

EXTRACTION AND CHARACTERIZATION OF CELLULOSE NANOCRYSTALS FROM CORN STOVER

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The aim of this study was to explore the utilization of corn stover as a source of raw material for the production of cellulose nanocrystals. Corn stover was first purified using chemical extraction and bleaching and then separated into nanocrystals by acid hydrolysis. The chemical composition and characteristics of corn stover were studied before and after purification according to a TAPPI standard and by X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR) and thermogravimetric analysis (TGA). Morphological features of the nanostructures were studied using transmission electron microscopy (TEM). The chemical composition of the employed corn stover was 45.5 wt% cellulose, 27.6 wt% hemicellulose and 6.8 wt% lignin. The crystallinity of the corn stover was 25.3%, and it increased to more than 43% after the acid hydrolysis. Changes in the peaks of the FTIR spectrum at 1731, 1556 and 1244 cm^{-1} indicated that the alkali treatment partly removed hemicelluloses and lignin from the fiber surface. The corn stover had better thermal stability than those of the cellulose obtained after the bleaching treatment and of the nanocrystals isolated following acid hydrolysis. The TEM study indicated that the nanosized crystals had an average diameter and length of 6.9 nm and 356 nm, respectively, leading to an aspect ratio of approximately 54.7. Therefore, the produced corn stover nanocrystals possessed great potential as strengthening agents in nanocomposites.

Keywords: corn stover, cellulose nanocrystals, acid hydrolysis

INTRODUCTION

Cellulose is the most abundant polymer available on earth and can be obtained from numerous resources, such as wood, eucalyptus, sisal, cotton, coconut fibers, and non-plant sources, including forms produced by bacteria and found in tunicates.¹ Cellulose is a carbohydrate formed by both crystalline and disordered amorphous domains. Its structure is organized into fibrils, which are surrounded by a matrix of lignin, extractive inorganics and hemicellulose.² Thus, these components must be extracted and cellulose chains must be deconstructed to extract cellulose nanocrystals from plant fibers.

Cellulose nanocrystals consisting of highly crystalline rod-shaped nanoparticles can be obtained through controlled acid hydrolysis or

enzymatic hydrolysis of different cellulose sources.³ The main benefits linked to this type of nanoparticles include exceptional mechanical properties (high specific strength and modulus), large specific surface area, high aspect ratio, low environmental impact, and reduced production cost.⁴⁻⁶

At present, acid hydrolysis (using sulfuric or hydrochloric acid) is the preferred route for obtaining cellulose nanocrystals, because it leads to a preferential digestion of the amorphous domains of the material and cleavage of the nanofibril bundles.⁷ In turn, this process results in the breaking down of the hierarchical structure of the material into crystalline nanofibers usually referred to as cellulose nanocrystals.

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Various agro-industries generate large amounts of cellulosic waste yearly. There is a great demand to find other end uses for these “agricultural cellulosic wastes”.^{8,9} Corn stover is an example of such an agro-industrial by-product that requires new end uses. Although corn crops have great importance in global agribusiness, with an annual production of approximately 870.5 million tons, corn stover is currently used only for the production of cigarettes and candy packaging and in basketry and doll crafting.¹⁰ In this context, this lignocellulosic fiber is an interesting, actively researched, starting material for production of cellulose nanocrystals for use as reinforcement in different polymer matrixes.

This study addresses the isolation of cellulose nanocrystals from bleached corn stover. The chemical composition of the residue used was evaluated and calculated based on the TAPPI standard. Fibers and cellulose nanocrystals were characterized by X-ray diffraction, FTIR spectroscopy and thermogravimetric analysis (TGA). The morphological features of the nanostructures were studied with transmission electron microscopy (TEM).

EXPERIMENTAL

Materials

The corn stover was provided by the company Juarez Francisco da Cruz Ltda. (Salvador, Bahia, Brazil). The chemical agents used for fiber surface modifications and bleaching (sodium hydroxide, sodium chlorite, sulfuric acid and acetic acid) were purchased from Vetec Química Fina (Duque de Caxias, Brazil). Cellulose membrane dialysis tubing D9777-100 FTO was purchased from Sigma-Aldrich (Saint Louis, MO, USA). All of the chemicals were reagent grade or higher in purity and were used as received without further purification.

Chemical composition

The determination of the main constituents of the corn stover was performed according to the method of Van Soest,¹¹ and the following TAPPI standards: TAPPI T222 om-88 for lignin and TAPPI T19 m-54 for hemicellulose and cellulose.

Pre-treatment of corn stover

Corn stover was subjected to a washing pre-treatment to remove the impurities and waxy substances covering the external surface of the fibers. First, fibers were ground in a mill (Tecnal, TE-645, Piracicaba, SP, Brazil) and sieved through a 40-mesh sieve. Then, these fibers (30 grams) were washed with NaOH solution 2% (1.200 mL) under agitation for 4 hours at 80 °C (Ika, C-Mag HS4, Campinas, SP,

Brazil). The resulting solution was filtered and washed with water to obtain the pulp. The washing process was repeated four times for the complete removal of agents soluble in water.^{8,12}

As lignin hinders fiber separation by acid hydrolysis, partial delignification (bleaching) was performed to facilitate further nanocrystals extraction. Partial delignification was carried out using a mixture of sodium hypochlorite 1.7% (300 mL) and buffer solution (300 mL). The resulting solution was placed under constant agitation (Ika, C-Mag HS4, Campinas, SP, Brazil) at a temperature of 80 °C for 6 hours and then filtered and dried in an oven (Tecnal, TE 394/2, Piracicaba, SP, Brazil). The final product of this procedure was the pulp.

Sulfuric acid hydrolysis

Sulfuric acid hydrolysis of the corn stover was performed as described in the literature, with minor modifications.^{13,14} Briefly, after bleach treatment of the fiber using NaOH and NaClO₂ (sodium chlorite), the resulting material was ground until a fine particulate was obtained. Then, 10.0 g of cellulose was added to 160.0 mL of 55 wt% sulfuric acid under strong mechanical stirring (Ika, RW 20, Campinas, SP, Brazil). Hydrolysis was performed at 50 °C for approximately 15 min as described by Silva *et al.*¹⁵ with modifications. After hydrolysis, the dispersion was diluted twofold in water, and the suspension was washed using three repeated centrifuge cycles (Eppendorf, 5702R, Hamburg, Germany). The last washing was conducted using dialysis against deionised water until the dispersion reached ~ pH6. A stable suspension of cellulose nanocrystals was obtained through sonication (Tecnal, USC 1850, Piracicaba, SP, Brazil) for approximately 5 min

Characterization

To determine the fiber length, width and aspect ratio (length-width) and to indicate the aggregation state of the nanocrystals, highly diluted samples of the hydrolyzed suspension were analyzed by transmission electron microscopy (TEM). The fibril solution was mixed in equal volumes with 2% aqueous uranyl acetate (UA). A 10 mL drop of the UA-nanocrystals mixture was dispensed onto a 400 mesh copper grid and allowed to stand for 30-60 s, and the excess fluid was wicked off with Whatman No. 1 filter paper. The grid was air dried and viewed in a CM12 scanning-transmission electron microscope (STEM) (FEI Co., Inc., Hillsboro, OR) operating in the bright field mode at 80 kV. Digital images were captured with the STEM's associated XR41 CCD camera system (AMT, Danvers, MA). The lengths and widths were measured directly from transmission electron micrographs using Image Tool 6.3 (Media Cybernetics, Inc., Bethesda, MD), and 30 measurements were used for each condition to determine the average and standard deviation values. Data were collected and analyzed.

X-ray powder diffraction analyses were performed with a Shimadzu diffractometer (XRD-6000, USA) operated at 40 kV and 30 mA with graphite filtered CuK ($\lambda = 1.5433 \text{ \AA}$) radiation. Data were acquired on a 2θ scale from 5 to 40° . In addition to corn stover and nanocrystals, the cellulose obtained after the bleaching process was also examined. The crystalline index of cellulose, C_{Ir} , was determined using Segal's empirical method:¹⁶

$$C_{Ir(\%)} = (I_{200} - I_{am})/I_{200} \times 100 \quad (1)$$

where I_{200} is the peak intensity corresponding to crystalline cellulose I, and I_{am} is the peak intensity of the amorphous fraction.

The Fourier transform infrared (FTIR) spectroscopy measurements (Shimadzu, IRPrestige-21, USA) were made with KBr pellets in the $400\text{-}4000 \text{ cm}^{-1}$ region with a 4 cm^{-1} resolution and 50 scans (Shimadzu, IRPrestige-21, USA). The mixture of KBr and fibers was dried (100°C , 1 h), and the samples were prepared immediately before measurement. The background spectra were collected by employing spectroscopic grade KBr.

Thermogravimetric analyses (TGA) of corn stover, cellulose obtained after bleaching treatment, and nanocrystals were performed in a Pyris 1 thermal analyzer (Perkin Elmer, USA). The temperature ranged from room temperature to 800°C at a heating rate of $10^\circ\text{C}/\text{min}$, and the samples were maintained under an inert atmosphere of nitrogen with a flow rate of 20 mL/min. The derivative of each TGA curve (DTG) was obtained using the program Origin 8.1 (OriginLab, USA).

RESULTS AND DISCUSSION

Chemical composition

This study verified that the corn stover used to obtain the cellulose nanocrystals displayed the following chemical composition: cellulose 45.5 wt%, hemicellulose 27.6 wt%, lignin 6.8 wt%, extractives 8.5 wt%, polyoses 3.3 wt%, ash 1.5 wt% and moisture 6.8 wt%. These values were similar to those reported in the literature.^{15,17} After the sequence of chemical treatments, cellulose content increased to 62.1 wt%, while the levels of hemicellulose and lignin were reduced to 13.5 wt% and 3.2 wt%, respectively. This trend was expected because the alkali treatment swells the fiber structure, thereby increasing its surface area and making the polymeric components more easily hydrolysable.⁹

Morphology of cellulose nanocrystals

The corn stover nanocrystals, prepared by acid hydrolysis, were examined by TEM to determine their size and shape. Figure 1 shows the arrangement of the cellulose nanocrystals

extracted after acid hydrolysis of bleached corn stover. The appearance is a three-dimensional lattice formed by nanocelulose. We infer that the structure is three-dimensional due to intertwining of dark and bright tones that could indicate proximity or remoteness. This appearance could be created by multiple layers overlapping each other.

Monocrystals of cellulose extracted from corn stover showed lengths that ranged from 168-610 nm and diameters between 4.3 and 10.1 nm; these dimensions produced an aspect ratio of 54.7, which is considered high when compared with the results reported by other authors. Silva *et al.* obtained an aspect ratio of 24 for nanocrystals of eucalyptus in 2012,¹⁸ while in 2006 Bondeson *et al.* already achieved an aspect ratio of 20 for nanocrystals obtained from microcrystalline cellulose derived from Norway spruce.¹⁹ In general, the aspect ratio depends on the cellulose source and the preparation conditions for nanocrystals. As reported in the literature, a high aspect ratio provides enhanced mechanical properties because shear deformations in the interfacial fiber-matrix region are responsible for the transfer of stresses in the composite material.¹⁸

Thus, nanocrystals obtained from corn stover can be used as mechanical reinforcement in a matrix of starch providing improved properties, such as greater module and elongation, reduction in gas permeability and increased resistance to water when compared with the polymer without the addition of nanocrystals.

X-ray diffraction

Figure 2 presents the X-ray diffraction patterns of lyophilized cellulose nanocrystals, corn stover and cellulose obtained after bleaching treatment. corn stover exhibited three main reflection peaks at $2\theta = 16.5^\circ$, 22.1° and 34.8° . These peaks are characteristic for the crystal form of cellulose I polymorph, this behavior has been reported by other authors.^{20,21} The peak at $2\theta = 16.5^\circ$ corresponds to the (110) crystallographic plane, and the peaks at $2\theta = 22.1$ and 34.8° correspond to the (002) and (023) or (004) planes, respectively. As a result of the bleaching treatment that removed the lignin fraction of the fiber, narrower and more intense crystalline peaks were observed for the bleached fiber, with main reflections at $2\theta = 16.2^\circ$, 22.7° and 34.9° . Note the existence of a double peak in the diffractogram of the nanocrystals. Literature ascribes the merging of these two peaks to the

presence of lignin, hemicellulose and amorphous cellulose.²² However, the main reflections were at approximately 11.4° and 20.7°, which are assigned to cellulose II. Therefore, alkali treatment results in a lattice transformation from cellulose I to cellulose II, which was also observed by other researchers.²³

The percent crystallinity of corn stover, cellulose and isolated nanocrystals was previously determined by Segal and others.¹⁶ The results show that the crystallinity increases in the series of transformations from untreated corn stover (25.3%) to bleached corn stover (28.6%) and

subsequently to corn stover cellulose nanocrystals (45.0%). Similar values of percent crystallinity in the range from 40 to 50 wt% have been reported by other researchers for nanocrystals isolated from other sources by acid hydrolysis.^{18,24}

FTIR spectroscopy analysis

Figure 3 displays the FTIR spectra of corn stover, cellulose obtained after bleaching treatment and cellulose nanocrystals. These spectra show absorption bands of chemical groups characteristic of the corn stover components cellulose, hemicellulose and lignin.

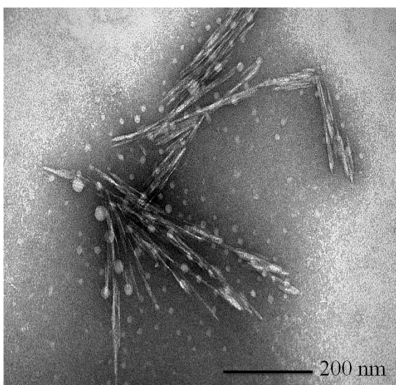


Figure 1: TEM micrograph of corn stover cellulose nanocrystals

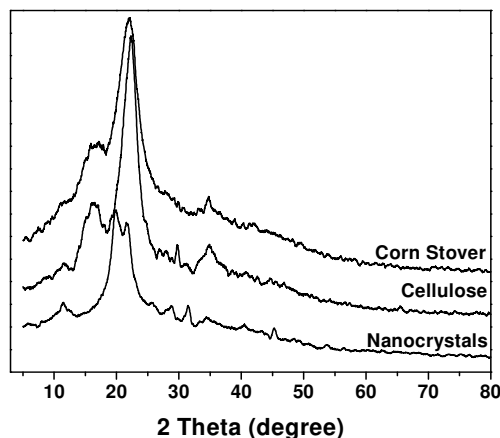


Figure 2: X-ray diffraction patterns for corn stover, cellulose obtained after the bleaching treatment, and corn stover cellulose nanocrystals

The region characterized by wide bands in the wavenumber range between 3100 and 3700 cm^{-1} corresponds to cellulose, hemicellulose and lignin. These bands are assigned to axial vibration of hydroxyl groups in these macromolecules. It is observed that this signal undergoes changes due to the alkaline treatment, reaching a frequency of

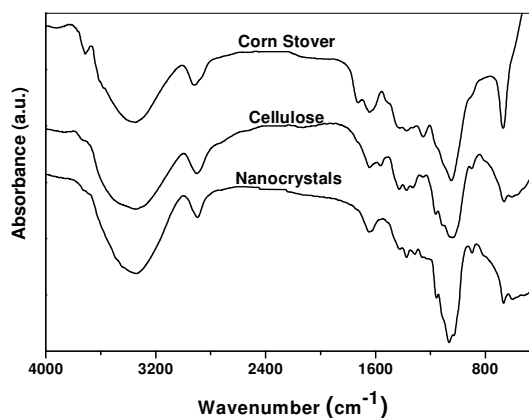


Figure 3: FTIR spectra of corn stover, cellulose obtained after bleaching treatment, and corn stover cellulose nanocrystals

3351 cm^{-1} for corn stover treated with 2% NaOH and 3364 cm^{-1} for cellulose nanocrystals obtained by acid hydrolysis. COOH bending peaks (670 cm^{-1}) are present in all fibers.

The presence of a band at 2928 cm^{-1} in the spectrum of corn stover is attributed to the stretching of C-H aliphatic bonds. The spectrum

also highlights the peak at 1731 cm^{-1} that is assigned to ester acetyl and uronic acid groups in hemicelluloses or to the ester group of ferulic and p-coumaric acids in lignin and/or hemicelluloses.^{25,26} This peak practically disappears in the spectra of cellulose and cellulose nanocrystals due to the removal of hemicelluloses after purification by acid hydrolysis. The authors also observed the disappearance of the signal at 1730 cm^{-1} after alkaline treatment and ascribed it to the removal of hemicelluloses.²⁷ The peaks at 1634 and 1244 cm^{-1} are assigned to the C=C stretching vibration of the aromatic ring of the lignin and the C-O-C stretching of hemicellulose, lignin and cellulose, respectively.⁸

The peak at 1556 cm^{-1} in the spectrum of cellulose is indicative of the presence of lignin and is attributed to the C=C vibration of the aromatic skeleton.^{26,28} This peak decreases in the spectrum of nanocrystals, indicating partial removal of lignin. This minimal change in the spectra indicates that the alkaline treatment did not completely remove this macromolecule, but promoted partial removal on the surface of the fiber. The presence of lignin even after the alkaline treatment agrees with the results of X-ray diffraction where the amorphous area (I_{am}) used in the calculation of the crystalline fraction

corresponds largely to the presence of this compound in the fibers.

The thin and small peak at 1374 cm^{-1} in the spectrum of cellulose is related to the C-H vibration due to asymmetric deformation of cellulose and lignin, as reported in the literature.²⁴ The cellulose spectrum also displays a band at 1647 cm^{-1} characteristic of water molecules adsorbed by cellulose.²⁹

Thermogravimetric analysis

Thermal decomposition properties were determined from the TG and DTG curves, as described below, at a heating rate of $10\text{ }^{\circ}\text{C}/\text{min}$. Figure 4 shows the TG and DTG curves of corn stover, cellulose obtained after bleaching treatment and corn stover cellulose nanocrystals. Table 1 summarizes the onset temperature and the corresponding degradation peak positions obtained from the DTG curves.

All of the TG curves showed an initial small drop between 25 and $150\text{ }^{\circ}\text{C}$, which represented a weight loss of approximately 5% and corresponded to the absorbed moisture on the surfaces of these materials, including chemisorbed water and/or the inter-molecularly H-bonded water.

Table 1
Degradation onset and peak decomposition temperatures of corn stover, cellulose and cellulose nanocrystals

| Material | Degradation onset temperature (T_o) | Peak degradation temperature (T_{max}) |
|------------------------|---|--|
| Corn stover | 344 | 372 |
| Cellulose | 261 | 349 |
| Cellulose nanocrystals | 211 | 302 |

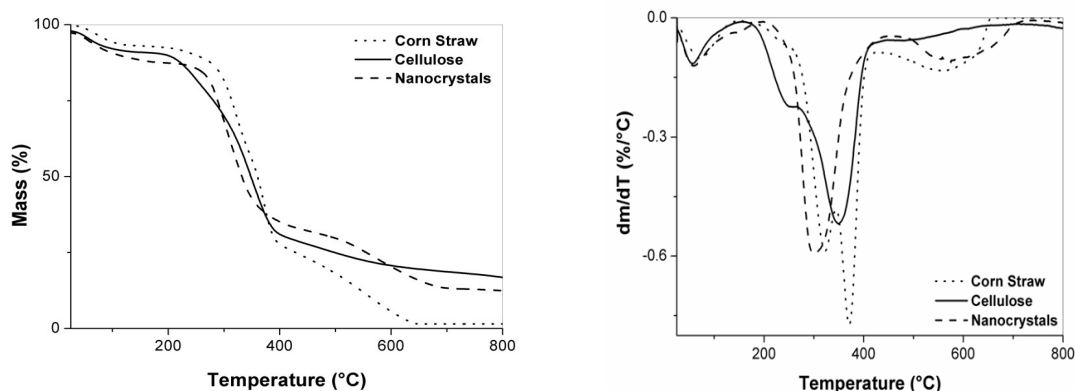


Figure 4: TG and DTG curves of corn stover (···), cellulose obtained after bleaching treatment (—), and corn stover cellulose nanocrystals (- - -)

Thermal degradation of the corn stover cellulose nanocrystals started at approximately 211 °C in a N₂ atmosphere, while for the corn stover, cellulose degradation began at approximately 261 °C. The replacement of hydroxyl groups by acid sulfate (O-SO₃H) groups in the hydrolysis step decreased the activation energy for the degradation of cellulose nanocrystals, making the sample less resistant to pyrolysis. Therefore, dehydration reactions occurred that led to the release of water and catalyzed nanocrystal decomposition.²⁹ It is quite interesting to note that the DTG curve of the corn stover consisted of two peaks at approximately 247 °C and 344 °C. The first peak was most likely due to the decomposition of hemicelluloses, and the second peak corresponded to losses of the volatile components of the pulp. Comparing the three components of the fiber, lignin was the most difficult to decompose; its decomposition occurred slowly, with a maximum decomposition rate at 558 °C. These results agree with the values reported in the literature.³⁰⁻³³

CONCLUSION

The main goal of this study was to investigate the viability of the corn stover as a simple and low-cost raw material for nanocrystals. The chemical composition was measured and was found to be 45.5 wt% cellulose, 27.6 wt% hemicellulose and 6.8 wt% lignin. The crystallinity of the corn stover was 25.3% before the bleaching treatment, and it increased to more than 43% after acid hydrolysis.

The changes in the peaks of the FTIR spectrum at 1731, 1556 and 1244 cm⁻¹ indicated that the alkali treatment partly removes hemicelluloses and lignin from the fiber surface. The corn stover had better thermal stability than those of the cellulose obtained after the bleaching treatment and of the nanocrystals isolated with acid hydrolysis.

The TEM study showed that the resulting nanosized cellulose crystals displayed lengths ranging from 168 to 610 nm and diameters between 4.3 and 10.1 nm, which produce an aspect ratio of 54.7.

These results demonstrate that cellulose nanocrystals can be successfully prepared from corn stover by acid hydrolysis. This study should stimulate further research on the use of this fiber as a novel renewable source for the production of cellulose nanocrystals that possess great potential as reinforcing agents in nanocomposites.

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