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Fractional isolation and physico-chemical characterization of alkali-soluble polysaccharides from sugar beet pulp

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Abstract

Pectin rich polysaccharide fractions were extracted with 2% sodium hydroxide at 45°C for 0.5, 1, 2, 3, 4 and 5 h, respectively, from sugar beet pulp. The neutral sugar rich polysaccharide preparations were obtained by treatment of the alkali extracts with pectinase. The pectin rich alkali-soluble polysaccharide–lignin complexes contained 36.7%–46.7% galacturonic acid, 13.7%–16.0% neutral sugars, 9.2%–14.8% protein, 3.6%–9.1% lignin and 18.9%–22.9% ash. The hemicellulose–lignin complex preparations were composed mainly of neutral sugars, in which galactose, rhamnose and arabinose were detected as the major sugar constituents. The weight-average molecular weights of the alkali-soluble polysaccharide–lignin complex fractions (38400–184900 Da) were much higher than those of the hemicellulose–lignin complex preparations (21100–26400 Da). The acetyl groups and methyl esters were found to be fully saponified during the alkali treatments, while feruloyl and *p*-coumaroyl esters appeared to be more resistant to the alkali conditions. Besides ferulic and *p*-coumaric acids, six other phenols were also firstly identified in the nitrobenzene oxidation products from all the isolated 12 fractions. These findings indicated that coumaryl, coniferyl and sinapyl alcohols are tightly associated with hemicelluloses and pectin in sugar beet pulp cell walls.

Keywords: Hemicellulose–ligin complex; Pectin; Sugar beet pulp; Alkali-soluble polysaccharides; Sugars; *p*-coumaric acid; Ferulic acid; Molecular weight

1. Introduction

Sugar beet pulp (SBP) is a raw material from the sugar refining industry and is mainly used for animal feeding (Michel et al., 1988). About 10^7 tonnes of SBP are produced every year in Western European (Dinand et al., 1996). On a dry weight basis, SBP contains 75%–80% polysaccharides, consisting roughly of 22%–24% cellulose, 30% hemicelluloses, mainly arabinans and (arabino) galactans and 25% pectin. Small amounts of fat, protein, ash and lignin contents in SBP are 1.4%, 10.3%, 3.7% and 5.9%, respectively.

Weibel and Myers (1990) in their detailed review have shown that SBP cellulose could have a strong potential for a number of applications in which rheology is important. Unlike most cellulose originating from secondary wall fibres, the cellulose from SBP is a typical primary wall cellulose, also called parenchymal cell cellulose. This kind of cellulose could be described as a 'dispersed membranous product' which upon shearing leads to 'expanded or hairy membranes' and is now used to improve comestibles. Arabinans from SBP can be used as gelling products and fat replacers, after enzymatic treatment to reduce branching (Oosterveld et al., 1996).

The pectins of SBP are complex heteropolysaccharides containing galacturonic acid, rhamnose, arabinose and galactose as the major sugar constituents. Sugar beet pectins have a relatively low viscosity and a poor gelling capacity compared to citrus and apple pectins, which limits their application. These poor physico–chemical properties were attributed to their high number of acetyl groups and the relatively low molecular weight (Oosterveld et al., 1996). Ferulic acid is associated almost exclusively with the pectic side chains of sugar beet, and is found ester-linked to either the C-2 of arabinofuranose or to the C-6 of galactopyranose residue. The presence of ferulic acid makes it possible to cross-link sugar beet pectins. Recent studies suggested that the ferulic acid is distributed about equally between the arabinan and galactan moieties (Kroon and Williamson, 1996). The structure of feruloylated galactose disaccharide, obtained by enzymatic degradation or mild acid hydrolysis of SBP, was confirmed as *O*-(6-*O*-*trans*-feruloyl-*β*-D-galactopyranosyl)-(1 → 4)-D-galactopyranose (Colquhoun

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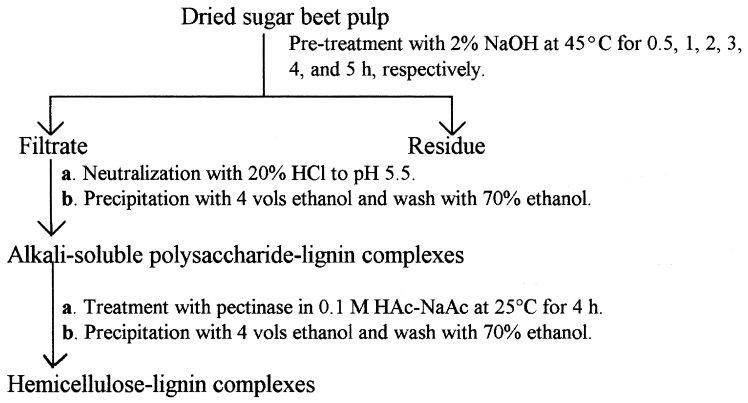

Fig. 1. Scheme for isolation of polysaccharides from the hydrolysates of alkali treatment of SBP.

et al., 1994). From these results the authors suggested that feruloyl groups in SBP are esterified to the arabinofuranosyl residues of the main core of α-(1 → 5) linked arabinan chains and to the galactopyranosyl residues of the main core of β-(1 → 4) linked type I galactan chains, in which 50%–55% of the feruloyl groups are linked to arabinose residues and 45%–50% to galactose residues (Ralet et al., 1994). These chains of arabinan and galactan polysaccharides are thought to play an important role in connecting rhamnogalacturonan to other cell wall components. Further, ferulic acid substituents can dimerize to diferulate bridges between side chains, between different rhamnogalacturonan molecules and possibly between other feruloylated cell wall components such as arabinoxylans, thus, increasing the molecular weight (Oosterveld et al., 1996). Cross-link polysaccharides through diferuloyl bridges have also been identified in cell walls of bamboo shoots (Ishii, 1991).

Traditionally, commercial pectins are being extracted from the soft tissues of citrus and apples under acidic conditions or using hot water. This process generally results in degradation of the arabinan side chains and therefore in a loss of feruloyl groups. Arabinan rich polysaccharides are generally extracted using hot alkali, releasing the feruloyl groups by saponification (Oosterveld et al., 1996). Recent work performed at our laboratory has focused on the development of isolated or chemically modified hemicelluloses, obtained from the agricultural wastes such as straw and SBP, for the new industrial applications. The native hemicelluloses, isolated from wheat straw, were processed through into decorative paints and show good properties from which real commercial systems may be developed. The acetylated, stearoylated and oleoylated straw hemicelluloses are more hydrophobic than the native hemicelluloses and were shown to dissolve in most of the common organic solvents, e.g. acetone, chloroform. The results obtained also show the modified hemicelluloses can be cast into films from simple solvents and could therefore be used as raw materials for preparation of films. To investigate the potential of SBP polysaccharides in this area, a thorough study and characterization of alkali-soluble polysaccharides is necessary. The aim of this study was to examine the effect of 2% sodium hydroxide treatment duration on the physico–chemical properties of alkali-soluble polysaccharides from SBP. Special attention is paid to their lignin association and composition.

2. Material and methods

2.1. Materials

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 kept at 5°C before extraction.

2.2. Alkali treatment of SBP

SBP (20 g) was dispersed for 0.5, 1, 2, 3, 4 and 5 h, respectively, into 800 ml 2% NaOH solution at 45°C under stirring. The alkali-soluble polysaccharide–lignin complexes were recovered by precipitation of the neutralized hydrolysate in 4 vols of ethanol. The hemicellulose–lignin complex preparations were isolated by treatment of the previous complexes with pectinase in 0.1 M HAc-NaAc solution at 25°C for 4 h (Fig. 1).

2.3. Characterization of alkali-soluble polysaccharides

Neutral sugar composition in isolated polysaccharide fractions was determined as alditol acetates (Blakeney et al., 1983). Alkaline nitrobenzene oxidation of residual lignin from alkali-soluble polysaccharide–lignin complex fractions and hemicellulose–lignin complex preparations was performed at 170°C for 3 h. The methods of uronic acid analysis and determination of phenolic acids and aldehydes with HPLC in nitrobenzene oxidation mixture were described in previous papers (Lawther et al., 1995; Sun et al., 1996). All the analyses including nitrobenzene oxidation results and neutral sugar analyses represent the mean of at least triplicate determinations and each mixture was chromatographed twice. The standard errors or deviations were

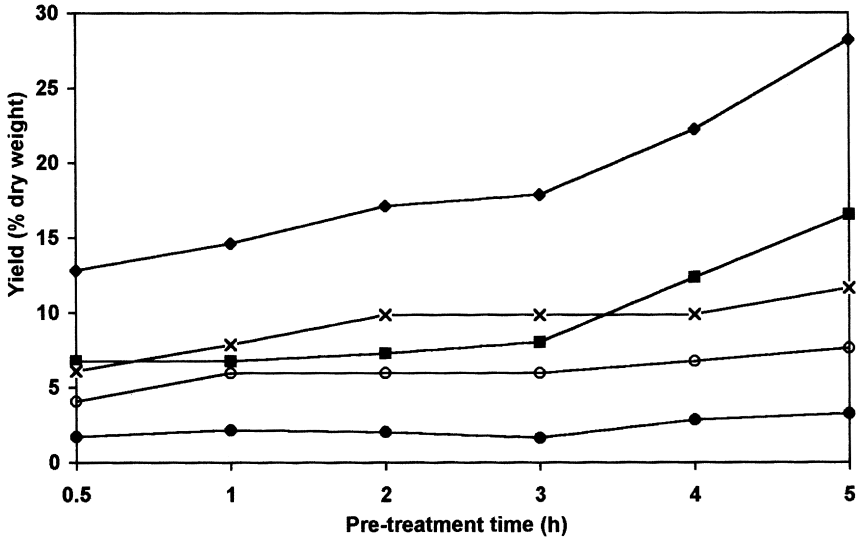

Fig. 2. The effect of treatment time on the extraction yields (% dry weight of sugar beet pulp) of alkali-soluble polysaccharide–lignin complexes (♦), hemicellulose–lignin complexes (×), pectin (■), protein (●) and lignin (○) during 2% NaOH treatment at 45°C.

observed to be lower than 5.5% except for the variations among the triplicate nitrobenzene oxidations (7–16%). FT-IR spectra were obtained on an FT-IR spectrophotometer (Nicolet) using a KBr disc containing 1% finely ground samples.

The weight-average molecular weight of alkali-soluble polysaccharide fractions were determined by gel permeation chromatography on a PL aquagel-OH 50 column. The samples were dissolved with 0.02 N NaCl in 0.005 M sodium phosphate buffer, pH 7.5 at a concentration of 0.1% and 200 μl sample in solution was injected. The column was operated at 40°C and eluted with 0.02 N NaCl in 0.005 M sodium phosphate buffer, pH 7.5 at a flow rate of 0.3 ml min^{-1}. The column was calibrated with PL pullulan polysaccharide (peak average molecular weights 738, 5900, 23700, 100000, 380000 and 600000).

3. Results and discussion

3.1. Yield of released polysaccharides, protein and lignin

The effect of treatment duration on the yield of released alkali-soluble polysaccharide–lignin complexes, hemicellulose–lignin complexes, pectin, protein and lignin during 2% NaOH treatment at 45°C is shown in Fig. 2. The dry matter losses during the treatment time at 0.5, 1, 2, 3, 4 and 5 h with 2% NaOH at 45°C were 15.8%, 19.9%, 22.4%, 23.2%, 27.9% and 34.1%, respectively. The yields of alkali-soluble polysaccharide–lignin complexes, hemicellulose–lignin complexes, pectin and lignin ranged between 12.9%–28.2%, 6.1%–11.7%, 6.8%–16.6% and 2.9%–5.9%, respectively, during treatment time from 0.5 to 5 h. This trend showed that the release of pectin and lignin paralleled the yield of alkali-soluble polysaccharide–lignin complexes. The solubility of hemicellulose–lignin complexes was higher than that of pectin during the treatment between 1 and 3 h. At treatment for 3 h, about 10% hemicellulose–lignin complexes was obtained, while only 8.0% of pectin was dissolved. However, with the increase of treatment time from 3 to 5 h, the yield of released pectin increased significantly from 8.0% to 16.6%, while the yield of hemicellulose–lignin complexes increased slightly from 10% to 11.7%. This phenomenon indicated that extension of extraction duration favoured the release of pectic substances. One can therefore predict that if this trend continues during the further treatment process, there will be more pectins released.

It is well known that alkali treatment can completely or partially cleave the ester bonds, such as linkages between ferulic acid and arabinan or galactan in SBP cell walls, resulting in the dissolution of pectins and hemicelluloses. Release of the relatively pure lignins (2.9%–5.9%) in alkali media resulted from the cleavage of aryl ether bonds in poly-phenolic units or ether linkages between lignin and hemicelluloses or pectins. The pure dissolved lignins, obtained by precipitation of the supernatant with 20% HCl at pH 1.5 after isolation of the alkali-soluble polysaccharide–lignin complexes, will be studied in detail in a forthcoming paper (Sun and Hughes, 1997).

3.2. Content of neutral sugars and galacturonic acid

The neutral sugar composition and content of galacturonic acid in alkali-soluble polysaccharide–lignin complexes are given in Table 1. As can be seen, the complexes consisted of galacturonic acid, neutral sugars, ash, protein and lignin. Polysaccharides accounted for 56.1%–60.9% of the dry weight samples. The high content in galacturonic acid (36.8%–46.7%) suggested the presence of a significant proportion of pectic substances. Arabinose

Table 1
The contents (% dry sample, w/w) of neutral sugars, anhydrogalacturonic acid, protein, lignin and ash in the alkali-soluble polysaccharide–lignin complexes obtained during 2% NaOH treatment of SBP at 45°C for various periods

Composition[a]	Treatment time (h)					
	0.5	1	2	3	4	5
Rhamnose	2.1	1.9	1.9	1.8	1.8	2.1
Arabinose	9.1	8.1	7.6	7.3	6.8	7.8
Xylose	0.5	0.4	0.3	0.3	0.3	0.5
Mannose	0.9	0.4	0.4	0.4	0.7	0.6
Glucose	1.7	0.9	0.7	0.7	0.9	1.1
Galactose	5.1	3.7	3.3	3.3	3.2	3.8
Total of sugars	19.3	15.3	14.2	13.8	13.7	16.0
Anhydrogalacturonic acid	36.8	42.4	46.7	46.4	42.1	40.1
Protein	13.2	14.8	11.9	9.2	12.8	11.6
Lignin	9.1	5.6	4.0	3.6	5.2	6.3
Ash	19.7	18.9	19.5	22.8	22.9	23.0

[a]Data is expressed on a dry basis and represents the mean of duplicate or triplicate runs.

(6.8%–9.1%), galactose (3.2%–5.1%) and rhamnose (1.8%–2.4%) were the major non cellulosic neutral sugars. Small amounts of xylose, mannose and non-cellulosic glucose were also present. Ash content was high, 18.9%–23.0% and the protein (N × 6.25) represented 9.2%–14.8%. No significant trend towards an increase or a decrease in the yields of protein was noticeable. Only minor changes in the yields of galacturonic acid, neutral sugars and ash were observed. Increase of treatment time from 0.5 to 2 h resulted in an increase of galacturonic acid content from 36.8% to 46.7%, while it decreased from 46.7% to 40.1% during the further treatment period between 2 and 5 h. As the alkali treatment time increased from 0.5 to 4 h, a negative correlation in the yield of neutral sugars and a positive correlation in ash content was noted. Interestingly, the content of associated lignin in alkali-soluble polysaccharides reduced significantly from 9.1% to 3.6% during the treatment time between 0.5 and 3 h, suggesting that more ether bonds between lignin and hemicelluloses or pectins were cleaved as the extraction progressed. The high content of lignin (5.2%–6.3%) in the 4 and 5 h extracted fractions was probably caused by lignin condensation during the longer alkali treatment processes.

In this study an alkali extraction of SBP was used to extract arabinose and galactose rich pectic polysaccharides and the results were reproducible. As compared to acid extracted sugar beet pectins (Michel et al., 1985; Phatak et al., 1988; Arslan, 1995), high amounts of arabinans were present in all the alkali-soluble polysaccharide–lignin complex fractions, which may affect their functional properties. The reason for the lower content of arabinans in acid extracted sugar beet pectins is probably that some glycoside bonds, mainly those involving arabinose and rhamnose, were split. A few ester linkages and a few bonds between galacturonic acid residues may also have been split (Guillon and Thibault, 1988). Further, based on the studies of alkali extracted pectic polysaccharides from SBP, Oosterveld et al. (1996) mentioned that the arabinosyl linkage compositions of the extracted pectins were similar to those found in acid and alkali extracted pectins and in arabinans from other sources.

It was found that rhamnose was involved in the pectic backbone and may constitute the point of attachment of the arabinose and galactose sugar side chains. Degrees of methylation and acetylation were not measured since alkali-soluble fractions would be totally or partially saponified by the conditions of extraction. The high yield of released pectic polysaccharides (16.6%), during the 2% NaOH treatment at 45°C for 5 h, was probably caused by increased cleavage of linkages between pectin and other cell wall components. With the studies of changes in the pectic substances of apples during development and postharvest ripening, Fischer et al. (1994) suggested that non-esterified carboxylic groups of the galacturonic acid residues may be ester-linked to hydroxyl groups of the hemicellulosic sugars and that these ester linkages would be saponified by the alkali solvent, thus, releasing the pectic polymers in solution. Clearly, the high yield of pectic substances found in the current study is caused by saponification of ester bonds between pectins and hemicelluloses.

The occurrence of 3.6%–9.1% bound lignin in extracted alkali-soluble polysaccharides indirectly indicates that there is an associative interaction between polysaccharides and lignin. The presence of linkage between hemicelluloses and lignin in the monocotyledons was extensively studied. In our previous studies (Sun et al., 1995) it was mentioned that the majority of wheat straw lignin is directly linked to arabinose side-chains of xylan by ether bonds in the cell walls. The results obtained also showed that some amounts of glucuronic acid or 4-*O*-methylglucuronic acid are also directly esterified to lignin, which was confirmed by $^{13}C-NMR$ spectroscopy. In the Chenopodiacees, the presence of associated lignin in extracted pectic polysaccharides from cell walls of SBP and suspension-cultured spinach was reported by Ishii (1994) and Guillon and Thibault (1988). The authors state that in the purified pectins, the presence of ferulic acid, *p*-coumaric acid and other phenols was demonstrated by the colorimetric measurements.

Table 2
The neutral sugar composition (relative %) and contents of anhydrogalacturonic acid and lignin (% sample, w/w) in the hemicellulose–lignin complex fractions obtained by treatment of the alkali-soluble polysaccharide–lignin complexes with pectinase in 0.1 M HAc-NaAc at 25°C for 4 h

Composition[a]	Treatment time (h)					
	0.5	1	2	3	4	5
Rhamnose	26.0	26.8	27.3	28.4	28.8	28.6
Arabinose	11.4	12.4	12.9	12.7	12.4	12.4
Xylose	6.4	3.9	3.7	3.7	3.9	3.6
Mannose	10.3	9.8	9.7	9.1	9.0	9.0
Glucose	5.3	5.1	5.1	4.7	4.6	4.3
Galactose	40.6	42.0	41.2	41.5	41.4	42.1
Anhydrogalacturonic acid	13.0	13.0	13.1	11.3	11.1	10.9
Lignin	4.2	5.5	5.1	2.3	1.9	1.5

[a]Data is expressed on a dry basis and represents the mean of duplicate or triplicate runs.

The appearance of small amounts of lignin in the pectin rich polysaccharide fractions (Table 1) suggested that some amounts of lignin were probably linked to pectic substances. These phenomena were in good agreement with Sugino et al. (1994), showing that coumaryl, coniferyl and sinapyl alcohols associated with hemicelluloses and pectin.

The presence of some proteins was previously reported in highly purified hemicelluloses and pectins (Akiyama and Kato, 1977; Guillon and Thibault, 1988). In hemicellulose–protein complexes, most of the hydroxyproline residues are glycosylated by a tri or tetraarabinoside (Lamport et al., 1973). Many of the serine residues are also glycosylated but with galactose rather than arabinose (Lamport and Miller, 1971). In sugar beet pectins, proteins are rich in hydroxy-amino-acid, acid amino-acids, serine and threonine. This amino-acid composition is comparable to that of some glycoproteins isolated from different plant tissues, in which the presence of uronic acid and rhamnose, probably from pectic material, was reported. Further investigation suggested that these proteins may be linked to the pectic backbone through their carbohydrate parts which are mainly composed of arabinose and galactose (Guillon and Thibault, 1988). The relatively high content of protein in alkali extracts (Table 1) suggested that proteins in SBP cell walls are tightly bound to hemicelluloses and pectins.

Treatment of the alkali extracts with pectinase (EC 3.2.1. 15 from *Aspergillus niger*), in 0.1 M HAc-NaAc buffer at 25°C for 4 h, resulted in fractions rich in hemicelluloses with a few amounts of bound lignin. The neutral sugar composition and contents of galacturonic acid and lignin in these hemicellulose–lignin complex fractions are shown in Table 2. As seen, all the fractions appeared to have high content in neutral sugars. Galactose, rhamnose, and arabinose were the major neutral sugars. Xylose and glucose were found in small amounts. A significant decrease of galacturonic acid content from about 40% in alkali extracts (Table 1) to 10%–13% in hemicellulose–lignin complex fractions (Table 2), indicates that the pectinase, which is a polygalacturonase, mainly broke down the glycoside linkages between the galacturonic acids in a 'smooth region' of pectin, subsequently resulting in rich neutral sugars in the hemicellulose–lignin complex fractions. These increasing amounts of neutral sugars indicate that they are located in the 'hairy fragments' of pectic polysaccharides. A decrease in lignin content from 3.6%–9.1% in alkali extracts to 1.5%–5.5% in pectinase treated fractions again suggests the presence of esterified linkages between galacturonic acid and lignin in SBP cell walls.

Table 3
The yield (% sample, w/w) of phenolic acids and aldehydes from alkaline nitrobenzene oxidation of lignin in the alkali-soluble polysaccharide–lignin complexes obtained during 2% NaOH treatment of SBP at 45°C for various periods

Phenolic acids and aldehydes[a]	Treatment time (h)					
	0.5	1	2	3	4	5
p-Hydroxybenzoic acid	0.19	0.13	0.11	0.11	0.15	0.16
p-Hydroxybenzaldehyde	0.095	0.059	0.043	0.035	0.057	0.058
Vanillic acid	0.045	0.022	0.0088	0.005	0.015	0.021
Syringic acid	0.055	0.042	0.022	0.011	0.031	0.033
Vanillin	0.11	0.052	0.042	0.032	0.035	0.092
Syringaldehyde	0.042	0.020	0.012	0.005	0.009	0.045
p-Coumaric acid	0.088	0.063	0.045	0.042	0.063	0.065
Ferulic acid	0.058	0.031	0.021	0.026	0.026	0.042
Total	0.68	0.42	0.30	0.27	0.39	0.47

[a]Data is expressed on a dry basis and represents the mean of triplicate analyses.

Table 4
The yield (% sample, w/w) of phenolic acids and aldehydes from alkaline nitrobenzene oxidation of lignin in the hemicellulose–lignin complex fractions obtained by treatment of the alkali-soluble polysaccharide–lignin complexes with pectinase in 0.1 M HAc-NaAc at 25°C for 4 h

Phenolic acids and aldehydes[a]	Treatment time (h)					
	0.5	1	2	3	4	5
p-Hydroxybenzoic acid	0.028	0.021	0.021	0.0063	0.0044	0.0075
p-Hydroxybenzaldehyde	0.072	0.098	0.088	0.078	0.058	0.049
Vanillic acid	0.024	0.042	0.049	0.0089	0.0071	0.0067
Syringic acid	0.020	0.030	0.032	0.0061	0.0060	0.0030
Vanillin	0.082	0.092	0.078	0.034	0.030	0.020
Syringaldehyde	0.021	0.037	0.024	0.0079	0.0072	0.0032
p-Coumaric acid	0.038	0.050	0.050	0.016	0.015	0.015
Ferulic acid	0.021	0.038	0.034	0.013	0.012	0.010
Total	0.31	0.41	0.38	0.17	0.14	0.11

[a]Data is expressed on a dry basis and represents the mean of triplicate analyses.

3.3. Composition of phenolic acids and aldehydes

To further verify the presence of lignin, nitrobenzene oxidation of isolated alkali-soluble polysaccharide–lignin complex fractions and hemicellulose–lignin complex preparations was performed. This method provided an estimate of the amount of lignin and an indication of the composition of the associated lignin. As shown in Table 3 and Table 4, the nitrobenzene oxidation produced approximately twice the amounts of vanillin than of syringaldehyde, suggesting that the majority of the pectins and hemicelluloses were linked to lignin with guaiacyl units. No significant difference between vanillin/syringaldehyde molar ratios was found among the 12 isolated complexes. A relatively high amount of *p*-hydroxybenzoic acid in the alkali extracts was partially caused by the oxidation of associated *p*-coumaric acid. Other phenolic monomers, such as *p*-hydroxybenzaldehyde, vanillic acid and syringic acid were also identified in all the mixtures of nitrobenzene oxidation. With the study of acid and alkali-soluble pectins from SBP, Guillon and Thibault (1988) mentioned that the presence of other phenols was indicated by the colorimetric measurements. However, these unidentified phenolic acids and aldehydes were not recovered in the hairy fragments, probably because they were eliminated with the oligogalacturonides during gel filtration. Therefore, the authors concluded that the presence of phenolics other than *p*-coumaric and ferulic acids in the isolated pectic polysaccharides may be an artifact of the isolation procedure of pectic substances. This is the first indication of the presence of such other phenolics in the nitrobenzene oxidation products of associated lignin in alkali extracted polysaccharides from SBP.

As mentioned earlier, both acid and alkali-soluble pectins contained some amounts of ferulic acid and *p*-coumaric acid. These hydroxycinnamic acids were found to be esterified to the 'hairy fragments' of sugar beet pectin and were known to be linked both to arabinose and galactose (Guillon and Thibault, 1988). Despite the alkaline conditions in this study, ferulic acid and *p*-coumaric acid were still present in all the alkali extracts (Table 3) and the pectinase treated fractions (Table 4), indicating the greater resistance of *p*-coumaroyl and feruloyl esters to alkali conditions than of acetyl groups and methyl esters. This observation agreed with the results reported by Oosterveld et al. (1996). After extraction with methanol for 16 h and air drying, the residue of SBP was found to contain 1.2 mg hydroxycinnamic acids per gram of dry cell walls. The molar ratio of *p*-coumaric, ferulic and diferulic acid, as determined by HPLC, was 1:165:0.6 (Ishii, 1994). Further characterization with NMR spectroscopy enabled the structure of feruloyl arabinobinose to be identified as *O* (2-*O*-*trans*-feruloyl-α-L-arabinofuranosyl)-(1 → 5)-L-arabinofuranose (Ishii, 1994).

3.4. Distribution of molecular weight

The weight-average (M_w) and number-average (M_n) molecular weights and the polydispersity (M_w/M_n) of the alkali-soluble polysaccharide–lignin complex fractions and hemicellulose–lignin complex preparations are given in Table 5. Obviously, resulting from the associated pectic substances, the alkali extract fractions, isolated with 2% NaOH at 45°C for 0.5–5 h from SBP, had a high degree of polymerization with weight-average molecular weights between 184900 and 38400 Da. This was high compared to the literature values for SBP pectins (Arslan, 1995; Phatak et al., 1988) and was also much higher than those of hemicellulose–lignin complex preparations, obtained by hydrolysis of the alkali extracts with pectinase at 25°C for 4 h. Increase of treatment duration from 0.5 to 5 h resulted in a decrease of molecular weight of alkali extracts from over 180000 to below 40000. These results suggest that extraction of alkali-soluble polysaccharides with 2% NaOH at 45°C over longer periods might result in degradation of polysaccharides. One can therefore, see that treatment duration has a strong effect on the molecular size of the isolated polysaccharides. On the other hand, as seen in Table 5 2% NaOH treatment duration showed only a small effect on molecular-weight, as decreasing from 26400 at 0.5 h to 21100 at 5 h for the hemicellulose–lignin complex fractions.

Table 5
The weight-average (M_w), number-average (M_n) molecular weights and the polydispersity (M_w/M_n) of the alkali-soluble polysaccharide–lignin complexes and hemicellulose–lignin complex fractions obtained from SBP

Treatment time (h)	$\overline{M_w}$		$\overline{M_n}$		$\overline{M_w}/\overline{M_n}$	
	A[a]	B[b]	A	B	A	B
0.5	184 900	26 400	11 000	12 200	16.8	2.2
1	85 600	22 700	10 500	10 300	8.1	2.2
2	84 300	21 700	10 600	9400	8.0	2.3
3	75 100	21 700	10 400	9500	7.2	2.3
4	47 900	21 200	8300	9900	5.8	2.1
5	38 400	21 100	8000	10 100	4.8	2.1

[a]Represent the alkaline soluble polysaccharide–lignin complexes.
[b]Represent the hemicellulose–lignin complex fractions.

The elution profiles of hemicellulose-lignin fraction, isolated from the hydrolysate of 2% NaOH treatment at 45°C for 2 h, showed two significant peaks and two small peaks (Fig. 3). The molecular weight distribution ranged between 363100 and 1400 Da. The small peak I eluted in the volume (8.50 ml) and had a molecular weight equal to 50800 Da. Two major peaks II and III had a molecular weight around 17600 and 6600, respectively. The low molecular weight eluted at peak IV was probably caused by the fragmentation of hemicelluloses or the associated lignins.

3.5. FT-IR spectra

The FT-IR spectra of the three alkali-soluble polysaccharide–lignin complexes are shown in Fig. 4. As seen, no significant difference in the main absorption intensity was observed among the three fractions. The pectic substances belong to a class of carboxypolysaccharides which differ from neutral polysaccharides, with an intense band in the region 1740 cm^{-1} (for salts around 1610 cm^{-1}) related to vibrations of the carboxyl group (Filippov, 1992). From this point, the alkali-soluble polysaccharide–lignin complexes contained much higher amounts of bound pectic substances as shown by a significant absorption at 1625 cm^{-1} (salt), which corresponded with the chemical analysis. Disappearance of the ester band at 1740 cm^{-1} is undoubtedly caused by the fully saponification of acetyl groups and methyl esters, indicating that the alkali-soluble pectic polysaccharides obtained in this study are fully deesterified. The prominent bands at 1017 and 1054 cm^{-1} can be attributed to the C-OH bending. The band at 895 cm^{-1} corresponds to the glycosidic C_1-H deformation with ring vibration contribution and OH bending. The low intensities of the bands at 956 and 1160 cm^{-1} represent C-O, C-O-C, stretching and C-OH bending (Kacurakova et al., 1994). The small band at 1098 cm^{-1} indicates the ring vibration and C-OH bending. The bands at 1241, 1322 and 1417 cm^{-1} represent C-H bending; CH wagging and OH bending; and CH, OH bending, respectively (Kacurakova and Mathlouthi, 1996).

The FT-IR spectra of three hemicellulose–lignin

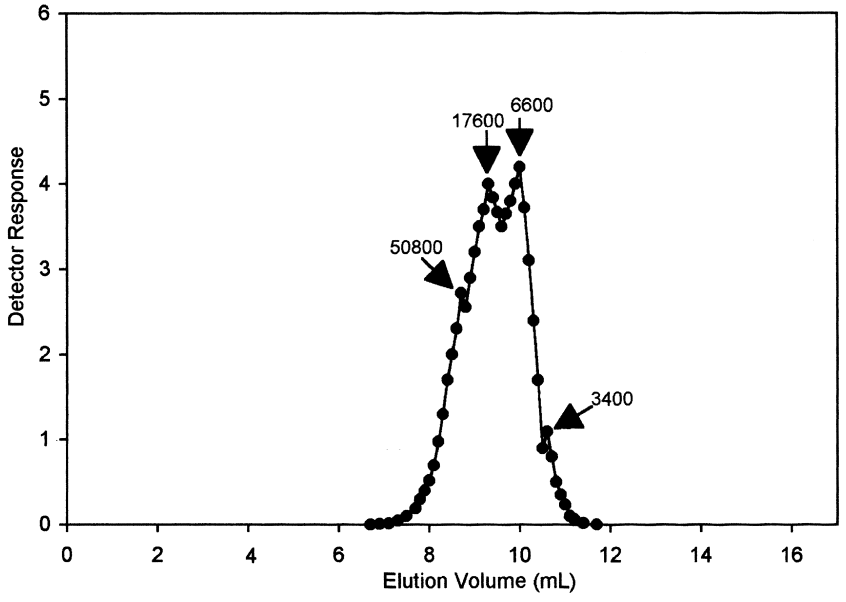

Fig. 3. GPC molecular weight distribution of hemicellulose–lignin complex isolated from the hydrolysate of 2% NaOH treatment at 45°C for 2 h.

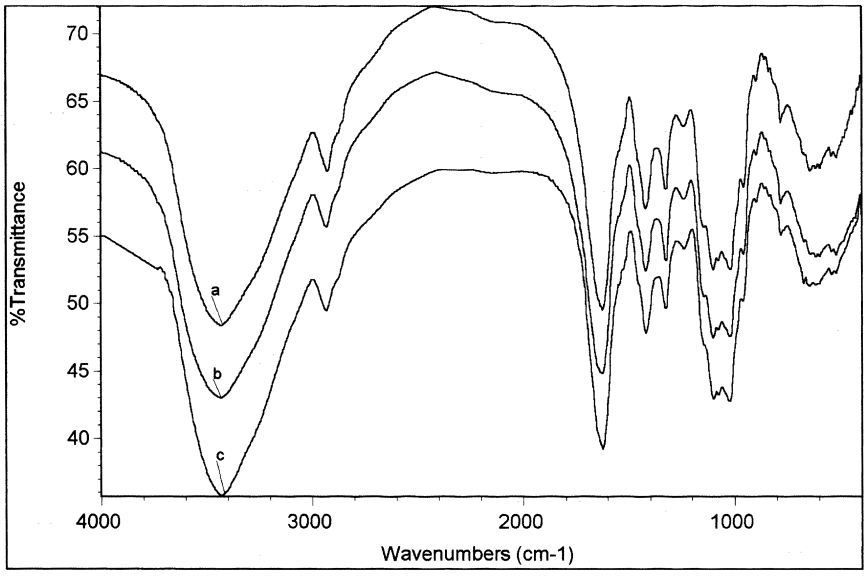

Fig. 4. FT-IR spectra of alkali-soluble polysaccharide–lignin complexes extracted with 2% NaOH at 45°C for: (a) 0.5 h; (b)2 h; and (c) 5 h.

complex fractions (Fig. 5) initially appeared rather similar to the spectra of corresponding three alkali-soluble polysaccharide–lignin complexes (Fig. 4). However, on closer examination, the spectra of hemicellulose–lignin complexes can be clearly distinguished from the spectra of alkali-soluble polysaccharide–lignin complexes by the increase of intensity at 1625 and 1322 cm^{-1}. An increase of intensity at 1625 cm^{-1} was probably caused by the presence of water or residual pectinase (salt) in the isolated hemicellulose–lignin complex preparations, while the, to be found, increase of intensity at 1322 cm^{-1} can be attributed to the C-H wagging and OH bending in galactose, which is in accordance with the results obtained by sugar analysis.

Based on the previous results, it can be concluded that 2% NaOH extraction of SBP resulted in pectin rich polysaccharide–lignin complex fractions, which were composed mainly of galacturonic acid (36.8%–46.7%), neutral sugars (13.7%–16.0%), protein (9.2%–14.8%) and lignin (3.6%–9.1%). Arabinose and galactose were detected as the major neutral sugar components, while rhamnose, xylose, mannose and glucose were identified as minor constituents. Increasing amounts of neutral sugars were obtained by treatment of the alkali extracts with pectinase, indicating that neutral sugars are located in the 'hairy fragments' of pectic polysaccharides. The results also showed that the alkali-soluble fractions were deesterified by saponification of the acetyl groups and methyl esters, while feruloyl and *p*-coumaroyl esters were more resistant to the alkali conditions. Both ferulic acid and *p*-coumaric acid were found to be esterified to arabinose and galactose in the 'hairy fragments' of pectic polysaccharides. The

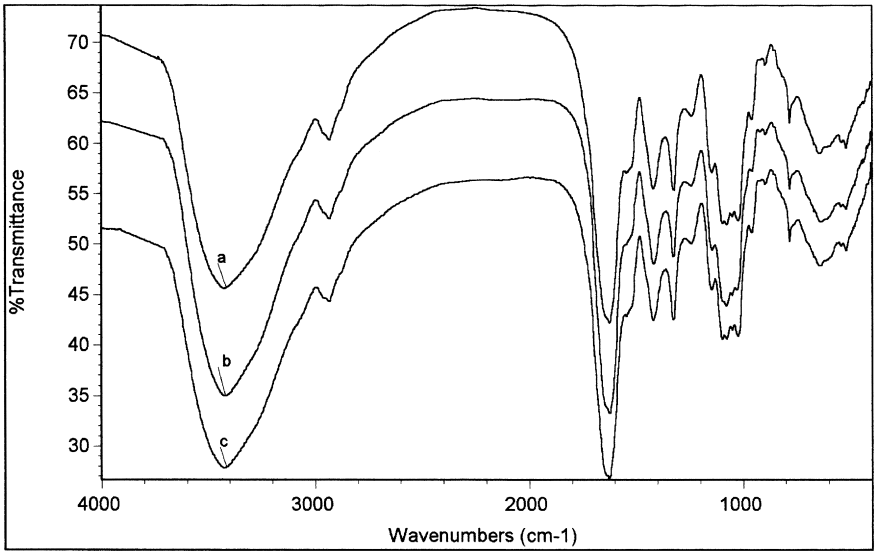

Fig. 5. FT-IR spectra of hemicellulose–lignin complex fractions isolated from the hydrolysates of 2% NaOH treatment at 45°C for: (a) 0.5 h; (b) 2 h; and (c) 5 h.

findings further confirmed that lignin is tightly associated with hemicelluloses and pectin in SBP cell walls.

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