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Analytical Methods

Development of novel nano-biocomposite antioxidant films based on poly (lactic acid) and thymol for active packaging

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ABSTRACT

Novel nano-biocomposite films based on poly (lactic acid) (PLA) were prepared by incorporating thymol, as the active additive, and modified montmorillonite (D43B) at two different concentrations. A complete thermal, structural, mechanical and functional characterization of all nano-biocomposites was carried out. Thermal stability was not significantly affected by the addition of thymol, but the incorporation of D43B improved mechanical properties and reduced the oxygen transmission rate by the formation of intercalated structures, as suggested by wide angle X-ray scattering patterns and transmission electron microscopy images. The addition of thymol decreased the PLA glass transition temperature, as the result of the polymer plasticization, and led to modification of the elastic modulus and elongation at break. Finally, the amount of thymol remaining in these formulations was determined by liquid chromatography (HPLC-UV) and the antioxidant activity by the DPPH spectroscopic method, suggesting that the formulated nano-biocomposites could be considered a promising antioxidant active packaging material.

1. Introduction

Poly (lactic acid) (PLA) is one of the most important commercially available bio-based and biodegradable thermoplastic polyesters (Inkinen, Hakkarainen, Albertsson, & Sodergard, 2011). PLA can offer a sustainable alternative for food packaging across a wide range of commodity applications in response to consumers' demands and market trends in the use of renewable resources (Hughes, Thomas, Byun, & Whiteside, 2012). PLA is a highly transparent and rigid material with a relatively low crystallization rate, making it a promising candidate for the fabrication of biaxial oriented films, thermoformed containers and stretch-blown bottles (Inkinen et al., 2011). However, some characteristic properties of pure PLA are inadequate for food packaging applications, such as weak thermal stability, low glass transition temperature, low gas barrier properties, and low ductility and toughness (Hwang et al., 2012). Recently, these poor PLA intrinsic properties have been improved by the reinforcement of the polymer matrix with layered silicates (Fukushima, Tabuani, & Camino, 2009; Gamez-Perez et al., 2011; Lagaron & Lopez-Rubio, 2011; Picard, Espuche, & Fulchiron, 2011). In this sense, the incorporation of lamellar nanofillers with high aspect ratio, such as montmorillonites, has significantly enhanced mechanical, gas barrier, and optical properties (Rhim, Hong, & Ha, 2009).

Current innovations in food packaging research include the development of active packaging systems based on materials, which can include a variety of additives such as antioxidants, antimicrobials, vitamins, flavours and colourants with the aim of improving their appearance and to extend foodstuff shelf-life (Álvarez, 2000; Del Nobile et al., 2009; Gómez-Estaca, Giménez, Montero, & Gómez-Guillén, 2009). The increasing demand for natural additives has resulted in studies based on natural active compounds, such as plant extracts or essential oils, which are categorized as Generally Recognised as Safe (GRAS) by the US Food and Drug Administration as well as the current European Legislation for materials intended to be in contact with food (EU N10/2011 Regulation) (Ramos, Jiménez, Peltzer, & Garrigós, 2012).

The addition of natural antioxidant additives allows their continued release during storage and distribution, extending food shelf-life by decreasing lipid auto-oxidation, which is recognized as a major cause of deterioration affecting both sensory and nutritional quality (Manzanarez-López, Soto-Valdez, Auras, & Peralta, 2011). In this sense, thymol is a phenolic compound obtained from thyme and oregano essential oils that has been reported to be an effective antioxidant to reduce or eliminate lipid oxidation (Al-Bandak & Oreopoulou, 2007). Thymol antioxidant properties are due to its ability to donate H-atoms from phenol hydroxyl groups, which could react with peroxyl radicals to produce stabilized

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phenoxyl radicals and, consequently, terminate lipid peroxidation chain reactions (Mastelic et al., 2008; Viuda-Martos, Navajas, Zapata, Fernández-López, & Pérez-Álvarez, 2010). Several methods can be used to evaluate the antioxidant activity of natural additives as pure compounds or plant extracts, which are based on the measurement of the free radical scavenging ability (Sánchez-Moreno, 2002).

The development of different nanocomposites based on PLA with nanoclays (Fukushima, Tabuani et al., 2009; Gamez-Perez et al., 2011; Picard et al., 2011) or active additives (Byun, Kim, & Whiteside, 2010; Hwang et al., 2012; López-Rubio & Lagaron, 2010) has been extensively reported by several authors in the last years. However, few works have reported the combination of natural active additives and nanofillers in biopolymer matrices resulting in nano-biocomposites with antioxidant properties and functionalities for use in food packaging applications. The use of these materials could be a promising alternative to enhance mechanical and gas barrier properties and extend foodstuff shelf-life.

This study focused on the development of antioxidant biodegradable films based on PLA reinforced with an organically modified montmorillonite [Dellite 43B (D43B)] and a natural additive (thymol) to obtain nano-biocomposites based on renewable resources with antioxidant activity and enhanced properties for active packaging applications. A full characterization was carried out including the determination of thermal, structural, mechanical and functional properties. Finally, the presence of thymol in the nano-biocomposites was determined by HPLC-UV analysis and antioxidant activity assessed by using the DPPH method.

2. Materials and methods

2.1. Materials

Poly (lactic acid) (PLA) 4060D was purchased in pellets from Natureworks Co., (Minnetonka, MN, USA). Thymol (99.5%), 2,2-diphenyl-1-picrylhydrazyl (DPPH, 95%) and methanol (HPLC grade) were supplied by Sigma–Aldrich (Madrid, Spain).

The nanoclay used was Dellite®43B (D43B) (Laviosa Chimica Mineraria S.p.A. Livorno, Italy). This nanoclay is a dimethyl-benzyldihydrogenated tallow ammonium modified montmorillonite; and it has a cation exchange capacity (CEC) of 95 meq/100 g clay, a bulk density of 0.40 g cm^{-3} and a typical particle size distribution between 7 and 9 μm.

2.2. Nano-biocomposites preparation

The different nano-biocomposites were obtained by melt-blending in a Haake Polylab QC mixer (ThermoFischer Scientific, Walham, MA, USA) with a mixing time of 20 min at 160 °C. Two different rotor speeds were used: 150 rpm in the loading and mixing steps and 100 rpm for the last 5 min, when thymol was added in order to limit degradation and to ensure the presence of the active additive in the final blends. Prior to the mixing step, PLA and the nanoclay were dried for 24 h at 80 and 100 °C, respectively. Thymol was used as received.

Five different formulations were obtained by adding thymol at one concentration level (8 wt%) and D43B at two different loadings (2.5 and 5 wt%), as described in Table 1. An additional sample without any additive was also prepared and used as control (neat PLA).

Films were obtained by compression-moulding at 180 °C in a hot-plates press (Carver Inc 3850, Wabash, IN, USA). Blends were kept at atmospheric pressure for 5 min until melted and pressed at 2 MPa for 1 min, 3.5 MPa for 1 min and finally 5 MPa for 5 min to eliminate the trapped air bubbles. Transparent films were obtained with average thickness 210 ± 1 μm measured with a Digimatic Micrometer Series 293 MDC-Lite (Mitutoyo, Japan) at five random positions.

2.3. Thymol quantification

The actual amount of thymol in PLA films after processing was determined by solid–liquid extraction followed by liquid chromatography coupled to ultraviolet spectroscopy (HPLC-UV) analysis. 0.05 ± 0.01 g of each film were extracted with 10 mL of methanol at 40 °C and 50% relative humidity (RH) for 24 h in a climate chamber (Dycometal CM-081, Barcelona, Spain), as previously reported (Manzanarez-López et al., 2011).

Thymol was determined with a Shimadzu LC-20A liquid chromatograph (Kyoto, Japan) equipped with a UV detector at 274 nm. The column used was a LiChrospher 100 RP 18 (250 mm × 5 mm × 5 μm, Agilent Technologies, USA). The mobile phase was composed of acetonitrile and water (40:60) at 1 mL min^{-1} flow rate. 20 μL of the extracted samples were injected and analyses were performed in triplicate. Quantification of the active additive was carried out by comparison of the chromatographic peak areas with standards in the same concentration range. Calibration curves were run at five concentrations from 100 to 500 mg kg^{-1} using appropriately diluted standards of thymol in methanol.

The antioxidant activity of thymol was analysed using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as previously reported (Byun et al., 2010). This method is based on colour decay when the odd electron of the nitrogen atom in the DPPH radical is reduced by receiving one hydrogen atom from antioxidant compounds (Scherer & Godoy, 2009).

500 μL of extracts were mixed with 2 mL of a methanolic solution of DPPH (0.06 mM) in a capped cuvette. The mixture was shaken vigorously at room temperature and the absorbance of the solution was measured at 517 nm with a Biomate-3 UV–VIS spectrophotometer (Thermospectronic, Mobile, AL, USA). DPPH radical absorbs at 517 nm but, upon reduction, its absorption at this particular wavelength decreases. The decay in absorbance was measured at 1 min intervals until it was stabilized (200 min). All analyses were performed in triplicate.

The scavenging ability of the stable radical DPPH was calculated as percentage of inhibition (I %) with the Eq. (1):

$$I\ (\%) = \left[(A_{\mathrm{Control}} - A_{\mathrm{Sample}})/A_{\mathrm{Control}}\right] \times 100 \qquad (1)$$

where A_{Control} is the absorbance of the blank sample at $t = 0$ min and A_{Sample} is the absorbance of the tested sample at $t = 200$ min.

2.4. Thermal analysis

Thermogravimetric analysis (TGA) tests were performed with a TGA/SDTA 851 Mettler Toledo thermal analyzer (Schwarzenbach, Switzerland). Approximately 5 mg samples were heated from 30 to 700 °C at 10 °C min^{-1} under nitrogen (flow rate 50 mL min^{-1}).

Differential scanning calorimetry (DSC) tests were used to determine glass transition temperatures (T_g) of all materials using a TA DSC Q-2000 instrument (New Castle, DE, USA) under nitrogen atmosphere (flow rate 50 mL min^{-1}). 3 mg samples were heated from −30 to 200 °C at 10 °C min^{-1} (3 min hold), then cooled at 10 °C min^{-1} to −30 °C (3 min hold) and further heating to 200 °C at 10 °C min^{-1}.

2.5. Mechanical properties

Tensile properties of all films were determined with a 3340 Series Single Column System Instron Instrument, LR30K model