Characteristics of Cellulose Isolated by A Totally Chlorine-Free Method from *Caragana korshinskii*

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ABSTRACT: The chemical composition and fiber morphology of *Caragana korshinskii* were investigated in this study. Isolation of cellulose was performed in a nonsulfur acetic acid/nitric acid system under various conditions. The influence of three factors, i.e., nitric acid concentration (0, 2, 4, 6, 8, or 10%), temperature (95, 100, 110, 115, 120, or 130°C), and reaction time (30, 40, 50, 60, or 90 min) on the cellulose properties (viscosity, yield, and molecular weight) was studied. The cellulose isolated was characterized by using Fourier transform infrared, gas chromatography, high performance liquid chromatography, solid-state cross-polarization magic angle spinning carbon-13 nuclear magnetic resonance, wide-angle X-ray diffraction, and thermogravimetric analysis/differential scanning calorimetry techniques. The results showed that the treatment using 80% acetic acid and nitric acid as a catalyst under the given conditions resulted in slight acetylation of the cellulose and increased the degree of crystallinity of cellulose except for significant degradation of lignin and hemicellulosic polymers. The thermal stability of the cellulose declined with an increase in nitric acid concentration. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 101: 3251–3263, 2006

Key words: *Caragana korshinskii*; cellulose; acetic acid; nitric acid; characteristics

INTRODUCTION

Over the years, an increasing preoccupation regarding rational use of abundant and renewable forest and agricultural residue has occurred. These residues, such as shrub, essentially consisted of three different polymer entities (cellulose, hemicelluloses, and lignin). About 40–50% of the dry residue is the glucose polymer cellulose, much of which is in a crystalline structure. Another 25–35% is hemicelluloses, an amorphous polymer usually composed of xylose, arabinose, galactose, glucose, and mannose. The reminder is mostly lignin and lesser amounts of minerals, waxes, and other compounds.[1] *Caragana korshinskii*, a relatively new and potential wood fiber source planted in the desert region in China to prevent wind erosion and control desertification, has a considerable economical and ecological importance. This shrub generates a large amount of residues, because its stems are cut once every 3 years to make it flourish. At present, only small amount of the stubble is used for the production of fiberboard, with the remainder being underutilized.[2] Usually, these residues are burnt as firewood, which causes serious environmental pollution. As we believe, these residues could and should find a rational way of utilization, namely, as a source of cellulosic material to help offset the growing shortage of wood resources in the pulp and paper production in China.

The large worldwide market for pulp or cellulose is dominated by that which is produced by means of the kraft process, which represents more than half of the total production and almost two-thirds of the chemical pulp produced. However, the current challenges to pulp and paper industry are increased shortages of capital, energy, chemicals, wood fiber, and water. The kraft process shows limited flexibility regarding modifications to meet the requirements of future situations. For these reasons alternate pulping methods are continually under

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investigation. One of the alternative processes currently being developed is delignification with organic solvents. Acetic acid has proved to be an effective lignin solvent for both wood and nonwood. The use of acetic acid in pulping has been investigated in HCl-catalyzed media (Acetosolv process), formic acid-catalyzed media (Formacell process), and uncatalyzed media (Acetocell process).[3–5] The reports on the delignification of rice straw in an acetic acid/water medium using H_2SO_4 as catalyst have been published.[6,7] This pulping method, in comparison with the more traditional methods, results in satisfactory mechanical characteristics of the pulp as well as the retention of a large part (75%) of the silicon derivatives in the unbleached pulp. Previous studies reported on *Eucalyptus globules* wood processing with hydrochloric acid-catalyzed acetic acid solutions also showed that both extensive and selective delignification could be achieved under a variety of experimental conditions. However, the pulp produced is not white and it needs further bleaching by totally chlorine-free chemicals.[8] In acetic acid and water medium, nitric acid is used as a catalyst to increase the efficiency of wood deligninfication and bleaching processes at a relatively high concentration (8.5%).[9]

In this work, the simultaneous delignification and removal of noncellulose polysaccharides of *C. korshinskii* in an acetic acid–nitric acid mixture were mainly carried out. The nitric acid concentration, reaction temperature, and time were chosen as the reaction parameters, other less important factors such as liquor-to-wood ratio (7 : 1) and time to maximum temperature being held constant. The influence of reaction parameters on cellulose yield, intrinsic viscosity, molecular weight, contents of residual hemicelluloses, and lignin was investigated. The cellulose isolated was also characterized by Fourier transform infrared (FTIR) and solid-state cross-polarization magic angle spinning carbon-13 nuclear magnetic resonance (CP/MAS ^{13}C NMR) spectroscopy, gas chromatography, alkaline nitrobenzene oxidation, wide-angle X-ray diffraction, thermogravimetric analysis, and differential scanning calorimetry.

EXPERIMENTAL

Materials

Stems of *C. korshinskii* were supplied by Shalin arboretum in Yikezhao League of Inner Mongolia, China. It was harvested in October 2002, with an average stem height of 3.5 m. The leaves and the capitula were removed and only the stalks were collected. To evaluate the fiber morphology on paper behavior, a number of stems were cut into $2.5 \times 2.5 \times 30\text{-mm}^3$ strips manually. For the isolation experiments, all material was disintegrated manually up to the approximate size of a match stick ($15 \times 1 \times 1\ \text{mm}^3$). For chemical analysis, a small portion of the chips was ground and screened to obtain a uniform particle size, which was kept on a 0.30-mm screen and passed through a 0.45-mm screen.

Chemical analysis

The analysis of the chemical compositions, including cellulose, hemicelluloses, lignin, extractives, and ash, were performed on debarked material according to the Chinese standard methods in the papermaking industry (GB/T 2677).

Light microscopy

The samples were first treated with acetic acid and 30% hydrogen peroxide (1 : 1, v/v) at 60°C for 24 h to effect cell dissociation. When the samples turned to white color, the separated fiber was taken off from the reactor and thoroughly washed with water. Then, the dissociated fiber was stained with Herzz reagent and mounted on the microscope slides. The fiber morphological characteristics were observed and measured (fiber length) using Nikon E200 light microscopy (LM) at different magnifications.

Scanning electron microscopy

Blocks of *C. korshinskii* exposing the transverse section surface were prepared to determine the fiber width and cell wall thickness. Stems were first cut into 3-cm-small blocks longitudinally by using razor blades. After preparation, sections of 1–2 mm thickness were coated with a 10-nm layer of gold on a sputter coater. Both the fiber diameter and the wall thickness were measured from scanning electron microscopy images, using the UTHSCSA image tool program.

Isolation of cellulose by an acetic acid and nitric acid mixture

An experimental design was employed, using nitric acid concentration, isolating temperature and time as the reaction parameters. Sample (100 g) was weighed into a 1000-mL stainless steel reactor. Subsequently, 700 mL 80% (w/w) aqueous acetic acid (AA) containing 0, 2, 4, 6, 8, or 10% nitric acid (w/w) was added. The reactor was sealed and placed into an oil bath set at the required temperature (95, 100, 110, 115, 120, or 130°C). Following the reaction for the required time (30, 40, 50, 60, or 90 min), the stainless steel reactor was removed from the oil bath. Once cooled, distilled water was added, and the reagent was decanted off. The residue was then thoroughly washed with distilled water and 95% ethanol to remove the nitric acid and extraction breakdown products. Sample was then dried in an oven at 60°C for 16 h prior to reweighing. The oven-dry sample was weighed to determine the

weight losses by degradation of lignin and hemicellulosic polymers or yield of cellulose on the basis of initial oven-dry measurements.

Characteristics of cellulose

Viscosity of the pulps was determined by Chinese Standard Methods for determination of limiting viscosity number of cellulose in dilute solutions (cupri-ethylene-diamine (CED), method, Chinese standard/GB1548–89). The viscosity average DP (degree of polymerization) of the cellulose samples was estimated from their intrinsic viscosity [η] in cupri-ethylene-diamine hydroxide (cuene) solution, $P^{0.905} = 0.75$ [η]/mL g^{-1}, where P is an indeterminate average DP.[10] Molecular weight of the cellulose was then calculated from their P multiplying by 162, molecular weight of an anhydroglucose.

The neutral sugar composition of the cellulose isolated was determined by gas chromatography (GC) analysis of the corresponding alditol acetates. 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 neutralized with 0.32 mL 15*M* ammonia.[11] After reduction, the resulting alditols were acetylated for GC analysis, as described by Blakeney et al.[12] The GC column for the sample is a fused silica capillary column (12QC3/BPX5 0.25).

The method for the determination of phenolic acids and aldehydes in nitrobenzene oxidation mixtures of residual lignins associated in the cellulosic preparations with high performance liquid chromatography (HPLC) has been described in a previous paper.[13] The lignin oxidation products were analyzed by HPLC on a SPHERECLONE 5u ODS-2 column of 250 × 4.6 mm^2). Separations were obtained using a linear gradient of two solvents A (water–methanol–acetic acid, 89 : 10 : 1) and B (methanol–water–acetic acid, 90 : 9 : 1). A linear gradient was run over 31 min from 0 to 40% B at a flow rate of 1 mL min^{-1}. Products were detected at 280 and 320 nm. Peak areas (280 nm) were calculated relative to the internal standard using Kontron MT 450 software. Calibration curves were established from appropriate mixtures of authentic phenolic acids and aldehydes.

The Fourier transform infrared (FTIR) spectra of the raw material and cellulose were recorded from KBr pellets containing 1% finely ground samples on a Nicolet-510 FTIR spectrophotometer (Warwick, England). Solid-state cross polarization magic angle spinning carbon-13 nuclear magnetic resonance (CP/MAS ^{13}C NMR) spectra were recorded using a Bruker DRX-400 spectrometer at 25°C and 100 MHz with 5 mm MAS BBO probe. About 250 mg of sample was packed into zirconia rotors for MAS. The delay time was 2 s, the special width 30,303 Hz, the proton 90° pulse time 4.85 μs, acquisition time 0.034 s, and total acquisition time 3 h.

Wide-angle X-ray diffraction (WAXRD) measurements of the raw material and cellulose crystallinity were performed with Ni-filtered Cu Kα_1 radiation (λ = 0.154 nm) from a Rigaku (Japan) D/max-1200 diffractometer equipped with a scintillation counter and a linear amplifier. The operating voltage and current were 40 kV and 30 mA, respectively. The scattering angle range was from 6° to 40° with the step angle 0.1°. The step time measurement was 3 s. The sealed Cu anode X-ray tube (line focus) was powered by a generator. Degree of crystallinity (K_c) was computed by the following equation:[14]

$$Kc = \frac{\int_0^\infty S^2 I_c(s)ds}{\int_0^\infty S^2 I(s)ds} \quad (1)$$

where S is the magnitude of the reciprocal lattice vector and is given by

$$S = \frac{(2\sin\theta)}{\lambda} \quad (2)$$

where θ is one half the angle of deviation of diffracted rays from the incident X-rays, λ is the X-ray wave length, I(S) is the intensity of coherent X-ray scatter from a specimen (both crystalline and amorphous), and I_c(S) is the intensity of coherent X-ray scatter from the crystalline region. The crystallite size was calculated according to the Scherrer equation[15]

$$L = \frac{K\lambda}{\beta\cos\theta} \quad (3)$$

where λ is the X-ray wave length, θ is half the peaks of diffraction angle, L is crystallite size, β is the breadth of the pure diffraction profile on the 2θ scale in radians, and K is a constant ($\approx$0.89). Crystallite size was calculated along the (101), (10$\bar{1}$), and (002) plane directions.

The thermal analysis of the pulp samples was performed using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) on a simultaneous thermal analyzer (SDT Q600). 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}.

RESULTS AND DISCUSSION

Chemical composition

The chemical composition of *C. korshinskii* is shown in Table I. The marked concern is the high amount of

TABLE I
The Chemical Compositions (%) of *Caragana korshinskii* Compared with China White Poplar, Wheat Straw, and Red Pine

		Extractives					
	Ash	Hot water	1% NaOH	Benzene–ethanol	Cellulose	Hemicelluloses	Lignin
Caragana korshinskii	0.66	5.12	19.47	5.89	45.12[a]	23.22	18.26
China white poplar	0.32	n/a	15.61	4.45	43.24[a]	22.61	17.1
Wheat straw	6.44	n/a	43.64	5.15	41.30[a]	22.85	18.03
Red pine	0.42	4.15	17.55	n/a	53.12[b]	10.46	27.69

n/a, not available.
[a] Nitric acid–ethanol cellulose.
[b] Cross and Bevan cellulose.

cellulose and hemicelluloses. The results of the chemical analysis show that the cellulose content (47.74%) of *C. korshinskii* is higher than that of both the China white poplar (43.24%) and wheat straw (41.30%), but lower than that of the red pine (53.12%). The values found for cellulose were also higher than those obtained for other hardwood like fast-growing poplar (43.80%),[16] a new raw material for pulping in China today. The hemicelluloses content (23.22%) of *C. korshinskii* is very similar to both wheat straw (22.85%) and China white poplar (22.61%), but much higher than red pine (10.46%). It is clear that the hemicelluloses content matches those found in hardwood (19–25%). Furthermore, the ratio of hemicelluloses to cellulose obtained during this study for *C. korshinskii* is about 1 : 2, which is common to other vegetal species. This ratio is an important parameter if one considers the capital role, since hemicelluloses play as a part of material in papermaking.[17] The lignin content (18.26%) of *C. korshinskii* is comparable to that of China white poplar (17.10%) and wheat straw (18.03%), but considerably lower than that of red pine (27.69%). The cellulose-to-lignin ratio of *C. korshinskii* is about 2.5. Ash content (0.66%) of *C. korshinskii* was slightly higher than that of both China white poplar (0.32%) and red pine (0.42%), but much lower than wheat straw (6.44%). Lower lignin and ash content meant normal alkali consumption and fewer problems at spent liquor recovery. There is also a difference in the extractives content of the compared materials. The quantity of extractives in benzene–ethanol and water was relatively high. The content of benzene–ethanol extraction (5.89%) is the highest in all of these raw materials under investigation. Hot water extraction of *C. korshinskii* is 5.12%, being higher than that of red pine. 1% NaOH extraction (19.47%) is higher in comparison with that of China white poplar (15.61%) and red pine (17.55%), but much lower than wheat straw (43.64%). Overall the chemical analysis data showed that *C. korshinskii* is suitable for preparing cellulose or pulp manufacture, mainly because of its relatively low lignin and high cellulose and hemicelluloses content.

Fiber morphology

The fibers of *C. korshinskii* make up 69.8% of its total cells. As observed under light microscopy, several cell types could be distinguished (Fig. 1). In addition to the fibers, the other cell types that were evident include axial parenchyma cells, ray parenchyma cells, and vessel elements. The vessel elements are usually long, thin-walled and with or without pitting, and with open ends. The parenchyma cells are abundant and fairly uniform when compared with the common

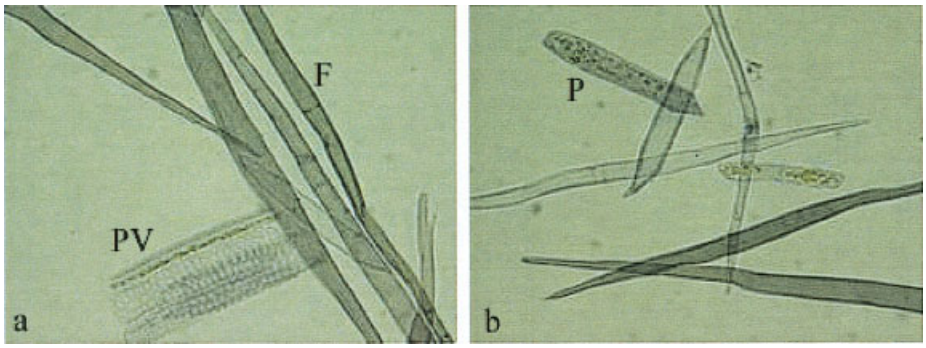

Figure 1 Light micrographs of *C. korshinskii* fibers (a and b, LM ×400). The different cell types identified are F, fiber; PV, pitted vessel element; P, parenchyma. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE II
The Fiber Morphological Characteristics of *Caragana korshinski* Compared with China White Poplar, Wheat Straw, and Red Pine

Species	Length (mm)		Width (μm)		Length to width ratio	Wall thickness (μm) W	Runkle ratio ($2W/d$)
	mean	range	mean	range			
Caragana korshinskii	0.53	0.38–0.65	8.9	6.2–12.7	60	1.7	0.30
China white poplar	1.18	n/a	21.0	n/a	56	2.4	0.37
Wheat straw	1.32	1.03–1.60	12.9	9.3–15.7	102	5.2	4.16
Red pine	3.62	2.45–4.10	54.3	39.2–3.8	67	3.9	0.37

n/a, not available

hardwood, and similar in dimensions with most of them being claviform shaped.

It is well known that wood samples with longer fiber length, higher flexibility coefficient (length-to-width ratio >33), and/or lower-wall-to-lumen ratio (<1) are suitable for isolating cellulose, pulping, or papermaking. As can be seen from Table II, *C. korshinskii* retains short-length fiber, and the mean fiber length is 0.53 mm. The fiber cell wall (1.7 μm) is thinner than that of China white poplar (2.4 μm), wheat straw (5.2 μm), and red pine (3.9 μm). Although the flexibility coefficient (60) is lower than that of wheat straw (102) and red pine (67), it is enough for preparing cellulose for industrial use. However, Runkle ratio (wall-to-lumen ratio, 0.30) is similar to that of China white poplar (0.37) and red pine (0.37), but significantly lower than that of wheat straw (4.16). The lower Runkle ratio indicated that the fiber of *C. korshinskii* should have the ability to collapse easily and form good fiber-to-fiber bonding, which is important for paper strength. From the data above we can deduce that the morphological indices of *C. korshinskii* are adequate for preparing cellulose or pulping, although it belongs to short-length fiber.

Yield, intrinsic viscosity (η), the viscosity average DP (P), and molecular weight of the cellulose

In acetic acid and nitric acid system, it seems that the structure of an oxidation product depends on the condition of the reaction (especially the temperature and the nitric acid concentration).[18] At the initial stage of the carbon oxidation, the oxidizing agent is a nitric acid solution (HNO_3, $(OH)_2NO^+$, NO_2^+, NO_3^-, H_3O^+), which during reduction, forms, among others, nitrogen oxides.[19] It was reported that the reactions of nitric acid with biomass can be largely limited to lignin, and the damage to cellulose can be minimized.[20]

As shown in Table III, during the treatment of *C. korshinskii* at 110°C with 80% AA containing 8.0% nitric acid as a catalyst, the yield of cellulose decreased from 50.41 to 50.32, 49.08, 49.00, and 38.70% with an increase in reaction time from 30 to 40, 50, 60, and 90 min, respectively. The reason for this decrement in the yield of cellulose from 50.41 to 38.70% by prolonging the reaction time from 30 to 90 min, with 8.0% nitric acid as a catalyst, is a direct consequence of the favorable effect of time on diffusion and adsorption of the reactants between the acetic acid–nitric acid and *C. korshinskii* lignin and/or hemicelluloses.

The effect of nitric acid concentration (% AA, w/w) on the rate of degradation of *C. korshinskii* polymers was investigated with concentrations between 0.0 and 10.0% at 110°C for 60 min. In comparison with a control sample 6, it is apparent that nitric acid does accelerate the rate of degradation. Use of 2 and 4% nitric acid as a catalyst led to a 17.05 and 35.20% *C. korshinskii* degradation (sample 7 and 8), mainly lignin and hemicelluloses. It is very likely that an increase in nitric acid concentration from 6.0 to 8.0% showed only a slight decrease in cellulose yield by 1.67%. Similarly, an increase of nitric acid concentration to 10.0% yielded cellulose with 46.75% (sample 11), which was slightly higher than the content of cellulose in *C. korshinskii* (45.12% dry matter). Such phenomena implied that treatment of *C. korshinskii* with a relatively higher concentration of nitric acid such as over 10% in 80% acetic acid at 110°C for 60 min and with 8.0% nitric acid at a higher temperature (over 110°C) also resulted in a slight degradation of cellulose from *C. korshinskii*. In other words, although the main aim of the treatment with a mixture of acetic acid–nitric acid is to degrade or oxidize lignin and hemicelluloses, cellulose also reacts with it. During the treatment with acetic acid–nitric acid medium, nitric acid is degraded into nitrogen dioxide, oxygen, and water at a relatively high temperature ($2HNO_3 \rightarrow 2NO_2 + O + H_2O$). In general, the various substitutions, side chain cleavage, and oxidation reactions are observed with nitric acid. In addition, free-radical-initiated reactions involving the addition of nitrogen dioxide to the aromatic ring in lignins, hydrogen abstraction from hemicelluloses, cellulose, and lignin, and electron transfer (mainly from phenolic groups) should also take place.[20]

In this study, the treatment of *C. korshinskii* in acetic acid–nitric acid media was also performed under different temperatures and various concentrations of ni-

TABLE III
The Yield (% Dry Matter), Intrinsic Viscosity (η), Viscosity Average DP (P), and Molecular Weight (Mw) of the Cellulose Preparations Obtained by Treatment of *Caragana korshinskii* with 80% Acetic Acid (AC) and Various Concentrations of Nitric Acid (NC) at a Liquid to Solid Ratio of 7 : 1 (mL/g) under Different Conditions

Reaction conditions			Cellulose preparations				
Temperature (°C)	Reaction time (min)	% NC in 80% AC (w/w)	Sample no.	Yield (%)	η (mL/g)[a]	P[b]	Mw[c]
110	30	8.0	1	50.41	354.2	477.3	77,322
110	40	8.0	2	50.32	345.5	464.4	75,232
110	50	8.0	3	49.08	336.0	450.3	72,949
110	60	8.0	4	49.00	335.7	449.8	72,387
110	90	8.0	5	38.70	315.6	420.2	68,067
110	60	0.0	6	92.43	517.9	726.3	117,660
110	60	2.0	7	83.95	421.8	578.9	93,782
110	60	4.0	8	64.80	386.3	525.3	85,102
110	60	6.0	9	50.67	353.7	476.6	77,203
110	60	8.0	10	49.00	335.7	449.8	72,387
110	60	10.0	11	46.75	306.4	406.7	65,878
95	60	8.0	12	51.60	388.8	529.1	85,711
100	60	8.0	13	51.40	386.0	524.9	85,033
110	60	8.0	14	49.00	335.7	449.8	72,387
115	60	8.0	15	49.20	323.0	431.1	69,833
120	60	8.0	16	44.51	320.4	427.2	69,212
130	60	8.0	17	43.70	315.0	419.3	67,927

[a] Determined by Chinese Standard Methods for determination of limiting viscosity number of cellulose in dilute solutions.
[b] Calculated by $P^{0.905} = 0.75$ [$\sqrt{}$[η], P represents the viscosity average DP (degree of polymerization).
[c] Calculated by $P \times 162$.

tric acid. As the data shown in Table III, an increase of treatment temperature from 95 to 100, 110, 115, 120, and 130°C resulted in a decrement of cellulose yield from 51.60% (sample 12) to 51.40% (sample 13), 49.00% (sample 14), 49.20% (sample 15), 44.51% (sample 16), and 43.70% (sample 17), respectively. This indicated that the *C. korshinskii* fiber's ultrastructure is also another important factor that affects the rate of degradation of the polymers in the cell walls of *C. korshinskii*, particularly lignin and hemicelluloses, since the acetic acid and nitric acid molecules have to diffuse through the fiber matrix to reach the polymers. The diffusion is, therefore, a dominant factor to affect the treatment kinetics. During the organosolv treatment process, the fiber swells as the treatment proceeds, requiring disruption of the hydrogen bonds network. In general, increasing temperature favored breaking such hydrogen bonds, swelling the fiber, diffusing the degrading agent, and moving the degraded molecules, thus enhancing the degradation rate.

The viscosity average DP, P, of the cellulose is conveniently estimated from the intrinsic viscosity of its solution in cuene by application of the equation $P^{0.905} = 0.75[\eta]/\text{mL g}^{-1}$. In practice, cellulose solution intrinsic viscosity is often estimated from a single viscosity measurement.[10] Molecular weight of the cellulose was then estimated multiplying by 162, a molar mass of anhydroglucose. Table III also gives the intrinsic viscosity [η], viscosity average DP (P), and molecular weight (Mw) of 17 cellulosic preparations. As can be seen, an increase in treatment time from 30 to 90 min at 110°C with a nitric acid concentration of 8.0%, in treatment temperature from 90 to 130°C with a nitric acid concentration of 8.0% for 60 min or in nitric acid concentration from 0.0 to 10.0% at 110°C for 60 min, led to a decrease in intrinsic viscosity from 517.9 to 421.8, 386.3, 353.7, 335.7, and 306.4 mL/g, and Mw from 117,660 to 93,782, 85,102, 77,203, 72,387, and 65,878 g mol^{-1}, respectively. This suggested that addition of nitric acid in the 80% acetic acid treatment also resulted in a degradation of the cellulose macromolecules at higher temperatures and relatively higher concentrations of nitric acid except for a substantial degradation of lignin and hemicellulosic polymers from *C. korshinskii*.

Contents of residual hemicelluloses and their neutral sugar composition

The neutral sugar composition of the isolated cellulose preparations was determined by GC analysis of the corresponding alditol acetates. The results are given in Table IV. GC analysis of the monosaccharides present in the liquors obtained in the quantitative acid hydrolysis of the cellulose samples showed that glucose is the predominant neutral sugar component, comprising 68.45–93.75% of the total neutral sugars. A small amount of xylose (4.87–11.02%) and minor proportions of arabinose (0.25–1.10%), mannose (0.25–0.61%), and galactose (0.55–0.97%) were also identi-

TABLE IV
Content of Residual Hemicelluloses and Their Neutral Sugar Composition (%) in Isolated Cellulose Samples

Sample no.	Temperature (°C)	NC in 80% AC (w/w)	Reaction time (min)	Arabinose	Xylose	Mannose	Galactose	Glucose
AC1	110	8	30	0.73	7.48	0.43	0.71	90.63
AC2	110	8	40	0.66	7.48	0.41	0.69	90.77
AC3	110	8	50	0.62	7.31	0.41	0.67	90.97
AC4	110	8	60	0.57	6.90	0.36	0.63	91.55
AC5	110	8	90	0.49	4.87	0.26	0.64	93.75
AC6	110	0	60	2.34	26.58	0.71	1.92	68.45
AC7	110	2	60	1.90	19.30	0.61	1.63	76.55
AC8	110	4	60	0.92	11.02	0.61	0.89	86.54
AC9	110	6	60	0.67	7.09	0.40	0.69	91.13
AC10	110	8	60	0.57	6.90	0.36	0.63	91.55
AC11	110	10	60	0.30	6.80	0.26	0.58	92.86
AC12	95	8	60	1.10	8.92	0.42	0.97	88.58
AC13	100	8	60	0.68	6.86	0.33	0.69	91.42
AC14	110	8	60	0.57	6.90	0.36	0.63	91.55
AC15	115	8	60	0.45	6.80	0.26	0.63	91.85
AC16	120	8	60	0.25	6.16	0.25	0.58	92.76
AC17	130	8	60	0.25	6.11	0.25	0.55	92.85

fied in 17 samples except for the sample 6 and 7, which contained 68.45 and 76.55% glucose, 26.58 and 19.30% xylose, 2.34 and 1.90% arabinose, 1.92 and 1.63% galactose, and 0.71 and 0.61% mannose, respectively. The current data revealed that the treatment of *C. korshinskii* with a mixture of acetic acid–nitric acid under the conditions given provides cellulose approaching a high purity, that is, the treatment with 80% acetic acid with nitric acid as a catalyst substantially degraded the hemicellulosic polymers from *C. korshinskii*. A slight increase in glucose with an increment in treatment time and temperature and nitric acid concentration corresponded to higher cellulose content and the lower residual hemicelluloses in cellulose samples, which reversed to the yield of cellulose.

Content of residual lignin and its phenolic composition

Table V lists the contents of associated lignin and its phenolic composition obtained by nitrobenzene oxidation of bound lignin in the isolated cellulose samples. As expected, a substantial degradation of lignin poly-

TABLE V
Content of Phenolic Composition of Residual Lignin in the Cellulosic Preparations

	Phenolic acids and aldehydes[a]								
Sample no.	HBA	HBAL	VA	VAN	SA	SYAL	AV	AS	Total
AC1	0.19	0.18	0.25	0.31	0.37	0.86	0.26	0.42	2.84
AC2	0.15	0.16	0.21	0.24	0.29	0.72	0.22	0.32	2.31
AC3	0.13	0.19	0.18	0.25	0.22	0.61	0.15	0.36	2.09
AC4	0.11	0.14	0.13	0.23	0.23	0.58	0.16	0.31	1.89
AC5	0.086	0.062	0.12	0.21	0.15	0.11	0.029	0.086	0.85
AC6	0.33	0.56	0.14	5.12	0.23	5.57	0.43	0.55	12.93
AC7	0.28	0.32	0.59	2.78	0.85	4.25	0.61	0.86	10.54
AC8	0.27	0.15	0.25	2.36	0.29	2.69	0.39	0.46	6.86
AC9	0.12	0.14	0.11	0.26	0.22	0.85	0.25	0.52	2.47
AC10	0.11	0.14	0.13	0.23	0.23	0.58	0.16	0.31	1.89
AC11	0.16	0.12	0.15	0.18	0.10	0.11	0.13	0.068	1.02
AC12	0.45	0.22	0.58	1.60	0.33	0.63	0.23	0.35	4.39
AC13	0.41	0.16	0.56	1.60	0.33	0.61	0.20	0.34	4.21
AC14	0.11	0.14	0.13	0.23	0.23	0.58	0.16	0.31	1.89
AC15	0.12	0.15	0.13	0.22	0.24	0.51	0.17	0.30	1.84
AC16	0.10	0.12	0.12	0.20	0.22	0.43	0.12	0.32	1.63
AC17	0.089	0.10	0.11	0.13	0.14	0.29	0.10	0.23	1.19

[a] HBA, HBAL, VA, VAN, SA, SYAL, AV, and AS represent *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, vanillic acid, vanillin, syringic acid, syringaldehyde, acetovanillone, and acetosyringone, respectively.

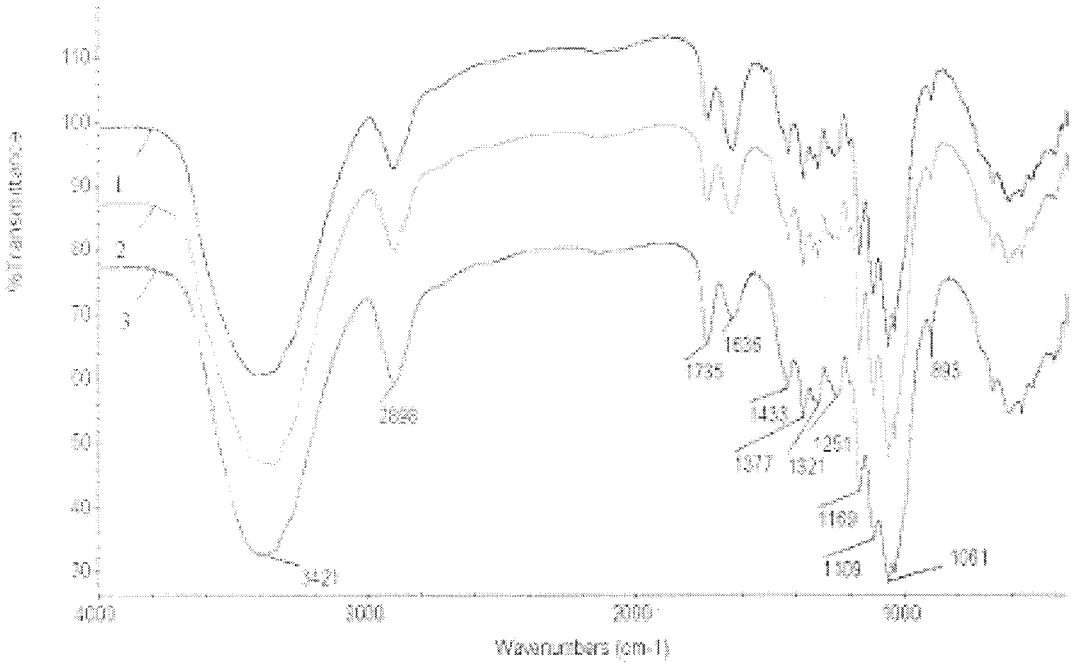

Figure 2 FTIR spectra of the cellulose samples obtained by treatment of *C. korshinskii* with 80% acetic acid and 8% nitric acid as a catalyst at 110°C for 30 (spectrum 1, sample 2), 60 (spectrum 2, sample 4), and 90 min (spectrum 3, sample 5).

mers also occurred during the treatment with a mixture of acetic acid–nitric acid under the conditions used as shown by minor amounts of total phenolics (0.85–6.86%) in the nitrobenzene oxidation mixture of the isolated cellulosic preparations except sample 6 and 7, indicating that the treatment with 80% acetic acid and 4–10% nitric acid as a catalyst is an effective technique for isolation of a large proportion of cellulose in a totally chlorine-free (TCF) process. Clearly, an increase in treatment time from 30 to 90 min and nitric acid concentration from 2 to 10.0% and temperature from 90 to 130°C resulted in a decrease in the associated lignin in cellulose samples as shown by the total phenolics from 2.84 to 0.85% (samples 1–5), 12.93 to 1.02% (samples 6–11), and 4.39 to 1.19% (samples 12–17), respectively. The major products were identified to be syringaldehyde, syringic acid, vanillin, and acetosyringone. A relatively small amount of *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, vanillic acid, and acetovanillone were also detected in the nitrobenzene oxidation mixture. Interestingly, relatively little of total phenolics obtained in the isolated cellulose samples illustrated that the lignin polymers were also significantly degraded during the treatment with acetic acid–nitric acid mixture.

In pulping and papermaking industry, the environmental risks associated with the traditional bleaching using elemental chlorine fostered the development of new bleaching sequences free from elemental chlorine or TCF.[21] In acetic acid–nitric acid treatment, wherein acidolysis and oxidation are the main mechanisms of lignin and hemicellulose degradation, good bleachability of cellulose is obtained. Considering the high degree of both lignin and hemicellulose degradation and removal achieved in an acetic acid–nitric acid mixture, this kind of process shows a remarkable potential for pure cellulose production. The isolated cellulose would denote the feedstock used in the manufacture of man-made fibers, cellophane, carboxymethylcellulose, sponges, plastics, photographic films, lacquers, and other cellulose derivatives.

FTIR spectra

The efficiency of the treatment procedure and the almost absent deleterious cellulose degradation were reflected in the FT-IR spectra of the samples. Figure 2 shows the FT-IR spectra of the cellulose samples obtained by treatment of *C. korshinskii* with 80% acetic acid and 8.0% nitric acid as a catalyst at 110°C for 30 (spectrum1, sample 2), 60 (spectrum 2, sample 4), and 90 min (spectrum 3, sample 5). Obviously, the treatment procedure removed all of the lignin-associated absorbances (1600, 1510, and to a lesser extent, 1440 cm^{-1}) and almost completely eliminated the noncellulosic polysaccharides associated absorbances (1210 cm^{-1}).[22] The absorption at 3421 cm^{-1} relates to stretching of —OH groups and the one at 2896 cm^{-1} to the C-H stretching. The band at 1636 cm^{-1} corresponds to the bending mode of the absorbed water.[23] Each spectrum presents a peak at 1433 cm^{-1}, which is attributed to the CH_2 bending, and one at 1377 cm^{-1}, to the O-H bending.[24] The absorbance at 1321 cm^{-1}

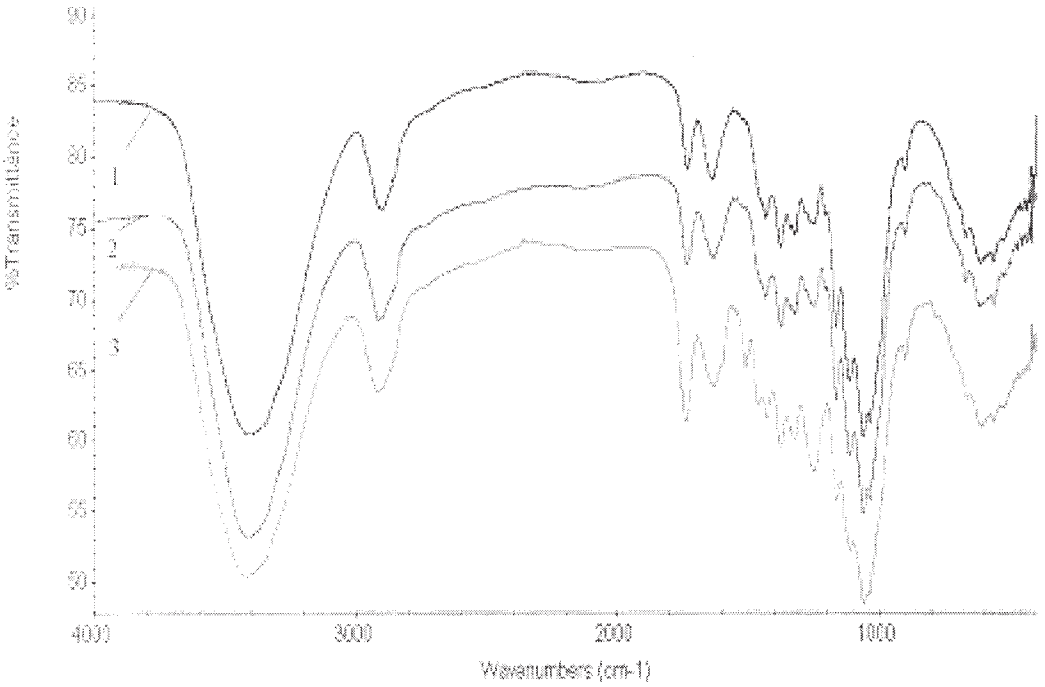

Figure 3 FTIR spectra of the cellulose samples obtained by treatment of *C. korshinskii* for 60 min at 110°C with 80% acetic acid and nitric acid concentration of 2% (spectrum 1, sample 7), 6% (spectrum 2, sample 9), and 10% (spectrum 3, sample 10).

arises from the C-C and C-O skeletal vibrations.[25] The peak at 1251 cm^{-1} is originated from the OH in-plane bending cellulose. The absorption band at 1169 cm^{-1} is due to C-O antisymmetric bridge stretching. A strong peak at 1061 cm^{-1} is attributed to C—O—C pyranose ring skeletal vibration. The sharp peak at 893 cm^{-1}, which represents the C_1 group frequency or ring frequency vibration, is characteristic of β-glycosidic linkages between the glucose units.[26] Interestingly, it is significant that there are no peaks unique to the spectra of the isolated cellulose, indicating that little or no cellulose degradation occurred. On the other hand, it should be noted that the treatment using 80% acetic acid and nitric acid as a catalyst under the conditions given also resulted in slight acetylation of cellulose, which is reflected in the presence of the three acetyl ester bands at 1745 (C═O ester), 1377 (C—H bond in an —O(C═O) —CH_3 group), and 1251 cm^{-1} (—CO— stretching of acetyl group).[27]

Figure 3 illustrates the FTIR spectra of the cellulose preparations obtained by treatment of *C. korshinskii* with 80% acetic acid and nitric acid concentration of 2% (spectrum1, sample 7), 6% (spectrum 2, sample 9), and 10% (spectrum 3, sample 10) at 110°C for 60 min. The similar spectral profiles indicated the similar structures of the cellulose samples. However, on close comparison of the spectra, a small difference is observed. The signal intensity for an ester peak at 1745 cm^{-1} increased slightly from spectrum 1 to 2, and to 3, which corresponds to a decrement of cellulose yield, suggesting that a higher concentration accelerates the acetylation of cellulose during the degradation of *C. korshinskii* lignin and hemicelluloses using a mixture of acetic acid–nitric acid. Similar increasing trend of the ester peak intensity with an increment in temperature was observed in the samples 12, 14, and 16 in Figure 4, thereby providing evidence for accelerated acetylation of cellulose at high temperature.

CP/MAS ^{13}C NMR spectrum

Cellulose is a semicrystalline biopolymer with ordered crystalline and disordered amorphous regions. The crystalline cellulose is known to crystallize in several different polymorphs. On the basis of their X-ray diffraction patterns and ^{13}C NMR spectra, four major polymorphs of cellulose have been reported, namely, celluloses I, II, III, and IV. The most abundant native crystalline form is celluloses I.[28] The samples obtained by treatment with 80% acetic acid and 4% nitric acid as a catalyst (spectrum 1, sample 8), and 8% nitric acid (spectrum 2, sample 4) for 60 min at 110°C were examined by CP/MAS ^{13}C NMR spectroscopy to confirm the structural feature of celluloses, and the spectra are shown in Figure 5. As shown in spectrum 1, the peaks identified represent the cellulose at 100.2 ppm for C-1, at 83.9 and one at 79.0 ppm for C-4 and at 59.8 and 58.0 ppm for the C-6. The resonances of C-2, C-3, and C-5 occur at 70.1 and 67.5 ppm. Based on the results described in the literature,[28] a peak at 83.9 ppm is attributed to crystalline cellulose and 79.0 ppm is assigned to disordered cellulose. A similar trend can be seen in the signals assigned to C-6 in crys-

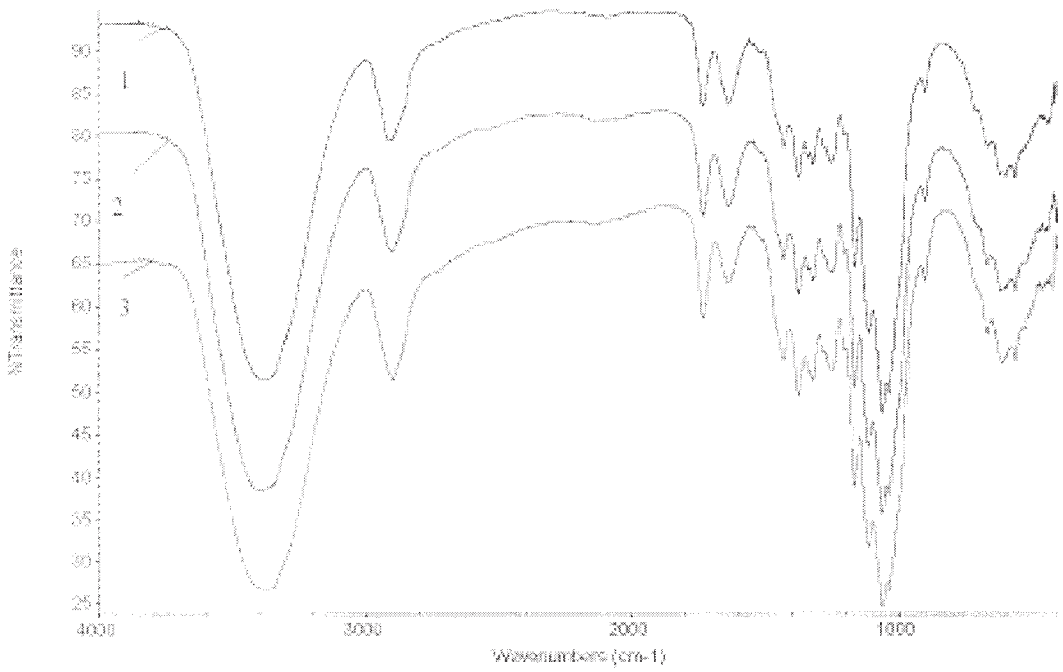

Figure 4 FTIR spectra of the cellulose samples obtained by treatment of *C. korshinskii* with 80% acetic acid and 8% nitric acid as a catalyst for 60 min at 95°C (spectrum 1, sample 12), 110°C (spectrum 2, sample 14), and 120°C (spectrum 3, sample 16).

talline cellulose (59.8 ppm) and on crystal surfaces or disordered cellulose (58.0 ppm). Similar intensities of the signals between 83.9 and 79.0 ppm, and between 59.8 and 58.0 ppm were observed at spectrum 2 of cellulosic sample 4. However, in comparison of the *C. korshinskii* material (not shown in spectra), an increase in the peak intensity at 83.9 and 59.8 ppm demonstrated that the treatment with a mixture of acetic acid–nitric acid under the conditions given increased the degree of crystallinity of cellulose. In addition, the spectra of the cellulosic samples in Figure 5 also provide evidence for the occurrence of the side reaction of acetylation as indicated by two small signals at 168.1 ppm (C═O in esterified acetyl group) and at 16.1 ppm (CH_3 in acetyl group).

X-ray diffraction and N-*O'KI* index analysis

Wide-angle X-ray scattering (WAXS) is a well-established method for determination of the average size of the crystallites and the crystallinity.[29] The fiber texture

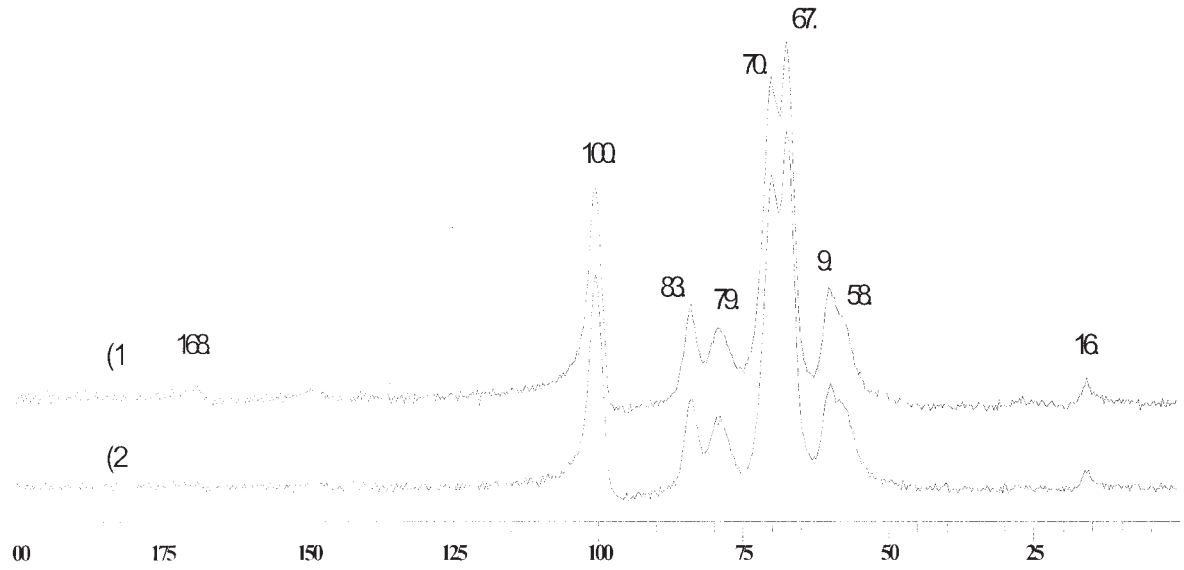

Figure 5 CP/MAS ^{13}C NMR spectra for the cellulose preparations obtained by treatment with 80% acetic acid and 4% nitric acid as a catalyst (spectrum 1, sample 8), and 8% nitric acid (spectrum 2, sample 4) for 60 min at 110°C.

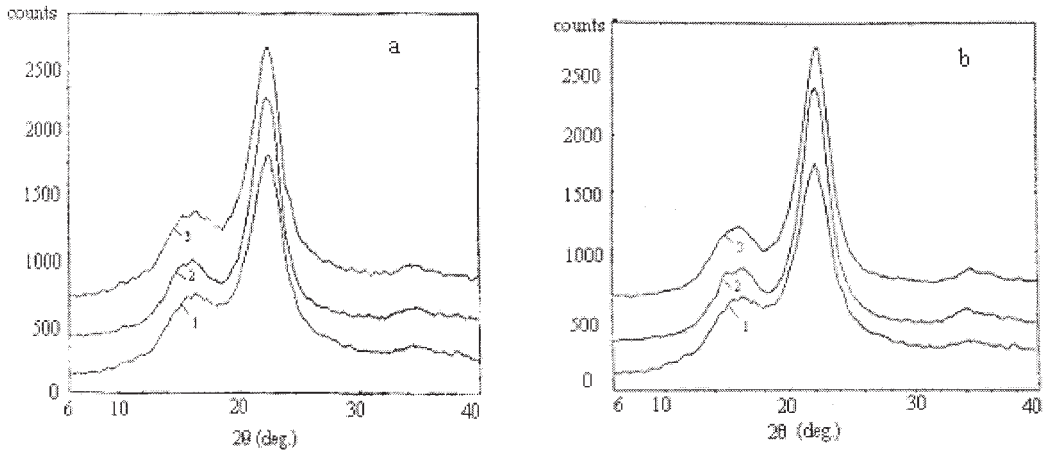

Figure 6 (a) The X-ray diffraction patterns of *C. korshinskii* material (spectrum 1) and cellulosic preparations obtained by treatment with 80% acetic acid and 8.0% nitric acid as a catalyst at 110°C for 40 (spectrum 2, sample 2) and 60 min (spectrum 3, sample 4). (b) The X-ray diffraction patterns of *C. korshinskii* material (spectrum 1) and cellulosic preparations obtained by treatment with 80% acetic acid and 0.0% (spectrum 2, sample 6) and 6% (spectrum 3, sample 8) nitric acid as a catalyst at 110°C for 60 min.

and the complex chemical composition of wood complicate the crystallinity determination. Furthermore, the separation of amorphous background from the diffraction pattern of cellulose crystallites is difficult because the cellulose crystallites are small. For these reasons the absolute crystallinity of wood cannot be determined accurately.[30] Most of the studies have aimed at determining the relative crystallinity of wood.[31,32]

Meanwhile, FTIR spectra can not only be used to analysis the change of cellulose chemical structure, but also to study the crystallinity index of N-*O'KI*. The value of N-*O'KI* index can be calculated using the formula N-*O'KI* = a_{1372}/a_{2900}, in which a_{1372} and a_{2900} are the absorbance intensities of IR. Each spectrum presents a peak at 1372 cm^{-1}, which is attributed to the O-H bending, and one at 2900 cm^{-1}, to the C—H and CH_2 stretching.

Figure 6(a) shows the X-ray diffraction patterns of *C. korshinskii* material (spectrum1) and the cellulose samples obtained by treatment with 80% acetic acid and 8.0% nitric acid as a catalyst at 110°C for 40 min (spectrum 2, sample 2) and 60 min (spectrum 3, sample 4). Figure 6(b) shows the X-ray diffraction patterns of *C. korshinskii* material (spectrum 1) and cellulosic preparations obtained by treatment with 80% acetic acid and 0.0% (spectrum 2, sample 6) and 6% nitric acid as a catalyst (spectrum 3, sample 8) at 110°C for 60 min. The X-ray diffraction patterns indicated that the samples were cellulose I, consisting of both crystalline and amorphous phases. It is clear that the intensity of the X-ray diffraction peak increased, which corresponds to increase in treatment time or nitric acid concentration, suggesting that a longer treatment time or higher concentrations increases the crystillinity of the cellulose.

Table VI shows the N-*O'KI* index, the crystallinity degree, and the crystallite size of the cellulosic prep-

TABLE VI
Crystallinity Degree and N-*O'KI* Index of the Cellulose Samples

Sample no.	Crystallinity degree (%)	L_{hkl} (10^{-10} m)			FTIR absorbance			
		L_{101}	$L_{10\bar{1}}$	L_{002}	Basic line	a_{1372}	a_{2900}	N-*O'KI* Index
AC1	53	52	44	32	0.073	0.169	0.160	1.102
AC2	57	52	48	34	0.082	0.213	0.194	1.167
AC3	61	52	52	34	0.085	0.245	0.261	1.228
AC4	65	51	51	36	0.089	0.262	0.223	1.299
AC5	66	52	52	37	0.087	0.239	0.203	1.310
AC6	50	41	41	34	0.088	0.254	0.240	1.089
AC7	52	40	41	35	0.096	0.199	0.187	1.123
AC8	59	41	42	35	0.087	0.232	0.206	1.214
AC9	62	41	42	36	0.093	0.271	0.232	1.281
AC10	65	41	43	38	0.105	0.203	0.179	1.325
AC11	67	40	44	39	0.093	0.274	0.229	1.328

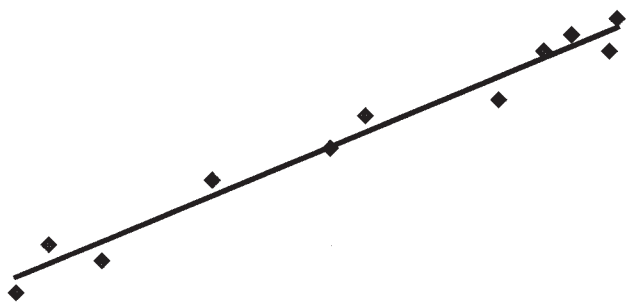

Figure 7 The relationship between N-*O'KI* index and crystallinity degree.

arations obtained from samples 1 to 11. When compared with the *C. korshinskii* raw material (1.077, 49%), both the N-*O'KI* index and the degree of crystallinity of the cellulosic preparations increased after treatment by an acetic acid and nitric acid mixture. The N-*O'KI* index increased from 1.102 to 1.310 with an increase in reaction time from 30 to 90 min, and from 1.089 to 1.328 with an increase in nitric acid concentration from 0 to 10%, respectively. Furthermore, the degree of crystallinity of the cellulosic preparations increased from 53 to 66% with an increase in reaction time from 30 to 90 min, and from 50 to 67% with an increase in nitric acid concentration from 0 to 10%, respectively. Since less ordered cellulose fractions and impurities (pectins, waxes, etc.) were removed during the reactions. Thus, the amount of crystalline cellulose increased in the cellulosic preparations. The crystalline sizes of (101) plane were almost constant with an increase in reaction time from 30 to 90 min and in nitric acid concentration from 0 to 10%, respectively. But the crystalline sizes of $10\bar{1}$ and 002 planes were increased with an increase in reaction time from 30 to 90 min and in nitric acid concentration from 0 to 10%, respectively.

In addition, the data in Table VI also show that the change of N-*O'KI* index act in accord with the degree of crystallinity. The relationship between N-*O'KI* index and degree of crystallinity is shown in Figure 7 and can be expressed as unitary linear regression equation:

$$Y = 64.738X - 19.524$$

$$R^2 = 0.9716$$

In other words, N-*O'KI* index gave an accurate account at the given reaction conditions.

Thermal analysis

Thermal analysis is convenient and reproducible, and is a useful method for characterizing heterogeneous organic material. In particular, it is a valuable analytical method to investigate the physicochemical properties of macromolecules such as cellulose. Figure 8 illustrates typical TGA/DSC curves of cellulose preparations AC8, AC4, and AC11 obtained from *C. korshinskii* subjected to 80% acetic acid and 4.0% (curves 1, sample 8), 8.0% (curves 2, sample 4), and 10.0% (curves 3, sample 11) nitric acid as a catalyst at 110°C for 60 min with liquor-to-solid ratio of 7 : 1 (mL/g), respectively. The endothermic peak due to the loss of absorbed water is observed at about 68°C at DSC curves. As shown in Figure 8, the three cellulose preparations started to decompose at 329°C (AC8), 321°C (AC4), and 311°C (AC11), respectively. At 50% weight loss, the decomposition temperature of the two cellulose preparations occurred at 350°C (AC8 and AC4). This same temperature implied that there are no sig-

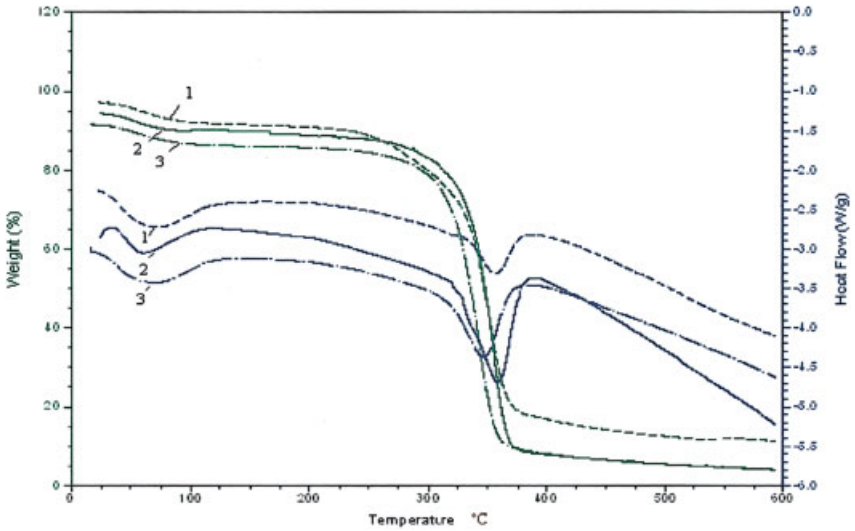

Figure 8 TGA/DSC curves of three cellulose preparations obtained by treatment with 80% acetic acid and 4.0% (curves 1, sample 8), 8.0% (curves 2, sample 4), and 10.0% nitric acid as a catalyst for 60 min at 110°C (curves 3, sample 11). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

nificant differences in the thermal stabilities of the cellulose preparations obtained by treatment with 80% acetic acid and 4.0 or 8.0% nitric acid as a catalyst at 110°C for 60 min. However, at 50% weight loss, the decomposition temperature of the cellulose preparation (AC11) occurred at 340°C. This decreasing trend with declining yield of cellulose indicated that the thermal stability of cellulose decreased with an increase in nitric acid concentration from 8.0 to 10.0%. In addition, the three DSC thermograms of the cellulose samples gave similar exothermic peak.

CONCLUSIONS

The results obtained from this study suggested that the method described here is a promising separation process that can selectively degrade lignin and hemicellulosic polymers, with reasonable yields and purity of cellulose from *C. korshinskii* in a system mixture of acetic acid–nitric acid. Special care has to be taken in selecting reactive conditions so as to avoid excessive degradation of the cellulosic polymers. The method is simple and was performed rapidly, requiring only the minimal quantity of nitric acid used (2.0–8.0% (w/w) in the extractant). The color of cellulosic preparations obtained is brighter than that obtained from the other published methods. The isolation of highly pure cellulose from *C. korshinskii* had not been addressed in relation to TCF technologies. This method represents a TCF process, which brings little risk of environment pollution, compared with the use of chlorite-containing reagent for delignification.

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