

Subcritical water extraction for recovering cellulose fibres from *Posidonia oceanica* waste

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Abstract

Posidonia oceanica (PO) is an abundant aquatic plant in the Mediterranean Sea that produces a great accumulation of leaves on the coast when detaching off the plant. PO waste collected at landfills could be used as a source of cellulose due to the considerable content of this component (30–40%). In this study, cellulose extraction from PO waste was studied in order to reduce chemicals in the process, in line with the green chemistry principles. Thus, subcritical water extraction (SWE) was applied to promote the separation of non-cellulosic compounds, such as hemicellulose and lignin, followed by bleaching treatments using hydrogen peroxide, alternatively to the usual sodium chlorite. Two SWE temperatures (150 and 170 °C) were tested, while hydrogen peroxide was used at 4 and 8% (v/v) at pH 12 in four one-hour bleaching cycles. This treatment was also carried out with sodium chlorite for comparison purposes. SWE efficiently reduced hemicellulose and lignin content in the solid extraction fraction, mainly at 170 °C, which yielded 63 wt. % of solid fraction, with 51% of cellulose content. This highest temperature also promoted the efficiency of the subsequent bleaching step. Using H₂O₂ as the bleaching agent, alternatively to chlorine agents, was effective at purifying cellulose but partially altered the cellulose structure through oxidative mechanisms. A combination of SWE at 170 °C and bleaching with H₂O₂ at 4 or 8% (v/v) yielded 24 wt. % bleached material from PO waste, with a high cellulose richness (near 90%).

1. Introduction

Posidonia oceanica (PO) is the most abundant aquatic plant species in the Mediterranean Sea, and forms wide meadows, covering approximately 40000 Km² of the seabed (Cebrian and Duarte 2001). During the PO lifecycle (starting from September to October), PO leaves and part of the rhizomes detach off the plant and reach the beaches, generating a residue (Cocozza et al. 2011). This waste represents an environmental and socioeconomic problem for the potential use of the coast for recreational and touristy purposes (Duarte et al. 2004).

PO waste, which consists of partially decomposed leaves, rhizomes, and balls of fibrous materials, accumulates in coastal areas and needs to be removed and dumped in landfills, which generates a considerable cost and loss of organic matter. PO leaves have a cellulose content that ranges, depending on the season, from 31 to 40%, whereas the hemicellulose and lignin content are in the range of 21–35% and 26–30%, respectively (Ncibi et al. 2009; Khiari et al. 2010; Bettaieb et al. 2015; Benito-González et al. 2019). Thus, PO waste represents a low-cost source of valuable components, such as cellulose, for further valorisation.

Cellulose contains hundreds to over ten thousand β-1,4 linked D-glucose monomer units in the form of unbranched straight chains, known as micro-fibrils (present in crystalline and amorphous form), interconnected with van der Waals forces and hydrogen bonds and entangled with hemicellulose and lignin in the cellular structures (Gupta et al. 2019). Hemicellulose is more easily removed from lignocellulosic biomass due to its branched and irregular structure compared to other biopolymers and,

therefore, relatively mild treatments such as hot water extraction may allow its removal (Zhuang et al. 2016; Verdini et al. 2021). In contrast, lignin resists this kind of treatment, and stronger chemical treatments are required to separate it from cellulose (Bhatia et al. 2020). Among these treatments, alkaline delignification is the most used (Kim et al. 2016). Additionally, bleaching treatments based on the use of oxidants such as sodium or potassium chlorite are applied to eliminate residual lignin while enhancing the hydrolysis of the remaining hemicelluloses, thus increasing the degree of whiteness and purity of the obtained cellulose fibres. These treatments generate toxic effluents that are corrosive and demand amounts of water to clean the fibres (Boonterm et al. 2016). Moreover, when lignin reacts with the elemental chlorine generated, it is transformed into the water-soluble fractionated chloro-lignin, which gives an intense colour to the obtained effluent. This waste is very recalcitrant to biodegradation, which implies severe problems for aquatic flora and fauna. Given the problems concerning chlorine-based bleaching processes, there is a tendency to replace them with more environmentally friendly treatments, partially or totally chlorine-free (Kaur et al. 2018), and sustainable cellulose recovery from lignocellulose residues is a challenge. Alternative oxidizing agents, such as ozone, which can break down the aromatic structure of lignin without affecting cellulose, have been exploited as biomass pretreatment at ambient temperatures. However, ozonolysis is not the most convenient method since it requires huge amounts of ozone (Verdini et al. 2021). Hydrogen peroxide has also been explored as a bleaching agent that can effectively remove lignin from cellulosic fractions combined with sodium hydroxide and sodium silicate for lignin separation (Zeronian and Inglesby 1995). The compositional complexity of the substrate can lead to different interactions with hydrogen peroxide and different efficiencies, depending on the bleaching mechanisms promoted in each case, which can modify the properties of the purified cellulose, making adjusting to the process conditions in each case essential (Li et al. 2011). Hydrogen peroxide-based treatments have been previously used to extract *α -cellulose* from PO waste (Coletti et al. 2013). This process, which has been also used to extract α -cellulose from industrial and agricultural waste by-products, such as wheat straw, involved a two-step oxidative process, in which the delignification and solubilisation of the non-cellulosic components were performed by using acetic acid, formic acid, and hydrogen peroxide, with a final alkaline extraction (Vismara et al. 2009). However, to the best of our knowledge, hydrogen peroxide bleaching treatments have not been used yet to recover cellulose fibres from PO in combination with a sustainable pre-treatment, such as subcritical water extraction.

Subcritical water extraction (SWE) has been explored as an environmentally friendly, inexpensive, and scalable tool for cellulose recovery due to its high extraction capacity, avoiding chemical agents. The possibility of tuning the properties of water at elevated temperatures (100–374 °C) and pressures up to 22 MPa makes subcritical water a promising reaction medium in short residence times and high rates of conversion (Álvarez-Viñas et al. 2021). The unique values of viscosity, diffusivity, solvent capacity, density, and dielectric constant of water at subcritical conditions allow for increasing its extractive capacity of low or medium polarity compounds, such as active compounds or hemicellulose and lignin (Requena et al. 2019). Through the selective extraction of different components, this treatment makes

the lignocellulosic substrate more accessible to other purification agents, and thus, it can enhance the efficacy of further purification treatments (Freitas et al. 2023a, 2024).

In this sense, the aim of this study was to analyse the efficiency of a two-step sustainable process based on SWE and bleaching with hydrogen peroxide for obtaining cellulose fibres from PO waste supplied by a landfill of the plant collected from the beaches. Two extraction temperatures in the SWE step were applied to remove a great part of the non-cellulosic components. Afterwards, the extraction residues were submitted to a bleaching process to improve cellulose purification via oxidation of the non-extracted organic substances accompanying cellulose, such as lignin. The composition of the different fractions in terms of cellulose, hemicellulose, lignin, and ashes was analysed to evaluate the degree of cellulose purification. Purified cellulose fibres were characterised as to their thermal stability and morphology as a function of the process conditions. Bleaching was also carried out with the usual sodium chlorite for comparison purposes.

2. Material and methods

2.1 Chemicals

Sodium hydroxide (NaOH), glucose, arabinose, and sodium chlorite (NaClO_2) were obtained from Sigma-Aldrich (USA). Ethanol (98%), hydrogen peroxide (H_2O_2 , 30%), sulphuric acid (H_2SO_4 , 98%), sodium carbonate (Na_2CO_3 , 99.5%), and acetic acid were purchased from Panreac Quimica S.L.U (Castellar del Vallés, Barcelona, Spain). Phosphorous pentoxide (P_2O_5 , 98.2%) was obtained from VWR Chemicals (Leuven, Belgium). D(+)-Xylose was supplied by Merck KGaA (Darmstadt, Germany). Sodium acetate trihydrate was provided by Fluka™ (Steinheim, Germany).

2.2 Plant material preparation

Posidonia oceanica (PO) waste was supplied by a landfill located in Denia (Alicante, Spain) in February 2023. PO was washed with distilled water and dried at 50 °C for three days in a forced-air oven (S. P. Selecta, s. a., Barcelona, Spain). After that, other plants, algae, PO rhizomes, and balls of fibrous materials were manually separated from the PO leaves.

PO leaves were ground using a mill (Model SM300 stainless, Retsch GmbH, Haan, Germany) and sieved to obtain particles under 0.50 mm, which were stored in plastic bags at 4 °C until use.

2.3 Subcritical water extraction

Subcritical water extraction (SWE) was performed to separate non-cellulosic, soluble compounds from the PO leaves using a pressure reactor (Model 1-TAP-CE, 5 L capacity, Amar Equipment PVT. LTD, Mumbai, India) with a PO: distilled water ratio of 1:15 (w/v), using two extraction temperatures (pressure), 150 °C, (5 bar) and 170 °C (9.5 bar), both at 150 rpm, for 30 min. After the extraction, the dispersion was filtered (Filterlab, Barcelona, Spain, pore size < 0.5 mm), and the solid residue was

washed with distilled water to eliminate water-soluble compounds retained in the solid fraction. The obtained residues, R150 and R170, were dried at 40 °C for 48 h, and the extraction yield was determined. The yield in extracted soluble solids was also determined gravimetrically by sampling two aliquots of the liquid extracts and drying them at 105 °C until constant weight to determine the solid: water ratio. The total solids extracted were obtained by multiplying this ratio by the total water mass in the reactor.

2.3 Bleaching treatments

Bleaching treatments were carried out on both R150 and R170 residues obtained from SWE to purify the cellulose in the fibres, as described by Freitas et al. (2023b). Each sample (R150 and R170) was submitted to bleaching with 4 and 8% (v/v) hydrogen peroxide solutions, using a solution-solid ratio of 30:1, at pH 12 (adjusted with NaOH) and 40 °C, in 4 cycles of 1 hour. After each cycle, the sample was filtered and washed with distilled water to remove the bleaching agent. Afterwards, the samples were dried at room conditions and stored in desiccators containing P₂O₅ to ensure dryness. Thus, four different bleached residues (BR) were obtained for each bleaching cycle and treated with each H₂O₂ concentration (4% v/v and 8% v/v): BR150-1, BR150-2, BR150-3, BR150-4, BR170-1, BR170-2, BR170-3, BR170-4. To monitor the progress of the bleaching process, these samples were analysed as to the mass yield and whiteness index.

The bleaching yield was determined gravimetrically from the dry sample mass before and after the bleaching step. The whiteness index (WI) was determined from the CIEL*a*b* coordinates, applying Eq. (1). Colour coordinates were obtained from the surface reflectance spectra of the samples, determined from 400 to 700 nm with a spectrophotometer CM-5 (Konica Minolta Co., Tokyo, Japan), using D65 illuminant and 10° observer.

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (1)$$

Likewise, for comparison purposes, both R150 and R170 samples were bleached with sodium chlorite, as reported by Freitas et al. (2022). The bleaching solution was obtained by mixing equal parts of acetate buffer solution (2 N), sodium chlorite (1.7%, w/v), and distilled water. Each extraction residue was mixed with the bleaching solution (5%, w/v) and treated under reflux for four hours. A total of 5 cycles, 4 hours each, were carried out till the sample was white. After each cycle, the sample was filtered and washed with distilled water to remove the bleaching agent. Afterwards, samples were dried at room conditions and stored in desiccators containing P₂O₅ to ensure dryness. Thus, two bleached residues, BR150-C and BR170-C, were obtained and characterised as to their yield and WI, as previously described.

2.4 Characterisation of cellulosic fraction

2.4.1 Compositional analysis

The raw material (PO), the two extraction residues (R150 and R170), and all bleached samples were analysed in duplicate as to their composition in terms of structural carbohydrates and lignin, using the

standard NREL method (NREL/TP-510-42618-2008) (Sluiter 2008a). The ash content was obtained by incineration of the samples in an oven at 575 °C for 24 h.

The raw material (PO) and solid residues (R150 and R170) were first submitted to the analyses of extractives in water and ethanol as described in the standard NREL method (NREL/TP-510-42619-2008) (Sluiter 2008b). These analyses consisted of a two-phase Soxhlet set-up: a first extraction using distilled water for 8 h, and a second extraction using ethanol for 8 h at 60 °C. Afterwards, the samples were submitted to a two-step acid hydrolysis with H₂SO₄. Klason lignin content was determined gravimetrically from the insoluble fraction, whereas the soluble fraction was used to determine the monosaccharide content (glucose, xylose, and arabinose) through high-performance liquid chromatography. An HPLC equipment (Agilent Technologies, model 1120 Compact LC, Waldbronn, Germany), with RezexTM RCM-Monosaccharide Ca²⁺ column (150 × 7.8 mm) and an evaporative light scattering detector (ELSD Agilent Technologies 1200 Series, Waldbronn, Germany) was used. Double distilled water was used as a mobile phase in an isocratic mode at a 0.4 mL·min⁻¹ flow rate. The detector conditions were 40 °C, 3.0 bar of N₂, and a gain of 3. ChemStation software (version LTS 01.11, Agilent Technologies, Waldbronn, Germany) was used for data acquisition. Results were evaluated by applying a Gaussian model for peak area determination with Origin software (version OriginPro 2021, OriginLab Corporation, Northampton, MA, USA). The hemicellulose content was calculated from the sum of the xylose and arabinose contents concerning the initial solid fraction, and the cellulose content was obtained from the glucose concentration, as described by Sluiter (2008a).

2.4.2 Thermogravimetric analysis (TGA)

The thermal behaviour of the samples was analysed in duplicate using a thermogravimetric analyser (TGA 1 Stare System analyser, Mettler-Toledo, Greifensee, Switzerland). Samples were previously conditioned in a desiccator containing P₂O₅ at 25 °C for two weeks to ensure dryness and then weighed (3–4 mg) in aluminium pans. The samples were heated from 25 °C to 900 °C under a constant flow of nitrogen (10 mL·min⁻¹) at 10 °C·min⁻¹. The weight loss *versus* temperature and their corresponding derivative curves were obtained using the STARE Evaluation Software (version V12.00a, Mettler-Toledo, Inc., Greifensee, Switzerland).

2.4.3 Fourier transform infrared spectroscopy (FTIR)

The vibrational profile of the functional groups present in the samples was obtained in duplicate using an FTIR spectrometer (Agilent Cary 630 FTIR Spectrometer) in the wavelength range of 4000 – 650 cm⁻¹, at a resolution of 6 cm⁻¹, and 128 scans for each spectrum. The analysis was performed in triplicate for each sample.

2.4.4 Morphology of lignocellulosic materials

The microstructure of the raw material (PO), the two solid residues (R150 and R170), the 4-cycle bleached samples with 4% and 8% H₂O₂ (BR150, BR170) and those bleached with sodium chlorite

(BR150-C and BR170-C) were analysed using High-resolution field emission scanning electron microscopy (HRFESEM, GeminiSEM 500, Zeiss, Oxford Instruments, UK). The samples were coated with a platinum layer using an EM MED020 sputter coater (Leica BioSystems, Barcelona, Spain). The micrographs were taken at a 2 kV acceleration voltage.

Likewise, the particle size distribution was analysed in defibrillated samples, as described by Freitas et al. (2022). To this end, the samples were dispersed at 0.5% (m/v) in double distilled water. Then samples were sonicated at 25°C (by immersion in an ice bath) for 30 min, using a probe high-intensity ultrasonic homogeniser (Vibra Cell™ VCX750, 750 W power, Sonics & Material Inc., Newtown, CT, USA), operating at a frequency of 20 kHz, 40% sonication amplitude, and continuous mode. The sonicated samples were analysed as to their particle size distribution by laser diffraction (Mastersizer 3000, Malvern Instruments, UK), based on the Mie theory. Values of refractive and absorption indexes were taken as 1.52 and 0.1, respectively. The samples were diluted, stirred at 1900 rpm, and fed into the system until an obscuration rate of 10% was achieved. Measurements were carried out in triplicate for each sample.

2.5 Statistical analysis

Analysis of variance (ANOVA) at 95% confidence level was carried out to identify the significance of sample differences in terms of the different parameters quantified, using Statgraphics Centurion XIX. Differences between treatments were determined by the Fisher test, using the least significant difference of 5% ($\alpha = 0.05$).

3. Results and Discussion

3.1 Yield and purity of cellulose fibres reached in the process steps

Figure 1a shows the flow chart diagram of the combined subcritical water extraction-bleaching process used to obtain cellulose fibres from PO waste. The images of the different products, raw material, extraction residues (R150 and R170), and bleached fibres (BR150 and BR170) using the different bleaching treatments are shown in Fig. 1b, as well as the final yield of each step. SWE promoted the release of 18 and 30% of PO components at 150 and 170 °C, respectively, giving rise to a yield of the extraction residues of 79 and 63 g.100 g⁻¹ dried PO, respectively. The solid mass balance implied a total recovery of 97% and 93% of total initial mass, which suggests that at the highest temperature, a small part of organic matter could be degraded, although no changes in the reactor pressure were observed due to the CO₂ generation. The low solid yield obtained at 170 °C suggests that insoluble cellulose was more purified at this temperature than at 150 °C, since more non-cellulosic compounds were removed. Other authors also observed higher extraction yields and cellulose purification when the extraction temperature rose (Erşan et al. 2018; Çalhan et al. 2023. In contrast, Freitas et al. (2023b) obtained a similar insoluble solid yield (55–56%) from almond skin submitted to SWE at 160 °C and 180 °C. The differences in yield values at different extraction temperatures are related to the chemical components

of each lignocellulosic matrix and their solubility and sensitivity to hydrolyse chemical bonds under the used extraction conditions.

The cellulose purification degree obtained in SWE at each temperature can be observed in Table 1, where the content of cellulose, hemicellulose, lignin, and ashes of R150 and R170 samples can be compared with the respective contents of raw material (PO). The cellulose content of PO was near 30%, in the range reported by other authors (Bettaieb et al. 2015; Benito-González et al. 2019; Tarchoun et al. 2019). Nevertheless, it is worth mentioning that the chemical composition of PO can be highly affected by the season and origin (Cebrian and Duarte 2001). The cellulose contents in R150 and R170 samples increased in comparison with the values for the PO samples due to the removal of non-cellulosic compounds ($p < 0.05$). The greater efficiency of the highest temperature at purifying cellulose was clearly observed. Nevertheless, the ratio of hemicellulose and lignin in these samples was still high since only hydrolysed structural compounds were mainly extracted by SWE. The amount of extractives in water (WE) and ethanol (EE) was determined for the different samples before the analyses of structural components. The WE values were 17 ± 3 , 10 ± 1 and $18 \pm 1\%$ wt., respectively for PO, R150, and R170 samples. These values suggest that SWE provokes the opening of the plant matrix, which promotes the subsequent extraction of water-soluble compounds, even after the previous removal of compounds by SWE, especially at $170\text{ }^{\circ}\text{C}$. Other authors reported the hydrolysis capacity of water under subcritical conditions (Erşan et al. 2018; Gabaston et al. 2018), which would enhance the water solubility of compounds released from the matrix during the hydrolysis occurred in the SWE step. No significant differences were obtained for EE of PO, R150, and R170 samples, the mean values being 5% ($p > 0.05$). The remaining non-cellulosic compounds would be eliminated during the applied bleaching treatments, aimed to oxidise lignin and other organic compounds that would be leached to the bleaching solution.

Table 1
Composition of *Posidonia oceanica* (PO) waste and the SWE residues obtained at $150\text{ }^{\circ}\text{C}$ (R150) and $170\text{ }^{\circ}\text{C}$ (R170)

Treatment	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ashes (%)
PO	29.6 ± 0.6^c	8.9 ± 0.4^a	13.6 ± 0.3^c	20.0 ± 3.0^a
R150	39.0 ± 4.0^b	10.6 ± 1.0^a	20.6 ± 1.2^b	8.4 ± 0.2^b
R170	51.1 ± 0.7^a	4.6 ± 0.1^b	23.5 ± 0.4^a	10.3 ± 0.3^b

Different letters in the same column indicate significant differences between treatments by Fisher test ($\alpha = 0.05$).

As concerns treatments with hydrogen peroxide, Fig. 2 shows the bleaching yield (BY) and whiteness index (WI) of the bleached samples after the different cycles using 4 and 8% (v/v) H_2O_2 . As expected, the BY decreased ($p < 0.05$) as the number of cycles rose since more non-cellulosic compounds were being eliminated from the sample. Coherently, the WI rose as the coloured compounds were oxidised or

leached to the bleaching solution ($p < 0.05$). In terms of BY, the cycles were more effective at removing non-cellulosic compounds (BY decrease) for sample R170, which, in turn, was richer in cellulose. The effectiveness of the first cycle was the highest ($p < 0.05$), whereas no remarkable changes in BY or WI occurred after the third cycle. Similar behaviour was observed for sample R150, but with higher BY values that implied a lower elimination of non-cellulosic compounds. This could be attributed to lower alteration of the lignocellulosic complex during the SWE, which reduces the accessibility of oxidants to the target compounds in the matrix. In fact, the hemicellulose content of the sample R150 was higher than that of the sample R170 (Table 1) ($p < 0.05$), which suggests better structural preservation in this sample. WI developed coherently with BY, increasing more quickly during the first cycle and reaching a practically asymptotic value in the third cycle onwards. Sample R170 became less white than sample R150 ($p < 0.05$), which can be explained by the formation of more recalcitrant brown compounds during the SWE treatment at the highest temperature. Sugar caramelisation and Maillard compounds are formed during SWE to a greater extent when temperature increases, as reported by other authors (Plaza et al. 2010a, b). The neo-formed compounds could be more resistant to the bleaching process. At 8% (v/v) H_2O_2 , lower BY and higher WI were observed at each bleaching cycle for the respective samples, indicating better efficacy at higher concentrations ($p < 0.05$). Nevertheless, the influence of the oxidant concentration on the bleaching efficiency was only remarkable for the BY during the first and second cycles. This can be attributed to the fast decomposition of H_2O_2 , depending on the initial concentration and different agents present in the media that can accelerate or moderate the decomposition reaction (Li et al. 2011).

Taking the final BY values into account, more purified cellulose would be obtained with the R170 sample using four cycles with 4 or 8% (v/v) H_2O_2 , since a greater amount of non-cellulosic compounds have been removed. Considering the yield after SWE and final BY, 24% of cellulose-rich material could be obtained from PO using SWE at 170 °C, whereas 47% of purified cellulose would be obtained at 150 °C. Nevertheless, different purity degrees of cellulose would be expected in each case, which was determined by the NREL method.

A bleaching treatment with $NaClO_2$ (the typically used bleaching agent in cellulosic materials) was carried out for comparison purposes. The BY and WI of the blanched samples are shown in Fig. 2. As occurs with samples bleached with H_2O_2 (BR150 and BR180), samples bleached with $NaClO_2$ (BR150-C and BR170-C) showed higher BY and lower WI when submitted to SWE at 170 °C (BR170-C) than when extracted at 150 °C (BR150-C) ($p < 0.05$).

The yields obtained with the bleaching treatment with sodium chlorite were 20.5 and 21% of raw PO for BR150-C and BR170-C, respectively. These yield values agrees with those obtained in previous studies with PO carried out by Tarchoun et al. (2019), where a 23% yield of starting dried raw material was obtained.

Figure 3 shows the compositional analysis in terms of cellulose, hemicellulose, lignin, and ashes of both SWE residues (R150 and R170) and the corresponding samples bleached with sodium chlorite and at the

different cycles with H_2O_2 at 4 and 8%. Regarding the cellulose content, all bleached samples have a higher cellulose content than the starting residue (R150 and R170) ($p < 0.05$). For the samples bleached with H_2O_2 , there is a progressive enrichment in cellulose with the cycles, coherently with the progressive elimination of non-cellulosic compounds, for both treatments with 4 and 8% of H_2O_2 . In general, the bleached samples from R150 sample, had less cellulose content than those R170 that contained less hemicellulose ($p < 0.05$). The effect of H_2O_2 concentration on the cellulose content reached at each bleaching cycle was only significant in the first cycle applied to R150 samples ($p < 0.05$) but did not significantly affect the other cases ($p > 0.05$). The cellulose contents in the samples treated with SWE at 150 °C were 63–67% after four bleaching cycles, whereas these were 84–96% in samples treated at 170 °C. In contrast, bleaching with sodium chlorite yielded 90 and 97% cellulose for samples treated at 150 and 170 °C, respectively.

The hemicellulose contents in BR150 and BR170 samples did not change significantly during the successive bleaching cycles with 4 or 8% (v/v) H_2O_2 , with values about 16 and 7%, respectively, which indicates no significant removal of hemicellulose during the bleaching steps ($p > 0.05$). The increased hemicellulose content, in comparison with their respective non-bleached samples, can be attributed to the major elimination of other compounds during the first bleaching cycle. Nevertheless, a selective hemicellulose removal occurred in the R150 sample (richer in hemicellulose) during bleaching with sodium chlorite, thus leading to a similar content (13%) in both BR150-C and BR170-C final samples.

The lignin content of both R150 and R170 samples was notably reduced ($p < 0.05$) during the bleaching cycles with 4 and 8% (v/v) H_2O_2 , the final content being similar (13–15%) in every 4-cycles-bleached sample. The bleaching with sodium chlorite was more effective at removing lignin from R150 and R170 samples ($p < 0.05$), which reached a final content of $\approx 4\%$. Likewise, the ash contents determined in these samples were much lower ($\approx 1.5\%$) than those obtained for non-bleached samples and those bleached with H_2O_2 . These values were near to that previously reported (0.65%) for bleached PO fibres with chlorite (Tarchoun et al. 2019).

Based on the results commented on above, SWE at 170 °C followed by four 1h-bleaching cycles with H_2O_2 at 4% (v/v) and pH = 12 could provide cellulose fibres from the PO waste with good yield (24% with respect to the initial dried PO) and purity (near 90%), although the residual lignin and ash contents would be higher than those obtained using sodium chlorite as a bleaching agent. Other physicochemical analyses were carried out on the fibres obtained using different treatments in order to achieve a better comparison between them.

FTIR spectra of the different lignocellulosic fractions with different purification degrees have been obtained with the typical bands described in Table 2, with different relative intensities, depending on the cellulose purification level. Figure 4 shows the spectra of the extraction residue obtained at 150 °C (R150) and its respective bleached samples with H_2O_2 at 4% (v/v) in the different cycles. The successive bleaching cycles led to changes in the relative intensity of different peaks. An increase and narrowing of

the peak related to the O-H stretching ($3000\text{--}3680\text{ cm}^{-1}$), which is an indicator of cellulose purification, can be observed. Likewise, the band at 1630 cm^{-1} attributed to the vibration of adsorbed water decreased in intensity, in line with the removal of the amorphous hemicellulose. The progressive extraction of phenolics and pectin was reflected in the decrease in the relative intensity of the peak at 1420 cm^{-1} . The peak at 898 cm^{-1} corresponding to the β -glucosidic bond of cellulose gained intensity and definition while the intensity of the bands at 875 and 711 cm^{-1} decreased due to the removal of sugars from the lignocellulosic matrix. Similar changes have been reported by other authors during cellulose purification from PO (Tarchoun et al. (2019); Ferchichi et al. (2022)).

In samples treated at $150\text{ }^{\circ}\text{C}$ (Fig. 4a), the abovementioned changes were more evident from the third cycle onwards when bleached with 4% (v/v) H_2O_2 and from the second cycle onwards when treated with 8% (v/v) oxygen peroxide. Figure 4b allows for comparison between FTIR spectra of raw material (PO), non-bleached R150 and R170 samples, and all bleached samples (4-cycle bleached BR150 and BR170 with 4 and 8% H_2O_2 , and BR150-C and BR170-C). The spectra development and the above-described changes could also be observed, allowing to deduce that bleached fibres from the R170 sample were more purified than those obtained at $150\text{ }^{\circ}\text{C}$ while bleaching with sodium chlorite was more effective at purifying cellulose, according to the compositional analyses.

Table 2
FTIR characteristic band assignments to the lignocellulosic fractions of *Posidonia oceanica*

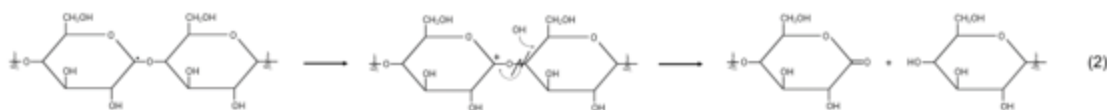
Wavenumber (cm ⁻¹)	Band assignment	Reference
3000–3680	ν_{O-H} : Stretching vibration of the O-H bonds of the primary and secondary hydroxyl groups present in cellulose, hemicellulose, and lignin.	Barbosa et al. (2008); Tarchoun et al. (2019)
2850–2990	$\nu_{as}CH_2$ and $\nu_{s}CH_2$: asymmetric and symmetric stretching vibration of CH ₂ group present in cellulose and hemicellulose.	Barbosa et al. (2008)
1730	$\nu_{C=O}$: C = O stretching vibration of acetyl uronic ester present in hemicelluloses and carboxylic groups present in phenolic acids of lignin and hemicellulose.	Cengiz et al. (2012)
1630	δ_{OH} : O-H bending vibration of adsorbed water.	Barbosa et al. (2008); Moslemi et al. (2020)
1420	ν_{C-O-H} , $\nu_{C=O}$: Stretching vibration of phenolic O-H and C = O present in carboxylates.	Schulz and Baranska (2007); Meseguer et al. (2016)
1370	ν_{C-H} , ν_{C-O} : Stretching vibration related to the C-H or C-O found in polysaccharides aromatic rings.	Tarchoun et al. (2019)
1240	ν_{C-O} : stretching vibration out of the plane of the aryl groups present in lignin molecules.	Tarchoun et al. (2019)
1160, 1021	$\nu_{C-O-C-O-C}$: Stretching vibration of C-O-C-O-C present in secondary alcohol (lignin, hemicelluloses, and cellulose) and acetal bonds of hemicellulose and cellulose.	Barbosa et al. (2008); Tarchoun et al. (2019)
898	ν_{C-O-C} : stretching vibration of the β -glycosidic bond present in the cellulose.	Barbosa et al. (2008); Freitas et al. (2022)
875	Stretching vibration of mannose and galactose pyranose rings	Gaber et al. (2021)
711	Related to the existence of xylans-type polysaccharides	Gaber et al. (2021)

3.2. Thermal behaviour of the materials

Thermal behaviour and stability of the lignocellulosic materials also reflected the cellulose purification degree and were analysed in the different fractions obtained at each purification step. Figure 5 shows the TGA curves and their derivative curves (DTGA) for the different lignocellulosic residues from the different process steps. All samples showed an initial weight loss (3–9%) below 150 °C, attributed to the loss of adsorbed water in the material, as also observed by other authors (Tarchoun et al. 2019; Freitas et al. 2023b). Afterwards, samples exhibited the typical pattern of lignocellulosic complexes with successive weight loss steps associated with the degradation of hemicellulose (220–315 °C), cellulose (315–400 °C), and lignin (150–900 °C), as previously described (Yang et al. 2007). According to Yang et

al. (2007), cellulose pyrolysis exhibited the maximum weight loss rate at 355 °C (T_p in DGTA curves) and minimal solid residue, whereas hemicellulose and lignin showed a final residual mass at 900 °C. Figure 5a shows the TGA and the DTGA curves of PO, the SWE residues (R150 and R170), the fourth cycle bleached samples (BR150 and BR170) with 4 and 8% (v/v) H_2O_2 , and those bleached with sodium chlorite (BR150-C and BR170-C), for comparison purposes. In every case, the degradation of hemicellulose, cellulose, and lignin can be observed with different overlapping and intensity depending on the purification step. Notable changes in the pattern of TGA curves were observed throughout the bleaching cycles (Fig. 5b).

The typical weight loss steps previously described by Tarchoun et al. (2019) for the PO sample can be observed, associated with water loss (T_p : 100 °C), hemicellulose and cellulose degradation (T_p : 324 °C), and lignin degradation in a wide temperature range, which is overlapped with the subsequent degradation of formed intermediate compounds. The SWE process modified the TGA pattern according to the partial removal of hemicellulose and lignin from the material. Specifically, the temperature of the maximum degradation rate of the main step associated with the cellulose/hemicellulose degradation shifted to a higher temperature (330 and 334 °C for R150 and R170, respectively). At the same time, this peak became thinner due to the lower content in hemicellulose of samples, mainly for R170. Likewise, the mass loss associated with this degradation step increased (from 46% in PO to 56 and 80%, respectively, in R150 and R170 samples). Nevertheless, the successive bleaching steps with H_2O_2 did not promote the peak shift towards higher temperatures or its thinning, as would be expected from the progressive purification of the cellulose. In contrast, T_p was about 300 °C in BR150 bleached samples and about 327 °C in BR170 samples. These temperatures suggest that cellulose could be partially degraded by treatment with H_2O_2 , with chain depolymerization. This kind of cellulose degradation with H_2O_2 has been previously described by Vismara et al. (2009) based on previous studies of Gilbert et al. (1981) in glucose. The proposed oxidative mechanisms are based on the generation of alpha hydroxyalkyl radicals, as summarized in Eq. 2 (Vismara et al. 2009).



The progress of this depolymerization reaction could also be deduced from the comparison of TGA curves of the samples submitted to the successive bleaching cycles, as shown in Fig. 5b for non-bleached and bleached R170 samples with 4% (v/v) H_2O_2 in the successive cycles. After 1, 2, or 3 cycles, the TGA curves showed different degradation patterns, suggesting the presence of degraded compounds with different thermal stability than the originally present compounds in the raw PO. These could be attributed to oligomers of cellulose and hemicellulose with carbonyl groups in the end glucose ring with greater thermal stability. After 4 cycles, the TGA curves recovered the more typical shape of the lignocellulosic residues, thus indicating that the oxidized/degraded material was released to the

bleaching medium and that the bleached material contains the residual cellulose, hemicellulose, and lignin. This particular behaviour was observed for both R150 and R170 samples treated with 4 or 8% of H_2O_2 , but the development of TGA curves throughout the different cycles was faster with 8% H_2O_2 , especially in R170 samples. However, in the TGA curves of 4-cycles-bleached, the onset and peak temperature of the main degradation step, attributed to the cellulose, were lower than in the non-bleached samples, indicating cellulose depolymerization occurred during bleaching. The peak temperature was about 300 °C for the BR150 samples and 327 °C for the BR170 sample. These values are in the range reported for PO cellulose by Tarchoun et al. (2019), although they were slightly lower.

In contrast, R150 and R170 samples bleached with sodium chlorite exhibited a sharp mass loss step of cellulose degradation with higher temperature peaks at 325 and 331 °C for BR150-C and BR170-C, respectively. which reveals the higher purification degree of the fibres without degraded-depolymerized compounds. However, a residual lignin content could also be deduced from the degradation curves, thus reflecting the recalcitrance of this material for cellulose purification, as also observed for other lignocellulosic biomasses (Ramírez-Estrada et al. 2022).

Therefore, SWE at 170 °C allows for obtaining a more cellulose-enriched material than at 150 °C. Purifying this material with a non-chlorine bleaching agent, such as H_2O_2 , produced cellulose fibres. Nevertheless, a certain degree of cellulose degradation/depolymerization occurred through oxidative mechanisms of glucose units promoted by free radicals. This degradation did not occur when fibres were bleached with sodium chloride, giving rise to better thermal performance of the obtained cellulosic material.

3.3 Morphogeometric properties of the cellulose fibres

The morphological changes that occurred in the initial PO biomass throughout the different cellulose purification steps at different conditions were analysed by HRFSEM. Figure 6 shows the micrographs of the initial PO particles, where some fibre bundles can be observed closely packed in the tissue. After the SWE treatments, a more eroded surface can be appreciated in the bundles, especially for the samples treated at 170 °C. This observation is according to the higher extraction efficiency of this treatment, mainly for hemicellulose, which will contribute to the bundle disaggregation. The removal of different compounds by bleaching with 4 or 8% H_2O_2 was also reflected on the surface structure of the fibre bundles that exhibited a smoother surface than non-bleached samples. The influence of the SWE temperature was also reflected in the bleached samples. A higher disaggregation of the bundles could be observed for bleached samples submitted to SWE at 170 °C, thus revealing the impact of the first extraction treatment on the fibre's purification efficiency. The higher removal of hemicellulose in the SWE process positively affected the subsequent bleaching treatment, possibly by increasing the exposure area of the bundles to the bleaching agent. Samples bleached with sodium chloride showed a practically total bundle separation, thus reflecting the greater efficiency of the bleaching process in removing non-cellulosic components. Therefore, SWE a 170 °C was an adequate pretreatment to obtain cellulose fibres

from PO, which can be purified to quite an extent by H_2O_2 at 8% (v/v) if non-chlorine bleaching agents are to be used.

Laser diffraction was also used to analyse the morpho-geometric properties of CF in terms of particle size distributions derived from their hydrodynamic volume. Samples submitted to SWE at 170 °C exhibited an almost bimodal distribution with two main peaks at 26 and 79 μm that hardly changed after bleaching. This dimension can be attributed to the particle length since the laser diffraction measurements provide the radius of the gyration of the particles. In contrast, particles from R150 samples exhibited a main peak at 105 μm , with shoulders at 28 and 373 μm . Bleaching treatment with H_2O_2 did not reduce particle size but promoted the percentage volume of the biggest particles. In contrast, bleaching with sodium chlorite promotes particle size reduction, increasing the volume percentage of particles with lower size (peak at 26 μm). Changes in the particle size would be related to removing material throughout the bleaching step that alters the initial particle size. These could lead to the disappearance of small particles if all their components are oxidised and released to the bleaching medium. In other cases, the material release will imply changes in their shape and size. The observed changes agree with the different elimination degree of material in the particles depending on their richness in cellulose and the action of the bleaching agent. In sample R170, with higher initial cellulose content, bleaching treatments did not modify the particle size distribution, but remarkable changes occurred in the R150 sample, depending on the bleaching agent, in line with the different cellulose purification degree. The removal of small particles seems to occur with H_2O_2 , while a predominant decrease in particle size was observed with sodium chlorite.

The morphological analysis also reflects the better results obtained with SWE at 170 °C to purify cellulose in the subsequent bleaching stage due to the higher extraction of hemicellulose, increasing the exposure area of the lignocellulosic complex to the bleaching agent. Likewise, bleaching with H_2O_2 allows cellulose purification but causes its partial degradation/depolymerisation, which may affect its properties.

Conclusions

Subcritical water extraction was an efficient treatment for obtaining cellulose from PO waste without using chemicals, with the subsequent application of a bleaching step that avoids chlorine effluents. The highest temperature of SWE, at 170 °C, was more effective than 150 °C for removing non-cellulose components, increasing the efficiency of the subsequent bleaching step. The yield in the extraction residue was 63 wt. % of the dry PO waste with a cellulose content of 51%. The use of H_2O_2 as a bleaching agent, an alternative to chlorine agents, was effective at purifying cellulose but partially altered the cellulose structure through oxidative mechanisms with free radicals, causing depolymerisation. Sodium chlorite was more effective at purifying and preserving cellulose from the SWE residue but using the longest treatment and generating more toxic and corrosive effluents. A combination of SWE at 170 °C and bleaching with H_2O_2 at 4 or 8% (v/v) with four 1 h-cycles yielded 24 wt. % cellulose purified fibres

from the PO waste, with a high cellulose richness (near 90%), but lower than that obtained with sodium chlorite (five 4 h-cycles). Further studies are needed to evaluate the obtained cellulose characteristics relevant to different applications.

Declarations

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Author Contributions

All authors contributed to the conceptualization, methodology, formal analysis, investigation, writing-original draft preparation, writing-review and editing. All authors read and approved the final manuscript.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Founding

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Figures

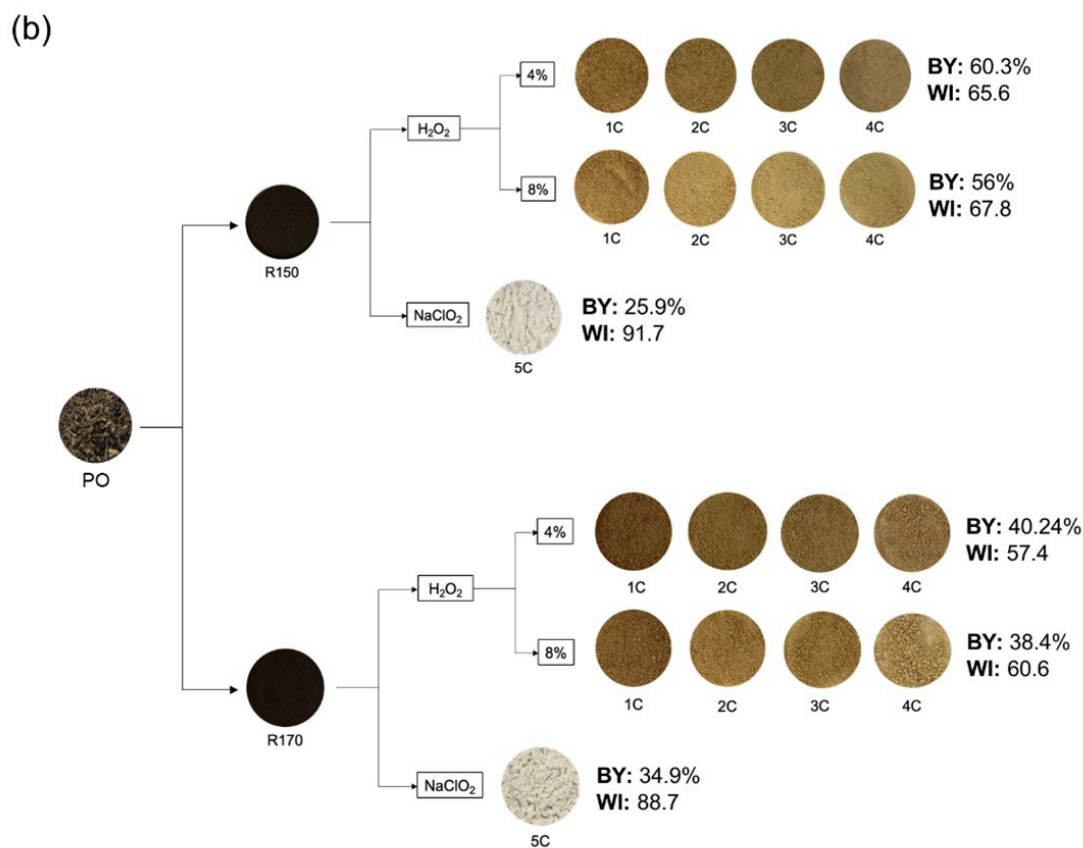
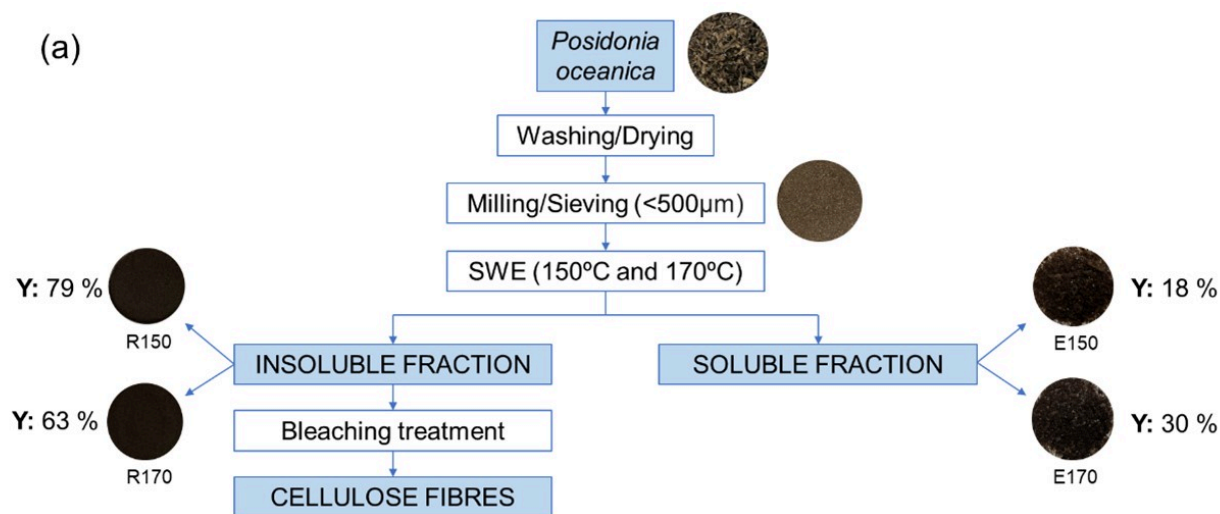


Figure 1

a) Flow chart diagram of the process used to fractionate *Posidonia oceanica* (PO) using subcritical water extraction (SWE) and bleaching process to obtain cellulose fibres from PO waste. b) Images of the lignocellulosic fibres at the different steps of the bleaching process with their yields of the last bleaching cycle (BY) and whiteness index (WI)

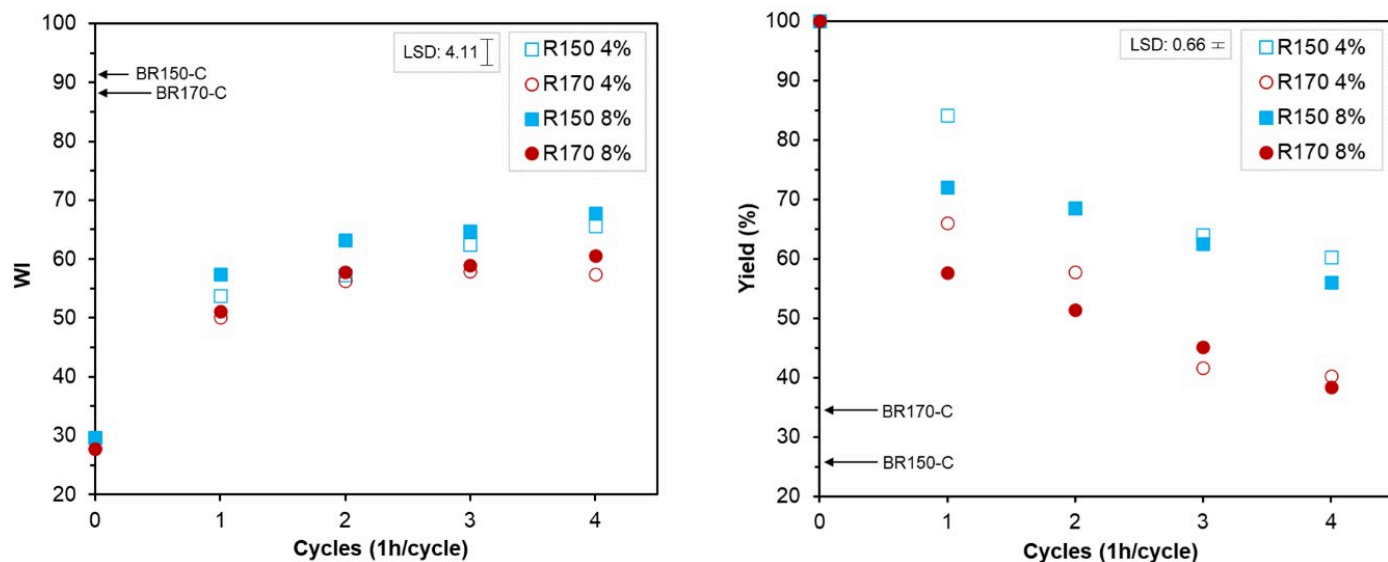


Figure 2

Whiteness index (WI) and bleaching yield (BY) of the SWE residues R150 and R170 after the successive 1h-bleaching cycles with hydrogen peroxide at 4 and 8 % (v/v). Arrows on Y-axis indicate the WI or yield values obtained for their bleaching with sodium chlorite (C)

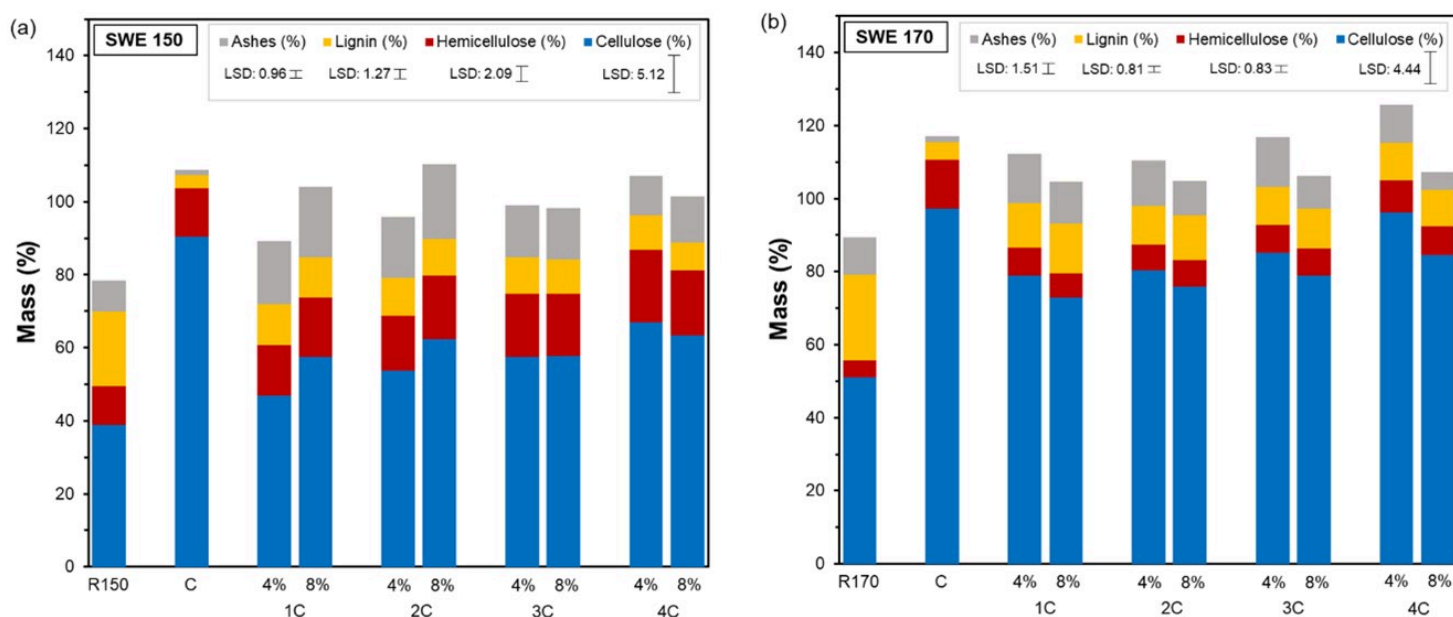
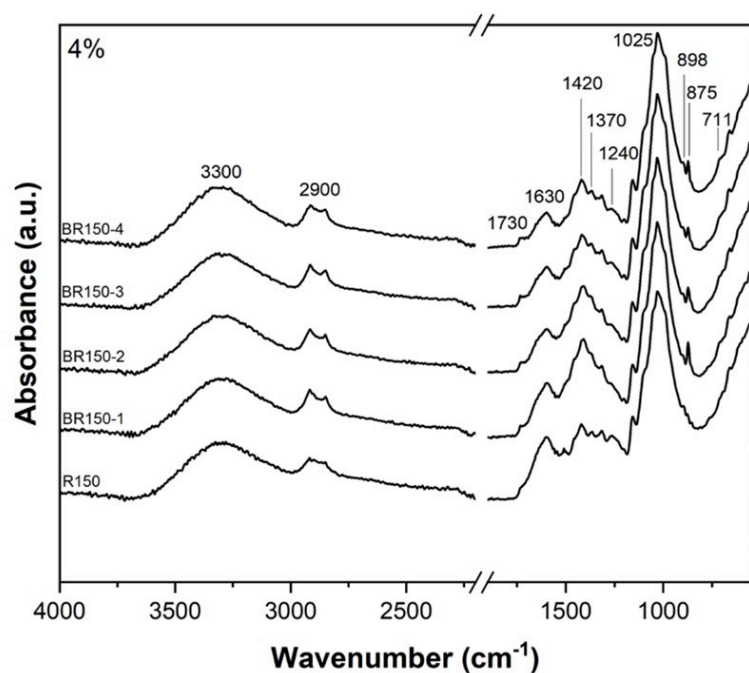


Figure 3

Composition of the different lignocellulosic residues before and after the different bleaching steps with hydrogen peroxide at 4 and 8 % (v/v) or sodium chlorite (C)

(a)



(b)

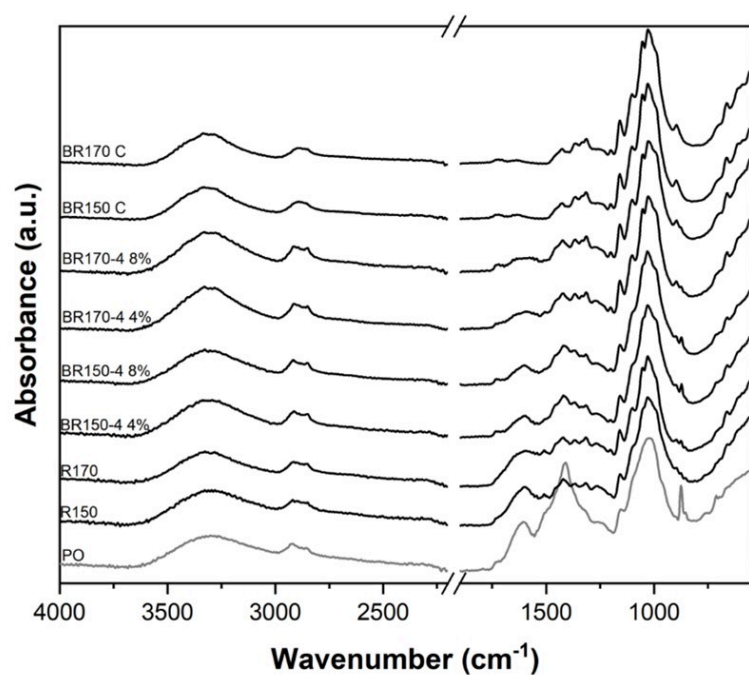


Figure 4

Comparison of FTIR spectra for a) non-bleached extraction residue obtained at 150 °C (R150) and bleached with 4 % (v/v) hydrogen peroxide in different cycles (BR150-1, BR150-2, BR150-3, BR150-4), and b) the different lignocellulosic residues from PO waste (R150 and R170) and bleached samples (BR) with the different treatments

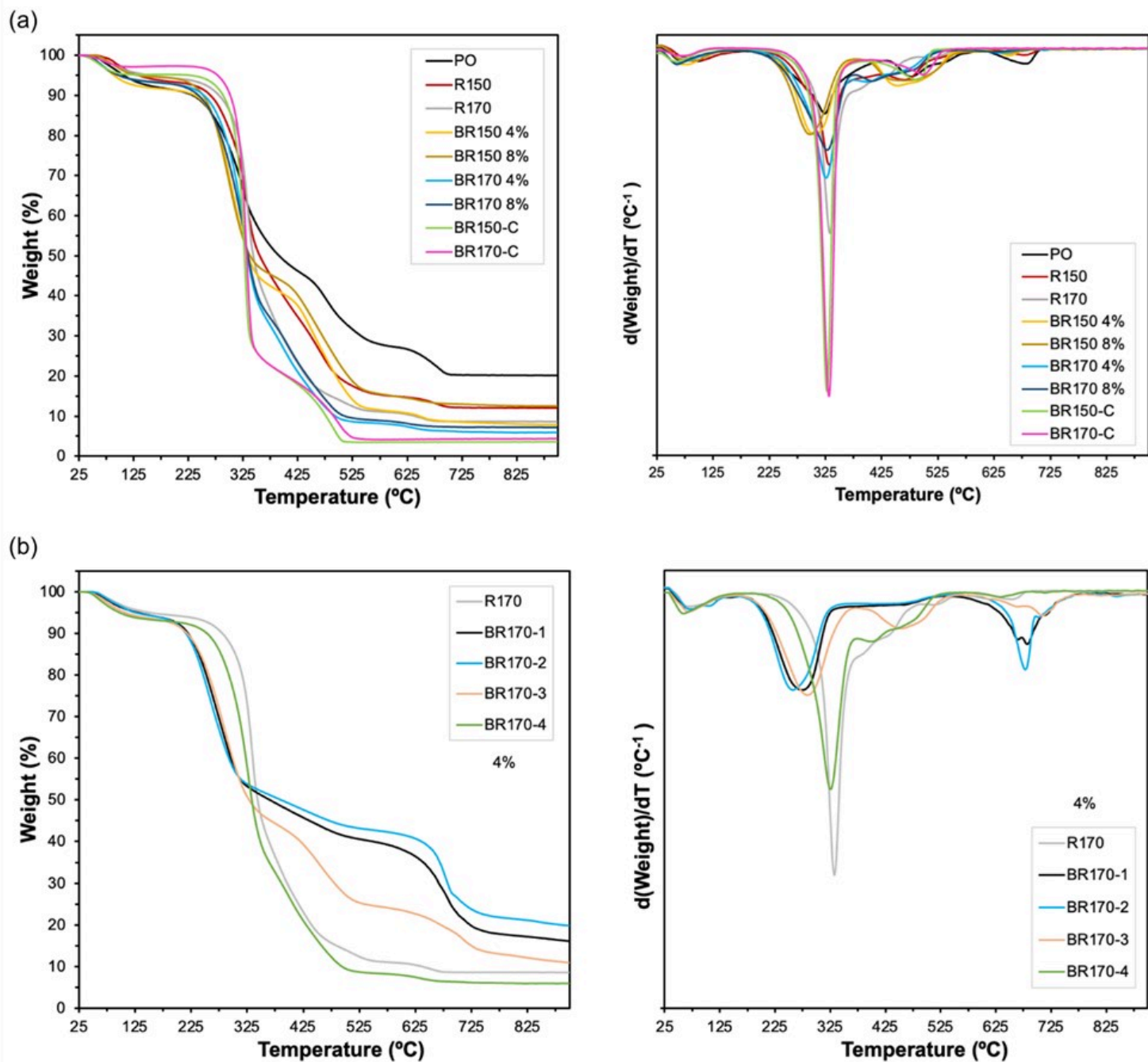


Figure 5

TGA and DTGA curves of different lignocellulosic residues. a) initial PO waste, SWE solid residues (R150 and R170), and bleached samples with H₂O₂ (4th-cycle) at 4 and 8 % (v/v) and with sodium chlorite (C); b) the extraction residue obtained at 170 °C (R170) before and after the different bleaching cycles with hydrogen peroxide at 4 % (v/v)

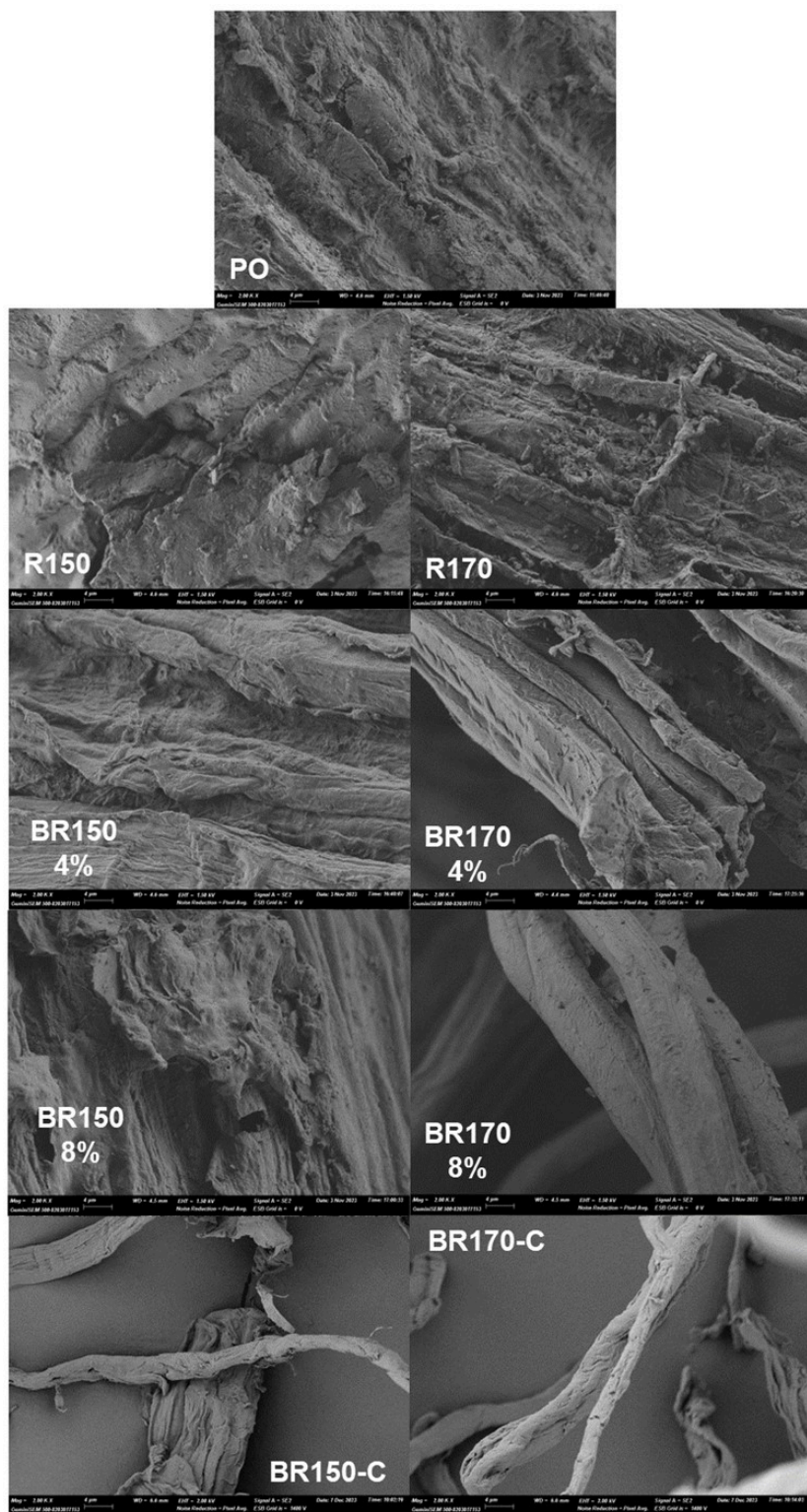


Figure 6

HRFSEM images of the different lignocellulosic residues: PO, SWE extraction residues (R150 and R170) and 4th cycle bleached samples with hydrogen peroxide at 4 and 8 % (v/v) (BR150 and BR170) and sodium chlorite (BR150-C and BR170-C)

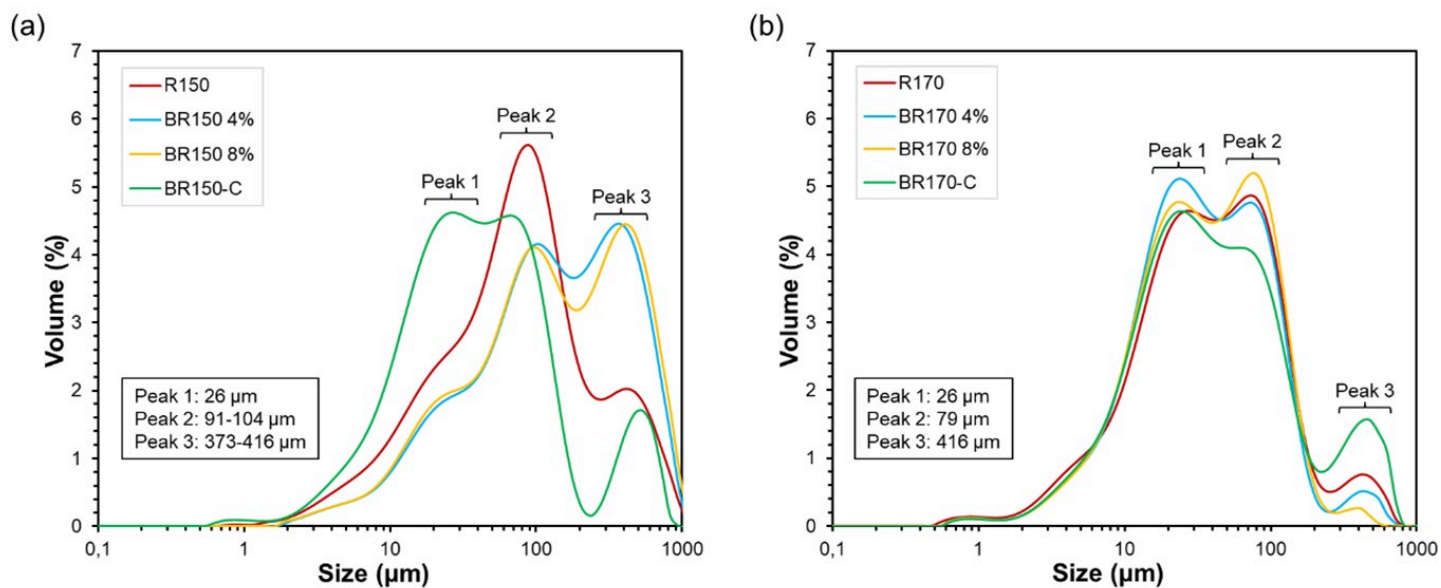


Figure 7

Particle size distribution of the extraction residues (a) R150 and (b) R170 before and after bleaching (BR150 and BR170) with hydrogen peroxide at 4 % and 8 % v/v and sodium chloride (BR150-C and BR170-C)