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Procurement and characterization of cellulose nanocrystals from cassava bagasse (*Manihot esculenta* Crantz)

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ABSTRACT

Objective: To procure and characterize cellulose nanocrystals from cassava bagasse.

Design/methodology/approach: Cellulose nanocrystals were obtained from cassava bagasse by acid hydrolysis (HCI), ultrasonication, centrifugation, dialysis, deep freezing and lyophilization. The cassava bagasse and the cellulose nanocrystals obtained were physicochemically characterized by Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD) and Scanning Electron Microscopy with Coupled Elemental Analysis (SEM-EDS). As an additional technique, Atomic Force Microscopy (AFM) was used.

Results: The analyses performed show that the cellulose obtained was type Iβ This study reports a percentage of crystallinity of the cassava bagasse cellulose of 37.1%, increasing the percentage to 48% crystallinity in cellulose nanocrystals. The diameters of the cassava bagasse fibers were reported to be 2 μm and their elemental composition (SEM-EDS) mainly constituted by carbon (C), oxygen (O) and traces of nitrogen (N). The morphology observed through AFM of the nanocrystals of cassava bagasse (*Manihot esculenta*) was rod-shaped, with helicoidal appearance without residual charge, with diameters between 8.7 and 9.3 nm.

Limitations on study/implications: The acid hydrolysis process showed a low percentage of crystallinity, although higher than other works reported for cassava bagasse.

Findings/conclusions: The results obtained confirm the possibility of obtaining cellulose nanocrystals from cassava bagasse (*Manihot esculenta*).

Keywords: Cassava, cellulose nanocrystals, physicochemical characterization.

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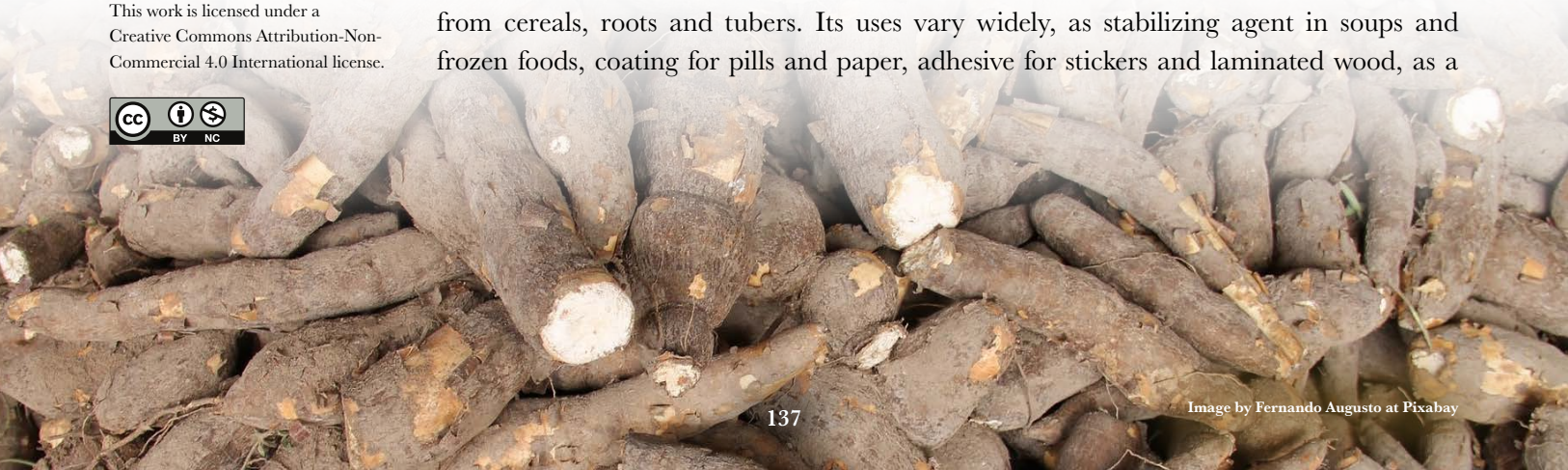
INTRODUCTION

Worldwide, approximately 60 million tons of starch are extracted annually derived from cereals, roots and tubers. Its uses vary widely, as stabilizing agent in soups and frozen foods, coating for pills and paper, adhesive for stickers and laminated wood, as a



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finishing agent on textiles, raw material in the production of ethanol, and even as cohesion agent in concrete. About 10% of this starch is derived from cassava roots (FAO, 2006). Cassava root starch is the cheapest food source to be found in the world, and it is used in over 300 industrial products (Rivera-Hernández *et al.*, 2012). Its composition is made up of glucose particles that are divided into two types: amylose or simple glucose, and amylopectin, a branching form of glucose. This is the quality that makes starch such a good source of energy for the organism (Villalobos and Thorpe, 1984). The solid residues produced during starch extraction are: peels, damaged tips, bran, and residual starch stillage (Cereda, 2001). Cassava bran or bagasse (Sriroth *et al.*, 2000; Pandey *et al.*, 2000) is a semisolid fibrous material with a high moisture content, resulting from the process of starch granule separation during the sifting stage (Cereda, 1994). According to Breuninger *et al.* (2009), for every ton of starch processed, approximately 350 kg of cassava bagasse are obtained. However, the high moisture content in the bagasse makes storage and transport difficult, as well as causing leaching processes which can affect the environment, which is why it is advisable to identify an alternate way to use it (Marmolejo *et al.*, 2008). Cellulose nanocrystals (whiskers) are very thin monocrystals (1-100 nm) that offer many advantages as reinforcement/barrier particles in polymer matrix compounds (Dufresne, 2006). They possess a high degree of crystalline perfection, high rigidity (the traction resistance of a single cellulose crystal is theoretically estimated at between 0.3 and 22 GPa), and mechanical properties, so they have exceptionally elevated resistance. Additionally, they are derived from a renewable source and, being biodegradable, can potentially offer low environmental risks (Lahiji *et al.*, 2008). With the aim of contributing to the sustainable management of the environment, and tending to a better handling and incorporation of the residues into the productive cycle, by reusing or transforming them into other products with a high added value, the study proposes the exploitation of cassava bagasse for its transformation into cellulose nanocrystals.

MATERIALS AND METHODS

The cassava bagasse was obtained from the pilot plant at Universidad Popular de la Chontalpa for cellulose extraction; it was dried in an oven (Ecoshel® model HV-50) at 60 °C for 2 days, pulverized with the help of a mortar, and then stored in hermetically sealed bags.

Procurement of cellulose nanocrystals (CNC)

A 350 mL solution of HCl was mixed at 4N with 10 g of cassava bagasse, agitating continuously for 225 min at 60 °C, and distilled water was added at a 1:5 ratio (with the purpose of halting the reaction). Then an ultrasonication cycle was applied at 750 Watts (with 50% amplitude) during 3 min. The solution obtained was subjected to repeated washing with deionized water at a centrifuge speed of 10,200 rpm during 10 min, until pH=4 was reached (Araki *et al.*, 1998), in order to collect a cloudy whitish supernatant which was stored and then concentrated with the help of a BUCHI® brand rotary evaporator until a colloidal suspension was reached. The nanocellulose concentrate was purified through dialysis membranes (12-14 kD) during 5 days, until a pH equal to that

of the deionized water used was reached. This suspension was deep frozen for 3 days at −18 °C for its subsequent lyophilization and storage.

Physicochemical characterization

To determine the functional groups of the cassava bagasse and of the CNC, a Nicolet Nexus 670 spectrometer was utilized, on absorbency mode, with a resolution of 4 cm^{-1} and 100 scans, using 1 mg sample pellets on 100 mg of KBr.

The crystallinity of the cassava bagasse and the CNC were determined via the powder X-ray diffraction method (PXRD), using a Bruker D-8 Advance diffractometer, in Bragg-Brentano geometry, exposure time of 0.5 sec, size of exposure 0.02 degrees, spectrum of CuKβ(α=1.5418 Å and 8.047 keV energy). The percentage of crystallinity of the cellulose obtained was calculated using the method described by Segal *et al.* (1959), using the equation (1).

$$X_C\% = 100\left[1-\left(\frac{I_1}{I_2}\right)\right] \qquad \text{Eq. (1)}$$

Where: I_1 is the minimum intensity of the crystalline peak and I_2 is the maximum intensity of the crystalline peak, respectively (taken from the diffractogram results generated during the XRD analysis).

In order to analyze the morphology of the cassava bagasse and perform its elemental analysis, a Phillips and XL-30 ESEM JEOL JSM-7600F scanning electron microscope with energy-dispersive spectroscopy (SEM-EDS) was utilized. The cellulose CNC were characterized with an NX10 Biometer atomic force microscope, combined with analysis of the images in order to determine the different sizes of particles, using an intermittent reading mode, scanning the surface areas on planes (X-Y).

RESULTS AND DISCUSSION

Chemical characterization of the functional groups via FTIR

Figure 1 (a and b) shows the interferograms (FTIR) of cassava bagasse (*Manihot esculenta*) and the cellulose nanocrystals, respectively.

In the spectra we observe peaks at certain frequencies that are characteristic of different groups: the wide peak that spans 3500-3200 cm^{-1} corresponds to the stretches of the OH group of the free and bonded hydroxyl, intra- and inter- molecularly present in the anhydrous glucose units of amylose and amylopectin (Enríquez *et al.*, 2010). On the other hand, both spectra demonstrate the characteristic C-H stretching vibration around 2900 cm^{-1} (Rosa *et al.*, 2012). A C=O bond was observed from 1730-1740 cm^{-1}, which is characteristic of lignin and hemicellulose (Abraham *et al.*, 2011). Figure 1a shows a peak at 1645 cm^{-1} which is associated with bending of the OH group of the adsorbed water (Alemdar and Sain, 2008; Abraham *et al.*, 2011), as well as bending curves in the C-OH plane on the peaks close to 1340 cm^{-1} corresponding to holocellulose (Faix and Beinhoff, 1988) and the 1147 cm^{-1} band is assigned to C-O-C stretching, since it is an acceptor of

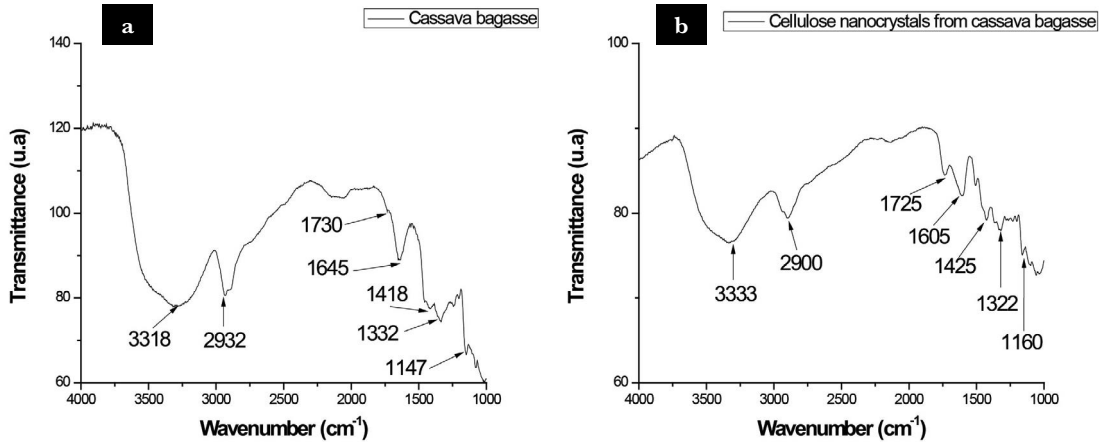

Figure 1. Interferograms (FTIR) of: a) cassava bagasse (*M. esculenta*) and b) cellulose nanocrystals.

protons capable of forming hydrogen bonds with proton donors, such as OH in cellulose nanocrystals and cellulose nanofibers (Kakade *et al*., 2007). Figure 1b shows the peaks of absorption close to 1605 cm^{-1} associated with the C-C aromatic bond in the symmetric stretching to the vibration plane of the aromatic ring present in lignin (Garside and Wyeth, 2003; Wang *et al*., 2009). Villar (2010), Bourtoom and Chinnan (2008), and Kim and Lee (2002) report that the 1425 and 1322 cm^{-1} peaks (of nanocrystals) correspond to bending vibrations of C-H, and the 1160 cm^{-1} band is attributed to the asymmetric stretching of cellulose C-O-C (Grande, 2014), specifically cellulose nanocrystals.

Physical characterization via X-ray diffraction (XRD)

In the X-ray diffractogram (XRD) of the cassava bagasse (Figure 2a) the following peaks are identified: 15.1, 17.1, 18.1 and 23.1°, which represent the typical diffraction pattern of type A crystals.

This type of crystallinity is more susceptible to enzymatic hydrolysis and is found in the starches of cereals and some roots, as well as in tubers such as cassava, potato and jicama

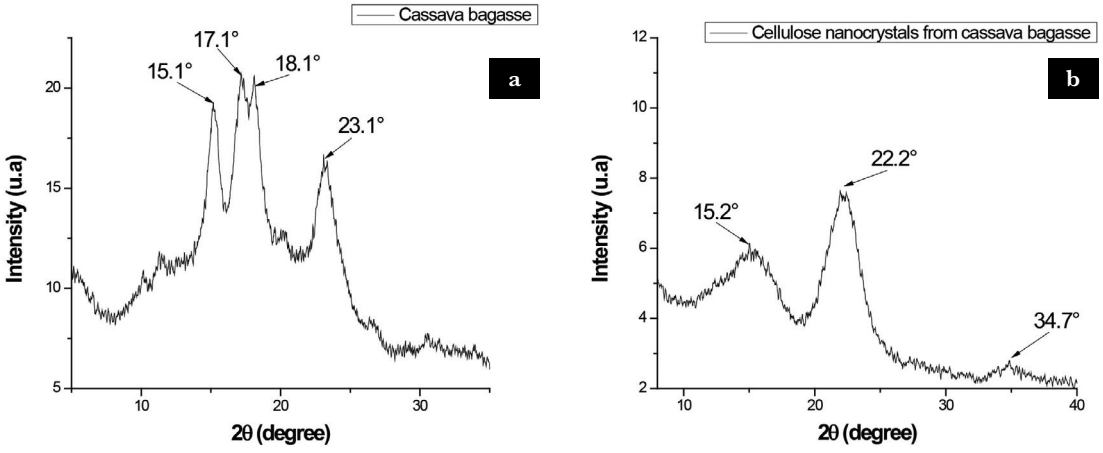

Figure 2. Diffractograms (XRD) of: a) cassava bagasse (*M. esculenta*) and b) cellulose nanocrystals.

(Flores, 2004; Atichokudom *et al.*, 2001). The percentage of crystallinity obtained from Eq. 1 was 37.1%, which is higher to that reported in cassava bagasse obtained from the Purbalingga, industry in Indonesia (14.52%) (Wicaksono *et al.*, 2013), savannah cassava (23%) and emerald (26%) starch (Vargas, 2015), and lower to that from sugarcane bagasse (43.6%). In the 15.2, 22.2 and 34.7° peaks present in the cassava cellulose nanocrystals (*M. esculenta*) (Figure 2b), an increase in intensity is observed, indicating greater crystallinity (47.9%), due to the elimination of amorphous material (hemicellulose and lignin). The 22 and 34° peaks belong to the reflection of the lattice on different planes (Sassi and Chanzy, 1995). Sugiyama *et al.* (1991), mention that values similar to 2=14.9°, 16.7°, 20.6°, 22.8° and 34° exhibit the type I crystalline structure which is found in nature and commonly known as native cellulose (Iβ, which is predominant in plants.

Morphological characterization and elemental analysis (SEM-EDX)

Figure 3a presents SEM micrographs obtained, where average diameters of 2 μm can be observed in cassava bagasse (*M. esculenta*). Moron *et al.* (2017) report diameters that range from 0.5 to 2.52 μm for this same material, while Versino *et al.* (2015) found

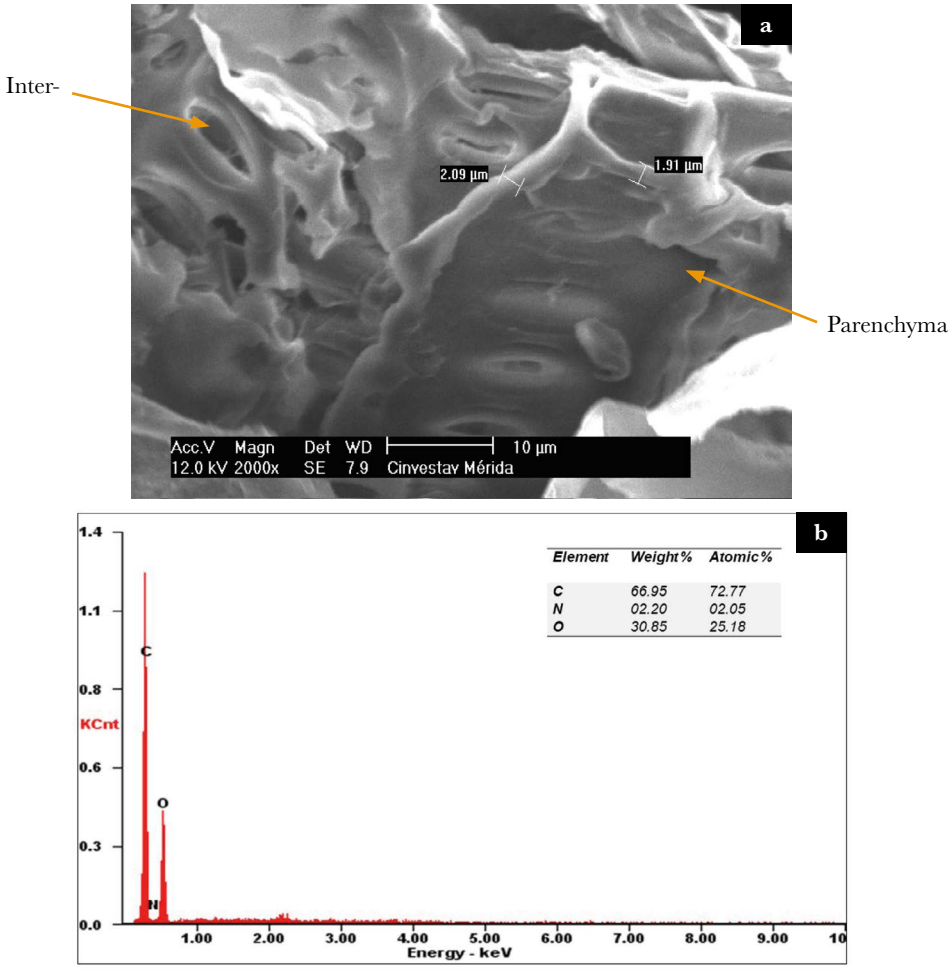

Figure 3. a) SEM micrographs of cassava bagasse and b) elemental analysis.

that the peels had bigger particles (mainly 300 μm) compared to the bagasse (particles smaller than 53 μm) while conducting studies with cassava (*M. esculenta*) bagasse and peel. The variations found can be attributed to the grinding and sifting processes prior to the micrograph sampling. The images clearly show the parenchyma, as well as the detachment of some of the fibers as a result of the grinding.

The SEM-EDS elemental analysis carried out on cassava bagasse (Figure 3b) demonstrates as principal components: carbon (C), oxygen (O) and traces of nitrogen (N). Cours *et al.* (1961) report the percentage in detail (in dry cassava) of N, K, P, and Ca in cassava leaf blades, petioles and branches, wood, and phelloderm of stems. Their results indicate that the percentages of nitrogen range from 3.84 to 0.76%. Lozano *et al.* (1981) report that the leaf blades have a higher content of N and P, the petioles of K and Ca, and add that the nutrient content in the same tissue changes as the plant ages, since in the case of cassava the contents of N, P and K decrease while the contents of Ca and Mg increase during the growth cycle.

Characterization of the diameter and amplitude of cellulose nanocrystals with an atomic force microscope

The AFM micrograph carried out on CNC (Figure 4), obtained via hydrolysis with HCI, shows rod-shaped nanocrystals with a helical appearance and no residual charge, having

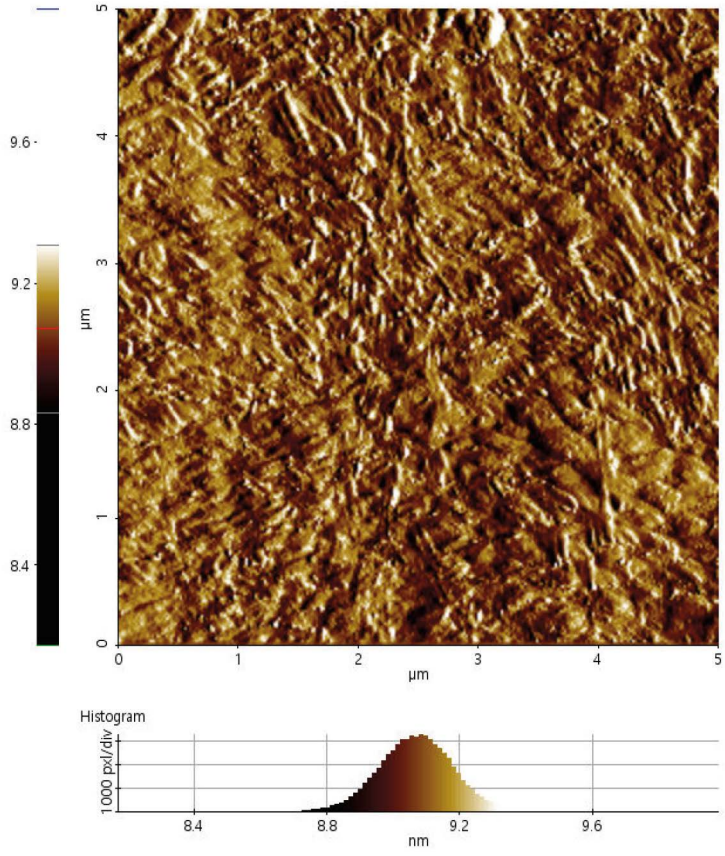

Figure 4. AFM micrograph of the cassava bagasse CNC.

a diameter of 8.7 and 9.3 nm. Teixeira *et al.* (2009) report values of 25±7 nm in cassava bagasse cellulose nanofibers, obtained through H_2SO_4, hydrolysis, while Wicaksono *et al.* (2013) report smaller diameters (5-8 nm), with the combination of chemical treatments (alkaline solutions to hydrolyze pectin and hemicellulose), as well as mechanical.

It is important to point out that the characteristics of cellulose CNC can be affected primarily by the raw material employed, the methodology (parameters like time, temperature, and reagents), types of treatments (chemical and mechanical) and the equipment used during the process. The cellulose CNC prepared with HCI have a limited capacity to be dispersed and their aqueous suspensions tend to flocculate (Araki *et al.*, 1998), while those prepared with H_2SO_4 lead to more stable aqueous whisker suspensions, presenting a higher negative charge on their surface, due to the formation of sulfate groups during the acid treatment in comparison to those prepared with HCl (Gardner *et al.*, 2008; Peng *et al.*, 2011).

CONCLUSIONS

Characteristic functional groups were identified, as well as the bonds present in cassava bagasse and in the cellulose nanocrystals obtained from the anhydrous glucose units of amylose and amylopectin, lignin, hemicellulose, holocellulose, and water; the presence of cellulose type I was corroborated, which is found in nature and commonly known as native cellulose ($I\beta$). The crystallinity percentages of the nanocrystals indicate that the amount of lignin and hemicellulose present decreased; however, they also suggest that the amorphous cellulose dominions remain to a greater extent. It was possible to obtain cellulose nanocrystals from cassava bagasse (*M. esculenta*), with rod shape, helical appearance and no residual charge. However, it would be interesting to attempt their formation with different methodologies, managing to increase the percentage of crystallinity and with this, its field of application.

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