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Separation and Characterization of Cellulose from Wheat Straw

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ABSTRACT

Highly purified cellulose was separated from wheat straw by sequential treatments of dewaxed straw with 0.5-M aqueous KOH at 35°C for 2.5 h under ultrasonic irradiation time of 0 to 35 min, 2% H_2O_2–0.2% TAED at pH 11.8 for 12 h at 48°C, and with 80% acetic acid–70% nitric acid (10/1, v/v) at 120°C for 15 min. The yield of crude cellulose preparations obtained by first two-stage treatments ranged between 45.3% and 46.9% of the dry weight straw which contained 7.3 to 7.9% residual hemicelluloses and 3.3 to 3.7% residual lignin, and had molecular weights ranging from 269,960 and 258,280 g mol^{-1} determined by their viscosity, while the purified cellulose samples separated by a further treatment of the

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corresponding crude cellulose with 80% acetic acid–70% nitric acid, are relatively free of bound lignin (0.1 to 0.2%) and contained minor amounts of associated hemicelluloses (~3%), but gave much lower molecular weights ranging between 42,300 and 44,650 g mol^{-1} estimated by GPC in DMAc/LiCl system indicating that the final step treatment with 80% acetic acid–70% nitric acid resulted in a noticeable degradation of the cellulose except for removal of residual hemicelluloses and lignin. However, there was no evidence of increased acid or aldehyde by oxidation in all the purified cellulosic preparations. Both crude and purified cellulose samples were characterized by FT-IR and CP/MAS ^{13}C-NMR spectroscopy. The thermal stability of the purified cellulosic preparation was higher than that of the crude cellulosic sample.

Key Words: Wheat straw; Cellulose; Viscosity; Molecular weight; FT-IR; CP/MAS ^{13}C-NMR; TGA; DSC.

INTRODUCTION

Lignocellulosic material from agricultural waste such as wheat straw represents an abundant renewable energy source for bioconversion processes as well as a raw material for the paper and pulp industry.[1] In Europe, 184 million tons of wheat were produced in 2000.[2] The average yield of straw is 1.3 to 1.4 kg per kg of grain, which results in a considerable amount of surplus straw.[3] Wheat straw is a biocomposite that consists mainly of cellulose (38 to 42%), hemicelluloses (34 to 40%), and lignin (15 to 18%)[4] and this relatively high amount of cellulose stresses the need for utilizing the cellulose as novel polymers for industries, except for paper and pulp production. In other words, cellulose comprises almost one-third of the weight of all tress, vines, grasses, and straws. In addition, it is constantly replenishing itself by photosynthesis and growth, with estimates of annual world biosynthesis of 10^{11} tons.[5] This represents a vast potential feedstock for a number of industries and has created a great deal of research interest. The use of cellulose and its derivatives in a diverse array of applications, such as fibers, films, plastics, coatings, suspension agents, and composites, continues to grow on a world-wide basis.[6] As a regenerative raw material, cellulose can be also converted by chemical modification into water-soluble derivatives that are often put to industrial use (toothpaste, flow enhancers, drilling fluid, shampoos, foodstuffs, etc.).[7]

There is an increased interest in the development of techniques for fractionation of lignocellulosic materials, for example, to be used in the production of environmental-friendly polymers.[8] The techniques available for the extraction of α-cellulose from whole wood are well established and have been described by Green.[9] The method adopted for this study was based upon

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the acidified sodium chlorite delignification as an initial step in the extraction of cellulose. However, environmental concerns associated with chlorinating agents (e.g., $NaClO_2$) have led to the increased use of more environmentally benign agents for delignification, such as hydrogen peroxide or an acetic acid–nitric acid mixture, in both elemental chlorine-free (ECF) and totally chlorine-free (TCF) separation sequences.[10,11] In this study, we describe a TCF procedure for cellulose isolation based on the separation of lignocelluloses into three major constituents (cellulose, hemicelluloses, and lignin) using a sequential extraction by alkali, alkaline peroxide, and an acetic acid–nitric acid mixture. The separated crude and purified cellulosic preparations were characterized by their yield, content of lignin and hemicelluloses, viscosity, molecular weight, and thermal stability. In particular, solid-state cross polarization magic angle spinning carbon-13 nuclear magnetic resonance (CP/MAS ^{13}C-NMR) was used to investigate the structural changes of the cellulosic polymers.

EXPERIMENTAL METHODS

Materials

Wheat straw (variety *Riband*) was kindly supplied by B. Lloyd Co., Llangefni, Wales. The composition (percentage, w/w) of the straw is cellulose 38.8%, hemicelluloses 39.5%, lignin 17.1%, ash 1.8%, and wax 2.2% on a dry weight basis.[4] 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.

Three-Stage Method for Separation of Crude and Purified Cellulose

Prior to ultrasound-assisted alkaline treatment, the dried powder of the straw was first extracted with toluene-ethanol (2 : 1, v/v) in a Soxhlet extractor for 6 h. To a 9.78-g dewaxed sample in a 500-mL beaker, 300-mL 0.5-M KOH aqueous solution was added. The irradiation was carried out using the sonic system SOMERSET (England, 20 kHz) provided with a sonator of 100 W and sonication time of 0, 5, 10, 15, 20, 25, 30, and 35 min, respectively. The mixture was then successively treated with the remaining 0.5-M KOH aqueous solution at 35°C for a total period of 2.5 h, respectively, under continuous agitation. After filtration on a nylon cloth, the hemicelluloses and lignin solubilized were isolated from the hydrolysates by a two-step precipitation method and investigated in detail as described in our previous articles.[4,12] The residues rich in cellulose were washed with water and

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ethanol, then dried at 60°C for 16 h. The remaining hemicelluloses and lignin were extracted from the eight corresponding residues with 2% H_2O_2–0.2% TAED (tetraacetylethylene diamine) at pH 11.8 for 12 h at 48°C, respectively. The ratio of liquor to dry matter was 24/1 (mL/g). Solubilized residual hemicelluloses and lignin were isolated according to the two-step precipitation method as described. The sub-residues (~150 mg) were weighed into 30-mL Pyrex tubes. Subsequently, 5.0 mL of 80% (v/v) acetic acid and 0.5 mL of concentrated nitric acid (70%, v/v) were added. To react completely, any residue left adhering to the inner wall of the test tube was washed downward to aid complete extraction. The tubes were sealed using screw-caps fitted with Teflon liners and placed into a preheated oil bath to 120°C for 15 min. Once cooled, the supernatant was then carefully decanted and pellets were washed sequentially with 95% ethanol (20 mL), distilled water (30 mL), and 95% ethanol (20 mL) to remove extraction breakdown products and traces of nitric acid. Finally, the purified cellulosic preparations were dried in an oven at 60°C for 16 h. The scheme for separation of crude and purified cellulose from wheat straw is illustrated in Fig. 1. All experiments were performed at least in

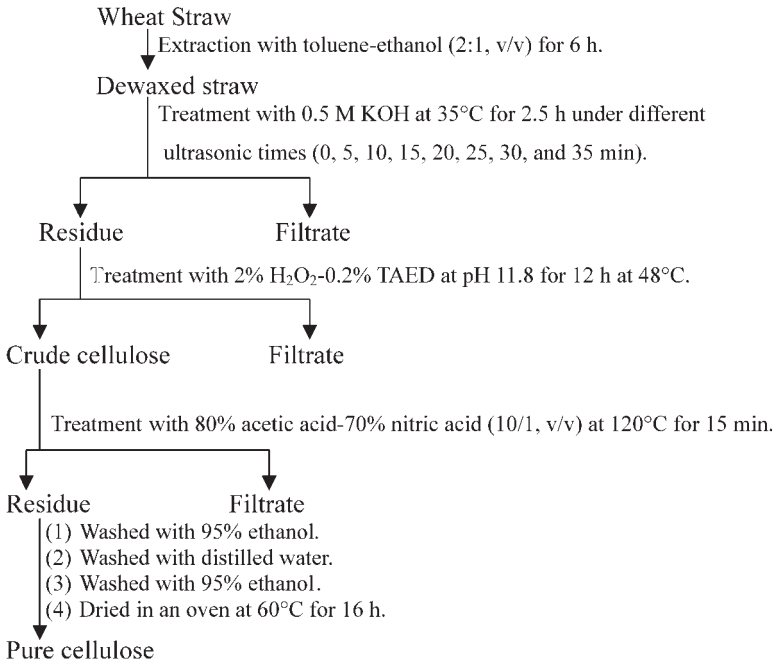

Figure 1. Scheme for isolation of crude and purified cellulose from wheat straw.

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duplicate. Yields of the crude and purified celluloses are given on a dry weight basis related to the wheat straw (Table 1).

Characterization of Crude and Purified Celluloses

The neutral sugar composition of the separated crude and pure cellulosic preparations was determined by gas chromatography (GC) analysis of the corresponding alditol acetates as described by Blakeney et al.[13] The method for the determination of phenolic acids and aldehydes in nitrobenzene oxidation mixtures of lignins associated in the separated crude cellulosic preparations with high performance liquid chromatography (HPLC) was described in a previous article.[14] The lignin content in crude cellulosic preparations was calculated by multiplying the yield of phenolics obtained from alkaline nitrobenzene oxidation by 1.64.[4,12] Klason lignin content in purified cellulose samples was determined according to the Tappi method T 249 cm-85.

The viscosity of the crude 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 (P) of the crude cellulose samples was estimated by $P^{0.90} = 1.65\ [\eta]$.[15] Molecular weight of the crude cellulosic preparations was then calculated from their P by multiplying by 162, molecular weight of an anhydroglucose. For determination of molecular weight of the purified cellulose sample by gel permeation chromatography (GPC), the purified cellulose sample ($\sim$7 mg) was first suspended in 3.5 mL of DMAc/4% LiCl (dimethylacetamide/lithium chloride), followed by

Table 1. Yield of crude cellulose and purified cellulose.

	Ultrasonic time (min)							
	0	5	10	15	20	25	30	35
Crude cellulose (CC)	46.9	46.8	46.7	46.7	46.4	46.4	45.8	45.3
Content of hemicelluloses in CC	7.9	7.7	7.7	7.9	7.5	7.3	7.3	7.3
Content of lignin in CC	3.7	3.6	3.6	3.4	3.4	3.4	3.3	3.3
Purified cellulose	37.5	37.4	37.2	36.9	36.8	37.0	36.6	36.4

Note: Percentage of dry matter isolated with 2% H_2O_2–0.2% TAED at pH 11.8 for 12 h at 48°C and sequentially with 80% acetic acid–70% nitric acid (10/1, v/v) at 120°C for 15 min from 0.5-M KOH treated (35°C, 2.5 h) wheat straw under different ultrasonic times.

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sequential heating to 120°C for 2 h and 80°C for 5 h under stirring and cooling to room temperature. It was found that direct application of this procedure in our laboratory was successful for dissolution of all the pure cellulosic preparations from wheat straw. The mobile-phase solvent for GPC was DMAc/0.5% LiCl prepared by raising the temperature of 1000 mL of DMAc to 100°C and then adding 5 g of LiCl (dried). After the salt was stirred until it dissolved, the solvent was filtered through a Teflon filter (0.5 μm, Millipore) with a glass filter apparatus. Filtered samples were analyzed on a Knauer GPC system (Berlin, Germany) consisting of a manual sampler with an HPLC pump (Model 64) and a Knauer differential refractometer detector. Molecular weight determination was performed on a PL gel mixed A column (Polymer Laboratories Ltd., UK), 10 μ, 0.75 cm i.d. × 30 cm. It was protected with a guard column, 0.7 cm i.d. × 5 cm, packed with 10 μ 100 Å packing material (Polymer Laboratories Ltd., UK). The column and guard column were placed inside a high temperature column oven Model 60C (Knauer, Berlin, Germany) set at 80°C to reduce solvent viscosity for greater mass transfer and to enhance column efficiency. The mobile phase of DMAc/0.5% LiCl was pumped through at 1.0 mL/min. Injection volume was 200 μL. Before injection, the column was equilibrated for 1 day in the circulating eluent. Five number of pullulans from Polymer Laboratories (Shropshire, UK), were used to calibrate the molecular weight. The pullulans are polymeric carbohydrates consisting of polymaltotriose units, which can be obtained in fractions with different narrow distributions of molecular weights. The pullulans range from 5800 to 853,000 in molecular weight. The pullulan standards were dissolved in DMAc/0.5% LiCl and chromatographed under the standard conditions of the analysis. Data were processed with Microsoft Excel.

The Fourier transform infrared (FT-IR) and solid-state cross polarization magic angle spinning carbon-13 nuclear magnetic resonance (CP/MAS ^{13}C-NMR) spectra and thermal analysis of the crude and purified cellulosic samples were performed as described previously.[16]

RESULTS AND DISCUSSION

Yield of Crude and Purified Cellulose

In this study, crude cellulose was isolated from wheat straw by an environmentally friendly method, ultrasound-assisted alkaline pretreatment following alkaline peroxide post-treatment. The yield of crude cellulose separated from wheat straw at different ultrasonic times, are given in Table 1. All values are calculated on the basis of mass of untreated starting straw. These results showed that the yield of crude cellulose in wheat straw is 45.3 to

46.9%, which contained 7.3 to 7.9% residual hemicelluloses and 3.3 to 3.7% residual lignin. The yield of crude cellulose decreased slightly with an increase in ultrasound-assisted time from 0 to 35 min, indicating that application of sonication resulted in raising solubility of hemicelluloses and lignin in comparison to the alkaline extraction procedure without ultrasound assistance. Remarkably, the two-stage treatments together solubilized 90.6, 90.9, 90.9, 90.6, 91.1, 91.4, 91.6, and 91.6% of the original hemicelluloses and 89.9, 90.1, 90.1, 90.6, 90.8, 90.8, 91.2, and 91.4% of the original lignin from wheat straw without ultrasonic irradiation and with ultrasonic times for 5, 10, 15, 20, 30, and 35 min, respectively. The reason for this higher solubility of hemicelluloses and lignin is probably due to the fact that hemicelluloses and lignin are present mainly on outer surface, from where they dissolve easily in the liquor during the two-stage treatments. On the other hand, the long cellulose chains located in the inner parts of the fibers and, therefore, are not easily dissolved. Furthermore, cellulose is a semicrystalline biopolymer with ordered crystalline and disordered amorphous regions. This partly crystal structure also reduces its solubility.[17] In contrast, the difficulty of removing the residual hemicelluloses and lignin, even by this two-stage treatment, suggested that the sorption is not limited at the outer fiber surface, and some amounts of hemicelluloses or lignin may be distributed near the outer fiber surface and on, or near, the lumen surfaces and pores.[18]

Organosolv pulping (solvent-based delignification) provides an interesting alternative to the commercial technologies used for chemical pulp manufacture. Among the variety of organic solvents proposed in published research,[19] acetic acid has received significant attention owing to its ability to achieve extensive and selective delignification in a single-step operation. The utilization of acetic acid in pulping has been investigated in HCl-catalyzed media (Acetosolv process),[20] formic acid-catalyzed media (Formacell process),[21] and no catalyzed media (Acetocell process).[22] In all the cases, a high degree of both lignin and hemicellulose removal was achieved in acetic-based pulping. However, in acetic acid pulping, where acidolysis is the main mechanism of lignin degradation, good bleachability of pulps is expected. In this study, a totally chlorine-free (TCF) bleaching of the crude cellulose by using nitric acid was performed.

As can be seen from the Table 1, the yield of purified cellulose, obtained by treatment of the crude cellulosic preparations with 80% acetic acid–70% nitric acid (10/1, v/v) at 120°C for 15 min, decreased significantly to 36.4 to 37.5%, indicating that 3.4 to 6.2% of the original cellulose was degraded during the treatment, and this is particularly true for the purified cellulosic preparations obtained from the sample of initial ultrasound irradiation for a longer period. An increase in ultrasonic time from 5 to 35 min led to a slight decrease in the yield of purified cellulose from 37.4% to 36.4%.

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During the bleaching, the various substitution, side chain cleavage, and oxidation reactions observed with nitric acid are all likely to occur with nitrogen dioxide. In addition, free-radical-initiated reactions involving the addition of nitrogen dioxide to the aromatic ring (in lignins), hydrogen abstraction (from cellulose and lignin), and electron transfer (mainly from phenolic groups) should also take place. In general, nitric acid or nitrogen dioxide is more reactive toward lignin than toward the carbohydrates. However, nitric acid or nitrogen dioxide treatment does damage the carbohydrates and, depending on the conditions of bleaching, the pulp viscosity can decrease.[23] Furthermore, in contrast to alkali hydroxides, nitric acid is a multifunctional reagent with respect to cellulose. It is capable of nitrating, oxidative, and hydrolyzing actions. Taking this into account, a low concentration of nitric acid (8.5%, w/v) was used in this study. However, this viewpoint did not provide an explanation of the degradation of cellulose observed in this study. On the other hand, the overall this three-step separation sequence is very interesting from a circuit, leading to the total effluent-free approach.

Content of Hemicelluloses

The content of hemicelluloses in crude and purified cellulose was estimated by GC determination of the sum of noncellulose sugars contained in the acid hydrolysates. As shown in Table 2, glucose was the predominant sugar component in all of the eight crude cellulosic preparations, comprising 88.1 to 88.8% of the total sugars, indicating a higher content of cellulose. While appearance of noticeable amounts of xylose (9.6 to 10.2%) and minor

Table 2. Composition of neutral sugars.

	Ultrasonic time (min)							
Neutral sugars	0	5	10	15	20	25	30	35
Arabinose	1.22	1.05	1.08	1.09	1.06	0.97	0.98	1.06
Xylose	9.72	9.98	10.04	10.22	9.91	9.57	9.63	9.59
Mannose	0.12	0.11	0.11	0.14	0.15	0.14	0.14	0.15
Galactose	0.58	0.48	0.49	0.46	0.43	0.39	0.42	0.48
Glucose	88.10	88.30	88.30	88.10	88.50	88.60	88.80	88.70

Note: Relative percentage of crude cellulosic sample, (w/w) in isolated crude cellulosic preparations obtained by extraction with 2% H_2O_2–0.2% TAED at pH 11.8 for 12 h at 48°C from 0.5-M KOH treated (35°C, 2.5 h) wheat straw under different ultrasonic times.

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quantities of arabinose (1.0 to 1.2%) and galactose (0.4 to 0.6%), as well as trace of mannose (0.1%), demonstrated that the crude cellulose contained noticeable amounts of associated hemicelluloses. Interestingly, further treatment of the crude cellulose with 80% acetic acid–70% nitric acid (10/1, v/v) at 120°C for 15 min resulted in a considerable increase in glucose content to 96.7 to 97.1%, implying a higher purity of the cellulose (Table 3). The presence of minor sugars other than glucose in purified cellulosic preparations could be the result of incomplete removal of contaminating hemicelluloses. Meanwhile, a significant improvement in brightness was also observed in treatment with 80% acetic acid–70% nitric acid. Eight purified cellulosic preparations showed a remarkable brightness gain. The fact that the amount of hemicelluloses is essentially lower in the purified cellulosic preparations (see noncellulose sugar content, ~2.9 to 3.3%, in Table 3) than in the corresponding crude cellulosic fractions (see noncellulose sugar content, ~11.3 to 11.9%, in Table 2) can be taken as evidence of the substantial disruption of the strong associative interaction between cellulose and hemicelluloses under the 80% acetic acid–70% nitric acid action.

Content of Bound Lignin and the Composition of Phenolic Acids and Aldehydes

To verify the presence of associated lignin in crude cellulosic preparations, nitrobenzene oxidation of bound lignin was performed at 170°C for 2.5 h.

Table 3. Neutral sugar composition.

Neutral sugars/ Klason lignin	Ultrasonic time (min)							
	0	5	10	15	20	25	30	35
Arabinose	0.6	0.5	0.5	0.5	0.5	0.4	0.4	0.5
Xylose	2.4	2.4	2.5	2.4	2.3	2.2	2.2	2.2
Mannose	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Galactose	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.2
Glucose	96.7	96.7	96.7	96.8	96.9	97.1	97.1	97.0
Klason lignin	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1

Note: Relative percentage of purified cellulosic sample, w/w and content of Klason lignin (percentage of purified cellulosic sample, w/w) of purified cellulosic preparations obtained by treatment with 80% acetic acid–70% nitric acid (10/1, v/v) at 120°C for 15 min from 0.5-M KOH treated (35°C, 2.5 h, under different ultrasonic times) and sequentially 2% H_2O_2–0.2% TAED extracted (pH 11.8, 12 h, 48°C) wheat straw.

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This method provides an estimate of the total amount of chemically linked lignin and yields of an indication of the phenolic monomer composition. As summarized in Table 4, the eight crude cellulosic preparations contained small amounts of linked lignin, 3.25 to 3.66%. Clearly, an increase in irradiation time from 0 to 5, to 10, to 15, to 20, to 25, to 30, and to 35 min resulted in a slight decrease in the associated lignin from 3.66, to 3.62, to 3.61, to 3.43, to 3.38, to 3.39, to 3.28, and to 3.25%, respectively. The major products obtained from alkaline nitrobenzene oxidation were identified to be syringaldehyde (0.92 to 1.03%) and vanillin (0.84 to 0.93%). This indicated that the associated residual lignin in the crude cellulosic preparations was mainly composed of noncondensed syringyl and guaiacyl units. In addition, small amounts of *p*-hydroxybenzoic acid (0.08 to 0.11%) and syringic acid (0.09 to 0.11%) together with a trace of *p*-hydroxybenzaldehyde (0.03 to 0.05%) and vanillic acid (0.01%), were also detected among the products of alkaline nitrobenzene oxidation. On the other hand, the occurrence of small amounts of bound lignin in the crude cellulose strongly suggests that lignin in the cell wall of wheat straw is tightly linked to hemicelluloses or cellulose and is more alkali resistant. Interestingly, a relatively free of associated lignin in all the purified cellulosic preparations (0.1–0.2%, Table 3) revealed that the mixture of 80% acetic acid–70% nitric acid is a powerful agent for removal of residual lignins from wheat straw, which coincides with the findings by Brendel et al.[11] They mentioned that approaching 100% purity of cellulose can be provided from plant cell wall materials by treatment with 80% acetic acid–70% nitric acid at 120°C for 20 min.

Intrinsic Viscosity (η), Viscosity Average DP (P), and Molecular Weight (M_w)

In general, pulp viscosity is used as a measure of cellulose degradation in pulping processes, although the viscosity determination is a relative method. Table 5 gives the results of viscosimetric analysis carried out on eight crude cellulosic preparations. Data in Table 5 show that viscosity decreased slightly from 484.2 mL g^{-1} without ultrasonic treatment condition to 465.3 mL g^{-1} at a severest condition, with ultrasonic irradiation time for 35 min. The data suggested that ultrasonic irradiation would cause a proportional decrease of viscosity of the crude cellulose. Similar results have previously been reported by Aliyu and Hepher[24] in a study of effects of ultrasound energy on degradation of cellulose materials. It should be noted that the presence of free radicals from metal induced decomposition of peroxide could also induce drastic scission in the glycosidic linkages of the cellulose that could cause lower viscosities.

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Table 4. Content (percentage of crude cellulosic sample, w/w) of phenolic acids and aldehydes from nitrobenzene oxidation of the associated lignin in isolated crude cellulosic preparations.

Phenolic acids and aldehydes	Ultrasonic time (min)							
	0	5	10	15	20	25	30	35
p-Hydroxybenzoic acid	0.110	0.100	0.110	0.090	0.088	0.084	0.086	0.082
p-Hydroxybenzaldehyde	0.045	0.041	0.039	0.036	0.035	0.036	0.033	0.031
Vanillic acid	0.012	0.011	0.011	0.013	0.013	0.011	0.012	0.010
Syringic acid	0.100	0.110	0.100	0.088	0.085	0.086	0.091	0.092
Vanillin	0.930	0.910	0.910	0.880	0.880	0.890	0.860	0.840
Syringaldehyde	1.020	1.030	1.020	0.960	0.950	0.960	0.920	0.920
p-Coumaric acid	T[a]	T	T	0.010	0.011	T	T	T
Ferulic acid	0.012	0.012	0.011	0.010	T	T	T	T
Total	2.23	2.21	2.20	2.09	2.06	2.07	2.00	1.98
Lignin content (%)	3.66	3.62	3.61	3.43	3.38	3.39	3.28	3.25

Note: Obtained by extraction with 2% H_2O_2–0.2% TAED at pH 11.8 for 12 h at 48°C from 0.5-M KOH treated (35°C, 2.5 h) wheat straw under different ultrasonic times.
[a]Trace.

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Table 5. Intrinsic viscosity (η), the viscosity average DP (P), and molecular weight (M_w) of the isolated crude cellulosic preparations obtained by extraction with 2% H_2O_2–0.2% TAED at pH 11.8 for 12 h at 48°C from 0.5-M KOH treated (35°C, 2.5 h) wheat straw under different ultrasonic times.

	Ultrasonic time (min)							
	0	5	10	15	20	25	30	35
Intrinsic viscosity (η, ml/g)[a]	484.2	475.1	472.2	471.6	470.0	469.2	468.1	465.3
Viscosity average DP (P)[b]	1666.4	1631.7	1620.6	1618.3	1612.3	1609.3	1605.1	1594.3
Molecular weight (M_w)[c]	269,960	264,340	262,540	262,160	261,190	260,710	260,030	258,280

[a]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).
[b]Calculated by $P^{0.90} = 1.65\ [\eta]$, P represents the viscosity average DP (degree of polymerization).
[c]Calculated by [DP] × 162.

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Changes in the viscosity average degree of polymerization (P) and molecular weight (M_w) of crude cellulose obtained under different ultrasonic irradiation times were observed by viscosity measurements, and the results are listed in Table 5. Similarly, as a result of ultrasonic irradiation, P and M_w of crude cellulose decreased from 1666.4 and 269,960 to 1594.3 mL g^{-1} and 258,280 g mol^{-1}, respectively, with an increase in ultrasonic treatment time from 0 to 35 min.

Gel permeation chromatography (GPC) is a technique that can provide detailed information on both the molecular size and the polydispersity of the high and low molecular weight components in a sample, separately. For purified cellulose, this will make it possible to obtain detailed information concerning degrading reactions during the treatment with 80% acetic acid–70% nitric acid at 120°C for 15 min. The problem of using GPC for cellulose analysis has been to find a nondegrading solvent system for the cellulose that is compatible with the chromatographic resins.[25] *N,N*-Dimethylacetamide (DMAc) in combination with lithium chloride in varying concentrations represents a solvent system that is very common in cellulose chemistry.[26] Table 6 summarizes the weight-average ($\bar{M}_w$) and number-average ($\bar{M}_n$) molecular weights and polydispersity ($\bar{M}_w/\bar{M}_n$) of eight purified cellulosic preparations. Obviously, the eight purified cellulosic preparations showed no significant difference in their $\bar{M}_w$ values, which ranged from 42,300 to 44,650 g mol^{-1}, but gave much lower $\bar{M}_w$ values than those of the corresponding crude cellulose samples. This indicated that treatment of the crude cellulose with 80% acetic acid–70% nitric acid (10/1, v/v) at 120°C for 15 min did degrade the macromolecular structure of cellulose to a noticeable extent except for removal of residual lignin and hemicelluloses. In addition, the eight purified cellulosic polymers exhibited a fairly analogous elution pattern, as shown by a similar value of $\bar{M}_w/\bar{M}_n$ ratios between 1.48 and 1.56.

Table 6. Weight-average ($\bar{M}_w$) and number-average ($\bar{M}_n$) molecular weights and polydispersity ($\bar{M}_w/\bar{M}_n$) of purified cellulosic preparations obtained by treatment with 80% acetic acid–70% nitric acid (10/1, v/v) at 120°C for 15 min from 0.5-M KOH treated (35°C, 2.5 h, under different ultrasonic times) and sequentially 2% H_2O_2–0.2% TAED extracted (pH 11.8, 12 h, 48°C) wheat straw.

	Ultrasonic time (min)							
	0	5	10	15	20	25	30	35
$\bar{M}_w$	44,650	43,820	43,570	43,380	43,180	43,250	42,800	42,300
$\bar{M}_n$	28,620	28,830	28,660	28,920	29,180	29,030	28,530	28,010
$\bar{M}_w/\bar{M}_n$	1.56	1.52	1.52	1.50	1.48	1.49	1.50	1.51

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FT-IR Spectra

The FT-IR spectra of crude cellulosic preparations obtained by extraction with 2% H_2O_2–0.2% TAED at pH 11.8 for 12 h at 48°C from 0.5-M KOH treated (35°C, 2.5 h) wheat straw without ultrasonic assistance (spectrum 1) and with ultrasonic assistance for 25 (spectrum 2), and 35 min (spectrum 3), are illustrated in Fig. 2. Clearly, this similar feature of the spectra verified that ultrasonic irradiation under the conditions used had little effect on the structure of cellulose. Figure 3 illustrates three FT-IR spectra of purified cellulosic preparations obtained by extraction with 80% acetic acid–70% nitric acid (10/1, v/v) at 120°C for 15 min from 0.5-M KOH treated (35°C, 2.5 h) without ultrasonic assistance (spectrum 1) and with ultrasonic assistance for 25 (spectrum 2), and 35 min (spectrum 3) and sequentially 2% H_2O_2–0.2% TAED extracted (pH 11.8, 12 h, 48°C) wheat straw. In comparison with the spectra of crude cellulose in Fig. 2, the spectra of purified cellulose show two important ester bands at 1739 (C═O ester) and 1261 cm^{-1} (–C–O– stretching). These carbonyl groups are undoubtedly due to the acetylation of hydroxyl groups in cellulose during the purifying crude cellulose with 80% acetic acid–70% nitric acid, which does not agree with the findings by

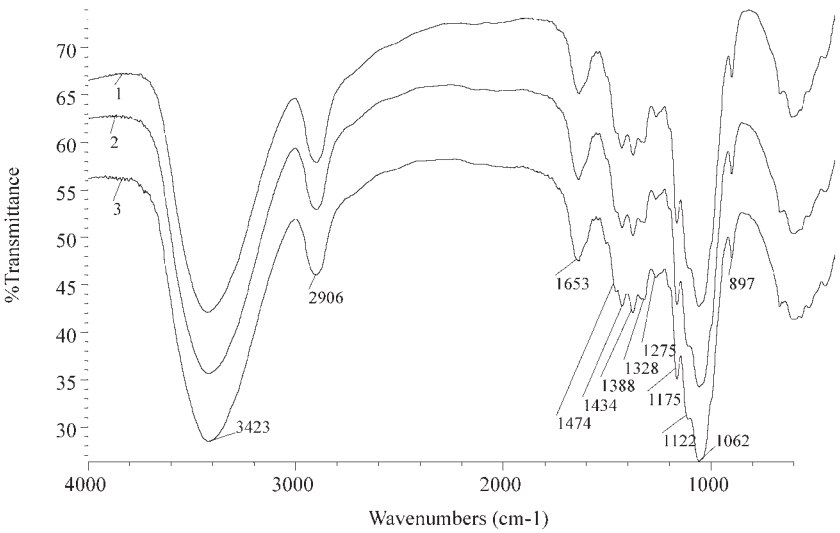

Figure 2. FT-IR spectra of crude cellulosic preparations obtained by extraction with 2% H_2O_2–0.2% TAED at pH 11.8 for 12 h at 48°C from 0.5-M KOH treated (35°C, 2.5 h) wheat straw without ultrasonic assistance (spectrum 1) and with ultrasonic assistance for 25 (spectrum 2) and 35 min (spectrum 3).

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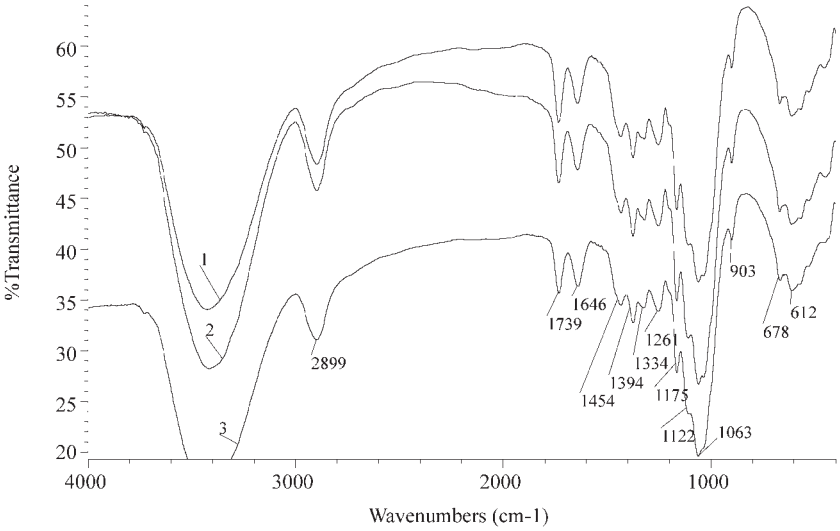

Figure 3. FT-IR spectra of purified cellulosic preparations obtained by extraction with 80% acetic acid–70% nitric acid (10/1, v/v) at 120°C for 15 min from 0.5-M KOH treated (35°C, 2.5 h) without ultrasonic assistance (spectrum 1) and with ultrasonic assistance for 25 (spectrum 2) and 35 min (spectrum 3) and sequentially 2% H_2O_2–0.2% TAED extracted (pH 11.8, 12 h, 48°C) wheat straw.

Brendel et al.[11] The investigators reported that the acetyl esters would be derived from the cell wall hemicelluloses. The lack of peak for the carboxylic group at 1700 cm^{-1} indicated that the purified cellulose is free of the unreacted acetic acid. In particular, there is no evidence of increased acid or aldehyde absorbances at 1660 to 1600 cm^{-1}, the presence of which would indicate oxidative degradation—a possible consequence of exposure to aqueous nitrogenous oxidants.[11]

CP/MAS ^{13}C-NMR Spectra

To confirm the structural changes between crude cellulose and purified cellulose, the crude cellulosic fraction obtained by extraction with 2% H_2O_2–0.2% TAED at pH 11.8 for 12 h at 48°C from 0.5-M KOH treated (35°C, 2.5 h) wheat straw with ultrasonic assistance for 25 min, and the purified cellulosic fraction obtained by extraction with 80% acetic acid–70% nitric acid (10/1, v/v) at 120°C for 15 min from the corresponding crude cellulosic preparation were analyzed by CP/MAS ^{13}C-NMR spectroscopy and their spectra are shown

in Fig. 4. According to the literature, the resonances of carbon-13 of cellulose appear at 106.4 ppm for the C-1, at 89.9 and 84.2 ppm for C-4, and at 66.4 and 64.3 ppm for the C-6. The resonances of C-2, C-3, and C-5 overlap each other and occur in the 70 to 80 ppm region.[27] A near disappearance of a broader peak, centered on 102 ppm, attributed to C-1 in disordered hemicelluloses,

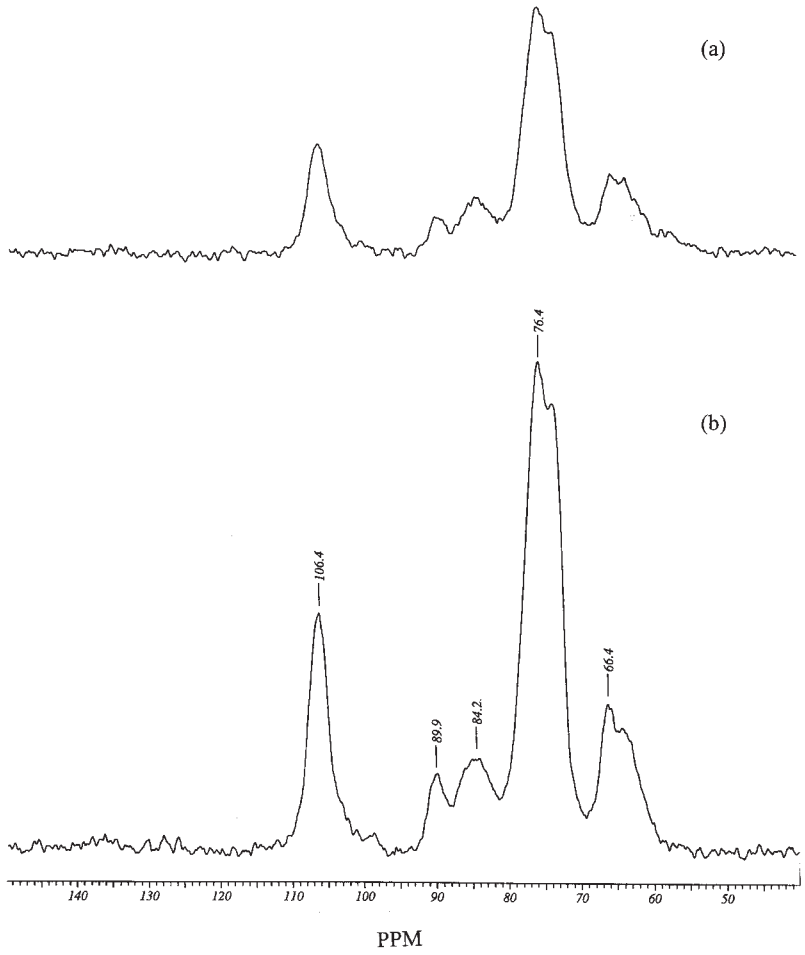

Figure 4. CP/MAS ^{13}C-NMR spectra of crude cellulosic fraction (a) obtained by extraction with 2% H_2O_2–0.2% TAED at pH 11.8 for 12 h at 48°C from 0.5-M KOH treated (35°C, 2.5 h) wheat straw with ultrasonic assistance for 25 min and purified cellulosic fraction (b) obtained by extraction with 80% acetic acid–70% nitric acid (10/1, v/v) at 120°C for 15 min from the corresponding crude cellulosic preparation.

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implied that both crude and purified cellulosic preparation contained only small or minor amounts of bound hemicelluloses.[28] In addition, both spectra show signals for C-4 in crystalline cellulose (89.9 ppm) and on disordered cellulose (84.2 ppm). A similar phenomenon can be seen in signals to C-6 in crystalline cellulose (66.4 ppm) and on amorphous cellulose (64.3 ppm).

Thermal Analysis

Both crude and purified cellulosic preparations were further subjected to derivative thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) in an N_2 atmosphere to determine their thermal nature. The thermal decomposing pattern of the crude cellulosic fraction (a) obtained by extraction with 2% H_2O_2–0.2% TAED at pH 11.8 for 12 h at 48°C from 0.5-M KOH treated (35°C, 2.5 h) wheat straw with ultrasonic assistance for 25 min, and the purified cellulosic fraction (b) obtained by extracted with 80% acetic acid–70% nitric acid (10/1, v/v) at 120°C for 15 min from the corresponding crude cellulosic preparation gave additional evidence to the relatively higher stability of the purified cellulosic preparation (Fig. 5). As illustrated in the figure, the TGA curves of both crude cellulose and purified cellulose started to decompose at 214°C [see Fig. 5(a)] and 276°C [see Fig. 5(b)], respectively. At 10% weight loss, the decomposition temperature of the crude cellulose and the purified cellulose occurred at 257 and 298°C, respectively. Similarly, at 50% weight loss, the decomposition temperature was observed at 312°C for crude cellulose and 330°C for purified cellulose. This revealed that the thermal stability of the cellulose increased with its purity. In other words, the purified cellulose had a higher thermal stability than the crude cellulosic sample. In addition, the DSC thermogram of the crude cellulosic sample gave two big exothermic peaks at 305 and 442°C. However, the purified cellulosic preparation exhibited only a small exothermic peak, and it shifted from 305°C in the crude cellulosic sample to 343°C in the purified cellulosic preparation, indicating again that the thermal stability of the purified cellulose increased. The reason for this relatively higher thermal stability of purified cellulose is probably due to the substantial removal of less stable hemicellulosic polymers from the crude cellulose during the treatment with 80% acetic acid–70% nitric acid under the conditions given.

CONCLUSION

In short, the two-stage separation processes of dewaxed wheat straw with 0.5-M aqueous KOH at 35°C for 2.5 h under ultrasonic irradiation for 0 to

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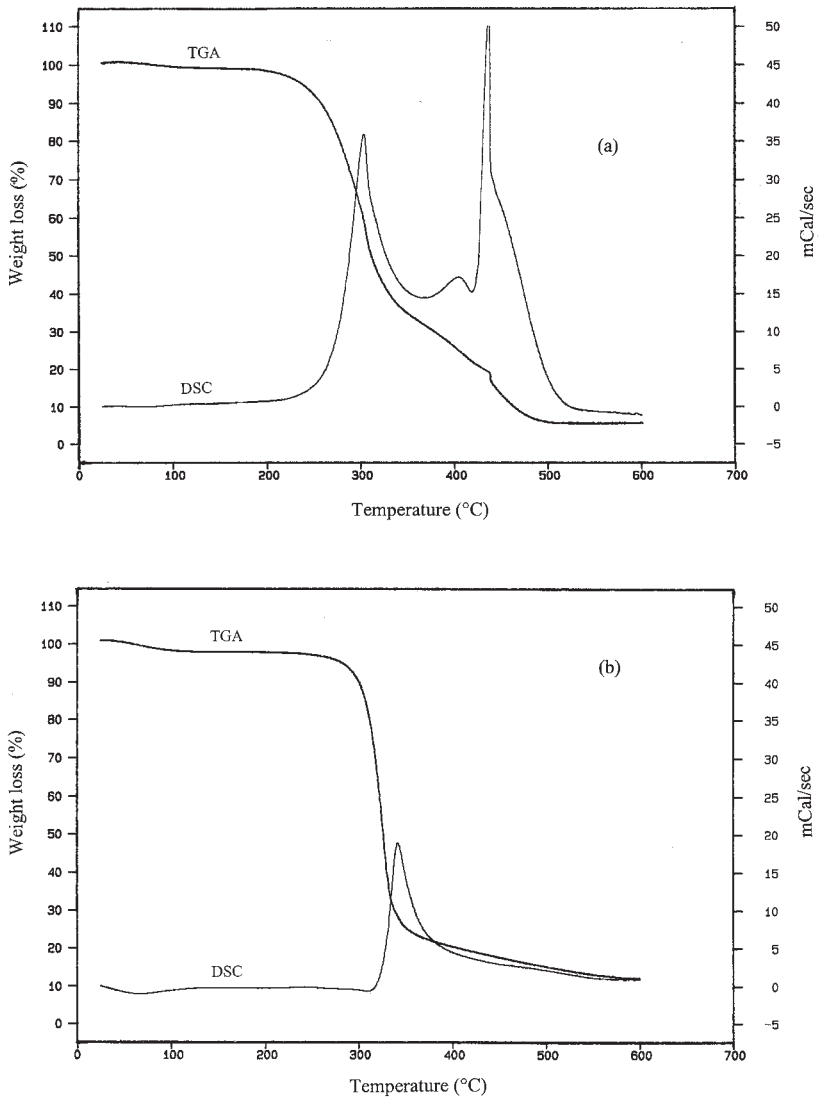

Figure 5. TGA/DSC curves of crude cellulosic fraction (a) obtained by extraction with 2% H_2O_2–0.2% TAED at pH 11.8 for 12 h at 48°C from 0.5-M KOH treated (35°C, 2.5 h) wheat straw with ultrasonic assistance for 25 min and purified cellulosic fraction (b) obtained by extracted with 80%acetic acid–70% nitric acid (10/1, v/v) at 120°C for 15 min from the corresponding crude cellulosic preparation.

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35 min and sequential with 2% H_2O_2–0.2% TAED at pH 11.8 for 12 h at 48°C, together solubilized 90.6 to 91.6% of the original hemicelluloses and 89.9 to 91.4% of the original lignin. This sequential separation process yielded 45.3 to 46.9% crude cellulose, which contained 7.3 to 7.9% residual hemicelluloses and 3.3 to 3.7% residual lignin. Further separation of the residual hemicelluloses and lignin from the corresponding crude cellulose with 80% acetic acid–70% nitric acid (10/1, v/v) at 120°C for 15 min resulted in 3.4 to 6.2% of the original cellulose degradation, with a yield of 36.4 to 37.5% purified cellulose. The purified cellulose is relatively free of bound lignin (0.1 to 0.2%) and contained minor amounts of associated hemicelluloses (~3%). More importantly, while negating the risk of toxic chlorine dioxide gas inherent in the use of chlorite for delignification, the quantity of nitric acid used is minimal and the waste composition (approximately 53% ethanol, 41% water, 5.3% acetic acid, and 0.7% nitric acid) should reduce disposal problems. The technique described here, therefore, may be considered an improvement upon other published methods for separation of cellulose from wood or other plant materials.

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