EFFECT OF HYDROLYSIS TIME ON PROPERTIES OF CELLULOSE NANOCRYSTALS FROM OIL PALM FRONDS

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ABSTRACT

The oil palm fronds (OPF) are available throughout the year in Malaysia. Since they are abundant and highly fibrous in nature, the OPF are suitable to be used as a starting material for the production of cellulose nanocrystals (CNC). Therefore, this study investigated the properties of cellulose nanocrystals isolated from oil palm fronds by using chemo-mechanical treatment. The OPF were hydrolysed using 64% (v/v) sulphuric acid for 45, 60 and 75 min. The CNC samples were denoted as CNC-45, CNC-60 and CNC-75 according to the hydrolysis time subjected to the samples during the treatment respectively. The properties of the CNC were characterized using thermogravimetric analyser (TGA), x-ray diffraction (XRD), transmission electron microscope (TEM) and fourier transform infrared spectroscopy (FTIR). The results showed that CNC that underwent acid hydrolysis for 60 min had the best thermal property based on the onset temperature of 308.4 °C in comparison with CNC-45 and CNC-60. Meanwhile, CNC-60 had the highest crystallinity index which was 65.8% compared to CNC-45 (63.1 %) and CNC-75 (63.8 %) respectively. The TEM picture confirmed the size of the CNC by having dimension of 8.94 nm in width and 498.15 nm in length. The FTIR spectroscopy of the samples were almost similar to one another because there was no chemical changes between the three samples. The findings indicated that the CNC which was hydrolysed with 64 % (v/v) sulphuric acid for 60 min had the best thermal property and crystallinity index. The CNC produced in this work can be used for various applications such as for tissue engineering, drug delivery, wound healing as well as for packaging purposes.

Keywords: Acid hydrolysis; cellulose nanocrystals; oil palm fronds.

INTRODUCTION

As a major contributor to agricultural waste in Malaysia, oil palm biomass has gained attention in the field of research to be utilized for some beneficial end product rather than being left in the plantation area without proper management. The oil palm plantation area in Malaysia has mounted to 5.74 million hectares making it the second largest oil palm plantation in the world [1]. The oil palm biomass is usually in the form of fronds, trunks, leaves, empty fruit bunches (EFB) and mesocarp fibres and the oil palm fronds (OPF) contribute to about 47 % out of the total biomass. The OPF are obtained during pruning when harvesting fresh fruit bunch (FFB), therefore they are available daily throughout the year [2]. Their abundance finally created environmental hazard as the fronds were left in the plantation area for soil conservation. Since the OPF contain high percentage of cellulose, they have become one of the potential materials for the production of cellulose nanocrystals (CNC).

In these recent years, the production of CNC from different types of materials have been carried out aiming for different purposes. Cellulose is one of the ubiquitous natural biopolymers available in nature. Major sources of celluloses are trees, which comprise around 42-45 % of dry weight of cellulose. Other than that, bacteria and algae also generate celluloses but not as much as can be found in trees [3]. Cellulose is a linear molecule consisting of glucose residues attached by the β-1,4 linkages to form a long chain. Cellulose has gained attention in various researches due to its renewability, biodegradability, good mechanical strength and non-toxicity. The CNC can be isolated using mechanical, chemical and enzymatic treatment. There are pros and cons for each type of treatment that would lead to different properties of the CNC. The most effective way to remove hemicellulose and lignin is by using acid hydrolysis and sulfuric acid was chosen to be used in this work. Acid hydrolysis of cellulose is a well-known process used to remove amorphous regions and enable isolation of crystallites. Disordered or para-crystalline regions of cellulose are preferentially hydrolyzed, whereas the crystalline regions with higher resistance to the acid attack remain intact. Sulfuric acid hydrolysis of native cellulose fibres would cause a breakdown of the cellulose structure into rod-like fragments [4].

During acid hydrolysis treatment, the sulfuric acid would attack the amorphous structure of cellulose resulting in a shorter and smaller cellulose fragments. The sulfate groups attached on the cellulose structure caused by esterification of free hydroxyl ensued a stable cellulose suspension [5]. There are four factors to be considered during acid hydrolysis; acid concentration, hydrolysis time, hydrolysis temperature and acid to fibre ratio. The right acid hydrolysis condition would result in the improvement of CNC properties while too harsh treatment would result in degradation of cellulose structure. These parameters would affect the physical and chemical properties of the CNC. Therefore, this study was aimed to investigate the morphology, thermal properties and crystallinity of CNC isolated from OPF at different hydrolysis time.

MATERIALS AND METHODS
Materials

The OPF were obtained from oil palm plantation in Kuala Selangor, Selangor, Malaysia. The fronds were debarked and cut to small pieces prior to drying in the oven at a temperature of 50 °C for 24 h to avoid fungal attack. After drying, the fronds were ground and sieved using a 70 mesh. The samples that passed through the sieved were used for the isolation of cellulose nanocrystals.

Extraction of Raw Oil Palm Frond

A total of 80 g of OPF samples were used for the extraction to remove extractives using the soxhlet extractor. A mixture

of ethanol and toluene were used in the ratio of 2:1. The extraction was done for 6 h at a temperature of 60 °C ± 5 until the colour of the solvent became clear. The extracted samples were washed with distilled water prior to drying. The samples were then dried in the oven of 50 °C before being further used [6].

Isolation of Cellulose Nanocrystals
Twenty grams of extractive free OPF samples were used for the isolation. They were soaked in sodium chlorite ($NaClO_2$) and 10 % (v/v) acetic acid alternately for four times, at a temperature of 70 °C to remove lignin. The bleaching treatment were done for 4 h and the bleached samples were subsequently washed with distilled water. Removal of hemicellulose was carried out by soaking the samples in 6 % of potassium hydroxide (KOH) solution under 20 °C for 24 h. After that, the fibres were washed with distilled water until it reached pH 7. The suspensions were subjected to acid hydrolysis with the ratio of fibre to acid 1:10 by adding 200 ml of 64 % (v/v) sulphuric acid at 45 °C under strong agitation for 45, 60 and 75 min. The hydrolysis was terminated by adding 400 ml of cold distilled water to the solution. The precipitate was resuspended in water and centrifuged at 10000 rpm to remove the excess water. The suspensions were neutralized using dialysis tube to obtain suspension of CNC at pH 6. The acid hydrolysed samples were homogenized, sonicated and freeze dried before they were ready to be used for analysis [7].

Transmission Electron Microscope Study
The structure and size of the CNC were observed by transmission electron microscopy using a Philips CM 12 electron microscope. A drop of diluted CNC suspension was deposited on a carbon-coated grid and allowed to dry at room temperature. The diameter of the fibres were measured manually using an image analyser program, XL Docu. A total of 10 single fibres of each sample were measured, and the result was calculated as the mean value of the data from each set of measurements.

X-ray Diffraction Analysis
The X-ray diffraction analysis was performed to check the crystallinity index of the CNC. Structural and phase analyses of the samples were measured by using an X-ray diffractometer, Bruker Advance 8 with Cu Kα radiation (wavelength of 1.5406 Å) generated at an operating voltage and current of 40 kV and 30 mA, respectively. The CuKα radiation was filtered electronically with a Ni-filter. A 2θ angle range from 5° to 60° in reflection mode was scanned at 2°/min. The crystallinity index was calculated based on the following equation 1:

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

where I_{200} is the peak intensity corresponding to crystalline and I_{am} is the peak intensity of the amorphous fraction [8].

Thermal Gravimetric Analysis
Thermogravimetric analysis was performed to determine the thermal properties of the CNC samples hydrolysed at different hydrolysis time. The thermal stability data were collected on a Perkin Elmer TGA 7 thermogravimetric analyzer under linear temperature conditions. The temperature was swept from 50 °C to 800 °C for samples of 10-15 mg placed in an aluminium pan at a heating rate of 10 °C/min under nitrogen atmosphere.

RESULTS AND DISCUSSION
The structure of nanocrystals was observed using transmission electron microscope. Cellulose nanocrystals have the dimension of 7-20 nm in width and length up to several micrometers [9]. The TEM analysis was not conducted for all samples because typically the CNC would have the same morphology and possessed a little difference in its dimension. The structure of CNC in Figure 1 showed that acid hydrolysis subjected to the OPF fibres managed to remove impurities as well as hemicellulose and lignin, which finally resulted in having individual nanocrystals. It can be observed that the CNC were scattered and did not agglomerate. The good dispersion of CNC can be attributed to the sulfate groups attached on the CNC that causes repulsion between the nanocrystals and thus prevented agglomeration to occur. The average dimension of CNC hydrolysed at 75 min was 8.94 nm in width and 498.15 nm in length. Based on the dimension, it fits the category of cellulose nanocrystals.
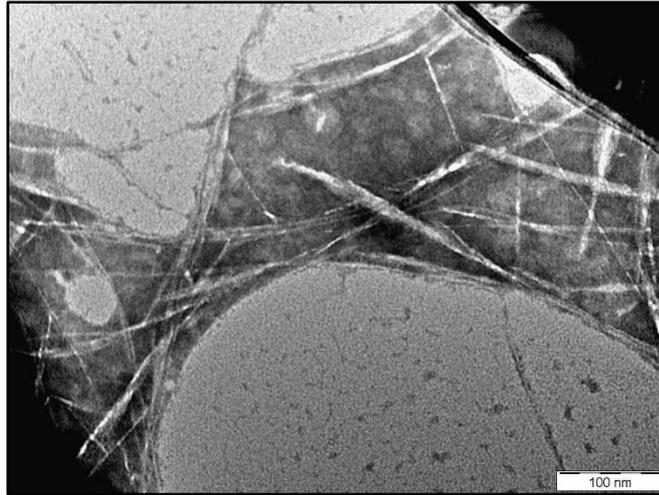
Figure 1. Transmission electron microscopy of oil palm fronds cellulose nanocrystals hydroysed for 75 min.

Thermal properties of CNC was observed by thermogravimetric analysis. The TGA graph in Figure 2 showed decomposition of samples at elevated temperature up to 800 °C. It can be seen that CNC-60 had the highest onset temperature which was 308.4 °C while the initial degradation temperature for CNC-45 was 290.7 °C and 252.8 °C for CNC-75. The reason for variation in the onset temperature was postulated due to the lignocellulosic components in the samples.
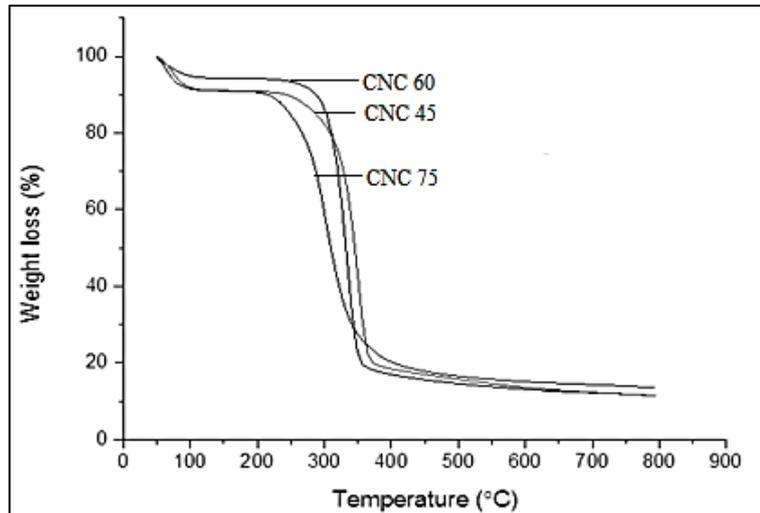
Figure 2. Thermogravimetric analysis of oil palm fronds cellulose nanocrystals at different hydrolysis time
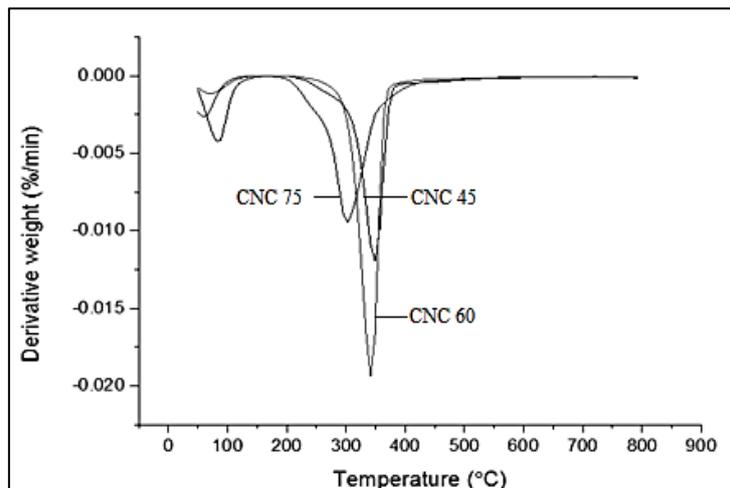
Figure 3. Derivative weight of oil palm fronds cellulose nanocrystals at different hydrolysis time.

Generally, hemicellulose and lignin started to degrade at temperature of 150° C – 250 °C while cellulose started to degrade between 300 - 350 °C [10]. The derivative thermogravimetric (DTG) curves in Fig. 3 represents the rate of change

mass. The sharp peak at temperature between 250 – 350 °C confirmed the degradation of cellulose crystalline structure for all three samples. It can be concluded that CNC-45 started to degrade at lower temperature because it contains hemicellulose and lignin, which indicated that 45 min of hydrolysis time was still inadequate to remove those materials. Meanwhile for CNC-75, it had the lowest temperature because longer hydrolysis time had led to the partial degradation of cellulose crystalline structure thus caused a significant decrease in its thermal property. Overall TGA results showed that 60 min of acid hydrolysis time was sufficient to remove hemicellulose and lignin without diminishing the cellulose structure.

The crystallinity index of the CNC showed variations in accordance to its hydrolysis time. As can be seen in Figure 4, the diffractogram was presumed to represent typical cellulose I because it showed a single peak at $2\theta = 22°$ and a shoulder in the region $2\theta = 19°$ [11]. The crystallinity index improved from CNC-45 (63.1%) to CNC-60 (65.8%) and showed a decrease for CNC-75 (63.8%). A significant decrease can be observed in CNC-75 indicating the possibility of the reaction to progress from amorphous region to the crystalline domains during acid hydrolysis treatment. Generally, celluloses are surrounded by non-celluloses polysaccharides such as hemicelluloses and lignin matrix which were amorphous in nature. During acid hydrolysis treatment, strong acid would attack the amorphous structure, causing cleavage of glycosidic bonds thus leaving the shorter chain of crystalline cellulose structure. Based on the diffractogram in Figure 4, the crystallinity index decreased for CNC-75.
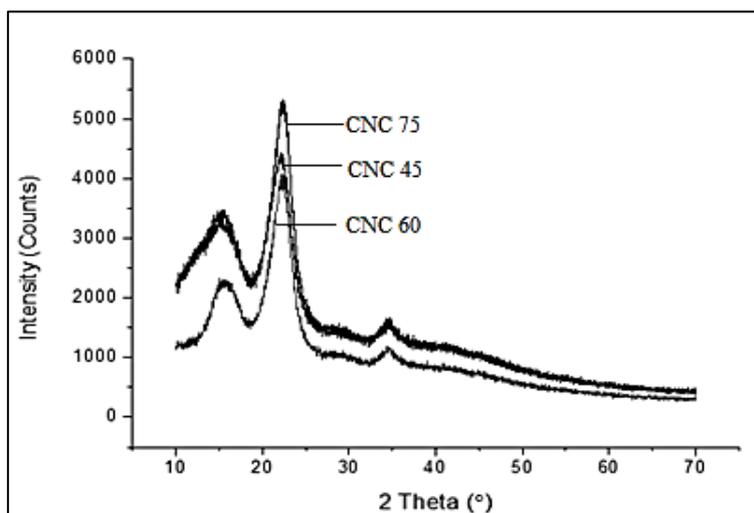
Figure 4. X-Ray diffraction of oil palm fronds cellulose nanocrystals at different hydrolysis time.

In accordance with the result of TGA, it can be concluded that degradation of crystalline structure took place during acid hydrolysis which finally caused a decrease in its crystallinity index. A higher crystallinity is desired for CNC as it would affect the mechanical properties of targeted applications.

The functional groups of CNC were observed by FTIR analysis. The peaks for all samples in Fig. 5 showed similar trends with some differences in its intensities. Based on the fingerprint region, it showed that there were no chemical changes for all samples hydrolyzed at different reaction time. The peaks in the 3,344–3,415 and 1,635–1,645 cm^{-1} region were attributed to O–H stretching and bending vibrations, respectively, of hydrogen bonded hydroxyl (OH) groups of cellulose and absorbed water [12].
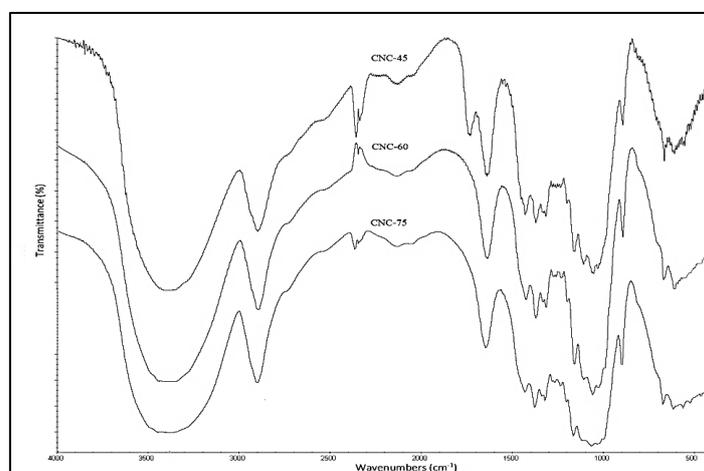
Figure 5. Fourier Transform Infrared spectroscopy of oil palm fronds cellulose nanocrystals at different hydrolysis time.

The peaks at 1,701–1,737 cm^{-1} was observed in CNC-45 which corresponded to the C=O stretching of hemicellulose and lignin but was absent in CNC-60 and CNC-75 [13]. This indicated that CNC-45 still had some amount of hemicellulose and lignin. Therefore, 45 min of acid hydrolysis is insufficient to remove major parts or the amorphous structure.

CONCLUSION

The finding in this study showed that the time for acid hydrolysis played an important role during the treatment. Increasing hydrolysis time from 45 min to 60 min caused some improvements in the crystallinity and thermal properties. However, a longer hydrolysis time (75 min) finally caused partial degradation of cellulose structure thus decrease its crystallinity index and thermal property. It was proven by the results from TGA and supported by the XRD results. A 64 % (v/v) sulfuric acid and 60 min of hydrolysis time was found suitable to be adapted for isolation of cellulose nanocrystals from oil palm fronds.

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