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Effect of alginate and λ-carrageenan on tensile properties and water vapour permeability of sodium caseinate–lipid based films

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ABSTRACT

Tensile properties and water vapour permeability (WVP) of edible films obtained from blends of sodium caseinate, polysaccharides (alginate or λ-carrageenan) and lipids (oleic acid and beeswax) were investigated. In lipid-free films with plasticizer, polysaccharides improved the tensile properties of films, although water vapour permeability values increased; this effect being more notable for λ-carrageenan and dependent on the polysaccharide concentration. Lipid mixture incorporation in the film resulted in more flexible films as compared to those prepared with pure beeswax, but the increase in beeswax contents implied a decrease in WVP. When lipids (oleic acid and beeswax) were present λ-carrageenan modify lightly the films properties, but the addition of alginate produce less flexible, less stretchable and more permeable films. In the absence of plasticizer, λ-carrageenan promoted stiffness and resistance to elongation of the caseinate films and reduce their WVP at low ratio of BW in the lipid mixtures (below 50%), showing the opposite effect at greater BW ratio.

1. Introduction

Edible films and coatings have been used in food domain in order to prevent mass transfer between the foodstuff and surrounding medium or between different phases of composite food products and therefore to avoid food quality deterioration due to physico-chemical changes, texture changes or chemical reactions. In this sense, this protective barrier can be formulated to prevent the transfer of moisture, gases, flavour or lipids, and to maintain or to improve food quality and to increase food product shelf life (Krochta & De Mulder-Johnston, 1997). In an effort to produce more environmentally friendly materials, renewable and biodegradable biopolymers have been investigated as coating materials (Chen, 1995; Guilbert, 1986; Han & Krochta, 2001; Krochta & De Mulder-Johnston, 1997; Tharanathan, 2003). Polymers derived from natural monomers, like proteins and polysaccharides, offer the greatest opportunities since their biodegradability and environmental compatibility are assured. Biopolymer films, which contain both protein and polysaccharide ingredients, may advantageously use the distinct functional characteristics of each film-forming ingredient. Some works indicate that incorporation of polysaccharides into globular protein matrices may extend the functional properties of these ingredients (Turgeon & Beaulieu, 2001; Zaleska, Ring, & Tomasik, 2000). Proteins and polysaccharides generally form films with good mechanical properties but they form poor moisture barriers, because of their hydrophilic nature. Conversely, lipids give rise to good moisture barriers, but have less effective mechanical properties and undesirable sensory properties (Guilbert, 1986).

Little information exists in the literature on the film-forming ability of such combinations of protein and polysaccharide components. Milk proteins, such as caseinates, have special properties which make them highly suitable for obtaining edible films. Their excellent nutritional value and their numerous functional properties such as their solubility in water and ability to act as emulsifiers are important factors for the formation of edible films (Arvanitoyannis, Psomiadou, & Nakayama, 1996; Chen, 1995, 2002; Khwaldia, Perez, Banon, Desobry, & Hardy, 2004; Mc Hugh & Krochta, 1994).

Carrageenans, biopolymers extracted from algae, are used extensively in the food industry to make milk gels and to stabilize products such as milk fat emulsions, ice cream and milk shakes. The three main carrageenans differ only in the number of sulphate groups. λ-Carrageenan is the most sulphated one and adopts a coil conformation whatever the ionic and temperature conditions. While kappa and iota carrageenans form gels, lambda is unable to do it and is used as a pure thickener (Langendorff et al., 2000; Lizarraga, De Piante Vicin, González, Rubiolo, & Santiago, 2006). Alginates are known as potential biopolymer films or coating components due to their unique and well studied colloidal properties, which include thickening, stabilizing, suspending, film-forming, gel producing and emulsion stabilizing properties (Rhim, 2004). Alginates are natural substances extracted from brown algae and

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composed of 1–4 β-D-mannuronic acid (M) and α-L-guluronic acid (G). In the polymer chain, the monomers are arranged alternately in GG and MM blocks together with MG blocks. The chemical composition and sequence of the M and G blocks depend on the biological source and growth and on seasonal conditions (Smidsrød, 1974).

The aim of this paper is to analyse the effect of λ-carrageenan and alginate on tensile and water permeability properties of sodium caseinate based films. This effect was analysed both in matrices without lipids and also in those where lipids are present in different ratio and composition (oleic acid and beeswax mixtures). The effect of the plasticizer was also considered.

2. Methods and materials

2.1. Materials

Alanate 110 sodium caseinate (Llorella, S.A., Barcerlona, Spain), λ-carrageenan (Biochemika, Fluka, Steinheim, Germany) and sodium alginate (Sigma–Aldrich, Steinheim, Germany) were used as film-forming components of the hydrophilic continuous phase for emulsion-based edible films. Beeswax (Brillocera, S.A., Valencia) and oleic acid (Panreac quimica, S.A., Castellar Del Vallés, Barcelona, Spain) were used as the hydrophobic dispersed phase, and glycerol (Panreac quimica, S.A., Castellar Del Vallés, Barcelona, Spain) was added as plasticizer.

2.2. Method of preparation of the film-forming emulsions

A first experimental series of films containing sodium caseinate–polysaccharide–glycerol was studied by using two polysaccharides (alginate or λ-carrageenan) at different protein–polysaccharide ratios (0.08 and 0.67 mg polysaccharide/g NaCas). λ-Carrageenan (negatively charged) was chosen because of its interactions with caseinates (Langendorff et al., 2000) which could lead to cross-linking among polymer chains, thus reducing film permeability, and alginate was chosen as an alternative negatively charged polymer where non-favourable interactions with caseinates have been described. The polysaccharide concentration was established on the basis of previous experiments, where higher concentration levels of alginate gave rise to non-homogenous film. The protein–plasticizer ratio was 1:0.3.

A second series made up of three groups of films with different sodium caseinate:lipid ratios (1:0.25 and 1:0.5) were prepared, also containing 0.3 glycerol–protein ratio. The lipid fraction was composed of oleic acid (OA) and beeswax (BW) in different OA:BW ratios (100:0; 95:5; 85:15; 70:30; 50:50; 30: 70 and 0:100). One group was prepared without polysaccharides and the other two groups included 0.67 mg polysaccharide/g NaCas of each one of the polysaccharides.

A third series was prepared by using 0.25 lipid–protein ratio with and without λ-carrageenan using 0.67 mg polysaccharide/g NaCas. This series was prepared without plasticizer.

The film-forming aqueous dispersions for the first series were prepared by dispersing 8% (ww) sodium caseinate in water and the amount of plasticizer required to obtain protein:plasticizer ratio of 1:0.3 (control film dispersion). The aqueous protein dispersions were homogenized for 1 min at 13,500 rpm, followed by 3 min at 20,500 rpm, using an Ultra-Turrax homogenizer (Ultra-Turrax T25, Janke & Kunkel, Germany). Separately, 1% (w/w) of polysaccharide was dispersed in deionised water under stirring for 10 min at room temperature and then heated at 85 °C until polysaccharide was perfectly solubilised. Different aliquots of this solution were added to the protein aqueous dispersions to give the required final protein–polysaccharide ratios in the mixtures.

For the second series, water dispersions of sodium caseinate, glycerol and polysaccharide were heated at 85 °C and the amount of beeswax required was incorporated, which then melted in the hot solution, and the mixture was homogenized for 1 min at 13,500 rpm, followed by 1 min at 20,500 rpm. The emulsions were cooled at room temperature and oleic acid was added in the amount required for each film formulation. Each emulsion was homogenized again with a vacuum high-shear probe mixer (Ultra-Turrax T25, Janke & Kunkel, Germany) for 2 min at 20,500 rpm. The film-forming dispersions were degasified at room temperature with a vacuum pump. The films prepared without beeswax (100:0 OA:BW ratio) were homogenized as explained previously but at room temperature.

For the third series, film-forming dispersions were prepared as for the second series, but using λ-carrageenan as the only polysaccharide and without adding glycerol.

Table 1 showed the mass fraction of the different film components reached in the dried film formulations for the three experimental series.

2.3. Film formation method

Films were prepared by weighing the amount of the degasified film-forming dispersion that would provide 2 g of total solids on a 120 × 120 mm casting plate resting on a level surface (138.9 g solids/m^2). The solution was spread evenly over the whole surface area and films were dried for approximately 24 h at 45% RH and 20 °C. Afterwards dry films were peeled from the casting surface.

2.4. Mechanical properties

A universal test Machine (TA.XTplus model, Stable Micro Systems, Haslemere, England) was used to determine tensile strength (TS), film elastic modulus (EM) and elongation (E) properties, according to ASTM standard method D882 (ASTM, 1992). EM, TS and E properties were determined from stress–strain curves, estimated from force-deformation data. After drying, two samples of each obtained film were selected for the tensile property measurements. At least eight replicates of each sodium caseinate film formulation were tested. The cut film samples had a rectangular section of 2.5 cm wide and 10 cm long. Before testing, all samples were equilibrated for 4 days at 58% RH in a cabinet using magnesium nitrate saturated solution at 5 °C. Equilibrated film specimens were mounted in the film-extension grips of the testing machine and stretched at a rate of 50 mm min^{-1} until breaking. The relative humidity and temperature of the testing environment was controlled before and after each testing session with a thermohygrometer (testo 60-H2, Testo GmbH & Co., Lenzkirch, Germany) and was held constant at 58 ± 2% RH and 20 ± 1 °C, respectively.

Table 1
Mass fraction of film components

Films	Protein:lipid ratio	Protein	Glycerol	Lipid
First series	Control (without lipid)	0.77	0.23	0
		0.77	0.23	0
		0.77	0.23	0
Second series	1:0.25	0.65	0.19	0.16
		0.64	0.19	0.16
	1:0.5	0.55	0.17	0.28
		0.56	0.17	0.28
Third series	1:0.25	0.80	0	0.20
		0.80	0	0.20

$x_{\text{polysaccharide}} = x_{\text{protein}} * \alpha$ ($\alpha = 0.8 * 10^{-4}, 6.7 * 10^{-4}$).
$x_{\text{OA}} = x_{\text{lipid}} * \beta$ ($\beta = 1, 0.95, 0.85, 0.70, 0.50, 0.30, 0$).
$x_{\text{BW}} = x_{\text{lipid}} * \gamma$ ($\gamma = 0, 0.05, 0.15, 0.30, 0.50, 0.70, 1$).

2.5. Water vapour permeability measurements

A modification of the ASTM E96-92 (Mc Hugh, Avena-Bustillos, & Krochta, 1993) gravimetric method for measuring WVP of flexible films was employed for all films with Payne permeability cups (Elcometer SPRL, Hermelle /s Argenteau, Bélgica). Nine films were cast from each solution. Upon drying, six films were chosen for WVP testing based on lack of physical defects such as cracks, bubbles or pinholes. Distilled water (15 mL) was placed in each test cup to expose the film to high relative humidity on one side through a circular opening of 6 cm in diameter. The film side in contact with the casting plate surface was exposed to the conditions inside the test cups. Once the films were secured, the cups were placed in a preequilibrated cabinet fitted with a variable-speed fan to provide a strong driving force across the film for water vapour diffusion. Thus, the free film surface during film formation was exposed to the RH of the cabinet. The environment within the cabinet was held constant at 58% RH using over-saturated magnesium nitrate-6-hydrate (Panreac quimica, S.A., Castellar del Vallés, Barcelona). The cabinets were placed at controlled temperature of 5 °C. The cups were weighed periodically after steady state was reached using an analytical balance (±0.0001 g). Water vapour transmission (WVTR) was determined from the slope obtained from the regression analysis of weight loss data as a function of time, once the steady state was reached,

$$\mathrm{WVTR} = \frac{\mathrm{Slope}}{\mathrm{Film\ area}}. \tag{1}$$

From WVTR data, the values of the vapour pressure on the film's inner surface (p_2) was obtained taking into account the method proposed by Mc Hugh et al. (1993) (Eq. (2)) to correct the effect of concentration gradients established in the stagnant air gap inside the cup,

$$\mathrm{WVTR} = \frac{P \cdot D \cdot \mathrm{Ln}[(P - p_2)/(P - p_1)]}{R \cdot T \cdot \Delta z}, \tag{2}$$

where P, total pressure; D, diffusivity of water through air at 5 °C; R, gas law constant, T, absolute temperature; Δz, mean stagnant air gap height, considering the initial and final z value; p_1, water vapour pressure on the solution surface; p_2, corrected water vapour pressure on the film's inner surface in the cup.

Water vapour permeance was calculated using Eq. (3) as a function of the vapour pressure on the inner surface of the film (p_2) and on the film's outer surface in the cabinet (p_3). Permeability was obtained by multiplying the permeance by the average film thickness,

$$\mathrm{Permeance} = \frac{\mathrm{WVTR}}{P_2 - P_3}. \tag{3}$$

2.6. Film thickness measurements

Film thickness was measured with a Palmer digital micrometer to the nearest 0.0025 mm at six random positions around the film used for WVP measurements and at four positions along the strips used for the mechanical properties. Average values were used in all WVP and tensile property determinations.

2.7. Statistical analysis

Statistical analysis of data was performed through analysis of variance (ANOVA) using Statgraphics Plus for Windows 5.1 (Manugistics Corp., Rockville, MD). Fisher's least significant difference (LSD) procedure was used.

3. Results and discussion

3.1. Effect of polysaccharide type and content

3.1.1. Tensile properties

Table 2 shows the tensile parameters, tensile strength (TS), elastic modulus (EM) and elongation (E) of control film (1:0.3 NaCas:Gly) and films containing alginate or λ-carrageenan at different ratios (0.08 and 0.67 mg polysaccharide/g NaCas). TS represents film resistance to elongation. EM indicates the stiffness of the material and *E* is a measure of the film's capacity for stretching. In general, the incorporation of polysaccharides produced a decrease in TS and EM and an increase in *E* values, depending on the polysaccharide–protein ratio. So, the films prepared with polysaccharides were more flexible and more stretchable than the films prepared without them. Similar results were observed by other authors working with whey protein, soy protein and caseinate films including alginate, pectin and/or carboxymethylcellulose in the film matrix (Parris, Coffin, Joubran, & Pessen, 1995; Sabato et al., 2001; Yu, Sabato, D'Aprano, & Lacroix, 2004). Table 2 also shows the homogeneous groups of tensile parameters, from a LSD analysis, for different characteristic parameters of the films. It can be observed that he films prepared with λ-carrageenan had significantly lower values of TS and EM than the control films whereas those prepared with alginate, especially those with the highest ratio, show very similar values to the control film. No significant differences in *E* values were observed for different films although those containing polysaccharide tend to be longer and the values showed greater variability. The differences in elastic modulus and tensile strength values with respect to those of the control film tend to decrease when the polysaccharide content increases; the TS and EM values of films with alginate at 0.67 mg polysaccharide/g NaCas were similar to the values for control film and they also showed greater variability. This aspect could be attributed to the heterogeneity introduced in the film structure by the incorporation of polysaccharides. The results show that the introduction of polysaccharide in the film matrix supposes an increase in the chain mobility that modifies the mechanical response leading to a more plasticized structure, especially at low concentrations in the matrix and depending on the protein–polysaccharide interactions; λ-carrageenan had a greater depressing effect on tensile properties of sodium caseinate films than alginate. Different interactions have been described for caseinates and these two polysaccharides: λ-carrageenan, the most highly charged form of carrageenans, shows attractive interaction with casein at a wide range of temperatures (Langendorff et al., 2000; Martin, Goff, Smith, & Dalgleish, 2006), whereas the poor thermodynamic interactions in sodium caseinate–alginate systems usually lead to the

Table 2
Values of tensile properties and water vapour permeability of sodium caseinate films (without lipids) containing glycerol and polysaccharides

Polysaccharide (mg polysaccharide/g NaCas)	Tensile parameters			Water vapour permeability
	EM (MPa)	TS (MPa)	E (%)	WVP (g mm/kPa h m^2)
Control	850 ± 1[a]	14.4 ± 0.6[a]	4.0 ± 0.6[a]	4.1 ± 0.3[a]
λ-Carrageenan				
0.08	637 ± 30[b]	12.4 ± 0.7[b]	6 ± 2[a,b]	6.1 ± 0.2[b]
0.67	678 ± 32[b]	11.7 ± 1.2[b]	7.8 ± 1.9[b]	5.9 ± 0.4[b]
Alginate				
0.08	723 ± 92[b]	12 ± 2[a,b]	6 ± 2[a,b]	5.1 ± 0.2[c]
0.67	899 ± 106[a]	14 ± 3[a]	6.0 ± 1.2[b]	5.00 ± 0.15[c]

[a–c] Homogeneous groups.

phases separating into an alginate rich phase and a sodium caseinate rich phase (Simeone, Alfani, & Guido, 2004; Simeone, Molè, & Guido, 2002). The interaction between λ-carrageenan and sodium caseinate could contribute to increase the sodium caseinate particle size giving rise to a more open structure, thus increasing flexibility and the ability to extend. In contrast, the more rigid and less extensible nature of the sodium caseinate:alginate composite films could be due to the biopolymers' ability to produce gels by phase separations (Rhim, 2004). Similar results were observed by Coughlan, Shaw, Kerry, and Kerry (2004) for films formed from whey protein isolate (WPI) and alginate which had slightly higher EM and TS than WPI and pectin or carrageenan films.

3.1.2. Water vapour permeability (WVP)

Table 2 shows the water vapour permeability values of the control film and films containing alginate or λ-carrageenan at different ratios. The incorporation of polysaccharides produced an increase of water vapour permeability values, according to the described structural effects deduced from mechanical behaviour. Films containing alginate exhibit better water barrier properties than those containing λ-carrageenan. These differences in WVP may be attributed, as commented on above for mechanical behaviour, to differences in gel formation and interactions between sodium caseinate and each polysaccharide (Langendorff et al., 2000; Martin et al., 2006). Similar results were observed by other authors mixing different proteins and polysaccharides such as soy protein isolate, whey protein isolate, caseinate, carboxymethylcellulose (CMC), alginate or pectin (Coughlan et al., 2004; Parris et al., 1995; Sabato et al., 2001; Yu et al., 2004); i.e. Parris et al. (1995) formed films from a combination of whey protein and alginate or pectin and reported lower WVP in films containing alginate than for films containing pectin. They attributed this effect to an increase in intermolecular hydrogen bonding between polymer molecules in the alginate-containing films when compared with those formed with pectin. As a result, spacing between the macromolecules may be decreased, leading to a reduction in WVP.

3.2. Effect of lipid incorporation: Type and content

Blends of polysaccharides and proteins with different ratios of polymers can be useful to improve tensile properties but are rather sensitive to moisture, due to the hydrophilic nature of these components. The incorporation of lipids tends to improve barrier properties of edible films composed of hydrocolloids (Avena-Bustillos & Krochta 1993; Gontard, Duchez, Cuq, & Guilbert, 1994). This strongly depends on the film structure (bilayer formation) and on the nature and distribution of fat in the hydrocolloids matrix.

The study of composite films produced by blending sodium caseinate, polysaccharide, oleic acid and beeswax mixtures could be very interesting because this approach permits the different functional properties of the film-forming components to be used advantageously. In this sense, the highest ratio of polysaccharide (0.67 mg polysaccharide/g NaCas) and two lipid:protein ratios (0.25 and 0.50) were chosen, always varying the OA:BW ratios in the lipid phase. This polysaccharide:protein ratio was chosen because the addition of the highest concentration of polysaccharides improved the mechanical properties whereas the water vapour barrier properties was not affected notably by polysaccharide concentration.

3.2.1. Tensile properties

Figs. 1 and 2 show the EM and TS values of the considered films. The results show that EM and TS values were significantly affected ($p < 0.05$) by the lipid content and its nature and by the type of polysaccharide. Nevertheless, lipid content and type affected these values more intensely. Generally, oleic acid reduced elastic modulus and tensile strength and increased elongation of films, thus producing more flexible and stretchable films than the control one. This behaviour was more pronounced when lipid content increased and was attributed to the discontinuities introduced in the polymer network (Bravin, Peressini, & Sensidoni, 2004; Pérez-Gagó & Krochta, 2000; Shellhammer & Krochta, 1997) and to the specific interactions with caseinates, especially for OA (Fabra, Talens, & Chiralt, 2008). Similar results were reported by Shellhammer and Krochta (1997) in whey protein based films containing different lipids (anhydrous milkfat fractions, beeswax, carnauba and candelilla wax). The tensile test showed that the lipid had a weakening effect on the films, hence showing the interruption effect of the lipid in the protein network which is what provides most of the tensile strength (Khwaldia, Banon, Desobry, & Hardy, 2004; Pérez-Gagó & Krochta, 2000; Shellhammer & Krochta, 1997).

The effect of OA:BW mixtures on tensile properties of sodium caseinate films has been described in a recent work (Fabra et al., 2008). The addition of lipid mixtures at different ratios let to a progressive decrease of EM and TS values till 30% of beeswax was reached in the lipid mixture, although a subsequent increase or stabilization of the values were observed for a greater ratio of

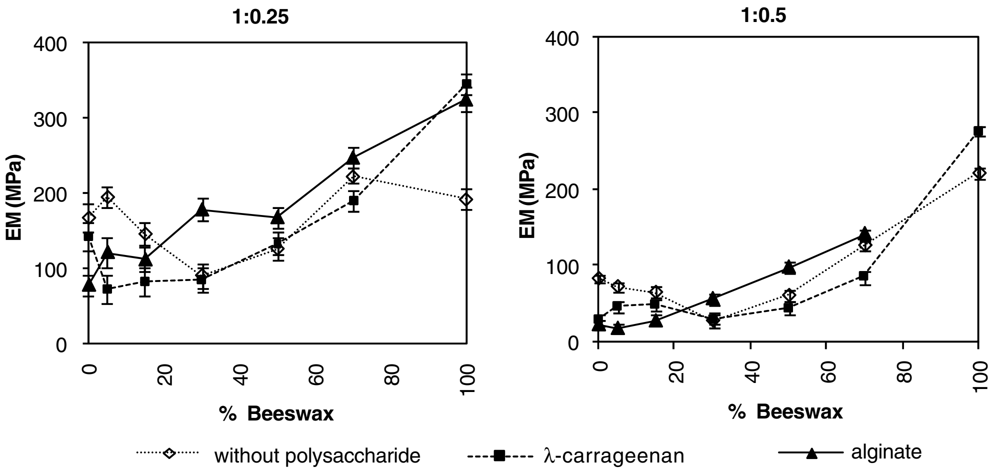

Fig. 1. Mean values (and LSD intervals) of elastic modulus (EM) of sodium caseinate films, with and without polysaccharide (alginate or λ-carrageenan), at different protein:lipid ratio (1:0.25 and 1:0.5).

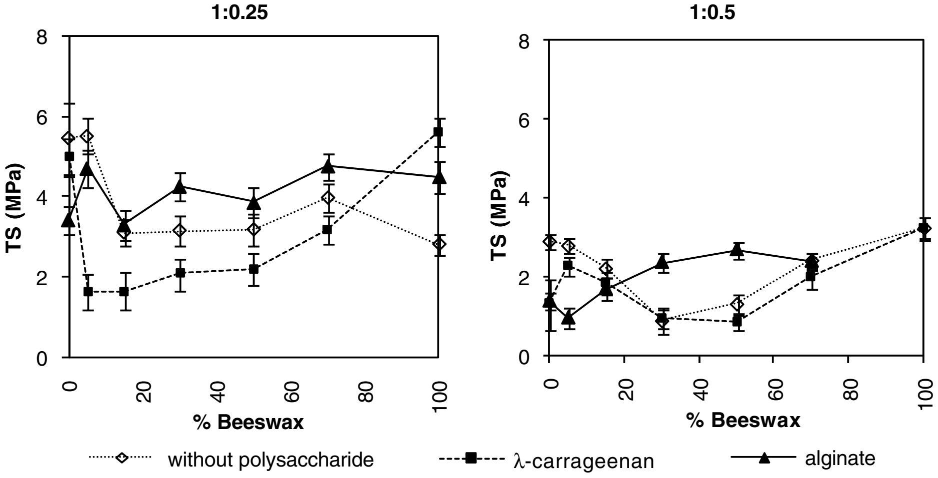

Fig. 2. Mean values (and LSD intervals) of tensile strength (TS) of sodium caseinate films, with and without polysaccharide (alginate or λ-carrageenan), at different protein:lipid ratio (1:0.25, 1:0.5).

BW. This effect was similar for both 1:0.25 and 1:0.5 protein:lipid ratio, the latter always showing lower values of TS and EM.

The described behaviour can be attributed to structural differences in the formed matrix and to the different molecular interactions. In previous studies (Fabra et al., 2008), the different effect of OA and BW in caseinate matrices was observed. OA had a great plasticizing effect (TS and EM decreased and *E* increased) on caseinate matrices, this being dependent on its concentration. Nevertheless, BW induced a decrease of TS and EM values but no notable changes in the film deformability (*E*) were observed. Consequently, the different lipid–lipid and lipid–protein interactions can lead to different microstructures in the film as a function of lipid mixture composition, thus showing a different mechanical response.

The presence of polysaccharide modified the described behaviour as a function of the lipid mixture composition, depending on its molecular structure. For alginate-containing films, the greater the beeswax content, the higher the EM and TS values (Figs. 1 and 2), which were generally greater than those observed for films without polysaccharide or with λ-carrageenan at the same lipid mixture composition. Deformability (*E*) of these films was also slightly higher (Fig. 3). All this indicates that the presence of alginate made the lipid containing films less flexible but more deformable; the more beeswax the greater the effect.

When comparing films with and without λ-carrageenan, significant differences ($p < 0.05$) were observed for tensile strength both at the two levels of protein:lipid ratio (Fig. 2) and also when the lipid had a different composition. For a determined lipid composition, the values were generally lower for films containing λ-carrageenan, except in the case of 100% BW where TS and EM values were greater. Nevertheless, the differences were greatly attenuated when the lipid ratio increased. For elastic modulus, the differences were not significant between 30% and 70% of BW, and outside this range the values were lower at 1:0.5 protein:lipid ratio. At the same time, elongation values (*E*) were lower for these films except for those containing 100% BW, where no significant differences were observed for the different polymers. The main effects introduced by λ-carrageenan in the caseinate–OA–BW films appeared at low lipid content and supposed a considerable reduction of TS when BW was present at low concentration. The presence of λ-carrageenan gave rise to slightly less deformable films than the control one at intermediate levels of BW, which was the opposite to what was observed when no lipids were present in the network.

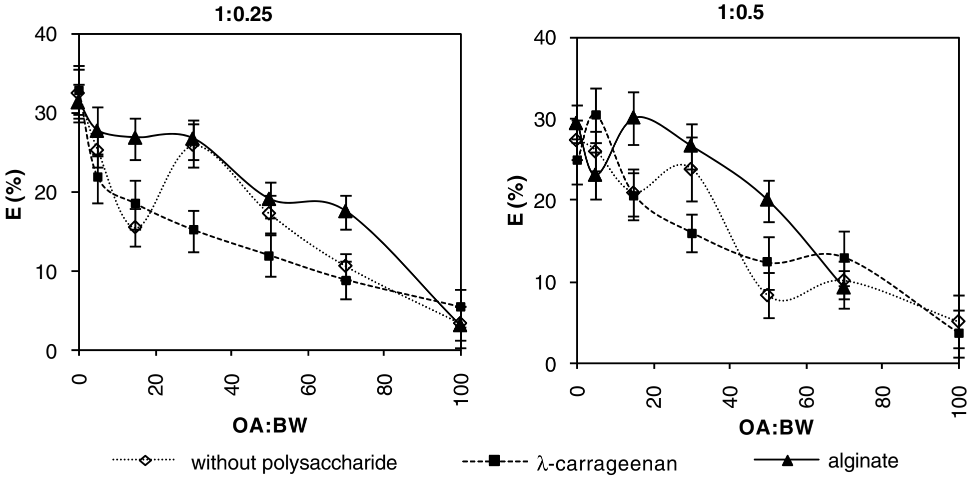

Fig. 3. Mean values (and LSD intervals) of elongation at break (*E*) of sodium caseinate films, with and without polysaccharide (alginate or λ-carrageenan) at different protein:lipid ratio (1:0.25, 1:0.5).

In lipid containing films, the effect of polysaccharides on protein matrix is different from the lipid-free films, thus indicating the interaction among all the components. A weakening effect of λ-carrageenan in the caseinate matrix was observed (TS decrease as compared to caseinate films) but not for alginate films, which became stiffer. As regards the ability to deform, films containing alginate are longer and those containing λ-carrageenan are shorter than the control films, especially at intermediate levels of BW. When BW is present at high levels, the films show the greatest stiffness and the lowest deformability. Elongation at break (E) was not influenced by the protein:lipid ratio.

3.2.2. Water vapour permeability (WVP)

Fig. 4 shows WVP of the studied films with different protein:lipid ratio (1:0.25 and 1:0.5) with and without polysaccharide. It should be noted that water vapour barrier efficiency depends on the type and amount (Donhowe, 1992; Kester & Fennema, 1989; Koelsch & Labuza, 1992; Shellhammer & Krochta, 1997) and physical state of the lipid (Martin-Polo et al., 1992). As expected, increasing the lipid content of the samples caused a decrease in WVP of all tested films. Adding BW to the lipid phase significantly decreased ($p < 0.05$) the water vapour permeability of the control films as compared to those containing only oleic acid. Increasing the amount of beeswax decreased WVP (Fig. 4) and the lowest asymptotic value was obtained with 30% of beeswax. Whereas oleic acid has greater polar character that favours water diffusivity (Gontard et al., 1994), beeswax is characterized by a dense structure with a high content in esters and long chain alkanes, which limits water diffusion (Debeaufort, Quezada-Gallo, Delporte, & Voilley, 2000; Kester & Fennema, 1989; Kristo, Biliaderis, & Zampraka, 2006; Morillon, Debeaufort, Blond, Capelle, & Voilley, 2002). Nevertheless, the incorporation of BW in amounts greater than 30% of the total lipid content did not further reduce the water vapour permeability, which could be associated to structural effects in the network; e.g. the formation of a more open structure due to the greater incompatibility of components. Similar behaviour of composite films has been reported in previous works (Avena-Bustillos & Krochta, 1993; Pérez-Gagó & Krochta, 2001).

The effect of λ-carrageenan on WVP of the films was only significant at a protein:lipid ratio of 1:0.5 when OA is present at 100% and 95% in the lipid mixture, where greater WVP values were observed. The increase of WVP promoted by λ-carrageenan in lipid-free caseinate films was mitigated when lipids were present except when OA was the main compound. However, the increase of WVP promoted by alginate in lipid-free caseinate films was also observed when lipids were present. The most significant difference was appreciated when both the lipid content and BW levels in the mixture were low. It is remarkable that the lowest value of WVP for alginate-containing films was obtained for the OA:BW ratio of 70:30, which is the ratio where a minimum asymptotic value was also obtained for control and λ-carrageenan containing films.

The greater compatibility of caseinates and λ-carrageenan probably implies the formation a matrix structure in caseinate and caseinate–λ-carrageenan films which offers similar interactions with lipids, which suppose a similar behaviour to water transmission. Nevertheless, phase separation of alginate and caseinate in the film matrix made the lipid action less effective and a part of the water molecules could be preferably transmitted through hydrophilic zones caused by a more heterogeneous distribution of components.

3.3. Characterization of sodium caseinate and λ-carrageenan containing films without plasticizer

As shown previously, incorporation of a low melting point lipid (oleic acid) into composite films provides an alternative means of plasticizing films whilst limiting water vapour transfer. In every case, oleic acid, beeswax and mixtures of both produce films which are more flexible than those formulated without lipids. Although the two polysaccharides studied had hydrophilic character, it seems that λ-carrageenan created a more compact structure with less free volume, increasing water barrier efficiency and improving tensile properties (Figs. 1–3). So, these films were formulated without plasticizer (glycerol) in order to evaluate the possibility of improving the water barrier properties without worsening the mechanical properties. In this sense, it was impossible to obtain films with high protein:lipid ratio (1:0.5), with or without λ-carrageenan, which seems to indicate that, although lipids (especially oleic acid) improve the flexibility and extensibility of films, above a determined level and in the absence of polar plasticizers, they cause an excessive discontinuity of the protein network, weakening the matrix structure. So, the characteristics of caseinate–λ-carrageenan–lipid composite films were analysed at the lowest protein–lipid ratio (1:0.25).

3.3.1. Tensile properties

Fig. 5 compares tensile parameters (EM, TS, E) of films with and without λ-carrageenan prepared without plasticizer, showing the

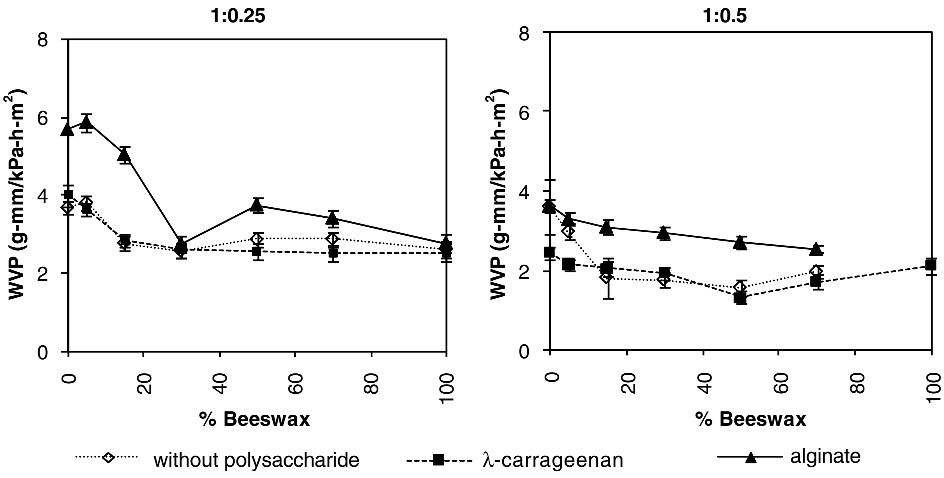

Fig. 4. Mean values (and LSD intervals) of WVP of sodium caseinate films, with and without polysaccharide (alginate or λ-carrageenan) at different protein:lipid ratio (1:0.25, 1:0.5).

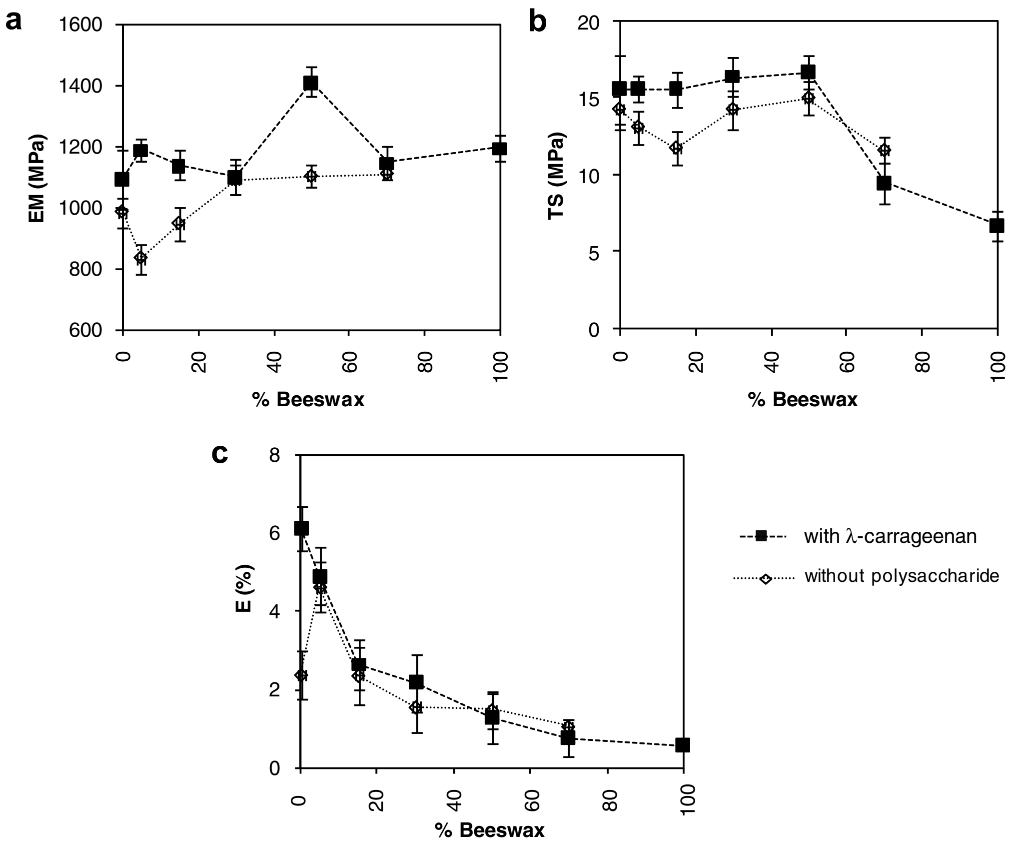

Fig. 5. Mean values (and LSD intervals) of tensile parameters (EM, TS, *E*) of sodium caseinate films prepared without glycerol.

loss of elasticity, flexibility and extensibility as compared with those containing glycerol (they are about ten times stiffer). The gain of stiffness and resistance imparted by beeswax in plasticized films was not now observed in absence of glycerol probably due to the greater resistance of the matrix which made the BW effect less appreciable. The presence of λ-carrageenan significantly affected TS, *E* and EM ($p < 0.05$) giving rise to more elongation resistant and stiffer films. Differences were affected by the BW ratio in the lipid mixture; in general greater values of TS and EM were observed for films containing λ-carrageenan up to 70% of BW in the lipid mixture, above which level no differences were detected. Deformability differences are only significant when no BW was present in the mixture; the films with λ-carrageenan being more deformable in this case.

The λ-carrageenan effect is now different from that observed in the absence of glycerol which could be attributed to the interactions of both components in the film network which in turn affect the protein–polysaccharide interactions. In this sense, a more intense protein–polysaccharide interaction seems to be deduced in the absence of glycerol which strengthens the film matrix, showing an increase in the film resistance to fracture and the elastic modulus. The influence of λ-carrageenan on the mechanical properties of caseinate films mitigated as the BW content in the film increased. This fact could be explained by the strong apolar character of this lipid which can limit the possibilities of interactions between the polymers.

It is remarkable that while it was not possible to obtain caseinate films using beeswax as pure lipid phase, it was possible if these films were formulated with λ-carrageenan, which shows the interaction among all the components in defining the film matrix structure.

3.3.2. Water vapour permeability (WVP)

Fig. 6 compares the water vapour permeability of glycerol-free films with and without λ-carrageenan. As expected, when plasticizer was not added, a sharp reduction ($p < 0.05$) in WVP was observed (comparing Figs. 4 and 6) which has been attributed to the fact that hydrophilic plasticizers reduce polymer chain to chain hydrogen bonding and increase intermolecular spacing (Pérez-Gagó, Nadaud, & Krochta, 1999).

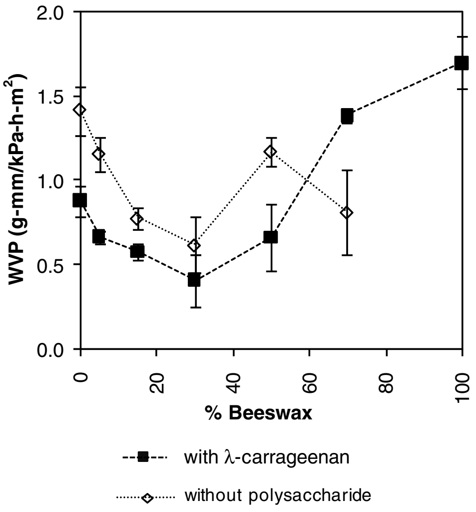

Fig. 6. Mean values (and LSD intervals) of WVP of sodium caseinate, films prepared without glycerol.

As regards the influence of λ-carrageenan on WVP of the glycerol-free films, significantly lower values were observed for the film containing polysaccharide up to when the content of BW in the lipid mixture reached 70%. Above this value, the opposite behaviour was observed. From the obtained results, the best water barrier properties were obtained for films containing λ-carrageenan and 30% BW in the lipid mixture.

4. Conclusion

The effect of polysaccharide addition on tensile properties and water vapour permeability of sodium caseinate films was dependent on film composition: lipids and plasticizer. In lipid-free films with plasticizer, polysaccharides improved the tensile properties of films, although water vapour permeability values increased; this effect being more notable for λ-carrageenan and dependent on the polysaccharide concentration. When lipids (oleic acid and beeswax) were present λ-carrageenan modify lightly the film properties, but the addition of alginate produce less flexible, less stretchable and more permeable films. In the absence of plasticizer, λ-carrageenan promoted stiffness and resistance to elongation of the caseinate films and reduce their WVP at low ratio of BW in the lipid mixtures (below 50%), showing the opposite effect at greater BW ratio. The obtained results show the important role of the specific interactions among the film components in defining its functional properties and put in evidence the difficulties to model the film behaviour as a function of composition. Taking into account the relative humidity conditions in which the films have been characterized, their behaviour when applied on a particular product could be predicted if product water activity was about 0.60.

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