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ORIGINAL PAPER

# Water retention value for characterizing fibrillation degree of cellulosic fibers at micro and nanometer scales

**Feng Gu · Wangxia Wang · Zhaosheng Cai · Feng Xue · Yongcan Jin · J. Y. Zhu**

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**Abstract** This study examined in detail the utility and validity of water retention value (WRV) for characterizing the extent of fibrillation of micro and nanofibrils. A bleached pulp fiber sample was either refined to different degrees using a PFI mill or milled for different time periods using a SuperMassColloider (SMC), to produce micro and nanofibril samples. These fibril samples were then characterized by electronic microscopic imaging (SEM and TEM), degree of polymerization (DP), Canadian Standard Freeness (CSF), enzymatic adsorption (EA), enzymatic cellulose digestibility (ED), as well as WRV measured under different relative centrifugation forces and durations. The results showed that WRV characterization is consistent with SEM and TEM imaging and correlate to sample DP, EA, and ED. CSF is a good measure for fiber samples, but is not sensitive and not suitable for characterizing microfibrils and nanofibrils. DP is a good measure of cellulose chain length or fiber cutting, but is not a sensitive measure for extensive fibrillation. The results also indicate that the centrifugation condition did not affect the effectiveness of WRV for characterizing fibril samples, as long as all samples were tested under the same conditions. A mild centrifugation condition, e.g., 3000*g* for 15 min, appears to have better sensitivity for characterizing fibril samples.

**Keywords** Water retention value · Cellulose · Cellulose nanofibrils · Refining · Milling · Fibrillation

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F. Gu · W. Wang · Z. Cai · F. Xue
School of Chemistry and Chemical Engineering, Yancheng Institute Technology, Yancheng 224000, China
e-mail: gufeng@ycit.edu.cn

W. Wang
e-mail: wangwangxia@ycit.edu.cn

F. Gu · W. Wang · J. Y. Zhu (✉)
USDA Forest Service, Forest Products Laboratory, Madison, WI 53726, USA
e-mail: jzhu@fs.fed.us

Y. Jin
Jiangsu Provincial Key Lab of Pulp and Paper Science and Technology, Nanjing Forestry University, Nanjing 210037, China

## Introduction

Cellulose, a linear polysaccharide of 1,4 linked β-D-glucose, is the most abundant renewable natural biopolymer on earth and having remarkable physical, optical, and mechanical properties. Cellulose nanomaterials, such as cellulose nanocrystals (CNCs) and cellulose nanofibrils (CNFs), with different morphologies could be widely used in various applications

(Fukuzumi et al. 2008; Nogi et al. 2010; Moon et al. 2011; Nair et al. 2014). During production of CNFs through mechanical fibrillation, the sources of cellulose and the pretreatment methods (chemical/mechanical/biological) can result in varied morphologies and sizes (Wang et al. 2012b; Jia et al. 2017; Qing et al. 2013; Abdul Khalil et al. 2014; Henriksson et al. 2007; Qin et al. 2016). The characterization of the morphologies of CNFs is often carried out using electron or atomic force microscopy. These microscopic imaging methods have many advantages; however, they are time consuming and expensive to obtain statistically meaningful information (Hirn and Bauer 2006). Developing a quantitative method with an adequate resolution for quick analyses of cellulosic fibrils is challenging. This is because cellulosic fibrils have lengths and width spanning from micro to nanometer scales (Gary 2013; Qin et al. 2016; Wang et al. 2012b).

Water retention value (WRV) measures the ability of a fiber sample to retain water and has been widely used in the pulp and paper industry to characterize the extent of fiber fibrillation. WRV of a fiber sample increases with refining (or beating) because of increased fiber swelling due to internal fiber fibrillation through delamination (Dekker 2003; Hartman 1985; Kerekes 2005b; Roffael and Kraft 2012). Refining also increases external surface area through external fibrillation (Hartman 1985, Kerekes 2005a) which increases fiber water retention. Several standards, such as TAPPI UM256, SCAN-C 62:00, and ISO 23714: 2012, have been developed to measure WRV. Typically, the WRV fixture includes a metal basket with a fine wire screen bottom that fits in an outer jacket having a shaped bottom for collecting expelled water; a plastic cylindrical adapter may be used depending on the size of the centrifuge (Fig. S1). Currently, WRV is mainly used in the pulp and paper industry to characterize fibrous materials with lengths in millimeters and radial dimensions of tens of micrometers (Scallan and Carles 1972; Luo et al. 2011; Mayr et al. 2017). Although WRV has been used to indirectly evaluate the degree of fibrillation of cellulose micro and nanofibrils with lengths in micrometers and diameters in the sub-micrometer range (Hu et al. 2015), its validity for such characterizations has never been fully explored and justified. Using existing equipment to measure WRV of cellulose micro and nanofibrils (or briefly fibrils) can result in cellulose loss during centrifugation through the wire screen, which affects measurement accuracy and therefore the proper characterization of the extent of fibrillation.

In this study, we used a membrane with a pore size of 0.1 μm in the WRV measurement unit to avoid cellulosic material loss through the bottom membrane during centrifugation. We compared the WRV obtained using different centrifugal forces and residence times against other methods for characterizing degree of cellulosic fiber fibrillation, i.e., degree of polymerization (DP), enzymatic adsorption capacity (EA), cellulose enzymatic digestibility (ED), Canadian Standard Freeness (CSF), scanning electron microscopy (SEM) and transmission electron microscopy (TEM) imaging. The objective of this study is to provide a careful evaluation of the effectiveness of WRV for characterizing cellulose nanomaterials. Because WRV testing is nondestructive, relatively simple to implement in both laboratory and commercial facilities, uses relatively inexpensive equipment, and is fairly quick (less than 30 min) to accomplish, this study has practical significance.

## Materials and methods

### Materials

A dry lap bleached kraft eucalyptus pulp (BEP) from Fibria (Aracruz, Brazil) was used as the feedstock for fibrillation study. The chemical composition of the BEP fibers was 78.1% glucan, 15.3% xylan, and 0.7% Klason lignin (Wang et al. 2012b). Dry lap BEP was first soaked in distilled water at solid consistency of 10% for 24 h, and then fiberized with a lab disintegrator (TMI, Ronkonkoma, NY). The disintegrated pulp fibers were collected by vacuum filtration and stored in a freezer until use. The commercial enzyme Cellic® CTec2 was used for enzymatic adsorption and hydrolysis studies and was kindly provided by Novozymes, North America (Franklinton, NC).

### Mechanical refining

A PFI mill (No. 160, Testing Machines, Inc., Amityville, L.I., NY) and a disk mill (SuperMassColloider, Model: MKZA 6-2, Disk model: MDGA 6-80#, Masuko Sangyo Co., Ltd, Japan) were used for BEP

fibrillation. A BEP suspension at 10% solids was prepared for PFI refining according to TAPPI standard procedure T248 (TAPPI 1996). Different revolutions (rev) of PFI milling were conducted to obtain pulp samples with varied degrees of fibrillation as measured by Canadian Standard Freeness (CSF), i.e., PFI10 (1500 rev): CSF = 437 mL, PFI30 (4500 rev): CSF = 206 mL, PFI51 (7500 rev): CSF = 147 mL. The freeness of the initial BEP pulp fibers was CSF = 630 mL. A BEP suspension at 2% solids of 4 L was prepared for SuperMassColloider (SMC) milling to generate cellulose micro and nanofibrils (Wang et al. 2012b; Qin et al. 2016; Hu et al. 2015). The SMC disk rotational speed was set at 1500 rpm with an initial disk plate gap of − 100 microns (the presence of fibers prevented the two disk touching each other during milling). SMC milling time was varied from 15 to 180 min.

### Degree of polymerization

The degrees of polymerization (DP) of the fiber or fibril samples were measured according to TAPPI Standard Test Method T230 om-99 (TAPPI 1999a). The DP of the cellulose was calculated using the following expression (Mazumder et al. 2000): $DP^{0.905} = 0.75(954 \log X - 325)$, where $X$ is the measured viscosity.

### Canadian Standard Freeness

The Canadian Standard Freeness of pulp fibers was measured according to TAPPI Standard Test Method T 227 om-99 (TAPPI 1999b) by recording the volume of water discharged from the side orifice of freeness tester.

### Enzymatic adsorption

Enzyme adsorptions to the milled BEP fibril samples were measured in slurries of pH 5.0 sodium acetate buffers containing 1% (w/v) fibrils (0.5 g in oven dry weight). The commercial cellulase enzyme, Cellic® CTec2 (81.7 mg protein/mL), was added to the slurries of 50 mL at a loading of 100 mg protein/g substrate (or briefly mg/g). The slurries were incubated on a shaking bed (MaxQ™ 4450, Thermo, Waltham, MA) at 20 °C and 200 rpm for 15 min. The slurries were then centrifuged (10 min, 5000 rpm) and the amount of enzyme adsorbed was determined by analyzing the amount of protein in the supernatant using the Bio-Rad protein method (Bradford 1976; Liu et al. 2011).

### Enzymatic digestibility

Enzymatic hydrolysis was performed in pH 5.0 sodium acetate buffer with a fibril consistency of 1% (w/v) on the same shaking bed incubator operating at 50 °C and 200 rpm for 48 h. Cellic® CTec2 was again used and dosed at a very low dosage of 1 mg protein/g substrate (or equivalently, 1.8 FPU/g substrate). The hydrolysate was separated through centrifugation at 5000 rpm for 10 min and analyzed for glucose using an YSI 2700 Biochemistry Analyzer (2700S, YSI Inc., Yellow Springs, OH, USA).

### Water retention value

Water retention value (WRV) was measured over a range of centrifugation conditions, including the condition specified in the Scandinavian test method SCAN-C 62:00 (Luo et al. 2011). A filtration membrane of pore size = 0.1 μm (JVWP14225, Millipore Corporation, USA) was used to eliminate cellulose loss through filtration during centrifugation. Because the samples from SMC milling was at 2% solids, we used this solids level in centrifugation for WRV measurements. This solids level is consistent with TAPPI UM256 (TAPPI 2011) of 1%. Fourteen gram fibril suspension was wrapped in the filtration membrane and placed in a centrifuge tube with a metal mesh underneath to leave space for water accumulation at the bottom of the tube (Fig. S1). Samples were centrifuged (International Centrifuge EXD 4871, International Equipment Co., Chattanooga, TN, USA) under relative centrifuge forces (RCF) of 1000, 3000, 6000 and 12,000$g$ for 15, 30 and 60 min. The centrifuged cellulosic samples were weighed before and after oven drying at 105 °C to determine WRV as follows:

$$\mathrm{WRV}\,(\%) = (m_{wet} - m_{od})/m_{od} \times 100$$

The $m_{wet}$ and $m_{od}$ are the mass of the centrifuged cellulosic sample before and after oven drying, respectively.

### Electron microscopy imaging, tensile test of mechanical fibrillated fiber films

The scanning electron microscopy (SEM) and transmission electron microscopy (TEM) imaging, the preparation of mechanically fibrillated fiber films, and film tensile testing were carried out as described previously (Wang et al. 2015a, b). Reported sheet mechanical properties were averages of test results from 12 specimens.

## Results and discussion

### Morphology of mechanically milled cellulosic fibrils

Electron microscopy provided direct observations of the morphologies of cellulosic fibers and fibrils resulting from two different mechanical fibrillation methods. Un-fibrillated BEP fibers had relatively uniform diameters with an average length of approximately 1 mm as shown in Fig. 1a. PFI refining reduced fiber length without substantially affecting fiber diameters as shown in Fig. 1b–d. Extended PFI refining resulted in increased fiber external surface area from the formation of fibrils at the fiber surface due to the shear and friction forces (Hartman 1985; Kerekes 2005a).

In addition to the shear and cutting forces, compression force plays a very important role in SMC milling (Wang et al. 2012b). Compression force drives internal fiber fibrillations by breaking the crosslinks between fibrils and leads to fiber delamination (Dekker 2003; Hartman 1985; Kerekes 2005b). Only 15 min of SMC refining (one pass, SMC15) resulted in a substantial amount of fines (the term used in the pulp and paper industry to describe microfibers of typically a couple of hundred micrometers in length) as shown in Fig. 1e. After 30 min of SMC milling (two passes, SMC30), almost all fibers were fibrillated into fine fibrils (Fig. 1f). Increasing SMC milling time to 60 min (SMC60) resulted in complete fibrillation of fibers into micro or nanofibrils (Fig. 1g). TEM imaging (inset in Fig. 1g) showed entangled nanofibrils. Further increasing SMC milling time did not result in finer fibrils or substantial changes in fibril morphology (comparing Fig. 1g with h), suggesting SMC fibrillation had reached the limit for the disk plate pattern used. These results are consistent with detailed SEM and TEM characterizations of the micro and nanofibrils from SMC milling of the same BEP fibers as published in our earlier study (Wang et al. 2012b).

### Measurements of cellulosic fibril water retention values

Different standards use different centrifugation conditions for measuring WRV of fibrous materials. According to the TAPPI standard UM 256, a relative centrifuge force (RCF) of 900*g* for a period of 30 min (TAPPI 2011) should be used. The Scan-C 62:00 method suggests a RCF of 3000*g* for 15 min (Luo et al. 2011). The ISO/FDIS 23714 standard suggests a RCF force of 3000*g* but with a longer time of 30 min. All these standards were developed for measuring WRV of fibers or fibrillated fibers, but not for micro or nanofibrils. WRV is a measure of the amount of water retained by the fiber or fibrils. It includes the amount of water in pores as well as in the thin layer on fiber or fibril external surfaces, especially those in the inter-fiber or inter-fibril meniscus (Luo et al. 2011). The idea of using centrifugation in WRV measurements is to reduce the amount of the bulk water on fiber or fibril surfaces so that WRV more accurately represents the amount of water in the fiber pores or the total fiber surface area. A higher force and longer centrifugation time can force out more water from fibrous materials than lower force and shorter time (Lips et al. 2009). WRV was found to correlate well with the fiber pore volume (Jaturapiree et al. 2008) and the amount of water in the fiber pores (Hui et al. 2009; Weise et al. 1996). Micro or nanofibrils have substantially more external and inter-fibril space than fibers. The extent of centrifugation certainly can have a substantial effect on the measured WRV of micro or nanofibrils. Early studies using WRV to characterize micro or nanofibrils did not provide sufficient data to support and validate the centrifugation conditions used for WRV testing (Hu et al. 2015).

Here we measured WRVs of both fiber and fibril samples with different degrees of fibrillation under different RCFs and durations. As expected, WRV varies with centrifugation RCF as well as duration for different sample types evaluated (Fig. 2). However, WRV can achieve an asymptotic value at a high RCF with extended centrifugation. For example, for the

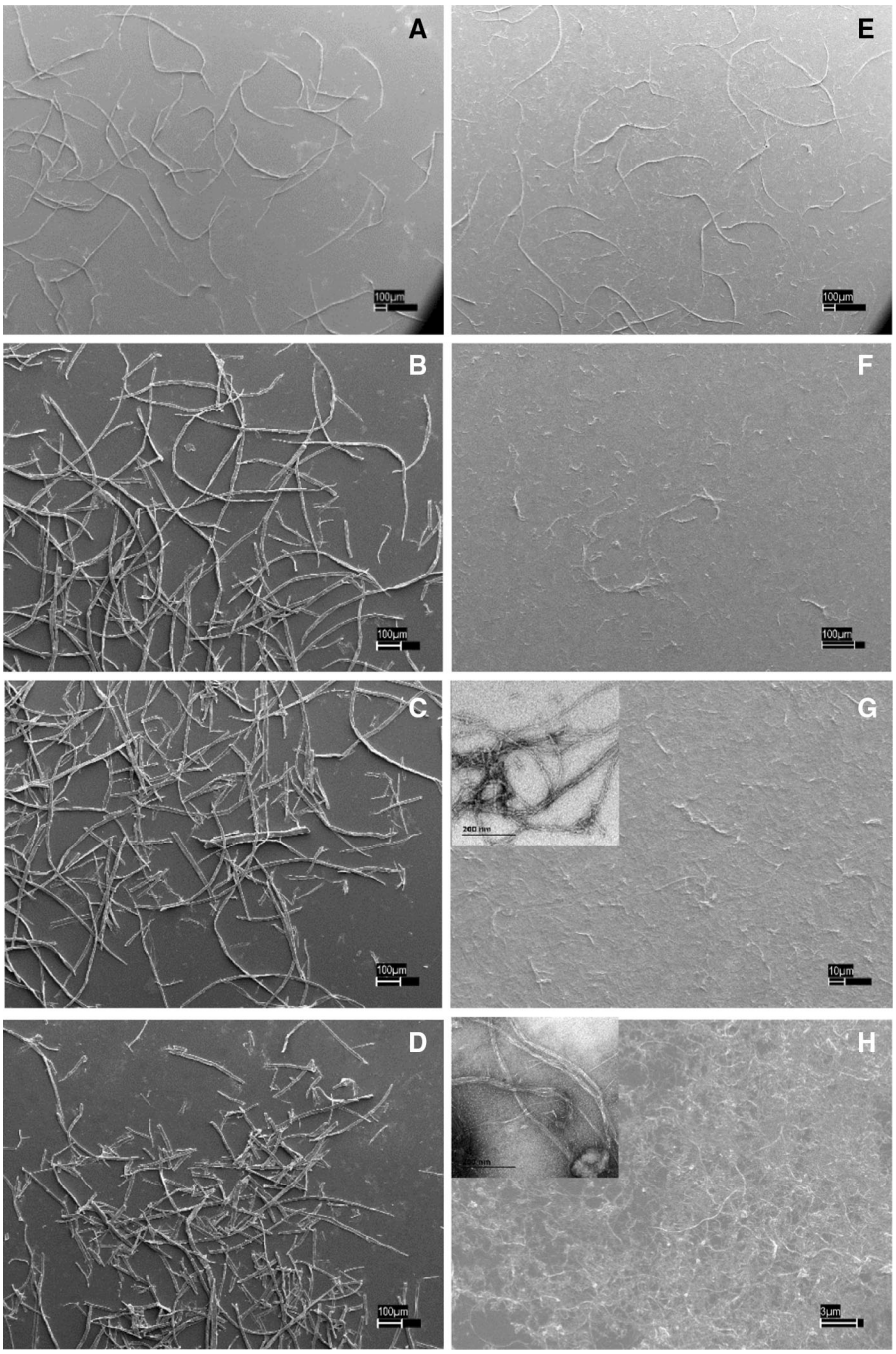


**Fig. 1** SEM and TEM images of unfibrillated BEP fibers and the fibrillated fibers and fibrils. **a** Unfibrillated BEP fibers; **b**–**d** PFI refined fibers: PFI10 (**b**); PFI30 (**c**); PFI51 (**d**); **e**–**h** SMC milled fibrils: SMC15 (**e**); SMC30 (**f**); SMC60 (**g**); SMC180 (**g**). **a**–**f** scales = 100 µm; **g**–**h** SEM scale = 10 µm; inset TEM scale = 200 nm

BEP fibers, an RCF of 6000*g* for 15 min was sufficient to achieve this asymptotic WRV of approximately 80% (Fig. 2a), suggesting most excess bulk water on fiber external surfaces can be removed under this centrifugation condition. A stronger centrifugation was needed for fibrillated fibers. For example, an RCF of 12,000*g* for 30 min was required to achieve an asymptotic WRV of approximately 130% for the well

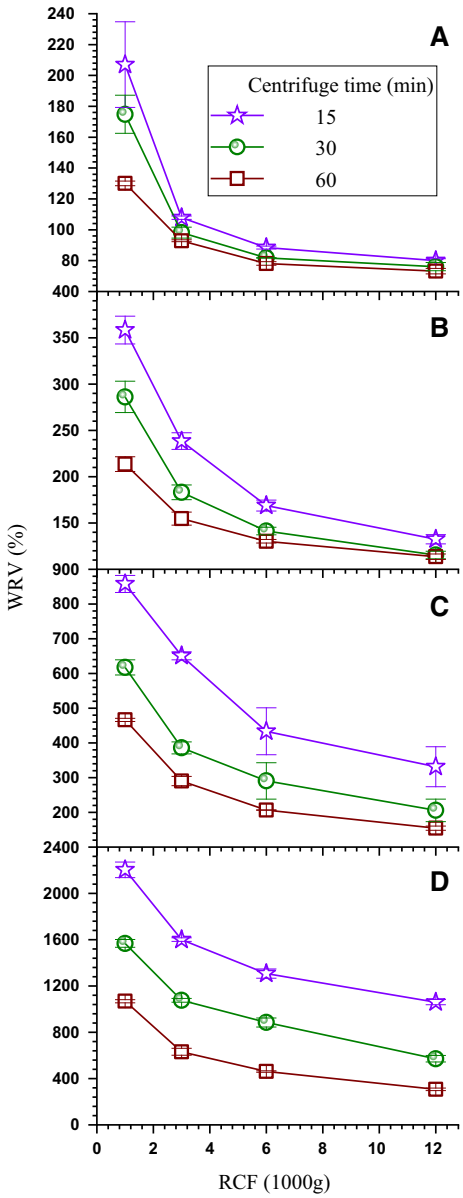


**Fig. 2** Effects of centrifugation condition on measured WRV of fiber and fibril samples. **a** BEP; **b** PFI51; **c** SMC15; **d** SMC180

fibrillated fiber sample PFI51 (Fig. 2b). As expected, an even stronger centrifugation condition of at least 12,000*g* for 60 min was needed to achieve an asymptotic WRV for microfibril sample SMC15 and nanofibril sample SMC180 as shown in Fig. 2c, d, respectively.

The results also indicate that a high RCF is more effective to achieve an asymptotic WRV for fibers. For example, even when extending the centrifugation time from 15 to 60 min (4 times), an asymptotic WRV was not achieved in measuring the initial BEP fibers, but increasing the RCF from 1000*g* to 3000*g* was sufficient as shown in Fig. 2a. However, an extended centrifugation time is needed for measuring fibril samples. For example, 15 min was not sufficient to achieve asymptotic WRV for sample SMC15 and SMC180 even at 12,000*g* as shown in Fig. 2c, d. When the centrifugation time was extended to 60 min, it appears that an asymptotic value can be achieved for these two fibril samples at RCF of 6000*g*. These results indicate that WRV of a fiber or fibril sample is highly dependent on the centrifugation condition used. Furthermore, the proper centrifugation condition varies with the degree of fibrillation of the sample.

To further illustrate the above discussion, we fitted the WRV data for the BEP and 8 refined samples with centrifuge force RCF $G$ and time $t$, using the below equation,

$$WRV = W \cdot \exp[-(A \cdot G^{\alpha} + Bt)] + WRV_o$$

where $G$ is RCF force in 1000*g*, $t$ is centrifugation time in min, $WRV_o$ is the lowest WRV value measured for the sample under the most severe centrifugation condition (theoretically it should the asymptotic WRV value and need to be fitted, but that would add one more fitting valuable). The fitting results were listed in Table S1. The SMC fibril samples were more difficult to obtain good fit than the PFI milled samples, which may well be due to the limited data points of 12 WRV values for each sample and the difficulties to make an accurate estimation of the functionality between WRV and centrifugation conditions. This difference is inherently the difference in milling process. For relative comparisons among each subset samples, we listed two sets of fitting result for BEP in Table S1. The coefficient of centrifuge time, $B$, did not vary substantially among the subset samples, suggesting a constant centrifuge time for different samples is a valid. The coefficient of centrifuge force, $A$, decreased slightly for the SMC milled fibril samples with continued milling, suggesting, nanofibril samples may require a slightly higher centrifuge force than testing fiber samples. $W$ value, related to the water holding capacity without centrifugation, was increased with milling.

## Quantitative characterization of mechanically fibrillated cellulose fibrils

Various measures, i.e., DP, EA, ED, and CSF of the unfibrillated BEP and fibrillated samples, were used to characterize the extent of fibrillation by PFI and SMC. WRVs measured according to the SCAN standard at RCF = 3000*g* for 15 min were also used. DP is the number of monomeric glucose unit along the cellulose chain length direction, a measure of fiber or fibril length. Increasing the extent of PFI refining or SMC milling resulted in a reduced cellulose DP. DP was reduced from 1040 (BEP) to 919 (PFI51), or by 12%, after extensive PFI refining. DP reduction is most rapid in the first 10 min of PFI refining as shown in Fig. 3a. DP reduction was 15% to 884 after 15 min SMC milling (SMC15). Extended SMC milling to 180 min did not result in substantial reduction in DP, or only a reduction of 20% from 1040 to 803 (SMC180). The DP data suggest that SMC was efficient in reducing cellulose chain length within the first 15 min and may have reached its limit in cutting cellulose chain length as the rate of DP reduction was substantially decreased beyond 15 min (Fig. 3b). However, substantial morphological changes and extensive fibrillation took place between 15 to 60 min SMC milling as can be seen from SEM imaging (comparing Fig. 1e with f) while DP reduction was minimal from 884 to 854, or only 3%. This suggests that SMC milling was very effective in fibrillation rather than cutting fibers, and that DP does not adequately represent the extent of fibrillation which took place during this time period. Comparing the DP values of the PFI refined fiber samples with those SMC milled micro and nanofibril samples revealed no great differences (Fig. 3a, b), although SEM images (Fig. 1) showed substantial differences in morphology and the extent of fibrillation, which again indicates that DP is not a good measure of degree of fibrillation. Fiber cutting that can be characterized by DP is different from fiber fibrillation. As fiber and fibril cutting always accompany fibrillation in the mechanical processing of fibers to produce fibrils, DP, to a small extent, could be used to characterize fibrillation, at least at the beginning stages.

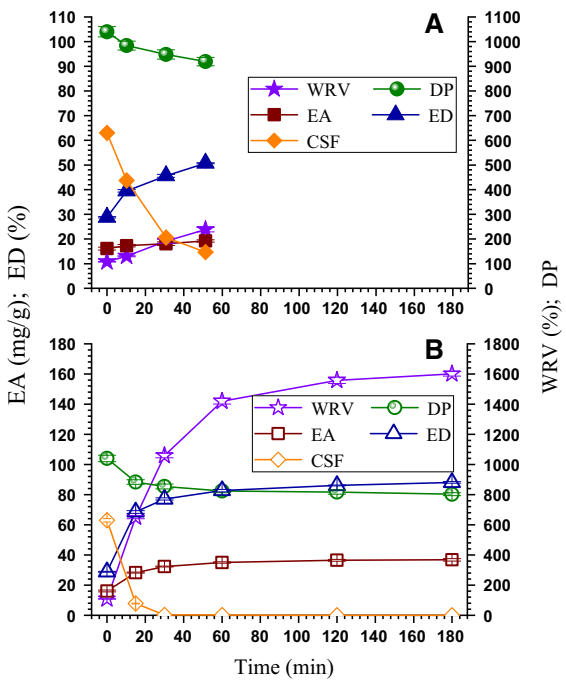


**Fig. 3** Using WRV (measured at 3000*g* for 15 min) and other measures to characterize the extent of fiber fibrillation through PFI refining (**a**) and SMC milling (**b**)

It should be noted that unfibrillated BEP fibers, over 1 mm long (Fig. 1a), had an average DP of only approximately 1040 while SMC15, containing a substantial amount of fines (a couple of hundreds of micrometer in length), had a DP of 884. This supports the fiber longitudinal model we proposed previously (Qin et al. 2016), i.e., cellulosic fibrils aggregate longitudinally to form a fiber, and therefore, the DP of a fiber is not much different from that of typical uncut fibrils. This again indicates that DP is not well suited for characterizing the degree of fibrillation.

CSF is a measure of water drainage rates from fibers and is often used to characterize the degree of fiber fibrillation in pulp and paper science. The freeness has been shown to be related to the surface conditions and swelling of the fibers (TAPPI 1999b). In our testing, CSF decreased from 630 mL for unfibrillated BEP fibers to 147 mL after 51 min (7500 rev) of PFI refining (PFI51). It appears that CSF reduction was rapid in the first 10 min (1500 rev) of PFI refining (Fig. 3a), in agreement with DP reduction. However, CSF apparently loses sensitivity and is not a good measure for characterizing the degree of fibrillation of micro or nanofibril samples that have very low water drainage rates, as shown in Fig. 3b. In fact, CSF was zero for fibril samples obtained from SMC milling longer than 30 min.

Similar to that observed for DP and CSF characterizations, WRV of fiber samples measured at RCF = 3000*g* for 15 min appears to linearly correlate with the PFI refining time, as shown in Fig. 3a. WRV moderately increased from 108% for the unfibrillated BEP fibers to 238% after 51 min of PFI refining (PFI51). WRV was substantially increased with only 15 min of SMC milling, i.e., from 108 to 651% (6 times, Fig. 3b). Unlike PFI refining that mainly cuts fibers without substantial morphological changes (Fig. 1a–d) as discussed above, the BEP fibers went through substantial morphological changes by 15 min of SMC milling as can be seen by comparing Fig. 1a with e. Most of the fibers were fibrillated into micro or nanofibrils. The fibril morphology continued to change with continued SMC milling to 60 min (Fig. 1e–g). This is also reflected by WRV with continued increase to over 1400% after 60 min SMC milling (Fig. 3b). However, further extending SMC milling time beyond 60 min did not result in substantial increase in WRV. This is in agreement with that observed in SEM imaging (Fig. 1g with h). This suggests that SMC has achieved its limitation after 60 min milling due to the fixed disk plate pattern used. Furthermore, WRV tracks the extent of fibrillation well during the entire course of SMC milling according to SEM imaging.

EA and ED can characterize the cellulose accessibility to cellulase enzyme (Wang et al. 2012a), which is dependent on the sum of the internal and external surface areas, similar to porosity, and therefore should correlate to the degree of fibrillation. EA of BEP was 16.1 mg protein/g substrate (or briefly mg/g) with ED at 28.8%. PFI refining for 50 min moderately improved cellulose accessibility EA to 19 mg/g and therefore ED to 51% (PFI50), or increases of 19 and 76%, respectively. EA and ED measurements also indicated that the extent of fibrillation was much more substantial by SMC milling (Fig. 3b). EA and EA were increased to 28.3 mg/g and 68.5% after only 15 min SMC milling, or increases of 76 and 138%, respectively. The increases in EA and ED continued with further milling, but plateaued after 60 in SMC milling, in agreement with the characterizations using WRV, and consistent with that observed from SEM imaging (Fig. 1).

Above discussion indicates that EA and ED can be good measures of the degree of fibrillation of cellulose fibers, in particular, EA can be considered as a measure of fibril total surface area, similar to porosimetry but using an enzyme as the probe molecule. Therefore, we correlated WRV with all measures including EA and ED (Fig. S2). We fitted the EA and ED data sets ($y$) with WRV ($x$) from RCF = 3000*g* for 15 min using a quadratic equation for quantitative predictions (Fig. S2B).

$$x - a = k(y - b)^2; \quad \text{or} \quad y = \sqrt{(x - a)/k} + b$$

where $a$ = 46 and 108, $b$ = 10.77 and 32.09, and $k$ = 2.263 and 0.477, for EA and ED, respectively. The quadratic equation indicates that WRV is more sensitive to fibrillation than EA or ED. This is because water molecule used in WRV measurements is much smaller than enzyme (45 kDa for CTec2 used in this study). WRV measurements are also much simpler than measuring EA and ED.

As discussed in the previous section, WRV of a sample depends on the centrifugation condition used. To further illustrate the utility of WRV for characterizing the degree of fibrillation of micro and nanofibrils, we used the WRVs measured at four centrifugation conditions, i.e., the mildest condition at RCF = 1000*g* for 15 min, a moderate condition at RCF = 3000*g* for 15 min (results presented in Fig. 3), a severe condition at RCF = 6000*g* for 30 min, and the most severe condition at RCF = 12,000*g* for 60 min, to characterize the PFI refined fiber and the SMC milled fibril samples. The results indicate that the extent of fibrillation can be well characterized by WRV obtained at each of the four centrifugation condition as shown in Fig. 4a. Despite the absolute values of WRV differing for any sample when using different centrifugation conditions, the ranking of the extent of fibrillation between the 8 samples measured by WRV at any one centrifugation condition, was the same. This suggests that one can use WRV values measured at any centrifugation condition to characterize or compare the extent of fibrillation of fiber or fibril samples, as long as all samples are tested under the same centrifugation condition. WRV values from the four different centrifugation conditions correlated well with other measures, i.e., DP, CSF, EA, ED for both the fibers from PFI refining and fibril samples from SMC milling. The results clearly show monotonic proportional correlations (Fig. S2).

To determine the optimal centrifugation condition under which WRV can provide the most sensitive

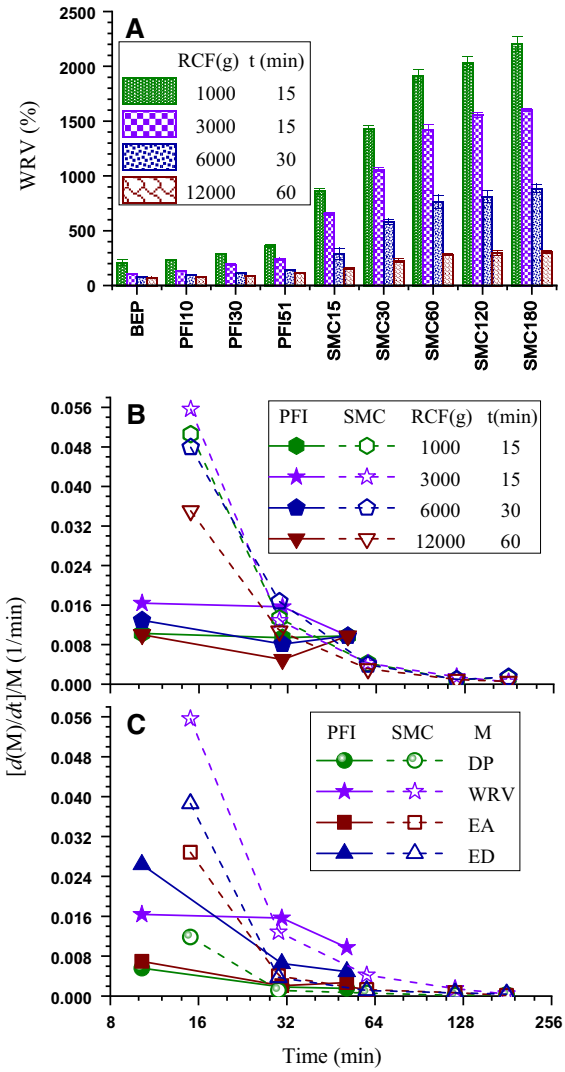


**Fig. 4** Comparisons of WRVs measured at four different centrifugation severities for characterizing the time progression of fiber fibrillation through PFI refining and SMC milling. **a** WRV; **b** comparison of sensitivity of WRV measured under different centrifugation conditions for characterizing fibrils during fibrillation; **c** comparison of sensitivity of WRV with DP, EA, and ED for characterizing fibrils during fibrillation. *M* in *y* coordinate represents WRV for **b** and DP, WRV, EA, and ED for **c**

characterization of the degree of fibrillation for micro or nanofibrils, we calculate the time derivatives of normalized WRV, i.e., $\frac{d(WRV)}{dt} \cdot \frac{1}{WRV}$, for both PFI refining and SMC milling as each process proceeds. The relative WRV was used to normalize the variations in WRV values measured under different centrifugation conditions. The time derivative was used to observe the effect of milling (duration in this case) on the change of the degree of fibrillation of the samples. The results indicate that mild to moderate centrifugation conditions are preferred to obtain good measurement sensitivity, i.e., a high $\frac{d(WRV)}{dt} \cdot \frac{1}{WRV}$ value. From the data shown in Fig. 4b, RCF = 3000*g* for 15 min produced the highest sensitivity for most fiber and fibril samples. The results also indicate that the effectiveness of PFI refining to fibrillate fibers was substantially lower than initial SMC milling (within 15 min or one pass). However, the effectiveness SMC milling reduced after the first 30 min because the extent of fibrillation was substantial and SMC milling may have reached its limit for the fixed disk plate pattern used.

We used the same approach to compare WRV with other measures, i.e., DP, EA, ED, for characterizing the extent of fibrillation. As shown in Fig. 4c, WRV obtained at RCF 3000*g* for 15 min produced the highest sensitivity for most of the samples evaluated. DP had the least sensitivity, which is understandable as DP is a measure for characterizing fiber or fibril cutting and is not a good measure for characterizing fibrillation as discussed previously. EA is also a poor measure especially for fiber samples, perhaps due to the fact that only those pores accessible to cellulase (Mw = 45 kDa, much larger than water of 18) are accounted for in EA measurements. This comparison further supports using WRV for characterizing the degree of fibrillation of micro and nanofibrils.

## Mechanical properties of cellulose fibril sheets

To further demonstrate the utility of WRV for characterizing micro and nanofibrils, we plotted the mechanical properties, i.e., density (ρ), strain (ε), specific modulus of tensile elasticity MOE (E/ρ), and specific tensile stress (σ/ρ), of the sheets made from the fiber and fibril samples with respect to the WRVs of the samples. Density of the sheets were determined by measuring the sheet weight, area, and thickness using a digital caliper (Sehaqui et al. 2011). As shown in Fig. 5, densities of cellulose sheet increased rapidly with either PFI refining or SMC milling. Further SMC milling beyond 15 min resulted in much less increase in sheet density, which is consistent with the slowed increase in the degree of fibrillation observed in SEM imaging. This is also in agreement with a previous

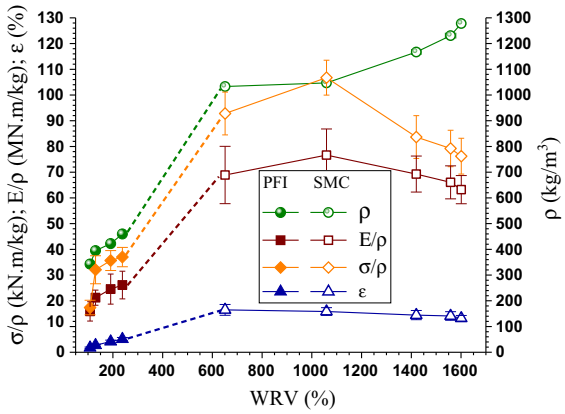


**Fig. 5** Using WRV to characterize the mechanical properties of sheets made of fibrillated BEP fiber and fibril samples with varied degrees of fibrillation

studies in SMC milling of the same BEP fibers (Wang and Zhu 2016) and mixed office paper that contained filler ($CaCO_3$) (Wang and Zhu 2015). The density increased from 343 kg/m$^3$ for the sheet made from unfibrillated BEP fibers to 1278 kg/m$^3$ for the sheet made from fibrils with 180 min SMC milling. The effectiveness of SMC milling can be clearly seen from the rapid density increase with only 15 min milling due to the delamination of fibers into microfibrils which reduced the voids in the sheet. Specific MOE of the sheets increased rapidly with milling, going from 1.6 MNm/kg for unfibrillated BEP up to 7.7 MNm/kg after 30 min of SMC milling. Further milling did not improve MOE. The variations among samples from milling over 30 min were within measurement errors, again in agreement with the previous study in SMC milling of mixed office paper (Wang and Zhu 2015, 2016). Similarly, specific tensile strength increased rapidly with initial milling, especially during the first 15 min of SMC milling. Specific tensile increased from 17.4 kNm/kg for the unfibrillated BEP sheet to 92.8 kNm/kg for the SMC15 sheets, or an increase of over 5 times, while sheet density increased by threefold after 15 min of SMC milling. This indicates that improved fibrillation densified the sheet, which substantially improved hydrogen bonding. The highest specific stress was 106.7 KNm/kg for SMC30. Continued SMC milling resulted in the shortening of fibrils as reflected from the measured DP (Fig. 3), but without substantial improvement in the extent of fibrillation, which can cause the reduction in tensile strength (Fig. S3) and therefore specific tensile strength. Strain to failure of cellulose film ranges from 1.8 to 16.5%; the highest strain was reached with SMC15. Therefore, SMC milling also substantially improve sheet tensile energy absorption or toughness.

## Conclusions

WRV is a reliable measure to characterize the extent of fibrillation of micro and nanofibrils. Characterization of the degree of fibrillation of fibrils using WRV is consistent with the observation from SEM images. In measuring WRV of micro and nanofibrils, a fine membrane of pore size in 0.1 μm is recommended to eliminate cellulose loss through centrifugation in measuring micro and nanofibrils. Furthermore, a moderate centrifugation condition, such as a RCF force of 3000*g* for 15 min as specified in SCAN-C 62:00, is preferred to obtain better sensitivity. Enzyme adsorption (EA) and enzymatic digestibility (ED) mimics porosity measurements to represent the total fibril surface area. EA or ED (y) can be scale to WRV (x) using a quadratic equation. However, comparing with WRV measurements EA and ED have low sensitivity and are more difficult to implement. Furthermore, ED measurements are destructive. CSF, a good measure of fiber refining, is not suitable for characterizing the extent of fibrillation of fibrils. DP can provide good measure of the cellulose chain length reduction through refining or milling, but cannot provide good measure of the degree of fibrillation. Based on DP and WRV measurements, SMC milling produced good fibrillation with low degree of fiber cutting.

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