

Study of steam explosion pretreatment and preservation methods of commercial cellulose

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### Highlights

- Steam explosion is a promising pre-treatment for compact cellulose
- Treatment at 150°C reduced crystallinity, but at 200°C favoured recrystallization
- Despite polymerization reduction, steam explosion reduced enzymatic accessibility
- Acetone counteracted crystallinity and accessibility changes and reduced viscosity
- Freezing caused severe degradative effects in accessibility and crystallinity

**Abstract**

Steam explosion (150 – 200 °C, 5 – 30 min) was performed on a commercial cellulose presented in two configurations (fiberized and compact sheet) and its effect on their chemical and physical properties was studied, along with the influence of two different preservation methods (acetone drying and freezing) after pretreatment. No degradation compounds were produced during pretreatment, although solid recovery ( $R_s$ ) decreased with temperature from 90% to 62%. Similar particle size and surface conditions (increased porosity) were found for both types of pretreated samples despite the extremely different initial configuration. Crystallinity diminished for 150 °C samples, but 200 °C pretreatment promoted recrystallization. Pretreatment also reduced polymerization degree, although enzymatic accessibility did not improve. Both acetone and freezing processes extremely affected cellulose properties. Acetone drying counterbalanced crystallinity and enzymatic accessibility variations of pretreated samples, while decreasing polymerization degree to 302. Freezing dramatically decreased enzymatic accessibility of pretreated samples down to 15.8%.

**Keywords**

Natural fiber, crystallinity, enzymatic hydrolysis, degree of polymerization, acetone drying, freezing.

## 1. Introduction

Fossil sources depletion has increased prices of petrol-derived polymers and natural fibers are starting to be considered a suitable alternative due to their easy processing and eco-friendly advantages. Significant amounts of natural fibers are currently considered a waste, and their disposal creates harmful environmental problems (Oliveira et al., 2013), so they can be a promising renewable raw material due to their abundance and low cost. Among the new alternative uses, polymeric materials based on natural fibers are emerging in the last decades for numerous diary and important purposes, such in biomedical or automotive industries (Thakur & Thakur, 2014).

Natural fibers are a well-known source of cellulose, since it is its principal component ( $\geq 60\%$ ). Usually, chemical methods are used to produce cellulose rich pulp, which is commercialized as roll-sheets of compacted fibers, being transformed back into fibers by mechanical processes such as carding.

A pretreatment like steam explosion could be a useful step in cellulose production processes for both substituting physical opening of cellulose rolls and improving cellulose fibers versatility, opening its structure and increasing its porosity, reactivity and specific surface, with minimum degradation. It is an innovative and effective pretreatment which has provided interesting results for lignocellulosic biomass. It consists in applying high-pressure saturated steam to biomass for short times, followed by a sudden decompression. Its advantages over chemical methods are low environmental impact and capital investment, energy efficiency, and the possibility of treating larger amounts of biomass (Jacquet et al., 2012). Commonly, steam explosion processes take place at temperatures between 150 °C and 250 °C for times varying from 2 to 60 min. Severity factor (SF) of the process is calculated from these two variables by Eq. 1 (Jacquet et al., 2011):

$$SF = \log_{10} [t_{SE} * \exp((T-100)/14.75)] \quad (1)$$

Where  $t_{SE}$  is reaction time (min) and  $T$  is the steam temperature ( $^{\circ}\text{C}$ ). The steam breaks and modifies the structure and could even provoke the hydrolysis of the glycosidic bonds, producing organic acids due to shearing forces during decompression (Medina et al., 2016). The global effect of both processes involves modifications on physical and chemical properties of the biomass such as reactivity (related with specific surface area), viscosity or crystallinity among others (Sui & Chen, 2016), which can be beneficial for ulterior industrial uses of cellulose fibers. When applied to lignocellulosic materials, steam explosion treatment lead to opened structures, modification of physicochemical properties such as the specific surface area, depolymerization and solubilization of hemicellulose, and increase of cellulose enzymatic hydrolysis degree, assisting following valorization processes. (Medina et al., 2016; Neves, Pitarelo, & Ramos, 2016),

However, scarce information about its effects on commercial cellulose fibers could be found. (Jacquet et al., 2011) studied the effect of different conditions during the steam explosion on degradation of pure cellulose, finding degradation products as 5-hydroxymethyl furfural (HMF) for severity factors higher than 4.0. In their subsequent study, (Jacquet et al., 2012) reported no improvement on enzymatic hydrolysis rate after steam explosion. Nevertheless, they observed modification on physicochemical properties of cellulose fibers, such as an increase in water retention and crystallinity index values. Water retention after treatment might play a significant role not only during pretreatment but afterwards as well, even accelerating cellulose degradation (Sui & Chen, 2016).

(Yamashiki et al., 1990) used acetone instead of the traditional oven drying, to avoid drastic structural changes due to temperature. Nevertheless, they did not consider

that acetone might also have some other effects. After drying, samples are usually stored in -20 °C freezers, but the formation of ice crystallites might harm microfibrils structure. To the best of authors' knowledge, no study has been conducted for systematically evaluating the influence of steam explosion in compacted cellulose pulps or the influence of the preservation methods on physical properties.

The aim of this work was to study steam explosion as a pretreatment for commercial cellulose samples presented in two different handlings, to explore the possibilities of this treatment as a substitute for other mechanical pretreatments and its effects on the modification of several physicochemical properties of cellulose fibers regarding a future use. To that end, several analyses were performed such as chemical composition, particle size distribution, crystallinity rate, limiting viscosity number and degree of polymerization. In addition, two different preservation methods of pretreated samples (acetone washing and freezing) were tested to study the effects of preservation methods could have on the stability and quality of pretreated samples.

## **2. Materials and methods**

### *2.1. Raw material*

One bleached commercial cellulose was used in this work, obtained 100% from softwoods pulp, purified from lignin and hemicellulose and presented in two different handlings: Compacted Cellulose (CC) was provided as a compacted sheet and cut in small squared pieces (10x30mm approx.) for better manipulation. Fiberized Cellulose (FC) was obtained by mechanical fiberization of the sheet.

Chemical composition (moisture, cellulose, hemicellulose, lignin, extractives and ash content), particle size distribution, enzymatic hydrolysis kinetics, optical and electronic

scanning microscopy analysis, soluble impurities (short-chain organic acids, HMF, furfural, and phenols), crystallinity index and limiting viscosity number were analyzed.

## 2.2. Steam explosion experiments

Steam explosion experiments were conducted in a 5L stainless-steel reactor, connected on top to a steam generator. Reactor bottom discharged through an electrovalve to a flash vessel partially open to the atmosphere. The reactor was loaded with 50.0 g of cellulose, tightly closed and filled with saturated steam at the desired temperature and pressure. After reaching experimental conditions (this period was called “demand time”,  $t_d$ ), a countdown for the defined reaction time ( $t_{SE}$ ) started. Once it finished, the bottom electrovalve automatically opened, discharging the reactor content into the flash vessel. A set of four experiments was conducted for each sample, combination of two reaction temperatures (150 or 200 °C) and two reaction times (5 or 30min).

Resulting pretreated cellulose was vacuum-filtrated in a Buchner funnel to eliminate excess water, weighed and stored in plastic flasks at 4 °C before analysis. Samples of pretreatment liquid fraction were also stored for chemical analysis to determine possible solubilization and degradation due to pretreatment.

The term “Severity Factor” (SF), calculated by Eq. (1), defined the intensity of the pretreatment, and the term “Solids recovery” ( $R_s$ ) provided the mass losses due to treatment or manipulation, referred to the initial amount of raw material. Due to the configuration of the steam explosion system,  $t_d$  never exceeded 100 seconds, and it has not been considered for SF calculations. Solids recovery (w/w %) was calculated through the ratio of the recovered and initially loaded mass, both in dry-matter base.

Pretreated samples were analyzed for chemical composition, particle size distribution, enzymatic hydrolysis kinetics, optical microscopy, crystallinity index, limiting viscosity number, and SEM.

### *2.3. Preservation methods assays*

Two different preservation methods were evaluated: (1) acetone-drying step and (2) freezing at -20 °C.

For (1), samples were profusely washed with acetone after filtration. Then, acetone-washed cellulose was dried for 48h at room temperature and stored in plastic flasks. Chemical composition, enzymatic hydrolysis kinetics, optical microscopy, crystallinity index and limiting viscosity number were conducted to evaluate composition and structural changes caused by acetone wash.

For (2), pretreated samples were frozen at -20 °C for 24h after water removal by filtration. After that time, they were defrosted and analyzed (chemical composition, enzymatic hydrolysis kinetics, optical microscopy and crystallinity index).

### *2.4. Physicochemical characterization*

#### *2.4.1. Chemical characterization*

The chemical composition analysis procedures followed NREL (National Renewable Energy Laboratory – USA) analytical procedures (TP-510-42618). Total and volatile solids (TS/VS), moisture, water extractives and ashes content were determined by gravimetric analysis. Cellulose and hemicellulose content were determined by HPLC analysis of acid hydrolysis liquid phase of samples. A Bio-Rad HPX-87H ion-exclusion column installed in a Waters e2695 separation module equipped with Waters 2414 refractive index detector was used to quantify the concentration of glucose. The mobile phase of 0.025 M H<sub>2</sub>SO<sub>4</sub> was eluted at a flow ratio of 0.6 mL/min and 50 °C. CC samples

needed three successive acid hydrolysis for their correct characterization. Acid Soluble Lignin (ASL) was determined by spectrophotometric method and Acid Insoluble Lignin (AIL) gravimetrically after acid hydrolysis of the samples. All results correspond to the mean value of two measures (Travaini, Barrado, & Bolado, 2016).

Solubilized sugars and soluble impurities (HMF, furfural and short-chain organic acids) were determined by HPLC analysis of the liquid fraction of a cellulose-in-water suspension (at 1 g/L under continuous stirring for 24h), using the same conditions as previously indicated. Results correspond to the mean of two measures. Total phenolic content (TPC) were determined by spectrophotometric method at a wavelength of 765 nm according to an internal protocol of the Laboratory of Instrumental Analysis of University of Valladolid, based on the Folin reaction (Toquero & Bolado, 2014).

#### *2.4.2. Physical characterization*

Particle size distribution was determined in triplicate on cellulose-in-water suspensions by Dynamic Laser Scattering (DLS) using a Mastersizer 2000 (Malvern Instruments Ltd), with an initial obscuration of 5%. This instrument reports particle size as a volume equivalent sphere diameter. While this is not an absolute measure, given that the fibre thickness can be considered uniform, it gives a relative value about the effect of the pre-treatment on the fibre length. Percentiles  $dp_{10}$  (equal to the value below which 10% of the observations may be found) and  $dp_{90}$  (equal to the value below which 90% of the observations may be found) were obtained, as well as particle size of the peaks.

Optical microscopy was conducted with a Leica optical microscope at 100, 200 and 400 magnifications. Scanning Electron Microscopy was performed according to internal procedures, for magnifications from 70 to 30000.

Crystallinity index ( $I_{Cr}$ ) was studied by X-ray diffraction (angular interval from  $10^\circ$  to  $40^\circ$ , 0.05 of path).  $I_{Cr}$  was calculated through the ratio of intensities  $I_{am}$  (minimum intensity in  $2\theta$  between  $18^\circ$  and  $19^\circ$ ) and  $I_{002}$  (maximum intensity of the crystalline peak in  $2\theta$  between  $22^\circ$  and  $23^\circ$ ) (Buschle- Diller & Zeronian, 1992).

Limiting viscosity number ( $[\eta]$ ) was measured in triplicate according to ISO 5351:2012, using a Cannon-Fenske viscometer. Cellulose samples were dissolved in 0.5M cupriethylenediamine solution at 0.003 g/mL and agitated for 30 minutes before solution viscosity was recorded. The degree of polymerization (DP) of samples was estimated using Immergut's equation (Immergut, Ranby, & Mark, 1953).

$$DP^{0.905} = 0.805 * [\eta] \quad (2)$$

#### 2.4.3. Enzymatic hydrolysis kinetics

Enzymatic hydrolysis was conducted using a commercial enzymatic cocktail kindly provided by Novozymes©, with a dosage of 10 FPU/g (Celluclast 1.5L, cellulase from *Trichoderma reesei*) and 40 CBU/g (Novozyme 188), performed at  $50^\circ\text{C}$  using 3% (w/w dry-matter base) cellulose suspensions for 48h. pH was adjusted at 4.9 with 5mM sodium acetate buffer (Travaini, Barrado, & Bolado-Rodríguez, 2016). Experimental time courses of glucose release were tracked every 4 hours, and glucose concentration was measured by HPLC as reported. Considering prior work, these values were fitted according to a single hyperbola (Nidetzky, Steiner, Hayn, & Esterbauer, 1993):

$$X_G = at / (b+t) \quad (3)$$

where  $X_G$  is the degree of cellulose hydrolysis (% w/w of cellulose), a and b factors describing cellulose hydrolysis kinetics, and t is time (h). Criteria for selecting the model for curve fitting were the sum of least squares, mean square error, and normal distribution of residuals. Kinetic factors were obtained through curve fitting of cellulose

conversion vs time values, and two enzymatic hydrolysis kinetic parameters were defined: Accessibility ( $a$ ) (% w/w of cellulose) was directly defined from Eq. 3; and a parameter describing reaction kinetics “ $k$ ” ( $\text{h}^{-1}$ ) was defined as the inverse value of factor “ $b$ ” ( $k = 1/b$ ).

### 3. Results and discussion

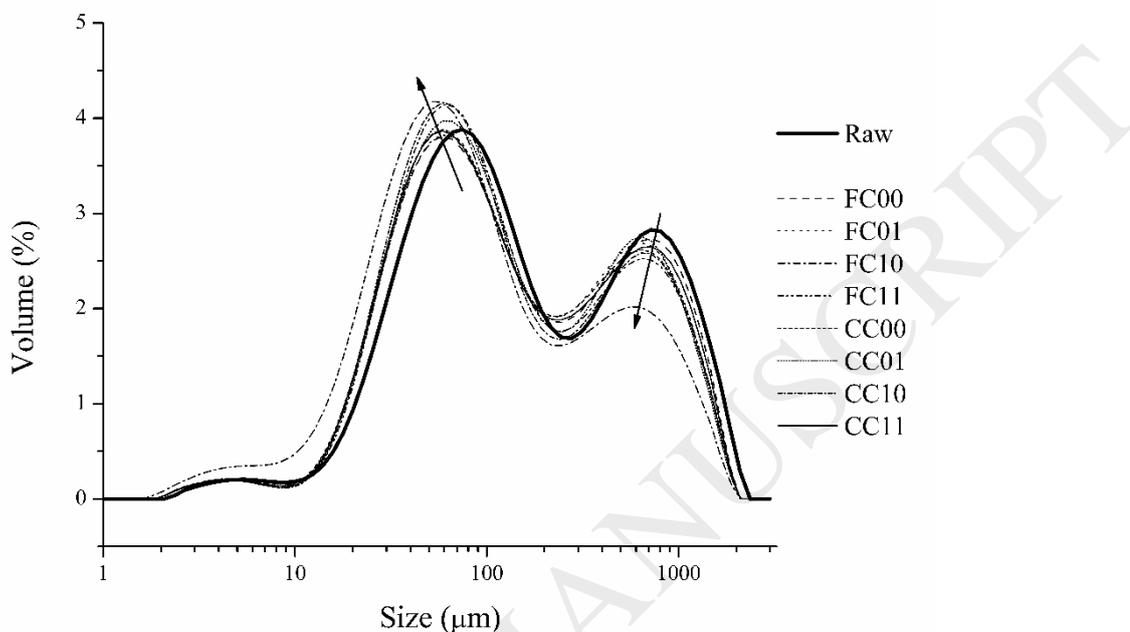
#### 3.1. Composition and properties of untreated fibers

Results of initial characterization of raw cellulosic materials (FC and CC) are shown in this section, concerning chemical composition, physical characteristics and enzymatic hydrolysis assays.

As expected, both raw materials have identical composition, with average 7.0% w/w of moisture. Cellulose is the main component (91.9 % w/w), with low concentrations of hemicellulose (4.2% w/w), acid soluble lignin (0.6% w/w) and extractives (0.7% w/w) (all these compositions in dry basis). The samples did not contain ashes or AIL amount was detected for any of the celluloses since the material had been previously delignified by the manufacturer. Three successive acid hydrolysis were needed to completely determine the cellulose content of CC samples, configuration. Its compactness hindered sulfuric acid access, causing a first incomplete acid hydrolysis (efficiency was around 50%) and leading to a cellulose content underestimation. On the other hand, TPC and HPLC analysis of liquid fraction of cellulose suspensions did not reveal cellulose solubilization or degradation (no simple sugars, short-chain organic acids, phenols, HMF and furfural were detected).

Unless otherwise indicated, physical analysis were conducted only for fiberized cellulose. Due to its compact configuration, particle size distribution, crystallinity index, SEM and limiting viscosity number protocols could not be applied to CC, and these

properties were assumed to be the same as for fiberized cellulose. Particle size distribution (% volume) of raw FC presented a bimodal particle size distribution, with peaks maxima around 75 and 720  $\mu\text{m}$ , and average size of 120  $\mu\text{m}$  (continuous line in Fig. 1).



**Fig.1.** Particle size distribution. Particle size distribution obtained by Dynamic Laser Scattering (DLS) of samples before (raw material) and after steam explosion pretreatment of fiberized (FC<sub>ij</sub>) and compacted (CC<sub>ij</sub>) celluloses.

Short fibers (peak at 75  $\mu\text{m}$ ) were predominant in cellulose-in-water suspension compared to the lower presence of long fibers (peak at 720  $\mu\text{m}$ ). This could be explained since it would be easier for smaller fibers to be disentangled under agitation compared to longer ones. Values for  $d_{p10}$  and  $d_{p90}$  were 29.1  $\mu\text{m}$  and 938.5  $\mu\text{m}$ , respectively.

Optical microscopy and SEM were conducted to visually analyze the surface, dimensions and porosity of FC before pretreatment. It was observed that fiber was partially twisted in a pseudo-helical structure, causing a high entanglement. It also presented a flat shape and irregular ends. Its surface was uneven, presenting some visible pores and an irregular arrangement of microfibrils bundles, probably due to

delignification process (Annex, Fig. A.1). The arrangement of polymer chains, expressed through crystallinity index, was found to be 78.7% (Fig. 2). Furthermore, degree of polymerization was studied through cellulose limiting viscosity number, which were found to be 1080 (dim.-less) and 690 mg/L, respectively (Fig. 3). All these results agreed with values provided in specification sheets from common suppliers of equivalent celluloses, as well as results reported by other authors (Jacquet et al., 2012).

Enzymatic hydrolysis of raw FC and CC showed high values of cellulose degree of hydrolysis at 48 hours, 69.1% for FC and 70.1% for CC samples (Fig. 4). Despite the different morphology and the difficulties found when performing the acid hydrolysis for chemical characterization of CC, enzymatic hydrolysis of both fiberized and compact samples was very similar. This equivalence was observed since reaction began, as shown by the similarity on calculated fitting parameters from Eq. (3) with identical  $k$  values and very similar enzymatic accessibilities (Table 1).

	FC	CC	FC00	FC01	FC10	FC11	CC00	CC01	CC10	CC11
$R_s$ (%)	-	-	89.1	91.4	64.0	61.9	88.5	96.3	72.0	72.6
$a$ (%)	77.1	80.7	37.7	34.2	57.5	56.2	40.2	38.3	66.3	61.3
$k$ ( $h^{-1}$ )	0.135	0.135	0.233	0.233	0.140	0.140	0.244	0.244	0.127	0.127
$R^2$	0.984	0.993	0.937	0.948	0.967	0.958	0.962	0.963	0.969	0.972

**Table 1.** Solids recovery ( $R_s$  (%)) and enzymatic kinetic parameters (accessibility  $a$  (%), kinetic coefficient  $k$  ( $h^{-1}$ ), and regression coefficient  $R^2$ ) of raw material (FC: Fiberized and CC: Compact) and steam-exploded samples (FC<sub>ij</sub>, CC<sub>ij</sub>).

The high accessibility found could be related with the moderate polymerization degree and the predominantly small fiber size. These values are considerably higher than those reported by (Jacquet et al., 2012), who working with microcrystalline cellulose under very similar conditions, just obtained 25% of glucose release yield.

### 3.2. Steam explosion pretreatment

Steam explosion was applied to cellulose samples FC and CC at four different severity factors, to evaluate it as an alternative pretreatment for opening cellulose sheets, as well as its effects on fiberized and compact samples. (Jacquet et al., 2011) proved that cellulose fibers subjected to steam explosion had limited thermal degradation if a SF below 4.0 was applied. Hence, the combination of temperature (150 or 200 °C) and reaction time (5 or 30 minutes) was selected to be under this limit, except for the hardest conditions, which were selected to be beyond this thermal degradation limit. Hence, the applied SFs (Eq. (1)) varied between 2.3 and 4.5. The eight resulting pretreated samples were named FC<sub>ij</sub> and CC<sub>ij</sub>, where i and j could be 0 or 1, depending if it is the low or the high value of temperature and time, respectively (FC<sub>01</sub> would mean steam-exploded fiberized cellulose at 150 °C and 30 minutes, etc.). As shown in Table 1, solids recovery ( $R_s$ ) decreased with increasing temperature, with no effect of reaction time. For both FC and CC samples, solid recovery was high (~90%) for 150 °C pretreatments. Steam explosions at 200 °C provided lower  $R_s$ , around 60% for FC samples and 70% for CC experiments. The greater intensity of the decompression and the most probable smaller size and lower density of cellulose fibers after treatment at 200°C could have caused a probable dragging of these particles to the atmosphere during the explosion, explaining the greater  $R_s$  losses at 200°C than at 150°C. Probably, the compacted configuration provided some protection to cellulose fibers, observed in their slightly higher  $R_s$  values. (Medina et al., 2016) also observed a decrease in solids recovery with increased severity factor, being more important the effect of temperature than that of reaction time, thus thermal effects e.g. volatilization of low molar mass components such as terpenes, aliphatic alcohols or aldehydes (Neves et al., 2016) or steam stripping when decompressing could be additionally considered.

### 3.2.1. Chemical composition of pretreated samples

Moisture values were found to be similar for all pretreated samples, around 70% after filtration step, suggesting that retained water was independent from steam explosion conditions. Ashes, ASL and extractives content remain mostly unvaried compared to raw materials. Cellulose content increased around 5% for both types of exploded samples, up to an average 96.0 wt.%. Pretreated CC samples did not require this time anymore acid hydrolysis for quantifying its cellulose content, and these values were comparable to those obtained for pretreated FC samples. On the contrary, hemicellulose content slightly decreased from 4.2 wt.% to 3.2 wt.%, independent of SF or cellulose configuration. These variations were most probably caused by relative changes due to the mass losses during pretreatment. (Medina et al., 2016) further reduced hemicellulose content of oil palm empty fruit bunches down to 1.8 wt.% when applying steam explosion to this lignocellulosic biomass at 195 °C for 10 minutes, which corresponded to a 68% reduction.

HPLC analysis of pretreatment liquid fractions showed absence of glucose, furfural and HMF, concluding that cellulose did not solubilize or degrade in appreciable quantities. Nevertheless, some traces of phenolic compounds (6 ppm on FC00 and 12 ppm on FC10), as well as xylose (0.1% w/w) and acetone (0.05 - 0.1 g/L), were found. Phenolic compounds presence in those two samples (which correspond to the shortest reaction time) might correspond to an early formation, followed by degradation for longer reaction times.

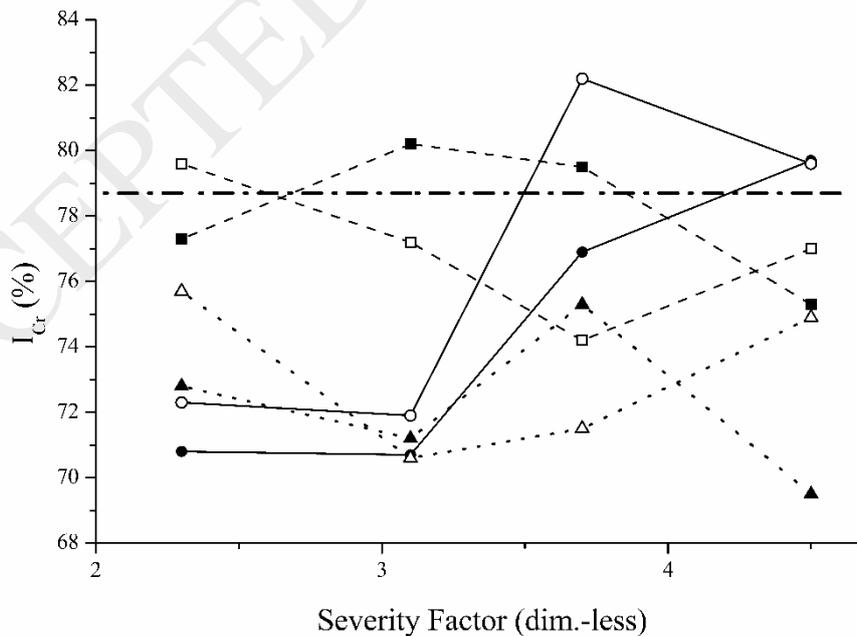
### 3.2.2. Physical properties

Steam explosion transformed the non-fiberized CC sample into a material very like FC which allowed the application of particle size distribution, crystallinity index, SEM and limiting viscosity number protocols to pretreated CC samples. Particle size

distribution were very similar for pretreated CC as for exploded FC samples, with  $dp_{10}$  and  $dp_{90}$  around 30 and 930  $\mu\text{m}$ , respectively. Pretreatment reduced particle size for both types of raw material, being FC10 the most affected (Fig. 1). Peaks values were around 60 and 640  $\mu\text{m}$  for all samples. Pretreatment reduced fiber size, but considering the extremely different initial handling of raw samples, it can be concluded that steam explosion has successfully overcome the need of other mechanical conditioning pretreatments.

Both optical and electronic scanning microscopy images corroborated the observed effects of steam explosion on cellulose fibers, revealing surface changes but without significant degradation, although some debris was detected. Steam explosion visually increased the number and size of pores, and surface got more irregular and crumpled (Annex, Fig. A.2 and A.3).

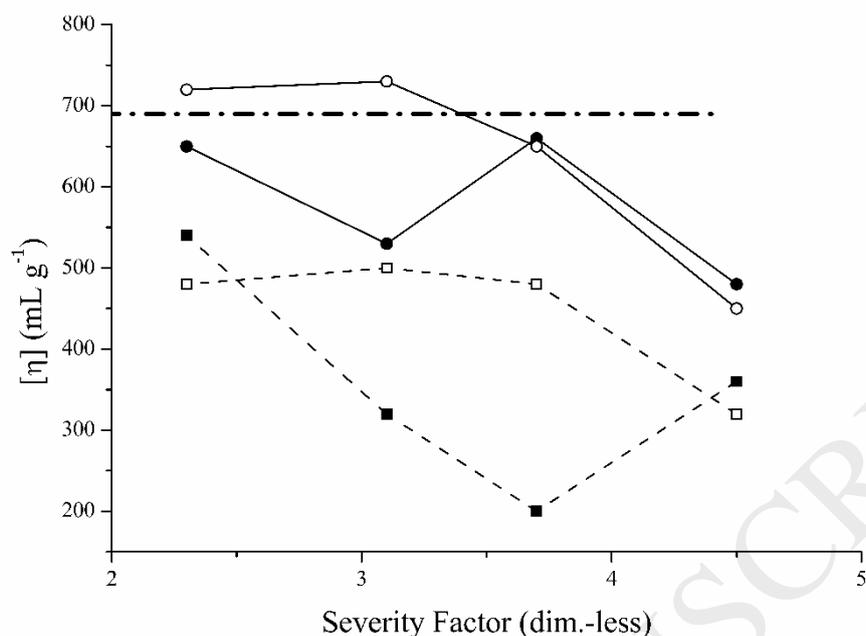
Crystallinity index of steam exploded samples suffered a variation up to 12 percentage points due to pretreatment (Fig. 2).



**Fig. 2.** Crystallinity index. Crystallinity index ( $I_{Cr}$ ) obtained from X-ray spectrometry of samples before (raw material (— · —)) and after steam explosion (FC (●), CC (○)); and after acetone drying (DFC (■), DCC (□)) or freezing process (FFC (▲), FCC (△)) as a function of the severity factor.

X-ray patterns of all samples corresponded to typical crystalline structure of raw cellulose. The presence of crystalline fraction was corroborated with the moderately high values (around 3.0) of the ratio between intensities at 22.7 ° and 20.4 °, which indicates the relative proportion between types of cellulose I and II. Pretreatment at 150 °C decreased  $I_{Cr}$  of samples, being the greatest reduction compared to raw material (8 percentage points) for pretreated FC. However, 200 °C steam explosions did not significantly modify  $I_{Cr}$ , with maximum  $I_{Cr}$  value of 82.2% for sample CC10. These results could indicate that steam explosion reduced the crystalline fraction, but high-temperature (and hence higher pressure) treatment promoted recrystallization, partially cancelling out or exceeding the downgrading effect. Some protective effect of compact configuration could be observed, which is not effective at the most severe conditions. Reaction time was only significant at high temperature experiments. (Wang, Jiang, Xu, & Sun, 2009) did not find significant variation on microcrystalline structure of *Lespedeza* fibers even at the most severe conditions.

Differently, limiting viscosity number (and hence the degree of polymerization) of pretreated samples decreased for all the fiberized samples to values ranging from 480 to 660 mL/g, and showing this time a greater influence of pretreatment time than that of temperature (Fig. 3).

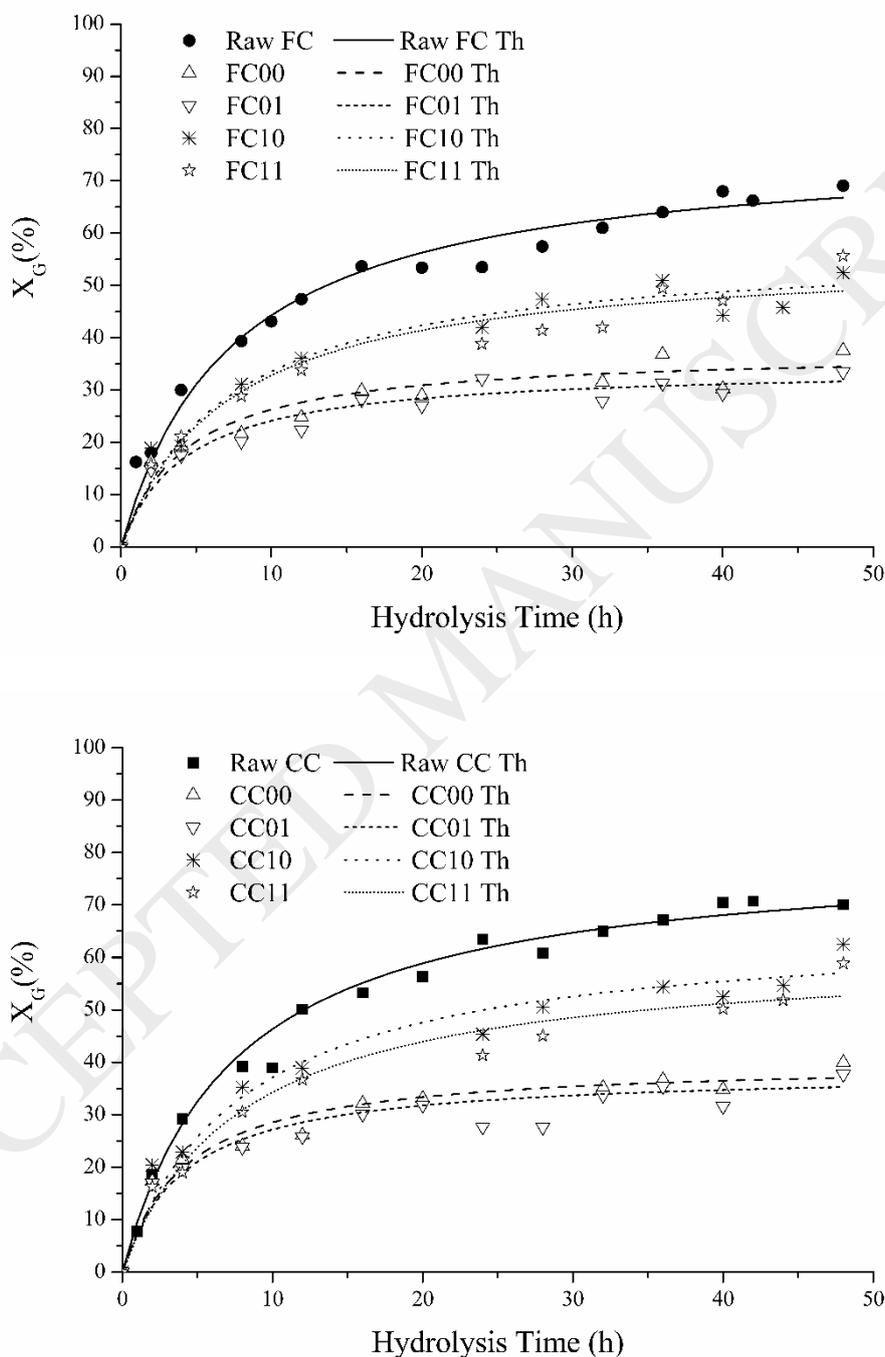


**Fig. 3.** Limiting viscosity number. Limiting viscosity number ( $[\eta]$ ) of samples before (raw material (---)) and after steam explosion treatment (FC (●), CC (○)); and after acetone drying (DFC (■), DCC (□)) as a function of the severity factor.

In the case of CC samples, 150 °C pretreatments did not influence  $[\eta]$  and, so, polymerization of cellulose. The compactness of CC configuration provided a certain protection to cellulose polymer when working at low SFs. Nevertheless, at 200 °C both FC and CC samples showed equivalent behavior, reaching similar  $[\eta]$  values. This dependence with severity factor was also observed by (Yamashiki et al., 1990), who reported a combined effect of temperature and time in degree of polymerization, but in this case applying severity factors over 4.45. They also reported a reduction in degree of polymerization as well as a shortening effect for steam-exploded cellulose fibers with temperatures below 230 °C.

### 3.2.3. Enzymatic hydrolysis

Table 1 shows the fitted parameters of enzymatic hydrolysis kinetics of FC and CC cellulose pretreated samples applying (Eq. 3), and cellulose hydrolysis courses with time are shown in Figs. 4a and b.



**Fig. 4a and b.** Enzymatic kinetics of raw and exploded materials. Enzymatic hydrolysis kinetics of raw cellulose and steam exploded samples of both fiberized (up) and compacted (down) handling celluloses.

Experimental results are shown as discrete points, lines represent the theoretical model of eq. 3 using the fitting parameters on Table 1.

As before, CC and FC-exploded samples showed an analogous behavior and similar reaction courses, accessibilities ( $a$ ) and kinetic parameters ( $k$ ). Enzymatic hydrolysis of pretreated samples provided lower degree of hydrolysis of cellulose than raw materials, but increasing with the pretreatment temperature. On the other hand, the pretreatment reaction time had not a decisive effect. For all samples, 48h of enzymatic hydrolysis was enough to achieve the maximum liberation of the accessible cellulose in samples. Cellulose degree of hydrolysis at 48h were almost up to accessibility ( $a$ ) values, being slightly higher for compact samples than for fiberized ones. Nevertheless, a clear reduction on accessibility was observed, represented by a generalized decrease in hydrolysis efficiency. This decrease was especially remarkable for low-temperature pretreated samples, increasing for 200 °C experiments but never reaching the values obtained for raw materials. The evolution trend of both accessibility and  $I_{Cr}$  is very similar with increasing SF, suggesting a correlation between these two properties.

Kinetic parameter ( $k$ ) showed an opposite trend to accessibility. Enzymatic hydrolysis rate was high for low temperature pretreated samples, but 200 °C pretreatments returned similar  $k$  values to those from the raw materials. The results shown no influence of pretreatment reaction time, with identical fitting parameter values for each sample configuration and temperature. This increase in  $k$  values could mean that, even if enzyme action was limited, the accessible cellulose was likely to be hydrolyzed, suggesting that steam explosion probably modified internal porosity and structure without extremely changing fiber physical properties.

### *3.3. Preservation methods study*

The influence of acetone drying (samples called DFC and DCC) and freezing (FFC and FCC) and their effect on physical and chemical properties of pretreated cellulose were evaluated.

### 3.3.1. Acetone-dried preservation

Chemical composition of acetone-dried samples remained almost unchanged, obtaining comparable values (data not shown) to pretreated samples from section 3.2. Acetone effectively dried steam-exploded samples for experiments conducted at 150 °C of temperature for both compacted and fiberized pretreated samples, obtaining moisture values around 5%. Acetone-dried samples from 200 °C pretreatments still retained notable amounts of water, observing significant variations depending on sample configuration and reaction time: moisture oscillated from 17.3 for DCC10 sample to 30.5% for DFC11. This significant water retention in 200 °C pretreated samples could be related with the higher accessibility found for enzymatic hydrolysis of these samples.

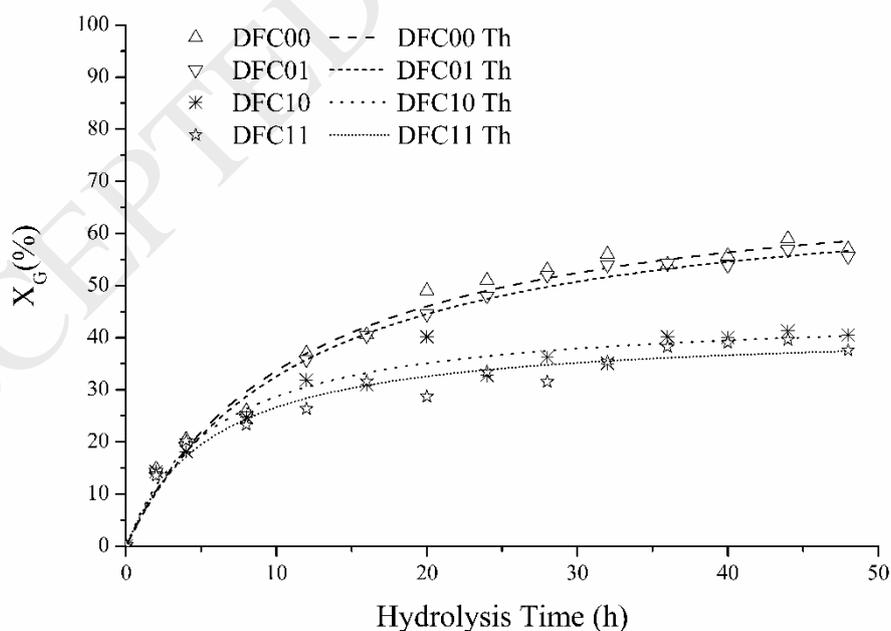
Optical microscopy images did not reveal significant changes on acetone-dried pretreated cellulose fibers. Surface appearance was similar to pretreated samples from section 3.2 (increased number and size of pores) but fibers were flat and more crumpled after acetone drying process.

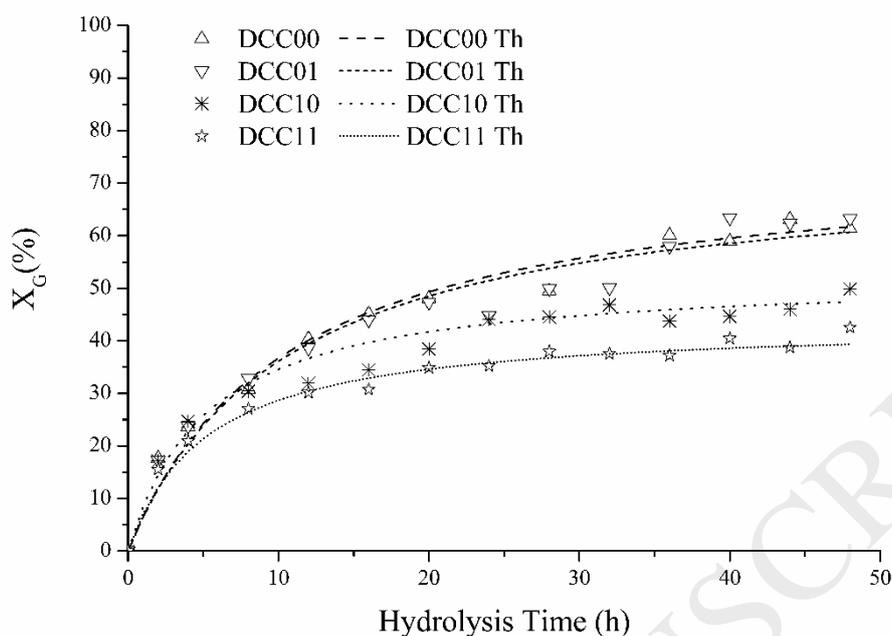
Surprisingly, acetone counterbalanced the variations of crystallinity index caused by steam explosion on pretreated samples (section 3.2) (Fig. 2). Crystallinity index of samples pretreated at low SFs, where  $I_{Cr}$  was reduced (FC00, FC01, FC10, CC00 and CC01), increased after acetone washing, and vice versa. This suggested that samples that recrystallized after 200°C treatment might suffer a rearrangement during acetone drying, losing crystallinity. In addition, retained water might have an effect, i.e. polymeric chains from samples with higher moisture had more flexibility and presented less crystallinity.

On the other hand, limiting viscosity number was considerably reduced by acetone washing, obtaining a maximum  $[\eta]$  value of 540 mL/g and a minimum of 200 mL/g (Fig. 3) but with no clear dependence on pretreatment conditions. Thus, polymerization degrees (from Eq. 2) ranged from 818 to 302. These results indicated that acetone did not just remove inner water but also affected the structure of cellulose polymeric chains, probably due to a complex interaction between several properties of pretreated cellulose samples, like structure, polymerization degree or moisture content.

	DFC00	DFC01	DFC10	DFC11	DCC00	DCC01	DCC10	DCC11
a (%)	72.2	71.1	45.8	42.0	75.2	73.9	51.5	44.4
k (h <sup>-1</sup> )	0.083	0.083	0.172	0.172	0.095	0.095	0.192	0.192
R <sup>2</sup>	0.986	0.991	0.957	0.963	0.983	0.965	0.969	0.982
	FFC00	FFC01	FFC10	FFC11	FCC00	FCC01	FCC10	FCC11
a (%)	21.4	15.8	22.0	17.6	30.8	21.36	31.6	23.0
k (h <sup>-1</sup> )	0.166	0.272	0.166	0.272	0.135	0.199	0.135	0.199
R <sup>2</sup>	0.946	0.944	0.981	0.964	0.977	0.936	0.983	0.976

**Table 2.** Enzymatic hydrolysis of preserved samples. Enzymatic kinetic parameters (accessibility a (%), kinetic coefficient k (h<sup>-1</sup>), and regression coefficient R<sup>2</sup>) of pretreated samples after acetone drying (DFCij, DCCij) and pretreated samples after freezing (FFCij, FCCij)





**Fig. 5a and b.** Enzymatic kinetics of acetone dried samples. Enzymatic hydrolysis kinetics of steam explosion pretreated samples of both fiberized (up) and compacted (down) handling celluloses, after acetone drying process. Experimental results are shown as discrete points, lines represent the theoretical model of equation 3 using the fitting parameters of Table 2.

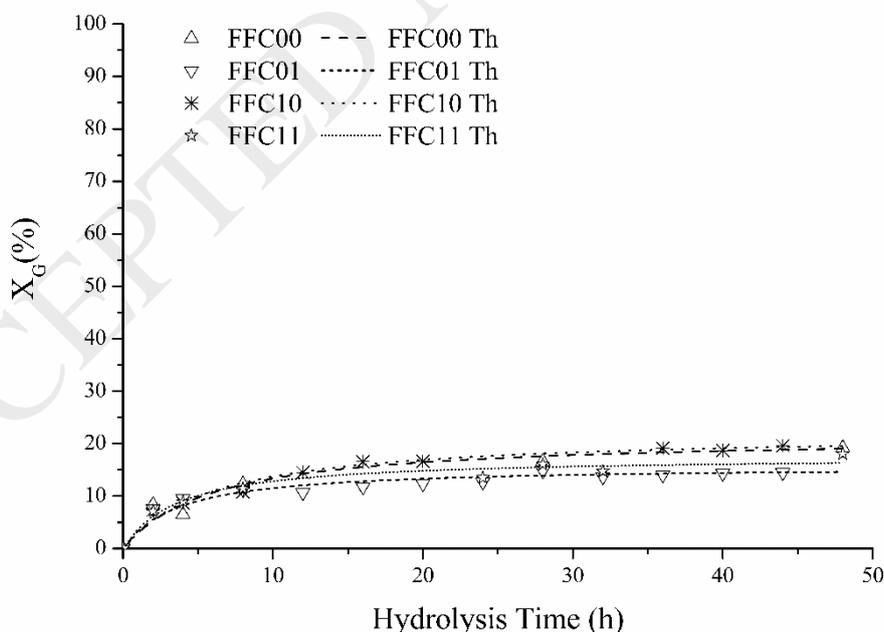
Enzymatic hydrolysis kinetics showed identical counterbalance effect as observed for  $I_{Cr}$  (Fig. 5). Acetone wash increased  $X_G$  of samples pretreated at 150 °C, but still not reaching raw cellulose results, and decreased for 200 °C pretreated samples (Table 2). As observed in section 3.2, hydrolysis extension was softly higher in CC samples than in FC samples, even after acetone drying. On the other hand, kinetic parameter  $k$  was remarkably affected, increasing as accessibility decreased. Again, results showed no influence of reaction time, with identical fitting parameter values for each sample configuration and temperature.

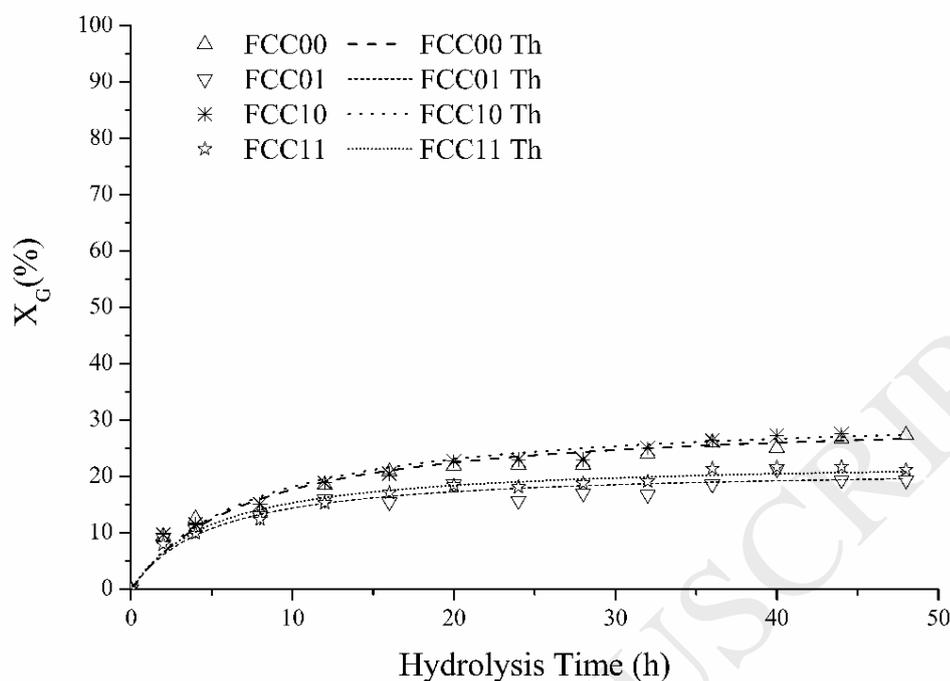
### 3.3.2. Freeze preservation

Chemical composition did not show changes due to freezing (data not shown), but the rest of the properties were indeed affected, as it was also observed for acetone-dried samples.

All frozen pretreated samples showed lower  $I_{Cr}$  values than for non-treated cellulose, decreasing between 3.0 (FCC00) and 9.2 (FFC11) percentage points compared to raw cellulose. Compared to pretreated samples, only  $I_{Cr}$  of FFC00, FFC10 and FCC00 samples (corresponding to low SFs) slightly increased, around 3 percentage points by freezing. Maximum decrease was observed for samples from high SFs pretreatments, those which suffer recrystallization, with  $I_{Cr}$  reductions down to 10 percentage points due to freezing.

Optical microscopy has shown that freezing caused slight macro-structural changes on fibers, appearing grooved and cracked and an important presence of debris, probably due to formation of ice crystallites.





**Fig. 6 a and b.** Enzymatic kinetics of frozen samples. Enzymatic hydrolysis kinetics of steam explosion pretreated samples of both fiberized (up) and compacted (down) handling celluloses after freezing process. Experimental results are shown as discrete points, lines represent the theoretical model of equation 3 using the fitting parameters of Table 2.

A generalized decrease in cellulose degree of hydrolysis was observed as well (Fig. 6). In this case, freezing process caused a more remarkable effect than acetone, resulting in a drastic decrease on cellulose degree of hydrolysis to values ranging from as down as 15.7% to only 27.3%. This meant an average reduction of more than 40 percentage points compared to raw cellulose fibers. Additionally, the effect of reaction time is more relevant than temperature for samples which underwent freezing. Accessibility and kinetic parameter are near identical for pairs of samples with same reaction time and handling (Table 2). The remarkable reduction in accessibility is not sufficiently counteracted by the high increase of kinetic coefficients. In these results, the degradation effect due to ice formation was clearer than it was observed for crystallinity.

However, it could not be possible to identify in which way or how much this process conditioned these changes. No clear relationship between the decrease of enzyme accessibility and the other properties such as crystallinity index was revealed to explain the effects of freezing. Water states and moisture could have great effects when pretreating lignocellulosic biomass (Sui & Chen, 2016) and further research should be done in this sense.

#### 4. Conclusions

The aims of this work were to perform a characterization of a commercial cellulose with two different configurations (fiberized /non-fiberized sheet), and to study the effect of steam explosion and two different preservation methods (acetone-washing and freezing). Steam explosion pretreatment was conducted at two different temperatures and reaction times (150-200 °C, 5-30 min), and the influence of severity factor and cellulose configuration on chemical and physical characteristics was analyzed. Initial characterization of commercial celluloses returned expected values for all studied properties, with high cellulose content (91.9%) and no soluble compounds. Despite compact cellulose configuration needed three consecutive acid hydrolysis for its complete solubilization during cellulose content analysis, it showed high  $X_G$  value at 48h, similar to that obtained for fiberized cellulose (~70%). Particle size measurements showed a bimodal distribution, with two peaks at 75 and 720  $\mu\text{m}$ , despite a completely homogeneous water suspension for fiberized cellulose was difficult to achieve due to its entanglement and complex format.

In general, steam explosion of compacted commercial cellulose seemed to be a good substitute of other mechanical treatments. No thermal degradation was observed

even at the strongest conditions, and pretreated compact cellulose presented comparable characteristics as pretreated fiberized cellulose.  $R_S$  decreased with increasing temperature, with no effect of reaction time. For samples pretreated at 150 °C,  $R_S$  was high (~90%), whereas at 200 °C  $R_S$  was lower (~63% for FC and ~72% for CC). This reduction might be attributed to thermal effects or stripping. Compact configuration provided some protection to pretreatment, as observed on the higher  $R_S$  values.

Evaluation of pretreated samples from fiberized and non-fiberized sheet cellulose returned similar values for properties such as crystallinity or particle size. Compact handling protective effect to thermal effects was observed at low-temperature experiments, but behavior of FC and CC samples pretreated at 200 °C was equivalent. Physically, pretreatment slightly reduced particle size (peaks at 60 and 640  $\mu\text{m}$ ), increased number of pores and crumpled fibers surface. It degraded cellulose crystalline structure at low temperature reducing  $I_{C1}$  to 70.7%, but high-temperature pretreatment promoted its recrystallization to values up to 82.2%. Steam explosion also reduced the DP, especially at the highest severity factors, to 725 for FC samples and 678 for CC samples. Pretreatment did not improve enzymatic hydrolysis despite the decrease on DP. This was especially noticeable in experiments at 150 °C (with values around 37%), increasing up to 60% for 200 °C experiments, where DP was lower. Steam explosion also slightly increased enzymatic reaction rates.

For their part, acetone and freezing process profoundly affected pretreated cellulose structure and properties, especially for samples treated at the highest severity factors. A thermal effect in cellulose structure was observed in moisture of acetone-dried samples, which increased from 5 % at 150 °C samples to values ranging from 17.3 to 30.5% for 200 °C samples. Acetone washing counterbalanced the variations observed on

$I_{Cr}$  and enzymatic accessibility for pretreated samples before acetone washing, and decreased remarkably the polymerization degrees to values from 818 to 302. Freezing also decreased  $I_{Cr}$  of samples pretreated with high severity factors (around 10 percentage points) and caused slight macro-structural changes on fibers, but the most important effect was the decrease of enzymatic accessibility, with very low values ranging from 15.8 to 31.6%. In general, acetone drying and freezing reverted the recrystallization caused by 200 °C pretreatment. On enzymatic hydrolysis kinetics, acetone drying mainly reduced accessibility of samples treated at 200 °C increasing the reaction rate, but freezing has significantly diminished accessibility of all pretreated samples, suggesting some damage related with ice formation.

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