Perdones Montero, A.; Vargas, M.; Atarés Huerta, LM.; Chiralt Boix, MA. (2014). Physical, antioxidant and antimicrobial properties of chitosan cinnamon leaf oil films as affected by oleic acid. Food Hydrocolloids. 36:256-264. doi:10.1016/j.foodhyd.2013.10.003.

Physical, antioxidant and antimicrobial properties of chitosan-cinnamon leaf oil films as affected by oleic acid

Ángela Perdones, Maria Vargas*, Lorena Atarés, Amparo Chiralt

Instituto Universitario de Ingeniería de Alimentos pare el Desarrollo – Universitat Politècnica de València, Spain. mavarco@tal.upv.es

Abstract

The physical, antioxidant and antimicrobial properties of chitosan (CH)-cinnamon leaf essential oil (C) films, containing or not oleic acid (OA), were evaluated. The addition of OA led an increase in surface charge and particle size of the film-forming dispersions. This is in agreement with a greater CH adsorption on the droplets containing OA and the entrapment of C compounds in the non-polar core of the OA molecule associations. OA contributed to a better retention of C in the film during its drying, diminished the changes in colour parameters provoked by C addition and reduced the film transparency. Water vapour permeability of CH films was reduced by OA incorporation while it increased when they contained only C. Every film containing C showed antioxidant and antifungal properties, depending on the C content (the higher the C content, the greater the effect). OA reduced the antifungal effectiveness of C containing films in line with its encapsulating effect on C compounds. All the coatings were effective in extending the shelf-life of cold-stored strawberries, mainly when CH was combined with C at the ratio 1:0.5.

Keywords: coating; TEAC; antifungal; strawberry

1. Introduction

Chitosan is a biopolymer that has film-forming ability and shows antimicrobial activity (No, Park, Lee & Meyers, 2001; Tharanathan & Kittur, 2003). One of the most important drawbacks to the application of chitosan films to fresh food products is their poor water vapor barrier properties (Vargas, Sánchez-González, Chiralt, Cháfer & González-Martínez, 2012), which can be improved by the incorporation of lipid compounds, such as oleic acid (Vargas, Pastor, Chiralt, McClements & Gonzalez-Martinez, 2008).

Essential oils (EOs) are natural antioxidant and antimicrobial substances, extracted from vegetables. Most of them consist of a mixture of terpens, terpenoids and other aromatic and aliphatic compounds (Bakkali, Averbeck, Averbeck & Idaomar, 2008), but their composition can vary markedly depending on the origin. Cinnamon leaf essential oil has shown not only antifungal and antibacterial properties against a broad spectrum of food spoilage microorganisms but also antioxidant activity (Singh, Maurya, de Lampasona & Catalan, 2007). The main compound of cinnamon leaf essential oil is eugenol (70-95%), followed by cinnamaldehyde which can be present in a proportion of 1 to 5% (Vangalapati, Satya Prakash & Avanigadda, 2012).

The use of EOs in food preservation is often limited because of their application costs and other drawbacks, such as their intense aroma and potential toxicity. An interesting approach to reduce the doses of essential oils, while maintaining their effectiveness, could be to incorporate these compounds into the formulation of edible coatings (Sánchez-González, Vargas, González-Martínez, Chiralt & Cháfer, 2011a). In this sense, Sánchez-González, Cháfer, Chiralt & González-Martínez, C. (2011b) developed antibacterial composite films based on chitosan and different EOs (lemon, tea tree or bergamot), which were proved to inhibit the growth of bacteria (*E. coli*, *L. monocytegenes* and *S. aureous*) in an *in vitro* study. Wang et al. (2011) prepared

chitosan films incorporated with cinnamon, clove and anise essential oils. Cinnamon oil-chitosan films exhibited a synergistic effect, which was related to the constant release of cinnamon essential oil.

The antifungal effect of bioactive coatings prepared with chitosan and essential oils, such as peppermint and lemon, on the fungal decay of cold-stored strawberries has recently been evaluated (Vu, Hollingsworth, Leroux, Salmieriv & Lacroix, 2011; Perdones, Sánchez-González, Chiralt & Vargas, 2012). Nevertheless, there are no published studies on the antifungal effect of chitosan-cinnamon essential oil composite coatings applied to strawberry. In addition, the studies into film development based on chitosan and cinnamon leaf oil are scarce.

The aim of this work was to characterize the film forming dispersions and physical, antioxidant and antimicrobial properties of chitosan-cinnamon leaf essential oil films, containing or not oleic acid. The potential application of such coatings to control the fungal decay of strawberries was also evaluated.

2. Materials and Methods

2.1. Reagents

High molecular weight chitosan (Batch MKBD1916V, 0.8 Pa·s viscosity, at 1% w/w in 1% w/w glacial acetic acid, acetylation degree: 22%), ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman- 2-carboxylic acid) and potassium persulfate was provided by Sigma-Aldrich Quimica (Madrid, Spain). Cinnamon leaf essential oil was supplied by Herbes del Moli (Alicante, Spain). Acetic acid, oleic acid and magnesium nitrate were purchased from Panreac Química, S.A. (Castellar del Vallés, Barcelona, Spain).

2.2. Preparation and characterization of the film-forming dispersions

Chitosan (CH) was dispersed at 1 wt% in an aqueous solution of acetic acid (1% v/w) and Tween 80 (0.1% w/w). After at least 8h of magnetic stirring, chitosan solution was vacuum-filtered. Cinnamon leaf oil (C) or oleic acid (OA) or both compounds were added at different concentrations as described in Table 1. Film-forming dispersions (FFDs) were prepared by means of a rotor-stator homogenizer (Ultraturrax DI 25 basic-Yellowline, Janke & Kunkel, Staufen, Germany). After homogenization, the formulations were degassed at room temperature and at 7 mbar with a vacuum pump (Wertheim, Germany).

2.2.1 Particle size distribution, zeta potential and rheological behaviour

The particle size analysis of the FFDs was carried out by means of a laser diffractometer (Mastersizer 2000, Malvern Instruments, Worcestershire, UK). The samples were diluted in the acetic acid solution (pH = 4.8) at 2,000 rpm until an obscuration rate of 10% was obtained. Mie theory was applied considering a refractive index of 1.47 and 1.50 for C and OA, respectively, and 0 absorption in both cases. Three replications per formulation were made. ζ-potential was measured in triplicate by Laser-Dopler electrophoresis performed with a Zetasizer nano-Z (Malvern Instruments, Worcestershire, UK). The electrophoretic mobility of the droplets was transformed into ζ-potential values using the Smoluchowsky model. The samples were diluted to a droplet concentration of 0.02% with an acetic acid solution (pH 4.8).

The rheological behaviour of FFDs was analyzed in triplicate at 25ºC using a rotational rheometer (HAAKE Rheostress 1, Thermo Electric Corporation, Karlsruhe, Germany) with a sensor system of coaxial cylinders, type Z34DIN Ti. Samples were left to rest for 5 minutes before the measurements were taken. The shear stress (σ) was obtained as a function of shear rate ($\dot{\gamma}$) between 0 and 300 s−1, taking 3 minutes for each (up and

down) cycle. Experimental data were fitted to the Ostwald de Waale model (Eq. 1) in order to determine the consistency (K) and the flow behaviour indexes (n).

$$\sigma = K \cdot \dot{\gamma}^{n} \qquad (1)$$

2.3. Preparation and characterization of the films

FFDs were casted in Teflon® plates (diameter = 15 cm), so as to keep CH amount constant in the dry films (28 g/m^2). The films were dried at room temperature and 60% relative humidity (RH) and were conditioned in desiccators with an oversaturated salt solution of magnesium nitrate at 20ºC or 5ºC. Film thickness was determined with a Palmer digital micrometer (Comecta, Barcelona, Spain) to the nearest 0.001 mm.

2.3.1. Microstructure

Microstructure was observed by SEM in cross-sectioned cryofractured film specimens, using a JEOL JSM-5410 (Japan) electron microscope. The films (2 samples per formulation) were equilibrated in P_2O_5 to eliminate water, cryofractured by immersion in liquid nitrogen, and then mounted on copper stubs perpendicularly to their surface. After gold coating, the images were captured using an accelerating voltage of 10kV.

2.3.2. Optical and mechanical properties

Gloss of the films was measured with a gloss meter (Multi Gloss 268, Minolta, Germany) on their shiny side, using a black matte background and at an incidence angle of 60º (ASTM D523, 1999). Nine replicates were made per each formulation. Results were expressed as gloss units, relative to a highly polished surface of black glass standard with a value near to 100.

Colour of the films was determined through the surface reflectance spectra with a spectrocolorimeter CM-3600d (Minolta Co, Tokyo, Japan) with a 10 mm illuminated sample area. Measurements were taken from nine replicates per formulation by using

125 both a white and a black background and Kubelka-Munk theory for multiple scattering
126 was applied to the sample reflection spectra. Internal transmittance (Ti) was calculated
127 from the reflectance of the sample layer backed by a known reflectance and the
128 reflectance of the film on an ideal black background (Hutchings, 1999). Moreover, CIE-
129 L^* a^* b^* coordinates, (CIE, 1986) were obtained by the infinite reflection spectra of the
130 samples, using D65 illuminant/10º observer in order to calculate the whiteness index
131 (WI) of the samples (Eq. 2).

132 $$WI = 100 - \left((100 - L^*)^2 + a^{*2} + b^{*2}\right)^{0.5} \quad \text{(Eq. 2)}$$

133 Mechanical properties were analysed by means of tensile tests (ASTM D882, 2001), to
134 obtain the true stress (σ) vs. Hencky strain (ε_H) curves. The mechanical parameters:
135 elastic modulus (EM), tensile strength at break (TS) and elongation percentage at break
136 (%E) were obtained. A Universal Testing Machine (TA.XT plus model, Stable Micro
137 Systems, Haslemere, England) with a 500 N load cell was used to perform the tests.
138 Film specimens were mounted in the film-extension grips and stretched at 50 $mm \cdot min^{-1}$
139 until breakage. Nine to twelve replicates of each formulation were tested.

140 2.3.3 Water vapour and oxygen permeability

141 Water vapour permeability (WVP) was determined gravimetrically at 5ºC and 20ºC and
142 58-100% and 54-100%, RH gradient, using a modification of the ASTM E96-95
143 gravimetric method (1995) for hydrophilic films (Gennadios, 1994). Payne permeability
144 cups of 3.5 cm in diameter (Elcometer SPRL, Hermelle/s Argenteau, Belgium) were
145 filled with 5 mL of distilled water (100% RH). The films were secured and the cups
146 were placed in pre-equilibrated cabinets fitted with a fan, at 5ºC or 20ºC. The RH of the
147 cabinets was held constant at 58% or 54% RH using oversaturated solutions of
148 $Mg(NO_3)_2$. The cups were weighted periodically after the steady state had been reached.

WVP was calculated with the equations described by Vargas, Perdones, Chiralt, Cháfer & González-Martínez (2011). Six replicates per formulation were made.

The oxygen permeability (OP) was measured according to the ASTM D3985-05 (2002). The oxygen barrier performance of the films was evaluated by measuring the oxygen transference rate with an Ox-Tran 1/50 system (Mocon, Minneapolis, USA) at 10ºC or 20°C. One side of the film was exposed to pure nitrogen and the other to pure oxygen flow. The tests were performed in continuous mode at 58% or 54%RH, depending on the temperature. OP was calculated by dividing the oxygen transmission rate by the difference in oxygen partial pressure between the two sides of the film, and multiplying by the average film thickness. Two replicates per formulation were made.

2.3.4. Antioxidant activity

The Trolox Equivalent Antioxidant Capacity (TEAC) of cinnamon essential oil and chitosan-based films was determined using a modification of the original TEAC method (Re et al., 1999). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), which is a vitamin E analogue, was used as a standard of antioxidant capacity. A 7mM aqueous solution of ABTS (2,2'-azinobis(3-ethylbenzothiazoline- 6-sulfonic acid) diammonium salt) was allowed to react with a 2.45 mM potassium persulfate solution in the dark, for 16 h. During this period, ABTS radical cation ($ABTS^{\bullet+}$), a blue chromophore, was produced. The tests were performed with aqueous dilutions of the $ABTS^{\bullet+}$ solution whose absorbance at 734 nm was 0.70 (± 0.02). The films (0.075 g) were diluted in 25 ml of an acetic solution (1% v/w) prior to the determination. This dilution was adjusted so that the addition of a 10 μl-aliquot to 990 μl of $ABTS^{\bullet+}$ dilution would produce a 20–80% absorbance decrease within 6 min. Absorbance at 734 nm was registered every minute during the test. For calibration, Trolox standards of between 60

and 500 mg/l were prepared following the procedure described above. The TEAC of cinnamon essential oil and film samples was determined by comparing the corresponding percentage of absorbance reduction to the concentration–response curve and was expressed as μg of sample that show the same antioxidant activity as 1mM of Trolox. All the determinations were carried out at least six times using a spectrophotometer (Beckman Coulter DU 730, England) and distilled water as the blank.

2.4. Antifungal assays

The antifungal effect of the stand-alone films and the FFDs was evaluated in vitro and in vivo against *Aspergillus niger* (CECT20156), *Botrytis cinerea* (CECT20516) and *Rhizopus stolonifer* (CECT2344) which were supplied by Colección Española de Cultivos Tipo (Burjasot, Spain). The stock culture were kept frozen (–25 ºC) in Potato Dextrose Agar (Scharlab, Barcelona, Spain) supplemented with 30% glycerol (Panreac, Barcelona, Spain) and was inoculated on potato dextrose agar (PDA) and incubated at 25 ºC until sporulation.

To perform the *in vitro* assays, the surface of PDA plates was seeded with 0.1 mL of a spores suspension (10^4 spores/mL). Pieces of film (diameter = 2.4 cm) were placed at the centre of the PDA plates, which were incubated at 25ºC for 6 days or 14 days (*Botrytis cinerea*). The diameter of the zone of growth inhibition was measured at different incubation times with a digital calliper to the nearest 0.01 mm. The activity of cinnamon essential oil was evaluated by diluting the oil with dimethyl sulphoxide (Sigma-Aldrich Química, Madrid, Spain) to obtain different oil concentrations as described by Saccheti et al. (2005). A constant amount of the serial diluted essential oil solutions was deposited on sterile paper discs (diameter = 2.4 cm) that were placed in the centre of the inoculated Petri dishes as described above.

The *in vivo* studies were carried out using 25 strawberries per formulation, which were inoculated with *Rhizopus stolonifer* by sample immersion in a spore suspension (10^5 spores/mL). Experiments were performed in duplicate. Inoculated and non-inoculated samples were dipped in the FFDs for 1 minute. FFDs containing the highest lipid proportion were not tested to avoid the possible phytotoxic effects of cinnamon essential oil. Samples were dried by natural convection for 1 hour at 20ºC and were stored on perforated plastic trays, where the pieces did not come into direct contact with each other. Non-coated inoculated strawberries were used as control treatment. The trays were kept at 10ºC-70 RH% or 20ºC-80 RH% in a storage chamber (EC1400, Radiber, Barcelona, Spain). The strawberries that showed any sign of surface mycelia development were considered decayed. Results were expressed as percentage of infected strawberries. Total aerobial, moulds and yeasts and coliforms counts of strawberries inoculated with spore suspension of *Rhizopus stolonifer* and stored at 10ºC-70%RH were also determined as a function of storage time.

3. Results and discussion

3.1. Properties of film-forming dispersions (FFDs)

FFDs showed monomodal particle size distributions. Mean particle sizes (d_{43} and d_{32}) are shown in Table 2. Very small particles were obtained for all formulations, but their mean size increased for 1% lipid content when C was present. When OA-C blends were incorporated, an increase in the particle size was observed with respect to those containing only C. However, CH_1-OA_1 exhibited relatively small particles. This different behaviour could be due to the surfactant properties of OA which will lead to the formation of micellar structures in aqueous media, which does not occur for C compounds. These could be included in the non-polar core of the OA micelles, thus

leading to an increase in their size, mainly when the amount of C increases in the blend. All the FFDs had high positive values of ζ-potential, in accordance with the values detected for chitosan-based solutions in acid media (Vargas, Albors, Chiralt & González-Martínez, 2009). Every sample containing OA showed the same value of ζ-potential, suggesting that interactions of surfactant molecules with CH on the droplet surface lead to a similar degree of polymer adsorption, which is responsible for the positive charge. When OA was not present, ζ-potential values were lower, mainly for the greater ratio of C. This suggests that, in this case, CH adsorption on the droplet surface was more in agreement with the different lipid composition and affinity with CH chains.

FFDs showed shear-thinning, non-time dependent rheological behaviour. Flow curves were fitted to the Power Law model and rheological parameters are shown in Table 2. Apparent viscosities ranged between 90 and 148 mPa·s depending on their composition. CH_1:OA_1 was the most viscous and showed the lowest flow behaviour index (Vargas et al. 2009; Perdones et al. 2012), while samples CH_1:C_1 are the least viscous with the higher flow behaviour index. Differences in flow behaviour can be explained in terms of the differences in total volume of dispersed phase, particle size distribution and droplet surface charge (McClements, 2005). In this sense, it is remarkable that dispersions with 1% lipid were more viscous when they showed higher zeta-potential, while the opposite effect was observed for 0.5% lipid.

The observed differences in the FFDs properties will affect the stability of the systems during the film formation. The water loss during film drying could lead to droplet flocculation, coalescence and creaming phenomena which in turn will affect the final microstructure of dry film. In this sense, the FFD with higher zeta potential, lower particle size and greater viscosity will be the most stable during film drying step. From

249 the obtained values, CH_1:C_1 FFD could suffer destabilization phenomena to a greater
250 extent.
251
252 3.2. Properties of the stand-alone films
253 3.2.1. Microstructure and thickness
254 SEM images of the cross-sections of films are shown in Figure 1. Pure CH film was
255 homogenous with a compact and regular continuous matrix. When C, OA or OA:C
256 blends are incorporated to the CH matrix, lipid particles can be distinguished and a
257 coarse microstructure can be appreciated, especially at the highest lipid content.
258 Samples containing 1% of lipid (OA or OA:C blend) showed the coarsest matrix.
259 Samples with 1% C showed finer structure probably due to a great loss of essential oil
260 during the film drying step, which lead to a less rich lipid film than that expected from
261 the initial ratios. Similar results were observed in chitosan-bergamot essential oil films
262 (Sánchez-González, Chafer, González-Martínez, Chiralt, & Desobry, 2011c). The
263 partial loss of cinnamon essential oil seems to be limited in the OA:C blends, which is
264 coherent with the values of the film thickness (Table 3); for the same theoretical surface
265 solid density, thickness was lower when OA was not present in the formulation.
266 The thickness of the films is shown in Table 3. All composite films were thicker than
267 CH films, which is coherent with their greater solid content per surface unit, since all
268 the films were prepared by keeping the amount of polymer per surface area constant.
269 CH_1:C_1 films were significantly thinner than CH_1:OA_1, which points to the partial
270 evaporation of the volatile compounds of the essential oil during film drying and
271 coincides with that observed by Sánchez-González, Cháfer, Chiralt & González-
272 Martínez (2010). The addition of OA seems to contribute to retain a greater amount of
273 cinnamon leaf EO into the film matrix. In fact, although the detected differences were

not statistically significant, a clear tendency was observed: CH:OA:C films were thicker than CH:C for the same total lipid amount. The quantification of C losses during film drying trough gravimetric balance reveals losses of: 39%, 46%, 37%, 33% with respect the initial added amount for $CH_1:C_1$, $CH_1:C_{0.5}$, $CH_1:OA_{0.5}:C_{0.5}$, $CH_1:OA_{0.25}:C_{0.25}$ films, respectively. The corresponding losses referred per 100 g of dry chitosan (constant basis in all films) were 39%, 23%, 18%, 8%. The results showed that the addition of higher amount of C led to higher absolute losses during film drying. Nevertheless, when comparing films with the same amount of C with or without oleic acid, a greater retention of cinnamon EO in the film matrix was observed, which pointed out to a certain degree of encapsulation effect of OA.

3.2.2. Mechanical properties

As shown in Table 3, the addition of OA or C promoted a decrease in tensile strength at break and elastic modulus, as compared with pure CH films, as previously reported for other films containing lipids (Vargas et al. 2009; Perdones et al. 2012; Sánchez-González et al, 2011b). This has been related with the presence of discontinuities in the polymer matrix which reduce the matrix cohesion forces, thus implying a loss of mechanical resistance. In the case of pure OA, a significant reduction of the film extensibility was obtained, which seems to indicate that essential oil contribute to plasticize the polymer matrix, thus inhibiting the usual brittleness effect that the lipid discontinuities of the matrix cause. At both total lipid concentrations, the presence of OA led to an increase in the elongation and tensile strength at break, although the effects were not significant. EM values were not significantly affected by OA addition.

3.2.3. Optical properties

Optical properties of the films are shown in Table 4. Gloss of the films containing lipids did not significantly differ from that of the CH films, except for the samples

CH_1:$OA_{0.5}$:$C_{0.5}$ and CH_1:OA_1 which showed a significant decrease. Film gloss is related with its surface roughness which, in turn, is affected by the progress of coalescence and creaming phenomenon of the lipids to the film surface. In this sense, it is remarkable that samples containing the greater amount of total lipid (except CH_1:C_1 which loss a great ratio of lipid during film drying) in the film were the less glossy, in agreement with the greater progress of the destabilization phenomena during film formation.

The internal transmittance (Ti) spectra of the films are plotted in Figure 2. Pure CH and CH:OA spectra were similar in shape, although OA provoked a decrease in the internal transmittance of films. Cinnamon leaf essential oil promoted a selective decrease in the film internal transmittance between 460 and 520 nm due to the selective absorption of the coloured components of cinnamon essential oil. Ti curves of samples containing only C at different ratios are almost parallel, as occurs with samples containing C:OA blends. In both films, the higher the lipid ratio, the lower the Ti values. Nevertheless, it is remarkable that, for the same total lipid content, the lipid blend provoked a greater Ti decrease than pure C, which points to a greater capacity of OA to reduce film transparency. In fact, the lowest internal transmittance was detected for CH_1:OA_1 films.

From the reflectance spectra of an infinite thickness film, lightness (L^*), hue (h^*_{ab}) and chroma (C^*_{ab}) and whiteness index (WI) of each film were obtained as well as the total colour difference with respect to the pure CH film (ΔE) (Table 4). CH_1:OA_1 films showed higher lightness and WI than pure CH films, coherent with their lower internal transparency. The incorporation of increasing amounts of C led to a significant reduction of WI and lightness values, which was mitigated by OA addition. C yielded films with a yellower, more saturated colour (lower hue values and higher chroma). At both total lipid contents, the addition of OA reduced the colour changes induced by C and the total colour difference with respect to pure CH films.

3.2.4. Equilibrium moisture content and barrier properties

Equilibrium moisture content (EMC), WVP and OP values of films at 5ºC and 20ºC are shown in Table 5. As expected, the increase in EMC with the decrease in temperature was observed, which is in agreement with the endothermic nature of the adsorption phenomenon. Changes in temperature could also affect the crystallization degree of polymer chains due to differences in molecular mobility and so the water adsorption capacity of the CH matrix since this is different for amorphous and crystalline regions. Several authors reported the crystalline structure of CH in films. Zhang, Ding, Ping & Yu (2006) described the development of crystallinity in CH matrices due to the formation of hydrogen bonds between flexible chains. The more dominated polymorph corresponds to hydrated crystals where water molecules are incorporated in to the crystal lattice (Wan, Wu & Wen, 2006).

The incorporation of lipid into the film matrix seems to reduce its water adsorption capacity. Nevertheless, when the water content was referred to a free-lipid basis, two groups of samples can be observed at both temperatures. One group consisted of CH_1 and CH_1:OA_1 films, without significant differences in moisture content (free-lipid basis: 16.0 ± 0.6 % and 12.6 ± 0.6 % at 5ºC and 20ºC, respectively). The other group was composed by the films containing C, which showed slightly lower values (free-lipid basis: 15.0 ± 1.6 % and 10.1 ± 0.8 % at 5ºC and 20ºC, respectively). This result suggested that C molecules partially interact with CH chains blocking some active groups for water adsorption, especially at the highest temperature where hydrophobic interactions are promoted (Fabra, Talens & Chiralt, 2010).

The increase in temperature led to higher OP values whereas WVP values were significantly reduced. The decrease in barrier properties at higher temperatures is explained by the increase in molecular mobility and diffusion phenomena. Nevertheless,

the opposite effect observed for WVP could be explained by the effect of the higher equilibrium moisture content reached at 5ºC. An increase in water content also provokes higher molecular mobility, which favours mass transfer phenomena. This effect was also observed by Jiménez, Fabra, Talens & Chiralt (2010) in hydroxypropil-methylcellulose films containing fatty acids. At both temperatures, WVP values of CH films were significantly reduced by OA incorporation, which coincides with the results previously reported by Vargas et al. (2009). However, the addition of C significantly increased the WVP of the films ($p<0.05$). This suggests that particular interactions of the EO compounds with CH, as deduced from water sorption data, make the matrix more open to the transport of water molecules, despite the theoretical increase of the hydrophobic nature of the matrix due to the presence of lipids. This agrees with their plasticizing effect commented on above. Similar effects were found by Bonilla, Atarés, Vargas & Chiralt (2012) in CH films containing basil or thyme essential oils. WVP of $CH_1:OA_{0.25}:C_{0.25}$ was in the range of pure CH films while $CH_1:OA_{0.5}:C_{0.5}$ showed significantly lower values ($p<0.05$).

At both temperatures, the addition of OA or C led to a significant increase in OP of the pure chitosan films, especially at the highest lipid content, as was observed for hydroxypropyl methylcellulose films with ginger essential oil (Atarés, Pérez-Masiá, & Chiralt, 2011). The liquid state of OA and C, together with their hydrophobic character, facilitates the oxygen transport in the film due to the increase of the oxygen solubility in the matrix. However, it is remarkable that pure OA seems to promote OP of the films to a greater extent than C or lipid blends, which again points to specific interactions of the EO compounds with CH molecules in the matrix that affect transport properties.

3.2.5. Antioxidant activity

The trolox equivalent antioxidant capacity (TEAC) of cinnamon essential oil, expressed as μg of sample that show the same activity as 1 mM of Trolox, was 1.09 ±0.11. Table 6 shows the TEAC values for dry films. Pure CH films show very low antioxidant activity (the highest TEAC values), which was not significantly affected by OA addition. On the other hand, all films containing C showed higher antioxidant activity. The higher the C content in the dry film, the lower the TEAC value and so, the greater antioxidant power. As expected, OA addition did not significantly affect the antioxidant activity of C in the films, since CH_1:$C_{0.5}$ and CH_1:$OA_{0.5}$:$C_{0.5}$ showed similar TEAC values. Therefore, the antioxidant capacity of the films was attributed essentially to this ingredient and, thus, TEAC values were referred to the added C content in the film (Table 6) to compare the different samples. These TEAC values were in the same range but they were higher than that obtained for pure cinnamon essential oil, which indicates a loss of antioxidant capacity in the films. This can be explained by the losses in C essential oil during the film preparation, drying and extraction processes, as well as by the partial oxidation of its compounds. However, TEAC values of the CH_1:$OA_{0.5}$:$C_{0.5}$ film were significantly lower than those of CH_1:$C_{0.5,}$ in agreement with the greater retention of C essential oil compounds during film formation and handling when OA is present in the formulation.

As shown in Table 7, cinnamon essential oil showed antifungal activity (inhibition halum was detected) against *Aspergillus niger*, *Botrytis cinerea* and *Rhizopus stolonifer*, providing C content is higher than 0.25%. Above this concentration, the higher the cinnamon essential oil content, the greater the diameter of the inhibition halum. In the case of *B. cinerea*, total inhibition (no growth) was detected when C content was higher than 0.25% (w/w). When films were tested, no inhibition zone was observed when pure CH or CH_1:OA_1 films were placed in the inoculated agar plates or when the CH:C essential oil ratio was lower than 0.5. For the three fungi, the addition of cinnamon oil at

398 a CH:C ratio 1:0.5 and 1:1 led to a fungal growth inhibition zone, which was greater
399 when C oil ratio increased in the film and was not affected by OA addition. The highest
400 inhibition was detected for *B. cynerea*, which showed no growth, for CH_1:$OA_{0.5}$:$C_{0.5}$ and
401 CH_1:C_1 films.
402 3.2.6. Antimicrobial properties
403 Figure 3 shows the development of the fungal decay of the non-coated (control) and
404 coated strawberries, inoculated with a spore suspension of *R. stolonifer*, throughout
405 cold storage (10ºC-70% RH) or when stored under ambient conditions (20ºC-80% RH).
406 As expected, fungal decay was faster at higher temperature and RH. The effect of
407 coating was more significant at low temperature, which can be explained by the
408 combined effect of temperature and antimicrobial coatings.
409 At 20ºC and 80%RH (Figure 3a) the use of chitosan-based coatings, promoted an initial
410 significant decrease in fungal decay, as compared to control samples. The percentage of
411 damaged strawberries decreased when cinnamon essential oil was incorporated at a
412 CH:C ratio higher than 1:0.25, which is in agreement with the *in vitro* test. No
413 significant effects in terms of antifungal effect were promoted by OA addition at 20ºC.
414 Whereas non-coated samples lasted for 2 days, coated samples showed a long shelf-life.
415 In cold storage (Figure 3b), the lowest percentage of damaged strawberries was detected
416 in samples coated with CH_1:$C_{0.5}$. In these, fungal decay remained almost constant
417 throughout the whole period of storage (14 days). This effect was not observed in
418 samples coated with CH_1:$OA_{0.5}$:$C_{0.5}$, despite containing theoretically greater amount of
419 C in line with the greater retention promoted by OA. This could be due to the
420 entrapment of C oil compounds in the non-polar core of OA micelles which make their
421 diffusion and antimicrobial action difficult. In this sense, it is remarkable that from

about 9 days CH_1:$OA_{0.5}$:$C_{0.5}$ films were more effective than CH_1:$OA_{0.25}$:$C_{0.25}$ in line with the possible greater release of the C compounds.

Figure 4 shows the total aerobial, coliform, moulds and yeast counts for cold-stored samples. All coatings reduced the microbial counts, but the addition of C led to an increase of the antimicrobial effect of CH coatings. The higher the C ratio, the lower the microbial counts. OA addition had no notable effect on the antimicrobial effect of the coatings, although it reduced the effectiveness in the case of coliform counts.

4. Conclusions

Chitosan-cinnamon leaf essential oil films showed antifungal activity against *Aspergillus niger*, *Botrytis cinerea* and *Rhizopus stolonifer* and allowed for a significantly increase in the self-life of strawberries infected with *R. stolonifer*. Nevertheless, these films showed worse barrier properties than pure chitosan films. The addition of oleic acid to chitosan-cinnamon leaf essential oil films promoted essential oil retention into the film matrix during film drying. Oleic acid incorporation led to a significant reduction in the water vapour permeability and a diminution of the changes in colour and mechanical properties that were promoted by essential oil addition. Oleic acid slightly reduced the antifungal efficacy of the CH:C films probably due to the encapsulation of the essential oil, which in turn can limit the release of cinnamon's active compounds to the surface of the coated product. These results suggest that it is possible to formulate antifungal edible films based on chitosan and cinnamon leaf essential oil with adequate properties by the incorporation of oleic acid into the film-forming dispersion.

Acknowledgements

The authors acknowledge the financial support provided by Universitat Politècnica de València (UPPTE/2012/183) and Ministerio de Ciencia e Innovación (AGL2010-20694). Author A. Perdones acknowledges Universitat Politècnica de València for a doctoral grant.

References

ASTM. (1995). Standard test methods for water vapor transmission of materials. Standard designations: E96-95. In *Annual Book of ASTM Standards* (pp. 406-413). Philadelphia: ASTM.

ASTM. (1999). Standard test method for specular gloss. Standard designation D523. In *Annual Book of ASTM Standards* (Vol.06.01). Philadelphia: ASTM.

ASTM. (2001). Standard test method for tensile properties of thin plastic sheeting. Standard D882. In *Annual Book of ASTM Standards* (pp. 162-170). Philadelphia: ASTM.

ASTM. (2002). Standar test method for oxygen gas transmission rate through plastic film and sheeting using coulometric sensor. Standard designation D 3985-05. In *Annual Book of ASTM Standards* (pp. 472-477). Philadelphia: ASTM.

Atarés, L., Pérez-Masiá, R., & Chiralt., A. (2011). The role of some antioxidants in the HPMC film properties and lipid protection in coated toasted almonds. *Journal of Food Eng.*, *104*, 649-656.

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M., 2008. Biological effects of essential oils – A review. *Food Chem. Toxicol. 46*, 446-475.

Bonilla, J., Atarés, L., Vargas, M., & Chiralt, A. (2012). Effect of essential oils and homogenization conditions on properties of chitosan-based films. *Food Hydrocoll.*, *26*, 9-16.

CIE (1986). *Colorimetry***.** (2nd ed.). Paris: Commission Internationale de L'Eclairage. (Technical Report CIE 15.2).

Fabra, M. J., Talens, P., & Chiralt, A. (2010). Water sorption isotherms and phase transitions of sodium caseinate–lipid films as affected by lipid interactions. *Food Hydrocolloid.*, 24, 384-391.

Gennadios, A., Weller, C. L., & Gooding, C. H. (1994). Measurements errors in water vapour permeability of highly permeable, hydrophilic edible films. *J. Food Eng., 21*, 395-409.

Hutchings, J. B. (1999). *Food and colour appearance*. (2nd ed.). Gaithersburg: Aspen Publication.

Jiménez, A., Fabra, M. J., Talens, P., & Chiralt, A. (2010). Effect of lipid self-association on the microstructure and physical properties of hydroxypropyl-methylcellulose edible films containing fatty acids. *Carbohydr. Polym.*, *82*, 585-593.

McClements, D. J. (2005). Emulsion Rheology. In D. J. McClements (Ed.), Food emulsions. Principles, practices, and techniques (pp. 341–388). Boca Raton: CRC Press.

No, H. K., Park, N. Y., Lee, S. H., & Meyers, S. P. (2001). Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. *Int. J. Food Microbiol. 74*, 65-72.

Perdones, A., Sánchez-González, L., Chiralt, M., & Vargas, M. (2012). Effect of chitosan-lemon essential oil coatings on storage-keeping quality of strawberry. *Postharvest Biol. Technol., 70*, 32-41.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical catión decoloration assay. *Free Radic. Biol. Med.*, *26(9-10)*, 1231-1237.

Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, M., & Bruni, R. (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chem., 91*, 621-632.

Sánchez-González, L., Cháfer, M., Chiralt, A., & González-Martínez, C. (2010). Physical properties of edible chitosan films containing bergamot essential oil and their inhibitory action on *Penicillium italicum*. *Carbohydr. Polym., 82*, 277-283.

Sánchez-González, L., Vargas, M., González-Martínez, C., Chiralt, A., & Cháfer, M. (2011a). Use of Essential Oils in Bioactive Edible Coatings. *Food Eng. Rev., 3*, 1-16.

Sánchez-González, L., Cháfer, M., Chiralt, A., & González-Martínez, C. (2011b). Effect of essential oils on properties of film forming emulsions and films based on hydroxypropylmethylcellulose and chitosan. *J. Food Eng., 105(2)*, 246-253.

Sánchez-González, L., Chafer, M., González-Martínez, C., Chiralt, A., & Desobry, S. (2011c). Study of the release of limonene present in chitosan films enriched with bergamot oil in food stimulants. *J. Food Eng.*, 105, 138-143.

Singh, G., Maurya, S., de Lampasona, M. P., & Catalan, C. A .N. (2007). A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. *Food Chem. Toxicol., 45*, 1650-1661.

Tharanathan, R.N., & Kittur, F.S. (2003). Chitin - The undisputed biomolecule of great potential. *Crit. Rev. Food Sci. Nutr., 43(1)*, 61-87.

Vangalapati, M., Satya S. N., Prakash D. V. S., & Avanigadda, S. (2012). A Review on Pharmacological Activities and Clinical effects of Cinnamon Species. *Res. J. Pharm., Biol. Chem. Sci., 3*, 653-663.

520 Vargas, M., Pastor, C., Chiralt, A., McClements, D. J., & Gonzalez-Martinez, C.
521 (2008). Recent advances in edible coatings for fresh and minimally processed fruits.
522 *Crit. Rev. Food Sci. Nutr., 48*, 496-511.
523 Vargas, M., Albors, A., Chiralt, A., & González-Martínez, C. (2009). Characterization
524 of chitosan–oleic acid composite films. *Food Hydrocoll., 23*, 536-547.
525 Vargas, M., Perdones, A., Chiralt, A., Cháfer, M., & González-Martínez, C. (2011).
526 Effect of Homogenization Conditions on Physicochemical Properties of Chitosan-
527 Based Film-Forming Dispersions and Films. *Food Hydrocoll., 25*, 1158-1164.
528 Vargas, M., Sánchez-González, L., Chiralt, A., Cháfer, M., & González-Martínez, C.
529 (2012). Edible chitosan coatings for fresh and minimally processed foods. In K.L.
530 Yam & D.S. Lee (Eds.), *Emerging food packaging technologies: Principles and*
531 *practice.* (pp. 66-95). Cambridge: Woodhead Publishing Limited.
532 Vu, K. D., Hollingsworth, R. G., Leroux, E., Salmieri, S. & Lacroix, M. (2011).
533 Development of edible bioactive coating based on modified chitosan for increasing
534 the shelf life of strawberries. *Food Res. Int., 44*, 198-203.
535 Wan, Y., Wu, H., & Wen, D. (2006). Biodegradable polylactide/chitosan films blend
536 membranes. *Biomacromolecules, 7*, 1362-1372.
537 Wang, L., Liu, F., Jiang, Y., Chai, Z., Li, P., Cheng, Y., Jing, H., & Leng, X. (2011).
538 Synergistic Antimicrobial Activities of Natural Essential Oils with Chitosan Films. *J.*
539 *Agric. Food Chem., 59*, 12411-12419.
540 Zhang, C., Ding, Y., Ping, Q., & Yu, L. L. (2006). Novel chitosan derived
541 nanomaterials and their micelle-forming properties. *J. Agric. Food Chem., 54*, 8409-
542 8416.

543

544 **Table 1.** Composition of the Film Forming Dispersions (FFDs). CH: chitosan. OA:
545 oleic acid. C: Cinnamon leaf essential oil. Subscripts indicate the ratio of film
546 components.

FFDs	Chitosan (% w.b.)	Oleic Acid (% w.b.)	Cinnamon Leaf EO (% w.b.)	TOTAL LIPID (% w.b.)
CH_1	1	-	-	-
$CH_1:OA_{0.25}:C_{0.25}$	1	0.25	0.25	0.5
$CH_1:C_{0.5}$	1	-	0.5	0.5
$CH_1:OA_{0.5}:C_{0.5}$	1	0.5	0.5	1
$CH_1:C_1$	1	-	1	1
$CH_1:OA_1$	1	1	-	1

547

548

549

550 **Table 2.** Particle size, ζ-potential and rheological properties of the film-forming
551 dispersions (FFDs). Mean values and standard deviations, in brackets. CH: chitosan.
552 OA: oleic acid. C: Cinnamon leaf essential oil. Subscripts indicate the ratio of film
553 components.

FFDs	Particle Size (μm)		ζ-Potential (mV)	n	k (Pa·s)	$\eta_{100s^{-1}}$ (Pa·s)
	d_{43}	d_{32}				
CH_1	-	-	63.4 (0.6) [a]	0.722 (0.002)[a]	0.488 (0.007)[a]	0.1355 (0.0012)[a]
CH_1:$OA_{0.25}$:$C_{0.25}$	0.222 (0.006)[a]	0.158 (0.004)[a]	75.9 (0.4)[d]	0.77010 (0.00106)[b]	0.317 (0.003)[b]	0.1098 (0.0004)[b]
CH_1:$C_{0.5}$	0.178 (0.004)[b]	0.127 (0.002)[b]	72 (2)[c]	0.726623 (0.001004)[c]	0.473 (0.005)[c]	0.1342 (0.0009)[ac]
CH_1:$OA_{0.5}$:$C_{0.5}$	0.479 (0.002)[c]	0.393 (0.002)[c]	76 (2)[d]	0.7373 (0.0013)[d]	0.443 (0.008)[d]	0.132 (0.002)[c]
CH_1:C_1	0.402 (0.005)[d]	0.327 (0.004)[d]	66 (2)[b]	0.8228 (0.0008)[e]	0.2039 (0.0011)[e]	0.0902 (0.0002)[d]
CH_1:OA_1	0.2297 (0.0006)[a]	0.1627 (0.0006)[a]	75.1 (0.6)[d]	0.7080 (0.0006)[f]	0.488 (0.007)[f]	0.1477 (0.0006)[e]

554 [abc]Different superscripts in the same column indicate 95% significant differences among FFDs.

555

556

557

558

559 **Table 3.** Thickness and elastic modulus (EM), elongation (E) and tensile strength at
560 break (TS) of the films. Mean values and standard deviation, in brackets. CH:
561 chitosan. OA: oleic acid. C: Cinnamon leaf essential oil. Subscripts indicate the
562 ratio of film components.

Film	Thickness (μm)	TS (MPa)	E (%)	EM (MPa)
CH	42 (12)^{a}	44 (6)^{a}	20 (5)^{a}	1674 (49)^{a}
CH_1:$OA_{0.25}$:$C_{0.25}$	51 (5)^{b}	43 (9)^{a}	21 (6)^{ac}	1421 (80)^{b}
CH_1:$C_{0.5}$	48 (4)^{b}	37 (3)^{b}	17 (3)^{ad}	1455 (89)^{b}
CH_1:$OA_{0.5}$:$C_{0.5}$	64 (3)^{c}	39 (4)^{ab}	29 (5)^{b}	1047 (161)^{c}
CH_1:C_1	60 (3)^{c}	38 (6)^{ab}	27 (4)^{bc}	962 (68)^{cd}
CH_1:OA_1	74 (3)^{d}	33 (4)^{b}	11 (5)^{d}	848 (24)^{d}

563 ^{abcde}Different superscripts in the same column indicate 95% significant differences among film
564 formulations.

565

566 **Table 4.** Gloss values at 60º, colour coordinates: Lightness (L^*), Chroma (C^*_{ab}) and
567 Hue (h^*_{ab}), whiteness index (WI) and total color difference (ΔE) with respect to CH
568 films of the obtained films. Mean values and standard deviations (in brackets) of CH:
569 chitosan. OA: oleic acid. C: Cinnamon leaf essential oil. Subscripts indicate the ratio of
570 film components.

Film	Gloss 60º	L^*_{ab}	h^*_{ab}	C^*_{ab}	WI	ΔE
CH_1	67 (5)[a]	79.6 (0.6)[a]	91.0 (0.3)[a]	19.0 (0.4)[a]	72.12 (0.19)[a]	-
CH_1:$OA_{0.25}$:$C_{0.25}$	61 (11)[a]	75 (1)[b]	81.44 (0.17)[b]	28 (2)[a]	63.8 (0.6)[b]	9.6 (0.4)[a]
CH_1:$C_{0.5}$	69 (12)[a]	74 (1)[c]	79.3 (0.4)[c]	32 (2)[b]	59 (1)[c]	14 (1)[b]
CH_1:$OA_{0.5}$:$C_{0.5}$	28 (10)[b]	72 (1)[d]	78.8 (0.7)[d]	31.1 (0.8)[c]	58.1 (0.9)[d]	15.3 (0.9)[c]
CH_1:C_1	68 (15)[a]	69 (1)[d]	76.6 (0.4)[e]	35.6 (0.6)[c]	53.0 (0.8)[e]	20.6 (0.7)[d]
CH_1:OA_1	46 (4)[c]	81.9 (0.8)[e]	89.9 (0.6)[f]	18.9 (0.5)[d]	73.8 (0.9)[f]	2.4 (0.7)[e]

571 [abcde]Different superscripts in the same column indicate 95% significant differences among films.

572 **Table 5.** Equilibrium moisture content (EMC), water vapor permeability (WVP) at RH
573 gradient of 100-58% (T = 5ºC) or 100-54% (20ºC) and oxygen permeability (OP) at
574 10ºC-58% RH and 20ºC-54% RH. Mean values and standard deviation, in brackets. CH:
575 chitosan. OA: oleic acid. C: Cinnamon leaf essential oil. Subscripts indicate the ratio of
576 film components.

Film	EMC (wt%)		WVP ($g \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$)		OP ($cm^3 \cdot mm \cdot m^{-2} \cdot atm^{-1} \cdot day^{-1}$)	
	T = 5ºC	T = 20ºC	T = 5ºC	T = 20ºC	T = 10ºC	T = 20ºC
CH_1	20.6 (0.4)^{a*}	16.20 (0.12)^{a*}	239 (47)^{a*}	177 (23)^{a*}	0.043 (0.002)^{a*}	0.14 (0.03)^{a*}
CH_1: $OA_{0.25}$:$C_{0.25}$	16.1 (0.8)^{b*}	11.66 (0.17)^{bc*}	228 (41)^{a*}	190 (29)^{a*}	0.0986 (0.0012)^{b*}	0.176 (0.014)^{b*}
CH_1:$C_{0.5}$	17.6 (0.9)^{c*}	12.4 (1.3)^{c*}	242 (29)^{ac}	211 (38)^{a}	0.13 (0.01)^{bc}	0.162 (0.008)^{ab}
CH_1:$OA_{0.5}$:$C_{0.5}$	13.0 (0.3)^{d*}	9.8 (0.4)^{d*}	187 (32)^{b*}	105 (28)^{b*}	0.16 (0.02)^{c*}	0.344 (0.017)^{c*}
CH_1:C_1	15.5 (0.8)^{c*}	10.8 (0.8)^{bd*}	274 (54)^{c*}	213 (7)^{a*}	0.15 (0.02)^{c*}	0.217 (0.005)^{d*}
CH_1:OA_1	12.1 (0.4)^{d*}	9.9 (0.9)^{d*}	172 (27)^{b*}	72 (4)^{b*}	0.223 (0.014)^{d*}	0.44 (0.03)^{e*}

577 ^{abcde}Different superscripts in the same column indicate 95% significant differences among
578 formulations.*95% significant differences between temperatures.
579

580 **Table 6.** Antioxidant activity expressed as μg of sample that
581 show the same antioxidant activity as 1 mM of Trolox. Mean
582 values and standard deviations, in brackets. CH: chitosan. OA:
583 oleic acid. C: Cinnamon leaf essential oil. Subscripts indicate
584 the ratio of film components.

Sample	TEAC (μg)	
	Dry film	Cinnamon Essential oil in the dry film
CH_1	54 (8)^{d}	-
CH_1:$OA_{0.25}$:$C_{0.25}$	19.7 (1.6)^{c}	3.3 (0.3)^{b}
CH_1:$C_{0.5}$	13.4 (1.5)^{b}	4.4 (0.5)^{c}
CH_1:$OA_{0.5}$:$C_{0.5}$	11.0 (1.8)^{b}	2.8 (1.8)^{a}
CH_1:C_1	5.1 (0.9)^{a}	2.5 (0.9)^{a}
CH_1:OA_1	58 (16)^{d}	-

585 ^{abcd}Different superscripts in the same column indicate 95% significant
586 differences between formulations.
587

588 **Table 7.** Antifungal effect of cinnamon essential oil and films (expressed as diameter of
589 the inhibition zone). Mean values and standard deviations, in brackets. CH: chitosan. OA:
590 oleic acid. C: Cinnamon leaf essential oil. Subscripts indicate the ratio of film
591 components.

	Inhibitium halum (cm)						
	Aspergillus niger		*Botrytis cinerea*			*Rhizopus stolonifer*	
Time (days)	1	3	1	3	6	1	3
$C_{0.25}$	nh	nh	NG	6.4 (0.5)^{a}	nh	nh	nh
$C_{0.5}$	5.0 (0.2)^{a*}	4.0 (0.4)^{a*}	NG	NG	NG	2.9 (0.3)^{a*}	1.8 (0.2)^{a*}
C_1	7.5 (0.5)^{b}	7.3 (0.6)^{b}	NG	NG	NG	3.5 (0.3)^{b}	3.2 (0.2)^{b}
CH_1	nh	nh	NG	nh	nh	nh	nh
CH_1:$OA_{0.25}$:$C_{0.25}$	nh	nh	NG	5.1 (0.4)^{a*}	nh	nh	nh
CH_1:$C_{0.5}$	2.2 (0.2)^{a}	1.93 (0.09)^{a}	NG	8.70 (0.13)^{b*}	8.50 (0.05)^{b*}	2.8 (0.2)^{a*}	2.4 (0.2)^{a*}
CH_1:$OA_{0.5}$:$C_{0.5}$	2.0 (0.5)^{a}	2.0 (0.2)^{a}	NG	NG	NG	2.6 (0.5)^{a*}	2.0 (0.4)^{b*}
CH_1:C_1	4.1 (0.12)^{b}	3.9 (0.4)^{b}	NG	NG	NG	3.92 (0.12)^{b*}	3.3 (0.6)^{c*}
CH_1:OA_1	nh	nh	NG	nh	nh	nh	nh

592 ^{abc}Different superscripts in the same column indicate 95% significant differences between formulations. *95%
593 significant differences between incubation times. NG (no growth), nh (no halum).
594

595 **Figure captions**

596 **Figure 1.** SEM micrographs of cross-sections of the films. Magnification is 2000x. CH:
597 chitosan. OA: oleic acid. C: Cinnamon leaf essential oil. Subscripts indicate the ratio of
598 the components in the film.

599 **Figure 2.** Average internal transmittance (Ti) spectra of the films conditioned at 20ºC-
600 54% RH. Subscripts indicate the ratio of film components. OA: oleic acid. C: Cinnamon
601 leaf essential oil. Subscripts indicate the ratio of the components in the film.

602 **Figure 3.** Fungal decay (expressed as percentage of infected samples) of strawberries
603 inoculated with spore suspensions of *Rhizopus stolonifer* and stored at (a) 20ºC-80%
604 RH and (b) 10ºC-70% RH. OA: oleic acid. C: Cinnamon leaf essential oil. Subscripts
605 indicate the ratio of film components.

606 **Figure 4.** (a) Total aerobial, (b) moulds and yeasts and (c) coliforms counts of
607 strawberries inoculated with spore suspensions of *Rhizopus stolonifer* and stored at
608 10ºC-70% RH. OA: oleic acid. C: Cinnamon leaf essential oil. Subscripts indicate the
609 ratio of film components.