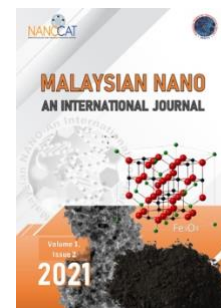




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Acid Hydrolysis Process of Mengkuang Cellulose

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Abstract

Cellulose nanocrystals (CNC) are involved in many applications due to their unique properties. Thus, the understanding hydrolysis process help to control the preparation conditions and the chemical reactions during the different uses. Cellulose from mengkuang leaves (*Pandanus tectorius*) was used in this research to prepare CNC. Sulfuric acid (H₂SO₄ 60 wt.%) hydrolysis was carried out over different times to produce the nanocrystals. The effects of hydrolysis time on the morphology, chemical structure, and crystallinity index of CNC were examined using transmission electron microscopy (TEM), Fourier transforms infrared spectroscopy (FTIR), and X-ray diffraction (XRD). The degradation process is dependent on the time of hydrolysis and the crystalline domains of cellulose fibers. The process starts by expanding the cellulose chains. FTIR results showed that no change in the chemical structure of the nanocrystals during the hydrolysis reaction. The results demonstrated that the optimum hydrolysis time to produce cellulose nanocrystals from mengkuang cellulose, with a crystallinity index of about 74% and aspect ratio of 15, was 45 min at 45 °C using 60 wt% H₂SO₄.

Keywords: Acid hydrolysis; Cellulose; Cellulose nanocrystals; Aspect ratio; Crystallinity index.

1. Introduction

Cellulose microfibril is a flexible material made up of crystal domains linked by amorphous domains. It is reported that the diameter of elementary fibrils (protofibrils) is about 5 nm, and the microfibrillated (also called nanofibrillated) cellulose has a diameter of 20–50 nm [1-3]. There are three methods to break up the macroscopic cellulosic fibers into nanoscale fibrils. They are strong acid hydrolysis, enzymatic hydrolysis, and the application of high shear forces [4]. The acid hydrolysis method is used for the isolation of cellulose nanocrystals (CNC). This method allows the removal of the amorphous domains of the fibrils while keeping the crystalline domains intact in the form of crystalline nanoparticles; the process of hydrolysis is carried out under controlled conditions. Sulfuric, hydrochloric, phosphoric, and hydrobromic acids were used to prepare CNC [5]. The use of sulfuric acid for CNC preparation leads to a more stable aqueous suspension than that prepared using hydrochloric acid. Indeed, the H₂SO₄ prepared nanocrystals present a negatively charged surface, whereas the HCl prepared nanocrystals are not charged [5, 6]. The nature of the resulting cellulose nanocrystals depends on the source and isolation conditions. Under the conditions employed, most amorphous regions could be removed and leaving cellulose nanocrystals [7]. But, further, hydrolysis could degrade the polycrystalline domains [8]. Different sources have been used to prepare nanocellulose, such as tunicate [9], tomato plant residue [10], cotton [11], sugarcane straw [12], and industrial denim [13].

The present work reported details of the acid hydrolysis process of cellulose extracted from Mengkuang leave. The cellulose at different hydrolysis times was examined using transmission electron microscopy (TEM), Fourier transforms infrared spectroscopy (FTIR), and X-ray diffraction (XRD).

2. Materials and Methods

2.1 Materials

Mengkuang cellulose fibers with diameters between 5–80 μm were used [14, 15]. Sulfuric acid (95-98%) was purchased from System.

2.2 Preparation of cellulose nanocrystals (CNC)

The CNC was prepared by acid hydrolysis of the cellulose extracted from mengkuang leaves. The hydrolysis process was carried out with 60 wt.% of H₂SO₄ solution at T= 45 °C (preheated), and the periods of hydrolysis were 15, 30, 45 & 60 min (H1, H2, H3 & H4, respectively). To remove the excess acid solution, the hydrolyzed cellulose suspensions were washed several times by centrifugation (10,000 rpm, 10 min & 10 °C).

The suspensions were then dialyzed against distilled water using dialysis tubing cellulose membrane to reach a constant pH. The resulting suspensions were sonicated for 30 min and then stored in the refrigerator. It is suggested to add several drops of acetone to avoid bacterial growth.

The yield of cellulose nanocrystals prepared was calculated in dry form as a percentage ($Y\%$) of the weight after hydrolysis ($wt.h$) to the initial weight ($wt.o$), as shown in the equation below. The cellulose was dried in an oven for 1 h at 100°C and then cooled down in a desiccator until a constant weight was recorded. The initial weight was taken from the dried cellulose ($wt.o$), and then it was hydrolyzed using acid as described earlier. After hydrolysis, the suspension of cellulose nanocrystals was frozen and then placed in a freeze-drying tube connected to the freeze dryer's vacuum chamber. After drying the samples (24 h), they were taken from the freeze dryer and kept in the desiccator after which the weight of the cellulose nanocrystals was taken ($wt.h$). The process of calculating the dry yield was duplicated to get the average.

$$Y\% = \frac{wt.h}{wt.o} \times 100 \quad (1)$$

2.3 Characterization

2.3.1 Transmission electron microscopy (TEM)

The Philips CM12 TEM microscope was used to measure the dimensions of CNC (the operation was at 80 kV).

2.3.2 Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra of CNC samples were recorded using a Perkin Elmer Spectrum 2000 ATR- FTIR spectrometer in the range of $4000\text{--}650\text{ cm}^{-1}$ (the scanning resolution was 4 cm^{-1}).

2.3.3 X-ray diffraction (XRD)

X-ray diffractometer (D8 Advance, Bruker AXS) was used to study the crystallinity index ($Xc\%$) of the CNC samples. The empirical method [16] was applied to obtain the $Xc\%$ of the samples, as shown in the equation below,

$$Xc\% = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad (2)$$

where I_{002} and I_{am} are the peak intensities of crystalline and amorphous regions of CNC, respectively.

3. Results and discussion

3.1 Morphological analysis

The ensuing suspensions of isolated cellulose nanocrystals prepared from mengkuang cellulose at different hydrolysis times are shown in Figure 1. The average yields of CNC were measured at different hydrolysis times as a percentage of the weight before and after hydrolysis; the yield percentages are reported in Table 1. The yield of nanocrystals decreased when the hydrolysis time increased. That is because the cellulose degradation increases with increasing the acid hydrolysis time. The acid breaks cellulose's glycosidic bonds, resulting in cellulose nanocrystals and a sugar molecule (glucose). The dramatic reduction was during the first 45 minutes of the hydrolysis process, after which there was no significant change. This observation is attributed to the rate of the degradation process. Hydrolysis of cellulose is dependent on crystallinity. There are two different rates. The degradation of the amorphous regions is a rapid process, and further hydrolysis would degrade the polycrystalline domains, which is a slow hydrolysis process [8, 17].



Figure 1: Aqueous suspensions of CNC at different hydrolysis times.

Table 1: The average of the yield (Y), degraded domain ($D = 100 - Y$), length (l), diameter (d), and aspect ratio ($AR = l/d$) of mengkuang cellulose nanocrystals

Samples	l (nm)	d (nm)	AR	Y (%)	D (%)
15 min hydrolysis (H1)	-	-	-	71 ± 1	29
30 min hydrolysis (H2)	202 ± 81	15 ± 4	14	60 ± 1	40
45 min hydrolysis (H3)	179 ± 84	12 ± 3	15	28 ± 0.9	72
60 min hydrolysis (H4)	155 ± 35	12 ± 4	13	25 ± 0.7	75

TEM images of extracted cellulose nanocrystals at different times of hydrolysis are shown in Figure 2. Figure 2-H1 shows the unhinged morphology of cellulose after 15 min hydrolysis, and it is difficult to recognize individual nanocrystals. This observation indicates that the acid treatment disturbed the interconnecting cellulose chains without destroying the structure [3]. Thus, hydrolysis for 15 min is not enough to produce the isolated nanocrystals. It might be due to the high percentage of amorphous domains, which can be estimated from the yield percentage listed in Table 1. After 15 minutes of hydrolysis, only 29% of the amorphous was removed; the elimination of this amount was not enough to take out the isolated nanocrystals. After 30 min hydrolysis, 40% of amorphous regions were hydrolyzed, and individual rod-like nanoparticles can be observed in TEM (Figure 2-H2). Another 32% were removed after 45 minutes of hydrolysis, and Figure 2-H3 shows the isolated cellulose nanocrystals. From these results, cellulose extracted from mengkuang leaves consists of about 72% amorphous domains and about 28% nanocrystals. Further acid hydrolysis (60 min) removed just 3% (Figure 2-H4). Some aggregation of the nanocrystals can be seen, which is due to the evaporation step during sample preparation

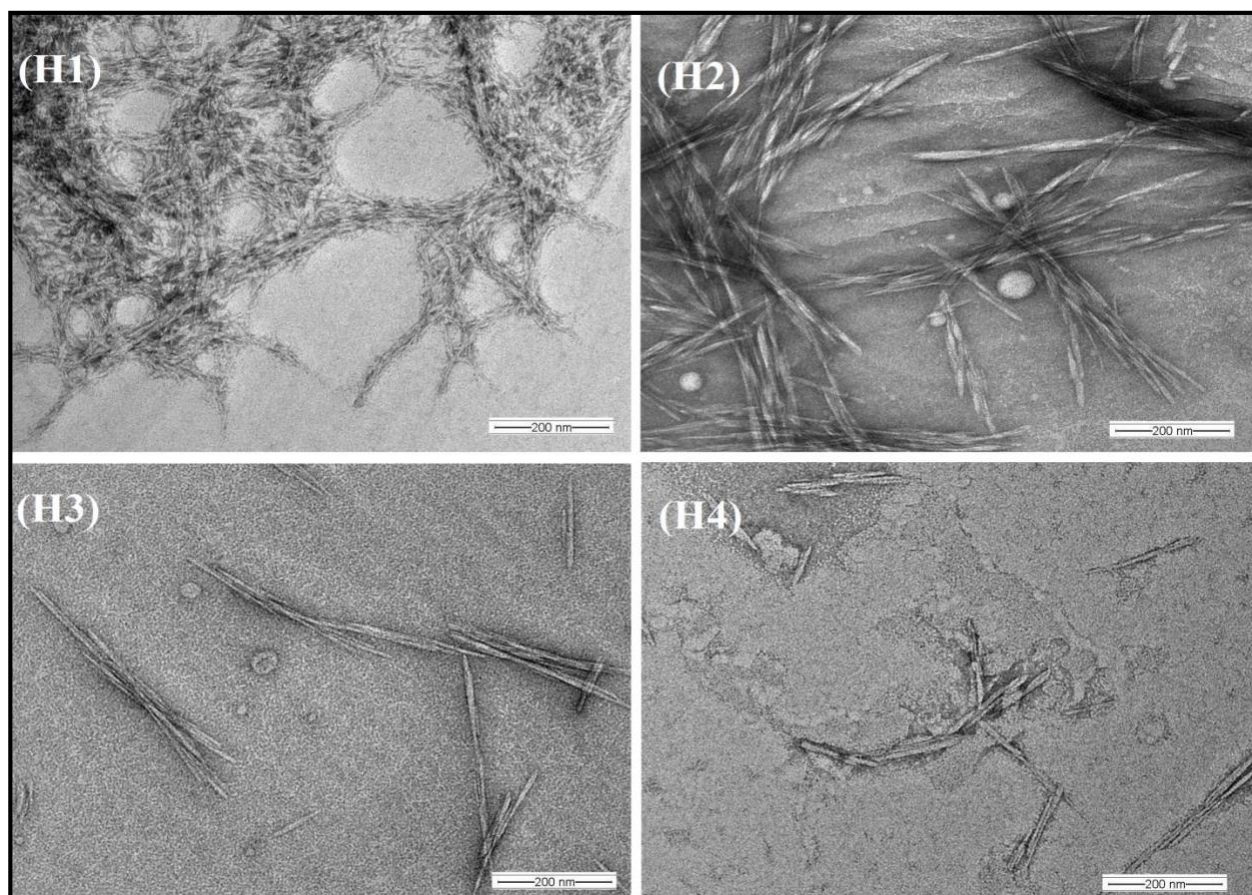
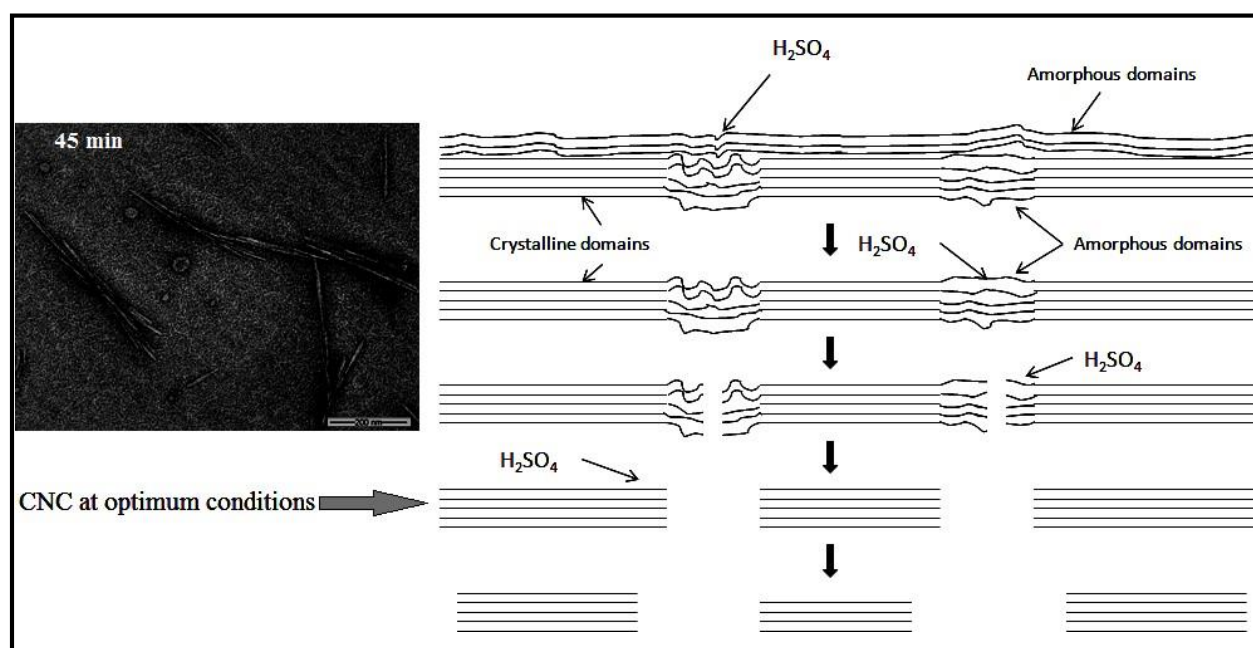


Figure 2: TEM images (magnification 28000) of cellulose nanocrystals at different times of hydrolysis (H1) 15 min, (H2) 30 min, (H3) 45 min and (H4) 60 min.

Mengkuang nanocrystals had a length of 50 – 400 nm (with an average value around 200 nm) and a diameter in the range of 5 – 25 nm. The distributions of the diameter and aspect ratio were reported in previous works [14, 15]. In the present discussion, the average of the dimensions and aspect ratio with the standard deviation will be used to show the change during the hydrolysis process. Note that the standard deviation is not indicating the range of nanocrystals dimensions; it shows the dispersion from the average. The average length (*l*), diameter (*d*), and aspect ratio (AR) of CNC are reported in Table 1. Mengkuang cellulose nanocrystals had an average length value around 202 nm, and the diameter was about 15 nm after 30 minutes of hydrolysis. During the acid hydrolysis process, the length degraded with increasing the hydrolysis time. There was no significant change in the diameter up to 45 minutes of hydrolysis, and no change can be observed as the duration of acid hydrolysis increases to more than 45 minutes. The length decreased from about 202 nm at 30 min to 155 nm at 60 min. The diameter decreased from 15 nm at 30 min to 12 nm at 45 min. It is worth to notes that, with increasing hydrolysis time, no significant change in the standard deviation of the diameter distribution while that of the length distribution decreased after 60 min. This shows the trend of degradation and a large change in the length compared to the diameter. The number of shorter nanocrystals increases with increasing hydrolysis time. A similar trend was informed by other researchers for cotton CNC with increasing hydrolysis temperature [18].

The aspect ratio was calculated as a ratio of the length and diameter average. The mengkuang cellulose nanocrystals had an aspect ratio of about 14 at 30 minutes of hydrolysis time. It was slightly increased as the hydrolysis time increased to 45 minutes, after which it was decreased. Thus, 45 minutes was taken as optimum hydrolysis time as the nanocrystals had the highest aspect ratio. At the optimum time, cellulose nanocrystals had an aspect ratio of about 15 with a yield of 28%.

Acid hydrolysis of cellulose could be summarised in several steps after the penetration of the sulfate groups to cellulose chains, as shown in Scheme 1. It concludes TEM investigation and the reported studies by other researchers [3, 4, 8, 19]. Firstly is the degradation of the amorphous domains which surround the flexible strand of cellulose microfibrils. Secondly is the nanofibrils extraction by amorphous domains degradation, which linked the crystalline domains along the microfibril. The third step is the degradation of all amorphous domains around the crystals. Finally, further hydrolysis leads to degrading the crystals' domains.



Scheme 1: The simplified scheme of likely acid hydrolysis steps of extraction cellulose nanocrystals.

3.2 Fourier transform infrared spectroscopy (FTIR) analysis.

Figure 3 shows FTIR spectra of cellulose nanocrystals at different times of hydrolysis. They are the typical absorption bands of cellulose [20, 21]. The absorbance peaks in the region around 3300 and 2900 cm^{-1} indicate the stretching of the O–H and C–H groups in cellulose, respectively. The bands between 895 and 1156 are associated with the C–O stretching and CH_2 rocking vibrations. An absorbance peak of $-\text{CH}_2$ symmetrical bending can be seen at about 1426 cm^{-1} as well as the absorbance bands of CH, and COH bending are in the region between 1200 – 1275 cm^{-1} . These different bands can be seen in all spectra of CNC. Up to 60 minutes of hydrolysis time, no difference was found in the spectra of cellulose nanocrystals compared to that of mengkuang cellulose [15]. This suggests that at the present conditions of hydrolysis, the molecular structure of cellulose was not changed.

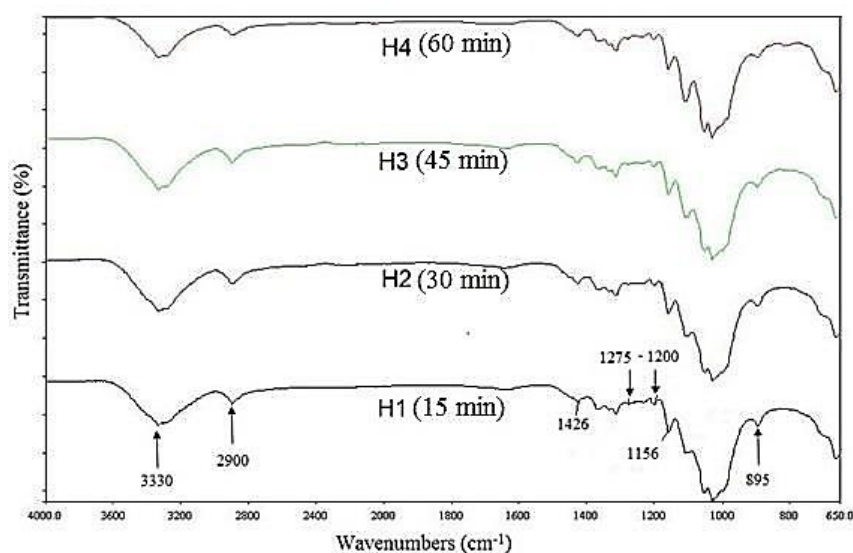


Figure 3: FTIR spectra of cellulose nanocrystals at different times of hydrolysis.

3.3 X-ray diffraction (XRD) measurements

It is known that acid hydrolysis breaks down the cellulose chains [22, 23]. This degradation starts with amorphous domains of cellulose; because of breaking down the cellulose chains, the crystallinity index increases. Further acid hydrolysis would degrade the crystalline domains [8]. XRD patterns of cellulose nanocrystals at different times of acid hydrolysis are shown in Figure 4. The X-ray diffractograms show that the three intensity peaks are located at 2θ values around 16° , 22.6° , and 34.5° , which are related to the crystalline structure of cellulose I. There is a shoulder peak at a 2θ value around 20° in the XRD diffractogram of the cellulose sample after 15 minutes of hydrolysis time (H1). It is related to the crystalline structure of cellulose II. Therefore, a mixture of cellulose I and cellulose II is produced in this duration of hydrolysis. It is worth comparing the observation with results reported by Sèbe et al. [24], although sulfuric acid hydrolysis's preparation conditions were different. Sèbe et al. [24] obtained cellulose II nanocrystals at narrow conditions, and a mixture of cellulose I and cellulose II was produced in some cases. There are two possibilities for this phenomenon. Firstly, the sulfuric acid acts as a swelling agent: cellulose I can be transformed to cellulose II after an intermediate swelling and subsequent removal of the agent. The second possibility is about recrystallization of cellulose; it occurs simultaneously with the chain splitting during the hydrolysis process [23- 25]. Based on the results in our research, the form of cellulose II is observed only after a short time of hydrolysis (15 min), but it is not observed with increasing the hydrolysis time. One possibility is that the penetration of sulfate groups into cellulose's amorphous domains leads to expanding the cellulose chains to begin the acid degradation.

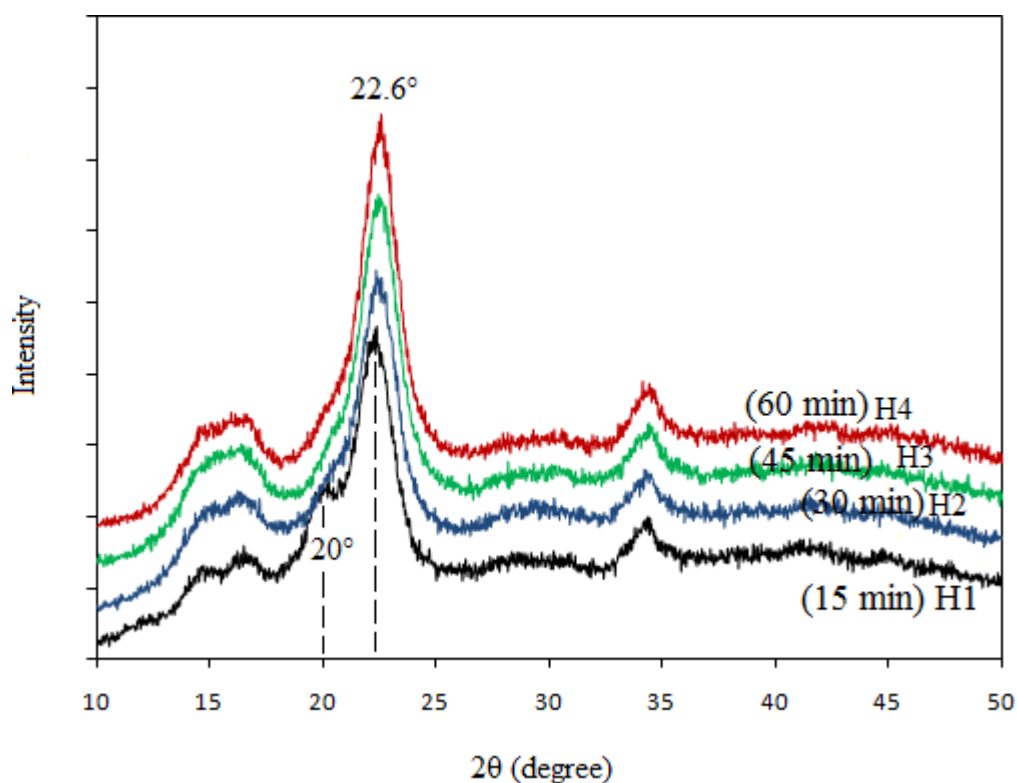


Figure 4: The X-ray diffractograms of cellulose nanocrystals at different times of hydrolysis.

Compared to the XRD pattern of the extracted cellulose [15], there is no significant change in the intensity of the peaks at about 22.6° up to 30 min hydrolysis. It increases while the time of the hydrolysis process increases, and the peaks tend to be sharper. The crystallinity index of cellulose nanocrystals was calculated by the Segal method [16], as mentioned earlier, and the values are listed in Table 2. There is no significant change in the crystallinity index of cellulose nanocrystals at 15 and 30 min of hydrolysis compared to one of the cellulose fibers extracted from mengkuang fiber [15]. The crystallinity index of CNC trends to increase at 45 min of hydrolysis. These findings could attribute to the time of CNC isolation and the degradation of amorphous domains. These results were supported by TEM observation, which indicates that 45 min of acid hydrolysis degrades the amorphous domains of mengkuang cellulose.

Table 2: The crystallinity index percentage ($X_c\%$) of cellulose nanocrystals.

Sample	2θ at am* ($^\circ$)	2θ at 002 ($^\circ$)	$X_c\%$
15 min hydrolysis (H1)	18	22.4	69.4
30 min hydrolysis (H2)	18.4	22.3	68.5
45 min hydrolysis (H3)	18.3	22.4	73.6
60 min hydrolysis (H4)	18.1	22.6	77.3

* amorphous background

4. Conclusion

Isolation of cellulose nanocrystals from mengkuang cellulose was performed with sulfuric acid hydrolysis (60 wt.%) at 45 °C (preheated). The hydrolysis was carried out at different times: 15, 30, 45, and 60 minutes. The results showed that the cellulose hydrolysis process is dependent on the percentage of amorphous and crystalline domains. They revealed that the optimum time of hydrolysis is 45 min. Cellulose from mengkuang leaf consists of about 28% cellulose nanocrystals with an aspect ratio of about 15 at optimum conditions. On the other hand, the crystallinity index of the cellulose nanocrystals increased with increasing the hydrolysis time. The index was about 74% at optimum conditions.

Competing interest

The authors declare no conflict of interest.

Acknowledgments

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