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Properties of PLA films with cinnamic acid: Effect of the processing method



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ABSTRACT

Poly(lactic acid) (PLA) is a sustainable material for food packaging applications. The incorporation of cinnamic acid (CA) into PLA films was studied to obtain antimicrobial films, using two different techniques for film production, namely casting and thermal processing (TP). Whereas CA could be successfully incorporated at 5 and 10% w/w into TP films, only 3% w/w could be used in cast films without observing any crystallisation of CA. The acid reduced the glass transition temperature in TP films, while also improving their thermal resistance. Cast films retained a little proportion of the casting solvent which contributed to their plasticisation. The processing method greatly affected the mechanical properties of the films, with the cast films being significantly more plastic and stretchable. CA reduced the film's stiffness and resistance to break and improved their water vapour and oxygen barrier capacity. Antibacterial assays with *Listeria innocua* did not show significant growth inhibition for any film, but the observed inhibition could be related to the acid concentration. This suggested that only a small amount of acid near the film surface could be released. Further research to promote the superficial concentration of cinnamic acid into PLA films is required to obtain active materials.

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1. Introduction

As the environmental global crisis escalates, the consumption of single-use plastic increases. Traditional plastics still figure as the most commonly used material for food packaging (Harnkarnsujarit et al., 2021). It is estimated that less than 30% of the plastic produced in Europe is recycled (Gong et al., 2020). Alternatives, such as biodegradable polymers, with suitable properties for foodstuff preservation purposes have been widely studied and are beginning to have a small presence in the market (Harnkarnsujarit et al., 2021). Creating more applications for these materials could help increase their demand and economic viability (Mangaraj et al., 2018). In this sense, active packaging represents an alternative means of providing biodegradable polymers with improved capacity to preserve the safety and quality of food products.

Active compounds from renewable sources, with known antioxidant or antimicrobial capacities, have been studied in the few past years as possible substitutes for synthetic food preservatives (Guil-

Guerrero et al., 2016). Several phenolic compounds are a promising alternative since they are abundant in nature, have low minimal inhibitory concentrations (MIC) for several pathogens, and do not possess marked organoleptic properties, unlike widely studied essential oil compounds (Choi et al., 2018; Miyague et al., 2015). Cinnamic acid (CA) is the precursor of the prevalent family of phenolic acids, known as hydroxycinnamic acids, and can be found in a wide range of vegetable sources (Rashmi and Negi, 2020). Cinnamic acid exhibits antimicrobial properties, as different studies have demonstrated (Miyague et al., 2015; Sánchez-Maldonado et al., 2011), being especially active against *Listeria*, which constitutes a dangerous food pathogen due to its persistency at cold temperatures (Tasara and Stephan, 2006).

Cinnamic acid has been previously incorporated into thermoplastic starch matrices to obtain active biodegradable films, which exhibited growth inhibition of both Gram-positive and negative bacterial strains (Ordoñez et al., 2021). Nevertheless, starch and other hydrophilic polymers present limited applications given their high degree of sensitivity to water (Zhong et al., 2020). Hydrophobic biodegradable or com-

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postable polyesters, such as polylactic acid (PLA), could be the base for water-resistant materials suitable for high moisture environments. PLA has been widely studied as a potential carrier for active compounds in food packaging applications as recently reported by Rojas et al. (2021). Different essential oils and their compounds, as well as other phenolic compounds, have been incorporated into the PLA matrices, provoking different changes in the film functional properties while providing them with antioxidant or antimicrobial capacity, depending on the film processing conditions and the nature (volatility, solubility or thermo-sensitivity) of the active agent (Velásquez et al., 2021).

A previous study (Ordoñez et al., 2022) showed no effective antimicrobial activity when cinnamic acid was incorporated at low concentrations (up to 2% w/w) into amorphous PLA matrices obtained by thermal processing (TP). This was attributed to the limited molecular mobility of the compound inside the glassy matrix, limiting its diffusion and release into the culture medium. Nevertheless, the release of active compounds from PLA films can highly be affected by the film processing method (Rojas et al., 2021; Velásquez et al., 2021). Considering the great potential of PLA with CA incorporated as possible active packaging material, several alternative means of enhancing the rates and ratio of the release of CA into the food system should be studied, such as the incorporation of a higher dose of active compounds in the films or plasticising the films to favour the mass transfer phenomena.

Polymeric films studied for food packaging applications have mainly been obtained by two types of methods: thermal processing (TP) techniques (such as melt-blending, compression-moulding, or extrusion) or solvent casting (Suhag et al., 2020). Casting consists of preparing polymeric solutions in suitable solvents and casting a certain amount of the solutions on levelled plates, letting the solvent evaporate in order to obtain thin film layers. While casting has been widely used in research, its use on an industrial scale is not realistic, given the energy requirements and process disadvantages for solvent evaporation. Therefore, TP methods are the standard industrial method, whereas casting could be used to coat thermo-processed materials with a thin layer of film-forming solution carrying active compounds (Tampau et al., 2020b). Casting does not apply high temperatures that can alter thermosensitive active compounds, while cast films tend to present more plastic behaviour (Muller et al., 2017a; Rhim et al., 2006), which could potentially favour the migration of active compounds, such as C, to the food substrate. Requena et al. (2018) obtained cast PLA-PHBV blend films with carvacrol with a great capacity to inhibit bacterial growth on inoculated aqueous culture media. Despite the proven antimicrobial properties of cinnamic acid, no previous studies have analysed the incorporation of cinnamic acid into PLA films by casting or thermoprocessing in a wide range of the compound concentration, in order to obtain active films with an effective compound release and antimicrobial action.

This study focused on incorporating cinnamic acid into PLA matrices at concentrations up to 10%, analysing the effect of the processing method (thermal processing or casting) on the antimicrobial activity of the films against *Listeria innocua*, as well as on the thermal behaviour and functional (mechanical, barrier and optical) properties of these materials.

2. Materials and methods

2.1. Materials

Amorphous PLA 4060D (Nature Works, MN, USA; 106 kDa average MW) was used to obtain films with and without cinnamic acid (Sigma-Aldrich, Saint Louis, USA) at different concentrations. Magnesium nitrate and phosphorus pentoxide (Panreac Química, Barcelona, Spain) were used to balance the sample relative humidity (RH) at 53% and 0% respectively. Ethyl acetate (EtAc) from Indukern (Barcelona, Spain) was used as food-grade solvent. *L. innocua* strain (CECT 910) supplied by Spanish Type Collection (Valencia, Spain) was used in antimicrobial *in vitro* assays. Tryptic soy broth (TSB), tryptic soy agar

(TSA), buffer peptone water, palcam agar base (PAB), and palcam selective supplement for *Listeria* were all supplied by Scharlab (Barcelona, Spain).

2.2. Film preparation

Different CA concentrations, up to 10% w/w, were tested both for cast and thermally processed (TP) PLA films. The cast films were produced as described by Muller et al. (2017a), using ethyl acetate (EtAc) as solvent. Film-forming solutions (FFS) with PLA at 10 wt.% were obtained, by stirring overnight at room temperature. To obtain 4 g films, 40 g of FFS were poured into 15 cm diameter Teflon plates and left to dry overnight. A formulation without CA was produced (Cast-PLA), and CA concentrations in the cast films ranged from 2% to 10% w/w in solids (2C–10C).

To obtain thermo-processed films, PLA pellets were first dried at 60 °C overnight to prevent hydrolysis during the process. TP films were obtained by adding the components in an internal mixer (Haake PolyLab QC, Thermo Fisher Scientific, Germany) where a melt blending process was taking place at 50 rpm and 160 °C for 5 min. The obtained pellets were cold ground employing an M20 mill (IKA, Staufen, Germany). To produce the films, 4 g pellet samples were placed in circular Teflon moulds, and 15 cm diameter films were obtained through compression moulding with a thermal-hydraulic press (LP20, Lactech Engineering, Thailand). The samples were preheated at 200 °C for 4 min before the compression step at 10 MPa for 4 min. Finally, the films were cooled down to 70 °C in 3 min. The concentrations of CA in TP films were 5 and 10% (w/w), giving rise to formulations TP-5C and TP-10C. A control formulation without CA was also obtained (TP-PLA).

2.3. Film characterisation

2.3.1. Thermal analysis

The thermal behaviour of the film formulations was analysed using a differential scanning calorimeter (DSC, 1 StareSystem, Mettler-Toledo, Switzerland) while thermal degradation was studied with a thermogravimetric analyser (TGA/SDTA 851e, Mettler-Toledo, Switzerland). Both tests were duplicated for each formulation. Before the analyses, the samples were conditioned at 0% RH. For the DSC, the samples (about 10 mg) were sealed inside aluminium pans and placed into the calorimeter. The temperature was initially lowered from 25 °C to –10 °C and then raised to 200 °C at 10 °C/min. Following a 5 min isothermal step at 200 °C, a fast-cooling step took place at 50 °C/min until –10 °C was reached. This temperature was maintained for 5 min and, finally, a second heating to 200 °C took place at 10 °C/min. For the TGA analyses, the samples (about 10 mg) were placed into aluminium crucibles in the TGA system and maintained under a nitrogen flow of 50 ml/min while heating took place from 25 to 600 °C at 10 °C/min. The initial degradation temperature (T_{onset}), (mass loss of 5%) and the maximum degradation rate temperature (T_{peak}) were registered.

2.3.2. Retention of cinnamic acid

The retention of CA in the films was quantified in three samples per formulation via methanolic extraction and spectrophotometric quantification. Dry film samples of around 100 mg were immersed in vials with 10 ml of methanol and magnetically stirred for 48 h at room temperature. The methanolic extracts were filtered and properly diluted to measure their

absorbance at 270 nm in a UV-vis spectrophotometer (Thermoscientific Evolution 201, USA). The concentration of CA was calculated with a previously obtained calibration curve and the results were expressed as g CA per 100 g of CA initially incorporated into the film sample.

2.3.3. Mechanical, barrier, and optical properties

The tensile properties of the films were determined using a texture analyser TA-XT plus (Stable Micro Systems, Haslemere, England), following the ASTM D882 method (American Society for Testing Materials, 2002). Eight film strip samples (25 × 100 mm) per formulation were tested. An electronic micrometer (Comecta S.A., Barcelona, Spain) was used to determine the thickness of each strip at six random points. Strips were placed on tensile grips with 50 mm of separation and were stretched at 50 mm/min to obtain the stress–Henky strain curves, from which tensile strength at break (TS), elastic modulus (EM) and percent elongation at break ϵ were obtained.

The ASTM E96-95 (American Society for Testing Materials, 1995) method was followed to measure the water vapour permeability (WVP), using 3.5 cm diameter Payne permeability cups (Elcometer SPRL, Hermelle/s Argeteau, Belgium). Three circular film samples per formulation were cut to match the diameter of the cups, and the thickness was measured at six different random points. The cups were filled with 5 ml of distilled water, the films were mounted and sealed before placing the cups into desiccators with MgNO₃ saturated solution and kept at 25 °C. The cup mass was monitored using an analytical balance (± 0.00001 g), and the mass-loss rate was used to calculate the WVP once the stationary state had been reached, according to Andrade et al. (2020).

The oxygen permeability (OP) was determined following ASTM D3985-05 (American Society for Testing Materials, 2010), using Ox-Tran 1/50 equipment (Mocon, Minneapolis, USA). The film samples (two per formulation, the thicknesses of which were measured at six random points) were cut to fit the equipment setup, with 50 cm² of exposed area. The tests were performed at 25 °C and 53% RH to obtain the oxygen transmission rate (OTR). The OP was calculated by dividing the OTR by the gradient of oxygen partial pressure on both sides of the film and multiplying it by the film thickness.

A CM-5 Spectrocolorimeter (Konica Minolta Inc., Japan) was used to evaluate the optical properties of the films. Reflection spectra (400–700 nm) were obtained by measuring three films per formulation at three different points, backed by black and white backgrounds. The CIEL*a*b* coordinates were calculated from the reflectance of an infinitely thick layer of material, considering D65 illuminant and 10° observer (Cano et al., 2014). The psychometric coordinates of lightness (L*), chroma (C_{ab}*), and hue (h_{ab}*) were calculated (Mahy et al., 1994). Kubelka–Munk's theory of multiple dispersion was used to determine the internal transmittance (T_i) of the film samples (Hutchings, 1999).

2.3.4. Antibacterial activity

In vitro bacterial growth inhibition tests were carried out against *L. innocua* according to the methodology used by Ordoñez et al. (2021). Circular film samples were cut to match the inner diameter of a 55 mm Petri dish and sterilised on both faces with UV light in a laminar flow cabinet (Bio II advance, Telstar, Spain). 10 ml of TSA were poured into Petri dishes and left to solidify before being inoculated with 100 μ l of 10⁶ UFC/ml *L. innocua* using an L-rod to evenly distribute the inoculum on the medium's surface. Afterwards, the inoculated

medium was covered with the different films. The dishes were closed and sealed with Parafilm™ and left to incubate for 6 days at 10 °C. After the incubation, the medium and films were homogenised in 100 ml of peptone water employing a masticator paddle blender (IUL Instruments, Barcelona, Spain). Serial dilutions were plated and covered with PAB selective media and left to incubate for 48 h at 37 °C before counting the colonies. Two film samples were included per formulation and all of the dilutions were plated in duplicate. An uncoated growth control sample was included as a growth reference.

2.4. Microscopic observations

Optical microscopy (B-353PL, Optika, Italy) was used to observe the CA crystalline structures in cast films. Field emission scanning microscopy (FESEM) was used to analyse the potential presence of crystals in TP films. To this end, the samples were cryofractured and platinum-coated. The cross-sectional observations were carried out under a 2 kV acceleration voltage in an Ultra 55 Zeiss, UK microscope.

2.5. Statistical analysis

Statistical data analysis was performed through analysis of variance (ANOVA) using Statgraphics Centurion XVII software. Fisher's least significant difference was used at a 95% confidence level.

3. Results and discussion

The films obtained by TP (C ratio up to 10% w/w) were homogeneous and transparent to the naked eye (Fig. 1), and FESEM cross-sectional images (Fig. 2) revealed that no crystalline structures were formed in any TP film despite the high concentration of cinnamic acid. The films obtained by casting (Fig. 1) with CA up to 3% w/w were homogeneous and translucent as well. However, cast films with CA concentrations above 3% presented visible crystalline formations whose number and size increased accordingly with the proportion of C. Optical microscopy observations of the formed crystals (Fig. 2) revealed the characteristic monoclinic structure of cinnamic acid crystals, as reported by Hsieh et al. (2005). The crystallisation of cinnamic acid during the casting process suggests that compound oversaturation occurred during solvent evaporation and film formation. The crystallisation was not inhibited by the viscous effects of the PLA solution or any polymer-compound interactions that could limit the nucleation and growth of cinnamic acid. In contrast, the high viscosity of the PLA-acid melt blend inhibited the crystallisation of cinnamic acid, which remains well dispersed in the film matrix. Likewise, more intense interactions between the polymer chains and acid molecules could be promoted at the high temperature used in TP conditions. In this sense, in the absence of a solvent, the acid could be bonded to the hydroxyl end chain groups of PLA at high temperature, and form hydrogen bonds with carbonyl groups of the chains. Therefore, the processing procedure would determine not only the proportion of CA that can be incorporated into the film but also all of its final properties derived from the different polymer-acid interactions. Given the heterogeneous structure of the cast films with more than 3% CA, which hinders their real application, the physico-chemical characterisation of cast films was limited to 2C and 3C formulations.

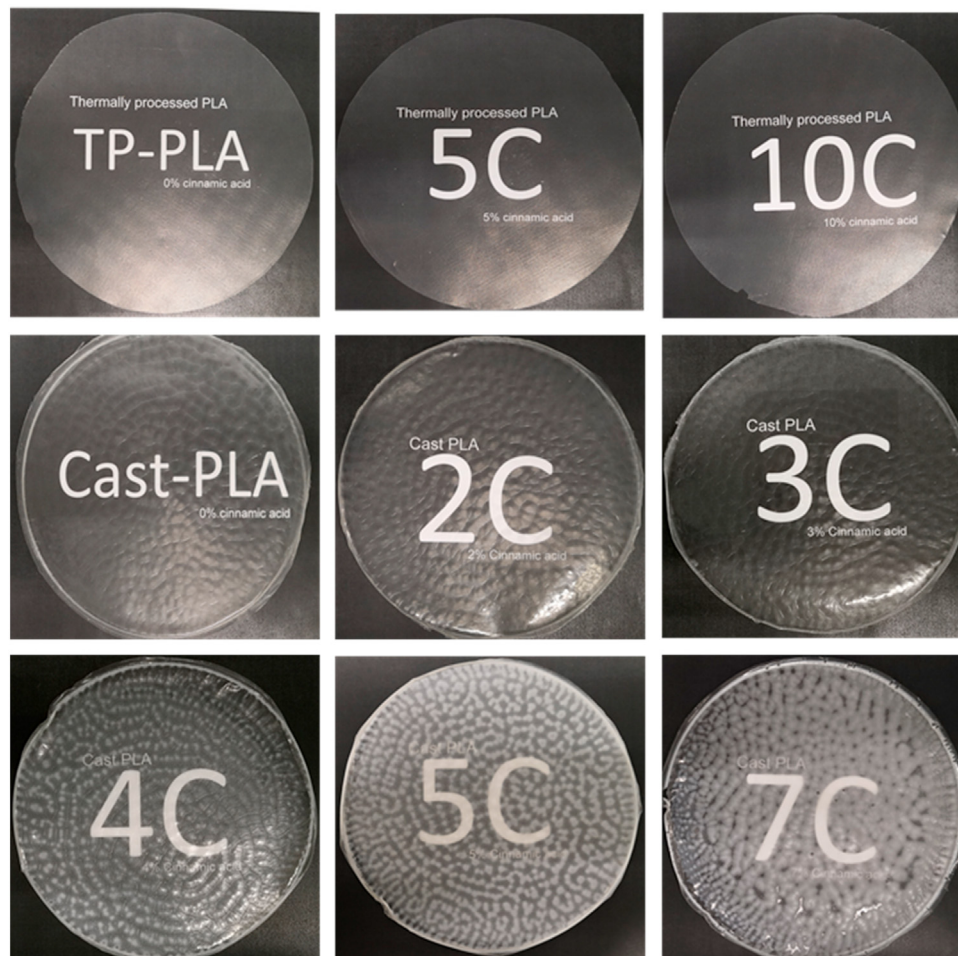


Fig. 1 – Thermally processed (first row) and cast (second and third rows) PLA films with different cinnamic acid concentrations.

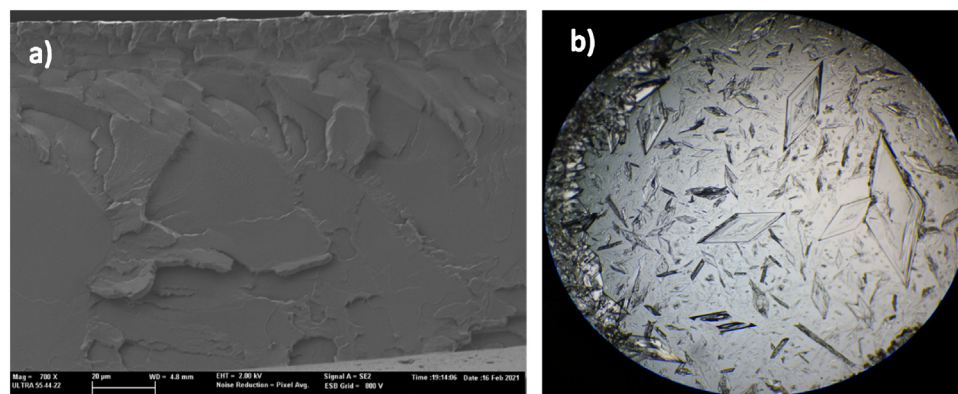


Fig. 2 – (a) FESEM micrograph of TP-10C film cross-section at 700 \times . (b) Crystals of cinnamic acid observed by optical microscopy ($\times 10$) in cast PLA films with 5%w/w CA.

3.1. Thermal behaviour and degradation

Fig. 3 shows the first and second heating thermograms obtained by DSC analyses for the different films obtained by casting and thermoprocessing with differing concentrations of cinnamic acid. Coherently with the amorphous nature of PLA, no melting peak was detected but only the glass transition, in some cases followed by the subsequent relaxation endotherms (only in the first heating step) associated with the effects brought about by matrix ageing. In cast films, small endotherms can be observed at about 85 °C that may be attributed to the total or partial evaporation of the sol-

vent retained (EtAc) in the film during the film formation step, which was released during the thermal scan. The integration of the peaks permits the estimation of the amount of solvent evaporated by comparing it with the evaporation enthalpy of the pure solvent (404 J/g). This amount was estimated as about 2% w/w of the film samples, with a certain variability between samples.

Table 1 shows T_g values (mid-point) obtained for the different samples from the first and second heating scans of DSC analyses. For the TP films, the incorporation of 5 and 10% CA promoted a significant plasticisation of the PLA matrix: the higher the CA concentration, the lower the T_g values. This

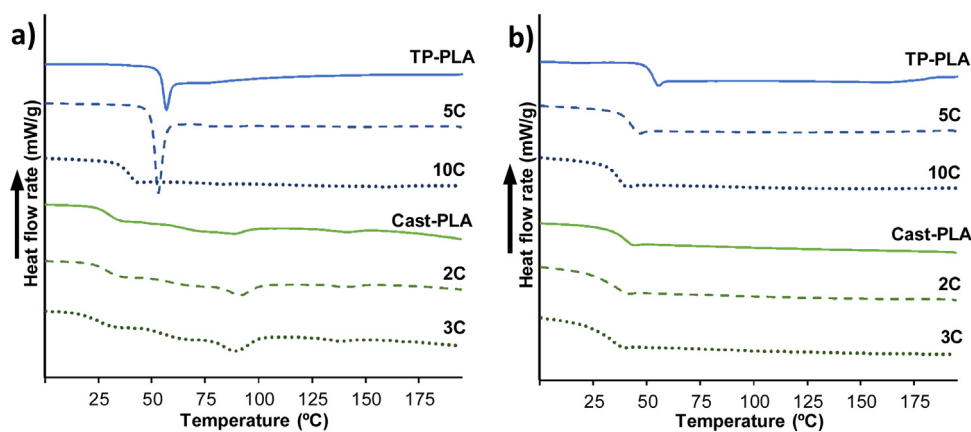


Fig. 3 – DSC heating thermograms of PLA films obtained by thermal processing (TP) or casting. Effect of cinnamic acid (CA). (a) First heating (b) second heating. 2–10C stands for the mass percentage of CA in the dry film.

Table 1 – Thermal properties of thermoprocessed (TP) and cast PLA films with different cinnamic acid ratios. Glass transition temperatures in the first and second DSC heating scans (T_{g1} and T_{g2} , respectively). Onset and peak degradation temperatures from TGA.

	DSC		TGA	
	T_{g1} ($^{\circ}\text{C}$)	T_{g2} ($^{\circ}\text{C}$)	T_{onset} ($^{\circ}\text{C}$)	T_{peak} ($^{\circ}\text{C}$)
TP-PLA	54.1 \pm 0.9 ^f	54.1 \pm 0.9 ^d	265 \pm 2 ^a	310 \pm 2 ^a
5C	49.1 \pm 0.1 ^d	41.5 \pm 0.1 ^c	312 \pm 2 ^b	363 \pm 7 ^b
10C	38.9 \pm 0.2 ^c	35.3 \pm 0.2 ^b	310 \pm 9 ^b	355 \pm 5 ^b
Cast-PLA	28.6 \pm 0.3 ^b	37.4 \pm 0.2 ^b	333 \pm 17 ^b	368 \pm 14 ^b
2C	22.0 \pm 0.5 ^a	31.0 \pm 2.0 ^a	311 \pm 2 ^b	354 \pm 7 ^b
3C	22.6 \pm 0.4 ^a	35.1 \pm 1.8 ^b	323 \pm 13 ^b	365 \pm 9 ^b

Different super indices in the same row indicate significant differences between film formulations ($p < 0.05$). 2–10C stands for the mass percentage of CA in the dry film.

could be attributed to a hydrolytic effect of the acid under thermal conditions, favouring the formation of oligomers that contribute to plasticising the PLA matrix. The second heating step did not reveal the significant changes in the T_g values obtained in the first scan, but as expected, no relaxation endotherms were observed after glass transition since the first heating erased the sample's thermal history. This plasticising effect was also observed (Ordoñez et al., 2022), but to a lesser extent, in thermo-processed PLA films with 1 and 2% cinnamic acid, which indicates that the effect was proportional to the compound concentration.

The cast films with cinnamic acid exhibited lower T_g values than TP PLA (28 vs. 54 $^{\circ}\text{C}$), which could be attributed to the solvent retained in the matrix, which plasticises the polymer. The incorporation of the acid at 2 and 3% also reduced the T_g values to the same extent (a reduction of approximately 7 $^{\circ}\text{C}$), which points to additional effects of cinnamic acid on polymer plasticisation. The solvent evaporation (at least in part) during the first heating scan led to higher T_{g2} values, as compared to T_{g1} , while the T_{g2} values of cast films were still lower than those of TP films. Muller et al. (2017a) reported slightly higher values of T_g for the cast films of amorphous PLA also obtained from EtAc solutions, as well as a plasticisation effect of cinnamaldehyde. Tampau et al. (2020a) also report that the EtAc absorption in cast PLA matrices took place to a different extent, seriously affecting the plasticisation level of the polymer.

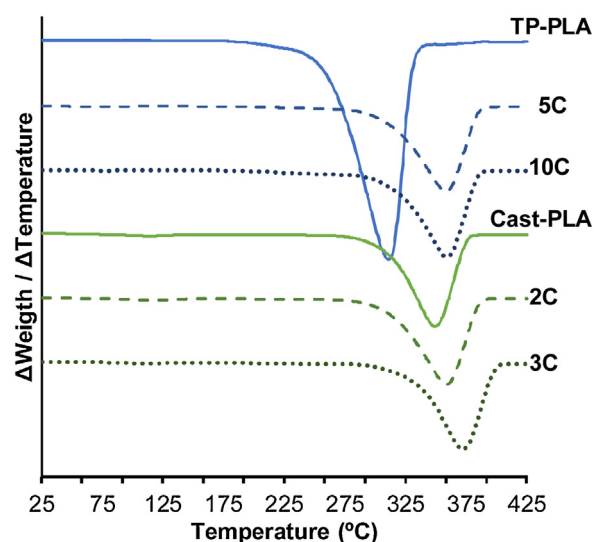


Fig. 4 – DTGA curves of PLA films obtained by thermal processing (TP) or casting with different ratios of cinnamic acid (CA). 2–10C stands for the mass percentage of CA in the dry film.

Fig. 4 shows the typical derivative curves of TGA analyses for the different film formulations and Table 1 gives the onset and peak temperatures in each sample. In TP films, only the thermal degradation of the polymer was observed, whereas in cast films small weight loss events were observed at about 115 $^{\circ}\text{C}$, which can be attributed to the solvent evaporation (1–3 wt.), as observed in DSC analyses. The thermal degradation of PLA is due to a random main-chain scission reaction, as well as depolymerisation, oxidative degradation and transesterification reactions. Reactive end groups and impurities have been reported to enhance thermal degradation (Signori et al., 2008). As observed in Fig. 4, the differing film-forming methods and proportions of CA affected the thermal degradation behaviour of PLA. The thermal resistance of TP PLA films was significantly lower than that of cast PLA films. This can be explained by the different packing of PLA chains in each treatment, with differing thermal resistance, or by the partial degradation that occurred in TP films during thermo-processing. Thermo-processing could give rise to the formation of oligomers which can reduce the onset and peak temperatures of the polymer matrix degradation. The incorporation of CA at 5 and 10% exerted a thermal protective effect on TP films, thus delaying degradation by more than 40 $^{\circ}\text{C}$. The

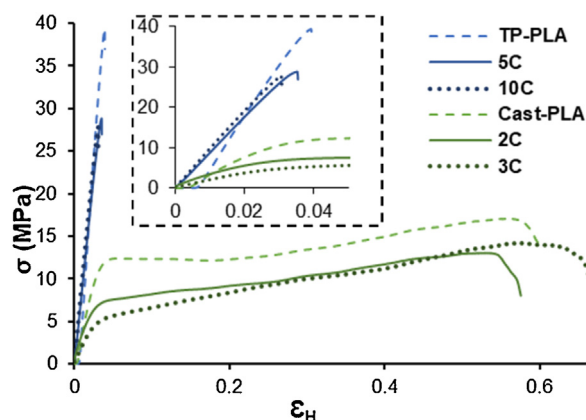


Fig. 5 – Typical stress–strain curves of the PLA films obtained by thermal processing (TP) and casting methods. 2–10C stands for the mass percentage of cinnamic acid in the dry film.

protective effect of CA on TP PLA films was previously observed at lower CA concentrations (Ordoñez et al., 2022). Tran et al. (2015) successfully obtained novel PLAs with the conjugation of both chain terminals with CA and its derivatives and proved the significant improvement in their thermal properties. In contrast, cinnamic acid did not exert a thermo-protective action in cast films which suggests no effective interactions of this compound with PLA chains. This was coherent with the observed crystallisation of CA in cast films, which suggested poor interactions between CA and PLA chains during the solvent casting process, which led to phase separation and compound crystallisation, as previously commented on. Therefore, the bonding of cinnamic acid to PLA chains during thermo-processing could be deduced from the thermal analyses.

3.2. Cinnamic acid retention

The results of CA retention in the films are shown in Table 2. The casting method preserved CA in the films to a remarkable extent, and percentages close to 100% were reached for both 2C and 3C formulations. The complete recovery of CA is probably due to the non-thermal nature of this method, where the films are formed at room temperature by the evaporation of the solvent. Under these conditions, CA is not oxidized or degraded by thermal stress. In contrast, CA extraction from TP films showed lower CA recovery values (89, 75%). This points to the partial losses of the compound caused by the thermal stress undergone by the matrix in both melt blending and compression moulding steps. In fact, previous studies reported the initial degradation of CA at 180 °C under a nitrogen atmosphere (Ordoñez et al., 2021), whereas in thermal processing occurred at lower temperatures.

3.3. Mechanical, barrier and optical properties

Table 2 shows the obtained values for thickness (t), tensile strength at break (TS), elastic modulus (EM), and percent elongation at break (%E) for all of the formulations and Fig. 5 shows the typical stress–strain curves of the different films. Despite having the same mass of solids (PLA + C), the TP films were significantly thinner than the cast films ($p < 0.05$), which suggests a different structural arrangement of the polymer chains, depending on the production method. A more com-

pact film structure would, hence, be obtained by TP, and the chain arrangement caused by thermal treatment may favour the cohesiveness of the matrix (Rhim et al., 2006). The previously commented on solvent retention in the cast films would also contribute to the swelling and greater thickness of the film. In no case was the film thickness significantly affected by the incorporation of cinnamic acid.

The film tensile behaviour was also barely affected by the production method. Compared to TP films, the cast films were less stiff and resistant to break with barely enhanced plastic deformability (Fig. 5). This agrees with that reported in a previous study (Rhim et al., 2006). The high degree of cohesiveness of the TP films led to their reduced stretchability and increased stiffness. Additionally, the solvent retained in the cast films would promote their plastic behaviour, facilitating the molecular mobility of the polymeric chains, as previously observed by Muller et al. (2017a). The incorporation of cinnamic acid modified the mechanical performance of both the TP and cast films, reducing the TS and EM while having no significant effect on the %E. This could be attributed to the plasticising effect of CA on the amorphous PLA matrices, as described for organic acids in previous studies (Azeredo and Waldron, 2016). The mechanism for this effect was attributed to the interruption of interchain polymer interactions by acid molecules, hence promoting a decrease in the matrix cohesion forces. In the TP films, the weakening effect provoked by CA could also be related to the potential hydrolytic effect of the acid during the melt-blending step, causing some degradation of PLA to yield oligomers, leading to a reduction in the material cohesiveness (Mangeon et al., 2017; Signori et al., 2008).

The obtained water vapour permeability (WVP) values are shown in Table 2. Additionally, specific arrangements in the polymer matrix could further improve this capacity. Both control PLA films (TP-PLA and Cast-PLA) exhibited similar water barrier capacity, as has been reported in previous studies (Muller et al., 2017a). The incorporation of cinnamic acid greatly reduced the WVP of the films, mainly in TP films; the greater the CA concentration the higher the WVP reduction. This could be related to the bonding of acid molecules to PLA chains through the end-chain OH groups and to the carbonyl groups inside the chains, forming hydrogen bonds (Collazo-Bigliardi et al., 2019; Madhavan Nampoothiri et al., 2010). This would contribute to a reduction in the little water affinity of the polymer matrix, thus reducing the solubility and permeability of water molecules through the polymer. The improvement in the water barrier properties brought about by the incorporation of CA was also observed in other more hydrophilic polymer matrices, such as sodium caseinate; in this case, the observed reduction in WVP was even significant when compared with what was promoted by the incorporation of ferulic acid (Fabra et al., 2011). The milder effect of CA on the WVP reduction in the cast films agrees with the inhibition of CA-PLA interaction, in the presence of the solvent, as commented on above.

A high degree of oxygen permeability is a problem for the application of PLA films in food packaging (Muller et al., 2017b). The processing method had a significant effect on the oxygen barrier capacity of the control films, and the TP-PLA presented a lower OP than the Cast-PLA films (Table 2). This could be related to the more compact structure attained by TP films, in part due to the solvent retention in the cast films, as deduced from the tensile behaviour. For both the TP and cast films, the oxygen barrier capacity was improved by the incorporation of C; the greater the proportion of C, the bigger the improvement

Table 2 – Functional properties of PLA films obtained by thermal processing (TP) and casting with different ratios of cinnamic acid (CA). CA retention (%), film thickness (t), tensile strength at break (TS), elongation at break (E), elastic modulus (EM), water vapour permeability (WVP), oxygen permeability (OP), lightness (L*), hue (h_{ab}*), chroma (C_{ab}*) and internal transmittance (T_i) at 400 and 700 nm.

	TP-PLA	5C	10C	Cast-PLA	2C	3C
C retention (%)	–	89 ± 7 ^b	75 ± 5 ^a	–	98 ± 3 ^c	98 ± 3 ^c
t (μm)	203 ± 9 ^a	213 ± 16 ^a	204 ± 31 ^a	247 ± 13 ^b	246 ± 22 ^b	251 ± 11 ^b
TS (MPa)	33 ± 5 ^d	30 ± 3 ^{cd}	28 ± 2 ^c	21 ± 7 ^b	13 ± 3 ^a	15 ± 2 ^a
EM (MPa)	1254 ± 71 ^e	912 ± 113 ^d	905 ± 97 ^d	466 ± 104 ^c	296 ± 53 ^b	208 ± 62 ^a
E (%)	3.0 ± 0.5 ^a	3.6 ± 0.3 ^a	3.3 ± 0.3 ^a	67 ± 25 ^c	52 ± 12 ^b	65 ± 9 ^c
WVP (g mm kPa h m ²)	0.28 ± 0.06 ^c	0.15 ± 0.03 ^b	0.11 ± 0.02 ^a	0.27 ± 0.08 ^{cd}	0.38 ± 0.06 ^d	0.18 ± 0.11 ^{bc}
OP (×10 ¹⁴ cm ³ /m s Pa)	187 ± 8 ^c	185 ± 6 ^c	141 ± 2 ^a	246 ± 15 ^d	185 ± 2 ^c	162 ± 3 ^b
L*	83.1 ± 0.6 ^{bc}	83.5 ± 0.6 ^{bc}	82.6 ± 0.4 ^{ab}	80.6 ± 3.0 ^b	84.7 ± 2.8 ^c	83.5 ± 0.3 ^{bc}
h _{ab} *	97.3 ± 1.0 ^d	74.7 ± 2.0 ^a	73.3 ± 0.9 ^a	83.6 ± 2.8 ^{bc}	84.3 ± 9.0 ^c	79.4 ± 1.0 ^b
C _{ab} *	8.2 ± 0.4 ^c	7.1 ± 0.2 ^{bc}	7.8 ± 0.2 ^b	5.9 ± 1.3 ^a	6.4 ± 1.9 ^{ab}	6.58 ± 0.3 ^{ab}
T _i 400 nm (×1000)	882 ± 3 ^b	883 ± 3 ^b	882 ± 2 ^b	858 ± 9 ^a	864 ± 2 ^a	863 ± 1 ^a
T _i 700 nm (×1000)	907 ± 1 ^b	906 ± 1 ^b	906 ± 1 ^b	891 ± 8 ^a	898 ± 1 ^a	897 ± 1 ^a

Different super indices in the same row indicate significant differences between film formulations ($p < 0.05$). 2–10C stands for the mass percentage of CA in the dry film.

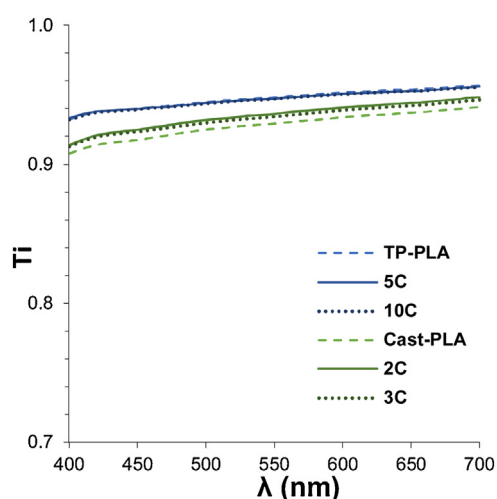


Fig. 6 – Internal transmittance (T_i) spectra of thermoprocessed (TP) films (TP-PLA, 5C and 10C), and cast films (Cast-PLA, 2C and 3C).

in the oxygen barrier capacity. Despite the lower proportion of C, the cast films experienced the most remarkable OP reduction since the C-loaded cast films exhibited similar OP values to those of the TP films, despite the higher OP of the cast PLA. This suggests that cinnamic acid could act as an oxygen scavenger in the films, as observed for other phenolic compounds (Bonilla et al., 2013).

The internal transmittance (T_i) spectra between 400 and 700 nm are shown in Fig. 6. The TP films exhibited higher T_i values than the cast films over the whole wavelength range, similar to those obtained by Collazo-Bigliardi et al. (2019) for amorphous TP PLA films; this indicates that the TP films were more transparent than the cast films. This could be attributed to the more compact and homogenous structure of the TP films. The solvent retention in the cast films and their greater thickness can help to make them less transparent. The incorporation of cinnamic acid did not significantly affect the transparency of either the TP or cast films.

The colour parameters of the films are shown in Table 2. As a result of the different light interactions with the films, the cast PLA samples tended to show lower hue values, with lower colour saturation than the TP films. In the cast films, the addition of CA set a trend towards greater saturation, which

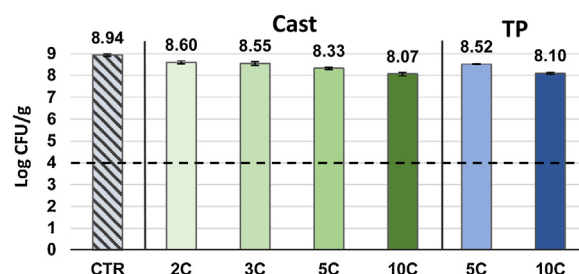


Fig. 7 – Microbial growth of *Listeria innocua* after 6 days of incubation at 10 °C in TSA in contact with cast (green bars) and thermally processed (TP, blue bars) PLA films with different cinnamic acid (CA) wt%. CTR: non-covered growth control. The dotted line represents the initial inoculum (10⁴ CFU/g). 2C–10C stands for the mass percentage of CA in the dry film. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

matches the values reported by Muller et al. (2017a), for cast PLA films with cinnamaldehyde. In the case of the TP films, the incorporation of CA caused a considerable reduction in hue along with a small decrease in chroma, indicating that films became slightly brownish. This effect can be attributed to the partial oxidation of CA during the melt-blending process, as discussed above. However, none of these changes were appreciable to the naked eye.

3.4. Antibacterial activity of the films

Fig. 7 shows the growth of *L. innocua* in the TSA medium in contact with the different PLA films, in comparison to the uncoated TSA control growth sample. Despite the crystalline formations found for 5C and 10C cast films, these were also submitted to the antimicrobial test in order to elucidate a possible relationship between the concentration of CA and *L. innocua* growth inhibition. All of the samples exhibited growth of over 4 logs(CFU/g) compared to the initial inoculum (10⁴ CFU/g). Despite the high proportion of C, the films proved to have a very little antimicrobial effect. A slight tendency linking growth inhibition and the ratio of CA in the film could be observed. In a previous study, Ordoñez et al. (2021) determined the minimal inhibitory concentration (MIC) of CA for *L.*

innocua, which was 0.65 mg/ml. This value would have already exceeded in the culture medium for 2C films if a total release of the CA incorporated into the film has occurred. However, not even 10C formulations reached a growth inhibition of 1log. This indicates that CA was retained tightly in the polymer matrix in all cases, thus being unable to exert its antimicrobial capacity. Despite the high proportion of active presence in the films to enhance the mass transfer driving force, the lack of polymer swelling and relaxation when in contact with the aqueous culture medium greatly limited the release of cinnamic acid from the film to exert its antimicrobial action against *L. innocua*. The obtained results suggest that only the compound near the film surface was delivered to the medium, but in too small a quantity to be able to significantly inhibit the bacterial growth. Therefore, other strategies, such as the surface application of the active compound, compound solution spraying or electrospinning, could yield more efficient active materials that may inhibit the bacterial growth in aqueous media such as those present in many foods.

4. Conclusions

Over 3% w/w cinnamic acid could not be homogeneously incorporated in cast PLA films, whereas 10% acid was successfully integrated by melt blending with PLA. Thermo-processed PLA films were significantly stiffer and had greater resistance to break than the cast films, whereas the latter exhibited a high degree of plastic deformability, in part due to the solvent retention in the cast films. The incorporation of phenolic acids reduced the film's stiffness and resistance to break in both the TP and cast films while it improved the water vapour and oxygen barrier capacity of the films. The TP films were more transparent than the cast films and turned slightly brownish after the addition of cinnamic acid.

Despite the high concentration of the active compound in the films, no significant antibacterial activity was observed against *L. innocua* in the *in vitro* assays, although a positive effect was observed when the acid concentration increased. This suggests that only the acid molecules near the film surface were released into the medium to exert their antibacterial action. Given the limited release of the acid from both the cast and thermo-processed PLA matrices, future research should focus on promoting it through the surface application of the active compound, such as compound solution spraying or electrospinning, which could yield active materials that are more efficient at inhibiting the bacterial growth in aqueous media, such as those present in many foods, thus helping to extend the shelf life of sensitive foodstuffs.

Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Ramón Ordoñez: Investigation, Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing –

review & editing. **Lorena Atarés:** Conceptualization, Methodology, Data curation, Writing – original draft, Writing – review & editing, Supervision. **Amparo Chiralt:** Conceptualization, Methodology, Data curation, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors report no declarations of interest.

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