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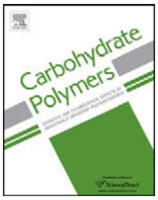

Structure and properties of glycerol-plasticized chitosan obtained by mechanical kneading

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ABSTRACT

This paper reports the elaboration of plasticized chitosan by thermo-mechanical treatment, as a possible alternative route to solvent casting, and the corresponding material properties. Glycerol was used as plasticizer with a concentration fixed at 25 wt.%. The water uptake, crystallinity evolution, mechanical and dynamic-mechanical thermal properties were studied during the post-processing ageing at different relative humidities. The properties of plasticized chitosan were compared with those of glycerol-free samples. For instance, increased elongation at break (+40%) is observed with plasticized chitosan. Like most plasticized polysaccharides, the material properties are strongly impacted by water content. The water uptake measurements evidenced that the hydrophilic character of the material is enhanced by the addition of glycerol. Both tensile tests and DMTA results confirm the plasticizing effect of glycerol and water. The analyses evidenced that several weeks of controlled storage are required to reach stabilized properties. The best results are obtained for materials stabilized in a medium relative humidity atmosphere (57% RH).

1. Introduction

In the last years, agropolymer-based materials have attracted great attention due to their large availability, renewability and biodegradability. Particularly, interesting results concerning the great potential of chitosan as biomaterial were reported (Ravi Kumar, 2000; Rinaudo, 2006). Chitosan has applications in biomedical and drug delivery systems (Ravi Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004) due to its biocompatibility, antimicrobial and good mucoadhesive properties.

Water-soluble chitosan salts are filmogenic (Chen & Horng-Dar, 1996; Dhanikula & Panchagnula, 2004; Muzzarelli, 1996; Ouattara, Simard, Piette, Bégin, & Holley, 2000) and thus found applications in the food packaging industry, especially as edible films or coatings. These films may improve food conservation and quality by forming a barrier against moisture (Caner, Vergano, & Wiles, 1998), oxygen and CO_2 (Hosokawa, Nishiyama, Yoshihara, & Kubo, 1990). The film properties depend on several parameters such as chitosan molecular weight and degree of deacetylation, organic acid used and the possible presence of plasticizer.

Plasticized chitosan present improved elasticity (Suyatma, Tighzert, Copinet, & Coma, 2005; Ziani, Oses, Coma, & Maté, 2008) and the addition of a plasticizer allows to overcome chitosan films brittleness. Main non-volatile plasticizers are glycerol, sorbitol, propylene glycol or polyethylene glycol (Kolhe & Kannan, 2003; Ziani et al., 2008). Glycerol is the most used plasticizer due to its good plasticization efficiency, large availability and low exudation.

Generally, two methods are used to produce films based on polysaccharide, namely the wet and dry methods. The wet route, also called solvent casting method, is at the present the unique process to obtain chitosan films. This method is used, for instance, to prepare chitosan films for food applications (Dutta, Tripathi, Mehrotra, & Dutta, 2009).

The dry-process usually implies the melt processing (extrusion, kneading, etc.) under thermo-mechanical treatment with plasticizers. This method is used for instance for plasticized starch (Avérous, 2004). Chitosan like many other polysaccharides has very low thermal stability, degrades without melting and thus is considered as infusible. Thus, even if the melt processing method is more convenient and highly preferred for industrial production, its adaptation for polysaccharides based materials remains very difficult.

Since plasticized chitosan is generally obtained by casting with strong limitations, the aim of this work was to develop an alternative approach for the elaboration of plasticized chitosan-based on thermo-mechanical processing (mechanical kneading) as done for conventional thermoplastics. The obtained materials were characterized and the influence of the plasticizer, the effect of the post-processing ageing and the relative humidity storage on the water uptake, crystallinity, mechanical properties and dynamic-mechanical thermal analysis (DMTA) have been investigated and compared to unplasticized chitosan.

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2. Materials and methods

2.1. Materials

Chitosan with degree of deacetylation DD = 97% and Mw = 250–300 kDA was purchased from Primex (trade name ChitoClear®). It is provided as white powder (93% dry matter content) with particles' diameter lower than 1 mm (100% through mesh 18). Deionized water was used for the samples preparation. Glycerol (99.5% purity, from Sigma–Aldrich) and acetic acid (Fluka) were used as received.

2.2. Samples preparation

2.2.1. Chitosan-based materials elaboration

Both plasticized and unplasticized chitosan-based materials were elaborated. In a first step, solid-state chitosan is destructured. For plasticized chitosan materials, a chitosan/glycerol ratio of 75/25 (wt./wt.) was considered. As a typical procedure, glycerol was first incorporated in the chitosan powder and manually mixed. Then, acetic acid aqueous solution (2%, v/v) was added to the chitosan/glycerol mixture to obtain a paste with final chitosan concentration of 20 wt.%. For unplasticized formulation, acetic acid aqueous solution was directly added to the chitosan powder. The chitosan/water/glycerol or chitosan/water pasty mixtures were then mechanically blended in a Haake Rheocord 9000 internal batch mixer at 80 °C for 15 min, with a rotor speed of 100 rpm. Finally, the resulting materials were hot-pressed in two steps. The blend was firstly hot-pressed at 110 °C, 250 bar pressure for 15 min and immediately cooled at room temperature for 3 min. Then, a second hot-pressing at 110 °C, 250 bar pressure for 10 min followed by a room temperature pressing was performed. After compression molding, plasticized and unplasticized chitosan plates of 1 mm thickness are obtained.

2.2.2. Post-processing ageing

Like many other polysaccharides, before reaching stabilized properties, chitosan-based materials show some post-processing ageing that needs to be monitored. After compression molding, and in a first step, both plasticized and unplasticized samples are kept for 32 h at 33% RH (relative humidity). In a second stage, dumbbell-shaped bars or rectangular tensile bars were cut into the plates and then stored at 33, 57 and 75% RH and ambient temperature (23 °C). The samples properties were monitored vs. time until stabilization.

2.3. Characterization

2.3.1. Water uptake

The water uptake behavior of plasticized and unplasticized chitosan samples was monitored. After compression molding, tensile bars (10 mm × 60 mm × 1 mm) were dried at 50 °C under vacuum, for 48 h in order to avoid glycerol volatilization and to obtain a "zero" value. The dried samples were immediately stored in a desiccator with P_2O_5 until the samples reached the room temperature. After being weighed, to obtain the "zero" weight value after drying, the samples were stored at different specific relative humidities (33, 57 and 75% RH) and then weighed vs. time. Three replicates of each sample were measured. The water content W (%) was calculated according to Eq. (1) where M_t is the weight at time t and M_d is the weight after drying.

$$W(\%) = \frac{M_t - M_\mathrm{d}}{M_\mathrm{d}} \times 100 \tag{1}$$

2.3.2. X-ray diffraction analysis

X-ray diffraction (XRD) was performed on powder and films. XRD patterns were obtained at room temperature, on a powder diffractometer Siemens D5000. Cu Kα radiation ($\lambda = 1.5406$ Å) at a voltage of 35 kV was used. The scattering range was $2\theta = 7$–$36°$ by step size of 0.02° and a scanning speed of 2 s/step. The relative crystallinity percentage X_c was estimated based on Eq. (2) where A_c and A_a represent the area of the crystalline peaks and amorphous halo, respectively (Wan, Creber, Peppley, & Bui, 2003).

$$X_\mathrm{c} = \frac{A_\mathrm{c}}{A_\mathrm{c} + A_\mathrm{a}} \times 100 \tag{2}$$

2.3.3. Tensile properties

Tensile tests were performed with an Instron 4204 tester on dumbbell-shaped bars at room temperature with a constant deformation rate of 5 mm/min. The true tensile modulus, stress at break and elongation at break were determined from five specimens for each formulation.

2.3.4. Dynamic-mechanical thermal analyses (DMTAs)

DMTA was performed on rectangular tensile bars of plasticized samples. The dynamic storage modulus (E'), loss modulus (E'') and loss tangent ($\tan\delta = E'/E''$) were obtained from stabilized samples, after 35 days of storage at 33, 57 and 75% RH and room temperature. To prevent water evaporation during the tests, the specimens were coated with paraffinic grease. The tests were performed on a Rheometrics Solid Analyser RSAII with dual cantilever bending mode from −80 to 100 °C, with a heating rate of 1.5 °C/min, a frequency of 1 Hz and a strain value of 0.05%.

3. Results and discussion

Preliminary studies (not reported here) allowed us to optimize the elaboration protocol (mixing time, rotor speed, operating temperatures, amount of water, glycerol content, etc.) in order to obtain a visually good material. Optimal glycerol content for chitosan plasticization was obtained for 25 wt.%, since higher amounts resulted in heterogeneous and sticky materials. The addition of acetic acid aqueous solution (2%, v/v) allowed partial protonation and destructuration of chitosan plain powder which is favorable for the mixing and plasticization. During the mechanical kneading process a large amount of water is lost from the chitosan gel mixture, but the water content of the material is still very high and the excess water is mostly removed during the hot-pressing step. The resulting material is translucent but shows a yellowish color resulting from the inevitable thermal degradation and Maillard reaction. For the glycerol-plasticized samples, the material is soft and rubbery.

The water uptake of glycerol-plasticized and unplasticized chitosan was monitored during storage at 33, 57 and 75% RH. From Fig. 1 we observe that unplasticized and plasticized chitosan materials present similar water uptake evolutions. The process is very fast since in 24 h the water content stabilization is reached. Similar behavior was reported for chitosan/glycerol films obtained by solvent casting (Suyatma et al., 2005). As usual for polysaccharide-based materials, we can see that the water content is influenced by the non-volatile plasticizer (glycerol) and by the storage RH.

As a general behavior, we observe that both plasticized and unplasticized samples have a great water sorption capacity through hydrogen bonds with some chitosan sites. It is worth pointing out that the values obtained for unplasticized chitosan are in very good agreement with previously reported values (Srinivasa, Ramesh, & Tharanathan, 2007). The highest values were obtained with plasticized samples since glycerol interacts through hydrogen bonds with chitosan and with water molecules, leading to higher water contents. Moreover, water sorption logically depends on the

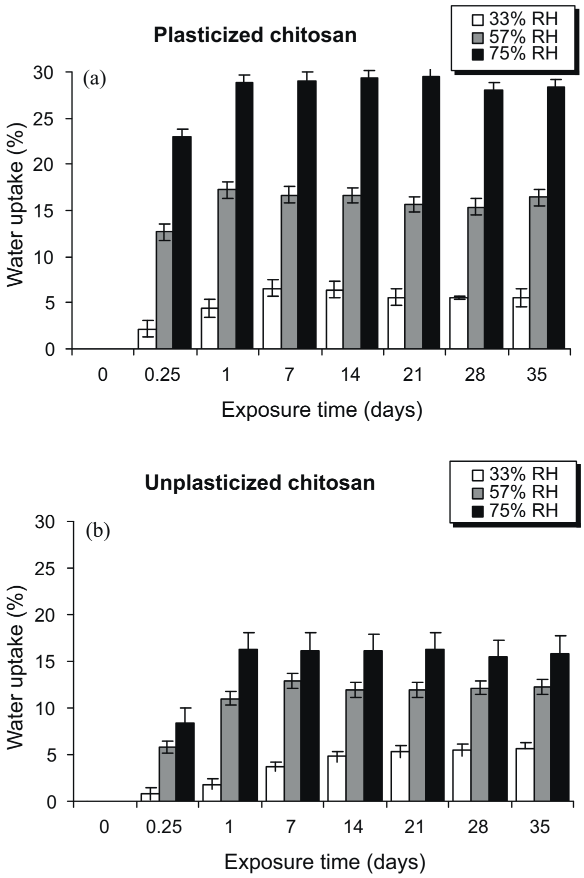

Fig. 1. Evolution of water uptake for (a) plasticized chitosan and (b) unplasticized chitosan vs. exposure time at 33, 57 and 75% RH.

relative humidity. For unplasticized chitosan, the water uptake increases perfectly linearly with the RH. Such linear evolution is no longer observed for plasticized chitosan. The water uptake value for plasticized chitosan stored at 75% RH is quite high, reaching almost 30%, due also to the glycerol hydrophilic character.

Elongation at break and tensile strength at break of glycerol-plasticized and unplasticized chitosan samples stored at different relative humidities were studied vs. time (Figs. 2 and 3). The mechanical properties were monitored only for 35 days of ageing since no significant evolution was observed after 35 days.

Since water also acts as a volatile plasticizer and thus increases the polymer chains mobility, the influence of the water content is clearly observed on the elongation (Fig. 2). Unplasticized chitosan samples are brittle and fragile when stored at low RH (less than 10% elongation after 35 days at 33% RH). The elongation at break values of both the plasticized and unplasticized samples stored at 33% RH decrease with time due to the material drying. On the contrary, these variations are more limited at 57% RH and the elongation values stay constant at 75% RH. After 35 days of storage at 33 and 57% RH, the elongation at break of the plasticized samples is higher than that of the unplasticized ones, demonstrating the efficacy of glycerol as a non-volatile plasticizer. The highest elongation at break values observed for plasticized and unplasticized chitosan materials stored at 57% RH clearly attest that, at this RH, optimal properties are obtained. Similar results and trends on the elongation at break were reported for chitosan/glycerol films obtained by solvent casting (Suyatma et al., 2005).

A particular behavior was observed for chitosan samples stored at 75% RH. The elongation at break is almost constant with time and close values were observed for both unplasticized and glycerol-plasticized chitosan. These trends demonstrate that, for such a high RH, the plasticizing effect of water predominates and thus glycerol has lower influence.

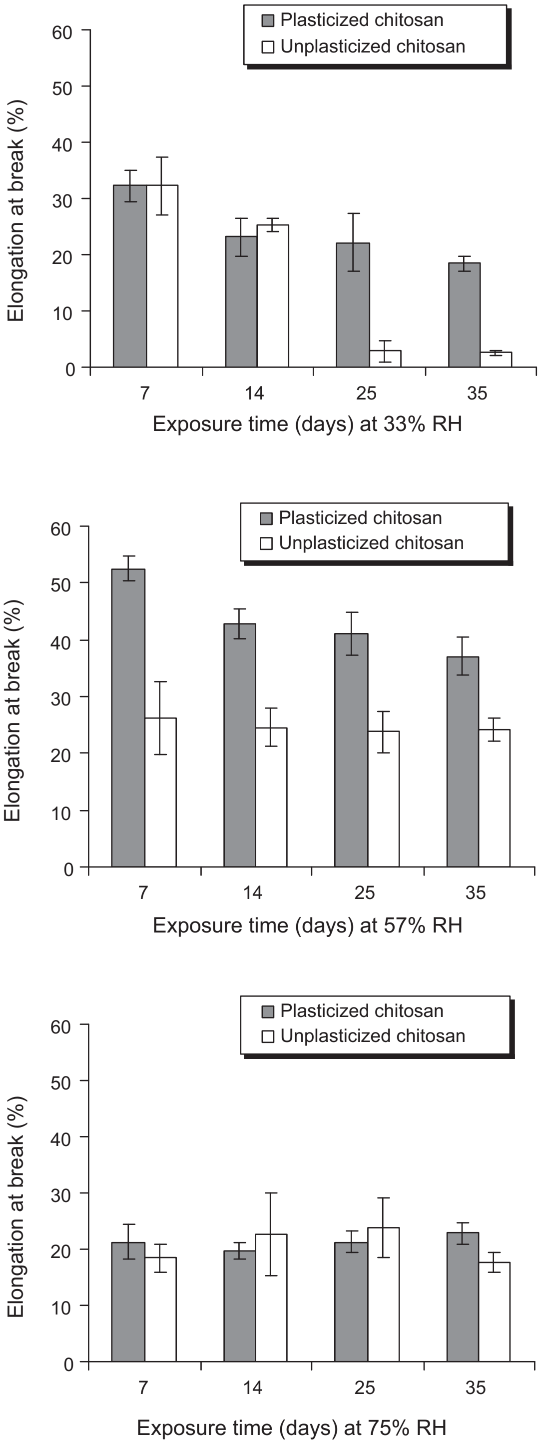

Fig. 2. Time evolution of the elongation at break for plasticized and unplasticized chitosan samples stored at 33, 57 and 75% RH.

Fig. 3 shows that at 33 and 75%RH an increase in tensile strength is observed during the storage for both glycerol-plasticized and unplasticized chitosan. At 57% RH, the tensile strength values of plasticized chitosan remain constant with time and display the highest value. This trend confirms that this medium humidity is the most appropriate storage atmosphere tested for the post-processing stabilization of these materials.

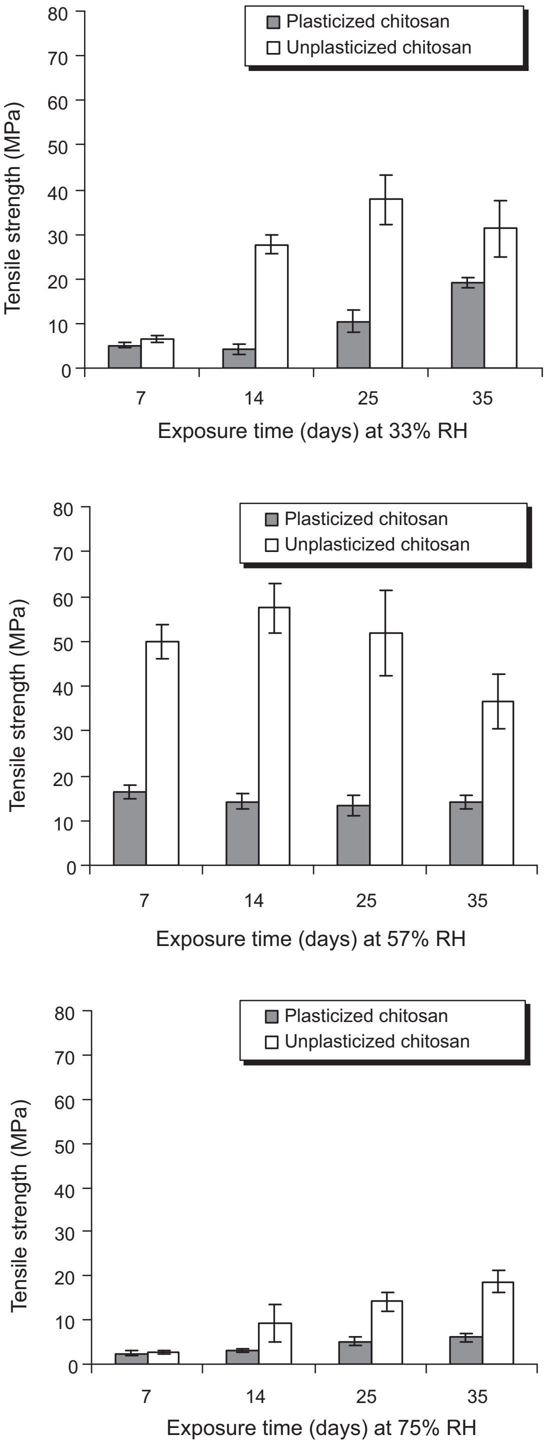

Fig. 3. Time evolution of the tensile strength for plasticized and unplasticized chitosan samples stored at 33, 57 and 75% RH.

Taking into account the standard deviations, whatever the relative humidity and the mechanical parameters (elongation at break or tensile strength) for glycerol-plasticized or unplasticized chitosan, the post-processing ageing seems to be stabilized after 25 days of storage. Between 25 and 35 days of storage, the variations are not significant.

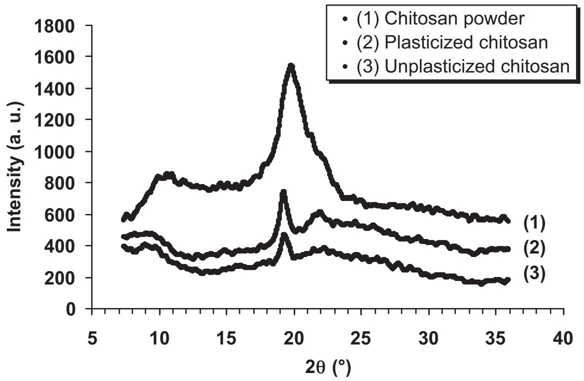

Fig. 4. XRD patterns of (1) chitosan plain powder compared to (2) plasticized chitosan and (3) unplasticized chitosan XRD patterns.

XRD analyses were performed to determine the influence of the process and the plasticizer content on the crystalline structure of the chitosan films. The influence of storage time and RH on the crystallinity type and percentage (X_c) were studied. As observed in Fig. 4, diffraction patterns and peak intensities of the glycerol-plasticized and unplasticized chitosan films strongly differ from the diffractogram of plain chitosan powder.

Fig. 4 shows that the plain chitosan powder displays a relatively high crystallinity (estimated to be over 80%) with two main peaks at around 10° and 20°. The peak at 10° is assigned to the hydrated crystals due to the integration of water molecules in the crystal lattice and the peak located at 20° is attributed to the regular crystal lattice of chitosan (Kittur, Vishu Kumar, & Tharanathan, 2003). Unplasticized chitosan films prepared by thermo-mechanical treatment and hot-pressing show these two peaks at slightly lower angles, 9.5° and 19.5°, respectively. Moreover, the intensity of this spectrum is largely inferior to the previous one. This is not unexpected, a decrease of the chitosan crystallinity was observed for films elaborated by solvent casting (Kittur et al., 2003). From the XRD data collected before and after 35 days of ageing, the time evolution of the relative crystallinity was estimated and values are reported in Table 1.

As it can be observed from Fig. 4 and from Table 1, a decrease in chitosan crystallinity is observed for the processed materials compared to the plain chitosan powder. This decrease is likely due to the modification of inter- and intra-chains hydrogen bonds and the modification of the structure after the chitosan destructuration. Interestingly, the plasticized chitosan film initially shows slightly higher crystallinity value (Table 1) compared to the unplasticized chitosan. This observation demonstrates that glycerol favors the chains mobility and thus the chitosan crystallization process in the early stage of the post-processing ageing. The long storage time modifies the degree of crystallinity of the unplasticized and plasticized chitosan films. The crystallinity increased except for glycerol-plasticized chitosan sample stored at 75% RH which displays a slight decrease in crystallinity. This latter material shows the highest water content and the resulting modification in hydrogen bond interactions may inhibit the crystallization process. These

Table 1
Time evolution of the relative crystallinity (X_c) for unplasticized and plasticized chitosan stored at 33, 57 and 75% RH. t_0 and t_{35} mean just after processing and after 35 days of storage (after material stabilization), respectively.

Sample	RH (%)	X_c (%)	
		t_0	t_{35}
Unplasticized chitosan	33	7	18
	57	7	13
	75	7	15
Plasticized chitosan	33	12	15
	57	12	16
	75	12	9

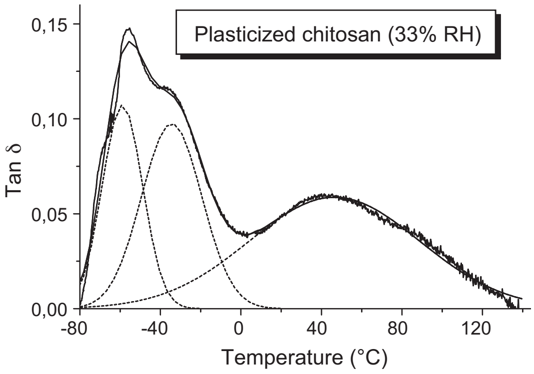

Fig. 5. Curve fitting and deconvolution of the DMTA loss tangent vs. temperature curves for plasticized chitosan after 35 days at 33% RH.

behaviors are in agreement with most of the mechanical property evolutions.

Regarding the DMTA measurements, it is worth noting that the properties of the glycerol-free samples could not be tested because of their extreme fragility. Fig. 5 shows, as a typical example, the raw experimental DMTA loss tangent curves as a function of temperature for glycerol-plasticized chitosan after 35 days of storage at 33% RH. To determine more precisely the temperatures associated to the main relaxations, curve fitting and deconvolution were performed on the tan δ plotting. Similar operations were performed on the tan δ curves obtained for plasticized samples stored at 57 and 75% RH (not shown here).

For the sake of clarity, the temperatures associated to the relaxation processes are summarized in Table 2. All samples display three main relaxations (α, β_1 and β_2).

For each RH, a first peak maximum of tan δ is observed at temperatures ranging from −70 to −55 °C. According to the values presented in Table 2, the temperature T_{β_1} associated to the secondary relaxation slightly shifts towards lower values as the water content increases (i.e. as the storage RH increases). Nevertheless, the water content dependence of this temperature is very limited and this β-relaxation is mainly attributed to chitosan chain interactions with glycerol. This is in good agreement with previously reported studies on chitosan/glycerol dried films produced by solvent casting for which the authors also identified a glycerol-induced β-relaxation with a clear dependence of T_β with the glycerol content (Quijada-Garrido, Laterza, Mazon-Arechederra, & Barrales-Rienda, 2006). This β-relaxation has been interpreted as motions of the side chains or lateral groups of chitosan interacting with glycerol by hydrogen bonding (Quijada-Garrido et al., 2006; Quijada-Garrido, Iglesias-González, Mazón-Arechederra, & Barrales-Rienda, 2007). In addition, similar T_β transition was reported for glycerol-plasticized starch elaborated by melt processing (Averous & Boquillon, 2004; Teixeira, Da Róz, Carvalho, & Curvelo, 2007).

A second maximum peak of tan δ curve is observed at temperatures (T_{β_2}) ranging from −55 to −30 °C depending on the storage RH (Table 2). This transition was attributed to water-induced β-relaxation process, and highlights the water/polymer interactions. The presence of such water-induced β-relaxation process has been previously reported for water plasticized chitosan films obtained by solvent casting (Mucha & Pawlak, 2005; Ratto, Chen, & Blumstein, 1996). Authors observed that even the presence of very low water amounts induces this relaxation. Moreover, they confirmed the water-induced nature of this relaxation by observing its disappearance during the second DMTA scan (after water removal). From the values reported in Table 2 it is obvious that T_{β_2} shows a clear and marked dependence with the water content, T_{β_2} shifting towards lower temperature when water content increases.

Table 2
Relaxation temperatures obtained from tan δ curves of plasticized chitosan stabilized for 35 days at 33, 57 or 75% RH.

Sample	RH (%)	T_{β_1} (°C)	T_{β_2} (°C)	T_α (°C)
Plasticized chitosan	33	−58	−34	46
	57	−66	−54	−1
	75	−68	−52	−13

These trends observed on T_{β_1} and T_{β_2} attest for an increase in molecular mobility of the chitosan/glycerol/water system — at temperatures below the chitosan glass transition temperature — with an increase in water content. Such behavior is in agreement with previously reported studies on different polysaccharides (Averous & Boquillon, 2004; Lazaridou & Biliaderis, 2002; Montes, Mazeau, & Cavaille, 1997; Mucha & Pawlak, 2005; Quijada-Garrido et al., 2006, 2007; Ratto et al., 1996; Teixeira et al., 2007).

Finally, a third relaxation is observed for all the samples with tan δ peaks maxima varying from −13 to 46 °C (Table 2). This characteristic temperature (T_α) corresponds to the α-relaxation process and is commonly associated to the glass transition temperature (T_g) of the chitosan. These temperatures are in good agreement with the T_α values ca. 65 °C previously reported for glycerol-plasticized chitosan films intensively dried (Quijada-Garrido et al., 2006, 2007). As seen from Table 2, the T_α values are strongly affected by the storage RH, and thus by the material water content, and clearly show the plasticizing effect of both plasticizers, glycerol and water.

The high T_α value observed for the samples stored at 33% RH is most likely due to the low water content but could also result from the slightly higher crystallinity of the sample, diminishing the mobility of the chitosan chains.

The important decrease observed on T_α with increasing water content was also reported for sorbitol-plasticized chitosan films obtained by solvent casting (Lazaridou & Biliaderis, 2002) with T_α values varying from −30 to 60 °C. At high water content (30 wt.%) the authors measured a T_α of ca. −25 °C for chitosan plasticized with 30 wt.% of sorbitol. This value is in good agreement with our results since we have measured a T_α value of −13 °C for the glycerol-plasticized chitosan stored at 75% RH, corresponding to a water content of ca. 30 wt.%. The slight discrepancy can easily be explained not only by the different nature and content of plasticizers but also by various other factors such as the degree of deacetylation and the molecular weight of chitosan or the experimental conditions used for the material preparation.

According to the values reported in Table 2, the T_α variation is more pronounced at low water content. This trend is also in perfect agreement with the behavior reported for sorbitol-plasticized chitosan films obtained by solvent casting (Lazaridou & Biliaderis, 2002). Authors stated that the polyol plasticizer efficiency is greater at low water contents, the plasticizing effect of water becoming predominant for high water contents, as it is also observed for other polysaccharides.

4. Conclusions

This paper clearly demonstrates the great potential of mechanical kneading process as an alternative way to elaborate plasticized chitosan. As it is observed for other polysaccharides, chitosan properties show a great sensitivity towards water content. Chitosan-based materials stored in different relative humidity atmospheres evidenced that the water uptake process is quite fast, around 1 day in this case. The glycerol-plasticized samples logically display the highest water content due to the highly hydrophilic character of glycerol. The addition of 25 wt.% of glycerol resulted in a decrease of the tensile strength but a significant improvement in chitosan elongation properties is shown. Ductile materials are obtained whereas the glycerol-free products are extremely brittle. The evaluation of mechanical properties vs. time has shown that these materials reach stable properties after 25–35 days of storage. The post-processing ageing of these materials is comparable of what is obtained for plasticized starch obtained by similar thermo-mechanical treatment (Avérous, 2004). Interestingly, the uniaxial tensile test results obtained on glycerol-plasticized chitosan highlighted that optimal mechanical properties are obtained for an ageing conducted with intermediate value of relative humidity, namely 57% RH. The XRD analyses have shown that all materials display a slight increase in crystallinity with time. Nevertheless, the final crystallinity extents remain lower than 20% and are not significantly influenced by the addition of glycerol which however controls the final water content.

Both tensile tests and DMTA highlighted the efficacy of glycerol for chitosan plasticization and the influence of water content. Indeed, from the $\tan\delta$ curves, two β-relaxation processes are observed on the glycerol-plasticized materials, evidencing some molecular mobility for chitosan in the glassy state. The relaxation process occurring at lower temperatures can be attributed to chain motions or lateral groups of chitosan interacting with glycerol through hydrogen bonding. It has been shown that the second β-relaxation process is a water-induced phenomenon and is attributed to the chitosan/water interactions. Finally, in addition to the enhanced elongation at break, the DMTA actually confirmed the benefit of glycerol with the α-relaxation, associated to the chitosan glass transition, reaching sub-ambient temperatures.

The present study thus demonstrates that thermo-mechanical treatment (mechanical kneading) is an efficient method to obtain plasticized chitosan-based materials displaying properties as good as those obtained by the existing wet method. More important, this route is also an attractive alternative to the traditional solvent casting method since it allows the preparation of thermoplastic-like chitosan materials using the conventional tools for polymer processing (extruder, kneader, injection molding, etc.). Such materials could be obtained in a wide variety of forms and shapes and not only as casted thin films. Moreover, this method paves the way for the elaboration of chitosan composites or blends with different thermoplastic polymers.

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