Isolation and Characterization of Nanocrystalline Cellulose from Sugar Palm Bunch (*Arenga pinnata* (Wurmb) *Merr*.)

Sumaiyah[1,], B. Wirjosentono[2], Karsono[1], M. P. Nasution[1], S. Gea[2]*

[1]Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Sumatera Utara Medan, Indonesia; sumaiyah7777@gmail.com
[2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Sumatera Utara Medan, Indonesia

Abstract. Nanocrystalline celluloses (NCCs) isolated from α-cellulose of sugar palm bunch (SPB) (*Arenga pinnata* (Wurmb) *Merr*.) by hydrolysis method using sulphuric acid with the fixed concentration of 54% have been done. The α-cellulose of SPBs put into flask containing 50 mL sulphuric acid and stirred by magnetic bar for 45 min. To remove solvent, the white suspension was carried out by centrifugation at 10,000 rpm for 15 min. The precipitate was dialyzed for 48 h. Characterization of NCCs was performed using Fourier transform infrared (FTIR) spectroscopy, transmission electron microscopy (TEM), X-ray diffraction (XRD) and thermogravimetric analysis (TGA) to functional group, morphology, structure crystalline and thermal degradation, respectively. FTIR Spectra indicated that absorption peak at 1022 cm^{-1} was derived from sulfate ester bond on the cellulosic chain and from TEM obtained that the NCC had nanoscale dimension with diameter less than 100 nm. XRD diffractogram showed enrichment in the proportion of crystalline cellulose in NCC. TGA showed that nanocrystalline cellulose degraded earlier than microcrystal cellulose but left higher residue.

Keywords: Nanocrystalline cellulose; α-cellulose; sugar palm bunches; sulphuric acid hydrolysis; characterization

Introduction

Cellulose is renewable and one of the most abundant bioploymer in the world. Production of cellulose in nature is about 100 billion tons annually. Pure cellulose is found in cotton seed hair, but usually it combines with lignin and other polysaccharides such as hemicellulose normally found in cell walls of woody plants, both in soft and hard wood. In addition, high purity cellulose is produced by culturing *Acetobacter xylinum* into glucose-based medium well-known as bacterial cellulose [1,2]. This compound is also found in single-celled plankton or algae in the oceans as well as fungi and bacteria [3,4]. As a chemical raw material, cellulose has been used in fiber form or its derivatives for about 150 years [5].

Cellulose is a relatively stable polymer. It is because the hydrogen bonds that are not soluble in aqueous solvent and does not have melting point. Also, cellulose fibers have good flexibility and elasticity. These properties lead cellulose to maintain a high aspect ratio in the manufacturing process [6].

In recent years cellulose is developed in nanocrystalline. NCC is a reinforcement in nanocomposite materials with low density (1.6 g/ml) and high mechanical strength (10 GPa). This material can also be used in the manufacture of biomedical devices, implants, textiles [6], additives in food and cosmetics [7], and drug delivery in the form of liposomes, micelles, microgel, carriers in the targetted delivery [8]. There are some important factors to consider in determining quality of the nanocrystalline cellulose, *i.e.* diameter, aspect ratio, shape and surface properties. A very large surface area is an advantage of cellulose nanocrystal which allows drug to bind and interact with the surface [9].

NCCs could be prepared from cellulose fibers or microcrystalline celluloses (MCCs). At the stage of hydrolysis, there will be wide degradation of cellulose structure and remove the amorphous structure, and crystalline is obtained. The isolation process of NCCs either from fiber cellulose or MCCs by acid hydrolysis involves few steps namely centrifuge, dialysis and ultrasonic treatment [9,10] or homogenize with high pressure [11].

Bunches of Palmae plants generally have a high fiber content in addition to other substances. As in the palm empty fruit bunches (EFBs) that have high fiber content in the base and edges are hard and rigid, i.e. 72.67% on the base and 62.47% at the end [12]. Unlike the corn cob fiber content is only 34.02% [13]. Fiber or cellulose consists of crystalline and amorphous. Crystalline of cellulose causes fibers become hard and stiff [12].

SPBs are kind of organic solid waste from kolang-kaling home industry in North Sumatera, Indonesia. EFBs of sugar palm were allowed to dry and only used as firewood. In this study, the bunches could be a source of cellulose by delignification process we isolated NCC from α-cellulose of SPB and investigated the characterization.

Experimental

Materials

SPBs were obtained from kolang-kaling home industry in Langkat, North Sumatera (Indonesia). Avicel® (MCC as a comparison) were purchased from Kimia Farma Industry. Dialysis tubing with a molecular weight cut off (MWCO) of 12,000-14,000 was purchased

from Fisher Scientific (Japan). All reagents used were analytic grade.

Isolation α-cellulose of SPB

SPB was chopped into small pieces, dried, and crushed (SPB powder). The amount of 75 g treated SPB with 1 L of 3.5% nitric acid containing 10 mg of sodium nitrite for 2 h in a beaker glass immersed in a water bath at 90°C. Followed by washing and filtrating, it digested with a 750 ml solution contain of 2% w/v each of sodium hydroxide and sodium sulfite at 50°C for 1 h. Washing and filtrating were repeated until pH of the filtrate to be neutral. Then it bleached with 250 ml of 1.75% sodium hypochlorite solution at boiling temperature for 0.5 h. After washing and filtrating were done, material was treated with 500 ml 17.5% sodium hydroxide solution for 0.5 h at 80°C. The obtained α-cellulose was washed by using tap water. The final step was whitened using 10% hydrogen peroxide solution at a temperature of 60°C for 5 min and washed with distilled water subsequently until the filtrate to be clear. The cellulose material filtered and dried at 60°C in an oven for 1 h, and used for further investigation [14].

Preparation of NCCs

In a chamber that contained 250 ml of 54% sulfuric acid solution was added 12,5 g of α-cellulose SPB and stirred at high speed for 45 min at 45°C. The white suspension was diluted with distilled water and allowed to settle overnight. The clear top layer was decanted and the remain of white cloudy layer was washed with distilled water and centrifuged several times to remove solvent and other organics soluble. The white precipitation was diluted with distilled water and was dialyzed using activated dialysis tubing with a 12,000–14,000 molecular weight cutoff (MWCO) for 48 h. The suspension from the dialysis tubes dispersed by ultrasonication for 10 min at 60% power. NCCs were dried in vacuum oven at 25°C and used for characterization.

Characterization of NCCs

The dried NCCs sample were mixed with KBr powder and examined with a FTIR (Shimadzu-IR Prestige 21) unit with the scanning region of 4000-500 cm^{-1} at 16 cm^{-1} resolution and averaging of 45 scans. The actual size of NCCs was observed by using TEM. Sample for TEM was dripped with 1% ammonium molibdat, then embedded in polymethylmethacrylate (PMMA) resin. Ultra thin sections of NCCs was sectioned using an ultramicrotom fitted with a sharp diamond knife. The sections were mounted on coated carbon grids and examined with a LEICA EM UC7-NT TEM. Wide-angle X-ray diffraction data were collected using a Shimadzu XRD-7000 X-ray diffractometer MAXima equipped with Cu K_ radiation at 40 kV and 30 mA to investigate the XRD

spectra of the cellulosic sample. Scattered radiation was detected in the range 2θ = 10-40 at a speed of 2^{o} min^{-1}. Moreover, the thermal degradation behaviors of NCC and Avicel were carried out using TGA (DTG-60/60H Shimadzu) with a $20^{\circ}C$ min^{-1} heating rate, and a temperature range of 40-800°C, in a nitrogen atmosphere with gas flow rate 15 ml min^{-1}.

Results and Discussion

Results of NCC of SPB preparation

NCCs have been prepared from α-cellulose of sugar palm bunches (Fig. 1a) using sulfuric acid with concentration of 54%. The yield of NCC SPB (Fig. 1b) is about 68 – 74.4%.

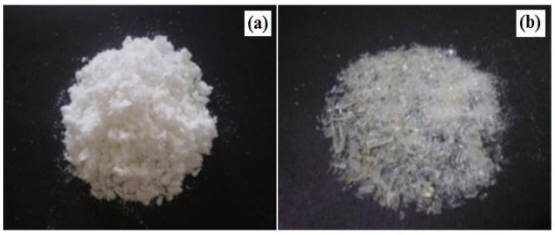

Figure 1. α-cellulose (a) and nanocrystalline cellulose (b) of sugar palm bunches.

Hydrolysis of cellulose is affected by the concentration of sulfuric acid. The amorphous parts of cellulose is preferred to undergo acid hydrolysis rather than the crystalline. Part Amorphous cellulose part is easier to be hydrolyzed compare to the crystalline. Treatment of cellulose with sulfuric acid involves esterification of the hydroxyl groups by sulfate ions as shown in Fig. 2. The introduction of sulfate groups on the surface of the crystalline will produce a negative charge on its surface when the pH increases. Anionic stabilization through the repelling force of the electric double layer on the crystal is a possible reason for the stability of the crystalline suspension [15]. This process resulted in a reduction of long-chain α-cellulose, from α-cellulose to microcrystlalline cellulose then nanocrystalline cellulose [16].

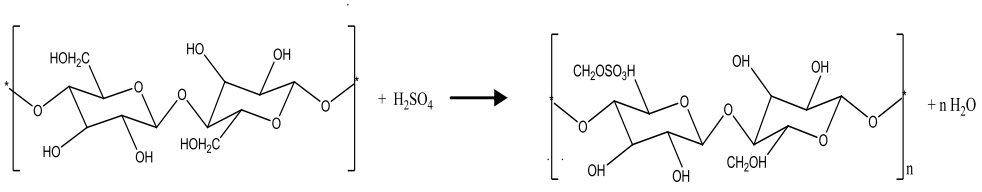

Figure 2. Esterification of hydroxyl groups by sulfate ions from sulfuric acid treatment of cellulose [15].

Beside the effect of concentration, time and temperature of hydrolysis are other important factor for the isolation process of NCCs. It has been reported that the increased time

also increased the surface charge and the number of sulfate groups [15]. After the hydrolysis step, the excess of the sulfuric acid in the suspension has to be removed by centrifugation. During centrifugation process, the sediment was kept and supernatant was separated. The precipitate was mixed with distilled water and centrifuged. This procedure was repeated until the pH 5-7 obtained. Then the suspension was dialyzed against distilled water in 48 hours using dialysis membranes that have been activated. Activation was done by immersing the membrane in distilled water for 20 min then rinsed with clean distilled water. Activation of membrane was made to open the pores which were present in the membrane. The purpose of dialysis is a process for separating nanometer sized particles of non-nanometer sized particles. Nanoparticles will diffuse out of the membrane, whereas the non nanometer-sized will remain in the membrane. For further dispersion, the particles in suspension form was treated by ultrasonic for few minutes. The ultrasonic treatment can be carried out in an ice bath to prevent overheating which might cause desulfation of the sulfate groups on the surface of the crystallines [15,17].

Characterization of NCC

Analysis of functional groups

Analysis of functional groups of NCC performed by FTIR and compared with microcrystalline cellulose Avicel. Spectra of the sample wave numbers were in the range 4000-500 cm^{-1}. Avicel and NCC of SPB had similar spectra as shown in Fig. 3. The peaks were at 3441, 2893, 1624 and 1022 cm^{-1} indicating the presence of OH groups, alkane CH, OH from water absorption, and CO (glycosidic bond) between glucose units in cellulose[2]. Hydrolysis treatment with H_2SO_4 did not alter the functional groups of cellulose, but only discontinued the cellulose chain.

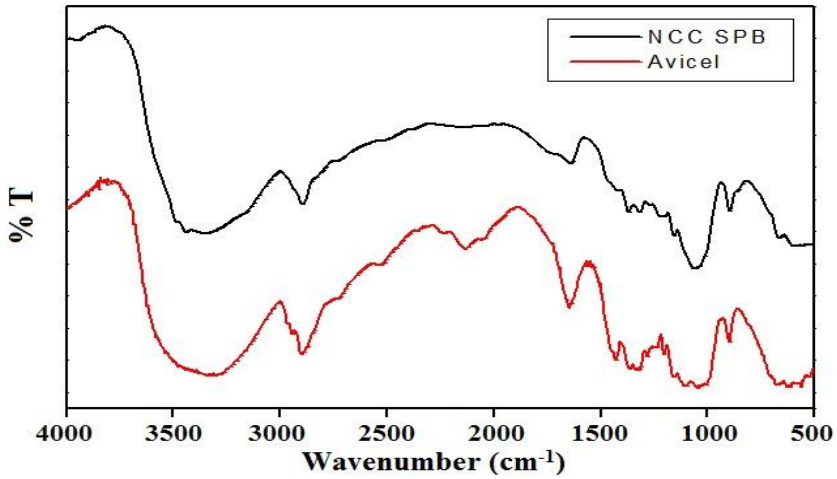

Figure 3. Infrared spectra of NCC SPB and Avicel.

Analysis morphology

Morphological analysis of NCCs were resulted using TEM. As shown in Fig. 4 that the particles were mutually single crystalline separated from another with average diameter of 15-20 nm. The TEM image showed that NCCs obtained from SPB, because the particle sizes were in the range from 0.2 to 100 nm [18]. NCC of SPB forms obtained were spherical. It was different with the NCC obtained from sisal and bamboo that had needle or rod shapes [5,19].

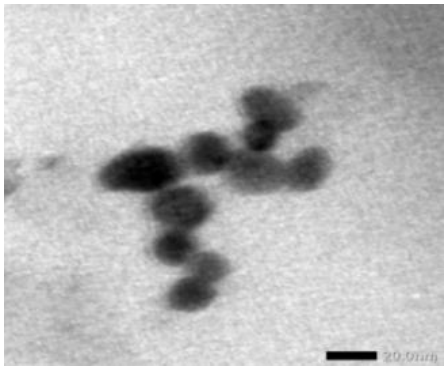

Figure 4. TEM image of NCC SPB.

Analysis structure cellulose

The structure and the degree of crystallinity both NCCs SPB and Avicel were analyzed by X-ray diffractometry. Fig. 5 showed that degree of crystallinity of NCCs of SPB (97,57%) is higher than Avicel's (82,77%) as indicated by the sharp peaks of the spectra of the sample NCC. At the stage of the isolation process α-cellulose from SPB, NaOH at 17.5% concentration used for the purification of α-cellulose, because α-cellulose is insoluble in NaOH 17.5%, while the β-cellulose and γ-cellulose will dissolve. Treatment with NaOH on cellulose changed the structure of cellulose from cellulose I to cellulose II[2]. In Fig. 5, the peaks of the spectra Avicel were at $2\theta = 14.56^{o}$, 20.14^{o}, and 22.2^{o}. This suggests that an Avicel was cellulose I. Peaks in the spectra of NCC of SPB were at 11.84^{o}, 19.76^{o}, and 21.82^{o}. This suggests that the NCC of SPB was cellulose II [2,4,20].

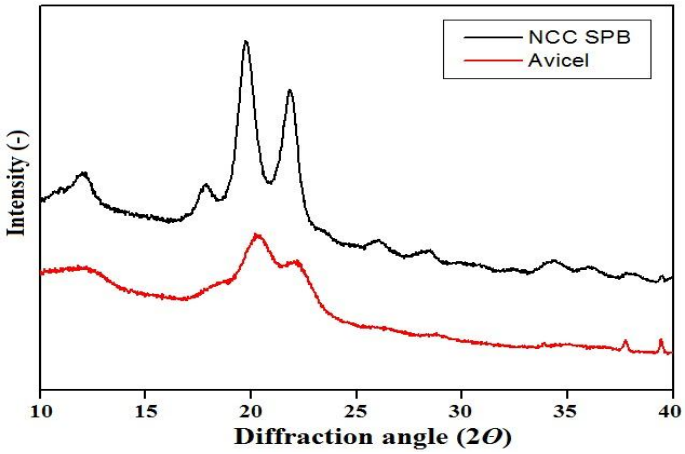

Figure 5. X-ray diffraction pattern for Avicel and NCC of SPB.

Thermal stability

Thermal gravimetric analysis (TGA) was performed to characterize changes due to heating and to determine the phase change due to the decomposition process. In Fig. 6 can be seen that thermogram mass change of the samples during heating temperature 0°C to 800°C. First mass loss occured at temperatures below 100°C for Avicel and NCC of SPB. This decrease was caused by the evaporation of water from the samples that are hydrophilic.

TGA thermogram showed that Avicel degraded at temperatures of 317°C, and NCC of SPB 173°C. The residue mass of Avicel and NCC of SPB were 5.4% and 11.25%, respectively at 800°C. TGA thermograms showed that NCC degraded faster than Avicel, but left more residue. Presence of sulfate groups on the cellulose caused cellulose degradation at low temperature [21]. Smaller particle size of NCC caused more free chain ends. The end chains started decomposition at lower temperature and causing an increase in the char yield of this hydrolyzed sample [22].

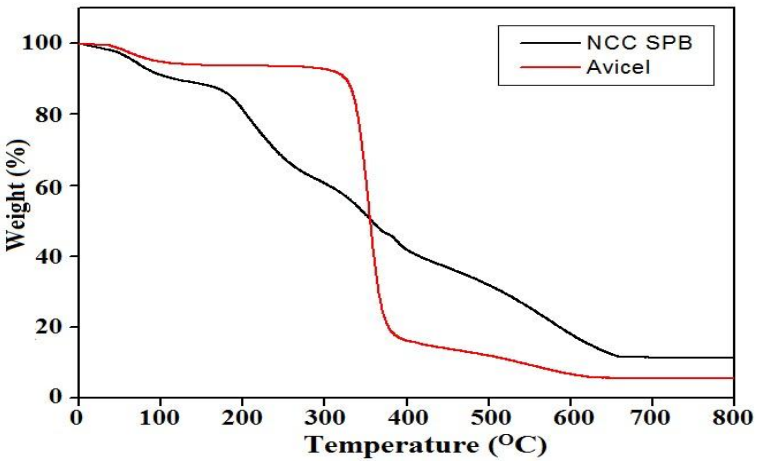

Figure 6. TGA thermograms of NCC of SPB and Avicel at a heating rate 20°C min^{-1} heating rate and a temperature range of 0-800°C.

Conclusion

The NCC isolated from sugar palm bunches by hydrolysis method using sulfuric acid with the fixed concentration of 54%. Spectra of both NCC and MCC are comparable. From TEM image can be concluded that the dimension of NCC of SPB is nanosize with the has spherical shape. XRD diffractogram showed that the type of NCC is cellulose II and containing higher crystalline cellulose compared to Avicel. From TGA data confirmed that NCC started to degrade earlier than MCC but left higher residue.

Acknowledgments

Sumaiyah would like to acknowledge home industry of kolang-kaling in Langkat, North Sumatera, Medan, for providing SPB sample.

[1] D. Klemm, B. Philipp, T. Heinze, U. Heinze, W. Wagenknecht, "*Comprehensive Cellulose Chemistry, Volume 1:Fundamentals and Analytical Methods*", Wiley-VCH Verlag GmbH, Jerman **1998**.
[2] S. Gea, C.T. Reynolds, N. Roohpour, B. Wirjosentono, N. Soykeabkaew, E. Bilorti, T. Peijs, *Bioresource Technol.* **2011**, *102*, 9105.
[3] A. Potthast, T. Rosenau, P. Kosma, *Adv. Polym. Sci.* **2006**, *205*, 1.
[4] P. Zugenmaier, "*Crystalline Cellulose and Derivatives"* 2008, Heidelberg, Springer Verlag, p.7-8.
[5] Y. Habibi, L.A. Lucia, O.J. Rojas, *Chem Rev*. **2010**, *110*, 3479.
[6] E. Benavides, "Cellulose *Nanocrystals Properties and Applications in Renewable Nanocomposites*", Clemson University, USA, 2011.
[7] Y. Boluk, "*Nanocellulose An Investigative Summary, Impact of Nanotechnology In Alberta Thecis Nanotechnology and Bio-Industries Workshop*", 2010.
[8] B.L. Peng, N. Dhar, H.L. Liu, K.C. Tam, *T. Can. J. Chem. Eng.* **2011**, *9999*, 1.
[9] J.K. Jackson, K. Letchford, B.Z. Wasserman, et al., *Int. J. Nanomedi*. **2011**, *6*, 321-330.
[10] K. Oksman, A. Mathew, "*Processing and Properties of Nanocomposites Based on Cellulose Whiskers, 9th International Conference on Wood and Biofiber Plastic Composites*" 2007.
[11] H. Liu, D. Liu, F. Yao, Q. Wu, *Biosource Technol*. **2010**, *101*, 5685.
[12] G.P. Darnoko, A. Sugiharto, S. Sugerti, *Proceding Oil Palm Trunk and Other Palm Wood* **1995**, 83.
[13] Y. Darliah, *Script* **2008**, 2.
[14] F.O. Ohwoavworhua, T.A. Adelakun, *Trop. J. Pharm. Res.* **2005**, *2*, 509.
[15] D. Bondeson, I. Kvein, K. Oksman, *ACS Symposium* **2005**, *938*, 12.
[16] D.N.S. Hon, S. Nobuo, *Wood and Cellulosic Chemistry*, Marcel Dekker, Inc., New York 1991, p.997 .
[17] X.M. Dong, J.F. Revol, D.G. Gray, *Cellulosa* **1998**, *5*, 19.
[18] D. Gardner, *J. Adhes. Sci. Technol*. **2008**, *22*, 545.
[19] M. Yu, R. Yang, L. Huang, X. Cao, F. Yang, D. Liu, *Bioresource* **2012**, *7*, 1802.
[20] G.K. Li, X.F. Li, Y. Jiang, M.Z. Zeng, E.Y. Ding, *Chinese Chem. Lett.* **2003**, *9*, 977.
[21] Z. Man, M. Nawshad, S. Ariyanti, A. Mohammad, K. Vignesh, R. Sikander, *J. Polym. Environ.* **2011**, *19*, 726.
[22] P. Krishnamachari, R. Hashaikeh, M. Chiesa, K.R.M. Gad El Rab, *Cell. Chem. Technol.* **2011**, *46*, 13.