Films from xylan/chitosan complexes: preparation and characterization
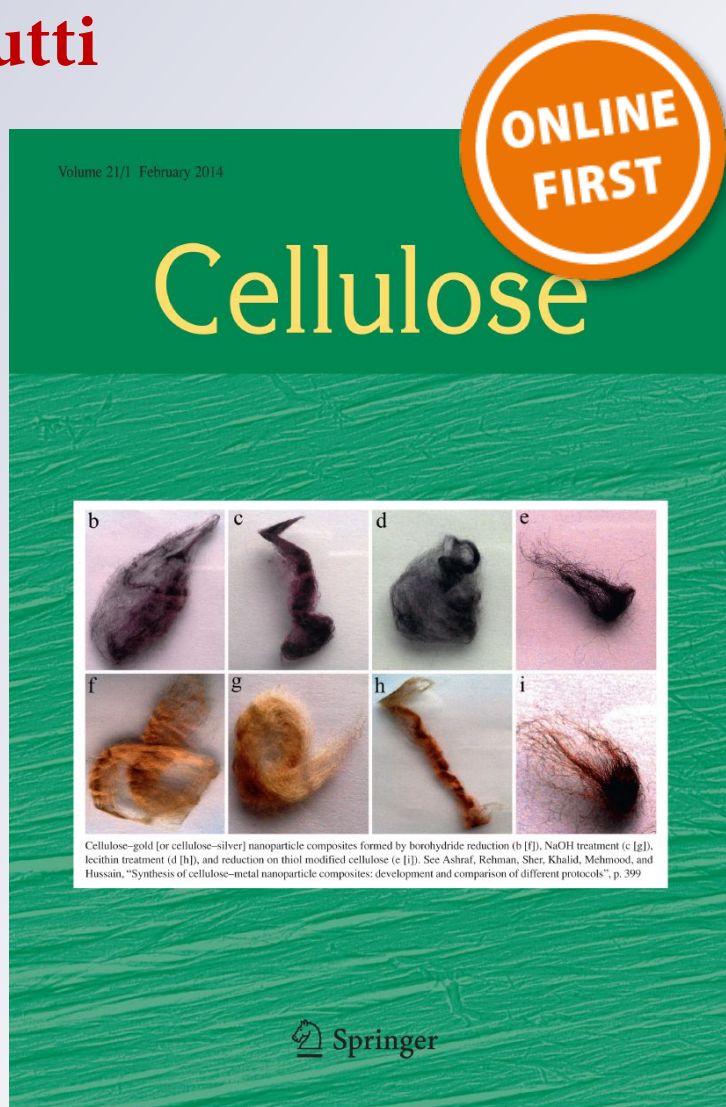
Carla N. Schnell, María V. Galván,
María S. Peresin, María C. Inalbon,
Jari Vartiainen, Miguel A. Zanuttini &
Paulina Mocchiutti

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Cellulose–gold [or cellulose–silver] nanoparticle composites formed by borohydride reduction (b [f]), NaOH treatment (c [g]), lecithin treatment (d [h]), and reduction on thiol modified cellulose (e [i]). See Ashraf, Rehman, Sher, Khalid, Mehmood, and Hussain, "Synthesis of cellulose–metal nanoparticle composites: development and comparison of different protocols", p. 399

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Films from xylan/chitosan complexes: preparation and characterization

Carla N. Schnell · María V. Galván · María S. Peresin · María C. Inalbon ·
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Abstract Films from colloidal suspensions of cationic polyelectrolyte complexes of xylan (Xyl) and chitosan (Ch) were formed and characterized by measuring: oxygen and water vapor barrier properties, solubility, and mechanical properties. High xylan content (Xyl/Ch mass ratio: from 70/30 to 85/15) was used, and a low amount of plasticizer (10 wt% glycerol) was always added. At 50% relative humidity (RH), all films had promising oxygen barrier properties (<0.3 μm cm^3/day m^2 kPa) and good mechanical properties for applications such as food packaging (stress at break of 10 MPa and strain at break of 12.2% for 70/30 Xyl/Ch film). All films showed a moderate water vapor transmission rate: 19.6–21.3 g/(h m^2). Although solubility was relatively high (26.5–35.4%), the integrity of the films was maintained after immersion in pH 5.0 buffer solution for 24 h. The 70/30 film was the most transparent, uniform, and deformable of the formulations evaluated, showing low crystallinity according to X-ray diffraction results. Dynamic mechanical analysis revealed a high correlation between its viscoelastic behavior and changes in RH (from 0 to 80% RH). Studies conducted using an agar disc diffusion protocol showed antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* bacteria only in the contact area under the film discs.

Keywords Natural polyelectrolyte complexes · Barrier properties · Relative humidity effect · Antimicrobial effect · Mechanical properties

C. N. Schnell · M. V. Galván · M. C. Inalbon ·
M. A. Zanuttini · P. Mocchiutti (✉)
Instituto de Tecnología Celulósica, Facultad de Ingeniería
Química (FIQ-CONICET), Universidad Nacional del
Litoral, Santiago del Estero 2654, S3000AOJ Santa Fe,
Argentina
e-mail: paulinam@fiq.unl.edu.ar

M. S. Peresin · J. Vartiainen
VTT Technical Research Centre of Finland Ltd,
Biologinkuja 7, P.O. Box 1000, 02044 VTT Espoo,
Finland

Present Address:
M. S. Peresin
Forest Products Development Center, School of Forestry and
Wildlife Sciences, Auburn University, 3301 Forestry &
Wildlife Sciences Bldg., 602 Duncan Dr., Auburn,
AL 36849-54185, USA

Introduction

To enable a more sustainable use of widely available natural resources, it is necessary to replace non-biodegradable plastic-based packaging materials with renewable and biodegradable polymers. Food industry waste is a significant source of pollution (Aider 2010), and in for this reason, the production of biodegradable films for food packaging is of great interest. The biorefinery industry offers a viable alternative, utilizing lignocellulosic biomass components as material

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sources, such as xylan from trees, grasses, cereals, and other plants (Hansen and Plackett 2008).

In general, an optimal film for food packaging should be easy to handle and should exhibit acceptable mechanical properties under dry or wet conditions. Such films should also have a high water vapor transfer rate (WVTR) if the material is going to be used for vegetable or fruit packaging, and should be resistant to relative humidity (RH, low WVTR) if the material must be protected against humid environments (Goksu et al. 2007). Additionally, films must have good barrier properties against gases such as oxygen to avoid the degradation of the packaged goods (Gröndahl et al. 2004).

To date, synthetic polymers used commercially for food packaging have largely been copolymers of ethylene and vinyl alcohol (EVOH) and low-density polyethylene (LDPE). EVOH has the advantage of having low oxygen and carbon dioxide permeability (Muramatsu et al. 2003), while LDPE demonstrates good water vapor barrier properties (Mauer and Ozen 2004). Multilayer films obtained by combining these two polymers (EVOH and LDPE) have been reported, thus taking advantage of both properties (Ferguson et al. 1987).

Xylan (Xyl) is an abundant component in lignocellulosic fibers that can be isolated and potentially used in the production of packaging films and other materials. However, because of its low molecular weight, high glass transition temperature, and low solubility in water, its film-forming capacity is poor, resulting in brittle films (Gabrielii et al. 2000; Gröndahl et al. 2004).

Several alternatives have been reported for the use of xylan in the production of films with acceptable mechanical and gas barrier properties (Hansen and Plackett 2008). These feature the combination of xylan with a high amount of plasticizers or with other polymers, such as wheat gluten, in the presence of a glycerol plasticizer (Hansen and Plackett 2008). More recently, researchers have reported the use of xylan together with nanofibrillated cellulose, utilizing high amounts of glycerol or sorbitol (Hansen et al. 2012), as well as the combination of xylan with polyvinyl alcohol, using citric acid as a plasticizer (Wang et al. 2014).

Another abundant polymer that can be isolated from renewable sources for potential use in food packaging materials is chitosan. The combination of xylan with chitosan offers an interesting 100% bio-based option. Strong ionic interaction between oppositely charged polyelectrolytes can be expected at specific pH, which could provide additional advantages in terms of the film's properties. Chitosan, which consists of β-1,4 linked glucosamine and *N*-acetyl glucosamine, is a natural, renewable polyelectrolyte derived from chitin. It is water-soluble, and behaves as a cationic polyelectrolyte when its amino groups are ionized, with a pK_a of 6.0–6.5, according to the condition of the liquid medium (Rinaudo et al. 1999). It has excellent properties including biocompatibility, biodegradability, and non-toxicity (Kumar 2000), as well as good film-forming capacity under acidic conditions (Kim et al. 2006). Chitosan has also shown antimicrobial activity (Rao et al. 2010).

In a study by Gabrielii et al. (2000), films were prepared by mixing xylan and chitosan—in dry form—followed by dissolution in acidic medium, and their capacity to act as a hydrogel was successfully determined. However, films with more than 20% of chitosan were found to gradually dissolve during swelling measurements.

Karaaslan et al. (2010, 2012) produced hydrogels of xylan and chitosan by rapidly mixing the xylan and chitosan solutions under acid conditions. The authors reported stress values of up to 29 MPa, but low (<3.6%) strain values (Karaaslan et al. 2010).

More recently, Luo et al. (2014) reported the preparation of 100% chitosan films in acidic medium. After neutralization, the films were soaked in xylan solutions at different concentrations (1–3% w/v). The authors reported that xylan-coated chitosan films showed increased tensile strength as well as improved oxygen barrier properties when compared to 100% chitosan films. However, none of these studies characterized the colloidal xylan/chitosan system prior to film formation, or considered the addition of a low amount of plasticizer.

In the present work, films were prepared from stable colloidal suspensions of cationic polyelectrolyte complexes (CatPECs) of xylan and chitosan. Different xylan/chitosan mass ratios (70/30, 75/25, 80/20, and 85/15) and a low amount of glycerol (10 wt%, relative to the total dry weight) as plasticizer were investigated. First, CatPECs were formed at pH 5.0 by the dropwise addition of xylan solution to chitosan solution (final ionic strength: 0.03 M). The PEC suspensions were characterized by measuring

charge density and particle size. Films were then prepared from these PEC suspensions using a solvent/casting method. The oxygen and water vapor barrier properties, solubility, and mechanical properties of these films were studied. In addition, the crystallinity of the films was evaluated using X-ray diffraction (XRD), and the effect of RH changes on mechanical properties was determined using dynamic mechanical analysis (DMA). Finally, the antimicrobial activity of the films against *Escherichia coli* and *Staphylococcus aureus* bacteria was analyzed, and the results are reported herein.

Experimental

Preparation of xylan and chitosan solutions

4-*O*-Metyl-glucuronoxylan isolated from beechwood by alkaline extraction was supplied by Sigma Aldrich (X-4252). The average molar mass was M_w 13.4 kDa, with polydispersity of 1.9, determined by size exclusion chromatography (SEC). Fresh xylan solutions were prepared daily, at a concentration of 4.0 g/L, according to Mocchiutti et al. (2016).

Chitosan was supplied by Sigma-Aldrich (product number 448877), with a viscosity average molecular mass of M_v: 190 kDa and deacetylation of $79.6 \pm 0.7\%$, as reported in our previous work (Mocchiutti et al. 2016). A solution of 2.5 g/L of chitosan in a low concentration of acetic acid (0.25 %w/v) was prepared. As the dissolution of chitosan was not complete, it was sonicated for 10 min in an ultrasonic bath, stirred for 30 min, and finally filtered using a glass filter with a pore size of 1.5 μm. The amount of chitosan retained in the filter was recorded and was used to calculate the final concentration of chitosan solution.

The charge densities of the chitosan and xylan solutions at pH 5.0 and 0.01 N NaCl were determined by polyelectrolyte titration. Streaming current measurements (Chemtrac ECA 2100 equipment, USA) were performed to determine the equivalence point (Mocchiutti et al. 2016). The xylan charge density at this pH was -0.39 ± 0.04 meq/g (which corresponds to a xylose:methyl-glucuronic acid ratio of 12.4:1), while the chitosan charge density was $+4.03 \pm 0.01$ meq/g (tenfold higher than the xylan charge density).

Preparation of the cationic polyelectrolyte complex (CatPEC) suspensions

Xyl/Ch cationic complexes were prepared by the dropwise addition of the xylan solution to the chitosan solution at a rate of 90 mL/h, under magnetic stirring. The pH values of the solutions were adjusted to 5.0 prior to mixing. This pH was selected to maximize the electrostatic interaction between xylan and chitosan, since both are partially ionized under these conditions. CatPECs with different xylan/chitosan mass ratios (70/30, 75/25, 80/20, and 85/25) were prepared. A glycerol solution (10 wt%, relative to the total dry weight of the polymers) was used as plasticizer and was added to the chitosan solution prior to the addition of xylan. Finally, CatPEC suspensions were sonicated for 2 min using a Sonics & Materials ultrasonic homogenizer (750 W, 50% amplitude).

Determination of CatPEC charge densities and particle sizes

The charge densities of the CatPECs were determined by polyelectrolyte titration at pH 5.0 and 0.01 N NaCl. A 200 μN potassium polyvinyl sulphate (KPVS) solution supplied by AppChem (UK) was used as the titrant.

The particle sizes of the CatPECs were determined using a Zetasizer Nano ZS90 apparatus (Malvern Instruments, UK). All measurements were made at 25 °C and pH 5.0. The refractive index of water (1.334) was used due to the high water content of the CatPEC particles (Cegnar and Kerč 2010; Mocchiutti et al. 2016).

Preparation of films

CatPEC suspensions (Xyl/Ch mass ratio: 70/30, 75/25, 80/20, and 85/15 with 10 wt% glycerol, relative to the total dry mass) were cast into silicone molds and heated for 10 min in a water bath at 75 °C in order to remove air. The samples were then placed in a fan-forced air circulation oven at 40 °C for approximately 48 h for drying. Then, the films were allowed to slowly dry in a conditioned room at 50% RH and 23 °C. When necessary, they were stored in a desiccator containing a saturated solution of magnesium salt, $Mg(NO_3)_2$, in order to obtain a RH of 50%.

Reference films with only one component—i.e. 100% xylan films (100/0) and 100% chitosan films (0/100)—in the presence of 10 wt% glycerol, relative to the total dry weight, were also prepared from xylan and chitosan solutions at pH 5.0, respectively.

Barrier properties

Water vapor transmission rate (WVTR)

The WVTR of the films was determined gravimetrically according to ASTM E96-98. According to this standard, the test was carried out under controlled conditions (23 °C, 50% RH), using a cup of polypropylene with a vapor interchange area of 39.6 cm^2 and containing 10 mL of distilled water (water method). The film was mounted on the top of the cup and its weight was monitored every 30 min for 5 h. A weight–time curve was constructed, and linear regression was used to estimate the slope of the straight line. An average of three measurements is reported for each sample.

Oxygen permeability (OP)

The oxygen permeability of the films was determined at 23 °C and at 50% and 80% RH after conditioning the samples for 24 h. The ASTM D 3985-02 procedure was followed using a Systech 8001 oxygen permeation analyzer (Systech Instruments Ltd., UK), with 100% oxygen as the test gas. At 50% RH, the area used was 50 cm^2, and at 80% RH, a metal mask was used to reduce the area to 5 cm^2.

Solubility

The conditioned films at 50% RH and 23 °C were cut into samples of 1.2 cm^2. They were weighed with a precision of ± 0.1 mg and their moisture content simultaneously determined gravimetrically in a vacuum oven at 60 °C for 24 h (W_{dry}). The samples were immersed in a buffer solution at pH 5.0 for 24 h. After this period of time, the samples were removed from the solution, and the excess liquid was mildly removed by pressing them between two blotters. The films were vacuum-dried at 60 °C for 24 h and their weight recorded (W_{final}). Solubility (S) was calculated according to Eq. (1). An average of five measurements is reported.

$$S = \left(\frac{W_{dry} - W_{final}}{W_{dry}} \right) * 100 \qquad (1)$$

Mechanical properties

Tensile strength and strain at break

The tensile strength and strain at break of the films were determined using an Instron® 3344 universal testing machine (Instron, USA), with a load cell of 1000 N, at an extension rate of 5 mm/min and an initial grip distance of 22 mm. The evaluation was performed according to the ASTM D882 standard test for tensile properties of thin plastic sheeting. For each treatment, six bone-shaped samples were cut according to ASTM D1708-9, similar to Pereda et al. (2008).

The mechanical properties of the films after immersion in a buffer solution at pH 5.0 for 24 h and re-drying were also studied.

Effect of relative humidity on storage modulus

The effect of RH on the films' storage modulus (E') was studied using DMA. Measurements were made using a TA Instruments Q800 equipped with an external humidity element (Kep Technologies Wetsys Setaram Instrumentation) connected to the measuring chamber. DMA measurements used a constant frequency of 1 Hz and amplitude of 15 μm. Film samples of 10 × 5.3 mm^2 were exposed to 0% RH followed by 80% RH, with each humidity level maintained for 60 min at 25 °C. This procedure was cycled three times. The E' values were continuously registered as the RH was changed.

Surface morphology

The upper surface morphology of the 70/30 and 85/15 films was studied using scanning electron microscopy (SEM; Phenom ProX, Phenom-World, Netherlands) with a temperature-controlled sample holder. The samples were mounted on metal grids using double-

sided adhesive tape. The morphological studies were conducted under a vacuum.

Crystallinity

The crystallinity of the films was analyzed using X-ray diffraction (XRD) on a Philips Analytical X-ray diffractometer with Cu Kα radiation at 45 kV and 30 mA. Diffractograms were recorded over a range of 5° to 30° (2θ) at a rate of 1.5°/min.

Antimicrobial assay

Antimicrobial activity tests were carried out using agar diffusion. The test organisms used were *E. coli* and *S. aureus* (coagulase +), and they were grown on nutrient agar (Biokar). Film discs 20 mm in diameter were placed on agar plates that had been seeded with a volume of inoculum containing indicator microorganisms in a range of 10^6–10^8 CFU/mL (Dehnad et al. 2014). The microorganisms were incubated at 37 °C for 24 h. Experiments were carried out in duplicate.

Results and discussion

Characterization of the cationic polyelectrolyte complexes (CatPECs)

Charge densities, particle sizes, and polydispersity indexes of the CatPECs prepared at different xylan/chitosan mass ratios were measured and the results are summarized in Table 1. Results show that all prepared CatPECs had low cationic charge densities. Taking into account the charge densities of the polyelectrolytes, they showed lower charge densities than expected, probably due to deviations from 1:1 stoichiometry during complex preparation, as was previously reported by others using poly(allylamine hydrochloride) and xylan (Galván et al. 2015). The table also shows that with increasing amounts of xylan, the charge density of the CatPECs decreased. The charge densities of the CatPECs prepared at 80/20 and 85/15 xylan/chitosan mass ratios were near zero.

On the other hand, a mono-modal distribution with small particle size was observed at a 70/30 mass ratio, while two populations were observed in those samples with increasing amounts of xylan (75/25). The population of larger particle size indicates that some aggregates were formed.

At 80/20 and 85/15 mass ratios, unstable complexes were formed, and the size of the CatPECs could not be determined by the technique used (DLS). For both cationic and anionic particles, irreversible flocculation generally occurs close to neutrality, as previously reported (Galván et al. 2015; Korhonen et al. 2013).

Characterization of films

Barrier properties

The WVTR of the films was measured at 50% RH, while OP was measured at 50% RH and 80% RH. As shown in Table 2, all films showed moderate WVTR

Table 1 Charge density, average particle size, and polydispersity (PdI) of the different complexes prepared at pH 5.0 and ionic strength of 0.03 M followed by sonication

Xylan/chitosan ratio (w/w)	Charge density meq/g	Average particle size[a] (nm)	PdI
70/30	+0.55 ± 0.03	492 ± 44 (100%)	0.17
75/25	+0.33 ± 0.03	663 ± 81 (84%)	0.21
		4447 ± 583 (16%)	
80/20	+0.11 ± 0.01	ND	ND
85/15	+0.06 ± 0.01	ND	ND

ND = values could not be determined using DLS

[a] The intensity particle size distribution (PSD) curve was used for reporting the size of each peak, and the volume PSD distribution curve was used for reporting the relative amounts of each peak in the distribution, according to the instrument operating instructions. Standard deviations of the means from six replicates of the trial are indicated

Table 2 Water vapor transmission rate (WVTR) and oxygen permeability (OP) of the films measured at 23 °C and 50% RH and 80% RH

Xylan/chitosan ratio (w/w)	WVTR (50% RH)[a] (g/h m^2)	OP (50% RH)[b] (μm cm^3/day m^2 kPa)	OP (80% RH)[b] (μm cm^3/day m^2 kPa)
70/30	21.3 ± 0.8	0.265 ± 0.001	626 ± 29
75/25	21.1 ± 1.9	0.133 ± 0.07	556 ± 21
80/20	18.9 ± 1.3	0.054 ± 0.02	551 ± 19
85/15	19.6 ± 0.3	<0.01	311 ± 1

[a] The standard deviations of the means from three replicates of the trial are indicated

[b] The standard deviations of the means from two replicates of the trial are indicated

and low OP results (at 50% RH), which decreased with increasing amounts of xylan. This behavior was attributed to the tight hydrogen bond networks formed between the xylan chains that contribute to good packing of the material.

The low OP values obtained were on the same order as those reported for commercial synthetic polymeric films of ethylene vinyl alcohol (EVOH): Hansen and Plackett (2008) reported OP values of 0.3 cm^3 μm/m^2 day kPa, at 23 °C and 50% RH for EVOH films. On the other hand, compared to the results obtained by Luo et al. (2014) using 100% chitosan films soaked in 3% w/v xylan solution (OP of 7.3 μm cm^3/day m^2 kPa at 0% RH and 23 °C), the films obtained in this work showed better oxygen barrier properties, even though the RH used here was higher (50% RH).

The table also shows that the OP was increased by three orders of magnitude when RH was increased from 50 to 80%, indicating that these films are highly sensitive to RH changes. These results were expected, considering the hydrophilicity of xylan (Mikkonen and Tenkanen 2012) as well as chitosan due to the high content of hydroxyl groups. EVOH films are also highly sensitive to humidity, a property that impairs its performance in high-RH environments (Faisant et al. 1998).

Due to their excellent oxygen barrier properties, the films developed in the present work could be used to extend the shelf life of fatty dry foods such as crisps or potato chips, and others such as cereal bars. However, to achieve a commercial-level product, the moisture resistance of the films should be further improved. Another alternative for extending the use of the films is to prepare multi-layer films, combining Xyl/Ch and other water vapor-resistant materials such as LDPE.

Solubility

Solubility is an important property for characterization of films. In the case of edible films, high water solubility is beneficial; however, for applications such as packaging, low water solubility is required to maintain product integrity over time (Goksu et al. 2007).

Table 3 shows the solubility values as well as the amounts (percentages) of sodium acetate/acetic acid, sodium chloride, and glycerol present in the films before immersion in buffer solution. All solubility values obtained were relatively high (26.5–35.4%). Nevertheless, all films maintained their integrity after 24 h of immersion, and their mechanical properties were determined and are reported in the following section. The table also shows that increasing the amount of chitosan in the films resulted in slightly increased solubility. Wang et al. (2014) obtained higher solubility results (35–50%) for composite polyvinyl alcohol/xylan films with citric acid as plasticizer after immersion in water at 23 °C and for 24 h.

Taking into account that the content of inorganic compounds and plasticizer present in the film before immersion in buffer solution are in agreement with the experimental solubility results, it could be suggested that these compounds were dissolved in the buffer solution.

Tensile strength and strain at break

Figure 1a shows the stress–strain curves of the films before immersion in buffer solution at pH 5.0 for 24 h. Increasing the amount of chitosan resulted in a

Table 3 Percentages of inorganic compounds and plasticizer present in the films, and solubility values of films at 23 °C, pH 5.0, and for 24 h

	Solubility (%)	Content of sodium acetate/ acetic acid, sodium chloride, and glycerol in the film (%)
70/30	33.2 ± 1.4	36.3
75/25	35.4 ± 2.2	34.2
80/20	33.4 ± 6.8	31.8
85/15	26.5 ± 1.6	29.3
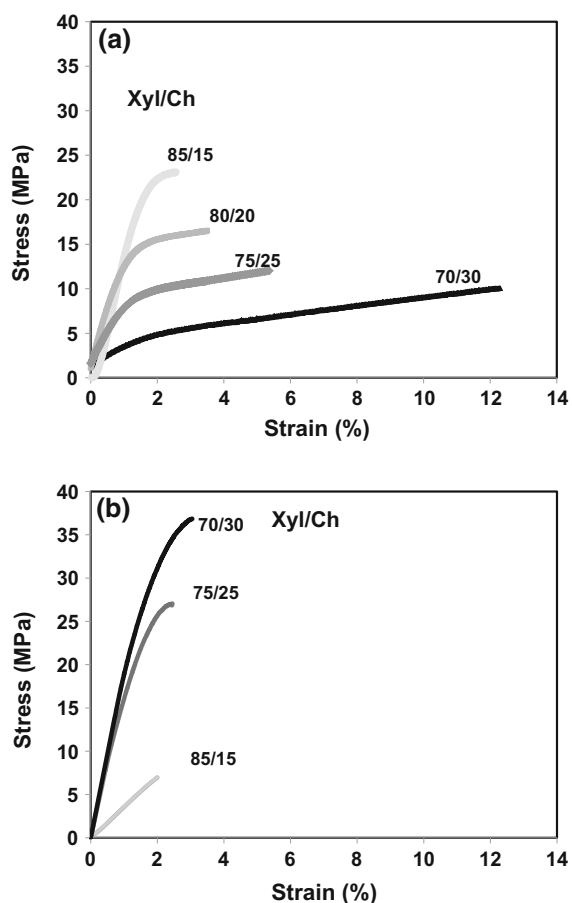
Fig. 1 Stress–strain curves of dried films **a** before and **b** after immersion in buffer pH 5.0 for 24 h and re-drying. The properties were measured at 23 °C and 50% RH. Film thickness was between 50 and 120 μm

reduction in the stress response (from 23.1 to 10 MPa), accompanied by a significant increase in the strain at break, reaching 12.2% in the case of the 70/30 film. Crystallization is easier to achieve in linear and low-molecular mass polymers, such as xylan, than in linear

and high-molecular mass polymers such as chitosan. Thus, increasing the amount of xylan in the film would be expected to lead to a notable increase in the intermolecular hydrogen bonds and the crystallinity of the films. As a consequence, the resulting films exhibit higher stress at break, but with a marked decrease in ductility (lower strain at break).

When compared with the mechanical properties reported by Bastarrachea et al. (2011) for common plastic films used for food packaging, the developed films showed tensile strength values similar to LDPE films (tensile strength of 7–25 MPa) and higher strain at break than polystyrene films (strain at break of 2–3%).

After immersion in buffer solution for 24 h and re-drying (Fig. 1b), the strain at break decreased significantly for all films, and the stress at break increased, as expected, due to the removal of the glycerol plasticizer. The results also show that the mechanical response of the films was opposite that obtained in Fig. 1a. One possible explanation is that the Cat-PECs—in particular, the free chitosan chains—swell during immersion of the film in buffer solution, while glycerol is dissolved and removed from the films. When the films are re-dried, a rearrangement of the chitosan chains occurs, with subsequent creation of new bonds that generate increased stress.

Figure 2a, c shows a photograph and a SEM image of the 70/30 film, respectively. This particular film was not only the most deformable and flexible, but also the most transparent and uniform of all those evaluated. Figure 2b, d shows a photograph and a SEM image, respectively, of the film prepared using the highest amount of xylan (85/15). In this film, visible aggregates can be observed. According to Fig. 1a, the 85/15 film showed a higher stress at break than the 70/30 film, but its strain at break was very low (2.6%) and the film was brittle.

It is worth mentioning that the mechanical properties of the reference films (100% xylan films and 100% chitosan films, both with 10 wt% glycerol added) were also investigated. The 100% chitosan films were opaque and they could not be tested, as they presented several wrinkles at 50% RH. Other authors have reported good film formability of 100% chitosan films (Kaaraslan et al. 2010; Kim et al. 2006), as was described in the introduction section. However, in most of these cases, the processing of chitosan was radically different from the methodology described in
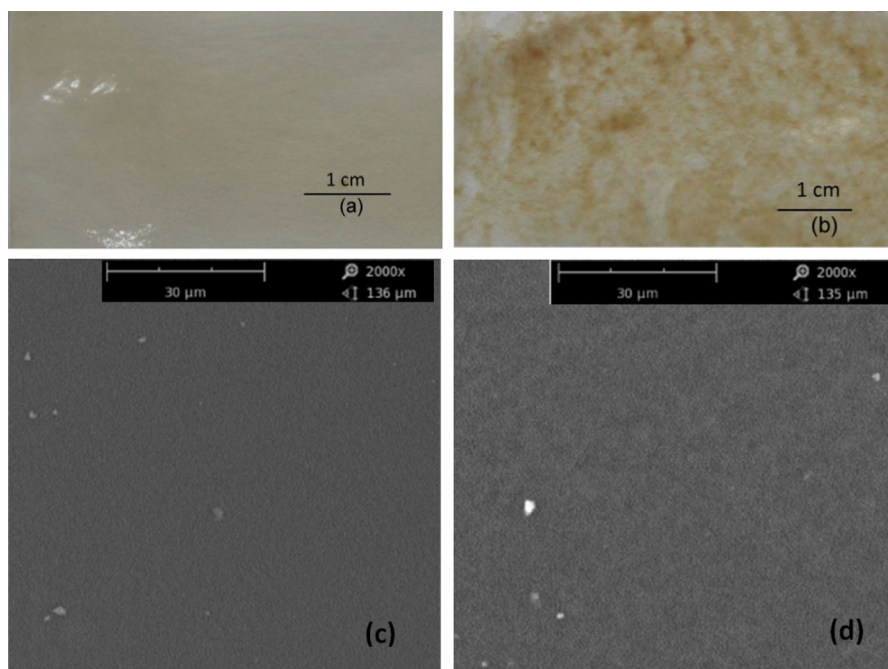
Fig. 2 Photographs (**a**, **b**) and SEM images (**c**, **d**) of xylan/chitosan films at different mass ratios: Xyl/Ch 70/30 (**a**, **c**) and Xy/Ch 85/15 (**b**, **d**). All films were formed in the presence of 10 wt% glycerol, relative to the total mass

this work. The 100% xylan films were too brittle and too fragile to undergo mechanical testing, similar to observations reported by others (Gröndahl et al. 2004).

Taking into account the mechanical properties, visual aspects, and the ability to handle the films without breakage, the 70/30 film (Fig. 2a) was selected for more detailed characterization than the other films. For comparison purposes, the films prepared with the highest xylan content (85/15) were also characterized (Fig. 2b).

Effect of relative humidity on storage modulus

The effect of humidity cycles on the viscoelastic properties of the 70/30 and 85/15 films was studied using DMA. Figure 3 shows the E' values, which are related to the elastic behavior of the film, as a function of time when the RH was continuously changed from 0 to 80% in cycles. Results show that the storage moduli of the two films were similar and were highly dependent on environmental conditions. At 0% RH, E' values were between 208 and 230 MPa. As RH was increased to 80%, the E' values of the films exhibited a sharp decrease to values near zero, indicating that the viscous behavior of the material was increased. Based
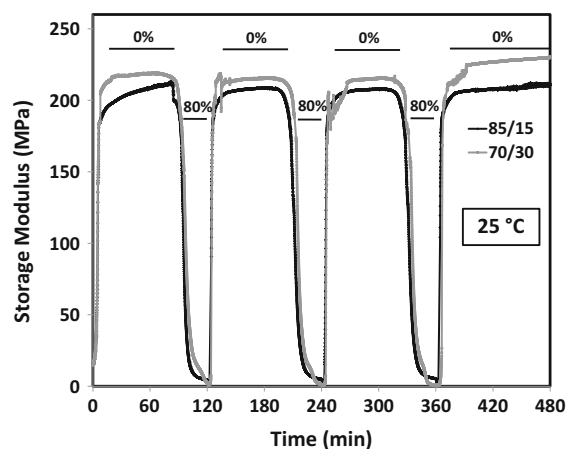

Fig. 3 Storage modulus of the films as a function of time when the relative humidity was continuously changed from 0 to 80%

on these results, the increase in the ability of the Xyl/Ch films to take up water at high RH conditions impedes the mechanical performance of the films, with the water molecules acting as plasticizers of the material. However, the E' values are fully recovered when humidity is reduced to 0% RH after each 0–80% RH cycle, suggesting that the films can withstand high humidity conditions.

Crystallinity

Figure 4 shows the XRD patterns of 100% xylan (100/0) and xylan/chitosan films (70/30 and 85/15). The diffractogram shows five distinct crystalline peaks at 2θ: 10.9, 12.4, 19.2, 22.6, and 25.2, where the two main peaks were at $2\theta = 19.2°$ and $2\theta = 22.6°$. These results are in agreement with those published by Horio and Imamura (1964) for membranes of beech xylan, which showed crystalline peaks at 2θ: 11.2, 12.6, 19.4, 22.6, and 25.3. According to Šimkovic et al. (2014) and Horio and Imamura (1964), increasing the content of 4-*O*-methyl-D-glucuronic acid in the xylan results in a decrease in the intensity of the crystalline peaks, and slight shifts can also be detected in the diffractogram.

Peng et al. (2011) also reported the observance of two main peaks in the XRD pattern for films of xylan isolated from bamboo holocellulose, but at $2\theta = \sim 18°$ and $2\theta = \sim 22.7°$. The authors suggested that these peaks could be attributed to the reorganization and association (e.g. hydrogen bonds) of the xylan chains during film formation, resulting in crystalline regions in the film.

Nieduszynski and Marchessault (1972) analyzed the structure of hydrated xylan from white birch by X-ray fiber diffraction and computer-aided chain-packing. They found that water was required to stabilize the lattice structure of xylan, and thus the c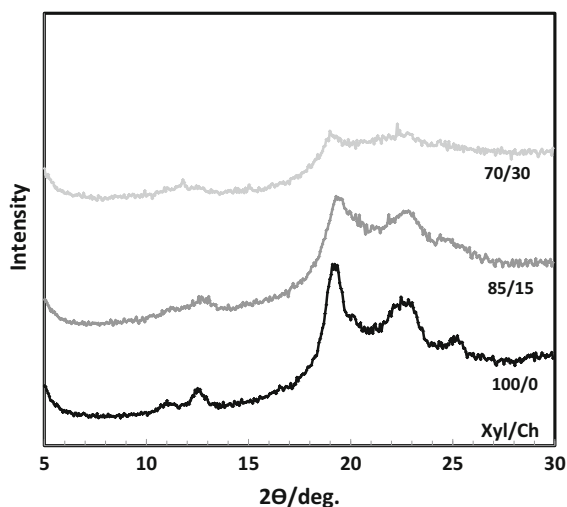onformation adopted by xylan hydrate was determined by the threefold screw helix, for which intermolecular forces play a dominant role.

On the other hand, the XRD patterns (Fig. 4) also reveal that the formation of complexes reduced the crystallinity of the 100% xylan film. As the amount of chitosan was increased, the film crystallinity decreased. Comparable behavior has been observed in similar systems by other authors (Gabrielii et al. 2000; Karaaslan et al. 2010). The film prepared at the 70/30 mass ratio in this work showed the widest and most amorphous XRD peaks. The low crystallinity, together with the strong ionic interaction between oppositely charged polyelectrolytes, could explain the acceptable dry strength of the film and its integrity in buffer solution.

Antimicrobial activity

The antimicrobial activity of the films (70/30 and 85/15) against *E. coli* (gram-negative) and *S. aureus* (gram-positive) bacteria was also tested. Figure 5a, b shows that the 70/30 Xyl/Ch film exhibited inhibition of both microorganism only in the contact area under the film discs. Neither film revealed a zone of inhibition surrounding the discs, suggesting that neither component with antimicrobial properties migrated (diffused) to the agar medium. A similar inhibitory behavior was found by Dehnad et al. (2014) using the agar disc diffusion method to evaluate the antimicrobial activity of chitosan-nanocellulose films against *S. aureus*, *E. coli*, and *S. enteritidis.*

According to Kong et al. (2010), the antibacterial action of chitosan depends on the media pH, among other conditions. When pH values are below the pK_a of chitosan, the electrostatic interaction between the cationic amino groups of chitosan and the negatively charged microbial cell membranes are primarily responsible for the antibacterial activity (Kong et al. 2010). Papineau et al. (1991) and Dutta et al. (2009) reported that this interaction resulted in the escape of proteinaceous and other intracellular constituents of the microorganisms, causing the cells to become inactive.

Considering that the films prepared in this work were obtained from CatPECs at pH 5.0, it is likely that the free amino groups of chitosan caused the inhibitory effect against the microorganisms.
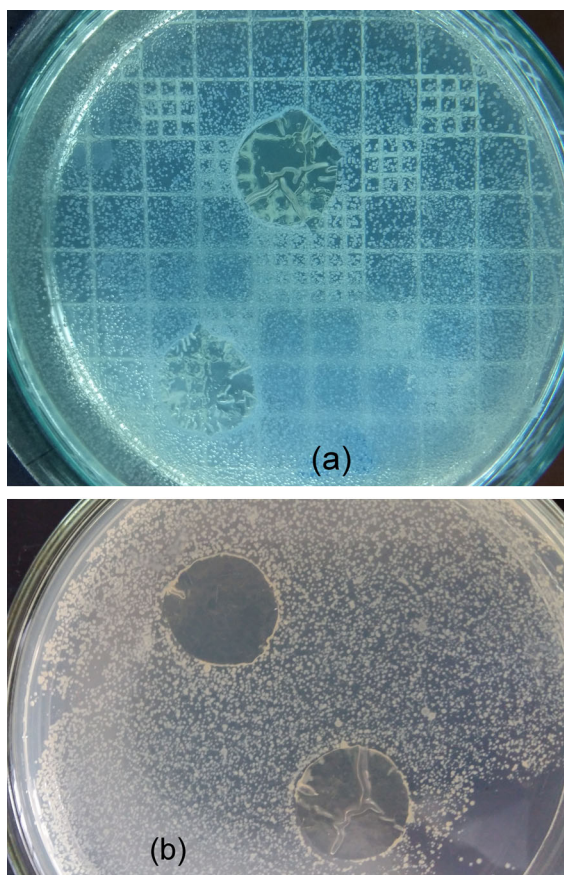

Fig. 4 XRD patterns of the films with different xylan/chitosan mass ratios

Fig. 5 Inhibition area of 70/30 films against **a** *Escherichia coli* and **b** *Staphylococcus aureus*

The 85/15 films did not cause inhibition of the microorganisms in either the contact area or the zone surrounding the discs (data not shown), probably due to the lack of sufficient cationic free amino groups.

Conclusions

In this work, a simple-stage procedure was described for obtaining natural xylan-rich films from colloidal suspensions of polyelectrolyte complexes of xylan from beechwood and chitosan.

All films showed promising mechanical properties. The film prepared with a xylan/chitosan mass ratio of 70/30 and in the presence of low amounts of glycerol was the most transparent, uniform, and deformable, and the easiest to handle. It also showed antimicrobial activity when tested against *E. coli* and *S. aureus* bacteria.

At 50% RH, all films showed a moderate WVTR and high oxygen barrier properties. When environmental RH values were increased to 80% RH, however, the oxygen permeability was significantly affected.

The DMA results revealed that the elastic behavior of the 70/30 film was markedly reduced by the plasticizing action of the water taken up in high humidity conditions (80% RH). However, this effect was largely reversed when humidity was eliminated (0% RH).

Considering the results obtained in this work, xylan/chitosan-based films appear to be a promising, environmentally friendly alternative for use in food packaging materials.

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