Development of PHA/fiber-based composites with antimicrobial performance for active food packaging applications

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1 July 2017 to 30 September 2017

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1 Introduction

The use of agro-food residues for the preparation of polymer composites is gaining a significant attention because of their high availability and low price, being at the same time a sustainable strategy for waste valorization. Natural fibers, particularly those obtained from plants, represent a suitable choice to reinforce bioplastics due to their relative high strength and stiffness.[1] The latest innovation in this field is the substitution of oil-derived polymers with bio-based polymers as the matrix component. These materials are habitually named "green composites",[2] a term that indicates that the composite as a whole, *i.e.* both matrix and reinforcement, originates from renewable resources. In this regard, the incorporation of natural fibers such as jute, sisal, flax, hemp, and bamboo fibers into biopolymers has been recently intensified.[3]

Polyhydroxyalkanoates (PHAs) are a well-known family of biodegradable bacterial polyesters with the highest potential to replace polyolefins in a wide range of applications, including food packaging.[4] Among PHAs, poly(3-hydroxybutyrate) (PHB) and its copolymer with 3-hydroxyvalerate (HV), *i.e.*, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), have received so far the greatest attention in terms of pathway characterization and industrial-scale production. Several plant-derived natural fibers have been applied as a reinforcement to produce newly developed composite materials with PHAs, this includes regenerated and/or recycled cellulose,[5-7] pineapple leaf fibers (PALF),[8, 9] wheat straw fibers,[10] wood floor,[11, 12] jute fibers,[13] flax fibers,[14-18] banana, sisal, and coir fibers,[17] hemp fibers,[19] abaca fibers,[20] bamboo fibers,[21] sugarcane bagasse fibers,[22, 23] kenaf and lyocell fibers,[24] wood powder,[25, 26] and pita (agave) fibers.[27, 28] These research works have suggested that the incorporation of natural fibers can definitely strength the mechanical performance of PHAs and the most promising techniques to process them are extrusion and compression molding. In particular, the molding methodologies present the advantage of minimizing filler attrition or breakage during processing since these associate lower mixing energy inputs and shear peaks.[29, 30]

Coconut husk is a good example of agro-industrial nonfood feedstock that is still considered waste for which important industrial new end uses are currently pursued.[31] Coconut husk fibers are characterized by a high toughness and durability and an improved thermal stability due to its high lignin content (~40%) and low cellulose content (~32%).[32] In addition, these natural fibers are exceptionally hydrophilic, as they contain high amounts of strongly polarized hydroxyl groups on their surface.[33] As a result, these fibers are inherently capable to adsorb and hold moisture up to 7–9 times their weight and have been proposed as biosorbents for water treatment.[34] This opens up attractive opportunities for uses as vehicle for active substances in polymer composites.

2 Objective

This research study will be focused on the preparation and characterization of green composites sheets made of PHA and natural fibers functionalized with an antimicrobial substance for the formulation of novel active materials of interest in packaging. For this, the natural fibers will be used as vehicle to adsorb an essential oil, *i.e.* aromatic and volatile oily liquid obtained from herbs and spices, with antimicrobial action. After this, the resultant functionalized fibers will be melt compounded with a PHA and shaped into sheets by compression molding to develop active food packaging materials.

3 Experimental

3.1 Materials

A bacterial aliphatic copolyester, commercially available in the form of off-white pellets, was used. The biopolymer resin presents a true density of 1.23 g/cm^3 and bulk density of 0.74 g/cm^3, as determined by ISO 1183 and ISO 60, respectively, and a melt flow index (MFI) of 5–10 g/10 min (190 °C, 2.16 kg) as determined by ISO 1133. The natural fibers were obtained from ripe coconuts collected, which were de-husked from the hard shell manually by driving the fruit down onto a spike to split it. An essential oil extract with 100% purity was purchased in liquid form.

3.2 Functionalization of natural fibers

The natural fibers were initially chopped to a length of a few millimeters using a Granulator. The chopped fibers were sieved using and placed in oven trays for drying. Resultant bulk density the fibers was 0.14 g/cm^3, as determined by ISO 60.

After drying at 80 °C for 4 h, the natural fibers were functionalized by coating them with a 2:1 (vol./vol.) food-grade paraffinic oil/essential oil solution by means of an air spray gun inside a rotating drum.

Figure 1 Granulator (left image) used to chop the fibers and the resultant fibers after sieving prior to drying in a tray (right image).

Figure 2 Overview of the setup used for the fibers functionalization (upper image) with detail of the air spray gun (bottom left image) and of the rotating drum (bottom right).

3.3 Preparation of green composites

Different PHA formulations were melt compounded varying the fiber content (1–10 wt.-%) in a co-rotating twin-screw extruder with a length (L) to diameter (D) ratio, *i.e.*, L/D, of 29. The biopolymer pellets were fed into the main hopper while fibers were introduced through the side feeder. Three different screw speeds were selected (low, intermediate, and high) and a decreasing temperature profile was set, resulting in a melt temperature of about 180 °C. Residence times were studied with a blue color masterbatch. The strand was cooled in a water bath at 15 °C and then pelletized using an air-knife unit.

Figure 3 Twin-screw extruder used to prepare the green composite pellets.

The compounded pellets were shaped into sheets of approximately 500 µm by compression using a hydraulic press equipped with two plates of iron steel. For this, about 10 g of material was placed in a hollow aluminum mold of 10 x 10 cm^2 and the introduced into the hot-press. Materials were initially preheated at 190 °C for 1 min without pressure in order to homogenize temperature and then hot-pressed for 3 min at 4 tons. Once the shaping process was accomplished, samples were cooled down to 25 °C by means of an internal water circulating system and taken out of the hot-press machine.

Figure 4 Hydraulic press used to prepare the green composite sheets.

3.4 Materials characterization

The thicknesses of the compression-molded sheets were measured with a digital micrometer, having ±0.001 mm accuracy, at five random positions. Samples were aged for at least 15 days prior to physical characterization in a desiccator at 25 ºC and 0% RH.

Dispersion of the natural fibers in the green composites was observed using a light transmission optical microscope. Fiber attrition was investigated by monitoring fiber length distribution on the composite green samples at different fiber contents and processing conditions. This was performed by immersing a small sample of each specimen in an organic solvent, only dissolving the biopolymer, and centrifuging and filtering the resultant solution. The fibers were also examined after functionalization by fluorescence microscope. For this, the samples were previously dyed with Nile Red and analyzed at an emission wavelength between 470 and 490 nm.

Figure 5 The light transmission optical microscope used to analyze the morphology of the natural fibers and their dispersion in the green composite sheets.

Thermal analysis was carried out by differential scanning calorimetry (DSC) on a DSC analyzer equipped with a refrigerating cooling accessory. A heating step from −30 to 200 °C was followed by a cooling step to −30 °C under a nitrogen atmosphere with a flow-rate of 20 mL/min. Thermogravimetric analysis (TGA) was performed in a TG-STDA thermobalance. The samples, with a weight of about 15mg, were heated from 50 to 900 ºC at a heating rate of 10 ºC/min under a nitrogen flow-rate of 50 mL/min.

Dumbbell 500 µm-thick samples were die-cut from the compression-molded sheets and conditioned to ambient conditions, *i.e.* 25 ºC and 50% RH, for 24 h. Tensile tests were carried out at room temperature in a universal mechanical testing machine in accordance with ASTM D638 (Type IV) standard.

The water vapor permeability (WVP) was determined according to the ASTM 2011 gravimetric method. For this, 5 mL of distilled water was placed inside Payne permeability cups (∅ = 3.5 cm). The films were placed in the cups so that on one side they were exposed to 100% RH, avoiding direct contact with water. The cups containing the films were then secured with silicon rings and stored in a desiccator at 25 °C and 0% RH. Identical cups with aluminum films were used as control samples to estimate water loss through the sealing. The cups were weighed periodically using an analytical balance of ±0.0001 g accuracy. For limonene permeability (LP), 5 mL of D-limonene was similarly placed inside the Payne permeability cups. The cups containing the films were placed at controlled conditions, *i.e.* 25 °C and 40% RH. Cups with aluminum films were also used as control samples to estimate solvent loss through the sealing. Oxygen permeability (OP) was obtained from the oxygen transmission rate (OTR) measurements recorded, in duplicate, using an Oxygen Permeation Analyzer at 25 °C and 60% RH. The samples were previously purged with nitrogen in the humidity equilibrated samples and then exposed to an oxygen flow of 10 mL/min. The exposure area during the test was 5 cm^2.

3.5 Antibacterial assay

The antibacterial activity of the green composite sheets was evaluated against *Staphylococcus aureus* (*S. aureus*) ATCC 6538P. The bacterial strain was obtained from the Spanish Type Culture Collection (CECT).

A modification of the Japanese Industrial Standard (JIS) Z 2801:2010 was performed to evaluate the bacterial efficiency on the sheets surface. Briefly, a bacterial suspension of *S. aureus* of about 5×10^5 colony forming units (CFU)/mL was spread uniformly on the surface sheets with dimensions of 2×2 cm^2 and then covered by an inert 10-μm piece of low-density polyethylene (LDPE) of 1.5×1.5 cm^2, which was previously sterilized by ultraviolet (UV) radiation. After 24 h of incubation at 95% RH, bacteria were recovered with phosphate-buffered saline (PBS), inoculated onto tryptone soy agar (TSA) plates, and incubated at 37 °C for 24 h to quantify the number of viable bacteria by conventional plate count. PHA sheets without fibers and different fiber contents, both without essential oil, were used as the negative control. The antimicrobial activity was evaluated as synthesized (0 days) and after 15 days of preparation. Surface reduction (R) was calculated by determining $\log(N_0/N_t)$, where N_0 is the average of the number of viable bacteria on the untreated test sample after 24 h and N_t is the average of the number of viable bacteria on the antimicrobial test sample after 24 h.

4 Results

4.1 Processing optimization

The green composite sheets presented a continuous and uniform surface in which the natural fibers were randomly distributed within the PHA matrix. In general, the fibers were relatively well dispersed in PHA though some areas without fibers were also observed, particularly in sheets with low contents. The brown color intensity was increasing as the fiber content was increased. Alternatively, the processing conditions showed a minor influence on both the aspect of the green composite sheets and the dispersion. The sheets with the highest fiber contents can be aesthetically suitable and attractive to replace solid wood parts such as boards or boxes in rigid packaging.

Figure 6 Green composite sheets with low (left) and high (right) natural fiber contents.

Residence time and fiber attrition were evaluated in order to select the most optimal processing conditions. The residence time during the compounding process in the extruder was gradually reduced with increasing the screws speed. The fibers incorporation slightly increased the residence time.

The length of the fibers in the sheets was significantly affected by both the fiber content and screws speed during the compounding process. The mean fiber length decreased with increasing the fibers content, which can be related to an increase of the energy input, *i.e.* the thermo-mechanical energy applied during compounding increased due to an increase in melt viscosity and, more importantly, to the intensification of filler-to-filler collisions.[29] In addition, particularly for high fiber contents, length also decreased with increasing the screws speed, which can be ascribed to a further increase of mixing energy input into the system.

These results indicated that the optimal condition to process the green composites was based on the use of intermediate screws speed. This processing condition delivered the most balanced condition in terms of fibers dispersion and length. It also offered low-to-intermediate residence times during compounding, which can be beneficial to both process PHA and the essential oil.

4.2 Physical properties

The compression-molded sheets obtained from green composites melt compounded at intermediate screws speeds were studied in terms of thermal, mechanical, and barrier properties.

In relation to their thermal properties, all PHA sheets presented similar values of thermal transition, with a glass transition temperature (T_g), crystallization temperature (T_c), and melting temperature (T_m) of about -3 °C, 121 °C, and 173 °C, respectively. Cold crystallization was not observed and all the here-studied samples crystallized from the melt in a single peak. However, the incorporation of the fibers significantly affected the biopolymer crystallization. On the one hand, low fiber contents reduced the degree of crystallinity while, on the other, the highest content resulted in an increase of crystallinity. This result suggests that crystal growth in the green composites was controlled by two competing factors related to the natural fibers, *i.e.* nucleation and confinement.

Thermal decomposition analysis indicated that all samples were stable up to 270 °C, presenting a degradation temperature close to 300 °C. The incorporation of fibers slightly reduced the thermal stability of the neat PHA but it also delayed the amount of mass loss during degradation. Therefore, the processing window of the green composite sheets was relatively wide since the samples were thermally stable up to approximately 100 °C more than their processing temperature.

The mechanical response of the PHA sheets indicated that these were rigid and brittle with a high modulus and very low plastic deformation. The fibers increased the tensile modulus of the sheets but reduced both the tensile strength and elongation at break, indicating that the green composites gained elastic properties against tensile efforts. The more balanced mechanical performance was observed for the green composite sheets with intermediate fiber contents and containing the longest fibers.

Finally, the barrier properties against water vapor, limonene, and oxygen were determined. The barrier performance is, in fact, one of the main parameters of application interest for food packaging. Although the permeability values to both water and limonene vapors slightly increased when the fiber content was increased, oxygen permeability was improved. In a packaging context, it was observed that the green composites still presented a WVP in the same order of magnitude as, for instance, polyethylene terephthalate (PET), which is widely used for rigid packaging applications,[35] and also showing a low limonene permeance that can be useful for aroma barrier applications.

4.3 Active properties

The essential oil was homogenously distributed on the fibers surface and the antibacterial activity of the resultant green composite sheets containing the functionalized fibers was studied. While the unfilled PHA sheets showed no inhibition effect on the bacterial growth ($R \leq 0.5$), the incorporation of essential oil-containing fibers into the PHA sheets exhibited significant antibacterial effect against *S. aureus*. At the initial day, *i.e.* for the tests carried out 1 day after the green composites production, the antibacterial activity of the functionalized sheets gradually increased with the functionalized fiber content. At the lowest content, the green composite sheets presented a slight antibacterial activity ($R = 0.97$) while, for higher contents, all sheets generated a significant surface reduction ($R \geq 1$ and < 3). Although none of the sheets produced a strong reduction ($R \geq 3$), very low fiber contents successfully inhibited the bacterial growth. For the tests performed after 15 days, though the antibacterial effect was reduced, the green composite sheets still kept significant values of reduction. This suggests that part of the extract was released from the sheets but the fibers were still able to retain a significant amount.

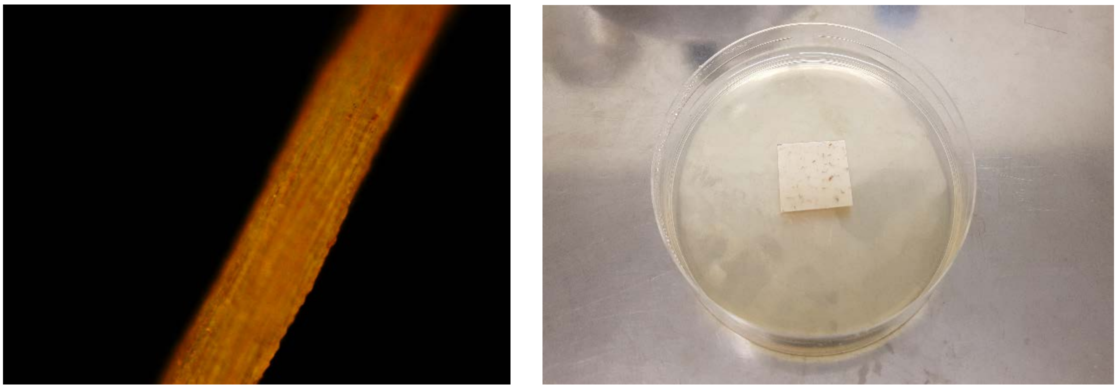

Figure 7 Fluorescence optical microscopy image of natural fiber coated with essential oil (left) and a green composite sheet during the antimicrobial analysis (right).

5 Conclusions

The incorporation of natural fibers into a PHA matrix by extrusion and subsequent compression molding to produce green composite sheets has shown several advantages, such as good thermal stability, high rigidity, good processability, improved oxygen barrier, and potentially low costs. The resultant green composite sheets are also highly sustainable because of their whole biodegradability and renewable characteristics. In addition, the use of these natural fibers as functional fillers in PHA matrices shows a great deal of potential in active food packaging, particularly for their use as vehicles to entrap natural extracts. Through this approach, it has been possible to achieve a great efficiency due to the antimicrobial active substance was released slowly to the packaging surface, remaining at effective concentrations for an extended period of time. Resultant essential oil-containing PHA composite materials can be applied as monolayers, in bio-based packaging materials, for instance trays and lids, or even boards to control undesirable microorganisms on surfaces in contact with foodstuff.

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