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ORIGINAL ARTICLE

Treatment of chenevotte, a co-product of industrial hemp fiber, by water or hydrochloric acid: impact on polymer mobility in the lignified cell walls

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Abstract The impact of water- and of mild acid impregnation with hydrochloric acid (HCl) on the viscoelastic and breaking properties of woody hemp core (chenevotte) was studied from the polymer to the macroscale level. We have shown that transition temperature (T_g) values of lignin and hemicelluloses were not affected by the acid treatment, whereas the relaxation mechanism of hemicelluloses, expressed by the apparent activation energy, was modified. The opposite impacts were observed in the water extraction. Changes in the sample strength were also noted, as the chenevotte treated with mild HCl became more brittle, as opposed to the samples extracted with water. It is suggested here that the moderate HCl treatment impacts chenevotte micro- and macro-properties through acid depolymerization of polysaccharides and extraction of low molecular mass entities, whereas water immersion contributes solely to the later mechanism.

Keywords Acid hydrolysis · Lignin · Hemicellulose · Micromechanics · Glass transition temperature

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Introduction

Under natural conditions, wood is exposed to different types of microbial decay. Amongst them are brown rot fungi, which attack the cell wall carbohydrates, leading to a rapid decrease in the strength properties of wood. These fungi are able to produce organic acids, such as oxalate, which results in local acidification, favors polysaccharide hydrolysis and promotes Fenton-like reactions involving transition metals and hydrogen peroxide (Green and Highley [1]). As such, fungal or bacterial colonization considerably modify the structures involved in fiber cohesion, particularly at the level of amorphous polymers, lignins and hemicelluloses. This opens several possibilities and strategies for biological pretreatment of wood in the pulp and paper industry for energy and chemical savings (Wolfaardt et al. [2]). The use of oxalic acid to pretreat wood chips was suggested several years ago for paper making to save energy during defiberization and mechanical pulping and to enhance fiber properties during refining (Meyer-Pinson et al. [3], Kenealy et al. [4]). The principle is based on the modification of cohesion between wood cell walls, and within the cell wall, in view of its natural composite and lamellar organization (Salmen [5]). The effects are assumed to be a consequence of chemical changes of wood constituents like hydrolysis of hemicelluloses and partial lignin polymerization (Meyer-Pinson [6]).

We recently demonstrated that oligomeric chemical entities removed from cell walls by solvent extractions do not modify the global chemistry of wood, but clearly have a strong influence on in situ mobility of the amorphous polymers (Bag et al. [7]). Such modification at the polymer level could then play a significant role in the defiberization mechanism in technical processing where mechanical

stresses are applied at high temperature and humidity (pulping, thermoforming of wood, etc).

Our data suggested that, in the presence of water and organic acids, wood can be modified simultaneously at different levels, affecting its chemical and functional properties that could drive technical transformations.

The objective of this work is to delineate the relationship between polymer mobility in woody material, chemical modification under mild acidic conditions, and the fracture mechanism leading to the individualization of wood fibers in a process involving temperature and moisture. For the latter purpose, the softening temperature (T_g) was chosen to evaluate the mobility properties of each amorphous polymer chain in the cell wall. The activation energy was then calculated to describe the softening mechanism for hemicelluloses. Finally, the fracture behavior of the sample was investigated in relation to the modifications caused by the treatments.

The studies were carried out on a non-conventional woody material originating from stem of *Cannabis sativa* (Fig. 1). Hemp is a dicotyledonous plant from the order of Rosales, from the family of Cannabaceae, genus *Cannabis*. The material used in this study, chenevotte, is a co-product of the hemp stem after an industrial defiberization process (Fig. 1). It is constituted from the xylem tissue of the stem (Cronier et al. [8]). Apart from recent interest in using chenevotte in new applications for biosourced composites (Islam et al. [9], Placet [10], Pickering et al. [11]), this material is also particularly suitable to this study due to its high porosity, amorphous polymer content and high sensitivity to water.

As stated above, oxalic acid may be involved in the natural process of wood decay, but is not suitable herein to investigate the impact of acidity on the wood components. Indeed, oxalic acid is a particular diacid with low p*K*a, good esterifying properties and strong chelating ability (Kenealy et al. [12], Kenealy et al. [13]). Acidification was then performed in our study with dilute HCl, allowing us to investigate specifically and separately the impacts of water and acidity on the chenevotte cell wall components and properties.

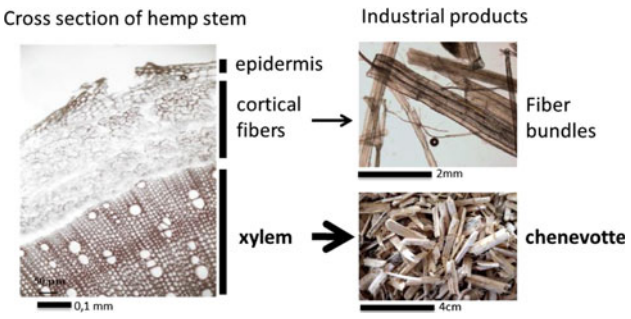

Fig. 1 Cross section of hemp stem and morphological aspect of the industrial products obtained from defiberization process (micrographs from B. Chabbert and B. Kurek, INRA Reims)

Materials and methods

Hemp material

The woody hemp core came from the industrial defiberizing factory operated at La Chanvrière de l'Aube (LCDA) in Bar-sur-Aube (France). A single hemp cultivar, Fedora 17, was used throughout the study. Sticks of woody hemp core with no visible cracks or damage were selected manually and retained for the experiments. They were further selected on the basis of geometrical shape ($\sim$2 mm wide, $\sim$1 mm thick, 10–40 mm length). For the dynamic mechanical analysis (DMA) and dielectric analysis (DEA, see below), it was necessary to erase the natural variability of the sticks, related to their initial different position within the hemp stem (Bag et al. [7], Cronier et al. [8]). For this purpose, the above selected and undamaged samples were carefully split into two identical pieces along the long axis to allow unambiguous perfectly matched paired comparisons (Fig. 2). One half of the stick was impregnated with water or HCl and the other half (hereafter defined as the reference sample) was left untreated.

Treatments

The samples (30 g) were impregnated with a solution (200 mL) of hydrochloric acid (0.1 mol/L) or in ultrapure water for 5 h with magnetic stirring, at room temperature (21 °C).

Biochemical analysis of the extracted products

The extracts contained in the soluble part (HCl or water media) were first concentrated by evaporation and then freeze-dried. The dry matter obtained was weighed and then analyzed.

The total and monosaccharide contents of the extractives were determined by acid hydrolysis of 10 mg of dry matter (DM) using a solution of 1 M H_2SO_4 for 2 h at 100 °C. After hydrolysis, the released monosaccharides were separated by high performance anion-exchange chromatography (HPAEC) as described previously (Beaugrand et al. [14]). Both water and HCl fractions were analyzed in triplicate.

The free glucose extracted from the cell wall was monitored by thin-layer chromatography (TLC), as described previously (Zilliox and Debeire [15]). To evaluate the hydrolysis potential of the 0.1 M HCl solution,

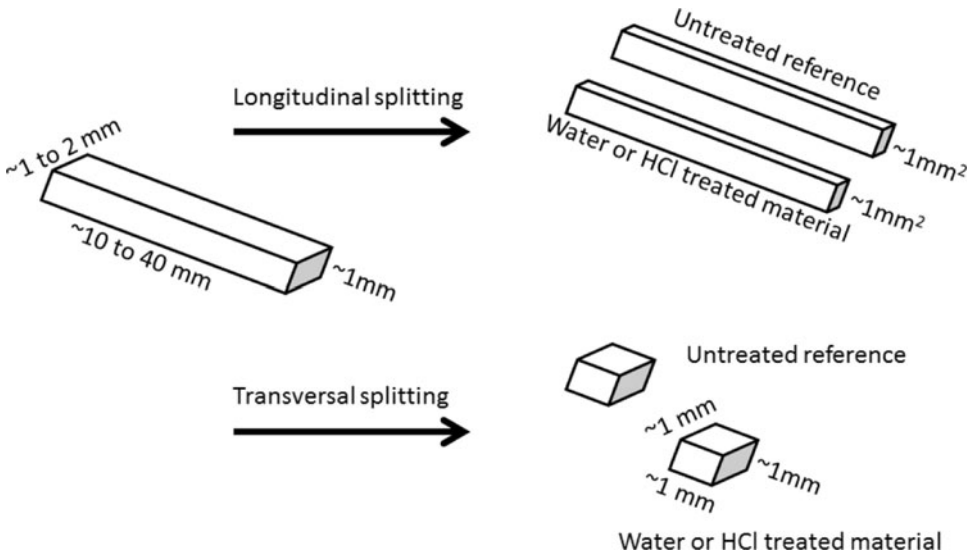

Fig. 2 Initial geometry and preparation of the chenevotte paired samples for the treatments and analysis. For DMA analysis, the matched reference- and treated- sample sizes were approximately 1 × 1 × (10–40)mm after longitudinal splitting of the original chenevotte stick; for DEA analysis, the matched reference and treated samples were split transversally to approximately (1 × 1 × 1)mm size

70 mg of cellulose from a hand cut Whatman filter (VWR), was impregnated with 1 mL of solution and compared with an ultrapure water immersion. Fifteen microliters of the solutions were spotted onto silica gel plates. The chromatography chamber was saturated with vapor from the solvent system, which consisted of butan-1-ol/acetic acid/water (2:1:1 by volume), before plate development. The solvent was allowed to migrate until 2 cm from the top of the plate. The plate was then removed and air-dried for half a day. The separated sugars were revealed using an orcinol–sulphuric acid spray reagent (200 mg orcinol/100 mL 20 % sulfuric acid) heated for 10 min at 130 °C.

The lignin content of the freeze-dried extractives was determined by the acetyl bromide method, slightly adapted: (1) 10 mg of samples were hydrolyzed at 70 °C for half an hour with a 5 mL mix of acetyl bromide (98 %), acetic acid (99.8 %) solution and 0.2 mL perchloric acid. (2) 5 mL of 2 M NaOH were added to 2 mL of this mixture (1) and adjusted to 20 mL with acetic acid. (3) The mixture [2] was left for 30 min in a dark place. (4) The absorbance of dissolved lignins was measured at 280 nm by analysis of triplicates (Day et al. [16]).

The protein content of the freeze-dried extractives was calculated by measuring the UV absorbance at 595 nm using a Bradford protein assay (Beaugrand et al. [14]).

The ash content was determined by thermogravimetric analysis (TGA) by incinerating 10 mg of the lyophilized extractive samples at 550 °C until a plateau was reached on the curve of mass versus temperature.

The contents of minerals, notably Ca, Cu, Fe, K, Mg, Mn, Na and Zn, were measured by comparison to calibrated standard curves with an ICP atomic emission spectrometer (Varian Liberty Series II), using 10 mg of dried extracts dissolved in 25 mL of ultrapure water, and filtered (0.45 µm).

Determination of the viscoelastic properties of woody hemp core

The polymers constituting the cell wall exhibit viscoelastic responses during deformation that are dependent upon frequency, temperature and water content. The techniques used to determine the T_g values of lignin and hemicelluloses were DMA and DEA, respectively (see below).

DMA in tension mode in immersion

DMA is specifically used to characterize in situ lignin relaxation; the peak signals observed in the range of 80–110 °C can be attributed to its glassy transition (Salmén et al. [17], Sun and Frazier [18]).

The sample geometry was described in "Hemp material" (see also Fig. 2). The instrument was set in single frequency mode (1 Hz), the amplitude of the oscillation constant was set at 12 µm in "autostrain method" with a static force of 112 %. Wood impregnated with ethylene glycol has been previously demonstrated to exhibit softening behavior similar to water-saturated wood (Salmén et al. [17], Bouajila et al. [19]). We therefore performed the experiments in ethylene glycol, to better determine the softening temperature, and the measurements were made above 100 °C with a heating rate of 2 °C/min. The storage modulus (E'), loss modulus (E''), and tanδ responses for woody hemp core were plotted as a function of temperature at a given frequency solicitation. As

with synthetic polymers, the values of E' decreased from a glassy plate to a rubbery state, and E'' and tanδ showed the same evolution with a maximum peak when the material softened.

DEA in controlled humidity

DEA is specifically used to characterize the in situ chain mobility of hemicelluloses. Some materials, including wood polymers, have a dielectric constant (ε') indicating to what extent polarization occurs in an electrical field. If an alternating electrical field is applied, the polarization lags behind the field by a phase angle, as a partial dissipation of the stored energy. The dissipated energy is proportional to the dielectric loss (ε'') and the stored energy to the dielectric constant (ε'). The values of ε'' and ε' measured by DEA are generally associated with different relaxations of polysaccharide (James [20]) depending on the frequency and relative humidity at a given temperature. They allow further determination of tanδ as in DMA analysis (see above; Lenth and Kamke [21], Sugimoto and Norimoto [22]).

For our analyses, the samples were first equilibrated and stored in a constant relative humidity box (65 % RH). They were then placed in a modified sample holder for the analysis to prevent water evaporation during the measurements, as described by Bag et al. [7]. No variation in water content was observed in samples that did not exceed 20 % water content, up to 150 °C. A constant force of 350 N was applied during the experiment to ensure good contact between the specimen and electrodes. The frequencies examined were 1–5–10–50–100 Hz, within the appropriate range for observing the softening effects. A heating ramp of 2 °C/min was used.

Plotting tanδ versus temperature allowed the determination of the softening temperature T for each frequency solicitation (f) used (Bag et al. [7]). Increasing the solicitation frequency led to an increase in the softening temperature. By considering the coupled values of the frequency (f) and of the softening temperature (T), a time/temperature diagram where log(f) is a function of $1/T$ can be drawn (Bag et al. [7], Sun and Frazier [18], Lenth and Kamke [21]). Acknowledging softening to be an Arrhenian-type mechanism, the activation energy E_a can be obtained from the slope of the straight line obtained, using the following equation

$$f = A^{-E_a/RT} \quad (1)$$

where f is the frequency, in Hz; A, a pre-exponential factor; E_a, the apparent activation energy in J mol^{-1}; R, the gas constant ($R = 8.31$ J mol^{-1} K^{-1}); T, the softening temperature in K.

Determination of the fracture behavior and properties of hydrolyzed woody hemp core

Mechanical tests were performed on a Testwell machine model 108.2Kn.H. Strain measurements were obtained by placing sensors between the fixed plate and the moving one. The displacement rate was 15 mm min^{-1}. The experiment was stopped when the force applied to samples attained 50 % of the maximum force just after crack initiation. The curve obtained by plotting the load (force in N) against displacement was analyzed with Test Winner 922 software. The elastic modulus, E (obtained from the slope of the curve), the strain, the stress σ_{max} (obtained from the maximum load at break), the drop, d_{max} (corresponding to the displacement after a 20 % decrease in the maximum force) and the fracture energy, $W_{20\ \%}$ (corresponding to the integration of the curve up to d_{max}), were calculated. The experiments were done using a three-point bending test under a controlled atmosphere with 50 % relative humidity and a temperature of 20 °C.

Fracture observations by scanning electron microscopy (SEM)

Micrographs of fracture sections from untreated and treated woody hemp core were obtained with a scanning electron microscope, model MEB XL 30 from PHILIPS. The samples were fixed on carbon adhesive bearings and coated with gold–palladium using a plasma sputtering apparatus, model SCD 040 from BALZERS, prior to SEM investigation.

Results and discussion

Impact of HCl and water on the chenevotte and on the extracted products

Raw material characterization

Table 1 gives the monosaccharide composition of raw woody hemp core. The monomers consisted of 36.7 % glucose, probably mostly from crystalline and amorphous cellulose, but also from hemicellulosic chains harboring glucose. Woody hemp core contained 15.6 % xylose, probably from xyloglucans, the principal hemicellulose present in hemp (Cappelletto et al. [23]). Less-represented hexoses (galactose, mannose, arabinose) were also found, implying the presence of other types of hemicellulose such as galactoglucomannans or arabinoxylans, as mentioned in the literature (Buchanan et al. [24]).The presence of rhamnose and galacturonic acid could also be attributed to pectic substances, such as rhamnogalacturonans as reported

Table 1 Biochemical composition of untreated raw material

Component	% of dry matter
Lignin	20.7 ± 0.6
Ash	2.7 ± 0.2
Total monosaccharides	57.8
Fucose	0.03 ± 0.01
Rhamnose	0.33 ± 0.01
Arabinose	0.39 ± 0.05
Galactose	0.86 ± 0.02
Glucose	36.78 ± 0.37
Xylose	15.57 ± 0.26
Mannose	1.33 ± 0.01
Galacturonic acid	1.96 ± 0.07
Glucuronic acid	0.53 ± 0.04

Expressed in % (weight)

Table 2 Biochemical composition of extractives issued from HCl or water treatments of chenevotte

	HCl extracts	SD	Water extracts	SD
Ash	36.3 (1.0)	2.7	45.5 (1.1)	0.2
Protein	0.30 (0.01)	0.01	2.1 (0.05)	0.4
Lignin	43.9 (1.3)	0.6	23.5 (0.6)	2.2
Total monosaccharides	16.6 (0.5)	2	7.2 (0.2)	0.6
Fucose	0.03 (0.01)	0.00	NQ	–
Rhamnose	1.29 (0.04)	0.14	0.60 (0.02)	0.05
Arabinose	0.42 (0.01)	0.02	0.56 (0.01)	0.00
Galactose	2.38 (0.07)	0.29	1.14 (0.03)	0.09
Glucose	10.1 (0.3)	1.22	1.45 (0.04)	0.08
Xylose	0.55 (0.02)	0.07	1.02 (0.03)	0.23
Mannose	0.93 (0.03)	0.11	0.35 (0.01)	0.03
Gal acid	0.53 (0.02)	0.13	0.68 (0.02)	0.05
Glu acid	0.31 (0.01)	0.07	1.40 (0.04)	0.08

Expressed in % (weight) of extractives; in brackets: expressed in % (weight) of initial chenevotte sample dry matter

SD standard deviation, *NQ* nonquantifiable

by (Ridley et al. [25]). In any case, the exact attribution of each sugar to a particular component would require specific fractionation methods that were not attempted here.

Products extracted from chenevotte by water and by dilute HCl

The impregnation by dilute HCl led to the removal of 2.9 % of the initial dry matter from the samples (Table 2). This is the same magnitude obtained after water impregnation (2.5 % of the raw material). In both cases, the extractives were composed from various components of the cell wall, as discussed below. The HCl extracts contained nearly two times more monosaccharide and lignin than the water extracts, which contained more ash and protein (1.5 and 8 times more, respectively). The higher amount of saccharide and lignin extracted with HCl confirms the hypothesis that acid treatment simultaneously extracts lignin and polysaccharides as either isolated components or covalently linked structures, such as LCC (Choi et al. [26]).

Origin of the products found in the water and HCl extracts

Table 2 also shows the amount of total monosaccharide analyzed in the HCl and water extracts. Under our experimental conditions, water should not split linkages within the cell wall. Therefore, the glucose found as one main component in the extracts may come from low molecular weight hemicelluloses containing glucose side chains and monomers of residual glucose produced by plant primary metabolism (Cosgrove [27]). In contrast, the significantly higher amount of glucose in the HCl extracts suggests that a part of it originates from degraded cellulose. This hypothesis is further supported by the data demonstrating that glucose was liberated from amorphous or crystalline cellulose free of hemicelluloses, when HCl impregnation was performed under the same experimental conditions with chenevotte. Indeed, TLC (Fig. 3) revealed a higher amount of glucose when cellulose was treated with HCl than with water (spots 2 and 1, respectively). A higher amount of glucose was also apparent in extracts from acid-treated woody hemp core (spot 4). Moreover, the water solubilized products of the woody hemp core contained more than monomeric glucose, presumably dimers or trimers of glucose compounds (spot 3), whereas the HCl extracts displayed only monomeric glucose on TLC, as seen by the single spot corresponding to glucose (spot 4). This demonstrated that the other oligosaccharides compounds solubilized in the presence of the dilute HCl used herein were further hydrolyzed in the medium and/or in the impregnated chenevotte.

Glucuronic and galacturonic acids accounted for nearly 30 % of the total amount of water extractives (Table 2), indicating the presence of acidic pectins. They may also include rhamnogalacturonans, ramified with short chains composed of the arabinose (0.56 %), rhamnose (0.6 %) and galactose (1.14 %) identified in the extracts. These last three monosaccharides are known to be abundant in the primary cell wall and middle lamellae of plants (Ridley et al. [25]). Glucuronoxylans may also be present, with chains of xylose (1.02 %) abundant in hemp fibers (Cronier et al. [8]).

Galactose was the main non-cellulosic monomer removed from woody hemp core by both extractions. A small percentage of xylose was also removed, supposedly derived from xyloglucans which are known to be quite abundant in the primary cell wall of dicotyledons

(Buchanan et al. [24]). The amount of mannose (representing ~5 % of the total extractives for each solvent) suggested the presence of glucomannans which are easily isolated from water-soluble extractives of flax fibers (Jacobs et al. [28]). The larger proportion of arabinose extracted with water, compared to HCl solution, indicates the occurrence of arabinogalactans (AG). The simultaneous presence of proteins in extracts and AG oligosaccharides (Table 2) indicate that arabinogalactan protein can be present in the recovered products (Parsons and Hogg [29], Girault et al. [30]).

Significant amounts of inorganic compounds were also found in the extracts (Table 3). The minerals mainly consisted of potassium among several other species. The woody hemp core was used in our experiment as received from the factory, without a prewashing step to avoid any prior extraction by water. Therefore, the high concentration of potassium in the extracts might be derived from residual fertilizers present in the soil or dust deposited on the plant stem and chenevotte either in the field or at the factory.

Excluding the exogenous potassium assumed to be mostly a contaminant, only calcium and magnesium were extracted in relatively significant amounts during water and dilute HCl impregnation. Calcium is known to be a structural mineral, which contributes to the conformation of pectic polygalacturonic acids. Even though the amount of pectin in the secondary cell wall is low, a slight removal of pectic calcium may occur during acid treatment, therefore destabilizing pectins and leading to greater modification of the cell wall structure. Transition metals like Cu, Fe, Mn and Zn are equally extracted by water and dilute HCl, suggesting that a presumed abiotic oxidative degradation of cell walls may not occur in our case (Paszczynski et al. [31], Xu and Goodell [32]).

In conclusion, the main difference between the HCl and water extractions was the amount of simultaneous removal of glucose and lignin. This was probably a consequence of: (1) the partial degradation of cellulose; (2) the removal of some LCC complexes; and (3) the extraction of cell wall-bound hemicelluloses and pectin-related substances by the moderate HCl treatment. All of these modifications may destabilize the global polysaccharide and lignin network of the cell wall, and may also modify their environment, as confirmed below by the study of the viscoelastic properties of chenevotte.

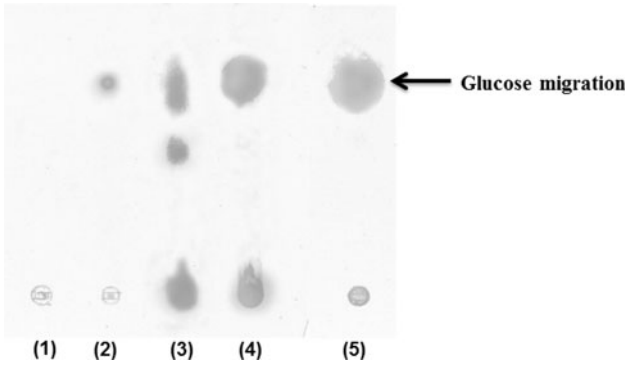

Fig. 3 Thin-layer chromatography of water-impregnated cellulose (*1*), HCl-impregnated cellulose (*2*), water extracts from woody hemp core (*3*), HCl extracts from woody hemp core (*4*) and glucose (*5*)

Variation of lignins and hemicelluloses *T*g

Figure 4 depicts the changes occurring in lignin T_g, whereas Fig. 5 shows the Arrhenius plot of the hemicellulose T_g after HCl (a) or water (b) impregnation. When chenevotte was impregnated by HCl, we observe either a positive or negative variation of the lignin T_g, depending on the stick (Fig. 4a). This suggests that the impact of the HCl treatment on the softening behavior of the polymer is probably governed by several interacting factors. These later include the ability of the reactants to impregnate the sample, which is related to the thickness and/or microstructure of the chenevotte, which further depends on the origin of the stick (stem and relative place in the stem). All these parameters may then impact the local reactivity and resulting local chemistry, which seemed to be associated to the natural variability of the industrial products used. In contrast, water extraction led to a systematic net increase of T_g, suggesting a better wettability and/or homogeneous behavior (Fig. 4b). However, in all cases, the average of pooled lignin T_g values were not significantly different for either the moderate HCl treatment (109 ± 10 °C for the treated and 112 ± 12 °C for the untreated portions) or the water treatment (106 ± 5 and 110 ± 7 °C in the same order). This points out the usefulness here of comparing perfectly matched paired sticks to increase the signal/noise ratio and to clearly describe the effect of water and acid treatments on the in situ polymers.

Table 3 Mineral composition of extractives issued from HCl or water treatments of chenevotte

	Ca	Cu	Fe	K	Mg	Mn	Na	Zn
HCl	3.97 (0.12)	(−)	0.05 (−)	22.14 (0.64)	0.88 (0.03)	0.01 (−)	0.52 (0.02)	0.01 (−)
Water	1.19 (0.03)	(−)	0.04 (−)	21.19 (0.53)	0.24 (0.01)	(−)	0.18 (−)	0.01 (−)

Expressed in % (weight) of extractives; in brackets: expressed in % of initial chenevotte sample dry matter; (−) trace amounts (below 0.01 %)

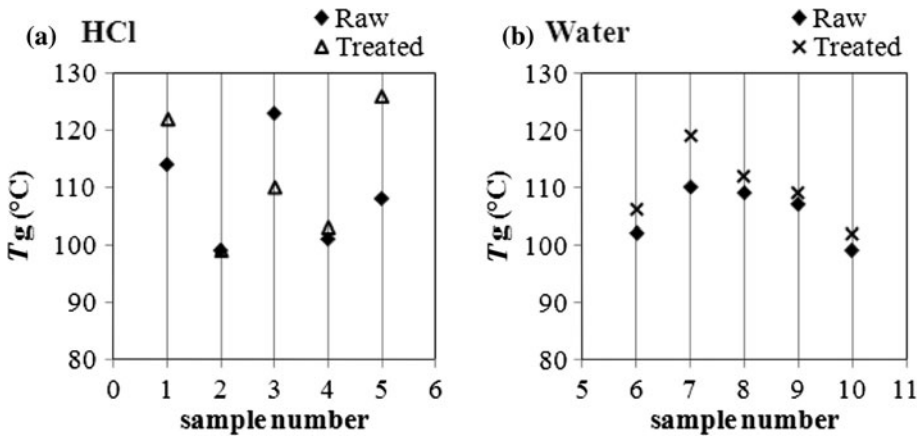

Fig. 4 Softening temperatures of lignin (T_g) after HCl treatment (**a**) and water treatment (**b**). *Filled diamond* raw, *empty triangle* treated with HCl and *cross* treated with water

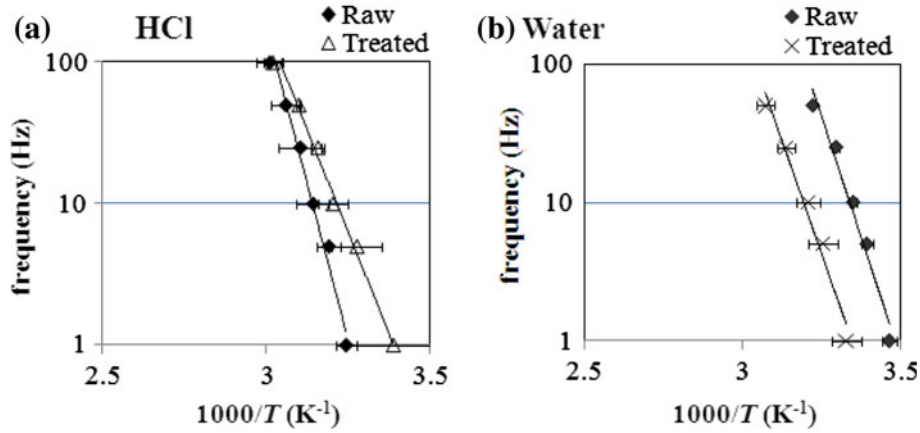

Fig. 5 Arrhenius plot of hemicelluloses after **a** HCl treatment and **b** water treatment. *Filled diamond* raw, *empty triangle* treated with HCl and *cross* treated with water

In contrast, hemicelluloses were affected unambiguously by water or acid impregnations, but not in the same way (Fig. 5). With moderate HCl treatment, the T_g values were slightly decreased (data not shown), and the apparent activation energy required to enhance polymer mobility was lower after the extraction. This decrease of E_a (from 389 to 254 kJ/mol) revealed a significant change in the softening mechanism. With water treatment, the mechanism of hemicellulose relaxation remained the same, as the slope of the curve after impregnation was unchanged, whereas the T_g values clearly increased. Again, the paired comparison of perfectly matched half parts of several sticks clearly showed the impact of the treatments, despite variable net values of the activation energy of the raw samples used.

The removal of low molecular mass hemicelluloses and lignin-like components from the cell wall during water impregnation seemed to have a uniform 'deplasticizing' effect on lignin and hemicelluloses, since their T_g values were slightly but systematically higher after extraction. In contrast, the chenevotte impregnation by HCl showed a more complex modification pattern: the T_g of hemicelluloses which decreased the softening mechanism was altered, and the lignin T_g exhibited variable behavior. Such marked differences compared to the water treatment would be due to the combination of several direct and indirect effects on the lignins. Indeed, whereas a restrained hydrolysis of some hemicellulose fragments or domains was demonstrated, dragging of the lignins associated with these saccharidic fragments during extraction is likely to occur (see discussion above and Fig. 4). Next, the lignin may be repolymerized under these mild acidic conditions, as demonstrated previously (Meyer-Pinson et al. [3], Pouteau et al. [33]), leading to the increase of the T_g observed for some samples. Finally, acid washing of Ca^{2+} ions might also alter the pectin structure and prompt changes in the viscoelastic properties of the material (Lootens et al. [34]).

Altogether, our data strongly suggest that the simultaneous removal of low amounts of linked or interacting lignin fragments with oligosaccharides are the basic cause of the disturbance of the physicochemical environment of the remaining amorphous polymers of the cell wall. There is also strong evidence that the mild acidic treatment acts as a water impregnation, modifying mostly the environment, combined with specific molecule degradation related

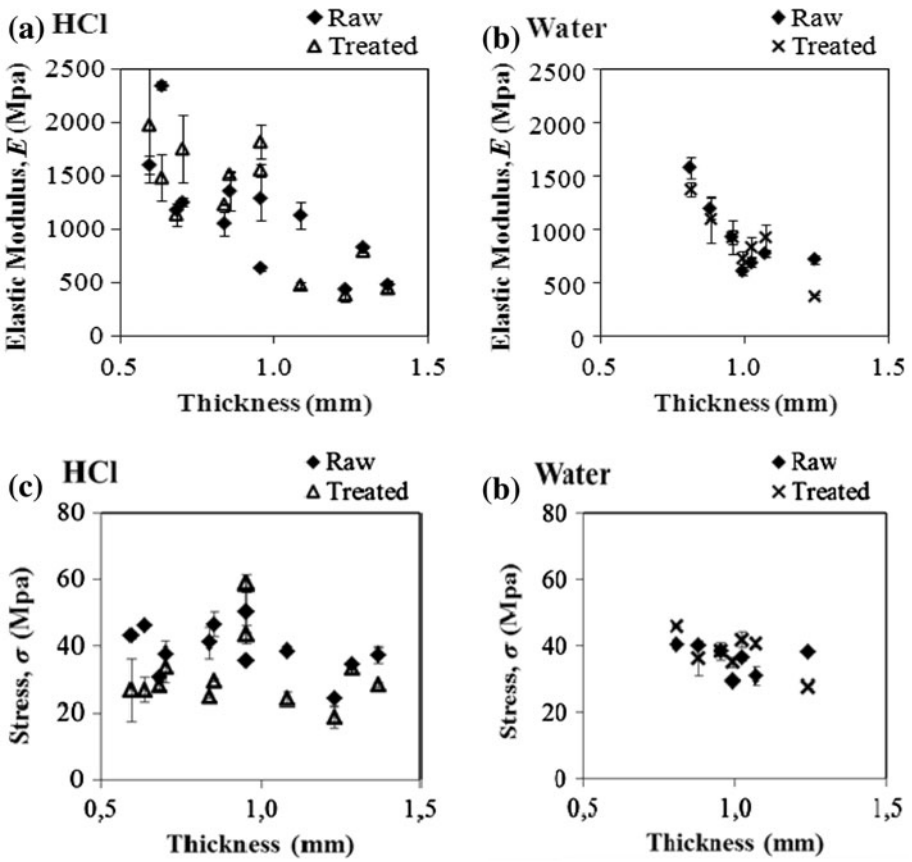

Fig. 6 Mechanical properties representing the elastic modulus, E in MPa (**a**, **b**) and the stress, σ in MPa (**c**, **d**) as a function of sample thickness (in mm). *Filled diamond* raw, *empty triangle* treated with HCl and *cross* treated with water

to acid hydrolysis. This in turn modifies the lignin and polysaccharide structures with simultaneous changes in the softening properties of the matrix chains and mobility.

Breaking properties

Figure 6 represents the shifts in elastic modulus and stress properties of chenevotte sticks after HCl (a) or water (b) impregnations, relative to their paired untreated part (raw). It appears first that the elasticity modulus decreased when sample thickness increased, which is an unexpected result. The modulus is indeed generally not dependent on sample geometry, but relies on the intrinsic properties of the material (nature and origin of wood; microstructure; chemistry (Goncalez et al. [35])). Based on our experience, we believe that thicker samples were more likely from the basal region of the plant, and the thinner ones from the apical region (Cronier et al. [8]). Such an assumption is, however, unverifiable without suitable markers for the bulky samples retained from the experiments. However, as both differences in chemical composition (Cronier et al. [8]) and density (Duval et al. [36]) have been reported for hemp fibers originating from various positions in the stem, we can assume that the observed dependency of the modulus on thickness reflects such natural structural variability combined with random sampling.

The data obtained also show that acid or water treatments did not alter the modulus significantly. Instead, the strength criterion (Fig. 6c, d) $\Delta\sigma_{max}$ decreased after mild acid impregnation, but was unchanged by water treatment. The student t test performed for the variations of the modulus and stress confirmed that HCl treatment only led to significant differences of the maximal stress at break, compared to raw sticks (0.002—on 12 samples; Table 4). It also showed that the average difference was negative, confirming that the HCl-treated samples were less resistant to force.

Figure 7a, b shows the drop value (corresponding to the displacement in mm after a 20 % decrease of the maximum force) as a function of sample thickness before (raw) and after treatments (water, HCl). The drop was always positively correlated to the sample thickness, independent of the treatment applied (water or dilute HCl). However, the drop decreased after acid treatment (Fig. 7a), regardless of the sample thickness. This means that the first failure cracks occurred earlier in the test and that the sample plasticity had changed. No modification was observed in the water-treated samples (Fig. 7b) relative to the raw material, as confirmed by the student t test (0.22 in Table 4) and the average values of Δd_{max} (0.1 ± 0.1).

The energy needed to reach the 20 % displacement was also determined ($W_{20\ \%}$; Fig. 7c, d). It was shown to be

Table 4 Student *t* test values for E, σ_{max}, d_{max} and $W_{20\ \%}$ and average values of ΔE (difference in elastic modulus), $\Delta\sigma_{max}$, (difference in stress), Δd_{max} (difference in drop) and $\Delta W_{20\ \%}$ (difference in fracture energy) for pairs of samples treated with HCl or water relative to raw material

	E	σ_{max}	d_{max}	$W_{20\ \%}$
Student *t*				
HCl	0.248	0.002	1.6×10^{-6}	6.9×10^{-5}
Water	0.329	0.269	0.223	0.066
	ΔE (MPa)	$\Delta\sigma_{max}$ (MPa)	Δd_{max} (mm)	$\Delta W_{20\ \%}$ (J/m^2)
Mean value				
HCl	85 ± 261	−7.4 ± 4.7	−0.3 ± 0.1	−2.3 ± 1.2
Water	−33.5 ± 78.9	1.7 ± 2.8	0.1 ± 0.1	−1.4 ± 1.1

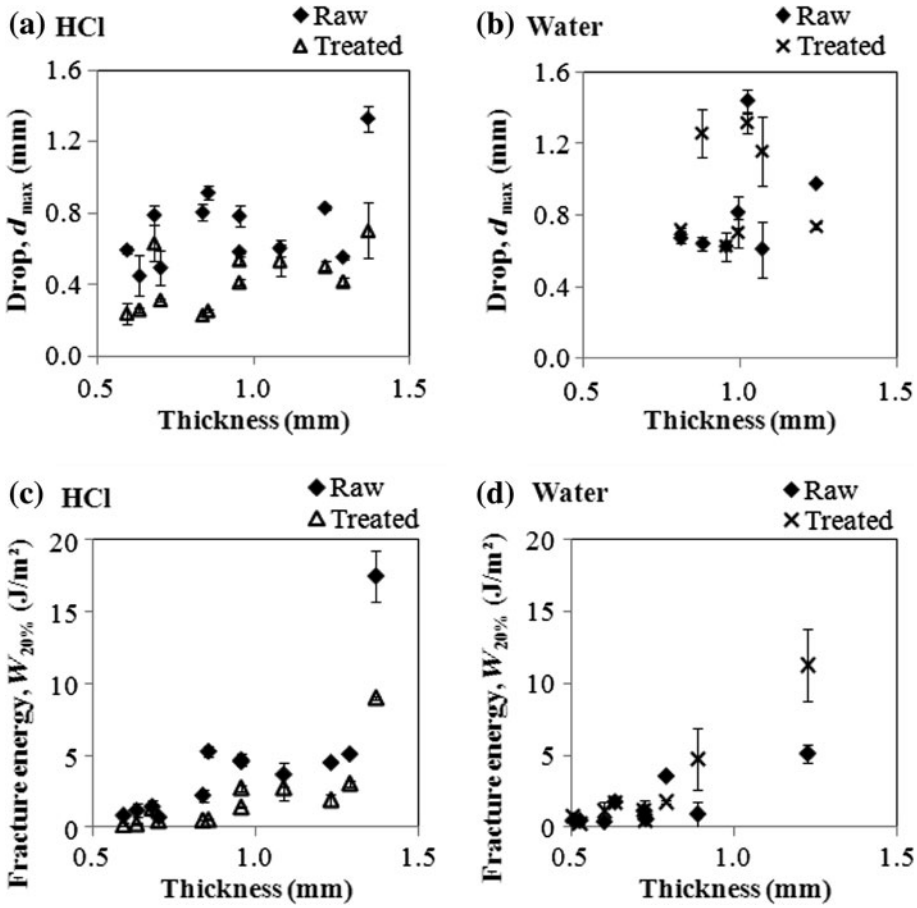

Fig. 7 Mechanical properties representing the drop, d_{max} in mm (**a**, **b**) and the fracture energy, $W_{20\ \%}$ in J/m^2 (**c**, **d**) as a function of sample thickness (in mm). *Filled diamond* raw, *empty triangle* treated with HCl and *cross* treated with water

lower in all HCl-treated samples, proving that they were more brittle than in the water test. In this latter case, the fracture energy after water impregnation (Fig. 7d) stayed constant relative to the raw material, as confirmed by the student *t* test value (0.066 in Table 4) and the average value of $\Delta W_{20\ \%}$ (-1.4 ± 1.1 in Table 4). These results demonstrate that the HCl treatment led to a decreasing of both the drop value and the related fracture energy, whereas water extraction had no impact on energy evolution.

The mechanical properties of the woody hemp core were clearly affected by the mild HCl treatment but not by the water extraction. Wood rigidity is mainly dependent on the amount of crystalline cellulose and orientation of the fibril angle (Åkerholm et al. [37], Wang et al. [38], Stanzl-Tschegg [39]). In our case, the increasing weakness in mechanical properties compared to the water impregnation would be mainly due to cellulose degradation, combined with the other modifications discussed in "Impact of HCl and water on the chenevotte and on the extracted products".

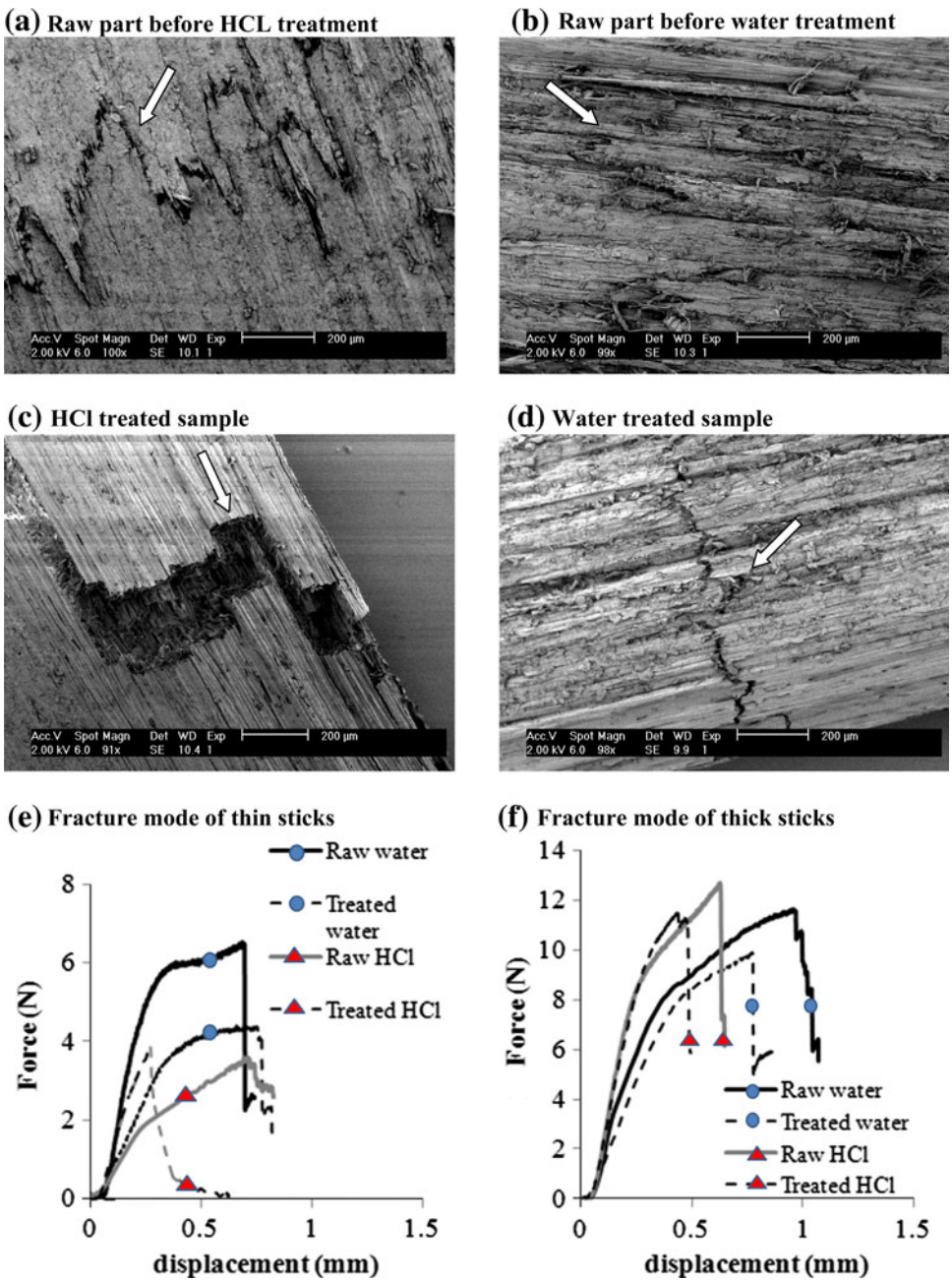

Fig. 8 SEM images of the fracture (*arrows*) of the raw parts (**a**, **b**) and of their treated parts with HCl (**c**) and water (**d**); corresponding fracture curves obtained for HCl and water treatment of thin (**e**) or thick (**f**) samples

Fracture behavior of chenevotte after water and HCl extraction

Structural modification of chenevotte was directly observed by SEM imaging (Fig. 8) of the HCl-treated pairs (Fig. 8a, c) and the water-treated pairs (Fig. 8b, d) of hemp core sticks. The surface of the HCl-treated sample (Fig. 8c) seemed to be smoother than that of the water-impregnated chenevotte (Fig. 8d), as if it had been cleaned. The breaking surface (arrows in figures) showed that failure was sharper and sometimes transverse or parallel to the fiber direction (c), which could explain the smaller drop values. Propagation of the fracture did not seem to be as straightforward in the untreated or water-treated samples (a, b, d), which might explain the higher drop values of the raw material. The fracture was more likely to be cubic and straight in the HCl-treated sample and more random in the untreated samples. This breaking aspect is typical cubic cracking, both with and across the grain observed in wood degradation made by brown rot fungi (Schwarze [40]).

Analysis of the fracture curves of paired samples in Fig. 8e, f revealed a more ductile breaking behavior of the raw sample, with two elastic and plastic domains distinguishable. The fracture was different in the thin

HCl-treated sample (e), occurring sooner and indicative of the brittle nature of the material. These results are consistent with the structural modification of woody hemp observed by SEM (Fig. 8a–d).

Under our experimental conditions, water extraction of chenevotte only affected polymer chain mobility of lignin and hemicelluloses. This modification could be due to the plasticizing effect of the water-soluble entities or extractives, as previously suggested (Bag et al. [7], Obataya, Eiichi and Norimoto [41], Obataya et al. [42]). However, no measurable or observable consequences on the breaking mechanism could be evidenced here. In contrast, after a mild acid impregnation leading to slight hydrolysis, the fracture mode, breaking region and surface appearance of the chenevotte stick were different. The changes in strength properties were mainly due to the removal and depolymerization of structural entities from the cementing matrix which induced sample brittleness, whereas with water extraction, only polymer chain mobility was generated. A separate and predominant effect of depolymerization over extracted entities on the modification of the fracture mechanism and matrix properties is demonstrated.

Nevertheless, it must be noted that the use of a non-ideal raw material derived from the mechanical separation of fibers from hemp stem may enhance some specific reactions or effects. Indeed, several structural defaults are already present inside the chenevotte sticks due not only to natural growth but also to the industrial separation process. The occurrence of such defaults is likely to be a predominant factor determining the site of acid solution attack, particularly on thinner specimens, rendering the samples more fragile at these sites (Fig. 8e, f). Thus, the macromechanical properties of the woody hemp core may be influenced not only by viscoelastic properties but also by the presence of macroscopic 'faults', which can be different in thick or thin sticks.

Conclusions

The mild chemical modification by HCl involved less than 3 % of the total sample mass, but clearly affected mechanical properties of the chenevotte. The acidic treatment extracted lignins and slightly hydrolyzed cellulose and hemicelluloses, disturbing the polymer networks. The viscoelastic behavior of the in situ amorphous polymers was then consequently impacted, with variations of their T_g and activation energy. At the upper scale, mild HCl modified the fracture behavior of chenevotte, which became more brittle. Controlled acid catalysis could then be a good strategy for biomass pretreatment, where control of mechanical properties and/or accessibility to specific reaction sites is needed.

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