

Genotypic Variations in Characteristics of Nano-Fibrillated Cellulose Derived from Banana Pseudostem

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A laboratory study was undertaken to extract nano-fibrillated cellulose (NFC) from the pseudostem (a waste from the fruit harvest) of two commercial banana cultivars ('Grand Naine' and 'Poovan') in Tamil Nadu using a novel approach: a one-step method with a bleaching agent and alkali-free acid hydrolysis coupled with ultrasonication. The acid hydrolysis was performed using nitric acid and acetic acid (1:10 ratio). The treatment was effective in the depolymerization and defibrillation of banana pseudostem fiber and in the formation of NFC, which was confirmed by several physico-chemical techniques. The average diameters of the nanofibrils were 6 to 8 nm and 4 to 6 nm for 'Grand Naine' and 'Poovan', respectively. The XRD analysis revealed an increased cellulose crystallinity of almost 20% in the NFC compared with the respective raw banana fibers. The Fourier transform infrared (FT-IR) spectroscopy and thermal gravimetric analysis (TGA) confirmed the absence of lignin, hemicelluloses, and pectin in the nano-fibrillated samples. The thermal analysis showed the increased thermal stability of the NFC.

Keywords: Lignocellulosic waste; Banana pseudostem; Nano-fibrillated cellulose; Acid hydrolysis; Ultrasonication; XRD; SEM; TEM

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INTRODUCTION

Cellulose is one the most ubiquitous and almost inexhaustible sources of polysaccharides on earth, contributing to approximately 1.5×10^{12} tons of annual biomass production (Klemm *et al.* 2005). Because of increasing ecological concerns, interest in the rational use of agricultural residues, and because of properties such as abundance, biodegradability, and renewability, cellulose and cellulose-derived novel materials have gained unprecedented attention in the past few decade (Gañán *et al.* 2008; Li *et al.* 2012). Nanocellulose is one such material that has attracted attention, with properties including high surface area, high aspect ratio, high axial Young's modulus, low density, modifiable surface properties, and higher biodegradability than its macroscopic form, as well as the availability and abundance of its precursor (Brinchi *et al.* 2013; Rajan *et al.* 2014). Because of the abundance of cellulose in various agricultural residues, which otherwise would go to waste, an intelligent approach to producing nanocellulose would be to disrupt the hierarchical structure to obtain a highly crystalline nanocellulosic material (Eichhorn *et al.* 2010).

Plant wastes from sources such as palm, pineapple, banana, and coconut are renewable, available in abundance, and could be a principal source for fibers in India,

Colombia, and China (Rowell *et al.* 2000; Khalil *et al.* 2006). India leads the world in the production of banana. Among the Indian states, Tamil Nadu ranks first in the production and cultivation of this fruit. The fruit contributes to only 12% of the plant's weight, and the effective utilization of the agricultural biomass generated (30 million tons), which otherwise raises great environmental concerns, is still in its initial stages of development (Baig *et al.* 2005; Elanthikkal *et al.* 2010).

Banana pseudostem can serve as a good source of nanocellulose (cellulose content, 47%; lignin, 13.0%) (Saraiva *et al.* 2012). Various attempts have been made to isolate nanocellulose from banana lignocellulosic waste using acid hydrolysis (Zuluaga *et al.* 2007; Pereira *et al.* 2010) and steam explosion (Cherian *et al.* 2010; Abraham *et al.* 2013), employing sodium or potassium hydroxide as the alkali, sodium hypochlorite, sodium chlorite, or hydrogen peroxide as the bleaching agent, and sulfuric acid, nitric acid, and acetic acid for the hydrolysis. All of the above synthesis procedures report the isolation of nanocellulose after an initial alkaline mercerization step followed by a bleaching procedure and a final treatment with acid. The procedure is time-consuming and cumbersome. A procedure that promises equally good results with less time, effort, and chemicals is desirable. The present work reports the successful isolation of nano-fibrillated cellulose from the pseudostem of two commercial banana cultivars (Grand Naine, AAA; Poovan, AAB) widely grown in Tamil Nadu, India using acid hydrolysis followed by ultrasonication.

The cultivated bananas were domesticated from the wild progenitors *Musa acuminata* (where the haploid genome is designated as 'A') and *Musa balbisiana* (the B genome). Banana varieties that are hybrids with AAB and ABB genome constitutions are a staple food for a billion people in Asia and Africa and have $2n=3x=33$ chromosomes. Approximately 15% of the world's banana production is for the export trade and is based on a single variety, the 'Cavendish'. This sweet banana has the genome constitution AAA. The AAA genotypes are completely derived from *Musa acuminata* and are the most familiar variety in Western supermarkets. These bananas dominate world trade, but globally they are not the most important bananas. AAB genotypes are far more important in Africa and Asia, where they are grown by hundreds of thousands of small farmers and are a staple food for millions. In India, the 'Grand Naine' (AAA) and 'Poovan' (AAB) cultivars constitutes 50% and 18%, respectively, of the total banana production in the country.

The study describes the isolation of nanofibrillated cellulose (NFC) using acid hydrolysis with nitric acid and acetic acid in combination with ultrasonication from two distinct cultivars with AAA and AAB genomes. The differences between the two were investigated both qualitatively and quantitatively. No pre-treatment employing bleaching agent or alkali was performed prior to acid hydrolysis. The structural, physico-chemical, and thermal properties of the as-synthesized NFC were studied using zeta potential analysis, FT-IR, X-ray diffraction (XRD), scanning electron microscope (SEM), transmission electron microscope (TEM), and TGA.

EXPERIMENTAL

Banana pseudostem from two cultivars ('Grand Naine' and 'Poovan') was collected after the fruit bunch harvest from the orchard at Tamil Nadu Agricultural University, Coimbatore. The outermost sheaths were peeled off and the inner pseudostem was cut into

slices. The weight of the fiber-extractable pseudostem was recorded, and the fiber was extracted after decortification using a custom-made Raspador machine at the university. The as-extracted fibers were weighed and transported to the lab. They were allowed to dry for 48 h; half of which was under shade at atmospheric conditions with temperature ranging from 35 to 40 °C and the rest was at 70 °C in an oven. The dry mass was recorded after complete drying of the fibers under shade. The oven-dried samples were used for the analysis of chemical composition. The fibers were then cut into pieces of 1 to 4 cm in length.

Polyvinyl alcohol (PVA) (M_w 85,000 to 124,000, 99% hydrolyzed) was purchased from Sigma Aldrich, India. All other chemicals were analytical reagents or High Performance Liquid Chromatography (HPLC)-grade and were obtained from Merck, India.

Fiber Yield, Recovery Percentage, and Chemical Composition Analysis

Fiber recovery percentage refers to the amount of fiber that can be recovered or extracted from the biomass (in this case, banana pseudostem).

The recovery percentage and fiber yield were estimated prior to any further analysis and were calculated using the following formula (Syracuse University 2011):

$$\text{Recovery (\%)} = \frac{\text{Weight of the fiber(gm)}}{\text{Weight of fiber extractable pseudostem(gm)}} \times 100 \quad (1)$$

Fiber yield percentage refers to the percentage of lignocellulosic material, mainly consisting of polysaccharides, cellulose micro-fibrils, hemicelluloses, lignin, pectin, and water-soluble components.

$$\text{Fiber yield} = \frac{\text{Weight of the dried fiber(gm)}}{\text{Weight of the fiber after decortified(gm)}} \times 100 \quad (2)$$

The pseudostem fibers were analyzed to determine their holocellulose, lignin, and ash contents. The holocellulose content was estimated using the sodium chlorite method. 2.5 gm of the raw sample was used for analysis; the detailed procedure has been described elsewhere (Rowell 2012). The lignin was analyzed using the modified ASTM D-1166-84 method (2001). The ash was quantified using TAPPI T211om-93 (1993). All the experiments were carried out in sets of three, and the standard deviation was calculated.

Isolation of NFC Cellulose from Banana Pseudostem Fibers

After drying and cutting the banana pseudostem fibers from the two cultivars ('Grand Naine' and 'Poovan') to the desired size, the fibers were washed repeatedly with distilled water at 50 °C, the excess water was drained, and then they were dried at 50 °C for 8 h. Precisely 2 g of each fiber was transferred into a beaker and treated with 10 mL of 69% nitric acid and 80% acetic acid in a 1:10 ratio (V/V) and kept in an oven preheated to 120 °C for 20 min. After cooling, 20 mL of 99.9% ethanol was added to the above, which was then vortexed and washed repeatedly with ethanol by centrifugation at 5000 rpm for 10 min. The pellet obtained was then washed twice with MilliQ water by centrifuging at 5000 rpm. Washing with 99.9% ethanol was carried out twice at 5000 rpm for 10 min (Brendel *et al.* 2000). The pellet was suspended in 10 mL of ethanol. To determine the appropriate ultrasonication time, the 'Poovan' cultivar suspension (pellet in ethanol) was split equally between three vials, and ultrasonication was carried out for the three samples

for time periods of 10, 15, or 20 min. Subsequent observations in TEM were carried out to determine the extent of defibrillation caused at various time intervals. Based on our requirements (nano-fibrillated cellulose) and TEM observations, the ultrasonication time was fixed for both cultivars, and the ultrasonication of the suspension (pellet in ethanol) was carried out at 25 °C for 15 min, with pulsation every 10 s (VCZ 1500, 1500W, 20 kHz, Sonics and Materials, Inc.). Finally, 10 mL of acetone was added to the suspension, which was centrifuged at 10,000 rpm for 10 min. The residue was suspended in 5 mL of MilliQ water and centrifuged in the MilliQ water to remove the remnants of acetone. The suspension was preserved in MilliQ water until further use. Gravimetric measurements in sets of three were made to calculate the yield of the NFC cellulose using the following formula:

$$\text{Nanofibrillated cellulosic yield} = \frac{\text{Weight of dried NFC cellulose (g)}}{\text{Weight of the fiber used for extraction (g)}} \times 100 \quad (3)$$

NFC yield refers to the amount of NFC extracted/obtained from the fiber of each variety.

Characterization

Zeta potential analysis

The surface charges of the NFC derived from the Grand naine and Poovan varieties were determined using a particle size analyzer (Horiba Scientific Nano Particle Analyzer SZ-100 S). The NFC suspension was prepared (0.05% NFC / H₂O w/v) and sonicated for 30 min and analyzed at room temperature at a voltage range of -200 to +200 mV. Ultrapure water at a conductivity of 18.2 MΩ•cm (25°C), a TOC value below 5 ppb and a pH of 6.9 was used for the above. Three measurements were made for each sample.

UV-Vis transmittance studies

The light-transmitting properties of the NFC samples were determined using a NFC - PVA mixture in Millipore water (1% PVA+ 1% NFC w/v) using a UV-Vis spectrophotometer (Analyticjena Specord Plus 210, Germany), and the spectra were acquired between 200 and 800 nm.

FT-IR

FT-IR analyses on the raw fibers, acid-treated samples, and NFC were performed on a Shimadzu IRAffinity-1 spectrometer. The samples were dried at 60 °C for 8 h. Raw fibers from the two cultivars were ground into a fine powder using a Wiley mill, while the NFC samples and the acid-treated samples were used as such. All samples were blended with KBr and compressed into pellets, and the instrument was operated in transmittance mode.

XRD

The X-ray diffraction analyses were performed on the samples using a Seifert JSO Debyeflex with CuK_α radiation at 40 kV and 30 mA. The scattered radiations were detected in the 2θ range of 10° to 70° at a speed of 0.04°/min. The degree of crystallinity of the samples was determined empirically using the formula for Crystallinity Index (CrI) as follows,

$$CrI = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad (4)$$

where I_{002} is the maximum intensity of the 200-lattice diffraction and I_{am} is the intensity of diffraction at $2\theta = 18^\circ$ (Segal 1959).

TGA

The thermal stability and decomposition temperature of the samples were assessed by thermo gravimetric analysis (TG) and derivative thermogravimetric analysis (DTG) (TA Q50, TA Instruments). Approximately 5 mg of each sample was placed on platinum crucibles, and the samples were heated from 30 to 800 °C at 10 °C/min under nitrogen atmosphere at a flow rate of 40 mL/min.

Scanning electron microscopy (SEM)

The external morphology of the raw fibers and the fiber samples after acid treatment were analyzed using a SEM (FEI QUANTA 250). Banana fibers from the two cultivars were cut to a size of 0.3 to 0.4 cm and placed vertically onto double-sided conductive C tape on an Al SEM stub. A quantity of 0.1 mg of the acid-treated sample was used for analysis.

Transmission electron microscopy (TEM)

The ultrasonicated samples ('Poovan' cultivar, time periods of 10, 15, and 20 minutes, respectively) and NFC samples from the two banana cultivars were observed under TEM (FEI Technai Basic at 100kV) in bright field mode. Approximately 10 µL of diluted sample (0.05% w/v) was dropped over the carbon-coated copper grid, and before it was completely dried, 2 µL of a 2% solution of uranyl acetate was dropped over it and allowed to stay for 1 min. The excess was blotted using Whatman No.1 filter paper, and the sample was allowed to dry completely at room temperature.

RESULTS AND DISCUSSION

Fiber Yield, Recovery Percentage, Chemical Composition, and NFC Yield

The fiber yield and recovery percentage differed between the two cultivars. The 'Poovan' cultivar (2.80%) recorded a 32% higher fiber recovery than the 'Grand Naine' (1.79%) (Table 1). Banana pseudostem fibers are characterized by a higher percentage of cellulose and lower lignin and higher ash contents (Khan *et al.* 2013). A higher percentage of holocellulose and lower contents of lignin and ash were recorded for 'Poovan' in comparison to 'Grand Naine'. These data are in agreement with the results of other investigators who have shown higher cellulosic values for 'Poovan' relative to other cultivars tested (Preethi and Balakrishna 2013). This can be explained by the fact that fiber yield is higher in cultivars with the AAB genome, represented by 'Poovan', as compared with those with the AAA genome, namely the 'Grand Naine' (Uma *et al.* 2005). The ash content of banana cultivars ('Grand Naine' 11.8%; 'Poovan' 8.7%) recorded in the present study is in agreement with the reports of earlier investigations as reported in literature (Khan *et al.* 2013). Slight variations may be attributed to the edapho-climatic conditions. The percentage composition in both the cases does not sum to 100%. This could be attributed to the fact that they had not been normalized with respect to all the constituents,

and with respect to such factors as the loss of any sugars or proteins, the various methods used for the determination of the analytes, the presence of non-quantified matter, the presence of nitrogenous or other material in the extract, *etc.* (Guimarães *et al.* 2009). The recovery of NFC was higher from 'Poovan' (4.57%) than from 'Grand Naine' (3.83%), which was in agreement with the reported high holocellulose content in 'Poovan' (Table 1).

Table 1. Average Values and Standard Deviations of Recovery, Fiber Yield Percentage and Chemical Composition of Banana Pseudostem Fiber from Two Banana Cultivars

No	Banana cultivars	Recovery (%)	Fiber yield (%)	Holocellulose (%)	Klason Lignin (%)	Ash (%)
1.	'Poovan'	2.80±0.136	0.86±0.103	59.5±0.38	14.7±1.02	8.7±0.67
2.	'Grand Naine'	1.79±0.098	0.59±0.074	56.9±0.58	16.8±0.84	11.8±0.42

Steps involved in the extraction of NFC cellulose from the pseudo-stem of two banana cultivars are shown in Fig. 1. Raw banana fibers were hydrolyzed with nitric acid and acetic acid. The delignified matrix was further defibrillated upon ultrasonication to form NFC.



Fig. 1. Images of (a) raw 'Grand Naine' banana pseudostem fiber, (b) raw fibers of 'Grand Naine' and 'Poovan' after addition of the acid mix, (c) fibers after the first wash with ethanol, and (d) 'Grand Naine' and 'Poovan' NFC

UV-VIS Studies

The transmittance values for 1 wt% of NFC derived from 'Grand Naine' and 'Poovan' mixed with 1% PVA were 37% and 42%, respectively. The difference in the transmittance values as depicted in the UV-VIS spectra (Fig. 2) may be attributed to the difference in the morphological characteristics of the NFC isolated from the two cultivars, which is further evident in the TEM images. Although optical transparency as high as 90% has been reported for TEMPO-oxidized NFC cellulose films (Klemm *et al.* 2005), it has

been postulated that transparency in films prepared from nanocellulose may be high if the interstices between the fibers are small enough to prevent light scattering (Fortunati *et al.* 2012). However, in the case of NFC prepared from the two cultivars, addition of NFC affects the otherwise transparent nature of PVA (93% transmittance). This property of low levels of transmission in the UV range upon the addition of NFC to PVA is quite important for developing food packaging material where the NFC in the film serves as a barrier to UV and minimizes the UV light-induced lipid oxidation in the skins of fruits, which may extend the shelf-life of perishables (Fortunati *et al.* 2012). PVA plays a role in affecting the dispersion rate of the NFC. The entanglement of fibers can affect the UV-vis transmittance. This property could be beneficial for the use of NFC as an additive in food packaging material.

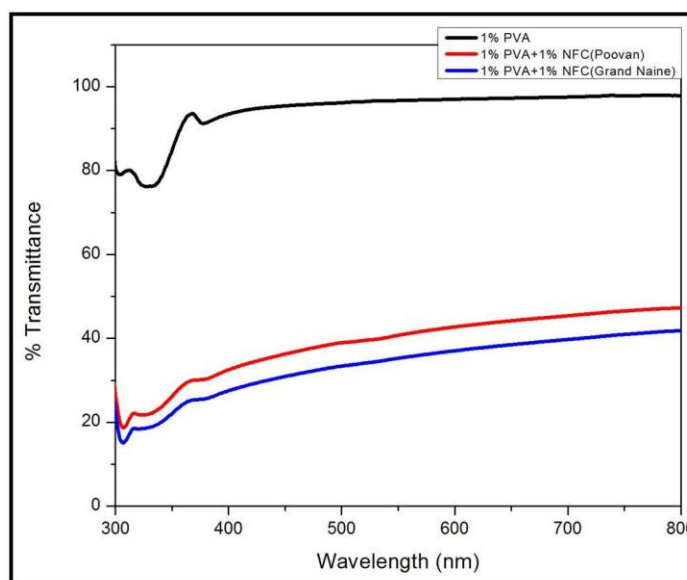


Fig. 2. UV-Vis transmittance studies of PVA and NFC ('Grand Naine' and 'Poovan') mixed with PVA

Zeta Potential Measurement

The zeta potential values of the NFC in MilliQ showed a negative zeta potential (minus 25 mV), indicating the stability of the suspension (Morais *et al.* 2013). The zeta potentials of 'Grand Naine' NFC showed higher negative values (-48.43 ± 0.73 mV), indicating higher dispersion capabilities, while the NFC from Poovan showed less negative values (-38.3 ± 0.41 mV), indicating the entanglement of fibers (Tonoli *et al.* 2012). These observations were evident in the TEM images. The low zeta potential could also have been a result of any residual pectin. Zeta potential measurements have been carried out in Millipore water.

Chemical Treatment

In the present study, the NFC obtained after treatment with a high concentration of nitric acid (69%) and no alkali or bleaching agent was white in color, which may have been due to the fact that nitric acid is a strong oxidizing agent and protein denaturant. A combination of acetic acid and nitric acid has been documented to be successful in the removal of all traces of lignin, hemicelluloses, and xylosans from lignocellulosic matter (Updegraff 1969). A similar acid mixture has been used for the removal of lignin and

isolation of cellulose (Xu *et al.* 2006). Cellulose extraction from barley straw was accomplished using an acid mixture (nitric acid and acetic acid in 1:10 ratio) with a pretreatment of hydrogen peroxide. The adopted one-step protocol is highly efficient for minimizing cellulose losses attributable to mercerization and bleaching. The procedure is not only effective in the delignification and removal of non-cellulose polysaccharides, but also reduces the environmental risks associated with the traditional delignification and bleaching procedures (Sun *et al.* 2004). The chemistry behind the effective delignification may be explained by the fact that at high temperatures, the concentrated nitric acid renders the lignin yellow and ultimately dissolves it, such that the resultant formed is soluble in acetic acid (Thomson 1838).

FT-IR

The FT-IR spectra of raw fibers and NFC are shown in Fig. 3. The main peaks observed in the FT-IR spectra are presented in Fig. 3. The stretching of hydrogen bonds in the OH groups of cellulose is represented by peaks around 3300 and 3400 cm^{-1} for ‘Grand Naine’ and ‘Poovan’ respectively. Shifts were detected in the peaks between the raw fibers and the NFC cellulose. The absence of the peak at 1728 cm^{-1} (resulting from the C=O bond vibrations of lignin or hemicelluloses) in the NFC sample suggest complete/partial removal of lignin. Shifts were detected in peaks between the raw fibers and NFC. Some occluded water was present in all samples. This was difficult to remove because of the strong interaction between cellulose and water, which is clearly exhibited in the FT-IR spectra (1635 cm^{-1}) and is supported by other reports in the literature (Abraham *et al.* 2011). The peak in the range 1435 cm^{-1} in the ‘Poovan’ cultivar may be attributed to $-\text{CH}_2$ bending (Pappas *et al.* 2002). The vibration peaks detected at 1381 and 1327 cm^{-1} in the case of ‘Poovan’ NFC and at 1373 and 1327 cm^{-1} for the ‘Grand Naine’ NFC could be related to the C-H and C-O bond vibrations, respectively, in the polysaccharide aromatic rings (Mandal and Chakrabarty 2011). The absence of the peak at 1157 cm^{-1} in the NFC samples could be suggestive of removal of amorphous cellulosic portions after the chemical treatment and formation of crystalline nanocellulose (Fan *et al.* 2012). The peak observed at 1064 cm^{-1} in all the samples may be attributed to the C-O-C pyranose ring stretching vibration.

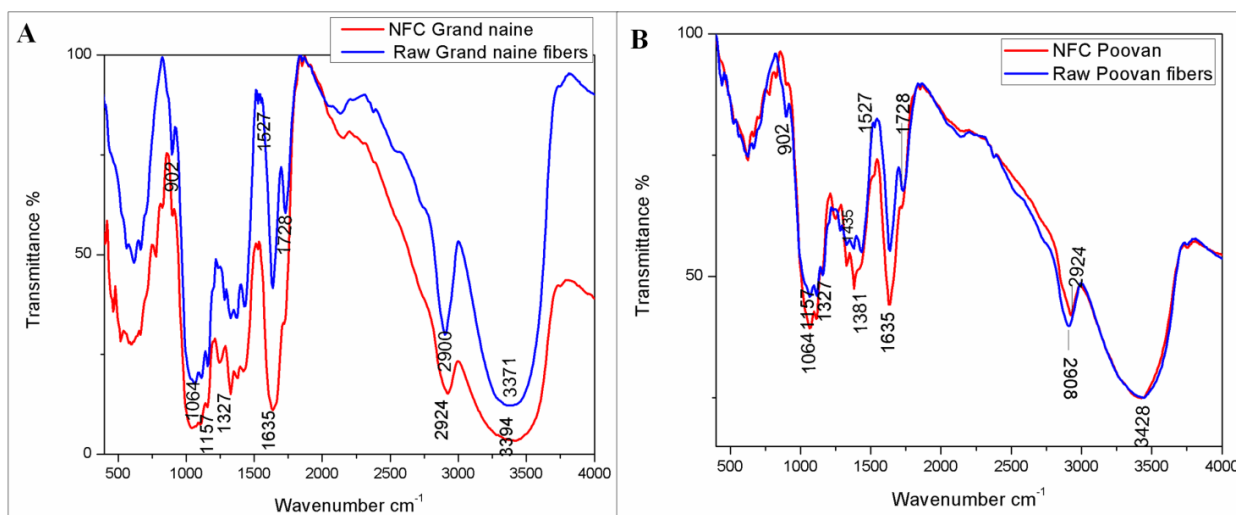


Fig. 3. FT-IR spectra of (a) ‘Grand Naine’ raw fibers and NFC (b) ‘Poovan’ raw fibers and NFC

The small peak at 902 cm^{-1} in the raw fiber samples may be related to the β -glycosidic linkages in cellulose (Jiang and Hsieh 2013; Mandal and Chakrabarty 2011; Pappas *et al.* 2002). The low intensity of the peaks in the raw fibers in comparison to the NFC depicted the removal of amorphous portions of the cellulose (Kumar *et al.* 2014). FT-IR analysis of the two samples after acid treatment was also carried out in order to confirm the efficacy of the adopted method in the removal of lignin. The FT-IR spectra have been depicted in Fig. 3. The present results confirmed the removal of lignin along with non-cellulosic material and the effectiveness of the adopted procedure in delignification.

Table 2. Infrared Transmittance Peaks (cm^{-1}) of the Raw Fibers and NFC

S.No	Cultivars	Stretching OH	C-H stretching	C=O bond vibrations of lignin	Absorbed water	C=C plane symmetric stretching of lignin	C-O-C pyranose ring stretching	C-O-C bond stretching vibration in amorphous cellulose
1.	'Grand Naine'	3371	2900	1728	1635	1527	1064	1157
2.	'Grand Naine' NFC	3394	2924	-	1635	-	1064	-
3.	'Poovan'	3428	2908	1728	1635	1527	1064	1157
4.	'Poovan' NFC	3428	2924	-	1635	-	1064	-

XRD

The XRD patterns of the raw banana fibers and the NFC are represented by Fig. 4. The crystallinity index of the samples was calculated by the peak height method as suggested, and the data are furnished in Table 3.

The degree of crystallinity in cellulose is an important factor that influences various properties, including compactability and the absorption of water (Azubuike and Okhamafe 2012). The NFC had a higher crystallinity than their raw counterparts, which was represented by its higher crystallinity index (CrI). The data on CrI values closely coincided with those of other reported literature (Guimarães *et al.* 2009). The 2θ angle around 22° in the raw fibers and the NFC corresponded to the plane in the sample, with the Millers indices 200 indicating the crystalline region of the cellulose (Terinte *et al.* 2011). A peak at $\sim 18^\circ$ in the raw fibers of the Poovan and Grand naine suggested the presence of amorphous or non-crystalline cellulose (Wulandari *et al.* 2016). The NFC did not have a peak at 18° , which reflected the removal of the lignin and hemi-celluloses during the acid treatment and the preservation of the crystallite species. The peaks at 2θ degree of approximately 15° and 16° corresponded to the planes in the sample with Miller indices 1-10 and 110, thereby indicating a predominance of the crystalline cellulose I structure. Similar results have been stated elsewhere in a recent work (Claes Nilsson 2017). Cellulose I structure is composed of two distinct crystal phases, namely I_α and I_β (Nishiyama *et al.* 2002). The I_β crystal phase is the major part of plant-fibers-derived cellulose. The diffraction peaks situated at about 15° and 16° were further attributed to d-spacing of 0.61 to 0.62 nm and 0.53 to 0.54 nm, respectively (Okita *et al.* 2010). A unit cell structure of cellulose I contains eight cellobiose moieties. Anhydroglucose units linked together by intermolecular hydrogen bonds form layers, which are held together by weak Van der Waal's forces (Pettersen 1984). Even after acid hydrolysis, there is no shift from cellulose I to cellulose II, as suggested by the main

peak, located at $\sim 22^\circ$ in both the raw fibers and the NFC. The release of hydronium ions during acid hydrolysis and their penetration into the amorphous regions of cellulose promoted the hydrolytic cleavage of the glycosidic bonds, thereby releasing the crystalline cellulose fraction.

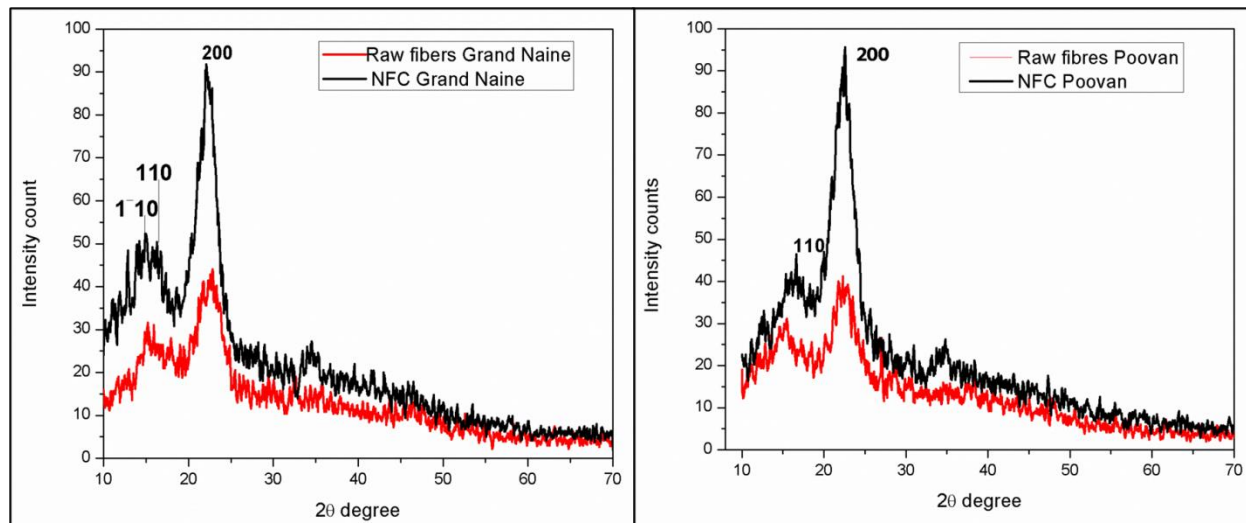


Fig. 4. XRD patterns of (a) 'Grand Naine' raw fibers and NFC and (b) 'Poovan' raw fibers and NFC

Table 3. Crystallinity Index and 2θ Values of Raw Fibers and NFC from 'Poovan' and 'Grand Naine' Cultivars

No	Sample	Crystallinity Index	2θ degree
1	'Grand Naine' raw fiber	32.86	22.6, 16.0, 14.9, 17.9
2	'Grand Naine' NFC	56.99	22.0, 16.6, 14.1
3	'Poovan' Raw Fiber	40.07	22.2, 15.5, 17.8
4	'Poovan' NFC	57.72	22.5, 16.7

TGA

The TG and DTG curves for raw fibers and NFC are presented in Fig. 5. The TGA curves of the raw 'Grand Naine' and 'Poovan' fibers showed a multi-stage decomposition process, which could be attributed to the presence in the fibers of various compounds, such as lignin, hemicelluloses, and cellulose, that decompose at different temperatures. The onset temperatures for the degradation of 'Grand Naine' raw fiber and NFC were 132 and 243 $^\circ\text{C}$, respectively, while for 'Poovan' raw fiber and NFC the temperatures were 149 and 343 $^\circ\text{C}$, respectively. A small weight loss, at 144 $^\circ\text{C}$ for the 'Grand Naine' and at 153 $^\circ\text{C}$ for the 'Poovan' cultivar, may have been related to the decomposition of the low molecular weight organics. The peaks at 192 and at 195 $^\circ\text{C}$ for the raw 'Grand Naine' and 'Poovan' fibers, respectively, could be related to the decomposition of lignin, which started well below 200 $^\circ\text{C}$ and lasted to about 700 $^\circ\text{C}$, and may be attributed to the different activities of the various chemical bonds in lignin (Abraham *et al.* 2011, 2013). The absence of these peaks in the NFC samples indicated the complete removal of lignin, which further confirmed the FT-IR spectra. The peak at 312 and 301 $^\circ\text{C}$ in the DTG curves for the raw 'Grand Naine' and 'Poovan' fibers denoted α -cellulose. The higher thermal stability of the 'Grand Naine' and 'Poovan' was depicted by peaks at 385 and 349 $^\circ\text{C}$, respectively, while such values were lower in their respective raw fibers. The higher thermal stability could be

of importance in exploiting nanocellulose as a reinforcement material and in processing thermoplastic polymers. The peaks at 443 and 458 °C for the raw ‘Grand Naine’ and ‘Poovan’ fibers, respectively, were associated with lignin decomposition and with the breakage of proto-lignin bonds (Guimarães *et al.* 2009).

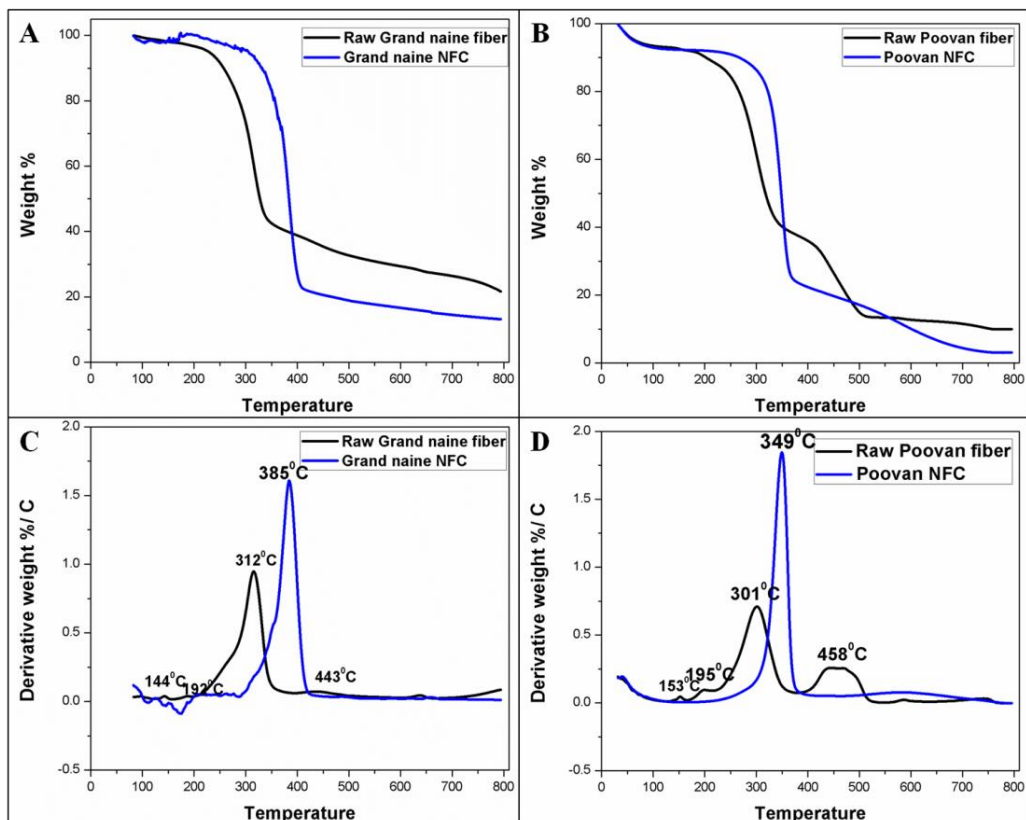


Fig. 5. TGA: (a) TG curve of raw ‘Grand Naine’ fiber and ‘Grand Naine’ NFC; (b) TG curve of raw ‘Poovan’ and ‘Poovan’ NFC; (c) DTG curve of raw ‘Grand Naine’ fiber and ‘Grand Naine’ NFC; (d) DTG curve of raw Poovan and Poovan NFC

Electron Microscopy

The SEM images of the raw fibers confirmed the multi-cellularity of the structure (Figs. 6 a and b). The spherical fiber cells composed of primary and secondary cell walls and lumen are clearly visible in the images. The central part of the fiber “lumen”, which is elliptical or nearly circular in shape, varied in size (14 to 23 μm), but no major differences between the two cultivars were observed in the SEM micrographs. However, the cell wall of the ‘Grand Naine’ was thicker ($0.629 \pm 0.15 \mu\text{m}$) than was the cell wall of the Poovan ($0.357 \pm 0.18 \mu\text{m}$) cultivar. Such variations in cell wall thickness could be attributed to the higher lignin content in ‘Grand Naine’ relative to ‘Poovan’. The mechanical characteristics of the fibers depend mainly on the thickness of the cell wall, number of fiber cells, and fiber cross-sectional area (Ernestina *et al.* 2013). Figure 6 (c and d) shows the SEM micrographs of the two banana fiber samples after acid treatment, depicting micro fibrils with diameter of 4 to 5 μm for ‘Grand Naine’ and 2 to 3 μm in the case of ‘Poovan’. The micro-fibrillar structure obtained after acid treatment explains the effectiveness of the procedure in delignifying the banana fibers and downsizing the fiber from the macro to the micro range. The higher entanglement of ‘Grand Naine’ fibers in comparison to ‘Poovan’ was also clearly visible.

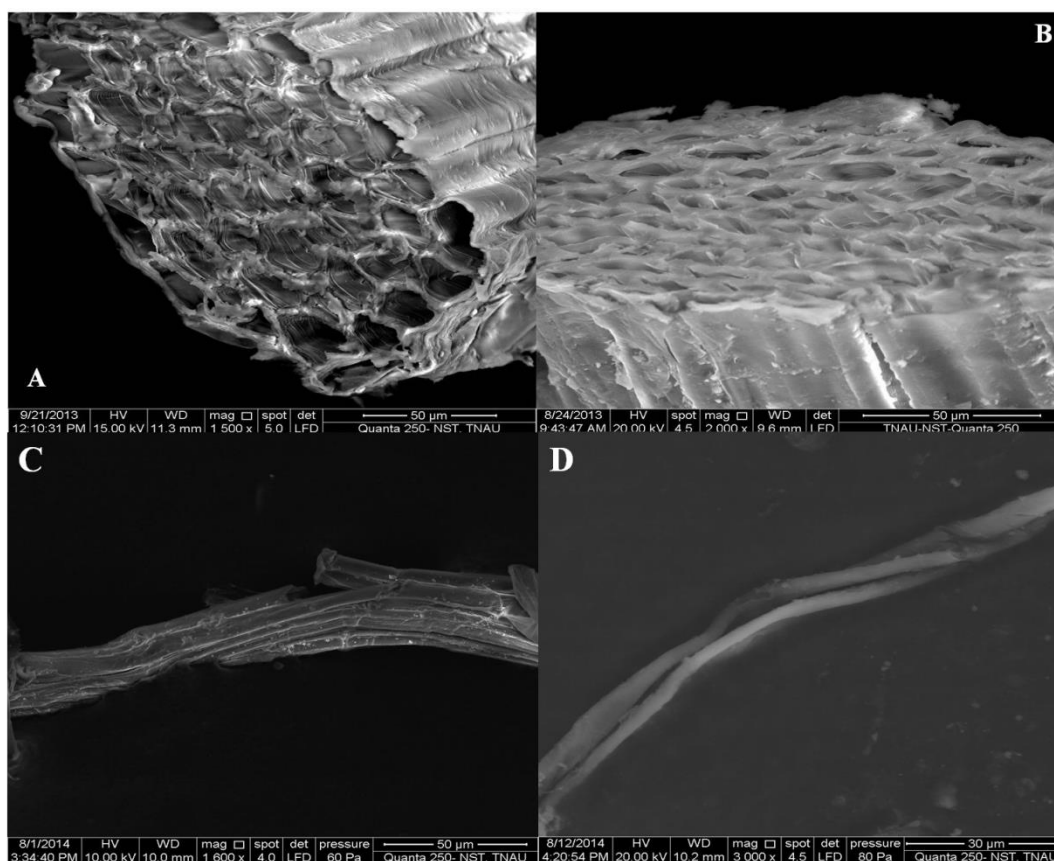


Fig. 6. Electron micrographs: SEM image of (a) raw 'Grand Naine' banana fiber, (b) raw 'Poovan' banana fiber, (c) 'Grand Naine' after acid treatment, and (d) 'Poovan' after acid treatment

The TEM images suggested that the chemical and mechanical treatments were successful in removing the cementing materials around the fiber-bundles. The effect of ultrasonication at various time intervals for the 'Poovan' cultivar is clearly depicted in the TEM micrographs in Fig. 7.

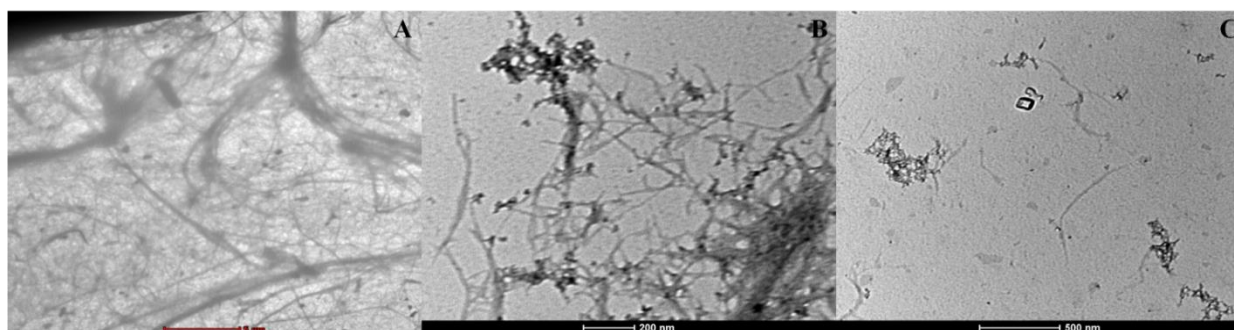


Fig. 7. TEM images of 'Poovan' after ultrasonication for (a) 10 min, (b) 15 min, and (c) 20 min

The hierarchical downsizing and onset of defibrillation from microfibrils to nanofibrils is clearly visible in the TEM micrograph in Fig. 7(a) (10 min). Increasing the ultrasonication time by 5 min led to the formation of nanofibrillated cellulose (Fig. 7(b)), while ultrasonication for 20 min led to the complete disruption of the structure, as is

depicted in Fig. 7(c). TEM micrographs of the suspensions revealed that NFC displayed some long entangled cellulosic filaments initially but ultra-sonication resulted in fibers individualization, culminating in more separate network (Pelissari *et al.* 2014). The time for ultrasonication was therefore fixed at 15 min, and the same was used for the ‘Grand Naine’ cultivar.

The analysis of the TEM micrograph revealed that the aqueous suspension of NFC consists of rod-like nanoparticles, whereby some of them agglomerated in the form of bundles while some others seemed to be well separated. The particle size distribution curve depicts the width of the nano-fibrils, ranging from 4 to 6 nm for ‘Poovan’ and from 6 to 10 nm for ‘Grand Naine’ (Fig. 8 (a) and (b)). The data agreed with the earlier reports of crystalline cellulose microfibrils measuring a width of 5 nm in textile celluloses like cotton and ramie (Sturcová *et al.* 2004). Long and slender fibrils are found as bundles or singles in both cultivars. The clustered nature of the fibrils in ‘Grand Naine’ may be a result of the formation of interfibrillar hydrogen bonds. However, this may also be attributed to the Van der Waals attraction forces between nanoparticles (Lani *et al.* 2014; Othman *et al.* 2012). Similar data have been reported for fibrils with diameters of 5 nm derived from banana rachis by acid hydrolysis (Zuluaga *et al.* 2007). The high aspect ratio (fibril length to diameter ratio) of these fibrils is also depicted in the TEM images. The aspect ratio is an important factor that may increase the mechanical strength of the fiber (Fortunati *et al.* 2012). An aspect ratio in the range 36 to 44 was calculated from the TEM micrographs. The exact length of the fibril was not visible in many cases, and only the visible length was considered, as suggested by Cherian *et al.* (2010). Other reports suggest that the cellulose fibrils were in the range of 2.5 to 5 nm in width and several micrometers in length. The width may be calculated from small angle XRD or TEM analysis, but the determination of length has experimental limitations (Wojtaszek 2011).

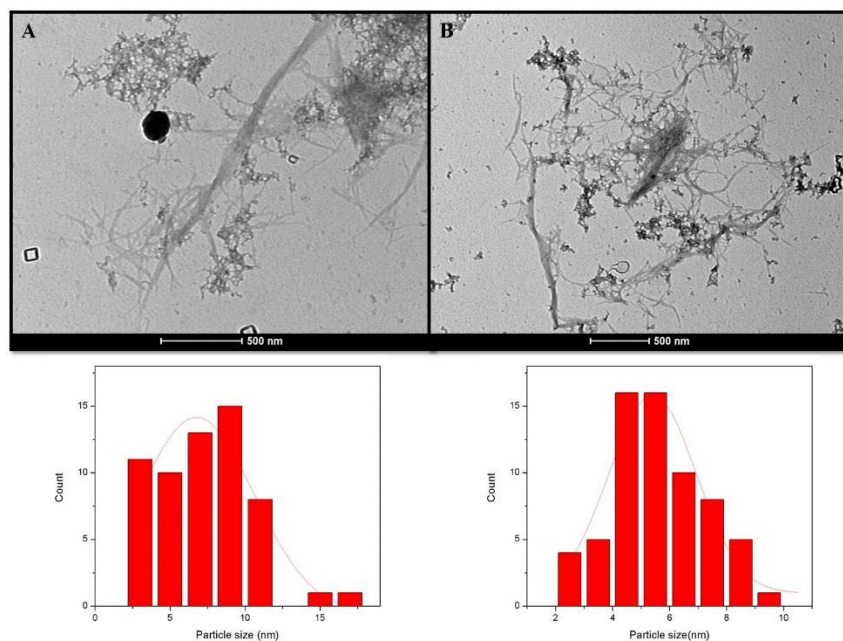


Fig. 8. TEM image of (a) ‘Grand Naine’ NFC (b) ‘Poovan’ NFC with respective particle size distribution curves below them

The fiber and NFC characteristics of the banana genotypes clearly showed that both the ‘Grand Naine’ and ‘Poovan’ possess desirable attributes that can be considered for developing biodegradable nano-packaging material. The ‘Poovan’ banana pseudostem was shown to carry more fiber content (2.8%) and NFC content (4.57%) in addition to higher crystallinity, while having a thinner cell wall (0.357 μm) and wider nano-fibrils (6 to 10 nm) in comparison to the ‘Grand Naine’. On the other hand, ‘Grand Naine’ banana was shown to have a high ash content (11.8%), lower NFC (3.83%), and higher thermal stability up to 385 °C. This study brought out genotypic variations between two predominant banana cultivars (‘Grand Naine’ and ‘Poovan’). These results open up a new possibility to exploit banana pseudostem as an agro-waste and to develop smart packaging materials to minimize post-harvest losses in perishables, particularly fruits and vegetables. While people are worried about the persistence of plastics in nature, cellulosic nano-films may be an alternative to derive wealth out of waste while ensuring environmental safety.

CONCLUSIONS

1. Nano-fibrillated cellulose (NFC) was successfully extracted from two commercial banana cultivars by employing a one-step, totally chlorine- and alkali-free method coupled with ultrasonication. This method was efficient in the removal of non-cellulosic material, and nanofibrils with diameters in the range of 4 to 8 nm were extracted. The hierarchical downsizing of the fibers from macro to micro to nano sizes has been clearly depicted.
2. The isolated NFC was almost 20% more crystalline than the raw fibers, as depicted from the XRD results, which could be attributed to the removal of the matrix components.
3. Based on our study, the ‘Poovan’ cultivar demonstrated superior properties in comparison to the ‘Grand Naine’ in terms of nanocellulose yield, cellulose content, and crystallinity of the NFC, while the thermal properties of the ‘Grand Naine’ NFC were better, as depicted in the DTG curves.
4. The agricultural biomass is readily available, and the extraction of cellulose from the above could introduce a new perspective to agricultural waste management. The authors hope to extend its limits and possible application to the extraction of nanocellulose from other plant fibers.
5. The prepared NFC cellulose may also prove to be a good reinforcement material in packaging material for the enhanced preservation of fruits possibly reducing the risk of damage caused by UV rays.

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