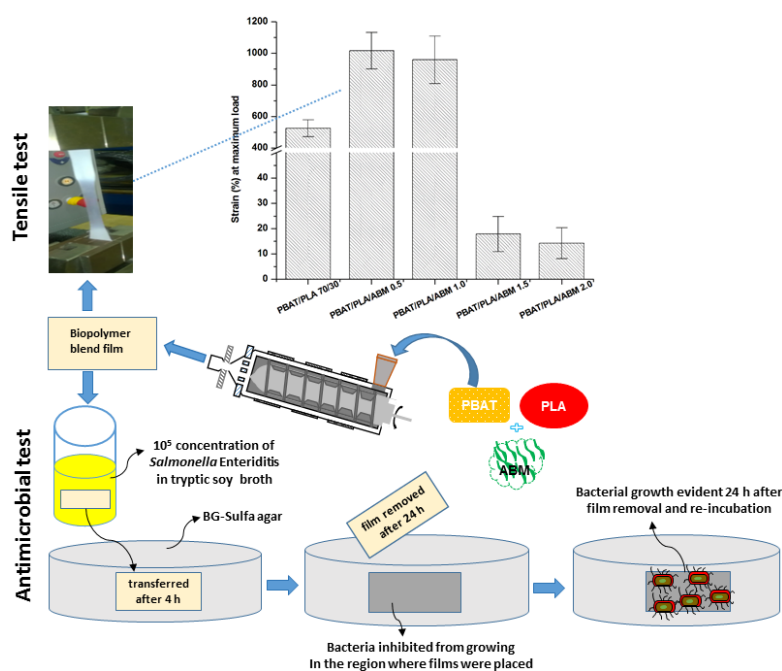


Highlights

- PBAT/PLA/egg white biodegradable flexible films produced through extrusion
- Egg white has an effect on microstructure, mechanical and antimicrobial properties of PBAT/PLA blend
- Egg white significantly increased the thermal stability and toughness of PBAT/PLA blend
- The inclusion of egg white in PBAT/PLA film show significant bacteriostatic effect when in contact with bacteria

Table of Content Graphic



Tough aliphatic-aromatic copolyester and chicken egg white flexible biopolymer blend with bacteriostatic effects

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KEYWORDS: bacteriostatic films, biopolymer blends, immiscible polymers, melt-extrusion

ABSTRACT

Extruded 70/30 poly (butylene adipate-co-terephthalate) (PBAT)/ polylactic acid (PLA) blends incorporated with 0.5, 1.0, 1.5 and 2 wt. % of egg white [albumen (ABM)] were investigated to determine the effect of ABM on the microstructure and antimicrobial properties of PBAT/PLA blend. Microstructural analysis revealed immiscibility in the two polymers, due to the presence of distinct melting points, heterogeneous phase and vibrational frequencies unique to each polymer. Thermal and tensile testing also suggests that the inclusion of ABM tailored the blend and significantly improved its thermal stability and flexibility, since 0.5 and 1.0 % (wt. /wt.) ABM led to 490 and 432% improvement in elongation, respectively. Furthermore, the ternary blend films in contact with *Salmonella enteritidis* (*S. enteritidis*) showed significant inhibition to growth for 24 h, but some bacterial cells grew when the film were removed. This study demonstrates the fabrication of ABM tuned potential biopolymer packaging flexible blend with bacteriostatic properties.

1. Introduction

The need for biodegradable polymer packaging systems in recent times has led to increased interest in research on substitutes to the recalcitrant polymer systems, especially in the current situation of surging plastic usage. Polymers from renewable sources currently being explored include starch, proteins and cellulose, synthetic polymers obtained from natural monomers like polylactic acid (PLA) and polymers obtained from microbial fermentation (Yu, Dean, & Li., 2006). In comparison with petroleum-based polymers, the properties of renewably sourced polymers are inferior and require structural tuning for use in similar fields of application. This tuning has mostly been advanced in thermoplastics through polymer blending.

PLA, a widely used high strength and modulus polymer is easily processed with other biopolymers with similar melting temperatures to design biomaterials. This helps in offsetting its extreme brittleness which limits its application (Li & Micheal, 2011; Garlotta, 2001; Al-Itry, Lamnawar, & Maazouz., 2014). Hence, blending PLA with elastomers and plasticizers (Tabasi, Najarzadeh, & Ajji., 2015) makes possible, the design of soft biomaterials and flexible biodegradable packaging systems. PBAT can suitably blend with PLA, offering good thermal stability, flexibility, exceptional toughness and biodegradability (Ki & Park, 2001). Blending PBAT with soy meal (Zhou, Mohanty, & Misra., 2013), starch (Brandelero, Yamashita, & Grossmann., 2010) and corn gluten meal (Reddy, Misra & Mohanty., 2014) has been reported as approach that could balance the cost and properties of PBAT for consideration in a wider field of applications.

ABM also has good thermal stability and conversion into plastic structure. Re-orientation of its proteins at about 135 °C led to the formation a bioplastic material (Sharma, Hodges, & Luzinov., 2008). Besides, a clinical study of antimicrobial activity of ABM showed that it inhibits microbial growth, due to the presence of the lysozyme which lethally interacts with bacteria (Baron &

64 Rehault, 2007; Jones, Mandal, & Sharma., 2015). These attractive properties make ABM a
65 promising biomaterial for polymer structural tuning to enhance performance, bioactivity,
66 biodegradability, barrier properties and antimicrobial activity.

67 Most PLA binary blends show inferior mechanical properties, due to immiscibility. Attempted
68 blends such as PLA/poly (ether-b-amide) (PEBA) (Zhang, Nagarajan, Misra, & Mohanty, 2014),
69 PLA/PBAT (Arruda, Magaton, Bretas, & Ueki., 2015), PLA/PCL (Tabasi, Najarzadeh, & Ajji.,
70 2015) and PLA/PHB blends (Jandas, Mohanty, & Nayak., 2014) mostly revealed poor mechanical
71 properties, due to differences in chemical structural of the constituent polymers in each case.

72 Hence, compatibilization or modification of the microstructure is a necessity in most PLA
73 biopolymer blends (Ojijo, Ray, & Sadiku, 2013; Li & Shimizu, 2009; Dong et al., 2015). Also,
74 blending with a third polymer possible means of obtaining desirable properties not attainable in
75 binary blends. Ternary blends of PLA, poly (3-hydroxybutyrate-co-hydroxyvalerate) (PHBV), and
76 poly (butylene succinate) (PBS) (PLA/PHBV PBS) revealed excellent toughness and thermal
77 stability due to developed favorable morphology and synergistic effects of each polymer (Zhang,
78 Mohanty, & Misra., 2012). Additionally, a blend of PLAs with poly (L-lactide) (PLLA), poly (D-
79 lactide) (PDLA), and poly (methyl methacrylate) (PMMA) revealed that 30-40% of PMMA
80 enhanced crystal nucleation and miscible morphologies, subsequently leading to superior storage
81 modulus and resistance to thermal deformation in the ternary blends, as compared to the binary
82 PLLA/PMMA blends (Samuel et al., 2013). These emphasize the essence of structural tuning in
83 binary blends. The study reported here probes the effect of ABM on the microstructure,
84 mechanical and antimicrobial properties of PBAT/PLA 70/30 immiscible blends. We
85 hypothesized that the weakness of the interface of PBAT/PLA binary blend could be minimized

by blending with small amount of ABM to produce biodegradable, bioactive and potential antimicrobial PBAT/PLA/ABM ternary system with synergistic properties.

2. Materials and methods

2.1 Chemicals

PBAT (Ecoflex F blend C 1200 was obtained as a research sample from BASF Corporation, Villa Park, IL, USA. PLA (Ingeo™ biopolymer 3051D) was purchased from NatureWorks LLC, Minnetonka, MN, USA. Egg white [albumen (ABM)] was purchased from Barry Farms, Wapakoneta, OH, and used as received. Chloroform (CHCl_3 , $\geq 99\%$), used to dissolve the pelleted polymers, was of HPLC grade, and methanol (CH_3OH , $\geq 99.9\%$), used to precipitate the blend from the solvent were bought from Sigma Aldrich, St. Louis, MO. Brilliant Green Sulfa Agar (BG-Sulfa) and Tryptic Soy Broth (TSB) were purchased from Thermo Fisher Scientific, Lenexa, KS, USA and Acumedia-Neogen Cooperation, MI, USA respectively. The bacterium, *Salmonella enteritidis* (*S. enteritidis*) (ATCC 13076) was purchased from American Type Culture Collection (ATCC), Manassas, VA, USA.

2.2 Incorporation of ABM in the blends

In order to homogeneously incorporate the ABM with the polymer blend, 150 g of 70/30 PBAT/PLA was blended in 500 mL of CHCl_3 for at least 12 h at 400 rpm on magnetic stirrer (CIMAREC, Barnstead International). ABM was then added to make 2, 1.5, 1.0 and 0.5 wt. /wt. % of the blends by first dispersing in 60 mL CHCl_3 on a magnetic stirrer (Sigma Aldrich, IKA WORKS, Inc.) at 600 rpm for 30 min before adding to each melt blend of PBAT/PLA 70/30 and then mixing for 4 hrs. After this, the mixture was further homogenized by mechanical stirring for 5 min, before excess (800 mL) CH_3OH was added in steps of 100 mL while mechanically stirring

to precipitate the mixture. The precipitate was vacuum filtered (100 mm Whatman™ filters) for 20 min and the powder dried at 40 °C for at least 6 h in an oven (Isotemp 200 Series).

2.3 Extrusion of polymer blend films

About 150 g of each of the precipitated blends containing ABM was dried for 12 h at 60 °C in a hopper (DRI-AIR Industries Inc., model RH5). This was then fed into a table top single screw extruder (Wayne SN: 8001) driven by 2 hp motor. Thermostat controlled heating zones (five) and rotation of the screw facilitated mixing and formation of continuous viscous melts for the extrusion of plastic sheets. Three heating zone are located on the barrel while two are in the die zone. The optimum working temperatures were 160, 160, 160, 157 and 156 °C, for the barrel and die zones respectively. The high barrel temperature causes the polymer to melt and randomly orient the particulates within the flowing matrix while the screw rotation induces high velocity in the matrix, causing large shear forces which contribute to the random distribution of the particles. Typically, 35 mm (w) × 0.2 mm (t) blend specimens were collected at the die orifice at a screw speed of 20 rpm and feed rate of 4.4 g/min. The collected sheets were drawn through water stationed at the orifice of the die for quenching. The PD-mini extrusion die (SN # 13-33386) which is 6-inch deep, 3-inch tall, 6.5-inch wide with 4-inch lip opening was purchased from Premier Die Corp. Chippewa Falls, WI. These sheets were then stored in a high vacuum desiccator (JEOL, EMDSC-U10A) for later characterization. **At least six specimens were used for each characterization.**

2.4 Determination material properties and characterization

2.4.1 Raman analysis

Molecular vibrational spectroscopy analysis of the pristine and blended polymer systems were achieved through the use of DXR Raman microscopy (Thermo Scientific). This test was performed

using a 532 nm laser (5.0 mW power), filter and grating. OMNIC Software was used for data acquisition and analysis.

2.4.2 X-Ray diffraction (XRD) analysis

The XRD analysis of the specimens was performed using a Rigaku diffractometer (DMAX 2100) equipped with Cu K α radiation. The equipment was operated at step size of 0.02°, scan rate of 1°/min, Bragg's angle of $2\theta = 3^\circ$ to 80° , tube voltage and current of 40 kV and 30 mA respectively.

2.4.5 X-Ray photoelectron spectroscopy (XPS)

The surface composition of the blends containing ABM was analyzed using XPS. The XPS spectra were acquired using K-alpha surface analysis system (Thermo Scientific) equipped with Al monochromator as radiation source operated at 12 keV and 12 mA.

2.4.6 Transmission electron microscopy (TEM)

A JEOL 2010 TEM was used to analyze ABM. One (1) mg of the ABM specimen was dispersed in 5 mL CH₃CH₂OH for 10 min in an ultrasonic bath and a drop of the colloidal solution was deposited on a copper grid and analyzed.

2.4.7 Differential scanning calorimetry (DSC)

A TA Q 2000 DSC was used to study the thermal transitions of the specimens. Samples of 10.0 ± 0.1 mg were used in the test. Each specimen was cooled at 20.0 °C/min from room temperature to -40 °C and subsequently heated from -40 °C to 200 °C at 5 °C/min and held at constant temperature for 2.0 min. It was again cooled from 200 °C to -40 °C at 20.0 °C/min before finally scanning at 5.0 °C/min from -40 °C to 200 °C.

2.4.8 Thermogravimetric analysis (TGA)

Thermogravimetric analysis was carried out with TA Q 500 equipment. Samples of 14 ± 0.2 mg were placed in platinum pans. An empty platinum pan was used as a reference. Each sample was

heated from 30 °C to 600 °C in a 50 mL/min flow of N₂. A heating rate of 5 °C/min was used to collect data of the changes in mass as the sample was heated until it turns to char.

2.4.9 Tensile testing

Measurement of tensile mechanical properties was performed using Zwick Roell Z2.5 mechanical testing system equipped with TestXpert data acquisition and analysis software, accordance to **ASTM D 882**. Crosshead speed of 500 mm/min, 2.0 kN load cell, wedge grips and 20 mm gauge length were used to collect the mechanical performance data of each specimen. Specimens were cut from the extruded polymer blend sheets to 19 mm x 0.2 mm x 120 mm and tested. At least 12 specimens of each category were tested, averaged and reported.

2.4.10 Light transmission and film transparency

The light transmission and transparency of the films was measured in the visible range (380-800 nm) using a visible spectrophotometer (Vernier SpectroVis Plus, Beaverton, OR) according to the method of **Shiku et al. (2004)**.

2.4. 11 Scanning electron microscopy (SEM)

Microstructure and blend morphologies were probed using a JEOL JSM-5800 SEM. Samples were placed on a carbon tape fastened onto the sample holder of the SEM and sputter coated with Au-Pd for 5 min in a Hummer 6.2 sputtering system. The sputtering was done in a N₂ atmosphere at 20 millitorr, 5 V and 15 mA. Fractured surfaces of the tensile specimens were examined using Hitachi S-3400N SEM.

2.5 Antimicrobial studies of the blend systems

2.5.1 Film preparation for antimicrobial study

The extruded films were cut to $1.5 \times 1.5 \text{ cm}^2$ (2.25 cm^2 area) with sterile scissors and exposed to UV light for 30 min in a PCR Workstation (Air Clean® 600, Serial # 42991). These film pieces were then stored in sterile petri dishes for later use in antimicrobial testing.

2.5.2 Antimicrobial testing

Brilliant green sulfa (BG-Sulfa) agar and tryptic soy broth (TSB) were used in the antimicrobial study of the films. In order to assess the antimicrobial activity, bacterial isolate was streaked onto BG-Sulfa agar plates and incubated at 37°C to obtain single colonies. Using sterile disposable inoculating loops, about 3 to 5 isolated colonies were transferred from each plate into sterile tubes containing 1.5 ml of TSB. The bacterial culture was vortexed and incubated at 37°C on a shaker at 350 rpm for about 2 h. Sterile TSB was used to adjust the turbidity of the *S. enteritidis* suspensions to obtain approximately equal optical density (OD) to that of 0.5 McFarland Standard. The OD of each bacterial suspension was measured at 600 nm (OD_{600}) using UV-VIS spectrophotometer (Nanodrop 2000c, Wilmington, DE, USA). The starting bacterial concentrations measured at OD_{600} ranged from 0.098 to 0.112.

Antimicrobial properties of the films were tested qualitatively by directly immersing the films in $5.9 \times 10^6 \text{ CFU/mL}$ concentration of bacterial suspension for 4 h and then removing the films to allow all liquid TSB to dry for 10 minutes under the biosafety hood. The dried films were placed on BG-Sulfa agar plates to assess the ability of the bacteria to adhere on the film surfaces and whether their growth will be inhibited. *S. enteritidis* was chosen based on common prevalence of these organisms as foodborne pathogens, their ability to survive and multiply in most food storage temperatures ($+ 4^\circ\text{C}$) and as representative pathogens of gram negative bacteria. In this experiment, tests were done in triplicates using *S. enteritidis* bacteria. The treated dry films were placed on agar plates and incubated at 37°C . After 24 h, films were removed with sterile forceps

and discarded, while the plates were re-incubated for additional 24 h. All plates were visually inspected after 24 h of incubation for any growth. Images of the plates were collected using an Alpha Innotech Multiimager II instrument (SN#: 510133; model: AlphaImager HP, Alpha Innotech Corp. San Leandro CA, USA). Control plates were inoculated with sterile TSB and growth control plates were inoculated with 20 μ L of the bacteria. The PBAT/PLA binary film was used as a control to check the effect of ABM on the antimicrobial properties.

2.6 Statistical analysis

The statistical significance of differences in thermal properties was determined with a one-way analysis of variance (ANOVA) and Tukey's multiple-comparison tests. In all cases, a value of $p < 0.05$ was considered to be significant. GraphPad Prism 7.03 software was used to do this analysis.

3. Results and discussion

3.1 XPS and TEM analysis of ABM

Figure 1 shows (a) an XPS spectrum (b) and TEM micrograph of ABM. The composition and chemical states of the ABM are shown by the XPS spectrum in **Figure 1a**. The material consists of S, Cl, C, N, O and Na. The peaks at 164, 198, 285, 400, 532, 976.2-978.0 and 1071 eV are due to S2p, Cl2p, C1s, N1s, O1s, O_{KLL} and Na1s transitions respectively (Demri & Muster, 1995; Rouxhet et al., 2008; Aeimbhu, James, & Singjail., 2005). The atomic percentages of each element suggest that C (64.35 %), O (18.74 %) and N (14.93 %) are the main constituents of ABM, while the minerals (S, Cl and Na) detected in the surface analysis constitute the remaining 1.98 %. The C, N and O could be due to organic molecules such as proteins and carbohydrates in the albumen. Reports show that ABM has a complex protein content (ovalbumens, ovotransferrin, ovomucoid, globulins, and lysozyme), low fat, minerals (chloride, sodium, Sulphur, potassium, calcium, magnesium and iron) and carbohydrates (glucose, galactose, mannose, glucosamine,

galactosamine and sialic acids) (Baron et al., 2016; Brand, Dachmann, Pichler, Lotz, & Kulozik., 2016; Mine, 1995). This supports our XPS result for ABM. The micrograph in **Figure 1b** reveals a network bridge-like structure of ABM. The structure is typically amorphous. This submicron (0.5 μm wide) giant and complex assembly of molecules has an elongated morphology which suggests that in the right environment, it can possibly interconnect or bridge different molecules at the interface. This has the potential to be used as an interfacial modifier needed to offset the weakness in immiscible polymer blends.

3.2 Raman microanalysis

Raman spectroscopy helps in structural elucidation of molecules at the functional group level through laser induced molecular excitations (Furukawa et al., 2006). This test helped in the identification and assignment of the functional groups in the structure of the blend and due to its individual components. **Figure 2** shows the micro-Raman spectra of polymer systems in the region of 2500 to 500 cm^{-1} using 532 nm laser excitation. It is evident that the vibrational bands for the binary (b) and ternary (a) blends in **Figure 2** are similar. The Raman spectra revealed distinct vibrational frequencies due to PBAT in the blend that appeared at 637, 1720, 1618, 1185 cm^{-1} . These are due to aromatic ring vibrations, -C=O stretching, aromatic -C=C , and -C-O-C stretching in the structure of the soft PBAT polymer respectively. These spectral assignments are in agreement with previous reports in this system (Nobrega, Olivato, Muller, & Yamashita., 2012; Furukawa et al., 2006; Kister, Cassanas, & Vert., 1998). The presence of the ABM in the ternary blend is revealed through the bands at 1010 and 1670 cm^{-1} , due to phenyl alanine aromatic breathing and amide I vibrations (Vandenabeele et al., 2000). The bands at 709 and 860 cm^{-1} are due to -C=O out-of-plane deformation and -C-COO stretching for PLA respectively. Bands at 1050 and 1109 cm^{-1} are ascribed to -C-CH_3 and -COC- stretching while 1395 and 2950 cm^{-1} are

due to $-\text{CH}_3$ symmetric deformation, 1461 and 3004 cm^{-1} are attributed to $-\text{CH}_3$ asymmetric deformation, and 1290 cm^{-1} is as a result of $-\text{CH}$ bending in PLA (Furukawa et al., 2007; Kister, Cassanas, & Vert., 1998). The spectrum for the ABM (Figure 2c) reveals the existence of amide I ($-\text{C}=\text{O}$, 1670 cm^{-1}), amide III (N-H , C-N-H , $1100\text{-}1280\text{ cm}^{-1}$), $-\text{CH}_2$ scissoring (1482 cm^{-1}), and aromatic breathing (1010 cm^{-1}) bands, as reported elsewhere (Vandenabeele et al., 2000). The identification of bands attributed to each individual polymer suggests that the interaction between the components of the blend is largely physical in nature.

3.3 XRD analysis of polymer blend systems

The morphology of the blends and the pure systems analyzed by X-ray diffraction is shown in Figure S1. Diffraction patterns are for; PBAT/PLA 70/30 (Figure S1 a), PBAT/PLA 70/30 composites (Figure S1 b-e), and ABM (Figure S1 f). A semicrystalline diffraction pattern within which an amorphous curve lies below four major crystalline peaks at diffraction angles of $2\theta = 17.5^\circ$, 20.0° , 23.1° and 24.3° was observed in the 70/30 blend and in the ternary system. These crystalline peaks are due to PBAT. The amorphous peak of PLA merges with the amorphous region of PBAT in the blend. Similar diffraction pattern has been reported for PBAT/PLA blends with different ratios (Arruda, Magaton, Brestas, & Ueki., 2015). ABM also revealed a semicrystalline pattern, shown in Figure S1 f. The semicrystalline microstructure of the ternary blend is critical to morphology-related structural properties, especially, stiffness and flexibility. The presence of ABM in the ternary blend is attributed to the maximum intensity in the range of $2\theta = 9\text{-}12^\circ$. Crystallinity has been found to alter mechanical properties in polymer blends (Li et al., 2015; Samuel et al., 2013). Hence, the addition of semicrystalline ABM into the binary 70/30 blend may potentially alter the blend morphology and microstructure to enhance the interfacial weakness and hydrophilicity of the immiscible blend as well as potentially imparting antimicrobial activity into

the biofilm. Past studies have demonstrated the influence of nanomaterials on the tuning of polymer structures and their resulting mechanical and thermal properties (Mofokeng & Luyt, 2015). ABM can potentially serve a similar purpose in the PBAT/PLA blend.

3.4 Differential scanning calorimetry

DSC heating curves of ABM, PBAT/PLA 70/30 and PBAT/PLA/ABM ternary blends incorporated with 0.5 to 2 wt. % of ABM after crystallization from melts obtained at 5 °C/min is shown in **Figure S2**. The PLA in the blend displayed a glass transition temperature at 60.20 °C, cold crystallization at 104.83 °C, and a double melting point at 148.75 °C and 156.48 °C. The PBAT melted at 122.74 with a thermal signature that merges with that of ABM, which shows a broad endothermic peak at ~ 137 °C. The denaturation point of ABM is consistent with the finding elsewhere for ABM proteins (Sharma, Hedges, & Luzinov., 2008) Comparing the curves in **Figure S2**, it is evident that the cold crystallization temperature of PLA is shifted by to lower temperature by 3-4 °C in the ternary blends, indicating enhanced cold crystallizability of the PLA due to the ABM, as observed in other studies (Labarbe Grau, Gadenne, Alfes, & Cramail., 2015). The occurrence of two distinct melting temperatures in all the blends, attributed to the individual polymers, indicates immiscibility in the blends as revealed in the microstructural analysis and past observations (Arruda, Magaton, Bretas, & Ueki., 2015). The double melting peaks of the PLA is attributed to less perfect crystals which melt at lower temperatures and more structurally perfect crystals melt at higher temperature (Jiang, Walcott, & Zhang., 2006). A second possible origin of the double melting peaks could be wide molecular weight distribution in the PLA. Lower molecular weight moieties of PLA will melt at low temperature compared to higher molecular weight moieties in the same polymer. This double melting peak is dramatically reduced in the ternary blend, indicating that albumen enhances the crystallization of the imperfect crystals of PLA

in the blend during the cold crystallization process, leading to a single melting temperature at 157 °C.

3.5 Thermogravimetric analysis (TGA)

The results on the thermal stability of the polymer blend systems by TGA are shown in **Figure S3** and **Table 1**. As evident in **Figure S3**, the inclusion of ABM to the 70/30 blend led to significant improvement in the thermal stability. Generally, the curves of the blends (**Figure S3**) show two-step degradation, due to less stable PLA (first) and more stable PBAT (second). The following improvements due to ABM were observed; onset of degradation, 7-25 °C, T_{d50} , 9-11 °C, T_{dmax2} , 5-7 °C, and residual yield of about 3-4 % from the binary blend. The study of PVDF/PMMA blend revealed improved T_{donset} and $T_{50\%}$ by 10.5 and 30 °C respectively, due to the addition of 6 % nanocellulose crystals (Zhang et al., 2015). Also, in PLA/PHB blend incorporated with nanoclays, the thermal stability improved significantly, due to decrease in oxygen permeability as a result of to the “crooked path” effect in the altered microstructure which delays the infiltration of oxygen and volatile degradation products which usually accelerate combustion (Jandas, Mohanty, & Nayak., 2013). These previous observations are in agreement with our findings for blends incorporating a small amount of ABM in the 70/30 blend, as depicted in the residual yields in **Table 1**.

The degradation temperature of the PLA (T_{dmax1}) in the ternary blend was observed to decrease in the ternary blend. This is probably due to the amorphous nature of the PLA domains serving as an avenue for permeation of volatile combustions products, and since there is no order in the PLA structure the inclusion of ABM might have increased the entropy in its domains to promote combustion.

3.6 Tensile Analysis

The tensile analysis of the PBAT/PLA and PBAT/PLA/ABM blend systems is shown in **Figure 3**. **Figure 3** presents the stress-strain curve representing the general mechanical behavior of the polymeric systems under tensile force (2.0 kN). **Table 2** summarizes the tensile strength, elastic modulus, and strain at maximum load respectively. The binary PBAT/PLA 70/30 blend showed moderate ductility. **Figure 3** reveals that the binary blend fails at 526% strain. The addition of 0.5 and 1.0% of ABM into the matrix of the binary blend produced a ternary blend with a large increase in elongation with a strain at maximum loads of 1016% and 958%, respectively. These are improvements of 490% and 432% in comparison to the binary blend for 0.5 and 1.0 % additions of ABM in the ternary blend, respectively. The addition of 1.5% and 2% ABM reduced the ductility of the blend to 372% and 506% strain, respectively, and also reduced the elastic modulus. The ductility and modulus of the high-ABM-concentration blend are lower than the binary blend, suggesting that the addition of higher concentrations of the ABM led to compromise in the structural properties due to the detrimental agglomeration effects of ABM in the matrix of the blend. Also, the blends showed distinct yielding, followed by considerable cold drawing during the tensile test, indicating significant transformation of the microstructure to favor ductile fracture due to blending with the ABM.

The yield strength in ternary blends with 0.5%, 1.0% and 1.5% of ABM rather lower than that of the binary blend. With 2.0% of ABM, however, similar yield strength was realized for both binary and ternary blends. The tensile strength and modulus (**Table 2**) were slightly less in the ternary blends, compared to the binary blend.

The ternary blends revealed essentially the same strength and modulus, regardless of the amount of ABM. This is because ABM has much weaker strength and stiffness but is better in toughness than PLA. The strain at maximum load in **Table 2** revealed that specimens with 0.5% and 1.0%

ABM could endure more load while being stretched compared to the binary and ternary blends with higher content of ABM. Similar trends in property modification have been reported with the use of nanomaterials in immiscible blends (Mofokeng, & Luyt, 2015), reactive and non-reactive compatibilized polymer blends (Ojijo, Ray, & Sadiku, 2013; Li & Shimizu, 2009; Dong et al., 2015) and in composites (Rahman, Netravali, Tiimob, & Rangari, 2014; Tiimob, Rangari, & Jeelani, 2014; Tiimob, Rangari, & Jeelani, 2016). Hence, ABM tuned the microstructure of the binary blend to improve the strain to failure of the biodegradable ternary blend.

3.7 SEM analysis

The morphology of the PBAT/PLA 70/30 binary blend revealed a heterogeneous phase, with ellipsoids domains of PLA dispersed in a continuous phase of PBAT (**Figure S4 a**). The domain sizes of about 10-40 μm . The observation of coarse domains in the phase separated “sea-island” structure indicates that the PBAT and PLA are immiscible. This is similar to findings in previous reports on PPC/PMMA (Li & Shimazu., 2009), PLLA/ABS (Dong et al., 2015), PCL/PLA (Chen et al., 2014), PLA/PBSA (Ojijo, Ray, & Sadiku, 2013), and PLA/PHBV/PBS (Zhang, Mohanty & Misra., 2012) where phase segregated morphologies have been reported.

The toughening effect of ABM on the ternary blend investigated through SEM analysis of the fractured surfaces after tensile tests are shown in the micrograph in **Figure S4 c**. The fractured surface of the pure 70/30 blend (**Figure S4 b**) reveals a microstructure which suggests a pull out of one phase from another. The deep crack created as a result of the pull out of the phase-segregated PLA in PBAT matrix affirms the immiscibility and poor interfacial interaction existing in the binary blend. However, the fractured surface of the PBAT/PLA/ABM ternary blend (**Figure S4 c**) reveals several necked regions, suggesting some resistance to the tensile force. This surface (**Figure S4 c**) show rough and tortuous morphologies with discontinuous crack paths diverted

around irregular fibrils of the matrix. This explains the enormous improvement in the toughness of the PBAT/PLA blends with 0.5% and 1.0% ABM. Interfacial interaction between dispersed domains and the matrix plays a crucial role in the toughening of a blend. High toughening effect is achieved when the dispersed phase strongly interacts with the matrix, inhibiting crack growth (Zhang, Nagarjan, Misra, & Mohanty., 2014). Hence, phase segregation in immiscibility helps in the toughening of materials through crack path interruption which delays failure, as observed in toughened blends of PLA/PEBA (Zhang, Nagarjan, Misra, & Mohanty., 2014), PLA/PCL (Tabasi, Najarzadeh, & Ajji., 2015), PLA/PBAT (Arruda, Magaton, Brestas, & Ueki., 2015) and PLA/PHB (Jandas, Mohanty, & Nayak., 2014) which revealed similar morphologies to that of PBAT/PLA/ABM.

3.8. Light transmission analysis on films

The transmission of visible light at wavelength range between 380-800 nm of PBAT/PLA neat film and a PBAT/PLA/ABM ternary films with different percentages of ABM are presented in Table 3. The neat blend (PBAT/PLA) generally showed high light transmittance, and a value of 60.17 % transmission at 600 nm but the ternary films generally revealed much less (less than half of that transmitted through the neat blend) transmission of visible light due to the inclusion of ABM. Though, no trend was observed as the amount of ABM increased in the ternary blend films, it is evident that the significant reduction in light transmission between neat blend and the ternary system is due to the included ABM in the films being able to absorb or scatter significant amount of visible light. This suggests that ABM is very effective at impeding light transmission through the film. Elsewhere (Ahmad, Arfat, Al-Attar, Auras & Ejaz), a reducing trend in transmittance was observed due to the inclusion of ZnO nanoparticles as UV blocking and light impeding agent in a linear low-density polyethylene films, but this ZnO was added in large amount than in our present study.

3.9 Antimicrobial Studies

The effects on the adhesion and growth of *S. enteritidis* bacteria on $1.5 \times 1.5 \text{ cm}^2$ PBAT/PLA and PBAT/PLA/ABM films are shown in **Figure 4**. In the control specimen (**Figure 4a**), 20 μL of TSB was dispensed in the middle of the plate. In another control, 20 μL of bacterial concentration $5.9 \times 10^6 \text{ CFU/mL}$ was dispensed on the agar without treatment with any film (**Figure 4b**). As expected, the bacterial control revealed growth of *S. enteritidis* colonies after 24 h while the TSB showed none (**Figures 4a** and **4b**, respectively). The absence of growth on the TSB negative control plates indicates that the broth was not contaminated, while growth from the positive control 20 μL inoculum suggests that the bacterial cells are viable.

When the films were immersed in $5.9 \times 10^6 \text{ CFU/mL}$ *S. enteritidis* suspension, then dried and placed on agar and incubated for 24 h (**Figure 4c₁-e₁**), bacterial growth was evident in the PLA/PBAT binary blend as shown in **Figure 4c₂** immediately after film removal. However, no growth was observed on those of PBAT/PLA/ABM as shown in **Figure 4 d₂** and **4 e₂** immediately after films were removed. After removal of the films and re-incubating the plates for additional 24 h, bacterial growth was now evident on all the plates, including those treated with PBAT/PLA/ABM (**Figures 4c₃, 4d₃ and 4e₃**). The bacterial cells were too many to count in the region where PLA/PBAT binary blend (neat film) was placed were as countable number of colonies were observed in the films containing ABM. This suggests that bacteria had adhered to all the films during the 4 h immersion in the bacteria suspension at 25 °C. While the adhesion was relatively high in the binary blend film, not much adhered to the ternary films. The survival of bacteria while the PBAT/PLA/ABM films were in contact with the agar suggests that the films most likely possess bacteriostatic properties as observed in eggshell/silver tailored PBAT/PLA blends (Tiimob et al., 2017). As evident in **Figure 4d₂-e₂**, after the films were

removed, the regions previously covered by the films revealed substantial inhibition of bacterial growth after 24 h of film contact with the agar. In agreement previous report (Tiimob et al. 2017), the 70/30 blend possessed no bactericidal effects since it is just a neat polymer blend. The blending of the 0.5% and 2.0% albumen with the 70/30 system showed some antimicrobial effects, probably due to the blockage of oxygen by particulate albumen components or the presence of some lysozyme in ABM.

Reduced permeation of gaseous materials prevents moisture-dependent microbial growth in food (Beltran et al., 2014). Nanocomposites based on poly (ϵ -caprolactone) (PCL), hydroxytyrosol (HT) (natural antioxidant) and nanoclay [cloisite 30B (C30B)], were reported to have improved the poor PCL intrinsic barrier properties, leading to significant improvement in oxygen barrier in ternary nanobiocomposites containing C30B and 10 wt. % HT (Beltran, Valente, Jimenez, & Garrigos., 2014). Lysozyme protein which is inherent in albumen is responsible for its antimicrobial activity (Baron et al., 2016; Brand, Dachmann, Pichler, Lotz, & Kulozik., 2016; Mine, 1995). The bacteriostatic effects (instead of bactericidal) observed with the ternary blends could as well be associated with the existence lysozyme in minute amounts, due to the quantity of ABM used in this study, leading to insufficient lysozyme in the films. It is also possible that lysozyme may have degraded due to high temperature and chemical treatment of the albumen during the film fabrication process.

4. Conclusion

Extruded biodegradable poly (butylene adipate-co-terephthalate) (PBAT)/agro-based polylactic acid (PLA) binary and ternary blend films with 0.5-2.0% ABM were studied to determine the effect of the ABM on microstructure, thermal and tensile properties of the blend. The results revealed that the two polymers are immiscible, due to the presence of distinct melting points and

phase segregated morphologies in the blend and composite structures. Also, the PLA was amorphous while PBAT is semicrystalline, resulting in a semi crystalline immiscible blend. The tensile and SEM results showed that 0.5-1.0 % of ABM led to significant enhancement in the toughness due to heterogeneous phased matrices with interrupt cracks paths, diverting crack propagation and delaying failure in the ternary systems, whereas thermal analysis showed that ABM enhanced the cold crystallization and thermal stability of the ternary blends. These revealed that the PBAT/PLA/ABM 70/30/0.5 ternary blend possessed the desirable balance strength and flexibility for flexible designs and applications. *In vitro* antimicrobial study on *S. enteritidis* showed significant inhibition of bacterial growth during 24 h on contact with films, but the bacteria grew after the films were removed and the plate re-incubated for additional 24 h, suggesting bacteriostatic effects due to the inclusion of ABM in the films.

Acknowledgements

The authors very grateful for the financial support from NSF-CREST #1137681, NSF-RISE #1459007, NSF-MRI-1531934 and AL/NSF EPSCoR # 1158862 grants. The authors gratefully acknowledge use of facilities and instrumentation supported by NSF through the University of Wisconsin Materials Research Science and Engineering Center (DMR-1121288). The authors are very grateful to Tuskegee University shared instrumentation biomedical research core research facility (NIH grant G12MD007585).

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Figure captions

Figure 1. Structural analysis of albumen. (a) X-ray photoelectron spectrum, (b) transmission electron micrograph.

Figure 2. Raman spectra of polymer systems: (a) PBAT/PLA/albumen 70/30/1 blend (b) PBAT/PLA 70/30 blend and (c) albumen.

Figure 3. Stress-strain curves of PBAT/PLA/albumen polymer blends from tensile test.

Figure 4. Antimicrobial effect of PBAT/PLA/albumen polymer blend: (a) Brilliant Green Sulfo Agar Control, (b) 20 μ L of *S. enteritidis* placed on agar as control, (c₁-c₃) PBAT/PLA 70/30 blend against *S. enteritidis*, (d₁-d₃) PBAT/PLA/albumen 70/30/0.5 blend against *S. enteritidis*, (e₁-e₃) PBAT/PLA/albumen 70/30/2.0 blend against *S. enteritidis*

Tables

Table 1. Thermal degradation of blended PBAT/PLA with albumen (ABM) determined using onset of degradation temperature (T_{donset}), temperature at 50 % degradation (T_{d50}), maximum degradation temperature T_{dmax} , and residual yield.

Specimen	T_{donset}	T_{d50}	T_{dmax1}	T_{dmax2}	Residue (%)
PBAT-PLA 70/30	288.06±5.88 ^{ab}	341.52±0.95 ^a	307.61±1.43 ^a	355.59±0.11 ^a	1.17±0.37 ^a
PBAT-PLA-ABM 0.5	295.52±1.79 ^{abe}	350.06±1.03 ^{bcd}	288.74±2.20 ^b	360.79±1.19 ^{bde}	4.39±0.35 ^{bcd}
PBAT-PLA-ABM 1.0	313.81±2.26 ^{cd}	352.54±1.41 ^{bcd}	282.94±1.34 ^c	362.80±0.47 ^{cde}	4.76±0.61 ^{bcd}
PBAT-PLA-ABM 1.5	308.42±1.85 ^{cd}	350.29±0.68 ^{bcd}	277.34±0.67 ^{de}	362.10±0.50 ^{bcd}	5.04±0.66 ^{bcd}
PBAT-PLA-ABM 2.0	299.68±2.36 ^{be}	346.91±2.34 ^{bde}	275.89±1.01 ^{de}	361.60±0.81 ^{bcd}	5.33±0.35 ^{bcd}

^a Mean values with different letters in the same column represent significant differences ($p < 0.05$) among the samples according to a one-way analysis of variance (ANOVA) and Tukey's multiple-comparison tests.

Table 2. Tensile properties of PBAT/PLA/albumen polymer blend systems.

Specimen	Tensile strength (MPa)	Elastic modulus (MPa)	Strain (%) at maximum load
PBAT/PLA 70-30	17.41 ± 0.46 ^{abcd}	333.43 ± 78.17 ^{abcde}	525.69 ± 53.46 ^a
PBAT/PLA/ABM 0.5	14.14 ± 1.87 ^{abcde}	242.82 ± 53.29 ^{abcde}	1016.24 ± 116.02 ^{bc}
PBAT/PLA/ABM 1.0	14.81 ± 1.85 ^{abcde}	259.30 ± 56.42 ^{abcde}	958.56 ± 150.45 ^{bc}
PBAT/PLA/ABM 1.5	14.02 ± 1.03 ^{abcde}	298.46 ± 46.24 ^{abcde}	17.89 ± 6.95 ^{de}
PBAT/PLA/ABM 2.0	13.16 ± 1.79 ^{abcde}	292.17 ± 49.72 ^{abcde}	14.29 ± 6.12 ^{de}

^a Mean values with different letters in the same column represent significant differences ($p < 0.05$) among the samples according to a one-way analysis of variance (ANOVA) and Tukey's multiple-comparison tests.

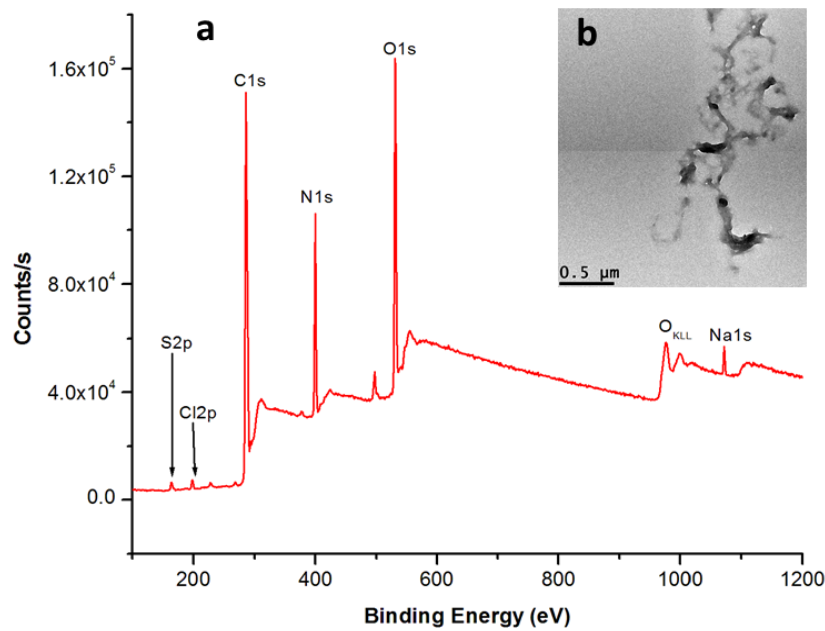
635 **Table 3.** Light transmittance values of PBAT/PLA/albumen polymer blend systems

636

Specimen	Transmittance (%)					
	380 nm	400 nm	500 nm	600 nm	700 nm	800 nm
637 PBAT/PLA-70-30	40.25 ± 3.13	18.54 ± 1.93	39.81 ± 2.48	60.17 ± 2.57	65.25 ± 2.29	69.24 ± 1.89
PBAT/PLA/ABM 0.5	11.24 ± 0.58	4.95 ± 0.18	11.86 ± 0.12	21.93 ± 0.44	24.74 ± 0.80	27.39 ± 1.21
638 PBAT/PLA/ABM 1.0	11.44 ± 0.92	5.03 ± 0.43	12.81 ± 0.95	23.94 ± 2.10	27.05 ± 2.86	30.10 ± 3.57
PBAT/PLA/ABM 1.5	11.44 ± 0.55	5.03 ± 0.18	12.81 ± 0.53	23.94 ± 0.88	27.05 ± 1.03	30.10 ± 0.97
639 PBAT/PLA/ABM 2.0	9.68 ± 0.36	4.3 ± 0.17	11.08 ± 0.36	20.16 ± 0.69	29.89 ± 0.89	33.50 ± 1.13

640 **Figures**

641



642

643 **Figure 1.**

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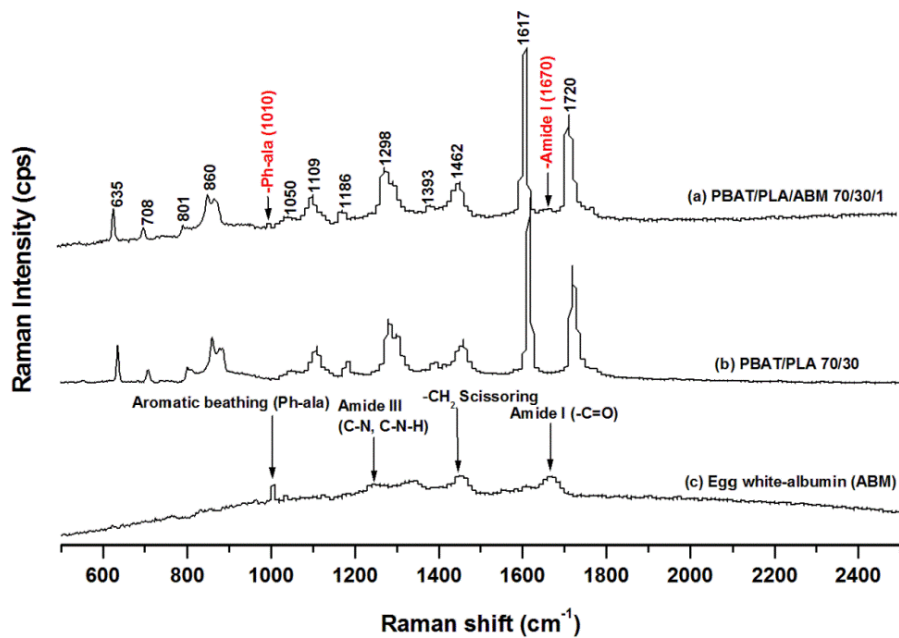


Figure 2.

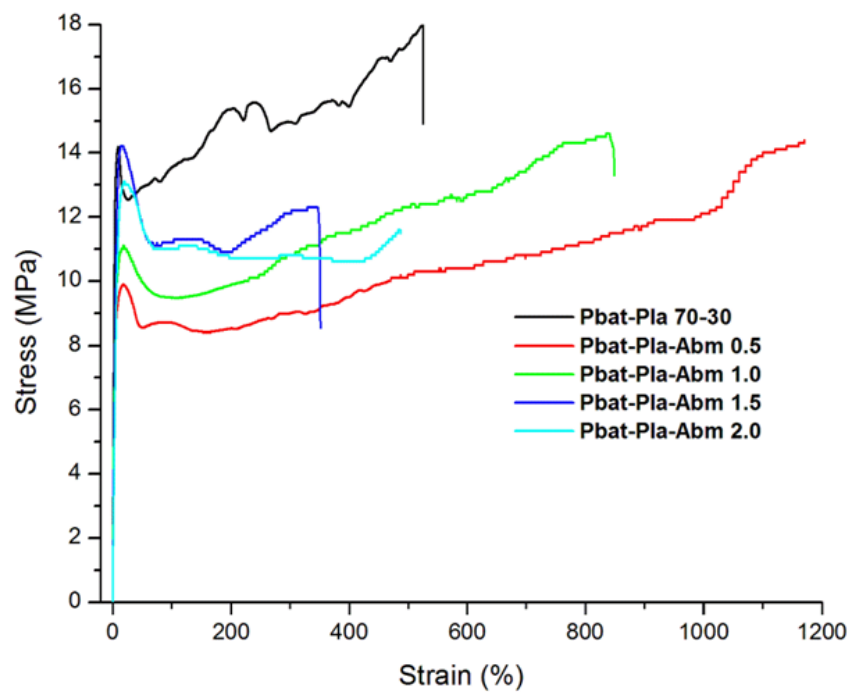


Figure 3.

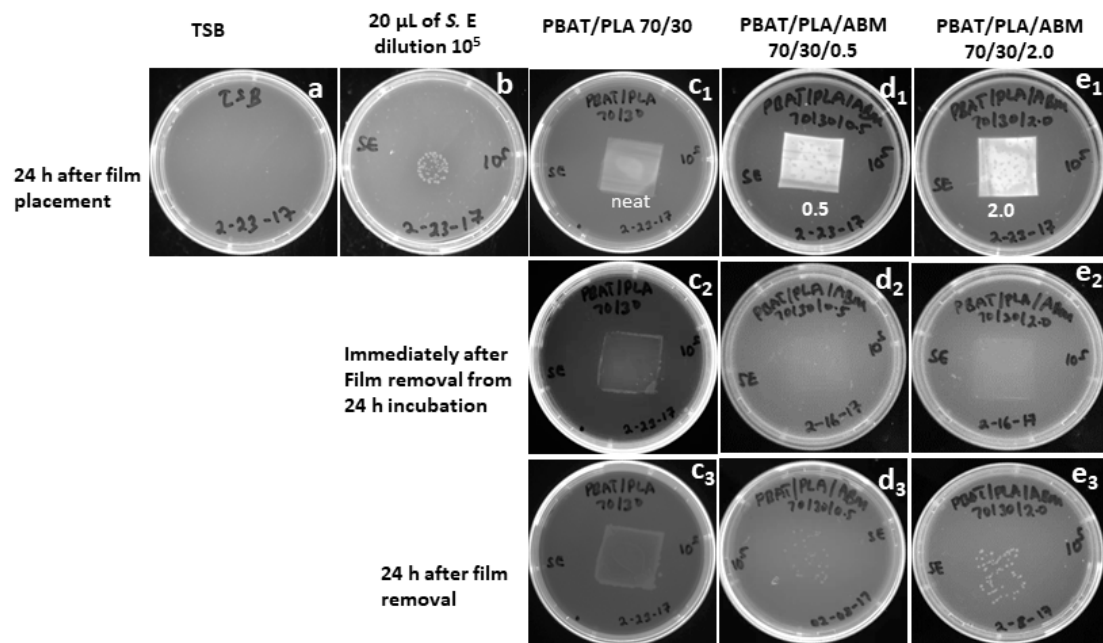


Figure 4.

Supplementary information

Tough aliphatic-aromatic copolyester and chicken egg white flexible biopolymer blend with bacteriostatic effects

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Figure S1. X-ray diffraction patterns: (a) PBAT/PLA 70/30 (b) PBAT/PLA/ABM 70/30/0.5 (c) PBAT/PLA/ABM 70/30/1 (d) PBAT/PLA/ABM 70/30/1.5 (e) PBAT/PLA/ABM 70/30/2 (f) ABM

Figure S2. DSC curves for ABM and PBAT/PLA/ABM blend composites.

Figure S3. Thermal degradation of PBAT/PLA/ABM polymer blends.

Figure S4. SEM micrographs:(a) PBAT/PLA 70/30 blend morphology; fractured surfaces after tensile analysis :(b) PBAT/PLA 70/30 binary blend, (c) PBAT/PLA/ABM 70/30/1ternary blend.

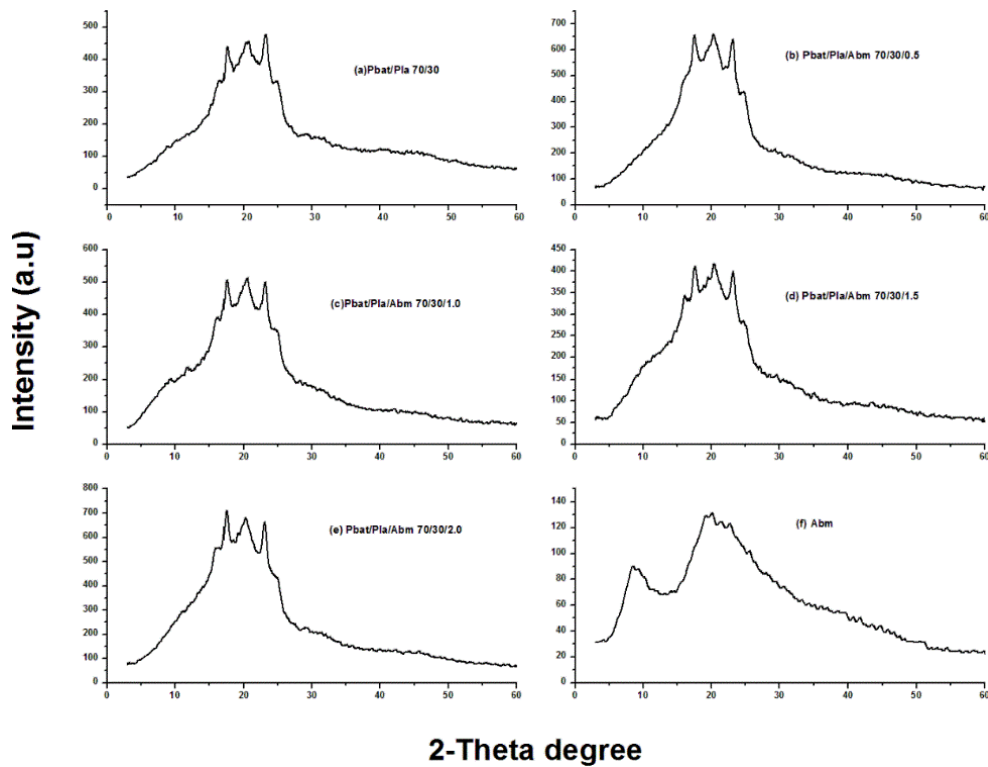


Figure S1. X-ray diffraction patterns: (a) PBAT/PLA 70/30 (b) PBAT/PLA/ABM 70/30/0.5 (c) PBAT/PLA/ABM 70/30/1 (d) PBAT/PLA/ABM 70/30/1.5 (e) PBAT/PLA/ABM 70/30/2 (f) ABM.

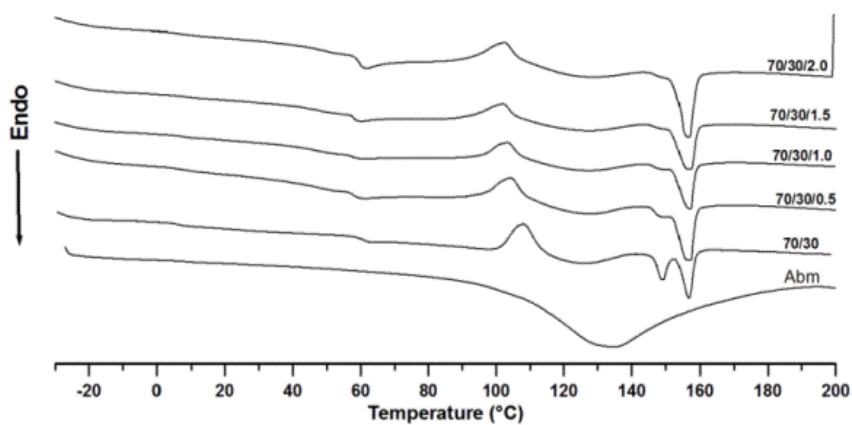


Figure S2. DSC curves for ABM and PBAT/PLA/ABM blend composites.

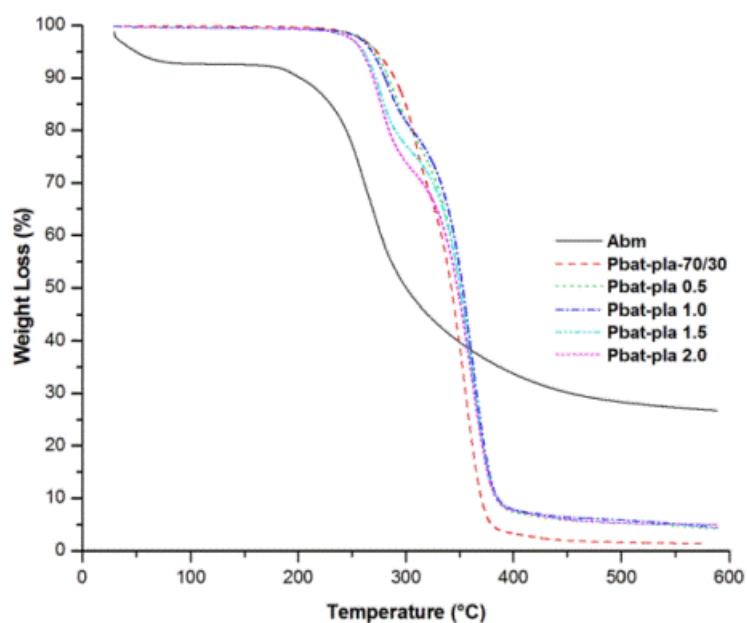


Figure S3. Thermal degradation of PBAT/PLA/ABM polymer blends.

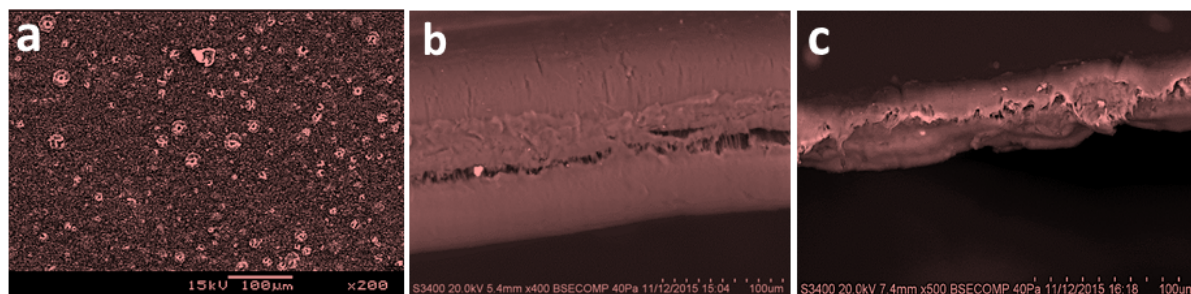


Figure S4. SEM micrographs:(a) PBAT/PLA 70/30 blend morphology; fractured surfaces after tensile analysis : (b) PBAT/PLA 70/30 binary blend, (c) PBAT/PLA/ABM 70/30/1% ABM ternary blend.