Effects and kinetics of a novel temperature cycling treatment on the *N*-deacetylation of chitin in alkaline solution

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ABSTRACT

The influences of alkaline concentration, cold-treatment temperature, cold- and heat-treatment method, reaction time and the ration of chitin powder to NaOH solution on the *N*-deacetylation of shrimp chitin were investigated. The effects of the alkaline concentration, cold- and heat-treatment method and reaction time were significant; nevertheless, the effects of temperature and the ration of chitin to solution were insignificant. The *N*-deacetylation followed the pseudo-first-order kinetics. The apparent rate constants of the reaction ranged from $3.3 \times 10^{-3} \, h^{-1}$ to $22.8 \times 10^{-3} \, h^{-1}$, and the apparent activation energy was preliminary estimated about 9.76 kJ/mol at aqueous 35% NaOH solution, in the temperature range of -5 to $-35 \, ^\circ C$, which was much lower than that of previous literatures. The effect of novel temperature cycling treatment on the reaction process and structure of chitin, compared with the single alkali-freezing treatment, was greater. So the novel temperature cycling treatment could be regarded as a more efficient novel pretreatment method for further modification of chitin.

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1. Introduction

Chitin is an ideally (1–4)-linked linear polysaccharide composed of 2-acetamino-2-deoxy-β-ᴅ-glucopyranosyl residues. Chitosan composed of 2-amino-2-deoxy-β-ᴅ-glucopyranosyl residues is the *N*-deacetylated derivative of chitin (Shigemasa, Matsu-ura, Sashiwa, & Saimoto, 1996) and usually prepared from chitin. Chitin is the second most abundant natural biopolymer on earth after cellulose, and found mainly in invertebrates, insects, marine diatoms, algae, fungi, and yeasts. Approximately 10 billion tons of chitin is produced by these organisms each year (Rege & Block, 1999; Tolaimate et al., 2000). Chitin and chitosan are bio-renewable, biocompatible, environmentally friendly, biodegradable, and bio-functional such as antibacterial activity, hypocholesterolemic acitivity, antitumor activity, immuno-stimulating effect, antioxidant activity and antihypertensive activity (Chae, Jang, & Nah, 2005; Je, Cho, & Kim, 2006), and these unique properties offer much potential applications in many fields. Recently, it has been widely applied in the fields of agriculture, medicine, pharmaceuticals, dentistry, functional food, food processing, textile production, environmental protection, biotechnology industry, cosmetic/personal care, and so on (Baxter, Zivanovic, & Weiss, 2005; Tan, Khor, Tan, & Wong, 1998; Sandford, 1988).

However, chitin is insoluble in water, acid, alkaline solution, and common organic solvents because of its strong intra- and inter-molecular hydrogen bonds. Its derivative, chitosan, prepared by deacetylation of native chitin, is only soluble in some specific dilute acids because of free amino groups in its chemical structure, such as organic acids including formic, acetic, propionic, lactic, citric, and succinic acid, and a very few inorganic solvents, such as hydrochloric, phosphoric, and notric acid (Wang, Turhan, & Gunasekaran, 2004), but it is still insoluble in the neutral or basic range (Koide, 1998). The water-insoluble property of chitin/chitosan is disadvantageous to its widespread application. Therefore, improving the solubility of chitosan is crucial if this plentiful resource is to be utilized across a wide pH range (Chung, Kuo, & Chen, 2005). To fully improve the solubility of chitin, many methods can be used, such as homogeneous phase reaction (Kurita, Kamiya, & Nishimura, 1991; Sannan, Kurita, & Iwakura, 1976), reducing the molecular weight of chitosan (Chang, 1996), introducing a hydrophilic functional group to the chitosan (Holme & Perlin, 1997). Alkali-freezing treatment of chitin is one of the methods, which has already been chosen as a convenient precursor for efficient modifications (Sannan, Kurita, & Iwakura, 1975; Sannan et al., 1976; Dong, Wu, Wang, & Wang, 2001; Feng, Liu, & Hu, 2004; Tokura & Tamura, 2001).

The kinetics of homogeneous alkaline deacetylation of α-chitin was reported to be a pseudo-first-order reaction (Sannan, Kurita, &

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Iwakura, 1977). Similar results were obtained from heterogeneous deacetylation at 150 °C (Castelli, Bergamasco, Beltrame, & Focher, 1996). However, Chang, Tsai, Lee, and Fu (1997) has reported that under different concentrations of alkali heterogeneous deacetylation of shrimp chitin appeared to be more complicated than a pseudo-first-order reaction. It might be controlled by a higher-order reaction and a diffusion controlled reaction (Chang et al., 1997). Methacanona, Prasitsilpa, Pothsreea, and Pattaraarchachaib (2003) reported that heterogeneous N-deacetylation of squid chitin also followed pseudo-first-order kinetics at the initial period and leveled off after 1 h (Methacanona et al., 2003).

However, deacetylation kinetics of chitin in alkaline solution at low-temperature was still in blank. In this study, we used a novel temperature cycling treatment and the N-deacetylation reaction conditions were controlled. The temperature we chose combined the industrial possibility of producing water-soluble chitosan and the properties of alkali solution. Because the normal low-temperature cold storage was usually kept at −35 °C, and most of the domestic refrigerator was at −18 °C. Here, we chose the two temperatures. In fact, we measured the approximate frozen temperature of aqueous 35% NaOH, and it began to turn to ice crystal below −13.5 °C (it can not be regard as physical concept "freezing point"), so we selected the −15 °C, since ice stress might be stronger when the temperature was close to the ice crystal forming temperature. We also selected −5 °C only for detecting the case when the chitin was in low-temperature but not low enough to freeze. The aim of this study was to investigate the effects of alkali-temperature-cycling treatment on the solid state structure of chitin, and the kinetics of the process was also investigated.

2. Materials and methods

2.1. Materials

Chitin derived from shrimp shells with a degree of deacetylation (DDA, %) of 21.11 ± 0.59%, as determined by alkalimetric estimation, was purchased from Zhejiang Yuhuan Biochemical Co. (PRC). All other commercially available solvents and reagents (analytical grade) were used without further purification. Double distilled water was used throughout unless otherwise specified.

2.2. Alkali-temperature-cycling treatment of chitin

Chitin powder was soaked in different concentration aq NaOH (35, 40, 45, and 50%, wt.%) at room temperature for 8 h, chitin powder to NaOH solution ratio was 1:4, 1:6, and 1:10 (w/V). The mixture in the sealed containers were kept freezing at different temperature (−5, −15, −18, and −35 °C) for about 22 h, the samples processed at low-temperatures were then taken out and heated by various methods: (I) processed at room temperature; (II) 320 W microwave (WP800TL23-K1, Galanz, China) assisted process for 5 min; (III) heated for 15 min in a boiling water bath; (IV) heated at 40 °C for 2 h. The total time of low-temperature treatment and heat-treatment was designed as 24 h (1 day), after the first cold-treatment and heat-treatment cycle, the sample was coded as 1st day. Repeated the temperature cycling treatment for 2, 3, 4, 5 or 6 times, the samples obtained were coded as 2nd, 3rd, 4th, 5th, and 6th day correspondingly, and the total treatment time was 48 h, 72 h, 96 h, 120 h, and 144 h, respectively. The rate of heating will depend not only on temperature but also the quantity of material being treated. All the samples treated with the same type of container, and the total mass of samples was the same except for 1:6 and 1:10 (the ratio of chitin and alkali solution) samples. And all the data used for kinetic analysis and regression analysis was obtained under the same conditions.

After the temperature cycling treatment, the heat-treated samples were then dispersed in 70% (V/V) aqueous ethanol (EtOH) and neutralized using diluted HCl in ice-water bath carefully. The precipitated samples were filtered and washed with 70% EtOH until no Cl⁻ remained (determined by AgNO₃ method), finally the samples were dried at 60 °C under reduced pressure. Each sample was carried out in triplicate.

The reference sample prepared as the following method and was coded as V: chitin was soaked in aq 45% (wt%) NaOH at room temperature, the chitin to solution ratio was 1:10, and then kept freezing at −18 and −35 °C for various time, ranging from 1 to 5 days, the sample was thawed at room temperature without further treatment (Feng et al., 2004).

2.3. Determination of degree of deacetylation (DDA)

The acid-base titration method (Gu, Wang, Liu, & Xia, 2003; Wu, Zeng, Zeng, & Zhang, 2004) with a little modification was used to determine the degree of deacetylation of the samples. All the samples were desiccated for 24 h at 80 °C under reduced pressure, and then 0.1500 g of the various samples were dissolved in 20 ml 0.1 mol/L HCl standard solutions, and stirred for 3 h at 25 ± 0.1 °C. The mixture was diluted with 20 ml double distilled water, and then titrated with 0.1 mol/L NaOH of the standard solution using two drops of 0.1% methyl orange–aniline blue (1:2, V/V) water solution as an indicator (Chen & Guo, 1990). All experiments were carried out in triplicate. DDA was calculated using the following equation:

$$\text{DDA}(\%) = \frac{(C_1 V_1 - C_2 V_2) \times 0.016}{G(1 - W) \times 0.0994} \times 100\%$$

where C_1 and C_2 are the concentration of the standard solution of HCl and NaOH (mol/L), respectively. V_1 was the volume of HCl (ml), and V_2 was the volume of NaOH at the end point of titrated (ml). 0.016 was the –NH₂% content equal to 1 ml 1 mol/L HCl standard solution (g), 0.0994 was the ideal –NH₂% content of chitin (16/161), G was the sample mass (g), W was the water content (%).

2.4. FT-IR spectra analysis

The deacetylated samples were ground and blend well with KBr, the sample to KBr ratio was 1:60, the mixture were dried overnight at 60 °C under reduced pressure, and then KBr discs were prepared under 27 MPa pressure for 2 min, each sample was prepared 6 discs. The KBr pellets of the mixed powder were desiccated for 24 h at 110 °C under reduced pressure, and then their IR spectra were recorded on a Nicolet NEXUS 470 FT-IR spectrophotometer. At 32 accumulate scans with a resolution of 4 cm⁻¹ were averaged and referenced against air. The intensity of selected IR absorption bands were determined by the baseline method using the OMNIC software package of the instrument (Brugnerotto et al., 2001; Duarte, Ferreira, Marvao, & Rocha, 2002; Shigemasa et al., 1996). Crystalline Index (CrI) was calculated from the ratio of absorbance at A_{1382}/A_{2920} cm⁻¹ (Focher, Beltranme, Naggi, & Torri, 1990).

2.5. Statistical analysis

All collected data were expressed as mean ± SD. The statistical analysis method such as ANOVA was adopted to analyse the data obtained from the experiments, and the least significant difference (LSD) at 5% was applied to define significant difference between mean values. Polymultiple non-linear regression analysis was also used to search the optimal condition for N-deacetylation reaction, the backward method was used. G3D model was employed to gen-

erate three-dimensional graphs. The data were statistically analysed by using the SAS System for Windows V8 (SAS Institute Inc., Cary, NC 27513, USA, 2000).

3. Results and discussions

3.1. Alkaline N-deacetylation of chitin

In traditional methods, abundant high concentration NaOH solution was usually employed to make chitin deacetylating, which caused environmental pollution and made the following purification of chitosan more difficult. Therefore, it was meaningfulness to develop a method to reduce the amount of NaOH. Alkali-freezing method was usually adopted, −18 °C was a common used temperature (Dong et al., 2001; Feng et al., 2004).

Effects of chitin to solution ratio, NaOH concentration, cold-treatment temperature, heated methods and reaction time on the N-deacetylation reaction were investigated. By using acid-base titration method for determination of DDA, the process of N-deacetylation could be monitored. Table 1 showed the change of DDA in several conditions. The effect of chitin to solution ratio was significant (chitin:solution < 1:6, $p < 0.05$), but it was insignificant when there was an excess of NaOH solution (chitin:solution > 1:6, $p > 0.05$). At high concentration of NaOH with long reaction time, there were no significant changes on the alkali N-deacetylation of chitin, while it was significant with a short reaction time. The effects of cold-treatment temperature and heated methods were significant not only at the initial treatment, but also in the later reaction period. The greatest impact on the DDA was microwave-assisted heat method and the second greatest was boiling water treatment with short times reaction such as the 1st day treatment. Nevertheless, long time of treatment such as heat method of 5 days at the 40 °C became the greatest. An interesting result showed that under −15 °C cold-treatment temperature, DDA increased significantly, and it might be related to the freezing concentration effects, which might made the NaOH infiltrate more easily. In a word, the DDA of most samples was still not increased too much even with long time of alkali-temperature-cycling treatment, which provided the possibility for preparing partially deacetylated water-soluble chitin using alkali-temperature-cycling treatment as a pretreatment method.

3.2. Optimal conditions for N-deacetylation

In order to investigate the optimum conditions of N-deacetylation, DDA of samples (Nos. 4–7 and 10–12, the No. is the same as Table 1) obtained from various conditions was analysed with the RSREG procedure of SAS and the Backward Elimination Stepwise method was employed, and the results were shown in Table 2. Reaction time, NaOH concentration, interaction of temperature and time, interaction of time and NaOH concentration played dominant role on the degree of deacetylation ($p < 0.05$). The quadratic term of temperature, NaOH concentration and time (($Temp.)^2$, $(Con.)^2$ and $(Time)^2$) also showed significant effects on the DDA ($p < 0.05$). However, the effect of other factors including the cold-treatment temperature, the interaction of temperature and NaOH concentration were insignificant ($p > 0.05$). Response surface for the change in DDA as a function of temperature and time, time and NaOH concentration, and temperature and NaOH concentration were shown in Fig. 1a–c, respectively. The best-fit regression equation for the optimum deacetylation within the experiment range was obtained from the statistical analysis.

$$DDA(\%) = -260.2205 + 12.7571 \times Con. + 0.6936 \times Time$$
$$+ 0.0019 \times Temp. \times Time - 0.0074 \times Con. \times Time$$
$$- 0.0053 \times (Temp.)^2 - 0.1337 \times (Con.)^2 - 0.0010$$
$$\times (Time)^2$$

where DDA was the degree of deacetylation (%); Temp., cold-treatment temperature (°C); Con., NaOH concentration (%); Time, temperature cycling treatment time (h).

3.3. Kinetics of N-deacetylation

3.3.1. Effects of alkaline concentration on N-deacetylation

The effect of alkaline concentration on N-deacetylation was shown in Fig. 2a, b. It seemed that at a low concentration condition, such as 35% (wt%), the N-deacetylation followed the pseudo-first-order kinetics at the first 5 days treatment, and the apparent reaction rate constant was 4.3×10^{-3} h^{-1} (R^2 value = 0.9879). Nevertheless, with the increasing of NaOH concentration, the deacetylation process did not followed the first-order kinetics at the total treatment. There was a sudden jump point on the first

Table 1
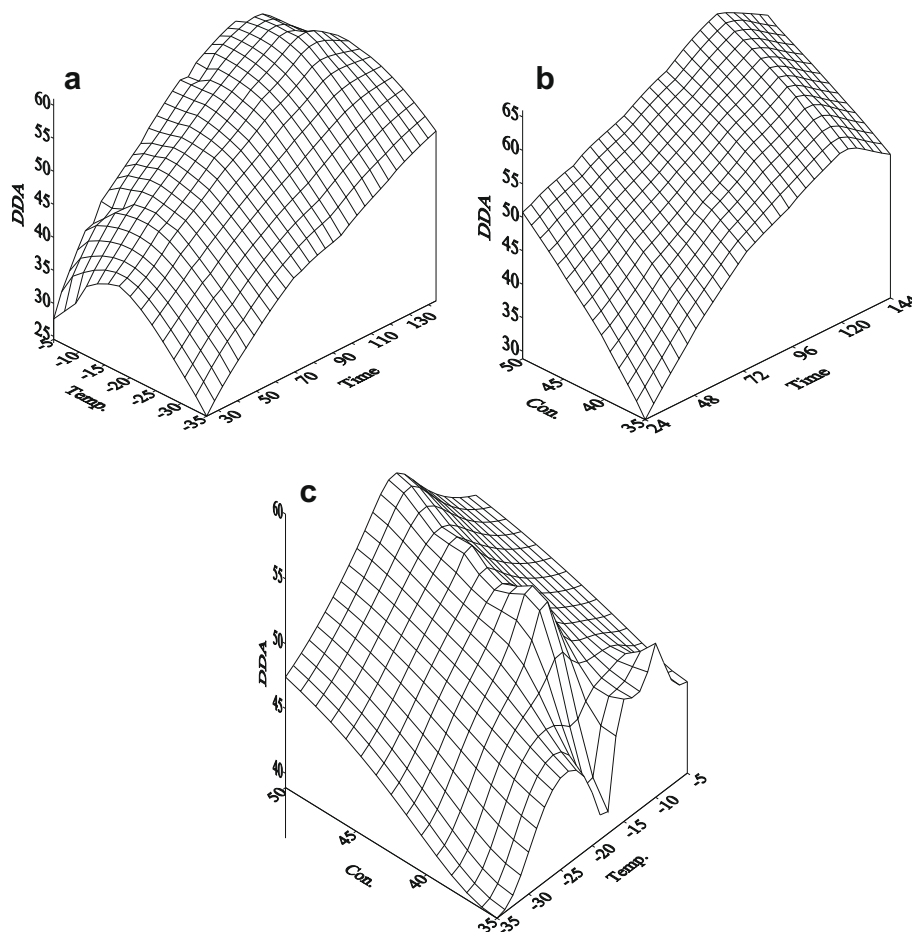Degree of N-deacetylation of chitin obtained from various conditions.

No.	RCS. (w/V)	He.	Con. (wt%)	Temp. (°C)	DDA(%)				
					24 h	48 h	72 h	96 h	120 h
1	1:4	IV	35	−18	35.41 ± 0.64	42.17 ± 0.65	48.12 ± 0.40	53.69 ± 1.24	62.59 ± 0.43[a]
2	1:6				–	56.10 ± 0.74[b]			
3	1:10				–	55.36 ± 0.15[b]			
4	1:4	I	35	−18	25.73 ± 0.25[b]	33.46 ± 1.15[c]	42.45 ± 0.18[c]	47.6 ± 0.25[c]	51.31 ± 0.46[b]
5			40		45.58 ± 6.57[a]	49.37 ± 0.66[b]	52.07 ± 0.71[b]	61.77 ± 0.74[a]	64.75 ± 0.42[a]
6			45		44.10 ± 0.11[a]	50.41 ± 0.21[a]	55.83 ± 0.23[a]	60.33 ± 0.44[b]	63.94 ± 1.14[a]
7			50		49.33 ± 0.53[a]	52.83 ± 0.52[a]	56.64 ± 0.68[a]	59.87 ± 0.77[b]	64.81 ± 0.85[a]
4	1:4	I	35	−18	25.73 ± 0.25[d]	33.46 ± 1.15[d]	42.45 ± 0.18[d]	47.60 ± 0.25[d]	51.31 ± 0.46[d]
8		II			47.24 ± 0.39[b]	53.79 ± 0.59[a]	60.12 ± 0.78[a]	64.55 ± 0.70[a]	60.49 ± 1.87[b]
9		III			54.32 ± 0.30[a]	49.77 ± 0.41[b]	45.74 ± 0.98[c]	50.32 ± 0.57[c]	56.85 ± 0.89[c]
1		IV			35.41 ± 0.64[c]	42.17 ± 0.65[c]	48.12 ± 0.40[b]	53.69 ± 1.24[b]	62.59 ± 0.43[a]
10	1:4	I	35	−35	23.44 ± 0.68[d]	32.69 ± 1.86[c]	39.27 ± 1.05[d]	41.34 ± 1.81[d]	46.38 ± 0.15[d]
4				−18	25.73 ± 0.25[c]	33.46 ± 1.15[c]	42.45 ± 0.18[c]	47.60 ± 0.25[c]	51.31 ± 0.46[c]
11				−15	35.05 ± 0.64[a]	44.30 ± 0.57[a]	49.38 ± 0.98[a]	53.30 ± 0.51[b]	59.67 ± 0.84[a]
12				−5	27.54 ± 0.58[b]	41.51 ± 0.61[b]	47.69 ± 0.84[b]	55.21 ± 0.22[a]	57.81 ± 0.41[b]
13	1:10	V	45	−35	23.03 ± 0.34[b]	23.11 ± 0.69[b]	24.12 ± 0.64[a]	22.43 ± 0.74[b]	23.65 ± 0.67[a]
14				−18	24.29 ± 0.30[a]	25.18 ± 0.52[a]	23.91 ± 0.32[a]	24.23 ± 0.77[a]	23.91 ± 1.41[a]

The values were presented as mean ± SD, $n = 3$; values with different superscripts within a column indicate significant differences ($p < 0.05$). RCS., ratio of chitin and NaOH aq. solution; He., heat methods; Con., NaOH concentration (wt%); Temp., cold-treatment temperature (°C), respectively.

Table 2
Regression analysis between the degree of *N*-deacetylation and the alkaline *N*-deacetylation variables.

Variable	Parameter estimate	Standard error	Type II SS	*F* value	Pr > *F*
Intercept	−260.2205	30.7185	647.1957	71.7600	<.0001
Con.	12.7571	1.4678	681.2426	75.5400	<.0001
Time	0.6936	0.0801	675.4753	74.9000	<.0001
Temp. × Time	0.0019	0.0009	43.0119	4.7700	0.0316
Con. × Time	−0.0074	0.0017	175.4392	19.4500	<.0001
(Temp.)^2	−0.0053	0.0017	85.9516	9.5300	0.0027
(Con.)^2	−0.1337	0.0173	541.1623	60.0000	<.0001
(Time)^2	−0.0010	0.0003	105.2717	11.6700	0.0010
R^2	0.9289				

Temp., cold-treatment temperature (°C); Con., NaOH concentration (wt%); Time, temperature cycling treatment time (h).

Fig. 1. Effects of (a) temperature (Temp.) and Time; (b) Time and NaOH concentration (Con.); (c) Temp. and Con. on the degree of *N*-deacetylation of chitin.

day's treatment, but the reaction reduced gradually and followed the first-order kinetics again at the subsequently treatment, and the reaction rate constant decreased as a function of concentration rapidly ($R^2 = 0.9985$). It could be seen, at the 1st day's treatment, the higher NaOH concentration led to the faster the loss of acetyl group. For example, at 50% concentration, the reaction rate was four times more than that of at 35% concentration, but at the subsequently treatment it was lower than the latter. The heterogeneous *N*-deacetylation of chitin was a typical solid–liquid phase reaction, and deacetylation reaction first occurred in the chitin particles surface and shallow surface. At the beginning, the DDA was low, many acetyl groups on the chitin particles surface. With increasing the NaOH concentration, more NaOH contacted with the acetyl groups, the reaction rate was bigger. Thereafter, the surface acetyl groups decreased and the infiltration of NaOH into the

chitin particles was farther more difficult, thus the reaction rate decreased. In a sense, it was not an effective method to improve the deacetylation rate by only increasing the concentration of NaOH.

3.3.2. Effects of cold-treatment temperature on N-deacetylation

Fig. 3 showed the effects of cold-treatment temperature on *N*-deacetylation. At the same alkaline concentration, the *N*-deacetylation process also followed the pseudo-first-order kinetics, and the reaction rate constants declined as a function of temperature decreased. At 35% NaOH conditions, the apparent activation energy was preliminary estimate as about 9.76 kJ/mol ($R^2 = 0.9247$) with Arrhenius equation and plot method. This is lower than the 35.56–57.74 kJ/mol reported for heterogeneous *N*-deacetylation between 80 and 120 °C (Castelli et al., 1996), 35.63 and 46.02 kJ/
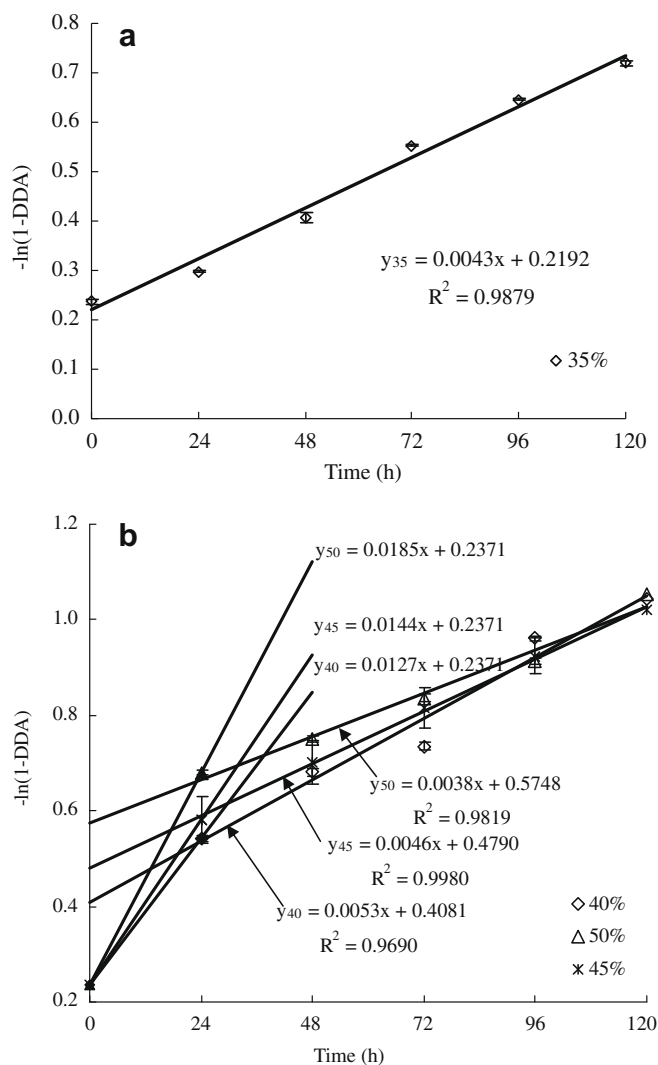
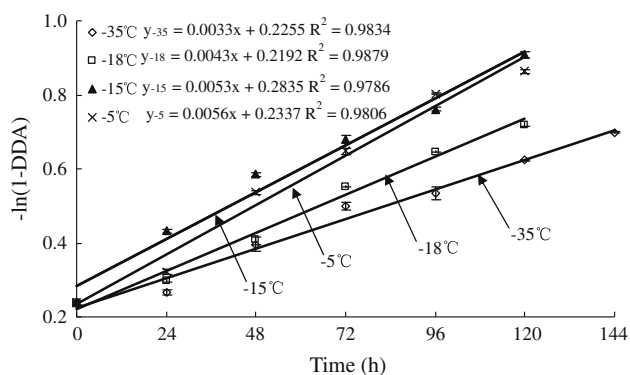**Fig. 2.** Effect of NaOH concentration on the alkali *N*-deacetylation: (a) 35%; (b) 40%, 45%, and 50%.

Fig. 3. Effect of cold-treatment temperature on the alkali *N*-deacetylation.

tion rate constants at −5 and −15 °C, but was higher than the constants at −18 °C and −35 °C, respectively. At approximately −15 °C, which was 1–2 °C below the freezing temperature of chitin–alkali mixture, there was a so called freezing concentration effect. The mechanical force induced by the repeatedly formed and recrystallized of ice crystals during the slow cold-treatment and low-temperature heat-treatment cycle process, might destruct the condensed matter especially the crystalline state structure, so that the mass-transfer process was aggrandizement. NaOH was forced to penetrate into the chitin particles, and the rate constants were larger. When the temperature deviated from the freezing temperature, most ice microcrystals formed rapidly, the crystalline
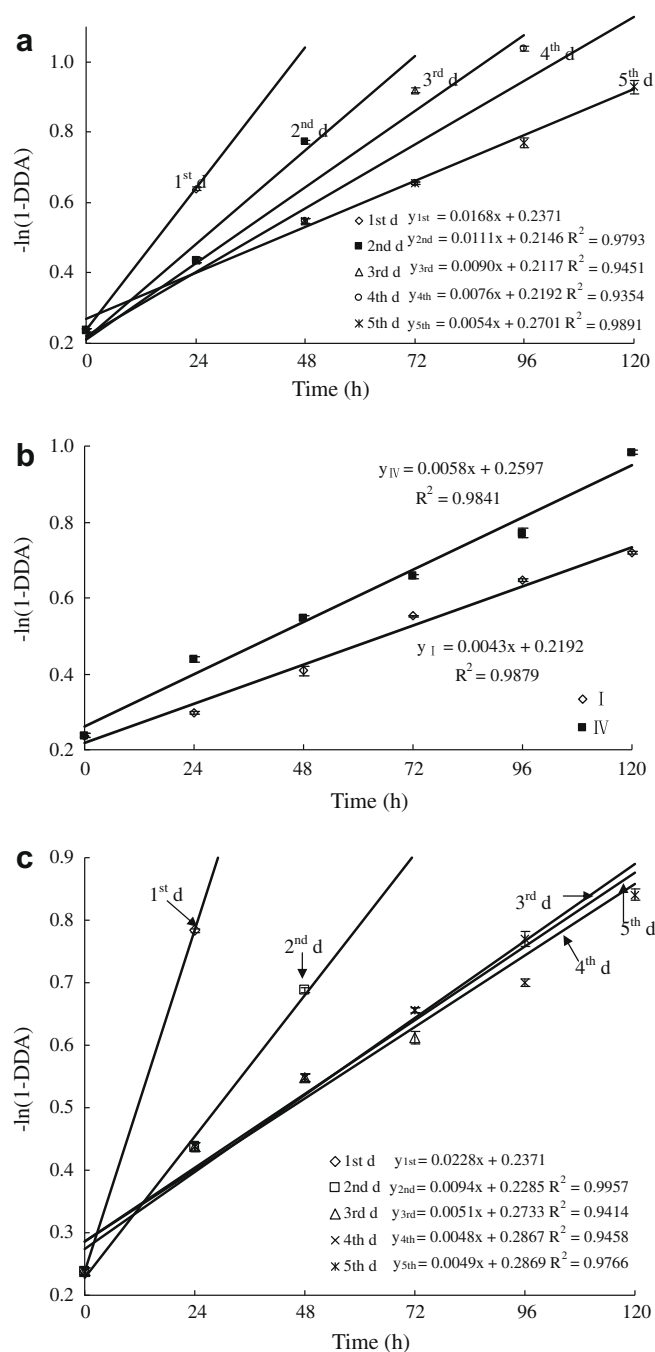

Fig. 4. Effects of different heat methods on the alkali *N*-deacetylation: (a) 15 min heat in boiling water bath; (b) room temperature treatment (I) and heat at 40 °C with 2h′ treatment (IV); (c) microwave-assistantted heat.

mol reported for heterogeneous *N*-deacetylation between 51.0 and 82.5 °C, and 80–100 °C, respectively (Chen, 1992; Focher et al., 1990). Furthermore, this is also lower than the 92.05 kJ/mol for homogeneous *N*-deacetylation in the temperature range 25–40 °C reported by Sannan et al. (1977). So it means that repeated cold- and heat-treatment may break the crystal structure of chitin and improve the process of deacetylation. It was similar with the reac-

destructed effect decreased, and then the concentration effect transferred as cryogenic effect, the resistance of mass-transfer decreased only to an extent far from enough, the rate of mass-transfer were lower than that cold-treatment at $-15\,^{\circ}\text{C}$, so the reaction rate constants were lower than that cold-treatment at $-15\,^{\circ}\text{C}$.

3.3.3. Effects of heating methods on N-deacetylation

The effects of heating methods on the *N*-deacetylation were shown in Fig. 4a–c. All the deacetylation process followed pseudo-first-order kinetics. The results showed the effects of heating methods on *N*-deacetylation were significant (Table 1, $p < 0.05$), and the more intense heating methods were, the higher DDA were. Fig. 4a showed the effects of boiling water treatment, it could be seen clearly that on the 1st day of treatment (1st day), the DDA increased fast, the rate constant was $16.8 \times 10^{-3}\,\text{h}^{-1}$, which was much higher than the treatment of the following days, because *N*-deacetylation was a typical nucleophilic substitute reaction, the contents of substrates and products could affect the reaction rate. At the initial day, the DDA was lower, and increased with the boiling water treatment rapidly. On the following days, the free acetyl-content of products in the reaction solution was increased with the increasing of DDA, while the acetyl residues on the chitin molecular chain was decreased, and it was difficult for nucleophilic substitutions reaction. With the increasing of resistance of mass-transfer, the inhibitory effect of deacetylation became the main resistance to improve the DDA, so the reaction rate declined and slower than the initial day's. Fig. 4c showed the effects of microwave-assisted heating method, it was similar to the effects of boiling water treatment. Nevertheless, the initial day's rate constant $(22.8 \times 10^{-3}\,\text{h}^{-1})$ was higher than that of the boiling water treatment $(16.8 \times 10^{-3}\,\text{h}^{-1})$, for the microwave heating model is a direct and internal heating model, and it could produce thermal-effect as well as non-thermal-effect. Microwave heating is the use of constantly rotating of molecular dipole moment, heating the materials internal and external simultaneously. It is more effective than traditional heating methods, which rely on thermal conduction and radiation model heating the materials from the surface to internal. Subsequently, the rate constants were lower than the boiling water method. On 1st day, the initial DDA was low, efficient microwave-assisted method can significantly improve the deacetylation process. DDA was already high in the following days, which could not improve too much on the basis of the original even use of microwave-assisted method. The rate constants decreased with longer time reaction, however, it was still higher than that of using room temperature treatment and 40 °C heated method, respectively. Of particular interests, the DDA of referenced samples had a very little change throughout the whole treatment process, it indicate that the heating method and the heating process was very important for improving the reaction rate of *N*-deacetylation at a low-temperature. It was also interesting that using this novel temperature cycling treatment could obtain a homogeneous chitin alkali solution with 4% chitin and 10% NaOH, which could be employed to prepare partially deacetylated water-soluble chitin with homogeneous deacetylation. Compared with traditional homogeneous method, the chitin concentration increased 3 times while the use of NaOH reduced to ¼ (Sannan et al., 1975).

3.4. FT-IR spectra analysis

Infrared spectroscopy is a useful method to study hydrogen bonding and other interactions. Fig. 5 showed an FT-IR spectrum of chitin and baselines (Duarte et al., 2002) for determining CrI with FT-IR spectroscopy. FT-IR spectra of samples treated with alkali-temperature-cycling were shown in Fig. 6. It could be seen, the band at $3448\,\text{cm}^{-1}$ became broader and moved to a lower number-waves due to the O–H stretching vibrations and the absorption of intra-hydrogen bonds. The concealed of the N–H stretching vibration (Amide III) absorption appeared at about 3264 and $3104\,\text{cm}^{-1}$ with increasing DDA, which could also indicate the reduction of inter-molecular $C(2_1)NH\cdots O=C(7_3)$ and $C(6_1)OH\cdots HOC(6_2)$ hydrogen bonds, respectively (Cho, Jang, Park, & Ko, 2000). It meant this novel temperature cycling treatment could break the chitin strong intra- and/or inter-molecular hydrogen bonds, which could also explain the increasing reaction rate displayed in the kinetics study. The absorption bands of chitin at about 1662 (1628),
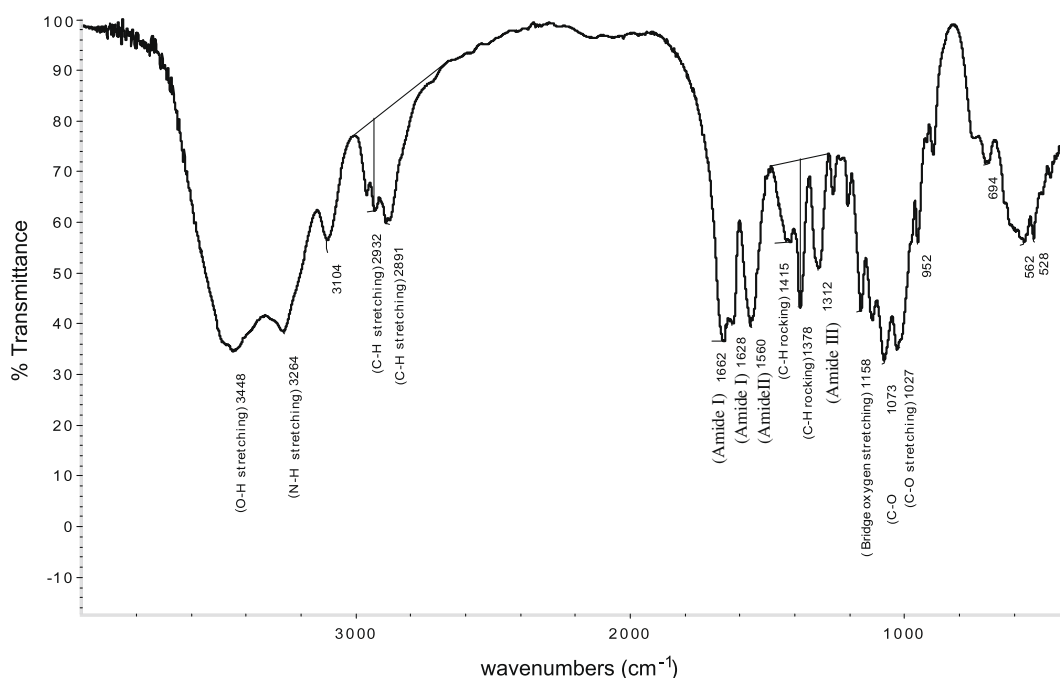

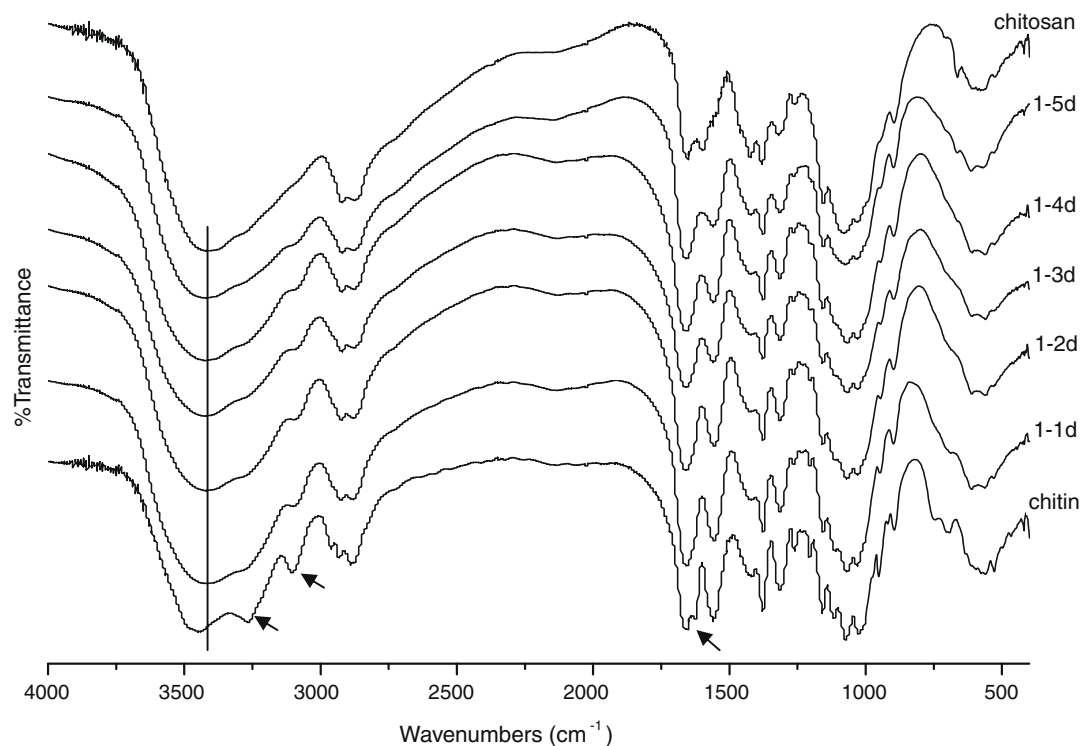**Fig. 5.** FT-IR spectrum of chitin and baselines for determining absorbance.

Fig. 6. FT-IR spectra of samples treated with alkali-temperature-cycling-treatment. The sample number is described below Table 1.

1560, and 1312 cm^{-1} were, respectively, referenced as amide I, amide II, and amide III. The absorption band at about 1597 cm^{-1} in chitosan was ascribed to N—H bending mode in the primary amine. The decrease of relative absorption intensity of amide II and amide III suggested the increase of DDA. The absorption bands of chitin at about 2932, 2891, 1415, and 1378 cm^{-1} were assigned to the C—H stretching and rocking bands, the relative absorption intensity varied with the CrI, and the 1415 cm^{-1} peak became acuity with increasing DDA. The absorption band at about 1158 cm^{-1} was assigned to the bridge oxygen stretching, the absorption bands at about 1073 and 1026 cm^{-1} was considered as the contribution of the C—O stretching, and the bands became broader with the further treatment.

Fig. 7 Showed the CrI of the treated chitin samples. During the temperature cycling treatment period, the crystallinity decreased rapidly in the initial 3 days and then underwent a few changes. It showed the initial days were useful for small molecules to permeate and make further reaction to take place more thoroughly; this was coincided with the kinetics study.
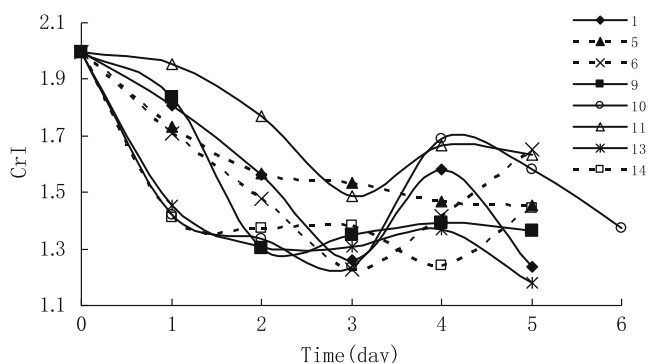
4. Conclusion

Under most conditions, semi-logarithmic plots between the amount of N-acetyl-D-glucosamine residues and the deacetylation time had well correlation coefficients ($R^2 > 0.9$). The N-deacetylation process followed the pseudo-first-order kinetics, and the apparent activation energy was preliminary estimated to be 9.76 kJ/mol which was lower than the activation energy reported for heterogeneous N-deacetylation at high temperature and for homogeneous N-deacetylation at low-temperature reported by Sanan et al., respectively. It means it has much higher reactively with this novel temperature cycling treatment.

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Fig. 7. The infrared crystalline index (CrI) of samples treated with alkali-temperature-cycling-treatment. The sample numbers are described below Table 1.

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