Characterisation of Non-Cellulosic Heteropolysaccharide from Sugarcane Bagasse - Sequential Extraction with Steam Pretreatment and Alkali

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Abstract: ***Two-stage treatments of sugarcane bagasse with steam and alkaline peroxide were performed and the hemicelluloses extracted were characterized by chemical methods, SEC-MALLS, FT-IR and ^{13}C NMR. The hemicellulose from steam pretreatment was found to contain galacto-arabinoxylans while alkaline peroxide extraction yielded predominately linear arabinoxylans.The present studyshowed 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: Alkaline peroxide extraction, Arabinoxylans, Degree of branching, Steam pretreatment, Sugarcane bagasse

1. Introduction

In the recent years agro-industrial residues are gaining potential interest as raw materials for industrial applications. 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 the sugar industry. About 54 million dry tons of bagasse is produced annually throughout the world (Rodrigues etal., 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. Thus, in view of large availability of sugarcane bagasse and gaining importance of hemicelluloses in different industrial sectors like pharmaceuticals, packaging, paint etc, it would be of great interest to isolate and characterize hemicelluloses from different biomasses in a biorefinery concept.

2. Experimental

2.1 Isolation of the Hemicelluloses

The ground sample was extracted with ethanol and toluene (2:1) in accordance with Tappi Method T204 om-88. Extractive free sugarcane bagasse was then subjected to steam pretreatment at 200ºC for 10 min. The extract was concentrated to one-third of its volume at 40 ºC under reduced pressure and precipitated in 95% EtOH, washed first with acetone and then with MTBE. The residue left after steam pretreatment was subjected to alkaline peroxide treatment at 40ºC for 12 hours, filtered, neutralized to pH 6.0 by 6M HCl, concentrated and precipitated in 95%EtOH. The precipitate was first washed with acetone and then with MTBE. The precipitate from steam pretreatment ($\mathbf{H_{ST}}$) and from alkaline peroxide treatment ($\mathbf{H_{AP}}$) was dried under vacuum at 40ºC for 10 hours.

2.2Characterisation of the Hemicelluloses

Monosaccharide analysis was carried out by methanolysis followed by GC according to Sundbergetal (1996); Banerjeeetal (2007, 2014).Molecular weight was determined by HPSEC using RI as well as MALLS detector and the results were analyzed using software provided by Wyatt Technology Corporation.Lignin was analysed by the ACBr method as described by Iiyamaetal(1998).^{1}H and ^{13}C NMR was recorded on a 600 MHz Bruker advance at 70ºC in D_2O. FT-IR was recorded in Bruker spectrophotometer in the range of 4000 – 400 cm^{-1}.

3. Results and Discussion

The sugar composition of $\mathbf{H_{ST}}$ and $\mathbf{H_{AP}}$are shown in Table 2.Evidently, xylose, arabinose and glucose are the major sugar components in $\mathbf{H_{ST}}$while $\mathbf{H_{AP}}$contains predominantly xylose, arabinose and 4-O-MeGlcA indicating the presence of arabino(4-O-methyl-D-glucurono)xylans.The arabinose to xylose ratio (Ara/Xyl) in $\mathbf{H_{ST}}$ is 0.48 while in $\mathbf{H_{AP}}$ it is0.13. These data indicates that steam pretreatment probably released more branched galactoarabinoxylanand β-glucanwhile linear arabinoxylans were predominantly released during alkaline peroxide treatment.Weight average ($\overline{M}_w$) and number average ($\overline{M}_n$) molecular weights and polydispersity ($\overline{M}_w$ / $\overline{M}_n$) of ($\mathbf{H_{ST}}$) and ($\mathbf{H_{AP}}$) are shown in Table 1.Steam pretreatment released high molarmass hemicellulose with low index of polydispersity indicating a narrow molar mass distribution. Lignin content in ($\mathbf{H_{ST}}$) and ($\mathbf{H_{AP}}$) was found to be 14% and 11% by AcBr method.(data not shown). FT-IR of both ($\mathbf{H_{ST}}$) and ($\mathbf{H_{AP}}$) shows peaks similar to those of typical hemicelluloses. In comparison with spectra of steam pretreated hemicellulose, a much weaker band at the region 1503 to 1515 cm^{-1} demonstrated that the alkaline treatment substantially cleaved the ester and ether bond between lignin and hemicelluloses. The ^{13}C NMR of $\mathbf{H_{AP}}$ shows strong signals at 102.3, 78.0, 77.5, 73.1 and 62.5 ppm, which are, respectively attributed to C-1, C-4, C-3 C-2 and C-5 of (1→4)-linked β-D-Xylp units. The peak at 175.9 is indicative of the carbonyl signal of the esterified ferulic or *p*-coumaric acids in the hemicelluloses. In comparison to ^{13}C NMR spectra of $\mathbf{H_{AP}}$, an extra peak at 17.3 ppm in the ^{13}C NMR spectra of $\mathbf{H_{ST}}$is indicative of acetyl group.

Table 1: Weight average ($\overline{M}_w$) and number average ($\overline{M}_n$) molecular weights and polydispersity ($\overline{M}_w$ / $\overline{M}_n$) of ($\mathbf{H_{ST}}$) and ($\mathbf{H_{AP}}$)

	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$
$\mathbf{H_{ST}}$	9855	7149	1.37
$\mathbf{H_{AP}}$	8284	5451	1.52

Table 2: The composition of sugars (relative % dry hemicelluloses, w/w) in hemicellulosesreleased during steam pretreatment ($\mathbf{H_{ST}}$) and alkaline peroxide treatment($\mathbf{H_{AP}}$)

	Ara	Rha	Xyl	Man	Gal	Glc	GlcA	GlaA	4-O-Me-GlcA
$\mathbf{H_{ST}}$	21.3	0.1	44.0	1.8	2.7	17.1	0.06	0.09	0.25
$\mathbf{H_{AP}}$	9.6	0.18	73.8	0.64	1.08	1.16	1.18	0.51	2.1

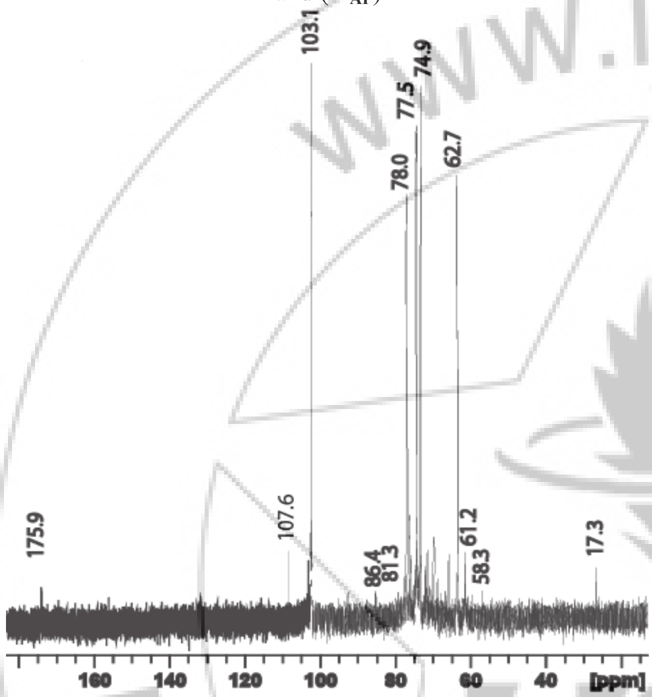

Figure 1: ^{13}C NMR spectra of the hemicellulose isolated by steam pretreatment(H_{ST})

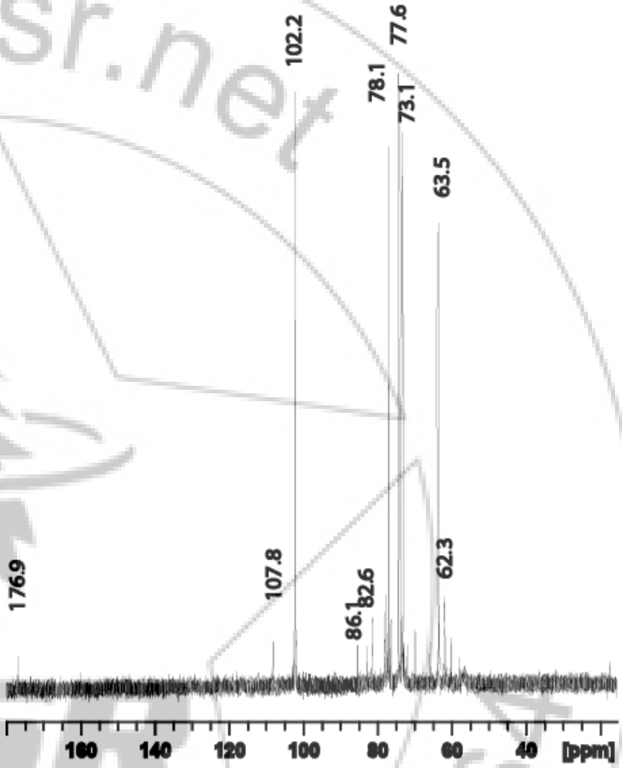

Figure 2: 13CNMRspectra of the hemicellulose extracted withalkaline peroxide (H_{AP})

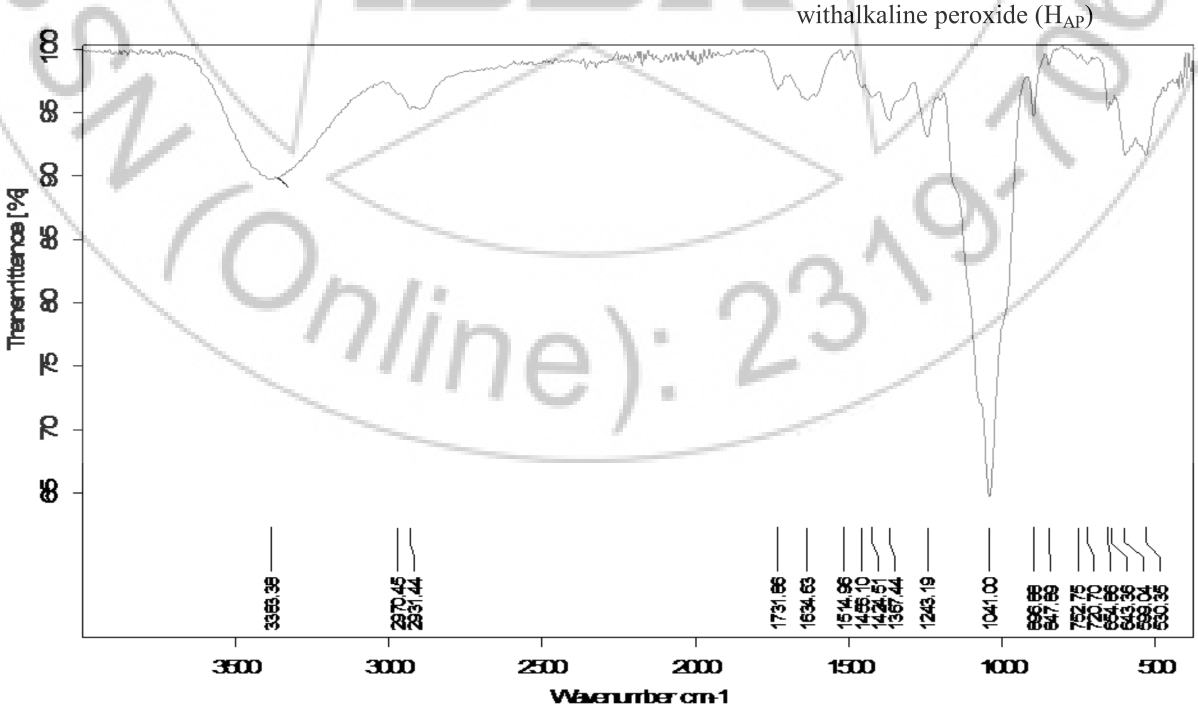

Figure 3: IRspectra of the hemicellulose isolated with steam pretreatment (H_{ST})

4. Conclusion

The present study showed that hemicelluloses with higher molecular weight with more branching can be extracted by steam treatment while more linear hemicelluloses were extracted by alkaline treatment. Based on the sugar composition, FT-IR and ^{13}C NMR, 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 differences occurs in the distribution of branches along the xylan backbone

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