

Three-Layer Sheets Based on Gelatin and Poly(lactic acid), Part 1: Preparation and Properties

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ABSTRACT: Glycerol-plasticized gelatin (Ge-30Gly) and poly(lactic acid) (PLA) films were prepared by heat-compression, molded, and then piled to produce a biodegradable three-layer sheet with PLA as outer layers and Ge-30Gly as the inner layer. Lamination with PLA increased the moisture resistance and reduced the total soluble matter with respect to a single gelatin layer, while keeping transparency. The tensile strength of the multilayer sheet (36.24 ± 4.27 MPa) increased 16 folds when compared to that of Ge-30Gly. Lamination also exerted beneficial effects on the barrier properties. The WVP of the multilayer sheet ($1.2 \pm 0.1 \cdot 10^{-14}$ kg·m·Pa⁻¹·s⁻¹·m⁻²) decreased with reference to that of Ge-30Gly, while oxygen permeability (17.1 ± 2.3 cm³(O₂)·mm·m⁻²·day⁻¹) was reduced with respect to that of neat PLA, and the value obtained was compara-

ble to that of Ge-30Gly layer. The presence of plasticized gelatin in the multilayer increased the energy at crack initiation (1.4 ± 0.3 J·m⁻¹) with respect to that of PLA. The improvement attained in all these properties was ascribed to the good compatibility and adhesion of the individual layers featured through hydrogen interactions between the carbonyl group from PLA and the hydrogen from the peptide bonds in gelatin. Compatibility was corroborated by scanning electron microscopy observations at PLA/Ge-30Gly interface and by the absence of additional peaks in the tan δ curve of dynamic-mechanical analysis. © 2010 Wiley Periodicals, Inc. *J Appl Polym Sci* 118: 3102–3110, 2010

Key words: biopolymers; proteins; multilayer; barrier; mechanical properties

INTRODUCTION

The production and consumption of polymeric materials for packaging applications are subjected to all the constraints and regulations concerning primary and postconsumer plastic-waste management. This has encouraged the search for environmentally sound materials based on naturally occurring biodegradable polymers, such as carbohydrates, lipids, and proteins, which unless heavily modified are biodegradable and compostable, thus promoting an environmentally friendly waste management system.¹

Gelatin is an animal protein attainable by the controlled hydrolysis of the fibrous insoluble collagen present in the bones and skin generated as waste during animal slaughtering and processing.² It is abundant, economic, safe, and renewable; and exhib-

its proper film forming properties for biopackaging materials production.³ Gelatin-based films constitute very efficient barriers to oxygen and aromas at low and intermediate relative humidity (RH). Yet, due to their hydrophilic features, they are not such good barriers to water vapor.^{2,4–11} As a consequence, several strategies have been proposed to improve the moisture resistance of gelatin-based films including chemical, physical, and enzymatic crosslinking^{6–8,12–15} and compositing with other moisture resistant polymers and biopolymers.^{9,16–20} Another way of overcoming such drawback is by creating multilayer structures. Lamination is used to improve polymeric films performance by combining the properties of several types of films into one sheet.^{21,22} In general terms, the outer layers impart moisture resistance and mechanical stability, while the inner one acts as a gas barrier in the multilayer.²³ To such an end, one of the most promising biopolymer is the poly(lactic acid) (PLA) obtained from sugar feedstock, corn, etc., which constitute renewable and readily biodegradable resources.^{24–26} This thermoplastic aliphatic polyester features high strength, high modulus, and good processability. Besides, it is completely biodegradable. PLA has been studied not only for medical purposes but also for food packaging applications, being classified as GRAS (Generally Recognized As Safe, GRAS).²⁷ Currently scant

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information is available about biodegradable multilayer films based on proteins and biodegradable polymers in the literature. Rhim et al. (2006, 2007)^{21,28} reported the preparation of multilayer films based on PLA and plasticized soybean protein isolate (SPI) films. The multilayer provided desirable barrier properties and improved the mechanical properties when compared to those of SPI. However, the individual components and the multilayer film were produced with a time-consuming solvent-casting technique, given the difficulty entailed in controlling some parameters, such as film thickness.²⁹ The development of multilayer films using thermal formation of protein-based materials by means of techniques generally implemented with synthetic thermoplastic polymers (extrusion, injection, etc.) is the next step in an attempt to develop marketable packaging films and sheets. Generally speaking, sheets compression molding has been studied as a precursor to extrusion, to demonstrate material flowability and fusion and identify conditions suitable for extrusion.²⁹ Gelatin can be thermoplastically processed under the plasticization of small molecules.^{6,30,31} In a previous work by these authors, biodegradable three-layer films were developed based on modified-bovine gelatin produced by compression molding with improved barrier properties.^{15,32} The outer layers were based on plasticized gelatin crosslinked with dialdehyde starch (DAS),¹⁴ while the inner gas barrier layer was a bionanocomposite film based on plasticized gelatin and 5%w/w of sodium montmorillonite. However, the tensile strength of such multilayer films was considered unsuitable for practical applications.

In view of the fact that PLA combined with a plasticized gelatin film into a multilayer structure can be beneficial for both materials, the aim of our research work was to determine the effect that laminating glycerol-plasticized gelatin films (inner layer) with PLA (outer layers) by compression molding exerted on the moisture resistance, mechanical properties (tensile, dynamic-mechanical and impact), and barrier properties (water vapor and oxygen) of three-layer sheets.

MATERIALS AND METHODS

Materials

Bovine hide gelatin (Ge) type B was kindly supplied by Rousselot (Argentina); Bloom 150, isoionic point (Ip) 5.3. Poly (lactic acid) (CML PLA) was purchased from Hycail Finland Oy (Turku, Finland) and used as received. Glycerol analytical grade (Gly, 98%) was supplied by DEM Chemicals (Mar del Plata, Argentina).

Preparation of laminated sheets

Multilayer sheets were prepared by combining PLA films as the outer layers and plasticized – gelatin film as the inner layer through a two-step process. The first step comprised the separate production of the individual layers by heat - compression molding. Additionally, the layers were stacked and heat-compressed into sheets.

PLA pellets were oven dried under vacuum at 60°C for 4 h. PLA (4 g) was processed by compression molding at 180°C in a hot press (E.M.S., Buenos Aires, Argentina). An aluminum frame was placed between the two plates to mark the edges of the film and control thickness. The material was kept between the plates at atmospheric pressure for 5 min and then successively pressed under 3 MPa for 1 min, 5 MPa for 1 min, and 10 MPa for 3 min.²⁷ After cooling to room temperature under pressure (10 MPa), the PLA film was removed from the mould and stored in a dessicator prior to use.

The plasticized gelatin film was prepared in agreement with the method above described.¹⁴ Gelatin powder was mixed with 30% w/w glycerol (gelatin dry basis) using a kitchen mixer (M.B.Z., San Justo, Buenos Aires, Argentina) at low speed (150 rpm) for 24 h and at ambient temperature. The blended Ge-30Gly was transferred to a stainless steel mould (30 cm × 30 cm) and kept between plates at atmospheric pressure and at 120°C for 5 min. Pressure was raised to 50 kg/cm² for 10 min to obtain homogeneous sheets. Samples were kept between the plates at atmospheric pressure and water cooled to room temperature.

The multilayer sheet was prepared by stacking an outer PLA layer, a Ge-30Gly inner layer and a second outer PLA layer subjected to thermomolding at 100°C and 50 kg·cm⁻² for 10 min with a further cooling step at room temperature and atmospheric pressure. The obtained specimens were stored under controlled temperature and humidity conditions prior to further testing (65 ± 2% HR; 25 ± 2°C).

Average film and sheets thickness

Average thickness was measured with a digital 10-micron resolution micrometer (Vernier, China). Overall thickness was expressed as the average of five measurements randomly taken around the individual and multilayer sheet testing areas. The obtained data were used to assess opacity, barrier, and mechanical properties.

Opacity

Opacity was determined following the procedure described by Gontard and Cuq (1992).³³ Samples were cut into rectangles (3 × 1 cm) and placed on

the internal side of a spectrophotometer cell. The absorbance spectrum (400–800 nm) was recorded for each specimen using a UV-Visible spectrophotometer Shimadzu 1601 PC (Tokyo, Japan). Opacity was defined as the area under the recorded curve and expressed as absorbance units (nanometers) per thickness unit (mm) ($\text{AU} \cdot \text{m} \cdot \text{mm}^{-1}$). Thickness values were reported as the average of five measurements for each material.

Scanning electron microscopy

The cross-sectional morphology of cryo-fractured samples of individual films and laminate sheets were observed with a scanning electron microscope (SEM) (JEOL, model JSM-6460 LV) with an operating voltage of 15 KV. All specimens were previously sputter-coated with gold.

Total soluble matter

Total soluble matter (TSM) was determined as the percentage of sample dry matter solubilized after 24 h immersion in distilled water.^{34,35} Samples (2 cm × 2 cm) from each type of film were weighed and immersed in a 50 mL beaker containing 30 mL of distilled water with sodium azide (0.02%) to prevent microbial growth. After 24 h storage in an environmental chamber at 25°C with occasional agitation, the specimens were recovered, gently rinsed with distilled water and then oven dried at 105 for 24 h so as to establish the weight of dry matter not dissolved in water. TSM was determined as the weight difference between the initial dry matter and the undissolved dry matter after 24 h immersion; and was expressed as a percentage of the initial dry matter. Proteins are susceptible to heat-induced crosslinking^{6,35}; therefore, to avoid heat curing, film specimens were immersed without previous drying at 105°C. The initial dry matter of conditioned films, in turn, was determined on different specimens (three from each film) by drying in an air-circulating oven (105°C for 24 h).

Moisture absorption at 65% relative humidity

Specimens were preconditioned in an oven at ambient temperature before testing. The exact weight (m_0) of the predried film samples was conditioned in an environmental chamber at a relative humidity (RH) of $65 \pm 2\%$ and at 25°C. Samples were removed at specific intervals (t) and reweighed (m_t). The moisture content (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:

$$w_t = \frac{(m_0 - m_t) * 100\%}{m_t}, \quad (1)$$

where w_t is the moisture content as a function of time (%), m_t is the sample weight after being exposed, and m_0 is the initial sample weight. The moisture absorption was reported as the average of five replicates.

Moisture absorption data were correlated with the empirical equation by Peleg,³⁶ which has proven its potential in the prediction of the sorption process of many food systems, such as milk powders, rice and sago starches,³⁷ and edible polymeric films.³⁸ This expression relates the instantaneous moisture content (w_t) to the initial moisture content (w_0), as shown in the equation:

$$w_t = w_0 + \frac{t}{k_1 + k_2 * t}, \quad (2)$$

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 (min/(% w/w water/% w/w solids)) is related to mass transfer. The lower the k_1 , the higher the initial water absorption rate. k_2 (1/% w/w water/% w/w solid) is associated to the maximum water absorption capacity, therefore the lower the k_2 , the higher the absorption capacity.^{36,37}

Barrier properties

Water vapor permeability (WVP)

The films WVP ($\text{Kg} \cdot \text{m} \cdot \text{Pa}^{-1} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$) was estimated as:

$$\text{WVP} = \frac{(\text{WVT} * e)}{\Delta P}, \quad (3)$$

where WVT ($\text{Kg} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$) is the vapor transmission rate through the film thickness e (m) and ΔP is the partial water vapor pressure difference (Pa) across the two sides of the film specimens. The films WVP was determined gravimetrically in line with the modified ASTM method E96–95 (ASTM, 1995). Film-covered cups were placed in an environmental chamber at 25°C and $65 \pm 2\%$ HR, and their weight change (± 0.0001 g) versus time was recorded at specific intervals (t) and then plotted. Linear regression was used to calculate the slope of a fitted straight line, which represented WVT, as follows:

$$\text{WVT} = \frac{\Delta m}{t * A}, \quad (4)$$

where Δm is the mass change of the cell test (Kg), t is the time (s) and A is the test area (0.028 m^2). Permeability was calculated according to:

$$\text{WVP}(\text{Kg} \cdot \text{m} \cdot \text{s}^{-1} \cdot \text{Pa}^{-1} \cdot \text{m}^{-2}) = \frac{\text{WVT}}{S * (\text{RH}_1 - \text{RH}_2)} * e, \quad (5)$$

where e is the film thickness (m), S is the saturation pressure (Pa) at the test temperature, RH_1 is the relative humidity in the test and RH_2 is the relative humidity inside the cell test. At least five repetitions were conducted per experiment.

Oxygen permeability

Oxygen Permeability (OP) was measured with an Oxygen Permeation Analyzer from Systech Instruments, model 8500 (Metrotec S.A, Spain) on circular samples (14 cm diameter) conditioned at $50 \pm 3\%$ RH before testing. The films were mounted between the upper lid and rubber ring with silicon lubricant and fixed to the diffusion chamber. 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. All experiments were conducted under the same conditions: pressure of 2.5 atm and at 25°C. The gas volumetric flow rate per unit area of the membrane OT ($\text{cm}^3(\text{O}_2) \cdot \text{day}^{-1} \cdot \text{m}^{-2}$) was continuously monitored until a steady state was reached (OT_∞). The permeability coefficient OP was determined by Eq.

$$OP(\text{cm}^3(\text{O}_2) \cdot \text{mm} \cdot \text{Pa}^{-1} \cdot \text{day}^{-1} \cdot \text{m}^{-2}) = \frac{OT_\infty \cdot e}{\Delta P} = \frac{Q}{\Delta P}, \quad (6)$$

where e is the specimen thickness (mm) and ΔP is the partial pressure gradient across the polymer film (Pa). For a constant ΔP throughout all experiments (2.5 atm), OP is proportional to $OT_\infty \cdot e$ ($\text{cm}^3(\text{O}_2) \cdot \text{mm} \cdot \text{day}^{-1} \cdot \text{m}^{-2}$).

Dynamic-mechanical analysis

The dynamic-mechanical analysis was conducted using a Perkin- Elmer dynamic-mechanical analyzer (DMA 7-e) under dynamic mode at 5°C/min under N_2 atmosphere. The experiments were performed under tensile mode with a specimen dimension of 15 mm \times 0.5 mm \times 0.4 mm. All the samples were preconditioned at $65 \pm 2\%$ HR before testing. Results were the average of three replicates.

Mechanical properties

Tensile tests

The film Young's modulus (E), tensile strength (TS), and elongation at break (%EB) were assessed in accordance with the ASTM D638-94b standard method (ASTM, 1995) using a Instron Universal Testing Machine (Instron, Darmstadt, Germany) equipped with a 0.5 KN cell and at a crosshead speed of

TABLE I
Opacity and Mean Thickness of Individual Films and Multilayer Sheet

Sample name	Opacity ($\text{UA} \cdot \text{nm} \cdot \text{mm}^{-1}$)	Thickness (mm)
Ge-30Gly	489.7 ± 53.7^a	0.428 ± 0.018^a
PLA/Ge-30Gly/PLA	343.5 ± 57.3^b	0.465 ± 0.046^a
PLA	257.9 ± 13.1^b	0.416 ± 0.028^a

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

3 mm/min. Dog-bone-shaped specimens of each film (30 mm \times 4.5 mm \times 0.2 mm) were conditioned at 25°C and $65 \pm 2\%$ HR before testing. Seven specimens of each film were analyzed.

Fracture puncture tests

Instrumented Falling Weight Impact (IFWI) tests were performed using a falling weight impact tower (Fractovis, Ceast SRL, Illinois). Samples with diameters of 14 cm, clamped on a supporting ring of 75 mm diameter, were impacted with a hemispherical tipped dart. The force-deformation curves were recorded during 80 msec. At least five specimens were impacted for each material. From the IFWI fractograms the energy at crack initiation (E_i) normalized by thickness was determined as follow:

$$E_i = \frac{1}{e} \int_0^{X_{\max}} F dx, \quad (7)$$

where e is the sample thickness (m) and X is the strain reached during the deformation of the material up to rupture.

Statistical analysis

Data values obtained in the experiments were statistically analyzed by one-way analysis of variance (ANOVA). Differences between pairs of means were assessed on the basis of confidence intervals using the Tukey test. The level of significance was $P < 0.05$.

RESULTS AND DISCUSSION

Macroscopic and microscopic aspect of the three-layer sheet

The multilayer sheet exhibited good handling properties and homogeneity, with neither bubbles nor cracks. In addition, the upper and lower surfaces were smooth for being in contact with the mold anti-adherent surface. All the assayed samples were visually homogeneous and transparent. Table I shows

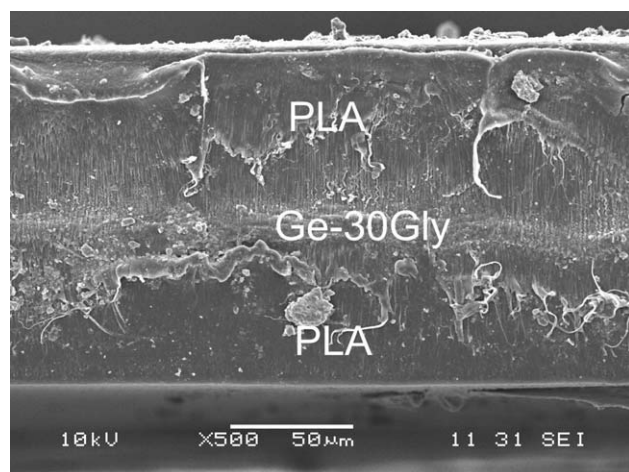


Figure 1 Cross section micrographs of three-layer sheet. Magnification: x500.

that the Ge-30Gly film accounted for the highest opacity value ($489.7 \pm 53.7 \text{ UA}\cdot\text{nm}\cdot\text{mm}^{-1}$) with respect to the values reported by Rivero et al. (2010)³⁹ for plasticized gelatin films. The opacity value of the multilayer sheet did not differ extensively ($P > 0.05$) from that obtained from the neat PLA film. Given the fact that no significant differences ($P > 0.05$) were reported in thickness values, the reduction in opacity of Ge-30Gly by PLA lamination could be indicating a likely compatibility between layers.^{13,21} Indeed, if interfaces between Ge-30Gly and PLA exist, when light reaches these interfaces, light scattering occurs and transmission is reduced, thereby increasing the sample opacity. SEM observations of the cryo-fractured surface of the compression molded multilayer sheet revealed that the outer PLA layers were tightly bonded to the inner Ge-30Gly layer, yielding a relatively smooth and compact morphology with no clear interfaces between layers (Fig. 1). Moreover, manual peeling of PLA layers from the inner Ge-30Gly was not possible. Therefore it was postulated that thermal compression molding favored the bond strength between layers by means of hydrogen interaction through the carbonyl groups from PLA and hydrogen from peptide bonds in gelatin or hydroxyl groups in glycerol.

The existence of such interactions has already been suggested for multilayer films, based on SPI and PLA produced by casting.²¹

Total soluble matter and moisture absorption at 65% RH

The effect of laminating Ge-30Gly with PLA on moisture resistance was evaluated by total soluble matter (TSM) and moisture absorption at 65%RH. TSM results for PLA/Ge-30Gly/PLA sheet and parent films are summarized in Table II. The TSM value of Ge-30Gly was significantly higher ($P < 0.05$) than those obtained for PLA and multilayer sheets due to the inherent hydrophilic features of plasticized gelatin films.^{5-7,15,40} The low TSM value of the multilayer sheet (about $8.99 \pm 1.53\%$) was ascribed to the solubilization of the Ge-30Gly inner layer and to the hydrophobic nature of PLA. This could be attributed to the penetration of water molecules into the interfacial zone. When water molecules reach the inner and more hydrophilic gelatin layer through the unprotected edges of the sample, gelatin swells and eventually dissolves conducting to the separation (delamination) of the external PLA layers.

Figure 2 illustrates the water absorption curves of individual and multilayer sheets at 23°C and $65 \pm 2\%$ HR. As expected, the Ge-30Gly film absorbed moisture rapidly up to reaching an apparent saturation value of $10.9 \pm 0.2\%$. Conversely, neat PLA yielded the lowest equilibrium saturation value (EMC%), i.e., $0.3 \pm 0.1\%$ in agreement with its hydrophobic nature.⁴¹⁻⁴⁵ The equilibrium saturation value of Ge-30Gly decreased to $3.7 \pm 0.2\%$ by laminating with PLA, thereby demonstrating that lamination was effective in restricting gelatin water absorption (Table II). Experimental data were fitted by using Peleg's equation.³⁶ Table II summarizes the fitting parameters and the correlation coefficients (R^2). It should be noted that the absorption curve slope of the laminate in Figure 2 changed after 740 min due to delamination. Therefore the ultimate value of water absorption used in the fitting process was taken at 740 min. Figure 2 indicates that Peleg's empirical equation fits well in terms of experimental

TABLE II
Total Soluble Matter (TSM), Equilibrium Moisture Content (EMC%) and Peleg Parameters (k_1 , k_2) of Control and Multilayer Sheet

Sample name	TSM (%) 24 h	EMC (%)	Peleg Parameters		
			k_1 ($\text{min}\%^{-1}$)	$k_2 \times 10^2$ ($\%^{-1}$)	R^2
Ge-30Gly	100.0 ± 0.0^a	10.9 ± 0.2^a	5.8 ± 0.7^a	8.4 ± 0.2^a	0.99
PLA/Ge-30Gly/PLA	8.99 ± 1.53^b	3.7 ± 0.2^b	99.7 ± 16.4^b	63.4 ± 6.3^b	0.98
PLA	0.02 ± 0.04^c	0.3 ± 0.1^c	141.2 ± 21.9^c	494.2 ± 16.6^c	0.99

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

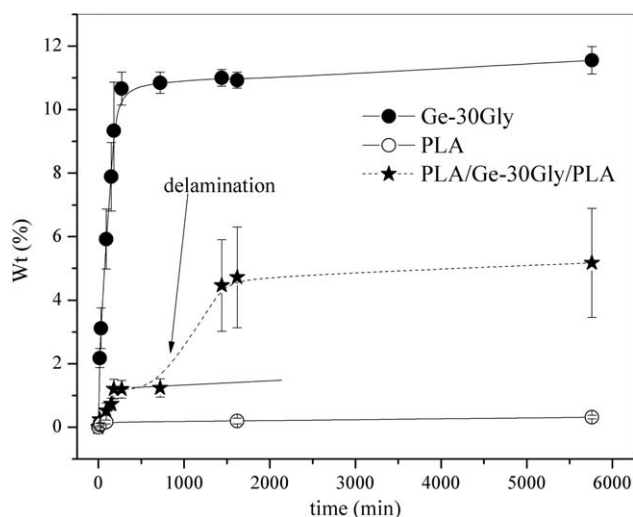


Figure 2 Moisture absorption curves at 65%RH (●) Ge-30Gly, (○) PLA and, (*) PLA/Ge-30Gly/PLA. The solid curves are the result of the fitting process with Peleg equation.

data, as noticed from the coefficient of determination, R^2 values (Table II). The estimated k_1 value was lower for Ge-30Gly film and higher for the neat PLA film, when compared to the corresponding value for the three-layer sheet, indicating that the moisture absorption of the multilayer was at a lower initial rate than that of neat Ge-30Gly. It is postulated that PLA outer layers may have contributed to slow down water uptake by sealing off pores on gelatin film surface during processing.^{21,23} Moreover, the low k_2 value of the Ge-30Gly clearly indicates that this sample possesses the highest final absorption capacity, thus being the most hydrophilic film, as previously concluded from the TSM tests. The intermediate k_2 value of the laminate suggested that, despite some degree of delamination (particularly on the edges of the sample) the constituent layers were still bonded together.

Water vapor and oxygen barrier properties

Table III shows the experimental WVP values measured at $65 \pm 2\%$ HR. It is well known that the WVP of polysaccharides and proteins usually shows thickness effect, that is, WVP increases when film thickness does.^{46,47} To restrict such an increase, mean thickness values of the tested specimens were about 0.4 mm without significant differences ($P > 0.05$). The WVP value of Ge-30Gly film was high ($13.6 \pm 3.9 \cdot 10^{-14} \text{ kg}\cdot\text{m}\cdot\text{s}^{-1}\cdot\text{Pa}^{-1}\cdot\text{m}^{-2}$) due to the hydrophilic nature of gelatin; and was comparable to that reported in the literature for gelatin films added hydrophilic plasticizers.¹⁴ Both the PLA neat film and the laminate yielded similar ($P > 0.05$) WVP values, being 10-times lower than those of Ge-30Gly. These results reveal that Ge-30Gly resistance to

water vapor is negligible with respect to PLA. The permeability value of the multilayer was comparable to that reported for cellulose acetate ($0.5\text{--}1.6 \cdot 10^{-14} \text{ kg}\cdot\text{m}\cdot\text{s}^{-1}\cdot\text{Pa}^{-1}\cdot\text{m}^{-2}$).²¹ As far as synthetic polymers are concerned, multilayer films accounted for higher WVP values compared to those of high-density polyethylene (HDPE) ($2.4 \cdot 10^{-16} \text{ kg}\cdot\text{m}\cdot\text{s}^{-1}\cdot\text{Pa}^{-1}\cdot\text{m}^{-2}$), polyvinyl chloride (PVC) ($0.7\text{--}2.4 \cdot 10^{-16} \text{ kg}\cdot\text{m}\cdot\text{s}^{-1}\cdot\text{Pa}^{-1}\cdot\text{m}^{-2}$),⁴⁸ and low density polyethylene (LDPE) ($3.6\text{--}9.7 \cdot 10^{-16} \text{ kg}\cdot\text{m}\cdot\text{s}^{-1}\cdot\text{Pa}^{-1}\cdot\text{m}^{-2}$).^{21,48}

The oxygen barrier was quantified by the oxygen transmission rate until a steady state (OT_{∞}) per thickness (e) was attained, which is proportional to the oxygen permeability coefficients (OP). So, when the $OT_{\infty}\cdot e$ of a polymer film packaging is low, the oxygen pressure inside the container drops to the point in which oxidation is retarded, thereby prolonging the product shelf life.⁴⁵ The final $OT_{\infty}\cdot e$ values for multilayer and its single components are summarized in Table III. As observed, lamination significantly reduced the $OT_{\infty}\cdot e$ value of PLA. This could be explained by the oxygen barrier role played by the inner Ge-30Gly film.^{14,15,23,28} For comparison purposes, tests with similar samples of LDPE and polyethylene terephthalate (PET) were also carried out. The $OT_{\infty}\cdot e$ results corresponding to the laminate sheet were still lower than those derived from LDPE under similar conditions ($OT_{\infty}\cdot e = 160 \text{ cm}^3\cdot\text{mm}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$). As this material is currently used in films manufacturing, the use of multilayer sheets could be suitable for food packaging with reduced oxygen permeation. On the other hand, PET film yielded $OT_{\infty}\cdot e$ values as low as $3 \text{ cm}^3\cdot\text{mm}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$, which are considerably low for this material. This was expected since the oxygen barrier properties of PET have been well documented and reported as better than those of pure and plasticized gelatin films.^{27,43,44}

Dynamic-mechanical properties

Figure 3 depicts the temperature dependence of the storage modulus (E') and loss factor ($\tan \delta$) for the

TABLE III
Water Vapor Permeability (WVP) and Oxygen Permeability ($OT_{\infty}\cdot e$) of Ge-30Gly, PLA, and Multilayer Sheet

Sample name	Barrier properties	
	$OT_{\infty}\cdot e$ ($\text{cm}^3\cdot\text{mm}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$)	WVP $\times 10^{14}$ ($\text{kg}\cdot\text{m}\cdot\text{Pa}^{-1}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$)
Ge-30Gly	13.3 ± 3.0^a	13.6 ± 3.9^a
PLA/Ge-30Gly/PLA	17.1 ± 2.3^a	1.2 ± 0.1^b
PLA	29.5 ± 6.4^b	1.3 ± 0.1^b

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

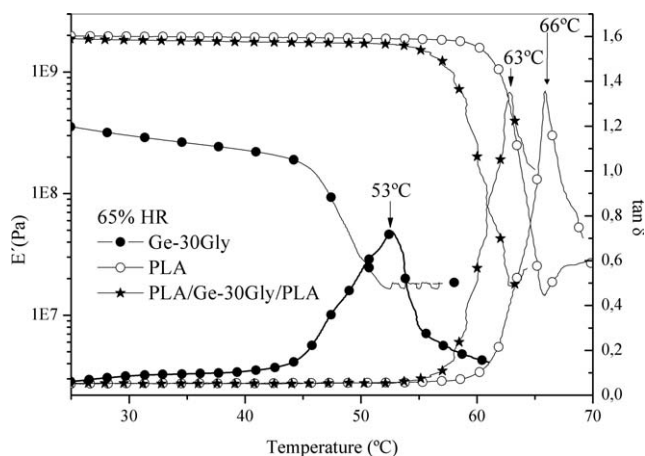


Figure 3 Dynamic mechanical analysis of (●) Ge-30Gly, (○) PLA, and (*) PLA/Ge-30Gly/PLA.

individual components and the three-layer sheet specimens stabilized at $65 \pm 2\%$ HR. The behavior displayed by the Ge-30Gly film was that of an amorphous polymer, considering that the decrease in the storage modulus and the $\tan \delta$ correspond to a typical glassy to rubber transition. The drop in E' with temperature is usually reported for proteinaceous materials and can be related to physical or chemical crosslinks inside the matrix.^{6,33} On the other hand, the E' value was significantly increased with PLA ($P > 0.05$) at 30°C , about from 0.3 GPa for Ge-30Gly to 1.83 GPa for the multilayer sheet. These results indicate that the storage modulus was governed by PLA sheets, since its behavior bore strong resemblance to that of neat PLA (Fig. 3).

With regard to proteins and agropolymers, the glass transition temperature (T_g) is large while that of heat capacity change is small; DMA measurements being more appropriate than differential scanning calorimetry.³³ The broadening in the $\tan \delta$ peak of Ge-30Gly when compared to that of PLA is explained by the wide distribution of protein molar masses.⁴⁹ The T_g values occurred at 53, 66, and 63°C for Ge-30Gly, neat PLA and PLA/Ge-30Gly/PLA, respectively. It is worth mentioning that no additional peaks different from those assigned to T_g were observed in the $\tan \delta$ curve of the multilayer sheet (at least within the temperature range ana-

lyzed), thereby providing indirect evidence for the compatibility between the individual layers.^{15,21} As a result, lamination increased the T_g of Ge-30Gly individual layer and enhanced its modulus at ambient temperature.

Tensile properties

Tensile properties were measured to assess the differences in ductile behavior between individual components and the multilayer sheet. The results are also summarized in Table IV. The tensile strength (TS), elongation at break (EB) and Young's modulus (E) values of Ge-30Gly film were 2.31 ± 0.59 MPa, $124.0 \pm 26.8\%$ and 7.39 ± 1.84 MPa, respectively. The low mechanical strength of plasticized gelatin films is one of the reasons that prevents their use in food packaging or related applications.⁶ On the other hand, amorphous neat PLA behaved as a brittle material with a high modulus (around 2.0 GPa) and small deformation at break (about 9%).^{27,28} The combination of such films into a three-layer sheet increased E and TS and drastically reduced EB ($P < 0.05$) with respect to the values obtained for Ge-30Gly. The fact that the mechanical response of the multilayer sheet did not answer to a simple mixing rule is also worthy of mention. Thus any deviation could be assigned to interactions between co-components, i.e., hydrogen interactions established at Ge-30Gly/PLA interface. The low EB of the multilayer could be enhanced by plasticizing PLA with polyadipates.²⁷ Other studies are currently under way to evaluate the final properties of multilayer films and sheets using PLA plasticized with 15–20% of polyadipates.

Puncture fracture test

Impact properties of PLA improved owing to the presence of the plasticized gelatin layer (Fig. 4). The maximum load was almost the same but the curve slope after the load reached its maximum became gentle, indicating that crack growth behavior becomes relatively ductile. This was evidenced by the increase of E_i value (from 0.3 ± 0.0 J·m⁻¹ for

TABLE IV
Tensile Properties of Ge-30Gly Film, PLA Film, and PLA/Ge-30Gly/PLA Sheet

Sample name	Tensile properties		
	E (MPa)	EB (%)	TS (MPa)
Ge-30Gly	7.39 ± 1.84^a	123.98 ± 26.80^a	2.31 ± 0.59^a
PLA/Ge-30Gly/PLA	1583.3 ± 189.4^b	7.76 ± 0.75^b	36.24 ± 4.27^b
PLA	2073.5 ± 174.2^c	8.57 ± 0.95^b	45.01 ± 1.43^c

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

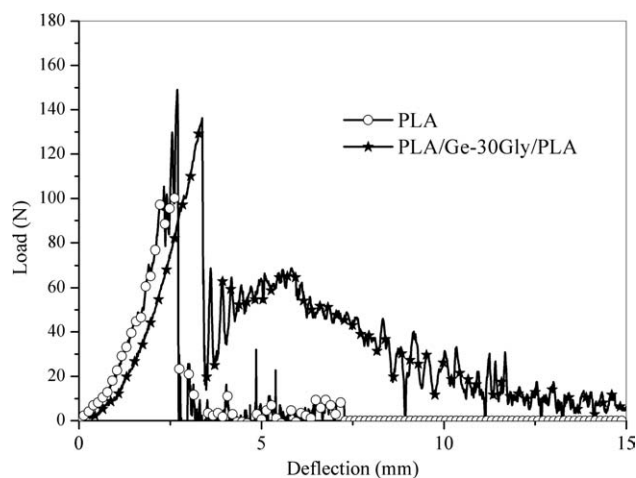


Figure 4 Impact behavior of (○) PLA films and (*) PLA/Ge-30Gly/PLA sheet.

PLA to $1.4 \pm 0.3 \text{ J}\cdot\text{m}^{-1}$ for multilayer sheet). The sudden drop of the load obtained for PLA suggests that crack growth behavior is brittle and that the material suffers a catastrophic failure preventable by the presence of the plasticized gelatin layer. Also worthy of mention is the fact that the initial curve slope becomes gentle due to the gelatin layer, corresponding to the specimen stiffness reduction.

CONCLUSION

A three-layer sheet with PLA as outer layers and glycerol-plasticized gelatin as the inner layer was produced by thermal compression molding without adding compatibilizing agents, adhesives, or chemically modifying the film surfaces. The systematic study of moisture resistance, mechanical (tensile and fracture puncture tests), dynamic-mechanical and barrier properties together with the morphological observations allows to suggest that the existence of hydrogen interactions between gelatin and PLA, particularly at the interface, accounts for the improved properties of the multilayer sheet. The synergetic effect of lamination was evidenced by the increased tensile strength (16-folds) and Young's modulus (214-folds). Lamination also had a beneficial effect on the barrier properties. The WVP of the multilayer sheet ($1.2 \pm 0.1 \cdot 10^{-14} \text{ kg}\cdot\text{m}\cdot\text{Pa}^{-1}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$) decreased when compared to that of Ge-30Gly, while the oxygen permeability ($17.1 \pm 2.3 \text{ cm}^3(\text{O}_2)\cdot\text{mm}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$) was reduced with respect to that of neat PLA and was comparable to the value of Ge-30Gly layer. All these results indicate that the combination of PLA's mechanical strength and hydrophobicity with the gas barrier properties of plasticized gelatin film could lead to a multilayer sheet with suitable packaging properties.

Since both materials are environmentally sound, the three-layer sheet could be anticipated to be biodegradable. Extensive research efforts should be conducted to investigate the biodegradability and ecotoxicity of such multilayer sheet in biotic natural environment.

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