



National Institute of Oceanography and Fisheries
Egyptian Journal of Aquatic Research

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FULL LENGTH ARTICLE

The production of fully deacetylated chitosan by compression method



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Received 31 July 2015; revised 22 September 2015; accepted 23 September 2015

Available online 17 November 2015

KEYWORDS

Preparation;
 Fully deacetylated chitosan;
 Compression method;
 Characterization;
 Low alkali condition

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.

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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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Peer review under responsibility of National Institute of Oceanography and Fisheries.

<http://dx.doi.org/10.1016/j.ejar.2015.09.003>

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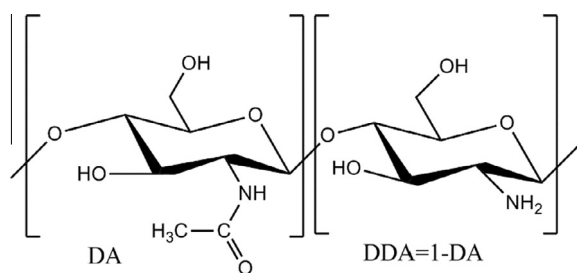


Figure 1 Structure of chitosan.

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 (Yaghoobi, 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 ($\lambda = 0.154 \text{ nm}$) 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.

Table 1 The effect of different reaction conditions on DDA value of chitosan.

C/%	CS:NaOH	RT/min	T/°C	Productivity/%	DDA/%	Mw/kDa
2	1:20	120	120	84.50	86.01	961
5	1:20	120	120	83.18	93.83	814
8	1:20	120	120	83.67	95.46	677
10	1:20	120	120	80.40	96.60	741
13	1:20	120	120	82.27	98.89	680
15	1:20	120	120	79.41	100.00	901
20	1:20	120	120	76.31	100.00	886
25	1:20	120	120	78.08	99.42	739
30	1:20	120	120	72.95	99.94	715
35	1:20	120	120	81.84	99.34	700
40	1:20	120	120	82.68	100.00	A
10	1:5	120	120	A	A	1090
10	1:10	120	120	86.12	98.09	849
10	1:15	120	120	86.08	98.12	773
10	1:20	120	120	87.08	97.70	851
10	1:25	120	120	86.32	97.93	900
10	1:10	40	120	77.12	92.62	1041
10	1:10	60	120	82.84	95.00	1239
10	1:10	80	120	85.22	95.68	910
10	1:10	100	120	83.78	97.68	1013
10	1:10	120	120	81.29	96.65	1017
10	1:10	100	80	86.84	87.42	1388
10	1:10	100	100	87.75	92.09	1192
10	1:10	100	110	84.79	93.15	1320
10	1:10	100	120	86.31	95.50	1091
10	1:10	100	130	80.74	97.25	894

^A Not determined.

Result and discussion

The production of ultra-high chitosan

Through compression method, the chitosan with 100% DDA value was produced from low DDA chitosan in low-concentration alkali conditions, which only required 15% alkali solution and 1:10 chitosan powder to NaOH solution ratio at 120 °C for 120 min. The pressure of the autoclave ranged from 0.11 to 0.12 MPa. In order to save energy and improve reaction efficiency, we dissolved chitosan with NaOH particles together. Because deacetylation is an endothermic reaction and the dissolution of NaOH is exothermic. The heat released in the process of dissolving sodium hydroxide promotes deacetylation of chitosan. When the alkali concentration varied from 5% to 15%, a solid–liquid ratio 1:8–1:15, 110–130 °C and the reaction times 100–180 min, the ultra-high chitosan was produced. Compared to the raw materials, ultra-high chitosan's Mw decreased moderately. When the chitosan's DDA varied from 80% to 100%, the chitosan's Mw fluctuated between 900 and 600 kDa. The yields of resultant are about 80% and all data shown in Table 1. The C% means alkali concentration and CS:NaOH means the ratio of chitosan powder and NaOH solution with various concentration.

The characterization of resultant chitosan

Fig. 2 indicated that the resultant chitosans with different DDA values have similar Mw. Chitosan with medium (400–800 kDa) Mw is able to enhance the transnasal absorption of peptides and other polar drugs, such as insulin, calcitonin and morphine metabolites (Aspden et al., 1996).

In addition, we chose different methods for determining degree of chitosan, and made a simple comparison in Table 2. Compared to the titration and infrared spectrometry methods, ultraviolet spectrometry method is accurate, reproducible and easy to operate. The observation of the end-points would introduce large systematic error in the titration method. In FT-IR, the measure is complex involving the baseline calibration and calculation. Moreover, different formulas caused a greater difference between the results of DDA value. We choose the following two formulas (Brugnerotto et al., 2001; El-Sherbiny, 2009). For example, A_{1655} means the degree of Absorbance appeared at 1655 cm^{-1} Wavenumber. We repeated each experiment under same conditions for five times numbered from 1 to 5 and each sample has been measured for 3 or 2 times.

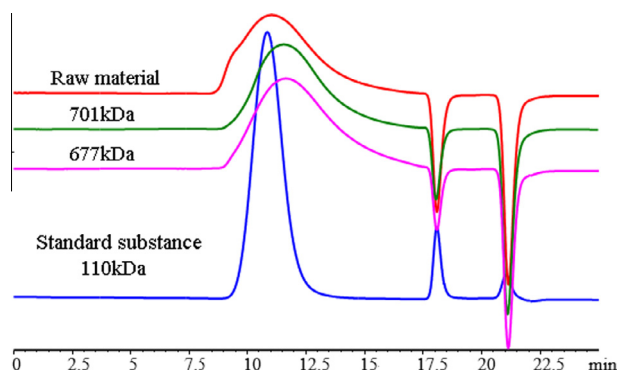


Figure 2 The molecular weight of chitosan, 99.34% DDA and 95.46% DDA chitosan's Mw is 677 kDa and 701 kDa respectively.

Table 2 The comparison of DDA value measured by different methods.

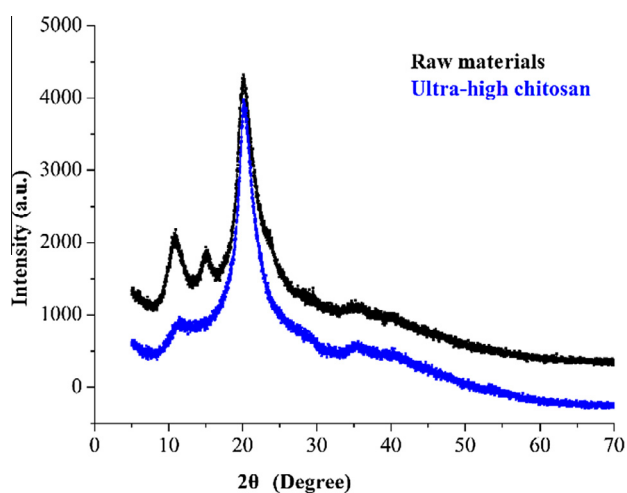
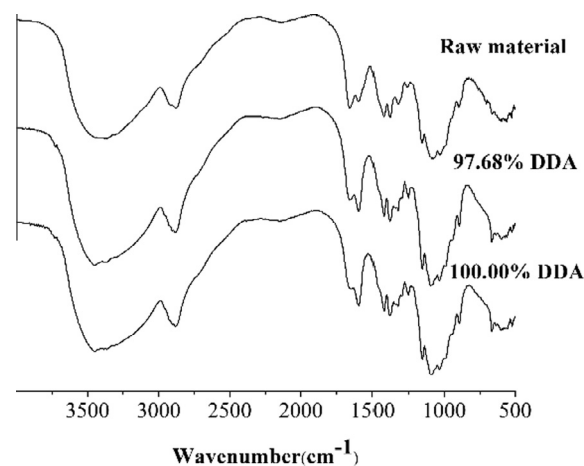
Method		1 (%)	2 (%)	3 (%)	4 (%)	5 (%)
Part-I	DDA _{Titration1}	90.82	90.57	94.93	93.04	92.48
	DDA _{Titration2}	92.85	94.62	93.97	95.40	94.99
	DDA _{Titration3}	93.10	94.45	94.86	94.35	94.66
	DDA-UV1	95.97	95.96	99.42	98.18	99.34
	DDA-UV2	94.44	94.87	97.72	97.07	97.00
	DDA-UV3	95.02	95.36	98.07	97.42	97.99
Part-II	DDA-UV1	82.00	95.96	97.68	100.00	100.00
	DDA-UV2	82.27	95.86	97.73	100.00	100.00
	DDA _{1658/3450}	80.43	94.50	94.60	95.37	96.33
	DDA _{1320/1420}	87.64	100.00	100.00	100.00	100.00

$$\text{DDA} = \left(1 - \frac{A_{1655}}{A_{3450}} \times \frac{1}{1.33}\right) \times 100\% \quad (1)$$

$$\text{DDA} = \left(1 - \frac{A_{1320}}{A_{1420}} \times 0.3192\right) \times 100\% \quad (2)$$

In order to confirm the consistency of structures between raw materials and products, we characterized the ultra-high DDA chitosan by XRD (Fig. 3) and FT-IR (Fig. 4). The results indicated that no chemical structure has changed under high temperature and high pressure.

X-ray diffraction is commonly used to determine the crystal planes and provide strong evidence for polymorphic differences. In addition, it provides accurate measurements of the crystalline content, which greatly affects physical and biological properties of the polymer (Fai et al., 2011). The XRD pattern of raw materials exhibited its characteristic crystalline peaks at $2\theta = 10.75^\circ$ and 20.14° . Similarly, the ultra-high DDA chitosan's peak appeared at $2\theta = 10.97^\circ$ and 20.14° . Moreover, chitosan as a semi-crystalline polymer, the degree of crystallinity is a function of the DDA value (Suh and Matthew, 2000). The intensity decreased with the increase in DDA value in ultra-high chitosan which is consistent with Jaworska's research (Jaworska et al., 2003).

**Figure 3** The X-ray diffraction pattern of chitosan raw materials and ultra-high DDA chitosan.**Figure 4** The infrared spectra of raw material, the 97.68% DDA chitosan and the 100% DDA chitosan.

In Fig. 4, we found that the C=O stretching vibration peak at about 1658 cm^{-1} in the acetyl group disappeared, accompanying with the DDA value increase. On the contrary, the bending vibration peak at 1597 cm^{-1} of the free amino group increased. This confirmed the structure of ultra-high chitosan and its high DDA value.

The effect of alkali concentration, temperature, and pressure, and power-solution ratio on production of chitosan

We illustrated the changes of DDA value during the changes of pressure, reaction time and alkali concentration, and solid-liquid rate in Fig. 5. Through the Origin software, we derived the fitted curve, and subsequently the fitted formula and R square. As shown in Fig. 5A, we found that the NaOH concentration has a great impact on the DDA value of chitosan, especially in low-concentration alkali stage (less than 15%). When the alkali concentration increased, the DDA value increased and has a linear relationship. When the concentration reached 10%, the DDA value grew smoothly, and when it arrived to 15%, the deacetylation of chitosan was completed and did not distinctly change any more. Therefore, the reasonable alkali concentration in industrial production to produce ultra-high chitosan is 10%, while 15% for full chitosan under high temperature and pressure. If the alkali was added excessively, it just means a waste and more pollution. Furthermore, the higher the concentration of alkali was, the more serious the corrosion of equipment was.

Compared to the alkali concentration, the solid-liquid ratio had no significant effect on the DDA value under the preconditions of complete submergence (Fig. 5B). However, the extension of reaction time and the rise of temperature resulted in a striking increase in DDA value (Fig. 5C and D). The prior stage of this reaction generally agreed with Tsaih's finding that the DDA value increased along with reaction time and/or reaction temperature (Tsaih and Chen, 2003). The temperature increased the DDA value markedly and it certified that the reaction temperature plays the dominant role in achieving higher DDA (Tahtat et al., 2007). But in the late stage, about reaction time, the results supported Baskar's research (Baskar and Kumar, 2009), that the DDA didn't vary significantly

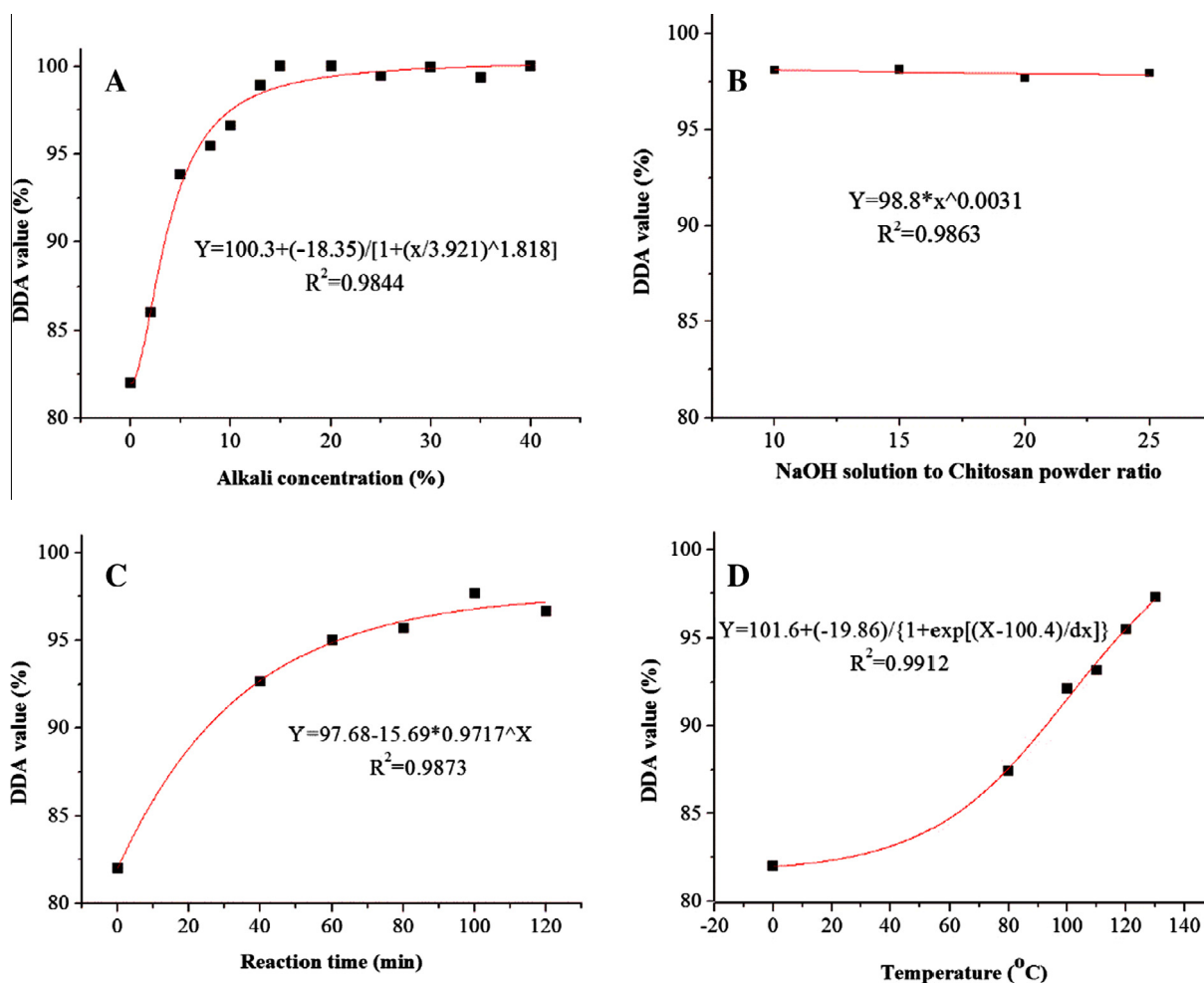


Figure 5 The effects of alkali concentration (A), solid–liquid ratio (B), reaction time (C) and temperature (D) on deacetylation of chitosan.

beyond optimal times. However, in this method, the DDA values didn't decrease apparently as in concentrated alkaline reaction after a certain time because in concentrated alkaline reaction, free amino groups were conducive to forming cations with water. That increased the viscosity and consequently, hindered the reaction processes.

The controlled chemical modification which was achieved by the molecular design has surely extended chitosan's applications. The production of ultra-high chitosan, especially the full chitosan, will guarantee these modifications by providing more active sites in raw materials. Chitin as the main source of chitosan is a natural polymer containing hydroxy and a low content of amino groups. Currently, there are three challenges in N-deacetylation of chitin to produce chitosan. First, the insolubility of chitin; second, the concentrated alkali solutions needed for N-deacetylation; third, high temperature treatment (Tahtat et al., 2007). To explore how to use chitin with lower DDA to produce ultra-high DDA chitosan with low-concentration alkali, we designed experiments at 5%, 10%, 15%, 20%, 25%, 30% alkali concentration and reacted at 120 °C for 120 min (Table 3). The results were consistent with

Table 3 The preparation of chitosan with high DDA value from chitin.

C/%	CT:NaOH	RT/min	T/°C	Productivity/%	DDA/%
5	1:20	120	120	88.95	A
5	1:20	240	120	90.48	A
10	1:20	120	120	88.41	A
10	1:20	240	120	90.88	A
15	1:20	120	120	86.76	A
15	1:20	240	120	88.73	A
20	1:20	120	120	87.66	A
20	1:20	240	120	86.94	A
25	1:20	120	120	85.69	A
25	1:20	240	120	85.88	A
30	1:20	120	120	85.19	A
30	1:20	240	120	86.53	A
30	1:20	360	120	85.44	A
35	1:20	120	120	84.17	85.05
40	1:20	120	120	82.00	90.13
40	1:20	120	120	81.27	94.26
40	1:20	120	120	81.93	89.19

^A Undissolved in HCl.

previous results—when the alkali was less than 30%, no matter how high the reaction temperature and how long the reaction time were, only less acetyl group was removed. Even when the time was extended to 240 min and the strip chitin was crushed into flakes, the DDA value didn't improve obviously. However, when the alkali concentration rose to 40%, the acetyl group of chitin was removed and achieved about 90% at 120 °C for 120 min in autoclave.

Conclusion

In this paper, we introduced a new method for producing ultra-high DDA chitosan in low-concentration alkali condition under high temperature and pressure. The resultants were characterized by XRD and IR. Low dosage alkali and shortened reaction time are excellent advantages over traditional methods. Moreover, compared to the enzymatic and organic solvent treatment method, the compressed method used to produce full chitosan was cheap and convenient without further purification. More importantly, the excellent repeatability and the simplified operation increased its availability in chitosan's large-scaled production and application. In order to produce ultra-high DDA chitosan by chitin with more effective and environmental friendly ways, we expect to employ the multistage method for chitin's deacetylation in low-concentration alkali in our future research. It would adopt the alkali recycling model by using the alkali wastes in the next deacetylated stage. In this model, we could add alkali according to different demands in each phase and timely scavenge acetyl, in case that the large concentration of acetyl group inhibited the deacetylation. Above all, the multistage alkali treatment will be effective not only in controlling the degree of deacetylation but also in controlling the molecular weight of the resultant. Yaghobi's results showed that the number of treatments and the duration of each step of deacetylation significantly affected molecular weight, so two samples were obtained with same DDA and different Mw (Yaghobi, 2012). Of course, various industrial sources of chitin and different production processes and conditions lead to distinct physical and chemical properties of chitosan (Kumirska et al., 2009).

Acknowledgements

The study was supported by Qingdao Science and Technology Plan (No. 14-2-3-47-nsh), the Public Science and Technology Research Funds Projects of Ocean (No. 201305016-2 and 201405038-2), the Science and Technology Development Program of Shandong Province (No. 2012GHY11530).

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