

# Toward a circular bioeconomy: extracting cellulose from grape stalks <sup>†</sup>

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**Abstract:** The purpose of this study was to assess the extraction of cellulose from stalks of vines using auto- auto-hydrolysis and characterize it. As results obtained, the colorimeter readings, displayed a final yellow color of the fiber, demonstrating that the bleaching process was insufficient and that multiple bleaching processes might be required. According to the FTIR (Fourier-transform infrared spectroscopy) data, the stretching and deformation vibrations of the C-H group in the glucose unit. Although lignin and hemicellulose were partial removed according to the FTIR. Distinct cellulose yields were achieved for each fraction, including fractions 500, 300, 250, 150  $\mu\text{m}$  and retain, with values of 21.98, 12.70, 7.20, 5.74, and 3.11 %, respectively. In sum, we were able to extract cellulose from the stalk vine, although the last step still needs to be optimized for better whitening.

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## 1. Introduction

In recent times, there has been an increase in the demand for cellulose fibers, where the main source is wood pulp, however, due to the depletion of natural resources and global warming, several kinds of research are being developed to replace this scarce resource, as alternative sources such as vegetable residues from agricultural activities and agro-industries are being sought to avoid deforestation [1,2]. Among these residues, highlighted in this study is the grape stem, which is obtained after destemming. The grape stem can represent up to 5 – 7 % of the raw material used in processing, are the skeletons of the grape bunch and are composed mainly of lignocelluloses [3,4]. According to recent studies, grape stems are intrinsically dangerous; however, they have a high content of organic matter and the fact that production is concentrated at one time of the year presents potential pollution problems [4,5], that is, the discharge of grape stems into the soil leads to the inhibition of the germinative properties of the soil, due to the biological demand of oxygen, carbon and phenolic compounds [4,6]. Thus, to obtain this polymer of great importance from the grape stem, there are numerous methods of extraction among them acid hydrolysis and autohydrolysis are the most used approaches to the extraction of cellulose. The most commonly used acids are acetic acid, sulfuric acid, and phosphoric acid, for acid hydrolysis [7]. Factors to be considered as advantages of acid hydrolysis are the low release of lignin fragments and the efficient hydrolysis of amorphous polysaccharides. However, the high cost of acid recovery and the corrosion of equipment are considered the disadvantages of acid hydrolysis [8]. Autohydrolysis is acidic hydrolysis without the use of any external acid. Because hydrogen ions come from the autoionization of water in situ and acetic acid generated from the acetyl substituent of hemicellulose. The latter

contributes much more to hydrolysis [8–10]. The acid-base method is the most used method for cellulose extraction, its advantages include a simple extraction process, high extraction efficiency, good thermal stability, good crystallinity, easy control of reaction conditions and low cost. The beneficial thing about this method of pulp extraction is to achieve large-scale industrial production and has ample development space [11]. Therefore, in the present study, the objective was to evaluate the extraction, by means of acid hydrolysis, alkaline hydrolysis and bleaching of cellulose from residues of the wine industry, focusing on the valorization of the grape stem ('Vinhão') and to characterize the effects of granulometric fractions and Retain (500, 300, 250, 150, 150)  $\mu\text{m}$  in relation to color, FTIR and light microscopy. The final application of the cellulose will be the creation of membranes/coatings through a sustainable and environmentally friendly process for the protection of heritage buildings from environmental conditions.

## 2. Materials and Methods

### 2.1. Material

Grape stalks from 'Vinhao' variety were kindly provided by Quinta de Mascate (Vila Verde, Portugal). The vine stalks were collected from (meter local) and frozen until used. The samples were dried at 60 °C for 48 hours in an oven with air circulation. The dried biomass was then micronized with a bimby (TM5) and sieved for grain-size classification within the range from 500  $\mu\text{m}$  to 150  $\mu\text{m}$  (500, 300, 250, 150)  $\mu\text{m}$  and retain Fig 1. The milled by-products were packed in sealed plastic bags, protected from light until use. To better understand the extraction and bleaching of the by-products all fractions were used in this study.

### 2.2. Cellulose extraction

Cellulose was extracted based on the work of Bassani et al., [12], using the process of auto-hydrolysis, with some modifications. The dried micronized by-product was weighted 20 g and proceeded to an acid hydrolysis, carried out in a Schott with a ratio of 1 : 20 (m/V) of 5 % acid sulfuric, in autoclave (121 °C, 45 min at 1.2 atm). The samples were cooled at room temperature and washed with distilled water until waster become clear. The samples were dried at 50 °C for 24 h.

The samples were submitted to 4 mol/L NaOH at a ratio 1 : 20 (m/V) and put into orbital shaker at room temperature with 180 rpm for 24 h. The samples were cooled at room temperature and washed with distilled water until waster become clear. The samples were dried at 50 °C for 24 h.

Lastly, the bleaching process was carried out by adding 1 : 20 (m/V) of 5 %  $\text{H}_2\text{O}_2$  (the pH was adjusted to 11.5 with NaOH), in an orbital shaker for 8 h at 45 °C. The sample were taken out of the orbital shaker and cooled for 24 h at room temperature. The samples were cooled at room temperature and washed with distilled water until waster become clear. The samples were dried at 50 °C for 24 h. The isolated cellulose was stored in flask and shelter from the light. The steps for cellulose extraction are represented in Fig. 1.

### 2.3. Characterization of Cellulose

#### 2.3.1. Optical Microscopy

A high-resolution digital microscope was used to collect visual data (Dino-lite Edge AM7915MZT). The extracted cellulose was topographically characterized using a 5-megapixel camera (2592x1944).

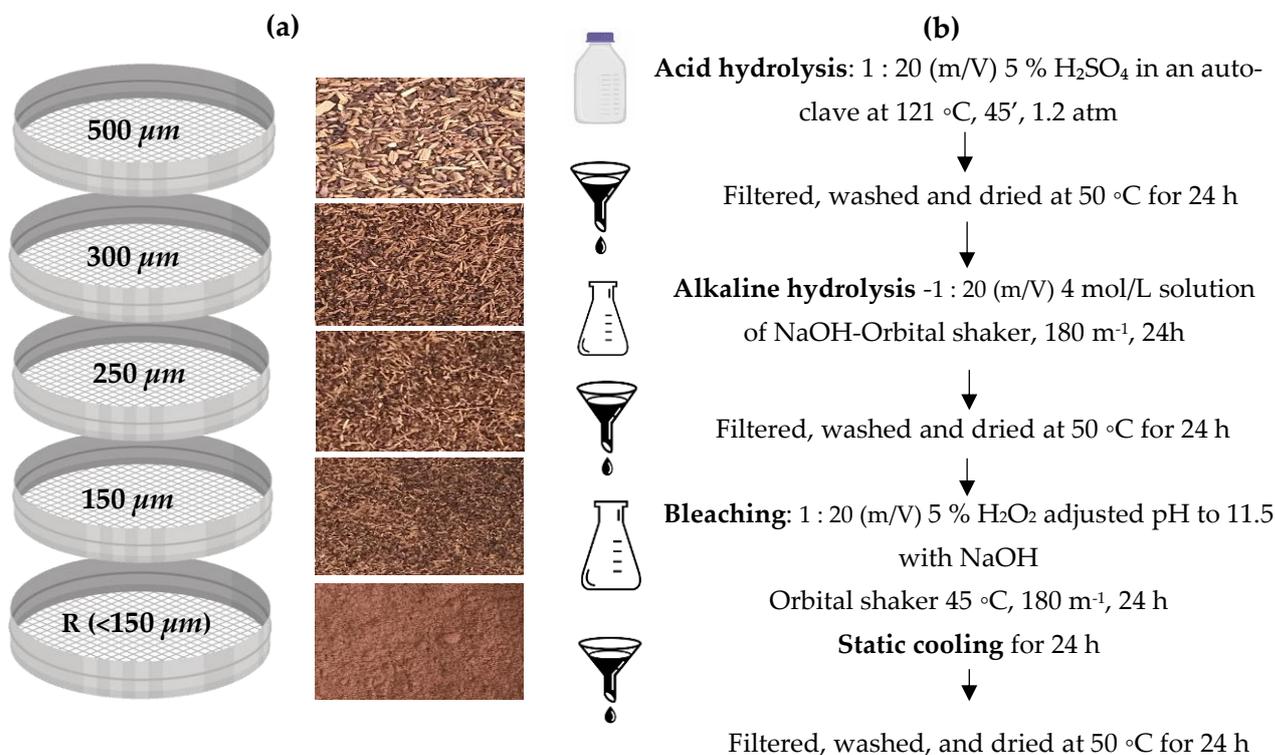
#### 2.3.2. Color analysis

The color of the samples was determined in a digital colorimeter model Chroma Meter CR-700 (Konica Minolta, Osaka, Japan), using the CIE Lab scale to determine  $L^*$ ,  $a^*$  and  $b^*$  color parameters [13]. The sample was poured in a Petri dish with 5 cm diameter covering the entire bottom of the dish, and the reading was performed in 10 different points.

The total color difference ( $\Delta E^*$ ) was calculated in relation to fresh vine stalks, according to Eq. (1).

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad 1$$

In this study,  $L^*0$ ,  $a^*0$  and  $b^*0$  stand for the values of the color parameters of fresh stalk vine and  $L^*$ ,  $a^*$  and  $b^*$  for the values of the color parameters of the sample in each step.



**Figure 1.** Schematic representation of the treatment steps of Stalk grape “Vinhão”, Steps 1-Granulometric fractions of vine stalks (a) and Steps 2- treatment (b).

### 2.3.3. Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance (FTIR-ATR)

The samples were analyzed on a PerkinElmer Paragon 1000 FTIR (Waltham, MA, USA) with an ATR accessory (Diamond/ZnSe). Spectra were obtained in the wavenumber range of 4000-550  $\text{cm}^{-1}$ . with a resolution of 4  $\text{cm}^{-1}$ . accumulating 16 scans. Based on the literature, the FTIR-ATR vibrational bands were identified [14].

## 3. Results

During the extraction of cellulose from stalk vines, cellulose extracted from granulometric fractions with different particle sizes (500, 300, 250, and 150  $\mu\text{m}$  and retain (<150)) was characterized (Figure 1), to determine whether particle size influences cellulose extraction or the type of cellulose extracted. It was found that the observation with the Dino-Lite microscope was extremely important, both in the evaluation of the state of conservation and in the measurement of sizes. Fig. 2 shows the photographs of the grape stalk fiber treated with an autohydrolysis (acid, alkali and bleached) at 500  $\mu\text{m}$  (a), 300  $\mu\text{m}$  (b), 250  $\mu\text{m}$  (c), 150  $\mu\text{m}$  (d) and retain(e). All grape stalk fibers in the different diameters presented opaque tint, after undergoing all treatments, presenting yellowish-brown coloration (Fig. 2). The white color desired during the process was not observed in the final product, in order to be able to reach an indication of high purity of the cellulosic material. Thus, in this method used, another hydrogen peroxide step is required for color change and

removal some traces of lignin and hemicellulose. Figure 2 depicts the topographic characterization of cellulose using a high-resolution digital microscope.



Figure 2. Photograph taken with Dino-Lite microscope of cellulose extracted from granulometric fractions with particle sizes of (a) 500 μm (b) 300 μm (c) 250 μm (d) 150 μm and (e) Retain.

Table 1 displays the length of the vine stalks as well as the yield of cellulose extracted from granulometric fractions with different particle sizes.

As shown in Figure 3, the bleaching procedure of cellulose obtained from different fractions was also assessed using a colorimetric analysis using the CIELab scale as well as the determination of the total color difference (ΔE\*) in reference to fresh vine stalks to further characterize the cellulose and analyze the influence of the particle size. Also, shown in figure 3(d) a represented color from the CIELab values, from the stalk vine and the bleached cellulose. Noticeably, based on figure 3(d) the bleaching process was not sufficient, and more cycles of hydrogen peroxide is needed.

Table 1. Yield of cellulose extracted from vine stalks with different particle size and length.

	Diameter (μm)	Length (mm)	Yield (%)
15	500	2.59 ± 0.13	21.98
16	300	0.82 ± 0.01	12.70
17	250	0.56 ± 0.04	7.20
	150	0.38 ± 0.01	5.74
18	Retain	0.22 ± 0.01	3.11

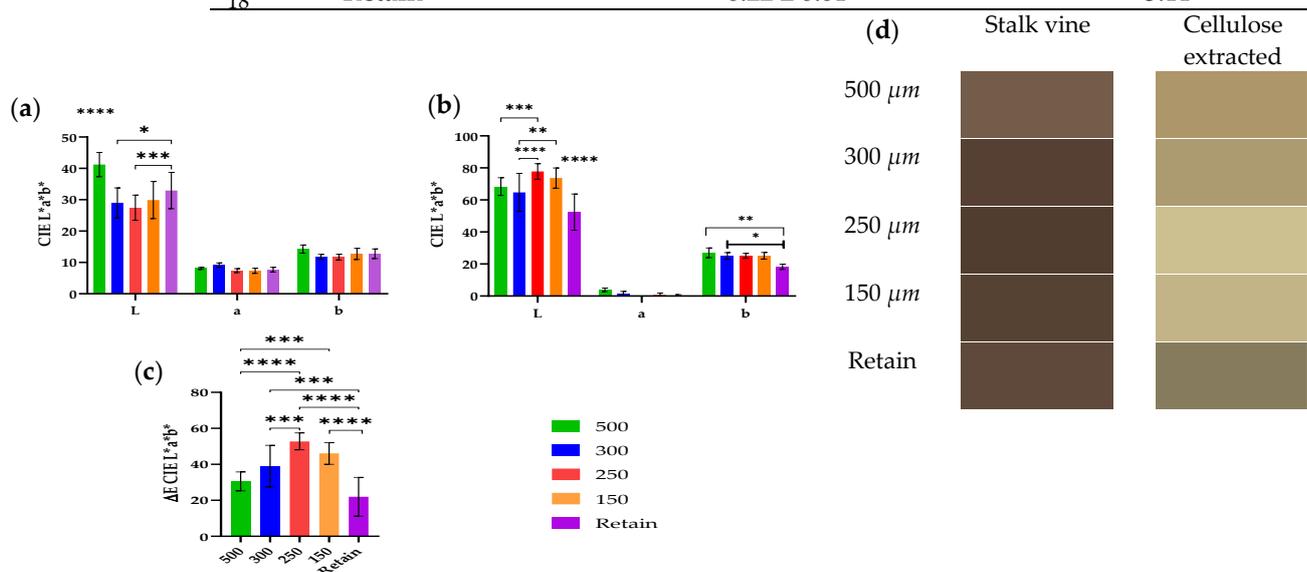


Figure 3. Analyses of color from colorimetry from different particle sizes of 500 μm, 300 μm, 250 μm, 150 μm and retain. The colorimetry was assed with CIELab and was evaluated the color from the raw material (stalk vines) (a) and from the cellulose extracted after bleaching (b). The ΔE was

calculated based on the difference of color between raw material and the cellulose extracted (c). the values obtain from  $L^*a^*b^*$  was converted into a represented color and showed in figure d.

### 3.1. Structural characterization

**Figure 4.** Structural characterization by Fourier Transform Infrared Spectrometry.

Cellulose fractions, from the vines stalks, obtained from the production of wine treatment including bleaching processes were characterized in terms of functional groups using FT-IR (Fig. 4). The spectra showed characteristic bands of the functional groups that make up cellulose 2900, 1500 and 1200  $\text{cm}^{-1}$ . According to the literature, it is confirmed that bleaching indicated the partial removal of lignin, leaving some traces, due to the yellowish-brown color, requiring a later stage of bleaching to obtain a final solid fraction of cellulose. Comparing the data obtained with the literature with cellulose from sugarcane bagasse where they obtained an increase in the cellulose content in the final solid fraction, using hydrogen peroxide at 35 % [15].

## 4. Discussion

In this work was demonstrated the extraction of cellulose from stalk vines using a modified auto-hydrolysis method. The use of vine stalks with different particle size allowed us to better understand the influence that the size as in the obtention of cellulose.

The observation with the Dino-Lite microscope and binocular magnifying glass illustrated in Figure 2 was found to be particularly important in determining the level of conservation as well as measuring sizes. The morphological structure of the micro cellulose extracted appear to be long fibers in bigger particles such as 500, 300 and 250  $\mu\text{m}$ . However, particles smaller than 250  $\mu\text{m}$  appear to have two distinctive shapes, rod-like and long fibers.

Essentially, particles with larger particle sizes yielded more cellulose extracted. As demonstrated in table 1, 500  $\mu\text{m}$  particles produced cellulose yields of about 22 %, while particles of less than 150  $\mu\text{m}$  produced cellulose yields of only 3.1 %.

The fractionation process of cellulose, hemicellulose and lignin from stalk vine based on the colorimetry and FTIR appeared to be insufficient. The fibers after bleaching shown a yellow color, the author Vallejo *et al.*, 2021 that also studied the extraction of cellulose from the some agro-industrial residue obtained a final cellulose with a yellow under tone. Even though the extraction method was different, it was generally challenging to separate out well-delignified cellulose fractions from this residue.

It is also crucial to note that the way lignin molecules are arranged within the cell walls of raw materials, such as their associations with other cell wall determines the effective of the extraction process. Accordingly, the bleaching conditions must be optimized and tailored to the characteristics of the agro-industrial residue if color and cellulose purity are significant characteristics [2].

Based on the results obtained on FTIR (figure 4) and the color (figure 3) the yellow obtained was in fact residual lignin. Also, interment particles (250 and 150  $\mu\text{m}$ ) had the highest  $\Delta E$  value indicating that the bleaching process was more effective.

It is expected that future research will yield an optimized method for extracting cellulose with a whiter color. Additionally, the optimized cellulose will be used to create membranes/coatings for the protection of heritage buildings. The cellulose coatings will be designed to shield buildings from external conditions such as heat.

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