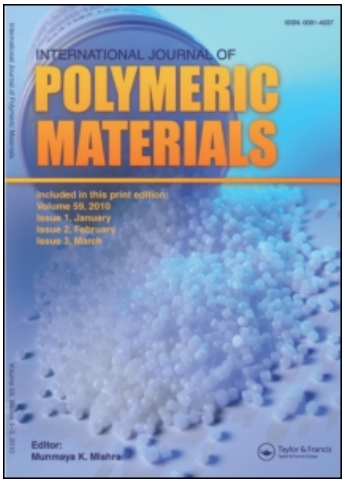

International Journal of Polymeric Materials
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713647664

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To cite this Article Sun, Runcang and Hughes, Sara(1998) 'Physico-chemical and Structural Characterization of Alkali-soluble Lignins from Sugar Beet Pulp', International Journal of Polymeric Materials, 42: 3, 181 — 193
To link to this Article: DOI: 10.1080/00914039808033869
URL: http://dx.doi.org/10.1080/00914039808033869

Intern. J. Polymeric Mater., 1998, Vol. 42, pp. 181 – 193
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Physico-chemical and Structural Characterization of Alkali-soluble Lignins from Sugar Beet Pulp

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(Received 21 November 1997)

Two alkali-soluble lignin fractions were extracted from wax, protein, and pectin free cell wall residues of sugar beet pulp with 10% KOH and 7.5% NaOH both at 15°C for 16 h. The physico-chemical properties and structural features of the two isolated pure lignin fractions (LA) were characterized by UV, FT-IR, and ^{13}C-NMR spectroscopy. Both of the lignin fractions contain rather low amounts of neutral sugars (0.89 – 1.03%) and pectic substances (1.75 – 1.80%). Their molecular-average weights ranged between 1830 – 1920 Da. The results obtained by alkaline nitrobenzene oxidation showed that the two lignins contain large amounts of non-condensed guaiacyl units, together with only a few non-condensed syringyl and *p*-hydroxyphenyl units. The lignin fraction, isolated by 7.5% NaOH at 15°C for 16 h, is mainly composed of β—O—4 ether bonds. The less common β-β, β-5, β-1, and 5-5' carbon – carbon linkages are also found to be present between the lignin structural units. Further studies suggested that hydroxycinnamic acids, such as *p*-coumaric and ferulic acids, and galacturonic acid are tightly linked to lignin molecules.

Keywords: Sugar beet pulp; lignin; phenolic acids and aldehydes; alkaline nitrobenzene oxidation; molecular weight; polysaccharides; infrared spectra; ^{13}C-NMR spectroscopy

INTRODUCTION

Sugar beet pulp (SBP), the main by-product of the sugar refining industry, has been reported to contain large amounts of polysaccharides (75 – 80%), together with small amounts of protein (~ 10%) and lignin (3 – 6%). The fractional characterization of pectic substances,

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hemicelluloses, and cellulose from SBP has been extensively studied in our laboratory [1–3]. Lignin is a plant cell·wall component and is tightly associated with other cell wall materials [4]. The study of this cell wall component is complicated by its large molecular size and difficult isolation. Furthermore, the structures of purified lignins often vary with the methodologies used for preparation and isolation.

Although the presence of lignin has been reported, there are no reports on the physico-chemical properties and structural features of lignin isolated from SBP. The present study was undertaken to isolate and characterize some of these properties in two lignin fractions extracted from SBP.

EXPERIMENTAL

General Methods

Sugar beet pulp (SBP) was obtained from the Danisco Sugar Development Centre, Denmark. The dried SBP was ground in a Christie Laboratory Mill to pass a 60 mesh size screen and stored at 5°C until use. Protease (EC 3.4. 24. 31) and pectinase (EC 3.2.1. 15) were purchased from the Sigma Chemical Co. St. Louis, Mo., USA.

Neutral sugar composition in isolated lignin fractions was determined as alditol acetates [5]. Alkaline nitrobenzene oxidation of lignin of alkali lignins was performed at 170°C for 3 h. Methods of uronic acid analysis and determination of phenolic acids and aldehydes with HPLC from nitrobenzene oxidation mixture were described in previous papers [6–9].

UV spectra were recorded on a Hewlett-Packard 8452A Diode Array spectrophotometer. A lignin sampe (5 mg) was dissolved in 95% (v/v) dioxane-water (10 mL). A 1 mL aliquot was diluted to 10 mL with 50% (v/v) dioxane-water, and the absorbances between 240 and 350 nm were measured. FT-IR spectra were obtained on an FT-IR spectrophotometer (Nicolet, 750) using a KBr disc containing 1% finely ground samples.

The molecular-average weights of lignin fractions were determined by gel permeation chromatography on a PLgel 5μ Mixed-D column. The samples were dissolved in tetrahydrofuran at a concentration of

0.2%, and a 200 μL sample in solution was injected. The columns were operated at 40°C and eluted with tetrahydrofuran at a flow rate of 1 mL min^{-1}. The column was calibrated using polystyrene standards.

The solution-state of ^{13}C-NMR spectrum was obtained on a Brucker 250 AC spectrometer operating in the FT mode at 62.4 MHz under total proton decoupled conditions. It was recorded at 25°C from a 250 mg sample dissolved in 1.0 mL DMSO-d_6 after 30000 scans. A 40° pulse flipping angle, a 3.0 μs pulse width and 0.85 s acquisition time were used.

Preparation of Wax, Protein and Pectin Free Sample

Crude lipids were extracted using chloroform–methanol (2:1, v/v) in Soxhlet for 6 h. Proteolysis was performed by addition of protease into 0.1 M sodium phosphate buffer (pH 7.5) containing dewaxed SBP for 2 h at 37°C. The defatted and protein free SBP was further extracted with 0.2% disodium ethylenediaminetetraacetic acid (EDTA) at pH 3.3, 85°C for 1 h. After filtration through a 20 μ nylon screen to remove the EDTA-soluble pectins, the residue was treated with pectinase under stirring in 0.1 M acetate buffer, pH 4.0 to remove the residual pectins. After incubation at 25°C for 2.5 h, the solution was again filtered through a 20 μ nylon screen. The pectin free residue was air-dried and used for further extraction of lignin.

Extraction of Alkali-Soluble Lignins

Two lignin fractions were extracted with 10% KOH and 7.5% NaOH (4.5 g residue/100 ml of extractant) both for 16 h at 15°C. After filtration, the extracts in each of the fractions were acidified to pH 5.0 with 20% HCl, concentrated with a rotary evaporator under reduced pressure at 40°C, and then mixed with 4 vols. ethanol. The precipitated hemicellulose–lignin complexes were filtered, washed with 75% ethanol, and air-dried. The alkali-soluble pure lignin fractions (LA) were then precipitated at pH 1.5 with 20% HCl from the supernatant solution and washed with acidified water (pH 2.0). The air-dried alkali lignins were kept in a refrigerator for further analysis (Fig. 1).

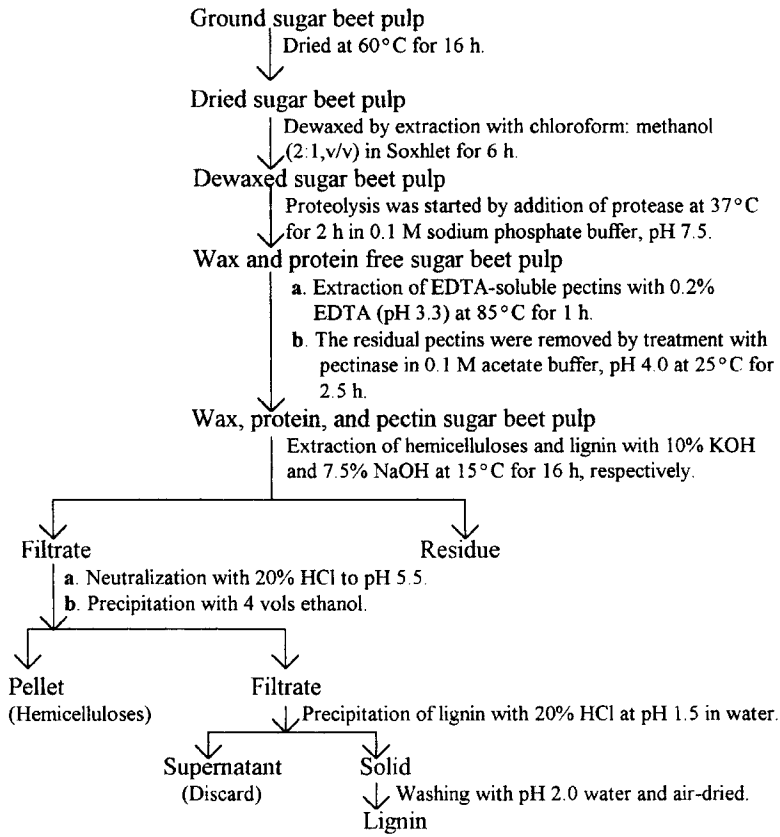

FIGURE 1 Scheme for extraction and isolation of alkali-soluble lignin from sugar beet pulp.

RESULTS AND DISCUSSION

The wax, protein, and pectin free cell wall residues were submitted to alkali extraction of lignins at 15°C for 16 h. The yields of total, pure lignin (LA), and co-precipitated in hemicellulose–lignin complexes (LB), are given in Table I. As expected, the lignin yield of LA was much higher than of LB, as shown by the ratios of LA/LB between 7.74 and 5.09. This indicated that both 10% and 7.5% NaOH treatments

TABLE I The yield (% dry weight of sugar beet pulp) of alkali-soluble lignin fractions extracted with 10% KOH and 7.5% NaOH at 15°C for 16 h, respectively, from the wax, protein, and pectin free cell wall residues of sugar beet pulp

Extraction conditions	*Total*	*LA* [a]	*LB* [b]	*LA/LB*
10% KOH, 15°C, 16 h	2.71	2.40	0.31	7.74
7.5% NaOH, 15°C, 16 h	3.47	2.90	0.57	5.09

[a] Obtained by re-precipitation of the supernatant solution with 20% HCl at pH 1.5 after isolation of hemicellulose–lignin complexes.
[b] Co-precipitated in the hemicellulose–lignin complexes.

can peel the lignins from most their neighbouring polysaccharide moieties at 15° for 16 h. A relatively higher yield of the lignin fraction, isolated by 7.15% NaOH, suggested that this treatment has more of an effect on the delignification from the cell wall residues of SBP than does 10% KOH extraction. A study of isolation and characterization of polysaccharides from SBP showed that the SBP contained 5.63% lignin [10]. Vogel [11] reported that the SBP is mainly composed of water-insoluble polymers like pectin, hemicelluloses, and cellulose, together with small amounts of lignin (2–4%) and crude protein (~8%). A similar result was obtained by Vaccai *et al.* [12], who proposed that SBP contained about 3% lignin. Our previous experiments showed the pretreatment of SBP with 2% NaOH at 45°C for 5 h yielded 7.63% lignin [1]. Taking into account the difference in lignin yield, the relatively lower yield of isolated lignins obtained in the current study can be explained by some lignin having been dissolved during the proteolysis and pectin extraction, or is due to the variety of isolation methods used, such as extraction temperature and duration. A much lower extraction temperature, as used in the current study, might also result in the partial isolation of lignin from the cell wall residues.

The purity of the lignins obtained was determined by the UV spectra (Fig. 2). As shown in the diagram, the two lignin fractions exhibit the basic UV spectrum typical of lignins, which have a absorption maximum at λ 280 nm, originating from non-condensed phenolic groups (aromatic ring) in lignin [13]. The relatively lower absorption of the lignin fraction, isolated by 10% KOH at 15°C for 16 h, is probably due to the slightly higher amounts of co-precipitated non-lignin materials such as ash and salts.

The contents of neutral sugars and galacturonic acid in isolated lignin fractions of LA are given in Table II. Obviously, the two lignin

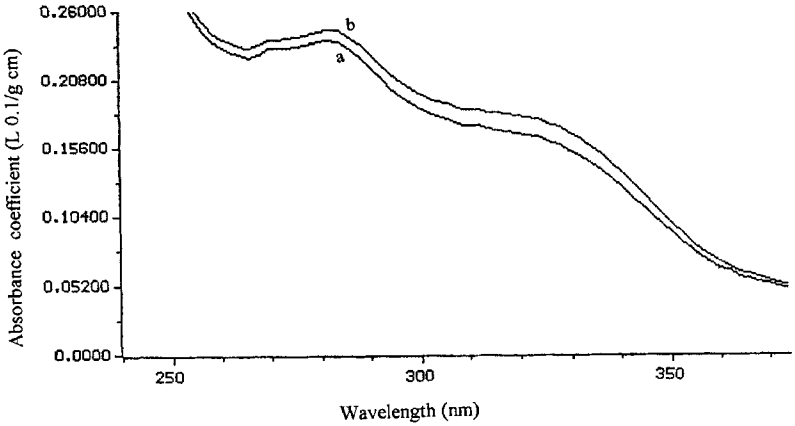

FIGURE 2 UV spectra of lignin fractions extracted with 10% KOH; (a) and 7.5% NaOH; (b) at 15°C for 16 h from depectinated sugar beet pulp.

fractions contained rather low levels of associated neutral sugars (0.89–1.03%) and galacturonic acid (2.69–2.78%). The results observed indicated again that the chemical bonds between lignin and polysaccharides in the two lignin preparations LA are mostly cleaved during the alkali treatment processes. Glucose and arabinose were found to be the only two sugar components. The presence of galacturonic acid as a major sugar component in the LA fractions is probably due to the ester bonds between lignin and galacturonic acid

TABLE II The content (% dry lignin, w/w) of neutral sugars and galacturonic acid in alkali-soluble lignin fractions LA extracted with 10% KOH and 7.5% NaOH at 15°C for 16 h respectively, from the wax, protein, and pectin free cell wall residues of sugar beet pulp

Composition [a]	*Extraction procedures*	
	10% *KOH*, 15°C, 16 *h*	7.5% *NaOH*, 15°C, 16 *h*
Rhamnose	Trace	Trace
Arabinose	0.27	0.21
Mannose	Trace	ND [b]
Glucose	0.76	0.68
Galactose	Trace	ND
Galacturonic acid	1.75	1.80
Total	2.78	2.69

[a] Data are expressed on a dry basis, and represent the mean of duplicate runs.
[b] ND = Not detected.

in SBP cell walls; and this was confirmed by ^{13}C-NMR spectroscopy (Fig. 5).

Table III gives the results concerning the yield and composition of phenolic acids and aldehydes from the alkaline nitrobenzene oxidation of the two alkali lignin fractions LA. The same trends were observed in both fractions. The predominant component of the phenolic monomers was found to be vanillin, resulting from the degradation of non-condensed guaiacyl (G) units. The second major degradation product, syringaldehyde, resulting from the degradation of non-condensed syringyl (S) units. The occurence of low amounts of *p*-hydroxybenzaldehyde is generally considered to be indicative of *p*-hydroxyphenyl (H) units with the lignin 'core', or partially results from the degradation of *p*-coumaric acid. The occurrence of a large amount of guaiacyl units, together with small amounts of syringyl and *p*-hydroxyphenyl units in LA, indicated that the two lignin fractions can be justified as a GSH-lignin, such as 'straw or grass type lignin'. Five phenolic acids such as *p*-hydroxybenzolic acid, vanillic acid, syringic acid, *p*-coumaric acid, and ferulic acid were also identified as minor quantities in all the nitrobenzene oxidation products. The presence of these phenols, excluding *p*-coumaric and ferulic acids, was also reported by Guillon and Thibault [14] in the colormetric measurement of phenols from SBP. However, none of these phenols was recovered and identified in the studies of Guillon and Thibault [14]. The lower

TABLE III The yield (% lignin, w/w) of phenolic acids and aldehydes from alkaline nitrobenzene oxidation of alkali-soluble lignin fractions LA extracted with 10% KOH and 7.5% NaOH at 15°C for 16 h, respectively, from the wax, protein, and pectin free cell wall residues of sugar beet pulp

Phenolic acids and aldehydes [a]	*Extraction procedures*	
	10% *KOH*, 15°*C*, 16*h*	7.5% *NaOH*, 15°*C*, 16*h*
p-Hydroxybenzoic acid	0.48	0.64
p-Hydroxybenzaldehyde	1.04	0.96
Vanillic acid	0.38	0.42
Syringic acid	0.56	0.60
Vanillin	9.52	9.36
Syringaldehyde	1.20	1.28
p-Coumaric acid	0.62	0.48
Ferulic acid	0.64	0.56
Total	14.44	14.30

[a] Data are expressed on a dry basis, and represent the mean of duplicate runs.

yields of nitrobenzene oxidation of the two alkali lignin fractions indicated a higher degree of condensation of the alkali lignins, compared to the corresponding yields of wood lignins.

The recovery yields of ferulic and *p*-coumaric acids, detected in the products of the alkaline nitrobenzene oxidation, decreased with increase in temperature and reaction time for both wheat straw internodes and leaves. Ferulic acid was not detected among the oxidation products after 4 h at 170°C or 2 h at 180°C, and the molar content in ferulic acid corresponded to an equivalent molar increase in vanillin [15]. These results suggested that some amounts of ferulic acids were quantitatively oxidized to vanillin by nitrobenzene under the reaction conditions used in out studies (170°C, 3 h). Similarly, part of the *p*-coumaric acid appeared to be quantitatively oxidized to *p*-hydroxybenzaldehye under the conditions of the alkaline nitrobenzene oxidation. However, the continuing occurence of small amounts of *p*-coumaric and ferulic acids in the alkaline nitrobenzene oxidation products of the two lignin fractions was due to the conditions of nitrobenzene oxidation at the relatively low temperature (170°C) and the relatively short oxidation period (3 h) which were used in the current study. This phenomenon indicated that *p*-coumaric and ferulic acids are tightly associated to lignin molecules in SBP cell walls.

The weight-average (M_w) and number-average (M_n) molecular weights, and the polydispersity of the two lignin fractions were computed from their chromatograms and are given in Table IV. The data in Table IV showed that the alkali-soluble lignins obtained from SBP had low molecular-average weights (M_w = 1830 – 1920). A slightly lower molecular weight of the lignin fractions, isolated by 7.5% NaOH at 15°C for 16 h, suggested that 7.5% NaOH has a greater effect on the cleavage of the ether bonds between the lignin precursors does 10% KOH.

TABLE IV The weight-average (M_w), number-average (M_n) molecular weights, and the polydispersity (M_w/M_n) of the alkali-soluble lignin fractions LA extracted with 10% KOH and 7.5% NaOH at 15°C for 16 h, respectively, from the wax, protein, and pectin free cell wall residues of sugar beet pulp

Extraction procedures	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$
10% KOH, 15°C, 16 h	1920	1620	1.19
7.5% NaOH, 15°C, 16 h	1830	1540	1.19

The gel permeation chromatogram of alkali-soluable lignin fraction, isolated by 7.5% NaOH at 15°C for 16 h, is shown in Figure 3. The elution maximum corresponded to polystyrene molecular weight 1710. The elution profile showed a wide polymolecularity, ranging from oligomers up to polystyrene of molecular weight over 10000 (polystyrene standards). These data were in accordance with our previous studies on alkali lignins from wheat straw [9].

The FT-IR spectra of the two lignin fractions showed minor changes in the peak intensities (Fig. 4), which confirmed that the 'core' of the two lignin structures does not change dramatically during the two cases of alkali extraction processes. The intensive bands of the carbonyl groups appear in the range between 1650 and 1720 cm^{-1}. The exact position of the band is dependent on whether the C = O groups are in conjugation with the aromatic ring (position below 1700 cm^{-1}, such as 1650 cm^{-1}) or not (position above 1700 cm^{-1}, such as 1716 cm^{-1}). The band for unconjugated carbonyl groups at 1716 cm^{-1} is more intensive in the spectrum of 10% KOH extracted lignin fraction than in that of 7.5% NaOH extracted lignin fraction. The aromatic skeleton vibration in two lignin fractions is assigned at

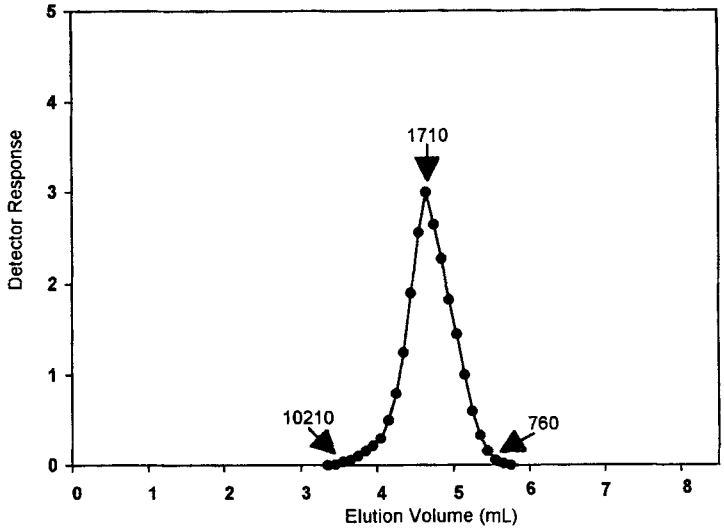

FIGURE 3 GPC molecular weight distribution of alkali-soluable lignin extracted with 7.5% NaOH at 15°C for 16 h.

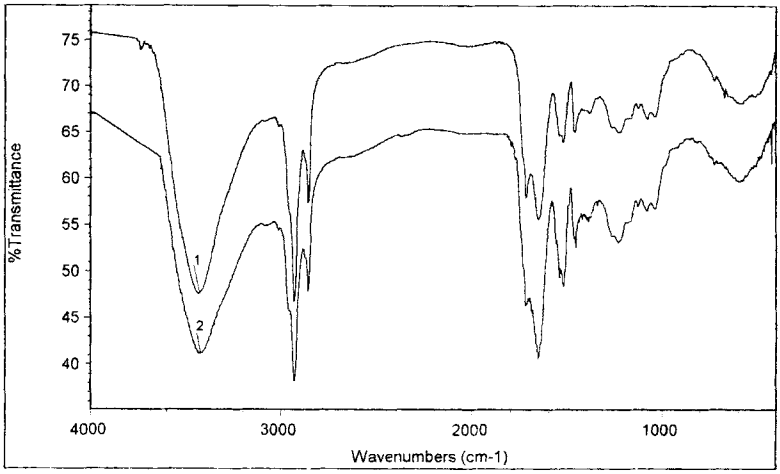

FIGURE 4 FT-IR spectra of 10% KOH (15°C, 16 h) soluble lignin (1) and 7.5% NaOH (15°C, 16 h) soluble lignin (2).

1512 cm^{-1}. Absorption at 1456 cm^{-1} indicates the C —H deformations and aromatic ring vibrations. A small band at 1380 cm^{-1} corresponds to aliphatic C —H stretch in CH_3. The bands at 1270 and 1030 cm^{-1} indicate the guaiacyl ring breathing with CO stretching and the aromatic CH in-plane deformation.

The lignin fraction, isolated by 7.5% NaOH at 15°C for 16 h, was also studied by ^{13}C-NMR spectoscopy (Fig. 5). Most of the observed signals have been previously assigned from straw and wood lignin spectra [13, 16–22]. As expected, the spectrum shows the near disappearance of typical neutral polysaccharide signals between 57 and 103 ppm. The only signal for the associated neutral polysaccharides is at 73.7 ppm, and the peak intensity is rather weak.

The region between 104 and 160 ppm is assigned to be aromatic moiety of the lignin. The guaiacyl residues are indicated by signals at 149.3 (C-3, G etherified), 148.0 (C-4, G), 131.5 (C-1, G non etherified), 115.0 (C-5, G), and 111.2 ppm (C-2, G), and *p*-hydroxyphenyl residues by two signals at 127.9 and 127.9 ppm (C-2/C-6, H). The syringyl residues are identified by a signal at 105.7 ppm (C-2/C-6, S), and peak intensity is rather weak. These signals confirm that the lignin fraction could be justified as a GSH-lignin. The signals at 144.6 (C-α, PC ester),

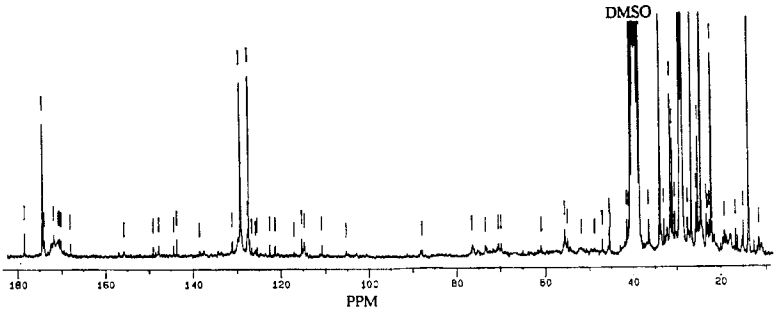

FIGURE 5 ^{13}C-NMR spectrum of alkali lignin extracted with 7.5% NaOH at 15°C for 16 h from depectinated sugar beet pulp (in DMSO-d_6).

129.7 and 129.6 (C-2/C-6, PC ester), 125.8 (C-1, PC ester), and 115.6 and 115.0 ppm (C-3/C-5, PC ester) indicate the presence of esterified *p*-coumaric acid in the isolated lignin fraction. Etherified ferulic acid is observed with signals at 168.1 (C-γ, FE ether), 143.9 (C-α FE ether), 121.6 (C-6, FE ether), and 117.3 ppm (C-β, FE ether). The esterified ferulic acid is identified by the signal at 122.8 ppm (C-6, PE ester). Therefore, it seems very likely that the *p*-coumaric acid is linked at the lignin side chains *b* ester bonds, whereas the ferulic acid is linked at the lignin side chains by both ether and ester bonds.

The signal at 55.7 ppm corresponds to the OCH_3 in guaiacyl and syringyl units. The γ-methyl, α- and β-methylene groups in *n*-propyl side chains appear as intensive signals between 13.9 and 33.8 ppm. The ester bonds between lignin and galacturonic acid may contribute to signals 174.4, 174.1, 172.0, and 171.0 ppm (C-6 in methyl uronates). The major ether linkages between lignin structural units are identified to be β—O—4 ether bonds by signals at 88.2 (C-β in β—O—4), 72.2(C-α in β—O —4), and 61.0 ppm (C-γ in β—O —4). The absence of any signal between 82 and 80 ppm indicates the lack of a α—O—4 ether bond between the lignin structural units. The less common β-β (C-β in β-β units, 55.1 ppm; C-γ in β-β units, 71.6 ppm), β-5 (C-4 in β-5 units, 143.9 ppm, overlapped with C-α, Fe ether C-β in β-5 units, 52.0 ppm), β-1 (C-α in β-1 units, 76.8 ppm), and 5-5' (C-5/C-5' in 5-5' units, 126.2 ppm) carbon-carbon linkages are also identified as being present between lignin structural units.

The data reported here show that the two alkali lignin fractions LA have similar chemical compositions and physico-chemical properties.

Both of the then are relatively free of polysaccharides, and are composed of large amounts of guaiacyl units with fewer syringyl and *p*-hydroxyphenyl units. The predominant linkages of β—O—4 ether bonds together with the less common carbon-carbon linkages, such as β-β, β-5, β-1, and 5-5' are found to be present between the lignin structural units. *p*-coumaric acid and galacturonic acid are identified as esterified to lignin, while ferulic acid is linked to lignin by both ether and ester bonds, which have not been previously reported.

Acknowledgements

The authors are grateful for the financial support of this research from the Danisco Sugar Development Centre, Denmark, for the 'Potential Utilization of Sugar Beet Pulp in Non-Food Areas' project, and also to Dr. James Bolton, Director of the BioComposites Centre, for the award of a research fellowship to R.-C. Sun.

References

[1] Sun, R. C. and Hughes, S. (1997). Fractional isolation and physico-chemical characterization of alkali-soluble polysaccharides from sugar beet pulp. *Carbohydrate Polymers*, (in press).
[2] Sun, R. C. and Hughes, S. (1997). Extraction and physico-chemical characterization of pectins from sugar beet pulp. *Polymer J.*, (in press).
[3] Sun, R. C. and Hughes, S. (1997). Fractional extraction and physico-chemical characterization of hemicelluloses and cellulose from sugar beet pulp. *Carbohydrate Polymers*, (in press).
[4] Selvendran, R. R. (1984). *Am. J. Clin. Nutr.*, **39**, 320.
[5] Blakeney, A. B., Harris, P. J., Henry, R. J. and Stone, B. A. (1983). *Carbohydrate Research*, **113**, 291.
[6] Lawther, J. M., Sun, R. C. and Banks, W. B. (1995). *J. Agric. Food Chem.*, **43**, 667.
[7] Sun, R.-C., Lawther, J. M. and Banks, W. B. (1995). *Industrial Crops and Products*, **4**, 127.
[8] Sun, R.-C., Lawther, J. M. and Banks, W. B. (1995). *Industrial Crops and Products*, **4**, 241.
[9] Sun, R.-C., Lawther, J. M. and Banks, W. B. (1997). *Holzforschung*, **51**, 244.
[10] Wen, L. F., Chang, K. C., Brown, G. and Gallaher, D. D. (1988). *J. Food Sci.*, **53**, 826.
[11] Vogel, M. (1991). *Zuckerind.*, **116**, 265.
[12] Vaccai, G., Nicolucci, C., Mantovani, G. and Monegato, A. (1994). *Zuckerind.*, **118**, 855.
[13] Scalbert, A., Monties, B., Guittet, E. and Lallemand, J. Y. (1986). *Holzforschung*, **40**, 119.
[14] Guillon, F. and Thibault, J. F. (1988). *Lebensm. Wiss. u. -Technol.*, **21**, 198.
[15] Billa, E., Tollier, M. T. and Monties, B. (1996). *J. Sci. Food Agric.*, **72**, 250.

[16] Kondo, T., Hiroi, T., Mizuno, K. and Kato, T. (1990). *Can. J. Plant. Sci.*, **70**, 193.
[17] Himmelsbach, D. S. and Barton II, F. E. (1980). *J. Agric. Food Chem.*, **28**, 1203.
[18] Nimz, H. H., Robert, D., Faix, O. and Nemr, M. (1981). *Holzforschung*, **35**, 16.
[19] Liu, X.-A., Lee, Z.-Z. and Tai, D.-S. (1989). *Cellulose Chem. Technol.*, **23**, 559.
[20] Imamura, T., Watanabe, T., Kuwahara, M. and Koshijima, T. (1994). *Phytochemistry*, **37**, 1165.
[21] Pan, X., Lachenal, D., Neirinck, V. and Robert, D. (1994). *J. Wood Chem. Technol.*, **14**, 483.
[22] McElroy, R. D. and Lai, K. (1988). *J. Wood Chem. Technol.*, **8**, 361.