Effect of Alkaline Peroxide Treatment on the Chemical Compositions and Characteristics of Lignocellulosic Nanofibrils

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Effects of alkaline peroxide (AP) treatment on chemical compositions and characteristics of lignocellulosic nanofibrils (LCNFs) were investigated. The AP treatment was conducted on a wood powder (*Liriodendron tulipifera*) at 80 °C for 1 or 5 h with different hydrogen peroxide concentrations (0.2 to 12 wt%) to selectively remove hemicelluloses and lignin. The treated wood powder was then defibrillated using wet-disk milling (WDM). The hemicelluloses and lignin in the LCNFs were successfully reduced to the ranges of 6.0% to 23.6% and 13.0% to 30.0%, respectively, depending upon the AP treatment conditions. The defibrillation efficiency improved as the hemicelluloses and lignin were removed, which reduced the LCNF dimensions. The filtration time increased with decreasing lignin and hemicellulose contents. The cellulose crystallinity and surface area of the LCNFs increased with decreasing lignin and hemicellulose contents. The tensile strength and water contact angles of the nanopaper handsheets increased, and the paper's color became whiter as the lignin content decreased.

Keywords: Lignocellulosic nanofibril; Alkaline peroxide treatment; Nanopaper; Defibrillation; Wet disk-milling

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INTRODUCTION

Cellulose microfibrils, which exist in the cell walls of lignocellulosic biomass, are often called cellulose nanofibrils (CNFs). They typically have diameters of approximately 15 nm and lengths of a few microns. These CNFs can commonly be liberated using mechanical defibrillation methods, such as wet-disk milling (WDM), microfluidization, ball milling, cryo-pulverization, and ultrasonication to the biomass once various chemical or biological pretreatments have been performed (Hideno *et al.* 2009; Chen *et al.* 2011; Zhu *et al.* 2011; Li *et al.* 2014)

The main role of the pretreatment is to disrupt the cell wall structure by removing hemicelluloses and lignin. Thus, pretreatment can be critical to improving the efficiency of defibrillation. The pretreatment can also remove minerals and extractives. Many different pretreatment methods have been developed over the years, and each method affects different components within the lignocellulosic biomass. Lee *et al.* (2010) pretreated eucalypt wood powder using hot-compressed water to remove hemicelluloses, which improved defibrillation efficiency when using wet-disk milling. Jang *et al.* (2014)

also reported using an ozone pretreatment method on Korean pine to selectively remove lignin. Ruan *et al.* (2017) used peroxymonosulfate ($2KHSO_5•KHSO_4•K_2SO_4$) and sodium bromide to improve the defibrillation efficiency of a high-shear homogenizer; the procedure produced CNFs that had widths of approximately 10 nm and lengths of a few hundred nm. The authors' results showed that more translucent CNF dispersions could be obtained after the oxidation pretreatment when compared to the samples produced without pretreatment. Chen *et al.* (2014) pretreated cotton fibers to remove hemicelluloses and other impurities using an acidified sodium chlorite solution, which yielded CNFs with widths of approximately 10 to 30 nm after mechanical defibrillation. Hu *et al.* (2018) reported increased nanofibrillation of a softwood kraft pulp using ultrasonification once the pulp had been pretreated by the synergistic reactions of endoglucanase, lytic polysaccharide monooxygenase, and xylanase. Espinosa *et al.* (2017a) reported the effect of pretreatment methods, such as mechanical, enzymatic, and TEMPO-mediated oxidation, on nanofibrillation yield, dimension, thermal stability and structure of lignocellulosic nanofibrils (LCNFs), which were significantly different among pretreatments. Espinosa et al. (2017b) also reported about the suitability of soda cereal straw pulps to produce LCNFs, showing that the chemical composition, particularly hemicellulose content, played a key role on the defibrillation efficiency, and lignin content contributed a greater thermal stability than pure cellulose nanofibrils.

Alkaline peroxide (AP) has been used at temperatures of 40 to 100 °C to bleach cellulosic pulps used for papermaking. This process uses readily available and environmentally compatible chemicals at low concentrations and at atmospheric pressure; the process does not require the use of a specialized chemical reactor (Cabrera *et al.* 2014). Furthermore, AP treatment does not leave chemical residues in the substrate because hydrogen peroxide degrades into oxygen and water (Rabelo *et al.* 2008). These benefits of AP treatment has led to much research on the use of AP treatment as a pretreatment step in bioenergy processes with the goal to improve enzymatic saccharification of lignocellulosic biomass (Gould 1984; Sun *et al.* 2000; Yang *et al.* 2002; Cara *et al.* 2006; Banerjee *et al.* 2011; Da Costa Correia *et al.* 2013).

In this study, AP was used as a pretreatment method to improve the defibrillation efficiency of wet-disk milling for the production of LCNFs with specific properties. For example, the presence of lignin on LCNFs decreases its hydrophilicity, whereas the presence of hemicelluloses on the surfaces of CNF increases hygroscopicity. Ferrer *et al.* (2012) addressed the impact of lignin on the properties of CNF, such as fibril morphology, chemical composition, nanopaper strength, and water absorbency. Spence *et al.* (2010) investigated the effect of chemical composition on the mechanical and physical properties of CNF films. The presence of lignin appreciably increased the film's toughness, tensile index, and elastic modulus. Park *et al.* (2017) also investigated the effect of hemicelluloses and lignin removal on the properties of LCNF, as well as how it affected the nanopaper produced from the LCNF; the authors reported that partial removal of these components can improve mechanical defibrillation efficiency, which decreased LCNF dimensions and tensile strength of the formed nanopaper. Jiang *et al.* (2018) also reported the effects of residual lignin on mechanical defibrillation process for LCNF production, showing that residual lignin eventually facilitate the defibrillation efficiency and greatly reduce the diameters of the resultant LCNFs. They concluded that lignin hinder the aggregation of defibrillated fibrils through reducing the inter-fibrillar hydrogen bonding presumably, resulting in the better eventual defibrillation.

EXPERIMENTAL

Materials

Liriodendron tulipifera was obtained from the Experimental Forest of Kangwon National University (Chuncheon, Republic of Korea). The wood was milled with a laboratory cutter mill (KF-20, Korea Medi Co., Ltd., Daegu, Republic of Korea) to produce a 40-mesh wood powder. Hydrogen peroxide, sulfuric acid, sodium chlorite, acetic acid, and tert-butyl alcohol were purchased from Daejung Chemicals and Metals Co., Ltd. (Siheung, Republic of Korea). All chemicals were of reagent grade.

Alkaline peroxide treatment

For alkali pretreatment, a wood meal suspension (400 mL) was prepared with 0.4% sodium hydroxide solution (1.6 g) at 5% solids concentration of wood powder (20 g). The slurry was stirred at 170 rpm for 1 h, during which the temperature was kept at 60 °C with a water bath. The insoluble residue was separated from the filtrate by vacuum filtration; the solid was washed with distilled water. Then, the alkali pretreated sample (20 g) was added to the solution (980 mL) with various concentrations of hydrogen peroxide (0.2, 1.0, 2.0, and 12.0%). After adjusting the pH of the suspensions to 11.5 using a 50% sodium hydroxide solution, the treatment was conducted at 80 °C for 1 h or 5 h. The solids were washed with distilled water and vacuum filtered until the pH of the wash filtrate was neutral.

Chemical composition

The lignin content in the untreated and AP-treated samples was determined by the Klason method. Wood powder (1 g) was added to a 72% sulfuric acid solution (20 mL) at 20 ± 3 °C for 2 h while gently stirring. Distilled water (345 mL) was subsequently added to the mixture to dilute the sulfuric acid concentration to 3%. The diluted solution was refluxed at 100 °C for 4 h. The acid-insoluble residue was separated from the supernatant using a 1G4 glass filter; the insoluble lignin was washed in the glass filter with excess distilled water until the wash filtrate had a neutral pH.

Wood powder was delignified *via* the Wise acid chlorite delignification method to produce holocellulose (Wise *et al.* 1946). Either the untreated or AP-treated sample (10 g) was added to the reaction flask with distilled water (600 mL) and sodium chlorite (4 g). The mixture was placed in a water bath at 80 °C while stirring the contents at 170 rpm. The suspension was acidified with acetic acid (800 μL), and equal amounts of sodium chlorite and acetic acid were added every hour over an 8 h period. The holocellulose residue was washed in a vacuum filter with distilled water until the wash filtrate had a neutral pH.

Holocellulose (1 g) obtained *via* acid chlorite delignification was treated with 17.5% NaOH solution (25 mL) at room temperature while stirring at 100 rpm. After 30 min, 10% acetic acid (25 mL) was added to the mixture to terminate the reaction. The resulting residue was vacuum filtered and washed with distilled water until the wash filtrate had a neutral pH. The contents of Klason lignin, holocellulose, cellulose, and hemicellulose were determined from mass measurements after each processing step.

Preparation of LCNF

Suspensions (1 wt% in 1500 mL or 5 wt% in 3000 mL) of AP-treated or untreated samples were prepared. The solids were defibrillated using a wet disk-mill

(Supermasscolloider, MKCA6-2, Masuko Sangyo Co., Ltd., Kawaguchi, Japan). Each WDM operation was repeated 15 times at 1800 rpm; the clearance between the two ceramic nonporous disks was set between 80 μm and 150 μm.

Methods

LCNF morphology

The LCNF (100 g) was made into a 0.001 wt% water suspension. The suspension was dispersed with an ultrasonicator (VCX130PB, Sonics and Materials, Inc., Newtown, USA) for 90 s. After vacuum filtration, the residue was left on a polytetrafluoroethylene (PTFE) membrane filter (ADVANTEC®, Toyo Roshi Kaisha, Ltd., Tokyo, Japan) and was subjected to solvent exchange using tert-butyl alcohol for 20 min. Solvent exchange was performed three times, and the resulting sample was dried in a freeze dryer (FDB-5502, Operon Co., Ltd., Gimpo, Republic of Korea) at -55 °C for 2 h to prepare it for SEM imaging. The samples were coated with iridium (Ir) with a vacuum sputter coater (EM ACE600, Leica, Seoul, Republic of Korea). The prepared samples were examined using a field emission scanning electron microscope (FE-SEM; S-4800, Hitachi, Tokyo, Japan) located at the Central Laboratory of Kangwon National University. The diameters of the LCNFs were measured on more than 100 nanofibrils using an image processing program (ImageJ, U.S. National Institutes of Health, Bethesda, MD, USA).

X-ray diffraction (XRD)

An X-ray diffractometer (X'Pert PRO MPD, PANalytical, Almelo, Netherlands) was used to compare crystallinity changes and average crystal size of the untreated and AP-treated samples. The scanning range of 2θ was 5° to 35°, and the scanning speed was 2°/min with Cu Kα radiation (λ = 0.1542 nm). The crystallinity index and average crystallite size were calculated by the Segal *et al.* (1959) method (Eq. 1) and by the Scherrer method (Eq. 2), respectively,

$$\text{Crystallinity index } I_c\,(\%) = [(I_{200} - I_{am}) / I_{200}] \times 100 \quad (1)$$

where I_{200} is the height of the [002] crystalline lattice plane peak (2θ = 22.5°) and I_{am} is the peak intensity of the amorphous material (2θ = 18.5°).

$$\text{Average crystallite size } D = K\lambda / \beta cos\theta \quad (2)$$

In Eq. 2, K is 0.94, λ is the X-ray wavelength (0.1542 nm), β is the full width at half maximum (FWHM) of the diffraction band, and θ is the Bragg angle corresponding to the [002] crystalline lattice plane.

LCNF nanopaper handsheet preparation

A 220 mL water suspension of LCNF (0.2 wt% solids concentration) was dispersed with an ultrasonicator for 90 s and vacuum filtered onto a silicone-coated filter paper (Whatman® No. 2200 125, GE Healthcare, Ltd., Buckinghamshire, UK) to prepare a LCNF nanopaper handsheet. After filtration, a second silicone-coated filter paper was overlaid on top of the filtrated LCNF pad, which was then pressed for 5 min at 15 MPa and 105 °C in a hot-press machine. The weight and grammage of the round nanopaper handsheets was 0.44g and 0.01g/cm^2, respectively.

LCNF nanopaper handsheet characterization

To evaluate the tensile strength of the nanopaper, the prepared handsheets were cut to 5 mm wide and 50 mm length. The thickness was measured, and then the sheet density was calculated. The specific tensile strength and elastic modulus were measured for each sample (seven replicates) with a universal testing machine (H50K, Hounsfield, UK) using a 50 mm span length and a 5 mm/min cross-head speed.

The contact angle of the nanopaper was measured using distilled water that was dropped onto the surface by a 1 mL syringe. The measurement was repeated 30 times, and the average contact angle was measured using an optical tensiometer (CAM 101, KSV Instruments Ltd., Espoo, Finland).

The color of the LCNF nanopaper handsheets was analyzed by using a colorimeter (NR110, 3NH Technology Co., Ltd., Shenzhen, China) and compared against a white color plate (L = 94.76, a = 0.07, and b = 0.87). Lightness value (L), red-green value (a), and yellow-blue value (b) were measured for each sample (five replicates), and the average total color difference (ΔE) was calculated,

$$\Delta E = [(\Delta L)^2+(\Delta a)^2+(\Delta b)^2]^{0.5} \quad (3)$$

where ΔL, Δa, and Δb are the difference in lightness, red-green, and yellow-blue values between the LCNF nanopaper *versus* the reference white color plate standard.

RESULTS AND DISCUSSION

Table 1 shows the chemical composition of the AP-treated wood powder. The initial contents of hemicelluloses and lignin in *L. tulipifera* were 26.3% and 32.1%, respectively. As the concentration of hydrogen peroxide was increased from 0.2% to 12% for the samples prepared over 1 h and 5 h reaction times, the content of these two constituents decreased to 6.0% and to 13.0%, respectively, whereas the cellulose content increased. In particular, the decreasing trend for the hemicelluloses became more drastic when the sample was treated with greater than 5% hydrogen peroxide for 5 h. This resulted in a 77% and a 60% reduction of the hemicelluloses and the lignin, respectively, in relation to their initial levels. Five samples were selected for defibrillation using WDM; their characterization is shown in Table 1. Gould (1984) examined the delignification of rice straw using AP treatment with 1% hydrogen peroxide at 25 °C for 24 h at 11.5 pH; the treatment resulted in 50% lignin removal, along with most of the hemicelluloses being removed as well. Yang *et al.* (2002) investigated the AP treatment of steam-exploded softwood powder with 1% hydrogen peroxide at 80 °C for 45 min at the same pH value; these investigators observed approximately 90% lignin removal. Cara *et al.* (2006) reported 80% lignin removal from steam-exploded olive wood using AP treatment at the same conditions as those employed by Yang *et al.* (2002).

Figure 1 shows the relationship between the WDM time *versus* the number of WDM passes. The WDM time increased as the number of WDM passes increased in all samples. As the contents of hemicelluloses and lignin decreased, the WDM time increased for a given number of WDM passes. This result may have been due to a viscosity increase that resulted from increased defibrillation efficiency and from increased hydrophilicity in the LCNFs as the lignin and hemicelluloses were removed. Park *et al.* (2017) reported similar observations, which indicated that the required WDM time was longer for CNFs that had lower lignin contents.

Table 1. Chemical Compositions of AP-treated Wood Powder

Sample Code	Hydrogen Peroxide Concentration (%)	Reaction Time (h)	Constituents (%)		
			Cellulose	Hemicellulose	Klason Lignin
LCNF-32	-	-	45.4	26.3	32.1
LCNF-30	0.2	1	53.9	22.7	30.4
LCNF-25	1.0	1	54.9	22.6	25.3
LCNF-20	2.0	1	56.2	22.4	19.9
-	5.0	1	55.5	21.6	17.9
-	5.0	5	59.6	17.0	16.7
-	8.0	5	74.8	15.9	15.7
-	10.0	5	78.8	7.0	13.5
LCNF-13	12.0	5	79.1	6.0	13.0
Note that the NaOH concentration was 0.4%, pH was 11.5, and reaction temperature was 80 °C.					

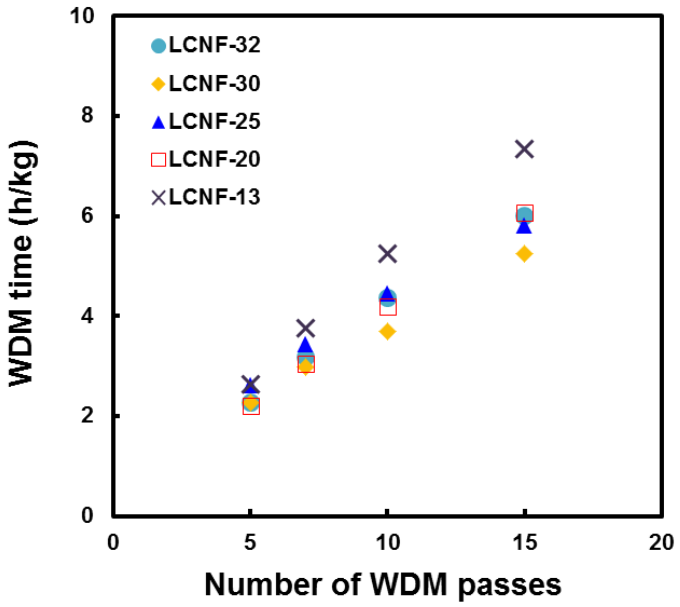

Fig. 1. The WDM time *versus* the number of WDM passes; the data for WDM time of the LCNF-32 sample were taken from the Park *et al.* (2017) study.

Figure 2 shows the SEM images of LCNFs prepared with different WDM times; Table 2 summarizes the average LCNF diameter as determined from the SEM images. In the LCNF-30 sample (from raw *L. tulipifera* without AP treatment) that was defibrillated for 2.28 h/kg, the partially un-defibrillated fibers with greater than 100 nm thickness were present due to the high levels of lignin and hemicelluloses within the cellulosic matrix.

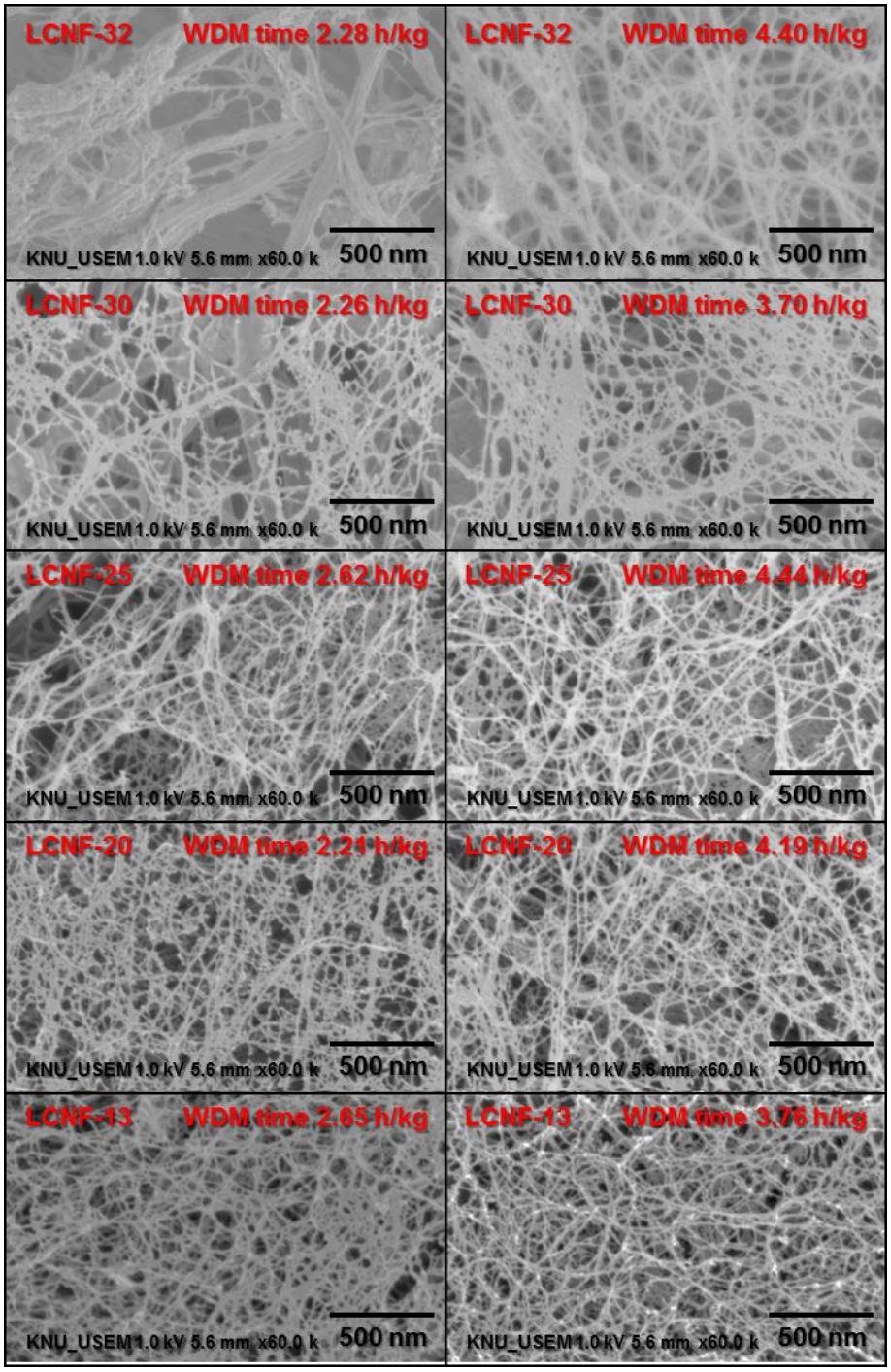

Fig. 2. SEM images of LCNFs pretreated at different chemical compositions and prepared with different WDM times; the images of LCNF-32 were taken from the Park *et al.* (2017) study.

As the WDM time was increased to 4.37 h/kg, the LCNF diameter became more uniform in size, which decreased to an average value of 40.2 nm. However, all AP-treated LCNFs showed a more uniform morphology with smaller average diameters of 22 to 30 nm at both WDM times *versus* the control (LCNF-32 without AP treatment). This observation indicated that the removal of lignin and hemicelluloses by AP treatment can improve the defibrillation efficiency.

Lee *et al.* (2010) proposed that the improved mechanical defibrillation efficiency is due to the formation of nanospaces created by extracting the hemicelluloses and lignin that are between the cellulose microfibrils. Park *et al.* (2017) reported that the defibrillation efficiency was improved when non-cellulose matrix polymers were removed; this resulted in CNFs with diameters that increased in the following order: pure cellulose nanofibril < holocellulose nanofibril < LCNF.

Table 2. Average Diameters of LCNFs Pretreated at Different Chemical Compositions and Prepared with Different WDM Times

Sample Code	WDM Time (h/kg)	Diameter (nm)
LCNF-32	2.28	53.4 ± 45.8
	4.40	33.4 ± 21.2
LCNF-30	2.26	30.6 ± 5.4
	3.70	29.1 ± 3.0
LCNF-25	2.62	26.6 ± 7.3
	4.44	25.8 ± 4.2
LCNF-20	2.21	25.5 ± 5.3
	4.19	23.4 ± 2.8
LCNF-13	2.65	23.4 ± 3.4
	3.76	22.1 ± 2.4
Note that the data of LCNF-32 were taken from the Park et al. (2017) study.		

Figure 3 shows the XRD patterns obtained from the various LCNFs; their crystallinity indices and the average crystallite diameters are summarized in Table 3. The XRD patterns from all samples showed the presence of the native cellulose I crystalline structure; however, the peak intensity at 2θ of 22.5° increased due to the AP treatment when compared to that of LCNF-32. This increasing trend became obvious as the contents of hemicelluloses and lignin decreased. The crystallinity index of LCNF-32 was 44.3 %, which increased to 61.2% in LCNF-13 that had lower amounts of non-cellulose constituents.

There were no noticeable differences in the average crystalline diameters with regards to AP treatment conditions. These results are natural because AP treatment removes the hemicelluloses and the lignin with more amorphous structure compared to cellulose. Wang *et al.* (2013) reported that the cellulose crystallinity index of furfural residue increased from 36.2% to 53.3% due to AP treatment (180 °C for 2 h). The AP treatment is known to not change the cellulose polymorphic structure. Morán *et al.* (2008) reported that AP treatment does not convert cellulose I to cellulose II; the authors observed that the AP-treated sisal fiber had a crystallinity index of approximately 75%.

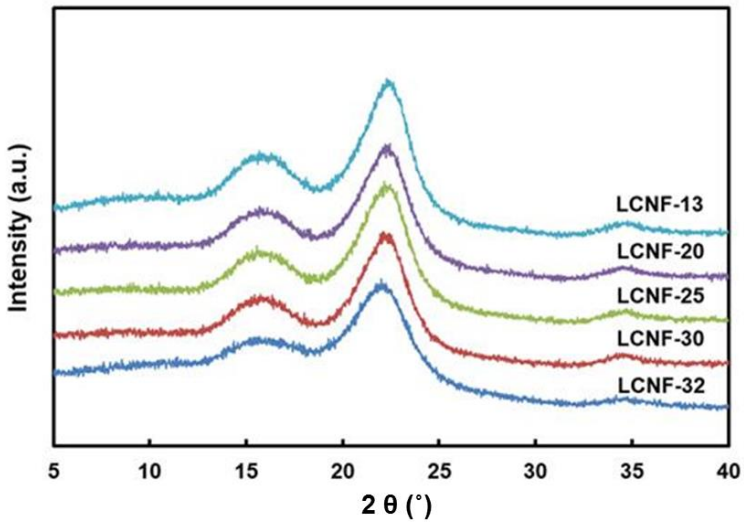

Fig. 3. XRD patterns from various LCNFs prepared using different AP treatment conditions

Table 3. Crystallinity Indices and Average Crystallite Diameters of LCNFs Prepared with Different AP Pretreatment Conditions

Sample Code	Crystallinity Index (%)	Average Crystalline Diameter (nm)
LCNF-32	44.3	3.3
LCNF-30	46.4	3.3
LCNF-25	49.5	3.4
LCNF-20	56.9	3.3
LCNF-13	61.2	3.2

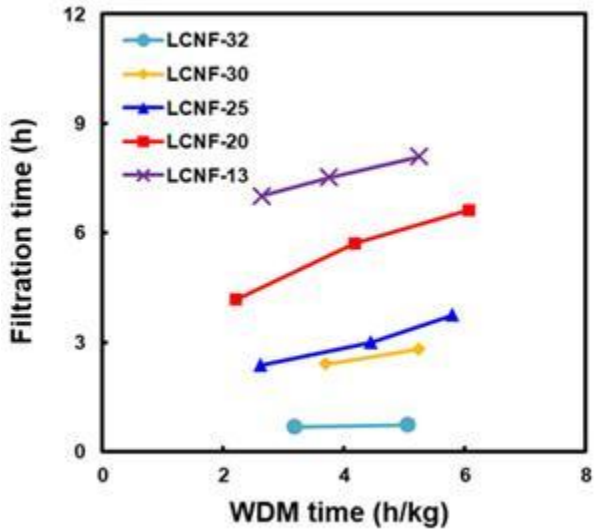

Fig. 4. Filtration time of LCNF suspensions prepared with different WDM times; the filtration time data of LCNF-32 were taken from the Park *et al.* (2017) study.

Figure 4 shows the filtration time for various LCNF suspensions prepared with different WDM times. The filtration time of LCNF prepared after AP treatment was longer than that of LCNF-32. As discussed earlier, the dimensions of LCNFs decreased as the non-cellulosic components were removed *via* the AP treatment. Interactions between water and surfaces of carbohydrates are affected by the chemical constituents comprising the LCNFs. Thus, the reduction in hydrophobicity as a function of lignin removal may have been the primary factor responsible for the increasing filtration time. In all LCNFs, the filtration time became longer as the WDM time increased. This is natural because the increased surface area by longer WDM time will increase the capacity to hold water. Chang *et al.* (2012) proposed the filtration time of CNFs as an indirect measure of degree of defibrillation because the filtration of finer CNFs requires more time. The high surface area of those CNFs will increase their water retention ability due to their high hydrophilicity.

Figure 5 shows the effect of WDM time on the specific tensile strength and modulus of nanopaper handsheets prepared from LCNFs. Both phenomena increased in all LCNF handsheets as the WDM time increased. Both measured parameters were higher in the nanopaper handsheets made from AP-treated LCNFs *versus* LCNF-32 at all WDM times. The specific tensile strength increased as the content of non-cellulosic constituents decreased due to AP treatment; however, there was appreciable differences in the specific moduli of handsheets made from the AP-treated LCNFs. Removal of non-cellulosic polymers due to severe AP treatment increased the exposed cellulose surface area, which resulted in stronger hydrogen bonding among the nanofibrils. This was possibly responsible for the observed tensile strength increase. In particular, lignin removal will increase the hydrophilicity of the purified material. Jang *et al.* (2015) reported an increased tensile strength (120 MPa) in handsheets made with LCNFs delignified by ozone treatment when compared with the handsheet made with untreated LCNF (85 MPa).

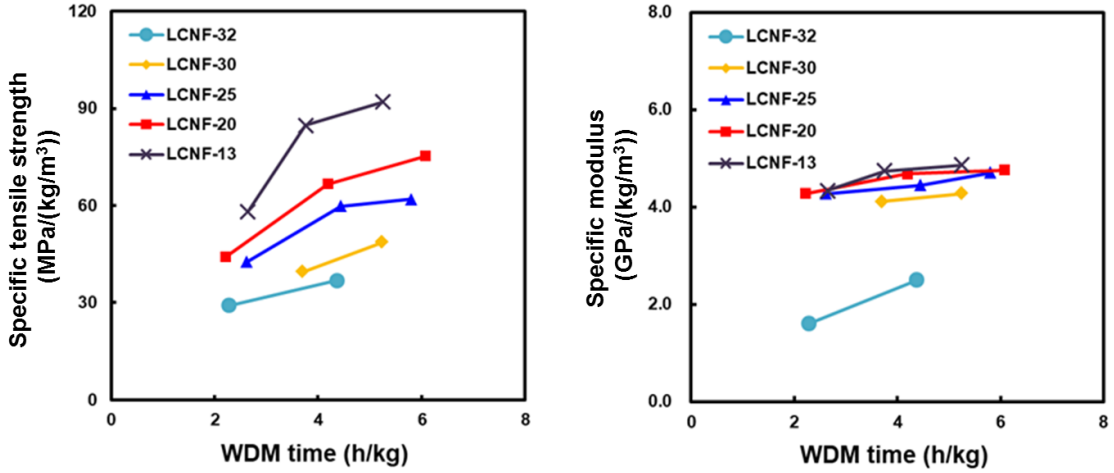

Fig. 5. Specific tensile strength and specific modulus of nanopaper handsheets fabricated from LCNFs with different chemical compositions and WDM time; the tensile strength and elastic modulus data of LCNF-32 were taken from Park *et al.* (2017) study.

Figure 6 shows the effect of lignin content on the contact angles of LCNF nanopaper handsheets. Some parameters, such as roughness, affected the contact angle of nanopaper handsheets, but chemical composition would be the more important parameter,

because of the same paper making process. For the LCNF-32 sample, the contact angle was 92°, which was attributed to its high lignin content. However, the contact angle decreased to 66° for the LCNF-13 sample due to its lower lignin content. It was inferred that the hydrophilicity of the nanopaper handsheets increased due to lignin removal during the AP treatment.

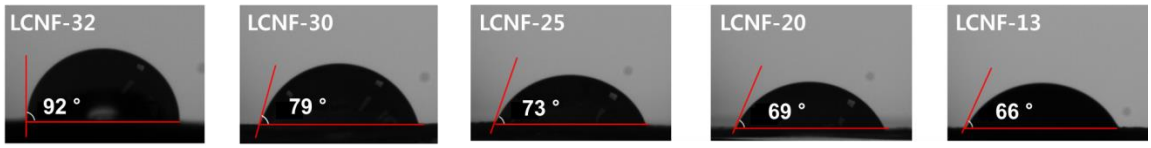

Fig. 6. Contact angles of LCNF nanopaper handsheets containing different lignin concentrations

Table 4. Color Measurements of LCNF Nanopaper Handsheets with Different Concentrations of Chemical Constituents

Sample Code	*L*	*a*	*b*	Δ*E*
LCNF-32	69.87 ± 1.85	5.54 ± 0.88	23.73 ± 1.66	34.26 ± 2.31
LCNF-30	80.13 ± 1.14	5.94 ± 0.84	22.12 ± 1.38	27.06 ± 0.91
LCNF-25	84.68 ± 0.46	2.53 ± 0.10	17.71 ± 0.34	19.78 ± 0.45
LCNF-20	88.99 ± 0.33	0.93 ± 0.15	12.39 ± 0.55	12.91 ± 0.64
LCNF-13	91.35 ± 0.76	0.16 ± 0.09	5.35 ± 0.89	4.84 ± 1.79

Table 4 summarizes the color properties of the LCNF handsheets. The total color difference (Δ*E*) with respect to the white color plate standard was 34.26 ± 2.31 for the LCNF-32 sample, and the total color difference for the LCNF-13 sample was 4.84 ± 1.79. The results showed that the lightness (*L*) value of the LCNF handsheet increased, and the red-green (*a*) value and yellow-blue (*b*) value decreased, which was attributed to lignin removal and its decolorization during the AP treatment. Jang *et al.* (2015) reported that the lightness of LCNF nanopapers was increased from 45 to 94 after delignification by ozone treatment.

CONCLUSIONS

1. Hemicelluloses and lignin in LCNFs were partially removed using AP treatment with different hydrogen peroxide concentrations (0.2 to 12 wt%) and reaction times (1 or 5 h) at mild reaction temperature (80 °C) and reaction pH (11.5), which was followed by WDM defibrillation.
2. Defibrillation efficiency improved as the amount of hemicelluloses and lignin were removed, which resulted in reduced LCNF dimensions. The filtration time, cellulose crystallinity, and surface area of the LCNFs increased as the lignin and hemicellulose contents decreased.

3. Tensile strengths and water contact angles on the nanopaper handsheets increased when AP treatment was used. The lightness (*L*) value became whiter as the lignin content was decreased by AP treatment.
4. Overall, the AP treatment described herein is an environmentally compatible and efficient method to produce LCNF.

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