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Bio-based PLA_PHB plasticized blend films. Part I: Processing and structural characterization

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Abstract

Bio-based films formed by poly(lactic acid) (PLA) and poly(3-hydroxybutyrate) (PHB) plasticized with an oligomer of the lactic acid (OLA) were used as supporting matrices for an antibacterial agent (carvacrol). This paper reports the main features of the processing and physico-chemical characterization of these innovative biodegradable material based films, which were extruded and further submitted to filmature process. The effect of the addition of carvacrol and OLA on their microstructure, chemical, thermal and mechanical properties was assessed. The presence of these additives did not affect the thermal stability of PLA_PHB films, but resulted in a decrease in their crystallinity and in the elastic modulus for the active formulations. The obtained results showed the effective presence of additives in the PLA or the PLA_PHB matrix after processing at high

temperatures, making them able to be used in active and bio-based formulations with antioxidant/antimicrobial performance.

Keywords: Poly(lactic acid); poly(3-hydroxybutyrate), carvacrol; lactic acid oligomers; active packaging.

1. Introduction

The high social demand for sustainable biomaterials in the production of consumer goods has lead to increased attention in “green” technologies, in particular by developing environmentally-friendly packaging systems (Reddy et al., 2013; Takala et al., 2013; Álvarez-Chávez et al., 2012).

The proposal of new structures coupling adequate functionalities and sustainability is one of the current challenges in the research for new packaging materials. Important efforts have been recently devoted to obtain high performance bio-based materials, including polymer matrices, nanostructures and additives, fully derived from renewable resources (Arrieta et al., 2014; Habibi et al. 2013).

Poly(lactic acid) (PLA) is one of the most promising polymers obtained from renewable resources, with similar properties to polystyrene and poly(ethylene terephthalate) (Armentano et al., 2013; Siracusa et al., 2008). PLA has attracted considerable attention by its inherent biodegradability and possibilities to be composted in an industrial environment (Fortunati et al., 2012a), while it can be extruded into films, injection-molded into different shapes or spun to obtain fibers (Jamshidian et al., 2010). However, PLA practical applications are often limited by its inherent brittle nature and low thermal stability. Blending strategies to toughen PLA with polymers and/or plasticizers have been recently proposed to overcome these limitations. (Armentano et al., 2015; Arrieta et al., 2013; Arrieta et al., 2014).

Poly(3-hydroxybutyrate) (PHB), an aliphatic polyesters, synthesized by microorganisms, with high cristallinity and high melting point, is often used as components for blending with PLA, and leads to materials with interesting physical, thermal, and mechanical properties compared to neat PLA.

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50 It is known that PLA plasticization is required to improve ductility (Hassouna et al., 2011) and a
51 large variety of plasticizers, either commercial or synthesized ad-hoc, have been tested. The
52 oligomeric lactic acid (OLA) has proved as one of the most promising possibilities to improve PLA
53 properties without compromising biodegradation performance. OLA is a bio-based plasticizer
54 capable to improve the PLA processability into films and their ductility. Burgos et al. (2013) found
55 that OLA was an efficient plasticizer for PLA, able to prepare flexible films which maintained their
56 homogeneity, thermal, mechanical and oxygen barrier properties for at least 90 days at 25 °C and 50
57 % relative humidity. In addition, the relatively high molar mass of OLA (compared to other
58 monomeric plasticizers), its renewable origin and similar chemical structure with PLA resulted in
59 highly homogeneous films (Burgos et al., 2013; Burgos et al., 2014).

60 Food active packaging is a research area with high interest by the increasing consumer's demands
61 and market trends. In particular, the addition of antimicrobial compounds to food packaging
62 materials has recently received considerable attention. Antimicrobial systems form a major category
63 in the active packaging research since they are very promising structures to inhibit or reduce the
64 growth rate of microorganisms in food while maintaining quality, freshness, and safety (Ramos et
65 al., 2014a; Ramos et al., 2014b; Tawakkal et al., 2014). In addition, the rising demand for the use of
66 natural additives has produced an increase of studies based on antimicrobial compounds obtained
67 from plant extracts, such as carvacrol, thymol, olive leaf extracts and resveratrol, which are all of
68 them generally recognized as safe by the food industry and authorities. These active compounds
69 provide antioxidant and/or antimicrobial properties to packaging materials, acting as potential
70 alternatives to food synthetic preservatives (Gómez-Estaca et al., 2014; Wu et al., 2014).

71 The incorporation of bioactive compounds into a polymer matrix could affect the morphological,
72 thermal, mechanical and gas barrier properties of films. In this sense, some authors reported the
73 effect of carvacrol in different polymers, such as polypropylene, low-density polyethylene,

polystyrene and edible films (Albunia et al., 2014; Arrieta et al., 2013; Higuera et al., 2015; Persico et al., 2009; Ramos et al., 2014).

To the best of our knowledge this is the first approach to incorporate active carvacrol into plasticized blend films based on PLA and poly(3-hydroxybutyrate) (PHB), and named: PLA_PHB films. The main aim of this work is to develop innovative antimicrobial plasticized PLA_PHB blends by using processing strategies common in an industrial level polymer transformation. In this work, different films were obtained by extrusion combined with a filming procedure and their structural, chemical, thermal and mechanical properties were evaluated to assess the effect of carvacrol and OLA addition on PLA_PHB based films. These strategies were developed with technologies suitable for immediate industrial application to obtain perspective antimicrobial/antioxidant active packaging films.

2. Materials and Methods

2.1. Materials

Commercial poly(lactic acid), PLA 3051D, was purchased from NatureWorks[®] Co. LLC (Blair, NE, USA). This PLA grade (96 % L-LA) is recommended for use in injection moulding and shows specific gravity 1.25 g cm⁻³, number molar mass (M_n) 1.42×10^4 g mol⁻¹, and melt flow index (MFI) 7.75 g 10 min⁻¹ tested at 210 °C and 2.16 kg loading. Poly(3-hydroxybutyrate) (PHB) was supplied by NaturePlast (Caen, France), with a density of 1.25 g cm⁻³ and MFI 15-30 g 10 min⁻¹ tested at 190 °C and 2.16 kg loading.

The selected plasticizer is an oligomer of lactic acid (OLA) synthesized and provided by Condensia Química S.A (Barcelona, Spain) by following a licensed method (Fiori & Ara, 2009). OLA was produced as a slightly colored liquid with number molar mass (M_n) 957 g mol⁻¹ (determined by size exclusion chromatography) and glass transition temperature around -37 °C (determined by

differential scanning calorimetry, DSC). Carvacrol (Carv - 98 % purity, Sigma-Aldrich, Madrid, Spain) was selected as antimicrobial and antioxidant additive for the film formulations. The chemical structures of OLA and carvacrol are shown in Figure 1.

2.2. Processing of antimicrobial flexible films

PLA and PHB were dried in an oven prior to processing to ensure the elimination of moisture traces in their composition and to avoid any undesirable hydrolysis reaction. PLA was heated at 98 °C for 3 h, while PHB was dried at 70 °C for 4 h. OLA was pre-heated at 100 °C for 5 min to ensure the liquid condition during the extrusion processing, while carvacrol was used as received. The selected content of PHB was 15 wt% (PLA_15PHB) and they were processed in a twin-screw microextruder (Dsm Explore 5&15 CC Micro Compounder) as well as neat PLA as reference material. Processing parameters (screw speed, mixing time and temperature profile) were optimized in all cases.

The carvacrol content was fixed at 10 wt% in agreement with previous works (Ramos et al., 2014a) and it was compounded with PLA_PHB blends and with OLA. **Table 1** shows the composition of all produced formulations.

PLA and PLA_15PHB based films with thicknesses between 20 and 60 µm were obtained by extrusion with the adequate filming die. Screw speed at 100 rpm was used to optimize the material final properties, while the temperature profile was set up at 180-190-200 °C in the three different extruder heating zones to ensure the complete processing of all systems. The total processing time was established at 6 min. Neat PLA and PLA_15PHB blend were mixed for 6 min, while in the case of active films, PLA_15PHB blends were previously mixed for 3 min and carvacrol was added for the last 3 min. Finally, in the case of PLA_15PHB_10Carv_15OLA films, the PLA_15PHB blend was previously mixed for 3 min, OLA was further mixed with the blend and finally carvacrol was added for the last 3 min (**Table 1**).

2.3. Film characterization

2.3.1. Determination of carvacrol

Carvacrol is a volatile compound, so the assessment of the remaining amount after processing in those formulations where it was added is essential to evaluate their possibilities to be used as active additive with antimicrobial/antioxidant performance. The total content of carvacrol after processing was determined by liquid chromatography coupled to ultraviolet spectroscopy detector (HPLC-UV) after a solid-liquid extraction of the selected films. Rectangular sheets (0.05 ± 0.01 g) of each film were immersed in 10 mL of methanol and kept in an oven at 40 °C for 24 h (per triplicate), as previously reported for the extraction of thymol in PLA-based films (Ramos et al., 2014a). Extracts were further analyzed by HPLC-UV (Agilent-1260 Infinity, Agilent Technologies, Spain) with the UV detector working at 274 nm wavelength. A C18 capillary column (100 mm x 4.6 mm x 3.5 μ m, Zorbox Eclipse Plus) was used to obtain the adequate separation of carvacrol from all other extracted components. Mobile phase consisted of an acetonitrile/water (40:60) solution, with a flow rate of 1 mL min⁻¹ and 20 μ L of sample were injected in each test. A calibration curve was obtained after preparation and further analysis of six carvacrol standards in methanol (100 to 700 mg kg⁻¹) by triplicate. The amount of carvacrol in the extracted solutions was calculated as weight percentage by comparison of the chromatographic peak areas for samples with those for standards.

2.3.2. Infrared spectroscopy (FT-IR)

Infrared spectroscopy analysis (FT-IR) was performed at room temperature in transmission and reflection modes by using a JASCO FT-IR 615 spectrometer at a wavenumber range 4000-400 cm⁻¹.

2.3.3. Thermal Analysis

Differential scanning calorimetry (DSC, TA Instruments, Mod. Q200) tests were performed to determine the carvacrol and OLA effect on the thermal parameters of PLA and PLA₁₅PHB

blends. Tests were performed from -25 °C to 200 °C at 10 °C min⁻¹ under nitrogen flow rate, with two heating and one cooling scans. The first heating scan was used to erase all thermal history. All measurements were performed in triplicate and data were reported as the mean value ± standard deviation. DSC analysis was used to evaluate thermal parameters in all formulations.

Thermogravimetric analysis (TGA, Seiko Exstar 6300) was performed on PLA_15PHB_10Carv and PLA_15PHB_15OLA_10Carv films and results were compared with those for neat PLA and the PLA_15PHB blend already reported (Armentano et al., 2015). Experimental conditions for all tests were selected for 5 ± 1 mg samples with nitrogen atmosphere (250 mL min⁻¹ flow rate). Tests were carried out from 30 °C to 600 °C at 10 °C min⁻¹. Thermal degradation temperatures (T_{max}) for each formulation were evaluated.

2.3.4. Mechanical Properties

The mechanical behavior of PLA, PLA_15PHB, PLA_15PHB_10Carv and PLA_15PHB_15OLA_10Carv films was evaluated. Tensile tests were performed at room temperature with rectangular probes (dimensions: 50 x 10 mm²) by following the procedure indicated in the UNI EN ISO 527-5 standard with a crosshead speed of 1 mm min⁻¹ and a load cell of 50 N. Tests were carried out in a digital Lloyd Instrument LR 30K and the initial grip separation was 25 mm. Tensile strength (σ_b), failure strain (ϵ_b), yield strength (σ_y), yield strain (ϵ_y) and elastic modulus (E) were calculated from the stress-strain curves. At least six samples were tested for each specimen.

2.3.5. Morphological analysis

The transparency of films was evaluated by visual observation, while microstructures of the cryo-fracture surfaces were observed by field emission scanning electron microscope (FESEM Supra 25, Zeiss, Germany).

3. Results and Discussion

3.1. Transparency

Optical properties of films with potential applications in food packaging are important since transparency is highly estimated by consumers and it is a valuable feature for commercial distribution. In this case, homogeneous, flexible, free-standing and transparent PLA-based films were successfully obtained after extrusion. Their visual appearance was evaluated and Figure 2 shows images of the processed films based on PLA_15PHB blends with OLA and carvacrol as well as those for pristine PLA. All films, with thicknesses ranging from 40 to 60 μm , maintained the transparency of PLA_15PHB films regardless of the presence of additives. The differences in optical properties between samples were not perceptible to the human eye, with good transparency even at high additive concentrations. This result suggested that multifunctional systems based on PLA with carvacrol and OLA were suitable for the manufacture of transparent films for food packaging.

3.2. Determination of carvacrol content in PLA_PHB films

The amount of carvacrol remaining in the plasticized PLA_15PHB films after processing was determined by HPLC. It was observed that approximately 25 % of the initial amount of carvacrol loaded to the blends before extrusion (10 wt%) was lost during processing, but 75 % remained in its chemical form and could be used for active functionalities in these blends. These results are similar

to those obtained for thymol in other PLA-based formulations (Ramos et al., 2014a). In addition, no significant differences in the loss of carvacrol between plasticized and unplasticized samples were observed, meaning that the presence of OLA is not conditioning the loss of the active compound during processing.

It is important to remark that the thermogravimetric analysis of carvacrol denoted a value for the maximum temperature for degradation ($T_{\max}$) at 205 °C (data not shown), slightly higher than the processing temperatures applied in this study. Therefore, the loss of this additive in the extruder could be associated to evaporation or some thermal degradation during processing, as it was reported by other authors (Jamshidian et al., 2012; Ramos et al., 2014a). These results ensure that a significant amount of this volatile additive is still present after processing and it could be used in food packaging applications by promoting release at a controlled rate from the polymer surface to the target food, improving food shelf-life and overall quality (Jamshidian et al., 2012). Therefore, carvacrol can be considered as a suitable active component in PLA-PHB formulations since a significant amount remained after processing.

3.3. Infrared spectroscopy (FT-IR)

Figure 3 shows the infrared spectra of PLA, PHB, PLA_15PHB, PLA_15PHB_10Carv and PLA_15PHB_15OLA_10Carv systems in the 4000-2000 cm^{-1} (a), 1900-1500 cm^{-1} (b) and 1050-650 cm^{-1} (c) regions.

All spectra displayed the characteristic bands of PLA-based materials. The spectrum of PLA contains peaks and bands for the carbonyl group ($\text{C}=\text{O}$) vibration at 1755 cm^{-1} , C-O-C asymmetric vibration at 1180 cm^{-1} , C-O-C symmetric vibration at 1083 cm^{-1} , and C- CH_3 vibration at 1043 cm^{-1} . Some differences were observed for these peaks and their wavenumbers between PLA and PHB, since differences in the initial crystallinity of both biopolymers should be considered. PLA is primarily amorphous whereas PHB is highly crystalline, and consequently the $\nu(\text{C}=\text{O})$ band widths

for PLA and PHB differed significantly (Vogel et al., 2007). FT-IR spectra for PLA based films (Figure 3b) showed an intense peak at 1755 cm^{-1} , which is related to the carbonyl vibration in polyesters. On the other hand, a sharp peak centered at 1723 cm^{-1} and attributed to the stretching vibrations of the crystalline carbonyl group was observed for neat PHB. Therefore, spectrum of PLA_15PHB blends showed the two major carbonyl stretching bands one due to PLA and the other to PHB, respectively, with their intensity ratio changing with the composition of blends.

Carvacrol showed characteristic FT-IR peaks in the $3630\text{--}3100\text{ cm}^{-1}$ range, due to the broad O-H stretching band, 2960 cm^{-1} (-CH stretching), 1459 cm^{-1} , 1382 cm^{-1} and 1346 cm^{-1} (-CH deformation) and 866 and 812 cm^{-1} (aromatic ring), observed in the multicomponent blends, giving new evidence of carvacrol presence in the bio-based films after processing, without signs of degradation. The intensity of the -CH stretching peak centered at $2870\text{--}2959\text{ cm}^{-1}$ increased significantly (Figure 3a), reflecting again the presence of carvacrol in the films, as previously reported (Keawchaoon & Yoksan, 2011).

3.4. Thermal Properties

DSC analysis was conducted in order to determine the carvacrol and OLA effect on the glass transition temperature (T_g), cold crystallization (T_{cc}) and melting (T_m) phenomena of PLA_15PHB based systems. DSC curves for the first and second heating scan are reported in Figure 4a and b, respectively, while the thermal parameters are summarized in Table 2. The DSC curve of neat PLA during the first heating scan displayed the glass transition temperature at $60\text{ }^\circ\text{C}$ as expected, while the exothermic cold crystallization showed its maximum at T_{cc} ($95\text{ }^\circ\text{C}$) and the endothermic melting peak was observed at T_m ($150\text{ }^\circ\text{C}$) (Figure 4a) (Armentano et al., 2015). DSC curves for the PLA_15PHB blend showed a slight shift of T_g to lower values than those obtained in neat PLA as well as a multi-step melting process with the first and second peaks (T'_m and T''_m) due to the PLA component of the blend and the third one (T'''_m) corresponding to the melting of the PHB

component at 173 °C, suggesting some lack of miscibility between both polymers, as already reported (Armentano et al., 2015). Finally, the double melting peak at around 145-150 °C, could be attributed to the formation of different crystal structures during heating, as reported in other PLA based blends and composites (Burgos et al., 2013; Martino et al., 2011). A similar behavior for PLA and PLA_15PHB systems was then detected during the second heating scan, not showing the cold crystallization and just a single melting peak (Figure 4b).

As expected, a clear reduction in the T_g value was detected at the first heating scan for the PLA_15PHB_10Carv ternary system, highlighting the plasticizer effect of carvacrol in these blends. This behavior was more evident in the case of the PLA_15PHB_10Carv_15OLA system, showing the lowest values of T_g if compared with all formulations studied in this work (Table 2), underlining the synergic effect of OLA and carvacrol on the thermal parameters of these multifunctional systems. OLA and carvacrol could act both as plasticizer agents, increasing the chain mobility of the macromolecules in the PLA_15PHB blend. Moreover, the OLA-carvacrol multifunctional systems did not display the signal related to the cold crystallization during the first heating scan, suggesting the ability of OLA-carvacrol based system to recrystallize after processing. However, a different behavior was detected for the PLA_15PHB_10Carv_15OLA film in the second heating scan (Figure 4b and Table 2), where a cold crystallization and a multi-step melting process was registered. This behavior could be attributed to the loss of carvacrol during the test and the effect of OLA that influence the re-crystallization processes in PLA_15PHB films. The presence of OLA increased the ability of PLA to crystallize (Armentano et al., 2015) and this result is confirmed here by the shift to lower temperatures of the T_{cc} value in the PLA_15PHB_10Carv_15OLA during the second heating scan (Table 2). Finally, no exothermic crystallization peaks were detected for all formulations during the cooling scan (data not shown).

Thermogravimetric analysis was also performed to evaluate the effect of OLA and carvacrol addition on the thermal stability of PLA_15PHB blends. The weight loss and derivative curves for all formulations are reported in Figure 5. Neat PLA film degraded in a single step process with a

maximum degradation peak ($T_{\max}$) centered at 361 °C, in agreement with values previously reported (Fortunati et al., 2012b) while a two-step degradation behavior was observed in the case of the PLA_15PHB blend with a first peak at temperatures around 280 °C corresponding to the PHB thermal degradation and a second degradation peak attributed to the PLA degradation, but shifted to lower temperatures ($T_{\max} = 345$ °C). The addition of carvacrol did not result in a significant change in the PLA_15PHB thermal stability ($T_{\max} = 345$ °C and 352 °C, respectively). However, a slight weight loss at around 130 °C, corresponding to the partial decomposition of carvacrol, was detected (Ramos et al., 2013). Nevertheless, the absence of significant degradation peaks at temperatures below the applied processing temperatures ensured the wide processing window for these formulations with no risk of significant or dangerous thermal degradation. In addition, some increase (about 10 °C) was detected for the first degradation peak (280 °C and 290 °C for PLA_15PHB and PLA_15PHB_10Carv, respectively). On the contrary, a shift to lower temperatures (about 10 °C) with some increase in the intensity of the first degradation peak was detected for the PLA_15PHB_10Carv_15OLA film due to the presence of OLA (Armentano et al., 2015). We can conclude that the introduction of OLA and carvacrol, at the selected content, affects the first thermal degradation peak of the PLA_PHB blend, but without resulting in a significant change in the thermal stability, with no risk of thermal degradation.

3.5. Mechanical Properties

The mechanical behavior of PLA, PLA_15PHB, PLA_15PHB_10Carv and PLA_15PHB_10Carv_15OLA films was evaluated by determining their tensile properties. Figure 6 shows the stress-strain curves for all materials, while Table 3 shows the results from tensile tests as average values with their standard deviation. The evaluation of these properties is an important issue, since films intended for food packaging require flexibility to avoid breaking during the packaging procedure, while a minimum value of hardness is also a requirement to avoid perforations during their transport and exposition lifecycle.

PLA showed an elastic modulus of 1300 MPa with an elongation at break of 90 % as shown in Table 3, similar results were obtained with analogous processing conditions (Fortunati et al., 2012b). The addition of 15 wt% of PHB to the PLA matrix did not significantly modify the elastic modulus, while maintaining the elongation at break at around 100 %, considering the persistence of the drawing phenomenon as visible in Figure 6 (red curve for PLA_PHB and black for neat PLA). In both PLA and PLA_15PHB films, a gradual drawing process located in the strain range between 50 and 100 % was, in fact, clearly visible; moreover, after yielding, both these formulations showed a strain-hardening phenomenon, which was related to strain orientation and crystallization mechanism. Such behavior is desirable in industrial thermoforming processes because it helps to obtain high quality pieces with small thickness variations (Armentano et al., 2015).

Table 3 shows that the addition of 10 wt% carvacrol preserved the elastic modulus at high values (1130 MPa), suggesting that the antimicrobial additive does not interfere with the stress-strain behavior of the PLA_PHB film. In fact the addition of carvacrol did not affect the elongation at break of the extruded films. Furthermore small reductions in σ_y in neat PLA and more evident decreases in PLA_15PHB films were clearly observed.

A completely different behavior was detected for the PLA_15PHB_10Carv_15OLA film, as underlined by the stress-strain curve in Figure 6, that deviated from linearity at a relatively small stress: 12 MPa; the stress increased further until the maximum value, then dropped and leveled off. The yield point, below 15 MPa and less pronounced than for the other materials, was followed by the gradual increase of the flow stress. Finally, the PLA_15PHB_10Carv_15OLA film showed the lowest elastic modulus value for all the studied formulations. These effects could be due to the increase in the chain mobility of PLA_15PHB films after the addition of two plasticizing additives, such as OLA and carvacrol, as confirmed in the DSC analysis by the clear decrease observed in T_g values caused by their presence (Table 2). These results suggested that the plasticizer effect in these blends was not only given by OLA, but also by carvacrol, whose presence affected interactions between macromolecular chains in the polymer matrices (Arrieta et al., 2013). However, although a

slight increase in the elongation at break was registered, this increase was not as expected for a plasticized system. It was previously demonstrated that the addition of the OLA plasticizer to PLA_PHB based systems caused a substantial decrease in the materials toughness (Armentano et al., 2015) and this effect was mainly evident in the system with 30 wt% of OLA showing the OLA content dependence. As consequence, the increase in the OLA content up to 20 or 30 wt% could positively affect the elongation at break increasing the ϵ_b values (Armentano et al., 2015). It has been proposed that OLA can act as impact modifier at concentrations below 15% partially counteracting the plasticizer effect (Fiori & Tolaguera, 2013) but in order to avoid a drastic decrease in the yield parameters that could negatively affect the final application of these films, OLA concentration was fixed at 15 wt% to guarantee, in combination with carvacrol, a useful elastic and plastic mechanical response of the developed films.

3.6. Microstructure

Figure 7 shows the FESEM micrographs obtained after cryo-fracture of PLA, PLA_15PHB, PLA_15PHB_10Carv and PLA_15PHB_10Carv_15OLA films at different resolutions, in order to compare the fracture morphology of carvacrol and OLA based systems with the polymer matrices. Neat PLA showed a smooth and uniform surface typical of amorphous polymers, while PLA_PHB blend images showed that the dispersed PHB phase had relatively small average diameter. This effect was possibly caused by the partial miscibility between PLA and PHB forcing their macromolecules to separate in two different phases. Based on images in Figure 7, the addition of carvacrol to PLA_PHB blends led to changes in the films microstructure, with the observation of micro-voids on the fracture surfaces of the ternary systems. In the case of antimicrobial packaging as proposed for these films, the presence of micro-voids and channels can facilitate the release of the antimicrobial agent through the bulk and to the films surface, helping to prevent food from spoilage. Furthermore, on the fracture surfaces of PLA_15PHB_10Carv blends the micro-voids

showed some internal defects induced by the presence of PHB aggregates. On the other hand, the fracture surfaces of blends with OLA and carvacrol showed a completely different aspect when compared to the ternary systems: PLA_15PHB_10Carv and PLA_15PHB_OLA (data not shown), highlighting a compact structure with no voids and holes. Moreover, in the fracture surface of these blends with OLA and carvacrol, two different regions were clearly visible with ductile and brittle behavior, as shown in Figure 7.

Shear-yield and plastic deformation formed on the fracture surfaces of blends with OLA were observed. Plastic deformation and the different fracture directions required more energy and consequently those materials with OLA should show higher toughness. Nevertheless, no apparent phase separation was observed in systems with carvacrol and OLA, underlining that plasticizer and antibacterial agent were well incorporated to the PLA polymer matrix.

4. Conclusions

This study focused on the processing and characterization of plasticized bio-films based on PLA_PHB blends with active functionalities offered by the carvacrol addition with antioxidant/antimicrobial properties to be used in active food packaging applications. Bio-based films with homogeneously dispersed OLA and carvacrol were successfully prepared by extrusion followed by a filming procedure, showing good processability at the selected additive content. The plasticizer effects of carvacrol and OLA were evidenced by decreasing the glass transition temperature of the unplasticized films. The ternary films with carvacrol maintained the mechanical properties of the PLA_15PHB blend, while the addition of OLA caused a reduction of the modulus with a slight increase in the elongation.

The present study showed the possibility of the addition of carvacrol to PLA_PHB matrices for fresh food preservation. Further work is currently on-going to evaluate the antimicrobial function of these films in order to ensure their potential as an alternative to synthetic plastic packaging

materials. It is expected that these formulations will restrict the growth of pathogenic and spoilage microorganisms, hence extending shelf life and preserving the quality and safety of food products during transportation and storage.

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Figure and Table Captions

Figure 1: Chemical structures of the two additives used in this work.

Figure 2: Visual observation of PLA, PLA_15PHB, PLA_15PHB_10Carv and PLA_15PHB_10Carv_15OLA sample films.

Figure 3: FT-IR spectra of PLA, PHB, PLA_15PHB, PLA_15PHB_10Carv and PLA_15PHB_10Carv_15OLA samples in the 4000-2000 cm^{-1} (a), 1900-1500 cm^{-1} (b) and 1050-650 cm^{-1} (c) regions.

Figure 4: DSC thermograms at the first and second heating scan for PLA, PLA_15PHB, PLA_15PHB_10Carv and PLA_15PHB_10Carv_15OLA films.

Figure 5: Residual mass and its derivative curves of PLA, PLA_15PHB and carvacrol based systems in nitrogen atmosphere.

Figure 6: Representative stress-strain curve for PLA, PLA_15PHB, PLA_15PHB_10Carv and PLA_15PHB_10Carv_15OLA films.

Figure 7: FESEM images of PLA, PLA_15PHB, PLA_15PHB_10Carv and PLA_15PHB_10Carv_15OLA films at different magnifications and OLA concentrations.

Table 1: Material formulations and process parameters.

Table 2: Thermal properties of PLA, PLA_PHB and carvacrol based systems at the first heating and second heating scan, after processing.

Table 3: Results from tensile test for PLA, PLA_PHB, PLA_15PHB_10Carv and PLA_PHB_10Carv_15OLA systems.

Table 1: Material formulations and processing parameters.

<i>Formulations</i>	<i>COMPONENT CONTENTS</i>				<i>MIXING PARAMETERS</i>		
	PLA (wt%)	PHB (wt%)	OLA (wt%)	Carvacrol (wt%)	Speed (rpm)	Mixing time (min)	Temperature Profile (°C)
<i>PLA</i>	100	0	0	0	100	6	180-190-200
<i>PLA_15PHB</i>	85	15	0	0	100	6	180-190-200
<i>PLA_15PHB_10Carv</i>	75	15	0	10	100	3+3*	180-190-200
<i>PLA_15PHB_10Carv_15OLA_</i>	60	15	15	10	100	3+3**	180-190-200

*3min mixing PLA_PHB and 3min mixing PLA_15PHB_10Carv

**3min mixing PLA_PHB, 3min mixing PLA_15PHB_10Carv_15OLA

Table 2: Thermal properties of PLA, PLA_15PHB and carvacrol based systems at the first and second heating scan.

<i>Formulations</i>	T_g (°C)	T_g' (°C)	T_{cc} (°C)	T_{cc}' (°C)	ΔH_{cc} (J g⁻¹)	T_m (°C)	T_m' (°C)	T_m''' (°C)	ΔH_m (J g⁻¹)
FIRST HEATING SCAN									
PLA		60.1±1.0	95.1±0.8	95.1±0.8	15.8±0.7	150.0±0.5	-	-	27.6±0.4
PLA_15PHB		55.4±1.2	103.6±0.5	103.6±0.5	9.3±0.6	144.0±0.9	150.2±0.5	170.4±1.0	25.4±0.5
PLA_15PHB_10Carv		47.2±0.4	113.2±1.0		16.7±0.7	145.0±0.5		166.2±0.2	32.1±1.4
PLA_15PHB_10Carv_15OLA	-7.6±0.5/4.5±1.2	35.9±1.6	-	-	-	139.9±0.5	160.6±0.5		34.3±0.8
SECOND HEATING SCAN									
	T_g (°C)	T_g' (°C)	T_{cc} (°C)	ΔH_{cc} (J g⁻¹)	T_m (°C)	T_m' (°C)	T_m''' (°C)	T_m'''' (°C)	ΔH_m (J g⁻¹)
PLA		59.3±0.3	126.2±0.5	2.5±0.2		151.2±0.4			3.1±0.1
PLA_15PHB		54.7±0.2	127.8±0.4	2.5±0.4		148.9±0.7			6.3±0.7
PLA_15PHB_10Carv		54.4±0.6	123.0±1.4	10.7±0.4	149.5±0.1	168.2±0.2			18.7±0.7
PLA_15PHB_10Carv_15OLA	-7.8±0.1/4.7±1.0	36.7±1.8	115.9±0.3	7.2±0.2	137.1±1.4	145.7±0.4	153.8±0.6	163.1±1.3	34.7±1.5

Table 3: Results from tensile test for PLA, PLA_15PHB, PLA_15PHB_10Carv and PLA_15PHB_10Carv_15OLA systems.

<i>Formulations</i>	σ_Y (MPa)	ε_Y (%)	σ_b (MPa)	ε_b (%)	E_{YOUNG} (MPa)
PLA	37±5	3.4±0.4	35±6	90±30	1300±180
PLA_15PHB	42±3	3.8±0.5	31±5	100±40	1220±140
PLA_15PHB_10Carv	33±3	3.3±0.4	24.3±1.7	105±26	1130±160
PLA_15PHB_10Carv_15OLA	16±2	19.5±1.8	14.8±1.7	150±30	330±60

OLA

Carvacrol

PLA

PLA_15PHB

PLA_15PHB_10Carv

PLA_15PHB_10Carv_15OLA

a)

b)

c)

Highlights

Bio-based plasticized films were developed by extrusion followed by a filming process

Poly(lactic acid) (PLA) and poly(3-hydroxybutyrate) (PHB) blends were considered

PLA and PHB were plasticized with an oligomer of the lactic acid (OLA)

PLA_PHB blends were used as supporting matrices for the carvacrol antibacterial agent

The addition of 10 wt% carvacrol preserved the elastic modulus at high values