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Cellulose derivative based active coatings: Effects of nisin and plasticizer on physico-chemical and antimicrobial properties of hydroxypropyl methylcellulose films

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ABSTRACT

Bioactive composite coatings based on hydroxypropyl methylcellulose (HPMC), broad-spectrum food preservative nisin (Nisaplin®), and hydrophilic plasticizer glycerol were evaluated for mechanical, barrier (O_2, H_2O), transparency and microbiological effectiveness. Incorporation of Nisaplin® into cellulose derivative, i.e. HPMC-based films strongly increased the film thickness due to salt crystallization while glycerol had normalized it by homogenous dispersibility. The tensile strength of composite films decreased, however ultimate elongation was increased significantly. The dynamic vapour sorption experimental data fitted by different models had shown lesser values of respective energy constants for composite films. The transparency and water permeability of HPMC films were negatively affected by the additives as an effect individual but conversely as combined effect for film transparency. Film bioactivity demonstrated efficacy against *Listeria* > *Enterococcus* > *Staphylococcus* > *Bacillus* spp. These cellulose derivative based active films may thus be a key approach towards eradicating post-process contamination of healthy foods.

1. Introduction

Post-process contamination caused by product mishandling and faulty packaging is responsible for about two-thirds of all microbiologically related class I recalls in the USA with most of these recalls originating from contamination of ready-to-eat food products (Cagri, Ustunol, & Ryser, 2004). Over the last few years, consumer demand for food stuff of natural origin (termed as "Bio"), high quality, elevated safety, minimally processed, longer shelf-life, ease-to-eat with a fresh taste and appearance have turned out to be the cardinal importance (Sobrino-Lopez & Martin-Belloso, 2008). Currently, there is an escalating tendency to employ environmental friendly materials with the intention of substituting non-degradable materials, thus reducing the environmental pollution resulting from waste accumulation. To address the environmental issues, and concurrently extend the shelf-life and food quality, reducing packaging waste has catalysed the exploration of new bio-based packaging materials such as edible and biodegradable films (Burke, 2006; Tharanathan, 2003). One of the approaches is to use renewable biopolymers such as polysaccharides, proteins, gums, lipids and their complexes, derived from animal and plant origin (Ray & Bousmina, 2005). Such biodegradable/edible packaging not only ensures food safety but at the same instant are good source of nutrition (Reppas, Swidan, Tobey, Turowski, & Dressman, 2009).

Cellulose-based materials are being widely used as they offer the advantages like edibility, biocompatibility, barrier properties, aesthetic appearance, being non-toxic, non-polluting and having low cost (Vasconez, Flores, Campos, Alvarado, & Gerschenson, 2009). Hydroxypropyl methylcellulose edible films are attractive for food applications because it is a readily available non-ionic edible plant derivative shown to form transparent, odourless, tasteless, oil-resistant, water-soluble films with very efficient oxygen, carbon dioxide, aroma and lipid barriers, but with moderate resistance to water vapour transport (Villalobos, Chanona, Hernandez, Gutiaerrez, & Chiralt, 2005). HPMC is used in the food industry as an emulsifier, film former, protective colloid, stabilizer, suspending agent, or thickener. HPMC is approved for food uses by the FDA (21 CFR 172.874) and the EU (EC, 1995); its safety in food use has been affirmed by the JECFA (Burdock, 2007). The tensile strength of HPMC films is high and flexibility neither too high nor too fragile, which make them suitable for edible coating purposes (Brindle & Krochta, 2008).

Within the scope of natural food preservation, the application of antimicrobial peptides from lactic acid bacteria (LAB) in bioactive packaging films has received great attention (Cleveland,

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Montville, Nes, & Chikindas, 2001). Nisin has been increasingly used as 'bio-preservative' for direct incorporation in food as well as in active/edible films. Nisin effectively inhibits Gram-positive bacteria and outgrowth spores of *Bacillus* and *Clostridium*. Structurally, it is a 34 amino acid polypeptide with a molar mass of 3500 Da. This lantibiotic contains unusual amino acids responsible for the important functional properties, i.e. acid tolerance, thermo stability at low pH and a specific bactericidal mode of action (de Arauz, Jozala, Mazzola, & Vessoni Penna, 2009). Nisaplin® is the commercially available form containing 2.5% nisin, 74.5% NaCl and 23.8% denatured milk solids and 1.7% moisture (Dawson, Hirt, Rieck, Acton, & Sotthibandhu, 2003). In a recent study for edible films (Sebti, Chollet, Degraeve, Noel, & Peyrol, 2007) using HPMC/chitosan and incorporating the pure nisin (Aplin and Barrett Ltd. now merged in as Danisco), the author had evaluated the effect of nisin on physical characteristics of films. However, the pure nisin is not available for commercial/industrial use and is provided in very low amount for research purposes (under conditions). For this reason the use of Nisaplin® (heterogeneous blend of nisin) in food industry becomes essential due to its commercial availability, broad spectrum against food borne pathogen, only approved bacteriocin by FDA, i.e. status as GRAS (EU, 2004; FDA, 2001) and its 'bio-additive' notion for edible packaging. Thus it is imperative to verify the contribution, either positively or negatively, played by commercially available Nisaplin® on the various physico-chemical characteristics of biodegradable/edible films of HPMC.

Plasticizers impressively affect the physical properties of biopolymer films (Zhang & Han, 2008). The plasticizers help to decrease inherent brittleness of films by reducing intermolecular forces, increasing the mobility of polymer chains, decreasing the glass transition temperature of these materials and improving their flexibility (Galdeano, Mali, Grossmann, Yamashita, & Garcia, 2009; Zhang & Han, 2008). Thus it is important to study the effect of commonly used polyol 'glycerol' on the homogenous dispersion of Nisaplin® (nisin, salt and milk solid) for the formation of composite active films of improved quality. However, plasticizer generally causes the increased water permeability so it must be added at a certain amount to obtain the films with improved flexibility, thickness and transparency without significant decrease of mechanical strength and barrier property to mass transfer (Brindle & Krochta, 2008; Jongjareonrak, Benjakul, Visessanguan, & Tanaka, 2006; Möller, Grelier, Pardon, & Coma, 2004). No information regarding the sorption behaviour, light transmission, tensile characteristics and bioactivity spectrum against potential pathogens of HPMC-Nisaplin®-plasticizer composite film has been reported. Therefore, the objective of this investigation was to study the effect of Nisaplin® or various concentrations of plasticizer on HPMC-based edible film individually, and to further analyse their simultaneous utilization for improving the above-mentioned physico-chemical attributes and antimicrobial efficacy against a spectrum of bacteria.

2. Materials and methods

2.1. Materials

2.1.1. Reagents

HPMC powder with hydroxypropoxyl content ~9% and viscosity ~15 mPa s (2% in H_2O, 25 °C) was obtained from Fluka-Biochemika, Japan. Ethanol 96.2% (Pharmaceutics CARLO Erba) was used to improve hydration of HPMC, it also helped in reduction of air bubbles in film forming solution (FFS) and eventually the drying of film. Glycerol (>97% purity) was used as a plasticizer and was purchased from Merck (Darmstadt, Germany). Nisin solution was prepared by using Nisaplin® (Sigma Chemical Co.) that contains 2.5% nisin and the rest are mainly salt and milk proteins. According to the supplier, the activity of 1 g Nisaplin® is ~10^6 IU.

2.1.2. Microorganism and culture media

Listeria, *Staphylococcus*, *Enterococcus* and *Bacillus* strains were purchased from different institute collections (*Staphylococcus aureus* CIP 677, *S. aureus* CIP 76.25, *S. aureus* CIP 4.83, *S. aureus* CIP 57.10, *Listeria seeligeri* SLCC 3954^T, *L. grayi* CIP 6818^T, *L. monocytogenes* CIP 7831, *L. monocytogenes* CIP 82110^T, *L. innocua* CIP 12511, *L. ivanoii* CIP 12510, *Bacillus cereus* CIP 6624, *B. licheniformis* CIP 5271, *B. subtilis* CIP 5265, *Enterococcus facium* DSM 20477^T, *E. durans* CIP 55125^T). The strains from these genera were cultivated in trypticase soy broth (Biokar Diagnostics, Beauvais, France) supplemented with 6 g L^{-1} of bacto-yeast extract (Biokar) (TSB-YE) except *Enterococcus* strains for which Ellikar (Biokar) medium was utilized. Incubation was performed at 37 °C except for *Bacillus* (30 °C). All strains were stored in appropriate culture medium supplemented with glycerol (10%) at −30 °C and propagated twice before use. Agar medium was prepared by addition of 12 g L^{-1} of bacteriological agar.

2.2. Film preparation

FFS were prepared by dissolving 6 g of HPMC in solution of ethanol (35 mL) and distilled water (65 mL). For better dissolving the Nisaplin®, the pH of solvent was adjusted to ~3 with HCl 0.1 N. The solutions were mixed for 40 min at 65 °C using a heating magnetic stirrer (Fisher Bio-block Scientific). Composition of the HPMC biodegradable active film formulations and variables of concern, such as concentration of plasticizer and Nisaplin® amount are shown in Table 1. Selected amounts of glycerol and Nisaplin® were added during heating and stirring. Nisaplin® concentration

Table 1
Mechanical properties and thickness of HPMC-Nisaplin®-plasticizer composite films (mean of triplicate analysis).

Film composition	Thickness, *X* (μm)	Tensile strength, TS (MPa)	Ultimate elongation, UE (%)	Young's modulus, *Y* (MPa)
HPMC	47 ± 2	63 ± 8	13 ± 1	2334 ± 99
HPMC + 10% G	49 ± 3^{NS}	47 ± 11*	33 ± 8*	1462 ± 63***
HPMC + 20% G	53 ± 1^{NS}	27 ± 10***	33 ± 3*	1098 ± 165***
HPMC + 30% G	57 ± 5^{NS}	21 ± 2***	41 ± 13**	961 ± 92***
HPMC + 50% G	56 ± 3^{NS}	16 ± 3***	50 ± 6***	421 ± 16***
HPMC + N	70 ± 12**	43 ± 9*	26 ± 14^{NS}	856 ± 229***
HPMC + N + 10% G	71 ± 12**	28 ± 4***	30 ± 13^{NS}	783 ± 269***
HPMC + N + 20% G	72 ± 11**	23 ± 6***	41 ± 4**	656 ± 335***
HPMC + N + 30% G	64 ± 7^{NS}	20 ± 4***	30 ± 1^{NS}	591 ± 209***
HPMC + N + 50% G	58 ± 2^{NS}	20 ± 3***	31 ± 3^{NS}	722 ± 97***

HPMC = hydroxypropyl methylcellulose; G = glycerol; N = Nisaplin® 1%, i.e. 10^4 IU.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.
^{NS} $p > 0.05$.

was adjusted to give a final activity of 10^4 IU. Its solution was prepared by taking 1/10th part of solvent for a formulation (H_2O and ethanol—pH 3) before adding HPMC or glycerol, the solution was centrifuged at 4000 rpm for 15 min at 4 °C and the supernatant recovered. As a homogenous solution was achieved after mixing, it was degassed at 50–60 °C under vacuum (Yamato®). Films were made by pouring approximately 5 g FFS in the lids of Petri-dishes (Optilux - Nunclon™ Fisher, DK-4000 Roskilde, Denmark) and left to dry them at room temperature (20 °C) and relative humidity (~50%) for 24–48 h. Films were either stored under similar conditions of drying or at approximately zero relative humidity using phosphorus pentaoxide (P_2O_5) depending upon film characterization experiment.

2.3. Film characterization

2.3.1. Film thickness measurement

The thickness of films was measured using the standard NF Q 03-016 with a manual micrometer (Messmer, London, England) equipped with a head measuring 1 cm in diameter and a sensitivity of 2 μm. The thickness was measured in 10 randomly selected points on each film prepared by using identical amount (5 g) of FFS and then an average value was determined.

2.3.2. Tensile properties

The mechanical characteristics of films were evaluated at 20 ± 1 °C and 50 ± 2% RH. It consists of tensile strength (TS, MPa), ultimate elongation (UE, percent at break point), and Young's modulus (*Y*, MPa). Maximum tensile strength is the largest stress that a film is able to sustain. Ultimate elongation is the maximum percentage change in the length of a film before breaking. Young's modulus, calculated from the slope of the initial linear region of the stress–strain curves, reflects the film stiffness. The tests were performed using the tension testing machine Lloyd instrument (Hants, United Kingdom) according to standard NF T 54-102 (1971) on 6 specimens previously stored for 7 days at 20 ± 1 °C and 50 ± 2% RH. Sample films of approximately 5 cm × 2 cm (analysed area = 3 cm × 2 cm) uniaxially stretched (sensor of force of 5 kN and constant speed of 20 mm/min). The stress–strain curves were computer-recorded and exploited with Nexygen software.

2.3.3. Water sorption isotherms

Sorption isotherms of films were obtained using a dynamic vapour sorption system (DVS, SMS Ltd., UK). The sample is equilibrated at a constant temperature for different relative humidity values. Film pieces were cut into small pieces (5 mm × 5 mm) and dried in a vacuum dessicator at 20 °C over phosphorus pentoxide (P_2O_5) for 2 weeks. The programmed relative humidities were from 0 to 95%, divided in 10% increments (10 points). The temperature was set at 25 °C. The samples were considered to be at equilibrium when the value d*m*/d*t* (slope of the changing in mass with time) was set to be <0.002 mass%/min.

The modelisation of the sorption isotherms was done using BET (Brunauer–Emmett–Teller) (Eq. (1)), GAB (Guggenheim–Anderson–de Boer) (Eq. (2)), and TSS (three sorption stage) (Eq. (3)) models. The procedure used for estimating the parameters for different models was non-linear regression (curve fitting), using Origin 6.1 software (Origin Lab Corporation, USA).

$$X = \frac{X_m \cdot C_{BET} \cdot a_w}{(1 - a_w)(1 - a_w + C \cdot a_w)} \quad (1)$$

$$X = \frac{X_m \cdot C_{GAB} \cdot K \cdot a_w}{(1 - K \cdot a_w)(1 - K \cdot a_w + C \cdot K \cdot a_w)} \quad (2)$$

$$X = \frac{X_m \cdot C_{TSS} \cdot K \cdot a_w \cdot h_{TSS}}{(1 - K \cdot a_w)(1 + (C \cdot h_{TSS} - 1) \cdot K \cdot a_w)} \quad (3)$$

where *X* is the mass of water adsorbed at p/p_0 (a_w), X_m is the monolayer value (% db). C_{BET} is a temperature-dependent constant, and p/p_0 is the water partial pressure (p = water partial pressure; p_0 = water vapour pressure at saturation). Constant C_{GAB} and C_{TSS} are related to the energy associated with the binding between the water molecules and the matrix primary interactions sites or monolayer. There is also a temperature-dependent value (*K*) related to the heat of sorption of the multilayer. The parameter h_{TSS} is a correction factor concerning the information related to sorption at high relative humidity (≥90%) and the energies (heat of sorption) involved during the sorption process.

2.3.4. Measurement of transparency/light transmission

Film transparency against ultraviolet (UV) and visible light was measured for a wavelength spectrum between 200 and 900 nm, using a UV–visible recording spectrophotometer (Ultrospec 4000 UV/visible, Pharmacia Biotech, UK) according to the procedure given by Fang, Tung, Britt, Yada, and Dalgleish (2002). The transparency of the films was calculated by the equation: transparency = $-\log T_{600}/X$ (Han & Floros, 1997), where T_{600} is the transmittance at 600 nm and *X* is the film thickness. Three replicates of each treatment were tested.

2.3.5. Water vapour permeability (WVP)

Water vapour permeability of the composite films was determined with the gravimetric method described in the AFNOR NFH00-030 standard (1974). The film was sealed in a permeation cell containing a desiccant (silica gel). The glass permeation cells were 5.8 cm (i.d.) × 7.8 cm (o.d.) × 3.6 cm deep with an exposed area of 26.42 cm^2. The permeation cells were placed in a controlled temperature (38 ± 1 °C) and RH (~100%) chamber via ventilation. The water vapour transport was determined from the weight gain of the cell. Three replicates were made from each film composition. Water vapour transmission rate (WVTR) and WVP of the films were calculated as follows (Khwaldia, Banon, Perez, & Desobry, 2004). CWVT was determined from the slope obtained from the regression analysis of weight gain data as a function of time, once the steady state was reached.

$$\mathrm{WVTR} = \frac{dm}{dt}A \quad (\mathrm{g\ h^{-1}\ m^{-2}}) \quad (4)$$

$$P = \frac{\mathrm{WVTR}}{\Delta p\, 3600} \quad (\mathrm{g\,s^{-1}\,m^{-2}\,Pa^{-1}}) \quad (5)$$

$$\mathrm{WVP} = P \times X \quad (\mathrm{g\ m^{-1}\ s^{-1}\ Pa^{-1}}) \quad (6)$$

where d*m* is the weight gain of the cup over time (d*t*), *A* is the area of exposed film, Δp is the vapour pressure differential across the film, and *X* is the film thickness.

2.3.6. Microbiological analysis

To find out the antibacterial activity of films, 1 cm diameter disks were cut from different composite bioactive films and placed on inoculated nutrient medium. The method was previously standardized by adjusting the microbial inoculation rate (0.1%, v/v) and the volume of the agar medium layer (12 mL). Dishes were refrigerated at 4 °C for 4 h to allow the process of bacteriocin diffusion without microbial growth and then incubated at 37 °C (*Listeria*, *Staphylococcus*, *Enterococcus strains*) or 30 °C (*Bacillus* strain). Data was expressed as growth inhibitory zone diameter (cm) and measured at the nearest 1 mm for three replicates.

2.3.7. Statistical analysis

Statistical analyses were carried out by using the software KyPlot version 2.0 (Koichi Yoshioka, Department of Biochemistry

and Biophysics, Graduate School of Allied Health Sciences, Tokyo, Japan). For comparison between HPMC film and films containing either glycerol or Nisaplin®, a parametric multiple test (Dunnett test with HPMC film as control) was performed. Furthermore composite films containing both Nisaplin® and glycerol were compared with their respective formulations without Nisaplin® using Tukey parametric multiple test.

3. Results and discussion

3.1. Film thickness measurement

Film thickness depended greatly on film nature and composition (Table 1). In the first step the addition of plasticizer alone (10–50%, w/w, d.m.) produced the HPMC films with thickness statistically indifferent (Dunnett test, $p > 0.05$). The narrow range variation in thickness might be there because elevated glycerol content maintained higher moisture content at the end of film drying (Chen & Lai, 2008). However, the incorporation of Nisaplin® radically increased the film thickness (70 ± 12 μm) due to the formation of salt crystals (salt present in Nisaplin® formulation) in course of drying and the film was non-homogenous. Previous studies had demonstrated that the film thickness depended primarily on the biopolymer (nature, concentration) and/or the additives incorporated (e.g. nisin, glycerol, ...) in the biodegradable films (Mali, Grossmann, Garcia, Martino, & Zaritzky, 2004; Sebti et al., 2007). In the present study, the composite films of Nisaplin® with 30 and 50% glycerol normalized the crystals effect by homogenous dispersibility because plasticizer could reduce the intermolecular forces and increase the mobility of polymer chains.

3.2. Tensile properties

One of the primary tasks of bioactive packaging is either provision of physical shield to food or slow release of active agent. The capacity of these composite bioactive films for preserving the integrity of food stuff was evaluated by measuring the tensile strength (TS), Young's modulus (Y) and ultimate elongation at break (%UE).

As expected the concentration of plasticizer had profound influence on the tensile strength (Table 1). While the concentration of glycerol increased 20% or more, the TS decreased very significantly (Dunnett test, $p \leq 0.001$) to 16 ± 3 MPa for glycerol content 50% (w/w, d.m.). The drop in TS with increasing concentration of plasticizer is in accordance with the results presented by previous studies for plastic and oat starch films (Galdeano et al., 2009; Guiga et al., 2009). However, when Nisaplin® was added in HPMC formulation the TS was decreased (Dunnett test, $p \leq 0.05$) due to the role of milk solids and salt as hydrophilic compounds. On the other hand the composite film containing both antimicrobial and plasticizer had shown significant decrease in tensile strength as compared to HPMC film, but pair-wise comparison of TS for films containing both Nisaplin® and glycerol with respective formulations without Nisaplin® presented non-significant variations (Tukey test, $p > 0.05$). The observed behaviour could be related to the structural modification of HPMC network. The plasticizer and hydrophilic compounds resulted in less dense film matrix, facilitating movements of polymer chains under stress, hence declining the film resistance.

An inverse correlation was observed between the TS and % elongation at break (%UE) characteristics for active composite films. With increasing concentration of the glycerol, the %UE increased very significantly up to 50 ± 6% for glycerol content 50% (Dunnett test, $p \leq 0.001$). Thus the films prepared with glycerol were more flexible and more stretchable than non-plasticized formulation.

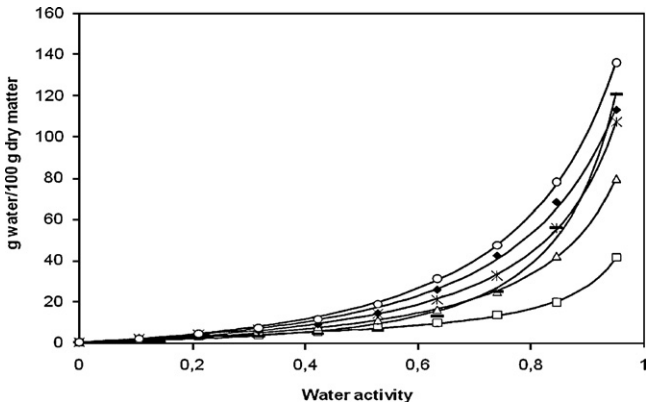

Fig. 1. Moisture sorption isotherms of HPMC, Nisaplin® and glycerol composite films at 25 °C. Experimental DVS values were averaged and fitted by isotherm equation; solid lines represent the GAB model fitted to the data. (□) HPMC film, (△) HPMC + 30% glycerol, (✳) HPMC + 50% glycerol, (—) HPMC + 10^4 IU Nisaplin®, (◆) HPMC + 30% glycerol + 10^4 IU Nisaplin®, (○) HPMC + 50% glycerol + 10^4 IU Nisaplin®.

Nisaplin® had a non-significant increase in the %UE but variation was quite high caused by random break due to presence of salt crystals of Nisaplin® once the film were dried. Concerning the Nisaplin® films with 30% or 50% glycerol, the homogenous dispersion of Nisaplin® by plasticizer resulted in formation of a film network normalized towards moderate elasticity (Dunnett test, $p > 0.05$).

Nisaplin® incorporation in non-plasticized HPMC film reduced the Young's modulus (Y) significantly indicating a decrease in film rigidity. NaCl, milk proteins and carbohydrates present in the Nisaplin® preparation could have interacted with HPMC and modified its mechanical conduct. In addition, these compounds have high affinity for water, known to be very effective plasticizer for most biopolymers, which induces lowering of elastic modulus. With glycerol incorporation, Y decreased significantly (Dunnett test, $p \leq 0.001$) which is in accordance to the results obtained earlier (Pushpadass, Marx, & Hanna, 2008) with starch coatings. Hence the key function in decreasing the film rigidity for composite film was carried out by plasticizer.

3.3. Water sorption isotherm

The isotherms acquired presented a slow initial increase in moisture content with water activity (a_w) increase up to 0.6 and a quick augmentation in film water adsorption with further rise of a_w implying a swelling phenomenon as water activity is increased (Fig. 1). Such a negligible convexity obtained at low a_w was related with type III sorption isotherm (Villalobos, Hernandez-Munoz, & Chiralt, 2006), a characteristic of components rich in hydrophilic components and are frequently reported in literature (Guiga et al., 2009; Kristo, Biliaderis, & Zampraka, 2007; Kristo, Koutsoumanis, & Biliaderis, 2008; Müller, Laurindo, & Yamashita, 2009; Sebti, Delves-Broughton, & Coma, 2003).

For plasticized films, glycerol contributed to an increase in moisture uptake for RH > 60%. At low vapour pressures, hydrogen bonding is the main force involved in the adsorption mechanism (Enrione, Hill, & Mitchell, 2007), thus the first step of DVS corresponds to the fixation of water molecules on the specific hydrophilic groups of polymer; afterwards, the amount of sorbed water depends on the swelling capacity of the polymer (Fringant et al., 1996). That is why incorporation of glycerol modified the water sorption only at high a_w. Similar behaviour was detected for active films with Nisaplin® due to its heterogeneity. Na salt is well acknowledged as hygroscopic reagent (Rougier, Bonazzi, & Daudin, 2007) for the biopolymers, and as a matter of fact it accounts for 74.5% in Nisaplin® formulation.

Table 2
Parameter values obtained from the curves fitted to various composite films with BET, GAB and TSS models.

Model	Formulation	X_m	C	K	h_{TSS}
BET	HPMC	4.30	2.86	–	–
	HPMC + 30% G	7.88	1.61	–	–
	HPMC + 50% G	10.88	1.35	–	–
	HPMC + N	3.67	2.68	–	–
	HPMC + N + 30% G	13.69	0.80	–	–
	HPMC + N + 50% G	19.61	0.70	–	–
GAB	HPMC	4.14	6.23	0.94	–
	HPMC + 30% G	14.51	0.70	0.89	–
	HPMC + 50% G	19.45	0.65	0.90	–
	HPMC + N	27.1	0.23	0.91	–
	HPMC + N + 30% G	41.17	0.38	0.82	–
	HPMC + N + 50% G	51.38	0.35	0.82	–
TSS	HPMC	4.33	4.45	0.94	0.71
	HPMC + 30% G	14.58	0.71	0.89	0.96
	HPMC + 50% G	19.39	0.63	0.90	0.99
	HPMC + N	26.84	0.33	0.91	0.68
	HPMC + N + 30% G	44.36	0.41	0.82	0.88
	HPMC + N + 50% G	50.97	0.38	0.83	0.90

HPMC = hydroxypropyl methylcellulose; G = glycerol; N = Nisaplin® 1%, i.e. 10^4 IU.

3.3.1. Modelling of the sorption isotherms

The experimental sorption data of biodegradable films was first fitted with the two parameter BET model (Eq. (1)), between 0 and 50% RH. GAB and TSS models (Eqs. (2) and (3)) were fitted to the whole range data (0–95% RH). For all the formulations, the coefficient of determination R^2 was 0.99 (except for GAB fitted HPMC containing Nisaplin® film with 0.98 R^2), verifying the suitability of equations in explaining the data.

The X_m value is of high concern, as it refers to strongly adsorbed water to specific sites and is considered as optimum value at which the film is most stable. For all models, X_m value of 4 ± 0.3 g/100 g for HPMC film was observed (Table 2) which is close to the previous findings (Villalobos et al., 2006). Glycerol increased the X_m values obtained from BET, GAB and TSS models for plasticized films. For active films containing nisin, the values of X_m were varied for BET and GAB or TSS models. The X_m values of BET and TSS were generally lower than those from the GAB fitted value. Even for the BET the monolayer value was slightly lower than HPMC which is possibly due to the fact that denatured milk solids in Nisaplin® adsorb water significantly when a_w is >0.5. As the BET model is applied to 0–50% RH, the presence of denatured milk solids with nisin effect the sorption behaviour in contrary manner by engaging the active sites of HPMC. Nonetheless, TSS model normalizes the higher elevation for X_m value by GAB for nisin incorporated films through its extra correction factor adjusting the rapid increase at higher a_w region.

The thermodynamic parameter C is represented in Table 2 after model fitting. A marked decrease in C_{BET}, C_{GAB} and C_{TSS} values was observed for different plasticizer concentrations. Its decrease suggested that nisin or polyol may perhaps occupy sorption sites of polymer and in consequence reduced the bonding energy.

On the other hand, the larger difference of K value from 1 would point towards a decrease in sorption energy of the multilayer. As a result, lower value of K suggested the higher water content in multilayers (Quirijns, Van Boxtel, Van Loon, & Van Straten, 2005). Thus Nisaplin® and glycerol molecules had reduced the interaction energies between the water molecules, on the second and higher water layers, and the polymer. When the h_{TSS} parameter value appears to be 1, it means that TSS model is reduced to GAB expression (Timmermann, 1989). The relative lower values (<1) for this constant observed were conceivably due to taking into account the solubility or quick sorption behaviour in third sorption stage.

Table 3
Light transparency of composite active films as affected by nisin, plasticizer or both (mean of triplicate analysis).

Formulation	Transparency = −log(T600/X)
HPMC	3.19 ± 0.01
HPMC + 10% G	2.90 ± 0.50^{NS}
HPMC + 20% G	2.85 ± 0.50^{NS}
HPMC + 30% G	2.73 ± 0.43^{NS}
HPMC + 50% G	2.81 ± 0.48^{NS}
HPMC + N	2.41 ± 0.35**
HPMC + N + 10% G	2.93 ± 0.01^{NS}
HPMC + N + 20% G	2.84 ± 0.08^{NS}
HPMC + N + 30% G	2.80 ± 0.13^{NS}
HPMC + N + 50% G	2.63 ± 0.36**

G = glycerol; N = Nisaplin® 1%, i.e. 10^4 IU.
** $p < 0.01$.
^{NS} $p > 0.05$.

3.4. Measurement of transparency/light transmission

Transparency of the film is relevant property of film since it has a direct impact on the appearance of packaged product. Compared to the effect of each mixture component on transparency, Nisaplin® was the primary factor reducing the film transparency (Table 3). Plasticizers are compounds used to increase film transmission (Jongjareonrak et al., 2006) but the control HPMC film showed such an excellent transparency characteristic that even addition of glycerol at higher content resulted in slight decrease in the light transmission. This attribute of the HPMC edible film justified its use as edible film biopolymer primarily to fulfil the consumer eagerness to see food through packaging. Transmission percentage had depended on the concentration of plasticizer (glycerol) and addition of active agent Nisaplin® (Fig. 2). The transparency of HPMC film was inversely proportional to the Nisaplin® addition and glycerol concentration as an effect individual.

Glycerol played a positive role to improve the transparency of films containing Nisaplin® due to improved dispersibility of Nisaplin® in network of HPMC film and thus provided homogenous film which had higher transparency values. HPMC film containing Nisaplin® only had given transmission value close to bottom which was improved with the addition of glycerol. But higher addition of plasticizer decreased the transparency, as HPMC films containing both Nisaplin® (10^4 IU) and glycerol (50%, db) showed minimum transmission value second to Nisaplin® addition alone. This result was in accordance with previous findings (Villalobos et al., 2005), which observed that lower ratios of surfactants infer lesser anisotropy degree in the physical properties throughout the matrix. In this logic, the observed effect of glycerol on transparency could be related with the smaller number of discontinuities in the refractive index through the internal homogenous film structure.

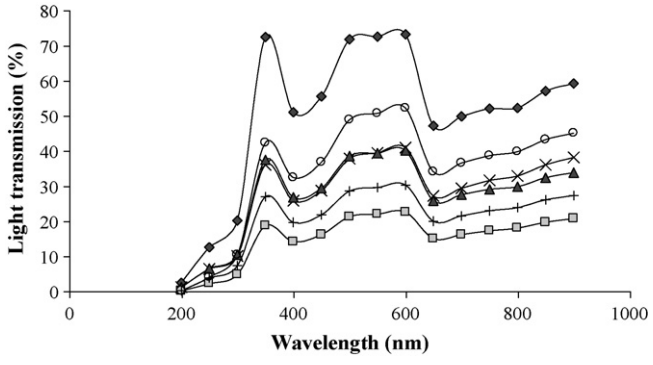

Fig. 2. Light transmission (%T) of UV, visible and NIR for HPMC composite/active films (♦) HPMC film, (×) HPMC + 30% glycerol, (▲) HPMC + 50% glycerol, (■) HPMC + 10^4 IU Nisaplin®, (○) HPMC + 30% glycerol + 10^4 IU Nisaplin®, (+) HPMC + 50% glycerol + 10^4 IU Nisaplin®.

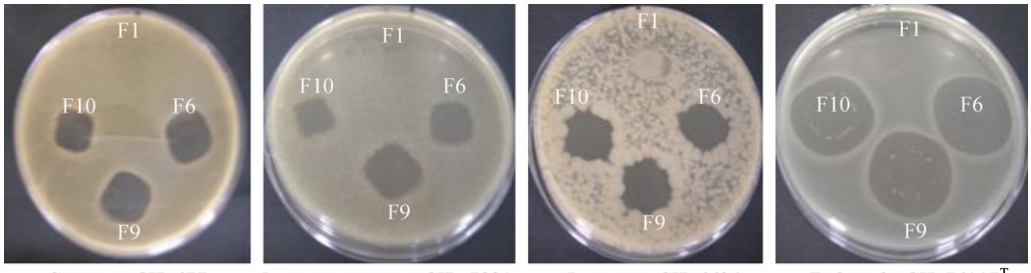

Fig. 3. Inhibition zone of composite active films against bacteria of food origin F1 = HPMC film, F6 = HPMC + 10^4 IU Nisaplin®, F9 = HPMC + 30% glycerol + 10^4 IU Nisaplin®, F10 = HPMC + 50% glycerol + 10^4 IU Nisaplin®.

Table 4
Water vapour permeability of HPMC films as a function of nisin and glycerol concentration at ~ 100% RH gradient (mean of triplicate analysis).

Films	C.W.V.T ($g\,m^{-2}\,h^{-1}$)	Water vapour permeability ($g\,m^{-1}\,s^{-1}\,Pa^{-1}$) × 10^{-10}
HPMC	32 ± 1	4.2 ± 0.1
HPMC + 30% G	48 ± 2***	6.5 ± 0.3***
HPMC + 50% G	57 ± 2***	8.8 ± 0.3***
HPMC + N	25 ± 1*	4.9 ± 0.1NS
HPMC + N + 30% G	58 ± 4***	10.4 ± 0.7***
HPMC + N + 50% G	59 ± 4***	9.5 ± 0.6***

G = glycerol; N = Nisaplin® 1%, i.e. 10^4 IU.
* $p < 0.05$.
*** $p < 0.001$.
NS $p > 0.05$.

3.5. Water vapour permeability (WVP)

WVP estimate the ease with which moisture penetrates through a barrier. Among the plasticized films containing 30% or 50% glycerol (w/w, d.b.) showed higher WVP values (Table 4). As expected, glycerol is an effective plasticizer with a high capacity to interact with water, facilitating its solubilisation and permeation through film. These results are similar to the findings of WVP using plasticizer for skin gelatine film (Jongjareonrak et al., 2006), oat starch film (Galdeano et al., 2009), tapioca starch (Chen & Lai, 2008) and sodium caseinate edible films (Schou et al., 2005). While the concentration of glycerol increased 30% or more, the WVP increased very significantly (Dunnett test, $p \leq 0.001$).

Regardless of the ever-increasing interest in the incorporation of antimicrobial compounds in edible films the literature is rather scarce on findings about their effect on modifying film structure and properties. Water permeation through edible films is the sum of three phenomena: (i) sorption already investigated with isotherms, (ii) water diffusion inside the polymer, and (iii) water desorption. Measurements of WVTR for HPMC plus Nisaplin® and HPMC plus Nisaplin® and glycerol were in total accordance with water sorption isotherms. The WVP of films made from HPMC with Nisaplin® were slightly more but not statistically different ($p < 0.05$). According to a prior study (Khwaldia, Linder, Banon, & Desobry, 2005), the re-structuring of biopolymer inside film matrix due to active agent/plasticizer incorporation significantly affect WVP which may explain the relative increase of WVP in composite active films.

3.6. Microbiological analysis

To identify whether using plasticizer for improving physicochemical properties of film, would influence negatively or not by inhibiting nisin release from composite film network, microbial test were used. The common method for testing antimicrobial activity of active film is to measure the inhibition zone of pre-inoculated agar gel containing the indicator strain. As expected, HPMC film had not shown any antimicrobial activity against 16 bacterial strains from food origin (*Listeria*, *Staphylococcus*, *Bacillus* and *Enterococcus*). Glycerol had not affected the release of nisin from films and thus the inhibition zone from active film was similar to the plasticized active films (Table 5). The only difference observed for antimicrobial effectiveness was due to the respective sensibility of specific strain against nisin (Galvez, Abriouel, Lopez, & Omar, 2007). The interesting fact was to observe that not only the active films had

Table 5
Inhibition zone of composite active films against food borne pathogens.

Bacterial strains	HPMC + N (cm)	HPMC + N + 30% G (cm)	HPMC + N + 50% G (cm)
Staphylococcus aureus CIP 677	1.4 ± 0.10	1.6 ± 0.05	1.5 ± 0.16
S. aureus CIP 76.25	1.4 ± 0.08	1.4 ± 0.10	1.4 ± 0.05
S. aureus CIP 4.83	1.7 ± 0.12	1.9 ± 0.02	1.9 ± 0.07
S. aureus CIP 57.10	1.4 ± 0.22	1.6 ± 0.10	1.6 ± 0.17
Listeria seeligeri SLCC 3954T	1.7 ± 0.12	1.9 ± 0.10	1.8 ± 0.08
L. grayi CIP 6818T	2.2 ± 0.15	2.1 ± 0.22	2.3 ± 0.08
L. monocytogenes CIP 7831	1.4 ± 0.22	1.7 ± 0.07	1.6 ± 0.15
L. monocytogenes CIP 82110T	1.5 ± 0.05	1.7 ± 0.02	1.7 ± 0.07
L. innocua CIP 12511	1.3 ± 0.22	1.3 ± 0.23	1.3 ± 0.25
L. ivanoii CIP 12510	3.5 ± 0.98	3.4 ± 0.17	3.5 ± 0.14
Bacillus cereus CIP 6624	1.5 ± 0.08	1.6 ± 0.16	1.5 ± 0.02
B. licheniformis CIP 5271	1.6 ± 0.18	1.6 ± 0.14	1.6 ± 0.23
B. subtilis CIP 5265	1.6 ± 0.17	1.4 ± 0.36	1.5 ± 0.14
Enterococcus facium DSM 20477T	2.5 ± 0.02	2.6 ± 0.10	2.5 ± 0.02
E. durans CIP 55125T	2.1 ± 0.18	2.4 ± 0.56	2.4 ± 0.20
E. faecalis CIP 76117T	2.6 ± 0.05	2.6 ± 0.07	2.7 ± 0.13

G = glycerol; N = Nisaplin® 1%, i.e. 10^4 IU.
CIP: Collection of Institute Pasteur, Paris, France.
DSM: Deutsche Sammlung von Mikio-Orgonismer and Zellkuturen, Göttingen, Germany.
SLCC: Special *Listeria* Culture Collection, University of Wurzburg, Germany.

inhibited bacteria below its surface but nisin had also diffused in the inoculated media to hamper bacterial survival (Fig. 3).

4. Conclusion

The imperative aim of this study was to provide information that may escort to the development of antimicrobial containing HPMC bio-packaging by means of commercially available form of nisin (Nisaplin®). It was demonstrated in the study that alongwith nisin the presence of salt and denatured protein in Nisaplin® greatly affected the transparency, thickness and water sorption behaviour of active films. In addition, the presence of plasticizer substantially improved the stretch-ability and transparency but adversely altered the permeability and tensile strength. Furthermore, the results clearly demonstrated that formulation containing HPMC, nisin and 30% glycerol is a promising bioactive film due to its transparent and homogenous matrix, stable structure with good stretch-ability, moderate water sorption and good antimicrobial efficiency.

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