

Pressurized Fluid Processing: From by-products to high value-added products

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ABSTRACT

Canada generates annually million tonnes of by-products, such as potato cull, lupin hull, canola straw among others. These by products have valuable components, such as starch, cellulose, sugars and phenolics, which can be used as sweeteners, stabilizers, bioethanol, and in packaging applications. Using two of these by-products, our laboratory produced bioactive starch films, phenolics, and nanocellulose with subcritical water (SCW) and pressurized fluid technology. All experiments were performed using a subcritical fluid reaction system. The effects of temperature and pressure were studied for all reactions. Bioactive films produced were characterized by mechanical, physico-chemical, optical and functional properties. Total phenolics were analyzed by spectrophotometer methods, while individual hemicellulosic sugars were analyzed by HPLC. Other characterizations include morphology and particle size. For the bioactive films, 100% of elongation was obtained. Bioactive films also showed high antioxidant activity. Using pressurized fluids, temperature was the most important process parameter for straw hydrolysis due to the break of ether and ester interactions between lignin and hemicellulose. At optimized conditions, hemicellulosic sugars were removed, providing a rich cellulose residue, which was successfully converted to nanocellulose using pressurized fluid followed by ultrasonication. Therefore, SCW is a suitable technology to produce bioactive starch films, and other high value-added compounds like nanocellulose.

1. INTRODUCTION

Worldwide, there is an increasing trend towards the use of by-products to produce high value added compounds. In Canada, huge amounts of potato by-products (potato peel and potato cull) are generated due to the growing production of processed potato products. Potato peel is a good source of phenolic compounds [1], while potato cull is a rich source of starch. Potato is the largest vegetable crop in Canada, with a 4.8 million tons production in 2017, from which 40-50% are not suitable for human consumption. Potato starch (natural biopolymer, abundant, inexpensive) is an attractive promising resource for packaging applications as it can be easily chemically modified. To date, most plastics are made of petroleum sources that require hundreds of years to decompose, becoming challenging to dispose in landfills in a sustainable way. There is an urgent need to develop alternative biodegradable plastics. Recently, bioactive films have attracted considerable attention due to their antioxidant, antimicrobial, antibrowning, and gas barrier, by incorporation of bioactive compounds in the biopolymer-based films that modify the film structure and functionality for food applications.

Bioactive films based on starch have some disadvantages, such as high water solubility, poor water barrier properties [2] and mechanical properties [3], limiting its application in the food industry. Various chemical approaches have been used to overcome these disadvantages like chemical modification. Potato starch film cross-linked by 5wt% citric acid showed improved tensile strength [4]. Also, oxidized potato starch film with 1.5wt% active chlorine had less water vapor permeability, but resulted in lower tensile strength [5]. Therefore, modification of bioactive starch films is of great importance to explore their applications in the food packaging area. Traditional methods used to produce starch films are casting, extrusion blowing, injection, and thermo-compression. However, the poor miscibility of bioactives with starch and films with poor consistency remain a challenge using these techniques.

Canada is also well known by generating another by-product, canola straw. In general, straws are composed of cellulose (35–50%), hemicellulose (20-35%), and lignin (10–25%).

Recently, nanocellulose with unique properties of biocompatibility, and tensile strength is being developed for applications in the pharmaceutical, biomedical, and food packaging. Currently, cellulose is removed by mechanical, chemical, physicochemical and biological treatments. Also, 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 $>180\text{ }^{\circ}\text{C}$, where the addition of ethanol is recognized to be a suitable solvent to improve lignin removal [6-7]. Earlier, Buranov & Mazza [7] fractionated flax straw using pressurized aqueous ethanol (PAE), extracting hemicelluloses and lignin with 30% (v/v) ethanol concentration where cellulose degradation was negligible. As cellulose is insoluble in water and most organic solvents, the use of nanocellulose (1–100 nm) has increased due to its high surface area, high tensile strength, low thermal expansion, and non-toxicity [8]. Currently, to produce nanocellulose, intensive mechanical treatments, such as high-pressure homogenization [9], grinding [10], cryocrushing [11] and high intensity ultrasonication [12-13] are required. Although chemical treatment favored delignification, long processing time and extensive water consumption are needed.

Our laboratory has pioneered the use of subcritical water technology for bioactive film production, where subcritical water acts as a catalyst and reaction medium to promote interactions between phenolics and starches. We also demonstrated that gallic acid solubility in water increased at subcritical water conditions, improving the loading capacity of gallic acid in starch films. Recently, we developed bioactive films made of potato by-products and gallic acid using SCW technology to improve cross-linking and antioxidant activity. We also treated canola straw and lupin hull using a combination of pressurized fluid processing followed by ultrasonication to produce nanocellulose. The effect of ethanol concentration for removal of maximum hemicellulose and lignin were studied. Then, the disintegration of the cellulose enriched-residue was achieved by ultrasonication (1200 W). Nanocellulose was characterized for crystallinity, stability, water retention value and size.

2. MATERIALS AND METHODS

Red potato was purchased from the local supermarket (Edmonton, AB, Canada). Canola straw was kindly provided by Dr. Barry Irving (University of Alberta). Glycerol ($>95\%$ purity, certified ACS grade) was purchased from Fisher Scientific (Ottawa, ON, Canada). Sodium acetate trihydrate (99%), glacial acetic acid (99.7%), hydrochloric acid (ACS reagent, 37%), ferric chloride hexahydrate (ACS reagent, 97%), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (HPLC, $\geq 98\%$), ethanol ($>95\%$), 2,2'-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Folin–Ciocalteu's phenol reagent, calcium chloride (96%, anhydrous), sodium carbonate (anhydrous powder), gallic acid (97.5-102.5% titration), potassium persulfate (ACS reagent, $\geq 99\%$), 2,4,6-tris(2-pyridyl)-s-triazine ($\geq 98\%$), sulfuric acid (72%, ACS reagent) and sugar standards ($\geq 96\%$ purity) were acquired from Sigma Aldrich (Oakville, ON, Canada). Purified water from a Milli-Q system (Millipore, Bellerica, MA) was used.

2.1. Production of bioactive films

Bioactive films were produced using a subcritical fluid system [14]. First, potato peel was added as the only resource of phenolics at different ratios of potato cull (0-1.3 g peel/g cull) until the maximum loading of peel was reached. The reaction was conducted at 120 bar and 125°C . Then, the influence of the glycerol/cull starch ratio (0-2 g glycerol/g starch) on the mechanical properties was investigated at highest potato peel loading (1.3 g peel/g cull) to find the optimized condition. To further improve antioxidant/antimicrobial activity of the film, gallic acid was selected to replace the peel as previous studies demonstrated the stability

of gallic acid under subcritical water conditions. Gallic acid/cull starch ratios of 0-0.3 g gallic acid/g starch were evaluated.

2.2. Film characterization

Mechanical properties

Films were cut into rectangle strips of 5 cm x 1cm for tensile strength (TS) and percent elongation at break (%E) using a texture analyzer (5960 Dual Column Tabletop Testing system with Instron® Bluehill® Software, Instron, Norwood, MA, USA) according to the ASTM standard method D882. Equilibrated film specimens were mounted between the grips with an initial separation of 30 mm, and the cross-head speed was set at 4 mm/min. At least six samples for each type of film were evaluated and averages were reported.

Physico-chemical properties

Water activity measurements were performed with a water activity meter (Aqualab 4TE, Pullman, WA, USA). Bioactive films were cut into 2cm x 2cm squares and evaluated in triplicate.

Water vapor permeability (WVP) of the film was determined according to the ASTM E96-00 method, with details of the methodology reported by Zhao et al. [15]. Each film sample was evaluated at least in triplicate.

Total phenolic content in the film extract was determined spectrophotometrically according to a modified method of Sarkar et al. [16]. All measurements were performed at least in duplicate.

Optical properties

The contact angle of film samples was measured using a Dynamic Angle and Tension Analysis instrument (FTA200, First Ten Angstroms, Inc. Portsmouth, VA, USA) following the methodology described by Zhang [17].

Functional properties

For determination of antioxidant activity, film (0.2g) was cut into small pieces of approximately 0.5 x 0.5 cm, and extracted using a mixture of 5 mL of water and 5 mL of ethanol under constant shaking for 24 h. Then, the film+solvent mixture was centrifuged at 5000 \times g for 10 min and the supernatant was used for the antioxidant activity measurement. The Ferric Reducing Antioxidant Power (FRAP) method was previously described by Benzie and Strain [18] and recently reported by Zhao et al. [15].

2.3. Pressurized aqueous ethanol treatment followed by ultrasonication

Pressurized aqueous ethanol (PAE) treatment of canola straw was performed in a semi-continuous flow type subcritical fluid system previously described [6]. 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. Then, canola straw cellulose-rich residue was ground to <106 μ m. 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.

2.4. Physico-chemical characterization

Raw and pressurized treated samples were analyzed for lignin, hemicellulose and cellulose contents following the NREL standard analytical procedures [19]. Total cellulose in the hydrolysates was 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.

Crystallinity index (CI), and Water retention value (WRV)

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.

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. [20] where the WRV is defined as the percent ratio of water retained in the sample after centrifugation (900g for 30 min) to the dry weight (dried at 105°C) of the sample.

Microscopic analysis

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. Approximately, 50 measurements were done using 5 TEM images.

3. RESULTS & DISCUSSIONS

Fig 1 shows data on the mechanical properties of bioactive films based on potato by-product with different potato peel/cull ratios and gallic acid/cull starch ratios. The tensile strength of films increased significantly from 2.5 to 9.0 MPa with increasing potato peel content up to 1.3 times the potato cull, whereas the elongation at break decreased from 28.5 to 10.2% (Fig. 1a). An important increase of 3.6 times in tensile strength was observed with the highest potato peel loading possibly due to the presence of fiber. Elongation showed an opposite trend compared to the tensile strength. Cross-linking of starch molecules with gallic acid limits the mobility of the starch molecules, leading to lower elongation. The addition of gallic acid influenced film mechanical properties (Fig. 1b). The increase of gallic acid/cull starch ratio from 0 to 0.3 g/g resulted in the decrease of TS from 3.0 to 1.6 MPa. However, film elongation increased to the highest value at 28.2% when 0.2 g/g of gallic acid was added, followed by a slowly decrease with the extra gallic acid addition. A possible explanation is that gallic acid has a small size molecule that could be between starch chains, just like glycerol, increasing even chain mobility and enhancing the initial plastic effect. The hydrophilic part of the gallic acid could interact with glycerol or water facilitating its presence between starch chains.

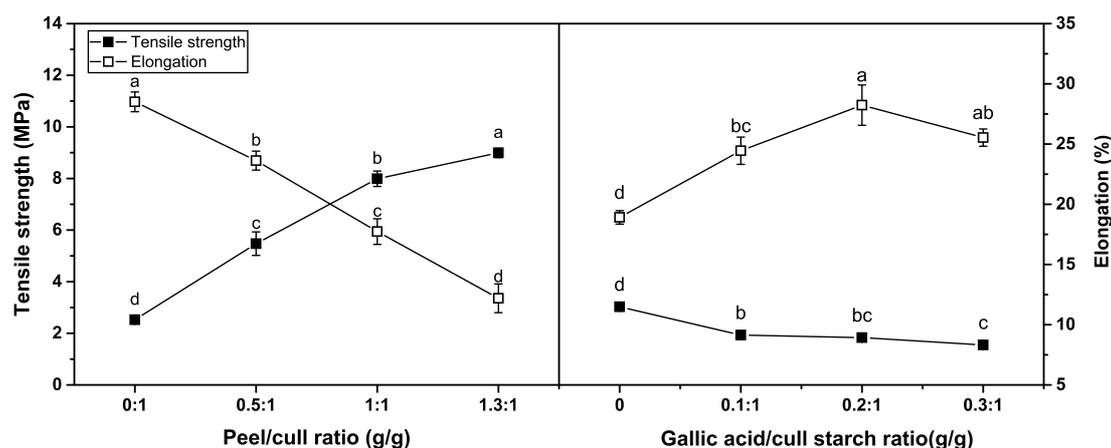


Fig. 1. Tensile strength and elongation at break for bioactive films based on potato by-products of (a) different potato peel/cull ratios (0-1.3 g/g), and (b) different GA/potato cull starch ratios (0-0.3 g/g)

Table 1 shows the water activity and WVP of bioactive films based on potato by-products. Water activity decreases with the increase of potato peel content. Values of water activity for different starch based films have been reported as 0.473 for native cassava starch films and 0.492 for sodium trimetaphosphate modified cassava starch films [21]. All bioactive films

based on potato by-products produced in this study had water activity >0.09 , preventing water to act as a solvent to favor chemical/biochemical reactions and growth of microorganisms. The initial WVP reduction in the gallic acid added film is the result of cross-linking and formation of ester linkages between starch and gallic acid. When excess gallic acid (0.3 g GA/g cull starch) was added, more active sites for water binding were introduced, therefore the affinity for water molecules increased in these films, resulting in higher water diffusion, providing films with higher WVP. The bioactive films produced in this study showed better water vapor barrier properties than the films prepared by Kang & Min [22] using potato peel and soy lecithin (3.0-5.3 g mm/kPa h m²) and the thymol loaded potato starch films (1.22 g mm/kPa h m²) produced by Davoodi et al. [2]. Also, the increase addition of potato peel had some influence on the surface contact angle of films. An increase of gallic acid content caused a significant decreased in contact angle values for both top and bottom surfaces of films due to the hydrophilicity of gallic acid.

Table 1. Water activity and WVP of bioactive films based on potato by-products.

Potato peel:cull ratio (g/g)	Water activity	WVP ($\times 10^{-4}$ g.mm/m ² .h.Pa)	Contact angle (°)	
			Top	Bottom
0:1	0.0891 \pm 0.0012 ^a	13.92 \pm 1.02 ^a	83.46 \pm 2.58 ^a	26.71 \pm 0.82 ^a
0.5:1	0.0822 \pm 0.0012 ^b	10.34 \pm 0.44 ^{ab}	77.35 \pm 1.58 ^{ab}	26.34 \pm 3.00 ^a
1:1	0.0727 \pm 0.0002 ^c	9.32 \pm 0.78 ^b	71.42 \pm 2.16 ^b	21.12 \pm 2.01 ^{ab}
1.3:1	0.0671 \pm 0.0027 ^d	5.41 \pm 0.22 ^c	76.94 \pm 3.03 ^{ab}	16.75 \pm 1.53 ^b
GA:potato cull starch ratio(g/g)	Water activity	WVP ($\times 10^{-4}$ g.mm/m ² .h.Pa)	Contact angle (°)	
			Top	Bottom
0:1	0.0893 \pm 0.0027 ^a	13.92 \pm 1.02 ^b	73.40 \pm 3.00 ^a	40.50 \pm 1.43 ^a
0.1:1	0.0868 \pm 0.0017 ^a	6.49 \pm 0.20 ^d	70.50 \pm 1.56 ^a	31.19 \pm 1.49 ^b
0.2:1	0.0870 \pm 0.0018 ^a	10.81 \pm 0.41 ^c	63.54 \pm 2.00 ^b	28.90 \pm 1.25 ^{bc}
0.3:1	0.0851 \pm 0.0036 ^{ab}	19.83 \pm 0.61 ^a	58.79 \pm 3.97 ^b	23.68 \pm 2.39 ^c

WVP: water vapour permeability. Data shown as mean \pm standard deviation ($n = 3$).^{a-d}Different lowercase letters in the same column indicate significant differences ($p < 0.05$).

Fig. 2 shows the total phenolic content and antioxidant activity of bioactive films based on potato by-products. Increasing the amount of potato peel during film formation could provide the film with high total phenolic content along with the increasing of antioxidant activity for FRAP and ABTS. The more gallic acid used for film formation, the higher antioxidant activity of the film. Due to the loading limitation, bioactive films with potato peel added provided with lower antioxidant activity than that of gallic acid added films.

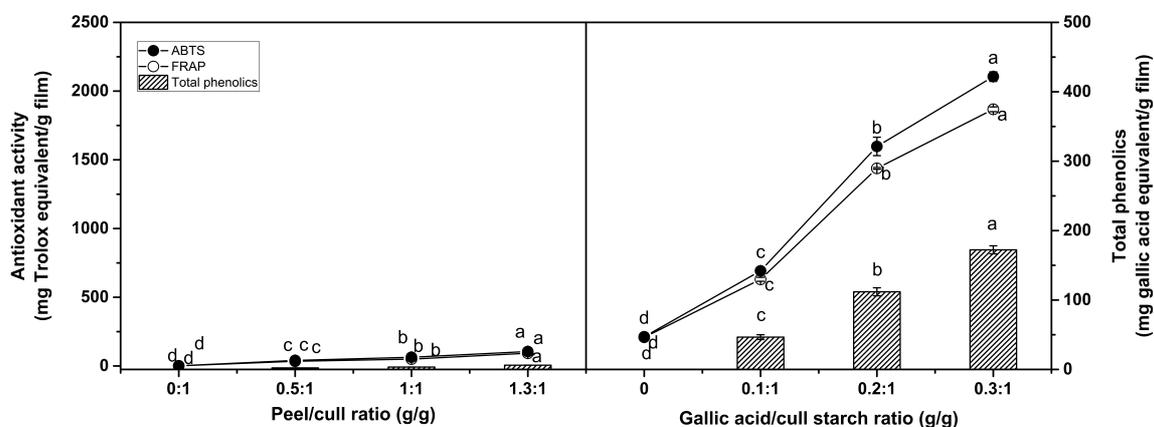


Fig. 2. Total phenolic content, and antioxidant activity by ABTS and FRAP methods of bioactive films based on potato by-products with different: (a) potato peel/cull ratios (0-1.3 g/g), and (b) GA/potato cull starch ratios (0-0.3 g/g).

For the other by-product, Fig. 3a shows the cellulose of untreated and PAE treated canola straw. Untreated canola straw consisted of $32.6 \pm 0.15\%$ cellulose. The pressurized water extracted 62% of hemicelluloses, which increased the cellulose (48%) contents in pre-treated canola straw through a concentration effect. Similarly, the pressurized water treatment at 180 °C, 50 bar and 30 min extracted 85% of hemicelluloses from lupin hull, increasing cellulose content up to 79% [23]. With the addition of 20% ethanol allowed the extraction of hemicelluloses from canola straw, yielding a solid residue mainly composed of 68% cellulose. The CI values of untreated and pressurized aqueous ethanol (20%) treated canola straw were 20.5% and 32.9%, respectively (Fig. 3b). 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 [23] reported that after subcritical water (180 °C 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.

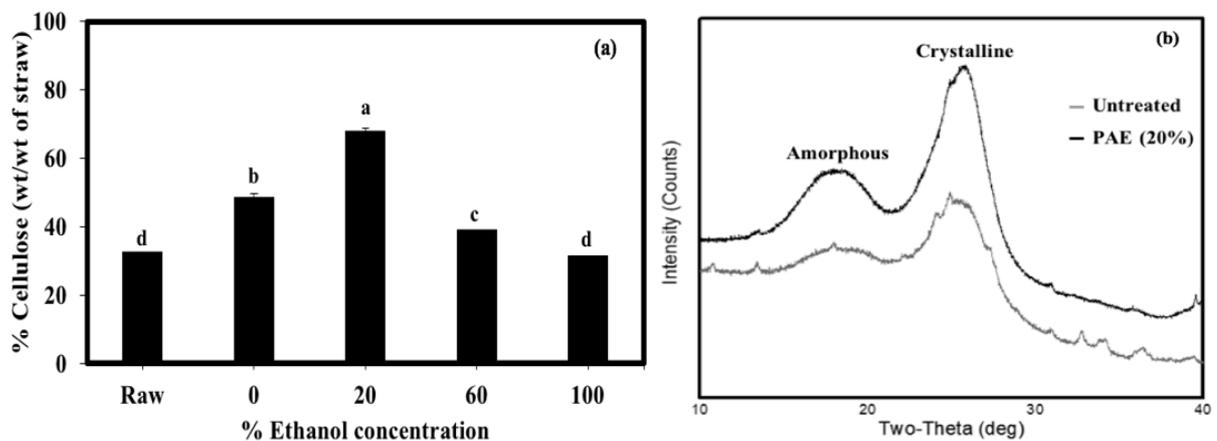


Fig. 3. Cellulose concentration (a) and XRD patterns (b) of canola straw before and after pressurized fluid treatments at 180 °C, 50 bar, 5 mL/min for 40 min.

Using mechanical forces, micro-sized cellulose fibers can be disintegrated to nano cellulose. An increase of swelling capacity is expected with the deconstructed cellulose fiber bundles into nanosized due to the increase of surface area. The variation in the WRV was explored for PAE (20%) treated canola straw before and after ultrasonication (Fig. 4). PAE (20%) treated canola straw before ultrasonication had the lowest WRV, possibly due to the residual lignin. WRV of PAE (20%) treated canola straw increased 1.3 times after ultrasonication, which are expected due to extensive degree of fibrillation.

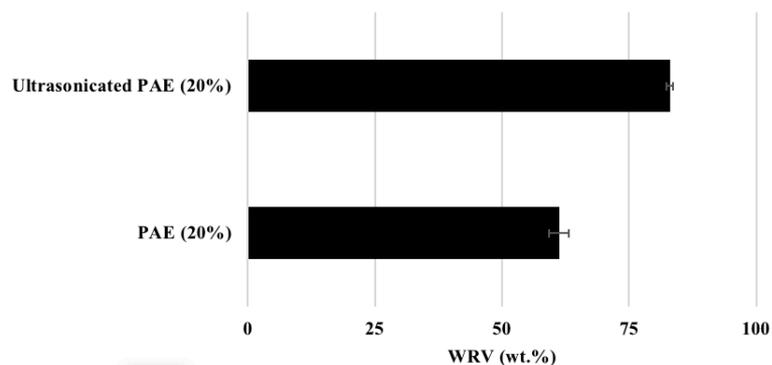


Fig. 4. Water retention value (WRV) of cellulose and PAE (20%) treated canola straw before and after ultrasonication.

The transmission electron microscopy (TEM) analysis and diameter distribution (Fig. 5) revealed that the mechanical process using high intensity ultrasound disintegrated the treated canola straw into nanocellulose. The nanofibrils presented diameters between 3-30 nm, with an average diameter of approximately 11 nm, and the lengths of several micrometers.

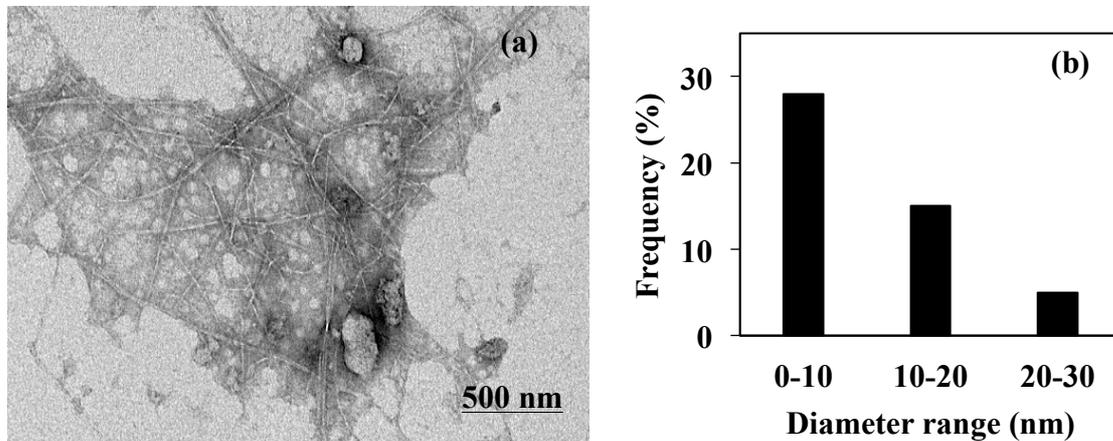


Fig. 5. Transmittance Electron Microscopic analysis of ultrasonicated canola straw residue after treatment (a) with diameter size distribution (b).

4. Conclusions

By-products of potato (peel and cull) were successfully utilized to produce bioactive films with unique antioxidant properties using subcritical fluid technology. A new approach on utilization of by-products and new data on the use of a green technology is reported. Gallic acid incorporated in the films formed cross-linking through ester bonds, creating a smooth surface and homogenous film, acting as a plasticizer. More importantly, all bioactive films had low water activities, preventing potential microbial growth. With the increasing amount of gallic acid incorporated in the film, higher antioxidant activity was achieved. Bioactive films made of potato by-products can offer an array of applications in the food industry. Also, the use of the by-product canola straw with PAE combined with ultrasonication resulted in the high value added nanosize cellulose. Pressurized aqueous ethanol (20%) removed 81% hemicelluloses, leading to an enriched cellulose material with 3-30 nm after ultrasonication. Nanocellulose is a promising biomaterial for reinforcement of starch-based packaging.

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