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

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Abstract

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

Keywords: *Posidonia oceanica*, lignocellulosic fractions, food packaging, biopolymers, starch.
1. Introduction

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

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

An additional drawback of starch as packaging material is related to the consumption of food sources for its production. With the increasing rate of the world population, the competition between forestry and farmlands and the current restriction laws against the use for feed purposes of transgenic organisms, food sources must be primarily used as food. An alternative to the use of land biomass is the utilization of marine resources,
such as algae and aquatic plants, which are available in large quantities. One of the most abundant aquatic plant species in the Mediterranean Sea, is *Posidonia oceanica*. During its lifecycle, *Posidonia* leaves detach off the stems and are transported towards the sea shores by marine currents, hence generating a residue that affects the quality of the beaches and, as a result, it is detrimental for tourism [5]. This also implies a considerable disposal cost to local authorities, since these marine residues need to be collected and they are usually disposed to landfill. Several studies have been carried out for the valorization of *Posidonia* residues, with the potential use as a low-cost adsorbent for the removal of dyes or phenol [6, 7], or for biofuel production [8, 9]. However, the presence of cell wall components limits the access of enzymes or chemicals towards the cellulosic fraction of *Posidonia* and as a result, the conversion rates are usually quite low. In a recent research work, *Posidonia* leaves have been used to produce cellulose nanocrystals by acid hydrolysis [10], which were then incorporated into poly-lactic acid (PLA) to produce nanocomposite films [11]. Although the cellulose nanocrystals presented high crystallinity and aspect ratio, they had to be modified with a commercial surfactant in order to overcome their low compatibility the PLA matrix, similarly to a previous work in which surface-modified microcrystalline cellulose (MCC) was incorporated into PLA [12]. Furthermore, the solvent casting method used to produce the materials would make industrial scaling unfeasible.

In this work, valorizing the residues from *Posidonia* leaves as a natural source of lignocellulosic materials is proposed. By using a sequential extraction method different lignocellulosic fractions were generated and characterized. Subsequently, 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 90ºC, 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).
For the composite films, the required amount of material (which was partially hydrated) was also incorporated and mixed manually until a paste was made. This paste was then melt-mixed in a Brabender Plastograph internal mixer at 130 °C and 60 rpm for 4 min. Subsequently, 4 g of the blends were spread evenly on a Teflon film and placed in a compression mold (Carver 4122, USA) at a pressure of 16 tons and 130 °C for 2 min to form one film. The samples were coded as follows: Starch (pure starch film), 10% F1 (starch + 10% F1), 10% F2 (starch + 10% F2) and 10% F3 (starch + 10% F3).

2.2 Chemical analysis

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

2.3 Scanning electron microscopy (SEM)

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

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

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

Combined small and wide angle X-ray scattering (SAXS and WAXS, respectively) experiments were carried out in the Non Crystalline Diffraction beamline, BL-11, at ALBA synchrotron light source (http://www.albasynchrotron.es) in order to characterize the starch films, loaded with lignocellulosic fractions. The experimental setup conditions, data reduction and WAXS data fitting procedures were the same as described in a previous work ([14]). Additionally, XRD measurements of the freeze-dried lignocellulosic fractions were carried out on a D5005 Bruker diffractometer. The instrument was equipped with a Cu tube and a secondary monochromator. The configuration of the equipment was 0–20, and the samples were examined over the angular range between 3°–60° with a step size of 0.02° and a count time of 200 s per step. Peak fitting was carried out by using the Igor software package (Wavemetrics, Lake Oswego, Oregon) as described in a previous work [15]. The crystallinity index was determined by the method reported by Wang et al. [16].
Where $A_{Total}$ is the sum of the areas under all the diffraction peaks and $\Sigma A_{Crystal}$ 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 Iβ by employing the well-known Scherrer equation:

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

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 1000ºC with a heating rate of 10ºC/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 24ºC. 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 ºC. 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
as control samples to estimate the weight gain through the sealing. The tests were done at least in triplicate.

2.8 Water uptake

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

2.9 Contact angle measurements

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

2.10 Optical properties

The transparency of the films was determined through the surface reflectance spectra in a spectrocolorimeter CM-3600d (Minolta Co., Tokyo, Japan) with a 10 mm illuminated sample area. Measurements were taken in duplicate for each sample by using white and black backgrounds. Film transparency was evaluated through the internal transmittance \((T_i)\) (0-1, theoretical range) by applying the Kubelka-Munk theory for multiple scattering to the reflection data. The internal transmittance \((T_i)\) of the films was quantified by using Eq. (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 ($\Delta E$) were calculated by using Eq. (6) being $L*a*b*$ the color attributes obtained by a D65 illuminator and a $10^\circ$ observer.

\[ T_i = \sqrt{(a - R_0)^2 - b^2} \]  
\[ a = \frac{1}{2} \left( R + \frac{R_0 - R + R_g}{R_0 R_g} \right) \]  
\[ b = (a^2 - 1)^{\frac{1}{2}} \]  
\[ \Delta E^* = \sqrt{(\Delta a^*^2) + (\Delta b^*^2) + (\Delta L^*^2)} \]

2.11 Mechanical properties

Tensile tests were carried out at ambient conditions of typically 24 ºC and 50 % RH on a Mecmesin MultiTest 1-i (1 kN) machine (Virginia, USA) with the Emperor\textsuperscript{TM} 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.

2.12 Statistics
All data have been represented as the average ± standard deviation. Different letters show significant differences both in tables and graphs \((p\leq0.05)\). Analysis of variance (ANOVA), followed by a Tukey-test, were used when comparing more than two data sets.

3. Results and discussion

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

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

In order to investigate the structural changes undergone by the material after each purification step and to assess the progressive removal of the cell wall components, FT-IR analyses were carried out and the results are shown in Fig. 1A. As observed, several characteristic bands, which appear in the raw biomass, are progressively 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].
Figure 1. (A) FT-IR spectra of the residue from *Posidonia* leaves and the extracted lignocellulosic fractions. The most intense bands are marked by discontinuous lines. The spectrum from *Posidonia* has been offset for clarity. (B) XRD patterns of the freeze-dried samples.

Cellulose is the crystalline component providing integrity in plant cell walls, while components such as hemicelluloses and lignin provide the amorphous matrix needed to adapt the plant tissue properties to their specific requirements [27]. Thus, the progressive removal of lignin and hemicelluloses from the biomass is expected to yield a material with increased crystallinity. To corroborate this, the XRD patterns of the raw *Posidonia* biomass and the different lignocellulosic fractions were collected, and the results are displayed in Fig. 1B. The first clear observation is that the shape of the diffraction patterns was strongly affected by the purification processes. All the samples presented the three diffraction peaks located at ca. $2\theta = 15.0^\circ$, $16.6^\circ$ and $22.7^\circ$, ...
corresponding to the (1-10), (110) and (200) crystalline planes from the cellulose Iβ crystalline allomorph [28-30]. However, the relative intensity of these peaks was very weak in the case of the native Posidonia biomass (where the first two peaks were completely overlapped) and the F2 and F3 fractions. Furthermore, the Posidonia and the F1 samples presented a peak located at $2\theta = 29.1^\circ$, which disappears after the purification treatment with sodium chlorite. This peak has been detected in the XRD patterns from residual lignin and was seen to disappear upon thermal treatment at temperatures higher than 600ºC [31], when lignin thermal degradation takes place (see TGA results, Fig. 2).

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

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

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.
3.2. Development of pure lignocellulosic films

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

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

The morphology of the cryo-fractured cross-sections of the films was analyzed by SEM. As evidenced in Fig. S1C, S1D and S1E, the F3 film presented the most compact 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 ($\varepsilon_b$) 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.
<table>
<thead>
<tr>
<th></th>
<th>E (MPa)</th>
<th>Tensile Strength (MPa)</th>
<th>εb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>F2</td>
<td>771.5 ± 46.4a</td>
<td>4.6 ± 0.1a</td>
<td>1.1 ± 0.3a</td>
</tr>
<tr>
<td>F3</td>
<td>4260.2 ± 416.2b</td>
<td>45.9 ± 6.9b</td>
<td>2.1 ± 0.4b</td>
</tr>
</tbody>
</table>

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

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

As water permeability is due to the combination of sorption and diffusion phenomena, the susceptibility of the different materials to sorb water was evaluated through gravimetrical 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].
Figure 3. (A) Water vapor permeability and (B) water sorption for the pure lignocellulosic films. Values with different letters are significantly different (p ≤0.05).

3.3. Development of starch films loaded with lignocellulosic fractions

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

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

The morphology of the pure starch and composite films as well as the degree of dispersion of the incorporated fractions were analyzed by observation of the films’ cryo-fractured cross-sections by means of SEM. As observed in the representative
micrographs shown in Fig. S3, the presence of the lignocellulosic fractions was pertinent in all the composite materials, which showed large particles evenly distributed across the whole cross-sections, as opposed to the smooth surface observed in the control starch film. Although the size of the aggregates seemed to be smaller for the film containing F3 (i.e. pure cellulose), an adequate filler dispersion was not attained for any of the samples, which is a consequence of the strong self-aggregation phenomenon typically undergone by lignocellulosic materials when present in the dry state [43].

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

<table>
<thead>
<tr>
<th></th>
<th>E (MPa)</th>
<th>Tensile Strength (MPa)</th>
<th>εb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>1039.8 ± 88.0b</td>
<td>12.8 ± 1.2ab</td>
<td>2.1 ± 0.2a</td>
</tr>
<tr>
<td>10% F1</td>
<td>1120.9 ± 132.0b</td>
<td>6.0 ± 2.7c</td>
<td>0.9 ± 0.2c</td>
</tr>
<tr>
<td>10% F2</td>
<td>1233.7 ± 79.4b</td>
<td>11.2 ± 2.8bc</td>
<td>1.4 ± 0.2b</td>
</tr>
<tr>
<td>10% F3</td>
<td>1919.9 ± 96.1a</td>
<td>17.7 ± 1.1a</td>
<td>1.4 ± 0.2b</td>
</tr>
</tbody>
</table>

Values in the same column with different letters are significantly different (p≤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 ≤0.05). (C) Images of water drops taken on the different film surfaces.
The fact that F3 was not able to reduce the water permeability of starch to a greater extent than the F1 and F2 fractions, despite its more hydrophobic behavior, can be attributed to the different effect of the lignin, the hemicelluloses and the cellulose on the gelatinization and retrogradation processes undergone by starch during the melt mixing processing and subsequent storage of the films. In order to investigate the structure of the different materials, X-ray scattering experiments were carried out and the WAXS and SAXS patterns obtained are shown in Fig. 5A and 5B, respectively. From the WAXS patterns (cf. Fig. 5A), it can be observed that the corn starch presented a V-type crystalline structure, in particular, the V_H type. This transition from the original A-type structure in corn starch to the V-type structure, has been previously reported to take place as a result of the melt mixing process at temperatures lower than 180°C and relatively high water contents [14] The crystallinity values calculated were: 26.2 ± 0.1% for the pure starch, 33.0 ± 0.1% for the film loaded with 10% F1, 28.8 ± 0.1% for the 10% F2 composite and 31.1 ± 0.1% for the 10% F3 composite. Thus, the incorporation of the lignocellulosic fractions led to a slight increase on the crystallinity of the films and this effect is similar for the three fractions in spite of the higher crystallinity of the pure cellulose (i.e. F3). As observed in Fig. 5B, the SAXS patterns from the pure starch and composite films display a broad shoulder which is centred at ca. 0.06 Å⁻¹, corresponding to a real distance of ca. 10.5 nm. This scattering feature has been previously reported for extruded starches and is characteristic of the heterogeneous semicrystalline structure into which starch re-organises after the extrusion process [14, 47, 48]. Within the low q region, the intensity presents a power-law behaviour, with an exponent of ca. -1.5 for pure starch. This value is much lower than those typically reported for native starches (ranging between ca. -4 to -3 [47, 48],
reflecting the disruption of the starch lamellar structure during extrusion. Higher power-law exponents of -3.7, -2.8 and -2.7 are observed for the 10% F1, 10% F2 and 10% F3 films, respectively. The lower deviation of the composite films from the -4 slope (corresponding to the Porod’s ideal two-phase system) may be indicative of an effect of the lignocellulosic fractions, especially the less purified F1 fraction, in preventing to some extent the disruption of the starch lamellar structures. This hypothesis is supported by the greater crystallinity values estimated for the composite films and may explain the water permeability results. The impurities present in the F1 fraction seem to have preserved the structure of starch during the melt mixing process to a higher extent than the pure cellulose. The more ordered structure in the 10% F1 sample is expected to result in a lower diffusion of water vapour molecules through the film, hence leading to permeability reductions greater than expected.
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.
The removal of the lignin and hemicellulose components, yielded a consistent improvement in the transparency, mechanical and water vapor barrier properties of the lignocellulosic films. The pure cellulose film obtained presented promising properties for its application in food packaging, showing better mechanical and water barrier performance than a commercial corn starch film.

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

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

**Acknowledgements**

Synchrotron experiments were performed at NCD beamline at ALBA Synchrotron with the collaboration of ALBA staff (2017022002 project). Marta Martinez-Sanz is recipient of a Juan de la Cierva (IJCI-2015-23389) contract from the Spanish Ministry of Economy, Industry and Competitiveness.
References


N. Abidi, L. Cabrales, C.H. Haigler, Changes in the cell wall and cellulose content of developing cotton fibers investigated by FTIR spectroscopy, Carbohydrate Polymers 100 (2014) 9-16.


P. Satyamurthy, P. Jain, R.H. Balasubramanya, N. Vigneshwaran, Preparation and characterization of cellulose nanowhiskers from cotton fibres by controlled microbial hydrolysis, Carbohydrate Polymers 83(1) (2011) 122-129.


Supplementary Material

A

B

C

D

E

33
**Figure S1.** (A) Visual aspect and (B) spectral distribution of internal transmittance (Ti) of the lignocellulosic films. SEM images from the cryo-fractured cross-sections of the lignocellulosic films: (C) F1 film, (D) F2 film and (E) F3 film. Scale markers correspond to 300µm (C) and 100µm (D and E).

![Graph showing spectral distribution of internal transmittance (Ti) for starch and composite films with different F1, F2, and F3 concentrations.](image)

**Figure S2.** Spectral distribution of internal transmittance (Ti) of the pure corn starch and composite films.
Figure S3. SEM images of the cryo-fractured sections from the corn starch film (A), 10% F1 (B), 10% F2 (C) and 10% F3 (D). Scale markers correspond to 100 µm.

Figure S4. Contact angle values for the pure corn starch and composite films. Values in the same column followed by different letters are significantly different (p ≤0.05).