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Optical and mechanical properties of bi-layer biodegradable films from poly lactic acid and bovine gelatin

Fatma Tuba Pirinc[1], Adnan Fatih Dagdelen[1], Furkan Turker Saricaoglu[*1]

[1] Department of Food Engineering, Faculty of Engineering and Natural Sciences, Bursa Technical University, Bursa, Turkey

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ABSTRACT

In this study, the development of bilayer films with bovine gelatin (BG) and poly lactic acid (PLA) was investigated, and the effect of gelatin layer thickness on optical and mechanical properties of bi-layer films was evaluated. BG incorporated PLA films didn't separate from each other during mechanical analyses, which means uniform film structure was obtained. The *L** values significantly decreased, whereas *a** and *b** values increased when compared with neat PLA film. The color differences of bi-layer films were classified between "very distinct and great" depending on the thickness of BG layer. The lowest opacity was obtained from neat PLA film, and increasing thickness of BG layer increased opacity due to yellowish color. The lamination of neat PLA with BG in varied thickness caused a significant decrease in UV and visible light transmission. Tensile strength of neat PLA film was significantly decreased by the lamination with BG, whereas elongation at break values increased, probably due to plasticizer addition to BG film. Similar tendency was observed for puncture force and puncture deformation values. It can be concluded from these results that the lamination of PLA films with BG decreased the mechanical properties, whereas improved the UV light barrier properties of neat PLA film.

1. Introduction

Packaging applications for food, medical and other industries use polymeric materials, especially petroleum-based materials due to good barrier and mechanical properties. However, constraints and regulations related with primary and post-consumer plastic waste management are restricting the utilizing of these synthetic materials, and hence, many researchers have focused on eco-friendly packaging materials from polysaccharides, proteins, lipids and/or their composites (Murrieta-Martínez, et al., 2018; Nilsuwan, Benjakul, & Prodpran, 2018). Packaging materials produced from eco-friendly substances are not widely used in the food industry due to lower mechanical and poorer gas barrier properties than synthetic packaging materials. Therefore, researchers are trying to improve these properties of eco-friendly materials with using novel technologies such as high pressure homogenization (Saricaoglu, Tural, Gul, & Turhan, 2018) and ultrasound (Gul, Saricaoglu, Besir, Atalar, & Yazici, 2018), oil incorporation (Rojas-Graü, et al., 2007) and lamination (Rakotonirainy & Padua, 2001).

Gelatin, a significant animal-based protein source found in skin, bone and connective tissue, is produced via thermal denaturation or partial hydrolysis of collagen (Lacroix & Vu, 2014). It is widely used in the production of gel desserts for the food industry, hard/soft capsules for pharmaceutical applications and edible films in packaging technology (Nilsuwan, et al., 2018). The edible films from gelatin have high tensile strength and low deformation at break, and also have low barrier properties against water vapor due to hydrophilicity, whereas gelatin films are a good barrier against oxygen and UV light (Rivero, García, & Pinotti, 2009). The improvement of mechanical and barrier properties of gelatin based films can be achieved by chemical and enzymatic cross-linking (Bigi, Cojazzi, Panzavolta, Rubini, & Roveri, 2001; de Carvalho & Grosso, 2004). The another way to improve these properties of gelatin films is the lamination with a different eco-friendly material such as oils, carbohydrates etc., which result in low water vapor permeability and good mechanical properties, respectively.

Poly lactic acid (PLA), a promising eco-friendly polymer, is obtained from sugar feedstock, corn, etc., and it is also renewable and completely bio-degradable polymer (Cabedo, Luis Feijoo, Pilar Villanueva, Lagarón, & Giménez, 2006). High strength, high modulus, good process ability and bio-degradable properties of PLA cause a promising role for packaging applications instead of petroleum based materials. Moreover, PLA is being classified as Generally Recognized as Safe (GRAS) (Martino, Jiménez, & Ruseckaite, 2009). Owing to these superior properties of PLA films, they still need to be improved in terms of optical, elongation and oxygen barrier properties, and production bi-layer film with PLA and another bio-degradable polymer, such as bovine gelatin (BG), can be useful for enhancing weak properties of PLA films. Rivero, et al. (2009) produced gelatin and chitosan bi-layer films and they reported that bi-layer films had better mechanical and barrier properties than mono-layer or composite films. It was reported in another study that the tensile strength of sugar palm starch films was increased from 7.74 MPa to 13.65 MPa by the incorporation of PLA layer, whereas elongation values were decreased (Sanyang, Sapuan, Jawaid, Ishak, & Sahari, 2016). Researchers also reported that the water vapor permeability of sugar palm starch films was decreased significantly when bi-layer films produced with PLA.

Most of the literature about bi-layer films describe the systems where a lipid forms a second layer over a polysaccharide and/or protein layer. Synthetic packaging materials are commonly produced using bi-layer film combinations to not only improve mechanical properties, but also enhance barrier properties against gaseous such as, water vapor, oxygen and ethylene. Therefore, the aims of this study are to produce bio-degradable bi-layer films from PLA and BG, and to characterize the effect of BG layer thickness on optical and mechanical properties of bi-layer films.

*Corresponding author
E-mail address: furkan.saricaoglu@btu.edu.tr

2. Materials and methods

2.1. Materials

Bovine gelatin at 250 bloom was kindly provided from Bursa Gelatin Inc. (Bursa, Turkey). Poly lactic acid neat resin (PLA, L175, low viscosity resin suitable for film extrusion) in the pellet form was purchased from a local supplier in Bursa, Turkey. Chloroform and glycerol were purchased from Merck (Darmstadt, Germany). All the chemicals were of analytical grade.

2.2. Preparation of film forming solutions

Bovine gelatin (BG) solution was prepared at 3.5% (w/v) concentration. For this purpose, 3.5 g of BG was added on 100 mL of distilled water in a glass jar, and then the jar was closed and hold 30 min for completely dissolving on a heating magnetic stirrer at 70 °C. After cooled to room temperature, glycerol at a concentration of 30% (w/w, based on BG amount) was added into solution. BG film forming solution was used as the first layer of the bi-layer film. For the preparation of poly lactic acid film forming solution, 5 g of PLA pellet was dissolved in chloroform to obtain a final concentration of 5% (w/v). The solution was stirred on a magnetic stirrer (Daihan, MSH-20D model, Seoul, South Korea) until completely dissolve and then used as the second layer of the bi-layer film.

2.3. Production of bi-layer films

Teflon pans with a diameter of 20 cm were used to produce biodegradable films from film forming solutions. Firstly, different amounts (59 (G1), 90 (G2), 121 (G3) and 153 (G4) mL) of BG film forming solutions were poured on to Teflon pans and dried in a fume hood overnight. The amount of BG film forming solutions was estimated from the plot between the volume of film forming solution and thickness of the final film. After drying the first layer, PLA solution at a fixed amount (43.5 mL) was poured on to BG film layer and held for overnight in a fume hood for drying (Nilsuwan, et al., 2018). The PLA film forming solution was used as control film for comparison. All films were peeled off and conditioned at 54% relative humidity for 3 days in a desiccator containing saturated magnesium nitrite solution.

2.4. Characterization of bi-layer films

Thickness

Film thickness was measured in 20 different locations of each film, using a digital micrometer (293-IP-54 model, Mitutoyo, Kawasaki, Japan).

Optical properties of films

Film color parameters (L^*, a^* and b^*) were measured using a colorimeter (CSM 3 model, PCE Instruments, UK). For measurements, five different locations were used and films were placed on a white standard plate (L^*=92.82, a^*= -1.24, b^*= 0.46). The total difference of color (ΔE^*) was calculated with the following equation:

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

The film opacity was calculated according to the method of Kurt and Kahyaoglu (2014) and the film opacity was calculated by:

$$Opacity = \frac{Abs_{600}}{x} \quad (2)$$

where Abs_{600} is the absorbance value at 600 nm and x is the thickness of the film.

Light transmission in ultraviolet (UV) and visible ranges of films (four samples of each treatment) was determined at selected wavelengths between 200 and 800 nm, using a UV–Visible spectrophotometer (K-Lab Inc. Optizen Pop model, South Korea).

Mechanical properties of films

Tensile strength (TS) and elongation at break (EAB) values of bi-layer films were measured with TA-HD Plus Texture Analyzer (Stable Micro Systems Co, Ltd., Godalming, UK) based on the ASTM standard method (ASTM, 2001). The films were cut in strips (2 cm width x 5 cm length) and fixed to the tensile grips of the device. The force and distance were recorded during the extension of the film strips at 2 mm/s until break. The measurements were performed for five film strips.

The puncture force (PF) and puncture distance (PD) values were measured with a film support ring equipment of texture analyzer. Film samples were cut into round shape with a watch glass at 50 mm diameter, and then mounted on to film support ring. The extension of film samples was performed by a spherical probe at a speed of 0.2 mm/s and during the test, the maximum force to rupture the film sample was recorded, as well as the maximum distance prior to rupture. Three different measurements were done for each film sample.

2.5. Statistical analyses

All experiments were run at least in triplicate with duplicated films. Data were subjected to analysis of variance (ANOVA) and mean comparisons were carried out by the Duncan's multiple range test at 95% confidence interval. Analysis was performed using the SPSS package program (SPSS for windows, SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Chemical properties

The thickness effect of the BG layer on the color and opacity values of PLA/BG bilayer films is illustrated in Table 1. Neat PLA films used as control for the comparison showed the highest L^*, the lowest a^* and b^* values, as well as lowest ΔE^* (p<0.05). As the BG thickness increased, L^* values did not significantly decrease. However, the increasing BG thickness caused a significant increase in a^* and b^* values (p<0.05). This was mainly because of the yellowish color of BG films. Similar results were also observed by Lee and Song (2017) in PLA/fish skin gelatin bilayer films, and by Nilsuwan et al. (2018) in fish gelatin/PLA bilayer films. In addition, control and PLA+G1 films showed the lowest ΔE^* values, which meant that BG layer at the lowest thickness did not significantly affect the color difference of PLA films. However, the increasing BG film thickness significantly increased the ΔE^* values of films (p<0.05), and it was not significantly increased when the PLA films coated with BG at the highest thickness levels (p>0.05). The ΔE^* values can be classified as "imperceptible (between 0 and 0.2)", "very small (between 0.2 and 0.5)", "small (between 0.5 and 1.5)", "distinct (between 1.5 and 3.0)", "very distinct (between 3 and 6)", "great (between 6 and 12)", and "a very great (>12.0)" (Silva & Silva, 1999). Based on this classification, the bilayer films from PLA and BG can be classified between "very distinct and great" color difference depending on the thickness of BG layer which caused a high difference in a^* and b^* values.

Regarding the opacity of bilayer films from PLA and BG, there was a significant difference between samples (p<0.05). The lowest opacity, which means the highest transparency, was observed from the neat PLA films. It is well known that PLA films have high transparency when compared with other biodegradable polymer films (Gupta & Kumar, 2007). The increasing BG layer thickness caused a significant increase in opacity, except for PLA+G4 sample, which decreased the opacity when compared with other BG laminated films. This could be attributed to better interactions of PLA and increased BG thickness. The increasing light transmission of bilayer film with increasing thickness might be proper application for see-through packaging material for fresh foods such as meat and fish. The transparency values of PLA and fish gelatin bilayer films were found between 0.39 and 0.56 by Nilsuwan et al. (2018), and it was also reported that the incorporation with fish gelatin layer of PLA films

Table 1. Optical properties of poly lactic acid and gelatin based bi-layer films

Films	*L**	*a**	*b**	*ΔE**	Opacity
C	95.48±1.03^{a}	-0.94±0.13^{c}	0.80±0.03^{e}	3.06±0.51^{c}	0.501±0.028^{e}
PLA+G1	93.05±0.98^{bc}	0.63±0.01^{b}	2.72±0.06^{d}	3.03±0.12^{c}	1.835±0.021^{a}
PLA+G2	88.32±2.91^{c}	0.64±0.04^{b}	3.37±0.07^{c}	4.99±0.98^{b}	0.925±0.007^{c}
PLA+G3	88.45±1.32^{c}	0.69±0.06^{b}	4.06±0.06^{b}	5.66±0.46^{ab}	1.065±0.035^{b}
PLA+G4	88.76±2.57^{c}	0.90±0.07^{a}	4.60±0.03^{a}	6.70±0.73^{a}	0.785±0.106^{d}

Values are Means ± Standard deviations. C: Control, neat PLA film; PLA: Poly lactic acid; G: Gelatin. Different subscript letters in the same column refer to significant statistical difference (p<0.05)

caused a significant decrease of transparency.

The protection of foods from light and UV radiation is an important property for packaging materials, because light and UV radiation could induce some physio-chemical changes on food products, and hence the product performance during shelf life and consumer acceptance could be negatively influenced. The UV barrier and visible light transmission results between 200 and 800 nm wavelength ranges of neat PLA and PLA/BG bilayer films are summarized in Fig. 1. It is obvious that control film showed the highest UV and visible light transmission due to low opacity values of neat PLA film (Table 1). The production of bilayer films with PLA/BG decreased the UV and visible light transmission values, however the increasing BG layer did not significantly affect the light barrier properties of bilayer films. It was reported earlier that protein films have high UV light barrier properties (Tongnuanchan, Benjakul, & Prodpran, 2013). The UV light barrier properties of protein based films are mainly dependent on the aromatic amino acid contents (Jongjareonrak, Benjakul, Visessanguan, Prodpran, & Tanaka, 2006). This is a key factor for packaging industry, because UV lights induce the lipid oxidation in foods (Wu, Sun, Guo, Ge, & Zhang, 2017). The blocking of UV light can be achieved by laminating of films and/or producing bilayer films at which the second layer has high barrier against UV light, but in these case the film transparency decreases which is a sensorial problem for consumers because they cannot see the product in the package. The increasing UV barrier properties of packaging materials are a major goal for packaging industry without increasing opacity, thickness and decreasing mechanical properties (Cárdenas, Díaz, Meléndrez, & Cruzat, 2008).

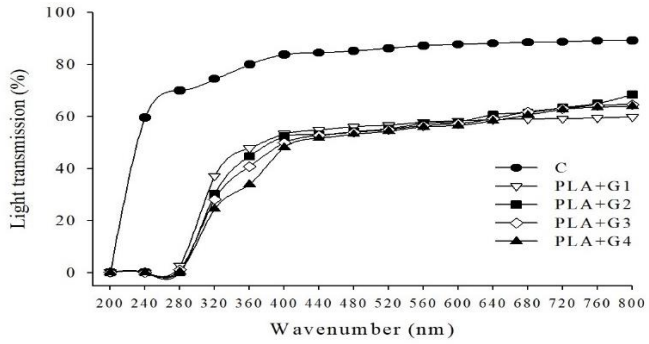

Figure 1. Effect of bovine gelatin thickness on light transmission values of bi-layer films

The increasing thickness of BG layer caused a significant decrease of UV and visible light transmission and resulted in good light barrier properties without increasing opacity. In general, all tested bilayer films showed significant differences for film color, opacity and light transmission values. Therefore, the production of PLA/BG bilayer films could be a promising packaging material for food applications.

3.2. Mechanical properties

Mechanical properties of films represent the ability of keeping the integrity and endure the external stress during the processing, transportation, handling and storage of the packaged materials. For food packaging applications the mechanical stress and film extensibility of films must be sufficient (Haghighi et al., 2019). Tensile strength (TS) and elongation at break (EAB) values of PLA/BG bilayer films, as well as thickness, are given in Table 2, and the curves of calculated TS and EAB also illustrated in Fig. 2. As expected, the thickness of bilayer film significantly increased with increasing the thickness of BG layer. The highest TS (33.22 MPa) and lowest EAB (6.96 %) values were observed for control sample, and the BG lamination of PLA films significantly decreased the TS values, whereas increased the EAB values when compared with control (p<0.05). It is well known that neat PLA films have high TS and low EAB values (Martino et al., 2009). However, gelatin based films plasticized with glycerol and/or other plasticizers generally had low TS, but high EAB values (Jongjareonrak et al., 2006). The increasing BG layer thickness caused a significant decrease in TS values (p<0.05), however PLA+G4 film showed similar TS value with PLA+G1 film (p>0.05). The thickness of BG layer did not significantly affect the EAB values of bilayer films, which meant that the elongation of BG films was not significantly dependent on the thickness. The increasing PLA layer thickness was caused an increase of TS values of sugar palm starch and PLA bilayer films (Sanyang et al., 2016). Nilsuwan et al. (2018) reported that the bilayer film with fish gelatin/PLA layer thickness ratios of 6/4 and 5/5 had higher TS, when compared to fish gelatin films. It is well known that the higher the TS and EAB values of films, the better the packaging properties of films are. When considering this explanation, PLA/BG bilayer films are not proper for food packaging applications due to lower TS values, and needs to be improved. It is an important situation for producing bilayer films that all layers should not separate from each other during testing the mechanical properties, handling, packaging applications, transportation etc.

Table 2. Thickness and mechanical properties of poly lactic acid and gelatin based bi-layer films.

Films	Thickness (mm)	TS (MPa)	EAB (%)	PF (N)	PD (%)
C	0.082±0.004^{d}	33.22±2.79^{a}	6.96±0.69^{b}	74.29±1.46^{b}	99.30±0.76^{b}
PLA+G1	0.137±0.014^{c}	10.04±1.20^{b}	265.26±0.57^{a}	44.55±2.83^{c}	110.01±0.09^{a}
PLA+G2	0.201±0.017^{b}	6.35±0.62^{bc}	312.57±16.50^{a}	44.60±1.06^{c}	110.22±0.15^{a}
PLA+G3	0.238±0.037^{b}	5.17±0.78^{c}	256.62±62.94^{a}	84.56±7.85^{a}	110.75±1.21^{a}
PLA+G4	0.312±0.016^{a}	7.51±0.01^{bc}	325.23±28.09^{a}	80.76±1.32^{ab}	109.94±0.06^{a}

Values are Means ± Standard deviations. C: Control; PLA: Poly lactic acid; G: Gelatin; TS: Tensile strength; EAB: Elongation at break; PF: Puncture force; PD: Puncture deformation. Different subscript letters in the same column refer to significant statistical difference (p<0.05).

As seen in Fig. 2, all samples, except for PLA+G3, showed only one breaking point, which resulted in a homogeneous film structure. However, PLA+G3 sample displayed two different breaking point, probably the first one was the tensile of PLA layer and the second was BG layer. This means that PLA+G3 sample had not homogeneous film structure when compared with others.

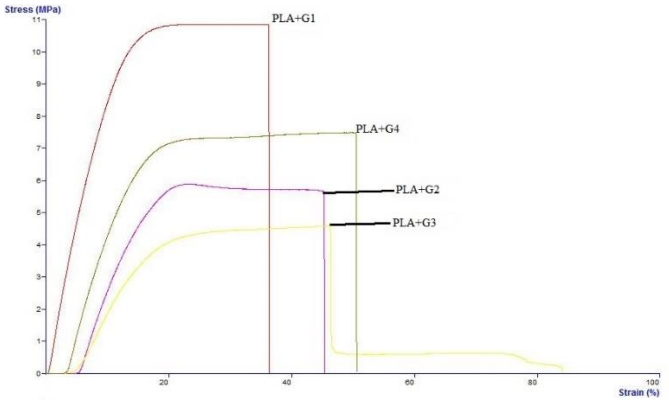

Figure 2. Stress vs strain curves of bi-layer films

Puncture properties such as force and deformation of packaging materials are another important mechanical property. Puncture force denotes the ability of a packaging material to inhibit the intrusion of a foreign object, and the puncture deformation is the proportional expression of the amount of elongation before the blast of packaging by this foreign substance (Sarıcaoğlu & Turhan, 2019). Puncture properties of bilayer films are summarized in Table 2, and the force and distance curves of films are illustrated in Fig. 3.

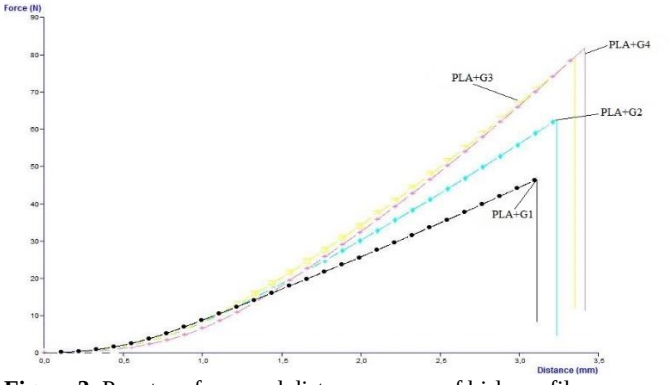

Figure 3. Puncture force and distance curves of bi-layer films.

The highest PF values were observed from the control sample, whereas BG layer incorporation of PLA layer caused a significant decrease of PF values up to PLA+G3 sample, after which PF increased significantly. In addition, PD values of bilayer films were significantly increased when compared with control (p<0.05), but the thickness of BG layer did not significantly affect the PD values (p>0.05). The decreasing trend of PF values could be related with the BG layer which produces films having lower tensile and puncture strengths. However, PD values were similar with the EAB values, probably due to higher EAB values of BG layer than PLA. Moreover, the decreasing and increasing tendency of PF and PD values, respectively, could also be attributed to plasticizing effect of glycerol which was added to BG layer for better film production. Glycerol disrupts the order of protein aggregates within the BG film matrix, and results in a more heterogeneous distribution of junction zones and higher free volume in film matrix. Hence, the polymeric chain mobility could be increased. The stabilizer hydrogen bonds between water and protein molecules also displace with the glycerol addition. All these plasticizer effects of glycerol cause further increase of free volume between protein molecules, and therefore, the puncture resistance of films decrease (Gontard, Guilbert, & Cuq, 1993; Janjarasskul & Krochta, 2010). As a result of glycerol addition to BG layer, the bilayer films became more flexible and weaker, however, when thickness increased PF values of bilayer films increased, probably due to increasing solid content of film matrix. The puncture properties of bilayer films revealed that BG layer could be used at a specified level for better PF values, and these films have a promising effectiveness for packaging applications.

4. Conclusions

The optical and mechanical properties of bilayer films from PLA and BG were influenced by the various thickness of BG layer. The increasing BG layer thickness caused more yellowish and higher opacity bilayer films, whereas better UV-light barrier properties were obtained with incorporation of BG layer. However, BG layer thickness has no effect on visible light transmission of bilayer films. Bilayer films behaved as monolayer films during mechanical analyses and a homogeneous film structure, such as monolayer, was an important result for packaging applications. The TS values significantly decreased with increasing BG layer, and EAB values increased due to plasticizer addition to BG layer. PF and PD values had a similar tendency with TS and EAB results, but PF values increased when G3 and G4 thickness were applied on PLA layer. The results of this study revealed that PLA and BG films could be used for bilayer film production in order to obtain better mechanical properties than monolayer films, and gas barrier properties of bilayer films need to be investigated with further studies.

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