Crystallinity of nanocellulose isolated from the flower waste of pine tree (Pinus merkusii)

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Abstract. Pine flower is an agricultural waste that has high cellulose content. Cellulose is a major material for making nanocellulose. Nanocellulose has been isolated from the flower waste of the pine tree (Pinus merkusii). The process was initiated by delignification and bleaching process with sodium hydroxide 6% and sodium hypochlorite 6% followed by hydrolysis with acetic acid under stirring at 45°C for 1 hour. Three different concentrations of acetic acid (10%, 30%, and 60%) were studied toward nanocellulose crystallinity. Nanocellulose was characterized by FTIR, XRD spectroscopy, and TEM analysis. The result shows that the % yield of nanocellulose was 87.4%, 94.2%, and 91.8% respectively. Nanocellulose has high crystallinity as indicated by the crystallinity index. Besides, the nanocellulose width is 12.82–21.36 nm and the length is 267.12–326.87 nm.

1. Introduction

Pine flower is an agricultural and industrial waste that is rarely used [1]. Pine flower contain cellulose and secondary metabolites. The previous study shows secondary metabolites of pine flower can be extracted in water and used as a reagent for synthesis copper-nanoparticles [2]. On the other hand, the main content of pine flower is cellulose (±43%) [3]. Cellulose is a linear polymer consisting of β-D-glucose monomers. Each monomer linked with the β-(1,4)-glycosidic bond and the degree of polymerization is more than 20000 units [4].

Cellulose is a precursor to produce nanocellulose. Nanocellulose is a promising material as a polymer matrix nanocomposite produced from a natural biopolymer [5]. Nanocellulose has unique structural and remarkable physicochemical properties such as biocompatibility, biodegradability, renewability, adaptable surface chemistry, high surface area, and many hydroxyl groups contents for modification, also environmentally friendly [6-7]. Nanocellulose can be applied in many sectors such as in biomedical, pharmaceuticals, electronics, barrier films, nanocomposites, membranes, texturing agents in cosmetics, paper, and supercapacitors [8-9].

Nanocellulose can be obtained with various methods, such as hydrolysis with acid, oxidative hydrolysis, and hydrolysis with sonication assisted [10-11]. The most acid widely used for hydrolyzing cellulose is sulphuric acid, hydrochloric acid, and phosphoric acid [12]. Previously, Lusiana et al. [13] reported nanocellulose can be produced by hydrolyzing cellulose from pine flower using different concentrations of sulfuric acid at 45°C for 60 min. The sulfuric acid concentration affects the crystallinity and the particle size of nanocellulose. Sulfuric acid 10% and 30% obtain high crystallinity nanocellulose and the particle size is 15 nm wide and 185 nm length. However, sulfuric
acid 60% obtain amorphous nanocellulose. The strong acid is corrosive, toxic, non-environmentally friendly, and cause decomposition of cellulose. In this paper, we report hydrolysis cellulose with weaker acid (acetic acid) by conventional stirring to prevent cellulose decomposition and produced high crystallinity of nanocellulose. The purpose of this study is to determine the effect of cellulose hydrolysis from pine flower (Pinus merkusii Jungh et De Vrise) with acetic acid in various concentrations on its crystallinity.

2. Methods

2.1. Material and chemicals
Pine flower is (Pinus merkusii Jungh et De Vrise) from Batu, East Java, Indonesia. Chemicals used in this research are sodium hydroxide (Merck), sodium hypochlorite 12% w/v (technical grade), and acetic acid 99.7% (SMART-LAB).

2.2. Method

2.2.1. Sample preparation
Pine flower was washed thoroughly with water then soaked in hot water for 2 h before drying in an oven. The dried pine flower was ground using grinding mill. The powder of pine flower obtained was soaked in hot water for 4 h to remove the sap and other contaminants [3].

2.2.2. Cellulose isolation
Cellulose isolation followed the reported procedure [3, 10] with modification. The powder of pine flower was added with sodium hydroxide 6% at 70 °C for 4 h. The residue was washed until neutral pH with distilled water. The neutral residue was bleached with sodium hypochlorite 6 % at 70 °C for 2 h. The bleaching process was repeated until white cellulose obtained then, the cellulose washed until neutral pH with distilled water and dried in an oven.

2.2.3. Nanocellulose production
Nanocellulose production followed the reported procedure [3] with modification. Cellulose produced from step 2.2.2 was hydrolyzed with acetic acid 10%, 30% and 60% at 45°C for 60 min. The ratio of cellulose and acetic acid was 1:10 w/v. The cold water was added to the mixture to stop the reaction. Then, it was centrifugated to separate the acid and product. The % yield of nanocellulose was calculated by the equation below.

\[
\%\text{Yield} = \frac{\text{Sample weight after hydrolysis (g)}}{\text{Sample weight before hydrolysis (g)}} \times 100 \tag{1}
\]

2.3. Characterization

2.3.1. Fourier Transform Infrared (FTIR) analysis
The FTIR spectra of pine flower and nanocellulose were recorded with Fourier Transform Infrared Spectrometer SHIMADZU 8400s. The sample was crushed with potassium bromide then pressed until thin pellets were formed and measured in the wavenumber of range 4000-400 cm\(^{-1}\).
2.3.2. X-Ray Diffraction (XRD) analysis

The X-ray diffractogram of nanocellulose was collected by XRD PanAnalytical type E’xpert Pro at room temperature and ranging from 10° to 90° with Cu Kα radiation (1.5406 Å). The crystallinity index (CI) of nanocellulose was determined by Seghal’s method [14] as indicated below.

\[
CI = \left[ \frac{I_{002} - I_{amorph}}{I_{002}} \right] \times 100\%
\]

In equation 2, CI is the crystallinity index, \(I_{002}\) is the intensity of the 0 0 2 lattice diffraction at \(2\theta = 22.8°\), and \(I_{amorph}\) is the intensity of diffraction at \(2\theta = 18°\). \(I_{002}\) represents both crystalline and amorphous regions, while \(I_{amorph}\) represents the amorphous region.

2.3.3. TEM Analysis

The morphology and particle size of nanocellulose analyzed using a transmission electron microscope (TEM) HT7700 operated in 120 V. The sample is coated with carbon before analysis.

3. Result and discussion

Cellulose in the cell walls of pine flower was covered by lignin, hemicellulose, and other components such as waxes, pectin, and secondary metabolites [12]. The hot water pretreatment removes the dirt, waxes, and secondary metabolite in pine flower. The alkaline treatment with sodium hydroxide 6% can completely remove hemicellulose and pectin, while the bleaching process with sodium hypochlorite 6% can reduce lignin to a very small amount. The several treatments make the brown pine powder turn to yellowish-white, indicated more pure cellulose has been successfully isolated [3,10].

Cellulose isolated from pine flower was hydrolyzed with 10%, 30%, and 60% acetic acid, produced yellowish-white powder labeled AA 10, AA 30 and AA 60 showed in Figure 1. The % yield of AA 10, AA 30, and AA 60 is 87.4%, 94.2%, and 91.8% respectively (calculated from equation 1). The highest % yield was obtained from hydrolysis with acetic acid 30% (AA 30).

![Figure 1](image1.png)

**Figure 1.** The product of cellulose hydrolysis with acetic acid. AA 10 (a), AA 30 (b), and AA 60 (c).

Cellulose hydrolysis is affected by acid strength. Acetic acid as a Brownsted acid produced hydronium ion in aqueous media, it acts as a catalyst to hydrolyze the glycosidic bond in cellulose. Acetic acid can hydrolize cellulose to nanocellulose. The particle size (width and length) of nanocellulose is determined with the direct measurement from the TEM image (Figure 2). The width of three nanocellulose sample are around 12.82 – 21.36 nm and the length range are 267.12 – 326.87 nm (shown in Table 1).
Figure 2. The transmission electron microscope image of AA 10 (a), AA 30 (b), and AA 60 (c).

Figure 3 shows the infrared spectra of pine flower and all products of nanocellulose. All samples have similar peak patterns. The peak at 3100 - 3700 cm$^{-1}$ was identified as stretching vibration of O-H groups [12]. Stretching vibration of C-H cellulose was indicated by the peak at 2894 cm$^{-1}$ [3]. The peak at 1643 cm$^{-1}$ shows stretching vibration of C=C aromatics, indicated a small amount of lignin remained in nanocellulose sample [10]. The pine flower has a peak at 1700 cm$^{-1}$ indicating C=O stretching from hemicellulose [10]. After hot water pretreatment, alkaline treatment, bleaching and followed by hydrolysis with acetic acid, hemicellulose was not identified in AA 10, AA 30 and AA 60. Pyranose rings in the cellulose are shown by the peak at 1160-1027 cm$^{-1}$ (bending vibration of C-O-C) and the glycosidic bonds by the peak at 896 cm$^{-1}$ [3].

Figure 3. Fourier Transform Infrared (FTIR) spectra of pine flower, AA 10, AA 30, and AA 60.
The X-ray diffraction pattern of AA 10, AA 30, and AA 60 are shown in Figure 3. AA 10, AA 30, and AA 60 have similar diffractogram profiles. Three peaks at $2\theta = 16^\circ$ (plane 101), $22^\circ$ (plane 002), $34^\circ$ (plane 004) indicated cellulose [15,16].

The crystallinity index of AA 10, AA 30, and AA 60 were calculated using Seghal’s equation (equation 2). The crystallinity and crystallite size of AA 10, AA 30, and AA 60 are showed in Table 1. The highest crystallinity index obtained from AA 10 (cellulose hydrolyze with acetic acid 10%), whereas the lowest crystallinity index is AA 60 (cellulose hydrolyze with acetic acid 60%). The data show that the crystallinity index decreased while the concentration of acetic acid for hydrolyzed the cellulose increased.

The high crystallinity index of nanocellulose indicated that the amorphous region in cellulose has been reduced by hydrolysis procedures. The amorphous region is less stable than the crystalline regions, it can be dissolved after hydrolysis with acid [6]. Hydrolyzed with strong acid at a high concentration not only remove the amorphous region but also break the crystalline region on cellulose. The crystallinity index of cellulose isolated from pine flower hydrolyzed with acetic acid 60% (AA 60) is higher than cellulose hydrolyze with sulfuric acid 60%. Lusiana et al. reported nanocellulose produced from hydrolysis using 60% of sulfuric acid is amorphous because sulfuric acid makes the cellulose from pine flower decomposed [13].
4. Conclusion
This study concludes that the nanocellulose sample with AA 10 gave the best crystallinity index (CI) of 55.60%. The high concentration of acetic acid makes the crystallinity index of nanocellulose decrease.

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References
[1] Ayrilmis A Buyuksari U Avci E and Koc E 2009 Forest Ecol. Manag. 259 65–70
[2] Masruri M Pangestin D N Ulfa S M Riyanto S Srihardystutie A and Rahman M F 2018 IOP Conf. Ser.: Mater. Sci. Eng. 299 012072
[3] Rambabu N Panthapulakkal S Sain M and Dalai A K 2015 Ind. Crop. Prod. 10 1-9
[4] Malladi R Nagalakshmaiah N Robert M and Elkoun S 2018 ACS. Sus. Chem. Eng. DOI: 10.1021/acssuschemeng.7b03437
[5] Brinchi L Cotana F Fortunati E and Kenny J M 2013 Carb. Pol. 94 154-169
[6] Trache D Hussin M H Haafiz M K M and Thakur V K 2017 Nanoscale DOI:10.1039/c6nr09494e
[7] Phanthong P Reubroycharoen P Hao X Xu G Abudula A and Guan G 2018 Carbon Resour. Convers DOI: doi.org/10.1016/j.crcon.2018.05.004
[8] Islam M T Alam M M Torino P Patrucco A Montarsolo A and Zoccola M 2014 AATCC J. of Research. 1 5 17-23
[9] Li J Cha R Mou K Zhao K Long K Luo H Zhou F and Jiang X 2018 Advanced Healthcare Materials 71800334 Jhon Wiley and Sons Canada
[10] Liu C Lia B Duc H Lva D Zhang Y Yua G Mua X and Penga H 2016 Carb. Pol 151 716–724
[11] Hamid S B A, Zain S K, Das R and Centi G 2016 Carb. Pol 138 349-355
[12] Zhang P P Tong D S Lin C X, Yang H M Zhong Z K Yu W H Wang H and Zhou C H 2014 Asia-Pac. J. Chem. Eng. DOI: 10.1002/apj.1812
[13] Lusiana S E Srihardystutie A Masruri M 2019 J. Phys.: Conf. Ser. 1374 012023
[14] Segal L Creely J J Martin A E Jr and Conrad C M 1959 Tex. Res. J. 29 786–794
[15] Neto W P F Silvério H A Dantasb N O and Pasquinia D 2013 Ind. Crop. Prod. 42 480–488
[16] Henrique M A Silvério H A Neto W P F and Pasquini D 2013 J. Env.Man. 121 202-209