



Environmentally friendly films based on chitosan and tetrahydrocurcuminoid derivatives exhibiting antibacterial and antioxidative properties

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ABSTRACT

Environmentally friendly films exhibiting both antibacterial and antioxidative properties were elaborated from chitosan and tetrahydrocurcuminoids (THCs). Two tetrahydrocurcuminoids, THC1 (5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hept-4-en-3-one) and THC2 (5-hydroxy-1,7-bis(4-hydroxy-3,5-dimethoxyphenyl)hept-4-en-3-one), were incorporated into a chitosan film. THC1 could be prepared from natural curcumin extracted from turmeric roots (*Curcuma longa* L.). The resulting tetrahydrocurcuminoid-chitosan films exhibited a high free-radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol, which was due to a progressive release of the THCs into the solvent. The release kinetics was governed both by molecular interactions between chitosan and THCs and probably by electrostatic forces between the ammonium units in chitosan and the aromatic rings in THCs. These interactions were clearly evidenced by the presence of new absorption bands in the visible regions of the electronic absorption spectra of the THCs. The molecular nature of these interactions was shown using glucosamine, the main monomer of chitosan. When associated with THCs, chitosan retained its bioactivity against *Listeria innocua*; THCs alone were not bioactive enough against listerial strains.

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1. Introduction

Oxidative reactions and contamination by pathogenic microorganisms are among the main factors reducing the shelf life of perishable foods (Lee, An, Lee, Park, & Lee, 2004). Packaging is generally used to maintain the quality and extend the shelf life of food products (Almenar, Catala, Hernandez-Muñoz, & Gavara, 2009; Cutter, 2002; Del Nobile et al., 2008; Kerry, O'Grady, & Hogan, 2006; Kilcast & Subramaniam, 2000).

Renewed interest in food packages based on natural macromolecules in recent years has been due to concerns about the environment and to a need to reduce the amount of disposable packaging materials. Biomass is a naturally abundant source of sustainable biopolymers, and in the last years, increasing environmental awareness has led to growing interest in the development of green compounds with improved performance. Biopolymer films and coatings, which act as a barrier to external elements (bacteria, moisture, oil, gases, volatile organic compounds...) and thus protect the product and extend its shelf life, are generally made from biological materials such as polysaccharides, proteins, lipids, and derivatives (Arvanitoyannis, 1999; Guilbert, Gontard, & Gorris, 1996; Petersen et al., 1999; Phisalaphong & Jatupaiboon, 2008; Tharanathan, 2003). Edible films have been used to coat food sur-

faces to preserve food quality by acting as a barrier to external elements such as oil, oxygen, moisture, aroma, and can also be used to provide additional nutrients or quality-enhancing ingredients such as antioxidants (Gomez-Estaca, Montero, Gimenez, & Gomez-Guillen, 2007; Jung, Chung, & Lee, 2006). The potential use of polysaccharides such as chitosan in food packaging is of great interest owing to its specific properties. Chitosan is a linear aminopolysaccharide obtained by deacetylation of chitin, a major structural component of the exoskeleton of invertebrates (Rasmussen & Morrissey, 2007; Shahidi, 2007) and of the cell walls of fungi (Dai, Cao, Li, Zeng, & Zhao, 2004), representing one of the most abundant natural biopolymer. Chitosan is a very promising biopolymer because it is environmentally friendly due to its biodegradability, and it exhibits good film-forming properties (Bordenave, Grelier, Pichavant, & Coma, 2007; Bourtoom & Chinnan, 2008; Gaellstedt, Brottman, & Hedenqvist, 2005; Kumar, 2000; Mucha & Miskiewicz, 2000; Nada, El-Sakhawy, Kamel, Eid, & Adel, 2006; Ziani, Oses, Coma, & Maté, 2008) combined with antimicrobial activity. Both antibacterial (Chen, Yeh, & Chiang, 1996; Coma, Deschamps, & Martial-Gros, 2003; Coma et al., 2002; Fernandez-Saiz, Lagaron, & Ocio, 2009; Helander, Nurmiäho-Lassila, Ahvenainen, Rhoades, & Roller, 2001; Knowles & Roller, 2001; Moller, Grelier, Pardon, & Coma, 2004; Tsai & Su, 1999) and antifungal (Coma, 2008; Fimbeau, Grelier, Copinet, & Coma, 2006; Roller & Covill, 1999; Sebt, Martial-Gros, Carnet-Pantiez, Grelier, & Coma, 2005) properties have been observed. The potential of chitosan for extending the

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shelf life of food products as antimicrobial films and coatings has been reviewed (No, Meyers, Prinyawiwatkul, & Xu, 2007; Srinavasa & Tharanathan, 2007). In addition, chitosan-based microcapsules (Kosaraju, D'ath, & Lawrence, 2006; Parize et al., 2008) and chitosan-based films (Özmeric et al., 2000) could be used as carriers for active molecules such as antioxidants. Mathew and Abraham (2008) successfully incorporated ferulic acid into starch–chitosan blend films, and reported intermolecular interactions between the different components. Such ferulic acid-based films result in a reduced formation of lipid peroxide.

In response to a growing concern regarding food safety, natural antioxidants such as tocopherol derivatives are now used (Wesling, Nielsen, Leufven, & Jägerstad, 1999). Among other compounds, tetrahydrocurcuminoids (THCs) are derived from curcuminoids extracted from the roots of *Curcuma longa* L., commonly called turmeric. THCs are colorless and more potent than yellow curcuminoids as antioxidants (Portes, Gardrat, & Castellan, 2007); they could therefore be used in colorless foods and cosmetics commonly employing conventional synthetic antioxidants (Majeed, Badmaev, Shimakumar, & Rajendran, 1990; see www.tetrahydrocurcuminoids.com). Curcuminoids and tetrahydrocurcuminoids have very similar mechanisms of antioxidative action (Portes et al., 2007), but tetrahydrocurcuminoids are much more photostable than curcuminoids (Castellan et al., 2007). The potential antimicrobial activity of tetrahydrocurcuminoids against several Gram-positive bacteria (Venkateswarlu, Rambabu, Subbaraju, & Satyanarayana, 2000) has attracted interest because these compounds may be able to offer protection against *Listeria monocytogenes*, a human pathogenic bacterium (listeriosis is one of the more severe foodborne infections). Coatings exhibiting both antimicrobial and antioxidative properties appear very attractive and have already been developed by Lee et al. (2004) from a combination of nisin and α -tocopherol in a vinyl acetate–ethylene copolymer binder.

The main object of this study was to make films exhibiting both antibacterial and antioxidative properties from renewable resources through the use of protonated chitosan as film matrix, and through the incorporation of two smaller molecules – tetrahydrocurcuminoids THC1 and THC2 (Fig. 1) – potentially released in food. These objectives are a step forward in the preparation of sustainable carbohydrate-based films with advanced properties which can be used to protect food. Nevertheless, it was shown that chitosan dissolved in an aqueous solution exhibits antioxidative properties (Huang, Rajapakse, & Kim, 2006; Kim & Thomas, 2007; Park, Je, & Kim, 2004; Yen, Yang, & Mau, 2008), and this aminopolysaccharide has been mainly used as an insoluble matrix exhibiting antibacterial properties thanks to its protonated amino groups. First of all, the antioxidative properties of tetrahydrocurcuminoids incorporated into films were studied. The interactions between chitosan and tetrahydrocurcuminoids in films and in solutions, as revealed by UV–vis absorption spectrometry, were then discussed. Secondly, the bioactivity of THCs against *Listeria innocua* – selected as model bacterial strain due to its non-pathogenicity to humans and to a similar behavior to *L. monocytogenes* against several bio-

cides (Sebti & Coma, 2002) – was evaluated. Finally, the antibacterial properties of THC–chitosan films against the same selected strain were evaluated to know whether possible interactions between both biomolecules may lead to a reduced bioactivity of chitosan-based films.

2. Experimental

2.1. Materials and reagents

The syntheses of 5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hept-4-en-3-one (THC1) and 5-hydroxy-1,7-bis(4-hydroxy-3,5-dimethoxyphenyl)hept-4-en-3-one (THC2) were reported previously (Portes et al., 2007). It was shown that both compounds are in their respective enolic forms in protic solvents. Organic solvents (spectroscopic grade) were obtained from Sigma–Aldrich (Saint Quentin Fallavier, France) and were used as received. Water was prepared by osmotic deionization of tap water. Acetic acid (99.7%, ACS grade) was purchased from Sigma–Aldrich. Chitosan powder 652 (165 kDa; low viscosity; food grade; degree of deacetylation: 85%) was provided by France Chitine (Marseille, France). *Listeria innocua*, selected from a private collection (Unité Sécurité Microbiologique des Aliments, Institut des Sciences et Techniques des Aliments de Bordeaux, University Bordeaux 1, France) was grown in Difco 62176 tryptose broth (Fisher Scientific Bioblock, Illkirch, France) at 37 °C for 18 h.

2.2. Film formation and characterization

2.2.1. Homogeneous chitosan films

A 2% (w/v) film-forming solution was obtained by dispersing chitosan in a 1% (w/w) acetic acid aqueous solution (pH 3.5). The solution was filtered through a Büchner funnel and then degassed under reduced pressure for 1 h. Films were obtained by casting the solutions onto polystyrene plates which were dried for 12 h under a laminar-flow hood at room temperature. Thanks to degasification and drying under laminar-flow, even though at room temperature, most of the volatile acetic acid could be removed from the thin films. Film thickness was measured using a D20S micrometer (Lorentzen and Wettre, Stockholm, Sweden). Ten thickness values were taken randomly at different positions on each film. Film thickness was found to be $31 \pm 5 \mu\text{m}$.

2.2.2. Chitosan–THC films

Film-forming solutions (2%) were prepared by adding chitosan (2 g) and THC (20 mg) to 100 mL of a 1% acetic acid aqueous solution (pH 3.5) under vigorous agitation. The solutions were filtered and cast as in the procedure used to make homogeneous chitosan films. Ten thickness values were taken randomly at different positions on each film. Film thicknesses were found to be $30 \pm 4 \mu\text{m}$. A good repeatability of the absorbances (Lambda 18 Perkin–Elmer spectrometer UV–vis) – directly measured on different portions of the films – showed that the THCs were distributed homogeneously.

2.3. Assessment of the release of THCs from the chitosan film

The release of THC1 and THC2 from chitosan films was measured by UV absorption as above, in methanol, at 280 nm (quartz cell length: 1 cm; $\epsilon_{280, \text{THC1}} = 15,400 \text{ L mol}^{-1} \text{ cm}^{-1}$; $\epsilon_{280, \text{THC2}} = 13,800 \text{ L mol}^{-1} \text{ cm}^{-1}$) (Portes et al., 2007) for various time periods. The films were cut ($0.5 \times 1 \text{ cm}$; $(2.49 \pm 0.03) \times 10^{-3} \text{ g}$ for THC1 and $(2.38 \pm 0.02) \times 10^{-3} \text{ g}$ for THC2) and dipped in methanol (100 mL) under agitation at 350 rpm. The absorption spectra of the solutions were recorded every 10 min, and the initial desorption rates were

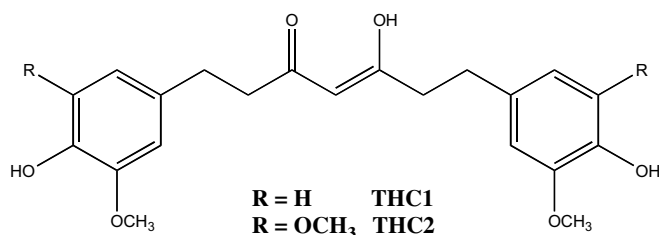


Fig. 1. Tetrahydrocurcuminoids THC1 and THC2.

determined by measuring the initial slopes of THC release. The experiments were repeated five times.

2.4. Assessment of the antioxidative properties of THC_s

The antioxidative activity of tetrahydrocurcuminoids incorporated into chitosan films was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical method according to a procedure previously described (Portes et al., 2007). H-transfer between THC_s in chitosan films and DPPH in a methanol solution was monitored by UV–vis absorption spectrometry at 515 nm (quartz cell length: 1 cm; solvent: methanol). The films were cut as above and dipped in the methanol–DPPH solution (200 mL; 2.5×10^{-4} mol L⁻¹) under agitation at 350 rpm. By monitoring the decay of DPPH absorption ($\lambda_{\text{max}} = 515$ nm, $\epsilon = 11,240$ L mol⁻¹ cm⁻¹) after release of antioxidant compounds from the chitosan film to the DPPH solution, the initial rates were determined by measuring the initial slopes of DPPH consumption. The experiments were repeated 5 times for the evaluation of each antioxidant.

2.5. Study of the interactions between chitosan and THC_s in acetic acid aqueous solutions

2.5.1. Chitosan/THC_s

Chitosan–THC films were solubilized in 1% acetic acid aqueous solutions and UV–vis absorption spectra were recorded. Absorptions were corrected for chitosan and THC_s at the same concentrations, giving only the absorption due to interactions between chitosan and THC_s.

2.5.2. Glucosamine/THC_s

Two solutions of THC_s (concentrations of 10^{-4} and 5×10^{-4} mol L⁻¹, respectively) were prepared in 1% acetic acid aqueous solutions. Glucosamine (10% w/w) was then added and absorption spectra were recorded. Absorptions were corrected for glucosamine and THC_s, giving only the absorption due to interactions between glucosamine and THC_s.

2.6. Bioactivity assessments

A bacterial pre-culture was obtained by inoculating 9 mL of tryptose broth with 1 mL of an overnight culture (18 hrs). The tubes were then incubated at 37 °C for 18 h.

2.6.1. Antilisterial properties of THC_s

The antilisterial properties of THC_s were determined using two methods. The first method is called “disk-diffusion method” (Method 1). The inhibition zone assay was conducted by inoculating a tryptose agar medium with 0.1 mL of a 10^{-4} diluted bacterial pre-culture. Paper disks (diameter: 5 mm), after UV sterilization, were soaked in solutions of THC_s in dimethyl sulfoxide (DMSO)–water mixtures (2/1 v/v) at various concentrations (50, 100, and 200 µg mL⁻¹). Disks were dipped in water as the negative control, and in DMSO–water mixtures (2/1 v/v) to evaluate the possible bioactive properties of the selected solvent. The disks were then placed on the inoculated Petri dishes. The dishes were cooled to 4 °C and maintained at this temperature for 4 h to allow biocide diffusion; they were then incubated at 37 °C for 48 h. The inhibition zones were measured with a tolerance of 1 mm. The experiments were repeated six times.

In the second method (Method 2), based on a coating technique, about 30–300 colony-forming units (CFU) of *L. innocua* per Petri dish were inoculated from the pre-culture onto a tryptose agar medium, and dried under a laminar-flow hood at room temperature for 30 min. The tetrahydrocurcuminoid in a tetrahydrofuran (THF) solution (200 µg/Petri dish) was coated on the surface and

incubated at 37 °C for 24–48 h prior to colony counting (test plates). Control plates with the solvent but without THC were conducted in parallel. The experiments were repeated six times. The percentages of inhibition were calculated using the following Eq. (1):

$$\text{Inhibition \%} = (\text{CFU on control plates} - \text{CFU on test plates}) / (\text{CFU on control plates}) \quad (1)$$

Method 2 was used to select the appropriate solvent.

2.6.2. Antilisterial properties of chitosan–THC films

The bioactivity of chitosan films incorporating THC_s was evaluated using Method 2, as described for the determination of the antilisterial effect of THC_s. THC–THF solutions were replaced by film-forming solutions of chitosan–THC in aqueous acetic acid (0.17%).

3. Results and discussion

3.1. THC release and antioxidative properties of chitosan–THC films

Incorporating antioxidants into food packaging materials to control the oxidation of fatty components and pigments may contribute to preserve the quality of food products. For this reason, chitosan films incorporating THC1 or THC2 at a concentration of 1% (w/w) were prepared. The release of the THC_s from the films was studied in methanol to ascertain whether the THC_s may be released under favorable solubility conditions. The kinetics was evaluated by selectively measuring the UV absorptions of THC_s (Fig. 2). Then, DPPH tests were conducted to see whether the THC_s retained their antioxidative potentials.

THC1 is more quickly released than THC2, and the initial slopes of antioxidant release were evaluated at $(0.080 \pm 0.005) \times 10^{-6}$ mol min⁻¹ for THC1 and $(0.023 \pm 0.005) \times 10^{-6}$ mol min⁻¹ for THC2, giving a THC1/THC2 rate ratio of 3.5. A plateau was reached after 4 h, with 30% of THC1 remaining in the film, whereas 20 h were necessary to reach a small plateau with 50% of THC2 released. The matter balance obtained by evaluating the amounts of THC1 and THC2 remaining in the films, using dissolution in acetic acid solutions (1%) and UV titration, and by measuring the amount of THC_s released as above, was satisfactory (98%) for both compounds. The differences observed in the kinetics and in the amounts of the two THC_s released from the chitosan films are probably due to stronger interactions between protonated chitosan and THC2 than between protonated chitosan and THC1. Protonated

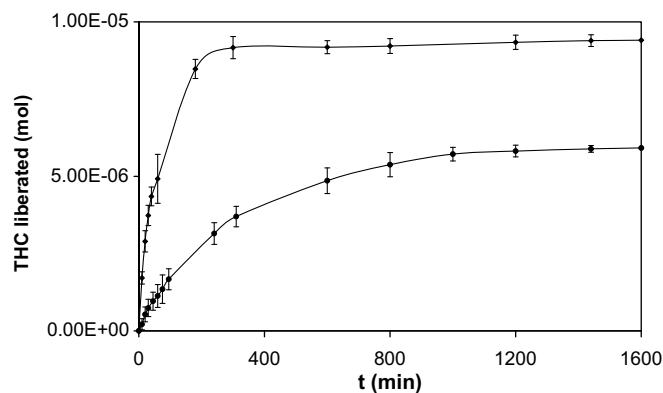


Fig. 2. THC_s released from chitosan–THC films in methanol (100 mL) versus time. THC1 (♦) and THC2 (●). 1% w/w THC_s–chitosan, methanol 100 mL (THC1: 13.4×10^{-6} mol; THC2: 11.6×10^{-6} mol; chitosan: 0.5 g; temperature: 25 °C).

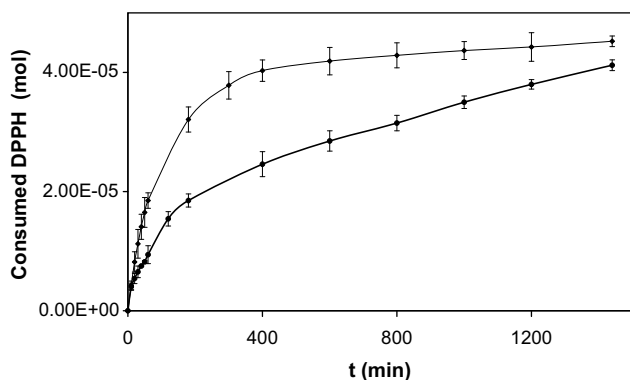


Fig. 3. DPPH consumed by THCs from chitosan–THC films in methanol versus time. THC1 (♦) and THC2 (●). 1% w/w THCs–chitosan, methanol 200 mL (THC1: 13.4×10^{-6} mol; THC2: 11.6×10^{-6} mol; chitosan: 0.5 g; DPPH: 2.5×10^{-4} mol L $^{-1}$; temperature: 25 °C).

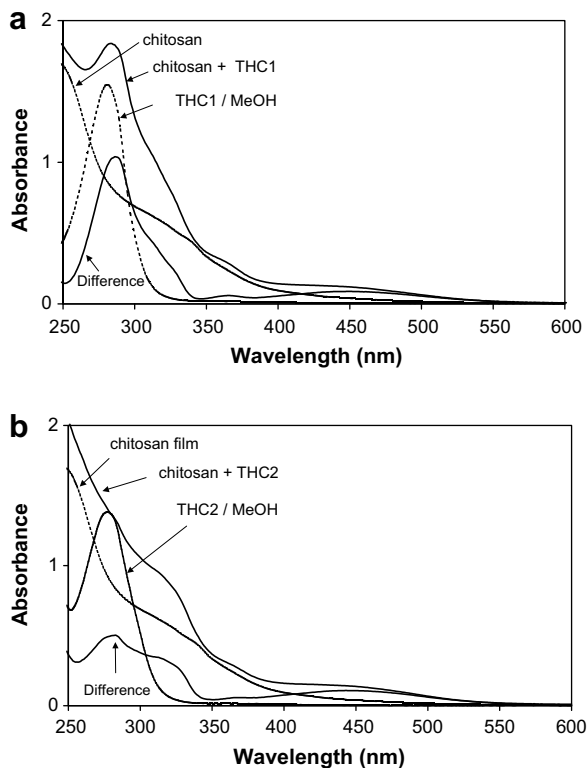


Fig. 4. UV–vis absorption spectra of chitosan–THC films (1% w/w THCs–chitosan with chitosan: 0.5 g); chitosan film (0.5 g); difference spectra (THC + chitosan)–(chitosan); THCs in a methanol solution ($\sim 10^{-4}$ mol L $^{-1}$). (a) THC1 (13.4×10^{-6} mol), (b) THC2 (11.6×10^{-6} mol).

chitosan is known to include electron-withdrawing groups (Kim & Thomas, 2007). The tetrahydrocurcuminoids studied included phenol and methoxy, two electron-donating groups, which increased the density of electrons on the benzene rings. Based on the assumption that the main interactions between chitosan and THCs are governed by electrostatic forces, it is not surprising that THC2 is less released than THC1 for the former includes two more methoxy groups than the latter. Consequently, the electronic absorption spectra of THCs are modified in the presence of protonated chitosan (*vide supra*).

The antioxidative properties of the THCs released from the chitosan-based films in methanol were evaluated by adding DPPH

to the solution. No antioxidative activity was found for chitosan films without THCs. The consumption of DPPH, as determined by the absorbance of the methanol solutions at 515 nm, versus time for chitosan–THC films is shown in Fig. 3.

The initial rates of DPPH consumption were calculated. These rates were $(0.31 \pm 0.03) \times 10^{-6}$ and $(0.16 \pm 0.02) \times 10^{-6}$ mol min $^{-1}$ for THC1 and THC2, respectively. The THC1/THC2 rate ratio for DPPH consumption, equal to 2, is still in favor of THC1. Antioxidative activity has usually been correlated to the rate constant for the first hydrogen atom abstraction, k_1 , between DPPH and the antioxidant. According to a previous study (Portes et al., 2007), the rate constant was found to be higher for THC2 than for THC1 ($k_1\text{THC2}/k_1\text{THC1} = 31$). Fig. 2 shows that THC2 was more retained in the film than THC1 and, as a consequence, THC2 was desorbed less quickly to react with DPPH.

3.2. Interactions between THCs and chitosan biopolymer

The lower release of THC2 comparatively to THC1 from chitosan films may be due to different interactions between tetrahydrocurcuminoids and chitosan. These interactions were studied by UV–vis absorption spectrometry by comparing the spectra of the THCs both in the solid state (chitosan films) and in solution, with chitosan or D-(+)-glucosamine (2-amino-2-deoxy-D-glucose) in 1% acetic acid. Glucosamine was not taken as a model of chitosan but it was used because it is the main monomer of chitosan, and it was then selected to determine whether the interactions were of polymer or of molecular nature. The studies took into account the impact of the steps taken to form the films on these interactions, and the impact of the association in a solid state.

The UV–vis spectra of chitosan films with and without THCs, and the difference spectra obtained after removing the contribution of chitosan to the absorbance of chitosan–THC films, are shown in Fig. 4a and b for THC1 and THC2, respectively.

The difference spectra were then compared to THC1 spectra in methanol (Fig. 4a). In chitosan films, THC1 exhibited a new absorption band centered at 455 nm, thus imparting an orange color to the film. In the UV region of the spectra, new bands appeared at 365 and 330 nm (shoulder), and a 5 nm shift to longer wavelengths was observed from the main band absorption of THC1 in a methanol solution (280 nm). The same observations were made for THC2, with a more intense band at 330 nm and vanishing of the shift to longer wavelength. The new absorption band in the visible region observed near 455 nm for the two THCs in chitosan films could be ascribed to interactions between chitosan and THCs. Solutions of chitosan and THCs in aqueous acetic acid (1% w/w) were prepared and analyzed by UV–vis absorption spectrometry. The spectra, corrected for chitosan and THC at the same concentrations, (Fig. 5) only corresponded to the absorption due to interactions between chitosan and THCs, which was extended at longer wavelengths for THC2.

To confirm the interactions existing between chitosan and THCs, mixtures of glucosamine (10% w/w) and THCs at two concentrations ($C1 = 10^{-4}$ mol L $^{-1}$, and $C2 = 5 \times 10^{-4}$ mol L $^{-1}$) were prepared in aqueous acetic acid (1%). The UV–vis spectra corrected for glucosamine and THCs are shown in Fig. 6a and b.

In both cases, absorption bands centered at about 310–320 and 370 nm appeared in the spectra. These bands were enhanced as THC concentrations increased. As a result, the molecular interactions obtained when glucosamine and THCs were mixed in solution with a large excess of glucosamine were not well defined. A study of the interactions between glucosamine and THCs was undertaken under the same experimental conditions using FTIR (Fourier transform infrared) spectroscopy, but the results were not conclusive. No significant change was observed on the spectra. Recently, several molecular chitosan complexes were reported in

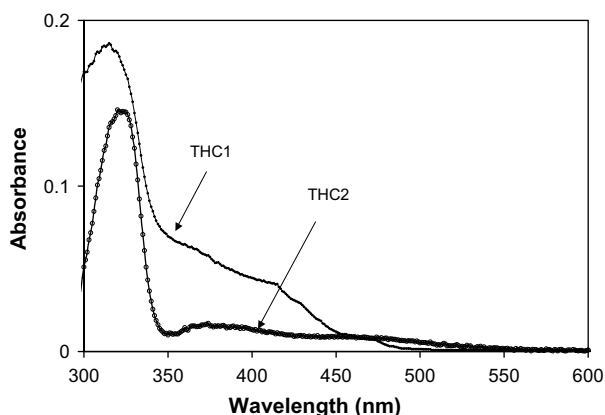


Fig. 5. UV-vis absorption spectra of THC-chitosan films dissolved in aqueous acetic acid (1%) at a concentration of 1% (w/w).

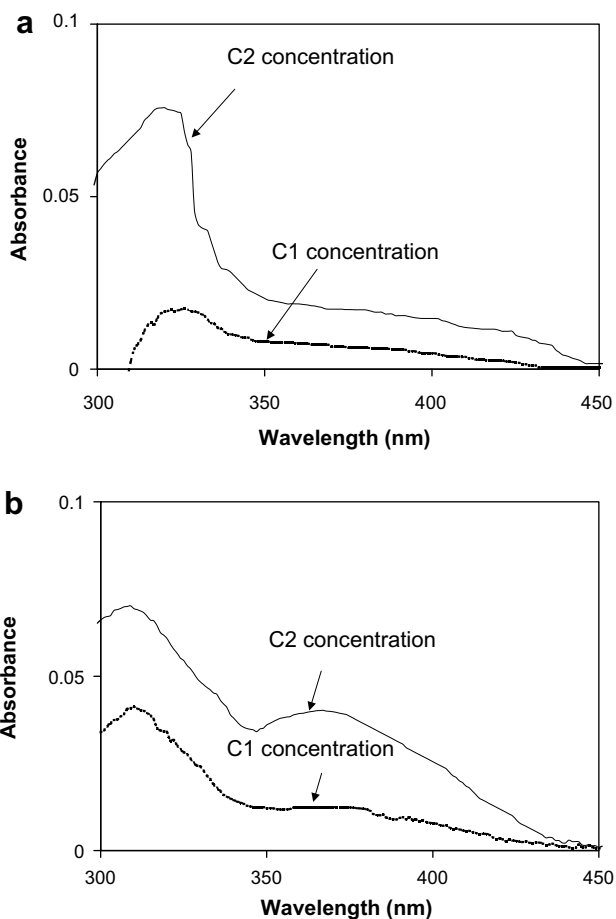


Fig. 6. UV-vis absorption spectra of THC-glucosamine mixtures in aqueous acetic acid (1%) (glucosamine concentration: 10% w/w; THC concentrations: C1 = 10^{-4} mol L $^{-1}$; C2 = 5×10^{-4} mol L $^{-1}$). Absorptions were corrected for glucosamine and THCs at the same concentrations. (a) THC1 (b) THC2.

3.3. Bioactivity of THCs and THC-associated chitosan

The bioactive effects of THCs were due, in particular, to their efficient antioxidative action on biological systems. These molecules showed an anti-inflammatory action in carrageenin-induced foot paw edema and cotton pellet granuloma models of inflammation in rats (Rao, Basu, & Siddiqui, 1982). To the best of our knowledge, only one study investigated the antibacterial activities of THCs obtained from natural curcumins (Venkateswarlu et al., 2000). Interestingly, the antibacterial activity of THCs was comparable or higher than that of the parent curcuminoids on *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Bacillus pumilis*. Nevertheless, no indication was given about the solvent used to dissolve the THCs and its bioactivity. After an evaluation of the bioactivity of various solvents to select the solvent that is less active against *L. innocua*, experiments were performed with THC1 and THC2 (Table 1).

DMSO and THF are suitable as solvents for THCs; they show a low bioactivity compared to ethyl acetate. DMSO was selected as solvent for the paper disk method (Method 1) because of its low volatility. THF was preferred for the coating method (Method 2) due to its high volatility, allowing rapid evaporation.

No inhibition zone was observed in assessing the bioactivity of THC1 and THC2 using Method 1. The proliferation of bacteria may be due to the lack of diffusion of THCs into the agar medium, or to their inability to control bacterial growth. A second series of microbial experiments was performed using Method 2 in order to test exclusively the bioactivity of THCs against *L. innocua* without any necessary diffusion steps. The results are reported in Table 2.

Unfortunately, THC1 only exhibited a low inhibition against *L. innocua* whereas THC2 did not show any significant inhibitory activity on the same bacterial strain. As a result, the bioactivity of the film produced is only induced by the chitosan part, whereas its antioxidative activity is provided by the THCs. A significant inhibition of listerial growth by protonated chitosan was previously shown (Chen et al., 1996; Coma et al., 2002; Helander et al., 2001; Knowles & Roller, 2001; Moller et al., 2004; Tsai & Su, 1999). However, the previously evidenced interactions between THCs and chitosan, which may involve the ammonium groups in chitosan, could lead to a decrease in the bioactive properties of the films. Method 2 was used to ascertain the bioactivity of chitosan when associated with THCs. A series of solutions containing chitosan, with or without THC1 or THC2 added, in aqueous acetic acid were tested. The bioactivity of the preparations against *L. innocua* is reported in Table 3 and shown in Fig. 7. *Listeria innocua*

Table 1

Bioactivity of some solvents against the growth of *L. innocua*.

Solvent	CFU/Petri dish (\pm SD)	Inhibition % (\pm SD)
Water	190 \pm 14	–
Ethyl acetate	102 \pm 9	46 \pm 9
Dimethylsulfoxide	160 \pm 9	16 \pm 6
Dimethylformamide	139 \pm 14	27 \pm 10
Tetrahydrofuran	160 \pm 10	16 \pm 6

Table 2

Bioactivity of THC1 and THC2^a against the growth of *L. innocua* by the coating method.

	CFU/Petri dish (\pm SD) ^b	Inhibition % (\pm SD)
Control	190 \pm 14	–
THC1	157 \pm 20	29 \pm 11
THC2	187 \pm 18	15 \pm 9

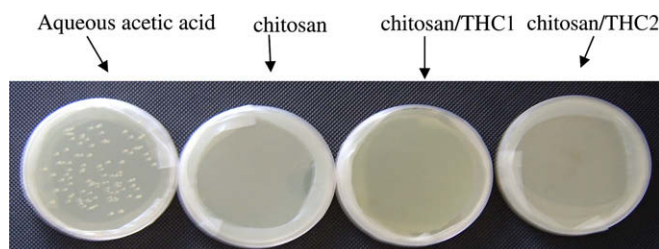
^a 200 μ g/Petri dish.

^b Water: 190 \pm 14 and tetrahydrofuran: 160 \pm 10.

the literature between chitosan and catechin (Zhang & Kosaraju, 2007), with both covalent and hydrogen bonds, and between chitosan and glucose (Kanatt, Chander, & Sharma, 2008), with covalent bonds. The nature of the interactions between THCs and chitosan remained unelucidated and will be studied further.

Table 3Bioactivity of chitosan associated with THC_s against *L. innocua*.

Petri dish	CFU/Petri dish (±SD)	Inhibition % (±SD)
Control growth	106 ± 10	/
Aqueous acetic acid	77 ± 4	27 ± 5
Chitosan 1 ^a	0	100
Chitosan 2 ^b	0	100
Chitosan 1 + THC1 ^c	0	100
Chitosan 2 + THC1 ^d	0	100
Chitosan 1 + THC2 ^c	0	100
Chitosan 2 + THC2 ^d	0	100

^a 3.3 mg mL⁻¹.^b 1.7 mg mL⁻¹.^c 37 µg mL⁻¹.^d 18 µg mL⁻¹.**Fig. 7.** Bioactivity of chitosan associated with THC_s against *Listeria innocua*.

could not develop with chitosan, nor with THC-associated chitosan. This reveals that films made from chitosan incorporating THC_s exhibit intact antibacterial properties.

4. Conclusion

For the first time, the association of chitosan and tetrahydrocurcuminoids allowed films exhibiting both antioxidative and antibacterial activities to be made. The interactions between chitosan and THC_s were evidenced by UV–vis absorption spectrometry. The molecular nature of these interactions was ascertained using glucosamine, but the exact nature of the complex remains unelucidated. The antioxidative properties of the films result from a progressive release of the THC_s into the medium. Chitosan retained its antimicrobial properties against the growth of *L. innocua* when associated with THC_s; THC_s alone are not bioactive enough. The association of natural antioxidants and bioactive biopolymers may be particularly useful to develop high-performance food packaging.

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