

CRYSTALLINITY OF CELLULOSE MICROFIBERS DERIVED FROM *Cistus ladanifer* AND *Erica arborea* SHRUBS

CRISTALINIDAD DE MICROFIBRAS DE CELULOSA OBTENIDAS DE *Cistus ladanifer* Y *Erica arborea*

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ABSTRACT

The effectiveness of the use of cellulose fibers as particulates/composite reinforcers involves the assessment of the crystallinity of such fibers. The aim of the present work is to provide information on the degree of crystallinity of the cellulose microfibrils obtained from the stems of *Cistus ladanifer* and *Erica arborea* shrubs through two different methods, namely an alkaline treatment and a microwave-assisted deep eutectic solvent (DES) method. The crystallinity indexes (CrI) obtained from X-ray powder diffraction patterns indicated that higher CrI were attained for cellulose obtained from the DES treatment. Complementary information on the degree of crystallinity was also retrieved from attenuated total reflection- Fourier transform infrared spectroscopy (ATR-FTIR) vibrational spectra, scanning electron microscopy (SEM) micrographs, and accessibility data for the DES-treated celluloses from the two species. The crystallinity results for the fibers derived from these two Mediterranean shrubs were within the range of the results for those derived from wood pulp, opening the door to their valorization for cellulose-derived packing applications or for their use as reinforcers in composite materials in combination with other biopolymers.

Keywords: Deep eutectic solvent, fibers, gum rockrose, mediterranean shrubs, tree heath.

RESUMEN

La efectividad del uso de las fibras de celulosa como reforzantes en materiales particulados/compuestos requiere el estudio de la cristalinidad de dichas fibras. El objetivo del presente trabajo es proporcionar información sobre el grado de cristalinidad de las microfibras de celulosa obtenidas de los tallos de dos especies arbustivas, *Cistus ladanifer* y *Erica arborea*, a través de diversos métodos y en concreto, mediante tratamiento alcalino y uso de disolventes eutécticos profundos (DES) conjuntamente con la aplicación de microondas. Los índices de cristalinidad (CrI) obtenidos a partir de los patrones de difracción de rayos X en polvo indican que el CrI es más alto para la celulosa obtenida mediante el tratamiento con DES. También se aporta información complementaria sobre el grado de cristalinidad a partir de otras metodologías: espectro vibracional de espectroscopía infrarroja con transformada de Fourier por reflexión total atenuada (ATR-FTIR), micrografías obtenidas mediante microscopía electrónica de barrido (SEM) y medidas de accesibilidad para la celulosa obtenida por tratamiento con DES para ambas especies. Los resultados de cristalinidad para las fibras de estas dos especies arbustivas mediterráneas se encuentran dentro del rango de los resultados obtenidos para la pulpa de papel, por lo que es factible su valorización para aplicaciones de empaquetado o para su uso como reforzantes de materiales compuestos junto con otros biopolímeros.

Palabras clave: Arbustos mediterráneos, brezo, disolventes eutécticos profundos, fibras, jara.

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INTRODUCTION

Cellulosic fiber is a polycrystalline aggregate containing crystalline and non-crystalline or amorphous components, *i.e.*, it is formed by crystalline regions separated by amorphous or less crystalline regions. The main chemical components of wood fiber are cellulose (approximately 40% in natural fiber), hemicelluloses, and lignin (approximately 20% to 30% each). Hemicelluloses and lignin are amorphous substances, whereas cellulose has ordered (crystalline) regions.

The content of ordered fibrils is a characteristic feature of cellulose molecules, as a consequence of their tendency to form intra-molecular and inter-molecular hydrogen bonds. In fact, the highly crystalline tertiary fibrillar structure, which confers cellulose with great resistance to solvents and reagents, has to be associated with the existence of such intermolecular hydrogen bonds. The smallest fibrillar unit that has been identified (referred to as 'elemental fibril') has an approximate cross-section of 3,0 nm to 3,5 nm. Elemental fibrils, separated by less ordered regions, are grouped into microfibrils with diameters greater than 25 nm (Stewart 1969, Ahtee *et al.* 1983, Kamide 2005).

Natural plant fibers, or biofibers, have been used as reinforcing material for different types of matrices. Attention has recently focused on their use for the reinforcement of natural polymer matrices and the attainment of environmentally-friendly composite materials (because they are produced from natural resources). The advantages of biofibers over traditional reinforcing fibers (such as fiberglass) are their low cost, low density (with good specific properties), low roughness, biodegradability, and the need for less processing equipment. Some of the natural fibers most widely used for obtaining reinforced materials are sisal, jute, flax, and cellulose fibers from different origins (Bledzki and Gassan 1999, Dante *et al.* 2014). Generally, fibers with high cellulose content (such as those from *C. ladanifer* wood) and low microfibrillar angle have better strength properties (Mwaikambo and Ansell 1999, Fakirov and Bhattacharyya 2007).

It is known that while *Pinus laricio* is the species with the highest relative content in lignin and *Cistus ladanifer* (gum rockrose) is one of the richest in cellulose (55% to 60%), *Erica arborea* (tree heath) is the plant species with the highest holocellulose (cellulose and hemicellulose) content (Barboni *et al.* 2010). These latter two species are evergreen small-diameter hardwood shrubs and constitute a significant proportion of Mediterranean forest vegetation, occupying surfaces of over 2100000 ha and 2400000 ha, respectively, in Spain (MAPAMA 2007). They represent a viable solution for oligotrophic and contaminated soils due to their resistance to pests and extreme environmental factors: their cultivation is possible in degraded sites, and thus involves no diversion of productive agricultural lands to the production of non-alimentary crops.

In spite of the aptitudes of these two fast-growing shrub species for phytochemical and biofuel industries (Carrión-Prieto *et al.* 2018), at present they do not have any regular economic exploitation (Raimundo *et al.* 2018). Thus, field studies aimed at the valorization of these neglected and underutilized natural resources, funded by the European Union through the LIFE+ and Joule programs, have been conducted in several municipalities in the province of Zamora (Spain) since 2012.

With a view to their valorization in cellulose-derived packing, a research topic that was included in the top 10 innovations for 2025, according to the Intellectual Property & Science business of Thomson Reuters (Thomson Reuters 2014), an assessment of the crystallinity of their cellulose fibers is required. Consequently, the study presented herein aims to gain insight by using different characterization techniques on the degree of crystallinity of the cellulose microfibrils obtained from the stems of the two aforementioned pyrophytes upon application of two different treatments (*viz.* an alkaline method and a microwave-assisted DES method).

MATERIALS AND METHODS

Materials

The samples used in this study were collected from a plot located in the municipality of Ayoó de Vidriales (42° 07' 10" N, 6° 06' 59" W), in the province of Zamora, Castilla y Leon, Spain. The chosen area (> 1,2 ha) is a mixed shrubland in which the dominant shrub species are *Cistus ladanifer* L. and *Erica arborea* L. (Carrión-Prieto *et al.* 2017a). Details on the sampling procedure, stem diameters, chemical composition and vegetal

components can be found in Carrión-Prieto *et al.* (2017b).

Choline chloride, urea, sodium hydroxide, and sodium thiosulfate were purchased from PanReac AppliChem (Barcelona, Spain). Titanium dioxide (TiO₂, anatase variety) and Avicel® were supplied by Sigma Aldrich Química S.L. (Madrid, Spain).

Treatments

Samples of the stem of *C. ladanifer* and *E. arborea* (200 mg) were ground in a blade mill and pre-dried at 70 °C for 24 h and then immersed in 4 mL of a NaOH solution (25% w/v) with vigorous stirring at ambient temperature for 24 h. The alkalinetreated fibers were subsequently washed with running tap water and then distilled water until no alkali was present in the wash, and finally dried under vacuum to a constant weight. The alkali wash was recycled to avoid environmental pollution.

Alternatively, a DES ionic liquids system, based on choline chloride-urea, was also used for the fibrillation of *C. ladanifer* and *E. arborea* wood cellulose. Choline chloride/urea DES was prepared by stirring the mixture of choline chloride and urea (mole ratio of 1:2) at 80 °C until a homogeneous colorless liquid formed (Sun *et al.* 2003), and then stored in a vacuum dryer. Subsequently, cellulose samples (200 mg) were treated by a mixture of choline chloride/urea+HNO₃ (1,18 mL), with TiO₂ (20 mg) as a catalyst, using a microwave digestion system - a Milestone (Sorisole, BG, Italy) Ethos-One microwave oven equipped with a magnetic stirrer system at 120 °C for an effective time of 30 min. The samples were allowed to cool down to room temperature. Then, the DES was removed by washing with deionized water (Li *et al.* 2011, Zhang and Zhao 2011, Da Silva Lacerda *et al.* 2015).

Characterization

For the determination of crystallinity *via* X-ray powder diffraction (XRPD), data were obtained with a Bruker (Billerica, MA, USA) D8 Advance Bragg-Brentano diffractometer, in reflection geometry, employing Cu K α radiation ($\lambda = 1,5406 \text{ \AA}$). Diffractograms were collected by continuous scanning over a diffraction angle $2\theta = 5^\circ$ to 80° . The crystallinity index (CrI) was calculated using Equation 1 from an internal reference method by Segal *et al.* (1959), Although Rietveld refinement would be the preferred method for sample crystallinity determinations of plant fiber samples (Thygesen *et al.* 2005), for wood based materials, as the ones studied herein, the method by Segal *et al.* 1959 is applicable. It should be clarified that Rietveld refinement in this case would be difficult, since there is contamination –and therefore superimposed peaks– from TiO₂, and increasing the number of phases would increase the ambiguity of the refined parameters. Further, if one cannot account completely for the preferential orientation, then the values for the FWHM will not be realistic. In any case, since none of the two is a gold standard, crystallinity was also determined by other procedures.

$$\text{CrI}(\%) = [1 - I_{am} / I_{002}] \times 100 \quad (1)$$

where I_{002} is the maximum intensity of crystalline scatter at the 002 reflection (in the case of cellulose, at $2\theta = 22,3^\circ$) and I_{am} corresponds to the intensity of the minimum or ‘valley’ between the peaks at $2\theta = 18^\circ$ and 22° . The amorphicity index (AmI) is $\text{AmI} = 1 - \text{CrI}$.

The vibrational spectra of the materials in the 400 cm^{-1} to 4000 cm^{-1} range were measured using a Thermo Scientific (Waltham, MA, USA) Nicolet iS50 FT-IR spectrometer, equipped with an in-built diamond attenuated total reflection (ATR) system. The absorption bands at 1430 cm^{-1} and 890 cm^{-1} were used for the calculation of the lateral order index (LOI) (O’Connor *et al.* 1958, Kljun *et al.* 2011).

The SEM micrographs were obtained with an FEI (Hillsboro, OR, USA) Quanta 200 FEG microscope with backscattered electrons (BSE) and secondary electrons (SE) detectors. Non-metalized samples were used.

Iodine absorption measurements were used to test the accessibility of cellulose, according to the ASTM D4607-14 method. The iodine adsorption value (ISV) (mg) was calculated according to Equation 2,

$$\text{ISV (mg)} = [(a - b) \times c \times 254] / w \quad (2)$$

where a is the consumable sodium hyposulphite solution with the control (mL), b is the consumable sodium hyposulphite solution with the samples (mL), c is the molar concentration of the sodium hyposulphite solution (mol/L), and w is the dry weight of the sample (g).

All determinations were performed with 20 replicates, except for the SEM analysis.

RESULTS AND DISCUSSION

Degree of crystallinity from X-ray powder diffraction data

The determination of the crystallinity indices from the intensities of the I_{002} and I_{am} peaks in the diffractograms of the cellulose fibers from *E. arborea* and *C. ladanifer* after alkaline treatment (Figure 1) resulted in CrI average values of 39% and 38%, respectively.

For the cellulose fibers from *E. arborea* and *C. ladanifer* subjected to the microwave-assisted DES process (Figure 2), the calculation of the CrI yielded average values of 51,2% and 51,6%, respectively. The error varied from 2% to 2,6% in all treatments, for both species, in good agreement with Park *et al.* (2010).

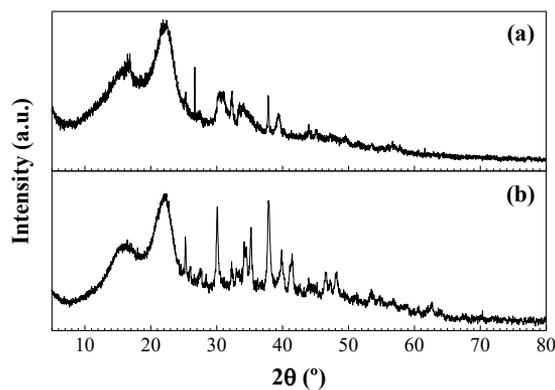


Figure 1: X-ray powder diffraction patterns for the cellulose fibers from (a) *E. arborea* and (b) *C. ladanifer* after alkaline treatment.

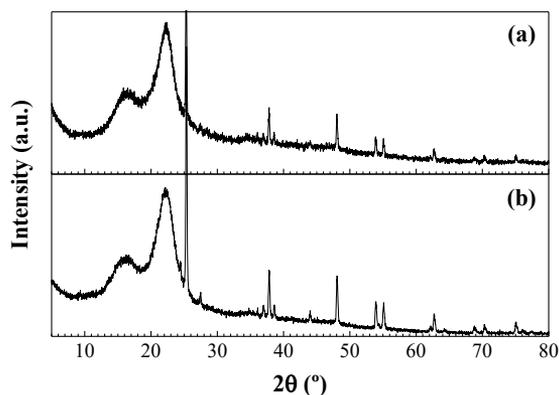


Figure 2: X-ray powder diffraction patterns for the cellulose fibers from (a) *E. arborea* and (b) *C. ladanifer* after microwave-assisted DES treatment for 30 min; the sharp peaks observed in both diffractograms for $2\theta > 25^\circ$ are spurious and can be unequivocally ascribed to some remaining TiO_2 , anatase variety (ICDD PDF # 04-002-8296), used in the treatment.

Degree of crystallinity from ATR-FTIR and SEM data

Having observed a higher CrI with the microwave-assisted DES method, compared to the alkaline treatment, the crystallinity of the DES-treated samples was assessed using alternative approaches. The comparison was made both by examination of the bands of the infrared spectra of the samples (calculation of the LOI) and from information derived from the SEM micrographs.

Firstly, information on the interferences responsible for the uncertainty that affected the CrI determinations from the X-ray powder diffraction patterns was obtained from the FTIR spectra (Figure 3). The observation of minimized peaks at 1730 cm^{-1} (associated with C=O bonds of non-conjugated ketones present in hemicellulose (Pandey 1999, Afanas'ev *et al.* 2007, Morán *et al.* 2008) evinced that the obtained samples had a low contamination of amorphous hemicellulose. However, the contamination with lignin was higher: in fact, bands of medium intensity at 1268 cm^{-1} and 1378 cm^{-1} attributed to guaiacyl units from lignin and to aromatic skeletal vibration in lignin were observed.

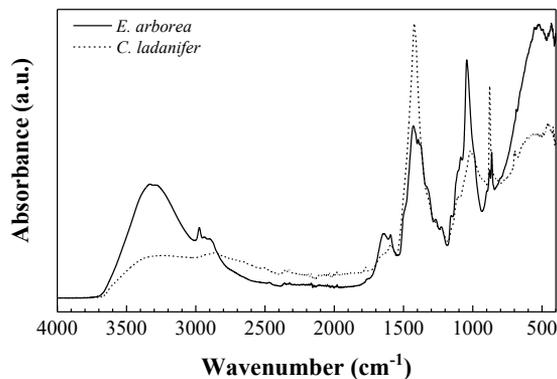


Figure 3: ATR-FTIR spectra of the cellulose fibers from *E. arborea* (solid line) and *C. ladanifer* (dashed line) after microwave-assisted DES treatment of the inner stem.

In relation to the crystallinity, the LOI based on the ratio of absorption bands at specific wavenumbers can be used to interpret qualitative changes in cellulose crystallinity. Generally, as the LOI decreases crystallinity also decreases (O'Connor *et al.* 1958). The absorption bands at 1430 cm^{-1} ('*crystallinity band*', assigned to a symmetric CH_2 bending vibration) and 893 cm^{-1} ('*amorphous band*', associated with C-O-C stretching at β -(1 \rightarrow 4)-glycosidic linkages) can be used to study the type of crystalline cellulose and the crystallinity changes (Oh *et al.* 2005, Kljun *et al.* 2011) because crystalline cellulose I spectrum clearly differs in this band from those of cellulose II and amorphous cellulose. From the intensities of the bands in the spectra, the LOI values for *C. ladanifer* and *E. arborea* samples were 2,2 and 2,9 respectively, which suggested a higher crystallinity for the treated cellulose from the *E. arborea*-derived sample than for the treated cellulose from *C. ladanifer*.

In contrast, according Kljun *et al.* (2011), if a cellulose fiber has a noticeable amount of crystalline cellulose I, the absorption band shifts toward 1430 cm^{-1} and the amounts of cellulose II and amorphous cellulose decrease. Because of a closer proximity of such band to 1430 cm^{-1} for *E. arborea* (1428 cm^{-1}) than for *C. ladanifer* (1422 cm^{-1}), a higher crystallinity was presumed for the cellulose obtained from the former.

Valuable information, complementary to previous results, was also obtained from the SEM micrographs of both samples. In the micrographs obtained for the fibers of the *C. ladanifer* inner stem (Figure 4a and Figure 4b), a structure comprised of cellulose fibrils agglomerated with each other, due to an incomplete removal of lignin, was observed. In contrast, *E. arborea* cellulose fibers (Figure 4c and Figure 4d) showed a more organized and regular morphology (more oriented) than those of *C. ladanifer*. From the micrographs obtained for the fibers of the inner stem of *E. arborea* it was also possible to recognize their dimensions: cylinders that ranged $100\text{ }\mu\text{m}$ to $110\text{ }\mu\text{m}$ in length and with diameters between $9,20\text{ }\mu\text{m}$ and $15,30\text{ }\mu\text{m}$. The aspect ratio ($L/d = 7$ to 10) of these microfibrils was high compared to that of untreated fibers ($L/d = 1,9$ to $3,3$) and made them suitable for sonication to obtain nanowhiskers, an application contemplated in the manufacture of composites.

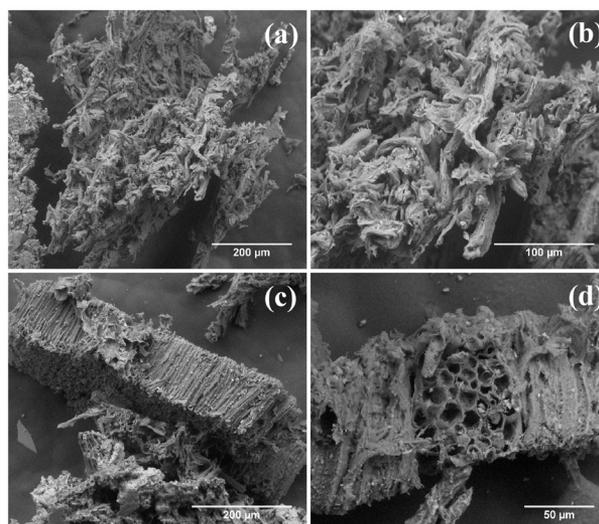


Figure 4: SEM micrographs of the fibers after microwave-assisted DES-based treatment: inner stem of *C. ladanifer* at 400× (a) and 1000× (b) magnification, and inner stem of *E. arborea* at 500× (c) and 1400× (d) magnification).

Crystallinity from accessibility data

The accessibility of cellulose concerns the degree of difficulty with which some low-molecular mass reagents are able to reach the hydroxyl groups of cellulose. Adsorption of various solutes onto fibers occurs mainly on the amorphous regions and usually to a lesser degree on the surfaces of crystallized areas. Thus, any method to measure the fiber accessibility can be used for the estimation of crystallinity (Roberts 1991, Schleicher *et al.* 1991). The accessibility of DES-treated cellulose fibers from *C. ladanifer* and *E. arborea* was studied in comparison with that of Avicel® microcrystalline cellulose by means of the iodine sorption method, and the results are summarized in Table 1. Accessibility percentages were estimated considering that the accessibility of amorphous cellulose (ISV of 412 mg I₂/g) was 100%.

Table 1: ISV values and accessibility and crystallinity estimations for treated cellulose fibers from different origins.

	Iodine Sorption Value (mg I₂/g cellulose)	Accessibility (%)	Crystallinity (%)
<i>C. ladanifer</i>	103,5	25,1	74,9
<i>E. arborea</i>	113,9	27,6	72,4
Avicel®	78,3	19,0	81,0

This indirect method led to remarkably higher crystallinity estimations than the other methods discussed above. Further, the value for *C. ladanifer*-derived cellulose would be higher than that for *E. arborea*, and both of them would be quite close to that obtained for the Avicel® reference material.

The crystallinity indices obtained from the X-ray powder diffractograms using the method proposed by Segal *et al.* (1959), suggested a higher effectiveness of the microwave-assisted DES treatment in comparison with the application of the alkaline treatment. Although this method is widely accepted for comparing the relative differences between samples, the obtained CrI values can only be taken as a rough approximation. A simple height comparison cannot be expected to provide a reasonable estimate of cellulose crystallinity, as it

excludes contributions from the other crystalline peaks and neglects variation in peak width, which can also be affected by crystallite size (Evans *et al.* 1995, Park *et al.* 2010, French and Santiago-Cintrón 2012, Ju *et al.* 2015). It should be remembered that Segal *et al.* (1959) only intended this method to be used as a “time-saving empirical measure of relative crystallinity”. The more accurate XRD deconvolution method could not be used for the samples studied herein, because the four/five peak fitting procedures are only reliable for purer cellulose samples (*e.g.*, filter paper, bleached kraft pulp, neutral sulfite semi-chemical pulp, *etc.*) (Hult and Berglund 2003, Garvey *et al.* 2005), which result in very clean diffraction patterns.

Regarding the results from the other indirect measurement techniques for DES-treated samples, the degree of crystallinity was more favorable for *C. ladanifer* than *E. arborea*, or *vice versa* depending on the chosen measurement method. Whereas the ATR-FTIR vibrational spectra and SEM micrographs suggested higher crystallinity values for *E. arborea* than for *C. ladanifer*, the indirect accessibility data from the ISV estimate pointed at a higher degree of crystallinity for *C. ladanifer*. In any case, the differences were not substantial.

The percentage of crystallinity for cellulose is a determining characteristic in terms of its possible applications. For example, the low crystallinity of cellulose from pineapple peel has been invoked to explain the excellent results obtained in the preparation of solutions of that material in polyethylene glycol and in the manufacturing of polyurethane foams (Vega-Baudrit *et al.* 2007). In contrast, the high crystallinity of the cellulose from ramie fibers (CrI = 0,89) or Avicel® (CrI = 0,81 to 0,83) (Moya-Portuguéz *et al.* 1992) lends itself to the reinforcement for plastics that could be used in place of glass (Mathijssen 2016).

In the case of the wood from the stems of *C. ladanifer* and *E. arborea*, the crystallinity of their celluloses, in the 52% to 75% range, would be within the interval established for wood pulp (60% to 85%) (Núñez 2008). Thus, it was anticipated that these treated cellulose fibers would be suitable for their application as reinforcers of composites, in combination with natural polymers, such as chitosan (De Mesquita *et al.* 2010, Akhlaghi *et al.* 2013), natural rubber (Bendahou *et al.* 2009), or soy protein (Wang *et al.* 2006).

CONCLUSIONS

The information resulting from XRD data established that the degree of crystallinity of the microwave-assisted DES-treated samples was higher than that of their alkaline-treated counterparts.

The complementary ATR-FTIR spectra and indirect accessibility data from ISV for the DES-treated samples suggested that there were no noticeable differences in the crystallinity of the cellulose microfibrils derived from these two Mediterranean shrubs.

The results of the present study indicated that the crystallinity of the treated fibers of both *C. ladanifer* and *E. arborea* were within the range established for wood pulp. Thus, they are suitable to be used in combination with natural polymers for the manufacturing of composite materials.

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