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LOCAL VARIATION IN THE DEACETYLATION OF CUTTLEFISH CHITIN STRIP

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Abstract

The effect of the deacetylation conditions has been investigated on the distribution of the percent degree of deacetylation (%DD) in the whole strip of cuttlefish chitin. The selected deacetylation conditions for this study were 40°C and 90°C and an alkali concentration of 50% (w/v) NaOH. Treatment time was chosen to achieve a %DD around 45% and 80%. After deacetylation, samples were dissected into 3 zones and 13 sections. The HPLC-acid hydrolysis method was used to analyze the %DD of each section.

For the samples partially deacetylated to around 45%DD, the deacetylation condition conducted at 40°C in 50% NaOH has a difference of 16%DD between the maximum and the minimum %DD of the 13 sections. For the partially deacetylation conducted at 90°C in 50% NaOH, this difference increases to 34%DD.

For those samples deacetylated to 80%DD, the deacetylation condition conducted at 40°C in 50% NaOH has a difference of less than 2%DD between the maximum %DD and the minimum %DD. For the condition carried out at 90°C in 50% NaOH, the difference is 9.5%DD.

Keywords : chitin deacetylation, distribution, chitosan quality and cuttlefish

Introduction

Chitin is the second most abundant natural biopolymer after cellulose. Its chemical structure is close to cellulose and has a $\beta(1\rightarrow4)$-linkage, but is composed of 2-acetamido-2-deoxy-β-D-glucose monomers (N-acetyl-D-glucosamine). Chitosan is the deacetylated form of chitin. Chitosan has a more reactive structure (Kurita, *et al.* 1993) and in contrast to chitin, can dissolve in wider range of solvent systems, e.g. diluted acetic acid. Additionally, when

exposed to an acidic environment, it carries a positive charge. Owing to its solubility and its charged nature, most of the industry applications at present have been focused on chitosan.

Chitosan is commonly prepared by heterogeneous deacetylation of chitin in concentrated alkaline solution. At present, high temperatures (80-140°C) are commonly applied in the industry for its production (Roberts, 1997), though the conditions used for the deacetylation may vary among factories. Many studies have tried to correlate between the deacetylation conditions and the degree of deacetylation under heterogeneous environment and models have also been generated (Wojtasz-Pajak, *et al.* 1997; and Castelli, *et al.* 1995). OttØy, *et al.* (1997), have demonstrated that deacetylation conducted at high temperature will accelerate the degradation of the polymeric chain.

Many reports at present show only the average value of their chitosans, few authors have examined the effect of the deacetylation condition to the distribution of the %DD in the chitosan matrix. Without the understanding of the distribution of %DD, chitosan even with the same %DD will be inconsistent and their applications will be limited.

Therefore, this paper carries the objectives of examining the distribution of the %DD in the chitosan and targeted to provide a guideline for controlling the deacetylation process. In this study, cuttlefish chitin was used to study the distribution of the degree of deacetylation owing to its relatively open structure and its physical shape.

Sample preparation and method of analysis

Preparation of the cuttlefish chitin strip

A common process was applied to produce cuttlefish chitin (Hall, 1996). The deproteination was conducted in 4% NaOH (w/v) at room temperature overnight. The decalcification was carried in 4% HCl (w/v) for 12 hours at room ambient temperature. No bleaching of the material has been conducted throughout the production process. All the deacetylation processes were conducted in 50% NaOH (w/v) at indicated temperature and time.

Determination of the degree of deacetylation (Stevens, *et al.* 1997; and Ng, *et al.* 1996)

Acid hydrolysis - Add 1.5mL of 12 M H_2SO_4 and 1mL of 126 mg/L oxalic acid solution to the ampoule containing a known amount of dry sample (20-30 mg). Seal the ampoule by an ampoule sealer and incubates it in a 110°C oven. The time needed for complete hydrolysis of cuttlefish chitin or chitosan is about 30 minutes. The completion of the hydrolysis is indicated by a golden light brownish clear solution. After hydrolysis, cool the ampoule in ice water for at least 1 hour before bringing it back to the

room temperature. Then dilute the sample with 10 times HPLC grade water. Filter the diluted sample by a 0.45 μm cellulose nitrate membrane filter before injecting into the HPLC.

HPLC Analysis - The analysis of the acetic acid was performed by a Waters HPLC with the aid of Millennium Chromatography Manager software program from Waters, version 2.15. The analytical system consists of a 300 x 7.8mm column ORH-801 cation exchange resin (Interaction Chromatography Inc., 2032 Concourse Drive, San Jose, CA 95131); 1 mM H_2SO_4; flow rate 0.8mL/min; pressure 1600psi, degas by helium at rate of 15 mL/min; column oven temperature 55°C; sample compartment 25°C; injection volume 15μL, auto-injection system and detection at UV 210 nm by Waters™ 486 tunable absorbance detector. Sulphuric acid used for sample hydrolysis and preparation of the eluent were GR grade reagent, Merck. HPLC grade water was used to prepare the eluent and dilution of the samples and standards.

Results and discussion

To investigate the phenomenon of deacetylation in cuttlefish chitosan, the cuttlefish strip is divided into 3 zones of equal width and 13 sections in total. Both the outer and the inner zones are dissected into 3 sections, while the middle zone is divided into 7 sections, as shown in Figure 1. The thickness of the cuttlefish chitin is about 1 mm in average.

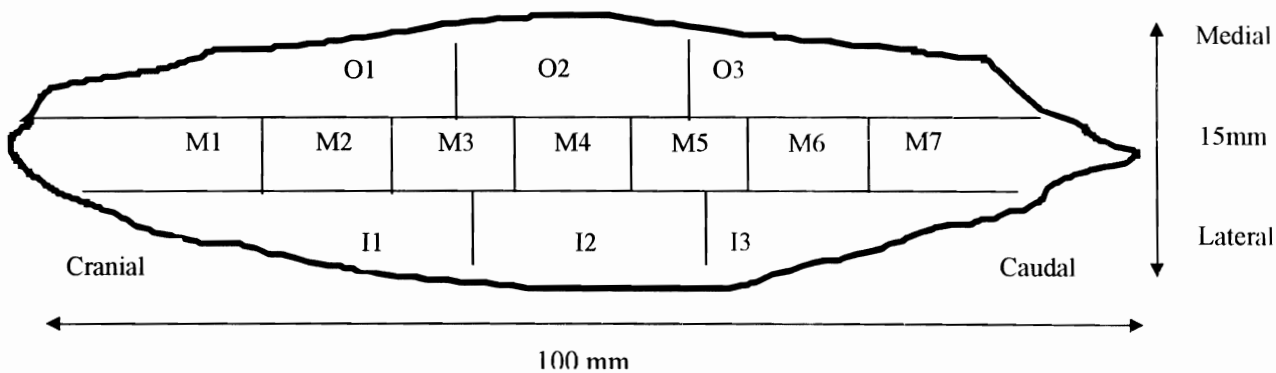


Figure 1 Determination of the distribution of %DD for the whole strip of cuttlefish chitosan. The analysis of the distribution of the %DD on a whole cuttlefish strip was obtained by dividing it into 3 zones of equal width (O = outer strip, I = inner strip and M = middle strip) and 13 sections. The size of the M2 through M6 is approximately 5 x 10 mm. The average thickness of the middle strip is 2mm, and is tapered towards both edges of the strip.

Figure 2 shows the natural distribution of the %DD in a cuttlefish chitin strip. In average, the size of the cuttlefish chitin is twice the size of the cuttlefish chitosan. The reduction in size of cuttlefish chitosan is due to the shrinkage of cuttlefish chitin in the deacetylation process. The distribution %DD in the cuttlefish chitosan was between 7.2% and 16.3%, with an average %DD of 13.6%. In most circumstances, it is observed that the inner edge of the strip has a %DD lower than the other parts. The outer strip has closer deacetylation values to the middle strip than the inner strip.

Range = 7.2-16.3 %DD

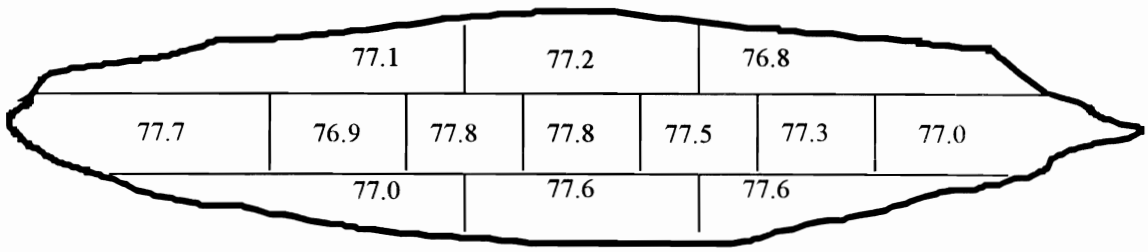
Average = 13.6 %DD

Figure 2 Distribution of %DD in cuttlefish chitin strip.

(I) The effect of deacetylation at low temperature on the distribution of %DD along the cuttlefish chitin strip

Figure 3 shows the distribution of the %DD along two cuttlefish chitosan strips after treatment by 50% NaOH at 40°C for 30 hours. It demonstrates that the distribution of the %DD is even throughout the strip. The range of the %DD for the 1st replicate is from 76.8 to 77.8%, with an average %DD of 77.4%. For the 2nd replicate, the range of distribution is between 77.1 and 78.9 and its average %DD for the whole strip is 77.9% deacetylation. The differences between the minimum and maximum %DD for the 1st and 2nd replicates are 1.0% and 1.8%, respectively. The minimum %DD for 1st and 2nd replicates are 98.7% and 97.7% to the maximum %DD, respectively.

Strip 1



Range = 76.8-77.8 %DD

Average = 77.9 %DD

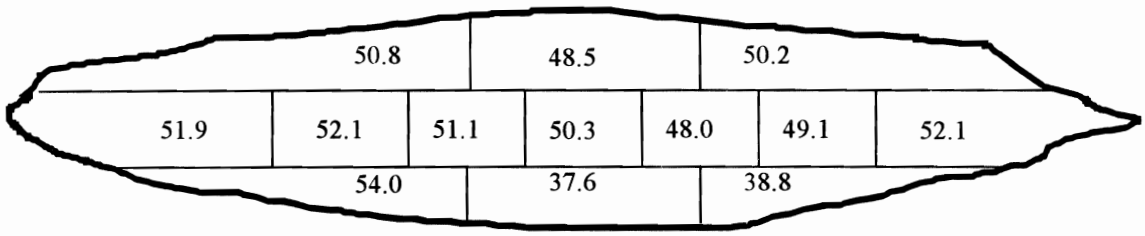
Strip 2 (replicate)

Range = 77.1-78.9 %DD

Average = 77.9 %DD

Figure 3 The effect of the distribution of %DD in the strip of cuttlefish chitosan strip under low Temperature condition. The treatment conditions were 40°C in 50% NaOH for 30 hours.

Figure 4 shows another strip of cuttlefish chitin at 40°C in 50% NaOH, however, for only 6 hours. The distribution of %DD ranges from 37.6 to 54.0%DD and with an average of 48.8%DD. In this case, a much larger localized difference in %DD is observed. The minimum and maximum %DD found in the strip differs for 16.4%DD. The minimum %DD deacetylated under this condition is 69.6% of the maximum %DD. Apparently, after longer duration of deacetylation (30 hours) about the same degree of deacetylation is attainted throughout the chitinous tissue. However, during short period of treatment, the latero-caudal region is deacetylated more extensively as compared to the other parts.



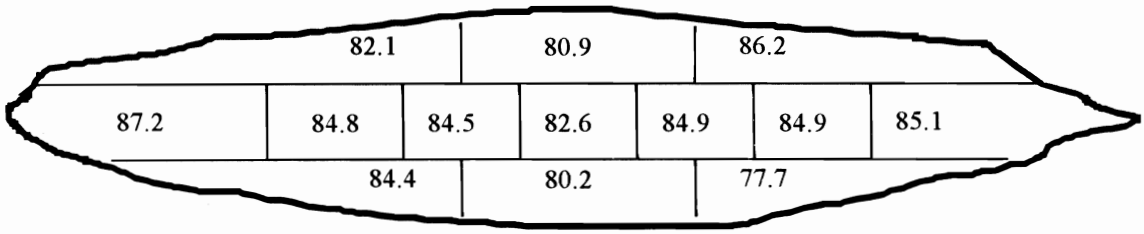
Range = 37.6-54.0 %DD

Average = 48.8 %DD

Figure 4 Distribution of %DD in cuttlefish chitosan strip, under low temperature condition. The treatment conditions were 40°C in 50% NaOH for 6 hours.

(II) The effects of deacetylation at high temperature on the distribution of %DD along the strip of cuttlefish chitin

The effect of the deacetylation conducted at high temperature of 90°C in 50% NaOH for 40 minutes on the distribution of %DD along the strip of cuttlefish chitosan is illustrated in Figure 5. The duration of the deacetylation was 40 minutes. This results in an average %DD of 83.4%, about equal to the deacetylation described in Figure 3. The range of distribution of %DD is between 77.7 and 87.2, with a difference of 9.5% between the minimum and maximum %DD of the 13 sections. The minimum %DD is 89.1% of the maximum %DD. Comparing to the deacetylation process conducted at low temperature for a period of 30 hours, deacetylation carried out at high temperature, which bring about fast deacetylation, is thus leading to more uneven distribution of the %DD along the strip of cuttlefish chitosan strip.



Range = 77.7-87.2 %DD

Average = 83.4 %DD

Figure 5 Distribution of %DD in cuttlefish chitosan strip, under high temperature condition. The treatment conditions were 90°C in 50% NaOH for 40 minutes.

Further evidence that fast deacetylation process, in condition of 90°C in 50% NaOh for 10 minutes, will cause larger gradient of %DD along the strip of cuttlefish chitosan than normal chitosan can be seen in Figure 6. The minimum %DD is only 42.4% of the maximum %DD. This confirms fast deacetylation conditions, especially during shorter treatment times, will bring about localized differences in deacetylation and may cause inconsistency in quality of the chitosan produced.

Range = 25.3-59.6 %DD

Average = 42.8 %DD

Figure 6 Distribution of %DD in cuttlefish chitosan strip, under high temperature condition. The treatment conditions were 90°C in 50% NaOH for 10 minutes.

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