



## Composite and bi-layer films based on gelatin and chitosan

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### ABSTRACT

The aims of this work were: to develop composite, bi-layer and laminated biodegradable films based on gelatin and chitosan, to determine film barrier and mechanical properties and to characterize their microstructure.

Gelatin and chitosan concentrations used were 7.5% and 1% (w/w), respectively. Glycerol (0.75%) was added as plasticizer.

Physicochemical properties such as moisture content, transparency and color were analyzed. Composite and bi-layer systems showed a compact structure indicating a good compatibility between components.

Water vapor permeability (WVP) was independent of film thickness up to 120  $\mu\text{m}$  for gelatin films and 60  $\mu\text{m}$  for chitosan ones. Both, bi-layer and laminated systems resulted effective alternatives to reduce WVP of composite films (at least 42.5%). Bi-layer systems showed better mechanical properties than laminated ones. The resistance at break increased from 54.3 for composite to 77.2 MPa for bi-layer films, whereas elongation at break values of both composite and bi-layer films were similar (2.2–5.7%).

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### 1. Introduction

Proteins are biopolymers that can be used successfully in food packaging formulations. Therefore they are an attractive alternative to synthetic plastic materials made from non-renewable resources that can be a hazard to the environment.

Gelatin obtained by partial degradation of collagen has gained more attention as edible films for its abundance and biodegradability. Gelatin has relatively low cost and excellent functional and filmogenic properties (Eastoe and Leach, 1997; Arvanitoyannis et al., 1997a; Arvanitoyannis et al., 1997b; Cao et al., 2007a). Gelatin film itself, as most protein films, does not have ideal water vapor barrier properties. Thus, some chemical treatments can be applied to modify the polymer network through cross-linking of the polymer chains to improve the hydrocolloid film functionality (Arvanitoyannis et al., 1997c; Cao et al., 2007b). Food, pharmaceutical and industries are the main users of gelatin, which has several other technical applications. Their most frequent uses in the biomedical field include hard and soft capsules, wound dressings and adsorbent pads for surgical uses, as well as three-dimensional tissue regeneration (Bigi et al., 2004).

Chitin is the second most abundant biopolymer (after cellulose) found in the exoskeleton of crustaceans and fungal cell walls

(Muzzarelli, 1996; Zhang et al., 2006a; No et al., 2007). Chitosan is a polysaccharide derived from chitin, and is mainly composed by 2-amino-2-deoxy- $\beta$ -D-glucopyranose repeating units. The importance of chitosan resides in its antimicrobial properties in conjunction with its cationic character and its film-forming properties (Khan et al., 2000; No et al., 2007).

A special property of chitosan is its bioactivity, which determines the medical and veterinary application of this polymer (Wiśniewska-Wrona et al., 2002).

In hydrocolloid films, plasticizers can be added to enhance the functional properties of films, improving both their film flexibility and their manipulation (Sothornvit and Krochta, 2001).

Composite edible films and coatings can be formulated to combine the advantages of each component. Whereas biopolymers, such as proteins and polysaccharides, provide the supporting matrix, lipids provide a good barrier to water vapor (Baldwin et al., 1997; García et al., 2000; Anker et al., 2001). Since gelatin and chitosan are hydrophilic biopolymers with good affinity and compatibility, they are expected to form composite films with good properties (Arvanitoyannis et al., 1998; Sionkowska et al., 2004).

Most of the cited literature about bi-layer films described systems where a lipid forms a second layer over the polysaccharide or protein support (Kamper and Fennema, 1984; Greener and Fennema, 1989; Weller et al., 1998; Perez-Gago, 2005). There are no references about bi-layer systems including two hydrocolloid layers. Previous literature related to characterization and applications of laminated films are scarce, although this is a common practice with synthetic films (Vidal et al., 2007). Besides, there is no

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distinction between bi-layer and laminated systems in the studies performed up to the present and these terms are used indistinctly (Park et al., 1994; Cho et al., 2002). In this work a distinction was made between them, calling bi-layer to a system prepared by the coating technique which is a two-step procedure and laminated to one formed by two independent films, placed one on the other.

The aims of this work were: to characterize the hydrocolloids used in the formulations and the rheological properties of the film forming solutions, to develop composite, bi-layer and laminated biodegradable films based on gelatin and chitosan, to analyze the effect of different film preparation techniques on film barrier and mechanical properties, to evaluate thickness effect on water vapor barrier properties and to characterize their microstructure.

## 2. Materials and methods

### 2.1. Reagents

Gelatins of two different sources were used for solution preparation: bovine gelatin type B provided by Gelico (Belo Horizonte, Brazil) (G1) and commercial one (G2) provided by Mallinckrodt Inc (New York, USA).

Commercial chitosan from crab shells with a minimum deacetylation degree of 85% was purchased from Sigma (St. Louis, MO, USA).

Glycerol (analytical grade) was used as plasticizer and was provided by J.T. Baker, (Xalostoc, México).

### 2.2. Hydrocolloids characterization

#### 2.2.1. Protein content

Protein content was measured by Kjeldahl method (AOAC, 1999). Approximately 0.5 g sample was weighed and poured into digestion tubes. Protein content was determined in duplicates. The nitrogen value determined allowed to compute the protein content multiplying by the nitrogen conversion factor for gelatin, which is 5.55 (AOAC, 1984).

#### 2.2.2. Ash determination

Porcelain crucibles were dried at 105 °C overnight to remove water. The crucibles were placed in a vacuum desiccator to reach room temperature and the weights were recorded. Approximately 1 g of sample was poured onto the porcelain crucibles. Ash content was determined in duplicates in a muffle furnace at 550 °C according to the method described by Harbers (1998).

### 2.3. Hydrocolloids solutions

Gelatin solutions (G1, G2) were prepared, using a concentration of 7.5% (w/w) in both cases by hydrating gelatin powder (G1, G2) with distilled water for 8 h and then samples were heated at 60 °C until complete dissolution.

Chitosan (CH) solution of 1% (w/w) was prepared by solubilizing in 1% (v/v) acetic acid solution as described in a previous work (García et al., 2006).

Glycerol was added as plasticizer (P); the concentration used was 0.75% (w/w).

Blends of G1 or G2, with and without plasticizer, and CH solutions were also prepared by mixing the corresponding solutions in the proportion 50:50 (w/w).

#### 2.3.1. Gel strength

The gel strength was determined according to the method described by Avena-Bustillos et al. (2006) with some modifications. Gelatin solutions were prepared as described previously using a

concentration of 6.67% (w/v) in both cases. Gels were formed by maintaining the solutions at 10 °C during 18 h. Gel strength was measured at 10 °C using a texturometer TA.XT2i – Stable Micro Systems (Surrey, England) equipped with a SMS P/0-5R plunger of 12.5 mm diameter. Gels were compressed 4 mm and the compression force was registered and expressed in grams.

#### 2.3.2. Rheological characterization of hydrocolloids solutions

Rheological characterization of the filmogenic solutions was performed in a Haake RheoWin 3.0 (Haake, Karlsruhe, Germany) rotational viscometer, at controlled constant temperature 25 °C. MV I P type sensor system of roughened coaxial cylinders was used. Rheological curves were obtained after a stabilization time of 3 min at 25 °C. The shear stress ( $\sigma$ ) was determined as a function of shear rate ( $\dot{\gamma}$ ) between 0 and 500 s<sup>-1</sup>, with the following program: 3 min to reach the maximum shear rate, then it was maintained during 1 min and finally it was allowed to reach 0 shear rate in 3 min. This program allows to evaluate the rheological behavior of the solutions and its time-dependence.

Rheological behavior was mathematically fitted using Newtonian ( $\tau = \eta \dot{\gamma}$ ) or Ostwald de Waele ( $\tau = k\dot{\gamma}^n$ ) model, where  $\eta$  is the viscosity of the Newtonian fluid;  $k$  is the consistency coefficient and  $n$  the flow behavior index. For non-Newtonian systems apparent viscosity was calculated at 500 s<sup>-1</sup>.

### 2.4. Hydrocolloids films

#### 2.4.1. Film preparation

Fig. 1 shows a scheme of the different types of films analyzed in this work: Composite, bi-layer and laminated ones. Gelatin films with (GP) and without (G) plasticizer were obtained; composite films formulated with gelatin and chitosan with (GCHP) and without (GCH) plasticizer were prepared. Both types of gelatins (G1 and G2) were used in single and composite film formulations. Besides, chitosan films (CH) were also analyzed. Film-forming solutions with the same dry matter, for guarantee a constant thickness, were poured onto rectangular acrylic plates (10 × 20 cm). The solutions were dried at 37 °C in an oven to constant weight. Films were stored at 20 °C and a relative humidity (RH) of 65% for barrier and mechanical property determinations.

Only G1 gelatin was used to formulate bi-layer and laminate films. Bi-layer films were formed by the coating technique which is a two-step procedure. Single or composite gelatin G1 based films were casted onto the acrylic plates and then dried in an oven at 37 °C until a firm surface but still with adhesive properties was obtained. In all the bi-layer tested formulations, a second layer of CH solution was added onto these pre-formed gelatin based films (Fig. 1) and finally the system was dried at 37 °C in the oven. Nomenclature used for the bi-layer systems was: G1-CH, G1P-CH and G1CH-CH (one layer of CH and other of G1, G1P or G1CH).

Besides, laminated films were also tested in order to compare their performance with those of bi-layer and composite films. These laminated films were formed by two individual films one on the other (Fig. 1). Nomenclature used for the laminated systems was: G1 + CH, G1P + CH, G1CH + CH (one film of CH and other of G1, G1P, or G1CH).

### 2.5. Film characterization

#### 2.5.1. Thickness measurement

Film thickness was determined using a coating thickness gauge Check Line DCN-900 (New York, USA) for non-conductive materials on non-ferrous substrates. The informed values correspond to the average of at least fifteen measurements at different positions for each specimen. Besides, thickness was also measured by Scanning

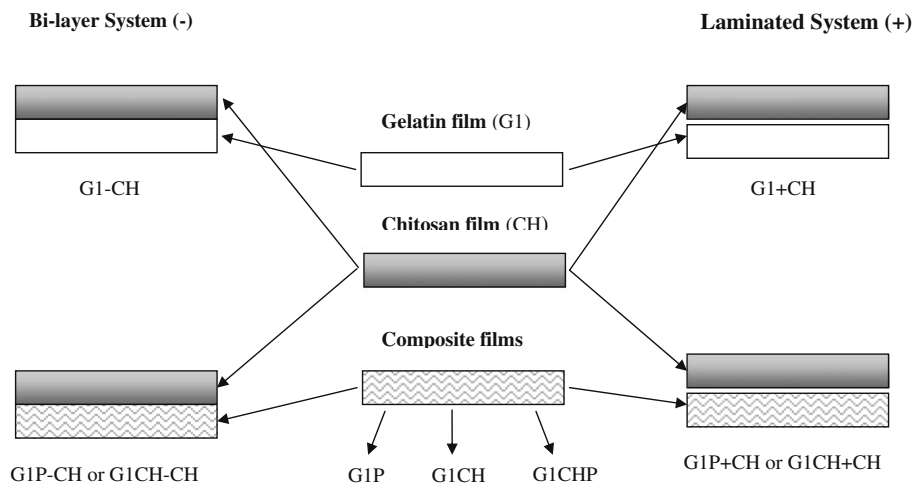


Fig. 1. Representative scheme of preparation of composite films, bi-layer and laminated systems.

Electron Microscopy (SEM) through cross-section film observations.

In order to evaluate thickness effect on water vapor barrier properties, solutions with different weight-plate area ratios were prepared to obtain films with different thicknesses.

#### 2.5.2. Moisture content

Moisture content of the films was determined by measuring the weight loss of them, upon drying in an oven at  $105 \pm 1$  °C until constant weight (dry sample weight). Samples were analyzed at least in triplicate and results were expressed as (%).

#### 2.5.3. Optical properties

Film opacity was determined using a procedure described by Cho and Rhee (2004) and film transparency was determined following the method described by Zhang and Han (2006). Film samples were cut into a rectangle and placed on the internal side of a spectrophotometer cell. The absorbance spectrum (400–700 nm) was recorded for each sample using a Beckman DU650 (Palo Alto, CA, USA) spectrophotometer. Film opacity was defined as the area under the recorded curve determined by an integration procedure. The opacity was expressed as absorbance units per nanometers and divided by film thickness ( $\text{AU} \times \text{nm}/\mu\text{m}$ ). Film transparency was calculated by the ratio between the absorbance at 600 nm ( $A_{600}$ ) and film thickness; it was expressed as ( $A_{600}/\text{mm}$ ). The measurement was repeated three times for each type of film, and the average value was informed.

Film color was determined by a Minolta colorimeter CR 300 Series (Osaka, Japan) calibrated with a standard ( $Y = 93.2$ ,  $x = 0.3133$ ,  $y = 0.3192$ ). The CIE Lab scale was used, lightness ( $L^*$ ) and chromaticity parameters  $a^*$  (red–green) and  $b^*$  (yellow–blue) were measured. Assays were performed placing the film samples over the standard. Samples were analyzed in triplicates, recording four measurements for each sample.

Color differences ( $\Delta E$ ) were also calculated by the following equation:

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

where

$$\Delta L^* = L^* - L_0^*$$

$$\Delta a^* = a^* - a_0^*$$

$$\Delta b^* = b^* - b_0^*$$

being:  $L_0^*$ ,  $a_0^*$ ,  $b_0^*$ , the color parameter values of the standard and  $L^*$ ,  $a^*$ ,  $b^*$ , the color parameter values of the sample.

#### 2.5.4. Microstructure studies by Scanning Electron Microscopy

Morphology of films was analyzed using a scanning electron microscope (Philips model SEM 505, Fuji, Japan) equipped with a Soft Imaging System ADDA II. Fractured films were rested vertically on the sides of an aluminum sheet and fixed on stubs using double sided adhesive carbon tape. Mounted samples were gold coated with a layer of 40–50 nm of thickness to permit the observation of the surface and the cross section of the films. SEM observations were performed using the followings magnifications: 200, 500, 1000 and 2000.

#### 2.5.5. Water vapor barrier properties

Assays were performed using a modified ASTM method E96 (1995) as described in a previous work using a specially designed cell with silica-gel (Mali et al., 2002). After steady-state conditions were reached, changes in the weight of the cell were recorded to the nearest 0.0001 g as a function of time, leading to the gain weight ( $\text{GW}$ ,  $\text{g m}^{-2}$ ) when it was divided by the cell area. Water vapor transmission rate ( $\text{WVTR}$ ,  $\text{g s}^{-1} \text{m}^{-2}$ ) was calculated from the slope of the straight line by linear regression ( $r^2 > 0.99$ ).

Permeance ( $\text{g Pa}^{-1} \text{s}^{-1} \text{m}^{-2}$ ) was calculated as the  $\text{WVTR}$  divided by the partial vapor pressure gradient across the film (1753.55 Pa at 20 °C). Water vapor permeability ( $\text{WVP}$ ) can be calculated as the product of permeance values and the film thickness ones (m), and was expressed as  $\text{g Pa}^{-1} \text{s}^{-1} \text{m}^{-1}$ .

$\text{WVTR}$  values of the bi-layer films were calculated as reported by Cooksey et al. (1999), using the following equation:

$$1/\text{WVTR} = 1/\text{WVTR}_1 + 1/\text{WVTR}_2 \quad (2)$$

where,  $\text{WVTR}$  ( $\text{g s}^{-1} \text{m}^{-2}$ ) is the water vapor transmission rate of bi-layer film, and the subscripts 1 and 2 denote CH layer and G1 single or composite layer (G1CH, G1P, G1CHP), respectively.

Water vapor barrier properties of laminated films were tested by placing individually films (CH and G1, G1P or G1CH) one on the other on the water vapor permeation cell. Both positions of CH film, top or bottom in the cell, with respect to the other film were tested.

#### 2.5.6. Mechanical properties

Tensile strength (TS) and elongation at break (EB) of the films were determined using a texturometer TA.XT2i – Stable Micro Systems (Surrey, England) equipped with a tension grip system A/TG,

according to the standard testing method ASTM D638-01 (2001). Film probes of 6 cm length and 0.7 cm width were used, a minimum of five probes was prepared from each film. Before analysis, the samples were conditioned for 72 h at 65% RH and 20 °C. The initial grip separation and velocity were fixed at 50 mm and 1 mm/s, respectively. The curves of force (N) as a function of deformation (mm) were recorded by the Texture Expert Exceed software. Tensile properties were calculated from the plot of stress (tensile force / initial cross-sectional area) versus strain (extension as a fraction of the original length), (Perez-Gago and Krochta, 2001). Tensile strength was calculated by dividing the required force for film rupture by the area of the transverse section (ASTM D638-01, 2001) and elongation at break was calculated from the ratio of increase in length to original length, expressed in percentage.

### 2.6. Statistical analysis

All experiments were performed at least in duplicates, with individually prepared and casted films as replicated experimental units as described previously in each determination. Systat-software (SYSTAT, Inc., Evanston, IL, USA) version 10.0 was used for multifactor analysis of variance. Differences in the properties of the films were determined by Fisher's least significant difference (LSD) mean discrimination test, using  $P < 0.05$  as level of significance.

## 3. Results and discussion

### 3.1. Hydrocolloid characterization

Table 1 summarizes the characterization performed on different gelatin types and chitosan powders assayed. With regard to chitosan, the known of the deacetylation degree is critical since it is synthesized by the deacetylation of chitin with NaOH (Wu et al., 2005) and its performance is strongly determined by this parameter. Physicochemical characterization of collagen and gelatin has been extensively reported previously (Johnston-Banks, 1990; Eastoe and Leach, 1997; Arvanitoyannis et al., 1998; Bigi et al., 2004). Gelatin physical properties are mainly determined by their type, source and preparation. According to Eastoe and Leach (1997) gelatin moisture content commonly varies between 9% and 14%, ash content is lower than 2%, and the remaining composition is protein. Carvalho and Grosso (2004) working with gelatin type B, have recently reported a contents of 88.92% protein, 0.78% ash and 10.3% moisture.

On the other hand, protein gels can be classified by measuring the required force for gel rupture as brittle, firm, weak and elastic. In this work, G1 showed higher gel strength than G2, being these values 220 g and 180 g, respectively. During gelatin preparation the characteristic triple-helical structure of collagen is lost, but it can be reformed below the gel melting temperature, leading to the typical network structure of gelatin (Bigi et al., 2004; Yakimets et al., 2005). Thus, G1 gelatin with high gel strength shows a more

**Table 1**  
Chemical compositions of different gelatin types and chitosan powders used

	G1	G2	CH
Ash content (%)	0.52	1.42	0.92
Moisture content (w/w dry basis)	12.7	12.9	4.73
Protein content (%)	82	87	na <sup>a</sup>
Deacetylation degree (%)	na	na	85 <sup>b</sup>

G1: 7.5% w/w, G2: 7.5% w/w, CH: 1% w/w.

<sup>a</sup> na: not applicable.

<sup>b</sup> Data provided by the supplier.

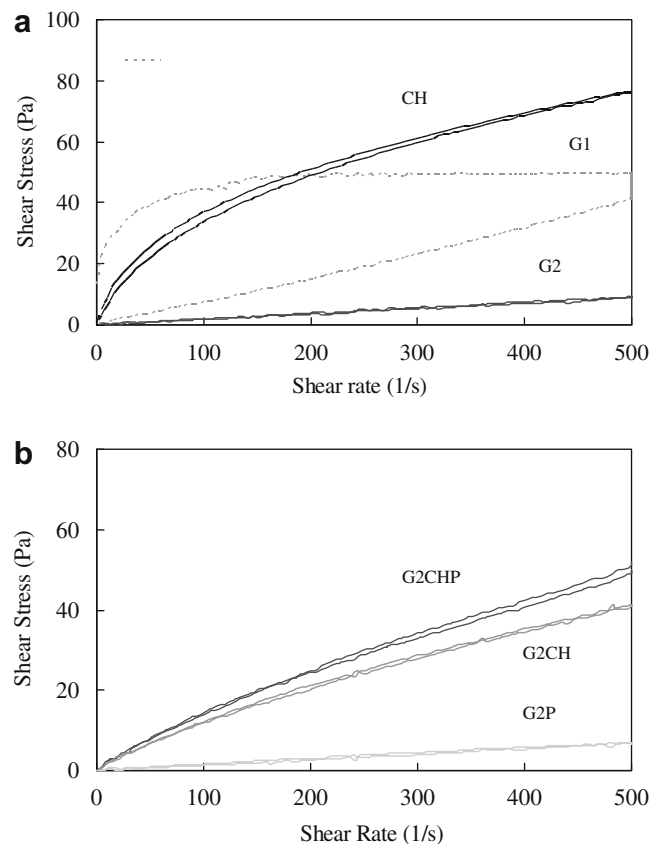
elevated level of renaturation than G2. Besides, the stiffness of gelatin gels is commonly expressed as the Bloom index, and it is commonly used to characterize different types of gelatins.

### 3.2. Rheological characterization of hydrocolloid solutions

Fig. 2a shows the typical flow curve of chitosan and gelatins (G1 and G2). CH solutions exhibited a pseudoplastic slightly thixotropic behavior. Gelatin solutions presented different rheological characteristics depending on their source. G2 solutions showed a Newtonian behavior, while those corresponding to G1 exhibited a rheopectic one (Fig. 2a). This response could be explained considering the setting time of both gelatins, since G1 developed a gel in a shorter time than G2. According to Avena-Bustillos et al. (2006) during gel formation gelatin molecules begin to form triple helical junction zones and partly revert to the collagen structure and this process depends on gelatin mammalian source.

The effect of plasticizer and chitosan addition on the rheological properties of gelatin solutions is shown in Fig. 2b for G2. Ostwald de Waele model fitted satisfactorily the experimental data ( $r^2 > 0.99$  in all cases); the corresponding fitting parameters are presented in Table 2. The addition of glycerol did not modify the rheological behavior of gelatin solutions, while chitosan blending changed it to a pseudoplastic behavior (Fig. 2b and Table 2).

CH solutions were more viscous than gelatin ones (Table 2). Apparent viscosity of G1 and their composite solutions exhibited higher values compared with those of G2 solutions and their blends. These results could be attributed to the changes in gelatin molecules conformation during the cooling process, as described previously (Avena-Bustillos et al., 2006). These results are in agreement with those obtained for hydrocolloid characterization.



**Fig. 2.** Rheological behavior of filmogenic solutions of: (a) Gelatin G1 and G2, and chitosan (CH) and (b) Gelatin G2 with glycerol (G2P), composite gelatin G2 and chitosan with plasticizer (G2CHP).

**Table 2**  
Rheological characterization of hydrocolloid solutions

Solution composition	Ostwald de Waele fitting parameters and correlation coefficient			Apparent viscosity at 500 s <sup>-1</sup> (mPa s)
	Consistency index (k)	Flow behavior index (n)	r <sup>2</sup>	
G1 <sup>a</sup>	0.45±0.03 <sup>g</sup>	1.24±0.21	0.978	89.9±13.9
G1P <sup>c</sup>	0.038±0.0004	1.32±0.25	0.999	84.0±25.5
G1CH <sup>e</sup>	1.50±0.46	0.61±0.07	0.994	135.8±12.9
G1CHP <sup>f</sup>	0.82±0.29	0.60±0.04	0.999	89.2±16.8
CH <sup>d</sup>	3.78±0.19	0.48±0.15	0.998	150.0±1.2
G2 <sup>b</sup>	0.021±0.005	1.01±0.06	0.997	16.8±1.7
G2P	0.008±0.001	1.11±0.07	0.993	18.0±6.0
G2CH	0.35±0.02	0.772±0.001	0.996	84.8±3.7
G2CHP	0.39±0.02	0.77±0.01	0.999	93.4±10.5

<sup>a</sup> G1: 7.5% w/w.

<sup>b</sup> G2: 7.5% w/w.

<sup>c</sup> P: 0.75% w/w glycerol.

<sup>d</sup> CH: 1% w/w.

<sup>e</sup> G1CH: Composite G1 and CH solutions.

<sup>f</sup> G1CHP: Composite G1 and CH solutions with glycerol.

<sup>g</sup> The informed value correspond to the ascending ramp of the flow curve.

Besides, CH addition increased significantly ( $P < 0.05$ ) apparent viscosity of both gelatin solutions while glycerol inclusion did not affect them (Table 2). Sionkowska et al. (2004) stressed that gelatin and chitosan are hydrophilic miscible biopolymers. This miscibility is assigned to specific interactions between polymeric components such as electrostatic attraction, covalent unions, hydrogen bonding and dipole between others. Moreover, being gelatin an ampholytic protein it was proposed to form a complex coacervate with chitosan (protein-colloidal particles stabilized by effective attraction forces) (Johnston-Banks, 1990; Kruif and Tuinier, 2001) and thus an increase in the composite solution viscosity is expected.

### 3.3. Characterization of hydrocolloid films

Films prepared with G1, G2 and their mixture with CH and glycerol were uniform, homogeneous and thin. The films were easily removed from the acrylic plate and showed smooth surfaces. Gelatin composite films (G1, G2), with or without glycerol and with the same total solid content, presented thicknesses between 60 and 90  $\mu\text{m}$ , while CH samples exhibited values between 30 and 45  $\mu\text{m}$ . With regard to bi-layer films the obtained values varied from 60 to 100  $\mu\text{m}$ .

Table 3 shows that gelatin, CH and composite films reached low moisture contents after drying. Optical properties are also presented in Table 3; as can be expected, opacity and transparency are inversely correlated.

**Table 3**  
Humidity content and optical properties of gelatin, chitosan with or without plasticizer films

Film composition	Humidity content (%)	Optical		Film color	
		Film transparency	Film opacity (Au $\times$ nm/ $\mu\text{m}$ )	Color differences( $\Delta E$ , Eq. (2))	Chromaticity parameter $b^*$
G1 <sup>a</sup>	11.44±0.69	0.62±0.04	0.18±1.34	2.11±0.17	3.41±0.18
G1CH	10.31±0.52	0.80±0.04	0.39±0.83	2.32±0.18	3.63±0.11
G1P <sup>c</sup>	8.46±0.59	0.92±0.38	0.17±0.09	1.32±0.04	2.71±0.38
G1CHP	10.19±0.92	0.82±0.08	0.39±1.95	1.82±0.23	3.08±0.29
G2 <sup>b</sup>	11.46±0.76	0.67±0.01	0.23±0.66	3.53±0.78	5.32±0.64
G2CH	10.63±0.58	0.99±0.06	0.47±0.94	2.94±0.30	4.08±0.26
G2P	7.90±0.40	0.68±0.05	0.22±1.27	1.87±0.20	3.33±0.24
G2CHP	10.23±0.61	0.73±0.04	0.28±0.85	2.44±0.41	3.66±0.14
CH <sup>d</sup>	8.49±0.42	1.13±0.05	0.54±1.10	1.66±0.15	3.04±0.32

<sup>a</sup> G1: 7.5% w/w.

<sup>b</sup> G2: 7.5% w/w.

<sup>c</sup> P: 0.75% w/w glycerol.

<sup>d</sup> CH: 1% w/w.

All assayed samples were transparent; gelatin film (G1, G2) transparency was  $0.60 \pm 0.02 A_{600}/\text{mm}$ . Glycerol addition did not modify this property while CH increased film transparency (Table 3). The obtained results indicated that films based on gelatin showed lower values than those of other polyol-plasticized films (Zhang and Han, 2006b) and some commonly used synthetic films such as low-density polyethylene ( $3.05 A_{600}/\text{mm}$ ) and oriented polypropylene ( $1.67 A_{600}/\text{mm}$ ).

Film opacities did not differ significantly ( $P > 0.05$ ). The obtained values were lower than those reported by Cho and Rhee (2004) for soy protein films and Park et al. (2007). These findings are important since film opacity is a critical property that conditioned film applications, particularly if the film will be used as a surface food coating or for improving product appearance.

Films based on G2 exhibited higher color differences ( $\Delta E$ ) and  $b^*$  values than those based on G1. Lightness ( $L^*$ ) did not differ significantly ( $P > 0.05$ ) regardless film formulation, exhibiting a value around  $95.77 \pm 0.60$ . A similarly trend was observed for  $a^*$ ;  $b^*$  parameter values decreased with both, glycerol and CH addition, independently of the gelatin source used (Table 3).

### 3.4. Scanning Electron Microscopy

The surface micrographs obtained by SEM revealed smooth matrix without any pores with good integrity for G1, G2 and CH films. Both, G1 and G2 films showed similar structures.

Fig. 3 a and b shows micrographs of cross-section of film composite by G1CH and G1CHP. Composite films with gelatin and chitosan were characterized by a compact, uniform, dense structure and homogenous appearance, independently of gelatin source. Plasticized composite films (Fig. 3b) showed smooth and compact structure indicating a good compatibility between the components.

G1-CH and G1CH-CH bi-layer films cross-section were less uniform than composite films (Fig. 3c and d). This could be due to a slight interaction between the components (G1 and CH or G1CH and CH) of the system. However in these micrographs the layers corresponding to individual components can be visualized (Fig. 3c and d).

### 3.5. Water vapor barrier properties of films

#### 3.5.1. Thickness effect on water vapor barrier properties

In contrast to hydrophobic synthetic polymeric materials, the WVP of films prepared from biopolymers depend on their thickness (McHugh et al., 1993; Gennadios et al., 1994a). The thickness effect is explained by the hydrophilic nature of most biopolymers since water vapor diffusivity of these materials is moisture dependent.

In this work, GW of gelatin films measured at a constant time, decreased linearly with film thickness until a constant value was reached at about 120  $\mu\text{m}$  (Fig. 4a). However, at thickness values higher than 120  $\mu\text{m}$ , GW became independent; thus, in these films a fictitious WVP increase could be obtained due only to the thickness, but not attributed to a real increase of GW. In these cases, water vapor permeabilities varied between  $3.32 \times 10^{-10}$  and  $5.16 \times 10^{-10} \text{ g s}^{-1} \text{ m}^{-1} \text{ Pa}^{-1}$  for film thickness ranged between 210 and 410  $\mu\text{m}$ , respectively.

Several explanations have been provided for these anomalous thickness effects, as film swelling due to attractive forces between films and water (McHugh et al., 1993). Bertuzzi et al. (2007) working with high amylose starch based films found that when relative humidity is greater than 52%, swelling is noticeable and the polymer matrix expands and water transport is facilitated.

Therefore, in this work to evaluate the WVP of gelatin film, thickness used was always lower than 90  $\mu\text{m}$ .

GW of chitosan films, measured at a constant time, decreased linearly for all thickness assayed ranging between 20 and 60  $\mu\text{m}$  (Fig. 4b); WVP values did not differ significantly ( $P > 0.05$ ) in the thickness range assayed, being  $6.35 \pm 1.3 \times 10^{-11} \text{ g s}^{-1} \text{ m}^{-1} \text{ Pa}^{-1}$ .

These findings about thickness effect was critical in the case of gelatin films since this polymer is highly hydrophilic and the thickness commonly obtained is higher than those of chitosan films as was previously described. Therefore it is essential for practical comparison purposes of water vapor permeability values get similar film thicknesses.

### 3.5.2. Water vapor permeability

As shown in Fig. 5, WVP of G1 and composite films showed the same behavior than G2 and their blends. WVP of G1 and G2 films, did not show significant differences ( $P > 0.05$ ). Both, CH and

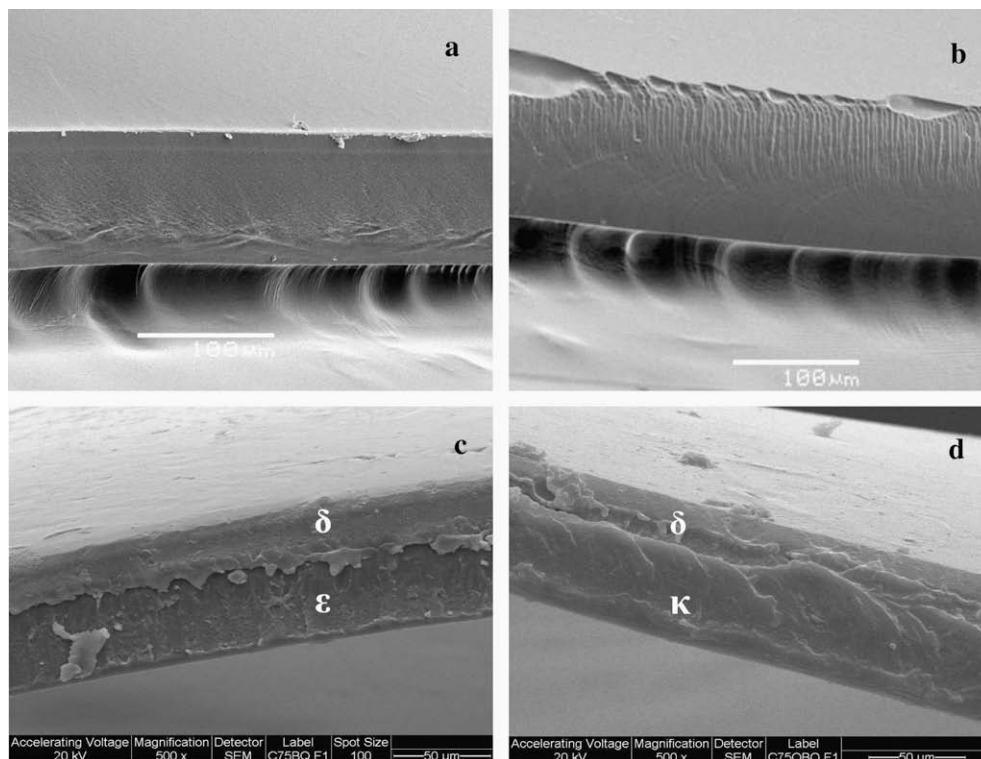
glycerol addition decreased WVP of gelatin films. These findings support those of Vanin et al. (2005) who studied gelatin plasticized films.

Besides, the obtained results are in agreement with those informed by Avena-Bustillos et al. (2006) for fish skin gelatin films but they are lower than those obtained from mammalian ones. A similar trend was observed when WVP results were compared with other protein films (McHugh et al., 1993; Gennadios et al., 1994b). Arvanitoyannis et al. (1998) found lower WVP values than those obtained in this work for a similar system. This could be explained considering that CH probably acts as cross-linking agent in gelatin matrix, modifying the polymer network and improve protein film functionality.

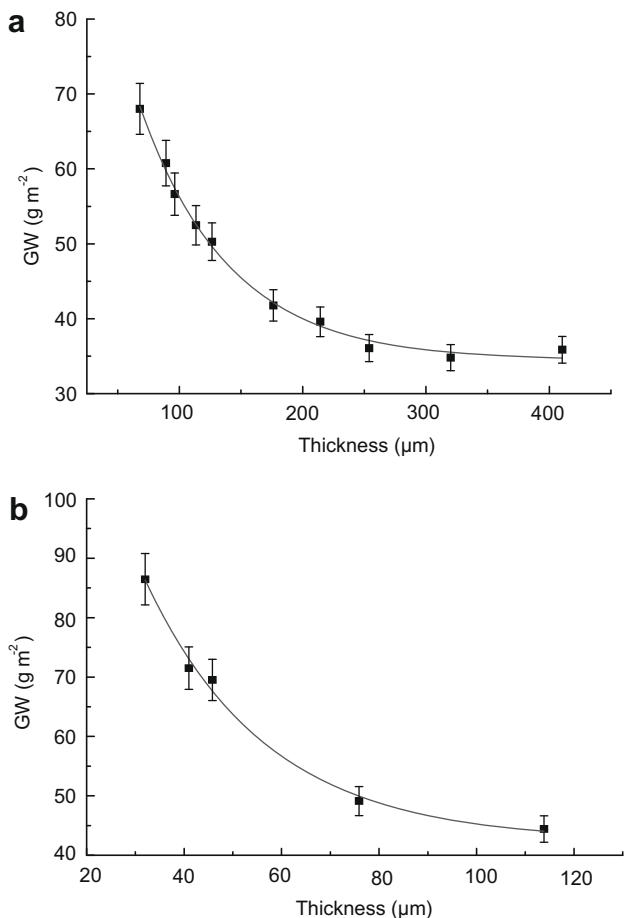
However, gelatin based films exhibited poor water-barrier characteristics compared to synthetic ones due to the hydrophilic character of the polymeric matrix. An alternative to enhance water vapor barrier properties of these films is to develop bi-layer systems. Many studies have researched the properties of bi-layer films (Kamper and Fennema, 1984; Park et al., 1994; Fang et al., 2005). In general, bi-layer systems are more effective barrier against water vapor transfer than composite films as was demonstrated by Perez-Gago (2005).

In this study, a similar trend was observed, regardless film formulation; Fig. 6a and b, shows the GW as a function of time as well as the WVTR for different film compositions. The performed assays indicated that WVTR of bi-layer or laminated films were independent of the film position onto the permeation cell; since the obtained results did not differ significantly ( $P > 0.05$ ).

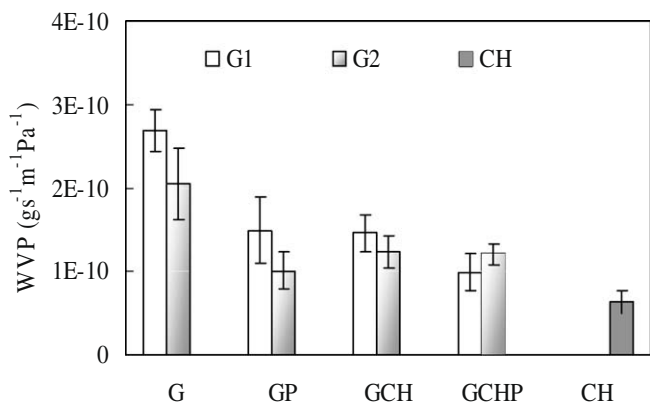
Bi-layer systems reduced the WVTR in 57.6% for G1-CH films (Fig. 6a) and 42.5% for G1P-CH ones (Fig. 6b) respect to the corresponding composite films. Besides, the obtained results with bi-layer systems were within the range of those obtained with lami-



**Fig. 3.** Cross-section micrographs of composite and bi-layer films: composite film of: (a) Composite gelatin G1 and chitosan film (G1CH) and (b) Gelatin G1 and chitosan with glycerol (G1CHP); bi-layer system consisting of: (c) Chitosan layer on gelatin G1 layer (G1-CH) and (d) Chitosan layer on composite gelatin G1 and chitosan film (G1CH-CH). In the micrograph  $\delta$  corresponds to chitosan layer,  $\epsilon$  to gelatin G1 and K to composite gelatin G1 and chitosan (G1CH) one respectively. Magnification is indicated in the micrographs.



**Fig. 4.** Gain weight (GW) of: (a) Gelatin film and (b) Chitosan film as a function of thickness. Error bars correspond to standard deviation.



**Fig. 5.** Water vapor permeability (WVP) of: chitosan film (CH), films of gelatin with (GP) and without (G) glycerol, composite films of gelatin G and chitosan with (GCHP) and without (GCH) glycerol, for both G1 and G2. Error bars correspond to standard deviation.

nated ones, being these WVTR values slightly lower than those of bi-layer systems. These findings are in agreement with the SEM observations reinforcing the idea that preparation technique allowed only a slight interaction between components.

**3.6. Mechanical properties**

High tensile strengths are generally necessary for films in order to withstand the normal stress encountered during their applica-

tion, subsequent shipping, and food handling. However, flexibility of biodegradable films, i.e. elongation at break should be adjusted according to the intended applications.

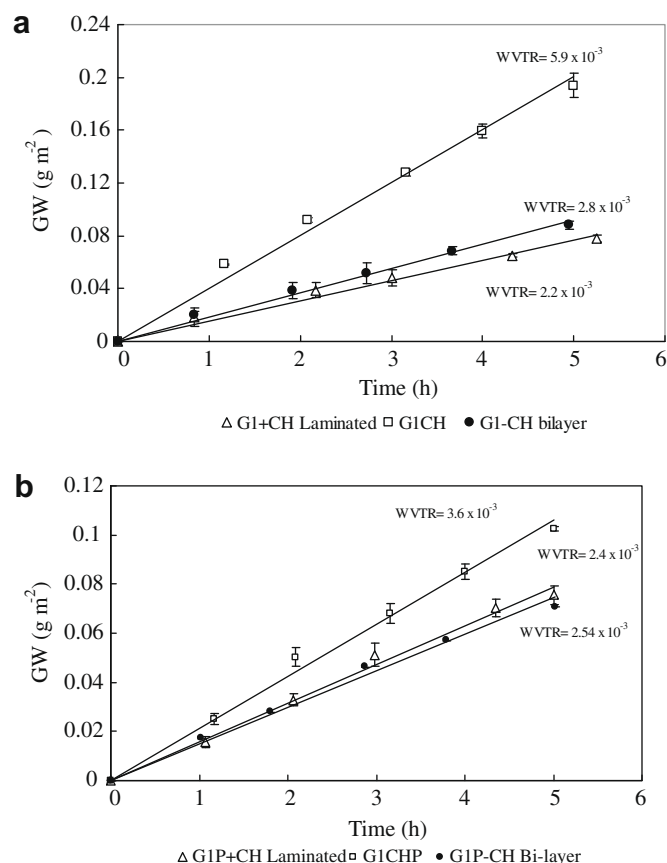
In general, protein films are brittle and susceptible to cracking due to the strong cohesive energy density of the polymer (Arvanitoyannis et al., 1998).

G1 and G2 films displayed lower TS values than CH ones, but similar to each other (Fig. 7a). Elongation at break values of CH films were 1.63 and 1.32 times higher than the corresponding G1 and G2 ones, respectively, (Fig. 7b).

Mechanical properties reported in literature for gelatin films were comparable to those obtained in this work (Park et al., 2007; Cao et al., 2007b).

CH films exhibited a TS value around  $94.9 \pm 8.5$  MPa and an EB of  $3.68\% \pm 0.7\%$ . The comparison with data of literature for tensile test is difficult due to different CH composition, solubilization method and film preparation (Butler et al., 1996; Caner et al., 1998). In despite of the use of similar CH concentrations, different mechanical properties were informed; Khan et al. (2000) found TS of 67.1 MPa and EB of 21.3% evaluating chitosan films for pharmaceutical uses. On the other hand, CH films for veterinary applications exhibited TS 13.3 MPa and a similar EB than those obtained in this work (Wiśniewska-Wrona et al., 2002).

As expected, when glycerol was added to gelatin films, higher EB and lower TS values were obtained, since plasticizer interfere with chain association facilitating their stretching and increasing film flexibility (Fig. 7). Glycerol effectiveness as plasticizer has been deeply studied in numerous protein films (Sothornvit and



**Fig. 6.** Gain weight (GW) as a function of time for: (a) Composite films (G1CH), bi-layer system (G1-CH) and laminated films (G1 + CH); (b) plasticized composite films (G1CHP), bi-layer system (G1P-CH) and laminated films (G1P + CH). Error bars correspond to standard deviation.

Krochta, 2001; Cho et al., 2002; Vanin et al., 2005; Park et al., 2007).

Carvalho and Grosso (2004) informed for bovine gelatin films plasticized with glycerol, values of TS and EB of 15.12 MPa and 39%, respectively.

In composite films, tensile strength increased with CH addition, leading to stronger films (Fig. 7a) exhibited TS data ranging between 27 and 53 MPa, and EB between 2.2% and 5.7%, which were lower than those reported by Arvanitoyannis et al. (1998) for a similar film formulation. From the results described above, it is evident that the incorporation of glycerol caused a deep effect on EB of gelatin films than CH.

The mechanical response of laminated films was conditioned by the more rigid component matrix which was determined by the composition of the individual films combined. For example in the case of G1CH + CH films, since both matrixes were brittle a single rupture peak was observed, while for G1 + CH and G1P + CH the patterns exhibited two individual rupture peaks where CH film determine the mechanical properties.

With regard to bi-layer films the stress–strain curves showed for G1-CH without plasticizer a typical pattern of brittle materials, since they exhibited high values of stress and low values of strain at maximum breaking force (Fig. 8), which are low EB values. Mechanical pattern of G1CH-CH bi-layer systems was characterized by a similar strain but higher stress at break than those of G1-CH films, increasing the system resistance of 54.3–77.2 MPa.

Plasticized bi-layer films G1P-CH showed the stress-strain behavior of ductile polymers. In these systems, the expected effect of plasticizer addition on mechanical properties was observed,

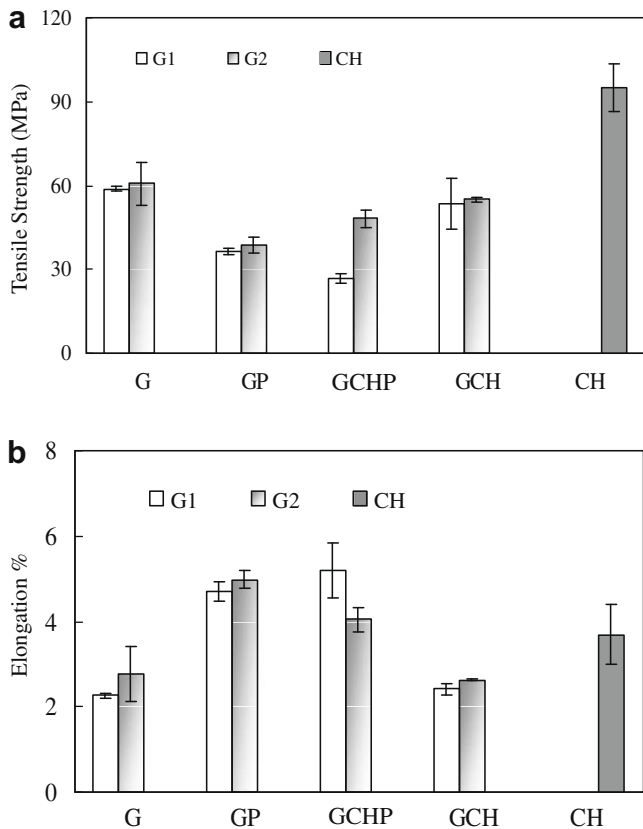


Fig. 7. Mechanical properties of chitosan film (CH), films of gelatin with (GP) and without (G) glycerol, composite films of gelatin G and chitosan with (GCHP) and without (GCH) glycerol, for both G1 and G2: (a) Tensile strength and (b) Percentage of elongation at break. Error bars correspond to standard deviation.

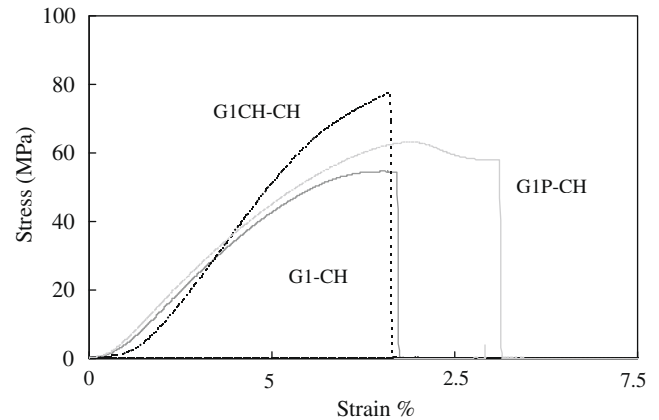


Fig. 8. Tensile mechanical behavior (stress vs. strain curves) of bi-layer systems formulated with chitosan layer on: Gelatin G1 film (G1-CH), gelatin G1 with plasticizer film (G1P-CH) and composite of gelatin G1 and chitosan film (G1CH-CH).

increasing strain at break and decreasing stress compared with unplasticized bi-layer films (Fig. 8).

According to Kamper and Fennema (1984) and Park et al. (2007) bi-layer films exhibited poor mechanical properties compared to composite films. In this work, tensile strength of bi-layer systems was significantly ( $P < 0.05$ ) higher than those of composite films, obtaining the high TS value for G1CH-CH (77.2 MPa). However, elongation at break values of composite and bi-layer films did not differ significantly ( $P > 0.05$ ), ranging between 2.2% and 5.7%. Similar results were reported by Weller et al. (1998), working with bi-layer film from zein, grain sorghum and wax.

Bi-layer systems exhibited a better mechanical performance than laminated ones. The mechanical response of laminated films was conditioned by the more rigid component matrix.

#### 4. Conclusions

Physical properties such as optical, water vapor barrier and mechanical properties of obtained films were similar regardless the gelatins types; however rheological behavior of G1 and G2 film forming solutions was different. The knowledge of rheological properties determines the conditions of manipulation of solutions and contributes to improve extensibility during the casting process. The management of film formulation and the function that each component plays, as well as their interaction, could allow to obtain tailor-made composite films.

In composite film, it was evident that the presence of CH led to stronger films while glycerol improved film flexibility. With regard to water vapor barrier properties both, glycerol (P) and CH improved WVP of composite films.

In this work it was demonstrated that GW presented a linear relationship with thickness until 120  $\mu\text{m}$  for gelatin and 60  $\mu\text{m}$  for chitosan films, while for thickness higher than those values, GW remained constant and WVP increased only due to the thickness effect. Thus, to evaluate barrier properties film based on gelatin or chitosan should require the knowledge of film thickness dependence of each particular hydrocolloid formulation.

On the other hand, bi-layer and laminated systems with CH addition, resulted effective alternatives to improve water vapor barrier properties of composite gelatin films. For example, a reduction of at least 42.5% was achieved with bi-layer systems of G1P-CH.

Although, the water vapor barrier capacity of both bi-layer and laminated systems is similar, the first one exhibited better mechanical properties. Thus, bi-layer systems had higher performance than both laminated and composite films.



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