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Fractional and physico-chemical characterization of hemicelluloses from ultrasonic irradiated sugarcane bagasse

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Abstract—The present study was undertaken to investigate the extractability of the hemicelluloses from bagasse obtained by ultrasound-assisted extraction methods. The results showed that the ultrasonic treatment and sequential extractions with alkali and alkaline peroxide under the conditions given led to a release of over 90% of the original hemicelluloses and lignin. This fact as well as the sugar composition and structural features of the isolated seven hemicellulosic fractions indicated that ultrasonication attacked the integrity of cell walls, cleaved the ether linkages between lignin and hemicelluloses, and increased accessibility and extractability of the hemicelluloses. Increasing alkali concentration from 0.5 to 2 M and alkaline peroxide percentage from 0.5% to 3.0% resulted in degradation of hemicellulosic backbone as shown by a decrease in their molecular weights from 43,580 to 14,470 and 30,180 to 18,130 g mol^{-1}, respectively. However, there were no significant differences in the structural features of the seven sequential alkali- or alkaline peroxide-soluble hemicellulosic fractions, which are composed mainly of L-arabino-(4-*O*-methyl-D-glucurono)-D-xylans. Ferulic and *p*-coumaric acids were found to be chemically linked with hemicelluloses.

Keywords: Ultrasound; Extraction; Hemicelluloses; Sugarcane bagasse

1. Introduction

Sugarcane bagasse (or, 'bagasse' as it is generally called) is a residue from the refining process of sugarcane. An absolute minimum of about 50% of bagasse is needed to generate heat and power to run the sugar milling process and the remainder can be stockpiled.[1] The stockpiled bagasse is of low economic value and constitutes an environmental problem to sugar mills and surrounding districts, especially if stockpiled for extended periods, due to the risk of spontaneous combustion occurring within the pile.[2,3] As a means of minimizing this hazard, there has been an increasing trend toward more efficient utilization of bagasse. Several processes and products have been reported utilizing bagasse as a raw material. These include electricity generation, pulp and paper production, and products of fermentation.[4]

Hemicelluloses, complex polysaccharides found in plant cell walls, are the second most abundant renewable materials after cellulose.[5] They are closely associated with cellulose and lignin in woody tissues. About 40–50% of dry bagasse is cellulose, much of which is in a crystalline structure. Another 25–35% is hemicelluloses, amorphous polymers. The remainder is mostly lignin plus lesser amounts of minerals, and other compounds.[6] Because of its low ash content (2.3%), bagasse offers numerous advantages in comparison to other crops residues such as rice straw, which has 13.3% ash content,[7] for usage as novel materials for industries.

Application of ultrasonic irradiation used during the isolation of plant materials has been found to improve significantly the extraction of polysaccharides, particularly for extracting low molecular weight substances.[8–10] Positive effect of ultrasound on the extractability of

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polysaccharides from corn bran,[11] buckwheat hulls,[12] and *Salvia officinalis* L.[13] has been reported. In addition, the ultrasonic treatment is simple and more rapid than other conventional techniques in the fractionation of plant materials.[14] The current work is focused on the fractionation and physico-chemical characterization of hemicelluloses from ultrasonic irradiated bagasse. The extraction techniques were also tested for yield of hemicelluloses. The isolated samples were studied by acid hydrolysis, nitrobenzene oxidation of associated lignins, thermal analysis, Fourier transform infrared (FT-IR) and hydrogen-1 and carbon-13 magnetic resonance (^{1}H and ^{13}C NMR) spectroscopy and gel permeation chromatography (GPC).

2. Results and discussion

2.1. Fractional yield

Alkaline peroxide, which is widely used in the pulp and paper industry to bleach lignin-rich pulps, is an effective agent for both delignification and solubilization of hemicelluloses from straw and grass. It is generally accepted that the hydroperoxide anion (HOO^-), formed in alkaline media, is the principal active species in hydrogen peroxide bleaching systems. In contrast, the decomposition products such as hydroxyl radicals ($HO\cdot$) and superoxide anion radicals ($O_2^-\cdot$) are thought to lead to cleavage of some inter-unit bonds between lignin and polysaccharides and eventually, the dissolution of hemicelluloses and lignin.[6,15] In this study, a sequential extraction of hemicelluloses with water, alkali, and alkaline peroxide was performed according to the scheme of Figure 1 under laboratory conditions. The yield of the extracts and soluble hemicelluloses are shown in Table 1. As can be seen, the application of

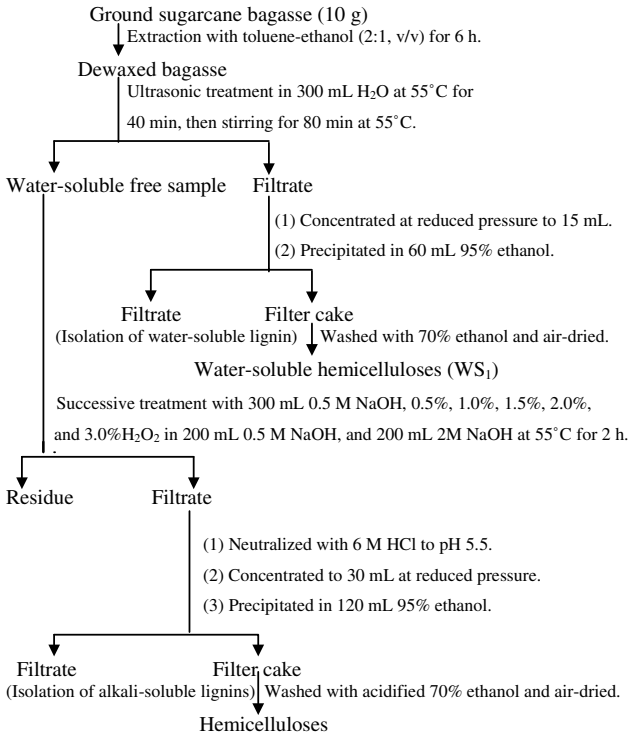

Figure 1. Scheme for fractional isolation of hemicelluloses from ultrasonic irradiated bagasse.

ultrasonic irradiation in distilled water at 55 °C for 40 min affect positively the yield of both hemicelluloses and lignin. Sequential extractions of the sonicated bagasse with water, 0.5 M NaOH, 0.5, 1.0, 1.5, 2.0, and 3.0% H_2O_2 at pH 11.5, and 2 M NaOH at 55 °C for 2 h yielded 4.5, 12.8, 4.0, 1.5, 3.0, 0.8, 0.4, and 3.8% hemicelluloses, corresponding to release of 13.4, 38.2, 11.9, 4.5, 9.0, 2.4, 1.2, and 11.3% of the original hemicelluloses, respectively. Meanwhile, as might be expected, the successive treatments also solubilized 3.3, 59.7, 15.5, 4.4,

Table 1. The yield of hemicelluloses and lignin (% dry matter) solubilized during the successive treatments of dewaxed bagasse with distilled water under ultrasonic irradiation, 0.5 M NaOH, various concentrations of alkaline peroxide, and 2 M NaOH at 55 °C for 2 h

	WS_1[a]	AS_1[b]	H_2O_2 concentration (%)					AS_2[d]	Total
			0.5[c]	1.0[c]	1.5[c]	2.0[c]	3.0[c]		
Hemicelluloses[e]	4.5	12.8	4.0	1.5	3.0	0.8	0.4	3.8	30.8
	(4.1)[f]	(12.0)[f]	(3.8)[f]	(1.4)[f]	(2.5)[f]	(1.0)[f]	(0.9)[f]	(4.6)[f]	(30.3)[f]
Lignin	0.6	10.8	2.8	0.8	1.0	0.3	0.1	0.1	16.5
	(0.5)[f]	(9.5)[f]	(2.7)[f]	(0.6)[f]	(1.0)[f]	(0.9)[f]	(0.5)[f]	(0.4)[f]	(16.1)[f]
Residue	94.2	70.0	63.1	60.9	56.6	55.5	55.0	44.7	
	(94.8)[f]	(71.4)[f]	(64.4)[f]	(62.4)[f]	(58.8)[f]	(56.8)[f]	(55.2)[f]	(45.9)[f]	

[a] Water-soluble hemicellulose and lignin fraction obtained by ultrasonic treatment of the dewaxed bagasse in distilled water at 55 °C for 40 min, then stirring for 80 min at 55 °C.

[b] Alkali-soluble hemicelluloses and lignin obtained by extraction with 0.5 M NaOH at 55 °C for 2 h from the ultrasonic irradiated and water treated bagasse.

[c] The fractions obtained by successive extractions of the 0.5 M NaOH treated bagasse with different concentrations of H_2O_2 at 55 °C for 2 h at pH 11.5.

[d] The fraction obtained by extraction with 2 M NaOH at 55 °C for 2 h from the 3.0% H_2O_2 treated bagasse.

[e] The hemicellulosic fractions obtained by precipitation of the neutralized extracts with 3 vol of 95% ethanol.

[f] Represents the yield obtained under the corresponding conditions without ultrasound.

5.5, 1.7, 0.6, and 0.6% of the original lignin, respectively. In comparison, the total of hemicelluloses was higher in the case of the ultrasound-treated bagasse. This is particularly true during the first five sequential treatments with distilled water, 0.5 M NaOH, 0.5, 1.0, and 1.5% H_2O_2 at pH 11.5, in which the yield of hemicelluloses was higher by 1.2, 2.4, 0.6, 0.3, and 1.5% of the original hemicelluloses than those of the hemicelluloses obtained under the corresponding conditions without ultrasound. The highest differences were seen in the yields of the 0.5 M NaOH extractable hemicelluloses. It is important to note that about 92% of the original hemicelluloses and 91% of the total lignin was released under the mild conditions from the ultrasound irradiated bagasse. As most of the hemicelluloses were solubilized during the first five sequential extraction processes, the hemicelluloses released in the latter three successive extraction steps with 2.0 and 3.0% H_2O_2 at pH 11.5 and 2 M NaOH at 55 °C for 2 h showed a lower yield. Interestingly, the yield of the total extracted hemicelluloses was 1.6% higher in comparison to that of the corresponding procedures without ultrasound. The yield of the final insoluble extraction residue (44.7%) are close to the cellulose content of bagasse (43.6%), indicating a substantial removal of the hemicelluloses and lignin during the sequential extractions of ultrasonic irradiated bagasse. Evidently, the extractability of the hemicelluloses was increased by the ultrasonic irradiation, which is thought to be due to the mechanical disruption of cell walls resulting in increased accessibility.[13] Such effects of sonication were shown to improve the isolation of extractives from various plant materials.[17]

2.2. Composition of neutral sugars

Further evidence of the cell wall disruption by ultrasonic irradiation was derived from the chemical composition of the isolated hemicellulosic fractions and their physico-chemical properties. Table 2 gives their content of neutral sugars and uronic acids. Obviously, in addition to xylose (37.4%) and arabinose (12.8%), the water-extractable fraction obtained from ultrasound-treated bagasse was rich in glucose (28.9%), galactose (11.6%), and mannose (8.1%). However, significant differences in the neutral sugar composition were observed for the alkali- and alkaline peroxide-soluble hemicelluloses in comparison with the water extracted ones, but there were no substantial differences between the sugar composition of the hemicellulosic fractions obtained by sequential extractions with alkali and alkaline peroxide. Xylose was the predominant sugar component ranging from 71.2% to 86.6%, suggesting the presence of a higher proportion of xylan, particularly in the two alkali extracts. Arabinose was the second major sugar constituent, consisting of 8.2–12.6% of the total sugars. Noticeable amounts of glucose (4.2–18.1%) and uronic acids (1.9–5.0%), mainly glucuronic acid or 4-*O*-methyl-D-glucuronic acid (MeGlcA) and minor quantities of galactose (0.4–3.0%), rhamnose (0–0.7%), and mannose (0–0.4%) were also identified in these fractions. These results implied that the seven alkali- or alkaline peroxide-soluble hemicellulosic fractions were composed mainly of glucuronoarabinoxylans or L-arabino-(4-*O*-methyl-D-glucurono)-D-xylans. It is very likely that arabinoxylans obtained at the initial extraction step were more highly substituted, as indicated by the lower Xyl/Ara ratio of 2.9:1 in the ultrasonic irradiated and water-extracted hemicellulosic fraction. The higher arabinose content indicated a higher degree of branching of the xylan chains and the higher solubility of the polymers. This phenomenon provided evidence that in bagasse cell walls arabinose, probably as a side chain in hemicelluloses, is easily solubilized during the initial extraction process, whereas this side chain was partially cleaved or degraded in the sequential alkali or alkaline peroxide treatments, as shown by higher Xyl/Ara ratios between 6.4:1 and 12.2:1. Similar results were observed in our previous study on wheat straw hemicelluloses.[16] We found that xylans from wheat straw containing a high degree of side chain substitution are more water

Table 2. The content of neutral sugars (relative % dry weight, w/w) and uronic acids (% dry weight, w/w) in isolated hemicellulosic fractions

Sugars (%)	WS_1^a	AS_1^b	H_2O_2 concentration (%)					AS_2^d
			0.5[c]	1.0[c]	1.5[c]	2.0[c]	3.0[c]	
Rhamnose	1.25	0.42	0.44	0.74	0.26	0.25	Tr[e]	ND[f]
Arabinose	12.83	12.57	10.40	10.48	10.38	10.59	8.23	7.09
Xylose	37.38	80.60	78.98	78.44	77.62	77.38	71.22	86.56
Mannose	8.07	0.40	0.32	0.22	0.18	0.18	Tr	ND
Galactose	11.61	1.83	1.95	2.35	2.49	3.02	2.45	0.44
Glucose	28.86	4.18	7.93	7.77	9.07	8.57	18.12	6.22
Uronic acids	5.38	3.50	4.00	4.65	5.04	4.65	4.34	1.87

[a,b,c,d]Corresponding to the hemicellulosic fractions in Table 1.

[e]Tr, trace.

[f]ND, not detectable.

soluble and bind less tightly to cellulose, whereas molecules with infrequent side chains are less water soluble and bind more tightly to cellulose.

2.3. Content of bound lignin and its phenolic composition

It is well know that lignin is tightly linked to polysaccharides in the cell walls of plants by various linkage types, and most commonly covalent linkage is the ether bond of the hydroxyl group at the α-position of the lignin side chain with alcoholic hydroxyl of sugar residue.[17] The phenolic acids and aldehydes, released from the eight hemicellulosic fractions by alkaline nitrobenzene oxidation of the associated lignin, were analyzed by high performance liquid chromatography, and the results are listed in Table 3. Clearly, all the hemicellulosic fractions contained relatively low amounts of associated lignins, ranging between 0.41% and 7.36%, which was lower than those of the corresponding hemicellulosic preparations obtained without ultrasound, 0.46–9.63% (data not shown). This low content of chemically linked lignin in hemicelluloses verified that the α-benzyl ether linkages between lignin and hemicelluloses in the cell walls of bagasse were substantially cleaved during the ultrasonic irradiation and the sequential alkali and alkaline peroxide treatments under the conditions given. In other words, the observed beneficial sonication effect on the extractability of the hemicelluloses can be explained by both the mechanical disruption of the cell walls and cleavage of the linkages between lignin and hemicelluloses. The content of bound lignin was maximized in the hemicellulosic fraction isolated with water from ultrasonic irradiated bagasse (7.36%) and minimized in the fraction of AS_2 (0.41%) extracted with 2 M NaOH at 55 °C for 2 h from 3.0% H_2O_2 treated bagasse. The major products, obtained from alkaline nitrobenzene oxidation, were detected to be vanillin and syringaldehyde, suggesting that the bound lignin in the hemicellulosic fractions contained almost equal amounts of noncondensed guaiacyl and syringyl units. A noticeable amount of *p*-hydroxybenzoic acid and *p*-hydroxybenzaldehyde, and traces of vanillic acid, syringic acid, acetovanillone, acetosyringone, *p*-coumaric acid, and ferulic acid were also found to be present in the nitrobenzene oxidation products.

Lignin in grasses are acylated with *p*-coumarate, which is attached primarily to syringyl units.[18] Although very small quantities of *p*-coumarate are esterified to arabinoxylans in immature tissues, most *p*-coumarate accretion occurs in tandem with lignification,[19] making *p*-coumarate accumulation a convenient indicator of lignification. During lignification, cell walls are further stiffened by oxidative coupling of ferulate monomers and dimers with monolignols, forming additional cross-links between structural lignin and hemicelluloses.[20] In grasses ferulate dimerization and the onset of lignification are associated with cessation of segmental elongation.[21] Based on study of lignin–carbohydrate complex from bagasse, Kato et al.[22] demonstrated that ferulic and *p*-coumaric acids released by alkaline treatment are esterified to the different molecular species, hemicelluloses and lignin moieties, respectively, and isolated a feruloylated trisaccharide, *O*-[5-*O*-(*trans*-feruloyl)-α-L-arabinofuranosyl]-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 4)-D-xylopyranose, from the enzymatic hydrolysate of the bagasse lignin–carbohydrate complex, in which ferulic acid is linked at O-5 of the L-arabinofuranose. As can be seen from Table 3, the occurrence of traces of ferulic and *p*-coumaric acids in the hemicellulosic fractions stated that these two phenolic acids are strongly linked with hemicelluloses or lignin in the cell walls of bagasse, since most of the *p*-coumaric acids and ferulic acids were

Table 3. The yield (% hemicellulosic sample, w/w) of phenolic acids and aldehydes from alkaline nitrobenzene oxidation of the associated lignin in isolated hemicellulosic fractions

Phenolic acids and aldehydes	WS_1[a]	AS_1[b]	H_2O_2 concentration (%)					AS_2[d]
			0.5[c]	1.0[c]	1.5[c]	2.0[c]	3.0[c]	
p-Hydroxybenzoic acid	0.59	0.10	0.012	0.026	0.030	0.023	0.031	0.033
p-Hydroxybenzaldehyde	0.67	0.062	0.064	0.11	0.10	0.13	0.10	0.036
Vanillic acid	0.037	0.008	0.014	0.036	0.037	0.037	0.021	0.010
Vanillin	1.05	0.36	0.35	0.72	0.70	0.68	0.35	0.10
Syringic acid	0.041	0.021	0.018	0.010	0.006	0.008	Tr[e]	ND[f]
Syringaldehyde	1.18	0.44	0.35	0.65	0.60	0.57	0.19	0.030
Acetovanillone	0.42	0.041	0.024	0.029	0.032	0.022	0.011	0.003
Acetosyringone	0.33	0.059	0.038	0.052	0.057	0.042	0.004	ND
p-Coumaric acid	0.10	0.011	0.011	0.008	0.007	0.007	0.003	ND
Ferulic acid	0.060	0.032	0.010	0.012	0.006	0.003	0.001	ND
Total	4.48	1.13	0.89	1.65	1.58	1.52	0.71	0.21
Content of klason lignin	7.36	2.18	1.81	2.70	2.28	2.17	1.34	0.41

[a,b,c,d]Corresponding to the hemicellulosic fractions in Table 1.

[e]Tr, trace.

[f]ND, not detectable.

quantitatively oxidized to *p*-hydroxybenzaldehyde and vanillin, respectively, by nitrobenzene under the reaction condition used. Obviously, ultrasonic irradiation and sequential extractions of bagasse with alkali and alkaline peroxide under the conditions given only cleaved partially these esterified or etherified linkages between hydroxycinnamic acids and hemicelluloses and/or lignin from the cell walls of bagasse.

2.4. Molecular weight

Weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) of the hemicellulosic fractions are shown in Table 4. The results indicated a significant decrease in the relative molecular weights of the hemicellulosic fraction obtained by ultrasonic irradiation and water extraction (M_w, 5220 g mol^{-1}) in comparison to those of other seven alkali- and alkaline peroxide-soluble hemicellulosic fractions (M_w, 14,470–43,580 g mol^{-1}). The results confirmed again that ultrasonic treatment not only attacked the integrity of the cell walls rendering their components more accessible to extraction, but also broke the inter- and intra-molecular hemicellulosic backbone linkages, resulting in a depolymerization of the hemicellulosic macromolecules into fragments soluble in water. In addition, as can be seen in Table 4, an increase in alkali concentration from 0.5 to 2 M and alkaline peroxide percentage from 0.5 to 3.0% resulted in a significant decrement of M_w from 43,580 to 14,470 and from 30,180 to 18,130 g mol^{-1}, respectively. Similar decreasing trend was also observed in their polydispersities, which reduced from 7.2 in AS_1 to 3.2 in AS_2 fraction and from 4.2 in 0.5% H_2O_2 soluble fraction to 3.8 in 3.0% H_2O_2 soluble hemicellulosic preparation. This may be due to alkali-catalyzed degradation reaction of hemicelluloses and splitting of the their main chains by reactions of the formed macroradicals during the alkaline peroxide treatments. These data were consistent with the studies on the ultrasonic degradation of corn cob xylans by Hromádková et al.[23]

2.5. FT-IR spectra

Figure 2 illustrates the FT-IR spectra of bagasse hemicellulosic fractions isolated by ultrasonic irradiation and water (spectrum 1), 0.5% H_2O_2 (spectrum 2), 2.0% H_2O_2 (spectrum 3), and 2 M NaOH (spectrum 4). Clearly, all the spectra show the typical signal pattern expected for a hemicellulosic moiety. The absorption at 3430 cm^{-1}

Table 4. Weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) of the hemicellulosic fractions

	WS_1[a]	AS_1[b]	H_2O_2 concentration (%)					AS_2[d]
			0.5[c]	1.0[c]	1.5[c]	2.0[c]	3.0[c]	
M_w	5220	43,580	30,180	28,000	26,230	25,420	18,130	14,470
M_n	4100	6070	7220	6030	5550	5880	4760	4510
(M_w/M_n)	1.27	7.18	4.18	4.64	4.73	4.32	3.81	3.21

[a,b,c,d]Corresponding to the hemicellulosic fractions in Table 1.

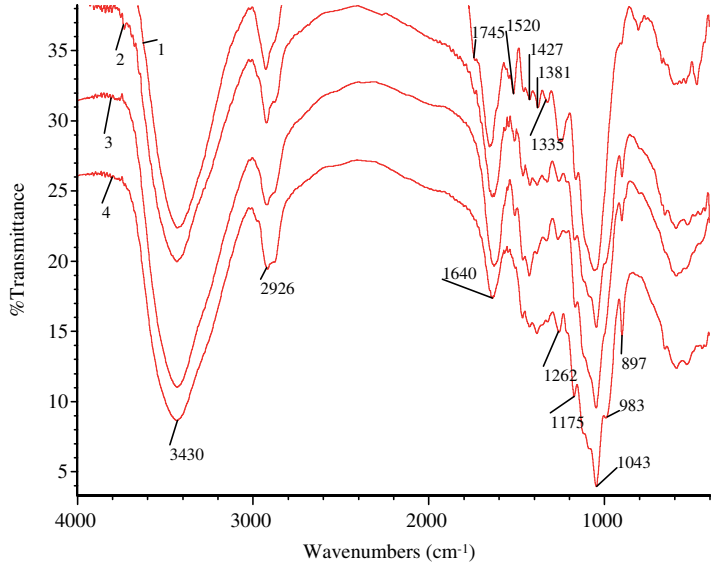

Figure 2. FT-IR spectra of bagasse hemicellulosic fractions isolated by ultrasonic irradiation and water (spectrum 1), 0.5% H_2O_2 (spectrum 2), 2.0% H_2O_2 (spectrum 3), and 2 M NaOH (spectrum 4).

arises from stretching of –OH groups and that at 2926 cm^{-1} from C–H stretching. The band at 1640 cm^{-1} is indicative of the bending mode of the absorbed water. An intensive and sharp band at 1043 cm^{-1} is attributed to the C–O–C stretching that is typical of xylans. This is particularly true in the spectra 2, 3, and 4 of hemicellulosic fractions, isolated with alkali or alkaline peroxide from the ultrasonic irradiated bagasse, which corresponded to the results obtained by sugar analysis. Each spectrum presents a peak at 1427 cm^{-1}, which is due to the CH_2 bending and that in 1381 cm^{-1} to the O–H bending. The absorbance at 1335 cm^{-1} arises from the C–C and C–O skeletal vibrations. The peak at 1262 cm^{-1} relates to the OH in-plane bending.[14] The presence of arabinosyl side chains is detected by the low intensity shoulder at 1175 cm^{-1}, corresponding to the C–O–C vibrations in hemicelluloses.[6] In the anomeric region (950–700 cm^{-1}), a small sharp band in spectra 2, 3, and 4 and a shoulder in spectrum 1 at 897 cm^{-1} is characteristic of β-glycosidic linkages in hemicelluloses, whereas a small peak at 792 cm^{-1} in spectrum 1 is indicative of α-anomers in side chains.[24] This demonstrated the presence of predominant β-glycosidic linkages between the sugar units in the hemicellulosic fractions extracted with alkali or alkaline peroxide, while the hemicellulosic fraction, solubilized during the ultrasonic irradiation and water treatment, have more α-anomers in side chains. The presence of a shoulder at 1745 cm^{-1} in spectrum 1 implies that the hemicellulosic fraction, solubilized during the ultrasonic irradiation and water treatment, contains small amounts of acetyl and uronic ester groups or the ester bonds of carboxylic groups of the ferulic or *p*-coumaric acid. In contrast, the absence of this signal in spectra 2, 3, and 4 indicates that both alkali and alkaline peroxide treatments under the conditions used completely saponifies these ester bonds from the hemicelluloses. The occurrence of an intensive band at 1520 cm^{-1} in spectrum 1 is due to the associated lignin in the hemicellulosic fraction, while it becomes rather weak in spectra 2, 3, and 4, indicating the presence of only small amounts of bound lignin in these hemicellulosic fractions, isolated by alkali or alkaline peroxide, which corresponded to the data obtained by alkaline nitrobenzene oxidation.

2.6. ^{1}H and ^{13}C NMR spectra

All the hemicellulosic fractions were found to be easily dissolved in D_2O at neutral pD. Figure 3 shows ^{1}H NMR spectrum of the hemicellulosic fraction isolated with 0.5% H_2O_2 at pH 11.5 from the ultrasonic irradiated and alkali treated bagasse. As can be seen, the spectrum gives the typical signal pattern expected for a hemicellulosic moiety. The chemical shifts of 3.3–

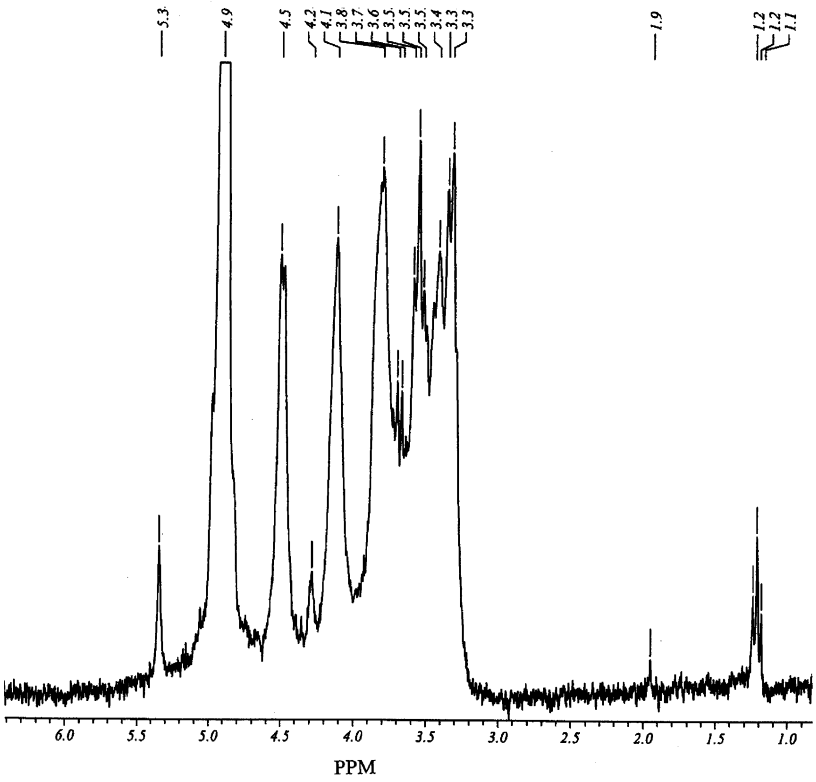

Figure 3. ^{1}H NMR spectra (in D_2O) of the hemicellulosic fraction isolated with 0.5% H_2O_2 at pH 11.5 from ultrasonic irradiated and alkali treated bagasse.

4.5 ppm are assigned to the equatorial proton and other protons of anhydroxylose units of hemicelluloses. The methyl protons of few amounts of acetyl group and 4-*O*-methyl-D-glucuronic acid give weak peaks at 1.9 and 1.2–1.1 ppm, respectively. Anomeric protons of terminal α-D-arabinofuranosyl residues occur at 5.3 ppm.[25] A strong signal at 4.9 ppm is indicative of the residual solvent (HDO).

^{13}C NMR spectrum (in D_2O) of the hemicellulosic fraction isolated with 0.5% H_2O_2 at pH 11.5 from the ultrasonic irradiated and alkali treated bagasse is given in Figure 4. Most of the major resonances were assigned by references to data in literature.[26,27] Signals in the anomeric region (109.5, 102.3, and 100.4 ppm) could be assigned to arabinofuranose, substituted xylopyranose, and 4-*O*-methyl-D-glucuronic acid unit, respectively. The five main signals at 102.3 (C-1), 73.3 (C-2), 74.9 (C-3), 75.9 (C-4), and 63.3 ppm (C-5) are attributed to (1→4)-linked β-D-Xyl residues. The signals at 109.5, 86.4, 80.3, 78.4, and 61.7 ppm originate from C-1, C-4, C-2, C-3, and C-5 of α-L-arabinofuranosyl residues linked to β-D-xylans, respectively. The C-4 and C-6 of glucose residue in the xylan exhibit two signals at 82.6 and 61.7 ppm. Two weak signals at 72.1 and 70.0 ppm are due to C-4 and C-2 of galactose residue in the xylan. Among others signals observed at 173.0 and 59.4 ppm, respectively, are characteristic signals of C-6 and methoxyl group of a 4-*O*-methyl-D-glucuronic acid residue in the xylan. The signal at 17.0 ppm arises from $-CH_3$ in $Ar-COCH_3$, indicating the associated lignins. Two small signals at 181.6, 177.1 ppm (data not shown) correspond to the carbonyl signal ($-CH_2COO^-$) of the esterified ferulic or *p*-coumaric acids in native hemicelluloses. The current results coincided with the findings by Kato et al.[22] They revealed that ferulic acid is linked at C-5 of the L-arabinofuranosyl residue, which is attached to the (1→4)-β-linked D-xylan backbone at C-3.

2.7. Thermal analysis

TGA and DSC thermograms of hemicellulosic fractions, isolated with 0.5 and 2 M NaOH at 55 °C for 2 h from the ultrasonic irradiated bagasse, are illustrated in Figure 5a and b. As observed, the two hemicellulosic fractions are stable up to 245 and 220 °C, respectively. Beyond these temperatures, thermal degradation takes place. Similarly, at 50.0% weight loss the decomposition temperatures of the two polymer samples occurred at 300 and 290 °C, respectively. These data implied that the hemicellulosic fraction, isolated with 0.5 M NaOH from

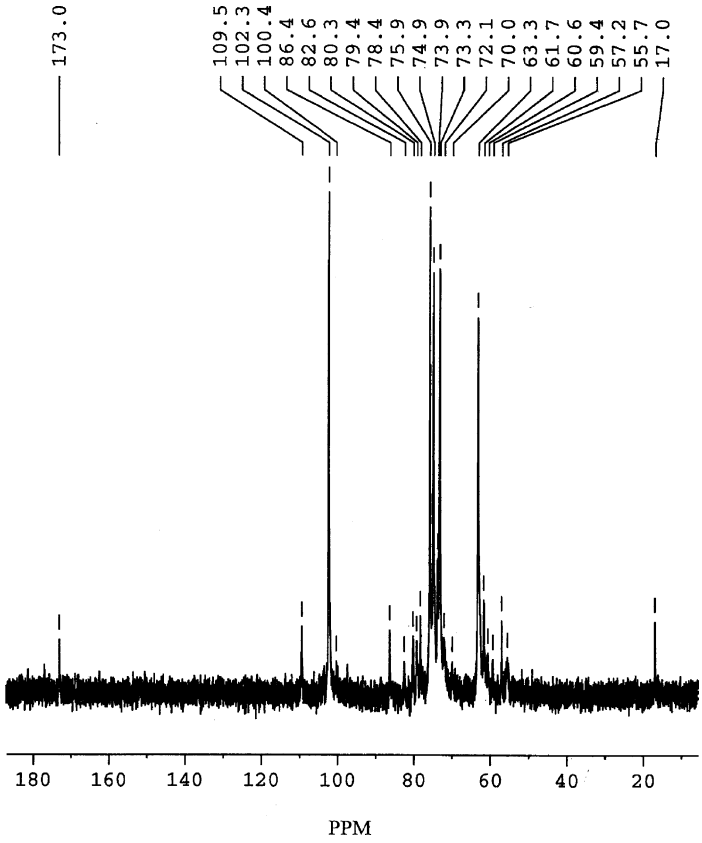

Figure 4. ^{13}C NMR spectra (in D_2O) of the hemicellulosic fraction isolated with 0.5% H_2O_2 at pH 11.5 from ultrasonic irradiated and alkali treated bagasse.

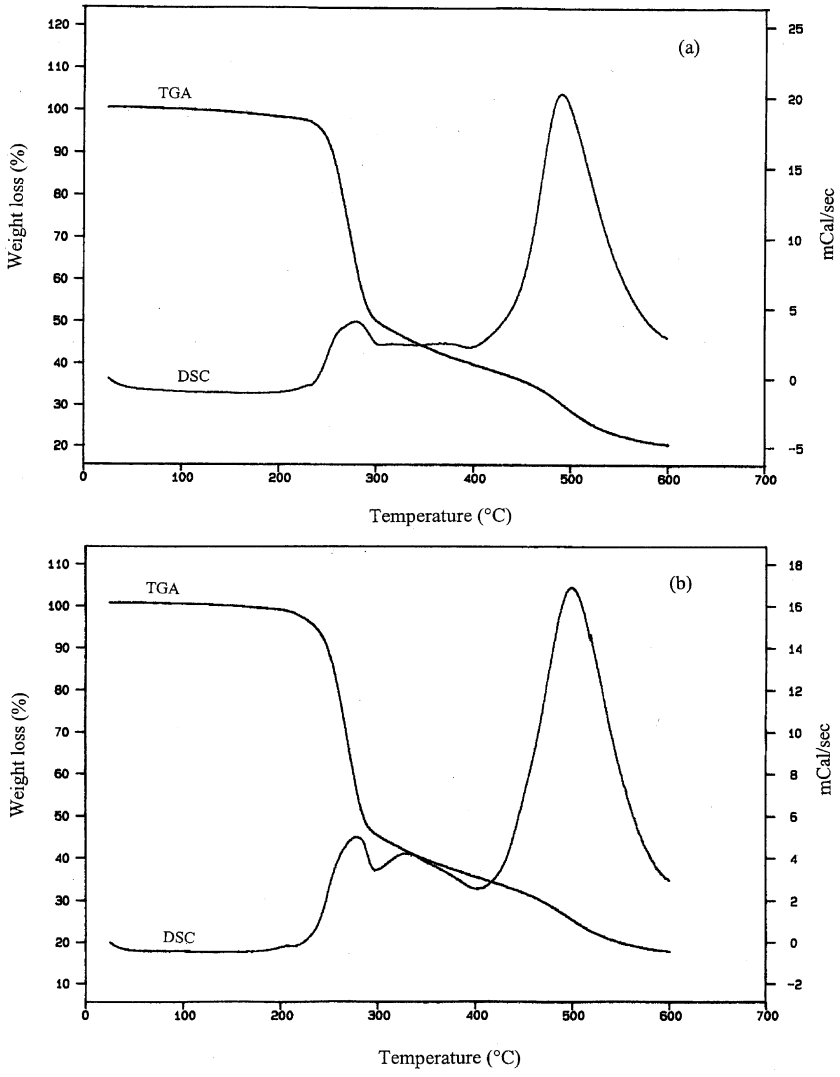

Figure 5. TGA/DSC curves of hemicellulosic fractions isolated with 0.5 M NaOH (a) and 2 M NaOH (b) from ultrasonic irradiated bagasse.

the ultrasonic irradiated and water treated bagasse, appeared to be more stable than the fraction, extracted with 2 M NaOH from the ultrasonic irradiated and sequential alkali and alkaline peroxide treated bagasse, which corresponded to the decreasing trends of their molecular weights in Table 4. In addition, although the two DSC thermograms of the two hemicellulosic fractions gave one similar big exothermic peak at 492 °C due to the disintegration of intra-molecular interaction and the decomposition of the polymer, their slight differences are observed between 243 and 400 °C in which the hemicellulosic fraction, isolated with 0.5 M NaOH, showed one smaller exothermic peak at 280 °C, while the fraction, isolated with 2 M NaOH, gave two smaller exothermic peaks at 280 and 330 °C. These slight differences in DSC curves were probably due to the variations in organization of the hemicellulosic molecules in different fractions.

3. Summary

The application of ultrasound pre-treatment during the sequential fractionation of hemicelluloses from bagasse had not only a positive effect on the extraction yield but affected also the purity of the obtained hemicelluloses. Except for the water-soluble fraction, obtained during the ultrasound-assisted water extraction, all of the seven hemicellulosic fractions, isolated successively with alkali and alkaline peroxide from the ultrasonic irradiated bagasse, contained lower amounts of associated lignins (0.41–2.90%) than those of the hemicellulosic preparation, obtained under the corresponding conditions without ultrasound treatment, 0.46–5.12%. The observed beneficial sonication effect on the extractability of the hemicelluloses can be explained by both the mechanical disruption of the cell walls and cleavage of the α-ether linkages between lignin and hemicelluloses.

Consequently, the accessibility, solubility, and diffusion of the dissolved hemicelluloses and lignin from the cell walls increased. In addition, there were no significant differences in the structural features of the seven alkali- and alkaline peroxide-soluble hemicellulosic fractions, which are composed mainly of L-arabino-(4-*O*-methyl-D-glucurono)-D-xylans. However, differences may occur in the fine structure of the polymers, that is, in the distribution of the branches along the xylan backbone.

4. Experimental

4.1. Materials

Sugarcane bagasse was obtained from a local sugar factory (Guangzhou, China). It was first dried in sunlight and then cut into small pieces (1–3 cm). The cut bagasse was ground to pass a 0.8-mm size screen. The dried powder (10 g) was first extracted with toluene/ethanol (2:1, v/v) in a Soxhlet apparatus for 6 h, and the meal was allowed to dry in an oven at 60 °C for 16 h. The composition (%, w/w) of the bagasse was cellulose 43.6%, hemicelluloses 33.5%, lignin 18.1%, ash 2.3%, and wax 0.8% on a dry weight basis.

4.2. Ultrasonic treatment

The extractive free bagasse (9.92 g) was soaked in 300 mL distilled water. The irradiation was then carried out using the Sonic system SOMERSET (England, 20 kHz) provided with a horn at sonic power of 100 W and sonication time for 40 min at 55 °C without additional stirring. The mixture was then stirred for 80 min at 55 °C.

4.3. Fractional extraction of hemicelluloses

The sequential treatment of bagasse from the ultrasonic irradiated residue and isolation of soluble hemicelluloses were carried out according to the scheme in Figure 1. After ultrasonic treatment for 40 min in distilled water and the remaining mixture was gently stirred for 80 min at 55 °C. The insoluble residue after filtration was washed with water (50 mL). The combined filtrate and washing water were evaporated to 15 mL at reduced pressure. The water-soluble hemicellulosic fraction was recovered by precipitation of the concentrated water extracts in 3 vol of 95% ethanol. The hemicelluloses from the water-insoluble residue were extracted sequentially with 300 mL 0.5 M NaOH, 200 mL 0.5%, 1.0%, 1.5%, 2.0%, and 3.0% H_2O_2 at pH 11.5, adjusted with 6 M NaOH, and 200 mL 2 M NaOH at 55 °C for 2 h. Each extract was neutralized with 6 M HCl solution to pH 5.5, and then concentrated to about 30 mL under reduced pressure. The released hemicelluloses were precipitated by pouring the concentrated supernatant fluid into 120 mL 95% ethanol. The precipitates that formed were recovered by filtration, washed with acidified 70% ethanol, and air-dried.

The constituent neutral sugars in isolated hemicelluloses were determined as their alditol-acetate derivatives by gas chromatography (GC) after hydrolysis with 2 M trifluoroacetic acid for 2 h at 120 °C.[28] Uronic acid content was determined by the automated colorimetric *m*-hydroxydiphenyl assay.[29] Methods for measurement of the hemicellulosic molecular weights and determination of chemical composition of phenolic acids and aldehydes liberated from alkaline nitrobenzene oxidation of the lignins associated in hemicellulosic fractions have been described in a previous paper.[30] Klason lignin content in hemicellulosic samples was determined according to Tappi method T 249 cm-85. FT-IR spectra were obtained on an FT-IR spectrophotometer (Nicolet 750) using a KBr disc containing 1% finely ground samples. The solution-state ^1H and ^{13}C NMR spectra were obtained on a Bruker MSL-300 spectrometer at 300 and 74.5 MHz. ^1H NMR spectrum was recorded at 25 °C from 20 mg of sample dissolved in 1.0 mL D_2O. The ^{13}C NMR spectrum was recorded at 25 °C from 80 mg of sample dissolved in 1.0 mL D_2O after 28,000 scans. A 60° pulse flipping angle, a 3.9 μs pulse width, and a 0.85 s delay time between scans were used. Thermal stability of hemicelluloses was performed using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) on a simultaneous thermal analyzer (NETZSCH STA-409). The apparatus was continually flushed with nitrogen. The samples weighed between 10 and 13 mg and were run from room temperature to 600 °C at a rate of 10 °C min^{-1}.

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