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% H_2O_2 treatment of water-soluble-free and dewaxed rye straw at pH 11.5 for 12 h at different temperatures

Sugars (%)	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]		
Arabinose	0.078	0.12	0.11	0.082	Tr[d]	Tr	0.11	0.10
Xylose	0.57	0.48	0.42	0.43	0.16	0.17	0.26	0.30
Mannose	ND[e]	ND	ND	ND	ND	ND	0.27	0.12
Glucose	0.40	0.40	0.35	0.36	0.21	0.20	5.49	1.39
Galactose	0.095	0.010	0.012	Tr	Tr	Tr	0.21	0.06
Total	1.14	1.01	0.89	0.87	0.37	0.37	6.34	1.97

[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] Tr, trace.
[e] ND, not detected.

when the alkaline peroxide performed at a relatively higher temperature. This observation indicated that the purity of PL was paralleled by the total yield of lignin solubilized during the peroxide treatment.

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 H_2O_2 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 3
The yield (percent lignin sample, w/w) of phenolic acids and aldehydes from alkaline nitrobenzene oxidation of the isolated pure lignin (PL) fractions

Phenolic acids and aldehydes	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]		
Gallic acid	0.13	0.12	0.18	0.14	0.13	0.13	0.12	0.11
Protocatechuic acid	0.17	0.15	0.23	0.18	0.18	0.16	0.14	0.12
p-Hydroxybenzoic acid	0.45	0.36	0.38	0.32	0.31	0.27	0.22	0.24
p-Hydroxybenzaldehyde	1.40	1.45	1.48	1.31	1.27	1.18	0.95	1.02
Vanillic acid	2.19	1.98	2.17	1.97	1.57	1.78	1.18	0.98
Syringic acid	1.81	1.55	1.62	1.47	1.29	1.38	0.90	0.89
Vanillin	12.29	11.22	11.28	10.49	10.80	10.68	8.91	7.48
Syringaldehyde	11.00	10.58	10.98	9.48	10.29	10.21	8.30	6.69
p-Coumaric acid	0.64	0.62	0.44	0.54	0.61	0.60	0.38	0.52
Ferulic acid	0.85	0.67	0.59	0.69	0.78	0.68	0.81	0.59
Total	30.93	28.50	29.35	26.59	27.23	27.07	21.91	18.64
Molar ratio (S:G:H)[d]	5:6:1	5:6:1	5:6:1	5:6:1	5:7:1	5:7:1	5:7:1	4:6:1

[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] 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 ($\bar{M}_w$) and number-average ($\bar{M}_n$) molecular weights and polydispersity ($\bar{M}_w/\bar{M}_n$) of the pure lignin (PL) fractions isolated with 2% H_2O_2 at pH 11.5 for 12 h in different temperatures from rye straw

	2% H_2O_2 (pH 11.5, 12 h) treating temperature (°C)						WS[b]	DAS[c]
	20[a]	30[a]	40[a]	50[a]	60[a]	70[a]		
$\bar{M}_w$	2420	2650	2930	3310	3480	3040	2830	2980
$\bar{M}_n$	620	660	690	920	1020	960	640	780
$\bar{M}_w/\bar{M}_n$	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% 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.

treatment, all the molecular weights of PL preparations were calculated from the GPC chromatograms, and the weight-average ($\bar{M}_w$) and number-average ($\bar{M}_n$) molecular weights and the polydispersity ($\bar{M}_w/\bar{M}_n$) of the lignin fractions are given in Table 4. As can be seen in Table 4, the eight lignin fractions showed no significant difference in their molecular-average weights, which ranged $\bar{M}_w$ from 2420 to 3480 g mol^{-1}. An increase in temperature from 20 to 60°C during the 2% H_2O_2 treatment at pH 11.5 for 12 h led to an increment of $\bar{M}_w$ 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 $\bar{M}_w$ 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 $\bar{M}_w$ between the water-soluble, dilute alkali-soluble, and 2% H_2O_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% H_2O_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% H_2O_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

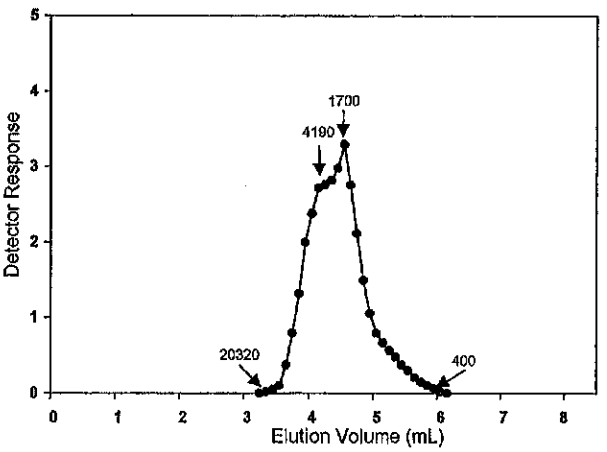

Fig. 3. GPC molecular weight distribution of pure lignin (PL) fraction isolated from the hydrolysate of 2% H_2O_2 treatment (40°C, pH 11.5, 12 h) of the dewaxed and water-extracted rye straw.