Table 1
The yield of lignin (percent dry matter) solubilized in water, dilute alkaline solution, and 2% H_2O_2 treatment of water-soluble-free and dewaxed rye straw at pH 11.5 for 12 h at different temperatures

Yield	2% H_2O_2 (pH 11.5, 12 h) treatment temperature (°C)						WS[b]	DAS[c]
	20[a]	30[a]	40[a]	50[a]	60[a]	70[a]		
Total solubilized lignin	7.8	11.2	12.1	12.3	12.7	13.0	2.8	1.1
Isolated pure lignin (PL)[d]	5.5	8.2	9.0	8.1	7.8	7.8	1.7	0.6
Lignin associated in the isolated hemicelluloses	1.0	1.0	1.2	1.2	1.3	1.3	0.8	0.2
Lignin solubilized in the supernatant (pH 1.5)[e]	1.3	2.0	1.9	3.0	3.6	3.9	0.3	0.3

[a] The lignin fractions obtained by treatment of water-soluble-free and dewaxed rye straw with 2% H_2O_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 H_2O_2 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).

conditions. They are recorded at 25°C from 250 mg of sample dissolved in 1.0 ml DMSO-d_6 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%).

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% H_2O_2 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% H_2O_2 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% H_2O_2 under the relatively high temperature could lead to substantial degradation of the solubilized lignin into small and acidic

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 ($HO^{\bullet}$) 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 ($O_2^{\bullet}$) 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:

$$H_2O_2 + HOO^- \rightarrow HO^{\bullet} + O_2^{\bullet} + H_2O$$

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

$$HO + O_2^{-\bullet} \rightarrow O_2^{\bullet} + HO^-$$

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 $MgSO_4$ and sodium silicate are well-known peroxide stabilisers or as a metal deactivator. The results obtained in our experiments showed that 2% H_2O_2 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 H_2O_2 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 O_2 evolution from the H_2O_2 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

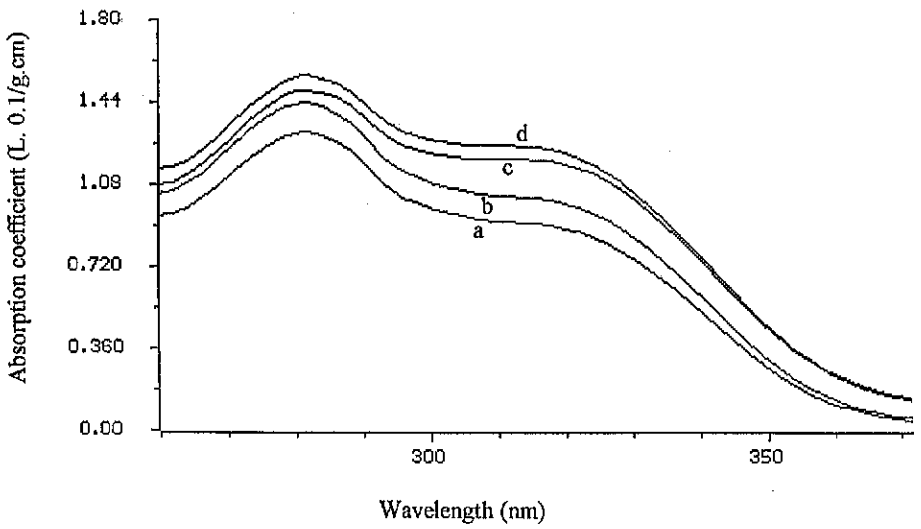

Fig. 2. UV spectra of lignin fractions obtained by treatment of the dewaxed and water-soluble-free straw with 2% H_2O_2 at pH 11.5 for 12 h at 30°C (spectrum a); 40°C (spectrum b); 50°C (spectrum c); and 70°C (spectrum d).