



Biodegradable three-layer film derived from bovine gelatin

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ABSTRACT

Biodegradable three-layer gelatin film was obtained by heat – compression of piled dialdehyde starch (DAS) – cross-linked and plasticized-gelatin films (Ge-10DAS) outer layers and sodium montmorillonite (MMt) – plasticized – gelatin film (Ge-5MMt) inner layer. Multilayer film displayed a compact and uniform micro-structure due to the highly compatible individual layers which could interact by strong hydrogen bonding. Lamination reduced moisture absorption and total soluble matter compared to the single layers while keeping transparency. Tensile strength and elastic modulus of the multilayer were 8.0 ± 1.3 MPa and 14.7 ± 2.4 MPa, which were significantly higher than values obtained for Ge-10DAS due to the contribution of the of the bio-nanocomposite inner layer. Elongation at break was not affected by lamination meanwhile it had a beneficial effect on barrier properties. Water vapor permeability (WVP) of the multilayer was $0.8 \pm 0.1 \times 10^{-13}$ kg m Pa⁻¹ s⁻¹ m⁻² which was lower than those of the individual components whereas oxygen permeability was similar to that of Ge-5MMt (10.5 ± 0.4 cm³(O₂) mm m⁻² day⁻¹) and lower than that of Ge-10DAS film.

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1. Introduction

Biopolymers, such as carbohydrates, proteins and lipids have traditionally been used primarily as a food source however they are becoming an increasingly important source of raw materials for industrial applications particularly as packaging materials. These materials are inexpensive, produced from renewable resources and biodegradable and their use in large-scale applications may contribute to a more sustainable future economy offering an outlet for overflowing solid-waste streams (Krochta and De Mulder-Johnston, 1997). Among them, proteins derived from vegetal or animal sources have potential for replacing at least a fraction of non-biodegradable polymers, mainly in those applications where biodegradability gives added value (Avella et al., 2005).

Gelatin is an animal protein obtained by a controlled hydrolysis of the fibrous insoluble collagen present in the bones and skin generated as waste during animal slaughtering and processing (Patil et al., 2000) or from the pharmaceutical industry (Chiellini et al., 2001) with appropriate film forming properties to produce bio-packaging materials (Jongjareonrak et al., 2006). As most proteins, gelatin based films are generally good barriers against oxygen and aromas at low and intermediate relative humidity (RH) and have fairly good mechanical properties, but their barrier against water vapor (WV) is poor, due to the hydrophilic nature of gelatin (Arvanitoyannis et al., 1998; Patil et al., 2000; Apostolov et al., 2002; Bigi

et al., 2002; Gennadios, 2002; de Carvalho and Grosso, 2004; Bertan et al., 2005; Avena-Bustillos et al., 2006; Arnesen and Gildberg, 2007). In many applications, a better barrier against WV is preferable since low levels of water activity must be maintained in low-moisture foods to prevent texture degradation and to minimize deteriorative chemical and enzymatic reactions (Kester and Fennema, 1989).

Different approaches have been proposed to overcome these limitations including cross-linking (Bigi et al., 2002; Gennadios, 2002; de Carvalho and Grosso, 2004; Lien et al., 2008; Martucci 2008; Martucci and Ruseckaite, 2009a), blending (Arvanitoyannis et al., 1998; Bertan et al., 2005; Karnnet et al., 2005), compounding with natural fibers (Chiellini et al., 2001) or with nanosized clay dispersed in the biopolymer matrix in order to produce bio-nanocomposites (Zheng et al., 2002; Martucci et al., 2007; Martucci and Ruseckaite, 2008).

Another strategy to solve this problem is lamination which improves the performance of polymeric films by combining the properties of several types of layers into one multilayer film (Fang et al., 2005). Studies on laminated biopolymer films are barely reported in the literature although this is a common practice with films based on non-biodegradable polymers. Lamination of plasma – treated linear low density polyethylene (LLDPE) with chitosan or zein protein was found to enhance mechanical and oxygen barrier properties of LLDPE films (Shin et al., 2002). Similarly, Elsabee et al. (2008) proposed the synthesis of corona treated polypropylene (PP) film covered with a multilayer of chitosan/pectin. The multilayer film did not suffer deterioration in terms of their mechanical

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properties and exhibited enhanced antibacterial and antifungal activity. Biodegradable laminates based on poly(lactic acid) (PLA) as external layer and modified starch (MS) as inner layer has been proposed by Fang et al. (2005), however little information about its performance was reported. Mechanical properties, water and oxygen barrier properties of soybean protein isolate (SPI) films were improved through lamination by solvent-casting with PLA as external layers (Rhim et al., 2006, 2007). Quite recently, bilayer films based on plasticized whey protein films and olive oil – plasticized zein films prepared by casting and subsequently laminated by compression – moulding were investigated (Ghanbarzadeh and Oromiehi, 2009). Authors attributed the improvement in tensile properties to favourable interactions between layers, as concluded from calorimetric studies.

In previous studies we reported moisture resistance, mechanical, optical and barrier properties as well as biodegradability in soil of dialdehyde starch (DAS)-cross-linked gelatin films (Ge-DAS) (Martucci and Ruseckaite, 2009a) and sodium montmorillonite (MMt) – reinforced gelatin films (Ge-MMt) (Martucci, 2008). Studies on the properties showed that none of these films fulfil all the requirements for packaging applications by their own. Therefore, the aim of this work was to prepare a three – layer film by combining Ge-DAS films as outer layers with Ge-MMt as inner layer through heat-compression molding, and to investigate the impact of lamination on tensile, optical and barrier properties by comparing with those of the individual layers.

2. Materials and methods

2.1. Materials

Bovine hide gelatin (Ge) type B was kindly provided by Rousset (Buenos Aires, Argentina), Bloom 150, isoionic point (Ip) 5.3. Sodium montmorillonite (MMt) nano-clay was purchased from Southern Clay Products Inc. (Texas, USA), under the trade name Cloisite Na⁺. The cation – exchange capacity (CEC) was 92.6 meq/100 mg clay (Technical Report) and the interlayer distance was 1.2 nm (as it was determined by X-ray diffraction on the dry powder). Dialdehyde starch (DAS, 81.8% starch oxidation degree) was purchased from Sigma–Aldrich (St. Louis, MO, USA) and used as received. Glycerol (Gly) (DEM Chemicals, Mar del Plata, Argentina) was used as film plasticizer.

2.2. Three-layer film preparation

Biodegradable three-layer films were prepared by combining the cross-linked gelatin films as the outer layers and bio-nanocomposite film as the inner layer through a two-step process. In the first step, the individual films were obtained separately. Furthermore, the layers were stacked together and heat-compressed.

Cross-linking gelatin films were obtained by heat – compression of a blend of gelatin, 30 wt.% glycerol as plasticizer and 10 wt.% DAS (on dry gelatin basis) as cross-linking agent, following the procedure previously reported (Martucci and Ruseckaite, 2009a). The obtained films were named **Ge-10DAS**. Un-cross-linked and glycerol-plasticized-gelatin films (**Ge-30Gly**) were prepared under similar conditions and used as control films.

The inner layer was produced by solution–intercalation method according to the procedure reported elsewhere (Martucci et al., 2007; Martucci and Ruseckaite, 2008, 2009b). Blend of gelatin plasticized with 30 wt.% glycerol was completely dissolved in distilled water at 50 °C and under constant stirring. The pH of gelatin solution was adjusted to 7 with NaOH 0.1 M. Subsequently, the aqueous gelatin solution was added drop-wise into an ultrasonically pre-treated suspension of MMt (5 wt.% on dry gelatin basis) and

mixed together at 50 °C under vigorous stirring for 15 min. The resulting suspensions were then poured on teflonated Petri dishes (diameter 15 cm) and dried at 50 °C in a convection oven. The obtained films were transparent and light yellow due to MMt. These samples were labelled as **Ge-5MMt**.

Three-layer films were prepared by stacking together an outer Ge-10DAS layer, a Ge-5MMt inner layer and a second Ge-10DAS layer followed by heat – compression. Processing conditions were 50 kg cm⁻² applied for 10 min at 70 °C with a further cooling step at room temperature and pressure. The obtained three-layer films were light brown and they were stored under controlled temperature and humidity conditions prior to further testing (65 ± 2% HR; 25 ± 2 °C).

2.3. Film characterization

2.3.1. Microstructure analysis

Microstructure was examined by Scanning Electronic Microscopy (SEM) on a Phillips 505 microscope (Eindhoven, The Netherlands) with an operating voltage of 15 KV. Samples were cryo-fractured and then the cross-sections were sputter-coated with gold.

2.3.2. Film thickness

Average thickness was measured at five random locations of the films by using a micrometer (Vernier, China). Samples were vacuum dried before testing. The average film thickness was used in assessing opacity, barrier and mechanical properties.

2.3.3. Opacity

The opacity of multilayer and control films was tested using a UV–Visible spectrophotometer Shimadzu 1601 PC (Tokyo, Japan). Two specimens were taken from each film and cut into rectangular strips and placed on the inner side of a transparent plastic 10 mm cell. The absorbance spectrum (400–800 nm) was recorded and the opacity was defined as the area under the recorded curve determined by an integration procedure and it was expressed as Absorbance units in nanometers per thickness unit (AU nm μm⁻¹) (Irissin-Mangata et al., 2001).

2.3.4. Thermogravimetric analysis

Dynamic experiments were carried out using a thermogravimetric analyzer TGA-DTGA Shimadzu 50 (Shimadzu Corp., Japan). Temperature was raised from 25 °C to 550 °C, at a heating rate of 10 °C min⁻¹ and under nitrogen (20 ml min⁻¹) in order to avoid thermo-oxidative degradation.

2.3.5. Thermomechanical properties

Dynamic mechanical analysis (DMA) was carried out in Perkin Elmer UNIX DMA 7 (Connecticut, USA) operating in tensile mode. Measurements were performed a heating rate of 10 °C min⁻¹ from –25 °C to 190 °C and a fixed frequency of 1 Hz. The maximum of the loss modulus (*E''*) vs. temperature curve was taken as measure of the glass transition temperature (*T_g*). Results were the average of three replicates.

2.3.6. Tensile properties

Tensile tests were performed according to ASTM D638-02b. Five dog-bone-shaped specimens (30 × 4.5 × 0.2 mm) were conditioned at 65% of relative humidity (RH) and at ambient temperature (25 ± 2 °C) in an environmental chamber. The equilibrated strips were clamped on a Universal Testing machine (Instron, Darmstadt, Germany) equipped with a 0.5 KN cell. The films were stretched at a crosshead speed of 3 mm min⁻¹ and the elastic modulus (EM), tensile strength (TS) and elongation at break (*E*) were

determined from the stress vs. strain experimental curves. Six replicates were run for each kind of film.

2.3.7. Total soluble matter determination

Total soluble matter (TSM) was determined according to the method proposed elsewhere (Rhim et al., 1998). Three specimens of each film were weighed (m_h) (± 0.0001 g) and subsequently dried in an air-circulating oven at 105 °C for 24 h. After this time films were recovered and re-weighed (± 0.0001 g) to determine their initial dry matter (m_0). Afterwards, the samples were immersed in 30 mL of distilled water in the presence of sodium azide (0.02%) in order to prevent the microbial growth. The flasks were stored in environmental chamber at 25 °C for 24 h. After this time, specimens were recovered, rinsed with distilled water and dried in an air-circulating oven at 105 °C until reaching constant weight (m_f). The TSM was calculated as:

$$\text{TMS}(\%) = 100 * \frac{m_0 - m_f}{m_0} \quad (1)$$

2.3.8. Moisture absorption at 65% relative humidity

Moisture absorption was determined in an environmental chamber at 65% RH and at a fixed temperature (23 ± 2 °C). Samples were vacuum dried before testing. The specimens were recovered from the chamber at regular time intervals and weighed with a precision of ± 0.0001 g. The moisture uptake (w_t) as a function of time t was obtained from the total and partial water mass balance over the sample as a function of time:

$$w_t(\%) = \frac{m_0 * w_0 + (m_t - m_0) * 100\%}{m_t} \quad (2)$$

where w_t is the moisture content as a function of time, m_t is the weight of the sample after exposition, m_0 is the initial weight and w_0 is the initial moisture content of the samples. This experiment was performed on four specimens of each sample to ensure the reproducibility of the results.

The empirical equation of Peleg (1988) is useful to describe the moisture absorption process of many biopolymers (Nashed et al., 2003). This expression relates the instantaneous moisture content (w_t) to the initial moisture content (w_0), as shown in Eq. (3)

$$w_t(\%) = w_0 + \frac{t}{k_1 + k_2 * t} \quad (3)$$

where w_0 is the initial moisture content and w_t is the moisture content as a function of time, t . The constants k_1 and k_2 are fitting parameters; k_1 ($\text{min}(\text{wt.\%water wt.\%solids}^{-1})^{-1}$) is the Peleg's rate factor is related to mass transfer, the lower k_1 , the higher the initial water absorption rate; k_2 ($(\text{wt.\%water wt.\%solid}^{-1})^{-1}$) is the Peleg's capacity parameter is associated to the maximum water absorption capacity, therefore the lower k_2 , the higher the absorption capacity (Peleg, 1998; Nashed et al., 2003).

2.3.9. Barrier properties

2.3.9.1. Water vapor permeability. The water-vapor transmission rate (WVTR) experiments were conducted according to the desiccant method described in the standard method ASTM E96-95. Each film was sealed onto a glass permeation cup containing silica gel (0% RH) with silicone vacuum grease and an O-ring to hold the film in place. The test cells were then placed in humidity chamber at $65 \pm 2\%$ RH and maintained at 25 °C. The RH gradient was 65:0 (RH outside: RH inside of cups). After steady state conditions were reached, the cells were weighed (± 0.0001 g) during 24 h. Each experimental point was the result of at least three replicates. Linear regression was used to calculate the slope of a fitted straight line. The slope of the variation of mass (Δm) of the test cells versus

time (t) was used to obtain the WVTR ($\text{kg s}^{-1} \text{m}^{-2}$). The water vapor permeability (WVP) ($\text{kg m Pa}^{-1} \text{s}^{-1} \text{m}^{-2}$) was calculated as:

$$\text{WVP}(\text{kg m Pa}^{-1} \text{s}^{-1} \text{m}^{-2}) = \frac{\text{WVTR}}{S * (\text{RH}_1 - \text{RH}_2)} * e \quad (4)$$

where WVTR is water-vapor transmission rate in the steady state ($\text{kg s}^{-1} \text{m}^{-2}$), e is the film thickness (m), S is the saturation pressure (Pa) at the test temperature, RH_1 is the relative humidity in the humidity chamber and RH_2 is the relative humidity inside the cell test.

Results were the average of five replicates.

2.3.9.2. Oxygen transmission rate. The oxygen transmission rate tests were assessed in an Oxygen Permeation Analyzer 8500 (Systech Instruments, Metrotec S.A, Spain). Circular samples (diameter 14 cm and thickness 0.61 ± 0.06 mm) were clamped in a diffusion chamber at 25 °C. Pure oxygen (99.9%) was introduced into the upper half of the sample chamber while nitrogen was injected into the lower half of the chamber where an oxygen sensor was placed. The gas volumetric flow rate per unit area of the membrane OT ($\text{cm}^3 (\text{O}_2) \text{day}^{-1} \text{m}^{-2}$) was continuously monitored until a steady state was reached (OT_∞). Thickness of films (e) was measured at 25 °C using a 0–25 mm manual micrometer (± 0.01 mm). The films were conditioned at 65% RH before testing. The permeability coefficient (OP) is proportional to $Q(\text{cm}^3(\text{O}_2) \text{mm} \cdot \text{day}^{-1} \text{m}^{-2}) = \text{OT}_\infty * e$.

2.3.10. Statistical analysis

Data for each test were statistically analyzed. The analysis of variance (ANOVA) was used ($\alpha = 0.05$) to evaluate the significance in the difference between factors and levels. Comparison of the means was done employing a Turkey test to identify which groups were significantly different from other groups ($P < 0.05$).

3. Results and discussion

Lamination was accomplished by hot-pressing the heat-sealable gelatin films together. Molding temperature was chosen from exploratory studies on the thermal properties of individual components. Multilayer film was produced at 70 °C under a pressure of 50 kg cm^{-2} applied for 10 min. According to TG/DTG curves (Fig. 1), Ge-5MMt and Ge-10DAS films were stable up to 200 °C. The main decomposition step was found at about 332 °C and was assigned to the thermal breakage of peptide bonds in the main chain of gelatin (Barreto et al., 2003; Martucci, 2008; Martucci and Ruseckaite, 2009b). On the other hand, the glass transition temperature of the single layers were 76 °C for Ge-5MMt and 83 °C for Ge-10DAS, therefore 70 °C was chosen as the optimal

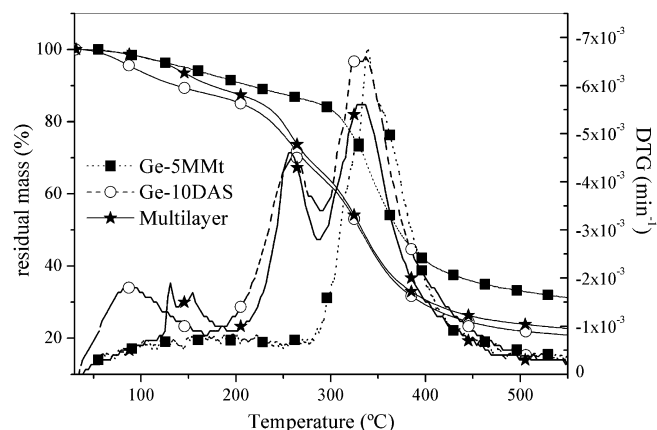


Fig. 1. Normalized TG/DTG curves of (●) Ge-5MMt, (○) Ge-10DAS and (★) multilayer films.

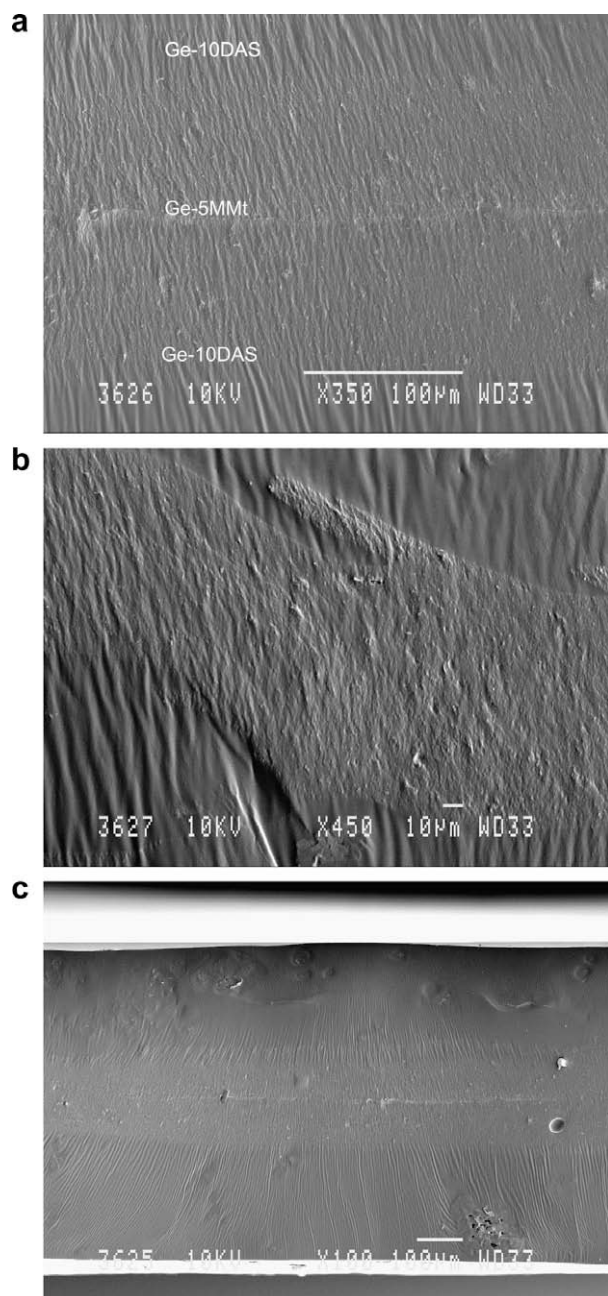


Fig. 2. Cross-section micrographs of three-layer film. Magnification: (a) $\times 350$, (b) $\times 450$ and (c) $\times 100$.

temperature to preheat the mold and also to produce homogeneous films during the pressing step. The compression-molding time was 10 min because lower times were not enough to produce a film and longer times led to protein and DAS thermal degradation (Barreto et al., 2003; Martucci and Ruseckaite, 2009a).

Multilayer film was flexible, transparent and light brown due to the presence of conjugated Schiff's bases in Ge-10DAS outer layers (Rhim et al., 1998; Martucci and Ruseckaite, 2009a). The microstructure of the cross-sectional area of the three-layer film revealed clearly the individual layers (Fig. 2a), with the outer Ge-10DAS layers firmly bonded to the Ge-5MMt inner one. This observation suggests that individual layers are highly compatible in the multilayer film. According to Jannerfeldt et al. (2000), three interfacial adhesion mechanisms might occur in multilayer systems: inter-diffusion of two different polymers; the crystallization of one polymer in the presence of the second (for semi-crystalline poly-

mers); and chemical and/or physical interactions at the interface. In the present study, the three layers are based on gelatin therefore they can easily interact through hydrogen bonding (Apostolov et al., 2002). Inter-diffusion among adjacent layers induced by heat-compression was also evidenced (Fig. 2b), favoring the formation of a more compact, dense and uniform structure, as observed in Fig. 2c (Rhim et al., 2006; Rivero et al., 2008).

Tensile properties of the multilayer and single films are summarized in Table 1. Results revealed that lamination performed by heat – compression may affect the tensile properties of the gelatin – based films. Significant differences ($P < 0.05$) in elastic modulus (EM) and tensile strength (TS) values were found between individual and multilayer films. Among the individual films, Ge-30Gly and Ge-10DAS films did not show significant differences ($P > 0.05$) in TS and EM values indicating that cross-linking with polymeric DAS did not improve considerably the tensile properties. Indeed, the reinforcing effect of cross-linkages induced by DAS is counterbalanced by the polymeric nature of DAS which may act as internal plasticizer, as it was already reported for this cross-linking agent (Gennadios et al., 1998; Rhim et al., 1998; Martucci and Ruseckaite, 2009a). Ge-5MMt film showed the highest TS and EM values. This finding was attributed to the high rigidity and aspect ratio of the nano-clay as well as the presence of strong hydrogen interactions between matrix and clay (Martucci and Ruseckaite, 2008). The low TS and EM values of control and Ge-10DAS films restricts their use in packaging applications therefore any improvement in such property is highly important for the intended application. The TS and EM values of the three-layer film were significantly increased ($P < 0.05$) increased compared with those of Ge-10DAS and control films (Table 1). This was attributed to the contribution of Ge-5MMt inner layer. It is important to note that E values did not vary significantly with lamination ($P > 0.05$), indicating that multilayer film retain extensivity and flexibility of the individual components. The mean TS value of the multilayer prepared in this study was in the same range of that of low density polyethylene (LDPE) (8.6–17 MPa) (Gennadios et al., 1994), but lower to that of multilayer film based on linear low density polyethylene (LLDPE), chitosan and EVA (LLDPE/chitosan/EVA) which was about 11 MPa (Shin et al., 2002). But, E% of multilayer film was higher than that of LLDPE/chitosan/EVA ($110.0 \pm 15.1\%$ vs. $27.16 \pm 8.41\%$, respectively) (Shin et al., 2002). It is important to remark that no separation of the layers was observed in the multilayer film upon tensile testing, indicating a strong adhesion between layers.

The opacity values of multilayer film along with those of single components and control films are summarized in Table 1. Opacity values of multilayer, Ge-5MMt and Ge-30Gly films did not differ significantly ($P > 0.05$) and can be considered transparent (Mali et al., 2004). However, these values were significantly lower ($P < 0.05$) than that of Ge-10DAS film. Film opacity is generally affected by additives, processing conditions, thickness as well as compatibility between layers in multilayer films (Cunnigham et al., 2000; Wang et al., 2000; Mali et al., 2004; Rhim et al., 2006, 2007). Since film thickness was almost invariable (Table 1) the higher opacity of Ge-10DAS film could be a consequence of some degree of phase separation due to the limited compatibility between the gelatin and polymeric DAS (Martucci and Ruseckaite, 2009a). The decreased opacity value of the multilayer films compared to that of Ge-10DAS film gave indirect evidence of the compatibility between neighboring layers. Multilayer opacity was lower than those reported by Park et al. (2008) for extruded gelatin and low density polyethylene (LDPE) films but higher than those of films produced by extrusion followed by heat-compression.

Total soluble matter (TSM) was determined to evaluate the integrity of the multilayer film in aqueous environment and results are summarized in Table 2. Ge-5MMt film disintegrated almost completely after soaking in water meanwhile Ge-10DAS exhibited

Table 1

Opacity and tensile properties of single components and three-layer film. For comparing purposes, values of gelatin film plasticized with 30 wt.% glycerol (Ge-30Gly) was also included.

| Film name | Thickness(μm) | Opacity(UA nm μm^{-1}) | EM(MPa) | TS(MPa) | E(%) |
|------------|------------------------------|------------------------------------|------------------------------|-----------------------------|-------------------------------|
| Ge-30Gly | 0.58 \pm 0.04 ^a | 215.8 \pm 13.8 ^a | 3.7 \pm 0.7 ^a | 3.9 \pm 1.0 ^a | 96.9 \pm 11.9 ^a |
| Ge-5MMt | 0.60 \pm 0.04 ^a | 218.3 \pm 11.9 ^a | 38.5 \pm 10.9 ^b | 12.1 \pm 1.7 ^b | 99.1 \pm 11.1 ^a |
| Ge-10DAS | 0.58 \pm 0.03 ^a | 391.7 \pm 9.0 ^b | 1.9 \pm 0.3 ^a | 3.5 \pm 0.3 ^a | 111.8 \pm 12.1 ^a |
| Multilayer | 0.59 \pm 0.02 ^a | 229.9 \pm 42.8 ^a | 14.7 \pm 2.4 ^c | 8.0 \pm 1.3 ^c | 110.0 \pm 15.1 ^a |

Any two means in the same column followed by the same letter are not significantly ($P > 0.05$) different according to Turkey test.

Table 2

Total soluble matter (TSM) and Peleg's parameters, k_1 and k_2 of individual and three-layers film.

| Film name | TSM(%) | $k_1(\text{min}^{-1})$ | $k_2 \times 10^2(\%^{-1})$ | r^2 |
|------------|------------------------------|------------------------|----------------------------|-------|
| Ge-5MMt | 100.0 \pm 0.0 ^a | 15.6 \pm 1.3 | 6.4 \pm 0.1 | 0.99 |
| Ge-10DAS | 31.2 \pm 1.4 ^b | 2.3 \pm 0.2 | 7.2 \pm 0.1 | 0.99 |
| Multilayer | 31.0 \pm 2.4 ^b | 23.6 \pm 2.0 | 7.5 \pm 0.1 | 0.99 |

Any two means in the same column followed by the same letter are not significantly ($P > 0.05$) different according to Turkey test.

a substantially ($P < 0.05$) lower solubility ($31.2 \pm 1.4\%$). The soluble fraction in Ge-10DAS film could be principally attributed to the loss of low molar mass compounds, such as oligopeptide chains that could not be cross-linked by DAS and to the exudation of glycerol out of the film (Martucci and Ruseckaite, 2009a). As expected, hydrogen interactions between glycerol, clay and gelatin matrix in the bio-nanocomposite film were less effective in preventing water solubility than cross-linking due to DAS (Martucci, 2008). The efficiency of DAS in stabilizing proteinaceous films against water was previously reported for zein protein obtained by compression molding (Spence et al., 1995), soy protein isolate (SPI) films obtained by solvent-casting (Rhim et al., 1998) and egg white films produced by casting (Gennadios et al., 1998). The TSM value of three-layer film did not show significant difference ($P > 0.05$) with that of Ge-10DAS film (Table 2), indicating that the cross-linked outer layers restrict water solubility of the multilayer film during short-time soaking experiments. Indeed, it was observed that multilayer film swells and eventually splits into layers after 48 h of immersion. It was assumed that most of the absorbed water probably entered from the cut edges of the multilayer film due the absence of the protective layer of Ge-10DAS. This facilitates the entrance of water and eventually, the dissolution of the un-cross-linked and more susceptible layer.

Moisture absorption at 65% RH was also evaluated. All absorption curves displayed fast absorption at short times and then the absorption rate hardly changed, indicating that samples became equilibrated with the storage environment (Fig. 3). Experimental data were fitted using the Peleg's empiric equation (Eq. (3)) (Peleg, 1988) and the constants k_1 and k_2 , which were derived from the linear fit, are shown in Table 2. The coefficients of determination were high (r^2 about 0.99); indicating a good agreement between predicted (dashed lines) and experimental (symbols) data (Fig. 3). The three-layer film exhibited higher k_1 and k_2 values compared to those of the individual components, indicating that multilayer film absorbed less moisture at slower rate. An explanation for this behavior may be proposed based on the structure of multilayer film. Since hydrogen interactions increase the bond strength between gelatin-based layers, reduction in water absorption could be related to the increased obstacle to water molecules to passing through a more closely packed structure in the multilayer film (Fig. 2c).

Water vapor permeability values of the multilayer, Ge-10DAS and Ge-5MMt films measured at 65% RH and 23 °C are reported in Table 3. The individual components of the multilayer film had lower WVP values than the control Ge-30Gly film ($P < 0.05$), indicating that

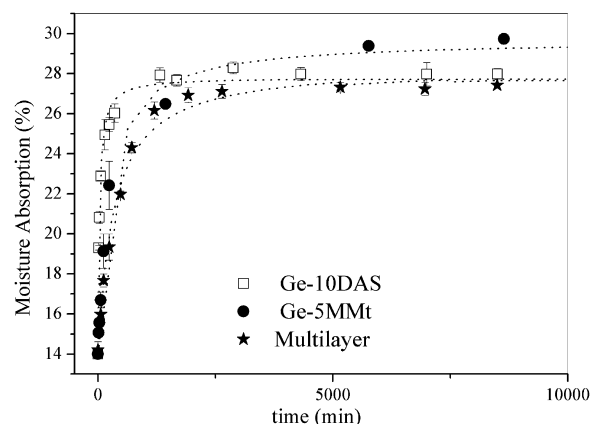


Fig. 3. Moisture absorption curves at 65% RH (●) Ge-5MMt, (○) Ge-10DAS and (★) multilayer films.

Table 3

Water vapor permeability (WVP) and oxygen permeability (Q) of single and three-layer films.

| Film name | Q ($\text{cm}^3(\text{O}_2) \text{ mm day}^{-1} \text{ m}^{-2}$) | WVP $\times 10^{13}$ ($\text{kg m Pa}^{-1} \text{ s}^{-1} \text{ m}^{-2}$) |
|------------|---|---|
| Ge-30Gly | 13.3 \pm 3.0 ^{ab} | 2.5 \pm 0.1 ^a |
| Ge-5MMt | 10.9 \pm 1.9 ^c | 1.4 \pm 0.1 ^b |
| Ge-10DAS | 12.1 \pm 2.6 ^b | 1.7 \pm 0.05 ^c |
| Multilayer | 10.5 \pm 0.4 ^c | 0.8 \pm 0.1 ^d |

Any two means in the same column followed by the same letter are not significantly ($P > 0.05$) different according to Turkey test.

cross-linking and compounding with nano-clay improve barrier properties. As it was stated before, cross-linking with 10 wt.%DAS reduce TSM and moisture binding capacity at 65% RH of plasticized-gelatin film (Martucci and Ruseckaite, 2009a). The Ge-5MMt showed lowest WVP among individual films. Due to its hydrophilic character, sodium montmorillonite was highly compatible and well dispersed in the plasticized-gelatin matrix (Martucci, 2008; Martucci and Ruseckaite, 2009a). It is well establish that layered structure of the nano-clays delays the diffusing water vapor through the film matrix due to tortuosity (Ray and Bousmina, 2005). A decrease in WVP has been observed for other biopolymer/clay systems (Hedenqvist et al., 2006; Kampeerappun et al., 2007; Zenkiewicz and Richert, 2008), but comparison between WVP values was rather difficult because of differences in film thickness and experimental conditions. Lamination improved significantly ($P < 0.05$) water vapor barrier properties, as evidenced by the decrease in WVP value of the multilayer (Table 3). When lamination is performed by heat-compression of the piled films, inter-diffusion between layers takes place (Fig. 2b), filling pores and irregularities and enabling the formation of a denser structure (Fig. 2c) which decreases vapor permeability (Rivero et al., 2008). The WVP value of the multilayer was comparable with those reported for cellulose acetate film (0.05–

$0.16 \times 10^{-13} \text{ kg m Pa}^{-1} \text{ s}^{-1} \text{ m}^{-2}$) (Briston, 1988) and values of poly(lactide) (PLA) film ($0.466 \pm 0.25 \times 10^{-13} \text{ kg m Pa}^{-1} \text{ s}^{-1} \text{ m}^{-2}$, thickness: ca. 90 μm , prepared by solvent-casting method) and multilayer PLA/SPI/PLA film ($0.66 \pm 0.27 \times 10^{-13} \text{ kg m Pa}^{-1} \text{ s}^{-1} \text{ m}^{-2}$) (Rhim et al., 2006). With regard to synthetic polymers, multilayer film had higher WVP values compared to those of high-density polyethylene (HDPE) ($2.4 \times 10^{-16} \text{ kg m Pa}^{-1} \text{ s}^{-1} \text{ m}^{-2}$), polyvinyl chloride (PVC) ($0.7\text{--}2.4 \times 10^{-16} \text{ kg m Pa}^{-1} \text{ s}^{-1} \text{ m}^{-2}$) and low density polyethylene (LDPE) ($3.6\text{--}9.7 \times 10^{-16} \text{ kg m Pa}^{-1} \text{ s}^{-1} \text{ m}^{-2}$) (Shellhammer and Krochta, 1997; Rhim et al., 2006). As expected, gelatin multilayer film showed significantly higher WVP value than LDPE. However, the association of modified-gelatin layers in a multilayer film have potential in reducing the water sensitivity of gelatin-based films.

The $Q(\text{cm}^3(\text{O}_2) \text{ mm day}^{-1} \text{ m}^{-2})$ values in the steady state are reported in Table 3. The increased Q values observed for Ge-10DAS and Ge-30Gly films was related to higher moisture absorption capacity of such films (Fig. 3). It is well reported that protein film as well as other biopolymer films are good oxygen barriers at low RH conditions, their OP values increase exponentially with RH increases above intermediate RH (Lieberman and Gilbert, 1973). At 65% RH, water molecules can act as plasticizer increasing the free volume of the protein network, thereby increasing polymer mobility and permeability (McHugh and Krochta, 1994). No significant differences ($P > 0.05$) were found between Q values of the multilayer and that of Ge-5MMt film. It is well known that compounding with clay enhance barrier effect because the hydrophobic oxygen molecules must transverse a tortuous path due to the presence of silicate particles distributed in the polymeric matrix (Ray and Bousmina, 2005). Hence the oxygen barrier of the multilayer was attributed to the Ge-5MMt inner layer. For comparing purposes, Q value of polyethylene terephthalate (PET), LDPE, cellulose acetate (CA) and PLA films tested with the same device in similar conditions were obtained. Q results were $2.5 \text{ cm}^3(\text{O}_2) \text{ mm day}^{-1} \text{ m}^{-2}$ for PET, $160 \text{ cm}^3(\text{O}_2) \text{ mm day}^{-1} \text{ m}^{-2}$ for LDPE, $44 \text{ cm}^3(\text{O}_2) \text{ mm day}^{-1} \text{ m}^{-2}$ for CA (Valente et al., 2007) and $29.5 \text{ cm}^3(\text{O}_2) \text{ mm day}^{-1} \text{ m}^{-2}$ for neat PLA (Martino et al., 2009). The Q values of multilayer films were lower than those obtained for LDPE, CA and PLA, thereby this films could be used in packaging formulation with reduce oxygen permeation requirements.

4. Conclusions

Three-layer films containing bovine gelatin as the main component were produced by compression molding of the single Ge-5MMt and Ge-10DAS layers under mild conditions. Lamination improved TSM and moisture resistance due to the presence of strong interaction between layers. Tensile modulus and strength values were intermediate than those of the individual components but higher enough for the intended application. Lamination resulted in desirable barrier properties with WVP values comparable to those reported for cellulose acetate and PLA and lower oxygen transmission rate values than those obtained for PLA and CA measured by the same method and for similar thicknesses. Results reported herein indicate that the association of gelatin films modified with environmentally safe reagents such as DAS, glycerol and MMT, can provide a new multilayer material with modulated properties and the added advantage of being biodegradable under soil burial condition (Martucci and Ruseckaite, 2009b).

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