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Water Barrier Arabinoxylan Hemicelluloses from Sugarcane Bagasse

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Abstract: The hemicellulose from sugarcane bagasse was extracted sequentially with steam treatment followed by alkali and were characterized by chemical methods, SEC-MALLS, FT-IR and 13C NMR. The hemicellulose from steam pre-treatment was found to contain gluco-arabinoxylans while alkaline peroxide extraction yielded predominately linear arabinoxylans with varying amount of lignin. These arabinoxylans with high lignin content were tested for barrier properties on cardboards, to be used as food packaging materials. Due to lignin content, these hemicelluloses were found to increase the water barrier properties of the cardboard.

Keywords: Arabinoxylans Polysaccharide, Water Barrier, Sugarcane Bagasse, Steam Treatment, Biopolymer and Renewable Polymer, Coating, Packaging, Films

1. Introduction

The hydrophobic nature of the polysaccharides can be increased by adding lignin to the isolated hemicellulose (Goksu etal 2010) or by chemical hydrophobication of the hemicellulose (Heinze etal2006). In addition to this, lignin is proved to increase antibacterial properties of fibers (Ling, H., 2010; Afrin, T. 2012). Ultrathin films from lignin were also produced for transducers (Volpati D., 2012). In packaging, oxygen, grease and water barriers can be made of modified or native polysaccharides (Hansen M., 2008). Edible coating of casein or chitosan can be applied onto food items to prevent water vapour transfer (Talens P, 2012), while lignin and lignin-phenol formaldehyde resin films are effective water barrier coatings (Doherty etal 2007). The millions of tons discarded plastic packaging worldwide contribute with substantial potential hazards to the environment and public health. Some of the current materials in food packaging may cause negative health effects on humans (Mazur H, 1990). Therefore, there is a definite need to produce food packaging with renewables, biodegradable, non-toxic and even health promoting materials from natural sources.

The present study aims in utilizing the hemicelluloses with lignin content that are extracted from sugarcane bagasse. The concept is not to remove lignin entirely from the hemicelluloses but to apply them directly as hydrophobic barrier for cardboard. To the best of our knowledge, sugar cane bagasse hemicelluloses with lignin have not been used so far as a water barrier on a solid substrate. The present study showed a simple way of isolation of hemicelluloses with different degree of branching, molar mass, functional groups, varying lignin content from sugarcane bagasse and demonstrated a significant water barrier property on cardboard.

2. Experimental

2.1. Materials

Sugarcane bagasse was washed with water, air dried and then dried at 65°C for 24 h. The oven dried bagasse was ground in a Wiley mill to particles passing a 20 mesh screen and extracted with ethanol and toluene (2:1 v/v) in accordance with Tappi Method *T204 om-88*. The sugarcane bagasse was found to contain cellulose (49%), hemicelluloses (23%), lignin (21%), extractives (3%), and ash (3%).

2.2. Isolation of the Hemicelluloses

Isolation of hemicelluloses by steam treatment

Extractive free sugarcane bagasse (10 g) was subjected to

steam at 200°C and 210°C for 10 min. The extracts were cooled, filtered and concentrated to one-third of its volume at 40°C under reduced pressure. The solubilized hemicelluloses (were isolated by precipitation of the concentrated filtrates with 3 vol of 95% EtOH, washed first with acetone and then with MTBE. The precipitated hemicelluloses were dried under vacuum at 40°C for 12 hours and are designated as H1 and H2.

Isolation of alkaline peroxide soluble hemicellulosic fragments

Each of the foregoing steam treated residues were post treated with 3% alkaline peroxide and magnesium sulphate (0.25%) with the pH adjusted to 11.6 with NaOH at 40°C for 12 hours. All the extracts were filtered off and washed with water. The combined supernatant fluids were neutralized to pH 6.0 with dropwise addition of 6M HCl over an ice bath. All the extracts were concentrated to one-third of its volume at 40°C under reduced pressure and precipitated with 3 vol of 95% EtOH. The precipitates were washed first with acetone and then with MTBE. The precipitated hemicelluloses were dried under vacuum at 40°C for 12 hours. These hemicelluloses are designated as H3 and H4. The procedure used to isolate steam treated and alkaline peroxide soluble hemicelluloses are illustrated in Figure 1.

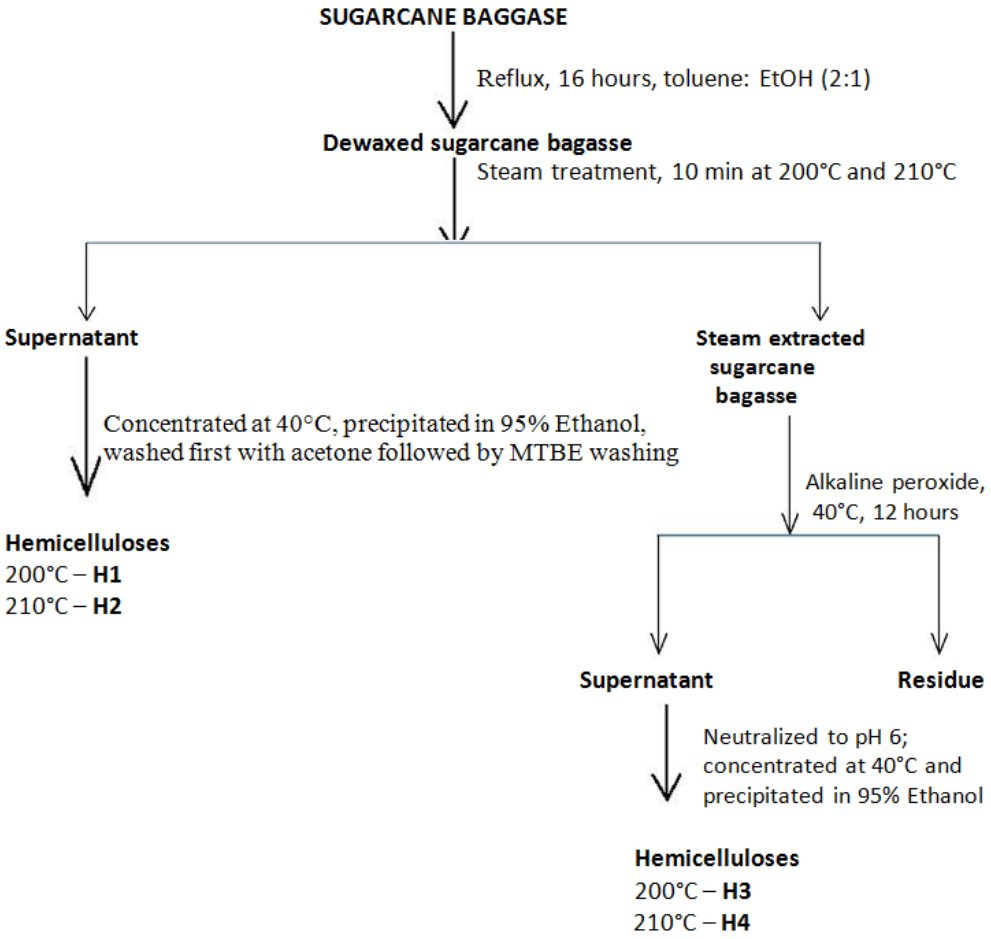

Fig. 1. *Scheme of isolation of hemicelluloses from sugarcane bagasse.*

2.3. Sugar Composition

Each hemicellulosic fraction (1 mg) was transferred to a pear shaped flask and dried in a vacuum oven at 40°C for 1 hour. Two mL of 2M HCl in anhydrous methanol was added to each flask and the samples were then kept at 105°C for 3 hours. A calibration solution containing equal amount (0.1mg/mL) of each sugar monomers and uronic acids (except 4-O-MeGlcA) was also subjected to methanolysis under similar condition. All samples were cooled to room temperature and neutralized by addition of 200 µL of pyridine. 1 mL of 0.1mg/mL sorbitol solution was added as internal standard to all the samples. The methanol was evaporated in stream of nitrogen, dried under vacuum at 40°C, silylated and analysed by GC according to Sundberg method (Banerjee, 2014).

2.4. HPSEC Analysis

Molecular weight of the hemicellulosic fractions was determined using high-performance size-exclusion chromatography (HPSEC) and refractive index detection (Reed, 1995). Four gel permeation ultra-hydrogel columns in series, with exclusion sizes of 7x106, 4x105, 8x104 and 5x103 Da, were used. The eluent was 0.1M aq. NaNO2 at 0.6 mL/min. The samples, previously filtered through a membrane (0.22µm), were injected at a concentration of 2

mg/mL. The dn/dc value was taken as 1.5 and the results were processed with software provided by the manufacturer (Wyatt Technology Corporation).

2.5. Methylation Analysis

Each hemicellulosic fraction (10 mg) was dissolved in 2 mL of dry DMSO and methylated by Hakomori method (Banerjee *etal*. 2007, 2014) The per-O-methylated derivative was extracted with chloroform (2x 5 mL), washed with water (3x 3 mL), evaporated under vacuum at 30°C and dried under vacuum at 40°C. The dried per-O-methylated samples were subjected to IR spectroscopy which showed no hydroxyl absorption and distinct peaks at 1740 cm-1 (C=O of ester) and 1125 cm-1 (C-O of ether). The per-O-methylated samples were then reduced with 1M superdeuteride (LiEt3BD) in THF at room temperature for 12 hours and then hydrolysed first with 50% v/v sulphuric acid at 30°C for 1 hour and then diluted to 5.0% and maintained at 120°C for 2 hours in an autoclave. The resulting mixtures of O-methyl aldoses was neutralized with BaCO3, filtered, reduced with sodium borohydride acetylated and analysed by GC-MS using column HP-1 (25m x 0.2mm x 0.11 µm) with a temperature of 80°C for 0.5 min and then increased to 300°C at a rate of 8°C/ min. Helium was used a carrier gas with a flow rate of 0.8 ml/ min (constant flow). The injector temperature was 300°C and the MS ionization mode was EI at 70 eV.

2.6. Lignin Determination

Lignin associated with the hemicelluloses was determined by the AcBr method according to Iiyama and Wallis, (1988). The structural composition of lignin was determined by pyrolysis GC–MS with tetramethylammonium hydroxide (TMAH) addition (Pranovich *et al*., 2005).

2.7. FT-IR Spectrometry

The infrared spectroscopy measurements were performed with a Bruker ALPHA series using the ALPHA platinum ATR single reflection diamond ATR module. The samples were directly placed on the ATR plate for measurement and the range was from 4000 to 400 cm^{-1} The results were evaluated using the software OPUS from Bruker.

2.8. NMR Spectrometry

The purified and dried samples were analysed by 1H NMR and 13C NMR measurements using a Bruker advance spectrometer (operation frequency: 1H: 600.13 MHz; 13C: 150.92 MHz). For the highly water-soluble hemicelluloses, D2O was used as a solvent while a D2O/DMSO-d6 mixture was applied for less soluble hemicelluloses to assure high concentration of the samples. For all the measurements, 50 mg of sample was dissolved in 1 mL of the respective solvent (concentration 50 mg/mL) and for the 1H NMR measurements 300 scans were made to assure a satisfactory peak to baseline ratio. For the 13C NMR measurements 18,000 scans were sufficient to obtain spectra with high resolution. When D2O was used as a solvent, DMSO-d6 was added as a standard for the chemical shift calibration. The temperature for all the measurement was set to 70°C and all the samples were saturated solution to assure a good signal to noise ratio in the 13C NMR spectra.

2.9. Preparation of the Coating Material

Solutions for coatings were prepared in the following way: Sorbitol (30% w/w of SCB) and sugarcane bagasse hemicelluloses were dissolved in water (12% (w/w)) for overnight. The solution was applied onto Stora Enso pre-coated Performa Natura 210 GSM cardboard with K-control bar-coater with 30.5 µm bars. Obtained coated cardboards were dried for ~7 seconds under an offline infrared heater (2 kW, IRT systems, Hedson Technologies AB) followed by 6 days drying in 50 (±) 3% relative humidity at 23°C.

2.10. Determination of Water Vapour Transmission

The water vapour transmission (WVTR) of the carton boards was evaluated using the NF ISO 2528 method. An aluminium cup with dried CaCl2 desiccant was sealed by the test carton boards (0.005m2). The edges were covered with wax. The duplicate samples were placed in the conditioning room at 23°C in 50% relative humidity. The weight of the cup was recorded in intervals for five times in two days.

2.11. Determination of Contact Angle

The apparent contact angles (three parallel measurements) were measured in ambient conditions (RH ~ 47%, T = 24 ± 1°C) using a CAM 200 contact angle goniometer (KSV Instruments Ltd). At least three different measurements were carried out. The apparent contact angles were calculated as a function of time using the One Attension software supplied with the instrument, which utilizes both a circular and a Laplace fit to the projected drop curvature.

3. Results and Discussion

3.1. Yield of Hemicelluloses

The steam treatment of sugarcane bagasse at temperatures between 200 and 210°C for 15 min resulted in the release of 30.9%, and 33.8% of polymeric hemicelluloses of the total hemicelluloses in the raw material (Table 1). The lignin associated with the steam treatment hemicelluloses was 11.3%, 9.4% which accounts for 4.2%, and 3.84% of the original lignin, respectively. The alkaline peroxide post-treatment of the residues resulted in the release of 51.4% and 53.86% polymeric hemicelluloses of the total hemicelluloses in the raw material (Table 1). The lignin associated with the hemicelluloses extracted with alkaline peroxide was 5.4%, and 5.0%, which accounts for 3.27%, and 3.1% of the original lignin, respectively. This result indicated that alkaline peroxide treatment significantly cleaved, probably, the a-ether and ester bonds between lignin and hemicelluloses.

Table 1. *Yield and composition of sugarcane bagasse hemicelluloses extracted sequentially by steam treatment and alkaline peroxide (H1 – H4).*

	Yield[a]	Total[b] sugar content	Hemicelluloses sugar units composition[c]									
			Ara	Rha	Xyl	GlcA	GalA	Man	Gal	Glc	4-O-Me GlcA	Ara/Xyl Ratio
H1	80	89.1	25.1	0.1	43.1	0.06	0.09	1.5	2.1	16.8	0.25	0.58
H2	86	90.5	24.3	0.09	48.0	0.05	0.08	0.6	2.0	15.2	0.2	0.50
H3	125	94.5	6.4	0.5	80.8	0.4	0.1	2.4	2.2	0.8	0.6	0.08
H4	130	95.3	6.7	0.1	81.2	0.7	0.7	2.1	2.4	0.7	0.7	0.08

[a]Expressed as mg/g of sugarcane bagasse
[b]Expressed as weight percent of total precipitated yield
[c]Expressed as weight percent of total precipitated yield

3.2. Non-cellulosic Carbohydrate Composition

The major sugar units from methanolysis of steam extracted hemicelluloses was xylose (43.1 and 48.0%), followed by arabinose (25.1 and 24.3%) and glucose (16.8 and 15.2%) (Table 1). The arabinose to xylose ratio was much lower at 210°C than at lower 200°C (0.58–0.5), indicating that the high-temperature extraction resulted in more linear structures, while extraction at 200°C resulted in the release of hemicelluloses with more branching. Thes data indicate that steam treatment probably released more branched galactoarabinoxylans and β-glucans. In case of alkali treated samples (Table 1), xylose was the predominant sugar (80.8 and 81.2%) followed by arabinose (6.4–6.7%), uronic acids, particularly glucuronic acid (0.4 and 0.7%) and 4-O-MeGlcA (0.6and0.7%), suggesting that the alkali-soluble hemicelluloses from sugarcane bagasse mainly consists of glucuronoarabinoxylans or L-arabino-(4-Omethyl-glucurono)-D-xylans. A low Ara/Xyl ratio would indicate a high degree of polymerization with little branching. The ratio of Ara/Xyl of the hemicelluloses obtained from alkaline treatment was lower (0.08) compared to the hemicelluloses obtained from steam treatment, indicating that the hemicelluloses obtained by alkali treatment were less branched than those from steam treatment.

3.3. Molar Mass Determination

The hemicelluloses from the steam treated extracts showed a relatively low degree of polymerization with molar-masses of 9900 and 10,500 g/mol (Table 2). On the other hand, high-molar-mass (17,000 and 23,000 g/mol) hemicelluloses were released during alkaline extraction. Additionally, the alkali-soluble hemicelluloses have broader molar-mass distribution with polydispersity indices from 3.1 and 2.2, while hemicelluloses from the steam treated extracts showed narrow molar-mass distribution with polydispersity indices of 1.2 and 1.5.

Table 2. *Weight average (Mw) and number average (Mn) molar mass and polydispersity (Mw/Mn) of hemicellulosic fractions released during extraction of sugarcane bagasse with steam treatment and alkaline peroxide.*

	H1	H2	H3	H4
M_w	10500	9900	23000	17000
M_n	8400	6600	7300	7500
M_w/M_n	1.2	1.5	3.1	2.2

3.4. Methylation Analysis

The partially methylated alditol acetates were subjected to GC–MS analysis and the results for H1 and H3 are shown in Table 3. Both hemicelluloses were dominated by (1-4)-arabinoxylan represented by a high percentage of 2,3-Me2-Xyl residues, substituted mainly at O-2 (3-Me-Xyl) and O-3 (2-Me-Xyl) by non-reducing end units of arabinofuranose (2,3,5-Me3-Ara). The uronic acids were determined by their respective m/z value, which is 2 units more than their corresponding neutral sugars. The appearance of 2,3,4,6-Me4Glc as glucuronic acid (confirmed from m/z value) in H3 suggest that the glucuronic acid moiety is present in the side chain and not in the main backbone as a-D-GlcpA or 4-O-Me-a-D-GlcpA. The steam extracted hemicelluloses is also dominated by (1-4)-glucose. The amount of 2,3,5-Me3-Ara was small in case of H3 (5.8%) corresponding to H1 (23.6%) corroborating that the alkaline extraction cleaves off the arabinose side chain significantly.

Table 3. *Partially O-methylated alditol acetates obtained from sugarcane bagasse hemicelluloses (H1 and H3).*

O-Me-alditol acetates	Linkages	H1(%)[a]	H3(%)[a]
2,3,5-Me_3-Ara	Ara*f*-(1→	23.6	5.8
3,5-Me_2-Ara	→2)-Ara*f*-(1→	1.2	0.7
2,3-Me_2-Ara	→5)-Ara*f*-(1→	0.3	0.1
2,3,4-Me_3-Xyl	Xyl*p*-(1→	0.2	0.1
2,3-Me_2-Xyl	→4)-Xyl*p*-(1→	40.6	76.3
2-Me-Xyl	→3,4)-Xyl*p*-(1→	1.6	2.7
3-Me-Xyl	→2,4)-Xyl*p*-(1→	1.0	1.5
2,3,4,6-Me_4-Glc[#]	Glc*p*-(1→	0.08	0.4
2,3,6-Me_3Glc	→4)-Glc*p*-(1→	17.1	0.7
3,4,6-Me_3Gal[#]	→2)-Gal*p*-(1→	0.1	0.2
2,4,6-Me_3Gal	→3)-Gal*p*-(1→	2.6	2.0
2,3,6-Me_3Man	→4)-Man*p*-(1→	2.0	2.6
2,4-Me_2Rha	→3)-Rha*p*-(1→	0.1	0.5

[a]Analysed by GC-MS
[#]Peaks from uronic acid moieties

3.5. Lignin Analysis

The hemicelluloses from steam treatment had higher lignin content (11.3–9.4%) than the corresponding alkali-soluble hemicelluloses (5.4 and 5.0%) suggesting that the a-benzyl ether linkage between lignin and hemicelluloses were significantly cleaved during alkaline peroxide treatment (Table 4). The associated lignin in the Hemicellulosic fractions were dominated by syringyl units, except inH2

indicating that syringyl units cleaved off significantly during steam treatment at higher temperatures (210°C).

Table 4. *Lignin content and p-hydroxyphenyl (H), guaiacyl- (G) and syringyl- (S) units determined in the Hemicellulosic fractions from sugarcane bagasse by pyrolysis GC-MS.*

	Lignin content[a]	H/G/S[#]
H1	11.3	0.8/1.0/1.2
H2	9.4	1.0/1.0/0.6
H3	5.4	0.9/1.0/1.2
H4	5.0	0.8/1.0/1.0

3.6. FT-IR Spectra

The presence of the absorption band at 1730 cm-1 in the spectrum of H1 (spectra not shown) and H2 might have originated from the acetyl, uronic, and ester groups of the hemicelluloses. The occurrence of a shoulder at 1514 cm-1 is due to the presence of associated lignin in the hemicelluloses (Pandey,1999), which corresponds to the data obtained by the AcBr method and pyrolysis GC–MS. The FT-IR spectra of hemicelluloses H3, and H4 (not shown) from alkaline peroxide extraction exhibited the absorbance bands associated with hemicelluloses. In comparison with the spectra of hemicelluloses released during steam extraction (H1, H2), the absence of a peak at 1730 cm-1 for carbonyl stretching demonstrated that acetyl groups and ester linkages between the hemicelluloses and the lignin cleaved during the alkaline extraction of the steam extracted residues.

3.7. ^{1}H and ^{13}C NMR Spectra

1H NMR spectra of H1 exhibited signals at 3.1–5.4 ppm that are due to protons of arabinose and xylose residues (spectra not shown). A strong signal at 2.6 ppm is evident of the acetyl groups that were not cleaved during steam extraction (very low signal was found in alkali-extracted fractions). In the 13C NMR spectrum of H3 (spectra not shown) the main (1-4)-linked b-D-xylopyranose units were evidenced by five strong signals at 102.1, 78.1, 77.4, 74, and 62.1 ppm, which are attributed to C-1, C-4, C-3, C-2, and C-5 of the β-D-Xylp units (Banerjee etal, 2014). The signals at 82.9 and 60.0 ppm are assigned to C-2 and C-5 of the a-L-Araf residues, respectively. The weak signal at 173.9 ppm is indicative of the carbonyl signal of the esterified ferulic or p-coumaric acids in the hemicelluloses. Moreover, a strong signal at 174.1 ppm is assigned to carbonyl group of the ester indicating the presence of lignin.

3.8. Determination of Water Vapour Transfer and Water Contact Angle

The dispersion of H1, H2 and H3 were prepared in water with sorbitol (30% (w/w)) as a plasticiser to enhance film forming properties of the coatings (Gounga M. E., 2008, Dai H. 2010). A thin coating was applied on a coated side of Stora Enso's Performa Natura cardboard with an inorganic pre-coated bar coater. Water vapour transfer (WVTR) was tested for sugar cane bagasse hemicellulose coated cardboards in 50% humidity at 23°C. Plain pre-coated cardboard gave the value of 277 g/ (m2 x 24h), and values for further coated samples were under 200 g/ (m^2 x 24h). Thus hemicellulose-lignin coating improved water barrier properties (Table 5). By taking into account the quantity of coating, H1 with highest lignin content gives slightly lower WVTR value than H2, which indicates increased effect of lignin as water barrier. However, H2 had higher contact angle than H1. Hemicellulose H3 has less branched structure than H1 and H2 and therefore it is less soluble in water. Hence solid and smooth coating has not been achieved with H3. The contact angle was lower for H3 than for H2. On a contrary to the WVTR values, the pre-coated cardboard itself had clearly higher water contact angle at the measured timescale than the ones with additional coating with hemicellulose lignin mixture.

Table 5. *The lignin content, coating weight, WVTR, apparent contact angle change in 2.5 seconds time for H1, H2 and H3.*

	Lignin % in Hemicellulose	Coating weight(g/m^2)	WVTR g/(m^2 x 24 h)	Apparent contact angle at 2.5 sec
H1	12.7	2.0	195	34.5+-1°
H2	11.0	2.8	182	38.5+-1°
H3	6.3	2.7	Not determined	24.5+-1°
Plain pre-coated cardboard	-	-	277	79.0+-2°

Comparison to earlier research is rather difficult, since solely polysaccharides applied for coatings as a function of water resistance is rare due to the hydrophilic nature of native polysaccharides. Comparison can be made to some independent films. The film made from chitosan – polylactic acid - polyethylene glycol 400 in a ratio of 90:8.3:1.7 gave WVTR value of 160 g/ (m2 x day) with film thickness of 31 µm (Sebastien F., 2012). To increase hydrophobicity of barriers, while maintaining the continuous coating network with lignin and hemicellulose needs further research. Nevertheless, we have established the novel biorefinery standpoint for much ignored hemicelluloses combined with lignin to improve barrier properties in food packaging applications. The natural source of these materials and image of the non-synthetic edible barrier renders value for sugarcane hemicellulose-lignin composites.

4. Conclusions

The present study showed a simple way of isolation of hemicelluloses with different degree of branching, molar mass, and functional groups from sugarcane bagasse. Consequently, products with a high aggregated value could be developed using this unmodified xylan-rich fraction as an ingredient for industrial products. In line with the known barrier properties of plant xylans, a significant water barrier

property was demonstrated for the isolated hemicelluloses with varying lignin content from sugarcane bagasse. Thus, the hemicelluloses containing lignin obtained from sugarcane bagasse can be of potential use as food packaging material in different industrial uses. Furthermore, the novel coating material shows promising properties for the development of sustainable barrier material in fibre-based packaging material to replace oil-based barriers.

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