycles as a new route used for chitin's deacetylation (Lamarque et al., 2005). However, only about 90% DDA value of α-chitosan was acquired. Moreover, although the microwave and gamma irradiation have accelerated the deacetylation, the alkali dosage used in the whole process is so high that it aggravates environmental pollution. Enzyme method as an environmental friendly method has attracted much attention, such as chitinase and xylan acetyl esterase (Morley et al., 2006). However, this enzyme is still used in the laboratory research regarding the expensive price. Considering all factors in chitosan's industrial production, such as the feasibility, economic costs and environmental protection, decreasing the dosage of alkali is a practical and necessary method to eliminate these disadvantages. Therefore, we improved a method to produce the chitosan with high DDA value and Mw in low-concentration alkali condition.

Since deacetylation process of chitosan involved the breakage of C—N bond by nucleophilic substitution, it required a certain activation energy which can be provided by high temperature and pressure. We took advantage of the autoclaves to promote the endothermic reaction to produce full chitosan in a large scale. Full chitosan as raw materials was useful for the preparation of amino-modified oligosaccharides and other applications. To optimize the production conditions and reduce consumption of raw materials, we carried out a series of experiments which focus on the alkali concentration, solid–liquid ratio, reaction time and temperature respectively. The structures and Mw of production were characterized by XRD, FT-IR, UV and HPLC.

Material and methods

Chitosan from shrimp shell, whose DDA value was 82%, was purchased from Qingdao Yunzhou Biochemical Corp. (China), as raw material (150 Yuan/kg). Sodium hydroxide (NaOH) and hydrochloric acid (HCl) are all analytical reagents. Sodium acetate and acetic acid are guaranteed reagent and high performance liquid chromatography (HPLC) reagent respectively. N-acetyl glucosamine was used as standard in ultraviolet detection (UV). They were all used without further purification. Fourier transform infrared (FT-IR) spectra of high DDA chitosan were measured in the 4000–400 cm^{-1} regions using a Thermo Scientific Nicolet iS10 FT-IR spectrometer in KBr discs. X-ray diffraction (XRD) patterns were obtained with a D8 Advance diffractometer (Bruker) with Cu target (l = 0.154 nm) at 40 kV. The scanning rate was 1.2°/min and the scanning scope of 2θ was 5–70°. The DDA value was measured by UV spectrum (Muzzarelli and Rocchetti, 1985) at 199 nm. The Mw of chitosan was measured by an Agilent 1260 gel permeation chromatography equipped with a refractive index detector. Chromatography was performed on TSK G5000-PWXL columns. Using 0.1 mol/L NaAc and 0.2 mol/L HAc aqueous solution as mobile phase at a flow rate of 0.6 mL/min with the column temperature at 30 °C (Shao, 2010; Li et al., 2012). The standards used to calibrate the column were dextran Mw 2000, 1100, 670, 410, 270, 133.8, 80, 50 kDa.

Preparation of full chitosan

Chitosan (10 g) and NaOH were dispersed in distilled water (the alkali concentration ranged from 2% to 40%) in a 500 ml measuring cup and stirred for 3–5 min. After chitosan dispersed completely, the measuring cup was put into vertical pressure steam sterilization pot, and the parameters were set as 120 °C, 120 min and 0.11–0.12 MPa. The resultant was washed by distilled water repeatedly until neutral by centrifugation or pouring. It was filtrated and then dried to constant weight in 105 °C in the oven.