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Fractional extraction and physico-chemical characterization of hemicelluloses and cellulose from sugar beet pulp

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Abstract

Four hemicelluloses and cellulose fractions were extracted with 10% KOH or 7.5% NaOH at 15°C for 16 h and with 24% KOH or 17.5% NaOH at 15°C for 2 h from defatted, protein and pectin free, lignified or delignified sugar beet pulp (SBP). There was no significant difference in the yield and sugar composition of isolated hemicelluloses and cellulose obtained from four different procedures. 7.5% NaOH extraction at 15°C for 16 h from lignified SBP gave a slightly higher yield of hemicelluloses (10.96%), while 24% KOH extraction at 15°C for 2 h from delignified SBP produced the highest yield of cellulose (18.35%). Molecular-average weights ranged from 88 850 to 91 330 Da for the hemicelluloses obtained from lignified SBP, and 21 620–21 990 Da for the hemicelluloses isolated from delignified SBP. The neutral sugar composition of the hemicelluloses consisted of glucose, arabinose, galactose, xylose, and minor quantities of rhamnose and mannose. The infrared spectra showed an absorption band at 900 cm^{-1}, indicating some amounts of β-linked polysaccharides. Besides ferulic and p-coumaric acids, six other phenolics were also identified in the mixture of alkaline nitrobenzene oxidation of associated lignin in the isolated hemicelluloses and cellulose fractions.

Keywords: Fractional extraction; Physico-chemical; Hemicelluloses; Cellulose; Sugar beet pulp

1. Introduction

Sugar beet pulp (SBP) is a byproduct from the beet sugar industry, produced annually in large quantities. About 10^7 tons of SBP in dry matter equivalent is left over by the sugar industry every year in Western Europe (Dinand et al., 1996). In countries, e.g. UK, with an intensive cattle-raising industry the pulp is used in feed manufacture. On a dry weight basis, SBP contains 65–80% polysaccharides, consisting roughly of 40% cellulose, 30% hemicelluloses, and 30% pectin (Weibel, 1989), and its valorization has become an important field of research. At the present time, the limited quantity of pulp used as dietary fibre has not led to any alternative utilization of beet pulp in bulk quantities. The low content of cellulose together with high levels of pectic substances and hemicelluloses, which are characteristics of the composition of SBP when compared with cane bagasse has always hindered the utilization of SBP for end use such as paper production (Konn and Zima, 1932; Frantisek et al., 1978; Vaccai et al., 1994).

The structure of the hemicelluloses, which is known as araban, is a group of branched chain compounds with the main chain composed of α-1,5-linked L-arabinose and the side chain by α-1,3-linked L-arabinose (Vogel, 1991). After enzymatic treatment to reduce branching, arabans from SBP can be used as gelling products and fat replacers (Oosterveld et al., 1996). However, the structure of the hemicelluloses often varies with the methodologies used for preparation and isolation (Wen et al., 1988). Besides arabinose and galactose, the hemicelluloses also contained xylose and glucose as the main sugar components (Kobayashi et al., 1993). Although the structural features of araban have been studied in detail, there is very little information on the physico-chemical properties of the isolated hemicelluloses from SBP.

The cellulose obtained from SBP was shown having a strong potential for a number of applications in which rheology is important. Unlike most celluloses originating from secondary wall fibres, the cellulose from SBP is a typical primary wall cellulose, also called parenchymal cell cellulose (PCC) (Weibel, 1986, 1989). In the words of Weibel and Myers (1990), PCC could be described as a 'dispersed membranous product' which, upon shearing, led to 'expanded or hairy membranes' (Dinand et al., 1996). The use of sugar beet cellulose fibrils containing some residual pectin as a cloudifier, and of cellulose isolates

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as creamer, has been reported recently (Voragen et al., 1997).

The aim of this study was to develop an extraction procedure for sugar beet hemicelluloses and cellulose with maximal yield and minimal degradation. The physico-chemical properties of isolated hemicelluloses and cellulose from SBP are reported. Special attention is also paid to their associated lignin composition, phenolic acids and aldehydes.

2. Material and 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. Crude lipids were extracted using chloroform–methanol (2:1, v/v) in Soxhlet for 6 h (1.0 g SBP/10 ml extractant). Proteolysis was performed by the addition of protease (1.0 g, 4.5 units mg^{-1}) into 0.1 M sodium phosphate buffer (pH 7.5, 1000 ml) containing dewaxed SBP (100 g) for 2 h at 37°C. The defatted and protein free SBP (80 g) was further extracted with 0.2% disodium ethylenediaminetetraacetic acid (EDTA, 2000 ml) at pH 3.3, 85°C for 1 h. After filtration through a 20μ nylon screen to remove the EDTA-soluble pectins, the residue (71 g) was treated with pectinase (10 ml, 10 000 units) under stirring in 0.1 M acetate buffer (2000 ml), pH 4.0, to remove the residual pectins. After incubation at 25°C for 2.5 h, the solution was filtered through a 20μ nylon screen again. The pectin free residue was air-dried and used for further extraction of hemicelluloses and cellulose.

In Route 1, two hemicellulosic fractions were extracted with 10% KOH and 7.5% NaOH, respectively (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 5 vol. ethanol. The precipitated hemicelluloses were filtered, washed with 75% ethanol, and air-dried. After it was recovered, washed with water and ethanol, and dried in an oven at 50°C for 16 h, the residue (2.0 g) was stirred with water (100 ml), 10% acetic acid (5 ml), and sodium chlorite (2.0 g) for delignification at 75°C. After a 1 h reaction, the residue was filtered out on a nylon cloth, washed with water and ethanol, and then dried in an oven at 50°C for 16 h. The weight of the residue remaining after sodium chlorite oxidation, corrected for ash content, was taken to be cellulose. In Route 2, the defatted, protein and pectin free residue was first delignified with sodium chlorite, and then extracted with 24% KOH and 17.5% NaOH, respectively (3.30 g residue/100 ml extractant) for the isolation of hemicelluloses at 15°C for 2 h with each extractant. The residue, after correction of ash content, was also taken to be cellulose.

The methods for chemical and physico-chemical analyses, including neutral sugar and uronic acid analyses, molecular weight measurement, and alkaline nitrobenzene oxidation of residual lignin in isolated hemicelluloses and cellulose, and determination of phenolic acids and aldehydes with HPLC, were described in previous papers (Lawther et al., 1995; Sun et al., 1995; Sun et al., 1996).

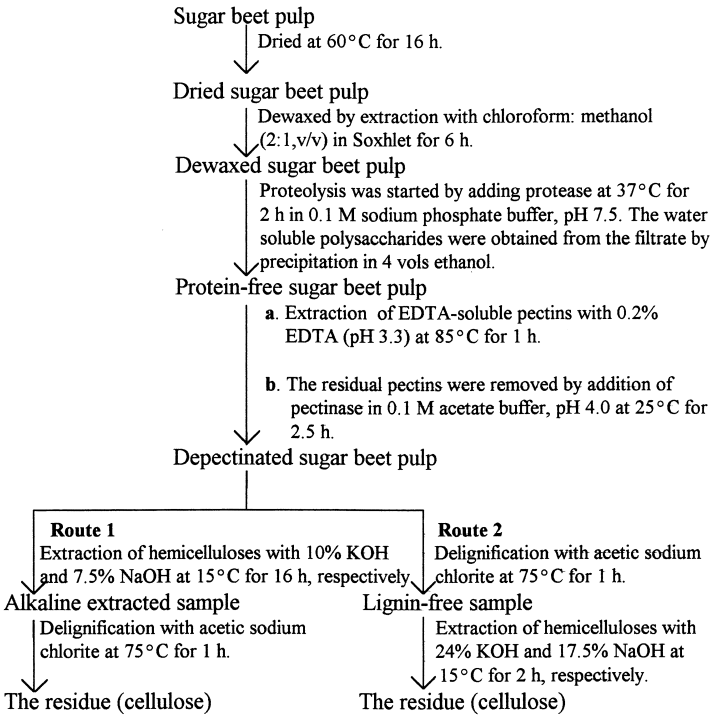

Fig. 1. Scheme for extraction of hemicelluloses and cellulose from sugar beet pulp.

FTIR spectra were obtained on an FTIR spectrophotometer (Nicolet, 750) using a KBr disc containing 1% finely ground samples. The extraction of hemicelluloses and cellulose, and chemical analyses of all the samples were performed in duplicate. The standard errors or derivations were always observed to be lower than 5% except for the variation observed in the triplicate nitrobenzene oxidation (7–15%).

3. Results and discussion

Fat, protein, ash, and lignin contents in SBP were 1.44%, 10.29%, 3.68%, and 5.85%, respectively. By difference, polysaccharide content amounted to 78.74%, and is the major component of SBP. It has been reported that SBP contains large amounts of hemicelluloses and cellulose with the exception of araban and galactan. The scheme for extraction and isolation of hemicelluloses and cellulose from defatted, protein and pectin free SBP is shown in Fig. 1. The procedure shown in Route 1 illustrates extraction of hemicelluloses from the lignified samples, whereas the method in Route 2 indicates extraction of hemicelluloses from the delignified samples, which are renamed holocelluloses. The yield (% dry weight of SBP) of the hemicelluloses and cellulose, and their monosaccharide composition, are given in Table 1. As seen, there was no significant difference in the yield of isolated hemicellulosic fractions. Extraction with 10% KOH and 7.5% NaOH at 15°C for 16 h from the lignified samples yielded hemicelluloses of 10.38% and 10.96%, respectively, in Route 1. In Route 2, extraction with 24% KOH and 17.5% NaOH at 15°C for 2 h from delignified SBP led to slightly lower yields of 9.29% and 9.80% hemicelluloses, respectively. Thus, extraction with 7.5% NaOH is more effective in the extraction of hemicelluloses than is extraction with 10% KOH. The results obtained were in agreement with our previous studies on extraction of wheat straw hemicelluloses (Lawther et al., 1996). We mentioned that sodium hydroxide and lithium hydroxide are more effective than potassium hydroxide for the removal of hemicelluloses from wheat straw. The yield of cellulose obtained in Route 1 and Route 2 ranged between 14.88–15.17% and 18.05–18.35%, respectively. The lower yield of cellulose in Route 1 was probably due to some degradation of cellulose during the 1 h delignification process from the hemicellulose free sample. Obviously, a short delignification time was sufficient to remove the residual lignin from the 10% KOH or 7.5% NaOH extracted SBP.

All the hemicellulosic fractions consisted mainly of glucose, arabinose, galactose, and xylose. Rhamnose, mannose, and uronic acid were present in relatively minor quantities. The hemicelluloses extracted with a relatively low concentration of alkali in Route 1 contained more arabinose and galactose, and less xylose and glucose, while the reverse trend occurred during the extraction with the high alkali concentration treatment in Route 2, indicating that the high alkali concentration treatment resulted in degradation of araban and galactan. Similarly, a relatively higher content of arabinose and galactose appeared in KOH extracted hemicelluloses than in those of NaOH extracts, suggesting that sodium hydroxide had a greater effect on the degradation of araban and galactan during the alkali extraction of hemicelluloses than did potassium hydroxide.

The pectin, isolated from protein free SBP with 0.2% EDTA at pH 3.3, 85°C for 1 h, yielded 6.70% of dry SBP and contained 62.35% galacturonic acid and 10.73% neutral sugars as well as 0.59% ferulic acid, in which arabinose and galactose were the predominant sugars (Sun and Hughes, 1997b). The results obtained were consistent with those of Rombouts and Thibault (1986), Phatak et al. (1988), and Kobayashi et al. (1993). The authors indicated that sugar beet pectin consists of large smooth regions and small hairy regions, in which arabinose and galactose are located in the hairy fragment, while the rhamnose is involved in the pectic backbone and may constitute the point of attachment of the

Table 1
The yield (% dry weight of sugar beet pulp), sugar composition (relative %), and uronic acid content (% dry hemicelluloses) of isolated hemicelluloses and cellulose from sugar beet pulp

Yield/Sugar[a]	Hemicelluloses				Cellulose			
	Route 1		Route 2		Route 1		Route 2	
	10% KOH extract	7.5% NaOH extract	24% KOH extract	17.5% NaOH extract	10% KOH extract	7.5% NaOH extract	24% KOH extract	17.5% NaOH extract
Yield	10.38	10.96	9.29	9.80	15.17	14.88	18.35	18.05
Rhamnose	9.77	9.59	5.07	3.47	ND[b]	ND	ND	ND
Arabinose	25.72	22.99	18.10	11.25	1.00	1.00	1.23	1.52
Xylose	13.34	13.10	20.18	16.88	ND	ND	ND	ND
Mannose	9.04	8.19	6.67	15.23	0.37	0.36	0.63	0.38
Glucose	23.95	31.99	38.18	44.22	98.52	98.53	98.02	98.00
Galactose	18.19	14.14	11.80	8.95	0.11	0.11	0.12	0.10
Uronic acid	5.50	5.62	4.00	3.00	–	–	–	–

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

neutral sugar side chains (Guillon and Thibault, 1988; Selevendran, 1985). The higher content of arabinose than of galactose, with a small amount of rhamnose in EDTA extracted pectins, indicated the presence of a rhamnogalacturonan with highly branched arabans and some galactose attached to it. This is in accordance with the results obtained from the studies of sugar beet pectin isolated with EDTA under alkaline conditions (Oosterveld et al., 1996), but in contrast to acid (HCl) extracted pectins, which contained galactose as a main neutral sugar since most of the arabinose is removed during the acid extraction. Further studies showed that ferulic acid is ester-linked to either the C-2 of arabinofuranose or to the C-6 of galactopyranose residue in the hairy region of sugar beet pectins (Colquhoun et al., 1994). McCready (1966) stated that large amounts of araban and galactan were present in SBP and they are difficult to separate from pectic substances, therefore, some amounts of araban and galactan remained in the residue of EDTA extracted samples. Further treatment of the residue with pectinase, a polygalacturonase, mainly cleaved the glycoside linkages between the galacturonic acid in smooth regions of pectin, subsequently resulting in rich in neutral sugars in the depectinated residues. These high amounts of arabinose and galactose in isolated hemicellulosic fractions also suggested that some of them were extracted from the hairy regions of the pectic substances (Sun and Hughes, 1997a).

The data in Table 1 shows that the isolated hemicelluloses contained a minor amount of uronic acid. This could be due either to the fact that in some cases hemicelluloses may contain residues of uronic acid, or because some residual pectic substances remained in the depectinated sample. As expected, the hemicelluloses, extracted with a relatively low concentration of alkali from lignified samples in Route 1, contained slightly more uronic acid (5.50–5.62%) than those in Route 2 (3.00–4.00%) which were isolated with a high concentration of alkali from delignified samples. The reason for this difference in uronic acid content was probably because some amounts of uronic acids were solubilized during the delignification process, resulting in a low content of uronic acid in isolated hemicelluloses from delignified samples.

The four cellulose fractions consisted of 98% glucose and trace amounts of other sugars such as arabinose, mannose, and galactose. The presence of sugars other than glucose in cellulose preparations could be due to incomplete extraction of pectins and hemicelluloses (Wen et al., 1988). However, after successive extractions of SBP with 80% methanol, 0.25% ammonium oxalate, 4% KOH, and 24% KOH, the cellulose obtained contained 56.8% glucose and 40% uronic acid and arabinose (Kobayashi et al., 1993). The cellulose preparation obtained by alkali extraction and delignification from the depectinated SBP contained 72% glucose and 28% other neutral sugars (Wen et al., 1988). These different sugar contents in isolated cellulose were probably due to the nature of the samples and various extraction procedures used.

The presence of lignin–hemicellulose linkages was studied in detail for straw, grass, and wood samples (Eriksson and Lindgren, 1977; Kondo et al., 1990; Sun et al., 1997). To further verify the presence of associated lignin, alkaline nitrobenzene oxidation of the residual lignin in the isolated hemicelluloses and cellulose preparations was performed. This method provided an estimate of the amount of lignin and an indication of its composition (Table 2). Due to the lignin–hemicellulose complex in the cell wall of SBP, the phenolic acids and aldehydes content (0.22–0.39%) in the hemicellulosic fractions which were isolated directly from lignified residue of SBP was about twice (0.11–0.20%) that of the hemicellulosic fractions extracted from delignified sample. Vanillin was found to be a major component of phenolic monomers in the mixtures of nitrobenzene oxidation of residual lignin from extracted hemicelluloses. In addition to ferulic and

Table 2
The yield (% lignin, w/w) of phenolic acids and aldehydes from alkaline nitrobenzene oxidation of residual lignin in isolated hemicelluloses and cellulose obtained from sugar beet pulp

Phenolic acids and aldehydes[a]	Hemicelluloses				Cellulose			
	Route 1		Route 2		Route 1		Route 2	
	10% KOH extract	7.5% NaOH extract	24% KOH extract	17.5% NaOH extract	10% KOH extract	7.5% NaOH extract	24% KOH extract	17.5% NaOH extract
p-Hydroxybenzoic acid	0.020	0.038	0.023	0.0076	0.10	0.20	0.083	0.076
p-Hydroxybenzaldehyde	0.050	0.093	0.0088	0.017	0.024	0.033	0.012	0.093
Vanillic acid	0.011	0.033	0.0082	0.020	0.0084	0.013	ND[b]	0.0042
Syringic acid	0.0095	0.021	0.0089	0.014	0.030	0.070	ND	0.024
Vanillin	0.078	0.11	0.045	0.080	0.067	0.15	0.026	0.019
Syringaldehyde	0.021	0.040	0.0049	0.0072	0.0086	0.014	0.0034	0.0036
p-Coumaric acid	0.015	0.034	0.0070	0.035	0.047	0.092	0.045	0.035
Ferulic acid	0.012	0.024	0.0061	0.022	0.026	0.071	0.022	0.014
Total	0.22	0.39	0.11	0.20	0.31	0.64	0.19	0.27

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

Table 3
The weight-average (M_w), number-average (M_n) molecular weights, and the polydispersity (M_w/M_n) of the hemicellulosic fractions isolated from sugar beet pulp

Hemicelluloses/extraction procedures	M_w	M_n	M_w/M_n
10% KOH, 15°C, 16 h	91330	6920	13.05
7.5% NaOH, 15°C, 16 h	88850	10650	8.34
24% KOH, 15°C, 2 h	21990	6320	3.48
17.5% NaOH, 15°C, 2 h	21620	6490	3.33

p-coumaric acids, another five phenols; p-hydroxybenzoic acid, p-hydroxybenzaldehyde, vanillic acid, syringic acid, and syringaldehyde, were identified in all the mixtures of nitrobenzene oxidation. With the study of acid and alkali soluble pectins from SBP, Guillon and Thibault (1988) reported that the presence of phenols other than ferulic and p-coumaric acids was identified by colorimetric measurements. However, these phenolic acids and aldehydes were not recovered and identified.

It has been generally accepted that blends of cellulose and lignin exist in nature in the form of wood, unbleached pulp and dietary fibre products (Kosikova et al., 1996). Table 2 also summarizes the phenolic composition of residual lignin from cellulose preparations obtained by nitrobenzene oxidation. Similarly, the content of phenolics in the cellulose preparations (0.31–0.64%) obtained from relatively low alkali extracted residue of SBP in Route 1 was about twice that of the cellulose fractions (0.19–0.27%) isolated from the residue of the high alkali concentration treatment of SBP in Route 2. The resistance to extraction with alkali and to oxidation with sodium chlorite suggested that lignin was also strongly associated with cellulose in the cell wall of SBP.

The weight-average (M_w) and number-average (M_n) molecular weights, and the polydispersity (M_w/M_n) of the hemicelluloses are given in Table 3. Obviously, the hemicelluloses extracted from the lignified residue of SBP with a low alkali concentration had a much higher degree of polymerization, with molecular-average weights ranged between 88 850 and 91 330 Da. This was about four times higher than of the hemicelluloses isolated with a high alkali concentration from delignified holocelluloses of SBP. These data indicated that extraction of hemicelluloses from SBP with a high alkali concentration might result in some degradation of hemicelluloses. Furthermore, as shown in Table 3, the hemicelluloses extracted with 10% KOH and 7.5% NaOH at 15°C for 16 h in Route 1, or with 24% KOH and 17.5% NaOH at 15°C for 2 h in Route 2 did not show any significant difference in their molecular-average weights, suggesting that type of alkali did not have a large effect on the molecular size of the isolated hemicelluloses.

The elution profiles for hemicelluloses extracted with 10% KOH at 15°C for 16 h from depectinated SBP (in Route 1) showed four peaks (Fig. 2). Peak I eluted in the void volume (6.88 ml) and had a molecular weight equal to 556 000 Da. Peaks II, III, and IV had molecular weights around 23 770, 6620, and 3080 Da, respectively. The low molecular weight eluted at peak IV was probably due to the fragmentation of hemicelluloses during 10% KOH extraction process.

The FTIR spectra of the four hemicellulosic fractions are shown in Fig. 3. As can be seen from the Fig. 3, all four spectra appeared to be rather similar. The only slight difference in the spectra is that the spectra of two hemicellulosic fractions extracted with 10% KOH (Fig. 3(a)) and 7.5% NaOH (Fig. 3(b)) from lignified SBP showed a band at 1546 cm^{-1} for associated proteins (Bartolome et al., 1995), which was not present in the other two spectra of hemicelluloses isolated with 24% KOH (Fig. 3(c)) and 17.5% NaOH (Fig. 3(d)) from delignified SBP. This

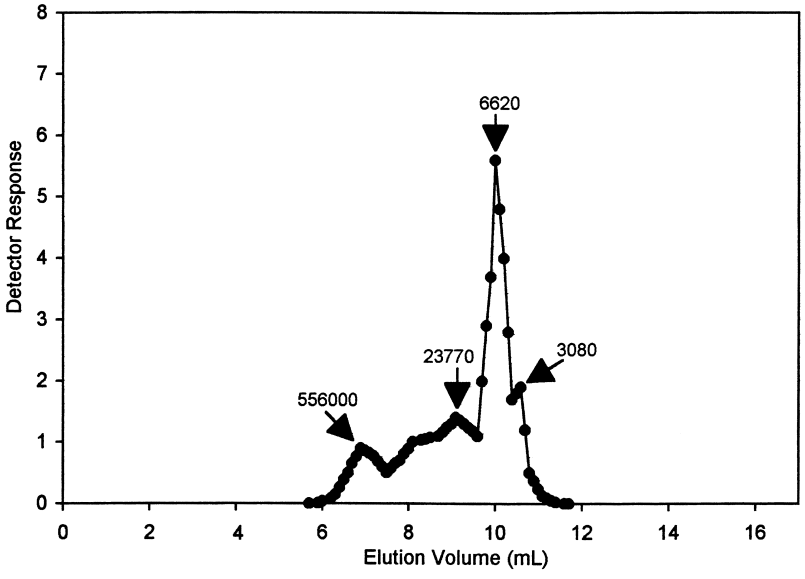

Fig. 2. GPC molecular weight distribution of hemicelluloses extracted with 10% KOH at 15°C for 16 h from the depedtinated sugar beet pulp (Route 1).

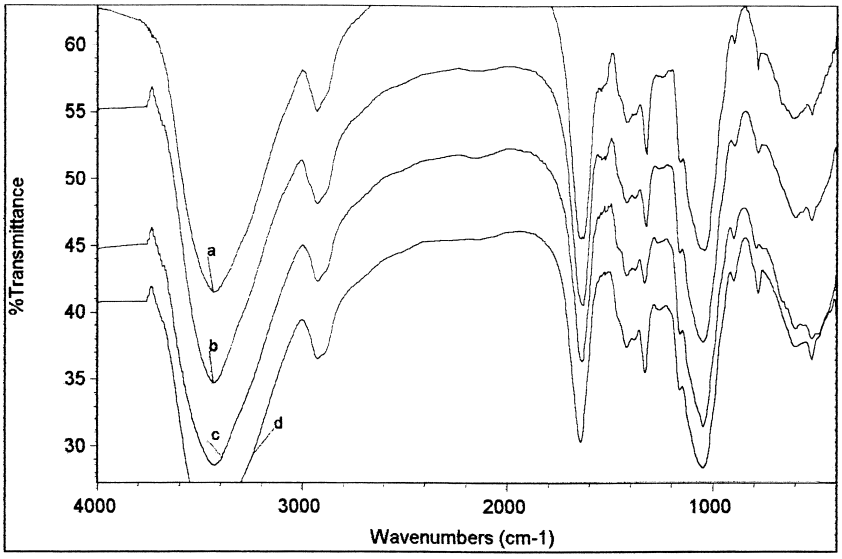

Fig. 3. FTIR spectra of hemicellulosic fractions extracted with 10% KOH at 15°C for 16 h (a), 7.5% KOH at 15°C for 16 h (b), 24% KOH at 15°C for 2 h (c), and 17.5% KOH at 15°C for 2 h (d).

suggests that treatment with protease at 37°C for 2 h only partially removed protein from the SBP, and that the residual protein can only be removed during the sodium chlorite treatment process. The band at 1640 cm^{-1} is probably due to the uronic acid (salt form) in the isolated hemicellulosic preparations. The small sharp band at 900 cm^{-1} is characteristic of β-glycosidic linkages between the sugar units, indicating some amount of β-polysaccharides in isolated hemicelluloses (Gupta et al., 1987; Bartolome et al., 1995). The prominent band at 1036 cm^{-1} is attributable to the C–OH bending. The band at 1164 cm^{-1} represents C–O, C–O–C stretching and some C–OH bending. The bands at 1322 and 1417 cm^{-1} originate from C–H bending and wagging, and OH bending (Kacurakova et al., 1994; Kacurakova and Mathlouthi, 1996).

As with the hemicellulose spectra, there is no significant difference in the main absorption intensity among the four FTIR spectra of cellulose preparations. As shown in Fig. 4, an absorption band at 900 cm^{-1} indicates the β-glycosidic linkages. The prominent band at 1064 cm^{-1} represents ring vibration and C–OH bending. The band at 1160 cm^{-1} is attributable to the C–O, C–O–C stretching, with some contribution from C–OH bending. The low intensities of the bands at 1322, 1370, and 1424 cm^{-1} indicates the C–H wagging and OH bending; CH_2 and OH bending; and C–H and OH bending, respectively. The presence of uronic acid (salt form) in the isolated cellulose was found to be much lower than in the extracted hemicellulosic fractions, as can be seen by the much lower intensity band at 1640 cm^{-1} in the cellulose spectra.

Based on the current results, it can be concluded that there is no significant difference in the yield or the sugar

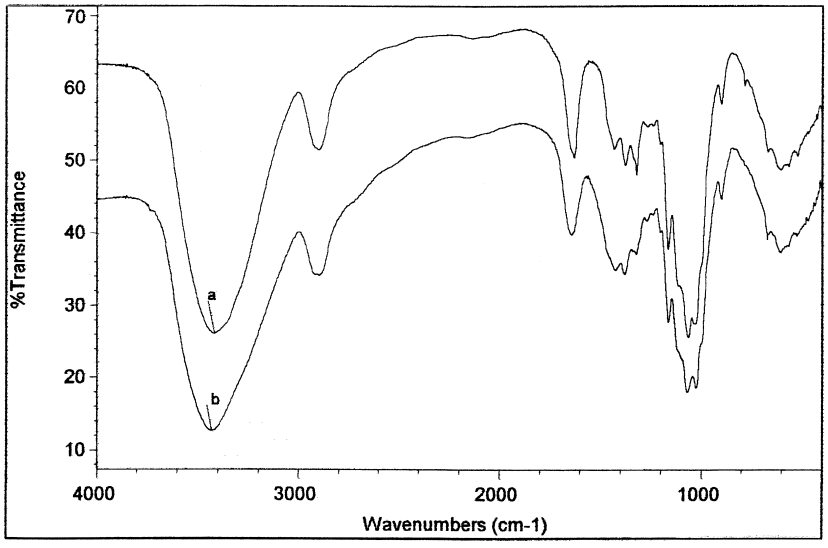

Fig. 4. FTIR spectra of cellulose fractions extracted by 7.5% NaOH extraction at 15°C for 16 h (a), 24% KOH at 15°C for 2 h (b).

composition of hemicelluloses, and cellulose extracted using four different procedures. The yield of hemicelluloses ranged from 9.29% to 10.96% and that of cellulose from 14.88% to 18.35% of dry SBP. Glucose, arabinose, galactose, and xylose were found to be the major sugar components of the extracted hemicelluloses, together with small amounts of rhamnose, mannose, and uronic acid. Glucose was the predominant sugar component (over 98%) in all the cellulose fractions. The yield of phenolic acids and aldehydes from alkaline nitrobenzene oxidation of associated lignin in the isolated hemicellulosic preparations, obtained from the lignified residue of SBP, was twice as high as that of the hemicellulosic fractions isolated from delignified residue of SBP. This suggests that lignin is strongly associated with hemicelluloses in the cell walls of SBP. The molecular-average weight of hemicelluloses extracted by 10% KOH or 7.5% NaOH at 15°C for 16 h from lignified SBP ranged from 88 850 to 91 330 Da, while it dropped to between 21 620 and 21 990 Da when the hemicelluloses were isolated by 24% KOH or 17.5% NaOH at 15°C for 2 h from delignified SBP. This indicates that a high concentration of alkali resulted in degradation of hemicelluloses.

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