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Preparation and characterization of sodium caseinate films reinforced with cellulose derivatives

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ABSTRACT

Edible composite films, based on glycerol-plasticized sodium caseinate (SC) and either carboxymethyl cellulose (CMC) or cellulose acetate (CA) fibers, were respectively prepared by suspension casting. The effects of mixing SC with very low amounts (up to 3 wt%) of CMC or CA were systematically investigated through changes in morphology, surface hydrophilicity, moisture sorption, water vapor permeability, opacity, dynamic mechanical response and mechanical properties of the films. Incorporation of 3 wt% cellulose derivatives into the protein matrix led to slight but measurable decreases of equilibrium moisture contents, achieving reductions of 7.5 and 14.4% for CMC and CA reinforced films, respectively. Besides, the addition of CA contributed to the decrease of water vapor permeability, leading to a 38% falloff at 3 wt% filler. Contact angle measurements using a polar solvent ranged from 40° to 29–30° as filler concentration increased, indicating that reinforced films had higher superficial hydrophilicity than neat caseinate ones. Transparency decreased as filler concentration increased: the opacities of the most concentrated samples were 1.9 (CMC) and 2.3 times (CA) higher than that of the SC matrix. Moreover, tensile tests revealed that the addition of cellulose derivatives enhanced the tensile modulus (i.e. an 80% increase with 3 wt% CMC) and strength (i.e. a 70% increase with 3 wt% CA) although the elongation at break decreased. The differences found in the performance of both cellulose derivatives were explained in terms of water-solubility, hydrophobic character, rigidity of the fibers and quality of the filler dispersion in the protein matrix.

1. Introduction

Edible films based on agricultural materials have received much attention as potential packaging materials, mainly because such biodegradable films are considered to be a promising solution to environmental impact of synthetic polymer packaging (Su, Huang, Yuan, Wang, & Li, 2010; Wang et al., 2008). Although these films are not meant to entirely replace synthetic packaging films, they do have the potential to substantially reduce the environmental burden due to food packaging, and to limit moisture, aroma and lipid migration between food components (Arvanitoyannis, 1999; Arvanitoyannis & Biliaderis, 1999; Krochta & De Muller-Johnson, 1997). Edible films can also be developed with many other functions such as carriers of substances (antimicrobial, aroma, pharmaceutical), coloring agents, or to improve mechanical handling of food (Langmaler, Mokrejs, Kolomamik, & Mladek, 2008). Among the naturally occurring edible materials, casein and casein derivatives have been extensively studied due to their low cost, availability, and complete biodegradability (Arvanitoyannis & Biliaderis, 1998). Caseinate presents thermoplastic and film-forming properties due to its random coil nature and its ability to form weak intermolecular interactions, i.e. the partially denatured peptide chains bond together primarily through hydrophilic and hydrogen bonds resulting in the formation of the protein matrix (Fabra, Talens, & Chiralt, 2010a; Rhim & Ng, 2007). Moreover, casein-based edible films are attractive for food applications due to their high nutritional quality, excellent sensory properties and potential to provide food products with adequate protection from their surrounding environment (Atarés, Bonilla, & Chiralt, 2010). However, as other protein-based films, the hydrophilic nature of casein and caseinate based films limits their moisture barrier ability when compared to the commonly used synthetic plastic films (Fabra, Talens, & Chiralt, 2010b; Han & Gennadios, 2005, chap. 15). The second inherent problem limiting usage of pure sodium caseinate films is their inadequate mechanical properties (Ibrahim, El-Zawawy, & Nassar, 2010; Li, Guo, Lin, Fan, & Song, 2010; Sothornvit & Krochta, 2001). Thus, to improve the mechanical properties and moisture resistance, one of the available options can be blending/reinforcing caseinate with other polymers. In this respect, protein/cellulose derivatives composites have the potential to replace conventional food packaging leading to edible and biodegradable films and coatings. However, to keep the edible

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properties, the additives must be safe to eat or generally recognized as safe (GRAS). The additives should be compatible with proteins and have the ability to form films (Su et al., 2010). Water-soluble cellulose derivatives are used for packaging because of their edibility and biodegradability (García, Pinotti, Martino, & Zaritzky, 2009). At the same time they offer good barrier properties, being non-toxic, non-polluting and having low cost (Vasconez, Flores, Campos, Alvarado, & Gerschenson, 2009). Carbomethoxy cellulose (CMC), one of the most important commercially produced derivatives of cellulose (Psomiadou, Arvanitoyannis, & Yamamoto, 1996), is a typical anionic polysaccharide that has been widely used as a stabilizer in food (Togrul & Arslan, 2004a, 2004b) and can be a suitable additive for enhancing the properties of protein films. CMC is one of the natural water-soluble cellulose derivatives that have no harmful effects on human health (Su et al., 2010; Ma, Chang, & Yu, 2008), therefore, it is used as a highly effective additive to improve product and processing properties in fields of application varying from foodstuffs, cosmetics, and pharmaceuticals to products for the paper and textile industries (Olaru, Olaru, Stoleriu, & Timpu, 1998; Togrul & Arslan, 2004a; Ma et al., 2008). CMC chains are linear β (1–4)-linked glucopyranose residues. Due to its non-toxicity, biocompatibility, biodegradability, hydrophilicity and good film-forming ability, CMC has been used in a high number of edible film formulations (Togrul & Arslan, 2004a). In the same way, cellulose acetate (CA), which is also made from different renewable resources of cellulose, has been widely used in oral pharmaceutical products and is regarded as a nontoxic, nonirritant and biodegradable material. Cellulose acetate had been used as a semi-permeable coating on tablets, which allows for controlled, extended release of actives (Zhou et al., 2006). It is also used in high volume applications ranging from fibers, to films, to injection molding thermoplastics due to its good physical properties, resistance to moulds and bacteria and relatively low cost (Aluigi, Vineis, Ceria, & Tonin, 2008; Chandra & Rustgi, 1998).

In a previous paper (Pereda, Amica, Rácz, & Marcovich, 2011), we studied the effect of adding low amounts of nanocellulose to sodium caseinate film forming solutions, with the aim of enhance the mechanical resistance and reduce the water vapor permeability (WVP) of the resulting composite films. However, the addition of non soluble cellulose particles produced only a marginal effect on WVP since there were two opposite factors affecting this behavior: both, the bubbles and pores content in the films and the path length for vapor diffusion increased as cellulose concentration increased. Consequently, the aim of this work is to enhance the dispersion of the filler into the sodium caseinate (SC) matrix by using water-soluble cellulose derivatives, in order to reduce the pore/bubble formation while maintaining the increased mechanical response. Thus, the effects of using CMC and CA to prepare reinforced films based on glycerol-plasticized sodium caseinate (SC) on the mechanical, optical and water barrier properties, microstructure and surface morphology are addressed.

2. Experimental

2.1. Materials and methods

2.1.1. 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^{-1} (Audic and Chaufer, 2005). The plasticizer used was glycerol (GLY) P.A., which was purchased from DEM Chemicals (Mar del Plata, Argentina). Microcrystalline cellulose powder (MCC) of 20 μm (Aldrich, cat. No. 31,069-7) was selected as the source raw material for producing the cellulose derivatives.

2.1.2. Preparation of cellulose derivatives

Acetilation of MCC: 5 g microcrystalline cellulose was suspended in 25 ml concentrated acetic acid and stirred at 70 °C for 60 min. After decanting the suspension, 25 ml acetic anhydride and 250 μl cc. sulphuric acid was added to the solid phase. The dissolved MCC was precipitated by pouring it onto water under vigorous stirring. The resulted powder was washed until neutralization and dried at 70 °C in vacuum oven, overnight. After that, 8 g of dried acetylated cellulose was added to a solution made from 0.4 g LiCl in 71.6 g DMAc in order to swell and separate the fibers. This blend resulted in a clear solution. The solution was stirred at 70 °C for 3 days and then poured onto water. The precipitating white powder was washed with distilled water, dried, and re-suspended again in distilled water. The suspension was sonicated at 70 °C for 180 min (18 steps of 10 min each) over a 3 days period of time. The final product was filtered and then freeze-dried.

Carboxymethylation of MCC: Solutions of monochloroacetic acid and sodium hydroxide were prepared, mixed and cooled down to 40 °C to achieve final concentrations of 0.66 mol/kg and 3 mol/kg, respectively. The solution was used 10 min after preparation. Microcrystalline cellulose was added to the solution using a 1:24 liquor ratio and the suspension was agitated at 40 °C for 20 min. The modified MCC was separated on a Büchner filter and washed with distilled water until neutralization was reached. The dried modified cellulose fibers were then swelled and separated using the same procedure described for separating cellulose acetate fibers but now, a suspension (instead a solution) was obtained by immersing the fibers into the LiCl–DMAc solution.

2.1.3. Film preparation

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. Appropriate amount of glycerol was added to achieve a glycerol/(sodium caseinate + glycerol) weight ratio of 0.21. Modified cellulose fibers were dispersed in distilled water by ultrasonication and then mixed with the film forming solutions to prepare composites containing 1, 2 and 3 wt% modified cellulose (dry weight). Films were prepared according to the usual casting method: the solutions were poured into Teflon Petri dishes (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 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 before each test.

2.2. Characterization techniques

2.2.1. Scanning electron microscopy (SEM)

The fracture surfaces (transversal area) obtained after immersing film samples in liquid air (fragile fracture) as well as the main surfaces of the films (upper and lower) were observed using a scanning electron microscope (JEOL, model JSM-6460 LV). For this purpose, selected 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.

2.2.2. Moisture sorption

Both, the modified cellulose fibers (CMC and CA) and the resulting films, dried at 40 °C for three days in a vacuum oven, were placed into 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 powders and films as a function of time were obtained from the total mass balance over the sample, as follows:

$$M_t = \frac{(W_t - W_0)}{W_0} \times 100 \tag{2}$$

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 reinforced films were fitted according to the Fick's equation for the unidimensional diffusion of a solute into a sheet (Crank, 1956):

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left[-D\frac{(2n+1)^2\pi^2 t}{l^2}\right] \tag{3}$$

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.

2.2.3. Water vapor permeability (WVP)

The water vapor transfer rate ($\mathrm{g\,s^{-1}\,m^{-2}}$) through the 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 73.5% RH and 18.5 ± 1 °C, to ensure equilibrium conditions. After that, film specimens were sealed on acrylic permeation cups (5 cm diameter) containing 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 $\mathrm{g\,s^{-1}}$. WVP of the films, expressed as ($\mathrm{g\,m/(Pa\,s\,m^2)}$), was calculated as follows:

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

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 ($\mathrm{m^2}$), 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 cellulose concentration.

2.2.4. Surface hydrophobicity (contact angle)

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. Seven replicate measurements were performed for each film at 24 °C, with a precision of ±2 °C.

2.2.5. Opacity

Film opacity was determined according to the method described by Irissin-Mangata, Bauduin, Boutevin, & 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.

2.2.6. Dynamic-mechanical analysis

The thermomechanical response of specific samples was determined using a rheometer (Anton Paar, Physica MCR 301) working in tension mode, using the film/fiber accessory. The specimens were cut to 80 mm × 10 mm, and the dimensions were measured with an accuracy of 0.01 mm. The frequency of the forced oscillations was fixed at 1 Hz. Tests were conducted using the temperature scan mode, applying a deformation of 0.05%. This strain value was sufficiently low to ensure that the mechanical response of the specimen was within its linear viscoelastic range. The set up allowed determining the storage modulus E', the loss modulus E'' and the ratio of these two parameters, $\tan\delta = E''/E'$.

2.2.7. Tensile

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. The ultimate strength (σ_b), elongation at break (ε_b) and elastic modulus (E) were calculated as described in ASTM D638-94b (ASTM, 1994).

2.2.8. 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 ± SD.

3. Results and discussion

3.1. Physical characterization

Crystalline cellulose is a relatively inert substance, due to the strong binding between adjacent cellulose molecules. Native cellulose swells in water, but is insoluble in both water and dilute acids. In order to obtain a solution of cellulose, concentrated acids that cause extensive hydrolysis of the polymer can be used (Krässing, Schurz, Steadman, Schliefer, & Albrecht, 1986). Chemically modified celluloses were developed primarily in order to overcome this insoluble nature of cellulose, thus extending the range of applications of the polymer. Commercial cellulose derivatives are usually ethers or esters that are soluble in water and/or organic solvents. They are produced by reacting the free hydroxyl groups in the anhydroglucose units with various chemical substitution groups. The introduction of substituents disturbs the inter- and intramolecular hydrogen bonds in cellulose, which leads to liberation of the hydrophilic character of the numerous hydroxyl groups and restriction of the chains to closely associate (Marchessault & Sundararajan, 1983). The properties of modified polysaccharides depend on several factors, such as the modification reaction, the nature of the substitution group, the degree of substitution (DS) and the distribution of the substitution groups. Derivatives with DS below 0.1 are generally insoluble, but if the DS is increased up to 0.2–0.5 (depending on the type of substituting group) the product becomes soluble in aqueous alkali. Water solubility is obtained above a DS of 0.4 for ionic ethers; for non-ionic ethers a DS of >1.0 is required (Richardson & Gorton, 2003). In this work, the degree of substitution of CMC and CA was not determined, but the visual inspection of the modified cellulose fibers when dispersed in distilled water by ultrasonication indicated that CA

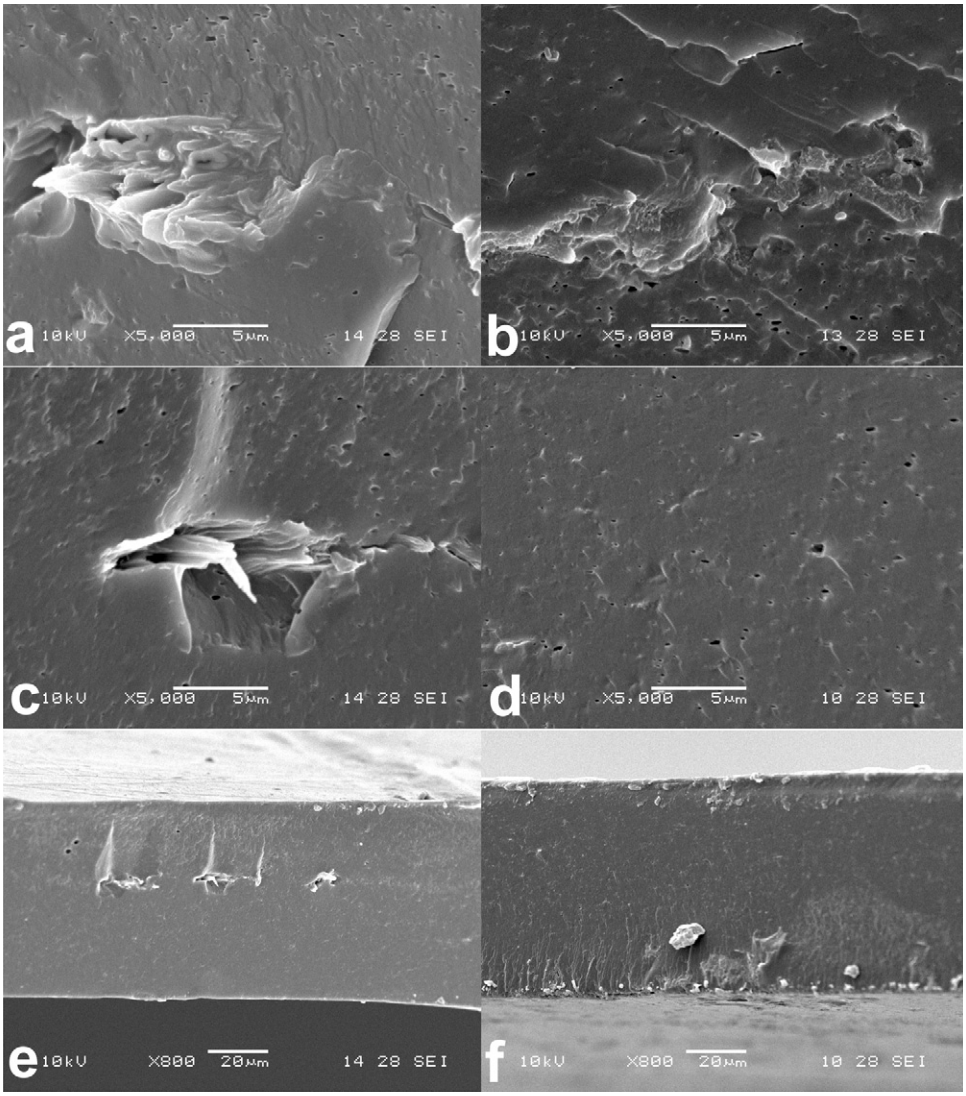

Fig. 1. Scanning electron micrographs from the cryogenic fractured surface (film thickness) of reinforced films. (a) CMC, 1 wt%; (b) CA, 1 wt%; (c) CMC, 3 wt%; (d) CA, 3 wt%; (e) CMC, 3 wt%, low magnification; (f) CA, 3 wt%, low magnification.

resulted almost completely soluble while part of the CMC particles remained as a homogeneous, stable suspension after the same treatment. However, a deeper observation of the reinforced film thickness (fractured in liquid nitrogen) by scanning electronic microscopy indicated that some fiber agglomerations were formed in both cases, even for the samples reinforced with only 1 wt% cellulose derivatives, as shown in Fig. 1a and b. In spite of that, the number and size of these aggregates are much larger in the films prepared from CMC in comparison to those made from CA, as can be clearly noticed from the SEM pictures took from the 3 wt% films, as revealed in Fig. 1c–f. Even though, the good compatibility between the modified polysaccharides and the caseinate matrix can be checked from the lack of empty spaces at the fiber–matrix interface.

Another important difference between both cellulose derivatives was found in their inherent hydrophilic character: while CA powder absorbed only 3.33% moisture when equilibrated in a 75% RH atmosphere, CMC reached a 6.86% increase in its initial weigh in the same conditions. In both cases, equilibrium conditions were attained in less than 2000 min of exposition to the controlled atmosphere. Thus, when analyzing the equilibrium moisture content of the reinforced films (presented in Table 1), it is observed that increasing concentrations of CA led to the expected reductions of the film hydrophilicity. Even more, it is also noticed that the addition of more than 1% of CMC produced the same behavior, which was quite unexpected. A plausible explanation for this performance can be found in the dispersion of the filler in the sodium caseinate matrix: very low amount of CMC (i.e. 1 wt%) can be quite successfully dispersed while higher contents should agglomerate, being the individual fibers connected trough hydrophilic sites and thus, reducing the points for absorbing water. Similar explanations were offered by other researchers that worked with

Table 1
Equilibrium moisture uptake and effective diffusion coefficients of sodium caseinate films reinforced with cellulose derivatives.

Property (wt%)	M_∞ (%)		$D \times 10^{12}$ (m^2/s)	
	CMC	CA	CMC	CA
0	19.13 ± 0.80^{a}		7.43 ± 0.13^{a}	
1	20.05 ± 1.65^{a}	18.01 ± 0.34^{a}	$7.69 \pm 1.27^{a,b}$	0.20 ± 0.019^{b}
2	$18.61 \pm 0.58^{a,b}$	$18.01 \pm 1.40^{a,b}$	8.65 ± 0.62^{b}	
3	17.71 ± 0.53^{b}	16.38 ± 1.10^{b}	12.40 ± 3.73^{b}	
100	6.86^{c}	3.33^{c}	–	–

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

Table 2
WVP of sodium caseinate films reinforced with carboxymethyl cellulose (CMC) and cellulose acetate (CA).

Filler (wt%)	WVP (g m/(Pa s m^2)) × 10^{10}	
	CMC	CA
0	8.56 ± 0.92^a	
1	9.88 ± 2.21^a	6.09 ± 0.61^b
2	11.60 ± 3.21^a	7.56 ± 0.18^a
3	9.87 ± 1.51^a	3.48 ± 0.89^c

$^{a-c}$Different superscripts within the same column indicate significant differences between formulations ($P < 0.05$).

Table 3
Contact angle values of sodium caseinate films reinforced with cellulose derivatives.

Filler (wt%)	θ (°)	
	CMC	CA
0	40.2 ± 2.2^a	
1	35.85 ± 1.45^b	35.02 ± 0.81^b
2	34.80 ± 2.70^b	33.22 ± 1.92^b
3	30.02 ± 1.45^c	28.84 ± 1.10^c

$^{a-c}$Different superscripts within the same column indicate significant differences between formulations ($P < 0.05$).

starch–cellulose fibers composites (Curvelo, de Carvalho, & Agnelli, 2001; Dias, Müller, Larotonda, & Laurindo, 2011; Müller, Laurindo, & Yamashita, 2009): they attributed the reduction of water sorption capability of composites to the interactions between fibers and the hydrophilic sites of starch chain, which substituted the starch–water interactions that predominate in films without fibers.

The moisture sorption behavior of the different films was modeled using the Fick's law, applying the effective diffusion coefficient as the fitting parameter (Table 1). The values obtained for CMC reinforced films indicate that the initial moisture absorption rate is slightly higher as the filler concentration increases, while the effective diffusion coefficients of the films reinforced with CA are independent of filler content, but much lower than that of the neat protein film, which is consistent with hydrophilic nature of the different cellulose derivatives.

Natural polymers are characterized by extensive water clustering and plasticization of their matrix, properties regarded as important shortcomings in packaging applications (Arvanitoyannis & Biliaderis, 1999). In fact, SC films become more permeable to water vapor with the addition of CMC, as can be noticed from Table 2, again due to its inadequate dispersion in the protein matrix added to the inherent hydrophilic character of this cellulose derivative. On the other hand, it is also noticed that the addition of CA produced a moderate reduction in the WVP values that reached a 38% decrease with only 3 wt% filler. As was reported elsewhere (Krochta & De Muller-Johnson, 1997; Ouattara, Canh, Vachon, Mateescu, & Lacroix, 2002), WPV values for casein and whey protein films are at least 10 times higher than the corresponding to methylcellulose, hydroxypropyl methyl cellulose, and cellulose acetate ones. However, films prepared with cellulose derivatives are almost completely opaque while mixing cellulose acetate with a protein-based matrix lead to quite transparent materials, i.e. a better coating appearance. Besides, the addition of CA and CMC probably introduced a tortuous path for water molecules to pass through, which should contribute to WVP reduction (Kristo & Biliaderis, 2007; Ma et al., 2008). But only the well-dispersed CA fibers were able to block the water vapor while the CMC ones, more easy to congregate, actually decreased the effective contents of filler and facilitated the water vapor permeation. Similar results were obtained by Ma et al. (2008) working with a glycerol-plasticized thermoplastic pea starch matrix reinforced with microcrystalline cellulose and CMC and by Psomiadou et al. (1996) who studied the behavior of corn starch/polyol blends filled with high contents of microcrystalline or methyl cellulose.

Table 3 shows the contact angle values of all the reinforced films. The contact angle is dependent on the relative magnitude of cohesive and adhesive molecular forces that exist respectively within the liquid (in this case ethylene-glycol) and between the liquid and the solid (Hambleton, Fabra, Debeaufort, Dury-Brun, & Voilley, 2009). Thus, a small contact angle implies an hydrophilic surface. Therefore, the values reported in Table 3 indicate that increasing contents of any of the modified polysaccharides cause an increase in the film surface hydrophilicity with respect to that of the neat caseinate film, being the CA reinforced samples slightly more hydrophilic that CMC films at each concentration, which seems to be in contradiction with the previous discussion. However, and as was reported previously, amphiphilic molecules like sodium caseinate (Pereda et al., 2011; Pérez, Carrera Sánchez, Pilosof, & Rodríguez Patino, 2009; Villalobos, Chanona, Hernández, Gutiérrez, & Chiralt, 2005) turn their hydrophobic parts mainly towards film–air and film–casting plate interfaces during film formation, thus, reducing their surface hydrophilicity in comparison with the global one. On the other hand, the cellulose derivative fibers do not have amphiphilic chemical characteristics and consequently contribute negatively to the superficial moisture sensitivity of the films.

Fig. 2 shows the relative opacity values of composites prepared with up to 3 wt% cellulose derivatives. Gloss and transparency (the opposite of opacity) are greatly relevant to the film's functionality due to their large impact on the appearance of the coated products (Fabra et al., 2010b). The opacity value of the neat plasticized SC film (without the addition of any cellulose derivative) is 1461.90 ± 35.91 AU nm/mm and all the values reported in Fig. 2 are higher than that, indicating clearly that the films are less transparent (more opaque) as the cellulose derivative content increases. Moreover, opacity values are higher for composites reinforced with CA, although the scatter in experimental determinations is higher for samples prepared from CMC. The different transparency of the films is related with the internal structure developed during film drying (Fabra, Talens, & Chiralt, 2009; Villalobos et al., 2005). As it is known, the presence of a disperse phase promotes opacity as a function of the differences in the refractive index of both phases (Villalobos et al., 2005). In addition, the degree of fiber/particle aggregation and a high compactness of the structure are associated to higher values of the refractive index and the subsequent

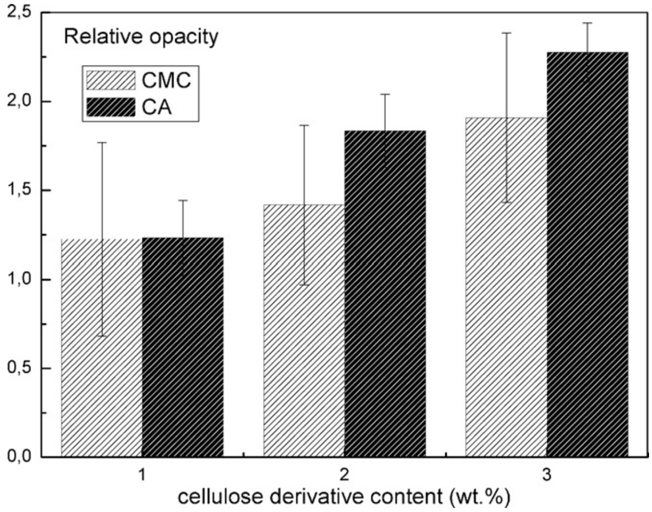

Fig. 2. Relative opacity values of caseinate films reinforced with modified polysaccharides.

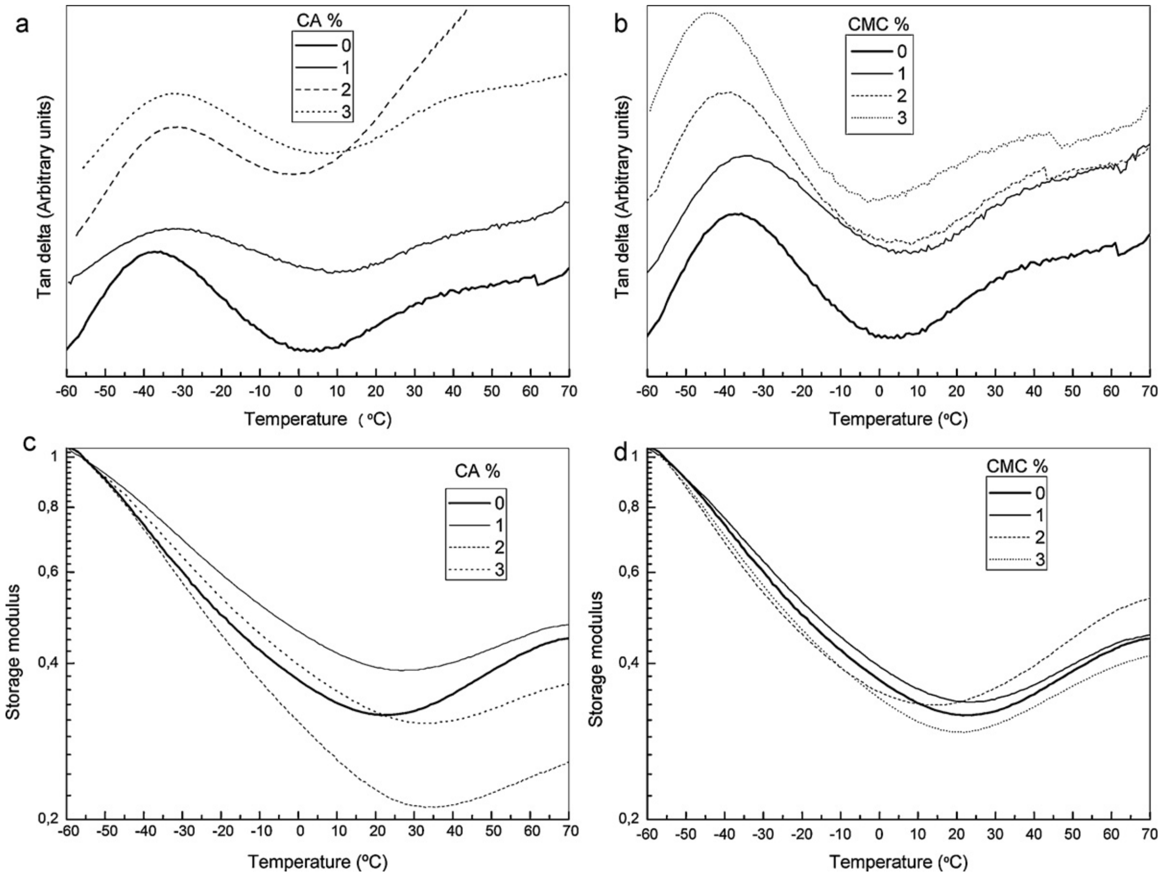

Fig. 3. Dynamic mechanical behavior of sodium caseinate reinforced films: (a) tan δ curves of films reinforced with CA; (b) tan δ curves of films reinforced with CMC; in both cases the curves were arbitrarily shifted to facilitate the comparison; (c) relative storage modulus curves of films reinforced with CA; (d) relative storage modulus curves of films reinforced with CMC.

increase in the reflected light (Fabra et al., 2010b). In our case, CMC was added to the SC film-forming solution mainly as a stabilized suspension, but during the 10-h drying step some kind of fiber association may took place, being this process enhanced by increasing fiber concentration. As individual opacity measurements were taken in random locations, the scatter of the data should indicate that the heterogeneity of the films reinforced with CMC due to fiber clusters, is higher than that of the composites based on CA.

3.2. Mechanical and thermo-mechanical characterization

As was reported previously (Pereda et al., 2011), the effect of temperature that can induce thermal cross-linkage in sodium caseinate films (Pereda, Aranguren, & Marcovich, 2010), coupled to the decreasing of the moisture content of samples (initially conditioned at 50% RH) as the test progress, are additional obstacles that difficult the exacting analysis of the thermo-mechanical behavior of protein based reinforced films at relatively high temperatures. Thus, in this paper, we focus on the analysis of the low temperature transition associated to the glycerol relaxation (Quijada-Garrido, Iglesias-González, Mazón-Arechederra, & Barrales-Rienda, 2007) that appears at about −38 °C (arbitrary measured as the temperature for the maximum in the tan δ curve) for the non-reinforced plasticized caseinate film, as noticed from Fig. 3a and b. Even the true glass transition of neat glycerol was found to occur at lower temperature (Quijada-Garrido et al., 2007), it is expected to move to higher temperature when it is used as a blend component for polymer systems (in this case a protein network), due to the decrease of its own mobility. This type of low-temperature transition is frequently observed in compatible polymer–diluent mixtures, and does not necessarily imply phase separation, being most frequently attributed to the onset of short-range motions, as supported by relevant literature (Kristo & Biliaderis, 2006). In this case it is noticed that the temperature of the glycerol relaxation increases as the CA concentration increases or when just 1 wt% of CMC is added to the films. On the other hand, it decreases when the CMC concentration of the films (more than 1 wt%) increases. This behavior could be associated to the glycerol adsorption on the filler surface: well-dispersed small fibers expose a larger superficial area than fiber clusters, promoting more intense glycerol–fiber interactions that constrain the plasticizer relaxation.

In Fig. 3c and d, the relative storage modulus, defined as the ratio between the actual modulus and the modulus in the glassy state (taken at −60 °C) of reinforced samples is shown. A continuous decrease of E' with temperature until reaching ambient temperature is noticed. After this temperature the modulus increased continuously up to reach a maximum value at about 70 °C. Beyond this temperature samples reached the flow region and failed. Since the rheometer used for the measurements cannot control the humidity of the testing chamber, the samples became frosty at the beginning of the test (ambient moisture solidified on sample surface); near ambient temperature, the ice melting led to the storage modulus depression and after that, the evaporation of the moisture contained in the own films (notice they were conditioned at 50% RH prior testing), caused the increased stiffness observed for all samples at relatively high temperatures. Moreover, there were no clear trends with respect to the filler content or type: even though caseinates are generally accepted as non-ordered polymers, containing mostly random coil chain segments (Kristo, Biliaderis, & Zampraka, 2007), at low temperature the protein based film is in

Table 4
Tensile properties of sodium caseinate films reinforced with cellulose derivatives.

Filler (wt%)	Young modulus (MPa)		Elongation at break (%)		Tensile strength (MPa)	
	CMC	CA	CMC	CA	CMC	CA
0	171.3 ± 20.1^{a}		23.5 ± 2.1^{a}		4.7 ± 0.7^{a}	
1	161.5 ± 20.5^{a}	195.3 ± 12.2^{a,b}	22.0 ± 3.7^{a,b}	17.4 ± 1.1^{b}	4.2 ± 0.8^{a}	6.5 ± 0.6^{b,c}
2	235.9 ± 62.0^{a,b}	224.4 ± 24.9^{b}	16.5 ± 2.4^{b,c}	12.2 ± 3.8^{c}	6.1 ± 1.2^{a,b}	6.0 ± 0.8^{a,b}
3	310.9 ± 76.2^{b}	229.6 ± 28.6^{b}	16.3 ± 1.0^{c}	6.1 ± 0.1^{d}	6.6 ± 0.6^{b}	8.0 ± 1.1^{c}

$^{a-d}$Different superscripts within the same column indicate significant differences between formulations ($P < 0.05$).

the glassy state and thus, it has a quite high storage modulus. On the other hand, cellulose derivatives have lost most of the crystalline structure of the non-modified cellulose fibers (Samios, Dart, & Dawkins, 1997) and consequently, their own rigidities are somewhat reduced.

Mechanical properties are important to edible films, because adequate mechanical strength ensures the integrity of the film and its freedom from minor defects such as pinholes (Murillo-Martínez, Pedroza-Islas, Lobato-Calleros, Martínez-Ferez, & Vernon-Carter, 2010). Thus, the tensile properties of the reinforced films were evaluated and the results are presented in Table 4. As the general trends, it is noticed that the tensile modulus of both composites increases as the concentration of the cellulose derivatives increases. The rigidity of the films based on CMC is higher than that of the CA counterpart, at least for filler concentrations higher than 1%. However, and as was previously discussed, the scattering of the values is also larger for CMC reinforced samples. In spite of that, at the maximum filler concentration (3 wt% fibers) the tensile modulus is 80% higher than that of neat caseinate film, which is, however, lower than the two-fold increase observed when cellulose fibers, instead cellulose derivatives, were used as reinforcement (Pereda et al., 2011). This is again, attributed to the decreased crystalline character of the modified celluloses, being the CMC fibers more crystalline (i.e. less soluble in water) than CA ones. On the other hand, the tensile strength increases and the elongation at break decreases as the concentration of modified polysaccharides increases, being the changes for CA samples more important that those corresponding to CMC ones. The addition of cellulose derivatives hardens the protein structure, decreasing the molecular mobility of the matrix. As a result of this, the reinforced materials become more stiff, more resistant to break and less stretchable than the neat protein film. Similar results (increase in tensile strength and decrease in elongation at break as carbohydrate concentration increases) were reported by Arvanitoyannis, Psomiadou, and Nakayama (1996) for their corn starch–sodium caseinate plasticized blends. Moreover, this behavior can be ascribed to adequate interfacial interactions (good compatibility) between filler and matrix due to their chemical similarities, as was also reported by other researchers (Chang, Jian, Yu, & Ma, 2010).

4. Conclusions

Carboxymethyl cellulose and cellulose acetate were prepared to be used as reinforcing fillers for a sodium caseinate matrix. Although the water-solubility of these modified polysaccharides resulted much higher than that of the insoluble nanocellulose fibers used in a previous work, it is not complete and thus, a certain degree of fiber agglomeration was still observed in the reinforced films. In spite of that, a measurable reduction of the equilibrium moisture content, respect to that of the neat caseinate film was obtained using very low concentrations of cellulose derivatives (i.e. 7.5% reduction at 3 wt% CMC), while the addition of CA contributed also to the decrease of water vapor permeability (almost a 38% decrease at 3 wt% filler). Reinforced films resulted more opaque than the protein one (up to 2.3 times higher at 3 wt% CA), although they were still transparent. Moreover, increasing concentrations of both cellulose derivatives contributed to the improvement of the tensile modulus (i.e. 80% increase with 3 wt% CMC) and strength (i.e. 70% increase with 3 wt% CA), while decreasing the elongation at break (most important reduction = 74% for 3 wt% CA films), which was the result of the good compatibility between filler and protein matrix.

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