Philippine e-Journal for Applied Research and Development 12(2022), 1-11 ISSN 2449-3694 (Online)
https://pejard2.slu.edu.ph/wp-content/uploads/2022.01.31.pdf

Comparative Characterization of Nanocellulose Produced from Kawayan Kiling (*Bambusa vulgaris* Schrad ex. Wendl) Pulp Using Different Pretreatment Methods

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Received, 05 June 2021; Accepted, 15 December 2021; Published, 31 January 2022

Abstract

Kraft pulp from *kawayan kiling* (*Bambusa vulgaris* Schrad ex. Wendl) were subjected to two separate pre-treatment procedures (namely, chemical and mechanical) prior to sulfuric acid hydrolysis for nanocellulose production. Experimental nanocellulose yields were 26.94% for the bleached and alkali pretreated pulp and 1.20% for the mechanically refined pulp. FTIR spectroscopy showed that the chemically pre-treated bamboo pulp yielded nanocellulose with no residual lignin while the ground, acid-hydrolyzed pulp retained some lignin. FIB-FESEM imaging showed that nanofibrils with average widths of 39.13 $\pm$ 34.11 nm were formed as hydrolysis products from the friction grinder treated material while nanometer-sized crystals with average widths of 134.2 $\pm$ 34.33 nm were produced from the chemically treated bamboo pulp. EDS analysis showed no impurities in the products from the chemical treatment, whereas the presence of silicon was detected in the mechanically refined pulp. Based on XRD analysis, the degree of crystallinity of the nanofibrils and crystals were 49.47% and 56.92%, respectively. In future works, *B. vulgaris* pulp that will be mechanically treated may require bleaching to further remove residual lignin and thus improve acid hydrolysis.

Keywords: methods of nanocellulose production, acid hydrolyzed production of nanocellulose, bamboo pulp bleaching and alkali digestion, friction grinder supermass colloider, nanofibrils

Introduction

Nanocelluloses have applications in nanocoats, bioimaging, biomedicine, gas barrier films and optically-transparent functional materials (Jiang & Hsieh, 2013). Nanocellulose can be obtained from plants especially from lignocellulosic-rich fibers found primarily in the stems and branches of trees and the culms of bamboos. Several plants have been explored for nanocellulose production like cotton stalks (Soni et al., 2015), grain straws (Oun & Rhim, 2016), pineapple leaf fibers (Cherian et al., 2010), corn stover (Costa et al., 2015), cacao pod husk (Hutomo et al., 2015), sugarcane bagasse (Wulandari et al., 2016), wood pulp (Thanh et al., 2019), and bamboo (Razal, 2016; Wang et al., 2015; Chitbanyong et al., 2018). In the Philippines, bamboos are a promising source of nanocellulose, as they are ubiquitous, easy to grow in almost all climate types, and have the ability to regenerate quickly when cut.

The common local varieties of bamboo used for pulping and nanocellulose production are the *kawayan kiling* (*Bambusa vulgaris* Schrad ex Wendl) and the *kawayan tinik* (*Bambusa*

blumeana J.A. & J.H. Schultes). *Kawayan kiling* culms contain a host of natural products with potential for commercial application (Fei et al., 2016), and several studies were conducted to determine the suitability of this for pulp and paper (Ameh et al., 2017; Sekyere, 1994; Escolano and Semana, 1970). Altenatively, Bayani et al. (2016) tested the use of acid-hydrolyzed cellulose nanowhiskers from *kawayan tinik* as reinforcement for xylan films. Villegas (2015) also attempted to incorporate cellulose nanowhiskers from *kawayan tinik* as a reinforcement in thermoplastic starch. Meanwhile, Jara (2019) used newly emerging bamboo shoots of *B. vulgaris* as feedstock for nanocellulose production without prior pulping, capitalizing on the low lignin content of young bamboo shoots.

Several preparatory steps such as pulping and removal of residual lignin are employed to separate cellulose from the lignocellulosic matrix. However, these methods commonly use chlorine containing compounds that could cause instability and irregularities in the properties of the nanocrystals produced (Dufresne, 2018). Moreover, the many steps required could affect production cost and energy requirement, and likewise have environmental impact. To address these problems, it would be helpful to search for methods of producing nanocellulose that can overcome the disadvantages and limitations of production processes that are highly dependent on the use of chemicals.

Acid hydrolysis is by far the most common method of producing nanocellulose from lignocellulosic materials, with either sulfuric acid (Mascheroni et al., 2016; Zhang et al., 2020) or hydrochloric acid (Hastuti et al., 2018), or both (Yu et al., 2013; Hutomo et al., 2015) serving as the inorganic catalyst to break down the isolated cellulose. Other reagents and methods tested are ammonium persulfate reaction (Mascheroni et al., 2016; Zhang et al., 2020), TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl radical) oxidation (Isogai et al., 2011), and $FeCl_3$-catalyzed hydrolysis assisted by ultrasonication (Lu et al., 2014).

This study aims to find a method of preparing nanocellulose from *kawayan kiling* that minimizes the issues attendant to the use of chemicals in pretreatment procedures prior to acid hydrolysis of cellulose. Towards this, the study will explore on two methods of pretreating the Kraft bamboo pulp prior to acid hydrolysis. The first will be an all-chemical sequence involving bleaching and alkali digestion while the second will dispense of the chemical treatments in favor of mechanical disintegration by multiple passes of the pulp in a friction grinder supermass colloider. The nanocellulose resulting from the two different pretreatment methods will then be analyzed and compared.

Materials & Methods

Materials

The kawayan kiling culms were obtained from the Makiling Forest Reserve, University of the Philippines Los Baños (UPLB). All reagent-grade chemicals were purchased from commercial sources and were used without further purification unless noted. The apparatus and equipment used in nanocellulose isolation are located at the Wood Chemistry Laboratory, Department of Forest Products and Paper Science, College of Forestry and Natural Resources, University of the Philippines Los Baños and the Institute of Chemistry, College of Arts and Sciences, UPLB.

Sample Preparation

Preparation of bamboo culms for pulping was done in accordance with the procedure reported by Razal (2016). Bamboo culms were examined for blemishes or damage and those found to be defective were discarded. The selected culm was cut into 1 to 1.5-inch rings, leaving out portions that contain the nodes. The bamboo skin was removed and then the bamboo rings were further subdivided into chunks using a utility cutter. The bamboo chunks were air-dried for three days and were made to pass through a Pallmann PHM3 Model 10180015 (Germany) hammermill for further breakdown.

Kraft Pulping

Weighed amounts of NaOH and Na_2S were sequentially dissolved in a predetermined volume of water and added to hammermilled bamboo chips in a large glass container. The quantity of

pulping chemicals used, and the water volume were based on calculations that provided 25% sulfidity, 13% effective alkali, and a liquor to bamboo chip ratio (ovendry basis) of 10:1,

where: $\%Sulfidity = \frac{Na2S}{(NaOH+Na2S)} \times 100$ Equation 1

and $Effective\ Alkali = \frac{NaOH}{1/2Na2S}$ Equation 2

and NaOH and Na_2S are the weights (g) of sodium sulfide and sodium hydroxide, respectively, expressed as Na_2O (Bajpai, 2018).

The improvised pulping digester that was assembled for the pulping of the bamboo chips consisted of a large glass vessel, a pressure cooker, and burner. The glass vessel containing the chips and "liquor" was covered with cheesecloth and placed inside the pressure cooker partially filled with distilled water, then sealed. It was heated up to a temperature of 121°C and 15 psi pressure, which was then maintained for an additional 2 hours. The pressure was released, and the cooked bamboo pulp was placed over cheesecloth and thoroughly washed with tap water with squeezing. The resulting pulp was then further disintegrated using an Oster (U.S.A.) commercial blender Model 4172 for at least 15 seconds.

Chemical Treatment

1. Bleaching and Holocellulose Preparation

Two-stage bleaching following the procedure of (Villegas, 2015) was employed using 0.5% (w/v) hypochlorite solution (3% consistency [dry mass to total solution], 70 °C, 30 min) followed by soaking in 5% (v/v) H_2O_2 solution (3% consistency, 70 °C, 120 min). The bleached pulp was washed several times with water under vacuum filtration to remove excess bleaching chemicals. Holocellulose was prepared from bleached pulp by acid chlorite delignification (Erickson, 1962). Solution A (60 mL CH_3COOH and 1.3 g NaOH in 1 L H_2O) was added to the bleached pulp in a flask at a ratio of 2 g pulp per 30 mL solution A. The flasks were placed in a water-filled metal pan mounted on a hot plate and heated to a temperature of 75°C monitored with a thermometer. Then, 3 mL of 20% sodium chlorite ($NaClO_2$) solution was placed in the flask with subsequent addition (3 mL each) of the $NaClO_2$ solution after 0.5, 1.0, 1.75, and 2.5 hr., with swirling after every addition. After 3.25 hr., the mixture was suction-filtered and washed with portions of chilled distilled water, four 75-mL quantities of 1% CH_3COOH and two 30-mL quantities of methanol.

2. Alkali-insoluble Cellulose Preparation

Cellulose was prepared from the holocellulose fraction following the modified TAPPI procedure for alpha-cellulose determination (TAPPI Test Method 203 cm-09, 2009). To the bamboo holocellulose (3 g oven-dry weight) preparation in a flask, 60 mL of 17.5% NaOH solution was added and heated at 20°C in a water bath for 30 min. The alkali-treated holocellulose was then suction-filtered and sequentially washed with 750 mL distilled water, 10% CH_3COOH (with prior soaking for 5 min), and 250 mL distilled water until the pH of the filtrate was neutral.

Mechanical Treatment

The Kraft pulp (without prior bleaching) was subjected to mechanical refining using a friction grinder supermass colloider Model MKCA6-2 (Masuko Sangyo Co. Ltd, Japan) for 15 passes at 1500 rpm. The grinder clearance was gradually decreased from -1 during the first pass, until the setting reached -10 in the 15th pass. After every pass, grinding was discontinued, and the grinder housing was opened. This was done to recover the residues stuck around the periphery of the grinding stones by washing with water. However, pulp retained on the outer surfaces of the grinding stones was discarded. This step was done to ensure that for the experiment, there was homogeneity in the ground pulp recovered from the colloider. By removing the pulp that were stuck outside the grinding discs and collecting only the pulp that was uniformly pressed between the discs, the experiment had minimized the variability in the pulp recovered for subsequent tests. However, this would result in low yield recovery from this experimental procedure. The opening of the grinder housing to recover undrained pulp was repeated for every pass until there were no observable differences between the pulp in and outside the grinding

stones. After 15 passes, 500 mL of the ground pulp suspension was obtained.

Acid Hydrolysis

In a two-neck round bottom flask, 1 g of air-dried alkali-insoluble cellulose was added, followed by 50 mL of 46% v/v sulfuric acid (Jara, 2019; Villegas, 2015). The flask was placed in a water bath on a hot plate and was constantly stirred with a magnetic stirrer. The reaction was carried out for 30 min while maintaining the water bath temperature at 45°C. After 30 min, 500 mL of cold distilled water was added to the mixture and subsequently neutralized using 10% NaOH. The mixture was transferred to a separatory funnel and allowed to settle until a distinct boundary between two layers was observed, where one layer contained the hydrolysis products. The acid-hydrolyzed cellulose produced from the chemically treated *kawayan kiling* pulp settled at the bottom layer and was subsequently separated by draining from the funnel.

For the colloider-treated pulps in water suspension, the water content was reduced in a Stuart RE301P rotary evaporator (Cole Parmer Ltd., USA). A portion of the thick suspension containing approximately 1 g of oven dried ground pulp was taken, and then concentrated H_2SO_4 was added to bring to a final concentration of 46% (v/v). The same conditions employed for the acid hydrolysis of the alkali insoluble cellulose and the recovery of the acid-hydrolyzed cellulose were followed for the colloider-treated Kraft pulp.

The solution containing the hydrolysis products was transferred in falcon tubes and was washed repeatedly with distilled water and centrifuged at 5°C and 9500 rpm for 10 min using Allegra X-22R centrifuge (Beckman Coulter Inc., USA). The pellets were combined and dialyzed, with stirring, using a dialysis tubing cellulose membrane (16 mm diameter, 14,000 molecular weight cut off) for 24 hr. Following dialysis, the samples were homogenized using Cole Palmer® ultrasonic cleaner Model 08895-20 at 50-60 Hz for 30 min. The yield of the resulting products (for both the alkali-insoluble cellulose and ground Kraft pulp) was determined and a portion of the product was freeze-dried for various characterization tests.

Characterization Tests

1. Focused Ion Beam - Field Emission Scanning Electron Microscopy (FIB-FESEM)

A drop of suspension containing the bamboo nanocellulose product was casted and spread on the surface of aluminum foil. The casted samples were dried in a covered glass petri dish with desiccant under dark condition before analysis using a Dual Beam Helios Nanolab 600i (Field Electron and Ion Company, OR, USA). The analysis was performed using an accelerated voltage of 2.0 kV (SE/TLD) and a beam current of 86 pA (SE/TLD). Data processing of the images was done through an image processing software using the Dual Beam Helios Nanolab 660i xT Microscope Server. The measurement of the widths of the nanocellulose was done using ImageJ software.

2. Energy Dispersive Spectroscopy (EDS)

This analysis was performed alongside FIB-FESEM imaging to determine the elemental composition of the sample. Both methods used the same equipment, with the EDS analysis being conducted using an accelerated voltage of 15.0 kV and a beam current of 0.69 nA. Data processing and elemental composition determination were done using the imaging software Oxford EDS AZtecEnergy.

3. Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectra of freeze-dried samples of kawayan kiling nanocellulose products Pin KBr pellets (1:20 w/w) were obtained using a Nicolet 6700 FT-IR spectrometer (Thermo Scientific, Waltham, MA, USA). The spectra were collected at ambient conditions in transmittance mode, from an accumulation of 16 scans at a 4 cm^{-1} resolution over the region of 4000-400 cm^{-1}.

4. Dynamic Light Scattering (DLS)

The Z-average (d.nm) and polydispersity indices (PdI) of the samples in pure water

were measured with Malvern® ZS90 Zetasizer (Malvern Panalytical Ltd, Worcestershire, UK). Suspensions of 0.01 wt. % concentration were previously homogenized using CP EW-08895-20 ultrasonicator (Cole-Parmer, Ltd. USA) for 5 min before analysis.

5. X-ray Diffraction (XRD) Analysis

The X-ray diffraction spectra of the Kawayan kiling nanocellulose products were measured in a Shimadzu Lab-X XRD-6000 diffractometer (Shimadzu, Columbia, MD, US) using a Ni-filtered Cu Kα radiation (λ = 1.54050 A˚) at an anode voltage of 40 kV and a current of 30 mA. Freeze-dried samples were compressed in a sample holder. Diffractograms were recorded from 10° to 80° at a scan rate of 2.00°/min. Crystallinity index (CrI) was calculated using Equation 3 (Segal et al., 1959).

$$CrI = \frac{I_{002} - I_{am}}{I_{002}} x100 \quad \text{Equation 3}$$

where I_{am} is the intensity of diffraction of the amorphous region at 2θ angle and I_{002} is the maximum intensity of the 002-lattice diffraction peak at 2θ.

Results and Discussion

Yield determination

The overall yield obtained from the chemically treated cellulose (i.e., bleached followed by alkali treatment) was 26.94% while for the pulp that was submitted to grinding in a refiner, the yield was only 1.20%. The low yield from the latter was largely due to the removal of unground material for every pass during the mechanical refining (16.1% yield). This was designed to ensure that only pulp that was uniformly ground was collected for further treatment and analysis. This ensured homogeneity of the ground material subjected to the property tests and this need not be the procedure in actual production. In comparison, Jara et al. (2020) found the acid hydrolyzed yield of cellulose nanocrystals from *kawayan kiling* shoots to be 9.16%; other studies reported yields from the hydrolysis or oxidation reactions to produce the nanocellulose. In our study, the yields of nanocellulose from acid hydrolysis were 61.76% and 11.24% from the solely chemically treated material and the mechanically treated pulp preparation, respectively.

The lower yield from the mechanically treated pulp can be attributed to the non-accessibility of the ground cellulose to the acid. Even after 15 passes through the colloider, the cellulose may not have been fully liberated from the hemicelluloses and lignin to allow the acid to penetrate the non-cellulosic matrices to reach the cellulose and break it down. This is evident in the observed color of the ground cellulose and the peaks obtained in the FTIR spectra corresponding to functional groups found in lignin, which suggests the presence of residual lignin in the preparation.

Nanocellulose particle size

Using dynamic light scattering, the Z-averages and polydispersity indices (PdI) of the nanocellulose products were determined to estimate the size of the particles. It can be seen in Table 1 that on average, the dimensions of the samples appear to exceed 100 nm. Most of the mechanically prepared nanocellulose appeared to be fibrillar which could be explained by the incomplete acid hydrolysis reaction. Refining could not fully release the cellulose from the associated lignin and hemicelluloses. Based on the PdI values, the samples were broadly polydisperse.

Table 1. Z-average and polydispersity index of acid-hydrolyzed nanocellulose products from the chemically and mechanically pretreated bamboo acid hydrolysis feedstock.

Pretreatment of the Bamboo Acid Hydrolysis Feedstock	Z-average (d.nm)*	Polydispersity Index*
Combined bleaching and alkali digestion	176.8 ± 7.5	0.501 ± 0.067
Refining with friction grinder supermass colloider	389.5 ± 74.7	0.561 ± 0.169

* Values are mean ± s.d. of 5 measurements.

Nanocellulose morphologies and elemental composition

Figure 1 shows the images of the nanocellulose products from the acid-hydrolysis of differently treated Kawayan kiling pulp. It was observed that the products from the combined bleaching and alkali digestion gave crystal-like structures which were aggregated, while those that underwent grinding were fibrillar in nature. The aggregation could be due to hydrogen bonding of the hydroxyl groups of cellulose as water was removed during drying prior to microscopy. This partially accounts for the broader widths (134.20 ± 34.33 nm) of the nanocrystals from the cellulose that was subjected to combined bleaching and alkali digestion as compared with that of the mechanically refined (39.13 ± 34.11 nm) product. In the latter, mechanical fibrillation greatly contributed to shearing the microfibrils but did not remove the lignin.

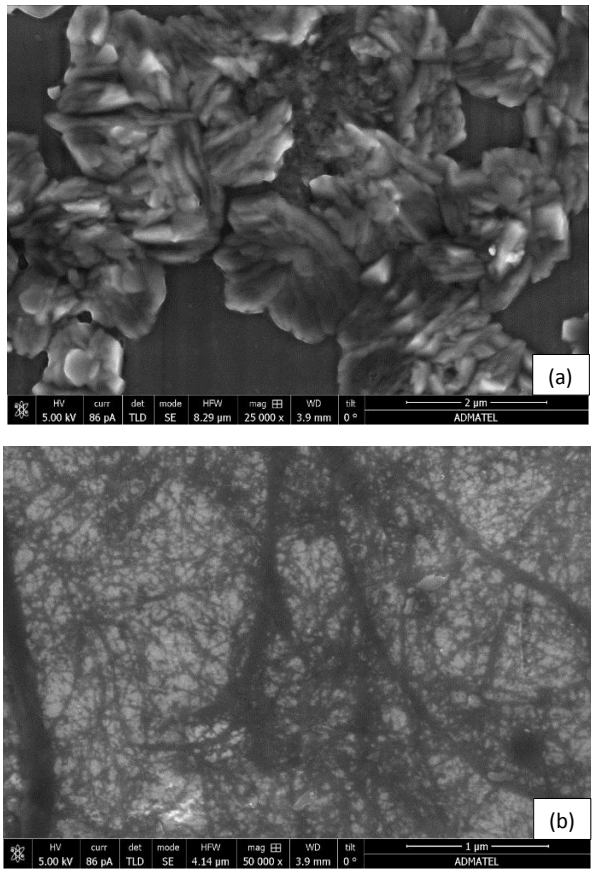

Figure 1. FESEM images showing the crystal-like morphology of *Bambusa vulgaris* nanocellulose products from (a) pulp subjected to bleaching and alkali digestion and (b) pulp refined in the friction grinder supermass colloider.

Table 2. Widths (nm) obtained by FESEM imaging of acid hydrolysis products from variously pretreated *kawayan kiling* (*Bambusa vulgaris* Schrad.) pulp.

Pretreatment of the bamboo acid hydrolysis feedstock	Mean ± SD	Min	Max
Combined bleaching and alkali digestion	134.2 ± 34.33	84.00	214.0
Refining with friction grinder supermass colloider	39.13 ± 34.11	12.00	171.0

Table 3. Elemental analysis by EDS analysis of acid hydrolysis products from variously pretreated *kawayan kiling* (*Bambusa vulgaris* Schrad.) pulp.

Pretreatment of the bamboo acid hydrolysis feedstock	% C	% O	% Si
Combined bleaching and alkali digestion	23.93	77.07	-
Refining with friction grinder supermass colloider	31.73	62.87	5.40

Elemental analysis (Table 3) showed that both nanocellulose products contained carbon and oxygen; however, the nanofibrils contained a significant amount of silicon. This could be attributed to the mechanical grinding process. It can be inferred that in the refining process, small fragments of the grinder stone, which is mainly composed of silicon carbide (SiC), could have been chipped away and mixed with the sample.

Chemical and crystalline structures of nanocellulose

Freeze-dried nanocellulose suspensions from the chemically treated *kawayan kiling* pulp produced white solids while the mechanically treated *kawayan kiling* pulp gave brownish flaky solids. As seen in Figure 2, both samples showed the same characteristic peaks for cellulose, such as the O-H vibration at 3413.29 cm^{-1} and 3439.93 cm^{-1} (Costa et al., 2015; Liu et al., 2016), C-O stretching vibration at 1063.67 cm^{-1} and 1064.92 cm^{-1}, aliphatic C-H bonds at around 2920.12 cm^{-1} and 2898.87 cm^{-1}, C-O-C stretching vibration of the pyranose ring at around 1064.36 cm^{-1} and 1063.67 cm^{-1} (Liu et al., 2016). Furthermore, the presence of the cellulose backbone was also confirmed by the peaks at 1645.54 cm^{-1} and 1633.55 cm^{-1} due to the O-H bending of the absorbed water and the peaks at 898.86 cm^{-1} and 896.92 cm^{-1} in the anomeric region which are attributed to the linkages between sugar units in cellulose (Jiang & Hsieh, 2013; Liu et al., 2016)

One notable FTIR peak for the hydrolysis product from the refined pulp is at 1727.47 cm^{-1} which is associated with acetyl ester groups and uronic groups of hemicelluloses or ester bond of

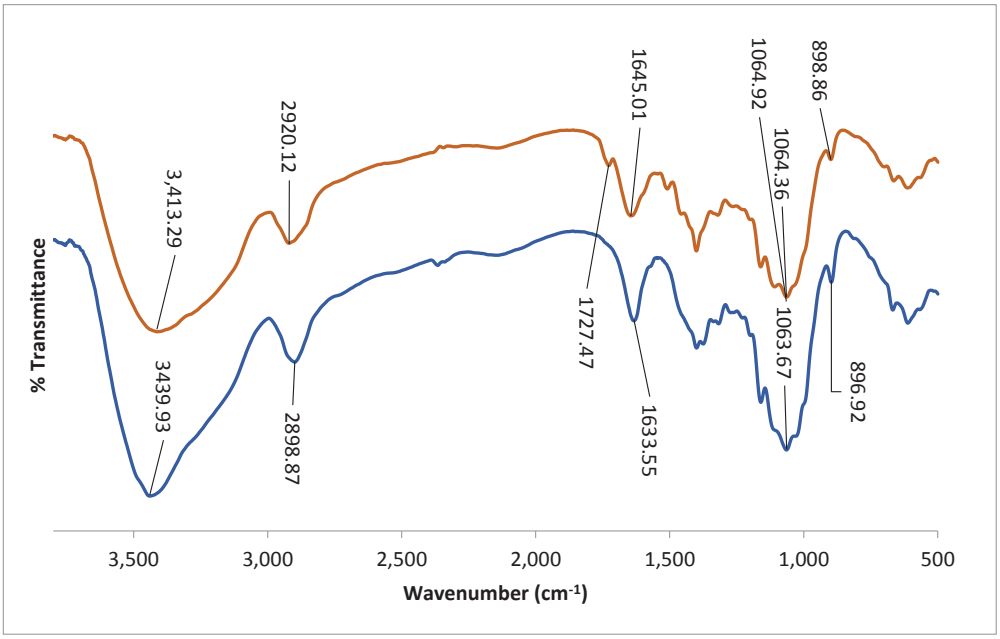

Figure 2. FTIR spectra of acid hydrolysis products from *Bambusa vulgaris* Schrad. pulp subjected to bleaching and alkali digestion (**in red**) and of pulp refined with the friction grinder supermass colloider (**in blue**).

carboxyl group of ferulic and *p*-coumaric acid of lignin and/or hemicellulose. This was not seen in the hydrolysis products of the bamboo pulp that was bleached and alkali digested. This indicates that residual hemicelluloses and/or lignin remained in the hydrolysis products from the friction grinder supermass colloider treated pulp; the absence of peaks associated with lignin in the chemically pretreated pulp suggests that the binding substance lignin, was completely removed from the chemically pretreated bamboo pulp.

The XRD spectra are shown in Figure 3. Based on the peak height calculation using Segal's (1959) method, the crystallinity indices (CrI) of the chemically and mechanically treated *kawayan kiling* pulps are 56.92% and 49.47%, respectively. The lower crystallinity of the nanocellulose from the mechanically treated pulp could be due to the presence of amorphous

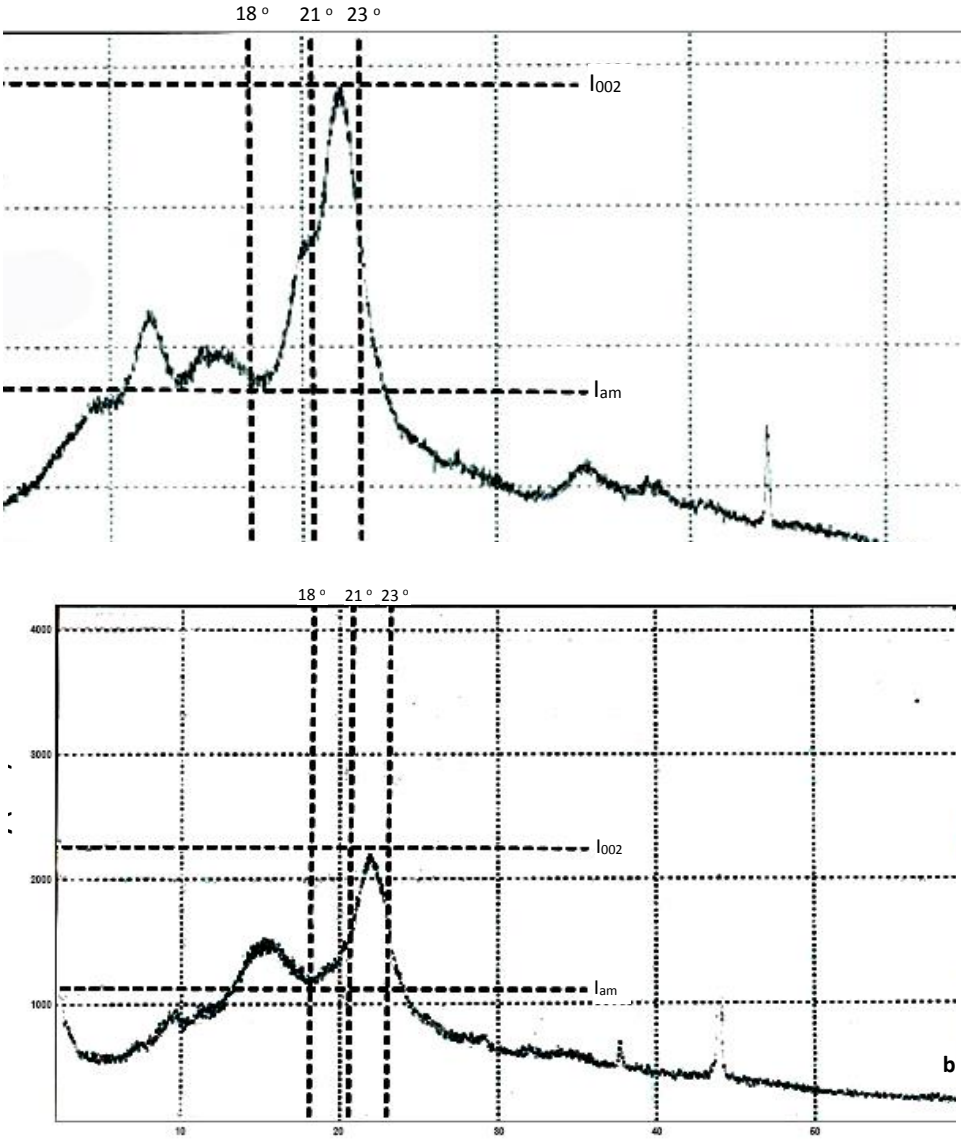

Figure 3. X-ray diffraction spectra of *Bambusa vulgaris* nanocellulose products from (a) combined bleaching and alkali digestion and (b) pulp subjected to grinding in a friction grinder supermass colloider.

residual lignin. In comparison, cellulose nanocrystals prepared by sulfuric acid hydrolysis of commercial bamboo pulp had a 71.98% crystallinity (Yu et al. 2012) while a recent study reported a 68.53% crystallinity for nanocellulose extracted from isolated bamboo parenchyma using deep eutectic solvent (Gu et al. 2021).

Conclusion

The study compared the nanocellulose from the kraft pulp derived from *kawayan kiling* culms subjected to two different pretreatment methods. FIB-FESEM analysis showed crystal-like structures of chemically treated acid-hydrolyzed nanocellulose while for the mechanically treated pulp, a fibrillar morphology of the acid-hydrolyzed nanocellulose product was obtained. Width and particle size measurements of the purported bamboo nanocellulose confirmed that the products were in the nanometer range. DLS analysis revealed that the acid-hydrolyzed chemically treated *kawayan kiling* pulp were smaller in size but had longer average widths as shown by FIB-SEM.

The presence of Si impurities was noted in the mechanically-ground nanocellulose. The nanocellulose from both treatments possess the cellulose backbone, although lignin was retained in mechanically-treated pulp even after several colloider passes. The acid hydrolyzed nanocellulose from chemically-treated pulp had a higher degree of crystallinity than the nanocellulose from the pulp subjected to mechanical treatment. In future works, *B. vulgaris* pulp that will be mechanically treated may require bleaching to further remove residual lignin and thus improve acid hydrolysis.

Potential applications of *B. vulgaris* nanocellulose fibrils would be as reinforcements for composites while the cellulose nanocrystals may find application as nanosensors for biomedical purposes or additives for food-grade packaging.

Acknowledgments

The authors are grateful to the staff of the Wood Chemistry Laboratory of the Dept. of Forest Products and Paper Science, University of the Philippines Los Baños College of Forestry and Natural Resources, College, Laguna for the assistance in sample preparation. Much appreciation for DOST-PCIEERD for funding the testing of FIB-FESEM and XRD analysis through the ADMATEL-EPDC Grant, and the staff of ADMATEL and MSD-ITDI for assistance in the characterization tests. The authors also acknowledge the UPLB Nanotechnology Laboratory for the use of the particle size analyzer for the DLS analysis and the De La Salle University Chemistry Department, Taft Ave., Manila for the FTIR analysis.

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