Delignification of rye straw using hydrogen peroxide

Run Cang Sun *, J.M. Fang, J. Tomkinson

The BioComposites Centre, University of Wales, Bangor, Gwynedd, Wales LL57 2UW, UK
Accepted 14 January 2000

Abstract

Alkaline peroxide delignification of rye straw has been first investigated in this paper. The results showed that treatment of dewaxed and water-extracted rye straw with 2% H₂O₂ at pH 11.5 for 12 h at 20, 30, 40, 50, 60, and 70°C resulted in a dissolution of 52.7, 75.7, 81.8, 83.1, 85.8, and 87.8% of the original lignin, and 44.2, 52.5, 70.0, 70.0, 71.3, and 71.9% of the original hemicelluloses, respectively. The isolated pure lignin fractions contained rather low amounts of neutral sugars, 0.4–1.1%, and had weight-average molecular weights between 2420 and 3480 g mol⁻¹. They contained almost equal amounts of noncondensed guaiacyl and syringyl units with fewer p-hydroxyphenyl units. The β–O–4 ether bonds together with β–β and β–5 carbon–carbon linkages were found to be present in the lignin structural units. Hydroxycinnamic acids such as p-coumaric and ferulic acids appeared to be strongly associated to lignin molecules. Comparison of these lignin samples indicated that the alkaline peroxide treatment of the straw under the conditions given did not affect the overall structure of lignin. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Rye straw; Hydrogen peroxide; Lignin; Hemicelluloses; Degradation

1. Introduction

World production of rye, one of the major cereal crops, exceeds 30 million tons per annum, which yields about 40–50 million tons of straw (Ghaly and Ergudenler, 1994). This straw and other agricultural residues exiting as waste streams from commercial crop processing plants have little inherent value and have traditionally constituted a disposal problem. These materials represent an abundant, inexpensive, and readily available source of renewable lignocellulosic biomass. However, their utilisation as a carbohydrate source for glucose and ethanol production, and as a metabolic energy source in ruminant feeds, has been severely hampered by the low efficiency with which organisms and enzymes are able to convert the polysaccharide portion of the residue into monomeric sugars (Gould, 1989). The low conversion efficiency for lignocellulosic materials and low digestibility is largely due to the lignin component of the cell wall and its association with other cell wall polysaccharides, which prevents the degradation of cellulose mainly by acting as a physical barrier between the cellulose enzyme and its substrate.
Lignin is an aromatic biopolymer, an integral cell wall constituent in all vascular plants including the herbaceous varieties. Lignin is unique among biopolymers in that there is limited control over its biosynthesis, and the factors guiding this process are not yet fully understood (Ammalahti et al., 1998). Lignin is built up by oxidative coupling of three major $C_6–C_3$ (phenypropanoid) units, namely, syringyl alcohol (S), guaiacyl alcohol (G), and $p$-coumaryl alcohol (H), which form a randomised structure in a tridimensional network inside the cell walls. The major interunit linkage is an aryl–aryl ether type. Besides the some 20 different types of bonds present within the lignin itself, lignin seems to be particularly associated with the hemicellulosic polysaccharides. Owing to its crosslinking, lignin in situ is usually insoluble in all solvents, unless it is degraded by physical or chemical treatments (Sun et al., 1999).

There is no known method for release of unaltered lignin from plant cell walls, and chemical or biochemical degradation methods produce low molecular weight products in modest yields only. However, numerous treatments have been developed in an effort to increase the removal of lignin from straw and grass. These processes utilise physical, chemical, and/or biological methods to remove lignin and decrease cellulose crystallinity. Among these, the processes such as autohydrolysis, alkaline cooking, and steam explosion require substantial energy input in the form of heat and tend to generate toxic side products. Other drawbacks typical of conventional treatments include loss of the hemicelluloses with the solubilized fraction (Gould, 1989). For example, in the cooking such as chemical pulping processes lignin is dissolved from the raw material at high pressures and temperatures under aqueous alkaline, neutral or acidic conditions. Important delignification reaction include the cleavage of phenolic $\alpha-O-4$ linkages, cleavage of non-phenolic $\beta-O-4$ linkages, and removal of residual lignin fractions, either by cleavage of carbon–carbon linkages or by carbohydrate degradation, releasing lignin-carbohydrate fractions, which are mainly oxidised into aliphatic carboxylic acids.

It is well known that hydrogen peroxide reacts with lignin under alkaline conditions and has widely been used for many years to bleach high-lignin wood pulps. The bleaching effect of hydrogen peroxide has been attributed to its ability to react with various coloured carbonyl-containing structures in lignin. This reaction has been explained through the reactions of the hydroperoxide anion ($\text{HOO}^-$), formed in an alkaline medium according to the equilibrium:

$$\ce{H2O2 + HO^- \leftrightarrow \text{HOO}^- + H2O}$$

where the $\ce{pK_a}$ is $11.6$ at $25^\circ\text{C}$.

This anion is a strong nucleophile that, during bleaching, preferentially attacks ethylenic and carbonyl groups present in lignin. As a consequence, such chromophores as quinones, cinnamaldehyde, and ring-conjugated ketones are converted to nonchromophoric species during the alkaline solution (Dence, 1996; Pan et al., 1998). On the other hand, hydrogen peroxide is unstable in alkaline conditions and readily decomposes, particularly in the presence of certain transition metals such as manganese, iron, and copper. This metal-catalysed decomposition of hydrogen peroxide is undesired in the bleaching process, since it leads to a loss of bleaching capacity and generates more active radicals such as the hydroxyl radicals ($\text{HO}^\cdot$) and superoxide anion radicals ($\text{O}_2^-\cdot$), which participate in the delignifying mechanism. This dual role of hydrogen peroxide in delignifying and bleaching has been investigated in detail by Gould (1984, 1985). Approximately one-half of the lignin and most of the hemicelluloses present in agricultural residues such as wheat straw and corn stover are solubilized when the residue is treated at $25^\circ\text{C}$ in an alkaline solution of $1\% \text{ H}_2\text{O}_2$. The delignification reaction is most efficient when the ratio of $\text{H}_2\text{O}_2$ to substrate is at least $0.25$ (w/w) and the pH is $11.5$ (Gould, 1984). In the process of alkaline peroxide treatment, wheat straw is delignified and bleached by alkaline peroxide solution and subsequently defibrated mechanically to pulp. As a high-yield pulping method, it is important to produce a pulp of acceptable brightness with a significant dissolution of lignin but a minimal degradation of cellulose. This paper reports the effect of alkaline peroxide treatment temperature
on the solubilization of lignin from rye straw in our newly developed process in which straws are sequentially treated with water and alkaline peroxide solutions. The isolated lignin preparations are physico-chemically characterised and the results are reported.

2. Material and methods

2.1. Material

Rye straw was obtained from Compak Co. (Gainsborough, UK), and was ground to pass a 0.7 mm size screen. The composition (w/w) of the rye straw used was cellulose 37.9%, hemicelluloses 36.9%, lignin 17.6%, protein 3.3%, ash 3.0% and wax 2.0%. All weights and calculations were made on an oven-dried (50°C, 16 h) basis. All chemicals used were of analytical or reagent grade.

2.2. Alkaline peroxide treatment

The dried powder was first extracted with toluene–ethanol (2:1, v/v) in a Soxhlet for 6 h. The dewaxed straw was then treated with water at 50°C for 2 h. After isolation of the water-soluble hemicelluloses by precipitation of water-extracts in three volumes ethanol, water-soluble lignin fraction was obtained by reprecipitation of water-extracts from the supernatant solution. Samples free of wax and water solubles (10.0 g) were added to 250 ml of distilled water containing 2% \( \text{H}_2\text{O}_2 \) (w/v) in a jacketed reaction vessel heated with water from a thermostat-controlled circulating bath. The suspension was adjusted to pH 11.5 with 4 M NaOH and allowed to stir gently for 12 h at 20, 30, 40, 50, 60, and 70°C, respectively. In comparison, one sample was treated with dilute alkaline solution at pH 11.5 for 12 h at 50°C in the absence of \( \text{H}_2\text{O}_2 \) from water-soluble free and dewaxed rye straw. During initial stages of stirring, oxygen evolution was active, and substantial frothing occurred, requiring that extractions were conducted in vessels with volumes two to three times those of extraction mixtures. No further adjustments in pH were made during the course of the treatment. Under these conditions, the reaction pH remained nearly constant for 2 h before slowly rising to a final value of ca 13.0. The insoluble residue was collected by filtration, washed with distilled water until the pH of the filtrate was neutral, and then dried at 60°C. The supernatant fluid was adjusted to pH 5.5 with 10% HCl and then concentrated. The released hemicelluloses were precipitated by pouring the concentrated supernatant fluid into three volumes of ethanol. The solubilized lignins were obtained from the corresponding supernatants as the method above (Fig. 1).

2.3. Characterisation of the lignin fractions PL

FT-IR spectra were obtained on a FT-IR spectrophotometer (Nicolet, 750) using a KBr disc containing 1% finely ground samples. The solution-state \(^{13}\text{C}\)-NMR spectrum was obtained on a Bruker 250 AC spectrometer operating in the FT mode at 62.4 MHz under total proton decoupled
3. Results and discussion

3.1. Lignin yield

The yield of lignin resulting from the various alkaline peroxide procedures was expressed as a percentage of dry starting material. Table 1 shows that treatment of dewaxed and water-extracted rye straw with 2% H₂O₂ at pH 11.5 for 12 h at 20, 30, 40, 50, 60, and 70°C resulted in a dissolution of 52.7, 75.7, 81.8, 83.1, 85.8, and 87.8% of the original lignin, and 44.2, 52.5, 70.0, 70.0, 71.3, and 71.9% of the original hemicelluloses, respectively. As expected, the isolated pure lignin (PL) preparation was the major fraction, comprising 60.0–74.4% of the total solubilized lignins, while the lignin fraction, associated in the solubilized hemicelluloses, was accounted only 8.9–12.8% of the total released lignins. This result indicated that treatment with 2% H₂O₂ under the conditions used significantly cleaved the ether linkages between lignin and hemicelluloses from the cell walls of rye straw. An increment in temperature from 20 to 40°C led to a significant growth of PL fraction from 5.5 to 9.0%. However, as the temperature was further increased to 70°C, a decrease of 13.3% yield of PL was found. On the other hand, as can be seen in Table 1, an increasing temperature from 20 to 70°C, the yield of lignin, solubilized in the supernatant (pH 1.5) enhanced by three-fold (from 1.3 to 3.9%), indicating that treatment with 2% H₂O₂ under the relatively high temperature could lead to substantial degradation of the solubilized lignin into small and acidic conditions. They are recorded at 25°C from 250 mg of sample dissolved in 1.0 ml DMSO-d₆ after 28 000 scans. A 60° pulse flipping angle, a 3.9 μs pulse width and 0.85 s acquisition time were used.

Methods of uronic acid analyses, UV spectra recording, measurement of the molecular-average weights of lignin fractions, and determination of phenolic acids and aldehydes with HPLC in nitrobenzene oxidation mixtures have been described in previous papers (Lawther et al., 1995; Sun et al., 1995). Neutral sugar composition in isolated lignin fractions was determined as alditol acetates (Blakeney et al., 1983). All nitrobenzene oxidation results represent the mean of at least triplicate samples and each oxidation mixture was chromatographed twice. Other experiments were performed in duplicate. The standard errors (S.E.) or deviations (S.D.) were observed to be lower than 6.2% except for the variation among the triplicate nitrobenzene oxidation (7.8–15.8%).

### Table 1

<table>
<thead>
<tr>
<th>Yield</th>
<th>2% H₂O₂ (pH 11.5, 12 h) treatment temperature (°C)</th>
<th>WSb</th>
<th>DASc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solubilized lignin</td>
<td>7.8 11.2 12.1 12.3 12.7 13.0 2.8 1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolated pure lignin (PL)d</td>
<td>5.5 8.2 9.0 8.1 7.8 7.8 1.7 0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lignin associated in the isolated hemicelluloses</td>
<td>1.0 1.0 1.2 1.2 1.3 1.3 0.8 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lignin solubilized in the supernatant (pH 1.5)e</td>
<td>1.3 2.0 1.9 3.0 3.6 3.9 0.3 0.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note:

- a The lignin fractions obtained by treatment of water-soluble-free and dewaxed rye straw with 2% H₂O₂ at pH 11.5 for 12 h at different temperatures.
- b The lignin fraction obtained by treatment of the dewaxed rye straw with water at 50°C for 2 h.
- c The lignin fraction extracted with dilute alkaline solution (pH 11.5) at 50°C for 12 h in the absence of H₂O₂ from water-soluble-free and dewaxed rye straw.
- d Represent for the lignin fraction obtained by precipitation of the supernatant solution at pH 1.5 after isolation of the hemicelluloses.
- e Represent for the lignin fraction which is still solubilized in the supernatant (pH 1.5) after precipitation of the pure lignins (PL).
water-soluble fragments. This increasing yield, therefore, offset the negative effect of temperature on the yield of PL decreased.

All in all, the data in Table 1 showed that the rate of alkaline peroxide delignification was strongly influenced by temperature. Increasing temperature from 20 to 70°C resulted in yield of total solubilized lignin from 7.8 to 13.0%. The reason for this high rate of delignification at high temperature is that hydrogen peroxide decomposition is strongly dependent upon temperature, which generates more active radicals such as hydroxyl radicals (HO') at high temperature, participating in degradation reactions of lignin and polysaccharides.

As mentioned above, the hydroperoxide anions (HOO') are principal active species involved in the elimination of chromophores in lignin structures, particularly conjugated carbonyl structures that are prone to react with the hydroperoxide anion. On the other hand, the peroxide decomposition products such as the hydroxyl radicals and superoxide anion radicals (O_2^-) are thought to cause the oxidation of lignin structures which leads to the introduction of hydrophilic (carboxyl) groups, the cleavage of some interunit bonds and, eventually, the dissolution of lignin even though they also may participate in the bleaching mechanism, at least to a small extent (Dence, 1996). At alkaline pH, these radicals are formed during the degradation of H_2O_2 in a reaction with hydroperoxide anion in the presence or in the absence of transition metals:

\[ \text{H}_2\text{O}_2 + \text{HOO}^- \rightarrow \text{HO}^+ + \text{O}_2^- + \text{H}_2\text{O} \]

In the absence of reactants for HO' and O_2^-, these radicals again react with each to form O_2^· and HO^·, giving an overall O_2 yield of 0.5 mol O_2/mol H_2O_2 (Gould, 1985), and subsequently increasing the reaction pH:

\[ \text{HO} + \text{O}_2^· \rightarrow \text{O}_2^· + \text{HO}^- \]

Our earlier studies on the solubilization of lignin from maize stem using hydrogen peroxide showed that the delignification reaction was strongly dependent on pH, with a sharp increase at pH 11.5 and a continuous increment up to pH 12.6 (Sun and Tomkinson, 2000). It was, therefore, not necessary to continuously regulate the reaction pH at 11.5, even though over the course of the treatment (12 h) the reaction pH rose from 11.5 to ca 13.0, since over 80% of the original lignin was solubilized during the treatment temperatures above 40°C. Interestingly, as the reaction pH became more alkaline, increasing amounts of hemicelluloses were solubilized as shown by over 70% of the original hemicelluloses released at 40–70°C. The current results obtained were in good agreement with the studies of delignification with alkaline peroxide from wheat straw by Gould (1984, 1985). The author showed that treatment of wheat straw with 1% H_2O_2 at room temperature and pH between 11.5 and 12.5 resulted in 60% degree of delignification.

In comparison, one sample of dewaxed and water-extracted residue was treated with a dilute alkaline solution (pH 11.5) at 50°C for 12 h in the absence of H_2O_2. As can be seen from Table 1, the yield of lignin, solubilized during this similar conditions but in the absence of peroxide, appeared only one eleventh of that, released during the treatment with 2% H_2O_2 at 50°C for 12 h at pH 11.5 from the dewaxed and water-soluble free straw. This once again indicated that alkaline peroxide had a marked effect on delignification from agricultural residues such as rye straw. Similar results were obtained by Gould (1984) from wheat straw. Very little lignin was solubilized at pH 11 even though as much as 30% of the lignin was solubilized at pH 13. However, in the presence of 1% H_2O_2, substantially more lignin (50%) was solubilized at room temperature and pH 11.5. Obviously, a much higher yield of lignin (~70%), solubilized during the 2% H_2O_2 treatment of rye straw at 30°C for 12 h at pH 11.5 in our experiment, was undoubtedly due to the increasing alkaline peroxide concentration to 2%. Hence at a substrate concentration of 10 g/250 ml, to achieve further increasing dissolution of lignin from rye straw, H_2O_2 levels greater than 2% are required.

With the studies on the efficient use of hydrogen peroxide as a chemical pulp delignification agent, Troughton and Sarot (1992) reported that increase in consistency from 10 to 25% had a positive effect on the extent of peroxide delignification. However, the H_2O_2 amount has to
be limited to a relatively low value, because of its high cost.

Another reason for this relatively high solubilization of lignin from rye straw during the alkaline extraction is probably due to water pretreatment prior to alkaline peroxide extraction, which removed the straw contaminants such as soil and metals by the washing contributes, at least in part, to enhance the rate of delignification, since these transition metals such as Fe, Cu, and Mn act as catalysts for decomposition of hydrogen peroxide under alkaline conditions. On the other hand, magnesium salts such as MgSO4 and sodium silicate are well-known peroxide stabilisers or as a metal deactivator. The results obtained in our experiments showed that 2% H2O2 was capable of reducing the lignin content from rye straw significantly at pH 11.5 for 12 h at 40–70°C. The addition of sodium silicate had no effect (or an adverse effect) on the straw delignification (data not shown). This observation was consistent with the finding that improvement in H2O2 stabilisation was not a pre-required condition for good delignification of soft wood even under particularly severe conditions (120°C) (Lachenal et al., 1980). Dence and Omori (1986) also mentioned that the use of silicate was deemed superfluous when sufficient sodium hydroxide was available.

The results obtained here were in disagreement with some of published conclusions. Studies based on the mechanism of alkaline peroxide delignification of agricultural residues, (Gould, 1985) indicated that removal of heavy metal contaminants from the wheat straw (for example, by acid prewash, by addition of chelators, or by alkaline precipitation) essentially eliminate O2 evolution from the H2O2 reaction mixture, even at very high straw concentrations [6–10% (w/v)]. Lachenal et al. (1992) reported that after ethylenediaminetetraacetic acid (EDTA) pretreatment of the pulp, not only was hydrogen peroxide delignification improved, but also bleaching was enhanced and cellulose degradation was less. At the same time, peroxide consumption was lower.

3.2. UV absorption

To verify the purity of the isolated PL fractions, they were studied by UV spectroscopy at λ 260–370 nm. As shown in Fig. 2, the four PL preparations exhibited the basic UV spectrum typical of lignins with a maximum at 280 nm, originating from nonconjugated phenolic groups in the lignin (Scalbert et al., 1986). Interestingly, as shown in the spectra, the absorption coefficient increased slightly with the increment of alkaline peroxide treatment temperature, suggesting that a more pure lignin preparation can be obtained.

Fig. 2. UV spectra of lignin fractions obtained by treatment of the dewaxed and water-soluble-free straw with 2% H2O2 at pH 11.5 for 12 h at 30°C (spectrum a); 40°C (spectrum b); 50°C (spectrum c); and 70°C (spectrum d).
Table 2
The content of neutral sugars (percent dry weight, w/w) in isolated pure lignin (PL) fractions solubilized in water, dilute alkaline solution, and 2% H2O2 treatment of water-soluble-free and dewaxed rye straw at pH 11.5 for 12 h at different temperatures

<table>
<thead>
<tr>
<th>Sugars (%)</th>
<th>2% H2O2 (pH 11.5, 12 h) treatment temperature (°C)</th>
<th>WSb</th>
<th>DASc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20°</td>
<td>30°</td>
<td>40°</td>
</tr>
<tr>
<td>Arabinose</td>
<td>0.078</td>
<td>0.12</td>
<td>0.11</td>
</tr>
<tr>
<td>Xylose</td>
<td>0.57</td>
<td>0.48</td>
<td>0.42</td>
</tr>
<tr>
<td>Mannose</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.40</td>
<td>0.40</td>
<td>0.35</td>
</tr>
<tr>
<td>Galactose</td>
<td>0.095</td>
<td>0.010</td>
<td>0.012</td>
</tr>
<tr>
<td>Total</td>
<td>1.14</td>
<td>1.01</td>
<td>0.89</td>
</tr>
</tbody>
</table>

a The lignin fractions obtained by treatment of water-soluble-free and dewaxed rye straw with 2% H2O2 at pH 11.5 for 12 h at different temperatures.
b The lignin fraction obtained by treatment of the dewaxed rye straw with water at 50°C for 2 h.
c The lignin fraction extracted with dilute alkaline solution (pH 11.5) at 50°C for 12 h in the absence of H2O2 from water-soluble-free and dewaxed rye straw.
d Tr, trace.
e ND, not detected.

3.3. Sugar composition of the associated hemicelluloses

As compared to the lignin fraction released during the water treatment, all the PL preparations contained rather low amounts of bound polysaccharides as shown by 0.4–1.1% neutral sugar content, indicating that treatment of the straw with alkaline peroxide under the conditions used significantly cleaved the ether bonds between lignin and hemicelluloses in the cell walls of rye straw in addition to saponification of hydroxycinnamic esters such as between p-coumaric acid and lignin/polysaccharides or between ferulic acid and hemicelluloses. Xylose and glucose were identified as the main sugar components. Obviously, an increase in the alkaline treatment temperature from 20 to 70°C resulted in a decrease in the level of associated polysaccharides from 1.1 to less than 0.4% in the PL fractions. These data implied that an increase in alkaline peroxide treatment temperature can peel more lignin from most of the neighbouring polysaccharide moieties (Table 2).

3.4. Content of phenolic acids and aldehydes

The standard procedures for analysing lignins by chemical degradative techniques such as alkaline nitrobenzene oxidation degradation result in information of degradative products, which can be used to derive information about the composition of the original polymer (Billa et al., 1996). In the case of alkaline nitrobenzene oxidation, the three constitutive monomeric lignin units p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) produce the corresponding p-hydroxybenzaldehyde, vanillin, and syringaldehyde. In order to gain insight into the lignin, the isolated eight lignin preparations were studied by alkaline nitrobenzene oxidation, and the contents of phenolic acids and aldehydes in each of the preparations are given in Table 3. The predominant oxidation products were found to be vanillin and syringaldehyde. The presence of fewer p-hydroxybenzaldehyde and p-hydroxybenzoic acid was considered most probably to be indicative of non-condensed p-hydroxyphenyl units, indicating the incorporation of p-hydroxycinnamoyl alcohol in rye straw lignin. The occurrence of almost equal amounts of non-condensed guaiacyl and syringyl units with relatively fewer p-hydroxyphenyl units implied that the eight lignin preparations can be justified
as SGH-lignin such as cereal straw and grass type lignin. The relative molar ratios of S (the relatively total moles of syringaldehyde and syringic acid) to G (the relatively total moles of vanillin and vanillic acid), and to H (the relatively total moles of p-hydroxybenzaldehyde and p-hydroxybenzoic acid) appeared to be of approximately the same order (4–5:6–7:1), indicating the same original lignin. These results were in partial agreement with the studies on degradation of lignin in natural substrates by xylotrophs and soil saprotrophs from rye straw. The authors (Babitskaya and Shcherba, 1994) reported that chromatographic analysis of the products of oxidation with nitrobenzene showed that rye straw lignin was guaiacyl–syringyl with a slight prevalence of syringyl structures (G:S:H = 43:53:1). In comparison, a relatively higher content of p-hydroxybenzaldehyde and p-hydroxybenzoic acid in the nitrobenzene oxidation products obtained in our experiments was presumed largely due to the partial oxidation of p-coumaric acid. Similarly, a slightly higher molar ratio of G suggested that a considerable proportion of ferulic acid was oxidised into vanillin or vanillic acid under the nitrobenzene oxidation conditions used in our experiment. As can be seen in Table 3, the lower yields of oxidation products, obtained in the absence of H2O2 from the water-soluble and dilute alkali-soluble lignin preparations, indicated a higher degree of condensation of the two isolated PL preparations, whereas the higher yields of oxidation products found in the cases of alkaline peroxide extractable PL preparations may be explained by a lower degree of condensation of PL fractions. This report is the first paper concerning the above details of lignin composition and it provides the quantitative values of a high proportion of aryl ether-linked guaiacyl and syringyl units in the lignin fractions, obtained from the alkaline peroxide treatment of rye straw.

3.5. Molecular weight distribution

To illustrate whether the extent of degradation occurred during the process of alkaline peroxide

<table>
<thead>
<tr>
<th>Phenolic acids and aldehydes</th>
<th>2% H2O2 (pH 11.5, 12 h) treatment temperature (°C)</th>
<th>WSb</th>
<th>DASc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20a</td>
<td>30b</td>
<td>40b</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>0.12</td>
<td>0.12</td>
<td>0.18</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>0.17</td>
<td>0.15</td>
<td>0.23</td>
</tr>
<tr>
<td>p-Hydroxybenzoic acid</td>
<td>0.45</td>
<td>0.36</td>
<td>0.38</td>
</tr>
<tr>
<td>p-Hydroxybenzaldehyde</td>
<td>1.40</td>
<td>1.45</td>
<td>1.48</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>2.19</td>
<td>1.98</td>
<td>2.17</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>1.81</td>
<td>1.55</td>
<td>1.62</td>
</tr>
<tr>
<td>Vanillin</td>
<td>12.29</td>
<td>11.22</td>
<td>11.28</td>
</tr>
<tr>
<td>Syringaldehyde</td>
<td>11.00</td>
<td>10.58</td>
<td>10.98</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>0.64</td>
<td>0.62</td>
<td>0.44</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>0.85</td>
<td>0.67</td>
<td>0.59</td>
</tr>
<tr>
<td>Total</td>
<td>30.93</td>
<td>28.50</td>
<td>29.35</td>
</tr>
</tbody>
</table>

a The lignin fractions obtained by treatment of water-soluble-free and dewaxed rye straw with 2% H2O2 at pH 11.5 for 12 h at different temperatures.  
b The lignin fraction obtained by treatment of the dewaxed rye straw with water at 50°C for 2 h.  
c The lignin fraction extracted with dilute alkaline solution (pH 11.5) at 50°C for 12 h in the absence of H2O2 from water-soluble-free and dewaxed rye straw.  
d S represents the relatively total moles of syringaldehyde and syringic acid, G represents the relatively total moles of vanillin and vanillic acid, and H represents the relatively total moles of p-hydroxybenzaldehyde and p-hydroxybenzoic acid.
Table 4
Weight-average (\(M_w\)) and number-average (\(M_n\)) molecular weights and polydispersity (\(M_w/M_n\)) of the pure lignin (PL) fractions isolated with 2% \(\text{H}_2\text{O}_2\) at pH 11.5 for 12 h in different temperatures from rye straw

<table>
<thead>
<tr>
<th>2% (\text{H}_2\text{O}_2) (pH 11.5, 12 h) treating temperature (°C)</th>
<th>WS(^a)</th>
<th>DAS(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20(^a)</td>
<td>2420</td>
<td>2650</td>
</tr>
<tr>
<td>30(^a)</td>
<td>2930</td>
<td>3310</td>
</tr>
<tr>
<td>40(^a)</td>
<td>3480</td>
<td>3420</td>
</tr>
<tr>
<td>50(^a)</td>
<td>3040</td>
<td>3060</td>
</tr>
<tr>
<td>60(^a)</td>
<td>2830</td>
<td>2920</td>
</tr>
<tr>
<td>70(^a)</td>
<td>2980</td>
<td>3200</td>
</tr>
</tbody>
</table>

\(M_w\) | 620 | 660 | 690 | 920 | 1020 | 960 | 640 | 780
---|---|---|---|---|---|---|---|---
\(M_n\) | 620 | 660 | 690 | 920 | 1020 | 960 | 640 | 780
\(M_w/M_n\) | 3.9 | 4.0 | 4.2 | 3.6 | 3.4 | 3.2 | 4.4 | 3.8

\(^a\) The lignin fractions obtained by treatment of water-soluble-free and dewaxed rye straw with 2% \(\text{H}_2\text{O}_2\) at pH 11.5 for 12 h at different temperatures.

\(^b\) The lignin fraction obtained by treatment of the dewaxed rye straw with water at 50°C for 2 h.

\(^c\) The lignin fraction extracted with dilute alkaline solution (pH 11.5) at 50°C for 12 h in the absence of \(\text{H}_2\text{O}_2\) from water-soluble-free and dewaxed rye straw.

As can be seen in Table 4, the eight lignin fractions showed no significant difference in their molecular-average weights, which ranged from 2420 to 3480 g mol\(^{-1}\). An increase in temperature from 20 to 60°C during the 2% \(\text{H}_2\text{O}_2\) treatment at pH 11.5 for 12 h led to an increment of \(M_w\) from 2420 to 3480 g mol\(^{-1}\), indicating an increase in solubilization of large molecular size lignins at higher temperature. In contrast, as the temperature was further increased to 70°C, the \(M_w\) slightly decreased to 3040 g mol\(^{-1}\), implying that a minimal degradation of the lignins occurred at a relatively higher temperature of 70°C. As expected, a similar level of \(M_w\) between the water-soluble, dilute alkali-soluble, and 2% \(\text{H}_2\text{O}_2\)-soluble lignin preparations demonstrated that the alkaline peroxide treatment under the conditions used did not degrade the macromolecular structure of lignin to any noticeable extent. In addition, the eight PL fractions also gave a fairly analogous elution pattern, and molecular weight distribution of the lignin fraction, obtained by 2% \(\text{H}_2\text{O}_2\) treatment (40°C, pH 11.5, 12 h) of the water-soluble free of rye straw, is shown in Fig. 3. As can be seen from the diagram, the molecular weight distribution showed two main peaks corresponding to polystyrene molecular weights of 4190 and 1700 g mol\(^{-1}\). The elution profile showed a wide polymolecularity, ranging from oligomer up to polystyrene of molecular weight over 20 000 g mol\(^{-1}\).

3.6. FT-IR spectra

The FT-IR spectra of PL preparations, extracted with 2% \(\text{H}_2\text{O}_2\) at pH 11.5 for 12 h at 20°C (spectrum a), 40°C (spectrum b), 50°C (spectrum c), and 70°C (spectrum d) from the dewaxed and water-treated rye straw are shown in Fig. 4. The
spectral profiles and the relative intensities of the bands were rather similar in four spectra, which confirmed that the ‘core’ of lignin structure did not change significantly during the alkaline peroxide treatment at the different temperatures. The band at 1706 cm\(^{-1}\) has been assigned to the unconjugated ketone and unconjugated carbonyl stretching, while the band at 1636 cm\(^{-1}\) has been attributed to carbonyl stretching conjugated with aromatic rings (Vazquez et al., 1997). Aromatic skeleton vibrations in the PL preparations are assigned at 1598, 1511, and 1425 cm\(^{-1}\). Absorption at 1464 cm\(^{-1}\) indicates C–H deformations and aromatic ring vibrations. The bands at 1334, 1270, and 1225 cm\(^{-1}\) have been assigned to ring breathing with C–O stretching. The 1334 cm\(^{-1}\) band has been associated with syringyl units, and 1270 cm\(^{-1}\) band with guaiacyl units. The bands at 1129 and 1033 cm\(^{-1}\) indicate the aromatic CH in-plain deformation for syringyl type and guaiacyl type, respectively. Aromatic C–H out of bending appears at 840 cm\(^{-1}\). Not surprisingly, it can be observed that a great similarity existed among the spectra of alkaline peroxide-soluble lignins and the lignin preparation, solubilized under analogous conditions (pH 11.5, 50°C, 12 h) but in the absence of peroxide, supporting the previous finding that alkaline peroxide treatment did not affect the overall structure of lignin from rye straw.

### 3.7. \(^{13}\)C-NMR spectrum

The PL fraction, obtained by treatment of the straw sample with 2% H\(_2\)O\(_2\) at 70°C for 12 h at pH 11.5, was also studied by \(^{13}\)C-NMR spectroscopy (Fig. 5). Most of the observed signals have been previously assigned in straw and wood lignin spectra (Nimz et al., 1981; Scalbert et al., 1986; Imamura et al., 1994; Kondo et al., 1995). As can be seen from Fig. 5, the most striking characteristic of the \(^{13}\)C-NMR spectrum is the almost absence of typical polysaccharide signals between 57 and 103 ppm. The spectrum does show a signal at 63.2 ppm (C-5, Xyl internal unit) for the associated hemicelluloses, however, the peak intensity is rather weak. The carbonyl resonances from uronic acids and esters may contribute to signal at 174.8 ppm, which indicates C-6 in methyl uronates (Himmelsbach and Barton, 1980).

The signals for aromatic part of the lignin appear in the region between 104.4 and 170.0 ppm. The syringyl (S) residues were indicated by signals at 152.3 (C-3/C-5, S), 138.2 (C-4, S etherified), 134.2 (C-1, S etherified), 133.4 (C-1, S nonetherified), 106.8 (C-2/C-6, S with \(\alpha\)-CO), and 104.4 ppm (C-2/C-6, S). Guaiacyl (G) residues gave signals at 149.8 and 149.3 ppm (C-3, G etherified), 148.0 and 147.1 (C-4, G etherified), 145.7 (C-4, G nonetherified), 134.2 (C-1, G etherified), 133.4 (C-1, G nonetherified), 119.3 (C-6, G), and 114.9 ppm (C-5, G). The \(p\)-hydroxyphenyl (H) residues appeared as two signals at 128.7 and 128.0 ppm (C-2/C-6, H). Esterified spectral profiles and the relative intensities of the bands were rather similar in four spectra, which confirmed that the ‘core’ of lignin structure did not change significantly during the alkaline peroxide treatment at the different temperatures. The band at 1706 cm\(^{-1}\) has been assigned to the unconjugated ketone and unconjugated carbonyl stretching, while the band at 1636 cm\(^{-1}\) has been attributed to carbonyl stretching conjugated with aromatic rings (Vazquez et al., 1997). Aromatic skeleton vibrations in the PL preparations are assigned at 1598, 1511, and 1425 cm\(^{-1}\). Absorption at 1464 cm\(^{-1}\) indicates C–H deformations and aromatic ring vibrations. The bands at 1334, 1270, and 1225 cm\(^{-1}\) have been assigned to ring breathing with C–O stretching. The 1334 cm\(^{-1}\) band has been associated with syringyl units, and 1270 cm\(^{-1}\) band with guaiacyl units. The bands at 1129 and 1033 cm\(^{-1}\) indicate the aromatic CH in-plain deformation for syringyl type and guaiacyl type, respectively. Aromatic C–H out of bending appears at 840 cm\(^{-1}\). Not surprisingly, it can be observed that a great similarity existed among the spectra of alkaline peroxide-soluble lignins and the lignin preparation, solubilized under analogous conditions (pH 11.5, 50°C, 12 h) but in the absence of peroxide, supporting the previous finding that alkaline peroxide treatment did not affect the overall structure of lignin from rye straw.

### 3.7. \(^{13}\)C-NMR spectrum

The PL fraction, obtained by treatment of the straw sample with 2% H\(_2\)O\(_2\) at 70°C for 12 h at pH 11.5, was also studied by \(^{13}\)C-NMR spectroscopy (Fig. 5). Most of the observed signals have been previously assigned in straw and wood lignin spectra (Nimz et al., 1981; Scalbert et al., 1986; Imamura et al., 1994; Kondo et al., 1995). As can be seen from Fig. 5, the most striking characteristic of the \(^{13}\)C-NMR spectrum is the almost absence of typical polysaccharide signals between 57 and 103 ppm. The spectrum does show a signal at 63.2 ppm (C-5, Xyl internal unit) for the associated hemicelluloses, however, the peak intensity is rather weak. The carbonyl resonances from uronic acids and esters may contribute to signal at 174.8 ppm, which indicates C-6 in methyl uronates (Himmelsbach and Barton, 1980).

The signals for aromatic part of the lignin appear in the region between 104.4 and 170.0 ppm. The syringyl (S) residues were indicated by signals at 152.3 (C-3/C-5, S), 138.2 (C-4, S etherified), 134.2 (C-1, S etherified), 133.4 (C-1, S nonetherified), 106.8 (C-2/C-6, S with \(\alpha\)-CO), and 104.4 ppm (C-2/C-6, S). Guaiacyl (G) residues gave signals at 149.8 and 149.3 ppm (C-3, G etherified), 148.0 and 147.1 (C-4, G etherified), 145.7 (C-4, G nonetherified), 134.2 (C-1, G etherified), 133.4 (C-1, G nonetherified), 119.3 (C-6, G), and 114.9 ppm (C-5, G). The \(p\)-hydroxyphenyl (H) residues appeared as two signals at 128.7 and 128.0 ppm (C-2/C-6, H). These signals confirmed that the lignin preparation could be justified as SGH-lignin. The signals at 168.2 (C-\(g\), PC ester), 159.9 (C-4, PC ester), 144.7 (C-\(a\), PC ester), 130.2 (C-2/C-6, PC ester), 125.9 and 125.3 (C-1, PC ester), and 115.9, 115.7, and 115.4 ppm (C-3/C-5, PC ester) represented the esterified \(p\)-coumaric acid. Esterified ferulic acid was observed with signals at 167.4 (C-\(g\), FE ether, data not shown in the spectrum), 144.4 (C-\(a\), FE ether), and 122.4 ppm (C-6, FE ether). Esterified
ferulic acid was identified with a signal at 122.9 ppm (C-6, FE ester). It seems clear that the p-coumaric acid is linked to lignin by ester bonds, while the ferulic acid is linked to lignin by ether and ester bonds. On the basis of study on the hydroxycinnamic acids, particularly ferulic and p-coumaric acids in the cell walls of wheat straw, we (Sun and Lawther, 1998) previously reported that p-coumaric acid was mostly esterified to lignin or polysaccharides, while ferulic acid appeared almost equally in etherified linkages with lignin and in esterified bonds to arabinose in hemicelluloses. Similarly, in the cell walls of rye straw, in addition to the etherified linkages between ferulic acid and lignin, ferulic acid at least in part, also esterified to hemicellulose.

The spectrum also indicated that β-O-4 linkages (C-α in β-O-4, 72.2; C-β in β-O-4, 86.1 ppm; C-γ in β-O-4, 60.1 ppm) were the major linkages between lignin structural units. The common carbon–carbon linkages such as β–β (C-γ in β–β units, 71.7 ppm, data not shown in the spectrum) and β-5 (C-4 in β-5 units, 144.7 ppm, overlapped with C-α, PC ester) were also present. These signals indicated that the linkages in this rye straw lignin is mainly composed of β–O–4 ether bonds together with small amounts of β–β and β-5 carbon–carbon linkages. These results suggested that alkaline peroxide under the conditions used here might not attack the β-aryl ether structure to a significant extent. The signals representing the γ-methyl, α and β-methylene groups in n-propyl side chains appeared in the spectrum between 14.1 and 33.8 ppm. A very strong signal at 56.0 ppm corresponded to the OCH₃ in syringyl and guaiacyl units.

On the basis of the foregoing data, it can be concluded that treatment by alkaline peroxide under the conditions given did not affect the overall structure of lignin from rye straw. Similar results have been reported by Dence (1996),

Fig. 5. ¹³CNMR spectrum of pure lignin (PL) fraction extracted with 2% H₂O₂ at 70°C for 12 h at pH 11.5 from the dewaxed and water-extracted rye straw.
Lachenal et al. (1992) in the studies on the behaviour of lignin in kraft pulp during hydrogen peroxide delignification. The authors stated that hydrogen peroxide was unable to attack phenols of the type present in lignin under alkaline conditions. That is, no degradation of the phenolic ring was observed during the alkaline peroxide treatment. However, at a relatively higher temperature such as 90°C, some depolymerization of lignin may occur and carboxyl groups are created (Dence, 1996).

In conclusion, the six lignin preparations, obtained by treatment of the dewaxed and water-extracted rye straw with 2% H₂O₂ at pH 11.5 for 12 h at 20–70°C, and one alkali lignin fraction, isolated under similar conditions but in the absence of peroxide, showed similar chemical composition and physico-chemical properties. They were relatively free of polysaccharides and contained almost equal amounts of noncondensed guaiacyl and syringyl units with fewer p-hydroxyphenyl units. They seem more condensed than wood lignins, but corresponded to the condensation degree of wheat straw lignins. Meanwhile, the lignin in rye straw cell walls appeared to be very closely associated to glucuronic acid or 4-O-methylglucuronic acid by ester bonds. p-Coumaric acid was found to be linked to lignin by ester bonds, while ferulic acid was linked by their phenolic groups via ether bonds to lignin and also principally linked by their carboxyl groups via ester bonds to hemicelluloses.

Acknowledgements

The authors are grateful for the financial support of this research from the European Community under the Industrial and Materials Technologies Programme (Brite-EuRam III)-Depolymerisation, Polymerisation and Applications of Biosustainable Raw Materials for Industrial End Uses.

References