

# Characterization of Chitosan/Caseinate Films

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**ABSTRACT:** Films made from sodium caseinate and/or chitosan, using glycerol as plasticizer, were prepared by casting from aqueous solutions. Films cryo-fractured surfaces were examined by SEM. FTIR spectra of the chitosan/caseinate films showed an increase in the intensity of the amide band, corresponding to the strong interactions developed by the polyelectrolytic complexation of both components. Addition of 28 wt% of glycerol to caseinate films, conditioned at 50% relative humidity (RH), reduced the modulus from 2908 to 250.9 MPa, while the ultimate elongation increased from 4 to 63.2%. Increment to 80% RH further reduced the modulus down to 52.8 MPa. Similar results were found for the glycerol plasticization of chitosan films. The complex films showed a moderate

improvement of the tensile strength (19.6 MPa) and an increase of the impact strength (35.6 GPa) with respect to those corresponding of chitosan films (17 MPa and 26.6 GPa, respectively). These properties were markedly improved with respect to those of caseinate (6.2 MPa and 13.4 GPa, respectively). The interactions developed between the cationic polymer chitosan, and the Na-caseinate carboxyl groups, lead to polyelectrolyte complexation in forming the film, which is proposed as the reason for improvement with respect to caseinate or chitosan films. © 2007 Wiley Periodicals, Inc. *J Appl Polym Sci* 000: 000–000, 2007

**Key words:** caseinate; chitosan; glycerol; edible films

## INTRODUCTION

In recent years, the interest in the development of edible films and coatings for foods has grown due to different reasons, such as consumer pressure raised by public health concerns, food quality, convenience, increased purchasing power, and more proactive attitudes toward reducing the environmental impact of packaging wastes.<sup>1</sup> An advantage of biodegradable packaging materials is that upon biodegradation or disintegration and composting they may act as fertilizer and soil conditioner, facilitating better yield of crops. Although somewhat expensive, biopackaging is the future for packaging, especially for a few value added food products.<sup>2</sup>

Proteins show properties that are advantageous in the preparation of packaging biomaterials, for example, their ability to form networks, their plasticity, and elasticity.<sup>3</sup> Casein and casein derivatives have been extensively used in the food industry (dairy, meat, and confectionery) and in medical and pharmaceutical applications.<sup>4</sup> Casein has also been used

in paints, leather finishes, and paper coatings, as well as in the preparation of biodegradable/edible films.<sup>3</sup>

Sodium caseinate is a commercially available water-soluble polymer obtained by acid precipitation of casein, the main protein in cow milk. Caseinate presents thermoplastic and film forming properties due to its random coil nature and its ability to form weak intermolecular interactions, i.e. the partially denatured peptide chains bond together primarily through hydrophilic and hydrogen bonds resulting in the formation of the protein matrix.<sup>3,5,6</sup> Because of its renewable and biodegradable nature, edible film wraps derived from caseinate could potentially replace some conventional synthetic packaging materials used to preserve and protect foods.<sup>5,7</sup>

Some authors have studied the effects of processing variables, such as type and content of plasticizers, and storage conditions (relative humidity and temperature) on rheological, mechanical, thermal and functional properties of sodium caseinate films.<sup>1,5,7,8</sup> Other publications have been focused on the addition of different polymers such as starch,<sup>9,10</sup> whey protein isolate (WPI), soy protein isolate (SPI),<sup>6,11</sup> or gelatin<sup>12,13</sup> to caseinate films in order to improve physical, barrier, and solubility properties.<sup>6</sup>

Plasticizers are low molecular weight agents, which can be combined with the film-forming biopolymers to modify physical properties (i.e. increasing film

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flexibility and processability) or to add functionality to films.<sup>3</sup> The use of plasticizers, such as glycerol, reduces the brittleness of the film by interfering with intra- and intermolecular hydrogen bonding in the protein films.<sup>2</sup>

Another interesting film forming material is the polysaccharide chitosan, commercially available as a fine powder derived from chitin, which is obtained from waste products of the shellfish industry.<sup>14</sup> Additional importance of this material resides in its antimicrobial properties in conjunction with its cationicity.<sup>15</sup> Chitosan also possess unique functional, nutritional and biomedical properties. Some of its applications include seed coatings, film-forming and controlled release of food ingredients, nutrients and drugs.<sup>16</sup> The major mechanism of film formation in the polysaccharide films is the breaking apart of polymer segments and reforming of the polymer chain into a film matrix or gel by evaporation of a solvent, creating hydrophilic and hydrogen bonding.<sup>3</sup>

Several researchers have coated fruits and vegetables with chitosan-based films and determined their effects on gas permeability, internal gas composition, and shelf-life of these fresh commodities. These studies indicated that chitosan coatings have the potential to extend the storage life of peeled litchi,<sup>17</sup> mango,<sup>18</sup> and banana fruit,<sup>19</sup> as they inhibited fungi growth, reduce ethylene production, increased internal CO<sub>2</sub> and decreased O<sub>2</sub> levels.

Several publications have reported studies on films based on chitosan such as chitosan-starch,<sup>14,16,20</sup> chitosan-pullulan,<sup>14</sup> chitosan-gelatin,<sup>15,21-24</sup> chitosan-PVA,<sup>25</sup> and chitosan-amylose starch<sup>26</sup> but, to our knowledge, reports on chitosan-caseinate or chitosan-casein films are rarely found.<sup>27</sup> In the production of these films, advantage can be taken from chitosan acting as a cationic polymer that can establish electronic interactions through the NH<sub>3</sub><sup>+</sup> groups with anionic groups, such as COO<sup>-</sup> groups, present in proteins or polyanions. These interactions are responsible for the formation of polymeric complexes.<sup>28,29</sup>

The objective of this work is to manufacture and characterize edible films made from sodium caseinate (NaCas) and chitosan (Chit), to take advantage of the availability of casein and the antimicrobial properties of chitosan. Glycerol was added as plasticizer to obtain flexible films that could be folded and manipulated without breakage. As in the case of water, glycerol competes for H bonding and forms electrostatic interactions with the caseinate chains, reducing the protein intermolecular interactions.<sup>7</sup> The characterization of a combined chitosan/sodium caseinate film and the effects of glycerol (Gly) concentration and moisture content on the mechanical and thermal properties of the films are presented.

## EXPERIMENTAL

### Materials

Sodium caseinate powder (NaCas), composed of 88.9 wt % of protein (Pro), the rest being lactose, lipids, attached moisture and ashes, was obtained from Lactoprot Deutschland GMBH, Germany. Chitosan (deacetylation degree 90%) was supplied by ACOFAR, Mar del Plata, Argentina. Glycerol was purchased from DEM Chemicals, Mar del Plata, Argentina.

### Methods and techniques

#### Film preparation

The procedure selected for the preparation of the films, solution casting, is similar to that used by other researchers.<sup>30,31</sup> NaCas solutions with protein concentrations of Pro—2.5% (w/v), were prepared by dispersing NaCas powder (2.81 g) in distilled water up to 100 mL, and stirring continuously during 3 h, at room temperature. Appropriate amounts of glycerol were added to achieve glycerol/protein (Gly/Pro) weight ratios of 0, 0.15, 0.28, and 0.5.

Chitosan solutions (2%, w/v)<sup>14</sup> were prepared by dispersing chitosan powder in acetic acid solution (1%, v/v). Different glycerol contents were added to achieve glycerol/chitosan (Gly/Chit) weight ratios of 0, 0.15, 0.28, and 0.5.

Samples prepared from NaCas/ Gly resulted in odorless and transparent films, while plasticized chitosan films were also odorless but presented a yellow hue.

Chitosan/NaCas composite films at a weight ratio of Chit/Pro = 0.8/1 were prepared by mixing 100 mL of the 2% (w/v) chitosan solution with 100 mL of 2.5% (w/v) sodium caseinate. These films were prepared with and without glycerol addition. In the case of films containing plasticizer, glycerol was added to achieve Gly/(Pro + Chit) weight ratio of 0.28, which represents the 20.8% of the total weight after water evaporation. Preparation of the polyelectrolyte complex between caseinate and chitosan required a careful control of the pH of the solutions. Sodium caseinate is remarkably heat-stable at pH = 6.5<sup>32</sup> and highly insoluble at the isoelectric point, pH between 3.8 and 4.0.<sup>33</sup> Chitosan shows best solubility in a 1% (v/v) acetic acid solution (pH = 4.4), but remains soluble at pH lower than 6.4. In this work the mixed solution had a pH of 5.0, and no phase separation was observed. Since chitosan is a cationic biopolymer, while caseinate acts as a macroanion at this pH, a complexation of the two polymers was expected as a result of their intimate mixture and conditions of film preparation.<sup>21</sup>

Once a solution was prepared, it was poured into Teflon-Petri dishes (diameter 14 cm) and dried at a

constant temperature for ~10 h in a convection oven, obtaining films with thicknesses between 50 and 90  $\mu\text{m}$ . The films were peeled off from the plates after the drying step and kept in a closed reservoir with controlled atmosphere for 5 days at  $23^\circ\text{C} \pm 2^\circ\text{C}$ . Three different relative humidity (RH) (40, 50, and 80%) conditions were used for conditioning the film prepared from the 2.5% (w/v) sodium caseinate solution and a Gly/Pro ratio of 0.28. The rest of the films were conditioned only at 50% RH.

#### Infrared spectroscopy characterization

FTIR spectra of the films were recorded by the attenuated total reflection method (ATR) using a Genesis II (Mattson) Fourier transform infrared spectrometer. Films used in the infrared tests were about 50–90  $\mu\text{m}$  thick. On the other hand, powder samples were mixed with dried KBr and prepared as pellets to be examined using transmission FTIR. In both cases, the spectra were recorded over a range of 600–4000  $\text{cm}^{-1}$  with a resolution of 2  $\text{cm}^{-1}$  and averaged over 32 scans.

#### Scanning electronic microscopy

The fracture surfaces of the films obtained after immersing them in liquid air (fragile fracture), as well as fracture surfaces obtained after tensile testing (ductile fracture) were observed using a scanning electron microscope (JEOL, model JSM-6460 LV). For this purpose, the pieces of the films were mounted on bronze stubs using a double-sided tape and then coated with gold, before being observed under the microscope.

#### Mechanical tests

A detailed analysis of the mechanical response of materials can be effectively used in the elucidation of the structure–property relationship of polymers.<sup>34</sup> Accordingly, in the present paper, tensile and impact testing was selected to evaluate the mechanical performance of the films, as follows.

**Tensile tests.** Film thickness was measured using a 0–25 mm manual micrometer, with a resolution of 0.01 mm. The reported values are the average of 10 readings taken randomly on each film sample.

Tensile tests were performed at room temperature ( $23^\circ\text{C} \pm 2^\circ\text{C}$ ) using an Instron Universal Testing Machine model 8501. The bone shape specimens were cut from the films using a scalpel, according to the ASTM D1708-93.<sup>35</sup> A minimum of three films for each composition were prepared and five specimens from each film were tested.

The film samples were clamped into the metal grips for tensile testing and stretched at an overhead

crosshead speed of 10 mm/min. The ultimate strength ( $\sigma_b$ ), elongation at break ( $\epsilon_b$ ) and elastic modulus ( $E$ ) were calculated as described in ASTM D638-94b.<sup>36</sup>

**Fracture puncture tests.** Instrumented falling weight impact (IFWI) tests were carried out using a falling weight impact tower (Fractovis, Ceast). Samples with diameters of 14 cm, clamped on a supporting ring of 75-mm diameter, were impacted with a hemispherical tipped dart at an incident speed of 1 m/s, using an impactor mass of 7.5 kg. The force-deformation curves were recorded during 16 ms. At least five specimens for each material were impacted.

The IFWI technique is widely accepted for the out of plane fracture response assessment of plastics and composites.<sup>37–39</sup> This method offers a quite truthful view of the material response against impact loads parallel to the thickness direction.

From the IFWI fractograms the following parameters were determined:

- The maximum strength ( $\sigma_d$ ), which is related to crack initiation:

$$\sigma_d = \frac{2.5 \cdot F_{\max}}{t^2} \quad (1)$$

where  $t$  is the sample thickness and  $F_{\max}$  is the maximum force applied during the test; and

- The energy at crack initiation ( $E_i$ ) normalized by thickness:

$$E_i = \frac{1}{t} \cdot \int_0^{X_{\max}} FdX \quad (2)$$

where  $X$  is the strain reached during the deformation of the material up to rupture.

#### Thermogravimetric analysis

Thermogravimetric measurements were carried out on a Shimadzu TGA- 50 thermogravimetric analyzer. Thermal degradation was performed under nitrogen atmosphere, from 25 to  $500^\circ\text{C}$ , using a heating ramp of  $5^\circ\text{C min}^{-1}$ . Samples of 4–10 mg were used for the thermogravimetric (TG) measurements.

## RESULTS AND DISCUSSION

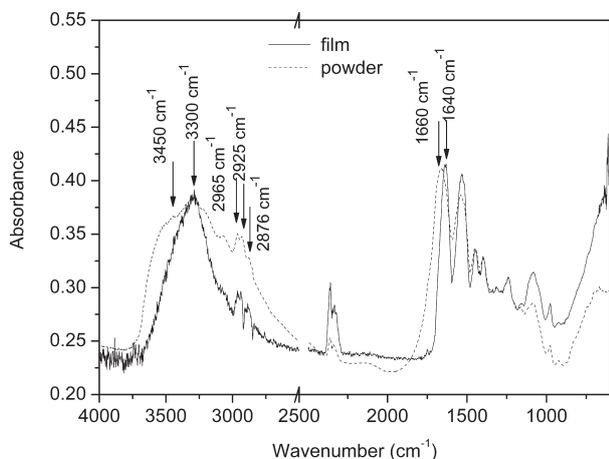
### Infrared spectroscopy

#### Caseinate films

FTIR spectrum of the materials and films was performed and analyzed. Figure 1 shows the FTIR spectrum corresponding to the NaCas powder and to the NaCas film prepared without additives.

The spectrum of NaCas powder shows a wider NH region, which covers the  $3400\text{--}3520 \text{ cm}^{-1}$ , indi-

F1



**Figure 1** FTIR spectra of NaCas film (without glycerol) and powder.

Both spectra show also bands in the 1600–1500  $\text{cm}^{-1}$  region corresponding to the amide I and amide II bands, common to proteins. There is a clear shift on the amide I band from 1660  $\text{cm}^{-1}$  for the caseinate powder to 1640  $\text{cm}^{-1}$  for the film, which may be related to changes ascribed to  $\text{NH}\cdots\text{O}$  hydrogen bonds. An example related to the shift of this band due to conformational changes has been discussed in the literature for poly-L-glutamate benzyl ester (from 1658 to 1630  $\text{cm}^{-1}$ ).<sup>40</sup>

FTIR spectra of glycerol modified films (not shown) show essentially the contributions of the individual spectra.

#### Chitosan Powder and Films

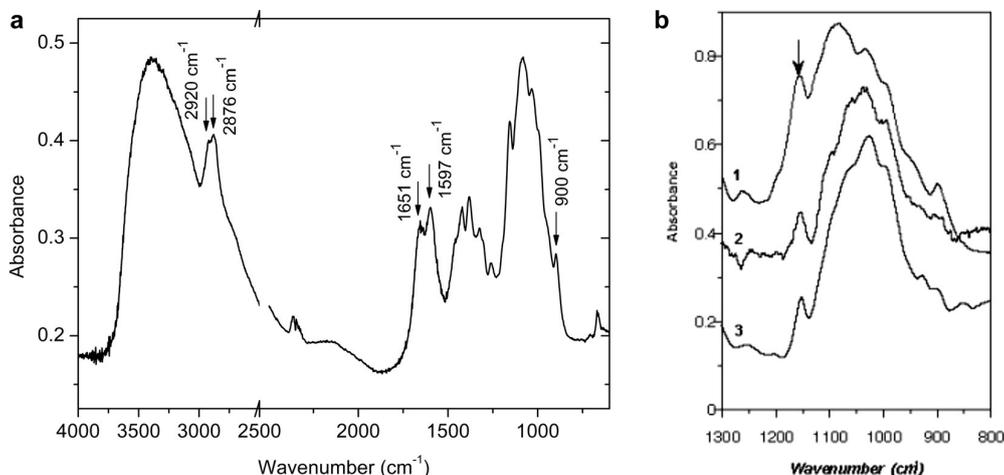
Figure 2(a) shows the FTIR curve corresponding to chitosan powder. Chitosan is an amino glucose polymer, containing a proportion of amide groups linked to acetic acid, which were not eliminated during the deacetylation of the mother chitin. The powder shows a broad band centered on 3400  $\text{cm}^{-1}$  assigned to OH hydrogen bonded with contribution of the NH absorption. No evidence of free OH bond stretch (3600  $\text{cm}^{-1}$ ) appears in this spectrum. The peaks at 2920 and 2876  $\text{cm}^{-1}$  correspond to  $\text{CH}_2$  and CH (tertiary), respectively. The band between 1550 and 1690  $\text{cm}^{-1}$  is indicative of the presence of amide groups, which remain from the incomplete deacetylation (in particular the 1651  $\text{cm}^{-1}$  corresponds to the  $\text{C}=\text{O}$  of the amide band).<sup>25,41</sup> It is also superposed to the absorption of  $\text{NH}_2$  of the amino groups (1597  $\text{cm}^{-1}$ ). The 1155–1025  $\text{cm}^{-1}$  band is the result of the C–O stretch and OH deformation vibrations of the primary and secondary alcohol groups in the chitosan

F2

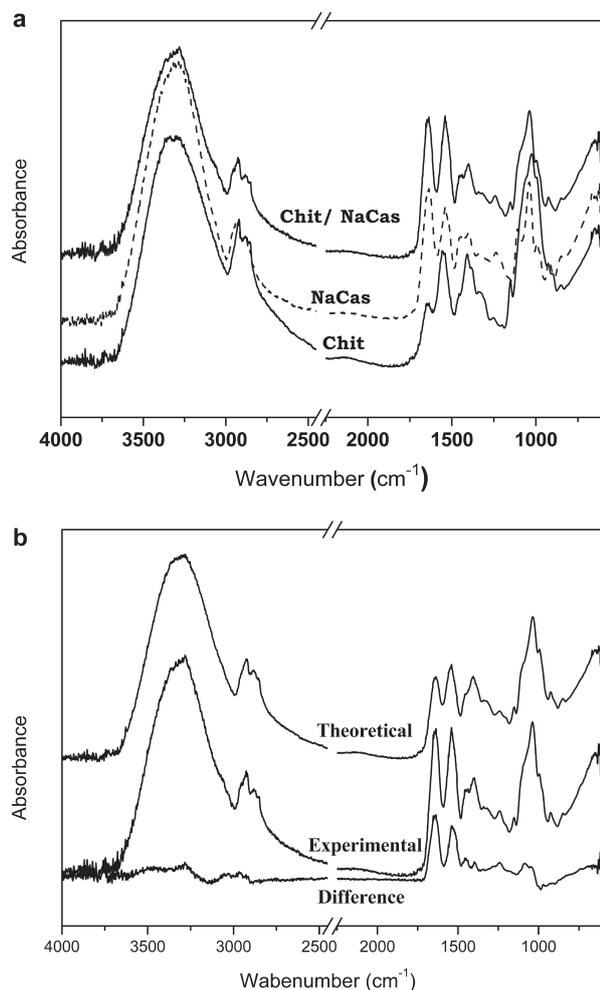
cating the presence of unbonded  $\text{NH}_2$  groups and suggesting that it is not possible for all the terminal  $\text{NH}_2$  groups to form hydrogen bonds while in the powder condition. Actually, the band around 3450  $\text{cm}^{-1}$  appears in isolated peptide chains,<sup>32</sup> and has been assigned to unbonded NH groups. The band centered around 3300  $\text{cm}^{-1}$  has been associated to intermolecular H bonds, although most probable, it is originated by intra and intermolecular H bonds. A small band at 3080  $\text{cm}^{-1}$  is also present which has been assigned to the NH stretch, probably an overtone of the amide II absorption.<sup>40</sup>

AQ3

The CH stretch region shows peaks corresponding to  $\text{CH}_2$  and  $\text{CH}_3$  (2925 and 2965  $\text{cm}^{-1}$ , respectively), with a very small peak coming from CH (tertiary) at 2876  $\text{cm}^{-1}$ .



**Figure 2** FTIR spectrum of (a) chitosan powder; (b) chitosan (1300–800  $\text{cm}^{-1}$  region): (1) Powder, (2) Film prepared without glycerol; (3) Film prepared with 28% glycerol weight ratio.



**Figure 3** FTIR spectra of (a) NaCas, chitosan and Chit/NaCas films prepared with 28% glycerol weight ratio; (b) Chit/NaCas films: theoretical, experimental and difference (experimental-theoretical).

molecules. The small peak at  $900\text{ cm}^{-1}$  corresponds to the  $\beta$  linkage of the glucoside rings.

Figure 2(b) shows the  $1300\text{--}800\text{ cm}^{-1}$  region for the chitosan powder and the films formulated without and with added glycerol. The bands due to glycerol contribute to the absorption in the region, with the peak at  $1150\text{ cm}^{-1}$  remaining invariant in the spectra. This will be useful in the analysis of the interactions appearing in the chitosan-caseinate films.

Chitosan/caseinate films prepared with glycerol as plasticizer

F3 Figure 3(a) shows the spectra of caseinate, chitosan and the Chit/NaCas films (28% glycerol weight ratio). To better illustrate the development of specific

interactions formed by complexation of the two biopolymers in the mixture Chit/NaCas, a theoretically calculated FTIR spectrum was obtained from the weighted sum of the experimental spectra of the individual films. The FTIR spectra of the Chit/NaCas film, experimental, calculated and difference (experimental-calculated), are shown in Figure 3(b). The calculated spectrum superposes very well with the experimental one (see the good agreement in the  $3000\text{ cm}^{-1}$  region corresponding to the CH stretch), with the exception of the amide region. A higher intensity in the  $1640$  and  $1550\text{ cm}^{-1}$  bands, assigned to the amide group, is observed in the experimental spectra with respect to the calculated one. This effect is indicative of the interaction of the amino groups of the chitosan with the negatively charged carboxyl groups of the caseinate in the complex film.

### Morphology of the films

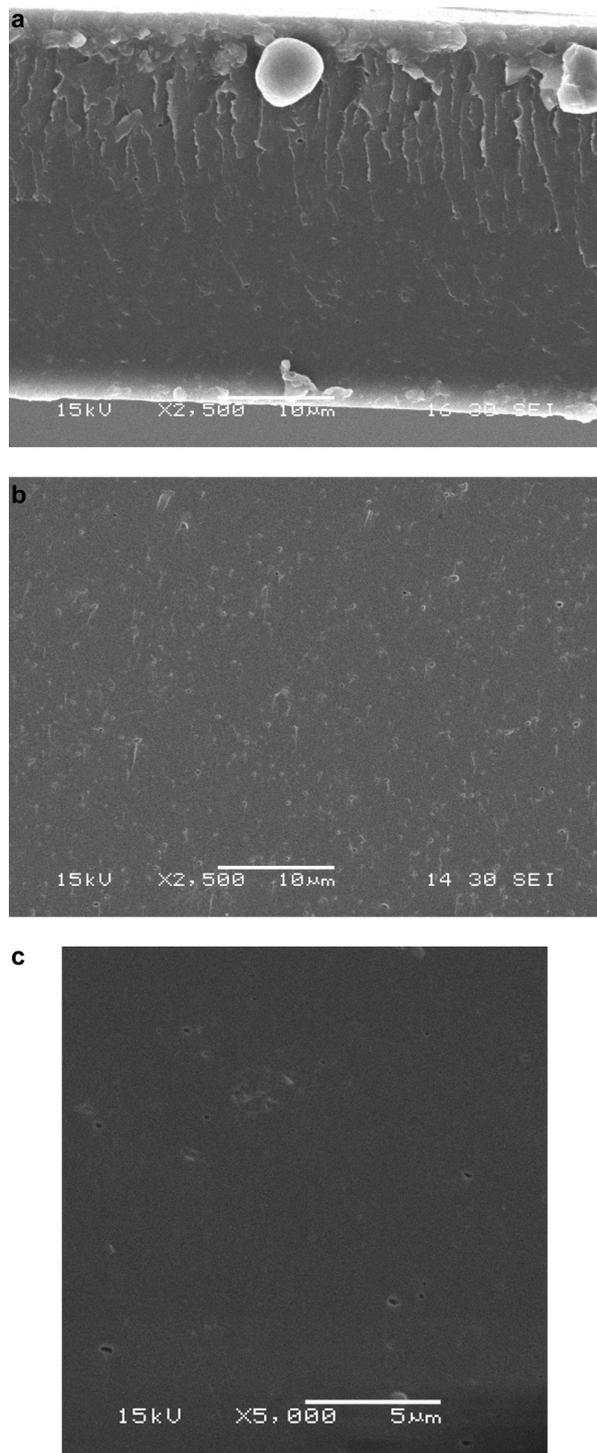
The surfaces of the films fractured at low temperature, were investigated to analyze the morphology of the undeformed materials. Figure 4(a,b) show fracture surfaces of caseinate films produced without and with glycerol, respectively. Some very small holes are observed in the interior of the films, having sizes in the order of the  $200\text{ nm}$ . These holes are not related to the addition of glycerol, since they also appear in the nonplasticized caseinate film with equal sizes and aspect. Instead, they may have been formed and trapped during the drying step by elimination of the water solvent. Apart from these features the micrographs obtained at these low temperatures show a featureless surface proper of undeformed homogeneous materials.

The low temperature fracture surface of Chit/NaCas films containing glycerol was also analyzed [Fig. 4(c)]. As expected from a film formed by complexation of the two polymers, there was not phase separation. As in the case of caseinate films, small holes can be visualized in the interior of the Chit/NaCas film.

### Mechanical properties

#### Caseinate films

F5 Figure 5(a) shows representative curves of the nominal tensile stress vs. deformation measured in NaCas films containing different concentrations of glycerol as plasticizer. At glycerol weight ratios of 0 and 15% the behavior corresponds to that of a relatively brittle material that cracks easily and breaks at deformations around 4 and 13%, respectively. This behavior is important for the envisioned application of the films, since at these glycerol concentrations the films cannot be folded without cracking. The most noticea-



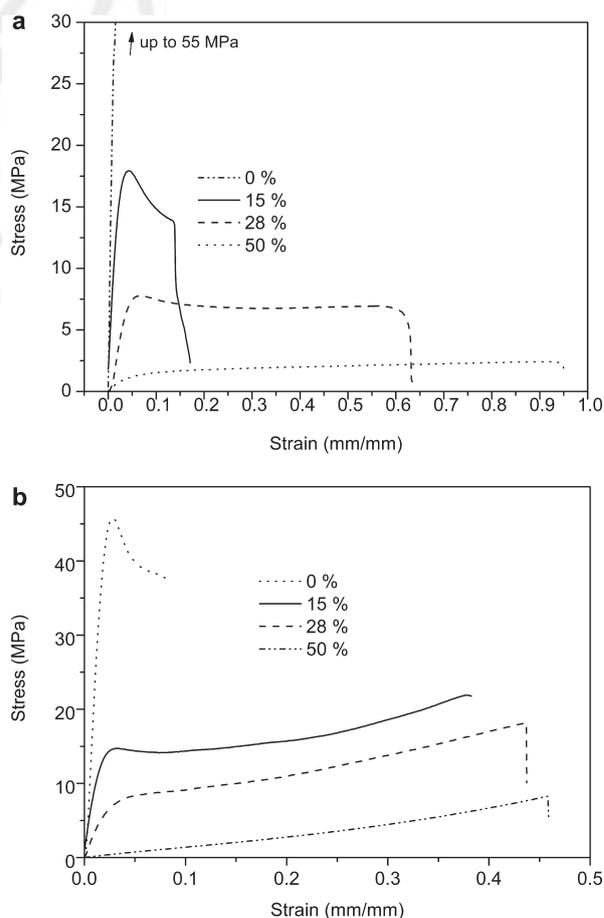
**Figure 4** SEM micrographs of cryo-fractured surfaces of (a) NaCas film without glycerol; (b) NaCas with glycerol; (c) Chit/NaCas film with glycerol.

ble change in tensile behavior occurs when changing the glycerol weight ratio from 15 to 28%. The sample containing 15% glycerol weight ratio shows macro-

scopic whitening after the yield point (development of microcracks that disperse light, producing the whitening effect in the film), in the region of stress concentration, until the sample finally breaks as it is further extended. The corresponding stress-strain curve shows a maximum stress after which the stress drops until rupture. At 28% weight ratio of glycerol it is still possible to identify a yield stress, but the 50% glycerol curve shows no clear yield stress and no stress hardening. Clearly, the addition of glycerol reduces modulus and maximum stress in tensile tests, while it largely improves the ultimate deformation of the films. The samples are less rigid and much more flexible by addition of glycerol. Table I summarizes the results obtained in the tensile testing of these samples.

T1

When comparing the results from mechanical tests it should be kept in mind that all samples were conditioned at 50% RH before testing, but because of the different glycerol content, the equilibrium moisture content of the films is also different. Glycerol is



**Figure 5** Stress-strain curves of films formulated with different glycerol content, conditioned at 23°C and 50% RH. (a) NaCas films; (b) Chitosan films.

**TABLE I**  
**Tensile Properties of NaCas, Conditioned at Different RH Ambient, and Chit Films Formulated with Different Glycerol Content**

Film	Weight ratio	RH (%)	$E$ (MPa)	$\sigma_b$ (MPa)	$\epsilon_b$ (%)
NaCas	Gly/Pro (%)				
	0	50	2908 $\pm$ 121.9	57 $\pm$ 0.3	4 $\pm$ 0.46
	15		802.8 $\pm$ 63.2	13.6 $\pm$ 1.3	11.6 $\pm$ 5.6
	28		250.9 $\pm$ 20.3	6.2 $\pm$ 0.2	63.2 $\pm$ 6
	50		32.5 $\pm$ 4.6	2.4 $\pm$ 0.2	91.6 $\pm$ 15.2
	28	40	315.4 $\pm$ 30.8	7.4 $\pm$ 0.5	60.7 $\pm$ 9.8
Chit	Gly/chit (%)				
	0	50	2386.7 $\pm$ 53.8	43.2 $\pm$ 1.5	2.7 $\pm$ 0.1
	15		819.2 $\pm$ 53.6	23.4 $\pm$ 2.1	39.5 $\pm$ 2.1
	28		229.8 $\pm$ 56.1	17.3 $\pm$ 2.8	44.2 $\pm$ 7.9
	50		13.8 $\pm$ 0.3	8.3 $\pm$ 0.9	45 $\pm$ 2.6

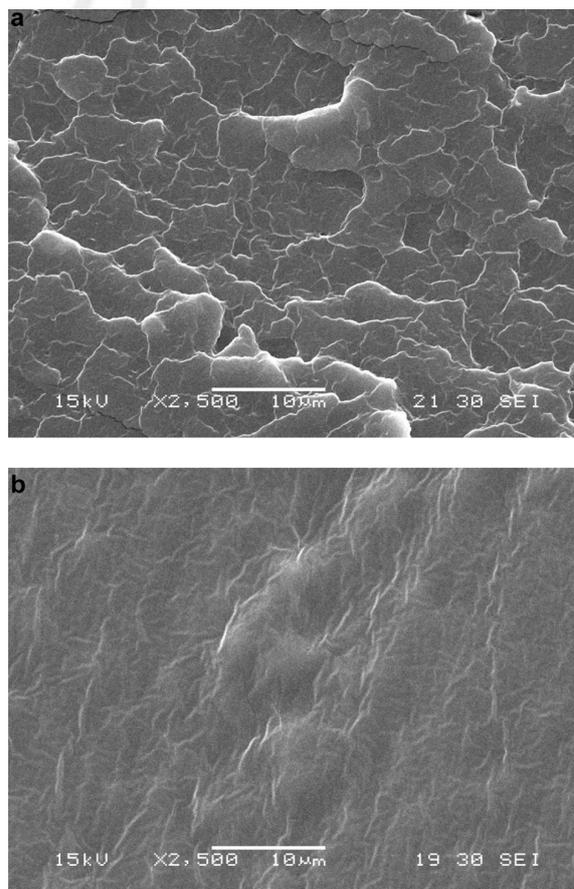
highly hygroscopic and so samples containing more glycerol also absorb more water. For this reason, the plasticizing effect of the additive is enhanced, and the material becomes more flexible and stretchable, at increasing glycerol contents. Other authors have reported this strong effect and have prepared caseinate films with as low as 8% glycerol weight ratio. Their findings indicated that these films were very delicate and cracked during manipulation.<sup>7</sup> However, these problems disappeared at glycerol weight ratios above 15%.

Table I also reports the tensile performance of NaCas films (containing 28% glycerol weight ratio) conditioned in environments with different % RH. The influence of the moisture in the tensile modulus and stress at break is clearly noticeable, they decreased when RH increased. The elongation at break increases as moisture content increases, thus films became more flexible and stretchable. This behavior is the result of water plasticization. Water molecules contain hydroxyl groups that interact with protein through hydrogen bonding, affecting the mechanical behavior of the films and leading to materials with a high molecular lubricity.<sup>5</sup>

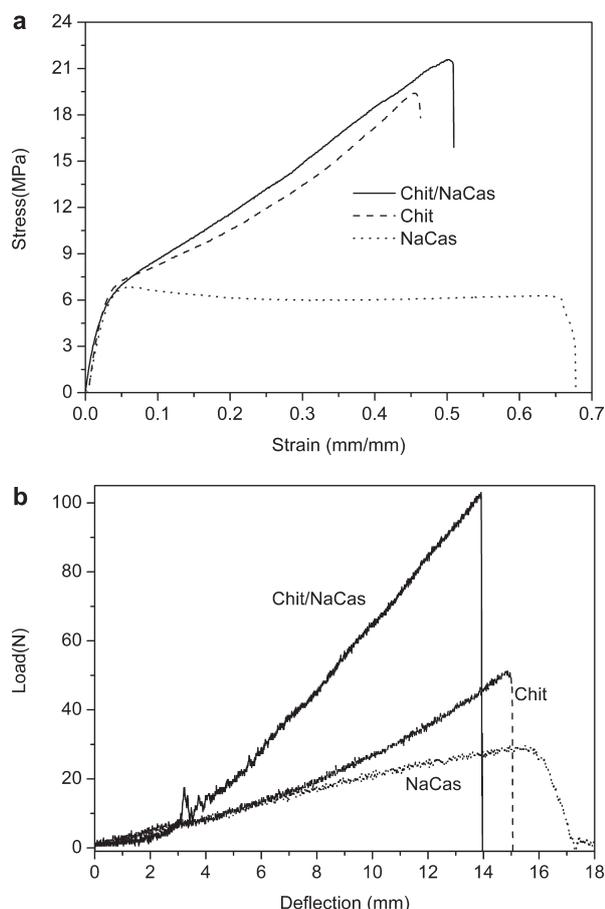
It is also interesting to analyze the tensile fracture surfaces of the films (at room temperature), by SEM. In this case, and as it could be expected, the appearance of the fracture surfaces is strongly affected by the humidity content of the samples. Thus, Figure 6(a,b) shows the fracture surface of glycerol plasticized caseinate films conditioned at 50 and 80% RH and tested under tensile loads up to breakage. Figure 6(b) shows that the planes of fracture are smoothed when samples were conditioned at higher RH. Surface fracture discontinuities are sharper in the low humidity sample, but they become smooth under the plastifying action of moisture in the material stored at 80% RH, due to the rapid relaxation of the material.

#### Chitosan films

The effect of glycerol content on films prepared from chitosan was also investigated. Figure 5(b) shows that the effect of the plasticizer addition on the



**Figure 6** SEM micrographs of tensile fractured surface of NaCas films containing glycerol. (a) Conditioned at 50% RH; (b) Conditioned at 80% RH.



**Figure 7** Mechanical behavior of NaCas, Chit, and Chit/NaCas films, prepared with 28% glycerol weight ratio and conditioned at 23°C and 50% RH. (a) Tensile and; (b) Impact behavior.

tensile behavior of Chit films is very similar to that observed for NaCas films. The rigidity of the films (elastic modulus) and yield stress decrease, while the ultimate strain increases with the glycerol weight rate up to 50%. The curves also show a clear yield point up to 15% glycerol weight ratio. It is to be noticed that Chit films seem to be more sensitive to the addition of the plasticizer than caseinate ones. The Chit film prepared with 15% weight ratio of glycerol reaches a maximum deformation (under tensile loads) of about 40%, while caseinate films

break at around 13% strain. Besides, Chit films present strain hardening up to the point of rupture (except at high glycerol weight rate), a feature that was not observed for the NaCas films. Table I also summarizes the tensile mechanical properties measured for these films.

#### Chitosan caseinate films

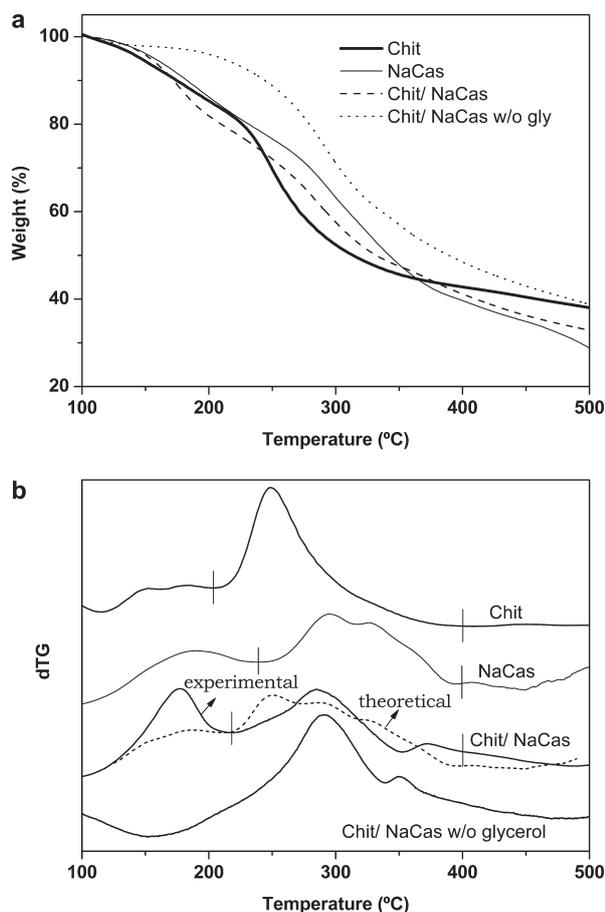
Figure 7(a) shows the comparative tensile behavior of NaCas, Chit films and the Chit/NaCas complex film. The combined material shows improved strength with respect to the simple films. It is quite interesting to notice that the behavior is not some average of the individual performances, but that the complexation contributes a synergistic effect that improves the behavior with respect to those of the individual components. This is the result of the anionic-cationic interactions that can be established between these two macromolecules, which result in the increase of the material strength. The Chit/NaCas film shows strain hardening as it did the Chit film, with a slight increment in extensibility, 8%, with respect to that of the Chit films, but decreased 24% with respect to NaCas films. It still reaches a 50% ultimate strain, which allows easy manipulation and folding without cracking. Table II summarizes the results of mechanical properties. The values of the elastic moduli are very similar for NaCas and Chit films, and as a result the complex film shows an almost identical value (considering the experimental errors). Studies on other complex films, gelatin/chitosan, have indicated that these films offer intermediate properties to those of the pure films.<sup>15</sup> In the present case, the interesting result is that the strength of the complex film is not a weighed average of the strengths of the individual films, but shows a tensile behavior comparable or slightly better than that of pure chitosan.

#### Puncture fracture tests

The desired property of a food packaging material depends on the application. In general, a food packaging may be an undeformable material to provide structural integrity or reinforce food structure, or on

**TABLE II**  
Tensile and Puncture Properties of NaCas, Chit, and Chit/NaCas Films

	Tensile properties			Puncture properties	
	$E$ (MPa)	$\sigma_b$ (MPa)	$\epsilon_b$ (%)	$\sigma_d$ (GPa)	$E_i \times 10^3$ (J/mm)
Chit	229.8 ± 56.1	17.3 ± 2.8	44.2 ± 7.8	26.6 ± 3.5	4.6 ± 0.7
Chit/NaCas	223.6 ± 63.8	19.6 ± 2.8	47.9 ± 5.7	35.6 ± 5.3	6.7 ± 0.6
NaCas	250.9 ± 20.3	6.2 ± 0.2	63.2 ± 6	13.4 ± 2.4	3.1 ± 0.2



**Figure 8** Thermograms of pure Chit, NaCas, and Chit/NaCas complex films. Glycerol percent weight ratio = 28%. (a) TG curves (b) dTG curves, shifted vertically for better visualization.

the contrary, a highly deformable film for applications such as wrapping foods.<sup>42</sup>

Figure 7(b) shows the IFWI fractograms for Chit, NaCas and Chit/NaCas films prepared with 28% glycerol weight ratio and conditioned at 50% RH. It is clear even from these raw data, the large improvement obtained by mixing the two polyanionic components. Table II shows the calculated values obtained from those fractograms. Chit/NaCas films show higher puncture strength ( $\sigma_d$ ) and energy at crack initiation ( $E_i$ ) than NaCas or Chit films. These results are in agreement with the improved tensile properties observed for the complex films, and constitute an important advantage for using this material over any of the individual film components.

### Thermogravimetric analysis

The thermal stability of the films was evaluated by TG analysis. Figure 8 shows TG [Fig. 8(a)] and derivative TG [dTG, Fig. 8(b)] curves obtained for pure

Chit, pure NaCas, and the Chit/NaCas complex films prepared without plasticizer and with 28% glycerol weight ratio. The three plasticized systems showed similar behavior with two main stages of weight loss. Water loss occurs below 105°C approximately in the three cases, so the experimental weight losses were recalculated on a dry basis (after water evaporation) and are presented in Figure 8. The first region in the Figure 8 (above 105°C and up to 200–240°C, depending on the specific sample considered) was related to the loss of the glycerol. This is clearly illustrated by comparison of the thermograms (derivative signal and weight loss) of the unplasticized and glycerol-containing complex film. The unplasticized film shows only the degradation region above 200°C.

The higher temperature degradation region (in each thermogram) is associated directly to the thermal degradation of the sodium caseinate and/or the chitosan.

Table III shows the temperature interval and the corresponding weight loss of Region I and Region II, as indicated in the Figure 8. The results from Region I indicate that the calculated values are in good correspondence with the glycerol content of the samples (thus glycerol is being eliminated in this region), at least in NaCas and Chit/NaCas films. In chitosan films the measured value (15%) is lower than the nominal glycerol content, suggesting that chitosan retains the plasticizer more strongly. Table III also summarizes the temperature position of the maximum of the degradation peaks ( $T_d$ ) for the investigated films in both regions, and the residual weight loss (char) measured at 500°C. The temperatures were determined from the dTG curves [Fig. 8(b)]. From the comparison of the curves, the degradation of the chitosan (Region II) appears shifted towards higher temperatures when complexed with NaCas, as a result of the strong interactions developed between the two components (already discussed in the analysis of the FTIR results) and that apparently improves the thermal resistance of the chitosan.

Figure 8(b) also shows the theoretical dTG curve for the degradation of the complex film, calculated from the weighed dTG curves of the individual components (NaCas and Chit). As it can be observed, both curves differ significantly as a result of the specific interaction that take place between the two main components of the films. The experimental results show that glycerol is easily eliminated in Region I (Table III), probably because of the unions formed between caseinate and chitosan, and reduce the number of groups available for interaction with the glycerol. In particular, the percentage of mass lost in Region I in the chitosan film add up only to 15%, which is lower than the nominal glycerol content, suggesting that chitosan retains the glycerol

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**TABLE III**  
**Temperature Intervals, Weight Loss and  $T_d$  of Region I and II; and Residual Weight Loss at 500°C, for Chit, NaCas, and Chit/NaCas Films, all Containing 28% Glycerol Weight Ratio**

Thermogravimetric parameters		Chit	NaCas	Chit/NaCas
Region I	Temp. interval	(105–204)°C	(105–239)°C	(105–218)°C
	$T_d$ (°C)	152/182,5	190	177
	% weight loss	15.43	21.32	21.51
Region II	Temp interval	(204–400)°C	(239–400)°C	(218–400)°C
	$T_d$ (°C)	249	295/326	285
	% weight loss	41.78	39.01	37.3
Residual char 500°C (%)		38.05	28.84	32.85

more strongly, due to the high concentration of OH groups in the polysaccharide. Further analysis shows that the degradation in the Region II appears at higher temperatures in the experimental curve than in the calculated one, indicating that the material is more resistant to thermal degradation than theoretically expected. This is explained by the specific interactions developed between the amino groups of the chitosan and the carboxyl groups of the caseinate in the complex film.

## CONCLUSIONS

NaCas, Chit and Chit/NaCas films, plasticized with glycerol, were successfully prepared and characterized in terms of mechanical properties.

The spectra of the complex film show intensities higher than the expected at 1640 and 1550  $\text{cm}^{-1}$ , in the bands associated to the amide groups, as a result of the formation of a polyelectrolyte complex between the cationic chitosan ( $\text{NH}_3^+$  groups) and the anionic caseinate (carboxylic groups), generating a strong interaction between two main components.

The mechanical properties, both in chitosan and caseinate films, are affected by glycerol and moisture content, that act as plasticizers, thus reducing modulus and strength, but increasing deformability of the films. Caseinate films, conditioned at 50% relative humidity (RH), showed a modulus reduction from 2908 to 250.9 MPa by addition of 28 wt% of glycerol (plasticizer), while the ultimate elongation increased from 4 to 63.2% elongation. Increment to 80%RH introduced a further modulus reduction down to 52.8 MPa. A similar response was found for the glycerol plasticization of chitosan films.

Additionally, the Chit/ NaCas film showed a moderate improvement of the tensile strength (19.6 MPa) and an important increase of the impact strength (35.6 GPa) with respect to the corresponding properties of the pure chitosan films (17 MPa and 26.6 GPa, respectively). These properties were markedly improved with respect to those of caseinate (6.2 MPa and 13.4 GPa, respectively). The complexation established between the two macromole-

cules is proposed as the reason for this improvement.

Chemical (FTIR), mechanical (tensile and fracture puncture tests) and thermogravimetric (TG) characterization of the complex Chit/ NaCas films were all coincident at indicating a strong interaction developed between the caseinate and the chitosan chains, which was ascribed to the polyelectrolyte complexation of the two main components of the combined film.

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