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Fractional isolation and structural characterisation of hemicellulosic polymers from delignified and ultrasonic irradiated sugarcane bagasse

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Abstract: Hemicellulose-type polysaccharides were isolated from the delignified and ultrasonic irradiated sugarcane bagasse by a sequential two-step alkaline extraction. It was found that the successive extractions with 15% and 18% KOH for 2 h, 15% and 18% NaOH for 2 h, 8% and 10% KOH for 15 h, and with 8% and 10% NaOH for 15 h resulted in a total dissolution of 89.6%, 92.8%, 94.9%, and 97.3% of the original hemicelluloses, respectively. Sugar analysis revealed that xylose was the predominant sugar composition of all the hemicelluloses, comprising 57.4-68.6% of the total sugars. Arabinose (12.3-18.4%) and glucose (10.8-14.6%) appeared as the second and third major sugar constituents. Galactose (3.9-8.7%), uronic acids, mainly 4-*O*-methyl-glucuronic acid (2.7-5.8%), rhamnose (1.2-2.6%), and mannose (0.2-1.3%) were observed as minor constituents. The structural studies by ^{13}C-NMR spectroscopy showed that L-arabino (4-*O*-methyl-D-glucurono) xylans were the major constituents of the hemicellulosic polymers. Furthermore, the current results also showed that the four alkali-soluble hemicellulosic fractions, isolated during the first step treatment with relatively lower concentrations of alkalis, were more branched and acidic, and had larger molecular weights (M_w, 23100-34500), but lower thermal stability than the other four alkali-soluble hemicellulosic preparations (M_w, 21700-28700), extracted during the second stage treatment with relatively higher concentrations of alkalis.

Introduction

In recent years, there is an increased interest in developing techniques for fractionation of lignocellulosic materials to be used in the production of environmental friendly chemicals and polymers. These renewable agro-industrial residues generally contain 15-25% of lignin, 25-38% of hemicelluloses and 35-45% of cellulose, which cannot be easily fractionated into the three major components due to their recalcitrant nature [1]. Sugarcane bagasse (SCB) is one of the most

important non-wood raw materials in developing countries. About 54 million dry tons of SCB, the fibrous by-product remaining after sugar extraction from sugarcane, is produced annually throughout the world [2]. During a typical cane crushing season, about 50% of this fibre is needed to generate heat and power to run the sugar milling process, the remainder is stockpiled. The stockpiled SCB is of low economic value and create an environmental problem to sugar mills and surrounding districts [3]. As bagasse fibre is constituted, mainly, of lignocellulosic material, considerable research effort has been dedicated on investigating fractional isolation of the pure polymers such as hemicelluloses and cellulose as biodegradable materials. In our program we are interested in isolation and chemical modification of hemicelluloses and cellulose components of SCB as novel materials for industrial utilization.

Hemicelluloses, unlike cellulose, which is a unique molecule differing only in degree of polymerization and crystallinity, are non-crystalline heteropolysacharides and classically defined as the alkali soluble material after removal of the pectic substances [4]. After cellulose, hemicelluloses constitute the second most abundant class of polysaccharides found in nature. They comprise roughly one-fourth to one-third of most plant materials. Hemicelluloses, however, are the most complex components in the cell wall of woods, straws, and grasses. They form hydrogen bonds with cellulose, covalent bonds (mainly α-benzyl ether linkages) with lignins and ester linkages with acetyl units and hydroxycinnamic acids. They are branched polymers of low molecular weight with a degree of polymerization of 80-200. Their general formulae are $(C_5H_8O_4)_n$ and $(C_6H_{10}O_5)_n$ and are called as pentosans and hexosans, respectively [5]. Hemicelluloses consist of various different sugar units, arranged in different proportion with different substituents [6-8]. The principal sugars are D-xylose, L-arabinose, D-glucose, D-galactose, D-mannose, D-glucuronic acid, 4-*O*-methyl-D-glucuronic acid, D-galacturonic acid, and to a lesser extent, L-rhamnose, L-fucose, and various O-methylated neutral sugars. The major hemicelluloses in Gramineae such as cereal straws and SCB have a backbone of (1$\rightarrow$4)-linked β-D-xylpyranosyl units. The chain may be linear, but is often branched and usually has other glycosidically bound sugar units. Some xylan chains have D-glucopyranosyluronic acid units attached, but the most important acidic hemicelluloses are *O*-acetyl-4-*O*-methyl-D-glucuronoxylans and L-arabino (4-*O*-methyl-D-glucurono)xylans [9,10].

Studies on utilization of hemicelluloses from cereal straws and SCB have demonstrated to be a potential fermentation feedstock in production of ethanol, acetone, butanol, and xylitol. The current uses of xylan on an industrial scale involve their conversion to xylose, xylitol and furfural. Xylitol is produced by hydrolysis of xylan, crystallization of xylose, and hydrogenation. This has been tested in a variety of food products [11]. In addition, some important applications for hemicelluloses have been discovered. These include uses in chiral separations [12], cholesterol depressant [13], table disintegrant [14], HIV inhibitor [15], and dietary fibre [16]. Evidently, hemicellulosic biopolymers have a very wide variety of direct food and non-food applications. In particular, some hemicelluloses from higher plants and herbs represent a potential source of pharmacologically active polysaccharides. Glucuronic acid-containing (acidic) xylans isolated from annual plant residues such as bamboo leaves, corn stalks, wheat straw as well as hardwood have been reported to inhibit markedly the growth of sarcoma-180 and other tumors, probably due to the indirect stimulation of the non-specific immunological host defense [17,18]. More recently, a growing interest in using hemicelluloses as a raw material for

various novel technological applications, e.g., the synthesis of cationic polymers [19], hydrogels [20], long-chain ester derivatives [21], and thermoplastic xylan derivatives [22], has developed. To this end, hemicelluloses have been isolated and characterized from the residues from the production of different agricultural materials e.g., wheat straw [23] and flax shive [24].

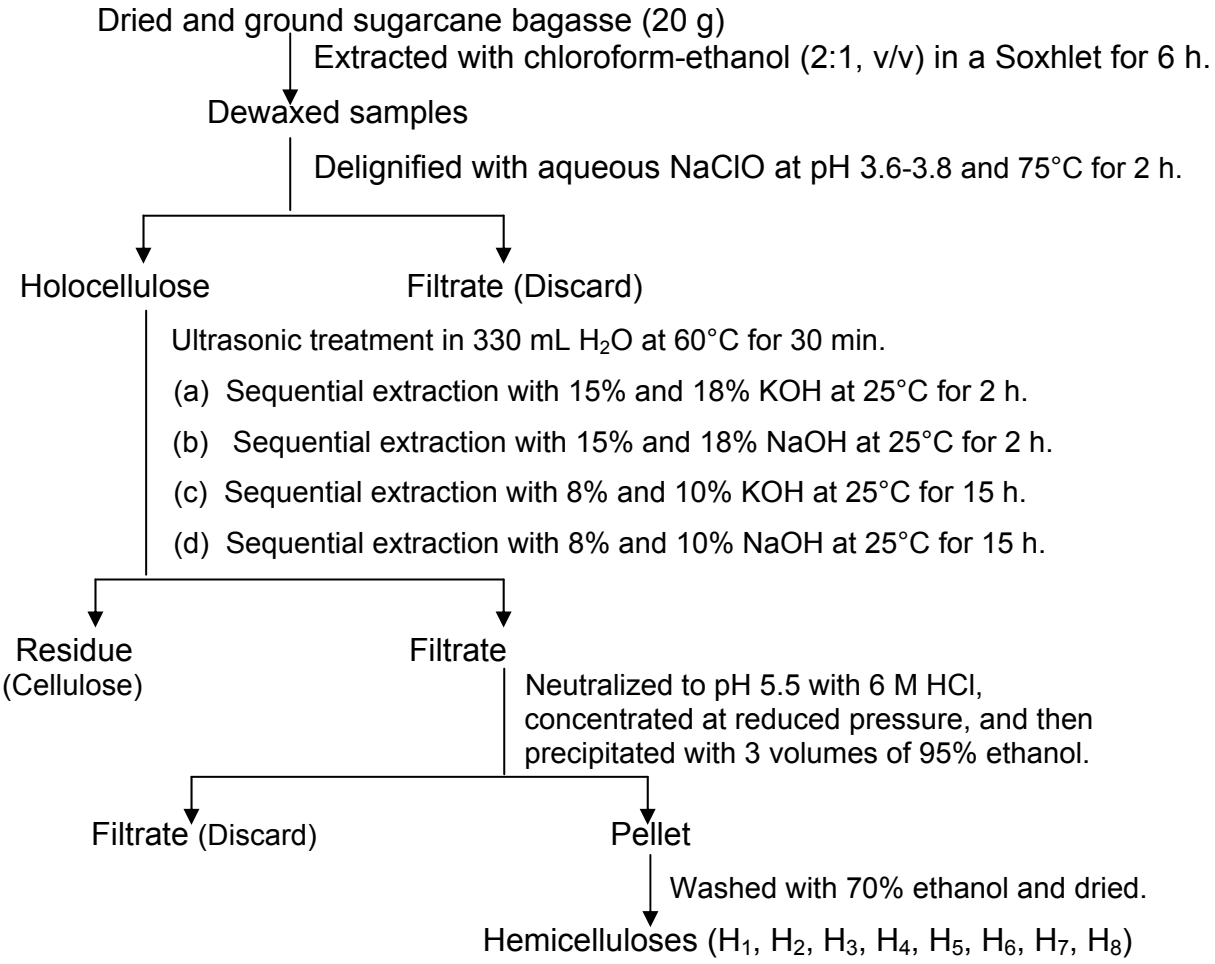

Fig. 1. Scheme for isolation of hemicelluloses from sugarcane bagasse.

The components in lignocellulose are tightly associated and in several processes it has been proved to be difficult to remove the lignin from hemicelluloses and cellulose without modifying the hemicelluloses [25]. Interestingly, the hemicelluloses from non-wood materials such as straw, grass, and SCB can be extracted directly from fully lignified materials with aqueous alkaline solution. The yields thus obtained vary widely for different types of the materials, probably reflecting differences in the structure of the cell walls [26]. The structure of the alkali-extracted hemicelluloses is thought to be quite similar to that of the native polysaccharides, except that under alkaline conditions the *O*-acetyl groups are removed, and the hemicelluloses contain noticeable amounts of associated lignin, which limit their usage in industries. Therefore, in order to obtain the hemicellulosic polymers having a high yield and purity, the material must be delignified and/or pre-treated in some way prior to extraction. The information available on the detailed structure of the hemicelluloses

from SCB is quite scarce. Also, the effect of the composition and structure of the hemicelluloses on the sequentially alkaline isolation process is far from being completely understood.

There are different ways to fractionate the hemicelluloses from lignocellulosic materials by pre-treatments including physical methods such as steam treatment [27], microwave irradiation [28], and sonication [1]. Pre-treatment is often combined with alkali extraction, which is crucial for the outcome due to different factors like solubility of the components, chemical reactions etc. [25]. In the present work, we have used sonication prior to alkali extraction in order to obtain a high yield and lignin-free hemicellulosic polymers. Scheme for the sequential treatments of SCB, and the isolation of hemicelluloses solubilized are depicted schematically in Fig. 1. The hemicelluloses obtained were physico-chemically and structurally characterized by both degradation methods such as acid hydrolysis and thermal analysis, and non-destructive techniques, e.g. Fourier transform infrared (FT-IR) and carbon-13 nuclear magnetic resonance (^{13}C NMR) spectroscopy, and gel permeation chromatography (GPC).

Results and discussion

Yield of hemicelluloses

Many attempts have been made to isolate hemicelluloses from various biomass sources, in which alkali extraction is the most efficient method for isolating large amounts of hemicellulosic polysaccharides [29]. In particular, a delignification step using chlorite prior to extraction of hemicelluloses can significantly facilitate the extraction. In this case most of the lignin can be removed without any noticeable degradation of hemicellulosic polymers, and thereafter the hemicelluloses can be extracted with alkali at room temperature [30]. Furthermore, the release of hemicelluloses from biomass may be assisted by pre-treatment procedures, such as sonication. Ultrasonic treatment is well established in the separation of plant materials, particularly for extraction of low molecular weight substances. The mechanical and chemical effects of ultrasound are believed to accelerate the extraction of organic compounds from plant materials due to disruption of cell walls and enhanced mass transfer of the cell wall contents [31].

As expected, the sequential alkali extractions of the delignified and ultrasonic irradiated SCB with 15% and 18% KOH for 2 h, 15% and 18% NaOH for 2 h, 8% and 10% KOH for 15 h, and with 8% and 10% NaOH for 15 h solubilized 23.2 and 6.8%, 24.1 and 7.0%, 26.0 and 5.8%, and 26.5 and 6.1% hemicelluloses (percent dry starting SCB), corresponding to a release of 69.3 and 20.3%, 71.9 and 20.9%, 77.6 and 17.3%, and 79.1 and 18.2% of the original hemicelluloses in SCB, respectively. Taken together, after ultrasonic treatment for 30 min at 60°C the successive extractions with 15% and 18% KOH for 2 h, 15% and 18% NaOH for 2 h, 8% and 10% KOH for 15 h, and with 8% and 10% NaOH for 15 h resulted in a total dissolution of 89.6%, 92.8%, 94.9%, and 97.3% of the original hemicelluloses, respectively. In comparison with the total yield of hemicelluloses (84.3, 87.6, 88.9, and 90.3% of the original hemicelluloses in SCB, data not shown) obtained under the corresponding conditions but without sonication, the application of ultrasonic treatment of the delignified SCB in water for 30 min affected positively the yield of

hemicelluloses, which represent an increase of hemicelluloses by 5.3, 5.2, 6.0, and 7.0%, respectively.

Monosaccharide composition of the hemicelluloses

To examine the solubilized hemicelluloses, eight preparations were performed for determination of their substituent sugars, and the results are given in Tab. 1. Clearly, xylose was the predominant sugar composition of all the hemicelluloses, comprising 57.4-68.6% of the total sugars. Arabinose (12.3-18.4%) and glucose (10.8-14.6%) appeared as the second and third major sugar constituents. Galactose (3.9-8.7%), uronic acids, mainly 4-*O*-methyl-glucuronic acid (2.7-5.8%), rhamnose (1.2-2.6%), and mannose (0.2-1.3%) were observed as minor constituents. Most of the xylose residues probably originated from the backbone of a xylan. The presence of a predominant amount of xylose, a noticeable amount of arabinose, and a minor quantity of uronic acids in all the eight hemicellulosic fractions indicated that L-arabino (4-*O*-methyl-D-glucurono) xylans were the major constituents of the hemicelluloses in the cell walls of SCB. Interestingly, the content of xylose was higher in the four hemicellulosic fractions (63.9-68.6%), post-extracted with 18% KOH (H_2), 18% NaOH (H_4), 10% KOH (H_6), and 10% NaOH (H_8) than in other four hemicellulosic preparations (57.4-62.0%), isolated firstly with 15% KOH (H_1), 15% NaOH (H_3), 8% KOH (H_5), and 8% NaOH (H_7) from the delignified and ultrasonic treated SCB, while the relative amounts of arabinose, galactose, uronic acids, rhamnose, and mannose in the hemicellulosic fractions (H_1, H_3, H_5, H_7), solubilized during the first step of alkaline extraction, were higher than those in the hemicellulosic fractions ((H_2, H_4, H_6, H_8), released during the corresponding second step of alkaline treatment.

Tab. 1. The content of neutral sugars (relative % dry weight, w/w) and uronic acids (% dry weight, w/w) in the isolated hemicellulosic preparations.

Sugars (%)	Hemicellulosic preparations							
	H_1[a]	H_2[a]	H_3[b]	H_4[b]	H_5[c]	H_6[c]	H_7[d]	H_8[d]
Arabinose	18.4	12.3	16.5	12.0	15.8	13.9	16.3	13.8
Rhamnose	2.5	1.5	2.6	1.2	2.1	2.0	1.9	1.6
Galactose	5.8	4.1	5.2	3.9	8.7	7.3	8.2	6.9
Glucose	10.8	13.5	12.8	14.1	14.6	12.4	13.6	11.8
Xylose	61.5	68.3	62.0	68.6	57.4	63.9	58.9	65.7
Mannose	1.1	0.2	0.9	0.3	1.3	0.6	1.1	0.3
Uronic acids	5.0	2.7	4.8	2.8	5.8	3.5	5.2	2.8

[a]H_1 and H_2 represent for the hemicellulosic preparations extracted sequentially with 15% and 18% KOH at 25°C for 2 h from the ultrasonic irradiated holocellulose, respectively.
[b]H_3 and H_4 represent for the hemicellulosic preparations extracted sequentially with 15% and 18% NaOH at 25°C for 2 h from the ultrasonic irradiated holocellulose, respectively.
[c]H_5 and H_6 represent for the hemicellulosic preparations extracted sequentially with 8% and 10% KOH at 25°C for 15 h from the ultrasonic irradiated holocellulose, respectively.
[d]H_7 and H_8 represent for the hemicellulosic preparations extracted sequentially with 8% and 10% NaOH at 25°C for 15 h from the ultrasonic irradiated holocellulose, respectively.

This phenomenon provides evidence that in SCB cell walls arabinose, galactose, uronic acids, rhamnose, and mannose, probably as side chains in hemicelluloses, are easily solublized during the first step treatment with alkali, whereas these side chains are partially cleaved or degraded in the relatively strong alkaline solution such as 18% KOH and 18% NaOH. In other words, these results indicated that the hemicelluloses solubilized during the first step of alkali extraction were more branched and acidic, but less tightly bound to cellulose than those of the hemicelluloses released during the corresponding post-treatment. This observation was consistent well with our previous studies on wheat straw hemicelluloses. It was found that the lower the arabinose content indicating a lower degree of branching of xylan chains, the lower the solubility of the hemicelluloses [9]. It should be noted that a relatively higher amount of glucose in H_2 (13.5%) and H_4 (14.1%) than that in H_1 (10.8%) and H_3 (12.8%) implied that the sequential treatment with a higher concentration of alkali (18% KOH, 18% NaOH) might degrade some amounts of cellulose even though the post-treatment was performed at a room temperature.

Molecular weight

The molecular weights of the eight hemicellulosic fractions were further determined by gel permeation chromatography (GPC), and their weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) are given in Tab. 2. The major elution peak in the hemicellulosic samples, corresponding to molecular weights around 20000-35000, is attributed to xylan (elution curves not shown) [9]. These figures are of the same order of magnitude of those found in the hemicelluloses from cereal straws [10].

Tab. 2. Weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) of the hemicellulosic preparations isolated from the delignified and ultrasonic irradiated sugarcane bagasse.

Sugars (%)	Hemicellulosic preparations[a]							
	H_1	H_2	H_3	H_4	H_5	H_6	H_7	H_8
M_w	25000	22400	23100	21700	34500	28700	33000	26600
M_n	3740	3100	3360	2910	4490	4620	4340	4130
M_w/M_n	6.67	7.23	6.89	7.46	7.69	6.21	7.61	6.45

[a]Corresponding to the hemicellulosic preparations in Tab. 2.

Clearly, the first four hemicellulosic fractions (H_1, H_2, H_3, H_4), solubilized during the treatment with higher concentrations of alkali (15% and 18% KOH, 15% and 18% NaOH), showed a lower degree of polymerisation with M_w values between 21680 and 24950 than those of the last four hemicellulosic preparations (H_5, H_6, H_7, H_8) with M_w values from 26630 to 34530, released during the treatments with relatively lower concentrations of alkali (8% and 10% KOH, 8% and 10% NaOH). In addition, as compared to the M_w values of the four hemicellulosic polymers (H_1, H_3, H_5, H_7) solubilized during the first step extraction with relatively lower concentrations of alkali (15% KOH, 15% NaOH, 8% KOH, 8% NaOH), the hemicellulosic preparations obtained during the sequentially second step extraction with relatively higher concentrations of alkali (18% KOH, 18% NaOH, 10% KOH, 10% NaOH) showed lower molecular weights. This observation suggested that the treatments with higher

concentrations of alkali under the conditions used resulted in a degradation of the macromolecular structure of hemicelluloses, since a partial depolymerization of the dissolved polysaccharides in alkali solution contributed to the lower molecular weights. That is, the lower molecular weights of the hemicelluloses in the present case can be explained by partial depolymerization during the treatments with relatively higher concentrations of alkalis. Furthermore, the analysis showed that all the polymeric hemicelluloses gave a broader molar mass distribution, corresponding to polydispersity indexes between 6.21 and 7.61.

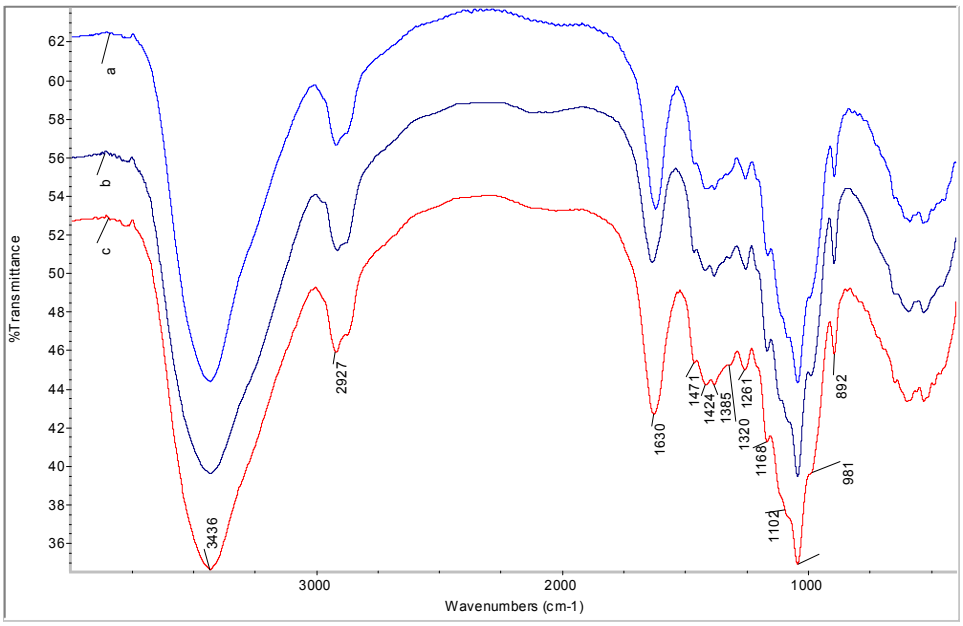

Fig. 2. FT-IR spectra of hemicellulosic preparations H_1 (spectrum a), H_2 (spectrum b), and H_3 (spectrum c).

As expected, all of the FT-IR spectral profiles of the isolated hemicellulosic fractions and their relative intensities of the bands were rather similar, indicating similar structures of the hemicelluloses isolated. Fig. 2 shows the FT-IR spectra of hemicellulosic preparations solubilized during the sequential treatments with 15% KOH (H_1, spectrum a) and 18% KOH (H_2, spectrum b), and 15% NaOH (H_3, spectrum c) from the delignified and ultrasonic irradiated SCB. The analysis of FT-IR data showed that all the three hemicellulosic preparations clearly illustrated the typical signal pattern for hemicellulosic moiety, and had a specific band maximum in the 1200-1000 cm^{-1} region. This region is dominated by ring vibrations (C-C stretching) and the glycosidic bond vibrations (C-O-C). The prominent band at 1043 cm^{-1} is attributed to the C-O stretching in C-O-C glycosidic bonds. The band at 1102 cm^{-1} is originated from the C-OH bending typical of xylans [9]. The presence of the arabinosyl side chains is clearly identified at 1168 cm^{-1} in all of the three spectra. A sharp peak at 892 cm^{-1}, which arises from the C_1 group frequency or ring frequency, is indicative of β-glycosidic linkages between the sugar units [29]. The bands at 1471, 1424, 1385, 1320, and 1261 cm^{-1} arise from C-H and O-H bending vibrations in hemicelluloses. The absorption at 1630 cm^{-1} is principally associated with absorbed water. Interestingly, the absence of a band at 1520 cm^{-1}, which represents the aromatic skeletal vibrations, demonstrated that all of the hemicellulosic fractions are free of associated lignins.

^{13}C-NMR spectrum

The structural features of the hemicelluloses were also investigated using carbon-13 magnetic resonance spectroscopy, and the samples were recorded in D_2O. All fractions showed very similar spectra and we show in Fig. 4 only the ^{13}C-NMR spectrum of H_5 isolated with 8% KOH at 25°C for 15 h. The spectrum gives five main signals at δ, 104.6 (C-1), 78.2 (C-4), 77.0 (C-3), 75.5 (C-2), and 65.6 (C-5) ppm, corresponding to (1→4) linked β-D-Xyl*p* residues. Other less intense signals observed at δ, 111.8, 88.8, 84.9, and 80.7 ppm are characteristic, respectively, of C-1, C-4, C-2, and C-3 of α-L-arabinofuranosyl residues in the side chain of xylan backbone. Two signals at δ, 82.4 and 64.0 ppm are attributed to C-4 and C-6 of glucose residues linked to β-D-xylans. The presence of glucuronic acid was also confirmed by the presence in ^{13}C-NMR spectrum with a characteristic signal at 72.2 (C-5) ppm. A signal at δ, 61.9 ppm originates from the 4-*O*-methoxyl groups of glucuronic acid residues in the xylan. Also the xylan backbone substituted with xylose is characterized by a small signal at 81.3 ppm. These NMR data are in good agreement with the structures of L-arabino (4-*O*-methyl-D-glucurono) xylans already described in a number of plants [1,9-11,32].

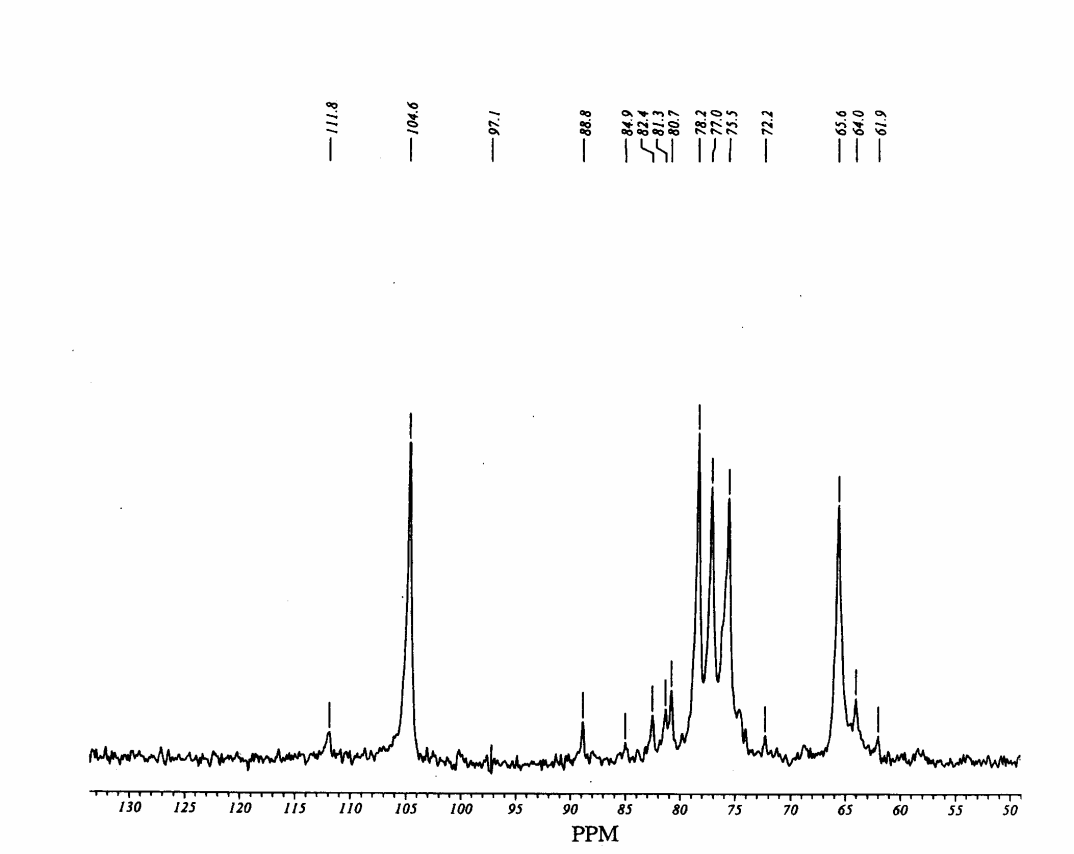

Fig. 4. ^{13}C-NMR spectrum of the hemicellulosic preparation H_5 extracted with 8% KOH at 25°C for 12 h from the ultrasonic irradiated holocellulose.

Thermal analysis

Fig. 5 illustrates the thermogravimetric analysis (TGA) and differential thermal analysis (DTA) curves of the hemicellulosic preparations H_5 (spectrum a) and H_6

(spectrum b) isolated sequentially with 8% and 10% KOH at 25°C for 15 h from the delignified and ultrasonic treated SCB. Four weight loss stages can be seen, which is coincided with other hemicellulosic polymer studies [9]. For all samples studied, the first weight loss occurred between 50 and 200°C, which is attributed to the evaporation of water. The pattern of the peaks suggested that water was not clathrated but probably adsorbed on the surface of the material. The second, much faster step spread between 200 and 270°C. It was followed by still faster step beginning at 270°C and ending at ~340°C, with a maximum weight loss rate at 322°C, which relates to the major decomposition of hemicellulosic molecules. The fourth stage of weight loss ranging from 340 to 600°C might be due to the further breakage of the hemicelluloses. At 50% weight loss the degradation temperature of the hemicellulosic fractions H_5 and H_6 occurred at 300 and 320°C, respectively. This phenomenon suggested that the hemicellulosic fraction H_6 post-extracted with a relatively higher concentration of alkali (10% KOH), had a slightly higher thermal stability than that of the hemicellulosic preparation H_5 isolated firstly with a relatively lower concentration of alkali (8% KOH). All hemicellulosic samples have a residual weight between 25 and 35 wt% at 600°C. In an inert atmosphere, the end-products of the decomposition of hemicelluloses are carbonaceous residues [33]. The inorganic compounds taken by the plants during growing, and the salts formed during the extraction and purification processes will contribute to the ash [34].

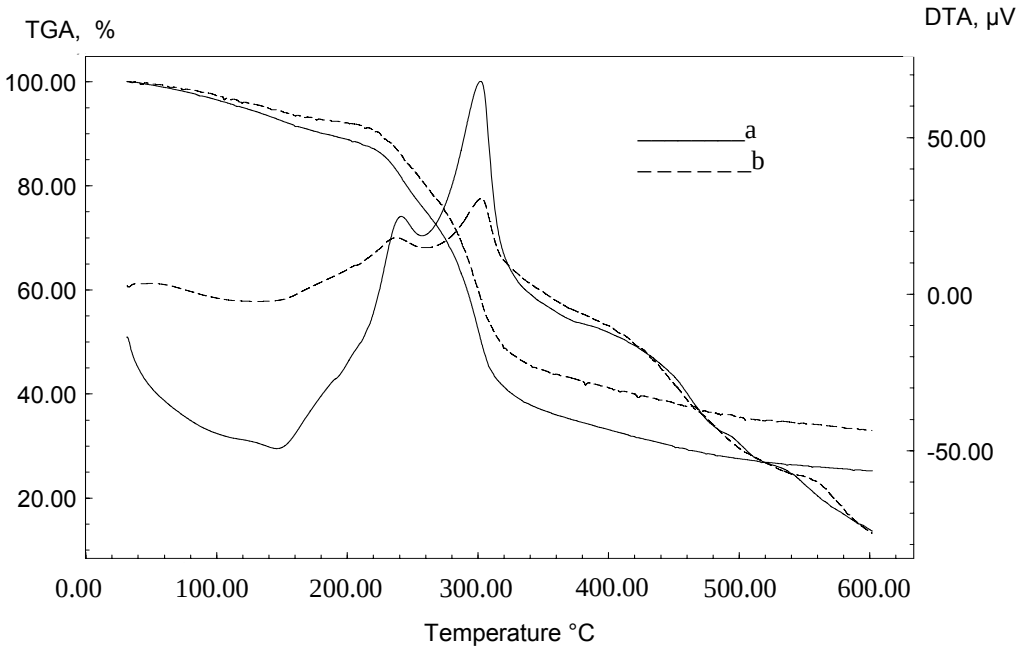

Fig. 5. Thermogram of the hemicellulosic preparations H_5 (spectrum a) and H_6 (spectrum b) extracted sequentially with 8% and 10% KOH at 25°C for 15 h from the ultrasonic irradiated holocellulose.

Conclusions

The application of sonication in the isolation of hemicellulosic polymers from the delignified SCB had a positive effect on the extraction yield. In comparison with the successive treatment processes without ultrasound, the total yield of hemicelluloses, obtained from the delignified and ultrasonic treated holocellulose, increased by 5.3-

7.0%, indicating that short ultrasonic treatment of the delignified holocellulose in distilled water prior to alkaline extraction was highly effective in disintegrating the hydrogen bonds between hemicelluloses and cellulose, but did not significantly change the structure of the dissolved hemicelluloses. The hemicelluloses proved to be composed mainly of L-arabino (4-*O*-methyl-D-glucurono) xylans, which were found to be free of bound lignin. The weight average molecular weights determined by GPC were between 21680 and 34530. These advantages implied that delignification with chlorite followed by ultrasound-assisted and alkaline extraction processes could be used for isolation of polysaccharides having a high yield and purity from renewable materials for industries.

Experimental

Materials

Sugarcane bagasse (SCB) was obtained from a local sugar mill (Guangzhou, China). It was first dried in sunlight and then ground to pass a 0.8 mm size screen. The ground SCB was further dried in a cabinet oven with air circulation for 16 h at 55°C. The chemical composition (%, w/w) of the SCB used in this work is cellulose 43.6%, hemicelluloses 33.5%, lignin 18.1%, ash 2.3%, and wax 0.8% on a dry weight basis. Prior to delignification, the dried powder of the SCB was first extracted with chloroform-ethanol (2:1, v/v) in a Soxhlet extractor for 6 h so as to dewax, and then air-dried.

Ultrasonic pre-treatment and sequential alkali extractions

The dewaxed powder (20 g) was delignified with 6% sodium chlorite at pH 3.6-3.8, adjusted with 10% acetic acid, at 75°C for 2 h [35]. The residue was subsequently washed with distilled water and ethanol, and then oven dried at 60°C for 16 h. The holocellulose obtained was then soaked in 330 mL distilled water. The irradiation was then carried out using the sonic system SOMERSET (German, 20 kHz) provided with a horn at sonic power of 100 W and sonication time for 30 min at 60°C without additional stirring. After cooling to room temperature, the mixture was successively extracted at 25°C with 15% and 18% KOH for 2 h, 15% and 18% NaOH for 2 h, 8% and 10% KOH for 15 h, and with 8% and 10% NaOH for 15 h by adding required amounts of alkali with a solid to liquid ratio of 1:20 (g mL^{-1}), respectively. The extraction flask was continuously purged with gaseous N_2. At the end of the extraction, the insoluble residue (cellulose) was collected by filtration, washed with distilled water and 95% ethanol, and then dried in an oven at 60°C for 16 h. Each of the supernatants was adjusted to pH 5.5 with 6 M HCl and then concentrated under reduced pressure. The hemicelluloses released were precipitated by pouring the concentrated supernatant fluid into 3 volumes of 95% ethanol. The solution containing the precipitated hemicelluloses was kept at room temperature for 3-5 h, and then the mixture was filtered. Finally, the hemicelluloses were washed with acidified 70% ethanol and air-dried. Note that the hemicelluloses solubilized during sequential extraction with 15% and 18% KOH, and 15% and 18% NaOH at 25°C for 2 h were labelled as hemicellulosic fractions H_1, H_2, H_3, and H_4, respectively, and the hemicelluloses released during the sequential extraction with 8% and 10% KOH, and 8% and 10% NaOH at 25°C for 15 h were named as the hemicellulosic fractions H_5, H_6, H_7, and H_8, respectively. All experiments were performed at least in duplicate.

Structural and physiochemical characterization of hemicelluloses

The neutral sugar composition in isolated hemicelluloses was determined as sugar alditol-acetate derivatives by gas chromatography (GC) after hydrolysis with 2 M trifluoroacetic acid for 2 h at 120°C [35,36]. Uronic acid content was determined by the automated colorimetric *m*-hydroxydiphenyl assay [37]. Method for measurement of the hemicellulosic molecular weights has been described in previous papers [21,35]. The hydrolysis and analyses were conducted in duplicate, and the values of individual monosaccharide residues were within ± 5%.

FT-IR spectra of the hemicellulosic samples were obtained on an FT-IR spectrophotometer (Thermo Nicolet 510, USA) in the range 4000-400 cm^{-1} using a KBr disc containing 1% finely ground samples. For solution-state ^{13}C-NMR analysis, a portion of the dried sample (80 mg) was dissolved in 1 mL D_2O (99.8% D). The spectrum was obtained on a Bruker DRX-400 spectrometer at 74.5 MHz. The spectrum was recorded at 25°C after 30 000 scans. A 60° pulse flipping angle, a 3.9 μs pulse width and a 0.85 s acquisition time were used.

Thermal analysis of the hemicellulosic samples was performed using thermogravimetric analysis (TGA) and differential thermal analysis (DTA) on a simultaneous thermal analyzer (SDT Q600, TA Instrument). The apparatus was continually flushed with nitrogen. The sample weighed between 9 and 11 mg and heated from room temperature to 600°C at a rate of 10°C per minute.

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