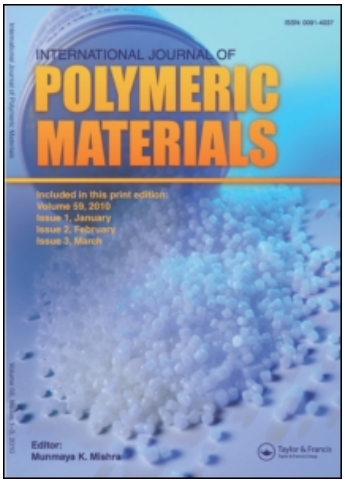

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Fractional Extraction and Structural Characterization of Hemicelluloses from Wheat Straw

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The chemical composition of 7 hemicellulosic fractions extracted directly from dewaxed wheat straw with sodium hydroxide at sequentially increasing strength from 0.125 to 1.50 M are reported. The structural features of the fraction 3 were evaluated by means of methylation analysis and ^{13}C-NMR spectroscopy. The hemicelluloses were confirmed to be a β-(1–4)-xylan with side chains consisting of L-arabinofuranosyl and D-xylopyranosyl groups attached in position 3, and D-glucopyranosyluronic acid or 4-*O*-methyl-D-glucopyranosyluronic acid group attached at position 2. For every 15 D-xylopyranosyl residues in the main chain, there was one L-arabinofuranosyl group. For 19 such D-xylopyranosyl residues, there was one D-xylopyranosyl group, and for ~26 such D-xylopyranosyl residues, there was one uronic acid unit.

Keywords: Wheat straw; hemicelluloses; sugars; uronic acids; molecular weight; structural determination; NMR; phenolic acids and aldehydes

INTRODUCTION

As part of our continuing study of wheat straw as a potential material for pulp and paper production, we have extensively investigated the polysaccharides. In our previous reports [1–4], various types and concentrations of alkali, as well as time and temperature conditions, have been used for the extraction of hemicelluloses from wheat straw holocelluloses. The present study were carried out on hemicelluloses

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extracted sequentially with increasing alkali strength (0.125–1.50 M) from dewaxed wheat straw, rather than from the holocelluloses, and undertaken to obtain detailed information on the structure of these polymers.

EXPERIMENTAL

General

IR spectra were obtained on an IR spectrophotometer (Mattson cygnus 100), using a KBr disc containing 1% finely ground samples. The solution ^{13}C NMR spectra (62.9 MHz) were measured at 25°C on the Bruker 250 AC spectrometer. The number of Scans was 40000. The dried hemicellulosic fraction 2 (200 mg/mL) were dissolved in 1 mL D_2O with M sodium deuteroxide.

Sample of hemicellulosic fraction 3 was methylated by a modified procedure (5,6). At room temperature, a stirred solution of sodium methylsulfinylmethanide (prepared from 1.70 g (60%) of sodium hydride and 25 mL of methylated sulfoxide under nitrogen) was added the solution of hemicellulosic fraction 2 (0.20 g) in methyl sulfoxide (5 mL). After stirring for 4 h, the solution of hemicellulosic alkoxide was cooled to 20°C in an ice-water bath and methyl iodide (4 mL) was added. Stirring was continued for 12 h, water (60 mL) was then added, and the mixture was extracted 3 times with 80 mL of chloroform. The combined extracts were washed 3 times with 30 mL of water, and the extracts in chloroform were concentrated to a yellow solid under reduced pressure at 40°C. Benzene (5 mL) was added to dissolve the yellow solid, and the solution was diluted with light petroleum to precipitated the methylated hemicelluloses (0.16 g), $[\alpha]_D^{17}$ −82°(c 0.1, chloroform). A portion (20 mg) of the material was hydrolysed with 3 N trifluoroacetic acid at 120°C for 3 h and the resulting sugars were converted into alditol acetates and analysed by GC(7).

The methods for chemical and physico-chemical analyses, including neutral sugar and uronic acid analysis, molecular weight measurement, and alkaline nitrobenzene oxidation of lignin and determination of phenolic acids and aldehydes with HPLC in extracted hemicellulosic fractions, were described in previous papers (1,2). All nitrobenzene

oxidation results represent the mean of at least triplicate and each oxidation mixture was chromatographed twice. Other experiments were performed in duplicate. The standard errors or deviations were always observed to be lower than 5% except the variations among triplicate nitrobenzene oxidation (8–16%).

Fractional Extraction of Hemicelluloses

The wheat straw (winter) was obtained from Silsoe Research Institute (Silsoe, Bedfordshire). Finely powdered straw was extracted (Soxhlet) with chloroform: methanol (2:1, v/v) to remove waxes. The air-dried dewaxed straw (48 g) was extracted sequentially in a thermostated reactor under nitrogen atmosphere and stirring with the extraction conditions below (Tab. I): (1) 0.125 M NaOH, 37°C, 2 h, 48 g/2000 mL; (2) 0.25 M NaOH, 37°C, 2 h, 39 g/2000 mL; (3) 0.50 M NaOH, 37°C, 2 h, 34 g/2000 mL; (4) 0.75 M NaOH, 37°C, 2 h, 31 g/2000 mL; (5) 1.00 M NaOH, 37°C, 6 h, 28 g/2000 mL; (6) 1.25 M NaOH, 60°C, 6 h, 26 g/2000 mL; (7) 1.50 M NaOH, 75°C, 6 h, 22 g/2000 mL.

After filtration, the extracts in each of the fractions were acidified to pH 5 with glacial acetic acid, concentrated with a rotary evaporator under reduced pressure at 40°C to about 800 mL, and then mixed with 5 volumes of 95% ethanol (24 h, 20°C). The crude hemicelluloses were filtered, washed with 75% ethanol, purified 3 times by dissolving in 500 mL water and re-precipitation with 4 volumes of 95% ethanol for 12 h at 20°C, respectively, and dried in an oven at 40°C.

RESULTS AND DISCUSSION

Yield and Composition of Hemicellulosic Fractions

As can be seen in Table I, 7 hemicellulosic fractions represented 33.4% of the dry weight of straw, which indicated that most of the hemicelluloses were extracted sequentially with sodium hydroxide of increasing strength from 0.125 to 1.50 M at the conditions chosen. It is of interest to note that hemicelluloses in wheat straw are more effective release by dilute alkali. With the successive increase of alkali concentration from 0.125 to 0.50 M, the yields of hemicelluloses were 6.4, 6.7

TABLE I The yield of hemicelluloses and the extraction conditions

Fraction No.	*NaOH concentration (M)*	*Dry material (g)/ extractant (mL)*	*Temperature (°C)/ times (h)*	*Yield (% dry straw)*
1	0.125	48/2000	37°C/2 h	6.4
2	0.25	39/2000	37°C/2 h	6.7
3	0.50	34/2000	37°C/2 h	6.3
4	0.75	31/2000	37°C/2 h	3.2
5	1.00	28/2000	37°C/6 h	3.5
6	1.25	26/2000	60°C/6 h	4.4
7	1.50	22/2000	75°C/6 h	2.9

and 6.3%, respectively, which showed more than 50% of hemicelluloses were released during the extraction above. In our previous study [2], treatment with 0.5% NaOH at 20°C for 6 h released more than 30% hemicelluloses. These high solubility of hemicelluloses in dilute alkali solution was probably due to the presence of phenolic acids in both alkali-labile (ester) and acid-labile (ether) linkages in wheat straw and interpreted in terms of a structure with hemicelluloses-ester-ferulic acid-ether-lignin bridges [8]. However, between fractions 4 and 7, the yields were quite low. This provides evidence that hemicelluloses in wheat straw cell wall material are either bound to or shielded by lignin, preventing extraction prior to delignification [9]. These foregoing data were in agreement with our previous report [4]. We mentioned that, in addition to the ester bond between ferulic acid and hemicelluloses, or *p*-coumaric acid and lignin, and ether bond between ferulic acid and lignin, the majority of lignin in atmospheric refined and alkaline pre-treated wheat straw was directly ether-linked to hemicelluloses.

The sugar composition and uronic acid content of each of the fractions are shown in Table II. Xylose was a major component sugar and comprised 60–80% of the total sugars in hemicellulosic fractions. A small amount of mannose was also present in these fractions. With sequential increase of alkali strength from 0.125 to 1.50 M, the relative content of arabinose decreased from 17.2 to 11.5%, which was, in general, parallel to the amounts of hemicelluloses released. These results obtained indicated that some amounts of arabinose in straw hemicelluloses were ester-linked to ferulic acid and easy to saponify, subsequently resulting to high solubility of hemicelluloses in dilute alkali solution during the first 3 extraction steps. Decrease of arabinose

TABLE II Neutral sugar composition (relative %) and uronic acid content (%) of hemicelluloses

Fraction No.	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acids*
1	17.2	66.4	2.6	9.8	4.1	3.8
2	13.5	77.1	1.4	5.3	2.8	3.6
3	12.7	79.3	1.3	4.6	2.1	4.0
4	12.4	77.8	1.2	5.6	3.0	4.5
5	11.9	77.8	1.0	6.1	3.2	4.5
6	11.7	75.9	1.0	7.8	3.6	5.1
7	11.5	66.8	0.9	12.9	8.0	5.1

and low yield of hemicelluloses during the last 4 extraction steps showed that lignin in wheat straw also appeared to be ether bond to hemicelluloses and impeded the extraction of hemicelluloses. It is obvious that higher contents of xylose and lower arabinose in hemicelluloses extracted from straw holocelluloses than those extracted directly from dewaxed straw suggested that arabinose was probable as side chain in hemicellulosic structure, while xylose was in the main chain of hemicelluloses and was prevented to extract prior to delignification. The content of uronic acids in hemicellulosic fractions was a minor change, 3.6–5.1%, and it was higher than that in the fractions ($\sim$3.0%) extracted from straw holocelluloses [3].

The Structural Features of Hemicellulosic Fraction 3

Methylation of hemicellulosic fraction 3 gave a product with $[\alpha]_D^{17}-82°$, indicative of β linkage, which was confirmed by the NMR spectra (δ 105.5 for C-1). After hydrolysis of methylated hemicellulosic fraction 3 with 3 M trifluoroacetic acid at 120°C for 3 h and reduction with $LiAlH_4$, the resulting methyl derivatives are summarized in Table III. The formation of 2,3-di-O-methyl-xylose in high proportion and 2-O-methyl-D-xylose as well as 3-O-methyl-D-xylose in relatively small proportion indicated that the hemicellulosic fraction 3 was essentially a (1$\rightarrow$4)-linked D-xylan having a few branches at C-3 and C-2. The formation of a small amount of 2,3,5-tri-O-methyl-L-arabinose and 2,3,4-tri-O-D-xylose suggested that the branch points consisted of L-arabinofuranosyl and D-xylopyranosyl groups. Monitoring the methylated glucuronic acid was not possible by g. c. on OV-17, but it was identified by the chemical shifts in ^{13}C-NMR spectrum

TABLE III Molar ratios of methyl monosaccharides from hemicellulosic fraction 3 and the corresponding chemical shifts in ^{13}C NMR data

Methyl derivative	*Mole %*	*Chemical shift (ppm)*					
		C-1	C-2	C-3	C-4	C-5	MeO
2,3,5-Me_3-Ara[a]	6.0	112.8	83.5	79.2	89.7	65.0	–
2,3,4-Me_3-Xyl	4.8	105.5	76.5	81.7	79.2	66.5	–
2,3-Me_2-Xyl	78.7	105.5	76.5	78.1	79.2	66.5	–
2-Me-Xyl	7.0	104.9	80.9	78.1	79.2	66.5	–
3-Me-Xyl	3.5	101.5	75.0	76.1	83.5	73.6	60.7

[a]2,3,5-Me_3-Ara = 2,3,5-tri-O-methyl-L-arabinose, etc.

(Fig. 1). The chemical shifts for D-glucuronic acid residues in ^{13}C-NMR spectrum appeared at 101.5(C-1), 76.1(C-2, C-3), 83.5(C-4), 75.0(C-5) and 180.5(C-6). Ehrenthal and co-workers [10] mentioned that it is possible that uronide-like substances were eliminated during the methylation step. Therefore, this loss of methylated glucuronic acid in our experiment was not surprising and indeed would lend support to the theory that glucuronic acid exists in the xylan as a terminal group. Wilkie [11] stated that non-endospermic xylans have substituent groups of D-glucopyranosyluronic acid, or its 4-methyl ether, or both. Aspinall and Meek [12] also reported that D-

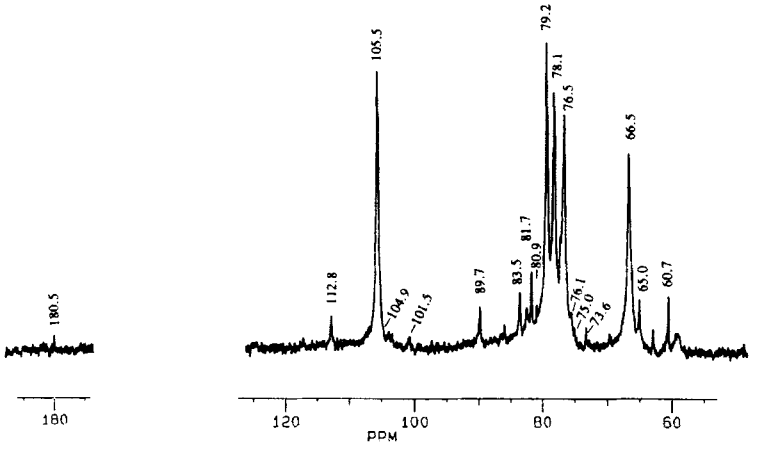


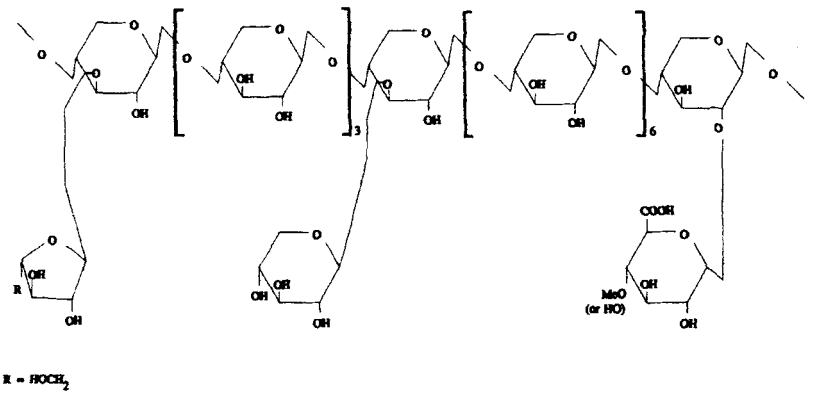
FIGURE 1 ^{13}C-NMR spectrum of hemicellulosic fraction 3 in D_2O.

glucuronic acid residues (partially present as the 4-methyl ether) are linked directly to the main chain through position 2 of xylose in wheat straw xylan, and D-xylopyranosyl and L-arabinofuranosyl groups attached at positions 3 in the main chain of xylan. In the continuing study of wheat straw xylan, Toman and Chimidcogzol [13] proved that 4-O-methyl-D-glucopyranosyluronic acid groups are attached to C-2 of D-xylopyranosyl units, and L-arabinofuranosyl residues are linked to C-3 of D-xylopyranosyl units in the main chain. In agreement with these previous studies and double content of 2-O-methyl-D-xylose than 3-O-methyl-D-xylose in methylated xylan fraction 3, we are of the opinion that L-arabinose and 4.8% D-xylose residues occurred as side-chains linked to the backbone of β-(1→4)-D-xylopyranosyl residues through position 3 of xylose, while D-glucuronic acid residues (or as the 4-methyl ether) are linked to the main chain through position 2 of xylose.

The structural features of hemicellulosic fraction 3 evaluated by methylation analysis are reflected by ^{13}C NMR spectrum (Fig. 1). The spectrum was interpreted (Tab. III) on the basis of reported data for structural defined arabinoxylan-type, glucuronoxylan-type and L-arabino-(4-O-methyl-D-glucurono)-D-xylan, as well as those of wheat straw hemicelluloses isolated after delignification (14–16). As expected, most signals of the spectrum (Fig. 1) are attributed to the corresponding carbon atoms of the L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan.

It is clear from the above study that hemicellulosic fraction 3 was shown to be essentially a (1→4) linked β-D-xylan with L-arabinofuranosyl and D-xylopyranosyl groups attached at positions 3, and D-glucopyranosyluronic acid (or 4-O-methyl-D-glucopyranosyluronic acid) groups attached at positions 2. For every 15 D-xylopyranosyl residues in the main chain, there was one L -arabinofuranosyl group. For 19 such D-xylopyranosyl residues, there was one D-xylopyranosyl group, and for ~26 such D-xylopyranosyl residues, there was one uronic acid unit.

Although no unique structure can be depicted for this xylan, the above experimental facts are consistent with the general type of structure shown (Scheme 1):



Scheme 1.

Physico-Chemical Characterization of Hemicelluloses

The hemicelluloses extracted directly from dewaxed straw before delignification had a high degree of polymerization with apparent mole- cular weights between 27800 and 63400 Da (Tab. IV), while the hemicelluloses extracted from straw holocelluloses had a low degree of polymerization with molecular-average weight valued at 13100 Da [16]. These data coincided with our earlier study [3]. We indicated that weak alkaline solution such as 0.125 M NaOH generally solubilized hemicelluloses B, more branched fraction, while hemicelluloses A, more linear fraction, could be extracted with high concentration of alkaline solution. With successive increase of sodium hydroxide concentration from 0.125 to 0.75 M, the molecular-average weight reduced from 42400 Da to less than 30000 Da. Interestingly,

TABLE IV Molecular-average weight of hemicellulosic fractions

Fraction No.	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$
1	42400	29900	1.42
2	34300	22600	1.52
3	30100	22300	1.35
4	27800	22000	1.26
5	45500	20900	2.17
6	50100	21100	2.37
7	63400	30200	2.10

during the last 3 extraction steps, the molecular-average weight did not decrease, while it increased from 45500 Da to over 60000 Da with the sequential growths of sodium hydroxide concentration from 1.00 to 1.50 M and temperature from 30°C to 75°C. The reason for this increasing tendency of molecular weight at high concentration of alkali and temperature was probable that more lignin-hemicellulosic complex was extracted in the hemicellulosic fractions.

The molecular weight distribution for fractions 2, 4 and 7 are illustrated in Figure 2. The molecular weight ranges of fraction 2 and 4, extracted with 0.25 and 0.75 M NaOH at 30°C for 2 h, respectively, appeared between 478600 and 6700 Da, whereas the range for fraction 7, extracted with 1.50 M NaOH at 75°C for 6 h, gave the elution profiles between 732800 and 6700 Da. In addition, the elution profiles of fractions 4 and 7 contained two major peaks. Peak I eluted in the void volume (15.7 mL) and had a molecular weight equal to or greater

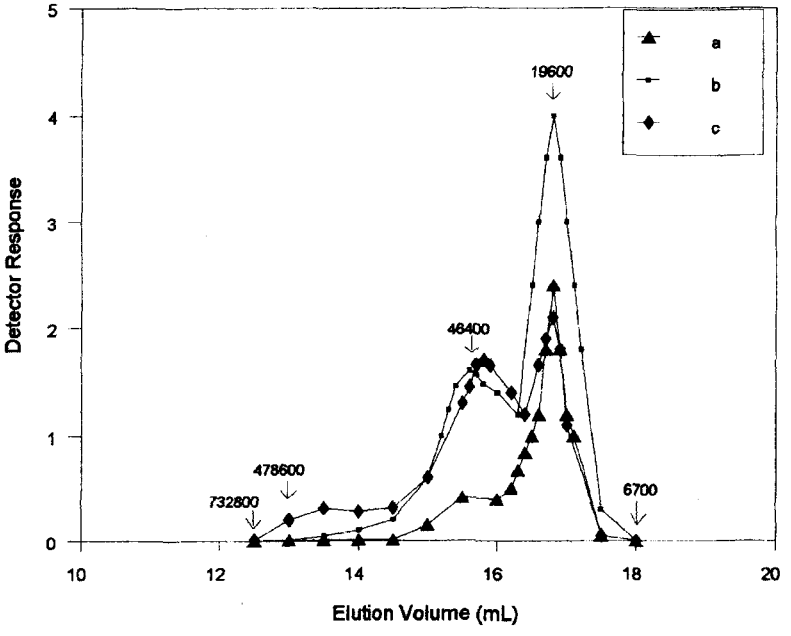


FIGURE 2 The molecular weight distribution of hemicelluloses (a) extracted with 0.25 M NaOH at 37°C for 2 h, (b) extracted with 0.75 M NaOH at 37°C for 2 h and (c) extracted with 1.50 M NaOH at 75°C for 6 h.

than 46400 Da. Peak II had a molecular weight around 19600 Da. The peak area of peak I was large with 1.50 M NaOH extraction than with 0.75 M NaOH. The elution profiles of fraction 2, however, yielded only one peak around 19600 Da.

The FT-IR spectra of hemicellulosic fractions 1, 3, 5 and 7 are shown in Figure 3 with the bands of interest being identified by their wavenumbers. As can be seen in the Figure, all the four spectra appeared to be rather similar. All of the spectra appeared sharp band at 890 cm^{-1}, which is characteristic of β-glucosidic linkages between the sugar units [17]. This suggested that the xylose residues forming the backbone of the macromolecular are linked by β form bonds. The other prominent bands in the spectra corresponded to hemicelluloses also appeared at 1370 and 1030 cm^{-1}. The broad band at 1635 cm^{-1} was probably due to linked water [18], and the small bands at 1510, 1460, 1420, 1245 and 1150 cm^{-1} showed low lignin content in these fractions. After alkaline nitrobenzene oxidation of each of the hemicellulosic fractions at 170°C for 2.5 h, the content of phenolic substances

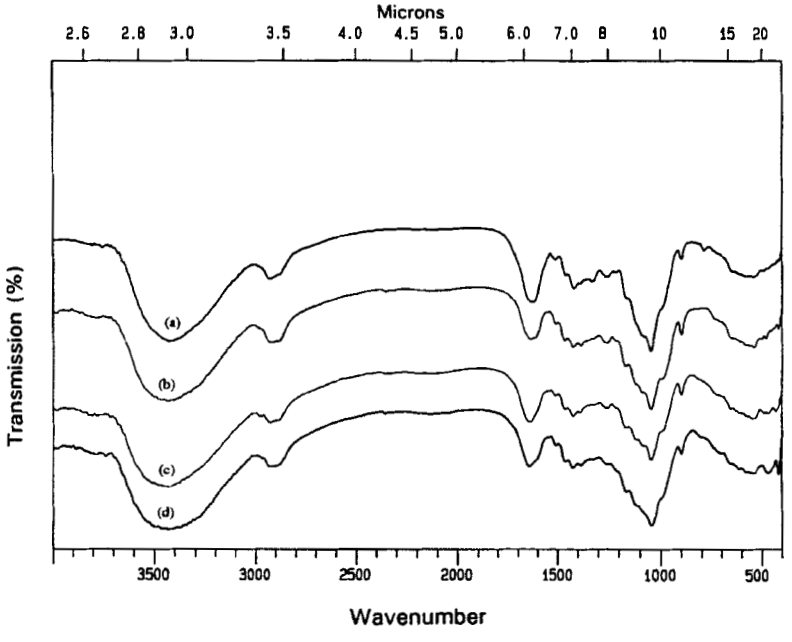


FIGURE 3 FT-IR spectra of hemicellulosic fractions, 1(a), 3(b), 5(c) and 7(d).

TABLE V The content of phenolic monomers in the products of alkaline nitrobenzene oxidation of lignin in hemicellulosic fractions

Phenolic monomers	*Fraction No.*						
	1	2	3	4	5	6	7
Gallic acid	0.11	0.24	0.23	0.14	0.20	0.14	0.27
Protocatechuic acid	0.016	0.14	0.025	0.018	0.009	0.005	0.026
p-Hydroxybenzoic acid	0.042	0.29	0.12	0.081	0.065	0.064	0.14
p-Hydroxybenzaldehyde	0.14	0.30	0.28	0.12	0.12	0.10	0.13
Vanillic acid	0.071	0.38	0.16	0.11	0.11	0.10	0.15
Syringic acid	0.16	0.68	0.41	0.30	0.33	0.28	0.43
Vanillin	0.96	1.45	1.23	1.13	1.11	0.91	1.20
Syringaldehyde	0.98	1.48	1.30	1.25	1.28	0.99	1.62
p-Coumaric acid	0.021	0.27	0.046	0.058	0.059	0.029	0.058
Acetovanillone	0.025	0.17	0.042	0.029	0.014	0.0071	0.029
Ferulic acid	0.028	0.35	0.15	0.14	0.085	0.070	0.28
Total	2.56	5.75	4.00	3.38	3.38	2.70	4.33

in the oxidation products is given in Table V. As mentioned earlier, due to the lignin-hemicellulose complex in cell walls of wheat straw, the content of phenolic acids and aldehydes in these fractions (2.6–5.8%) extracted directly from dewaxed straw was 5–10 times higher than that in the fractions (0.3–0.7%) extracted from straw holocelluloses. The major components of phenolic monomers in the alkaline nitrobenzene oxidation of residual lignin in extracted hemicellulosic fractions were found to be syringaldehyde and vanillin, and the content of syringaldehyde was always higher than vanillin in all the fractions.

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