Effects of Extraction Conditions and Alkali Type on Yield and Composition of Wheat Straw Hemicellulose

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SYNOPSIS

The utilization of various alkaline regimes for the optimal extraction and isolation of hemicellulose and cellulose from wheat straw was extensively examined. Factors investigated include varying concentrations of one alkali (KOH) and H_3BO_3, varying temperature and time of extraction, and varying the nature of the alkali: calcium hydroxide, sodium hydroxide, lithium hydroxide, and liquid ammonia were examined in this context. For example, a preferred extraction of hemicellulose from holocellulose preparations utilized a solution of 24% KOH/2% H_3BO_3 at 20°C for 2 h. This produced yields for hemicellulose and cellulose of 34.23 and 35.96%, respectively. The neutral sugar composition of the various hemicellulose fractions was found to vary slightly with treatment regime. In all hydrolysates of hemicellulose preparations, xylose was by far the predominant sugar, comprising around 80% of the material. Minor constituents were arabinose, galactose, glucose, and uronic acids. The composition of phenolic acids and aldehydes in extracted wheat straw hemicellulose was also studied. The average molecular weights of the hemicellulose isolates ranged from 12,000 for the 30% KOH/2% H_3BO_3 (20°C, 2 h) extract to 27,000 for the extract obtained using 5% KOH/2% H_3BO_3 (20°C, 2 h). © 1996 John Wiley & Sons, Inc.

INTRODUCTION

Wheat straw contains large amounts of cell wall polysaccharides and lignin.[1-3] Cellulose, hemicellulose, and pectin are the major structural polysaccharides. Hemicellulose ranks second to cellulose in abundance in wheat straw and its content changes with growth and maturity.[4] In addition, hemicellulose, which is rich in a number of neutral sugars, is a promising source of xylose, the predominant sugar, accounting for 80% of the monosaccharides and can readily be modified to give xylitol, a sugar substitute.[5] Hemicellulose, however, is the most complex of the fiber components of the straws and needs improved isolation procedures and detailed characterization.[6]

Hemicelluloses are heteropolysaccharides, made up of at least two to four different types of sugar residues depending upon the source.[7] For example, the lignified tissues of grasses and straws yield, on alkaline extraction after delignification, hemicelluloses containing D-xylose residues together with 5–10% of L-arabinose residues. In some hemicelluloses D-glucuronic acid residues are also present.[8]

In continuing studies of the polysaccharides from wheat straw, the hemicellulose is discussed in particular. Extraction of hemicellulose by potassium or sodium hydroxide has been investigated in some detail, but relatively little is known about the precise nature of hemicellulose extracted using different conditions and different alkalis. The objective of this study was to examine the effects of alkali extraction conditions, such as time, concentration, tempera-

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ture, and various alkali types, on the yield of wheat straw hemicellulose and its neutral sugar composition. The content of uronic acids in hemicellulose fractions and their molecular weights were also estimated.

EXPERIMENTAL

Materials

Wheat straw was obtained from Silsoe Research Institute (Silsoe, Bedfordshire) and was ground using a Christie Laboratory mill to a 60-mesh size screen. The ground straw was then dried in a cabinet oven with air circulation for 16 h at 60°C and stored at 5°C until use. All chemicals were of analytical or reagent grade. All experiments were conducted in duplicate and all weights and yields are given on a dry weight basis.

Determination of Moisture, Ash, and Protein

Moisture was determined by drying ground wheat straw in an air circulated oven at 105°C for 16 h. Ash content was measured by incinerating the sample for 5 h in a muffle furnace at 550°C. Proteins in wheat straw were measured by a Kjeldahl procedure.[9] A protein conversion factor of 6.25 was used.

Preparation of Wheat Straw Holocellulose

Dried wheat straw was defatted by refluxing with chloroform for 5 h in a Soxhlet apparatus. After drying, the defatted wheat straw was suspended in 0.1*M* phosphate buffer (KH_2PO_4—NaOH), pH 7.5, containing 0.02% sodium azide as a bacteriocide. Proteolysis was then started by the addition of trypsin (2.5 mg/g wheat straw). The suspension was incubated for 6 h at 40°C with continuous stirring. After filtration and washing with water, ethanol, and ether, the residue was gently boiled in distilled water (30 g/L) for 2 h, then filtered and washed. The treatment was repeated for another 2 h with further distilled water (30 g/L). The residue was recovered by filtration, extensively washed with water, and dried by solvent exchange through ethanol and diethyl ether. Pectic substances were isolated from the extracted straw using 0.25% ammonium oxalate solution for 4 h at 85°C. After filtration and drying, 80% of the ethanol (1.5 g straw/100 mL extractant) was added to the straw remaining and the mixture boiled gently under reflux for 3 h. The residue was recovered by filtration, washed twice with 80% ethanol and water, dried for 16 h at 60°C, and reweighed. The weight lost was defined as the 80% hot ethanol soluble materials (Fig. 1).

The 80% ethanol extracted straw sample was delignified according to the method described by Bagby et al.,[10] Collings et al.,[11] and Asensio and Seoane.[12] The residue extracted from the above procedures (3.00 g) was stirred with water (150 mL) and 10% acetic acid (10 mL), and delignified with sodium chlorite ($NaClO_2$, 5.0 g) in a flask with stirring. The mixture was heated for 1 h at 75°C, more acid (5 mL) and sodium chlorite (2.5 g) were then added, and the mixture heated for another hour. After 2 h of oxidation, the residue was filtered out on a nylon cloth and washed with water (three times), 96% ethanol (twice), and ether (once), then dried at 60°C for 16 h and reweighed. The difference in weight was defined as "sodium chlorite lignin." The supernatant was concentrated on a rotary evaporator under reduced pressure at 40°C. The dried supernatant was kept in a refrigerator at 5°C until analysis of polysaccharides solubilized during delignification was carried out.

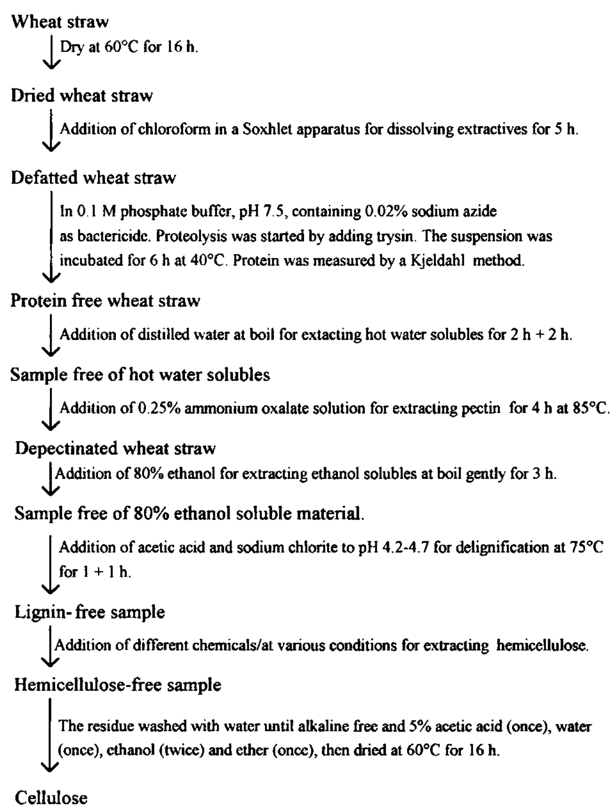

Figure 1 Scheme for extraction and isolation of hemicellulose from wheat straw.

Extraction of Hemicellulose from Holocellulose

The optimization of the yield of hemicellulose from wheat straw holocellulose required the systematic evaluation of a range of alkaline extraction regimes. These are detailed below.

The holocellulose was extracted (1.2 g holocellulose/100 mL extractant):

1. with 10% potassium hydroxide and 2% boric acid at 20°C in air for 4, 8, 12, 16, 21, and 26 h, respectively;
2. with 5, 10, 15, 20, 24, and 30% potassium hydroxide and 2% boric acid at 20°C for 2 h, respectively;
3. with 24% potassium hydroxide and 2% boric acid for 2 h at 0, 10, 20, 35, and 50°C;
4. using 24% potassium hydroxide without boric acid and with 1, 2, 5, 8, and 12% boric acid for 2 h at 20°C;
5. using 15% liquid ammonia, 15% calcium hydroxide, 15% potassium hydroxide, 15% sodium hydroxide, and 15% lithium hydroxide with 2% boric acid at 20°C for 2 h, respectively;
6. using 1*M* calcium hydroxide, 1*M* liquid ammonia, 1*M* potassium hydroxide, 1*M* sodium hydroxide, and 1*M* lithium hydroxide with 0.2*M* boric acid for 2 h at 20°C.

After filtration on a nylon cloth, the residues were recovered, washed three times with water, once with 5% acetic acid, once with distilled water, twice with 96% ethanol, and once with ether, then dried in an oven for 16 h at 60°C and reweighed. The weight lost was defined as hemicellulose. The weight of the residue that remained after the alkaline extraction, corrected for ash content, was taken as "cellulose." After the supernatant was neutralized with dilute acetic acid, it was concentrated on a rotary evaporator under reduced pressure at 40°C, and hemicellulose was slowly precipitated with 5 vol of 96% ethanol for 24 h at 20°C. After filtration and drying in an air circulated oven for 16 h at 60°C, the resultant white hemicellulose fraction was kept in a refrigerator until analysis.

Sugar Analysis by Gas Chromatography

See our previous reports.[13,14]

Uronic Acid Measurement by Spectrophotometry

Total uronic acids were assayed colorimetrically as glucuronic acid in hemicellulose using 3-phenylphenol color reagent according to the procedure outlined by Blumenkrantz and Asboe–Hanson[15] with a modification by Wedig and coworkers.[16] A Hewlett–Packard Diode Array 8452A spectrophotometer was used to measure glucuronic acid at a wavelength of 520 nm.

Average Molecular Weight Estimation by Gel Permeation Chromatography (GPC)

The hemicellulose (0.1 g) was dissolved in 0.005*M* sodium phosphate buffer, pH 7.5 (100 mL), containing 0.02*N* NaCl. The solution was chromatographed on a PL aquagel-OH 50 column (300 × 7.7 mm, Polymer Laboratories Ltd.), calibrated with PL pullulan polysaccharide standards (peak average molecular weights 667, 5,800, 12,200, 23,700, 48,000, 100,000, 186,000, and 386,000, Polymer Laboratories Ltd.), and eluted with 0.02*N* NaCl in the same buffer above at 0.1 mL/min. Eluate was detected using a Knauer differential refractometer. The column oven was maintained at 30°C.

Nitrobenzene Oxidation of Lignin in Extracted Wheat Straw Hemicellulose

The method for alkaline nitrobenzene oxidation of lignin remaining attached to or associated with hemicellulosic fractions and cellulose was as described previously.[17]

Infrared Spectroscopy for Extracted Hemicellulose

The various extracted hemicelluloses were thoroughly ground and pressed into potassium bromide (KBr) pellets. Spectra were run on a Mattson FTIR instrument.

RESULTS AND DISCUSSION

Moisture, Ash, Protein, and Extractives Contents and Fractional Yields of Polysaccharides and Lignin

Moisture, ash, protein, and extractive contents in wheat straw were determined at 9.18, 7.93, 1.73, and 1.65%, respectively. The ash content was high, presumably due to soil contamination.[18] The yields of water soluble polysaccharides during proteolysis and boiling water extraction were 2.92 and 4.76%, respectively.[17] The contents of pectin, 80% ethanol soluble materials, and lignin were 1.20, 1.27, and 14.13% (sodium chlorite lignin) of dry straw, respectively. Hemicellulose and cellulose are predominant components, which comprise about 70% of the dry straw. More details of the neutral sugar composition of water soluble polysaccharides obtained during proteolysis, boiling water extraction, and delignification, and in pectin and 80% ethanol soluble materials, are given elsewhere.[17,19]

Effects of Time and Temperature of Extraction, Concentrations of KOH and H_3BO_3, and Other Alkalis on Yields of Hemicellulose and Cellulose

The variation of hemicellulose and cellulose yields with extraction time using 10% potassium hydroxide and 2% boric acid at 20°C is shown in Figure 2. It can be seen that most hemicellulose was removed in the early part of the extraction procedure. The yield of hemicellulose was 31.80% using 10% KOH and 2% H_3BO_3 at 20°C for 4 h; this only increased to 34.22% after 26-h extraction. Therefore, more than 90% of the hemicellulose was solubilized during the first 4-h extraction; less than 10% further hemicellulose was removed during the subsequent prolonged extraction period up to 22 h.

Figure 3 shows the different yields of hemicelluloses and celluloses, depending on the potassium hydroxide concentration, at 20°C for 2-h extraction with 2% boric acid, plotting the yield versus potassium hydroxide concentration for extraction at 5, 10, 15, 20, 24, and 30% (w/v). As expected, at lower concentrations of KOH, the extracting yield increased quickly with potassium hydroxide concentration until a constant value was reached due to the depletion of remaining hemicellulose in the sample. These values were approximately 27.79, 32.57, 33.57, 34.21, 34.23, and 34.93% at the concentrations of 5, 10, 15, 20, 24, and 30% of KOH, respectively. If the extraction was to continue, one could assume that the yield of hemicellulose would give nearly the same value. In contrast to hemicellulose, the yield of cellulose was dramatically decreased at the low concentration of KOH and then dropped slowly to a constant value. The results obtained coincided with Wise and coworkers' study.[20] They demonstrated that, in general, the yield of hemicellulose obtained depended upon the alkaline strength of the extractant with alkaline solutions up to 10% concentration. Only small increases in yield were noted at high concentrations.

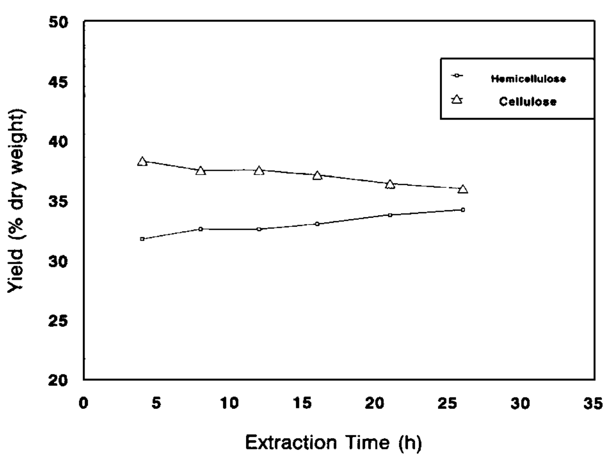

Figure 2 The effect of extracting time on the yield of hemicellulose and cellulose extracted using 10% KOH and 2% H_3BO_3 at 20°C.

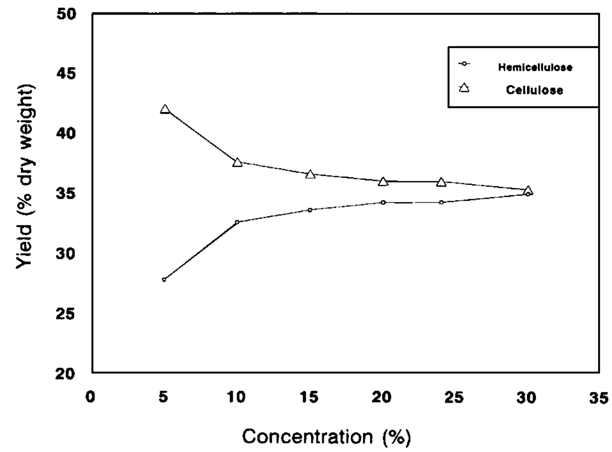

Figure 3 The effect of KOH concentration on the extraction yield of wheat straw hemicellulose and cellulose at 20°C for 2-h extraction with 2% H_3BO_3.

The yields of hemicellulose and cellulose extracted from wheat straw holocellulose using 24% potassium hydroxide with 2% boric acid for 2 h are plotted in Figure 4 against the extracting temperatures of 0, 10, 20, 35, and 50°C. The yields of hemicellulose were 31.01, 33.43, 34.23, 35.85, and 36.54% at the respective temperatures. The hemicellulose has a finite solubility in cold alkaline solutions, but, presumably, warm alkaline solutions lead to enhanced hemicellulose degradation; above 50°C further extraction occurred. The yield of cellulose decreased from 39.05 to 33.76% as the temperature increased from 0 to 50°C.

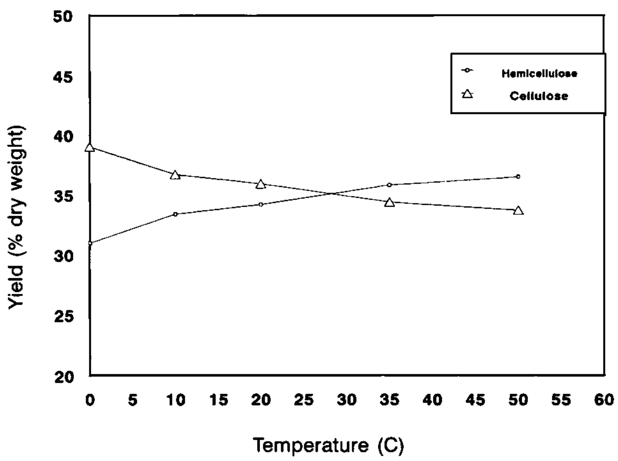

Figure 4 The effect of extracting temperature on the yield of wheat straw hemicellulose and cellulose with 24% KOH and 2% H_3BO_3 at 20°C for 2 h.

Hemicelluloses can be extracted from holocellulose by alkali; but a proportion of them, particularly the glucomannans, are exceedingly resistant to extraction. The addition of boric acid or borates to potassium hydroxide increases the dissolving power for glucomannans, mainly due to the formation of borate complexes with hydroxyl groups in the cis position.[21,22] The effect of boric acid concentration on the extraction yield of hemicellulose (20°C for 2 h) with 24% potassium hydroxide is shown in Figure 5. Significant effects can be seen. At the lower concentrations of boric acid, the yield of hemicellulose increased with acid concentration; this was particularly enhanced with the increase in [H_3BO_3] from 1 to 2%. Above 5%, the yield of hemicellulose decreased from 35.0 to 33.74% at 12% boric acid. This is partly ascribed to the effective neutralization of alkali as boric acid concentration increased to significant levels. The "optimum extraction concentration" of boric acid is therefore either 2 or 5%, depending upon which of the following criteria are considered most important: consumption of boric acid or total hemicellulose yield.

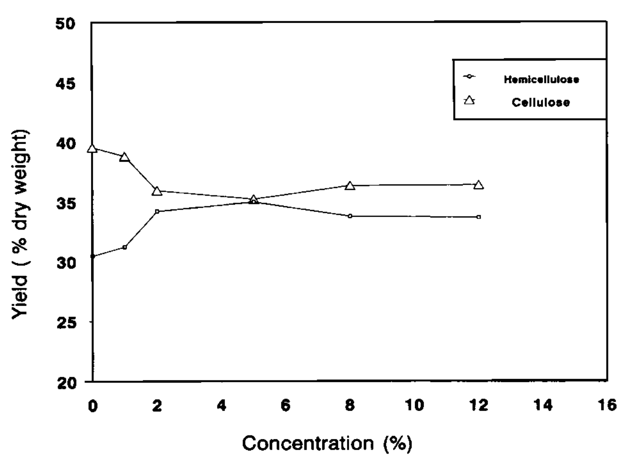

Figure 5 The effect of H_3BO_3 concentration on the extraction yield of wheat straw hemicellulose and cellulose at 20°C for 2-h extraction with 24% KOH.

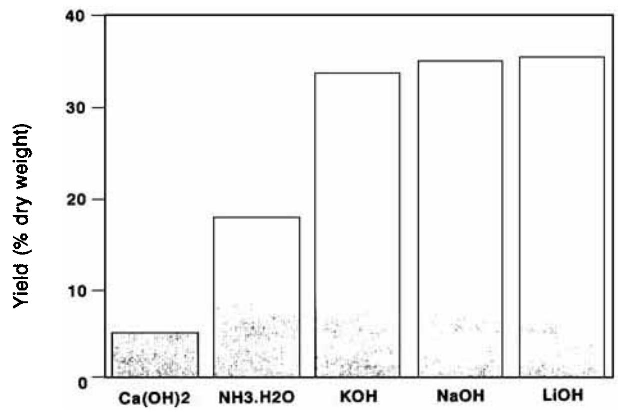

Figure 6 The effect of different alkalis on the extraction yield of wheat straw hemicellulose with 15% of each alkali and 2% H_3BO_3 at 20°C for 2 h.

Figures 6 and 7 clearly show wide variations in the "hemicellulose dissolving power" of the various alkalis at equivalent concentrations. The yields of hemicelluloses extracted with 15% $Ca(OH)_2$, $NH_3 \cdot H_2O$, KOH, NaOH, and LiOH at 20°C for 2 h were 5.46, 17.84, 33.59, 34.80, and 35.08%, respectively. In a comparison of the extracting power of 1*M* solutions of potassium, sodium, and lithium hydroxide, an approximately equal effect of all three hydroxides was found. This is in agreement with

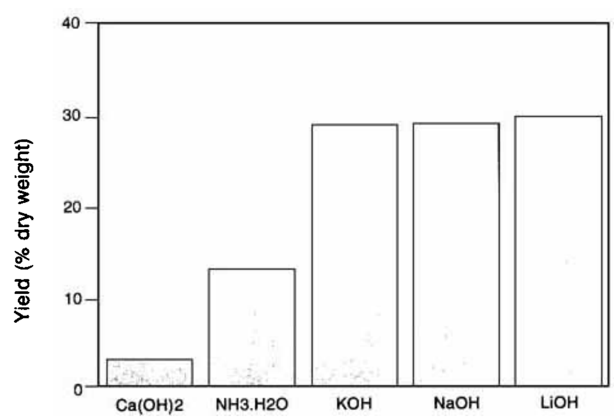

Figure 7 The effect of different alkalis on the extraction yield of wheat straw hemicellulose with 1*M* of each alkali and 0.2*M* H_3BO_3 solution at 20°C for 2 h.

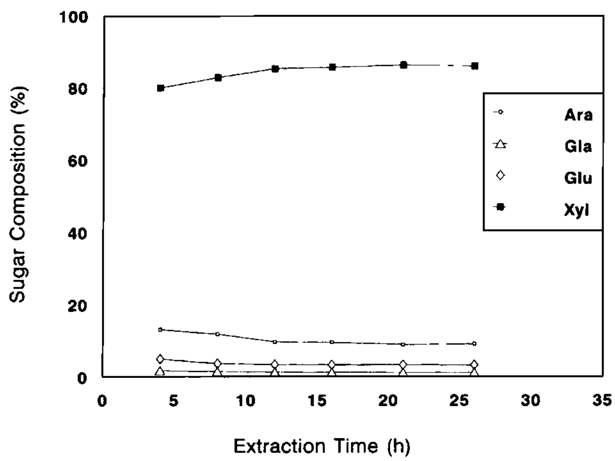

Figure 8 The effect of extracting time on the sugar composition (relative percent) of hemicellulose extracted with 10% KOH and 2% H_3BO_3 at 20°C from wheat straw.

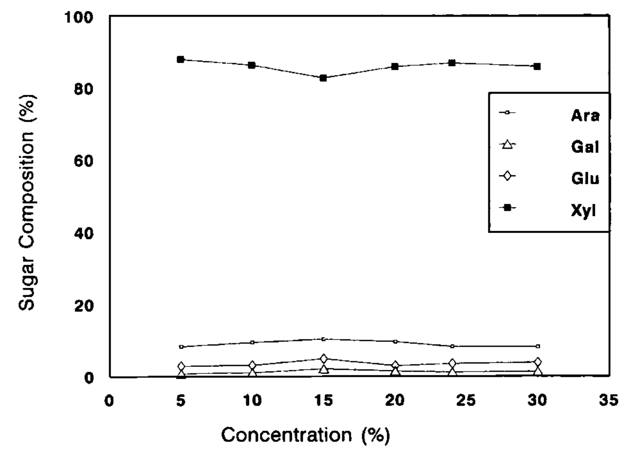

Figure 9 The effect of KOH concentration on the sugar composition (relative percent) of hemicellulose extracted from wheat straw at 20°C with 2% H_3BO_3.

studies of the extraction of xylans from softwood.[21] At equal concentrations, sodium hydroxide and lithium hydroxide are more effective than potassium hydroxide for the removal of hemicellulose. However, the preferred hydroxide is still potassium hydroxide, mainly because the potassium acetate formed during the neutralization of the alkali extract is more soluble in the alcohol used for precipitation than sodium acetate.[21] The yields of hemicellulose obtained using calcium hydroxide and liquid ammonia were markedly lower than for the alkali metal hydroxide solutions. In general, an increase in the concentrations of the various alkalis resulted in increased hemicellulose yields.

The studies demonstrated that the yield of hemicellulose depends strongly on a number of factors. These include concentration and type of alkali, concentration of boric acid, and temperature and time of extraction.

Effects of Time and Temperature of Extraction, Concentrations of KOH and H_3BO_3, and Various Alkalis on Sugar Compositions of Hemicellulose

Hemicellulose can be found in many structures and compositions. The most abundant in wheat straw are the arabinoxylans, which are heteropolymers of xylose units, linked by the $\beta(1 \rightarrow 4)$ glycosidic bond and arabinose residues linked to the main chain.[23] The relative sugar composition of hemicellulose fractions after hydrolysis are presented in Figures 8–11. The sugar analysis of hydrolysates showed that xylose was a predominant component sugar in wheat straw hemicellulose and that it comprised more than 80% of the total sugars in the material. Arabinose, galactose, and glucose were detected as minor sugar constituents of the hemicellulose. Mannose was also detected in trace amounts. These results accord with those of Hatfield's,[24] in which he stated that in grasses most of the xylose and arabinose would be found specifically in hemicellulose, because pectic polysaccharides are present in relatively small amounts.

Inspection of Figure 8 shows that no significant differences in neutral sugar contents were observed for the extractions at different periods with 10% KOH and 2% H_3BO_3. This suggests that extraction time does not affect the nature of the solubilized

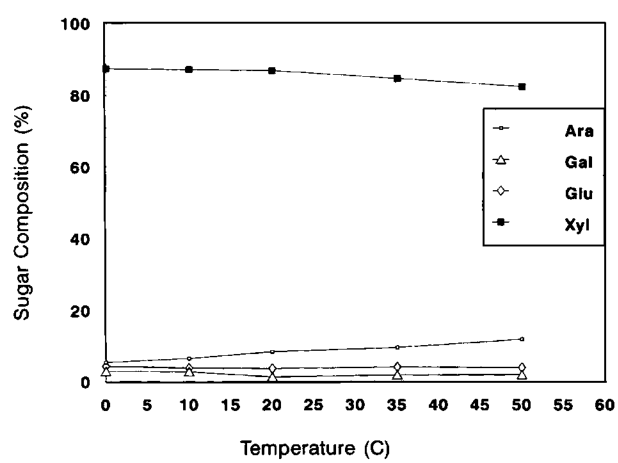

Figure 10 The effect of extracting temperature on the sugar composition (relative percent) of hemicellulose extracted with 24% KOH and 2% H_3BO_3 for 2 h from wheat straw.

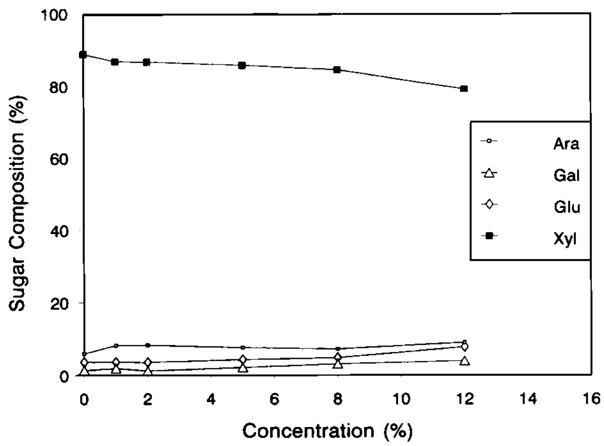

Figure 11 The effect of H_3BO_3 concentration on the sugar composition (relative percent) of hemicellulose extracted with 24% KOH at 20°C from wheat straw.

polysaccharides to a great extent. The yield of xylose was increased from 80.00 to 86.12% with the increase of extraction time from 4 to 26 h. The yields of arabinose, glucose, and galactose decreased from 13.18 to 9.23%, 5.00 to 3.33%, and 1.78 to 1.23%, respectively, with an increase in extraction time from 4 to 26 h. The data indicated that arabinose, glucose, and galactose were easily removed at lower extraction times.

The effect of increasing potassium hydroxide concentration from 5 to 30% on the neutral sugar composition of the hemicellulose fraction is shown in Figure 9. Inspection of this figure shows that, in general, the relative amount of xylose present in hemicellulose decreases as the KOH concentration is increased from 5 to 30%. Yields of arabinose, glucose, and galactose increase accordingly. The molar ratios of xylose : arabinose : galactose : glucose in hemicellulose extracted with 24% KOH and 2% H_3BO_3 for 2 h at 20°C were 58 : 5.6 : 0.7 : 2.0. It is interesting to note that a significant dip in the xylose yield curve occurs at a [KOH] of around 15%, with an obvious concomitant increase in relative yields of the other sugars. A hemicellulose fraction rich in arabinose, glucose, and galactose can hence be isolated using a specific extraction regime, nominally 15% KOH/2% boric acid at 20°C.

In Figure 10, the relative sugar compositions of hemicellulose extracted with 24% potassium hydroxide and 2% boric acid for 2 h are plotted against extracting temperatures. It is apparent that the amount of arabinose occurring in extracts increases significantly from 5.42 to 11.92% as the temperature increases from 0 to 50°C.

The relative content of sugars in the hydrolysates of hemicelluloses extracted with 24% potassium hydroxide and various concentrations of boric acid, at 20°C for 2 h, are plotted in Figure 11 as a function of the concentration of boric acid. As expected, as the boric acid concentration increased, the content of xylose dropped significantly from 88.87 to 79.29%. The contents of arabinose and galactose increased from 6.03 to 8.28% and 1.43 to 1.97%, respectively, in the boric acid free and 1% boric acid extraction media. Relative glucose content was generally noted to increase with the increase of boric acid concentration.

The relative neutral sugar compositions of hemicellulose extracted by the various alkalis with 2% boric acid at 20°C for 2 h (Table I) was very similar. In all cases xylose was the major component and arabinose, glucose, and galactose were the minor component sugars. The only significant difference between the hemicellulose fractions was that the content of xylose was relatively low, and that of ar-

Table I Sugar Composition (Relative %) of Hemicellulose

Chemical Concn	Ara	Xyl	Man	Gal	Glu
15% $Ca(OH)_2$ + 2% H_3BO_3	15.6	77.3		1.9	4.3
1*M* $Ca(OH)_2$ + 0.2*M* H_3BO_3	14.7	76.8		2.6	6.0
15% NH_3 + 2% H_3BO_3	7.4	87.9		1.3	3.3
1*M* NH_3 + 0.2*M* H_3BO_3	7.4	87.8		1.4	3.4
15% KOH + 2% H_3BO_3	10.4	82.6		2.2	5.0
1*M* KOH + 0.2*M* H_3BO_3	10.9	83.6		1.3	3.8
15% NaOH + 2% H_3BO_3	7.5	86.4	Tr	2.1	4.0
1*M* NaOH + 0.2*M* H_3BO_3	7.0	86.6		2.2	4.3
15% LiOH + 2% H_3BO_3	7.8	87.0	Tr	2.2	3.9
1*M* LiOH + 0.2*M* H_3BO_3	8.2	87.0		1.4	3.4

Tr, trace. Hemicellulose extracted from wheat straw holocellulose using various alkalis at 20°C for 2 h.

Table II Content (%) of Uronic Acids and Average Molecular Weight (M_w) of Wheat Straw Hemicellulose Fractions

Concn KOH (%)	Uronic Acids (%)	M_w
5	7.2	27,000
10	5.8	20,000
15	4.2	20,000
20	3.9	20,000
24	3.1	12,000
30	3.1	12,000

Fractions extracted using different concentrations of KOH at 20°C for 2 h with 2% H_3BO_3.

abinose high in the calcium hydroxide extract hemicellulose compared with other alkali media. The molar ratios of xylose : arabinose : galactose : glucose in 15% $Ca(OH)_2$ and 2% H_3BO_3 extracted hemicellulose were 51 : 10 : 1.1 : 2.4; in 15% Li(OH) and 2% H_3BO_3 extracts the corresponding values were 58 : 5.2 : 1.2 : 2.2, respectively. These results pointed to the presence of mainly xylan or arabinoxylan. In 1993 Fidalgo and coworkers[25] showed that wheat straw hemicellulose contains an arabinoxylan and that a percentage of arabinose units were linked to lignin. In addition, they revealed that in the alkali lignin fraction the percentage of arabinose units linked to lignin (as percent of total arabinose) was higher than the percentage of linked xylose. This is in agreement with the findings of Liu et al.[26] There is a certain amount of ester bounding between phenolic components of lignin and xylose, arabinose, and uronic acids in the heteroxylans of hemicellulose[27]; the amount of this bounding appears to increase with plant maturity.[28] Lignin inhibits digestion of hemicellulose by steric hindrance as well as by direct bonding to hemicellulose.[6,29] Overall, xylose is the most resistant chemical constituent of hemicellulose to alkali extraction.

Contents of Uronic Acids and Average Molecular Weights of Hemicellulose

The content of uronic acids and average molecular weights of hemicellulose fractions extracted using different concentrations of KOH at 20°C for 2 h with 2% H_3BO_3 are shown in Table II. Although there is a small component of uronic acids in hemicellulose, significant differences appeared at different concentrations of KOH. An increase of KOH concentration from 5 to 24%, led to a 4% decrease in uronic acid content in resultant hemicellulose fractions.

The GPC determined ranges of molecular weights of wheat straw hemicellulose extracted using 5, 15, and 24% KOH at 20°C for 2 h with 2% H_3BO_3 are shown in Figure 12. The mean molecular weights (M_w) of hemicelluloses extracted using 5, 15, and 24% KOH with 2% H_3BO_3 at 20°C for 2 h were 27,000, 20,000, and 12,000, respectively. This result is in approximate agreement with Aspinall and colleague's study.[8] They illustrated that a molecular weight determination by the isothermal distillation method gave a value of 8,000–11,800 ± 400 (degree of polymerization 47–76) for methylated wheat straw xylan. Wegener[21] stated that extraction with dilute alkali solutions (e.g., 5% KOH) removed the more soluble xylans and galactoglucomannans, while most of the glucomannan can be removed only with higher alkali concentrations of 16 or 24% potassium hydroxide or 17.5% sodium hydroxide, which is in accordance with our experimental results. With the increase of alkali concentration from 5 to 24%, the average molecular weights decreased from 27,000 to 12,000. According to Whistler and coworkers' study[30] in early 1948, weak alkaline solutions generally solubilize hemicellulose B, the more acidic and/or branched portion, to a greater extent than hemicellulose A, which is more linear and less acidic in nature. Therefore, hemicellulose B can be more or less selectively extracted from plant material with very weak alkaline solutions, such as saturated lime water or low percentage potassium hydroxide solution (e.g., 5%). Forty years later, Wen et al.[8] studied the iso-

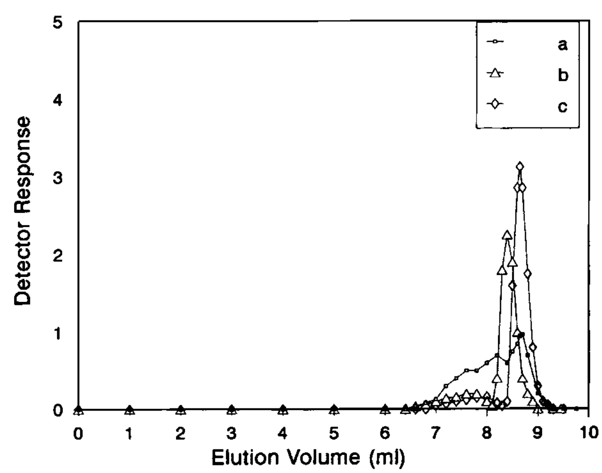

Figure 12 The range of the molecular weight of wheat straw hemicellulose extracted using (a) 5% KOH, (b) 15% KOH, and (c) 24% KOH at 20°C for 2 h with 2% H_3BO_3.

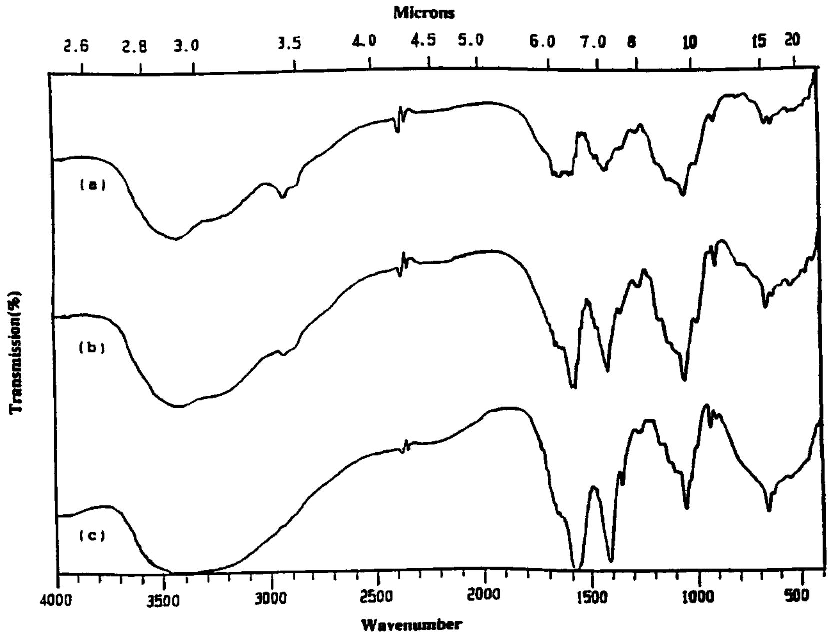

Figure 13 FTIR spectra of wheat straw hemicellulose extracted with (a) 5% KOH and 2% H_3BO_3, (b) 10% KOH and 2% H_3BO_3, and (c) 24% KOH and 2% H_3BO_3 at 20°C for 2 h.

lation and characterization of hemicellulose from sugar beet pulp and indicated that apparent molecular weight profiles of hemicellulose A and B showed two major carbohydrate peaks. The first one had a molecular weight equal or greater than 150,000 D. The second major carbohydrate peak had a molecular weight of 40,000 D. Furthermore, they also mentioned that the elution profiles of hemicellulose B extracted with 5% NaOH was similar to that extracted with 10% NaOH; but the first peak area was larger with 5% NaOH extraction than that for 10% NaOH, and there was less tailing of the peak. Hence, they concluded that higher concentrations of NaOH caused fragmentation of hemicellulose B. This result is in agreement with our experimental data that shows that the average molecular weights of hemicellulose decreased from 27,000 to 12,000 with increase of extraction concentration of KOH from 5 to 30%, mainly due to the fragmentation at higher KOH concentrations.

Neutral Sugar Composition of Cellulose

It is evident that α cellulose is contaminated with hemicellulose and pectic substances that have not been extracted during the previous fractionation procedures. Treatment with 72% H_2SO_4 (2 h, 20°C) and 3% H_2SO_4 (6 h, 100°C) hydrolyzed the "cellulose," producing a neutral sugar composition (relative percent) of arabinose 2.8, xylose 7.3, galactose 1.1, and glucose 88.8, with trace amounts of mannose. The resistance to extraction by 24% KOH suggests that hemicellulose and pectic substances are very strongly associated with the cellulose.

According to the model proposed by Preston,[31] hemicellulose and cellulose are closely connected and the cellulose mirofibrils are coated with hemicellulose polymers. Studies of sycamore suspension cell wall by Darvill et al.[32] suggested that the neutral and acidic pectic polysaccharides were covalently attached to the hemicellulose. In 1986, based on a study of hindrance of hemicellulose and cellulose hydrolysis by pectic substances using pectinase and cellulase, Ben-Shalom[33] confirmed that cellulose and hemicellulose in the cell wall are sterically masked by the pectic substances. This surrounding effect may sterically hinder hemicellulose and cellulose hydrolysis.

Content of Phenolic Acids and Aldehydes of Alkaline Nitrobenzene Oxidation Lignin in Extracted Wheat Straw Hemicellulose

The FTIR spectra of hemicellulosic fractions extracted with 5% KOH and 2% H_3BO_3, 10% KOH and 2% H_3BO_3, and 24% KOH and 2% H_3BO_3 at 20°C for 2 h (Fig. 13) appeared to be rather similar. However, on closer examination of the spectrum of

Table III Composition of Phenolic Acids and Aldehydes in Wheat Straw Hemicellulose

Phenolic Acids and Aldehydes	Free[a] (%)	Bound[b] (%)
Gallic acid	0.2	0.03
Protocatechuic acid		0.008
p-Hydoxybenzoic acid		0.2
p-Hydroxybenzaldehyde		0.02
Vanillic acid		0.2
Vanillin		0.09
Syringaldehyde		0.02
Ferulic acid		0.003
Cinnamic acid		0.03
Total	0.2	0.6

Hemicellulose extracted with 24% KOH and 2% H_3BO_3 at 20°C for 2 h.

[a] Determined by HPLC without alkaline nitrobenzene oxidation.

[b] Determined by HPLC after alkaline nitrobenzene oxidation at 170°C for 2.5 h in steel autoclaves.

the hemicellulosic fraction extracted with 5% KOH and 2% H_3BO_3 at 20°C for 2 h, it can be seen that there are two weak peaks at 1626 and 1562 cm^{-1}, whereas that fraction extracted with 24% KOH and 2% H_3BO_3 at 20°C for 2 h has a strongly flattened single peak at 1562 cm^{-1}. The absorbances around 1626 cm^{-1} is a carbonyl stretching band due to *para*-substituted ketone or aryl aldehydes. The small bands at 1330, 1220, 1155, and 840 cm^{-1} indicated syringl and guaiacyl ring breathing with CO stretching, aromatic CH in-plane deformation, and aromatic C—H out of plane bending vibrations in wheat straw lignin. This figure indicates that the extracted hemicellulosic fractions still contained some residual lignin.

The phenolic composition of the hemicellulosic fraction extracted with 24% KOH and 2% H_3BO_3 at 20°C for 2 h is summarized in Table III. The free and bound phenolic contents in the hemicellulosic fraction were 0.2 and 0.6%, respectively. The major components in bound phenolic acids and aldehydes were found to be *p*-hydroxybenzoic acid and vanillic acid, while the free phenolic content showed higher content of gallic acid. The characterization of wheat straw lignin and lignin–polysaccharide complexes is currently the subject of detailed further study in our laboratory.

SUMMARY

Various procedures for extracting and isolating hemicellulose and cellulose from wheat straw holocellulose were examined. Aqueous solutions of potassium, sodium, and lithium hydroxide are appropriate for isolation of hemicellulose from wheat straw holocellulose, but the preferred alkali was potassium hydroxide. The optimal time for extracting hemicellulose using 10% KOH and 2% H_3BO_3 at 20°C was found to lie between 21 and 26 h. The suitable concentrations of KOH for extracting hemicellulose at 20°C for 2 h were between 20 and 30%, which yielded hemicellulose from 34.21 to 34.93%. The optimum extraction concentration of H_3BO_3 in the extractant of 24% KOH at 20°C for 2 h was either 2 or 5%. The molar ratios of xylose : arabinose : galactose : glucose in 24% KOH and 2% H_3BO_3 for 2 h at 20°C extracted hemicellulose were 58 : 5.6 : 0.7 : 2.0 and in that the content of uronic acid was 3.1%. The average molecular weight of the hemicellulose was about 12,000, and the total content of phenolic acids and aldehydes in hemicellulose extracted with 24% KOH and 2% H_3BO_3 at 20°C for 2 h was 0.8%.

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