Characterization of Residues from Chilean Blueberry Bushes: A Potential Source of Cellulose

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Chile is the second largest global producer of blueberries, which are harvested in the south-central region. As a result of the exponential production growth, a large amount of lignocellulosic biomass is generated from pruning and left on the ground as waste. As an alternative to the current incineration practices and their negative air pollution effects, this study proposed value-added utilization of these agroindustry residues. The chemical compositions (cellulose, hemicellulose, lignin, extractives, and ash) of the pruning residues from blueberry branches and trunks were analyzed. The cellulose contents from the branches and trunks were similar at 52% and 51%, respectively. However, the X-ray diffraction analysis indicated important differences in their crystallinity index, with 52% and 84%, respectively. Compared with the cellulose obtained from the trunks, cellulose from the branches was less thermally stable, possibly due to the presence of residual lignin and hemicellulose. According to the results, it is expected that the agroindustrial residues from pruning of the Chilean blueberry bushes (branches and trunks) might be of use as a potential platform for bioproducts, such as cellulose materials in order to replace synthetic or unsustainable materials.

Keywords: Blueberry bushes; Pruning residues; Cellulose; Chemical compounds; Crystallinity

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INTRODUCTION

Blueberries (Vaccinium corymbosum) are round fruits that are typically dark in color and grown from medium-sized shrubs with deciduous leaves (Gayol 2012). Blueberries are commercially important as a fruit crop and are widely cultivated in America and Asia (Li et al. 2017). Regular consumption of blueberries is associated with health benefits (Johnson et al. 2015; USHBC 2015; Song et al. 2016). Given its climatological conditions, Chile has become a main producer and exporter of blueberries, second to the United States (García et al. 2013; Mayorga Ramos 2014). The location of Chile in the southern hemisphere makes it possible to ship high quality, fresh blueberries to countries in the northern hemisphere during winter (Gómez Martínez 2010; Retamales et al. 2014). The management of the pruning of bushes influences the quality of the blueberries. For example, the average fruit size can be increased if shrub pruning is applied systematically to allow for a balance between the annual growth of shoots and fruit production (San Martín 2009; Gómez Martínez 2010; Cline 2011; UCONN 2016).
Accordingly, between 3000 kg and 7500 kg (dry weight) of pruning waste are generated in a planted hectare (Pinochet et al. 2014). In the last decade, the south-central region of Chile has witnessed an exponential increase, specifically in the regions of Maule (35° S, 71° W) and Bio-Bío (36° S, 73° W). Currently, approximately 10,000 ha are planted in these regions, which corresponds to 56% of the total Chilean production (ODEPA 2016). A large amount of residue and underutilized biomass from pruning is available in these areas. Given the threats of reproduction and growth of pests and pathogens from residues that are left on fields after compaction, the only currently viable solution is to burn this lignocellulosic biomass (Abou Hussein and Sawan 2010). While this is the easiest and least expensive practice related to agricultural activities, the impact on the air quality cannot be ignored. It contributes to 40% of carbon dioxide, 32% of carbon monoxide, 20% of particulate matter, and 50% of polycyclic aromatic hydrocarbons emissions released into the environment globally (CEC 2014). Additionally, the incineration of agricultural residue causes severe land and water pollution (Kumar et al. 2015).

In this context, the chemistry of blueberry pruning is a key enabler to evaluate the potential of this material and its later integration to a circular bioeconomy as a platform for new biomaterials. Without chemical expertise and knowhow, it would be impossible to grow and utilize different types of biomass smartly and sustainably (Pohjakallio 2017). The bioeconomy can contribute in various ways to the circular economy, including the use of different residues from agriculture, forestry, fisheries, and food industries. The forestry industry is actively investigating new technologies to separate wood-based biomass into its constituent components, which can then be processed into products such as paper, cardboard, packaging, composite and textiles, reducing the volumes of substances and materials that have adverse impacts on the environment, including fossil oil and plastic. An ecological alternative to the incineration of blueberry agro-industrial waste was proposed in this study that takes advantage of the high cellulose content and potential for use in biopolymers (Malucelli et al. 2017).

Cellulose is a renewable material because of its natural origin and biological, chemical, and mechanical properties, and it is considered a source of raw material for the increasing demand for environmentally friendly and biocompatible products (Brinchi et al. 2013). It can be used as a raw material for the synthesis of biomaterials and biofuels because of its abundance, low weight, high strength, and biodegradability (Deng et al. 2015). Several reports discuss the extraction and use of cellulose from various sources, including agroindustry waste (Bismarck et al. 2002; Sun et al. 2004, 2005; Liu et al. 2006; Ibrahim et al. 2010; Moniruzzaman and Ono 2013). For example, Alemdar and Sain (2008) and Ferrer et al. (2016) isolated cellulose from wheat straw and soy hulls with the aim of producing nanofibers and to examine their potential as a reinforcing agent in biocomposites. Johar et al. (2012) evaluated the extraction of cellulose fibers from rice husk via alkali and bleaching treatments. The obtained cellulose was utilized to produce cellulose nanocrystals. Sung et al. (2017) used a by-product from coffee roasting to obtain cellulose fibers. Their methods included isolation by an alkaline treatment, which was followed by bleaching with sodium hypochlorite. Subsequently, the cellulose was used to obtain cellulose nanocrystals.

The increasing demand for sustainable materials in industrial applications has led to a focus on developing technologies that enable the use of biopolymers to improve the properties of given materials (Neto et al. 2013; Ghandi 2014; Ng et al. 2015). In the context of the Chilean blueberry agroindustry, these residues offer an interesting
opportunity for the generation of sustainable materials. Therefore, the aim of this study was to characterize the chemical and structural compositions of pruning residues and evaluate their potential as a new source of cellulose.

EXPERIMENTAL

Materials

Blueberry pruning residues (BPRs) were used as the raw material and were collected from a Cabrero plantation in Bío-Bío, Chile. The reagent grade chemicals that were used included sulfuric acid, sodium hydroxide, acetone, and sodium chlorite, which were acquired from Sigma-Aldrich (St. Louis, MO, USA).

Methods

Fiber morphology

The morphology structure of each BPR sample was assessed with scanning electron microscopy (SEM; JEOL JSM6610LV, JEOL, Tokyo, Japan). The alpha-cellulose samples were stirred into deionized water, and 50 μL of the dispersion was placed on a sample holder and dried in an oven at 40 °C for 30 min. The samples were coated with Au by the direct current-sputtering technique using a Denton vacuum desk V-sputter/etch unit (JEOL, Peabody, MA, USA) at 20 mA for 30 s. Afterwards, the samples were observed with an acceleration voltage of 10 kV (Aguayo et al. 2017).

Chemical composition

The BPRs were classified as branches and trunks, and their chemical compositions were characterized. The methodology used allowed the quantification of each of the cell wall components before cellulose isolation. The residues were cut into small pieces (3 cm to 5 cm in length), washed in water, and then dried at 30 °C in an oven. Both types of samples, branches and trunks, were milled in a knife mill (GmbH, FRITSCH, Idar-Oberstein, Germany) and sieved with a 45-mesh to 60-mesh screen. To determine the contents of the extractives, the milled samples were Soxhlet-extracted with acetone for 16 h, according to TAPPI T280 wd-06 (2015). The lignin content was determined using TAPPI T222 om-11 (2015). For this, 300 mg of extractives-free sample were subjected to hydrolysis with 3 mL of 72% (w/w) H₂SO₄ at 30 °C for 1 h. Then, the acid was diluted to 4% and the solution was autoclaved for 1 h at 121 °C. The residual material was cooled and filtered. The solids were dried to a constant weight at 105 °C and classified as insoluble lignin. The soluble lignin was determined by measuring the solution absorbance at 205 nm (Dence 1992). The total lignin content was calculated as the sum of the insoluble and soluble lignin contents.

The holocellulose and alpha-cellulose contents were determined according to TAPPI T9 wd-75 (2015). The holocellulose was prepared from 250 mg of extractives-free sample, which were added to 5 mL of deionized water, 2 mL of CH₃COOH, and 5 mL of 80% NaClO₂. The samples were immersed in a water bath at 90 °C for 1 h. After this period, an additional 4 mL of CH₃COOH and 10 mL of 80% NaClO₂ were added to the flask, and the reaction was continued for 1 h at 90 °C. The reaction was stopped by immersing the flask in a water bath at 10 °C. The solids were filtered and washed with deionized water, and then dried at 105 °C to a constant weight. The remaining weight was quantified as holocellulose. Afterwards, 100 mg of holocellulose were treated with 8

mL of 17.5% (w/v) NaOH for 30 min at room temperature with stirring every 10 min. Afterwards, 8 mL of distilled water were added to the solution, and the reaction was continued for an additional 30 min. The solids were filtered, washed with distilled water, and impregnated with 20 mL of 1.0 M CH$_3$COOH for 5 min. The residue was washed with excess water, dried at 105 °C to a constant weight, and quantified as alpha-cellulose. The ash content was determined with TAPPI T211 om-12 (2015), where approximately 1 g of sample in a porcelain crucible was burned in a muffle furnace at 525 °C for 6 h. The obtained ash was weighed, and the percentage content was calculated based on the initial weight of the dry sample. The experiments that determined the lignin, holocellulose, alpha-cellulose, and ash contents were all performed in triplicate.

Fourier transform infrared spectroscopy

The Fourier transform infrared (FTIR) spectra were obtained using a Nicolet 380 FT-IR spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) in the transmittance mode. Afterwards, 5 mg of alpha-cellulose sample from the branches and trunks were dispersed in a KBr matrix, which was followed by compression to form pellets. The samples were analyzed in the spectral region between 4000 cm$^{-1}$ and 400 cm$^{-1}$ with a 2 cm$^{-1}$ resolution and an average of more than 32 scans.

Degree of crystallinity

The X-ray diffraction (XRD) analysis was performed to determine the crystallinity of the samples from the branch and trunk BPRs. The alpha-cellulose of the branches and trunks in the form of milled powder was placed on the sample holder and leveled to obtain a total and uniform X-ray exposure. The samples were examined using wide angle X-ray scattering equipment in an X-ray diffractometer (Rigaku Smartlab, Tokyo, Japan) with the transmission XRD method. The beam size was controlled with one 10-mm horizontal slit, and the detector used was a single-photon counting HyPix-3000 (Rigaku Corp., Tokyo, Japan). Approximately 0.05 g was used for each experiment. Angular scanning was conducted from 5° to 50° at a rate of 5°/min with Cu Kα radiation ($\lambda = 0.154$ nm), and the generator was set to 45 kV and 200 mA. Background correction was performed because of the sampler holder film and air, and was done by subtracting the corresponding signal obtained without a sample. Microcrystalline analysis of the cellulosic material was performed and took into account the crystallographic data reported in the literature (Ford et al. 2010; Nam et al. 2016). The literature data were used as an initial step to optimize the deconvolution of the XRD patterns.

The crystallite size ($\tau$, nm) perpendicular to the lattice plane (2 0 0) for cellulose I and (0 2 0) for cellulose II was calculated with the Scherrer equation, which is given as Eq. 1 (Scherrer 1918),

$$\tau = \frac{K\lambda}{\beta \cos \theta}$$

(1)

where $K$ is the Scherrer constant (0.96), $\lambda$ is the wavelength of the X-ray radiation (nm), $\beta$ is the full width at half maximum of the diffraction peak (rad), and $\theta$ is the diffraction angle of the peak (°). The Segal crystallinity index (CI, %) was calculated according to Eq. 2 (Segal et al. 1959),

$$CI = \frac{t_{c} - t_{o}}{t_{c}} \times 100$$

(2)
where $I_1$ is the total intensity of the (2 0 0) peak for cellulose I and (0 2 0) peak for cellulose II, and $I_a$ is the amorphous intensity.

**Thermogravimetric analysis**

Thermogravimetric analysis (TGA) was conducted by means of a TGA Q50 (TA Instruments, New Castle, DE, USA) under nitrogen atmosphere with a gas flow rate of 50 mL/min from 25 °C to 600 °C at a heating rate of 10 °C/min. The weight loss rate was obtained from the derivative thermogravimetric data. Approximately 4.0 mg of the alpha-cellulose sample from the branches and trunks were used.

**Data analysis**

The normality of the distribution of chemical data, analysis of variance, and determination of significant differences were based on Fisher’s least significant difference (LSD) test and were calculated using the software (STATGRAPHICS centurion XV.II, Statpoint Technologies Inc., Virginia, USA). The deconvolution of the XRD patterns was done using PeakFit software (www.systat.com), according to Carrillo et al. (2018). Gaussian functions were fitted, and later graphing and data analysis software (Origin, OriginLab, Northampton, MA, USA) was used for plotting and calculating the procedures.

**RESULTS AND DISCUSSION**

**BPR Morphology**

Optical images of branch and trunk BPRs are shown in Figs. 1a and 1b, respectively.

![Image](Fig. 1. Images of BPRs: photograph of the branch waste (a), photograph of the trunk waste (b), SEM micrograph of the cellulose obtained from the branch BPR (c), and SEM micrograph of the cellulose obtained from the trunk BPR (d))
Scanning electron microscopy was used to identify the fiber structure after the acid treatment on cellulose isolation (Figs. 1c and 1d). A slight destruction of the fibers occurred mainly in the branches after acid treatment (Fig. 1c). This destruction might be due to the difference between the initial content of hemicellulose and lignin in the branch and in the trunk (analyzed in Table 1), since the elimination of the cell wall components directly affect the organization that connected the cellulosic fibrils.

Chemical Compositions of the BPRs

The alpha-cellulose, hemicellulose, lignin, extractives, and ash contents of the BPRs are given in Table 1. Compared with the trunk residue, the branch residue contained a larger amount of ash. The results also demonstrated significant differences between the insoluble lignin contents, which were of 26.6% and 22.1% in the branch and trunk residues, respectively. The lignin content in the trunk BPR was similar to those reported by Alemdar and Sain (2008) and Johar et al. (2012), who stated that the lignin contents for wheat straw and rice hulls were 22% and 23%, respectively. Sung et al. (2017) reported 29% lignin in coffee silverskin, which was similar to the lignin content in the branches in this study. Significant differences were observed between the branches and trunks for the holocellulose and hemicellulose contents. The hemicellulose content was higher in the trunk BPR (21.4%) compared with that in the branch BPR (11.9%).

Table 1. Chemical Characterization of the Analyzed BPRs

<table>
<thead>
<tr>
<th>Component</th>
<th>BPR</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Branches</td>
<td>Trunks</td>
</tr>
<tr>
<td>Extractives (%)</td>
<td>6.9 ± 0.3a</td>
<td>3.1 ± 0.4b</td>
</tr>
<tr>
<td>Holocellulose (%)</td>
<td>63.8 ± 0.5b</td>
<td>72.8 ± 1.1a</td>
</tr>
<tr>
<td>Alpha-cellulose (%)</td>
<td>51.9 ± 0.4a</td>
<td>51.4 ± 0.7a</td>
</tr>
<tr>
<td>Hemicellulose* (%)</td>
<td>11.9 ± 0.4b</td>
<td>21.4 ± 0.7a</td>
</tr>
<tr>
<td>Insoluble lignin (%)</td>
<td>26.6 ± 0.1a</td>
<td>22.1 ± 0.1b</td>
</tr>
<tr>
<td>Soluble lignin (%)</td>
<td>2.8 ± 0.1b</td>
<td>3.5 ± 0.2a</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>1.8 ± 0.1a</td>
<td>0.9 ± 0.1b</td>
</tr>
</tbody>
</table>

Values with different letters within a row indicate significant differences at p < 0.05 (LSD test); *Hemicellulose content was calculated as the alpha-cellulose content subtracted from the holocellulose content (Cruz et al. 2018)

The alpha-cellulose contents in both sample types were approximately 51%. These values were significantly higher compared with other agroindustry waste, such as 42% for garlic skin (Reddy and Rhim 2014), 24% for coffee silverskin (Sung et al. 2017), and 35% for rice husk (Johar et al. 2012). Similar cellulose content results to those obtained in the present study have been reported for cotton stalk (49%) (Abou Hussein and Sawan 2010).

These results indicated promising uses for BPR because of the high cellulose and low lignin contents, specifically in the trunk BPR (Table 1). Therefore, it is considered
that the development of new biologically based products is possible, as well as the potential isolation of nanocelluloses from these residues. The development of cellulose-fiber composites is light and have good performance and appearance, as well as better recyclability in comparison with glass fiber-reinforced polymer composites. The applications of BPR as biopolymers and biologically based materials can reduce air pollution that is generated when lignocellulosic biomass is burned.

**FTIR Analysis of the BPRs**

Figure 2 includes the FTIR spectra for the holocellulose and alpha-cellulose samples obtained from the BPRs. The results showed a typical cellulose spectrum in branches and trunks after the elimination of hemicellulose and lignin. However, the spectrum of the alpha-cellulose from the branch sample showed a small peak at approximately 1730 cm$^{-1}$ (C=O bond), usually associated with the stretching vibration of the carboxyl and acetyl groups in hemicelluloses (xyloglucan), (Ng et al. 2015; Du et al. 2016; Sofla et al. 2016). Additionally, the alpha-cellulose from the branch sample showed a small peak near 2850 cm$^{-1}$, the dominant peaks at 2900 to 2800 cm$^{-1}$ are C-H stretching vibrations present in cellulose molecules (C-H from $\text{-CH}_2$), commonly assigned to the lignin component (Ng et al. 2015), which suggested the presence of residual cell wall components or a higher recalcitrance of the cell wall components in the branch samples.

![FTIR spectra of the BPRs](image)

**Fig. 2.** FTIR spectra of the BPRs: (a) alpha-cellulose from the branches (CB); (b) alpha-cellulose from the trunks (CT); (c) holocellulose from the branches (HB); and (d) holocellulose from the trunks (HT)

In general, the alpha-cellulose samples from the branches and trunks exhibited normal peaks for cellulose type I. However, the peak at approximately 3330 cm$^{-1}$ had a broad band from 3500 cm$^{-1}$ to 3200 cm$^{-1}$ assigned to O-H stretching vibrations for strong inter- and intra-molecular H-bonding (Naduparambath et al. 2018). A weak peak appeared near 1640 cm$^{-1}$ that was related to O-H bending vibrations of absorbed water with some carboxylate groups (Du et al. 2016). The peaks at 1371 cm$^{-1}$ and 1319 cm$^{-1}$
were related to the bending vibrations of the C-H and C-O bonds in polysaccharide aromatic rings, respectively (Sofla et al. 2016), and the peak at approximately 890 cm\(^{-1}\) was from rocking vibrations of CH\(_2\) groups in the cellulose (Sharma et al. 2012).

Guaiacyl-type lignin absorbs near 1270 cm\(^{-1}\) and 1230 cm\(^{-1}\), and syringyl-type lignin absorbs at approximately 1230 cm\(^{-1}\) (Pandey 1999; Pandey and Pitman 2003). Figure 2 shows the removal of the 1230 cm\(^{-1}\) peak in the alpha-cellulose from the branches and trunks, which is indicated by dotted lines in Fig. 2. The peak at 2850 cm\(^{-1}\), which was associated with lignin (CH bond vibration) (Pandey 1999; Zhao et al. 2017), was present in the branch samples (holocellulose and alpha-cellulose) and indicated that the lignin was not completely removed from these samples, unlike the trunk samples, where a more crystalline material was obtained. The peaks at 1730 cm\(^{-1}\) were attributed to C=O stretching of the ketones and/or esters in the hemicellulose (Du et al. 2016), or the ester linkage of carboxylic groups of ferulic and p-coumaric acids in the lignin or hemicellulose (Chen et al. 2011; Sofla et al. 2016; El Achaby et al. 2018). Additionally, the alpha-cellulose trunk samples showed a weak peak at approximately 1730 cm\(^{-1}\), which revealed it was a more crystalline material.

**Crystallinity Analysis of the Alpha-cellulose**

The XRD tests were performed to investigate the crystalline structure of the alpha-cellulose in the fibers (Fig. 3).

![Fig. 3. Diffraction patterns of the (a) alpha-cellulose from the branches (black) and (b) trunks (red)](image)

The alpha-cellulose from the trunks had peaks at 2\(\theta\) values of approximately 14.7° (110) and 16.4° (101), which indicated the presence of cellulose I. Therefore, the alpha-cellulose from the trunks showed a higher crystallinity than the alpha-cellulose from the branches, which was possibly because of a greater removal of non-cellulosic components (hemicellulose and lignin). The alpha-cellulose from the branches presented a peak at a 2\(\theta\) of 11.9° (101), which indicated the presence of cellulose II (Oun and Rhim 2016).

The Segal CI and \(\tau\) values were then calculated using Eqs. 1 and 2, respectively (Table 2).
Table 2. Crystallinity Results for the Alpha-cellulose Samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>CI (%)</th>
<th>τ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-cellulose from the trunk BPR</td>
<td>84</td>
<td>3.5</td>
</tr>
<tr>
<td>Alpha-cellulose from the branch BPR</td>
<td>52</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Mechanical and thermal properties are improved with an increasing cellulose crystallinity (Tan et al. 2015). Additionally, a greater crystallinity of the cellulose fibers increases their stiffness and rigidity, and therefore their strength (Johar et al. 2012; Kallel et al. 2016). The cellulose from the trunk BPR had a greater crystallinity than the cellulose from the branch BPR. The lower crystallinity of the cellulose from the branches could be due to the presence of small peaks attributed to residual lignin and hemicellulose, as shown in Fig. 2. It is important to mention that the total amount of lignin in the final product should be negligible, since this is only composed of alpha-cellulose; therefore the peaks assigned to lignin might come from small residual antioxidants or chromophores groups as well (Dragović-Uzelac et al. 2010). Other waste fibers obtained from the agroindustry, such as bleached rice husk and soy hulls, reached CI values of 57% (Johar et al. 2012) and 73% (Neto et al. 2013), respectively. On the other hand, value-added byproducts from bio-waste such as cellulose microfibers from garlic skin and cellulose nanocrystals from garlic straw had CI values of 45% (Reddy and Rhim 2014) and 69% (Kallel et al. 2016), respectively.

**Thermal Analyses of the Alpha-cellulose from the BPRs**

The thermal stability of the alpha-cellulose from the branches and trunks was investigated (Fig. 4). The samples that were studied exhibited similar thermal behavior to what was reported by Yang et al. (2007) and Chan et al. (2013). According to the thermogram, the mass loss between 25 °C and 120 °C corresponded to the loss of absorbed moisture on the surfaces of these materials (Wang et al. 2007), which included chemisorbed water and/or intermolecular H-bonded water. The initial degradation temperature was approximately 230 °C and 250 °C for the branch and trunk BPRs, respectively (Fig. 4a).

![Fig. 4. Thermal analysis of the BPRs: weight loss vs. temperature (a); and derivative weight change vs. temperature (b)](image-url)
The maximum degradation temperature was 341 °C and 354 °C for the branch and trunk BPRs, respectively (Fig. 4b). The weight loss of the residues at 600 °C was 81.5% for the branches and 82.4% for the trunks. The decomposition steps at 290 °C to 390 °C were attributed to the decomposition of cellulose (Du et al. 2016). Compared with the trunk BPR, the branch samples presented a lower temperature for the maximum degradation of cellulose, which indicated that this sample had a decreased thermal stability. This might be explained due to the presence of small amounts of residual lignin and hemicellulose as it was hypothesized before. It has also been determined that when the crystallinity of a raw material is greater, then the thermal stability is greater (Du et al. 2016).

CONCLUSIONS

1. Both the branch and trunk blueberry pruning residues (BPRs) were found to have high cellulose contents. Specifically, the alpha-cellulose in the trunk samples displayed a higher crystallinity index (CI) and better thermal properties.

2. The production of cellulose from BPR is an alternative process that can alleviate air pollution that is generated by burning lignocellulosic biomass.

3. The BPRs show promising potential for the production of cellulose, opening the possibility to its use as a platform for the innovation and development of bioproducts in the context of a circular economy.

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