

INFLUENCE OF PARTICLE SIZE ON CELLULOSE EXTRACTION FROM CALAMAGROSTIS INTERMEDIA SPECIES

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ABSTRACT

The purpose of the research work was to determine the influence of the particle size in the extraction of cellulose from the Calamagrostis Intermedia species, since the extraction and commercialization of cellulose is one of the most versatile industries, however, the use of wood sources leads to an environmental impact that cannot be mitigated, considering that in extractive processes there is no rationing of resources within washing or bleaching. The analysis of the particle size of a non-timber endemic species of Ecuador was proposed, considering a pretreatment of the samples with a study of drying, grinding and screening after the taxonomic identification, an alkaline extraction was applied simulating the industrial conditions at the laboratory level. with the samples of defined particle size with an experimental design 22, to give way to the characterization of the extracted cellulose. It was determined that the particle size influences the amount of fiber mass and cellulose pulp extracted in the samples previously dried at 65°C and with particle sizes of 300 and 106 µm. Characterization tests reveal a variation in the color and texture of the extracted cellulose, caused by an inefficient delignification step within the fiber bleaching, variations in molecular weight corresponding to 126.32×10^3 g/mol between the samples, FTIR analysis shows a syringyl ring and stretching in lignin, light microscopy reveals the absence of well-defined regions in the fibers and an easily discernible crystal lattice.

Key words: Cellulose, Particle size, extraction, Calamagrostis Intermedia, characterization, polymers

1. Introduction

Cellulose is considered a biopolymer made up of hundreds, or even thousands, of carbon, hydrogen and oxygen atoms(1). Cellulose is the main substance of cell walls in the plant kingdom, helping plants to remain rigid and upright, functioning as a support or "skeleton"(2). In this way, cellulose is the most abundant material within the biomass of the earth, considering that the percentage of existence in each plant varies according to the species and stage. maturity(3).

Since as the plant adds a molecule of glucose to the polymer, a molecule of water is released, thus the natural polymerization process of cellulose occurs. When a plant grows and creates new cells. Cellulose is a polysaccharide consisting of long chains of beta glucose molecules linked by glycosidic bonds. The orientation of the sugar molecules is such that the OH groups protrude from the cadena in all directions(4). These OH groups can form hydrogen bonds with neighboring chains, thus establishing a kind of three-dimensional lattice structure that is quite different from The way N(5) starch molecules are constructed. Starch consists of a long chain of glucose molecules, but the chain is wound into a helix and the orientation of the glucose subunits is such that most of the OH groups capable of forming hydrogen bonds project inward. This means that there are no cross-links in starch and this is why it lacks the structural properties that cellulose possesses(6).

A single cellulose chain can contain up to 10,000 sugar units with a total length of 5 micrometers. The strength of the glycosidic bonds, along with the cross-links between adjacent chains, makes cellulose as strong as rubber. In the cell wall, groups of approximately 2000 cellulose chains come together to form ribbon-like microfibrils, each between 10 and 30 nm in diameter(7).

This biopolymer has versatile uses in many industries, such as veterinary food, wood and paper, fibers and clothing, cosmetic and pharmaceutical industry as an excipient. Cellulose has very semi-synthetic derivatives that are widely used in the pharmaceutical and cosmetic industries(8). Cellulose ethers and cellulose esters are two main groups of cellulose derivatives with different physicochemical and mechanical properties(9). These polymers are widely used in the formulation of dosage forms and health care products. These compounds play an important role in different types of pharmaceutical products, such as prolonged and delayed release coated dosage forms, prolonged and controlled release matrices, Osmotic drug delivery systems, bioadhesives and mucoadhesives, compression tablets as compressibility enhancers, liquid dosage forms as thickening and stabilizing agents, granules and tablets as binders, semi-solid preparations as gelling agents and many Other applications. These polymeric materials have also been used as fillers, flavor maskers, free-flowing agents, and pressure-sensitive adhesives in transdermal patches. Today, cellulose and cellulose-based polymers have gained great popularity in the pharmaceutical industries and become increasingly important in this field due to the production of new derivatives and Search for new applications for existing compounds by pharmaceutical researchers(10). Bioadhesives and mucoadhesives are polymeric films containing drugs with the ability to adhere to biological membranes after combining with wet or mucous compounds(11).

Cellulose derivatives, especially cellulose ethers are widely used in bioadhesives. They are used in several types of these formulations such as buccal, ocular, vaginal, nasal and transdermal formulations alone or with a combination of other polymers(12). Cellulose ethers most recently used in bioadhesives include nonionic cellulose ethers such as ethylcellulose (EC), hydroxyethyl cellulose, hydroxypropyl cellulose (HPC), methylcellulose (MC), carboxymethylcellulose (CMC) or hydroxypropyl methylcellulose (HPMC) and anionic ether derivatives such as sodium

carboxymethylcellulose (NaCMC)(13). The ability of the polymer to absorb water from mucus and the pH of the target site are important factors that determine the adhesive power of polymers. Some bioadhesive polymers, such as polyacrylates, show a very different adhesion capacity at various pH values, so the selection of the adhesive polymer should be made depending on the type of bioadhesive preparation(14). An advantage of cellulose ethers such as NaCMC and HPC is the lower dependence of adhesion time and adhesion strength of them on the pH of the medium compared to bioadhesive polymers of polyacrylate and thiolates. Cellulose ethers, alone or their mixtures with other polymers, have been studied in oral, buccal, ocular, aginal and transdermal bioadhesive treatments(15). In some studies, other groups of adhesive polymers or polysaccharides with cellulose ethers are used to improve their adhesion characteristics,

such as the time of adhesion and the strength of adhesion. The simultaneous use of polyvinylpyrrolidone (PVP), hydroxypropyl beta cyclodextrin, polycarbophil, carbopol(s), pectin, dextran and mannitol with HPMC, HEC or NaCMC has been reported in the literature(16).

The commercial importance, meaning and use of cellulose cannot fail to be emphasized. This is because cellulose has found use in various industrial raw materials, food components and household items. For example, cellulose provides the raw material for the production of paper, cellophane, celluloid and rayon, including several types of plastics(17). Cellulose derivatives such as cellulose nitrate are used in the manufacture of films, laces and explosives. Cellulose is almost pure cotton that requires very little treatment before being used in the manufacture of fabrics and other useful materials. Wood pulp, which is also a form of cellulose, is mainly obtained from spruce. The first stage involved in cellulose preparation involves the removal of lignin and other non-cellulosic materials(18). This is done by treating the wood pulp at high temperature and pressure with sulfur oxide and calcium bisulfite. The purified pulp that comes out of this process, which contains up to 97% pure cellulose, is dissolved and the cellulose is generated as synthetic fibers or sheets. Regenerated cellulose has enormous tensile strength as well as several protective properties. This makes cellophane ideal for wrapping, while rayon, an even stronger material, is used in the manufacture of industrial belts and tyre ropes(19).

On the other hand, the waste generated by the extraction of cellulose in timber sources contains many valuable components, including organic compounds such as macro and microelements essential for plants. The composition of the residues includes calcium and magnesium, which can occur in the form of carbonate, oxide and silicate, small amounts of calcium hydroxides and alkali metals, as well as. Environmental management of such residues could improve the physicochemical properties of the soil and create beneficial conditions for plant growth and development(20). However, due to the specific physicochemical properties of timber sources, the environmental use of pulp production residues is affected by the amount of heavy metals and other pollutants. Additionally, the problem is emphasized when using timber sources for pulp extraction, since the contamination of freshwater bodies is not an exclusively local, but a global problem(15).

The pulp industry is a profitable business that grows every year worldwide. It is one of the main activities responsible for the deterioration of water resources due to the amount of water required for the process and the amount of liquid industrial waste it generates, ranking worldwide as the sixth industry generating liquid pollutants(21).

Therefore, it is evident that although the extraction and commercialization of pulp is one of the most versatile and useful industries within the production processes, the use of timber or "typical" sources leads to an environmental impact that cannot be managed or mitigated correctly, on the other hand the problem indicates that this effect is caused by the methodology and technology applied in the extraction, since although the extractive processes are based on mechanical techniques or basic solutions there is no rationing of the resources of the washing or bleaching. In this way the present research work aimed to determine the influence of particle size within the process of extraction of cellulose in a non-timber source such as the species *Calamagrostis Intermedia*, this being a type of Andean straw endemic to Ecuador, thus applying an alkaline extraction method to identify the effects of particle size when handling the same variables in the extraction and correlate the information based on the characterization tests applied to the extracted cellulose.

2. Materials and methods

The study was carried out at the laboratory level considering the particularities in the extraction methodology at the industrial level, in this way a non-timber source such as the species *Calamagrostis Intermedia* is selected because this is an endemic species in Ecuador that has no commercial uses or defined extractive agriculture(22). In addition, this species is not considered a threat as it is not included in the Red Book of Endemic Species of Ecuador(23). An experimental design was proposed where the variables of interest are the particle size and the percentage of humidity related to the drying temperature in the samples to be treated for extraction and subsequent characterization.

2.1. Raw material

The samples of the Andean straw species *Calamagrostis Intermedia* were collected at the location - 1°28'13.225"S-78°45'1.001"W belonging to the parish Condor Samana within the canton Guano in the Province of Chimborazo, it is worth mentioning within the collection process the roots of the plants were not extracted, instead a pruning was carried out at a height of 5 cm from the base considering samples in which the leaves are at least 90 cm high, after the collection the samples were sent for their Botanical identification.

2.2. Pretreatment of the sample

When considering the percentage by weight of water contained in the sample as a reaction intermediate that can generate interruption within the specificity of the reagents in the cellulose extraction process, a drying study and then a particle size study that will clearly reveal the

influence of this variable in the extractive process. In this way 100 g of straw were weighed and placed in each tray, drying was carried out at a temperature of 65 ° C and 80 ° C until the weight is constant, recording the weight every hour, the dry straw was crushed and placed for 10 minutes in the sieve at 200 rpm.

2.2.1. Sample pretreatment equipment

- Electric tray dryer Aio-300
- Balance GRAM FS-220
- Shredder 0.5 Hp
- Tamizadora AS 200 RESTCH

2.3. Experimental design

In order to determine the influence of particle size in the extraction of cellulose from this non-timber plant species, an experimental design was proposed for the pretreatment of the sample before extraction simulating the methodological conditions at the laboratory level. Therefore, the drying temperature was considered as variable, because the water molecules linked to the cellulose portions are the result of the natural polymerization process in the plant and this substance in turn prevents a successful extraction by means. From a basic solution, the particle size after drying was additionally considered since grinding depends largely on the dry portion of the sample (17). In this way the experimental design 2 2 taking into account that the best analytical result is sought in percentage of cellulose and fiber, in addition to the physical characterization and chemistry of the extracted cellulose, thus being an experimental design in a methodological way can be seen in Table 1.

Table I. Factors and levels of experimental design 2 2

Factors	Levels
Particle size	A ₀ : 300 μm
	A ₁ : 106 μm
Drying Temperature	B ₀ : 65° C
	B ₁ : 80° C

2.4. Cellulose extraction

Considering the particularities at industrial level on the extractive process of cellulose was used an extraction in alkaline medium at laboratory level in this way, in a 10% solution of NaOH samples of Andean straw were placed and heated to a temperature of 90 ° C, maintaining this temperature 10 minutes, after this type was allowed to cool to room temperature, the solid phase was washed with plenty of water until it reached a neutral pH, then placed in a solution of sulfuric acid at 4% by volume, the mixture was brought to a boil for one hour and allowed to cool to room temperature, the solid phase was washed until it reached a neutral pH, the washed sample was placed in a 3.5% solution by volume of sodium chlorite, the mixture was left to stand for 3 hours and heated for 40 minutes at 95°C, the solid phase was washed to a neutral pH, the sample was placed in a 20% solution by volume of NaOH and heated to 50° C on a

griddle stirred at medium speed for 1 hour, the solid phase was washed to a neutral pH, the solid phase was placed in 0,5 % by volume solution of sodium chlorite and the previous step was repeated. Finally, the last washing was carried out until a pH 7 was obtained and dried for 6 hours at 70 ° C.

2.4.1. Materials and reagents cellulose extraction

- Electric tray dryer Aio-300
- Balance GRAM FS-220
- Reverbero PROCTOR SILEX
- Potenciómetro pH HANNA EDGE
- Magnetic stirrer VEVOR 79-1
- Sodium Hydroxide MERCK CAS 1310-73-2
- Sulfuric Acid MERCK CAS 7664-93-9
- MMS Sodium Chlorite
- 1000, 500, 250 and 100 mL beakers
- Glass Watch
- Spatula
- Clothes peg
- Thermometer
- Distilled water
- Mesh filter
- Timer
- Stirring rod
- Piseta
- 500mL volumetric flask
- Pipette of 10 and 25 mL
- Suction pear

2.5. Characterization of extracted cellulose

2.5.1 Molecular weight determination

Within this characterization test widely used at industrial and laboratory level recommended by the Spanish association of pulp, pulp and paper manufacturers (ASPAPPEL) (24), a viscosimetry analysis was applied in a viscometer of Ostwald being so prepared a solution of cupric sulfate pentahydrate with distilled water, sulfuric acid 50% by volume and ammonium hydroxide, to this solution was added 0.035 g of cellulose extracted thus preparing solutions of concentration 0.1-0.3-0.5-0.7 g / L, for each experimentation, then 15 mL of standard cupriethylenediamine were placed in the viscometer of Ostwald maintaining a temperature of 25 °C. The previous procedure was repeated with diluted cellulose concentrations in order to evaluate the difference in travel times within the instrument(17,25).

2.5.2 FTIR analysis

Fourier transform infrared spectroscopy provides information on the molecular structure of a polymer(26), which allows it to be identified according to the spectra obtained, thus the samples were crushed in a mortar to obtain a uniform fine powder and analyzed within the zone corresponding to 4000 to 600 cm^{-1} , the spectra obtained were reported using the SPECTRAL MANAGER software and the vibrations the peaks of each functional group with those reported in samples of cellulose extracted from cotton and wood(27).

2.5.3 Type of cellulose

The typology of structural cellulose obtained can be characterized by identifying the position of the hydroxyl group through which the glycidic bond was generated in the aldose(24), so for each cellulose sample its solubility was established using 0.1g of cellulose in 10 mL of NaOH at 17.5% and 8% by volume.

2.5.4 Optical Microscopy

This analysis is of interest because in the chemical industry and the field of manufacturing, polymers are observed under the microscope when observing the morphology of the crystal, the spherulites, the surfaces of the fracture planes, the process control, failure analysis, roughness, strain, quantitative and qualitative analysis of the microstructure and when interference patterns are observed(28), so direct observation was applied in an amplitude of 40 and 60 X on the samples of cellulose extracted, comparing the regions and images with those reported in samples of cellulose extracted in cotton and wood.

2.5.5 Materials and Equipment in characterization

- Mortar
- Spatula
- Distilled water
- Spatula
- Agitation Rod
- Precipitation glass 50mL
- Test tubes
- 50 mL test tube
- Cupric sulfate pentahydrate AVALCO
- Ammonium hydroxide BIOPACK
- Sodium Hydroxide MERCK CAS 1310-73-2
- Sulfuric Acid MERCK CAS 7664-93-9
- Balance GRAM FS-220
- Espectrofotómetro AND JASCO FT/IR-4100typeA
- Optical microscope UNICO M280
- Viscometer from Ostwald YUCHENGTECH

- Chronometer

3. Results and discussion

3.1. Taxonomic identification of samples

Based on the morphology and taxonomy analyzed in the collected samples compared and studied with patterns identified in the Alfredo Paredes Herbarium of the Central University of Ecuador, it was determined that the samples of the present study belonged to the Poaceae Family being of the species Calamagrostis Intermedia (J. Chair) Steud. Thus confirming that it is a non-timber species, endemic in Ecuador and not threatened.

3.2. Sample drying

Table II. Sample Drying

Drying at 65°C		Drying at 80°C	
Time (h)	Sample weight (g)	Time (h)	Sample weight (g)
0	100	0	100
1	98,3	1	97,5
2	96,6	2	96,4
3	95,8	3	95,6
4	95	4	92,8
5	93,5	5	91,9
6	92,8	6	90,3
7	92,3	7	89,8
8	92,2	8	88,6
9	91,2	9	88,8
10	91,1	10	88,8
11	91		
12	91		

Table 2 shows the mass of the sample recorded every 1 hour during the drying process at 2 different temperatures, while the sample at 65 ° C keeps its mass constant for 11 hours feels this the postcritical point in the drying kinetics, which is achieved 8 hours at a temperature of 80 ° C, however, 9 and 12.2 g are lost respectively at 65° and 80°C . So it can be seen that the sample contains about 10% water before the extraction process.

3.3. Screening

Table III. Sample screening

	Drying at 65°C	Drying at 80°C
Mesh light (µm)	Percentage retained (%)	Percentage retained (%)
425	1,65	2,29
300	45,93	40,67
212	1,21	3,01
150	1,42	9,67
106	44,18	38,11
53	1,76	3,13
38	0,99	2,34
Base	2,86	0,78

Table 3 shows the results of the screening study applied after grinding the dry sample, thus identifying that the particle size belonging to the mesh light 300 and 106 µm are the ones that retain the greatest amount of mass in the samples treated at 65 and 80 ° C.

3.4. Experimental design

Experimentation	Fiber (%)	Cellulose (%)
A ₀ B ₀	53,1	48,06
A ₀ B ₁	42,77	39,76
A ₁ B ₀	47,9	48,80
A ₁ B ₁	40,12	37,88

To determine the percentage of cellulose and fiber in each experimentation in which the experimental design was applied, the following formulas were used:

$$\text{Cellulose fiber} = \frac{X}{P} \times 100 \quad (1)$$

Where:

X=amount of fibre(g)

P=quantity of dry straw (g)

$$\text{Cellulose yield} = \frac{C}{Z} \times 100 \quad (2)$$

Where:

C=quantity of pulp (g)

Z= Cellulose fibre (g)

By virtue of this, it is established that the cellulose extraction process with the highest amount of fiber mass and cellulose pulp is in which samples previously dried at 65 ° C and with particle sizes of 300 and 106 µm were considered.

3.5. Cellulose extraction

Table V. Mass of cellulose fibre and pulp extracted in the final particle size samples

Definition	Sample with particle size 300 µm	Sample with particle size 106 µm	Difference
Dry straw mass (g)	40	40	-----
Cellulose fiber mass (g)	21,24	19,16	2,08
Amount of Cellulose (Bleached Pulp) (g)	10,21	9,35	0,86

Table 5 shows the difference in the amount of mass of fiber and cellulose pulp extracted in the samples of particle size 300 and 106 μm , considering that this difference is minimal however in Figure 1 there is a significant difference in the color and texture of the cellulose pulp extracted, Presumably it accuses of an inefficient delignification process which prevents an adequate swelling of the fibers and a bad release of cellulose, evidencing the influence of the particle size in the extraction.

Figure I. Cellulose pulp extracted from different particle sizes .



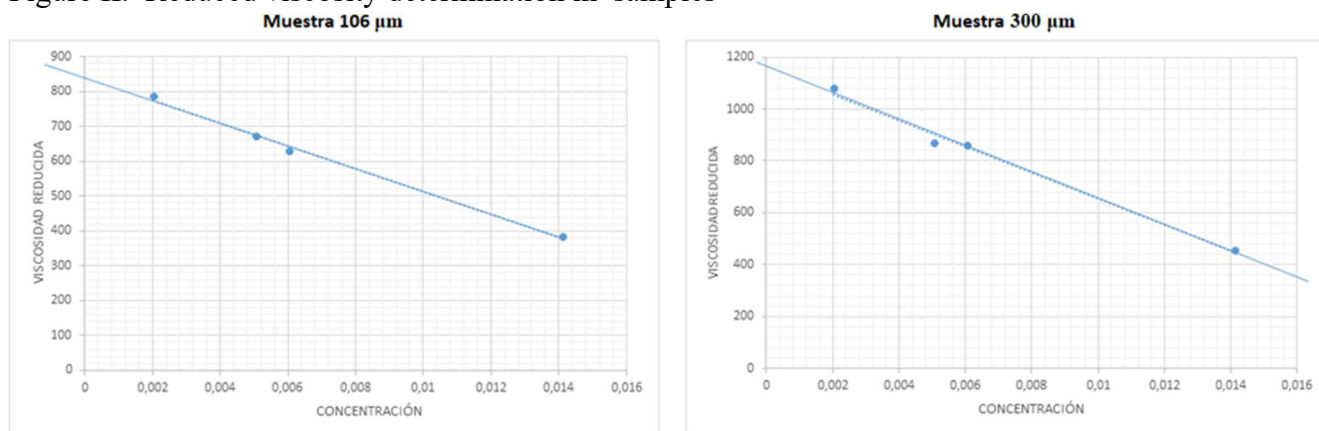
3.6. Cellulose characterization

Although the cellulose extracted from the 300 μm sample is shown with a yellowish color and with the typical structure of cellulose fiber, the tests Characterization was applied in both cases to determine the influence of particle size and its effect on the structural properties of the extracted cellulose.

3.6.1 Molecular weight

Figure 2 shows the extrapolation when the concentration tends to zero considering 5 experiments to determine the reduced viscosity of the sample, so it is possible to replace the value of this viscosity in the equation of Mark Houwink considering the constants and parameters described in the literature (25), in this way the molecular weight obtained in the cellulose extracted from the Sample size 106 and 300 μm is $292.83 \times 10^3 \text{ g/mol}$ and $419.15 \times 10^3 \text{ g/mol}$ respectively, in this characterization test there is a notable difference of about $126.32 \times 10^3 \text{ g/mol}$ This magnitude of difference is assumed to be related to the amount of lignin present in the fibers of the 300 μm sample. However, both samples are within the optimal standard molecular weight of cellulose at an industrial level.

Figure II. Reduced viscosity determination in samples



3.6.2 FTIR analysis

Figure 3 shows the spectra and vibrations obtained in the samples of cellulose extracted from defined particle sizes, so it is possible to characterize these polymers based on the percentage of transmittance present in each vibration assigned to a specific functional group, being that the functional groups provide precise structural information on the type of polymer obtained. In general, both spectra have very similar patterns in vibrations and transmittance percentages. The wavenumbers observed are similar for the functional groups Hydroxyl, Stretching C-H in methyl and methylene groups, unconjugated ester groups, folding in cellulose and hemicellulose, cellulose and hemicellulose C-O-C, CH AND C-O- deformations. However in the wavenumbers corresponding to the ring of syringyl and stretching C-O in lignin and xylan has a value of 1268.93 cm⁻¹ in the sample of 300 μm while this peak is not evident in the sample of 106 μm. Therefore the inefficiency of the delignification process in this sample is rectified.

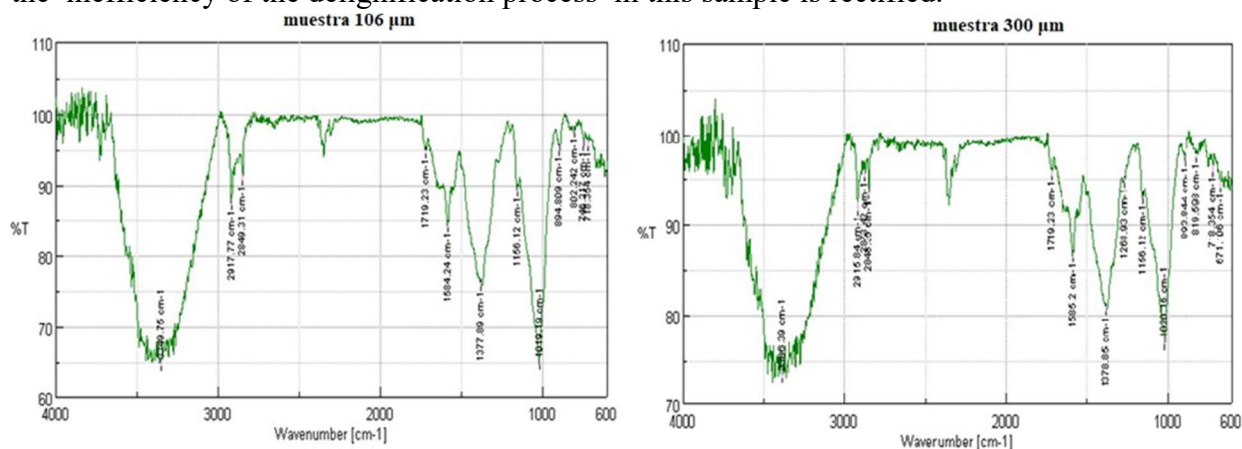


Figure III. FTIR analysis of extracted cellulose samples from particle sizes of 106 and 300 μm

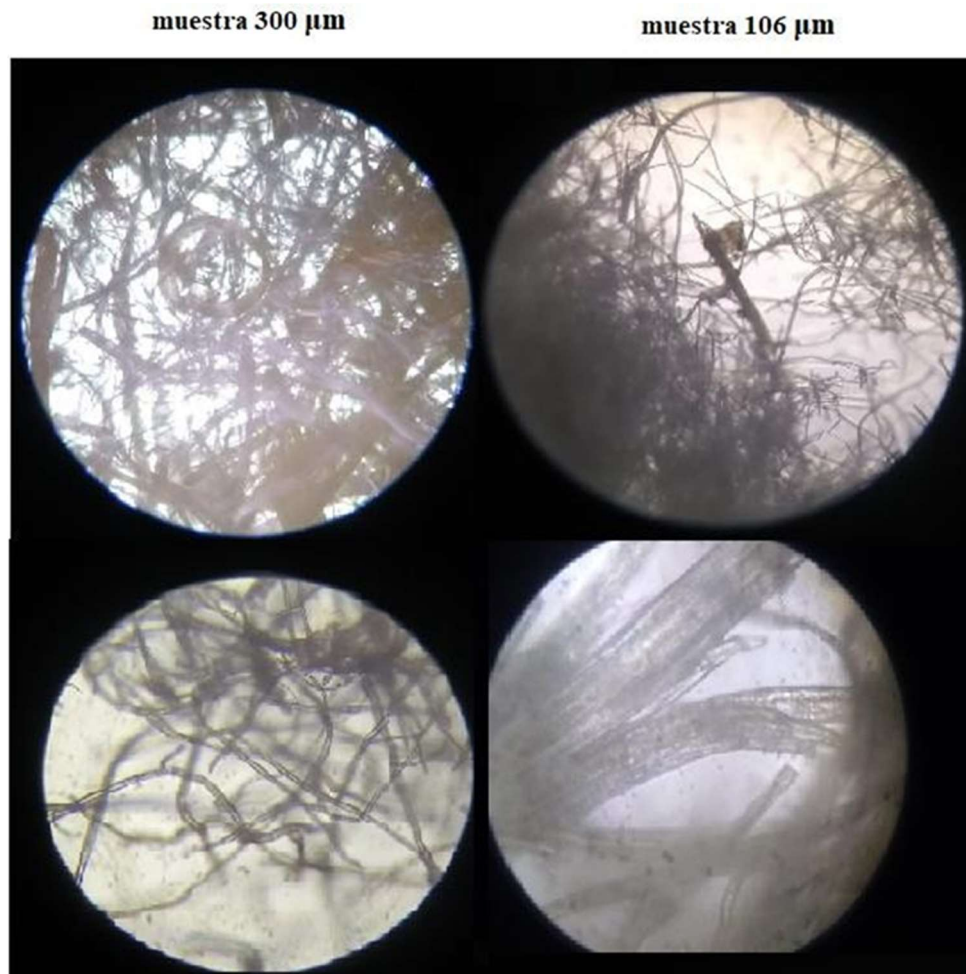
3.6.3 Type of cellulose

With relevance to the solubility of the samples in solutions of sodium hydroxide or 17.5 and 8% it was determined that the cellulose present in the plant species corresponds to the beta type since it is soluble in a concentration of 17.5% by volume of sodium hydroxide.

3.6.4 Optical Microscopy

After the application of this characterization analysis, fibers are observed very close between loops in both samples, however in the cellulose sample extracted from a particle size of 106 μm the contours of the fibers are even and present aclear crystallinity, which is expected in high purity polymers, However, in cellulose extracted from the particle size of 300 μm the fibers are appreciably thicker and no defined contours with crystallinity are observed.

Figure IV. Optical microscopy to cellulose samples extracted from particle sizes of 106 and 300 μm



4. Conclusions

For the present study, a non-timber plant species was selected and based on the morphology and taxonomy analyzed with patterns identified in the Alfredo Paredes Herbarium of the Central University of Ecuador, it was determined that they belong to the Poaceae Family being of the species *Calamagrostis Intermedia* (J. Presi) Steud, being an endemic species in Ecuador and not threatened. When determining the influence of particle size in the cellulose extraction process, it was established that the cellulose extraction process that presents the greatest amount of

fiber mass and cellulose pulp is in which the samples previously dried at 65 ° C and with Particle sizes of 300 and 106 µm. Against this background, characterization tests reveal in the first instance an indisputable variation in the colour and texture of the cellulose extracted, since cellulose extracted from the particle size of 300 µm has a yellowish colour caused by an ineffective delignification step within the bleaching of the fibre, this result is confirmed by characterisation tests, in which both samples are within the quantifiable and qualifiable parameters of cellulose at an industrial level, however there are variations in the molecular weight corresponding to 126.32×10^3 g / mol between the samples, in addition it is appreciated within the FTIR analysis a wavenumber of 1268.93 cm⁻¹ corresponding to the syringyl ring and stretching C-O in lignin and xylan, optical microscopy reveals that between the samples the cellulose extracted from

The particle size of 300 µm lacks well-defined regions in the fibers as well as an easily noticeable crystal lattice. In this way it is possible to say that the particle size directly influences the process of delignification and bleaching of the pulp, as well as the process of swelling of the fibers that allows to release the cellulose contained in the cell walls of the plants, this effect is directly found. related to the fact that the greater the surface area the chemical reaction has greater efficiency, that is, when the particle size generates a greater contact surface between the reactant and the reactant, the reaction yield increases, Considering even a determining factor within the properties and factors that influence the kinetics of the reaction, therefore the bleaching reaction will have a greater efficiency with greater surface area of contact in the experimental conditions of the present study. Finally, it was found that the plant species contains cellulose of the beta type.

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Conflicts of interest

No conflicts of interest are reported.

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