Isolation and Fractionation of Cellulose Nanocrystals from Kenaf Core
(Pemencilan dan Pemecahan Selulosa Nanohablur daripada Teras Kenaf)

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ABSTRACT

In this study, cellulose nanocrystals (CNC) were produced using acid hydrolysis method. Kenaf core was pretreated with 4 wt. % sodium hydroxide (NaOH), followed by bleaching using 1.7 wt. % sodium chlorite ($NaClO_2$) in acetate buffer. The bleached fiber was acid hydrolyzed for 45 and 55 min using 64 wt. % sulfuric acid (H_2SO_4). The size distribution of the CNC segregated via differential centrifugation with different speed was also investigated. The CNC suspension obtained was centrifuged at 3000, 6000, 9000 and 12000 rpm. The resultant CNC suspension collected was characterized using Fourier transform infrared (FTIR) analysis, X-ray diffraction (XRD) and transmission electron microscopy (TEM). FTIR results showed the progressive removal of non-cellulosic constituents for each subsequent treatment. It also showed that the CNC produced after hydrolysing for 55 min has the highest degree of crystallinity (81.15%). CNC produced from acid hydrolysis process of 45 min have lengths between 50 and 270 nm while CNC produced from acid hydrolysis process of 55 min have length around 40 to 370 nm.

Keywords: Acid hydrolysis; cellulose nanocrystal; kenaf core

ABSTRAK

Dalam kajian ini, selulosa nanohablur (CNC) dihasilkan menggunakan kaedah hidrolisis asid. Teras kenaf yang digunakan sebagai bahan mentah diprarawat menggunakan 4 bt. % natrium hidroksida (NaOH). Serabut yang telah dirawat kemudian dilunturkan menggunakan natrium klorit ($NaClO_2$). Hidrolisis asid telah dijalankan selama 45 dan 55 minit ke atas serabut yang telah dilunturkan menggunakan 64 bt. % asid sulfurik (H_2SO_4). Taburan saiz CNC yang dihasilkan dikaji menggunakan teknik pengemparan dengan kelajuan yang berbeza. Ampaian CNC yang terhasil diemparkan pada kelajuan 3000, 6000, 9000 dan 12000 rpm. Ampaian CNC yang terhasil dianalisis menggunakan spektroskopi Fourier inframerah (FTIR), pembelauan sinar-X (XRD) dan mikroskop elektron transmisi (TEM). Keputusan FTIR menunjukkan penyingkiran unsur-unsur bukan selulosa pada setiap rawatan. CNC yang terhasil selepas proses hidrolisis selama 55 minit mempunyai darjah kehabluran yang tertinggi pada 81.15% melalui analisis XRD. CNC yang telah dihidrolisis selama 45 minit mempunyai panjang antara 50 dan 270 nm, manakala CNC yang telah dihidrolisis selama 55 minit mempunyai panjang sekitar 40 ke 370 nm.

Kata kunci: Hidrolisis asid; selulosa nanohablur; teras kenaf

INTRODUCTION

Kenaf (*Hibiscus cannabinus*) attracts wide attention because it grows rapidly and has high fiber quality (Adamson & Bagby 1975; Nishino et al. 2003; Seo et al. 2008). Many previous studies proved that kenaf bast and core could be used to improve bioremediation (Boraziani & Diehl 1994), as adsorbents (Sajab et al. 2011, 2010), sound absorbers, and heat insulators (Sellers et al. 1994). Kenaf also has high capability in removing heavy metal ions via adsorption (Hasfalina et al. 2010; Sajab et al. 2010).

Cellulose is the most abundant and renewable biopolymer. Orderly, organized cellulose chains which are stabilized by inter-molecular and intra-molecular hydrogen bonding formed compact nanocrystal (Alemdar & Sain 2008). In plant cell wall structure, crystal and amorphous cellulose structure form nano/micro fibers, which together produce cellulose fiber (Eichhorn et al. 2010). Therefore, cellulose contains crystalline and amorphous segments. The amorphous segments can be removed to produce highly crystalline cellulose.

Cellulose nanocrystals (CNC) can be extracted from various resources such as wheat straw (Helbert et al. 1996), cotton (Elazzouri-Hafraoui et al. 2008), ramie (Moran et al. 2008), wood (Beck et al. 2011), rice husk (Johar et al. 2012), bamboo (Yu et al. 2012) and also soy hulls (Neto et al. 2013). Rod shaped CNC has lengths ranging from tens to hundred nanometer and its diameter ranging from one to 100 nanometer (Lima & Borsali 2004; Ruiz et al. 2000). Various methods were used to produce CNC, but acid hydrolysis is the most oftenly used method (Peng et al. 2011) to remove amorphous segment in order to produce cellulose with high degree of crystallinity (Bai et al. 2009; Elazzouri-Hafraoui et al. 2008; Hirai et al. 2009).

The aim of this study was to produce cellulose nanocrystal (CNC) with acid hydrolysis method and to investigate the size distribution of the CNC produced using centrifugation technique with different speed. At low speed, the CNC that are longer and heavier will precipitate first, while at high speed, CNC that are shorter and lighter will precipitate (Iammarino et al. 2007).

MATERIALS AND METHODS

Kenaf core pulp was obtained from Rice and Industrial Crops Research Centre (MARDI). Sodium hydroxide (NaOH) used for alkali treatment was purchased from Merck. Sodium chlorite (NaClO$_2$) and acetic acid used for bleaching process were purchased from Acros and Systerm, respectively. Sulphuric acid used for acid hydrolysis was purchased from JT Baker.

PREPARATION OF NANOCRYSTALLINE CELLULOSE

ALKALI TREATMENT

Kenaf core was first cooked with 14 wt. % of active alkali and 25 wt. % sulphidity at 170°C for 3 h (Chia et al. 2008) to produce kenaf core pulp. Then, the kenaf core pulp was washed where it was separated from the cooking liquor. The kenaf core pulp was kept in dried form before further treatment. 4 wt. % of NaOH solution was used to treat the kenaf core pulp. It was performed to remove the remaining hemicelluloses, lignin and other impurities. The treatment was done at 80°C for 2 h using a water bath.

BLEACHING PROCESS

Alkaline-treated pulp was then bleached with NaClO$_2$ (1.7 wt. %) in acetic acid buffer solution for 4 h at 80°C using a water bath. This step was repeated twice. Then, the bleached fibres was filtered and washed with distilled water until it reaches neutrality.

ACID HYDROLYSIS

Acid hydrolysis was done on the bleached fibres. The bleached fibres was grinded and sieved (50 micron) before hydrolysis. The sieved fibres were hydrolyzed using 64 wt. % H$_2$SO$_4$ (preheated). Two sets of hydrolysis were done for different reaction times, i.e. 45 and 55 min. Then, the hydrolyzed cellulose was washed with distilled water by centrifugation at 10000 rpm for 10 min. It was repeated several times before the cellulose suspension was dialyzed in deionized water until the pH reached around 5-6.

FRACTIONATION OF CNC SUSPENSION

The CNC suspension that was hydrolyzed for 45 min was first centrifuged at 500 rpm for 10 min. The precipitate was discarded because it contains impurities and most of the CNC had diameter over 100 nm (Bai et al. 2009).

The supernatant that was previously centrifuged at 500 rpm was used to centrifuge at 3000 rpm. The precipitate was collected and labelled as Sample 1. The supernatant that was previously centrifuged at 3000 rpm was used to centrifuge at 6000 rpm and the resultant precipitate was collected and labelled as Sample 2. These steps were repeated for Samples 3 and 4 which was centrifuged for 9000 and 12000 rpm. The same steps were repeated for CNC suspension that was hydrolyzed for 55 min and the precipitate was collected and labelled as Samples 5, 6, 7 and 8. Full variables are summarized in Table 1.

TABLE 1. Acid hydrolysis time, centrifugation speed and sample's label

Acid hydrolysis time (min)	Centrifugation speed (rpm)	Sample
45	3000	1
	6000	2
	9000	3
	12000	4
55	3000	5
	6000	6
	9000	7
	12000	8

CHARACTERIZATION

LIGNIN CONTENT DETERMINATION

Kenaf core, alkaline-treated kenaf core and bleached kenaf core were analyzed for lignin content using TAPPI T222 om-06. The samples were hydrolyzed with 72 wt. % H$_2$SO$_4$ at low temperature, 20°C for 2 h and boiled for 4 h. The precipitate was cooled, filtered and dried. The mass of dried lignin was determined.

FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY

Five samples (kenaf core, alkaline-treated kenaf core, bleached kenaf core, 45 min hydrolyzed cellulose and 55 min hydrolyzed cellulose) were analyzed using a Fourier transform infrared spectroscopy (FTIR) (Perkin-Elmer Spectrum 400) at wavenumber ranging from 4000 to 650 cm^{-1}.

X-RAY DIFFRACTION

Kenaf core, alkaline-treated kenaf core, bleached kenaf core, 45 min hydrolysed cellulose and 55 min hydrolysed cellulose were analysed using XRD (Bruker AXS D8 Advance) with monochromatic Cu Kα radiation source (λ = 0.1539 nm) from 2θ = 5° to 50° with a step size of 0.0250°. The crystallinity index, CrI was determined using Segal's equation. It was calculated using (1):

$$CrI\,(\%) = [(I_{002} - I_{AM})\,/\,I_{002}] \times 100\%. \qquad (1)$$

TRANSMISSION ELECTRON MICROSCOPY (TEM)

The CNC suspension was dropped on a copper grid and stained with uranyl acetate (3 wt. %). Images of the samples were captured using a transmission electron microscope (TEM, CM 12 Philips). At least 50 measurements of CNC length and diameter were taken for each sample.

RESULTS AND DISCUSSION

LIGNIN CONTENT DETERMINATION

Table 2 shows the lignin percentage for all the samples. Lignin content for alkaline treated pulp was 4.855%. After going through the bleaching process once, lignin percentage decreased to 0.565 wt. %. This showed that most of the lignin was expelled after bleaching process. This lignin content was still high for cellulose nanocrystals (CNC) production. Therefore, bleaching process was carried out once again so that the fibre that sticks together to form lumps were separated to produce individual fibre which helps in acid hydrolysis process for cellulose nanocrystals production (Johar et al. 2012). Lignin content in twice bleached pulp was 0.170%. Lignin percentage for twice bleached pulp was low enough for cellulose nanocrystals production because high lignin content in fibres caused the acid unable to hydrolyse amorphous region during acid hydrolysis process to produce CNC (Yu et al. 2012).

TABLE 2. Lignin percentage after every treatment

Sample	Lignin percentage (wt. %)
Alkaline-treated kenaf core	4.855
Bleached kenaf core (once)	0.565
Bleached kenaf core (twice)	0.170

FTIR

FTIR analyses were carried out to identify the chemical; functional groups of samples. Figure 1 shows the spectra for (a) kenaf core, (b) alkaline-treated kenaf core, (c) bleached kenaf core, (d) 45 min hydrolyzed cellulose and (e) 55 min hydrolyzed cellulose. Peak that was located around 1734 cm^{-1} was functional group of acetyl and ester in hemicellulose (Jonoobi et al. 2010). This peak was observed in the untreated fibre sample.

Peaks at around 1593 and 1503 cm^{-1} were representing symmetry and asymmetry stretching of aromatic ring in lignin (Atalla & Agarwal 2010; Chan et al. 2015; Yan et al. 2009). Peaks at 1734, 1593 and 1503 cm^{-1} were observed on the kenaf core but not from the alkaline treated fibre and the bleached fibre. Peak appeared at around 2900 cm^{-1} is due to the -CH vibration (Kisku et al. 2014), 1640 cm^{-1} was due to O-H bond resulted from water adsorption (Moran et al. 2008; Yang et al. 2007) and 1160 cm^{-1} was due to C-O-C from pyranose ring stretching (Kisku et al. 2014; Maiti et al. 2013). These peaks existed on all samples but less visible in kenaf core.
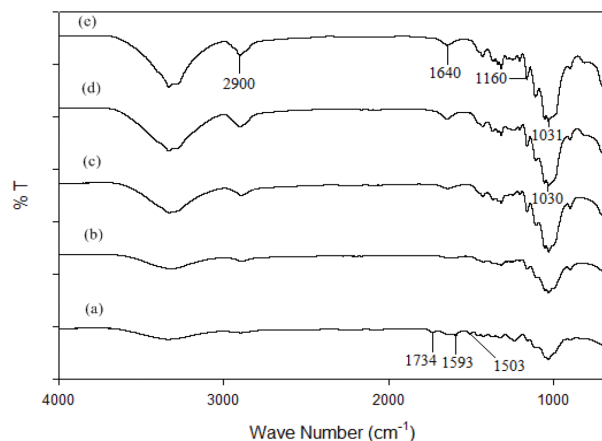

FIGURE 1. FTIR spectra for (a) raw kenaf core, (b) alkaline-treated kenaf core, (c) bleached kenaf core, (d) 45 min hydrolyzed cellulose and (e) 55 min hydrolyzed cellulose

On the other hand, vibration of functional groups C-H and stretching of C-O group (Johar et al. 2012) appeared at 1032 cm^{-1} for KC, 1030 cm^{-1} for alkaline treated pulp, 1029 cm^{-1} for bleached pulp, 1030 cm^{-1} for pulp hydrolyzed for 45 min and 1031 cm^{-1} for pulp hydrolyzed for 55 min. This shows that this peak was present in all samples and was not shifted even after the chemical treatment.

S-O-H bending of HSO$_4$ group (Periasamy et al. 2009) can be observed on the peak at around 813 cm^{-1} representing the sulphate functional group which stabilized the CNC suspension (Siqueira et al. 2010). This peak was visible after treatment. There were no peak changes between bleached pulp and hydrolyzed pulp. This showed that cellulose structure produced after bleaching process and acid hydrolysis remains unchanged.

XRD

Figure 2 shows the XRD diagrams for kenaf core, alkaline-treated kenaf core, bleached kenaf core, 45 min hydrolyzed cellulose and 55 min hydrolyzed cellulose. Table 3 shows the CrI for every sample. CrI increased after each chemical treatment, i.e. from 45.1 to 65.0% after the removal of amorphous region of hemicellulose after the alkaline treatment. After the bleaching process, CrI of the bleached pulp has no significant changes which can be attributed to the removal of most of the hemicellulose and lignin after pulping. Compared to the study that was carried out by Chan et al. (2013), the CrI of the sample increased after the bleaching process due to high lignin content of the fibre after the alkaline treatment.

Cellulose contains crystalline and amorphous segments. The amorphous segment of the cellulose has lower density than that of the crystalline region. During concentrated acid hydrolysis, amorphous segments of cellulose were hydrolysed to release individual crystallites or CNC (Peng et al 2011). After acid hydrolysis
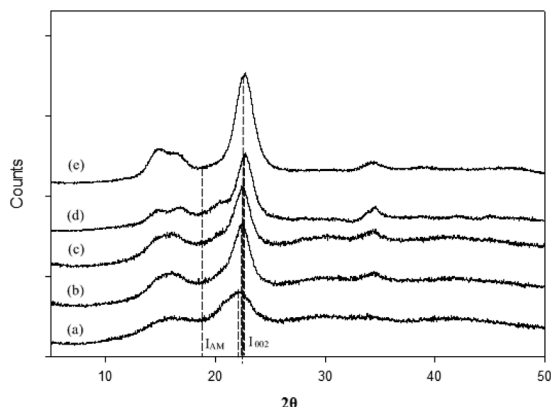
FIGURE 2. XRD intensities of (a) kenaf core, (b) alkaline-treated kenaf core, (c) bleached kenaf core, (d) 45 min hydrolyzed cellulose and (e) 55 min hydrolyzed cellulose

process for 45 min, CrI of the sample increased to 74.0%. The CrI increased to 81.2% for the bleached kenaf core that was hydrolyzed for 55 min. Penetration of hydronium ions into cellulose could remove amorphous region of cellulose and eventually freed individual cellulose crystal via hydrolytic cleavage of glycosidic bond (Lima & Borsali 2004).

The CrI for pulp that was hydrolyzed for 55 min is higher than that of the pulp that was hydrolyzed for 45 min. This shows that there was still amorphous cellulose presented in the sample which can be removed even though it has been hydrolyzed for 45 min. After being hydrolyzed for 55 min, more amorphous cellulose was removed and contributed to the increase in CrI.

TEM

Figures 3 and 4 show the TEM images of the CNC produced. Figure 3 presents the CNC produced after being hydrolyzed for 45 min which was precipitated at (a) 3000 rpm, (b) 6000 rpm, (c) 9000 rpm and (d) 12000 rpm while Figure 4 shows the CNC produced after being hydrolyzed for 55 min and precipitated at (a) 3000 rpm, (b) 6000 rpm, (c) 9000 rpm and (d) 12000 rpm. All CNC produced were in rod shape with different lengths and diameters.

CNC'S LENGTH AND DIAMETER ANALYSIS

A total of 50 measurements of CNC's length and diameter that precipitated at 3000, 6000, 9000 and 12000 rpm that had undergone acid hydrolysis process for 45 and 55 min were measured using Axio Vision Release software 4.8.2. From the 50 measurements taken, graph of length versus

TABLE 3. Cellulose intensity peak and its respective degree of crystallinity

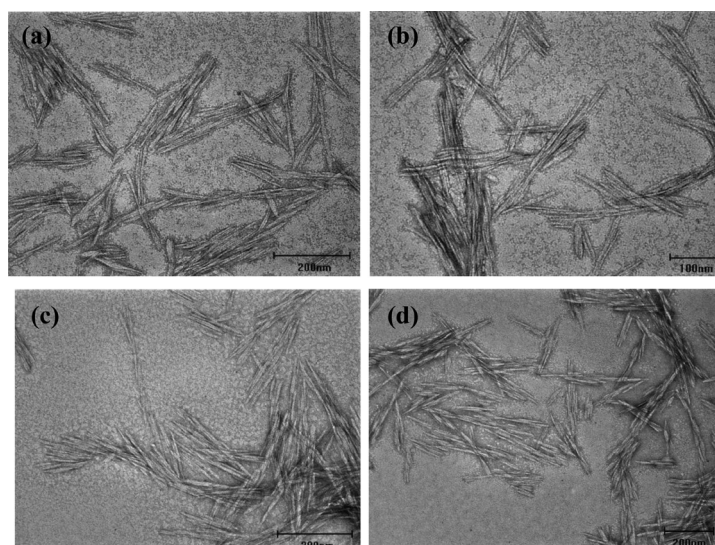| Samples	Cellulose intensity peak (2θ)	Degree of crystallinity (%)
Raw kenaf core	22.025	45.13
Alkaline-treated kenaf core	22.4	65.04
Bleached kenaf core	22.5	65.74
Hydrolyzed NCC (45 min)	22.675	73.98
Hydrolyzed NCC (55 min)	22.725	81.15

FIGURE 3. TEM morphology of CNC hydrolyzed for 45 min precipitated at
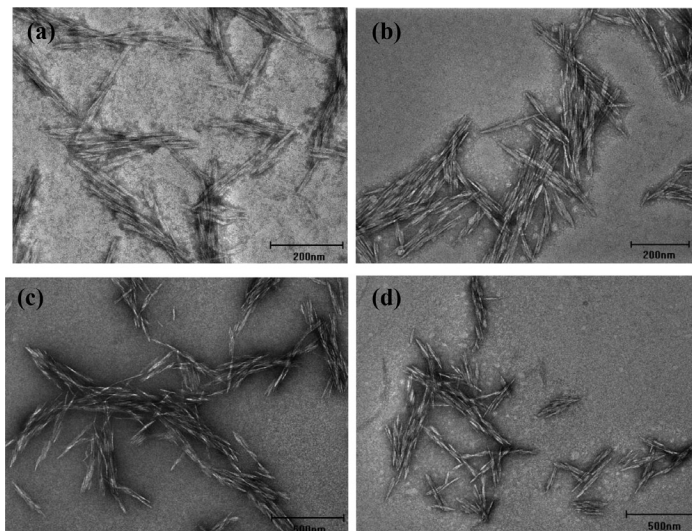(a) 3000 rpm, (b) 6000 rpm, (c) 9000 rpm and (d) 12000 rpm

FIGURE 4. TEM morphology of CNC hydrolyzed for 55 min precipitated at
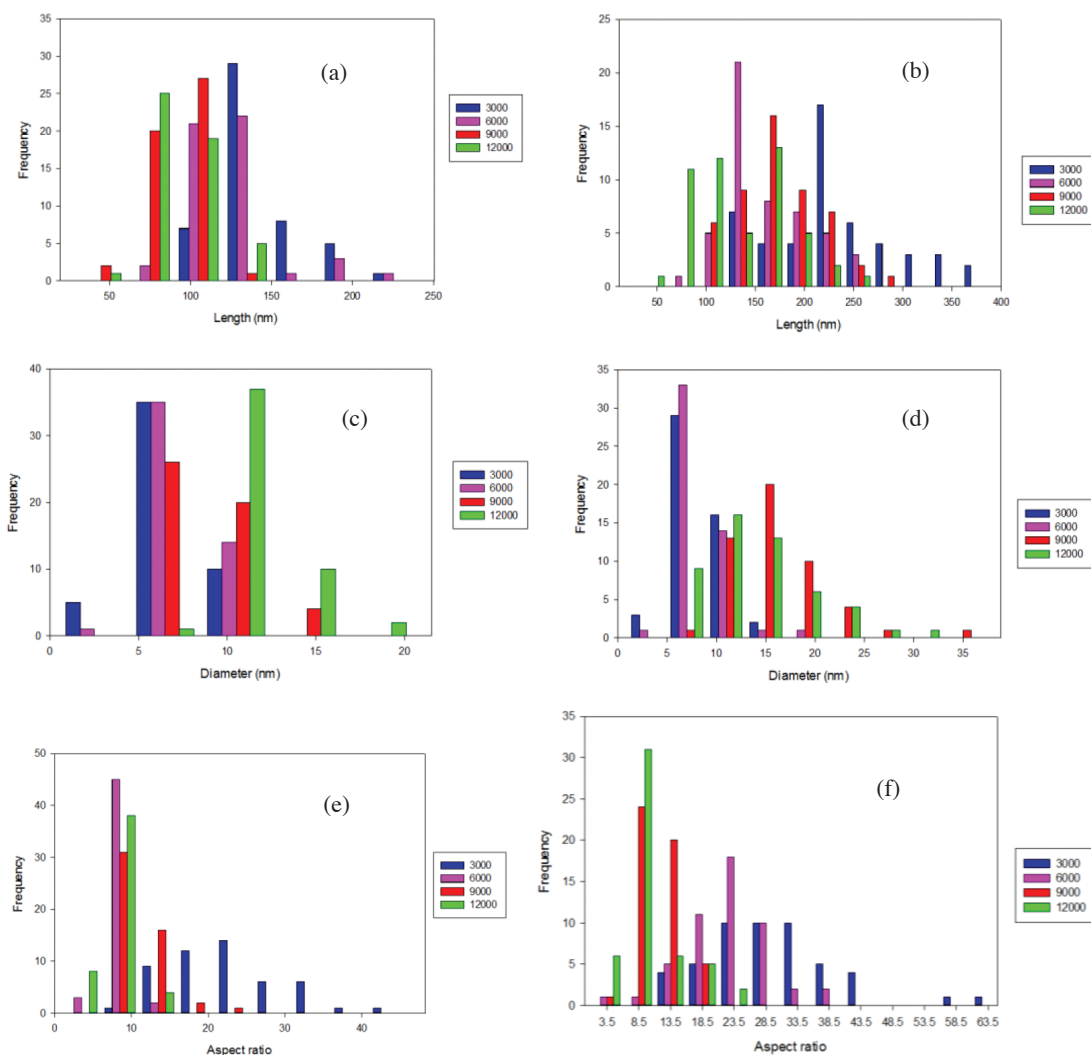(a) 3000 rpm, (b) 6000 rpm, (c) 9000 rpm and (d) 12000 rpm

FIGURE 5. Graph of (a) length vs frequency for 45 min CNC, (b) length vs frequency for 55 min CNC,
(c) diameter vs frequency for 45 min CNC, (d) diameter vs frequency for 55 min CNC,(e) aspect ratio vs
frequency for 45 min CNC and (f) aspect ratio vs frequency for 55 min CNC

frequency and diameter versus frequency were plotted. All the centrifugation speed rotation per minute (rpm) was converted to relative centrifugation force (rcf).

CNC that had undergone acid hydrolysis process for 45 min has length ranging from 50 to 270 nm. From Figure 5(a), the CNC that precipitated at 940 rcf was that longest with 265.2 nm in length and CNC that precipitated at speed 14910 rcf was the shortest with 51.1 nm.

CNC that had been undergone acid hydrolysis process for 55 min have length around 40 to 370 nm. From Figure 5(b), the CNC that precipitated at 940 rcf was that longest with 366.3 nm in length and CNC that precipitated at speed 14,910 rcf was that shortest with 37.6 nm. Both graphs show that the increase in centrifugation speed will produce CNC that are shorter in length. Longer CNC are heavier and will precipitate first at low centrifugation speed.

CNC that had been undergone acid hydrolysis process for 45 min have diameter around 3 to 21 nm. From Figure 5(c), the CNC that precipitated at 940 rcf have the shortest diameter with 3.4 nm and CNC that precipitated at speed 14910 rcf has the longest diameter with 20.1 nm.

CNC that had been undergone acid hydrolysis process for 55 min have diameter around 3 to 35 nm. From Figure 5(d), the CNC that precipitated at 940 rcf have the shortest diameter with 4.0 nm and CNC that precipitated at speed 8,400 rcf have the longest diameter with 34.7 nm.

Graph for CNC that have been undergone acid hydrolysis process for 45 min shows that the diameter increases with centrifugation speed. While graph for CNC that have been undergone acid hydrolysis process for 55 min show its diameter increase when speed centrifugation increased from 940 to 8400 rcf but declines when centrifugation speed was increased to 14910 rcf.

From 50 length and diameter readings taken, the mean length and diameter were calculated for every centrifugation speed. Table 4 shows that CNC collected at centrifugation speed 940 rcf were the longest and have the lowest diameter. Mean length for CNC that had been through acid hydrolysis process for 45 min show that it decreased from 163.5 to 91.9 nm and its average diameter increased from 7.6 to 11.8 nm when centrifugation speed were increased from 940 to 14910 rcf.

For CNC that had been through acid hydrolysis process for 55 min, the mean length decreased from 233.5 to 131.2 nm as the centrifugation speed increases. Its average diameter increased from 8.4 to 16.1 nm when speed was increased from 940 to 8400 rcf, but it drops to 13.8 nm when centrifugation speed was increased to 14910 rcf. Therefore, this showed that increased in centrifugation speed influence more on the CNC length than its diameter.

Figure 5(e) is the aspect ratio graph versus frequency for CNC that had been through acid hydrolysis process for 45 min. The aspect ratio values for CNC that precipitated

TABLE 4. Comparison of average volume, length, diameter and aspect ratio of CNC by varying RCF for CNC

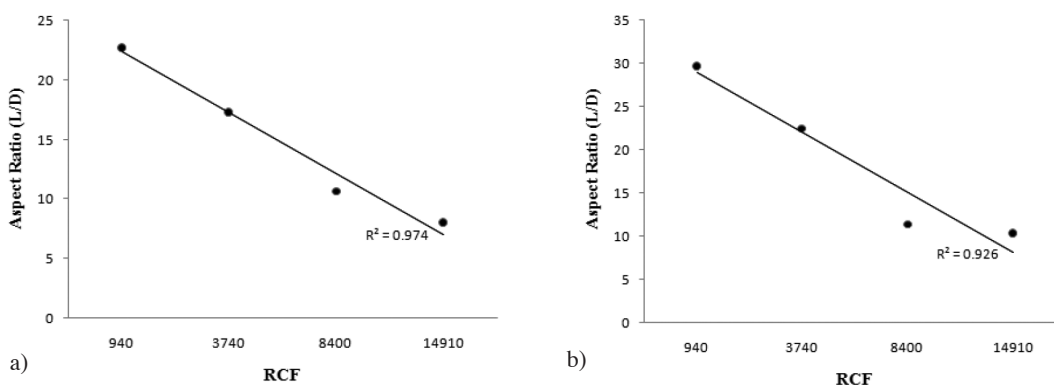| Sample	RPM	RCF	Average volume (nm^3)	Average length (nm)	Average diameter (nm)	Aspect ratio (L/D)
1	3000	940	7356	163	7.58	22.77
2	6000	3740	6803	133	8.07	17.32
3	9000	8400	6427	94	9.33	10.65
4	12000	14910	9925	92	11.78	8.02
5	3000	940	12820	233	8.37	29.75
6	6000	3740	10223	181	8.48	22.50
7	9000	8400	35001	173	16.05	11.42
8	12000	14910	19480	131	13.76	10.36

FIGURE 6. The relative centrifugal force (RCF) shows an inverse relationship towards the aspect ratio of CNC (a) 45 min of hydrolysis and (b) 55 min of hydrolysis

at 940 rcf ranges from 10 to 43. The aspect ratio values for CNC that precipitated at 3740 rcf ranges from 3 to 16, 8400 rcf ranges from 8 to 25 and 14910 rcf ranges from 6 to 25.

While Figure 5(f), is the aspect ratio graph versus frequency for CNC that had been through acid hydrolysis process for 55 min. The aspect ratio values for CNC that precipitated at 940 rcf ranges from 12 to 63. The aspect ratio values for CNC that precipitated at 3,740 rcf ranges from 6 to 39, 8,400 rcf ranges from 3 to 20 and 14910 rcf ranges from 3 to 26.

Linear trendline graph was plotted using average length, average diameter, average volume and average aspect ratio data for both CNC hydrolyzed for 45 and 55 min. This is to find the best-fit straight line. The most reliable trendline is when the R^2 value is on or near 1. The graph using average length, average diameter and average volume have R^2 values far from 1. Figure 6 shows the best-fit straight line obtained aspect ratio versus RCF. So this shows that the sedimentation of CNC during centrifugation was mostly affected by its aspect ratio compared to its length, diameter and volume. The aspect ratio of CNC produced was inversely proportional to RCF.

CONCLUSION

In summary, CNC were produced from kenaf core via acid hydrolysis after chemical pre-treatments. Most of the hemicelluloses were removed from the pulp by pulping and lignin was removed from bleaching process using the FTIR analysis. The highest crystallinity degree was reached at 81.2% by CNC that were hydrolyzed for 55 min. The TEM image shows that the CNC produced were rod-like shape. The mean length for CNC hydrolyzed for 45 and 55 min showed that the increase in centrifugation speed precipitates shorter CNC. CNC of different aspect ratio can be fractionated using a simple centrifugation method, in which, CNC with higher aspect ratio will sediment first followed by CNC of lower aspect ratio. This relatively simple technique of CNC fractionation of different aspect ratios will allow the fine tuning of pitch length of self-assembled CNC in future studies.

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