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Antimicrobial and Physicochemical Properties of Chitosan−HPMC-Based Films

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To prepare composite films from biopolymers with anti-listerial activity and moisture barrier properties, the antimicrobial efficiency of chitosan−hydroxy propyl methyl cellulose (HPMC) films, chitosan−HPMC films associated with lipid, and chitosan−HPMC films chemically modified by cross-linking were evaluated. In addition, the physicochemical properties of composite films were evaluated to determine their potential for food applications. The incorporation of stearic acid into the composite chitosan−HPMC film formulation decreased water sensitivity such as initial solubility in water and water drop angle. Thus, cross-linking of composite chitosan−HPMC, using citric acid as the cross-linking agent, led to a 40% reduction in solubility in water. The water vapor transfer rate of HPMC film, ~270 g·m^{-2}·day^{-1}·atm^{-1}, was improved by incorporating chitosan and was further reduced 40% by the addition of stearic acid and/or cross-linking. Anti-listerial activity of films was determined on solid medium by a numeration technique. Chitosan−HPMC-based films, with and without stearic acid, inhibited the growth of *Listeria monocytogenes* completely. On the other hand, a loss of antimicrobial activity after chemical cross-linking modification was observed. FTIR and ^{13}C NMR analyses were then conducted in order to study a potential chemical modification of biopolymers such as a chemical reaction with the amino group of chitosan. To complete the study, the mechanical properties of composite films were determined from tensile strength assays.

KEYWORDS: Antimicrobial films; chitosan; cellulose derivatives; *L. monocytogenes*; cross-linking

INTRODUCTION

Growing concerns regarding the safety of intermediate-moisture food warrant a greater emphasis on the development of active packaging. Bacterial foodborne pathogens such as *Listeria monocytogenes* are of special concern due to their recent implication in human infections and diseases. To prevent the development and spread of pathogenic microorganisms via food, more information is needed concerning potential alternative solutions such as antimicrobial packaging materials.

Combining biocide agents directly into a packaging material could provide several advantages. First, only the necessary amount of biocide would be used. Second, the agent would not be a direct additive to the food product. In addition, if the packaging materials were made of a biopolymer, there would be environmental advantages (*1*).

According to Cooksey (*2*), there are three basic categories of antimicrobial films. One involves the direct incorporation of the antimicrobial additive into the packaging film, whereas the second type is coated with a material that acts as a carrier for the additive. These categories of materials can release the antimicrobial agents onto the surface of the food (*3*−*8*). Third, antimicrobial macromolecules with film-forming properties, such as cationic amino-polysaccharides, can produce antimicrobial films (*9*−*12*). Release of biocide agent would not be required for this system. Moreover, legal issues and standards concerning the rate of migration of substances in packaging into food products do not limit the development of such bioactive materials. However, the limitation is the necessity of direct contact between the packaging and the food for these systems to be effective.

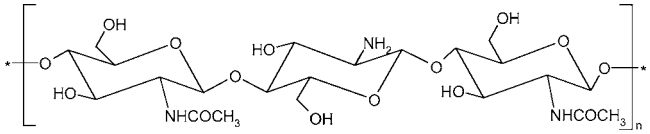

Figure 1. Structure of chitosan.

Chitosan, a polysaccharide derived particularly from crustacean chitin, is of particular interest in the food-packaging field. Both chitin and chitosan biopolymers are composed of glucosamine and *N*-acetylated glucosamine (2-acetylamino-2-deoxy-D-glucopyranose) units linked by (1−4) glycosidic bonds. Chitin is extensively acetylated, whereas chitosan is largely deacetylated (*13*) (**Figure 1**). Additionally, chitin and chitosan are biodegradable, bioabsorbable, and bioactive molecules in

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either their polymeric or oligomeric forms (*10*, *14*). As related by Cooksey (*2*), the positive charges of chitosan interfere with the negatively charged residues of the macromolecules at the bacterial cell surface, compete with calcium for electronegative sites on the membrane, and compromise the membrane's integrity, thus causing leakage of intracellular material, leading to bacterial cell death.

Cellulose derivatives such as hydroxy propyl methyl cellulose (HPMC) are promising raw materials for edible coatings or films, associated with antimicrobial entities. HPMC is a water-soluble polymer used in food industries as a gelling and stabilizing agent. However, cellulose films are poor water vapor barriers because of the inherent hydrophilic nature of polysaccharides. Hygroscopic characteristics of cellulose derivatives show that swelling by water leads to a loss of gas and vapor barrier properties. A way to improve the moisture barrier would be the incorporation of hydrophobic compounds, such as fatty acids, into the cellulose ether matrix to develop a composite film (*15*−*18*). Cross-linking by a polycarboxylic acid would be another interesting way to potentially decrease the hydrophilic characteristics and water solubility of cellulosic polymers. Consisting of the formation of covalent bonds between the chains of cellulose, this chemical modification leads to a decrease in the availability of hydroxyl groups, limiting polysaccharide−water interactions by hydrogen bonding. As previously described (*19*), cross-linking of HPMC films with citric acid resulted in a decrease in the affinity of the natural polymer toward water and produced non-water-soluble films associated with improved water vapor barrier properties. To develop bioactive environmentally friendly packaging materials, chitosan−HPMC composite films have been elaborated, with chitosan as antibacterial compound. The greatest obstacle to the development of chitosan-based packaging is cost. Therefore, association with a cellulose derivative has been conducted to reduce the cost of materials, because HPMC is not an expensive biopolymer to produce. To improve the barrier properties of these chitosan−HPMC films, a combination of hydrophilic polysaccharide with stearic acid and chemical modification of biopolymers from citric acid reactant were evaluated.

The overall objective of the current study was to investigate the antimicrobial efficiency and water sensitivity of chitosan−HPMC films, chitosan−HPMC films associated with lipid, and chemically modified chitosan−HPMC films from cross-linking in order to exhibit anti-listerial activity and moisture barrier properties. In addition, the physicochemical properties of composite films were evaluated to determine their potential for food applications.

MATERIALS AND METHODS

Materials. *Compounds.* Chitosan 244 (deacetylation degree >95%, low viscosity) was furnished by France Chitine (Marseille, France). HPMC (Culminal 50, Aqualon, Rueil Malmaison, France), polyethylene glycol 400 (PEG400, Merck, Darmstadt, Germany), and stearic acid (SA, 5376, Sigma Chemical Co. St. Louis, MO) were used, without further purification.

Methods. *Strains, Culture Media, and Growth Conditions. Listeria monocytogenes*, ATCC 15313, was stored at −20 °C in 70% glycerol. Overnight preculture was performed as follows: the strain was grown in tryptose broth (Difco 62176, Detroit, MI) at 30 °C and agitated at 140−160 rpm for 18−24 h.

Chitosan−HPMC Film Preparation. (a) Composite Chitosan−HPMC Films (Composite Film). Chitosan solution (2% w/v) was prepared by dissolving chitosan in 1% acetic acid as specified in Coma et al. (*10*). The HPMC solution was obtained using a modified procedure described by Kamper and Fennema (*20*), by dissolving 9 parts HPMC polymer in 200 parts distilled water, 100 parts absolute ethanol, and 1 part PEG400. To select a solvent not only deprived of activity inhibitory to the microbial strain but also allowing a good solubility of both polysaccharides, cast films elaborated from different solvents were studied. Water/ethanol (200:100 parts) was replaced by 1% acetic acid (300 parts) or distilled water (300 parts).

The composite film-forming solution, prepared from chitosan−HPMC 50:50 proportionally, was then poured in a uniform layer of 1 mm thickness onto a polypropylene plate. The optimum conditions for casting were 60 °C for 2.5 h in a drying oven at ambient relative humidity (RH). The dried composite films were peeled from the plate, and samples were conditioned at 20 ± 1 °C and 50 ± 5% RH before property measurements.

(b) Composite Chitosan−HPMC−Stearic Acid Film (Composite−SA). To elaborate lipid composite films, the same HPMC−chitosan film-forming procedure as above was used, associated with stearic acid incorporation prior to film formation. The quantity of hydrophobic compounds was expressed as a percentage of the polysaccharide part (w/w). The film-forming solution was heated to the melting point of the selected hydrophobic compound (72 °C) before fatty acid addition.

The composite film-forming solution was then poured in a uniform layer of 1 mm thickness onto a polypropylene plate and dried at 60 °C for 2.5 h in a drying oven. The dried composite films were peeled from the plate, and samples were conditioned at 20 ± 1 °C and 50 ± 5% RH before property measurements.

(c) Cross-Linked Films. Films were obtained after incorporation of citric acid (15% w/w HPMC) and NaH_2PO_4 (50% w/w citric acid) prior to film formation as described in Coma et al. (*19*). The pH of the film-forming solution was 4.8. The solution was then homogenized for 15 min and plated onto polypropylene support at a thickness of 1 mm. Films were dried at 60 °C for 2.5 h at ambient RH, peeled, and heated again at 190 °C for 15 min. Materials were conditioned for 5 days at 20 ± 1 °C and 50 ± 5% RH before property measurements.

A micrometer (Mitutoyo electronic micrometer) was used to measure film thickness to an accuracy of 1 μm. Ten measurements were made on each test sample and showed a mean final thickness of ∼30 ± 4 μm. Treatment means were separated using Student's *t* test at 95% probability ($p < 0.05$).

Bioactivity Assessments. Antibacterial activity of chitosan-based films was tested by an agar plate method as described by Sebti et al. (*21*). A known cell density per plate of an overnight *L. monocytogenes* preculture was deposited on tryptose agar medium and allowed to dry in a laminar flow hood at room temperature for 1 h. The initial cell number per Petri dish was evaluated from preliminary experiments on the microbial charge of an overnight culture in tryptose broth. Moreover, initial cell number was confirmed by the control assays conducted in parallel, without film. Two different initial microbial charges were used to determine the influence of cell density on the effectiveness of bioactive films. Petri dishes were inoculated with ∼300 cells.

Bioactive films were deposited on the surface of inoculated tryptose agar, and the plates were incubated at 30 °C. The numeration was performed after different incubation times.

Growth controls (without films) were conducted to ensure that viable organisms were present. In addition, contamination controls (film−agar medium experiments, without microbial cells) were performed on films deposited on an uninoculated tryptose agar to test their initial contamination. Finally, the bioactivity of homogeneous films, with chitosan replaced by HPMC (same content, same procedure), was determined in parallel to verify the non-antimicrobial activity of potential residual solvent previously used in the film-forming solution. All experiments were conducted in triplicate. Treatment means were separated using Student's *t* test at 95% probability ($p < 0.05$).

Water Vapor Transmission Rate (WVTR) Determination. The WVTR of biomaterials was evaluated using NF ISO 2528 (1989). An aluminum cup containing anhydrous $CaCl_2$ desiccant (assay cup) or nothing (control cup) 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 (50 ± 5% RH and 20 ± 1 °C). The WVTR ($g \cdot m^{-2} \cdot day^{-1} \cdot atm^{-1}$) was determined from the weight increase of the cup over time at a steady state of transfer. All

Table 1. Effect of the Solvent and Chitosan Content of Film-Forming Solutions on the Anti-Listerial Activity Evaluated by Numeration Experiments

film	solvent of the film-forming solution[a]	anti-listerial activity (%)
HPMC 2.5%	A	0
HPMC 2.5%	B	0
HPMC 2.5%	A + C (2:1)	0
HPMC 2.5%	A + B + C (2:2:1)	0
1% chitosan	A + B + C (2:2:1)	100
0.5% chitosan–1.5% HPMC	A + B + C (2:2:1)	91
1% chitosan–1.5% HPMC	A + B + C (2:2:1)	100
1.5% chitosan–1.5% HPMC	A + B + C (2:2:1)	100

[a] A, H_2O; B, 1% aqueous acetic acid; C, 95% ethanol.

experiments were conducted in triplicate. Treatment means were separated using Student's *t* test at 95% probability ($p < 0.05$).

Water Contact Angle Meter. A water drop was deposited on the surface of different films (size = 65 mm × 25 mm). The θ angle at the water/film interface was measured (Krüss instrument) after 5 s to the nearest degree. The θ value varied from 0 to 90° from hydrophilic (0°) to hydrophobic (90°) film nature. Five measurements on each film were performed at random positions. Treatment means were separated using Student's *t* test at 95% probability ($p < 0.05$).

Solubility in Water. The solubility in water of the different cellulose films was measured from immersion assays in 50 mL of distilled water for 24 h at 20 ± 1 °C. The solubility in water expressed as a percentage of the initial dry matter was determined from the residual dry weight after immersion compared to initial dry weight. The percentage of initial dry matter in film was determined by thermal processing at 103 °C for 1 h or by an infrared scale (Mettler instrument). All experiments were conducted in triplicate. Treatment means were separated using Student's *t* test at 95% probability ($p < 0.05$).

Spectroscopic Analysis. (a) FTIR Analysis. Infrared spectrometry using a Nicolet 210 apparatus with an attenuated total reflectance (ATR) accessory was selected because of saturation phenomena of transmission spectra. Infrared spectra were recorded in the 500−4000 cm^{-1} zone using 200 scans and with a resolution equal to 4 cm^{-1}.

(b) ^{13}C NMR Analysis. Solid state ^{13}C NMR spectra of powder samples were performed at room temperature on a Bruker DPX-400 NMR spectrometer, using MAS rates of 4 and 8 kHz, at a frequency of 100.61 MHz. Samples were packed in MAS 4 mm diameter zirconia rotors. All of the spectra were run for 15 h.

Mechanical Properties. The mechanical resistance of films was performed at 20 ± 1 °C and 50% ± 5 RH. It included tensile strength (TS, Pa), ultimate elongation (UE, percent at break point), and Young's modulus (*Y*, Pa). Tests were performed (Amadel Lhomargy instrument) according to AFNOR NF ISO 527-3 (1995) on nine films previously stored for 7 days at 20 ± 1 °C and 50 ± 5% RH. Films (analyzed area = 25 mm × 60 mm) were uniaxially stretched at a constant velocity of 3 mm/min. The stress−strain curves were computer-recorded. Treatment means were separated using Student's *t* test at 95% probability ($p < 0.05$).

RESULTS AND DISCUSSION

Preliminary Assays. First experiments were performed to determine the potential inhibitory activity of solvents on the selected bacterial strain. Bacterial growth was measured by the numeration method on tryptose agar medium. The control, in parallel, consisted of the same initial listerial charge without film. Second, the minimal content of chitosan necessary to produce strong anti-listerial materials was determined.

Results in **Table 1** indicated that none of the initial solvents influenced the anti-listerial activity of the films. The solvent combination A + B + C allowed the films to be formed more easily than with the other combinations as to the time needed to dry and peel the films from plates. The selected solvents were 1% aqueous acetic acid to dissolve chitosan, distilled water/ethanol (2:1) to dissolve HPMC, and PEG as plasticizer, without significantly ($p < 0.05$) inhibiting microbial growth.

After the selection of the solvent combination, different chitosan contents in films were tested for anti-listerial activity. Results proposed in the second part of **Table 1** show that 1% chitosan was sufficient to maintain a significant antimicrobial property within the biopackaging. Below 0.5%, film recovery was particularly difficult. As a result, the selected film composite, based on 1% chitosan and 1.5% HPMC, was used. The structure/property relationships were only measured on this combination, following stearic acid incorporation or the cross-linking process.

Table 2. Water Affinity and Anti-Listerial Activity of Composite, Composite–SA, and Cross-Linked Films[a]

	composite film	composite–SA film	cross-linked film
solubility in water (%)	100 ± 0	55 ± 7	40 ± 4
initial drop angle (deg)	65 ± 3	90 ± 6	59 ± 5
water content at 50% RH (%)	9.94 ± 0.86	11.08 ± 2,40	6.42 ± 1.17
WVTR ($g \cdot m^{-2} \cdot day^{-1} \cdot atm^{-1}$)	217 ± 7	134 ± 4	131 ± 13
anti-listerial activity			
growth control (UFC per Petri dish)	343 ± 6	278 ± 13	343 ± 6
listerial growth below the film (UFC per Petri dish)	0	0	247 ± 15
inhibition (%)	100	100	28

[a] SA, stearic acid; RH, relative humidity; WVTR, water vapor transfer rate; UFC, unit-forming colony. Data, followed by their standard deviations, are means of at least three experiments. Anti-listerial activity was investigated on the growth of *L. monocytogenes*.

Table 3. Kjeldahl Nitrogen Analysis of Composite, Composite–SA, and Cross-Linked Films[a]

	composite film (100 g)	composite–SA film (100 g)	cross-linked film (100 g)
N in the initial film (g)	2.40 ± 0.03	1.99 ± 0.03	2.28 ±0.03
solubilized N in immersion water (g)	2.38 ± 0.02	1.30 ± 0.03	0.04 ± 0.03
residual N in the film after immersion process (g)		0.66 ± 0.03	2.35 ± 0.03
solubilized N in water (%)	99	65	0

[a] SA, stearic acid. Data, followed by their standard deviations, are means of three experiments.

Structure−Property Relationships. Dehydration of food products can be limited by the use of edible films as moisture barriers. Because of the hydrophilic nature of HPMC films, a significant water vapor transfer was expected. To improve the moisture barrier, incorporation of edible hydrophobic compounds, such as fatty acid, and chemical modification on cellulose hydroxyl groups from cross-linking modification were studied. A previous study carried out on HPMC-based films had shown that a stearic acid content >15% (w/w HPMC) produced an optically unusable film with very poor mechanical properties (*19*). In the present study, 15% fatty acid content (w/w polysaccharide) was thus used, and the effects of stearic acid incorporation (composite−SA films) or cross-linking with

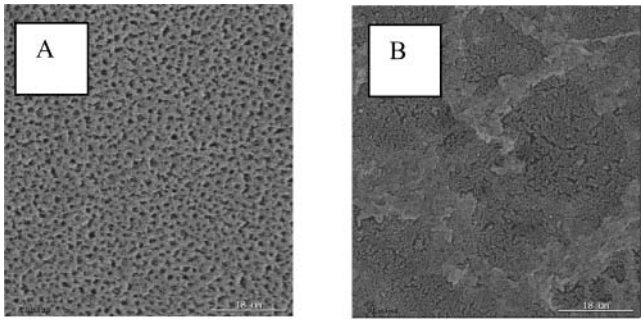

Figure 2. Scanning electronic microscopic views (JEOL 840A): (**A**) surface observation of composite film, magnification × 1500; (**B**) surface observation of cross-linked film, magnification × 1500.

citric acid (cross-linked films) were investigated on liquid−water interactions, moisture transfer, and antibacterial properties.

The first part of **Table 2** shows water sensitivity parameters of composite−SA and cross-linked films. Composite films were completely soluble in distilled water. Combination with stearic acid, under the present experimental conditions, decreased water solubility to 55%, and the solubility in water of cross-linked films was further reduced to 40%, due to interchain covalent bonds from cross-linking reactions, thus opening application possibilities for these materials. To determine the chitosan distribution between the aqueous phase and the insoluble film, Kjeldahl nitrogen analysis (Kjeltec 2200 apparatus) was conducted on initial films, immersion water, and residual material (recovered after immersion). Fatty acid incorporation decreased the fraction of solubilized chitosan (**Table 3**). Stearic acid thus decreased the accessibility of chitosan, and potential chitosan−fatty acid interactions could lead to a hydrophilic−hydrophobic "complex". Results obtained after cross-linking show that the fraction of solubilized film corresponded only to HPMC. This observation can be explained by the creation of covalent bonds primarily between polyacid and chitosan (and much more slightly with HPMC), leading to esters and/or amide groups.

Incorporation of SA into the film-forming solution strongly increased the initial drop angles, due to its hydrophobic nature (**Table 2**). Opposite results were obtained with cross-linked films. Moreover, for the composite−SA and cross-linked films the angle did not change for at least 20 s. The composite film, made from chitosan and HPMC, absorbed the water drop immediately after it was positioned. These results must be interpreted cautiously due to problems of experiment repeatability for the cross-linked films, concerning the very heterogeneous surface area structure of the chemically modified films (**Figure 2**).

To determine the water vapor barrier, the WVTR of films was calculated. The HPMC film, without chitosan, showed a significant WVTR near 270 $g \cdot m^{-2} \cdot day^{-1} \cdot atm^{-1}$. This strong coefficient was due to multiple interactions between water and the free hydroxyl group of HPMC. For composite films, a combination of HPMC with chitosan reduced this value by 20%. These results were further improved to 40% by the addition of SA and/or cross-linking. Water sorption in the different films was determined at 50 ± 5% RH and 20 ± 1 °C, and results showed no significant differences between water content of the composite and the composite−SA (**Table 2**). Cross-linked film had a water vapor sorption significantly weaker than those of both composite films. This latter result led to the assumption, taking into account the same WVTR obtained from composite−SA and cross-linked films, that the water diffusion coefficient through the chemically modified film could be higher than that of composite−SA.

Concerning antibacterial activity of films, results are given in the second part of **Table 2**. Chitosan−HPMC-based films totally inhibited listerial growth. The polycationic nature of the chitosan under the present experimental conditions was thus the bioactive compound responsible for the biological properties of the film. Homogeneous HPMC films, formulated from 1% aqueous acetic acid as solvent, were also tested to verify that a possible limitation of oxygen transfer by materials and/or an action of residual solvents still potentially present in films did not have consequences on the growth of *L. monocytogenes*.

In conclusion, the incorporation of stearic acid into the film-forming solution led to a decrease in the hydrophilic nature of the films and an increase in the moisture barrier properties with an inhibition of *L. monocytogenes* of 100%. The cross-linking improved the moisture barrier properties and, interestingly, the solubility in water, but the anti-listerial properties were lost.

The loss of the anti-listerial activity after chemical modification could possibly be attributed to a chemical reaction of the amino group, which is responsible for the anti-listerial activity.

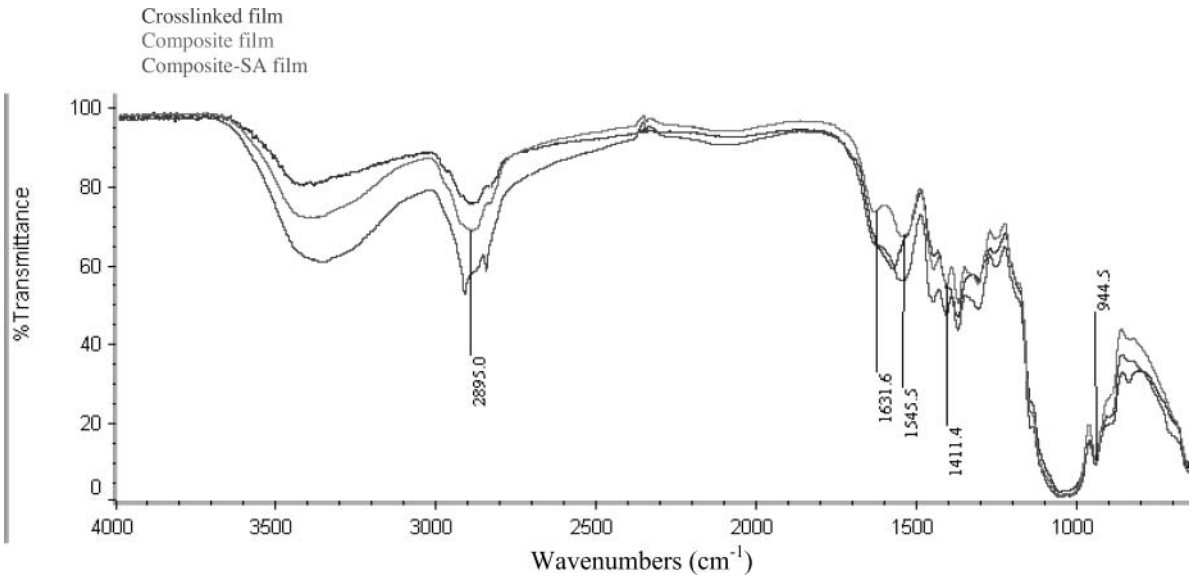

Figure 3. Infrared spectra of composite, composite–SA, and cross-linked films, from ATR analysis.

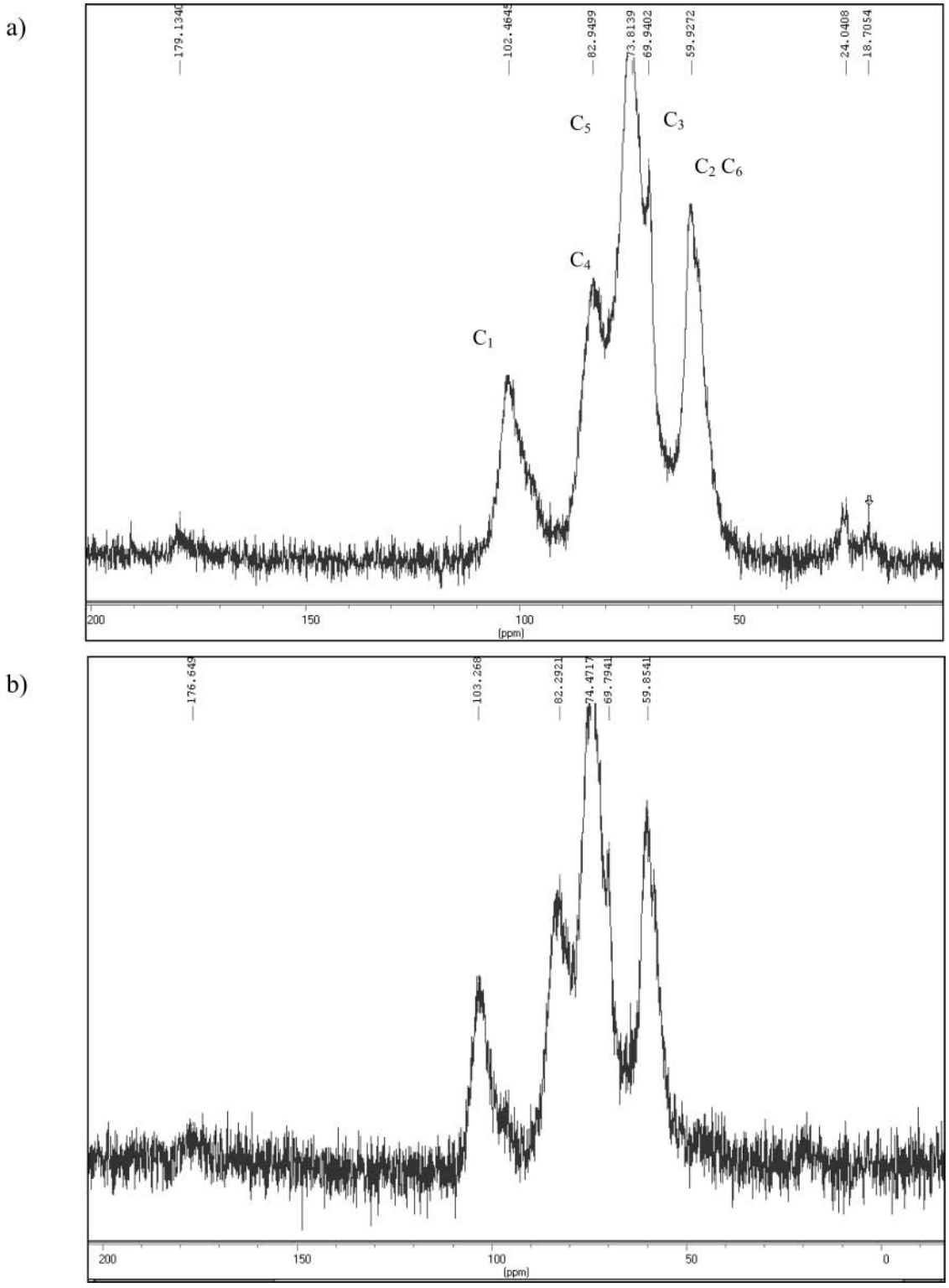

Figure 4. ^{13}C NMR spectra: (**a**) composite film chitosan–HPMC; (**b**) cross-linked film.

Between carboxylic functions of citric acid and amino groups of chitosan, a potential reaction was, indeed, an amide linkage.

To bear out this possibility, ATR-FTIR analysis was performed directly on the different films. The infrared spectra of the composite, composite−SA, and cross-linked films (**Figure 3**) showed characteristic polysaccharide bands at 1060 cm^{-1} (C−O−C stretching) as well as bands at 1550 cm^{-1} (A1) and 1407 cm^{-1} (A2), which, according to the literature, could be attributed to the −NH group (*22*). A1 and A2 could be attributed to $-NH_2$ or to the protonation of the primary amino group $-NH_3^+$. The band at 946 cm^{-1} (A3), anti-symmetric out of phase ring vibration, was used as an internal standard. Because of its variations with only the HPMC level, this reference band was not affected by the film thickness. Spectra of the composite−SA films showed bands at 2915 and 2849 cm^{-1}, due essentially to the CH_2 group of stearic acid. None of the infrared spectra clearly indicated the presence of carboxylic groups in the region near 1740 cm^{-1} or newly formed functional groups.

Table 4. Water Vapor Barrier and Inhibition Effectiveness of Cross-Linked Film, Thermally Treated HPMC–Chitosan Film, and HPMC–Chitosan Film with Citric Acid[a]

	cross-linked film	film without citric acid and with thermal processing	film with citric acid and without thermal processing
WVTR ($g \cdot m^{-2} \cdot day^{-1} \cdot atm^{-1}$)	131 ± 13	212 ± 10	194 ± 5
anti-listerial activity			
growth control (UFC per Petri dish)	343 ± 6	334 ± 12	278 ± 13
growth of listerial below the film (UFC per Petri dish)	247 ± 15	295 ± 13	8 ± 7
inhibition (%)	28	12	97

[a] WVTR, water vapor tranfer rate; UFC, unit-forming colony. Anti-listerial activity was investigated on the growth of L. monocytogenes.

Table 5. Mechanical Properties of Composite, Composite–SA, and Cross-Linked Films[a]

	film HPMC	composite film	composite–SA film	cross-linked film
UE (%)	6.63 ± 1.28	3.87 ± 0.50	1.79 ± 0.16	1.77 ± 0.16
Y (MPa)	19 ± 6	18 ± 2	31 ± 2	32 ± 3
TS (MPa)	34 ± 6	24 ± 2	30± 2	35 ±5

[a] SA, stearic acid; UE, ultimate elongation; *Y*, Young's modulus; TS, tensile strength. Data, followed by their standard deviations, are means of three experiments.

This led to the assumption that there could be a particular arrangement in the films: the hydrophobic hydrocarbon chains would be mostly oriented on the surface and the hydrophilic carbonyl groups would be directed inward into the film. Thus, it may be possible that the carbonyl groups are not seen on the infrared spectrum because of the ATR mode (the infrared beam penetrates only a few micrometers into the material).

To verify the absence of carbonyl groups in the cross-linked films, ^{13}C NMR measurements were performed on films first converted to powder by crushing in liquid nitrogen. The ^{13}C NMR spectrum confirmed the results of the infrared analyses (**Figure 4**): none of the signals could be attributed to carbonyl groups. No chemical reaction was observed during the cross-linking process. Citric acid incorporation and thermal processing may be involved in the loss of the anti-listerial activity.

Two different types of films were made and tested: thermally processed film without citric acid and film with citric acid but no thermal processing. Results given in **Table 4** show that only the thermal processing influenced the anti-listerial activity or, rather, caused the loss of the activity. It was difficult to determine the percentage of inhibition of the temperature-treated film (without citric acid) because the film did not stick to the agar medium. After deposition on the agar medium, the film partially came off. The colonies counted grew all over the agar medium, so it could be assumed that zones of the film that were not in contact with the medium, without anti-listerial activity, led to an undervaluation of the antimicrobial properties.

To complete the study, the mechanical properties of composite films were determined from tensile strength essays. **Table 5** shows the tensile strength, Young's modulus, and ultimate elongation of composite, composite−SA, and cross-linked films.

After stearic acid introduction and chemical modification, the UE decreased ~40%. Young's modulus increased ~70%. The films became less elastic and extensible as seen in a previous study (*21*). The incorporation of SA led to a decrease in mechanical resistance, which may have been due to the partial replacement of the polymer by lipids in the film matrix. This phenomenon led to discontinuities within the polysaccharide network. Concerning chemically modified films, the cross-linking led to extensive interchain bonding, which may have contributed to the limited flexibility of the films.

The present results indicate that HPMC−chitosan-based films show potential for preserving the microbial quality of perishable foods and, thus, extending their shelf life. It seems that the antimicrobial effectiveness of composite films depends on the mode of thermal processing. Mechanical properties would be improved by incorporation of a plasticizer. The impact of film deposition on the development of microbial resistance, especially on *L. monocytogenes* growth, should also be studied. Moreover, the antimicrobial effect of such composite films should be determined on an entire model food (surface and mass inhibition).

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