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Water vapour barrier and tensile properties of composite caseinate-pullulan films: Biopolymer composition effects and impact of beeswax lamination

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Abstract

The water sorption, water barrier properties and mechanical behaviour of pullulan (P) and sodium caseinate (SC), as well as their blend and bilayer films plasticized with sorbitol (25% dry basis), were investigated as a function of weight polymer ratio, water content and beeswax lamination. Very similar moisture sorption isotherms were obtained for blend and bilayer films with P/SC weight ratio of 1/3 and 3/1. Neither the type of film (blend or bilayer) nor the different P/SC ratio affected significantly ($P > 0.05$) the water vapour permeability (WVP) of the films. A mixture-process variable experimental design was applied to evaluate the effect of the proportion of the two polymers in relation with the relative humidity (RH, 53% and 75%) on the mechanical properties of the films. Increasing the P/SC ratio decreased the Young's modulus (E), the tensile strength (σ_{max}) and increased the % elongation at break (% EB), suggesting that P imparts flexibility and SC stiffness to the composite films. With moisture content increase from 5% to 8% most of the films exhibited an increase in E and σ_{max}, whereas a sharp decline in both parameters and an increase in % EB were observed above this moisture level. The brittle to ductile transition of P coincided with its glass to rubber transition, whereas SC exhibited a ductile behaviour within the glassy state. The tensile characteristics of bilayer films at moisture content greater than 8% were dominated by the component present in higher proportion, while films made with the biopolymer blends showed mechanical behaviour closer to that of plain P films. Beeswax lamination of plain, bilayer and blend films resulted in a drastic decrease in water vapour permeance, whereas its effect on E and σ_{max} and in % EB was related to the mechanical properties of the hydrocolloid layers used and varied according to the moisture content of the films.
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1. Introduction

Recently, studies on edible films have been intensified, since the potential benefits of these films are both environmental and cost-related. Edible films are primarily composed of polysaccharides, proteins and lipids, alone or in combination. Such films should possess suitable gas and aroma barrier and mechanical properties, to protect foodstuffs from deterioration. Combining polysaccharides and proteins in the form of blends or layers, with varying ratios of polymers, offers the possibility of creating different films with improved characteristics.

Pullulan (P) is a water-soluble microbial polysaccharide with excellent film-forming properties. Its films are colourless, tasteless, odourless, transparent, flexible, highly impermeable to oil and oxygen and heat-sealable (Yuen, 1974). The α $(1 \rightarrow 6)$ linkages that interconnect the maltotriose units are responsible for the irregularly ordered chains and for the resulting amorphous character of this polysaccharide (Gidley, Cooke, & Ward-Smith, 1993). Caseinates are generally accepted as non-ordered polymers, containing mostly random coil chain segments (Siew, Heilmann, East-

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eal, & Cooney, 1999). Consequently, sodium caseinate (SC) readily forms films, owing to its high water solubility, its random-coil structure and the capacity to form chain aggregates via electrostatic, van der Waal's forces and hydrophobic interactions (McHugh & Krochta, 1994).

Usually films made from polysaccharides or proteins show good mechanical properties, but are rather sensitive to moisture, due to the hydrophilic nature of these components. On the other hand, films made from lipids show good water vapour barrier properties, but are opaque, only slightly flexible and brittle (Greener, 1992). Thus, composite films that combine proteins or polysaccharides with fatty emulsions or fatty layers could be of particular interest, since the lipids help to lessen water vapour transmission and the proteins or polysaccharides give the necessary film strength and structural integrity. The incorporation of a lipid, in the form of an emulsion, with protein or polysaccharide to form a film (Avena-Bustillos & Krochta, 1993; Gontard, Duchez, Cuq, & Guilbert, 1994; McHugh, Avena-Bustillos, & Krochta, 1993; Möller, Grelier, Pardon, & Coma, 2004; Pérez-Gago & Krochta, 1999; Péroval, Debeaufort, Despré, & Voilley, 2002; Pommet, Redl, Morel, & Guilbert, 2003; Shellhammer & Krochta, 1997; Yang & Paulson, 2000) or as an additional layer onto a previously formed film (Debeaufort, Martin-Polo, & Voilley, 1993; Debeaufort, Quezada-Gallo, Delporte, & Voilley, 2000; Despond, Espuche, Cartier, & Domard, 2005; Greener, 1992; Greener & Fennema, 1989; Kamper & Fennema, 1984a, 1984b; Kester & Fennema, 1989) was explored in an attempt to improve the water barrier properties of hydrophilic films. Lipid-laminated films have 10–1000 times better barrier efficiency against water transfer than their emulsified counterparts (Debeaufort et al., 1993).

The purpose of the present work was to investigate the water sorption, water barrier and mechanical properties of composite blend and bilayer systems from pullulan and sodium caseinate, as they were affected by the polymer ratio, sample moisture content and beeswax lamination.

2. Materials and methods

2.1. Materials

Pullulan was a food grade preparation from Hayashibara Biochemical Laboratory, (Okayama, Japan). Sodium caseinate was from Wako Chemicals (Japan) and sorbitol (analytically pure) was obtained from Sigma Chemical (St. Louis, MO, USA). Inorganic salts (reagent grade) used for adjusting the relative humidity (RH) (saturated salt solutions) were from Merck KGaA (Darmstadt, Germany). Silica gel used as desiccant and beeswax (refined, yellow) were purchased from Sigma–Aldrich GmbH (Germany).

2.2. Film preparation

Pullulan, sodium caseinate, as well as their mixture in different ratios (according to the experimental design given in Table 1), were dissolved in distilled water under continuous stirring, to obtain casting solutions of 4% (w/w) concentration. Sorbitol at a concentration of 25% dry basis (db), was added as plasticizer to the polymer solutions and this concentration was kept constant throughout the study. Such a concentration of sorbitol was necessary, since films containing sodium caseinate were very difficult to handle without breaking, when lower plasticizer levels were used. The solutions were subsequently filtered to remove any undissolved material, vacuum degassed to remove air bubbles and 20 g of the solution were cast on plastic frames. The frames were then stored in an oven at 35 °C, allowing them to dry slowly. Bilayer films were prepared by successively casting of sodium caseinate solutions onto already preformed pullulan films. For bilayer films, different polymer ratios essentially resulted in differences in polymer layers. For example, in the bilayer film with P/SC ratio = 3/1, the pullulan layer was obtained by casting of 20 g of 3% (w/w) solution of pullulan (containing 25% db sorbitol, i.e., 1 g sorbitol in 100 g solution), whereas the sodium caseinate layer was obtained by casting 20 g of 1% (w/w) solution of sodium caseinate (containing 25% sorbitol, i.e., 0.33 g sorbitol in 100 g solution) on the same plate over the already prepared pullulan film.

Beeswax-laminated films were prepared by laminating molten beeswax onto preformed plain polymer films, as well as bilayer and blend films (P/SC ratio = 1). Molten beeswax (100 °C) was spread evenly over the hydrocolloid film with a prewarmed thin layer chromatography coater (Desaga Heidelberg, Germany). Film thickness was determined using a manual micrometer at five random positions on the film.

2.3. Experimental design

A mixture-process variable experimental design was employed, to investigate the effect of film composition

Table 1
Mixture-process variable experimental design with level of factors given in proportions

Run[a]	Factors in proportion		Process variable
	Pullulan	Sodium caseinate	RH (%)[b]
1	0.250	0.750	53 (−1)
2	0.625	0.375	53 (−1)
3	0.750	0.250	53 (−1)
4	0.375	0.625	53 (−1)
5	0.500	0.500	53 (−1)
6	0.750	0.250	75 (1)
7	0.625	0.375	75 (1)
8	0.500	0.500	75 (1)
9	0.375	0.625	75 (1)
10	0.250	0.750	75 (1)

[a] Experimental runs were performed in random order. Treatments corresponding to 5 and 8 denote reference mixture formulation.
[b] RH, relative humidity (%) at which samples were conditioned. Numbers in parenthesis are the coded levels of the process variable.

on the tensile properties of blend and bilayer films, comprised of pullulan and sodium caseinate and plasticized with 25% db sorbitol. Mixture experiments are a special class of response surface experiments in which the factors are the proportions of the components and the measured property response depends on such component ratios. Thus, the basic constraint for the mixture experimental design is

$$\sum_{i=1}^{n} x_i = x_1 + x_2 + \ldots x_n = 1$$

where x_i is the proportion of the ith component and n is the number of components in the mixture. Besides the relative proportions of the components, the response could be a function of process variables, which are not part of the mixture but may affect the mixture blending properties. In the present study, a mixture-process variable experiment was employed, with pullulan and sodium caseinate being the polymeric film components and the RH (53% and 75%), at which samples were conditioned, was the process variable. In bilayer films pullulan and sodium caseinate were not components of the same mixture. However, keeping the proportion of each polymer, as determined from the mixture design, allowed us to have in a bilayer film structure the same composition of biopolymers as in blends and the overall film thickness constant. The experimental design consisted of 10 experimental points (compositional protocols), as shown in Table 1. The experimental data were analyzed to fit polynomial models to the response variables, using MINITAB Statistical Software, Release 13.1. The analysis was performed using coded units.

Furthermore, statistical analyses were performed to detect differences in water vapour permeability and tensile properties of films with and without beeswax coating. The general linear model (GLM) procedure was utilized for analysis of variance (ANOVA). Tukey's multiple comparisons were used to determine any significant differences between specific means at a 95% confidence interval.

2.4. Moisture sorption isotherms

Moisture sorption isotherms were determined, according to Biliaderis, Lazaridou, and Arvanitoyannis (1999), for mix and bilayer films with P/SC ratio of 1/3 and 3/1. Film samples (~300 mg) were placed in previously weighed aluminum dishes and dried at 45 °C in an air-circulated oven over silica gel for 3 days (until constant weight). The samples were subsequently kept in desiccators over saturated salt solutions of known RH at 25 °C for 21 days, a time sufficient to reach constant weight and hence practical equilibrium. The moisture content of samples, after storage, was determined by drying at 110 °C for 2 h. The obtained data were fitted to the Brunauer–Emmett–Teller (BET) or Guggenheim–Anderson–DeBoer (GAB) sorption isotherm models. Measurements were performed in triplicate.

2.5. Water vapour permeability

Water vapour permeability (WVP) of films was performed gravimetrically at 25 °C, using the ASTM (E96-63T) procedure, modified for the vapour pressure at film underside, according to McHugh et al. (1993). Before WVP determination, all films were equilibrated at 53% RH at 25 °C for 48 h. The test films, cut in the form of disks, were sealed to cups containing distilled water and the cups were placed in an air-circulating oven at 25 °C. The oven was equilibrated at 53% RH, using a saturated solution of $MgCl_2 \cdot 6H_2O$. The air gap inside the cup was ~1.2 cm and the film area exposed for water vapour transmission was 13.8 cm^2. The lipid side of the beeswax-laminated film was exposed to the high humidity conditions. Film permeance was determined using the following equation:

$$\text{Permeance} = \frac{\text{Slope}}{A \cdot \Delta p}$$

where slope is given from weight loss vs. time, A is the exposed area of the film and Δp is the water vapour pressure differential across the film. WVP was calculated as follows:

$$\text{WVP} = \text{Permeance} \times \text{Thickness}$$

The steady-state vapour flow was reached within 2 h for films without wax and after about 10 h for beeswax laminated films. Slopes were calculated by linear regression and correlation coefficients for all reported data were >0.99. At least five replicates of each film type were tested for WVP.

2.6. Mechanical properties

Films were cut into dumbbell form strips and stored at appropriate RH for 10 days, to obtain films with different moisture contents. Film thickness was measured at three different points with a hand-held micrometer and an average value was obtained. Samples were analyzed with a TA-XT2i instrument (Stable Microsystems, Godalming, Surrey, UK) in the tensile mode, operated at ambient temperature and a cross head speed of 60 mm min^{-1}. Young's modulus (E), tensile strength (σ_{max}) and % elongation at break (% EB) were calculated from load-deformation curves of tensile measurements. Measurements represented an average of at least eight samples. The moisture content of samples, after storage, was determined by drying at 110 °C for 2 h.

3. Results and discussion

3.1. Moisture sorption isotherms

Moisture sorption isotherms were constructed for mix and bilayer films with P/SC ratio of 1/3 and 3/1. The specified polymer ratios were chosen to see if there was any possible significant effect of film composition on the moisture

sorption. The isotherms obtained were sigmoid in shape, showing a slow initial increase in moisture content with a_w increase up to 0.53 and a rapid increment in film water content with further increase of a_w (Fig. 1). Such a behaviour is characteristic of materials rich in hydrophilic polymers and has been reported extensively in literature (Biliaderis et al., 1999; Cho & Rhee, 2002; Cuq, Gontard, Aymard, & Guilbert, 1997; Diab, Biliaderis, Gerasopoulos, & Sfakiotakis, 2001; Gaurdin, Lourdin, Forssell, & Colonna, 2000; Gaurdin, Lourdin, Le Botlan, Ilari, & Colonna, 1999; Gontard, Guilbert, & Cuq, 1993; Hernández-Muñoz, Kanavouras, Perry, & Gavara, 2003; Hernández-Muñoz, Lagaron, Lopez-Rubio, & Gavara, 2004; Kim & Ustunol, 2001; Myllärinen, Partanen, Seppälä, & Forssell, 2002). No differences in the equilibrium water content were observed between blend and bilayer films with different polymer ratios; their moisture sorption isotherms were practically indistinguishable. It was probably the relatively high concentration of sorbitol that might obscure any differences in moisture uptake of films due to the effect of polymer ratio. In a previous investigation (Kristo & Biliaderis, 2006) it was shown that even unplasticized samples of pullulan and sodium caseinate exhibit very similar values of equilibrium moisture content. It was suggested that in the presence of sodium ions the tendency of caseinate toward water uptake increases (Ruegg & Moor, 1984) to a comparable degree with the more hydrophilic pullulan. Since both polymers absorb water to a similar degree, it is reasonable to assume that their blend or bilayer films would have comparable equilibrium water contents, independent of the polymer ratio.

The GAB and BET equations were fitted to experimental sorption data and the calculated parameters are given in Table 2. High r^2 values (>0.93) verified the suitability of both equations in explaining the experimental data. However, the three parameter GAB model is the more generally accepted equation, providing an extension of the BET equation to water sorption data up to an a_w of 0.94 and taking into consideration the properties of the sorbed water in the multilayer region (Kapsalis, 1987). In contrast, the

Table 2
Estimated parameters for water sorption data of pullulan–sodium caseinate bilayer and blend films using the BET and GAB isotherm models at 25 °C

Sample	GAB				BET		
	m_m [a]	K [b]	C [b]	r^2	m_m [a]	K [b]	r^2
P/SC[d] 1/3	6.22	0.93	5.81	0.95	5.56	6.45	0.99
P/SC 3/1	6.09	0.94	6.46	0.94	5.46	7.38	0.99
P:SC[c] 1:3	6.28	0.92	6.10	0.95	5.60	6.72	0.98
P:SC 3:1	6.10	0.93	6.95	0.93	5.46	7.89	0.98

[a] m_m, monolayer value.
[b] K and C – GAB and BET model constants.
[c] P/SC, pullulan/sodium caseinate bilayer films.
[d] P:SC, pullulan:sodium caseinate blend films.

BET model is mostly applicable for $a_w \leqslant 0.55$ (Mathlouthi, 2001). Monolayer moisture values obtained from the BET model were slightly lower than their respective counterparts derived from the GAB model. All types of films showed very close monolayer values in both models. Also bilayer or blend films with different P/SC ratios did not show appreciable variations. Lewicki (1997) concluded that when the K and C values of the GAB model fall within the intervals $0.24 \leqslant K \leqslant 1$, $5.67 \leqslant C \leqslant \infty$, the calculated monolayer values differ by no more than $\pm 15.5\%$ from the true monolayer capacity. In the current study, the K and C values fell within the aforementioned ranges, with K values being between 0.92 and 0.94 and C values from 6.09 to 6.28.

3.2. Water vapour permeability

Different ratios of P/SC (ranging from 0.33 to 3, w/w) were employed to establish any possible relationship between film composition and WVP in bilayer and blend films. The WVP values of the studied films, along with film thickness and RH estimates at the film underside are given in Table 3. No significant differences ($P > 0.05$) were observed in WVP, either within bilayer or blend films of different composition, or between two kinds of films with the same P/SC analogy. Also the difference between the WVP of plain pullulan and sodium caseinate film was statistically non-significant ($P > 0.05$). It was probably the relatively high concentration of sorbitol in combination with the high RH region of measurements (RH gradient 100/53%, inside/outside the cell) that might obscure the differences in film WVP due to the effect of polymer ratio. It is well documented in the literature that biopolymer plasticization by polyols brings about the increase of WVP (Biliaderis et al., 1999; Hernández-Muñoz et al., 2003; Hernández-Muñoz et al., 2004; Mali, Grossmann, Garcra, Martino, & Zaritzky, 2004; Myllärinen et al., 2002). At the high RH region, sorbitol absorbs readily water, which consequently increases the diffusion constant of water vapour and hence the WVP (Chang, Cheah, & Seow, 2000; Kester & Fennema, 1989). Also, the polyol molecules compete
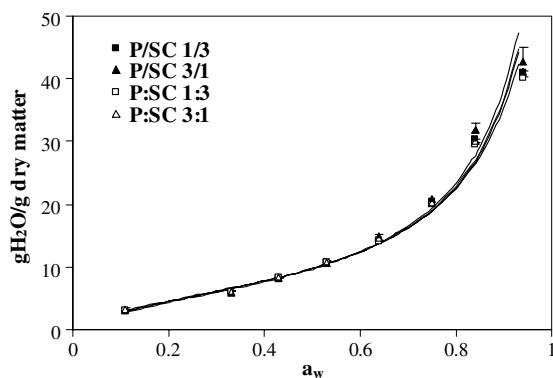

Fig. 1. Moisture sorption isotherms of pullulan–sodium caseinate bilayer (P/SC) and blend (P:SC) films with weight polymer ratio 1/3 or 3/1 at 25 °C; solid lines represent the GAB model fit to the data.

Table 3
Effect of polymer weight ratio on water vapor permeability (WVP) of plain, bilayer and blend films of pullulan and sodium caseinate

P/SC ratio	Bilayer films			Blend films		
	Thickness (µm)	WVP $(\text{g s}^{-1} \text{ m}^{-1} \text{ Pa}^{-1}) \times 10^{10}$	RH at film underside (%)	Thickness (µm)	WVP $(\text{g s}^{-1} \text{ m}^{-1} \text{ Pa}^{-1}) \times 10^{10}$	RH at film underside (%)
0.33	62 ± 2	9.41 ± 0.8	77.49 ± 0.9	69 ± 0	9.07 ± 0.1	79.49 ± 0.6
0.60	63 ± 2	10.59 ± 2.3	76.25 ± 2.2	64 ± 1	9.02 ± 0.3	78.35 ± 0.9
1.00	58 ± 4	9.22 ± 0.8	75.29 ± 3.1	62 ± 7	10.23 ± 0.9	76.18 ± 2.0
1.67	58 ± 1	9.98 ± 1.0	75.77 ± 1.5	65 ± 4	10.39 ± 0.9	76.54 ± 0.4
3.00	57 ± 3	9.33 ± 0.5	76.57 ± 0.8	61 ± 4	9.58 ± 0.9	76.76 ± 1.0
	Pullulan film			Na caseinate film		
	55 ± 0	9.94 ± 1.0	75.78 ± 1.4	70 ± 1.0	11.05 ± 0.7	75.27 ± 1.1

with water for active sites on the polymer, thus assisting in water clustering (Kilburn, Claude, Schweizer, Alam, & Ubbink, 2005), which, in turn, increases the free volume and polymer permeability (Hernández-Muñoz et al., 2003; McHugh & Krochta, 1994). The WVP values of the films $(9\text{--}11 \times 10^{-10} \text{ g s}^{-1} \text{ m}^{-1} \text{ Pa}^{-1})$ were similar to the WVP values of sodium caseinate films plasticized by glycerol $(\sim 8.3 \times 10^{-10} \text{ g s}^{-1} \text{ m}^{-1} \text{ Pa}^{-1})$ reported by Schou et al. (2005), soy protein films $(10.5\text{--}12 \times 10^{-10} \text{ g s}^{-1} \text{ m}^{-1} \text{ Pa}^{-1})$ reported by Kunte, Gennadios, Cuppet, Hanna, and Weller (1997) and soy protein isolate films $(8\text{--}8.5 \times 10^{-10} \text{ g s}^{-1} \text{ m}^{-1} \text{ Pa}^{-1})$ reported by Cho and Rhee (2004), all tested under similar conditions of temperature and RH gradient. The WVP value obtained for sodium caseinate film $(11.05 \times 10^{-10} \text{ g s}^{-1} \text{ m}^{-1} \text{ Pa}^{-1})$ was higher than the value of $4.11 \times 10^{-10} \text{ g s}^{-1} \text{ m}^{-1} \text{ Pa}^{-1}$ reported by McHugh et al. (1993) for unplasticized sodium caseinate films. The difference could be attributed to the presence of 25% db sorbitol as plasticizer in the present study as well as to the different RH gradient conditions employed. The 100/53% (inside/outside the cup) RH gradient applied in this work constitutes more severe conditions than the 100/0% (inside/outside the cup) RH gradient used in the study of McHugh et al. (1993). It has been reported in several investigations that the WVP of edible films are significantly greater when tested at 97/65% RH gradient than at 100/0% RH gradient (Greener, 1992; Greener & Fennema, 1989; Kester & Fennema, 1989).

Polysaccharide and/or protein components of edible films are characterized by an emphatic hydrophilicity, which is responsible for film-water interactions and structural changes in the polymeric film network. The non-linear sorption isotherms of such compounds make the water permeability through their films a complex phenomenon, which is accompanied by some previously reported anomalies, such as thickness effects (Banker, Gore, & Swarbrick, 1966; Hagenmaier & Shaw, 1990; Mali et al., 2004; McHugh et al., 1993; Park & Chinnan, 1995). In the current study, the effect of film thickness on WVP was investigated in the case of pullulan and sodium caseinate films. A linear relationship fitted well to the data of WVP as a function of thickness (Fig. 2) and was consistent
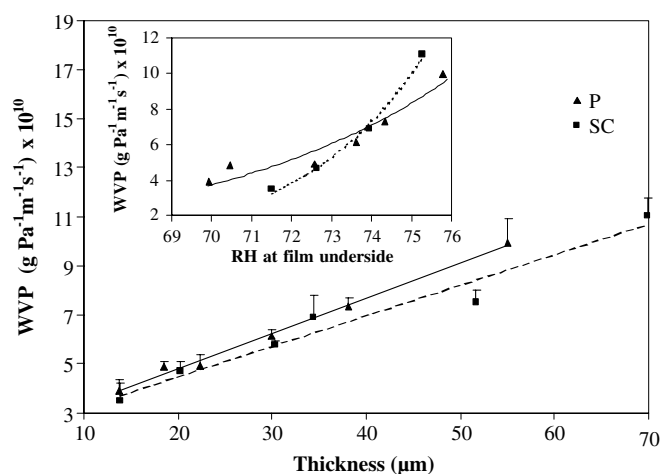

Fig. 2. Effect of film thickness and relative humidity conditions under the film (inset) on the water vapour permeability of pullulan (P) and sodium caseinate (SC) films at 25 °C.

with the results of Bravin, Peressini, and Sensidoni (2006), Hagenmaier and Shaw (1990), Mali et al. (2004), McHugh et al. (1993) and Park and Chinnan (1995). McHugh et al. (1993) attributed this phenomenon to the increased RH at film underside, as a consequence of the greater resistance that the film provides to mass transfer when its thickness increases. The higher RH at film underside produces the WVP augmentation due, to the exponential relationship between WVP and RH observed in hydrophilic films. Such a relationship has been described by McHugh et al. (1993) for sodium caseinate and whey protein isolate films, Bravin et al. (2006) for starch–methylcellulose films, Cuq et al. (1997) for myofibrillar protein-based films and is also observed in the present study (Fig. 2).

3.3. Tensile properties

Table 4 summarizes the estimated regression coefficients of the polynomial models, along with their r^2 values. Pullulan and sodium caseinate proportions in both blend and bilayer films showed a significant negative interactive effect on E, $\sigma_{\max}$ and % EB. Cox trace plots were generated for

Table 4
Regression coefficients of polynomial models for the response variables; analysis has been performed using coded units

Factors[a]	Bilayer films			Blend films		
	E^{b} (MPa)	$\sigma_{max}{}^{b}$ (MPa)	% EB[b]	E (MPa)	σ_{max} (MPa)	% EB
P	296	10.44	433.8	565	11.85	454
SC	1024	25.16	74.8	757	19.37	57
P × SC	−1391[*]	−36.16[**]	−770.4[**]	−1455[***]	−28.66[***]	−733[***]
P × RH	−126	−4.02	280[***]	−434[***]	−7.27[***]	462[***]
SC × RH	−435[*]	−12.69[***]	35.7	−190[***]	−6.99[***]	119[**]
P × SC × RH	34	10.19	−507.5[*]	67.28[*]	3.2	−1100[***]
r^2	0.997	0.997	0.994	0.999	0.999	0.994

[a] Factors: P, pullulan; SC, sodium caseinate; RH, relative humidity (%).
[b] E, Young modulus; σ_{max}, tensile strength; % EB, % elongation at break.
[*] $P \leqslant 0.05$.
[**] $P \leqslant 0.01$.
[***] $P \leqslant 0.001$.

all responses and representative plots for E and % EB of a blend sample conditioned at RH 75% are shown in Fig. 3. The Cox trace plots show the effect of changing one of the mixture components while keeping constant the ratio between components. In those plots, the fitted values of the responses were plotted while moving away from a reference mixture, which is the centroid of the design and is represented with 0 in the Cox plots. The composition in proportion of the centroid point is pullulan = 0.5 and sodium caseinate = 0.5. From the Cox plots (Fig. 3), it is obvious that increasing the pullulan content (and simultaneously decreasing sodium caseinate content) decreased E

Fig. 3. Cox trace plots for: (a) Young's modulus E and (b) % elongation at break of a blend film conditioned at 75% RH. A, pullulan; B, sodium caseinate.

and increased % EB suggesting that the polysaccharide provides a more flexible structure to the blend and bilayer films, in contrast to sodium caseinate, which contributes to increased stiffness and tensile strength. The distinct effect could arise from the substantially different structures of these polymers. The presence of $(1 \rightarrow 6)$ linkages on the pullulan chains allows for molecular motions to take place around the three inter-residue bonds providing chains with extra flexibility (Biliaderis et al., 1999; Bizot, Le Bail, Leroux, Roger, & Buleon, 1997; Gidley et al., 1993). On the other hand, electrostatic interactions involving the sodium cations in the sodium caseinate matrix could be involved in a chain ridification mechanism (Eisenberg & Navratil, 1973; Gidley et al., 1993). Similarly, Lee, Shim, and Lee (2004) observed that increasing the proportion of gelatin in a gellan/gelatin composite film (gelatin by itself formed more flexible films than gellan) resulted in a decrease of σ_{max} and in % EB increment. The observed curvature in the Cox trace plots originates from the significant interactive effect between pullulan and sodium caseinate, as well as the interaction with RH. The statistical data given in Table 4 indicated that the mechanical properties of bilayer films show notably lower dependence on factor interaction terms than their blend counterparts. Thus, besides the P × SC term, E and σ_{max} of bilayer films were significantly affected only by the interactive term SC × RH, whereas % EB was positively affected by P × RH and P × SC × RH. On the other hand, E, σ_{max} and % EB of blend films were significantly affected by almost all the terms of the model. This differentiation could arise from the distinct morphology of the layered films, where the interactions between components are focused at the interface of the layers, in contrast to blends, where the components' interface is much greater. Because of the difference in the moduli of components, both layered and blend systems would have stress concentrations at the interfacial regions when they are under tension. However, the blend systems have higher local stress concentrations than layered films. Moreover, the interface of layered films is aligned along the tensile direction, and so no strong stress concentration effect exists
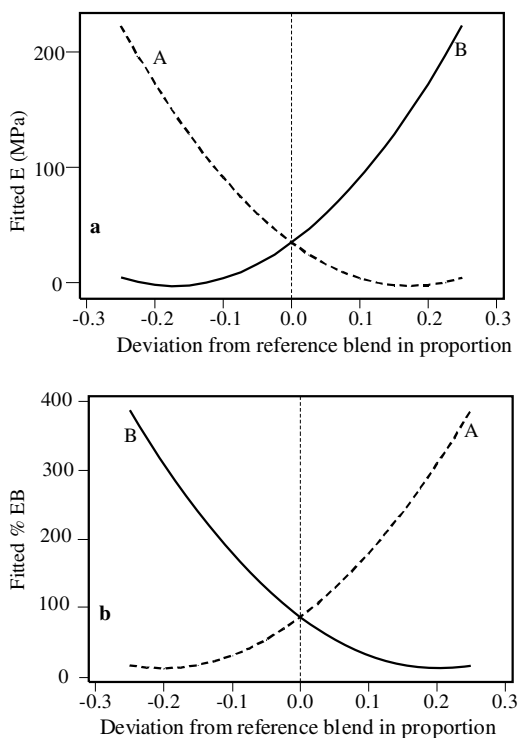
near the interface (Lee, Macosko, & Bates, 2005). Since the local stress conditions play an important role in the initiation of crazing and mechanical failure of the material, it is probable that the mechanical behaviour of blend films will depend to a greater extent on the interactive effects of both polymers.

Furthermore, to study the effect of moisture content on the mechanical properties of blends and bilayers with different P/SC ratios, films were conditioned at four RH levels (33%, 43%, 53% and 75%). The effect of water content on E, σ_{max} and % EB is represented in Figs. 4 and 5. For comparison, the curves of E and σ_{max} and % EB of plain pullulan and sodium caseinate films are also incorporated in both figures. In the moisture content range of 5–8%, most of the films exhibited an increase in E and σ_{max} with the addition of small amounts of water. A similar behaviour of biopolymer films has been reported in previous investigations (Biliaderis et al., 1999; Cheng, Abd Karim, Norz-iah, & Seow, 2002; Cuq et al., 1997; Diab et al., 2001; Fontanet, Davidou, Dacremont, & Le Meste, 1997; Gontard et al., 1993; Harris & Pelleg, 1996; Lazaridou & Biliaderis, 2002; Lazaridou, Biliaderis, & Kontogiorgos, 2003; Le Meste, Roudaut, & Davidou, 1996; Marzec & Lewicki, 2006; van Soest, Benes, de Wit, & Vliegenthart, 1996a; van Soest, de Wit, & Vliegenthart, 1996b). The initial strengthening in E and σ_{max} was attributed to water-induced cohesiveness and toughness of the brittle material at low a_w, allowing the structural elements to remain intact and continue to offer resistance to yield (Harris & Pelleg, 1996). Cheng et al. (2002) ascribed the phenomenon to antiplasticization by water, which makes the biopolymer matrix rigid rather than flexible. Furthermore, Chang et al. (2000) adopted the hypothesis of antiplasicization of synthetic polymers by diluents to give an explanation for the antiplasticization of water on the tensile strength of starch. According to this hypothesis, water molecules
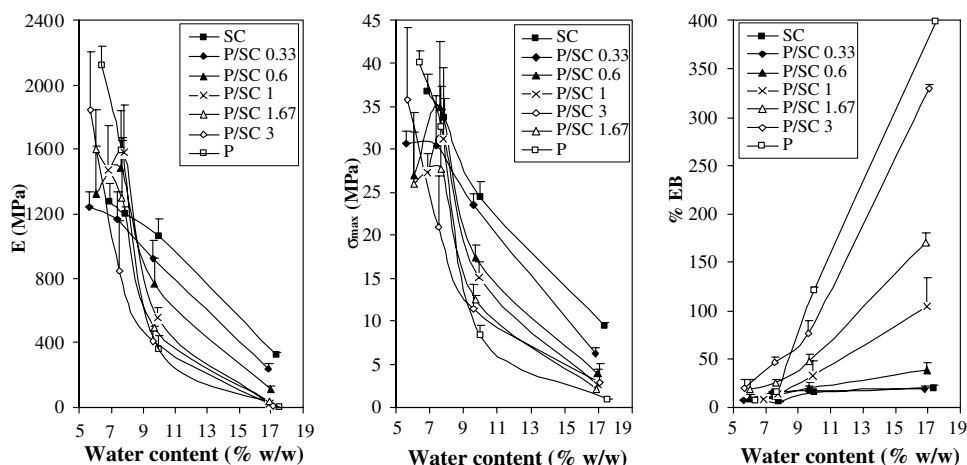

Fig. 4. Effect of water content on tensile modulus (E), tensile strength (σ_{max}) and % elongation at break (% EB) as determined from tensile tests of pullulan (P)/sodium caseinate (SC) bilayer (P/SC) films with various weight polymer ratios.
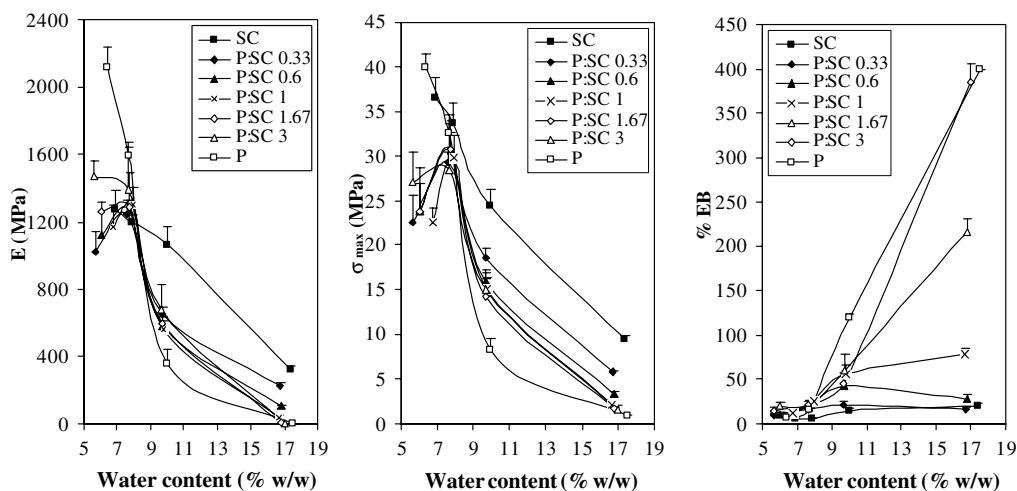

Fig. 5. Effect of water content on tensile modulus (E), tensile strength (σ_{max}) and % elongation at break (% EB) as determined from tensile tests of pullulan (P)/sodium caseinate (SC) blend (P:SC) films with various weight polymer ratios.

added in small amounts to the polymer facilitate polymer orientation and fill "holes" created in the polymeric system under large deformation, thus reducing "hole free volume" and increasing structural order. The further increase in water content of films was followed by a notable decrease in E and σ_{max} and an increase in % EB (Figs. 4 and 5), implying a moisture-induced brittle to ductile transition of the failure mode. The drop in E and σ_{max} was steep in the moisture content region of 8–11% for all films, with the exception of plain sodium caseinate and the bilayer film with P/SC ratio = 1/3. Considering that the Young's modulus drops steeply in the region around the glass transition (Ollett, Parker, & Smith, 1991; van Soest et al., 1996a, 1996b), the brittle to ductile transition of pullulan films could be attributed to the glass to rubbery transition, but this may not be the case for sodium caseinate films. In our previous investigation (Kristo & Biliaderis, 2006) it was noticed that, at moisture contents of 8–11%, the glass transition temperature (T_g) ranged from 33 to 21 °C (around the ambient temperature at which the tensile measurements were conducted) for pullulan and from 75 to 70 °C for sodium caseinate (T_g determined from the onset of E drop). Thus, the sodium caseinate films seemed to exhibit the brittle to ductile transition at temperatures well below their T_g, suggesting that considerable changes in their fracture properties happen within the glassy state. The smooth decline of the Young's modulus with the moisture content increase observed for sodium caseinate films may be attributed to the plasticizing action of water on the polymeric matrix and not to the transition from the glassy to rubbery state of this polymer. Similarly, Nicholls, Appelqvist, Davies, Ingman, and Lillford (1995) demonstrated that the brittle to ductile transition of gluten coincided with the glass to rubbery transition, whereas starch exhibited a ductile behaviour within the glassy state. Pullulan samples in the rubbery state are very weak and soft with low E and σ_{max}, but can be stretched to notably high ultimate elongations, reaching values of >399% when conditioned at RH 75%, as shown in Figs. 4 and 5. Note that the % elongation and tensile strength of the last samples correspond neither to the % elongation at break nor to maximum tensile strength because none of the tested samples broke within the experimental range of the mechanical analyzer used in this work. As is shown in Figs. 4 and 5, the curves of bilayer and blend films with varying ratio of both polymers are positioned between the curves corresponding to plain sodium caseinate and pullulan films. However, the tensile characteristics of bilayer films at moisture contents >8% are dominated by the component present in higher proportion (Fig. 4), while the tensile characteristics of blend films are closer to those of plain pullulan films (Fig. 5). Consequently, bilayer films containing a greater portion of sodium caseinate (i.e., P/SC ratio 1/3 and 1.5/2.5) showed fracture behaviour closer to that of plain sodium caseinate (gradual decline of E and σ_{max}, and smaller increase in % elongation at break). In both figures it is evident that increasing the pullulan fraction results in

decrease of E and σ_{max} and a drastic increase of % EB, supporting the notion that pullulan is mostly responsible for the film flexibility in the composite materials. It is worth noting that when increasing the P/SC ratio above 1, the elongation at break of the films increased drastically at 75% RH (e.g., blend film with P/SC ratio 3/1 at 17% moisture content showed an elongation at break of around 385%).

3.4. Water vapour permeance and mechanical properties of beeswax laminated films

Beeswax has been proposed as an excellent barrier to WVP (Kamper & Fennema, 1984a, 1984b; Kester & Fennema, 1989; Yang & Paulson, 2000) and was applied as a layer over the preformed hydrocolloid films, since previous studies (Greener & Fennema, 1989; Morillon, Debeaufort, Blond, Capelle, & Voilley, 2002) have shown that beeswax exhibited greater performance as a water barrier when applied in the form of a layer rather than used as a component of the blend. In general, waxes are the most efficient substances to reduce moisture permeability. Their high hydrophobicity is a consequence of a high content in esters of long chain fatty alcohols and acids, as well as long chain alkanes. Furthermore, solid fats are characterized by a dense structure, which limits the solubility and diffusion of water through them (Debeaufort et al., 2000; Greener & Fennema, 1989; Kamper & Fennema, 1984b; Kester & Fennema, 1989; Morillon et al., 2002). Water vapour permeances of beeswax-laminated pullulan, sodium caseinate and their blend and bilayer films were determined at a RH gradient 100/53% (inside/outside) at 25 °C and the results are presented in Fig. 6a. For comparison, in Fig. 6b the permeances of the same samples without beeswax are shown. The WV permeance values were chosen instead of permeability to avoid the incorporation of thickness into the calculations, since the thicknesses of unlaminated films (60–70 μm, Table 3) were significantly lower than the thickness of the beeswax laminated films (260–295 μm). Comparing both figures, the significantly ($P < 0.05$) lower permeance values of beeswax-coated films, as compared to those free of wax are obvious. The incorporation of beeswax brought about a reduction of permeance by 69 and 136 times for pullulan–sodium caseinate blend and bilayer films, respectively. Of the four wax laminated samples tested, the P/SC bilayer film displayed the lowest water vapour permeance ($P < 0.05$). It seems that beeswax-lamination of bilayer films provide better water barrier properties than that of blend samples. Thus, when pullulan and sodium caseinate are in the form of layers, the beeswax layer is spread over a homogeneous layer (that of sodium caseinate), in contrast to the blend films where a layer with regions of pullulan and sodium caseinate could be in contact with beeswax. During moisture absorption beeswax probably cracks, due to swelling of the hydrocolloid layer (Greener & Fennema, 1989; Kamper & Fennema, 1984b). The swelling of a homogenous sodium caseinate layer
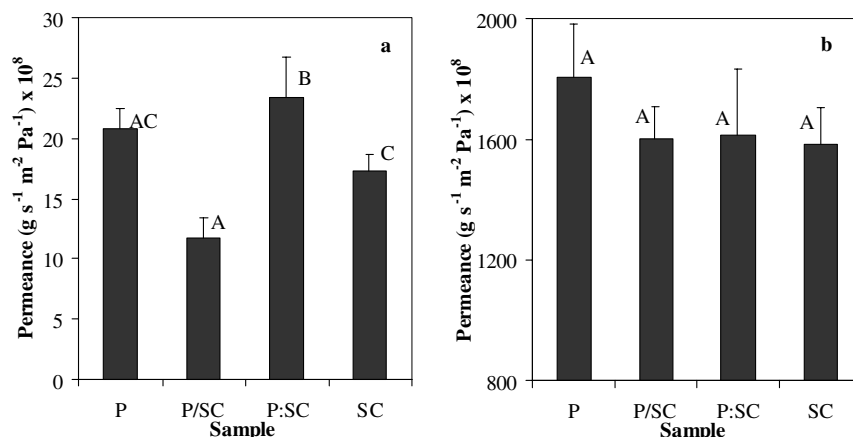
Fig. 6. Water vapor permeance of: (a) beeswax-laminated and (b) unlaminated films: P-pullulan, SC-sodium caseinate, P/SC-pullulan/sodium caseinate bilayer, P:SC pullulan:sodium caseinate blends. Means with the same capital letter are not statistically different ($P > 0.05$).

might be more uniform than the swelling of a heterogeneous blend layer, resulting in less defects of beeswax layer deposition in the first case. Beeswax laminated films maintained >99% RH at their inner surface (data not shown), due to the high resistance to moisture transfer of the beeswax layer. In comparison, the wax-free films, being less resistant to mass transfer, resulted in 75–76% RH (Table 3) at film underside instead of the expected 100% RH. The permeance values of beeswax laminated films (11.8–23.3×10^{-8} g s^{-1} m^{-2} Pa^{-1}) were in the same order of magnitude as the permeance of beeswax-laminated methylcellulose films (34×10^{-8} g s^{-1} m^{-2} Pa^{-1}) tested at 25 °C and a 97/65% RH gradient, as reported by Greener and Fennema (1989), and of zein films laminated with sorghum wax or carnauba wax (8.6–33.8×10^{-8} g s^{-1} m^{-2} Pa^{-1}), tested at 25 °C and a 50/100% RH gradient, as reported by Weller, Gennadios, and Saraiva (1998).

The water absorption by beeswax is expected to be very low compared to that of pullulan or sodium caseinate.

Thus, Greener (1992) reported that beeswax conditioned at RH up to 65% has a moisture content of ∼0.20% and wax conditioned at RH 80% a water content of ∼0.35% (dry beeswax basis). On the other hand, the hydrocolloid layers of films presented herein contain water ranging between ∼6% and 18% (sorption data of the wax-free films). Thus, the water content of the beeswax layer in the composite films would constitute only 2–3% of that of the hydrophilic polymeric layers. Considering these facts, the tensile properties of beeswax-laminated films are expressed as a function of water content of the hydrocolloid layer only. Beeswax lamination was performed on plain pullulan and sodium caseinate films, as well as on their bilayer and blend films at a P/SC ratio = 1. As shown in Fig. 7, increasing the water content of the samples caused a continuous decrease in E and σ_{max} and increase in % EB for beeswax-laminated samples, a similar behaviour to that found for the respective unlaminated films (Figs. 4 and 5). Similarly, Yang and Paulson (2000)
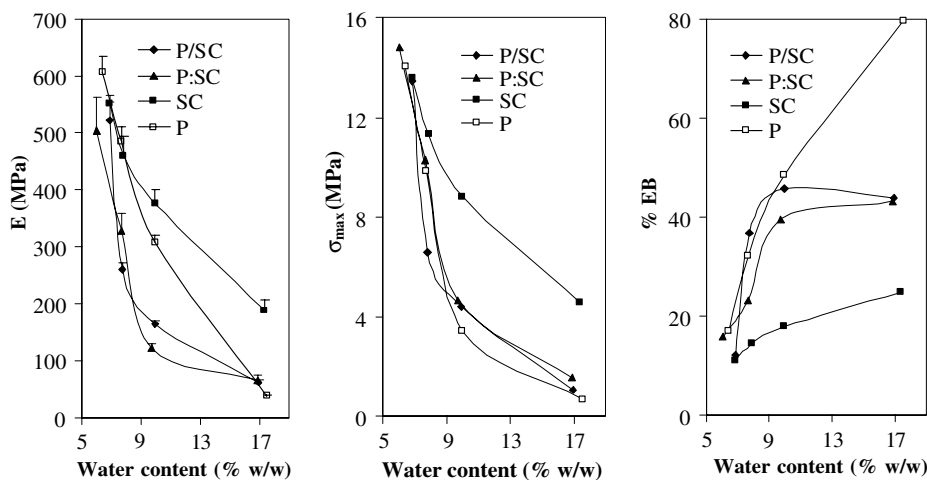

Fig. 7. Effect of water content on tensile modulus (E), tensile strength (σ_{max}) and % elongation at break (% EB) as determined from tensile tests of plain pullulan (P), sodium caseinate (SC) as well as their blend (P:SC) and bilayer (P/SC) films laminated with beeswax (weight polymer ratio 1:1).

reported a decline in E and σ_{max} of gellan/beeswax composite films, but no effect on % EB with increasing the water content of the samples.

Lipids are often incorporated in hydrocolloid films to improve the water vapour barrier properties of the matrix, while their use as free-standing films is limited, since they lack the sufficient structural integrity and durability (Greener, 1992). A decline in mechanical strength of polymeric films when lipid matter is incorporated mainly in the form of emulsion with the hydrocolloid component has often been reported (Bertan, Tanada-Palmu, Siani, & Grosso, 2005; Chen, 1995; Gontard et al., 1994; Möller et al., 2004; Park, Testin, Park, Vergano, & Weller, 1994; Péroval et al., 2002; Phan The et al., 2002; Shellhammer & Krochta, 1997; Yang & Paulson, 2000). In the present study, beeswax-laminated films (Fig. 7) showed significantly lower ($P < 0.05$) E (all films conditioned up to 53% RH) and σ_{max} values, as compared to those of unlaminated films conditioned at the same RH (Figs. 4 and 5). Thus, E and σ_{max} of pullulan films conditioned at 33% RH decreased from 2123 and 40 MPa to 610 and 14 MPa, respectively, when the film was laminated with beeswax. On the other hand, the E values of pullulan, blend and bilayer films conditioned at 75% RH decreased, whereas those of sodium caseinate increased, with lamination. As mentioned in Section 3.3, pullulan samples conditioned at RH 75% were in the rubbery state; they were very weak with very low Young's modulus, that could not be measured accurately. The opposite applied for sodium caseinate films, which have high E values under similar RH conditions. Probably the beeswax imparts some stiffness to films containing the weak pullulan matrix, increasing their modulus, but impairs the stiffness of already strong sodium caseinate films. More complicated was the whole picture with regard to the % EB. The % EB of all samples conditioned at RH up to 43% (moisture content ~6–8%) increased significantly ($P < 0.05$) with beeswax lamination. This is in agreement with the results reported by Park et al. (1994), for methylcellulose film laminated with a palmitic acid-corn zein layer, Anker (2000), for a whey protein isolate film laminated with acetylated monoglyceride, Weller et al. (1998), for zein films laminated with sorghum- or carnauba wax-medium chain triglyceride oil, Rakotonirainy and Padua (2001), for zein films coated with drying oils and Lai and Padua (1998), for zein films coated with linseed oil. Rakotonirainy and Padua (2001) suggested that coating with drying oils prevents crack propagation, thus improving the tensile properties of laminated films, whereas Lai and Padua (1998) indicated than the layer formed by polymerization of flax oil may form crazes that absorb energy before film breaking and delay sample failure. Despite the lack of structural integrity of lipid materials in general, it has been reported that beeswax exhibits viscoelastic properties, which make it flexible and less likely to crack (Shellhammer, Rumsey, & Krochta, 1997). On the other hand, lamination of pullulan films conditioned at 53 and 75% RH, caused a statistically significant decrease

($P < 0.05$) in their % EB (e.g., from 120% to 48% for samples conditioned at RH 53%), whereas no significant ($P > 0.05$) differences were noticed with lamination of the respective sodium caseinate films. Blend and bilayer films showed no significant change ($P > 0.05$) in % EB after lamination when conditioned at RH 53% but their % EB decreased due to beeswax lamination after conditioning at RH 75%. The pullulan samples conditioned at RH 75% were very soft and showed very high flexibility (% EB > 399%), thus imparting great flexibility to blend and bilayer samples too (% EB 78% and 104%, respectively). The beeswax layer added to these films made them unable to follow such high deformations and contributed to a much earlier film failure and, consequently in lower % EB (80%, 44% and 43% for beeswax-laminated pullulan, bilayer and blend films, respectively).

However, it should be noted that laminated films had greater thicknesses (250–320 μm) than their unlaminated counterparts (60–90 μm). Essentially, we compared the tensile properties of beeswax-laminated films with those of their plane hydrocolloid layers. Film thickness is in fact included in the calculations of E and σ_{max}, but not in % EB. Thus, it is important that tensile characteristics of films with different thickness be cautiously compared. To examine whether the observed differences in the tensile properties were due to the significant differences in thickness, pullulan and sodium caseinate films plasticized with 25% (db) sorbitol and having a thickness of ~400 μm were also tested after conditioning for 10 days at RH 43%, 53% and 75%. In agreement with the above discussion, these specimens showed greater E and σ_{max} values than their laminated counterparts, despite the greater thickness of the former (data not shown). Moreover, similarly to the aforementioned comparisons, the % EB of sodium caseinate thick samples (conditioned at RH 53 and 75%) was similar to that of their beeswax-laminated counterparts. For the pullulan film conditioned at RH 43% (7.7% moisture content) the % EB was lower ($P < 0.05$) than that of wax-laminated sample, whereas the opposite was observed for samples conditioned at RH 53 and 75% ($P < 0.05$). Consequently, the differences in E, σ_{max} and % EB between beeswax-laminated and wax-free films are largely due to the lipid layer.

4. Conclusions

Blend or bilayer films of pullulan and sodium caseinate with different weight polymer ratios were made. Varying the ratio of polymers did not significantly affect the water sorption capacity and the WVP of the films. Contrary, changing the ratio of the two polymers modified the mechanical properties of the composite films. Increasing the pullulan content (and simultaneously decreasing the sodium caseinate portion) decreased the Young's modulus (E), the tensile strength (σ_{max}) and increased the % elongation at break (% EB), suggesting that pullulan imparts flexibility and sodium caseinate stiffness to the composite film

materials. A sharp decline in E and σ_{max} and an increase in % EB followed the increase in moisture content of the samples above 8%. The brittle to ductile transition of pullulan coincided with the glass to rubbery transition, whereas sodium caseinate exhibited a ductile behaviour within the glassy state. Beeswax lamination largely improved the water barrier properties of the films, while its effect on E, σ_{max} and % EB was related to the characteristics of the hydrocolloid layer used and the moisture content of the films.

Acknowledgment

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