Isolation and Characterization of Hemicelluloses of Corn Hulls

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Received 27 December 1994

Hemicelluloses were prepared from hulls by two-step methods using alternating oxidative-alkaline treatments. The hemicellulose fractions comprised arabinoxylans differing in the amount of coextracted starch. The type of the delignification method afftects the extractibility of the arabinoxylan component, but not its sugar composition. The ^{13}C NMR spectra indicate a highly branched β-1,4-D-xylan backbone with α-L-Araf and β-D-Xylp units in terminal position of the side chains. The corn hull arabinoxylan represents a highly viscous xylan type.

The increasing production of corn starch has resulted in large amounts of hulls remaining in the starch mills. Chemical analysis of the hulls showed a significant proportion of arabinoxylan-rich hemicelluloses applicable as food gums [1]. However, they could not compete with the commercial gums. Xylans present in cereal products are well known dietary fibres due to their beneficial physiological effects in human nutrition and health [2, 3]. Recently, water-soluble xylans from various medicinal plants [4, 5] and corn cobs [6] have been reported to have immunomodulating activities. Moreover, xylans represent an important biopolymer source for industrial raw materials [7] and various technical applications [8].

The aim of this study was to investigate the extractibility of the hemicellulose component of industrial corn hulls and the effect of different extraction procedures on the composition and physicochemical properties of the isolated xylan fractions.

EXPERIMENTAL

Materials and Methods

Corn hulls from *Zea mays* L. were obtained as a by-product of the production of starch (Barby, Magdeburg, Germany). The hulls were ground and sieved to yield a material with particle size 0.25 mm. Its characteristics are given in Table 1.

Moisture content was determined by drying the sample at 105 °C to constant mass. Fat was estimated by extraction with a mixture of benzene and ethanol (φ_r = 2 : 1) under reflux for 8 h. Ash was determined as sulfates. Protein was calculated from the nitrogen content w(N) = 6.25 % assayed on the elemental analyzer Model 240 (Perkin—Elmer). The uronic acid content was determined by potentiometric titration [9]. Lignin was estimated by the conventional Klason method [10].

Paper chromatography (PC) was performed by the descending method on Whatman No. 1 in the systems S_1 ethyl acetate—pyridine—water (φ_r = 8 : 2 :1) and S_2 ethyl acetate—acetic acid—formic acid—water (φ_r = 18 : 3 : 1 : 4) and reducing sugars were detected with anilinium hydrogen phthalate. The polysaccharide fractions were hydrolyzed with 2 M-TFA under reflux for 2 h. The hydrolyzate was separated into neutral and acidic sugars by ion-exchange technique on Dowex 1 x 8 (acetate form) and the acidic sugars were analyzed by PC.

GLC was carried out on a Hewlett—Packard instrument, Model HP 5890. For the quantitative determination of sugars in the form of their alditol trifluoroacetates [11], a capillary column (25 mm x 0.32 mm i.d.) packed with PAS 1701, and temperature range 110 °C (1 min) to 125 °C (2 °C min^{-1}) were used. Viscosities

Table 1. Analytical Data of Original (H) and Defatted, Destarched (DSH) Corn Hulls

	w_i/%[a]	
	H	DSH
Humidity	9.2	9.1
Ash	1.5	1.4
Protein	7.8	6.7
Fat	2.0	0
Starch[b]	22.1	nd
Klason lignin	4.7	nd
Soluble lignin[c]	nd	3.7
Neutral sugar components[d]		
Rhamnose	3.9	2.7
Arabinose	10.5	11.4
Xylose	17.2	24.2
Mannose	2.1	2.6
Glucose	56.6	49.1
Galactose	3.2	3.7

a) On absolutely dry corn hulls; *b*) isolated by wet-sieving; *c*) as material loss during $NaClO_2$ delignification; *d*) from the Klason lignin hydrolyzate expressed as x_r (mole %).

were measured at 21 °C in water or 0.1 M-NaCl using an Ubbelohde viscometer. ^{13}C NMR spectra (75 MHz) of the samples (ρ = 2 mass % in D_2O) were recorded with a Bruker AM-300 spectrometer at 40 °C in the inverse gated decoupling mode. Chemical shifts are reported relative to internal MeOH (δ(TMS) = 50.15).

Extraction Procedures

I. The BA-extracted hulls (1000 g) were macerated with 70 % ethanol for 1 h and then the light starch-rich fraction was separated by decantation. This treatment was repeated four times and the obtained sediment was washed with ethanol and dried on air yielding the destarched corn hulls DSH (860 g). DSH (100 g) was delignified by $NaClO_2$ at pH 4.6 and 65 °C for 1 h [12]. The holocellulose was recovered by filtration and subsequently suspended in 2.5 % NH_4OH (1000 cm^3) and stirred for 3 h. The ammoniacal extract was filtered off and submitted to dialysis and lyophilization, yielding fraction HI-1D. The insoluble residue was extracted with 5 % NaOH (1000 cm^3) for 2 h. After filtration, the insoluble residue was washed with 5 % NaOH (500 cm^3) and water (500 cm^3). The extractions were performed at room temperature. The alkaline extracts and washings were combined and hemicellulose A was precipitated by acidification to pH 4 and then separated by centrifugation. Hemicellulose B was obtained from the supernatant by precipitation with three volumes of ethanol. Both hemicelluloses were dispersed in water, dialyzed and freeze-dried yielding fractions HI-2A and HI-2B. The alkaline-insoluble residue was acidified with dilute acetic acid, exhaustively washed with water and dried at 105 °C to constant mass (HI-R).

II. In the first step, DSH (10 g) was treated with 5 % NaOH (100 cm^3), containing H_2O_2 (0.3 g), K_2SO_4 (0.3 g), and $MgSO_4$ (0.08 g), at 50 °C for 1 h [13]. After cooling to room temperature, the insoluble residue was filtered off. Then, in the second step, it was subsequently extracted with 5 % NaOH (100 cm^3) for 2 h. After removal of the extract by filtration, the residue was washed with 5 % NaOH (50 cm^3). The extracts from both extraction steps were combined and hemicelluloses were isolated either by dialysis and lyophilization or by precipitation into three volumes of ethanol, yielding fractions HII-CD and HII-CE, respectively.

III. The procedure differs from the procedure II only in the first step where a saturated aqueous solution of $Ca(OH)_2$ (100 cm^3) was used as the alkali. Hemicelluloses were isolated from the extract of the first step (HIII-1D) and second step (HIII-2D) by dialysis of the acidified extracts and lyophilization.

IV. Original corn hulls (H, 10 g) were extracted under the experimental conditions of procedure II yielding the corresponding hemicellulose fractions HIV-CD and HIV-CE from the combined extracts.

V. In this experiment, 10 g of H was extracted under the conditions of procedure IV, but 96 % ethanol (300 cm^3) was added in the first step. The hemicelluloses from the extracts of the first and second step were isolated separately by neutralization with dilute HCl, dialysis and lyophilization yielding fractions HV-1D and HV-2D.

The alkali-insoluble residues from procedures II—V were recovered in the same way as described in procedure I.

RESULTS AND DISCUSSION

Analysis of the corn hulls (Table 1) showed a significant proportion of arabinose and xylose which comprised more than 35 % of the neutral sugar components in accordance with reported data [14]. The proportion of glucose is due not only to cellulose but also to the high amount of unremoved starch which could be partially separated from the hulls by wet-sieving. The hulls contained also small amounts of residual protein and fat.

As a standard method, the fractional extraction of defatted and destarched corn hulls (DSH) delignified with $NaClO_2$ was used. The applied extractants (2.5 % NH_4OH and 5 % NaOH) are known to solubilize easily-extractable hemicelluloses from hardwood and annual plants [15, 16]. The results of this extraction procedure are summarized in Table 2. The ammoniacal extraction yielded a small fraction (HI-1D) containing most of the residual protein of the corn hulls. By extraction with

Table 2. Isolation of Easily-Extractable Hemicelluloses of Destarched Corn Hulls after $NaClO_2$ Delignification (Procedure I)

Fraction	Yield	Neutral sugar composition *w*/%						
	w/%[a]	Rha	Ara	Xyl	Man	Glc	Gal	x_i(Ara—Xyl)
HI-1D[b]	3.1 (2.2)	–	24.0	30.9	15.9	28.6	0.6	0.78
HI-2A	1.9 (0.7)	0.7	11.4	20.2	2.1	60.6	5.0	0.56
HI-2B	20.7 (19.0)	1.2	32.1	50.7	–	8.4	7.6	0.63
HI-R	45.5	–	1.3	6.1	3.5	89.1	tr	0.21

a) On absolutely dry corn hulls; *b*) nitrogen content, 8.9 mass %, the other fractions are nitrogen-free. The values in brackets were calculated by subtraction of glucose from the neutral sugar portion.

5 % NaOH, most of the easily-extractable hemicelluloses (HI-2) were isolated. The hemicellulose A component of this fraction (HI-2A) which usually consists of low-branched polysaccharides [17] comprised glucan and arabinoxylan in the mole ratio $x_r = 2 : 1$. The glucan originates mainly from starch which was detected by the positive I_2/KI test. The hemicellulose B component (HI-2B) contained both components in the ratio $x_r = 1 : 9.8$. Sugar analysis of the alkali-insoluble residue (HI-R), shown in Table 2, indicated that similarly as in the case of other plant tissues [15], a portion of the hemicelluloses are strongly associated with the cell wall matrix.

In order to isolate the xylan component of corn hulls by a technically applicable procedure, the previously reported [12] two-stage extraction method was used. In the first step, partial oxidative delignification with alkaline hydrogen peroxide is connected with extraction of lignin and hemicelluloses. In the second step, the removal of the released components is completed. The results of the alkaline/oxidative extraction of DSH using 5 % NaOH (procedure II) or saturated $Ca(OH)_2$ (procedure III) in the first step are given in Table 3. The $NaOH/H_2O_2$ step yielded hemicelluloses by dialysis of the combined extracts from both steps (HII-CD). They contain arabinoxylan and glucan in the ratio $x_r = 2.2 : 1$. Hemicelluloses isolated from the combined extracts by ethanol precipitation (HII-CE) were less contaminated with glucan, but lower in yield. Probably, the highly-branched polysaccharide components were not precipitable under the used conditions. When saturated $Ca(OH)_2$ was used instead of 5 % NaOH in the delignification step, the solubility of hemicelluloses was low and the bulk of them were extracted in the second step (HIII-2D). However, the sugar composition of both fractions obtained by dialysis was essentially the same.

The extraction of hemicelluloses from the original corn hulls after the $NaOH/H_2O_2$ delignification is demonstrated in Table 4. In this case, the yield of the hemicellulose fractions is much higher due to the high amount of extracted starch. The ratio of arabinoxylan to glucan was about $x_r = 1 : 1$ in both fractions, isolated by dialysis as well as ethanol precipitation.

In procedure V, the delignification step was carried out in the presence of ethanol which was previously shown [18] to enhance delignification of mechanical pulps and suppression of hemicellulose extraction. This effect was confirmed by the results in Table 4 which show the highest yield of extracted hemicelluloses (HV-2D) and composition similar to the above-mentioned corn hull fractions.

The mole ratio of arabinose to xylose of the main hemicellulose fractions varied from 0.55 to 0.68. As seen in Table 5, the heteroxylans contain small proportions of glucuronic acid and its 4-*O*-methyl ether as well as traces of galacturonic acid (detected by PC of the acidic sugar components of the hydrolyzates). The uronic acid content, determined by potentiometric titration, varied between 4.0 and 4.7 mass %. It was only 2.1 mass % in the case of starch-rich fraction.

Table 3. Yield and Composition of Hemicelluloses Isolated from Destarched Corn Hulls after Alkaline/Oxidative Delignification (Procedures II and III)

Fraction	Yield w/%[a]	Neutral sugar composition w/%						
		Rha	Ara	Xyl	Man	Glc	Gal	x_r(Ara—Xyl)
HII-CD	33.2 (23.7)	–	23.2	41.3	–	28.7	6.8	0.56
HII-CE	23.7 (20.5)	–	29.3	50.9	–	13.7	6.1	0.58
HIII-1D	6.6 (4.5)	–	27.2	30.8	9.9	32.1	tr	0.88
HIII-2D	32.9 (23.1)	–	24.9	40.1	3.1	29.9	2.0	0.62

a) See footnote in Table 2.

Table 4. Yield and Composition of Hemicelluloses Isolated from Original Corn Hulls after Alkaline/Oxidative Delignification (Procedures IV and V)

Fraction	Yield w/%[a]	Neutral sugar composition w/%						
		Rha	Ara	Xyl	Man	Glc	Gal	x_r(Ara—Xyl)
HIV-CD	39.6 (22.3)	–	21.4	33.9	–	43.6	1.1	0.63
HIV-CE	36.8 (18.0)	–	15.8	27.9	–	51.2	5.9	0.56
HV-1D	2.8 (1.4)	–	19.7	29.5	–	50.8	tr	0.67
HV-2D	46.2 (24.5)	0.2	17.5	27.7	0.8	46.9	6.9	0.63

a) See footnote in Table 2.

Table 5. Analytical Characteristics of Corn Hull Xylan Fractions

	HI-2B	HII-CE	HIV-CE
Neutral sugars, x_i/mole %			
Rhamnose	1.1	–	–
Arabinose	33.1	30.4	16.6
Xylose	52.3	52.8	30.3
Mannose	–	–	–
Glucose	7.1	11.6	47.7
Galactose	6.4	5.2	5.4
w(Uronic acid)/%[a]	4.7	4.0	2.1
x_i(Ara—Xyl)	0.63	0.58	0.55
$[\eta]$/(100 cm^3 g^{-1})	125	121	98

a) Calculated as the Na^+ salt of 4-*O*-methyl-D-glucuronic acid, M_r = 213.

The ^{13}C NMR spectra of the heteroxylans showed a very complex character which resembles that of the water-soluble heteroxylan from rye bran [17] and corn cob [19] (Fig. 1). The anomeric region shows a large group of signals at δ = 107.5—109.9 corresponding to C-1 of α-L-Araf units linked at various positions. The signals at δ = 102.4, 102.8, and 103.7 were assigned

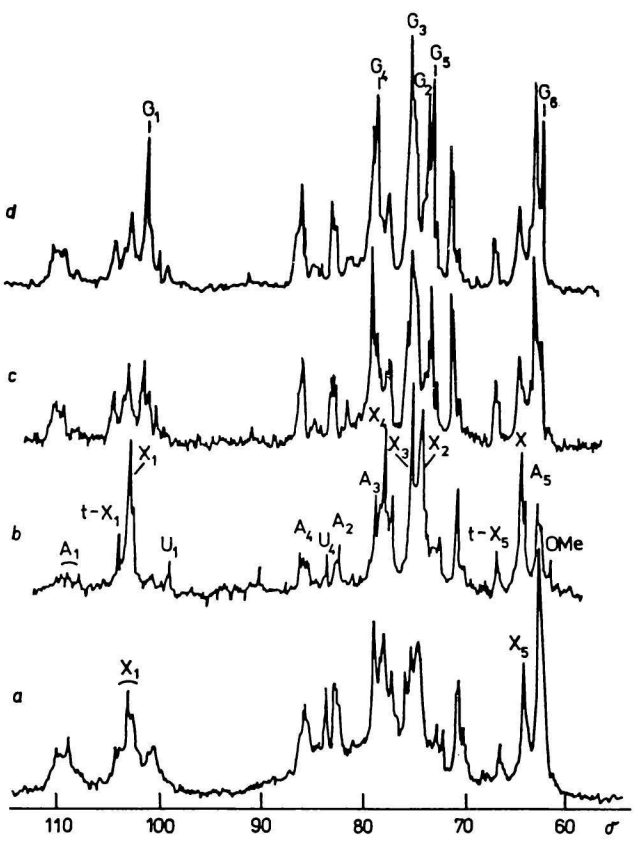

Fig. 1. ^{13}C NMR spectra (in D_2O) of various water-soluble heteroxylans. *a*) AX from rye bran [17]; *b*) AGX from corn cobs [19]; corn hull fractions *c*) HII-CD and *d*) HIV-CE. G – α-D-Glcp, X – β-D-Xylp, A – α-L-Araf, U – α-D-GlcpA, t – terminal nonreducing end unit.

Table 6. The Viscosity of Various Heteroxylans in Aqueous Solution

Xylan	Source	ρ^a	η_{sp}	η_{sp}/ρ^b
AGX	Corn cob [19]	2.06	5.56	2.70
GX	Beechwood [21]	2.05	2.73	1.33
AX	Rye bran [17]	2.09	17.40	8.32
HI-2B	Corn hull	2.07	12.52	6.05
HII-CE		1.02	8.41	8.24
HIII-2D		2.23	9.32	4.18
HV-2D		2.11	6.48	3.07

a) Concentration in g/100 cm^3; *b*) in 100 cm^3 g^{-1}.

to C-1 of substituted, internal, and nonreducing terminal β-D-Xylp units, respectively. The high proportion of the signal of substituted Xylp units indicated a degree of branching of the xylan chain which is higher than that of the aforementioned xylans [17, 19]. The signals at δ = 103.71, 70.02, and 66.54 attributed to C-1, C-4, and C-5 of nonreducing terminal Xylp units confirm this assumption. The results are in accord with the high specific viscosity of aqueous solutions of the xylans (Table 6) in comparison to other water-soluble xylan types.

The presence of starch is documented by the appearance of signals at δ = 100.84, 72.78, 74.59, 78.01, 72.39, and 61.73 corresponding to C-1—C-6 of α-1,4-linked D-Glcp residues [20]. The signals at δ = 98.78, 83.19, 175.51 (not shown in the spectra), and 61.01 related to C-1, C-4, C-6, and OCH_3 of 4-*O*-methyl-α-D-glucuronic acid residues [21] are very weak and in accord with the low uronic acid content.

By the presented extraction procedures, water-soluble arabinoxylan-starch fractions with different ratios of both polysaccharide components can be prepared from corn hulls. Most of the starch can be removed by wet-sieving of the hulls and used in other applications. Due to the interesting solution properties, further studies on the structural and molecular properties of the heteroxylans from corn hulls are in progress.

Acknowledgements. Thanks are due to the Slovak Grant Agency for financial support of the research.

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Translated by A. Ebringerová