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Fractionation and characterization of polysaccharides from abaca fibre

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Abstract

Abaca fibre polysaccharides were fractionated into water soluble, pectic, 1% NaOH soluble, hemicellulosic and cellulose fractions by extraction with hot water, dilute hydrochloric acid (pH 1.6), aqueous 1% NaOH and 17.5% NaOH, respectively. Cellulose (60.4–63.6%) and hemicelluloses (20.8%) were the major polysaccharides in abaca fibres. The hot water soluble polysaccharides contained noticeable amounts of pectic substances and a large proportion of neutral polysaccharides. The pectic polysaccharide preparation was enriched in both galacturonic acid and neutral sugars, including xylose, glucose, galactose, arabinose, and rhamnose. Extraction of the fibre with aqueous 1% NaOH produced the hemicellulose–lignin complex, which was enriched in xylose and, to a lesser extent, glucose-, arabinose- and galactose-containing polysaccharides, together with 7.6% associated lignin. Further extraction of the delignified fibre residue with aqueous 17.5%. NaOH removed the hemicellulose fractions, which were strongly enriched in xylose-containing polysaccharides. Besides ferulic and *p*-coumaric acids, six other phenolic monomers were also detected in the mixtures of alkaline nitrobenzene oxidation of associated lignin in all the polysaccharide fractions. The content of bound lignin in water soluble, pectic, and 1% NaOH soluble polysaccharides (Fractions 1, 2, and 3), isolated directly from the lignified fibres, was 12 times that of the hemicellulosic preparations (Fractions 4 and 5) isolated from the delignified fibre residues.

Keywords: Polysaccharides; Hemicellulose-lignin complex; Sugars; Lignin; Molecular weight

1. Introduction

The term ‘‘fibre’’ has several distinct meanings in the literature. Pulped fibres are individual cells—tracheids, vessels, elements and libriform fibres—used in the manufacture of various paper and cardboard products. Bast fibres and hard fibres are tissues composed of strands of overlapping libriform fibre cells. Bast fibres are obtained from the phloem of stems of dicots such as flax, hemp, jute, and ramie. Hard fibres are obtained from the leaves of monocots, including abaca, sisal, henequen, and New Zealand flax. Bast and hard fibres are used primarily in textiles and in cordage products, such as rope, mats, burlap bags, and brushes (Mclaughlin and Schuck, 1991).

Abaca (*Musa textlis* Nee.) fibre, is an agronomically important source of natural fibres, especially in areas where cotton cannot be grown. Its long fibre length, high strength, and fineness make it a superior material for the production of thin, light weight papers of high porosity and excellent tear, burst and tensile strengths (Peralta, 1996). To maximize the exploitation of this fibre, a more complete understanding of its chemistry is required. Although the chemistry of the cell wall of bast fibres has been the subject of intensive research (Geerdes and Smith, 1955; Gorshkova et al., 1996; Groot et al., 1994; Hazendonk et al., 1996; Maddern and French, 1995; McDougall, 1993; Pallesen, 1996; Stewart et al., 1995; Stewart and Morrison, 1995), little concomitant work has been done on hard fibres. The aim of this study was to develop a fractional extraction procedure for abaca fibre polysaccharides with maximal yield and minimal degradation. The chemical composition and physico-chemical properties of seven isolated polysaccharide fractions are reported. Further attention is also paid to the composition of associated lignin.

2. Experimental

2.1. Material

Abaca fibre was obtained as a gift from the Radcliffe Mill, England. The fibres were cut into 1–2 cm lengths by

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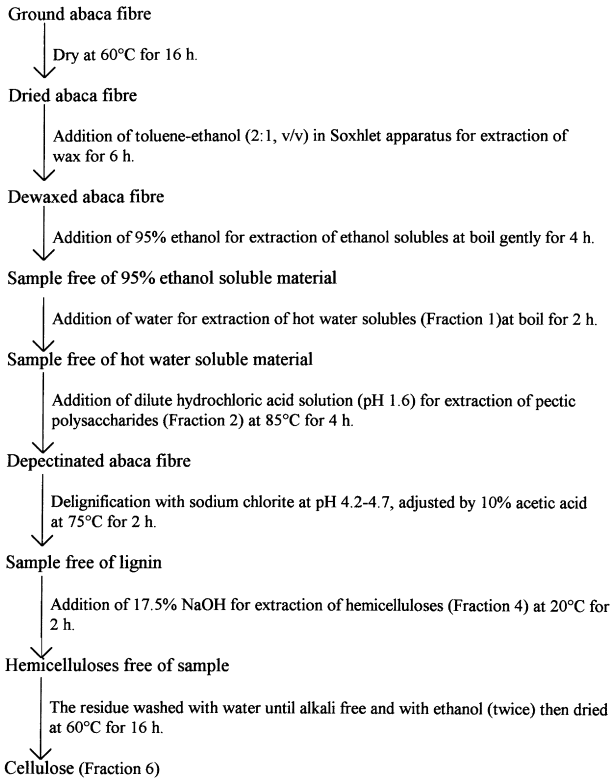
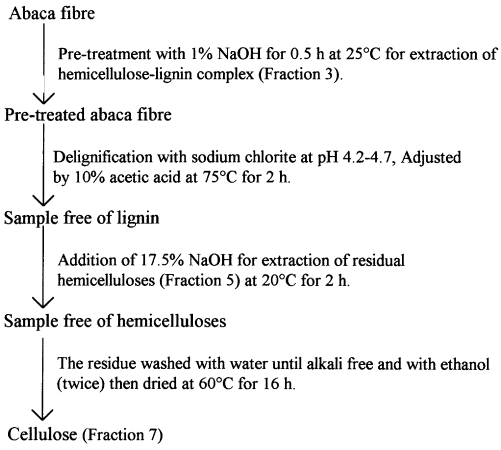


Scheme 1. Scheme for fractional extracion of polysaccharides from abaca fibre.

hand. After being dried in an oven at 50°C for 16 h, the fibre was ground to pass through a 0.7 mm screen and stored at 5°C until use.

2.2. Fractional isolation of polysaccharides

In Scheme 1, fibre was firstly dewaxed in toluene–EtOH (2:1, v/v) for 6 h in a Soxhlet apparatus. The dewaxed fibre was washed with EtOH then water, and dried at 60°C. Dewaxed fibre (50 g) was extracted with 95% EtOH (1500 ml) boiling gently for 4 h, which yielded 4.8% total of sodium chlorite lignin (result not shown). After drying of the fibres overnight at 50°C, the fibre was extracted using boiling water (2500 ml) for 2 h, and the hot water soluble polysaccharide preparation (Fraction 1) was isolated by precipitation of the extract in 4 vols ethanol. The residues (47 g) were extracted in 2500 ml of aqueous dilute hydrochloric acid (pH 1.6) at 85°C for 4 h. After filtration, the solubilized pectic polysaccharides (Fraction 2) were collected by precipitation of the concentrated filtrate in 4 vols ethanol, and washed twice with 70% ethanol. Delignification was performed using sodium chlorite in acidic aqueous solution (pH 4.2–4.7) at 75°C. After a 2 h reaction, the residue was filtered out on a nylon cloth, extensively washed with water and ethanol, and then dried in an oven at 50°C for 16 h. The hemicellulosic preparation (Fraction 4) was obtained by extraction with 17.5% NaOH from the above delignified residues for 2 h at 20°C (1 g residue/38 ml alkaline extractant). The extracted hemicelluloses were separated from the residue by filtration. The filtrate was brought to pH 5.5 using 20% HCl, concentrated using a rotary evaporator under reduced pressure at 40°C, and then mixed with 5 vols ethanol. The precipitated hemicelluloses were filtered, washed with 70% ethanol, and air-dried. The fibre residue was washed extensively with water until the eluant was neutral and then dried. After correction for corrected for ash content, the dry mass was taken to be cellulose (Fraction 6).



Scheme 2. Scheme for extraction of residual hemicelluloses from alkaline pre-treated abaca fibre.

In Scheme 2, the abaca fibre (10 g) was directly pre-treated with 1% NaOH (600 ml) for 0.5 h at 25°C. The extract was neutralized, concentrated eight-fold by rotary evaporation, and the solubilized hemicellulose-lignin complex (Fraction 3) was precipitated by the addition of 5 vols ethanol. The precipitated complex fraction was washed with 70% ethanol, and then air-dried. The remaining hemicelluloses were extracted with 17.5% NaOH (1 g residue/38 ml alkaline extractant) at 20°C for 2 h from the pre-treated and delignified residues, and isolated as Fraction 3. The isolated hemicellulosic preparation (Fraction 5) was washed with 70% ethanol and air-dried. The weight of the residue which remained after the 17.5% NaOH extraction, corrected for ash content, was considered to be cellulose (Fraction 7).

3. Characterization of polysaccharides

The neutral sugar composition of the isolated polysaccharides was determined by GC analysis of their alditol acetates (Blakeney et al., 1983). Polysaccharide-bound saccharidic components in the fractions were firstly subjected to hydrolysis in 2 M trifluoroacetic acid for 2 h at 120°C. Trifluoroacetic acid was removed by vacuum evaporation at 40°C. The dried monosaccharides were then reduced with sodium borohydride in dimethyl sulphoxide and the resulting alditols acetylated using

1-methylimidazole as the catalyst. Alkaline nitrobenzene oxidation of the lignin associated in the isolated polysaccharides was performed at 170°C for 3 h. Methods of uronic acid analysis and determination of phenolic acids and aldehydes in nitrobenzene oxidation mixtures with HPLC have been described in previous papers (Lawther et al., 1996; Sun et al., 1995; Sun et al., 1996). Fourier transform infra-red (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 the polysaccharides were determined by gel permeation chromatography (GPC) on a PL aquagel-OH 50 column. The samples were dissolved with 0.02 M NaCl in 0.005 M sodium phosphate butter, pH 7.5, at a sample concentration of 0.1%, and 200 μl of this solution was injected. The columns were operated at 40°C, and eluted with 0.02 M 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 using PL pullulan polysaccharide.

4. Results and discussion

4.1. Fractional yield of polysaccharides

The yields of the sequential extractions of the abaca fibres are shown in Table 1. All values are calculated on basis of the untreated starting material. These results show that abaca fibres contain 0.8% fatty substances (toluene–EtOH extract), 0.6% ethanol solubles (mainly solubilized lignin), 4.9% hot water soluble polysaccharides, 0.8% pectic material (HCl, pH 1.6 extract), 12.4% lignin, 20.8% hemicelluloses (17.5% NaOH extract), and 60.4% cellulose. This suggested that cellulose and hemicelluloses are the major constituents of the abaca fibre, and cellulose is the predominant polysaccharide. The total polysaccharides accounted for about 87% of the dry weight of the fibre. The low yield of pectic substances was consistent with our previous studies on wheat straw pectic polysaccharides (1.1%), extracted with aqueous dilute hydrochloric acid (pH 1.6, 85°C, 4 h) (Sun et al., 1998a). However, this yield was much lower than reported for the pectic material isolated from flax fibres, which contain 10–15% pectins, isolated with the Ca^{2+}-chelator ammonium oxalate (McDougall, 1993). Further studies showed that the pectic substances are particularly associated with the middle lamellae in cell walls of flax fibres, and their partial removal by a retting process allows the fibre bundles to separate from the surrounding cells of the stem (McDougall, 1993).

Table 1 (Scheme 2) also shows that about 27% of the total hemicelluloses were dissolved during the 1% NaOH pretreatment at 25°C for 0.5 h. The fast dissolution and high solubility of hemicelluloses were probably due to the cleavage of ester-bonds between hydroxycinnamic acids and hemicelluloses or lignin by alkali. Cleavage of non-phenolic *β-O-4* linkages, such as the ether bonds between hemicelluloses and lignin, is also an important factor during fast dissolution of hemicelluloses (Groot et al., 1994). The subsequent alkaline extraction (17.5% NaOH) was performed to determine the residual hemicellulose content, 15.3%. A slightly higher content of crude cellulose (63.6%) in Scheme 2 was probably due to incomplete extraction before isolation of cellulose as compared to Scheme 1.

4.2. Sugar composition and content of uronic acids

Table 2 shows the monosaccharide composition and content of uronic acids in seven recovered polysaccharide fractions. The hot water soluble fraction mainly comprised neutral chains, including glucose, xylose, galactose, and arabinose. This high percentage of glucose and xylose was taken to indicate correspondingly more glucans and xylans. The presence of hot water soluble polysaccharides in agricultural residues has been widely demonstrated. For example, Lawther et al. (1995) have reported that wheat straw yields 4.6% hot water soluble polysaccharides, in which galactose was a major sugar constituent.

Similarly, Fraction 2, pectic polysaccharides, contained high percentages of uronic acids, xylose, and glucose. Galactose and arabinose appeared as the other major sugar constituents. Rhamnose and mannose were detected in trace amounts. The neutral sugar data reported in Table 2 showed that hot water soluble polysaccharides and pectic polysaccharides were very similar in terms of contents of rhamnose, arabinose, and galactose, with molar ratios of 1:12:12 for Fraction 1 and 1:11:15 for Fraction 2. These data suggested that Fractions 1 and 2 resembled the so-called ''hairy regions'' of pectic polysaccharides. Furthermore, when compared to the commercial pectins, the

Table 1
The chemical composition of abaca fibre (% dry weight, w/w)

Scheme 1		Scheme 2	
Extractives	0.8		
Ethanol solubles	0.6		
Hot water solubles	4.9	1% NaOH soluble hemicelluloses	5.6
Pectic polysaccharide	0.8	Residual hemicelluloses isolated by 17.5 NaOH	15.3
Lignin	12.4	Cellulose	63.6
Hemicelluloses	20.8		
Cellulose	60.4		

Table 2
The composition of neutral sugars (relative % sample, w/w) and content of uronic acids (% sample, w/w) in the polysaccharide fractions isolated from abaca fibre

Polysaccharide Fraction[a]	Neutral sugar composition (relative %)						Uronic acids (%)
	Rha	Ara	Xyl	Man	Glc	Gal	
1	0.85	8.32	31.03	1.49	47.86	10.47	28.92
2	0.93	8.33	42.00	0.81	33.73	14.22	46.00
3	1.10	10.72	52.52	0.95	29.15	5.58	9.61
4	0.46	4.38	82.48	1.91	8.79	1.91	10.96
5	1.08	4.85	79.28	4.42	8.36	2.01	10.30
6	Trace	0.44	4.01	1.28	93.88	0.28	
7	Trace	0.38	2.48	1.20	95.90	Trace	

[a] Fraction 1 represents the water soluble polysaccharides, isolated with distilled water at boiling for 2 h from 95% ethanol extracted abaca fibre (Scheme 1); Fraction 2 represents the pectic polysaccharides, extracted with dilute hydrochloric acid (pH 1.6) from the boiling water extracted abaca fibre (Scheme 1); Fraction 3 represents the hemicellulose–lignin complex, isolated by 1% NaOH (25°C, 0.5 h) from abaca fibre (Scheme 2); Fraction 4 represents the hemicelluloses, extracted with 17.5% NaOH at 20°C for 2 h from untreated abaca fibre holocellulose (Scheme 1); Fraction 5 represents the residual hemicelluloses, extracted with 17.5% NaOH at 20°C for 2 h from 1% NaOH pre-treated (25°C, 0.5 h) abaca fibre holocellulose (Scheme 2); Fraction 6 represents the cellulose, obtained from untreated abaca fibre (Scheme 1); Fraction 7 represents the cellulose, obtained from 1% NaOH pre-treated abaca fibre (Scheme 2).

relatively low yields of rhamnose and uronic acids in these two fractions may be due to the incomplete hydrolysis of the GalA–Rha linkage by 2 M trifluoroacetic acid at 120°C for 2 h, inducing an under-estimation of rhamnose and galacturonic acid content. The pectic polysaccharides, isolated from retted hemp bast fibre bundles by sequential extraction with water and ammonium oxalate at 100°C, contain galacturonic acid, rhamnose and galactose units in variable ratios. Their chemical structure comprises a disaccharide repeating unit → 2)-α-L-Rhaρ-(1 → 4)-α-GalρA-(1 → backbone, with short side chains attached to the rhamnose residues. The β-D-Galactose residues are attached to *O*-4 of the rhamnosyl residues (Vignon and Jaldon, 1996). In comparison with the pectic polysaccharides, isolated from citrus peels, apple peels, sunflower residues, sugar beet pulp, and hemp fibres, the relatively higher contents of xylose and glucose in Fractions 1 and 2 were probably due to the presence of hemicelluloses, which were co-extracted during the isolation procedures. This could particularly be the case during the hot water extraction stage.

Table 2 shows that Fraction 3, obtained during the 1% NaOH treatment at 25°C for 0.5 h, was enriched in xylose and glucose, and the contents of arabinose (10.72%), glucose (29.15%), and galactose (5.08%) were higher than for Fractions 4 and 5, isolated with 17.5% NaOH at 20°C for 2 h. These were predominately enriched in xylose, comprising about 80% of the total sugars. These data provide evidence that in abaca fibre cell walls, galactose and glucose, probably present in side chains of hemicellulose, are bound to ferulic acid or directly to lignin and easily released in the weak alkaline extractions, while the xylose and mannose in the main chain of hemicelluloses are not readily extracted prior to delignification. It is very likely that the alkaline extracts are mainly composed of a xylan structure similar to that found in straw, grass and flax fibres. In addition, alkali has a strong swelling effect on crystalline cellulose; it could facilitate the liberation of these hemicelluloses because the hemicelluloses are, in general, associated to the surface of cellulose. The difference between the alkaline and the first two extracts is that the hemicelluloses in the former are de-*O*-acetylated by the alkaline treatment (Hazendonk et al., 1996). The molar ratios of arabinose, xylose, glucose, galactose, and uronic acids are very similar in the strong alkaline extracts (1:18.8:1.7:0.4:1.9), suggesting that Fractions 4 and 5 contain similar hemicellulosic structures. A relatively higher mannose content in Fraction 5 compared with Fraction 4 indicated that alkaline pre-treatment favoured release of mannose-containing polysaccharides, suggesting that aqueous sodium hydroxide can swell the fibre bundles sufficiently to extract material enriched in mannose during strong alkaline extractions. This effect is similar to the extraction of plant material with alkaline boric acid, which is known to favour the removal of mannose-containing polysaccharides (McDougall, 1993). The occurrence of xylans in plants has been also reported by Gorshkova et al. (1996) in studies of flax fibres. The authors showed that xylan was the major portion of the alkaline extract in flax fibrous tissues. Structural studies showed that the xylans contain β-(1 → 4)-D-xylosyl units branched exclusively at the xylosyl *O*-2 with (4-*O*-methyl)-glucosyluronic acid and (galacto) glucomannans (Gorshkova et al., 1996). The uronic acid values (9.61–10.96%) in three isolated hemicellulosic Fractions, 3, 4 and 5, were higher than those in alkaline extracts obtained from wood and straw samples (~3.0%).

The two cellulose fractions, isolated with 17.5% NaOH from untreated abaca fibre residues (Fraction 6) and from 1% NaOH treated residues (Fraction 7), were similar in sugar composition (Table 2). However, quantitative composition of Fraction 7 showed slightly higher glucose

(95.90%) and lower xylose (2.48%) contents than of Fraction 6. This probably resulted from the effect of alkaline swelling of cellulose, and subsequent increased a more dissolution of hemicelluloses from the cellulose microfibrills in the secondary wall.

4.3. Content of associated lignin and composition of phenolic acids and aldehydes

The lignin–hemicellulose complexes present in straw, grass and wood samples have been studied in detail (Sun et al., 1995; Eriksson and Lindgren, 1997; Kondo et al., 1990). To further verify the presence of associated lignin, alkaline nitrobenzene oxidation of bound lignin in the seven isolated polysaccharide preparations was performed at 170°C for 3 h. This method provides both an estimate of the total amount of lignin and an indication of the composition of phenolic units. In this case, the three constitutive monometric lignin units *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) are oxidized into corresponding benzaldehydes: *p*-hydroxybenzaldehyde, vanillin and syringaldehyde (Billa et al., 1996). *p*-Coumaric acid and ferulic acid in the particular case of the Gramineae are also oxidized to *p*-hydroxybenzaldehyde and vanillin, respectively, and therefore interfere with the analysis. However, at temperatures of oxidation as low as 120 and 160°C, these acids are only partially oxidized into the corresponding benzaldehyde, as demonstrated by Iiyama and Lam (1990). As can be seen in Table 3, the major product was identified to be syringaldehyde, which ranged between 41.1% and 63.7% of the total phenolic monomers. Small amounts of *p*-hydroxybenzaldehyde and vanillin, and traces of *p*-hydroxybenzoic acid, vanillic acid, syringic acid, *p*-coumaric acid, and ferulic acid were also found to be present in the nitrobenzene oxidation mixtures. This large amount of syringaldehyde suggested that the majority of the hemicelluloses are linked to lignin via syringyl units, which is consistent with our previous studies on abaca fibre lignins (Sun et al., 1998b). Furthermore, due to the hemicellulose–lignin complex in the cell walls of abaca fibre, the lignin content in the first three polysaccharide preparations (7.6–8.5%, Table 3), extracted directly from the lignified fibres, was 12 times that of the hemicellulosic preparations (Fractions 4 and 5) (0.63–0.64%, Table 3), extracted from the delignified fibre residues. A much lower content of associated lignin was observed in cellulose preparations (Fractions 6 and 7) ranging between 0.20% and 0.27%, indicating cellulose essentially free of lignin.

The association of *p*-coumaric and ferulic acids in the cell walls of grass (Uchiyama et al., 1983), wheat straw (Sun et al., 1995; Scalbert et al., 1986), and oil palm fibres (Sun et al., 1998c) has been studied in detail. 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 (Billa et al., 1996). These results implied that a large proportion of the ferulic acid was quantitatively oxidized to vanillin by nitrobenzene under the reaction conditions given in our studies (170°C, 3 h) as indicated by the small amount of residual ferulic acid in the nitrobenzene oxidation mixtures. Similarly, most of the *p*-coumaric acids appeared to be quantitatively oxidized to *p*-hydroxybenzaldehyde under the conditions of the alkaline nitrobenzene oxidation as shown by the trace of *p*-coumaric acid in the nitrobenzene oxidation products. The absence of ferulic acid and the relative lack of *p*-coumaric acid in the nitrobenzene oxidation products from the hemicellulosic preparations (Fractions 4 and 5), isolated with 17.5% NaOH from delignified fibre residues, and the two cellulose fractions indicated that a majority of these acids are linked to lignin in abaca fibre cell walls. Further studies have shown that *p*-coumaric acid is mainly linked to lignin by ester bonds, whilst ferulic acid is linked to lignin by ether bonds (Sun et al., 1998b).

Table 3
The yield (% sample, w/w) of phenolic acids and aldehydes from alkaline nitrobenzene oxidation of associated lignin in the isolated polysaccharide fractions

Phenolic acids and aldehydes	Polysaccharide fractions						
	1	2	3	4	5	6	7
p-Hydroxybenzoic acid	0.0087	0.0084	0.012	0.012	0.0058	ND[a]	0.0029
p-Hydroxybenzaldehyde	0.40	0.37	0.23	0.030	0.028	0.016	0.011
Vanillic acid	0.015	0.014	0.021	0.018	0.021	0.0050	0.0064
Syringic acid	0.087	0.079	0.091	0.0091	0.010	ND	0.0023
Vanillin	0.35	0.31	0.28	0.030	0.023	0.0087	0.0058
Syringaldehyde	1.33	1.26	1.30	0.074	0.074	0.039	0.023
p-Coumaric acid	0.018	0.016	0.023	0.0033	0.0046	0.0033	0.0016
Ferulic acid	0.067	0.072	0.086	ND	ND	ND	ND
Total	2.28	2.13	2.04	0.18	0.17	0.072	0.053
Lignin content	8.46	7.90	7.57	0.64	0.63	0.27	0.20

[a] Not detectable.

Table 4
The weight-average (M_w) and number-average (M_n) molecular weights, and the polydispersity (M_w/M_n) of polysaccharides isolated from abaca fibre

Polysaccharide fractions	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$
1	30090	12870	2.34
2	41930	14610	2.87
3	88634	22030	4.02
4	101940	23820	4.27
5	164950	18750	8.80

4.4. Distribution of molecular weight

The weight-average (M_w) and number-average (M_n) molecular weights as well as the polydispersities (M_w/M_n) of the first five polysaccharide preparations are presented in Table 4. As expected, the hot water soluble polysaccharides (Fraction 1) and pectic polysaccharides (Fraction 2) had lower M_ws; between 30 090 and 41 930 as compared to the hemicellulose–lignin complex (Fraction 3), indicating that the extractions of abaca fibre with hot water and dilute hydrochloric acid (pH 1.6) resulted in dissolution of low molecular size polysaccharides. The hemicellulose–lignin complex preparation (Fraction 3) clearly showed a relatively higher degree of polymerization with a M_w of 88 634. This is double that of Fractions 1 and 2. Furthermore, the hemicellulosic preparations (Fractions 4 and 5), isolated with strong alkali (17.5% NaOH) from the delignified fibre residues, showed much higher M_ws of 101 940 and 164 950, respectively, suggesting that extraction with concentrated alkali can result in dissolution of large hemicellulose molecules from delignified fibre residues, particularly from alkali pre-treated and delignified fibre residues. In this case, the degradation of polymers by strong alkali can be neglected.

The GPC molecular weight distribution of the pectic polysaccharides (Fraction 2) is shown in Fig. 1. As can be seen from the diagram, the polysaccharide fraction showed a wide polydispersity from 2010 to 130 3170. The main peak, Peak I, gave a molecular weight equal to 39 630. Peak II had a relatively lower molecular weight value of around 7850. Molecules eluting in peak III are presumed to be due to the fragmentation of polysaccharides during the dilute hydrochloric acid extraction process.

4.5. FT-IR spectra

A variety of polysaccharide chemistry problems have been tackled using FT-IR spectroscopy. In addition to X-ray and electron diffraction studies, FT-IR spectroscopy makes it possible, in particular, to solve the problems of identification of polysaccharides, to check their purity, to carry out semi-quantitative functional analyses, to determine structure, and to investigate complexing and intermolecular interactions (Filippov, 1992). Fig. 2 shows the FT-IR spectra of: hot-water soluble polysaccharides (a); pectic polysaccharides (b); and hemicelluloses (c); extracted with 17.5% NaOH from abaca fibre holocellulose according to Scheme 1. As expected, the spectral profiles and relative intensities of the bands of spectra a and b are rather similar, indicating similar structures for the polysaccharides. The pectic substances belong to a class of carboxypolysaccharides which differ from neutral polysaccharides, with an intense band in the region 1740 cm^{-1} (ester carbonyl absorbance, for salts at 1600 cm^{-1}) related to vibrations of the carboxyl group. The spectra of the pectic substances extracted from different plants are similar in the

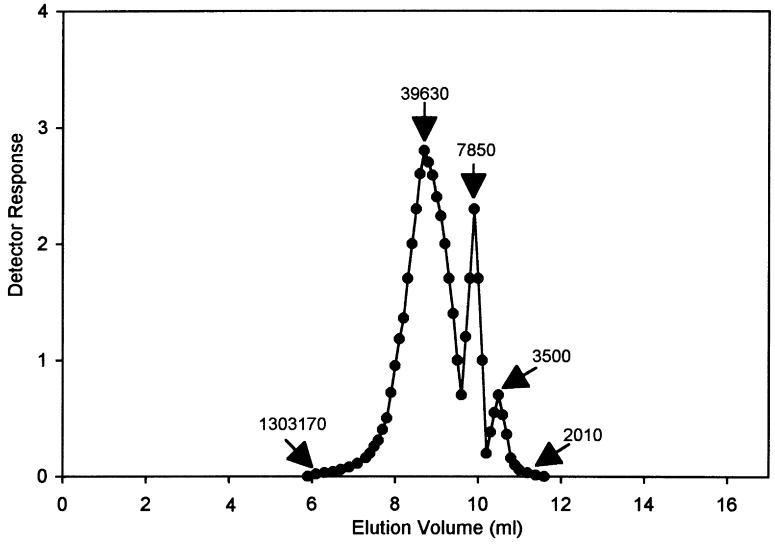


Fig. 1. GPC molecular weight distribution of pectic polysaccharides extracted with dilute hydrochloric acid (pH 1.6) at 85°C for 4 h from the boiling water extracted abaca fibre.

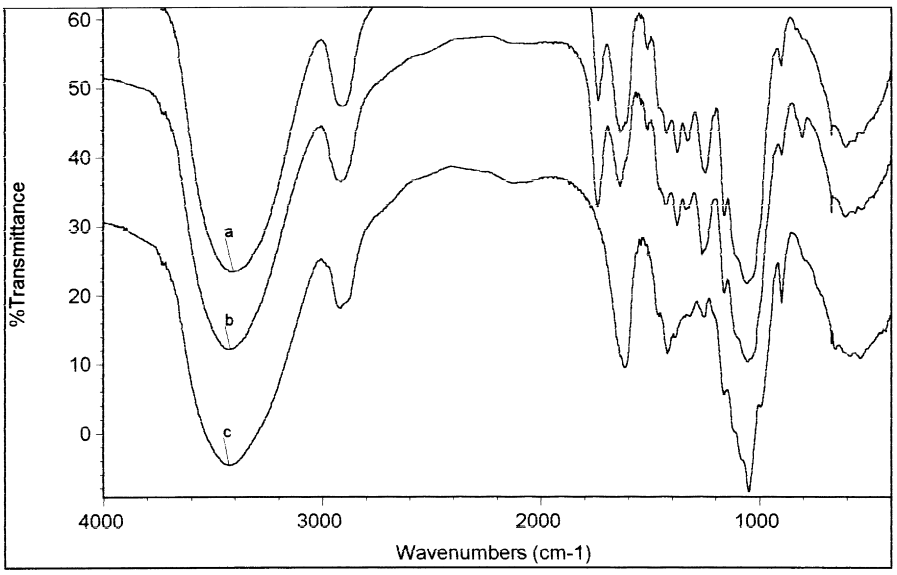


Fig. 2. FT-IR spectra of hot water soluble polysaccharides (a) extracted with water at boil for 4 h, pectin substances (b) extracted with HCl (pH 1.6) at 85°C for 4 h, and hemicelluloses (c) extracted with 17.5% NaOH at 20°C for 2 h from abaca fibre holocellulose in Scheme 1.

region 950–1200 cm^{-1} and differ depending on the state of the carboxyl group in the region 1200–1800 cm^{-1} (Filippov, 1992). As shown from Fig. 2, the spectra a and b clearly showed an intensive band at 1740 cm^{-1}, indicating that the hot water soluble polysaccharides contained some pectic substances. This high intensity at 1740 cm^{-1} indicates the pectin methyl and acetyl ester groups absorbance. The acetyl ester groups of hemicellulose residues also absorb at this band. A stronger absorption at 1740 cm^{-1} in spectrum b than in spectrum a implied a higher content of methylated or acetylated galacturonic acid in the pectic polysaccharide fraction than in the hot water soluble polysaccharide fraction, which corresponded with the results obtained by a colorimetric method (Table 2). The absorption at 1640 cm^{-1} in spectra a and b may be the C=O stretch of carboxylic anions (salt) for galacturonic acid in pectic substances. A prominent absorbance at 1255 cm^{-1} in spectra a and b also corresponds the general carbonyl absorbance for pectic polysaccharides, which is lacking in the other spectra. Additional evidence for the pectins comes from the absorbance at 1440 cm^{-1}, which can be assigned to methyl C–H wagging vibrations (pectin methyl ester). A low intensity band at 1166 cm^{-1} represents C–O, C–O–C stretching and C–OH bending in arabinoxylan structures. A band at 1050 cm^{-1} is typical of oxylans, indicating that some amounts of hemicelluloses are co-extracted with the pectic substances, and extraction with hot water solubilized both hemicelluloses and pectic polysaccharides. The sharp

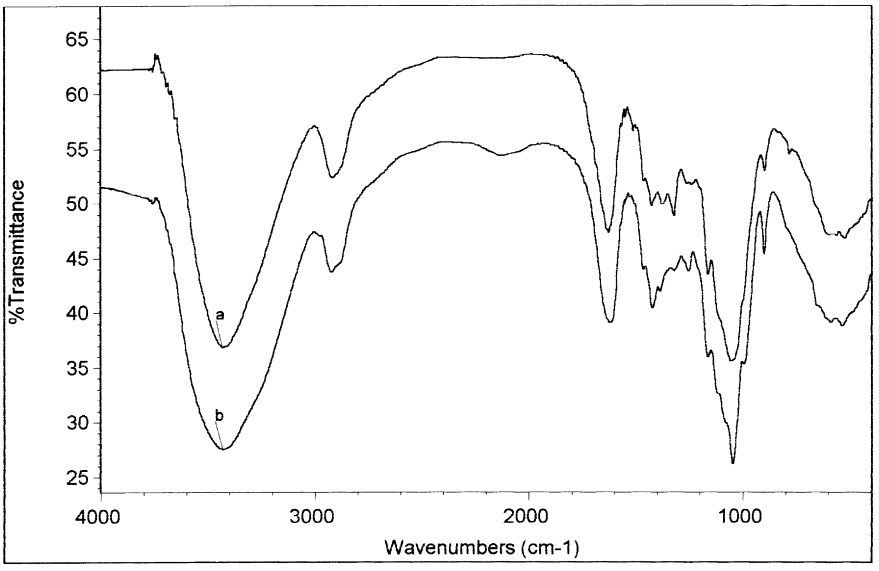


Fig. 3. FT-IR spectra of hemicellulose–lignin complex (a) extracted with 1% NaOH at 25°C for 0.5 h from abaca fibre and residual hemicelluloses (b) extracted with 17.5% NaOH at 20°C for 2 h from the 1% NaOH pre-treated and delignified abaca fibre in Scheme 2.

band at 904 cm^{-1} is characteristic of β-glycosidic linkages between the sugar units (Gupta et al., 1987). This band is strongest in the hemicellulosic fraction (Fraction 4, Fig. 2c) and weakest in pectic polysaccharides (Fraction 2, Fig. 2b), which is proportional to the content of co-extracted hemicelluloses. The lignin-related absorbances at 1520 and 1328 cm^{-1} are weak in spectra a and b, and poorly resolved in spectrum c, implying a low amount of associated lignin in hot water and dilute hydrochloric acid soluble polysaccharide fractions (Fraction 1 and 2), and a hemicellulosic fraction nearly free of bound lignin (Fraction 4).

The hemicellulosic Fractions 4 (Fig. 2c), 3 (Fig. 3a) and 5 (Fig. 3b) exhibit similar spectral profiles, indicating similar structures. The absorbances at 1636, 1470, 1383, 1254, 1166, 1125, 1048, 998, and 904 cm^{-1} seen in the spectra of hemicellulose–lignin complex and hemicelluloses are associated with hemicelluloses. The band at 1636 cm^{-1} is presumed due to the uronic acids (salt form) in the isolated hemicellulosic preparations. The small sharp band at 904 cm^{-1} is characteristic of β-glycosidic linkages between the sugar units, indicating β-linked hemicelluloses (Gupta et al., 1987). The prominent band at 1048 cm^{-1} is attributed to the C–OH bending. The very low intensity band at 998 cm^{-1} corresponds to C–O stretching in C–O–C linkages. The intensity changes of the bands at 1166 and 998 cm^{-1} can be suggested to reflect the arabinosyl substituent contribution and may, therefore, use for the identification of arabinoxylan structures (Kacurakova and Mathlouthi, 1996). Bands between 1125 and 1000 cm^{-1} are typical of xylans. The small band at 1125 cm^{-1} corresponds to the C–O and C–C stretching in xylans. The bands at 1254, 1383, and 1470 cm^{-1} represent C–H bending, CH_2 and OH bending, and CH_2 bending, respectively (Kacurakova et al., 1994). The hemicellulose–lignin complex fraction (Fig. 3a) exhibits signals at 1512 and 1328 cm^{-1}, due to the presence of associated lignin. The band at 1512 cm^{-1} indicates the aromatic skeleton vibrations in lignin. The syringyl ring breathing made with CO stretching appears at 1328 cm^{-1}. The near loss of these two bands in the two spectra of hemicellulosic fractions (Fig. 2c, Fig. 3b) suggests they are relatively free of associated lignin.

In conclusion, this study has shown that the major polysaccharides in abaca fibre are cellulose (60.4–63.6%) and hemicelluloses (20.8%). Small amounts of other polysaccharides components are hot water soluble polysaccharides (4.9%) and pectic substances (0.8%). Extraction with hot water yielded the polysaccharide Fraction 1, which was enriched in neutral chains, including glucose, xylose, galactose, and arabinose. The relatively high percentage of uronic acids in this fraction originated from co-extracted pectic substances. The pectic polysaccharide fraction contained 46% galacturonic acid. The neutral sugars in this fraction were mainly xylose, glucose, galactose, and arabinose. Xylose was found to be the major sugar constituent in the hemicellulose–lignin complex fraction, and the predominant sugar component in the two hemicellulose fractions, indicating similar structures. A major difference between the alkaline and the other two (Fractions 1 and 2) extracts is that the hemicelluloses in the former are completely de-*O*-acetylated by the alkaline treatment. The M_ws of hot water soluble polysaccharides and pectic polysaccharides were much lower than those of the hemicellulosic fractions. In addition, the polysaccharide Fractions 1, 2, and 3, extracted directly from the lignified fibres, were contaminated with small amounts of associated lignin (7.6–8.5%), while the polysaccharide Fractions 4, 5, 6 and 7, isolated from the delignified fibre residues, were relatively free of associated lignin. Further studies showed that polysaccharides in cell walls of abaca fibre are mainly associated with syringyl units in lignin molecules.

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