



Synthetic characterization and Structural Properties of Nanocellulose from *Moringa oleifera* seeds

A. F. Afolabi*, S. S. Oluyamo, I. A. Fuwape

Condensed Matter and Statistical Physics Research Unit, Department of Physics, The Federal University of Technology, P.M.B. 704, Akure, Nigeria.

Abstract

In this research, nanocellulose is isolated from *Moringa oleifera* seed using acid hydrolysis and the structural properties were determined. X-ray diffraction (XRD) and Fourier transform infrared (FTIR) spectroscopy were used for the characterization of the isolated nanocellulose. The most noticeable peak is observed at 22.53° and the value of the crystallinity index (C_{lr}) from the XRD pattern is 63.1%. The calculated values of hydrogen bond intensity (HBI), lateral order index (LOI) and total crystalline index (TCI) are 0.93, 1.17 and 0.94 respectively exhibited high degree of crystallinity and well arranged cellulose crystal structure. The isolated nanocellulose has an average length and diameter of 14.3 nm and 36.33 nm respectively. Furthermore, the FTIR peaks revealed the presence of C-H bending, C-O stretching and O-H stretching functional groups.

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1. Introduction

Moringa oleifera is a well known plant material with numerous potential uses which belong to the family of Moringaceae [1,2]. *Moringa oleifera* is a plant material composed of organic nutrients, lignin, hemicellulose and cellulose. One of the prominent structural compositions of different green plants cell wall is cellulose. Moreover, nanocellulose can be prepared from cellulose [3]. The fact has been established that cellulose with appearance of nanostructures (nanocellulose) is among the paramount organic materials of recent times [4]. Nanocellulose exhibits unique characteristics due to the nanoscale size. The properties of the nanocellulose can be tailored to increase their performance for specific applications [5,6]. Chemical method

of treating nanocellulose is based on the source, the resulting material can change in crystal arrangement (crystal structure), degree of crystallinity, morphology and surface chemistry [7]. Nanocellulose has been a research key in nanomaterial because it is a sustainable biomaterial which has low toxicity. Nanocellulose is isolated using various distinct approaches such as oxidative, acid hydrolysis, oxidative, enzymatic and mechanical treatments of cellulose. The most common approach for isolating nanocellulose from wood and other plant materials is acid hydrolysis [8,9].

Many researchers have investigated the isolation of nanocellulose from agricultural residues such as banana [10], sisal [11], tomato peels [12], calotropis procera fibers, onion waste, citrus waste, coconut [13], sesame husk [14], cotton, rice husk [15], oil palm [16], groundnut shells [17], macrophyte typha dominicensis, potato peel, jute, spruce bark, agave angustifolia fibers,

*Corresponding author tel. no:

Email address: agafolabi@gmail.com (A. F. Afolabi)

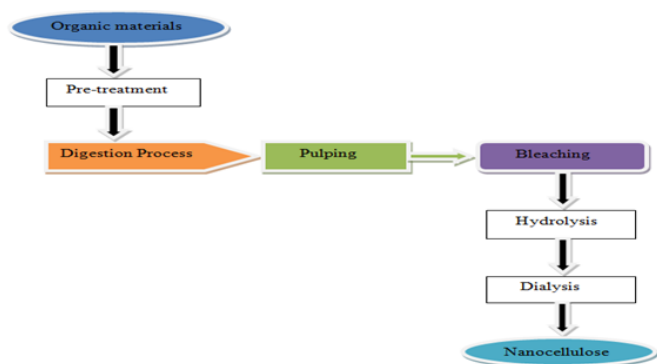


Figure 1. Schematic Diagram of Experimental Procedure of nanocellulose

mango seed, sugarcane bagasse, corncob, bamboo, straws, soy hulls, olive stones, miscanthus giganteus, kapok and flax fibers. The potential and industrial application of the isolated nanocellulose is based on the structural and other properties of the nanocellulose. The aim of this research is to synthesis, characterize and determine the structural properties of nanocellulose from moringa oleifera seed.

2. Materials and Methods

2.1. Materials

The locally sourced organic material (*Moringa oleifera* seeds) was removed from the shells, dried and grinded with a mixer grinder (Bajaj GX 10 DLX, Mumbai, India). It was sieved to obtain fine particles using a Pascal Engineering Wiley Mechanical Sieve Shaker, England. Analytical chemical reagents used are NaOH, NaClO₂, acetic acid and H₂SO₄. The chemical reagents were obtained from the Pascal Scientific Ltd. The schematic representation of experimental procedure is shown in Figure 1.

2.2. Methods

A liquor ratio of 15:1(V/W) cooking condition was employed, the *Moringa oleifera* seed particles was pulped with 20% of NaOH at a temperature of 90° for 1 hour 30 minutes. After digestion process, the cooked pulp was filtered, screened and cleaned by rinsing properly with water without alkali. The pulped was left in the oven at 105°C until the water was completely dried. Mixture of 200 mL hot water, 6 g of NaClO₂, 1.5 mL of acetic acid and 10g of bone dried sample of pulp in a titration flask were placed in the water bath at 70° and heated for 30 minutes. Another 6 g of NaClO₂ and 1.5 mL of acetic acid were added to the mixture and switched off the water bath after submitted to heat for the next 30 minutes. The sample was left in the water bath for 24 hours. After digestion, it was washed, filtered and cleaned by rinsing properly with water until the chlorine and the acid were washed away. The sample acquired was left in the oven at 105° until the water was completely dried to obtain the cellulose.

2.3. Preparation of Nanocellulose

The nanocellulose of the sample was prepared by acid hydrolysis in accordance with the method developed by Bondeson [18] with little change. The cellulose sample was treated with 60 % sulfuric acid (H₂SO₄). The hydrolysis was conducted by using a hot plate to heat the suspension in a round bottom flask with reflux condenser and intermittently stirred with a magnetic stirrer at an average temperature of 45° for 60 minutes. The hydrolyzed cellulose sample was distinctly washed and drained to remove excess H₂SO₄ until the sample was neutral and dried. The reflux condenser was used to cool the acid so that the acid will not escape.

3. Characterization

The crystallinity index of the isolated nanocellulose from *Moringa oleifera* seeds was acquired by making use of a Philips PW diffractometer with Cu-Kα monochromator at the voltage of 15kV, scanned at wavelength λ=1.54Å with 2θ angle range from 5° to 90°. The surface morphology was determined by scanning electron microscope using 15 kV accelerated voltage of JEOL/EO JSM-6390 and has a resolution up to 100μm. Fourier transform infrared (FTIR) Spectrophotometer was used to determine variation in functional groups induced by various treatments within a wavelength range of 700–4000cm⁻¹.

3.1. Theoretical background

The Interplanar spacing (d-spacing) was obtained as [19,20]

$$d = \frac{n\lambda}{2 \sin \theta} \quad (1)$$

where n is the order of reflection, d is the interplanar spacing of the crystal, θ is the angle of incidence and λ is the wavelength of the incident X-ray. The crystallinity index was determined using equation (2) [21,22]

$$C_{I_r} = \frac{I_{200} - I_{am}}{I_{200}} \times 100 \quad (2)$$

where, low intensity peak of the amorphous region is I_{am} and highest peak intensity of the crystalline fractions is I_{200} . The crystallite size (L) was calculated using Scherrer's equation [23]

$$L = \frac{K \times \lambda}{B \times \sin \theta} \quad (3)$$

where, constant value given as 0.91 is K , Bragg's angle (°) is θ , wavelength of the incident X-rays is λ and intensity of the full width at half maximum (FWHM) proportional to a high intensity peak of the diffraction plane is B .

The surface chains (X) is the proportion of crystallite interior chains [24] was calculated as

$$X = \frac{(L - 2h)^2}{L^2}, \quad (4)$$

where L is the crystallite size and $h = 0.57 \text{ nm}$ is the layer thickness of the surface chain.

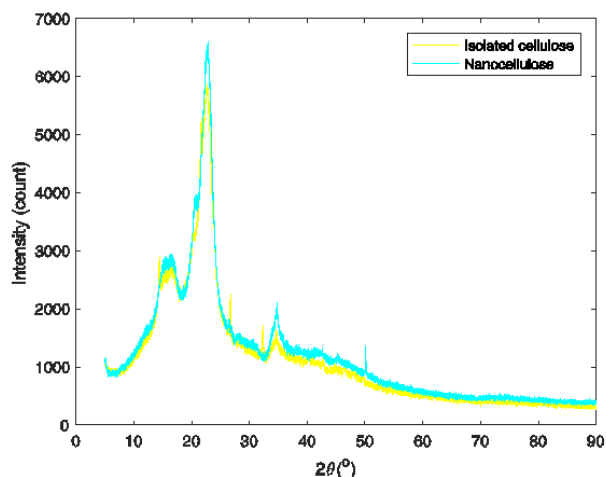


Figure 2. X-ray diffractogram of isolated nanocellulose from *Moringa oleifera* seeds.

4. Results and Discussion

4.1. X-Ray Diffraction (XRD) of Isolated Cellulose and Nanocellulose

The XRD pattern of the isolated cellulose in Figure 2 revealed crystalline characteristics peaks at $2\theta = 14.39^\circ$, 15.33° , 22.47° and 34.50° while nanocellulose has distinct peaks at $2\theta = 14.95^\circ$, 15.01° , 22.53° and 34.67° in agreement with isolation and characterization of cellulose nanocrystals from *Agave angustifolia* fibre [25]. The crystalline peaks indicate that the crystal structure is attributed to planes (1 $\bar{1}$ 0), (110), (200) and (004) respectively. Furthermore, there is a noticeable crystal peak observed at 50.12° similar to the peaks in the XRD results of cellulose and α -cellulose from date palm biomass waste [26]. The peaks at 21.58° , 24.88° and 32.24° in the pattern of the cellulose were not noticed in the pattern of nanocellulose in Figure 2. This is due to the fact that the bond of the cellulose was broken after the sulfuric acid hydrolysis. The most prominent peaks of the isolated cellulose and nanocellulose are 22.47° and 22.53° . The crystallinity index of isolated cellulose from *Moringa oleifera* seeds (62.6%) is lower than the crystallinity index of the nanocellulose (65.4%), this contributed to high degree of crystallinity of the nanocellulose [27,28,29,30]. Additionally, the high crystallinity of nanocellulose depends on the three hydroxyl groups in fundamental chemical structure of cellulose which have potential to instigate large intra and intermolecular hydrogen bonding included in the cellulose chains, granting the crystalline packing of cellulose chains into greatly compact system (crystal structure) [31]. The diffraction peaks of the nanocellulose were narrowed, longer and became sharper due to the efficient elimination of the amorphous parts. This shows that the nanocellulose is highly crystalline [32]. Table 1 showed the values of d - spacing (d), full width at half maximum (FWHM), crystallinity index (C_r), crystallite size (L), and surface chains (X) also known as the crystalline proportion of the crystallites of the isolated nanocellulose.

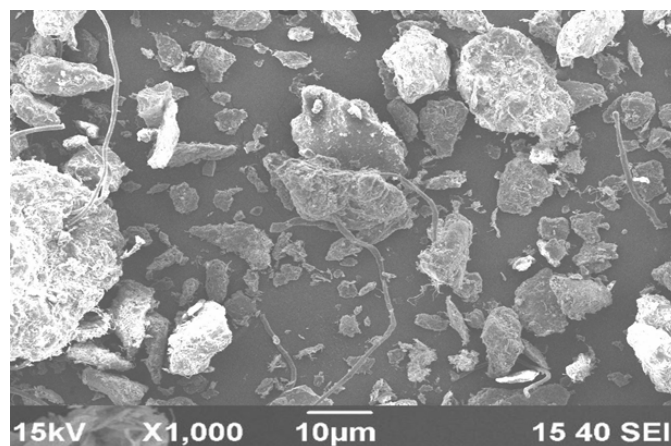


Figure 3. Scanning electron micrograph of cellulose from *Moringa oleifera* seeds

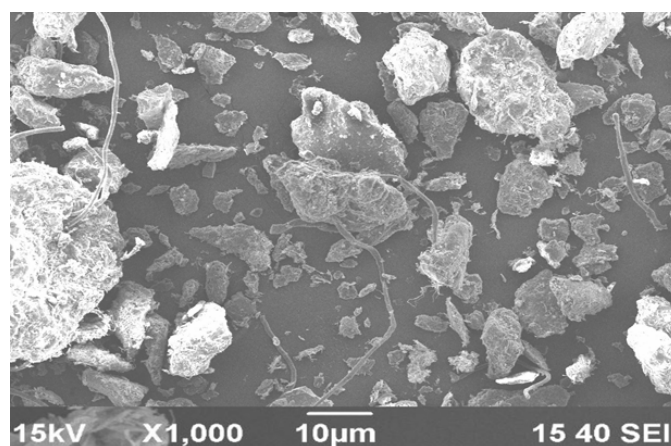


Figure 4. Scanning electron micrograph of nanocellulose from *Moringa oleifera* seeds.

4.2. Scanning Electron Microscopy (SEM) Analysis of Isolated Cellulose and Nanocellulose

Figure 3 shows the surface morphological features of the isolated cellulose. The surface of the isolated cellulose from *Moringa oleifera* seeds was rough due to amorphous nature of the materials [28]. The isolated cellulose from *Moringa oleifera* seeds has an average length of $46.20\ \mu\text{m}$ and diameter of $88.90\ \mu\text{m}$. The particles were dissociated from one another, indicating the elimination of hemicelluloses and lignin. This is similar to the report of Nazir *et al.* [22].

The surface morphology of the isolated nanocellulose from *Moringa oleifera* seeds in Figure 4 is predominantly rod-like with conical feature. In addition, the nanocellulose is clean, smooth and disjointed from one another owing to the removal of impurities and non-cellulosic components from the materials. Furthermore, non-agglomerated structure of the nanocellulose is expressed as highly porous with noticeable diameters, thus able to provide large surface areas [26]. The isolated nanocellulose has an average length and diameter of $14.30\ \text{nm}$ and $36.33\ \text{nm}$ respectively.

Table 1. Structural Analysis of the isolated nanocellulose from the XRD patterns

Sample	$2\theta(^{\circ})$	$d(\text{\AA})$	$L(\text{nm})$	FWHM	X	$C_r I(\%)$
Isolated Cellulose	22.43	3.95	1.95	0.07	0.17	62.60
Isolated Nanocellulose	22.53	3.90	2.13	0.06	0.22	65.40

4.3. Fourier Transform Infrared (FTIR) of Isolated Cellulose and Nanocellulose

The Fourier transform infrared (FTIR) spectra of the isolated cellulose and nanocellulose are shown in Figure 5. The prospect of the FTIR was to ascertain the functional groups of the cellulose and nanocellulose isolated from the *Moringa oleifera* seeds. Absorption bands in all spectra of the isolated cellulose were observed at 3335.43 cm^{-1} , 2913.17 cm^{-1} , 2345.16 cm^{-1} , 1577.27 cm^{-1} , 1426.66 cm^{-1} , 1156.49 cm^{-1} , 1015.50 cm^{-1} and 661.67 cm^{-1} . The spectra of the isolated cellulose showed wide band centered at 3335.43 cm^{-1} appointed to O-H stretching vibration of hydroxyl groups and absorbed water having strong intermolecular hydrogen bonding with alcohol compound class [33,34]. The wide absorption band of the isolated nanocellulose observed at 3340 cm^{-1} in Figure 5 is strong corresponded to the vibration of the O-H group having a compound class of alcohol. This is in agreement with preparation and characterization of novel microstructure cellulosic sawdust material [35]. This functional group commonly present in the cellulose. The characteristics spectra of C-H vibration occur at 2910 cm^{-1} . The absence of peak between 1740 cm^{-1} and 1726 cm^{-1} signifies that there is no ester linkage of lignin or ester group of the hemicellulose due to the sulfuric acid hydrolysis [32]. Furthermore, disappearance of the hemicellulose and lignin in the FTIR spectrum verifies that the cellulose is crystalline. The peaks in the region between $1025\text{ cm}^{-1} - 1321\text{ cm}^{-1}$ are associated to the C-O stretching [26]. Additionally, the band at 664 cm^{-1} is a characteristic associated with the C-H bending [36].

Total crystalline index (TCI), hydrogen bond intensity (HBI), lateral order index (LOI) and lateral order index (LOI) of the nanocellulose from *Moringa oleifera* seeds were obtained from the spectra of the FTIR spectroscopy. The calculated values of TCI and LOI are 0.93 and 1.17 respectively. The values signify more ordered cellulose structure and structure high degree of crystallinity. This result is similar to previous research on green solvent for water hyacinth biomass deconstruction [37]. The other indicator of high degree of intermolecular regularity and ordered nature of cellulose is HBI value. The HBI value of the isolated nanocellulose is 0.94 which indicates high degree of intermolecular regularity. This is in agreement with the result on native cellulose: structure, characterization and thermal properties [23]. This result show that there were additional chains of cellulose in a highly coordinated form which leads to higher hydrogen bond intensity among neighbouring chains of cellulose and produce a more packing structure of cellulose and higher crystallinity.

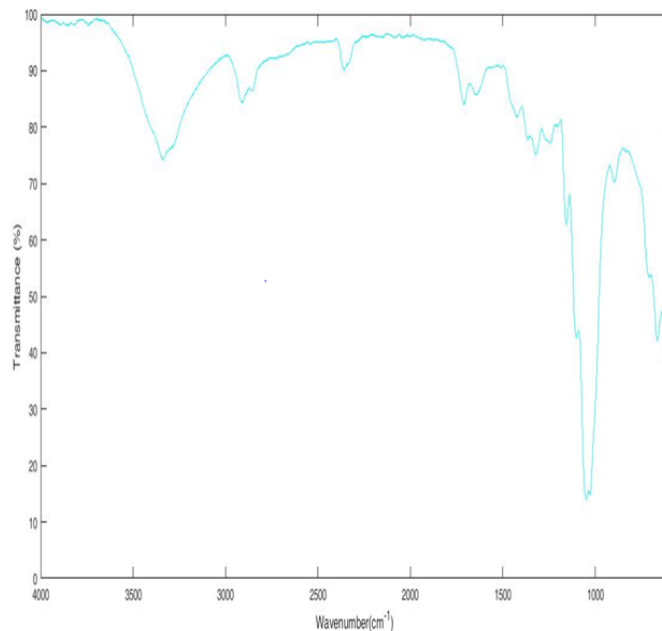


Figure 5. Fourier transform infrared (FTIR) spectra of isolated cellulose from *Moringa oleifera* seeds

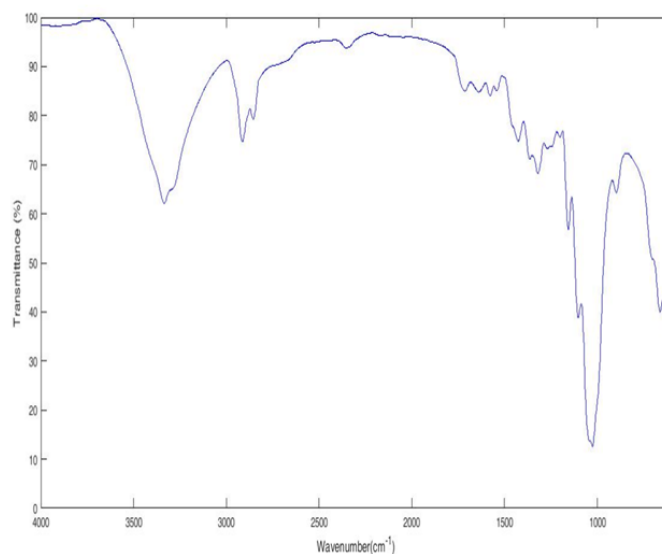


Figure 6. Fourier transform infrared (FTIR) spectra of isolated nanocellulose from *Moringa Oleifera* seeds

5. Conclusion

The structural properties of the isolated nanocellulose were successfully examined in this research. The isolated cellulose and nanocellulose from *Moringa oleifera* seeds revealed the

most prominent peaks at $2\theta = 22.47^\circ$ and 22.53° respectively. The crystallinity index values were 62.60% and 65.40%.

In addition, the nanocellulose is predominantly rod-like with conical feature. The isolated cellulose has an average length of $46.20 \mu\text{m}$ and diameter of $88.90 \mu\text{m}$ while the average length and diameter of the obtained nanocellulose are 14.3 nm and 36.33 nm respectively.

The FTIR revealed the presence of C-O stretching, O-H stretching and C-H bending functional groups. The TCI, LOI and HBI values of the nanocellulose from *Moringa oleifera* seeds were 0.93, 1.17 and 0.94. These results indicate more ordered cellulose structure and high degree of crystallinity.

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