The effect of the natural degradation process on the cellulose structure of Moroccan hardwood fiber: a survey on spectroscopy and structural properties

Abdellatif Boukir 1,*, Ikram Mehyaoui 1, Somia Fellak 1, Laurence Asia 2 and Pierre Doumenq 2

1 Laboratory of Applied Chemistry, Faculty of Sciences and Techniques of Fez, Sidi Mohamed Ben Abdellah University, B.P. 2202, Imouzzer road, Fez, Morocco
2 Laboratory of Environmental Chemistry, UMR CNRS 7376, MPO Team, Europôle Arbois BP 80, 13545Aix in Provence Cedex 04; Aix-Marseille University, France

Abstract: The aim of this work is to study the effect of natural degradation on the cellulose structure conformation changes of 2 ageing Moroccan hardwoods (400 and 500 years) compared to recent one considered as a reference; and to provide information on the polymorphs content variability from two-phases material (crystalline and amorphous) influenced by a long time of ageing and environmental degradation effects. In order to investigate the effects of both natural degradation conditions and a long time of exposure on cellulose structure conformation (examined samples) with estimating their content (crystalline and amorphous cellulose), three combined techniques XRD, ATR-FTIR and FT-Raman spectroscopy were used. XRD results associated with the crystallographic planes and Miller indices provide information on the presence of a mixture of cellulose polymorphs (crystalline cellulose I, II, I spouses and amorphous phase). The decrease in crystallinity-index values from recent to aged ones (38 to 19.5%) confirms well the occurred alteration of crystalline cellulose fibres and their evolution towards a high content of the amorphous form. The prominent regression in the intensities of three FTIR fingerprint cellulose regions evolving towards an overall increase in the intensities of C=O area (1733-1630 cm⁻¹) is a sign on the introduced changes on cellulose conformation and cellulose fibres degradation more accentuated in the case of the very aged sample (500 years). Similar results were confirmed by combining FT-Raman spectroscopy as a vibrational technique. No work has been done on this genus of degraded Moroccan hardwood and the relevance of this study is to investigate the compositional content and structural conformation, to determine the variability in the forms of both crystalline and amorphous cellulose phases with estimating the evolution of their polymorphism, and to monitor the degree of crystalline cellulose fibres deterioration.

Keywords: cellulose; crystallinity index and polymorphs; hardwood; X-ray diffraction (XRD); infra-red (ATR-FTIR), Raman spectroscopy.

Introduction

Cellulose component is the most abundant and renewable biopolymer in nature biosynthesized largely in the cell wall and constitute the key material for the wood, paper, textile, lumber and renewable biofuel industries 1. It is a linear polymer of D-anhydro glucopyranose units (AGU) linked by β-1,4-glycosidic bonds. Two types of AGU units exist at the end of the cellulose chain; the reducing and non-reducing end, and the cellobiose represents the repeating unit. Also, the monomer units can form either highly ordered (crystalline) or less ordered (amorphous) structures, which is due to the result of extensive interaction through intra- and intermolecular hydrogen bonding of the three hydroxyl groups present in each cellulose macromolecules 2-3.

In nature, crystalline cellulose exists in four allomorphs: native cellulose I, cellulose II (prepared regeneration or alkaline treatment), cellulose III (III and IIII forms) (prepared by liquid ammonia treatment) and cellulose IV.

These cellulose allomorphs are physically different, and they differ in reactivity. New evidence suggests that cellulose IV is a slightly disordered form of cellulose Iβ. Additionally, the native cellulose (crystalline fraction) presents a complex ultrastructure due to the presence of two forms for crystal phases; metastable triclinic Ia (one chain) and stable monoclinic Ib (two chain) phase 4. Generally, the cellulose Ia is the dominant form in algae and
bacteria, while the cellulose \( I_\alpha \) is the dominant form in higher plants (wood and cotton) and tunicates. The main differences between the \( I_\alpha \) and \( I_\beta \) structures are in crystal packing, hydrogen bonding, conformations of the anhydroglucose residues and the \( \beta-1,4 \) linkages. As reported by Bansal et al., the crystallographic structures and hydrogen bonding arrangements for cellulose \( I_\alpha \) and cellulose \( I_\beta \) provide important insights into cellulose stability and transformation and contribute toward a scientific basis for understanding cellulose bio-generation and reactivity. The relative amounts of celluloses \( I_\alpha \) and \( I_\beta \) vary with the source of the cellulose, with the \( I_\beta \) form being dominant in higher plants. It is in great importance to mention that the cellulose in its native state (cellulose I) has been reported to be more recalcitrant than its regenerated forms (cellulose II, III, etc).

The cellulose wood materials have been used for the production of novel objects in several fields due to this unique and useful property of crystallinity, because of the crystalline fraction can provide mechanical rigidity and toughness for composite materials, and constitutes a source of the recalcitrance of lignocellulosic biomass against deconstructive processes. Whatever, the effect of serious degradation of cellulose fraction can destroy the strong intermolecular bonding, resulting in a significant change in the chemical structure of cellulose compound as well as the decrease in its crystallinity. The loss of crystallinity amount results in an enhancement in properties of flexibility, dye sorption, moisture regain, swelling and chemical reactivity, whereas density, hardness, tensile strength and dimensional stability decrease. Therefore, the determination of wood crystallinity may be an approach for understanding the effect of weathering on wood properties.

Numerous non-destructive methods as X-ray diffraction (XRD), attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) spectroscopy, Raman spectroscopy, solid-state \( ^{13} \)C nuclear magnetic resonance (NMR) \( ^{1} \)C/CP-MAS measurements, and differential scanning calorimetry (DSC), were employed in several works to quantify the cellulose crystallinity and understand the structural roles of cellulose in the recalcitrant, mechanical and biological properties of plant cell walls. Lionetto et al., using X-ray diffraction and FTIR spectroscopy to quantify crystalline cellulose in lignocellulosic biomass, revealed that the crystallinity index (Cr.I.) of cellulose increased when wood was subjected to weathering accompanied by a considerable increase in the size of crystallites. However, the presence of hemicellulose in lignocellulosic materials as softwoods and hardwoods reduced the crystallinity amount, whereas the syringyl lignin generated higher crystallinity.

No work was meant to highlight the estimation of crystallinity feature and structural changes directly related to the cellulose fibers in Moroccan hardwood. Although, three non-destructive techniques incorporating ATR-FTIR, Raman and X-ray diffraction were employed in the present research to study cellulose polymer originated from argan Moroccan hardwood. Raman and Fourier transform infra-red (FTIR) spectroscopy technique for determining the crystalline absorption bands and functional groups interacting within the natural fiber and for investigating the effect of hydrogen bond on the cellulose fiber alteration. X-ray diffraction to quantify sample crystallinity based on calculated crystallinity index (Cr.I.)

Experimental

Materials

The present study was conducted on three wooden materials about different centuries originated from the Agadir region. The dimensions of wood samples are 200×200×100 mm\(^3\) (tangential × radial × longitudinal directions). The three characterized samples are listed in Table 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>A(_r)</td>
<td>recent</td>
</tr>
<tr>
<td>A(_d)</td>
<td>4 centuries</td>
</tr>
<tr>
<td>A(_5)</td>
<td>5 centuries</td>
</tr>
</tbody>
</table>

X-ray diffraction measurement

The X-ray diffraction analysis was performed at room temperature with a X’Pert Pro diffractometer using CuK\(_\alpha\) monochromatic wavelength radiation at \( \lambda = 1.5406 \) \( \AA \) and generator working at 40 kV and 30 mA. The angular scanning were measured in the area of \( 4^\circ < 2\theta < 60^\circ \), it changes 0.016° with a step of 40s. No background correction was made.

Crystallinity index Cr.I

The crystallinity index Cr.I. % (sometimes called Segal index) of hardwood samples was calculated from diffraction intensity data using the following empirical equation (1):

\[
\text{Cr. I(\%)} = \left( \frac{I_{200} \times \text{rad}}{I_{200}} \right) \times 100
\]
Where $I_{2θ}$ is the total intensity of diffraction of the (200) lattice peak at a $2θ$ of 16.0°–22.0° for cellulose I (Iβ) and of the (020) lattice peak for cellulose II at a $2θ$ of 16.0°–21.6°, and $I_{am}$ is the intensity of diffraction of amorphous cellulose content at a $2θ$ of 16.0°–18.0° for cellulose I (Iβ) and at a $2θ$ of 16.0° for cellulose II (minimum intensity in this region) 18.

**Crystalline size $D_{hkl}$**

The crystalline size $D_{hkl}$ was calculated by the Scherrer equation (2) 23 perpendicularly to different lattice planes $D_{hkl}$ from the 101, 110, 002 and 004 18,20,21,22,24.

$$D_{hkl} = \frac{K \lambda}{B_{hkl} \cos \theta}$$

(2)

where $D_{hkl}$ is the crystalline size (nm), $k$ is Scherrer constant: correction factor (0.89), $\lambda$ is X-ray wavelength (1.5406 Å), $B_{hkl}$ is the angular full width at half maximum FWHM (sometimes called PWHM) in radians of the (hkl) line profile, and $\theta$ is the Bragg angle corresponding to the 200 plane.

**Attenuated total reflectance infrared spectroscopy (ATR-FTIR)**

Fourier Transform infrared (FTIR) transmission spectra were carried out through a BRUCKER VERTEX 70® spectrometer coupled to a Hyperion® microscope. All samples were scanned using Platinum diamond ATR (Attenuated Total Reflectance) in the wavenumber region between 4000 and 400 cm$^{-1}$ with a resolution of 4 cm$^{-1}$. At each position, 16 scans were averaged. The temperature and humidity room were controlled during analysis.

**FT-Raman Spectroscopy**

The FT-Raman study was conducted with a Bruker (USA) MultiRAM Stand Alone FT-Raman Spectrometer. The instrument is equipped with a diode-pumped Nd: YAG excitation source with a large emission intensity at 1.064 nm. Furthermore, the signal was collected with a liquid nitrogen cooled germanium detector. For each FT-Raman measurement, 100 scans were averaged, with a resolution of 4 cm$^{-1}$ and time measurement of 3 min for each spectrum. All FT-Raman spectra were registered from 4000 to 250 cm$^{-1}$. Three analyses were performed on several locations for each sample.

**Results and Discussion**

**X-ray**

The cellulose of hardwood samples was analyzed by X-ray diffraction in order to determine both crystalline and amorphous phase as well as to estimate the amount of its crystalline fraction quantitatively. The XRD data from studied samples, over the $2θ$ range from 5° to 60°, have been plotted in Fig. 1. This range is more reliable to cover all of the significant intensities from both crystalline and amorphous fraction of cellulose, while the peak located in the $2θ$ area ranging from 34.5° to 35.2° was reserved to a mixture of the crystalline fraction (cellulose I, II, Iβ) 20,21,25.

![Figure 1. X-ray diffractogram of the hardwood samples](image)

Compared to the XRD profile of cellulose from Eucalyptus spp. Fibres 21 and others woods fibres such as Acacia 26 and Argania Spinosa 27, the recent wood profile (Fig. 1: A1) manifested some differences with presenting an intense broad hump between $2θ$ of 10° to 16°, a mixture of specific crystal sharp peak well resolved at $2θ$ of 16.5° (Carrillo-Varela et al., 2018 21) and an amorphous phase with a broad wide at the bottom ($2θ$ of 16° to 18°) (Nam et al., 2016 26) closely neighboring and covering the left end of the great extent hump located between $2θ$ area 20° and 25°. Moreover, we can note the high increase in the intensity of the
sharp peak at 2θ of 34.5° usually known by its very low intensity and characterize the crystalline phase.

Concerning the aged samples, a significant decrease in the intensities (2θ ranging from 10° to 18° and 34.5°) was observed from A₀ to A₅, more pronounced in the case of (A₅). According to Ling et al., 2019 25, in XRD feature the arising broad peaks instead of sharp ones, might be correlated to the contribution of small crystallite size which increases the breadth peak, or to the defects in the crystal lattice, and could be originated from the lacks of organization material responsible on the more diffusion in crystalline peaks.

Referring to past and recent literature data of French et Santiago Cintron 2013 18, Nam et al. 2016 20, Ling et al. 2019 25 and French 2014 28, all our X-ray pattern samples showed similar characteristic peaks at 2θ of 14.5°, 16.5°, 21.6°, 22.9° and 34.5° corresponding to the (101), (110) and (020), (200) and (004) crystallographic planes, respectively. The shoulder at 2θ=20.6° that ascribes the (102) lattice plane reflection can also be observed. According to the literature data, these diffractions correspond to the typical profile of the cellulose I allomorph 7,21,26,29. The trough peaks at 2θ=18 between the 110 and the 200 crystallographic planes were assumed to account for the amorphous portion of cellulose 22.

In XRD recent sample (Fig. 1: A₀), the X-ray patterns indicated the presence of four reflections related to a rich fraction of both cellulose I (2θ : 14.5°, 16.5°, 22.9° and 34.5°) 21 and cellulose II (2θ : 12°, 20°, 21.6° and 34.5°) 20,21. However, for the degraded sample, the decrease in the intensity of the peak at 22.9° (cellulose I) towards the formation of the new very weak peak at 2θ of 12° (A₀) and two resolved humps in the 2θ area between 20° and 23° (Aₓ, A₅) which resulting in arising shoulders, is an indication on their contribution to the content of cellulose II 25.

The consistent fraction of cellulose II was manifested by the presence of four reflections at 2θ of 12°, 20°, 21.6° attributed to the Miller indices of (110), (110) and (020) respectively, and at 2θ of 34.5° corresponding to the (004) crystallographic plane 28. The results obtained are in accordance with the past and more recent literature findings of French et Santiago Cintron, 2013 18, Nam et al., 2016 20, Carrillo-Varela et al., 2018 21, Ling et al. (2019) 22 and French, 2014 28. Recently and with careful XRD visual analysis, Edwards et al., 2018 30, assigned the peaks in the form of a shoulder at about 2θ of 12° accompanied by the peak at 2θ of 16.5° to the very small cellulose II, and the convex intensity in the 2θ area of 15°-17° to a mixture of three cellulosic: I (2θ area of 15°-17°), II (2θ of 12° and 2θ of 16.5°) and amorphous forms (2θ area of 18°). The assignment of the weak peak at 2θ : 12° to cellulose II was supported by numerous recent literature studies 20,21,30.

As presented in Fig. 1, from the recent sample (A₀) to the oldest one (A₅), all these peaks shifted to lower intensities suggesting the degradation of a crystalline form. Structural and chemical changes involved the crystalline and amorphous fraction of cellulose. Generally, amorphous cellulose is more susceptible (more reactive) than crystalline cellulose (stronger and less-reactive) to degradation process 22, since it is more accessible to water, microorganisms 14,31. An unusual increase for the feature peak typical of (004) crystallographic plane at 34.52° was manifested in the aged sample (A₅), generally presented by a very weak intensity peak. It indicates that a partial degraded cellulose might regenerate a native crystalline form and thus, contributing to the enrichment of the relative crystalline content upon time. Another proposition is that the crystalline structure cannot be destroyed, because decreasing elasticity due to higher thermal degradation causes a lack of sheer stress which destroys the hydrogen bonding of the crystalline structure, and consequently the increase in peak intensity related to crystalline cellulose fraction 22.

The changes that might occur in these fractions upon exposure to the unfavorable environment and the effect of deterioration on microcrystalline structure were also investigated based on two calculated parameters: crystallinity index and crystallite size. The obtained values of these parameters for studied samples are plotted in Table 2. It is worth to mention that the crystallinity index of cellulose I is given by comparing the minimum in intensity above baseline at 2θ =16.5° (Iₐ₀), and the maximum in intensity at 2θ =22.9° (Iₐ₀), accounting for the amorphous part and the crystalline part (major diffraction from the 200 plane), respectively 6,28,50,21,22. This parameter varies significantly depending on the choice of the measurement method. The crystallite sizes were calculated from 101, 110 and 002 lattice planes, respectively.

Table 2. Crystallite size and Crystallinity index values of cellulose from hardwood.

<table>
<thead>
<tr>
<th>Sample</th>
<th>D(101) (nm)</th>
<th>D(110) (nm)</th>
<th>D(020) (nm)</th>
<th>Cr.I (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₀</td>
<td>-</td>
<td>0.56</td>
<td>0.408</td>
<td>37.96</td>
</tr>
<tr>
<td>A₄</td>
<td>0.622</td>
<td>-</td>
<td>0.397</td>
<td>25.36</td>
</tr>
<tr>
<td>A₅</td>
<td>0.644</td>
<td>-</td>
<td>0.419</td>
<td>19.50</td>
</tr>
</tbody>
</table>
It is shown that all amorphous cellulosic samples possessed a lower value of the crystallinity index. This can be referred to the sensibility of the analyzed sample to degradation and/or the presence of amorphous components as lignin and hemicelluloses that influence the estimation of crystallinity amount. It is reported in the literature that the presence of lignin lowers the crystallinity estimation by X-ray diffraction.

The Cr.I index value was 37.96% for the non-aged sample A₄ and slightly decreased reaching 19.50% for the oldest sample A₁ (Table 2). This indicated that the crystalline region of cellulose fraction began to undergo chemical changes because of different natural degradation mechanisms as oxidation, hydrolysis. Also, the reduction of the crystalline amount of cellulose can refer to the destruction of hydrogen bonds in the crystalline cellulose fraction. The latter becomes small, and therefore, the cellulose microfibrils become harder and brittler efficiency causing efficient cut of hydrogen bonds, consequently, they become easy to destroy.

Generally, the crystalline cellulose structure is directly related to the hydrogen bonding between intermolecular hydroxyl groups and included water. Consequently, the loss of crystallinity is considered to result from opening of glucopyranose rings and destruction of their ordered packing. The results are by literature data, suggesting that the rapid evaporation of water causes limits the chain mobility of cellulose, and consequently, its alignment, which decreases the crystallinity feature. Thus, we can confirm that our samples underwent thermal degradation.

The small size differences may not be significant because of the calculations may have some uncertainties. So, the non-significant changes observed on D290 crystallite size (Table 2), mean that the microcrystalline structure was not significantly affected and broken by natural degradation process. Compared to the recent sample, the crystallite sizes of both A₄ and A₁ sample showed similar values (at around 0.644) in the (101) and (020) planes, while no value was mentioned for their (110) direction planes. It means that the degradation affects a crystalline zone more than other and/or some of these zones degraded as faster than others. For the recent sample A₄, the crystallite size in (101) lattice plane could not be estimated due to the lower intensity of these peaks and to that the crystalline regions underwent degradation at a similar extent to the amorphous regions, thus the resulting materials showed no crystallites size. A mechanism of cellulose degradation in the natural environments proceeds through a depolymerisation step, involving the amorphous regions of cellulose, until the size of oligomers becomes sufficiently small to allow metabolism by microorganisms or solubilisation by water. Consequently, the reduction in crystallite size is correlated with the decrease in the crystalline mass fraction. It should be mentioned that the Segal crystallinity index can be influenced greatly by crystallite size.

**FTIR spectroscopy**

The FTIR spectra of the three analyzed samples are reported in Fig. 2 and their assignments are summarized in Table 3.

As presented in Fig. 2, the strong and broad absorption band centred at 3330 cm⁻¹ is generally characteristic of the mixture of both crystalline fractions I₇ and I₆ and assigned to O5-H5···O3 intramolecular hydrogen bonds in carbohydrates (cellulose crystals). A mixture of intra- and intermolecular hydrogen bonds in cellulose are detected in the region between 3500 and 3000 cm⁻¹. Concerning the crystalline form of cellulose, the monoclinic cellulose I₇ was identified by the slight peak at 3278 cm⁻¹ related to O6-H6···O3 intermolecular hydrogen bonds in carbohydrates, while in triclinic I₆ cellulose this band was detected at 3240 cm⁻¹. According to Popescu et al. 2016, the O-H intramolecular hydrogen bonds of phenolic lignin appear at 3553 cm⁻¹. Additionally, there are significant contributions from adsorbed water (weak absorption) arising from 1640 to 1620 cm⁻¹ in the amorphous region of the sample and at 3553 cm⁻¹ when it is weakly bonded. A noticeable decrease in the intensities of these bands with increasing time was observed in Fig. 2. It indicates effectively the water desorption phenomenon followed by the decrease in the hydrogen bond strength between the cellulose chains, leading to the destruction of crystalline cellulose structure. Recently, Broda et Popescu 2019 reported that the decrease in temperature reinforce the hydrogen bonds in the crystalline cellulose and microfibrils become harder and present a brittler efficiency.
Also, the cellulose of wood spectra typically shows multiple C-H (sp\(^3\)) stretching vibrations at 2850 that assigned to \(\nu(CH)\) and 2919 cm\(^{-1}\) ascribed to \(\nu(CH_2)\) and C-H in cellulose, and to \(\nu(CH)\) (methoxyl group) in lignin. Recently, Ling et al. 2019 attributed the signal at 2944 cm\(^{-1}\) to cellulose \(I_B\) accompanied with a shoulder at 2968 cm\(^{-1}\). As shown in Fig. 2, each FTIR spectrum presents the main characteristic peaks related to the mixture of crystallized and amorphous cellulose fractions. The presence of crystalline cellulose is confirmed by the three following signals at 1456 cm\(^{-1}\), 1372 cm\(^{-1}\), and 1314 cm\(^{-1}\). (bending mode vibration of C-H), while the amorphous content is justified by the presence of centered peak at 1425 cm\(^{-1}\) corresponding to \(\delta(CH_2)\) bending vibration (scissoring). According to Song et al. 2015 and Zghari et al. 2018 the crystalline cellulose I and cellulose II appear as doublet peak at 1430 cm\(^{-1}\) (strong band) and at 1420 cm\(^{-1}\) (weak band), respectively. Furthermore, Hajji et al. 2015 and Broda et al. 2019 report that the decline in the intensities of the two bands at 1314 and 1163 cm\(^{-1}\) is correlated to the decrease of cellulose crystallinity (cellulose I). Usually in amorphous cellulose (disordered structure), these bands were located at 1336 and 1156 cm\(^{-1}\), respectively.

The two absorptions bands at 1163 and 1156 cm\(^{-1}\) are related to the crystalline and amorphous form of cellulose, respectively, characteristics of the C-O-C asymmetric bridge stretching vibration of the glycosidic ring. According to a recent study by Ling et al. 2019, the introduced transformation of the two bands is considered as a key of included changes on fingerprint cellulose area, informing well on both distortions of crystalline arrangements and smaller crystallinity index. Similar spectral changes have been observed in our case study from recent sample \(A_1\) to aged ones \((A_d\) and \(A_s\)) indicating the conversion of crystalline content towards an amorphous fraction. This finding supports the hypothesis of distortion in cellulose crystalline arrangements. The fingerprint region of cellulose with prominent signal centred at 1031 cm\(^{-1}\) (C-O) is gradually shifted to the lower intensity from recent to altered sample \(A_s\). Additionally, the visible and notable reduction indicate well the occurred changes in the structural conformation of the aged cellulose sample \((A_s)\) which is more affected after 500 years of exposure to natural environmental degradation process.

Concerning the band at 899 cm\(^{-1}\) ascribed to C-O-C stretching vibration mode of cellulose \(\beta-(1-4)\)-glycosidic linkage is related to the content of amorphous cellulose which is completely in accordance with published literature studies. However, this band is sensitive to changes, and so, a portion of amorphous cellulose content underwent crystallization due to mechanochemical processes and/or to natural environmental degradation conditions, which is subject of many controversies data.

A clear indication has been reported by Song et al. 2015 which attributed the weak and broad absorption band to cellulose I, while the strong and

**Figure 2.** FTIR spectra 4000-500 cm\(^{-1}\) range acquired from hardwood samples.
sharp one might be assigned to cellulose II and amorphous cellulose. The similar finding was confirmed by Hajji et al. 2016 and Zghari et al. 2018 which reported that the ageing process promote the breakage of the C-O-C bonds of crystalline cellulose I in the β-(1-4)-glycosidic linkage.

In recent literature data, Ling et al. 2019 correlated the weak signal at 720 cm⁻¹ to the presence of a small amount of cellulose Iβ and ascribed it to CH₂ rocking vibration, while the tiny peak at 750 cm⁻¹ attributed to a small amount of cellulose Iα, the latter is supported by the presence of O-H stretching vibration mode at 3240 cm⁻¹.

The FTIR crystalline and amorphous results correlate well with the crystallinity index values measured in XRD section (X-ray diffraction).

### Table 3. Assignments of the main vibrations of cellulose in the hardwood FTIR spectra.

<table>
<thead>
<tr>
<th>Wavenumber (cm⁻¹)</th>
<th>Assignment</th>
<th>Cellulose fraction</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3330</td>
<td>ν(OH) stretching vibration of alcohol-bonded hydroxyl groups</td>
<td>crystalline Iβ + Iα</td>
<td>22,38,39,37</td>
</tr>
<tr>
<td>3278</td>
<td>O6 H6⋯O3 intermolecular hydrogen bonds</td>
<td>cellulose Iβ</td>
<td>22,27,39,40</td>
</tr>
<tr>
<td>3000-2850</td>
<td>νas CH₂ and νs CH₂ in methylene and νas CH₃, νs CH₂ in methyl groups</td>
<td>crystalline and amorphous</td>
<td>39,40,44</td>
</tr>
<tr>
<td>2900</td>
<td>νC-H stretching vibration</td>
<td>crystalline</td>
<td>22, 39,43</td>
</tr>
<tr>
<td>1456</td>
<td>δCH₂ asymmetric bending mode (scissoring)</td>
<td>crystalline</td>
<td>27,37,39,42</td>
</tr>
<tr>
<td>1425</td>
<td>δCH₂ symmetric bending (scissoring) crystalline cellulose I at 1430 cm⁻¹ (strong) and crystalline cellulose II at 1420 cm⁻¹ (weak) O-C-H in plane bending vibration</td>
<td>amorphous</td>
<td>42,43,19,37,39,40,27,16</td>
</tr>
<tr>
<td>1372</td>
<td>δC-H bending vibration</td>
<td>crystalline</td>
<td>22,42,43,44,19,37,39,27</td>
</tr>
<tr>
<td>1314</td>
<td>δCH₂ rocking</td>
<td>crystalline</td>
<td>22,27,19,37,40,38,39,44</td>
</tr>
<tr>
<td>1163</td>
<td>C-O-C asymmetric bridge stretching vibration</td>
<td>crystalline</td>
<td>22,37,38,40,27,43</td>
</tr>
<tr>
<td>1156</td>
<td>C-O-H anti-asymmetric in-plane stretching vibrations</td>
<td>more, amorphous</td>
<td></td>
</tr>
<tr>
<td>1110</td>
<td>C-O-C of β-(1-4)-glycosidic linkage bond (weak and broad in cellulose I, but strong and sharp in cellulose II and amorphous cellulose) and references cited</td>
<td>amorphous + (a portion of this band is sensitive to change and might undergo crystallization)</td>
<td>16,19,22,27,37,38,43</td>
</tr>
<tr>
<td>899</td>
<td>νC₁-O-C of β-(1-4)-glycosidic linkage bond (weak and broad in cellulose I, but strong and sharp in cellulose II and amorphous cellulose) and references cited</td>
<td>amorphous + (a portion of this band is sensitive to change and might undergo crystallization)</td>
<td>37,39,42,44</td>
</tr>
<tr>
<td>720</td>
<td>CH₂ rocking vibration in cellulose Iβ (cellulose Iα appear generally as a tiny peak at 750 cm⁻¹ and 3240 cm⁻¹)</td>
<td>a small amount of cellulose Iβ</td>
<td>22,27,37,40</td>
</tr>
<tr>
<td>650</td>
<td>δO-Hₗₑₒ out of plane bending mode (weak and broad band)</td>
<td>cellulose crystalline</td>
<td>37,40</td>
</tr>
</tbody>
</table>

The feature of crystallinity was influenced by different types of degradation mechanism, such as oxidation and hydrolysis. It increases the intensity of the C=O band, especially in the sample dating from the 15th centuries (Fig. 2 A₂). This could be justified by the presence of a large and intense absorption band between 1650-1732 cm⁻¹ ascribed to the stretching vibration of the C=O group, informing on the pronounced alteration. As can be illustrated in Fig 3, the opening chain which is the result of oxidation mechanism occurred on cellulose can be undergoing to the other types of oxidation resulting in another type of compound.
According to Agarwal et al. 16, the presence of lignin and hemicelluloses influences the amount of the crystalline form of cellulose fibers, and the lignin fraction is the most responsible for this evolution. The presence of the later fraction in all spectra (Fig. 2) is manifested by the following absorptions bands at 1595 and 1504 cm⁻¹ assigned to the νC=O skeletal vibration in the phenolic ring (lignin motif type guaiacyl, ie coniferyl) and at 1236 cm⁻¹ attributed to νC=O in lignin 27,37,38. Generally, a weak 1595 cm⁻¹ band stands for non-polar aromatic compounds, while a strong one stands for polar aromatic compounds such as phenols 27,38,46. In our case study, the very strong 1595 cm⁻¹ absorption indicates well the presence of high polar aromatic compounds of lignin (aromatic ring substituted by hydroxyl and/or methoxy groups : guaiacyl, ie conifer). The obtained result showed an excellent correlation with FT-Raman data (next section).

The small sharp detected at 897 cm⁻¹ (Fig. 2) represents the glycosidic C₁-H deformation with ring vibration contribution, which is characteristic of β(1-4) glycosidic linkages between glucose in amorphous cellulose 14,16,19,27,37,40. According to S. Acharya et al. 39, the ratio of integrated peaks at 1427 and 899 cm⁻¹ (A₁427/A₈99) has been used in order to estimate the IR empirical crystallinity index of cellulose, while in recent study conducted by 22 various IR peak ratios have been proposed using the three following ratios (A₁372/A₈95, A₁430/A₈94- A₁372/A₂900). It should be noted that the amorphous area of the celluloseic component was less affected by the degradation process. According to Boukir et al. 46 and Zghari et al. 35, the broad signal at 650 was attributed to OH out of plane bending 10,27,39,40.

FT-Raman spectroscopy

The FT-Raman technique was used to obtain more structural information on the hardwood cellulose samples. The FT-Raman spectra of the three samples are illustrated in Fig. 4. The complexity of FT-Raman spectra is due to the presence of many signals related to the cellulose, hemicelluloses and lignin which create a polymeric matter that produce a complex wooden matrix. All FT-Raman bands assignment related to the analysed materials were summarized in Table 4.

The FT-Raman spectra of cellulosic fibers can be visually divided into three regions (Fig. 4): 3350-2700 cm⁻¹, 1750-800 cm⁻¹ and 610-150 cm⁻¹ that include, respectively, methyl and methylene stretching vibrations, methyl bending and rocking as well as C-O-H in-plane bending and the skeletal bending vibrations (e.g. C-C-C, C-O-C, O-C-C and O-C-O) 47,48.

The broad CH stretching vibration band at 2897 cm⁻¹ characterize the CH₂ group in the glucopyranose ring of cellulose Ip 10. The doublet band at 1094 cm⁻¹ and 1119 cm⁻¹ were assigned to the symmetric and asymmetric β-(1,4)-glycosidic linkage stretching modes, respectively 47,49. The intensity ratio between these later provides information on the hydrolytic cleavage of the glycosidic ether bonds of the cellulose chains. According to Kavkler and Demsar 50, this ratio can be used as an indicator of ageing in different historic specimens. While, the disappearance of these two bands is a sign on an advance cellulose degradation step 31. In recent literature studies, Ling et al. 2019 22, Makarem et al. 2019 52 and Agarwal et al. 2016 53 reported that the regression of the two main bands at 1096 cm⁻¹ (fingerprint cellulose: broad and very intense) and 2900 cm⁻¹ is an indication on the distortion of crystalline arrangements, and thus, resulting a reduction of C.I.. The same trends have been shown in our samples from recent (Aₙ) to aged one (Aₐ) but more pronounced until the disappearance of all the bands in the case of very aged one (Aₐ), confirming well the complete deterioration of cellulose (Fig. 4).

Additionally, the amorphous cellulose fraction is detected in the Raman spectra by the presence of the following signals at 1455 cm⁻¹ 22,47 assigned to H-C-H bending with the contribution of small H proportion of H-OC bending, and at 896 cm⁻¹ ascribed to the C-H deformation 47. The two latter signals (amorphous content) influenced by the degradation phenomena underwent the same fate such others absorption bands (1096, 2900 cm⁻¹).
Figure 4. FT-Raman spectra 3500-0 cm\(^{-1}\) range acquired from hardwood samples.

The detected absorptions between 1300 and 1410 cm\(^{-1}\) were characteristic of \(\delta CH_2\) modes. Generally, the bands at 1476, 1455 and 431 cm\(^{-1}\) (bending \(\delta CCO\) and \(\delta CCC\)) are typical for crystalline cellulose \(I^\beta\). The arising peak at 519 cm\(^{-1}\) informs on the presence of glycosidic linkage deformation in hemicelluloses (\(\delta COC\), \(\delta CCC\)). The decrease in the intensities of these bands ascribed to loss of the structural order when interchain hydrogen bonds are broken, followed by the cleavage of glycosidic linkages that shortened chains, leading to the decrease of crystallinity depending on the prolonged sample age. The results obtained by FT-Raman seem to be in complete agreement with the crystallinity index values measured in XRD section (X-ray diffraction, Table 2) and match well with those of FTIR data (FTIR spectroscopy section).

Table 4. Main bands vibrations in hardwood cellulose samples FT-Raman spectra.

<table>
<thead>
<tr>
<th>Wavenumber (cm(^{-1}))</th>
<th>Assignments</th>
<th>Fraction</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>2897</td>
<td>(vCH) and (vCH_2)</td>
<td>cellulose (I^\beta)</td>
<td>22,47,52,53</td>
</tr>
<tr>
<td>1455-1462</td>
<td>HCH bending and small proportion of HOC bending</td>
<td>amorphous cellulose</td>
<td>22,47,51,54</td>
</tr>
<tr>
<td>1378</td>
<td>(CH_2) wagging mode</td>
<td></td>
<td>47,55</td>
</tr>
<tr>
<td>1331 + 1119 + 990 +</td>
<td>bending vibrations of cellulose glycosidic linkages and/or Coupled C-O-C and CC stretching modes</td>
<td>amorphous cellulose</td>
<td>22,10,47,51,55</td>
</tr>
<tr>
<td>1094</td>
<td>Coupled C-O-C and CC stretching modes</td>
<td>crystalline cellulose</td>
<td>22,52,53,47,10,51,55</td>
</tr>
<tr>
<td>896</td>
<td>(CH) deformation</td>
<td>amorphous cellulose</td>
<td>47,51</td>
</tr>
<tr>
<td>763</td>
<td>(\beta)-anomer glucopyranose (very weak band)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>519</td>
<td>CCO deformations of (\beta)-anomer glucopyranose</td>
<td>cellulose</td>
<td>55</td>
</tr>
<tr>
<td>431</td>
<td>(\delta(CCO))</td>
<td>crystalline cellulose</td>
<td>54</td>
</tr>
<tr>
<td>378</td>
<td>symmetric bending vibration of pyranose rings and/or (\delta_s(CCC))</td>
<td>crystalline cellulose</td>
<td>22,52,53,47,51,54</td>
</tr>
</tbody>
</table>

It is noteworthy that the evolution of cellulose crystallinity was influenced by the presence of amorphous content, such as lignin and hemicelluloses. In our spectra, this can be confirmed by the typical bands at 1655, 1599 and 1267 cm\(^{-1}\) that correspond, respectively, to the conjugated C=C bonds.
stretching vibration of coniferyl alcohol (guaiacyl) overlapping with vC=O group of coniferyl acid after alcohol oxidation in side chain, and with polar aromatic C=C in phenolic compounds related to guaiacyl and syringyl monomers in lignin.  

The region bands between 300 and 600 cm\(^{-1}\) cannot be unambiguously attributed to certain vibrations, and might be assigned to δC-C deformation in crystalline fraction of cellulose \(^{22,47,50,52-55}\), while Dudek et al. \(^{35}\) have considered this region as a key of the information and signature of the absolute configuration of the anomeric centre of glycosidic sugar.

**Conclusion**

The present work developed a detailed evaluation of cellulose structure as a component of Moroccan hardwood materials as well as the changes that occur at its microcrystalline fraction. Based on the X-ray diffraction obtained results, the cellulose lost its property of crystallinity during exposure time to the natural degradation process. The FTIR spectroscopy and FT-Raman results converge towards the conclusion that the evolution of cellulose crystallinity was influenced by the presence of lignin and hemicelluloses. The used methods were considered as ones of the best vibrational techniques that can be applicable to analyze cellulose polymorphs and evaluate, exhaustively, crystallinity behaviors as well as structural changes on other wood components as lignin and hemicelluloses.

**Acknowledgement**

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**References**


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