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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.

Keywords: PLA; active packaging; thymol; montmorillonite; antioxidant film.

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, 2009b; 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 colorants with the aim of improving their appearance and to extend foodstuff shelf-life (Álvarez, 2000; Del Nobile, Conte, Buonocore, Incoronato, Massaro & Panza, 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 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 et al., 2009b; Gamez-Perez et al., 2011; Picard et al., 2011) or active additives (Byun, Kim & Whiteside, 2010; López-Rubio & Lagaron, 2010; Hwang et al., 2012) 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.

1. Materials and methods

1.1. 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 7-9 µm.

1.2. 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 ºC. 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 ºC and 100 ºC, 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 ºC 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 ºC 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 x 5 mm x 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 100-500 mg Kg^{-1} using appropriately diluted standards of thymol in methanol.

The antioxidant activity of thymol was analyzed 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 equation (1):

$$I(\%) = [(A_{Control} - A_{Sample})/A_{Control}] \cdot 100 \quad (1)$$

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 ºC to 700 ºC at 10 ºC 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 ºC to 200 ºC at 10 ºC min^{-1} (3 min hold), then cooled at 10 ºC min^{-1} to -30 ºC (3 min hold) and further heating to 200 ºC at 10 ºC min^{-1}.

2.5. Mechanical properties

Tensile properties of all films were determined with a 3340 Series Single Column System Instron Instrument, LR30K model (Fareham Hants, UK) equipped with a 2 kN load cell. The main tensile parameters, such as elastic modulus and elongation at break, were calculated from stress-strain curves according to ASTM D882-09 Standard procedure (ASTM D 882 - 09. 2009). Before testing, all samples were conditioned for 48 h at 25 ºC and 50 % RH. Tests were performed with 100 x 10 mm^2 rectangular probes and initial grip separation of 60 mm. The specimens were stretched at 10 mm min^{-1} until breaking. Results were the average of five measurements (± standard deviation).

2.6. Oxygen transmission rate (OTR)

OTR is defined as the quantity of oxygen circulating through a determined area of the parallel surface of a plastic film per time unit. An oxygen permeation analyzer (8500 model Systech, Metrotec S.A, Spain) was used for OTR tests. Pure oxygen (99.9%) was introduced into the upper half of the diffusion chamber while nitrogen was injected into the lower half, where an oxygen sensor was located. Films were cut into 14 cm diameter circles for each formulation and they were clamped in the diffusion chamber at 25 ºC before testing. Tests were performed in triplicate and mean values were expressed as oxygen transmission rate per film thickness (OTR·e).

2.7. Colour tests

Colour modifications on PLA films caused by the addition of the active additive and the nanoclay were followed by using a Konica CM-3600d COLORFLEX-DIFF2 colorimeter, HunterLab, (Reston, VA, USA). Colour values were expressed as L* (lightness), a* (red/green) and b* (yellow/blue) coordinates in the CIELab colour space. These parameters were determined at five different locations in films surfaces and the average values were calculated. Total colour difference (ΔE^*) was calculated according to Eq. (2).

$$\Delta E^* = [(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2} \quad (2)$$

where ΔL^*, Δa^* and Δb^* are the coordinate differences between control (neat PLA) and samples.

2.8. Morphological analysis

The nano-biocomposites structure, including the nanoclay dispersion, was studied by wide angle X-ray scattering (WAXS) patterns and transmission electron microscopy (TEM) micrographs.

WAXS patterns were recorded at room temperature in the scattering angle (2θ) 2-30° (step size: 0.01°, scanning rate: 8 s step^{-1}) using filtered Cu K_α radiation (λ: 1.54 Å). A Bruker D8-Advance model diffractometer (Madison, WI, USA) was used to determine the interlayer distance (d-spacing) and intercalation of the nanoclay.

TEM micrographs were performed using a JEOL JEM-2010 (Tokyo, Japan) with accelerating voltage 100 kV. Prior to analysis, films were ultra-microtomed to obtain slices of 100 nm thick (RMC, model MTXL).

3. Results and discussion

3.1. Determination of thymol in nano-biocomposite films and antioxidant activity

The amount of thymol present in formulations after processing is indicated in Table 1. Results showed that approximately 30 % of the initially thymol was lost during processing by evaporation or degradation due to the high temperature used during the polymer melting (Ramos et al., 2012). In this sense, other commonly used antioxidants in PLA-based formulations, such as butylated hydroxytoluene (BHT), suffer similar losses due to several factors, such as poor mixing in the extruder, evaporation, thermal degradation and the own antioxidant action of BHT to protect the polymer during processing (Ortiz-Vazquez, Shin, Soto-Valdez & Auras, 2011). Antioxidant volatility is desirable for food packaging to promote their migration from the polymer surface (Wessling, Nielsen, & Giacin, 2001). Therefore, thymol can be considered a good antioxidant in food packaging materials since most remains after processing and may be

released from the polymer matrix to improve food shelf-life. On the other hand, the amount of thymol after processing was slightly higher in nanocomposites containing D43B because the nanoclay can retard thymol evaporation during processing.

The antioxidant activity of the extracts obtained was estimated by scavenging activity against DPPH radicals. This test was performed to evaluate if the remaining thymol in the polymer matrix was enough to be considered an efficient antioxidant in these formulations. Results are also shown in Table 1. All extracts containing thymol showed an important antioxidant activity, as determined by the inhibition of the DPPH radical. The inhibition values are indicative of the amount of thymol remaining in the polymer matrix, which is able to act as an active (antioxidant) agent. In addition, thymol could protect the polymer matrix from oxidative degradation during processing and further the use of these nano-biocomposites (Ramos et al., 2012).

Table 1

3.2. Thermal analysis

Many authors have considered several molecular, as well as radical, mechanisms to explain PLA thermal degradation. The primary cause reported is a non-radical, 'backbiting' ester interchange reaction involving -OH chain ends. This reaction mechanism can, depending upon the size of the cyclic transition state, produce lactide, olygomers or acetaldehyde as well as carbon monoxide. However, other authors have proposed radical reactions, which start with either alkyloxygen or acyl-oxygen homolysis leading to the formation of several types of oxygen- and carbon-centred macroradicals and carbon monoxide (Fukushima, Abbate, Tabuani, Gennari & Camino, 2009a).

The thermal stability of all the materials was studied by TGA under nitrogen. Fig. 1 shows the weight loss (TG) and derivative curves (DTG) obtained for PLA and all nano-biocomposites. The main degradation peak for PLA was observed in all samples around 365-370 ºC. The first degradation step observed in those materials containing thymol was at 120 ºC and continued up to 280 ºC. Losses were associated with thymol degradation, as reported in previous works (Ramos et al., 2012). This result is another indication of the presence of thymol after processing. In this sense, the amounts of thymol, calculated by TGA (Table 2), were similar in all cases to values obtained from the quantification study discussed in the previous section. The initial degradation temperature (T_{ini}), determined at 5% of weight loss, and maximum degradation temperature (T_{max}) of the PLA degradation process are also shown in Table 2. No noticeable differences were observed for T_{ini} and T_{max} values in the materials studied. These results showed the addition of thymol and D43B did not significantly affect the nano-biocomposites thermal degradation profile.

Fig. 1

Glass transition temperatures (T_g) in all nano-biocomposites were determined by DSC (Table 2). This parameter is dependent upon the polymer structural arrangement and corresponds to the torsion oscillation of the carbon backbone (Hughes et al., 2012). T_g results showed the addition of D43B to PLA did not produce significant changes in the polymer structure, as reported by other authors (Lewitus, McCarthy, Ophir & Kenig, 2006). The effect of thymol on PLA was much more important; thymol caused a decrease of more than 10 °C in T_g values, regardless of the presence of D43B. Similar behaviour was reported in PLA formulations with other antioxidants (Byun, Kim &

Whiteside, 2010; Hwang et al., 2012). This could be explained by the plasticizing effect caused by thymol resulting in an increase in molecular mobility of the macromolecular chains. Parameters related with crystallization or melting phenomena of PLA nano-biocomposites were not observed in these formulations due to the amorphous structure of the PLA used in this study.

Table 2

3.3. Mechanical properties

The addition of nanoclays to polymer matrices usually improves mechanical properties, particularly when nanoclay exfoliation occurs. Tensile tests were performed with all the materials studied to evaluate the influence on ductile properties. Results from elastic modulus (MPa) and elongation at break (%) are shown in Table 3. The addition of thymol to PLA matrices resulted in slight modifications of tensile properties. Elastic modulus decreased around 15 % from the original values for neat PLA for those materials containing thymol. This modification could be explained, again, by the plasticizing effect caused by thymol. This result confirmed the observed decrease in T_g values already reported. Similar results have been reported for PLA and low-density polyethylene (LDPE) formulations with active compounds, such as resveratrol, carvacrol or α-tocopherol (Hwang et al., 2012; Persico, Ambrogi, Carfagna, Cerruti, Ferrocino & Mauriello, 2009).

Table 3

As expected, the addition of D43B to PLA increased the elastic modulus and decreased elongation at break. Therefore, the addition of nanoclays increased brittleness (Tabatabaei & Ajji, 2011). This behaviour is related to reinforcement provided by silicate layers and the high aspect ratio and surface area, good dispersion of clay layers throughout the polymer matrix, and strong interactions (Quilaqueo Gutiérrez, Echeverría, Ihl, Bifani & Mauri, 2012). However, this effect was not observed for ternary nano-biocomposites, where lower elastic modulus values were observed compared with pure PLA. This suggests the plasticizing effect of thymol in PLA may prevail over the reinforcement offered by the nanoclay.

3.4. Oxygen transmission rate (OTR)

Barrier properties to oxygen of PLA nano-biocomposites were studied by the determination of oxygen transmission rate per film thickness (e), OTR·e. Results are shown in Table 3. A slight decrease in OTR·e values for films containing D43B was observed. These results could be attributed to the effective intercalation of the nanoclay into the PLA matrix. This behaviour can be explained by considering that oxygen transmission rate is governed by two mechanisms: diffusion and sorption. In general, the efficient dispersion of D43B into the polymer matrix may form a tortuous pathway for oxygen molecules to permeate through the film. This more tortuous pathway results in oxygen molecules following a more complicated way through the polymer matrix. In general, the efficient dispersion of D43B into the polymer matrix may form a tortuous pathway for oxygen molecules to permeate through the film (Martino, Ruseckaite, Jiménez & Averous, 2010; Quilaqueo Gutiérrez et al., 2012).

Regarding PLA with thymol, a slight increase in the OTR.e value was observed. The addition of thymol could modify the properties of PLA by increasing the mobility of macromolecular chains, reducing the polymer orientation and, consequently, decreasing oxygen permeability (Jamshidian, Arab Tehrany, Cleymand, Leconte, Falher & Desobry, 2012).

Finally, no significant differences were found in OTR·*e* values for samples PLA/D43B2.5 and PLA/T/D43B2.5, containing D43B and thymol. However, sample PLA/T/D43B5 presented an OTR·*e* value near to that obtained for PLA and higher than the one observed for sample PLA/D43B5, which may be due to decreased oxygen permeability caused by incorporation of thymol.

In general, the use of nanoclays in these PLA nano-biocomposites effectively improved oxygen barrier properties for food packaging applications.

3.5. Optical properties

Colour and transparency are important factors to be considered in food packaging since they could influence consumer acceptance and commercial success of a food product. Fig. 2 shows the visual aspect of all formulations. All the films had high transparency. Moreover, no agglomeration effects were revealed confirming the efficiency of the processing of PLA nanocomposites. However, some differences in the CIELab coordinates (L*, a*, b*) and ΔE* between neat PLA and nanocomposites were observed (Table 3). These differences could be attributed to the intrinsic colour of the additives used (white for thymol and yellowish for D43B). In this sense, pure PLA had the lowest L* value, indicating that brightness increased with the addition of thymol and D43B. A yellowish-reddish tone was obtained for PLA/T, while PLA/T/D43B5 showed the higher value for ΔE*, as expected, due to the high concentrations of the additives

used (5 wt% D43B and 8 wt% thymol). The uniform distribution of the colour observed throughout the films (Fig. 2) also implies the additives were distributed uniformly within the polymer matrix. Similar tendencies in colour differences have been reported when using active additives such as α-tocopherol and resveratrol into PLA, where the presence of these compounds contributed to strength of colour of the films obtained (Byun et al., 2010).

Fig. 2

3.6. Morphological analysis

3.6.1. Wide Angle X-Ray scattering (WAXS)

WAXS is a useful technique to determine d-spacing in intercalated nanocomposites. The WAXS pattern of PLA is characterized by a broad peak approximately at $2\theta = 15^{o}$ (Fukushima et al., 2009a), confirming its amorphous structure. No significant differences were found from the WAXS patterns of all the formulations studied at this angle range, indicating the polymer structure and crystallinity were not influenced by the presence of D43B and/or thymol.

The most significant features in this study were found in the low angle range (2-10º), (Figure 3). D43B is characterized by a single diffraction peak at $2\theta = 4.6°$ corresponding to the (001) plane, accounting for a 19.2 Å interlayer distance. A shift of the clay diffraction peak to lower angles, corresponding to an interlayer distance of 35.6 Å, was observed for all nano-biocomposites suggesting good interaction of D43B with the polymer matrix. Moreover, a significant decrease in peak intensity was observed, accounting for the formation of a disordered structure. These results indicate the formation of intercalated nano-biocomposites with PLA chains in the galleries of the

D43B layers (Picard et al., 2011). The basal diffraction observed at 2θ around 5.2º (d-spacing = 17.0 Å) in the nano-biocomposites WAXS patterns could be attributed to the fraction characterized by a different alkylammonium chain arrangement in the interlayer space (Persico et al., 2009).

The lowest peak intensity in the WAXS study was obtained for PLA/T/D43B2.5. The presence of thymol could favour nanoclay exfoliation and the effective interaction between the silicate layers and thymol, which could promote the swelling of the nanoclay stacks (Persico et al., 2009). However, the formulations with 5 wt% of D43B showed more intense peaks. This behaviour could be due to the unfavourable effect of nanoclay swelling, produced by thymol at high nanoclay loading. In conclusion, WAXS results suggest effective nanoclay intercalation achieved by mixing PLA with 2.5 wt% of D43B and 8 wt% of thymol.

Fig. 3

3.6.2. Transmission Electron Microscopy (TEM)

The dispersion of nanoclays in PLA nano-biocomposites was evaluated by TEM. Figure 4 shows the micrographs obtained for PLA/T/D43B2.5, showing the nanoparticles partial exfoliation. Single dispersed clay layers (dark regions in Figure 3) were randomly distributed through the PLA matrix (clear areas) and some regions with complete exfoliation of nanoclay layers were noticed. TEM analyses also suggested the good dispersion of D43B and thymol through the PLA matrix, already asserted by WAXS patterns, since no important aggregates were observed. The interaction of nanoclays with PLA was attributed to the formation of hydrogen bonds between the

carbonyl group of lactide and the hydroxyl groups of the nanoclay organic modifier (Fukushima et al., 2009b).

Fig. 4

4. Conclusions

Different nano-biocomposite films, based on PLA, with antioxidant potential to be used in active packaging were processed and characterized. Several analytical techniques were used to evaluate the effects of incorporating the nanoclay (D43B) and thymol on the PLA nano-biocomposites properties. The addition of thymol did not significantly affect the thermal stability of PLA, but some decrease in the elastic modulus was observed due to the thymol plasticizing effect. The incorporation of D43B resulted in a clear enhancement of oxygen barrier and mechanical properties, due to the intercalation and partial exfoliation of the nanoparticles through the polymer matrix. Some differences in films colour were observed with the addition of thymol and D43B, but films remained mostly transparent. Most of the thymol added remained in the formulations after processing, which resulted in a significant antioxidant activity, as indicated by the high percentage of inhibition obtained using the DPPH test. In conclusion, the PLA nano-biocomposites studied, in particular those containing 8 wt% of thymol and 2.5 wt% of D43B, could be considered promising antioxidant active packaging materials with a biodegradable nature and able to increase foodstuff shelf-life.

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511 **Figure captions**

512 **Fig. 1.** TG (a) and DTG (b) curves obtained for pure PLA and nano-biocomposites
513 under nitrogen.

514 **Fig. 2.** Visual observation of PLA and nano-biocomposite films.

515 **Fig. 3.** WAXS patterns of D43B, PLA and nano-biocomposites.

516 **Fig. 4.** TEM images of PLA/T/D43B2.5 nano-biocomposite film.

Table 1

Quantification of thymol by HPLC-UV analysis and radical scavenging activity measured by DPPH assay obtained for the different formulations used in this work.

Sample	D43B (wt%)	Thymol (wt%)	Amount of extracted Thymol (wt%)[a]	Inhibition (%)[b]
PLA	-	-	n.d.	n.d.
PLA/D43B2.5	2.5	-	n.d.	n.d.
PLA/D43B5	5	-	n.d.	n.d.
PLA/T	-	8	5.57 ± 0.01	71.1 ± 0.2
PLA/T/D43B2.5	2.5	8	5.99 ± 0.03	84.3 ± 0.3
PLA/T/D43B5	5	8	5.78 ± 0.02	83.5 ± 0.1

[a](n =3, mean ± SD); [b](n =3, mean ± SD); (n.d.: not detected)

Table 2

TGA and DSC parameters obtained for all nano-biocomposites.

Sample	wt% weight loss (1^{st} step)	T_{ini} (ºC) (2^{nd} step)	T_{max} (ºC) (2^{nd} step)	T_g (ºC)
PLA	n.d.	335	369	57
PLA/D43B2.5	n.d.	334	363	57
PLA/D43B5	n.d.	340	369	57
PLA/T	6.6	331	366	43
PLA/T/D43B2.5	6.3	336	366	41
PLA/T/D43B5	7.1	339	369	44

(n.d.: not detected)

Table 3

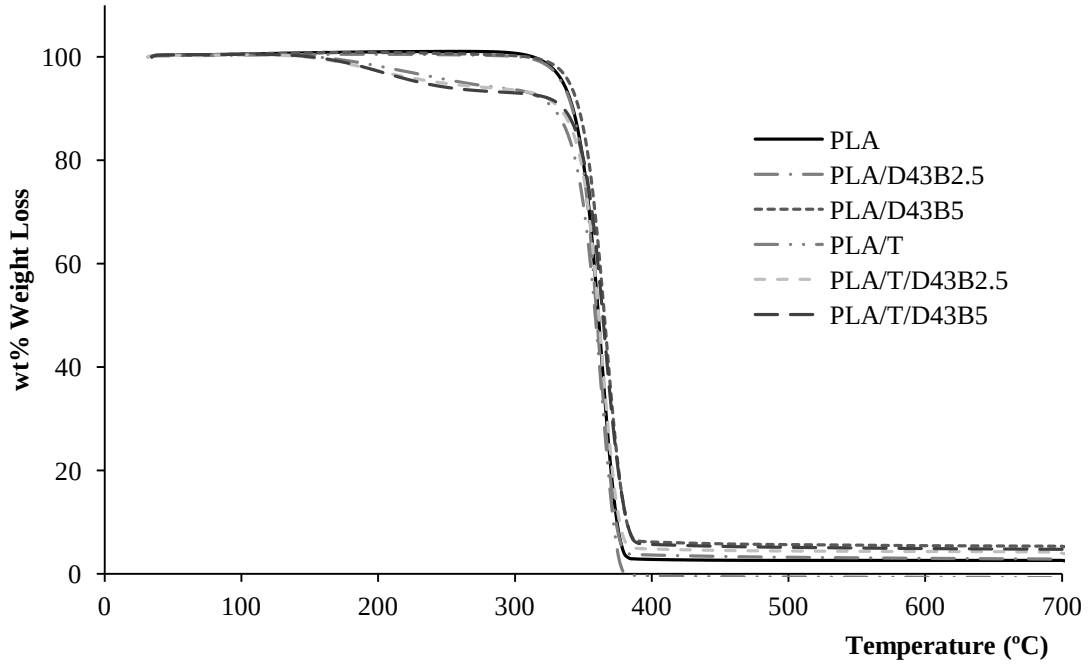
Tensile properties (ASTM D882-09), oxygen transmission rate and CIELab colour parameters obtained for all the studied formulations.

Sample	Elongation at break (%)[a]	Elastic modulus (MPa)[a]	OTR·e (cm^3 mm m^{-2} day)[b]	L*	a*	b*	ΔE*[c]
PLA	3.5 ± 0.1	2575 ± 76	22.1 ± 1.5	30.3	-0.11	-0.20	-
PLA/D43B2.5	2.1± 0.4	2739 ± 151	20.1 ± 2.0	30.7	0.02	-0.01	0.5
PLA/D43B5	1.5 ± 0.2	2715 ± 95	17.1 ± 2.3	32.0	-0.24	-0.81	1.9
PLA/T	4.3 ± 0.1	2167 ± 196	23.0 ± 0.2	33.3	-0.49	-1.10	3.2
PLA/T/D43B2.5	2.4 ± 0.1	2246 ± 135	20.1 ± 0.1	32.0	-0.22	-1.14	2.0
PLA/T/D43B5	2.4 ± 0.2	2140 ± 116	22.7 ± 1.3	34.4	-0.58	-1.48	4.4

[a] n = 5, mean ± SD
[b] n =3, mean ± SD (e: thickness, mm)
[c] PLA film was used as control

Fig. 1

(a)



(b)

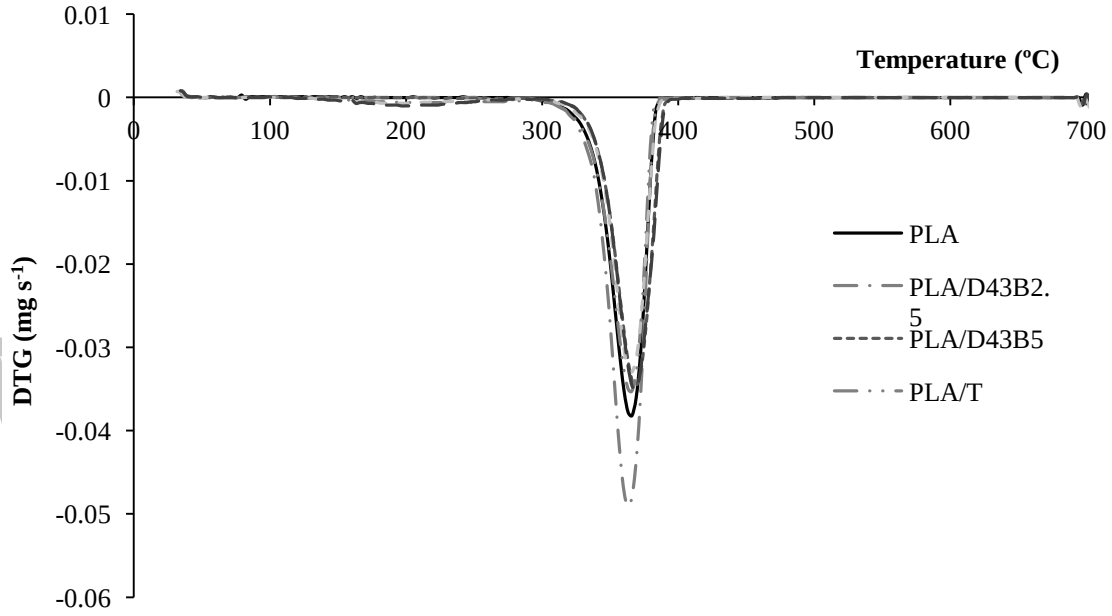


Fig. 2

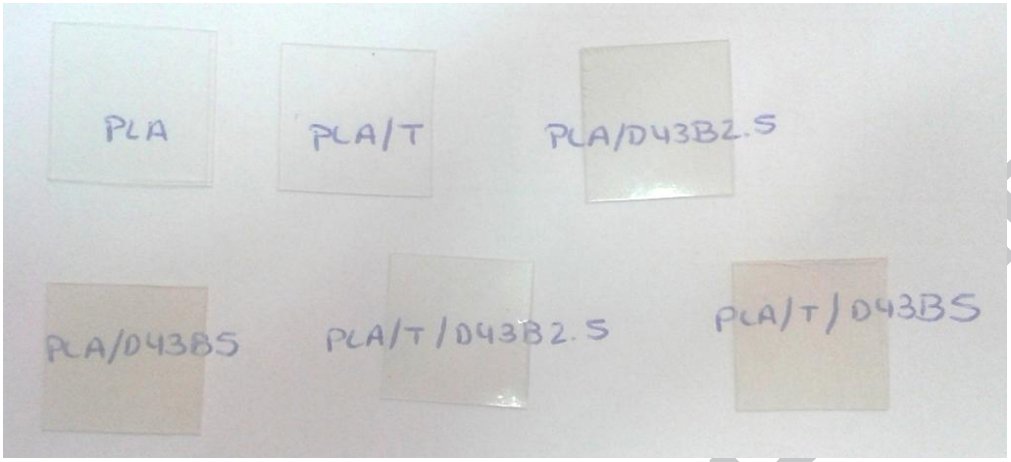


Fig. 3

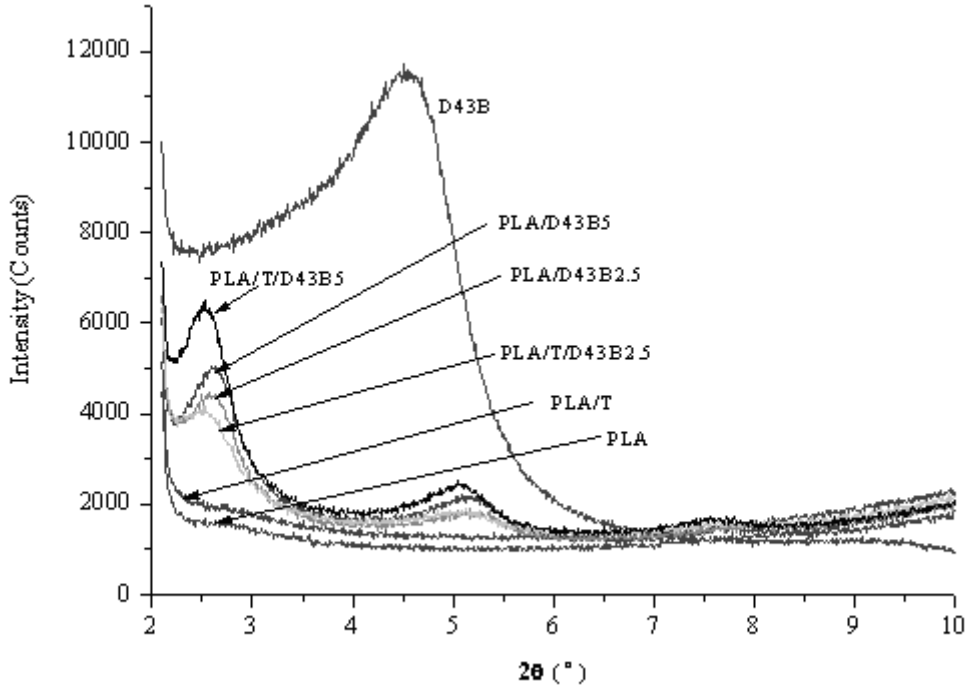
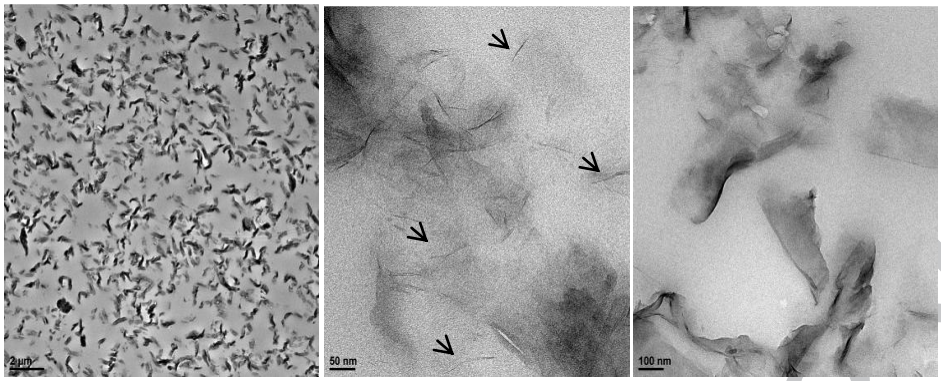


Fig. 4



Highlights

1. Thymol and D43B were added to PLA to get antioxidant nano-biocomposite films
2. A full characterization of all formulations was carried out
3. The presence of thymol in the films and its antioxidant activity were demonstrated
4. The addition of D43B improved oxygen barrier and mechanical properties of films
5. The film with thymol and 2.5 wt% of D43B was considered the best enhanced material