Development and characterization of edible chitosan/olive oil emulsion films

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ABSTRACT

The properties of plasticized chitosan–olive oil emulsion films prepared with increasing oil concentrations were investigated. Emulsifying nature of chitosan was enough to stabilize olive oil droplets in the film forming emulsions; hence homogeneous, thin and translucent films were obtained in all cases. The homogeneity of the lipid globules distribution in the films was confirmed by contact angle measurements and optical microscopy. All the tensile properties (Young Modulus, strength and maximum elongation) increased with olive oil concentration and were explained considering the interactions developed between lipid and carbohydrate phases in addition to the lubricant characteristics of the oil. Moisture sorption, water vapor permeation through the films and effective diffusion coefficients decreased as oil concentration increases, as a result of the non-polar nature of the lipid. Total soluble matter measurements were used to confirm the development of strong associations between chitosan and olive oil.

1. Introduction

Environmental concerns enhance and stimulate the use of renewable resources for producing economically convenient applications to maintain or even improve life quality (García, Pinottia, Martino, & Zaritzky, 2004). Usually, edible films are made of proteins or polysaccharides that can also help to maintain moisture, thereby improving shelf life of coated foods (Vu, Hollingsworth, Leroux, Salmieri, & Lacroix, 2011). Specifically, several polysaccharides have good film forming capacities, including cellulose derivatives, chitosan, starch, alginate, carrageenan and pectin (García et al., 2004).

In particular chitosan is a de-N-acetylated form of chitin, the second most abundant natural polymer obtained from the shells of crab, shrimp, etc. It consists mainly of β(1–4)-2 amino-2-deoxy-D-glucose units. This cationic polysaccharide, natural, non-toxic, biodegradable and available commercially, has been employed in a variety of applications (Tharanathan & Kittur, 2003). It is a well-known biopolymer with a broad antimicrobial activity against bacteria and fungi (Cagri, Ustunol, & Ryser, 2004; Rabea, Badawy, Stevens, Smagghe, & Steurbaut, 2003). Chitosan possesses high positive charge on NH^{+3} groups when dissolved in aqueous acidic solution, and therefore it is able to adhere to or aggregate with negatively charged lipids and fats (Wydro, Krajewska, & Hac-Wydro, 2007). Moreover, chitosan acts as stabilizer of hydrocolloids – lipids mixtures, promoting emulsion formation and interfacial stabilization, since its molecules are composed of both, hydrophilic and hydrophobic portions. Thus, it is frequently used as emulsifier to uniformly stabilize oil droplets in emulsion systems (Rodríguez, Albertengo, & Agullo, 2002; Schulz, Rodríguez, Del Blanco, Pistonesi, & Agulló, 1998). Thus, chitosan can be used to emulsify the oil added in film-forming solutions.

Chitosan films have a selective permeability to gases (CO_2 and O_2) and good mechanical properties. However, due to their hydrophilic nature, they are poor barriers to moisture, which limits their uses (Butler, Vergano, Testin, Bunn, & Wiles, 1996; Caner, Vergano, & Wiles, 1998; Ojagh, Rezaei, Razavi, & Hosseini, 2010). This is an important drawback since an effective control of moisture transfer is a desirable property for most foods.

Current approaches to extend functional and mechanical properties of this film include (i) incorporation of hydrophobic compounds such as lipids (fats and oils) to improve their resistance to water (McHugh & Krochta, 1994): lipids of different types have been successfully included into protein- and carbohydrate-based films by means of lamination and dispersion or emulsion (Prodpran, Benjakul, & Artharn, 2007) (ii) optimization of the interaction between polymers (carbohydrate–carbohydrate interactions, charge–charge electrostatic complexes between proteins or polysaccharides) and (iii) cross-linking or functionalization through physical, chemical, or enzymatic treatments (Pierro et al., 2006). Coming back to the first approach, Wong, Gastineau, Gregorski, Tillin, and Pavlath (1992), added saturated fatty acids and fatty acid esters to chitosan-based films, obtaining the higher resistance to water vapor transmission when lauric and butyric acids were used. On the other hand, Park and Zhao (2004) incorporated Vitamin E into the chitosan matrix obtaining a significant

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Table 1
Composition of chitosan/olive oil films based on the total dry mass.

Nomenclature (olive oil/chitosan, wt%)	Chitosan (wt%)	Glycerol (wt%)	Olive oil (wt%)
0	78.13	21.87	0
5	75.19	21.05	3.76
10	72.46	20.29	7.25
15	69.93	19.58	10.49

decrease in the water vapor permeability (WVP), at expenses of an increase in opacity and a significant reduction in the tensile strength of the composite films. Nevertheless, to the best of our knowledge, there are scarce (Vargas, Albors, Chiralt, & González-Martínez, 2006) reported data on the effect of unsaturated oils, such as olive one, on the properties of chitosan-based films and only few works dealing with the study of the interactions between chitosan and olive oil or chitosan and olive oil components (Ham-Pichavant, Sèbe, Pardon, & Coma, 2005; Muzzarelli, Frega, Miliani, Muzzarelli, & Cartolari, 2000; Vargas et al., 2006). Moreover, the study published by Vargas et al. (2006) focused in the improved physicochemical quality of the strawberries coated with the edible coatings and not in the physical and mechanical characterization of the chitosan based films themselves; the data reported by Muzzarelli et al. (2000) dealt with the capacity of chitosan to alter the composition of olive oil upon percolation of the oil through a bed of chitosan powder; and the paper published by Ham-Pichavant et al. (2005) was focused in the potential replacement of fluorocarbon-treatment of paper-based materials by chitosan coating, to produce oil barrier packaging.

Olive oil is extensively consumed due to its nutritional value and its organoleptic characteristics. It is a vegetable oil whose monounsaturated fatty acid content (MUFA) is very high. It contains 56.3–86.5% MUFA, particularly oleic acid and it is also rich in tocopherols and phenolic substances which act as antioxidants (Viola, 1970). Therefore, it is an obvious alternative to consider when dealing with edible films aimed reducing water vapor transmission. Hence, the aim of this work is to analyze the effect of olive oil incorporation on the functional characteristics of chitosan based films such as water sorption, moisture barrier behavior, transparency and mechanical properties.

2. Experimental

2.1. Materials

Chitosan (deacetylation degree 90%, Mv = 1.61×10^5 g/mol) was supplied by PARAFARM, Mar del Plata, Argentina. The plasticizer used was glycerol (Gly), which was purchased from DEM Chemicals (Mar del Plata, Argentina). The olive oil (purity 100%, extra virgin) was purchased at Plaza Vea markets (Mar del Plata, Argentina).

2.2. Emulsion preparation

Chitosan solutions (2%, w/v) (Lacroix et al., 2002) were prepared by dispersing chitosan powder in acetic acid solution (1%, v/v) at room temperature ($23 \pm 2\,°C$). Appropriate amounts of glycerol were added to achieve a glycerol/chitosan (Gly/CH) weight ratio of 0.28. Lipid fraction was incorporated in olive oil/chitosan weight ratios of 0.05, 0.1 and 0.15. Table 1 reports the composition of each emulsion, based on the total dry mass. After that, each emulsion was homogenized with a high speed Homogenizer (Thorbell, HS-30-FC) for 10 min at 20,000 rpm. The film-forming dispersions were defoamed at room temperature by filtering them with the aid of filter fabric; only air bubbles were removed by this procedure and thus, the emulsion concentration was unaffected.

2.3. Film preparation

Films were prepared by the casting technique, that is, 50 g of the film-forming dispersions were poured into each Teflon Petri dish (diameter = 14 cm) and dried at 35 °C for approximately 15 h in a convection oven (30% RH, average). After the excess of water was evaporated, the obtained films were peeled off from the plates and kept in a closed reservoir at constant relative humidity (RH) and temperature ($23 \pm 2\,°C$) for 3 days. The films were further characterized and tested.

2.4. Characterization of films

2.4.1. Film thickness

The film thickness was measured with a 0–25-mm manual micrometer with an accuracy of ± 0.01 mm in four random locations for each film.

2.4.2. Film opacity

Film opacity was determined according to the method described by Irissin-Mangata, Bauduin, Boutevin, and Gontard (2001) on rectangular strips directly placed in a UV-Visible spectrophotometer test cell. The absorption spectrum of the sample was obtained from 400 to 800 nm in a UV-Visible spectrophotometer Shimadzu 1601 PC (Tokyo, Japan). Film opacity was defined as the area under the curve divided by film thickness and expressed as Absorbance Units × nanometers/millimeters (AU nm/mm). Measurements were taken in triplicate for each sample.

2.4.3. Transmission optic microscopy (TOM)

The surfaces of the films were analyzed by TOM, using a microscope (Leica DMLB) coupled to a video camera (Leica DC100). For this purpose, the pieces of the films were mounted between glass-holder and glass-cover and pictures were taken at different magnifications.

2.4.4. Mechanical properties

Prior to running mechanical tests, films were conditioned for 48 h at $50 \pm 5\%$ RH at 25 °C. Tensile tests were performed at room temperature ($23 \pm 2\,°C$) using an Instron Universal Testing Machine model 8501. The specimens were cut according to the ASTM D1708-93 (ASTM, 1993). Five specimens from each film were tested from a minimum of three films per sample. Crosshead speed was set at 10 mm/min. The ultimate strength (σ_b), elongation at break (ε_b) and elastic modulus (E) were calculated as described in ASTM D638-94b (ASTM, 1994).

2.4.5. Surface hydrophobicity

The surface hydrophobicity of the films was estimated by the sessile drop method, based on optical contact angle method. Contact angle measurements were carried out with a homemade instrument. A droplet of ethylene–glycol (Aldrich Co.) (5 μL) was deposited on the film surface with an automatic piston syringe. The drop image was photographed using a digital camera. An image analyzer was used to measure the angle formed between the surface of the film in contact with the drop, and the tangent to the drop of liquid at the point of contact with the film surface. The measurements were performed within the first 15 s after dropping ethylene–glycol onto film surfaces, to avoid variations due to solvent penetration onto the specimens. All the samples were conditioned at 50% RH three days before the test. Seven measurements were performed for each film at 24 °C, with a precision of $\pm 2\,°C$.

2.4.6. Moisture sorption

The films, dried at 40 °C for three days in a vacuum oven, were placed inside an environmental chamber maintained at 75%

relative humidity (RH) and $23 \pm 2\,^{\circ}$C, to obtain water sorption kinetics. Samples were taken out of the chamber at regular time intervals and weighed with a precision of ± 0.0001 g.

The moisture content (M_t) of the films as a function of time was obtained from the total mass balance over the sample, as follows:

$$M_t = \frac{(W_t - W_0)}{W_0} \times 100 \tag{1}$$

where M_t is the moisture content of the sample at a fixed time expressed in dry basis [%]; W_t is the weight of the sample at a fixed time [g] and W_0 is the initial dry weight of the sample [g]. The curves were fitted according to the Fick's diffusion equation for the one-dimensional diffusion of a solute into a sheet (Crank, 1956):

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left[-D \frac{(2n+1)^2 \pi^2 t}{l^2} \right] \tag{2}$$

where M_∞ is the amount of water absorbed at equilibrium, D is the effective diffusion coefficient, t is the time and l is the average thickness of the film. Notice that this equation is only valid in the specific case of mass transport through a plane sheet with constant boundary conditions and uniform initial moisture distribution, in which the edge effects can be neglected.

To ensure the reproducibility of the results, four specimens for each sample were tested.

2.4.7. Water vapor permeability (WVP)

The water vapor transfer rate [g s^{-1} m^{-2}] through films was determined gravimetrically using the ASTM Method E96-95 (ASTM, 1995). Prior to the test, the films were placed in a chamber maintained at room temperature for 3 days at 67% RH, to ensure equilibrium conditions. After that, film specimens were sealed on acrylic permeation cups (5 cm diameter) containing distilled water (100% RH). The cups were weighed at 1 h intervals over a 6 h period. A fan located inside the chamber was used to move the internal air ensuring uniform conditions at all test locations. Linear regression was used to fit the data, weight vs. time, and to calculate the slope of the resulting straight line in g s^{-1}. WVP of the films, expressed as [g m/(Pa s m^2)], was calculated as follows:

$$\text{WVP} = \text{WVTR} \; y(p_2 - p_1)^{-1} \tag{3}$$

where WVTR, water vapor transmission rate $= \Delta W[A \, \Delta t]^{-1}$, ΔW is the weight of water absorbed in the cup (g), Δt is the time for weight change (thus $\Delta W/\Delta t$ is the slope calculated from a plot of cup weight vs. time), A is the exposed area of the film (m^2), y is the film thickness (m), and $p_2 - p_1$ is the vapor pressure difference across the film (Pa), which was calculated based on the chamber temperature and the relative humidity inside and outside the cup. Four specimens were tested for each film type.

2.4.8. Total soluble matter (TSM)

The total soluble matter (TSM) was expressed as the percentage of the film dry mass dissolved after 24 h immersion in distilled water. TSM determinations were carried out according to the "wet" method proposed by Rhim, Gennadios, Weller, Cezeirat, and Hanna (1998), using distilled water (30 ml) and in the presence of sodium azide (0.02%) to prevent microbial growth.

Three specimens of each film were weighed (m_h) (± 0.0001 g) and then directly immersed in distilled water under the conditions described above. After 24 h immersion, the samples were oven dried at 105 $^{\circ}$C during 24 h, to determine the dried remnant insoluble mass (m_f). The initial dry mass values (m_0) needed for the TSM calculations were obtained from different specimens cut from the same film and dried at 105 $^{\circ}$C during 24 h.
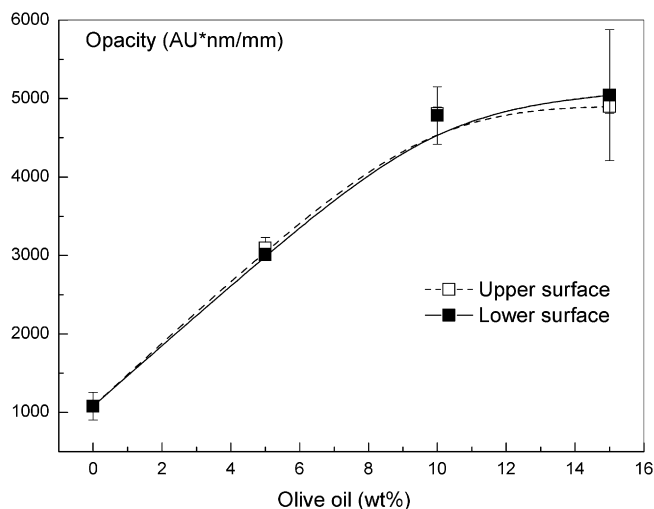

Fig. 1. Opacity values of composite films as a function of the olive oil content. Upper surface is the surface of the film forming dispersion that was in contact with air during drying. Lower surface is the surface in contact with the plate during drying.

The TSM was calculated as following:

$$\text{TSM} \;\; (\%) = \frac{m_0 - m_f}{m_0} \times 100 \tag{4}$$

2.4.9. Statistical analysis

Data for each test were statistically analyzed. The analysis of variance (ANOVA) was used to evaluate the significance in the difference between factors and levels. Comparison of the means was done employing a Tukey test to identify which groups were significantly different from other groups ($P < 0.05$). All data are presented as mean $\pm$ SD. Statistical analysis was performed using ORIGIN LAB 8 software.

3. Results and discussion

As expected, the emulsifying nature of chitosan (i.e., amphiphilic property) was enough to stabilize olive oil droplets in the film forming emulsions and thus, homogeneous, thin and translucent films were obtained from CH–olive oil mixtures, for all the oil concentrations used in this work. The films were also flexible, which was in part the result of using glycerol, a low molecular weight agent that acts as plasticizer, reducing the brittleness of the films by interfering with intra- and intermolecular hydrogen bonding in these carbohydrate-based films (Srinivasa & Tharanathan, 2007). Moreover, all the films were easily removed from the cast plates and showed smooth surfaces.

3.1. Opacity

Optical properties are essential to define the ability of films and coatings to be applied over a food surface, since these affect the appearance of the coated product, which is an important quality factor. CH–olive oil composite films were much more opaque than CH films, since oil droplets dispersed in the carbohydrate matrix affect the transparency by preventing the light transmission through the resulting film, as shown in Fig. 1. It is also interesting to notice that the transparencies measured in both film surfaces (i.e., upper surface, the one in contact with air and, lower surface, the one in contact with the Teflon mold during film drying) are actually similar for oil concentrations up to 10 wt% and only slightly different (3% higher opacity values for the lower side) for 15% oil, indicating that the oil dispersion in the thickness direction is homogeneous. Yang and Paulson (2000) also noticed an important sharp increase
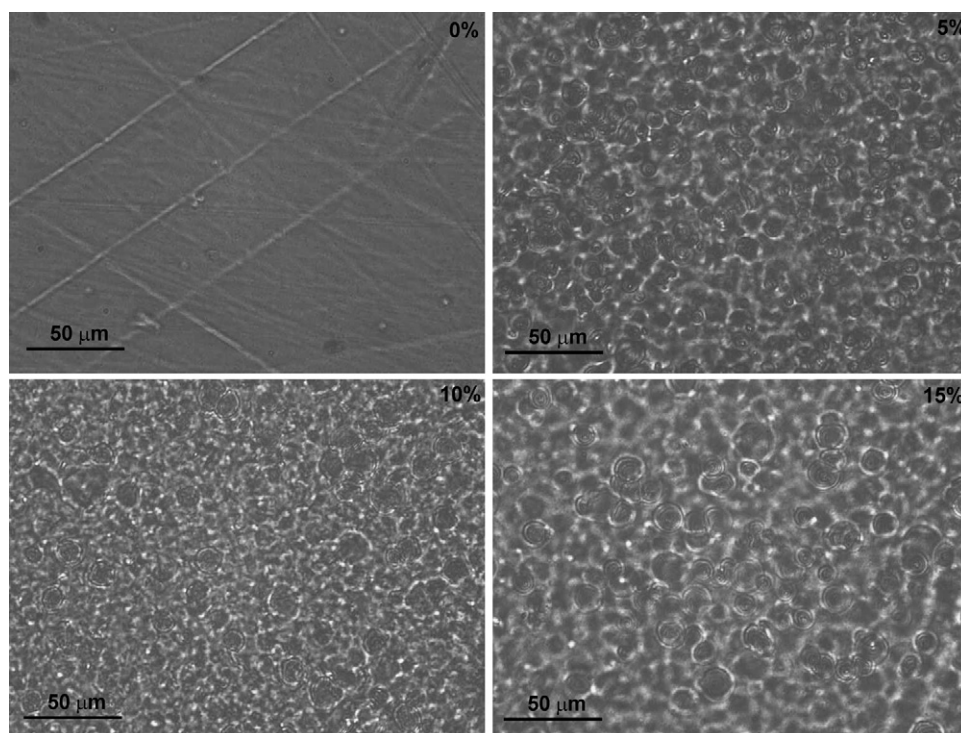
Fig. 2. Optical micrographs of film surfaces (500×).

in the film opacity as concentration of the lipids increased. Moreover, they reported that the differences in opacity of their films were determined by the optical properties of the lipids incorporated, i.e., beeswax incorporation led to more opaque films than the 1:1 blend of stearic and palmitic acids, at the same lipid concentration. On the other hand, Monedero, Fabra, Talens, and Chiralt (2009) and Villalobos, Chanona, Hernández, Gutiérrez, and Chiralt (2005) pointed out that the presence of a disperse, non-miscible phase promotes opacity as a function of the differences in the refractive index of the phases and the concentration and particle size of the dispersed phase. Other authors (Vargas, Albors, Chiralt, & González-Martínez, 2009; Villalobos et al., 2005) indicated that the different transparency level should be linked to the internal structure developed throughout drying process, which is greatly affected by the initial structure of the film forming dispersion, i.e., volume fraction of the dispersed phase and size of chitosan–oil aggregates. During drying, changes in the solvent properties of the continuous phase do occur, leading to coalescence and creaming phenomena as water is eliminated. At the same time, the viscosity of the continuous phase increases, thus limiting aggregation and creaming, which contributes to ensuring a specific dispersion level of lipid components in the polymer matrix and a certain degree of anisotropy in the physical properties throughout the matrix (Villalobos et al., 2005). In our case, it is obvious that the increase in film opacity arose from light scattering from lipid droplets that were dispersed in the emulsion and continuously distributed throughout the polymer network after the film formed. The well dispersion level, as well as the size of the olive oil droplets achieved in the composite films are shown in Fig. 2: while a smooth, continuous structure was observed for the neat chitosan film surface, the presence of oil caused discontinuities associated with the formation of two phases (lipid and polymer) in the matrix. It is noticed that the particle size of the globules due to olive oil do not change very much with lipid concentration, being the average sizes of oil droplet 10.7, 14.3 and 15 μm for emulsions having 5%, 10% and 15 wt% olive oil, respectively. Moreover, the droplet distribution is mostly unimodal for

each oil concentration, which is correlated with the high stability of the emulsions (Prodpran et al., 2007). The effect of chitosan on emulsion stability can be attributed to an increase in surface activity of a continuous phase (Speiciene, Guilmineau, Kulozik, & Leskauskaite, 2007).

3.2. Contact angle

Surface properties of the films (i.e., contact angle) give information about the phenomenon of wetting or non-wetting of a product surface by film forming dispersions and thus, about the uniformity of coating when applied to a particular solid surface (Vargas et al., 2009; Karbowiak, Debeaufort, & Voilley, 2006). Moreover, the contact angle method is a simple way to determine the superficial hydrophilicity of films since when using water or another polar solvent, contact angle will increase with increasing surface hydrophobicity (Hambleton, Fabra, Debeaufort, Dury-Brun, & Voilley, 2009; Pereda, Aranguren, & Marcovich, 2010; Zia, Zuber, Mahboob, Sultana, & Sultana, 2010). For these reasons, the contact angles of the superior and inferior sides of the films were measured, using ethylene-glycol as polar solvent, and the results are shown in Fig. 3. Again it is noticed that there are no significant differences ($P < 0.05$) in the measurements between both surfaces, up to 10% olive oil. At higher contents (15%) it seems that a little portion of the lipid phase tend to migrate from the lower surface to the air interface, which is favored by the density difference of the two phases. These results are in agreement with the amphiphilic character of chitosan (Prodpran et al., 2007), with the opacity/optical microscopy results discussed in the previous section, and confirm that exist a homogeneous distribution of the lipid globules in the films.

According to Fig. 3, increasing olive oil concentration causes a slight increase of contact angle values, from approximately 60° for 0% olive oil to 65° for 15% olive oil, which is due to the hydrophobic nature of the added lipid. Others authors found also similar behaviors, for example Ojagh et al. (2010) reported that the incorporation
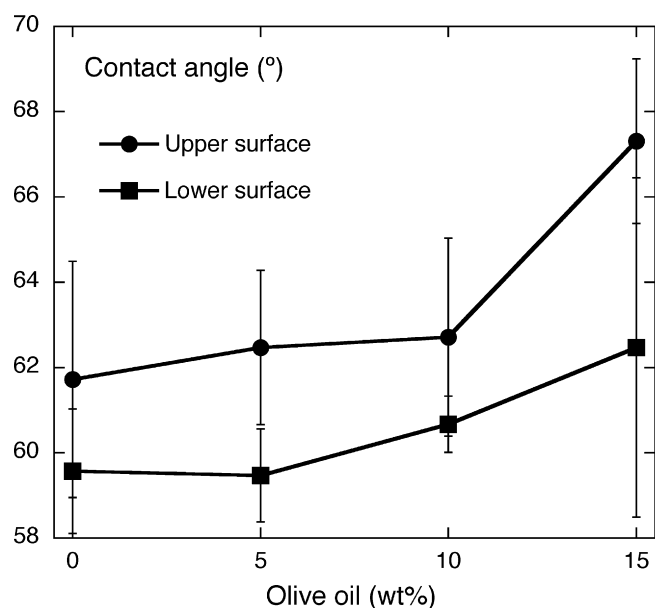
Fig. 3. Contact angle values of chitosan–olive oil films, measured within the first 15 s after dropping ethylene-glycol onto film surfaces. Samples conditioned at 50% RH and 24 ± 2 °C.

of cinnamon essential oils into chitosan films resulted in decreasing hydrophilicity of the composite films, which was attributed to the loss of free functional groups (amino and hydroxyl groups). Vargas et al. (2009) found an increment of 7° in the contact angle when 4% (v/w) oleic acid was added to the chitosan film, which was attributed to the development of molecular interactions among the components of the film forming suspension.

3.3. Mechanical properties

Table 2 shows the percentage of elongation at break, tensile strength and Young modulus of films equilibrated at 50% RH and 25 °C. Tensile strength indicates the maximum tensile stress that the film can sustain, elongation at break is the maximum change in length of a test specimen before breaking, and Young modulus is a measure of the stiffness of the film.

Chitosan control film had a tensile strength value of 8.4 MPa. Incorporation of olive oil into chitosan films increased tensile strength and tensile modulus significantly ($P < 0.05$). A strong interaction between the polymer and the olive oil produced a cross-linking effect, which decreases the free volume and the molecular mobility of the polymer; in this sense, this effect can also been considered as an important increase in the local viscosity of the oil in the vicinity of chitosan chains. This phenomenon was previously reported by other researches in similar systems, i.e., Vargas et al. (2009) reported that this cross-linking effect in chitosan–oleic acid films prepared at 1:1 ratio, resulted in an enhancement of the structural bonds in the polymer network that led to an increase in the tensile strength. Ojagh et al. (2010) found the same behavior in chitosan–cinnamon essential oil composite films and attributed it to the special microstructure noticed for the composite samples, which was composed by arrangements of stacking layers of olive oil added chitosan sheets. Prodpran et al. (2007) reported that interactions could enhance the structural bonds in the polymer network leading to an increase in the tensile strength but to a decrease in the stretchability of chitosan films modified with palm oil, although in their case, the oil was utilized as glycerol (plasticizer) substitution. Moreover, Vargas et al. (2009) confirmed by z-potential measurements the electrostatic interactions between the positively charged amino groups of chitosan and the carboxylate function of oleic

acid (main constituent of olive oil), which is negatively charged in the film forming dispersion. What is more, Kristo and Biliaderis (2006) indicated that sorbitol could function as antiplasticizer for their caseinate–pullulan bilayers and blends, when the moisture content of their films is low, by strongly interacting with polymer molecules and bringing up steric hindrance and reduction in free volume. In fact, in a later paper (Kristo, Biliaderis, & Zampraka, 2007) they strengthen this idea by asserting that the enhancement of the Young modulus and strength of their caseinate–pullulan composite films can be attributed to water induced cohesiveness and toughness of the brittle material at low water activity, allowing the structural elements to remain intact and continue to offer resistance to yield. Furthermore, Chang, Cheah, and Seow (2000) adopted the hypothesis of antiplasticization of synthetic polymers by diluents to give an explanation for the antiplasticization of water on the tensile strength of starch. According to this hypothesis, water molecules added in small amounts to the polymer facilitate polymer orientation and fill "holes" created in the polymeric system under large deformation, thus reducing "hole free volume" and increasing structural order. Summarizing, incorporation of relatively low amounts of liquid lipids into chitosan films led to more compact structures, with increased continuities within the polysaccharide network.

On the other hand, there are also reports indicating that a reduction of all tensile parameters (strength, modulus and elongation at break) of chitosan films is the expected output when a liquid lipid is incorporated in the system. These are the conclusions of the papers of Sánchez-González, Cháfer, Chiralt, and González-Martínez (2010), Sánchez-González, González-Martínez, Chiralt, and Cháfer (2010) and Sánchez-González, Chiralt, González-Martínez, and Cháfer (2011), who studied the effects of adding essential oils to chitosan based emulsion films and attributed the behavior to the presence of structural discontinuities in the polymer network caused by the lipid dispersed phase. Nevertheless, as far as we know, there are no reports that present an increase in elongation at break as the concentration of the lipid phase increases, as shown in Table 2. This different behavior can be attributed to the type of chitosan (degree of deacetylation and molecular weight) used and to the particular interactions developed with the olive oil components which, in turn, are affected by relative humidity, the presence of glycerol, the temperature during film drying, etc. Besides, the film microstructure proposed by Ojagh et al. (2010) could be used to explain this result since the olive oil layers located among chitosan ones would act as lubricant sheets, leading to increased deformation capacity of the composite. Moreover, being the olive oil a mixture of triglyceride esters, fatty acids, and other organic compound it is also plausible that some of its minor constituents contribute to the plasticization of the composite film.

3.4. Moisture sorption and water vapor permeability

The results of equilibrium moisture content (EMC) of the different composite films conditioned at 75% RH are shown in Table 3. The EMC of olive oil–chitosan films is significantly ($P < 0.05$) lower than the corresponding to the neat chitosan matrix. The diminished availability of amino groups of chitosan due to the electrostatic neutralization with the carboxylate groups of olive oil, added to the compactness of the film network may cause limited access of water molecules to hydrophilic chitosan sites, thus resulting in a decrease of equilibrium moisture content of composite films. However, there are no significant differences between samples prepared with increasing lipid concentrations, which constitutes a difference respect to the observations of Vargas et al. (2009), but it is consistent with the relatively high elongation values of our composite films.

Table 2
Tensile properties of chitosan–olive oil films. Samples conditioned at 50% RH and $25 \pm 2\,^{\circ}$C.

| Olive oil (wt%)	Young modulus (MPa)	Elongation at break (%)	Tensile strength (MPa)
0	76.2 ± 3.9[a]	19.55 ± 3.38[a]	8.41 ± 0.79[a]
5	99.6 ± 10.9[b]	21.91 ± 2.40[a]	8.52 ± 1.20[a]
10	107.1 ± 11.7[b]	31.28 ± 3.83[b]	11.07 ± 1.96[a]
15	139.6 ± 31.2[b]	32.90 ± 4.13[b]	14.69 ± 1.24[b]

Different superscripts letters (a,b) within the same column indicate significant differences between formulations ($P < 0.05$).

Table 3
Equilibrium moisture content (EMC) assessed at 75% RH and water vapor permeability (WVP) values measured with a relative humidity (RH) gradient of 67–100% (external to internal) of chitosan–olive oil films.

Olive oil (wt%)	EMC (%)	WVP (g m/Pa s m^2) $\times 10^{-9}$	WVTR (g/m^2 h)	Film thickness (mm)
0	25.08 ± 0.87[a]	1.35 ± 0.66[a]	-63.32 ± 18.97[a]	0.070 ± 0.007[a]
5	18.99 ± 2.17[b]	1.17 ± 0.22[a]	-56.42 ± 10.64[a]	0.070 ± 0.005[a]
10	21.85 ± 0.45[c]	1.14 ± 0.34[a]	-41.05 ± 15.16[a]	0.095 ± 0.006[b]
15	19.32 ± 3.11[b,c]	1.03 ± 0.36[a]	-38.49 ± 13.41[a]	0.090 ± 0.005[b]

Different superscripts letters (a–c) within the same column indicate significant differences between formulations ($P < 0.05$).

In order to determine the effective diffusion coefficients of water vapor in the composite films, the fractional mass absorbed, defined as M_t/M_∞, where M_t is the mass of water vapor entering into the material at time t and M_∞ is the water mass absorbed at infinite time, was plotted against the elapsed time. The absorption of water vapor from chitosan–olive oil films was gradual and followed an exponential model (Fick's Law) tending asymptotically to 1. A good fit was obtained between experimental data and the model, as can be noticed from Fig. 4. Equilibrium was reached after approximately 6 h of absorption and the final M_∞ values of the composite films were lower than that of the neat chitosan film, with no clear trend respect to the amount of olive oil, as the EMC values. The effective diffusion coefficients for water vapor were in the order of 10^{-13} m^2/s (Fig. 5) and decreased with the lipid concentration. Although these values can be considered quite low, it is essential to remark that water sorption in hydrophilic polymers is a rather complex phenomenon due to the presence of specific interactions between water molecules and the hydrophilic sites on the polymer backbone, which delays water diffusion through the film (Netti, Del Nobile, Mensitieri, Ambrosio, & Nicolais, 1996; Pereda, Aranguren, & Marcovich, 2009; Pereda et al., 2010). On the other hand, the decrease of the effective diffusion coefficients with oil concentration is related to the non polar character of the oil component that decreased the overall hydrophilicity of the composite material with respect to that of the neat chitosan matrix.
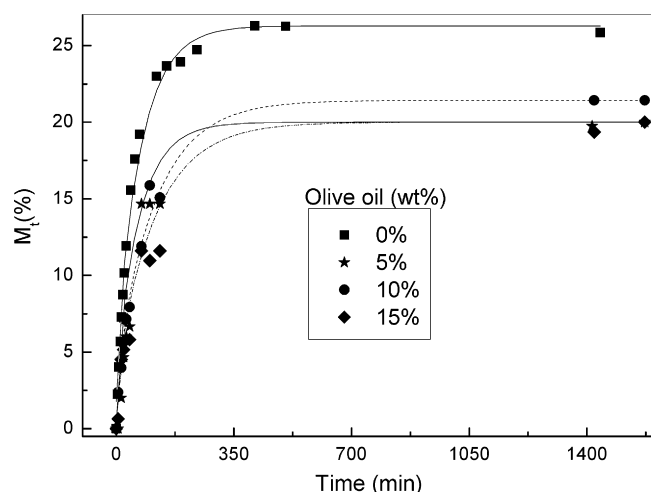
In the same way, WVP is considered a crucial property for films intended to be used as edible food coatings, because most natural biopolymers are very prone to water absorption (Arvanitoyannis, Nakayama, & Aiba, 1998; Prodpran et al., 2007). As shown in Table 3, the WVP decreases slightly as the lipid content of the composites increases, which strengthen the hypothesis of uniform distribution of the olive oil in the plasticized chitosan network. However, all the WVP values are in the order of 10^9 g/Pa s m^2, which could be quite high for preventing migration of moisture from the fruit, for example, to the environment during freezing and thawing. At this high level of relative humidity gradient (100: 67% RH), water acts as a strong plasticizer in hydrophilic polymers such as chitosan, decreasing their barrier properties, regardless of the presence of any organic oil. At this large hydration level, water molecules partially dissolve CH to form a gel and then, the advantages of the more compact structure obtained by oil addition are lost. In other words, the cationic nature of the chitosan seems to control the water vapor permeation, as was also reported in related papers (Srinivasa, Ramesh, & Tharanathan, 2007). Even more, it is reported (Cheng, Abd Karim, & Seow, 2008) that the addition of a hydrophobic material into an emulsion film does not guarantee reduced WVP, because permeability of emulsion films is influenced by the existence of steric hindrance and "tortuosity" for diffusion of water molecules (Cheng et al., 2008; Holton, ASP, & Zottola, 1994) but also by the existence of pores, voids, cracks and channelings (Cheng et al., 2008; Torres, 1994; Wong et al., 1992).
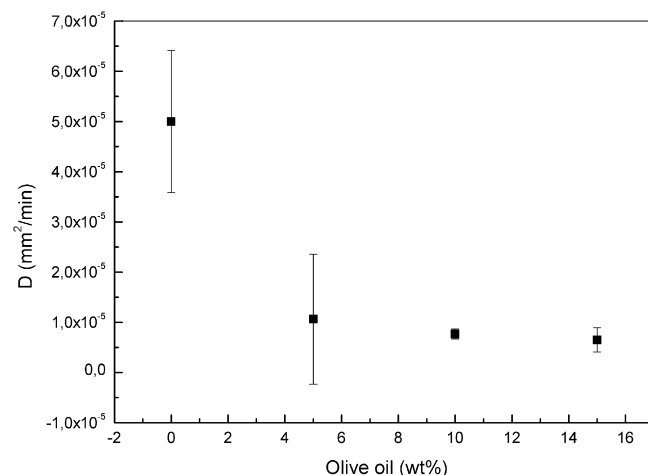

Fig. 4. Moisture sorption of chitosan–olive oil films at $23 \pm 2\,^{\circ}$C and 75% RH as a function of time, solid lines represent the Fickian model [Eq. (2)].

Fig. 5. Effective diffusion coefficients of composite films as a function of the olive oil concentration.

Table 4
Experimental and theoretical TSM values of chitosan–olive oil films.

Olive oil (wt% respect chitosan mass)	Experimental	[a]Theoretical 1	[b]Theoretical 2
0	20.60 ± 1.59[a]	21.9	21.9
5	17.63 ± 0.76[b]	21.0	24.8
10	18.02 ± 1.20[a,b]	20.3	27.5
15	14.82 ± 1.84[c]	19.6	30.0

Different superscripts letters (a–c) within the same column indicate significant differences between formulations ($P < 0.05$).

[a] Theoretical 1 values were calculated assuming all the glycerol moves to the water phase.

[b] Theoretical 2 values were calculated assuming both, the glycerol and the olive oil, move to the water phase.

3.5. Total soluble matter

A simple way to confirm the development of interactions between the main constituents of our films is to determine the water-soluble fraction as a function of olive oil concentration. Chitosan is a weak base and it is insoluble in water and organic solvents, although it is soluble in dilute aqueous acidic solutions (pH < 6.5), which can convert glucosamine units into soluble form $R–NHC_3$ (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004; Qin et al., 2006). Water-soluble chitosan can be obtained through a chemical modification in which the degree of substitution is controlled. For instance, it is known that water-soluble chitosan with about 50% DD can be obtained from chitin by hydrolysis with alkali (Kurita, Sannan, & Iwakura, 1977; Qin et al., 2006) or from chitosan by N-acetylation with acetic anhydride (Kubota, Tatsumoto, Sano, & Toya, 2000; Qin et al., 2006). Moreover chitosan oligomers, which can be achieved by degradation of chitosan polymer chain, are water-soluble too (Qin et al., 2006). However in this work, the relatively high molecular weight and high DD of the chitosan used, ensures its water insolubility. On the other hand, the glycerol used as plasticizer is completely soluble in water, that is why a theoretical total soluble matter of 21.9% for the chitosan–glycerol plasticized film (without olive oil) can be calculated, which is in agreement with the experimental determination. Conversely, it is noticed that the experimental TSM values (Table 4) for samples containing olive oil are systematically lower than the theoretical values for complete loss of glycerol and much more lower than those predicted considering that both, the olive oil plus the glycerol migrate from the film to the liquid during the test. Even though olive oil is not miscible with water, if there were no interactions at all between lipid and polysaccharide components, or even more, if the interactions developed were weak enough, as in the case of sodium caseinate–tung oil films reported in a previous work (Pereda et al., 2010), the complete migration of the oil component to the water phase is expected. In this case, the presence of oil not only leads to strong associations with chitosan but also entraps part of the plasticizer, avoiding its loss in aqueous medium. Thus, the calculations presented in Table 4 also confirm the development of strong interaction between both film constituents.

4. Conclusions

Homogeneous, thin and translucent films based on chitosan and olive oil emulsions were successfully prepared and characterized. The emulsifying property of chitosan was enough to stabilize olive oil droplets in the film forming emulsions at the lipid concentrations used in this work. Physical characterization confirmed that the lipid globules distribution in the films was homogeneous, which was associated to the emulsion stability. Tensile properties, including elongation at break, increased with olive oil concentration due to the lubricant characteristics of the liquid lipid in addition to the strong interactions developed between lipid and carbohydrate phases, which were also confirmed by TSM determinations. Moisture sorption, water vapor permeation through the films and effective diffusion coefficients decreased as oil concentration increases owing to the non-polar nature of the lipid.

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