Asian Journal of Applied Sciences (ISSN: 2321 – 0893)
Volume 03 – Issue 05, October 2015

SYNTHESIS OF NANOCELLULOSE FROM RUBBERWOOD FIBERS VIA ULTRASONICATION COMBINED WITH ENZYMATIC AND CHEMICAL PRETREATMENTS

Pradeep Kumar Poddar* ,Arun Gupta* Shima Saidatul Jamari, Ng Siew Kim, Tanveer Ahmed Khan, Swati Sharma, Mohd Aizudin Abd Aziz

Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300, Kuantan, Pahang, Malaysia
*Corresponding authors email: pradeeppoddarwst@ gmail.com / arun@ump.edu.my

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ABSTRACT— ***This paper presents synthesis of nanocellulose from rubber wood (Hevea brasiliensis) fibers via ultrasonication combined with enzymatic and chemical pretreatments. Cellulose nanofibers were separated from rubber wood fibers in three distinct stages. Initially, rubber wood fibers were subjected to a series of enzymatic and chemical pre-treatments to eliminate lignin and hemicellulose. The obtained chemical-purified cellulose fibers were then mechanically separated into nanofibers using ultrasonication. The diameter distributions of the resulting nanofibers were dependent on the output power of ultrasonic treatment. The extent of dispersion improved significantly with increasing output power of ultrasonic treatment. Microscopy study showed that the diameters of the nanofibers isolated ranged from 8.7 to 20 µm. The effectiveness of laccase and xylanase enzymes was also studied. The results obtained from FTIR and thermo gravimetric analyses indicated that there were consistencies between the studied enzymes to the thermal stability or chemical structure. FTIR spectroscopy confirmed that the prominent peaks indicating that most of the lignin and hemicellulose were removed during the step-wise chemical treatment were present in the spectrum. FTIR spectroscopy suggested that the spectrum of cellulose nanofibers obtained under different ultrasonic output powers and chemical-purified cellulose fibers were similar, signifying that the molecular structure of cellulose were unaffected by the ultrasonic treatment. TGA results revealed that the thermal properties of cellulose nanofibers were enhanced and the thermal degradation temperature increased to proximately 310 °C as compared to 240 °C of the untreated rubber wood fibers. Results from this work may be potentially applied in various fields such as bio-nanocomposites, filtration media packaging, tissue engineering scaffolds, and so on.***

Keywords— Cellulose nanofibers, chemical pre-treatment, enzyme pre-treatment, ultrasonication, rubber wood

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1. INTRODUCTION

Cellulose is one of the most ubiquitous and abundant polymers on the planet. In plants and trees, cellulose acts as a structural reinforcing agent that provides mechanical strength as well as chemical stability to plants. Cellulose is a classic example where the reinforcing elements exist as whisker-like microfibrils that are biosynthesized and deposited in a continuous manner [1].The development of low-cost, sustainable and renewable resources is critical to meet the growing environmental concerns and energy demands. Nanocellulose can be used as a filler to enhance the thermal and mechanical properties of wood composite. Previously the study of heat and mass transfer model [2] and vertical density profile model [3] was developed to improve the understanding of internal process of wood composite. To improve the strength of wood composite, researchers have added aluminium nanoparticles [4] , carbon particles [5,6] and multiwalled carbon nanotubes [7] to enhance the mechanical strength of wood composite. Earlier some work is done to remove lignin from wood fibers using laccase enzymes and to use lignin to synthesize bioadhesive and bioplastic.[8-12].

Numerous studies have been conducted to elucidate the synthesis of highly purified cellulose nanofibers from cellulosic materials via cryocrushing [13], grinding; [14-18], high pressure homogenizing [19,20], chemical treatments [21,22] biological treatments [23,24] as well as a combination of two or several of the aforementioned methods. Since cellulose nanofibers are embedded in matrix substances such as hemicellulose and lignin, chemical methods are the most common approach utilized in removing the matrix substances [25,26]. However, there has been inadequate research for ultrasonic treatment. Due to its environmental benefits and convenience of the operation, ultrasonication has been selected to isolate cellulose nanofibers. Recent work suggested that ultrasonication does not significantly affect the fiber properties [27]. The application of enzymes in fiber processing has been mainly directed towards the degradation or modification of hemicelluloses and lignin while preserving the cellulosic fraction [28]. In addition, past studies were conducted to investigate the use of enzymes in enhancing the bleaching of cellulose fibers. The study repealed that the final brightness of pulp was enhanced with the aid of xylanase) [29-32]. A combination of methods often yields preferable cellulose nanofibers. Hence, this study aims to synthesize nanocellulose from rubber wood fibers via ultrasonication combined with enzyme and chemical pretreatments.

2. MATERIALS AND METHODS

2.1 Chemicals

Thermo–mechanically processed rubber wood fibers were supplied by Robin Resources Sdn. Bhd, Temerloh (Malaysia). Laccase and xylanase enzyme was supplied by Novozyme, Malaysia. In this work, an enzyme activity of 70 U/g and 30 U/g were considered for laccase and xylanase respectively. Sodium chlorite, acetic acid, potassium hydroxide and sodium acetate with a purity of 25%, 99.5%, 99.9% and 99.99%, respectively, were obtained from Sigma Aldrich (Malaysia).

2.2 Preparation of nanofibers

The separation process consists of three distinct stages, namely enzymatic pre-treatment, chemical pre-treatment and ultrasonic treatment. 15 grams of well-dried fibres were suspended in 485 grams of deionized water to prepare a solution of 3% w/w consistency (mass pulp/mass suspension) [34]. A buffer solution of sodium acetate and acetic acid was added to maintain the solution at pH 5. Six Unit/g (U/g) of enzymes were added to obtain a desired lignin oxidation as per the method suggested by Lei, Lin, and Li (2008). All enzymatic treatments were carried out at 40 to 65 °C. In order to allow the best enzymatic reaction, the reaction was kept at uniform rotational speed of 160 rpm for 24 hours. Once the pre-treatment has been completed, the suspension was filtered out and the fibers were exposed to heat at 60°C overnight to stop the enzymatic reaction. This was done to avoid further reaction of enzyme with the fiber which might deteriorate the desired results for the subsequent treatments.

In the chemical pre-treatment stage, the wood fibers were purified to prepare the cellulose fibers. Initially, the remaining lignin from the after effect of enzymatic pre-treatment was removed from the samples using acidified sodium chlorite solution at 75 °C for an hour. Next, the samples were treated in 3% w/w potassium hydroxide at 80 °C for two hours, and then in 6% w/w potassium hydroxide at 80°C for two hours in order to leach hemicellulose and residual starch. After a series of chemical treatments, the samples were filtered and rinsed with distilled water until the residues were neutralized. As per suggested by Chen et al. (2011), the samples were kept in a water-swollen state throughout the chemical process in order to avoid strong hydrogen bonding among nano fibers being generated after matrix removal.

After chemical pre-treatment, the chemical-purified cellulose fibers were subjected to ultrasonic treatment. The subsequent ultrasonication was conducted 30 minutes to isolate the nanofibers. The output power of the ultrasonication was varied for 80, 100 and 200 W respectively in order to investigate the effect of ultrasonic intensity on the nanofibrillation of the chemical-purified cellulose fibers. The same procedure was repeated for enzymatic reaction using xylanase in combination with chemical and ultrasonic treatment. In this set of experiment, the optimum output power for ultrasonic treatment which can provide the largest extent of dispersion was chosen for the nanofibrillation step, for which in this case, 200 W output power was chosen.

The effects of the treatments on the morphology of the fibers after chemical and ultrasonic treatments were observed with a Field Emission Scanning Electron Microscope (FE-SEM, JSM-7800F). All samples were sputter-coated with platinum to avoid charging. Fourier Transform Infrared (FTIR) spectroscopy studies were performed using a Perkin-Elmer FTIR spectrophotometer (Perkin Elmer Spectrum 100) to track changes in functional groups that might have been caused by the treatments. The samples were analyzed in the transmittance mode within the wave number range of 4000 to 900 cm-1. Thermo gravimetric analysis was carried out to examine the extent of thermal decomposition of the fibers after each treatment. The thermal stability data of each sample were obtained using a thermo gravimetric analyzer (TA Universal Q500) under linear temperature conditions. The temperature was set from 100 to 800 °C at a heating rate of 10 °C/min in a nitrogen atmosphere.

3. RESULTS AND DISCUSSION

3.1 Field Emission Scanning Electron Microscopy (FE-SEM) analysis

FESEM is an effective media to investigate the morphologies of composites. It is believed that the chemo-mechanical treatment employed will alter the morphology of the fibers in terms of size, that has been changed from 71-80µm untreated to 8-13 µm treated. The variations in the fiber morphology are evident from the FESEM images (Figure 1). Figure1a and c correspond to surface of chemical-purified fibers, which suggests that clusters of individualized fibers can be seen on the surface and the reduction of fibers diameters are more pronounced. Here, individualized nanofibers can be obtained with ultrasonic treatment (Figure 1b and d).

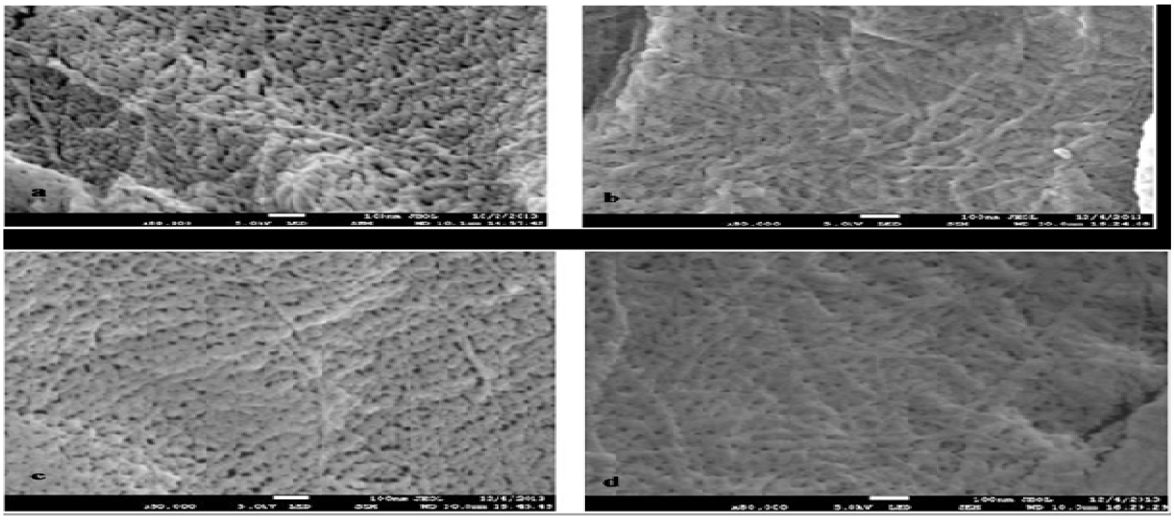

Figure 1: FE-SEM micrographs of (a) cellulose fibers and (b) nanofibers via combination of laccase and chemo-mechanically treated fibers; (c) cellulose fibers and (d) nanofibers via combination of xylanase and chemo-mechanically treated fibers

A comparison between the particle size diameter of the untreated and cellulose nanofibers illustrates that the diameters of the former ranged from 71 to 80µm whereas those of the latter ranged from 13 to 20 µm, 11.5 to 13 µm and 8.7 to 10 µm respectively for output power of 80 W, 100 W and 200 W. Figure 2 illustrates the comparison between the particle size distribution of untreated fibers with that obtained after ultrasonic output power at 200 W. The results emphasize that the sizes of the rubber wood fibers decreased as a result of the chemical and ultrasonic treatment. As a result, it is verified that the chemical treatments affected separation of micro-sized fibers from the fiber bundles.

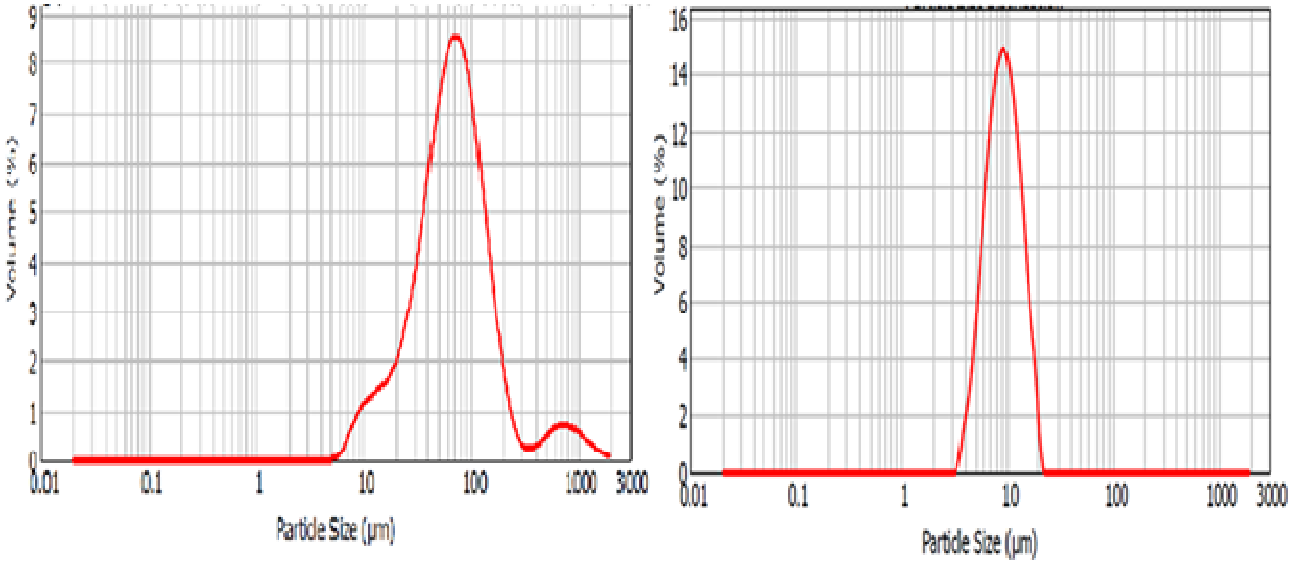

Figure 2: Particle size distribution of (a) untreated fibers and (b) cellulose nanofibers with output power of 200 W.

3.2 Fourier Transform Infrared Spectroscopy (FTIR) analysis

FTIR spectroscopy is an indispensable technique in establishing variations in the chemical structures of the isolated samples introduced by different treatments. Figure 3 compares the FTIR spectra of the untreated and chemo-mechanically treated fibers in combination with laccase enzyme.The results indicated the occurrence of chemical reactions during chemical treatments of the fibers. In Figure 3a, the dominant peaks observed in all samples in the regions 3335 cm-1and 2902cm-1 are due to stretching of the hydroxyl group and C-H groups of cellulose respectively[33,34]. When the spectra of the untreated rubber wood fibers are compared with those of the chemo-mechanically treated ones, the most evident difference is the disappearance of the vibration peak formerly observed at 1732 cm-1 from the spectra of the treated fibers. The disappearance of this prominent peak in the spectra of the is attributed to the C=O stretching in the acetyl and uronic ester groups of the hemicelluloses or the ester carbonyl groups in the p-coumaric units of the lignin [35-37]. The disappearance of this peak from the spectra of the treated fibers can be ascribed to treatment of the raw fibers with NaOH followed by bleaching as these treatments are known to remove most of the hemicelluloses and lignin from the rubber wood fibers [36,37,15]

On the other hand, the peak detected at 1593 cm-1 in the spectra of the untreated fibers is attributed to the C=C stretching of the lignin carboxyl groups while the peak at 1237 cm-1 in the same spectra corresponds to the C–O–C stretching of the aryl–alkyl ether linkage in lignin [38]. Disappearance of these two peaks from the spectra of the bleached pulp fibers and nanofibers is believed to be due to removal of lignin by the chemical treatments. In addition, appearance of a peak detected at around1642 cm-1 in the spectra of the purified fibers and nanofibers is most likely to be associated with H-O-H stretching for absorption of water [39-41]. Nevertheless, this peak was not detected in the spectra of the untreated fibers. According to Troedec et al. (2008), this is probably due to the reaction of sodium hydroxide with the hydroxyl groups of cellulose and subsequent formation of water molecules. The peak observed at 1428 cm-1 in the spectra of the bleached pulp fibers and nanofibers is ascribed to the CH2 symmetric bending [42]. Moreover, the peaks indicating O–H and C–O stretching were identified at 1061 cm-1 in all samples [39].

The FTIR spectra of the untreated and chemo-mechanically treated rubber wood fibers in combination with xylanase enzyme are as illustrated in Figure 3b. These spectra look very much similar to those treated using laccase enzyme. The dominant peaks observed in all samples in the regions 3334 cm-1 and 2903 cm-1 which is due to stretching of the hydroxyl group and C-H groups of cellulose respectively are also observed here [33,34]. The disappearance of C=O stretching frequency at 1731 cm-1 which corresponds to the carboxylic acid group is observed. The peak observed at1593 cm-1 in the untreated fibers signifies the presence of the carboxyl groups in the lignin [39]. This peak disappeared entirely from the spectra of the purified fibers and cellulose nanofibers due to the removal of lignin as a result of the chemical treatment applied. Moreover, the peak traced at 1237 cm-1 in the spectra of the untreated fibers is recognized as the stretching of C–O of the aryl group in lignin [42]. Absence of this peak from the spectra of the bleached pulp fibers and nanofibers confirms that lignin was successfully removed from the fibers with the combination of enzyme and chemical treatment.

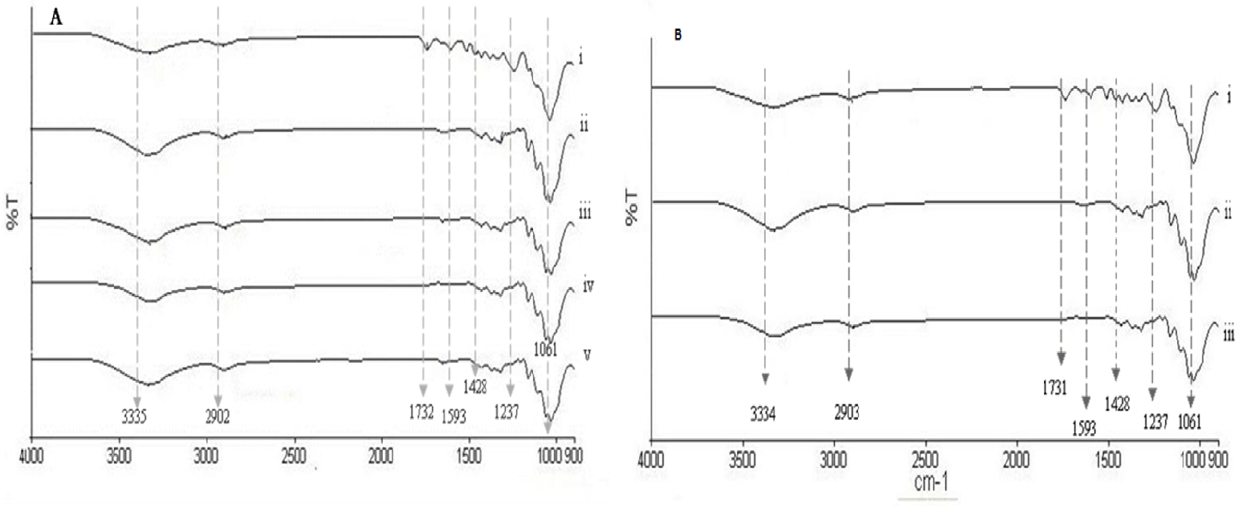

Figure 3: FTIR spectra using chemo-mechanical treatment in combination with (a) laccase enzyme for (i) untreated wood fibers, (ii) purified fibers, (iii) cellulose nanofibers with ultrasonic output of 80 W, (iv) 100 W, (v) 200 W; and (b) xylanase enzyme: (i) untreated wood fibers, (ii) purified fibers, (iii) cellulose nanofibers with ultrasonic output of 200 W.

3.3 Thermo gravimetric analysis (TGA)

Cellulosic materials are renowned for experiencing rapid thermal degradation at low to moderate temperatures, namely, below 400 °C[43]. Thermal degradation of lignocellulosic materials begins with an early decomposition of hemicelluloses, followed by an early stage of pyrolysis of lignin, depolymerization of cellulose, active flaming combustion and char oxidation [44]. The thermal stability of cellulose fibers were examined using thermo gravimetric analysis (TGA). In this test, the thermal stability was studied in terms of weight loss as a function of temperature in a nitrogen atmosphere. Figure 4 shows the TGA curves of the untreated fibers, purified cellulose fibers and cellulose nanofibers obtained after ultrasonic treatments in combination with enzymatic treatment. The TG curves of the untreated and chemo-mechanically treated fibers with laccase and chemo-mechanical treatment are shown in Figure 4a. It can be seen from the TG curves that cellulose fibers exhibited two distinct stages of decomposition. A small decrease in mass loss was observed in the range of 20 to 220°C, indicating evaporation of moisture in the fibers occurred. The second stage of decomposition which was due to the degradation of fibers occurred in the temperature range of 310 to 410 °C. The curve for untreated rubberwood fibers shows the onset of decomposition of the untreated fibers occurred at around 240 °C. This is a result of thermal depolymerization of hemicellulose or pectin since hemicellulose proved to be the lignocellulosic component having the lowest thermal stability [45]. Likewise, the lack of crystallinity may be the ultimate reason leading to its low thermal stability [46].

In addition to that, TG curves disclosed that the thermal properties of cellulose nanofibers were enhanced and the thermal degradation temperature increased to proximately 310 °C. Within this context, the higher temperature of degradation attained after chemo-mechanical treatment reflects an improved thermal behavior of the fibers. This is

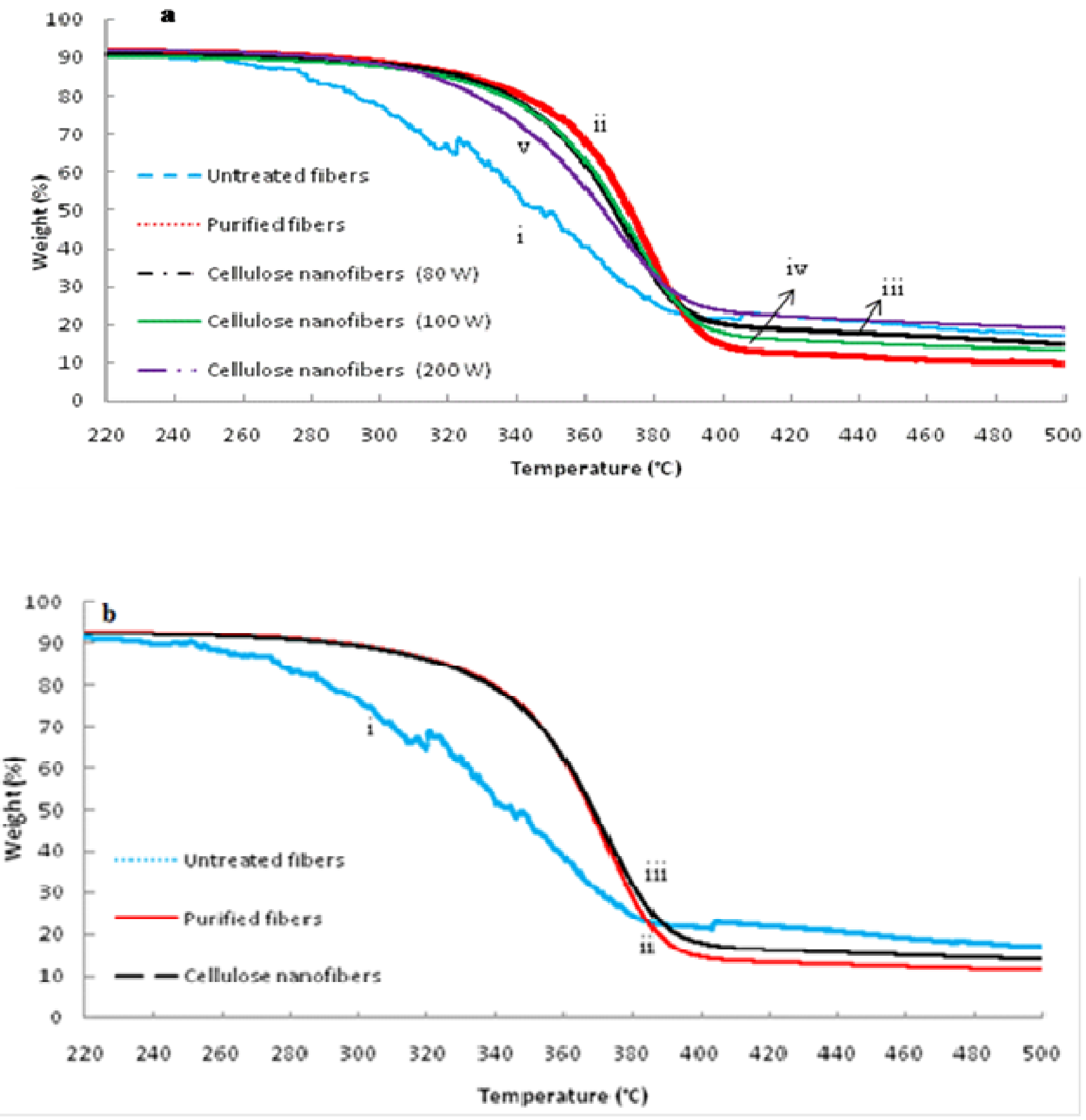

Figure 4: TG curves forchemo-mechanical treatment in combination with (a) laccase enzyme for (i) untreated wood fibers, (ii) chemical-purified fibers, (iii) cellulose nanofibers with ultrasonic output of 80 W, (iv) 100 W, (v) 200 W; and (b) xylanase enzyme: (i) untreated wood fibers, (ii) chemical-purified fibers, (iii) cellulose nanofibers with ultrasonic output of 200 W

attributed to the removal of hemicellulose, pectin and lignin from fibers during the chemical treatment. This additional thermal stability is affected by the crystalline structure of cellulose which had increased as a result of the chemical and mechanical treatments. This decomposition temperature holds for all cellulose nanofibers under different ultrasonic output powers. As a final remark, similar results were obtained for the rubberwood fibers separated using chemo-mechanical treatment in combination with xylanase. The TG curves of the latter before and after treatment are shown in Figure 4b. There was only slight difference between the curves for cellulose nanofibers obtained after ultrasonic treatments with that of purified cellulose fibers; such an effect is ascribed to the insignificant effect of ultrasonic treatment on thermal degradation of cellulose nanofibers. This analysis is consistent with the results obtained from FTIR analysis, implying that ultrasonic treatment had not influence cellulose chemical composition and thermostability. Apparently, only structural changes occurred. These results also show that the nanofibers isolated using chemo-mechanical treatment with the aid of laccase and xylanase had equal thermal stabilities and that they had better thermal behaviors than the untreated fibers and the purified fibers.

4. CONCLUSION

Separation of cellulose nano fibers from rubber wood fibers was achieved via enzymatic and chemical treatments in combination with ultrasonic treatment in this research. The extent of dispersion improved significantly with increasing output power of ultrasonic treatment. Microscopy study showed that the diameters of the nanofibers isolated ranged from 8.7 to 20 µm. The chemical structures of the fibers before and after chemo-mechanical treatments were monitored by FTIR spectroscopy. The FTIR spectra indicated that the removal of the lignin and most of the hemicellulose from rubber wood fibers after the enzyme and chemical treatments. Results of the thermogravimetric analysis demonstrated an improvement in the thermal properties of the fibers treated chemo-mechanically relative to the untreated fibers. The former exhibited enhanced thermal stability with its thermal degradation temperature increased to 310 °C as compared to 240 °C of the latter. The results obtained from FTIR and thermogravimetric analyses indicated that there were consistencies between the studied enzymes to the thermal stability or chemical structure. These results may be useful and can be potentially applied in various fields such as bio-nanocomposites, filtration media packaging, tissue engineering scaffolds, and so on.

5. ACKNOWLEDGEMENT

Authors are thankful to Universiti Malaysia Pahang for the Doctoral Scholarship Scheme.

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