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Physical properties of edible chitosan films containing bergamot essential oil and their inhibitory action on *Penicillium italicum*

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ABSTRACT

Chitosan-based films containing bergamot essential oil (BO) at 0.5%, 1%, 2% and 3% w/w were prepared to evaluate their physical and antifungal properties. Film-forming dispersions (FFD) were also characterized in terms of rheological properties, particle size distribution and ζ-potential. In order to study the impact of the incorporation of BO into the chitosan (CH) matrix, water vapour permeability (WVP), mechanical and optical properties of the dry films were evaluated. Furthermore, the antifungal effectiveness of CH–BO composite films against *Penicillium italicum* was studied. Results showed that the incorporation of BO provoked a decrease in the water vapour permeability, this reduction being around 50% when using a BO–CH ratio of 3:1. Concerning mechanical and optical properties, CH–BO composite films were less resistant to break, less deformable and less glossy. The load parameters (TS and EM) decreased more than 50% and the percentage of elongation at break was also dramatically reduced from 22% to 5%, as compared with the pure chitosan films. CH–BO composite films showed a significant inhibitory effect on the growth of *P. italicum*, which depended on the BO concentration. Chitosan films with the maximum bergamot oil content (3:1 BO–CH ratio) led to a total inhibition of the fungus growth during the first 5 days at 20 °C. Although the antifungal effectiveness of the films decreased throughout the storage time, a significant reduction of 2 logarithm units as compared with the control remained possible, after 12 days at 20 °C, using the highest BO content.

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1. Introduction

To design proper edible antimicrobial films to be used in food preservation can be considered as one of the major challenges for food technologists in the next few years. In the future, these films will be tailor made to solve some specific problems for a given product. It is important to know the influence of different factors on their properties in order to design the most suitable film for a determined use and functionality.

Among the active biomolecules, chitosan has a great potential for a wide range of food applications due to its biodegradability, biocompatibility, antimicrobial activity, non-toxicity and film-forming capacity (Arvanitoyannis, 1999; Li, Dunn, Grandmaison, & Goosen, 1992; Tharanathan & Kittur, 2003). Chitosan-based films have been proven to present moderate oxygen barrier properties and good carbon dioxide barrier properties but high water vapour permeability, due to their hydrophilic nature (Butler, Vergano, Testin, Bunn, & Wiles, 1996). Usually, hydrophobic compounds, such as lipids, are incorporated into this type of hydrophilic hydrocolloid films to improve their water barrier properties. One possibility is the use of essential oils (EOs), as hydrophobic constituents, which have also been demonstrated to present potential antimicrobial activity against a wide variety of bacteria, moulds and yeast (Fischer & Phillips, 2006). Generally, phenolic and terpene compounds are major contributors to these antimicrobial properties. The specific advantage of EO appears to be the synergistic effects of their compounds as evidenced in the greater activity when applied as natural EO, as compared with the sum of the effects of the individual substances (Duke & Beckstrom-Sternberg, 1992).

Recently, the application of citrus essential oils to food preservation has received increased attention because not only do they lend themselves to use in food products but are also generally recognized as safe at flavouring concentrations (GRAS). These factors made them into very promising compounds to be used as a natural alternative to chemical-based preservatives, in line with the changes in legislation and consumer trends (Brul & Coote, 1999).

Bergamot oil is a citrus oil (from *Citrus bergamia*), whose major chemical compounds are volatile, such as limonene (32–45%) and linalool (around 10.23%) (Moufida & Marzouk, 2003; Svoboda & Greenaway, 2003). The antimicrobial efficiency of BO, and its com-

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ponents, linaool and citral, has been found to be effective against *Campylobacter jejuni*, *Escherichia coli* O157, *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus*, *Arcobacter butzleri* and *Penicillium digitatum* (Fisher & Phillips, 2008), among others, both when oil is applied directly and when in contact with the oil vapour. The mechanisms by which essential oils bring about their antimicrobial effect are not clear but there are a number of proposed mechanisms (Holley & Patel, 2005): terpenes have the ability to disrupt and penetrate not only the lipid structure of the cell membrane, but also the mitochondrial membrane, leading to the denaturing of proteins and the destruction of cell membrane, cytoplasmatic leakage, cell lysis and eventually, cell death.

In composite chitosan–essential oil based films, the possible presence of interactions between the active antimicrobial agent and the chitosan should be taking into account, as they could affect the efficiency of the antimicrobial response. Chitosan might interact with terpens which are the major components of essential oils, mainly by weak interactions such as hydrogen bonding (Mayachiew, Devahastin, Mackey, & Niranjan, 2010). Hosseini, Razavi, and Moussavi (2009) found that the intensity of these interactions depended on the nature of the essential oil. These authors showed that the incorporation of essential oils into chitosan films led to a loose in the compactness of the film structure, this effect being more accused with cinnamon essential oil in comparison with thyme and clove oils. These interactions could affect the release of the added antimicrobial agent (Hosseini et al., 2009) and so, the antimicrobial response of the composite films.

On the other hand, films and coatings should be designed to fulfill a number of requirements, such as to have proper mechanical properties, good appearance (adequate gloss and transparency) and water and gas barrier properties. Thus, knowing how different factors influence these physical film properties is relevant to be able to improve and optimize the film functionality. Among these factors, the stability related properties of the film-forming dispersions (FFD), such as rheological behaviour, particle size and distribution and ζ-potential of the dispersed lipid particles, play an important role in the properties of lipid–hydrocolloid composite films. The control of the FFD properties could allow us to design films with determined functional properties (McClements, 2007)

The aim of this work was to evaluate how the functionality of chitosan-based films was affected by the incorporation of different ratios of bergamot essential oil, through the analysis of different physical and structural properties of the FFD and films. The antifungal properties of the films against *Penicillium italicum* were also evaluated. This fungus is one of the major causes of citrus fruit decay (blue mould) and films containing citrus essential oils could be used, without any impact on the fruit flavour, to prevent the microbial growth.

2. Materials and methods

2.1. Materials

High molecular weight chitosan (CH) with a deacetylation degree of 82.7% (Batch 10305DD, Sigma–Aldrich Química, Madrid, Spain), 98% glacial acetic acid (Panreac, Barcelona, Spain) and bergamot essential oil (BO) supplied by Herbes del Molí (Alicante, Spain) were used to prepare the film-forming dispersions.

2.2. Preparation of the film-forming dispersions

Chitosan (1% w/w) was dispersed in an aqueous solution of glacial acetic acid (0.5% w/w) at 25 °C. After stirring overnight, bergamot essential oil (BO) was added to chitosan (CH) solution to reach a final concentration of 0%, 0.5%, 1%, 2% and 3% (w/w). CH–BO mixtures were emulsified at room temperature (25 °C) using a rotor–stator homogenizer (Ultraturrax DI 25 basic-Yellowline, Janke & Kunkel, Staufen, Germany) at 13,500 rpm for 4 min. These emulsions were vacuum degasified at room temperature (25 °C) with a vacuum pump (Diaphragm vacuum pump, Wertheim, Germany). Sample nomenclature was CH–*n*BO, the *n* value being the ratio BO:CH in the film or FFD.

2.3. Characterization of the film-forming dispersions

The density of the FFD was measured by means of a digital densimeter DA-110M (Mettler Toledo, Barcelona, Spain). A pH-meter C831 (Consort, Tumhout, Belgium) was used to determine the pH of the FFD at 20 °C.

2.3.1. ζ-Potential measurements

In order to perform ζ-potential measurements, FFD were diluted to a droplet concentration of 0.02% BO using an aqueous solution of glacial acetic acid (0.5%w/w). ζ-Potential was determined using a Zetasizer nano-Z (Malvern Instruments, Worcestershire, UK). The Smoluchowsky mathematical model was used to convert the electrophoretic mobility measurements into ζ-potential values.

2.3.2. Particle size measurements

Particle size analysis of the FFD was carried out using a laser diffractometer (Mastersizer 2000, Malvern Instruments, Worcestershire, UK). The samples were diluted in deionised water at 2000 rpm until an obscuration rate of 10% was obtained. The Mie theory was applied by considering a refractive index of 1.52 and absorption of 0.1 for BO. Three samples of each FFD were measured in quintuplicate.

2.3.3. Rheological behaviour

The rheological behaviour of FFD was analysed in triplicate at 25 °C by means of a rotational rheometer (HAAKE Rheostress 1, Thermo Electric Corporation, Karlsruhe, Germany) with a sensor system of coaxial cylinders, type Z34DIN Ti. Rheological curves were obtained after a stabilization time of 5 min at 25 °C. The shear stress (σ) was measured as a function of shear rate ($\dot{\gamma}$) from 0 to 512 s^{-1}, taking 5 min to reach the maximum shear rate and another 5 min to attain zero shear rate. The power law model (Eq. (1)) was applied to determine the consistency index (K) and the flow behaviour index (n). Apparent viscosities were calculated at 100 s^{-1}.

$$\sigma = K \cdot \dot{\gamma}^n \tag{1}$$

2.4. Preparation of films

A casting method was used to obtain films. FFD were poured onto a framed and levelled polytetrafluorethylene (PTFE) plate (ϕ = 15 cm) and were dried in atmospheric conditions (25 °C, 60% relative humidity) for 48 h. Film thickness was controlled by pouring the amount of FFD that will provide a surface density of solids in the dry films of 56 g/m^2 in all formulations. Dry films were peeled off the casting surface and preconditioned in desiccators at 20 °C and 54.4% relative humidity (RH) prior to testing. A hand-held digital micrometer (Palmer-Comecta, Spain, ±0.001 mm) was used to measure film thickness at three different points of the same sample at least.

2.5. Water vapour permeability

Water vapour permeability (WVP) was measured in dry film discs (ϕ = 7 cm), which were equilibrated at 54.4% RH and 20 °C, according to the “water method” of the ASTM E-96-95 (ASTM, 1995), using Payne permeability cups (Elcometer SPRL, Hermelle/s Argenteau, Belgium). Deionised water was used inside the testing cup to achieve 100% RH on one side of the film, while an oversaturated magnesium nitrate solution was used to control the RH on the other side of the film. During WVP testing, the side of the film in contact with the PTFE plate was placed in contact with that part of the test cup having the highest RH. This situation tries to simulate the case of a film applied on the wet surface of a fresh cut vegetable or fruit. A fan placed on the top of the cup was used to reduce resistance to water vapour transport. Water vapour transmission rate measurements (WVTR) were performed at 20 °C. To calculate WVTR, the slopes of the steady state period of the curves of weight loss as a function of time were determined by linear regression. For each type of film, WVP measurements were replicated three times and WVP was calculated according to Villalobos, Hernández-Muñoz, and Chiralt (2006).

2.6. Mechanical properties

Mechanical properties were measured using a Texture Analyser TA-XT-plus (Stable Micro Systems, Surrey, UK), with a 50 N load cell equipped with tensile grips (A/TG model). Sample films, previously equilibrated at 54.4% RH and 20 °C, were cut into 25.4 mm wide and 100 mm long strips, according to the ASTM D-882 standard (ASTM, 2001). Grip separation was set at 50 mm and cross-head speed was 50 mm/min. Tensile strength (TS) and percentage of elongation (E%) at break, and elastic modulus (EM) were evaluated in eight samples from each type of film.

2.7. Optical properties

Gloss was measured using a flat surface gloss meter (Multi-Gloss 268, Minolta, Langenhagen, Germany) at an angle of 60° with respect to the normal to the film surface, according to the ASTM standard D523 (ASTM, 1999). Prior to gloss measurements, films were conditioned in desiccators at 20 °C and 54.4% RH. Gloss measurements were performed over a black matte standard plate and were taken in quintuplicate. Results were expressed as gloss units, relative to a highly polished surface of standard black glass with a value close to 100.

The transparency of the films was determined through the surface reflectance spectra in a spectrocolorimeter CM-3600d (Minolta Co., Tokyo, Japan) with a 10 mm illuminated sample area. Measurements were taken from three samples in each formulation using both a white and a black background. The transparency was determined by applying the Kubelka–Munk theory for multiple scattering to the reflection spectra. As each light flux passes through the layer, it is affected by the absorption coefficient (K) and the scattering coefficient (S). Transparency (K/S) was calculated, as indicated by Hutchings (1999), from the reflectance of the sample layer on a white background of known reflectance and on an ideal black background.

2.8. Microbiological analysis

2.8.1. Fungal strain

Stock culture of *P. italicum* (CECT 2294), supplied by Colección Española de Cultivos Tipos (CECT, Burjassot, Spain), was kept frozen (−25 °C) in Potato Dextrose Broth (Scharlab, Barcelona, Spain) supplemented with 30% glycerol (Panreac, Barcelona, Spain). The fungus was inoculated on Potato Dextrose Agar (PDA) and incubated at 25 °C until sporulation. The inoculums' concentration was adjusted by means of a haemocytometer at 10^5 spores per mL.

2.8.2. Antifungal effectiveness of films

The methodology followed for the determination of antimicrobial effectiveness of films was adapted from Kristo, Koutsoumanis, and Biliaderis (2008).

Aliquots of PDA (20 g) were poured into Petri dishes. After solidification of the culture medium, diluted spore solution was inoculated on the surface and different test films (containing or not antimicrobial substance) of the same diameter as the Petri dishes were placed on the inoculated surface. Inoculated uncoated PDA was used as control. Plates were then covered with parafilm to avoid dehydration and stored at 20 °C for 12 days. *P. italicum* counts on PDA plates were examined immediately after the inoculation and periodically during the storage period.

The agar was removed aseptically from Petri dishes and placed in a sterile plastic bag with 100 mL of tryptone phosphate water (Scharlab, Barcelona, Spain). The bag was homogenized for 2 min in a Stomacher blender (Bag Mixer 400, Interscience). Serial dilutions were made and then poured onto PDA. Plates were incubated for 5 days at 25 °C before colonies were counted. All tests were run in triplicate.

2.9. Statistical analysis

Results were analysed by multifactor analysis of variance with 95% significance level using Statgraphics®Plus 5.1. Multiple comparisons were performed through 95% Least Significant Difference intervals (LSD).

3. Results and discussion

3.1. Characterization of the film-forming dispersions

3.1.1. Density, ζ-potential and particle size distribution

Density and *ζ-potential* values of the different FFD are reported in Table 1. The incorporation of bergamot oil (BO) led to the expected decrease in the density of the film-forming dispersions (FFD). The values found for chitosan FFD were in the range of those reported by other authors at the same pH (Vargas, Albors, Chiralt, & González-Martínez, 2009) and of those predicted on the basis of the FFD composition, taking into account density of CH: ~1450 kg/m³ (Park & Zhao, 2004) and the value for bergamot oil: 897 kg/m³. The

Table 1
Density (ρ), Ostwald de Waale rheological parameters, apparent viscosity (η_{ap}), mean particle size (d_{43}) and ζ-potential values of film-forming dispersions at 25 °C. Mean values and standard deviation.

FFS	ρ (kg/m³)	$0 \leq \dot{\gamma} \leq 512\,s^{-1}$			$r^{2\,a}$	d_{43} (μm)	ζ (mV)
		n	k (Pa s)^n	η_{ap} (100 s^{-1}) (Pa s)			
CH	1004.69 (0.14)a	0.785 (0.007)a	0.58 (0.02)a	0.217 (0.002)a	0.975	–	101 (3)a
CH–0.5BO	1003.888 (0.005)b	0.777 (0.002)b	0.554 (0.116)a	0.179 (0.019)b	0.996	7.1(0.4)a	82 (3)b
CH–1BO	1002.55 (0.05)c	0.7936 (0.0116)ac	0.38 (0.04)b	0.150 (0.008)c	0.997	15.1(0.4)b	80.3 (1.4)bc
CH–2BO	1001.26 (0.17)d	0.82 (0.02)cd	0.29 (0.06)bc	0.139 (0.015)cd	0.997	22.1(0.2)c	77.60 (1.02)c
CH–3BO	1000.319 (0.107)e	0.842 (0.019)d	0.24 (0.05)c	0.122 (0.015)d	0.997	28.6(0.4)d	73.9 (1.8)d

Different letters (a, b, c, d, e) within a column indicate significant differences among formulations ($p < 0.05$).

[a] Correlation coefficient of the fitting.

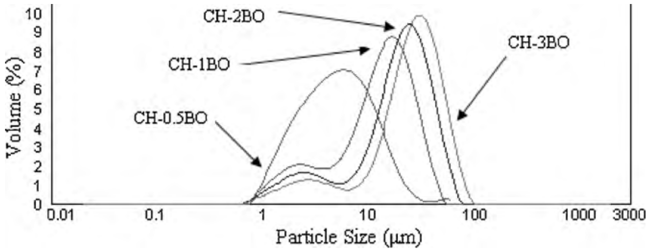

Fig. 1. Particle size distribution of CH–BO film-forming dispersions.

pH of FFD was around 4.3 at ambient temperature and did not vary significantly ($p < 0.05$) with the incorporation of BO.

The typical average particle size distributions of these FFD are plotted in Fig. 1. Particle size distributions were bimodal except for the formulation containing the lowest BO content, which showed a monomodal trend towards smaller particles.

The increase in BO content significantly ($p < 0.05$) increased the mean particle size and decreased the ζ-potential of the particles ($p < 0.05$) when compared to the values of the CH dispersion. Thus, the increase of BO content led to bigger droplets with lower electrical net charge. This is also reflected in Table 1, where the mean diameter was expressed as volume-length diameter (d_{43}). The reduction of the electrical net charge of CH–BO particles (decrease in ζ-potential) when BO content increased could be explained by the presence of electrostatic interactions between CH and BO components. At this pH, the amino group of chitosan (pKa NH^{3+}/NH_2, ≈6.5) is positively charged and could be partially neutralized through the interactions at the O/W interface with some polar groups of bergamot oil components, such as alcohols (linaool, methanol, isopropanol, etc.). To corroborate this assumption, an aqueous dispersion of BO (1% v/w) was prepared in distilled water and pH (4.2); particle size and ζ-potential were determined. In the absence of chitosan, BO droplets showed negative charge (ζ-potential = −61 ± 3 mV) and showed a monomodal distribution, with a significantly lower mean particle size ($d_{43} = 3.67 \pm 0.05$ μm) than that reported for CH–1BO FFD. Negative charge is usual in oil dispersions due to the predominant adsorption of the negative ions present in the aqueous system. Therefore, the hypothesis for the structure of CH–BO system is that chitosan chains are adsorbed on the BO droplets, leading to bigger, positively charged particles. This theory could also explain the presence of bimodal distributions when there is a considerable amount of BO (over 0.5%) as there is not enough chitosan to cover all oil particles, leading to some degree of droplet flocculation (bridging flocculation). Nevertheless, the steric stabilization promoted by the chitosan interfacial adsorption and viscosity of the system ensures the stability of the emulsified system (Roland, Piel, Delattre, & Evrard, 2003).

3.1.2. Rheological characterization

All FFD showed a shear-thinning behaviour ($n < 1$) and no thyxotropic effects were observed from the comparison of the up and down curves. Rheological data were fitted to the Ostwald de Waale model and the model parameters are shown in Table 1, together with the apparent viscosity (η_{ap}) values at a shear rate of 100 s^{-1}. Rheological parameters and apparent viscosity at 100 s^{-1} for pure chitosan FFD are in the order of those found by No, Kim, Lee, Park, and Prinyawiwatkul (2006), Rodriguez, Albertengo, and Agullo (2002) and Vargas et al. (2009) in chitosan solutions and chitosan–oleic acid dispersions, respectively.

The apparent viscosity values significantly ($p < 0.05$) decreased when the BO content increased, which was not how oil in water emulsions are expected to behave, as viscosity usually increases when the concentration of the dispersed phase increases. Nevertheless, in this case there are two contributions to the rheological behaviour of the BO emulsions: the viscosity imparted by CH molecules dissolved in the aqueous phase and the viscosity imparted by dispersed BO droplets. As deduced from droplet size and ζ-potential values, CH molecules adsorb on BO droplets, thus reducing their viscous contribution in the continuous phase. In this sense, the increase in the BO ratio led to a greater reduction not only of the CH available in the aqueous phase but also of the particle charge, while increasing the mean particle size. All these effects can explain the viscosity decrease when BO ratio increased in the emulsion. Droplet charge influences the rheology due to electroviscous effects (Larson, 1999; Pal, 1996, Chap. 3; Rubio-Hernández, Carrique, & Ruiz-Reina, 2004). So, the particles with a higher charge will lead to more viscous systems. This can be explained by the greater attraction of the cloud of counter ions surrounding the charged droplets, which makes the droplet movement more difficult and by the greater effective diameter of the droplets as they cannot get so close together due to electrostatic repulsions (McClements, 2005).

3.2. Characteristics of the films

3.2.1. Mechanical behaviour

Table 2 shows the mechanical characterization of the obtained films, in terms of percentage of elongation at break (E%), tensile strength (TS) and elastic modulus (EM), equilibrated at 54.4% and 20 °C. TS indicates the maximum tensile stress that the film can sustain, E% is the maximum change in length of a test specimen before breaking, and EM is a measure of the stiffness of the film.

Values of mechanical properties obtained for CH films agreed with those found by other authors when using similar chitosan–acetic acid ratio (Zivanovic, Chi, & Draughon, 2005: TS 105 MPa, E% 5%) but differed from those obtained in other works (Vargas et al., 2009: TS 12 MPa, E% 17%; Srinivasa, Ramesh, & Tharanathan, 2007: TS 39.10 MPa, E% 10.84%). The source of chitosan, the acid medium used to dissolve the polymer and the experimental conditions (pH, concentration, RH used during equilibration of the film, presence of emulsifiers) can explain the observed differences.

The incorporation of the BO dispersed phase leads to softer, less resistant to break and less stretchable films, almost regardless of the BO concentration added, since no significant differences were found between mechanical parameters of the different composite

Table 2

Elongation (E), tensile strength (TS), elastic modulus (EM), water vapour permeability (WVP) (100/54.4 RH gradient), equilibrium water content (We) estimated with GAB parameters ($a_w = 0.77$) and film thickness of CH and CH–BO composite films at 20 °C. Mean values and standard deviation.

Film	E (%)	TS (MPa)	EM (MPa)	WVP (g Pa^{-1} s^{-1} m^{-1}) × 10^{11}	We (g H_2O/g d.m.)	Thickness (μm)
CH	22 (5)a	113 (20)a	2182 (277)a	124 (12)a	0.235	52 (2)a
CH–0.5BO	7 (4)b	65 (10)b	766 (205)b	130.2 (0.3)a	0.189	56 (2)a
CH–1BO	5.5 (0.7)b	63 (21)b	799 (163)b	86 (5)b	0.174	41 (2)b
CH–2BO	6 (2)b	50 (8)bc	747 (225)b	92 (9)b	0.137	36 (3)c
CH–3BO	1.7 (0.4)b	22 (8)c	682 (196)b	65 (2)c	0.123	32 (2)c

Different letters (a, b, c) in the same column indicate significant differences among formulations ($p < 0.05$).

films. The load parameters (TS and EM) decreased more than 50% and the percentage of elongation at break was also dramatically reduced from 22% to 5%, in comparison with the pure chitosan films. This could be explained by discontinuities in the polymer matrix introduced by the BO incorporation and by changes in the polymer chain interactions when oil components are present, which lead to a weak mechanical response. Srinivasa, Ramesh, & Tharanathan (2007) and Vargas et al. (2009) found similar behaviour when analysing the mechanical properties of chitosan films blended with different fatty acids. On the other hand, Zivanovic et al. (2005) also found that the tensile strength decreased when introducing some essential oils into CH films but no changes in the elongation percentage were found. This different behaviour can be attributed to the type of chitosan (solvent and molecular weight) and particular interactions with the essential oil components which, in turn, are affected by relative humidity, the presence of surfactants, temperature, etc.

3.2.2. Water vapour permeability

The water vapour permeabilities (WVPs) of the films at 100/54.4 RH gradient and 20 °C are also reported in Table 2. The RH gradient was chosen to simulate the environmental conditions of coatings applied to fruit or vegetables stored at 20 °C. The thickness of pure and composite films is reported in Table 2. The composite films are not as thick as the pure CH films, suggesting the existence of a different arrangement of components (CH surrounding oil droplets), as commented on above. In pure CH or composite films containing low amounts of BO, the hydration layers of polymer will contribute to increase the film thickness, giving rise to a swelling effect and, subsequently, thicker films. Nevertheless, when the polymer ratio is reduced a smaller number of hydration water layers will be present and so film thickness is reduced.

Lipid compounds are known to enhance the water barrier properties of polymer based films due to their hydrophobic nature and to decrease the water sorption capacity of the films. This can be observed in Table 2, where the values of the equilibrium water content of the films are shown. Nevertheless, due to the greater polarity of essential oil components, this trend was only significant ($p < 0.05$) in CH–BO composite films when the BO ratio in the film was higher than 0.5. The WVP value decreased by 50% when using a BO–CH ratio of 3:1. The WVP values for the 100/54.4 RH gradient were in the range of those reported by Park and Zhao (2004) and Vargas et al. (2009) (160×10^{-11} g Pa^{-1} s^{-1} m^{-1} at 5 °C for pure chitosan) in chitosan–oleic acid films. No correlation was found between the WVP values and the equilibrium water content of the films (Table 2). A linear decrease of WVP as a function of the BO ratio in the film was observed (Fig. 2) which is coherent with the reduction of the hydrophilic phase (polysaccharide) ratio where water molecules diffuse preferentially. The oil phase introduces an increase in the tortuosity factor for water transfer in the matrix, thus increasing the distance travelled by water molecules diffusing through the film. The tortuosity factor is higher when the oil phase ratio increases or when the oil particle size is reduced (Pérez-Gagó & Krochta, 2001).

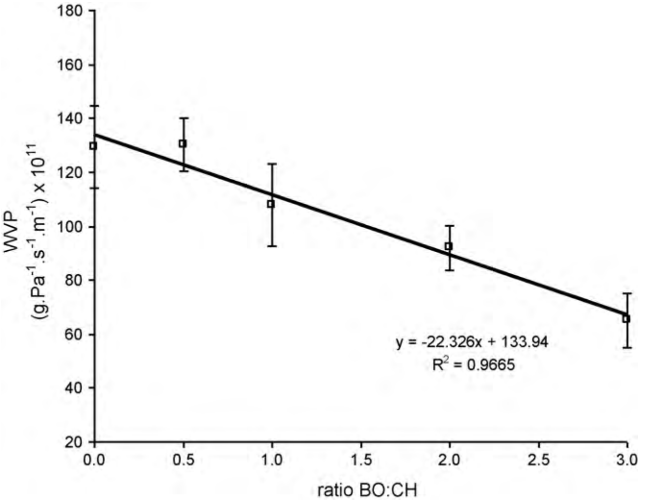

Fig. 2. Water vapour permeability of films at 20 °C (100/54.4 RH gradient) as a function of the BO ratio in the film matrix. Mean values and 95% LSD intervals.

3.2.3. Optical properties

The optical properties of the films were evaluated through their gloss and transparency since these properties have a direct impact on the appearance of the coated product. Film transparency was evaluated through the Kubelka–Munk *K*/*S* coefficient (Fig. 3a) at 450 nm, defined as the ratio between light absorption and scattering. Usually, an increase in *K*/*S* can be assumed as an increase in transparency (Hutchings, 1999). The gloss values of the films were measured at an incidence angle value of 60°. As can be observed in Fig. 3, relevant changes in the transparency and gloss of the films were detected due to BO incorporation into the CH matrix. BO–CH composite films become less opaque and less glossy and are hardly affected by the amount of BO. The different transparency level is related with the internal structure of the films. Chitosan films are considered to be constituted by densely packed chains which make them opaque. This structure is altered by the presence of the oil dispersed phase, where the lipid droplets are interrupting the continuous matrix of chitosan chains, leading to more open structure and so films become more transparent. This effect has been also observed in CH films containing oleic acid (Vargas et al., 2009).

On the other hand, the gloss of the films is linked to the morphology of their surface. In general, the smoother the surface, the glossier they are (Ward & Nussinovitch, 1996). The decrease of the

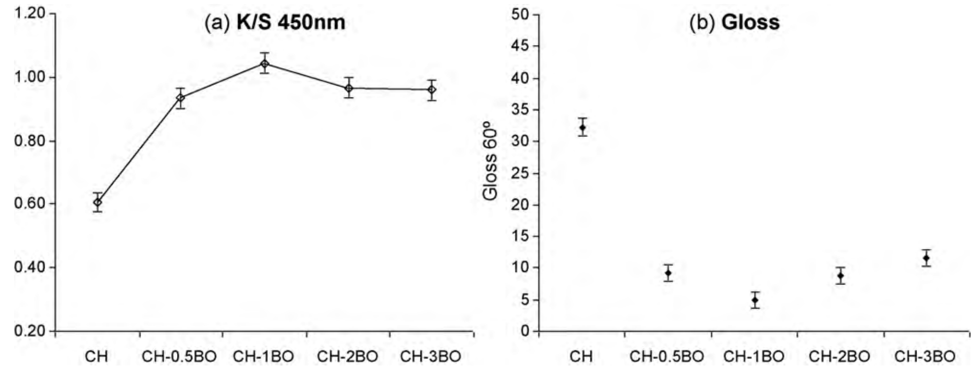

Fig. 3. (a) *K*/*S* values at 450 nm and (b) gloss at 60° of CH and CH–BO composite films at 54.4% relative humidity and 20 °C. Mean values and 95% LSD intervals.

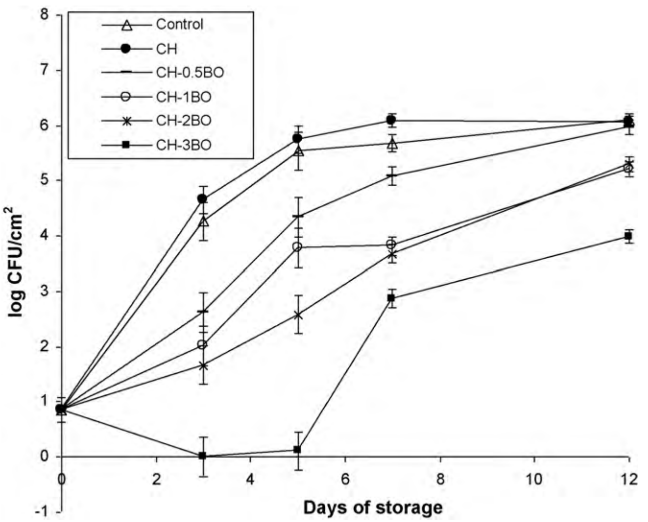

Fig. 4. Effect of CH and CH–BO composite films on the growth and survival of *Penicillium italicum* on PDA medium stored at 20 °C. Mean values and 95% LSD intervals.

gloss of the composite films could be related to the presence of discontinuities in the film matrix surface (oil dispersed droplets), which increase the surface roughness and lead to a decrease in the specular reflectance in the air–film interface. Similar behaviour was found by Sánchez-González, Vargas, González-Martínez, Chiralt, and Cháfer (2009) in HPMC-TTO composite films.

3.2.4. Antifungal activity

The possible antifungal effect against *P. italicum* at 20 °C of CH and composite films was determined on PDA medium and is shown in Fig. 4. Although some evaporation of BO components can occur during the preparation and drying of the films, the initial BO concentration in the FFD was considered for the discussion of the obtained results, by assuming that proportional losses will occur in the films containing different ratios of BO. Pure chitosan films, with no BO, served as a control to determine the potential antimicrobial effect of chitosan films per se, but CH films were found not to be effective against this fungus. Chitosan has been proven to have antimicrobial properties against numerous groups of microorganisms, mainly bacteria, but also against some moulds and yeasts (Tharanathan & Kittur, 2003). El Ghaouth, Arul, Grenier, and Asselin (1992) observed a fungistatic effect of chitosan against *Botrytis* and *Rhizopus* rather than a fungicidal. Nevertheless, some microorganisms have been shown to be insensitive to chitosan activity. In this sense, Roller and Covill (1999) found, in similar film studies, that the growth of *Aspergillus flavus*, *Cladosporium cladosporioides* and *Penicillium aurantiogriseum* was not affected by the presence of chitosan, either. Neither did Zivanovic et al. (2005) observe any inhibition of the tested bacteria (*E. coli* and *L. monocytogenes*) by the chitosan films.

The composite films showed an inhibitory effect on the growth of *P. italicum*, which depended on the BO concentration. The films containing the highest BO content (3:1 BO–CH ratio) exhibited the strongest power of inhibition. No fungal growth was observed throughout the first 5 storage days. Du Plooy, Regnier, and Combrinck (2009) also observed an inhibitory effect of different essential oils against *P. digitatum* in *in vitro* and *in vivo* assays. Nevertheless, the power of inhibition of the composite films decreased throughout the storage time. This behaviour has also been observed by other authors and has been explained by taking into account the changes in the availability of the antimicrobial compounds throughout time. In this sense, Cutter and Siragusa (1997), Ouattara, Simard, Piette, Begin, & Holley, 2000, Sebti and Coma (2002), Sebti, Carnet, Blanc, Saurel, and Coma (2003) and Kristo et al. (2008) observed that these antimicrobial compounds were more effective when applied on the product surface as a component of a film or gel than when sprayed on as a solution or directly spread on the product. This is because the active substances can evaporate or diffuse more easily into the culture medium if they are not embedded in a polymeric matrix. So, the antimicrobial effect of BO could be limited by the diffusion of the active compounds into the agar medium, decreasing their availability on the medium surface where the microorganisms are predominantly located, thus increasing the rate of microbial growth (Kristo et al., 2008; Ouattara et al., 2000).

4. Conclusion

The incorporation of bergamot essential oil into chitosan films offers the possibility not only of imparting antimicrobial activity against *P. italicum*, but also of improving the film's water vapour barrier properties. The most intense, longest-lasting antimicrobial effect against *P. italicum* was obtained in films with the maximum bergamot oil content (3:1 BO–CH ratio), which showed a reduction of 2 logarithm units, as compared with the control film, at the end of the storage. So, these films could be used to extend the shelf life of fruits, by controlling moisture losses and fungal decay, with no notable changes in the fruit appearance. Nevertheless, *in vivo* studies are necessary to take the possible interactions between the film constituents and the food matrix into account.

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