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Alginate and pectin composite films crosslinked with Ca^{2+} ions: Effect of the plasticizer concentration

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ABSTRACT

The manufacture of composite biofilms of alginate and LM-pectin crosslinked with calcium ions requires a two-step contact with Ca^{2+}: initially a low-structured pre-film is formatted which is further crosslinked in a second contact with a more concentrated Ca^{2+} solution containing plasticizer. This research evaluated the influence of the plasticizer (glycerol) concentration (1–15% w/v) in this finishing reticulation step on final films characteristics. The results indicated that the extent of the simultaneous Ca^{2+} crosslinking and plasticization with glycerol was determined by the level of structural organization obtained in the pre-reticulation. Increasing the glycerol concentration of the crosslinking solution increased film solubility in water, moisture content, volumetric swelling and flexibility and decreased the resistance to tensile stress. Transparent alginate and pectin composite films with acceptable mechanical properties, low solubility and limited degree of swelling were obtained with 10% glycerol in the second contact solution.

1. Introduction

The increasing consumers concern on environmental problems ensuing from the use of synthetic packaging materials, as well as the demand for higher quality foods have contributed to the development of packaging manufactured with natural sources such as polysaccharides, proteins and/or lipids. Although edible films are not meant to totally replace traditional synthetic packagings they do have the potential to reduce their use, as well as to function as controlled release systems of active substances, and to limit moisture, aroma, and lipid migration between food components (Krochta & De Mulder-Johnston, 1997).

The use of edible films in food applications depends on several features like cost, availability, functional attributes, mechanical properties (strength and flexibility), optical quality (gloss and opacity), barrier requisites (water vapor, O_2 and CO_2 permeability), structure resistance to water and sensorial acceptance. These characteristics are greatly influenced by parameters such as the type of material used as structural matrix (conformation, molecular mass, charge distribution), film manufacturing conditions (solvent, pH, concentration, temperature, etc.) and the type and concentration of additives (plasticizers, crosslinking agents, antimicrobials, antioxidants, etc.) (Debeaufort, Quezada-Gallo, & Voilley, 1998; Guilbert, Gontard, & Gorris, 1996).

A recent approach to edible and biodegradable films technology contemplates the production of composite films, by combining different polysaccharides, proteins and lipids in order to improve their functionality. Composite films may be designed to take synergistic advantages of the pure components features, even though, as with synthetic polymers, the mechanical and barrier properties of composite biofilms strongly depend on the constituting polymer characteristics and their compatibility (Garcia, Pinotti, Martino, & Zaritzky, 2004).

Pectin and alginate, both polyuronates, are two characteristic examples of natural ionic polysaccharides undergoing chain–chain association and forming hydrogels upon addition of divalent cations (e.g. Ca^{2+}) (Fang et al., 2008).

Alginates are structural polysaccharides extracted from brown algae. On a molecular basis alginates are composed of guluronic (G) and mannuronic (M) acid units forming regions of M-blocks, G-blocks and blocks of alternating sequence (MG-blocks), where the relative proportions of these sequential organizations depends on the source (Ertesvåg & Valla, 1998).

Pectin is a major structural component of cell walls, being one of the largest constituent in citrus by-products. Pectin is a complex anionic polysaccharide composed of β-1,4-linked d-galacturonic acid residues, wherein the uronic acid carboxyls are either fully (HMP, high methoxyl pectin, DE > 50%) or partially (LMP, low methoxyl pectin, DE < 50%) methyl esterified. Like alginates, low methoxyl pectins, form gel with divalent cations (Marudova, MacDougall, & Ring, 2004; Walkenström, Kidman, Hermansson, Rasmussen, & Hoegh, 2003).

Calcium induced gelation has been demonstrated to result from specific and strong interactions between calcium ions and guluronate and galacturonate blocks in alginate and pectin, respectively

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(Braccini & Perez, 2001; Fang et al., 2008). In this process the solvent is confined in the interstices of a three dimensional network linked by junction zones that involve cooperative association of extended segments of the polymer chains (Bryce, McKinnon, Morris, Rees, & Thom, 1974). The mechanism that better describes gel formation in alginate and LM-pectin in the presence of calcium ions is the so called "egg-box" model.

Pectin and alginate tend to form strong films, but with poor resistance to water due to their hydrophilic nature. Their ability to form strong and insoluble gels with divalent cations can be used to improve these shortcomings (Pavlath, Gossett, Camirand, & Robertson, 1999). Allen, Nelson, Steinberg, and Mcgill (1963) studying different calcium salts for alginate gel coating formation, classified calcium chloride as the most effective crosslinking agent.

The dehydration of these gelified structures produces strong cohesive films that require plasticizers. The addition of plasticizer leads to a decrease in intermolecular forces along polymer chains which improves the flexibility and chain mobility. They are added to enhance film flexibility, decrease brittleness, avoid shrinking during handling and storage and therefore make it easier for film to be peeled of from the support during manufacture (Guilbert et al., 1996). Several studies, however, have also evidenced adverse effects of plasticizers on edible film attributes. Most of them describe an increase in gas, solute and water vapor permeability and the decrease in cohesion affects mainly mechanical properties. The characteristics of films based on polyuronated matrices depend therefore, of an equilibrium between the degree of crosslinking with Ca^{2+} (necessary to reduce the solubility in water but induces brittleness) and the addition of plasticizers for better workability. Above a critical concentration the plasticizer can exceed the compatibility limit with the biopolymer and phase separation with plasticizer exclusion is usually observed. The more commonly used plasticizers in edible carbohydrate based films are polyols, mainly glycerol and sorbitol (Yang & Paulson, 2000).

The present work evaluated the effect of the plasticizer (glycerol) concentration on the solubility in water, swelling degree and mechanical properties of alginate/pectin composite films crosslinked with calcium ions.

2. Materials and methods

2.1. Materials

Medium viscosity sodium alginate, obtained from *Macrocystis pyrifera* seaweed, purchased from Sigma–Aldrich (USA), with an average molecular mass (M_w) measured by gel permeation chromatography of 1.61×10^6 Da, and low methoxy pectin kindly donated by CPKelco (Denmark), with M_w of 4.28×10^5 Da, were used as biopolymers matrices in the composite and single films. Calcium chloride dihydrate (Merck, Germany) was used as crosslinking agent and glycerol (Synth, Brazil) as plasticizer.

2.2. Film preparation

Alginate and pectin composite films were made by casting in a two-stage crosslinking procedure. In the first stage a low reticulated film was prepared as follows. Polysaccharide solution with equal mass fractions of alginate and pectin (0.75% w/v of each polysaccharide) was prepared in distilled water already containing 0.6 g glycerol/g biopolymer at room temperature. The solution was mechanically stirred at 1000 rpm (Fisatom, model 713, Brazil) for about 1 h to ensure homogeneity. Afterwards, the temperature of the system was raised to 70 °C and a dilute aqueous calcium chloride solution was slowly added to the biopolymer solution at a flow rate of 1 mL/min delivered by a peristaltic pump (Masterflex C/L, model 77120-70, USA) until a total amount of 0.02 or 0.04 g $CaCl_2{\cdot}2H_2O$/g biopolymers was transferred (approximately 30 min). The increase in temperature, the low flow rate and the strong agitation were necessary to avoid local gelation, and as a consequence, film heterogeneities. A defined amount (70 g) of the partially crosslinked film forming solution was poured into polystyrene Petri dishes (d = 14 cm) and dried in a convection oven (Nova Ética, 420D, Brazil) at 40 °C for 20 h. After detaching the resulting film from the support, the crosslinking was complemented in a second stage, by total immersion of the films in 50 mL of an aqueous calcium chloride solution (3% w/v) containing glycerol (1%, 3%, 5%, 7%, 10%, 12% and 15% v/v) for 30 min. The excess surface liquid was removed and the films were placed over inverted Petri dishes and dried in a ventilated ambient for about 6 h, at room temperature and RH > 60% having the borders fixed by Teflon® rings to avoid wrinkling of the film edges. All films were conditioned at room temperature and 52% RH inside desiccators for 3 days before characterization. Films prepared with 0.02 g $CaCl_2{\cdot}2H_2O$/g biopolymer in the first stage were denominated low calcium films (*LCa* film), whereas films prepared with 0.04 g $CaCl_2{\cdot}2H_2O$/g biopolymer were referred as high calcium films (*HCa* film).

Simple films manufactured only with alginate or pectin and using 0.04 g $CaCl_2{\cdot}2H_2O$/g biopolymer in the first stage (*HCa* film) and 5% and 10% of glycerol in the crosslinking solutions of the second stage were also prepared following the same procedure as for the composite films.

2.3. Film characteristics

2.3.1. Film thickness, δ

The film thickness was controlled by pouring a constant mass (70 g) of the film forming solution over the support. Thickness of the conditioned films was measured using a digital micrometer (Mitutoyo, MDC-25S, Japan). Measurements were taken at ten different positions of the film and the mean value was used in the calculations of the mechanical properties.

2.3.2. Solubility in water, S_w

The solubility in water of the films was measured as proposed by Irissin-Mangata, Bauduin, Boutevin, and Gontard (2001). The moisture weight fraction, ω, of the film was gravimetrically determined in a vacuum oven (Lab-Line, Squaroid, USA) at 105 °C for 24 h. Disks cut from the same film, were weighed (total mass m_o) and immersed in 50 mL of distilled water using a 250 mL beaker maintained under mild agitation (175 rpm) at 25 °C for 24 h (Shaker Bath Orbit, Lab-Line, USA). The final dry matter (m_f) of the sample was determined in the same vacuum oven (105 °C/24 h). The fractional solubilized matter (S_w) was calculated as a function of the initial dry matter using Eq. (1).

$$S_w = \frac{m_o(1-\omega) - m_f}{m_o(1-\omega)} \quad (1)$$

2.3.3. Swelling degree, SD

The swelling degree of the films was determined as described by Xu, Bartley, and Johnson (2003). The initial mass (m_o) of a circular cut (d = 2.5 cm) of the film was quantified and the sample was immersed in 100 mL of stirred distilled water at 25 °C for different periods of time. The wet sample was carefully blotted between filter paper to remove the excess water from the surface and reweighed. The swelling degree (SD) was measured in terms of the amount of absorbed water relative to the initial mass (Eq. (2)).

$$SD = \frac{(m_w - m_o)}{m_o} \quad (2)$$

where m_w is the swollen film mass.

The swelling behavior of the films was also monitored by measuring changes in the thickness of the swollen films.

2.3.4. Mechanical properties

Tensile strength (*TS*) and percentage tensile elongation at break (*E*) of the preconditioned films were determined at room temperature using a TA.XT2 (Stable Microsystems SMD, England) according to ASTM standard method D882 (ASTM, 1995). Films were cut into strips (10×2.54 cm) and mounted between the corrugated tensile grips of the instrument. The initial grip spacing and cross-head speed were set at 50 mm and 0.1 cm/s, respectively. The tensile strength was expressed as the maximum force at break divided by the initial cross-sectional area of the film strip and the elongation at break as a percentage of the original length.

2.3.5. Calcium content

The concentration of calcium in the crosslinked films was determined according to Sriamornsak and Kennedy (2008), using an atomic absorption spectrophotometer (PerkinElmer, model 373, USA) in the absorption mode with an air-acetylene flame detector. Film samples (about 8 mg) were dissolved in 10 mL of a 2% sodium citrate solution. Each determination was performed in triplicate.

2.3.6. Statistical analysis

Analysis of variance and Tukey test were used to determine statistically significant differences ($p < .05$) among averages, using the Software Statistica V.1.1.5.

3. Results and discussion

Alginate and pectin composite films produced by the two-stage crosslinking method were homogeneous, transparent and visually attractive. However, composite films made with the two higher concentrations of plasticizer (12% and 15%), showed vestiges of phase separation on the surface of the film and films with 3% or less glycerol were very brittle. The use of a limited amount of crosslinking agent (first stage) before the total consolidation of the film structure was necessary to allow a proper handling of the films. These partially reticulated films produced were very flexible and completely water soluble. The same characteristics were found for the partially reticulated pure alginate or pure pectin simple films, however alginate films were more transparent than pectin films, the latter showing slightly hazy appearance. Zactiti and Kieckbusch (2006) also obtained highly soluble alginate films manufactured without the final crosslinking contact. Completely soluble alginate films were also obtained by Pavlath et al. (1999) by adding up to 0.18 g $CaCl_2{\cdot}2H_2O$/g alginate in a single stage procedure.

3.1. Film thickness

The results of averaged thickness measurements are shown in Fig. 1. The thickness of the composite films increases steadily when the glycerol concentration in the second stage was increased leveling off at higher concentration, probably due to glycerol exudation. In all studied conditions the *HCa* composite films were thicker than *LCa* composite films. These tendencies at a macroscopic level can be attributed to an increase in molecular volume due to a more intense plasticizing effect of higher concentration of glycerol. The values in Fig. 1 also indicate that single component films were thinner compared to composite films and that pure pectin films produce thicker films compared to pure alginate films. The latter observation is probably due to a more organized structure formed in alginate simple films (Sriamornsak & Kennedy, 2008).

3.2. Moisture content and solubility in water

The moisture content and the water solubility of the alginate and pectin composite films (*LCa* and *HCa* film), treated with different concentrations of glycerol in the second stage are shown in Table 1. Low solubility of edible films is one of the most important requirement in food and pharmaceutical applications, however most of the published studies on biopolymer films deal mainly with film characteristics such as mechanical and barrier properties (Kim, Ko, & Park, 2002; Rhim, 2004).

The equilibrium moisture content and the solubility in water of the films conditioned at 52% RH increased consistently and significantly by increasing the glycerol content present in the crosslinking solution. Since the final reticulation conditions for the *LCa* and *HCa* films were the same it is reasonable to associate the variation in moisture content to the amount of glycerol present in the film.

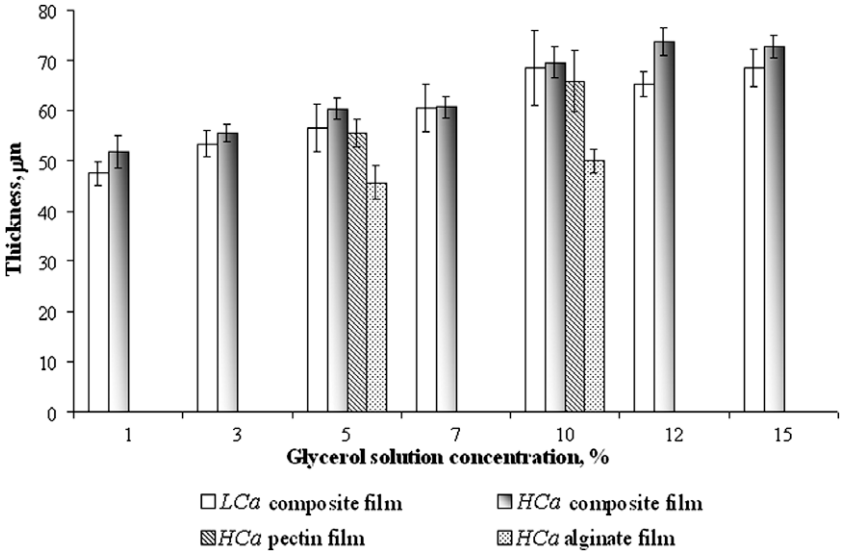

Fig. 1. Thickness of *LCa* and *HCa* composite films and *HCa* pectin and alginate simple films with different glycerol concentration in the second stage crosslinking solution. (Standard deviation is shown through error bars.)

Table 1
Moisture content (ω) and solubility in water (S_w) of *LCa* and *HCa* composite films equilibrated at 52% RH with different glycerol concentration in the second stage crosslinking solution.

Glycerol solution concentration, %	*LCa* composite film		*HCa* composite film	
	ω gH_2O/g film	S_w g/g dry matter	ω gH_2O/g film	S_w g/g dry matter
1	0.152 (0.003)[a]	0.121 (0.004) [a]	0.134 (0.006)[a]	0.088 (0.003)[a]
3	0.153 (0.003)[a]	0.170 (0.006)[b]	0.140 (0.002)[a]	0.151 (0.007)[b]
5	0.160 (0.008)[a]	0.230 (0.017)[c]	0.141 (0.005)[a]	0.199 (0.007)[c]
7	0.213 (0.007)[b]	0.246 (0.007)[c]	0.162 (0.008)[a]	0.237 (0.010)[d]
10	0.234 (0.008)[b]	0.296 (0.026)[d]	0.224 (0.004)[b]	0.234 (0.020)[d]
12	0.241 (0.004)[b]	0.288 (0.001)[d]	0.254 (0.001)[b]	0.233 (0.001)[d]
15	0.233 (0.001)[b]	0.379 (0.012)[e]	0.299 (0.008)[c]	0.372 (0.011)[e]

Average (standard deviation) of three experimental determinations.
Average with the same letter, in the same column, indicate no significant difference ($p < .05$).

Higher glycerol content unfastens the polymeric structure allowing more charged sites to be accessible to water molecules. More important, glycerol itself is very hygroscopic and therefore, probably responsible for most of the water uptake. The main contribution to the increase in S_w with increasing amount of plasticizer is the leaching of the glycerol, since after 24 h contact with water, the re-dehydrated films lost their flexibility. Schou et al. (2005) also observed a significant increase in the moisture content, with increasing plasticizer concentration in sodium caseinate films. These authors concluded that increasing the glycerol:protein ratio from 0.16 to 0.32 almost doubled the moisture content. The values in Table 1 indicate that *LCa* films showed higher solubility in water compared to *HCa* films. This is probably caused by the higher initial crosslinking degree obtained by the alginate and pectin chains when higher concentration of calcium was used in the first stage and the better aligned incipient polymeric structure assisted the strengthening action when additional Ca^{2+} was available at the second stage.

The moisture content and solubility of simple pectin and alginate *HCa* films treated with 5% and 10% of glycerol in the second stage crosslinking solutions are presented in Table 2. Differently from the composite films the equilibrium moisture content of the simple films did not significantly change with the increase in the glycerol concentration. The solubility in water, however, showed a statistically significant increase for both simple films when the plasticizer solution concentration was increased from 5% to 10%, in the second stage. Pure alginate films showed much lower solubility in water compared to pure pectin films. Because of the strong similarities of the molecular structures of the two polymers, most studies treat pectin and alginate as almost equivalent regarding their calcium binding mechanisms and gel formation abilities. Donati, Benegas, and Paoletti (2006), for example, formulated a common theoretical framework to describe the chain–chain associations of alginate and of pectin with calcium. In contrast, the literature that highlights the dissimilarities in the binding and gelation mechanisms of these two biopolymers is sparse. Braccini and Perez (2001) using molecular modeling pointed out significant differences in the gelation mechanisms and strength of pectin and alginate. In another work, Fang et al. (2008) found important differences between alginate and pectin with regard to their Ca-binding behaviors. These authors suggest that the different distribution patterns of binding sites, blockwise for alginates and randomly for pectin, could account for the differences with regard to their reactivity with Ca^{2+}. These structural distinctions could explain the higher solubility of pectin films and also the differences found in thicknesses (Fig. 1).

Table 2
Moisture content and solubility in water of pure pectin and alginate *HCa* films with 5% and 10% of glycerol in the second stage crosslinking solution.

Film	Glycerol solution concentration, %	ω gH_2O/g film	S_w g/g dry matter
HCa pectin film	5	0.183 (0.003)[a]	0.204 (0.006)[c]
HCa pectin film	10	0.176 (0.002)[a]	0.251 (0.007)[d]
HCa alginate film	5	0.202 (0.004)[b]	0.129 (0.003)[a]
HCa alginate film	10	0.196 (0.002)[b]	0.156 (0.007)[b]

Average (standard deviation) of three experimental determinations.
Average with the same letter, in the same column, indicate no significant difference ($p < .05$).

3.3. Swelling

Composite and simple polymer films were submitted to water uptake and swelling in contact with distilled water at 25 °C and the swelling kinetics obtained are showed in Fig. 2. For pure alginate film the water uptake at equilibrium was the lowest (SD = 0.85) and was reached after about 5 min of immersion in water for all treatments. Similar period of time was reported by Zactiti and Kieckbusch (2006) for alginate films. Pure pectin films showed the highest equilibrium SD, reached after more than 20 min of immersion and the composite films scored intermediate values. Sriamornsak and Kennedy (2008) evaluating the swelling of alginate and pectin films also crosslinked with Ca^{2+} found a hydration equilibrium of 2 h for both polymers. Their gelation and hardening method was different, and they did not use plasticizers. Sriamornsak and Kennedy (2008) also observed much higher swelling degree for pectin films compared to alginate films. These authors explained that a reduction in the extent of crosslinking would be expected to lead to a reduced retractive force and would allow more water to be absorbed. Therefore, the greater swelling of pectin films in water could be due to a lower number of crosslinking sites.

The striking feature of the results in Fig. 2 is the tendency of the swelling degree to decrease with an increase in the glycerol concentration of the crosslinking solution (second stage). This can be better observed by the asymptotic values of SD given in Table 3. The trend of the calculated SD values is misleading, however, since the leaching of higher amounts of plasticizers by the water during the experiments inversely affects the numerator of Eq. (2). The structure weakening effect of higher glycerol content can be better appreciated by observing the decay of the water uptake equilibration time with increasing plasticizer solution concentration. An increase in the calcium concentration used in the first stage seems to slightly decrease the extent of water uptake of the composite films and extends the equilibration time. These findings corroborate with the suggestion that the performance of the final film crosslinking depends on the quality of the pre-film structure.

All composite films kept their integrity even after 36 min of immersion in water and the general visual appearance was similar

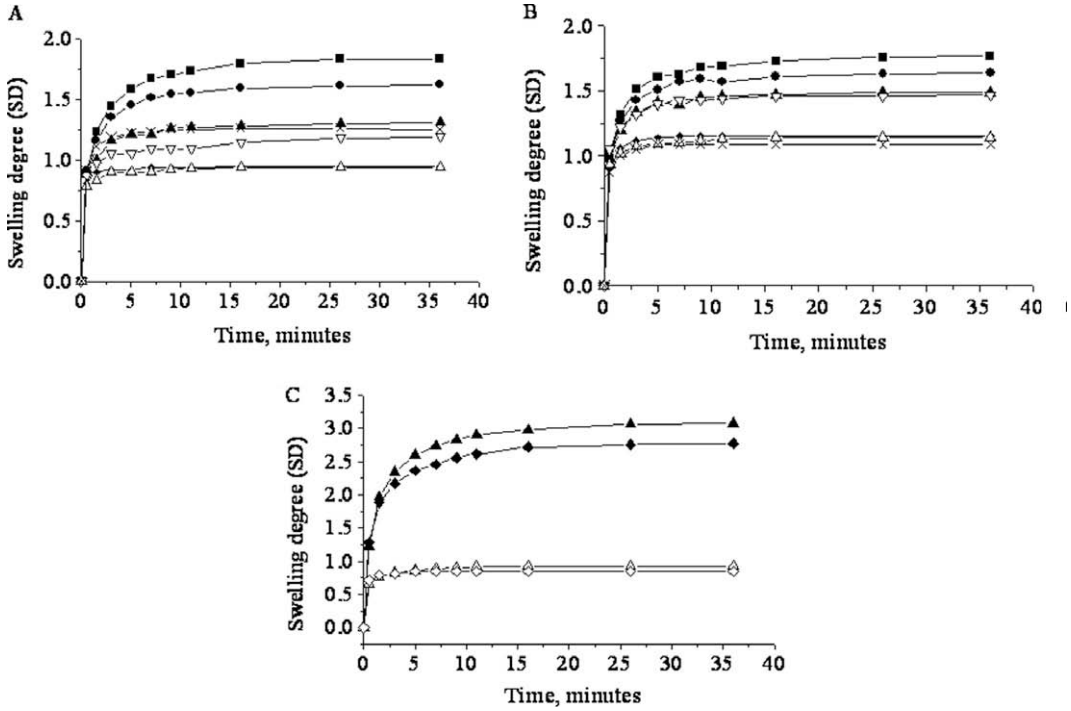

Fig. 2. Swelling degree: (A) *LCa* and (B) *HCa* composite films with 1% (■), 3% (●), 5% (▲), 7% (▽), 10% (♦), 12% (△), (×) 15% of glycerol in the crosslinking solution (second stage); (C) *HCa* simple films: pectin 5% (▲), pectin 10% (♦), alginate 5% (△), alginate 10% (◇) of glycerol in the crosslinking solution (second stage).

to the original film. However an increase in thickness was observed and is also given in Table 3. As can be seen, the thickness ratio increases with higher glycerol concentrations, and, therefore, should be considered a more adequate index to measure the structure relaxation by water.

3.4. Mechanical characteristics

The values of the mechanical properties of *LCa* and *HCa* composite films and of the *HCa* simple alginate and pectin films are given in Figs. 3 and 4. The tensile strength (*TS*) accounts for the film mechanical resistance due to the cohesion between the chains, while the elongation at break (*E*) measures its plasticity, which is the capacity of the film to extend before breaking. Due to the structural nature of these attributes, usually films with high *TS* show low *E* so that both properties should be analyzed simultaneously. Tensile strength values found ranged from 39.7 to 61.5 MPa for *LCa* composite films and from 41.6 to 75.7 MPa for *HCa* composite films and their variation according to the second stage glycerol solution content are given in Fig. 3. The values of *E* varied from 2.1% to 19.8% for *LCa* composite films and from 1.5% to 12.9% for *HCa* composite films and are shown in Fig. 4. According to the classification established by Krochta and De Mulder-Johnston (1997), the mechanical attributes obtained by the composite alginate and pectin films can be considered as moderate, compared to synthetic films.

TS of the simple alginate film in both concentration of glycerol evaluated (5% and 10%) were significantly higher than the respective pectin film and the composite films. Subsequently, the elongation for those alginate films were low. The remarkably higher *TS* values found for the simple alginate films can only be partially attributed to their denser molecular structure. The alginate films have the smallest thickness (Fig. 1) and according to Cuq, Gontard, Cuq, and Guilbert (1996) the tensile strength of films manufactured with the same formulation increases with the decrease in film thickness. A comparison between the experimental data of Figs. 3 and 4 confirms that variations in the plasticizer concentration exert an appreciable positive effect on film elongation but only a moderate reducing effect on tensile strength.

Figs. 3 and 4 also indicate that an increase in the calcium concentration in the first stage promoted an increase in *TS* and a decrease in *E* for almost all formulations. This *E* values tendency is even more significant than revealed by Fig. 3 since the film thicknesses of *HCa* films are larger *LCa* films, which would tend to depress *TS*.

Similar results were found by other authors. Rhim (2004) observed an increase in the *TS* and a decrease in the *E* by $CaCl_2$ treatment of alginate films, though the degree of change was dependent on the contact method and concentration of $CaCl_2$ treatment. Kang, Job, Leeb, Kwona, and Byunb (2005), studying pectin films, found a *TS* of 193 MPa and a *E* of 2.6% for pectin films containing glycerol (2.5% v/v) and crosslinked by total immersion of the films with a 5% $CaCl_2$ solution. Pavlath et al. (1999) obtained higher values of

Table 3
Equilibrium swelling degree and thickness ration of pure pectin, pure alginate and composite *HCa* films with 5% and 10% of glycerol in the second stage crosslinking solution.

Film	Glycerol solution concentration, %	$SD_{eq.}$	δ_f/δ_i*
HCa pectin film	5	3.08 (0.13)^{f}	2.69 (0.11)^{b,c}
HCa pectin film	10	2.77 (0.10)^{e}	3.15 (0.08)^{d}
HCa alginate film	5	0.92 (0.01)^{b}	1.98 (0.04)^{a}
HCa alginate film	10	0.85 (0.10)^{a}	2.84 (0.12)^{c}
HCa composite film	5	1.49 (0.08)^{d}	2.44 (0.06)^{b}
HCa composite film	10	1.15 (0.06)^{c}	3.08 (0.07)^{d}

Average (standard deviation) of three experimental determinations.
Average with the same letter, in the same column, indicate no significant difference ($p < 0.05$).
* Thickness variation: δ_f (final equilibrium thickness), δ_i (initial thickness).

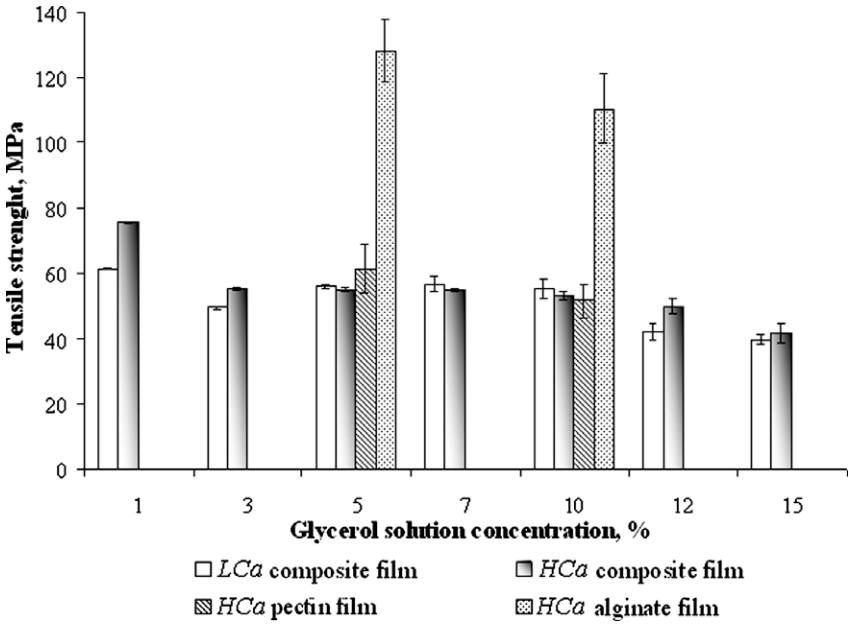

Fig. 3. Tensile strength of *LCa* and *HCa* composite films and of the *HCa* pectin and alginate simple films with different glycerol concentration in the crosslinking solution (second stage). (n = 10; standard deviation is shown through error bars.)

TS for alginate films crosslinked with $CaCl_2$ without plasticizers compared to the same film with addition of 40% (w/w) of glycerol, but the increase in the *E* values was not so pronounced.

3.5. Calcium content

The calcium content of *HCa* composite films, *HCa* alginate films and *HCa* pectin films containing 5% and 10% (w/v) of glycerol in the crosslinking solution of the second stage is presented in Table 4. As expected higher calcium contents, on dry basis, were observed for *HCa* alginate films, followed by *HCa* composite films and *HCa* pectin films. The concentration of glycerol used in the second stage crosslinking solution did not significantly influence the calcium content. These results correlate well with the tendency found for water uptake, solubility in water and mechanical properties of the corresponding films, reinforcing the assumption that a more effective crosslinking is obtained by alginate polymeric matrix compared to pectin.

Sriamornsak and Kennedy (2008) also found less calcium in pectin based films compared to alginate films. However their values of calcium content are approximately two times lower than the amount determined in the present work for the same type of alginate and pectin. The higher calcium contents can be attributed to the different crosslinking method used.

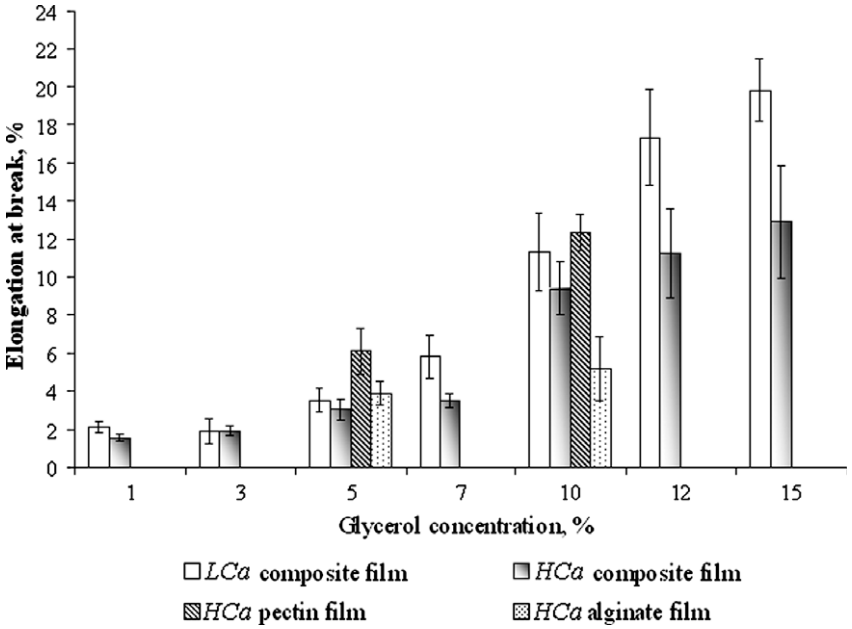

Fig. 4. Elongation at break of *LCa* and *HCa* composite films and of the *HCa* pectin and alginate simple films with different glycerol concentration in the crosslinking solution (second stage). (n = 10; standard deviation is shown through error bars; n = 8 for *LCa composite film* with 7% and 10% glycerol.)

Table 4
Calcium content of pure pectin, pure alginate and composite *HCa* films with 5% and 10% of glycerol in the secoond stage crosslinking solution.

Film	Glycerol solution concentration, %	Calcium content, μmol/mg dry film
HCa pectin film	5	2.251 (0.003)[a]
HCa pectin film	10	2.539 (0.022)[a]
HCa alginate film	5	4.354 (0.017)[c]
HCa alginate film	10	4.265 (0.005)[c]
HCa composite film	5	3.291 (0.025)[b]
HCa composite film	10	3.529 (0.012)[b]

Average (standard deviation) of three experimental determinations.
Average with the same letter, in the same column, indicate no significant difference ($p < .05$).

4. Conclusions

Transparent and homogenous alginate/pectin films were obtained after a low Ca^{2+} reticulated pre-film manufactured by casting was exposed for 30 min to 3% $CaCl_2{\cdot}2H_2O$ solutions containing glycerol.

The increase in glycerol concentration in the finishing crosslinking solution decreases the tensile strength (*TS*) of the alginate and pectin composite films, and increases the solubility in water, moisture content and their elongation at break. Alginate and pectin simple films, despite using the same crosslinking procedure showed different calcium content, indicating a less effective crosslinking for pectin films. Composite films tend to present characteristics properties values compared to films based on pure polymeric components.

As a compromise between film mechanical resistance and flexibility, keeping low solubility and swelling in water, the use of 5–10% glycerol in the finishing crosslinking step is recommended. Concentrations lower than 3% glycerol produce brittle films and phase separation was observed on the film surface when concentrations higher than 12% glycerol were used.

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