

Effects of chitosan characteristics on its thermal stability

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Abstract

Chitosan samples exhibiting different characteristics, namely average degree of acetylation ($7.5\% < DA < 25.0\%$), viscosity average molecular weight ($1.17 \times 10^5 \text{ g mol}^{-1} < Mv < 6.45 \times 10^5 \text{ g mol}^{-1}$) and crystallinity index ($19.1\% < CI < 52.7\%$), were produced by applying non-isothermal ultrasound-assisted deacetylation process (NIUSAD) to beta-chitin extracted from squid pens. The effects of these characteristics on the thermal stability of chitosan were investigated by performing thermal analysis in synthetic air atmosphere. The results showed that the thermal stability of NIUSAD chitosan depended on Mv , DA and CI , the last being the main factor affecting the onset degradation temperature (T_{onset}). Accordingly, the more stable NIUSAD chitosans ($T_{\text{onset}} > 280 \text{ }^\circ\text{C}$) corresponded to the samples whose crystallinity index was inserted in a quite narrow interval ($45\% < CI < 50\%$).

Keywords: Chitosan, degree of acetylation, thermal stability

1. Introduction

Chitin is a linear biopolymer whose repeating unit is a $\beta(1 \rightarrow 4)$ -linked disaccharide containing 2-acetamido-2-deoxy-D-glucopyranose (GlcNAc) and 2-amino-2-deoxy-D-glucopyranose (GlcN) units with high predominance ($> 80\%$) of the former units [1]. Chitosan is obtained when the deacetylation of chitin, the reaction corresponding to the hydrolysis of the acetamido group of its GlcNAc units - a heterogeneous process generally carried out by treating chitin with alkaline aqueous solution and whose main variables are alkali concentration, reaction time and temperature - results in the predominance of GlcN units.

Recently, new deacetylation processes, such as those employing microwave irradiation [2], flash treatment [3], freeze - pump out - thawing procedure [4] and the ultrasound-assisted deacetylation process (USAD) [5] have been proposed, the last two being claimed to be more efficient than the thermochemical deacetylation of chitin and yet suitable for preparing chitosan with controlled characteristics, including those exhibiting high molecular weight and low average degree of acetylation.

Indeed, the production of chitosan with controlled characteristics is a very important objective to ensure the reproducibility of the physicochemical properties of the polymer, such as solubility, viscosity, rheological and thermal behavior, since these are determined mainly by the average degree of acetylation and average molecular weight of chitosan [6]. Thus, several deacetylation processes and different reaction conditions can be used in order to produce chitosan with different average degree of acetylation, because once high molecular weight chitosan is available, its depolymerization, with the aim of producing lower molecular weight samples, can be carried out by using, for

instance, microwave irradiation [7], high intensity ultrasound irradiation [8] and enzymatic hydrolysis [9]. Because the thermal processing of polymers is largely used in industry, the study on the thermal behavior of chitosan and its dependence on the polymer's characteristics is a very important task as it may support its specific applications.

WANJUN et al. (2005) [10] reported that the lower thermal stability of chitosan, when compared to chitin, is directly linked to its lower average degree of acetylation, a conclusion which was well supported by Britto et al. (2007) [11] while Guinesi et al. (2006) [12] proposed that the average degree of acetylation can be determined with the use of DSC curves. Also, according to Zacaria et al. (2012) [13], the thermal behavior of chitosans films depends on the degree of acetylation of the polymer. The average degree of acetylation of chitosan, however, is not the single parameter affecting its thermal behavior and, accordingly, the proposal of this study is to investigate its influence alongside with viscosity average molecular weight (Mv) and crystallinity index (CI) on the thermal stability of chitosan.

2. Experimental

2.1. Extraction of beta-chitin

Beta-chitin was extracted from squid pens (*Loligo* sp.) by treating it with aqueous alkali according to the procedure reported by Chaussard et al. (2004) [14], the demineralization not being carried out due to its low content of inorganic compounds [15].

2.2. NIUSAD chitosan

The chitosan samples were produced by applying the NIUSAD process [5] to beta-chitin extracted from squid pens (*Loligo* sp.). Typically, beta-chitin was suspended in NaOH 40% (w/w), the suspension poured into a double-

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walled cylindrical glass reactor (internal diameter of 3.5 cm) and then submitted to ultrasound irradiation using a model 450 Branson Sonifier ($\nu = 20$ kHz) ultrasonic device coupled to a 0.5 in (1.3 cm) stepped probe. The ultrasonic device was set to intermittent irradiation and the NIUSAD process was performed according to the following conditions:

i) irradiation amplitude (A) was adjusted to low (L; $30\% < A_{max} < 50\%$), medium (M; $55\% < A_{max} < 70\%$) or high H; ($75\% < A_{max} < 95\%$);

ii) irradiation duration was set to short (S; 10 – 30 min.), medium (M; 35 – 45 min.) or long (L; 50 – 60 min.).

According to irradiation amplitude, the processing temperature increased from room temperature to approximately 95 °C after 25 - 30 min, and then remained constant for treatment duration up to 60 min. The reaction was interrupted by the cooling of the suspension to -15 °C followed by the addition of dilute hydrochloric acid to result in $\text{pH} \approx 8.0 - 8.5$. The resulting NIUSAD chitosan was isolated after extensive washing with deionized water and drying at room temperature.

Table 1: NIUSAD processing conditions and identification of chitosan samples.

Chitosan	Processing Conditions	
	Irradiation Amplitude	Irradiation Time
NILS	Low	Short
NILM	Low	Medium
NILL	Low	Long
NIMS	Medium	Short
NIMM	Medium	Medium
NIML	Medium	Long
NIHS	High	Short
NIHM	High	Medium
NIHL	High	Long

Each chitosan was identified by NI and two capital letters according to the processing conditions employed to produce it, the first and second capital letter standing for the irradiation amplitude (L, M or H) and the irradiation duration (S, M or L), respectively. Therefore, for instance, the NILS sample corresponds to chitosan generated by applying the non-isothermal USAD process set to low irradiation amplitude (L) and short irradiation (S). The processing conditions and the identification of the resulting NIUSAD chitosans are shown in Table 1.

2.3. Characterizations

The average degree of acetylation (DA) of chitosan was determined by hydrogen nuclear magnetic resonance spectroscopy ($^1\text{H-NMR}$) [16] while its intrinsic viscosity was determined from viscosity measurements in 0.3 mol.L^{-1} acetic acid/ 0.2 mol.L^{-1} sodium acetate buffer ($\text{pH} \approx 4.5$) [17]. The viscosity average molecular weight (Mv) was calculated from intrinsic viscosity by employing the values of K and α determined in the same solvent and temperature and according to the DA of the chitosan sample [18].

X-ray diffraction was used as described above [5] to determine the crystallinity index (CI) of NIUSAD chitosans by using the following equation:

$$\text{CI} = \frac{I_C - I_A}{I_C} \cdot 100$$

where I_C and I_A correspond to the intensity of the signals concerning the crystalline domains and the amorphous regions, respectively.

2.4. Thermal stability

The thermogravimetric curves of the NIUSAD chitosans were acquired with the use of a Shimadzu TGA-50 in synthetic air atmosphere at 50 mL min^{-1} as the flow rate. The samples were heated (heating rate = $10 \text{ }^\circ\text{C min}^{-1}$) from room temperature to 100 °C, the temperature kept constant for 20 min and then the heating continued at the same rate up to 900 °C. The temperature corresponding to the beginning of the thermal degradation of the NIUSAD chitosans (T_{onset}) was determined from the TG curve by intersecting the straight lines shown in Figure 1.

3. Results and Discussion

Chitosan samples exhibiting different characteristics ($7.5\% < \text{DA} < 25.0\%$; $1.17 \times 10^5 \text{ g mol}^{-1} < \text{Mv} < 6.45 \times 10^5 \text{ g mol}^{-1}$; $19.1\% < \text{CI} < 52.7\%$) were produced by applying the USAD process to beta-chitin extracted from squid pens [5].

The TG curve of NIMS chitosan (Figure 1), representing the whole set of NIUSAD chitosans, show a first step (25 °C – 100 °C) corresponding to the elimination of water molecules loosely bound to the polymer network followed by the main thermal degradation step, beginning at 300 °C, which is associated to the highest loss of weight ($\approx 56\%$) during the thermal degradation. It was also observed that the loss of weight during this degradation step was higher for chitosans exhibiting higher average degree of acetylation (data not shown), supporting the assumption that most of the loss of weight occurring in this step is due to the elimination of acetyl groups from GlcNAc units [10].

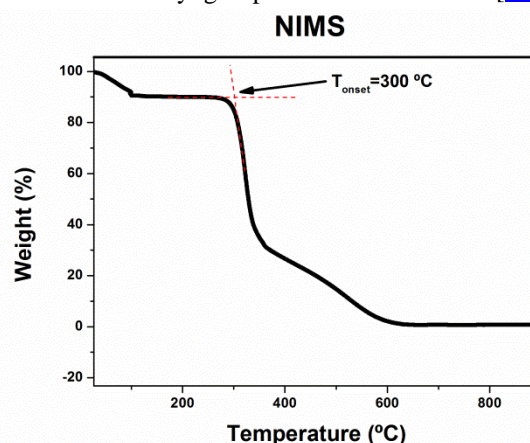


Figure 1. TG curve showing the graphical determination of T_{onset} of chitosan NIMS.

Table 2 shows that the NIUSAD chitosans exhibited different characteristics, namely average degree of acetylation (DA) and viscosity average molecular weight

(Mv), which in turn affected both crystallinity index (CI) and onset degradation temperature (T_{onset}). Indeed, it is well documented in the literature that characteristics, DA and Mv, affect the chitosan crystallinity, but it is important to consider that when NIUSAD chitosans are involved, the ultrasound irradiation itself affects the polymer's crystallinity besides DA and Mv [5]. Accordingly, since the NIUSAD chitosans were produced by using different processing conditions (Table 1), the parent beta-chitin was exposed to ultrasound irradiation of variable intensity and duration, resulting in less/more deacetylated NIUSAD chitosan exhibiting higher/lower viscosity average molecular weight the lower/higher the irradiation amplitude and the shorter/longer the irradiation duration, but the dependence of crystallinity index and onset temperature on the NIUSAD processing conditions is not so simply related.

Table 2: Degree of acetylation (DA), viscosity average molecular weight (Mv), crystallinity index (CI) and onset degradation temperature (T_{onset}) of the NIUSAD chitosans.

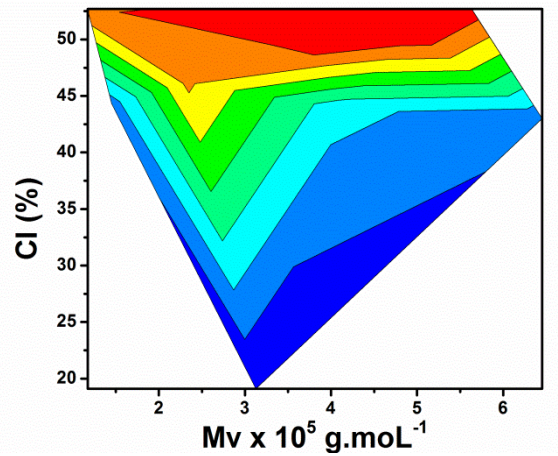
Chitosan	DA (%)	Mv x10 ⁵ (g mol ⁻¹)	CI (%)	T _{onset} (°C)
NILS	25.0	6.45	43.0	243
NILM	22.0	5.63	52.7	291
NILL	20.8	4.13	43.9	251
NIMS	15.7	4.94	50.6	300
NIMM	14.0	3.13	19.1	234
NIML	8.6	2.32	46.2	280
NIHS	12.5	3.85	48.7	286
NIHM	7.7	1.45	44.3	245
NIHL	7.5	1.17	52.6	285

According to the data in Table 2, NIUSAD chitosans can be classified into two main groups according to their crystallinity index and onset temperature:

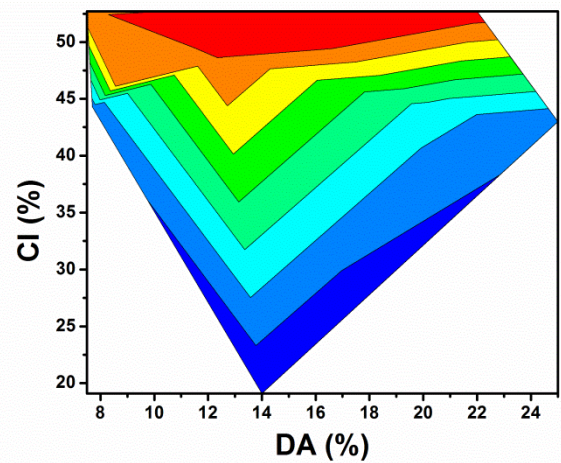
- 1) Samples NILM, NIMS, NIML, NIHS and NIHL display higher thermal stability ($T_{onset} \geq 280$ °C) and exhibit a critical crystallinity index ($CI > 46\%$);
- 2) Samples NILS, NILL and NIHM are less stable (240 °C $< T_{onset} < 250$ °C) and they are also less crystalline ($CI < 45\%$). Yet, the least stable sample ($T_{onset} = 234$ °C) is the NIMM chitosan, which also displays the lowest crystallinity index ($CI < 20\%$).

Such approach suggests that the crystallinity index is the main factor affecting the thermal stability of NIUSAD chitosans, an assertion which is well supported by the ternary contour plots relating T_{onset} to CI/Mv (Fig. 2a) and to CI/DA (Fig. 2b). Indeed, these plots show that the thermally more stable NIUSAD chitosans (red and orange contours in Fig. 2a and 2b) exhibit $CI > 45\%$ although DA and Mv range in wide intervals ($7\% < DA < 23\%$; $1.2 \times 10^5 \text{ g mol}^{-1} < Mv < 6.0 \times 10^5 \text{ g mol}^{-1}$), while the thermally less stable chitosans (deep blue contours in Fig. 2a and 2b) exhibits much lower crystallinity index. Additionally, when normalized values of T_{onset} , Mv and DA are plotted (Figure 2c), it is observed that, concerning the thermal degradation of NIUSAD chitosans, highly stable samples are

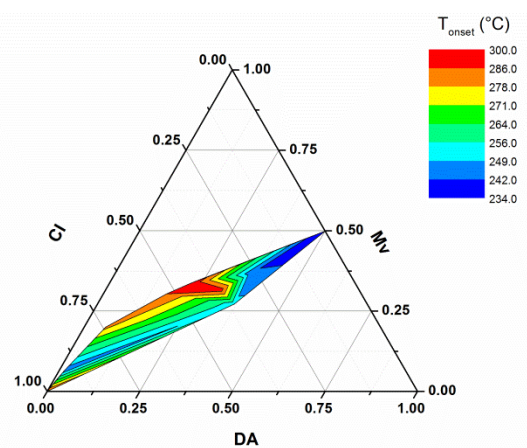
inserted in a quite narrow range of CI (red contour in Fig. 2c). Therefore, as already discussed, this behavior can be mainly attributed to the occurrence of ordered domains, which provide a resistance to thermal degradation which increases proportionally with the crystallinity index of NIUSAD chitosans.



(a)



(b)



(c)

Figure 2. Ternary plots relating the onset temperature (T_{onset}) to: (a) viscosity average molecular weight (Mv) and (b) average degree of acetylation (DA) of NIUSAD chitosans. The ternary plot in (c) expresses normalized values of T_{onset} , Mv and DA.

4. Conclusion

The application of the NIUSAD process to beta-chitin resulted in chitosan samples exhibiting different characteristics (DA and Mv) whose crystallinity index (CI) were strongly affected by them and also by the processing conditions. The thermal stability of NIUSAD chitosan was affected by the polymer characteristics as well as by its crystallinity index. It has been shown, however, that the second is the main factor affecting thermal stability, which increases proportionally with the CI (the higher the CI the more thermally stable the chitosan). Further work is in progress seeking a deeper understanding of the effect of USAD parameters on the characteristics and physicochemical properties of the resulting chitosans.

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