



Thermal, Structural and Morphological Studies of Cellulose and Cellulose Nanofibers Extracted from Bitter Watermelon of the Cucurbitaceae Family

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Abstract

This work presents a new way to prepare and isolate nanometric cellulose fibrils as extracted from *Citrullus Colocynthis* (*Cc*) seeds. The nanofibrils were then characterized using spectroscopic, thermal and morphological/structural techniques. IR-Raman spectra of cellulose fibers confirmed that the treatment they went through, effectively removed all non-cellulosic material (i.e. hemicellulose and lignin). In addition to that, TGA results indicated a good and higher thermal stability of the extracted cellulose. HPLC and ¹H NMR measurements were carried out to estimate the chemical composition of cellulose from *Cc* seeds. TEM confirmed the presence of nanofibers in the treated sample; XRD (WAXS-SAXS) data show that the cellulose nanofibers have a higher crystallinity. The preparation (extraction and treatment) of cellulose and cellulose nanofibers from the *Cc* seeds as well as their thermal, structural and morphological characterizations, were successful.

Keywords *Citrullus colocynthis* seeds · Cellulose · Cellulose nanofibers · Thermal · Structural · Morphological study

Abbreviations

Cc *Citrullus Colocynthis*

Introduction

The use of biomass for the production of energy and materials has been the object of academic and industrial research for a sustainable development thus mitigating environmental problems [1, 2]. Now, the natural fibers are used that are naturally produced by animals and plants because being more economical, renewable, non-toxic, easily processable and biodegradable. Among natural fibers, cellulose fibers constitute rich sources of cellulose and are found to be the most abundant biopolymer on earth [3].

In this context, cellulose is one of the most representative bio-material that is richly present in plants because this

biopolymer showed many advantages over other fibers [4, 5]. In the last decades, cellulose has given more importance in the field of nanotechnology which allows producing particles with nanometric dimensions [6]. Significant effort is being given to the development of Cellulose nanofibers as they are abundant in plants and woods, and their composites comprise an attractive contemporary research line [7] because of their unique characteristics, such as renewability, biodegradability, processing flexibility, low density, high specific strength and low-cost [3, 8]. Also, nanofibers have applications in various fields such as bioenergy industries, automobiles, paper manufacturing and textile owing to their properties and broad availability [9, 10]. Among various methods applied for nanocellulose and nanofibers production is acid hydrolysis. This treatment helps in the digestion of less ordered amorphous region of cellulose microfibrils leaving the nanocrystalline particles [9].

Several plants are rich in cellulose; besides, cellulose fibers have been extracted from several sources [8]. In the present study, the isolation of cellulose and cellulose nanofibers from *Citrullus Colocynthis* (*Cc*) (a renewable resource from many biomasses) is investigated.

Cc (Cucurbitaceae family) is an herbaceous perennial plant that is widespread in the sandy soils of the hot and arid regions [11, 12]. *Cc* has been studied for its biological activities [13] and for their wide range in traditional medicine

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uses. The fruits and most parts of *Cc* have been investigated for the presence of several compounds [14, 15].

This work aimed, firstly, to add value to *Cc* by using their seeds as raw material. Secondly, to produce cellulose from *Cc* seeds. Then, this cellulose was transformed into nanocellulose for its future use as nanofibers for biodegradable matrices. Through such a study, we aimed also to complete a previous work on the same materials (see Kouadri and Satha, 2018) [11] which focused on thermal and structural properties. Finally, the purified cellulose and cellulose nanofibers were examined by analytical techniques for shows its different aspect and properties.

Materials and Methods

Raw Material

The fruits of *Cc* of the family Cucurbitaceae was collected from the surroundings of the city Ouargla (located in the south-east region of Algeria). The seeds are recovered from the fruits and dried in the dark. The dried seeds are then ground into a fine powder (Fig. 1).

Isolation of Cellulose

The cellulose extraction method used in this work, has been fully described by Kouadri and Satha, 2018 [11]. In brief, A three-step extraction protocol was carried out: (i)

a deproteinization process with NaOH solution, followed by (ii) a lipid extraction process with a chloroform/methanol solution, then (iii) a bleaching process with an aqueous chlorite solution.

Nanofibers Production

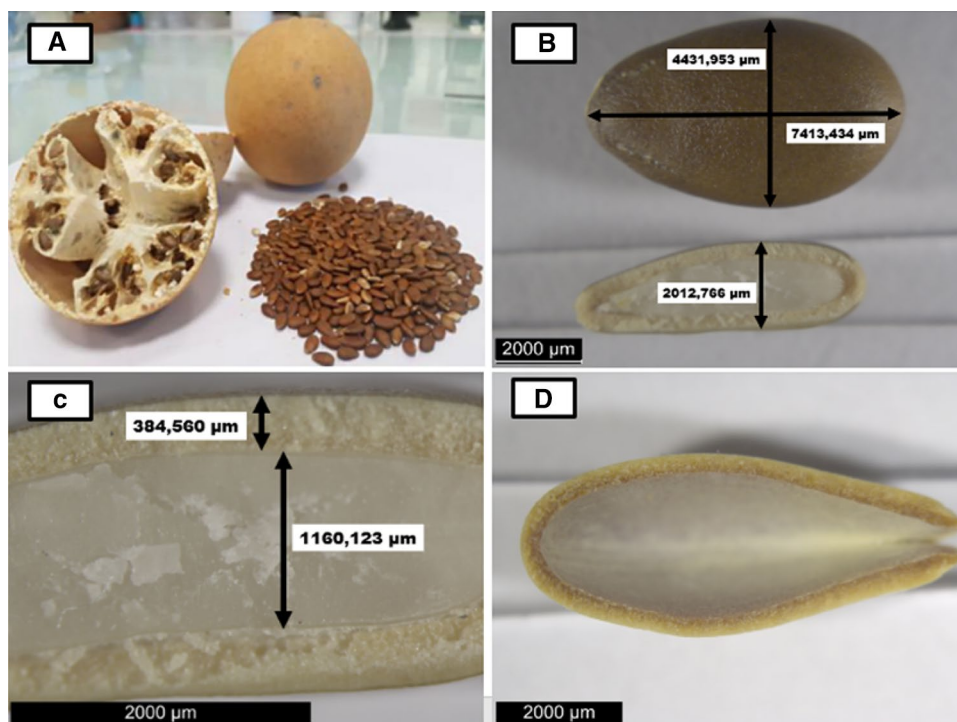
Nanofibers were prepared by the acid hydrolysis of obtained cellulose from *Cc* according to the acid-hydrolysis method of Kouadri and Satha, 2018 [11]. This step could be summarized as follows: the acid hydrolysis was carried out with sulfuric acid (H_2SO_4) solution 40 wt% at room temperature, 4 h in the ratio powder/ solution 20:1 (mg/mL), under vigorous stirring. The obtained product was referred to as cellulose nanofibers.

Characterization Methods

Infra-red Raman Spectroscopy Analysis (IR-Raman)

Infra-red Raman spectra were obtained using an Infra-red Raman spectrometer (Ramspec 1064 from Bayspec/Photon-Lines). The measurements were collected in the range from 0 to 4000 cm^{-1} with a resolution of 4 cm^{-1} ; each spectrum was an average of 4 scans. The laser ($\lambda = 1064\text{ nm}$) incident beam was focused on a spot diameter of about 1 mm on the sample.

Fig. 1 Pictures of; **a** *Cc* with its fruits and seeds, **b** Seed and its cross section with their dimension, **c** Shape and sizes of seed inside, **d** Outer shell of the seed without their pulp



Thermogravimetric Analysis (TGA)

Thermal degradation analysis of cellulose extracted from *Cc* was performed using a model TAG-Q500 (TA Instruments, New Castle, DE) instrument at a 10 °C/min heating rate from 25 to 800 °C under nitrogen flow. All the samples were vacuum oven-dried at 60 °C for 24 h before testing.

¹H NMR Spectroscopy

¹H NMR spectra were obtained using a Bruker DRX-300 MHz spectrometer (ULTRASHIELD) and Sulfuric acid-d₂ solution (D₂SO₄) as a solvent. For hydrolysis of the glycosidic bonds; 20 mg of cellulose of *Cc* is hydrolyzed by 1 mL of Sulfuric acid-d₂ solution (D₂SO₄/D₂O) (50%), at room temperature for 12 h, in closed tubes. The ¹H NMR spectra were treated with a TOPSPIN 3, 2 Software.

Transmission Electron Microscopy (TEM)

For imaging, a transmission electron microscope Philips CM120 at an accelerating voltage of 120 kV, was used. A droplet of 5 μl of "cellulose nanofibers" dispersion, was deposited onto a carbon film coated on a copper grid. To keep the sample onto the grid, excess solution was not blotted off, as usual, but was only let air-dried at room temperature.

High-Performance Liquid Chromatography (HPLC)

HPLC was used to determine the purity of extracted cellulose and to determine the monosaccharide composition simultaneously. Briefly, the polysaccharides samples were hydrolyzed with cellulase enzyme. After enzymatic hydrolysis, the monosaccharide was identified by analytical HPLC using a Shimadzu system equipped with an SPD-20A ultraviolet detector and a Shodex column. The mobile phase was a mixture consisting of a solvent A (Acetonitrile) and a solvent B (Water) at a ratio of 70%, 30% (v/v) respectively with a flow rate of 1 mL/min and UV absorption was measured at 195 nm. The injection volume was 20 μL and the column temperature was 30 °C. The sugar was identified by comparison to a reference monosaccharide (glucose). The same steps were used to label the standard monosaccharide, D-Glucose. The Samples for HPLC analysis were filtered through the 0.22 μm membrane filters.

Enzymatic Hydrolysis

Enzymatic hydrolysis pretreatments of *Cc* cellulose were carried out at solids consistency of 125 mg in a 5 ml of acetate buffer of pH 4.5. Enzyme charge of 300 μl cellulase was used. The slurry was incubated at 50 C in an incubator

shaker at 150 rpm for 6 h. This step aimed to attack the bonds present in the cellulose of *Cc*, cellulase helped to cleave the b-1,4 glycosidic bonds, also isolating the glucose monomers.

X-ray Diffraction (X-RD) (WAXS and SAXS)

SAXS measurements are widely used to analyze nanoscale dimensions, structures, and shapes of nanoparticles, biomolecules, polymers, and mesoporous solids [16]. In this study, Wide-angle and Small-angle X-ray scattering (i.e. WAXS and SAXS) were used to determine the crystalline structure of cellulose nanofibers from *Cc*.

Results and Discussion

IR-Raman Spectroscopy Analysis

Figure 2 shows the structural identification by IR-Raman spectroscopy analysis. The identification of the polysaccharide of cellulose, obtained by isolation from the raw materials of *Cc* in the range of 0–4000 cm⁻¹.

The characteristic IR-Raman signals as depicted in Fig. 2, characterized by the presence of vibrations signals about 1094 cm⁻¹, which are attributed to C–H and C–H₂ stretching and asymmetric stretching vibration of the C–O–C glycosidic linkage [17–19]. However, the peaks at 1479 cm⁻¹ indicate H–C–H bending; the characteristic peak at 1377 cm⁻¹ represents H–O–C bending respectively [11, 12, 20, 21]. According to Kouadri and Satha (2018) [11], the final spectrum is indicative of commercial microcrystalline cellulose. Finally, and according to these results, there is no difference between the spectra of samples of the cellulose of *Cc* and pure cellulose [11, 12].

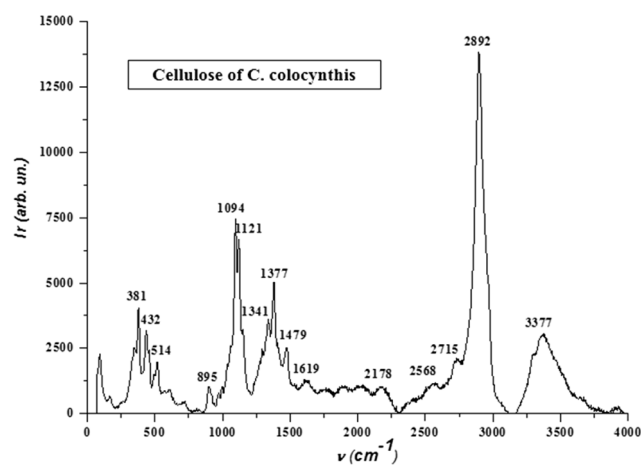


Fig. 2 IR-Raman spectroscopy of Cellulose from *Cc* seeds

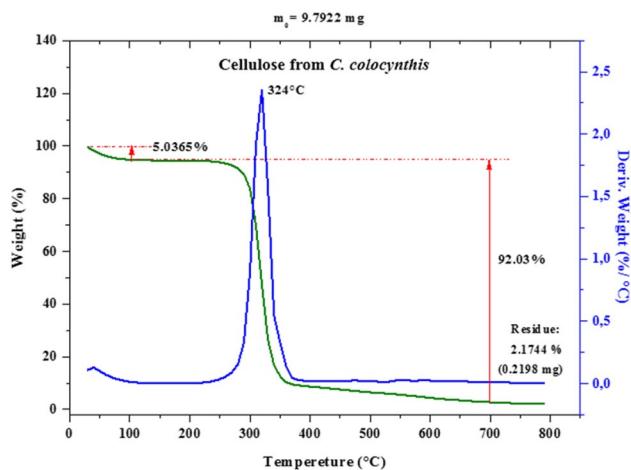


Fig. 3 ATG Thermograms of Cc Cellulose

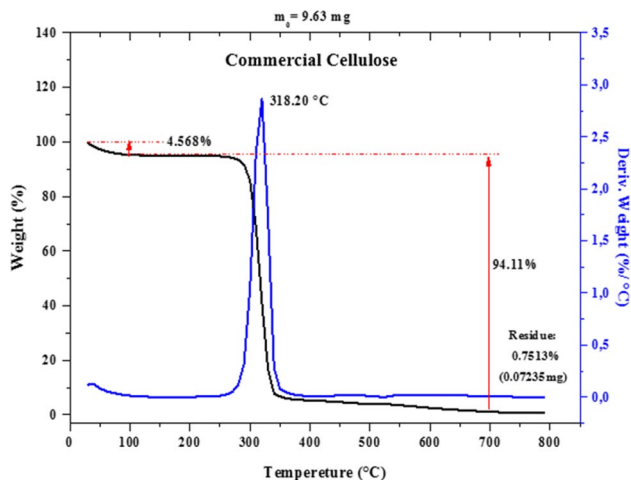


Fig. 4 ATG Thermograms of Commercial Cellulose

Thermogravimetric Analysis (TGA)

The thermal stability of commercial cellulose and cellulose extracted from *Cc* was studied by thermogravimetric analysis. The evolution of the percentage of the sample masses, as well as its derivatives in Figs. 3 and 4.

The thermograms show two main mass losses. The first, of the order of 4–5%, starts at room temperature up to 100 °C for both samples, and is characteristic of the loss of water, and the second from 100 to 700 °C, is characteristic

of the degradation of vegetal material. The maximum of the peak of the derivative is at 324 °C and 318 °C for Cc cellulose and commercial cellulose respectively. Above 700 °C, the residues represent about 1% and 2%. TGA data are summarized in Table 1.

Experimental results of the thermogravimetric analysis allow observing a behavior common to the degradation between the commercial cellulose and the cellulose of *Cc*. So, there is a similarity between the two samples; thus, the final extract is cellulose. The extracted cellulose is a homogeneous material. This result is verified by another study, for the purest cellulose [22]. Chemical purity increases the thermal stability of cellulose [22, 23]. Lignin, on the contrary, begins to degrade towards 180 °C, because of its heterogeneous structure having less stability. The thermal stability of the plant wall depends on the chemical structure of its main constituents: several studies [24] have shown that cellulose more stable in front of lignin and hemicellulose. The thermal stability of the plant wall depends mainly on cellulose and lignin. The results indicate that the extracted cellulose has the same thermal behavior of the commercial sample.

¹H NMR Spectroscopy

Figure 5 shows the ¹H NMR spectra of commercial glucose, commercial cellulose, and *Cc* Cellulose.

The ¹H NMR spectrum of the commercial cellulose was obtained in the D₂SO₄ solvent (50%). Also, the Cellulose of *Cc* was carried out under the same conditions to make comparisons. Regardless of the spectrum of commercial cellulose and commercial glucose, they show very similar peaks with the same chemical shift. In comparison with *Cc* cellulose, the spectrum also has characteristic signals that clearly distinguish cellulose from glucose. So, the spectra are identical, but they appear as small peaks in *Cc* Cellulose (are almost negligible); this may be due to impurities in the extract, or in the solvent. Thus, the results obtained by ¹H NMR confirm that the structure of extract obtained from *Cc* after bleaching is very similar to commercial cellulose and glucose, and also confirm the efficiency of solubility of cellulose with this method of acid hydrolysis with D₂SO₄ of 50% concentration, at room temperature for 12 h. The conclusion from the results obtained by ¹H NMR is that the insoluble final extract of *Cc* after bleaching is cellulose.

Table 1 Thermogravimetric data analysis

	1 ^{er} mass loss (%) (100 °C)	Degradation (°C)	2 nd mass loss (%) (700 °C)	Residues (%)
Commercial cellulose	4,568	318,20	94,11	≈ 1
<i>Cc</i> Cellulose	5,036	324	92,03	2

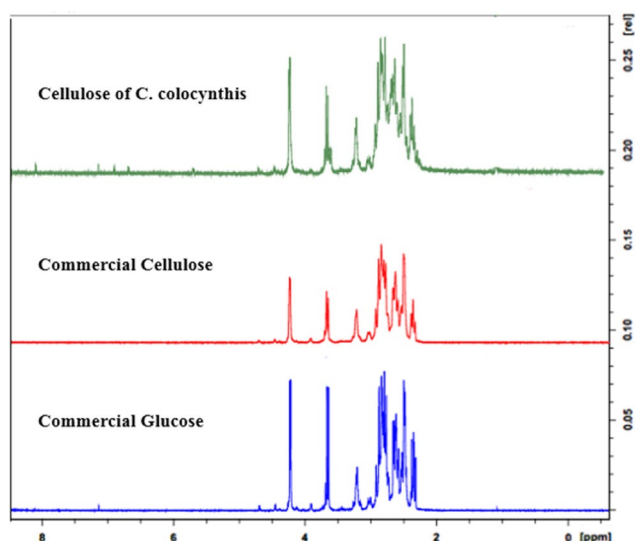


Fig. 5 ^1H NMR spectra of Cellulose, Glucose commercial and Cellulose of *Cc*

Transmission Electron Microscopy (TEM)

Morphological inspection in this study has been employed by Transmission electron microscopy (TEM). Figure 6 shows the TEM micrographics of the nanofibers of cellulose from *Cc* under various magnifications.

TEM images confirmed the presence of nanofibers obtained after the acid extraction and the cellulose nanofibers had nanometric diameters. The TEM images show that the chemical and mechanical treatments removed the amorphous components. The nanofibers comprised a network structure as entangled filaments are randomly oriented in arrangement and it have no regular surface because they consist of crystalline and amorphous regions. [25, 26]. The fibers underwent morphological alterations with all the treatment steps that changed the fibers structurally and chemically [27].

High-Performance Liquid Chromatography (HPLC)

Figure 7 shows the influence of chemical and enzymatic treatments on the crystalline nature of the resulting cellulose.

Enzymatic hydrolysis is a two-phase biochemistry process between cellulase and cellulose, and thus the greater the contact between the cellulases and the substrates, the higher the enzymatic hydrolysis efficiency [28]. The purpose of using enzymatic degradation is to assess the release of glucose unit after the treatment with cellulase. For compositional analysis, in Fig. 7(D) glucose is the predominant neutral sugar component in the raw cellulose samples, indicating a much higher purity compared to the corresponding raw cellulose preparations. This phenomenon revealed that, under the conditions given, the enzymatic medium could attack the bonds present in the cellulose of *Cc*, and the cellulase enzyme helped to cleave the β -1,4 glycosidic bonds. In other words, the enzyme cellulase had no difficulty in solubilizing cellulose; so, it was able to penetrate the cellulose chains, which made cellulose hydrolysis easy.

X-ray Scattering (SAXS/WAXS)

Figure 8 shows the WAXS and SAXS patterns of nanofibers cellulose after acid hydrolysis.

Approximately 6 well-defined diffraction peaks are observed. However, the behavior of the WAXS intensity depends on the range of the q value. In the 2D WAXS pattern that was measured from the precipitate after hydrolysis, several sharp diffraction rings appeared. In the azimuthally averaged WAXS profile, the corresponding diffraction peaks are visible underneath the diffuse scattering from the single particles. The peak positions can be explained by the formation of crystals. In other words, the crystallinity was improved. This may be attributed to the improvement in the integrity of the crystal structure. These results suggest that the structure of the amorphous phase of nanofibers cellulose changes after acid hydrolysis. The increase in crystallinity after hydrolysis is due to the

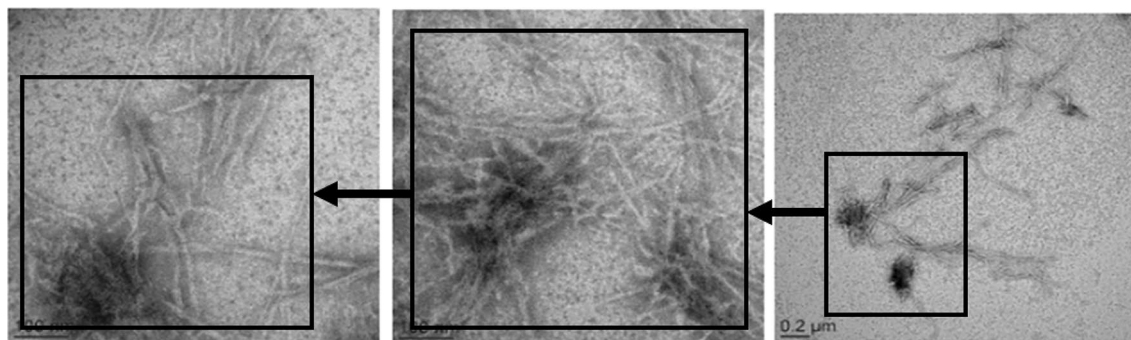


Fig. 6 Transmission electron micrograph (TEM) of cellulose nanofibers obtained from *Cc* seeds after acid hydrolysis

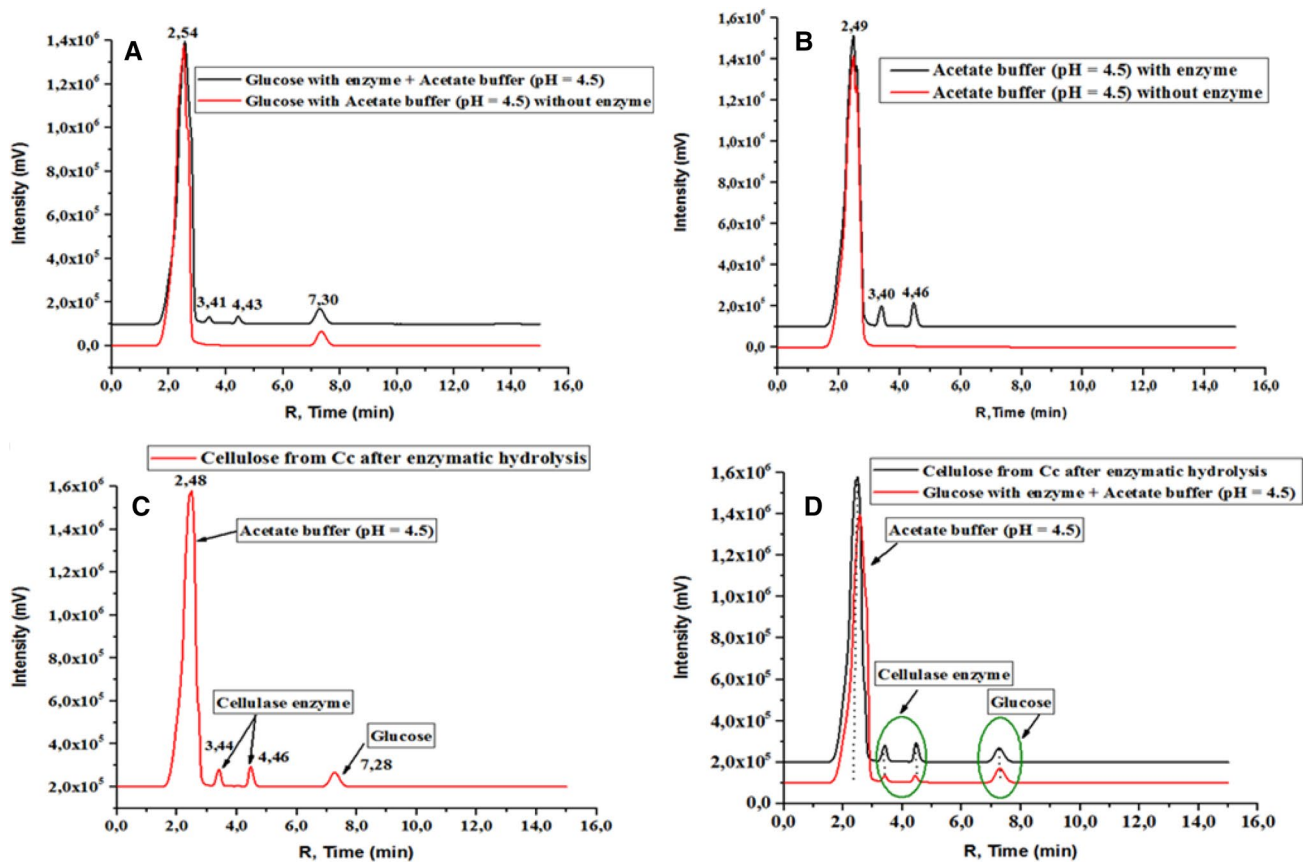


Fig. 7 High-Performance Liquid Chromatography (HPLC) analysis of Glucose commercial and Cellulose of Cc after enzymatic hydrolysis

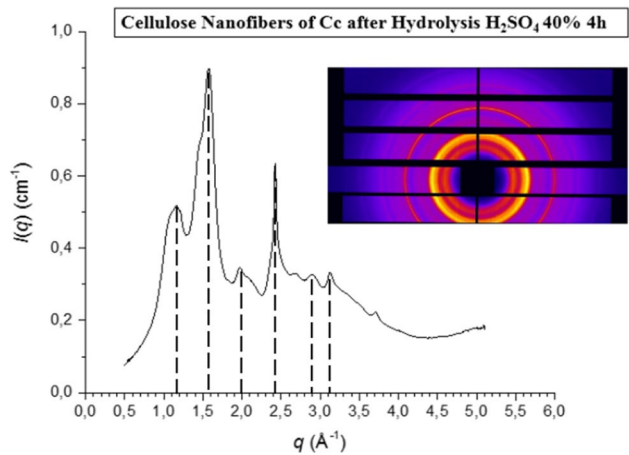


Fig. 8 X-ray scattering (SAXS/WAXS) analysis of Cellulose nanofibers of Cc after hydrolysis acid

different arrangements of the glucose chains after removal of the amorphous phase. Whereas the large increase of the crystallinity index highlights the preferential attack of the amorphous parts of cellulose during the sulfuric acid hydrolysis [16, 29–32].

Conclusion

The main goal of this work was to investigate the viability of Cc seeds as a novel, supportable, low-cost, renewable biosource, and the process of cellulose isolation and cellulose nanofibers are environmentally friendly. The purified cellulose and cellulose nanofibers were examined by analytical techniques for shows its different aspect and properties. Thermogravimetric analysis showed that, at 100 °C the water is evaporated from the surface and a maximum decomposition peak at 324 °C. The compositional analysis showed that formation of the molecular structure of cellulose from Cc seeds is that of glucose as confirmed by acid and enzymatic hydrolysis. Morphological analysis showed that cellulose nanofibers consist of uniform nanometer crystalline bundles and had a higher crystalline fraction. In conclusion, we successfully isolated the cellulose and cellulose nanofibers from the Cc seeds and these results are encouraging and could be translated to the use of this agroindustry residue as a renewable source of nanofibers. These findings indicate that cellulose nanofibers extracted to be used, in the future, as organic filler, with a good reinforcement effect in composite materials.

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