Characteristics and Properties of Cellulose Nanofibers Prepared by TEMPO Oxidation of Corn Husk

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This paper describes preparation of 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) mediated oxidized cellulose nanofibers (TOCNs) from corn husk. The process adopted for this work included alkaline treatment, TEMPO/NaBr/NaClO-mediated oxidation, and homogenization. The morphological investigation of TOCNs was performed using atomic force microscopy (AFM) and transmission electron microscopy (TEM). The chemical composition and structure of the material obtained after each stage of the treatments was characterized through Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) analysis. The thermal stability of the samples was investigated by thermogravimetric analysis (TGA). The obtained TOCNs possessed a narrow width, ranging from 8 to 10 nm, and a high aspect ratio (> 300). The crystallinity type of TOCNs was found to be cellulose-Iβ. Compared with the original fibers, the crystallinity of TOCNs gradually increased because of the removal of hemicellulose and lignin, while the thermal stability decreased.

Keywords: Corn husk; TEMPO-mediated oxidation; Cellulose nanofibers; Residue

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INTRODUCTION

Corn is one of the most cultivated crops in the world, with 969 million tons produced in 2014 (Baghestany *et al*. 2015). China was the second largest producer and consumption market, and it produced approximately 217 million tons for daily food, animal feed, and as a natural material for starch production (Lohmar 2015; Zhang *et al*. 2015). Corn husk, which is the thin cellulose-rich leafy sheath that covers corn cobs, can be obtained from this processing as a renewable, low-cost, and abundant lignocellulosic waste material. With the increasing concern for waste material’s appreciation, corn husk recently has been used for preparing starch or for extracting carboxymethyl cellulose (Mondal *et al*. 2015).

Nanofibers can be defined as nano-sized cellulose fibers < 100 nm wide or micron-sized fibers with nano-dimensional cross-sectional structures (Isogai *et al.* 2011). Cellulose nanofibers (CNFs), inherit the traits of natural cellulose, such as low density, easy biodegradability, reproducibility, and also have outstanding characteristics such as high surface area-to-volume ratio, excellent tolerable mechanical properties, high gas barrier property, and low coefficient of thermal expansion. All of these outstanding properties impel CNFs to gain attention in both academia and industry (Dufresne *et al.* 1997; Siró and Plackett 2010; Hamada and Bousfield 2010; Klemm *et al*. 2011). In recent years many efforts have been made worldwide to extract nanocrystalline cellulose from corn husk. Xiao *et al.* (2016) fabricated and characterized poly(vinyl alcohol) films that were reinforced by nanofibrillated corn husk using a combination of chemical pretreatments and ultrasonication; de Carvalho Mendes and his colleagues (2015) investigated the influence of the reaction time and the fiber/acid ratio on the properties of nanocrystalline cellulose isolated from corn husk.

The technological approach of 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)-mediated oxidation, which was pioneered by Isogai and co-workers, is one of the most widely used methodologies for CNF preparation (Saito and Isogai 2004; Isogai *et al*. 2011). During this oxidation process, the abundant primary hydroxyl groups present on each cellulose microfibril surface are selectively converted into carboxylate groups, which greatly contribute to an easy and fast fibrillation process because of the strong electrostatic repulsion, thus obtaining well-dispersed and individual nanofibers. The TEMPO-oxidized cellulose nanofibers (TOCNs) typically exhibit uniform widths of 3 to 4 nm and a high aspect ratio (> 100) without changing the original crystalline structure of cellulose (Okita *et al*. 2009). As far as we know, corn husk has not been extensively investigated as a raw material for the preparation of TOCNs. In this study, the possibility of preparing cellulose nanofibers from corn husk was investigated. Alkaline treatment was applied to remove hemicellulose and lignin, and a TEMPO/NaBr/NaClO-mediated oxidation system combined with homogenization was used to obtain nanofibers. The prepared TOCNs were fully characterized in terms of morphology, chemical structure and crystallinity, and thermal properties.

EXPERIMENTAL

Chemicals and Materials

Corn husk (CH) was provided by a local farm in Shandong Province (China). The chemical TEMPO was purchased from Aladdin Chemistry Co. Ltd., China. All other chemicals used in this study were of analytical grade and purchased from the Guangzhou Chemical Reagent Factory (Guangzhou, China). Deionized water was used for all of the experiments.

Methods

Corn Husk (CH) was milled using a plant disintegrator (FZ102, Tianjin Taisite instrument Co., Ltd., China) to pass through a 40-mesh sieve. To increase the yields of total extracted polysaccharides and improve the property of pretreated fibers, the milled CH was treated with 5 wt% NaOH solution at a solid-liquid ratio of 1:10 (w/v) and kept in a common ultrasonic generator (KQ-200VDE, Kunshan Ultrasonic Instrument Co., Ltd., China) with a constant power of 100 W at 50 °C for 2 h (Ebringerova and Hromadkova 2002). Then, the dark brown solution was filtered to collect the light yellow solid, which was washed repeatedly with deionized water.

Alkaline-treated CH powder (5 g) was dispersed in deionized water at 1% (w/v) consistency in a 1-L beaker containing TEMPO (0.075 g) and NaBr (0.5 g). The TEMPO-mediated oxidation of fibers was initiated by adding NaClO solution (12%, 5 mmol/g o.d.p.) and conducted at 25 °C. The mixtures were stirred at pH 9.5 ± 0.3 by addition of 0.5 M NaOH solution until no further decrease in pH was observed. The TEMPO-oxidized cellulose fibers (TOCs) thus obtained was thoroughly washed with deionized water. The TOCs were suspended in water to provide a solid content of 0.5% and homogenized by running three passes through a Nano DeBee high-pressure homogenizer (BEE International, Inc., USA) operating at a pressure of 1600 bar. A schematic representation of the isolation procedure is given in Fig. 1.

Characterization

The chemical composition of CH at each stage of treatment was determined according to the relevant ASTM standards [α-cellulose (ASTM D 1103-55T), hemicellulose (ASTM D 1104-56), and lignin (ASTM D 1106-56)]. Morphological investigation was carried out by atomic force microscopy (AFM) and transmission electron microscopy (TEM). For AFM analysis, a drop of TOCN suspension (0.005%) was dispersed on the surface of a mica plate and allowed to dry under air. The sample thus prepared was analyzed by scanning in tapping mode using a Veeco Nanoscope III atomic force microscope (USA) at ambient temperature. The TEM analysis was performed by a H7650 (Hitachi Ltd., Tokyo, Japan) with an accelerating voltage of 80 kV. Approximately 8 μL of 0.01% TOCN suspension was deposited on the surface of a clean copper grid and stained with a 3% solution of phosphor tungstic acid for 2 min and dried at room temperature for TEM analysis. Fourier transform infrared spectroscopy (FTIR) was performed using a VerTex70 spectrometer (Bruker Corp., Germany). Spectra in the range from 400 to 4000 cm^{-1} were obtained at a 4 cm^{-1} resolution. X-ray diffraction (XRD) analysis for the samples was accomplished on a diffractometer (D8 Advance, Bruker AXS, Germany) with Cu Kα radiation (k = 0.154 nm) at 40 kV and 40 mA in the 2θ range of 5° to 40° at a speed of 0.2°/min. The crystallinity index (CrI) was calculated as follows (Segal *et al*. 1959),

$$\text{CrI} = ((I_{002} - I_{\text{am}})/I_{002}) \times 100 \quad (1)$$

where I_{002} is the diffraction intensity of crystalline segments at the 2θ angle 22° and I_{am} is the diffraction intensity of amorphous material, which is taken at the 2θ angle 18°. The thermal stabilities were determined using a TGA Q500 (TA Instruments, USA) from room temperature to 600 °C with the heating rate of 10 °C/min under a N_2 atmosphere.

RESULTS AND DISCUSSION

Chemical Composition

The chemical composition of CH fibers was determined at each stage of the treatment and is summarized in Table 1. The results showed that CH is a residue with low lignin content (6.2%) and similar amounts of hemicellulose (49.6%) and α-cellulose (42.5%). Compared with CH fibers, there is a dramatic decrease in the proportion of hemicellulose and lignin for alkali-treated fibers. Such an obvious decrease can be ascribed to the cleavage of the ether linkages between lignin and hemicelluloses by alkaline treatment (Chen *et al.* 2011). The increased percentage of cellulose content improved the suitability of cellulose for TEMPO oxidation, which resulted in a stable TOCN suspension.

Morphological Analysis

The TOCs and TOCNs were isolated from CH fibers following alkaline treatment, TEMPO/NaBr/NaClO-mediated oxidation, and homogenization, as shown in Fig. 1. The surface morphology and dimension scale of TOCNs were determined using AFM and TEM image analysis. In Figs. 2a and 2b, the TOCNs were less than 10 nm wide and hundreds of nanometers to micrometers long. The high aspect ratio (> 300) is extremely favorable for unidirectional mechanical properties and for nanocomposite manufacturing (Espino *et al*. 2014). The TOCNs aggregated and formed larger particles in the AFM, which can be ascribed to the strong hydrogen bonding between the fibrils (Raposo *et al*. 2007; Chirayil *et al*. 2014). This aggregation is more pronounced when the dispersing medium is removed. The TEM analysis gave a clearer entwined fiber-like morphology of TOCNs, with an average width ranged from 5 to 8 nm, corresponding to the cellulose nanofibers extracted from bamboo pulp (Puangsin *et al*. 2013). Together, these results show the successful isolation of CH nanofibers by TEMPO-mediated oxidation and homogenization.

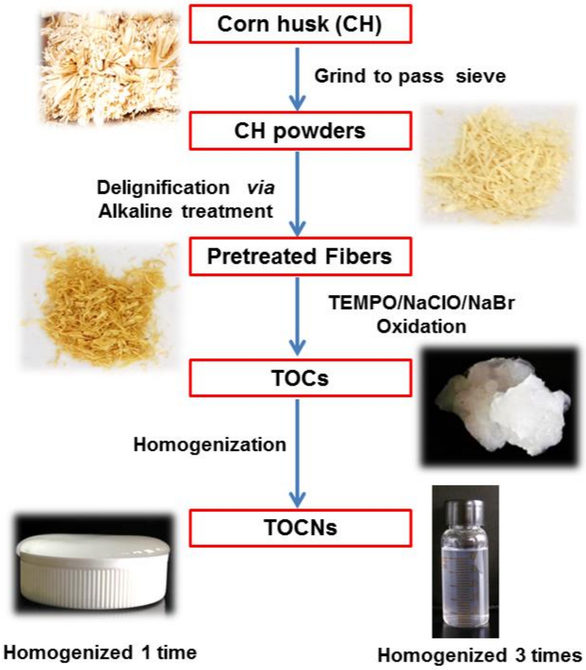

Fig. 1. Schematic representation of the isolation of cellulose nanofibers

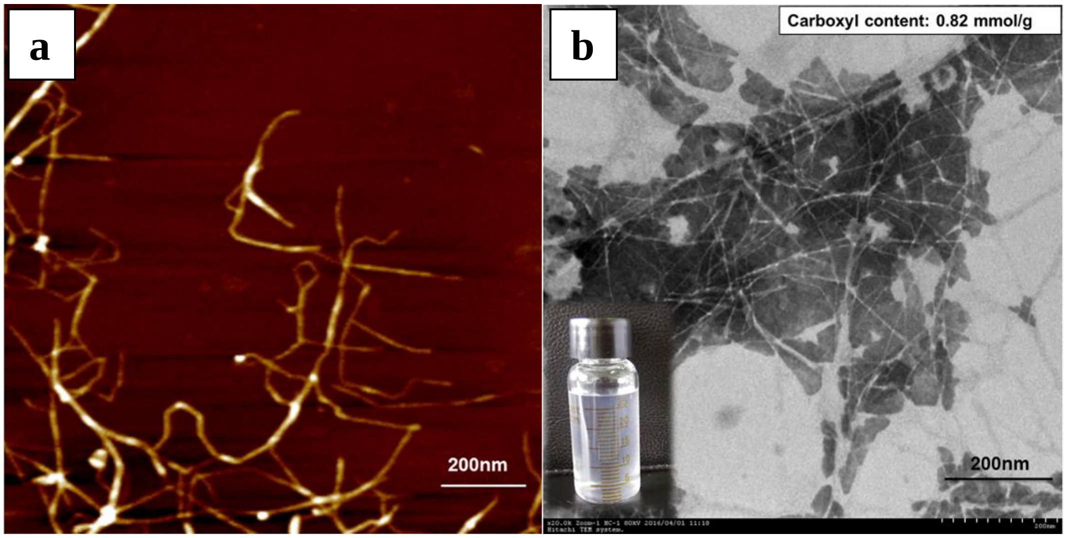

Fig. 2. (a) AFM and (b) TEM images of TOCNs from corn husk; inset is a photograph of the CH TOCN suspension at 0.3 % w/v

Table 1. Effect of Pretreatment on the Major Chemical Composition of Corn Husk

Samples	Cellulose (%)	Hemicellulose (%)	Lignin (%)
CH	42.5	49.6	6.2
Alkali-treated CH	66.5	29.3	2.6

FTIR Spectroscopy Analysis

The FTIR spectroscopy of CH fibers, alkali-treated CH fibers, and CH TOCNs are shown in Fig. 3. The similarity in the three spectra indicated that the cellulose component was not removed during the chemical treatment in all samples. The FTIR peaks observed in the samples included a broad band at 3300 cm^{-1} (–OH group stretching vibration) and a peak at 2900 cm^{-1} (C–H bond vibration), which can be attributed to the elemental functional groups found in lignocellulosic materials. The absorption peak at 1247 cm^{-1} is related to the C=O stretching vibration. The peak at 1640 cm$^{−1}$ is associated with the O–H bending vibration of the absorbed water with some contributions from carboxylate groups. The absorption peaks at 1058 cm^{-1} correspond to the C–O stretching vibrations. The prominent peaks at 1732 cm^{-1} in the origin fiber can be ascribed to C=O stretching from the ketones and/or esters of hemicellulose. The intensity of this peak was reduced dramatically after the isolation process, which demonstrates a heavy reduction of hemicellulose and lignin in Figs. 3(b) and 3(c). The removal of lignin was revealed by the decreased intensity of peaks at 1515 cm^{-1}, corresponding to aromatic C–O skeletal vibration in lignin (Chen *et al*. 2011).

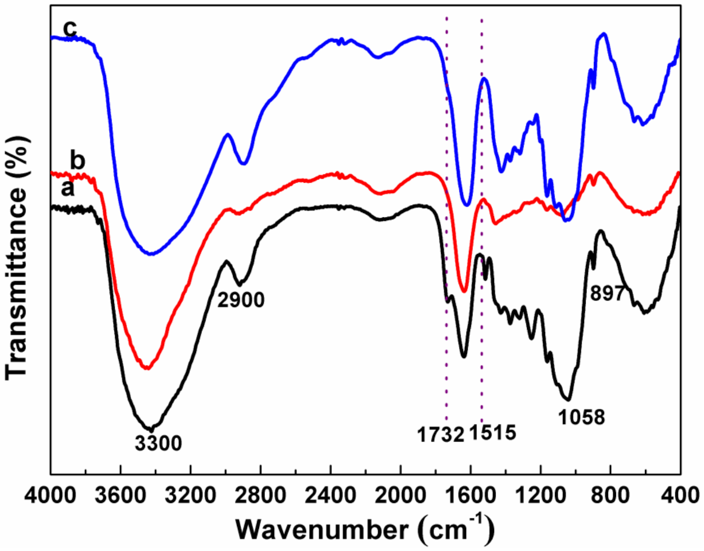

Fig. 3. FTIR spectra of (a) CH fibers, (b) alkali-treated CH fibers, and (c) CH TOCNs

XRD Analysis

The crystalline form and the crystallinity degree of fiber samples were investigated by X-ray diffraction, and the results are shown in Fig. 4. Similar diffraction patterns with diffraction peaks at 2θ values of approximately 14.7º, 16.5º, and 22.8º were characteristic of cellulose crystal assignments of the $1\bar{1}0$, 110, and 002 planes, respectively; these patterns resemble the typical cellulose-Iβ crystalline structure. The crystallinity indices (CrIs) of CH fibers, alkali-treated CH fibers, and CH TOCNs were 40.0%, 54.5%, and 63.0%, respectively (Table 2). Alkali treatment of the natural fiber will lead to the swelling of the fiber and subsequent increase in the absorption of moisture. Treatment with alkali leads to the removal of cementing materials like lignin, hemicellulose and pectin which will result in the increase of percentage crystallinity of the fiber (Abraham *et al.* 2011). The oxidation of hydroxyl groups also accelerates dissolution of the amorphous region and crystallinity improvement (Saito and Isogai 2004; Okita *et al*. 2009).

Table 2. Crystallinity Index (CrI) of CH Samples, Onset Temperature (T_{onset}), Maximum Degradation Temperature (T_{max}), and Char Residuals at 600 °C Obtained from TGA and DTG Curves

Samples	**CrI (%)**	**Cellulose Thermal Degradation**		
		T_{onset} (°C)	**T_{max} (ºC)**	**Char at 600ºC (%)**
CH fibers	40.0	210	329	21.9
Alkali-treated CH fibers	54.5	274	327	5.6
CH TOCNs	63.3	205	313	23.7

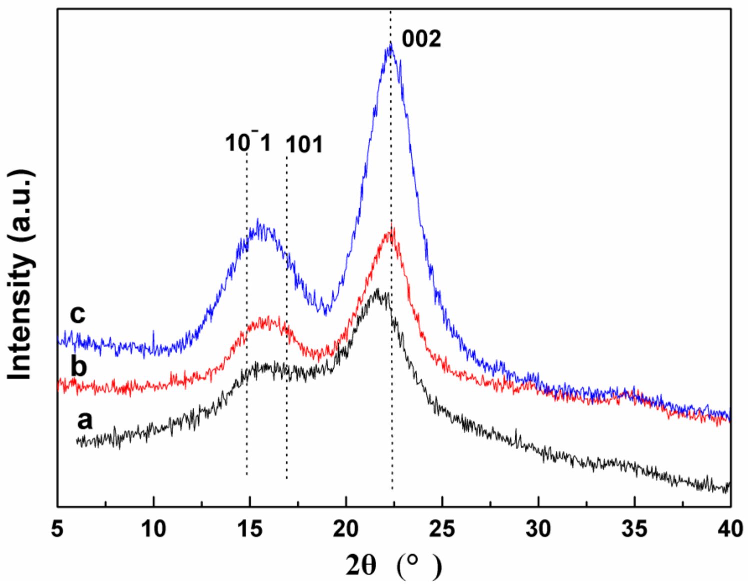

Fig. 4. XRD patterns of (a) CH fibers, (b) alkali-treated CH fibers, and (c) CH TOCNs

Thermal Stability of TOCNs

Figure 5 shows the TG and DTG curves of CH fibers, alkali-treated fibers, and TOCNs. Table 2 shows detailed information about the onset degradation temperature (T_{onset}), maximum degradation temperature (T_{max}), and char residuals at 600 °C. In Fig. 5, all samples showed a small weight loss below 150 °C, which was attributed to the evaporation of bound water in the cellulose samples. For the CH fibers, the DTG curves showed two decomposition steps, at 210 to 300 °C and 300 to 390 °C, respectively. These two weight loss steps were attributed to the decomposition of hemicellulose and cellulose, respectively (Wang *et al*. 2007). For the alkali-treated fibers, there was only the cellulose decomposition process at 290 to 360 °C, which was ascribed to the removal of hemicellulose and lignin during the chemical pretreatment process. For the TOCNs, the DTG curve seemed to consist of two peaks, near 240 °C and 310 °C. The first peak was ascribed to the T_{onset} point of the sodium anhydroglucuronate units. The second peak was obviously lower than the T_{max} of the CH and alkali fibers, indicating that the TOCNs possessed decreased thermal stability because of the introduction of carboxyl groups on the fiber surface during TEMPO-mediated oxidation (Shen and Gu 2009; Fukuzumi *et al*. 2010). Despite reduced thermal stability, the TOCNs displayed higher char residue of 23.7%, much higher than that from the CH and alkali fibers (21.9%, 5.6%), respectively. This high char yield for TOCNs may be due to the favored dehydration reaction at lower temperature. In addition, the decomposition of TOCNs would require less thermal energy and occur at low temperature due to their smaller lateral dimensions, which increased in the exposure surface area with heating (Jiang and Hsieh 2013).

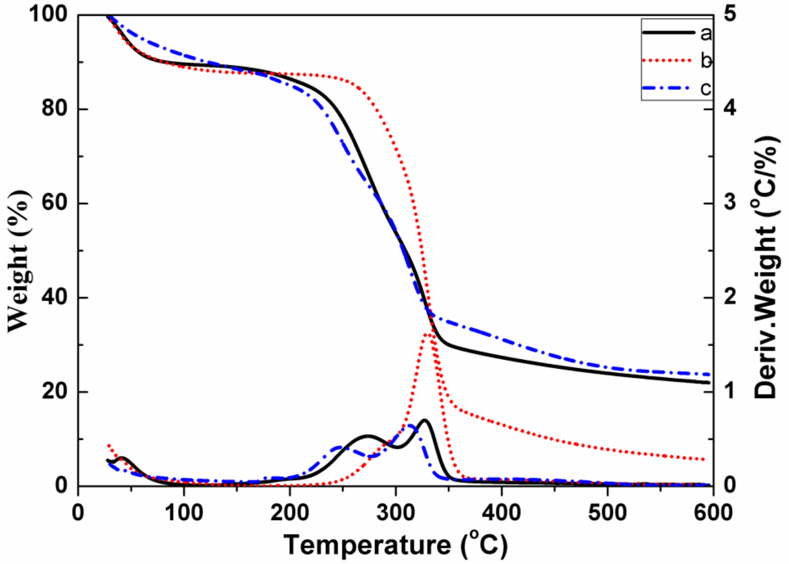

Fig. 5. TGA and its derivative thermograms for (a) CH fibers, (b) alkali-treated CH fibers, and (c) CH TOCNs

CONCLUSIONS

1. Cellulose nanofibers (CNFs) were successfully isolated from an agricultural by-product, corn husk, by alkaline treatment followed by TEMPO-mediated oxidation and subsequent homogenization.
2. Various characterization techniques were employed to determine the properties of corn husk CNFs. Results showed that the obtained TOCNs possessed a narrow width ranging from 8 to 10 nm and a high aspect ratio (> 300). The crystalline type of TOCNs was found to be cellulose-Iβ. Compared with the original fibers, the TOCNs' crystallinity gradually increased because of the removal of hemicellulose and lignin, while its thermal stability decreased. This work demonstrates the benefit of the transformation of waste to potentially useful products.

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REFERENCES CITED

Abraham, E., Deepa, B., Pothan, L., Jacob, M., Thomas, S., Cvelbar, U., and Anandjiwala, R. (2011). "Extraction of nanocellulose fibrils from lignocellulosic fibres: a novel approach," *Carbohydrate Polymers* 86(4), 1468-1475. DOI: 10.1016/j.carbpol.2011.06.034

ASTM D1103-55T. (1979). "Standard test method for α-cellulose in wood," American Society for Testing and Materials, Philadelphia, PA.

ASTM D1104-56. (1979). "Standard test method for holocellulose in wood," American Society for Testing and Materials, Philadelphia, PA.

ASTM D1106-56. (1979). "Standard test method for lignin in wood," American Society for Testing and Materials, Philadelphia, PA.

Baghestany, A. A., Yazdani, S., and Ahmadian, M. (2015). "World trade market structure for corn," *International Journal of Life Sciences* 8(5), 1-5. DOI: 10.3126/ijls.v8i5.11856

Chen, W., Yu, H., Liu, Y., Hai, Y., Zhang, M., and Chen, P. (2011). "Isolation and characterization of cellulose nanofibers from four plant cellulose fibers using a chemical-ultrasonic process," *Cellulose* 18(2), 433-442. DOI: 10.1007/s10570-011-9497-z

Chirayil, C. J., Joy, J., Mathew, L., Mozetic, M., Koetz, J., and Thomas, S. (2014). "Isolation and characterization of cellulose nanofibrils from *Helicteres isora* plant," *Industrial Crops and Products* 59, 27-34. DOI: 10.1016/j.indcrop.2014.04.020

de Carvalho Mendes, C. A., Ferreira, N. M. S., Furtado, C. R. G., and de Sousa, A. M. F. (2015). "Isolation and characterization of nanocrystalline cellulose from corn husk," *Materials Letters* 148, 26-29. DOI: 10.1016/j.matlet.2015.02.047

Dufresne, A., Cavaille, J.-Y., and Vignon, M. R. (1997). "Mechanical behavior of sheets prepared from sugar beet cellulose microfibrils," *Journal of applied polymer science* 64(6), 1185-1194. DOI: 10.1016/j.carbpol.2016.01.015

Ebringerova, A., and Hromadkova, Z. (2002). "Effect of ultrasound on the extractibility of corn bran hemicelluloses," *Ultrasonics sonochemistry* 9(4), 225-229. DOI: 10.1016/S1350-4177(01)00124-9

Espino, E., Cakir, M., Domenek, S., Román-Gutiérrez, A. D., Belgacem, N., and Bras, J. (2014). "Isolation and characterization of cellulose nanocrystals from industrial by-products of *Agave tequilana* and barley," *Industrial Crops and Products* 62, 552-559. DOI: 10.1016/j.indcrop.2014.09.017

Fukuzumi, H., Saito, T., Okita, Y., and Isogai, A. (2010). "Thermal stabilization of TEMPO-oxidized cellulose," *Polymer Degradation and Stability* 95(9), 1502-1508. DOI: 10.1016/j.polymdegradstab.2010.06.015

Hamada, H., and Bousfield, D. W. (2010). "Nanofibrillated cellulose as a coating agent to improve print quality of synthetic fiber sheets," *TAPPI Journal* 9(11), 25-29.

Isogai, A., Saito, T., and Fukuzumi, H. (2011). "TEMPO-oxidized cellulose nanofibers," *Nanoscale* 3(1), 71-85. DOI: 10.1039/C0NR00583E

Jiang, F., and Hsieh, Y.-L. (2013). "Chemically and mechanically isolated nanocellulose and their self-assembled structures," *Carbohydrate Polymers* 95(1), 32-40. DOI:

10.1016/j.carbpol.2013.02.022

Klemm, D., Kramer, F., Moritz, S., Lindström, T., Ankerfors, M., Gray, D., and Dorris, A. (2011). "Nanocelluloses: A new family of nature-based materials," *Angewandte Chemie International Edition* 50(24), 5438-5466. DOI: 10.1002/anie.201001273

Lohmar, B. (2015). "Will China import more corn?," *Choices* 30, 2 (http://purl.umn.edu/200300)

Mondal, M. I. H., Yeasmin, M. S., and Rahman, M. S. (2015). "Preparation of food grade carboxymethyl cellulose from corn husk agrowaste," *International Journal of Biological Macromolecules* 79, 144-150. DOI: 10.1016/j.ijbiomac.2015.04.061

Okita, Y., Saito, T., and Isogai, A. (2009). "TEMPO-mediated oxidation of softwood thermomechanical pulp," *Holzforschung* 63(5), 529-535. DOI: 10.1515/HF.2009.096

Puangsin, B., Yang, Q., Saito, T., and Isogai, A. (2013). "Comparative characterization of TEMPO-oxidized cellulose nanofibril films prepared from non-wood resources," *International Journal of Biological Macromolecules* 59, 208-213. DOI: 10.1016/j.ijbiomac.2013.04.016

Raposo, M., Ferreira, Q., and Ribeiro, P. (2007). "A guide for atomic force microscopy analysis of soft-condensed matter," in: *Modern Research and Educational Topics in Microscopy*, Vol. 1, A. Mendez-Vilas and J. Diaz (eds.), Formatex, Badajoz, Spain, pp. 758-769.

Saito, T., and Isogai, A. (2004). "TEMPO-mediated oxidation of native cellulose. The effect of oxidation conditions on chemical and crystal structures of the water-insoluble fractions," *Biomacromolecules* 5(5), 1983-1989. DOI: 10.1021/bm0703970

Segal, L., Creely, J., Martin, A., and Conrad, C. (1959). "An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer," *Textile Research Journal* 29(10), 786-794. DOI: 10.1177/004051755902901003

Shen, D., and Gu, S. (2009). "The mechanism for thermal decomposition of cellulose and its main products," *Bioresource Technology* 100(24), 6496-6504. DOI: 10.1016/j.biortech.2009.06.095

Siró, I., and Plackett, D. (2010). "Microfibrillated cellulose and new nanocomposite materials: A review," *Cellulose* 17(3), 459-494. DOI: 10.1007/s10570-010-9405-y

Wang, N., Ding, E., and Cheng, R. (2007). "Thermal degradation behaviors of spherical cellulose nanocrystals with sulfate groups," *Polymer* 48(12), 3486-3493. DOI: 10.1016/j.polymer.2007.03.062

Xiao, S., Gao, R., Gao, L., and Li, J. (2016). "Poly(vinyl alcohol) films reinforced with nanofibrillated cellulose (NFC) isolated from corn husk by high intensity ultrasonication," *Carbohydrate Polymers* 136, 1027-1034. DOI: 10.1016/j.carbpol.2015.09.115

Zhang, Y., Peng, M., Wang, J., Gao, Q., Cao, N., and Yang, Z. (2015). "Corn yield response to phosphorus fertilization in northeastern China," *Agronomy Journal* 107(3), 1135-1140. DOI: 10.2134/agronj14.0600

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