Water Cast Film Formability of Sugarcane Bagasse Xylans Favored by Side Groups

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ABSTRACT: Hemicellulose, one of the most abundant biopolymers next to cellulose, has been considered as a potential substitute to synthetic polymers. Film casting from water is the most basic route for material applications of xylan. However, depending on plant sources and separation methods, xylans do not always form films and the related mechanism is unclear, which significantly hinders their material applications. We extensively characterized various fractions of bagasse xylan to understand the molecular features promoting the film formation. The side groups of xylans or impurities contributed to the prevention of excessive aggregation or crystallization of xylan molecules, leading to the film-forming capacity. However, once the film is formed, the side groups do not seem to be necessarily contributing to the mechanical resistance.

Keywords: Xylan; Crystallization; Side group; Film formation; Mechanical property

Introduction

Polysaccharides in plant secondary cell walls are the most abundant renewable biopolymers on Earth, among which hemicelluloses are the second most abundant after cellulose (Huang et al., 2019a; Lin et al., 2019). Hemicelluloses are a group of hetero-polysaccharides with highly diversified structures incorporating various sugars. Hemicelluloses extracted from hardwood and graminaceous plants are primarily xylans, which is relatively easy to be separated and processed compared to other types of hemicellulose such as glucomannans from softwood. Generally, xylans have β -(1-4)-linked D-xylopyranosyl backbone but are heavily substituted in native state with substituents mainly including α -L-arabinofuranosyl, α -D-glucuronopyranosyl units or/and 4-*O*-methyl- α -D-glucuronopyranosyl units; most xylans are partially acetylated (Ebringerova and Heinze, 2000; Morais de Carvalho et al., 2017; Spiridon and Popa, 2008; Sun et al., 2000). From different plant sources, the chemical structure of xylans also varies. In hardwood, xylans do not have arabinofuranosyl substitutions, which are specifically termed as glucuronoxylans. The xylans from woody tissues of grasses and annual plants are specifically termed as glucuronoarabinoxylans; they have 2-linked (4-*O*-methyl)-glucuronopyranosyl units and 3-linked arabinofuranosyl units on the xylopyranosyl backbone. The xylans from cereal grains are usually termed as arabinoxylans, in which, the backbone xylopyranosyl units can be mono- or di-substituted at positions 2 or/and 3 by the arabinofuranosyl units (Ebringerova and Heinze, 2000; Morais de Carvalho et al., 2017; Spiridon and Popa, 2008).

As a naturally abundant and water dispersible biopolymer, xylan has been considered as a potential substitute to the petroleum-based synthetic polymers (Hansen and Plackett, 2008). However, xylan films generally have inferior mechanical properties, which usually leads to excessive chemical modifications with high cost and high environmental impact which leads to

no advantage over the petroleum-based synthetic polymers. The major hurdle in the application of originally separated xylans is their highly diversified chemical structures and our inadequate understanding of the mechanism between mechanical properties and their structures.

Film casting from water is the most basic route for material applications of xylan. However, depending on plant sources and separation methods, xylan does not always form films. For example, xylan from barley husk (Hoije et al., 2005) and distillers' grains (Xiang et al., 2014) always forms films, while xylan from hardwood requires the addition of plasticizer to form films (Grondahl et al., 2004); from our previous study and as revisited in this study, xylan extracted from sugarcane bagasse with 2% NaOH forms films very well, while xylan extracted with 4% or 6% NaOH does not form films even with the addition of a plasticizer (Jin et al., 2019). The unpredictable film formability of xylan greatly hinders its rational separation strategy for material applications. Works on enzymatically or chemically tailored xylan suggested that the presence of lignin promotes (Goksu et al., 2007), while the degree of substitution does not affect the film formability of xylan (Hoije et al., 2008; Sternemalm et al., 2008). However, many structural aspects still await investigation and a key question, examined here, is the relation between xylan molecular architecture and its film formability.

The side groups of xylan are known to prevent the intermolecular aggregation, which happens for a linear molecule such as cellulose that tends to crystallize. The side groups would thus greatly affect the properties of xylan (Dea et al., 1973; Hoije et al., 2008; Shrestha et al., 2019). The solubility of xylan in water decreased with decreasing number of side groups as the unsubstituted segments would aggregate into insoluble form (Andrewartha et al., 1979). The side groups of xylan can also be responsible for the high viscosity of xylan solution by restricting the mobility of xylan backbone (Andrewartha et al., 1979; Sternemalm et al., 2008).

Xylan is generally known as an amorphous polymer. However, with reduced number of side groups, xylan can form hydrous and anhydrous crystals (Dea et al., 1973; Hoijs et al., 2008; Horio and Imamura, 1964; Kobayashi et al., 2013; Marchessault and Timell, 1960; Nieduszynski and Marchessault, 1972), which greatly hinders the polymer internal rotation and thus increase the rigidity (Arinstein et al., 2007; De Rosa et al., 2006; Fama et al., 2005; Shrestha et al., 2019). A moderate degree of branching or an addition of external plasticizers may ensure flexibility of xylan films by increasing free volume (Grondahl et al., 2004; Hansen and Plackett, 2008; Hoijs et al., 2008). However, some studies also found that external plasticizer may promote the crystallinity of xylan films due to the increased chain mobility (Mikkonen et al., 2009). Our previous studies showed that xylans with higher molecular weight and smaller number of side groups produced films with higher tensile strength and modulus of elasticity; lignin can act either as an impurity (detrimental to the film strength) or as a reinforcement to the films depending on the molecular weights of xylan (Jin et al., 2019).

The addition of external plasticizers or biopolymers, e.g. sorbitol, xylitol, chitosan, gluten, often helps the film formation of xylan (Gabrielii and Gatenholm, 1998; Grondahl et al., 2004; Kayserilioglu et al., 2003). However, xylans with the same molecular weight and lignin content may or may not form a film and the underlying mechanism for film formability is unclear. In this study, we chose alkali-extraction as one of the scalable extraction techniques that can be easily implemented in the laboratory, though it inevitably removes the acetyl groups. We prepared various xylan fractions by modulating extraction and precipitation methods, i.e. alkali extraction method, sequential alkali extraction method, graded ethanol precipitation, I₂-KI precipitation and ammonium sulfate precipitation methods. We studied the chemical structures, solution properties, particle morphology, and film microstructures to relate with the film formability of xylans.

Materials and Methods

Separation of xylan

Grounded sugarcane bagasse sample sieved between 40 and 60 mesh was extracted in a Soxhlet extractor with 95% ethanol for 6 hours to remove extractives, fat and wax. The dewaxed bagasse was dried in an oven at 60 °C until moisture content reduced to ~4%. The types of separation methods of xylan used in this study are summarized in Tables 1.

Approximately 20 g dewaxed bagasse was extracted in 240 ml of 2%, 4% or 6% NaOH solution or sequentially by 2 and 4% or 2, 4 and 6% NaOH solutions. Each extraction was conducted in conical flasks for 3 h at 90 °C under continuous magnetic stirring. After extraction, the mixture was filtered and the solid residues were washed with 100 mL distilled water. The filtrates as well as the washed liquor were then adjusted to pH = 5.5 with 5 M HCl and concentrated under reduced pressure.

Table 1 Symbols of xylan fractions separated through different extraction and precipitation methods.

Extraction Methods	Precipitation Methods	Xylan Fractions	Yield %*
2% NaOH	100% Ethanol	H1	9.7
4% NaOH (successive after 2%)	100% Ethanol	H2	13.0
6% NaOH (successive after 2 and 4%)	100% Ethanol	H3	7.9
2% NaOH	70% Ethanol	H4	11.5
4% NaOH	70% Ethanol	H5	17.2
6% NaOH	70% Ethanol	H6	21.6
2% NaOH	Successive precipitation with 15%, 30% and 60% ethanol	H7、 H8、 H9	3.1, 2.9, 5.6
2% NaOH	I ₂ -KI Precipitation	H10、 H11	2.9, 6.1
2% NaOH	Ammonium sulfate	H12	7.8
Commercial xylan from sugarcane bagasse		H14	-

Note: *Yield was calculated based on the oven-dried weight of the raw sugarcane bagasse.

The concentrated liquor was slowly poured into three volume of 100% ethanol or 70% ethanol with constant stirring. Graded ethanol precipitation (liquor successively treated by 15%, 30% and 60% ethanol) (Bian et al., 2010), I₂-KI precipitation (Peng et al., 2012) and ammonium sulfate precipitation (Rao and Muralikrishna, 2006) methods were also used according to previous reports. The obtained xylan samples were named as H1-H12 (Table 1). For I₂-KI precipitation method, the first step was adding 0.1 volume of I₂-KI solution (3 g I₂ and 4 g KI in 100 mL water) to the concentrated liquor and the precipitated solid was denoted as solid part one (H10); the second step was taking the supernatant for dialysis (molecular weight cut off 3500) and the solid precipitated by adding three volume of 100% ethanol was denoted as solid part two (H11). The precipitated solids from each precipitation step or method were washed several times with 70% ethanol and freeze-dried. The commercial xylan (from sugarcane) purchased from Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China) was named as H14 and used for comparison.

Characterization of xylan samples

The xylan fractions were hydrolyzed according to previous protocols described by Min (Min et al., 2011; Min et al., 2013) and Ruiter (De Ruiter et al., 1992) in order to determine their lignin and neutral sugar, and uronic acid compositions. Neutral sugar and uronic acid concentration of the xylan hydrolysate was quantified by an Ion Chromatography system (IC-3000, Dionex, USA) equipped with an anion-exchange column (CarboPacTM PA20, Dionex, USA); the column was pre-equilibrated with 200 mM NaOH and gradiently eluted with water, 20 mM NaOH and 500 mM NaAc at a flow rate of 0.5 mL/min.

The weight average molecular weight (M_w), number average molecular weight (M_n) and polydispersity index (PDI) were obtained based on the previous method by Jin (Jin et al., 2019),

using NaNO_3 - Na_2HPO_4 solution (0.1M:0.001M) as the elution buffer. The measurement was conducted at a column temperature of 40 °C through a high performance liquid chromatography (HPLC, 1260 Infinity, Agilent Technologies, USA) equipped with GPC columns (PL aquagel-OH 60 and PL aquagel-OH MIXED-H, Agilent Technologies, USA).

The 2D-HSQC NMR spectra of selected xylan samples were analyzed by a 600 MHz NMR spectrometer (AVANCE III HD 600, Bruker, Germany). Approximately 20 mg of xylans was dissolved in 0.5 mL of DMSO-d_6 . The acquisition parameters used were 160 transients (scans per block) acquired using 1024 data points in the F2 (^1H) dimension with an acquisition time of 53 ms and 256 data points in the F1 (^{13}C) dimension with an acquisition time of 5.14 ms. Total running time was 18 h. The coupling constant ($^1\text{J}_{\text{C-H}}$) of 147 Hz was applied.

The morphology of xylan aggregates in film was observed by a polarizing microscope (BX53M, Olympus, Tokyo, Japan) equipped with a U-AN360P analyzer slider. X-ray diffraction (XRD) measurements were performed in symmetric reflection mode using X'Pert Powder (Panalytical, Eindhoven, Netherlands) equipped with a copper X-ray source ($\lambda = 1.5418 \text{ \AA}$) and operated at 40 kV and 40 mA. The precipitated and freeze-dried xylan samples were mounted on a powder sample holder with 200 μm -deep central well. The sample was gently pressed and the excess above the surface level was wiped out with a flat blade. An empty holder was also measured and the intensity was subtracted from the sample data, taking the X-ray attenuation by the sample into account.

Preparation and Characterization of xylan films

Exactly 0.51 g of xylan powder and 0.09g sorbitol as plasticizer were dispersed in 8 mL of deionized water with magnetic stirring for 30 min at 90 °C to make a 7.5 % (w/v) suspension. The suspension was poured into a Teflon mold (65 mm $\times$ 40 mm) and dried in an environmental

chamber regulated to a temperature of 50 °C and a relative humidity of 70 % for 24 h, allowing the formation and equilibration of xylan films. The films had a thickness of ~150 µm.

The film fracture surface was observed using a scanning electron microscope (SEM: Evo-18, Carl Zeiss, Germany). The tensile strength and modulus of elasticity (MOE) of the xylan films were measured using a universal tensile tester (Instron 5565, Instron, USA). At least two films were prepared from each hemicellulose fraction. Each film was cut into 4 strips (5 cm × 1 cm). The strip specimens were equilibrated and tested in a room with a constant temperature of 23 °C and a relative humidity of 50 %. Each of the 4 strip specimens was tested with a head speed of 5 mm/min and a span length of 30 mm.

Results and Discussion

Sugar composition

The types of extraction methods of xylan used in this study are summerized in Tables 1. All separated xylans mainly consisted of arabinose, xylose and glucuronic acid units (Table 2). As shown by the 2D-HSQC NMR spectra, arabinofuranosyl units linked to xylopyranosyl backbone through α -(1-3)-linkage; glucuronopyranosyl units were 4-*O*-methylated and linked to xylopyranosyl backbone through α -(1-2)-linkage (Fig. 1 and Table 3). The results are consistent with the chemical structure of glucuronoarabinoxylans extracted from graminaceous plants including sugarcane bagasse (Ebringerova and Heinze, 2000; Morais de Carvalho et al., 2017; Spiridon and Popa, 2008; Sun et al., 2000). The minor residues of galactose and galacturonic acid may come from the residual pectin component from sugarcane (de Souza et al., 2013), which was co-extracted with xylan. Some samples, e.g. H1, H4, H5, H9, H11 and H12, had high content of glucose (Table 2). 2D-HSQC spectra indicate that the glucose had its origins from

glucan instead of residual sucrose in the bagasse (Fig. 1 and Table 3); the existence of glucan could be due to the alkaline degradation of cellulose (Pavasars et al., 2003). No acetylation on the extracted xylan was observed, since alkaline treatment cleaves acetyl groups easily (Jaafar et al., 2019). It was also found that lignin was covalently bonded to xylan (Ralph et al., 1994) by phenyl glycoside and γ -ester linkages (Fig. 1 and Table 3), making a lignin-carbohydrate complex (LCC) (Dong et al., 2020; Huang et al., 2019b); therefore, the co-extraction of lignin is inevitable without extensive bleaching. The lignin and sugar contents do not sum up to 100% mass balance of the xylan samples, which could be due to the undetermined ash contents and incomplete hydrolysis or degradation to sugars during sample hydrolysis.

The successive alkaline treatments (H2 and H3) were able to separate xylans with high purity excluding some inherent impurities, e.g. lignin and glucose, compared to a single alkaline extraction (comparing between H1 and H3 in Table 2). High alkali concentration treatments (H2, H3, H5, and H6) resulted in extraction of low degree of substitution (Table 4), corroborating with the fact that xylan with smaller number of side groups is less soluble in water (Andrewartha et al., 1979). Low concentration of ethanol (H7 and H8) precipitated xylans with high purity and high molecular weight, but with low yield and low degree of substitution. By using the I₂-KI precipitation method to obtain two xylan fractions (H10 and H11), xylans was separated by degree of substitution.

Table 2 Chemical compositions of the xylan fractions separated through different extraction and precipitation methods.

Samples	Lignin %	Arabinose %	Galactose %	Glucose %	Xylose %	Galacturonic acid %	Glucuronic acid %
Bagasse	18.2 ± 0.3	1.4 ± 0.1	0.4 ± 0.0	33.9 ± 1.7	21.6 ± 1.3	1.4 ± 0.1	3.7 ± 0.2
H1	3.6 ± 1.7	8.0 ± 0.8	1.8 ± 0.1	11.4 ± 2.4	53.0 ± 0.6	2.2 ± 0.2	3.8 ± 0.2
H2	2.9 ± 0.0	6.5 ± 0.1	0.4 ± 0.1	3.5 ± 0.3	65.4 ± 0.7	1.4 ± 0.0	2.9 ± 0.3

H3	2.7 ± 0.3	5.7 ± 0.3	0.3 ± 0.0	3.1 ± 0.1	76.2 ± 0.9	1.3 ± 0.0	2.6 ± 0.1
H4	6.1 ± 1.0	7.1 ± 0.2	0.9 ± 0.0	9.0 ± 1.1	57.6 ± 0.3	2.3 ± 0.0	4.4 ± 0.3
H5	4.7 ± 1.6	5.2 ± 0.0	0.6 ± 0.0	7.3 ± 0.8	58.5 ± 1.3	1.8 ± 0.1	3.1 ± 0.2
H6	5.1 ± 1.0	5.3 ± 0.2	0.6 ± 0.0	3.3 ± 0.0	57.6 ± 0.3	1.5 ± 0.0	3.4 ± 0.2
H7	11.4 ± 0.4	6.0 ± 0.8	0.5 ± 0.0	3.8 ± 0.2	70.7 ± 1.1	1.8 ± 0.0	2.3 ± 0.3
H8	4.7 ± 0.0	7.3 ± 0.1	0.4 ± 0.0	4.8 ± 0.0	66.2 ± 0.2	2.4 ± 0.1	4.1 ± 0.1
H9	8.3 ± 0.9	8.4 ± 0.3	1.8 ± 0.3	9.9 ± 0.5	50.1 ± 0.1	1.4 ± 0.1	4.7 ± 1.2
H10	16.3 ± 0.9	4.3 ± 0.3	0.3 ± 0.1	3.4 ± 0.9	50.2 ± 0.7	1.3 ± 0.0	2.7 ± 0.0
H11	10.2 ± 1.3	8.5 ± 0.2	1.8 ± 0.0	18.0 ± 0.5	50.6 ± 1.4	2.3 ± 0.0	2.8 ± 0.0
H12	4.2 ± 0.1	7.0 ± 0.1	0.6 ± 0.1	7.8 ± 0.1	61.8 ± 0.2	2.0 ± 0.0	2.3 ± 0.2
H14	1.6 ± 0.3	7.5 ± 0.2	1.7 ± 0.0	1.4 ± 0.1	76.5 ± 1.9	1.3 ± 0.0	3.9 ± 0.1

Table 3 Peak assignments of the HSQC spectra of xylans associated with Fig. 1.

Labels	δ_C/δ_H	Assignment
Lignin structure		
-OCH ₃	55.9/3.73	C-H in methoxyls
A _{γ}	59.6-60.8/3.37-3.72	C _{γ} -H _{γ} in β -O-4 substructures(A)
A _{γ'}	63.6/3.86	C _{γ'} -H _{γ'} in γ -acylated β -O-4 substructures (A')
A _{β (S)}	86.0/3.91	C _{β} -H _{β} in β -O-4 substructures linked to a S unit (A)
A _{α}	72.8/4.86	C _{α} -H _{α} in phenylcoumaran substructures (C)
Associated carbohydrate and LCCs linkages		
X _{1+Glu1}	103.2/4.21	C ₁ -H ₁ in β -(1→4)-D-xylopyranoside C ₁ -H ₁ in β -(1→4)-D-Glucan
X ₂	72.5/3.02	C ₂ -H ₂ in β -(1→4)-D-xylopyranoside
X ₃	73.5/3.25	C ₃ -H ₃ in β -(1→4)-D-xylopyranoside
X ₄	75.4/3.50	C ₄ -H ₄ in β -(1→4)-D-xylopyranoside
X ₅	62.6/3.20	C ₅ -H ₅ in β -(1→4)-D-xylopyranoside
X _{NR4}	69.5/3.24	C ₄ -H ₄ in β -(1→4)-D-xylopyranoside with non-reducing end
X _{NR5}	65.5/3.01,3.45	C ₅ -H ₅ in β -(1→4)-D-xylopyranoside with non-reducing end
X2 ₁	99.4/4.42	C ₁ -H ₁ in β -(1→4)-D-Xylp-2-O-(4-O-methyl- α -D-GlcUA)
Ara ₁	106.9/5.32	C ₁ -H ₁ in α -(1→3)-L-arabinofuranoside
Ara ₂	81.6/3.79	C ₂ -H ₂ in α -(1→3)-L-arabinofuranoside

Ara ₃	77.1/3.62	C ₃ -H ₃ in α -(1→3)-L-arabinofuranoside
Ara ₄	85.9/3.89	C ₄ -H ₄ in α -(1→3)-L-arabinofuranoside
Ara ₅	61.9/3.52	C ₅ -H ₅ in α -(1→3)-L-arabinofuranoside
U ₁	97.2/4.98	C ₁ -H ₁ in 4- <i>O</i> -methyl- α -D-GlcUA
U ₄	81.9/3.08	C ₄ -H ₄ in 4- <i>O</i> -methyl- α -D-GlcUA
U ₅	72.9/4.28	C ₅ -H ₅ in 4- <i>O</i> -methyl- α -D-GlcUA
Glu ₄	73.8/3.62	C ₄ -H ₄ in β -(1→4)-D-Glucan
Glu ₅	73.4/3.45	C ₅ -H ₅ in β -(1→4)-D-Glucan
Est+A _{γ}	65-62/3.5-4.0	γ -ester and A' γ in LCC
PhGlc2	100.9/4.63	Phenyl glycoside-2 in LCC

Table 4 Molecular structures and film forming ability for the separated xylan fractions.

Xylan Fractions	Mn	Mw	PI	Degree of substitution*	Film Formable
H1	33500	61300	1.83	0.224 ± 0.016	Yes
H2	29700	61500	2.07	0.143 ± 0.005	No
H3	25100	66400	2.64	0.109 ± 0.004	No
H4	40200	63700	1.58	0.200 ± 0.004	Yes
H5	29800	52000	1.74	0.142 ± 0.007	No
H6	27500	52300	1.90	0.151 ± 0.000	No
H7	41300	67600	1.64	0.124 ± 0.003	No
H8	45500	71900	1.58	0.172 ± 0.003	Yes
H9	13800	60500	4.39	0.261 ± 0.017	Yes
H10	40300	64400	1.60	0.139 ± 0.008	No
H11	34200	61000	1.78	0.223 ± 0.002	Yes
H12	39500	61600	1.56	0.150 ± 0.001	Yes
H14	17500	58000	3.31	0.149 ± 0.002	No

Note: *Degree of substitution was calculated by the content ratio of (arabinose+ glucuronic acid)/xylose.

Figure 1 HSQC spectra of xylan fractions H1 (a), H3 (b), H7 (c), H9 (d), H10 (e), H11(f).

Film formation

When cast in a Teflon mold from aqueous solution/suspension, samples H1, H4, H8, H9, H11, H12 were able to form integral films without apparent cracks; we call these samples “film forming” (Fig. 2 & Table 4). Samples H5, H7, H10 formed films but with long cracks and samples H2, H3, H6, H14 broke into small fragments; we qualify both as not film forming.

Figure 2 The films casted in Teflon molds.

The xylans able to form films generally had high degree of substitution. A critical value of degree of substitution of about 0.15 can be identified above which an integral film is formed in our casting condition. Samples H6, H12 and H14 had degree of substitution values of 0.15, but only H12 formed a film. H12 had higher glucose content (Table 2), which corroborates the need for the addition of external plasticizers or biopolymers is helpful to the film formation of xylan (Gabrielii and Gatenholm, 1998; Grondahl et al., 2004; Kayserilioglu et al., 2003). The lignin contents in film-forming fraction were in the range of 1.6-16 %, while those in the other fractions were in range of 3.6-10 %, indicating lignin is not influencing xylan film formation. This contradicts the finding of the previous study that low content of lignin is necessary to xylan film formation (Goksu et al., 2007), and the previous result might have been a pure coincidence.

We do not know whether different side groups, i.e. arabinose and glucuronic acid, have specific influences on the film formability of xylans. The alkaline extracted xylans in this study were deacetylated, so the effect of acetyl groups on the film formability was not determined.

Figure 3 The molecular weight distributions of xylan samples extracted by different methods. The elution profile was normalized with the quantity injected, so the area is proportional to the soluble fraction in the elution buffer (0.1M NaNO₃-Na₂HPO₄ at 40°C).

The molecular weight distribution calibrated with dextran standard is shown in Figure 3. All samples except H9 (last fraction of sequential precipitation with increasing concentration of ethanol) and commercial xylan (H14) showed similar distribution. The elution profile was normalized with the quantity injected, so the area is proportional to the soluble fraction in the elution buffer (0.1M NaNO₃-Na₂HPO₄ at 40°C). The film forming samples are shown in thick lines in Figure 3. In each series, only the samples having high soluble fraction were forming films.

Film microstructure

The SEM images showed that the film fracture surface (at the natural cracking position for broken films) was smooth for film forming samples (Fig. 4 a&b). On the other hand, films that were cracked during drying showed flake-like granular morphology (Fig. 4 c-f) or spherical shape (Fig. 4 g&h) for the sample fragmented in small pieces. When observed under crossed polarizers, the films showed little birefringence (Fig. 5 a-c), while the broken films showed a large number of birefringent granular objects (Fig. 5 d-f).

The suspensions of xylan samples that showed fragmentation during cast-drying showed low viscosity and milky appearance (Fig. S1a and Table S1), in which some suspensions would settle after standing, indicating large xylan particles are already present in the suspension, and free chains are not abundant. The suspensions of xylan samples that form integral films showed high viscosity and gel-like property with some transparency (Fig. S1b and Table S1), indicating limited formation of xylan particles and presence of more free chains. The aggregates present already in the suspension probably limit the film formability.

Figure 4 SEM images of the film cross-section (from the natural cracking position for broken films) for (a)(b) H4-F, (c)(d) H5-NF, (e)(f) H7-NF, and (g)(h) H14-NF. Note: “F” means sample formed an integral film; “NF” means sample formed broken films.

Figure 5 Cross-polarized microscopic images of xylan films (a) H1-F, (b) H8-F, (c) H11-F, (d) H5-NF, (e) H7-NF, and (f) H14-NF. Scale bar = 100 µm. Note: “F” means sample formed an integral film; “NF” means sample formed broken films.

Crystallinity

Xylan is generally considered as an amorphous polymer, but the unsubstituted segment of xylan form hydrous and anhydrous crystals (Dea et al., 1973; Hoije et al., 2008; Horio and Imamura, 1964; Kobayashi et al., 2013; Marchessault and Timell, 1960; Nieduszynski and Marchessault, 1972). Indeed, in a sequential extraction the first extraction is quite amorphous while a successive extraction is more crystalline (Fig. 6a). In the single extraction, the fraction that can be extracted by 2% NaOH is less crystalline (Fig. 6b). The precipitation in the single extraction was done with 70% ethanol, so the 2% NaOH in this extraction was slightly more crystalline than in the sequential series. When xylan was sequentially precipitated with different ethanol concentration, from lower to higher, the first precipitates are crystalline while the last precipitate that can only be precipitated at high ethanol concentration is amorphous (Fig. 6c). Finally the direct precipitate with KI-I₂ was crystalline while the supernatant was amorphous. In

general the amorphous samples were film forming (thick lines in Fig. 6) whereas highly crystalline samples were cracked during drying. In addition, as each sample had slightly different peak positions indicating that the crystal structures are not exactly the same among samples, we did not calculate crystallinity index or crystallite sizes of xylan that would not be reliable. However, the general aspect of XRD profiles is sufficiently different among samples to allow for qualitative comparison.

The calculated diffraction profile based on the atomic coordinate proposed by Nieduszynski and Marchessault (1972) is given in Figure 6e (cif file of the structure is given in supplementary material). The general diffraction features agree roughly with the model, and the miller indices of peaks of the reflections mainly contributing to the peaks, as judged from the reported fiber diffraction diagram and calculated intensities are indicated in the Figure 6. This xylan in this structure form three-fold helix in contrast to the two-fold helix of cellulose or xylan adsorbed on cellulose surface (Falcoz-Vigne et al., 2017; Ling et al., 2020; Martínez-Abad et al., 2017).

The presence of side groups seems to play role in preventing the crystallization/excessive aggregation of xylan molecules (Andrewartha et al., 1979; Dea et al., 1973; Hoijs et al., 2008; Shrestha et al., 2019) (Table 4 and Figs. 4-6); for example, samples H1 and H9 had high degree of substitution and showed almost amorphous XRD diffraction patterns. We did not find evidence of different side groups, i.e. arabinose and glucuronic acid, having different influences on the crystallization of xylans. The carbohydrate impurities, e.g. galactose, glucose and galacturonic acid (Table 2), were also found to be related to the low crystallinity of xylans, e.g. sample H11; this is similar to other biopolymers, e.g. starch, for which artificial additives are also able to prevent the aggregation (Fama et al., 2005). The lignin content was not found to be very relevant to the xylan crystallization (Table 2). Its hydrophobic nature results in the

incompatibility to the hydrophilic xylan (Baumberger et al., 1998), and as a consequence, would be less effective in preventing the xylan from aggregation. The excessive aggregation of xylan molecules affects the solubility and solution/suspension viscosity (Andrewartha et al., 1979; Dea et al., 1973; Nieduszynski and Marchessault, 1972; Shrestha et al., 2019). Our results suggest that the prevention of excessive aggregation of xylan molecules, which is assisted by the xylan side chains or impurities, is highly related to the film forming capacity of xylans.

Figure 6 a-d) X-ray diffraction profiles of freeze-dried xylans after subtraction of air scattering and sample holder (thick lines indicate the film forming samples). The Miller indices of major peaks based on trigonal unit cell in the study of (Nieduszynski and Marchessault, 1972) are indicated. e) Simulated powder diffraction based on Nieduszynski's monohydrate structure with isotropic crystallite size of 10 nm (violet) or 100 nm (green).

Film mechanical properties

The six integral film samples were tested for their mechanical properties including tensile strength and modulus of elasticity (MOE). Samples H1, H4, and H11 had the highest tensile strength of 12~14 MPa and MOE of 150~180 MPa, while H8, H9, and H12 had much lower values (Table 5). Once the xylan is able to form a film, the film mechanical property is no longer positively related to the degree of substitution or negatively correlated to crystallinity. Samples H1, H4 and H11 had a medium degree of substitution, but had the highest mechanical properties, i.e. tensile strength and MOE; sample H9 had the highest degree of substitution of 0.261 and showed an almost amorphous XRD diffraction pattern, but had low mechanical properties. This is consistent with previous studies that moderate degree of substitution improved, while high degree of substitution was detrimental to the xylan film tensile strength (Hoijs et al., 2008; Jin et al., 2019). It can be concluded that the regulating mechanisms to xylan film formation and xylan film mechanical properties are different. The side groups of xylan molecules prevent their aggregation or crystallization, facilitating the film formation, but once the film is formed, the side groups do not seem to be necessarily contributing to the mechanical resistance.

Table 5 Xylan film mechanical properties ($\pm$ standard error).

Xylan Samples		Tensile Strength (MPa)	MOE (MPa)
H1	High	13.7 ± 1.8	182 ± 19
H4		13.2 ± 2.2	150 ± 15
H11		11.6 ± 1.7	171 ± 42
H9	Low	4.8 ± 0.4	99 ± 13
H12		4.5 ± 0.3	38 ± 5
H8		1.4 ± 0.1	14 ± 2

Conclusion

Bagasse xylan could be fractionated as function of side group densities using either severity of extracting conditions or precipitation conditions, with the higher density side groups being easier to extract and difficult to precipitate, with relatively constant molecular weight distribution as measured by gel permeation chromatography. The side group density was directly correlated with the crystallinity of freeze-dried samples. The excessive aggregation or crystallization of xylan, which can be effectively prevented by the side chains of xylans or impurities, *i.e.* lignin and glucan, is detrimental to the water-cast film forming ability of xylans. However, once a xylan film is formed, the crystallinity contributes to the higher stiffness and strength of the film. This finding would contribute to rational fractionation and xylan modification for material processing with controlled properties. We focused on alkaline extraction in this study, but will further evaluate milder extraction conditions such as hot-water extraction at neutral pH or DMSO extraction that can keep acetyl groups which might also contribute to the film formability.

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457