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Fractional extraction and structural characterization of sugarcane bagasse hemicelluloses

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Abstract

Over 90% of the original hemicelluloses in the cell walls of bagasse were sequentially extracted with distilled water, 0.5 M NaOH, 0.5, 1.0, 1.5, 2.0 and 3.0% H_2O_2 at pH 11.5, and 2.0 M NaOH at 55 °C for 2 h. Meanwhile, the successive treatments also released 89.0% of the original lignin. Chemical composition, physico-chemical properties, and structures of the eight hemicelluloses were elucidated by a combination of sugar analysis, nitrobenzene oxidation of bound lignin, molecular determination, Fourier transform infrared, and ^1H and ^{13}C NMR spectroscopy and thermal analysis and the results are reported. The results showed that the sequential treatments were very effective on the fractionation of hemicelluloses from bagasse, and the extraction strength, such as alkali and H_2O_2 concentration, had a great influence on the chemical and structural features of the hemicelluloses, e.g. content of associated lignin and molecular weight. The hemicellulosic fraction, isolated with 0.5% H_2O_2 at pH 11.5 for 2 h under 55 °C, had a backbone of xylose residues with β-(1 → 4) linkages and were branched mainly through arabinofuranosyl and 4-*O*-methyl glucopyranosyl units. Ferulic and *p*-coumaric acids were found to be esterified to the hemicelluloses.

Keywords: Bagasse; Hemicelluloses; Fractionation; Alkaline peroxide; Bound lignin; ^{13}C NMR

1. Introduction

Sugarcane bagasse is a residue produced in large quantities by sugar and alcohol industries. In general, 1 ton of sugarcane generates 280 kg of bagasse, the fibrous by-product remaining after sugar extraction from sugarcane. About 54 million dry tons of bagasse is produced annually throughout the world (Rodrigues, Felipe, Sil, & Vitolo, 2003; Rowell & Keany, 1991). This product represents a great morphological heterogeneity. It consists of fiber bundles and other structural elements like vessels, parenchyma, and epithelial cells (Sanjuan, Anzaldo, Vargas, Turrado, & Patt, 2001). Chemically, about 40–50% of the dry residue is the glucose polymer cellulose, much of which is in a crystalline structure. Another 25–35% are hemicelluloses, an amorphous polymer usually composed of xylose, arabinose, galactose, glucose, and mannose. The remainder is mostly lignin plus lesser amounts minerals, waxes, and other compounds (Jacobsen & Wyman, 2002). Application of agro-industrial residues in pulping process and other chemical production on the one hand provides alternative substrates, and on the other hand helps in solving pollution problems, which their disposal may otherwise cause.

Hemicelluloses, which occur in the cell wall, are heteropolysaccharides. Xylans are the most abundant of the hemicelluloses found in the cell walls of land plants, of which they can constitute more than 30% of the dry weight (Linder, Bergman, Bodin, & Gatenholm, 2003). Xylans from cereal straws are characterized by a β-(1 → 4)-D-Xyl*p*backbone to which arabinosyl, glucuronic acid, and acetyl substituents can be attached at the two free OH groups of carbons C-2 and C-3 of the xylopyranose residue (Puls, 1997). For example, the hemicelluloses from wheat straw were confirmed to be a (1 → 4)-linked β-D-xylan with D-glucopyranosyluronic acid or (4-*O*-methyl-α-glucopyranosyluronic acid) group attached at position 2, and L-arabinofuranosyl and D-xylopyranosyl groups attached at

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position 3. For every 26 D-xylopyranosyl residues in the main chain, there is one uronic acid unit. For 13, such D-xylopranosyl residues, there is one L-arabinofuranosyl group, and for 18 such D-xylopyranosyl residues, there is one D-xylopyranosyl group (Sun, Lawther, & Banks, 1996). The main xylan in softwoods is arabino-4-*O*-methyl glucurnoxylan, while 4-*O*-methyl glucurnoxylan free from arabinose substituents dominates in hardwoods (Sjöström, 1992). In other words, these saccharide residues can be bonded directly to the main chain or to the one side residue (Verbruggen, Beldman, & Voragen, 1998). Therefore, there are a great variety of xylans with different: degrees of polymerization of the poly-D-xylopyranose main chain, degrees of substitution, side residues, and the side chain length. This complex and wide variety of structures has a direct effect on the hemicellulosic properties (Sarbu, Goncalves, & Pinho, 2003).

In recent years, great interest has been occurred in hemicelluloses as polymers for chemical and pharmaceutical applications, e.g. for production of cationic biopolymers (Ebringerová, Hromádhová, kacuráková, & Antal, 1994), hydrogels (Gabrielli & Gatenholm, 1998; Gabrielli, Gatenholm, Glasser, Jain, & Kenne, 2000; Lindblad, Ranucci, & Albertsson, 2001), and long-chain alkyl ester derivatives (Fang, Sun, Fowler, Tommkinson, & Hill, 1999). However, the hemicelluloses from bagasse are much less studied in this respect. The aim in our laboratory is to develop a commercial process for fractionation of cereal straw components using an environmental friendly procedure for the extraction of hemicelluloses in a large scale with a light color. Based on our recent 10 years' study on hemicelluloses, we found that alkaline peroxide is an effective agent for both delignification and solubilization of hemicelluloses from straws and grasses. 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, hydrogen peroxide is unstable in alkaline conditions and readily decomposes into hydroxyl radicals (HO·) and superoxide anion radicals (O_2^-·). This is particularly true in the presence of certain transition metals such as manganese, iron, and copper. These radicals are thought to cause the oxidation of lignin structures, which lead to the introduction of hydrophilic (carboxyl) groups, cleavage of some interunit bonds and eventually, the dissolution of lignin and hemicelluloses (Fang, Sun, Salisbury, Fowler, & Tomkinson, 1999; Pan, Bolton, & Leary, 1998). The results obtained from our laboratory found that alkaline peroxide is an effective agent for both delignification and solubilization of hemicelluloses from cereal straws. The process is easy to handle, easily available, and environmentally friendly. The present communication describes the fractional isolation and physico-chemical characterization of hemicelluloses from bagasse. The effect of alkaline peroxide concentration on the yield and physico-chemical properties of the solubilized hemicelluloses are comparatively studied by Fourier transform infrared (FT-IR), gel permeation chromatography (GPC), carbon-13 nuclear magnetic resonance spectroscopy (^{13}C NMR) and the results are reported.

2. Experimental

2.1. 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 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.

2.2. Fractionation of hemicelluloses

In order to study structural differences in the hemicelluloses present in bagasse, hemicellulosic fractions were isolated by sequential extraction. 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 dewaxed bagasse meal was then soaked in 300 ml distilled water at 55 °C for 2 h under stirring. Water-soluble hemicelluloses were obtained by precipitation of concentrated aqueous extracts in 4 volumes of 95% ethanol. Sample free of wax and water solubles was successively treated 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 2.0 M NaOH at 55 °C for 2 h. After the indicated period of treatment, the insoluble residue was collected by filtration, washed with distilled water until the pH of the filtrate was neutral, and then dried at 60 °C. Each of the filtrates fluid was adjusted to 5.5 with 6 M HCl and then concentrated to about 30 ml under reduced pressure. The hemicelluloses released, were then 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 then air-dried. The scheme for fractionation of hemicelluloses from bagasse is illustrated in Fig. 1, and the yield of hemicelluloses is given on the basis of the dry weight of starting bagasse. All experiments were performed at least in duplicate. The relative standard deviation, determined by dividing the standard deviation by the mean value, was less than 3.8%.

2.3. Chemical analysis

The constituent neutral sugars in isolated hemicelluloses was determined as their alditol-acetate derivatives by gas chromatography (GC) after hydrolysis with 2 M trifluoroacetic acid for 2 h at 120 °C (Blakeney, Harris, Henry, & Stone, 1983). The sugars were reduced to their

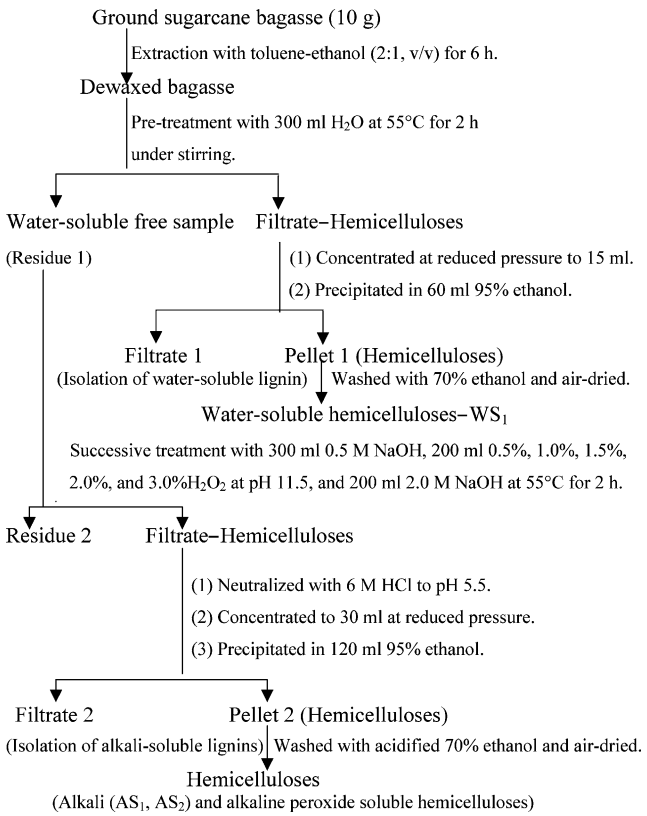

Fig. 1. Scheme for fractional isolation of hemicelluloses from dewaxed bagasse.

corresponding alditols by adding 5 M NH_3 containing 2% $NaBH_4$. Reduction was performed for 90 min at 40 °C. The excess of sodium borohydride was then destroyed by adding 0.1 ml glacial acetic acid. Acetylation was then performed with acetic anhydride (2 ml, 10 min at room temperature) in the presence of 1-methylimidazole (0.2 ml) as a catalyst. Acetylation was stopped with 5 ml deionized water and the acetylated alditols were partitioned between dichloromethane (1.0 ml) and water. After the phase had separated, the lower one was removed with a Pasteur pipette and stored in a 1-ml, septum-cap vial at −20 °C before analysis. Uronic acid content was determined by the automated colorimetric *m*-hydroxydiphenyl assay (Blumenkrantz & Asboe-Hansen, 1973). Method for measurement of the hemicellulosic molecular weights has been described in a previously paper (Lawther, Sun, & Banks, 1995). The chemical composition of phenolic acids and aldehydes liberated from alkaline nitrobenzene oxidation of the lignins associated in hemicellulosic fractions was determined on a Hichrom H5ODS HPLC column of dimensions 250×4.6 mm^2 (purchased from Phenomenex, Co., Beijing). The identification of the individual compounds were detected at 280 nm by computer comparison of the retention times and peak areas with the authentic phenolics. Klason lignin content in hemicellulosic samples was determined according to Tappi method T 249 cm-85 (Lignin in wood, 1994).

FT-IR spectra analysis was done on an FT-IR spectrometer (Nicolet 750) operating at 32 cm^{-1} resolutions and using a KBr disc containing 1% finely ground samples. The solution-state ^{1}H and ^{13}C NMR spectra were obtained using a Bruker MSL-300 spectrometer at 300 and 74.5 MHz. ^{1}H 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 30,000 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.

3. Results and discussion

3.1. Fractional yield

Extraction of hemicelluloses under alkaline conditions actually involves alkaline hydrolysis of ester linkages to liberate them from the lignocellulosic matrix followed by extracting them into aqueous media. However, the liberation of the hemicellulosic component from the plant cell walls is restricted by the presence of lignin network as well as ester and ether lignin–hemicellulose linkages. In addition, extensive hydrogen bonding between the individual polysaccharide cell wall components may impede isolation of hemicelluloses (Ebringerová & Heinze, 2000). Interestingly, as mentioned earlier, treatment of lignocellulosic materials with alkaline peroxide resulted in not only substantial lignin degradation but also significant hemicellulose solubilization, resulting from hydroperoxyl and hydroxyl radicals generated by the decomposition of hydrogen peroxide (Fang et al., 1999). As can be seen from Table 1, sequential treatment of the dewaxed bagasse with distilled water, 0.5 M NaOH, 0.5, 1.0, 1.5, 2.0 and 3.0% H_2O_2 at pH 11.5, and 2.0 M NaOH at 55 °C for 2 h released 4.1, 12.0, 3.8, 1.4, 2.5, 1.0, 0.9, and 4.6% hemicelluloses (% dry starting material), corresponding to the dissolution of 12.2, 35.8, 11.3, 4.2, 7.5, 3.0, 2.7, and 13.7% of the original hemicelluloses, respectively. Meanwhile, the successive treatment also solubilized 0.5, 9.5, 2.7, 0.6, 1.0, 0.9, 0.5, and 0.4% lignin (% dry starting material), corresponding to release of 2.8, 52.5, 14.9, 3.3, 5.5, 5.0, 2.8, and 2.2% of the original lignin, respectively. Obviously, total yield of eight hemicellulosic fractions accounted for 90.4% of the original hemicelluloses in the cell walls of bagasse, indicating that substantial amounts of hemicelluloses were extracted sequentially with sodium hydroxide and alkaline peroxide in the increasing concentration from 0.5 to 3.0% under the conditions used. These results also

Table 1
The yield of hemicelluloses and lignin (% dry matter) solubilized during the successive treatments of dewaxed bagasse with distilled water, 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[c]	Total
			0.5[d]	1.0[d]	1.5[d]	2.0[d]	3.0[d]		
Hemicelluloses[e]	4.1	12.0	3.8	1.4	2.5	1.0	0.9	4.6	30.3
Lignin	0.5	9.5	2.7	0.6	1.0	0.9	0.5	0.4	16.1
Residue	94.8	71.4	64.4	62.4	58.8	56.8	55.2	45.9	

[a] Abbreviation for the water-soluble hemicelluloses and lignin obtained by treatment of the dewaxed bagasse with distilled water at 55 °C for 2 h.
[b] Abbreviation for the alkali-soluble hemicelluloses and lignin obtained by extraction with 0.5 M NaOH at 55 °C for 2 h from the water treated bagasse.
[c] The fraction obtained by extraction with 2.0 M NaOH at 55 °C for 2 h from the 3.0% H_2O_2 treated bagasse.
[d] 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 under pH 11.5.
[e] The hemicellulosic fractions obtained by precipitation of the neutralized extracts with 4 volumes of 95% ethanol.

revealed that the sequential extraction of the bagasse was very effective, and the highest extraction yield was obtained with 0.5 M NaOH (12.0% w/w), implying that initial extraction with 0.5 M NaOH librated 35.8% of the total available hemicelluloses, which is selective to hemicelluloses. On the other hand, the 2.0 and 3.0% H_2O_2 extracts resulted in a yield of only 1.0 and 0.9%, respectively, accounting for 3.0 and 2.7% of the hemicelluloses present in the bagasse. Apparently, a part of the hemicelluloses is loosely attached within the cell walls, while a major part of the hemicelluloses are embedded firmly in the cell wall. It can be speculated that this difference in extractability of the hemicelluloses is a result of a different function of these polysaccharides in the cell wall.

3.2. Content of neutral sugars and uronic acids

To characterize the solubilized hemicelluloses, the eight fractions were hydrolyzed to determine their constituent sugars, and the results are given in Table 2. The major monosaccharide in water extract (WS_1) was xylose (55.2%), indicating the presence of an arabinoxylan. Glucose (20.4%) appeared as the second major sugar constituent, resulting from solubilized hemicelluloses and released starch. Additionally, relatively high amounts of uronic acid (7.0%) and rhamnose (1.8%) suggested that the water extract consisted of small amounts of pectic substances.

Knowing that alkali proved to be efficient in extracting most available hemicelluloses from secondary cell wall, the extraction process was carried out with 0.5 M NaOH. This concentration extracted 35.8% of the total hemicelluloses (AS_1) with a relatively low arabinose substitution (Xyl/Ara (6.8:1) ratio). Glucose (4.0%), uronic acid (3.5%), galactose (1.8%), mannose (0.5%) and rhamnose (0.5%) were observed as minor constituents. Successive treatments with 0.5, 1.0, 1.5, 2.0, and 3.0% H_2O_2 extracted the hemicelluloses rich in arabino (glucurono) xylan types as shown by the sugar order (from high to low): xylose (68.6–76.6%), arabinose (12.8–15.6%), glucose (7.4–13.1%), uronic acid (3.5–4.8%), galactose (1.9–3.0%), rhamnose (trace–1.1%), and mannose (trace–0.3%). It should be noted that an increase in glucose content in hemicellulosic fractions with an increment in alkaline peroxide concentration from 0.5 to 3.0% resulted partially from degradation of cellulose from bagasse under the conditions used. Similar phenomenon has been reported during the treatment of rice straw with alkaline peroxide in our previous studies (Sun, Tomkinson, Ma, & Liang, 2000). The data showed that treatment of the alkali-extracted rice straw under the similar alkaline conditions but in the absence of peroxide led to only 2.5% of the cellulose degradation. While the treatment with alkaline peroxide under the successive increase in H_2O_2 concentration from 0.5 to 5.0% resulted in a solubilization of 9.8–17.8% of the original cellulose.

Table 2 also showed that the final treatment with 2.0 M NaOH led to a release of 13.7% of the original hemicelluloses. This extract (AS_2) had comparable sugar

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[c]
			0.5[d]	1.0[d]	1.5[d]	2.0[d]	3.0[d]	
Rhamnose	1.84	0.48	1.03	1.12	0.46	0.16	Tr[e]	ND[f]
Arabinose	10.73	11.93	12.76	14.77	15.63	15.07	14.16	8.65
Xylose	55.20	81.37	76.56	70.38	69.28	68.60	69.82	85.02
Mannose	4.13	0.47	0.34	0.24	0.18	0.12	Tr	Tr
Galactose	7.68	1.78	1.93	2.71	2.89	3.02	2.89	0.46
Glucose	20.42	3.97	7.39	10.78	11.56	13.04	13.13	5.87
Uronic acids	6.95	3.50	3.52	4.50	4.75	4.83	3.84	1.75

[a] Abbreviation for the water-soluble hemicelluloses and lignin obtained by treatment of the dewaxed bagasse with distilled water at 55 °C for 2 h.
[b] Abbreviation for the alkali-soluble hemicelluloses and lignin obtained by extraction with 0.5 M NaOH at 55 °C for 2 h from the water treated bagasse.
[c] The fraction obtained by extraction with 2.0 M NaOH at 55 °C for 2 h from the 3.0% H_2O_2 treated bagasse.
[d] 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 under pH 11.5.
[e] Tr, trace.
[f] ND, not detectable.

compositions with 0.5 M NaOH extract (AS_1), consisting mainly of xylose (85.0%) and arabinose (8.7%). Based on sugar composition, this final extract consisted predominantly of arabinoxylans. The arabinose/xylose ratio of the two alkali extracts appeared to decrease from 0.15 (AS_1) to 0.10 (AS_2) with increasing extraction strength from 0.5 to 2.0 M NaOH, indicating that the arabinose rich hemicelluloses are relatively easy to extract. This suggested that the degree of branching of the arabinoxylans decreased in AS_2 fraction. Also the uronic acid and rhamnose content in AS_2 decreased, indicating that xylans containing a high degree of side-chain substitution are more alkali soluble and bind less tightly to cellulose, whereas molecules with infrequent side chains are less alkali soluble and bind more tightly to cellulose. The current result was consistent with the study on polysaccharides from green and roasted *Coffea arabica* beans (Oosterveld, Harmsen, Voragen, & Schols, 2003). The authors stated that the degree of branching of the arabinogalactans decreased with increasing extraction strength.

3.3. Content of associated lignin and its composition of phenolic acids and aldehydes

To verify the content of lignin and its phenolic composition, the isolated eight hemicellulosic fractions were carried out by nitrobenzene oxidation, and the phenolic acids and aldehydes released from the associated lignin are given in Table 3. Clearly, compared to the lignin content in water-soluble hemicelluloses (9.6%), the seven hemicellulosic fractions, isolated with alkali or alkaline peroxide, had a much lower content of associated lignin (0.5–5.1%). This is particularly true for the hemicelluloses obtained at relatively higher concentrations of alkali or alkaline peroxide. An increase in alkali concentration from 0.5 to 2.0 M and percentage of hydrogen peroxide from 0.5 to 3.0% led to a decrease in lignin content from 5.1 to 0.5% and from 3.8 to 0.8%, respectively, indicating that both alkali and alkaline peroxide treatments at a high concentration had a more positive effect on the cleavage of α-ether bonds between lignin and hemicelluloses from bagasse. This once again implied that the sequential alkali and alkaline peroxide treatment was an efficient method for fractionation of hemicelluloses having relatively low amounts of bound lignin. The major products, obtained by alkaline nitrobenzene oxidation, were identified to be vanillin and syringaldehyde, which ranged between 30.7–52.2 and 26.1–43.8% of the total phenolic acids and aldehydes, respectively. This indicated that the associated lignin in the hemicellulosic fractions contained roughly equal amounts of non-condensed guaiacyl and syringyl units. A noticeable amount of *p*-hydroxybenzaldehyde and acetosyringone, and trace of *p*-hydroxybenzoic acid, vanillic acid, syringic acid, acetovanillone, ferulic acid, and *p*-coumaric acid were also found to be present in the nitrobenzene oxidation mixtures. Occurrence of ferulic and *p*-coumaric acids in cell walls of bagasse has been reported by Kato, Azuma, and Koshijima (1987) as early as in the study of lignin–hemicellulosic complex containing hydroxycinnamic acids. The authors demonstrated that ferulic acid and *p*-coumaric acid are esterified to the hemicelluloses and lignin in the cell wall of bagasse, respectively. Further studies found that the site of feruloylation in bagasse hemicelluloses is exclusively the O-5 of L-arabinofuranosyl

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[c]
			0.5[d]	1.0[d]	1.5[d]	2.0[d]	3.0[d]	
p-Hydroxybenzoic acid	0.39	0.18	0.066	0.072	0.032	0.028	0.018	0.014
p-Hydroxybenzaldehyde	0.76	0.27	0.21	0.19	0.056	0.052	0.020	0.012
Vanillic acid	0.073	0.033	0.032	0.051	0.045	0.037	0.012	0.003
Vanillin	1.47	0.96	0.82	1.18	0.71	0.43	0.16	0.12
Syringic acid	0.049	0.011	0.008	0.005	0.004	0.002	Tr[e]	ND[f]
Syringaldehyde	2.18	1.25	0.85	1.21	0.58	0.47	0.17	0.060
Acetovanillone	0.22	0.047	0.056	0.066	0.012	0.010	0.003	0.002
Acetosyringone	0.28	0.25	0.14	0.12	0.061	0.052	0.014	0.012
p-Coumaric acid	0.22	0.061	0.027	0.025	0.008	0.005	0.003	0.002
Ferulic acid	0.032	0.058	0.012	0.015	0.008	0.006	0.003	0.004
Total	5.67	3.12	2.22	2.76	1.80	1.09	0.40	0.23
Content of klasson lignin	9.63	5.12	3.81	4.21	2.52	1.64	0.78	0.46

[a] Abbreviation for the water-soluble hemicelluloses and lignin obtained by treatment of the dewaxed bagasse with distilled water at 55 °C for 2 h.
[b] Abbreviation for the alkali-soluble hemicelluloses and lignin obtained by extraction with 0.5 M NaOH at 55 °C for 2 h from the water treated bagasse.
[c] The fraction obtained by extraction with 2.0 M NaOH at 55 °C for 2 h from the 3.0% H_2O_2 treated bagasse.
[d] 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 under pH 11.5.
[e] Tr, trace.
[f] ND, not detectable.

residue which is attached to the (1 → 4)-β-linked D-xylan backbone at O-3 (Kato et al., 1987).

3.4. Molecular weight

Recently, based on the study of molecular weight of arabinoxylans from wheat flour, Dervilly-Pinel, Thibault, and Saulnier (2000) stated that the chains of water-soluble arabinoxylans are semi-flexible. This feature allows these hemicelluloses to adopt a random coil conformation in solution. Therefore, the hydrodynamic volume of the macromolecule depends on the solvent characteristics. In addition, the results obtained from oat spelts xylan molecular weight estimation by size exclusion chromatography indicated that the values of xylan molecular weights increased with alkali concentration of the hemicellulosic solution and the eluent pH, while the xylan and NaCl concentrations had no significant effect on the determinations (Sarbu et al., 2003). In this study, the weight-average (M_w) molecular weights of eight hemicellulosic fractions were determined by GPC on a PL aquagel-OH 50 column. The hemicelluloses were dissolved with 0.02N NaCl in 0.005 M sodium phosphate buffer, pH 7.5 at a concentration of 0.1%. The eluents were 0.02N NaCl in 0.005 M sodium phosphate buffer, pH 7.5. Table 4 lists the weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) of the eight hemicellulosic fractions. Clearly, the fraction of water-soluble hemicelluloses showed a much lower degree of polymerization with a M_w value of 7380 g/mol than those two alkali-soluble hemicellulosic fractions and five alkaline peroxide-soluble hemicellulosic preparations, ranging from 18,960 to 45,370 g/mol. This suggested that the first treatment of dewaxed bagasse with distilled water in the absence of alkali or hydrogen peroxide solubilized the small molecular size of hemicelluloses. In other words, this indicated that the addition of alkali or alkaline peroxide during the extraction resulted in a higher molecular weight of the hemicelluloses released than the use of distilled water alone. As shown in Table 4, the following treatment with 0.5 M NaOH favored release the macromolecular hemicelluloses as shown by their M_w value of 45,370 g/mol. Interestingly, as the data shown in Table 4, an increase in alkaline peroxide concentration from 0.5 to 2.0% led to an increment of M_w value from 34,070 to 38,890 g/mol, indicating an increase in solubilization of large molecular size hemicelluloses as the H_2O_2 concentration rose to 2.0%. On the other hand, as the H_2O_2 concentration was further increased from 2.0 to 3.0%, the M_w decreased significantly from 38,890 to 23,340 g/mol, implying that a degradation of hemicelluloses occurred during the treatment with 3.0% H_2O_2. Similarly, a final treatment with 2.0 M NaOH resulted in further substantial degradation of the hemicellulosic polymers as shown by a much lower M_w value of 18,960 g/mol. Apparently, the lower molecular weight polysaccharides are relatively easy to extract and are released under mild extraction condition such as water treatment. Also, these data indicated that molecular weight of the hemicelluloses in the extracts are lower under high extraction strength, e.g. 3.0% H_2O_2 and 2.0 M NaOH treatments, probably as a results of degradation of these polysaccharides. In addition, the data also showed that the water-soluble hemicelluloses gave a more narrow molecular weight distribution, corresponding to polydispersity index of 1.5 as compared to those of the alkali and alkaline peroxide-soluble hemicellulosic fractions having a more broad molar weight distribution with polydispersity indexes between 4.4 and 8.1.

3.5. FT-IR spectra

The analysis of FT-IR data showed that all the eight hemicellulosic fractions clearly illustrated the typical signal pattern for hemicellulosic moiety, and had a specific band maximum in the 1200–1000 cm^{-1} region. Fig. 2 gives FT-IR spectra of hemicellulosic fractions solubilized in distilled water (spectrum 1) and 0.5 M NaOH solution (spectrum 2). The spectra are dominated with stretching and bending vibrations of C–O, C–C, C–OH, and C–O–C at 1049 cm^{-1}. Bands between 1175 and 1000 cm^{-1} are typical of xylans. Evidently, the presence of the arabinosyl side-chains is documented by the two low-intensity shoulders at 1175 and 990 cm^{-1}, which have been reported to be attached only at positions of the xylopyranosyl constituents (Sun & Tomkinson, 2002). The intensity changes of these two bands can be suggested to reflect the arabinosyl substituent contribution and therefore, used for

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[c]
			0.5[d]	1.0[d]	1.5[d]	2.0[d]	3.0[d]	
M_w	7380	45,370	34,070	34,330	38,820	38,890	23,340	18,960
M_n	4890	5600	5800	5870	6210	6020	4720	4330
M_w/M_n	1.51	8.10	5.87	5.85	6.25	6.46	4.94	4.38

[a] Abbreviation for the water-soluble hemicelluloses and lignin obtained by treatment of the dewaxed bagasse with distilled water at 55 °C for 2 h.
[b] Abbreviation for the alkali-soluble hemicelluloses and lignin obtained by extraction with 0.5 M NaOH at 55 °C for 2 h from the water treated bagasse.
[c] The fraction obtained by extraction with 2.0 M NaOH at 55 °C for 2 h from the 3.0% H_2O_2 treated bagasse.
[d] 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 under pH 11.5.

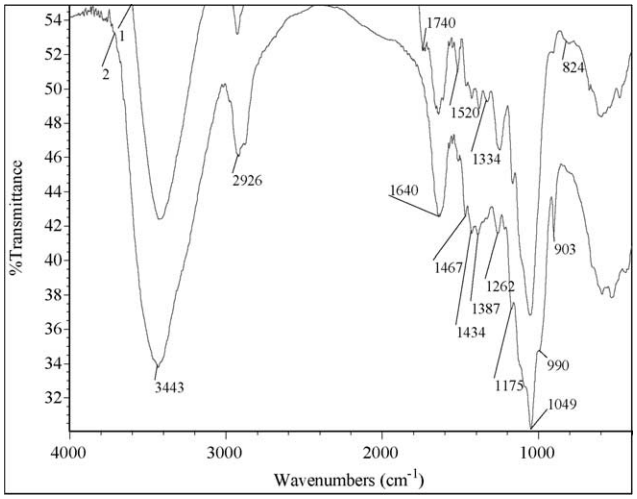

Fig. 2. FT-IR spectra of hemicellulosic fractions solubilized in distilled water (spectrum 1) and 0.5 M NaOH solution (spectrum 2).

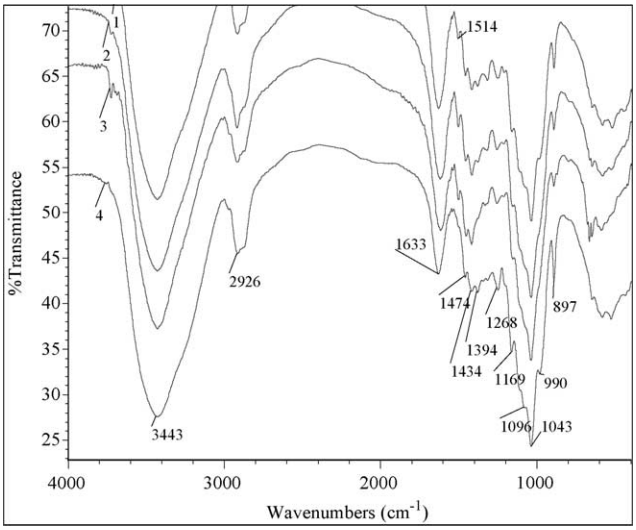

Fig. 3. FT-IR spectra of hemicellulosic fractions released during the sequential treatment with 0.5 (spectrum 1), 1.5 (spectrum 2) and 2.0% H_2O_2 (spectrum 3), and 2.0 M NaOH (spectrum 4).

the identification of arabinoxylan structures. That is, this band gives variation in spectral shape depending on the branches at the O-2 and O-3 positions. With an increase in the number of branches, a decrease in the intensity at 1175–990 is reported to occur (Kacuraková, Belton, Wilson, Hirsch, & Ebringerová, 1998). In the carbonyl stretching region, in addition to an intensive signal due to the absorbed water at 1640 cm^{-1}, a small band at 1740 cm^{-1} in water-soluble hemicellulosic fraction (spectrum 1) is assigned to the acetyl, uronic, and ferulic ester groups of the polysaccharides, whereas the absence of this signal in the spectrum of 0.5 M NaOH-soluble hemicellulosic fraction demonstrated that the alkali treatment under the condition used completely cleaved this ester bond from the hemicelluloses. A shoulder in spectrum 1 and a sharp band at 903 cm^{-1}, which is due to the C-1 group frequency or ring frequency, is characteristic of β-glycosidic linkages between the sugar units (Fang et al., 1999). Bands due to $-CH_2$ stretching vibrations were observed at 1467 and 1434 cm^{-1}. The bands at 1387, 1334, and 1262 cm^{-1} are originated from C–H, OH, or CH_2 bendings. The C–H stretching vibrations give a signal at 2926 cm^{-1}. The prominent band around 3443 cm^{-1} represents the hydroxyl stretching vibrations of the hemicelluloses and water involved in hydrogen bonding. The occurrence of an intensive band in spectrum 1 and a shoulder in spectrum 2 at 1520 cm^{-1} is undoubtedly due to the presence of small amounts of associated lignin in the hemicelluloses, which corresponded to the results obtained by alkaline nitrobenzene oxidation and klasson lignin determination.

Fig. 3 illustrates the FT-IR spectra of hemicellulosic fractions released during the treatment with 0.5% (spectrum 1), 1.5% (spectrum 2) and 2.0% H_2O_2 (spectrum 3), and 2.0 M NaOH (spectrum 4). The most obvious feature is the similarity of the spectra, indicating a similar structure of the hemicelluloses. As expected, the absence of a signal around 1720 cm^{-1} for carbonyl stretching in all the four spectra indicated that the sequential treatments with alkaline peroxide and alkali under the conditions given did not significantly attack or oxidize the glycosidic linkages and hydroxyl groups of hemicelluloses. The absorbances at 3443, 2926, 1474, 1434, 1394, 1268, 1169, 1096, 1043, 990, and 897 cm^{-1} in the spectra are associated with hemicelluloses. All the spectra have an intense absorbed water-related absorbance at 1633 cm^{-1}. The lignin-related absorbance at 1514 cm^{-1} is rather weak in the spectra 1–3, and poorly resolved in the spectrum 4. This is accordance with the content of bound lignin in the isolated hemicellulosic fractions.

3.6. ^{1}H and ^{13}C NMR spectra

The ^{1}H and ^{13}C NMR spectra of the hemicellulosic fraction isolated with 0.5% H_2O_2 were performed in D_2O. Fig. 4 shows ^{1}H NMR spectrum of the hemicellulosic fraction isolated with 0.5% H_2O_2 at pH 11.5 from the 0.5 M NaOH treated bagasse. As can be seen, the spectrum gives the typical signal pattern expected for a hemicellulosic moiety. The chemical shifts of 3.0–4.2 ppm are originated from 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 1.7 and 1.0–0.9 ppm, respectively. Anomeric protons of terminal α-D-arabinofuranosyl residues occur at 5.0 ppm (Teleman, Lundqvist, Tjerneld, & Stalbrand, 2000). A strong signal at 4.6 ppm is attributed to the residual solvent (HDO).

^{13}C NMR spectrum of the hemicellulosic fraction isolated with 0.5% H_2O_2 at pH 11.5 gives a complex spectrum (Fig. 5). Most of the major resonances were assigned by references to data in literature (Gabrielli et al., 2000; Imamura, Watanabe, Kuwahara, & Koshijima, 1994;

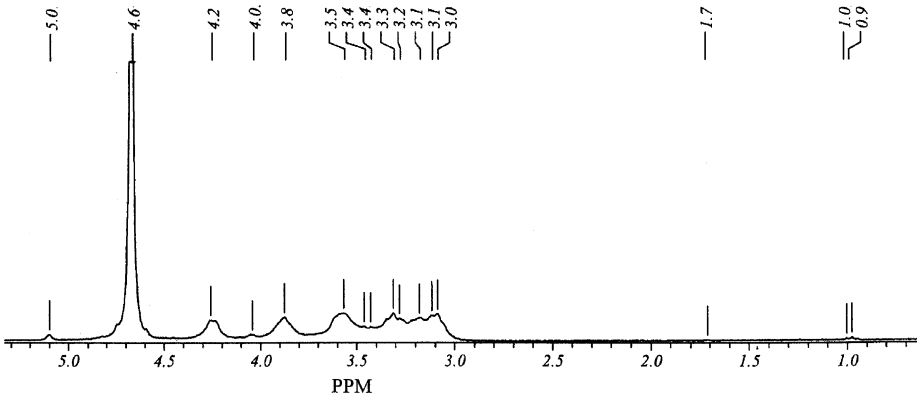

Fig. 4. ^{1}H NMR spectrum (in D_2O) of the hemicellulosic fraction isolated with 0.5% H_2O_2.

Sun et al., 1996). The main 1,4-linked β-D-Xyl*p* units are obviously characterized by five strong signals at 99.5, 73.2, 72.0, 70.5, and 60.6 ppm, which are attributed, respectively, to C-1, C-4, C-3, C-2, and C-5 positions of the β-D-Xyl*p* units. The signals at 106.7, 83.7, 77.5, 75.7, and 57.8 ppm are assigned to C-1, C-4, C-2, C-3, and C-5 of α-L-arabinofuranosyl residues linked to β-D-xylans, respectively. An intensive signal at 59.0 ppm is due to the 4-*O*-methoxyl group of glucuronic acid residue in the xylan. The carbonyl resonances from uronic acids contribute to a signal at 174.2 ppm, which indicates C-6 in methyl uronic acids. The C-4 and C-5 of the 4-*O*-methylglucuronic acid residues in the hemicelluloses occur signals at 79.8 and 69.0 ppm, respectively. The signal at 14.2 ppm relates to $-CH_3$ in Ar–$COCH_3$, indicating the associated lignins. The presence of fragments of lignin is also demonstrated by a signal at 56.8 ppm, arising from methoxyl groups, released after 0.5% H_2O_2 treatment. A small signal at 170.4 ppm represents the carbonyl signal ($-CH_2COO^-$) of the esterified ferulic or *p*-coumaric acids in isolated hemicelluloses. The current results coincided with the findings by Kato et al. (1987). They stated 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. In short, these results implied that the hemicellulosic fraction can be structurally defined L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan together with small amounts of associated lignin and esterified hydroxycinnamic acids.

3.7. Thermal analysis

Fig. 6 illustrates the thermograms of the hemicellulosic fractions, isolated with 1.0% H_2O_2 (Fig. 6(a)) and 2.0 M NaOH (Fig. 6(b)). As shown in the figure, the two

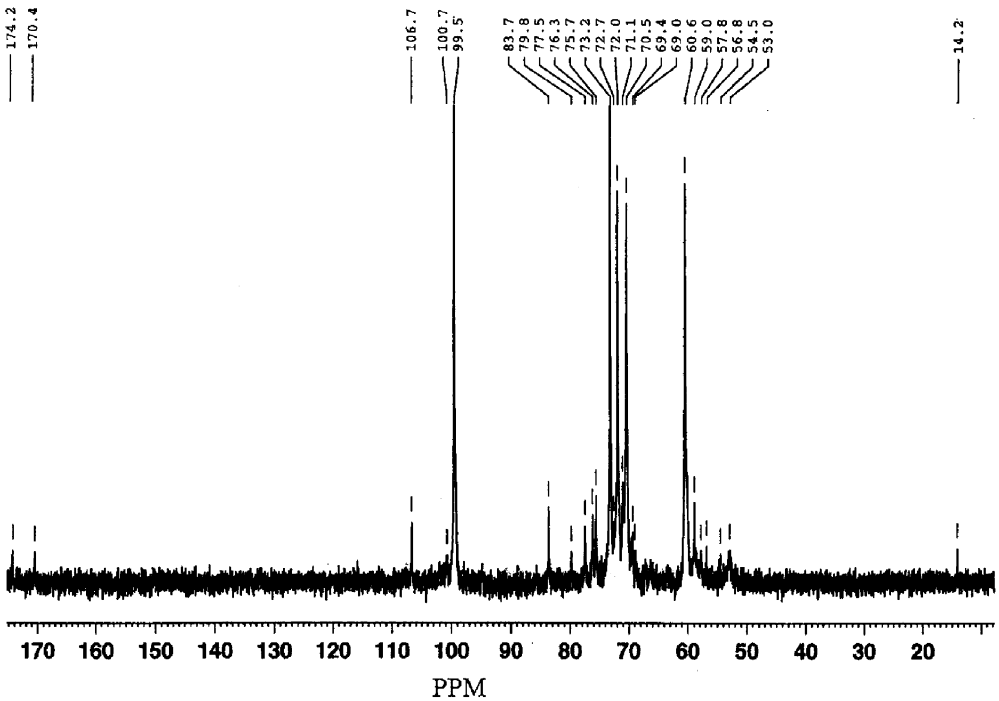

Fig. 5. ^{13}C NMR spectrum (in D_2O) of the hemicellulosic fraction isolated with 0.5% H_2O_2.

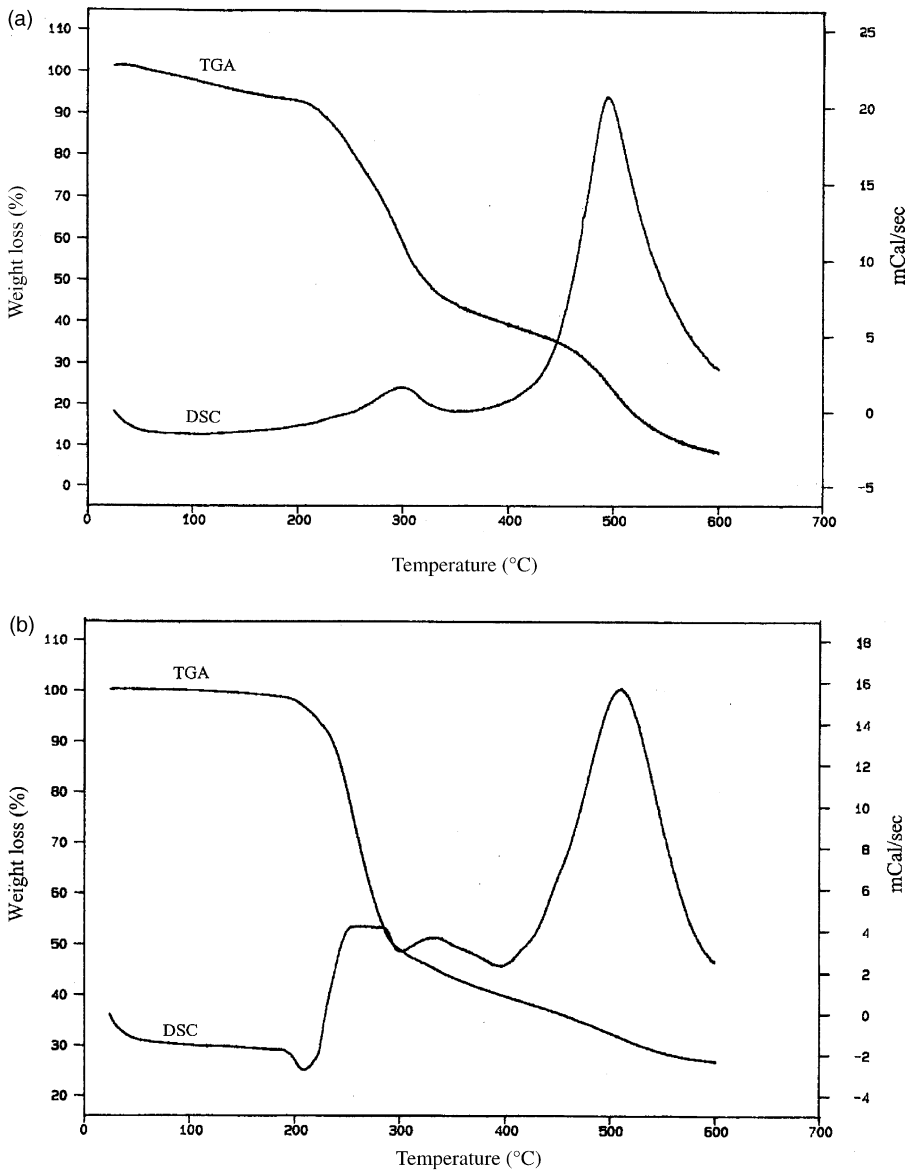

Fig. 6. TGA/DSC curves of hemicellulosic fractions isolated with 1.0% H_2O_2 (a) and 2.0 M NaOH (b).

hemicellulosic fractions are stable up to 214 and 196 °C, respectively. Beyond these temperatures, thermal degradation takes place. Similarly, at 50.0% weight loss the decomposition temperatures of the two polymer samples appeared at 324 and 290 °C, respectively. These data indicated that the hemicellulosic fraction, isolated with 1.0% H_2O_2, appeared to be more stable than the fraction, extracted with 2.0 M NaOH, which corresponded to the decreasing trends of their molecular weights from 34,330 to 18,960 g/mol in Table 4. There were no significant differences in DSC curves between the two fractions. Both of them showed a large exothermic peak around 500 °C due to the disintegration of intra-molecular interaction and the decomposing of the polymer.

As discussed above, the sequential treatment of dewaxed bagasse with alkali and alkaline peroxide is very effective on the fractionation of hemicelluloses, in which 90.4% of the original hemicelluloses and 89.0% of the total lignin in the cell walls of bagasse were released. This indicated that the successive extraction with alkali and alkaline peroxide significantly cleaved the linkages between lignin and hemicelluloses from the cell wall of bagasse. This was confirmed by rather low lignin contents (0.5–5.1%) in isolated seven hemicellulosic fractions. This was particularly true in hemicelluloses extracted with 2.0 and 3.0% H_2O_2 and 2.0 M NaOH, in which the associated lignin content was less than 2%. In addition, the hemicelluloses, which were released under alkali without addition of peroxide, had a lower degree of branching than the hemicelluloses, which were solubilized using alkaline peroxide as a extraction media. This suggested that there is a diversity of hemicelluloses

present in the cell wall of bagasse, which vary in their degree of branching and possibly in their molecular weight. Based on sugar analysis and ^{1}H and ^{13}C NMR study, the hemicellulosic fraction, isolated with 0.5% H_2O_2, can be structurally defined L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan. On the other hand, the resistance to extracting other 10% of the original hemicelluloses by this sequential alkali and alkaline peroxide treatment revealed that most of hemicelluloses in the cell wall of bagasse are more exposed and small amounts of them interact strongly with cellulose. It was also found that the degradation reactions of the hemicellulosic polymers occurred during the more strong extraction strength, such as 3.0% H_2O_2 and 2.0 M NaOH.

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