Isolation and Characterisation of Hemicelluloses Extracted by Steam and Alkaline Peroxide at Different Temperatures from Sugarcane Bagasse

Protibha Nath Banerjee

The hemicelluloses from sugarcane bagasse were sequentially extracted with steam treatment and alkali. The hemicellulose from steam treatment was found to contain galacto- arabinoxylans while alkali extraction yielded predominantly linear arabinoxylans. The isolated hemicelluloses were found to vary considerably in branching pattern confirmed from different spectroscopic and chemical methods. The hemicellulose from steam treatment was found to be more branched compared to those isolated after alkali treatment. The present study also showed a promising sequential extraction for isolating arabinoxylan hemicelluloses with different degree of branching, molar mass and functional group from sugarcane bagasse. Consequently, products with a high aggregated value could be developed using this xylan-rich fraction as an ingredient for industrial products.

Keywords: Steam treatment, Sugarcane bagasse, Methylation analysis, degree of branching, alkaline peroxide

*Contact information: a: Department of chemistry,School of physical sciences, The university of Dodoma, PO Box 259, Dodoma, Tanzania; *Corresponding author: protibha.banerjee@udom.ac.tz*

INTRODUCTION

In the recent years agro-industrial residues are gaining potential interest as raw materials for industrial application. Reusing and recycling of these residues will not only resolve the environmental issues associated with their build-up but will also help in adding value, creating employment and boosting socio-economic security of the rural people. Sugarcane bagasse is a kind of agricultural residue produced in large quantities by sugar industry. About 54 million dry tons of bagasse is produced annually throughout the world (Rodrigues et al., 2003 and Rowell & Keany, 1991) and about 80% of this is used in sugar and distillery plants as a source of energy(Pandey, Soccol, Nigam & Soccol, 2000) which is a misutilization of nature's precious material due to lack of proper biorefinery technologies. Bagasse generally contains 40-45% cellulose and 25-35% hemicellulose, an amorphous polymer usually composed of xylose, arabinose, galactose, glucose, mannose and smaller amount of 4-O-methyl glucuronic acid. The remainder is mostly lignin plus lesser amount of minerals, waxes and other compounds (Jacobsen & Wymen 2002). Thus, in view of large availability of sugarcane bagasse and its importance as a raw material from industrial waste, there is a great potential of producing value added chemicals from its lignocellulosic constituents, such as

hemicelluloses, in a biorefinery concept. To the best of our knowledge, only some earlier work on the characterization of hemicelluloses from sugarcane bagasse has been done. Xu et al. (2006) isolated the hemicelluloses from sugarcane bagasse after alkaline treatment followed by organic solvent extraction. Peng et al. (2010) reported the characterization of sugarcane bagasse hemicelluloses obtained by hot water extraction at one temperature only (i.e. 55°C), followed by alkaline extraction. Banerjee *etal* (2014) reported the characterisation of non-cellulosic heteropolysaccharide from sugarcane bagasse obtained by pressurised hot water at different temperatures followed by alkaline peroxide extraction. In the present study the hemicelluloses were first isolated by steam pretreatment at two temperatures followed by alkaline peroxide extraction and the hemicelluloses obtained were characterised by chemical and spectroscopic methods.

EXPERIMENTAL

Materials

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

Methods

Isolation of hemicelluloses by steam treatment

Extractive free sugarcane bagasse (10 g) was subjected to steam at 200°C and 210°C for 10 min. The extracts were cooled, filtered and concentrated to one-third of its volume at 40°C under reduced pressure. The solubilized hemicelluloses (were isolated by precipitation of the concentrated filtrates with 3 vol of 95% EtOH, washed first with acetone and then with MTBE. The precipitated hemicelluloses were dried under vacuum at 40°C for 12 hours and are designated as H_1 and H_2.

Isolation of alkaline peroxide soluble hemicellulosic fragments

Each of the foregoing steam treated residues were post treated with 3% alkaline peroxide and magnesium sulphate (0.25%) with the pH adjusted to 11.6 with NaOH at 40°C for 12 hours. All the extracts were filtered off and washed with water. The combined supernatant fluids were neutralized to pH 6.0 with dropwise addition of 6M HCl over an ice bath. All the extracts were concentrated to one-third of its volume at 40°C under reduced pressure and precipitated with 3 vol of 95% EtOH. The precipitates were washed first with acetone and then with MTBE. The precipitated hemicelluloses were dried under vacuum at 40°C for 12 hours. These hemicelluloses are designated as H_3 and H_4.

The procedure used to isolate steam treated and alkaline peroxide soluble hemicelluloses are illustrated in Figure 1.

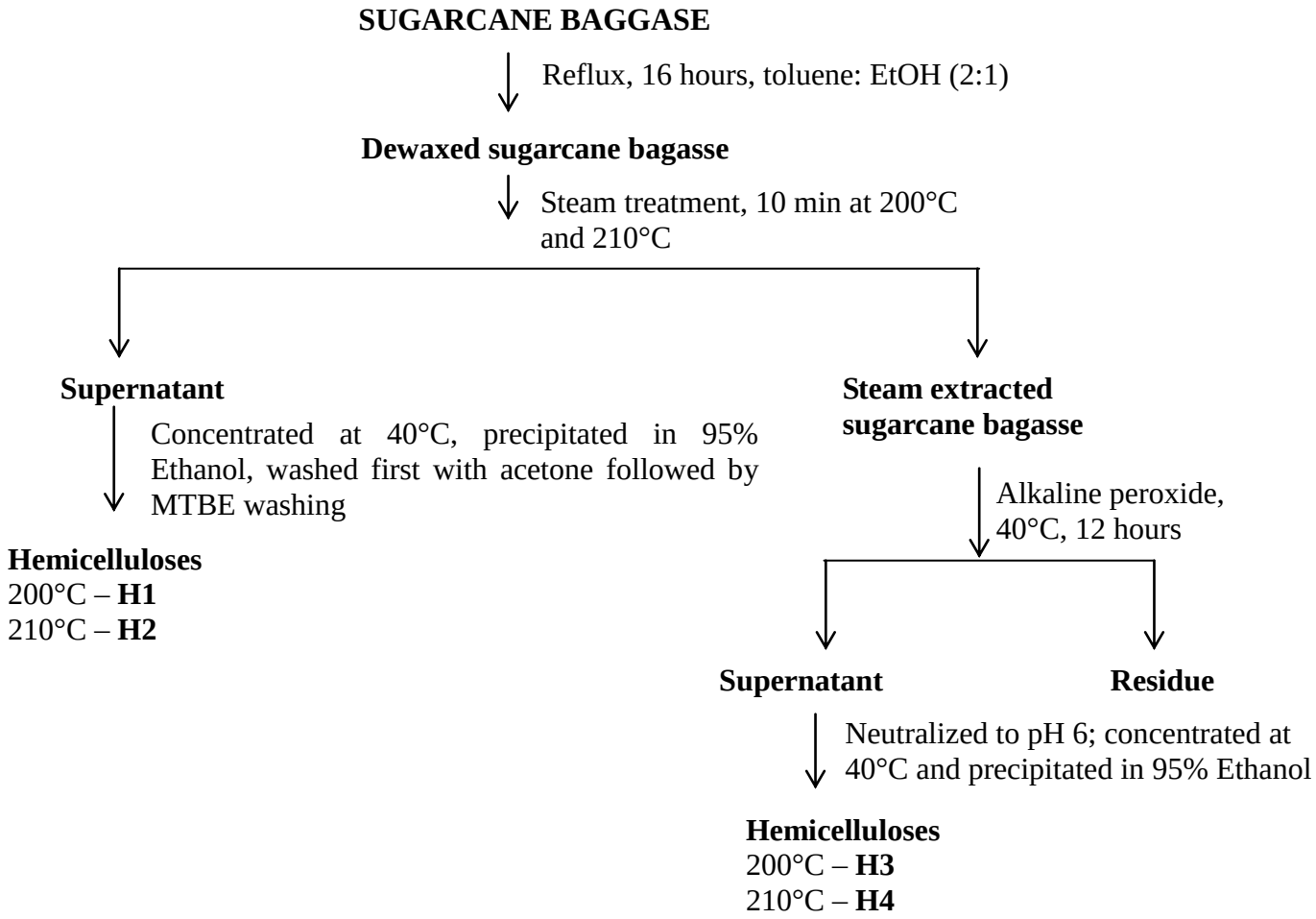

Fig.1. Scheme for the isolation of hemicelluloses by sequential extraction of sugarcane bagasse with steam and alkaline peroxide

Monosaccharide analysis

Each hemicellulosic fraction (1 mg) was transferred to a pear shaped flask and dried in a vacuum oven at 40°C for 1 hour. Two mL of 2M HCl in anhydrous methanol was added to each flask and the samples were then kept at 105°C for 3 hours. A calibration solution containing equal amout (0.1mg/mL) of each sugar monomers and uronic acids (except 4-O-MeGlcA) was also subjected to methanolysis under similar condition. All samples were cooled to room temperature and neutralized by addition of 200 µL of pyridine. 1 mL of 0.1mg/mL sorbitol solution was added as internal standard to all the samples. The methanol was evaporated in stream of nitrogen, dried under vacuum at 40°C, silylated and analysed by GC according to Sundberg method (Sundberg etal, 1996 and Banerjee, 2014).

HPSEC analysis

Molecular weight of the hemicellulosic fractions was determined using high-performance size-exclusion chromatography (HPSEC) and refractive index detection (Reed, 1995). Four gel permeation ultra-hydrogel columns in series, with exclusion sizes of $7x10^6$, $4x10^5$, $8x10^4$ and $5x10^3$ Da, were used. The eluent was 0.1M aq. NaNO2 at 0.6 mL/min. The samples, previously filtered through a membrane (0.22µm), were injected at a concentration of 2 mg/mL. The *dn/dc* value was taken as 1.5 and the results were processed with software provided by the manufacturer (Wyatt Technology Corporation).

Methylation analysis

Each hemicellulosic fraction (10 mg) was dissolved in 2 mL of dry DMSO and methylated by Hakomori method (Banerjee *etal*. 2007, 2014) The per-O-methylated derivative was extracted with chloroform (2x 5 mL), washed with water (3x 3 mL), evaporated under vacuum at 30°C and dried under vacuum at 40°C. The dried per-O-methylated samples were subjected to IR spectroscopy which showed no hydroxyl absorption and distinct peaks at 1740 cm^{-1} (C=O of ester) and 1125 cm^{-1} (C-O of ether). The per-O-methylated samples were then reduced with 1M superdeuteride ($LiEt_3BD$) in THF at room temperature for 12 hours and then hydrolysed first with 50% v/v sulphuric acid at 30°C for 1 hour and then diluted to 5.0% and maintained at 120°C for 2 hours in an autoclave. The resulting mixtures of O-methyl aldoses was neutralized with BaCO3, filtered, reduced with sodium borohydride, acetylated and analysed by GC-MS using column HP-1 (25m x 0.2mm x 0.11 µm) with a temperature of 80ºC for 0.5 min and then increased to 300 ºC at a rate of 8 ºC/ min. Helium was used a carrier gas with a flow rate of 0.8 ml/ min (constant flow). The injector temperature was 300ºC and the MS ionization mode was EI at 70 eV.

Content of bound lignin

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

NMR spectroscopy

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

all the samples were saturated solution to assure a good signal to noise ratio in the 13C NMR spectra.

Infrared spectroscopy

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

RESULTS AND DISCUSSION

Yield of Hemicelluloses

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

Non-cellulosic Carbohydrate Composition

The major sugar units from methanolysis of steam extracted hemicelluloses was xylose (43.1and48.0%), followed by arabinose (25.1and24.3%) and glucose (16.8 and 15.2%) (Table1). The arabinose to xylose ratio was much lower at 210°C than at lower 200°C (0.58–0.5), indicating that the high-temperature extraction resulted in more linear structures, while extraction at 200°C resulted in the release of hemicelluloses with more branching. Thes data indicate that steam treatment probably released more branched galactoarabinoxylans and β-glucans. In case of alkali treated samples (Table1), xylose was the predominant sugar (80.8and81.2%) followed by arabinose (6.4–6.7%), uronic acids, particularly glucuronic acid (0.4and0.7%) and 4-O-MeGlcA (0.6and0.7%), suggesting that the alkali-soluble hemicelluloses from sugarcane bagasse mainly consists of glucuronoarabinoxylans or L-arabino-(4-Omethyl-glucurono)-D-xylans. A low Ara/Xyl ratio would indicate a high degree of polymerization with little branching. The ratio of Ara/Xyl of the hemicelluloses obtained from alkaline treatment was lower (0.08) compared to the hemicelluloses obtained from steam treatment, indicating that the hemicelluloses obtained by alkali treatment were less branched than those from steam treatment.

Table 1. Yield And Composition For The Sugarcane Bagasse Hemicelluloses Extracted Sequentially By Steam Treatment And Alkaline Peroxide (H1 - H4).

Yield[a]		Total[b] sugar content	Hemicelluloses sugar units composition[c]								4-O-Me-GlcA	Ara/Xyl Ratio
			Ara	Rha	Xyl	GlcA	GalA	Man	Gal	Glc		
H_1	80	89.1	25.1	0.1	43.1	0.06	0.09	1.5	2.1	16.8	0.25	0.58
H_2	86	90.5	24.3	0.09	48.0	0.05	0.08	0.6	2.0	15.2	0.2	0.50
H_3	125	94.5	6.4	0.5	80.8	0.4	0.1	2.4	2.2	0.8	0.6	0.08
H_4	130	95.3	6.7	0.1	81.2	0.7	0.7	2.1	2.4	0.7	0.7	0.08

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

Molar Mass Determination

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

Table 2. Weight Average (Mw) and Number Average (Mn) Molar Mass and Polydispersity (Mw/Mn) of Hemicellulosic Fractions Released During Extraction of Sugarcane Bagasse with Steam Treatment and Alkaline Peroxide.

	H1	H2	H3	H4
$\overline{M}_w$	10500	9900	23000	17000
$\overline{M}_n$	8400	6600	7300	7500
$\overline{M}_w/\overline{M}_n$	1.2	1.5	3.1	2.2

Methylation Analysis

The partially methylated alditol acetates were subjected to GC–MS analysis and the results for H1 and H3 are shown in Table 3. Both hemicelluloses were dominated by (1-4)- arabinoxylan represented by a high percentage of 2,3-Me2-Xyl residues, substituted mainly at O-2 (3-Me-Xyl) and O-3 (2-Me-Xyl) by non-reducing end units of arabinofuranose (2,3,5-Me3-Ara). The uronic acids were determined by their respective

m/z value, which is 2 units more than their corresponding neutral sugars. The appearance of 2,3,4,6-Me4Glc as glucuronic acid (confirmed from m/z value) in H3 suggest that the glucuronic acid moiety is present in the side chain and not in the main backbone as a-D-GlcpA or 4-O-Me-a-D-GlcpA. The steam extracted hemicelluloses is also dominated by (1-4)-glucose. The amount of 2,3,5-Me3-Ara was small in case of H3 (5.8%) corresponding to H1 (23.6%) corroborating that the alkaline extraction cleaves off the arabinose side chain significantly.

Table 3. Partially *O*-Methylated Alditol Acetates Obtained from Sugarcane Bagasse Hemicelluloses (H3 And H4).

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

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

Lignin Analysis

The hemicelluloses from steam treatment had higher lignin content (11.3–9.4%) than the corresponding alkali-soluble hemicelluloses (5.4 and 5.0%) suggesting that the a-benzyl ether linkage between lignin and hemicelluloses were significantly cleaved during alkaline peroxide treatment (Table 4). The associated lignin in the Hemicellulosic fractions were dominated by syringyl units, except inH2 indicating that syringyl units cleaved off significantly during steam treatment at higher temperatures (210°C).

Table 4. Lignin Content and *P*-Hydroxyphenyl (H), Guaiacyl- (G) and Syringyl- (S) Units Determined in the Hemicellulosic Fractions from Sugarcane Bagasse by Pyrolysis GC-MS.

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

[a]Expressed as weight percent of total precipitated yield
[#]H= *p*-hydroxy phenyl
[#]G= guaiacyl
[#] S= syringyl

FT-IR Spectra

The presence of the absorption band at 1730 cm-1 in the spectrum of H1 (Fig1) and H2 might have originated from the acetyl, uronic, and ester groups of the hemicelluloses. The occurrence of a shoulder at 1514 cm-1 is due to the presence of associated lignin in the hemicelluloses (Pandey,1999), which corresponds to the data obtained by the AcBr method and pyrolysis GC–MS. The The FT-IR spectra of hemicelluloses H3, and H4 (not shown) from alkaline peroxide extraction exhibited the absorbance bands associated with hemicelluloses. In comparison with the spectra of hemicelluloses released during steam extraction (H1, H2), the absence of a peak at 1730 cm-1 for carbonyl stretching demonstrated that acetyl groups and ester linkages between the hemicelluloses and the lignin cleaved during the alkaline extraction of the steam extracted residues.

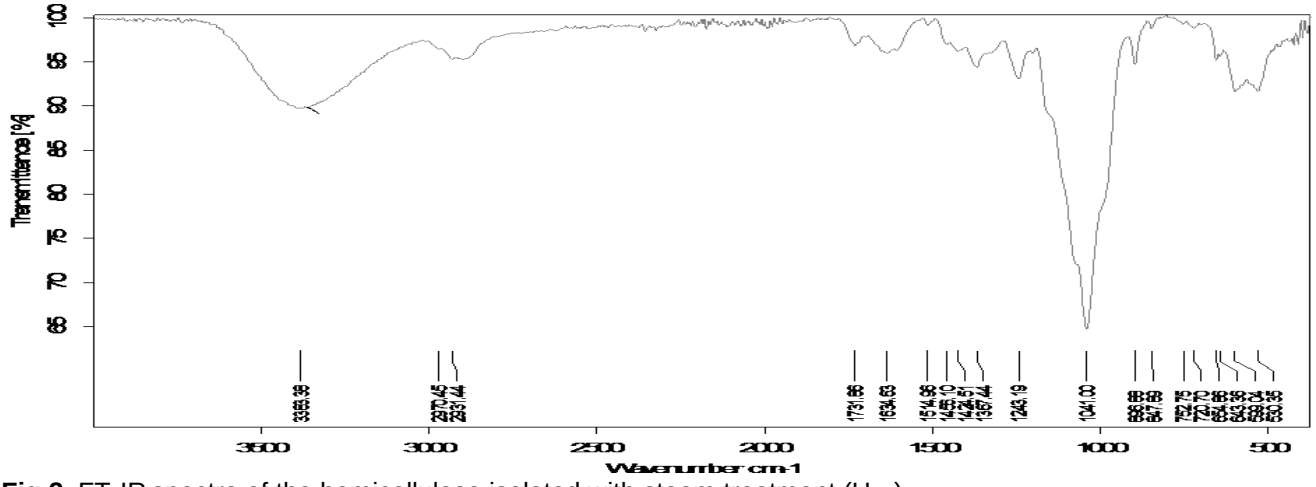

Fig.2. FT-IR spectra of the hemicellulose isolated with steam treatment (H_{ST})

1H and 13C NMR Spectra

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

CONCLUSION

The present study showed that Low-molar-mass hemicelluloses, with more branching, were more easily extracted by steam treatment, while high-molar-mass and more linear hemicelluloses were dissolved in the subsequent alkaline peroxide treatment. Moreover, noticeable differences in the chemical composition and molar-mass characteristics were observed among the hemicelluloses obtained from steam and alkaline peroxide treatment. Based on sugar composition, methylation analysis, FT-IR, 1H and 13C NMR studies, the hemicelluloses have classical structures with a backbone of β-(1-4)-linked xylosyl residues substituted with arabinose at C-2 and C-3 of the main chain, whereas the difference occurs in the distribution of branches along the xylan backbone.

ACKNOWLEDGMENTS

The author thanks Prof. R.B. Nigam (Ex-Director, National Sugar Institute, India) for providing the sugarcane bagasse sample and Ron Janzon (vTI, Hamburg) for steam pretreatment facility.

REFERENCES CITED

Banerjee, P.N., .Pranovich, A., Dax, D., Stefan Willfor ((2014). Non-Cellulosic heteropolysaccharide from sugarcane bagasse - Sequential extraction with pressurized hot water and alkaline peroxide at different temperatures, *Bioresource Technology*, vol – 155, 446-450

Banerjee, P.N. (2014). Characterisation of Non-Cellulosic heteropolysaccharide from sugarcane bagasse - Sequential extraction with Steam pretreatment and alkali, *International journal of Science and Research*, 3(9), 953-955

Banerjee, P.N. (2014). Comparative analysis of sugarcane bagasse using different methods, *International Journal of Scientific and Engineering Research*, 5(9), 953-955

Banerjee, P.N., and S. Bhatt (2007). *Structural studies of a new acidic polysaccharide of apricot seeds, Natural Product Research,* 21(6), 507 – 514

Iiyama, K., Wallis, A.F.A. W (1998). Wood Science & Technology, 22, 271-280

Jacobsen, S. E. and Wyman, C. E., Xylose monomer and oligomer yields for uncatalyzed hydrolysis of sugarcane bagasse hemicelluloses at varying solids concentration (2002). Ind. Eng. Chem. Res., 41,1454-1461

Pandey, A., Soccol, C.R., Nigam, P., & Soccol, V.T. (2000). Biotechnological potential of agro-industrial residues: Sugarcane bagasse. *Bioresource Technology*, 74(1), 69-80.

Pandey, K.K.(1999). A study of chemical structure of soft and hardwood polymers by FT-IR spectroscopy. *J. Appl. Polym. Sci.* 71 (12), 1965–1975.

Pranovich, A., Reunanen, M., Sjöholm, R., Holmbom, B. (2005). Dissolved lignin and other aromatic substances in thermomechanical pulp waters. *J. Wood Chem. Technol*. 25, 109–132.

Rodrigues, R.C.L.B., Felipe, M.G.A., Sil, J.B.A., & Vitolo, M. (2003).Response surface methodology for xylitol production from sugarcane bagasse hemicellulosic hydrolyzate using controlled vacuum evaporation process variables. *Process Biochemistry*, 38, 1231-1237.

Reed. (1995). Data evaluation for unified multi-detector size exclusion chromatography — molar mass, viscosity and radius of gyration distributions, 196(5), 1539-1575.

Standford, P.A., Conrad, H.E. (1966). The structure of the aerobacter aerogenes (A3 S1) polysaccharide. I. A Reexamination of the improved procedure for methylation analysis. Biochemistry, 5, 1508.

Sundberg, A., Sundberg, K., Lillandt, C., & Holmbom, B. (1996). Determination of hemicelluloses and pectin in wood and pulp fibres by acid methanolysis and gas chromatography. *Nordic Pulp and Paper Research Journal*, 11(4), 216-219.

Article submitted: September 23, 2014; Peer review completed: October 28, 2014; Revised version received and accepted: November 18, 2014; Published: December 25, 2014.