Isolation and characterization of Cellulose Nanofiber from Subang Pineapple Leaf Fiber waste produced using Ultrafine Grinding method

H Amirulhakim\textsuperscript{1}, A L Juwono\textsuperscript{1,*} and S Roseno\textsuperscript{2}

\textsuperscript{1}Master Program of Materials Science, Department of Physics, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Kampus UI, Depok 16424, Indonesia
\textsuperscript{2}Centre of Technology for Materials – BPPT, PUSPIPTEK, South Tangerang, 15314, Indonesia

*ariadne.laksimi@ui.ac.id

Abstract. Pineapple leaf fiber (PALF) is one of natural fibers that has high cellulose content. However, pineapple plants must be replaced with new plants once it is harvested, leaving the leaves as waste. In this research, the isolation and characterization of Subang based PALF were conducted. Chemical pre-treatments including alkaline treatment with Sodium Hydroxide and bleaching to remove nanocellulosic constituents such as lignin and hemicellulose were conducted. This process was followed by mechanical treatment using ultrafine grinder to produce cellulose nanofiber (CNF). Transmission Electron Microscopy (TEM) images showed that the CNF had 45-75 nm in diameters. The percentage crystallinity was determined by X-ray diffraction (XRD). The crystallinity values of raw PALF, treated PALF, and CNF were 74.97%, 76.29%, and 69.52% respectively. Fourier Transform Infrared (FTIR) spectroscopy analysis was carried out to investigate the chemical structure changes after both chemical and mechanical treatments. The presence of a peak that related to cellulose confirmed that the process was well conducted. These results indicated that PALF waste could become added value to agricultural waste and expected to become reinforcement agent in nanocomposite for structural application since PALF had low aspect ratio and had high percentage crystallinity values.

1. Introduction
Agriculture is the biggest economic driving sector of Indonesia. This causes an increase in biomass waste. As in pineapple plants, each harvest of pineapple plants must be replaced with new plants, leaving the leaves as waste [1]. The existence of such solid waste has no economic value. The utilization biomass waste has attracted academic and industrial research for materials and energy application [2].

Pineapple leaf fiber (PALF) is a natural fiber that shows high specific strength and stiffness. The fiber is very hygroscopic, relatively inexpensive and widely available. This superior mechanical property is associated with high cellulose content. Cellulose content in PALF reaches 71.5%. Pineapple leaf fiber is also a candidate for glass fiber replacement materials, especially in structural applications [3].

In most studies, nanocellulose has gained much attention due to its low cost, biocompatibility, biodegradability, nontoxicity, renewability, sustainability, strong surface reactivity, and favorable...
physical properties [4]. Nanocellulose also has great potential as a construction material and as a substitute for other structural materials (for example, GRP, steel, concrete) in many applications [4]. Compared to micro scale cellulose, nanocellulose materials are lighter in weight with higher surface area to volume ratio and higher strength and stiffness [5].

Cellulose nanofiber (CNF) consists of elementary nanofibril aggregates constructed from crystalline and amorphous regions. The diameter of CNF can be 20-100 nm and length 500-2000 nm. Generally, CNF is produced by mechanical treatments, which have passed the process of alkalization and bleaching to eliminate lignin and hemicellulose, with high pressure homogenizers (HPH) [6]. Another technique for separating cellulose fibers into cellulose nanofibrils is by grinding method.

In this research, grinding method was used to produce CNF from PALF waste originated in Subang, Indonesia. Microscale cellulose fibers are forced through a gap between the rotary and stator stone disks, is fast, simple, one-step and high-yield. Repeated cyclic pressure and shearing stresses result in the nano-fibrillation of the cellulose fibers [7]. The resulting products were characterized by X-ray diffraction (XRD) to determine the percentage of crystallinity, transmission electron microscopy (TEM), and Fourier-transform infrared spectroscopy (FTIR).

2. Materials and methods

2.1. Materials
Pineapple leaves were collected from Subang, Indonesia. The reagents used for chemical process were NaOH, and NaClO. Distilled water used in whole process. The apparatus used for pre-treatment of pineapple fiber was an Autoclave. The isolation of CNF was conducted using Ultra fine Grinder (Masulo Corp, Japan). Characterization were carried out using the Transmission Electron Microscope (TECNAI G2Spirit Twin), X-Ray Diffraction (XRD), and Fourier Transmission Infrared Spectroscopy (FTIR).

2.2. Methods

2.2.1. Chemical treatment. Pineapple leaf fibers were cut into pieces and washed by heating at 80°C. Afterwards, the fiber was alkaline treated by adding 5% NaOH 1:10 at a pressure of 20 psi. Then, the bleaching process was carried out by adding 10% 1:10 natrum hypochlorite (NaClO). To remove lignin and hemicellulose from the fiber. The fiber was washed to neutral pH and dried. The dried fibers were cut into smaller pieces using cutter and mechanical blender. The treated fiber was ready for mechanical treatment (Grinding).

2.2.2. Mechanical treatment (Grinding). Treated PALF was mixed in distilled water at a ratio of 1:10 (1wt% treated PALF in aquabidest). Then, it was put in the Ultrafine Grinder (Matsuko Corp, Japan) with a gap of +10, +5, 0, -5, -10, and -15 with 10 cycles of each gap. Finally, 1% CNF suspension was obtained. The suspension was dried in ambient air to undergo further characterization.

2.2.3. Characterization. XRD patterns were obtained using x-ray diffractometer with Cu-Kα radiation within 20 angle range 5°-80°. XRD patterns were used to determine the percentage of crystallinity of raw PALF, treated PALF, and CNF.

Fourier Transform Infrared Spectroscopy were recorded with Burker Infrared Spectrometer within range of 400-4000 cm\(^{-1}\). The sample was dried in ambient air until become dried powder. The powdered sample from all samples (raw, treated, and CNF) were pelleted and mixed with KBr.

Transmission Electron Microscopy used is FEI Tecnai G2 Spirit Twin. Cellulose nanofiber suspension was dropped to the film grid and dried to be examined.
3. Results and discussion

3.1. XRD Characterization

Pineapple leaf fiber consists of amorphous region and crystalline region. The higher the percentage of crystallinity, the more favorable to become better in reinforcement with the polymer matrix. According to George et al., percentage of crystallinity can be calculated from equation (1) [8].

\[
X_c = \frac{I_c}{I_c + I_a} \times 100\%
\]

where \( I_c \) and \( I_a \) is the integrated intensity of crystalline region and amorphous region respectively. Integrated intensity is the area under a curve [8]. The integrated intensities were calculated using origin pro software.

Based on Figure 1, all three samples (Raw, Treated, and CNF) showed the typical pattern of cellulose crystal. The XRD patterns of all three samples exhibited two major peaks. The sharp peak at \( 2\theta = 22.5 \), corresponds to the (002) lattice, which represents the crystalline region, and lower peak at \( 2\theta=15.6 \), which represents the amorphous region [9].

The percentage of crystallinity of all samples can be seen at Table 1. There was an increase in percentage of crystallinity after chemical treatment process. It analogous with the previous reports [10,11]. In the raw fiber, cellulose is surrounded by amorphous lignin and hemicellulose. The increase of crystallinity can be attributed to the removal of lignin and hemicellulose on account of alkaline treatment and bleaching.

On the other hand, the crystallinity was decreased after mechanical treatment (grinding). A 7% decrease in crystallinity can be associated with the agglomeration of cellulose nanofibril. Agglomeration occurred due to the purification and drying in sample preparation. Daicho et al reported that the crystallinity decreases as the microfibrils disperse with disintegration as CNFs. The interfacial molecules between bundled microfibrils are partly crystalline [12]. Meanwhile, according to Xu et al, reported that CNFs showed broadened and merged peaks. The mechanical grinding used in CNF
manufacturing could deform or even completely destruct cellulose crystals, leading to broadened and shifted diffraction peaks [13]. Compared to cellulose nano crystal with chemical and enzymatic treatments, CNFs typically have lower percentage of crystallinity. However, in terms of reinforcement to polymer matrix, Xu et al also reported that although CNFs have lower percentage of crystallinity, reinforcing polymer matrix with CNF leads to higher strength and modulus than CNCs’ due to higher aspect ratio and network entanglements.

Table 1. Percentage of Crystallinity of Raw, Treated, and CNF.

| Sample  | Xc % |
|---------|------|
| Raw     | 74.97% |
| Treated | 76.29% |
| CNF     | 69.52% |

3.2. FT-IR Characterization
Characterization using FT-IR were conducted to show the effect of alkaline and bleaching treatment in removing lignin and hemicellulose. The FT-IR spectra of raw, treated, and CNF from PALF are shown in Figure 2. The peak around 1500 cm\(^{-1}\) in treated and also in CNF was not found, while in raw fiber was appeared. The peak at about 3 1500 cm\(^{-1}\) is attributed to the C=C stretching vibration of the aromatic ring in lignin [14]. The increase of peak at around 2900 cm\(^{-1}\) indicated that the purity of cellulose is increased since it shows the presence of polysaccharide groups, which cellulose is made of linear polysaccharide. The peak around 800 cm\(^{-1}\) is due to crystal forms of cellulose [15]. The peak at 3300 cm\(^{-1}\) shows the presence of hydroxyl groups (-OH) [10]. It indicated that cellulose is hydrophilic. The band around 1640 cm\(^{-1}\) corresponds to the vibrational mode of the cellulose water, and the peak around 1738 cm\(^{-1}\) attributed to carbonyl groups (C=O) in hemicellulose was decreased in treated PALF and CNF [16]. This absorption may also be related to the acetyl ester groups of hemicelluloses or to the ester linkage of carboxylic acids present in hemicellulose and lignin [17]. In other word, hemicellulose and lignin removal was successfully done.

Figure 2. FT-IR Spectra of raw PALF, treated PALF, and CNF.

3.3. Transmission Electron Microscopy (TEM)
The size and morphology of CNF isolated from PALF were evaluated by Transmission Electron Microscopy (TEM). The TEM image is shown in Figure 2. The CNF average diameter is 45-75nm
calculated using imageJ software. Unlike CNC, CNF isolated from PALF has shape like entangled networks. Cellulose nanofiber has large amount of hydroxyl groups. This causes agglomeration tends to occur as the dark colored networks in Figure 3.

The TEM image also indicated that the fibrillation of cellulose after grinding was occurred as an individual fiber starts to separate from bundled fibers. The fibrillation occurred due to the friction force between ceramic disks. Alkaline and bleaching process also helped the fibrillation during grinding. Alkaline and bleaching process removed noncellulosic constituent. Therefore, the remaining constituent mainly cellulose and made grinding process smoothly run and reduce the diameter of the fiber from micron to nano scale.

Compared to Correia et al, the diameter of each individual fiber were 10-20 nm [16]. This result is due to 20 cycles of grinding the fibers gone through and heating to 70°C while bleaching process. While in this study, 10 cycles of grinding were carried out and the bleaching process was without heating. However, the TEM images are mostly similar. Thus, the amount of cycle carried out and the effectiveness in removing noncellulosic constituent affect the size and morphology of CNF.

![Figure 3. TEM image of Cellulose Nanofiber.](image)

4. Conclusion
The Isolation of cellulose nanofiber from pineapple leaf fiber waste was successfully done. The FT-IR results showed that alkaline treatment and bleaching removed most amorphous hemicellulose and lignin. The removal of hemicellulose and lignin has resulted the increase in percentage of crystallinity. The highest percentage of crystallinity was 76.29%. However, percentage of crystallinity of CNF was about 7% lower than that of the treated fiber due to the long mechanical treatment. The CNFs have an average diameter of 45-75 nm. The CNF has appeared like an entangled network and thus making it suitable as reinforcement in polymer matrix. One of the ways to improve the result, including increasing percentage of crystallinity, is by reducing the cycle of grinding, and applying heat treatment in bleaching process. Therefore, pineapple leaf fiber waste may become more valuable source of nanocellulose.

Acknowledgement
This work was supported by Hibah PUTI Prosiding 2020 funded by DRPM Universitas Indonesia.
References

[1] Setiawan A A, Shofiyani A and Syahbanu I 2017 Pemanfaatan Limbah Daun Nanas (Ananas comosus) Sebagai Bahan Dasar Aarang Aktif untuk Adsorpsi Fe (II) Jurnal Kimia Khatulistiwa 6

[2] Santos R M dos, Flauzino Neto W P, Silvério H A, Martins D F, Dantas N O and Pasquini D 2013 Cellulose nanocrystals from pineapple leaf, a new approach for the reuse of this agro-waste Industrial Crops and Products 50 707–14

[3] Cherian B M, Leão A L, de Souza S F, Thomas S, Pothan L A and Kottaisamy M 2010 Isolation of nanocellulose from pineapple leaf fibres by steam explosion Carbohydrate Polymers 81 720–5

[4] Zheng D, Zhang Y, Guo Y and Yue J 2019 Isolation and Characterization of Nanocellulose with a Novel Shape from Walnut (Juglans Regia L.) Shell Agricultural Waste Polymers (Basel) 11

[5] Lavanya D, P K Kulkarni, Dixit M, Raavi P K and Krishna L N V 2011 Sources of Cellulose and Their Applications 2 21

[6] Chakrabarty A and Teramoto Y 2018 Recent Advances in Nanocellulose Composites with Polymers: A Guide for Choosing Partners and How to Incorporate Them Polymers (Basel) 10

[7] Afra E, Yousefi H, Hadilam M and Nishino T 2013 Comparative effect of mechanical beating and nanofibrillation of cellulose on paper properties made from bagasse and softwood pulps Carbohydrate polymers 97 725–30

[8] George Thomas M, Abraham E, Parameswaranpillai J, Pothan L, Maria H and Thomas S 2015 Nanocellulosates from jute fibers and their nanocomposites with natural rubber: Preparation and characterization International journal of biological macromolecules 81

[9] Chen C, Bu X, Feng Q and Li D 2018 Cellulose Nanofiber/Carbon Nanotube Conductive Nano-Network as a Reinforcement Template for Polydimethylsiloxane Nanocomposite Polymers 10 1000

[10] Ravindran L, M.s. S and Thomas S 2019 Novel processing parameters for the extraction of cellulose nanofibres (CNF) from environmentally benign pineapple leaf fibres (PALF): Structure-property relationships International Journal of Biological Macromolecules 131 858–70

[11] Pelissari F M, Sobral P J do A and Menegalli F C 2014 Isolation and characterization of cellulose nanofibers from banana peels Cellulose 21 417–32

[12] Daicho K, Saito T, Fujisawa S and Isogai A 2018 The Crystallinity of Nanocellulose: Dispersion-Induced Disordering of the Grain Boundary in Biologically Structured Cellulose ACS Appl. Nano Mater. 1 5774–85

[13] Xu X, Liu F, Jiang L, Zhu J Y, Haagens D and Wiesenberg D 2013 Cellulose Nanocrystals vs. Cellulose Nanofibrils: A Comparative Study on Their Microstructures and Effects as Polymer Reinforcing Agents ACS Applied Materials & Interfaces 6

[14] Wulandari W T, Rochliadi A and Arcana I M 2016 Nanocellulose prepared by acid hydrolysis of isolated cellulose from sugarcane bagasse IOP Conf. Ser.: Mater. Sci. Eng. 107 012045

[15] Liu Y and Kim H-J 2017 Fourier Transform Infrared Spectroscopy (FT-IR) and Simple Algorithm Analysis for Rapid and Non-Destructive Assessment of Developmental Cotton Fibers Sensors (Basel) 17

[16] Correia V da C, dos Santos V, Sain M, Santos S F, Leão A L and Savastano Junior H 2016 Grinding process for the production of nanofibrillated cellulose based on unbleached and bleached bamboo organosolv pulp Cellulose 23 2971–87

[17] Prado K S and Spinacé M A S 2019 Isolation and characterization of cellulose nanocrystals from pineapple crown waste and their potential uses International Journal of Biological Macromolecules 122 410–6