

Caseinate films modified with tung oil

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ABSTRACT

Sodium caseinate/tung oil composite films were prepared through emulsification in order to reduce the water affinity of the protein-based film. Addition of lipid leads to milky films, resulting in a sharp increase in opacity. Uneven lipid distribution was evidenced by SEM in films prepared with 15% tung oil, which most likely arose from the limited dispersion capability of the lipids. Tung oil content also affects the film mechanical response, increasing tensile strength and elastic modulus but decreasing deformability. Contact angle measurements, besides water absorption tests, confirm lower hydrophilicity of the samples. However, tung oil incorporation into the caseinate matrix did not affect significantly the WVP at any of the three levels used, probably due to pore formation. The advantages of cross-linking composite films using heat were also addressed.

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1. Introduction

The use of films or coatings made from biobased materials, as an alternative to polymeric non-biodegradable ones, has arisen considerable interest from researchers and manufacturers (Fabra, Talens, & Chiralt, 2009). Among renewable biomass based films, those made from proteins and polysaccharides have generally good organoleptic and mechanical properties, also acting as good barriers to noncondensable gases (O_2 , CO_2 , N_2) and aromas (Fabra, Hambleton, et al., 2008). However, protein-based films display high water and moisture absorption, due to hydrophilicity of the amino acids that form the protein molecules (Anker, Berntsen, Hermansson, & Stading, 2002; Prodpran, Benjaku, & Artharn, 2007; Quezada Gallo, Debeaufort, Callegarin, & Voilley, 2000). The water-vapor permeability (WVP) values of protein films are normally 2–4 orders of magnitude higher than that of low-density polyethylene (LDPE). This is due to the presence of free hydroxyl groups in the matrix, which interact strongly with migrating water molecules (Ghanbarzadeh & Oromiehi, 2008). Additionally, hydrophilic plasticizers necessarily incorporated into the protein films to improve the film flexibility, further decrease their water-vapor barrier properties (Prodpran et al., 2007). Moisture considerably affects the WVP and oxygen permeability of protein films, which is attributed to the plasticization effect of water on protein films (Ghanbarzadeh & Oromiehi, 2008).

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In many applications, a better barrier against WV is desirable since low levels of water activity must be maintained in low-moisture foods to prevent texture degradation and to minimize deteriorative chemical and enzymatic reactions (Anker et al., 2002) (lipid oxidation, Maillard browning and enzymatic browning) as well as microbial growth, that proceed at rates strongly associated with water activity and water content of the foods (Yang & Paulson, 2000). Also, dry and crispy cereal foods may become soggy and lose their crispness upon water absorption, and soft-textured foods may become hard upon loss of water (Yang & Paulson, 2000). Composite films have also been used to retard dehydration in coated foods (Yang & Paulson, 2000).

Because of films made from a single film-forming polymer display good properties in some aspects but poor ones in other aspects, two or more film-forming polymers of different type are commonly used to prepare composite edible films by blending methods (Jia, Fang, & Yao, 2009). Indeed, to improve water-vapor barrier properties of protein films, hydrophobic substances such as lipids could be added. Conversely, lipid films are good moisture barriers, but they are usually opaque (Quezada Gallo et al., 2000). Fat and lipids (waxes, hydrogenated oils, triglycerides, oils, fatty acids) (Quezada Gallo et al., 2000) of different types have been successfully incorporated into protein- and carbohydrate-based films by means of lamination and dispersion or emulsion (Anker et al., 2002; Fabra, Talens, & Chiralt, 2008a; Fabra et al., 2009; Prodpran et al., 2007; Quezada Gallo et al., 2000; Yang & Paulson, 2000). The barrier properties of emulsion films are reported to be dependent on preparation techniques, component compatibility, type and quantity of lipids used, microstructural heterogeneity and

surface energy of the formed film (Ghanbarzadeh & Oromiehi, 2008; Fabra et al., 2008a). Improved film performances are obtained with a multicomponent system where hydrocolloids (proteins or polysaccharides) form a continuous and cohesive network, and the hydrophobic substances (lipids) provide the moisture barrier properties (Quezada Gallo et al., 2000).

Another way to improve water-vapor barrier properties is the introduction of cross-links within or between protein chains by physical (radiation or heat), enzymatic, or chemical modification (Gerrard, 2002; Han, Bourgeois, & Lacroix, 2009). Proteins have the ability to form intermolecular disulfide bonds during heat treatment (Gerrard, 2002; Maté & Krochta, 1996). They are formed by thiol–disulfide interchange and by the oxidative coupling of two cysteine residues that are adjacent within the protein matrix to produce disulfide cross-links (Gerrard, 2002; Han et al., 2009). This process is necessary to obtain a flexible film that retains its structural integrity in high moisture environments (Zinoviadou et al., 2009).

Although there has been extensive work on lipid–protein films (Fabra, Talens, & Chiralt, 2008b; Monedero, Fabra, Talens, & Chiralt, 2009; Shaw, Monahan, ÓRiordan, & ÓSullivan, 2002; Sosa-Herrera, Berli, & Martínez-Padilla, 2008), no information is available in tung oil–caseinate films. Tung oil is a vegetable oil extracted from the seeds of the tung tree. The main fatty acid component of this triglyceride is the α -elaeostearic acid, which contains three conjugated unsaturations (Sharma & Kundu, 2006). Moreover, tung oil is already approved by the US Food and Drug Administration (FDA) for use in all types of food-contact polymers.

On the other hand, commercial sodium caseinate has nutritional value and numerous functional properties, such as its solubility in water and its ability to act as emulsifier (Fabra et al., 2009). Sodium caseinate can easily form films from aqueous solutions due to its random coil nature and its ability to form extensive intermolecular hydrogen, electrostatic, and hydrophobic bonds. Films of milk protein are usually flavorless, tasteless, and flexible (Fabra, Hambleton, et al., 2008).

The objectives of this work are to develop sodium caseinate/tung oil composite films through emulsification and to evaluate the effect of the lipid content on the moisture sorption and permeability, as well as the mechanical and optical properties of the films. Additionally, the effect of thermal cross-linking on selected hybrid films (sodium caseinate–tung oil films) is also presented and analyzed.

2. Materials and methods

2.1. Materials

Sodium caseinate (SC) (88.9 wt% protein) was obtained from Lactoprot Deutschland GmbH (Kaltenkirchen, Germany). SC was used as the film-forming component of the hydrophilic continuous phase for emulsion based films. The average protein molecular weight is $22,600 \text{ g mol}^{-1}$ (Audic & Chaufer, 2005). Tung oil (TO) (84% α -elaeostearic acid (C 18:3)), supplied from Cooperativa Agrícola Limitada de Picada Libertad, Argentina, was used as the hydrophobic dispersed phase. The plasticizer used was glycerol (Gly), which was purchased from DEM Chemicals (Mar del Plata, Argentina).

2.2. Solution preparation

Sodium caseinate aqueous solutions with protein concentrations of 2.5% (w/v) were prepared by dispersion of the sodium caseinate powder in distilled water and continuous stirring for 3 h at room temperature. Appropriate amounts of glycerol were added to achieve a glycerol/protein (Gly/prot) weight ratio of 0.28. Concentrations of protein and glycerol were selected according to

the optimization of mechanical properties, which were discussed in previous publications (Pereda, Aranguren, & Marcovich, 2008, 2009a). Lipid fraction was incorporated in tung oil/protein weight ratios of 0.05, 0.1 and 0.15. 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 with the aid of filter fabric.

2.3. Film preparation

Films were prepared by casting technique, that is, 45 g of the film-forming dispersions were poured into each Teflon Petri dish (diameter = 14 cm) and dried at 35°C for approximately 10 h in a convection oven. After the excess of water was evaporated, the obtained films (final solid content ranged from 1.44 to 1.63 g for samples containing from 0 to 15 wt% tung oil) were peeled off from the plates and kept in a closed reservoir at constant relative humidity (RH) and temperature ($23 \pm 2^\circ\text{C}$) for 3 days. The films were further characterized and tested.

2.4. Analysis of film-forming mixtures

2.4.1. Emulsion stability

Emulsion stability was determined according to the method proposed by Rodríguez, Albertengo, and Agullo (2002). The film-forming mixtures were allowed to stand up during 48 h.

2.5. Properties of films

2.5.1. Film thickness

The film thickness was measured with a 0–25-mm manual micrometer with an accuracy of $\pm 0.01 \text{ mm}$ in four random locations for each film.

2.5.2. Film opacity and color

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 \times nanometers/millimeters (AU nm/mm). Measurements were taken in triplicate for each sample.

Color related properties of the film were determined using a NoviBond Colorimeter RT500 (Neu-Isenberg, Germany) with an 8 mm diameter measuring area. Measurements are expressed as L^* , a^* and b^* (lightness ' L ', red-green ' a ' and yellow-blue ' b ') coordinates of the color space CIELab (Gennadios, Weller, Hanna, & Froning, 1996; Kunte, Gennadios, Cuppett, Hanna, & Weller, 1997), that were used to measure lightness, redness, and yellowness.

The measured coordinates were used to calculate total color difference (ΔE) with respect to the control caseinate film and whiteness index (WI), as given by Eqs. (1) and (2) (Monedero et al., 2009):

$$\Delta E = \sqrt{(\Delta a^*)^2 + (\Delta b^*)^2 + (\Delta L^*)^2} \quad (1)$$

$$\text{WI} = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (2)$$

Film samples were placed on a white standard plate and results were expressed as the average of six samples.

2.5.3. Scanning electron microscopy (SEM)

The surface and the cross-section of the films (obtained by cryo-fracture after immersing samples in liquid nitrogen), were analyzed using a scanning electron microscope (JEOL, model JSM-6460 LV, Tokyo, Japan). For this purpose, the pieces of the films were mounted on bronze stubs using a double-sided tape and then coated with gold, before being observed under the microscope.

2.5.4. Infrared spectroscopy

FTIR spectra of the films were recorded using the attenuated total reflection method (ATR) in a Genesis II (Mattson) Fourier transform infrared spectrometer. On the other hand, oil was examined by transmission using smeared samples on NaCl windows. In both cases, the spectra were recorded over a range of 600–4000 cm^{-1} with a resolution of 2 cm^{-1} and averaged over 32 scans.

2.5.5. Mechanical properties

Prior to testing the mechanical properties, films were conditioned for 48 h at $50 \pm 5\%$ RH at 25 °C. Tensile tests were performed at room temperature (23 ± 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^{-1} . The ultimate strength (σ_b), elongation at break (ϵ_b) and elastic modulus (E) were calculated as described in ASTM D638-94b (ASTM, 1994).

2.5.6. 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. All the samples were conditioned at 50% RH three days before the test. Seven parallel measurements were performed for each film at 24 °C, with a precision of ± 2 °C.

2.5.7. 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 ± 2 °C, to obtain water sorption isotherms. 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:

$$M_t = \frac{(W_t - W_0)}{W_0} \times 100 \quad (3)$$

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 unidimensional diffusion of a solute into a sheet (Crank, 1956):

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp \left[-D \frac{(2n+1)^2 \pi^2 t}{l^2} \right] \quad (4)$$

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.

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

2.5.8. Water-vapor permeability (WVP)

The water-vapor transfer rate [g/s 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 64.5% RH, to ensure equilibrium conditions. After that, film specimens were sealed on acrylic permeation cups (5 cm diameter) containing dry Cl_2Ca (0% RH) or liquid 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 [$\text{g m}/(\text{Pa s m}^2)$], was calculated as follows:

$$\text{WVP} = \Delta W y [A \Delta t (p_2 - p_1)]^{-1} \quad (5)$$

where ΔW is the weight of water absorbed in the cup (g), Δt 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.

2.5.9. Total soluble mass (TSM)

The total soluble mass (TSM) was expressed as the percentage of the film dry mass solubilized 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). This test was carried out in distilled water (30 mL) and in the presence of sodium azide (0.02%) in order 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 °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 °C during 24 h.

The TSM was calculated as following:

$$\text{TSM (\%)} = \frac{m_0 - m_f}{m_0} \times 100 \quad (6)$$

2.5.10. 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.

3. Results and discussion

3.1. Emulsion and film formation

The appearance of the emulsion was milky, and no macroscopic phase separation (separation of liquid layers) was visually observed in any of the samples prepared from caseinate/tung oil after 48 h at room temperature, indicating the efficiency of caseinate in stabilizing the oil droplets in the system. The emulsifying ability of

caseinate in the oil-in-water emulsions has been reported in the literature and explained as a result of its amphiphilic chemical characteristics (Fabra et al., 2008a, 2008b, 2009; Sosa-Herrera et al., 2008).

Average film thickness was 0.11 ± 0.014 mm, and was unaffected by tung oil concentration.

3.2. Optical properties

Optical properties are, to a great extent, relevant to the film functionality because of its great impact on the appearance of coating or packaging (Mali, Grossmann, García, Martino, & Zaritzky, 2004; Yang & Paulson, 2000). Table 1 shows the opacity values of sodium caseinate–tung oil films. While caseinate film without lipids was rather transparent, the addition of lipid generally caused the films to become whitish/milky (Yang & Paulson, 2000). In the present case, addition of tung oil resulted in a sharp increase in the film opacity, which increased as the concentration of the lipids increased (Table 1). Our results are in agreement with those reported by Shaw et al. (2002), Fabra et al. (2009), Monedero et al. (2009) and Pérez-Mateos, Montero, and Gómez-Guillén (2009), for different protein–lipid systems. Yang and Paulson (2000) reported that the differences in opacity of films were determined by the optical properties of the incorporated lipids. Nevertheless, it is known 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 (Monedero et al., 2009; Villalobos, Chanona, Hernández, Gutiérrez, & Chiralt, 2005). Thus, this increase in film opacity arises from the light scattering produced by the lipid droplets dispersed in the emulsion and thoroughly distributed in the polymer network that constitutes the film (Yang & Paulson, 2000), reducing the overall light transmitted through it (Prodpran et al., 2007). The incorporation of tung oil in concentrations above 10 wt% lead to a marked opacity, which could be associated to a wide size distribution and larger droplets of tung oil in the caseinate emulsion.

In order to further evaluate the differences between neat sodium caseinate films and those prepared with different amounts of tung oil, color coordinates were calculated from the spectral distribution of the films obtained on a standard white plate, and are reported in Table 1. The differences in lightness (L^*) between the different samples are minor, with a slight decreasing trend as tung oil content increases. Red-green (a^*) and Whiteness (WI) values follow a similar decreasing general tendency. Concerning yellow-blue (b^*) coordinates and total color differences (ΔE), these parameters clearly increase as tung oil increases. Taking into account that the chromaticity components of the color space CIELab were obtained on a standard white plate, a decrease of WI and L^* values with respect to the control film may reflect a decrease of transparency or a gain of color of the films. In this sense, the incorporation of a yellowish lipid, contributes to strengthen the color of the films.

In order to study the film morphology and to investigate the formation and distribution of oil droplets in the films containing

tung oil, Fig. 1 shows SEM micrographs of the upper film surface (in contact with air during molding). No important differences were noticed in the cross-section of the different films (micrographs not shown).

On the contrary, the existence of the lipid-rich phase is clearly confirmed by this technique when the surface of the films is observed, as shown in Fig. 1: the control sample (28 wt% glycerol, without tung oil) shows a smooth and continuous surface. On the other hand, with the incorporation of tung oil, a heterogeneous structure, composed of a continuous protein matrix with inclusions of lipid globules, more or less homogeneously distributed, is formed. The amount of lipidic globules increases as oil concentration increases, as expected. A closer observation reveals that in the low concentrated samples (5 and 10 wt% oil) these globules are formed by spherical discrete droplets of 3–8 μ m average diameter, which result from the stability of the emulsion due to the ability of sodium caseinate. For the film containing 15 wt% tung oil, the droplet shape becomes somewhat irregular and larger droplets appear, being some of them larger than 10 μ m.

The micrographs confirm the results obtained in the absorbance spectrum, that is poor dispersion of oil droplets at concentration higher than 10 wt%. Films containing higher oil concentration show uneven lipid distribution that results from the limited dispersion capability of the oil and from the poor stability of the oil-concentrated emulsions, as it was also observed by Yang and Paulson (2000) in gelatin films containing more than 25 wt% lipid.

3.3. Infrared spectroscopy

Fig. 2a shows the FTIR spectra corresponding to the tung oil and to the unplasticized (without glycerol) SC films prepared with and without the addition of the lipidic additive.

The FTIR spectrum of the tung oil shows a negligible absorption at $3400\text{--}3500\text{ cm}^{-1}$ indicating an insignificant content of --OH groups, as expected. The peak at 3015 cm^{-1} corresponds to the *trans*–*trans* conjugated unsaturations and the peak at 1745 cm^{-1} to the carbonyl in the ester groups from the triglyceride molecules. The existence of a strong peak at 993 cm^{-1} is assigned to the presence of the *trans*–*trans* conjugation in the elaeostearic chains. The small peaks at 964 cm^{-1} and 735 cm^{-1} are the result of the presence of *cis* unsaturations.

The FTIR spectrum of the SC neat film was analyzed in detail in a previous publication (Pereda et al., 2008).

On the other hand, the spectrum of the film obtained from the emulsion prepared with 15 wt% of tung oil shows both, the distinctive peaks of the tung oil plus the characteristic bands attributed to SC. The peaks attributed to the *trans*–*trans* conjugated unsaturations of the tung oil appears shifted in the emulsion film spectra, at 988 and 3061 cm^{-1} . However, the emulsion film spectrum cannot be successfully constructed from the weighed contributions of the TO and SC spectra, which could be related to a certain degree of interaction between both phases.

Finally, Fig. 2b shows the $4000\text{--}2500\text{ cm}^{-1}$ region for sodium caseinate films prepared with both, glycerol (plasticizer) and tung

Table 1
Color coordinates and opacity values for plasticized (28% Gly/prot) caseinate–tung oil films.

TO/prot (%)	Opacity/thickness (AU nm/mm)	L^*	a^*	b^*	ΔE^a	Whiteness
0	1505.04 ± 150.50^a	87.95 ± 1.29^a	0.58 ± 0.08^a	-2.11 ± 1.09^a	—	88.49 ± 0.49^a
5	1787.66 ± 18.83^b	$86.62 \pm 1.09^{a,b}$	-0.32 ± 0.17^b	4.59 ± 2.78^b	6.88 ± 2.81^a	85.85 ± 1.53^b
10	2378.25 ± 389.57^c	87.43 ± 0.73^a	-0.85 ± 0.29^c	8.11 ± 1.69^c	$10.21 \pm 1.88^{a,b}$	85.06 ± 1.27^b
15	5220.95 ± 516.56^d	85.95 ± 0.69^b	-0.45 ± 0.15^b	12.2 ± 2.08^d	13.89 ± 1.91^b	81.88 ± 1.65^c

Different superscripts within a same column indicate significant differences among formulations ($P < 0.05$).

^a ΔE values calculated respect to caseinate films without tung oil (TO/prot = 0).

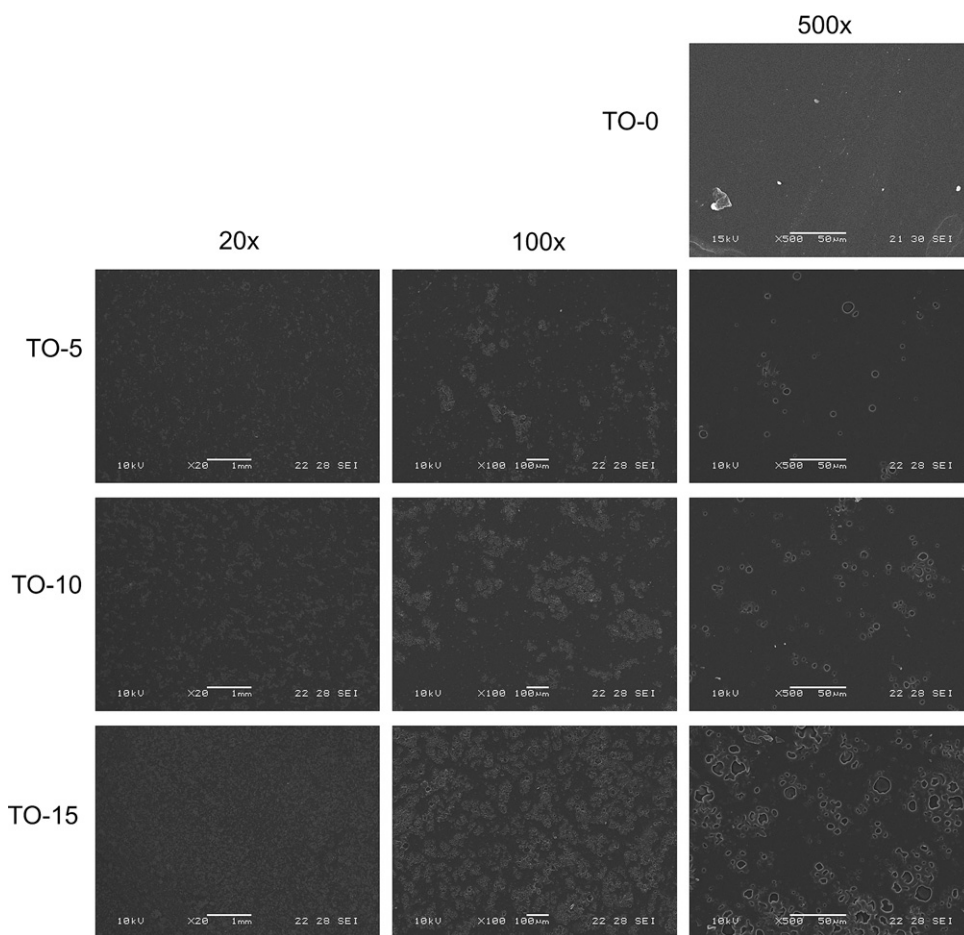


Fig. 1. SEM micrographs of sodium caseinate–tung oil films (0, 5, 10 and 15 wt% TO/protein).

oil (lipidic additive). The band centered at 3300 cm^{-1} (associated to intra and intermolecular H bonds) is reduced with tung oil incorporation, suggesting a reduction in the hydrophilic character of the films.

3.4. Mechanical properties

Mechanical strength and extensibility are generally required for a packaging film to withstand external stress and maintain its integrity. Additionally, good performance as barrier to moisture and gases is another usual requirement for applications in packaging (Yang & Paulson, 2000).

Since some oils can act as plasticizers in protein-based films (Andreuccetti, Carvalho, & Grosso, 2009; Bertan, Tanada-Palmu, Siani, & Grosso, 2005; Monedero et al., 2009; Monedero, Fabra, Talens, & Chiralt, 2010) the viability of the tung oil was evaluated in this respect. The first rows of Table 2 show the tensile behavior of SC films prepared without plasticizer, with 15 wt% of glycerol and with 15 wt% of tung oil. The non-plasticized film exhibits high modulus and ultimate stress at expenses of a reduced elongation. The opposite behavior (lower modulus, higher ultimate deformation) is registered for the sample plasticized with glycerol, confirming its plasticizing role. Moreover, the stress at break is also reduced due to the plasticizing effect of glycerol that could distribute uniformly throughout the film matrix, interacting with protein chains through hydrogen bonding and disrupting some of the primary protein–protein interactions. On the other hand, the film prepared with 15 wt% of tung oil displays reduced modulus

and ultimate stress in comparison with the neat SC film, but higher than the properties corresponding to the glycerol-plasticized film. It also shows a very low ultimate deformation, and the difference was not significant respect to the neat SC sample ($P < 0.05$). This feature determines that the films cannot be folded without cracking and thus, tung oil is not useful to increase the flexibility of the SC films. Consequently, for the following analysis, films with 28% of glycerol and different amounts of tung oil were prepared. The mechanical properties of these glycerol-plasticized films are also reported in Table 2. An increase in the elastic modulus and the ultimate strength can be observed when TO is added to glycerol-plasticized films. No significant differences were observed with the increase of oil concentration ($P < 0.05$). Since tung oil constituted a non-miscible emulsified phase in the film-forming solution, the protein chains segregated to form a protein-rich phase. The increasing protein aggregation favored protein–protein interactions to a greater extent and, as a consequence, higher tensile strength and modulus were observed in comparison with those found in the film without oil. Moreover, the glycerol (completely soluble in tung oil) could partially migrate to the lipidic phase during the film formation, decreasing its plasticizing effect and thus, increasing the rigidity of the films. These observations are consistent with the results reported by Prodpran et al. (2007) and Wang, Auty, Rau, Kerry, and Kerry (2009) for protein–palm oil and gelatin–corn oil systems, respectively.

At the same time, a decrease in the elongation at break is observed. The reduced continuity and cohesion of the protein network in the presence of lipid globules might be the reason of

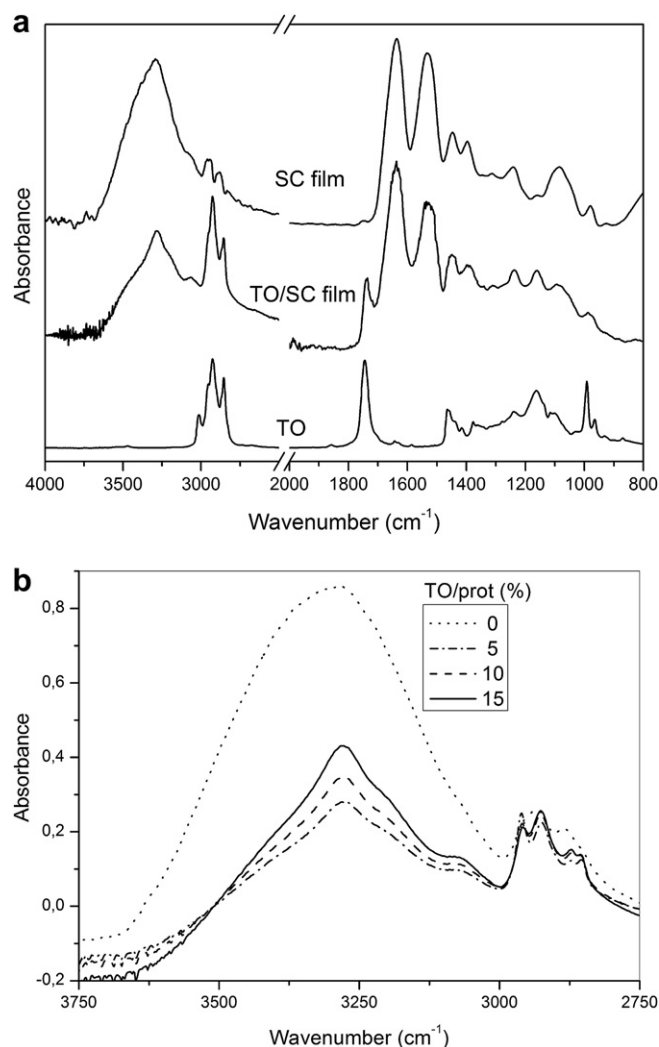


Fig. 2. FTIR spectra of a) neat TO, neat SC film and TO/SC film (15 wt% TO respect to protein); b) plasticized SC films with different concentrations of tung oil.

this behavior, as pointed out by Prodan et al. (2007) and Zinoviadou, Koutsoumanis, and Biliaderis (2009). The discontinuities introduced by the lipid addition may affect the stretching ability of the film depending on the characteristics of the lipids added. Wang et al. (2009) explained this behavior indicating that corn oil occupies the space within gelatin protein network, hence limiting the stretching capacity and mobility of the films. Since absorbed water acts as plasticizer in hydrophilic films (Quezada Gallo et al., 2000), the lower water content of film containing lipids (lower plasticizing effect) may also lead to the decreased

elongation of the film. It means that tung oil has an anti-plasticizing effect on sodium caseinate. Some authors have reported a similar effect on the mechanical response of methylcellulose films modified with liquid lipids (Debeaufort, Quezada-Gallo, Delporte, & Voilley, 2000), and in protein films from round scad containing palm oil (Prodpran et al., 2007). In contrast, other studies on the incorporation of fats or lipids into edible films have shown that the lipid layer could act as a plasticizer for biopolymeric films (Anker et al., 2002; Fabra et al., 2008b; Shaw et al., 2002; Yang & Paulson, 2000; Zinoviadou et al., 2009), what has been related to the lack of structural integrity of the lipid layer.

3.5. Contact angle

The main objective of this study was to reduce the hydrophilicity of films by the introduction of lipids in the system; therefore, the superficial hydrophilicity of films was determined by the contact angle method. In general, higher angles represent less hydrophilic surface. Table 3 reports the results of contact angle measurements using ethylene-glycol as the polar solvent. The measurements were performed in both film sides: the upper one, which was in contact with air during the drying step and the lower one, which was in contact with the Teflon mold. As it was expected, contact angle values increase with tung oil addition, which is due to the hydrophobic nature of the added lipid. Comparing the values measured for the lower surface, an increase of 78% with respect to the control sample is obtained with the addition of the minimum amount of oil (5 wt%) used in the study. Further addition (10 wt%) leads to further increase of the contact angle, but the incremental change is not as dramatic as the one just reported, probably because tung oil content is near the caseinate maximum emulsification capacity. However no significant changes ($P < 0.05$) are observed when TO concentration further increases to 15 wt%, which is also in agreement with the inhomogeneous dispersion of the lipid globules, observed previously by SEM. The values measured for the upper surface ranged from rather similar (if the standard deviation is considered) to a little higher compared with those of the lower surface for low oil concentrations, and much higher for the 15 wt% oil samples, indicating that the lipid phase tends to concentrate in that surface, since hydrophobic components tend to migrate to the air interface, which in this case is favored by the density difference of the two phases. Again, the observation is consistent with an oil saturated emulsion.

3.6. Moisture sorption

To investigate the effect of TO on the sodium caseinate films, a water-vapor sorption study was conducted at 75% RH and 25 °C. The absorption isotherms (at 24 °C) are shown in Fig. 3. Experimental humidity absorption data indicate a high rate of absorption at short times, to finally reach an equilibrium moisture content (EMC) that depends on the tung oil content, being lower as the oil concentration increases due to the hydrophobic nature of the lipid.

Table 2
Tensile properties of sodium caseinate–tung oil films.

TO/prot (%)	Gly/prot (%)	E (MPa)	σ_b (MPa)	ϵ_b (%)
0	0	2908.0 ± 121.9 ^a	57.0 ± 0.3 ^a	4.0 ± 0.5 ^a
0	15	802.8 ± 63.2 ^b	13.6 ± 1.3 ^b	11.6 ± 5.6 ^b
15	0	1202.4 ± 62.5 ^c	25.8 ± 2.7 ^c	3.0 ± 0.6 ^a
0	28	251.0 ± 20.3 ^d	6.3 ± 0.2 ^d	63.2 ± 6.0 ^c
5	28	298.4 ± 30.5 ^{d,e}	7.1 ± 0.4 ^e	45.1 ± 12.8 ^c
10	28	325.3 ± 47.8 ^e	8.3 ± 0.8 ^e	46.9 ± 17.2 ^c
15	28	330.2 ± 34.3 ^e	8.3 ± 0.4 ^e	45.9 ± 17.7 ^c

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

Table 3
Contact angle values of sodium caseinate–tung oil films.

TO/prot (%)	$\theta_{\text{ethylene-glycol}}$ (°)	
	Lower-side	Upper-side
0	26.31 ± 1.59 ^a	
5	46.57 ± 3.43 ^b	48.38 ± 3.80 ^b
10	58.96 ± 1.95 ^c	61.75 ± 3.91 ^c
15	55.93 ± 3.02 ^c	70.60 ± 3.59 ^d

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

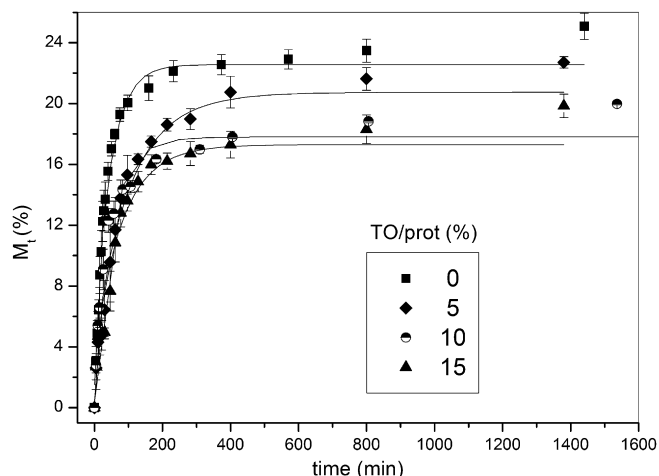


Fig. 3. Water-vapor absorption of sodium caseinate–tung oil films (75% RH, 24 °C).

The experimental moisture sorption data were modeled according to the Fick law (equation (4)), showing a reasonable agreement until about 400 min (see Fig. 3). At longer times the predictions deviate substantially from the experimental data. For this reason, the effective diffusion coefficients (fitting parameters, reported in Table 4) were obtained using the water absorption measured at 400 min as the equilibrium moisture content for Fickian behavior (FEMC). It should be noticed that this FEMC differs from the EMC reported in Table 4, since the last one was measured after 1500 min exposure.

As it was discussed in different publications, a dependence of the diffusion coefficient with moisture is expected due to the hydrophilic character of the sodium caseinate, which is not considered by the Fick law (Pereda et al., 2009a). Among the effective diffusion coefficients of the oil modified films, the sample with 5 wt% tung oil shows the lowest one (see Table 4). In this case, the water molecules are more retained in the film since it is less hydrophobic than the samples prepared with 10 and 15 wt% TO. This effect has been already discussed in previous works (Pereda et al., 2009a; Pereda, Aranguren, & Marcovich, 2009b). However, the control film is out of this trend, probably because it was cast from a single phase solution.

3.7. Water-vapor permeability

Table 5 reports WVP measurements of films containing tung oil. The observed behavior showed a moderate improvement to water-vapor permeation with the incorporation of lipid as a consequence of the reduced affinity to water and the increase in the tortuosity of the vapor diffusion-path through film due to the presence of lipid globules (Anker et al., 2002). However, tung oil incorporation in the caseinate matrix decreases only marginally the WVP at any of the three levels used. The same behavior was observed by Zinoviadou

Table 5
WVP of sodium caseinate–tung oil films.

TO/prot (%)	Thickness (mm)	WVP (% RH 0/64.5) (g m/Pa s m ²) × 10 ¹⁰
0	0.088	1.288 ± 0.083 ^a
5	0.104	0.923 ± 0.045 ^b
10	0.118	1.103 ± 0.058 ^b
15	0.118	0.986 ± 0.091 ^b

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

et al. (2009) on whey protein isolate films containing oregano oil. This illustrates that sometimes emulsion films are rather poor barriers against WV, since the water molecules still permeate through the non-lipid phase. In contrast, other studies on incorporation of fats or lipids into edible films, for example, gelatin films modified with *Brazilian elemi* (Bertan et al., 2005) and soy protein films with oleic acid and beeswax (Monedero et al., 2010); have shown improvements in water-vapor barrier properties (Bertan et al., 2005; Monedero et al., 2010); in these studies, however, higher concentrations of the lipid components were added to the films (protein:lipid 1:0.5).

It was noticed that defoaming of the emulsified film-forming solutions deteriorated, to some extent, the stability of the emulsion systems, leading to heterogeneous distribution of the lipids in the film matrix. This effect has been reported by Yang and Paulson (2000) on gelatin films with stearic and palmitic oils. On the other hand, although de-aeration has a deleterious effect on emulsification, it is useful to obtain non-porous films. Without this step, air trapped in the emulsion originates small pores within the final film, leading to high WVP values (Ghanbarzadeh et al., 2007; Wang & Padua, 2005). Actually, the pores observed inside the films, in the SEM micrographs (Fig. 1) would strongly contribute to this behavior, facilitating the transport of the water molecules through the film.

Moreover, it has been demonstrated that final WVP of composite films formed from different biopolymers and lipids strongly depend on the type, quantity and structure of the lipid incorporated (Bertan et al., 2005). Solid lipids (waxes) show better water barrier properties than liquid lipids (oils) (Bertan et al., 2005). This was explained in terms of the relative water-vapor solubility in the liquid/solid phase and/or of the molecular organization of the lipids.

From the previous results (tensile strength, opacity values, contact angle measurements, moisture absorption), it can be concluded that the maximum amount of tung oil that could be added to caseinate films in order to obtain improved properties appears to be 10 wt% with respect to the protein weight content. For this reason, this formulation was selected to be crosslinked by a physical treatment (heating), based on previous works on heated protein films, in which most of the properties were improved by this method (Kim, Weller, Hanna, & Gennadios, 2002; Micard, Belamri, Morel, & Guilbert, 2000; Pereda et al., 2009a; Pérez-Gago, Nadaud, & Krochta, 1999; Rhim, Gennadios, Handa, Weller, & Hanna, 2000). Preliminary results (Pereda, Aranguren, & Marcovich, 2009c) indicated that a heating procedure of 6 h at 95 °C leads to sodium caseinate films with well balanced properties, i. e: reduced solubility in water (measured as total soluble matter) and good mechanical stiffness. Thus, this treatment was performed on caseinate films containing 10 wt% of tung oil (TOH) and the resulting properties are compared in Table 6 with those corresponding to the non-heated film (TO). Heating produces the reduction of soluble mass (TSM = 58%) as a result of the sample cross-linking, which is due to protein–protein linkages formed at high temperatures, specifically disulphide bridges (Gerrard, 2002;

Table 4
Moisture absorption behavior of sodium caseinate–tung oil films.

TO/prot (%)	Thickness (mm)	EMC (%)	Fick Model D (m ² s ^{−1}) × 10 ¹³
0	0.09 ± 0.02	25.08 ± 0.87 ^a	2.83 ± 0.73 ^a
5	0.10 ± 0.01	22.71 ± 0.37 ^b	1.81 ± 0.21 ^b
10	0.08 ± 0.02	20.13 ± 0.83 ^c	2.47 ± 0.36 ^a
15	0.12 ± 0.00	19.84 ± 0.77 ^c	3.14 ± 0.41 ^a

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

Table 6

Mechanical and physical properties of TO/protein films (10 wt%), without (TO) and with thermal treatment (TOH).

Films	Mechanical properties					Color parameters				
	E (MPa)	σ_b (MPa)	ε_b (%)	TSM (%)	Opacity/ thickness (AU nm/mm)	WI	ΔE	Contact angle ($^\circ$)	WVP ^a $\times 10^{10}$ (g m/Pa s m ²)	EMC (%)
TO	325.3 \pm 47.8 ^a	8.3 \pm 0.8 ^a	46.9 \pm 17.2 ^a	100.0 \pm 0.0 ^a	2378.3 \pm 389.6 ^a	85.06 \pm 1.27 ^a	10.21 \pm 1.88 ^a	58.9 \pm 1.9 ^a	9.50 \pm 0.25 ^a	18.9 \pm 0.9 ^a
TOH	600.9 \pm 33.3 ^b	12.0 \pm 0.4 ^b	33.7 \pm 8.8 ^a	56.7 \pm 4.2 ^b	3600.3 \pm 353.5 ^b	74.19 \pm 1.48 ^b	22.46 \pm 2.00 ^b	57.8 \pm 1.3 ^a	7.60 \pm 1.17 ^b	16.9 \pm 0.6 ^b

Different superscripts within the same column indicate significant differences between formulations ($P < 0.05$).^a % RH inside–outside: 100/64.5.

Maté & Krochta, 1996). The cross-linking is accompanied by the improvement in mechanical properties (modulus and tensile strength) (see Table 6) and also by a reduction in equilibrium moisture content (from 19 to 17%), in agreement with the less hydrophilic nature of the heated film. The cross-linking and strong bonding generated between the protein chains during the thermal treatment, lead to reduced water affinity and final water content since less polar groups are available to interact with or to retain water molecules. Similarly the WVP, this time measured in the most unfavorable case (higher water-vapor pressure, 100/64.5 instead of 0/64.5 as in the previous measurements), decreases with the heating treatment. However, the opacity value increased 50% for treated films (TOH), due to the more yellowish color achieved, which was also observed by others authors in soy protein films (Kim et al., 2002) and gluten films (Micard et al., 2000). In the same way, WI decreases and total color difference increases appreciably. Surface hydrophilicity is not affected by this treatment, which is evidenced in the values of contact angles, being the difference between samples not significant ($P < 0.05$).

4. Conclusions

Sodium caseinate–tung oil films were successfully prepared and characterized. Addition of lipid causes the films to become whitish, resulting in a sharp increase in opacity. Uneven lipid distribution was evidenced by SEM in films prepared with 15% tung oil, which most likely arose from the limited dispersion capability of the lipids.

Tung oil content affects the film mechanical response, increasing tensile strength and elastic modulus but decreasing stretchability. The higher aggregation of protein molecules in the protein-rich phase and the partial migration of the plasticizer to the lipid phase of the film were presumed.

Contact angle measurements, besides water absorption tests, confirm lower hydrophilicity of the samples (higher θ and lower EMC). Tung oil incorporation into the caseinate matrix did not affect significantly the WVP at any of the three levels used, probably due to pore formation.

Tung oil–caseinate films crosslinked by heating (95 $^\circ$ C, 6 h) showed great improvements: higher integrity in water (TSM = 58%), lower EMC and better mechanical properties (σ_b and E), maintaining acceptable stretching capabilities.

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