

## Sodium caseinate films containing linseed oil resin as oily modifier



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### ABSTRACT

Modified sodium caseinate films were prepared by casting of film forming emulsions prepared by adding different contents of a linseed oil based resin (LOR) into the aqueous protein solution. Infrared spectroscopy of the formed films revealed that physical interactions between the main component of the film and the oily modifier were developed. The films were also characterized in terms of water vapor permeability, water absorption, optical, mechanical and thermal properties. Water vapor permeability and equilibrium moisture content decreased with the addition of LOR, reaching their minimum values (59% reduction in WVP and about 70% reduction in EMC, respect to the corresponding values of the neat SC film) with the incorporation of 10 and 15 wt% of LOR, respectively. As expected, contact angle measured using a polar solvent and opacity increase (respect to the neat SC film) when the lipid modifier is used in the formulation, although the increment in the last property is lower than the reported in similar works. The presence of LOR decreased tensile modulus and strength but the elongation at break is not significantly affected.

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### 1. Introduction

Naturally renewable films, based on biopolymers such as proteins, polysaccharides, lipids or their combination, have attracted a continuously increasing research interest over the last two decades because of the wide field of potential applications, their low cost, great availability as well as their biodegradable character that avoid ecological imbalance and esthetic deterioration of nature (Bonilla, Atarés, Vargas, & Chiralt, 2012; Matsakidou, Biliaderis, & Kiosseoglou, 2013; Pavlath & Orts, 2009; Tharanathan, 2003).

Specifically, sodium caseinate (SC), a family of phosphoserylated proteins ( $\alpha_{s1}$ -,  $\alpha_{s2}$ -,  $\beta$ -,  $\kappa$ -caseins) (Holt & Sawyer, 1993), presents film-forming ability, either on its own (Schou et al., 2005), or in combination with polysaccharides (Kristo & Biliaderis, 2006; Pereda, Aranguren, & Marcovich, 2008) and/or hydrophobic compounds (Fabra, Perez-Masia, Talens, & Chiralt, 2011; Pereda Aranguren, & Marcovich, 2010). Due to the structure and amino acid composition of caseins, it is likely that hydrogen bonds, electrostatic interactions and most probably hydrophobic forces are involved in the formation of casein-based edible films (Schou et al., 2005).

This protein, in general, produces flavorless, tasteless, flexible and biodegradable films with good gas barrier properties (Cuq,

Gontard, Aymard, & Guilbert, 1997; Park & Chinnan, 1995). In addition, it provides nutritional value to the final product if used as an edible coating, it is water soluble and may additionally act as an emulsifier due to its surface activity, heat resistance and water-holding properties (Khwaldia, Perez, Banon, Desobry, & Hardy, 2004). Because of all of these characteristics, sodium caseinate presents potential use in different applications, that can include their direct use in food packaging (Pavlath & Orts, 2009) or as a barrier coatings on paper packaging materials (Khwaldia, Arab-Tehrany, & Desobry, 2010; Khwaldia, Basta, & Houssni El-Saied, 2014).

However, water-soluble hydrocolloid films present poor water barrier properties (Quezada-Gallo, 2009). A number of studies have been published on the incorporation of hydrophobic substances into the biopolymer film matrix, such as vegetable oils (Pereda, Aranguren, & Marcovich, 2010), essential oils (Zinoviadou, Koutsoumanis, & Biliaderis, 2009), waxes (Perez-Gago & Krochta, 2001) and fatty acids, to bring about a decrease in film water vapor permeability (WVP). The incorporation of hydrophobic materials can effectively modify the properties of protein films, as reported by Krochta, Hudson, and Avena-Bustillos (1990) for their films made of SC and acetylated monoglycerides that had a 41% reduction in water vapor transmission rate in comparison with control films (without lipid). However, the emulsification of the initial biopolymer-oil mixture is required, to obtain a uniform droplet distribution with small-sized droplets so as to increase the tortuosity factor and enhance, effectively, the water barrier

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performance of the film (Debeaufort, Martinpolo, & Voilley, 1993; Fabra, Perez-Masia, et al., 2011; McHugh & Krochta, 1994; Perez-Gago & Krochta, 2001; Vargas, Perdones, Chiralt, Chafer, & Gonzalez-Martinez, 2011).

The affinity of hydrophobic modifiers with the protein that forms the film can be improved by chemical modification of the oils. In this way, vegetable oil can be reacted with glycerol (transesterification) followed by esterification with maleic anhydride, to produce a maleinated monoglyceride (Mosiewicki, Aranguren, & Borrajo, 2005). This final oil based resin presents lower molecular weight and higher polar structure in comparison with the original oil.

In this study, a linseed oil based resin was chosen as the lipid additive. Linseed oil, also known as flax seed oil, is a clear to yellowish oil obtained from the dried ripe seeds of the flax plant (*Linum usitatissimum*, Linaceae). As a renewable resource from agriculture, the use of the linseed oil in industrial products has received much attention. In particular, this oil has been widely used as a drying oil in the painting industry since it contains a high percentage of linolenic acid, with 3 unsaturations per fatty acid chain (Formo, Jungermann, Norris, & Sonntag, 1985; Mallégol, Lemaire, & Gardette, 2000; Yang & Luo, 2013).

Although the properties of films based on caseinates have been studied in several publications, there are very few published data on sodium caseinate films modified with alternative oil based resins. The aim of the present study is, therefore, to report the properties of films based on sodium caseinate (SC) and a linseed oil based resin (LOR) made by casting of film forming emulsions.

## 2. Materials and methods

### 2.1. Materials

Sodium caseinate (SC) powder, containing 88.9 wt.% protein (the rest being lactose, lipids, attached moisture, and ashes), was obtained from Lactoprot Deutschland GmbH (Germany). The average protein molecular weight is 22,600 g/mol (Audic and Chaufer, 2005). Linseed oil based resin (LOR) was obtained from non-refined linseed oil (Grainer S.A., Entre Rios, Argentina) by glycerolysis and maleinization reactions (Mosiewicki et al., 2005).

### 2.2. Methods

#### 2.2.1. Linseed oil resin (LOR) preparation

The LOR was prepared in two-steps reaction: 1) transesterification of linseed oil with glycerol to obtain mainly monoglycerides, b) chemical reaction of the monoglycerides with maleic anhydride to produce a final resin with maleated half esters. The details of synthesis and characterization of LOR were published previously (Mosiewicki et al., 2005). The main component of this linseed oil-based resin is the monoglyceride bis maleate, which chemical structure is represented in Scheme 1. However, the resin also contains minor proportions of diglyceride mono maleate,

monoglyceride monomaleate, glycerol mono and bis maleate, and glycerol tris maleate (Mosiewicki et al., 2005).

#### 2.2.2. Preparation of the film forming emulsion

Sodium caseinate aqueous solutions with protein concentrations of 2.5% (w/v) were prepared by dissolving the sodium caseinate powder in distilled water and stirring continuously for 3 h at room temperature. Then, the lipid fraction was incorporated in LOR/protein weight ratios of 0.05, 0.1, 0.15 and 0.2, and stable emulsions were achieved by using both homogenizer (Ultra-Turrax, 3 min treatment) and ultrasonic (Elma- Elmasonic P, 4 times of 5 min each) treatments. The film-forming dispersions were defoamed under rest for 1 h at room temperature.

#### 2.2.3. Film preparation

Films were prepared according to the usual casting method, i.e. 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 water was evaporated, the obtained films were peeled off from the plates and kept in a closed reservoir at 50% relative humidity (RH) and constant temperature (23 ± 2 °C) for 3 days.

### 2.3. Characterization of the film forming emulsions

#### 2.3.1. Transmission optic microscopy (TOM)

The film forming emulsions were analyzed by TOM, using a microscope (Leica DMLB) coupled to a video camera (Leica DC100). For this purpose, a drop of the selected emulsion was mounted between glass-holder and glass-cover and pictures were taken at different magnifications.

### 2.4. Characterization of the films

#### 2.4.1. Film thickness

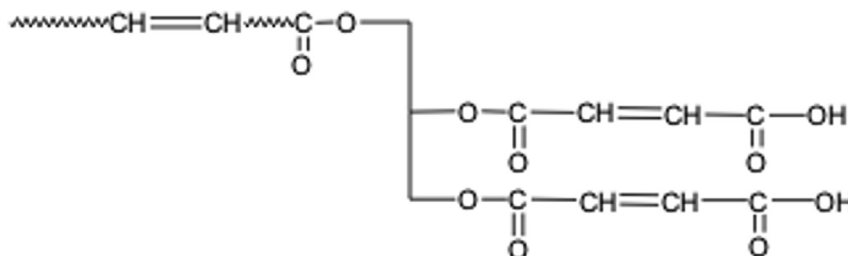
The film thickness was measured with a 0–25 mm manual micrometer with an accuracy of ±0.01 mm in four random locations for each film.

#### 2.4.2. 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. The 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<sup>-1</sup> with a resolution of 2 cm<sup>-1</sup> and averaged over 32 scans.

#### 2.4.3. Opacity and color properties

The color of the films was determined with a NoviBond Colorimeter RT500 (Neu-Isenberg, Germany) with an 8 mm diameter measuring area. A white standard color plate for the instrument calibration was used as a background for color measurement of the films. Results were expressed as *L\**, *a\** and *b\** (lightness 'L', red-



Scheme 1. Structure of the monoglyceride bis maleate.

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, respectively. The measured coordinates were used to calculate total color difference ( $\Delta E$ ) with respect to the control caseinate film and whiteness index ( $WI$ ), as given by Eqs. (1) and (2) (Monedero, Fabra, Talens, & Chiralt, 2009):

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

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

The opacity of a material is an indication of how much light passes through it. The higher the opacity, the lower the amount of light that can pass through the material (Casariego et al., 2009). 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 with a UV–Visible spectrophotometer Shimadzu 1601 PC (Tokyo, Japan). Film opacity was defined as the area under the curve divided by the film thickness and expressed as Absorbance Unit  $\times$  nanometer/millimeter (AU nm/mm).

Color results were expressed as the average of six samples while opacity measurements were taken in triplicate for each sample.

#### 2.4.4. Water vapor permeability (WVP)

This property was determined gravimetrically using the ASTM Method E96–95 (ASTM, 1995). Four specimens were tested for each film type. Prior to the test, the film was placed in a chamber maintained at room temperature for 3 days at 66% RH, to ensure equilibrium conditions. Then, the film specimen was sealed on acrylic permeation cup (5 cm diameter) containing distilled water (100% RH). The cup was 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.

#### 2.4.5. Surface hydrophobicity

This value 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  $\mu$ L) 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 for three days before the test. Seven parallel measurements were performed for each film at  $24 \pm 2$  °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  $23 \pm 2$  °C, to obtain water sorption isotherms. Samples were taken out of the chamber at regular time intervals and weighed with a precision of  $\pm 0.0001$  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:

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

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 absorption curves of the films 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  $M_\infty$  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.

#### 2.4.7. Dynamic mechanical analysis (DMA)

DMA tests were performed using a rheometer (DMTA IV, Rheometric Scientific) operated at rectangular torsion mode at 1 Hz, in the temperature range from  $-90$  to  $70$  °C, at a heating rate of  $2$  °C  $\text{min}^{-1}$ . The samples were subjected to a cyclic strain of 0.05%. This strain value was sufficiently small to ensure that the mechanical response of the specimen was within the linear viscoelastic range. All the samples were conditioned at 50% RH for three days before the test.

#### 2.4.8. Tensile properties

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 ( $\sigma_b$ ), elongation at break ( $\epsilon_b$ ) and elastic modulus ( $E$ ) were calculated as described in ASTM D638-94b (ASTM, 1994). Prior to running mechanical tests, films were conditioned for 48 h at 50% RH at 25 °C.

#### 2.4.9. Statistical analysis

Data values obtained in the experiments were statistically analyzed by one-way analysis of variance (ANOVA) employing OriginLab 8 software. Differences between pairs of means were assessed on the basis of confidence intervals using the Tukey test. The least significance difference was  $P < 0.05$ .

### 3. Results and discussion

The appearance of the film forming emulsions was milky, and no macroscopic phase separation was visually observed in any of the samples after 48 h at room temperature, confirming the efficiency of caseinate in stabilizing the linseed oil resin droplets in these systems. The emulsifying capability 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, Talens, & Chiralt, 2008a, 2008b; 2009; Sosa-Herrera, Berli, & Martínez-Padilla, 2008). In this sense, it is worth noting that casein is not a homogenous protein. Four different types of casein, namely  $\alpha_{S1}$ ,  $\alpha_{S2}$ ,  $\beta$  and  $\kappa$ , are found in bovine milk in the approximate ratio of 4:1:4:1 (Honda Chen, 2002; Hernandez-Izquierdo & Krochta, 2008). The major caseins ( $\alpha_{S1}$  and  $\beta$  caseins) contain neither cysteine nor cystine. The other two casein fractions contain only two half-cystine residues. The  $\beta$  and  $\kappa$  caseins are amphipathic proteins having hydrophobic and hydrophilic ends and, thus, are especially suitable for use as emulsifiers. Among the casein fractions,  $\beta$ -casein is the most hydrophobic and  $\alpha_{S2}$ -casein the most hydrophilic (Honda Chen, 2002).

The LOR resin also presents an amphiphilic character, which ensues from the hydrophobic characteristics of the fatty acid chain



(acyl residue) combined with the hydrophilic  $-\text{COOH}$  end groups (maleated half esters), as can be noticed from its chemical structure (Scheme 1), and thus, additionally contributes to the emulsion stability. To corroborate this assumption, film forming emulsions containing 10 and 20 wt% unmodified linseed oil were prepared on purpose and compared with those obtained with the modified resin. Accordingly, Fig. 1 shows the transmission optical micrographs of SC based emulsions containing 10 and 20 wt.% of unmodified linseed oil (Fig. 1a and b, respectively) and LOR (Fig. 1c and d). Fig. 1a and b denotes that the dispersion of linseed oil take place in spherical shaped beads of different sizes, but reasonably small ( $3\text{--}17\ \mu\text{m}$  for 10% oil;  $5\text{--}25\ \mu\text{m}$  for 20% oil) with the uneven presence of globules formed by the coalescence of drops, that increase in frequency and size as the linseed oil concentration increases. On the other hand, the micrographs of emulsions prepared from LOR show that the oily modifier drops are smaller and better dispersed through the aqueous SC, being the droplet distribution mostly unimodal, with almost no bead coalescence. Moreover, Fig. 1d reveals that with increasing LOR concentration the drops prefer change their shape to a more irregular (or less spherical) one instead of coalesce. This behavior is clearly associated to a higher affinity between SC and LOR in comparison with the original unmodified linseed oil.

The films obtained were homogenous, flexible and translucent in naked eye examination. Average film thickness was  $0.11 \pm 0.014\ \text{mm}$ , and was unaffected by the oil based resin concentration. All the films were completely dissolved in water after 24 h of immersion, indicating that no chemical bonds between SC and LOR were formed.

### 3.1. Optical properties

Optical characteristics are relevant to the final appearance of films, which can be an important factor to decide their final applications, for example, coating or packaging.

Table 1 shows the opacity values of the sodium caseinate films with different content of LOR. As was reported by other researchers (Fabra, Talens, & Chiralt, 2009; Pereda et al., 2010), the SC films

were visually moderately transparent, although the addition of LOR to the films provided them with a whitish appearance. This macroscopic observation agrees with the increase in their opacity values as the content of LOR in the film increases (see Table 1). In this present case, addition of 5 wt.% of LOR resulted in the sharpest increase in the film opacity (24% increment respect to the neat SC film, considering the average values), since the neat SC sample was prepared from a film forming solution while the 5% modified one was obtained from a film forming emulsion. As it was observed by several authors (Fabra et al., 2009; Monedero et al., 2009; Pereda et al., 2010; Pérez-Mateos, Montero, & Gómez-Guillén, 2009; Shaw, Monahan, ÓRiordan, & ÓSullivan, 2002), the lipid phase dispersed in a protein system causes a sharp increase in opacity because of differences in refractive index of the two phases. This augment arises from the light scattering produced by the lipid droplets thoroughly distributed in the polymer network that constitutes the film (Prodpran, Benjaku, & Artharn, 2007; Yang & Paulson, 2000). However, the increase in opacity depends not only on the lipid content but also on the size of dispersed oily drops in the films, and that is why the addition of 20 wt.% of LOR in the SC film increase only 64% its opacity value, which is a quite low change in comparison with the variation observed in other lipid-SC systems. For example, Pereda et al. (2010) reported an increase of 247% in the opacity with respect to the neat SC film with the addition of just 15 wt.% of tung oil.

Thus, this behavior is attributed to reduced droplet size and better droplet dispersion that was achieved prior to casting (and was maintained during film drying) using LOR of lower molecular weight and higher polarity respect to those of unmodified vegetable oils, as it was discussed above.

Color coordinates were determined from the spectral distribution of the films obtained on a standard white plate and the values are also shown in Table 1.

The differences in lightness ( $L$ ) among the different samples are small with a minor trend to increase with LOR incorporation if the average values are considered, but negligible if the statistical analysis is taken into account. Red-green ( $a^*$ ) coordinates increase with the addition of LOR, without significant differences with

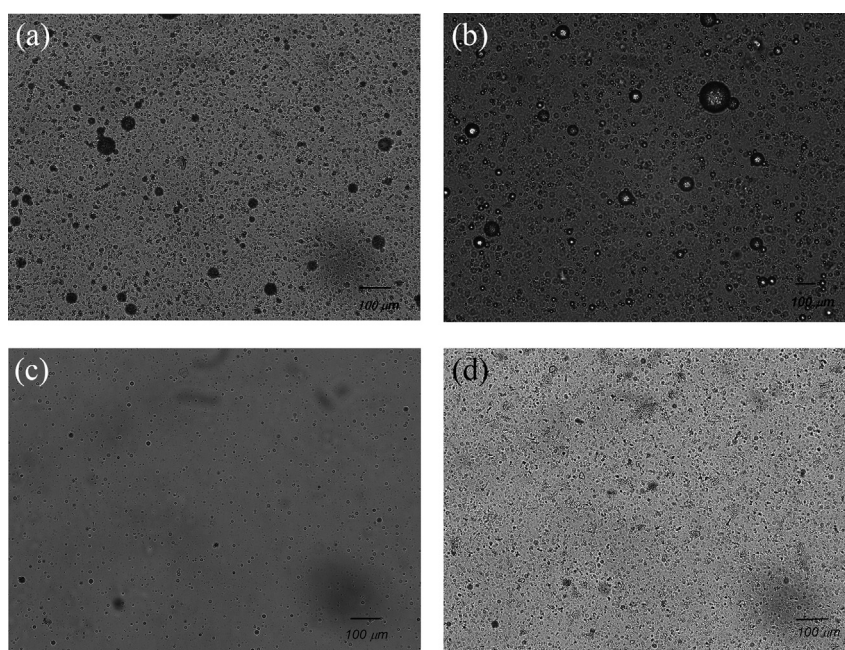


Fig. 1. Transmission optical micrographs of suspensions of SC with: (a) 10 wt.% linseed oil; (b) 20 wt.% linseed oil; (c) 10 wt.% LOR and (d) 20 wt.% LOR.

**Table 1**  
Optical properties. Opacity and color parameters of SC films with different contents of LOR.

LOR (%)	Opacity (UA*nm/mm)	$L^*$	$a^*$	$b^*$	$\Delta E$	WI
0	1695.17 ± 410.99 <sup>a</sup>	87.95 ± 1.29 <sup>a</sup>	0.58 ± 0.08 <sup>a</sup>	-2.11 ± 1.09 <sup>a</sup>	—	88.49 ± 0.49 <sup>a</sup>
5	2113.22 ± 191.51 <sup>a,c</sup>	88.90 ± 1.14 <sup>a</sup>	0.85 ± 0.22 <sup>a</sup>	0.53 ± 2.29 <sup>a</sup>	2.49 ± 0.67 <sup>a</sup>	88.69 ± 1.36 <sup>a</sup>
10	2360.45 ± 21.56 <sup>b,c</sup>	88.74 ± 1.16 <sup>a</sup>	0.83 ± 0.27 <sup>a</sup>	0.95 ± 2.64 <sup>a</sup>	2.66 ± 0.45 <sup>a</sup>	88.47 ± 1.52 <sup>a</sup>
15	2402.33 ± 181.31 <sup>c</sup>	87.68 ± 1.68 <sup>a</sup>	0.69 ± 0.15 <sup>a</sup>	1.26 ± 0.65 <sup>a</sup>	3.19 ± 0.83 <sup>a</sup>	88.19 ± 0.95 <sup>a</sup>
20	2773.16 ± 55.48 <sup>d</sup>	88.73 ± 0.85 <sup>a</sup>	0.86 ± 0.32 <sup>a</sup>	0.54 ± 2.99 <sup>a</sup>	2.09 ± 0.62 <sup>a</sup>	88.41 ± 1.26 <sup>a</sup>

Any two means in the same column followed by the same letter are not significantly ( $P > 0.05$ ) different according to Turkey test.

different amount of LOR added, indicating that the films containing LOR are slightly more red (or less green) than the neat SC one. *WI* of different samples are relatively similar without a clear effect produced by the addition of LOR. On the other hand, both yellow-blue ( $b^*$ ) coordinate and total color differences ( $\Delta E$ ) follow a similar increasing general trend. The increase in  $b^*$  (blue-yellow) denotes a tendency to yellowness in the films containing LOR. Color coordinates measured for films containing 20 wt.% LOR were found to be out of trend with respect to the samples containing less LOR but the  $b^*$  value is still higher than that of the neat SC film. However, according to the statistical analysis, none of these differences are of consideration, which is judged as an advantage since it indicates that the attractive visual properties of the SC films are not significantly modified by the addition of this linseed oil based resin.

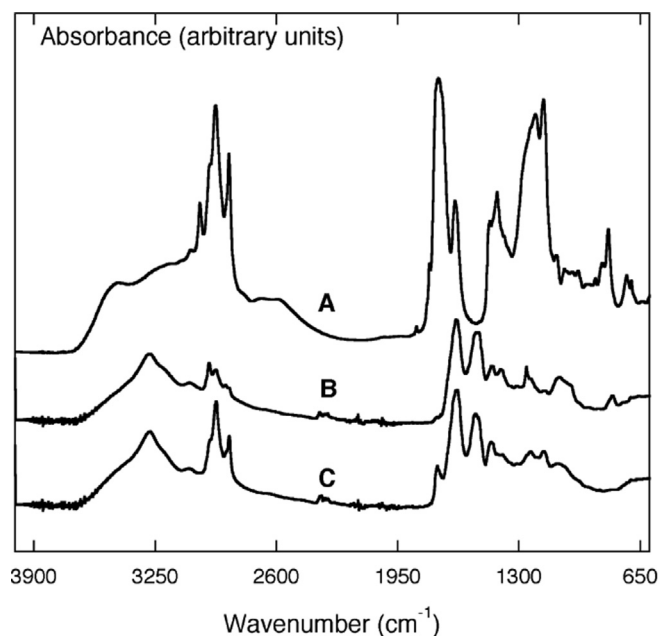
### 3.2. Infrared spectroscopy analysis

Fig. 2 shows the FTIR spectra of LOR (curve A), neat SC film (curve B) and SC film with the addition of 15 wt.% of LOR (curve C). The FTIR spectrum of the LOR (curve A) shows an absorption band located at 3450  $\text{cm}^{-1}$ , which is due to the O–H stretching. The band at 3010  $\text{cm}^{-1}$  is assigned to the absorption of C=C bonds, while the peak at 1730  $\text{cm}^{-1}$ , belongs to the stretch  $\nu(\text{C}=\text{O})$  in the ester groups. In addition, this infrared region is dominated by two strong bands at 2930 and 2850  $\text{cm}^{-1}$  resulting respectively from the asymmetric and the symmetric stretching vibrations of the CH, CH<sub>2</sub> and CH<sub>3</sub> groups. Another important contributions to this spectrum

came from the 1170  $\text{cm}^{-1}$  band attributed to the stretch  $\nu(\text{C}=\text{C})$  in maleates and from the 1420 and 820  $\text{cm}^{-1}$  peaks corresponding to  $-\text{H}_2\text{C}-\text{COOH}$  and  $=\text{CH}_2$  absorptions.

The SC spectrum (curve B) presents a band centered around 3290  $\text{cm}^{-1}$  associated to intra and intermolecular H bonds. A small band at 3070  $\text{cm}^{-1}$  is assigned to the NH structure, probably an overtone of the amide II absorption. The CH stretch region shows peaks at 2930 and 2960  $\text{cm}^{-1}$  corresponding to CH<sub>2</sub> and CH<sub>3</sub>, respectively, with a very small peak at 2870  $\text{cm}^{-1}$  from the CH (tertiary). The bands at 1630 and 1530  $\text{cm}^{-1}$  correspond to the amide I and amide II bands, typical of proteins, assigned to  $\beta$ -sheet and  $\alpha$ -helix structures (Barth, 2007; Dong et al., 1996; Kalnin, Baikolov, & Venyaminov, 1990).

From the spectrum corresponding to sodium caseinate/15wt% LOR film (curve C), it can be noticed a small shoulder at 3450 and a peak at 1740  $\text{cm}^{-1}$ , which were absent in the neat SC spectrum and clearly can be associated to the stretching vibration of the  $-\text{OH}$  groups and C=O in ester groups, respectively, coming from the modified oil. Moreover, the individual peaks corresponding to the individual contributions of CH<sub>2</sub> and CH<sub>3</sub> cannot be separated and now appeared at 2930  $\text{cm}^{-1}$  with higher intensity, due to the contribution of the LOR. Another peak that denotes the presence of the modified oil is the 1170  $\text{cm}^{-1}$  contribution, indicating that the C=C bonds are still available in the composite film. The absence of a band at about 1400  $\text{cm}^{-1}$  suggests that carboxylic groups of the LOR are not active in the composite film, probably involved in interactions with the amino acid side chains of the SC through the formation of hydrogen bonds, as reported in similar works (Fabra, Hambleton, et al., 2011; Fabra, Perez-Masia, et al., 2011).



**Fig. 2.** FTIR spectra of LOR, SC and SC film with 15 wt.% of LOR (curves A, B and C, respectively).

### 3.3. Contact angle

Comparing the wettability of different fluids on coatings or packaging films provides important information with respect to their expected interaction. For smooth surfaces, the most frequently applied approach for quantifying the wettability of plastic materials is the sessile drop method, whereby the contact angle is measured at equilibrium (Meiron and Saguy, 2007). The overall interaction between a plastic surface and a wetting liquid depends on the degree of contact achieved and the magnitude of the intermolecular forces involved. The latter are affected by several factors, including film surface free energy, liquid surface tension, and roughness. Typically, higher measured contact angles result in a reduced potential interaction of the fluid with the film (Meiron and Saguy, 2007). In particular, when using water or another polar solvent, contact angle will increase with increasing surface hydrophobicity (Hambleton, Fabra, Debeaufort, Dury-Brun, & Voilley, 2009; Pereda et al., 2010; Zia, Zuber, Mahboob, Sultana, & Sultana, 2010). Taking into account that one of the main objectives of this study was to reduce the hydrophilic character of protein films by the incorporation of a lipid modifier, ethylene glycol, a bipolar liquid (Ma, Tang, Yang, & Yin, 2013) with dispersive component similar to water (29 mN/m, Carré, 2007) and a relatively high polar component (19 mN/m, Carré, 2007) was chosen to

evaluate the ability of the edible films to resist liquids of moderate polarity. Water was discarded as the wetting liquid because the high solubility of caseinate films in water led to changes in the contact surface (due to solvation, hydration and swelling) from the initial time after deposition of the liquid and thus, the sessile drop did not reach its mechanical true equilibrium, a necessary condition for validate the measurements (Karbowski, Debeaufort, Champion and Voilley, 2006). Table 2 shows the contact angle of SC films with different content of LOR, using ethylene glycol as a solvent. The low contact angles, (lower than  $90^\circ$ ), measured for SC and SC-LOR films are typical of hydrophilic materials, like films based on proteins (Karbowski, Debeaufort, & Voilley, 2006). However, the addition of the oil resin into caseinate film-forming solutions was useful to decrease appreciably the hydrophilic character of the resulting film surface, as can be concluded from the comparison of contact angles presented by the modified films with that of the SC neat one. Notice that in this case the statistic analysis also confirms a favorable change by adding the lipid component to the SC solution. The addition of 5 wt.% of LOR increases the contact angle a 63% with respect to the value of the neat casein film, due to the relatively more non-polar character of the LOR that reduces the surface polarity of the films. The addition of higher percentages of LOR does not modify appreciably this property, as confirmed by statistical analysis, possibly because the film surface is already saturated with the lipid component. Similar results can be found in other related research works (Pereda et al., 2010).

### 3.4. Moisture sorption

Fig. 3 shows the moisture content of the films as a function of time performed at 75% RH and  $25^\circ\text{C}$ . The experimental values show the typical progress with high rate of absorption taking place at short times and then slowing down, to finally reach the equilibrium moisture content (EMC) after about 50 h exposition.

The moisture absorption data were fitted according to the Fick's diffusion equation for unidimensional diffusion of a solute into a sheet (equation (4)), obtaining from it the effective diffusion coefficient,  $D$ . The Fickian model was included for the samples with 0 and 10 wt.% of LOR as solid lines in Fig. 3. The curve corresponding to the neat SC (Fig. 3) presents a good fitting at the early stages of sorption, but at longer times the model prediction presents a deviation as was already discussed in a previous work: after a period of time in which the samples appeared to reach the equilibrium, they start to absorb water again although at a very slow rate. (Pereda, Aranguren, & Marcovich, 2009).

On the other hand, the addition of LOR introduces an additional deviation from the Fick's law that appears at short times of sorption. The LOR addition seems to delay the water sorption in the films during the first stage (below 1000 min), leading to a low-time pseudo plateau, to recover the sorption ability at longer times. As it is already known, the moisture absorbed by the films acts as

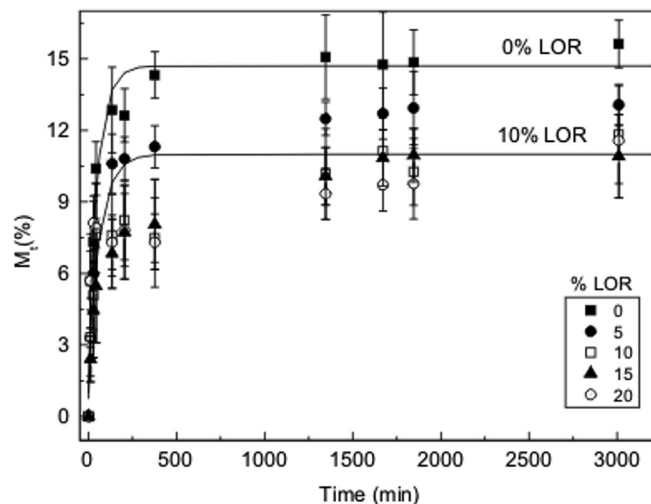


Fig. 3. Water absorption as a function of the time of SC films with different contents of LOR. Solid lines represent the Fickian model, applied to selected samples.

plasticizing agent and thus, allows an increase in the mobility of the polymeric chains that could lead to their reorganization. Probably the first plateau could be ascribed to the equilibrium moisture content of un-plasticized composite films, while the second corresponds to the equilibrium moisture contents of the water-plasticized samples.

The diffusion coefficients as well as the EMC values as a function of the LOR concentration are also shown in Table 2. From both, Table 2 and Fig. 3, it is clearly seen that the whole absorption process (including the EMC reached) depends on the LOR concentration in the films. The EMC decreases gradually with the increase of LOR content until reaching 15 wt% (comparison made on mean values), indicating an interesting reduction of the films ability to absorb water. However, higher lipid concentrations do not lead to any additional reduction. Nevertheless, a closer examination of these values in the light of the statistical analysis indicated that samples containing 10–20% LOR presented similar equilibrium moisture content, that is, that no additional reduction is obtained with more 10 wt% lipid. The same behavior is observed about the effective diffusion coefficient (notice values corresponding to 10, 15 and 20 wt% oil are in the same range), which indicates that the film forming emulsions are saturated with about 10 wt.% LOR.

The incorporation of lipids in hydrocolloid films usually change the overall hydrophobicity of the matrix as a consequence of the presence of more hydrophobic tails and the interactions among components that affect the total active points for water sorption (Fabra, Talens, & Chiralt, 2010). In this case, the more hydrophobic nature of the LOR added to the physical interactions between SC and LOR (as revealed by infrared spectroscopy) could reduce the availability of hydrophilic free groups to interact with water and, in this way, difficult the course of water through the film.

The effective diffusion coefficients also seem to decrease with the addition of LOR due to its more hydrophobic character, although the lipid concentration in the film does not influence further their values. Moreover, similar effects were reported in a previous work (Pereda et al., 2010), for the addition of tung oil to sodium caseinate films. The explanation provided by Fabra, Talens, and Chiralt (2008b, 2009), who reported that oleic acid seems to interact with the sodium caseinate matrix forming bonds through polar groups, thus modifying the interaction balances in the protein network and film functional properties, entirely match our observations.

Table 2

Contact angles, moisture absorption properties (at 75% RH) and water-vapor permeability of SC films with different contents of LOR.

LOR (%)	Contact angle ( $\theta$ )	EMC (%)	$D_{\text{eff}}$ ( $\text{m}^2/\text{s}$ ) $\times 10^{13}$	WVP ( $\text{g m}/\text{Pa s m}^2$ ) $\times 10^{10}$
0	$26.31 \pm 1.59^{\text{a}}$	$15.62 \pm 0.99^{\text{a}}$	$3.341 \pm 1.238^{\text{a}}$	$5.361 \pm 0.815^{\text{a}}$
5	$43.04 \pm 3.11^{\text{b,c}}$	$13.07 \pm 0.87^{\text{b}}$	$1.649 \pm 0.896^{\text{b}}$	$5.019 \pm 1.286^{\text{c}}$
10	$37.11 \pm 3.78^{\text{c}}$	$11.82 \pm 2.05^{\text{b,c}}$	$0.834 \pm 0.133^{\text{b}}$	$2.171 \pm 0.592^{\text{b}}$
15	$45.76 \pm 3.08^{\text{b}}$	$10.91 \pm 1.75^{\text{b,c}}$	$1.036 \pm 0.516^{\text{b}}$	$3.548 \pm 0.797^{\text{b,c}}$
20	$39.43 \pm 4.62^{\text{b,c}}$	$11.57 \pm 0.62^{\text{c}}$	$0.996 \pm 0.076^{\text{b}}$	$3.494 \pm 0.777^{\text{b,c}}$

Any two means in the same column followed by the same letter are not significantly ( $P > 0.05$ ) different according to Turkey test.



However considering the important standard deviation of the effective diffusivity mean values, which are attributed to the divergence of the sorption experimental curves from the Fick's theoretical law, we believe that a deeper analysis of this property is pointless.

### 3.5. Water-vapor permeability (WVP)

Table 2 also reports WVP of the SC films with different contents of LOR. The results showed a significant improvement (decrease in the WVP values) of this property with the incorporation LOR as a consequence of a reduced affinity to water of the lipid chains and the corresponding increase in the tortuosity of vapor diffusion-path through the film, as a result of the presence of oily resin droplets (Anker, Berntsen, Hermansson, & Stading, 2002). Others authors reported the same strong behavior for other protein-based films incorporating emulsified oil (Bertan, Tanada-Palmu, Siani, & Grosso, 2005; Fabra et al., 2008b; Fabra, Talens, Gavara, & Chiralt, 2012; Zahedi, Ghanbarzadeh, & Sedaghat, 2010). Pereda et al. (2010) reported moderate improvement to WVP with the incorporation of tung oil to sodium caseinate films (23% of reduction in the WVP value with the addition of 15 wt.% of tung oil). Although the LOR present higher affinity with water in comparison with the tung oil according to its more polar structure, the maleates in the LOR structure (see Scheme 1) can interact with the protein reducing the permeation of the water molecules. Moreover, Fabra, Hambleton, Talens & Debeaufort (2011) indicated that a more closed structure was formed when ferulic acid was incorporated into sodium caseinate films, due to the formation of hydrogen bonds between the carboxyl group and the with amino acid residues of the protein, which affected favorably the permeability values of the matrix. The high affinity between SC and LOR is denoted in a reduction in the WVP of 59% with the incorporation of 10 wt.% of LOR in the SC film, which is the lowest value reached in this work. Evidently, using 10 wt.% LOR the balance between the increased tortuosity of the path for water diffusion, the level of interactions between constituents and the size of the droplets is the optimum for this system.

### 3.6. Dynamic-mechanical properties

Fig. 4 shows curves of relative storage modulus (relative to the modulus of each sample at  $-60^{\circ}\text{C}$ ) and  $\tan \delta$  as a function of the temperature for SC films with different concentrations of LOR, that were conditioned at 50% RH before the tests.

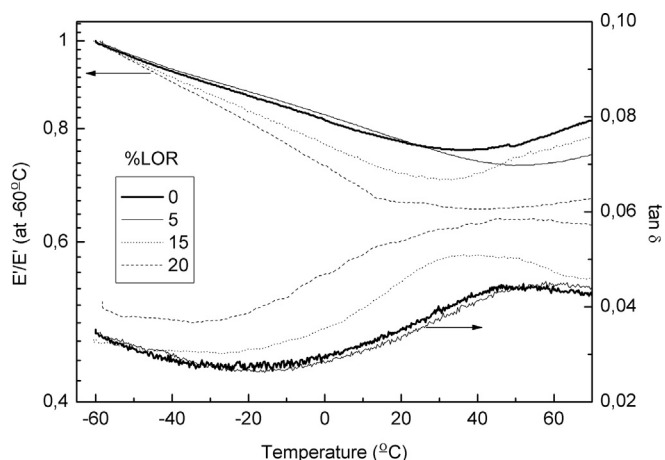


Fig. 4. Dynamic-mechanical properties of SC films with different contents of LOR.

All the curves show the typical reduction in the storage modulus as the temperature increases until temperatures close to  $30^{\circ}\text{C}$ . At higher temperatures, the modulus increases as a result of the evaporation of the water (plasticizing agent) that was initially present in the conditioned films. On the other hand, the incorporation of LOR in percentages higher than 5 wt.% decreases the relative storage modulus respect to the corresponding to neat SC films, in all the analyzed temperature range, indicating that the modified oil also acts as a plasticizer in this system. This fact is also observed in the maximum value reached by the loss factor ( $\tan \delta$ ), that increase with the LOR content in the SC films. As it is widely known,  $\tan \delta$  is the ratio between loss and storage modulus and it is an indicator of the viscoelasticity of a sample. As more oily modifier is incorporated in the films, the dissipation capacity of the samples becomes higher.

### 3.7. Tensile properties

Table 3 shows the tensile mechanical properties of films conditioned for 48 h at 50% RH at  $25^{\circ}\text{C}$ . As the LOR content in the film increases, the values of tensile modulus and strength decrease as a result of the presence in the protein matrix of the oily resin droplets.

On the other hand, the elongation at break does not have a clear trend regarding LOR concentration, globally it seems that the addition of the modifier lead to a slight reduction of the deformability of the films respect to that of the neat SC one. The mechanical behavior of these emulsion systems could be affected by different contrary effects: 1) the presence in the protein matrix of the easily deformable oil based resin droplets that could plasticize the material producing a decrease in rigidity and strength and an increase the elongation at break; 2) molecular interactions that could enhance the structural bonds in the polymer network leading to an increase in the tensile strength but decreasing its ability to stretch. These different interaction routes between oily phases and proteins have been reported by several authors (Fabra, Talens & Chiralt, 2008; Nylander, 2004).

Most of the times, when an oil in the form of emulsified droplets is incorporated into a biopolymer-based film, it become more flexible (higher elongation at break values) and less stiff (decrease of Young Modulus and tensile strength) (Bertan et al., 2005; Fabra et al., 2008b; Vargas et al., 2011). Such an effect is attributed to the development of a heterogenous matrix, featuring discontinuities that influence the stretching ability of the film. Since the modifier droplets in the film matrix are easily deformed upon application of tensile stress, the oil-incorporating film is expected to exhibit a higher extensibility compared with a film free from oil (Fabra, Talens, & Chiralt, 2008), which is obviously not the effect noticed here. Even though, the plasticizing effect of emulsified oil on protein-based films was observed even when the oil is

Table 3  
Tensile properties of SC films with LOR, glycerol and TO.

Modifier	Young modulus (MPa)	Tensile strength (MPa)	Elongation at break (%)
0% LOR	2908.0 ± 121.9 <sup>a</sup>	57.0 ± 0.3 <sup>a</sup>	4.0 ± 0.5 <sup>a,c,d</sup>
5% LOR	2269.2 ± 360.1 <sup>b</sup>	41.2 ± 6.7 <sup>b</sup>	2.7 ± 0.7 <sup>b,c,d</sup>
10% LOR	1844.4 ± 71.8 <sup>b</sup>	37.7 ± 4.1 <sup>b</sup>	3.7 ± 0.6 <sup>c,d</sup>
15% LOR	1859.4 ± 200.1 <sup>b</sup>	32.8 ± 2.4 <sup>b</sup>	4.7 ± 1.1 <sup>a,c,d</sup>
15% Glycerol	802.8 ± 63.2 <sup>a</sup>	13.6 ± 1.3 <sup>a</sup>	11.6 ± 5.6 <sup>a</sup>
15% TO	1202.4 ± 62.5 <sup>c</sup>	25.8 ± 2.7 <sup>c</sup>	3.0 ± 0.5 <sup>c</sup>
20% LOR	1349.2 ± 304.2 <sup>c</sup>	23.4 ± 2.7 <sup>c</sup>	2.9 ± 0.8 <sup>d</sup>

Any two means in the same column followed by the same letter are not significantly ( $P > 0.05$ ) different according to Turkey test.

incorporated at a relatively low concentration as in the case of the work of Zinoviadou et al. (2009).

Other times, different oil effects were also noticed: in a previous work (Pereda et al., 2010) showed that tung oil (TO) acted as a reinforcement when incorporated into a caseinate-glycerol based matrix. This antiplasticizing effect was attributed to the segregation of the protein molecules in the aqueous, protein-rich phase that resulted, in turn, in more extensive protein-protein aggregation. Moreover, Fabra, Hambleton, et al. (2011) and Fabra, Perez-Masia, et al. (2011) indicated that ferulic acid could promote a cross-linking effect in the polymer matrix (sodium caseinate) through the formation of hydrogen bonds between the carboxyl groups of the ferulic acid and the amino acid residues of the protein, which can contribute to the reinforcement of the polymer chains entanglements, thus increasing the film stiffness and resistance. In different works, these authors (Fabra, Hambleton, et al., 2011; Fabra, Perez-Masia, et al., 2011) indicated also that fatty acids like oleic acid interact with protein matrices, forming bonds through polar groups. Moreover, Pereda et al. (2010) reported that in presence of triglycerides the cohesion and continuity of the protein is reduced and this affect the stretching ability of the film.

In the present case, we believe that the interactions through polar groups between SC and LOR, that cause a reduction in the free volume and the molecular mobility of the polymer, could be the main aspect that determined the slight reduction of the deformability of the films containing LOR. On the other hand, the decrease of the tensile modulus, as well the tensile strength is mainly determined by the plasticizing effect of the oil based resin droplets.

Table 3 also presents the tensile properties of SC based films containing 15 wt.% with respect to the protein content of glycerol (Pereda et al., 2008), LOR or TO (Pereda et al., 2010), in order to evaluate the plasticizing ability of any additive. The modulus and tensile strength of the SC films containing glycerol are the lowest, at the same time that its elongation at break is the highest, being the more flexible and stretchable film in comparison with the other samples. This behavior can be attributed to several factors: the smaller size of glycerol added to its higher hydrophilic nature (it is the most common plasticizer for hydrocolloid systems) compared with the other two additives, resulted in higher water content in the films by increasing the moisture absorption capability, thus acting as co-plasticizer. On the other hand, all the tensile properties of the sample prepared with LOR are higher than those of the film containing TO, although the LOR has intermediate molecular size and hydrophilic character among the glycerol and the TO. In fact, Pereda et al. (2010) reported an anti-plasticizing effect of the TO when used in sodium caseinate films. In the light of these results, we believe that the LOR presents an intermediate and interesting behavior in this emulsion system, becoming a middle plasticizing (i.e. decreasing the stiffness of the neat SC films) although reducing the moisture sorption of the films like a middle anti-plasticizer due to the well balanced amount of polar (-OH) and -COOH groups that contains in its structure, which can interact strongly and in different modes with the amine groups of the protein.

#### 4. Conclusions

LOR modified sodium caseinate films were successfully by prepared by casting of film forming emulsions. The procedure selected for forming the emulsions allowed us to obtain homogenous, flexible and translucent films, with opacity values increased respect to the corresponding to the neat SC film, but reduced respect to the valued reported in similar works. Infrared spectroscopy and solubility tests revealed that physical interactions between the protein component of the film and the oily modifier were developed. Water vapor permeability and equilibrium moisture content decreased

with the addition of LOR, reaching their minimum values (59% reduction in WVP and about 70% reduction in EMC, respect to the corresponding values of the neat SC film) with the incorporation of 10 and 15 wt% of LOR, respectively. This behavior was explained in terms of the reduced affinity for water due to the addition of a lipid component in addition to the increase in tortuosity of vapor diffusion-path through the film due to the homogeneously dispersed oil droplets into the protein-based matrix. The contact angle increased with LOR addition, in concordance with the more non-polar character of the films surfaces. The presence of LOR decreased tensile modulus and strength, but the elongation at break was not significantly affected, which was ascribed to a combination of the plasticizing effect of the modifier and the physical interactions developed between sodium caseinate and LOR.

Overall, it was noticed that the main changes in the properties took place when low amounts of LOR were added to the formulations. Definitely, a 20 wt% LOR is a too high value for this system that does not provide additional benefits in any of the measured properties.

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