Morphological, Chemical, and Thermal Characteristics of Nanofibrillated Cellulose Isolated Using Chemo-mechanical Methods

Achmad Solikhin\textsuperscript{1,*}, Yusuf Sudo Hadi\textsuperscript{1}, Muh Yusram Massijaya\textsuperscript{1}, and Siti Nikmatin\textsuperscript{2}

1. Department of Forest Products, Faculty of Forestry, Institut Pertanian Bogor, Bogor 16680, Indonesia
2. Department of Physics, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor, Bogor 16680, Indonesia

*E-mail: achmad_solikhin@apps.ipb.ac.id

Received August 11, 2016 | Accepted December 30, 2016

Abstract

The objective of this research was to analyze the morphology, crystallinity, elemental components, and functional group changes, as well as thermal stability of nanofibrillated cellulose (NFC). Nanofibrillated cellulose has an irregular and aggregated shape with a diameter of about 100 nm. NFC self-aggregations were observed due to hydrogen bonding and Van-der Waals forces. The cellulose crystallinity index, atomic size, and polymorph of the NFC sample were found to be 63.57%, 2.2 nm, and cellulose I, respectively. The NFC sample was composed of various elemental components, such as C, O, Na, Al, Si, and K. IR analysis showed only small amounts of hemicellulose and lignin deposits, whereas cellulose functional groups appeared in several wavenumbers. Aromatic and oxygenated compounds, such as carboxylic acids, phenols, ketones, and aldehydes, were deposited as extractive on NFC; these compounds were associated with cellulose, hemicellulose, and lignin. The NFC thermal degradation process consisted of four steps: water evaporation (50-90 °C); hemicellulose degradation and glycosidic linkage cleavage (250-325 °C); amorphous cellulose and lignin degradation (325-429.29 °C); and cellulose crystalline degradation (above 429.29 °C).

Introduction

The utilization of cellulose nanofibers as a reinforcing agent has been tremendously interesting for versatile nanocomposite products in several industries. This is due to its excellent properties, such as the large and active surface area, high abundance, renewability, biodegradability, biocompatibility, low weight, high strength and stiffness, low thermal expansion, environmental friendliness, and low cost [1-4]. Different lignocellulosic wastes were used as the source of cellulose nanofibers, beneficial as nanocomposite reinforcing agents, such as...
oil palm empty fruit bunch [5], paper [6], cotton [7], flax [8], wood [9], wheat straw [10], sugarcane bagasse [11], and bamboo [12].

One of the forms of cellulose nanofibers is nanofibrillated cellulose (NFC), or microfibrillated cellulose (MFC), which can be isolated by mechanical methods, such as disk milling, high-pressure homogenization, microfluidizers, or sonication. The isolation of NFC has been reported in previous studies using different methods, such as high-pressure homogenization [13], ultra-fine grinder [4,14], disk milling [15], and ultrasonication [16]. To optimize the production of NFC, chemical pretreatment can be initially applied to obtain pure cellulose without deposited cementing agents (hemicellulose, pectin, extractives, and lignin). To obtain the purified cellulose, low molecular weight carbohydrate (hemicellulose and pectin) and extractives removal are indispensable. This process can be carried out, respectively, by hot water and organic solvent extraction [17,18], and enzyme or fungi retting method [19-21]. Certain lipophilic extractives associated with cellulose also contribute as inhibiting agents for cellulose purification, such as condensed tannin, fatty acid, wax, alcohol, sterol, glyceride, ketone, and other oxygen-containing compounds [22].

Besides those agents, recalcitrant amorphous lignin can be removed through delignification and bleaching process. Sodium hydroxide and sulfuric acid can be used for delignification and hemicellulose [23] removal, whereas sodium hypochlorite [24] and sodium chloride [25] are commonly used for the bleaching process of cellulose nanofibers. Sodium hydroxide is generally used for lignin and hemicellulose removal [26]. However, the acidified sodium chloride is not an eco-friendly reagent for delignification because it produces a chlorine radical (Cl•), which is toxic in nature. In addition, alkaline (KOH) and acid (HCl) can be used to remove lignin and hemicellulose [27, 28], and its combination can be used to remove silica before fabricating nanocellulose [29].

An eco-friendly chemical pretreatment used for delignification and bleaching requires the use of hydrogen peroxide and organic acids, such as acetic acid and formic acid. The utilization of these reagents has been reported by Nazir et al. [30], Rayung et al. [31], and Fatah et al. [5]. Hydrogen peroxide plays a role as an oxidizing bleaching agent that generates an obvious effect on the brightness of lignocellulose fibers, whereas formic acid is a less corrosive and more stable medium for monosaccharide than sulfuric acid. Besides the chemical pretreatment, ultrasonication is an effective mechanical method for synthesizing nanofibrillated cellulose with a diameter of between 5 nm and 15 nm [32-34]. High-intensity ultrasonication is also utilized with output power of between 1000 and 1200 W in the time range of 30-60 minutes [35,36]. Ultrasound energy induces cavitation, which can disrupt physical and chemical systems, and degrade polysaccharide linkages [2]. The disruption of polysaccharide linkages is brought about by sonochemistry energy with approximately 10–100 kJ/mol in which the energy is within the hydrogen bond energy scale [37].

From the above illustration, a combination method that includes eco-friendly chemical treatment, dry disk milling, high-speed centrifugation, and ultrasonication is an effective and promising method to isolate NFC. Dry disk milling pretreatment has not yet been utilized for isolating nanofibrillated cellulose but it has been used for obtaining microfibers before producing lignocellulose nanofibers [15,38,39]. In this study, sodium hydroxide, hydrogen peroxide, and formic acid were used as chemical purification agents for extracting cellulose prior to NFC isolation. After utilizing these reagents, centrifugation and ultrasonication will be harnessed to synthesize NFC. The resultant NFC was analyzed for its basic characteristics using SEM, TEM, PSA, XRD, EDS, FT-IR, GC-MS, and STA. The objective of the research was to analyze the morphology, crystallinity, elemental components, and functional group changes, as well as thermal stability.

Materials and Methods

Materials. Oil palm empty fruit bunch (OPEFB) fibers were taken from PT Perkebunan Kelapa Sawit Nusantara VIII, Bogor, West Java, Indonesia. Prior to utilization, the fibers were manually cut and separated into two major parts: spikelet and stalk. From these parts, the ratio of spikelet and stalk utilized for this study was 1:1 (wt%). The analytical grades of the chemicals were 99.8% ethanol, 100% acetone, 30% hydrogen peroxide, 98% formic acid, and sodium hydroxide (pellet), indirectly supplied from Merck KGaA, 64271 Darmstadt, Germany.

Preparation of dry disk milled OPEFB microfibers. OPEFB spikelet and stalk fibers were weighed at a ratio of 1:1 (wt%) and mixed. These mixed fibers were washed with water and detergent to leach impurities (oil, sand, soil, stones, etc.). They were subsequently air-dried under sunlight for 7 days. To easily obtain the micro-sized fibers of about 75 µm, the fibers were pretreated in a conventional oven (Memmert, Germany) at 100 °C for 15 min, and milled with a dry disk milling equipped with AC motor (Siemens TEC 112M, Germany) at 2895 rpm for 3-4 cycles [38]. The pulverized fibers were sieved with a 200-mesh (75 µm) test sieve analyis (ABM Test Sieve Analysis ASTM E:11, Indonesia).

Chemical treatment of OPEFB fibers. Since the extractives were dominantly deposited in the cell lumina [40] and cell walls [41] of the OPEFB fibers, the
obtained microfibers (20 g) were extracted using soxhlet in 300 mL of ethanol:acetonc at a ratio of 1:2 (v/v%) for 8 h. The extraction was intended to eliminate some lipophilic extractives (waxes, fatty acids, phenols, ketones, etc.) that might inhibit the isolation of cellulose [42,43]. This chemical method used for cellulose isolation was modified from Nazir et al. [30]. The mixtures of 5% of 100 mL sodium hydroxide and 5% of 100 mL hydrogen peroxide were used to remove lignin (delignification) encrusted in OPEFB microfibers (2 grams). Suspended OPEFB microfibers in the solution were then autoclaved (Autoclave ES-315 Tomy Kogyo Co Ltd, Japan) at 121ºC under a pressure of 1.5 bar for 1 h. The autoclaved microfibers were washed several times with deionized water until clear water was obtained. Another delignification process was carried out by immersing the microfibers in 10% hydrogen peroxide and 20% formic acid solution at a ratio of 1:1, and in a shaking wise bath (WSB-30, South Korea) at 85 ºC with a shaking rate of 75 rpm for 2 h. After the process, the microfibers were re-washed with deionized water. OPEFB microfibers were re-suspended in a mixture of 5% hydrogen peroxide and 5% sodium hydroxide, and heated in a shaking wise bath at 60 ºC with a shaking rate of 90 rpm for 90 min. Furthermore, the resultant cellulose derived from the OPEFB microfibers was re-washed with deionized water several times.

Mechanical method for NFC isolation. The obtained cellulose was centrifuged at high-speed for 20 min at 13000 rpm until supernatant and filtrate were completely separated. Cellulose supernatant (1% v/v) was diluted with deionized water (before ultrasonication). Ultrasonication (Ultrasonic Processor Cole Parmer Instrument, USA) process was undertaken in an ice water bath for 25 min with an amplitude of 40%, power of 130 Watt, and frequency of 20 kHz. The device was equipped with a 6 mm titanium probe as well as a tip and foot switch connector.

Characterizations Morphology and nanostructure analysis. External surface morphology and nanostructure of NFC were undertaken using a scanning electron microscope (JSM6510LV JEOL, Japan) and a transmission electron microscope (JEOL, Japan), respectively. For SEM, NFC was coated with gold using an autofine coater (JEOL JFC 1600, Japan) at an acceleration voltage of 10 kV with 900× magnification. A drop of diluted NFC suspension was deposited using a sputter coater (JEC 560, JOEL Japan) onto glow-discharged, 400-mesh carbon-coated TEM copper grid. From the analysis, the sample was not stained with uranyl acetate.

Particle size distribution. Particle size distribution of NFC was investigated with a particle size analyzer (VASCO FlexTM French) equipped with NanoQ software. In the investigation, the cumulants method was used over the statistical and Pade-Laplace methods. Prior to measurement, 1% (v/v) NFC supernatant was diluted with deionized water, and was analyzed in the operating system at pH 7.0 with a laser power of 100% at room temperature.

Crystallinity index and size. NFC crystallinity index (CrI) and atomic crystalline size (ACS), and cellulose polymorphs were analyzed using an X-ray diffraction analysis (MAXima X XRD-7000 Shimadzu, Japan). The instrument was also equipped with JCPDS ICDD Software 1997 to investigate the cellulose polymorph. The diffraction angle ranged from 20 = 10 ° to 20 = 40 ° at a speed of 2 °/min with monochromatic CuKα radiation (λ = 0.15418 nm) utilized as an initial measuring parameter. Crystallinity index (CrI) and size of the NFC samples were calculated based on Segal’s empirical method [44] and Scherrer Equation [45,46], respectively.

\[
CrI = \frac{IO_{2θ} - IO_{2θ}}{IO_{2θ}} \times 100
\]

(1)

where IO_{2θ} is the intensity value of crystalline cellulose (2θ = 22.2 °) and IO_m is the intensity value of amorphous cellulose (2θ = 17.2 °).

\[
D = \frac{\beta}{\cos \theta}
\]

(2)

where D is the atomic crystal size (nm), k is the medium form factor (0.94), λ is the X-ray radiation wavelength (1.5406 Å), β is the full width at half maximum (FWHM) of 002 reflection, and 0 is the highest peak in the diffraction angle.

Elemental components. The measurement of NFC elemental analysis was carried out using an energy dispersive x-ray spectroscopy (JEOL EDS, Tokyo, Japan) equipped with A ZAF Method Standardless Quantitative Analysis. The measurement was conducted at 10.0 kV accumulation voltage and 0-20 keV energy range along with the analysis of the microstructure of NFC using a scanning electron microscope (JSM6510 LV JEOL, Japan). X-ray probe was detected using a lithium-drifted silicon detector in a solid-state device.

Functional chemical groups. A Fourier transform – infrared spectroscopy (MB3000, ABB Canada) was used to analyze NFC chemical functional group changes. Wavenumber range used was between 4000 and 370 cm⁻¹ with KBr:NFC at a ratio of 1:1.

Bio-oil and extractives components. Extractives and chemical composition deposited on NFC was investigated by using a gas chromatography-mass spectrometry (GC-System 7890A/MS 5975 Agilent Technology, USA). The instrument was equipped with a medium polarity
capillary column (HP-5MS column (60 m × 0.25 mm) with a film thickness of 0.25 μm, Agilent)

with an Ultra High Purity helium flow rate of 1.0 mL/min. Diluted NFC (one microliter) dissolved in ethanol was injected using splitless mode (split ratio 10:1) with an injector temperature of 325 °C (100 °C for 0 min then 15 °C/min to 290 °C for 28 min), and a total runtime of 40.667 min. The scan mass range used was between 50 and 1000 m/z, and the potential of electron ionization was 70 eV, as well as solve ntim delay time at 6 min.

Thermal stability analysis. Thermal stability analysis of NFC was conducted using a simultaneous thermal analysis (PerkinElmer STA 6000, USA). In this study, the instrument was merged with TGA and DSC, and the acquired curve of the analysis was the change in weight and heat flow as a function of temperature. NFC sample (about 3.15 mg) was placed in an aluminum pan, and heated in the temperature range of 40-800 °C. The scanning rate of the measurement was 10 °C/min with a nitrogen purge gas at a scanning rate of 20 ml/min.

Results and Discussion

Morphology and nanostructure analysis. Figure 1 shows SEM and TEM images of NFC derived from OPEFB fibers. Generally, NFC fibers have different sizes ranging from micro-sized fibers (Figure 1a-b) to nano-sized fibers (Figure 1c). Due to the influence of oven drying on NFC (Figure 1a-b), NFC was self-aggregated into micro-sized fibers with irregular and uneven shapes. The process, known as hornification, occurs due to the strong interaction of hydrogen bonding and Van der Waals force. TEM photograph (Figure 1c) also depicts the aggregation of NFC dispersed in distilled water after the ultrasonication process. Some of the NFCs had irregular and aggregated shapes with diameters of about 100 nm. Previous studies also reported that the diameter and length of the NFCs varied depending on the isolation method and fiber sources [46,47].

To maintain good dispersion of NFC in the water, Mesquita et al. [9] recommended two indispensable strategies that involve the use of surfactants and chemical surface modification. In addition, the solvent exchange method was used to assist with freeze drying as it can prevent self-aggregation of NFC better than the oven-drying method. The OPEFB fibers were purified using eco-friendly chemical pretreatment that was effectively able to bleach and delignify some cementing agents, such as hemicellulose and lignin (Figure 1b). Some silica bodies disappeared because they were removed by dry disk milling and chemical treatment during NFC isolation. The pretreatment even produced a regular and smooth external surface of the fibers. In addition, the various sizes of the OPEFB fibers under 1 μm were successfully produced after chemical pre-treatment assisted with the auto-hydrolysis of autoclave.

The analysis referred to the cumulants method in which the Z-average (average mean particle diameter) of NFC sample was 245.08 nm ranging from 37.16 nm to 1778.75 nm. However, Dmean of NFC particle size distribution by number was 101.55 nm, and about 75% of the NFC particle size distribution was under 100 nm, ranging from 37.16 nm to 97.75 nm (Figure 1b). The alteration of NFC nano-sized fibers into microfibers during a PSA analysis was due to reversible self-aggregation. The aggregation occurs presumably because of the internanoparticle and water-nanoparticle interaction generated by hydrogen bonding and Van der Waals force [39].

Crystallinity index and size. X-ray diffraction was used to analyze the CRL and ACS of NFC sample in which JCPDS ICDD Software 1997 and JADE XRD Pattern Processing 1995-2016 were used to analyze the cellulose substance pattern and polymorph, respectively.
Morphological, Chemical, and Thermal Characteristics

Figure 2 depicts the NFC pattern of XRD with the highest peak at $2\theta = 22.21^\circ$. An initial peak ($2\theta = 19^\circ - 25^\circ$) and two small crystalline peaks ($2\theta = 14^\circ - 17.5^\circ$ and $26^\circ - 27^\circ$) indicate the improvement of NFC crystallinity. This phenomenon was similar in a previous study by Maiti et al. [7].

In addition, there was a noticeable increase of CrI derived from OPEFB raw materials and NFC, which were 41.4% [31] and 63.57%, respectively. The increase of CrI was presumably because of the removal of the amorphous region of cellulose and hemicellulose so it could enhance the CrI of NFC. In addition, the increase of CrI was due to the removal of silica bodies after dry disk milling [15, 38].

After the analyses using JCPDS ICDD Software 1997, NFC isolated with chemo-mechanical methods contains cellulose that consisted of pure galactose, xylose, glucose, arabinose, and polysaccharide phase (JCPDS Card No: 46-1932). From JADE XRD Pattern Processing 1995-2016, native cellulose ($C_6H_{12}O_6$)$_\times$ was still embedded in NFC with cellulose 1 polymorph. The polymorph was indicated with main XRD peaks at $2\theta = 15.66^\circ$, 16.48, 20.60, and 22.20°. Those peaks were in line with previous studies [15, 48, 49]. ACS of NFC was about 1.36 nm, which was smaller than that of a study by Deraman et al. [50] and Solikhin et al. [39], revealing that the raw OPEFB fibers were in the range of 2.42 and 4.69 nm.

**Elemental components.** Elemental components of NFC sample were analyzed using an energy dispersive x-ray that was used in conjunction with a scanning electron microscope (SEM). The result of this study shows that C (40.71%), O (29.66%), and N (28.69%) are the most dominant elemental components of NFC. Other predominant elemental components are Na, Al, Si, and K. These elements can be in the form of ash deposited on OPEFB fibers, which are essential for healthy plant growth. NFC extracted from OPEFB was a lignocellulose organic source so that C, O, and N were the initial elemental components. These components were composed chemical elements of NFC, including cellulose, hemicellulose, pectin, lignin, and extractives. However, those components were dominated by cellulose after delignification and bleaching processes were carried out. Besides these organic components, silica (Si) remained although chemical and mechanical treatments were given.

The existence of Si in NFC was presumably because the chemical pretreatment and mechanical method applied to the OPEFB fibers did not intensively damage the silica bodies. The presence of Si was imparted as a filler in the OPEFB fibers, and the removal of the component must be carried out by acid hydrolysis and ultrasonication as well as high-pressure steam [51].

**Functional chemical groups.** Figure 6 shows the FTIR spectrum of NFC, where the wavenumber of the transmittance band observed was in the range of 4000 cm$^{-1}$ and 390 cm$^{-1}$. From the analysis, the presence of cellulose was in the broad transmittance wavenumber of 3800-3000 cm$^{-1}$, indicating the O-H stretching vibration of cellulose hydroxyl groups and absorbed water [15, 39].
A peak at 2950 cm\(^{-1}\) was indicated with the presence of CH\(_2\) group of cellulose. The appearance of peak 2361 cm\(^{-1}\) was correlated with CO\(_2\) in which hygroscopic organic material and NFC were able to absorb CO\(_2\) and H\(_2\)O. The chemical interaction between moisture and NFC was associated with the presence of a transmittance peak at 1643 cm\(^{-1}\). A transmittance peak at 1520 cm\(^{-1}\) was attributable to lignin existence. In addition, a peak at 1744 cm\(^{-1}\) was designated to C=O acetyl group of hemicellulose or ester carbonyl groups of lignin [38].

**Thermal stability analysis.** Figure 7 and 8 show the graph of a simultaneous thermal analysis equipped with DSC and TGA of OPEFB NFC, respectively. From the thermograph (Figure 7), there are four steps of thermal degradation of NFC vapor and cementing agents. At the beginning of the decomposition, water evaporation (drying process) in the temperature ranged of 50 °C and 90 °C. The second decomposition was about 250 °C, while the maximum decomposing temperature was about 325 °C. These temperatures indicated the degradation of hemicellulose, amorphous cellulose, and cellulose glycosidic linkage breakage. Hemicellulose is easy to thermally degrade due to the presence of acetyl groups. Compared with the previous study [38], the lower decomposition of amorphous and glycosidic cellulose at 250 °C was due to the nano-size effect in terms of very small and uniform particle sizes (Z-average) as well as high surface-to-volume ratio [15, 39]. The lignin and crystalline cellulose region was depolymerized at a temperature of above 325 °C until it leveled off at 492.29 °C. At 492.29 °C (Delta H = 71.95 J/g), a melting point of NFC was at 498.84 °C (Delta H = 7.29 J/g). At the last degradation or melting temperature, crystalline cellulose was totally degraded.

From Figure 8, it can be observed that at the beginning of the evaporation phase, there was about 5% to 10% loss of NFC weight, which was in accordance with the loss of absorbed water in NFC. The highest loss (65-70%) of NFC weight occurred in the temperature range of 250 °C and 325 °C. A final NFC residue of 18% was attained at temperatures above 900 °C in which the residue was in the form of char.
Figure 6. Extractive Analysis and Abundance of NFC using GC-MS

Figure 7. Simultaneous Thermal Analysis – DSC Graph of OPEFB NFC

Figure 8. Simultaneous Thermal Analysis – TGA Graph of OPEFB NFC
Conclusion
Nanofibrillated cellulose (NFC) was successfully isolated by using mechanical methods assisted by eco-friendly chemical pretreatment. NFC has an irregular and aggregated shape with a diameter of about 100 nm. Based on the cumulants method, Z-average (average mean particle diameter) of NFC sample was 245.08 nm ranging from 37.16 nm and 1778.75 nm, whereas 75% Dmean of NFC particle size distribution by number was under 100 nm, ranging from 37.16 nm and 97.75 nm. The bigger size of NFC was presumably due to self-aggregation of NFC generated by hydrogen bonding and Van der Waals forces. Crystallinity index and size of NFC were 63.57% and 1.36 nm, respectively, with cellulose I polymorph. NFC belongs to native cellulose comprising of pure galactose, xylose, glucose, arabinose, and polysaccharide. C (40.71%), O (29.66%) and N (28.69%) were the most dominant elemental components, whereas Na, Al, Si, and K were predominant components of NFC. IR analysis showed only small amounts of hemicellulose and lignin were deposited on NFC due to the recalcitrant characteristics of these components, and several wavenumbers appeared, indicating the presence of cellulose chemical functional groups. Extractives were still imparted in NFC in the form of aromatic and oxygenated compounds, such as carboxylic acids, phenols, ketones and aldehydes. These extractives were also associated with the deposition of cementing agents of cellulose, hemicellulose, and lignin. There were four steps of NFC thermal degradation process, which were: water evaporation (50-90 °C), hemicellulose and amorphous cellulose degradation, and glycosidic linkage cleavage (250-325 °C), crystalline cellulose and lignin depolymerization (325-429.29 °C), and cellulose crystalline degradation (above 429.29 °C). The percentage of NFC residue was about 18% in the form of char.

Acknowledgements
The authors would like to thank the Doctoral Programme for Outstanding Undergraduate Students (PDSU) Secretariat, Directorate of Higher Education (DIKTI), Ministry of Research, Technology, and Higher Education of the Republic of Indonesia for its financial support (Grant No. 157/SPTi/PL/DirLitabmas/II/2015 February 5, 2015, PMDSU 2016-2017). We would also like to thank the laboratory staff at PT Perkebunan Kelapa Sawit Nusantara VIII, Indonesian Institute of Sciences, Forest Products Research and Development Centre, Institut Pertanian Bogor, Institut Teknologi Bandung, Universitas Gadjah Mada, and Universitas Indonesia for their assistance in this research.

References


