JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Microstructural Behaviors of Matrices Based on Polylactic Acid and Polyhydroxyalkanoates

Juan Carlos Alzate Marin,[†] Sandra Rivero,[*,†,‡] Adriana Pinotti,[†,§] Alejandro Caravelli,[†] and Noemí Elisabet Zaritzky[†,§]

[†]Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), CONICET, Universidad Nacional de La Plata (UNLP), Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CICPBA), 47 y 116 S/N, La Plata B1900AJJ, Buenos Aires, Argentina

[‡]Facultad de Ciencias Exactas, Universidad Nacional de La Plata (UNLP), La Plata B1900AJJ, Buenos Aires, Argentina

[§]Facultad de Ingeniería, Universidad Nacional de La Plata (UNLP), La Plata B1900AJJ, Buenos Aires, Argentina

ABSTRACT: Individual films of polyhydroxyalkanoates (PHA) and polylactic acid (PLA) and their blends were developed by solvent casting. PHA was obtained from activated sludges from a wastewater-treatment system at a laboratory scale. This work focused on analyzing the microstructural properties and thermal behaviors of individual films of PHA and PLA as well as those of their blends. The behaviors of the biodegradation processes of the individual films and blends were examined from a microstructural point of view. ATR-FTIR spectra indicated the existence of weak molecular interactions between the polymers. The formulation of blend films improved the crystallinity of PLA; additionally, it induced the polymer-recrystallization phenomenon, because crystallized PHA acted as a PLA-nucleating agent. This phenomenon explains the improvements in the films' water-vapor-barrier properties. The blends exposed to a biodegradation process showed an intermediate behavior between PLA and PHA, leading to a consistent basis for designing systems tailored to a particular purpose.

KEYWORDS: *biopolymers, polymer blends, infrared spectroscopy, crystallinity, modulated DSC, morphology*

INTRODUCTION

Polyhydroxylalkanoate (PHA) is a family of biopolyesters synthesized by microorganisms from various carbon sources; they are accumulated intracellularly under nutrient stress and act as carbon and energy reserves.[1] PHAs can be produced by both pure and mixed microbial cultures. Mixed cultures have the advantage that they do not need sterile conditions and are better able to adapt to complex substrates, such as industrial wastes, than pure cultures.[2] For this, wastes from the dairy industry, the sugar industry, forestry, and biodiesel production can be utilized.[3] Cheese whey is a surplus material from the dairy industry that is mainly discharged to both soils and water bodies, causing serious environmental problems. PHA production from cheese whey could revalorize this dairy subproduct and, at the same time, solve the waste-disposal problems.

A relevant characteristic of PHA is its versatility, because there are more than a hundred different monomers, including hydroxyvalerate and butyrate among others. The properties of the synthesized polymer are modified depending on the position of its functional groups and degrees of polymerization. This chemistry allows PHAs to be tailored to provide similar properties to traditional thermoplastics, such as polyethylene (PE) and polypropylene (PP), while maintaining biodegradability.[4]

Polylactic acid (PLA), a thermoplastic aliphatic polyester derived from lactic acid (2-hydroxy propionic acid), can be produced by condensation and polymerization directly from its basic building block, lactic acid, which is derived from the fermentation of sugars from carbohydrates sources, such as corn starch; cassava root, chips, and starch; and sugar cane.[5] Polylactic acid is produced at the largest industrial scale of all biodegradable polymers, and it is considered the most promising candidate for replacing conventional plastics.[6] It is being used in biomedical applications, for bottle production, for filament production in 3D printing, and for compostable-food-packaging production. It is also being evaluated as a material for tissue engineering. Mass production has reduced the cost of PLA production, making it an economically viable choice for the fabrication of containers, plastic bags, and fibers. Commercial-scale plants today produce hundreds of thousands of tons of PLA per year.[7]

By controlling the molecular architecture, suppliers are able to tailor the polymer to specific applications. Consequently, new and improved grades of bioplastic resins are constantly being introduced to the market. NatureWorks LLC focuses on a multistep procedure involving the condensation reaction of aqueous lactic acid for the production of a low-molecular-weight PLA prepolymer. It is converted into a blend of lactide stereoisomers in order to produce a large spectrum of PLA grades.[8] The films based on PLA have excellent optics and good machinability, barrier, and mechanical properties.[5]

PLA and PHA polymers are polyesters and are used in consumer products by a wide industrial sector as a result of their biocompatibility and sustainability.[9] They have thermal

Received: March 25, 2018
Revised: July 14, 2018
Accepted: July 23, 2018
Published: July 23, 2018

DOI: 10.1021/acs.jafc.8b01506
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

behaviors comparable to those of some conventional polymers, and this has generated much interest in exploring their physical and structural properties to identify potential applications.[10]

The combination of the two polymers allows the design of new materials with tailorable properties differing meaningfully from those of each component by adjusting the advantages of each polymer in order to obtain materials for different applications. In this sense, the formulation of blend systems is easier and faster than copolymerization methods.[11] There are many studies focused on PLA modification, such as the addition of modifiers and copolymerization.[12] The addition of a highly ordered stereochemical structure, such as that of PHA, could improve the film properties of PLA.[13,14] In this sense, blending PLA with another biobased and biodegradable material, PHA, in order to obtain tailor-made materials constitutes a promising alternative.

This work focused on analyzing the microstructural properties and thermal behaviors of PHA and PLA individual films as well as those of their blends. Furthermore, the biodegradation processes of the individual films and PLA/PHA blends were examined from a microstructural point of view.

MATERIALS AND METHODS

Polylactic acid (PLA), grade 4043D in pellet form (98% L-lactide with a D-isomer content of approximately 2%) and designed for the production of films, was purchased from Natureworks under the trademark Ingeo. As is well-known, the production of PLA involves the conversion of starch in dextrose via a hydrolysis process and fermentation by microorganisms to synthesize the chain of the polylactide polymer.

Polyhydroxylalkanoate (PHA) was obtained from activated sludges from a wastewater-treatment system at the laboratory scale (CIDCA). A two-stage biological system was proposed to produce PHA by aerobic mixed culture from hydrolyzed cheese whey. The first step involved the selection of PHA-accumulating organisms using an aerobic sequencing batch reactor (SBR) operated under a feast−famine regime. Then, there was the stage of PHA accumulation in an aerobic batch reactor that was inoculated with biomass taken from SBR enriched with PHA-accumulating bacteria.

Extraction and Quantification of PHA. Extraction and characterization of PHA from the batch-reactor biomass were performed following the method proposed by Venkateswar Reddy et al.[15] A hydroxybutyrate−hydroxyvalerate (HB−HV) copolymer with a purity of 96% and a molar relation 95:5 HB−HV was obtained.

Film Preparation. The films were prepared by dissolving PHA and PLA in chloroform at 1 and 2% (w/v), respectively, under stirring for 3 h. PLA/PHA mixtures were prepared in the following proportions: 20/80, 40/60, 60/40, and 80/20 (w/w). They were stirred for 30 min at 60 °C.

Filmogenic solutions of PLA, PHA, and their blends were cast into glass Petri dishes (9 cm diameter) that were left under a fume hood. Later, the samples were dried in a vacuum oven at 60 °C to ensure solvent removal from the matrices. When the solutions were cast, constant mass−molding-area ratios were maintained in order to ensure uniform thicknesses in the different samples, because the control solutions (PLA and PHA) had different concentrations.

Film thickness was measured by means of a coating-thickness gauge (Check Line DCN-900) for nonconductive materials on nonferrous substrates. For each specimen, at least 14 measurements at different positions were taken.

Water-Vapor Permeability. Water-vapor-permeability (WVP) tests were carried out according to the technique described by Rivero et al.[16] based on a modified version of ASTM[17] method E96. A permeation cell maintained at 20 °C was used for the assays. Eight measurements were performed once steady-state conditions were reached. All measurements were performed at least four times for each specimen.

Thermal Analysis by MDSC. Thermal analysis of the samples was conducted by the modulated-differential-scanning-calorimetry (MDSC) technique. Q100 (TA Instruments) controlled by a TA 5000 module (TA Instruments) and equipped with a cryogenic-quench-cooling accessory was used under a N_2 atmosphere. This technique allowed us to measure the calorific capacity of the sample and simultaneously differentiate between the reversible (dependent on the calorific capacity) and irreversible events (dependent on the time and temperature) that the sample experienced. Likewise, it allowed the resolution of complex transitions.

A standard heating ramp of 10 °C min^{-1} with a modulation-temperature amplitude of 0.5 °C and a modulation period of 60 s was used. The first scan (heating) was performed in order to delete the thermal story from −50 °C up to 200 °C. Once the first scan was completed, the sample was cooled to −80 °C, and then a second heating scan was recorded. Informed results were the averages of two replicates to ensure reproducibility of the results.

The results were analyzed by using the Universal Analysis V1.7F software (TA Instruments). Different parameters were determined from the obtained thermograms: melting temperature (T_m), enthalpy corresponding to the area of the endothermic peak (ΔH_m), crystallization temperature during the cooling stage (T_c), crystallization enthalpy (ΔH_c), and glass-transition temperature (T_g) determined from the reversible signal.

ATR-FTIR Spectroscopy. The interactions between the system components were analyzed using the ATR-FTIR technique. Spectra of the samples were registered by using the Thermo Scientific Nicolet iS10 FT-IR Spectrometer in the wavenumber region of 4000−400 cm^{-1}, performing 64 scans at a 4 cm^{-1} resolution. Specimens were placed onto the diamond ATR crystal (Smart iTX accessory), and the software Omnic 8 (Thermo Scientific) was used for the data-analysis process. To ensure the reproducibility of the results, the tests were performed in triplicate.

X-ray Diffraction. X-ray diffractograms of individual PVA and PLA films and their blends were evaluated by using an X'Pert Pro P Analytical Model PW 3040/60 (Almelo) operated at room temperature. The Cu Kα radiation (1.542 Å) was generated at 30 mA and 40 kV. Scattered radiation was detected in an angular range of 3−60° (2θ) at a step size of 0.02°. The crystallinity degree (CD) was calculated following the procedure described in previous works.[18,19]

Structural Studies by SEM. The microstructures of the matrices were examined by using an FEI model Quanta 200 scanning electron microscope. Individual films and blend matrices were immersed in liquid nitrogen and fractured cryogenically. Samples were mounted onto metal stubs by using double-sided tape and observed at low pressure and an acceleration voltage of 12.5 kV, without any metal or carbon coating.

Biodegradation Test. A series of plastic pots (400 cm^3) were used as soil containers, and the microflora present was used as a degrading medium. Square specimens of 3 × 3 cm with a thickness of approximately 25 μm on average were put into a plastic mesh to allow the access of moisture and microorganisms and the retrieval of degraded specimens. In order to guarantee aerobic degradation, the film samples were buried. The samples were conditioned at a relative humidity of 50% and a temperature of 20 °C. Containers were sprayed regularly with a defined and constant amount of water. Similar conditions were employed by Rivero et al.[20] At specific time intervals, specimens were recovered from the soil and cleaned with a brush to avoid any possible damage of the structure.

In accordance with the described methodology in ASTM D5988-03,[21] a ratio of 1 g of compost per 25 g of soil was used. Studies of the morphology of the degraded films were performed by means of SEM, MDSC, and ATR-FTIR analysis.

Statistical Analysis. For statistical analysis, version 10.0 of Systat-software (Systat, Inc.) was used. Analysis of variance (ANOVA), linear regressions, and Fisher's least-significant-difference test for mean comparison were performed. The significance level used was 0.05.

DOI: 10.1021/acs.jafc.8b01506
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

RESULTS AND DISCUSSION

Thermal Analysis. The thermograms obtained by means of MDSC from PLA, PHA, and their blends can be observed in Figure 1, where the stages of heating and cooling are shown. The temperatures of the thermal transitions and the enthalpy values (J g^{-1}) of the characteristic events during the heating and cooling stages obtained from MDSC curves are summarized in Table 1.

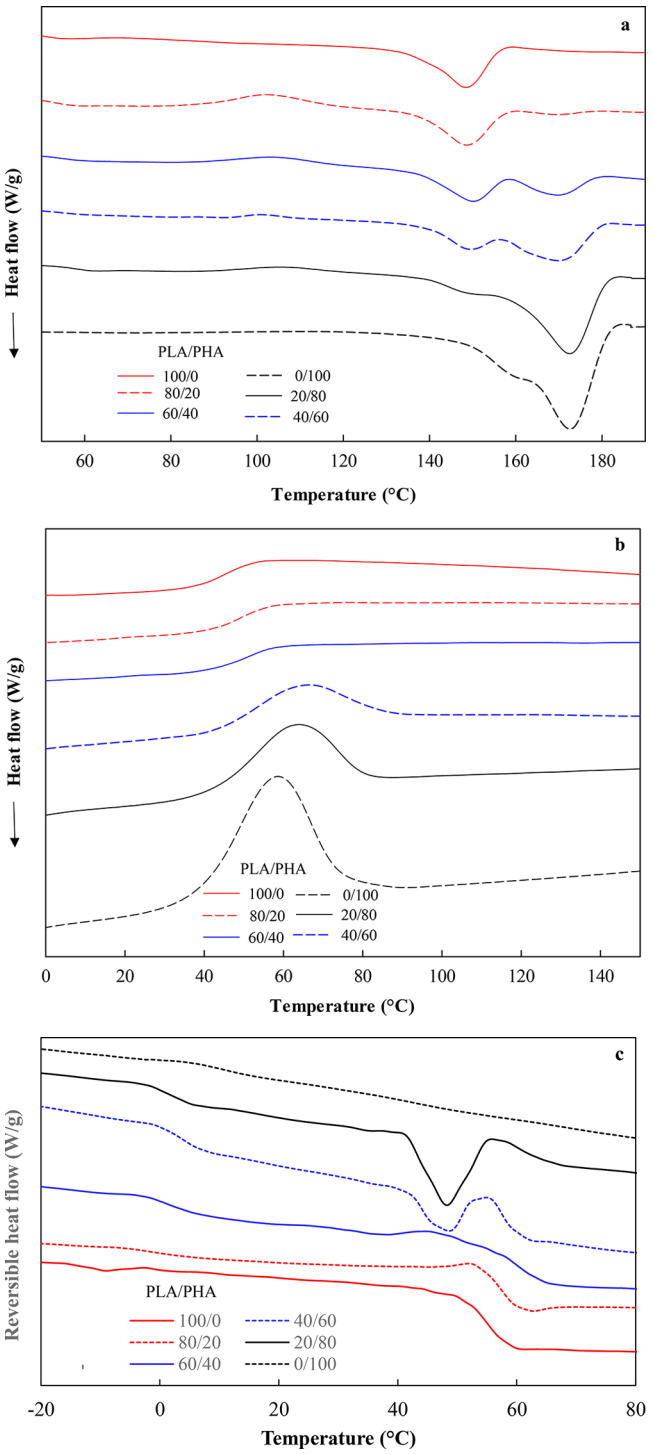

Figure 1. MDSC curves of individual (PLA and PHA) and blend films with different proportions of PLA/PHA showing (a) heating stage, (b) cooling stage, and (c) reversing heat flow (W g^{-1}), evidencing the glass-transition temperatures (T_g) of each component.

As it is possible to see in Figure 1a, the thermal analysis allowed us to observe a peak for each individual film, attributable to the melting of the crystalline domains corresponding to PLA and PHA at 147.7 and 172.7 °C, with the associated enthalpies of 20.1 and 86.6 J g^{-1}, respectively. The intensity of the melting peak is related to the number of crystals or crystalline populations (crystalline fraction) that exist in the material when the melting occurs. Using MDSC, Thellen et al.[4] reported that crystal melting is produced at higher temperatures in samples of PHA containing lower percentages of valerate, because as the valerate content increases, the onset of crystal melting occurs at lower temperatures. In the present study, the PHA thermograms evidenced the presence of a peak with a shoulder, which could represent the melting of crystal lamellae, as was reported by Thellen et al.[4] for extruded PHA.

On the other hand, PHA films exhibited a T_g at −4.7 °C, whereas PLA showed a marked transition at 58.1 °C (Figure 1c). Glass-transition temperatures ranging from 2.7 to −4.4 °C for a PHA matrix obtained by casting were reported by da Silva et al.[22]

The thermograms of all the mixtures exhibited PLA-recrystallization peaks at 107 °C; this transition was more evident with higher PLA proportions in the mixture (Figure 1a). These results indicate that the incorporation of PHA contributes to the process of recrystallization of PLA in all the blend formulations. This effect was more marked in the 80/20 blend, a behavior that could be explained by bearing in mind that a low proportion of crystalline domains of PHA dispersed in a continuous matrix of PLA induced the phenomenon of nucleation of this latter polymer. The presence of double melting temperatures in the PLA/PHA blends corresponds to the melting of both the "as formed" and recrystallized polymer.[10]

According to Abdelwahab et al.[10] and Ikehara et al.,[23] recrystallization strongly depends on the melting-temperature differences among the components of the mixture. When the T_m difference is very large, the component with the higher melting temperature crystallizes first, and its spherulites contribute to fill all the volume.

The 20/80 blend exhibited a peak at 171.9 °C because of the melting of the PHA crystalline fraction and a shoulder at 148.4 °C corresponding to the melting of PLA. The 80/20, 60/40, and 40/60 blends exhibited two endothermic transitions; the enthalpy associated with the first event diminished with the incorporation of a higher proportion of PHA to the blend, whereas the enthalpy of the endothermic peak, characteristic of the PHA-crystalline phase, became more prominent (Figure 1a and Table 1). These findings were in accordance with a higher crystalline degree.

From the obtained DSC curves, two T_g values were observed irrespective of the blend composition. These results suggest that the analyzed PLA/PHA blends are immiscible in the amorphous state (Figure 1c).

According to Lipatov and Alekseeva,[24] the appearance of two transitions associated with the glass-transition temperatures supports the existence of a partially miscible system (i.e., materials with a heterogeneous biphasic structure).

During the cooling step, PHA exhibited a peak of crystallization at 58.7 °C with an associated enthalpy of 48.4 J g^{-1}, whereas the thermogram of PLA did not show this

DOI: 10.1021/acs.jafc.8b01506
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Table 1. Thermal Analysis of Single and Blend Matrices with Different Proportions of PLA/PHA[a]

	heating stage				cooling stage	
	characteristic events of PHA		characteristic events of PLA		characteristic events of PHA	
PLA/PHA	T_m (°C)	ΔH_m (J g^{-1})	T_m (°C)	ΔH_m (J g^{-1})	T_c (°C)	ΔH_c (J g^{-1})
100/0	—	—	147.7(1.0) a	20.1(1.3) c	—	—
80/20	170.4(1.2) a	1.5(0.07) a	150.3(1.9) a	20.0(0.7) c	—	—
60/40	169.9(0.6) a	9.1(0.1) b	149.8(0.6) a	13.4(0.5) b	—	—
40/60	170.3(0.4) a	18.8(0.7) c	149.5(0.9) a	5.8(0.1) a	65.6(0.5) b	18.8(0.1) a
20/80	171.9(0.8) a	61.7(1.2) d	—	—	65.9(3.8) b	31.4(1.9) b
0/100	172.7(0.1) a	86.6(0.1) e	—	—	58.8(0.5) a	48.4(0.4) c

[a]Different letters in the same column indicate significant differences ($p < 0.05$) among samples.

transition (Figure 1b and Table 1). Taking these results into account, it is possible to infer that the crystallinity of PHA is greater than that of PLA.

The thermal analysis of the PLA/PHA mixtures showed that the peak of crystallization attributable to PHA was observed only in the 20/80 and 40/60 mixtures, with a minor associated enthalpy regarding a single PHA matrix (Figure 1b). On the other hand, the thermograms of the individual PLA film and the 60/40 and 80/20 mixtures did not exhibit this thermal transition (Figure 1b). Nevertheless, an event was observed at 57.7 °C attributable to the glass-transition temperature of the PLA-enriched phase. Similar results were informed by Arrieta et al.,[12] Furukawa et al.,[25] and Zhang and Thomas,[11] indicating that these results are a signal of the fact that the PHA in the mixtures does not crystallize during the cooling stage when it is the minor proportion.

Microstructural Analysis. *FTIR Analysis.* From the ATR-FTIR spectra, the functional characteristic groups of PLA and PHA were identified (Figure 2a,b). They are consistent with those reported in the literature.[5,26,27]

In the PLA spectrum, the band located at 1746 cm^{-1} is a strong peak that corresponds to the stretching of the carbonyls' amorphous phase.[5] The bands located at 1180 and 1080 cm^{-1} belong to the asymmetric and symmetric stretching vibrations of C−O−C. As is known, PLA is a hydrophobic polymer because of the presence of −CH side groups. The peaks at about 2997 and 2946 cm^{-1} correspond to the asymmetric and symmetric stretching vibrations of the −CH groups in the side chains, respectively (data not shown).

On the other hand, in the PHA spectrum, the peak at 1719 cm^{-1} is attributable to the stretching of the crystalline carbonyl group, and the stretching of the carbonyl group of the amorphous phase is virtually imperceptible. The 40/60 and 20/80 samples exhibited peaks at 1748 and 1755 cm^{-1} (Figure 2b), which were assigned to the amorphous and crystalline bands of PLA, respectively.[24]

Furthermore, Figure 2a,b depicts the ATR-FTIR spectra of the PLA/PHA blend matrices. The spectra of the 80/20 and 60/40 compositions followed a similar pattern to that of the pristine PLA, whereas the 40/60 and 20/80 mixtures were identified mainly with the PHA spectrum. All of them unfolded both bands corresponding to the stretching of carbonyls located at 1746 and 1719 cm^{-1} due to PLA and PHA phase, respectively (Figure 2a,b).

In the cases of the 80/20, 60/40, and 40/60 blends, the bands in the C−O−C and C−C stretching and CH deformation mode region (1300−1000 cm^{-1}) are visualized at about the same wavenumbers as those of pure PHA (Figure 2a,b).

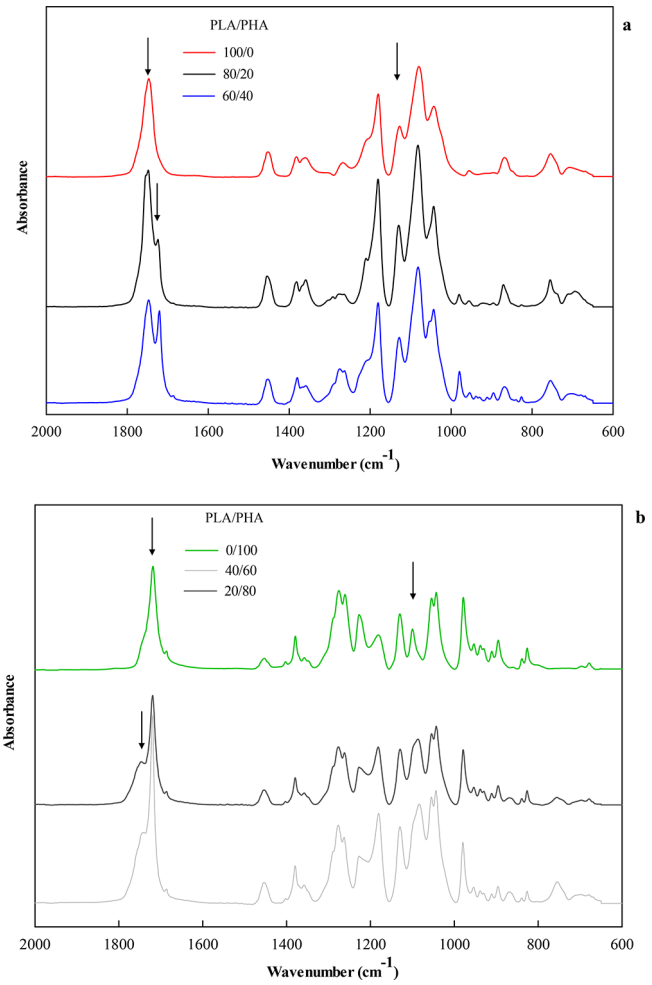

Figure 2. ATR-FTIR spectra of individual (PLA and PHA) and blend matrices with different proportions of PLA/PHA.

It is noteworthy that the intensities of these bands were interdependent and mutually dependent, one being increased at the expense of the other, meaning that the ratio of intensities of these two bands changed in agreement with the ratio of the individual components. These results allowed us to infer the immiscibility of these components and that there were no strong molecular interactions between the polymers.

X-ray Diffraction. The typical diffraction patterns of individual PHA and PLA and those of the composite films are depicted in Figure 3. The X-ray diffractogram of the PLA films exhibited a pattern corresponding to an amorphous material with a peak located at $2\theta = 17°$, which was associated with the reflection of the crystals of the polymer;[13,28] the

DOI: 10.1021/acs.jafc.8b01506
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

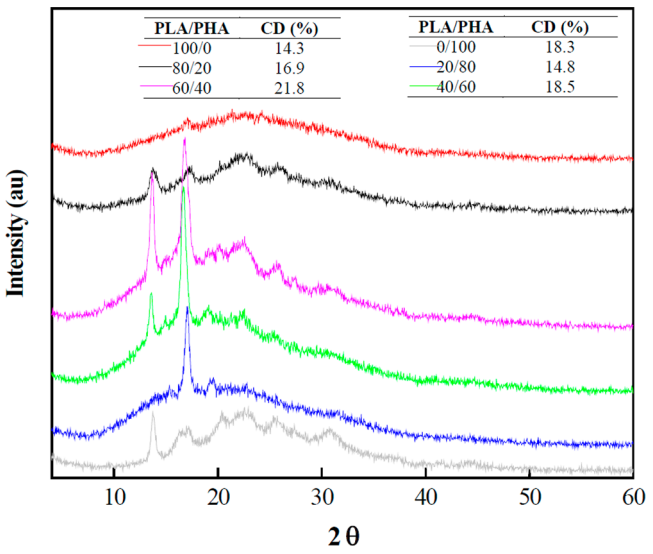

Figure 3. X-ray diffractograms of individual (PLA and PHA) and blend films with different proportions of PLA/PHA. Table inset shows the crystallinity degrees (%) of the samples.

estimated crystallinity degree was 14.3% (table insert in Figure 3). In contrast, PHA is a highly ordered polymer, and it is known to crystallize in an orthorhombic cell.[10]

X-ray-diffraction analysis was used to determine the crystalline structures and crystallinity degrees of the blends. The PHA spectrum showed diffraction peaks at 2θ = 13.8 and 17°, corresponding to the (020) and (110) planes, respectively.[11] Additionally, reflections at 2θ = 20.3, 22.4, 25.4, and 30.8° were detected, which were characteristics of the PHA polymer, its CD being 18.3%.[14]

The inclusion of PHA in the blends increased the crystallinity of the materials. The highest crystallinity degrees of the 60/40 and 40/60 blends were confirmed by means of X-rays (table insert in Figure 3). The diffractograms showed peaks of higher intensity in relation to individual matrices.

This behavior allows us to infer that PHA induces the recrystallization of PLA due to its capacity to act as an agent of nucleation. According to Furukawa et al.[24] and Zhang and Thomas,[11] it generates better packing density of the polymeric segments and promotes better adhesion and interaction at the interface level. However, the microstructure, porosity, and permeability of the matrix seem to depend on the PLA/PHA proportions, as presented below.

SEM Observations. From the macroscopic point of view, the blend of the two polymers appeared to be well mixed in the matrices with no apparent phase separation, although FTIR showed the immiscibility of both components. The average thickness of the films was 25 μm, which was corroborated by microscopic analysis. As it can be seen in Figure 4, all matrices presented homogeneous appearances irrespective of the PLA/PHA ratio.

The films showed important differences when analyzed in the cross-sections of single or blend matrices. PLA-only films showed a homogeneous appearance, without pores and with good structural integrity. Addition of the PHA polymer led to films whose cross-section was rough, giving a structure with a fibrous appearance (Figure 4).

The cross-sections and surfaces of the 20/80 and 40/60 mixtures revealed the presence of an orderly structure, attributable to the growth and crystallization of the spherulites

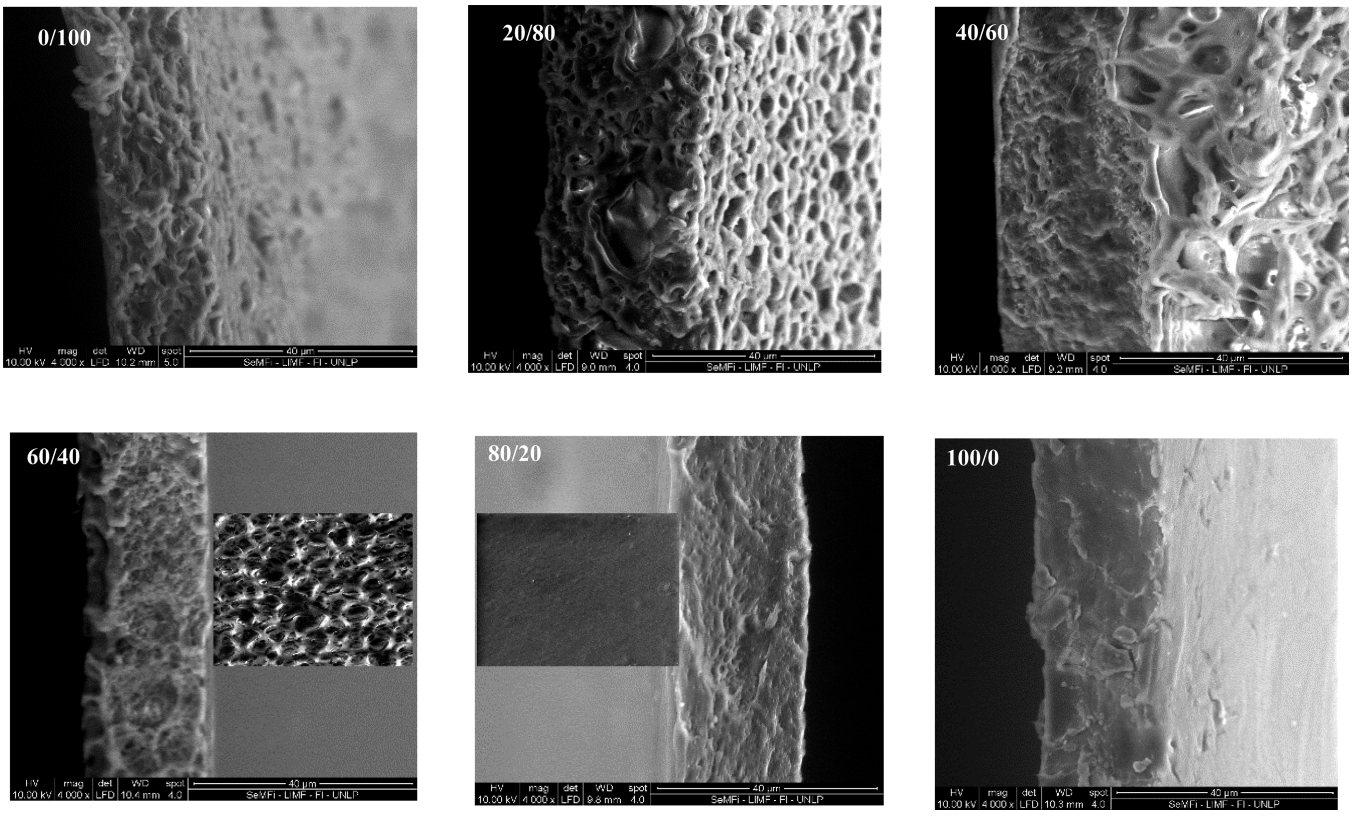

Figure 4. Cross-section SEM micrographs of individual (PLA and PHA) and blend films with different proportions of PLA/PHA.

DOI: 10.1021/acs.jafc.8b01506
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

of PHA, proving to be a network formed by codomains corresponding to the different polymers with a granular, interspersed appearance and a repetitive pattern. It is important to remark that the highest proportion of PHA in the blends (PLA/PHA = 20/80) evidenced a porous microstructure as a result of the structural arrangement between the polymers. According to Abdelwahab et al.,[10] the interface interactions between the PHA and PLA phases could influence the nucleation phenomenon as supported by the XRD results.

These micrographs reinforced the results of immiscibility obtained by other techniques. As the PLA proportion increased, the morphology of the matrices became more homogeneous and less rough, until the 80/20 mixture presented an appearance where the PLA-enriched phase formed a continuous domain in which the PHA-enriched phase was dispersed and homogeneously distributed, as can be seen in Figure 4.

Water-Vapor Permeability. Table 2 shows the WVP values of the individual films and PLA/PHA blends. The formulation

Table 2. Water-Vapor-Barrier Properties of the Individual (PLA and PHA) and Blend Films with Different Proportions of PLA/PHA[a]

PLA/PHA	WVP ($\times 10^{11}$ g s^{-1} m^{-1} Pa^{-1})
100/0	1.73(0.25) b
80/20	0.62(0.06) a
60/40	0.68(0.07) a
40/60	0.72(0.05) a
20/80	2.22(0.38) b
0/100	2.24(0.30) b

[a]Different letters indicate significant differences ($p < 0.05$) among samples.

of the blend matrices improved the barrier properties of the materials, obtaining a reduction of 40% in the WVP values for the 40/60, 60/40, and 80/20 blends compared with the values of the individual films (Table 2). Meanwhile, the 20/80 blend did not show significant differences ($p > 0.05$).

According to Arrieta et al.,[12] the crystallization of PLA reduces the water-vapor permeability because the crystals decrease the volume of the amorphous phase, generating a path of greater tortuosity and reducing mass matter transfer.

The improvement of the barrier properties is related to the higher crystallinity of the PLA/PHA blend matrices, as observed by the X-ray diffraction technique and the MDSC thermal analysis.

Soil-Biodegradation Studies. The influence of the microstructure developed during the formulation of the blends on the biodegradation process was examined. A morphological study by SEM during soil-biodegradation experiments was complementary to visual examination as it helped to confirm the existence of structural modifications in the films, allowing a detailed evaluation of the degradation process.

The results showed that the degradation behavior of the biobased polymers followed different patterns regarding the morphological characteristic of each matrix. As can be seen in Figure 5a, the biodegradation occurred in both the PHA and PLA phases.

The biodegradation assay revealed that the PHA films exhibited remarkable biodegradation after 30 days, whereas the blend matrices (PLA/PHA) showed changes around 50 days, which indicated a delay in the process of biodegradation (Figure 5a). According to Weng et al.,[27] a PHA polymer could be biodegraded at a high rate under composting as well as soil-environment conditions; in contrast, although PLA was biodegradable under composting conditions, its degradation rate in soil was slow.

SEM micrographs show the fractured surface of the PLA/PHA matrices after a biodegradation process of 50 days (Figure 5a). It can be seen that the biodegradation was intensified with higher proportions of PHA in the blend system. As the proportion of PHA increased, particularly from 40/60 to 20/80, the matrices showed greater interstices or cavities (Figure 4), which could facilitate the biodegradation process. Zhang and Thomas[11] explained that the inclusion of PHA improved the degradation degree of PLA at room temperature. The authors reported that PHA and PLA exhibit different degradation patterns. PHA is mainly degraded by the attack of various enzymes at the surface. According to Weng et al.[27] the degradation of PHA can be attributed to erosion catalyzed by bacteria from the surface to the interior.

In contrast, PLA degradation is produced throughout the whole sample, starting with a nonenzymatic hydrolysis that leads to a reduction in the molecular weight.[11] Then, low-molecular-weight PLA diffuses out of the bulk polymer and can be metabolized by microorganisms, producing water, carbon dioxide, and humus.[29]

On the other hand, the thermograms of the 20/80, 40/60 and 60/40 blend matrices exposed to the biodegradation process for 50 days showed a decrease of the transition enthalpy, corresponding to the melting of the PHA crystalline fraction being more marked in the blends with the greatest proportion of PHA (Figure 5b). In this sense, Dharmalingam[30] found that the changes of melting enthalpy (ΔH_m) for a composite material based on PLA/PHA blends represent depolymerization. Consequently, this phenomenon could indicate a higher sensitivity to the degradation process in the presence of higher proportions of PHA, independent of the rearrangement formed between both polymers.

The chemical-structure changes of the matrices exposed to the biodegradation process were investigated by ATR-FTIR (Figure 5c).

ATR-FTIR spectra corroborated the changes caused in the structure of the polymers. After 50 days of exposure to the biodegradation process, ATR-FTIR spectra of the PHA and blend films showed that the bands lost definition.

For all the samples assayed, the peaks in the 4000−3000 cm^{-1} region became broadened after the biodegradation process (data not shown). According to Weng et al.,[27] this fact was attributable to the formation of hydroxyl and carboxylic groups. This was also confirmed by the shift of the absorption peak of the C=O stretching vibration after biodegradation.

Ludueña et al.[31] pointed out that chemical and enzymatic hydrolysis are the main mechanisms of chain rupture during the degradation process. Then, biodegradation process depends on water availability, which produces hydrolysis, and microbial attack of the matrix. Water diffusion in the soil leads to a hydrolytic degradation of the material modifying the surface, resulting in a porous structure with evident signs of degradation. A similar explanation was proposed by Rocca-Smith et al.[32]

DOI: 10.1021/acs.jafc.8b01506
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

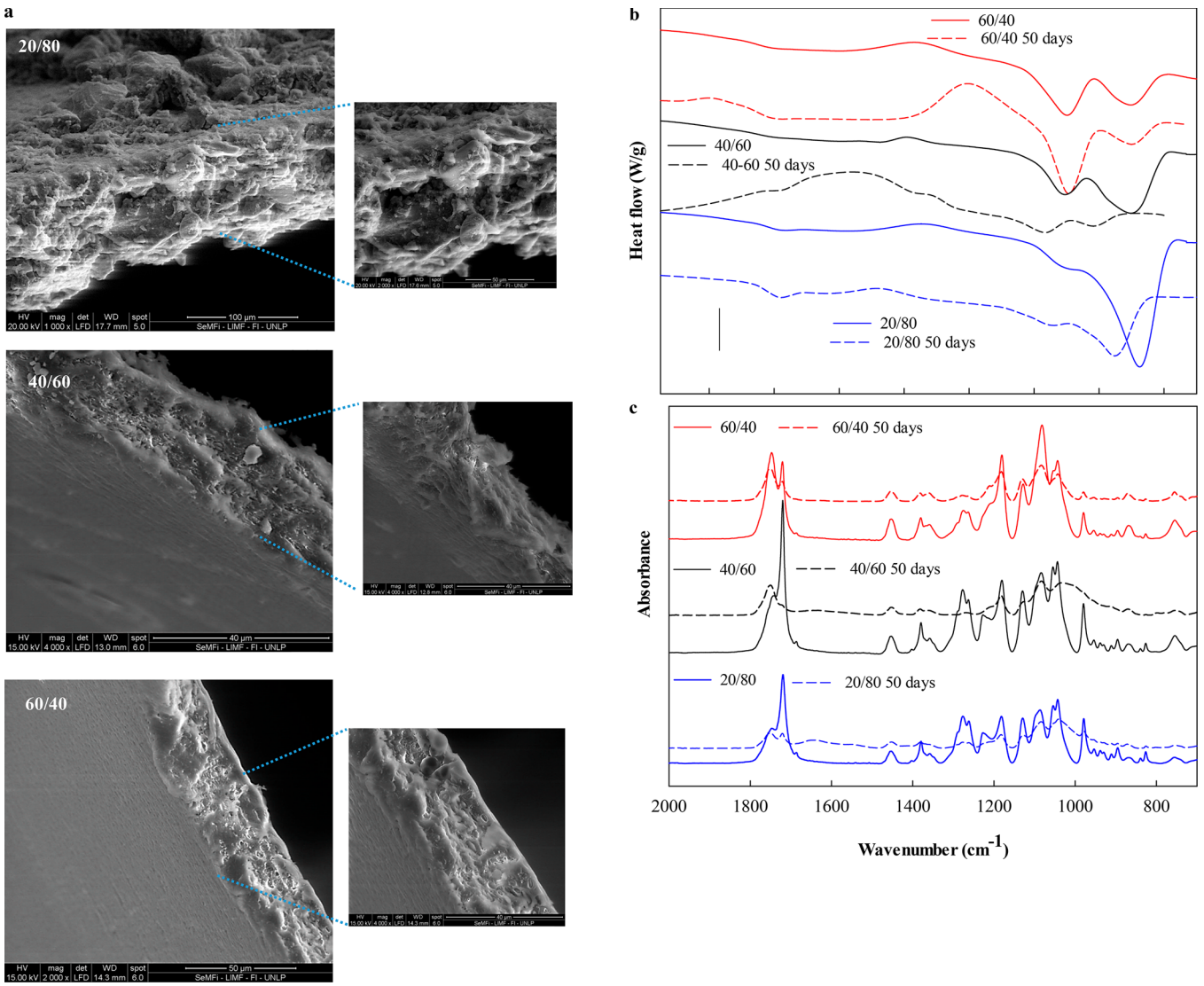

Figure 5. Monitoring of the behaviors of samples after they were submitted to biodegradation processes (buried for 50 days) through (a) SEM micrographs of the PLA/PHA blend matrices with enlarged sections showing the degradation (magnification is indicated in the micrographs), (b) MDSC analysis, and (b) FTIR-ATR studies.

The biodegradability of the blends was more marked in films formulated with increasing proportions of PHA; similar results were reported by Zhang and Thomas.[11]

Conclusions. Thermal and microstructural studies revealed that blends of PLA/PHA-based systems were partially miscible. The formulation of blend matrices improved the crystallinity of PLA; additionally, it induced the polymer-recrystallization process, because crystallized PHA acted as a PLA-nucleating agent. This phenomenon explains the improvements in water-vapor-barrier film properties. Moreover, the blends exposed to a biodegradation process showed an intermediate behavior between those of PLA and PHA, leading to a consistent basis for designing systems tailored to a particular purpose or use.

This work, therefore, contributes to the knowledge of microstructures, allowing one to develop and test materials with specific technological applications.

AUTHOR INFORMATION

Corresponding Author

*E-mail: sandra_gmr@yahoo.com. Tel. and Fax: +54 0221-424-9287, +54 0221-425-4853, or +54 0221-489-0741.

ORCID

Sandra Rivero: 0000-0002-9002-182X

Funding

This work was supported by the Argentinean Agency for the Scientific and Technological Promotion (ANPCyT; Project PICT 2012-0415, 2014-1620), the Argentinean National Research Council (CONICET, PIP 2013-0109), and the University of La Plata (Argentina).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors acknowledge Ing. Javier Lecot, Lic. Diana Velasco, and Daniel Russo for technical assistance.

REFERENCES

(1) Ren, J. *Biodegradable Poly (Lactic Acid): Synthesis, Modification, Processing*; Springer-Verlag: Berlin, 2011.

(2) Colombo, B.; Sciarria, T. P.; Reis, M.; Scaglia, B.; Adani, F. Polyhydroxyalkanoates (PHAs) production from fermented cheese

DOI: 10.1021/acs.jafc.8b01506
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

whey by using a mixed microbial culture. *Bioresour. Technol.* **2016**, *218*, 692−699.
(3) Mothes, G.; Schnorpfeil, C.; Ackermann, J.-U. Production of PHB from crude glycerol. *Eng. Life Sci.* **2007**, *7*, 475−479.
(4) Thellen, C.; Coyne, M.; Froio, D.; Auerbach, M.; Wirsen, C.; Ratto, J. A. A processing, characterization and marine biodegradation study of melt-extruded polyhydroxyalkanoate (PHA) films. *J. Polym. Environ.* **2008**, *16* (1), 1−11.
(5) Rivero, S.; Lecot, J.; Pinotti, A. Impregnation of kraft paper support with polylactic acid multilayers. *Adv. Mater. Lett.* **2017**, *8* (6), 741−751.
(6) Madhavan Nampoothiri, K.; Nair, N. R.; John, R. P. An overview of the recent developments in polylactide (PLA) research. *Bioresour. Technol.* **2010**, *101* (22), 8493−8501.
(7) Sin, L. T. *Polylactic Acid: PLA Biopolymer Technology and Applications*, 1st ed.; Elsevier, 2012.
(8) Murariu, M.; Dubois, P. PLA composites: From production to properties. *Adv. Drug Delivery Rev.* **2016**, *107*, 17−46.
(9) Armentano, I.; Fortunati, E.; Burgos, N.; Dominici, F.; Luzi, F.; Fiori, S.; Jiménez, A.; Yoon, K.; Ahn, J.; Kang, S.; Kenny, J. M. Processing and characterization of plasticized PLA/PHB blends for biodegradable multiphase systems. *eXPRESS Polym. Lett.* **2015**, *9* (7), 583−596.
(10) Abdelwahab, M. A.; Flynn, A.; Chiou, B. S.; Imam, S.; Orts, W.; Chiellini, E. Thermal, mechanical and morphological characterization of plasticized PLA−PHB blends. *Polym. Degrad. Stab.* **2012**, *97* (9), 1822−1828.
(11) Zhang, M.; Thomas, N. L. Blending polylactic acid with polyhydroxybutyrate: the effect on thermal, mechanical, and biodegradation properties. *Adv. Polym. Technol.* **2011**, *30* (2), 67−79.
(12) Arrieta, M. P.; López, J.; Hernández, A.; Rayón, E. Ternary PLA−PHB−Limonene blends intended for biodegradable food packaging applications. *Eur. Polym. J.* **2014**, *50*, 255−270.
(13) Arrieta, M. P.; Castro-Lopez, M. D. M.; Rayón, E.; Barral-Losada, L. F.; López-Vilariño, J. M.; López, J.; González-Rodríguez, M. V. Plasticized poly (lactic acid)−poly (hydroxybutyrate)(PLA−PHB) blends incorporated with catechin intended for active food-packaging applications. *J. Agric. Food Chem.* **2014**, *62* (41), 10170−10180.
(14) Arrieta, M. P.; Fortunati, E.; Dominici, F.; López, J.; Kenny, J. M. Bionanocomposite films based on plasticized PLA−PHB/cellulose nanocrystal blends. *Carbohydr. Polym.* **2015**, *121*, 265−275.
(15) Venkateswar Reddy, M.; Nikhil, G. N.; Venkata Mohan, S.; Swamy, Y. V.; Sarma, P. N. *Pseudomonas otitidis* as a potential biocatalyst for polyhydroxyalkanoates (PHA) synthesis using synthetic wastewater and acidogenic effluents. *Bioresour. Technol.* **2012**, *123*, 471−479.
(16) Rivero, S.; García, M. A.; Pinotti, A. Crosslinking capacity of tannic acid in plasticized chitosan films. *Carbohydr. Polym.* **2010**, *82* (2), 270−276.
(17) Standard test methods for water vapor transmission of material. In *Annual book of ASTM*, E96-95; American Society for Testing and Materials: Philadelphia, PA, 1995.
(18) Lamarra, J.; Giannuzzi, L.; Rivero, S.; Pinotti, A. Assembly of chitosan support matrix with gallic acid-functionalized nanoparticles. *Mater. Sci. Eng., C* **2017**, *79*, 848−859.
(19) Villarruel, S.; Giannuzzi, L.; Rivero, S.; Pinotti, A. Changes induced by UV radiation in the presence of sodium benzoate in films formulated with polyvinyl alcohol and carboxymethyl cellulose. *Mater. Sci. Eng., C* **2015**, *56*, 545−554.
(20) Rivero, S.; García, M. A.; Pinotti, A. Physical and chemical treatments on chitosan matrix to modify film properties and kinetics of biodegradation. *J. Mater. Physics Chem.* **2013**, *1* (3), 51−57.
(21) Standard Test Method for determining aerobic biodegradation in soil of plastic materials or residual plastic materials after composting. In *Annual book of ASTM*, D5988-03; American Society for Testing and Materials: Philadelphia, PA, 2003.
(22) da Silva, M. G. D.; Vargas, H.; Poley, L. H.; Rodriguez, R. S.; Baptista, G. B. Structural impact of hydroxyvalerate in polyhydroxyalkanoates (PHAscl) dense film monitored by XPS and photothermal methods. *J. Braz. Chem. Soc.* **2005**, *16* (4), 790−795.
(23) Ikehara, T.; Kimura, H.; Qiu, Z. Penetrating spherulitic growth in poly (butylene adipate-co-butylene succinate)/poly (ethylene oxide) blends. *Macromolecules* **2005**, *38* (12), 5104−5108.
(24) Lipatov, Y. S.; Alekseeva, T. *Phase-Separated Interpenetrating Polymer Networks*; Springer-Verlag: Berlin, 2007.
(25) Furukawa, T.; Sato, H.; Murakami, R.; Zhang, J.; Duan, Y. X.; Noda, I.; Ochiai, S.; Ozaki, Y. Structure, dispersibility, and crystallinity of poly (hydroxybutyrate)/poly (L-lactic acid) blends studied by FT-IR microspectroscopy and differential scanning calorimetry. *Macromolecules* **2005**, *38* (15), 6445−6454.
(26) Ashok, B.; Naresh, S.; Reddy, K. O.; Madhukar, K.; Cai, J.; Zhang, L.; Rajulu, A. V. Tensile and Thermal Properties of Poly(lactic acid)/Eggshell Powder Composite Films. *Int. J. Polym. Anal. Charact.* **2014**, *19*, 245−255.
(27) Weng, Y. X.; Wang, L.; Zhang, M.; Wang, X. L.; Wang, Y. Z. Biodegradation behavior of P (3HB, 4HB)/PLA blends in real soil environments. *Polym. Test.* **2013**, *32* (1), 60−70.
(28) Reis, M. O.; Olivato, J. B.; Bilck, A. P.; Zanela, J.; Grossmann, M. V. E.; Yamashita, F. Biodegradable trays of thermoplastic starch/poly (lactic acid) coated with beeswax. *Ind. Crops Prod.* **2018**, *112*, 481−487.
(29) Auras, R.; Harte, B.; Selke, S. An Overview of polylactides as packaging materials. *Macromol. Biosci.* **2004**, *4*, 835−864.
(30) Dharmalingam, S. *Biodegradation and photodegradation of polylactic acid and polylactic acid/polyhydroxyalkanoate blends non-woven agricultural mulches in ambient soil conditions*. Ph.D. Dissertation, University of Tennessee, Knoxville, TN, 2014.
(31) Ludueña, L.; Vázquez, A.; Alvarez, V. Effect of lignocellulosic filler type and content on the behavior of polycaprolactone based eco-composites for packaging applications. *Carbohydr. Polym.* **2012**, *87*, 411−421.
(32) Rocca-Smith, J. R.; Chau, N.; Champion, D.; Brachais, C. H.; Marcuzzo, E.; Sensidoni, A.; Piasente, F.; Karbowiak, T.; Debeaufort, F. Effect of the state of water and relative humidity on ageing of PLA films. *Food Chem.* **2017**, *236*, 109−119.

DOI: 10.1021/acs.jafc.8b01506
J. Agric. Food Chem. XXXX, XXX, XXX−XXX