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New Bioactive Biomaterials Based on Quaternized Chitosan

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Chitosan was chemically modified to produce quaternary ammonium salts in order to improve its antimicrobial activity and physicochemical properties. Quaternization of *N*-alkyl chitosan derivatives was carried out using alkyl iodide to elaborate water-soluble cationic polyelectrolytes (*N,N,N*-trimethylchitosan, TMC). TMC was characterized by ^{1}H NMR spectroscopy; the quaternization degree was determined from ^{1}H NMR spectra and by titration of iodide ion. The antibacterial activity of hydroxypropylcellulose (HPC) films or coatings associated with chitosan or TMC as biocide was evaluated against the growth of *Listeria monocytogenes* and *Salmonella typhimurium*. The HPC−chitosan and HPC−TMC coatings exhibited a total inhibition on solid medium of both bacterial strains. Experiments conducted in liquid medium showed that the inhibitory activity against the growth of *Listeria innocua* was improved after chemical modification. Moreover, physicochemical properties of films were evaluated to determine their potential for food applications. The addition of the antibacterial agents showed a significant impact on the moisture barrier and mechanical properties of HPC films.

KEYWORDS: Active packaging; chitosan; *N,N,N*-trimethylchitosan; antibacterial activity; chemical modification

INTRODUCTION

Due to recent outbreaks of contaminations associated with food products, as well as growing concerns regarding the safety of intermediate-moisture foods, active packaging has been greatly developed in recent years. Principal active-packaging systems involve oxygen scavenging, moisture absorption, carbon dioxide or ethanol generation, and finally antimicrobial systems.

In addition, due to environmental considerations, the elaboration of biopackagings from renewable resources constitutes a very interesting option complementary to recycling. Throughout the past decade, bioactive chitosan matrices have been an interesting research topic for applications in food preservation. This copolymer including both β-1,4-anhydroglucosamine and *N*-acetyl-β-1,4-anhydroglucosamine units is a biodegradable polysaccharide exhibiting bioactive amino groups (**Figure 1**).

Many papers have been published on the utilization of chitosan as a bioactive matrix (*1–4*). The chitosan acts on both Gram-positive and Gram-negative bacteria, but its action seems to be less significant against Gram-negative strains (*5, 6*). Helander et al. (*7*) showed that highly concentrated chitosan has a bactericidal effect against Gram-negative bacteria such as *Salmonella typhimurium* (20000 ppm). Ouattara et al. (*8*) reported that chitosan films exhibited inhibitory effect against *Serratia liquefaciens*. According to Moller et al. (*9*), composite films obtained by the combination in the same proportions of chitosan and hydroxypropylmethyl cellulose inhibited completely the growth of *Listeria monocytogenes*.

However, the nonsolubility of chitosan in neutral and alkaline aqueous solutions limits its applications as a food preservative or as bioactive matrices. The biopolymer is soluble only in weak acid solutions. Moreover, the bioactivity is due to the cationic charges on the macromolecular chain, controlled by the solvent pH. In acid solutions, at a pH of <6.2, amino groups are mainly protonated and the soluble polysaccharide is positively charged.

Therefore, several derivatives of the chitosan were synthesized to improve its antimicrobial activity and its solubility in water. For example, the bioactivity was improved by depolymerization of chitosan with a chitinase at 50 °C for 24 h (*10*). The chitooligosaccharides, with lower molecular weight, showed a greater antibacterial activity against *Actinobacillus actinomycetemcomitans* at 0.1% (w/w). The complexing nature of chitosan was also used to improve its bioactive properties. A complex of chitosan−Zn^{2+} exhibited a higher antibacterial activity against *Escherichia coli*, compared to native chitosan (*11*).

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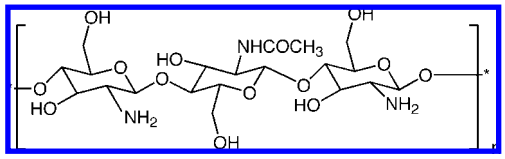

Figure 1. Ideal chemical structure of chitosan.

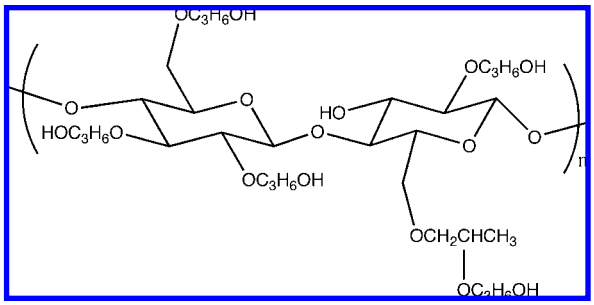

Figure 2. Ideal chemical structure of HPC.

To increase the solubility of chitosan in water, Yang et al. (*12*) substituted one NH_2 proton by a cellobiose unit. The derivative presented a significant inhibitory activity against *E. coli* and *Staphyloccocus aureus*. Although the disaccharide chitosan derivative showed less antimicrobial activity than the native chitosan at pH 6, the derivative exhibited a higher activity than native chitosan at pH 7. Xie et al. (*13*) synthesized multiple-derivated chitosan by etherification of chitosan with propylene epoxide followed by the graft copolymerization of maleic acid sodium in alkaline medium. The grafted hydroxypropyl chitosans also presented a bactericidal effect on *S. aureus* and *E. coli*. Muzzarelli et al. (*14*) studied the antifungal activity of the *N*-carboxymethylchitosan, *N*,*N*-dicarboxymethylchitosan, and *N*-phosphonomethylchitosan. The *N*-carboxymethyl derivative exhibited a significant antifungal activity, whereas the *N*-phosphonomethyl one allowed the growth of some molds. Moreover, the approach based on the introduction of an ammonium group allowed chitosan solubilization, whatever the pH solution, and an increase of the antimicrobial activity. Lim and Hudson (*15*) synthesized the *O*-acrylamide methyl-*N*-[(2-hydroxy-3-trimethylammonium)propyl] chitosan (NMA−HTCC) and specified that this product showed an antibacterial effect against *S. aureus* and *E. coli* growth. In addition, quaternization of chitosan by methyl groups (*N*,*N*,*N*-trimethylchitosan) improved antibacterial activity (*16, 17*). Indeed, to increase the solubility in water of chitosan and to produce permanent cationic charges, the protonation of amine groups by trimethylation is an interesting approach.

To improve the mechanical properties of chitosan-based films, the elaboration of biocomposite chitosan films with other polysaccharides was often investigated (*9, 18*). Film-forming capacities of hydroxypropylcellulose (HPC, **Figure 2**) have been largely studied to elaborate films or coatings due to the peculiar thermoplastic properties of this cellulose derivative. Films based on HPC showed suitable optical and mechanical properties. HPC is biodegradable, abundant, and inexpensive, and it belongs to renewable raw materials. Chitosan and HPC composite films can lead to improved mechanical and physical properties because these two polysaccharides have compatible structures.

This paper deals with the synthesis of *N*,*N*,*N*-trimethylchitosan (TMC) as an antibacterial agent. The antimicrobial effects of TMC against *L. monocytogenes* and *S. typhimurium* were compared to that of chitosan. In addition, antimicrobial HPC-based films were elaborated by adding chitosan or TMC as a biocide. The impact of biocide addition on moisture barrier properties, wettability, and mechanical properties of biocomposite films was determined.

MATERIALS AND METHODS

Materials. HPC (KLUCEL GF-EP) was provided by Hercules (France). Chitosan 244 (deacetylation degree > 95%, molecular mass = 400 kDa) was furnished by France Chitine (Marseille, France). Acetic acid (purity ≥ 99.5%) was provided by Sigma-Aldrich (France). Formaldehyde, sodium borohydride, sodium hydroxide, *N*-methyl-2-pyrrolidinone, sodium iodide, methyl iodide, bromine (Aldrich, Germany), diethyl ether (Fischer Chemicals, United Kingdom), potassium iodide (Prolabo, France), acetone (Xilab, France), and sodium bisulfite (SDS, France) were used, without further purification.

Organisms and Maintenance. *Listeria innocua* 430 (USMA collection, University Bordeaux 1, France) and *L. monocytogenes* (ATCC 15313) were grown in tryptose broth (DIFCO 62176), whereas *S. typhimurium*(IP 5858) was grown in nutritive broth (DIFCO 3178), at 37 °C and agitated at 140−160 rpm for 18−24 h..

Methods. *1. Synthesis and Characterization of N,N,N-Trimethylchitosan (TMC). 1.1. Synthesis of N-Methylchitosan.* Chitosan (4 g) was dissolved in 1% (v/v) aqueous acetic acid (400 mL). The solution was then filtered to eliminate the impurities. Formaldehyde was added (3-fold excess to amine of chitosan). The solution was stirred at ambient temperature for 30 min. $NaBH_4$ (0.33 g) was then added, and the solution was stirred at ambient temperature for 60 min. The pH was adjusted to 10 using 1 M NaOH. After filtration, the system was washed to reach pH 7. Finally, the excess of reagent was eliminated by extraction with a Soxhlet, using ethanol/diethyl ether (80:20 v/v). The product was dried at ambient temperature for 24 h.

1.2. Synthesis of N,N,N-Trimethylchitosan. Previously prepared *N*-methylchitosan was dispersed in 120 mL of *N*-methyl-2-pyrrolidinone with NaI (5 g) under vigorous agitation at 60 °C for 1 h. Fifteen percent NaOH (22 mL) and methyl iodide (10-fold excess to amine of chitosan) were then added. The mixture was stirred at 60 °C for 6 h. Finally, the quaternary ammonium salt of chitosan was precipitated using acetone (3-fold excess to the volume of *N*-methyl-2-pyrrolidinone). The product was dried under P_2O_5.

1.3. NMR Analysis. TMC (40–50 mg) ^{1}H NMR spectra were registered on a Bruker Avance 300 NMR spectrometer using in D_2O.

1.4. Determination of the Quaternization Degree of TMC. The quaternization degree of TMC was evaluated both by the titration of iodide ions (*19*) and by ^{1}H NMR spectra as described by Snyman et al. (*20*).

2. Film Preparation. 2.1. Homogeneous HPC Films. HPC (9 g), water (200 g), and ethanol (96%, 100 g) were mixed for 1 h under 500 rpm magnetic agitation. Film-forming solutions (30 g) were then degassed under reduced pressure, cast on polypropylene support, and then dried overnight at room temperature and room relative humidity. The films were peeled from the support, and samples were conditioned at 23 ± 1 °C and 50 ± 5% relative humidity for 7 days. Ten random measurements were carried out to measure film thickness (Micrometer Lorentzen & Wettre, Saint-Germain-en-Laye, France): the films were homogeneous and showed a thickness of 30 ± 2 μm.

2.2. Biocomposite Films Based on HPC Associated with Chitosan or TMC. The film-forming solutions were prepared with the same quantity of active moieties. Chitosan or TMC solutions (with 6×10^{-3} mol L^{-1} of glucosamine unit or trimethylglucosamine unit, respectively) were added to the HPC film-forming solution prepared as described under Homogeneous HPC Films at a ratio of 50:50 (v/v). The mixture was then degassed, cast (20 g), dried, and stored at 23 ± 1 °C and 50 ± 5% relative humidity for 7 days.

The film thickness was equal to 29 ± 2 μm for HPC−chitosan and 29 ± 1 μm for HPC−TMC.

3. Bioactivity Assessments. 3.1. Bacterial Preculture. Precultures were obtained by the inoculation of 9 mL of tryptose or nutrient broth for listerial strains and *S. typhimurium*, respectively, with 1 mL of a 18-h-old culture. The precultures were incubated at 37 °C for 18 h.

3.2. Antimicrobial Activity of Films. About 30–300 colony-forming units (CFU) of microbial strains per Petri dish were inoculated from the preculture on the surface of a tryptose or nutrient agar medium for

listerial or *S. typhimurium* strains, respectively. HPC-based films, associated with chitosan (HPC−chitosan) or with *N*,*N*,*N*-trimethylchitosan (HPC−TMC), were deposited on the surface medium and incubated at 37 °C for 24−48 h prior to numeration.

Control plates without film were conducted in parallel. Percentages of inhibition were calculated using the following equation:

$$\frac{\text{CFU number in control plates} - \text{CFU number in test plates}}{\text{CFU number in control plates}} \times 100$$

The experiment was repeated six times.

3.3. Antimicrobial Activity of Coatings. As specified above, agar medium in Petri dishes was inoculated with the target strain. Selected film-forming solution (30 g of HPC solution and 20 g of HPC−chitosan or HPC−TMC) was deposited on the inoculated surface to produce a coating of about 30 μm, after 5 h of drying in a flow hood at room temperature prior to the incubation at 37 °C for 24–48 h. The experiment was repeated six times.

The percentages of inhibition were calculated using the same expression as specified for film bioactivity.

3.4. Antimicrobial Properties of Chitosan and TMC against L. innocua Growth in Liquid Medium. Chitosan or TMC was added during the lag phase, with the same concentration in active moieties, that is, glucosamine and trimethylglucosamine unit. *L. innocua* preculture (1 mL) and chitosan or TMC solution with 6×10^{-3} mol L^{-1} in active units (10 mL) were added to tryptose broth (100 mL, time = 0 h), corresponding to a final concentration of 0.54×10^{-3} mol L^{-1} in active moieties in the culture medium. The culture was then incubated at 37 °C and agitated at 140 rpm. The bacterial growth was evaluated by periodic numeration on tryptose agar, after sequential dilutions. Four repetitions were carried out.

4. Film Characterization. 4.1. Water Content. The films were stored at 23 ± 1 °C and 50 ± 5% relative humidity for 7 days prior to the determination of the initial mass. The moisture content was determined by drying the films at 105 °C, until a constant mass. The moisture content was calculated as

$$\text{moisture content of film (\%)} = [(m_0 - m_1)/m_1] \times 100$$

with m_0 = mass of film after storage at 23 ± 1 °C and 50 ± 5% relative humidity and m_1 = mass of dried film (105 °C). Five repetitions were performed.

4.2. Solubility in Water. The solubility in water of the different films was measured from immersion assays in distilled water (50 mL) for 24 h at 23 ± 1 °C. The water solubility, expressed as a percentage of the initial dry matter, was determined from residual dry weight after immersion compared to initial dry weight. The percentage of initial dry matter in film was determined after drying at 105 °C until constant mass. All tests were conducted in triplicate.

4.3. Water Contact Angle. The contact angle between the material and a distilled water drop was measured according to TAPPI T458 cm-94 (1994) and using a goniometer Krüss DSA 10Mk2 (Krüss, Palaiseau, France) equipped with a camera and a recording system. The resulting angle was calculated after five measurements. A water drop was deposited on the surface of the different films. The θ angle in the interface water/film was measured at the nearest 1°. Three measurements on each film were performed at random positions.

4.4. Water Vapor Transmission Rate (WVTR). The WVTR of biomaterials was evaluated using NF ISO 2528 (1989). Briefly, 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. It was placed in an environment of controlled humidity and temperature (50 ± 5% relative humidty and 23 ± 1 °C). The WVTR (g m^{-2} 24 h^{-1} atm^{-1}) was determined from the weight increase of the cup over time at a steady state of transfer. All tests were conducted in triplicate.

4.5. Mechanical Properties. The mechanical resistance of films was performed at 23 ± 1 °C and 50 ± 5% relative humidity. It included tensile strength (TS, Pa), elongation at break (EB, %), and Young's modulus (*Y*, Pa). Tests were performed on an Adamel Lhomargy instrument according to AFNOR NF ISO 527-3 (1995) on five films previously stored for 7 days at 23 ± 1 °C and 50 ± 5% relative humidity. 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.

All experiments were replicated at least three times. Treatment means were compared using the Student confidence interval at 95% probability ($p > 95\%$).

RESULTS AND DISCUSSION

Synthesis of TMC. The quaternization of chitosan was carried out to improve the solubility of chitosan in water or other solvents and to generate non-pH-dependent positive charges while increasing its antimicrobial activity. The quaternization was performed according to a modified method of Jia et al. (*17*) using iodomethane. This method allows the synthesis of TMC with a high degree of quaternization (Dq > 90%). According to Britto and Assis (*21*) various methods have been used to synthesize quaternary chitosan salts. TMC with a quaternization degree equal to 52.5% has been achieved by reacting chitosan with dimethylsulfate in *N*-methyl-2-pyrrolidone at room temperature. This method led to a less depolymerized chitosan than usual quaternization reactions. However, the quaternization degree of chitosan obtained in this reaction was lower than that with iodomethane.

TMC synthesis using iodomethane was also selected in this paper to study, for a second time, the influence of the nature of alkyl chains potentially grafted on the polyglucosamine.

In this study, chitosan quaternization was performed in two steps (**Figure 3**): step 1, monoalkylation of the amine group; step 2, quaternization of the alkyl chitosan.

Aldehydes and ketones form hemiaminals with amine groups. The hemiaminals, resulting from primary amines of anhydroglucosamine units of chitosan, easily lose water, inducing a double carbon−nitrogen bond. The reduction of the double bond allows the formation of *N*-alkyl chitosan. As already mentioned, the synthesis of TMC with iodomethane was chosen for its general applications to synthezise various *N*-alkyl and *N*,*N*-dimethyl chitosans in order to further study the influence of the alkyl chain length on antimicrobial properties. Formaldehyde was selected for the chitosan alkylation. The quaternization of *N*-methyl chitosan was then obtained using methyl iodide with sodium hydroxide at 60 °C for 6 h under vigorous agitation. With regard to the method used by Jia et al. (*17*), in which alkyl chitosan was dried under vacuum conditions for 12 h at 40 °C, the drying at ambient temperature for 12 h allowed better ability to react with iodomethane and reduced the reaction time from 24 to 6 h, for practically the same quaternization degree.

First, TMC was characterized by ^{1}H NMR (**Figure 4**). In addition to the signals of glucopyranose proton, the spectrum revealed an intense signal at 3.16 ppm corresponding to the trimethylammonium group. The formation of 3-*O*(CH_3) (3.55 ppm) and 6-*O*(CH_3) (3.45 ppm) alkylkated chitosan side products was also observed (*22*). Sieval et al. (*23*) reported that TMC with a high degree of quaternization (Dq > 85%) exhibited a complete O-methylation. The quaternization degree of TMC was determined from the dosage of iodide ions (*18*) and from the ^{1}H NMR spectrum as described by Snyman et al. (*19*). Both methods showed a quaternization degree of 95%.

In addition, TMC showed a total solubility in acid-free water at any pH value (a mass of 4 g of TMC was totally and quickly soluble in 100 mL of water).

Bioactivity of the Chitosan-Based Materials. *Antibacterial Activity of Films.* Inhibitory activity of HPC-, HPC−chitosan-, or HPC−TMC-based films was measured against *L. monocytogenes* or *S. typhimurium* growth. The results are presented in **Table 1**. The incorporation of TMC in HPC-based matrices

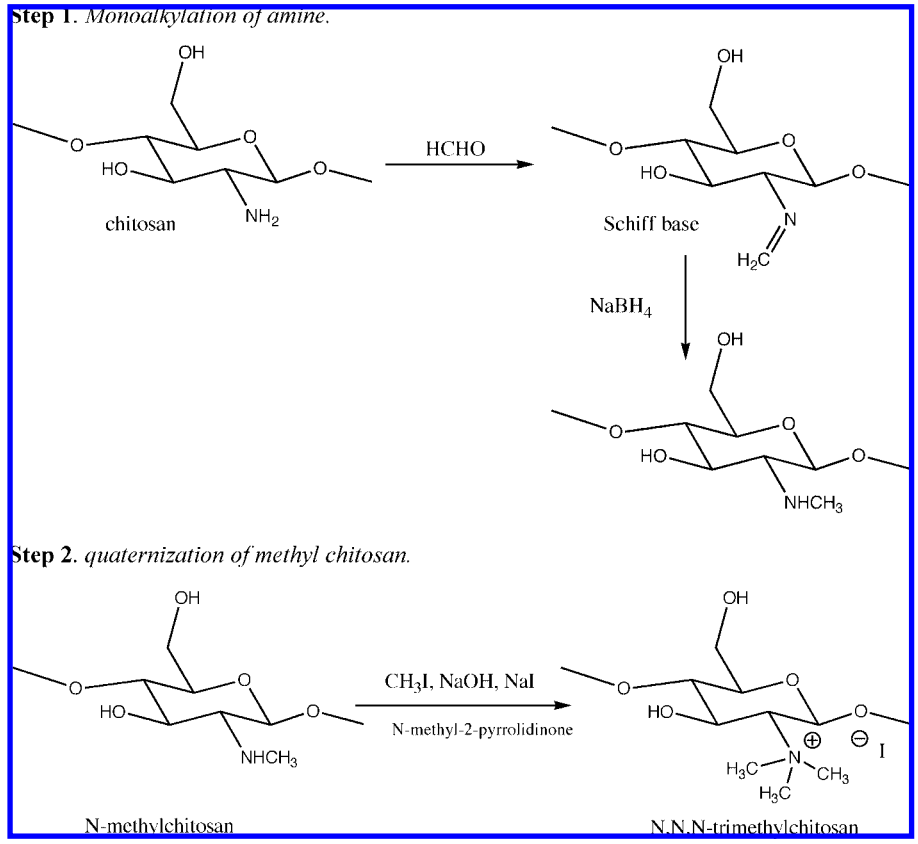

Figure 3. Mechanism of chitosan quaternization.

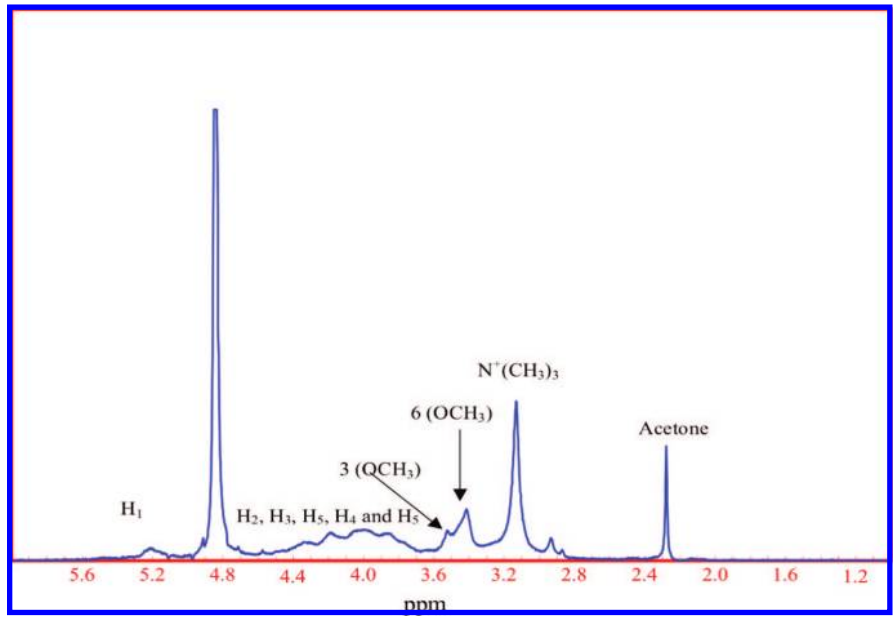

Figure 4. ^{1}H NMR spectrum of TMC.

Table 1. Antibacterial Activity (Inhibition Percent) of Different Films against *L. monocytogenes* and *S. typhimurium* Growth

	film		
	HPC	HPC−chitosan	HPC−TMC
L. monocytogenes	0	ND[a]	91 ± 1
S. typhimurium	0	ND	100 ± 0

[a] Not determined.

allowed a strong antibacterial activity against both bacterial strains, with an inhibition of 90 or 100% of the listerial or *S. typhimurium* development, respectively. The impact of the incorporation of the unmodified chitosan in the HPC film matrix could not be determined due to the development of film opacity after incubation, which did not allow any bacterial numeration. As a result, to study the influence of the chemical modification on the antibacterial activity, the experiments were then conducted with the coatings of HPC, HPC−chitosan, and HPC−TMC.

Antibacterial Activity of Coatings. The antibacterial activity of the coatings against *L. monocytogenes* and *S. typhimurium* was determined on solid medium by a numeration technique.

Table 2. Antibacterial Activity (Inhibition Percent) of the Coatings Based on HPC, HPC−Chitosan, and HPC−TMC on *L. monocytogenes* and *S. typhimurium* Development

	coating		
	HPC	HPC−chitosan	HPC−TMC
L. monocytogenes	0	96 ± 10	96 ± 1
S. typhimurium	0	99 ± 3	100 ± 0

The results are presented in **Table 2**. The coatings with unmodified and modified chitosan exhibited a significant antibacterial activity against both target pathogen strains, with an inhibition close to 100%. According to Helander et al. (*7*), a key feature of chitosan is the positive charge of the amino group at C-2 below its pK_a (6.3). This creates a polycationic structure, which can be expected to interact with the predominantly anionic components (lipopolysaccharides, proteins) of the cell surface. The comparable strong bioactivity of chitosan and its derivative could be due to the weak initial bacterial charge, essential for direct bacterial numeration (from 30 to 300 CFU per Petri dish). To compare the activities of chitosan and TMC using a higher contamination level, a study in liquid medium was conducted on *L. innocua*. *L. innocua* was used instead of *L. monocytogenes* because it is nonpathogenic to humans and it behaves similarly to the pathogen strain with respect to many biocides.

Influence of the Chemical Modification on the Inhibitory Activity against L. innocua. The antibacterial activity of quaternized chitosan and chitosan against *L. innocua* was compared in liquid medium. As shown in **Figure 5**, TMC exhibited a superior antibacterial activity compared to chitosan. The cell number in the control culture increased from $10^{8.97}$ to $10^{10.51}$ CFU after 3 h of incubation. After the same time of incubation, a reduction of 31.6% was obtained with TMC compared to 17.6% with chitosan. Moreover, after 9 h of incubation, the inhibition percentage with chitosan was lower than 2.5%, whereas 26.8% of inhibition was maintained with TMC and with a number of bacteria cells lower than the initial microbial charge ($10^{8.97}$ CFU).

First, the higher bioactivity of TMC could be due to the permanent positive charges on the chitosan chain, as a consequence of the quaternization of the amino groups in the C-2 position. As already specified, the bioactivity of chitosan was the result of ionic interactions between the positive charges of the chitosan and the negatively charged cell surface of bacteria (*7, 20*). The nonstable inhibition of the unmodified chitosan could be due to their dependence on the pH and to potential resistant bacteria. Indeed, Roller and Covill (*24*) showed that a solution of chitosan with 0.5 g L^{-1} in active moieties produced morphological abnormalities on the cellular membrane of *Zygosaccharomyces bailii* molds. However, this morphological change disappeared after 10 min of incubation. The authors mentioned that these fungal strains developed a resistance against the bioactivity mechanisms of chitosan.

In contrast to the bioactivity of chitosan, the inhibitory activity of TMC was maintained during the incubation time. After quaternization, the chitosan became a water-soluble polyelectrolyte, with a permanent cationic charge density. Jia et al. (*17*) also found that the antibacterial activity of quaternized chitosan against *E. coli* was stronger than that of chitosan. This different behavior could also be due to the lower polymerization degree of TMC compared to the starting polymer. As already mentioned, the quaternization led to a reduction in chitosan molecular weight due to temperature and alkaline synthesis conditions (*19*). TMC could then penetrate through the cellular membrane of bacteria and act on the intercellular material, leading to an improvement of its antibacterial action. Indeed, Chi et al. (*25*) suggested that chitosan−*N*-2-hydroxypropyltrimethylammonium chloride compounds with low molecular weights are able to pass the outer membrane of the cell surface of microorganisms and absorbs the cytoplasm with anion to disturb the microbial growth.

TMC demonstrated improved antilisterial activity when compared with chitosan and offers a great advantage in preventing pathogen strain growth, particularly Gram-positive bacteria.

Film Characterization. *Affinity of Biopackagings to Water Vapor.* The incorporation of the active agents had a significant impact on the water vapor transfer of HPC-based films (**Table 3**). The addition of chitosan led to an increase of about 18% in WVTR values. The WVTR further increased after the addition of TMC (30%). The decrease of the moisture barrier properties after incorporation of the aminopolysaccharides could be due to the hydrophilic character of both biocides. The negative impact on the WVTR of the chemical modification of the chitosan could be also due to the presence of voluminous moieties on the macromolecular chain, leaving spaces between chains and allowing the diffusion of water molecules (*26*). The incorporation of bioactive agents could also influence the arrangement of the polymer chain. In addition, a partial miscibility of the biopolymers, even if both components of the blend are polysaccharides and have similar chemical structures, could also increase the transfer (*27*). An improvement of the moisture barrier properties could be obtained, for example, by the addition of more hydrophobic compounds (*28*).

Wettability of Biopackagings toward Liquid Water. The sensitivity of the film surface toward liquid water was first estimated by the water drop angle contact. The results showed that introduction of chitosan in HPC films did not change the natural hydrophilic character of the cellulose films (**Table 3**). On the other hand, TMC slightly increased the contact angle (7%), but without significant modification of the hydrophilic character of HPC films. Britto and Assis (*20*) showed that the affinity to water was increased in quaternized derivatives, from 100° for an unmodified chitosan to <40° for a quaternized chitosan in acidic conditions. According to these authors, there are two opposing phenomena occurring after quaternization. Quaternization of chitosan increases the hydrophilic character due to the formation of permanent positive charges. In contrast, O-methylation and N,N-dimethylation reduce the hydrophilic character. Indeed, the expected higher affinity to water of the TMC would be balanced by both side reactions.

The second parameter to estimate the liquid water sensitivity of biopackagings is the solubility in water, a parameter that can select applications in food preservation. The films of HPC, HPC−chitosan, and HPC−TMC showed the same moisture content, close to 4% (w/w), and were totally soluble in water (**Table 3**).

Mechanical Properties. The mechanical properties of the films were determined, and the results of tensile strength, Young's modulus, and elongation at break are presented in **Table 4**.

The mechanical properties of HPC films are in accordance with Almeida et al. (*29*). However, the confidence intervals were significant and the impact of biocide incorporation on mechanical properties was not so clear due to the high variability, which could be due to a nonuniform distribution of the biocide within HPC matrices. Nevertheless, tendencies could be observed. Homogeneous HPC films showed a plastic deformation, with

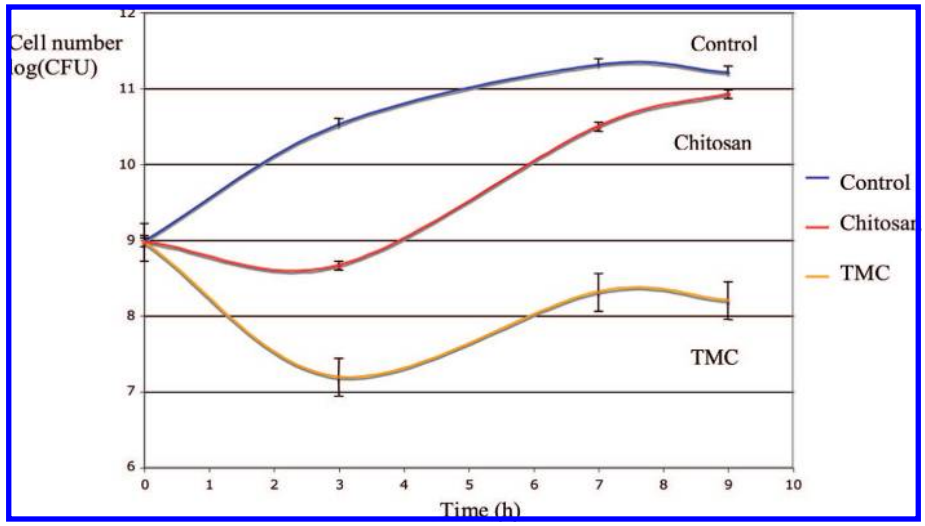

Figure 5. Antimicrobial properties of chitosan and TMC against *L. innocua* growth in liquid medium.

Table 3. Physicochemical Properties of Chitosan-Based Films

	film		
	HPC	HPC−chitosan	HPC−TMC
thickness (μm)	30 ± 2	29 ± 2	29 ± 1
WVTR (g m^{-2} 24 h^{-1} atm^{-1})	144 ± 10	170 ± 15	188 ± 12
water drop angle contact (deg)	59 ± 5	59 ± 6	63 ± 5
water content (%)	3.8 ± 0.1	4.0 ± 0.7	4.0 ± 0.4
solubility in water (%)	100	100	100

Table 4. Mechanical Properties of the HPC, HPC−Chitosan, and HPC−TMC Films

	film		
property[a]	HPC	HPC−chitosan	HPC−TMC
Y (MPa)	291 ± 28	439 ± 24	337 ± 87
TS (MPa)	18 ± 3	18 ± 4	16 ± 3
EB (%)	94 ± 6	60 ± 12	110 ± 13

[a] *Y*, Young's modulus; TS, tensile strength; EB, elongation at break.

90% elongation, which was reduced after chitosan incorporation. Addition of chitosan or TMC led to an increase of Young's modulus of about 50 or 15%, respectively, whereas the elongation decreased. Tensile strength remained practically unchanged after the incorporation of the bioactive agents. The decrease in mechanical strength of HPC films after chitosan or TMC incorporation could be due to a not so good miscibility of the components in the systems (*24*).

Conclusion. The non-pH-dependent quaternization of chitosan led to a water-soluble bioactive agent. Moreover, the antibacterial activity was improved and TMC bioactivity was found to last longer. Incorporation of chitosan and of TMC in HPC matrices allowed elaboration of effective antibacterial biopackagings, with a weak impact of the biocide addition on moisture barrier properties, on liquid water interaction, and on mechanical properties. Association of TMC and a biopolymer such as HPC to develop high-performance food packaging is promising. Nevertheless, studies will be pursued to determine the impact of TMC on Gram-negative bacteria in liquid medium. In addition, the effect of the biocide molecular weight will be further examined. Finally, the impact of the alkyl chain length will be determined in the future. Indeed, a higher lipophilicity could confer to TMC an ability to penetrate through the cell wall, particularly for less sensitive Gram-negative bacteria.

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