Isolation of Nanocrystalline Cellulose from Empty Fruit Bunch (EFB) via Microwave Assisted Technique

Utaibah Mustafa and Noor Fitrah Abu Bakar

Faculty of Chemical Engineering, Universiti Teknologi MARA

***Abstract*— Nanocrystalline cellulose (NCC) was isolated from cellulose and holocellulose of palm oil empty fruit bunch with microwave assisted technique. The microwave assisted acid hydrolysis process was conducted for 10 min with various power of 100 W, 200 W and 300 W. 11 wt.% of oxalic acid dehydrate was used to performed the acid hydrolysis. Ultrasonication was applied to break up aggregates of micron-sized colloidal particles in the formed NCC. NCC particles was characterized by using Malvern Zetasizer, X-Ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR) was used to determine zeta potential and average particle size, crystallinity of the sample and the functional groups. Cellulose and holocellulose samples treated at 300 W showed the highest value of zeta potential with value of -42.9 mV and -40 mV respectively. Analysis by FTIR showed that both of the samples had the functional groups that represent cellulose chemical structure. XRD analysis showed that holocellulose sample treated at 300 W had the highest value of crystallinity index.**

Keywords*— Nanocrystalline cellulose (NCC), Palm Oil Empty fruit bunch (EFB), Cellulose, Holocellulose

I. INTRODUCTION

Concern regarding the scarcity of renewable resources, it is important to find another alternative material. Besides that it is also crucial to take a serious look on it impact toward the environment. The emerging of one of nanomaterials which is nanocrystalline cellulose (NCC) has received increasing interest in nanotechnology due to its advantages of abundance, renewability, biodegradability, and excellent mechanical properties [1]. NCC is truly a promising renewable materials, NCC is made of crystalline particles that is structurally organize hence made it naturally strong.

Extensive studies have shown that NCC has great application potential in fields such as regenerative medicine, optics and composite materials [2]. Nanocrystalline cellulose (NCC) acquired by acid hydrolysis from a wide source of natural materials, has been acknowledged as another class of nanomaterials [3-5]. Empty fruit bunch (EFB) from palm oil is one of the natural source materials that been used to isolate the NCC. EFB mainly composed of cellulose, hemicellulose and lignin. Removal of amorphous region in the cellulose will generated the cellulose nanocrystals.

Contrasted with cellulose fibres, NCC has numerous favorable properties such as nanoscale dimension, high surface area, high specific strength and unique optical properties. These astonishing physicochemical properties and wide application prospects have pulled in critical enthusiasm from both researchers and industrialists. Besides that, NCC is a captivating nanoparticle because they have low environmental, health, and safety risks, are inherently renewable, sustainable, and carbon-neutral like the sources from which they are isolated, and have the viable to be processed in industrial-scale portions at low prices. The isolation of NCC via microwave assisted technique reduced the total production time by directly interacts with the molecules in the reaction mixture [6]. The thermal effect in microwave heating resulted from the interactions between material and the presence of electromagnetic field [1].

A microwave (MW) is a form of electromagnetic energy that falls at the lower frequency end of the electromagnetic spectrum (300-300000 MHz). Microwave heating (dielectric heating) is a very efficient process due to the microwave couple directly with the molecules that are present in the reaction mixture, leading to a fast rise in temperature, faster reactions and cleaner chemistry [7].

Previously, most of the researches have reported that the isolation of NCC is optimum at sulphuric acid concentration between 50-60% and the reaction temperature mostly revolved around at 70℃. Besides that, the microwave-assisted technique was mainly involved in the pretreatment method where it was mainly applied to perform the delignification of hemicellulose and lignin under alkaline condition only [8]. Then it was further treated with acid hydrolysis to isolate the NCC [9]. Based on previous study by [10, 11], the microwave-assisted technique also is applied to enhance the accessibility of enzymatic reaction. Study by [12] also used the sulphuric acid between 50-60% and temperature between 70-90℃ under microwave condition to isolate the NCC from MCC.

Thus, by applying the organic acid and weak acid, oxalic acid to perform the acid hydrolysis on cellulose and holocellulose with combined of microwave assisted technique it can produced NCC at higher crystallinity since NCC yield may lower if too much harsh condition is adopt such as high concentration of acid, high temperature and long treatment time. This research study is conducted to isolate nanocrystalline cellulose (NCC) via microwave assisted technique from holocellulose and cellulose. The microwave power was varied for power at 100 W, 200 W and 300 W.

II. MATERIAL AND METHOD

A. Materials

The material used for this research study includes the starting samples of cellulose (Fig. 1) and holocellulose (Fig. 2) that was provided by WARIS NOVE SDN BHD where both of the materials were obtained from empty fruit bunch (EFB) of palm oil. Oxalic acid was purchased from Sigma Aldrich, Malaysia.

Fig. 1: Cellulose from EFB of Palm Oil


Fig. 2 : Holocellulose from EFB of Palm Oil

B. *Isolation of Nanocrystals*

Cellulose and holocellulose was treated with 11 wt.% of oxalic acid dehydrate. The samples were in a microwave where the samples to solvent ratio was kept 1:20, *i.e.* 1 g of holocellulose and cellulose was treated with 20 mL of 11 wt.% of oxalic acid. The samples were treated in a microwave at power of 100W, 200W and 300W for 10 minutes. The treated samples were immersed in a cold water to stop the reaction. Then all of the treated samples were let to cool to room temperature. After the samples were cooled off, further centrifugation was carried out to basically separate the excess oxalic acid from the suspension at 10 000 rpm for 10 minutes. Dialysis using distilled water was conducted until the pH become 7. Then the samples were subjected to ultrasonication for 30 minutes at 20 kHz. After that, all of the samples were dried out in an oven at temperature of 100℃ for 6 hours.

Table 1: Varied Parameter for Isolation of NCC

Microwave Power	Sample	
	Cellulose	Holocellulose
100 W	C-1	H-1
200 W	C-2	H-2
300 W	C-3	H-3

C. *Fourier Transform Infrared Spectroscopy*

The FTIR spectra for all of raw and treated samples were analyzed between 4000 and 400 cm^{-1}. FTIR was conducted to determine the chemical structure in the samples.

D. *Zeta Potential and Particle Size Measurement*

The zeta potential and the particle size measurement of the samples were determined by employing Malvern Zetasizer Nano-ZS. Three measurements were conducted for each sample. It was conducted to determine the stability behavior of samples suspension and average particle size measurement.

E. *X-ray diffraction (XRD)*

The XRD patterns for treated was analyzed at 40 kV and 40 mA. Powder samples were scanned in 2θ range varying from 2° to 50°. XRD was conducted to determine the crystallinity of sample analyzed. Equation (1) was used to calculate the crystallinity index of the fibers:

$$Crystallinity\ Index = \frac{I_{200} - I_{am}}{I_{200}} \times 100\% \qquad (1)$$

I_{200} is the height of the [200] peak at 2θ of 22˚ which represents both crystalline while I_{am} is the lowest height between the [200] and [110] peaks at 2θ of 18˚, which represents amorphous material.

III. RESULTS AND DISCUSSION

A. *Fourier Transform Infrared Spectroscopy Analysis*

Fig. 3 shows the FTIR analysis for cellulose. The analysis was performed to determine the O-H bond and C-H bond and β-glucosidic linkages between sugar units. The absorbance ranging between 1314 cm^{-1} and 1315 cm^{-1} referred to C-H bending present in cellulose. Meanwhile the absorbance at peak of 896 cm^{-1} referred to the β-glucosidic linkages between sugar units [13, 14]. Besides that, the peak with absorbance value ranging between 1160 cm^{-1} and 1159 cm^{-1} referred to O-H bonding which also known as hydroxyl group that presence in cellulose structure. it can assured that there are no significance behavioral difference between the raw and treated which indicates that the cellulose composition retained in the tested sample [15].

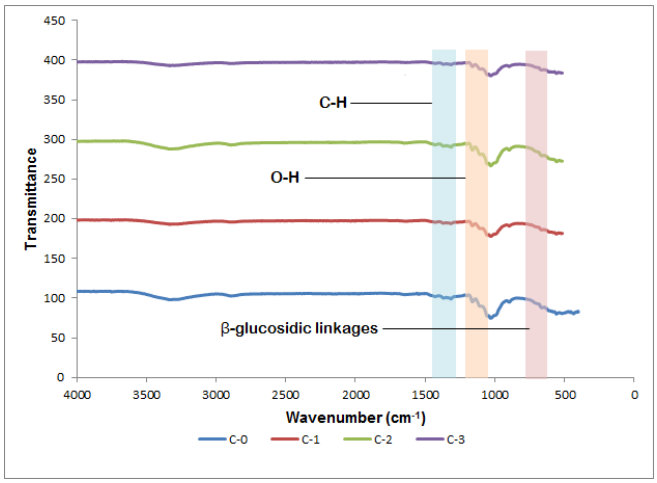

Fig .3: FTIR Spectrum for Cellulose

FTIR analysis for holocellulose is shown in Fig. 4. The analysis was performed to determine C=O, C-H and O-H bond in the samples. The holocellulose mainly composed of hemicellulose and cellulose. These substances are usually composed of alkane, esters, aromatics, ketones and alcohols with different oxygen containing functional groups[13]. FTIR analysis for the absorbance

range between 1733 cm^{-1} and 1731 cm^{-1} in all four of the spectra is referred to stretching band of C=O where it is likely stretching in the acetyl and uronic ester groups of hemicelluloses [15, 16]. This indicated that hemicellulose existed in all sample tested however, as the microwave power increase, the amount of hemicellulose decrease due to partial removal of hemicellulose [17]. Meanwhile, the absorbance between 1370 cm^{-1} and 1368 cm^{-1} in Fig. 4 represent the units of CH_2 and C-H of cellulose respectively [15]. Besides that, the absorbance between 1314 cm^{-1} and 1315 cm^{-1} also referred to C-H bending present in cellulose [13]. Besides that, the peak with absorbance value ranging between 1160 cm^{-1} and 1161 cm^{-1} referred to O-H bonding also known as hydroxyl group that presence in cellulose structure. Based on all of these four spectra, the cellulose and nanocellulose obtained in this study consisted of high cellulose and small amount of hemicellulose.

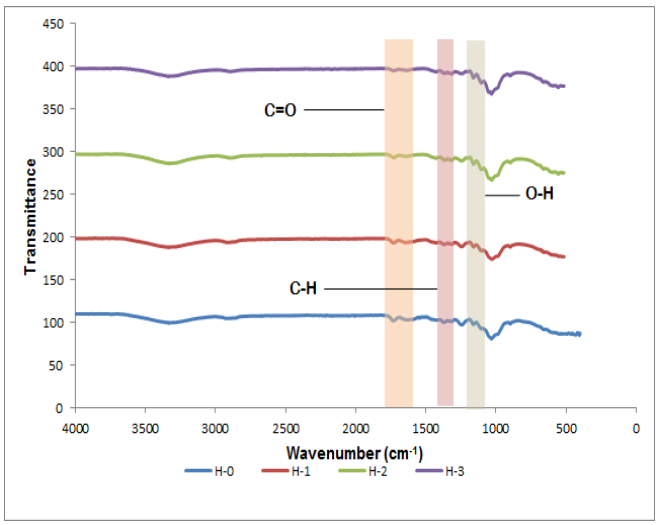

Fig. 4: FTIR Spectrum for Holocellulose

B. *Zeta Potential and Particle Size Measurement*

Table 2 shows the zeta potential values for all samples. Zeta potential values before sonication increased as the microwave power increased except for sample C-2 and C-3. From observation sample C-2 and C-3 with zeta potential value of-1.58 and -6.43 respectively aggregated and sedimented after applying microwave, thus it shows the lowest value of zeta potential. According to Gong et al. [18] rapid coagulation or flocculation of suspension may occur when the zeta potential value fall between 0 to -10 mV. However, holocellulose sample was more stable than cellulose because the zeta potential value increased as the microwave increase where the value of H-1, H-2 and H-3 were -20.9, -26 and -31.4 mV respectively. According to Gong et al. [18] nanocellulose suspension might had moderate stability when the zeta potential value fall between -30 to -40 mV. From observation also, holocellulose suspension aggregated slower than cellulose sample.

Analysis for zeta potential after sonicate showed that the value increased as the microwave power increased for all sample tested. The sample of C-1 and H-1 valued -28.6 and -20.6 mV respectively had the lowest value of zeta potential measurement. Previous study by Gong et al. and Hanaor et al. [18, 19] emphasize that zeta potential value of suspension that fall between -10 to -30 mV might had incipient instability. Besides that, zeta potential for all samples after sonicated increased where this issue could related to their particle size distribution obtained [20]. Meanwhile, sample C-3 and H-3 had the highest value of zeta potential valued -42.9 and -40 mV respectively.

Table 2: Data Analysis for Zeta Potential

Sample	Zeta Potential (mV)	
	Before Sonicate	**After Sonicate**
C-1	-27.3	-28.6
C-2	-1.58	-32.6
C-3	-6.43	-42.9
H-1	-16.7	-20.9
H-2	-26	-36.6
H-3	-31.4	-40

The particle size measurements for samples before sonicated is shown in Fig. 3. The measurement shows that the particle size fall into two group, nanoparticle and microparticle. Particle size measurement of C-2 is not shown in Fig. 3 because zetasizer could not measure the particle size as it is more than 10000 nm. It was because the microwave irradiation could not completely hydrolyze cellulose and holocellulose sample. The size distribution shows that the samples were not evenly treated as both microparticles and nanoparticles obtained. The data assured that all of the samples need to be sonicated to break up the aggregates of micron-sized colloidal particles and to disperse the nanoparticles.

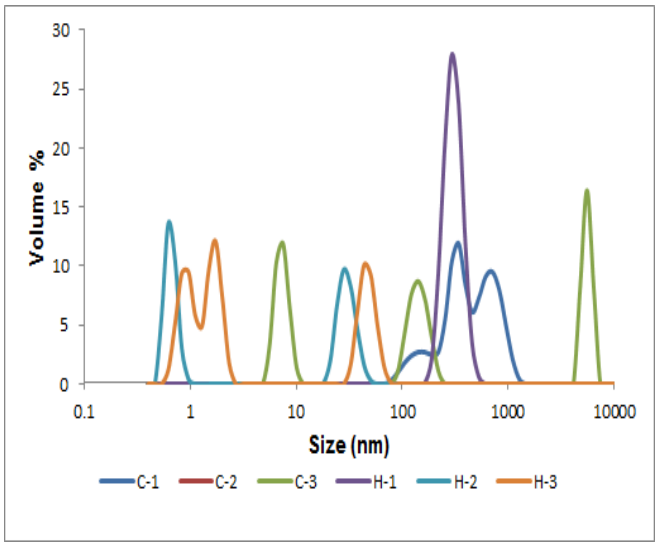

Fig. 3: Particles Size Distribution Before Sonicate

Particle size distribution for samples after sonicated is shown in Fig. 4. The measurement shows that the particle size also fall into two group, nanoparticle and microparticle. However, the volume percentage for size below than 1000 nm had increased in Fig. 4 as compared to Fig. 3 after applied the sonicated. Particle size of C-2 is shown in Fig. 4 indicated that the sonicated had reduce it size as it is included in the measurement. This related to FTIR result where sample C-2 had absorbance at 896 cm^{-1} representing β-glucosidic linkages between sugar units. It indicated that hydrolysis by oxalic acid cannot penetrated completely into amorphous region of cellulose thus, resulting a slightly large particle size.

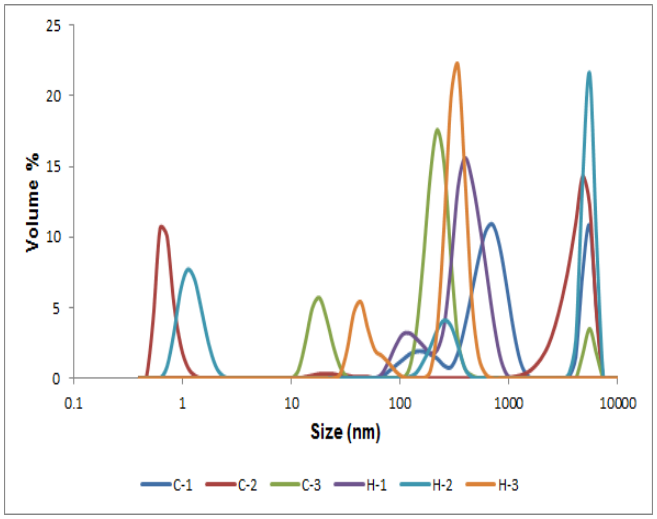

Fig. 3: Particles Size Distribution After Sonicate

C. X-Ray Diffraction (XRD)

Fig. 5 shows the X-ray diffraction patterns for all treated samples with diffraction peaks at around 2θ = 22˚ and 2θ = 18˚, representing the presence of crystalline and amorphous cellulose structure, respectively. Sample C-3 showed the highest peak at 2θ = 22.5˚ while sample H-1 showed the lowest peak at 2θ = 22.45˚, both representing crystalline cellulose structure. However, XRD patterns for samples of cellulose and holocellulose obtained were compared and sample H-3 had the highest crystallinity index as listed in table 4. The crystallinity index was calculated using equation (1). Meanwhile sample C-2 had the lowest peak for cellulose as listed in table 4 and crystallinity index for sample C-2 was higher than sample C-3. According to Lani et al. [16], the higher crystallinity of NCC was due to the peeling reactions that take place in the amorphous areas during the hydrolysis process. Because of the ability of oxalic acid to penetrate into the amorphous region, the glycosidic bonds were cleaved under hydrolytic conditions and finally releasing individual crystallites. FTIR result for sample C-2 in Fig. 3 shows presence of β-glucosidic linkages between sugar units thus it shows the lowest value of peak in table 4 for sample C-2 due to the amorphous region in the cellulose that did not degrade.

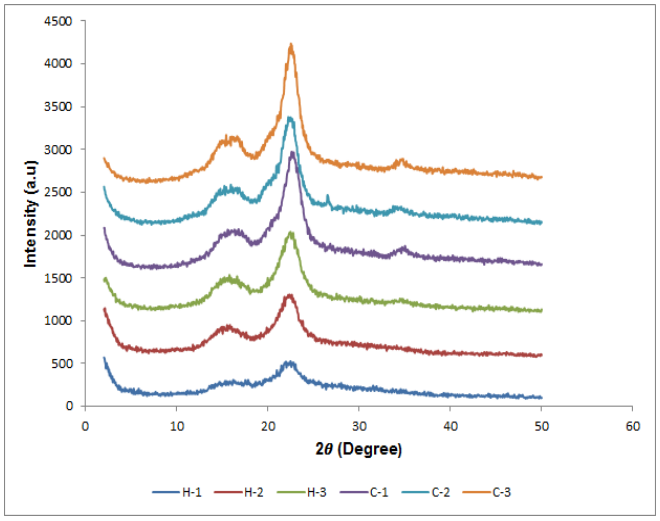

Fig. 5: XRD Data for All Treated Samples

Table 4: Data of X-Ray Diffraction Analysis

Sample	Highest Peak	2Θ (Deg)	Crystallinity Index (%)
C-1	1474	22	74.4
C-2	1379	22	72.2
C-3	1737	22	77.7
H-1	518	22	58.9
H-2	806	22	66.3
H-3	1039	22	69.6

Based on this present study, the concentration of acid used may be increase to enhance the hydrolysis of cellulose and holocellulose and can increase the yield of NCC. FTIR results, shows hemicellulose existed in the treated sample of holocellulose and β-glucosidic linkages between sugar units existed in treated cellulose. Eventhough microwave assisted techniques was able to isolate the NCC however acid hydrolysis process was not evenly hydrolyze all sample as the shows in analysis performed. Addition of stirring mechanism during acid hydrolysis may be an aid to enhance the hydrolysis process.

IV. Conclusion

NCC was isolated from two different samples, cellulose and holocellulose of EFB by performing acid hydrolysis in a microwave where the power varied at 100 W, 200 W and 300 W. NCC was successfully isolated by using 11% concentration of oxalic acid dehydrate at different microwave power in 10 min. The analysis of zeta potential shows that samples C-2 and C-3 had the lowest value which are-1.58 mV and -6.43 mV respectively. Particle size distribution shows a wide range of value ranging between 1nm and 10 000 nm. Besides that, the XRD analysis result shows crystallinity index between 58.9% and 77.7%. Analysis of FTIR shows that hemicellulose still existed in treated holocellulose and β-glucosidic linkages between sugar units still existed in treated cellulose. However hemicellulose content decreased as the microwave power increase and the β-glucosidic linkages only present in sample C-2. From the analysis obtained, it revealed that microwave assisted technique was an efficient approach to isolate the NCC from material of EFB in shorter time however due to some limitations generate low yield of NCC.

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