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1 **Meaning of xylan acetylation on xylan-cellulose**
2 **interactions: a quartz crystal microbalance with**
3 **dissipation (QCM-D) and molecular dynamic**
4 **study**

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18

19

20 **ABSTRACT**

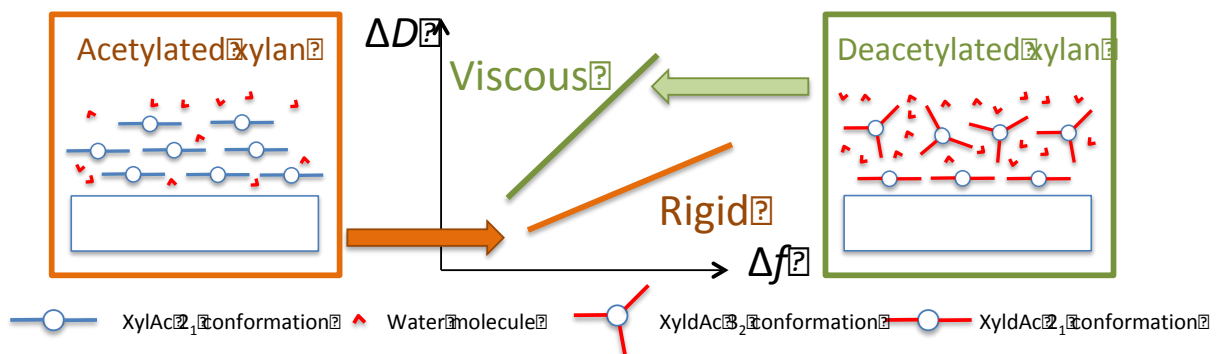
21 In plant cell walls, xylan chains present various substituents including acetate groups. The
22 influence of the acetyl substitution on the organization of xylan-cellulose complexes remains
23 poorly understood. This work combines *in vitro* and *in silico* approaches to decipher the
24 functional role of acetyl groups on the xylan/cellulose interaction. Acetylated xylans were
25 extracted from apple pomace with dimethyl sulfoxide-lithium chloride (DMSO-LiCl) and
26 deacetylated using a mild alkali treatment. The adsorption behavior of acetylated and
27 deacetylated xylan fractions was investigated using quartz crystal microbalance with
28 dissipation (QCM-D) and molecular dynamics. Acetylated xylans form a dense and poorly
29 hydrated and rigid layer on cellulose with xylan chains that have two residues per helical turn
30 conformation, whereas the deacetylated fraction forms a swollen and more viscous layer in
31 which only the xylan chains in direct contact with the cellulose surface have two residues per
32 helical turn conformation. The other chains have three residues per turn conformation.

33 **Keywords:**

34 Xylan; acetyl group; cellulose nanocrystal; quartz crystal microbalance with dissipation
35 (QCM-D); molecular dynamics; hemicellulose.

36

37 **Graphical abstract**



41 **INTRODUCTION**

42 Plant cell walls are highly complex materials that consist of an intricate network of
43 biopolymers whose main components are polysaccharides. These components include
44 cellulose, pectin and hemicellulose. As a result of their many uses, their structure and function
45 *in planta* has been the focus of many studies (Rojas, 2016; Voragen, Schols, & Visser, 2003).
46 Hemicellulose represents a group of various polysaccharides traditionally extracted by
47 alkaline treatment from plant cell walls. These polysaccharides in dicots are based on a β -(1-
48 4) linked backbone of xylose or glucose and/or mannose, forming xyloglucan, xylan, mannan
49 and glucomannan families (Scheller & Ulvskov, 2010). They exhibit large variations in
50 composition and structure depending on their botanical origin, tissue type and developmental
51 stage. This is particularly true of their backbone substitutions with side chains of saccharide
52 units, hydroxycinnamic acids, methyl ether and acetyl ester groups (Brummell & Schröder,
53 2009; Gatenholm & Tenkanen, 2004; Scheller & Ulvskov, 2010). Specific substitution
54 patterns and their role *in planta* remain unclear. Acetyl esterification is a ubiquitous
55 substitution within hemicellulose families. It usually occurs on O-2 and/or O-3 of xylose in
56 xylan, on O-6 of galactose and/or mannose, and O-2 and/or O-3 of mannose in
57 mannan/glucomannan (Melton, Smith, Ibrahim, & Schröder, 2009; Sims, Craik, & Bacic,
58 1997), and on fucose and/or O-6, O-4 or O-3 of galactose in xyloglucan, although the exact
59 position has yet to be fully established (Quemener et al., 2015; York, Oates, van Halbeek,
60 Darvill, & Albersheim, 1988). The alteration of xylan acetyl esterification *in planta* severely
61 impacts the plant phenotype and its resistance to biotic stresses (Escudero et al., 2017;
62 Grantham et al., 2017; Kumar, Campbell, & Turner, 2016; Yuan, Teng, Zhong, & Ye, 2016).
63 From the applied point of view, decreasing xylan acetyl esterification improves enzymatic

64 saccharification in the lignocellulosic biomass biorefinery (Kumar et al., 2016; P. M. Pawar et
65 al., 2016).

66 Accordingly, understanding the role of the hemicellulose structure in terms of its interactions
67 with cellulose and its consequences on cell wall architecture and properties remains a key
68 question in plant development and applications. The present study focuses on the role of
69 acetyl ester decorations on xylan interactions with cellulose by combining *in vitro* and *in*
70 *silico* experiments to assess the adsorption features of acetylated and deacetylated xylans.

71 In the case of *in vitro* experiments, a major challenge is the availability of unmodified xylan
72 fractions since acetyl esters are lost when using classical alkaline hemicellulose extractions.
73 However, acetylated xylans can be recovered from wood biomass using DMSO extraction,
74 steam explosion or organosolv pulping (Gatenholm & Tenkanen, 2004). In rich primary cell
75 wall biomass such as fleshy fruit, acetyl-esterified hemicellulose is recovered after the partial
76 removal of pectin in the cell wall via dimethyl sulfoxide doped by lithium chloride (DMSO-
77 LiCl) (Assor, Quemener, Vigouroux, & Lahaye, 2013; Ray, Vigouroux, Quemener, Bonnin,
78 & Lahaye, 2014).

79 In this study, interactions of acetylated and deacetylated xylan-enriched fractions obtained by
80 this approach from apple pomace were assessed on a cellulose model surface using quartz
81 crystal microbalance and dissipation (QCM-D). QCM-D is a highly effective mass-sensing
82 technique based on the piezoelectric properties of quartz crystals. It has been successfully
83 used to monitor the hemicellulose adsorption on the cellulose nanocrystal model surface. In
84 fact, it provides information about the mass adsorbed on the surface as well as the mechanical
85 behavior of the layer through the measurement of the dissipation factor (Bensselfelt et al.,
86 2016; Eronen, Osterberg, Heikkinen, Tenkanen, & Laine, 2011; Kohnke, Ostlund, & Brelid,
87 2011; Villares, Bizot, Moreau, Rolland-Sabate, & Cathala, 2017; Villares, Moreau, Capron, &
88 Cathala, 2014; Villares, Moreau, Dammak, Capron, & Cathala, 2015). Recently, Villares et

89 al. reported the kinetically-driven xyloglucan adsorption on the cellulose surface and
90 described a two-regime mechanism (Villares et al., 2015). A similar approach was applied to
91 investigate the adsorption behavior of acetylated and deacetylated xylan-enriched fractions
92 referred to as XylAc and XyldAc, respectively, which display different behaviors. As a
93 complement to the *in vitro* experiment, molecular dynamics (MD) were used to explore the
94 conformation of xylan chains at the surface of cellulose crystals and to assess their
95 interactions with water.

96

97 MATERIAL AND METHODS

98 Chemicals

99 Dimethyl sulfoxide (DMSO), lithium chloride (LiCl), 37% hydrochloric acid (HCl), orcinol,
100 standard monosaccharides, sodium tetraborate, 1-methylimidazol, acetic anhydride, sodium
101 borohydride, ammonia 25%, hydrogen peroxide and dichloromethane were provided by
102 Sigma-Aldrich Chimie (Saint-Quentin Fallavier, France). Poly(allylamine hydrochloride)
103 (PAH) was purchased by PolySciences (Mw 120-200 000 g mol⁻¹; sodium hydroxide (50%)
104 (NaOH) by Fluka (Sigma-Aldrich Chimie, Saint-Quentin Fallavier, France); m-
105 hydroxydiphenyl (MHDP, 3 phenylphenol) by Acros Organics (Geel, Belgium); sulfuric acid
106 by Fisher Scientific, Illkirch, France; and ethanol 99% by VWR (Fontenay-sous-Bois,
107 France).

108

109 *Cellulose Nanocrystal (CNC) Suspension*

110 Cellulose nanocrystals (CNCs) were obtained by sulfuric acid hydrolysis of cotton linters
111 according to Revol et al. (Revol, Bradford, Giasson, Marchessault, & Gray, 1992). Cotton
112 linters were hydrolyzed with 65% wt H₂SO₄ at 65°C for 35 min (12 g cotton linters/170 mL
113 H₂SO₄). Immediately following hydrolysis, the suspension was diluted 10-fold to stop the

114 reaction and then washed by centrifugation at 10000 rpm for 10 min in order to concentrate
115 the cellulose and to remove the excess aqueous acid. The resulting precipitate was repeatedly
116 rinsed **with ultrapure water** and recentrifuged until a colloidal suspension was obtained. The
117 colloidal suspension was then dialyzed again with distilled water until neutrality (dialysis
118 membranes MWCO 12-14kDa, Fisher Scientific), after which a mixed-bed research-grade
119 resin (1 g resin/400 g CNC suspension; Sigma-Aldrich TMD-8) was added to the suspension
120 for 48 h to remove residual ions. The final aqueous suspension was 2% concentration by
121 weight (~45% yield). The average crystal dimensions (length and cross-section) measured by
122 tapping-mode atomic force microscopy (AFM) were 100-200 nm x 6-8 nm. Charges
123 determined by conductometric titration were equal to 0.098 e/nm².

124

125 *Hemicellulose extraction*

126 Native hemicellulose enriched fractions were sequentially extracted from apple pomace
127 obtained from the “Institut Français des Produits Cidricoles” (IFPC, Le Rheu, France),
128 according to the procedure described by Ray et al. (Ray et al., 2014). First, the pectin was
129 partially extracted from fresh apple pomace (15.6 kg) with 37.5 L of a potassium oxalate
130 solution (60 mM, pH 4.7, 16 h) at room temperature (~20 °C) and with 3 x 24 L of water (2 x
131 3 h and 16 h) at 80 °C. After each extraction, the insoluble residue was recovered by manually
132 pressing through a nylon mesh. The final residue was freeze-dried, weighed and ground
133 (yield: 79% initial DW). The insoluble residue (200 g) was then incubated with 4 L of
134 DMSO-LiCl (8.4 wt%; 5 h at 100 °C) under gentle agitation and nitrogen flow. After
135 centrifugation (11000 g, 15 min, 20 °C), the supernatant was evaporated to dryness using a
136 rotary evaporator at 70 °C and the dried residue was dissolved in 300 mL of water. Native
137 hemicellulose was then precipitated with four volumes of ethanol at 4 °C for 16 h. After
138 centrifugation (11000 g, 15 min, 20 °C), **the dried pellet** was weighed and the yield of

139 DMSO-LiCl extraction was calculated as 5% initial DW. Sixteen g of native hemicellulose
140 were fractionated by anion exchange chromatography (DEAE- Sepharose Fast Flow column,
141 5 cm × 15 cm, GE Healthcare, Uppsala, Sweden) (Ray et al., 2014). Elution was performed at
142 a flow rate of 2 mL min⁻¹ with water (two column volumes, 600 mL) to recover the unbound
143 fraction. Bound polysaccharides were eluted using a gradient (six column volumes) from 0 to
144 1.0 M NaCl. XylAc was recovered in the second carbohydrate eluting peak (~400 mL
145 elution, ~0.25 M NaCl) determined by colorimetry (see below), dialyzed and freeze-dried
146 (yield: 500 mg, i.e., 0.14% initial DW). An additional removal of pectin was then performed
147 by pectin lyase (0.12 U, 3 h at 40 °C, [EC 4.2.2.10] purified from Peclyve Lyven, France)
148 (Ralet et al., 2012). The XylAc fraction (100 mg) was deacetylated by saponification with 20
149 mL of 0.5 M NaOH (1 h at 4 °C). After neutralization with HCl, the **deacetylated fraction,**
150 **referred to as XyldAc,** was desalted by dialysis against deionized water (MWCO 3500 Da,
151 CelluSep H1) and freeze-dried in order to obtain (the yield is greater than 90% DW of the
152 XylAc fraction).

153

154 *Hemicellulose characterization*

155 The total neutral sugar content was determined by colorimetry with an automated
156 orcinol/sulfuric acid assay (Tollier & Robin, 1979). Glucose was used as a standard.

157 Uronic acid content was measured by colorimetry using m-hydroxydiphenyl and concentrated
158 sulfuric acid hydrolysis (Blumenkrantz & Asboe-Hansen, 1973; Thibault, 1979). The
159 distinction between galacturonic acid and glucuronic acid was made by comparing
160 colorimetric responses with and without the addition of sodium tetraborate in the acid,
161 according to the procedure used by Filisetti-Cozzi, & Carpita (1991). Galacturonic and
162 glucuronic acids were used as the standard.

163 Identification and quantification of neutral sugars were performed by gas-liquid
164 chromatography on a TG-225 GC Column (30 x 0.32 mm ID) using a TRACE™ Ultra Gas
165 Chromatograph (Thermo Scientific™; column temperature: 205 °C; split injector
166 temperature: 220 °C; flame ionization detector temperature: 250 °C; carrier gas: H₂) after
167 sulfuric acid degradation and derivatization as alditol acetates (Dheilly et al., 2016).

168 Acetyl ester content was measured by HPLC as the amount of acetic acid released by
169 saponification of a 5-mg sample in 1 mL 0.5 M NaOH for 1 h at 4 °C. HPLC was carried out
170 on a C18 column (4 mm × 250 mm, Lichrospher 100 RP-18e (5 μm), Interchim, France)
171 thermostated to 25 °C using a refractometric detector (Waters, 2414). An isocratic elution of
172 4 mM H₂SO₄ was used at a flow rate of 1.0 mL min⁻¹ (Levigne, Thomas, Ralet, Quemener, &
173 Thibault, 2002).

174

175 *Surface preparation*

176 Piezoelectric AT-cut quartz crystals coated with gold electrodes on each side (QSX301, Q-
177 Sense) were used for QCM-D experiments. Silicon wafers were used for AFM experiments.

178 Quartz crystals and silicon wafers were cleaned with H₂SO₄/H₂O₂ (7:3, v/v), rinsed
179 exhaustively with Milli-Q water, and dried under a nitrogen stream. Prior to use, QCM-D
180 quartz sensors were subjected to a plasma-etching device (Harrick Plasma). CNC surfaces on
181 quartz crystals were prepared by the spin-coating method, as previously described (Villares et
182 al., 2015). A CNC dispersion at 3 g L⁻¹ was dropped onto a pre-coated substrate with
183 poly(allylamine hydrochloride) at 1 g L⁻¹ in water and accelerated from 180 rpm to 3600 rpm
184 after 5 min of adsorption and maintained for 60 s at that speed using an expressly designed
185 spin-coater. The thickness of the CNC layer, evaluated by ellipsometry, was 9 nm ± 5 nm, in
186 agreement with previous reports (Villares et al., 2017; Villares et al., 2015). Xylan solutions

187 at 1 g L^{-1} were then dropped onto wafers pre-coated with CNCs in order to perform AFM
188 experiments.

189

190 *Quartz crystal microbalance with dissipation (QCM-D) experiments*

191 QCM-D experiments were carried out using a Q-Sense E4 instrument (AB, Sweden). Spin-
192 coated CNC surfaces were mounted in the QCM-D cells at $20 \text{ }^\circ\text{C}$ and equilibrated with water
193 at a flow rate of $50 \text{ } \mu\text{L min}^{-1}$ until the resonance response was stable. The frequency and
194 dissipation signals were then offset to zero just before the measurement.

195 Frequency ($\Delta f_n/n$) and dissipation (ΔD_n) changes were simultaneously registered at 5 MHz
196 fundamental resonance frequency ($n = 1$) and its several overtones as a function of time ($n =$
197 $3, 5, 7, \text{ etc.}$). The third overtone ($15 \text{ MHz}, n = 3$) was used to evaluate the QCM-D data.

198 Xylan solutions at different concentrations (0.3 to $20 \text{ } \mu\text{g mL}^{-1}$) were injected at $50 \text{ } \mu\text{L min}^{-1}$
199 and allowed to adsorb for 40 min before being exposed again to a water flow in order to
200 remove loosely-bound material. Each xylan solution at the desired concentration was
201 adsorbed on a freshly-prepared CNC modified surface and the experiments were repeated at
202 least three times. All QCM-D experiments were conducted at a constant temperature of $20 \text{ }^\circ\text{C}$.
203 The surface concentration (Γ) can be calculated from the mass variation during adsorption
204 obtained from the frequency change ($\Delta f_n/n$) by using the Sauerbrey equation (Sauerbrey,
205 1959):

206
$$\Delta m = - C \frac{\Delta f_n}{n}$$

207 where C is the Sauerbrey constant: constant for the mass sensitivity of the quartz crystal
208 ($0.177 \text{ mg m}^{-2} \text{ Hz}^{-1}$ for 5-MHz crystals); and n is the overtone number. Strictly speaking, the
209 Sauerbrey equation is valid for ultrathin rigid films whose frequency and dissipation changes
210 only depend on the mass of the film, especially for adsorbed polymers. A thin film can be
211 considered to be fully elastic and rigid when $\Delta D \leq 1 \cdot 10^{-6}$ and no clear spreading of the

212 overtones in the $\Delta f_n/n$ and ΔD_n graphs can be detected. Under such conditions, the Sauerbrey
213 equation is valid for estimating the mass changes on the sensor surface.

214 A complete description of the kinetic modeling approach is described in Appendix 1 of the
215 Supplementary Materials (SM).

216

217 *Characterization of CNC surfaces coated with xylan fractions*

218 AFM: Topographical images of silicon wafers were recorded by atomic force microscopy
219 (AFM) using an Innova AFM (Bruker). The images were collected in tapping mode under
220 ambient air conditions (temperature and relative humidity) using a monolithic silicon tip
221 (TESPA, Bruker) with a spring constant of 42 N m^{-1} and a nominal frequency of 320 kHz.
222 Image processing was performed using WSxM 5.0 software.

223

224 *Molecular dynamics (MD) simulations*

225 The models considered in this study consisted of three molecular species: a cellulose
226 microfibril, xylan chains and water molecules. The cellulose microfibril model contained 18
227 chains of cellulose (Kubicki et al., 2018) in the I β organization and exposed the (110), (1-10)
228 and (100) surfaces (Mazeau, 2011). The two primitive cell chains of the I β allomorph
229 (Nishiyama, Langan, & Chanzy, 2002) were first duplicated through space, and a P₁ periodic
230 super-crystal was redefined, which consisted of 6x6 cellulose chains. Each individual chain
231 consisted of six anhydroglucose units and was covalently bonded to its own periodic image,
232 mimicking a chain of infinite length. The supercell was then equilibrated using NPT-MD
233 (constant number of particles, pressure and temperature) at 300 °K and 1 atm. The **a** and **b**
234 parameters of the periodic cell were then increased to 50 Å and the excess cellulose chains
235 were removed to retain only 18 chains. The **c** parameter was maintained at its equilibrium
236 value of 31.4 Å. A charged sulfate group (SO₃⁻) was added on an O-6 oxygen atom of the (1-

237 10) surface to model a cellulose crystal prepared by sulfuric acid, and a Na⁺ cation was also
238 inserted in the simulation box to ensure the neutrality of the system. The model is infinitely
239 long in the longitudinal direction but finite in the perpendicular direction. This model was
240 then hydrated using the simple three-point-charges water model (Berendsen, Postma, Van
241 Gunsteren, & Hermans, 1981) and equilibrated by 50 ns of NVT-MD (constant number of
242 particles, volume and temperature) at 400 °K. This equilibration resulted in a conformational
243 change of the hydroxyl and hydroxymethyl groups exposed to the water environment, the
244 hydroxyl groups created hydrogen bonds with the water molecules, and the surface
245 hydroxymethyl groups rotated from the *tg* orientation to a mixture of the *gg* and *gt*
246 orientations. The water molecules were then removed to generate the initial models of the
247 cellulose-xylan complexes.

248 Xylan chains consisted exclusively of six skeletal anhydroxylose units. They are either non-
249 substituted or per-acetylated. In the latter case, all of the units were O-acetylated at the O-2
250 and O-3 oxygen atoms. Two helical conformations, 2_1 and 3_2 , of the xylan skeleton were
251 considered. Their coordinates were extracted from a previous study (Mazeau, Moine, Krausz,
252 & Gloaguen, 2005). For the two helical conformations of the per-acetylated xylan chain, the
253 initial orientation of the acetyl groups corresponded to the lowest energy extracted from a
254 Monte-Carlo sampling of 1000 conformations on an isolated chain with a rigid backbone
255 (SM, Appendix 2).

256 The xylan chains were inserted in the simulation box around the cellulose microfibril. They
257 were aligned with the microfibril, either parallel or antiparallel at random. Similar to the
258 cellulose chains, the xylan chains were linked to their periodic images by covalent bonds in
259 order to mimic infinitely long chains. The two acetylated models, XylAc, consisted of 20
260 chains of fully acetylated xylan chains (total mass: 25920 g mol⁻¹), whereas the two non-
261 acetylated models, XyldAc, consisted of 33 chains of non-substituted xylan chains (26136 g

262 mol⁻¹). Water molecules were then added in the remaining empty spaces of the simulation
263 boxes.

264 The models were then subjected to energy minimizations followed by MD simulations. The
265 first energy minimization was performed while maintaining the cellulose and xylan chains
266 frozen; only the positions of the water molecules were refined at this step. All the atoms were
267 free to move in the second minimization step. The MD simulations were performed in the
268 NVT ensemble at 400 °K (50 ns), and then at 298 °K (10 ns). The coordinates of the cellulose
269 microfibril were frozen during the MDs. MD simulations were long enough to reach
270 equilibration when the energy, location and conformation of the xylan chains were stable. The
271 last ns of the room-temperature trajectories were used for analysis.

272

273 *Computational details*

274 Material Studio modeling software (v5.5) was used (Accelrys, Inc., San Diego, CA, USA).
275 Energy calculations were performed using the Universal Force Field (Rappe, Casewit,
276 Colwell, Goddard, & Skiff, 1992). This all-atoms force field was applied in our previous
277 study of cellulose-xylan complexes (Mazeau & Charlier, 2012) and it was also used for the
278 development of a coarse grain force field (Li, Perre, Frank, & Mazeau, 2015). The charge
279 equilibration method was used to calculate partial charges for each atom (Rappe & Goddard,
280 1991). The long-range interactions were calculated using the particle-mesh Ewald summation
281 method (Darden, York, & Pedersen, 1993) with a cut-off distance of 9 Å.
282 Energy minimization was performed by the steepest descent method and then by the
283 conjugated gradient method. The convergence criterion was a root-mean-square (rms) force of
284 less than 0.1 kcal.mol⁻¹.Å⁻¹. The integration of Newton's laws of motion in the MD
285 simulations was performed using the standard Verlet algorithm (Verlet, 1967) with a time step

286 of 0.001 ps. The total external pressure was maintained at 1 atm (Berendsen et al., 1981) and
287 a Hoover algorithm (Evans & Holian, 1985) was used to keep the cell temperature constant.

288

289

290 **RESULTS**

291 *XylAc and XylAc fractions extraction and characterization*

292 Investigation of the hemicellulose function in the plant cell wall requires careful extraction in
293 order to provide a structure representative of those naturally occurring *in planta*. However,
294 hemicellulose is classically extracted with alkali, leading to the hydrolysis of the acetyl ester
295 groups. In a previous study, a non-aqueous polar aprotic solvent, namely DMSO doped with
296 LiCl, was used to efficiently extract acetyl-esterified hemicelluloses (Assor et al., 2013; Ray
297 et al., 2014). Applying this extraction procedure to apple pomace after partial removal of
298 pectin followed by anion exchange chromatography allowed the recovery of a xylan-enriched
299 fraction (XylAc). Fig. S1 reviews the overall extraction process. The extract was composed in
300 decreasing order of weight proportion as follows: xylose, uronic acid, arabinose and acetyl
301 esters, as well as low amounts of glucose, galactose and mannose and traces of fucose (Table
302 1). Mild alkali treatment of the fraction succeeded in removing acetyl esters with limited
303 changes in the sugar composition (Table 1). **The extract was thus mainly enriched in**
304 **arabinoxylan (GAX), whose composition was close to that reported for Gala GAX (Ray et al.,**
305 **2014) except for the presence of glucuronic acid, though comparatively richer in acetyl esters**
306 **(7.2% vs. 3.8% in Gala). The presence of low amounts of glucose, mannose, galactose and**
307 **fucose indicates the likely contamination of the AX by some galactoglucomannan and**
308 **xyloglucan, whereas the galacturonic acid and rhamnose content suggests the presence of**
309 **remaining pectin.** Assuming that all acetyl esters were related to the xylan and were linked to
310 the xylose residue, there would be one acetyl group for approximately two xylose residues, in

311 agreement with the average distribution of acetyl ester in plant xylan (Busse-Wicher et al.,
 312 2014; Chong et al., 2014; Grantham et al., 2017). Glucuronic acid and arabinose are present in
 313 the proportion of one uronic acid for approximately three xyloses, instead of one to six
 314 xyloses in the *Arabidopsis* primary wall and one arabinose for approximately 3.4 xylose
 315 residues, which could account in part for the pentose unit linked to glucuronic acid in the
 316 *Arabidopsis* primary wall xylan (Mortimer et al., 2015).

317

318 Table 1. Chemical composition of the acetylated (XylAc) and deacetylated (XyldAc) xylan
 319 fractions. Total sugars (TS) and the acetyl group contents are expressed in wt% and
 320 monosaccharide content is expressed in mol%.

	<i>Acetyl</i>	<i>TS</i>	<i>Glc</i>	<i>Gal</i>	<i>Man</i>	<i>Xyl</i>	<i>Ara</i>	<i>Fuc</i>	<i>Rha</i>	<i>GalA</i>	<i>GlcA</i>
	(wt%)		(mol%)								
XylAc	7.2	78.3	5.4	5.1	2.3	51.9	16.5	0.2	1.0	16.5	1.0
XyldAc	0	77.6	4.9	5.0	2.7	53.6	16.9	0.3	1.0	14.8	0.9

321

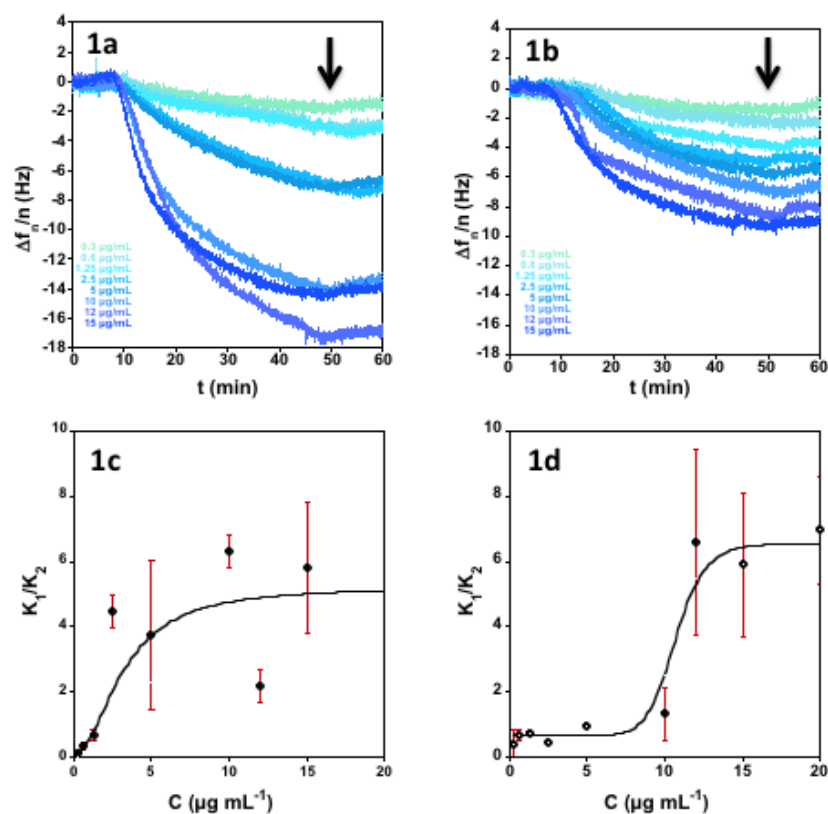
322 *Adsorption of XylAc and XyldAc fractions on the cellulose model surface*

323 Spin-coated CNCs prepared from cotton linters were used as a model surface. They displayed
 324 the expected rod-like morphology with dimensions of approximately 175 nm in length, 15 nm
 325 in width and 7 nm in thickness, consistent with previous reports (Cherhal, Cousin, & Capron,
 326 2015). The ability of XylAc and XyldAc-enriched fractions to adsorb on the CNC surface was
 327 evaluated and compared by QCM-D. This technique relies on the properties of piezoelectric
 328 quartz sensors submitted to an electric field that induces mechanical shear wave of the crystal.
 329 Adsorption of any material on the quartz surface induces a shift in the frequency resonance
 330 (Δf_n), allowing real-time monitoring of polymer adsorption on the quartz surface. Thanks to
 331 its very high sensitivity, the technique has been successfully used to monitor a wide range of

332 adsorption processes. The second piece of information that can be obtained from the QCM-D
333 analysis is the dissipation factor ΔD . This value provides an indication of the mechanical
334 (viscoelastic) behavior of the layer adsorbed on the quartz since it reflects how the layer will
335 dissipate the energy of the oscillation waves of the quartz sensor. QCM-D has already been
336 used for the investigation of adsorption of xylan and xylan derivatives (Dammak et al., 2013;
337 Kohnke et al., 2011; Sang Hoon Lee, Lee, & Youn, 2014; S. H. Lee, Lee, & Youn, 2015).
338 Prior to QCM-D analysis, the xylan fractions were adsorbed on the CNC surfaces and the
339 morphology was analyzed by atomic force microscopy (Fig. S2). In all the cases, the CNCs
340 were easily visible and the complexes showed no clear differences with the initial CNC
341 surface. This indicates that xylan is adsorbed as an individual polymer chain or, at most, as
342 very small aggregates undetectable by AFM. In the following section, we report the frequency
343 variations for different hemicellulose fractions, followed by an investigation of the dissipation
344 patterns.

345 *Frequency variation during XylAc and XyldAc adsorption and kinetic modeling of the*
346 *adsorption behaviors*

347 Prior to injection, the CNC surfaces were stabilized with water and the xylan fractions were
348 injected at different concentrations ranging from $0.3 \mu\text{g mL}^{-1}$ up to $20 \mu\text{g mL}^{-1}$ to explore a
349 large panel of ratios between polymers and adsorption sites. For the two fractions, acetylated
350 and deacetylated, the decrease of frequencies observed, regardless of the concentration
351 injected, indicated adsorption of xylan fractions on the cellulose surface. For each xylan
352 concentration, adsorption was repeated at least three times to assess the reproducibility, and a
353 typical frequency variation against time is reported in Fig. 1. Adsorption was irreversible
354 since the rinsing steps applied after adsorption (indicated by arrows on Fig. 1) did not change
355 the frequency values.



356

357 **Fig. 1.** Frequency (Δf_n) (3rd overtone) for the adsorption of XylAc (1a) and XyldAc (1b)
 358 fractions and evolution of the k_1/k_2 ratio as a function of the XylAc (1c) or XyldAc (1d)
 359 concentration (solid lines are visual guides). Arrows indicate the beginning of the rinsing step
 360 with water.

361

362 For the XylAc and XyldAc fractions, different maximal frequency values were obtained for
 363 all concentrations studied, indicating that the final amount of xylan adsorbed depended on the
 364 sample concentration applied, as previously reported for the xyloglucan adsorption on CNC
 365 surfaces (Villares et al., 2017; Villares et al., 2015). Frequency variations were in the same
 366 range (tens of Hz) for both xylan fractions, suggesting that the amounts adsorbed are similar,
 367 contrary to Kabel *et al* results that reported a higher adsorption of the deacetylated fraction.
 368 (Kabel, van den Borne, Vincken, Voragen, & Schols, 2007). This finding can be assigned to

369 a lower steric hindrance due to the removal of acetyl groups to increase the self-associative
370 capacity of xylans, as reported earlier by Linder (Bosmans et al., 2014; Linder, Bergman,
371 Bodin, & Gatenholm, 2003). Since both the XylAc and XyldAc fractions did not desorb from
372 the surface during the time scale of the experiment, the maximal frequencies cannot be
373 attributed to a dynamic equilibrium between adsorption and desorption of the polymer.
374 Moreover, the continuous flow of XylAc and XyldAc on the surface guaranteed that the
375 maximum coverage possible for each condition was reached. Thus, this pattern suggests that
376 the final amount of polymer adsorbed depended on the competition between adsorption,
377 packing optimization and potential rearrangement, suggesting that the adsorption process is
378 therefore not the result of thermodynamic equilibrium but, instead, is kinetically-driven.

379 We previously described such behavior by using a kinetic model that comprises two steps: the
380 first one describes the adsorption of hemicellulose onto an uncovered surface associated with
381 the rearrangement of the chains to cover the surface, and the second corresponds to adsorption
382 on a covered surface on which the polymer must diffuse through a pre-adsorbed
383 hemicellulose layer (Villares et al., 2017). This kinetic model was used to determine k_1 and k_2
384 for the XylAc and XyldAc fractions that are the kinetic constant for the first and second
385 adsorption steps, respectively. The detailed mathematical expressions of the kinetic model
386 and typical fits obtained are given in the Supplementary Material (SM, Appendix 1). The
387 experimental fractional coverage was calculated to obtain values of the kinetic constants k_1
388 and k_2 , and there was a good fit between experimental and calculated values (Fig. S3). The
389 evolution of the ratio k_1/k_2 against the XylAc and XyldAc fraction concentration is plotted in
390 Fig. 1c and d, respectively. The fractions display different patterns with two domains of
391 concentrations.

392 At low concentration, the k_1/k_2 ratio is lower or close to one indicating that the kinetic
393 adsorption and lateral rearrangement occur at a similar time scale, leading to large amounts of

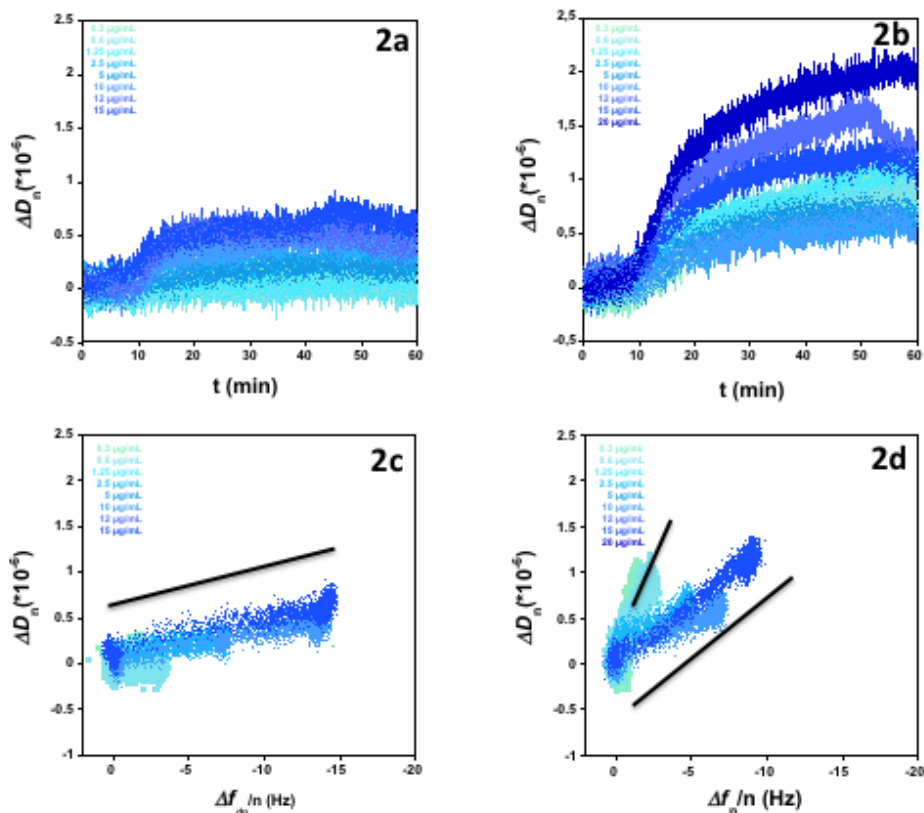
394 extended xylan conformation or trains. At higher concentrations, adsorption occurs through
395 diffusion on the pre-adsorbed layer, and the crowding of the surface limits diffusion and
396 rearrangement mechanisms. Thus, the k_1/k_2 ratio increases since the adsorption process on a
397 bare surface (k_1) dominates, while (k_2) decreases dramatically. The increase of the k_1/k_2 ratio
398 associated with a higher amount of polymer adsorbed can be interpreted either by a denser
399 packing of the molecules when the concentration increases or by limited rearrangement
400 possibilities. A clear difference can be seen between acetylated and deacetylated xylan. In the
401 case of the XylAc fraction, the k_1/k_2 ratio increases very rapidly, suggesting that even at low
402 concentrations, the acetylated sample is able to efficiently cover the surface and the
403 possibility of rearrangement seems to be strongly limited, suggesting efficient interactions
404 between XylAc and the cellulose surface. In the case of XyldAc, a transition between the two
405 regimes (i.e., the concentration at which the k_1/k_2 ratio increases) is clearly seen at around 5-
406 $10 \mu\text{g mL}^{-1}$. This suggests that chain rearrangements can occur at low surface concentrations,
407 whereas surface crowding may limit conformation modification at higher concentrations.
408 Nevertheless, the presence of acetyl groups changes the adsorption behavior of the xylan
409 chains and the acetylated fraction seems to have a greater ability to cover the surface than the
410 deacetylated one.

411 *Comparison of the viscoelasticity behaviors of adsorbed layers*

412 To correlate the evolution in dissipation with changes in frequency, the ΔD was plotted as a
413 function of Δf (hereafter referred to as ΔD vs. Δf plots) to eliminate time as an explicit
414 parameter (Fig. 2). The raw dissipation data for both fractions at different concentrations are
415 illustrated in Figs. 2a and 2b. The ΔD vs. Δf plots revealed different slopes of the curves that
416 are related to the overall viscoelasticity of the layer. A rigid layer will behave similarly to the
417 quartz dissipation and the ΔD will present low values per mass addition and, accordingly, the
418 ΔD vs. Δf slope will be low. In the case of a more viscoelastic layer, the ΔD vs. Δf slope will

419 display higher values. As shown in Figs. 2c and 2d, the two xylan fractions display strikingly
420 different behaviors. The slope obtained for XylAc is constant ($0.03 \cdot 10^{-6} \text{ Hz}^{-1}$) regardless of the
421 concentration studied, indicating that irrespective of concentration, the adsorbed acetylated
422 xylan layer on cellulose shares a similar organization. Moreover, the very low value of the
423 slope suggests that the adsorbed layer is rigid. This finding is consistent with the kinetic
424 analysis that displays only one regime of adsorption in which the k_t constant is dominant and
425 which suggests that the XylAc layer is strongly adsorbed with limited diffusion and
426 rearrangement processes. It should be emphasized that the slope value of the ΔD vs. Δf of
427 XylAc is significantly lower compared to the values found in the literature. For example,
428 Eeronen et al. (Eronen et al., 2011) reported a value of $0.25 \cdot 10^{-6} \text{ Hz}^{-1}$ for wheat arabinoxylans
429 and of $0.1 \cdot 10^{-6} \text{ Hz}^{-1}$ for spruce galactoglucomanans, which are on the same order of magnitude
430 as the value measured for the XyldAc but almost ten times higher than the value of $0.03 \cdot 10^{-6}$
431 Hz^{-1} determined for the XylAc. This finding suggests that the mechanical properties of the
432 XylAc layer are quite similar to those of the cellulose surface and can probably be attributed
433 to flat and dense layers.

434 In contrast, the ΔD vs. Δf plot of XyldAc displays different slopes depending on the injection
435 concentration, indicating a more complex behavior (Fig. 2d). The ΔD vs. Δf plot pattern
436 evolved during the adsorption process, suggesting a change in layer organization during
437 adsorption. At low concentrations ($<5 \mu\text{g mL}^{-1}$), the slope value is around $0.25\text{-}0.5 \cdot 10^{-6} \text{ Hz}^{-1}$,
438 whereas at higher concentrations, the value decreases to $0.1\text{-}0.15 \cdot 10^{-6} \text{ Hz}^{-1}$. At low
439 concentrations, the slope value is high, which indicates a high viscosity of the layer, and then
440 starts to decrease to reach a stable value at a concentration of $5\text{-}10 \mu\text{g mL}^{-1}$. This suggests the
441 formation of a loose layer that becomes denser as the concentration increases.



442

443 **Fig. 2.** Dissipation (ΔD) (3^{rd} overtone) for the adsorption of XylAc (2a) and XyldAc (2b)
 444 fractions for different concentrations as a function of time and corresponding ΔD vs. Δf plots
 445 for XylAc (2c) and XyldAc (2d) (solid lines are visual guides).

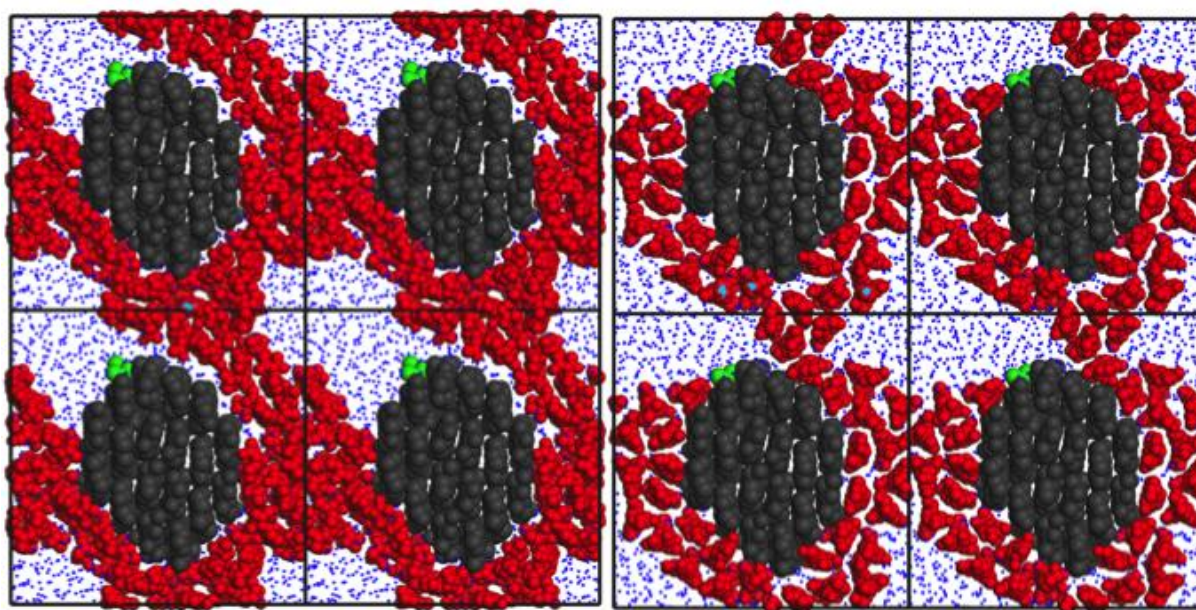
446

447 *Adsorption of XylAc and XyldAc fractions on the cellulose model surface by MD*

448 Four models of the hydrated cellulose xylan complex that differed by the chemical structure
 449 of their xylan chains and by their initial conformation were considered. The xylan chains were
 450 either acetylated or non-acetylated (corresponding to deacetylated sample). Repeat unit is
 451 shown in Fig S4. In addition, to account for the conformational polymorphism of xylan
 452 (Chanzy, Dube, & Marchessault, 1979; Larsson, 2004; Teleman, Larsson, & Iversen, 2001),
 453 the xylans differed by the initial helical conformation of their backbone: either two units per
 454 helical turn when the xylan chains contained three turns of the 2_1 helix in their initial state, or

455 three units per helical turn when they contained two turns of the left-handed 3_2 helix (Figs. S5
456 and S6).

457 The four models were subjected to MD simulations at two temperatures. During the high
458 temperature phase, the xylan chains explored the two helical conformations with rapid inter-
459 conversions between the two forms. This is not surprising since the two conformations have
460 similar energies and the energy barrier between them, estimated from the adiabatic potential
461 energy surface of xylobiose (Mazeau, Moine, Krausz, & Gloaguen, 2005), is lower than 2
462 kcal mol⁻¹. In contrast, during the room-temperature phase, each xylan chain stabilized in its
463 preferred conformation depending on its immediate environment and remained in that
464 conformation up to the end of the trajectory. As a consequence, the models that differed by
465 the initial conformation of the xylan chains converged into an identical structure of the
466 complex. Fig. 3 provides representative snapshots of the XylAc and XyldAc models.



467
468 Fig. 3: Cross-sectional views of the models of the hydrated cellulose-xylan complexes. 2x2
469 basic cells are displayed. Hydrogen atoms are omitted for clarity. Cellulose is in gray. Sulfate
470 group, in green, is located on a chain of the (1-10) surface of cellulose next to its top corner.

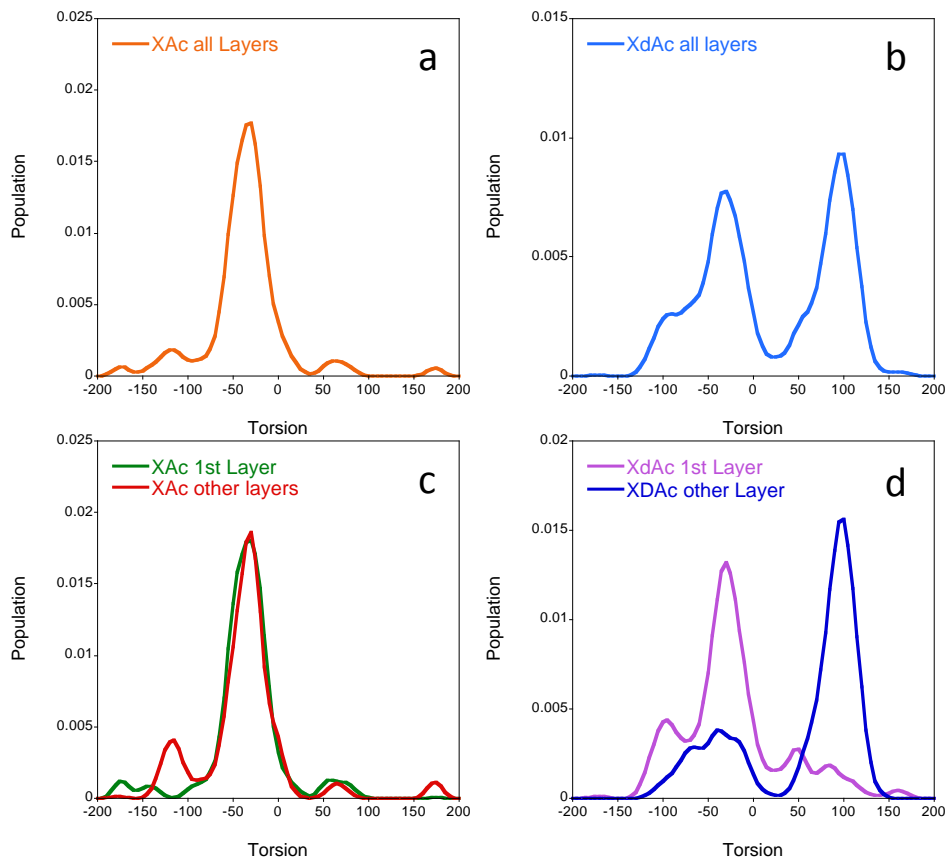
471 The xylan chains are in red and the water molecules in blue. Left: XylAc model; right XyldAc
472 model.

473

474 *Conformation of the xylan chains*

475 Analysis of the helical conformations of the xylan chains in the equilibrated models is
476 performed using the turn angle (T), which is defined by the sequence of atoms O-5''-C-
477 5''-C-4-C-5 (Fig. S4). The turn angle describes the orientation of a given unit with respect to
478 its second neighbor for those perfectly stereo-regular helices that were used to build the initial
479 models $T = -50^\circ$ for the 2_1 helix and $T=100^\circ$ for the 3_2 helix.

480 Fig. 4 gives the distributions of the turn angle of the xylan chains in the equilibrated XylAc
481 and XyldAc models. It shows the distributions of all the chains (denoted 'all'), together with
482 that of the chains in close proximity to the cellulose, denoted '1st layer', and that of the chains
483 far from the cellulose, denoted 'other layer'. For the purpose of comparison, the same
484 calculations were performed for arged cellulose, and similar distributions were obtained in all
485 cases (SM, Appendix 3 - Fig. S10 - S11-S12).



486

487 **Fig. 4.** Distribution of the turn angle in the XylAc (left) and XyldAc (right) simulated
 488 systems Global distributions from all the xylan chains (4a and 4b); and distributions from the
 489 xylan chains interacting with cellulose (orange in 4c and light blue in 4d) and from the bulk
 490 xylan (red in 4c and dark blue in 4d).

491

492 For XylAc, the distribution is almost monomodal and centered at $T = -35^\circ$. Considering the
 493 thermal motion and local conformational variability, this peak corresponds to helices with two
 494 residues per helical turn. The distribution of T also reveals other forms, non-canonical, less
 495 organized and of low occurrence, at $T = -120^\circ$, 60° and 180° . Conformations of the xylan
 496 chains in the XyldAc model differ substantially. The bimodal distribution of the turn angle
 497 centered at $T = -35^\circ$ and $+95^\circ$ shows that the deacetylated xylan chains explore the two main 2_1
 498 and 3_2 helical forms. It should be noted that most of the chains in direct interaction with

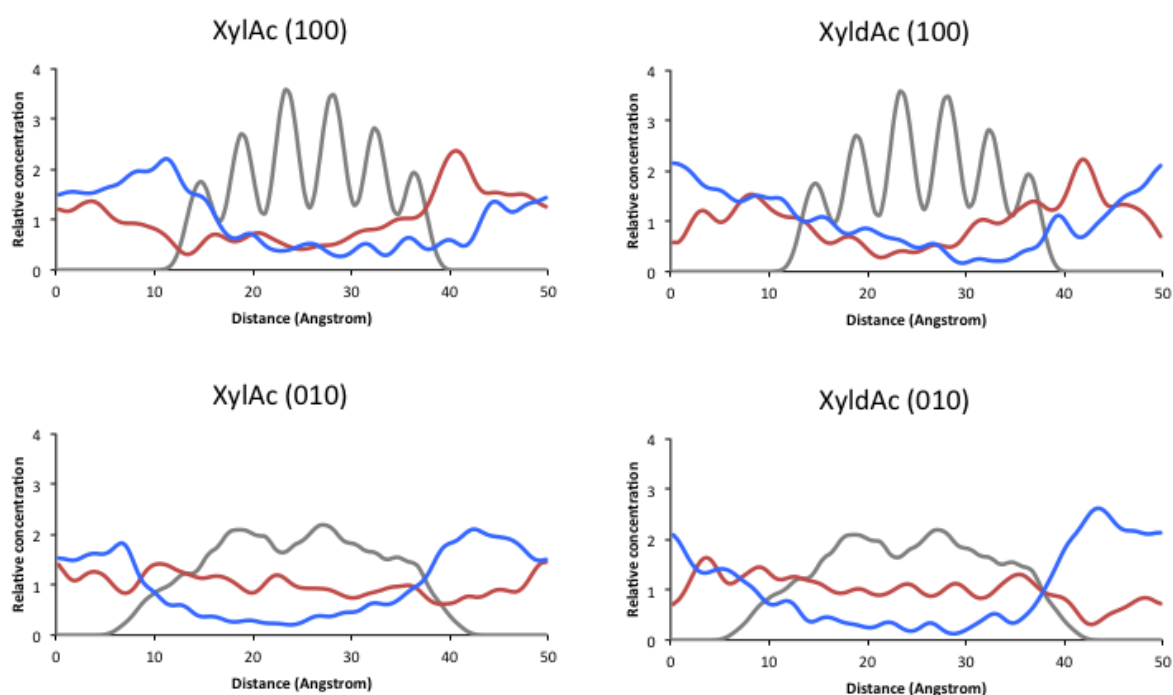
499 cellulose are mainly in the 2_1 conformation, whereas those far from the cellulose (in the bulk
500 phase of xylan) are in the 3_2 conformation. Here again, a few chains explored alternative
501 disorganized conformations, as suggested by the broad distribution of the turn angle.

502 *Concentration profiles.*

503 The concentration fluctuations of cellulose, xylan and water were determined along the 100
504 and 010 directions in both the immediate environment of the cellulose and in the bulk xylan
505 (Fig. 5).

506 At the interface with cellulose, the acetyl groups do not affect the adsorption behavior of
507 xylan. The concentration traces at 40 Å show a maximum for xylan and, concomitantly, a
508 minimum for water, which is particularly visible on the profiles along the 100 direction. This
509 reveals that xylan accumulates at the interface on the uncharged side of the cellulose crystal.
510 The concentration profiles of xylan and water at the interface with cellulose are, however,
511 dissymmetrical. At 10 Å in the plots, i.e., on the charged side of the cellulose, the xylan
512 concentration is minimal, whereas that of water is maximal, which suggests that the charged
513 sulfate group preferentially interacts with the surrounding water molecules rather than with
514 xylans. Additional modeling was performed with a native (uncharged) cellulose crystal in
515 order to estimate the exact effect of the charged sulfate group (see SM, Appendix 3, Figs. S11
516 and 12). The corresponding concentration traces with uncharged cellulose are in fact more
517 symmetrical and show a high concentration of xylan and a low concentration of water at both
518 interfaces. The charged sulfate group does not interact with the acetylated xylans but can
519 occasionally form a hydrogen bond with a hydroxyl group of the deacetylated xylan. The
520 charged sulfate group, along with its surrounding hydration shell, enhances the cellulose
521 hydrophilicity, increases the porosity of the total system, and lowers the total cellulose surface
522 covered by xylan.

523 In contrast, within the xylan phase, at around 0 or 50 Å in the concentration profiles, the
 524 acetyl groups have a strong influence on xylan organization. For XylAc, xylan concentration
 525 is similar to that of water, suggesting that the system is dense and weakly hydrated. This type
 526 of organization is obviously facilitated by the dominant 2_1 helical conformation of their
 527 backbones whose compact packing, similar to that of cellulose or chitosan, can be easily
 528 achieved. However, the multiple conformations explored by the acetyl groups disturb the
 529 perfection of the packing at the local level and create small cavities in which few water
 530 molecules can penetrate. For XyldAc, in contrast, xylan concentration is much lower than that
 531 of water, which suggests that the deacetylated xylan layer is loose and highly hydrated. The
 532 two free hydroxyl groups per repeat unit strongly interact with water molecules and the
 533 packing of the 3_2 helices promotes uninterrupted columns of water that appear in between the
 534 deacetylated xylan chains.



535
 536 Fig. 5: Concentration profiles of cellulose, xylan and water along directions perpendicular to
 537 the exposed crystallographic planes of the cellulose. In gray: cellulose; in red: xylan; in blue:
 538 water.

539 Our models are consistent with experimental data from X-ray diffraction and solid-state
540 NMR. The crystal structure of xylan diacetate is anhydrous and made of two-fold helices
541 (Gabbay, Sundararajan, & Marchessault, 1972), whereas that of deacetylated xylan contains
542 between one to three water molecules per xylosyl unit and the chains are in the left-handed
543 three-fold helix (Nieduszynski & Marchessault, 1971; Nieduszynski & Marchessault, 1972).
544 These deacetylated xylan chains also explore the three-fold conformation in a water solution
545 but change their conformation to the two-fold one when interacting with cellulose (Larsson,
546 Hult, Wickholm, Pettersson, & Iversen, 1999). It should be noted that such conformational
547 adaptation is limited to those xylan chains in direct contact with the cellulose surface (Falcoz-
548 Vigne et al., 2017).

549 In a modeling study, the two limiting cases were studied where xylan chains are either per-
550 acetylated or deacetylated, whereas Busse-Wicher et al. modeled the adsorption of a partly-
551 acetylated xylan chain in which the distribution of the acetyl groups is stereoregular, i.e.,
552 where every other xylose residue is acetylated (Busse-Wicher et al., 2014). Comparing the
553 two studies makes it possible to determine the effects of the degree of acetylation in the xylan
554 chain on its interaction with cellulose. The degree of acetylation does not affect the
555 conformation of xylan in direct contact with cellulose, adopting the two residues per helical
556 turn conformation. Acetylation also does not affect the adsorption features of xylan on the
557 hydrophobic surface of cellulose. Instead, it stacks on this surface by its aliphatic C-H groups.
558 The interaction on this particular cellulose surface is dominated by the Van der Waals forces
559 (Martinez-Abad et al, 2017; Mazeau & Charlier, 2012). In contrast, the degree of acetylation
560 affects the adsorption features of xylan on the hydrophilic surfaces of cellulose. The per-
561 acetylated xylan adsorbs flat to maximize the number of Van der Waals contacts. This
562 organization is obviously promoted by the amphiphilicity of the cellulose surfaces (Mazeau,
563 2011). On the contrary, the deacetylated xylan adsorbs in a tilted conformation to establish

564 hydrogen bonds between its hydroxyl groups and the hydroxyl groups protruding from the
565 cellulose surface. The regularly and alternately substituted xylan considered by Busse-Wicher
566 et al., which forms hydrogen bonds with cellulose, thus follows the adsorption of the non-
567 substituted xylan (Busse-Wicher et al., 2014). This similarity is understandable considering
568 that a xylan polymer in the 2_1 helical conformation in which only one residue out of two is
569 substituted has only one acetylated side. This is the non-substituted side, consisting only of
570 hydroxyl groups, that preferentially interacts with cellulose in the same way as a deacetylated
571 xylan.

572

573 **DISCUSSION**

574 Xylan presents a broad structural variability and is a major hemicellulose in plant biomass,
575 essentially encountered in secondary cell walls (Brummell & Schröder, 2009; Scheller &
576 Ulvskov, 2010). It is, however, a minor component of primary cell walls and particularly
577 those of fleshy fruit (Brummell & Schröder, 2009) such as apple where it occurs as
578 glucuronoarabinoxylan (GAX) (Ray et al., 2014). The proportions of uronic acids, arabinose
579 and acetyl ester substitutions determined for the apple pomace xylan fractions are similar to
580 those reported for Gala apple (Ray et al., 2014). Considering that apple pomace is not
581 lignified, it is therefore a convenient biological source for native xylan with a preserved
582 substitution pattern that can be used as a model polymer for *in vitro* investigation.

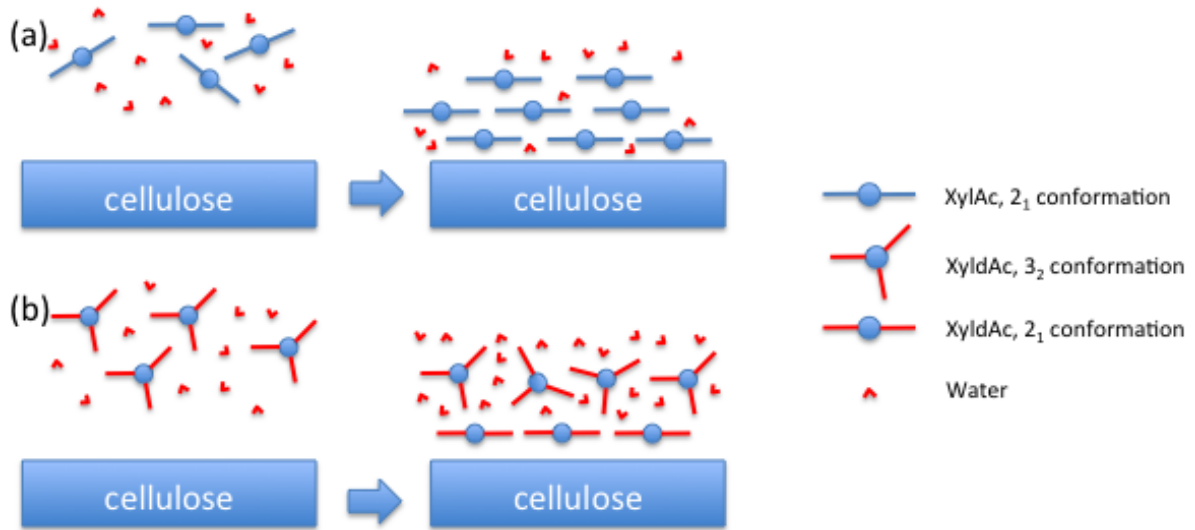
583 QCM-D experiments complemented by molecular modeling provide an atomistic-scale view
584 of the effect of the acetyl groups attached to xylan on their adsorption on cellulose.

585 Adsorption of xylan fractions followed by QCM-D demonstrated that the acetylated and
586 deacetylated fractions adsorption processes are both kinetically-driven but display different
587 behaviors. XylAc has an almost homogenous adsorption pattern for all concentrations (k_f

588 dominated), which suggests limited rearrangement after adsorption, whereas XyldAc displays
589 two clear concentration domains of adsorption (k_1/k_2 increased between 5 and 10 g L⁻¹). These
590 behaviors are also supported by the pattern of the ΔD vs. Δf representation. The ΔD vs. Δf plot
591 for XylAc displays a single slope, whereas that for XyldAc shows two slopes between 2.5 and
592 10 mg L⁻¹ of xylan injected. The slope change corresponds to the increase of the k_1/k_2 ratio
593 observed in the kinetic modeling analysis. For the higher slope (low concentration), k_1/k_2 is
594 close to one and increase when the slope become lower. The models revealed that adsorption
595 of XylAc is straightforward since the acetylated xylan does not need to modify its solution
596 conformation (two-fold helices). In contrast, adsorption of the very first molecules of XyldAc
597 requires a conformational change to adopt a form complementary to that of cellulose (from
598 three-fold to two-fold), whereas the additional molecules adsorb without conformation change
599 (three-fold).

600 Additionally, the XylAc ΔD vs. Δf slope displays a very low and homogeneous value ($0.03 \cdot 10^6$
601 Hz⁻¹) compared to XyldAc fraction values that are higher and variable according to the
602 concentration injected (from 0.5 to 0.1 10^6 Hz⁻¹). This suggests the formation of a stiff and
603 densely-packed XylAc layer, whereas the XyldAc fraction forms a more viscous layer that
604 can be densified with increasing XyldAc concentration. In agreement with the experimental
605 observations, the models also show that the acetyl groups affect the compactness and
606 hydration of the xylan layer surrounding the cellulose. It is compact and weakly hydrated for
607 acetylated xylan, but loose and highly hydrated for deacetylated xylan. Considering that water
608 plasticizes hemicellulose, complexes made with XylAc would be rigid, as revealed by the low
609 gradient of the ΔD vs. Δf plot, whereas those made with XyldAc would be softer, showing a
610 more viscous character, as suggested by the larger slopes of the ΔD vs. Δf plot. The adsorption
611 process of the acetylated and deacetylated xylans on cellulose can be illustrated with the
612 schematics shown in Fig. 6.

613



614

615 Fig. 6. Schematic representation of the adsorption process of XylAc (a) and XyldAc (b) on
616 cellulose. Adsorption of XylAc results in the formation of a rigid (almost) anhydrous layer,
617 whereas XyldAc forms a water-swollen soft layer.

618 Our findings indicated that the presence of acetyl esters influences the supramolecular
619 organization of the xylan and its interaction with the cellulose surface. Deacetylated and
620 acetylated xylans display different organization suggesting that the remaining substituent
621 groups are not enough to keep the xylan interaction with cellulose surface unchanged. Indeed,
622 in *Arabidopsis* mutants with decreased acetyl ester, compensation is provided by an increase
623 in glucuronic (Grantham et al., 2017). In our case, the removal of all acetyl groups cannot be
624 compensated by the other substituents.

625 The functional role of xylan in fleshy fruit remains unknown. In tomato, the trace amount of
626 xylan was localized in cell walls lining intercellular space (Ordaz-Ortiz, Marcus, & Knox,
627 2009) and particularly in the skin and inner epidermal cells of the pericarp (Takizawa et al.,
628 2014). In biomass, it is found in the secondary wall (Carafa, Duckett, Knox, & Ligrone, 2005)
629 and directly or indirectly contributes to the mechanical properties of the stems (Li et al., 2015;
630 Dammstrom, Salmen, & Gatenholm, 2009). The biological and technological roles of acetyl

631 groups are still unclear but it has been hypothesized that they limit the saccharification and
632 fermentation of biomass (Pawar et al., 2017). Besides the steric hindrance induced by acetyl
633 groups to access a xylanase catalytic site, the formation of a poorly hydrated and stiff layer
634 interacting with cellulose, as suggested by our study, can also be an explanation for the
635 limited xylanase accessibility and mechanical properties of plant cell walls.

636 **CONCLUSION**

637 Combined QCM-D and MD approaches were used to illustrate the impact of acetyl decoration
638 on the interaction between xylan and cellulose. Both *in vitro* and *in silico* results showed that
639 acetyl groups attached to the xylan chains significantly influence the conformational behavior
640 of xylan and the supramolecular organization of the cellulose-xylan complex. Acetylated
641 fractions adsorbed on cellulose form a dense and poorly hydrated layer, whereas the
642 deacetylated fraction forms a more hydrated layer. It should be noted that the acetylated
643 fraction has the two residue per turn conformation for all the adsorbed chains, whereas this
644 conformation is limited to the xylan chains in direct contact with the cellulose surface for the
645 deacetylated fraction. Our study therefore supports the assumption that the acetylation pattern
646 promotes different organizations and hydrations of xylan cellulose complexes that can
647 modulate the interaction strength and rheological properties of the cell wall xylan-cellulose
648 supramolecular complexes. Our future studies will focus on the evaluation of the influence of
649 the presence of the acetyl groups on the properties as well as the use of the modulation of
650 acetylation patterns to implement xylan/CNC-based composites.

651 **ASSOCIATED CONTENT**

652 **Supporting Information**

653 DOI:

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