Properties of Cellulose Nanofibril Produced from Wet Ball Milling after Enzymatic Treatment *vs.* Mechanical Grinding of Bleached Softwood Kraft Fibers

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Cellulose nanofibril (CNF) is a class of promising and renewable nanocellulosic material due to its unique dimensional characteristics and appealing properties. CNF preparations based on TEMPO pretreatment followed by high-pressure homogenization have been studied intensively, while the high energy consumption and the environmental issues remain challenges to their application. Mechanical refining processes have been commonly applied at the academic and industrial relevant scales for CNF production. In this study, bleached softwood kraft pulp was subjected to high-efficiency wet ball milling (following enzymatic pretreatment) and mechanical grinding to obtain CNF. The effects of ball milling time, grinding gap, and grinding passes on structure and properties of CNF were evaluated. Scanning electron microscopy images confirmed that the diameter of CNF was decreased with the increment of ball milling time and number of grinding passes. The results indicated that ball milling time, grinding gap, and grinding passes were important to increase the dispersity of CNF suspensions. The degree of polymerization and crystallinity index of CNF decreased with increasing ball milling time and grinding passes.

Keywords: Cellulose nanofibril; Ball milling; Mechanical grinding

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INTRODUCTION

Cellulose nanofibril (CNF), a nano-scale material obtained from cellulose, has been used as a source for the development of bio-based functional materials with high volume. The CNF has many interesting and useful properties including high strength and stiffness, optical transparency, high thermal stability, low density, thermal insulation, and mechanical resistance (Osong *et al*. 2016; Marinhoa *et al*. 2020). The CNF has been already evaluated for a wide range of areas in science and technology, such as the production of paper-based products, nanocomposites, flexible electrodes, automotive components, biomedical devices, and foods, as well as in water treatment (Abdul Khalil *et al*. 2014; Mondal 2017; Francoa *et al*. 2019; Qi *et al*. 2019).

Different techniques, such as high-pressure homogenization, chemical treatment, and ultrafine grinding methods, have been employed to produce CNF (Bian *et al*. 2016; Nechyporchuk *et al*. 2016; Long *et al*. 2017). However, the main drawback of mechanical processing is the high energy consumption (Berglund *et al*. 2016). Spence *et al*. (2011), and it has been concluded that ultrafine grinding is the most efficient approach in terms of

consumed energy for the CNF preparation. During the grinding, the cellulose slurry is passed between static and rotating grinding stones (disks). The distance between these disks can be precisely adjusted, which makes it possible to avoid the problem of clogging. By the shearing forces generated between the discs, the cell wall is delaminated and the CNF is individualized (Liu *et al*. 2018). Wang and Zhu (2016) reported on the effects of mechanical fibrillation time by disk grinding on the properties of resultant CNF films. They showed that it was possible to fractionate large networked CNF from small ones by centrifugation.

Ball milling is an environmentally friendly and low-cost method of producing CNF because of its ease of use, relatively inexpensive equipment, its combination of friction, collision, shear, and other mechanical actions to reduce the crystallinity of cellulose, and its applicability to essentially all classes of materials (Zhang *et al*. 2018; Ewulonu *et al*. 2019; Nagarajana *et al*. 2019). A planetary ball mill applies artificial gravitational force to the grinding medium by using a centrifugal force field. In a planetary mill, the grinding jar rotates around its own axis in a direction opposite to the direction of support plate rotation, which causes a non-uniform field of centripetal acceleration. Therefore, the balls in planetary mill have notably higher impact energies (Kim *et al*. 2013; Nobuta *et al*. 2016). Many researchers have used ball milling for the pretreatment of all classes of lignocellulosic materials prior to glucose production (Amiralian *et al*. 2015; Kwon *et al*. 2015, 2016; Du *et al*. 2017). Zhang *et al.* (2015) studied the process of CNF production from softwood pulp by ball milling. The effects of ball milling conditions including the ball-to-cellulose mass ratio, milling time, ball size, and alkaline pretreatment were investigated. Postproduction-treatments such as dimensional homogenization were also studied.

In this study, CNF was obtained from bleached softwood kraft pulp (BSKP), and the properties of CNF from wet ball milling and mechanical grinding were compared. Ball milling was combined with enzymatic pretreatment in order to decrease the energy consumption and to obtain high quality and homogeneity of the CNF. The process conditions, dispersity, morphology, degree of polymerization, and crystal structures of the resulting two kinds of CNF obtained were compared and discussed.

EXPERIMENTAL

Materials

Commercial dried BSKP was obtained from a paper mill (Guangdong, China). The pulp fibers were first soaked in deionized water overnight and then disintegrated in a lab Hollander beater for 10 min. Commercial endoglucanase (Banzyme 2900) was purchased from UPM-kymmene Co. Ltd. (Jiangsu, China). The optimum pH and temperature for the Banzyme 2900 were 5.5 and 50 °C, respectively. The cupriethylenediamine hydroxide solution was purchased from the China Pulp and Paper Research Institute (Beijing, China). Deionized water was used for all of the experiments.

Enzymatic Pretreatment

The BSKP fibers were pretreated with enzyme prior to ball milling under the following conditions: 50 °C, 7.0% pulp consistency, pH 5.5 (adjusted with citrate acid-sodium citrate buffer), enzyme dosage of 9 mg/g based on the weight of dried pulp, 3.0 h, at 200 rpm for mechanical stirring in a reactor. After treatment, the enzyme was deactivated

by increasing temperature to 100 °C for 15 min. Subsequently, the slurry was centrifuged to separate the solid and liquid phases. The solid material was washed with deionized water several times. The washed solids were used for analysis and subsequent mechanical fibrillation.

Ball Milling

Cellulose materials were wet ball-milled with a PQ-N2 planetary ball mill (Livingston, NJ, USA). The enzymatically treated fibers were diluted to 1.0 wt% and then agitated by a mechanical blender for 10 min. Then 150 g of the diluted fibril slurry were put into an agate jar of 500 mL capacity. One jar was loaded with a ball-to-cellulose mass ratio of 60:1. The weight ratio of the balls with 10 mm and 6 mm diameters was 5:3. The jar was rotated at a velocity of 30 Hz (387 rpm) with one rotation direction. The ball milling was carried out for 0.5, 1.0, 1.5, 2.0, and 3.0 h at room temperature in order to investigate the effect of ball milling time on the production of CNF. To prevent overheating of the cellulose, 10 min of interval was provided between every 5 min of milling. The preparation process is shown in Fig. 1b. The CNF produced from wet ball milling were marked as CNF-B.

Mechanical Fibrillation by Ultrafine Grinder

The raw BSKP fibers were immersed in deionized water for 2.0 h at 2% (w/v) solid consistency. The suspensions were mechanically fibrillated using a super mass-colloider MKCA6-2J (Masuko Sangyo Co. Ltd., Kawaguchi, Japan) at 2500 rpm. The gap between the grinding stones was gradually adjusted to -100 μm. It is well known that, due to the pressure exerted by the material being ground, a negative setting of gap does not necessarily imply clashing of the grinding surfaces. The motion zero position was defined at the contact position between the two grinding parts before loading fiber suspension. The samples were taken with the gap of +50, +20, 0, -20, -50, -80, and -100 μm for 5, 5, 10, 20, 20, 10, and 10 passes, respectively. The preparation process is shown in Fig. 1c. The CNF produced from mechanical grinding were marked as CNF-G.

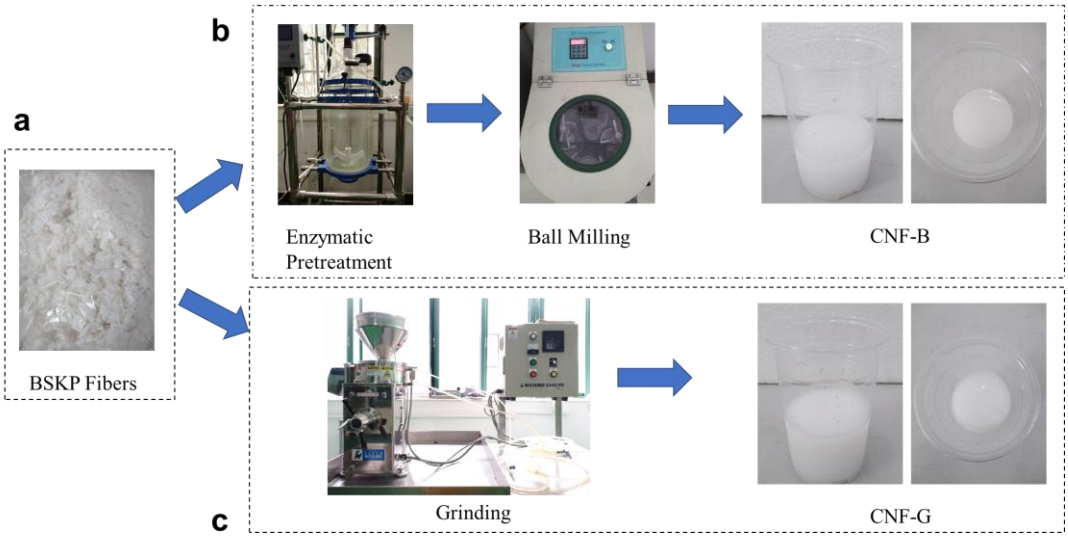

Fig. 1. Schematic flow diagram of CNF preparation. (a) BSKP fibers; (b) Representation of the enzymatic pretreatment and wet ball milling process used to obtain CNF-B; (c) Representation of the mechanical grinding process used to obtain CNF-G

Characterization

Scanning electron microscopy (SEM) observations

The surface morphologies of fibers were analyzed by a field-emission scanning electron microscopy (FE-SEM Merlin, Zeiss, Oberkochen, Germany). The samples were dried at room temperature and coated with a thin layer of gold to ensure the conductivity before testing.

X-ray diffraction (XRD) analysis

X-ray diffraction measurement was performed using an X-ray diffractometer (D8 ADVANGE, Bruker, Karlsruhe, Germany) using a Cu Ka radiation at 100, which was operated at a voltage of 40 kV and a current of 40 mA from 5° to 60° at a scanning rate of 4°/min. The sample was prepared for a thin sheet before testing. The crystallinity index (CrI) was calculated using Eq. 1 *via* the Segal method (Liu *et al.* 2018).

$$CI\ (\%) = \frac{I_{200} - I_{am}}{I_{200}} \times 100\% \quad (1)$$

where I_{200} was the maximum intensity of the crystalline peak at 2θ between 22° and 23°, and I_{am} was the minimum intensity of the amorphous cellulose at 2θ between 18° and 19°.

Degree of polymerization (DP)

The average degree of polymerization was determined by viscosity (25 °C) of fiber solution in 0.5 mol/L cupriethylenediamine hydroxide solution using an Ubbelohde viscometer. The DP value was determined from Eq. 2,

$$DP^{0.905} = 0.75[\eta] \quad (2)$$

The intrinsic viscosity $[\eta]$ (cm^3/g) was calculated according to an ASTM method (Wang *et al*. 2018).

RESULTS AND DISCUSSION

CNF Properties

Dispersion of the CNF suspension can be indirectly reflected through the optical properties of the suspensions (Nie *et al.* 2018). Photographs of CNF suspensions (0.5% w/v) from wet ball milling and mechanical grinding are shown in Fig. 2. The CNF suspensions were contained in glass tubes. A piece of green paper was used as background to visually demonstrate the opacity of the CNF suspension. The solution volume was limited to 8 mL and it was kept for 1.0 h standing. For each initial fiber sample, the CNF with various degrees of fibrillation had different levels of dispersion in water. As shown in Fig. 2a, the raw BSKP fibers did not present a homogeneous dispersion, being arranged as several groups of fibers enclosed by a cellulose matrix. The suspension prepared using enzymatic was almost completely precipitated. With the increment of ball milling time, the CNF-B suspensions showed better dispersion. As shown in Fig. 2b, the fibers (after 5 passes through the 50 μm) settled to the bottom of the tube. Fibrillation through grinding increased dispersity of the resultant fibrillated material, which can be seen from the increased opacities of the suspensions. After 20 passes through the -20 μm chamber, all samples were well dispersed in water, and became more and more transparent with increased numbers of passes of grinding. So the CNF-G was greater in thickness (a higher viscosity).

(a) (b)

Fig. 2. Photographs of the suspensions of (a) CNF-B; and (b) CNF-G

Morphological Characteristics of the CNF

The morphology of fibers was investigated by using scanning electron microscopy (SEM). Figures 3(b1-b6) indicated that when the pulps were ball-milled for longer time periods, the proportion of intact fibers and the diameter of fibrils decreased as a result of fiber fragmentation. Ball milling for 1.5 h induced defibrillation with an average diameter of 33 nm. The impact force, shear force, compression, and frictional force resulted in the reduction of size. After 3.0 h of ball milling, the diameter again was reduced to around 26 nm. During ball milling, the fibers collided with the agate balls inside the container and imparted reduction (Ravindran *et al*. 2019).

The grinding pass number had a significant influence on the CNF properties. Figure 3(c1-c8) shows the SEM images of the nanofibril from grinding of pulps. The nanofibril diameter decreased with increasing number of grinding passes and progression of the fibrillation. The CNF produced by the grinding process were highly kinked, twisted, and entangled networks with fibril diameters ranging from tens of nanometers to microns (Wang *et al*. 2016). With the increment of grinding passes, the CNF showed more uniform nano-micro dimensions and a more highly networked structure. The raw BSKP fibers had diameters as wide as several microns. The SEM images revealed that the BSKP fibers (after grinding with a gap of -50 μm for 10 passes) were very much fibrillated and no micro-fibers could be detected. In the case of the initial BSKP fibers (before grinding with a gap of -50 μm for 10 passes), non-fibrillated fibers were observed. After grinding with a gap of -50 μm for 10 passes, stable and homogeneous colloidal suspensions were obtained, which had a gel-like appearance, and no phase separation was observed during storage.

Average Degree of Polymerization

Mechanical properties of the CNF are strongly dependent on fibril length. The DP is a measurement of the length and branching of cellulose chains; therefore, DP can be an intrinsic parameter of the mechanical properties of CNF (Wang *et al*. 2015). The enzyme that was used for enzymatic pretreatment in this work is an extensive commercial cellulase (Wang *et al*. 2018). After enzymatic pretreatment, the fibers were loosened, and the DP decreased rapidly by 40%. Therefore, the application of enzymatic pretreatment to facilitate the fibrillation of cellulosic fibers is a cost-effective technology. As shown in Fig. 4a, the DP of CNF decreased rapidly at the initial stage of wet ball milling. For the ball milling time from 0 h to 0.5 h, the DP of BSKP fibers decreased by 25%. A moderate reduction in DP was observed with increasing the ball milling time.

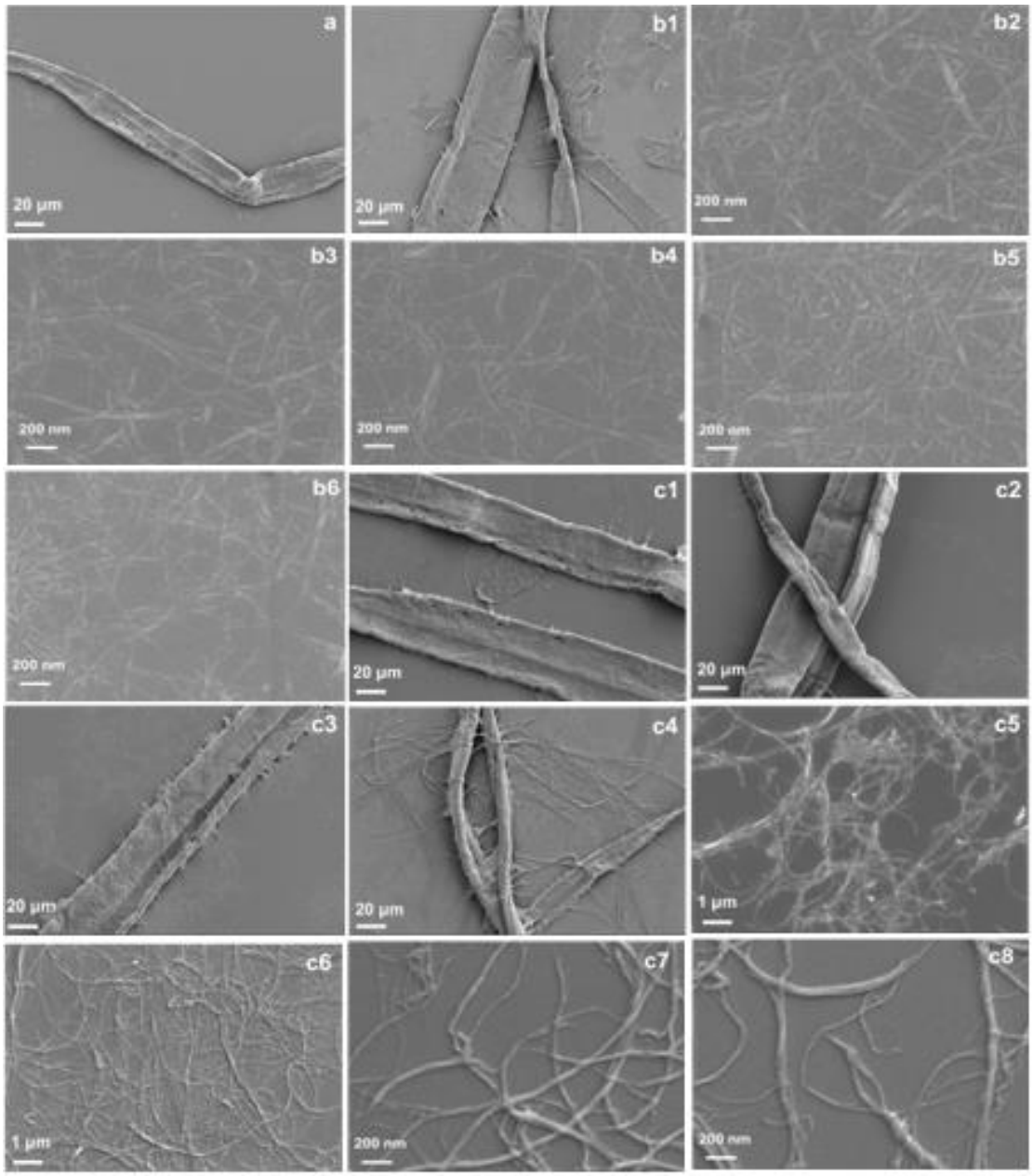

Fig. 3. The SEM images of fibrils produced from (a) BSKP fibers; (b1) BSKP fibers with enzyme pretreatment; (b2-b6) BSKP fibers with enzyme pretreatment followed by ball milling for 0.5 h, 1.0 h,1.5 h,2.0 h, and 3.0 h; (c1) BSKP fibers after grinding with a gap of +50 μm for 5 passes; (c2) BSKP fibers after grinding with a gap of +20 μm for 5 passes; (c3) BSKP fibers after grinding with a gap of 0 μm for 10 passes; (c4) BSKP fibers after grinding with a gap of -20 μm for 20 passes; (c5) BSKP fibers after grinding with a gap of -50 μm for 10 passes; (c6) BSKP fibers after grinding with a gap of 50 μm for 20 passes; (c7) BSKP fibers after grinding with a gap of -80 μm for 10 passes; and (c8) BSKP fibers after grinding with a gap of -100 μm for 10 passes.

The high energy collision between the balls reduced the length of cellulose. The reduction of the DP induced by the grinding treatment was seen in Fig. 4b, which is consistent with previous reports (Velásquez-Cock *et al*. 2016). Ultrafine grinding of the pulp to isolate the nanofibers exposes the fibers to very high shear forces. The length of cellulose chain decreased as a result.

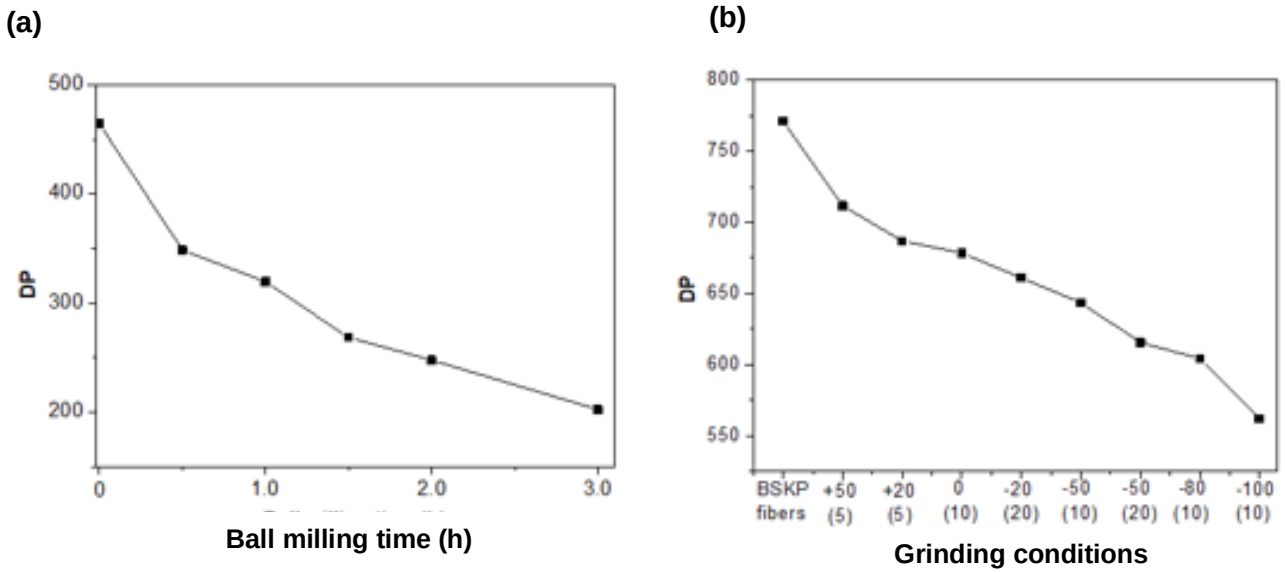

Fig. 4. (a) DP of BSKP fibers with enzyme pretreatment and BSKP fibers with enzyme pretreatment followed by different ball milling time; and (b) DP of BSKP fibers and BSKP fibers after different grinding conditions

Crystal Structures of CNF

The crystallinity of the CNF was analyzed by XRD. The XRD-profiles of BSKP fibers and fibrils prepared by ball milling and mechanical grinding are shown in Fig. 5. All the sheets showed cellulose-I structures with peaks at 2θ of 23° and 18°, which belong to diffraction from the (200) and (110) planes, respectively (Hassan *et al*. 2018).

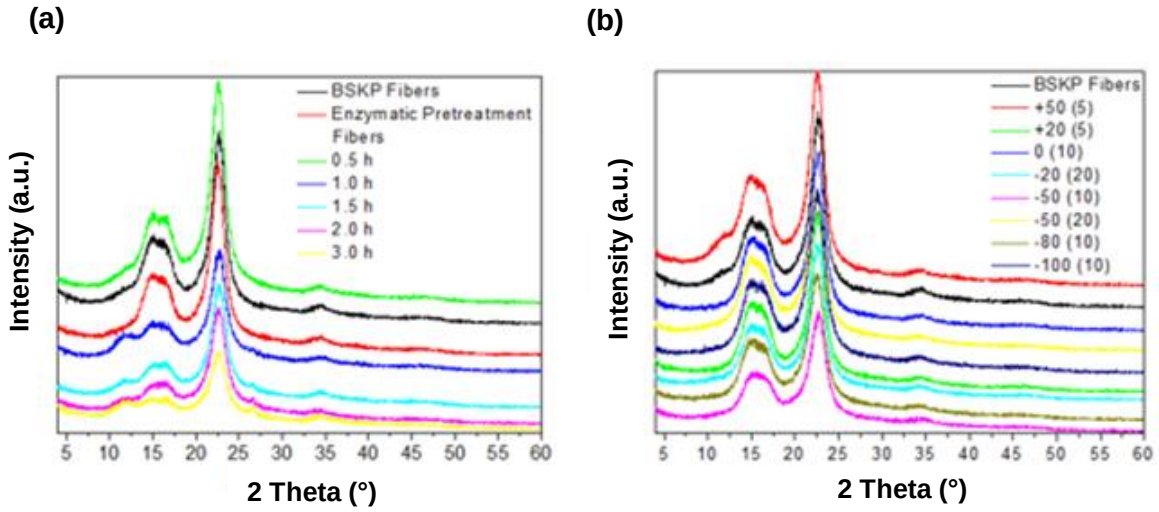

Fig. 5. X-ray diffraction patterns of (a) BSKP fibers; BSKP fibers with enzyme pretreatment and BSKP fibers with enzyme pretreatment followed by different ball milling time; (b) BSKP fibers and BSKP fibers after different grinding conditions

The enzymatic pretreatment and mechanical process had little effect on the crystalline structure of CNF. This indicated that the enzyme pretreatment and mechanical processes affected only the amorphous region and the surface of the crystalline region

within cellulose domains.

The CrI was calculated according to the Segal method, and the results are shown in Tables 1 and 2. It could be seen that the CrI increased with enzymatic pretreatment, changing from 76.3% to 78.6%. The enzyme treatment mainly loosened the amorphous area of cellulose and did not cause hydroxylation of the amorphous area (Wang *et al*. 2018). However, the sample treated only by ball milling showed completely contrary behavior, and the CrI decreased from 76.3% to 67.7%. This should be attributed to the effect of ball milling, which provided high shear force from the collision between the different sizes of balls and the friction of balls with the wall of jar, leading to weakening the hydrogen bonding networks within the crystalline cellulose. As a result, the crystallinity of CNF was reduced after the ball milling treatment (Phanthong *et al*. 2017).

The crystallinity of CNF decreased with the increasing number of passes through mechanical grinding equipment, which is similar to the values found by Wang and Zhu (2016). Specifically, the CrI of BSKP fibers changed from 76.3% to 69.9%. Before the grinding gaps down to -20, the crystallinity index decreases slightly. This is because the fibers were dispersed when the gap between the grinding stones was wide. The fibers were not destroyed too much. Mechanical grinding appeared to indiscriminately disintegrate the cellulose into fragments by damaging the crystalline region, which resulted in reduced CrI (Chen *et al*. 2017).

Table 1. Crystallinity Indices of BSKP Fibers, BSKP Fibers with Enzyme Pretreatment, and BSKP Fibers with Enzyme Pretreatment Followed by Different Ball Milling Time

Specimens	Crystallinity index (%)
Raw fibers	76.3
Enzyme pretreated fibers	78.6
0.5 h	77.2
1.0 h	76.2
1.5 h	74.7
2.0 h	72.5
3.0 h	67.7

Table 2. Crystallinity Indices of BSKP Fibers and BSKP Fibers after Different Grinding Conditions

Specimens	Crystallinity index (%)
Raw fibers	76.3
+50 (5)	75.8
+20 (5)	75.7
0 (10)	75.5
-20 (20)	75.4
-50 (10)	72.3
-50 (20)	71.8
-80 (10)	71.1
-100 (10)	69.9

CONCLUSIONS

The properties of cellulose nanofibril (CNF) produced from the cellulase-treated bleached softwood kraft pulp (BSKP) using various ball milling periods, grinding gaps, and numbers of grinding passes were evaluated. These conditions affected the morphology, mechanical, and physical properties of the CNF produced. The dispersity of the CNF increased with the increment of ball milling time and number of grinding passes. This tendency was proportional to the reductions in degree of polymerization and crystallinity. However, the CNF produced by grinding (CNF-G) was greater in thickness (a higher viscosity) and transparency than when the CNF was prepared by ball milling (CNF-B). The crystallinity indices of CNF-B were reduced more than that of CNF-G. This is attributed to the action of a different mechanism for the CNF-B preparation, where the CNF was disintegrated by the high energy collision between the balls. During the mechanical grinding, the multilayered structure and bonds were broken down by the shearing forces produced by the grinding stones, and the CNF-G can be isolated from the pulp. As a result, the CNF-G appeared as polydisperse and entangled networks.

ACKNOWLEDGEMENTS

This research was financially supported by National Key R&D Program of China (2017YFB0307902), Guangdong Provincial Science & Technology Plan Projects (2015B020241001), National Natural Science Foundation of China (No. 31600471), China Postdoctoral Science Foundation (2019TQ0100), Fundamental Research Funds for the Central Universities (2019MS085 and 2017YFB0307902), and State Key Laboratory of Pulp and Paper Engineering (201833).

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Article submitted: February 9, 2020; Peer review completed: March 23, 2020; Revised version received and accepted: March 30, 2020; Published: April 6, 2020.
DOI: 10.15376/biores.15.2.3809-3820