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Novel biodegradable films made from chitosan and poly(lactic acid) with antifungal properties against mycotoxinogen strains

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Abstract

Composite films from chitosan and poly(lactic acid) (PLA) were prepared by solution mixing and a film casting procedure. The main objectives of this study were the elaboration and the characterization of chitosan/PLA based bio-packaging for potential food applications and the study of antifungal activity of coatings and films on three mycotoxinogen fungal strains, *Fusarium proliferatum*, *Fusarium moniliforme* and *Aspergillus ochraceus*. The first part of the study was related to the impact of the PEG content, allowing an easy film recovery without considerably decreasing the moisture barrier properties of materials: 16.6% of plasticizer was selected. Difficulties were however encountered to produce miscible PLA and chitosan film forming solution, leading to heterogeneous films with high water sensitivity.

Although composite films offer a great advantage in preventing the surface growth of mycotoxinogen strains because of their antifungal activity, the physico-chemical properties of such heterogeneous films, dramatically limit their further usage as packaging materials.

Keywords: Chitosan; Poly(lactic acid); Antifungal films; *Fusarium*; *Aspergillus*; Mycotoxins; Bio-packaging

1. Introduction

Mycotoxins can be characterized as secondary metabolites of various toxigenic fungi. Mycotoxins occur in a wide variety of foods and feeds and have been implicated in a range of human and animal diseases (Coker, 1997). Exposure to mycotoxins can produce both acute and chronic toxicities ranging from death to deleterious effects upon, for example, the central nervous, cardiovascular and pulmonary systems. Their general teratogenicity, cancerogenicity and their toxicological properties constitute a high human and animal health risk. The mycotoxins also attract attention because of the significant economic losses associated with their impact on human health and animal productivity. Moulds and mycotoxins such as *Aspergillus parasiticus* (Aflatoxins B1, B2, G1, G2), *Aspergillus flavus* (Aflatoxins B1, B2), *Fusarium monoliforme* (Fumonisine B1), *Fusarium graminearum* (zearalenone) and *Aspergillus ochraceus* (Ochratoxin A) are currently considered to be of world-wide importance (Miller, 1994, 1995). Several approaches were adopted towards diminishing the human and animal exposure to these mycotoxins. According to Torres, Ramirez, Arroyo, Chulze, and Magan (2003), the use of cultivars less susceptible to toxin production, cultural practices and, moreover antifungal compounds has been examined.

The development of active materials based on antifungal coatings and films could be one solution to limiting the growth of these phytopathogens. In addition, due to environmental considerations, the elaboration of new edible or biodegradable bioactive packaging constitutes a very interesting option complementary to recycling. Chitosan was thus used as a polymeric matrix to produce films from renewable resources which exhibit potential antifungal properties on mycotoxinogen strains (*Fusarium, Aspergillus*, etc), because of its good film-forming properties and its recognized antimicrobial activity (Arai, Kinumaki, & Fujita, 1968; Begin & Van Calsteren, 1999; Chen, Yeh, & Chiang, 1996; El Ghaouth, Arul, Ponnampalam, & Boulet, 1991; El Ghaouth, Arul, Wilson, & Benhamou, 1994; Muzzarelli et al., 1990; Shahidi, Arackchi, & Jeon, 1999). Many researchers have studied the antimicrobial action of this polymer on fungal strains (Allan & Hadwiger, 1979; Fang, Li, & Shih, 1994; Kendra, Christian, & Hadwiger, 1989; Sebti, Martial-Gros, Carnet-Pantiez, Grelier & Coma, 2005) or on bacterial strains (Coma et al., 2002;

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Möller, Grelier, Pardon, & Coma, 2004; Ouattara, Simard, Piette, Bégin, & Holley, 2000; Sudarshan, Hoover, & Knorr, 1992; Tsai, Wu, & Su, 2000). Chitosan is a chitin derived polysaccharide and is one of the more abundant natural polymers, largely widespread in living organisms such as shellfish, insects, and mushrooms. It is a linear binary heteropolysaccharide composed of (β1,4)-linked 2-acetamido-2-deoxy-glucopyranose (GlcNAc) and 2 amino-2-deoxy-glucopyranose (GlcN), and is determined as a non-toxic, a biodegradable and a biocompatible polymer. Chitosan is a multi-purpose material that has found a wide range of applications ranging from dietary regime constituents, food packaging material, drug release components and for environmental pollutants (Agullo, Rodriguez, Ramos, & Albertengo, 2003; Arvanitoyannis, 1999; Ravi Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). Unfortunately, the hygroscopic properties of the bio-packaging containing polysaccharides are responsible for their weak moisture barrier and thus have little or no influence on the dehydration/rehydration phenomena of the foodstuffs, a property crucial for maintaining organoleptic and microbiological food qualities. One strategy to overcome the drawback of the high sensitivity to moisture of chitosan is to associate the polysaccharide with a more moisture-resistant polymer, while maintaining the overall biodegradability of the product. The association of chitosan and polylactic acid was thus considered in the form of composite (blending) films. PLA belongs to the family of aliphatic polyester commonly made from lactic acid, which can be produced from renewable resources such as starch via fermentation processes (Garlotta, 2001). It is a thermoplastic, high strength, high modulus polymer and is considered biodegradable and compostable (Jarerat & Tokiwa, 2001). Moreover, it is possible to use it for food contact, i.e. it is classified as GRAS (Generally Recognized As Safe, GRAS) (Conn et al., 1995). Thanks to its relatively hydrophobic nature the use of this polyester could reduce the hydrophobilic nature of chitosan-based films and consequently improve their moisture barrier properties and decrease overall the water/matrix interactions. The main objectives of this study were (1) the elaboration and the characterization of chitosan/PLA based bio-packaging for potential food applications and (2) the study of the antifungal activity of coatings and films on three mycotoxinogen fungal targets.

Preliminary assays showed difficulties in film recovery when formulated without plasticizers, and, primarily for materials containing PLA, polyethylene glycol (PEG) was selected as the plasticizer. The first part of the study was related to the selection of the PEG level, allowing easy film recovery without decreasing the moisture barrier properties of materials too much. Experiments were conducted on film with various contents of PEG and films were characterized. Thereafter, preparation and study of the composite was carried out and their physicochemical and biological properties were determined. Inhibitory activity was evaluated on the *Fusarium proliferatum*, *Fusarium moniliforme* and *A. ochraceus* growth.

2. Materials and methods

2.1. Materials

Chitosan was obtained from crab shell (No. 244, low viscosity, Chitin France). Its degree of deacetylation was higher than 98% as the supplier specified. Poly(lactic acid) (PLA: 92% L-lactide and 8% *meso*-lactide) was provided in pellet form by Cargill-Dow (USA). The average molecular weight of 49,000 was determined by intrinsic viscosity measurements in chloroform at 25 °C. Polyethylene Glycol (PEG 400), acetic acid and chloroform were provided by Sigma (USA). Hydroxypropylmethylcellulose was provided by Hercules (HPMC Culminal 50, Aqualon, France).

2.2. Methods

2.2.1. Film elaboration

2.2.1.1. Chitosan films. The film forming solution of 1% (w/v) chitosan was obtained by dispersing chitosan in a 1% (v/v) aqueous acid solution prior to PEG400 incorporation. The pH was adjusted to 5.0 with NaOH 1 M. The preparation was filtered through 5.3 μm and then 0.65 μm membrane (Millipore) and degassed under reduced pressure. Film forming solutions were then poured in 1 mm thick uniform layer onto a polypropylene plate. The optimum conditions for casting were 25 °C at ambient relative humidity (RH) for 2 days. The dried films were then peeled from the plate and samples were conditioned at 20 ± 1 °C and 50 ± 5% relative humidity (RH) for 5 days before taking property measurements.

2.2.1.2. PLA films. One percent (w/v) PLA was dissolved in chloroform under magnetic stirring for 2 h. The various content of PEG 400 was then added. Films were obtained by casting on glass Petri dishes in 1 mm thick uniform layers. The casting conditions were 25 °C at ambient RH for 20 h. The dried films were then peeled from the plate and samples were conditioned at 20 ± 1 °C and 50 ± 5% RH for 5 days before taking property measurements.

2.2.1.3. Chitosan-PLA blends based films. Blends of chitosan with PLA were prepared as described by Nugraha, Copinet, Tighzert, and Coma (2003), with some modifications. Chitosan and PLA were dissolved separately in different solvents before blending. Chitosan (1% by weight) was dissolved in acetic acid 1% and PLA (1% by weight) was dissolved in chloroform as specified before. Then both solutions were blended for 5 min with electric drill homogenization prior to PEG 400 incorporation. The composite films were obtained by casting on glass Petri dishes (1-mm thickness) and evaporating at 25 °C and ambient RH for 2 days. The final compositions (chitosan/PLA) were 100/0; 90/10; 80/20; 70/30 (w/w).

2.2.2. Film analysis

2.2.2.1. Water vapor transmission rate (WVTR) determination. The WVTR of biomaterials was evaluated using NF ISO 2528

(1989). An aluminum cup containing either anhydrous $CaCl_2$ desiccant (assay cup) or nothing (control cup) was sealed with the test film (50 cm^2 exchange film area) and 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 water vapor transmission rate ($g\ m^{-2}\ 24\ h^{-1}\ atm^{-1}$) was determined from the weight increase of the cup over time at the steady state of transfer. All tests were conducted in triplicate.

2.2.2.2. Water contact angle meter measurements. The contact angles formed between a water droplet placed at the surface of a material and the kinetics of spreading are related to the surface hydrophobicity of the material. Contact angle measurements were performed with a Kruss G23 (Germany) apparatus. A water drop was deposited on the surface of different films (size 65 mm × 25 mm). The evolution of the droplet shape was recorded. A CCD video camera and image analysis software were used to determine the contact angle evolution, which may be used to determine the kinetics of water sorption (slope of the curve contact angle = f (time)), related to the hydrophobic character of the material. Five measurements on each film were performed at random positions.

2.2.2.3. Solubility in water assessments. The water solubility of the different cellulose films was measured from immersion assays in 50 mL of distilled water for 24 h at 20 ± 1 °C. The water solubility 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 the film was determined using an infrared scale (Mettler instrument). All tests were conducted in triplicate.

2.2.2.4. Tensile testing. The mechanical resistance of films was performed at 20 ± 1 °C and 50% ± 5 RH on a tensile testing machine (Adamel-Lhomargy, DY25 instrument) according to AFNOR NF ISO 527-3 (1995). It included tensile strength (TS, Pa), ultimate elongation (UE, percent at break point) and Young's modulus (Y, Pa). Experiments were conducted on 10 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. Sample width and thickness were measured before testing. The stress–strain curves were computer-recorded.

2.2.2.5. Bioactivity assessments. Spores of *F. monoliforme* (INRA collection, 61.Bt 9841.1.NT), *F. proliferatum* (INRA collection, 76.MUCL 1130) and *A. ochraceus* (INRA collection) were recovered from a 5-day-old PDA culture (DIFCO, Detroit, USA) pre-culture and suspended in 9 mL of sterile physiological water. The spore charge was counted on a Malassez cell before the inoculation of a PCA agar medium. Approximately 10^3 spores were deposited on agar medium and dried in a flow hood at room temperature for 1 h. Films were then deposited on the surface of inoculated PDA agar and the plates were incubated at 25 °C for 72 h. A visual observation was performed after different incubation times for 10 days.

Growth controls with the same spore charge were conducted in parallel to ensure that viable organisms were present. In addition, contamination controls (film-agar medium experiments, without microbial cell) were performed from films deposited on a non-inoculated Tryptose agar to test their initial contamination. Finally, the bioactivity of 1% HPMC (w/v) film as the negative control, elaborated according to the method described in Coma, Deschamps, and Martial-Gros (2003) using 1% aqueous acetic acid as the solvent, was conducted in parallel to verify the non-antifungal activity of potential residual solvent.

In order to test the antifungal activity of the chitosan film forming solution, a volume corresponding to a liquid thickness of about 1 mm was deposited on the surface of the inoculated PDA agar to lead to a coating with a final thickness of about 30 μm. After drying in a flow hood at room temperature (2 h), the plates were incubated at 25 °C and a visual observation was performed after different incubation times.

2.2.2.6. Statistical treatment. All experiments were replicated at least three times. Treatment means were separated using the Student's t-test at 95% probability ($p > 95\%$).

3. Results and discussion

3.1. 1-Effect of the PEG content on the physico-chemical properties of films

Preliminary tests were carried out to determine the plasticizer content (PEG 400) in the chitosan and PLA solutions, required to produce flexible and 'easy to recover' materials.

Both chitosan and PLA films were transparent and the variable thickness according to the quantity of incorporated PEG (Table 1). The higher the PEG concentration, the easier the films of chitosan were removed from polypropylene support and the more flexible they were. PLA film could be separated from the support only if a minimum of 5% PEG was incorporated. The effect of PEG content on vapor and liquid water sensitivity of films was first studied.

Table 1
Film thickness of the different films

Films	PEG content (%)	Film thickness (μm)
Chitosan	5% de PEG	46.6 ± 4.7
	8.3% de PEG	51.1 ± 7.3
	16.6% de PEG	43.3 ± 9.4
PLA	5% de PEG	40.0 ± 3.7
	8.3% de PEG	31.1 ± 7.3
	16.6% de PEG	35.5 ± 2.8
Composite films (chitosan/PLA)		
90/10	16.6% de PEG	67.1 ± 6.9
80/20	16.6% de PEG	56.7 ± 12.1
70/30	16.6% de PEG	47.7 ± 13.9

Data, followed by their standard deviations, are means of three experiments. Treatment means were separated using the Student's t-test ($p > 0.05$).

3.1.1. Water sensitivity

Water vapor barrier properties of chitosan and PLA films, prepared with various contents of PEG, were measured at 50% HR and 23 °C. These experimental conditions are closer to the current applications in food preservation than the standardized analysis conditions (90% HR and 38 °C). WVTR versus PEG content are given in Fig. 1. As expected, the greater the content of PEG was, the higher the WVTR, due to progressive film plasticization which is associated with modification of the hydrophilic character of PLA film. Both were responsible for the potential increase of the diffusion factor and water sorption in film, respectively. The PEG incorporation thus decreased the material cohesion by creating intermolecular spaces and increasing the water molecule diffusion coefficient. Moreover, PEG separation in the amorphous phase of PLA could also explain these results. In studies related to the mechanical properties of plasticized PLA, with a certain PEG content, dependent on its molecular weight, blends of PLA with PEG undergo a phase segregation. Kulinski and Piorkowska (2005), in a study devoted to the plasticization of the amorphous phase of semicrystalline PLA with PEG, mentioned that the crystallization of plasticized PLA was rather a side effect that occurred with a relatively high plasticizer where PEG separation in the amorphous phase was also observed. These authors showed that their PEG used decreased glass temperature as expected and, for the same plasticizer content, that a semi-crystalline PLA compared to an amorphous PLA exhibited non-uniform plasticization of the amorphous phase. While the plasticizing effect on a semi-cristalline PLA remains unclear, PLA film WVTR, according to the plasticizer content, were lower than those obtained for chitosan films, especially for 16.6% PEG. An association of both biopolymers could thus be interesting with the aim of reducing water sensitivity of chitosan films. The WVRT of PEG-free chitosan film was however relatively low for polysaccharide based bio-packaging (<50 g m^{-2} day^{-1} atm^{-1}). Fig. 1 shows also the effect of the PEG content on the water contact angle measurements of both chitosan and PLA films. The hydrophilic or hydrophobic nature of films was evaluated using the contact angle on the smooth side of the films (drying support side), which varies between 0° for the hydrophilic and 90° for the more hydrophobic matrix. Results did not show significant differences after the PEG incorporation in the chitosan film formulation. However, taking into account the hydrophilic nature of chitosan, the obtained values were surprising (90–100°). These results were due to the chitosan film solubilization into the water drop, a few seconds after its deposit, and led to unusable values.

The PEG incorporation in PLA films did not show a significant reduction in the water contact angle, which remained close to 70°. The rather hydrophobic character of this material was thus not dramatically modified. Results related to the water sensitivity of films led to an additional interest in associating both biopolymers in order to reduce water/chitosan matrix interactions.

Solubility in water is an important property, which governs potential applications of these materials to food preservation. Films with low water solubility are necessary for the protection of foodstuffs with high or intermediate water activity (aw). Results for both films versus PEG content are presented in Fig. 2. Pure chitosan films were completely soluble or very difficult to recover after the water immersion process. The more the content of PEG increased in the film forming solution, the more the water solubility decreased. However, only an improvement of 10% was obtained with 16.6% PEG content in chitosan film compared to a PEG-free chitosan film. The very low water solubility of PLA films was observed whatever the studied plasticizer content. Less than 5% was solubilized after a 24 h immersion process. However, this linear aliphatic

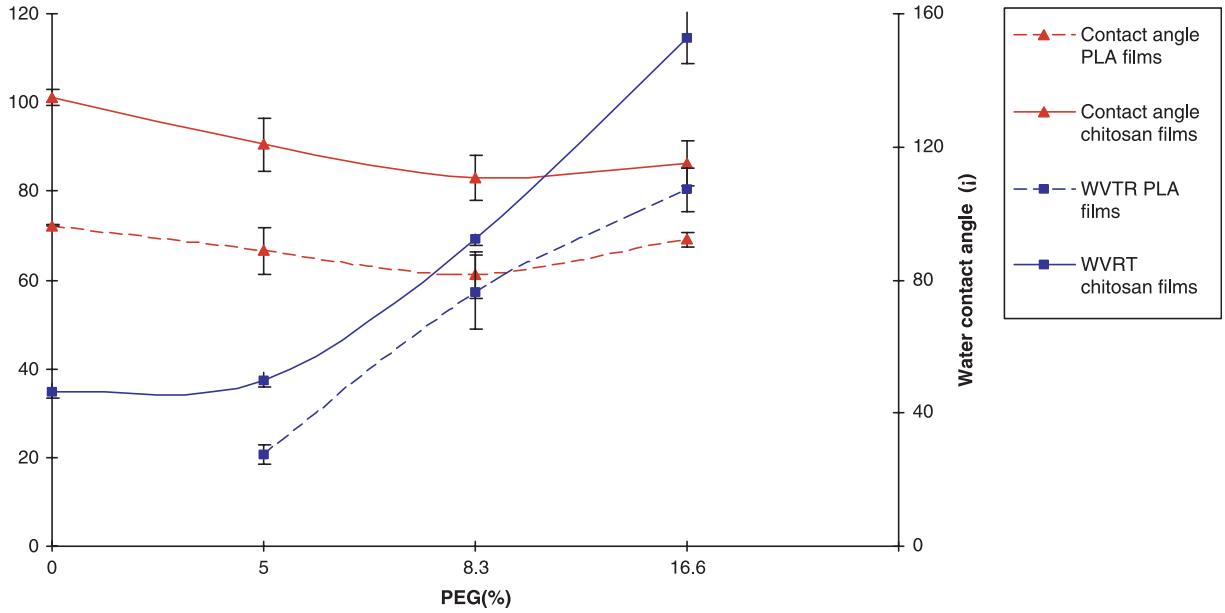


Fig. 1. Evolution of the water contact angle and the water vapor transfer rate (WVTR) of PLA and chitosan films versus PEG content. Each point of the graph is the mean of three experiments. Treatment means were separated using the Student's *t*-test ($p > 0.05$) and the standard deviations are vertical lines.

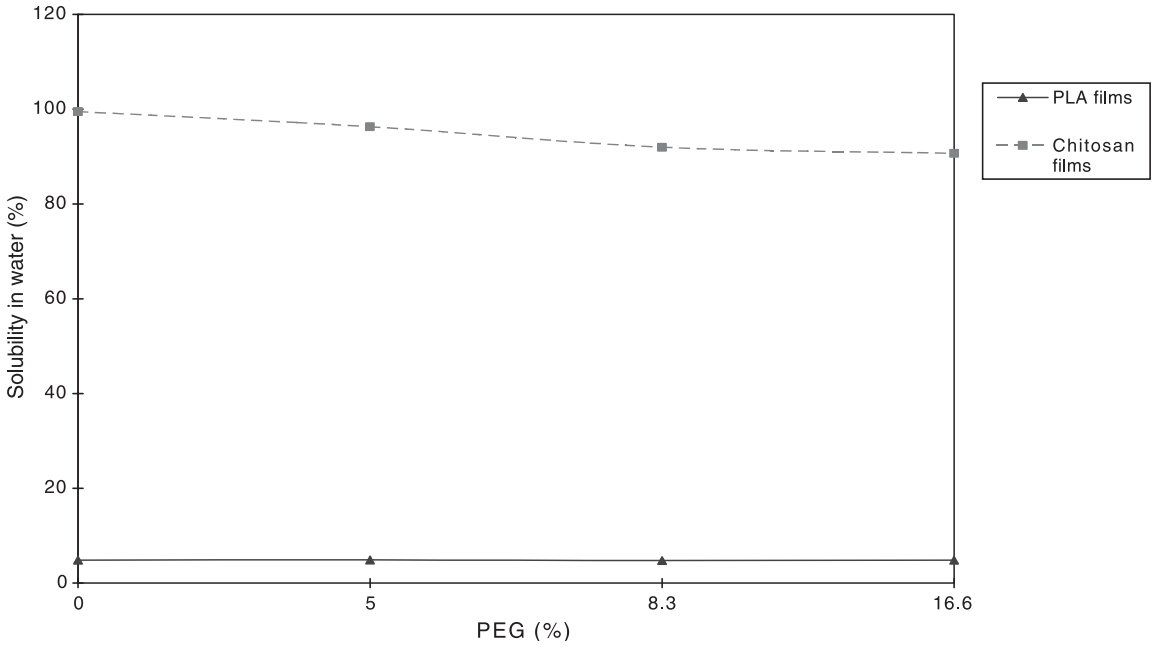


Fig. 2. Variation of the solubility in water of PLA and chitosan films versus PEG content. Each point of the graph is the mean of three experiments.

polyester degrades through hydrolytic scission of the ester groups and potential degradation of the mechanical properties could be thus critical.

3.1.2. Mechanical properties

The mechanical resistance of films was studied according to three parameters: tensile strength (TS), Young's modulus (Y) and ultimate elongation at break (UE). With 16.6% of PEG in chitosan films, the parameters TS and Y decreased by about 23 and 40%, respectively, compared to the films without plasticizers (Fig. 3). On the other hand, an increase from 7.5 to 22% was obtained using 16.6% of PEG in the chitosan film forming solution. The influence of the plasticizer on the mechanical properties of PLA films led to the same tendency (data not shown). These results are obviously due to the plasticization of films, decreasing the rigidity and the brittleness of materials, thus improving their mechanical properties and their recovery. As specified before, potential degradation of the mechanical properties could be due to hydrolytic reactions by water absorption. Renouf-Glauser, Rose, Farrar, and Cameron (2005) showed that hydration of amorphous PLLA resulted in a dramatic change in the development of the craze structure on deformation, but with little effect on the bulk mechanical properties. In crystalline annealed PLLA, absorbed water plasticized the movement of crystallites resulting in a change of the bulk mechanical properties.

3.1.3. Biological properties

Chitosan possesses several biological properties in addition to its ability to form a semi-permeable film due to its polymeric nature. Its antifungal activities Agullo et al. (2003), observed that the use of a chitosan-based coating delayed Alternaria sp, Penicillium sp and Cladosporium sp growth. This behavior is similar to the action of synthetic preservatives such as calcium propionate (0.15 wt%) and potassium sorbate (0.15 wt%). On the other hand, chitosan showed little activity on Aspergillus sp growth. Preliminary tests were carried out to verify the antifungal activity of the chitosan on F. proliferatum, F. moniliforme and A. ochraceus growth. Inhibitory effect of a 1% (w/v) chitosan film forming solution was observed using a coating process of an inoculated agar medium (data not shown). However, the solvent of the chitosan solution (1% aqueous acetic acid) revealed this same antifungal activity. One percent aqueous acetic acid was then diluted to 1/2, 1/3,1/5 and 1/6 and antifungal properties were evaluated on the three selected strains. This solvent completely loses its

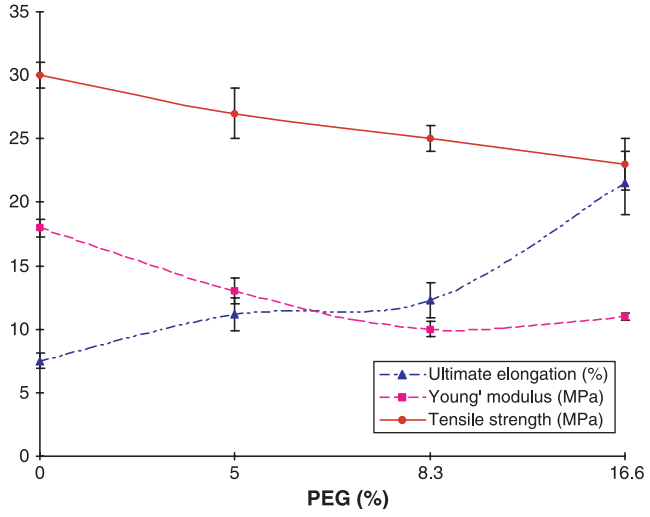


Fig. 3. Evolution of the mechanical properties of chitosan film versus PEG content. Each point of the graph is the mean of three experiments. Treatment means were separated using the Student's t-test ($p > 0.05$) and the standard deviations are vertical lines.

Table 2
Antifungal activities of chitosan based-coatings and composite films

	Fusarium moniliforme	*Fusarium proliferatum*	*Aspergillus ochraceus*
Part I: inhibitory properties of the coatings			
Positive control	+	+	+
2.5% Chitosan coating diluted to 1/5	−	−	−
1% HPMC coating	+	+	+
Part II: inhibitory properties of the films			
Films PLA-PEG 16.6	+	+	+
Composite films with the different studied PLA contents (Chitosan-PLA-PEG 16.6)	−	−	−
1% HPMC film	+	+	+

Visual observations, after 72 h at 25 °C of incubation led to the following results: +, fungal growth and −, growth inhibition. Three repetitions were conducted.

inhibitory activity for the 1/5 dilution. To ensure that the potential antifungal properties were due to the aminopolysaccharide activity, a film forming solution was thus prepared from an aqueous acetic acid diluted to 1/5 to obtain a final concentration of 1% (w/v) in chitosan without inhibitory activity of the solvent (pH 4.7). The chitosan coating of inoculated agar mediums caused a total growth inhibition of the three fungi as shown in the (First part of the Table 2). This result showed that a pulverization of chitosan could be directly used on plants to limit the development of mycotoxinogen fungi. The polycationic property allows chitosan to interact with negatively charged substances, thereby exhibiting antimicrobial activity on molds. As specified by Agullo et al. (2003), three mechanisms have been proposed for chitosan's antifungal property. The biopolymer appears to work both by interfering directly with fungal growth and also, on plants, by activating many defense responses. The chelating properties of chitosan make it an antifungal agent (Allan & Hadwiger, 1979; Fang et al., 1994; Kendra et al., 1989; Sebti et al., 2005). Moreover, as specified by Sebti et al. (2005), some authors obtained a strong inhibition of Aspergillus niger spore germination on a solid medium supplemented with chitosan and observed that chitosan produced spore aggregation and morphological anomalies (Planscencia-Jatomea M., Viniegra G., Olayo R., Castillo-ortega M.M., & Shirai K., 2003). In a previous study, related to A. niger by chitoan-based matrices, we observed through epifluorescence analysis that chitosan probably acts on nucleic acids (Sebti et al., 2005).

Inhibitory properties of chitosan and PLA films, with 16.6% (w/w) of PEG, were then studied on inoculated solid medium (Second part of the Table 2). The growth of *F. moniliforme*, *F. proliferatum* and *A. ochraceus* was inhibited by chitosan film whereas the PLA film did not cause any inhibition of growth. Moreover, HPMC film allowed a normal fungal growth. Bioactivity of chitosan film did not come from the potential antifungal activity of the residual solvent.

Finally, this first study showed that the PEG improved the mechanical properties of the chitosan and PLA films by increasing the flexibility of materials but decreases, as expected, their water vapor barrier properties. The minimal content of PEG to be incorporated was fixed at 16.6% (w/w), a quantity leading to a compromise between the different properties and allowing flexible and 'easy to recover' films. Also, taking into account the interesting mechanical and barrier properties of PLA films, due to the relatively hydrophobic nature of the polymer, PLA would be a good candidate to associate with the chitosan. Composite films were therefore studied a second time.

3.2. 2-Elaboration and study of composite films

As specified in Table 1, thickness values were very dispersed, probably due to the heterogeneity of films. Only the most homogeneous and the most regular films were recovered and stored in a climatic chamber at 50 ± 5% RH and 23 ± 1 °C before analysis.

3.2.1. IR spectra

The infra-red spectra of several composite film samples with 10, 20 and 30% (w/w) of PLA were carried out (data not shown). As already mentioned, the bands 1555 cm^{-1} (A2) and 1407 cm^{-1} (A3) were attributed, respectively, to the chitosan group—NH_3^+ and—NH_2. The 1755 cm^{-1} (A1) band corresponds to the carbonyl group of the PLA. The ratio of peak intensities was calculated to study the mixture of both polymers and to test the homogeneity of composite film. Important variations of the ratios A1/A2 and A1/A3, calculated from different spectra of the same sample, were obtained (Fig. 4). These results are characteristic of a non-miscible or partially miscible mixture, leading to the heterogeneous aspect of composite films. The ratio A2/A3, related to the polycationic

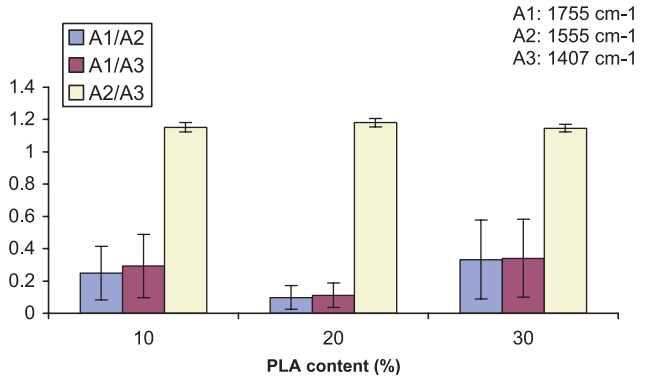


Fig. 4. Ratio of different peak intensities evaluated by FTIR measurements in function of the PLA content.

nature of the chitosan and thus to its antimicrobial activity, remained constant. This would explain the inhibitory properties of composite films further observed. These results were in accordance with those of Peesan, Supaphol and Rujiravanit (2005) obtained from a FTIR study. These results indicated that no significant interaction between chemically modified chitosan (hexanoyl chitosan) and PLA molecule was observed, even if a same solvent was used.

3.2.2. Water sensitivity

Concerning the solubility, the addition of PLA decreased the solubility in water by approximately 70% compared to homogeneous chitosan films (Table 3). The water solubility of composite films was close to 30–35%, depending on the PLA content. Yew, Mohd Yusof, Mohd Ishak, and Ishiaku (2005), in a study related to the properties of PLA/rice starch, suggested that it is expected that the exposed starch granules absorb moisture faster than those in the interior parts, i.e. starch granules which are entrapped or coated by PLA. But for short exposure times, the water molecules could saturate the surface of the composites easily and also penetrate into the composite through voids. In our study, even if the composite was a chitosan-based matrix, the PLA used as an 'additive' led to a strong reduction of the water solubility. The relative hydrophobic and non-soluble character of PLA would decrease the availability of hydroxyl groups of the chitosan matrix and explain this strong sensitivity to water. Contact angle measurements showed two type of film-liquid water behavior from the composite films. Indeed, a few seconds after the deposit, some film areas exhibited the sorption phenomenon of the film by the water drop such as that already observed for the homogeneous chitosan matrix. For other experiments, a type of behavior similar to that of homogeneous PLA films was noted. These observations confirmed the heterogeneity of the structure of composite films with relatively hydrophobic zones, rich in PLA and hydrophilic areas and low in PLA. In spite of this poor repeatability, the contact angle for composite films showed higher θ when the concentration in PLA increased. That would indicate that the introduction of PLA into a chitosan solution would decrease the absorbent character of these films.

The results of the kinetic study, i.e. θ versus time for composite films are illustrated in Fig. 5. Composite films 90/10 and 80/20 chitosan/PLA led to a progressive sorption of the film by the water drop (contact angles increased with time). From 70/30 chitosan/PLA films, the drop was spread out gradually over the film, in spite of its solubility in water close to that of the other composites.

Table 3
Some properties of the composites films chitosan/PLA 90/10, 80/20, 70/30

Composite films (Chitosan/PLA-PEG 16.6)	90/10	80/20	70/30
WVTR (g m^{-2} d atm)	165±7	218±18	221±4
Solubility in water (%)	35±1	33±1	30±2
Contact angle θ (°)	54.1±18.5	68.9±9.9	68.3±13.6

Data, followed by their standard deviations, are means of three experiments. Treatment means were separated using the Student's t-test ($p > 0.05$).

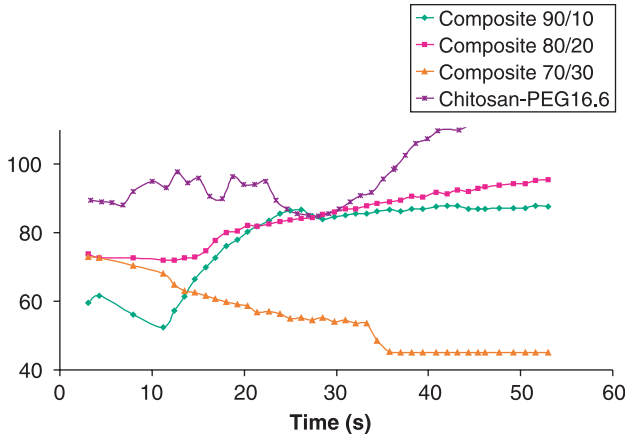


Fig. 5. Evolution of the water contact angle of composite films chitosan/PLA 90/10, 80/20, 70/30 and homogeneous chitosan films with 16.6% of PEG. Each point of the graph is the mean of three experiments.

The WVTR of composite films, measured at 50±5% and 23±1 °C (Table 3), with various contents of PLA corresponded to a very high moisture transfer. Unexpectedly, the addition of the PLA to a chitosan film forming solution did not improve moisture barrier properties. Moreover, an increase of 10–30% of the WVTR was observed after the PLA adding compared to chitosan-PEG (16.6%) films. Heterogeneity could be the result of a phase separation of the blend systems, due to an incompatible chitosan/PLA association, giving films with a low structural cohesion, potentially leading to an easy diffusion of the water vapor molecules. Moreover, film-making conditions, including solvent pH and type of solvent, are parameters which could lead to the modification of membrane porosity. For example, Ravi Kumar et al. (2004) mentioned that chitosan films made with lactic acid exhibited a lower tensile strength and a higher flexibility and bioadhesive character than those made with acetic acid. The mixture of solvent, i.e. acetic acid suitable for the aminopolysaccharide and chloroform suitable for the polyester, could thus decrease the cohesion of the film by reducing the solubility of the polymer and the inter- and intra-molecular electrostatic

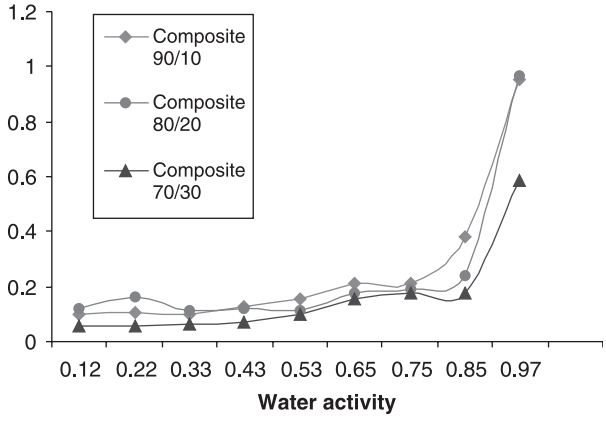
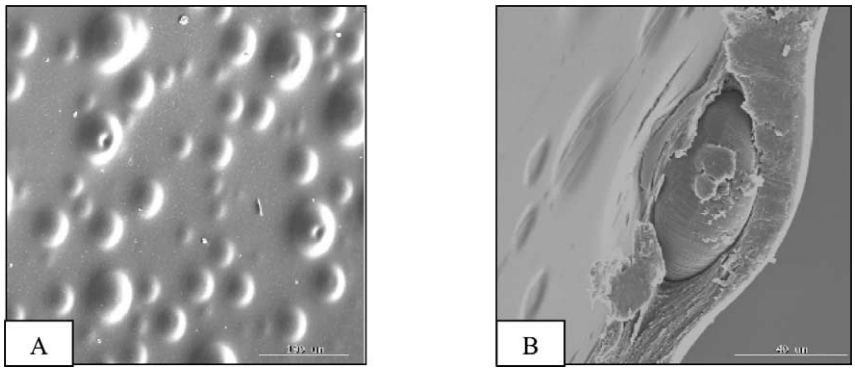


Fig. 6. Sorption isotherms of composite films chitosan/PLA 90/10, 80/20, 70/30. Each point of the graph is the mean of three experiments.



Photographs 1 and 2. Microscopic views of a composite film chitosan/PLA 70/30 w/w (Joel 840A). (A) Surface observation (magnification 100). (B) Section observation (magnification 600).

repulsion between chitosan chains. Due to the abundance of hydrophilic functional groups, chitosan is not soluble in most organic solvents. In order to solve this problem, some chemical modifications to introduce a hydrophobic nature to chitosan could be considered. Peesan et al. (2005) have synthetized hexanoyl chitosan and produced blend films of modified chitosan and PLA with the chloroform as the sole solvent. All the blend films exhibited one composition-dependent glass transition temperature, suggesting, in their conditions, that partial miscibility between hexanoyl-chitosan and PLA molecules might be possible in the bulk amorphous phase.

3.2.2.1. Sorption isotherms. The sensitivity to water vapor was also studied using the sorption isotherms to compare behavior of the composite and the homogeneous films with respect to the relative humidity of the medium. The nature of composite films isotherms depended on the percentage of the built-in PLA (Fig. 6). The smaller the PLA concentration was, the more the moisture sensitivity of films increased. The films with 30% of PLA presented the lowest moisture absorption. However, these results did not explain the unexpected WVTR results. PLA would decrease the surface hygroscopic character of chitosan-based film while the polyester would increase the diffusion of water by creating low molecular cohesion. To confirm this assumption, the film macrostructure was analyzed by scanning electronic microscopy (Photographs 1 and 2). Composite film (70/30: w/w) showed a very rough surface, with asperities, which could correspond to PLA globules. The section analyses also showed a very heterogeneous structure. These observations would explain the weak moisture barrier properties of the composite films.

3.2.3. Biological properties

The inhibitory properties of films were studied on a solid medium for the three fungi: *F. proliferatum*, *F. moniliforme* and *A. ochraceus*. The antifungal activity of films is presented in the second part of Table 2. Similarly to homogeneous chitosan based film, it was difficult to depose composite films on the agar medium. The latter retracted after contact with the medium, preventing a 'coating' on the whole of the surface of the agar medium. However, as observed for chitosan films, composite films showed an inhibitory activity against the three fungal strains, only visible under the points of contact between the agar and the film.

To verify that the bioactivity of films was indeed due to the chemical structure of the chitosan and not to (1) potentially active residual solvents or (2) potential reduction in residual oxygen on the surface of the microflore, a control was carried out using HPMC films. The HPMC is a non-polycationic polysaccharide, without the amino group (mainly responsive to the antimicrobial activity of chitosan), exhibiting oxygen barrier properties close to chitosan based films (data not shown). The chitosan was then replaced by the HPMC (same solvent, same concentration and same procedure) and the results showed a total inactivity of HPMC films (last part of Table 2). The inhibitory activity of chitosan films was thus primarily due to the chemical nature of the chitosan and to the protonation of the amino group. These bio-packagings would be thus potentially used to reduce the development of mycotoxinogen fungal strains. They are interesting candidates in improving food safety of some foodstuffs, especially those sensitive to pathogenic fungi.

4. Conclusion

The objective of this study was to produce packaging materials from renewable resources with low moisture barriers and high inhibitory properties against mycotoxinogen fungal strains, which can lead to a considerable public health risk.

The chitosan and the PLA exhibited interesting qualities in the field of bioactive packaging, due to antimicrobial properties of chitosan films and excellent mechanical and moisture barrier properties associated with liquid water resistance of PLA films. Consequently, it appeared interesting to associate both polymers to combine the principal qualities. Difficulties were however encountered in producing miscible PLA and chitosan film forming solution, leading to heterogeneous films with high water sensitivity.

Due to their antifungal activity, the composite films offer a great advantage in preventing the growth of mycotoxinogen strains. However, the physico-chemical properties of such heterogeneous films dramatically limit their development as packaging materials. Further studies need to be carried out to improve the behavior of material with respect to liquid water and water vapor in order to limit the migration and

dehydration/rehydration phenomena of foodstuffs. In addition, further studies relating to the formulation of composite material have to allow an improvement of the compatibility between both biopolymers, using for example some chemical compounds, or the elaboration of a homogeneous film produced by grafting the PLA onto the chitosan polymer. Finally, bilayer matrices have to be studied.

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