



Stability of methylcellulose-based films after being subjected to different conservation and processing temperatures

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ABSTRACT

Methylcellulose films with and without sorbitol addition were developed. The major objective of this study was to attempt insights into the stability of the methylcellulose-based film properties after having been subjected to freezing, storage or a combination of both procedures. The importance of the sorbitol concentration and process temperature was also to be elucidated. As-prepared film solubility decreased at 100 °C, as a result of the methylcellulose thermogelation property when the samples were exposed to high temperatures.

By analyzing the film pattern behavior and its properties 0.25% w/v sorbitol concentration turned out to be an inflexion point. The moisture content as well as the mechanical and thermal properties made this fact evident. Moreover the elastic modulus (E_c) and glass transition temperature (T_g) did not undergo significant changes for higher plasticizer concentrations. The methylcellulose film properties remained more stable in the presence of sorbitol, which would act as a protective agent due to its hydrogen bonding capacity. This stability is crucial for film and coating applications in the food industry.

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1. Introduction

Cellulose is the most abundant polysaccharide, which occurs mainly within the cell wall of higher plants as a structural material [1,2]. Modification of cellulose with hydrophobic side groups disrupts the rigid crystalline structure stabilized by strong intermolecular hydrogen bonding and improves the polysaccharide's water affinity [3,4]. An optimum level of substitution is required for an appropriate water solubility, which is usually between 1.4 and 2.0 [5]. The simplest derivative of cellulose, methylcellulose (MC), which has methyl groups substituted for hydrogen, forms a clear viscous polymer in an aqueous environment [3]. Methylcellulose is useful for a variety of applications because of its low cost and its unique ability to gel at elevated temperatures [5]; it is also used in the pharmaceutical and food industry as a thickener, water binder and film forming agent [6–8]. According to their physico-chemical properties, MCs can be also employed as emulsifiers, medicine constituents, colloidal stabilizers, viscosity and flow controllers.

Even though water is a ubiquitous natural plasticizer, the effects of water on molecular mobility including plasticization are poorly understood mainly because molecular and structural analyses are scarce. Kilburn et al. [9] concluded that the plasticization effect of water in carbohydrates is via a complex mechanism involving both hydrogen bond formations and disruption and changes in the matrix free volume. In the

dry state, the hydrogen bonding between carbohydrate molecules leads to the formation of large molecular entities. When water is absorbed, it disrupts the hydrogen bonds between the carbohydrate chains [10]. Plasticizers, which are low molecular weight components, increase the free volume of the material or the macromolecular mobility of the polymer, and consequently the polymeric network becomes less dense due to the decrease in intermolecular forces, thus improving the extensibility and flexibility of the films [11–13]. The most effective plasticizers will more closely resemble the structure of the polymer they plasticize. Thus, the plasticizers most commonly used in starch and cellulose-based films are polyols, such as sorbitol [14,15]. Sorbitol is a common pharmaceutical excipient used to improve the stability of proteins and other drug molecules during freezing, drying and storage [16].

In order to avoid food deterioration, different methods are used. One of them is freezing, which is one of the most widespread preservation techniques. The use of edible coatings could have a beneficial effect on the quality of frozen food, since they act as a barrier against moisture transfer and oxygen uptake [17]. Several studies have shown that edible coatings help to control quality deterioration during frozen fish storage and to prolong its shelf life [18,19].

Although several researches have been conducted on methylcellulose films and films plasticized with sorbitol, their combination in the same system is not frequent [11,20,21]. To the best of our knowledge, studies on the properties of MC films after being submitted to freezing and storage have hardly been reported. Thus, the major objective of this study was to attempt insights into the stability of the methylcellulose-based film properties after having been subjected to freezing, storage or a combination of both procedures. The importance of the processing

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and conservation temperature and the sorbitol concentration was also to be elucidated.

2. Materials and methods

2.1. Hydrocolloid solutions

Methylcellulose (A4M, Methocel) was provided by COLORCON S.A. (Argentina). According to specifications of the supplier, the molecular weight was 65.10^3 Da. To prepare the hydrocolloid solution, 1.5 g methylcellulose (MC) was slowly dispersed in 50 mL of hot distilled water (80 °C) under constant stirring for 1 h. Once a homogeneous system was obtained, a total volume of 100 mL was made up with cold distilled water and the solution was kept under stirring until it attained room temperature.

Sorbitol (Merck, USA) was added as a plasticizer after MC was completely dissolved at different concentrations (0.25, 0.5, 0.75 and 1.0% w/v). Each experiment was made in triplicate.

2.2. Film preparation

The film-forming solution weight (about 20 g) with and without plasticizer casted onto Petri dishes (9 cm diameter) was controlled in order to guarantee a constant film thickness and dried at 37 °C in an oven until reaching a constant weight. The obtained films were removed from the dish and afterwards they were stored at 20 °C and 65% RH in a controlled room.

Film thickness was determined by using a coating thickness gauge Check Line DCN-900 (New York, USA) for non-conductive materials on non-ferrous substrates. The informed values correspond to the average of at least fifteen measurements at different positions for each specimen.

Samples were divided in different groups for the assays.

A: unplasticized and plasticized as-prepared samples.

B: unplasticized and plasticized samples stored at room temperature for 3 months.

C: unplasticized and plasticized samples frozen and stored for 3 months, to study both effects jointly.

D: unplasticized and plasticized samples frozen and immediately thawed to study the effect of the freezing process separately from the storage process.

Film will be named as-prepared, stored, frozen-thawed and frozen-stored or groups A, B, C and D from here onwards, interchangeably. Within every group, film nomenclature used will be: MC for methylcellulose films and MC-0.25S, MC-0.5S, MC-0.75S and MC-1S for films containing 0.25, 0.5, 0.75 and 1% sorbitol, respectively. In addition, as-prepared MC was used as a principal control, whereas films of group A were used as secondary controls (as-prepared MC-0.25S, MC-0.5S, MC-0.75S and MC-1S).

2.3. Physicochemical characterization

2.3.1. Moisture content

As-prepared film moisture contents were determined by measuring their weight loss, upon drying in an oven at 105 ± 1 °C until reaching constant weight (dry sample weight). Samples were analyzed at least in triplicate and results were expressed as grams of water per 100 g of dry sample.

2.3.2. Film solubility and water uptake

As-prepared MC-based films were cut in 30×30 mm pieces to determine both water uptake and film solubility. To determine the solubility, the samples were weighed, immersed into distilled water at 20 and 100 °C, and maintained under constant agitation for 1 h.

After soaking, the remaining pieces of the films were dried again in an oven at 105 ± 1 °C until reaching constant weight. Film solubility (%) was calculated as it was described by Rivero et al. [22].

Water uptake was measured by immersion of previously weighted film pieces in 80 mL distilled water. One hour after, samples were recovered and dried with filter paper to remove the excess of surface water and weighted again (film wet weight). The water uptake of the films was calculated as follows,

$$\% \text{ Water uptake} = \left[\frac{(W_t - W_i)}{W_i} \right] \times 100 \quad (1)$$

where: W_t is the weight of the swollen sample at time t and W_i is the weight of the film before immersion. Samples were analyzed at least in triplicate.

2.3.3. Water activity

Water activity of as-prepared samples was evaluated by using an AquaLab Water Activity Meter (Decagon Devices, Inc., Washington, USA) equipment. The measurements were performed in triplicate.

2.3.4. Water vapor barrier properties

Water vapor permeability (WVP, $\text{g m}^{-1} \text{s}^{-1} \text{Pa}^{-1}$) tests were performed on groups A, B, C and D, by using a modified ASTM method E96 [23] as it was described in previous work [21].

Each sample was sealed over a permeation cell which was maintained at 20 °C. A driving force of 1753.55 Pa, corresponding to a 75% RH gradient across the film was used. After steady state conditions were reached, the permeation cells were weighed (0.0001 g) at a 1 h interval for 8 h. The reported values correspond to the average of at least three determinations.

2.3.5. Differential scanning calorimetry (DSC)

Film thermal properties were determined by using a DSC model Q100 controlled by a TA 5000 module (TA Instruments, New Castle, USA), with a quench-cooling accessory, under a N_2 atmosphere (20 mL min^{-1}).

Film samples of 6–7 mg were weighed in aluminum pans and were hermetically sealed; an empty pan was used as reference. Samples were analyzed at a heating rate of 10 °C min^{-1} . The first scan was performed from -100 °C up to 250 °C to limit possible methylcellulose degradation. After the first scan was completed, the sample was cooled until -100 °C and then a second scan was recorded between -100 and 350 °C . The pans were punctured and dried until reaching constant weight at 105 °C to obtain the dry weight of the samples.

From the thermograms, glass transition temperature T_g was obtained using the Universal Analysis V1.7F software (TA Instruments).

2.3.6. Dynamic mechanical analysis (DMA)

DMA assays were conducted in a dynamic-mechanical thermal equipment Q800 (TA Instruments, New Castle, USA) using a tension clamp with a liquid N_2 cooling system. Film probes with a rectangular geometry (6 mm width and 30 mm length) were assayed. Amplitude sweep from 1 to 50 μm at a fixed frequency (5 Hz) was performed. Multi-frequency sweeps (1, 5, 10 and 15 Hz) at fixed amplitude from -100 to 250 °C at 5 °C/min were carried out, with an isotherm of 10 min at -100 °C . Storage (E'), loss (E'') modulus and $\tan \delta$ (E''/E') curves as a function of temperature were recorded and analyzed using the software Universal Analysis 2000. Temperatures of the relaxation processes associated to glass transition temperatures were determined through the inflexion point of the maximum peak in the $\tan \delta$ curves.

2.3.7. Film stress–strain behavior

Probes of 6×30 mm were used to analyze the tensile stress–strain behavior of the MC films with and without plasticizer through the DMA TA Instruments-Q 800, using the tension clamp as was described in previous work [21]. Tests were conducted on samples from groups A

at -20 , 5 , 25 , 100 and 180 °C, which are characteristic temperatures of food processing and conservation. Samples from groups B, C and D were studied at 5 and 100 °C, to analyze their behaviors at low and high temperatures after they were subjected to the different processes. At least, five replicates of each condition were measured.

2.3.8. Fourier transform infrared spectra of the films

The Fourier transform infrared (FT-IR) spectra of the films were recorded in an IR spectrometer (Nicolet, iS10, Thermo Scientific, Madison, USA) in the wavenumber range 4000 – 400 cm^{-1} by accumulation of 64 scans at 4 cm^{-1} resolution. The spectral deconvolution of the data was performed using the software Omnic 8 (Thermo Scientific, Madison, USA).

2.3.9. X-ray diffraction

Plasticized and unplasticized methylcellulose films were analyzed by X-ray diffraction in an X'Pert Pro P Analytical Model PW 3040/60 (The Netherlands). The $\text{Cu K}\alpha$ radiation (1.542 Å), operating at room temperature, was generated at 40 kV and 30 mA, and the relative intensity was recorded in scattering over an angular range (2θ) of 3 – 60° with step size 0.02 .

Crystallinity degree (CD) was evaluated on the basis of the main peak areas; it was calculated as the ratio between the absorption peaks area and the diffractogram total area and expressed as percentage (%) [24,25]. The reported values corresponded to the average of two determinations.

2.3.10. Microscope observations at low vacuum

Scanning electron microscopy (SEM) analysis was performed with an FEI model Quanta 200 electron microscope (The Netherlands). For low vacuum SEM, the samples were mounted onto bronze stubs using a double-sided tape and examined without any metal or carbon coating using the environment scanning electron microscopy (ESEM), at an acceleration voltage of 25 kV.

2.4. Statistical analysis

All experiments were performed at least in duplicates, with individually prepared and casted films as replicated experimental units as it was described previously in each determination.

Analysis of variance was conducted separately for the dependent variables of moisture, solubility, water activity, water vapor permeability, glass transition temperature and elastic modulus. The independent variables of the design were: treatment (frozen or unfrozen), storage (0 or 3 m) and sorbitol concentration (0 , 0.25 , 0.5 , 0.75 , 1%).

For simultaneous pairwise comparisons, LSD's test was chosen. Differences in means and F-tests were considered significant when $P < 0.05$. Confidence intervals of the means were also calculated at $P < 0.05$. All statistical procedures were computed using the SYSTAT software (SYSTAT, Inc., Evanston, USA) version 10.0.

3. Results and discussion

MC films with and without sorbitol were homogeneous, thin, and easy to handle in all concentrations assayed. The films were removed without difficulty from the acrylic plates. They had thickness values of 35 ± 5 μm .

3.1. Solubility and moisture content of as-prepared films

When as-prepared based-MC films were immersed in water at 20 °C, the samples underwent swelling and matrix dissolution, losing their structural characteristics and showing a gelatinous aspect. Although the fragments of the transparent gel remained in the medium, they were too soft to be quantified. These results were comparable to those obtained by Lim and Wan [26] working on chitosan films. Instead,

at 100 °C, unplasticized films showed only 8.7% of solubility, which increased with the increasing sorbitol concentration, ranging from 24 to 48% , for MC-0.25S and MC-1.0S, respectively (Table 1).

On the other hand, MC-based film moisture did not show significant differences among samples with plasticizer concentration higher than 0.5% . The average moisture value was almost 44% higher than the value obtained for MC film.

3.2. Film stress–strain tests

According to Mancini et al. [27] and Del Nobile et al. [28] a mathematical model able to describe the stress–strain curve is:

$$\sigma_T(\varepsilon_T) = E_C \varepsilon_T \exp(\varepsilon_T K) \quad (2)$$

where: ε_T and σ_T (MPa) are the true strain and the true stress, respectively, E_C (MPa) is the elastic modulus (the tangent to the stress–strain curve at the origin) and K is a constant considered as a fitting parameter.

The relationship between stress and strain, which corresponds to Young's modulus, was here affected by an exponential term acting as a damping factor [28]. The E_C parameter could be estimated, which allowed characterizing the elastic region of the curves, meaning that the points immediately before rupture were not considered to obtain a better fitting of the experimental points at initial and intermediate deformations.

The films were tested at different temperatures at a fixed value of sorbitol. In addition, the behavior with increasing sorbitol concentrations at a fixed value of temperature was evaluated. The model (Eq. (2)) used to estimate the elastic modulus from stress–strain curves fitted the experimental data satisfactorily ($R^2 > 0.99$) for unplasticized MC as well as for MC films containing sorbitol for all the temperatures assayed.

Fig. 1a shows as an example the mechanical properties of as-prepared MC-0.25S at -20 , 5 , 25 and 100 °C. The mechanical behavior of MC-0.25S films at -20 °C exhibited values of stress and strain of about 47 MPa and 0.04 , respectively. As temperature rose to 100 °C, the film strain increased significantly ($P < 0.05$) reaching a value of 0.2 . However, at a fixed strain value the stress dramatically decreased with the increase in the temperature. Films without sorbitol showed a similar trend; for samples with the highest concentrations of sorbitol, the stress value turned out to be half the value of MC-0.25S.

Fig. 1b exhibits the mechanical behavior at 5 °C of the frozen-stored films with sorbitol concentration comprised between 0 and 1% . A marked change in the mechanical profile was produced for a sorbitol concentration higher than 0.25% since more flexible materials were obtained. Concentrations of 0.5 and 0.75% almost tripled the strain values obtained without sorbitol whereas with the addition of 1% of plasticizer, it was six times as high as MC. When testing the samples at 100 °C, a similar trend was observed.

These findings are consistent with those reported by Srinivasa et al. [29], who observed that the addition of 0.5% sorbitol increased the percentage of elongation of chitosan films dramatically.

Taking into consideration the E_C values calculated from Eq. (2), a linear relationship of E_C with the temperature for as-prepared unplasticized

Table 1

Moisture and solubility values of as-prepared methylcellulose films with different sorbitol concentrations.

	Moisture (%)	Solubility (%) ^A
MC	10.09 ^a	8.69 ^a
MC-0.25S	11.40 ^{a, b}	24.04 ^b
MC-0.50S	14.35 ^b	34.20 ^c
MC-0.75S	14.55 ^b	42.40 ^d
MC-1.0S	14.58 ^b	47.98 ^e

^{a,b,c,d,e} Different letters within columns indicate significant differences ($P < 0.05$) between films with different sorbitol concentrations.

^A Determined at 100 °C.

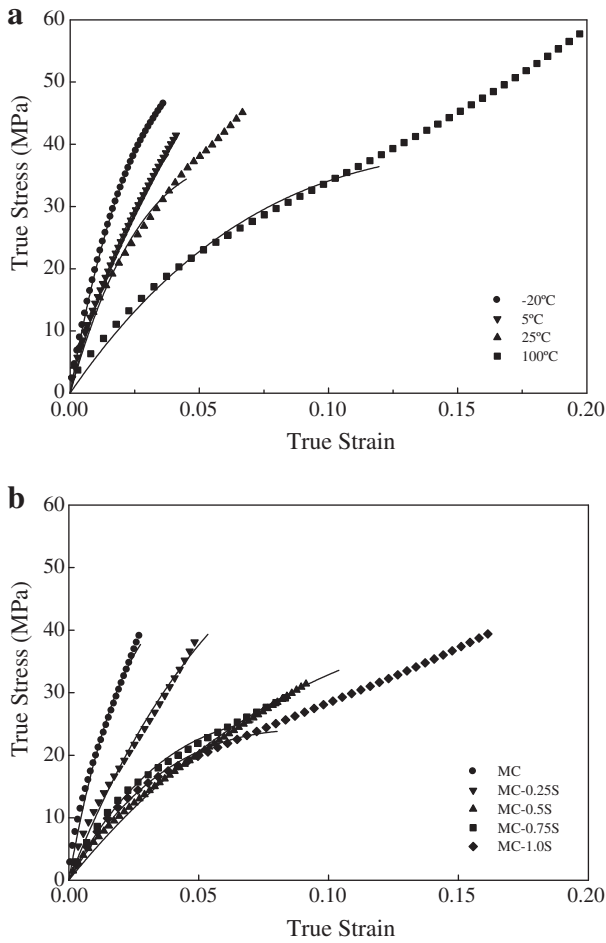


Fig. 1. Mechanical properties of: a) as-prepared methylcellulose films with 0.25% sorbitol (MC-0.25S) tested at different temperatures by DMA and b) frozen-stored methylcellulose films without and with sorbitol evaluated at 5 °C by DMA.

films as well as for films plasticized with sorbitol was found. As expected, the higher the temperature and the sorbitol concentration, the lower the E_c value (Fig. 2). At 180 °C the calculated values decreased several orders of magnitude and the linear proportionality between the modulus and the temperature was not observed.

When the sorbitol concentration exceeded 0.25% the differences between E_c for each temperature were not significant ($P > 0.05$), as it can be seen in Fig. 2.

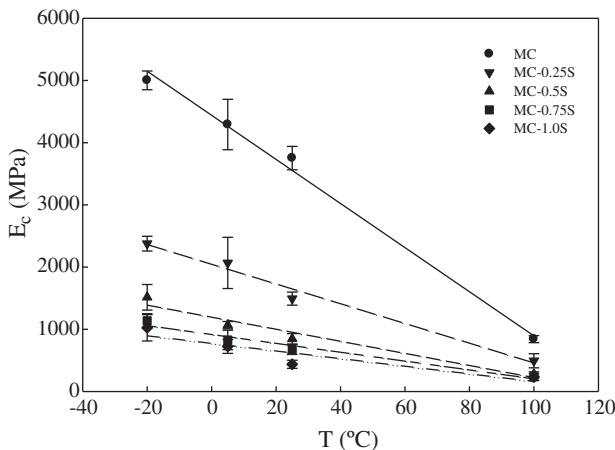


Fig. 2. Dependence of elastic modulus E_c with the temperature for as-prepared methylcellulose films with different sorbitol concentrations.

Eq. (3) described by Pintye-Hódi et al. [30], was used with some modifications to explain the effect of the sorbitol concentration on the elastic modulus of the films.

$$E_c = a + b \left[1 - \exp\left(-c \frac{c_p}{c_p + c_{MC}}\right) \right] \quad (3)$$

where: c_p is the sorbitol concentration (% w/v) in the film-forming solution, c_{MC} is the methylcellulose concentration in the film-forming solution (1.5%, w/v), and a , b and c are constants.

As was explained, A factorial design was employed to assess the effects of treatment (unfrozen: groups A and B, and frozen: groups C and D), storage (0 for groups A and D, and 3 months: groups B and C) and sorbitol concentration (0, 0.25, 0.50, 0.75, 1.0% w/v). The statistical analysis demonstrated the existence of significant differences among treatments ($P < 0.05$) up to sorbitol concentration of 0.5%.

Fig. 3a and b exhibits the elastic modulus obtained from the Eq. (3) for all samples assayed at 5 and 100 °C, respectively; as it was expected, the higher the sorbitol concentration the lower the elastic modulus.

By graphing E_c as a function of the plasticizer concentration, it could be observed that the curves tended to the same value for the highest amount of sorbitol irrespective of the treatment (Fig. 3). The theoretical data calculated from Eq. (3) were in good agreement with the experimental data ($R^2 > 0.99$). The E_c curves as a function of the sorbitol concentration presented an inflexion point at a sorbitol content between 0.25 and 0.50%.

Compared with other biodegradable materials the E_c values for methylcellulose films were higher than those obtained for chitosan-based

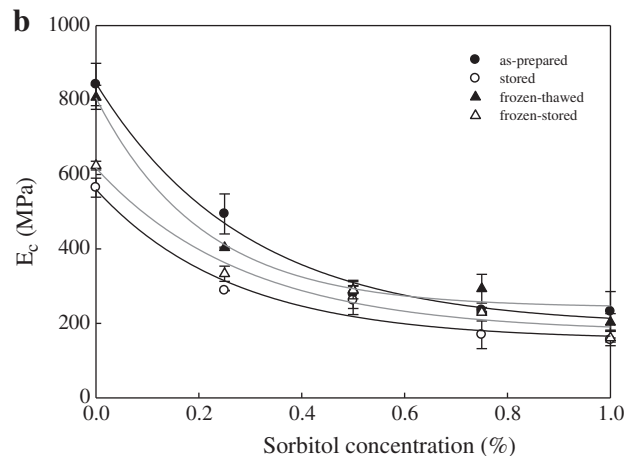
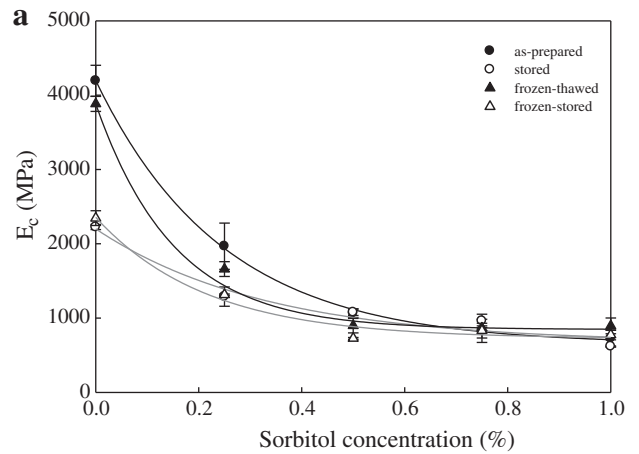


Fig. 3. Elastic modulus E_c as a function of sorbitol concentration for assayed temperatures of: a) 5 and b) 100 °C for films previously submitted to different treatments.

films [31] and gellan-based matrices [32], measured under the same conditions and equipment used in this work. Similar behavior was observed by Gupta and Jabrail [33] working on PET at different temperatures.

As it can be seen in Fig. 3a and b, at 5 °C the effect of storage on E_c was more marked than at 100 °C, considering the unplasticized samples. Stored (groups B and C) E_c value of MC film turned out to be nearly half the value of non-stored one (groups A and D). With the addition of sorbitol, the differences between both stored and non-stored samples were softened.

3.3. Thermal properties by DMA and DSC

MC films were tested by DMA in order to study the effect of the different treatments on the thermal properties. Immediately after the drying process (as-prepared), two relaxations, β and α were found in the obtained $\tan \delta$ curve for MC control films with increasing temperatures (Fig. 4), located around -12 °C and 218.5 °C, respectively. Park and Ruckenstein [34] informed values of 26.8 and 199.5 °C working with methylcellulose films. The second peak in $\tan \delta$ curves of dynamic mechanical spectra corresponds to α relaxation. The temperature at which it takes place can be labeled as dynamic glass transition temperature T_g . These transitions are related to relaxation phenomena due to mobility activation processes of non crystalline local regions, macromolecular backbone, specific macromolecular parts or macromolecular chain terminal groups [35].

Neto et al. [36] and Mucha and Pawlak [37] reported that chitosan films exhibited an event associated to β relaxation at around -20 (T_{β}), characteristic of the local motion of side groups in polysaccharide. Other authors [38,39] considered this β relaxation in hydrophilic materials as a typical water relaxation, as a consequence of hydroxyl motions favored by water molecules.

Plasticizers interfere with the hydrogen bonds among the hydroxyl groups of the polymer molecules, hence T_g slightly shifted to lower values, but there were no discernible differences ($P > 0.05$) with increasing sorbitol concentrations above 0.25%. These findings may be explained in relation to the moisture content results obtained. The same trend was

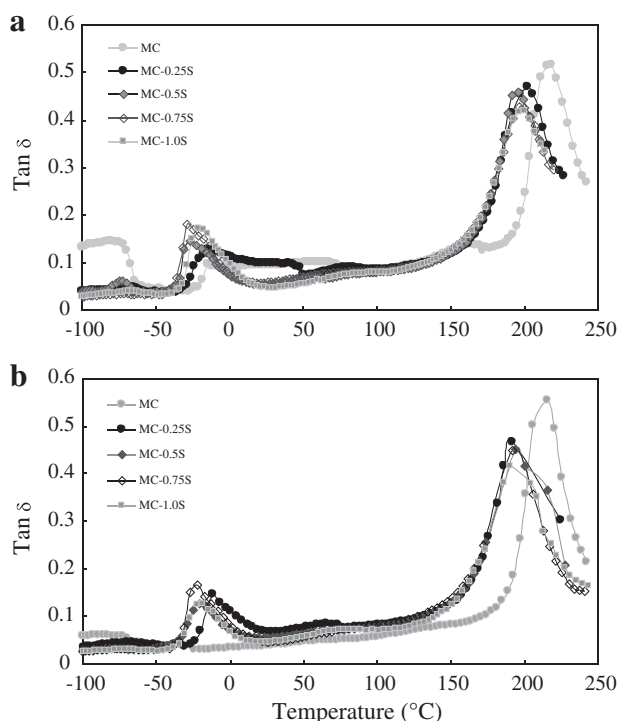


Fig. 4. Effect of the sorbitol concentration on the dynamic mechanical spectra of: a) as-prepared and b) frozen-stored MC films.

observed in the case of β relaxation, which underwent a displacement toward a lower temperature but also a better peak definition (Fig. 4a). According to Park and Ruckenstein [34] the shift of the T_g would prove the compatibility between MC and the plasticizer.

Unplasticized MC samples belonging to groups B, C and D exhibited similar glass transition temperatures than those obtained for MC control film, being T_g values 201, 215 and 211.6 °C, respectively, whereas β relaxation almost disappeared. Concerning plasticized samples, $\tan \delta$ curves turned out to be similar to those obtained for plasticized samples of group A. Fig. 4b shows $\tan \delta$ curves of films subjected to freezing and storage (group C).

3.4. Water vapor permeability

Water vapor permeability (WVP) of as-prepared MC film exhibited a value of $8.7 \cdot 10^{-11} \text{ g s}^{-1} \text{ m}^{-1} \text{ Pa}^{-1}$ (Fig. 5), which was similar to that found by Khan et al. [40]. Stored MC films did not show significant differences in WVP values with respect to as-prepared MC samples meanwhile permeability values of frozen-thawed MC increased and frozen-stored decreased.

The addition of 0.25% sorbitol decreased the WVP significantly ($P < 0.05$) but higher sorbitol concentration increased again the WVP values. In all cases, 0.25% sorbitol addition allowed obtaining the lowest WVP value (Fig. 5), which was constant for all assayed treatments ($P > 0.05$), indicating that this concentration might protect the structure against dramatic changes. These results are in accordance with those found by Srinivasa et al. [29].

The major differences in permeability values were observed with sorbitol concentrations greater than 0.5% for as-prepared and to a lesser extent in the case of frozen-thawed films. For these last samples the short period of the process would not allowed a matrix rearrangement fast enough, persisting major WVP for films formulated with the highest sorbitol concentrations. Instead films stored (unfrozen and frozen) long term might be favorable to an aging process of the matrix that led to the lowest permeability values. The aging range is bounded by T_g as the upper bound and by the highest secondary transition temperature (T_{β}) as the lower [41]. These temperatures turned out to be about 212 and -20 °C, respectively. According to Struik [41], the reduction of free volume that accompanies the aging process leads to a decrease in the mobility of the chain segments and hence to a decrease in the diffusivity of gases in the polymer matrix.

Several authors showed a decrease in WVP during the storage and attributed this phenomenon to the decrease of free volume and the matrix crosslinking over time [42,43].

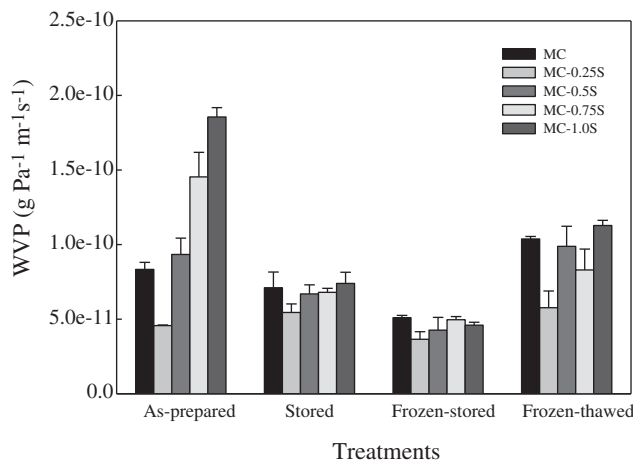


Fig. 5. Water vapor permeability (WVP) of methylcellulose films with different sorbitol concentrations submitted to different treatments: as-prepared, stored, frozen-stored and frozen-thawed.

3.5. X-ray analysis

As prepared MC films (Fig. 6) exhibited an amorphous–crystalline structure, with a sharp peak in the region of $2\theta = 8^\circ$ (d_{101} spacing, 10.7–11.2 Å) and a broad peak with a maximum at $2\theta = 21.5^\circ$ (d_{101} spacing 4.33 Å) [44]. Kato et al. [45] found that the presence of the sharp d_{101} peak in the diffraction patterns of MC samples corresponds to the trimethylglucose-type crystalline order in these films. The appearance of the peak at 8° is evidence of the generation of disorder when native cellulose is modified. Films containing plasticizer exhibited similar X-ray diffraction, but the occurrence of a more marked peak at $2\theta = 13^\circ$ indicated the existence of a more hydrated structure. MC without the presence of plasticizers is brittle in nature because of weak inter- and intramolecular hydrogen bonds. With the addition of the plasticizer, the crystalline size decreased. According to Parameswara et al. [46] this fact essentially implies that there was a decrease in the number of weak hydrogen bonds.

In the case of films submitted to freezing for 24 h the obtained pattern turned out to be similar to those obtained for as-prepared samples, with sorbitol addition as well as without plasticization. Even though the samples stored (unfrozen and frozen) showed a marked decrease of the peak located at $2\theta = 8^\circ$, the presence of sorbitol had a damping effect, making the change softer. The X-ray pattern was barely altered by the 24 h freezing process.

As can be seen in Table inserted in Fig. 6, as-prepared MC samples showed a crystallinity degree of 22.9%, decreasing slightly to 19.8% in the presence of 0.25% sorbitol. In the case of samples subjected to freezing and thawing, CD remained constant around 21%, irrespective of the addition of the plasticizer. Although the storage of MC samples without sorbitol decreased the crystallinity to 16.3%, this reduction was not revealed in films with the addition of sorbitol (MC-0.25S), probably because of its presence exerted a protective effect of the structure.

3.6. FT-IR spectroscopy of films

Figs. 7 and 8 depict the IR absorption spectra in the region between 1520 and 1720 cm^{-1} , which corresponds to the bending mode of water molecules contained in the film. The maximum of as-prepared MC control was located at 1640 cm^{-1} . With sorbitol addition it could be observed that the absorption maximum of the peak shifted to higher wavenumber (Fig. 7a), signal of a stronger hydrogen bond.

On the other hand, the peak maximum of group B stored samples was located at 1646 cm^{-1} , independently of the amount of sorbitol for concentrations higher than 0.25% (Fig. 7b). Taking the frozen-thawed

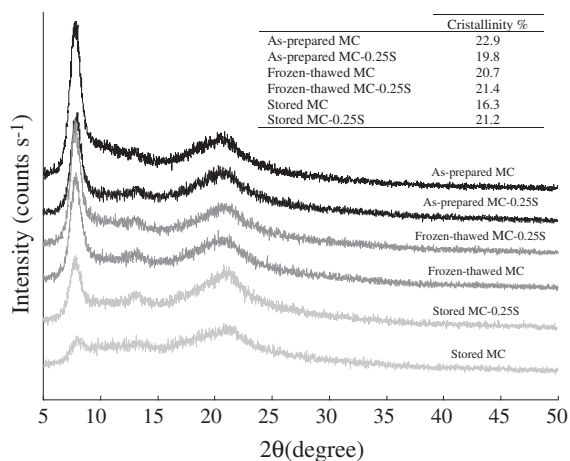


Fig. 6. X-ray diffractograms of methylcellulose films without sorbitol (MC) and with 0.25% sorbitol concentration (MC-0.25S) for as-prepared, frozen-thawed and stored samples.

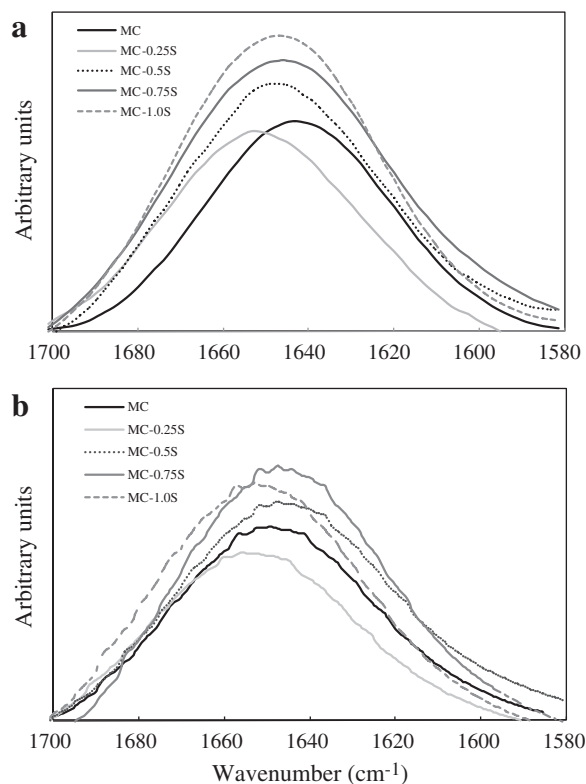


Fig. 7. FTIR spectra of: a) as-prepared and b) stored methylcellulose films with different sorbitol concentrations in the wavenumber region 1700–1580 cm^{-1} .

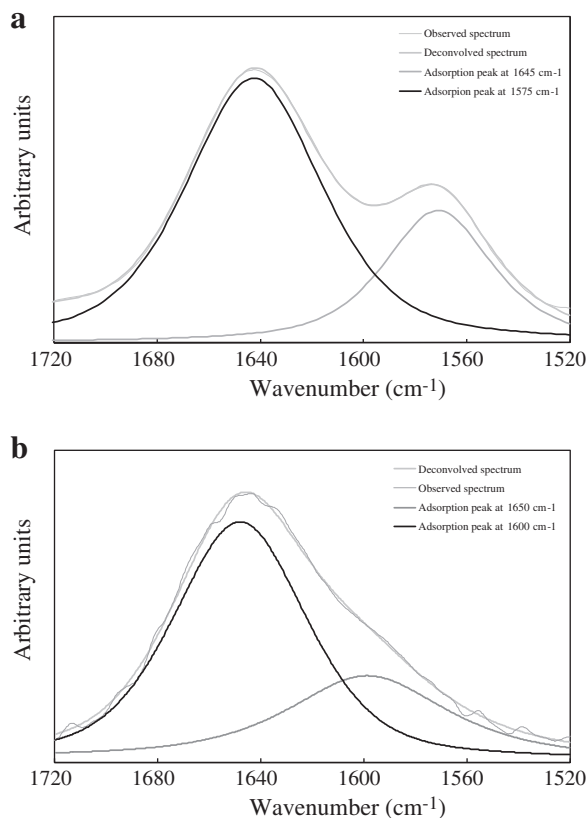


Fig. 8. Deconvolution of FTIR spectra in the wavenumber region 1720–1520 cm^{-1} for: a) frozen-stored MC films and b) frozen-stored MC-0.5S films.

samples into account, FTIR spectra showed the appearance of two small peaks at 1650 and 1638 cm^{-1} , exhibiting a shoulder at 1630 cm^{-1} . When unplasticized film belonging to group C was analyzed by deconvolution, two well defined peaks were observed at wavenumbers 1645 and 1575 cm^{-1} (Fig. 8a). According to Laporta et al. [47] and Lasagabaster et al. [48] these bands might correspond to water association by strong and weak hydrogen bonds, respectively. The addition of sorbitol produced a shift of the maximum to 1650 cm^{-1} and the occurrence of a shoulder at 1600 cm^{-1} (Fig. 8b). Consequently, sorbitol would prevent the separation of the weakly bonded water, owing to its extensive hydrogen bonding capacity that is believed to contribute to its protective properties [16]. With concern to these findings, FTIR spectra of those films submitted to freezing for long periods of time provided evidence of the existence of different types of water molecules that coexist simultaneously, their proportion being variable with film moisture since freezable water is associated to free water.

3.7. SEM observations

Fig. 9a–f shows SEM surface morphologies of as-prepared (a and b), frozen (c and d) and frozen-stored (e and f) based-MC films with and without 0.25% sorbitol addition.

Unplasticized as well as plasticized samples presented a dense and compact structure, showing that a continuous matrix was formed. The surface became more homogeneous because of the presence of sorbitol (Fig. 9b, d and f). The differences observed in frozen structures were intensified when freezing and storage were used together, probably due to the separation between free and bound water, which was facilitated by the freezing process (Fig. 9e). The “vesicles” visibly marked in MC samples frozen and stored were attenuated in the presence of sorbitol, almost disappearing (Fig. 9f).

4. Conclusions

The flexibility of the films increased as a result of the decrease in the number of hydrogen bonds among the hydroxyl groups of the polymer molecules because of the interference of sorbitol. As-prepared film solubility decreased at 100 °C, as a result of the MC thermogelation property when the samples were exposed to high temperatures.

Films subjected to different treatments (freezing, storage or a combination of both processes) exhibited the highest barrier properties at a sorbitol concentration of 0.25%, which demonstrated to be an inflexion point for mechanical and thermal properties. Moreover, elastic modulus and glass transition temperature did not undergo significant changes for

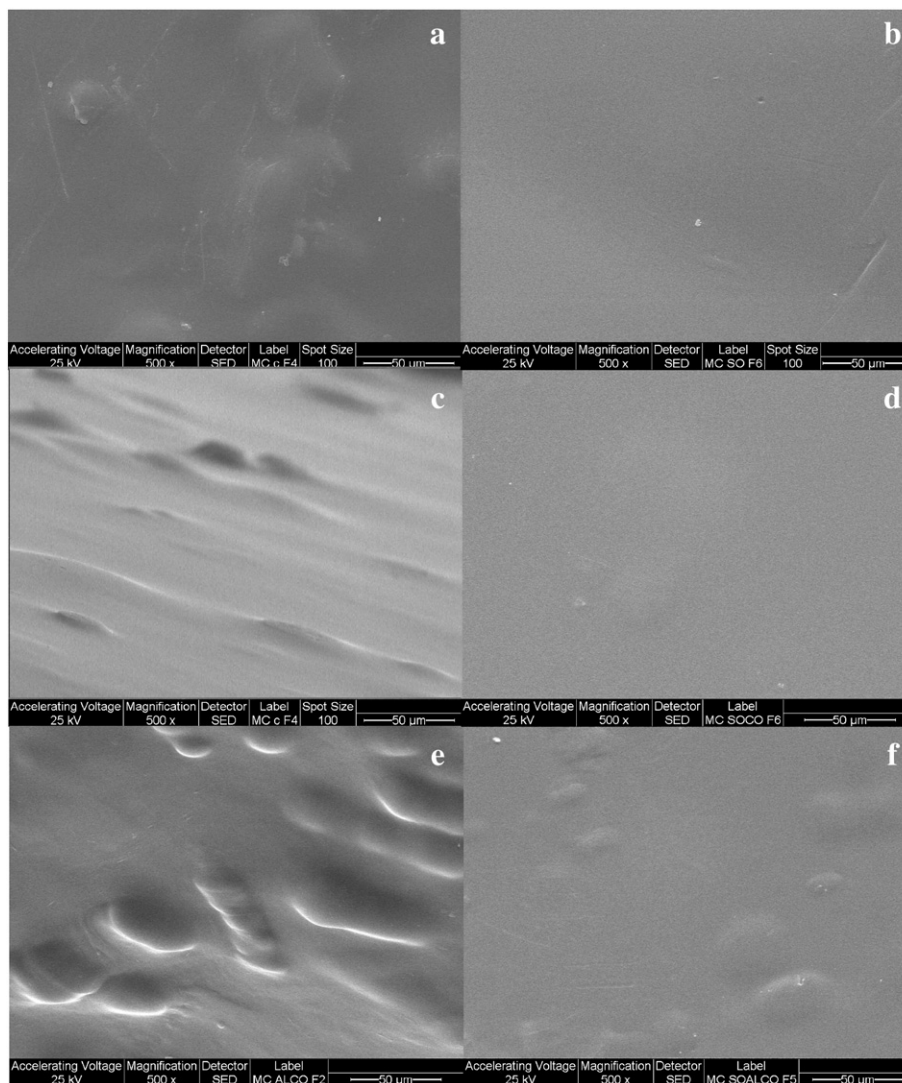


Fig. 9. SEM micrographs of: (a, b) as-prepared, (c, d) frozen-thawed, (e, f) frozen-stored of MC and MC-0.25S films, respectively. Scale bars are indicated in each image.

higher plasticizer concentrations. The most noticeable changes of film properties were a consequence of the combination of both freezing and storage processes, probably due to the separation of the freezable and non-freezable water. The above mentioned results were confirmed through SEM and FTIR techniques. Nevertheless, the presence of sorbitol smoothed the observed changes in all properties.

In summary, after operations involved in food processing and conservation such as freezing, refrigeration and storage, the methylcellulose film properties remained stable, without significant changes in the presence of sorbitol, which would act as a protective agent due to its hydrogen bonding capacity. This stability is crucial for film and coating applications in the food industry.

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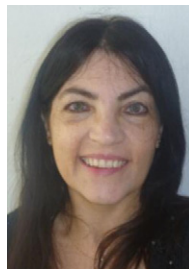
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