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Biorefining of canola straw using pressurized aqueous ethanol: nanosized cellulose

Raquel Razzera Huerta[a] and Marleny D.A. Saldaña[a,*]
[a]Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada T6G 2P5
*marleny@ualberta.ca

ABSTRACT

Canada produces in average 16 million tons of canola seed every year, generating large amounts of straw, commonly utilized for bedding and plant nutrient recycling. Canola straw is mainly composed of cellulose (32%), hemicellulose (23%) and lignin (16%). The production of nanosized cellulose is gaining attention because of its unique mechanical and thermal properties, being a suitable material for biocomposites, food packaging, and biomedical applications. The objective of this study was to investigate the production of nanosized cellulose using environmentally friendly approaches. Canola straw was treated using pressurized aqueous ethanol followed by mechanical disintegration to optimize cellulose size. The effect of ethanol concentration (0-100%) at 180°C and 50 bar was studied for the maximum removal of non-cellulosic compounds, and the effect of ultrasonication treatment, for 30 min and output power of 1200 W, was evaluated for the disintegration of enriched cellulose residue. Samples were characterized for their chemical composition, crystallinity, thermal degradation, morphology, water retention value and size. Extensive removal of total hemicellulose and partial removal of lignin were obtained at 180 °C, 50 bar and 20% ethanol, leading to a cellulose enriched treated residue with 70% of cellulose. Furthermore, the surface morphology studies showed clear rupture of the material confirming the removal of hemicellulose and lignin after the pressurized treatment process. Disintegration of the cellulose enriched residue using ultrasonication resulted in uniformly dispersed nanosized cellulose. The morphology analysis showed nanocellulose fibers with diameters in a range of 3-30 nm the lengths of several micrometers. The results suggest that the biorefining of canola straw using pressurized aqueous ethanol followed by mechanical disintegration can be an environmentally friendly alternative for nanosized cellulose production with no residual chemicals for its application in food packaging and as a reinforcement material for biocomposites.

1. INTRODUCTION

Lignocellulose is the most abundant renewable biomass. The available sources of lignocellulosic biomass include hardwood, softwood, grasses and agricultural waste, such as stalks, straws, hulls and husks [1]. Canada is known by the large production of canola seed every year (an average of 16 million ton/year), generating large amounts of straw. Worldwide, there is an increasing trend toward the biorefinery of lignocellulosic biomass to produce fuels and high value added compounds. One of the most important goals of lignocellulosic biomass refining is to fractionate lignocellulosic biomass into its main components: cellulose (35–50%), hemicellulose (20-35%), and lignin (10–25%).

For the past few years, the fractionation of biomass towards cellulose isolation has received increasing attention due to its large availability, biocompatibility, sustainable production and potential uses within different industries, including paper making, building materials, pharmaceuticals, cosmetics, insulation, food, animal feed and liquid fuel production

[1]. Cellulose is a polymer of glucose linked together by β-1-4-linkages and hydrogen bounds, where the molecules are aggregated into long straight fibrils with diameter that range from 2 to 20 nm, and a length of more than a few micrometers [2]. Cellulose can also be expressed as glucan content, as it covers all glucose polymers in which the glucoses are linked by glycosidic bonds [3].

Various treatments, such as mechanical, chemical, physicochemical and biological methods, were reported to fractionate lignocellulosic biomass into valuable cellulose material. Pressurized fluids technology is an environmentally friendly approach which has been studied for treatment of agricultural residues such as cereal and oilseed straws [4, 5, 6, 7, 8], lupin hull [9] and sugarcane bagasse [10]. Pressurized fluids at subcritical conditions exhibit unique properties due to the change of dielectric constant, ionic product, density, viscosity and diffusivity. Hemicelluloses are easily solubilized and hydrolyzed in subcritical water at temperatures above 180 °C, where the addition of ethanol is recognized to be a suitable solvent to improve lignin removal [3, 7, 8]. Buranov & Mazza [8] fractionated flax straw using pressurized aqueous ethanol (PAE) at temperatures of 160–220 °C, ethanol concentrations of 0–95% (v/v), a pressure of 50 bar, flow rates of 1.5–7 mL/min, sample sizes of 2.5–25 g and liquid-to-solid ratios of 27–100 mL/g. The optimal condition for simultaneous extraction of hemicelluloses (80%) and lignin (78%) was obtained at 30% (v/v) ethanol concentration, 3 mL/min flow rate, and 18 mL/g liquid-to-solid ratio. Under these extraction conditions, cellulose degradation was negligible. Literature suggested that ethanol induces hydrolysis of esters and ethers bonds of lignin, increasing the formation of soluble lignin in ethanol [5].

As cellulose is insoluble in water and most organic solvents, the application of nanosized cellulose, which means that at least one dimension is in nanoscale (1–100 nm), has increased due to its high surface area, high tensile strength, low coefficient of thermal expansion, non-toxicity and excellent mechanical properties [11]. The production of nanosized cellulose requires intensive mechanical treatments, such as high pressure homogenization [12], grinding [13], cryocrushing [14] and high intensity ultrasonication [2, 15, 16]. As an efficient method, the ultrasonication consists in exposing a liquid to ultrasonic waves (~ 20 kHz), leading to the formation, growth, and violent collapse of cavities in water. This process called cavitation, provides energy of approximately 10–100 kJ/mol, which is within the hydrogen bond energy scale [17, 3]. Some researchers have used the combination of pressurized fluids, chemical treatment and ultrasonication to obtain nanofiber cellulose [16]. In the experimental conditions used by Ciftci et al. [16], lupin hull was fractionated using subcritical water at 180°C, 50 bar and 5 mL/min followed by bleaching treatment using 1.7% acidified sodium chlorite at 75 °C for 4h to obtain purified cellulose fibers. The disintegration of cellulose fibers was induced by ultrasonication for 35 min at 80% amplitude and output power of 700 W resulting in nanofibers with an average diameter of 15 nm. Although the chemical treatment favored delignification, a long processing time is required, and extensive water consumption is needed to wash the recovered cellulose.

Therefore, the main objective of this study was to evaluate treatment of canola straw using a combination of pressurized fluid processing followed by mechanical fibrillation with ultrasonication as an environmentally friendly alternative for sustainable nanosized cellulose production, with no residual chemicals. The effect of ethanol concentration (0–100%) at 180 °C, 50 bar and flow rate of 5 mL/min for 40 min on removal of maximum hemicellulose and lignin were studied. Then, the disintegration of the cellulose enriched residue at 1 wt.% was induced by ultrasonication for 30 min and an output power of 1200 W. Scanning electron microscopy (SEM), X-ray diffraction (XRD) and thermo-gravimetric analysis (TGA) of treated sample at optimum conditions were performed to investigate the impact of pressurized treatment on fractionation of canola straw. Furthermore, transmission electron microscopy

(TEM) and water retention value (WRV) analysis were used to evaluate the the production of nanosized cellulose using high intensity ultrasonication.

2. MATERIALS AND METHODS

Canola straw was kindly provided by Dr. Barry Irving (University of Alberta). The sample was ground and sieved to <1 mm particle size in a centrifugal mill (Retsch, Haan, Germany). All chemicals used, such as sodium carbonate anhydrous (99%, ACS reagent), sulfuric acid (72%, ACS reagent), and sugar standards (≥ 96% purity) were obtained from Sigma Aldrich (Oakville, ON, Canada) and used as received without further purification.

2.1. Pressurized aqueous ethanol treatment

Pressurized aqueous ethanol (PAE) treatment of canola straw was performed in a semi-continuous flow type subcritical fluid system previously described [7]. The experiments were conducted at 180°C, 50 bar and ethanol concentrations of 0–100% (v/v) for 40 min. The solid residue left in the high-pressure vessel after each experiment was dried in an oven at 30 °C.

2.2. High intensity ultrasonication treatment

Canola straw cellulose-rich residue was ground and sieved to a particle size of <106 mm. The aqueous dispersion (1 wt.%) was treated with a high intensity ultrasonicator (Model FS-1200N, Shanghai Sonxi Ultrasonic Instrument Co., Shanghai, ZJ, China) equipped with a cylindrical titanium alloy probe tip of 2 cm in diameter. The ultrasonication of the samples was carried out at output power of 1200 W for 30 min. Ultrasonic treatments were performed in an ice/water bath to prevent heat generation.

2.3. Characterization

2.3.1. Chemical composition

Raw and pressurized treated samples were analyzed for lignin, hemicellulose and cellulose contents following the NREL standard analytical procedures [18]. The total lignin contents of the samples were expressed as the sum of the acid insoluble lignin and acid soluble lignin. Total hemicellulose (xylose, arabinose, and mannose) and cellulose (glucose) in the hydrolysates were determined using an Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, CA, USA) with an UV detector and Aminex sugar HPX-42C column (300 mm x 7.8 mm; Bio-Rad, Hercules, CA, USA) operated at 85 °C. A HPLC grade water was used as the mobile phase at a flow rate of 0.6 mL/min.

2.3.2. Crystallinity index (CI)

The untreated and pressurized treated canola straw were analyzed by an X-ray diffractometer (XRD Rigaku Ultima IV, Tokyo, Japan) with a D/Tex detector using a Fe filter, over the 2θ range of 10°-40°. Cu tube was used at the conditions of 40 kV and 40 mA. The scanning speed was 0.6 degrees 2θ per minute with a 0.01 step size.

2.3.3. Thermo-gravimetric analysis (TG)

Thermo-gravimetric analysis was performed using a thermogravimetric analyzer TGA Q50 (TA Instrument, New Castle, DE, USA). About 10 mg of sample was placed in a platinum pan and heated from room temperature to 600 °C at a heating rate of 10 °C/min under a nitrogen flow of 60 mL/min.

2.3.4. Water retention value (WRV)

The water retention values (WRV) of pressurized treated residue before and after ultrasonication and commercially pure cellulose (1 wt.%) were measured according to Cheng et al. [19] where the WRV is defined as the percent ratio of water retained in the sample after centrifugation (900*g* for 30 min) to the dry weight (dried at 105 °C) of the sample.

2.3.5. Microscopic analysis

The morphology of untreated and pressurized treated fibers were analyzed using Zeiss Sigma Field Emission SEM (Carl Zeiss AG, Oberkochen, BW, Germany). The nanocellulose dispersions were analyzed by a transmission electron microscope (TEM) (H7500 TEM,

Hitachi, Tokyo, Japan) operated at 80 kV, where the diameters of nanocellulose were calculated using the ImageJ processing software IJ1.46.

2.4. Statistical analysis

Data are presented as mean ± standard deviation based on at least duplicate experiments and analysis. Statistical analyses were carried out using RStudio 3.4.1 (RStudio Inc., Boston, MA, USA) at 95% confidence interval.

3. RESULTS & DISCUSSIONS

Figure 1 shows the chemical composition of untreated and PAE treated canola straw under different conditions. Untreated canola straw consisted of 32.6±0.15% cellulose, 23.3±0.03% hemicellulose and 15.9±0.10% lignin. These results are similar to those reported by Wi et al. [20], where canola straw presented 31.7% cellulose, 24.7% hemicellulose and 16.5% lignin.

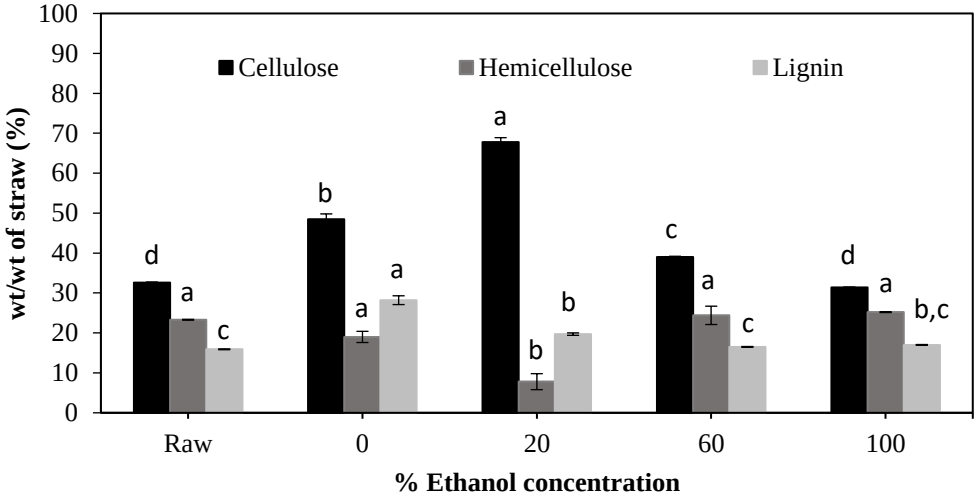


Figure 1. Chemical composition of canola straw before and after pressurized fluid treatments at 180 °C, 50 bar, 5 mL/min for 40 min.

The pressurized treatment with 0% ethanol (pressurized hot water) extracted 62% of hemicelluloses, which increased the cellulose (48%) and lignin (28%) contents in pretreated canola straw through a concentration effect (Figure 1). At mild operating temperatures up to 185 °C, hemicellulose cleaving that takes place during pressurized hot water treatment. The breaking of bonds between the monomeric sugars of hemicellulose produces shorter oligomers that have a length low enough to be extracted, but lignin oligomers cannot be dissolved in water at mild temperatures due to their hydrophobic features [21, 22].

Similar results of pressurized fluid treatment on removal of hemicelluloses and subsequent increase of cellulose and lignin content was previously reported for a variety of lignocellulosic biomasses [4, 6, 9, 10]. Vallejos et al. [10] during pressurized hot water treatment of sugarcane bagasse at 170 °C for 60 min and a liquid-to-solid ratio of 6 mL/g, extracted 69% of hemicellulose (xylan), resulting in a residue rich in cellulose (64%) and lignin (26%). Pronyk & Mazza [4] hydrolyzed triticale straw using pressurized hot water at 184 °C for 60 min and a liquid-to-solid ratio of 40 mL/g. The hydrothermal treatment yielded a hydrolysate containing more than 90% of the initial hemicellulose and a solid residue containing 68% cellulose and 20% lignin. Similarly, the pressurized hot water treatment at 180 °C, 50 bar and 30 min, extracted 85% of hemicelluloses from lupin hull, increasing cellulose and lignin content up to 79 and 15%, respectively [9]. According to Takada et al. [22], the lignin in the secondary wall is rich in ether type linkages and easily removed, while the lignin in the middle lamella is not removed due to intermolecular condensation during the pressurized hot water treatment, resulting in a enriched cellulose material with residual lignin.

Canola straw was hydrolyzed by Pronyk & Mazza [6] using a flow-through reactor, where the water was pumped at 165 °C with a flow rate of 115 mL/min and a liquid-to-solid ratio of

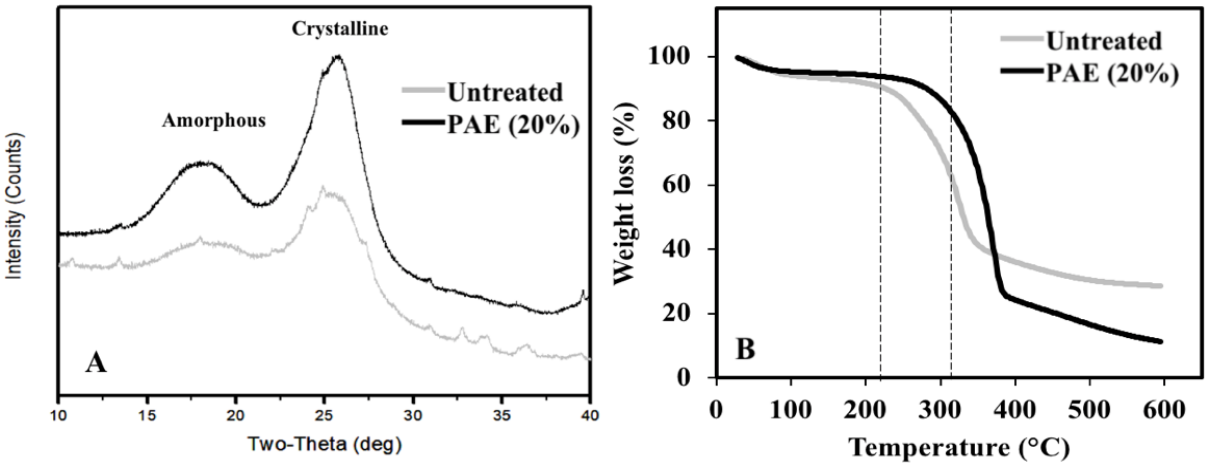
60 mL/g, during 60 min. The treatment extracted 81% of the initial hemicellulose, yielding a solid residue mainly composed of 64% cellulose and 23% lignin, which are similar to values obtained in this study (53% cellulose and 28% lignin) at 180 °C, 50 bar, 5 mL/min for 40 min. This can be explained by the macromolecular structure, crystallinity and the formation of intramolecular H-bonds on cellulose structure and the good thermal stability of lignins, which enhances their stability for hydrothermal experiments, requiring elevated temperatures for its hydrolysis [3]. Kim & Lee [23] reported almost no reduction in glucan content of corn stover treated with pressurized hot water at temperatures of 170–220 °C. According to Sasaki et al. [24], pressurized hot water at temperatures below 280 °C gradually reduced the cellulose particles with increasing reaction time but at high temperatures (300-320 °C) cellulose particles depolymerize in about 10 seconds.

In the present study, the addition of 20% ethanol was beneficial for the extraction of hemicelluloses from canola straw (Figure 1). PAE (20%) extracted 81% of the initial hemicellulose, yielding a solid residue mainly composed of 68% cellulose and 20% lignin. Previously, PAE was used to fractionate flax straw in order to determine the effect of ethanol concentration on the level of simultaneous extraction of hemicellulose (xylan) and lignin [8]. One-stage and two-stage extractions involving pressurized hot water followed by PAE treatment was also carried out to determine the extent of cellulose (glucan) degradation. Extractions were studied at constant pressure of 50 bar and temperatures of 160–220 °C, ethanol concentration between 0–95% (v/v), flow rates of 1.5–7 mL/min, sample sizes of 2.5–25 g and liquid-to-solid ratios of 27–100 mL/g. The optimal condition for PAE extraction was 180 °C, 30% ethanol, liquid-to-solid ratio of 18 mL/g, and flow rate of 3 mL/min for 25 g of flax straw. Under these conditions, one-stage process removed about 80% of the hemicellulose (xylan) and 78% of the lignin, where the two-stage extraction was efficient for removing xylan (100%) but much less efficient for removing lignin (37%). Aqueous ethanol treatment, also known as organosolv treatment, has been recognized as an efficient delignification process in the the paper and pulp industry, where it typically results in up to 50% lignin removal through cleavage of lignin-carbohydrate bonds and β-O-4 interunit linkages and its subsequent solubilization in the organic solvent [25].

The X-ray diffraction (XRD) patterns and TG thermograms (Figure 2A and B) of canola straw changed significantly after the hemicellulose removal and delignification achieved with the pressurized aqueous ethanol (20%) treatment. The CI values of untreated and pressurized aqueous ethanol (20%) treated canola straw were 20.5% and 32.9%, respectively, representing an increase of 1.6 times after treatment (Figure 2A). The results suggest that a significant portion of the amorphous material, such as hemicellulose and lignin were removed during the pressurized treatment, increasing the relative amount of crystalline part of the canola straw. Ciftci & Saldaña [9] reported that after subcritical water (180 ºC and 50 bar) treatment, the CI value of lupin hull increased 1.5 times from 38.2% to 58.6%. Such difference of CI values can be related to the type of lignocellulosic biomass used and the content of amorphous material. According to Yang et al. [26], a grater crystalline structure requires higher degradation temperatures. In Figure 2B, the thermo-gravimetric (TG) analysis clearly showed that the PAE (20%) treatment increased the thermal stability of canola straw. Hemicellulose is substantially decomposed at 220-315°C, while the thermal stability of cellulose is higher (315-400°C) due to its structure that consist of highly ordered polymer of glucose without branches [26]. The thermal stability of lignin covers an extremely wide range due to its chemical bonds, which led to the degradation of lignin occurring at a temperature range of 100–900°C [26]. The weight loss achieved for the untreated and PAE (20%) treated canola straw between 220 and 315°C were 28 and 11%, respectively, which can be attributed to the high removal of hemicellulose. The weight loss between 315-400°C was higher for PAE (20%) treated straw compared to the untreated canola straw, that could be related to the increase of cellulose content on the straw

after pressurized treatment. Also, the residual weight at 600°C was higher for untreated canola straw (28%) compared to PAE (20%) treated sample due to the high lignin content.

Using mechanical forces, micron-sized cellulose fibers can be disintegrated to nanosized cellulose. An increase of swelling capacity is expected with the deconstructed cellulose fiber bundles into nanosized due to the increase of surface area. Water retention value (WRV) can be applied to determine changes in cellulose fibers swelling capacity. The variation in the WRV was explored for PAE (20%) treated canola straw before and after ultrasonication and also for commercially available pure cellulose (not mechanically treated) (Figure 3). In the present study, cellulose presented WRV of 84.6±1.9%, which agrees with Cheng et al. [19] that reported WRV of 95% for microcrystalline cellulose. PAE (20%) treated canola straw before ultrasonication presented the lowest WRV, which can be due to the residual lignin. Between the three major materials in lignocellulosic biomass (cellulose, hemicellulose and lignin) the lignin has the lowest affinity with water [22]. WRV of PAE (20%) treated canola straw increased 1.3 times after ultrasonication, which are expected due to extensive degree of fibrillation. For instance, to have nanosized cellulose with high WRV it needs to be either free or having low residual lignin content. Ciftci et al. [16], produced nanosized cellulose from lupin hull using subcritical water (180°C, 50 bar and 5 mL/min) followed by bleaching treatment (1.7% acidified sodium chlorite at 75 °C for 4 h) and ultrasonication at output power of 700 W, 80% amplitude for 35 min. The WRV of bleached cellulose before and after ultrasonication



increased almost 2 times, from 157 to 305%, respectively. Chlorite delignification under acid conditions is the most popular and established laboratory method for removal residual of lignin from biomass. Besides delignification, bleaching treatment increases the porosity of the cellulose and swelling capacity of the fiber.

Figure 2. XRD patterns (A), and TG thermograms (B) of canola straw before and after PAE (20%) treatment at 180 °C, 50 bar, 5 mL/min for 40 min.

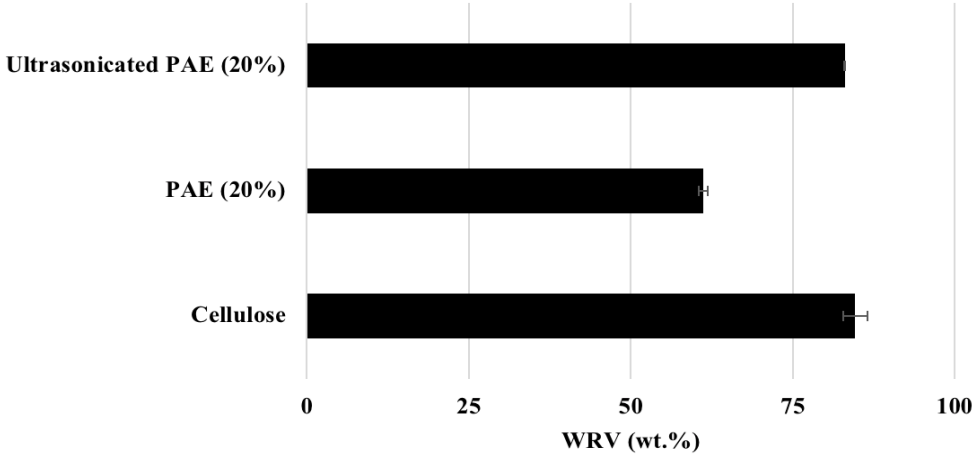
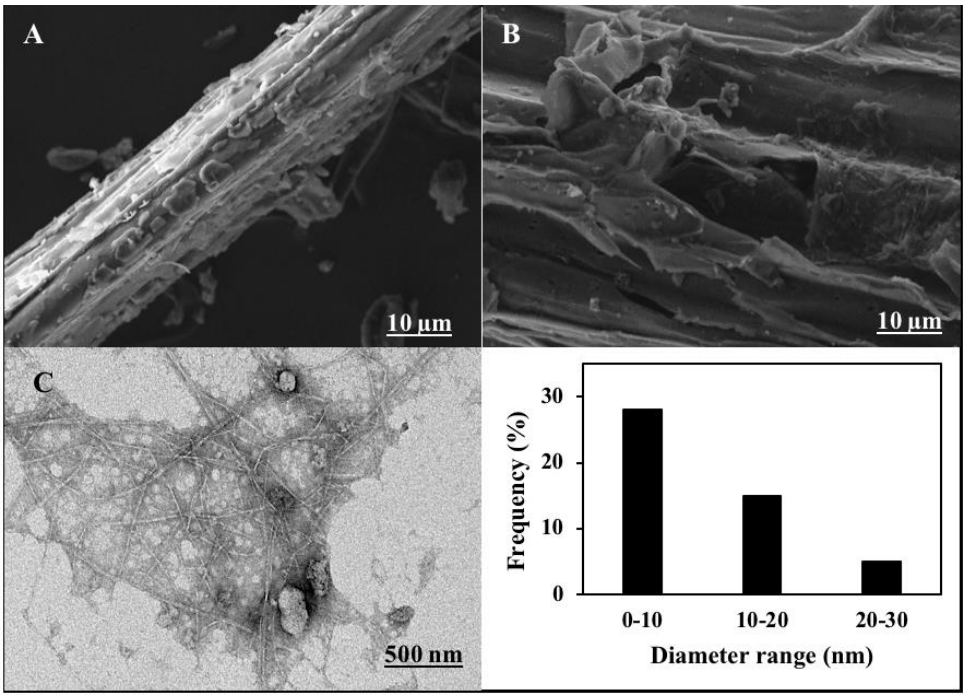


Figure 3. Water retention value (WRV) of cellulose and PAE (20%) treated canola straw before and after ultrasonication.

To further characterize how PAE (20%) treatment and mechanical disintegration affect the structure of canola straw, the scanning electron microscopy (SEM) (Figure 4A and B) and transmission electron microscopy (TEM) (Figure 4C) were performed. The untreated canola straw exhibited intact morphology with material deposition on the surface structure (Figure 4A). Microscopic structures after PAE (20%) treatment (Figure 4B) revealed that the cell walls were disintegrated, and the removal of non-cellulosic materials resulted in a rough surface with exposure of its internal structure. The TEM analysis (Figure 5C) and the diameter distribution results revealed that the mechanical process using high intensity ultrasound, disintegrate the treated canola straw into nanosized cellulose. The aqueous dispersion (1 wt.%) contained nanometer scale fibers (namely nanofibrils) is comprised of a large number of elemental fibrils,



some fibril bundles, and a small amount of aggregated bundles. The nanofibrils presented diameters between 3-30 nm, with an average diameter of approximately 11 nm, and the lengths of several micrometers. The nanosized cellulose obtained in this study had a similar result to Xie et al. [27], where the cellulose aqueous suspension (0.2 wt%) isolated from bamboo presented nanofibers with diameter in the range of 2–30 nm.

Figure 5. Microscopic analysis of untreated canola straw (A), pressurized aqueous ethanol (20%) treated canola straw (B), and ultrassonicated canola straw residue after treatment (C) with diameter size distribution.

4. CONCLUSIONS

Nanosized cellulose has been successfully obtained from canola straw using environmentally friendly alternatives. Pressurized aqueous ethanol (20%) at 180 °C and 50 bar removed 81% of hemicelluloses and almost 30% of total lignin, leading to an enriched cellulose material (~70% cellulose). Removal of non-cellulosic compounds from canola straw after treatment was confirmed by SEM, XRD and TG analysis. Then, disintegration of cellulose fiber into nanofibers with diameter of 3-30 nm was obtained using ultrasonication treatment. The TEM analysis and WRV values provided supporting evidence for the formation of nanosized cellulose. Nanosized cellulose obtained by the biorefining of canola straw using pressurized aqueous ethanol followed by ultrasonication is a promising biomaterial for reinforcement of polysaccharide based thermoplastic and biocomposites.

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