

Title Slide (1 slide) :

- **Title:** Recovery and evaluation of cellulose from agroindustrial residues of corn, grape, pomegranate, strawberry-tree fruit, and fava
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Introduction (2-3 slides):

- **SOP:** Wood pulp remains the most popular source of cellulose due to its abundance and cost-effective extraction which enable large-scale production. **However, the processes involved entail several environmental impacts, namely forest devastation and consequent contribution to global warming, which have driven researchers to seek environmentally friendly and biocompatible materials as sustainable alternatives to wood-derived cellulose (Pennells et al. 2020).**
- **Objectives:**
 - To offer an attractive alternative to wood pulp through the use of non-wood cellulosic biomass sources, such as agricultural and industrial wastes for the extraction of cellulose and production of cellulosic fibers.
 - To offer inexpensive, readily available and renewable, and promising cellulose and cellulosic fibers through the use of non-wood sources, as these have lower lignin and higher hemicellulose contents when compared to forestry materials, leading to lower energy and chemical consumption in delignification processes and fiber purification.
 - To offer significant opportunities to convert biomass resources into valuable raw materials, considering the low economic value of the existing applications and the high value of potential cellulose products there is.
 - To further boost research on sustainable biomass procurement, and further promote circular economy practices to achieve environmentally sustainable economic growth, as the pulp industry follows the current trend of pursuing sustainable practices.
- **Hypothesis:**
 - If non-wood sources are used for cellulose extraction, the cellulose fibers will be more inexpensive, available, and renewable.
 - If non-wood sources are used for cellulose extraction, the cellulose fibers will provide significant improvements for biocomposites, such as nontoxicity, biocompatibility, biodegradability, low cost and high mechanical modulus.
 - If non-wood sources are used for cellulose extraction, the cellulose fibers will be more environmentally friendly.

- **Significance:** In the upcoming years, the demand for cellulose is expected to exceed the available supply, considering the continual research that has led to the development of novel cellulose-based products, particularly in the food and biomedical fields (Hindi 2017; Abdul Khalil et al. 2020).
- **Scope:** The focus of the present study was to evaluate the cellulose isolation suitability from the renewable agroindustrial residues available in Portugal through following a multi-step solvent extraction procedure. The isolated cellulose samples were characterized and compared to wood-derived microcrystalline cellulose, considering their further application in the biomedical field as a reinforcing material of composite scaffolds.

Methodology (1 slide):

Materials

This study will require the following materials: the raw materials that will be used for the extraction of cellulose and lignin (pomegranate peels) to turn into fibers. The pomegranates will be bought from local markets residing around Angeles City, Pampanga.

Preparation of Fibers

1. Raw Material Preparation

The gathered ripened pomegranates will be cleansed with tap water. The fruits will be cut into pieces, and the arils will be extracted from the peels through the use of a grape stem removing machine. The pomegranate peels will then be stored at a temperature of 20°C until further processing.

2. Multi-Step Cellulose and Lignin Isolation Process

The fractionation process to be used for cellulose isolation is based on various research studies. As lignin acts as the “glue” that connects cellulose and hemi-cellulose, it may be extracted during the extraction process of the cellulose, as well. Therefore, both of these polymers may be obtained simultaneously in one process. The four consecutive cellulose and lignin extraction steps are schematically represented in Figure 1:

2.1. Dewaxing

In the first fractionation step, the pomegranate peels will be dewaxed using a 2:1 (v/v) toluene-ethanol solvent mixture and a solid-to-solvent ratio of 1:20 (w/v) in a Soxhlet apparatus for six hours. This will then be followed by the rinsing of the dewaxed peels with ethanol for under 30 minutes under vacuum filtration, then dried in an oven at a temperature of 105 °C until the material reaches the persistent weight.

2.2. Bleaching

The following procedure is the bleaching process of the dried dewaxed peels for delignification purposes using a 1:1 (v/v) mixture of acetate buffer and sodium chlorite aqueous solution (17 g/L) for 4 hours, at 100 °C, using a solid-to-solvent ratio of 1:40 (w/v). This is the step in which the lignin content from the pomegranate peels will be extracted. After the removal of lignin, the delignified celluloses will be rinsed with a solution of sodium hydrogen sulfate (20 g/L), deionized water and ethanol under vacuum filtration. Successively, the rinsed and delignified celluloses will be dried at the temperature of 105 °C.

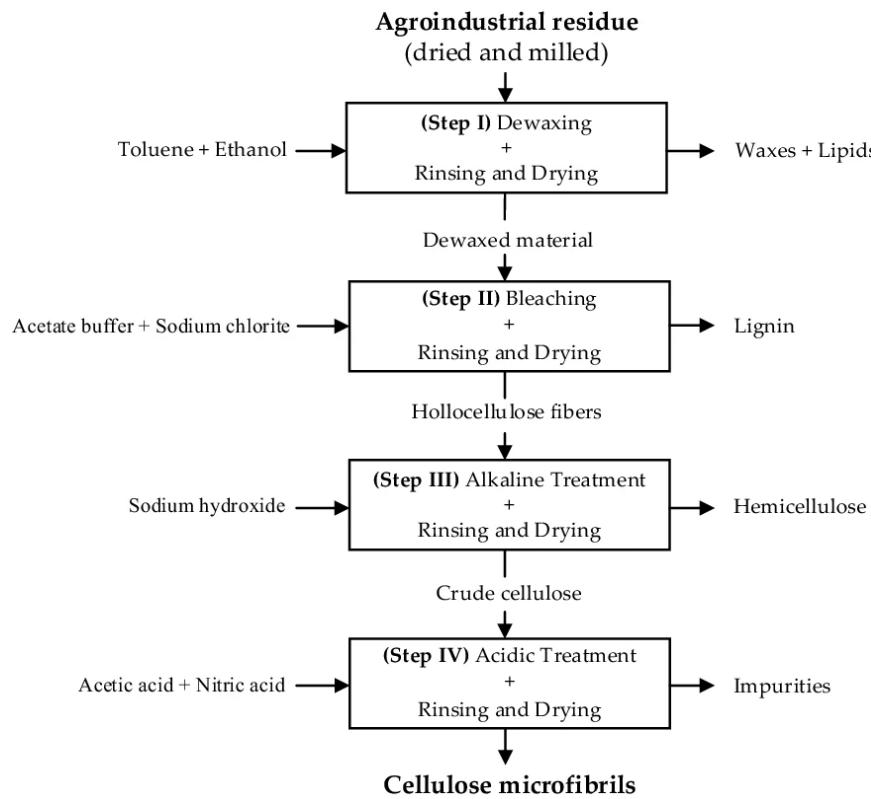
2.3. Alkaline Treatment

As the second step is executed, the researchers will be able to extract holocelluloses, or the total polysaccharide fraction of lignocellulosic biomass which is composed of cellulose and all of the hemicelluloses. The extracted crude holocelluloses will then undergo alkaline treatment with a sodium hydroxide solution (175 g/L) in an orbital shaker for 45 minutes at room temperature and 60 rpm using a solid-to-solvent ratio of 1:50 (w/v). The crude cellulose fibers will then be rinsed with 10% acetic acid, followed by deionized water.

2.4. Acidic Treatment

After being dried at 105 °C, the crude cellulose fibers will be put through an acidic treatment to remove impurities, which is the last step. A 10:1 (v/v) mixture of acetic acid (80%, v/v) and nitric acid (70%, w/v) will be used at 120 °C in an oil bath for 15 min with a solid-to-solvent ratio of 1:40 (w/v). Again, the fibers will be rinsed with ethanol twice, before and after the use of distilled water to remove the remaining traces of acid. Next, the purified cellulose fibers will be dried at 105 °C, together with the extracted lignin from step 2, to make the fiber more rigid and sturdy. This multi-step extraction process will be repeated at least three times for each agroindustrial residue considered in this study, and as the pomegranate peel extracts have antibacterial properties, the resulting product of this procedure will be antibacterial cellulosic fibers.

- **Flow chart:**



Results and Discussion (Tables, Charts, Diagrams) (2-3 slides):

The moisture content of the agroindustrial residues at the end of the drying and milling processes that preceded the multi-step extraction procedure is reported in Table 1. The relative high moisture content of pomegranate peel (13.3%) may be related to its high content of soluble carbohydrates (sugars) (Gullón et al. 2020), that are usually associated with higher hygroscopicity and consequent difficulties during drying (Roos 1995).

Table 1 (Raw materials' moisture content)

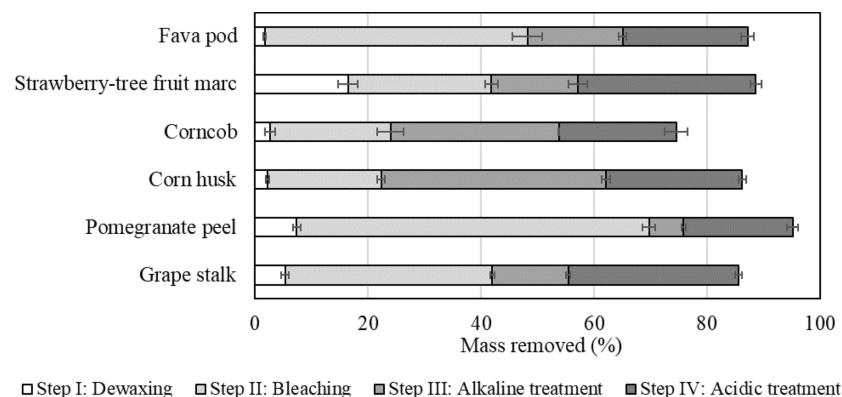
Raw material	Moisture (%)
Fava pod	11.3 ± 0.97
Strawberry-tree fruit marc	10.1 ± 0.03
Corn cob	6.64 ± 0.10
Corn husk	7.48 ± 0.11
Pomegranate peel	13.3 ± 0.33
Grape stalk	8.04 ± 0.06

3. 1. Multi-step cellulose isolation process

To evaluate and compare the fractionation process for each agroindustrial residue, the percentage of mass removed at each extraction step was quantified and is presented in Fig. 2.

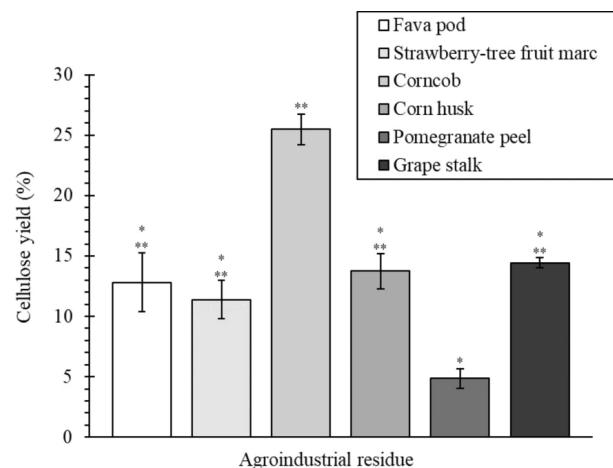
Based on previous studies (Sun et al. 2004; Morán et al. 2008; Maheswari et al. 2012; Madureira et al. 2018), it is expected that the fractions obtained at the end of steps I, II and III of the extraction procedure are rich in waxes/lipids, lignin and hemicellulose, respectively, leaving the purified cellulose isolated from each agroindustrial residue, after a final acidic treatment (Fig. 1).

Fig 2



The cellulose yield, obtained at the end of the multi-step extraction process for each agroindustrial residue, is shown in Fig. 3. The influence of the rinsing and drying phases that were performed in between the extraction steps (Fig. 1) on cellulose yield was considered, and it was found that the mass losses during those operations varied between 0.2 and 0.4 g (dry basis) which may have possibly decreased cellulose yields from 2 to 4%.

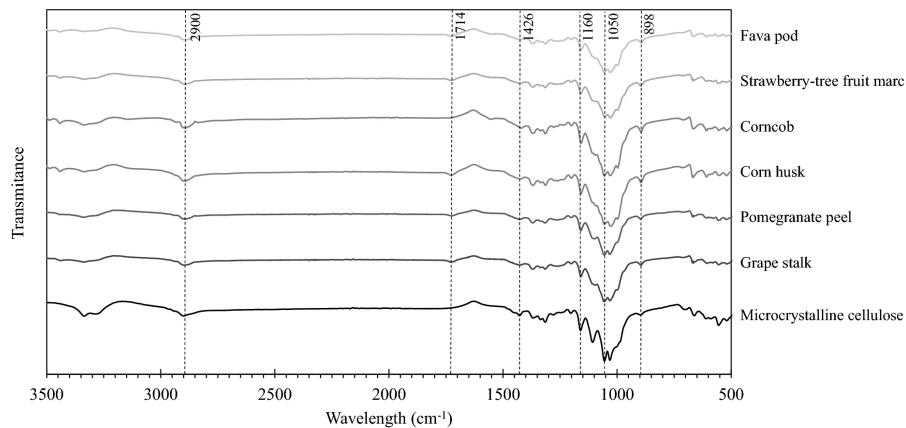
Fig 3



3.2. ATR-FTIR spectroscopy analysis

Figure 4 shows the FTIR spectra of the samples obtained in this study and of the wood-derived commercial MC. All spectra presented roughly the same peaks, indicating the presence of the same functional groups and confirming that the multi-step extraction process was successful in isolating purified cellulose from the six agroindustrial residues.

Fig 4



3.3. TGA and DSC analyses of cellulose

Figure 5 shows the TGA and DSC curves for all cellulose samples, including MC, and Table 2 reports the data obtained from these curves. The TGA curves of the MC and of the six cellulose samples obtained in this study were similar. A small weight loss was found in the range of 30–150 °C, due to the evaporation of low molecular weight compounds and water, and ranged from 4.1 to 5.3%, being of the same order of magnitude of the one observed for the commercial MC ($5.61 \pm 0.11\%$) (Table 2). No noticeable thermal events were observed after water evaporation and up to 306.4–316.7 °C, depending on the agroindustrial residue, confirming the effectiveness of the extraction procedure in removing hemicellulose and lignin from the raw materials. However, the variation of the onset temperatures between 306.4 and 316.7 °C (Table 2) might be associated with residual amounts of hemicellulose and lignin, confirming the hypothesis presented in the FTIR analysis.

Fig 5

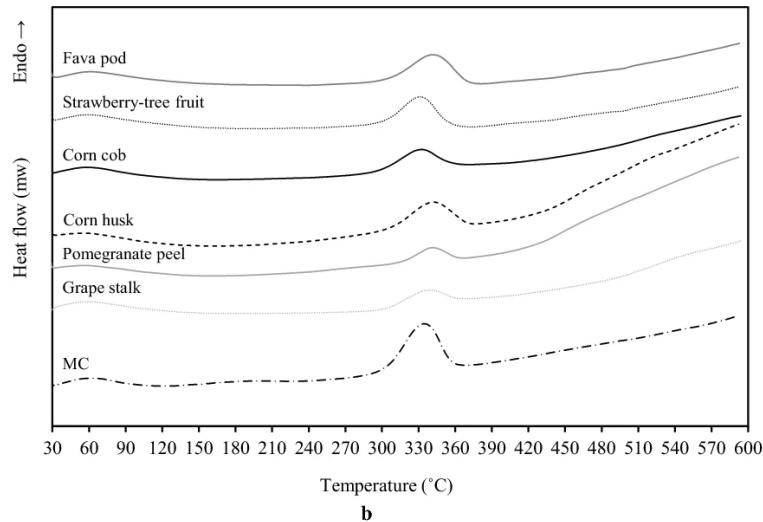
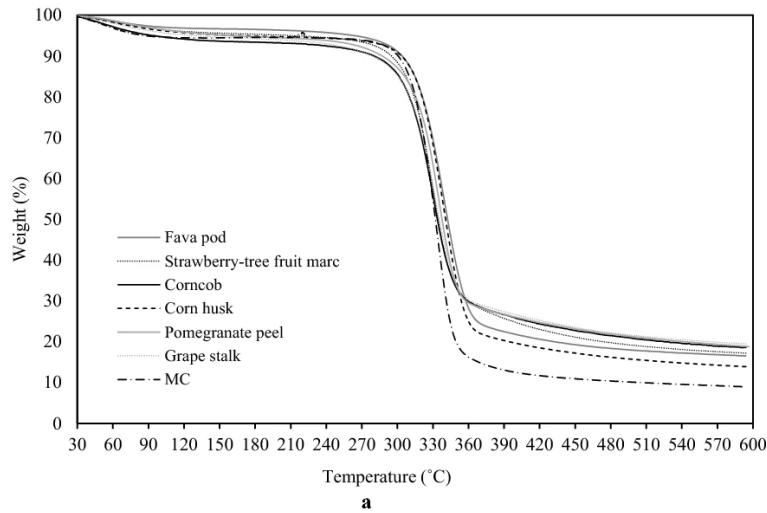


Table 2 (Water loss, onset temperature (T_{Onset}), degradation temperature (T_D), decomposition enthalpy (ΔH_{Decomp}), and total mass loss (TML) obtained from TGA and DSC analyses of the six cellulose samples)

Raw material	Water loss (%)	$T_{\text{Onset}} (\text{°C})$	$T_D (\text{°C})$	$\Delta H_{\text{Decomp}} (\text{kJ/kg})$	TML (%)
Fava pod	4.95 ± 0.11	314.02 ± 0.89	339.65 ± 0.73	306.04 ± 8.38	86.71 ± 0.63
Strawberry-tree fruit marc	4.69 ± 0.17	309.11 ± 0.34	329.62 ± 0.09	254.60 ± 9.13	82.73 ± 0.25
Corn cob	5.19 ± 0.29	307.17 ± 1.60	330.31 ± 0.19	166.55 ± 4.36	82.14 ± 1.47
Corn husk	4.98 ± 0.19	316.69 ± 1.34	339.40 ± 0.43	259.66 ± 8.88	84.83 ± 1.42
Pomegranate peel	$4.11 \pm 0.24^*$	311.24 ± 0.62	333.65 ± 0.32	89.75 ± 2.45	80.05 ± 0.44
Grape stalk	5.29 ± 0.37	306.38 ± 0.53	330.79 ± 0.74	125.47 ± 5.94	81.97 ± 1.66
MC	5.61 ± 0.11	312.24 ± 0.58	332.50 ± 0.46	$413.74 \pm 9.84^*$	$91.15 \pm 0.47^{**}$

3.4. Morphological analysis of cellulose

The macroscopic and microscopic images of the cellulose samples obtained in this study indicate noticeable structural differences among the cellulose samples obtained from the different agroindustrial residues. The most evident differences were the color and brittleness (Fig. 6a-f) which are two features mainly associated with the residual presence of lignin (Rojas et al. 2015) that was possibly not properly removed during the bleaching step. The obtained celluloses can be classified from yellow to white, according to their origin, by the following order: grape stalk, fava pod, strawberry-tree fruit marc, pomegranate peel, corncob and corn husk. These observations suggest that the bleaching process was more efficient in the case of corncob and corn husk. A previous work that investigated the fractionation of cellulose, hemicelluloses and lignin from grape stalk, already mentioned a general difficulty in obtaining well delignified cellulose fractions from this residue, highly influenced by the cultivar (Spigno et al. 2008). It is also important to refer that the organization of the lignin molecules within the raw material cell wall structure, such as the associative interactions with other cell wall components like cellulose, is determinant to make the extraction process more or less efficient. According to this, if color and cellulose purity are important features, the bleaching conditions must be optimized and adapted to the nature of the agro industrial residue.

Fig 6

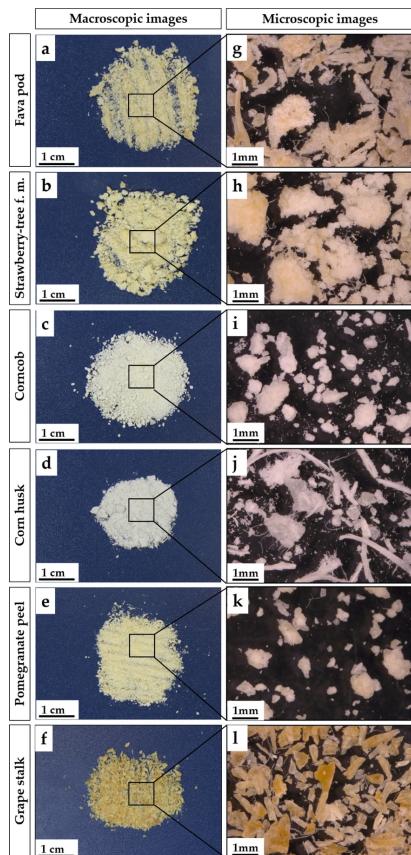
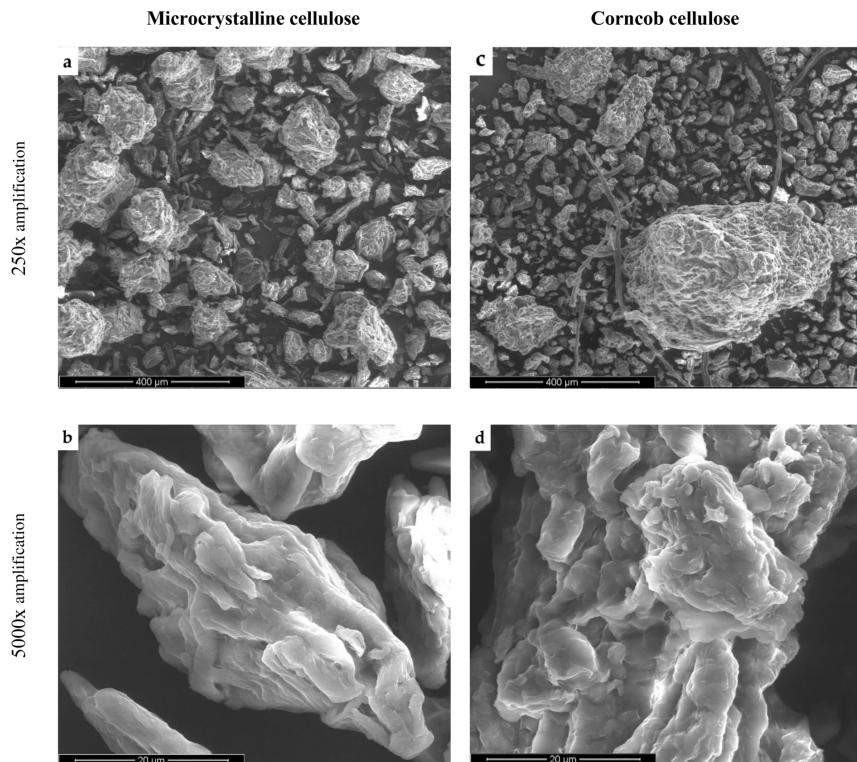


Figure 7 shows the SEM images under two different magnifications and reveals differences in the morphology of the two samples.

Fig 7



Conclusion and Recommendations (1 slide):

In conclusion, this study suggests novel non-wood cellulosic biomass sources, thus contributing to the development of new trade opportunities for the involved processing industries, and following the current worldwide tendency of recycling agroindustrial residues.

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