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Optimization of Cellulose Nanocrystal Isolation from Ayous Sawdust Using Response Surface Methodology

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Abstract

This study focuses on the extraction of cellulose nanocrystals (CNC), from microcrystalline cellulose (MCC), derived from Ayous sawdust. The process involves multiple steps and a large amount of chemical products. The objective of this research was to determine the effects of factors that impact the isolation process and to identify the optimal conditions for CNC isolation by using the response surface methodology. The factors that varied during the process were the quantity of MCC, the concentration of sulfuric acid, the hydrolysis time and temperature, and the ultrasonic treatment time. The response measured was the yield. The study found that with 5.80 g of microcrystalline cellulose, a sulfuric acid concentration of 63.50% (w/w), a hydrolysis time of 53 minutes, a hydrolysis temperature of 69˚C, and a sonication time of 19 minutes are the ideal conditions for isolation. The experimental yield achieved was (37.84 ± 0.99) %. The main factors influencing the process were the sulfuric acid concentration, hydrolysis time and temperature, with a significant influence ($p < 0.05$). Infrared characterization results showed that nanocrystals were indeed isolated. With a crystallinity of 35.23 and 79.74, respectively, for Ayous wood fiber and nanocrystalline cellulose were observed by X-ray diffraction, with the formation of type II cellulose, thermodynamically more stable than native cellulose type I.

Keywords

Ayous Sawdust, Lignocellulosic Waste, Isolation, Cellulose Nanocrystals, Optimization, Response Surface Methodology

1. Introduction

Industrial timber production in Cameroon is characterized by increasing volumes of selective and extensive logging. *Triplochiton scleroxylon* (Ayous) is the most exploited species. It accounts for more than half of the total volume of the species most used to supply the domestic market [1] [2].

Local processing of this species is generally based on sawmilling, which generates a large volume of waste, not including wood that is felled and left in the forest. In 2012, the volume generated was estimated at 5.08 million·m^3 [3] [4].

Wood is a porous, heterogeneous material composed of natural fibers primarily made of polysaccharides [5]. These fibers, known as cellulosic fibers, are commonly used as reinforcement in manufacturing composite materials due to their abundant, bioavailable, and biodegradable nature [6].

Cellulose is a semi-crystalline polymer found in plant fibers in the form of fibril bundles consisting of a cluster of cellulose microfibrils composed of crystalline parts and amorphous zones. Crystalline cellulose regions can be isolated as single crystals known as whiskers, nanowhiskers or simply nanocrystals. In addition, different morphologies can be obtained depending on the method used and the duration of the treatment [7].

Cellulose nanocrystals (CNCs), are unique nanomaterials due to their mechanical, optical, chemical and rheological properties. They are finding new applications in regenerative medicine, optics, composite films, hydrogels, coatings, pharmaceuticals and food packaging [8].

CNCs have been extracted from a wide range of natural fibers, such as those derived from cotton, ramie, and wood. These extractions, are influenced by several parameters such as the type of tannin extraction solvent, acid concentration, hydrolysis temperature, time, type of bleaching agent, etc. [9].

Acid hydrolysis is the best-known chemical process for obtaining and isolating CNC. The protons supplied by the acid diffuse into the fibers and react with the disordered parts of the cellulose fibrils, breaking the glycosidic bonds. However, since the crystalline zones are more resistant, their delayed degradation allows only the crystalline parts to be isolated. The acid cleaves the cellulose chains at the glycosidic bond, gradually reducing the degree of cellulose polymerization until CNCs are obtained [10].

However, the isolation of cellulose nanocrystals requires a large amount of microcrystalline cellulose, a large amount of acid, and an extremely long isolation time [11]. Thus, the nature of the source, the nature of the acid, its concentration, the temperature of the mixture, the duration of the reaction, and the type of stirring of the reaction medium could have a direct relationship with the nature of the CNCs isolated by acid hydrolysis.

Nanocrystal isolation can be evaluated through experimental design, so that isolation can be predicted by modelling, interactions between factors can be studied, and optimal conditions for cellulose nanocrystal isolation can be determined. The principles of design and experimentation allow us to study parame-

ter influences, interactions, and confounding effects on the desired response. The design of experiments method is more effective than the usual approaches to conducting tests because it allows more precise expected results to be obtained with a reduced number of tests [12] [13].

In this sense, the response surface methodology was used to optimize the isolation of Ayous sawdust nanocrystals. The aim of the present work is to determine the degree of influence of the extraction parameters and to search for the optimal conditions for the isolation of nanocrystalline cellulose from sawdust (Ayous). More specifically: to search for the experimental ranges of each factor; to determine the levels of influence of the factors and to search for the optimal conditions for isolation of cellulose nanocrystals.

2. Materials and Methods

2.1. Materials

The Ayous sawdust used in this study was sampled in January 2023 at a sawmill located in the city of Ngaoundere with GPS coordinates latitude: 7.33/longitude: 13.58, in the department of Vina, Adamawa region. Ayous, whose scientific name is Triplochiton scleroxylon, belongs to the Sterculiaceae family. Ethanol, sodium chlorite, sodium pellets, sulfuric acid and toluene, were obtained from Sigma Aldrich. All chemicals purchased were used as received without further purification.

2.2. Experimental Methods

2.2.1. Preparation of Isolated Cellulose Nanocrystals from Ayous Sawdust

The method previously reported by [14] and [15], was used to isolate cellulose nanocrystals by multi-step extraction process with a few modifications. Firstly, the sawdust from Ayous wood with a particle size ≤ 500 μm was previously washed with distilled water and dried at 105˚C in the oven for 24 hours. After that, the waxes, resins, fats and tannins were removed by soxhlet extraction with ethanol/toluene solvent 1:2 (v/v). Then, the extraction was carried out after six hours and then the hemicelluloses were eliminated with 20% (w/v) of NaOH at room temperature under magnetic agitation for 24 hours. After filtration, the sample was washed with distilled water to remove any trace of NaOH and dried in the oven at 60˚C for 24 hours. In addition, the lignin is eliminated with 10% (v/v) sodium chlorite in reflux heating for 2 hours at pH 4. Then the sample was treated with a mixture of 18% NaOH solution—H_2O_2, 2:1 (v/v), at 40˚C to remove the lignin residues still present in the cellulose. After this washing step, cellulose microcrystals are obtained.

In the second step of the extraction, a mass M_1 g of microcrystalline cellulose (MCC) extracted from Ayous wood was dispersed in 40 mL of distilled water in an Erlenmeyer flask, then sulfuric acid (H_2SO_4) of 98% purity was added to the mixture and heated and kept under magnetic stirring for some time. After acid hydrolysis, the mixture was stirred by sonication and washed several times with

distilled water by centrifugation to separate the crystalline from the amorphous part dissolved in the water-acid mixture [16].

2.2.2. Determination of the Individual Influence of the Factors on the Isolation Yield of the Cellulose Nanocrystals and Definition of the Field of Study

It has been reported in the literature that several factors can influence the isolation of cellulose nanocrystals by acid hydrolysis [17] [18] [19]. A study of the individual influence of the factors on the isolation performance of nanocrystals was first carried out to determine the low (−1) and high (+1) values of the factors and to define the scope of the study. Five factors were identified as the main factors influencing the isolation of cellulose nanocrystals (CNC) obtained as an aqueous suspension during hydrolysis with sulfuric acid: amount of cellulose (2 - 15 g), sulfuric acid concentration (20% - 80%), hydrolysis time (5 - 120 min), hydrolysis temperature (30˚C - 100˚C), and sonication time (5 - 30 min). For the purpose of this study, the factor constants were: cellulose amount 5 g; hydrolysis concentration 50%; hydrolysis time 60 min; hydrolysis temperature 70˚C and sonication time 15 min.

2.3. Experimental Design

2.3.1. Optimization Study Using Response Surface Methodology

Response surface methodology was used to model the effect of various factors and interactions between factors on the isolation process of cellulose nanocrystals from Ayous wood. The study focused on the influence of the following factors: cellulose amount (X_1), acid concentration (X_2), hydrolysis time (X_3), temperature (X_4), and sonication time (X_5) on the isolation of cellulose nanocrystals. The response monitored during acid hydrolysis for cellulose nanocrystal isolation was the yield expressed as a percentage according to Equation (1). Yield (%)

$$\text{Yield}(\%) = \frac{M_1}{M_0} \times 100 \tag{1}$$

where: M_0: initial mass of microcrystalline cellulose; M_1: mass of cellulose after hydrolysis with sulfuric acid.

In order to better study the influence of factors on the isolation yield of cellulose nanocrystals, the Centered Composite Plane method was used here. It consists of three parts, with a total of 48 tests distributed as follows: A factorial design with five factors at two levels (32 trials), six experimental points located in the center of the study area (6 trials), and axial points ($+\alpha$ and $-\alpha$) (10 trials), which are the experimental points located on the axes of each of the factors and constitute the star design. To meet the criterion of near orthogonality, the $-\alpha$ and $+\alpha$ values for each factor were calculated using the following formula.

$$\alpha = \left[\frac{N_f \left(\sqrt{N} - \sqrt{N_f} \right)^2}{4} \right]^{\frac{1}{4}} \tag{2}$$

where: $N = 2k + 2k + N_0$; k = number of factors; $N_f = 2k$ = number of trials in the factorial design; N = total number of trials. This equation gives a distance $\alpha = 1.90$ between the star points. Calculations were also performed to switch from coded to real variables using Equation (3).

$$X_i = \frac{x - \left[x_{\max} + x_{\min}\right]/2}{\left[x_{\max} - x_{\min}\right]/2} \tag{3}$$

where X_i is the coded variable, x is the natural variable and $x_{\max}$ and $x_{\min}$ are the maximum and minimum values of the natural variable.

2.3.2. Suggested Model

Isolation yield was taken as the response (Y_{yield}) and a second-degree polynomial model was postulated for five variables. The model had the following form:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j + \beta_{ijk} x_i x_j x_k + \varepsilon \tag{4}$$

where; Y_i is the predicted response, β_0 is the constant, β_1, β_2 and β_3 are the linear coefficients (β_i), β_{11}, β_{22} and β_{33} are the squared coefficients (β_{ii}), β_{12}, β_{13}, β_{23} are the interaction coefficients (β_{ij}), and X_1, X_2, X_3; X_1X_2, X_1X_3, X_2X_3 and X_{12}, X_{22}, X_{32}: are the levels of the independent variables (X_i, X_iX_j and X_{i2}). ε is the associated error. ANOVA was used to model the system represented by independent parameters and dependent output response and to optimize the system by estimating the statistical parameters.

2.3.3. Model Validation

In order to equate the observed phenomenon and predict Y_{yield} responses in the domain defined for the study, it was important to validate the empirical models obtained. To this end, model performance was measured by comparing predicted and observed response values. In addition to the linear regression coefficient (R^2).

$$R^2 = \frac{\sum_{i=0}^{p}\left(Y_{i\,theo} - \overline{Y_{i\,exp}}\right)^2}{\sum_{i=0}^{p}\left(Y_{i\,exp} - \overline{Y_{i\,exp}}\right)^2} \tag{5}$$

Other mathematical procedures and tools were used to validate the mathematical model. One such tool is the absolute average deviation (AAD) analysis, which provides information on the average error of laboratory manipulations. The AAD is calculated by the following equation:

$$\text{AAD} = \frac{\sum_{i=1}^{p}\left(\frac{\left|Y_{i\,exp} - Y_{i\,theo}\right|}{Y_{i\,exp}}\right)}{p} \tag{6}$$

With: $Y_{i\,exp}$ the experimental value for trial i; $Y_{i\,theo}$ the theoretical value calculated from the model equation for trial i; p the total number of experiments. Each experiment was carried out in triplicate. Statgraphics plus, Origin 9 and Sigmaplot software were used to analyze the obtained data.

2.4. Characterization of Samples

The extracts obtained from Ayous sawdust, under the optimum preparation condition of microcrystalline cellulose (MCC) and cellulose nanocrystals (CNC), both were characterized by Fourier transform infrared (FT–IR) and X–ray diffraction (XRD). The Fourier transform infrared spectroscopy used was the Thermo Scientific Nicolet 6700. The measurements were carried out in transmission between 400 and 4000 cm^{-1} on powders of microcrystalline and nanocrystals of dried cellulose. Wide angle X–ray diffraction patterns of microcrystalline and nanocrystals cellulose were obtained using a PANalytical X'pert MPD-PRO diffractometer, equipped with a secondary monochromator, a changer for 3 × 15 samples and an X'celerator detector with an angular range of $2\theta = 8^\circ$ - 80°. The Cu–Kα X–ray source is generated at 45 kV and 40 mA (λ = 0.15188 nm). The crystallinity index (C_rI) of sample, is obtained by carrying out the ratio of diffraction patterns according to the following equation.

$$C_rI(\%) = \frac{\text{Total area} - \text{Amorphous part area}}{\text{Total area}} \times 100 \quad (7)$$

3. Results and Discussion

3.1. Effect of Experimental Parameters on the Isolation Yield of the Cellulose Nanocrystals

Study of the Effect of Microcrystalline Cellulose Content on Isolation Performance

Figure 1(a) shows a significant decrease in microcrystalline cellulose (MCC) content at a threshold of 5%. At low masses, such as 2 g, the cellulose is completely destroyed. This is due to the destruction of the amorphous phase by sulfuric acid, which reacts with the glycosidic bonds of the cellulose chains, reducing the degree of polymerization of the cellulose and increasing the degree of crystallinity until CNC is obtained. This increase was facilitated by the conversion of certain surface hydroxyl groups into sulfate groups [20]. On the other hand, a constant plateau is observed for amounts of 10 to 15 g of cellulose. Equilibrium is reached for cellulose levels ≥ 10 g. Taking into account other factors, the experimental range for cellulose is: [5 - 10] g.

3.2. Effect of Sulfuric Acid Concentration on Isolation Yield

Figure 1(b) shows a significant decrease in cellulose content at the 5% threshold with increasing acid. The protons supplied by the acid diffuse into the fibers and react preferentially with the amorphous parts of the cellulose fiber, breaking the glycosidic bonds and facilitating the formation of cellulose nanocrystals (CNC), whose degradation is slower due to their crystallinity. This would be due to the adsorption of sulfate groups present in the solution, allowing a good interaction with water and a better dispersion of the CNC in solution, phenomenon shown in **Figure 2** [21]. It has been also observed that the higher the concentration, the greater the degradation of the fibers, leading to the destruction of the crystalline

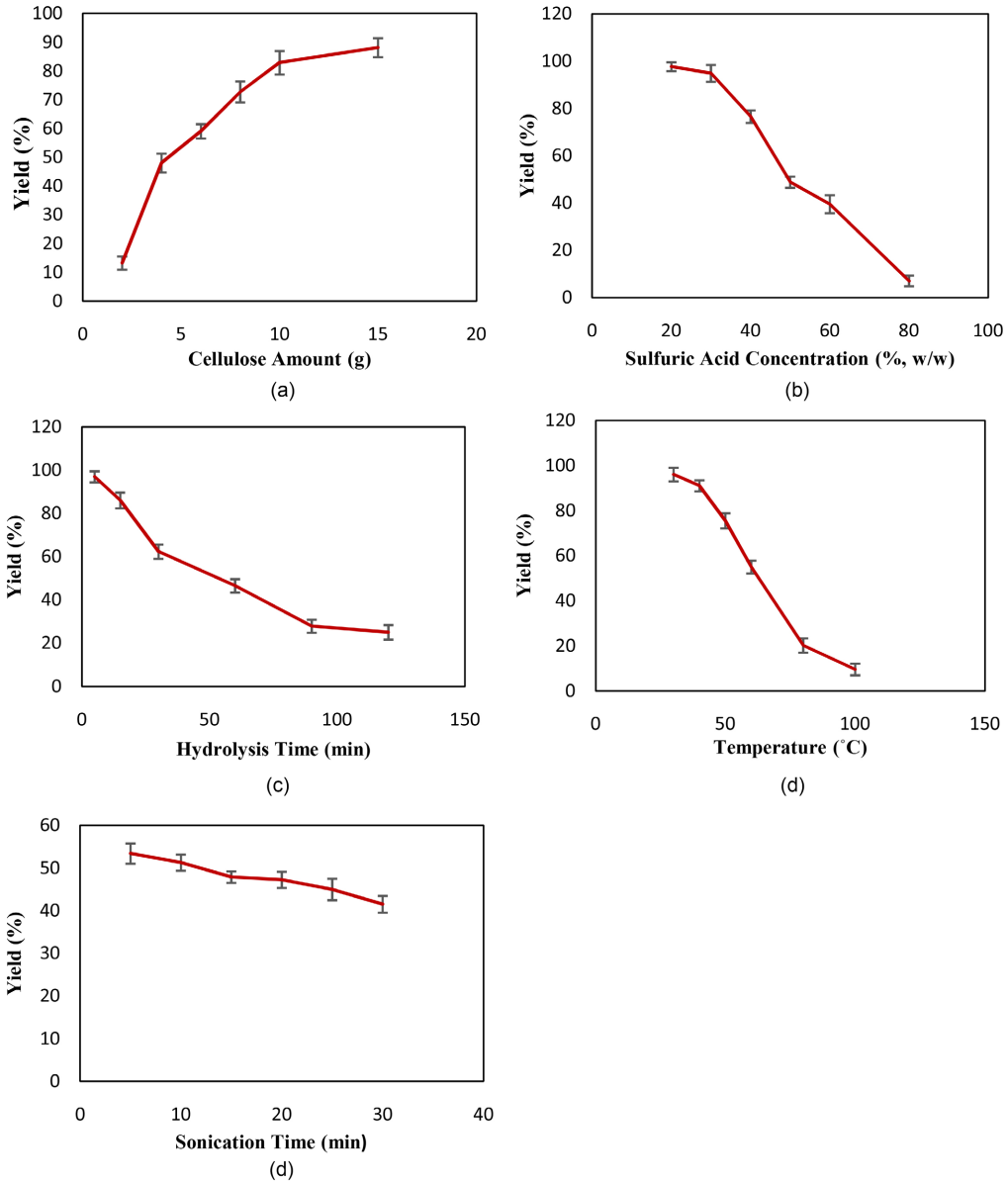

Figure 1. (a): Effect of cellulose amount on the isolation yield of cellulose; (b): Effect of sulfuric Acid concentration on the isolation yield of cellulose nanocrystal; (c): Effect of hydrolysis time on the isolation yield of cellulose nanocrystals; (d): Effect of temperature on isolation yield of cellulose nanocrystal; (e): Effect of sonication time on cellulose nanocrystal isolation yield.

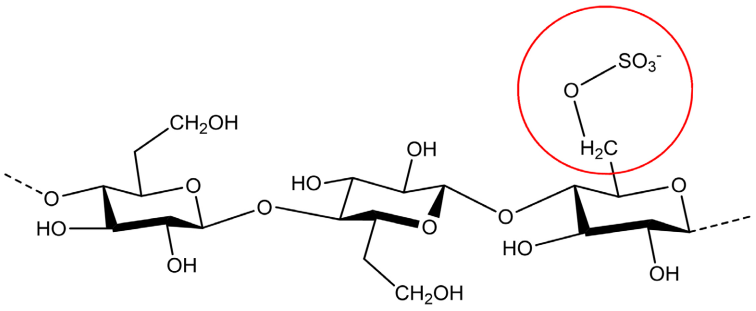

Figure 2. Cellulose chain surface functionalization.

part of the fibers. The range between [40 - 65]% (w/w), was chosen as the experimental range of sulfuric acid concentration for the study of the centered composite plane with response surface.

3.2.1. Effect of Hydrolysis Time on the Isolation Yield of Cellulose Nanocrystals

The CNC content decreases significantly at the 5% threshold as a function of hydrolysis time. Figure 1(c) shows a rapid decrease in isolation yield, the longer the contact time between acid and fiber, the lower the CNC isolation. However, between 80 and 120 minutes, a stabilization plateau is observed for cellulose nanocrystal yields of 20%. At this level, hydrolysis concentration and time no longer affect cellulose fiber degradation. On the other hand, in the same Figure for times between 5 - 10 min, little degradation of microcrystalline cellulose is observed. This may be due to the short contact time, which does not allow the acid to hydrolyze the amorphous parts of the cellulose. The hydrolysis time range thus selected for the composite plan was [10 - 90] min, during which a clear degradation of the cellulose fibrils was observed, with a constant yield plateau beginning to form after 90 min.

3.2.2. Effect of Temperature on the Isolation Performance of Cellulose Nanocrystals

Like hydrolysis time, hydrolysis temperature has a significant effect on the acid hydrolysis degradation of microcrystalline cellulose at the 5% threshold. Figure 1(d) shows that at low temperatures between 20˚C - 40˚C, no significant degradation of microcrystalline cellulose is observed during hydrolysis. However, when the temperature reaches 60˚C, a clear evolution of degradation is observed with a decrease in the yield of isolated particles, and if it is prolonged at temperatures above 90˚C, the cellulose is practically destroyed. Sulfuric acid hydrolysis is favored by temperature and accelerates the cellulose depolymerization process. Therefore, the hydrolysis temperature range chosen for the composite design was [40 - 80]˚C.

3.2.3. Effect of Sonication Time on the Isolation Yield of Cellulose Nanocrystals

The effect of sonication on the isolation of cellulose nanocrystals is shown in Figure 1(e). Nevertheless, a decrease in yield was observed after 5 - 30 min of sonication. In fact, during sonication, the partially hydrolyzed amorphous parts detach under the effect of vibration, thus reducing the isolation yield. The longer the sonication time, the higher the crystallinity of the cellulose will be, but the crystals obtained after sonication will be smaller in size. The selected sonication range for the composite plane was [10 - 30] min.

3.3. Model and Optimum Conditions for Cellulose Nanocrystal Isolation

The experimental range of factors and the corresponding orthogonal range are shown in Table 1. The range was obtained after preliminary tests on the individual

Table 1. Experimental area with orthogonal extensions.

Parameters	Notations	Experimental field				
		$-\alpha$	Low level (−1)	Midrange level (0)	High Level (+1)	$+\alpha$
Cellulose Amount (g)	X_1	2.76	5	7.50	10	12.24
Sulfuric Acide Conc % (w/w)	X_2	28.80	40	52.50	65	76.20
Hydrolyse Time (min)	X_3	10.00	10	50	90	125.85
Hydrolyse Temperature (˚C)	X_4	22.10	40	60	80	98.00
Sonication Time (min)	X_5	1.00	10	20	30	39.00

influence of hydrolysis factors. With, the lower levels noted ($-\alpha$; −1), the levels in the middle (0) and the upper levels ($+\alpha$; +1), where α corresponds to the orthogonal extensions.

The result of the evaluation of the influence of the factors on the isolation performance of CNCs is presented in Table 2. This table shows the decoded experimental centered composite design with the associated experimental and theoretical responses, as well as the calculated residuals. Residuals correspond to the portion of the response that is not explained by the highlighted effects. They are calculated for each trial by determining the difference between the measured response and the theoretical response. The sum of the residuals is always equal to zero, which makes it possible to check that there have been no errors in the calculations performed [22].

Table 2 also shows that the residuals, which represent the natural variance of the process and the variance of the fits between experimental and theoretical responses, have a weak, non-significant influence on the isolation performance of cellulose nanocrystals. Thus, uncontrolled factors such as hygrometry and ambient temperature do not affect the CNC isolation process.

3.3.1. Statistical Analysis and Model Fitting

The postulated model makes it possible to predict optimal isolation conditions and to determine the influence of factors on the isolation process of cellulose nanocrystals from Ayous sawdust. Maximum efficiency is preferred, but preliminary laboratory experiments have shown that values above 40% are not reasonable and values below 20% are not acceptable. STATGRAPHICS 16.1 software was used to propose a model that integrates the global and individual desirability function for the isolation performance of cellulose nanocrystals. The postulated model is represented by the Equation 5 below.

$$Y_{\text{yield}} = 18.7 - 0.06X_1 + 4.97X_2 + 3.45X_3 + 2.60X_4 - 0.40X_5 - 0.08X_2X_3 - 0.06X_2X_4 - 0.01X_2X_5 + 0.20X_1^2 - 0.01X_2^2 + 0.06X_5^2 \quad (8)$$

where, Y_{yield}, X_1, X_2, X_3, X_4, and X_5 are CNC yield, cellulose amount, sulfuric acid

Table 2. Experimental and theoretical responses of Centered composite level of acid hydrolysis for cellulose Nanocrystal isolation.

N°	Real Values of Factors					Cellulose Nanocrystal Isolation Yield		
	Amount of Cellulose	Sulfuric Acid Concentration	Hydrolysis Time	Hydrolysis Temperature	Sonication Time	Experimental responses	Theoretical Responses	Residues
	X_1 (g)	X_2 (%. w/w)	X_3 (min)	X_4 (˚C)	X_5 (min)	Y_{yield} (%)	Y_{yield} (%)	r (%)
1	10	65	90	40	10	61.22	64.49	−3.27
2	10	40	10	80	10	96.26	93.22	3.04
3	7.5	52.5	126	60	20	51.39	57.93	−6.54
4	5	65	10	40	10	90.10	84.57	5.53
5	5	40	90	40	30	89.01	92.11	−3.10
6	10	65	10	40	10	84.17	87.03	−2.86
7	5	40	10	80	10	94.58	91.40	3.18
8	7.5	52.5	10	60	20	62.02	67.69	−5.67
9	7.5	52.5	50	60	20	57.78	59.76	−1.98
10	7.5	52.5	50	60	20	59.17	59.76	−0.59
11	10	40	90	40	30	96.27	91.47	4.80
12	7.5	52.5	50	60	20	54.02	59.76	−5.74
13	5	40	90	40	10	95.34	91.47	3.87
14	5	65	90	40	10	62.34	61.00	1.34
15	10	65	10	40	30	77.19	81.84	−4.65
16	5	65	10	80	30	15.81	15.60	0.21
17	10	40	10	40	30	97.01	95.45	1.56
18	12.2	52.5	50	60	20	67.12	63.83	3.29
19	10	40	90	80	30	82.81	87.62	−4.81
20	10	65	10	80	30	12.76	13.74	−0.98
21	5	65	90	80	10	5.85	0.75	5.10
22	7.5	76.2	50	60	20	3.52	5.14	−1.62
23	7.5	52.5	50	22	20	90.47	88.56	1.91
24	10	65	10	80	10	27.08	20.91	6.17
25	5	65	10	40	30	88.26	83.30	4.96
26	5	40	10	80	30	94.10	88.23	5.87
27	7.5	52.5	50	60	1	81.25	85.94	−4.69
28	5	40	90	80	10	92.21	90.02	2.19
29	5	40	10	40	10	97.04	98.31	−1.27
30	7.5	52.5	50	60	39	76.32	79.75	−3.43

Continued

31	10	40	90	40	10	96.14	94.74	1.40
32	5	65	90	40	30	59.64	61.55	−1.91
33	7.5	28.8	50	60	20	96.19	102.68	−6.49
34	10	40	10	80	30	85.00	86.14	−1.14
35	7.5	52.5	50	97.9	20	12.58	22.61	−10.03
36	7.5	52.5	50	60	20	66.81	59.76	7.05
37	10	65	90	80	10	2.48	3.83	−1.35
38	5	40	10	40	30	96.31	97.13	−0.82
39	10	65	90	40	30	61.31	61.13	0.18
40	10	65	90	80	30	3.25	−1.52	4.77
41	10	40	90	80	10	93.17	92.88	0.29
42	5	40	90	80	30	95.31	88.67	6.64
43	7.5	52.5	50	60	20	67.07	59.76	7.31
44	5	65	90	80	30	1.12	−0.69	1.81
45	5	65	10	80	10	13.78	18.86	−5.08
46	10	40	10	40	10	98.04	100.54	−2.50
47	7.5	52.5	50	60	20	69.19	59.76	9.43
48	2.8	52.5	50	60	20	51.09	62.50	−11.41

concentration, hydrolysis time, hydrolysis temperature, and wash sonication time, respectively.

Table 3 shows the calculated coefficients of the model, including the degrees of freedom, probabilities (p-value), related to the postulated second order model. The lack-of-fit test was also performed to determine whether the postulated second-order model is adequate to describe the process of sulfuric acid isolation of cellulose nanocrystals. The probability of the adequacy test for the observed model was $p = 0.5505$ ($p > 0.05$). Statistical analysis showed that the response values would fit a second-degree polynomial model.

In addition, the significance of the effects on isolation performance was determined by comparing the root mean square of each effect to an estimate of the experimental error to assess the significance of the factors studied on the observed response. Effects with a probability of less than 0.05 are considered significant at the 95% confidence level. It can be seen that the main factors: sulfuric acid concentration (X_2), hydrolysis time (X_3), hydrolysis temperature (X_4), have a direct linear effect with a significant contribution at the 95% confidence level on the isolation performance of cellulose nanocrystals.

On the other hand, the main effects of cellulose amount (X_1) and sonication time (X_5) do not have a significant effect on the desired response. However, a

Table 3. Analysis of the variance (ANOVA), significance of different effects.

	Y_{yield}			
	Coefficient	**Degree of freedom**	**F-value**	**p-Value**
X_1: Amount of Cellulose	−0.0622	1	0.13	0.7255
X_2: Sulfuric acide Concentration	**4.9715**	1	675.83	**0.0000**
X_3: Hydrolysis Time	**3.4497**	1	27.7	**0.0000**
X_4: Hydrolysis Temperature	**2.5453**	1	308.96	**0.0000**
X_5: Sonication Time	−0.3670	1	2.72	0.1105
X_1^2	0.1515	1	0.6	0.4464
X_1X_2	0.0018	1	0	0.9603
X_1X_3	0.0026	1	0.06	0.8145
X_1X_4	−0.0020	1	0.01	0.9266
X_1X_5	−0.0031	1	0.8	0.3798
X_2^2	−0.0104	1	1.76	0.1957
X_2X_3	**−0.0842**	1	14.6	**0.0007**
X_2X_4	**−0.0588**	1	180.25	**0.0000**
X_3^2	0.0015	1	2.49	0.1260
X_3X_4	0.0017	1	1.55	0.2232
X_4^2	−0.0029	1	0.9	0.3517
X_5^2	**0.0642**	1	27.45	**0.0000**
Lack-of-Fit Test	-	**22**	**1.02**	**0.5505**
Pure error	-	**5**	**-**	**-**

significant quadratic effect of sonication time (X_5^2) on cellulose isolation performance was observed. Significant second-order factor interactions are also observed: sulfuric acid concentration/hydrolysis time interaction (X_2X_3) and sulfuric acid concentration/hydrolysis temperature interaction (X_2X_4), whose contributions are significant at the 95% confidence level.

Table 4 below gives the criterions of evaluation and validation of the postulated model. The analyses of variance give regression coefficient (R^2), adjusted regression coefficient (adjusted R^2) and absolute average deviation analysis (AAD). According to [22] and [23], a model can be valid if the model explains at least 80% of the variability of the response expressed by the (adjusted R^2). Similarly, [24], considered that a model is valid if the AAD is between 0 and 0.3, which provides information on the average error of laboratory manipulations. In addition, if the P-value of model, it's less than 0.0001 also indicates that the model terms are significant.

Table 4. Criterion of evaluation of the model.

Validation Indicator	Y_{yield}	Reference Value
R^2	97.88%	≥95%
Adjusted R^2	96.31%	≥80%
AAD	0.037	$0 \leq AAD \leq 0.3$

The regression coefficient $R^2 = 0.9788$ of model, is close to 1, it's indicating that the postulated model explains 97.88% of the variability in cellulose nanocrystal isolation yield, for 2.12% of unexplained effects. The thus postulated model appears to be adequate for the observed data at the 95.0% confidence level, with an adjusted R^2 of the model (96.31% > 80%), which allows the postulated model to be considered valid.

3.3.2. Graphic Analysis

The Pareto chart displays each of the estimated effects in descending order of importance. The Pareto plot can be used to examine the individual effects of different parameters and their interactions. **Figure 3** shows the Pareto chart, which ranks of the absolute values of the coefficients in descending order. In this diagram, standardized effects below 2/30% are irrelevant, while effects above 2/30% have a significant influence on the isolation performance of cellulose nanocrystals. The diagram shows that the main factors X_2, X_3, X_4 have a significant influence on the isolation performance of cellulose nanocrystals with a negative percentage contribution of 26/30%, 17.5/30% and 5.5/30%, respectively. This reflects a destruction of the amorphous part of the cellulose, thus facilitating the isolation of the crystalline part of the cellulose.

However, this graph also shows that the amount of cellulose (X_1) and the sonication time (X_5) do not affect the isolation yield of CNCs and have a non-significant contribution to the hydrolysis. However, the quadratic effect of sonication time is significant with a percentage contribution of 5.5/30%, and the positive contribution effect of this exponential increase in sonication time facilitates the elimination of amorphous parts of microcrystalline cellulose that dissolve in the acid, allowing the colloidal formation of cellulose nanocrystals.

On the other hand, the interactions of the main effects X_2X_3 and X_2X_4 have a significant influence, with a negative percentage contribution effect on the isolation on CNC, 4/30% for X_2X_3 and 14.5/30% for X_2X_4, respectively. The objective, which was to degrade the amorphous zones as much as possible without affecting more the crystalline part of the extracted microcrystalline cellulose, can be seen on the response surface and isoresponse curves in **Figure 4**.

Increasing the sulfuric acid concentration and the hydrolysis time reduces the size of the microcrystalline cellulose and favors the isolation of CNC. On the other hand, when the sulfuric acid concentration is low, between 30% - 40%, the acid concentration has no effect on the degradation of the amorphous zones of microcrystalline cellulose **Figure 4** with the factors cellulose amount, hydrolysis

temperature and sonication time held constant. It also observes that the interaction of concentration and hydrolysis time led to an increased degradation of the amorphous part, facilitating the isolation of nanocrystals. An increase in isolation yield was also observed when the degrading power of the acid was coupled to the hydrolysis temperature Figure 5 for temperatures above 70˚C, hydrolysis was possible with sometimes complete degradation of microcrystalline cellulose at high acid concentrations. Thus, the esterification of hydroxyl groups by sulfate ions is highly dependent on processing conditions.

Therefore, it would be possible for CNCs to be completely degraded by oxidation in a highly acidic environment at high temperatures. As the sulfuric acid concentration, hydrolysis time, and temperature were increased, more and more cellulose degradation was observed, reducing the possibility of having a good content of cellulose nanocrystals. To optimize the isolation of cellulose nanocrystals (CNC), it would be advisable to work under moderate, controlled conditions.

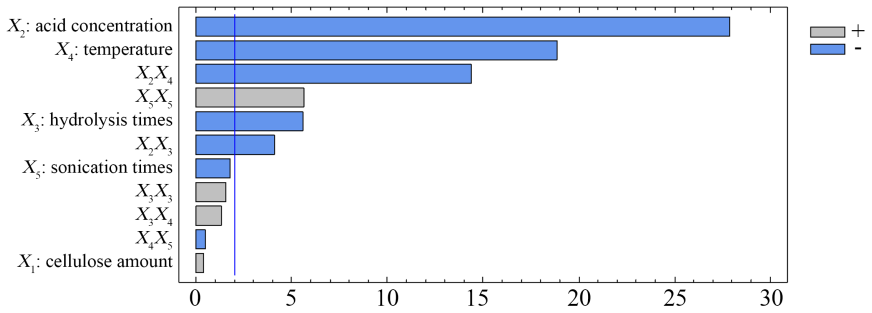

Figure 3. Contribution of model factors.

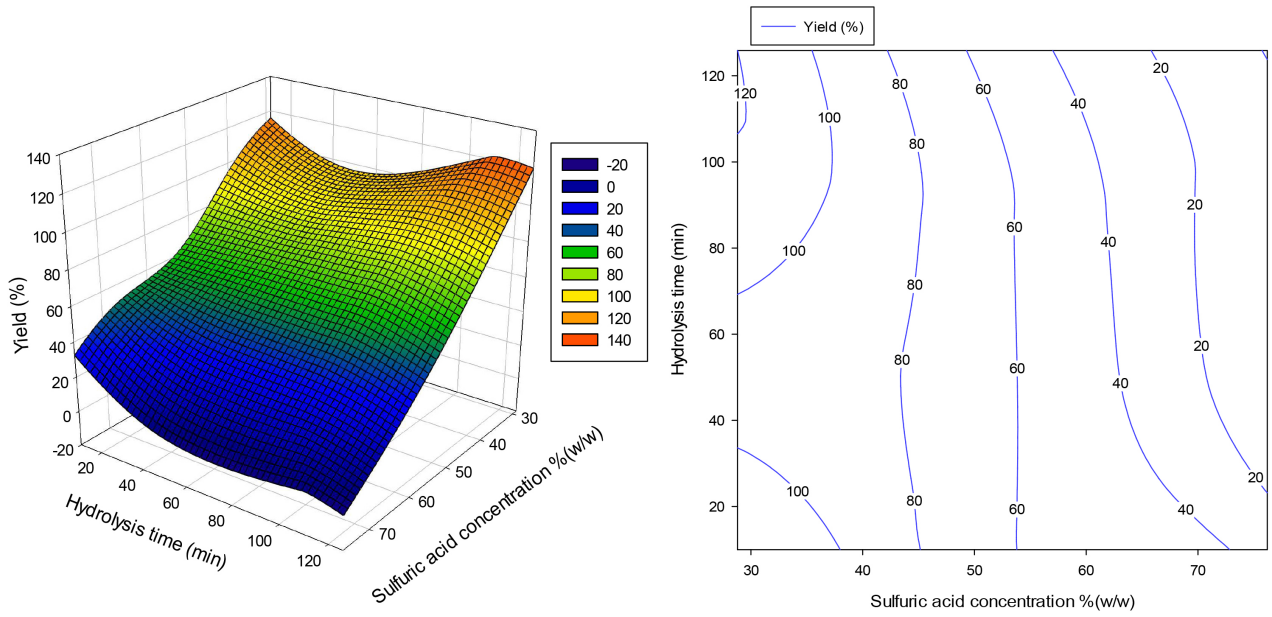

Figure 4. Response surfaces and isoresponse curves for CNC isolation of Interaction sulfuric acid concentration and time of hydrolysis.

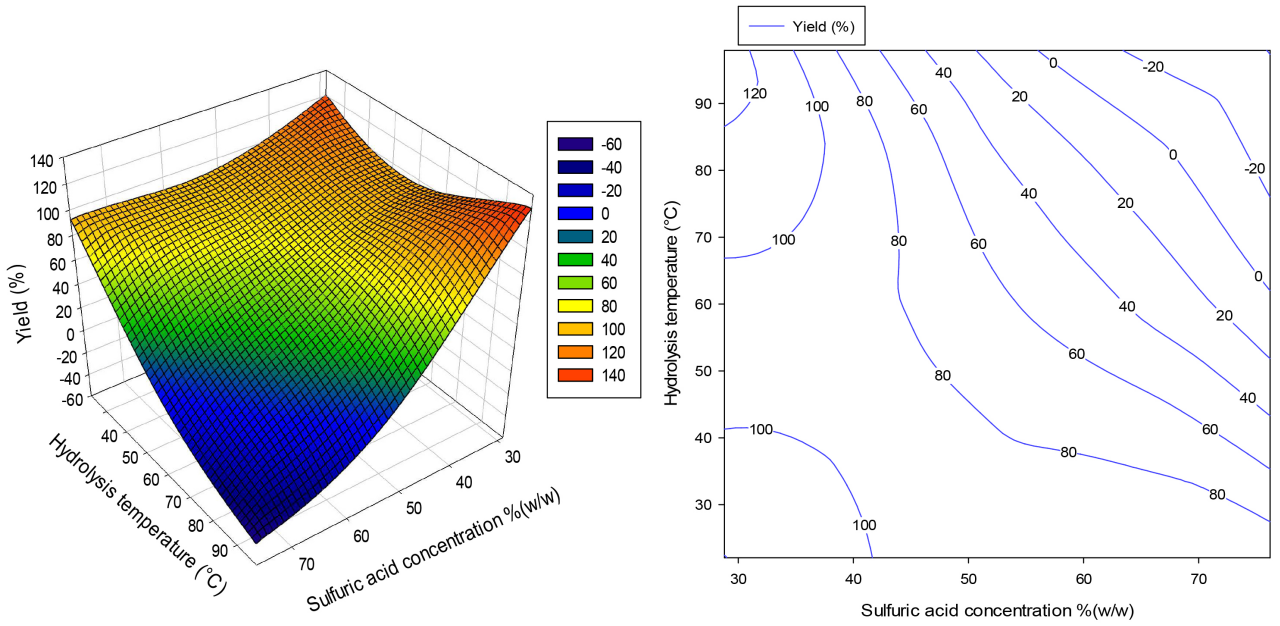

Figure 5. Response surfaces and isoresponse curves for CNC isolation of Interaction sulfuric acid concentration and hydrolysis concentration.

3.3.3. Verification of the Postulated Model

In order to verify the isolation efficiency of nanocrystals under optimal conditions (reduced acid concentration, short relative hydrolysis time and non-aggressive temperature), laboratory tests were conducted to validate the optimal isolation conditions according to the desirability factor (d = 40%). The experimental tests were repeated three times to obtain an average value of the experimental optimum in real values, which was compared with the calculated theoretical optimum. The results of the optimization of cellulose nanocrystal isolation by acid hydrolysis are shown in Table 5.

3.4. Characterisation of Samples

3.4.1. FTIR Spectra Analysis of Ayous Sawdust and Nanocrystals of Cellulose

Figure 6 shows the FTIR spectrum of Ayous sawdust and nanocrystalline cellulose derived from it. Table 6 shows the different functional peaks found in the sawdust and nanocrystalline cellulose. For tropical wood materials, peaks in the 3000 - 3600 cm^{-1} region represent stretching vibrations (ν) of OH functions, and peaks between 2750 - 3000 cm^{-1} represent water adsorption. The band at 1733 cm^{-1} represents the vibration due to C=O stretching of acetyl ester groups or ester linkage of hemicellulose carboxyl groups [25] [26].

However, this characteristic hemicellulose peak is not present in nanocrystalline cellulose because the latter is obtained after a multiple extraction that removes lignin, hemicelluloses, and the amorphous portion of microcrystalline cellulose. The band at 1508 cm^{-1}, related to aromatic C=C stretching, and the band at 1226 cm^{-1}, related to the vibration of the C–O–C bonds of aryl-alkyl ethers, were attributed to the carboxylic and ether groups of lignin. The peaks

present between 1421 - 1030 cm^{-1} in Ayous sawdust disappear completely in the spectrum of nanocrystalline cellulose, due to the different acid and alkaline extractions that the sawdust has undergone [27] [28].

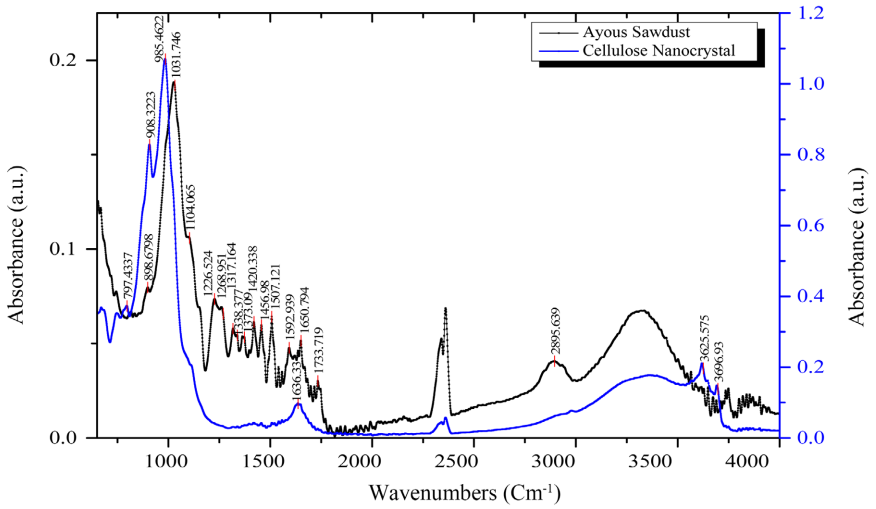

Figure 6. FTIR spectra of raw material and cellulose nanocrystal.

Table 5. Optimum conditions.

Factors	Values					Responses	
	X_1 (g)	X_2 (% w/w)	X_3 (min)	X_4 (°C)	X_5 (min)	Calculated (%)	Trials (%)
Experimental Conditions	5.80	63.50	53	69	19	40	37.84 ± 0.99

Cellulose amount (X_1); Acid concentration (X_2); Hydrolysis time (X_3); Hydrolysis temperature (X_4); Sonication time (X_5).

Table 6. The typical functional groups.

Accounted Band for the Absorption	Wavenumbers (Cm^{-1})	
	Ayous Sawdust	CNC
O–H Stretching Intramolecular Hydrogen Bond	3600 - 3200	3600 - 3200
C–H stretching	2891	/
C=O Stretching of the Carbonyl and Acetyl Groups	1733	/
O–H Bending of Water	1642	1648
C=C Stretching Vibration Aromatic of Lignin	1508	/
–C–O–C stretching	1456	1453
C–H Asymmetric Deformation	1421	1416
C–H Bending	1373	/
–C–O–C Aryl-Alkyl Ether	1226	/
C–O–C Glycoside Ether Band of Hemicellulose	1156	/
C–O–C Pyranose Ring	1031	986
β–Glycosidic	899	909

3.4.2. XRD Spectra Analysis of Raw Material (Ayous Sawdust) and Nanocrystals of Cellulose (CNC)

Figure 7 shows the XRD spectra of Ayous sawdust and extracted nanocrystalline cellulose. In this figure, two significant peaks clearly appear around $2\theta = 16.07^{\circ}$ and 22.02˚, which represent the characteristic peaks of native type I cellulose for the Ayous sawdust spectrum. However, very few differences have been observed between the native celluloses of certain botanical sources such as: flax, hemp, cotton and wood Fibers [29] [30].

The spectrum of nanocrystalline cellulose is obtained after a sequential multi-stage extraction that removes the various components of the wood fiber: tannins, hemicelluloses, lignin and the amorphous parts of microcrystalline cellulose, with enhancement of the crystallinity of type II cellulose. In the XRD spectrum of nanocrystalline cellulose, several peaks are observed at $2\theta = 12.02^{\circ}$, 20.08˚, 22.02˚ and 34.97˚ whose crystallographic planes are: (101), $(10\bar{1})$, (200) and (004).

The crystallinity indices of amorphous sawdust and nanocrystalline cellulose are shown in Table 7. These indices clearly show that the crystallinity of the material increased significantly after removal of hemicelluloses, lignin and amorphous cellulose by sequential chemical extraction. These results are in agreement with the observations of [14] [29].

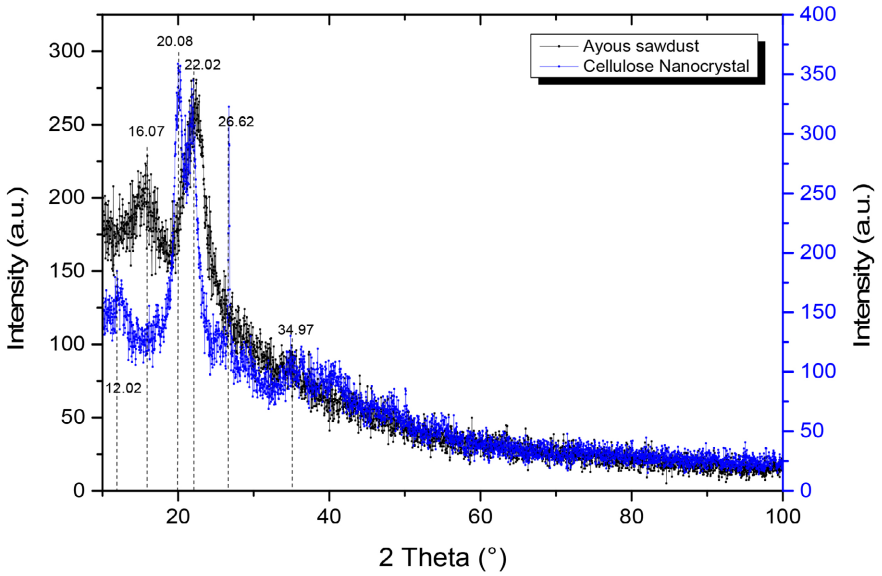

Figure 7. XRD spectra of raw material and cellulose nanocrystal.

Table 7. Crystallinity index of Ayous sawdust and Cellulose nanocrystal.

Samples	2θ (amorphous) (˚)	2θ (crystalline) (˚)	CrI (%)
Ayous Sawdust	16.07	22.02	35.23
CNC	12.02	20.08	79.74

4. Conclusion

In this work, the isolation of cellulose nanocrystals (CNC) was optimized by experimental design to predict the model using response surface methodology. The optimum was obtained for the following conditions: 5.8 g microcrystalline cellulose, 59% (w/w) of sulfuric acid concentration, 53 minutes hydrolysis time, 69˚C hydrolysis temperature and 19 minutes sonication time, for a calculated yield of 20% and an experimental three-trial yield of (37.84 ± 0.99)%, were isolated. Under the significant influence of the main parameter's acid concentration, hydrolysis time and hydrolysis temperature on the isolation of cellulose nanocrystals. Similarly, the interactions of acid concentration/hydrolysis time and acid concentration/hydrolysis temperature had a significant influence on the isolation of cellulose nanocrystals. The protons supplied by the sulfuric acid diffuse inside the fibers and react preferentially with the amorphous parts of the cellulose, which are more accessible, breaking the glycosidic bonds. The crystalline zones, on the other hand, are more resistant and their degradation is delayed, allowing the isolation of type II CNC, which is thermodynamically more stable than native type I cellulose, with a crystallinity of 79.74% observed by X-ray diffraction.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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