

Low-energy extraction of lignocellulose nanofibers from fresh *Musa basjoo* pseudo-stem

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Abstract

This study presents a novel approach for the extraction of cellulose nanofibers (CNF) and lignocellulose nanofibers (LCNF) from *Musa basjoo* pseudo-stems, a relative of bananas, without the need for extensive drying. Instead, wet pseudo-stems were compressed and treated with NaOH solutions at varying temperatures and durations. The extracted material exhibited the characteristic peaks of cellulose I in X-ray diffraction (XRD) patterns, similar to those obtained from dried pseudo-stems. Fourier-transform infrared (FT-IR) spectroscopy confirmed the presence of cellulose I in the treated material and lignocellulose nanofiber clearly shown at 1600-1500, 1421, 1365, and 1161 cm^{-1} . Composition analysis by Van Soest fiber analysis revealed a higher cellulose content in the treated material of wet pseudo-stems compared to that obtained from dried pseudo-stems, indicating the effectiveness of this low-energy extraction method. Meanwhile, field-emission scanning electron microscopy (FE-SEM) images demonstrated clear LCNF in the nanometer scale fibers after NaOH treatment. Overall, this study successfully demonstrated the extraction of LCNF from wet pseudo-stems of *Musa basjoo* with NaOH treatment at 70°C for 3 hours with 80% extraction result, providing a more efficient and low-energy approach for utilizing waste from *Musa basjoo* and bananas.

Keywords: Lignocellulose nanofiber; *Musa basjoo*; banana relative; low-energy extraction; wet raw material

1. Introduction

In recent years, interest in the use of cellulose nanofibers (CNF) and lignocellulose nanofibers (LCNF) has increased with a large number of published literatures. As bio-based materials, CNF and LCNF have many advantages such as related to their abundance, affordability and renewability. They are also available at a lower cost than other advanced or nano-sized materials, non-toxic, durable, and lightweight [1–3]. CNF and LCNF have many applications in the following areas: nanocomposite absorbents [4,5], packing materials [6], electronics [7], energy conversion [3], memory devices [8–10], and biomedicine [11,12].

CNF and LCNF are commonly extracted from plants such as trees, and agricultural wastes as the promising raw materials such as pampas grass, silk thread, coconut, *Phormium tenax* [13], *Muntingia calabura* [14], lemongrass [15], lettuce peel [16], sugarcane bagasse [17], corn straw [18], *Eucalyptus* [19], pineapple crown [20], algae *Gracilaria sp.* [21], banana peel [22, 23], and banana pseudo-stem [24].

The proper utilization of agricultural waste as a potential source for extracting cellulose nanofibers holds a great promise; however, certain waste materials, such as banana

stems, have not been effectively utilized. Indonesia, one of the globally largest banana-producing countries, ranks third in production after India and China, boasting a wide variety of banana species [25]. This abundance of banana stems presents an increasingly challenging opportunity for further research in the field of banana stem fiber in Indonesia. Moreover, recent findings have revealed that the pseudo-stem derived from *Musa basjoo* exhibits significant potential as a source of cellulose nanofibers (CNF) that can be utilized in various industries. The development of effective and low-energy extraction methods for CNF from *M. basjoo* holds promise for diverse applications.

Various extraction methods of extracting CNF and LCNF, including alkaline, acid-based [24, 26], enzymatic [27], and combinations of chemical/biological with mechanical ones [28, 29] are commonly employed for the production of cellulose nanofibers. This research focuses on a modified approach to extract cellulose using NaOH at lower temperatures and shorter extraction times, building upon previous successful extraction of LCNF in one step from *Musa basjoo* [30]. Alkaline methods, which involve combining NaOH with other compounds, have been extensively utilized by researchers. Isogai et al. [28] for instance employed the TEMPO/NaBr/NaClO approach to oxidize cellulose in an alkaline environment. However, the high cost of TEMPO limited the effectiveness of this method. The excessive use of acidic compounds also can lead to the increase the acid waste production, and prolonged extraction

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times result in inefficiency. In turn, the primary objective of this study is to achieve the effective and efficient extraction of cellulose nanofibers from wet *Musa basjoo* samples using NaOH within a shorter timeframe, facilitating easy extraction with reduced energy requirements. The combination of NaOH treatment and physical treatment serves as two crucial steps in the energy-effective production of cellulose nanofibers through this simplified approach.

Recently, we succeeded in extracting LCNF from dried pseudo-stems of *Musa basjoo*, a banana relative, by using a one-step alkalization method using NaOH that combined with a mechanical process at 90°C within 24 hours [30]. It is usually hard to extract CNF and LCNF from plants by NaOH treatment alone. The success of this extraction was attributed to the specific structure of the pseudo-stem microfibrils of *Musa basjoo*, which have been arranged in simple thin sheets rather than in bundles, as seen in trees. Since *Musa basjoo* is a related species of banana, the research on extracting LCNF from *Musa basjoo* at low energy at 70°C for 1 and 3 hours will contribute 8 times the efficient and effective utilization of banana pseudo-stem wastes.

2. Materials and Methods

2.1. Extraction of lignocellulose nanofibers

Pseudo-stems of *Musa basjoo* were collected from Uchiko town, Ehime Prefecture, Japan. In a previous study [30], the collected *Musa basjoo* pseudo-stems were dried in the sun for one month and used as samples. The abundance of banana stems presents an increasingly challenging opportunity for further research in the field of banana stem fiber in Indonesia.

This treatment aimed to obtain more LCNF in one extraction. However, since the pseudo-stem of *M. basjoo* contains more than 90% water, it requires a long time to dry. In this study, instead of drying, the pseudo-stems were compressed using a press to reduce the water content to about 1/10 (w/w), which dry chips *M. basjoo* and NaOH solution ratio. This could significantly reduce the time for LCNF extraction. Also, since the sample was in a wet state, it was expected that the reaction between the raw material and the aqueous NaOH solution proceeded more effectively than in the case of the previous dry sample. Figure 1 shows the resulting wet raw material (WRMA). The WRMA was cut into 1 cm in length and used as a sample. The sample was mixed with distilled water or NaOH solution in an alkali-resistant bottle at a mass ratio of 1:1, sealed, and heat-treated in an oven at 50°C and 70°C for 1, 3, and 24 hours. In the previous study, the conditions with the dried pseudo-stems were 1 mol L⁻¹ NaOH at 90°C within 24 hours. The solution after the treatment was as black as the previous one. It was filtered with distilled water until becoming transparent and reaching a neutral pH. The WRMA and treated samples were dried and subjected to characterization.

2.2. Characterization of lignocellulose nanofibers

The crystal structures of the samples were analyzed by means of X-ray diffractometry (XRD) with an Ultima IV instrument manufactured by Rigaku, Japan. CuK α radiation served as the X-ray source with a 2 θ range spanning from 3° to 60°. The X-ray setup employed an acceleration voltage of 40 kV and a current of 40 mA. To calculate the crystallinity index

(CI), Equation (1) was utilized, where I_{200} denotes the diffraction peak height corresponding to the cellulose (200) plane, and I_{am} represents the peak height of the amorphous region.

$$C_i (\%) = (1 - I_{am}/I_{200}) \times 100 \quad (1)$$

The investigation of fiber components and functional groups involved Fourier Transform Infrared Spectroscopy (FT-IR) analysis conducted on Fourier transform infrared spectroscopy (FT-IR) ATR analysis conducted on a spectrum two, Perkin Elmer UATR Two, Japan. The attenuated total reflection method was employed with a diamond crystal, covering the wavenumber range of 400 – 4000 cm⁻¹.

Field-emission scanning electron microscopy (FE-SEM), using a JSM-7610F instrument by Jeol, Japan, was carried out to examine the fiber morphology and changes in fiber diameter resulting from the alkali treatment. Before FE-SEM imaging, the samples underwent a treatment with tertiary butyl alcohol to disrupt hydrogen bonds between cellulose fibers, followed by freeze-drying and osmium coating. Secondary electron images were acquired using an acceleration voltage of 2 kV.

2.3. Composition analysis of lignocellulose nanofibers

The composition analysis of LCNF was conducted by Van Soest fiber analysis. The determination of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) contents in the samples was performed. The cellulose, hemicellulose, and lignin contents were subsequently calculated using equations (2) to (4) according to Liu et al. [31] (equation 2-4):

$$\text{Cellulose} = \text{ADF} - \text{ADL} \quad (2)$$

$$\text{Hemicellulose} = \text{NDF} - \text{ADF} \quad (3)$$

$$\text{Lignin} = \text{ADL} \quad (4)$$

In brief, the fiber samples underwent boiling with sodium sulfite and a neutral detergent solution with the addition of α -amylase to eliminate starch. The NDF content was derived by deducting the ash amount from the quantity of insoluble matter present in the neutral detergent solution.

Furthermore, the fiber samples were boiled in an acidic detergent solution containing sulfuric acid and detergent to remove hydrolyzed hemicellulose. The ADF content was determined by subtracting the ash amount from the quantity of insoluble matter in the acidic detergent solution. Subsequently, the insoluble matter obtained from the acidic detergent solution underwent a treatment with 72% sulfuric acid to decompose and eliminate cellulose. The total ADL content was determined by subtracting the ash amount from the residue obtained after the treatment with 72% sulfuric acid. Analytical grade reagents were procured from FUJIFILM Wako Pure Chemical Industries, Japan.

3. Results and Discussion

3.1 Characterization of WRMA and LCNFs

The wet raw material (WRMA) of banana pseudo-stem became a novelty topic, and decreasing temperature and time of lignocellulose extraction may decrease the energy. After the treatment of WRMA with NaOH solutions, the contents of the

container turned blackish brown. The degree of blackish-brown color increased with the increase of NaOH concentration. The repeated filtration with distilled water removed the blackish-brown component, and the remaining material was nearly white. The dark brown components were lignin and ash. Lignin had the property of binding cellulose. Here, the more lignin hydrolyzed, the more cellulose freed from its binding to lignin [32]. The extraction conditions used in this study failed to extract LCNF when the concentration of NaOH was below 1 mol L⁻¹ and the treatment temperature was below 70°C. The case of NaOH concentration of 1 mol L⁻¹ and a heating temperature of 70°C is discussed in the following parts.

The XRD patterns of WRMA and treated WRMA (Figure 1) showed two peaks characteristic of LCNF: peaks at 15.8 degrees and 22.2 degrees, characteristic of cellulose I. These were similar with the data for dried *Musa basjoo* [30]. Marais et al. [24] also observed XRD peaks at 16 and 22 degrees in extracts from *Musa acuminata* and *Musa balbisianal*.

The CI value was 67.3% for WRMA, 77.2% for 1-hour treatment (LCNF-1h), and 75.1% for 3-hour treatment (LCNF-3h), and the CI value increased even after 1-hour treatment. In the previous report, the CI value was decreased by NaOH treatment of dried pseudo-stems of *Musa basjoo*. This might be due to the destruction of the cellulose structure by long-term treatment at a high temperature. This study showed that the destruction of the cellulose structure was suppressed by using a wet sample and making the treatment temperature and time milder. These indicated that cellulose I was concentrated in the sample by treatment with NaOH at 70°C.

The FT-IR data in Figure 2 show important information about the chemical structure of the WRMA and the processed material. WRMA, like other biomass, is mainly composed of cellulose, hemicellulose, and lignin. Furthermore, the main functional groups involved in these three components are alkanes, esters, aromatics, ketones, and alcohols with different oxygen-containing functional groups. The region between 3000 and 3700 cm⁻¹ is the strong O–H stretching frequency of the intra- and intermolecular hydrogen bonds of cellulose. This is also enhanced by the fingerprint area in the range 900–1400 cm⁻¹ [33]. The strength of this hydrogen bond is closely related to the crystal system and crystallinity. The peak at 1365 cm⁻¹ corresponded to the bending vibrations of the C–H and C–O groups of the polysaccharide. The peak at 1161 cm⁻¹ was related to the C–O–C asymmetric stretching vibration of CNF. The peak of the C–H stretching vibration was approximately 2890 cm⁻¹. The peak at 896 cm⁻¹ corresponded to C–O–C deformation and stretching of cellulose. The regions ranging from 1420–1430 cm⁻¹ and 890–900 cm⁻¹ were associated with the number of crystalline structures and amorphous regions in cellulose, respectively. A weak peak at 776 cm⁻¹ in WRMA (Figure 2(a)), due to C–H out-of-plane deformation of lignin, was not found in the XRD patterns of LCNF-1h and LCNF-3h, indicating the hydrolysis of lignin with the NaOH treatment. The lignin wavenumber showed at 3343, 2891, 1600–1500, 1421, 1365, 1161 cm⁻¹ in Figure 2 (a, b)—the data supported by Pereira et al. [35].

The XRD and FT IR data presented above for wet pseudo-stems treated with NaOH showed the ability of NaOH to extract LCNF from wet pseudo-stems at 70°C for 1 hour and 3 hours.

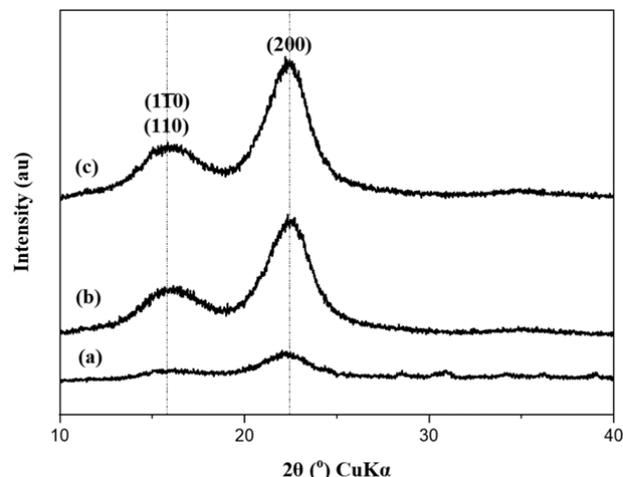


Fig. 1. Characterization of WRMA and LCNFs. The XRD pattern of (a) WRMA; (b) LCNF-1h; (c) LCNF-3h

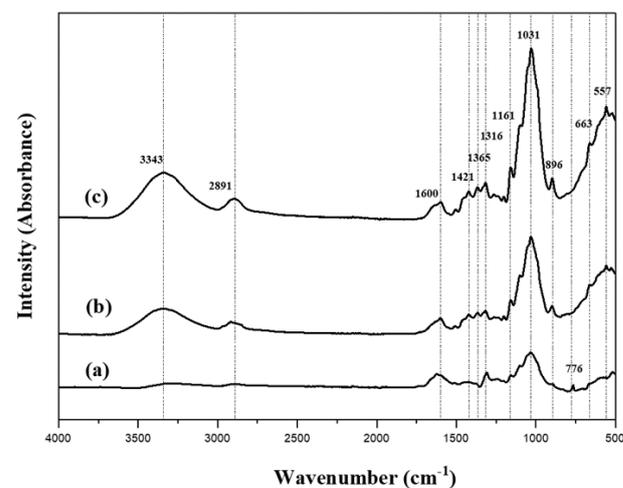


Fig. 2. Characterization of WRMA and LCNFs. The FTIR-ATR pattern of (a) WRMA; (b) LCNF-1h; (c) LCNF-3h

3.2 Composition analysis of WRMA and LCNFs

Table 1 shows composition analysis data of the WRMA and treated materials. The cellulose content of the sample treated with NaOH for 3 hours was 74%, and that of the product treated at 90°C for 24 hours using the previously dried pseudo-stems was 71%. This indicated that using the wet pseudo-stem as a raw material has made it possible to obtain a treated product with a higher cellulose content at a lower temperature and in a shorter time. In addition, in the case of this treatment for 1 hour, the cellulose content was 66%, indicating that the treatment time was insufficient. Pereira et al. reported that *Musa sp cv pavan* had 61.4 % of alpha-cellulose content in the outer, middle, and inner sheaths [34].

Cellulose in plants is typically present in and between the mixtures of hemicellulose, lignin, pectin, and other substances. The unique molecular structure of cellulose makes it attractive properties such as chirality, hydrophilicity, degradability, and high reactivity due to the presence of donor OH groups. This material exhibits a crystalline fibrous structure as a result of the strong hydrogen bonds present in cellulose. More interestingly, unlike other polysaccharides, cellulose can maintain a semi-crystalline state of aggregation in aqueous environments. From

a microstructural point of view, cellulose is composed of fibrils with crystalline and amorphous domains.

Figure 3 shows the morphology of WRMA and treated products using FE-SEM. Figure 4(a) shows a section of the surface of the *M.basjoo* as wet raw material. No nanofibers were observed on the surface of WRMA (Figure 3(a)). This was because the nanofibers were embedded in lignin, hemicellulose, and the like. It was a series of parallel units made of cellulose, hemicellulose, and lignin. It indicated of very rigid structure and was not easy to break. On the other hand, fiber morphology was confirmed in both processed products. Alkaline treatments for 3 hours (Figure 3(c)) showed a clearer structure of lignocellulose nanofibers compared to those treated for 1 hour (Figure 3(b)). This indicated that impurities such as lignin were removed more by prolonging the treatment time.

Both Figures 3(b) and 3(c) demonstrate the efficacy of alkalization extraction, revealing well-separated, fine fibers that underwent cellulose degradation. By immersing the material in tertiary butyl alcohol, and employing shaking and centrifugation, the breaking of hydrogen bonds was accomplished. Unlike the wet raw material, Figures 3(b) and 3(c) exhibit distinct surface areas, indicating the effectiveness of the alkalization process using NaOH as a sole chemical compound, and achieving results in a shorter period (1h and 3h). Patil et al. conducted a study revealing that delignification was more efficient when NaOH was utilized compared to NH_3 . These advantages render NaOH more environmentally friendly and highly valuable for biomass pre-treatment. It is advantageous due to its low-energy extraction, dilute concentrations, and normal air pressure [35].

Table 1. Composition analysis results (wt%)

Material	Cellulose	Hemicellulose	Lignin
WRMA	54	10	9.8
LCNF-1h	66	11	12
LCNF-3h	74	8.6	9.7

These results indicated that NaOH treatment of wet pseudo-stems of *Musa basjoo* could extract LCNFs at a lower temperature and shorter time compared to the dried pseudo-stems. Hydrogen bonds between nanofibers are usually reversible, and in pulps, they are dispersed when dried and immersed in water. In contrast, since *Musa basjoo* pseudo-stems are composed of sheet-like CNFs [30], the strong hydrogen bonds, when dried, would have required higher temperature and longer time for re-dispersion. The *Musa basjoo* pseudo-stem possesses commendable characteristics and holds great potential as a low-energy source for lignocellulose nanofibers.

4. Conclusion

The extraction of LCNF from wet pseudo-stems of *Musa basjoo* using NaOH at 70°C for 3 hours resulted in a lower-energy process compared to previous methods. This novel approach yielded higher cellulose I content and crystallinity than the conventional treatment of dry pseudo-stems of *Musa basjoo* at 90°C within 24 hours. The higher cellulose I content suggested a greater abundance of crystalline cellulose, which is

desirable for the production of LCNF. Additionally, the increased crystallinity indicated a more organized and tightly packed cellulose structure, further enhancing the quality of the extracted LCNF. These findings highlighted the potential of utilizing wet pseudo-stems of *Musa basjoo* and optimizing extraction conditions to achieve the higher yields of LCNF with improved cellulose content and crystallinity.

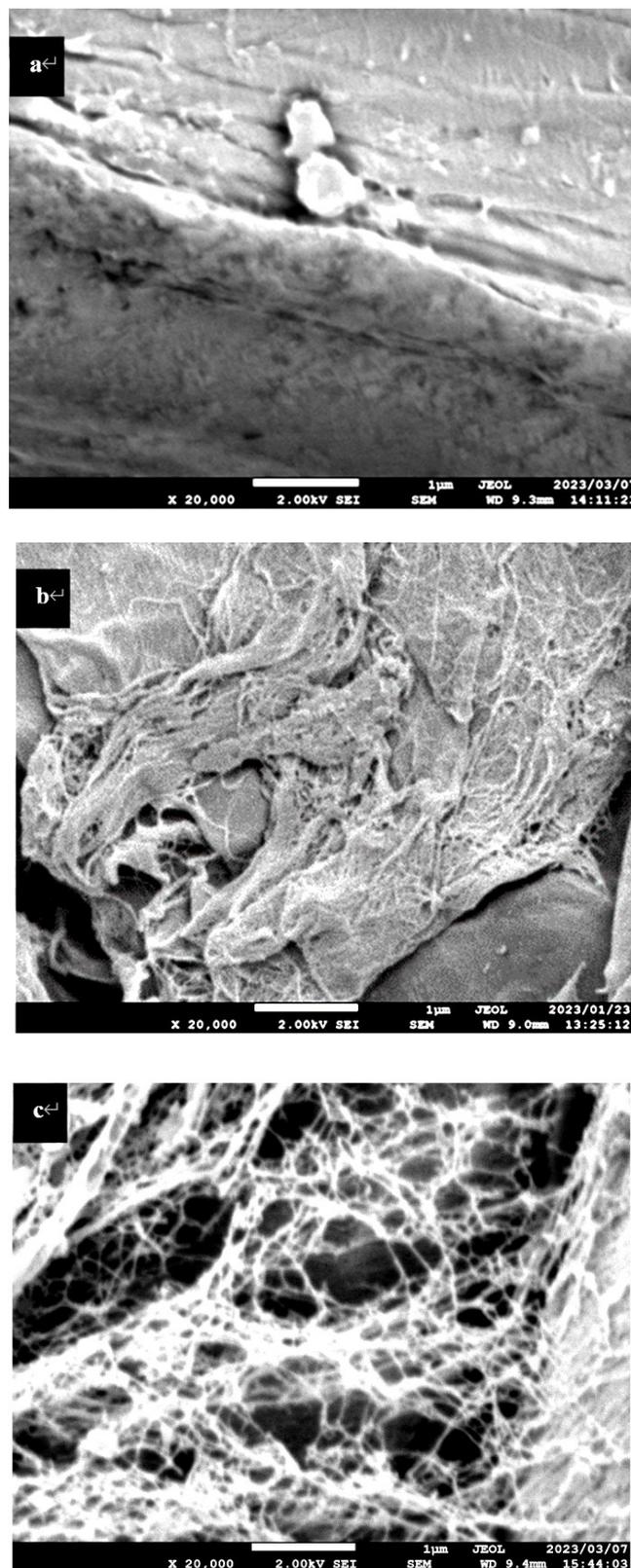


Fig. 3. FE-SEM images of WRMA and LCNFs of (a) WRMA; (b) LCNF-1h; and (c) LCNF-3h

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