1 **POTENTIAL OF LIGNOCELLULOSIC FRACTIONS FROM *Posidonia***
2 ***oceanica* TO IMPROVE BARRIER AND MECHANICAL PROPERTIES OF**
3 **BIO-BASED PACKAGING MATERIALS**

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15 **Abstract**

16
17 This work reports on the valorization of residues from *Posidonia oceanica* leaves for
18 the purpose of obtaining lignocellulosic fractions of interest for the development of
19 bio-based materials for food packaging. The lignocellulosic fractions were
20 characterized, thereby confirming the purification of cellulose and showing increases
21 in crystallinity and thermal stability after the consecutive extraction steps.
22 Subsequently, pure lignocellulosic films were obtained and characterized and the pure
23 cellulose film showed the best properties in terms of mechanical performance and
24 water vapor permeability. Finally, composite starch films containing lignocellulosic
25 fractions were developed by melt compounding and characterized. Although the film
26 containing the pure cellulose additive showed the optimum improvement in terms of
27 mechanical properties (with an increase of 85% in the elastic modulus and 38% in the
28 tensile strength), similar water vapor permeability reduction (~40%) was achieved
29 with the least purified fractions, explained by their effect on starch gelatinization, as
30 evidenced by SAXS/WAXS.

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38 **Keywords:** *Posidonia oceanica*, lignocellulosic fractions, food packaging,
39 biopolymers, starch.

40   **1. Introduction**

41   Due to the steady depletion of fossil fuels and the growing concerns about the negative
42   impact of plastic materials on the environment, great efforts are currently being
43   focused on the development of bio-based polymers (also known as biopolymers or
44   bioplastics). This implies a transition from the utilization of conventional petroleum-
45   based resources to renewable resources and the application of circular economy
46   principles.

47   Amongst the range of bioplastics currently available, thermoplastic starch, obtained
48   from resources, such as corn and rice, has attracted a great deal of interest due to its
49   abundance, low cost and good processability. However, it presents several drawbacks,
50   such as low mechanical performance and high hygroscopicity when compared to the
51   benchmark synthetic polymers, as well as retrogradation upon storage. In this sense,
52   the incorporation of fillers has been proven to be an efficient strategy to enhance the
53   properties of bio-based polymers and the development of composite materials that are
54   competitive for their intended applications [1, 2]. Lignocellulosic biomass, such as
55   forestry and agro-industrial wastes [3], provide cheap feedstocks for the extraction of
56   different biopolymers (i.e. cellulose, lignin, hemicelluloses, etc.) which have a wide
57   range of industrial applications, such as: rheology modifiers, food additives, feedstock
58   for bioenergy production and fillers for biocomposite materials [4].

59   An additional drawback of starch as packaging material is related to the consumption
60   of food sources for its production. With the increasing rate of the world population,
61   the competition between forestry and farmlands and the current restriction laws against
62   the use for feed purposes of transgenic organisms, food sources must be primarily used
63   as food. An alternative to the use of land biomass is the utilization of marine resources,

64 such as algae and aquatic plants, which are available in large quantities. One of the
65 most abundant aquatic plant species in the Mediterranean Sea, is *Posidonia oceanica*.
66 During its lifecycle, *Posidonia* leaves detach off the stems and are transported towards
67 the sea shores by marine currents, hence generating a residue that affects the quality
68 of the beaches and, as a result, it is detrimental for tourism [5]. This also implies a
69 considerable disposal cost to local authorities, since these marine residues need to be
70 collected and they are usually disposed to landfill. Several studies have been carried
71 out for the valorization of *Posidonia* residues, with the potential use as a low-cost
72 adsorbent for the removal of dyes or phenol [6, 7], or for biofuel production [8, 9].
73 However, the presence of cell wall components limits the access of enzymes or
74 chemicals towards the cellulosic fraction of *Posidonia* and as a result, the conversion
75 rates are usually quite low. In a recent research work, *Posidonia* leaves have been used
76 to produce cellulose nanocrystals by acid hydrolysis [10], which were then
77 incorporated into poly-lactic acid (PLA) to produce nanocomposite films [11].
78 Although the cellulose nanocrystals presented high crystallinity and aspect ratio, they
79 had to be modified with a commercial surfactant in order to overcome their low
80 compatibility the PLA matrix, similarly to a previous work in which surface-modified
81 microcrystalline cellulose (MCC) was incorporated into PLA [12]. Furthermore, the
82 solvent casting method used to produce the materials would make industrial scaling
83 unfeasible.

84 In this work, valorizing the residues from *Posidonia* leaves as a natural source of
85 lignocellulosic materials is proposed. By using a sequential extraction method
86 different lignocellulosic fractions were generated and characterized. Subsequently,
87 their suitability as film-forming components was evaluated. Moreover, their

performance as fillers was investigated by incorporating the different fractions into starch films through the melt compounding method, partially replacing starch with marine biomass waste and obtaining materials with improved properties for their application in food packaging.

2. Materials and methods

2.1 Materials

2.1.1 Raw materials

Biomass waste material consisting of *Posidonia oceanica* leaves was collected directly from the shore in Calpe, Alicante (Spain) between February-March 2017. The material was washed vigorously with water in order to remove sand and salts. It was stored in the fridge until its use. Corn starch (27-28% amylose) was supplied by Roquette (Roquette Laisa España, Benifaio, Spain) and glycerol was purchased from Panreac Quimica, S.A. (Castellar Del Vallés, Barcelona, Spain).

2.1.2 Preparation of lignocellulosic fractions

A purification procedure previously applied to vegetal resources [13], was carried out to sequentially remove cell wall components and obtain different lignocellulosic fractions. Firstly, in order to remove wax, pigments and oils, 40 g of ground wet *Posidonia* leaves (approximately 4 g of dry material) were subjected to Soxhlet extraction with 800 ml of toluene/ethanol 2:1 (v/v) during 24 h. The resulting material was dried at room temperature overnight, constituting the first fraction (referred to as F1). Subsequently, in order to dissolve lignin, the de-waxed material was grounded into powder and treated for 5h at 70°C with 700 ml of 1.4 % NaClO₂ solution, having

the pH adjusted to 3 with acetic acid. After that, the reaction was stopped by quenching with ice, and the excess liquid was decanted. The yellow solid was collected and washed repeatedly with distilled water by vacuum filtration until the filtrate became approximately neutral, hence obtaining the second fraction (referred to as F2). The delignified material was then treated with 400 ml 5% KOH solution for 24 h at room temperature, followed by 2 h at 90°C, in order to remove the hemicelluloses, yielding pure cellulose (referred to as F3). A small amount of each fraction was subjected to freeze-drying for further analyses and the rest was stored in the fridge as a partially hydrated material.

2.1.3 Production of pure lignocellulosic films

Pure lignocellulosic films were producing by adding between 0.25-0.5 g of lignocellulosic materials (F1, F2 and F3) to 50 mL of distilled water and dispersing them by mild sonication, followed by ultra-turrax homogenization until homogeneous suspensions were obtained. These were then vacuum-filtered by using PTFE filters with 0,2 µm pore size to remove water. The solid material remaining in the filter was then dried at room temperature overnight. The formed films were peeled off the filters and stored at 0% RH.

2.1.4 Production of starch films loaded with lignocellulosic additives

Pure corn starch and composite films with 10 wt.-% (relative to the starch weight) of the three different lignocellulosic additives (F1, F2 and F3) were prepared. Corn starch and glycerol, as plasticizer, were dispersed in water by using a polymer:glycerol:water ratio of 1:0.3:0.5 (w/w/w).

136 For the composite films, the required amount of material (which was partially
137 hydrated) was also incorporated and mixed manually until a paste was made. This
138 paste was then melt-mixed in a Brabender Plastograph internal mixer at 130 °C and 60
139 rpm for 4 min. Subsequently, 4 g of the blends were spread evenly on a Teflon film
140 and placed in a compression mold (Carver 4122, USA) at a pressure of 16 tons and
141 130 °C for 2 min to form one film. The samples were coded as follows: Starch (pure
142 starch film), 10% F1 (starch + 10% F1), 10% F2 (starch + 10% F2) and 10% F3 (starch
143 + 10% F3).

144

145 **2.2 Chemical analysis**

146 The standard methods TAPPI T211 om-07, T222 om-06 and ASTM D1104-56 were
147 used to determine the ash, Klason lignin and holocellulose contents, respectively, in
148 the *Posidonia* biomass waste.

149

150 **2.3 Scanning electron microscopy (SEM)**

151 SEM was conducted on a Hitachi microscope (Hitachi S-4800) at an accelerating
152 voltage of 10 kV and a working distance between 8-16 mm. The freeze-dried powder
153 from the different fractions, the pure films plus its mixtures with corn starch were
154 sputtered with a gold–palladium mixture under vacuum before their morphology was
155 examined. In the case of the films, the samples were cryo-fractured after immersion in
156 liquid nitrogen in order to observe the cross-sections.

157

158 **2.4 Fourier Transform Infrared Spectroscopy (FT-IR)**

159 In order to examine the different lignocellulosic fractions by FT-IR, samples of ca. 1.2
160 mg were grounded and dispersed in 120 mg of spectroscopic grade KBr. A pellet was
161 then formed by compressing the sample with 10 tons. FT-IR experiments were
162 recorded in transmission mode in a controlled chamber at 21°C and dry air in order to
163 avoid humidity and CO₂ by using a Thermo Nicolet Nexus (GMI, USA) equipment.
164 The spectra were taken at 4 cm⁻¹ resolution in a wavelength range of 400-4000 cm⁻¹
165 and averaging a minimum of 32 scans.

166

167 **2.5 Small and Wide Angle X-ray scattering (SAXS/WAXS) and X-ray diffraction** 168 **(XRD)**

169 Combined small and wide angle X-ray scattering (SAXS and WAXS, respectively)
170 experiments were carried out in the Non Crystalline Diffraction beamline, BL-11, at
171 ALBA synchrotron light source (<http://www.albasynchrotron.es>) in order to
172 characterize the starch films, loaded with lignocellulosic fractions. The experimental
173 setup conditions, data reduction and WAXS data fitting procedures were the same as
174 described in a previous work ([14].

175 Additionally, XRD measurements of the freeze-dried lignocellulosic fractions were
176 carried out on a D5005 Bruker diffractometer. The instrument was equipped with a Cu
177 tube and a secondary monochromator. The configuration of the equipment was θ - 2θ ,
178 and the samples were examined over the angular range between 3°-60° with a step
179 size of 0.02° and a count time of 200 s per step. Peak fitting was carried out by using
180 the Igor software package (Wavemetrics, Lake Oswego, Oregon) as described in a
181 previous work [15]. The crystallinity index was determined by the method reported by
182 Wang et al. [16].

$$X_c(\%) = \frac{\sum A_{Crystal}}{A_{Total}} \times 100 \quad (1)$$

Where A_{Total} is the sum of the areas under all the diffraction peaks and $\sum A_{Crystal}$ is the sum of the areas corresponding to the three crystalline peaks from cellulose I. The crystallite sizes were estimated from the three different lattice planes of cellulose I β by employing the well-known Scherrer equation:

$$D_{(hkl)} = \frac{k \cdot \lambda}{B_{(hkl)} \cdot \cos \theta} \quad (2)$$

2.6 Thermogravimetric analyses (TGA)

Thermogravimetric curves (TG) were recorded with a Setaram Setsys 16/18 (SETARAM Instrumentation, France). The samples (ca. 10 mg) were heated from 30 to 1000°C with a heating rate of 10°C/min under nitrogen atmosphere. Derivative TG curves (DTG) express the weight loss rate as a function of temperature.

2.7 Water vapor permeability (WVP)

Direct permeability to water was determined from the slope of the weight gain versus time curves at 24°C. The films were sandwiched between the aluminum top (open O-ring) and bottom (deposit for the silica) parts of a specifically designed permeability cell with screws. A Viton rubber O-ring was placed between the film and bottom part of the cell in order to enhance sealability. These permeability cells containing silica were then placed in an equilibrated relative humidity cabinet at 75% RH and 25 °C. The weight gain through a film area of 0.001 m² was monitored and plotted as a function of time. Cells with aluminum films (with thickness of ca. 11 μm) were used

205 as control samples to estimate the weight gain through the sealing. The tests were done
206 at least in triplicate.

207

208 **2.8 Water uptake**

209 The water uptake was estimated from sorption experiments at 25°C and 75% RH by
210 means of weight gain by using a Precisa Gravimetrics AG SERIES 320XB analytical
211 balance (Dietikon, Switzerland). The assays were carried out at least in triplicate.

212

213 **2.9 Contact angle measurements**

214 Contact angle measurements were carried out at 23 ± 2 °C and ambient relative
215 humidity (ca. 60% RH) in a Video-Based Contact Angle Meter model OCA 20
216 (DataPhysics Instruments GmbH, Filderstadt, Germany). Contact angle values were
217 obtained by analyzing the shape of a distilled water drop after it had been placed over
218 the film for 15 s. Image analyses were carried out by using SCA20 software.

219

220 **2.10 Optical properties**

221 The transparency of the films was determined through the surface reflectance spectra
222 in a spectrophotometer CM-3600d (Minolta Co., Tokyo, Japan) with a 10 mm
223 illuminated sample area. Measurements were taken in duplicate for each sample by
224 using white and black backgrounds.

225 Film transparency was evaluated through the internal transmittance (T_i) (0-1,
226 theoretical range) by applying the Kubelka-Munk theory for multiple scattering to the
227 reflection data. The internal transmittance (T_i) of the films was quantified by using Eq.
228 (3). In this equation, R_0 is the reflectance of the film on an ideal black background.

Parameters a and b were calculated by using Eqs. (4) and (5), where R is the reflectance of the sample layer backed by a known reflectance R_g . Additionally, total color differences (ΔE) were calculated by using Eq. (6) being $L^*a^*b^*$ the color attributes obtained by a D65 illuminator and a 10° observer.

233

$$T_i = \sqrt{(a - R_0)^2 - b^2} \quad (3)$$

$$a = \frac{1}{2} \left(R + \frac{R_0 - R + R_g}{R_0 R_g} \right) \quad (4)$$

$$b = (a^2 - 1)^{\frac{1}{2}} \quad (5)$$

$$\Delta E^* = \sqrt{(\Delta a^*)^2 + (\Delta b^*)^2 + (\Delta L^*)^2} \quad (6)$$

238

2.11 Mechanical properties

Tensile tests were carried out at ambient conditions of typically 24 °C and 50 % RH on a Mecmesin MultiTest 1-i (1 kN) machine (Virginia, USA) with the Emperor™ software. Pre-conditioned rectangular-shaped specimens with initial gauge length of 8 cm and 1 cm in width were cut directly from the films. A fixed crosshead rate of 10 mm/min was utilized in all cases. Elastic Modulus (E), Tensile Strength (TS), and Elongation at Break (EAB) were determined from the stress-strain curves, estimated from force–distance data obtained for the different films. At least, three specimens of each film were tensile tested in order to obtain statistically meaningful results.

248

2.12 Statistics

250 All data have been represented as the average $\pm$ standard deviation. Different letters
251 show significant differences both in tables and graphs ($p \leq 0.05$). Analysis of variance
252 (ANOVA), followed by a Tukey-test, were used when comparing more than two data
253 sets.

254

255 **3. Results and discussion**

256 **3.1. Extraction and characterization of lignocellulosic fractions from *Posidonia*** 257 ***oceanica* residues**

258 The extraction of lignocellulosic fractions from residues of *Posidonia oceanica* leaves
259 by using the sequential process, described in section 2.1.2, was investigated. The raw
260 biomass material was determined to contain $13 \pm 2\%$ ash, $18 \pm 2\%$ lignin and $59 \pm 1\%$
261 holocellulose. These values are similar to those previously reported for *Posidonia*
262 leaves [10, 17]. The extraction yields for the different lignocellulosic fractions were
263 estimated to be $77 \pm 5\%$ for F1, $53 \pm 5\%$ for F2 and $24 \pm 3\%$ for F3. These yields are
264 quite consistent with the raw material composition and a good evidence of the
265 efficiency of the applied processes to sequentially purify lignocellulose, holocellulose
266 and cellulose fractions. The cellulose extraction yield obtained is higher than the
267 values reported for different seaweed species [18, 19], highlighting the potential of this
268 aquatic biomass source for the production of pure cellulose.

269 In order to investigate the structural changes undergone by the material after each
270 purification step and to assess the progressive removal of the cell wall components,
271 FT-IR analyses were carried out and the results are shown in Fig. 1A. As observed,
272 several characteristic bands, which appear in the raw biomass, are progressively
273 removed after the applied extraction processes. The first clear observation is that the

raw *Posidonia* biomass showed two peaks, located at 2918 and 2850 cm^{-1} , corresponding to the CH_2 asymmetrical and symmetrical stretching usually displayed in fatty acids [20], which were absent in the material after the Soxhlet treatment. This confirms the removal of lipidic compounds after this first purification step. It is also worth noting that the band centered at 1729 cm^{-1} , corresponding to the esters and acetyl groups from hemicelluloses and lignin [21], which overlapped with the one corresponding to bound water (located at ca. 1630 cm^{-1}) in the spectrum from the raw material, becomes visible in the F1 fraction. The intensity of this band was gradually reduced with the applied treatments and it was absent in the spectrum from the F3 fraction, confirming the fact that pure cellulose was obtained. The spectra from F3 was similar to those reported in the literature [13, 22, 23] for cellulose extracted from other resources, with characteristic bands, such as those located at 1430, 1375 and 1318 cm^{-1} corresponding to the CH_2 scissoring, C-H bending and CH_2 rocking, respectively [20], and the bands at 1165, 1061 and 1034 cm^{-1} , which are also seen in other carbohydrates arising from the highly coupled modes, corresponding to the C-C, C-O, C-H stretching and C-OH bending modes [24-26].

291

292 **Figure 1.** (A) FT-IR spectra of the residue from *Posidonia* leaves and the extracted
 293 lignocellulosic fractions. The most intense bands are marked by discontinuous lines.
 294 The spectrum from *Posidonia* has been offset for clarity. (B) XRD patterns of the
 295 freeze-dried samples.

296

297 Cellulose is the crystalline component providing integrity in plant cell walls, while
 298 components such as hemicelluloses and lignin provide the amorphous matrix needed
 299 to adapt the plant tissue properties to their specific requirements [27]. Thus, the
 300 progressive removal of lignin and hemicelluloses from the biomass is expected to yield
 301 a material with increased crystallinity. To corroborate this, the XRD patterns of the
 302 raw *Posidonia* biomass and the different lignocellulosic fractions were collected, and
 303 the results are displayed in Fig. 1B. The first clear observation is that the shape of the
 304 diffraction patterns was strongly affected by the purification processes. All the samples
 305 presented the three diffraction peaks located at ca. $2\theta = 15.0^\circ$, 16.6° and 22.7° ,

306 corresponding to the (1-10), (110) and (200) crystalline planes from the cellulose I_β
307 crystalline allomorph [28-30]. However, the relative intensity of these peaks was very
308 weak in the case of the native *Posidonia* biomass (where the first two peaks were
309 completely overlapped) and the F2 and F3 fractions. Furthermore, the *Posidonia* and
310 the F1 samples presented a peak located at $2\theta = 29.1^\circ$, which disappears after the
311 purification treatment with sodium chlorite. This peak has been detected in the XRD
312 patterns from residual lignin and was seen to disappear upon thermal treatment at
313 temperatures higher than 600°C [31], when lignin thermal degradation takes place (see
314 TGA results, Fig. 2).

315 The estimated crystallinity index and crystallite cross-sectional dimensions are
316 summarized in Table 1. As observed, upon applying the purification steps and
317 gradually removing the amorphous components, more crystalline materials were
318 obtained, reaching a final value of 51.4% crystallinity for the F3 fraction (i.e. pure
319 cellulose). This value is slightly higher than that previously reported for cellulose
320 extracted from *Posidonia* leaves [10] and very similar to the value obtained from other
321 marine sources such as the algae *Gelidium elegans* ($X_C \sim 51\%$) [19], while lower than
322 that from plant vegetal resources such as cotton ($X_C \sim 68\%$) or flax ($X_C \sim 70\%$) [32]. The
323 dimensions of the crystalline/paracrystalline regions, especially those in the direction
324 perpendicular to the well-resolved (200) peak, also increased with the purification
325 processes, which is a consequence of the removal of amorphous cell wall matrix
326 components.

327

Table 1. Crystallinity index (X_C) and cross-sectional dimensions of crystallite sizes in the direction perpendicular to the (1-10), (110) and (200) planes ($D_{(1-10)}$, $D_{(110)}$ and $D_{(200)}$), determined from the XRD patterns.

	<i>Posidonia</i>	F1	F2	F3
X_C (%)	17.3	39.1	34.8	51.4
D_{1-10} (nm)	---	2.7	2.2	3.0
D_{110} (nm)	---	2.3	2.6	1.9
D_{200} (nm)	2.6	3.1	3.1	4.1

Additionally, TGA characterization was carried out in order to determine the thermal stability of the raw *Posidonia* biomass and the extracted lignocellulosic fractions. The derivative thermogravimetric profiles obtained are displayed in Fig. 2. The results show evidence that the thermal stability of the different materials was related to their degree of purity and crystallinity, and the F3 being the most thermally stable fraction, whereas the native *Posidonia* biomass was the most thermally unstable. While F3 presented a one-step degradation profile, the other materials exhibited multiple-step degradation processes. It should be noted that all the fractions presented a small peak between ca. 90-100 °C, corresponding to the evaporation of the water held by the materials. The degradation process taking place at temperatures between 290 and 350°C, which was detected in all the samples, has been ascribed to cellulose degradation processes, such as depolymerization, dehydration and decomposition of glycosyl units [33-35]. The associated peak became sharper and shifted towards higher temperatures as the cell wall matrix components were removed and the cellulose was purified. F1 presented a second degradation step, occurring at temperatures between

400 and 460 °C, which has been previously attributed to the degradation of lignin [36-38]. Interestingly, after removing lignin from the material (obtaining F2), an additional peak appeared at around 260 °C. This peak might correspond to the degradation of hemicelluloses, which must have been released from the complex network provided by lignin after the sodium chlorite treatment. Finally, the F3 fraction mainly presented a narrow and defined peak corresponding to the degradation of cellulose at around 334°C. Thus, the TGA results, together with the FT-IR characterization (cf. Fig. 1A), confirm that the purification process yielded pure cellulose with high thermal stability, partly due to its relatively high crystallinity index. Another important implication is that the native *Posidonia* biomass as well as all the extracted fractions are thermally resistant enough to undergoing the typical temperatures applied during the processing of polymeric materials, such as starch without suffering thermal degradation.

Figure 2. Derivative thermogravimetric (DTG) curves of residues from *Posidonia* leaves and the extracted lignocellulosic fractions.

363

364 **3.2. Development of pure lignocellulosic films**

365 As previously mentioned, one of the aims of this work was to evaluate the suitability
366 of the *Posidonia* lignocellulosic fractions obtained for the development of
367 biopolymeric materials for packaging applications. For this purpose, the different
368 fractions were initially used to generate pure films by means of vacuum filtration and
369 the properties of the materials obtained were investigated.

370 Optical properties (including transparency and color) are important attributes in
371 packaging, especially in the case of food packaging, which is the intended application
372 of the materials developed in this work. As shown in Fig. S1A, the visual aspect of the
373 films obtained was substantially different, depending on the fraction utilized and it is
374 evident that the transparency increased along the purification process. While the F1
375 film appeared completely opaque and showed a brownish coloration, the F2 and F3
376 films were translucent and presented no evident coloration. The transparency was also
377 quantitatively assessed by means of internal transmittance (T_i) measurements. As
378 shown in Fig. S1B, the internal transmittance markedly increased as the lignin and
379 hemicelluloses were removed from the material, with the pure cellulose film (F3)
380 being the most transparent of all. Additionally, color differences (ΔE) between the
381 three different films were assessed. F1 presented the highest values according to its
382 brown tonality ($\Delta E_{F1-F3} = 42.58$ and $\Delta E_{F1-F2} = 31.91$), although F2 and F3 were also
383 significantly different between them ($\Delta E_{F2-F3} = 10.96$).

384 The morphology of the cryo-fractured cross-sections of the films was analyzed by
385 SEM. As evidenced in Fig. S1C, S1D and S1E, the F3 film presented the most compact
386 and uniform morphology. This is due to the presence of the amorphous components in

the F1 and F2 fractions, which are interspaced with the cellulose microfibrils and prevent the formation of a densely packed structure upon drying.

The mechanical performance of the different films was assessed by tensile testing and the calculated values for the Young's modulus (E), tensile strength and elongation at break (ϵ_b) are summarized in Table 2. As observed from the results presented in Table 2, the three parameters were significantly improved by purifying the cellulose fraction. The F1 film presented very poor mechanical performance and it was not possible to determine its properties since it broke down very quickly. In contrast, the mechanical properties of the F3 film, in terms of tensile strength and elongation at break, outperformed those reported on other biomass-derived cellulosic materials such as microfibrillated cellulose (MFC) extracted from carrot [39]. The increased Young's modulus and tensile strength, indicative of greater stiffness, are related to the higher crystallinity of F3 as compared with F2 (cf. Table 1). Interestingly, the F3 film also presented greater elongation at break than sample F2, indicating the fact that the materials also became more ductile with the purification of cellulose. The poorer mechanical performance of the F1 and F2 films may also be explained by the greater difficulty in obtaining homogeneous aqueous dispersions from these fractions due to the presence of cell wall matrix components interacting with the cellulose hydroxyl groups, which thus hindered their accessibility to water molecules [15]. It is worth highlighting the fact that the F3 film presented superior mechanical properties than a benchmark biopolymer, such as corn starch (cf. Table 3).

Table 2. Mechanical properties (Young's modulus, tensile strength and elongation at break) from the lignocellulosic films.

	E (MPa)	Tensile Strength (MPa)	ϵ_b (%)
F1	---	---	---
F2	771.5 ± 46.4^a	4.6 ± 0.1^a	1.1 ± 0.3^a
F3	4260.2 ± 416.2^b	45.9 ± 6.9^b	2.1 ± 0.4^b

411 Values in the same column followed by different letters are significantly different (p
412 ≤ 0.05).

413

414 Water vapour permeability (WVP) of the films was also measured and the results
415 obtained are shown in Fig. 3A. The results show evidence that the permeability of the
416 films strongly decreased as the fractions produced were progressively purified, with
417 74% and 91% WVP decreases for the F2 and F3 films, respectively with regards to the
418 F1 film. This may be a consequence of the more densely packed structure attained with
419 the F3 fraction (cf. Fig. 3E) and its higher crystallinity index (cf. Table 1), hence
420 blocking more efficiently the transport of water vapour through the film. The WPV
421 value for the F3 film ($1.25 \cdot 10^{-13}$ Kg·m/s·m²·Pa) is even lower than those reported for
422 films from regenerated cellulose and nanocellulose (ca. $2.5 \cdot 10^{-13}$ Kg·m/s·m²·Pa,
423 measured at >25 %RH) [40] and for corn starch ($2.59 \cdot 10^{-13}$ Kg·m/s·m²·Pa, cf. Fig. 4),
424 highlighting the promising characteristics of the generated material in terms of water
425 vapour barrier.

426

427 As water permeability is due to the combination of sorption and diffusion phenomena,
428 the susceptibility of the different materials to sorb water was evaluated through
429 gravimetric tests and the results, expressed as weight gained per exposed film surface

area, are displayed in Fig. 3B. As observed in the graph, the F1 film presented the highest water sorption value, suggesting that this material presented the most hygroscopic behavior. On the contrary, F3 presented the least water uptake value. This is again, related to the more crystalline structure of F3 (since crystalline regions are less accessible to moisture), but may also be explained by the strong self-association of cellulose microfibrils upon drying, thus limiting the amount of free hydroxyl groups available for water to interact with the cellulose composing the film [41].

437

438

439 **Figure 3.** (A) Water vapor permeability and (B) water sorption for the pure
440 lignocellulosic films. Values with different letters are significantly different ($p \leq 0.05$).
441

442 **3.3. Development of starch films loaded with lignocellulosic fractions**

443 After characterizing the different extracted fractions and the pure films, the next step
444 was to incorporate the lignocellulosic additives into starch matrices in order to produce
445 composite films. The aim was to reduce the amount of starch while improving the
446 properties of the materials obtained. Corn starch films containing 10 wt.-% of
447 lignocellulosic additives were produced by means of the melt compounding method,
448 followed by compression molding and the properties of the materials obtained were
449 evaluated. The additive concentration was selected based on the previous tests
450 performed on corn starch films with cellulosic additives, which presented optimum
451 properties with loadings close to 10 wt.-% [42].

452 The effect of the incorporation of the lignocellulosic additives on the transparency of
453 the starch films was evaluated through their transmittance values, (cf. Fig. S2). It can
454 be observed that while the addition of F1 caused a remarkable decrease in the
455 transmittance (i.e. the transparency of the pure starch film was reduced), F2 and F3
456 fractions had minor impact on the film transparency. Additionally, the total color
457 differences (ΔE) between the three different composite films and the control starch
458 film, were measured. The results ($\Delta E_{F1-Starch} = 43.15$, $\Delta E_{F2-Starch} = 12.10$ and $\Delta E_{F3-Starch}$
459 $= 16.25$), indicate that the film containing F1 presented the highest color difference,
460 due to its brown tonality.

461 The morphology of the pure starch and composite films as well as the degree of
462 dispersion of the incorporated fractions were analyzed by observation of the films'
463 cryo-fractured cross-sections by means of SEM. As observed in the representative

464 micrographs shown in Fig. S3, the presence of the lignocellulosic fractions was
465 pertinent in all the composite materials, which showed large particles evenly
466 distributed across the whole cross-sections, as opposed to the smooth surface observed
467 in the control starch film. Although the size of the aggregates seemed to be smaller for
468 the film containing F3 (i.e. pure cellulose), an adequate filler dispersion was not
469 attained for any of the samples, which is a consequence of the strong self-aggregation
470 phenomenon typically undergone by lignocellulosic materials when present in the dry
471 state [43].

472 Mechanical properties of the pure starch and the three composites were evaluated, and
473 the results are summarized in Table 3. From the results it was observed that the
474 incorporation of F1 and F2 fractions did not significantly improve the mechanical
475 properties of starch and moreover, the elongation at break was reduced. This indicates
476 that the materials became less ductile with the addition of those two lignocellulosic
477 additives, just as it had been previously described for PLA films loaded with cellulose
478 nanocrystals extracted from *Posidonia* [44]. This effect is believed to be a consequence
479 of the presence of large and stiff particles of lignocellulosic additives, disrupting the
480 starch matrix and impairing the plastic mechanical performance of the material. In
481 contrast, the addition of F3 significantly increased the elastic modulus (85% increase
482 when compared to the pure starch), while the elongation at break was not further
483 reduced if compared to the composite containing F2. It is also evident from the results
484 that, as the cellulose was sequentially purified, the additives conferred better
485 mechanical properties (i.e. higher elastic modulus, tensile strength and elongation at
486 break). Therefore, F3 was the best additive in terms of mechanical performance.
487 Although direct comparisons with other works are not straightforward considering the

variability in properties derived from starch source and processing conditions, our results are rather promising since relatively significant improvements have been observed when compared with previous works. For instance, F3 films presented a lower decrease in elongation at break than composite starch films loaded with 3% (dry weight) cellulose nanocrystals from sugarcane bagasse (33% vs. 79% drop) [45] without a significant improvement in the tensile strength values (Table 3).

Table 3. Mechanical properties (Young's modulus, tensile strength and elongation at break) of the pure starch and composite films.

	E (MPa)	Tensile Strength (MPa)	ϵ_b (%)
Starch	1039.8 ± 88.0^b	12.8 ± 1.2^{ab}	2.1 ± 0.2^a
10% F1	1120.9 ± 132.0^b	6.0 ± 2.7^c	0.9 ± 0.2^c
10% F2	1233.7 ± 79.4^b	11.2 ± 2.8^{bc}	1.4 ± 0.2^b
10% F3	1919.9 ± 96.1^a	17.7 ± 1.1^a	1.4 ± 0.2^b

Values in the same column with different letters are significantly different ($p \leq 0.05$).

WVP was also measured and the results are shown in Fig. 4A. The incorporation of the three types of lignocellulosic additives led to improved water vapor permeability when compared to the control starch film (ca. 39%, 38% and 43% WVP decrease with the incorporation of F1, F2 and F3, respectively), with no significant differences between them. This marked decrease in the WVP is due to a combination of factors: (i) the incorporation of fillers with a more hydrophobic behavior than the native starch and (ii) a decrease in the hydroxyl groups available for interacting with water as a

consequence of the strong intermolecular hydrogen bonds established between starch and the lignocellulosic materials [46]. Similar WVP reductions (ca. 52%, measured at 100% RH), have been reported for corn starch films loaded with 10% and 20% bacterial cellulose nanowhiskers [42]. Therefore, and from a cost perspective, F1 would be the best additive choice in terms of WVP properties, since it leads to optimum barrier enhancement with a minimal number of processing steps.

Water sorption and contact angle measurements were additionally carried out in order to evaluate the effect of the different lignocellulosic fractions on the water affinity of the films. From the water sorption results (cf. Fig. 4B), it is clear that the addition of the lignocellulosic fractions led to a decrease in the water uptake, having a maximum decrease of ca. 44% with the addition of pure cellulose. The more hydrophobic behavior of the 10% F3 film was also confirmed by the contact angle measurements (cf. Fig. 4C). The calculated contact angle tended to increase (i.e. the materials became more hydrophobic) with the progressive purification of the added fractions (cf. Fig. S3). However, only the 10% F3 film presented a significant increase in the contact angle (65°) when compared with the pure starch (47°). These results suggest that the pure cellulose presents a more hydrophobic behavior than the unpurified fractions, judging from its greater crystallinity and strong self-association, as supported by the water permeability and water sorption results, previously obtained for the pure films.

Figure 4. (A) Water vapor permeability and (B) water uptake of the pure starch and composite films. Values with different letters are significantly different ($p \leq 0.05$). (C) Images of water drops taken on the different film surfaces.

534 The fact that F3 was not able to reduce the water permeability of starch to a greater
535 extent than the F1 and F2 fractions, despite its more hydrophobic behavior, can be
536 attributed to the different effect of the lignin, the hemicelluloses and the cellulose on
537 the gelatinization and retrogradation processes undergone by starch during the melt
538 mixing processing and subsequent storage of the films. In order to investigate the
539 structure of the different materials, X-ray scattering experiments were carried out and
540 the WAXS and SAXS patterns obtained are shown in Fig. 5A and 5B, respectively.
541 From the WAXS patterns (cf. Fig. 5A), it can be observed that the corn starch
542 presented a V-type crystalline structure, in particular, the V_H type. This transition from
543 the original A-type structure in corn starch to the V-type structure, has been previously
544 reported to take place as a result of the melt mixing process at temperatures lower than
545 180°C and relatively high water contents [14] The crystallinity values calculated were:
546 $26.2 \pm 0.1\%$ for the pure starch, $33.0 \pm 0.1\%$ for the film loaded with 10% F1, $28.8 \pm$
547 0.1% for the 10% F2 composite and $31.1 \pm 0.1\%$ for the 10% F3 composite. Thus, the
548 incorporation of the lignocellulosic fractions led to a slight increase on the crystallinity
549 of the films and this effect is similar for the three fractions in spite of the higher
550 crystallinity of the pure cellulose (i.e. F3). As observed in Fig. 5B, the SAXS patterns
551 from the pure starch and composite films display a broad shoulder which is centred at
552 $ca. 0.06 \text{ \AA}^{-1}$, corresponding to a real distance of $ca. 10.5 \text{ nm}$. This scattering feature
553 has been previously reported for extruded starches and is characteristic of the
554 heterogeneous semicrystalline structure into which starch re-organises after the
555 extrusion process [14, 47, 48]. Within the low q region, the intensity presents a power-
556 law behaviour, with an exponent of $ca. -1.5$ for pure starch. This value is much lower
557 than those typically reported for native starches (ranging between $ca. -4$ to -3 [47, 48],

558 reflecting the disruption of the starch lamellar structure during extrusion. Higher
 559 power-law exponents of -3.7, -2.8 and -2.7 are observed for the 10% F1, 10% F2 and
 560 10% F3 films, respectively. The lower deviation of the composite films from the -4
 561 slope (corresponding to the Porod's ideal two-phase system) may be indicative of an
 562 effect of the lignocellulosic fractions, especially the less purified F1 fraction, in
 563 preventing to some extent the disruption of the starch lamellar structures. This
 564 hypothesis is supported by the greater crystallinity values estimated for the composite
 565 films and may explain the water permeability results. The impurities present in the F1
 566 fraction seem to have preserved the structure of starch during the melt mixing process
 567 to a higher extent than the pure cellulose. The more ordered structure in the 10% F1
 568 sample is expected to result in a lower diffusion of water vapour molecules through
 569 the film, hence leading to permeability reductions greater than expected.

570

571

Figure 5. (A) WAXS and (B) SAXS patterns of the pure starch and its composite films.

4. Conclusions

Three different lignocellulosic fractions (F1, F2 and F3) were obtained from the residues of *Posidonia oceanica* leaves by sequentially removing the cell wall components. The extraction treatments applied proved to be efficient in removing components, such as lignin and hemicelluloses, thereby obtaining a final fraction F3 with a yield of ca. 24%, which consisted of pure cellulose. The crystallinity and thermal stability of the lignocellulosic fractions increased progressively with the extraction processes, and hence obtaining pure cellulose with a crystallinity index of ca. 51% and thermally stable at temperatures at around 250 °C. The three lignocellulosic fractions were subsequently utilized either as base materials or as additives to generate films.

587 The removal of the lignin and hemicellulose components, yielded a consistent
588 improvement in the transparency, mechanical and water vapor barrier properties of the
589 lignocellulosic films. The pure cellulose film obtained presented promising properties
590 for its application in food packaging, showing better mechanical and water barrier
591 performance than a commercial corn starch film.

592 Regarding the composite starch films prepared by melt mixing, incorporation of 10
593 wt.-% of the three lignocellulosic additives led to a drop between 30-40% in the water
594 vapor permeability with respect to the control starch. In terms of the mechanical
595 performance, the addition of pure cellulose was optimal since it was able to increase
596 the stiffness of the material (with an increase of ca. 85% in the elastic modulus), while
597 preventing an excessive reduction of its ductility.

598 These results demonstrate the potential of lignocellulosic fractions extracted from the
599 residues of *Posidonia* leaves for the development of sustainable bio-based materials
600 for food packaging applications, while providing an added value to the unutilized
601 waste material, either by producing pure lignocellulosic films with superior
602 mechanical and water barrier performance or by using them as additives to produce
603 starch composite films with improved properties.

604

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610

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 765

766 Supplementary Material

770 **Figure S1.** (A) Visual aspect and (B) spectral distribution of internal transmittance
771 (Ti) of the lignocellulosic films. SEM images from the cryo-fractured cross-sections
772 of the lignocellulosic films: (C) F1 film, (D) F2 film and (E) F3 film. Scale markers
773 correspond to 300 μ m (C) and 100 μ m (D and E).

774

775

776 **Figure S2.** Spectral distribution of internal transmittance (Ti) of the pure corn starch
777 and composite films.

778
779 **Figure S3.** SEM images of the cryo-fractured sections from the corn starch film (A),
780 10% F1 (B), 10% F2 (C) and 10% F3 (D). Scale markers correspond to 100 μm.

781
782 **Figure S4.** Contact angle values for the pure corn starch and composite films. Values
783 in the same column followed by different letters are significantly different ($p \leq 0.05$).