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Film properties from crosslinking of cellulosic derivatives with a polyfunctional carboxylic acid

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Abstract

In order to decrease water sensitivity of packaging materials based on water-soluble cellulose derivatives, and especially to avoid partial solubilisation of films into food products, crosslinking of hydroxy propyl methyl cellulose (HPMC) with citric acid was carried out using different contents of polycarboxylic acid and catalyst. To determine reticulation rate or ester bond rate in crosslinked cellulosic derivatives, FTIR in transmittance, based on of the ester band (1735 cm^{-1}) was compared to acid–base titration. Infra-red spectroscopy had advantages over time consuming titration. Crosslinking of the hydrophilic polymers induced a strong influence on water solubility and an improvement in the water vapor barrier of about 34%. © 2003 Elsevier Science Ltd. All rights reserved.

Keywords: Cellulosic based packaging; Crosslinking; Moisture barrier properties

1. Introduction

Water-soluble cellulose derivatives are mainly used for non-food applications, especially packaging because of their edibility and biodegradability. A limitation of these packaging materials is their water sensitivity, producing a loss of barrier properties when the degree of hydration increases and also, a solubilisation of bio-coatings into food with a significant water activity. In previous publications, we reported a study of bioactive edible packaging material based on hydroxy propyl methyl cellulose (HPMC). It was found that the moisture barrier was improved by stearic acid incorporation into the film forming solution, however there were difficulties in preparing a homogenous composite film with both hydrophobic and hydrophilic compounds (Sebti, Pichavant, Pardon, & Coma, 2000; Coma, Sebti, Pardon, Deschamps, & Pichavant, 2001). For these reasons, an homogeneous packaging film was prepared from HMPC by chemical modification allowing an increase in hydrophobicity. Crosslinking of HPMC can be considered a useful approach to prepare non-water soluble cellulose derivatives with interesting moisture barrier properties, which could be used as biodegradable packaging materials. Keslter and Fennema (1986) reported that, crosslinking would decrease polymer chain mobility which, in synthetic polymers, increases the resistance to vapor and gas transport. An attempt was made to enhance barrier characteristics by crosslinking modified polysaccharides chains. Highly water-soluble HPMC crosslinked to various extents should offer the possibility to modulate its biodegradability.

Glutaraldehyde has been frequently used to crosslink polysaccharides (Kawase, Michibayashi, Nakashima, Kurikawa, Yagi, & Mizaoguchi, 1997) but it is reported to contain cytotoxic chemical species (Dal Pozzo et al., 2000). Polyethyleneglycol (PEG) was also used as linking factor, especially on chitosan (Dal Pozzo et al., 2000). According to Zhou, Luner, and Caluwe (1995), the effectiveness of carboxylic acids to crosslink papers increased with their functionality in the order of 1,2,3,4 butane tetra carboxylic acid (BTCA) > Tricarballylic acid > Succinic acid. This phenomenon could be due to their ability to form highly reactive cyclic anhydride under thermal reaction conditions.

These previously results showed higher crosslinking effectiveness with polycarboxylic acid with at least three reactive groups. As a result, citric acid was selected, taking into account its non-toxicity and its wide use in the food industry as a safe natural additive.

The present work was aimed at introducing a certain degree of crosslinking in cellulosic derivative via a citric

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acid crosslinker and at improving the moisture resistance of cellulosic packaging material. The influence of crosslinking on the film water solubility and water vapor transfer were evaluated.

2. Materials and methods

Chemicals. Citric acid, Sodium dihydrogenophosphate (NaH_2PO_4) and Ethanol were supplied by Sigma (St Quentin Fallavier, France).

Film formation. Films were prepared using a modified procedure described by Kamper and Fennema (1984, 1985) by dissolving 9 parts HPMC polymer (HPMC-Culminal 50, Aqualon, Alizay, France) in 200 parts distilled water, 100 parts absolute ethanol and 1 part polyethyleneglycol (PEG 400-Merck, Darmstadt, Germany). PEG-400 was used as plasticizer. Citric acid and NaH_2PO_4 at different concentrations were added prior to film formation. The pH of the film forming solution is about of 2.65. The solution was then homogenized for 15 min, degassed for 30 s and plated onto a polypropylene support at a thickness of 1 mm. The films were dried at 60 °C for 60 min. Films were then peeled. For crosslinked material, films were heated again at 190 °C for 15 min. A 25–35 μm final thickness was obtained. Crosslinked and non-crosslinked films were stored at 50 ± 5% relative humidity and 23 ± 1 °C for 5 days before use.

Film thickness. Film thickness was measured to the nearest 1 μm (MITUTOYO electronic micrometer). Ten measurements were performed at random positions.

2.1. Assessment of the degree of crosslinking

Titration. A 0.1 g of film was deposited in 40 ml of distilled water. An excess of NaOH (0.01 M) was added, and titration with sulphuric acid (0.01 M) was conducted. Taking into account the initial introduced citric acid (Initial COOH mole/g dry film), the crosslinking rate was calculated from the residual citric acid that did not react with the free hydroxyl groups of HPMC.

The free moles of COOH/g of dry film and the moles of COOH included in the ester group (initial moles number of COOH/g − free moles number of COOH/g) were calculated.

The percentage of crosslinking was calculated as follows:

$$\text{Crosslinking}(\%) = \frac{\text{COOH included in ester bond (mole/g)}}{\text{Initial COOH (mole/g)}}100$$

Experiments were replicated 15 times.

FTIR analysis. Infrared spectra were recorded with FTIR (NICOLET 210), to determine the crosslinking rate from the increase in absorption intensity of the ester band A1 (1735 cm^{-1}) using the absorbance of the 1371 cm^{-1} band, CH bending vibration of methyl group (A2) or 948 cm^{-1} band, antisymetric out of phase ring vibration, (A3) as internal standards. The standard bands do not vary with crosslinking, allowing the effect of film thickness to be eliminated. The ratio of these two bands was compared with the titration value of each sample. The films were directly analyzed in transmittance. Films with different content of esterified citric acid (triethylcitrate) were analyzed to work out calibration curves. The % of crosslinking was calculated from the ratio between these two bands and from the triethylcitrate calibration curve as follows, taking into account the film volume crossed by the infra-red beam:

$$\text{Crosslinking (\%)} = \frac{\text{COOR per film volume}}{\text{Theoretical COOR per film volume}}100$$

COOR per film volume was calculated from the calibration curve after FTIR analysis using the ratio A1/A2 or A1/A3. For clarity, only results from the ratio A1/A2 will be given. Theoretical COOR per film volume was calculated by supposing that the totality of introduced COOH functions by citric acid were included in esters bonds. Experiments were replicated 15 times and treatment means were separated using the Student's t-test ($p < 0.05$).

Water solubility. The water solubility of crosslinked or non-crosslinked cellulosic films was measured from immersion assays in 50 ml of distilled water for 24 h at 25 °C. The water solubility expressed as a percentage of the initial dry matter, was determined from the residual dry weight after immersion compared to the initial dry weight. The percentage of initial dry matter in film was determined by an infrared drying (Mettler instrument). All tests were conducted in triplicate. Film solubility was modeled with the Statgraphic program 4.1 (Sigma-plus) using a multifactor linear regression method based on a variance comparative study. The factor means were represented by the Pareto chart (95% probability). The objective of these calculations was to represent the HPMC water solubility through the crosslinking parameter (variations of the citric acid and NaH_2PO_4 contents)

Water vapor transmission rate (WVTR). The WVTR of edible films was evaluated using method NF ISO 2528 (1989). An aluminum cup containing anhydrous $CaCl_2$ dessicant was sealed by the test film (50 cm^2 exchange film area) with paraffin wax at 50 °C. It was placed in an environment of controlled humidity and temperature (90% RH and 38 °C or 50% RH and 23 °C). The water vapor transmission rate ($g\,m^{-2}\,d^{-1}$) was determined from the weight increase of the cup over time at the steady state of transfer using the following equation:

$$\text{WVTR} = \frac{\Delta m \times 24}{\Delta t \times S}$$

where Δm = amount of H_2O vapor passing through a film of area S during time Δt determined from weight change of the

Table 1
Crosslinking rate calculated from the titration method with different citric acid content taking into account thermal processing. Data are means of 15 experiments

Initial acid content (%)	Thermal treatment	Titrated acidity (mole/g)	Introduced COOH (mole/g)	RETICULATION RATE (%)
0		0	0	
	X	0	0	0
5		2.2×10^{-4}	2.2×10^{-4}	
	X	1.4×10^{-4}	2.2×10^{-4}	35
10		4.4×10^{-4}	4.3×10^{-4}	
	X	2.1×10^{-4}	4.3×10^{-4}	50
15		7.0×10^{-4}	6.7×10^{-4}	
	X	2.4×10^{-4}	6.7×10^{-4}	65

cup vs. time. Plotted-linear regression was used to calculate the slope of a fitted straight line.

Control cups, without the anhydrous $CaCl_2$ dessicant were conducted in parallel.

The WVTR was given for a mean film thickness of about 29 ± 8 μm, at atmospheric pressure (1 atm) with two partial water vapor pressure difference across the two sides of the film specimen corresponding to 0–90% RH and 0–50% RH.

All tests were performed in triplicate and treatment means were separated using the Student's *t*-test ($p < 0.05$).

3. Results and discussion

Different mechanisms have been proposed in the literature to explain the crosslinking of cellulosic polymers. According to Zhou et al. (1995), the two main stages of the reaction of polyfunctional carboxylic acids with paper are firstly attachment of the polyfunctional carboxylic acid via esterification with a cellulosic hydroxyl group and its further reaction—via esterification—with another cellulosic hydroxyl group producing the bridging crosslinks of the cellulose fiber. These authors proposed a mechanism based on anhydride intermediate formation. Attachment of the carboxylic acid moiety to HPMC polymer via esterification reaction of the first cyclic anhydride would expose a new carboxylic acid unit in citric acid, which has the proper chemical connectivity to form a new intra molecular anhydride moiety with the adjacent carboxylic acid unit. Further reaction with cellulose ether can then lead to crosslinking.

Because of the implication of hydroxyl groups of HPMC in ester bond, crosslinking of this hydrophilic polymer was conducted to limit the affinity of films toward water.

From preliminary study, 10 min at 190 °C was the lowest time/temperature necessary to produce colourless and non water-soluble films. The films produced were transparent and clear. Prior to the assessment of film properties, two methods intended to evaluate the rate of reticulation were performed.

3.1. Assessment of the rate of crosslinking:

From titration measurements. Titration was carried out on films of which the water solubility differs according to the degree of crosslinking. For non water-soluble films, grinding with liquid nitrogen was ineffective. The films were then cut into pieces and titration was carried out on film suspensions. The mole number of titrated acid related to the mole number of theoretical introduced acid are represented Table 1. Indeed the acid content of the non-crosslinked films (without thermal processing) corresponded to the introduced acid content, in contrast to crosslinked films. Acid content in free citric acid films submitted to the thermal processing was zero. These experiments were conducted to eliminate the effect of thermal processing alone on HPMC films, which could cause chemical modifications giving acidity. The differences obtained between acidity titrated before and after thermal processing on films containing increasing quantities

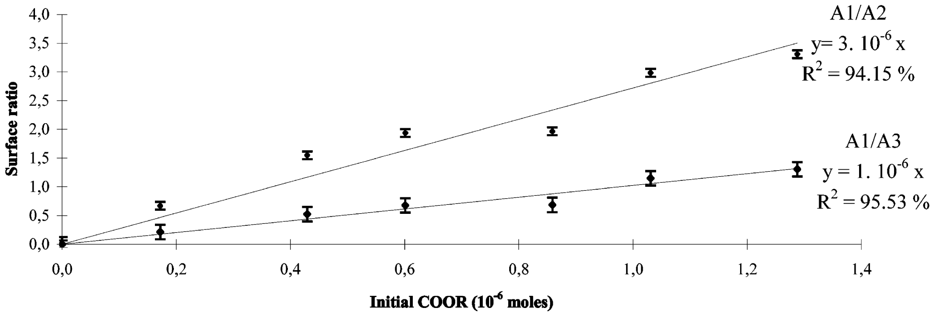

Fig. 1. FTIR calibration curves from triethylcitrate with both A2 (1371 cm^{-1}) and A3 (948 cm^{-1}) bands as internal standards. Each point of the graph is a mean of 15 experiments and the standard derivations are vertical lines.

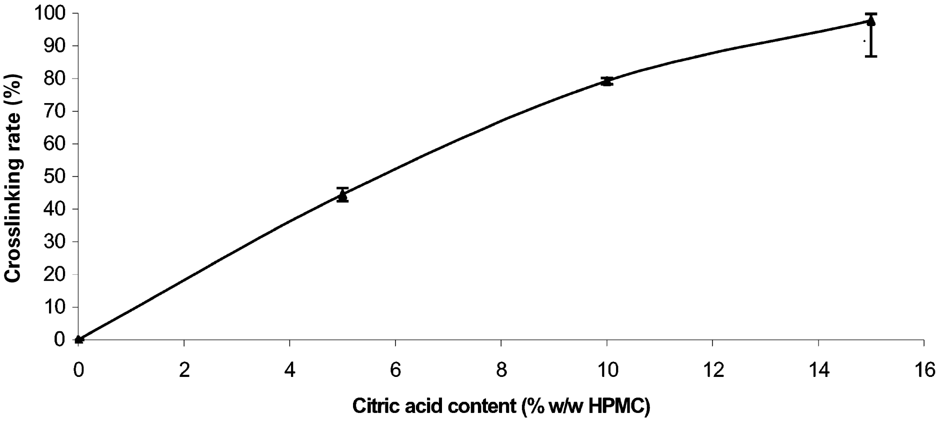

Fig. 2. Crosslinking rate calculated from FTIR method using A2 band (1371 cm^{-1}) as internal standard versus initial citric acid content. Each point of the graph is means of 15 experiments and the standard derivations are vertical lines.

in citric acid, showed a strong reduction in the acid functions available due to the formation of ester bonds. The percentage of crosslinking was also calculated and the data are proposed Table 1.

FTIR analysis. With the crosslinking proceeding, the absorbance value of ester band around 1735 cm^{-1} was significantly increased.

Before determination of the percentage of crosslinking in films, a standard curve from triethylcitrate was established by comparing two bands as internal standards A2 (1371 cm^{-1}, CH bending vibration of methyl group) and A3 (948 cm^{-1}, antisymetric out of phase ring vibration). The characteristic peak of the ester bond vibration (1740 cm^{-1}) increased according to the percentage of acid or triethylcitrate (Fig. 1). Taking into account the high correlation between the ratios A1/A2 or A1/A3, crosslinking percentages were then calculated from the 1371 cm^{-1} bond as internal standard. The linearity of the calibration curve was satisfactory and showed that this easy to implement and rapid method could be used at first approximation for the determination of the crosslinking rate.

Fig. 2 shows the change of crosslinking rate with initial acid content using calibration curves with triethylcitrate. The curve showed an asymptote towards 100%, which represented either saturation of the infrared signal or saturation as for the percentage of crosslinking. The rate of crosslinking calculated from both titration method and FTIR analysis was compared (Fig. 3). These two methods showed a positive linear relationship ($R^2 > 98\%$). Even though wide scatters are shown in some data, this equation is acceptable as an easy method for the determination the approximate degree of crosslinking of cellulosic derivatives rather than the time-consuming titration method.

Solubility.In order to study the efficiency of crosslinking on water solubility, experiments were conducted with various content of NaH_2PO_4, (Factor A) for its catalytic effect from 0–50% (w/w citric acid) and of citric acid (Factor B) from 0–15% (w/w HPMC). The analysis of the effects of factors A, B and their interactions AB using a multifactorial linear regression, based on variance analysis, was presented Fig. 4. Only both factors B and BB conducted to a significant effect on the reduction of the solubility of crosslinked films. A model was established giving the following equation:

$$\text{Solubility} = 96.51 - 18.19B + 0.80B^2$$

An *R*-squared coefficient higher than 97% validate the equation of the model. An increasing citric acid content decreased the solubility of films with an optimum located at about 10%. However, 80% of the initial dry matter became non water soluble even if only 5% of citric acid was used in the film forming solution.

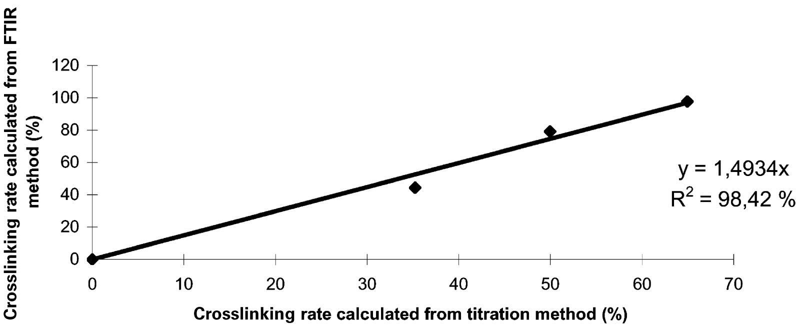

Fig. 3. Crosslinking rate calculated from FTIR method using A2 band (1371 cm^{-1}) as internal standard versius reticulation rate calculated from titration method.

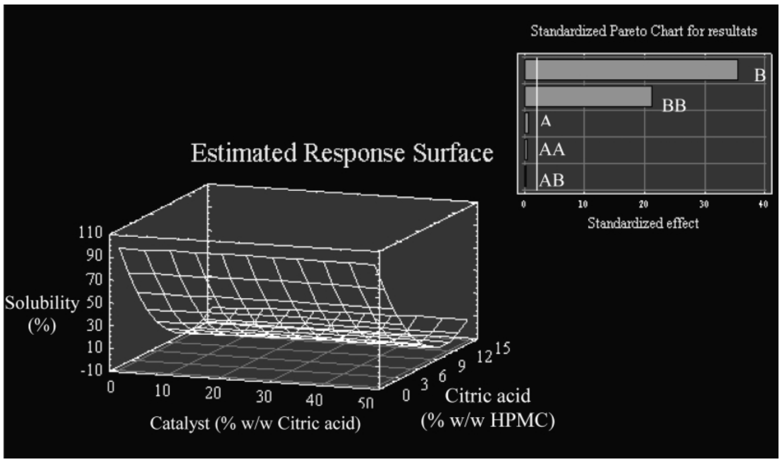

Fig. 4. Effect of initial citric acid and catalyst contents on the film water-solubility and standardized Pareto Chart of results. Model was obtained using a multifactor linear relationship ($p < 0.05$).

Moreover, there was no significant influence of the catalyst on the reaction between the polyfunctional carboxylic acid and the cellulosic polymer. This latter result was not in accordance with conclusions of Zhou et al. (1995). These authors mentioned an important catalytic effect of NaH_2PO_4 on the rate of anhydride formation from the polyfunctional carboxylic acid. The mechanism of the catalyst is unknown, although the catalyst may simply be functioning as buffering agents, keeping the pH levels within the desired ranges (Payne 1998). It is also hypothesized that during the heat cure, the polycarboxylic acid forms cyclic anhydrides which then add the alkali metal hypophosphite to form acylphosphinates which may react with cellulose to yield the desired crosslinked esters. Experimentally it is found that the catalyst is effective at concentration as low as 0.3% by weight (Welch and Andrew, 1989). Nevertheless, authors mentioned that applicants have discovered that the crosslinking reaction can be accomplished without the use of a catalyst if the pH of the solution containing the crosslinking agent is kept within the ranges of pH 1.5 and 5 (Herron, Hanser, Cooper and Hersko, 1990).

Water vapor transmission rate (WVTR). Upon attainment of steady state vapor flow, Fick's law of diffusion applies. Even if there is significant interaction between the film and the diffusing substance due to hydrophilic nature of cellulosic compound, previously equation was used to evaluate moisture barrier properties of film, using two Relative Humidity (RH) conditions, 90% RH and 38 °C and especially 50% RH and 25 °C. This latter condition is more relevant to situations that would be encountered in practical use.

Crosslinking of HPMC enhanced moisture barrier of the cellulose derivatives, especially from 50% RH and 25 °C experiments (Table 2). The acid content had a real effect on the reduction of the transfer coefficient whereas the catalyst did not have any significant effect. In order to check that the thermal processing had no influence on WVTR, possibly due to a structure modification performed by high temperature, the experiments were conducted on HPMC

Table 2

Effect of citric acid and catalyst initial contents on the WVTR (g m^{-2} day^{-2}) from both 38 °C to 90% RH and 25 °C to 50% RH experiments. Effect of thermal treatment on the WVRT of free citric acid and catalyst films specimens. Data are means of three experiments and the standard deviations were calculated using student-*t*-test (95% probability)

Thermal treatment: 190 °C for 15 min					
Citric acid (% w/w HPMC)	50% RH and 23 °C			90% RH and 38 °C	
	Catalyst (% w/w citric acid)				
	0	20	50	0	20
0	269 ± 14	268 ± 8	238 ± 58	4421 ± 222	
5	221 ± 2	190 ± 18	229 ± 1	3400 ± 144	2989 ± 156
15	184 ± 15	177 ± 3	168 ± 21	3400 ± 156	2612 ± 83
Without thermal treatment	50% RH and 23 °C			90% RH and 38 °C	
0		316 ± 26			4415 ± 218

films without citric acid and catalyst. At some critical temperature, a three-dimentional gel structure will form from cellulosic polymers. This phenomenon is attributable to increased intermolecular hydrophobic interactions caused by thermal disruption of hydration shells surrounding polymer chains (Whistler and Daniel, 1985). Experiments were therefore conducted on crosslinked HPMC films and on free citric acid HPMC films following the thermal treatment. Results (Table 2) showed that the simple thermal treatment had no significative effect on moisture barrier ($p < 0.05$).

As expected, at 90% RH and 38 °C, the moisture barrier dramatically decreased. The solubility coefficient increases with water vapor pressure because of the shape of the sorption isotherm and the diffusion constant increases with vapor pressure due to plasticization of the film by absorbed water (Keslter and Fennema, 1986). Consequently, permeability of water vapor through a polar film matrix increases greatly as vapor pressure is elevated.

In tropical conditions, citric acid had a significant effect on the reduction of the WVTR. However, there was no significant difference between moisture transfer from 5 and 15% of citric acid. As previously mentioned for 50% RH and 23 °C, the non significant influence of thermal processing on WVTR was verified (Table 2). It is well known that the efficiency of films with regard to their barrier properties strongly depends on the solubility and diffusivity of the penetrant in the polymer. The greater the sorption of the penetrant, the greater is the permeability. In the absence of cracks, pinholes or other flaws, the primary mechanism for gas and vapor flow through a film is by activated diffusion, i.e. the penetrant dissolves in the film matrix at the high concentration side, diffuses through the film driven by a concentration gradient and evaporates from the other surface. The second step of the process, i.e. diffusion, depends upon size, shape, and polarity of the penetrating molecule as well as polymer chain segmental motion within the film matrix. Factors affecting segmental motion of polymers chains include degree of crosslinking (Keslter and Fennema, 1986).

Though the hydrophilic character appeared to be reduced, crosslinking did not induce a reduction in the WVTR as significant as the one obtained after addition of hydrophobic compounds such as stearic acid. An addition of lipid compound of about 15% (w/w HPMC) decreased the WVTR by about 74% under conditions of 50% RH and 23 °C (Sebti et al., 2000; Coma et al., 2001). At best, the crosslinking decreased the WVTR by about 34% in the same temperature and humidity conditions. The weak decrease in the WVTR could be caused by weakened hydrogen bonding strengths between inter and intra molecules and by a spacing of the cellulose chains by citric acid, thus increasing the diffusion of the water molecules in the polymeric matrix. The removal of voids in cellulosic films due to the bulking by acyl groups allowed an increase in water absorption, which induced a plasticization of the matrix. This latter phenomenon increased the diffusion rate of water vapor. To support this hypothesis, crosslinking introduces high swelling capacity according to the length of the crosslinker agent (Dal Pozzo et al., 2000) while decreasing hydrophily. To verify it, DSC measurements were conducted to determine modifications of the percentage of film crystallinity but HPMC films are amorphous and no glass transition temperature appeared. Therefore, a study of the water mobility by measuring relaxation times from low resolution NMR spectroscopy experiments (Labbé, De Jeso, Lartigue, Daudé, Petraud and Ratier, 2002) will be conducted in our future studies.

4. Conclusions

FTIR based method could be used to determine to a first approximation the ester bond rate formation directly on crosslinked cellulosic films from polycarboxylic acid in the place of time-consuming titration method. This study shows that crosslinking offers great advantages in decreasing water solubility of ether cellulosic films while improving moisture barrier properties by about 34%. As a result, these homogeneous materials could be used to allow a reduction in dehydration phenomena or alternatively moisture adsorption of some foodstuffs. The weaker decrease in the WVTR after crosslinking reaction compared to composite films with hydrophobic compounds, could be caused by an increasing distance between polymer chains due to the incorporation of citric acid, involving a reduction in matrix cohesion and inducing an increase in water vapor diffusion coefficient. The effect of crosslinking on hydrophobicity and mechanical properties of cellulosic matrix must still be evaluated as well as their biodegradability.

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References

Coma, V., Sebti, I., Pardon, P., Deschamps, A., & Pichavant, F. H. (2001). Anti-microbial edible packaging based on cellulosic ethers, fatty acids and nisin incorporation to inhibit Listeria innocua and Staphylococcus aureus. *Journal of Food Protection*, *64*(4), 470–475.

Dal Pozzo, A., Vanini, L., Fagnoni, M., Guerrini, M., De Benedittis, A., & Muzzarelli, R. A. A. (2000). Preparation and characterization of poly(ethylene glycol)-crosslinked reacetylated chitosans. *Carbohydrate Polymers*, *42*, 201–206.

Herron, C. M., Hanser, T. R., Cooper, D. J., & Hersko, B. S (1990). Process for preparing individualized, polycarboxylic acid crosslinked fibers European Patent 0, 427, 317, A2.

Kamper, S. L., & Fennema, O. R. (1984). Water vapor permeability of an edible, fatty acid, bilayer film. *Journal of Food Science*, *49*, 1482–1485.

Kamper, S. L., & Fennema, O. R. (1985). Use of edible film to maintain water vapor gradients in foods. *Journal of Food Science*, *50*, 382–384.

Kawase, M., Michibayashi, N., Nakashima, Y., Kurikawa, N., Yagi, K., & Mizaoguchi, T. (1997). Application of glutaraldehyde-crosslinked chitosan as a scaffold for hepatocyte attachment. *Biological Pharmaceutical Bulletin*, *20*, 708–710.

Keslter, J. J., & Fennema, O. R. (1986). Edible films and coatings: a review. *Food Technology*, *48*, 47–59.

Labbé, N., De Jeso, B., Lartigue, J. C., Daudé, G., Petraud, M., & Ratier, M. (2002). Moisture content and extractive materials in Maritime Pine wood by low field ^{1}H NMR. *Holzforschung*, *56*, 25–31.

Payne, M (1998). Fluid acquisition and distribution member for absorbent core US Patent, 5, 716, 703.

Sebti, I., Pichavant, F. H., Pardon, P., & Coma, V. (2000). *Active edible packaging materials based on cellulosic derivatives and bacteriocins incorporation*. *Proceedings of the Sixth European Workshop on lignocellulosics and pulp, Bordeaux, France*, pp. 563–566.

Welch, C. M., & Andrew, B.K (1989). Catalyst and processes for formaldehyde-free durable press finishing of cotton textiles with polycarboxylic acids US Patent, 4, 820, 307.

Whistler, R. L., & Daniel, J. R. (1985). In O. R. Fennema (Ed.), *Carbohydrates* (2nd ed.) pp. 69, *Food Chemistry*.

Zhou, Y. J., Luner, P., & Caluwe, P. (1995). Mechanism of crosslinking of papers with polyfunctional carboxylic acids. *Journal of Applied Polymer Science*, *58*, 1523–1534.