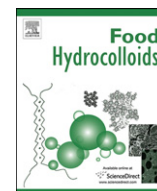


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# Food Hydrocolloids

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## Effect of glycerol and corn oil on physicochemical properties of polysaccharide films – A comparative study

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### ABSTRACT

The aim of this work was to evaluate the influence of glycerol and corn oil on physicochemical properties of polysaccharide-based films. The polysaccharides used were galactomannan from *Gleditsia triacanthos* and chitosan. Fourier-transform infrared spectroscopy, differential scanning calorimetry and thermogravimetric analysis were performed, together with determinations of moisture content, solubility, water vapor permeability and mechanical properties. Structure–properties relationships were established, relating the two polysaccharides' structures with the way they interact with water, other film's constituents (glycerol and oil) and the resulting properties. The presence of glycerol and corn oil originated a more hydrophilic structure and a decreased affinity of the film matrix to water, respectively, in both polysaccharides. However, the two polysaccharides presented different behaviors in terms of glass transition temperature, water vapor permeability and elongation-at-break that have been related with the particularities of their structure: while for the galactomannan the specific sorption sites for water are the O–H groups, for chitosan those are O–H and/or NH<sub>2</sub> groups.

The present work provides insight regarding the physicochemical properties of polysaccharide-based films and established relationships with polymers' structure, showing that the two polysaccharides studied here have adequate properties to be used as packaging materials for specific food applications.

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### 1. Introduction

In the most recent years, industries have been joining efforts to reduce the amount of synthetic materials used. Therefore research on new materials from renewable resources to be used in textile, pharmaceutical, biomedical, cosmetics and food industries has progressed in order to decrease the problem of plastic waste disposal due to lack of biodegradability (Prashanth & Tharanathan, 2007; Siracusa, Rocculi, Romani, & Rosa, 2008). In this context edible films based on polysaccharides and/or proteins appear as potential substitutes of synthetic packaging, thus playing a decisive role in the improvement of the shelf-life of food through controlling gas transfer and being carriers of functional compounds (Srinivasa, Ramesh, & Tharanathan, 2007).

Polysaccharides are natural polymers composed of monosaccharide residues that are connected by O-glycosidic linkages and, depending on their source, they can be neutral, positively or negatively charged. They either act as energy reserve in plants and animals, or have structural roles in plant cell walls or in the tough

outer skeleton of insects and other animals (Nelson & Cox, 2000). The great diversity of structural features of polysaccharides have origin from differences in the monosaccharide composition, linkage types and patterns, chain shapes, and degree of polymerization, influencing their physicochemical properties.

Galactomannans are present in the endosperm of numerous plants, and they have several functions, e.g. as a reserve of carbohydrates. Galactomannans are polysaccharides built up of a  $\beta$ -(1–4)-D-mannan backbone with single D-galactose branches linked  $\alpha$ -(1–6) (Kök, Hill, & Mitchell, 1999). Galactomannans can often be used in different forms for human consumption. Featuring different physicochemical properties, galactomannans are a versatile material used for many applications: they are excellent stiffeners and stabilizers of emulsions, and the absence of toxicity allows their use in the textile, pharmaceutical, biomedical, cosmetics and food industries (Srivastava & Kapoor, 2005). *Gleditsia triacanthos* belongs to the family *Leguminosae* and grows in America, Middle Europe and Mediterranean area (Üner & Altınkurt, 2004). Seeds of *G. triacanthos* were used as source of the galactomannan used as raw material for edible films production.

Chitosan is a natural polymer derived by deacetylation of chitin, the second most abundant biopolymer in nature after cellulose

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(Shahidi, Arachchi, & Jeon, 1999). When compared with other polysaccharides, chitosan has several important advantages such as biocompatibility, biodegradability and no toxicity; several studies indicated chitosan as bacteriostatic and fungistatic (Yi et al., 2005). The polycationic properties of chitosan provide the possibility of film formation by the breakage of polymer segments and subsequent reforming of the polymer chain into a film matrix or gel; this can be achieved through the evaporation of the solvent thus creating hydrophilic and hydrogen bonding and/or electrolytic and ionic crosslinking.

Natural biopolymers present several advantages over synthetic polymers but their application is limited by their high affinity to water, leading to textural transformations that have a strong impact on their mechanical, transport and solubility properties. In a previous publication the development of edible films based in chitosan and galactomannan was discussed (Cerqueira, Lima, et al., 2009), however no relationship was established between their structure and their properties. To our knowledge no work has been published comparing the physico-chemical properties of these two polysaccharides and very few publications do it for other polysaccharides. Further, the understanding of their structure–properties relationship in order to predict and control their function is one of the major flaws in this area, especially when mixtures with other substances are considered (which is often the case). In fact, the incorporation of other compounds, such as plasticizers and lipids, is common in order to improve mechanical and transport properties of edible films (Bergo & Sobral, 2007), but research focused on e.g. polysaccharides' interaction with other film components is needed in order to understand the influence of such components in films' properties.

Plasticizers are commonly used to facilitate processing and/or to increase films flexibility. Water, oligosaccharides, polyols, and lipids are different types of plasticizers widely used in hydrocolloid-based films (Suyatma, Tighzert, Copinet, & Coma, 2005). Their combination could give rise to synergistic effects between components improving the properties of edible films. The lubrication theory postulates that plasticizers, by interspersing themselves, act as internal lubricants by reducing frictional forces between polymer chains. The gel theory postulates that the rigidity of the polymer network comes from its three-dimensional structure, and plasticizers take effect by breaking polymer–polymer interactions (e.g., hydrogen bonds and van der Waals or ionic forces). The free volume theory states plasticization as a way to increase free volume (Santosa & Padua, 1999; Suyatma et al., 2005). Glycerol is a major by-product of biodiesel production which has significantly increased, thus creating a significant surplus and is often regarded as a waste stream with an associated cost (Fountoulakis & Manios, 2009; Gu & Jérôme, 2010). The use of glycerol as plasticizer in these films can be a way to help solving the existing surplus of this co-product from biodiesel production. Lipids, due their hydrophobic behavior, are added to polysaccharide films aiming at decreasing their hydrophilicity, consequently decreasing their water affinity (Vargas, Albors, Chiralt, & González-Martínez, 2009). From all the commercial oils, corn oil has shown to be one of the most effective, in comparison with others, in decreasing the water vapor permeability of polysaccharide and protein films (Ekthamasut & Akesowan, 2001; Tanaka, Ishizaki, Suzuki, & Takai, 2001).

The aim of this study was to evaluate the influence of glycerol and corn oil presence in the properties of the films. This was achieved by relating the information gathered from Fourier-transform infrared spectroscopy, thermal analyses (DSC and TGA), solubility measurements, moisture content determinations, water vapor permeability measurements and mechanical tests.

## 2. Materials and methods

### 2.1. Films preparation

Chitosan film-forming solutions were prepared dissolving chitosan (deacetylation degree of 90% approximately, Aqua Premier Co., Thailand) (1.5% w/v) in a lactic acid (1.0% v/v) solution (Merck, Germany) with agitation using a magnetic stirrer (at 200 rpm) overnight at room temperature (20 °C); Tween 80 (0.2%) (Acros Organics, Belgium) was also added as surfactant. Galactomannan film-forming solutions were prepared by dissolving *G. triacanthos* galactomannan (obtained as described in Cerqueira, Pinheiro, et al. (2009)) (1.5% w/v) in distilled water, followed by the same conditions as for chitosan. Glycerol (87%, Panreac, Spain) was added at three different concentrations (0.5, 1.25 and 2.0% w/v). Corn oil (Sovena, Portugal) was added at three different concentrations (0.25, 0.5 and 0.75% w/v) under agitation during 20 min at 60 °C, to films with constant concentrations of 1.5% of polysaccharide and 0.5% of glycerol. To produce the films, a constant amount (13 mL) of film-forming solution was cast onto a 5.7 cm diameter Petri plate. The films were dried in an oven at 35 °C during 16 h. Films were maintained at 23 °C and 54% RH at least 24 h before performing the tests (these conditions were obtained in a desiccator through a saturated salt solution of Mg(NO<sub>3</sub>)<sub>2</sub>).

### 2.2. Moisture content

To determine the moisture content of films about 50 mg of film were dried at 105 °C during 24 h (until the equilibrium weight was attained). The weight loss of the sample was determined, from which the moisture content was calculated using the following equation:

$$\text{Moisture content} = \frac{(M_i - M_f)}{M_i} \times 100 \quad (1)$$

where  $M_i$  and  $M_f$  are the masses of initial and dried samples, respectively.

### 2.3. Fourier-transform infrared (FTIR) spectroscopy

The IR spectra of the films were determined using an infrared spectrometer (FTIR) (Perkin–Elmer 16 PC spectrometer, Boston, USA), in Attenuated Total Reflectance mode (ATR) between 400 and 4000 cm<sup>-1</sup>, using 16 scans at a resolution of 4 cm<sup>-1</sup>. Before film analysis, an open bean background spectrum of clean crystal was recorded. Data analysis of each film was performed with Peak Fit 4.12 (SYSTAT Software Inc., Richmond, CA, USA) program. Spectra of films have been deconvoluted with the second derivative method with a smoothing filter set at 15%.

### 2.4. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA)

Differential scanning calorimetry (DSC) measurements were performed with a Shimadzu DSC-50 (Shimadzu Corporation, Kyoto, Japan) calibrated with Indium as standard. Ca. 10 mg of the sample was placed in aluminum DSC pans (Al crimp Pan C.201-52943). The measurements were performed between –100 and 250 °C at a heating rate of 10 °C min<sup>-1</sup> under a nitrogen atmosphere. In a first heating scan the enthalpy of melting ( $\Delta H_m$ ) and the melting peak ( $T_m$ ) were determined; a second heating allowed the measurement of glass transition temperature ( $T_g$ ). Thermogravimetric analysis (TGA) was completed with a Shimadzu TGA-50 (Shimadzu Corporation,

Kyoto, Japan). Samples were placed in the balance system and heated from 20 °C to 580 °C at a heating rate of 10 °C min<sup>-1</sup> under a nitrogen atmosphere.

### 2.5. Water solubility

The films' solubility in water was determined according to the method reported by Cuq, Gontard, Cuq, and Guilber (1996). Solubility is defined as the content of dry matter solubilized after 24 h immersion in water. The initial dry matter content of each film was determined by drying to constant weight in an oven at 105 °C. Disks of film (2 cm diameter) were cut, weighed ( $M_i$ ), and immersed in 50 mL of water. After 24 h of immersion at 20 °C with agitation (60 rpm), the pieces of film were taken out and dried to constant weight ( $M_f$ ) in an oven at 105 °C, to determine the weight of dry matter that was not solubilized in water. The solubility of the films was then determined as follows:

$$\text{Water solubility} = \frac{(M_i - M_f)}{M_i} \times 100 \quad (2)$$

where  $M_i$  is the initial mass and  $M_f$  is the final mass of the sample.

### 2.6. Film thickness

The film thickness was measured with a digital micrometer (No. 293-561, Mitutoyo, Japan). Five thickness measurements were taken on each testing sample in different points and the mean values were used to calculate permeability and mechanical properties.

### 2.7. Water vapor permeability (WVP) measurement

The measurement of water vapor permeability (WVP) was performed gravimetrically based on ASTM E96-92 method (Guillard, Broyart, Bonazzi, Guilbert, & Gontard, 2003; McHugh, Avena-Bustillos, & Krochta, 1993). The film was sealed on the top of a permeation cell containing distilled water (100% RH; 2337 Pa vapor pressure at 20 °C), placed in a desiccator at 20 °C and 0% RH (0 Pa water vapor pressure) containing silica. The cells were weighted at 2 h intervals during 10 h. Steady-state and uniform water pressure conditions were assumed by keeping the air circulation constant outside the test cell by using a miniature fan inside the desiccator (Guillard et al., 2003). The slope of weight loss versus time was obtained by linear regression. The measured (WVP) of the films was determined as follows:

$$\text{WVP} = \frac{\text{WVTR} \cdot L}{\Delta P} \quad (3)$$

where WVTR is the measured water vapor transmission rate through a film,  $L$  is the mean film thickness (m), and  $\Delta P$  is the partial water vapor pressure difference (Pa) across the two sides of the film. Three replicates were obtained for each film.

### 2.8. Tensile strength (TS) and elongation-at-break (EB)

TS and EB were measured with an Instron Universal Testing Machine (Model 4500, Instron Corporation) following the guidelines of ASTM Standard Method D 882-91 (ASTM-D-882-91, 1991). The initial grip separation was set at 30 mm and the crosshead speed was set at 5 mm min<sup>-1</sup>. TS was expressed in Pa and calculated by dividing the maximum load (N) by the initial cross-sectional area (m<sup>2</sup>) of the specimen. EB was calculated as the ratio of the final length at the point of sample rupture to the initial length of a specimen (30 mm) and expressed as a percentage. According to the ASTM standard, film strips with a length of 45 mm and a width of 20 mm were used. TS and EB tests were replicated at least three times for each type of film.

### 2.9. Statistical analyses

Statistical analyses were performed using Analysis of Variance (ANOVA) and linear regression analysis. The Tukey test ( $\alpha = 0.05$ ) was used to determine any significance of differences between specific means (SigmaStat, trial version, 2003, USA).

## 3. Results and discussion

### 3.1. Fourier-transform infrared (FTIR) spectroscopy

The effect of glycerol and oil in chitosan and galactomannan films was initially evaluated by FTIR analyses. Fig. 1a shows the FTIR spectra of the chitosan (CH) films containing 0, 0.5, 1.25 and 2.0% of glycerol (Gly). The broad band ranging between 3500 and 3100 cm<sup>-1</sup> is attributed to O–H stretching vibration that overlaps the N–H stretching vibration in the same region. The broad band between 2800 and 3000 cm<sup>-1</sup> is attributed to C–H stretching vibration. The peak at 1574 cm<sup>-1</sup> was due to the N–H bending (amide II); and the peak at 1733 cm<sup>-1</sup> suggests the presence of a carbonyl group (C=O) in the film matrix (Xu, Kim, Hanna, & Nag, 2005; Ziani, Oses, Coma, & Maté, 2008). When compounds are mixed, physical bonds and chemical interactions are reflected by changes in characteristic spectra peaks. By deconvolution of the area band between 800 and 1200 cm<sup>-1</sup> of FTIR spectra seven bands appear. Peak positions and areas of bands between 800 and 1200 cm<sup>-1</sup> for chitosan films with increasing glycerol concentrations after the application of deconvolution are presented in Table 1

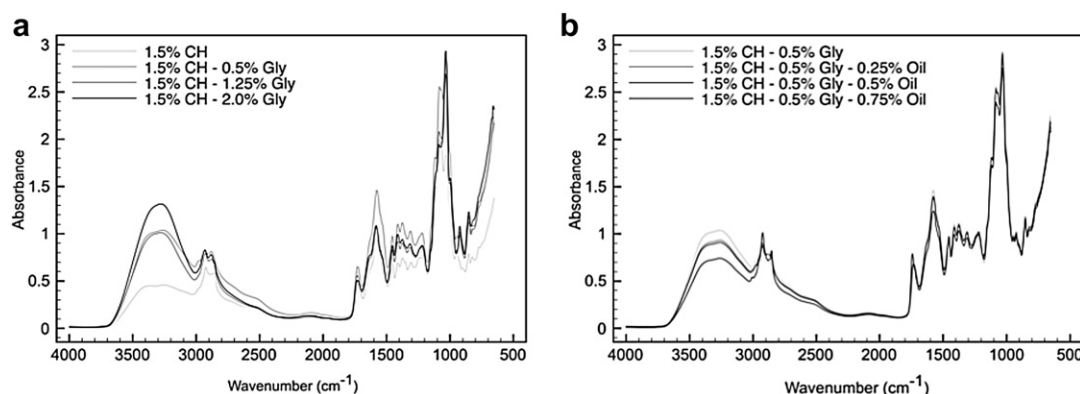


Fig. 1. FTIR spectra of chitosan films for increasing glycerol (a) and oil (b) concentrations.

of the Supplementary data. Glycerol incorporation leads to a shift of the peak  $942.9\text{ cm}^{-1}$ , presented in chitosan films without glycerol, to peak + bands of  $929.2$ ,  $923.1$  and  $920.5\text{ cm}^{-1}$  for chitosan films with 0.5, 1.25 and 2.0% of glycerol, respectively. This shift can be related with the symmetric stretching vibrations of the alcoxyl group (C–O–C), resulting of the glycerol presence (Jamróz et al., 2007). Also the shift of the peak  $999.9\text{ cm}^{-1}$ , of the chitosan films without glycerol, to  $994.1$ ,  $991.2$  and  $990.2\text{ cm}^{-1}$  for chitosan films with 0.5, 1.25 and 2.0% of glycerol, respectively, can be related with the asymmetric stretching vibrations of the alcoxyl group (C–O–C) (Jamróz et al., 2007). Moreover, the broad band that corresponds to the O–H stretching vibration ( $3500\text{--}3100\text{ cm}^{-1}$ ) was more intense for higher glycerol concentrations. Table 2 of the Supplementary data shows the peaks + areas of bands after the application of deconvolution of the FTIR spectra to the area band between 2500 and  $3500\text{ cm}^{-1}$  for chitosan films with increasing glycerol concentrations. The increase of glycerol concentrations leads to a higher number of bands after the deconvolution of the spectra. For chitosan films with 2.0% of glycerol the deconvolution origins two more peaks when compared with the other chitosan films; furthermore the area of the bands between  $3500$  and  $3100\text{ cm}^{-1}$  increased with the increasing of glycerol concentrations, confirming the higher intensity of O–H bonds. Also evident is the decrease of the area of the bands close to those at ca.  $2720\text{ cm}^{-1}$  and ca.  $2900\text{ cm}^{-1}$  that correspond to the C–H stretching vibration. The increase of glycerol concentration leads to higher numbers of O–H bonds and consequently higher band areas, also resulting in the decrease of the areas that correspond to the C–H bonds (Table 2 of the Supplementary data).

Fig. 1b shows FTIR spectra of chitosan films for increasing oil concentrations. FTIR spectra of chitosan films with oil also show a high and more intense number of peaks in the frequency range between  $2800$  and  $3100\text{ cm}^{-1}$ . By the deconvolution of FTIR band ranging between  $2500$  and  $3500\text{ cm}^{-1}$  a different number of peaks appear for chitosan films without and with oil. These results are presented in Table 3 in the Supplementary data, where two new peaks appear when oil is added to chitosan films. The most relevant peak, ca.  $2925\text{ cm}^{-1}$ , can be related with the symmetric and asymmetric stretching vibration of the aliphatic group ( $\text{CH}_2$ ) (Vlachos et al., 2006), with a more intense area (27%) for chitosan films with 0.75% of oil. Table 4 in the Supplementary data presents the results for the deconvolution of the FTIR area band ranging between  $1600$  and  $1800\text{ cm}^{-1}$ ; it shows that the oil incorporation leads to the presence of a new band at ca.  $1740\text{ cm}^{-1}$ , that is only observed for chitosan films when oil is added. Table 4 in the Supplementary data also shows an increase of the area from 30.2 to 31.0 and 40.9% for 0.25, 0.5 and 0.75% of oil, for the band ca.

$1740\text{ cm}^{-1}$ . This peak corresponds to the C=O stretching vibration, that can be explained by the presence of the carbonyl radical in the ester functional group of the triglycerides, associated with the presence of corn oil, at  $1746\text{ cm}^{-1}$  (Vlachos et al., 2006).

Fig. 2a and b shows FTIR spectra of galactomannan films for increasing glycerol and oil concentrations, respectively. The broad band ranging between  $3500$  and  $3100\text{ cm}^{-1}$  is attributed to O–H stretching vibration formed by the hydroxyl group of galactomannan and water and the broad band around  $2800\text{--}3000\text{ cm}^{-1}$  is attributed to C–H stretching vibration (Yuen, Choi, Phillips, & Ma, 2009). Table 5 in the Supplementary data shows the peak + areas of bands between  $2500$  and  $3500\text{ cm}^{-1}$  for galactomannan films with increasing glycerol concentrations, after the deconvolution of FTIR spectra. The increasing glycerol concentration increases the FTIR spectra area corresponding to the region of the O–H stretching vibration ( $3500\text{--}3100\text{ cm}^{-1}$ ), decreasing the number of bands/peaks from eight to four. This phenomenon is related with the hydrophilic behavior of glycerol and due to the hydrogen bonds formed by the hydroxyl groups of both galactomannan and glycerol structures. The presence of glycerol also leads to changes in the band region ranging between  $800$  and  $1200\text{ cm}^{-1}$ , which is giving information about the alcoholic (C–O) stretching bands and the asymmetric and symmetric (C–O–C) stretching vibrations bands (Jamróz et al., 2007). Table 6 in the Supplementary data shows the peaks + areas of bands between  $800$  and  $1200\text{ cm}^{-1}$  for galactomannan films with increasing glycerol concentrations, after the application of deconvolution process. The band with the peak at  $1078.7\text{ cm}^{-1}$  shifts to  $1083.2$ ,  $1094.8$  and  $1089.2\text{ cm}^{-1}$  for chitosan films with glycerol, that can result from the stretching vibration of C–O in C–O–H bonds. The broad band at  $1017.2\text{ cm}^{-1}$  that corresponds to the stretching vibration of C–O in C–O–C bonds appears in all galactomannan film samples, however presenting a shift to  $1022.8$ ,  $1034.7$  and  $1030.5\text{ cm}^{-1}$  for galactomannan films with 0.5, 1.25 and 2.0% of glycerol, respectively. Moreover, the broad band at ca.  $920\text{ cm}^{-1}$  that also corresponds to the stretching vibration of C–O in C–O–C bond, only appears in the films containing glycerol, confirming the more intense C–O stretching vibration in these films' structure (Jamróz et al., 2007; Pelissari, Grossmann, Yamashita, & Pineda, 2009).

Also changes in the frequency range between  $2800$  and  $3500\text{ cm}^{-1}$  are observed when oil is added to galactomannan films. Table 7 in Supplementary data shows the shift of the band at  $2905.5\text{ cm}^{-1}$  to a peak + band of ca.  $2850\text{ cm}^{-1}$  when oil is added. Also a new peak + band at ca.  $2925\text{ cm}^{-1}$  appears in the FTIR spectra of galactomannan films with oil. These two peaks + bands are related with symmetric and asymmetric stretching vibration of the aliphatic C–H group ( $\text{CH}_2$ ) (Vlachos et al., 2006). By other side, the broad band ranged between  $3500$  and  $3100\text{ cm}^{-1}$ , associated to

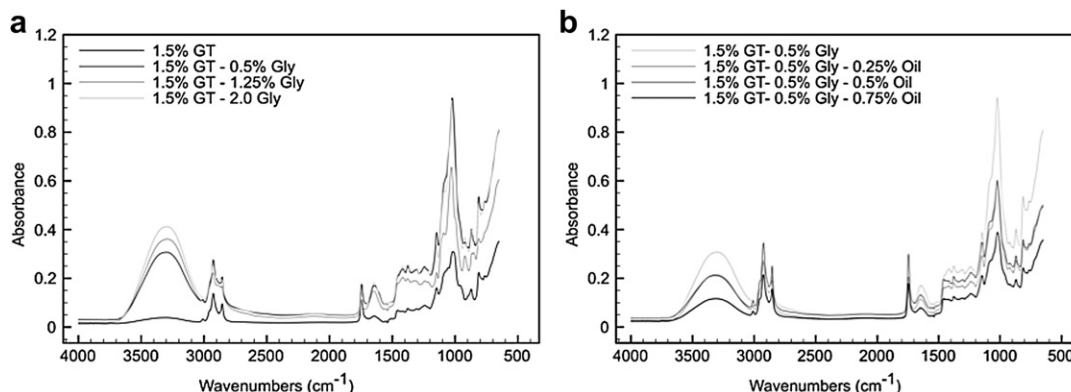


Fig. 2. FTIR spectra of the galactomannan (GT) films for increasing glycerol (Gly) (a) and oil (b) concentrations.

the O–H stretching vibration, shows a lower intensity that can be associated with hydroxyl bonds of the films that decrease when oil is added to the film (Yuen et al., 2009), leading to a more hydrophobic matrix. Also in the area band between 1600 and 1800  $\text{cm}^{-1}$  changes were observed in the FTIR spectra for galactomannan films with increasing oil concentrations. By the application of deconvolution to this area band six peaks appear, decreasing for five peaks for a concentration of 0.75% of oil (Table 8 in the Supplementary data). With the increasing of oil concentration it is evident the increase of the area of the peak + band around 1744  $\text{cm}^{-1}$ , associated with the carbonyl radical in the ester functional group of triglycerides (Vlachos et al., 2006).

### 3.2. Moisture content and thermal analyses

The determination of moisture content gives information on the amount of water present in the films, while the differential scanning calorimetry (DSC) analyses were used to measure the glass transition temperature ( $T_g$ ), the enthalpy of melting ( $\Delta H_m$ ) and the peak melting temperature ( $T_m$ ) of the films.  $T_g$  is a parameter associated with the system mobility, and is defined as a physical change from the glassy to the rubbery state in amorphous materials promoted by heat (Roos & Karel, 1991), while  $\Delta H_m$  and  $T_m$  can be associated with the crystallinity of the films samples.

Fig. 3a and b shows the variations of the moisture content in chitosan films for increasing concentrations of glycerol and oil, respectively. The presence of water in chitosan films is highly dependent on glycerol concentration. Glycerol, due to its hydrophilic nature, retains water in the film matrix. Higher concentrations of plasticizer favor the adsorption of water molecules, which is mainly attributed to the predisposition of plasticizers to form hydrogen bonds (O–H), confirmed by FTIR spectra analyses. On the other hand, it is observed that moisture content decreases for films with oil (Fig. 3b) when compared with films without oil. However, the values do not present a statistically significant difference ( $p > 0.05$ ) between chitosan films with different oil concentrations.

Fig. 4a and b shows the variations of the moisture content in galactomannan films for increasing concentrations of glycerol and oil, respectively, and presents a similar behavior to chitosan films. Water is not only associated with the galactomannan film's structure, but also with the glycerol hydrophilic nature that retains water in the matrix. Furthermore, a decrease in the water content is observed for galactomannan films with oil, (Fig. 4b), in agreement with FTIR spectra analyses.

Fig. 3a shows  $T_g$  values for chitosan films with different glycerol concentrations. Glycerol, due its plasticizing effect, decreases glass transition temperatures of polysaccharide films, which is in agreement with the free volume theory of plasticization. The increase of glycerol concentration leads to an increase of the free

volume and mobility of molecules, changing the physical structure of the chitosan film, which is in agreement with the decrease of the  $T_g$  values. Moreover,  $T_g$  values are inversely associated with the moisture content of chitosan films; in fact, also water acts as a plasticizer increasing the molecular mobility (lower  $T_g$  values) of the chitosan films. Glycerol changes the polymer network creating mobile regions with greater interchain distances, promoting water clustering (Diab, Biliaderis, Gerasopoulos, & Sfakiotakis, 2001; Olivas & Barbosa-Cánovas, 2008), thus increasing the moisture content in the films. The thermograms present a great decrease of  $T_g$  values for glycerol concentrations of 2.0%, that can be explained by the value reported for the  $T_g$  of glycerol ( $-75^\circ\text{C}$ ) (Mathew & Dufresne, 2002).  $T_g$  values obtained here are in agreement with those reported in other works (Dong, Ruan, Wang, Zhao, & Bi, 2004; Suyatma et al., 2005). Oil incorporation in chitosan films decreases the mobility of the chitosan matrix, as confirmed by the increase ( $p < 0.05$ ) of  $T_g$  values (Fig. 3b). However, the increase of oil concentrations does not lead to statistically significant differences ( $p > 0.05$ ) between  $T_g$  values. This behavior is in agreement with the moisture content of the films containing oil, that also does not present a statistically significant difference ( $p > 0.05$ ) for different oil concentrations.

Fig. 4a shows  $T_g$  of galactomannan films for different glycerol concentrations. Results show that the presence of glycerol decreases ( $p < 0.05$ ) the values of  $T_g$ . Higher glycerol concentrations lead to the increase of the free volume of the polymer matrix and consequently to the increase of the mobility of molecules, thus changing the physical structure of the film and decreasing  $T_g$  (Roos & Karel, 1991). Moreover,  $T_g$  values are inversely associated with the water content of the galactomannan films; also, water itself acts as a plasticizer increasing the molecular mobility (lower  $T_g$  values) in the galactomannan films. The presence of glycerol contributes to the establishment of more hydrogen bonds in the film matrix, as confirmed by FTIR analyses. The  $T_g$  values obtained for galactomannan films are in agreement with reported results for galactomannan films from other sources (Mikkonen et al., 2007). Oil incorporation decreases the mobility of the galactomannan matrix (increase of  $T_g$  values) that can be related with the oil structure (e.g. aliphatic C–H group and carbonyl radical in the ester functional group) and also with the decrease of the moisture content of films (Fig. 4b).

Fig. 5a and b shows the changes of  $\Delta H_m$  and  $T_m$  of chitosan and galactomannan films, respectively, with the increase of glycerol concentration. For chitosan films the increase of glycerol concentration leads to an increase of  $\Delta H_m$ , and to a decrease of  $T_m$  values. The higher values of  $\Delta H_m$  are possibly explained by the increase of the crystallinity of chitosan films (Sperling, 2006). When glycerol concentration increases, a greater polymer mobility (lower  $T_g$  values) is obtained that favors the formation of crystalline domains

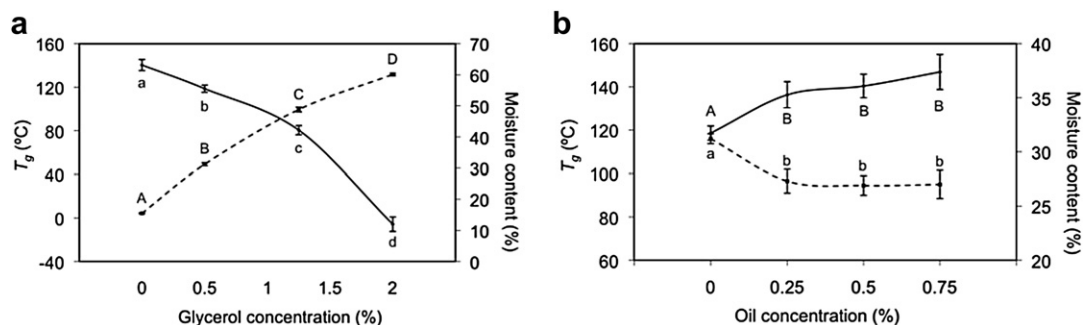
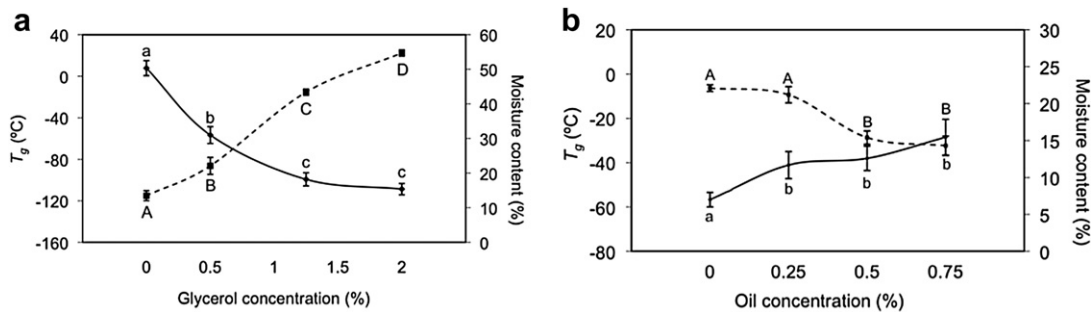


Fig. 3. Glass transition temperature ( $T_g$ ) (—) and moisture content (---) of chitosan films for increasing glycerol (a) and oil (b) concentrations. a–d; A–D Means with different superscripts are significantly different ( $p < 0.05$ ).



**Fig. 4.** Glass transition temperature ( $T_g$ ) (—) and moisture content (---) of galactomannan films for increasing glycerol (a) and oil (b) concentrations. <sup>a-d;A-D</sup>Means with different superscripts are significantly different ( $p < 0.05$ ).

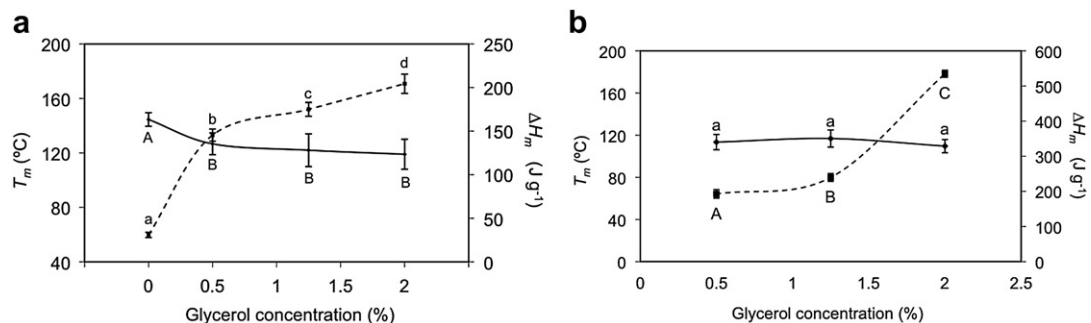
(Fabra, Talens, & Chiralt, 2010; Mathew & Dufresne, 2002). Also the increase of the moisture content in the film matrix when more hydrogen bonds are available can influence the intensification of films' crystallinity (Chen, Liu, Chen, Chen, & Chang, 2008). The presence of oil does not provoke statistically significant differences ( $p > 0.05$ ) on the values of  $\Delta H_m$  and  $T_m$  for chitosan films (results not shown).

The melting peak was not observed for galactomannan films without glycerol and for films with oil concentrations above 0.5%. The thermograms (results not shown) had a flat shape indicating an amorphous structure of these films (Mathew & Dufresne, 2002; Yakimets et al., 2007). Fig. 5b shows that the increase of glycerol concentration leads to higher values of  $\Delta H_m$ , that can be explained by the formation of crystalline domains, favored by the increase of the polymer mobility (lower  $T_g$  values) how was already explained elsewhere (Fabra et al., 2010; Mathew & Dufresne, 2002; Sperling, 2006). Also, the increase of the moisture content in the film matrix when more hydrogen bonds are available can influence the crystallinity of the films (Chen et al., 2008).  $T_m$  values were not statistical significance influenced ( $p > 0.05$ ) by the increase of glycerol concentration.

Thermogravimetric analysis determine the changes in weight of the films samples with the increase of the temperature. Tables 9 and 10 in the Supplementary data show the peak values of thermal events and the corresponding weight loss for each event, for chitosan and galactomannan films, respectively. Thermal analysis shows that chitosan films began the dehydration process at 60 °C (results not shown), being stable below that temperature. They present at least three thermal events, however for samples with oil a fourth event was observed. Peak 1 is related with the evaporation process, a characteristic phenomenon of a polysaccharide with a hydrophilic

nature. The differences in weight loss due to the presence of glycerol (peak 2) are also very marked, where an increase of the weight loss for higher glycerol concentrations is observed, related with the loss of chemisorbed water through hydrogen bonds and the elimination reaction of  $\text{NH}_3$  (Quijada-Garrido, Iglesias-González, Mazón-Arechederra, & Barrales-Rienda, 2007). Peak 3 (around 290 °C) is related with the dehydration, depolymerization and pyrolytic decomposition of the polysaccharide backbone (Zohuriaan & Shokrolahi, 2004). In films containing oil, where the plasticizer content is constant, the weight loss at peak 2 (related with the presence of glycerol) does not present statistically significant differences when the oil concentration was increased; however, there is an increase in the size of the peak associated with oil decomposition (peak 4), related with the aromatic structures present in corn oil with decomposition temperatures above 380 °C (Pelissari et al., 2009).

Thermal analyses show that galactomannan films without glycerol are stable up to 40 °C, and when glycerol is added this stability increases up to 59 °C (results not shown). Galactomannan films show at least three thermal events, the first being attributed to water evaporation, the second (around 200 °C) attributed to the presence of glycerol, and the third related to polysaccharide decomposition (Zohuriaan & Shokrolahi, 2004). As for chitosan films, for samples containing oil a fourth event was observed related with the aromatic structures present in corn oil with decomposition temperatures above 380 °C (Pelissari et al., 2009). Peak events show that the increase of glycerol concentration leads to a more significant weight loss associated with two thermal events: water evaporation (peak 1), and chemisorbed water through the hydrogen bonds favored by the presence of glycerol (peak 2) (Quijada-Garrido et al., 2007). In films containing oil



**Fig. 5.** Melting temperature peak ( $T_m$ ) (—) and enthalpy of melting ( $\Delta H_m$ ) (---) of chitosan (a) and galactomannan (b) films for increasing glycerol concentrations. <sup>a-d;A-C</sup>Means with different superscripts are significantly different ( $p < 0.05$ ).

a weight loss associated with oil decomposition is observed (peak 4); this loss was more substantial for samples with higher corn oil concentration.

### 3.3. Water solubility

The water solubility of edible films indicates their water resistance when applied, e.g., on water-rich foods such as peeled fruits. It is also related to the biodegradability of films when used as packaging materials (Gnanasambadam, Hettiarachchy, & Coleman, 1997). Table 1 shows that water solubility of chitosan films increases for higher glycerol concentrations ( $p < 0.05$ ). This is related with the hydrophilic behavior of glycerol and with the increase of O–H bonds on chitosan films matrix with the increase of glycerol concentration, that are more available to interact with the water molecules, in agreement with FTIR spectra analyses, values of moisture content and thermal events detected during TGA. On the other hand, the presence of oil and the increase of its concentration lead to a statistically significant decrease ( $p < 0.05$ ) of the solubility. The decrease of the number of O–H bonds, the appearance of aliphatic groups in the film when oil is added (as shown by FTIR results), and the corresponding increase of the hydrophobic portion of the film originated a less soluble material (Morillon, Debeaufort, Blond, Martine, & Voilley, 2002).

Table 2 shows the solubility values of galactomannan films and indicates that both glycerol and oil influence their water solubility. The presence of oil and the increase of glycerol concentration show different effects. The increase of glycerol concentration leads to an increase ( $p < 0.05$ ) of the solubility, motivated by the corresponding increase of the hydrophilicity of the film matrix. FTIR analyses showed an increase of O–H bonds number with the increase of glycerol content; this means that glycerol fostered the interaction of the film matrix with water. Also the higher mobility of the matrix (lower  $T_g$ ) has a great influence in these results: in a system with improved mobility, water molecules enter more easily in the film matrix which results in an increase of its solubility.

Oil addition (from 0 to 0.25%) does not cause a statistically significant difference ( $p > 0.05$ ) in water solubility values. However, for oil concentrations of 0.75% a significant decrease of the solubility ( $p < 0.05$ ) occurs. As explained before, for chitosan films, the hydrophobic character of oil changes the film structure leading to a less soluble film (i.e. decreases the number of O–H bonds and the presence of an aliphatic groups). The results obtained are in agreement with the solubility values for other polysaccharide-based films (Casariego et al., 2009; Mehyar & Han, 2004; Piermaria, Pinotti, Garcia, & Abraham, 2009).

### 3.4. Water vapor permeability (WVP)

Water vapor permeability is the most extensively studied property of edible films. Table 1 shows that oil and glycerol have

a distinct influence in WVP of chitosan films; while the WVP values increase for increasing glycerol concentrations, the presence of oil leads to a decrease of WVP. These results are in agreement with other works where the increase of plasticizer concentration has increased the values of WVP (Caner, Vergano, & Wiles, 1998; Ziani et al., 2008). As already explained above, the increase of glycerol concentration leads to higher moisture contents of chitosan films. The plasticizer action increases the free volume and chain movements (lower  $T_g$ ), reducing the rigidity and increasing the molecular mobility of films, thus allowing a higher water vapor diffusion through their structure.

The presence of oil changes the properties of the films, decreasing the affinity for water. The decrease of the WVP values in chitosan films with the addition of oil can be explained by the diminution of the hydrophilic portion of the film (Hernandez-Munöz, López-Rubio, Del-Valle, Almenar, & Gavara, 2004), that reduces its affinity for water molecules and consequently decreases WVP (Table 1). As already stated, higher glycerol concentrations lead to an increase of the moisture content in films, while for different oil concentrations the moisture content values do not present statistically significant differences ( $p > 0.05$ ). So, if in the case of films without oil the glycerol effect was emphasized by the water influence (increase of moisture content), for films with oil this does not happen. The range of values obtained is in agreement with other reported works (Casariego et al., 2009; Wong, Gastineau, Gregorski, Tillin, & Pavlath, 1992).

Galactomannan films present a similar behavior to chitosan films; however when 0.5% of glycerol is added to the galactomannan film no statistically significant difference ( $p > 0.05$ ) of WVP values is observed (Table 2). The presence of the plasticizer decreases the occurrence of cracks and pores, improving the dispersion and flexibility of the film and thus decreasing gas permeability (Garcia, Martino, & Zaritzky, 2000). Moreover, when glycerol concentration increases from 0.5% to 1.25% and then to 2.0% the WVP values increase significantly. Glycerol and its plasticizing effect increase the molecular mobility and decrease the rigidity of polysaccharide chains. In this case, the higher concentrations of plasticizer favor molecular mobility (decreasing  $T_g$  values) and the adsorption of water molecules, increasing WVP values (Diab et al., 2001). As observed for chitosan films, also for galactomannan films the increase of oil concentration leads to lower WVP values, however these differences are statistically significant ( $p < 0.05$ ) only for oil concentrations higher than 0.25% (Table 2). The hydrophobic character of the oil blended with galactomannan polysaccharide changes the film properties decreasing their WVP.

### 3.5. Tensile strength (TS) and elongation-at-break (EB)

Tensile strength is the ability of a material to resist under tensile stress until it breaks and is one of the most important and widely

**Table 1**

Values of water solubility, water vapor permeability (WVP), tensile strength (TS) and elongation-at-break (EB) for chitosan (CH) films with variation of glycerol (Gly) and corn oil concentrations.

| Films                      | Solubility (%)                | WVP $\times 10^{-11}$<br>( $\text{g m}^{-1} \text{s}^{-1} \text{Pa}^{-1}$ ) | TS (MPa)                      | EB (%)                         |
|----------------------------|-------------------------------|---|-------------------------------|--------------------------------|
| 1.5% GT–0.0% Gly           | 42.25 $\pm$ 0.33 <sup>a</sup> | 5.07 $\pm$ 0.23 <sup>a</sup>  | 21.45 $\pm$ 2.82 <sup>a</sup> | 16.18 $\pm$ 3.53 <sup>a</sup>  |
| 1.5% GT–0.5% Gly           | 51.86 $\pm$ 0.16 <sup>b</sup> | 8.61 $\pm$ 0.38 <sup>b</sup>  | 7.72 $\pm$ 0.75 <sup>b</sup>  | 71.75 $\pm$ 4.27 <sup>b</sup>  |
| 1.5% GT–1.25% Gly          | 63.77 $\pm$ 0.77 <sup>c</sup> | 10.16 $\pm$ 0.18 <sup>c</sup>   | 3.64 $\pm$ 0.63 <sup>c</sup>  | 86.18 $\pm$ 4.88 <sup>c</sup>  |
| 1.5% GT–2.0% Gly           | 69.94 $\pm$ 0.72 <sup>d</sup> | 11.40 $\pm$ 0.45 <sup>d</sup>   | 1.17 $\pm$ 0.06 <sup>c</sup>  | 99.52 $\pm$ 2.84 <sup>d</sup>  |
| 1.5% GT–0.5% Gly–0.25% oil | 48.98 $\pm$ 1.07 <sup>e</sup> | 8.28 $\pm$ 0.33 <sup>b</sup>  | 3.66 $\pm$ 0.66 <sup>c</sup>  | 65.08 $\pm$ 2.72 <sup>e</sup>  |
| 1.5% GT–0.5% Gly–0.5% oil  | 45.20 $\pm$ 0.95 <sup>f</sup> | 7.78 $\pm$ 0.11 <sup>b</sup>  | 3.05 $\pm$ 0.15 <sup>c</sup>  | 63.01 $\pm$ 3.34 <sup>ef</sup> |
| 1.5% GT–0.5% Gly–0.75% oil | 42.25 $\pm$ 0.10 <sup>a</sup> | 6.68 $\pm$ 0.31 <sup>e</sup>  | 3.00 $\pm$ 0.17 <sup>c</sup>  | 56.01 $\pm$ 5.77 <sup>f</sup>  |

Values reported are the means  $\pm$  standard deviations. <sup>a–f</sup>Different letters in the same column indicate a statistically significant difference ( $p < 0.05$ ).

**Table 2**  
Values of water solubility, water vapor permeability (WVP), tensile strength (TS) and elongation-at-break (EB) for galactomannan (GT) films with variation of glycerol (Gly) and corn oil concentrations.

| Films                      | Solubility (%)                  | WVP $\times 10^{11}$<br>(g m <sup>-1</sup> s <sup>-1</sup> Pa <sup>-1</sup> ) | TS (MPa)                       | EB (%)                        |
|----------------------------|---------------------------------|---|--------------------------------|-------------------------------|
| 1.5% GT–0.0% Gly           | 22.00 $\pm$ 0.70 <sup>a</sup>   | 8.38 $\pm$ 0.57 <sup>a</sup>  | 18.55 $\pm$ 4.43 <sup>a</sup>  | 3.77 $\pm$ 0.09 <sup>a</sup>  |
| 1.5% GT–0.5% Gly           | 44.35 $\pm$ 0.47 <sup>bef</sup> | 7.87 $\pm$ 0.13 <sup>ac</sup>   | 13.25 $\pm$ 0.74 <sup>ac</sup> | 11.94 $\pm$ 0.47 <sup>b</sup> |
| 1.5% GT–1.25% Gly          | 56.41 $\pm$ 1.17 <sup>c</sup>   | 12.32 $\pm$ 0.17 <sup>b</sup>   | 2.58 $\pm$ 0.30 <sup>b</sup>   | 34.22 $\pm$ 1.10 <sup>c</sup> |
| 1.5% GT–2.0% Gly           | 65.43 $\pm$ 1.53 <sup>d</sup>   | 13.33 $\pm$ 0.44 <sup>b</sup>   | 1.70 $\pm$ 0.06 <sup>b</sup>   | 38.72 $\pm$ 3.78 <sup>c</sup> |
| 1.5% GT–0.5% Gly–0.25% oil | 48.39 $\pm$ 3.87 <sup>e</sup>   | 7.80 $\pm$ 0.34 <sup>cd</sup>   | 11.95 $\pm$ 1.79 <sup>cd</sup> | 14.35 $\pm$ 1.18 <sup>b</sup> |
| 1.5% GT–0.5% Gly–0.5% oil  | 41.84 $\pm$ 1.12 <sup>f</sup>   | 7.14 $\pm$ 0.35 <sup>de</sup>   | 8.34 $\pm$ 1.05 <sup>d</sup>   | 17.17 $\pm$ 1.37 <sup>d</sup> |
| 1.5% GT–0.5% Gly–0.75% oil | 34.11 $\pm$ 2.84 <sup>g</sup>   | 6.60 $\pm$ 0.26 <sup>e</sup>  | 3.38 $\pm$ 0.39 <sup>e</sup>   | 21.27 $\pm$ 4.40 <sup>d</sup> |

Values reported are the means  $\pm$  standard deviations. <sup>a–g</sup>Different letters in the same column indicate a statistically significant difference ( $p < 0.05$ ).

measured properties of materials used in structural applications. Elongation-at-break of a material is the percentage increase in length that occurs before it breaks under tension (Sperling, 2006).

The increase of plasticizer concentration exerts a great influence over TS values, and leads to a decrease of approximately 64% when 0.5% of glycerol was added to chitosan films (Table 1). For the same amount of added glycerol, EB values were 4.4-fold those of chitosan films without glycerol. Furthermore, when glycerol concentration increased from 0.5% to 2.0%, this behavior was more evident with a decrease of TS values of approximately 85% and an increase of EB values of 39%. Plasticizers interfere with chitosan chains: they decrease intermolecular forces, soften the rigidity of the film's structure and increase polymer mobility (in agreement with the  $T_g$  values observed), thus decreasing TS and increasing EB. The presence of glycerol leads to a ductile and flexible material. Furthermore, also the water content of the films when glycerol is added affects TS and EB, and accentuates the effect of the glycerol content (Ziani et al., 2008). These results are in agreement with those reported in the literature that show a decrease of TS values and an increase of EB values with the presence and increasing concentrations of plasticizer (Caner et al., 1998; Ziani et al., 2008).

Results in Table 1 show that the presence of oil influences both TS and EB values. Oil incorporation leads to a decrease of TS and EB values when compared with films without oil. Increasing oil concentrations from 0.5 to 0.75% did not have statistically significant influence ( $p > 0.05$ ) on TS and EB values for chitosan films, suggesting that the chitosan matrix is not able to incorporate oil concentrations above 0.5%. The oil presence leads to a less rigid film structure, being the structural discontinuities provoked by the oil incorporation possibly responsible for the decrease of their flexibility and their resistance to fracture (Sánchez-González, Vargas, González-Martínez, Chiralt, & Cháfer, 2009).

Results in Table 2 show how glycerol concentrations affect TS values of galactomannan films. Galactomannan films without glycerol present a typical behavior of an unplasticized film: high values of TS and a great variability due to their non-homogeneous structure. It is important to note that galactomannan films without glycerol at room temperature are in the glass–rubber transition zone (Fig. 4a), which from a molecular point of view involves molecular motion and the beginning of reptation, leading to a non-uniform structure (Roos & Karel, 1991; Sperling, 2006). The increase of glycerol concentration (from 0.5% to 2.0%) had a statistically significant influence ( $p < 0.05$ ) on the values of TS and EB. This increase led to TS values 87% lower and values of EB 3.2-fold higher than those found in films without glycerol. As previously mentioned, plasticizers interfere with galactomannan chains where, by decreasing intermolecular forces, they reduce the rigidity of the film structure and increase the polymer mobility; this facilitates film elongation, in accordance with the  $T_g$  values obtained in the present work.

Table 2 shows that the presence of oil influences both TS and EB values for the galactomannan films. The oil incorporation leads to a decrease of TS and an increase of EB values when compared with

the film without oil. This decrease only presents statistical significance ( $p < 0.05$ ) for oil concentrations above 0.25%. The results seem to indicate that oil acts as a plasticizer leading to a less rigid film structure, increasing its flexibility and decreasing its resistance to fracture. In the present work, the highest values of TS were obtained for the formulation containing 1.5% of galactomannan (18.6 MPa); and the highest value of EB was obtained for the film formulation containing 1.5% of galactomannan and 2.0% of glycerol (38.7%). These results are in agreement with other reported results for galactomannan films (Martins, Cerqueira, Souza, Avides, & Vicente, 2010; Mikkonen et al., 2007).

### 3.6. Comparison between structure–properties relationships of chitosan and galactomannan films

The study of the physicochemical properties of polysaccharide films shows how the presence of glycerol and/or corn oil can influence their structural reorganization. Water adsorption on the films' matrix for different glycerol and corn oil concentrations varies for both chitosan and galactomannan films, as shown by FTIR spectra, moisture content and thermal analyses results. The major differences between the used polysaccharides, from a structural point of view, lay in the substitution of the O–H group by an N–H function in the case of chitosan. This means that glycerol and oil will influence the structure of the films in a different way. While for the galactomannan the specific sorption sites for water are the O–H groups, for chitosan those are O–H and/or NH<sub>2</sub> groups (Despond, Espuche, Cartier, & Domard, 2005), thus changing their capacity to interact with water.

Chitosan and galactomannan films present a similar behavior when glycerol is added to the film. Glycerol leads to an increase of the moisture content; however, the obtained values for chitosan films are higher and statistically different ( $p < 0.05$ ) from the values for galactomannan films. For films without glycerol moisture content values are 15.5 and 13.5% for chitosan and galactomannan, respectively; however, when glycerol is added to the film-forming solutions, the resulting chitosan films present a moisture content at least 12% higher than galactomannan films. This behavior can be explained by the presence of Tween 80 in all chitosan films. Tween 80 presents a hydrophilic–lipophilic balance (HLB) of 15 that indicates that the surfactant is readily soluble in water (Carneiro-da-Cunha et al., 2009), increasing the ability of the chitosan matrix to adsorb water molecules. The water sorption occurs in two main steps: the water sorption on polymer sites and the water clustering surrounding the firstly sorbed water molecules (Fringant et al., 1996). The plasticizing effect of glycerol leads to a film matrix with more mobile regions with larger interchain distances, thus allowing the sorption of more water molecules to polysaccharide sites. Besides, glycerol's structure creates more hydroxyl bonds that promote the sorption of water molecules to the film.

Both polysaccharides are strongly water content dependent, however they present different behaviors when their thermal, transport and mechanical properties are compared. The major



differences are observed in the glass transition temperature ( $T_g$ ), water vapor permeability (WVP) and elongation-at-break (EB) values.  $T_g$  values for the both polysaccharides films show the well-known plasticizing effect of glycerol, which results in a decrease of  $T_g$ . However, they are in different states at room temperature. While chitosan films are in the glassy state, with  $T_g$  values higher than 20 °C (with exception of chitosan films containing 2.0% of glycerol), galactomannan films are in the rubbery state (with exception of galactomannan films without glycerol, that present a brittle behavior). Galactomannan films without glycerol, at room temperature, are in the glass–rubber transition zone leading to a non-uniform structure (Roos & Karel, 1991) meaning that glycerol is necessary for a correct processing of these films. TS values of galactomannan films without glycerol show an unplasticized film behavior with a great variability due to their non-homogeneous structure. However, for films containing glycerol (0.5%) it is observed that TS values for galactomannan films are higher than those obtained for chitosan films. This behavior can be justified by the linear and neutral nature of the polymer chains of the galactomannan films, which can associate more easily through intermolecular hydrogen bonding (Nieto, 2009). Chitosan films present higher values for EB than galactomannan films, being this difference more evident for higher glycerol concentrations. The chitosan structure is more flexible due to the presence of N–H bonds and this may be the reason for the observed behavior. Also, when oil is added chitosan and galactomannan films present distinct behaviors for EB values. While for chitosan the presence of oil leads to lower values of EB, for galactomannan films this presence increases the EB values. This behavior is possibly related with the emulsifying capacity of the chitosan film-forming solutions, which is higher than that of galactomannan film-forming solutions; when the films are cast, oil increases the structural discontinuities, decreasing film flexibility.

#### 4. Conclusion

The presence of glycerol and corn oil leads to changes in the polysaccharide films structure, with the formation of new and/or the increase of the number of existing bonds in the films' structure. These bonds influence the water affinity of the films, and consequently change their properties. It has been indirectly shown how the structure of the films is influenced by the presence of plasticizer and/or oil through measurement of thermal properties (DSC and TGA) and chemical structure (FTIR), and such influence has been confirmed by the results for transport and mechanical properties, solubility and opacity. Moreover, chitosan and galactomannan films evaluated in this work provide water vapor permeability and elongation-at-break values in the range of e.g. cellophane films; they also show in some cases tensile strength values close to those reported for high-density polyethylene and low-density polyethylene (Han & Gennadios, 2005), thus positioning them as possible alternatives to those synthetic materials.

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#### Appendix. Supplementary data

Tables showing the results of FTIR and thermogravimetric analyses can be found, in the online version, at doi:10.1016/j.foodhyd.2011.07.007.

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