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Isolation and characterisation of cellulose obtained by a two-stage treatment with organosolv and cyanamide activated hydrogen peroxide from wheat straw

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Abstract

Seven wheat straw cellulose preparations were isolated by a two-stage acidic organosolv treatment followed by cyanamide activated hydrogen peroxide bleaching. The effects of concentration of acetic and formic acids on the yield of cellulose and degradation of lignin and non-cellulose polysaccharides were investigated. Organic acids were more effective than alcohols on the degradation of lignin and hemicelluloses. Formic acid/acetic acid/water (30/60/10, v/v/v) system was found to be the most effective in delignification and removal of non-cellulose polysaccharides from the straw and did not have any undesirable effects on cellulose properties such as its intrinsic viscosity. In this case, the treatment removed 94.1% of the original lignin and 76.5% of the original hemicelluloses using 0.1% HCl as a catalyst at 85 °C for 4 h. Cyanamide activated hydrogen peroxide bleaching degraded substantial amounts of residual hemicelluloses and lignin, produced the cellulose samples having a relatively high purity. Under a best condition, a cellulose relatively free of lignin (0.7%) and with intrinsic viscosity of 393 ml g^{-1} and favourable molar mass (213,940 g mol^{-1}) was obtained. Both unbleached and bleached cellulose preparations were further characterised by FT-IR and CP/MAS ^{13}C NMR spectroscopy, and thermal stability.

Keywords: Cellulose; Wheat straw; Organosolv; Cyanamide activated hydrogen peroxide; Intrinsic viscosity; FT-IR; ^{13}C NMR

1. Introduction

Cellulose is a linear polymer of anhydroglucose units linked at C-1 and C-4 by β-glycosidic bonds. This is confirmed by the presence of three hydroxyl groups with different acidity/reactivity, secondary OH at the C-2, secondary OH at the C-3, and primary OH at the C-6 position, and, accordingly, by the formation of strong various intermolecular and intramolecular hydrogen bonds (Kadla & Gilbert, 2000). Despite its simple molecular structure, cellulose shows a large complexity and variability in its supermolecular arrangement in cellulose fibrils. This variability is due to its biosynthetic origin and is shown in, for example, lateral fibril dimensions and the degree of order (Wickholm, Hult, Larsson, Iversen, & Lennholm, 2001). Cellulose is organised into fibrils, which are surrounded by a matrix of lignin and hemicelluloses. On the basis of X-ray diffraction patterns and ^{13}C NMR spectra, four major polymorphs of cellulose have been reported, namely cellulose I, II, III, and IV. Cellulose I is the native and predominate crystalline structure of alga, bacterial, some animal and most higher plants, and can be converted into the other polymorphs through a variety of treatments (Hayashi, Kon, Takai, Hatano, & Nozawa, 1987). At present, it seems generally accepted that cellulose I has a parallel chain orientation, while in cellulose II, the chains are anti-parallel (Kadla & Gilbert, 2000). Cellulose I can be converted to cellulose II by strong alkali treatment or by regeneration of dissolved cellulose. Small amounts of cellulose II has been observed to form also during kraft pulping (Lennholm & Iversen,

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1995). The cellulose fibril is partly crystalline, with two different crystal forms, cellulose Iα and cellulose Iβ. Cellulose Iα has one-chain triclinic structure and cellulose Iβ has two-chain monoclinic structure (Sugiyama, Vuong, & Chanzy, 1991), and they differ in hydrogen bonding (Sugiyama, Persson, & Chanzy, 1991). Cellulose Iα has been reported the dominant polymorph in bacterial and alga cellulose, while cellulose Iβ is predominant in higher plants such as cotton and wood. It is also known that cellulose Iα can be irreversibly converted to cellulose Iβ by the application of heat (Atalla & VanderHart, 1984). This has also been noticed to happen during pulping (Newman & Hemmingson, 1995). Non-crystalline cellulose forms are also present in the fibril: paracrystalline cellulose and cellulose at inaccessible and accessible fibril surfaces (Duchesne et al., 2001; Newman, 1998).

Cellulose represents a vast potential feedstock for a number of industries and has created a great deal of research interest. Researchers now develop efficient methods for the isolation of cellulose from wood and other organic materials. The cellulose isolation requires the removal of other substances such as hemicelluloses and lignin from wood and cereal straws. However, a protocol originally described by Green (1963) using acidified sodium chlorite is frequently applied to delignify wood as an initial step in the isolation of cellulose, which causes serious environmental concerns. In other words, to obtain cellulose fibres from wood and agricultural residues, using traditional paper producing procedures, consists of degrading a large amount of lignin and hemicelluloses and making them soluble in the aqueous medium. For economically viable exploitation of this biomass, the first and important stage must be the efficient isolation of its major fractions: cellulose, hemicelluloses, and lignin.

The interest in agricultural residues such as cereal straws, as an alternative source of cellulose fibres for the paper industry is growing, in part resulting from shortage of wood fibres in some countries of Asia or from agricultural overproduction in Europe (Moore, 1996; Shatalov & Pereira, 2002). The processes currently employed for commercial straw pulping, which use inorganic reagents, achieve high cellulose extraction efficiency only at the expense of the hemicellulosic fraction, which undergoes hydrolysis and degradation. These processes not only underexploit the lignin, but also cause serious environmental problems. For these reasons, intensive research is being carried out on the development of environmentally friendly approaches, which generally involve the use of organic solvents for efficient separation of the three major components (Baeza et al., 1991; Vazquez, Antorrena, & Gonzalez, 1994).

The organoslv pulping processes involve the treatment of lignocellulosic substances with organic solvent–water media in the presence or absence of a catalyst since they have lower environmental impact and lower energy consumption. The fractions of cellulose, hemicelluloses, and lignin obtained have different characteristics depending on the specific process conditions (Cordeiro, Neto, Rocha, Belgacem, & Grandini, 2002). Cellulose fibre, suitable for either papermaking or enzymatic conversion to glucose; hemicellulose sugars, which may be used for various fermentation processes or as chemical feedstocks; and solid low-molecular weight lignin, usable either as fuel or as feedstock for chemical conversions (Sarkanen, 1980). Acetic acid pulping has been proved to be an effective method to delignify and fractionate wood and non-wood (Davis, Young, & Deodhar, 1986; Nimz & Casten, 1986; Pan & Sano, 1999; Sano, Nakamura, & Shimamoto, 1990). An advantage of delignification with acetic acid is that it can be followed immediately by bleaching, since addition of hydrogen peroxide yields the bleaching agent peracetic acid. Except for paper, the acetic pulp could be also used as raw material of cellulose derivatives because of the high content of cellulose (Uraki, Hashida, & Sano, 1997). Recently, one of the developments in acetic acid pulping is the FORMACELL process, based on the addition of 5–10% formic acid to aqueous acetic acid, resulting in improved selectivity of delignification (Lehnen, Saake, & Nimz, 2002). Besides their role in delignification, organic acids actively take part in the hydrolysis of hemicelluloses. Correspondingly, organic acid based pulping processes include the option for manufacture of dissolving pulps as a feedstock for cellulose derivatives and cellulosic fibres (Abad, Saake, Puls, & Parajo, 2002).

There is a growing concern, from an environmental perspective, about chlorinated organic compounds present in pulp mill effluents. Oxygen-containing chemicals such as oxygen, hydrogen peroxide and ozone have been used as non-chlorine bleaching agents for commercial kraft pulp. Elemental chlorine-free (ECF) and totally chlorine-free (TCF) bleaching have been applied to oragnosolv pulps in laboratory experiments (Kishimoto, Tsuji, Uraki, & Sano, 2003; Ooi & Ni, 1998). In this case, the reported results confirm the favourable behaviour of these kinds of pulps (Abad et al., 2002).

In this study, wheat straw was treated by sequential extractions with various organic solvents under acid catalyst (0.1% HCl) at 85 °C for 4 h and with 1.8% H_2O_2–0.18% cyanamide at 50 °C under pH 10.0 for 4 h, respectively. The cellulosic preparations isolated under TCF conditions before and after bleaching were subject to analysis their content of associated hemicelluloses and lignin, intrinsic viscosity, molecular weight, and thermal stability. The structural changes between unbleached and bleached cellulosic samples were investigated by using Fourier transform infrared (FT-IR) and solid-state cross-polarisation magic angle spinning carbon-13 nuclear magnetic resonance (CP/MAS ^{13}C NMR) spectroscopy.

2. Experiments

2.1. Materials

Wheat straw (*Variety Riband*) was kindly supplied by B Lloyd Co., Llangefni. The composition (%, w/w) of the straw is cellulose 39.0%, hemicelluloses 38.7%, lignin 17.0%, ash 1.8%, and wax 1.9% on a dry weight basis. After being dried at 60 °C in an oven for 16 h, the straw was ground to pass through a 0.7 mm screen and stored at 5 °C until use.

2.2. Organosolv delignification and cyanamide activated hydrogen peroxide bleaching

Prior to organosolv delignification, the dried powder of the straw was first extracted with toluene–ethanol (2:1, v/v) in a Soxhlet extractor for 6 h. Organosolv delignification was carried out in a 500 ml glass reactor at atmospheric pressure. The extractive free powder (10.0 g) was first treated with acetic acid–H_2O (65/35, v/v) to give preparation C_{1a}, acetic acid–H_2O (80/20, v/v) (C_{2a}), acetic acid–H_2O (90/10, v/v) (C_{3a}), formic acid–acetic acid–H_2O (20/60/20, v/v/v) (C_{4a}), formic acid–acetic acid–H_2O (30/60/10, v/v/v) (C_{5a}), methanol–H_2O (60/40, v/v) (C_{6a}), and ethanol–H_2O (60/40, v/v) (C_{7a}) using 0.1% HCl as a catalyst at 85 °C for 4 h with a liquor-to-solid ratio of 20:1 (ml g^{-1}), respectively. The residue was subsequently washed with distilled water and ethanol, and then oven dried at 60 °C for 16 h. The cyanamide activated hydrogen peroxide bleaching of the above residue was performed by post-treatment with 1.8% H_2O_2–0.18% cyanamide (residue:extractant, 1:30, g ml^{-1}) at 50 °C under pH 10.0 for 4 h, respectively. The bleached cellulose obtained was filtered and washed with water and ethanol. Finally, it was dried in an oven at 60 °C for 16 h. Note that the bleached cellulose sample obtained from the corresponding organosolv delignified straw residue C_{1a}, C_{2a}, C_{3a}, C_{4a}, C_{5a}, C_{6a}, and C_{7a} was labelled as cellulose preparation C_{1b}, C_{2b}, C_{3b}, C_{4b}, C_{5b}, C_{6b}, and C_{7b}, respectively. Scheme for organosolv delignification of wheat straw and cyanamide activated hydrogen peroxide bleaching is shown in Fig. 1. All experiments were performed at least in duplicate. Yields of the unbleached cellulose (C_{1a}–C_{7a}) and bleached cellulose (C_{1b}–C_{7b}) are given on a dry weight basis related to the wheat straw (Table 1).

2.3. Characterisation of the unbleached and bleached cellulosic preparations

The neutral sugar composition of the unbleached and bleached cellulosic preparations was determined by gas chromatography (GC) analysis of the corresponding alditol acetates. For the cellulosic sample, hydrolysis was performed as follows: the sample (10 mg) was treated with 72% H_2SO_4 (0.125 ml) for 45 min at room temperature by agitation on a vortex mixture. The solution was then diluted to 1.475 ml, heated at 100 °C for 2.5 h, cooled, and neutralised with 0.32 ml 15 M ammonia. After reduction, the resulting alditols were acetylated for GC analysis as described by Blakeney, Harris, Henry, and Stone (1983). Method for the determination of phenolic acids and aldehydes in nitrobenzene oxidation mixtures of lignins associated in the unbleached and bleached cellulosic preparations with high performance liquid chromatography (HPLC) has been described in a previous paper (Geng, Sun, Sun, & Lu, 2003). Klason lignin content in the unbleached

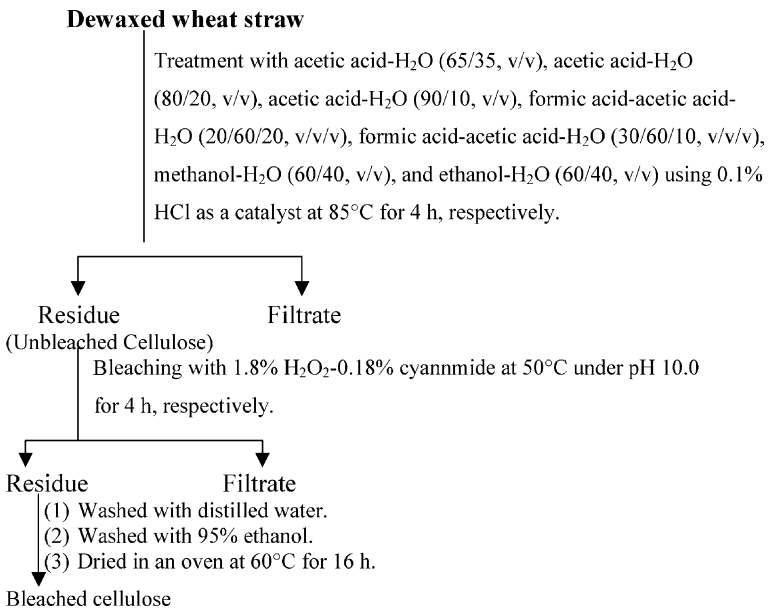


Fig. 1. Scheme for isolation of unbleached and bleached cellulose from wheat straw.

Table 1
The yield (% dry matter), intrinsic viscosity (η), the viscosity average DP (P), and molecular weight (M_w) of unbleached and bleached cellulosic preparations obtained from wheat straw

Sample no.[a]	Yield (%)	η (ml g^{-1})[b]	P[c]	M_w[d]
C_{1a}	64.3	465.2	1594.0	258,230
C_{2a}	54.8	509.4	1762.9	285,590
C_{3a}	50.4	558.4	1952.3	316,270
C_{4a}	51.1	617.4	2182.4	353,550
C_{5a}	47.2	644.5	2289.2	370,850
C_{6a}	84.2	445.4	1519.1	246,090
C_{7a}	82.2	402.0	1355.5	219,590
C_{1b}	45.1	386.5	1297.6	210,210
C_{2b}	39.7	356.0	1184.7	191,870
C_{3b}	39.1	337.8	1117.3	181,000
C_{4b}	43.5	400.2	1348.7	218,490
C_{5b}	38.4	392.6	1320.6	213,940
C_{6b}	63.7	343.3	1137.5	184,280
C_{7b}	59.1	363.1	1210.7	196,130

[a] C_{1a}, C_{2a}, C_{3a}, C_{4a}, C_{5a}, C_{6a}, and C_{7a} represent the unbleached cellulosic preparations obtained by treatment of dewaxed wheat straw with acetic acid–H_2O (65/35, v/v), acetic acid–H_2O (80/20, v/v), acetic acid–H_2O (90/10, v/v), formic acid–acetic acid–H_2O (20/60/20, v/v/v), formic acid–acetic acid–H_2O (30/60/10, v/v/v), methanol–H_2O (60/40, v/v), and ethanol–H_2O (60/40, v/v) using 0.1% HCl as a catalyst at 85 °C for 4 h, respectively, while the C_{1b}, C_{2b}, C_{3b}, C_{4b}, C_{5b}, C_{6b}, and C_{7b} represent the bleached cellulosic preparations obtained by bleaching of the corresponding unbleached cellulosic preparations with 1.8% H_2O_2–0.18% cyanamide at 50 °C under pH 10.0 for 4 h, respectively.

[b] Determined by British Standard Methods for determination of limiting viscosity number of cellulose in dilute solutions, Part 1. Cupri-ethylene-diamine (CED) method.

[c] Calculated by $P^{0.90} = 1.65[\eta]$, P represents the viscosity average DP (degree of polymerization).

[d] Calculated by $P \times 162$.

and bleached cellulose samples was determined according to Tappi method T 249 cm-85.

Viscosity of the unbleached and bleached cellulosic preparations was determined by British Standard Methods for determination of limiting viscosity number of cellulose in dilute solutions, Part 1. Cupri-ethylene-diamine (CED) method (BS 6306: Part 1: 1982). The viscosity average DP (degree of polymerisation) of the cellulose samples was estimated from their intrinsic viscosity [η] in cupri-ethylene-diamine hydroxide (cuene) solution, $P^{0.90} = 1.65[\eta]/\text{ml g}^{-1}$, where P is an indeterminate average DP (Evans & Wallis, 1989). Molecular weight of the cellulosic preparations was then calculated from their P multiplying by 162, molecular weight of an anhydroglucose.

The FT-IR spectra of unbleached and bleached cellulose were recorded on a Nicolet 510 spectrophotometer (Warwick, England) using a KBr disc containing 1% finely ground sample. CP/MAS ^{13}C NMR spectra were recorded using a Bruker MSI-300 spectrometer at 25 °C and 62.9 MHz for carbons. The MAS rate was 3 kHz. Each spectrum was obtained with an accumulation of 5000 scans. The delay time was 60 s, the proton 90° pulse width was 9 μm, and the contact time for cross-polarisation was 2 ms.

Thermal analysis of the unbleached and bleached cellulose samples was performed using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) on a simultaneous thermal analyzer (STA 625). The apparatus was continually flushed with nitrogen. The sample weighed between 9 and 11 mg. Each sample was heated from room temperature to 600 °C at a rate of 10 °C min^{-1}.

3. Results and discussion

3.1. Yield of unbleached and bleached cellulose

The effect of various organic solvents on the yield of unbleached cellulose is given in Table 1. These results showed that the treatment with acetic acid–H_2O (80/20, v/v), acetic acid–H_2O (90/10, v/v), formic acid–acetic acid–H_2O (20/60/20, v/v/v), and formic acid–acetic acid–H_2O (30/60/10, v/v/v) gave relatively lower yields of unbleached cellulose (C_{2a}, 54.8%; C_{3a}, 50.4%; C_{4a}, 51.1%; and C_{5a}, 47.2%). The yield of C_{1a} obtained by treatment with acetic acid–H_2O (65/35, v/v) accounted for 64.3%. Treatment with methanol–H_2O (60/40, v/v) and ethanol–H_2O (60/40, v/v) yielded higher amounts of unbleached cellulose (C_{6a}, 84.2% and C_{7a}, 82.2%). Lower yields of C_{2a}, C_{3a}, C_{4a}, and C_{5a} revealed that the treatment under the organic solvents used significantly degraded the polymers of lignin and hemicelluloses. Further analysis confirmed that the treatment of dewaxed wheat straw with acetic acid–H_2O (65/35, v/v), acetic acid–H_2O (80/20, v/v), acetic acid–H_2O (90/10, v/v), formic acid–acetic acid–H_2O (20/60/20, v/v/v), formic acid–acetic acid–H_2O (30/60/10, v/v/v), methanol–H_2O (60/40, v/v), and ethanol–H_2O (60/40, v/v) using 0.1% HCl as a catalyst at 85 °C for 4 h led to 78.2, 80.0, 88.2, 89.4, 94.1, 23.5, and 37.4% of the original lignin removal, and 42.4, 58.7, 70.0, 65.1, 76.5, 14.2, and 22.2% of the original hemicelluloses degradation, respectively (data not shown). This higher solubility of lignin than hemicelluloses indicated that lignin is dissolved or degraded more easily in the organosolv treatment.

In addition to the main variables that affect the extent of delignification and hemicelluloses degradation such as extraction temperature and time, and hydrogen ion concentration, solvent systems often play dominant roles in the rate of delignification and degradation of hemicelluloses (Lora & Aziz, 1985). It has been known for a long time that acetic acid acts as a solvent for lignin (Schuerch, 1952). In this case, the acid catalyses hydrolysis of lignin and hemicelluloses, and its aqueous solution, i.e. acetic acid and water dissolves the lignin fragments. In addition, a high concentration of acetic acid is necessary in the treating liquor since the pH in the acetic acid solutions must be low

enough to accelerate lignin hydrolysis, the solvation of lignin fragments must hold the key to lignin removal in the acetic acid pulping process (Young & Davis, 1986). In the present experiments, the yield of degradation of hemicelluloses and lignin increased from 16.4 and 13.3% (% dry straw) to 22.7 and 13.6%, and to 27.1 and 15.0% (data not shown) as the acetic acid concentration increased from 65 to 80, and to 90% (% acetic acid by volume), respectively. As a result, the yield of unbleached cellulose decreased from 64.3 to 54.8, and to 50.4%. Furthermore, due to an excellent swelling agent of acetic acid for lignin preparation, less swelling of the carbohydrates would mean that the hemicelluloses would be less accessible for hydrolysis and dissolution. More of the lignin could be attacked with less degradation of the hemicelluloses. This observation is consistent with the results obtained by Schuerch (1952) during the study of acetic acid pulping of wood samples.

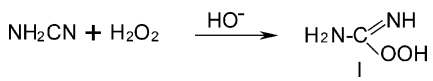
To improve selectivity of delignification, formic acid was added in the acetic acid treatment of this study. The results in Table 1 show that a composition of formic acid/acetic acid/water of 20/60/20 is superior in delignification of wheat straw than that of acetic acid/water (80/20) as shown by an increase in release of the original lignin from 80.0% (C_{2a}) to 89.5% (C_{4a}). Interestingly, an increment in formic acid concentration during the aqueous acetic acid treatment from 20 to 30% resulted in an increase of degradation of lignin and hemicelluloses from 89.4 and 65.1% (C_{4a}) to 94.1 and 76.5% (C_{5a}). The reason for this increasing trend of degradation of lignin and hemicelluloses is that the formic acid essentially plays the role of a proton donor, which has the effect of hydrolysing the lignins and hemicelluloses. While the acetic aid serves as the solvent of the lignin and hemicelluloses fragments. The water intervenes at the level of the dissociation of the organic acids. It also participates in the hydrolysis of the hemicelluloses (Lam, Bigot, Delmas, & Avignon, 2001).

In comparison with organic acid, aqueous solutions of volatile solvents such as alcohol are easily recovered by simple flash evaporation and condensation of the vapours formed for recycle use. In this study, treatment of the straw with methanol–H_2O (60/40, v/v) and ethanol–H_2O (60/40, v/v) using 0.1% HCl as a catalyst at 85 °C for 4 h released only 23.5 and 37.4% of the original lignin and 14.2 and 22.2% of the original hemicelluloses. This is an unexpected result and further increase of treating temperature is therefore needed. However, a relatively higher yield of lignin than hemicelluloses released implied that lignin condensation is considerably retarded at alcohol concentrations of 60%, resulting in a higher rate of delignification, but discouraging hemicellulose degradation. Obviously, under acidic conditions, α-ether cleavage occurs to a great extent, and the likelihood of β-ether cleavage is greater in a more strongly acidic system (Sarkanen & Hoo, 1981). In addition, when part of the original hemicelluloses is hydrolysed by acid, accessibility of the cell wall is opened up through creation of macropores, subsequently resulting in increase of delignification during the organosolv treatment under acidic conditions. Furthermore, in the case of organic acid treatment, where the composition of the residual lignin is closely related with that of the native lignin owing to the nature of the depolymerisation reactions involved in lignin break down, the residues are usually fairly susceptible to bleaching.

Recently, environmental concerns have heightened interest in chlorine-free bleaching sequences. Alkaline peroxide, oxygen–alkali, ozone, and peroxyacetic acid systems are of particular interest since the by-products are environmentally benign (Guay, Cole, Fort, Genco, & Hausman, 2000). In general, chemical pulp bleaching involves mainly electrophilic reactions of hydrogen peroxide or peroxyacids as delignifying agents and mechanical pulp bleaching involves nucleophilic reactions and lignin-retaining bleaching (Pan, Spencer, & Leary, 2000). The release of hemicelluloses and lignin during the alkaline peroxide bleaching process was suggested to be due to direct attack on α-aryl ether bonds between lignin and hemicelluloses. Although they are strong bleaching reagents, they are, however, limited in their effectiveness in degrading lignin (Ek, Gierer, Jansbo, & Reitberger, 1989). To increase their reactivity, strong reaction conditions are being employed. Although high temperature and chemical charge facilitates delignification, it also promotes polysaccharide degradation, particularly in the presence of transition-metal ions (Kadla, Chang, Chen, & Gratzl, 1998). For this reason the activation of oxygen-based reagents is being extensively studied. One particular area of interest has been in the activation of hydrogen peroxide by cyanamide (H_2NCN) (Hamilton, Senior, Sartiogo, Szwee, & Ragauskas, 1996; Kadla & Chang, 2002; Sturm & Kuchler, 1993). This activator improves peroxide bleaching by increased lignin removal and results in an increased brightness (Kordsachia, Patt, & Sturm, 2001). Therefore, the present study has also been extended to the bleaching of the organosolv treated wheat straw with cyanamide activated hydrogen peroxide under alkaline conditions. The results showed that the bleaching of C_{1a}, C_{2a}, C_{3a}, C_{4a}, C_{5a}, C_{6a}, and C_{7a} residues with 1.8% H_2O_2–0.18% cyanamide at 50 °C under pH 10.0 for 4 h released 17.4, 12.5, 9.8, 11.0, 7.6, 12.5, and 14.5% of hemicelluloses (% dry starting material), corresponding to dissolution of 45.0, 32.3, 25.3, 28.4, 19.6, 32.3, and 37.5% of the original hemicelluloses, respectively. Meanwhile, the bleaching degraded 1.7, 1.5, 1.0, 1.3, 0.8, 6.8, and 5.3% lignin (% dry starting material), yielding 45.1, 39.7, 39.1, 43.5, 38.4, 63.7, and 59.1% of the bleached cellulose (% dry starting material), respectively.

Although the reaction of alkaline hydrogen peroxide with cyanamide is not well understood, the reactions proceed ionically via a peroxyimidic acid intermediate I which is isoelectronic with peroxyacids, have been well documented

during the study of the degradation of lignin model compounds (Kadla et al., 1998). In the absence of added substrate, I reacts with hydrogen peroxide or another intermediate I to give the corresponding amide and oxygen (McIsaac, Ball, & Behrman, 1971). It is obvious that the reactivity of the cyanamide–hydrogen peroxide system is quite unique in relation to other traditional peroxide regents. The results obtained by Kadla et al. (1998) provide evidence of two competing reaction systems, radical and ionic, in which the majority of the chemistry proceeds via radical mechanisms.



3.2. Intrinsic viscosity, the viscosity average DP (P), and molecular weight (M_w)

The viscosity average DP (degree of polymerisation), P, of a cellulose sample is conveniently estimated from the intrinsic viscosity of its solution in 0.5 M cupriethylenediamine hydroxide (cuene) by applying the equation $P^{0.90} = 1.65\,[\eta]/\mathrm{ml\ g^{-1}}$, according to the method of Evans and Wallis (1989). Molecular weight of the cellulose was estimated multiplying by 162, a molar mass of anhydroglucose. Table 1 lists the intrinsic viscosity (η), the viscosity average DP (P), and molecular weight (M_w) of the seven unbleached and bleached cellulosic preparations. As can be seen, the intrinsic viscosity (η), the viscosity average DP (P), and molecular weight (M_w) of the unbleached cellulose increased with the decrease in its yield. The reason for this decrease is probably due to the removal of some more amounts of low-molecular weight of hemicelluloses and lignin under the organosolv treatment conditions given, thereby increasing the viscosity and molecular weight. Interestingly, these results revealed that the organosolv treatment did not degrade the macromolecule of cellulose under the solvent concentrations. This is particularly true for the unbleached cellulose obtained during the treatment with formic acid/acetic acid/water system since either C_{4a} or C_{5a} has a higher intrinsic viscosity (617.4, 644.5 ml g^{-1}). More importantly, as the data shown in Table 1, an increase in acetic acid concentration from 65 to 80, and to 90% resulted in an increasing value of η from 465.2 to 509.4, and to 558.4 ml g^{-1}, respectively. Similarly, the viscosity of C_{4a} and C_{5a} raised from 617.4 to 644.5 ml g^{-1} when the formic acid concentration increased from 20 to 30%. As is obvious from the sugar analysis (Table 2), no cellulose loss occurred during the organosolv treatment under any solvent concentrations. A similar effect of improved cellulose stability was also noted for alkaline systems in the presence of low-molecular alcohols, such as ethanol (Shatalov & Pereira, 2002).

To obtain detailed information concerning the degradation reactions during the bleach with 1.8% H_2O_2–0.18% cyanamide, the seven bleached cellulose samples were also performed to determine their intrinsic viscosity, the viscosity average DP, and molecular weight, and results are given in Table 1. Clearly, the seven bleached cellulosic preparations showed no significant difference in their η and M_w values which ranged from 337.8 to 400.2 ml g^{-1} and from 181,000 to 218,490 g mol^{-1}, respectively, but gave lower η and M_w values than those of the corresponding unbleached cellulose samples. This indicated that treatment of the unbleached cellulose with 1.8% H_2O_2–0.18% cyanamide at 50 °C under pH 10.0

Table 2
The neutral sugar composition (relative% cellulosic sample, w/w) of the unbleached and bleached cellulosic preparations

Sample no.[a]	Neutral sugars					
	Rhamnose	Arabinose	Xylose	Mannose	Galactose	Glucose
C_{1a}	0.3	2.6	22.5	3.6	4.9	66.1
C_{2a}	0.2	1.0	22.4	2.2	3.7	70.6
C_{3a}	0.1	0.5	21.7	0.7	0.2	76.8
C_{4a}	0.2	0.8	24.2	1.0	0.4	73.3
C_{5a}	0.1	0.3	18.1	0.7	0.2	80.5
C_{6a}	0.1	5.8	29.2	0.8	2.7	61.4
C_{7a}	0.3	5.4	27.5	1.0	2.5	63.2
C_{1b}	ND[b]	0.5	9.5	0.3	0.6	90.1
C_{2b}	ND[b]	0.2	8.5	0.2	0.4	90.8
C_{3b}	ND[b]	0.2	3.4	0.8	0.3	95.2
C_{4b}	ND[b]	0.2	7.0	0.6	0.3	92.0
C_{5b}	ND[b]	0.2	3.4	0.5	0.2	95.8
C_{6b}	0.2	3.2	27.2	0.3	0.5	68.4
C_{7b}	ND[b]	3.1	22.3	0.3	0.4	73.7

[a] Corresponding to the cellulosic fractions in Table 1.
[b] ND, not detectable.

for 4 h did degrade the macromolecular structure of cellulose to a noticeable extent except for removal of residual lignin and hemicelluloses.

3.3. Content of hemicelluloses and their neutral sugar composition

Based on the study of association between hemicelluloses and cellulose, Tokoh, Takabe, Fujita, and Saiki (1998) stated that hemicelluloses play the very important role of regulators during the association of cellulose chains, and influence the pattern of aggregation of cellulose into fibril and fibril aggregates during the biogenesis of bacterial cellulose and the cell wall of higher plants. This association has been proposed to be physical, in terms of co-aggregation (Hackney, Atalla, & Vanderhart, 1994) or based on chemical linkage (Isogai, Ishizu, & Nakano, 1989). Hemicelluloses have also been suggested to be intimately integrated into the structure of the cellulose, and located within and between the cellulose fibrils (Whitney, Brigham, Darke, Reid, & Gidley, 1998). In this study, GC analysis of the monosaccharides present in the liquors obtained in the quantitative acid hydrolysis of the seven unbleached cellulosic preparations showed that cellulose accounted for 61.4–80.5%, estimated in glucose. Obviously, the unbleached cellulose, obtained by organoslv treatment contained a noticeable amount of non-cellulose sugars such as xylose (18.1–29.2%) and arabinose (0.3–5.8%). This indicated that the main contribution to the higher yield of unbleached cellulose during the organosolv treatment comes from the preservation of non-cellulosic polysaccharides. The improved stability of hemicelluloses during methanol or ethanol/water treatment can be explained by the positive action of the alcohol on suppression of peeling reaction of polysaccharides in acidic medium (Shatalov & Pereira, 2002). On the other hand, as seen from Table 2, a rise in acetic acid concentration in the cooking liquor from 65 to 80, and to 90% led to a decrease in hemicellulose stability and, consequently, to a decrease in content of hemicelluloses. This is undoubtedly due to the increase of hydrolysis of non-cellulose polysaccharides by the acid. A similar decreasing trend of hemicelluloses has been observed during the treatment with formic acid/acetic acid/water system in C_{4a} and C_{5a} samples.

Analysis of the corresponding seven bleached cellulose samples revealed that the cellulose preparations contained only small amounts of hemicelluloses (4.2–9.9%) except for the samples C_{6b} and C_{7b}, which contained noticeable amounts of non-cellulose polysaccharides (26.3–31.6%, Table 2). The resistance to extraction with cyanamide activated hydrogen peroxide under alkaline condition implied that the hemicelluloses in the cell walls of wheat straw are not only associated to the surface of cellulose or not limited at the outer fibre surface. Scott (1984) came to the conclusion that the extraction of hemicelluloses with alkali is highly dependent on the hemicelluloses distribution, near outer fibre surfaces and on, or near, the lumen surfaces and pores. Another likely explanation for the sorption of hemicelluloses on the cellulose framework is through hydrogen bonds, which can retain the hemicelluloses on the fibrils network during the alkali extraction (Mora, Ruel, Comtat, & Joseleu, 1986).

3.4. Content of residual lignin and its phenolic composition

Lignin is a complex aromatic polymer which appears to function both as a matrix between the fibres in the composite wood structure and as a component which contributes to the resistance of wood towards attack by microorganisms and decay (Roberts, 1996). In order to produce high quality paper with good strength properties it is necessary to remove the lignin from the wood or straw matrix, since it hinders the formation of hydrogen bonds between fibres, restricts the swelling of fibres and makes them stiff (Maximova, Osterberg, Koljonen, & Stenius, 2001). During the chemical pulping, wood or straw fibres undergo severe modification. More than half of the total amounts of hemicelluloses and nearly all the lignin are dissolved from the fibres, while the cellulose is partly degraded but not dissolved. Cellulose crystallinity increases during the pulping by heat (Hult, Larsson, & Iversen, 2000; Newman, Hemmingson, & Sucking, 1993).

The degree of organosolv delignification is affected strongly by organic solvents (Table 3). Evidently, organic acids had a much stronger effect on the delignification than alcohols. With an increase in acetic acid concentration from 65 to 90%, the content of residual lignin in unbleached cellulose reduced from 5.8 (C_{1a}) to 4.0% (C_{3a}). Similarly, an increment in formic acid concentration from 20 to 30% resulted in a decrease in lignin content from 3.0 (C_{4a}) to 2.0% (C_{5a}). Addition of organic acids, particularly formic acid, to the acidic solution has, therefore, a beneficial effect on lignin removal during the organosolv treatment. This positive effect of organic acid addition can be ascribed to the substantial cleavage of the α-benzyl ether linkages between lignin and hemicelluloses in the cell walls of wheat straw, while the positive effect of alcohol addition can be considered to the improved solubility of the dissolved lignin fragments or to a reduced tendency toward lignin condensation (Sarkanen, 1990).

As mentioned above, the activators such as cyanamide improve peroxide bleaching by increasing lignin removal and result in an increased brightness (Kordsachia et al., 2001). Results concerning the residual lignin in bleached cellulose are also given in Table 3. The data showed that the seven bleached cellulosic preparations contained lower amounts of associated lignin, ranging between 0.74 and 9.24%, in comparison with that of the unbleached cellulose samples. This is particularly true when the formic acid was added in the acetic acid/water system during the organosolv treatment. In this case, the bleached cellulose obtained contained only 0.74–1.2% lignin (C_{4b} and C_{5b} samples). The major products, obtained from alkaline nitrobenzene

Table 3
The yield (% cellulosic sample, w/w) of phenolic acids and aldehydes from nitrobenzene oxidation of the associated lignin and content of klason lignin in the unbleached and bleached cellulosic preparations

Sample no.	Phenolic acids and aldehydes[a]												KL[b]
	HBA	HBAL	VA	VAN	SA	SYAL	AV	AS	PCA	FA	CA	Total	
C_{1a}	0.068	0.16	0.062	1.11	0.066	1.06	ND[c]	ND	0.007	0.077	0.023	2.63	5.79
C_{2a}	0.062	0.14	0.055	1.01	0.052	0.98	ND	ND	0.005	0.062	0.016	2.38	5.46
C_{3a}	0.051	0.081	0.036	0.65	0.045	0.63	ND	ND	0.004	0.047	0.011	1.56	3.96
C_{4a}	0.047	0.042	0.008	0.43	0.027	0.44	ND	ND	0.009	0.040	0.008	1.05	3.02
C_{5a}	0.021	0.021	0.003	0.24	0.022	0.22	ND	ND	0.004	0.012	0.004	0.55	2.01
C_{6a}	0.27	0.41	0.10	2.70	0.083	3.69	0.14	0.32	0.096	0.13	0.085	8.02	15.0
C_{7a}	0.22	0.32	0.11	2.20	0.10	2.92	0.11	0.31	0.075	0.16	0.051	6.58	12.2
C_{1b}	0.19	0.14	0.13	0.64	0.11	0.82	0.06	0.02	0.26	0.024	0.020	2.44	4.39
C_{2b}	0.26	0.063	0.12	0.41	0.20	0.54	0.04	0.01	0.22	0.028	0.010	1.90	3.80
C_{3b}	0.18	0.045	0.066	0.29	0.17	0.35	ND	ND	0.18	0.018	0.008	1.31	2.71
C_{4b}	0.035	0.024	0.022	0.16	0.049	0.18	ND	ND	0.024	0.036	0.005	0.54	1.16
C_{5b}	0.032	0.022	0.031	0.07	0.047	0.06	ND	ND	0.010	0.012	0.004	0.29	0.74
C_{6b}	0.10	0.26	0.13	2.04	0.18	2.43	0.22	0.05	0.03	0.18	0.053	5.67	9.24
C_{7b}	0.086	0.25	0.11	1.92	0.14	2.35	0.17	0.06	0.04	0.10	0.059	5.29	8.31

[a] HBA, HBAL, VA, VAN, SA, SYAL, AV, AS, PCA, FA, and CA represent *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, vanillic acid, vanillin, syringic acid, syringaldehyde, acetovanillone, acetosyringone, *p*-coumaric acid, ferulic acid, and cinnamic acid, respectively.
[b] KL represents klason lignin content.
[c] ND, not detectable.

oxidation, were identified to be vanillin and syringaldehyde. This suggested that the lignin linked with hemicelluloses in both unbleached and bleached cellulose is composed mainly of non-condensed guaiacyl and syringyl units. Relatively small amounts of *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde and syringic acid, vanillic acid, *p*-coumaric acid and ferulic acid were also detected in the nitrobenzene oxidation mixture.

3.5. FT-IR spectra

Fig. 2 shows the FT-IR spectra of the unbleached cellulosic preparations C_{2a} (spectrum 1), C_{3a} (spectrum 2), C_{4a} (spectrum 3), and C_{5a} (spectrum 4). The major features of the spectra are the occurrence of three ester bands at 1732, (C=O ester), 1381 ($-C-CH_3$), and $-C-O-$ stretching band at 1255 cm^{-1} due to partial acetylation of hydroxyl

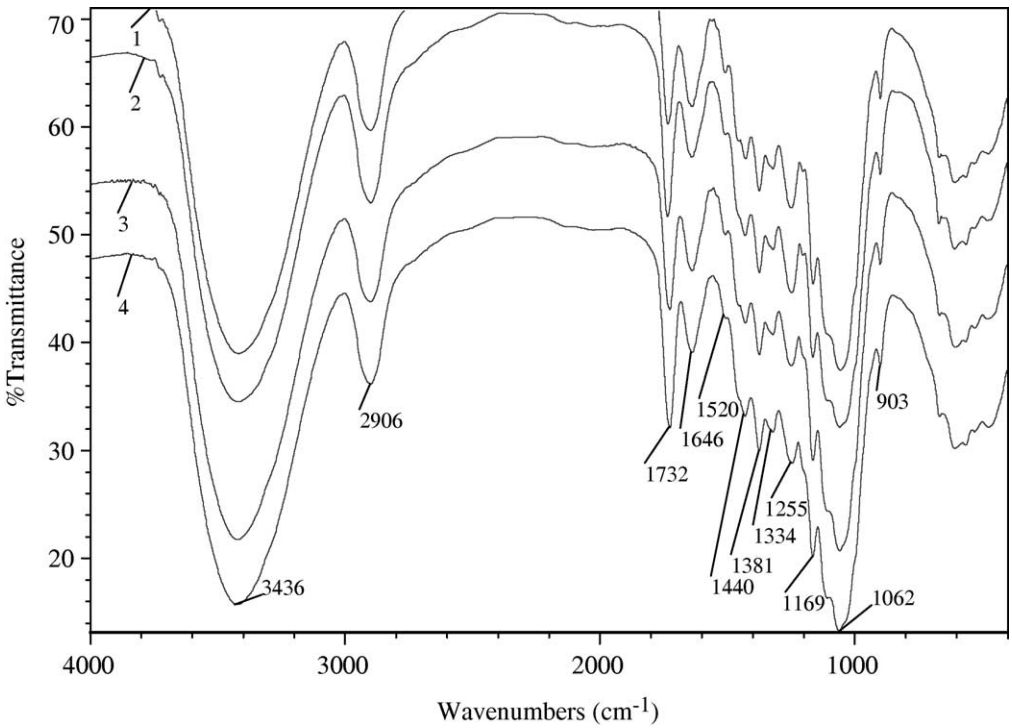


Fig. 2. FT-IR spectra of the unbleached cellulosic preparations C_{2a} (spectrum 1), C_{3a} (spectrum 2), C_{4a} (spectrum 3), and C_{5a} (spectrum 4).

groups in both polysaccharides and residual lignins (Sun & Sun, 2002), indicating that the unbleached cellulose was acetylated to a noticeable degree during the treatment with acetic acid/water or formic acid/acetic acid/water system. The absorption at 3436 cm^{-1} is assigned to stretching of –OH groups and that one at 2906 cm^{-1} to the C–H stretching. The band at 1646 cm^{-1} relates to the bending mode of the absorbed water. A noticeable peak at 1440 cm^{-1} is due to the CH_2 bending. The absorbance at 1334 cm^{-1} originated from the C–C and C–O skeletal vibrations (Sun, Fang, Goodwin, Lawther, & Bolton). The peak at 1169 cm^{-1} arises from C–O anti-symmetric bridge stretching. The absorption band at 1122 cm^{-1} (data not shown) is attributed to C–OH skeletal vibration. The C–O–C pyranose ring skeletal vibration gives a prominent band at 1062 cm^{-1}. A small sharp band at 897 cm^{-1} represents the glycosidic C_1–H deformation with ring vibration contribution and OH bending, which is characteristic of β-glycosidic linkages between glucose in cellulose. In addition, it should be noted that a shoulder at ~1520 cm^{-1} in all the spectra is indicative of aromatic skeletal vibrations in bound lignin, indicating that the unbleached cellulosic preparations contained small amounts of bound lignins, which corresponded to the results obtained by lignin analysis.

In the FT-IR spectra of unbleached cellulose samples (C_{6a} and C_{7a}) obtained in the presence of alcohol (Fig. 3), small changes were observed only at 1739 cm^{-1} and in the bands corresponding to an aromatic ring stretch from residual lignin components (1513 cm^{-1}). In comparison with the spectra of unbleached cellulose samples obtained with the presence of acetic acid in Fig. 2, occurrence of a smaller peak at 1739 cm^{-1} in the unbleached cellulose samples obtained without acetic acid is characteristic of aliphatic esters in residual hemicelluloses or lignin. An increase in band intensity at 1513 cm^{-1} corresponds to noticeable amounts of residual lignin in C_{6a} and C_{7a} (15.0 and 12.2%). After the alkaline peroxide treatment, the FT-IR spectra of bleached cellulose preparations (Fig. 4) do not show the carbonyl band at 1739 cm^{-1} due to the saponification. The disappearance of the band at 1513 cm^{-1} in the spectra of bleached cellulose samples C_{2b} and C_{4b} indicates that the two cellulose preparations are relatively free of residual lignin, while the presence of a shoulder of this band in spectrum 3 (Fig. 4) is indicative of small amounts of associated lignin in the bleached cellulose sample C_{7b}, which corresponds to their residual lignin content. These results suggested again that the lignin polymers associated to the hemicelluloses or cellulose are so tightly bound, that they resist attack even by strong ethanol/water acidic and cyanamide activated hydrogen peroxide alkaline media.

3.6. CP/MAS ^{13}C NMR spectra

CP/MAS ^{13}C NMR spectroscopy has proved particularly useful for examining changes in the nature of cellulose subjected to degradation processes. In contrast to wet chemical analyses, NMR spectroscopy provides information about molecular orders in cellulose as well as a description of any changes in the structure or content of hemicelluloses and lignin (Kim & Newman, 1995; Leary, Morgan, & Newman, 1987). CP/MAS ^{13}C NMR spectroscopy therefore complements the traditional chemical

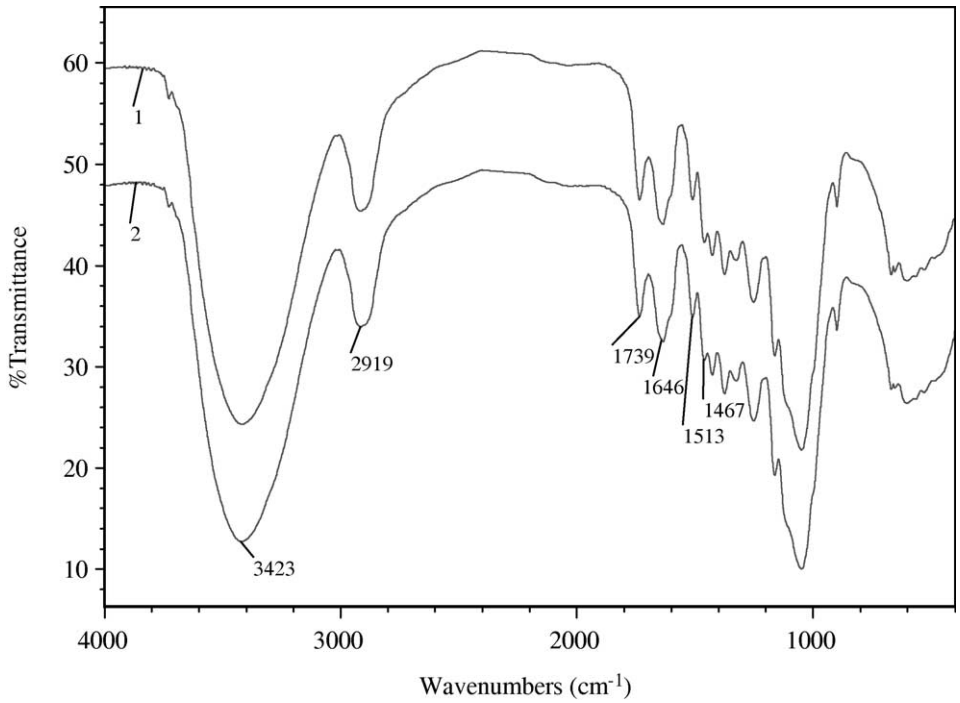


Fig. 3. FT-IR spectra of the unbleached cellulosic preparations C_{6a} (spectrum 1) and C_{7a} (spectrum 2).

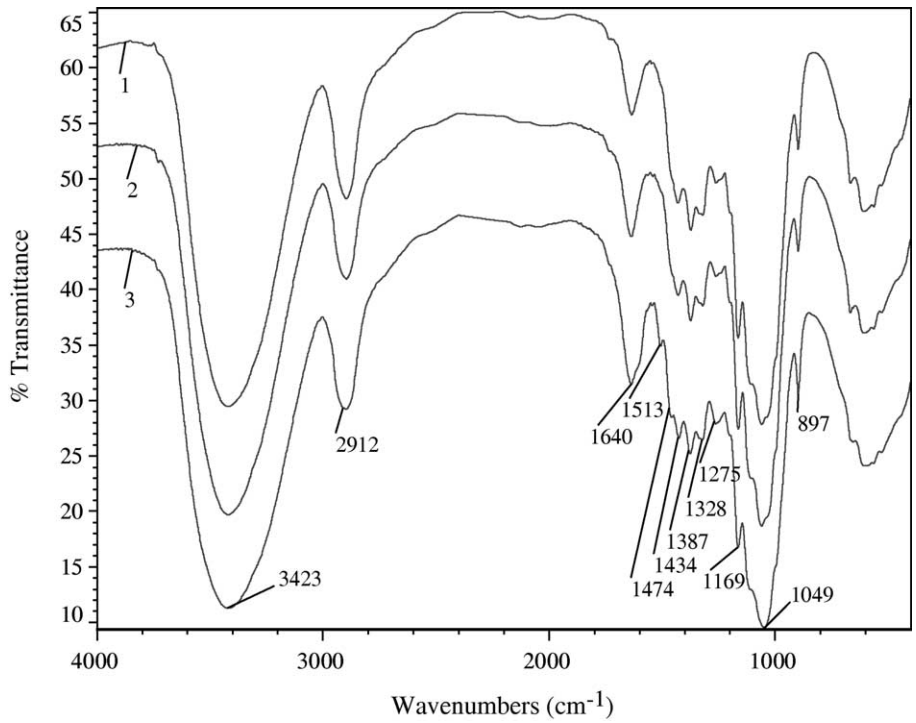


Fig. 4. FT-IR spectra of the bleached cellulosic preparations C_{2b} (spectrum 1), C_{4b} (spectrum 2), and C_{7b} (spectrum 3).

methods of cellulose analysis with minimal sample preparation. In addition, it has the advantage of simultaneously and quantitatively detecting signals from crystalline and less ordered states of cellulose (Larsson, Wickholm, & Iversen, 1997).

The CP/MAS ^{13}C NMR spectra of bleached celluloses C_{2b} (spectrum a) and C_{5b} (spectrum b) are shown in Fig. 5. As can be seen, they are similar in the cellulose region (60–110 ppm), namely C-1 (104.9 ppm), C-4 (88.8–88.9 ppm, crystal-interior cellulose, and 81.3–82.8 ppm, crystal-surface cellulose), C-2, C-3 and C-5 (74.9–72.4 ppm), and C-6 of cellulose (64.8 ppm, crystalline cellulose, 62.2 ppm, amorphous cellulose). This indicated that the cellulose was affected to a much smaller extent by a two-stage organosolv treatment followed by cyanamide activated hydrogen peroxide bleaching. As expected, the resonances in the 105–160 ppm from aromatic carbons in lignin, 20–33 ppm from methylenes in lignin, at 56 ppm from $-OCH_3$ groups in lignin and hemicelluloses, and at 173 ppm from acetyl groups in hemicelluloses completely disappeared, indicating that the bleached cellulose preparations are relatively free of bound lignin and hemicelluloses. This observation seems consistent with the results obtained by FT-IR spectroscopy and chemical analysis.

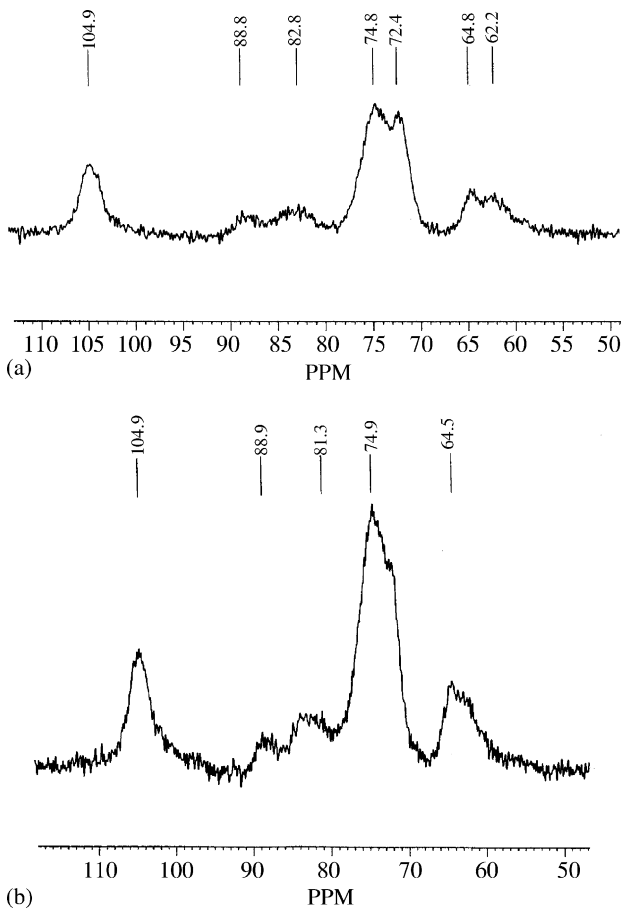


Fig. 5. CP/MAS ^{13}C NMR spectra of bleached cellulose samples C_{2b} (spectrum a) and C_{5b} (spectrum b).

3.7. Thermal analysis

Fig. 6 gives the TGA/DSC curves of the unbleached cellulosic preparation C_{2a} (Fig. 6a) obtained by treatment with acetic acid–H_2O (80/20, v/v) using 0.1% HCl

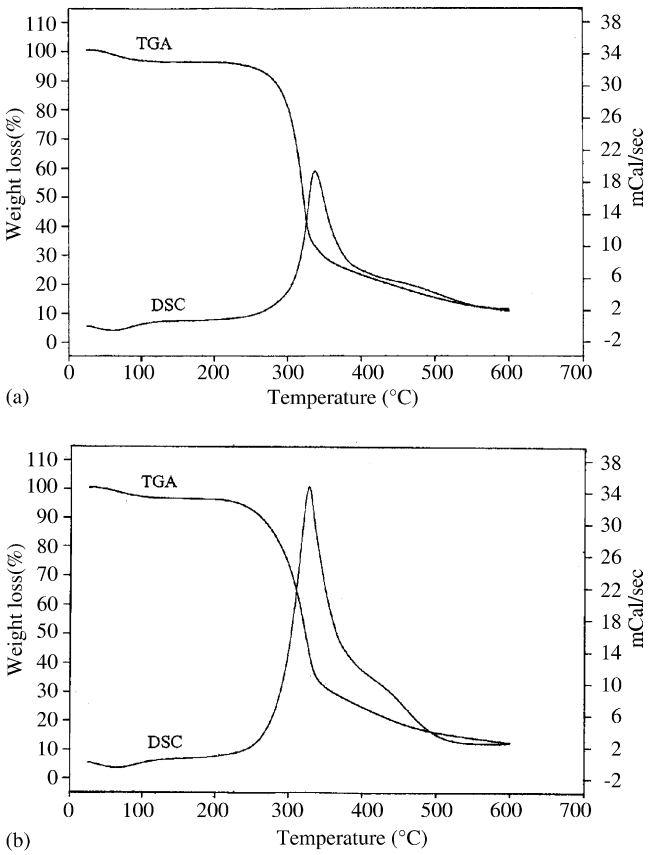


Fig. 6. TGA/DSC curves of the unbleached cellulosic preparation C_{2a} (a) and the bleached cellulosic sample C_{2b} (b).

as a catalyst at 85 °C for 4 h, and the bleached cellulosic sample C_{2b} (Fig. 6b) obtained by bleaching with 1.8% H_2O_2–0.18% cyanamide at 50 °C under pH 10.0 for 4 h from the corresponding unbleached cellulose C_{2a}. As shown in the figure, the TGA curves of the unbleached and bleached cellulose started to decompose at 267 (Fig. 6a) and 240 °C (Fig. 6b), respectively. At 10% weight loss the decomposition temperature of the unbleached cellulose and the corresponding bleached cellulose occurred at 287 and 267 °C, respectively. At 50% weight loss the decomposition temperature was observed at 325 °C for either unbleached cellulose or bleached cellulose. This implied that the unbleached cellulose had a higher thermal stability than the corresponding bleached cellulosic sample. In addition, the DSC thermogram of the unbleached cellulosic sample showed a small exothermic peak at 334 °C. However, the corresponding bleached cellulosic preparation gave a big exothermic peak, and it shifted to 327 °C. This indicated again that thermal stability of the cellulose decreased after bleaching, which corresponded to their decrease in intrinsic viscosity and molecular weight from 509.4 ml g^{-1} and 285,590 g mol^{-1} in C_{2a} to 356.0 ml g^{-1} and 191,870 g mol^{-1} in C_{2b}, respectively.

In conclusion, the results obtained in this study suggest that the optimum condition for isolation of cellulose by a TCF method from wheat straw is using formic acid/acetic acid/water (30/60/10, v/v/v) treatment followed by cyanamide activated hydrogen peroxide bleaching. The cellulose in this case obtained contained approximately 4% hemicelluloses and 0.7% lignin, and had a intrinsic viscosity of 392 ml g^{-1}. The use of different organic acids and alcohols led to the conclusion that organic acid/water systems promote both removal of lignin and non-cellulose polysaccharides such as hemicelluloses and the preservation of degradation of cellulose. Another interesting observation was that the linkages between lignin and hemicelluloses/-cellulose matrix are relatively strong and can only be cleaved under the organic acid/water system, not in the alcohol/water condition.

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References

Abad, S., Saake, B., Puls, J., & Parajo, J. C. (2002). Totally chlorine free bleaching of *Eucalyptus globulus* dissolving pulps delignified with peroxyformic acid and formic acid. *Holzforschung*, *56*, 60–66.

Atalla, R. H., & VanderHart, D. L. (1984). Native cellulose: A composite of two distinct crystalline forms. *Science*, *223*, 283–285.

Baeza, J., Urizar, S., de Magalhaes, N., Freer, J., Schmidt, E., & Duran, N. (1991). Organicsolv pulping-V: Formic acid delignification of *Eucalyptus globulus* and *Eucalyptus grandis*. *Bioresource Technology*, *37*, 1–6.

Blakeney, A. B., Harris, P. J., Henry, R. J., & Stone, B. A. (1983). A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydrate Research*, *113*, 291–299.

Cordeiro, N., Neto, C. P., Rocha, J., Belgacem, M. N., & Grandini, A. (2002). The organosolv fractionation of cork components. *Holzforschung*, *56*, 135–142.

Davis, L. L., Young, R. A., & Deodhar, S. S. (1986). Organic acid pulping of wood (3). Acetic acid pulping of spruce. *Mokuza Gakkaishi*, *32*, 905–914.

Duchesne, I., Hult, E. L., Molin, U., Daniel, G., Iversen, T., & Lennholm, H. (2001). The influence of hemicellulose on fibril aggregation of kraft pulp fibres as revealed by FE-SEM and CP/MAS ^{13}C-NMR. *Cellulose*, *8*, 103–111.

Ek, M., Gierer, J., Jansbo, K., & Reitberger, T. (1989). Study on the selectivity of bleaching with oxygen-containing species. *Holzforschung*, *43*, 391–396.

Evans, R., & Wallis, A. F. A. (1989). Cellulose molecular weights determined by viscosity. *Journal of Applied Polymer Science*, *37*, 2331–2340.

Geng, Z. C., Sun, R. C., Sun, X. F., & Lu, Q. (2003). Comparative study of hemicelluloses released during two-stage treatments with acidic organosolv and alkaline peroxide from *Caligonum monogoliacum* and *Tamarix* spp. *Polymer Degradation and Stability*, *80*, 315–325.

Green, J. W. (1963). In R. L. Whistler (Ed.), (*Vol. III*) (pp. 9–21). *Methods of carbohydrate chemistry*, New York: Academic Press.

Guay, D. F., Cole, B. J. W., Fort, R. C., Genco, J. M., & Hausman, M. C. (2000). Mechanisms of oxidative degradation of carbohydrates during delignification. 1. Reaction of methyl β-D-glucopyranoside with photochemically generated hydroxyl radicals. *Journal of Wood Chemistry and Technology*, *20*, 375–394.

Hackney, J. M., Atalla, R. H., & Vanderhart, D. L. (1994). Modification of crystallinity and crystalline structure of *Acetobacter xylinum* cellulose in the presence of water-soluble β-1,4-linked polysaccharides: 13C NMR evidence. *International Journal of Biological Macromolecules*, *16*, 215–218.

Hamilton, J., Senior, D., Sartiogo, A., Szwee, J., & Ragauskas, A. (1996). Improvements in ECF bleaching: Use of activated oxygen species and xylanase. *Tappi*, *79*, 23–231.

Hayashi, J., Kon, H., Takai, M., Hatano, M., & Nozawa, T. (1987). The structure of cellulose. *ACS Symposium Series*, *340*, 134–136.

Hult, E. L., Larsson, P. T., & Iversen, T. A. (2000). Comparative CP/MAS ^{13}C-NMR study of cellulose structure in spruce wood and kraft pulp. *Cellulose*, *7*, 35–45.

Isogai, A., Ishizu, A., & Nakano, J. (1989). Residual lignin and hemicellulose in wood cellulose, analysis using new permethylation method. *Holzforschung*, *43*, 333–338.

Kadla, J. F., & Chang, H. M. (2002). Reactions of lignin with cyanamide activated hydrogen peroxide. Part 3. The degradation of pine kraft lignin. *Holzforschung*, *56*, 76–84.

Kadla, J. F., Chang, H. M., Chen, C. L., & Gratzl, J. S. (1998). Reactions of lignin with cyanamide activated hydrogen peroxide. Part 1. The degradation of lignin model compounds. *Holzforschung*, *52*, 506–512.

Kadla, J. F., & Gilbert, R. D. (2000). Cellulose structure: A review. *Cellulose Chemistry and Technology*, *34*, 197–216.

Kim, Y. S., & Newman, R. H. (1995). Solid state ^{13}C NMR study of wood degraded by the brown rot fungus *Gloeophyllum trabeum*. *Holzforschung*, *49*, 109–114.

Kishimoto, T., Tsuji, H., Uraki, Y., & Sano, Y. (2003). Ozone bleaching of atmospheric acetic acid hardwood pulp from *Betula platyphylla var. japonica* Hara. *Holzforschung*, *57*, 181–188.

Kordsachia, O., Patt, R., & Sturm, W. (2001). Chlorine-free pulp bleaching by using cyanamide and dicyan-diamide. *Wochenblatt Fur Paplerfabrikation*, *129*, 941–942.

Lam, H. Q., Bigot, Y. L., Delmas, M., & Avignon, G. (2001). A new procedure for the destructuring of vegetable matter at atmospheric pressure by a catalyst/solvent system of formic acid/acetic acid. Applied to the pulping of triticale straw. *Industrial Crops and Products*, *14*, 139–144.

Larsson, P. T., Wickholm, K., & Iversen, T. (1997). A CP/MAS ^{13}C NMR investigation of molecular ordering in cellulose. *Carbohydrate Research*, *302*, 19–25.

Leary, G. J., Morgan, K. R., & Newman, R. H. (1987). Solid-state carbon-13 nuclear magnetic resonance study of *Pinus radiata* wood. *Appita*, *40*, 181–184.

Lehnen, R., Saake, B., & Nimz, H. H. (2002). Impact of pulping conditions on FORMACELL aspen: Investigation of methoxyl and ester groups, carbohydrates, molar mass and glass transition temperatures. *Holzforschung*, *56*, 498–506.

Lennholm, H., & Iversen, T. (1995). The effects of laboratory beating on cellulose structure. *Nordic Pulp and Paper Research Journal*, *10*, 104–108.

Lora, J. H., & Aziz, S. (1985). Organosolv pulping: A versatile approach to wood refining. *Tappi*, *68*, 94–97.

Maximova, N., Osterberg, M., Koljonen, K., & Stenius, P. (2001). Lignin adsorption on cellulose fibre surfaces: Effect on surface chemistry, surface morphology and paper strength. *Cellulose*, *8*, 113–125.

McIsaac, J. E., Jr., Ball, R. E., & Behrman, E. J. (1971). The mechanism of the base-catalysed conversion of nitriles to amides by hydrogen peroxide. *Journal of Organic Chemistry*, *36*, 3048–3050.

Moore, G. (1996). Nonwood fibre applications in papermaking. *Pira International, UK*, 1–4.

Mora, F., Ruel, K., Comtat, J., & Joseleu, J. P. (1986). Aspect of native and redeposited xylans at the surface of cellulose microfibrils. *Holzforschung*, *40*, 85–91.

Newman, R. H. (1998). Evidence of assignment of ^{13}C NMR signals to cellulose crystalline surface in wood, pulp and isolated cellulose. *Holzforschung*, *52*, 157–159.

Newman, R. H., & Hemmingson, J. A. (1995). Thermal conversion between crystalline forms of cellulose during pulping. *Proceedings of Eighth International Symposium on Wood Pulp and Chemistry, Helsinki, Finland*, *1*, 519–525.

Newman, R. H., Hemmingson, J. A., & Sucking, I. D. (1993). Carbon-13 nuclear magnetic resonance studies of kraft pulping. *Holzforschung*, *47*, 234–238.

Nimz, H. H., & Casten, R. (1986). Chemical processing of lignocellulosics. *Holz Roh-Werkstoff*, *44*, 207–212.

Ooi, T., & Ni, Y. (1998). Development of an ozone-based TCF sequence for bleaching hardwood ALCELL pulp. *Tappi Journal*, *81*, 255–259.

Pan, X. J., & Sano, Y. (1999). Atmospheric acetic acid pulping of rice straw IV: Physico-chemical characterisation of acetic acid lignins from rice straw and woods. *Holzforschung*, *53*, 590–596.

Pan, G. X., Spencer, L., & Leary, G. J. (2000). A comparative study on reactions of hydrogen peroxide and peracetic acid lignin chromophores. *Holzforschung*, *54*, 144–152.

Roberts, J. C. (1996). *The chemistry of paper*. Cambridge: The Royal Society of Chemistry, pp. 190–192.

Sano, Y., Nakamura, M., & Shimamoto, S. (1990). Pulping of wood at atmospheric pressure II: Pulping of hardwoods with aqueous acetic acid containing a small amount of sulfuric acid. *Mokuza Gakkaishi*, *36*, 207–209.

Sarkanen, K. V. (1980). Acid-catalysed delignification of lignocellulosics in organic solvents. *Progress in Biomass Conversion*, *2*, 127–145.

Sarkanen, K. V. (1990). Chemistry of solvent pulping. *Tappi Journal*, *73*, 215–219.

Sarkanen, K. V., & Hoo, L. H. (1981). Kinetics of hydrolysis of erythro-guaiacylglycerol β-(2-methxyphenyl) ether and its veratryl analogue using HCl and aluminum chloride as catalysts. *Journal of Wood Chemistry and Technology*, *1*, 11–27.

Schuerch, C. (1952). The solvent properties of liquid and their relation to the solubility, swelling, isolation and fractionation of lignin. *Journal of American Chemical Society*, *74*, 5061–5067.

Shatalov, A. A., & Pereira, H. (2002). Ethanol-enhanced alkaline pulping of *Arundo Donax* L. reed: Influence of solvent on pulp yield and quality. *Holzforschung*, *56*, 507–512.

Scott, R. W. (1984). Hemicellulose distribution in pulp fibres and alkaline extraction rates. *Journal of Wood Chemistry and Technology*, *4*, 199–218.

Sturm, W., & Kuchler, G. (1993). The nitrilamine reinforced hydrogen peroxide bleaching of kraft pulps. *Non-Chlorine Bleaching Conference Proceedings, Hilton Head*, pp. 31–41.

Sugiyama, J., Persson, J., & Chanzy, H. (1991). Combined infrared and electron diffraction study of the polymorphism of native cellulose. *Macromolecules*, *24*, 2461–2466.

Sugiyama, J., Vuong, R., & Chanzy, H. (1991). Electron diffraction study on the two crystalline phases occurring in native cellulose from alga cell wall. *Macromolecules*, *24*, 4168–4175.

Sun, R. C., Fang, J. M., Goodwin, A., Lawther, J. M., & Bolton, J. (1998). Isolation and characterization of polysaccharides from abaca fibre. *Journal of Agricultural and Food Chemistry*, *46*, 2817–2822.

Sun, X. F., & Sun, R. C. (2002). Comparative study of acetylation of rice straw fibre with or without catalysts. *Wood and Fibre Science*, *34*, 306–307.

Tokoh, C., Takabe, K., Fujita, M., & Saiki, H. (1998). Cellulose synthesized by *Acetobacter xylinum* in the presence of acetyl glucomannan. *Cellulose*, *5*, 249–261.

Uraki, Y., Hashida, K., & Sano, Y. (1997). Self-assembly of pulp derivatives as amphiphilic compounds: Preparation of amphiphilic

compounds from acetic acid pulp and its properties as an inclusion compound. *Holzforschung*, *51*, 91–97.

Vazquez, G., Antorrena, G., & Gonzalez, J. (1994). Kinetics and mechanism of acetic acid pulping of detannined Pinus pinaster bark. *Wood Science and Technology*, *28*, 403–408.

Whitney, S. E. C., Brigham, J. E., Darke, A. H., Reid, J. S. G., & Gidley, M. J. (1998). Structural aspects of the interaction of mannan-based polysaccharides with bacterial cellulose. *Carbohydrate Research*, *307*, 299–309.

Wickholm, K., Hult, E. L., Larsson, P. T., Iversen, T., & Lennholm, H. (2001). Quantification of cellulose forms in complex cellulose materials: A chemometric model. *Cellulose*, *8*, 139–148.

Young, R. A., & Davis, J. L. (1986). Organic acid pulping of wood. Part II. Acetic acid pulping of aspen. *Holzforschung*, *40*, 99–108.