Fractional Characterization of Wheat Straw Lignin Components by Alkaline Nitrobenzene Oxidation and FT-IR Spectroscopy

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A method was developed for the isolation and fractional characterization of phenolic monomers in wheat straw lignin using (a) methanol/toluene, ethanol/toluene, or chloroform extraction for isolation of free phenolic monomers; (b) treatments with various alkalis and hydrogen peroxide for different lengths of time to extract loosely bound phenolic acids and aldehydes; and (c) alkaline nitrobenzene oxidation of lignin in the residue of alkali-treated wheat straw, extracted hemicellulose, and cellulose for determination of tightly bound phenolics. The yield of free phenolic monomers ranged from 0.007 to 0.031% of dry straw. The predominant components of the alkali-labile free monomers were found to be ferulic and *p*-coumaric acid, which together comprised 65−82% of the total. The results of alkaline nitrobenzene oxidation indicated a high guaiacyl content in the original wheat straw and a high relative syringyl content in treated wheat straw. A comparison of FT-IR spectroscopic data of untreated and treated wheat straw illustrated that the most obvious features in the spectra of alkaline treated wheat straw were the disappearance of the ester linkage absorption and reduced lignin content in treated wheat straw.

Keywords: *Wheat straw; lignin; phenolic monomers; alkaline nitrobenzene oxidation; Fourier transform infrared spectroscopy (FT-IR)*

INTRODUCTION

Our previous work (Sun et al., 1995a) indicated that pretreatment of wheat straw with 1.5% sodium hydroxide for 6 days at 20 °C dissolved about 60% of lignin and more than 80% of hemicellulose. The possible mechanisms for the solubilization of Gramineae lignin by alkali include the release of phenolic acids from cell walls and the release of lignin resulting from cleavage of ester linkages in lignin−polysaccharide complexes (Kondo et al., 1992). In addition, the solubilisation of Gramineae lignin by dilute aqueous alkali under mild conditions should not cause chemical modification much beyond saponification of esterified hydroxycinnamic acids. Therefore, the method for isolation of lignins through alkaline extraction has been recommended as particularly suitable for Gramineae lignin (Schwarz et al., 1989).

Alkaline nitrobenzene oxidation is established as an important method for the cleavage of lignins into monomer units and for qualitative and quantitative determination of the building units of lignins (Iiyama and Lam, 1990). Oxidative hydrolysis of lignin macromolecules in heterogeneous basic media containing nitrobenzene results in the production of simple phenolic acids and aldehydes, consisting mainly of hydroxycinnamic and hydroxybenzoic moieties (Galletti et al., 1989a). In contrast to the nitrobenzene oxidation method, which requires degradation of the phenolic macromolecules into small fragments suitable for determination by common chromatographic techniques (HPLC etc.), spectroscopic techniques such as Fourier transferorm infra red (FT-IR) and solid state ^{13}C NMR can be used to investigate "polymeric" lignin either extracted from straw or "in situ" within the native straw substrate (Galletti et al., 1989b).

Jung et al. (1983) have fractionated the phenolic acids and aldehydes from alfalfa hay, soybean stover, smooth bromegrass hay, and corn stalk into free, loosely bound and tightly bound fractions. There have been several fractionation studies of wheat straw lignin (Scalbert et al., 1986a; Galletti et al., 1989b; Jung and Himmelsbach, 1989; Iiyama and Lam, 1990; Kondo et al., 1992; Cheng, 1993). However, the phenolic monomers among these fractions from wheat straw have not yet been thoroughly characterized. The objectives of this study were to determine (1) the phenolic monomer content of wheat straw by alkaline nitrobenzene oxidation, (2) the distribution of monomers among various fractions (free, loosely bound, and tightly bound), and (3) the effects of various mild chemical pretreatments of wheat straw on the composition of phenolics in the oxidation products of the residual material. In addition, FT-IR spectroscopy has been used to further characterize the wheat straw fractions obtained.
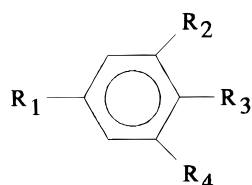
MATERIALS AND METHODS

Materials. Wheat straw (winter wheat) was obtained from Silsoe Research Institute (Silsoe Bedfordshire) and was ground in a Christie Laboratory Mill to pass a 1 mm screen. In straw, cellulose and hemicellulose make up about 70% of the total dry weight. Lignin makes up around 14% (sodium chlorite lignin) of the dry weight (Lawther et al., 1995). Phenolic compounds used as reference standards were of analytical or reagent grade.

Fractionation of Phenolic Monomers. The phenolic monomers were extracted and fractionated using the procedure of Jung et al. (1983) with some modifications. To determine the free phenolic fraction, samples of dried wheat straw (26.0 g) were extracted with 800 mL of methanol−toluene (1:2) for 5 h, ethanol−toluene (1:2) for 22 h, and chloroform for 5 h, respectively. Each filtrate was evaporated to dryness under reduced pressure at 40 °C and redissolved in 20 mL of methanol for HPLC analysis.

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Table 1. Alkaline Nitrobenzene Oxidation Products of Wheat Straw Lignin

	structure					
compd	R_1	R_2	R_3	R_4	MW	retention time,[a] min
gallic acid (GA)	COOH	OH	OH	OH	170	1.86
protocatechuic acid (PA)	COOH	OH	OH	H	154	3.34
p-hydroxybenzoic acid (PHBA)	COOH	H	OH	H	138	5.66
p-hydroxybenzaldehyde (PHBAL)	CHO	H	OH	H	122	7.40
vanillic acid (VA)	COOH	H	OH	OCH_3	168	8.01
vanillin (VAN)	CHO	H	OH	OCH_3	152	10.12
syringic acid (SYA)	COOH	OCH_3	OH	OCH_3	198	11.05
p-coumaric acid (PCA)	CH=CHCOOH	H	OH	H	164	11.54
syringaldehyde (SYAL)	CHO	OCH_3	OH	OCH_3	182	12.44
acetovanillone (AV)	$COCH_3$	$OCH3$	OH	H	166	13.20
ferulic acid (FA)	CH=CHCOOH	OCH_3	OH	H	194	14.55
acetosyringone (AS, standard)	$COCH_3$	OCH_3	OH	OCH_3	196	15.42
cinnamic acid (CA)	CH=CHCOOH	H	H	H	148	27.70

[a] On a Hichrom H5ODS column.

The solvent-insoluble residues were extracted (2.5 g of straw/100 mL of solution) to determine the loosely bound phenolic fraction as follows: (a) with 1.5% sodium hydroxide at 20 °C in air for 0.5, 1, 2, 3, 4, 6, 12, 24, 48, 72, 96, and 144 h, respectively; (b) with 1.5% hydrogen peroxide, liquid ammonia, calcium hydroxide, potassium hydroxide, sodium hydroxide and lithium hydroxide for 6 h at 20 °C, respectively. After filtration on a nylon cloth, the filtrates were neutralized with dilute hydrochloric acid and concentrated on a rotary evaporator under reduced pressure at 40 °C. The hemicellulose fractions were recovered from the above solution by ethanol precipitation (pH 6.5), and the alkali lignin fractions (bound phenolic monomers) were then precipitated (pH 1.5) from the supernatant solution. For determination of free phenolics in the alkali-labile phenolic monomers, the concentrated filtrates (10 mL) were acidified to pH 1 with 20% HCl and extracted three times with 30 mL of chloroform. The aqueous phase was then discarded and the chloroform was removed by evaporation at 40 °C. Samples were then redissolved in 2 mL of methanol for HPLC analysis.

The tightly bound fractions were determined by alkaline nitrobenzene oxidation of alkali-insoluble residues and extracted hemicellulose and cellulose. The detailed methods for these oxidation and HPLC analytical procedures were described in our previous paper (Sun et al., 1995a). The weights and yields are given on a dry straw weight basis. All nitrobenzene oxidation results represent the mean of at least triplicate analyses, and each oxidation mixture was chromatographed twice.

RESULTS AND DISCUSSION

Composition of Wheat Straw Lignin. The separation of lignin-related phenolic monomers has been accomplished by high-performance liquid chromatography using an ultraviolet detector. Peaks corresponding to components in the alkaline nitrobenzene oxidation products of wheat straw were identified with reference to the following standards: gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, vanillic acid, vanillin, syringic acid, *p*-coumaric acid, syringaldehyde, acetovanillone, ferulic acid, and cinnamic acid. The structures, molecular weights, and retention times for each peak are illustrated in Table 1. With the exception of cinnamic acid, all other phenolic monomers in the samples were eluted in less than 15 min. Recoveries of the standard phenolic

Table 2. Content (Grams per 100 g of Dry Straw) of Free Phenolic Acids and Aldehydes Extracted Using a Range of Solvents from Wheat Straw

compd	methanol–toluene (1:2, 5 h)	ethanol–toluene (1:2, 22 h)	chloroform (5 h)
GA	0.0027	0.00018	0.00091
PA	0.0010	0.00097	0.00081
PHBA	0.0020	0.0013	0.00017
PHBAL	0.00042	0.00014	0.00064
VA	0.0097	0.0040	0
VAN	0.00021	0.00016	0.0013
SYA	0	0	0.0016
PCA	0.0048	0.0080	0.00089
SYAL	0.0094	0.0061	0
AV	0.00026	0.00012	0
FA	0.00014	0.00050	0.00046
CA	0.00010	0.00014	0.00021
total	0.031	0.022	0.0070

monomers in the sample preparation and HPLC procedure were high (90–92%). Variation throughout samples obtained from triplicate nitrobenzene oxidation of wheat straw, however, was high (10–22%) and yields were generally low. This observation was in a good agreement with those of Galletti et al. (1989a), who state that incomplete removal of nitrobenzene and its byproducts can seriously interfere with the HPLC elution profile and that phenolic compounds can be underestimated due to losses during extraction into the organic phase.

Free Phenolic Monomers. The content (percent dry straw weight) of free phenolic acids and aldehydes extracted with different solvents in a Soxhlet apparatus from wheat straw is given in Table 2. The total yields of phenolic compounds in methanol–toluene (1:2, 5 h), ethanol–toluene (1:2, 22 h), and chloroform (5 h) fractions were 0.031, 0.022, and 0.0070%, respectively. The major components were found to be vanillic acid, *p*-coumaric acid, and syringaldehyde in the methanol–toluene and ethanol–toluene extracts, while in the chloroform extract syringic acid and vanillin were the predominant compounds. The very low content of phenolic monomers detected in the chloroform extract indicated that wheat straw lipids are largely free of this class of compound. Vanillic acid, syringaldehyde, and

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Table 3. The Relative Composition (Percent) of Free Phenolic Acids and Aldehydes in the Hydrolysates of NaOH Treatments from Pre-extracted Wheat Straw

	extraction with 1.5% NaOH at 20 °C for											
compd	0.5 h	1 h	2 h	3 h	4 h	6 h	12 h	24 h	48 h	72 h	96 h	144 h
GA	0	0	0	0	0	0.2	0.7	1.1	1.5	1.0	0.8	0.5
PHBA	13.6	7.0	7.0	0	0	0	0	1.6	0	0	0	0
PHBAL	2.7	1.9	1.3	0.6	0.6	0.6	0.5	0.5	0.9	0.5	0.5	0.7
VA	15.0	6.9	4.8	4.6	4.4	4.2	4.8	2.2	3.7	3.6	3.5	3.5
VAN	0	1.1	2.8	3.4	5.6	7.3	8.6	9.9	6.3	5.9	5.6	6.5
SYA	3.7	4.3	7.3	14.2	13.3	3.8	5.6	6.6	6.8	8.5	9.3	10.0
PCA	8.0	39.6	34.8	32.6	27.8	20.0	18.1	17.3	20.1	22.6	24.6	23.2
SYAL	0	0	0	0	0	0.7	0.9	1.5	1.3	1.2	0.9	0.6
AV	0	0	0	0	0	0.4	1.1	1.1	1.4	1.2	0.6	0.3
FA	57.0	39.2	42.0	44.9	48.8	62.8	60.3	57.8	57.8	55.4	54.5	54.3
CA	0	0	0	0	0	0	0.08	0.4	0.2	0.2	0.04	0

acetovanillone were not identified in the chloroform extract, while they appeared as major components in the methanol−toluene and ethanol−toluene extracts. These data agreed with those in the study of Newby et al. (1980) in which only small amounts of free phenolic monomers were isolated from alfalfa. However, during an investigation of alfalfa hay, soybean stover, smooth bromegrass hay, and corn stalk, Jung et al. (1983) established that no free phenolic monomers could be extracted from the above materials using ether as a solvent and state that the phenolic monomers generally occur in the bound form in plant cells.

Loosely Bound Phenolic Monomers. It was readily established that ferulic and *p*-coumaric acid were the predominant components in the alkali-labile free phenolic monomers fractions. Small amounts of free *p*-hydroxybenzaldehyde, vanillic acid, vanillin, and syringic acid and traces of free gallic acid, *p*-hydroxybenzoic acid, syringaldehyde, acetovanillone, and cinnamic acid were also detected in the extracts. The presence of free phenolic aldehydes in the alkaline extracts from wheat straw agrees with the observations of Fritz and Moore (1987). The characterization of bound phenolic monomers in the alkaline extracts will be given elsewhere (Sun et al., 1995b).

The relative compositions of the loosely bound phenolic acids and aldehydes extracted using 1.5% NaOH (20 °C for different lengths of time) from wheat straw are presented in Table 3. Protocatechuic acid was not detected in any of the samples, which was not in agreement with the findings of Jung et al. (1983). The authors found that protocatechuic acid was the third most abundant component in the alkali-labile phenolic monomer fractions of alfalfa hay, soybean stover, smooth bromegrass hay, and corn stalk, and this was consistent for all of the studied materials. Gallic acid, syringaldehyde, acetovanillone, and cinnamic acid were detected only after 4 or 6 h treatments, whereas *p*-hydroxybenzoic acid was not in samples treated for more than 2 h (with the exception of the 24 h treatment sample). In general, legumes (alfalfa, soybean stover) have been shown to contain lower amounts of alkali-labile phenolic monomers than grasses (smooth bromegrass, corn stalk) (Jung et al., 1983). Hartley and Jones (1977) have also reported that larger quantities of *p*-coumaric and ferulic acid can be extracted with alkali from grass cell walls than from those of legumes. The authors indicated that the phenolic acid components of water-soluble carbohydrate esters from these plants released by treatment with sodium hydroxide contained *trans*-ferulic acid as the main component with smaller amounts of *cis*-ferulic, *cis*- and *trans*-*p*-coumaric, and diferulic acids. In a study of the distribution of free and covalently bound phenolic acid in various fractions of Lucerne, Newby et

al. (1980) reported that in the covalently combined forms of phenolic acid, the *trans* forms predominated in every case. Data displayed in Table 3 show that ferulic and *p*-coumaric acid were the predominant compounds and comprised 65−82% of the total free phenolic monomers in all of the 1.5% NaOH extracts (20 °C for various times). Free syringic acid, vanillic acid, vanillin, and *p*-hydroxybenzaldehyde were present as minor constituents. These results were in accordance with the study of Fritz and Moore (1987), in which the content of ferulic and *p*-coumaric acid in alkali-liable phenolic monomers extracted from orchardgrass cell wall was found to be 22.01 g kg^{-1}, whereas that of vanillin was only 0.15 g kg^{-1}. Other phenolic acid and aldehydes were not detected in the alkali extract from orchardgrass cell walls.

In contrast to the high contents of ferulic and *p*-coumaric acids, the low level of other phenolic monomers in the 1.5% NaOH extracts is not surprising, as NaOH treatment would be expected to saponify the hydroxycinnamic esters that have been hypothesized to cross-link lignin to a cell wall hemicellulose (Schwarz et al., 1989). Scalbert and Monties (1986) reported that the phenolic and carboxylic groups, resulting from the hydrolysis of ether and ester bonds, are ionized in the alkaline solution. From a study of the soluble lignins from untreated and ammonia-treated wheat straw, Kondo et al. (1992) have stated that in both straw samples a large proportion of the detected *p*-coumaric acid was originally present in the ester-linked form, whereas more than 50% of the ferulic acid was involved in ether links. The authors also indicated that marginally higher levels of total *p*-coumaric and ferulic acid were found in the untreated straw. For three lignin preparations isolated from wheat straw, Scalbert et al. (1985) have suggested that *p*-coumaric acid was mainly ester-linked, whereas 35−75% of the recovered ferulic acid was ether-linked to milled straw lignin or enzyme lignin. In addition, ester-linked *p*-coumaric acid is probably exclusively associated with lignin and the majority of *p*-coumaric acid molecules in grass lignins are linked to the γ-position of the side chain of lignin molecules (Shimada et al., 1971). Ferulic acid, on the other hand, is both etherified to lignin and (mainly) esterified to arabinoxylans and forms an alkali-labile cross-link between these two cell wall polymers (Scalbert et al., 1986b). Such a relatively higher content of alkali-labile cross-linkages within the lignin network or between lignin and polysaccharides might explain the high solubility of straw lignin in soda. In our previous study, treatment with 1.5% NaOH for 6 days at 20 °C released about 60% lignin and 80% hemicellulose. More details of the effect of NaOH treatment on the cell wall

Table 4. Relative Concentration (Percent) of Free Phenolic Acids and Aldehydes in the Hydrolysates of KOH, NaOH, and LiOH Treatments (at 20 °C for 6 h) from Pre-extracted Wheat Straw

compd	1.5% KOH hydrolysate	1.5% NaOH hydrolysate	1.5% LiOH hydrolysate
GA	0.1	0.2	0.3
PHBA	0.09	0	0
PHBAL	0.4	0.6	0.2
VA	2.8	4.2	4.9
VAN	7.4	7.3	6.4
SYA	4.8	3.8	6.4
PCA	19.4	20.0	18.7
SYAL	0.9	0.7	1.3
AV	0.6	0.4	0.2
FA	63.5	62.8	61.6

Table 5. Content (Grams per 100 g of Dry Treated Straw) of Tightly Bound Phenolic Acids and Aldehydes Resulting from Alkaline Nitrobenzene Oxidation of Wheat Straw Treated with a Range of Chemicals

compd[a]	treatment chemicals (1.5%, at 20 °C for 6 h)					
	H_2O_2	$NH_3 \cdot H_2O$	$Ca(OH)_2$	KOH	NaOH	LiOH
GA	0	0.19	0.20	0.18	0.11	0.079
PA	0	0.034	0	0	0	0
PHBA	0	0.050	0.074	0.052	0.047	0
PHBAL	0.25	0.17	0.15	0.21	0.11	0.053
VA	0.11	0.081	0.089	0.084	0.051	0.010
VAN	2.23	2.16	1.87	1.69	1.69	1.20
SYA	0	0.085	0	0	0	0
PCA	0	0.050	0	0	0.010	0
SYAL	3.69	2.31	1.96	1.85	1.89	1.60
AV	0.15	0.058	0.086	0.089	0.083	0.071
FA	0.20	0.26	0.12	0.18	0.17	0.15
CA	0.0061	0.0042	0.040	0.039	0.023	0.060
total yield	6.64	5.45	4.59	4.37	4.18	3.22

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

components of wheat straw have been described (Sun et al., 1995a).

The relative concentrations of free phenolic acids and aldehydes in the hydrolysates resulting from 1.5% KOH, NaOH, and LiOH treatments (20 °C for 6 h) of wheat straw are summarised in Table 4. As can be seen, the net percentage of free ferulic and *p*-coumaric acid was over 80% in each case. The distribution of the free phenolic monomers in each of the extracts was similar.

Tightly Bound Phenolic Monomers. Alkali-labile phenolic monomers represent relatively loosely bound compounds in wheat straw. In contrast, nitrobenzene oxidation has been regarded as a technique for degrading lignin, which can be described as a "tightly bound" material. The tightly bound phenolic acids and aldehydes obtained by alkaline nitrobenzene oxidation of wheat straw treated with 1.5% of the various reagents at 20 °C for 6 h are shown in Table 5. The major products of alkaline nitrobenzene oxidation were found to be syringaldehyde, vanillin, *p*-hydroxybenzaldehyde, and ferulic acid. Vanillin, syringaldehyde, and *p*-hydroxybenzaldehyde are the usual products reported from the alkaline nitrobenzene oxidation of grass lignin and are considered a diagnostic test for lignin (Schwarz et al., 1989). *p*-Hydroxybenzaldehyde has always been found to be the least abundant component of the three, and this was also the case in our study. As can be seen from Table 5, the content of syringaldehyde was higher than that of vanillin in all treated wheat straw samples, and the molar ratios of vanillin to syringaldehyde were 0.72, 1.12, 1.14, 1.09, 1.07, and 0.90, respectively, in 1.5% H_2O_2-, $NH_3 \cdot H_2O$-, $Ca(OH)_2$-, KOH-, NaOH-, and
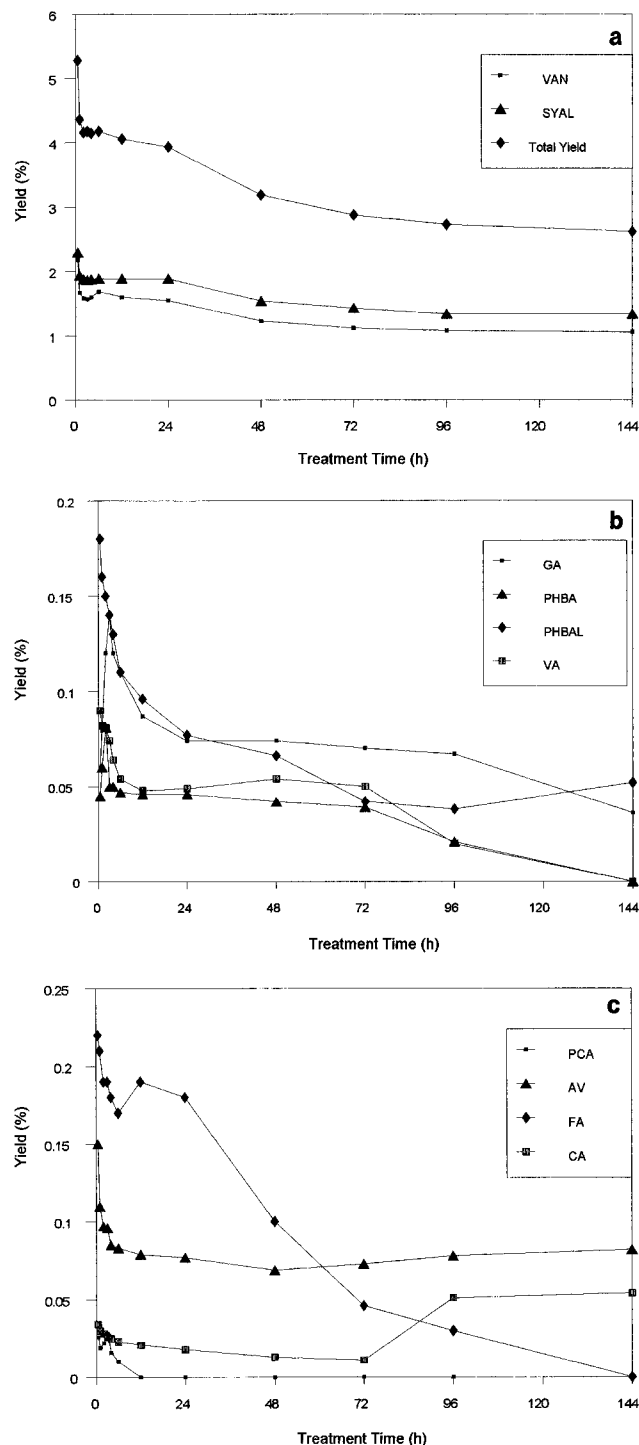
LiOH-treated (20 °C, 6 h) wheat straw. In contrast, untreated wheat straw examined in this study yielded more vanillin than syringaldehyde and the molar ratio of vanillin to syringaldehyde was found to be 1.26 (data not shown). Three previously unreported phenolic monomers, gallic acid, protocatechuic acid, and acetovanillone, were also identified as products of alkaline nitrobenzene oxidation of wheat straw in this study. Higuchi et al. (1967) have shown that vanillin and syringaldehyde in grasses vary in their relative proportions to each other. Reeves (1985) found that the relative amounts of vanillin and syringaldehyde in the nitrobenzene oxidation products of untreated wheat straw were 43.5 and 30.7%, respectively, which is in approximate agreement with our study. Scalbert et al. (1986b) have reported that lignin preparations from wheat straw contained equivalent amounts of guaiacyl- and syringylpropane units and low amounts of *p*-hydroxyphenylpropane units. In addition, by analyses of alkaline nitrobenzene oxidation products of Björkman lignin from wheat straw internodes before and after saponification, Iiyama et al. (1990) observed no differences, suggesting that the aromatic structures in these lignins were not altered during saponification. These differences in apparent composition of wheat straw lignin are probably due to the different sample sources.

According to the data displayed in Table 5, the yield of nitrobenzene oxidation and the composition of tightly bound phenolic monomers in the oxidation products were very dependent on the various chemical treatments. Due to the high solubility of lignin in the 1.5% KOH, NaOH, and LiOH treatments (20 °C for 6 h), the yields of oxidation products from these three treated wheat straw samples were low, 4.37−3.22%. There was no loss of lignin in the treatments with 1.5% calcium hydroxide and liquid ammonia for 6 h at 20 °C, while for the treatment of straw with 1.5% hydrogen peroxide at 20 °C for 6 h, more than 7% of lignin was dissolved. This is most likely due to the oxidizing action of H_2O_2. Interestingly, compared to the yield from untreated wheat straw, 6.00% (data not shown), the yield of nitrobenzene oxidation products was increased by treatment with hydrogen peroxide and generally decreased as a result of the other chemical regimes. These results demonstrate that nitrobenzene oxidation of H_2O_2-treated wheat straw leads to higher levels of total phenolic monomers than for the other treated straws. However, no correlation with lignin content was observed because $Ca(OH)_2$- and $NH_3 \cdot H_2O$-treated wheat straw had high lignin contents but lower total concentrations of phenolic monomers. In addition, the yield of phenolic acids and aldehydes from alkaline nitrobenzene oxidation is much lower than that expected on the basis of the acidic sodium chlorite result (14−15%). This could also be due to a higher degree of condensation of wheat straw lignin aromatic nuclei. Kondo et al. (1992) indicated that the soluble lignin from ammonia-treated wheat straw contained less *p*-coumaric acid and much more ferulic acid than that obtained from untreated straw. However, the results in this study show that the content of *p*-coumaric acid in the nitrobenzene oxidation from untreated wheat straw was 0.07% and that this decreased dramatically to 0.01% in 1.5% NaOH-treated wheat straw, whereas the concentration of ferulic acid in the nitrobenzene oxidation mixture reduced only from 0.25 to 0.17% between untreated and 1.5% NaOH-treated samples. The release of lignin fragments from wheat straw by alkali treatment may be ascribable to

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cleavage of ester linkages both in ferulic acid and in *p*-coumaric acid bridges. Ether linkages between lignin and ferulic acid are less available and hence remain. Therefore, it can be concluded that about 90% of the *p*-coumaric acid in wheat straw was present in the ester-linked form to lignin and to polysaccharides, whereas about 50% of ferulic acid was ester-linked to polysaccharides. This observation is in agreement with the result obtained by Scalbert et al. (1985), who concluded that at least 93% of *p*-coumaric acid is alkali-labile and thus linked by an ester bond with lignin. However, ferulic acid might be cross-linked between lignin and hemicellulose by esterification of its carboxyl group with the hydroxyl group of arabinose residue in hemicellulose and etherification of its phenolic hydroxyl group of lignin.

The effect of 1.5% NaOH treatment time on the variation in lignin composition, examined with the yield of tightly bound phenolic acids and aldehydes of alkaline nitrobenzene oxidation of treated wheat straw, seemed to depend on the treatment time (Figure 1). The total yield of oxidation products appeared always to decrease with the increase of treatment time from 0.5 to 144 h. The contents of the major components syringaldehyde and vanillin were parallel to the total yield of oxidation products. The concentration of syringaldehyde was higher than that of vanillin in all of the 1.5% NaOH-treated samples (room temperature). Gallic acid, *p*-hydroxybenzaldehyde, and ferulic acid showed the largest concentration changes with treatment time, although these compounds were not the most abundant. As the treatment time increased from 0.5 to 144 h, the relative contents of *p*-hydroxy benzaldehyde and ferulic acid dropped from 0.18 to 0.05% and from 0.22 to 0%, respectively. Because the cinnamic acids should remain in the nitrobenzene oxidation products mixture after 2 h at 170 °C, the reduced ferulic acid content after a 24 h period of treatment and its disappearance after 144 h suggested that ether linkages between ferulic acid and lignin might be also cleaved after longer periods of treatment. Liu et al. (1989) state that phenolic acid ester and phenolic α-*O*-4 ether linkages are easily hydrolyzed in alkaline medium (1 N NaOH, 25 °C) and nonphenolic α-benzylaryl ether bonds might be hydrolyzed in the temperature condition of acidolysis. Furthermore, no cleavage of β-aryl ether bonds occurs under either set of conditions. This suggested that the ferulic and *p*-coumaric acid were released during 1.5% NaOH treatments from either ester or phenolic α-*O*-4 ether bonds or from both of them.

The composition of tightly bound phenolic monomers in the alkaline nitrobenzene oxidation products of wheat straw hemicellulose and cellulose, extracted with 24% KOH and 2% boric acid at 20 °C for 2 h from untreated and 1.5% NaOH-treated (20 °C for 6 and 144 h) wheat straw, is given in Table 6. With the increase of treatment time from 0 to 144 h, the contents of phenolic acids and aldehydes in extracted hemicellulose and cellulose were observed to decrease from 0.26 to 0.067% and from 0.20 to 0.10%, respectively. The major components of phenolic monomers in the alkaline nitrobenzene oxidation of residual lignin in extracted hemicellulose and cellulose were vanillic acid, vanillin, and syringic acid. Interestingly, the yields of *p*-coumaric acid, ferulic acid, and cinnamic acid in the alkaline nitrobenzene oxidation products from untreated wheat straw hemicellulose and cellulose were much higher than those from 1.5% NaOH-treated wheat straw hemi-

Figure 1. Effect of 1.5% NaOH treatment time at 20 °C on the yield (percent dry NaOH treated straw) of tightly bound phenolic acids and aldehydes of alkaline nitrobenzene oxidation lignin from treated wheat straw.

cellulose and cellulose, suggesting that ester linkages of *p*-coumaric and ferulic acid were cleaved during the treatment with 1.5% NaOH at room temperature. In contrast, the resistance to extraction of *p*-coumaric and ferulic acid by 1.5% NaOH, acidic sodium chlorite, and 24% KOH indicated that some amounts of ferulic acid and *p*-coumaric acid also appeared in aryl ether linkages in wheat straw cell walls. These results support models involving bridges between wheat straw polysaccharide and lignin through ferulic acid (Scalbert et al., 1985).

FT-IR Spectra. The FT-IR spectra of untreated wheat straw and of wheat straw treated with 1.5%

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Table 6. Content (Grams per 100 g of Dry Hemicellulose or Cellulose) of Tightly Bound Phenolic Acids and Aldehydes Resulting from Alkaline Nitrobenzene Oxidation of Cellulose and Hemicellulose Fractions Isolated from both Untreated and 1.5% NaOH-Treated (20 °C, 6 and 144 h) Wheat Straw

compd[a]	hemicelluloses (treated wheat straw time)			celluloses (treated wheat straw time)		
	0 h	6 h	144 h	0 h	6 h	144 h
GA	0.0086	0.0048	0.0040	0.025	0.0054	0.0040
PCA	0	tr	0.0030	0	0	0
PHBA	0.0054	0.0042	0.0033	0	tr	0.023
PHBAL	0.0075	0.0065	0.0034	0.0074	0.0043	0.0045
VA	0.075	0.026	0.0087	0.037	0.026	0.016
VAN	0.047	0.025	0.018	0.036	0.028	0.019
SYA	0.056	0.014	0.013	0.054	0.030	0.029
PCA	0.014	0.0050	0.0038	0.0037	0.0022	0.0011
SYAL	0.023	0.0012	0.0020	0.015	0.0062	0.0010
AV	0.0012	0.0012	0.0011	0.0013	tr	0
FA	0.0058	0.0036	0.0032	0.0056	0.0017	tr
CA	0.010	0.0042	0.0011	0.014	0.0090	0.0053
unknown	0.0048	0.0038	0.0024	0.0033	0.0032	0.0019
total	0.26	0.10	0.067	0.20	0.12	0.10

[a] Determined by HPLC after alkaline nitrobenzene oxidation at 170°C for 2.5 h in steel autoclaves.
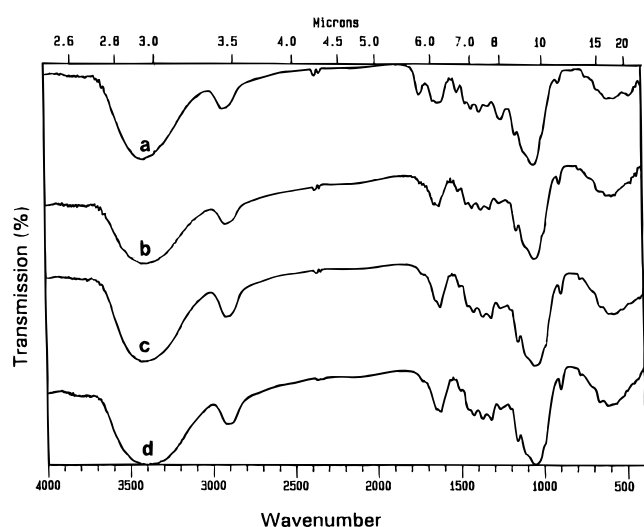
Figure 2. FT-IR spectra of untreated wheat straw (a) and 6 h (b), 72 h (c), and 144 h (d) treated (1.5% NaOH, 20 °C) wheat straw.

NaOH at 20 °C for 6, 72, and 144 h are shown in Figure 2 with the bands of interest being identified by their wavenumbers. All four spectra appear to be similar; however, on closer examination it is apparent that the spectra of treated wheat straw (b, c, d) can be clearly distinguished from the spectrum of untreated wheat straw (a) by the disappearance of the ester linkage absorption at 1720 cm^{-1}. This corresponds to ester linkages between cinnamic acids and lignin/polysaccharides or of cutin from the waxy cuticular layer. This indicated that 1.5% NaOH treatment at 20 °C results in thorough de-esterification. In addition, the ratios of the absorptions at 1311 (syringyl units) and 1250 cm^{-1} (guaiacyl units) to that at 1510 cm^{-1} are similar among the three treated wheat straw samples. This is in agreement with the results of the alkaline nitrobenzene oxidation, which indicate high syringyl content in all of the treated wheat straw samples. In contrast, in untreated straw (a) the intensity of the absorption at 1250 cm^{-1} was much stronger than in treated wheat straw (b, c, d), suggesting a high guaiacyl content in the original straw. With the increase of treatment time from 6 to 144 h, the intensity of the band at 1311 cm^{-1} increased, while that at 1250 cm^{-1} was observed to decrease, illustrating that substantially more guaiacyl

units than syringyl units were released during the longer period of alkaline treatment. Another distinct difference appeared at 1510 cm^{-1} due to aryl-H vibrations in wheat straw lignin (Stewart and Morrison, 1992). Not surprisingly, this absorption was considerably more intense in the untreated sample, reflecting the higher level of lignin in this substrate. In accordance with our earlier findings, the intensity of the peak at 1510 cm^{-1} was observed to decrease as the treatment time increased from 6 to 144 h. It has been reported previously that the ester-linked feruloyl and *p*-coumaroyl groups contribute to both the 1720 and 1510 cm^{-1} absorbances (Stewart and Morrison, 1992). The loss of the 1720 cm^{-1} absorbance and reduction in the 1510 cm^{-1} absorbance in the treated wheat straw spectra indicated that amounts of ester-linked ferulic and *p*-coumaric acid were released during the alkaline treatments. It can also be seen that there are two approximately equal weak absorptions at 1637 and 1600 cm^{-1} in the spectrum of untreated wheat straw, whereas the treated wheat straw has a weak absorption at 1637 cm^{-1} and a strong absorbance at 1600 cm^{-1}. The 1637 cm^{-1} band is a carbonyl stretching band due to para-substituted ketone or aryl aldehydes (Jung and Himmelsbach, 1989). Besides aromatic skeleton vibrations, the 1600 cm^{-1} band also contains components due to the polysaccharide-derived vibrations, suggesting high polysaccharide content in treated wheat straw. Another obvious effect of alkali treatment was the partial dissolution of SiO$_2$ and indeed the stretching Si$-$O bands at 460 and 800 cm^{-1} are scarcely detectable in the spectra of alkali treated wheat straw. Methyl, methylene, and methine group vibrations appear at 2924 cm^{-1} and were present as shoulders in all straw spectra. The bands at 2330 and 2366 cm^{-1} in the untreated wheat straw spectrum are relatively more intense than the corresponding absorptions in the treated wheat straw spectra. The strong bands in the 1200$-$870 cm^{-1}, region can be ascribed to the polysaccharides present in all samples examined. It was also noted that the very strong broad band at 1035 cm^{-1} which probably derives from polysaccharide vibrations, appeared in all of the straw spectra.

The results presented indicate the presence of 12 simple phenolic monomers in wheat straw after methanol$-$toluene, ethanol$-$toluene, chloroform extraction, alkali treatments, and nitrobenzene oxidation. The

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content of free phenolic acids and aldehydes was quite low, 0.0070−0.031% of dry straw. Alkali treatments cleave ester linkages both in ferulic acid and in *p*-coumaric acid bridges. Ferulic acid and *p*-coumaric acid comprise 65−82% of the total free phenolic monomers in the alkali extracts. About 90% of the *p*-coumaric acid in wheat straw is present in the ester-linked form to lignin and to polysaccharides, whereas approximately 50% of ferulic acid is ester-linked to polysaccharides. The major products of alkaline nitrobenzene oxidation were found to be syringaldehyde, vanillin, and *p*-hydroxybenzaldehyde. The content of syringaldehyde was higher than that of vanillin in all alkali-treated wheat straw samples, while untreated straw yielded more vanillin than syringaldehyde in the alkaline nitrobenzene oxidation products. The relationship between chemical or physical properties of lignin and monomer composition needs to be identified, and this work is currently underway in our laboratory.

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