1 # Multilayer Structures Based on Annealed Electrospun Biopolymer Coatings of Interest in

2 # Water and Aroma Barrier Fiber-Based Food Packaging Applications

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9 **ABSTRACT:** In this research work, for the first time, a fiber-based packaging material was coated by annealed

10 electrospun ultrathin fibers of poly(3-hydroxybutyrate) (PHB), polyvinyl alcohol (PVOH), and polylactide

11 (PLA). The resultant mono- and multilayer structures self-adhered to the paper substrate and were

12 characterized in terms of morphology, optical, and barrier properties. Additionally, the use of a static flat

13 plate and rotating mandrel collector as well as the application of different electrospinning deposition times

14 were analyzed. The thermally treated electrospun biopolymers yielded totally transparent films while, due

15 to the opaque nature of the uncoated paper substrate, the developed packaging materials were also

16 opaque but with a glossier surface finish provided by the bioplastic coating. The annealed films obtained

17 from random electrospun fibers, *i.e.* the mats of ultrathin fibers collected on the static plate, presented

18 higher transparency and thickness and also enhanced barrier performance. On the overall, the developed

19 annealed electrospun biopolymer coatings resulted in a significant improvement of the paper barrier

20 properties to water and limonene vapors, being the paper/PVOH/PHB film the best performing multilayer

21 packaging structure.

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23 **KEYWORDS:** Barrier properties; electrospinning; paper packaging; polyhydroxyalkanoates

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## INTRODUCTION

Lightweight, renewability, and recyclability represent the main advantages of fiber-based packaging, which is highly used in the food packaging industry. The concept of fiber-based packaging, habitually referred for simplicity as paper, includes different types of materials such as sulfite paper, Kraft paper, grease-free paper, paperboards, and laminated paper.[1] This material is mainly made of cellulosic pulp fibers that are derived from renewable resources, including wood and nonwood lignocellulosic materials.[2] After processing, the paper surface habitually remains rather rough and porous. Filling paper with a color-containing pigment is an excellent method to improve certain qualities, including weight, surface smoothness, opacity, gas permeability, and ink absorbency.[3] However, the porosity and hydrophilic nature of paper, which are intrinsically ascribed to the hydroxyl groups (O-H) of cellulose, creates some limitations for its use on food packaging applications. These are mainly related to the high permeation of moisture, organic vapors, and gases, and to the adsorption of oils. This certainly makes uncoated paper unable to retaining its proper shape, resulting in a loss of quality and organoleptic properties for the packaged products.[4]

The limitations described above can be reduced by the application of plastic coatings to paper that can advantageously increase paper stiffness too. Nevertheless, these plastic materials are habitually based on polymers obtained from monomers derived from petroleum that certainly limits intrinsic sustainability aspects of fiber-based packaging materials such as recyclability, biodegradability, carbon footprint, etc.[5] In contrast to traditional petroleum-based plastics, biopolymers certainly represent a promising alternative as paper coatings due to their environmentally friendly nature both in terms of natural origin and biodegradability.[6] Indeed, the association of biopolymers to paper provides interesting functionalities while maintaining the environmentally friendly characteristics of the packaging material.[7] In particular, biodegradable polymers can enhance compostability, recyclability, nontoxicity, and even biocompatibility of paper.[8,9] Biodegradable polymers can be divided into four categories according to their origin: 1)

Polymers directly extracted from biomass, including both polysaccharides (*e.g.* chitosan, starch, cellulose, etc.) and proteins (*e.g.* zein, whey protein, collagen, etc.); 2) Synthetic polymers based on petrochemical monomers such as poly(ε-caprolactone) (PCL), poly(butylene adipate-*co*-terephthalate) (PBAT), and polyvinyl alcohol (PVOH); 3) Synthetic polymers based on renewable monomers, for instance polylactide (PLA); 4) Natural polymers produced by microorganisms, typically polyhydroxyalkanoates (PHAs) such as poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-*co*-3-valerate) (PHBV).[10,11] Among them, PLA is undoubtedly considered the front runner in the emerging bioplastics market since its basic building block, lactic acid, can be easily obtained from the fermentation of starch sources. In addition, PLA shows excellent processability, well-balanced mechanical properties, and high transparency.[12,13] More recently, PHAs, including PHB and its copolymers, are also receiving a great deal of attention in terms of production and characterization due to their excellent sustainable profile.[14] This is related to the fact that PHB-based materials are easily compostable, *i.e.* these can be enzymatically degraded in controlled compost soil, and fully bio-based, which allows achieving the so-called closed-loop sustainable model.[15] Additionally, in contrast to most biopolymers, because of its high crystallinity, PHB presents a relatively high gas and water vapor barrier so that it can replace polyolefins for a large number of food packaging applications.[16-18]

The main objective for paper coatings based on bioplastics is currently to perform, in terms of both transparency and barrier properties, similarly as polyolefins and polyethylene terephthalate (PET).[19] For this, the most challenging improvement of biopolyesters is related to reduce the water vapor and oxygen permeability.[20] While the study of both water vapor and oxygen barrier properties is of interest in food preservation, limonene transport properties are also important because this is usually used as a standard permeant to test aroma barrier. In this context, the barrier performance of biopolymers can be potentially enhanced through the application the electrohydrodynamic processing (EHDP) technology, including both electrospinning and electrospraying.[21]

72    In particular, electrospun mats of biopolymer nanofibers can be applied as mono- and multilayers, layer-

73    by-layer assemblies, and similar structures in packaging materials.[22] Recent advances in this field have

74    shown that multilayer systems containing electrospun ultrathin fibers, *i.e.* both submicron fibers and

75    nanofibers, can significantly contribute to improve the barrier performance of biopolymers.[23] Additionally,

76    more remarkably, the electrospinning technology provides novel routes to develop novel active and

77    bioactive functionalities, for instance packaging materials with antimicrobial performance and control

78    release capacity of health promoting functional ingredients.[24]

79    In the first work, the oxygen barrier properties of PLA films made by wet casting were originally improved

80    by electrospun ultrathin zein fibers laminated in a sandwich-type structure.[25,26] Later on, interlayers of

81    electrospun zein, pullulan, whey protein isolate (WPI) films were used to improve the barrier properties of

82    compression-molded PHBV films.[27,28] More recently, wheat gluten films were coated by post-processed

83    electrospun PHB and PHBV fibers.[29,30] Therefore, these previous research works have added a new insight

84    into the potential of the so-called electrospinning coating technology to develop packaging structures of

85    higher quality and safety, showing a high capacity to extend the shelf life of food products. In the case of

86    the electrospun PHB films, interestingly, it was previously found that the storage time at dry conditions did

87    not significantly modify the degree of crystallinity of the homopolyester.[31] In particular, the optical and

88    barrier properties of PHB films stored for 3 months remained nearly constant.

89    This study describes, for the first time, the preparation of a fiber-based packaging material that is coated

90    by films of different biopolymers, namely PHB, PVOH, and PLA, obtained by electrospinning. It further

91    explores the influence of the electrospun coatings on the optical and barrier performance of the resultant

92    paper-coated structures. To carry this out, different electrospun biopolymer mono- and multilayers were

93    deposited on the paper substrate by means of two types of collectors, *i.e.* a flat plate and a rotating drum,

94    and the processing time during electrospinning was varied. Finally, the resultant multilayers were annealed

95 in order to obtain transparent continuous electrospun films with improved adhesion to the paper substrate

96 and enhanced barrier performance for their potential application in the field of fiber-based food packaging.

## EXPERIMENTAL

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### Materials

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99 Bacterial aliphatic homopolyester PHB was supplied by Biomer (Krailling Germany) as P226F. According to

100 the manufacturer, this is certified both as compostable and food contact, presenting a density of 1.25 g/cm$^3$

101 and a melt flow rate (MFR) of 10 g/10 min at 180°C and 5 kg. Semi-crystalline PLA was Ingeo™ 2002D, which

102 is an extrusion-grade resin produced by Natureworks (Minnesota, USA) with a D-isomer content of ~2%.

103 This has a number-average molecular weight (M$_N$) of *ca.* 130000 g/mol and a weight-average molecular

104 weight (M$_W$) of *ca.* 150000 g/mol. PVOH, 363065 grade, with M$_W$ in the range of 146000-186000 g/mol and

105 degree of hydrolysis of >99% was purchased from Sigma-Aldrich S.A. (Madrid, Spain). 2,2,2-trifuoroethanol

106 (TFE) with 99% purity and D-limonene with 98% purity were obtained from Sigma-Aldrich S.A. too. N,N-

107 dimethylformamide (DMF) and trichloromethane (TCM), both with 99% purity, were supplied by Panreac

108 Quimica S.A. (Barcelona, Spain). All raw materials were used as received without further modification.

109 The fiber-based packaging substrate was prepared using commercial bleached Kraft eucalyptus pulp as raw

110 material, which was kindly provided by Ence-Celulosas y Energía S.A. (Madrid, Spain). Briefly, the pulp was

111 disintegrated in a pulp disintegrator for 1h at 3000 rpm to achieve a consistency of 1.5%. Paper sheets of

112 700 x 16 mm$^2$ with a final grammage of 75 g/m$^2$ were fabricated in an isotropic Rapid-Köthen sheet former

113 and conditioned at 23°C and 50% of relative humidity (RH) according to ISO standard 187. The grammage

114 and thickness were evaluated following ISO standards 536 and 534, respectively. Further details can be

115 found in previous research.[32]

### Film Preparation

116 Film Preparation

117 The biopolymers solutions for electrospinning were prepared by dissolving, under continuous stirring

118 conditions, PHB and PLA in TFE and a TCM/DMF mixture 85:15 (wt./wt.), respectively, at room temperature

119 while PVOH was dissolved in distilled water at 80°C. The weight content (wt.-%) of each biopolymer in the

120 solutions is included in **Table 1**.

121 Electrospinning was then performed using a Fluidnatek® LE10 lab line from Bioinicia S.L. (Valencia, Spain)

122 with a variable high-voltage 0-30 kV power supply. This device was equipped with a motorized injector that

123 was scanning horizontally towards a metallic collector, aiming to obtain a homogeneous electrospun

124 deposition. The different biopolymers solutions were transferred to a 30-mL plastic syringe, which was

125 coupled by means of a Teflon tube to a stainless-steel needle (∅=0.9 mm) whereas the needle tip was

126 connected to the power supply. The paper substrate was placed either on a flat plate collector or a rotating

127 mandrel turning at 1000 rpm to achieve fiber orientation. The biopolymer solutions were electrospun in a

128 controlled environmental chamber at room conditions, *i.e.* 23°C and 40% RH, for a given processing time

129 and in optimal conditions to achieve steady fiber formation. **Table 1** also summarizes the parameters used

130 during electrospinning.

131 Finally, the obtained electrospun mats were subjected to annealing process using a hydraulic press 4122-

132 model from Carver, Inc. (Indiana, USA). This was optimally performed at 160°C, without pressure, for 5 ± 1

133 s, based on a previous study.[33] The resultant films were air cooled at room temperature. **Figure 1** shows a

134 scheme illustrating the prepared mono- and multilayer structures based on paper coated by different

135 combinations of electrospun fibers and their annealed films. Prior to thermal treatment, the electrospun

136 mats were equilibrated in desiccator at 25°C and 0% RH by using silica gel for at least 1 week.

**Film thickness and conditioning**

138 Before the tests, the whole thickness of all structures was measured using a digital micrometer series

139 S00014, having ±0.001 mm accuracy, from Mitutoyo Corporation (Kawasaki, Japan). Measurements were

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140  performed at three random positions and values were averaged. ==Films were stored in a desiccator at 25°C==

141  ==and 0% RH for 15 days before characterization.==

### Scanning electron microscopy

143  The morphology of the electrospun fibers and the fracture surfaces of the annealed films was observed by

144  scanning electron microscopy (SEM) using an S-4800 from Hitachi (Tokyo, Japan). Cryo-fractures of the

145  films were obtained from the frozen samples using liquid nitrogen. Prior to examination, all samples were

146  fixed to beveled holders using a conductive double-sided adhesive tape, sputtered with a mixture of gold-

147  palladium under vacuum, and observed using an accelerating voltage of 5 kV. Fiber sizes and layer

148  thicknesses were determined by means of the Aperture software from Apple (California, USA) using the

149  SEM micrographs in their original magnification. At least 25 micrographs were used for the measurements.

### Optical properties

151  Film transparency was evaluated through the surface reflectance spectra using a spectrocolorimeter CM-

152  3600d from Minolta Co. (Tokyo, Japan). Film transparency was evaluated through the internal

153  transmittance ($T_i$) in a 0–1 theoretical range by applying the Kubelka–Munk theory[34] for multiple scattering

154  to the reflection, following **Equation 1**:

155

$$T_i = \sqrt{(a - R_0)^2 - b^2} \quad (1)$$

156

$$a = \frac{1}{2}\left( R + \frac{R_0 - R + R_g}{R_0 R_g} \right) \quad (2)$$

157

$$b = \sqrt{(a^2 - 1)} \quad (3)$$

158

159  where $R_0$ is the reflectance of the film on an ideal black background while $a$ and $b$ parameters are calculated

160  from the reflectance of the sample ($R$) and the layer backed by a known reflectance ($R_g$) according to

161 **Equations 2** and **3**, respectively. Measurements were taken, in triplicate, for each sample by using both a

162 white and black background.

### Water vapor permeability

164 The water vapor permeability (WVP) was determined using the ASTM 2011 gravimetric method. To this

165 end, 5 mL of distilled water was placed inside a Payne permeability cup ($\varnothing$=3.5 cm) from Elcometer Sprl

166 (Hermalle-sous-Argenteau, Belgium). The films were placed in the cups so that on one side they were

167 exposed to 100% RH, avoiding direct contact with water. The cups containing the films were then secured

168 with silicon rings and stored in a desiccator at 25°C and 0% RH. Identical cups with aluminum films were

169 used as control samples to estimate water loss through the sealing. The cups were weighed periodically

170 using an analytical balance of ±0.0001 g accuracy. Water vapor permeation rate (WVRT), also called water

171 permeance when corrected for permeant partial pressure, was determined from the steady-state

172 permeation slope obtained from the regression analysis of weight loss data per unit area *vs*. time, in which

173 the weight loss was calculated as the total cell loss minus the loss through the sealing. Permeability was

174 obtained by correcting the permeance by the average film thicknesses. Measurements were performed in

175 triplicate. In the case of multilayers, the coated layer was facing the permeant.

### Limonene vapor permeability

177 Permeability to limonene vapor was measured as described above for WVP. For this, 5 mL of D-limonene

178 was placed inside the Payne permeability cups. The cups containing the films were placed at controlled

179 conditions, *i.e.* 25°C and 40% RH. Cups with aluminum films were used as control samples to estimate

180 solvent loss through the sealing. Limonene permeation rates were obtained from the steady-state

181 permeation slopes and weight loss was calculated as the total cell loss minus the loss through the sealing.

182 Limonene permeability was calculated taking into account the average film thickness in each case. Three

183 replicates per sample were measured. In the case of multilayers, the coated layer was facing the permeant.

184    **Oxygen permeability**

185    The oxygen permeability coefficient was derived from the oxygen transmission rate (OTR) measurements

186    recorded, in duplicate, using an Oxygen Permeation Analyzer M8001 from Systech Illinois (Thame, UK) at

187    25°C and 60% RH. The samples were previously purged with nitrogen in the humidity equilibrated samples

188    and then exposed to an oxygen flow of 10 mL/min. The exposure area during the test was 5 cm$^2$. In order

189    to obtain the oxygen permeability, film thickness and gas partial pressure were considered.

190    **RESULTS AND DISCUSSION**

191    **Morphology**

192    **Tables 2** and **3** summarize the thicknesses of the here-obtained mono- and multilayer structures obtained

193    from the random and aligned electrospun fibers of biopolymers, respectively. As it can be seen in **Figure 1**,

194    these were based on different combinations of electrospun biopolymer fibers and their resultant annealed

195    films, which were applied as coatings on paper. The tables include information about the mean thickness

196    of each layer and of the whole structure.

197    The cross-sections of the fiber-based packaging structures are shown in the SEM images included in **Figures**

198    **2** to **4**. In particular, **Figure 2** shows the SEM images of the monolayer structures based on paper and

199    electrospun PHB fibers and their annealed films. In **Figure 2a** it can be observed that the neat paper was

200    based on micro-sized fibers with a rough surface. These cellulosic fibers presented a mean diameter of 17

201    ± 2 μm, which clearly contribute to the high level of porosity of paper. In contrast, as shown in **Figure 2b**,

202    the electrospun PHB fibers collected on the plate were based on an ultrathin fiber morphology with a mean

203    diameter of 220 ± 20 nm. As expected, these nanofibers mostly presented a random orientation due to the

204    use of a static flat collector. Alternatively, **Figure 2c** shows the morphology of the electrospun PHB fibers

205    mat for which the mandrel collector was employed. In this image, it can be clearly seen that the PHB

206    nanofibers, with a mean diameter of 215 ± 15 nm, were effectively aligned in the turning direction of the

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207 drum. Therefore, no significant differences can be observed between the average fiber diameter in the

208 randomly oriented electrospun fibers and in the aligned fibers, which is in agreement with a recent research

209 work.[35] In any case, this confirms that through the use of a mandrel collector is possible to control locally

210 the alignment pattern of the deposited fibers during electrospinning. A similar morphological effect has

211 been previously reported.[36-38] However, visual comparison of both SEM micrographs, *i.e.* random (**Figure**

212 **2b**) and aligned (**Figure 2c**) PHB nanofibers mats, suggests that the electrospun mat density increased for

213 the same deposition time as a result of the fiber orientation process. As it can be seen in **Tables 2** and **3**,

214 while the layer thickness of the random PHB nanofibers was 258 µm, the thickness of the equivalent

215 electrospun mat made of oriented nanofibers was 114 µm. In **Figure 2d** it can be seen that the annealing

216 process applied to the random PHB random nanofibers resulted in a film with a more continuous structure

217 due to coalescence of nanofibers at the interphase.[33] The annealed film obtained from the aligned PHB

218 fibers, shown in **Figure 2e**, presented a similar cross-section. Additionally, it is also worthy to note that fiber

219 orientation also had a similar effect on the annealed films thicknesses. In particular, film thickness was

220 reduced from 38 µm, for the PHB film obtained from the random nanofibers (**Figure 2d**), to 26 µm, from

221 the aligned nanofibers (**Figure 2e**).

222 **Figure 3** gathers the morphology of the multilayer structures obtained from the electrospun nanofibers

223 using the plate-type collector. As it can be seen from **Table 2**, for all these structures, the thickness of the

224 paper layer was kept at 125 µm. **Figure 3a** particularly shows the bilayer structure based on electrospun

225 PHB nanofibers applied as a coating on the paper. This electrospun mat presented a mean thickness of 269

226 µm. Even though both layers were based on a fibrilar structure, the SEM micrograph clearly revealed that

227 these presented morphologies with contrasting porosities. It can be also observed the large gap between

228 the two layers, which resulted after cryo-fracture procedure, indicates that the electrospun mat may not

229 be strongly adhered to the paper layer. **Figures 3b** to **3d** show the bilayer structures made of paper coated

230 with annealed films of PHB, PVOH, and PLA. In these films one can observe that the PHB layer thickness

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231 (**Figure 3b**) was considerably thicker than those of both PVOH (**Figure 3c**) and PLA (**Figure 3d**), *i.e.* 37 µm *vs.*

232 10 µm and 7 µm, respectively. This can be directly related to the deposition time during the electrospinning

233 process that was significantly lower for PVOH and PLA, which was 1h in both cases, than for PHB, *i.e.* 2h.

234 **Figures 3e** and **3f** show the multilayer structures of paper/PVOH/PHB film and paper/PLA/PHB film,

235 respectively. Both structures, based on three layers, presented a similar thickness than the bilayer structure

236 of paper/PHB film, *i.e.* around 160-180 µm. Interestingly, all biopolymer layers based on the annealed films

237 presented good adhesion to the paper layer as expected from our previous works[6,27]. This observation

238 proved that, regardless of the chemistry of the contacting materials, the electrospun fibers coated very

239 efficiently the paper substrate surface during annealing due to their large surface-to-volume ratio, resulting

240 in a strong physical adhesion.

241  Similar morphologies can be observed in **Figure 4** for the multilayer structures obtained from the aligned

242 electrospun mats. However, interestingly, the electrospun mat of oriented PHB nanofibers appeared to be

243 more adhered to the paper layer, as shown in **Figure 4a**. In this sense, the rotating movement of the

244 mandrel could favor the physical adhesion of both layers. In addition, all annealed multilayer films based

245 on aligned electrospun fibers presented lower values of thickness than those films based on equivalent

246 random fibers, which can be seen in **Table 3**. This is related the above-described higher density of the

247 aligned electrospun fibers mats.

**Optical Appearance and Transparency Properties**

249 **Figure 5** shows the visual aspect of the mono- and multilayer structures. Simple naked eye examination of

250 this figure indicates that the annealed films obtained from the electrospun PHB nanofibers produced highly

251 transparent materials. Another general observation is that, as paper was opaque, the resulting multilayers

252 were also opaque materials but with a glossier surface finish. Transparency was seen somewhat lower for

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253    the PHB film obtained from the oriented electrospun fibers. In particular, these PHB films showed

254    somewhat higher heterogeneity across the surface.

255    The opacity of the mono- and multilayer structures was measured in terms of $T_i$, which is proportionally

256    related to the sample transparency. Samples spectra of $T_i$ for the mono- and multilayer structures are given

257    in **Figure 6**. These graphs show that, in general, the structures obtained from the electrospun fibers

258    collected on the plate (**Figure 6a**) presented similar transmittance behavior than those based on fibers

259    collected on the drum (**Figure 6b**). However, as previously shown in **Tables 2** and **3**, it is worthy to mention

260    that the annealed films obtained from the random electrospun fibers presented higher thicknesses. In

261    particular, both PHB mono-layer films showed in the 75-85% range of $T_i$, indicating that these films present

262    a single and relatively homogeneous refractive index. However, values of $T_i$ were slightly higher for the

263    annealed PHB film obtained from the random electrospun fibers. As expected, the PHB fibers presented

264    the lowest transparency with $T_i$ values in the range of 5-10% due to the high inherent porosity of the

265    electrospun mat. In general, all multilayer structures showed poor transparency as a result of the opaque

266    nature of paper, being in the 10-25% range of $T_i$. From these results, it is inferred that the here-prepared

267    biopolymer coatings do not alter the transparency properties of paper due to both the intrinsically high

268    opacity of paper and the high transparency achieved in the annealed electrospun films during thermal

269    treatment.[21] In any case, it is apparent that a lower surface roughness can be anticipated for the

270    biopolymer-coated paper materials. A full characterization of these samples in terms of typical paper

271    industry characterization standards it is currently underway and will be published elsewhere.

272    **Barrier Properties**

273    Initially, the effect of the nanofibers deposition time was related to the thickness of the PHB monolayer

274    film, for a given flow-rate. As shown in **Figure 7**, higher processing times during electrospinning led to

275    thicker electrospun mats that, in turn, resulted in annealed films with higher thickness. In particular, the

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276 increase of the electrospinning deposition time from 1h to 4h increased the PHB film thickness. This

277 specifically increased from 29 μm to 50 μm and from 19 μm to 41 μm for the PHB films obtained from

278 random and aligned electrospun fibers, respectively. Therefore, as expected, the resultant thicknesses of

279 the PHB films can be successfully controlled by electrospinning deposition time. However, this did not

280 follow a linear trend, suggesting that some nanofibers losses inside the electrospinning chamber occurred

281 with deposition time.

282 The barrier properties of the monolayer PHB films in relation to water, limonene, and also oxygen

283 permeability are shown in **Figure 8**. Permeability is formally independent of samples thickness,[39] however

284 in practice film thickness has usually an impact in permeability specially for very thin films or heterogeneous

285 materials. McHugh *et al.*[40] also found relationships between film thickness and permeability properties for

286 sodium caseinate films. It was observed that, as film thickness increased, the film provided an increased

287 resistance to mass transfer across it. Since the present materials were annealing-induced coalesced fiber

288 mats, one can expect variations in permeability across thickness, especially for the smallest non-

289 condensable permeants. As **Figure 8** shows, the permeability values were significantly affected by variances

290 in the PHB film thickness. In particular, it can be observed that PHB films exhibited negative slope

291 relationships between thickness and gas permeability. A lower permeability was seen for the films based

292 on electrospun fibers collected on the static plate, which showed a tendency to *plateau* in thickness

293 dependency beyond 30 μm for water but specially for limonene. It seems that, although alignment of the

294 fibers in a rotating mandrel should in principle be expected to reduce porosity since the fibers are more

295 efficiently packed, it probably also created some more heterogeneous materials in the current

296 experiments. This is mostly likely because the trapped remnant solvent cannot so easily evaporate from

297 the strongly aligned fibers hence creating some more surface heterogeneities (see previous **Figure 5**).

298 In relation to WVP, which is shown in **Figure 8a**, the annealed films obtained from the aligned electrospun

299 PHB fibers presented higher WVP values than those obtained from the random fibers. Thus, as water

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300 sorption in the biopolymer is relatively low, *i.e.* PHB is highly hydrophobic, WVP is known to be mainly

301 diffusivity driven.[41] Therefore, it can be considered that heterogeneities within the PHB films do have, as

302 expected, a strong effect on permeability. Interestingly, Sanchez-Garcia *et al.*[42] reported in an earlier work

303 carried out in our laboratory that compression-molded PHB films in the range of 100-700 $\mu$m presented a

304 WVP value of 1.70 x 10$^{-15}$ kg m/s m$^2$ Pa, which is just slightly lower than the one here obtained for 48 $\mu$m,

305 *i.e.* 2.05 x 10$^{-15}$ kg m/s m$^2$ Pa. Interestingly, the WVP value of the present PHB films is in the same order of

306 magnitude as PET films, *i.e.* 2.30 x 10$^{-15}$ kg m/s m$^2$ Pa, and approximately 10-12 times lower than commercial

307 films made of polyamide 6 (PA6), *i.e.* 2.06 × 10$^{-14}$ kg m/s m$^2$ Pa, and ethylene-vinyl alcohol copolymer with

308 32-mol% ethylene (EVOH32), *i.e.* 1.70 × 10$^{-14}$ kg m/s m$^2$ Pa.[43]

309 Regarding limonene permeability, the type of deposition was less relevant, as it can be seen in **Figure 8b**.

310 Since limonene is a strong plasticizing component for the biopolymer, solubility plays a stronger role in

311 permeability than diffusion. For example, it has been previously reported that limonene uptake in PHBV

312 films of around 100 $\mu$m prepared by solvent casting was 12.7  wt.-%, leading to a limonene permeability

313 value of 1.99 x 10$^{-13}$ kg m/s m$^2$ Pa.[44] In another study, Fabra *et al.*[27] recently reported the limonene

314 permeability of PHB and PET films of approximately 75 $\mu$m prepared by compression-molding, showing

315 values of 6.38 x 10$^{-15}$ kg m/s m$^2$ Pa and 6.43 x 10$^{-15}$ kg m/s m$^2$ Pa, respectively. These permeability values

316 are higher than the here-obtained annealed PHB films above 35 $\mu$m, which presented a *plateau* at 3.89 x

317 10$^{-15}$ kg m/s m$^2$ Pa. However, it is worthy to mention that the previously studied films were also plasticized

318 with 5 wt.-% of polyethylene glycol (PEG).

319 A similar behavior to that observed above for WVP, but much more intense, was found for oxygen

320 permeability. Since oxygen is a non-condensable small permeant, the degree of porosity and sample

321 heterogeneities will serve as preferential passage ways, particularly for very small permeants. **Figure 8c**

322 shows that only thicker coatings, *i.e.* beyond 80 $\mu$m, exhibited relatively low values of oxygen permeability.

323 In particular, the oxygen permeability showed a value of 1.20 x 10$^{-18}$ m$^3$ m/m$^2$ s Pa for a PHB film thickness

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324 of 142 µm. This is closer to the values typically reported for conventional 100-µm PHB films prepared by

325 compression molding, *i.e.* 2.24 x $10^{-19}$ m$^3$ m/m$^2$ s Pa.[42] In addition, to put these results into a more practical

326 context for food packaging applications, these values are slightly higher than the oxygen permeability for

327 PET films, *i.e.* 1.35 × $10^{-19}$ m$^3$ m/m$^2$ s Pa, but considerably lower than those values for polyolefins such as

328 polypropylene (PP), *i.e.* 6.75 × $10^{-18}$ m$^3$ m/m$^2$ s Pa, and low-density polyethylene (LDPE) films, *i.e.* 2.15 × 10$^-$

329 $^{17}$ m$^3$ m/m$^2$ s Pa.[43]

330 Finally, **Figure 9** shows the values of permeance, since we deal with multicomponent materials, of water

331 and limonene for the here-obtained mono- and multilayer structures. In both graphs, it can be observed

332 that the neat uncoated paper clearly showed the highest values of permeance. This confirms the inherent

333 low barrier performance of uncoated paper for food packaging applications. Since to gain oxygen barrier,

334 thicker and double side coatings were needed, we focused this preliminary study on achieving barrier to

335 limonene and water vapor. Still oxygen permeability tests were attempted by measuring at 60% RH on the

336 paper coated side and 0% RH on the paper uncoated side, but the multilayers yielded very high permeability

337 values for the coating thicknesses used (results not shown).

338 Regarding water barrier, **Figure 9a** indicates that all multi-layer structures showed significantly lower values

339 of water vapor permeance than the uncoated paper. The lowest permeance values were observed for the

340 multilayer films containing PHB. In particular, the multilayer structures of paper/PLA/PHB film and, more

341 significantly, of paper/PVOH/PHB film presented the highest barrier performance. Regarding limonene,

342 **Figure 9b** shows that a significant decrease in aroma barrier can be also observed for the mono- and

343 multilayer structures based on PHB films. The PHB films obtained from the electrospun fibers collected on

344 the plate presented higher limonene barrier performance than the equivalent films based on aligned fibers.

345 As previously described, this is based on the higher thickness achieved for the films obtained from random

346 electrospun fibers and also to the more heterogeneity observed in the aligned ones. Surprisingly, PLA did

347 not yield barrier performance to the aroma vapor, perhaps due to insufficient coating thickness. Even more

15

348 surprisingly, PVOH coatings, which are known to present high barrier to organic vapors when dry, also did

349 not result in enhanced aroma barrier most likely because of insufficient sample thickness and plasticization

350 by moisture during the test. On the other hand, the multilayer structures made of paper/PLA/PHB film but

351 especially the paper/PVOH/PHB film showed the highest aroma barrier performance. Interestingly, a

352 synergy between the PVOH and PHB coatings were, therefore, obtained for both permeants.

353 CONCLUSIONS

354 Thermally post-processed electrospun biopolymer coatings over fiber-based packaging materials are very

355 promising systems since they self-adhere during annealing as a result of the high surface-to-volume ratio

356 of the fibers and do, furthermore, provide enhanced barrier to gases and vapors when built with sufficient

357 thickness. It was observed that the minimal thickness of PHB coating required for high barrier to moisture

358 and aroma was 30 $\mu$m while for oxygen it was 80 $\mu$m. These multilayer systems are also likely to provide

359 potential environmental advantages over traditional paper coatings based on non-biodegradable

360 petroleum-based materials.

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423 FIGURE CAPTIONS

424 **Figure 1.** Scheme of the mono- and multilayer structures based on paper and annealed electrospun fibers

425 of poly(3-hydroxybutyrate) (PHB), polyvinyl alcohol (PVOH), and polylactide (PLA).

426 **Figure 2.** Scanning electron microscopy (SEM) images of monolayer structures of: a) Paper; b) Electrospun

427 poly(3-hydroxybutyrate) (PHB) fibers collected on the plate; c) Electrospun PHB fibers collected on the

428 mandrel; d) Annealed PHB film obtained from electrospun fibers collected on the plate; e) Annealed PHB

429 film obtained from electrospun fibers collected on the mandrel.

430 **Figure 3.** Scanning electron microscopy (SEM) images of multilayer structures of: a) Paper/poly(3-

431 hydroxybutyrate) (PHB) fibers; b) Paper/PHB film; c) Paper/polyvinyl alcohol (PVOH) film; d)

432 Paper/polylactide (PLA) film; e) Paper/PVOH/PHB film; f) Paper/PLA/PHB film. The electrospun fibers were

433 collected on the plate.

434 **Figure 4.** Scanning electron microscopy (SEM) images of multilayer structures of: a) Paper/poly(3-

435 hydroxybutyrate) (PHB) fibers; b) Paper/PHB film; c) Paper/polyvinyl alcohol (PVOH) film; d)

436 Paper/polylactide (PLA) film; e) Paper/PVOH/PHB film; f) Paper/PLA/PHB film. The electrospun fibers were

437 collected on the mandrel.

438 **Figure 5.** Contact transparency of the mono- and multilayer structures based on paper and electrospun

439 fibers and annealed films of poly(3-hydroxybutyrate) (PHB), polyvinyl alcohol (PVOH), and polylactide (PLA).

440 **Figure 6.** Spectral distribution of internal transmittance ($T_i$) at the range of 400-700 nm of the mono- and

441 multilayer structures based on paper and annealed films of poly(3-hydroxybutyrate) (PHB), polyvinyl

442 alcohol (PVOH), and polylactide (PLA) obtained from electrospun fibers collected on: a) Plate; b) Mandrel.

443 **Figure 7.** Resulting thickness values of films made from annealed electrospun poly(3-hydroxybutyrate)

444 (PHB) fibers as a function of the electrospinning deposition time.

445 **Figure 8.** Effect of film thickness of annealed electrospun poly(3-hydroxybutyrate) (PHB) fibers on: a) Water

446 vapor permeability (WVP); b) Limonene permeability; c) Oxygen permeability.

447 **Figure 9.** Permeance of the mono- and multilayer structures based on paper and electrospun fibers and

448 annealed films of poly(3-hydroxybutyrate) (PHB), polylactide (PLA), and polyvinyl alcohol (PVOH) to: a)

449 Water vapor; b) Limonene. The electrospun fibers were collected on the plate and mandrel.

450    **Table 1.** Optimal electrospinning conditions for poly(3-hydroxybutyrate) (PHB), polyvinyl alcohol (PVOH),

451    and polylactide (PLA).

| Biopolymer | Solution content (wt.-%) | Voltage (kV) | Flow-rate (ml/h) | Tip-to-collector distance (cm) | Time (h) |
|---|---|---|---|---|---|
| PHB | 10 | 15 | 6 | | 1-4 |
| PVOH | 12 | 18 | 1 | 15 | 1 |
| PLA | 5 | 18 | 2 | | 1 |

452

453    **Table 2.** Layer thickness of the mono- and multilayer structures based on paper, poly(3-hydroxybutyrate)

454    (PHB), polyvinyl alcohol (PVOH), and polylactide (PLA). Annealed films were obtained from electrospun

455    fibers collected on the plate.

| Structure | Layer thickness (μm) | | | | |
|---|---|---|---|---|---|
| | Paper | PVOH | PHB | PLA | Total |
| Paper | 125 ± 5 | | | | 125 ± 5 |
| PHB fibers | | | 258 ± 7 | | 258 ± 7 |
| PHB film | | | 38 ± 3 | | 38 ± 3 |
| Paper/PHB fibers | 125 ± 5 | | 269 ± 7 | | 394 ± 6 |
| Paper/PHB film | 125 ± 5 | | 37 ± 3 | | 162 ± 4 |
| Paper/PVOH film | 125 ± 5 | 10 ± 2 | | | 135 ± 3 |
| Paper/PLA film | 125 ± 5 | | | 7 ± 2 | 132 ± 3 |
| Paper/PVOH/PHB film | 125 ± 5 | 48 ± 3 | | | 173 ± 4 |
| Paper/PLA/PHB film | 125 ± 5 | | 45 ± 3 | | 170 ± 5 |

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457

22

458    **Table 3.** Layer thickness of the mono- and multilayer structures based on paper, poly(3-hydroxybutyrate)

459    (PHB), polyvinyl alcohol (PVOH), and polylactide (PLA). Annealed films were obtained from electrospun

460    fibers collected on the mandrel.

| Structure | Layer thickness (µm) | | | | |
|---|---|---|---|---|---|
| | Paper | PVOH | PHB | PLA | Total |
| Paper | 125 ± 5 | | | | 125 ± 5 |
| PHB fibers | | | 114 ± 4 | | 114 ± 4 |
| PHB film | | | 26 ± 3 | | 26 ± 3 |
| Paper/PHB fibers | 125 ± 5 | | 114 ± 4 | | 239 ± 6 |
| Paper/PHB film | 125 ± 5 | | 26 ± 3 | | 151 ± 5 |
| Paper/PVOH film | 125 ± 5 | 6 ± 2 | | | 131 ± 4 |
| Paper/PLA film | 125 ± 5 | | | 5 ± 2 | 130 ± 3 |
| Paper/PVOH/PHB film | 125 ± 5 | 32 ± 2 | | | 157 ± 2 |
| Paper/PLA/PHB film | 125 ± 5 | | 31 ± 3 | | 156 ± 2 |

461

Figure 1

Paper

Paper/PHB fibers

Paper/PVOH film

Paper/PVOH/PHB film

From random and aligned electrospun fibers

PHB fibers

Paper/PHB film

Paper/PLA film

Paper/PLA/PHB film

PHB film

Figure 2

Click here to download Figure Figure 2.tif ±

Figure 3

Figure 4

Figure 5

Paper

Paper/PVOH/PHB film

Paper/PVOH film

Paper/PHB fibers

PHB fibers

Paper/PLA/PHB film

Paper/PLA film

Paper/PHB film

FILM

FILM

PHB film

From random and aligned electrospun fibers

Figure 6

Figure 7

Figure 7

Figure 8

Figure 9