CHARACTERIZATION OF CELLULOSE NANOCRYSTAL ISOLATED FROM OIL PALM EMPTY FRUIT BUNCH USING FORMIC ACID HYDROLYSIS

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ABSTRACT

Cellulose nanocrystals (CNCs) is a renewable and biodegradable nanomaterial which has wide application values and can be isolated from various sources such as plant, animal and microorganism. Oil palm empty fruit bunch (OPEFB) is a potential biomass produced from extraction of oil palm in oil palm mill. In this work, CNCs from OPEFB undergo two types of treatment which are pre-treatment using sodium hydroxide (NaOH) and sodium hypochlorite (NaClO) to remove lignin and hemicellulose followed by 90% formic acid (FA) hydrolysis by using different temperature and time. Fourier Transform Infrared (FTIR) analysis showed peak at 1742.47 cm^{-1} indicate that carbonyl stretching in the acetyl and uronic ester groups of hemicelluloses or the ester carbonyl groups in the p-coumaric units of the lignin were successfully removed. Furthermore, the functional group of cellulose were retained by observing peak at 1056.72 cm^{-1}. The morphological analysis of CNC with different temperature isolated from EFB by using Field Emission Scanning Electron Microscope (FESEM) displayed clear needle like structure while in different time is globular structure. The diameter of needle like structure in EFB-CNC decrease when temperature increase. The X-Ray Diffraction (XRD) shows the crystallinity index of EFB-CNCs with different temperature and different time which are 66-69% and 9-13% respectively. CNCs isolated from OPEFB using 90% FA hydrolysis at 45 °C have similar properties compared to isolation using strong acid hydrolysis.

Keywords: Cellulose nanocrystal, oil palm empty fruit bunch, acid hydrolysis, crystallinity.

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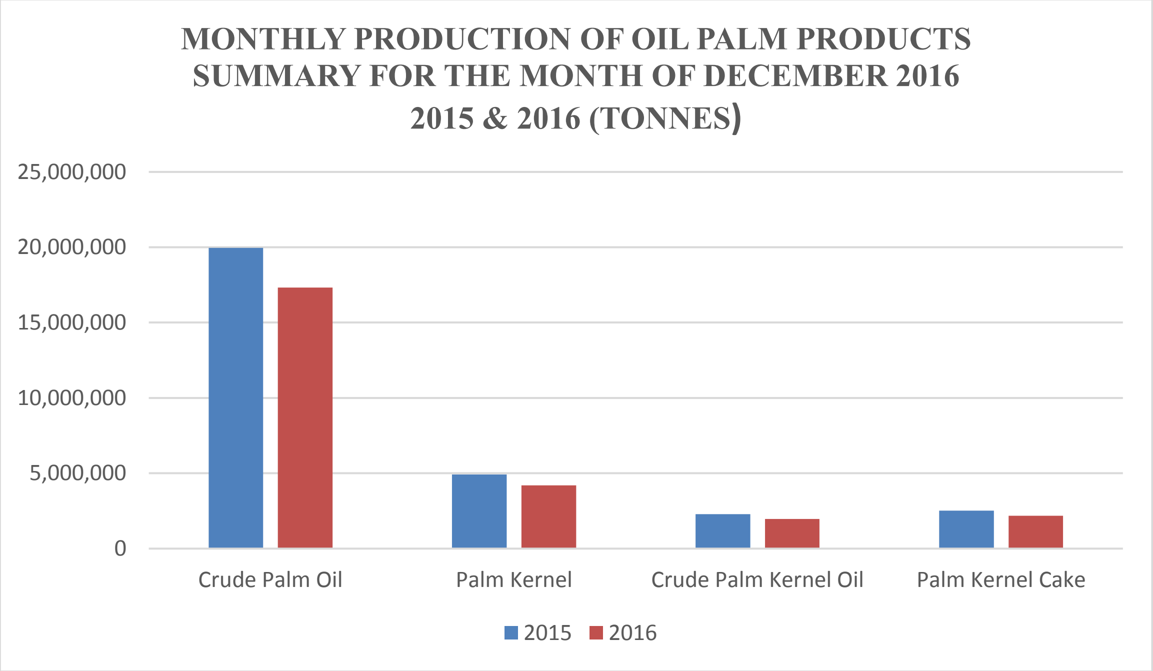
1. INTRODUCTION

In recent year, focus has been shifted to nanomaterial component due to its unique features such as specific surface area, high aspect ratio, good mechanical properties and easily to be modified to obtain desired characteristic (Wang *et al*., 2013). Nanomaterial can be obtained from various sources either through natural or synthetic means. However, due to environmental concerns and stewardship programme organised by the authority has made natural sources more reliable and practical due to its renewability and biodegradability properties. One of the sources that have seen prominent rise in researches is lignocellulosic biomass and according to Tye *et al.,* (2016) the production of lignocellulosic biomass will increase by about 0.9–1.6% per annum and forecasted to increase by 38–67% in 2050.

Malaysia is one of the largest palm oil producer (51% of the worldwide production), and oil palm production is an important economic resource for the country (Ferrer *et al.*, 2012). The large production of palm oil contributes massive amounts of biomass causing environmental problems such as biodiversity loss and pollution. Some methods have been carried out to reduce the abundance biomass such as biogas production, composts manufacturing, and electric generation by burning (Saswattecha *et al*., 2016). Oil palm empty fruit bunch (EFB) is one of the main biomass with high cellulose content of up to 65% (Palamae *et al,* 2016).

George and Sabapathi (2015) mentioned that cellulose is tough, fibrous and water-insoluble polymers that support the structure of plant cell walls. Cellulose has high-molecular-weight and linear homopolymer with the molecular formula $(C_6H_{10}O_5)_n$ that derived from repetition monomers of β-ᴅ-glucopyranosyl joined together by (1-4) glucosidic linkage (Fahma *et al.,* 2010). Since cellulose is renewable, many research has been carried out focusing on finding new alternative method to procure cellulose from biomass in environmental friendly manners (Jonoobi *et al.,* 2015).



Source: Bepi.mpob.gov.my, (2017).

CNC is nanocellulose with the 2 – 15 nm width and 100 – 500 nm length. CNC is a suitable nanomaterial for a wide range of applications, such as enzyme immobilization, synthesis of medical materials, green catalysis, and biosensing (Peng *et al.,* 2011).CNC is mainly isolated through chemical treatment by treating the cellulose with aqueous acid at high concentration with low temperature and using wide treatment time range. Sulphuric acid that are prominently used for this method can cause several environmental issues like huge generation of effluents, high water consumption and products that have high content of sulphur. The conditions during hydrolysis heavily affect the yield of CNC; extreme conditions and high presence of pectin and hemicellulose might decrease the production yield (García *et al.,* 2016).

Formic acid hydrolysis was found to be able to isolate CNC that is longer rod-like structure and high in crystallinity resulting in good thermal stability. Fahma *et al.,* (2010) in their study found that increasing hydrolysis time will produce higher yield of dispersed CNC; whereas the degree of crystallinity decreased with increasing hydrolysis time. In this study, the comparison between cellulose nanocrystals (CNC) that isolated using formic acid hydrolysis with different hydrolysis time and temperature.

2. METHOD

2.1 Materials

Oil palm empty fruit bunch was obtained from Sime Darby Berhad (Labu, Negeri Sembilan, Malaysia). Formic acid and sodium hypochlorite were purchased from Sigma-Aldrich Sdn Bhd (Kuala Lumpur, Malaysia).

2.2 Preparation of oil palm empty fruit bunch

OPEFB fiber was washed using distilled water to remove impurities. The fiber was then dried at 100 °C overnight. The fiber was ground using crusher (Model SY-50) at Forest Research Institute Malaysia.

2.3 Pre-treatment

Ground OPEFB fiber were treated with 100 ml of 3% sodium hydroxide (NaOH) solution for 3 hours at 100 °C and continuously stirred. The treated OPEFB fiber was made to neutral pH using concentrated hydrochloric acid. The treated fiber then further bleached with 40 ml sodium hypochlorite (NaClO not less than 10% active chlorine) at 80 °C for 2 hours and continuously stirred. This bleaching process was repeated for three (3) times until the color of the fiber change from dark brown to white fiber (removal of lignin). The OPEFB fiber was filtered and washed again with distilled water. The cellulose obtained was air-dried and stored at room temperature prior for isolation.

2.4 Formic acid hydrolysis with different temperature.

CNC was isolated from bleached OPEFB was done by using acid hydrolysis method of Oun and Rhim (2015) with slight modification. The bleached fiber hydrolyzed with pre-heated 60% CH_2O_2 with fiber to acid ratio of 1:20 at 65 °C for 90 minutes with strong agitation. The hydrolysis was stopped by 10-fold of cold deionized water. The excess of CH_2O_2 was removed from the suspension by washed with deionized water until achieve neutrality. The suspension of CNC then was sonicated using ultrasonic (Starsonic 60) for 30 minutes in ice water bath to obtain well dispersed CNC colloidal suspensions. The suspension was filtered using filter paper and freeze dry prior to characterization. This method was repeated by using different temperature, 75 °C and 85 °C. Then the product was label as CNC-75 °C, and CNC-85 °C.

2.5 Formic acid hydrolysis with different hydrolysis time

Oil palm biomass were hydrolyzed with 90 % formic acid with fiber to acid ratio of 1:10 at 45 °C for 30, 90 and 150 minutes with strong agitation. The hydrolysis was stopped and quenched by adding 10-fold of deionized water. All the CNC suspensions were sonicated using sonicator (Starsonic 60) for 90 minutes in ice water bath to obtain well dispersed CNC colloidal suspensions. The suspensions were filtered and the residues are frozen overnight. The frozen residues are dried using freeze dry method and the products were characterized. Then the product was label as CNC-30, CNC-90, and CNC-150.

3. RESULTS AND DISSCUSSION

3.1 Isolation of CNC from OPEFB

The raw oil palm biomass was first pre-treated with NaOH and NaClO to remove non-cellulosic constituent such as hemicellulose and lignin. Initially raw OPEFB is brown in colour and turned dark brown when treated with NaOH. Repetitive bleaching with NaClO produced white powder as shown in figure 3.1. This process of pre-treatment was supported by Kallel *et al.,* 2016), where NaOH and NaClO were used as efficient chemicals to remove lignin and hemicellulose, resulting with high yield of 87% of cellulose remained as product.

Isolation of treated OPEFB using formic acid hydrolysis produce agglomerated and less dispersed white colloidal suspension. During the formic acid hydrolysis process, the amorphous region was digested and the resulting product become smaller in size with large surface area so that it can easily agglomerate (Maiti *et al.,* 2013). Various temperature and hydrolysis time used did not affect the products observed under naked eyes.

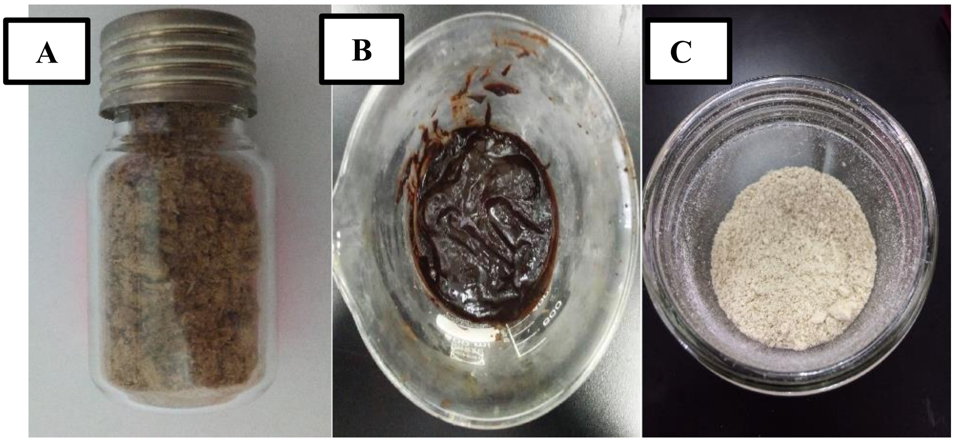


Figure 3.1 **(A) Raw EFB, (B) EFB with NaOH, (C) Bleached EFB.**

3.2 FTIR spectroscopic analysis

The FTIR spectroscopic analysis in figure 3.2 shows the absorption spectra of oil palm biomass for raw, bleached and isolated CNC using formic acid hydrolysis at different temperature and time. The peak for hemicellulose and lignin at wavenumber 1742.47 cm^{-1} (C=O stretching) and 1244.31 cm^{-1} (C-O-C stretching) were found absence after pre-treatment and hydrolysis process indicating that both components have been successfully removed from the fibres (Haafiz *et al.,* 2013), The general cellulose functional groups after pre-treatment and isolation of CNC were found to be preserved and similar to the patterns in previous reported studies (Kargarzadeh *et al.,* 2012; Oun and Rhim, 2016). The spectrum peak at 1718.78 cm^{-1} for C=O stretching showing the present of formate ions that weakly attached through hydrogen bonding on the surface of nanocellulose structure (Li *et al.,* 2015). The FTIR spectra showed the structure of cellulose based on the functional groups in the product, proving that CNC can be isolated using the method in the study.

3.3 X-ray diffraction (XRD) analysis

The XRD diffractogram spectra as shown in figure 3.3 and 3.4 was scanned from 10-60º angle at 40kV and Kα: λ= 1.54443 Å. From XRD spectrum, the crystallinity index (CI) value of CNC at different isolation temperature show the reading of percentage ranging from 66-69%, while different isolation time is at 18-70%. Nearly all of the isolated CNC in this study conform to the CI of CNC isolated by Estela and Luis (2013) at 40-95%. The 002 plane at value 2θ = 23º in all XRD spectra signal that isolated products are cellulose I as no characteristic peaks of cellulose II (12.1◦, 20.0◦, 21.7◦) were observed in the spectra (Rambabu *et al.,* 2016). From figure 3.3, XRD spectrum shows the CNC-75 ºC have high intensity compare to CNC-65 ºC and CNC-85 ºC because most of the amorphous region was hydrolysed by reaction with formic acid. Thus CNC isolated at 75 ºC is considered as optimum isolation temperature condition. Previous studies performed by Rosa *et al.,* (2010) explained that the isolation time affect the CI of the CNC and thus using too high isolation time will destroy the crystalline region.

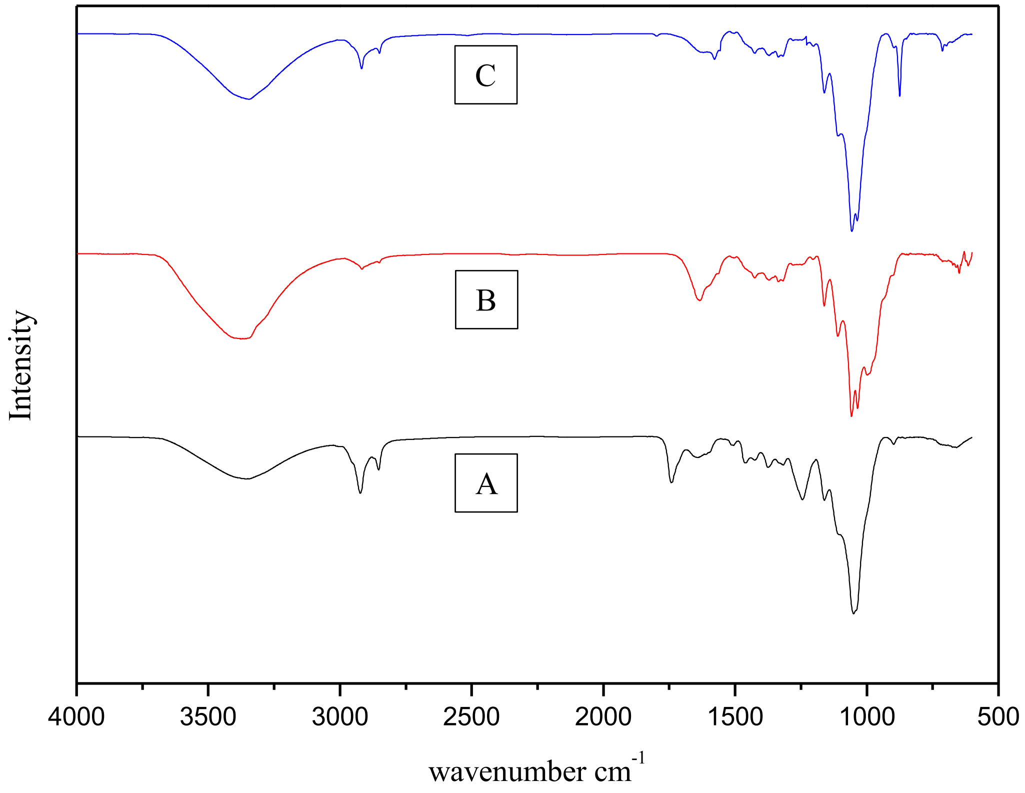


Figure 3.2 FTIR result for (A) Raw EFB (B) EFB Bleach (C) CNC.

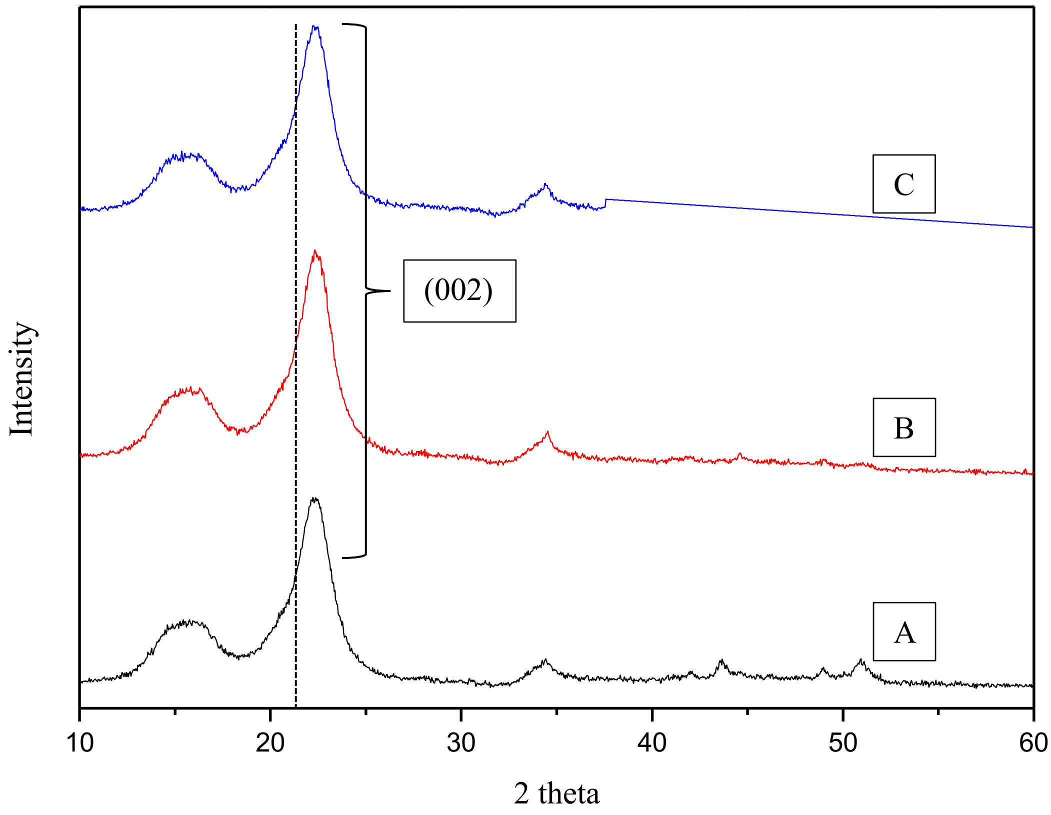


Figure 3.3 **XRD result for (A) CNC-65 °C (B) CNC-75 °C (C) CNC-75 °C.**

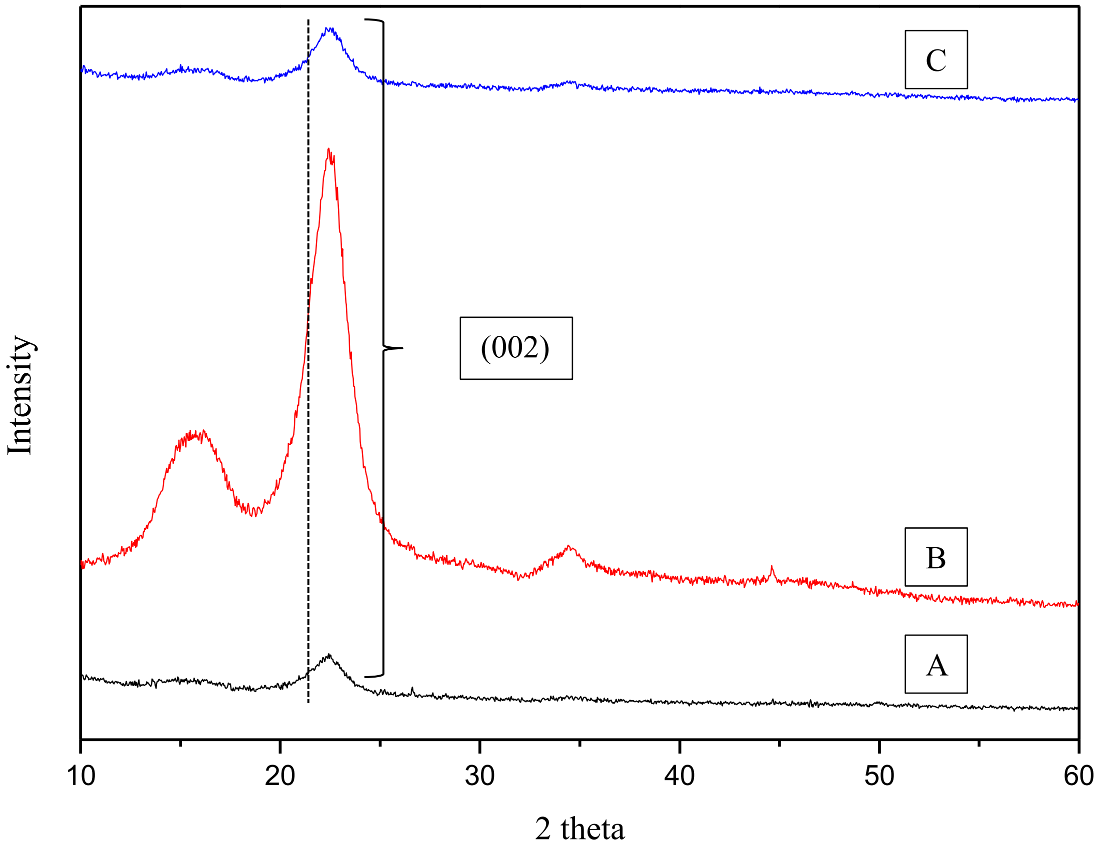


Figure 3.4 **XRD result for (A) CNC-30 (B) CNC-90 (C) CNC-150.**

Table 3.1 Crystallinity index of CNC

	Crystallinity index (%)
CNC-65 ºC	66.53
CNC-75 ºC	69.82
CNC-85 ºC	68.94
CNC-30	23.53
CNC-90	70.73
CNC-150	18.34

3.4 Morphology of the isolated CNC

The morphology of CNC from oil palm biomass was examined by field emission scanning electron microscopy (FESEM) and sample was coated used platinum because of CNC is natural polymer that can't transmit the electron. The CNC-EFB exhibit the needle like structure at all different temperature product and CNC-90. According to Oun and Rhim (2016), the product of acid hydrolysis produced the needle like with 79% of CI.

The figure below is the morphology of EFB in raw, and treated CNC. From the observation, diameter of needle-like structure in CNC-90 and CNC-65 ºC is bigger compare to CNC-75 ºC and CNC-85 ºC for the oil palm EFB biomass. CNC-30 under FESEM, show that some needle-like structure started to be visible while retaining the shape of raw EFB This show that cellulose has started to break down into CNC but not fully isolated. CNC-150 show globules structure with some needle-like structure indicating that while CNC is isolated the integral structure of the CNC has started to break down. For CNC with different temperature parameter, the needle-like structure is decrease in diameter with increasing temperature.

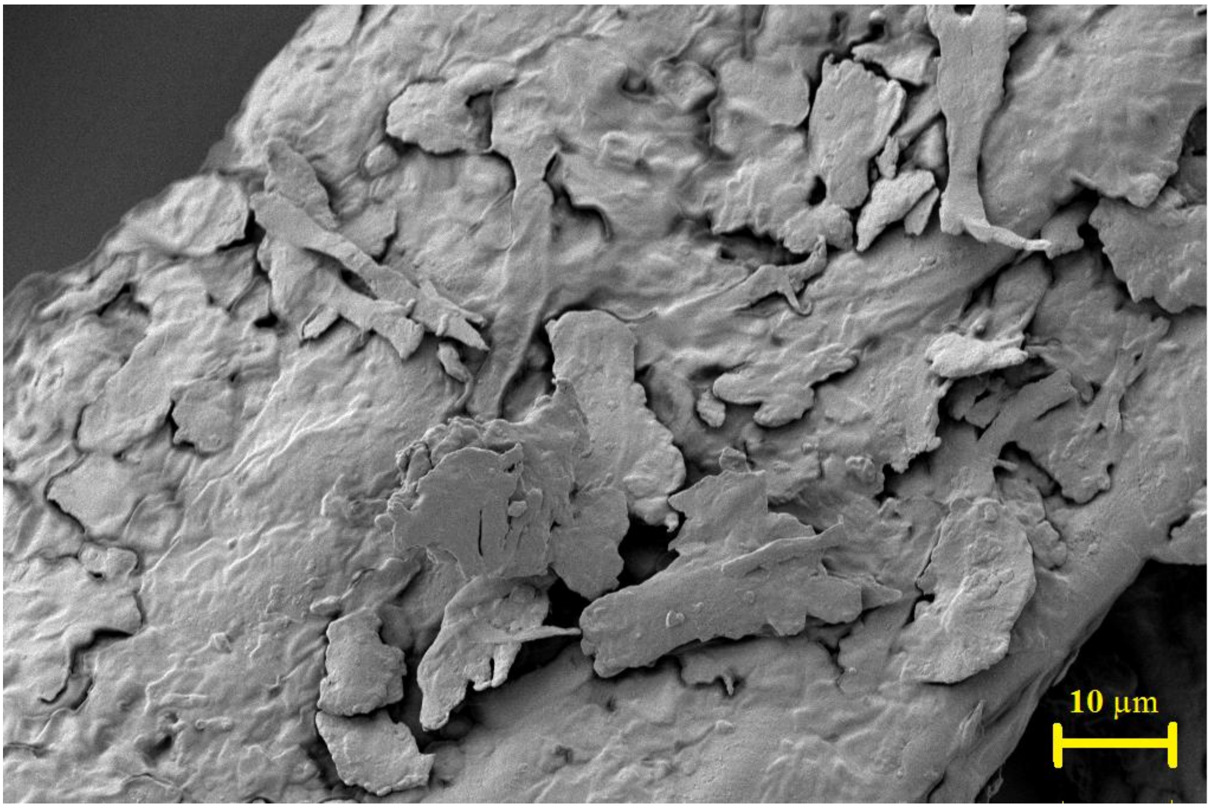


Figure 3.5 Raw EFB.

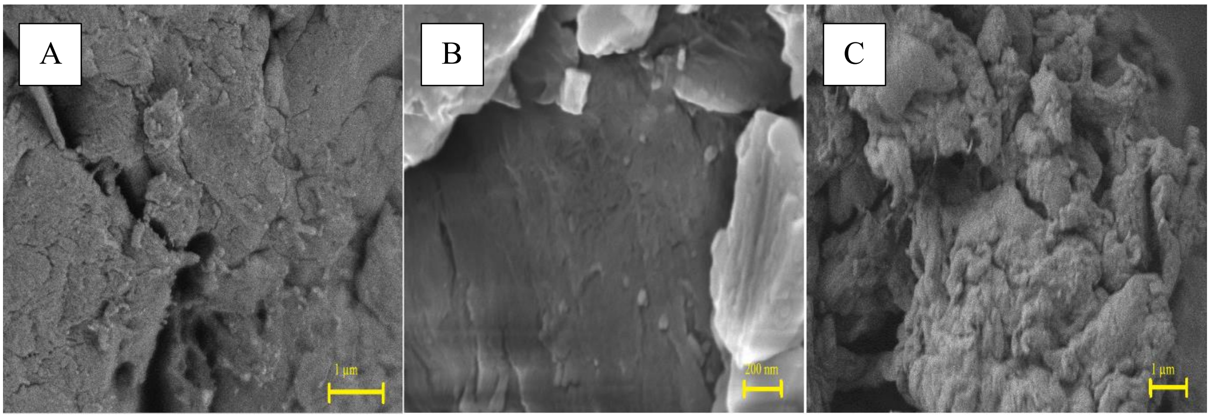


Figure 3.6 (A) CNC-30 (B) CNC-90 (C) CNC-150.

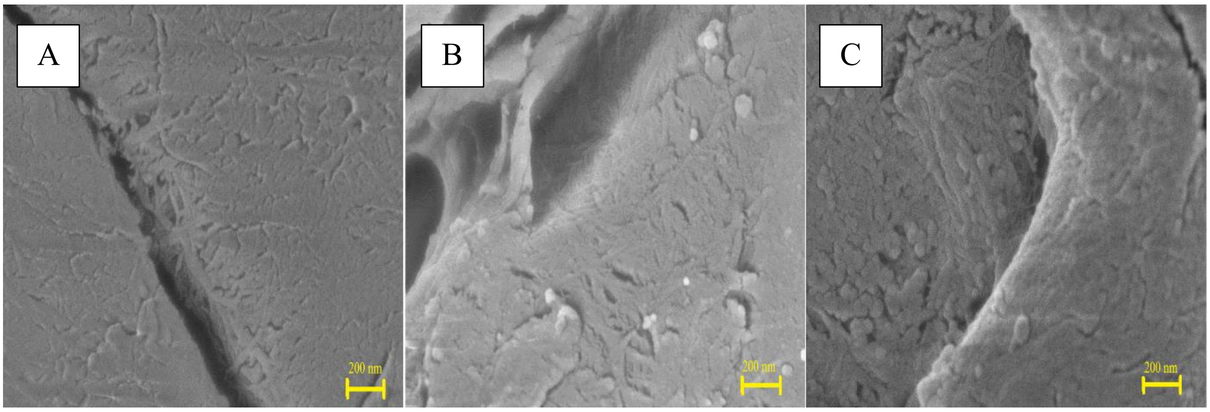


Figure 3.7 (A) CNC-65 ºC (B) CNC-75 ºC (C) CNC-85 ºC.

4. CONCLUSION

Cellulose nanocrystals (CNCs) were successfully isolated from OPEFB fiber after chemical treatment through alkali treatment, bleaching and formic acid hydrolysis. Analysis by FTIR showed that the lignin and hemicellulose of OPEFB was efficiently removed after the bleaching process. XRD analysis indicated that crystallinity index of CNC-90 (70.73%) was superior compared to others CNC. Furthermore, CNC-75 ºC shows the expected morphology of CNC under FESEM which are needle-like structure. In the isolation time procedure, increasing hydrolysis timing will increase the CI. However, after the optimum isolation time (90 minute) obtained, crystallinity of the CNC structure can be disrupted. The formic acid used in acid hydrolysis of OPEFB is highly potential chemical, simple and efficient way for isolation of CNC from palm oil biomass.

To further study the effect of hydrolysis conditions towards the properties of CNC isolated from OPEFB, more instrument such as transmission electron microscopy (TEM) can be used to observe the morphology of CNC with more individualized structure. Surface modification of the CNC can also be done to utilize the potential of CNC in various industries such as nanocomposites, biomedical, food packaging and paper industries.

Based on the characterization conducted, the modified acid hydrolysis method is viable for isolation of CNC from OPEFB. However, the method needs to be further optimized to find the best condition for CNC isolation and making it cost-effective for industrial production. South East Asia is the region that acts as the main force behind palm oil production and the biomass in the form of OPEFB can be utilized as the source of nanocellulose. This will reduce the needs for landfills, burning of the biomass and reduce the contamination from biomass into water. This method of nanocellulose isolation can propel a new pathway for oil palm biomass utilization, nanocellulose production industry in the region, creating jobs and increasing the economy of the region. Government can also enforce laws and regulations to ensure that a sustainable way of disposing oil palm biomass must be applied in the industry, else it could lead to wastage of a highly potential resource.

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