e-ISSN 2320 –7876 www.ijfans.org
Vol.11, Iss 7, 2022

Research paper UGC CARE Listed (Group -I) Journal

Effect Of Combined Enzymatic And Chemical Treatment On Production Of Nanocellulose From The Banana Pseudostems

[1]Parvinder Kaur, [1]A A Broadway, [2]Tanweer Alam, [3]Harinder Singh, [4]Jyoti Jain

[1]Warner College of Dairy Technology, SHUATS, Allahabad, Uttar Pradesh, India

[2]Director, Government of India, Indian Institute of Packaging, 110 092, Delhi, India

[3]Department of Chemical Engineering, MNNIT, Allahabad, India

[4]Department of Chemical Engineering, Indian Institute of Technology, Roorkee, India

Abstract

Banana plant waste pseudostems were exploited as a favorable cellulose source for the production of nanocellulose via combined enzymatic and chemical treatments. Four different processing strategies to develop banana nanocellulose were utilized. The developed nanocellulose samples has been experimentally assessed by determining their functional characteristics such as its functional groups, morphology, crystallinity index, and thermal stability. The FTIR results displayed that the cellulose and nanocellulose spectra had no substantial differences. The XRD analysis has shown a high degree of crystallinity (60.13%) with enzymatic hydrolysis (70 units/gram of fiber) followed by mild acid treatment (5% oxalic acid). After comparing different methods for producing nanocellulose, it can be concluded that alkali pretreatment at room temperature for a longer duration (25 °C for 14 h) leads to high crystallinity with a more symmetric crystal structure as compared to alkali treatment at a higher temperature for a short duration under steam (121 °C for 1 hr in an autoclave). Typically, higher temperatures and long reaction times lead to decreased crystallinity. The produced nanocellulose from banana-derived cellulose shows great potential for using banana pseudostems as a raw material for nanocellulose production.

Keywords: Banana pseudostems; nanocellulose; crystallinity; enzymatic hydrolysis.

Introduction

Cellulose is one of the most abundant, useful and biodegradable polymer having multiple applications. There is active research going into exploration of new sources for cellulose extraction. Plant fiber cell walls are made of this compound, which looks like a synthetic polymer. It's an excellent source for the production of nanocellulose(*Kaushik, Singh, and Verma, 2010; Wu et al., 2019*).Nanocellulose can be utilized for food packaging or as a reinforcement phase in composites due to its good mechanical properties, interesting thermal properties, sustainability, low environmental impact, and a high level of crystallinity (*Radakisnin et al., 2020).*

Banana pseudostems fibers are broadly exist as agro waste in the tropical countries of the world. Around 12% of the banana plant (fruit) is edible, while the rest ends up as agricultural waste which create a huge disposal problem(*Priyadarshana* et al., 2020). Banana plant waste includes both fibrous and non-fibrous parts. The pseudostems, rachis, and leaf sheath are all part of the fibrous component. The pseudostems is the fruit-bearing stem of a banana plant. As pseudostems never bears banana fruit again, it is cut down from the base and becomes waste biomass after harvesting banana fruit. In the next cycle, a new

pseudostems grows from the true stem and bears fruit. In terms of cellulose content, it is known to be one of the most incredible natural fibers(Paramasivam et al., 2020). The profitable utilization of banana nanocellulose derived from the pseudostems of the banana plant has the potential to benefit both the local and national economies*(Krishnan and Ramesh, 2017; Komal, Lila, and Singh, 2020)*which will also solve environmental issues. Some researchers *(Deepa et al., 2011; Meng et al., 2018; Pelissari et al., 2014; Tibolla et al., 2016)* have already investigated the nanocellulose isolation from banana fibers such as banana rachis and peels. Due to their high tensile strength, low density, and modulus, banana fiber nanocellulose can be used as a filler in a variety of polymer composites*(Erdogan et al., 2017; Nandi & Guha, 2018; Zuluaga et al., 2009)*.

Higher plants cellulose has a complex, varied morphological and physical structure. *(Leite, Zanon, and Menegalli, 2017).* The isolation procedure involves structural breakdown through pretreatment methods usually alkaline hydrolysis followed by enzymatic or acid hydrolysis *(Chen et al., 2011; Krishnadev et al., 2020; Syafri et al., 2018*). These processes produce nanocellulose with high crystallinity while reducing amorphous material. It is possible to use mechanical disintegration, high-pressure processing, or ultrasonic processing for its isolation. However, since the cellulose in plant cell walls are closely bounded by hydrogen bonds, preparing nanocellulose purely through mechanical treatments is challenging. Thus isolation of nanocellulose from banana pseudostems involves many processes depending on the level of separation and the intended function. Lignin and hemicellulose (non-cellulosic components) are removed from the fiber by combination of physical, chemical, thermal, or biological techniques. Recently, researchers have successfully used both chemical and enzymatic methods to synthesize and isolate nanocellulose.(*de Amorim et al., 2020; Radakisnin et al., 2020; Shreedhana & Ilavarasi, 2020; Wang et al., 2015*). Enzymatic treatment is a less harmful alternative to chemical treatment and can be used to produce nano cellulose with comparatively less energy. Researchers used ligninases, xylanases, and other enzymes to degrade non cellulosic components (*Pelissari et al., 2014; Tibolla et al., 2017).*While enzymatic hydrolysis utilizing cellulolytic enzymes (cellulases) helps in hydrolyzing cellulosic fibers to produce nanocellulose. The amorphous areas of the cellulose substrate are hydrolyzed by enzymatic treatments in the same manner as acid hydrolysis while maintaining the crystalline regions alone(*Kumari et al., 2019; Santos et al., 2016).*

The exploitation of agricultural waste based bio-fibers sources and improving the efficiency of its extraction process will play an important role in the evolving green economy. Although banana pseudostems fibers are exist in abundance still its extraction processes have not been studied fully yet. It can be utilized in a varied kind of applications by converting to nanocellulose form. Thus the current study focuses on utilization of four different processing strategies to develop banana pseudostems nano cellulose and its characterization for various industrialized utilization. Experimental analysis has been done to determine how these methods affect the developed nanocellulose.

Materials and methods

Materials and chemicals:

Banana pseudostems was obtained from Prayagraj, U.P India. All of the chemicals used in this research work were of the reagent grade. Cellulase enzyme from *Aspergillus Niger (C 1184-5KU)* was procured from Sigma–Aldrich Chemical Co., USA having a specific activity (3.57G, 1.4 units/mg solid) and used for hydrolysis of banana fiber.

Preparation of nanocellulose

Nanocellulose was prepared from banana pseudostems, adapted from the methodology described by*(Pelissari et al., 2014)* with some modifications. The banana pseudostems fibers were washed, cut into uniform size and dried in a hot air oven at 70 °C. Dried banana fibers were ground and sieved using a willy mill, with a 500 µm sieve (trapezium-shaped stainless steel) followed by ethanol washing. After washing banana fiber was dried which was followed by sieving to get banana fiber bran. A combination of alkaline treatment, enzyme hydrolysis, and acid hydrolysis was used to prepare nanocellulose from banana psudostems bran. *(Beltramino et al., 2018; Deepa et al., 2011; Tibolla et al., 2017*), with some modifications. Banana pseudostems cellulose bran (yield = 64.15%) was subjected to pretreatment (alkali treatment) under steam with 2% NaOH (1:10 w/v) using an autoclave at 121 °C for 1 h and at 25 °C for 14h followed by successive washings with distilled water and centrifugation (5 °C,15 min at 10,000 rpm). The main objective of alkali treatments was to hydrolyze non-cellulosic components of banana cellulose i.e. hemicellulose, lignin, and pectin. Before enzymatic treatment, banana cellulose bran was maintained at 55 °C in 0.1 M sodium acetate buffer (pH 5.0) for 10 minutes in a thermostatic shaking incubator (*1HC-2542, Remi, Mumbai-India*). Cellulase enzyme at 70 and 20 units/gram of bran was added slowly to the bran suspension and incubated for 24 hours at 150 rpm. After enzymatic hydrolysis, it was denaturated for 30 minutes in an 80 °C water bath. The resulting suspension was washed and centrifuged (5 °C, 15 min at 10,000 rpm) to remove any traces of enzymes. After enzymatic treatment, the resulting suspension was subjected to controlled acid hydrolysis under steam with 5 % oxalic acid solution, 1:15 w/v for 3 h at121 °C in an autoclave and 25 °C for 14h. Washing and centrifugation were repeated for the complete removal of acid. After all these treatments, obtained suspension was subjected to mechanical stirring for 6 h. After these steps, a colloidal nanocellulose suspension was formed and kept at 4 °C in a sealed container for further analysis.

Table 1 Treatment combination for synthesis of Nanocellulose

Treatments	Alkali treatment	Enzymatic treatment	Acid hydrolysis
CEUT	-	-	-
CE01	2%NaOH(1:10w/v),25 °C for 14h	20unit/g of fiber, 55 °C for 24h,150 rpm	5% oxalic acid (1:15w/v), 25 °C for 14h
CE02	2%NaOH(1:10w/v),25 °C for 14h	70unit/g of fiber, 55 °C for 24h,150 rpm	5% oxalic acid (1:15w/v), 25 °C for 14h
CE03	2%NaOH(1:10w/v), 121 °C for 1hr in an autoclave	20unit/g of fiber, 55 °C for 24h,150 rpm	5% oxalic acid (1:15w/v), 121 °C for 3h
CE04	2% NaOH(1:10w/v), 121°C for 1hr in an autoclave	Without enzymatic hydrolysis	5% oxalic acid (1:15w/v), 121 °C for 3h

Characterization of banana nanocellulose

Field Emission Scanning electron microscopy (FESEM)

FESEM was used to investigate the surface morphology and microstructure of nanocrystalline cellulose. The sample was double-sided taped to an aluminium stub and then coated with a gold layer. A FESEM (*JSM 7610FPlus*) with an acceleration voltage of 5 kV was used to view the coated sample.

Attenuated total reflection Fourier transform infrared spectroscopy

FTIR was conducted using an FTIR spectrophotometer *(Bruker)* equipped with an ATR device. Analysis was performed with 16 scans with a spectral resolution of 4 cm^{-1} in wavenumbers ranging from 4000 to 500 cm^{-1}.

X-ray diffraction

An X-ray diffractometer *(Bruker, D8Advance)* operating at a voltage of 40 kV with an input current of 30 mA and a Cu target with a scanning speed of 1.2°/min was used to conduct the X-ray diffraction investigation. In a range of 5 to 40°, the diffractograms were examined at an angle of 2θ and crystallinity index was determined using the method of (Lopez-Rubio et al., 2008).

Thermal properties:

The thermal profile of the samples (TGA and DTGA curves) was obtained in an *EXSTAR TG/DTA*, model 6300. Samples weighing between 5 and 10 mg were heated from 25 °C to 600 °C at a rate of 10 °C min^{-1}. The measurements were performed under nitrogen with a flow rate of 100 $ml.min^{-1}$.

Results and Discussions

Field Emission Scanning electron microscopy (FESEM)

The comparison of the FESEM images of untreated banana pseudostems fiber bran and nanocellulose after enzymatic hydrolysis was shown in Fig.1. The presence of an external non-cellulosic surface, which consists of cementing materials such as lignin and hemicelluloses, offers a smooth appearance to the raw cellulose surface. FESEM images of treated nano cellulose showed the removal of hemicelluloses and lignin with some other extractives during the chemical and enzymatic treatments as apparent from the FTIR spectra. This fact was also confirmed by XRD analysis due to an increase in crystallinity.

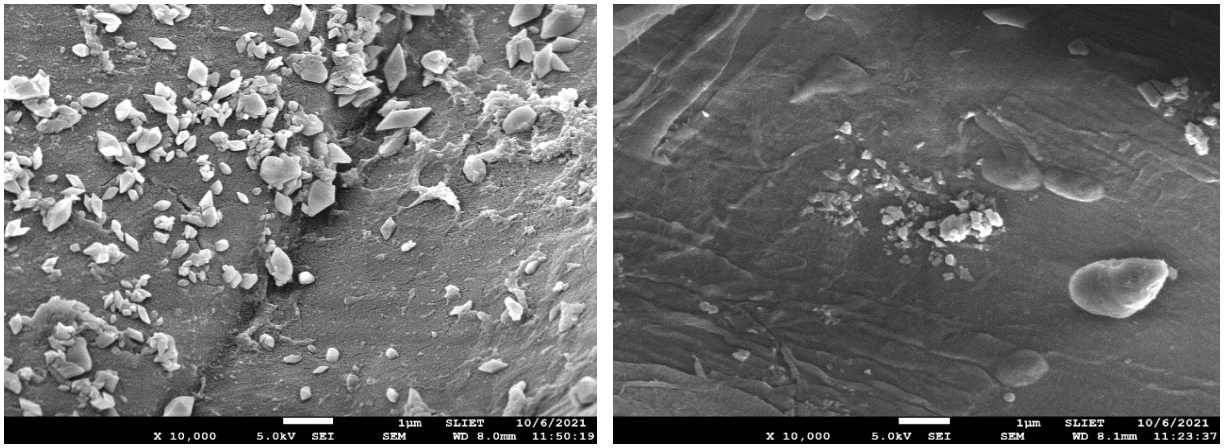


Fig. 1 Field Emission Scanning electron micrographs of treated and untreated samples: 10,000 × magnification.

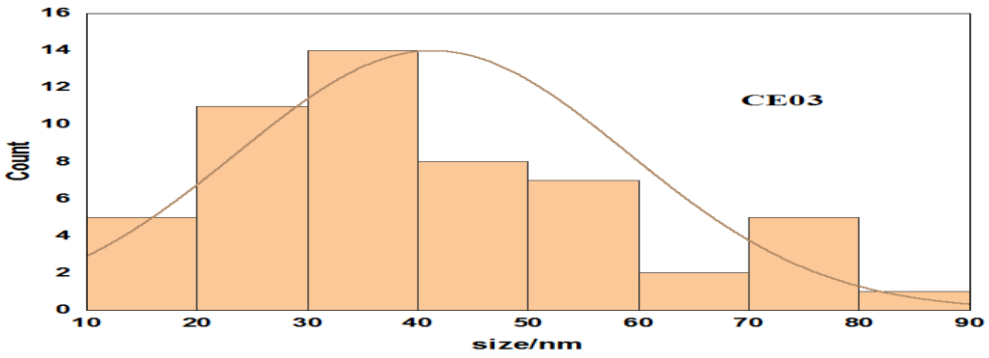


Fig. 2 Size ratio in CE03

The size of particles CEUT and CE03 was determined using Image J and Origin Pro 8.5 software. As depicted in the histogram the size of nano cellulose was in nanometeric range. These micrographs evidenced the size reduction that takes place. Thus chemical and enzymatic treatment lead to the purification of nano cellulose along with a reduction in size as depicted in the above images.

Attenuated total reflection Fourier transform infrared spectroscopy

The diffuse reflections of FTIR spectra and results for untreated banana pseudostems fiber and nanocellulose over 4000 to 500 cm^{-1} wavenumbers are represented as CEUT and CE03 respectively.

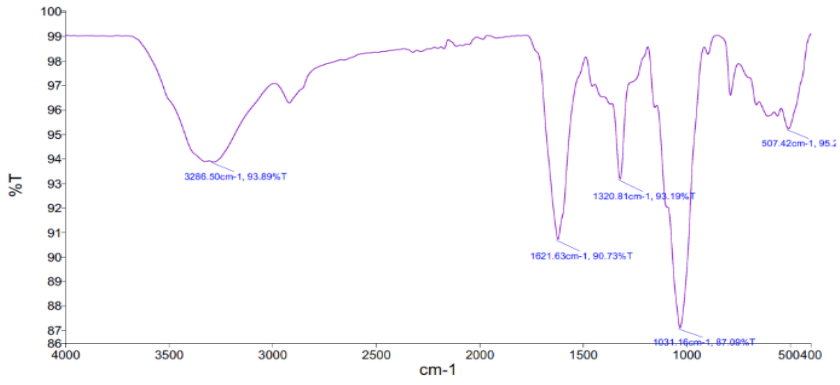


Fig. 3a: FTIR Spectra of CEUT

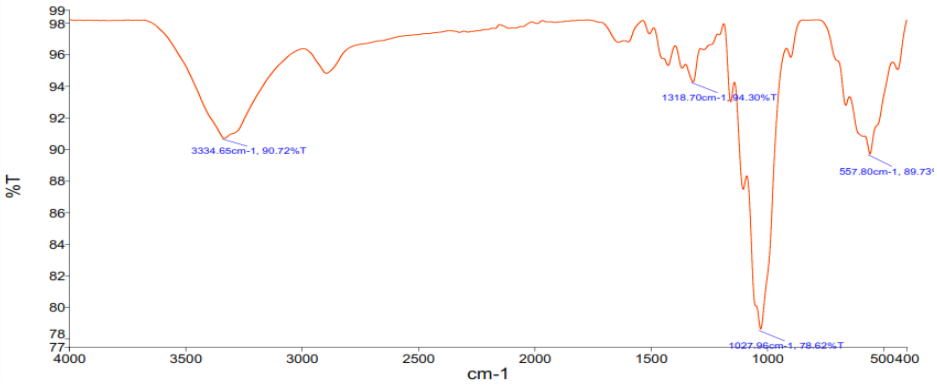


Fig. 3b: FTIR Spectra of CE03

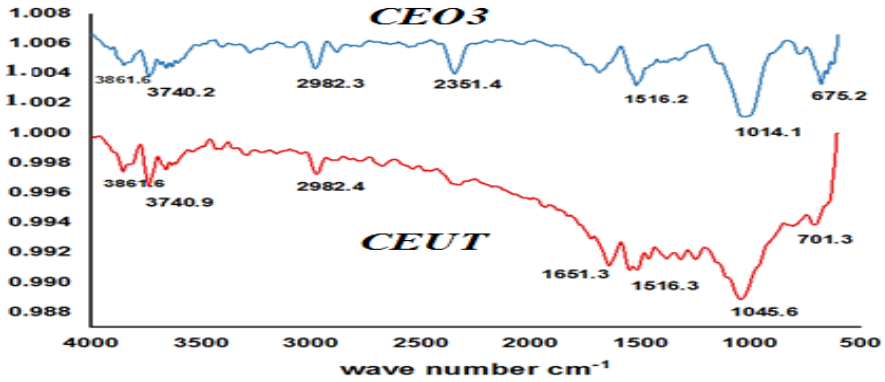


Fig. 3c: FTIR graph of CEUT and CE03

The absorbance of the hydrogen-bonded OH group in the CEUT and CE03 spectra was 3740 cm^{-1} and 3861 cm^{-1}. This band is specific for cellulose and reflects the hydrophilic tendency of cellulose. The CEUT spectrum showed a tiny band around 2982 cm^{-1}, which strengthened in the CE03 spectrum. The aliphatic saturated C-H stretching vibration of methylene and methyl groups in hemicellulose and cellulose created this band (Wu et al., 2019). In the CEUT spectrum, there was a band at 1651 cm^{-1}, associated with aromatic rings and conjugated carbonyl groups found in polyphenols of the lignin structure along with some contribution from the bending mode of absorbed water. This same band disappeared in the spectrum of CE03, demonstrating the delignification. The absorptions at 1516 cm^{-1} were associated with the stretching vibration of aromatic C=C in lignin. The band in the region of 1014-1045 cm^{-1}, referred to the residues of xyloglucans associated with hemicellulose and lignin. The overall decline in peak strengths in the region 1014 to 1516 cm^{-1} was observed in banana nanocrystalline cellulose indicating removal of hemicellulose during the chemical and enzymatic treatments. Due to a combination of enzymatic treatment, changes in the morphology of the nanofibers were observed. In addition, pyranose rings vibrations (1014-1045 cm^{-1}), and rocking vibrations of C-H (701 cm^{-1}) linked to the characteristic values of cellulose(Meng et al., 2018; Pelissari et al., 2014).

X-ray diffraction Analysis

XRD pattern of untreated banana fiber (CEUT) and different treatments named CE01, CE02, CE03, and CE04 respectively was shown in (Fig. 5 and Table 2). CEUT graph (banana fiber untreated) showed a straight line which indicates the amorphous nature of the compound. CE01 graph showed two main peaks 15.23° and 22.23° at the angle of 2θ. The same peaks were observed in the remaining samples of CE02, CE03, and CE04. Different treatments resulted in an increase in crystallinity due to the delignification and removal of hemicellulose. The diffractograms for the CE02 present strong peaks at around 2θ =15.57°, 22.69°, and 34.59°, which revealed on the crystallographic planes of (021), (112), and (211), respectively. It confirm that the nanocellulose samples are in the cellulose Iβ crystal structure, as mentioned in literature(Radakisnin et al., 2020; Tibolla et al., 2017). These findings also show that the nanocellulose crystal integrity was preserved during the enzymatic and chemical treatment processes, as well as asymmetric crystalline structure. The percentage crystallinity in the original untreated banana fibers was 43.67. It was noticeably raised in the CE02 due to enzymatic pretreatment. While a slight increase in crystallinity (53.09) was found in the acid-treated samples without enzymatic treatment (CE04). During the chemical treatments, little amorphous material was removed. While enzymatic treatment followed by acid treatment leads to an increase in crystallinity in CE01, CE02, and CE03 samples. The

Research paper UGC CARE Listed (Group -I) Journal

best results were obtained in the CE02 sample, in which 70 units of cellulase enzyme were used per gram of fiber treatment was given. Thus above results depict that the synthesis of nanocellulose was influenced by temperature as well as enzyme concentration. Alkali and acid treatment at room temperature improved the crystallinity while treatment at higher temperature(121 °C in autoclave) lead to cellulose degradation which reduce the crystallinity, clearly depicted by hkl values of CE03 samples at 2θ =15.77 corresponding to the crystalline plane of (131) as compared to CE02 having a crystalline plane of (021).

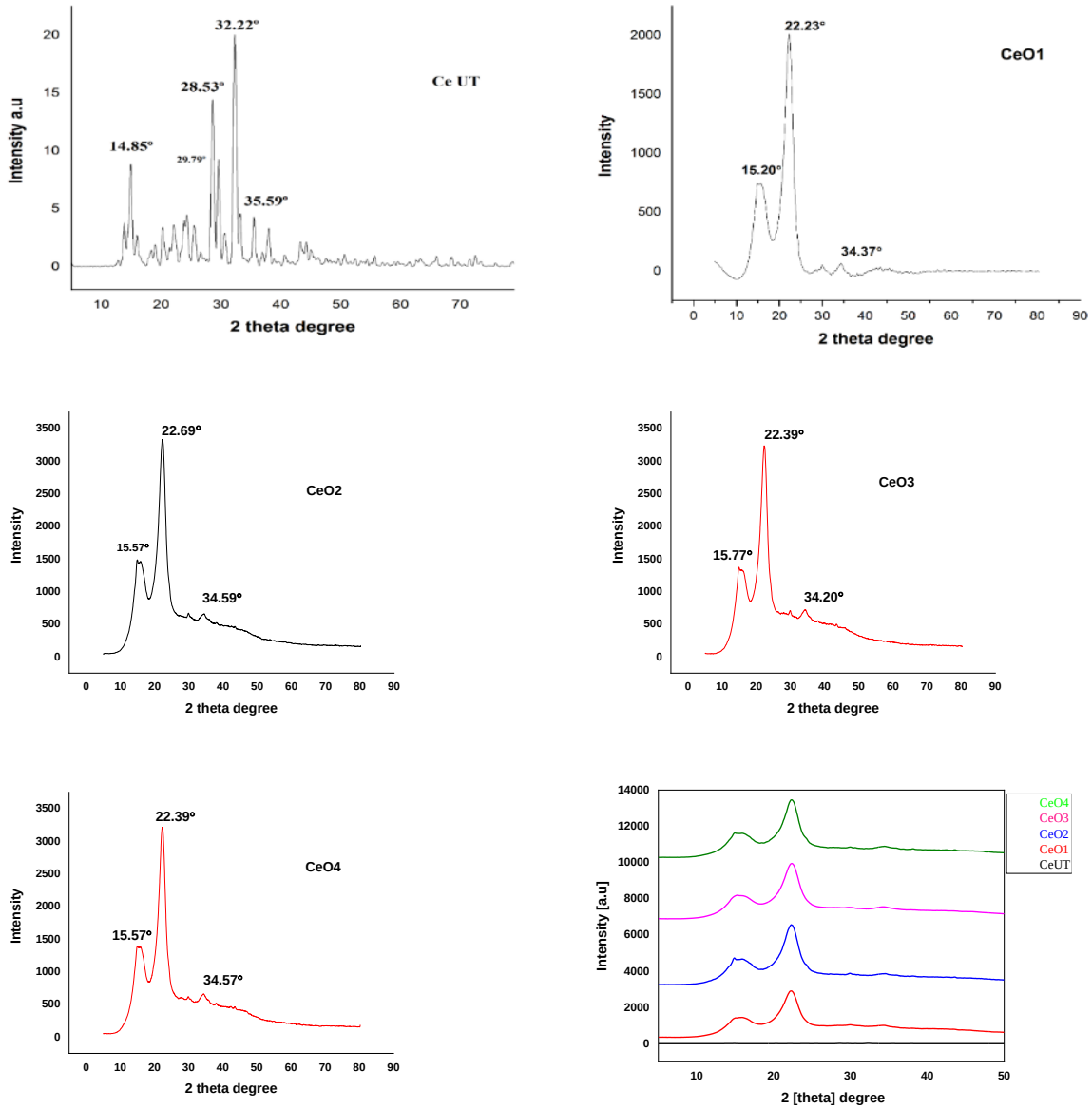


Fig. 4. XRD pattern for CEUT, CE01, CE02, CE03, and CE04

Table 2 XRD Profile of CEUT, CE01, CE02, CE03, CE04 (Peak indexing from d-spacing)

Samples	Peak Position 2θ	FWHM of peak β (average cross-sectional dimension) radians	Crystallite size (D)	hkl	Crystallinity index
	Θ	Θ	Nm		
CEUT	14.85	0.615	13.60090	211	43.67
	28.53	0.599	14.31037	321	
	29.79	0.493	17.41370	102	
	32.22	0.633	13.66309	220	
	35.59	0.058	150.75141	402	
CE01	15.2	2.301	12.13576	120	56.04
	22.22	3.321	8.493742	110	
	34.37	2.909	9.958936	021	
CE02	15.57	3.307	8.44771	021	60.13
	22.69	1.957	14.4255	112	
	34.59	1.516	19.12004	211	
CE03	15.77	3.593	7.77714	131	55.54
	22.39	1.868	15.10386	111	
	34.2	1.632	17.74466	211	
CE04	15.57	4.551	6.13855	112	53.09
	22.39	1.6579	17.0191	231	
	34.59	1.345	21.55376	103	

Table 2 shows the crystallinity index values of CEUT, CE01, CE02, CE03, and CE04 which varied depending on the conditions applied. An increase in the peak intensity was observed with different treatments. This also depicts that the combined enzymatic and chemical treatment increased the crystallinity of the nanocrystalline cellulose. The maximum crystallinity obtained for the 70 unit/g of the enzyme-treated sample (CE02) was 60.13%.

Thermal Properties:

The degradation parameters of chemically and enzymatically treated and untreated banana pseudostems fiber specimens were compared using thermogravimetric analysis. It have three distinct weight-loss zones. Evaporation of adsorbed water caused the initial weight

loss in the 50–100 °C range. Thermal depolymerization and breakage of glycosidic links of cellulose are primarily responsible for the weight loss in the temperature range of 220–300 °C. At a temperature of around 263 °C, a dramatic drop in weight loss occurs, due to degradation of hemicellulose. The presence of acetyl groups in hemicellulose causes its low heat stability. The thermal breakdown of untreated cellulose causes a peak at 303 °C on the DTG curve while treated nanocellulose sample showed peak at 365 °C due to removal of non-cellulosic constituents such as lignin and hemicellulose. There's also a difference between the amounts of residues left after heating at 700 °C in both untreated and treated cellulose. The removal of hemicelluloses and lignin, as well as increased accessibility of the cellulose in the treated fiber, may be responsible for low amount of carbon residue in the treated nanocellulose sample. The thermal stability of the treated cellulose samples were higher than untreated sample, with a greater stability was obtained for acid-treated fibers.

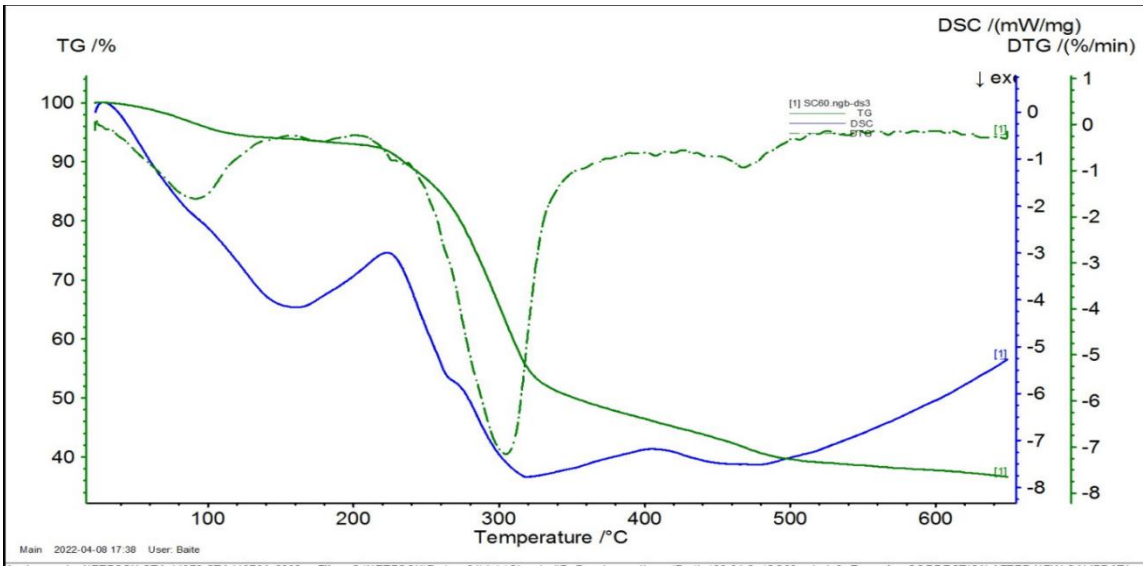


Fig. 5 a: Thermo gravimetric curves for the raw banana pseudo stem fiber

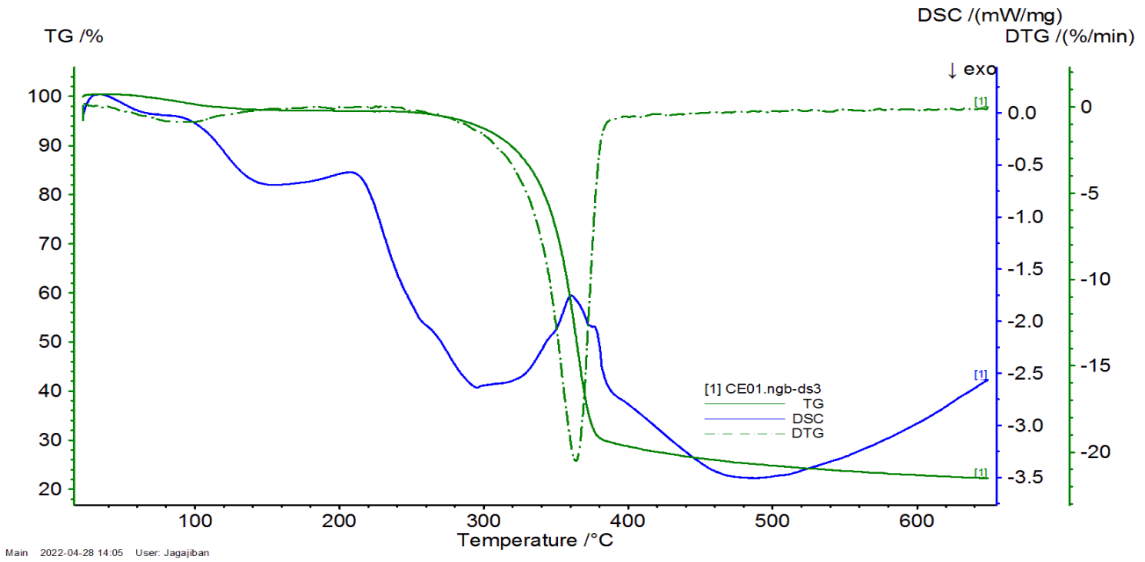


Fig. 5 b: Thermogravimetric curves for the CE01 (treated banana fiber at room temp.)

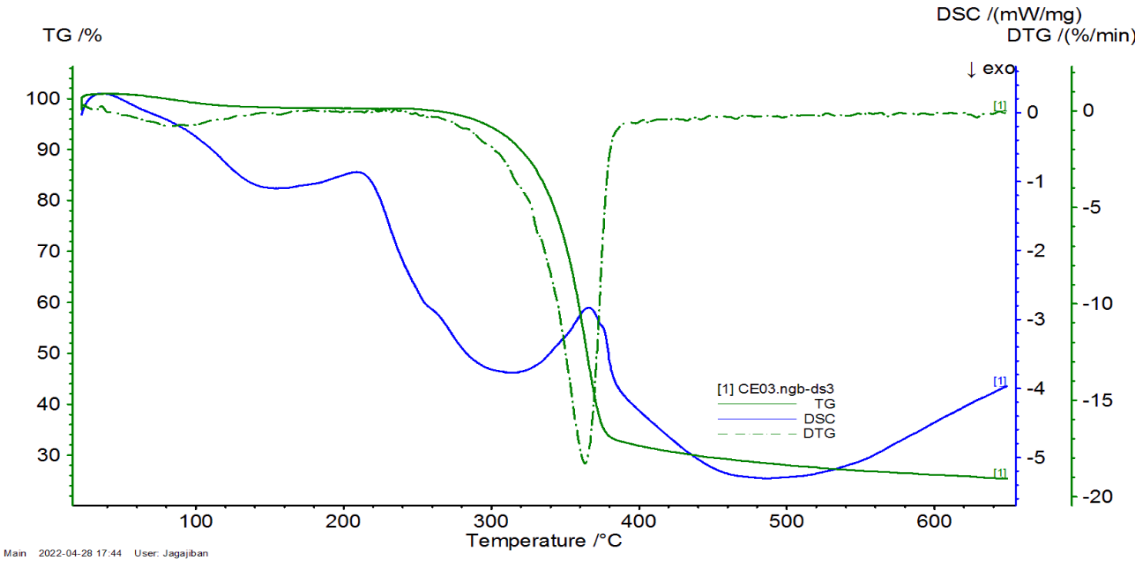
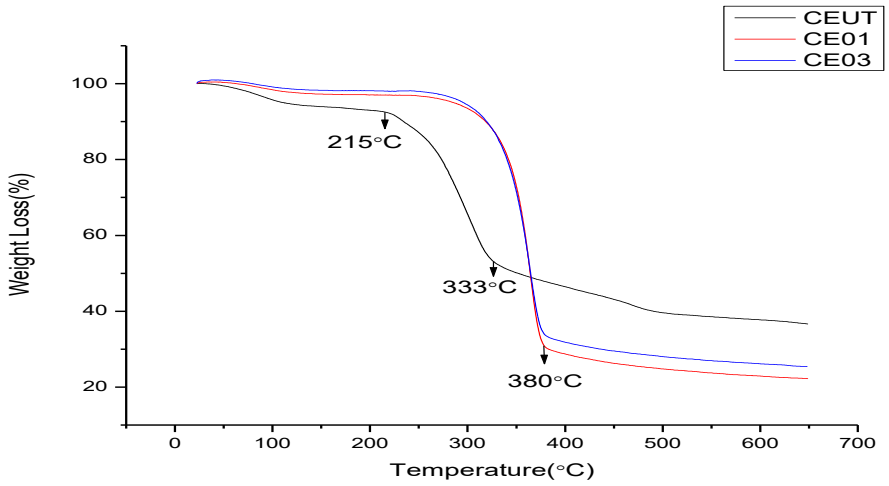


Fig. 5 c: Thermogravimetric curves for the CE03 (treated banana fiber at 121 °C)



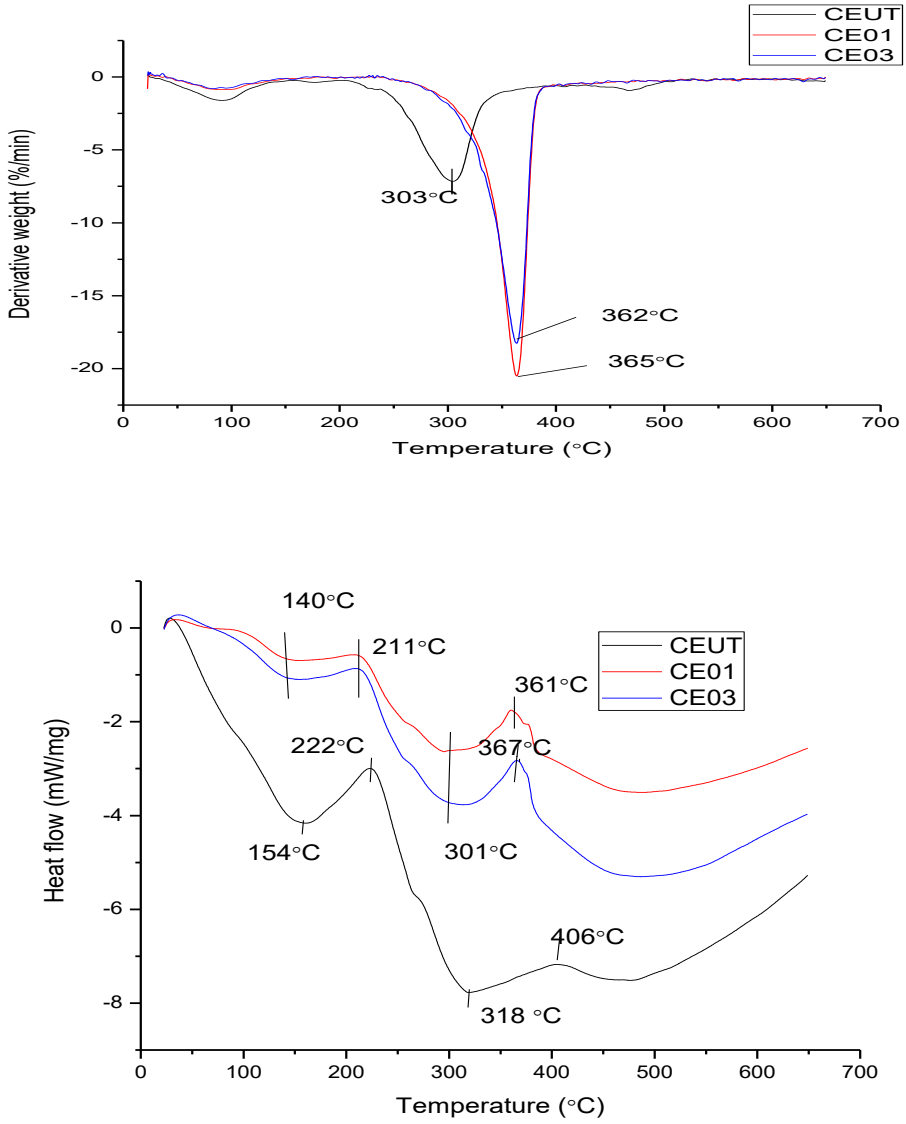


Fig. 6: Thermogravimetric curves for the CEUT, CE01 and CE03 samples (a) TGA curves (b) DTG curves (c) DSC curves

CONCLUSIONS

Nanocellulose can be successfully isolated from banana pseudostems (agricultural waste) using combined enzymatic hydrolysis and chemical treatment. This work has demonstrated the potential application of banana pseudostem as a raw material for nanocellulose production. Enzymatic hydrolysis with cellulose enzyme followed by mild acid treatment is a new approach for producing nanocellulose from banana pseudostems. The synthesis of nanocellulose was influenced by time, temperature as well as enzyme concentration. After comparing different methods for producing nanocellulose, it can be concluded that alkali pretreatment at room temperature for a longer duration (25 °C for 14h) leads to high crystallinity (60.13%) as compared to alkali treatment at a higher temperature for a short duration under steam(121 °C, for 1hr in an autoclave). It might be due to the slow crystal development process. Further enzymatic hydrolysis (dose-dependent manner) before mild acid treatment showed a high level of crystallinity. Additionally crystal structure is more

symmetrical as evidenced by hkl index. As compared to strong acid hydrolysis, enzymatic hydrolysis before mild acid treatment (5% oxalic acid) reduces the use of harmful chemicals. As oxalic acid is a dicarboxylic acid, it produces less toxic compounds than strong mineral acids. Although different treatments increased the crystallinity of nanocellulose. Nanocellulose obtained after pretreatment of enzymatic hydrolysis had improved properties as compared with acid-treated nanocellulose alone. TGA data demonstrated that the produced nanocellulose have enhanced thermal stability, which make them less likely to degradation and thus allowing them to be processed at higher temperatures than their untreated nanocellulose. These findings proposed the potential application of banana pseudostems for the production of nanocellulose.

References

Beltramino, F., Blanca Roncero, M., Vidal, T., & Valls, C. (2018). A novel enzymatic approach to nanocrystalline cellulose preparation. *Carbohydrate Polymers*, *189*(November 2017), 39–47. https://doi.org/10.1016/j.carbpol.2018.02.015

Bhattacharya, D., Germinario, L. T., & Winter, W. T. (2008). Isolation, preparation and characterization of cellulose microfibers obtained from bagasse. *Carbohydrate Polymers*, *73*(3), 371–377. https://doi.org/10.1016/j.carbpol.2007.12.005

Chen, W., Yu, H., Liu, Y., Hai, Y., Zhang, M., & Chen, P. (2011). Isolation and characterization of cellulose nanofibers from four plant cellulose fibers using a chemical-ultrasonic process. *Cellulose*, *18*(2), 433–442. https://doi.org/10.1007/s10570-011-9497-z

de Amorim, J. D. P., de Souza, K. C., Duarte, C. R., da Silva Duarte, I., de Assis Sales Ribeiro, F., Silva, G. S., de Farias, P. M. A., Stingl, A., Costa, A. F. S., Vinhas, G. M., & Sarubbo, L. A. (2020). Plant and bacterial nanocellulose: production, properties and applications in medicine, food, cosmetics, electronics and engineering. A review. *Environmental Chemistry Letters*, *18*(3), 851–869. https://doi.org/10.1007/s10311-020-00989-9

Deepa, B., Abraham, E., Cherian, B. M., Bismarck, A., Blaker, J. J., Pothan, L. A., Leao, A. L., de Souza, S. F., & Kottaisamy, M. (2011). Structure, morphology and thermal characteristics of banana nano fibers obtained by steam explosion. *Bioresource Technology*, *102*(2), 1988–1997. https://doi.org/10.1016/j.biortech.2010.09.030

Erdogan, U. H., Selli, F., & Duran, H. (2017). Banana plant waste as raw material for cellulose extraction. *Vlakna a Textil*, *24*(3), 48–52. https://doi.org/10.13140/RG.2.2.25941.22245

Kargarzadeh, H., Ioelovich, M., Ahmad, I., Thomas, S., & Dufresne, A. (2017). Methods for Extraction of Nanocellulose from Various Sources. *Handbook of Nanocellulose and Cellulose Nanocomposites*, 1–49. https://doi.org/10.1002/9783527689972.ch1

Kaushik, A., Singh, M., & Verma, G. (2010). Green nanocomposites based on thermoplastic starch and steam exploded cellulose nanofibrils from wheat straw. *Carbohydrate Polymers*, *82*(2), 337–345. https://doi.org/10.1016/j.carbpol.2010.04.063

Komal, U. K., Lila, M. K., & Singh, I. (2020). PLA/banana fiber based sustainable biocomposites: A manufacturing perspective. In *Composites Part B: Engineering* (Vol. 180). Elsevier Ltd. https://doi.org/10.1016/j.compositesb.2019.107535

Krishnadev, P., Subramanian, K. S., Janavi, G. J., Ganapathy, S., & Lakshmanan, A. (2020). Synthesis and characterization of nano-fibrillated cellulose derived from green agave Americana L. fiber. *BioResources*, *15*(2), 2442–2458. https://doi.org/10.15376/biores.15.2.2442-2458

Krishnan, V. N., & Ramesh, A. (2017). Synthesis and Characterization of Surface Modified Cellulose Nanofibers from Banana Peel. *IOSR Journal of Applied Chemistry*, *10*(01), 15–19. https://doi.org/10.9790/5736-1001021519

Kumari, P., Pathak, G., Gupta, R., Sharma, D., & Meena, A. (2019). Cellulose nanofibers from lignocellulosic biomass of lemongrass using enzymatic hydrolysis: characterization and cytotoxicity assessment. *DARU, Journal of Pharmaceutical Sciences*, *27*(2), 683–693. https://doi.org/10.1007/s40199-019-00303-1

Leite, A. L. M. P., Zanon, C. D., & Menegalli, F. C. (2017). Isolation and characterization of cellulose nanofibers from cassava root bagasse and peelings. *Carbohydrate Polymers*, *157*, 962–970. https://doi.org/10.1016/j.carbpol.2016.10.048

Lopez-Rubio, A., Flanagan, B. M., Gilbert, E. P., & Gidley, M. J. (2008). A novel approach for calculating starch crystallinity and its correlation with double helix content: A combined XRD and NMR study. *Biopolymers*, *89*(9), 761–768. https://doi.org/10.1002/bip.21005

Meng, F., Wang, G., Du, X., Wang, Z., Xu, S., & Zhang, Y. (2018). Preparation and characterization of cellulose nanofibers and nanocrystals from liquefied banana pseudo-stem residue. *Composites Part B*. https://doi.org/10.1016/j.compositesb.2018.08.048

Nandi, S., & Guha, P. (2018). A Review on Preparation and Properties of Cellulose Nanocrystal-Incorporated Natural Biopolymer. *Journal of Packaging Technology and Research*, *2*(2), 149–166. https://doi.org/10.1007/s41783-018-0036-3

Paramasivam, S. K., Panneerselvam, D., Sundaram, D., Shiva, K. N., & Subbaraya, U. (2020). Extraction, Characterization and Enzymatic Degumming of Banana Fiber. *Journal of Natural Fibers*, *00*(00), 1–10. https://doi.org/10.1080/15440478.2020.1764456

Pelissari, F. M., Sobral, P. J. D. A., & Menegalli, F. C. (2014). Isolation and characterization of cellulose nanofibers from banana peels. *Cellulose*, *21*(1), 417–432. https://doi.org/10.1007/s10570-013-0138-6

Priyadarshana, R. W. I. B., Kaliyadasa, P. E., Ranawana, S. R. W. M. C. J. K., & Senarathna,

K. G. C. (2020). Biowaste Management: Banana Fiber Utilization for Product Development. *Journal of Natural Fibers*, *00*(00), 1–11. https://doi.org/10.1080/15440478.2020.1776665

Radakisnin, R., Majid, M. S. A., Jamir, M. R. M., Jawaid, M., Sultan, M. T. H., & Tahir, M. F. M. (2020). Structural, morphological and thermal properties of cellulose nanofibers from napier fiber (Pennisetum purpureum). *Materials*, *13*(18). https://doi.org/10.3390/ma13184125

Santos, F. A. dos, Iulianelli, G. C. V., & Tavares, M. I. B. (2016). The Use of Cellulose Nanofillers in Obtaining Polymer Nanocomposites: Properties, Processing, and Applications. *Materials Sciences and Applications*, *07*(05), 257–294. https://doi.org/10.4236/msa.2016.75026

Shreedhana, K., & Ilavarasi, R. (2020). Fabrication of nanocrystalline cellulose from banana peel obtained from unripe plantain bananas. *Journal of Physics: Conference Series*, *1644*(1). https://doi.org/10.1088/1742-6596/1644/1/012002

Syafri, E., Kasim, A., Abral, H., Sudirman, Sulungbudi, G. T., Sanjay, M. R., & Sari, N. H. (2018). Synthesis and characterization of cellulose nanofibers (CNF) ramie reinforced cassava starch hybrid composites. *International Journal of Biological Macromolecules*, *120*, 578–586. https://doi.org/10.1016/j.ijbiomac.2018.08.134

Tibolla, H., Pelissari, F. M., Rodrigues, M. I., & Menegalli, F. C. (2017). Cellulose nanofibers produced from banana peel by enzymatic treatment: Study of process conditions. *Industrial Crops and Products*, *95*, 664–674. https://doi.org/10.1016/j.indcrop.2016.11.035

Wang, H., Zhang, X., Jiang, Z., Li, W., & Yu, Y. (2015). A comparison study on the preparation of nanocellulose fibrils from fibers and parenchymal cells in bamboo (Phyllostachys pubescens). *Industrial Crops and Products*, *71*, 80–88. https://doi.org/10.1016/j.indcrop.2015.03.086

Wu, J., Du, X., Yin, Z., Xu, S., Xu, S., & Zhang, Y. (2019). Preparation and characterization of cellulose nanofibrils from coconut coir fibers and their reinforcements in biodegradable composite films. *Carbohydrate Polymers*, *211*(November 2018), 49–56. https://doi.org/10.1016/j.carbpol.2019.01.093

Zuluaga, R., Luc, J., Cruz, J., Vélez, J., Mondragon, I., & Gañán, P. (2009). Cellulose microfibrils from banana rachis : Effect of alkaline treatments on structural and morphological features. *Carbohydrate Polymers*, *76*(1), 51–59. https://doi.org/10.1016/j.carbpol.2008.09.024