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Distribution of D-glucosamine moieties in heterogeneously deacetylated cuttlefish chitin

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Abstract

The distribution of D-glucosamine moieties was investigated in partially deacetylated chitins prepared under various conditions but with nearly the same degree of deacetylation in the range of 30–65%. The presence of adjacently located (blocked) D-glucosamine moieties was demonstrated by the formation of 2,5-anhydro-D-mannose using the nitrous acid deamination method. Both the alkali concentration and the deacetylation temperature affected the distribution of the D-glucosamine moieties. Within the range of deacetylation mentioned, the amount of released 2,5-anhydrous-D-mannose may vary up to 6-fold, highly depending on the conditions applied during the deacetylation. The effect of reaction conditions on other characteristics, swelling indices, crystallinity, thermal properties and the formation of acid insoluble material, supports the hypothesis that external conditions during thermochemical deacetylation can control the distribution of D-glucosamine in partially deacetylated chitin.

Keywords: Chitin; Chitosan; Deacetylation; D-Glucosamine; Distribution

1. Introduction

Chitin, a natural copolymer of β (1–4) linked *N*-acetyl-D-glucosamine and D-glucosamine, is found abundantly in crustacean shell wastes. Chitosan is a derivative of chitin prepared by deacetylating chitin. The term 'chitosan' is generally used to cover a wide range of deacetylated chitins, which are soluble in diluted organic acid solution, e.g. 1% w/w acetic acid. (Roberts, 1992) Although chitosan can be produced in concentrated alkali solution by either a heterogeneous or a homogeneous deacetylation process (Kurita, Sannan, & Iwakura, 1977; Sannan, Kurita, & Iwakura, 1975, 1976), chitin industry generally applies heterogeneous deacetylation at high temperatures (80–140 °C) (No & Meyers, 1997; Roberts, 1997) taking advantage of the speed and low cost of the production process.

It is known that the deacetylation conditions have a considerable effect on the physico-chemical properties of chitin and chitosan such as degree of deacetylation (DD), molecular weight and crystallinity (Chen, Wang, & Qu, 2004; Lamarque, Viton, & Domard, 2004; No & Meyers, 1997; Roberts, 1997). The physico-chemical properties affect the functionalities of the biopolymers in many applications (Chen & Tsaih, 1997; Demarger-André & Domard, 1995; Trung, Ng, & Stevens, 2003). A physico-chemical characteristic that often overlooked is the distribution pattern of the *N*-acetyl-D-glucosamine (NAG) and D-glucosamine (GlcN) residues in partially deacetylated chitin (PDC).

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The NAG–GlcN distribution pattern is a crucial factor in solubility and crystallinity of the final product. (Kurita et al., 1977; Vårum, Anthonsen, Grasdalen, & Smidsrød, 1991a) In addition, it can directly affect the biodegradability and chitooligosaccharide production (Aiba, 1989, 1991; Shigemasa, Saito, Sashiwa, & Saimoto, 1994). Therefore, it is important to understand the distribution of these residues in relation to the deacetylation conditions.

The presence of block and random copolymers of NAG and GlcN was first demonstrated by X-ray diffraction. It was concluded that the heterogeneous deacetylation process tends to result in block copolymer, whereas the homogeneous deacetylation process gave more random copolymer (Kurita et al., 1977). On the contrary, data obtained by nitrous acid deaminative hydrolysis (Sashiwa, Saimoto, Shigemasa, Ogawa, & Tokura, 1991, 1993) and NMR (Ottøy, Vårum, & Smidsrød, 1996; Vårum et al., 1991a, Vårum, Anthonsen, Grasdalen, & Smidsrød, 1991b) showed the random distribution of GlcN residues in partially deacetylated chitin from both the homogeneous and heterogeneous deacetylation processes. Recently, both block and random distribution of NAG or GlcN have been observed in heterogeneous deacetylation of chitin. (Lamarque et al., 2004) These discrepancies among research findings may be caused by variation in the deacetylation conditions. A slow deacetylation process conducted at 40 °C in 50% (w/v) NaOH showed a nearly even distribution of DD throughout the cuttlefish chitosan strip, while in a fast deacetylation process, at 90 °C in 50% (w/v) NaOH, a large local variation of deacetylation was observed (Ng, Chandrkrachang, & Stevens, 2000).

In the present study, the distribution of GlcN moieties in partially deacetylated chitin (PDC) was investigated using the nitrous acid deaminative hydrolysis method (Sashiwa et al., 1991, Sashiwa, Saimoto, Shigemasa, Ogawa, & Tokura, 1993). The term PDC refers to deacetylated chitomaterials with DD in the range between 30% and 65%. To observe the effect of deacetylation conditions on the distribution pattern, chitin was deacetylated using various temperatures, reaction times and alkali concentrations. Further physico-chemical analyses were conducted to support the research findings.

2. Materials and methods

2.1. Preparation of chitin

The chitin used in this study was obtained by treating cuttlefish pen with 4% sodium hydroxide (w/v) at 30 °C for 24 h and was subsequently decalcified by 4% hydrochloric acid (w/v) at 30 °C for 24 h. The weight to volume ratio of wet cuttlefish pen to alkali or acid was 1:10. After the decalcification process, the material was washed to neutral pH and dried in a vacuum oven at 40 °C. The chitin was ground and screened through a 30-mesh sieve before deacetylation. This cuttlefish chitin has a protein and ash content of less than 0.5%.

2.2. Heterogeneous deacetylation of chitin

Before the deacetylation, both chitin (5 g) and NaOH solution (50 mL) were separately pre-heated to the designated temperature. Then, the chitin and NaOH solution were mixed into an air-tight polypropylene bottle. The deacetylation process was conducted in a reciprocal shaking bath at the speed of 100 strokes/min. Four main deacetylation conditions were compared:

(a) 90 °C and 50% NaOH, abbreviated as 90(*x*)50,
(b) 90 °C and 40% NaOH, abbreviated as 90(*x*)40,
(c) 80 °C and 30% NaOH, abbreviated as 80(*x*)30,
(d) 30 °C and 50% NaOH, abbreviated as 30(*x*)50.

The “*x*” denotes the deacetylation time (h) as specified per experiment. A number of other deacetylation conditions were also investigated; some of them are reported for supplementary information. Without exception all non-reported samples support the evidence presented in this paper. All concentrations of NaOH and HCl used are expressed in weight by volume (w/v). After deacetylation, the NaOH solution was removed instantaneously by filtering through a 200-mesh sieve and the deacetylated chitin was washed extensively with water until neutral pH. All materials were dried in a vacuum oven at 40 °C.

2.3. Determination of the distribution of D-glucosamine groups

The deamination method of Sashiwa et al. (1991, 1993) was used with a slight modification. Briefly, 1 g of material obtained after deacetylation at different conditions was mixed with 100 mL of 100 g/L acetic acid solution. Then, 15 mL of 50 g/L aqueous sodium nitrite solution was added to 50 mL of the mixture. The mixture was stirred at ~4 °C for 3 h, followed by standing at room temperature for 40 h. Insoluble materials were removed by filtration through Whatman GF/C filter paper. The pH of the supernatant was adjusted to 5.5 with Amberlite IRA-400 (OH^- form, 50 mL), followed by addition of 5 mL 60 g/L aqueous sodium borohydride. The deaminated sample was analyzed by a Waters HPLC system equipped with a GPC column (Hitachi-W520, Japan) and a RI detector (Waters, USA). The eluent was HPLC grade water, flow rate was 0.3 mL/min and column temperature was 32 °C. As reported by Sashiwa et al. (1993), the first peak, in reverse elution sequence, was 2,5-anhydro-D-mannose, followed by the NAG oligomers with 2,5-anhydro-D-mannose at their terminal end (Fig. 1). The weight fraction of each deaminated fragment was calculated by dividing the peak height over the total peak height as in Eq. (1).

$$\text{Weight fraction}(\%) = (\text{peak height})/(\text{total peak height}) \times 100 \qquad (1)$$

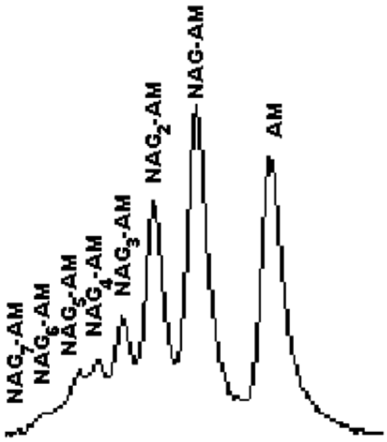


Fig. 1. Typical GPC chromatogram of the deaminative hydrolysate of a partially deacetylated chitin. NAG, *N*-acetyl-D-glucosamine; GlcN, D-glucosamine; AM, 2,5-anhydro-D-mannose.

2.4. Physico-chemical characterization of chitomaterials

The degree of deacetylation was analyzed by acid hydrolysis-HPLC method. (Ng, Hein, Chandrkrachang, & Stevens, 2006) The presence of insoluble chitinous material in PDC that did not dissolve in diluted acid was performed as described by Hein, Ng, and Stevens (2003).

The swelling index was measured by weight gain after immersing in water. Cuttlefish chitin strip was cut to a size of 2 × 2 cm and deacetylated under the conditions as specified. The weight of samples before (W_d) and after (W_h) immersion in distilled water for 5 h, was recorded. The swelling index was calculated as: $(W_h - W_d)/W_d \times 100\%$.

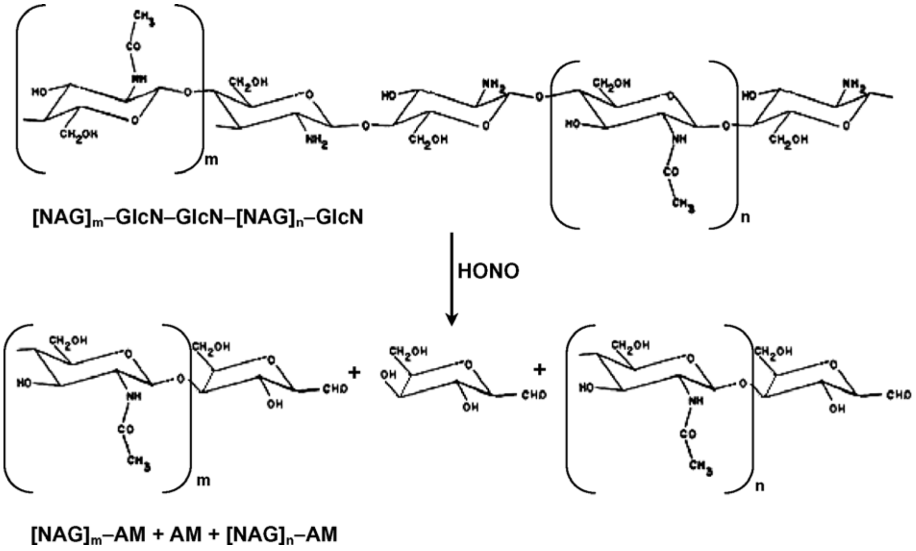
Samples for X-ray diffraction studies were ground to pass a 200 mesh sieve and were incubated in a helium atmosphere before analysis, and they were X-rayed under helium atmosphere at 100% relative humidity with a Rigaku Geigerflex X-ray diffractometer employing Ni-filtered CuKα radiation generated at 40 kV and 15 mA. The diffraction pattern was recorded on an imaging plate (R-AXIS, Rigaku) using a flat-film camera. Derivative thermogravimetry (DTG) analysis was conducted using a Netzsch Thermal Analyzer, Model 409, Germany. An amount of 50 mg sample (ground to pass 40 mesh and vacuum-dried at 65 °C overnight) was used for pyrolytic analysis. The range of temperature applied was from room temperature to 600 °C, heating rate 10 °C/min. The sample was finally held at 600 °C for 5 min. The whole process was conducted in a nitrogen atmosphere.

3. Results and discussion

3.1. Nitrous acid deaminative hydrolysis

Nitrous acid selectively deaminates the amine groups and cleaves the C-1 glycosidic bonds of D-glucosamine (GlcN) moieties to form 2,5-anhydro-D-mannose (AM) (Scheme 1). Nitrous acid does not affect the C1 glycosidic bond of the *N*-acetyl glucosamine moieties. The hydrolysis products of chitomaterials are mainly composed of free 2,5-anhydro-D-mannose and mono-, di-, tri-, and higher oligosaccharides of *N*-acetyl glucosamine (NAG) C4–C′1 bound to 2,5-anhydro-D-mannose (AM) (Hirano, Kondo, & Fujii, 1985; Sashiwa et al., 1991, 1993). A typical elution pattern of a hydrolysate is shown in Fig. 1.

By deaminative hydrolysis, free AM will be produced when and where sequences of GlcN–GlcN are present in the chitomaterial. In other words, more blockwise distribution of GlcN moieties in PDC results in a higher amount of 2,5-anhydro-mannose (AM) released. In a chitin with low DD having purely randomized GlcN moieties, GlcN–GlcN sequences will be absent and AM will not be produced upon deaminative hydrolysis.



Scheme 1. Deaminative hydrolysis of a chitomaterial by nitrous acid. NAG, *N*-acetyl-D-glucosamine; GlcN, D-glucosamine; AM, 2,5-anhydro-D-mannose.

The presence of random or block distribution of GlcN moieties can be explained as follows. During the deacetylation of chitin, *N*-acetyl groups are gradually removed. If the deacetylation process acts equally throughout the chitin matrix, the distribution of GlcN will be random and the chance for formation of a block of GlcN will be small. However, due to localized differences in crystallinity or insufficient time for the alkali to diffuse into the solid chitin matrix, the deacetylation process may not act equally throughout the chitin matrix. This leads to localized differences in the extent of deacetylation.

3.2. Heterogeneous deacetylation of chitin

The deacetylation profiles of cuttlefish chitin deacetylated under four different conditions are presented in Fig. 2. These conditions were selected after a series of preliminary observations to allow to make the following comparisons: (i) deacetylation at the same NaOH concentration [90(*x*)50 and 30(*x*)50], (ii) deacetylation at the same temperature [90(*x*)50 and 90(*x*)40] and (iii) deacetylation conditions that result in the same rate of deacetylation [30(*x*)50 and 80(*x*)30]. Of the four conditions reported here, the condition 90(*x*)50 showed the fastest deacetylation rate, followed by 90(*x*)40. The slowest rates were observed when 30(*x*)50 and 80(*x*)30 were applied. Deacetylation proceeded in a pseudo-first order reaction.

3.3. Effect of deacetylation conditions on the distribution of D-glucosamine moieties

Fig. 3(a) shows that all conditions that lead to a higher DD also result in release of more AM after nitrous acid deamination. For samples with (about) the same degree of deacetylation or samples with about the same release of AM additional rules apply.

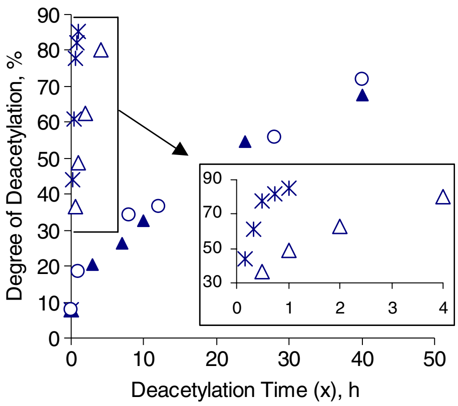


Fig. 2. Deacetylation profiles of cuttlefish chitin at different deacetylation conditions. (△) 90(*x*)40 deacetylation of chitin at 90 °C with 40% (w/v) NaOH for '*x*' hour. (▲) 30(*x*)50 deacetylation of chitin at 90 °C with 50% (w/v) NaOH for '*x*' hour. (✳) 90(*x*)50 deacetylation of chitin at 30 °C with 50% (w/v) NaOH for '*x*' hour. (○) 80(*x*)30 deacetylation of chitin at 80 °C with 30% (w/v) NaOH for '*x*' hour.

The PDCs inside the solid-lined box (Fig. 3(a)) have a DD in the narrow range of 55–63%, nevertheless the amounts of AM are quite different. The PDC prepared at higher temperature using condition 90(*x*)50 produced ~2 times as much AM as PDC prepared by 30(*x*)50. PDC prepared at a lower alkali concentration at condition 90(*x*)40 produced ~2-fold the amount of AM as PDC prepared by 90(*x*)50. These data reveal that increasing the deacetylation temperature or decreasing the alkali concentration results in higher amounts of AM after deaminative hydrolysis even though the PDCs had a comparable DD.

The PDCs within the dotted lined box have been deacetylated over a broad range of 30–80%DD but produce about the same amounts of AM. Again, this demonstrates the effect of reaction conditions. At lower alkali and higher temperature, a lower DD value reached the same AM production after deamination as compared with samples deacetylated with higher alkali concentration and lower temperature.

These results indicate that both the concentration of NaOH and the deacetylation temperature determine the formation of blockwise distribution of GlcN moieties in PDC. An extreme example was demonstrated by comparing the PDCs prepared by 80(*x*)30 and 30(*x*)50. Both deacetylation conditions showed the same rate of deacetylation as shown in Fig. 2. Despite the DD of both PDCs were 55%, the PDC of 80(*x*)30 released ~6 times more AM as compared to the PDC prepared by 30(*x*)50 (Fig. 3(a), inside the solid-lined box). These findings repeatedly suggested that the decrease of the alkali concentration or the increase of the deacetylation temperature can produce PDCs with blockwise distribution of GlcN moieties.

3.4. Formation of insoluble material

Supportive evidence was found when the acid insoluble fraction of the PDCs were examined (Fig. 3(b)). The PDCs having high amounts of block GlcN moieties (as concluded from high production of AM) appear also to contain high amounts of insoluble materials. In contrast, the PDCs with low AM production contain low amount of insoluble materials. It is tempting to speculate that these insolubles represent the regions of low deacetylation (high NAG content) that are also caused by block deacetylation. In a parallel study, FTIR, X-ray diffraction and acid hydrolysis-HPLC data showed that the materials comprised the acid insoluble fraction were of low DD (close to 20%) with high crystalline order (Hein et al., 2003). So, it can be stated that the higher the amounts of insolubles, the less randomly is NAG in the deacetylated PDC. Since the insoluble parts have a higher incidence of blockwise distributed NAG moieties. The PDCs (Fig. 3(b), inside the oval dotted-line) yielded different amounts of insoluble although their DD values are quite close (55–63%). The PDC from a condition with higher temperature and lower alkali 80(*x*)30 had the amounts of insolubles ~3 times more than the PDC from 30(*x*)50. Thus, the acid insolubility data confirm with data on AM

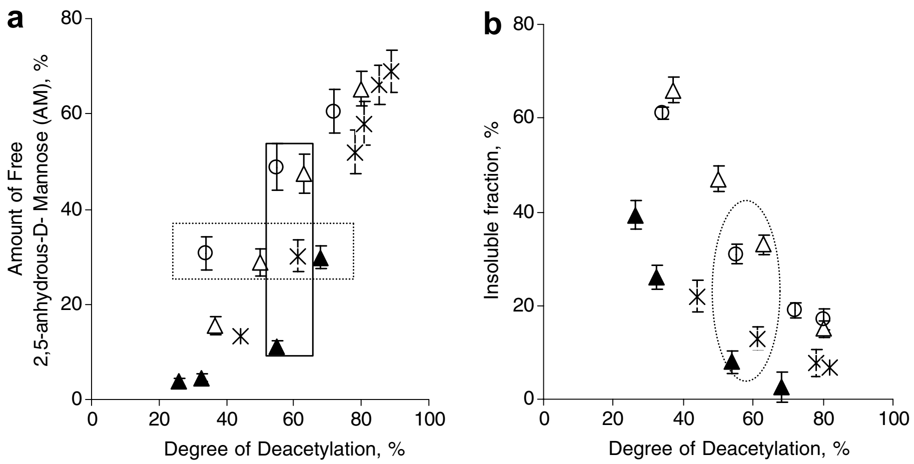


Fig. 3. (a) The amount of released 2,5-anhydro-D-mannose (AM) as a function of degree of deacetylation. (b) Amount of acid insoluble fraction as a function of degree of deacetylation. (△) 90(*x*)40 deacetylation of chitin at 90 °C with 40% (w/v) NaOH for '*x*' hour. (▲) 30(*x*)50 deacetylation of chitin at 90 °C with 50% (w/v) NaOH for '*x*' hour. (✳) 90(*x*)50 deacetylation of chitin at 30 °C with 50% (w/v) NaOH for '*x*' hour. (○) 80(*x*)30 deacetylation of chitin at 80 °C with 30% (w/v) NaOH for '*x*' hour. Data on duration of the deacetylation treatment are shown in Fig. 2.

release. The increasing order of the chance of getting block PDC copolymer depending on deacetylation conditions is 30(*x*)50 < 90(*x*)50 < 90(*x*)40 < 80(*x*)30 (Fig. 3(b)), which is the same trend as the AM data in Fig. 3(a).

3.5. HPLC analysis of oligochitins

Further evidence for the effect of the reaction conditions on the mechanism of deacetylation has been obtained by HPLC analysis of AM and chitin-AM oligomers after nitrous acid deamination. Three sets of PDCs are compared with almost the same %DD obtained through five different deacetylation conditions (Fig. 4). The amount of AM released after the deaminative hydrolysis is presented as the ratio of the amount over the DD value of the sample (AM/DD) in order to compensate for the higher amount of GlcN in more deacetylated samples. Similarly, the NAG oligomeric data are presented as (NAG_x–AM)/DD, where '*x*' ranges from 0 to 7. The AM/DD increased by 1.6-fold when the alkali concentration decreased from 50% to 40% at 90 °C (Fig. 4(a)). When alkali concentration was maintained at 40%, the AM/DD increased by 2.6-fold when deacetylation temperature increased from 40 to 90 °C (Fig. 4(b)). There was a 6.6-fold increase of AM/DD when deacetylation condition was increased from 30 °C to 80 °C and the alkali concentration was decreased from 50% to 30% (Fig. 4(c)).

Furthermore, the distribution of GlcN in the deaminated products has been compared with what may be expected on basis of Bernoulian statistics for a random distribution (Eq. (2)) (Sashiwa et al., 1993),

$$\text{Weight fraction}(\%) = n \times \text{DD}^2 \times (1 - \text{DD})^{n-1} \times M_n \times (n \times M_o)^{-1} \qquad (2)$$

where *n* is the degree of polymerization of the oligomers, DD is the degree of deacetylation, M_n is the molecular

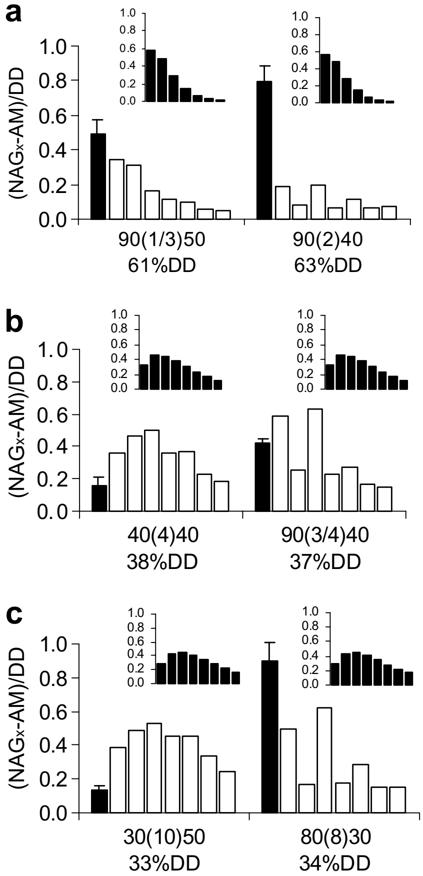


Fig. 4. Comparing the release of 2,5-anhydro-D-mannose (AM) and oligomers between paired deacetylation conditions after deamination. The amount of AM and NAG_x–AM are normalized by their respective DD and collectively presented as (NAG_x–AM)/DD, '*x*' ranges from 0 to 7. The results of all paired treatments are significantly different at $p < 0.05$. The Bernoulian statistics for respective DD are included in the graph for comparison. The black columns represent the amount of AM, the following white columns represent NAG–AM linked oligomers in order of increasing size (NAG–AM dimer to NAG_7–AM octamer).

weight of the *n*th oligomer and M_o is its respective average molecular weight.

Fig. 4(a) shows that the distribution of the deaminated oligomer pattern for 90(1/3)50, as compared to 90(2)40, is closer to its Bernoulian prediction, suggesting a more random character of the former pattern. Likewise, the samples treated with 40(4)40 and 30(10)50 had a more random character compared to their paired treatments, i.e. 90(3/4)40 and 80(8)30, respectively.

Based on these findings, the distribution of GlcN moieties in heterogeneously deacetylated PDC can either be blockwise or random (Bernoullian) depending on the deacetylation conditions. Contradictory data presented in previous investigations are probably due to PDC prepared by one deacetylation condition only (Kurita et al., 1977; Sashiwa et al., 1991; Vårum et al., 1991a, 1991b) or unknown history of deacetylation conditions (i.e. commercial samples) (Ottøy et al., 1996). Present findings suggest that either blockwise or randomized (Bernoullian) distribution of GlcN can take place depending on deacetylation conditions even in the heterogeneous deacetylation process.

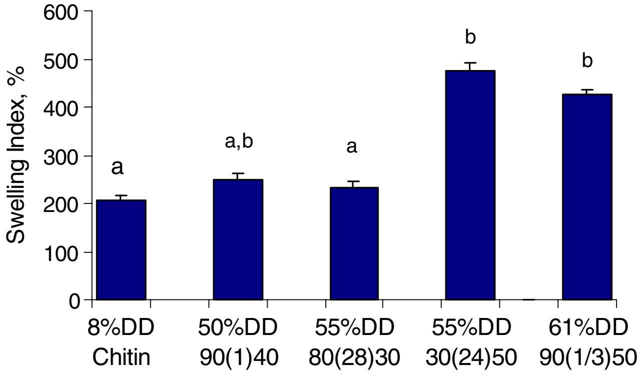


Fig. 5. Swelling indexes of chitin and PDC from different deacetylation conditions. Note: Different characters (a,b) indicate the significant difference at $p < 0.05$.

3.6. Physical and thermal properties of PDC with different GlcN distribution

3.6.1. Shrinkage and swelling

The physical appearance of chitomaterials was compared before and after four different deacetylation conditions. PDC and chitosan obtained from 30(*x*)50 and 90(*x*)50 were highly shrunk, as compared to the size of

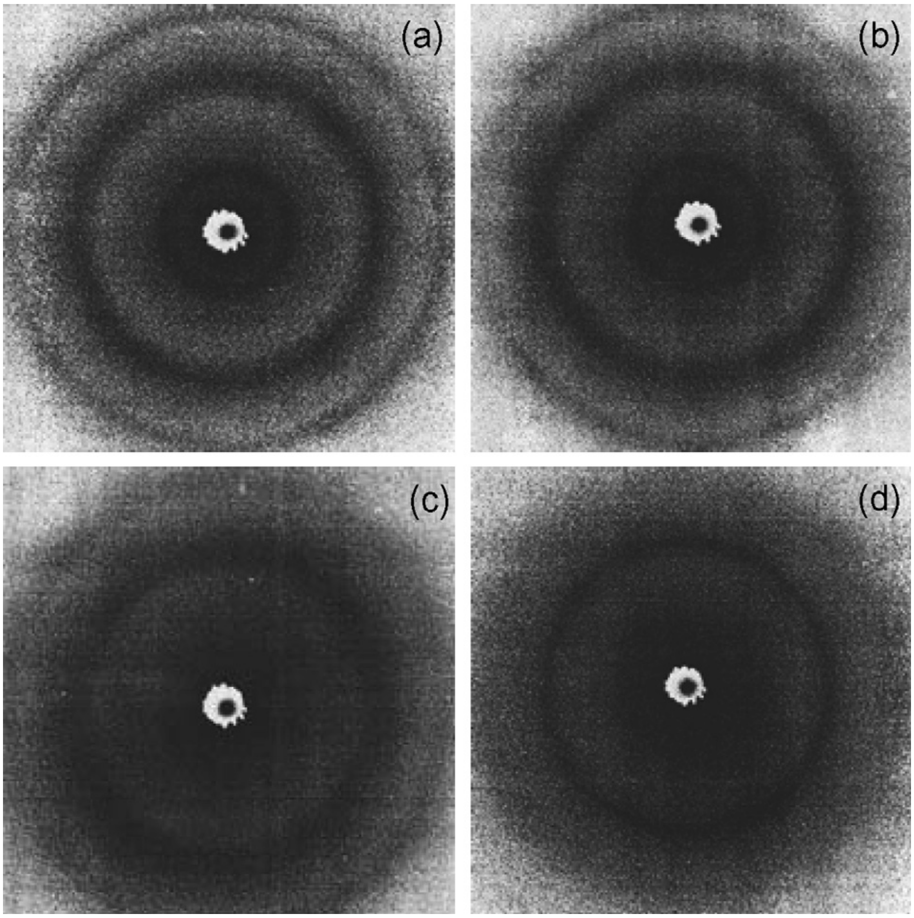


Fig. 6. X-rays diffractogram of chitin and PDC prepared from different deacetylation conditions. (a) Cuttlefish chitin (8%DD, 29% crystallinity). (b) PDC prepared from 90(1)40 (50%DD, 19% crystallinity). (c) PDC prepared from 80(28)30 (55%DD, 17% crystallinity). (d) PDC prepared from 30(24)50 (55%DD, 7 % crystallinity).

the original chitin. However, hardly any physical change was observed on the PDCs after the 90(x)40 and 80(x)30 treatments. These observations comply with the swelling indexes: the shrunken samples showed most swelling. Fig. 5 shows that the swelling indexes of PDCs from 90(x)50 and 30(x)50 were significantly higher ($p < 0.05$) than PDCs from 90(x)40 and 80(x)30 although these preparations were in a close range of DD. It demonstrated that PDC with blockwise GlcN distribution did swell less.

3.6.2. *X-ray crystallography*

Data from X-ray crystallography demonstrated that the crystalline nature of PDC with block NAG and GlcN moieties is similar to that of chitin. Block PDC copolymers have higher crystallinity than random PDC copolymers (Kurita et al., 1977). Since diffraction rings of PDCs from 80(x)30 and 90(x)40 were sharper than those from 30(x)50 PDC, the crystallinity of the former is higher than the latter although they had close DD (50–55%) (Fig. 6). These observations are in concordance with the interpretation of the results from the analysis by the nitrous acid deaminative hydrolysis method.

3.6.3. *First-derivative thermogravimetric analysis*

The first-derivative thermogravimetric (DTG) analyses of cuttlefish chitin, chitosan and PDC showed peak temperatures of decomposition for chitin 8%DD (CT_p) and chitosan 89%DD (CTS_p) at 390 ± 3 °C and 340 ± 3 °C, respectively. The peak shape and peak temperature of the PDC samples appeared to be highly related to the deacetylation conditions. In the thermograms of PDCs with a 33–37%DD, shouldered peaks were observed in the 340-390 °C range. Some peaks or shoulders were closer to CT_p, others were shifted to CTS_p having more chitosanous character with a peak temperature at lower temperature. In DTG of samples with DD values of 70% and higher only one CTS_p peak was observed in all treatments studied (data not shown).

Among four treatments selected as shown in Fig. 7, the DTG shift from chitinous (CT_p) to chitosanous (CTS_p) occurred in the order of PDCs prepared at 80(8)30 > 90(1/2)40 > 30(10)40 > 30(10)50. In other words, PDC prepared at 30(10)50 had a more chitosanous nature than prepared at 80(8)30. This sequence is the same sequence as observed in the order of decreasing block deacetylation, as well as in the X-ray diffraction measurements. This suggests that a high alkaline concentration and a low deacetylation temperature are required to prepare PDC with a more chitosanous character. Further verification was obtained by comparing the PDCs within a DD range of 50–55% (Fig. 8). Although the PDCs treated by 30(25)50 and 80(28)30 have the same degree of deacetylation, the former PDC showed only one peak close to CTS_p and no peak or shoulder in the CT_p area. The latter PDC showed a broad peak with a shoulder closer to CT_p.

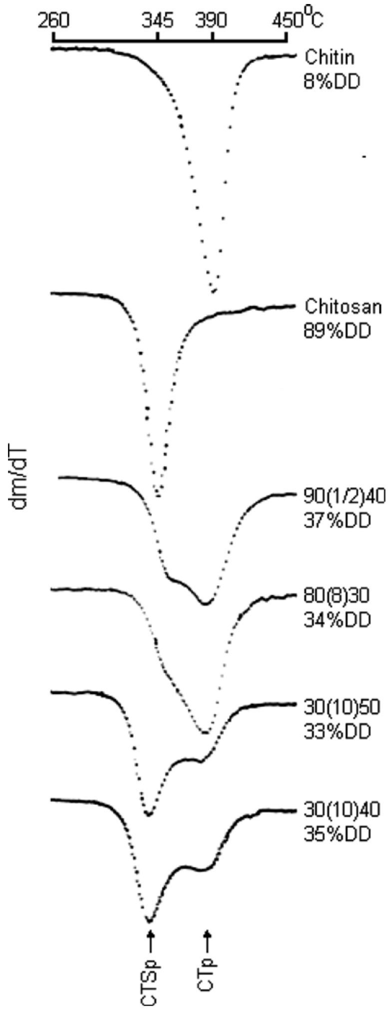


Fig. 7. The first derivative thermogravimetric analyses of chitin, chitosan and PDC prepared from different deacetylation conditions in range of 33–37%DD. CT_p, peak temperature of decomposition for chitin; CTS_p, peak temperature of decomposition for chitosan; dm/dT, mass change over temperature change.

The shape of the DTG thermograms can be explained as follows. The deacetylation of a solid piece of chitin occurs from the outer to the inner part of the material, or from more amorphous to less amorphous regions. This will most likely lead to the formation of a heterogeneous distribution of NAG and GlcN moieties in the PDC. The chitinous microareas will be rich in blocked NAG and decompose closer to CT_p; whereas chitosanous microareas are rich in GlcN and decompose at lower temperature. If PDC has more chitin microarea's than chitosan microarea's, DTG will show more shift to CT_p and vice versa for CTS_p. When the deacetylation process proceeds, the number of acetylated moieties will decrease, resulting in a more homogeneous product with a majority of GlcN moieties, giving the product the characteristics of chitosan.

4. Conclusion

The heterogeneous thermochemical deacetylation of cuttlefish chitin was investigated to elucidate the effect of deacetylation conditions on distribution of GlcN moieties in partially

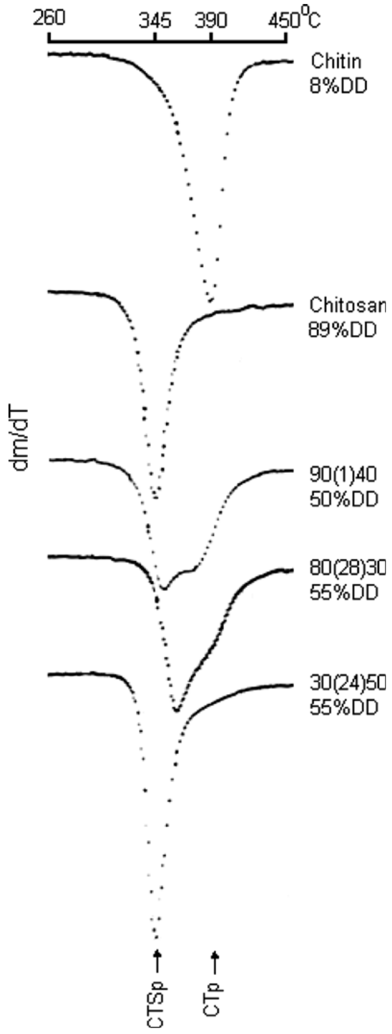


Fig. 8. The first derivative thermogravimetric analyses of chitin, chitosan and PDC prepared from different deacetylation conditions in range of 50–55%DD. CT_p, peak temperature of decomposition for chitin; CTS_p, peak temperature of decomposition for chitosan. dm/dT, mass change over temperature change.

deacetylated chitin. The distribution of GlcN was determined by the deaminative hydrolysis of nitrous acid followed by gel permeation chromatography. Three deacetylation conditions (constant deacetylation temperature, constant alkali concentration, and constant rate of deacetylation) were chosen to demonstrate their effects on the distribution pattern. When chitin is deacetylated with higher temperature or lower NaOH concentration, the resultant PDCs are in blockwise distribution of GlcN and NAG compared to lower temperature and higher NaOH concentration.

This result was confirmed by analysis of several other physical properties including the swelling index, the degree of crystallinity, the amount of acid insoluble material and the first-derivative thermogravimetric behavior. Especially the latter showed to be informative for demonstrating the presence of chitinous and chitosanous microareas in the PDC sample, using the peak temperature (CT_p and CTS_p) as reference. All observations presented in this paper support the conclusion that the heterogeneous thermochemical deacetylation of β-chitin from cuttlefish can occur either more random or more blockwise, highly depending on the combination of reaction temperature and NaOH concentration during the deacetylation.

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