Enhancement of PLA-PVA surface adhesion in bilayer assemblies by PLA aminolization

Alina Tampau [a,1,*], **Chelo González-Martínez** [b,2], **António A. Vicente** [c,3], **Amparo Chiralt** [d,4]

[a, b, d] Instituto Universitario de Ingeniería de Alimentos para el Desarrollo, Ciudad Politécnica de la Innovación, Universitat Politécnica de Valencia, Camino de Vera, s/n, 46022 Valencia, Spain.

[c] CEB – Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

[1] altam@upv.es, [2] cgonza@tal.upv.es, [3] avicente@deb.uminho.pt, [4] dchiralt@tal.upv.es

ABSTRACT

Poly(lactic acid) (PLA) and poly(vinyl alcohol) (PVA) present complementary barrier properties and their combination in multilayer assemblies (laminates) could provide materials with more effective barrier capacity for food packaging purposes. However, their low chemical affinity compromises adequate polymer adhesion. Surface free energy modification of thermo-processed PLA films through treatment with 1,6-hexanediamine was used to enhance adhesion with polar PVA aqueous solutions. Treatments of 1 and 3 min increased the polar component of the solid surface tension, while treatments above 10 min provoked a corrosive effect in the films' structure. Extensibility analyses of PVA solutions loaded with carvacrol (15 wt. %) and different Tween 85 ratios on PLA activated surfaces allowed the selection of the 1 min aminolyzed surface for obtaining PLA-PVA bilayers, by casting PVA solutions on the PLA films. This study revealed that despite aminolization enhancing the PLA surface affinity for aqueous PVA solutions, casting-obtained bilayers presented limited oxygen barrier effectiveness due to heterogeneous thickness of PVA layer in the laminates.

KEYWORDS: aminolization; surface activation; poly(lactic acid); poly(vinyl alcohol); carvacrol; bilayer assembly.

DECLARATIONS

---

[*] Corresponding author. E-mail address: altam@upv.es (Alina Tampau).

Conflicts of interest/Competing interests: The authors declare that they have no conflict of interest.

Ethics approval: Not applicable.

Consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and material: The raw/processed data required to reproduce these findings cannot be shared at this time due to legal or ethical reasons.

Code availability: Not applicable.

Authors' contributions: A. Tampau*: Writing - original draft, review & editing; Data curation; Investigation Methodology.* C. González-Martínez: *Supervision; Writing - review & editing.* A. A. Vicente: *Supervision; Writing - review & editing.* A. Chiralt: *Supervision; Writing - review & editing.*

1. Introduction

Biodegradable polymers have generated an increased interest in the current packaging field, as the massive use of conventional petroleum-based plastics has led to a negative environmental impact world-wide. Some biobased aliphatic polyesters, such as the polylactides, constitute a very promising group of materials for applications in the packaging area (Petersen et al., 1999). Poly(lactic acid) (PLA) is biocompatible (Food and Drug Administration approved) (Muller, González-Martínez, & Chiralt, 2017a), compostable (Farrington, Lunt, Davies, & Blackburn, 2005) and made from renewable sources. PLA presents potential as packaging material, as it can produce highly transparent, rigid films that exhibit low water vapour permeability (WVP) (Bonilla, Fortunati, Vargas, Chiralt, & Kenny, 2013; Collazo-Bigliardi, Ortega-Toro, & Chiralt, 2018). Nevertheless, PLA also presents shortcomings, as it exhibits mechanical fragility/brittleness and limited oxygen barrier capacity (Bonilla et al., 2013).

A useful approach to offset drawbacks of individual films is by emulating the multilayer structure of commercial films. These are currently made of several sheets of polymers (3 to 12 layers according to http://polymerdatabase.com/) imparting complementary properties to the final material (e.g. European Patent EP0175451A2). Some layers provide moisture resistance, while others act as gas barriers or provide mechanical support, creating materials more suitable for the intended applications.

This strategy of pairing polymer sheets is already ongoing in the field of biopolymers. Several authors have reported obtaining multilayers with improved physical properties, based on starch and polyester combinations. Requena, Vargas, & Chiralt (2018) obtained bilayers by thermo-sealing cassava starch sheets, and polylactic acid-polyhydroxybutyrate-co-hydroxyvalerate blend films, with low WVP and good oxygen barrier capacity. Muller et al. (2017a) also obtained bilayer assemblies from thermo-processed cassava starch and amorphous PLA with improved barrier capacity with

3

respect to the corresponding monolayers. Rhim, Mohanty, Singh, & Ng, (2006) developed multilayer films based on a middle sheet of soy protein isolate (SPI) and exterior layers of polylactide obtained by casting. The tensile strength and barrier properties of the laminates also benefited from pairing the two polymers.

Poly(vinyl alcohol) (PVA) is a polar biopolymer with complementary barrier properties with respect to PLA, since its films possess a high barrier capacity to oxygen, with good tensile strength and flexibility (Cano et al, 2015). PVA has already been approved by the USDA to be used in the packaging of meat and poultry products. (DeMerlis & Schoneker, 2003). However, PVA exhibits high WVP due to its hydrophilic nature. Thus, the laminated assembly with hydrophobic polymer sheets, such as PLA, could yield a multilayer material with enhanced mechanical and barrier features.

One possible strategy to obtain multilayer films is the casting of a polymeric solution on the surface of another polymeric layer support. This method requires a good extensibility of the solution on the polymeric film, which is greatly affected by the surface interactions of the polymer solution with the supporting polymer that, in turn, depends on the molecular interactions between the components in the solution and those on the contact surface (Sapper & Chiralt, 2018). However, when using polymers with complementary properties, such as PVA and PLA, these are hydrophilic and hydrophobic in nature, respectively. Therefore, chemical affinity between them or their solutions is low, which compromises the surface interactions to obtain adequate polymer adhesion. To address this shortcoming, a potential strategy is the functionalization of the PLA surface through the aminolization technique. This method uses a diamine solution to introduce radicals carrying free amino moieties onto the surface of the PLA film (Zhu, Gao, Liu, & Shen, 2002). The ester (-COO-) groups in the polylactic acid chain would interact with one of the diamine's $-NH_2$ groups forming a covalent bond (O=C-NH-) (**Figure 1**). Thus, the diamine's structure carrying a free $-NH_2$ moiety would become attached to the polymer surface. The surface modifications in the

hydrophilic-lipophilic balance produced by the diamine can easily be monitored by observing the change in the surface energy components, through contact angle measurement. Then, the positively charged amino moieties (activated by immersion in a hydrochloric solution) could interact with a negatively charged polymer (such as partially acetylated PVA) in aqueous solution, generating a well-adhered coating on top of the PLA support material. This surface modification approach has found applications not only in constructing layer-by-layer assemblies for food coating application (Carneiro-da-Cunha et al., 2010; Pinheiro, Bourbon, Quintas, Coimbra, & Vicente, 2012; Medeiros, Pinheiro, Vicente, & Carneiro-da-Cunha, 2012; Medeiros et al., 2014; Fabra, Flores-López, Cerqueira, de Rodriguez, Lagaron, & Vicente, 2016), but also in live cell immobilization on the activated polyester surface (Zhu et al., 2002; Noel et al., 2013). In the previous studies for food packaging applications (Medeiros et al. 2012; Medeiros et al., 2014; Fabra et al. 2016; Souza et al. 2018) aminolysis functionalization technique was applied on a non-biodegradable polymer, polyethylene terephthalate (PET), to increase the adhesion of subsequently applied layers of polysaccharides or proteins containing active molecules. No previous aminolization studies on biodegradable polymeric supports to this end have been found.

The addition of antimicrobial or antioxidant molecules into the multilayer structure could provide additional benefits when used as packaging material, exerting an active protection on the packaged foodstuffs. Several studies have been carried out using different polymers and active compounds to obtain antimicrobial materials for food packaging. Anwar, Sugiarto and Warsiki (2018) reviewed previous studies, analyzing different incorporation methods (direct mixing, polishing, and encapsulation) of active compounds into the polymeric material in this packaging-making concept.

Carvacrol (CA) is a natural monoterpenoid present as a main constituent in the essential oils of oregano or thyme (Burt, 2004; Can Baser, 2008). It has proven antimicrobial and antioxidant effects, as reported by many authors (Kamimura, Santos,

123 Hill, & Gomes, 2014; Mascheroni, Guillard, Gastaldi, Gontard, & Chalier, 2011; Ramos,

124 Beltrán, Peltzer, Valente, & Garrigós, 2014). The inclusion of CA in a carrier polymeric

125 matrix could be a useful way to deliver its effects (Turek and Stintzing, 2013). In this

126 sense, previous studies reported very slow release kinetics of these kinds of

127 compounds from PLA films (Muller, Casado Quesada, González-Martínez, & Chiralt,

128 2017b), whereas their encapsulation in more hydrophilic polymers (zein, chitosan,

129 cassava and corn starch) favours the release, providing greater effectiveness as

130 antimicrobial agents (Fabra et al., 2016; Muller et al., 2017b; Tampau, González-

131 Martínez, & Chiralt, 2018).

132 Multilayer assemblies could incorporate the active compound into the polymer layer

133 where the active compound is more effectively delivered in the target food to exert the

134 antimicrobial or antioxidant action. The incorporation of the active compound in the

135 polymer matrix modified barrier and mechanical properties of the film to a different

136 extent, as reported by Requena et al. (2018) and Tampau et al. (2018) when carvacrol

137 was incorporated into starch-polyester-blend bilayers and starch-PCL-starch

138 multilayers, respectively.

139 Obtaining polyester sheets by the usual thermoprocessing and casting on their surface

140 a film forming solution with a polar polymer ,such as PVA, with complementary barrier

141 properties, is a feasible process to prepare laminates for food packaging purposes. The

142 incorporation of the active compounds, such as carvacrol in the film forming solution,

143 allows to avoid the thermal treatment of the active, preserving better its properties.

144 The aim of this study was to analyse the effect of the aminolization of thermo-processed

145 PLA films on their surface energy (polar and dispersive components) and its impact on

146 the extensibility of PVA aqueous solutions containing or not CA as active component,

147 and surfactant. The functional properties of the bilayer assemblies as packaging

148 material were also characterized to analyse the aminolization effect on the laminate

149 properties.

2. Materials and methods

2.1. Materials

Poly(lactic acid) (PLA) 4060D (density 1.24 g/cm^3; D-isomer content 12 % wt.) was acquired from Natureworks (Minnetonka, MN, USA) in the form of pellets. 1,6-diamino-hexane (for synthesis) was purchased from Merck Millipore Corporation (France), and 2-propanol, hydrochloric acid and dimethyl sulfoxide from PanReac AppliChem (Castellar del Vallès, Barcelona, Spain). Ethyl acetate was acquired from Indukern (El Prat de Llobregat, Barcelona, Spain). Poly(vinyl alcohol) (PVA) (M_w 13,000-23,000; 87-89% hydrolysed), polyoxyethylene sorbitan trioleate Tween 85 (S), carvacrol and phosphorous pentoxide (P_2O_5) were purchased from Sigma-Aldrich (Sigma–Aldrich Chemie, Steinheim, Germany). Ultrapure water (resistivity of 18.2 MΩ cm) was prepared using Milli Q Advantage A10 equipment from Millipore S.A.S., Molsheim, France.

2.2. Mono- and bi- layer preparation

PLA support monolayers

Polylactic acid sheets were obtained by compression moulding. To this end, 4 g of the amorphous PLA pellets were placed on Teflon sheets in a hot plate press (Model LP20, Labtech Engineering, Thailand) and preheated for 4 min at 160 ºC. Compression was applied for 4 min at 100 bar and 160 ºC, followed by a cooling cycle of 3 min. The films thus obtained were kept in a desiccator with SiO_2 until used.

PLA /aminolization

Surface aminolization of PLA was carried out as described by Zhu et al. (2004). Briefly, the PLA films were immersed in ethanol-water (1:1 v/v) solution for 2 h at room temperature to remove possible fatty residues adhered during manipulation. Afterwards, they were washed with abundant deionized water and dried in a vacuum oven at 30 ºC for 24 h, until constant weight. The sheets were then immersed for different times (1, 3,

5, 10, 15 and 30 min) at 50 ºC in 0.25 mol·L^{-1} 1,6-hexanediamine solution in 2-propanol. A control sample immersed only in 2-propanol at 50 ºC for the different times was also prepared. The treated surfaces were thoroughly rinsed with deionized water for 24 h at room temperature to remove traces of the unreacted diamine and dried in a vacuum oven (30 ºC for 24 h). Similarly to Pinheiro et al. (2012) and Madeiros et al. (2012), the aminolized sheets were then immersed in a 0.1 mol·L^{-1} hydrochloric acid solution for 3 h at room temperature, in order to positively charge the amino moieties grafted onto the surface. Then, the films were washed with abundant deionized water and dried again under vacuum at 30 ºC for 24 h. The weight and thickness (measured by a Palmer digital micrometre from Comecta, Barcelona, Spain) of the dried materials were recorded. All the dried PLA films (treated and activated) were then stored in desiccators with SiO$_2$ until their use.

PVA /incorporation of actives

PVA film-forming dispersions (FFDs) were prepared by dissolving the polymer (15 wt. %) in ultrapure water, under heating at 80 ºC and constant magnetic stirring for one hour. Carvacrol (CA) was added at 15 wt. % with respect to the PVA content. These aqueous formulations were prepared with and without Tween 85 surfactant (S), using three different S:CA weight ratios: 0.3:100, 0.4:100 and 0.5:100. The first ratio value was considered based on an expected CA droplet diameter of 10 μm (Tampau, González-Martínez, & Chiralt, 2020) and a surfactant excess surface concentration of 5 mg/m^2, in the range of the previously reported values (Owusu Apenten & Zhu, 1996). This initial ratio was increased in order to determine possible benefits of an excess of surfactant on the wettability of PVA solutions. Afterwards, the dispersions were homogenized at 12,000 rpm for 3 min using an Ultra Turrax rotor–stator homogenizer (Model T25D, IKA Germany). CA-free FFD containing only PVA were also prepared as control. The FFDs were degassed under vacuum and allowed to rest at room temperature for 24 h to check their stability.

Bilayer assembly

204 The bilayer assemblies were obtained by coating the support PLA sheet (aminolized or

205 not) with the selected PVA solutions. For this purpose, the aqueous solution, equivalent

206 to 1.5 g of PVA polymer, was spread onto each PLA sheet by means of a manual spiral

207 bar coater (100 μm, Elcometer, UK). This implied a PVA/PLA wt. ratio of about 0.35 in

208 the bilayer. The bilayers were allowed to dry at 21 °C and 55-60 % relative humidity

209 (RH). Then, the dry materials were conditioned at 25 °C in desiccators with saturated

210 solutions of $Mg(NO_3)_2$ ensuring 53 % RH, until their characterization. The weights of the

211 films were carefully recorded before and after the coating application to check the

212 polymer wt. ratio. The prepared bilayers were labelled based on the untreated (PLA) or

213 aminolized (am-PLA) support, and the applied PVA coating, carrying or not active (C)

214 and/or surfactant (S) (*e.g.* am-PLA-PVA-C/S describes the bilayer obtained with PLA

215 aminolized support and PVA layer containing both CA and S).

216 **2.3. Analyses of surface properties**

217 **Surface free energy of PLA films**

218 The surface free energy of the PLA sheets was assessed pre- and post-aminolization

219 using the sessile drop method (Kwok & Neumann, 1999) by means of a contact angle

220 meter (OCA 20 Dataphysics, Filderstadt, Germany). This involved measurement of the

221 contact angle θ between the analysed surface and different standard liquids of different

222 polarities and known surface tensions. Dimethylsulfoxide (DMSO), ultrapure water, and

223 ethyl acetate were used for this purpose. Their surface tension and dispersive and polar

224 components are, respectively, 44.0, 36.0 and 8.0 mN·m^{-1} for DMSO, 72.8, 22.1 and

225 50.7 mN·m^{-1} for water, and 23.9, 23.9 and 0.0 mN·m^{-1} for ethyl acetate

226 (www.accudynetest.com/surface_tension_table.html). According to Hejda, Solař, &

227 Kousal (2010), the surface free energy of the solid (γ_S) can be expressed by Young's

228 equation **(equation 1)**:

$$\gamma_S = \gamma_{SL} + \gamma_L \cdot \cos\theta \tag{1}$$

where:

-γ_{SL} is interfacial tension solid-liquid;

-γ_L is the surface tension of the liquid, and

-θ is the contact angle formed between the liquid-air interface and the solid surface.

If the polar and dispersive components acting at the interfacial tension are considered, this surface interaction can be described by **equation 2.** Then, from the values of the contact angle of pure solvents, with known dispersive (γ_L^d) and polar (γ_L^p) components, on a determined surface, the plot of the variables according to equation 2 permits the estimation of dispersive (γ_S^d) (from the intercept of linear plot) and polar (γ_S^p) (from the slope of linear plot) components of the surface free energy (Sapper & Chiralt, 2018). Solvent drops of 2 µL were placed on the PLA surface and the contact angle was determined at contact times of 0, 30 and 60 s. Ten replicates of the angle measurements were carried out at room temperature (21±1 ºC) for each film sample and solvent.

$$\frac{1+\cos\theta}{2} \cdot \frac{\gamma_L}{\sqrt{\gamma_L^d}} = \sqrt{\gamma_S^p} \cdot \sqrt{\frac{\gamma_L^p}{\gamma_L^d}} + \sqrt{\gamma_S^d} \tag{2}$$

Surface tension of PVA solutions

All PVA solutions were characterized in terms of their liquid-vapour surface tension (γ_{LV}) using the same equipment as described for contact angle measurement. The surface tension was estimated by applying the pendant drop method, using the Laplace-Young approximation, as described by Ribeiro, Vicente, Teixeira, & Miranda (2007) and Carneiro-da-Cuhna et al. (2009). Briefly, 10-12 µL drops of the PVA solutions were suspended at the flat end of a vertical needle and with the equipment's image

252　processing software the γ_{LV} was determined. At least thirty measurements at room

253　temperature (21±1 ºC) were taken for each solution.

254　**Wettability of PVA solutions on PLA surface**

255　Wettability of the different PVA solutions (containing or not CA and S) was determined

256　on the PLA film surface (aminolized or not). Wettability is assessed by calculating the

257　spreading coefficient (W_s) (**equation 3**) that represents the balance of the adhesion

258　(W_a) and cohesion (W_c) works, as a function of the surface tension of the different PVA

259　solutions and their contact angle on the PLA films (**equation 4**) (Sapper & Chiralt,

260　2018). This parameter only yields a negative or zero value. The closer the value of W_s is

261　to zero, the better that surface will be coated.

262
$$W_s = W_a - W_c \tag{3}$$

263
$$W_s = \gamma_L(\cos\theta - 1) \tag{4}$$

264　To this end, the measurement of the contact angle between PVA solutions and PLA

265　films was taken, by using the sessile method described above. Ten measurements were

266　carried out for each solution.

267　**2.4. Surface microstructure**

268　The surface morphology of control and treated PLA was analysed by Field Emission

269　Scanning Electron Microscopy (FESEM Ultra 55, Zeiss, Oxford Instruments, UK). For

270　this purpose, the surface of monolayers was observed without any additional treatment,

271　whereas bilayers were previously cryo-fractured by immersion in liquid nitrogen to allow

272　for cross-section view. Samples were mounted with carbon tape on stubs, and after

273　sputtering with platinum in an EM MED020 (Leica Microsystems, Germany), were

274　observed under an accelerating voltage of 1 kV.

275　**2.5. Analysis of functional properties of bilayer films**

Water vapour and oxygen permeability

Water vapour permeability **(WVP)** of bilayers was assessed at 25 ⁰C and 53-100 % RH gradient, using ASTM E96-95 gravimetric method (ASTM, 1995), and considering the modification proposed by Gennadios, Weller, & Gooding (1994). Briefly, previously conditioned samples were fitted onto Payne aluminium cups (diameter of 3.5 cm) (Elcometer SPRL, Hermelle/s Argenteau, Belgium) containing 5 mL of deionized water and were placed in desiccators with saturated $Mg(NO_3)_2$ aqueous solution. A small fan was placed on top of each cup to favour the water vapour transport. The cups were weighed periodically with an analytical balance (±0.00001 g) and weight loss vs. time was plotted to obtain the water vapour transmission rate (WVTR). Then, the WVP was calculated as described by Perdones, Chiralt, & Vargas (2016). Measurements were carried out in triplicate.

The oxygen permeability **(OP)** of conditioned samples was determined at 25 ⁰C and 53 % RH, following the guidance of Standard Method D3985-05 (ASTM, 2005). The equipment OXTRAN (Mocon Lippke, Neuwied,Germany) was used to determine the oxygen transmission rate (OTR). Film samples (area of 50 cm^2) were exposed to pure oxygen flow on one side and pure nitrogen flow on the opposite side. The oxygen transmission values were registered every 10 min until equilibrium was reached. Then the OP was determined as described by Cano, Jiménez, Cháfer, Gónzalez, & Chiralt (2014), taking into account the samples' thickness. At least two replicates per formulation were considered.

Mechanical properties

The tensile properties of the materials were assessed with a universal test machine (TA.XT plus model, Stable Micro Systems, Haslemere, England) following the guidelines of ASTM standard method D882 (ASTM, 2001), as previously described by Ortega-Toro, Contreras, Talens, & Chiralt (2015). Conditioned samples (25x100 mm)

302 were fixed in the machine's extension grips (positioned 50 mm apart) and stretched at

303 50 mm·min^{-1} until break. Tensile strength at break (TS), Young modulus (YM) and

304 elongation at break (ε%) were obtained from the stress-strain curves. A minimum of

305 eight measurements per sample were taken.

306 **Optical properties**

307 The samples were analysed in terms of their transparency, by means of internal

308 transmittance (T_i) assessed by applying the Kubelka-Munk theory for multiple scattering

309 (Hutchings, 1999), as previously reported by Ortega-Toro et al. (2015). A

310 spectrocolorimeter CM-3600d (Minolta Co., Tokyo, Japan) was used to determine the

311 surface reflectance spectra (wavelength range 400-700 nm) against black and white

312 backgrounds. The value of T_i at 460 nm was selected as the benchmark value for

313 sample comparison.

314 **2.6. Thermogravimetric analysis (TGA) and differential scanning calorimetry**

315 **(DSC)**

316 Previously P_2O_5 conditioned films were submitted to thermal analyses. Their thermal

317 degradation (TGA) was analysed with a thermo-gravimetric analyser (TGA/SDTA 851e,

318 Mettler Toledo, Schwarzenbach, Switzerland). To this end, approximately 10 mg of

319 samples were placed into alumina crucibles (capacity 70 μL) and heated from 25 to

320 600 ºC at a rate of 10 K/min, under nitrogen flow (20 mL/min). The thermal weight loss

321 curves (TA) and their derivatives (DTA) were obtained and the onset, peak and endset

322 temperatures for each degradation peak were extracted by using the STARe Evaluation

323 Software (Mettler-Toledo, Inc., Switzerland). For the DSC analyses, a triple step thermal

324 scan was applied using DSC equipment (1 StarE System, Mettler-Toledo, Inc.,

325 Switzerland). Samples were cooled and kept at -25 ºC for 5 min and then heated at

326 10 K/min from -25 to 200 ºC, where they were maintained for 2 min. Then, samples

327 were cooled from 200 to -25 ºC at 10 K/min, maintained at -25 ºC for 5 min and

328 afterwards heated from -25 to 250 ºC, at 10 K/min. As reference, an empty aluminium

329 pan was used. All thermal analyses were done in duplicate.

330 **2.7. Statistical analysis**

331 The analysis of the data was performed through variance analysis (ANOVA) using the

332 Statgraphics Centurion XVII.64 software (Statgraphics Technologies, Inc., Warrenton,

333 VA, USA). The Fisher Least Significant Difference (LSD) was used to evaluate the

334 differences ($p < 0.05$) among the samples' means.

335 **3. Results and Discussion**

336 **3.1. Changes in the PLA surface induced by aminolization**

337 The aminolization process of the PLA surface was followed through the changes in the

338 surface energy (SE) of the films (polar and dispersive components) and microstructural

339 observations of the film surface by FESEM. Contact angles of the different solvents on

340 the PLA surface were constant with time for all pure solvents, except for ethyl acetate

341 where changes occurred mainly due to the solvent volatility. Thus, only the contact

342 angle value for 0 seconds contact time were considered. From the contact angle

343 measurements, and taking into account the values of the dispersive and polar

344 components of the surface tension for the different pure solvents, the values of

345 dispersive and polar components of the surface energy of PLA films were obtained. To

346 this end, the linear fitting of eq. 2, according to the variable combinations shown in the

347 equation, was carried out. These obtained values are shown in **Table 1** for the surfaces

348 aminolized for different times. The total surface energy (sum of both components) was

349 also determined at each aminolization time. A notable increase (of almost 100 %) in the

350 polar component value was observed at the shortest treatment times (1 and 3 min). The

351 film submitted to the 5 min treatment did not show a similar trend, having values of the

SE components that were more similar to the untreated sample. The changes (% relative to the initial value) in mass and thickness of treated samples are also shown in **Table 1.** Both parameters increased with treatment time, probably due to solvent adsorption and retention in the polymer matrix and its subsequent swelling. In fact, samples immersed only in solvent at the same temperature also gained mass, but in a lower proportion, as shown in **Table 1** (values in brackets). In control treatments using only solvent, the mass gain was greater than in the reactive samples for all treatment times and reached a constant value of about 8 % from 5 min immersion onwards. This indicates that in the presence of reactive, mass change also involves other mechanisms related with the reaction, which could imply losses through the polymer solubilisation in the reactant media. In fact, the sample treated for 30 min exhibited a net weight loss, while the film thickness greatly increased, thus revealing the effective mass loss of the highly swollen film due to its partial solubilisation in the solvent-reactive system. Then, a prolonged contact with the diamine leads to a high degree of erosion/dissolution of the polylactide. Additionally, beyond the 10 min mark, the post-aminolization samples were visibly brittle. So, they were considered physically unfit for use in the subsequent coating step, as they would provide little mechanical support for the bilayers.

The FESEM micrographs in **Figure 2** show the surface aspect of the PLA films where the changes provoked by the aminolization process at different times can be observed. The initial relatively rough surface of the untreated polylactide films shows a gradual smoothening at short aminolization times (1 and 3 min), as also observed by other authors (Drobota et al. 2013). This can be attributed to the solubilisation of the more emerging zones of the rough surface overlapping with the aminolization of the surface chains, as revealed by the increase in the polar component of the film surface energy. However, a more irregular surface was observed at 5 min treatments where the surface tension polar component decreased. This suggests that aminolized chains could prevalently solubilise during treatment, generating an irregular film surface. For the 10

15

379   min treatment, visible voids can be observed in the films, confirming the internal action

380   of the 1,6-hexane diamine reactive and partial solubilisation of the film material. The

381   internal diffusion of the reactive and solvent into the films seems to produce a highly

382   porous structure at 15 and 30 min treatments, as revealed by the visible pores in the

383   film microstructure and the great increase in the film thickness. At the end of the latter

384   treatment, the intense erosion of the films structure can be clearly observed in **Figure 2**,

385   where a high ratio of voids and pores can be seen on the film surface, while the films

386   appeared highly deformed after drying. In fact, the measurements of contact angle were

387   not possible in these distorted films. Film immersion in pure solvent did not provoke a

388   great alteration in the film surface beyond the leaching of the most superficial chains

389   that changed the surface aspect of the films compared with the initial samples (**Figure

390   2**).

391   The obtained results indicate that aminolization of PLA films must be carried out for

392   short times (1 or 3 min) where the polar component of the film surface tension

393   increased. Longer treatment times provoked internal damage in the film structure

394   associated with the internal diffusion of reactants and solvent that provoked the swelling

395   of the polymer matrix and the partial solubilisation of the film components.

396   **Wettability of PLA with PVA liquid systems**

397   PLA films treated at 0, 1 and 3 min aminolization (when the polar component of surface

398   energy   increased)   were   submitted   to   the   wettability   test   with   the   PVA

399   solutions/emulsions prepared as described in section 2.2, containing or not carvacrol

400   and different ratios of surfactant. Contact angles obtained between PVA liquid systems

401   and the PLA films treated for different times are shown in **Table 2**, as well as the values

402   of surface tension of the different PVA liquid systems. All contact angles were below

403   90º, indicating that the surface can be wetted by the liquids. The surface tension (γ_L) of

404   the PVA liquid systems was significantly reduced by carvacrol incorporation, whereas

405   no significant effect of the surfactant was observed at any ratio. The effect of the

emulsified carvacrol on the surface tension of PVA solution can be attributed to the presence of small droplets of the compound at the liquid surface that modify the interfacial interaction forces due to its more hydrophobic nature. Likewise, the lack of a significant surfactant effect on the surface tension of the carvacrol emulsions could be due to its prevalent location at the oil-water interface and the main effect of carvacrol droplets at the surface level. This behaviour was also observed by Sapper, Bonet and Chiralt (2019) when thyme essential oil was emulsified in an aqueous solution of cassava starch-gellan with and without surfactant.

As concerns the contact angle, it decreased (from 51 to 33) for pure PVA solutions as the polar component of surface tension increased during aminolization, thus reflecting the greater chemical affinity between the aminolized PLA surface and PVA aqueous solutions. However, for the PVA solution with emulsified carvacrol, the contact angle of non-aminolized PLA was lower (44) and less sensitive to the aminolization time. This agrees with the greater surface affinity of the carvacrol-containing liquid with the initial PLA surface. In fact, the contact angle of this emulsion increased in the case of the 3 min aminolization treatment. The presence of surfactant provoked small changes in the contact angle, which could be attributed to specific interactions of the amphiphilic molecule with the PLA surface that affect the global surface forces at the solid-liquid-air contact. The small ratio of surfactant was more effective at decreasing the contact angle in non-aminolized PLA surfaces than the higher proportions, whereas it was not effective at reducing the contact angle in aminolized PLA surfaces.

The wettability parameter (W_s) values calculated for the different PVA formulations and PLA surfaces are also shown in **Table 2**. The further from zero the values of W_s are, the less likely it is for that formulation to spread well over the PLA surface. Therefore, the best extensibility was obtained for 3 min treated PLA films with pure PVA solutions, for 1 min treated PLA films with PVA solution with emulsified carvacrol and for 0 min treated PLA support when emulsions contained the lowest ratio of surfactant. Therefore, 1 min

17

aminolized PLA support could be a compromise point to achieve a good extensibility of

the different PVA liquid systems while the lowest ratio of surfactant was more adequate

to enhance the liquid phase extension. This choice implies an improvement in the

extensibility of the pure PVA solution on PLA films, while a similar extensibility of the

three PVA formulations (without and with carvacrol, containing or not surfactant) would

be achieved. Thus, from this study, the aminolization time selected for the subsequent

application was 1 min, and the PLA films treated for 1 min were coated with PVA,

PVA/C and PVA/C-S (0.3:100) formulations to obtain bilayer films whose functional

properties were characterized.

3.2. Functional properties of PLA-PVA bilayer films

The obtained bilayer films exhibited a thickness of 230-250 μm from which

approximately 22 % corresponded to the PVA layer (56±21 μm average value) and the

rest to the PLA sheet (195±11 μm average value). These values are coherent with the

mass ratio of each polymer in the laminate (approximately 0.27 for PVA). However, at

microscopic level a higher heterogeneity in the PVA thickness was detected, depending

on the film sample. **Figure 3** shows FESEM micrographs of some bilayer films where

differences in the overall thickness of the PVA sheet can be seen, as well as differences

in the thickness along a determined film, depending on the roughness of the PLA

surface.

When a small protuberance appeared in the PLA surface (dotted arrows, **Figure 3**), the

PVA layer was thinner, which can be critical when the coating was very thin. This aspect

could compromise the barrier capacity provided by the PVA layer in the zones where its

thickness was extremely thin (about 10 μm). Then, in the laminates obtained by casting

PVA solutions on PLA surface, the more hydrophobic PLA layer was thicker, whereas

the cast hydrophilic layer of PVA was thinner with great variability in thickness, which

could influence its effectiveness at controlling its barrier capacity (Debeaufort, Martin-Polo, & Voilley, 1993).

Given that most deteriorative processes in foods are caused by water or oxygen exchanges, food packaging materials require a good barrier capacity against moisture and oxygen to extend foodstuff shelf life (Cerqueira, Lima, Teixeira, Moreira, & Vicente, 2009). The WVP and OP of the obtained bilayers are shown in **Table 3,** together with the corresponding values of each monolayer. According to the Ideal Laminate Theory (Siracusa, 2012), the polymer layer with lower permeability usually determines the barrier capacity of laminates for water vapour or gases. As such, the WVP values of bilayers were expected to be around those of the PLA sheet that exhibited the lowest WVP. Indeed, the WVP of bilayers present similar values to those of the PLA monolayer with small differences between the different samples. Laminates with aminolized PLA exhibited a wider WVP range, the bilayer with the pure PVA sheet being the one with the lowest value.

However, the values of oxygen permeability were not in the range of that of the PVA sheet that should be the limiting layer for oxygen transmission. Both the aminolized bilayers and those non-aminolized with pure PVA showed similar OP values to those of the PLA monolayer. In contrast, non-aminolized laminates containing carvacrol (with and without surfactant) exhibited lower OP values. This could be attributed to the oxygen-blocking effect exerted by the antioxidant CA, but this was not observed in bilayers with aminolized PLA. The lack of effectiveness of PVA sheets in reducing the OP of laminates could be due to the heterogeneous coating at microscopic level that should generate regions in the films with very thin hydrophilic coating. Thus, oxygen transfer would take place, mainly limited by the PLA sheet.

The tensile properties (Young modulus (YM), tensile strength (TS) and elongation (ε) at break) of bilayers are also presented in **Table 3**. The bilayers exhibited tensile property values in the range of the stiffest PLA sheet, which also represents the thickest layer of

485 the assembly. The films containing CA with and without surfactant had the lowest

486 values of YM, regardless of the aminolization of the PLA sheet. This could be explained

487 by the partial carvacrol migration into the PLA sheet, weakening the inter-chain forces of

488 the PLA matrix. This effect was also appreciated in the values of the bilayer's resistance

489 to break (TS), which were higher when neither CA nor T85 were present, showing

490 values similar to those of the PLA monolayer. All materials had elongation at break

491 values of under 10 %, denoting a quite brittle behaviour, in the order of PLA films.

492 However, a slight increase in the film extensibility could be appreciated for the CA-

493 carrying laminates, in line with the plasticising effect of the CA that potentially migrated

494 into the PLA sheets.

495 The internal transmittance (T_i), also presented in **Table 3,** is directly related to the film

496 transparency. All of the aminolized sheets presented a cloudiness (photos not shown)

497 as result of the aminolization step. This explains the decrease in the T_i parameter for

498 these treated materials. However, this acquired opacity could be considered positive for

499 certain food packaging applications where a reduction in light-induced oxidative

500 reactions is required.

501 The results indicate that bilayer PLA-PVA films obtained by casting PVA aqueous

502 solutions onto the PLA surface did not provide additional benefits to the PLA

503 monolayers. The expected, the barrier capacity against oxygen potentially imparted by

504 the PVA sheet was not effective, while the PVA did not contribute to the mechanical

505 reinforcement of the PLA layer. Despite PLA aminolization enhancing the polar nature

506 of the PLA surface and thus the chemical affinity with the PVA aqueous solutions to be

507 extended, the obtained PVA coatings were heterogeneous in thickness and the thinner

508 regions seem to limit the barrier capacity of the coating against oxygen.

509 **3.3. Thermal behaviour as affected by the aminolization treatment**

A thermal analysis of the obtained materials was carried out in order to discover the effect of aminolization and lamination on the physical state of polymers, which, in turn, affects their functional properties. **Table 4** summarizes the degradation temperatures of the different samples, as obtained by thermogravimetric analysis. TGA thermographs (**Figure 4**) show an overlapping of the degradation steps of both polymers due to due to the proximity of its degradation temperature range. The aminolization process did not modify the degradation temperature of PLA, the peak being at 310 °C. PVA starts its degradation and exhibits the peak at a lower temperature (192/288 °C) than the PLA. Thus, bilayers showed notable T_{onset} values in the range of 240-255 °C, lower than that of pure PLA (269 °C). A second and third small degradation peak appeared in bilayers at about 390 and 500 °C, which could be attributed to the PVA degradation in subsequent steps as described by other authors (Bonilla et al., 2014; Cano et al., 2016). No significant differences were observed between the degradation behaviour of the different bilayers. No thermo-release of carvacrol from the corresponding bilayers was detected, probably due to the very small mass fraction of the compound in the laminate: about 11 g/g PVA, according to previous studies (Tampau et al., 2020). **Table 4** also shows the glass transition temperature (T_g) for the different materials obtained in the first and second heating scans. In the first heating step, two distinct T_g can be observed, corresponding to the two polymers present in bilayers. For samples without carvacrol, the first one, at a slightly lower temperature, corresponds to the PLA, while the second one must be attributed to the PVA. For laminates without carvacrol, both transitions practically overlap, but these are more decoupled for bilayers with carvacrol due to the plasticizing effect of the compound that shifts the transition of the PVA sheet to a lower temperature, below the T_g of PLA. The DSC thermograms (**Figure 5**) also revealed the typical relaxation of PLA after T_g in the first heating step, associated with polymer ageing (Muller, Jiménez, González-Martínez, & Chiralt, 2016). However, as the thermal history of the materials is erased and they become melt blended in the first step, only a

single T_g was observed in the second heating, without relaxation enthalpy. This T_g value was lower for laminates with carvacrol, thus indicating the plasticizing effect of the compound on the melt blended polymer matrix.

Likewise, PVA crystallizes in the laminate and the melting temperature and enthalpies are shown in **Table 4.** Polymer melting was observed from about 160 ⁰C, with a peak at about 185⁰C, in agreement with other studies (Tampau et al., 2020). However, a high degree of variability was observed in the melting enthalpy of PVA when expressed per g of polymer, in both the first and second (values not shown) heating steps, assuming a constant mass fraction of PVA in the bilayer film. Nevertheless, the variable thickness observed in the layers could imply notable differences in the PVA ratio in the DSC analysed samples. This could explain the variability in the melting enthalpy and corroborate the heterogeneous coating in the bilayer obtained by casting.

Therefore, DSC analyses revealed the amorphous state of PLA and the semicrystalline nature of PVA in the laminates, without any appreciable effect of the PLA aminolization. However, the variability in the PVA melting enthalpy points to the heterogeneity of the coating on the laminate obtained by casting, even in the case of aminolized PLA, where wettability was enhanced.

4. Conclusions

The aminolization technique applied for less than 3 min yields PLA surfaces with a greater polar component of the surface free energy (100% increase), thus enhancing wettability with polar solutions of PVA. The prolonged (>3 min) exposure to 1,6-hexanediamine had negative effects on the polar component and microstructure of the PLA films. Extensibility of the liquid phase containing carvacrol on the PLA sheet was not improved by aminolization. Mechanical and barrier properties of PLA-PVA bilayers containing, or not, carvacrol were not significantly affected by the aminolization treatment and were very close to the properties of the major PLA monolayer. Therefore,

563 the PVA sheet did not provide the expected oxygen barrier capacity to the laminate.

564 Carvacrol promoted the bilayer stretchability due to its plasticising effect on both PVA

565 and PLA sheets. Thus, coating PLA sheets (aminolized or not) with PVA solutions is not

566 recommended for the purposes of obtaining laminates for food packaging applications,

567 since neither the barrier nor the mechanical properties were an improvement on those

568 of PLA films, mainly due to the heterogeneous thickness of the PVA coating.

573 **Conflict of Interest:** The authors declare that they have no conflict of interest.

5. References

575 ASTM. (1995). Standard test methods for Water Vapor Transmission of materials.
576   Standard designations: E96-95. Annual book of ASTM standards, Philadelphia,
577   PA: American Society for Testing and Materials (pp. 406–413).

578 ASTM. (2001). Standard test method for tensile properties of thin plastic sheeting.
579   Standard D882. Annual book of American standard testing methods. Philadelphia,
580   PA: American Society for Testing and Materials (pp. 162–170).

581 ASTM. (2005). Standard test method for oxygen gas transmission rate through plastic
582   film and sheeting using a coulometric sensor. Standard designations: 3985-05.
583   Annual book of ASTM standards. West Conshohocken, PA: American Society for
584   Testing and Materials.

585 Bonilla, J., Fortunati, E., Vargas, M., Chiralt, A., & Kenny, J. M. (2013). Effects of
586   chitosan on the physicochemical and antimicrobial properties of PLA films. *Journal
587   of Food Engineering*, *119*(2), 236–243.

588 Bonilla, J., Fortunati, E., Atarés, L., Chiralt, A., & Kenny, J. M. (2014). Physical,

589   structural and antimicrobial properties of poly vinyl alcohol-chitosan biodegradable

590   films. *Food Hydrocolloids*, *35*, 463–470.

591 Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in

592   foods - A review. *International Journal of Food Microbiology*, *94*(3), 223–253.

593 Can Baser, K. (2008). Biological and pharmacological activities of carvacrol and

594   carvacrol bearing essential oils. *Current Pharmaceutical Design*, *14*(29), 3106–

595   3119.

596 Cano, A., Jiménez, A., Cháfer, M., Gónzalez, C., & Chiralt, A. (2014). Effect of

597   amylose:amylopectin ratio and rice bran addition on starch films properties.

598   *Carbohydrate Polymers*, *111*, 543–555.

599 Cano, A., Fortunati, E., Cháfer, M., Kenny, J. M., Chiralt, A., & González-Martínez, C.

600   (2015). Properties and ageing behaviour of pea starch films as affected by blend

601   with poly(vinyl alcohol). *Food Hydrocolloids*, *48*, 84–93.

602 Cano, A. I., Cháfer, M., Chiralt, A., & González-Martínez, C. (2016). Biodegradation

603   behaviour of starch-PVA films as affected by the incorporation of different

604   antimicrobials. *Polymer Degradation and Stability*, *132*, 11–20.

605 Carneiro-da-Cunha, M. G., Cerqueira, M. A., Souza, B. W. S., Souza, M. P., Teixeira, J.

606   A., & Vicente, A. A. (2009). Physical properties of edible coatings and films made

607   with a polysaccharide from *Anacardium occidentale* L. *Journal of Food

608   Engineering*, *95*(3), 379–385.

609 Carneiro-da-Cunha, M. G., Cerqueira, M. A., Souza, B. W. S., Carvalho, S., Quintas, M.

610   A. C., Teixeira, J. A., & Vicente, A. A. (2010). Physical and thermal properties of a

611   chitosan/alginate nanolayered PET film. *Carbohydrate Polymers*, *82*(1), 153–159.

612 Cerqueira, M. A., Lima, Á. M., Teixeira, J. A., Moreira, R. A., & Vicente, A. A. (2009).

613   Suitability of novel galactomannans as edible coatings for tropical fruits. *Journal of

614   Food Engineering*, *94*(3–4), 372–378.

Collazo-Bigliardi, S., Ortega-Toro, R., & Chiralt, A. (2018). Properties of micro- and nano-reinforced biopolymers for food applications. *Polymers for Food Applications* (T. J. Gutiérrez, ed.).

Collazo-Bigliardi, S., Ortega-Toro, R., & Chiralt, A. (2019). Using grafted poly(ε-caprolactone) for the compatibilization of thermoplastic starch-polylactic acid blends. *Reactive and Functional Polymers*.

Debeaufort, F., Martin-Polo, M., & Voilley, A. (1993). Polarity homogeneity and structure affect water vapor permeability of model edible films. *Journal of Food Science*, *58*(2), 426–429.

DeMerlis, C. C., & Schoneker, D. R. (2003). Review of the oral toxicity of polyvinyl alcohol (PVA). *Food and Chemical Toxicology*, *41*(3), 319–326.

Drobota, M., Persin, Z., Zemljic, L., Mohan, T., Stana-Kleinschek, K., Doliska, A., Coseri, S. (2013). Chemical modification and characterization of poly(ethylene terephthalate) surfaces for collagen immobilization. *Open Chemistry*, *11*(11).

Fabra, M. J., Flores-López, M. L., Cerqueira, M. A., de Rodriguez, D. J., Lagaron, J. M., & Vicente, A. A. (2016). Layer-by-layer technique to developing functional nanolaminate films with antifungal activity. *Food and Bioprocess Technology*, 9(3), 471–480.

Farrington, D. W., Lunt, J., Davies, S., & Blackburn, R. S. (2005). 6 - Poly(lactic acid) fibers. In R. S. B. T.-B. and S. F. Blackburn (Ed.), *Woodhead Publishing Series in Textiles* (pp. 191–220).

Gennadios A., Weller C. L, & Gooding C. H. (1994). Measurement errors in water vapour permeability of highly permeable, hydrophilic edible films. *Journal of Food Engineering*, *21*(4), 395-409.

Hejda, F., Solař, P., & Kousal, J. (2010). Surface free energy determination by contact angle measurements – A comparison of various approaches. (3), 25–30.

Retrieved online from: https://pdfs.semanticscholar.org/e5a0/e7dc916cfeb7f0b4a3e24027cf7421d5d5e0.pdf (September 2019)

Hsu J. C.-H., & Guckenberger, A. C. (1985). EP0175451A2. Retrieved from https://patents.google.com/patent/EP0175451A2/ru (September 2019)

Hutchings, J. B. (1999). Food and colour appearance. Chapman and Hall food science book, (2nd edition). Gaithersburg, Maryland: Aspen Publication.

Kamimura, J. A., Santos, E. H., Hill, L. E., & Gomes, C. L. (2014). Antimicrobial and antioxidant activities of carvacrol microencapsulated in hydroxypropyl-beta-cyclodextrin. *LWT - Food Science and Technology*, *57*(2), 701–709.

Kwok, D. Y., & Neumann, A. W. (1999). Contact angle measurement and contact angle interpretation. In *Advances in Colloid and Interface Science* (Vol. 81).

Medeiros, B. G. d. S., Pinheiro, A. C., Teixeira, J. A., Vicente, A. A., & Carneiro-da-Cunha, M. G. (2012). Polysaccharide/protein nanomultilayer coatings: construction, characterization and evaluation of their effect on "Rocha" pear (Pyrus communis L.) Shelf-Life. *Food and Bioprocess Technology*, *5*(6), 2435–2445.

Medeiros, B. G. d. S., Souza, M. P., Pinheiro, A. C., Bourbon, A. I., Cerqueira, M. A., Vicente, A. A., & Carneiro-da-Cunha, M. G. (2014). Physical characterisation of an alginate/lysozyme nano-laminate coating and its evaluation on "Coalho" cheese shelf life. *Food and Bioprocess Technology*, 7(4), 1088–1098.

Mascheroni, E., Guillard, V., Gastaldi, E., Gontard, N., & Chalier, P. (2011). Anti-microbial effectiveness of relative humidity-controlled carvacrol release from wheat gluten/montmorillonite coated papers. *Food Control*, *22*(10), 1582–1591.

Muller, J., Jiménez, A., González-Martínez, C., & Chiralt, A. (2016). Influence of plasticizers on thermal properties and crystallization behaviour of poly(lactic acid) films obtained by compression moulding. *Polymer International*, *65*(8), 970–978.

Muller, J., González-Martínez, C., & Chiralt, A. (2017a). Poly(lactic) acid (PLA) and starch bilayer films, containing cinnamaldehyde, obtained by compression moulding. *European Polymer Journal*, *95*(July), 56–70.

Muller, J., Casado Quesada, A., González-Martínez, C., & Chiralt, A. (2017b). Antimicrobial properties and release of cinnamaldehyde in bilayer films based on polylactic acid (PLA) and starch. *European Polymer Journal*, *96*(May), 316–325.

Noel, S., Liberelle, B., Yogi, A., Moreno, M. J., Bureau, M. N., Robitaille, L., & De Crescenzo, G. (2013). A non-damaging chemical amination protocol for poly(ethylene terephthalate) – application to the design of functionalized compliant vascular grafts. *J. Mater. Chem. B*, *1*(2), 230–238.

Ortega-Toro, R., Contreras, J., Talens, P., & Chiralt., A. (2015). Physical and structural properties and thermal behaviour of starch-poly(ε-caprolactone) blend films for food packaging. *Food Packaging and Shelf Life*, *5*, 10–20.

Owusu Apenten, R. K., & Zhu, Q.-H. (1996). Interfacial parameters for selected Spans and Tweens at the hydrocarbon—water interface. *Food Hydrocolloids*, *10*(1), 27–30.

Perdones, Á., Chiralt, A., & Vargas, M. (2016). Properties of film-forming dispersions and films based on chitosan containing basil or thyme essential oil. *Food Hydrocolloids*, *57*, 271–279.

Petersen, K., Væggemose Nielsen, P., Bertelsen, G., Lawther, M., Olsen, M. B., Nilsson, N. H., & Mortensen, G. (1999). Potential of biobased materials for food packaging. *Trends in Food Science and Technology*, *10*(2), 52–68.

Pinheiro, A. C., Bourbon, A. I., Quintas, M. A. C., Coimbra, M. A., & Vicente, A. A. (2012). K -carrageenan / chitosan nanolayered coating for controlled release of a model bioactive compound. *Innovative Food Science and Emerging Technologies*, *16*, 227–232.

693 Ramos, M., Beltrán, A., Peltzer, M., Valente, A. J. M., & Garrigós, M. del C. (2014).

694 Release and antioxidant activity of carvacrol and thymol from polypropylene active

695 packaging films. *LWT - Food Science and Technology*, *58*(2), 470–477.

696 Requena, R., Vargas, M., & Chiralt, A. (2018). Obtaining antimicrobial bilayer starch and

697 polyester-blend films with carvacrol. *Food Hydrocolloids*, *83*, 118–133.

698 Rhim, J. W., Mohanty, K. A., Singh, S. P., & Ng, P. K. W. (2006). Preparation and

699 properties of biodegradable multilayer films based on soy protein isolate and

700 poly(lactide). *Industrial and Engineering Chemistry Research*, *45*(9), 3059–3066.

701 Ribeiro, C., Vicente, A. A., Teixeira, J. A., & Miranda, C. (2007). Optimization of edible

702 coating composition to retard strawberry fruit senescence. *Postharvest Biology and*

703 *Technology*, *44*(1), 63–70.

704 Sapper, M., & Chiralt, A. (2018). Starch-based coatings for preservation of fruits and

705 vegetables. *Coatings*, 8(5).

706 Sapper, M., Bonet, M., & Chiralt, A. (2019). Wettability of starch-gellan coatings on

707 fruits, as affected by the incorporation of essential oil and/or surfactants. *Lwt*,

708 *116*(March), 108574.

709 Siracusa, V. (2012). Food packaging permeability behaviour: A Report. *International*

710 *Journal of Polymer Science*, *2012*(i), 1–11.

711 Souza, M. P., Vaz, A. F. M., Costa, T. B., Cerqueira, M. A., De Castro, C. M. M. B.,

712 Vicente, A. A., & Carneiro-da-Cunha, M. G. (2018). Construction of a biocompatible

713 and antioxidant multilayer coating by layer-by-layer assembly of κ-carrageenan and

714 quercetin nanoparticles. *Food and Bioprocess Technology*, 11(5), 1050–1060.

715 Tampau, A., González-Martínez, C., & Chiralt, A. (2018). Release kinetics and

716 antimicrobial properties of carvacrol encapsulated in electrospun poly-(ε-

717 caprolactone) nanofibres. Application in starch multilayer films. *Food Hydrocolloids*,

718 *79*, 158–169.

719 Tampau, A., González-Martínez, C., & Chiralt, A. (2020). Polyvinyl alcohol-based

720   materials encapsulating carvacrol obtained by solvent casting and electrospinning.

721   *Reactive and Functional Polymers, 153(April), 104603.*

722 Turek, C., & Stintzing, F. C. (2013). Stability of essential oils: A Review. *Comprehensive*

723   *reviews in food science and food safety*, *12*(1), 40–53.

724 Xiao, L., Wang, B., Yang, G., & Gauthier, M. (2012). Poly(lactic acid)-based

725   biomaterials: synthesis, modification and applications. In *Biomedical Science,*

726   *Engineering and Technology*.

727 Zhu, Y., Gao, C., Liu, X., & Shen, J. (2002). Surface modification of polycaprolactone

728   membrane via aminolysis and biomacromolecule immobilization for promoting

729   cytocompatibility of human endothelial cells. *Biomacromolecules*, *3*(6), 1312–1319.

730 Zhu, Y., Gao, C., Liu, X., He, T., & Shen, J. (2004). Immobilization of

731   biomacromolecules onto aminolyzed poly(L-lactic acid) toward acceleration of

732   endothelium regeneration. *Tissue Engineering*, *10*(1–2), 53–61.

733 www.accudynetest.com/surface_tension_table.html (page accessed in October 2018)

734 www.natureworksllc.com/What-is-Ingeo/How-Ingeo-is-Made (page accessed in May

735 2019)

736 http://polymerdatabase.com/Films/Multilayer%20Films.html (page accessed in October

737 2019)

738

739

$$-\overset{\displaystyle CH_3}{\underset{\displaystyle \underset{O}{\parallel}}{C}}-C-O- \quad + \quad H_2N-(CH_2)_6-NH_2 \quad \longrightarrow \quad \underset{\displaystyle HN-(CH_2)_6-NH_2}{-\overset{\displaystyle CH_3}{\underset{\displaystyle }{C}}-C=O} \quad + \quad H_2O$$
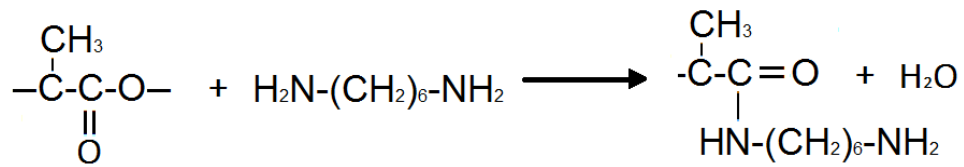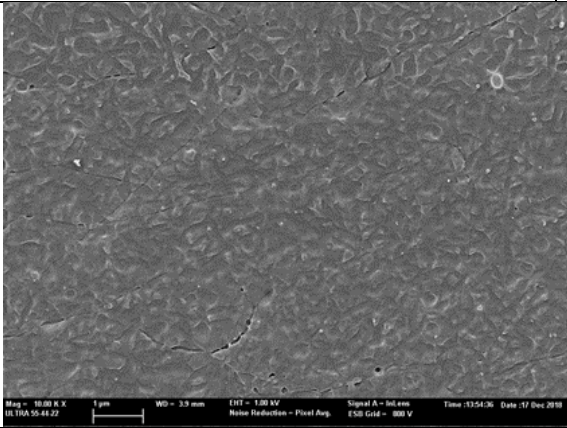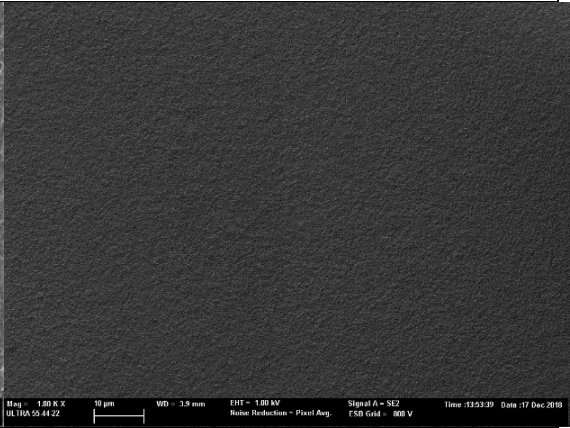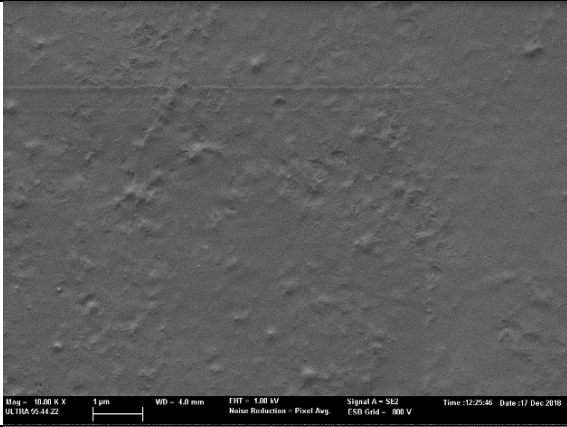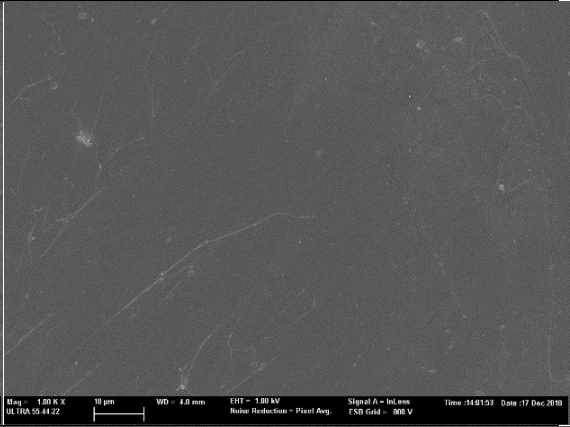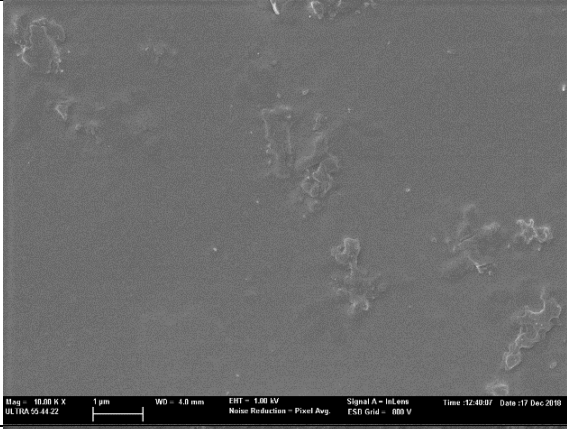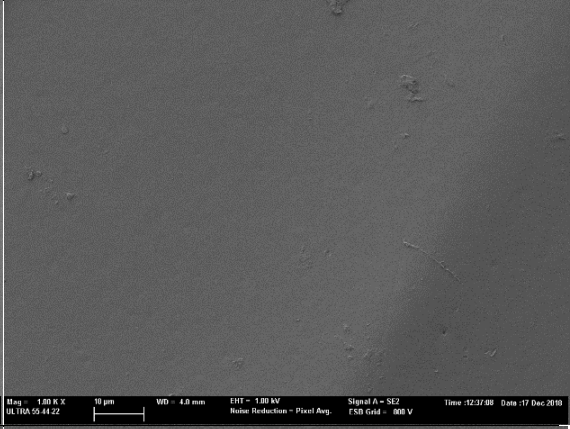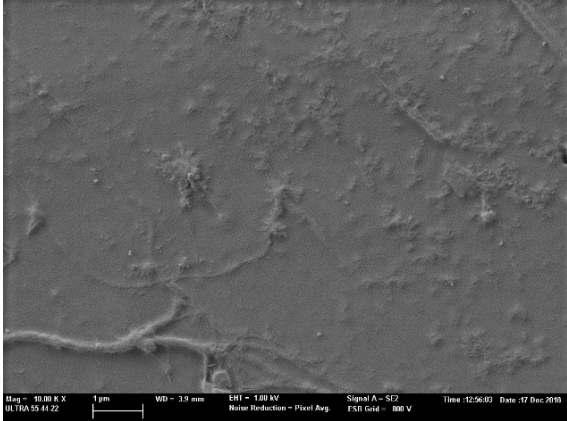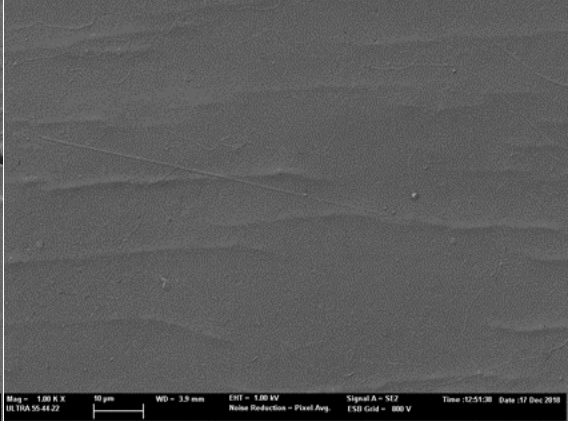
740   **Figure 1.** Mechanism of aminolization by 1,6-hexanediamine, occurring at the
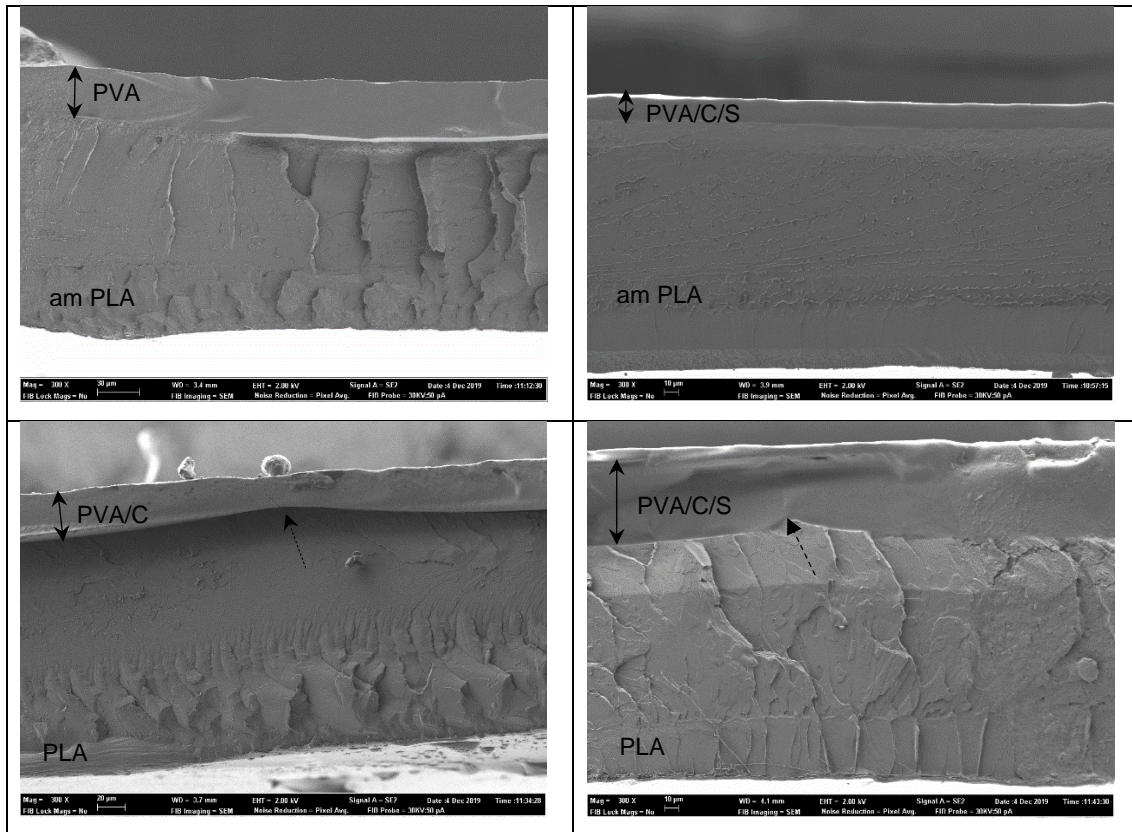
741   polylactide's surface.

742

| treatment time	Magnification factor	
	x 10 000	x 1000
0 min		
1 min		
3 min		
5 min		

Figure 2. FESEM micrographs of the PLA film surface submitted to the aminolization treatment (different times). Magnification of 10 000 x and 1000 x.

Figure 3. Micrographs of several bilayer cross-sections. Top row: aminolized PLA with PVA (left) and PVA/C/S (right) coatings. Bottom row: untreated PLA with PVA/C (left) and PVA/C/S (right) coatings. Dotted arrows indicate protuberances at PLA surface.
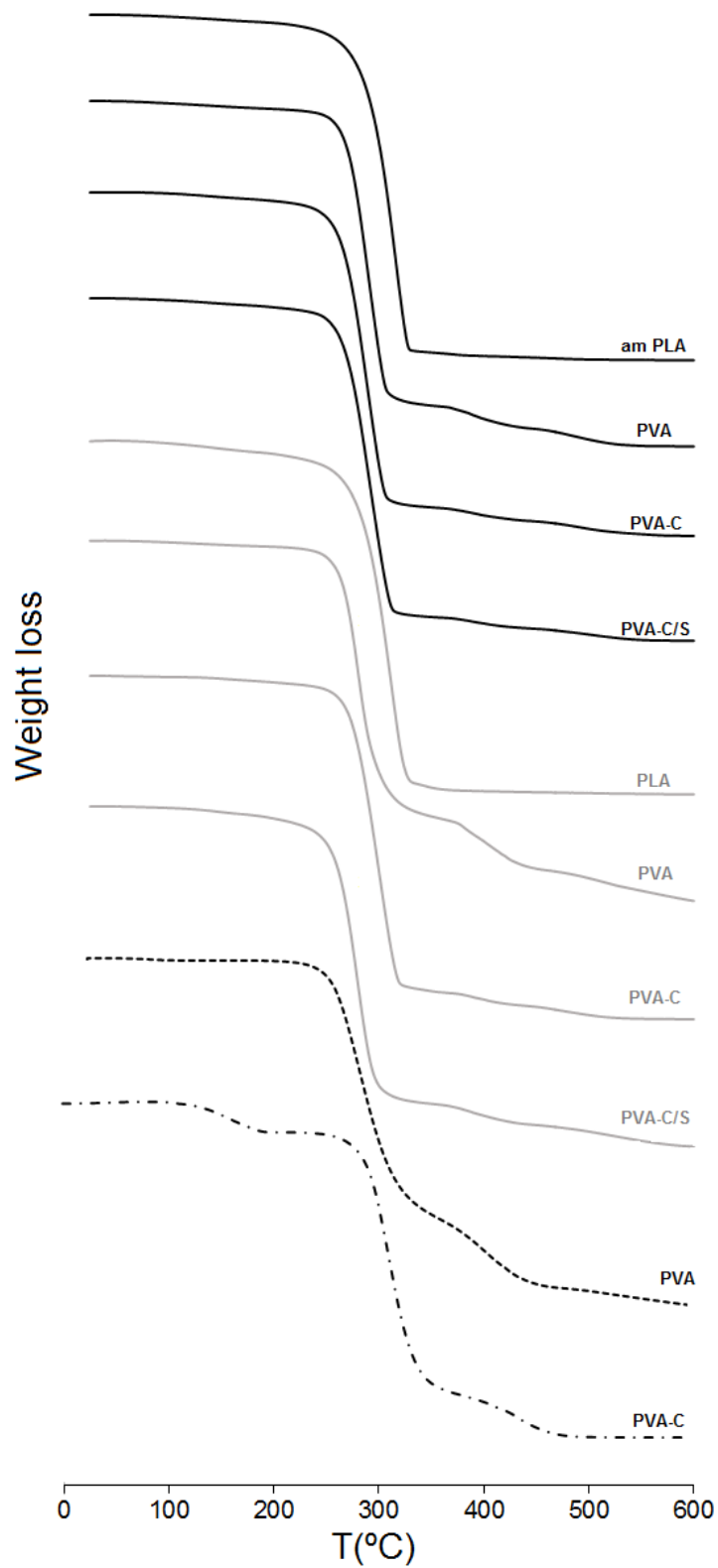
Figure 4. TGA curves of multilayer assemblies with PLA, aminolized (black curves) or not (grey curves), and PVA, containing or not carvacrol (C) and surfactant (S).
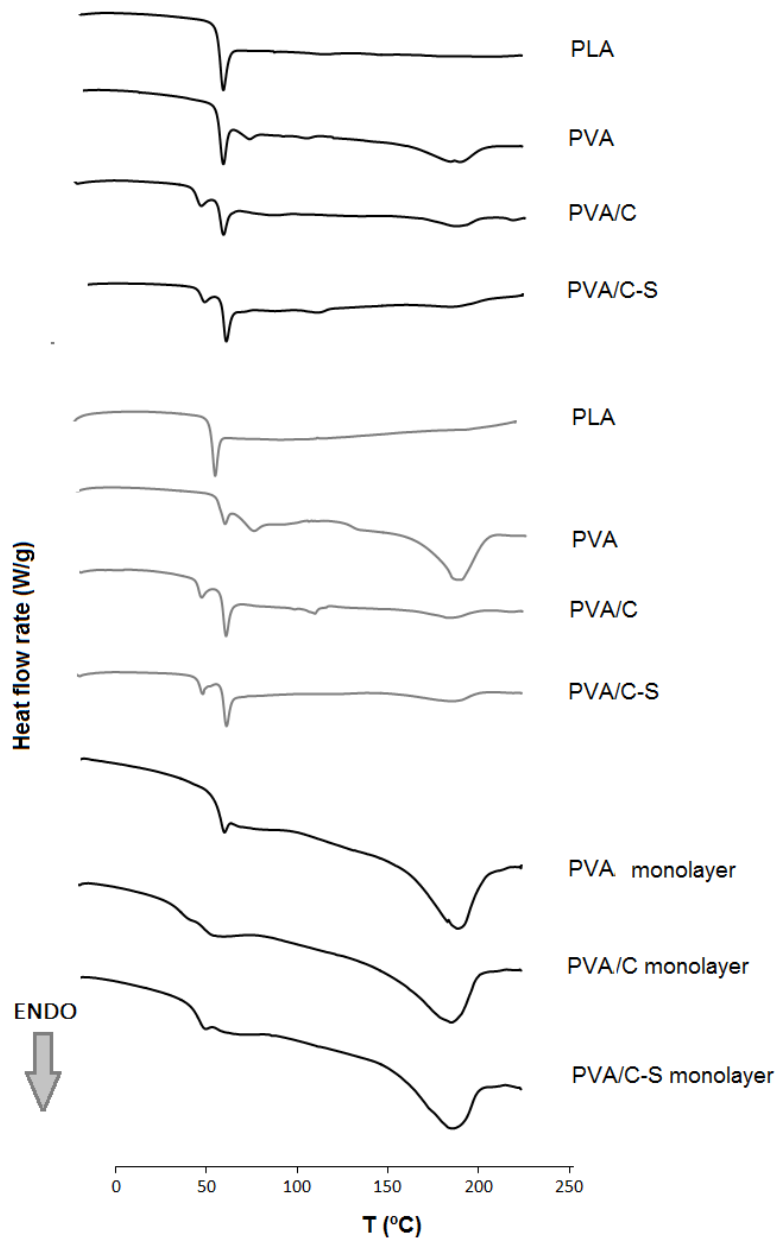
Figure 5. DSC curves of multilayer assemblies with PLA, aminolized (black curves) or not (grey curves), and PVA, containing or not carvacrol (C) and surfactant (S). Monolayers of aminolized PLA (solid black curve), untreated PLA (solid grey curve) and PVA also included.

761 **Table 1**. Surface energy (SE) and its polar and dispersive components of PLA films

762 submitted to different aminolization times. Relative change (%) with respect to the initial

763 value in mass (Δm) and thickness (Δe) provoked by the process are shown. In brackets,

764 the values of mass gain of films immersed only in solvent.

Aminolization time (min)	PLA surface free energy (mN/m)			Δm (%)	Δe (%)
	SE	Polar component	Dispersive component		
0	29.3	8.8	20.5	n/a	n/a
1	37.0	18.5	18.5	0.5±0.2 (1.2)	7.6
3	37.4	17.6	19.8	0.7±0.2 (5.9)	8.8
5	31.7	9.1	22.6	1.1±0.2 (7.9)	17.2
10	32.3	13.5	18.8	3.2±0.6 (8.4)	21.2
15	n/a	n/a	n/a	4.2±1.2 (7.1)	35.6
30	n/a	n/a	n/a	-5.49 (8.6)	419.2

765

766

Table 2. Surface tension (γ$_L$**)** of the PVA solutions (C/S wt. ratios are specified) and contact angles (**θ**) on PLA layer as a function of the aminolization time. Different superscript letters ([a, b, c]) and numbers ([1, 2, 3]) in the same column, and row respectively, indicate significant (p<0.05) differences between samples for the analysed parameter.

	PVA	PVA/C	PVA-C/S (0.3:100)	PVA-C/S (0.4:100)	PVA- C/S (0.5:100)
γ$_L$ (mN/m)	45.4±0.5[2]	36.0±0.3[1]	36.1±0.4[1]	36.0±0.2[1]	36.5±0.2[1]
Aminolization time (min)	Contact angles (θ)				
0	51±1[c,4]	44±2[a,23]	38±2[a,1]	41±2[a,2]	44±3[ab,3]
1	44±3[b,1]	42±1[a,1]	45±7[b,1]	44±7[ab,1]	46±5[bc,1]
3	33±3[a,1]	47±6[a,3]	49±3[b,3]	48±4[b,3]	42±3[a,2]
	Wettability parameter (W$_s$)				
0	-17.0±1.0[a1]	-10.0±1.0[a23]	-7.5±1.0[b4]	-9.0±0.7[b3]	-10.5±1.0[ab2]
1	-13.0±2.0[b1]	-9.5±1.0[a2]	-11.0±3.0[a12]	-11.0±3.0[ab12]	-11.0±2.2[a12]
3	-7.0±2.0[c3]	-12.0±3.0[a1]	-12.0±1.3[a1]	-12.0±2.0[a1]	-9.4±1.2[b2]

771

772

773 **Table 3**. Barrier and mechanical properties of bilayer PVA-PLA films. Film thickness and transparency (T_i) are also shown. Different superscript

774 letters in the same column indicate significant differences ($p < 0.05$) between samples.

Multilayer components		WVP x 10^{10} (g/Pa·s·m)	OP x 10^{12} (cm³/m·s·Pa)	Young Modulus (MPa)	Tensile Strength (MPa)	ε (%)	Thickness (mm)	T_i at 460 nm (%)
am PLA	PVA	0.28±0.03[a]	4.45±0.47[b]	1300±200[bc]	53±8[b]	4.9±0.5[a]	0.251±0.060[a]	56±13[a]
	PVA-C	0.36±0.05[ab]	5.02±0.32[bc]	1140±150[ab]	40±7[a]	5.4±0.4[ab]	0.231±0.019[a]	69±10[b]
	PVA-C/S	0.40±0.09[b]	5.89±1.07[c]	1100±300[a]	41±5[a]	6.7±2.5[bc]	0.236±0.013[a]	60±5[a]
PLA	PVA	0.37±0.05[ab]	4.07±0.16[b]	1400±50[c]	49±3[b]	5.8±0.9[ab]	0.230±0.018[a]	86.2±0.3[c]
	PVA-C	0.33±0.01[ab]	0.15±0.02[a]	1100±120[ab]	42±5[a]	7.9±2.1[cd]	0.247±0.022[a]	86±1[c]
	PVA-C/S	0.36±0.02[ab]	0.20±0.06[a]	1150±110[ab]	41±4[a]	8.9±1.0[d]	0.240±0.010[a]	85±4[c]
Monolayers								
	PVA	8.05±0.06	0.0015±0.02	54±5	46±6	97±6	0.065±0.002	86±1
	PLA	0.44±0.03	4.66±0.03	1370±34	53±2	4.3±0.2	0.22±0.01	87.7±0.2

775

776

Table 4. Thermal parameters of polymers in the multilayer assemblies. Values of pure, non-treated polymers are also included. Different superscript letters in the same column indicate significant differences ($p<0.05$) between specimens.

Bilayer/ monolayer		TGA*				DSC				2nd heating
						1st heating				
		$T_{onset\ 1}$ (ºC)	$T_{peak\ 1}$ (ºC)	$T_{peak\ 2}$ (ºC)	$T_{peak\ 3}$ (ºC)	$T_{g\ PLA}$ (ºC)	$T_{g\ PVA}$ (ºC)	T_{peak} (ºC)	ΔH (J/g PVA)	T_g (ºC)
am PLA	PVA	248±2[cd]	286±3[ab]	392±2[ab]	511±27[a]	56±1[b]	68.4±1.0[d]	185±1[ab]	34±4[a]	52±3[c]
	PVA-C	244.9±0.3[bcd]	286±1[ab]	391±1[a]	496±6[a]	56.2±0.4[b]	43.8±0.3[a]	187±1[b]	17±2[a]	44±1[a]
	PVA-C/S	246±1[bcd]	289±1[b]	393±0[ab]	508±1[a]	57.6±0.0[c]	45.2±0.2[b]	188±1[b]	33±28[a]	49±2[bc]
PLA	PVA	244±1[bc]	280±1[a]	398±2[b]	508±2[a]	56.0±1.0[b]	68.9±0.1[d]	189±2[b]	84±43[b]	53±2[c]
	PVA-C	251±4[d]	292±7[b]	396±1[ab]	504±28[a]	57.8±0.7[c]	44.3±0.7[ab]	183±1[a]	25±7[a]	47±2[ab]
	PVA-C/S	241±2[b]	279±2[a]	392±1[ab]	530±12[a]	58.2±0.0[c]	44.6±0.3[ab]	186±1[ab]	23±8[a]	45±1[ab]
am PLA		268±1[e]	310±1[c]	-	-	55±1[ab]	-	-	-	51.9±0.1[c]
PLA		269±5[e]	310±4[c]	-	-	54.4±0.2[a]	-	-	-	53±2[c]
PVA		192±1[a]	288±4[b]	398±6[b]	-	-	55.0±0.3[c]	-	50±3[ab]	63±3[d]

no residue was observed at 600 ºC.