BIODEGRADABLE POLYMER-BASED COMPOSITES FILLED WITH BIOCHAR FOR TUNABLE RELEASE OF CARVACROL

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Abstract: Bio-composites are commonly obtained by combining biodegradable polymers with fillers collected from natural resources. In this context, biochar (BC) is attracting high interest as filler for polymer-based composites due to its challenging properties, including eco-sustainability. This study aimed to prepare and characterize bio-composites with antimicrobial properties evaluating the role of the filler in the release kinetics. In particular, BC as filler and carvacrol (CRV) as antimicrobial agent, were incorporated via melt-compounding in poly(butylene adipate-co-terephthalate) (PBAT) samples. The rheological, tensile, and antimicrobial properties of the obtained bio-composites were evaluated paying particular attention to the influence of BC concentration, i.e., 5, 10, and 20 wt%, on the investigated properties. Moreover, the influence of BC content on the release kinetics of carvacrol was studied and mathematically modeled to evaluate the release mechanism. The results showed that the presence of biochar modified the carvacrol release in comparison with the unfilled system thus allowing to tune the release kinetics.

Keywords: PBAT; carvacrol; bio-composites; biochar

1. Introduction

In recent years, polymers and polymer-based systems have been widely investigated as materials for thin-film preparation. Usually, thermoplastic polymers offer low raw material costs and a well-established manufacturing process that can be easily scaled to large-scale production. However, major concerns for traditional thermoplastics used for film preparation, commonly derived from fossil fuels, refer to the non-renewability and non-biodegradability of the raw materials chosen for their production, thus causing environmental issues [1–3]. Due to the rising attention towards eco-sustainable products, it is unsurprising that more and more research groups and industries are exploring the possibility of using new biodegradable and compostable polymers suitable for different applications [4–6] .

A wide plethora of biodegradable polymers, such as polysaccharides, proteins, and lipids, were proposed as suitable materials for thin-film preparation [4,7,8]. In this context, poly(butylene adipate-co-terephthalate) (PBAT) is an aliphatic/aromatic copolyester that is biodegradable and compostable and, due to its interesting properties, is considered among the most promising biopolymers for packaging film, agricultural film, and compost bag fabrication [9–11].

Over recent years, several additives, including natural compounds, peptides, enzymes, metals, chelating agents, and antibiotics, were incorporated into bio-polymeric matrices to provide antimicrobial activity [12–14]. Among them, plant essential oils (EOs) are interesting natural antimicrobial agents to be incorporated into the biopolymeric films or membranes due

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to their high inhibitory potential against a wide spectrum of microorganisms [15,16]. Among the great variety of EOs, carvacrol (CRV), commonly present as the main compound in thyme and oregano EOs, has gained greater acceptance among food technologists due to its ability to inhibit undesired pathogenic and spoilage microorganisms [17,18]. Furthermore, CRV is a "generally recognized as safe" food additive and is approved by the U.S. Food and Drug Administration (FDA) for use in foods and drinks.

However, CRV release from thin biopolymeric films can lead to rapid (burst) release of the bioactive compound thus hindering a long-term release.

In order to mitigate the burst effect, it is usually preferable adding a third component, such as a filler. In fact, from a physical point of view, the presence of a filler may force the molecules to follow a tortuous path throughout the matrix before reaching the surface [12]. Moreover, from a chemical point of view the eventual strong interaction between filler and drug may decrease the amount released and the kinetics delivery.

In this context, biochar (BC) is attracting high interest as filler for polymer-based composites due to its challenging properties, such as high thermal and chemical stability combined with its cost-effectiveness and eco-sustainability [19,20]. BC is usually produced by the pyrolysis of wastes from the forestry and agricultural industries, and its structure can be modified by tuning the pyrolysis conditions [21].

Recently, several remarkable articles about the efficacy of BC as filler for the fabrication of green composites were published but its effect on the release kinetic of a natural antimicrobial compound was never reported so far [22,23].

Therefore, in this work, biocomposite films based on PBAT, biochar, and CRV were produced via melt mixing and filmed in hot press. The PBAT-based films were loaded with 20 wt% of CRV and filled with 5, 10, or 20 wt% of BC. The rheology of the melts was evaluated via frequency sweep analysis. The mechanical properties of the PBAT-based films were evaluated through tensile tests. The release profile of CRV from was evaluated via UV–Vis measurements and it was fitted with a power-law model.

2. Experimental part

2.1 Materials

PBAT (ecoflex® F Blend C1200, Basf, SE, Ludwigshafen, Germany) is a film grade with a melt flow rate (MFR) of 2.7–4.9 g/10 min (190 °C, 2.16 kg), a density in the range of 1.25–1.27 g/cm^3, and a melting temperature in the range of 110–120 °C.

Commercial biochar powder (hereafter coded as BC) used in the food industry (Special Ingredients) was chosen as filler. In particular, as reported in the technical data sheet of the supplier, this biochar was obtained from the pyrolysis of coconut shells.

Carvacrol (purity ≥98%) was purchased by Sigma Aldrich.

2.2 Film preparation

PBAT-based films were prepared by combining melt mixing and compression molding. More in detail, PBAT was fed to a batch mixer (Brabender PLE-330 T = 170 °C, n = 60 rpm) and processed

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for 4 min, then the additives (BC and CRV) were added and mixed with PBAT for 1 min in order to minimize the evaporation of CRV. The PBAT-based mixtures were then fed out and rapidly quenched in liquid nitrogen in order to stop the CRV evaporation due to the high temperature. BC concentration was 5, 10, and 20 PHR while a single CRV concentration (equal to 20 PHR) was added to each composite. Therefore, five materials were produced and coded as PBAT (pure PBAT), PBAT/CRV (without BC) or PBAT/CRV/BC_N where N is equal to the PHR of BC in the sample. PBAT-based systems were then filmed in a Carver laboratory press at 170 °C and 140 bar in 15 cm × 15 cm × 0.2 mm height square molds for 3 min. In order to avoid the loss of CRV due to evaporation during the storage stages, all the PBAT/CRV and PBAT/CRV/BC films were stored at 2 °C and characterized within 24 h of preparation.

2.3 Rheological characterization

Rheological investigations of the melts were carried out through a plate–plate rotational rheometer ARES-G2 (TA Instruments, New Castle, DE, USA) equipped with a parallel-plate geometry (25-mm diameter). Frequency sweep tests, from 0.1 to 100 rad/s, were performed at 170 °C. The PBAT-based samples for rheological tests were prepared via compression molding of the melt mixed systems in a 25-mm diameter and 1.5-mm thick stainless-steel mold at the same conditions above described. Before testing, all the samples were let to dry under vacuum overnight at 70 °C.

2.4 Tensile tests

All PBAT-based films were mechanically tested in uniaxial tensile mode by using an Instron 3365 (Instron, Norwood, MA, USA) universal testing machine equipped with a 1 kN load cell. The tests were performed on rectangular film specimens (10 × 90 mm) until fracture with a uniform crosshead speed of 1 mm/min. At least 7 specimens were tested for each sample.

2.4 Carvacrol release kinetics

It is well known that the maximum absorbance peak of CRV in water detectable by UV–Vis measurements can be detected at 272 nm [1]. A series of CRV/water solutions containing from 1 to 50 mg/L of CRV were prepared and analyzed via UV–Vis (model UVPC 2401, Shimadzu Italia s.r.L., Milan, Italy) in order to obtain a calibration curve correlating the absorbance peak intensity and the CRV concentration (mg/L). In this range of concentration, the calibration curve was found to be linear (ABS_{272nm} = 0.0142 [CRV]; R^2 = 0.99998). The release of the essential oils from the films was investigated by immersing a pre-weighed sample (a section of 3 × 20 × 3 mm, approximately 120 mg) in 10 mL of distilled water at 37 °C. The absorbance of the medium at 272 nm was measured at specific time intervals and converted CRV concentration by using the calibration line. After each measurement, the samples were immersed in 10 mL of fresh distilled water at 37 °C, and the cumulative release of CRV here reported was calculated by sequentially adding the CRV released after each step.

2.4 Antibacterial activity determination

PBAT-based films containing CRV and BC at different concentrations were tested for antibacterial activity applying the paper disc diffusion method [24] with a few modifications. Briefly, a water agar (2% w/v) base support [25] was overlaid with 7 mL of the optimal soft agar (0.7% w/v) medium for each indicator strain inoculated at approximately 10^7 CFU/mL. MB foams discs (6-mm diameter) containing CRV were placed onto the surface of the double agar layer.

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Sterile filter paper discs (Whatman no. 1) of the same diameter were soaked with streptomycin (10% w/v) and used as positive controls, while discs of MB foams without CRV represented the negative controls.

The inhibitory activity was evaluated after incubation at the optimal temperature (reported above) for each strain. The inhibitory activity was scored positive when a definite clear area was detected around the discs. The diameters of the inhibitory halos around the paper discs were measured. The experiments were performed in triplicate.

3. Results and discussion

3.1 Rheological properties

The effect of the filler and the essential oil on the rheological properties of the biocomposites was analyzed by measuring the storage modulus (G '), the loss modulus (G' ') and the complex viscosity (η *) as a function of frequency. In pure PBAT, a decrease of the complex viscosity with frequency was observed. More in detail, a pseudo-Newtonian behavior of the matrix at low frequencies was followed by a shear thinning behavior at high frequencies, as also reported by the studies of Adrar et al. [26]. Both the moduli and the viscosity decrease with the introduction of CRV, thus indicating the plasticizer action of this essential oil to PBAT, as already observed for other biopolymeric matrices. The addition of BC to the PBAT/CRV systems caused an increase of the complex viscosity at the lower frequencies. On the other hand, the complex viscosity of the melts containing or not BC were overlapped at the higher frequencies. Upon increasing the BC concentration, the shear thinning behavior of the melt became more evident reflecting a solid-like behavior. Similarly and coherently, the values of G 'and G'' increased at the lower frequencies upon increasing the content of BC while remained almost independent from the BC concentration at the higher frequencies. These results let us reasonably conclude that CRV has an evident plasticizer action on PBAT and that the further addition of BC slightly modified this behavior, in particular at the higher frequencies.

3.2 Mechanical properties

Tensile tests were performed to investigate the influence of BC and CRV addition on the mechanical behavior of PBAT-based films.

Table 1 displays the tensile properties investigated, and specifically the elastic modulus (E), the tensile strength (TS) and the elongation at break (EB).

Table 1. Tensile properties of the PBAT-based films

Sample	E [Mpa]	TS [Mpa]	EB [%]
PBAT	63 ± 4.0	26.0 ± 3.3	1245 ± 79
PBAT/CRV	18.6 ± 2.2	10.3 ± 1.3	759 ± 72
PBAT/CRV/BC_5	18.9 ± 2.2	7.1 ± 0.5	493 ± 52
PBAT/CRV/BC_10	22.3 ± 1.4	6.2 ± 0.4	288 ± 41
PBAT/CRV/BC_20	23.4 ± 2.2	5.4 ± 0.3	212 ± 33

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Pure PBAT is a polymer with a relatively low elastic modulus (~63 MPa), with a relatively high tensile strength (~26 MPa) and high deformation at break (~1245%), values consistent with those found in the literature [27,28]. The high tensile strength values are due to the fact that, when they are subjected to high elongations, the polymer chains of the PBAT reorganize to undergo a stress-induced crystallization, thus increasing the force required to break.

The addition of CRV leads to a worsening of the mechanical properties with respect to the neat matrix. Upon increasing the BC concentration a slight increase of the elastic modulus was observed. The addition of biochar gives greater rigidity and a reduction in the mobility of the polymer chains which results in an increase in the elastic modulus. At the same time, the tensile strength, and the elongation at break of the PBAT-based films decreased upon increasing the BC content. This phenomenon can be ascribed to the formation of clusters that acted as stress-concentrators thus reducing the elongation at break and the tensile strength of the samples containing BC.

3.3 Carvacrol release kinetics

In order to investigate the release mechanism of CRV in water from the PBAT-based films, the experimental data of the release kinetic were fitted using the power law model:

$$\frac{M_t}{M_\infty} = kt^n \quad (1)$$

where *t* is the release time, *k* is a kinetics constant and *n* is the diffusion exponent related with the release mechanism. In particular, the release is diffusion-controlled when *n* is lower than 0.5 (Fickian) and it is swelling-controlled when *n* is equal to 1.0. If *n* is in the range 0.5–1.0 it can be assumed a release due to the superposition of both phenomena that is defined as anomalous transport by Peppas et al. [29].

In Table 2 there are summarized n, k and the R^2 values for each system here investigated.

Table 2. Power law parameters obtained from the release kinetics of CRV.

Sample	I Stage			II Stage			III Stage		
	k [h^{-1}]	n	R^2	k [h^{-1}]	n	R^2	k [h^{-1}]	n	R^2
PBAT/CRV	0.172	0.922	0.993	0.254	0.491	0.989	0.588	0.139	0.970
PBAT/CRV/BC_5	0.168	0.915	0.997	0.245	0.496	0.993	0.579	0.137	0.966
PBAT/CRV/BC_10	0.200	0.883	0.997	0.299	0.448	0.990	0.691	0.096	0.953
PBAT/CRV/BC_20	0.207	0.896	0.996	0.316	0.440	0.984	0.687	0.106	0.995

For all the systems, the release of CRV was characterized by three phases: (i) a burst phase, (ii) a second phase characterized by slower release rate, and (iii) a release plateau.

The release can be defined as diffusion-controlled when n is lower than 0.5 (Fickian) and it is swelling-controlled when n is equal to 1.0. The superposition of both phenomena occurs if n is

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in the range 0.5–1.0. In this case, Peppas et al. defined this behavior as anomalous transport [29].

According to Peppas, the CRV release showed an anomalous mechanism of release during the first stage, and a Fickian release during the second and third release stages.

As highlighted by k values in table 2, the kinetic release of CRV was slowened by lowest concentration of BC while it increased upon furtherly increasing the BC concentration to 10 wt% and 20 wt%.

3.4 Antibacterial properties

Results of the screening of the antibacterial activity in vitro revealed that among the active materials tested the only ones to be effective on high concentrations of indicator strains were those produced at 10% and 20% of BC which also showed the faster kinetics of carvacrol release. In particular, the inhibitory effect was detected against spoilage bacteria, i.e. *Brochotrix thermosphacta* (SP10) and pathogenic bacteria, i.e *Stenotrophomonas maltophilia* (ICE272), while no effect was detected against pro-technological bacteria. While requiring further studies, this result highlights the potential use of this active material in applications in the food packaging sector. In fact, in a possible in vivo application, the presence of microorganisms with potential pro-technological and probiotic aptitudes in the food matrix in contact with the active material would not be compromised while the presence of alterative microorganisms responsible for reducing the shelf-life of the product would be inhibited.

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