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On the sonication of chitin: effects on its structure and morphology and influence on its deacetylation

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Summary

The pre-sonication provoked noticeable changes in the chitin morphology but its overall crystallinity was unaffected. The sonication of chitin seemed to improve its homogeneity and to increase the particles surface, enhancing the accessibility of reagents. Pre-sonicated chitin was more efficiently deacetylated (up to 95%) than commercial chitin (up to 88%) probably due to the mechanical action of ultra-sound since the previous stirring of solid chitin while suspended in hot or cold water for 1 hour also enhanced the deacetylation efficiency.

Introduction

The main factors affecting the efficiency of the heterogeneous deacetylation of solid chitin and the characteristics of the produced chitosans are: a)solid to liquor ratio; b)particle's average size and porosity; c)alkali concentration; d)temperature and reaction time; e)presence of oxygen and addition of reducing agents and oxygen scavengers to the reaction medium (1-3). It has been recently reported that pre-sonicated chitin is more completely deacetylated than untreated chitin, probably due to the enhanced accessibility promoted by the ultrasound treatment (4). In this work the effects of pre-treating solid chitin are investigated by monitoring its structural and morphological characteristics. Also, the average degrees of deacetylation and intrinsic viscosities of chitosans prepared from crude commercial chitin and from pre-treated chitin are compared.

Experimental

Chitin deacetylation and chitosan purification

The deacetylation reactions were carried out with crude commercial chitin (Sigma, from crab shells), with chitin previously submitted to ultrasound treatment and with chitin previously submitted to magnetic stirring for 60 minutes, at room temperature and at 55°C, while suspended in water. For the sonication of commercial chitin a Branson Sonifier 450 was employed in the intermittent mode with the pulse duration adjusted to 0.5 per second to minimize the concomitant heating of the suspension. The output power was adjusted to a fixed level and the sonication treatment proceeded for adjusted to 0.5 per second to minimize the concomitant heating of the suspension. The output power was adjusted to a fixed level and the sonication treatment proceeded for the desired time. In this manner, crude chitin was suspended in water (4.6g chitin/80mL of water), submitted to sonication inside a sound-proof box and after that the suspension was filtered, the chitin sample was allowed to dry and then submitted to the deacetylation reaction. To verify if the concomitant heating and shaking provoked by ultra-sound irradiation contributed to enhance the reactivity of chitin toward deacetylation, two additional previous treatments of chitin were tested. In this manner, the aqueous suspension of chitin (4.6g chitin/80mL of water) was submitted to magnetic stirring during 60 minutes at room temperature and at 55°C, maximum temperature attained by the chitin suspension upon sonication for equal period of time. The suspension was then filtered, the treated chitin was allowed to dry and then submitted to the deacetylation reaction.

In a typical deacetylation, a suspension containing 4.6g of chitin in 220ml of 40% aqueous sodium hydroxide solution was kept at constant mechanical stirring (350rpm) in a glass reactor thermostatized at 115°±2°C. After 6h the reaction was discontinued, the mixture was cooled to room temperature, filtered and the solid deacetylated product was repeatedly washed with water until the neutrality of washings was attained. Following washing with methanol, the deacetylated product was allowed to dry in ambient conditions. The deacetylated product was dissolved in dilute acetic acid solution, the resulting solution was filtered and a concentrated NaOH solution was carefully added to promote the precipitation of the polymer. The polysaccharide was recovered by filtration and exhaustively washed with water and then with methanol.

Characterizations

The X-rays diffraction analyses of chitin were carried out in a Rigaku Diffractometer employing CuKα radiation (λ =1.54Å) with a voltage of 40kV and current of 40mA. The range of investigation corresponded to $3° < 2\theta < 50°$ with a step scan of 0.02° at a scan rate of 1°/min. The degrees of crystallinity were estimated by using the method proposed by Challa et al. (5) which has been recently applied to the characterization of chitin and chitosan (6). For the morphological characterizations, the samples were previously stored during two days in a desiccator containing P_2O_5, covered with a thin layer (20µm) of gold and examined in a LEO-440 digital scanning electron microscope employing I=500pA and P=20kV. Infrared spectra were obtained from KBr/sample pellets by using a Bomen MB-102 FTIR spectrometer. The purified chitosans were dissolved in D_2O/HCl (100:1 v/v) for the acquisition of ^{1}H nmr spectra at 80°C, by using a Bruker 200 MHz spectrometer, which were employed for the determination of average degrees of acetylation, as described in more detail elsewhere (7). The intrinsic viscosities were determined at 25°C ± 0.01°C by capillary viscometry of chitosan dissolved in 0.3 mol/L acetic acid / 0.2 mol/L sodium acetate buffer (pH≅4.5) as described elsewhere (7). For these determinations the concentration of the chitosan's solutions were below Ig/L so that, in all cases, the range $1.2 < \eta_{rel} < 2.0$ was observed. The corresponding viscosity average molecular weights (M_v) were calculated by applying the values of K and a determined in the same solvent and temperature (8).

Results and Discussion

The composition of commercial chitin according to the average size of its particles was determined by sieving it through a set of sieves arranged in order of decreasing mesh size and by weighting the fractions retained in each sieve. It was observed from this analysis that the commercial chitin employed in this work is an heterogeneous powder but most of its solid particles are retained in the sieves whose mesh sizes are 0.29mm and 0.25mm (Figure 1). After the sonication treatment, it was visually evident that the dried chitin was formed by clusters of particles greaters than the particles previously present and they were manually disaggregated in a mortar before the deacetylation, taking care to avoid the grinding of the solid. The infrared spectra of commercial chitin and of sonicated chitin show that the sonication provoked no structural changes in chitin since the same main characteristic bands (1) are present in both spectra (Figure 2) but the comparison among chitosans obtained from crude and from pre-sonicated chitin (Table 1) reveals that the latter set of samples are more deacetylated, showing that the sonication treatment activated chitin for the deacetylation reaction.

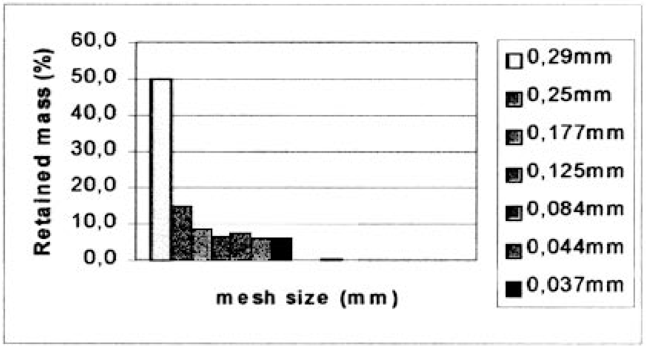

Figure 1: Fractionation of commercial chitin according to the average size of the solid particles.

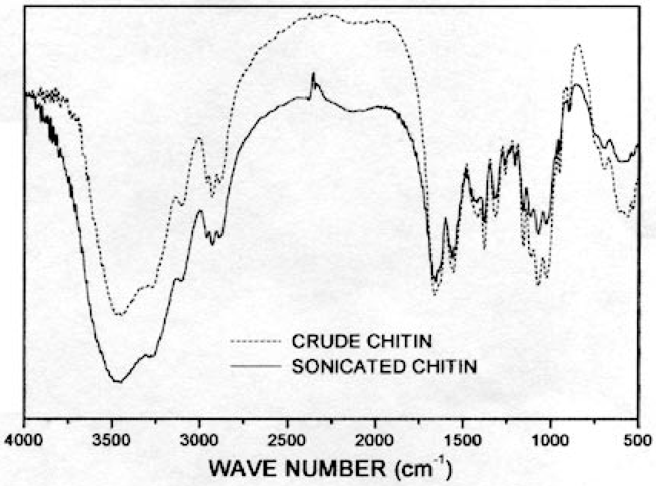

Figure 2: Infrared spectra of commercial and of sonicated chitin.

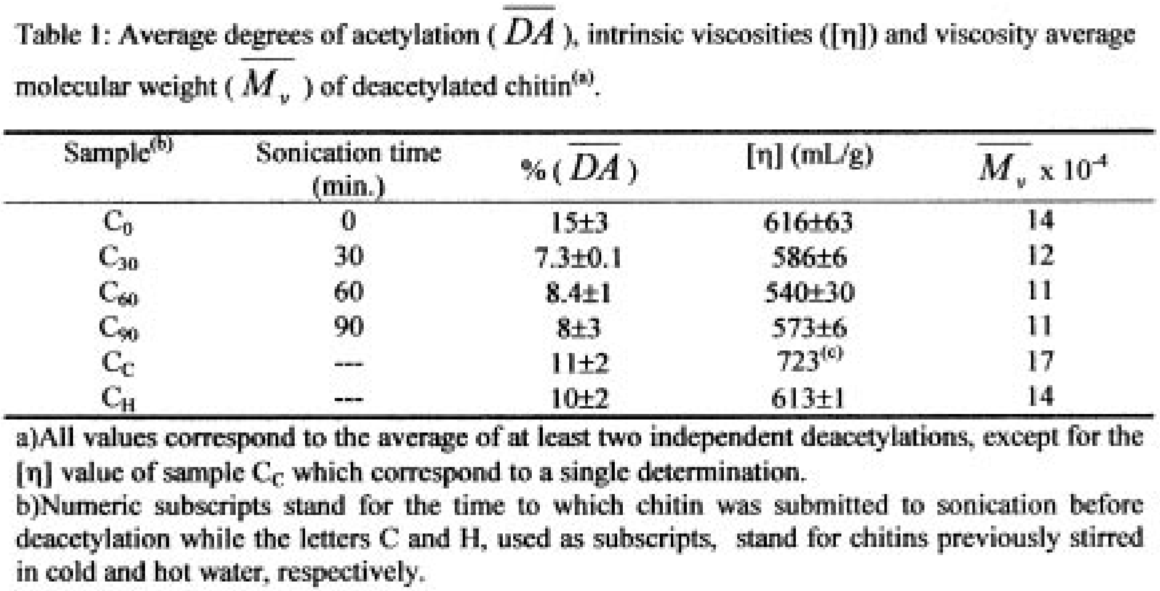

Table 1: Average degrees of acetylation ($\overline{DA}$), intrinsic viscosities ([η]) and viscosity average molecular weight ($\overline{M}_v$) of deacetylated chitin[(a)].

Sample[(b)]	Sonication time (min.)	% ($\overline{DA}$)	[η] (mL/g)	$\overline{M}_v \times 10^{-4}$
C_0	0	15±3	616±63	14
C_{30}	30	7.3±0.1	586±6	12
C_{60}	60	8.4±1	540±30	11
C_{90}	90	8±3	573±6	11
C_C	---	11±2	723[(c)]	17
C_H	---	10±2	613±1	14

a)All values correspond to the average of at least two independent deacetylations, except for the [η] value of sample C_C which correspond to a single determination.
b)Numeric subscripts stand for the time to which chitin was submitted to sonication before deacetylation while the letters C and H, used as subscripts, stand for chitins previously stirred in cold and hot water, respectively.

It may be also observed by comparing the values of intrinsic viscosity and viscosity average molecular weight of the chitosan samples (Table 1) that the previous sonication slightly increased the depolymerization which normally occurs simultaneously to the deacetylation reaction however it has been demonstrated (9) that adding sodium borohydride to the reaction medium reduced its occurrence. It was also observed that increasing the sonication time did not enhance the deacetylation efficiency but the previous stirring of the chitin suspension, independently of the temperature maintained during this treatment, did activate chitin for the deacetylation reaction. When a given medium is submitted to sonication, the vibrational motion of its molecular structure can, in some conditions, induce the generation of cavitation bubbles and the local liberation of a large amount of energy (10). This has been used, for example, to activate metallic particles suspended in the medium submitted to sonication, resulting in the removal of inert surface layers and in the reduction of the average size of the sonicated particles (11). The sonication of chitin, as employed in this study, did not result in mass losses but the possible reduction of the average size of the solid particles was not evaluated since sonicated chitin was formed by clusters of particles and they were carefully disaggregated to avoid the further grinding of the solid before submit it to the deacetylation. X-rays diffraction and scanning electron microscopy analyses were carried out aiming to understand the role of sonication as an activation treatment of chitin before its deacetylation. The comparison of crude commercial chitin and of a sample submitted to sonication during 60 minutes reveals that the pre-treatment provoked changes in the diffraction pattern (Figure 3). It may be observed as a small shift of all signals toward lower values of the Bragg's angle, more easily observed in the well-defined and more intense peak. If the crystallinity indexes are estimated by using the method described in the literature (5), it is observed that they are 84% and 83% for crude and sonicated chitin, respectively. However, considering that the average size of crystallites is inversely proportional to the line width at half-maximum intensity (WHMI was measured at $2\theta \cong 20°$, in this work) (12) it is observed that the crystallites are smaller in sonicated chitin than in crude commercial chitin. In fact, by measuring the line width of the more intense signal of each diffractogram at half height, a reduction of 10 % in WHMI is observed in the case of the sonicated chitin. These results suggest that the sonication treatment provoked changes in the crystalline domains of chitin, probably due to the reduction of the size of the crystallites which, in turn, increased its superficial area exposed to the reagents.

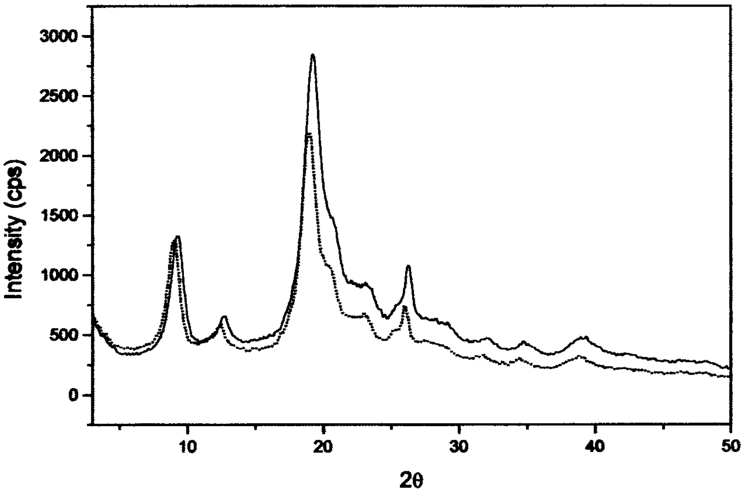

Figure 3: X-rays diffractograms of commercial chitin (solid line) and of a sample of chitin after being sonicated for 60 minutes (doted line).

When examined by scanning electron microscopy at relatively small magnification it was observed that crude and sonicated chitin (Figures 4 and 5, respectively) presented very different morphological constitutions. In fact, the commercial sample is much more heterogeneous, being mainly composed by sheet-like and filament-like elements (Figure 4) than the sonicated chitin (Figure 5) which contains only grain-like elements. Also, considering the average linear dimensions of the morphological elements, it is observed that they are much less uniform in commercial chitin than in sonicated chitin. When the commercial and sonicated samples of chitin were examined at a much higher magnification it was observed that their surfaces presented very different characteristics (Figures 6 and 7). The surface of the sheet-like element of the commercial sample is very smooth and seems to be flat (Figure 6) while the surface of the grain-like element of sonicated chitin is very rough and seems to present channels directed to its interior and projections toward the viewer (Figure 7), supporting the idea that sonication increased the accessibility of reagents by enhancing the superficial area of treated chitin. The whole set of results suggests that the sonication treatment enhanced the reactivity of chitin toward its heterogeneous deacetylation by the so-called mechanical effects, representing a case of "false sonochemistry" (13), since a similar reactivity was induced by stirring the aqueous suspension of chitin.

Figure 4: Scanning electron microscopy of commercial chitin; 75x magnification.

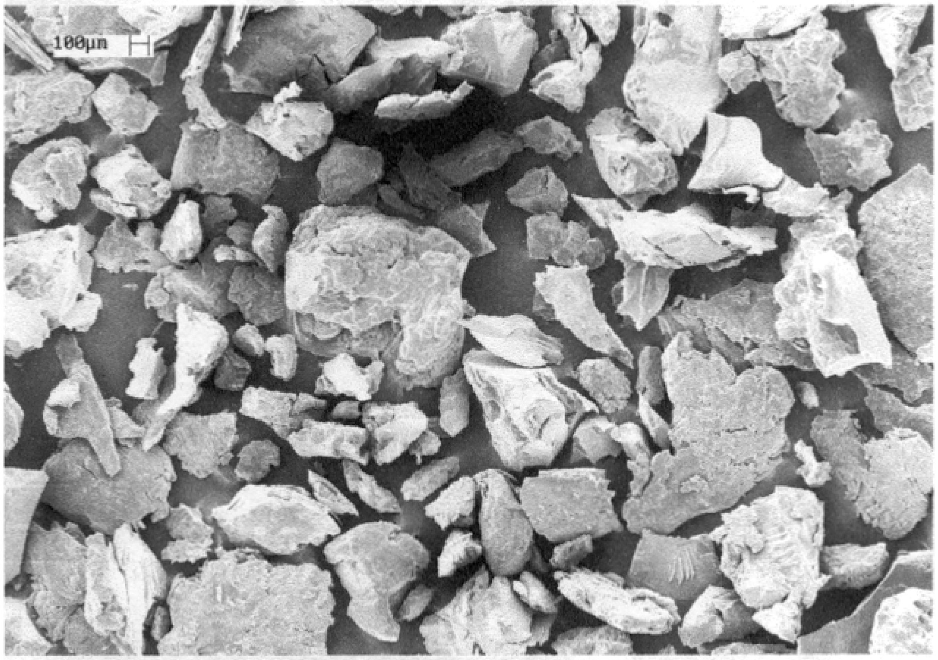

Figure 5: Scanning electron microscopy of sonicated chitin ; 75x magnification.

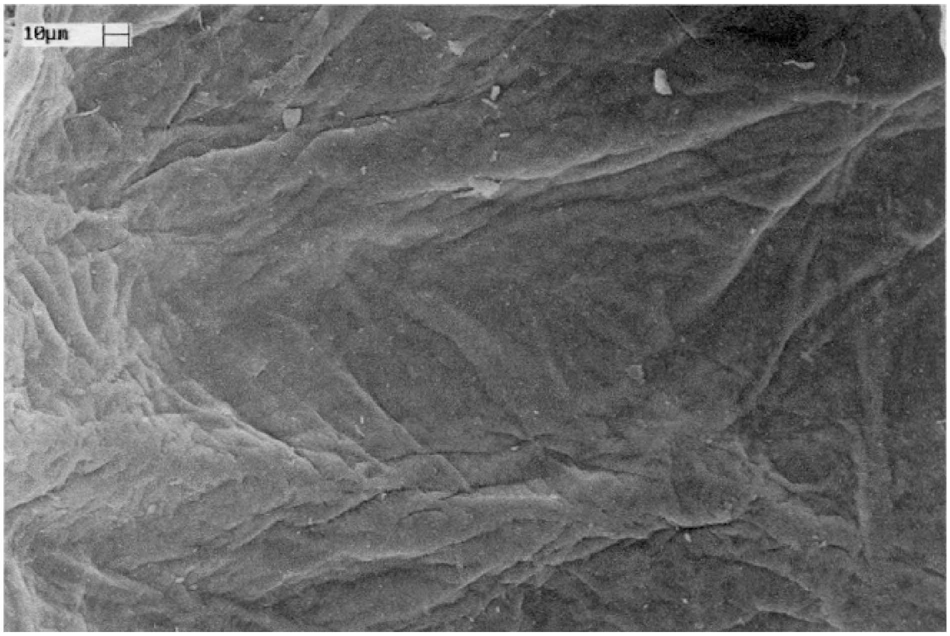

Figure 6: Scanning electron microscopy of commmercial chitin; 1000x magnification.

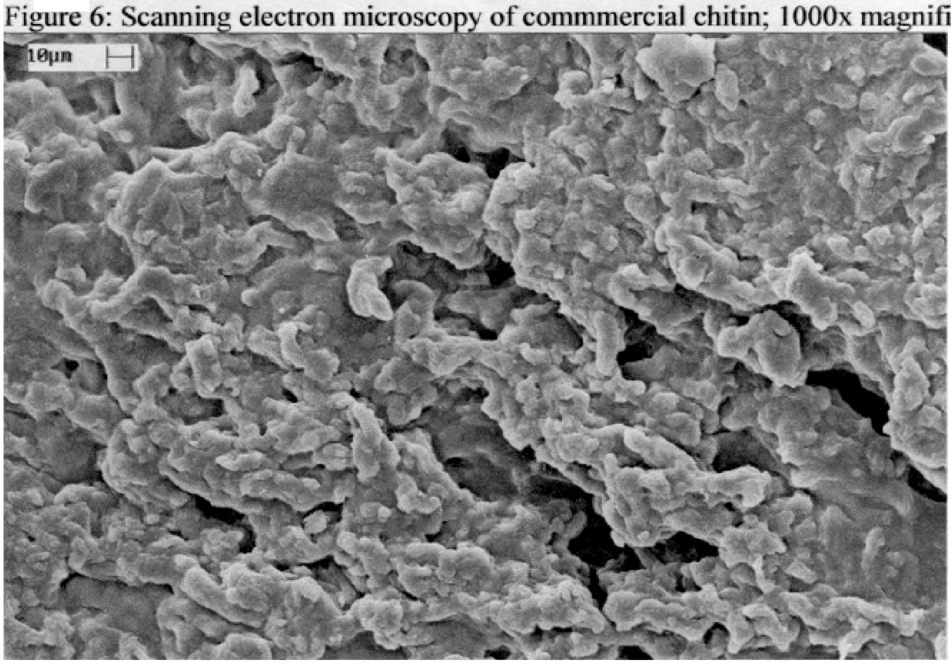

Figure 7: Scanning electron microscopy of sonicated chitin; 1000x magnification.

Conclusions

The results suggest that the sonication treatment provoked changes in the crystalline domains of chitin, enhancing the accessibility to its reactive sites, probably due to the size reduction of crystallites. The higher homogeneity and large surface area of sonicated particles of chitin may also be responsible for its higher reactivity toward deacetylation. The occurrence of defects, such as dislocations (14), may be induced by ultra-sound treatment and the search for the effects of sonication on suspended chitin should concern it and also the investigation of changes in its microporosity. The effects of employing efficient stirring systems and dispersing tools to previously treat the suspended chitin should be compared to the ultra-sound action to confirm the mechanical nature of the latter and to determine which treatment is more advantageous.

Acknowledgements.
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