

Characterization of Enzymatically Modified Cellulose Obtained From the From the Cocoa Pod Husk (Cph) Theobroma Cacao L. Clone Ccn51

Diana Carolina Meza Sepúlveda

dcmeza@utp.edu.co

Universidad Industrial de Santander

Katalina Ángel Valencia

Universidad Industrial de Santander

Mónica María Quintero Morales

Universidad Tecnológica de Pereira

Lucia Constanza Vasco Sepúlveda

Universidad Industrial de Santander

Jorge Iván Quintero Saavedra

Universidad Industrial de Santander

Research Article

Keywords: Cocoa pods, Cellulose, Enzymatic modification, Reducing sugars, Characterization

Posted Date: July 19th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4639072/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Water interactions with cellulose, hemi-cellulose, and Cocoa (*Theobroma cacao* L.) processing generates by-products such as shells, husks, placenta and leachates that cause environmental and phytosanitary problems. The husk is a lignocellulosic material composed mainly of cellulose, hemicellulose and lignin, which can be used to produce coproducts useful at the industrial level. The objective of this research was to characterize the enzymatically modified cellulose obtained from cocoa pod husk (CPH) Clone CCN51. For this purpose, physicochemical analyses such as pH, ethereal extract, ash, moisture, crude fiber and water retention were carried out to establish the differences and/or similarities presented with respect to a commercial cellulose, thus making it possible to establish its possible agroindustrial use. The results revealed that in the transformation process of the raw material by the action of the Celluclast 1.5 L enzyme, a yield of 29% was obtained. Likewise, significant differences were evidenced in the characterization tests performed between commercial cellulose and modified CPH cellulose, indicating that the latter presents better conditions for industrial uses, such as the production of bioplastic films.

Introduction

Cocoa cultivation is one of the fastest growing crops in the world, with an estimated production of 5024 million tons during the 2020–2021 harvest (Hernández-Mendoza et al., 2021). In this productive process, substantial residual biomass is generated in each of the links, such as husk, husk, placenta, leachates, among others, which cause environmental and phytosanitary problems, due to inadequate removal of the plantation and incineration that create environmental burdens, and that at the same time could be transformed into bioproducts with high added value (de Oliveira et al., 2022; Gallego et al., 2022; Ofori-Boateng & Lee, 2013; Vásquez et al., 2019).

Theobroma cacao L. pod husk (CPH) is the most abundant residue, representing 70 to 80% of the dry weight of the fruit; for each ton of beans, 10 tons of residual husk are generated (Akinjokun et al., 2021; de Oliveira et al., 2022; Kley Valladares-Diestra et al., 2022; Vásquez et al., 2019).

The CPH is a relatively hard structure, however, the endocarp is a soft structure and its color depends on the variety or clone; likewise, this is a lignocellulosic material composed mainly of cellulose, hemicellulose, lignin, pectins, oils and waxes (Campos-Vega et al., 2018); lignocellulosic residues that could be exploited in the generation of biomaterials, fertilizers, energies and extraction of bioactive compounds that can lead to a profitable product, with additional income for farmers, promoting economic development (Lu et al., 2018; Muharja et al., 2023; Valladares-Diestra et al., 2022).

The use of agro-industrial waste can be an element of innovation that provides alternatives to the socioeconomic and environmental problems of cocoa production in Colombia, becoming a source of additional income for productive units that derive their livelihood from cocoa cultivation, reducing the high volumes of waste generated in their activities.

From the available literature, a small number of studies have reported the presence of cellulose in the husk of *Theobroma cacao* L. (Alemawor et al., 2009; Lu et al., 2018). Although the presence of cellulose in the shell has already been identified, it has only been studied for use in the production of paper (Valero-Valdivieso et al., 2013). Production of bioplastic from starch from jackfruit (*Artocarpus heterophyllus*) seeds and cellulose from cocoa shell with glycerol (Lubis et al., 2018).

Likewise, different cellulose sources have been identified to obtain biofilms, such as sugar beet (Perzon et al., 2020), orange peel (Mayhuire et al., 2019). however, there are specific methodological gaps, such as the enzymes used, the specific temperature of the process, and other variables such as pH value, agitation and concentration, which are key parameters to carry out enzymatic action processes.

This paper presents the method of enzymatic modification of CPH *Theobroma cacao* L. Clone CCN51, carried out for the first time, in which the aim was to maintain controlled working conditions for the optimum performance of the Celluclast 1.5L enzyme. The product resulting from the modification underwent characterization tests such as pH, Ash, Ethereal Extract, Moisture, Crude Fiber and Water Retention in order to make a comparison with Commercial Cellulose and to know the physicochemical differences between both raw materials.

Materials and methods

Materials

The cellulose extracted from the cocoa pod shell (CPD) variety CCN51 from the department of Amazonas was provided by the Agroindustrial Development Research group of the Faculty of Agrarian Sciences and Agroindustry of the Technological University of Pereira. The following reagents were used in this work: Celluclast 1.5 L enzyme (Novozymes), sodium acetate trihydrate buffer (Loba Chemie 99.5%), glacial acetic acid (Loba Chemie 99.7%), 3,5-Dinitrosalicylic acid (Loba Chemie 98%), NaOH (Loba Chemie 99.5%), sodium potassium tartrate tetrahydrate (Loba Chemie 99%), distilled water.

Enzymatic modification

The assembly was carried out in a thermostated bath (20 liter WBE20 Polysciencie Ref. WBE20A11B), keeping the temperature constant at 45°C (optimum working temperature of the enzyme according to the technical data sheet). Subsequently, 2 grams of CPH-CCN51 cellulose was weighed into a beaker and an acetate buffer solution pH 4.8 was added at a sample/buffer ratio of 1:100.

The solution was introduced into the setup and subjected to pneumatic agitation generated by an air pump (Power Air pum-life p-500); once the optimal reaction conditions (pH: 4.8, temperature: 45°C and bubble agitation) were reached, the Celluclast 1.5 L enzyme was added and monitoring of the modification began for 12 hours, taking a 1mL aliquot every 3 hours. The system was constantly monitored to guarantee the stability of the variables.

Determination of Reducing Sugars

The aliquots taken during the follow-up at 3, 6, 9 and 12 hours were subjected to an enzymatic inactivation process in a water bath (90–95°C) for 20min. Subsequently, the concentration of reducing sugars was determined by the DNS method in order to establish cellulose cleavage over time.

For the measurement, previously labeled and protected from light test tubes were taken, in which 250 µL of each sample and 250 µL of the DNS reagent were deposited. Then, they were heated in a water bath (90–95°C) for 5 minutes, after this time they were subjected to an ice bath for 10 min and 2.5mL of distilled water was added to each tube. Finally, they were shaken manually and read in a spectrophotometer (Mapada; model: PV4), at a wavelength of 540 nm, (Miller, 1959). Calculating the percentage of saccharification by means of equation (Salcedo M. et al., 2011).

$$\% \text{Saccharification} = \frac{R - R_0}{S - R_0} \times 100$$

Characterization

The characterization tests pH, ash content, ether extract, moisture, crude fiber, water retention, and grain morphology were conducted in triplicate for both enzymatically modified CPH cellulose, CCN51 variety, and the commercial cellulose taken as a reference parameter.

pH, AOAC 10.041/84

The method consists of the potentiometric measurement of hydrogen ion activity with the use of an electrode.

One gram of sample was weighed and added to 20 mL of distilled water in a 100 mL beaker. It was shaken for 20 min and with the pH-meter (brand: Hanna, model: HI5222) previously calibrated the pH of the sample was measured. This procedure was carried out in triplicate.

Ashes (A), AOAC 923.03

The method consists of incineration to destroy the organic matter, obtaining an ash composed of carbonates.

A crucible was tared at 105°C in which 1 gram of CCN51 modified cellulose was weighed, then it was taken for 3 hours to the muffle (brand: Thermo scientific, reference: F6018) until reaching a temperature of 550°C, once this time was completed, it was waited for the temperature to drop to later put them in the desiccator and take their final weight. The % ash was calculated using the following equation.

$$\% \text{Ashes} = \frac{\text{final weight}}{\text{initial sample weight}} \times 100$$

Ethereal extract (Ee), AOAC 920.39

The method consists of the extraction of crude fat from a sample previously dried in an oven in soxhlet equipment with n-hexane, to later eliminate the solvent and gravimetrically determine the dry extract that represents the cellulose lipids.

Boiling beads were deposited in a balloon which was tared for 2 hours at 105°C and their weight was taken. For the soxhlet extraction, n-hexane was used as solvent; 2 grams of sample were weighed in a thimble, maintaining a sample: solvent ratio of 1:100 for 4 hours. Once this process was completed, the hexane was recovered and the ethereal

$$\% \text{Ethereal Extract} = \frac{W - W_0}{W} \times 100$$

Moisture (M), AOAC 925.10

The method consists of the determination of moisture or volatile substances by weight loss that the cellulose undergoes when heated at 105°C.

Two grams of sample were weighed in a porcelain capsule previously tared and labeled. Subsequently, drying was carried out in a forced convection oven (brand: THERMO SCIENTIFIC, REFERENCE: 51028121) at a temperature of 103 ± 2°C for 5 hours, taking the weight every hour on an analytical balance (brand: Optika Italy, reference: B124Ai) until a constant value was obtained or with a difference of less than 0.002 grams between the last measurement and the previous one. Finally, the moisture percentage was calculated using the following formula.

$$\% \text{Moisture} = \frac{W - W_0}{W} \times 100$$

Where:

IW = Initial weight of the sample with the capsule (wet sample)

FW = Final weight of sample with capsule (after drying time)

SM = Weight of wet sample

Weight of the wet sample = (Weight of the capsule + wet sample) - (Weight of the empty capsule)

Weight of evaporated water = (Weight of capsule + wet sample) - (Weight of tray + dry sample).

$$\frac{\text{Weight of wet sample}}{\text{Weight of capsule + wet sample} - \text{Weight of empty capsule}} \times 100 = \frac{\text{Weight of capsule + wet sample} - \text{Weight of tray + dry sample}}{\text{Weight of capsule + wet sample} - \text{Weight of empty capsule}} \times 100$$

Crude fiber (CF), Weende

The method is based on the solubilization of non-cellulosic compounds by sulfuric acid and sodium hydroxide solutions. The mass loss resulting from incineration corresponds to the mass of crude fiber in the enzymatically modified CCN 51 cellulose sample and the commercial cellulose.

One gram of cellulose was added into a tared crucible, then the FIWE Raw Fiber Extractors equipment (brand: VELD SCIENTIFICA, REFERENCE: SA30540200) was turned on and water was recirculated. Subsequently, the crucible was positioned and the voltage regulator was programmed between 6 to 7 V. Then, 150mL of hot 1.25% sulfuric acid plus 3 drops of n-Octanol were added, and once the boiling point was reached, 30min were timed, allowing air to pass every 10 min. After this time, a vacuum was performed to drain the sulfuric acid and 3 washes were carried out with 30mL of boiling distilled water. The crucible was removed and dried in an drying was carried out in a forced convection oven (brand: THERMO SCIENTIFIC, REFERENCE: 51028121) for 2 hours at 130°C (Wr), then an analytical balance (brand: Optika Italy, reference: B124Ai) the weight was taken once the temperature was stabilized in a desiccator and it was taken to a muffle (brand: Thermo scientific, reference: F6018) for 2 hours at 550°C ± 15°C. The crucible was allowed to cool, was transferred to the desiccator and the final weight was taken to calculate the percentage of crude fiber with the following equation.

$$\frac{\text{Weight of crude fiber}}{\text{Weight of sample} - \text{Weight of empty crucible}} \times 100 = \frac{\text{Weight of crude fiber}}{\text{Weight of sample} - \text{Weight of empty crucible}} \times 100$$

Water retention (WRV), Beauchat (1977)

The method consists of measuring the amount of water retained in the sample which depends on the interactions of hydrogen bonds between the water molecules and the polar groups of the cellulose polymeric chains.

Five mL of distilled water was added to a falcom tube to which one gram of sample was added. Subsequently, it was vortexed, and the volume was completed up to 10 mL, shaking again. The tube was left in rest for 30 minutes; then it was taken to the centrifuge (brand:marca POWERSPIN LX) at 3500 RPM for 30 minutes and the volume of the supernatant was measured to calculate the percentage of water retention with the following equation.

$$\frac{\text{Volume of supernatant}}{\text{Volume of sample} + \text{Volume of water}} \times 100 = \frac{\text{Volume of supernatant}}{\text{Volume of sample} + \text{Volume of water}} \times 100$$

Where:

Wm = sample weight after centrifugation.

Wd = absolute sample weight.

Cellulose Morphology

The sample of enzymatically modified CCN51 cellulose and commercial cellulose, before being analyzed, were ground in a NUTRIBULLET BX180F-02 blender and passed through a number 100 sieve. Subsequently, each sample was placed on a slide and stained with methylene blue. and its morphology was observed using a microscope with an Optika Italia Model B383 PLI camera, C-B5 camera, Brightfield observation mode.

Statistical analysis

The averages and standard deviations of the different analyses performed were determined from the three replicates of each of the samples evaluated. Using IBM SPSS Statistics Version 22 software, an analysis of variance was performed using Tukey's test to determine if there were differences and/or similarities between the commercial cellulose and the cellulose extracted from cocoa pod husk (CPH) variety in the characterization tests.

Results and discussion

Enzymatic modification

During modification, simple sugars are released from the polymeric structure that composes cellulose. The enzyme Celluclast 1.5 L attacks the internal sites of the low crystallinity regions of the cellulose fiber to transform it into free end chains by removing cellobiose units, which are converted into glucose. Enzymatic hydrolysis is inhibited due to abrupt changes in parameters such as pH, agitation, temperature, among others. Therefore, it is important to establish optimal working conditions to achieve high yields in the conversion of reducing sugars. In the enzymatic modification process of cellulose extracted from cocoa husk variety CCN51, an average yield of 29% was obtained, maintaining a pH of 4.8, constant agitation by bubbling, and a temperature of 45°C during the procedure, which is higher than that reported by other authors in the conversion of cellulose obtained from different plant materials (Carolina et al., 2011; Piñeros-castro et al., 2011).

Table 1 shows the results obtained from the concentration of reducing sugars during the monitoring of the enzymatic modification, where a progressive increase of the concentration is observed, indicating the hydrolysis of the cellulose structure. On the other hand, graph 1 shows the percentage of saccharification, which allows determining that at 3 hours of reaction a representative sugar conversion had already been carried out (18.69%), presenting the highest saccharification at 12 hours with a percentage of 33.21%. The results obtained allowed establishing that an enzymatic modification of cellulose was successfully carried out and the parameters established during the development of the process were optimal.

Table 1
Concentration of reducing sugars in ppm during enzymatic modification monitoring.

Follow-up of the modification (hours)	Concentration of reducing sugars in PPM (mg/L)
3	2076.94 ± 223.33
6	3025.88 ± 221.70
9	3587.56 ± 332.94
12	3689.68 ± 274.85

Graph 1. Concentration in parts per million (ppm) of reducing sugars during the hours of monitoring the enzymatic modification of cellulose extracted from cocoa pod husk variety CCN51.

Characterization

Table 2 shows the results obtained from the physicochemical characterization of the modified cellulose extracted from CPH and commercial cellulose (reference parameter), establishing that there are significant differences in all the parameters evaluated between the two celluloses.

Table 2
Results of physicochemical analysis of Commercial Cellulose and CPH Cellulose CCN51 modified.

Analysis	Commercial cellulose	Cellulose CPH- CCN51 modified
pH	8.21 ± 0.16 ^a	7.05 ± 0.15 ^b
%M	3.91 ± 0.16 ^a	15.78 ± 0.25 ^b
%Ee	0.16 ± 0.03 ^a	0.52 ± 0.03 ^b
%CF	74.73 ± 1.63 ^a	3.64 ± 0.92 ^b
%A	0.14 ± 0.01 ^a	42.35 ± 0.35 ^b
%WRV	22.4 ± 0.10 ^a	8.13 ± 0.43 ^b

a y b: Average with common letter not significantly different (p > 0.05).

pH

The pH is an expression of the acidic or basic character of an aqueous system. In exact terms, it is a measure of the "activity" of the hydrogen ion in a given sample (Rodríguez, 2009). The results obtained show that the modified cellulose has a lower pH (7.05) than the commercial cellulose (8.21), which may be due to the production methods used, the porosity and particle size of each of the raw materials. In addition, during the enzymatic modification, hydrolysis of some cellulose polymeric bonds is carried out in an acid medium, which can trigger the presence of free hydronium ions that can lower the pH of the sample.

% Moisture

The modified cellulose presents a higher moisture content with a value of 15.78% than the commercial cellulose with 3.91%. The increase in moisture can be attributed to the possible decrease in the degree of polymerization due to the acidic conditions used during the enzymatic hydrolysis, which makes the sample more susceptible to absorb water from the medium because of the possible increase in the crystalline regions (Osto, 2021). The percentage of moisture in the cellulose is fundamental for the determination of subsequent treatments or uses, since a higher value of this parameter would indicate the presence of hydrophilic groups that improve adherence, resistance and compatibility.

% Ethereal extract.

The content of lipid compounds in the samples evaluated was less than 1%, being lower in the commercial cellulose with a value of 0.16% than in the modified cellulose with 052%. Cellulose is a glucose polymer that has an insoluble structure in non-polar solvents. Therefore, the extraction of compounds of this chemical nature is reduced. Also, the results obtained allow establishing that the samples do not present high contents of impurities and the pretreatments carried out on the samples were efficient.

% Crude fiber.

Crude fiber is understood as all those non-nitrogenous organic substances that do not dissolve after successive hydrolysis; one in an acid medium and the other in an alkaline medium. The main component of FC is cellulose, hemicelluloses and lignin (Omar Eduardo García Ochoa, Ramón Benito Infante, 2008).

The results obtained allow determining that the modified cellulose presents a low percentage of crude fiber with a value of 3.64% in comparison with the commercial cellulose that has 74.73%, which can be an indication of a lower presence of impurities product of the structural modification to which the sample was submitted during the enzymatic process; being this result significant because it could extend its uses at industrial level. On the other hand, having a high crude fiber content, as is the case of commercial cellulose, indicates that this substance contains a significant proportion of non-cellulosic components such as lignin and hemicellulose, which are insoluble in water.

% Ashes

Commercial cellulose has a lower ash content (0.14%) than cellulose extracted from modified CPH variety CCN51 (42.35%), which may be due to the adhesion of some inorganic components within the interstices of the sample during the enzymatic hydrolysis process. The ash content is an important indicator to guide the subsequent use of cellulose, since depending on the total content of minerals, organic matter and microelements it can be established what type of metabolic functions they fulfill and the type of industry for which they could be relevant (H & Jim, 2009).

% Water retention

Cellulose contains hydroxyl groups (-OH) in its structure, making it highly hydrophilic, which means it has a strong affinity for water, forming hydrogen bonds. Therefore, the water retention capacity depends on the amount of hydroxyl groups available in the structure and how quickly saturation points are reached (Patural et al., 2011). In the obtained results, the modified cellulose shows a lower water retention percentage with a value of 8.13% compared to the commercial cellulose which has 22.4%. This difference may be due to the structural changes that occurred during the enzymatic hydrolysis process, where the hydrophilic groups, due to their crystalline structure, present a lower contact surface, reducing their ability to absorb water. Therefore, enzymatically modified cellulose, with a low water retention percentage, offers several advantages, including better stability, ease of handling, and efficiency in production processes. This makes them ideal for a wide range of industrial and commercial applications.

Cellulose morphology

Cellulose is a linear polysaccharide formed by glucose units linked by beta-1,4-glucosidic bonds. Each glucose unit forms intramolecular and intermolecular hydrogen bonds, which have a profound effect on the morphology of this raw material, forming fibrillar crystalline structures called microfibrils, which in turn aggregate to form larger fibers (Pérez et al., 2002). As shown in Figs. 1 and 2, commercial cellulose presents irregularly shaped fibrils and contours with a rough surface, while modified cellulose has a smooth fibril structure with defined ends that are observed repetitively. These results allow determining that there was successful enzymatic hydrolysis because one of the main indicators is the decrease in amorphous and paracrystalline structures.

Conclusion

From the enzymatic modification method, a transformation of the CPH *Theobroma cacao* L. clone CCN51 was achieved, obtaining an approximate yield of 29%, which means that the method presented to carry out the process is optimal to control the working parameters of the enzyme used; due to the little information found in this regard, this methodology can be very useful for the implementation of processes involving biological agents for the transformation of a raw material.

In the comparison of the results obtained in the characterization tests conducted, it was established that there are significant differences between the modified cellulose from CMC *Theobroma cacao* L. clone CCN51 compared to commercial cellulose. This indicates that the modification process enhances the characteristics of this raw material, which could broaden its agroindustrial uses, such as the generation of bioplastic films, and even lead to replacing the use of commercial cellulose, as it proves to be more efficient and stable in various processes.

Declarations

Authors' contribution

Diana Carolina Meza Sepúlveda: conceptualization, data collection, writing, revising and editing of the article.

Katalina Ángel Valencia: writing, revising, editing, data collection and data analysis.

Mónica María Quintero Morales: conceptualization, data collection, writing, revision and editing.

Lucia Constanza Vasco: data collection and data analysis.

Jorge Iván Quintero Saavedra: Conceptualization and data analysis.

All the authors mentioned above have contributed significantly to the development of this article.

Financing statement

This research was financed by the project: Increasing the competitiveness of the cocoa sector through the transformation of agroindustrial waste for innovation and development of nutraceuticals and bioproducts that generate added value to the cocoa bean in the Department of Amazonas. Approved OCAD: BPIN 2021000100226.

Statement on data availability

The data used are confidential.

Additional information

No additional information available for this article.

Ethical approval

No results of studies involving humans or animals are reported.

Consent for publication

Written informed consent for publication was obtained from all participants.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work presented in this article.

Acknowledgments

To the project DEVELOPMENT OF A BIOPELICULA FROM ENZYMATICALLY MODIFIED CELLULOSE EXTRACTED FROM THE COCOA POD HUSK OF *Theobroma cacao* L. OF RISARALDA, code 11-22-1 of the Technological University of Pereira, Risaralda, Colombia.

References

1. Akinjokun, A. I., Petrik, L. F., Ogunfowokan, A. O., Ajao, J., & Ojumu, T. V. (2021). Isolation and characterization of nanocrystalline cellulose from cocoa pod husk (CPH) biomass wastes. *Heliyon*, 7(4), e06680. <https://doi.org/10.1016/j.heliyon.2021.e06680>
2. Alemawor, F., Dzagbafia, V. P., Oddoye, E. O. K., & Oldham, J. H. (2009). Enzyme cocktail for enhancing poultry utilisation of cocoa pod husk MonoBG, Viscozyme ® L and Pectinex ® 5XL were observed as appropriate levels for supplementing CPH feedstuff. Among the enzyme combinations tested, the Pentopan ® MonoBG + Viscozyme ® L. *Scientific Research and Essay*, 4(6), 555–559. <http://www.academicjournals.org/SRE>
3. Campos-Vega, R., Nieto-Figueroa, K. H., & Oomah, B. D. (2018). Cocoa (*Theobroma cacao* L.) pod husk: Renewable source of bioactive compounds. *Trends in Food Science and Technology*, 81(November 2017), 172–184. <https://doi.org/10.1016/j.tifs.2018.09.022>
4. Carolina, L., Pérez, R., Piñeros-castro, Y., Enrique, M., & Lozano, V. (2011). Production of fermentable sugars from press fiber oil palm pre-treated biologically by *Pleurotus ostreatus* and *Phanerochaete chrysosporium*. *Revista ION*, 24(2), 29–35.
5. de Oliveira, P. Z., de Souza Vandenberghe, L. P., Rodrigues, C., de Melo Pereira, G. V., & Soccol, C. R. (2022). Exploring cocoa pod husks as a potential substrate for citric acid production by solid-state fermentation using *Aspergillus niger* mutant strain. *Process Biochemistry*, 113(December 2021), 107–112. <https://doi.org/10.1016/j.procbio.2021.12.020>
6. Gallego, A. M., Zambrano, R. A., Zuluaga, M., Camargo Rodríguez, A. V., Candamil Cortés, M. S., Romero Vergel, A. P., & Arboleda Valencia, J. W. (2022). Analysis of fruit ripening in *Theobroma cacao* pod husk based on untargeted metabolomics. *Phytochemistry*, 203(August). <https://doi.org/10.1016/j.phytochem.2022.113412>
7. H, K. V., & Jim, Y. (2009). Evaluacion de la incertidumbre en la determinacion gravimetrica de humedad , cenizas , grasa y fibra cruda Evaluation of the uncertainty in the gravimetric humidity determination , ashes , fat and crude fiber. *Revista Ingenieria UC*, 16(2). <http://www.redalyc.org/articulo.oa?id=70717501005>
8. Hernández-Mendoza, A. G., Saldaña-Trinidad, S., Martínez-Hernández, S., Pérez-Sariñana, B. Y., & Láinez, M. (2021). Optimization of alkaline pretreatment and enzymatic hydrolysis of cocoa pod husk (*Theobroma cacao* L.) for ethanol production. *Biomass and Bioenergy*, 154(March). <https://doi.org/10.1016/j.biombioe.2021.106268>
9. Kley Valladares-Diestra, K., Porto de Souza Vandenberghe, L., & Ricardo Soccol, C. (2022). A biorefinery approach for pectin extraction and second-generation bioethanol production from cocoa pod husk. *Bioresource Technology*, 346(December 2021), 126635. <https://doi.org/10.1016/j.biortech.2021.126635>
10. Lu, F., Rodriguez-Garcia, J., Van Damme, I., Westwood, N. J., Shaw, L., Robinson, J. S., Warren, G., Chatzifragkou, A., McQueen Mason, S., Gomez, L., Faas, L., Balcombe, K., Srinivasan, C., Picchioni, F., Hadley, P., & Charalampopoulos, D. (2018). Valorisation strategies for cocoa pod husk and its fractions. *Current Opinion in Green and Sustainable Chemistry*, 14(July), 80–88. <https://doi.org/10.1016/j.cogsc.2018.07.007>
11. Lubis, M., Gana, A., Maysarah, S., Ginting, M. H. S., & Harahap, M. B. (2018). Production of bioplastic from jackfruit seed starch (*Artocarpus heterophyllus*) reinforced with microcrystalline cellulose from cocoa pod husk (*Theobroma cacao* L.) using glycerol as plasticizer. *IOP Conference Series: Materials Science and Engineering*, 309(1). <https://doi.org/10.1088/1757-899X/309/1/012100>
12. Mayhuire, E. A., Cuadros Huamaní, Y., Zanardi, L. M., & Medina De Miranda, E. (2019). Biopelículas producidas con cáscara de naranja y reforzadas con celulosa bacteriana. *Rev Soc Quím Perú*, 85(2), 231–241.
13. Miller, G. L. (1959). Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry*, 31(3), 426–428. <https://doi.org/10.1021/ac60147a030>
14. Muharja, M., Darmayanti, R. F., Fachri, B. A., Palupi, B., Rahmawati, I., Rizkiana, M. F., Amini, H. W., Putri, D. K. Y., Setiawan, F. A., Asrofi, M., Widjaja, A., & Halim, A. (2023). Biobutanol production from cocoa pod husk through a sequential green method: Depectination, delignification, enzymatic hydrolysis, and extractive fermentation. *Bioresource Technology Reports*, 21(September 2022), 101298. <https://doi.org/10.1016/j.biteb.2022.101298>

15. Ofori-Boateng, C., & Lee, K. T. (2013). The potential of using cocoa pod husks as green solid base catalysts for the transesterification of soybean oil into biodiesel: Effects of biodiesel on engine performance. *Chemical Engineering Journal*, 220, 395–401. <https://doi.org/10.1016/j.cej.2013.01.046>
16. Omar Eduardo García Ochoa, Ramón Benito Infante, C. J. R. (2008). Hacia una definición de fibra alimentaria. *An Venez Nutr*, 21(1), 25–30. https://ve.scielo.org/scielo.php?pid=S0798-07522008000100005&script=sci_arttext
17. Osto, H. L.-S. (2021). Caracterización de la celulosa proveniente del lodo papelerero y su esterificación. *Revista de La Facultad de Ciencias Universidad Nacional de Colombia, Sede Medellín*, 10(2), 67–81.
18. Patural, L., Marchal, P., Govin, A., Grosseau, P., Ruot, B., & Devès, O. (2011). Cellulose ethers influence on water retention and consistency in cement-based mortars. *Cement and Concrete Research*, 41(1), 46–55. <https://doi.org/10.1016/j.cemconres.2010.09.004>
19. Pérez, J., Muñoz-Dorado, J., De La Rubia, T., & Martínez, J. (2002). Biodegradation and biological treatments of cellulose, hemicellulose and lignin: An overview. *International Microbiology*, 5(2), 53–63. <https://doi.org/10.1007/s10123-002-0062-3>
20. Perzon, A., Jørgensen, B., & Ulvskov, P. (2020). Sustainable production of cellulose nanofiber gels and paper from sugar beet waste using enzymatic pre-treatment. *Carbohydrate Polymers*, 230(November 2019), 115581. <https://doi.org/10.1016/j.carbpol.2019.115581>
21. Piñeros-castro, Y., Velasco, G. A., Cortes Ortiz, W. G., & Proaños, J. (2011). Producción de azúcares fermentables por hidrólisis enzimática de cascarilla de arroz pretratada mediante explosión con vapor. *Revista Ión*, 24(2), 23–28.
22. Rodríguez, J. (2009). Parámetros fisicoquímicos de dureza total en calcio y magnesio , pH , conductividad y temperatura del agua potable analizados en conjunto con las Asociaciones Administradoras del Acueducto , (ASADAS), de cada distrito de Grecia , cantón de Alajuel. *Revista Pensamiento Actual, Universidad de Costa Rica*, 9(12), 125–134. <file:///C:/Users/Miqueas/Downloads/2842-4409-1-SM.pdf>
23. Salcedo M., J. G., Galán, J. E. L., & Pardo, L. M. F. (2011). Evaluación de enzimas para la hidrólisis de residuos (hojas y cogollos) de la cosecha caña de azúcar. *DYNA (Colombia)*, 78(169), 182–190.
24. Valero-Valdivieso, M. F., Ortigón, Y., & Uscategui, Y. (2013). Biopolímeros: Avances y perspectivas. *DYNA (Colombia)*, 80(181), 171–180.
25. Valladares-Diestra, K. K., Porto de Souza Vandenberghe, L., Zevallos Torres, L. A., Zandoná Filho, A., Lorenci Woiciechowski, A., & Ricardo Soccol, C. (2022). Citric acid assisted hydrothermal pretreatment for the extraction of pectin and xylooligosaccharides production from cocoa pod husks. *Bioresource Technology*, 343(July 2021). <https://doi.org/10.1016/j.biortech.2021.126074>
26. Vázquez, Z. S., de Carvalho Neto, D. P., Pereira, G. V. M., Vandenberghe, L. P. S., de Oliveira, P. Z., Tiburcio, P. B., Rogez, H. L. G., Góes Neto, A., & Soccol, C. R. (2019). Biotechnological approaches for cocoa waste management: A review. *Waste Management*, 90, 72–83. <https://doi.org/10.1016/j.wasman.2019.04.030>

Graph 1

Graph 1 is available in the Supplementary Files section.

Figures

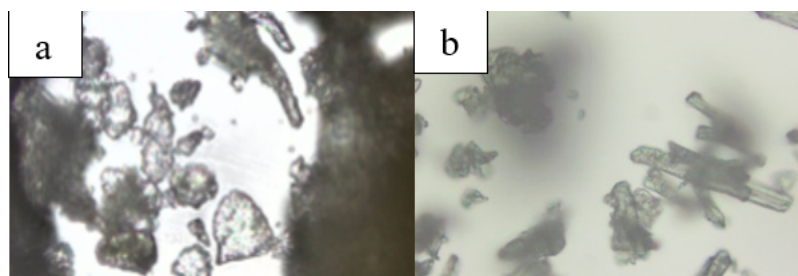


Figure 1

(a) Commercial cellulose y (b) Cellulose CCN51 modified with the lens 40x.

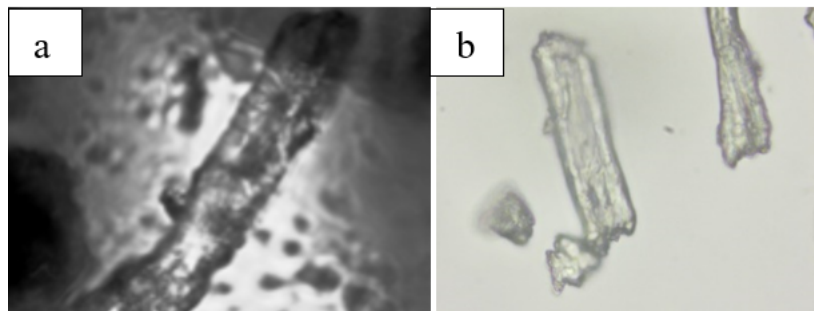


Figure 2

(a) Commercial cellulose y (b) Cellulose CCN51 modified with the lens 100x.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Graph1.png](#)