STRUCTURAL CHARACTERIZATION OF HEMICELLULOSES FROM BAMBOO CULMS (*NEOSINOCALAMUS AFFINIS*)

S. N. SUN, T. Q. YUAN, M. F. Li, X. F. CAO,* F. XU and Q. Y. Liu**

Institute of Biomass Chemistry and Technology, Beijing Forestry University, Beijing 100083, China
**State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, China*
***The BioComposites Centre, University of Wales, Bangor LL57 2UW, United Kingdom*

Received November 21, 2011

Dewaxed bamboo culms (*Neosinocalamus affinis*) were sequentially extracted with distilled water and 70% ethanol at 80 ºC for 3 h, 0.2 and 0.5 M NaOH, 70% ethanol containing 0.6 M NaOH, and 1.0 and 2.0 M NaOH at 50 ºC for 3 h using a solid to liquid ratio of 1:25 (g/mL). The hemicellulosic fractions obtained were characterized by high performance anion exchange chromatography (HPAEC), gel permeation chromatography (GPC), Fourier transform infrared (FT-IR) spectroscopy, ^{1}H and ^{13}C nuclear magnetic resonance (NMR), and 2D heteronuclear single quantum coherence (HSQC) NMR spectroscopies. Compared to the water-soluble fraction, the alkali-soluble hemicelluloses primarily consisted of xylose (73.5-92.7%), and had a more linear structure and larger macromolecules, evidenced by Xyl/Ara ratios (11.0-22.9) and higher average molecular weights (M_w = 41200-68770 g/mol). Increasing the alkaline concentrations from 0.2 to 2.0 M NaOH decreased the molecular weights from 68770 to 42790 g/mol, but increased the Xyl/Ara ratios from 11.0 to 22.9 in the hemicellulosic fractions. The fractions isolated with 0.5 and 1.0 M NaOH were found to be composed of a linear (1→4)-*β*-D-xylopyranosyl main chain substituted by a small amount of L-arabinofuranosyl at C-2 and/or C-3 together with a minor quantity of 4-*O*-methylglucuronic acid at C-2, representing typical polysaccharide structure in bamboo.

Keywords: bamboo culms, hemicelluloses, structural characterization, xylan, NMR

INTRODUCTION

Current shortages of natural energy sources and the need for replacing petroleum-based products, because of their contribution to global environmental pollution, are driving forces for research on lignocellulosic materials. Hemicelluloses are the second most abundant polysaccharides in lignocellulose, composed of non-cellulosic short-branched chains of sugars. In the plant cell wall, hemicelluloses form a complex network by hydrogen bonds to cellulose, covalent bonds (mainly *α*-benzyl ether linkages) with lignin, and ester linkages with acetyl units and hydroxycinnamic acids.[1] Unlike cellulose, hemicelluloses are a class of heterogeneous polymers containing xylans (arabinoxylans and 4-*O*-methyl-glucuronoxylans), galactomannans, glucomannans, *β*-D-glucans (3- and 4-linked), *β*-D-glucan-callose (3-linked), and xyloglucans (4-linked *β*-D-glucans with attached side chains).[2] Xylans, xyloglucans and galacto-arabino-glurono-xylan are the main hemicelluloses present in plants.[3] Generally, xylan consists of a (1→4)-*β*-D-xylopyranosyl main chain with random L-arabinofuranose residues, uronic acid, acetic acid and other side chains attached, such as ferulic and *p*-coumaric acids in the *O*-3 and/or *O*-2 position. Hemicelluloses can be applied in tissue engineering and drug delivery systems, such as hemicelluloses-based hydrogels.[4,5] The reported industrial applications for using plant hemicelluloses also include their use as viscosity modifiers, gelling agents, tablet binders, or wet strength additives.[6] The modification or derivatization of hemicelluloses is crucial to the utilization of these renewable polymers to extend and replace petrochemical-based materials. Current applications of hemicelluloses show that they are

a potential feedstock in fermentation for the production of xylitol, which can be applied in a variety of food industries.[7] Other chemicals and bio-based products, such as sweeteners, furfural and degradable packaging films, have also been produced from xylan.[8] Due to these applications, hemicelluloses may play an important role as a biopolymer in the future.

The isolation of hemicelluloses is an essential step for the production of a wide range of fuels and chemicals. A number of effective methods to obtain hemicellulosic polymers have been studied, including extraction using hot water (autohydrolysis),[9] alkali,[10] dimethyl sulfoxide (DMSO), and steam explosion-based treatment.[11] Alkali treatment of lignocellulosic materials, such as bamboo and cereal straw, disrupts the cell wall and cleaves the linkages between lignin and hemicelluloses. This extraction has been a promising and eco-friendly process to achieve complete utilization of lignocelluloses with a minimal impact on the environment. The main advantages of alkaline extraction are simple operation and cost-effectiveness. Hemicelluloses, together with other polysaccharides, could be used in the future as feedstocks for "green" advanced materials, if their structural features could be identified and well-characterized.

Bamboo (*Neosinocalamus affinis*) is widely distributed in China, Japan and South-East Asian countries, and represents a readily available renewable feedstock. In recent years, bamboo has been extensively used in the materials and energy fields. As an example, bamboo fibers are being considered for reinforcement of polymeric composites, due to their high strength, biodegradability and low price.[12] Additionally, bamboo starch is a versatile biopolymer with potential for application in non-food industries. The unique structure of this material, coupled with its reactivity, make the design of starch-based oil field chemicals a viable possibility. However, applications of bamboo hemicelluloses are currently limited, because of their complex structural features. Bamboo generally contains 22-35% hemicelluloses with high xylan content.[13] Although these hemicelluloses are considered to be an abundant renewable source for the production of xylose and xylooligosaccharides, their structural characteristics and physicochemical features have been poorly characterized. In the present work, hemicellulosic fractions were obtained by successive extractions from bamboo culms (*Neosinocalamus affinis*) with distilled water, ethanol and alkaline aqueous solutions at different concentrations. The physicochemical properties and structural features of these hemicellulosic fractions were then evaluated and characterized by high performance anion exchange chromatography (HPAEC), gel permeation chromatography (GPC), Fourier transform infrared (FT-IR), and ^{1}H and ^{13}C nuclear magnetic resonance (NMR), as well as by 2D heteronuclear single quantum coherence (HSQC) NMR spectroscopy.

EXPERIMENTAL

Materials

Bamboo culms (*Neosinocalamus affinis*) were harvested in Sichuan province, China. They were dried in sunlight, cut into small pieces (1-3 cm), then ground to pass through a 0.8 mm screen. The chemical composition of the dewaxed bamboo was the following: glucose – 50.82%, xylose – 22.94%, arabinose – 1.13%, galactose – 0.51%, mannose – 0.37%, rhamnose – 0.02%, glucuronic acid – 0.94%, lignin – 19.46% (Klason lignin – 16.97% and acid-soluble lignin – 2.49%), and ash – 2.52%. To remove the non-cell wall components, such as wax and chlorophyll, the powder was extracted with toluene/ethanol (2:1, v/v) in a Soxhlet apparatus for 6 h, and then over-dried at 60 ºC for 16 h. All chemicals used were of analytical or reagent grade.

Isolation of hemicelluloses

To study comparatively the structural differences in the bamboo hemicelluloses, the fractions were extracted sequentially with water, ethanol and an alkaline solution at different concentrations, as illustrated in Figure 1. The extractive-free powder (10 g) was successively extracted with distilled water and 70% ethanol at 80 ºC, 0.2 and 0.5 M NaOH aqueous solutions, 70% ethanol containing 0.6 M NaOH, and 1.0 and 2.0 M NaOH aqueous solutions at 50 ºC for 3 h using a solid to liquid ratio of 1:25 (g/mL). The cellulosic residues were filtered out with a nylon cloth, washed thoroughly with distilled water, and further dried in a cabinet oven under air circulation at 60 ºC for 16 h. The filtrates were concentrated to 40 mL at reduced pressure, and then poured into 2 volumes of 95% ethanol under vigorous stirring. The pellets were centrifuged, freeze-dried, and finally labeled from H_1 to H_7 according to the order of successive extractions. The filtrate obtained under alkaline conditions was adjusted to pH 5.5 with aqueous 6 M hydrochloric acid prior to concentration and precipitation. All samples were stored in a desiccator for further characterization. The experiments were performed in duplicate, and the deviation was below 4.8%. Note that the hemicellulosic fractions extracted with water at 80 ºC, with 0.2 and 0.5 M NaOH aqueous solutions, 70%

ethanol containing 0.6 M NaOH, and 1.0 and 2.0 M NaOH aqueous solutions at 50 ºC for 3 h were labeled as H_1, H_3, H_4, H_5, H_6 and H_7, respectively. The extraction with 70% ethanol at 80 ºC for 3 h yielded only trace amounts of the hemicelluloses, therefore, the corresponding fraction (H_2) was not characterized in this study.

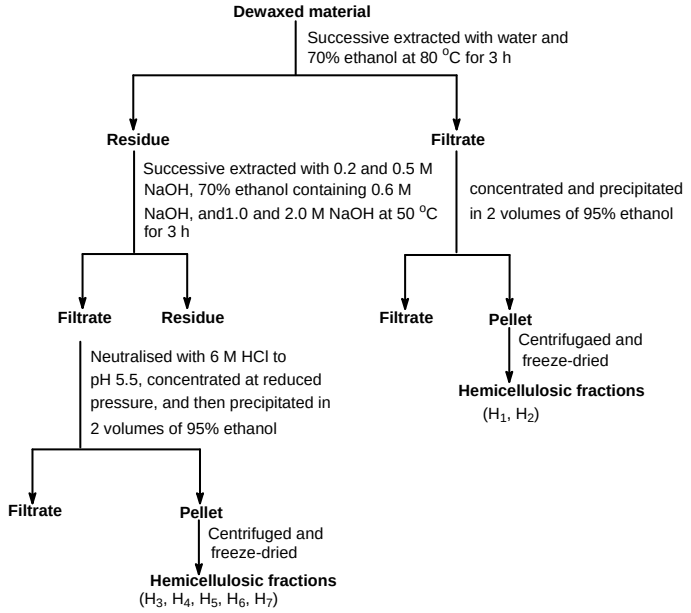

Figure 1: Scheme for successive isolation of hemicellulosic fractions from *Neosinocalamus affinis*

Sugar composition analysis by HPAEC

Each of the hemicellulosic fractions (~5 mg) was hydrolyzed in 10% sulphuric acid at 105 ºC for 2.5 h, and the sugar composition was analyzed by HPAEC. The analysis was performed as follows: the sample was hydrolyzed to its monosaccharide components and the solution was filtered, diluted 50-fold, and injected into the HPAEC system (Dionex ISC 3000) with an amperometric detector, an AS50 autosample, a CarbopacTM PA-20 column (4×250 mm, Dionex), and the guard PA-20 column (3×30 mm, Dionex). Neutral sugars and uronic acids were separated in a 5 mM NaOH isocratic (which was carbonate free and purged with nitrogen) for 20 min, followed by a 0-75 mM NaAc gradient in a 5 mM NaOH for 15 min. The columns were then washed with 200 mM NaOH for 10 min to remove carbonate, followed by a 5 min elution with 5 mM NaOH to re-equilibrate the column before the next injection. The total analysis time was 50 min, and the flow rate used was 0.4 mL/min. Calibration was performed with a standard solution of L-rhamnose, L-arabinose, D-glucose, D-xylose, D-mannose, D-galactose, glucuronic acid and galacturonic acids. The sugars present in the samples were identified by comparing their relative retention times with standards and were quantified by using response factors.

Molecular weight determination by GPC

The molecular-average weights and molecular weight distributions of all hemicellulosic fractions were examined using gel permeation chromatography (GPC), with a PL aquagel-OH 50 column (300×7.7 mm, Polymer Laboratories Ltd.), and with the column oven controlled at 30 ºC. The chromatographs were calibrated with PL pullulan polysaccharide standards (peak average molecular weights of 783, 12200, 100000, 1600000, Polymer Laboratories Ltd.). The detector used was a differential refractive index detector (RID). The eluent was 0.02 N NaCl in 0.005 M sodium phosphate buffer, at a pH of 7.5, and the flow rate used was 0.5 mL/min. All of the hemicellulosic samples were adjusted to a concentration of 0.1% before measurement.

FT-IR

The measurements were performed on a Tensor 27 FT-IR spectrophotometer by transmittance using a KBr pellet containing 1% finely ground samples. Each spectrum was recorded in 32 scans in the range from 4000 cm^{-1} to 400 cm^{-1} with a resolution of 2 cm^{-1}. KBr was previously oven-dried to avoid interferences due to the presence of water, and the background was collected before each sample scanning.

NMR spectroscopy

The soluble-state ^{1}H-NMR, ^{13}C-NMR and HSQC spectra were recorded on a Bruker AV III 400 MHz spectrometer operating in the FT mode at 100.6 MHz. The purified hemicelluloses (15 mg/mL in D_2O for ^{1}H, 80 mg/mL in D_2O for ^{13}C) were placed in the sample probe and the resonance spectra were obtained. The chemical shifts of ^{1}H-NMR spectra were calibrated with reference to D_2O, used as an internal standard at 4.70 ppm. The acquisition and relaxation times were of 3.9 s and 1.0 s, respectively. For ^{13}C-NMR analysis, the spectra were recorded at 25 ºC after 30000 scans. A 30^{o} pulse flipping angle, a 9.2 μs pulse width, 1.36 s acquisition time, and 1.89 s relaxation delay time were used. The spectral widths were of 2200 and 15400 Hz for the ^{1}H- and ^{13}C-dimensions, respectively. The heteronuclear single quantum coherence (HSQC) NMR experiment was obtained with 20 mg sample dissolved in 1 mL D_2O after 128 scans. The number of collected complex points was 1024 for the ^{1}H dimension with a relaxation of 1.5/5 s. The number of scans was 128, and 256 time increments were recorded in the ^{13}C-dimension. The $^{1}J_{C\text{-}H}$ used was 146 Hz. Prior to Fourier transformation, the data matrices were zero-filled up to 1024 points in the ^{13}C-dimension.

RESULTS AND DISCUSSION

Yield of hemicelluloses

Hemicelluloses form covalent bonds with lignin and ester linkages with acetyl units and hydroxycinamic acids, restricting the liberation of hemicelluloses from the cell wall matrix.[14] In addition, extensive hydrogen bonding between the individual polysaccharide and cell wall components may also impede the isolation of hemicelluloses.[12] Therefore, the yield of the hemicellulosic fraction is highly dependent on the characteristics of the medium used during the fractionation process. In the present study, dewaxed bamboo was sequentially extracted with neutral water and 70% ethanol, aqueous alkaline solution at low concentrations (0.2 and 0.5 M NaOH), aqueous alkaline ethanol solution (70% ethanol-0.6 M NaOH), and aqueous alkaline solution at high concentrations (1.0 and 2.0 M NaOH). The yields of hemicelluloses were of 2.0%, trace, 6.1%, 3.2%, 2.6%, 5.6%, and 2.7% of dewaxed bamboo culms in each step, respectively, accounting to nearly 22% of the total material. The yield of hemicelluloses obtained under alkaline conditions accounted for 91.0% of the total hemicelluloses liberated, indicating that the addition of alkali could effectively cleave the ester and/or ether bonds between lignin and hemicelluloses, leading to the release of hemicelluloses. In comparison, the treatment using water only released a small amount of hemicelluloses (2.0%). There was no hemicellulosic fraction isolated with 70% ethanol under the given conditions. This can be explained by the positive action of ethanol on suppression of the pelling reaction of the polysaccharides in a neutral organosolv medium.[15]

The addition of a small amount of NaOH (0.2 M) yielded the highest amount of hemicelluloses (6.1%), accounting for 27.7% of the total hemicelluloses isolated. It was also found that most of bamboo hemicelluloses could be released when the alkaline concentration was lower than 2.0 M, which agreed well with the results previously reported by Sun et al.[16] on the fractionation of hemicelluloses from fast-growing poplar wood. It was found that as the concentration of NaOH was increased to 2.0 M, the yield of the hemicellulosic fraction decreased to 2.7%. This reason may be the degradation in the end-wise peeling reaction at high alkaline concentrations, and lower hemicelluloses content in the residues after the previous extractions. These results suggest that alkaline extraction is an efficient process for the fractionation of hemicellulosic polymers from lignocellulosic materials as compared to the water and hydrogen peroxide treatments.[17,18]

Neutral sugar composition

Hemicelluloses are a mixture of a number of polysaccharides, which mainly consist of different sugar units containing pentoses (*β*-D-xylose, *α*-L-arabinose), hexoses (*β*-D-mannose, *β*-D-glucose, *α*-D-galactose) and/or uronic acids (*α*-D-glucuronic, *α*-D-4-*O*-methylgalacturonic and *α*-D-galacturonic acids). Other sugars, such as *α*-L-rhamnose and *α*-L-fucose, may also be present in small amounts and the hydroxyl groups of sugars may be partially substituted with acetyl groups.[19] The neutral/acidic sugar compositions of the hemicellulosic fractions are given in Table 1, and are expressed as a relative percentage of total sugars. The glucose and xylose are dominant in the water-soluble fraction (H_1) and alkali-soluble fractions (H_3-H_7), respectively. The high content of glucose (82.49%) in H_1 may originate from undigested starch and other polysaccharides, such as xyloglucans and *β*-glucans,[20] as well as pectic substances, which have been reported in previous studies.[21] Xylose and arabinose were the second major sugar, comprising 8.14% and 5.44% of the total sugar composition, respectively. Galactose (2.45%) and uronic acid (1.48%) were also

observed as minor constituents. This indicated that the hot water treatment probably released the hemicellulosic polymers with a more branched structure and a low molecular weight, which is discussed in subsequent sections. In contrast, xylose was the predominant sugar component (73.53-92.65%) in the alkali-soluble hemicellulosic fractions H_3-H_7. Glucose (1.92-17.87%) and arabinose (4.05-6.69%) were present in significant quantities, and uronic acids (0.84-1.29%), mainly 4-*O*-methyl-D-glucuronic acid (MeGlcA), and galactose (0.09-0.82%) were also observed as minor sugar constituents. These data suggest that bamboo hemicelluloses are primarily of the xylan and arabinoxylan-type. The high content of glucose likely resulted from the degradation of *β*-glucans, which has been proven to exist in bamboo by Wilkie and Woo.[22] The strong association between *β*-glucans and alkali-extractable arabinoxylans is caused by non-covalent interactions between them, as well as with other cell wall material, such as cellulose and lignin.[23] With the increment of alkaline concentration from 0.2 M to 2.0 M, the content of xylose increased significantly from 73.53% to 92.65%, whereas the content of glucose decreased from 17.87% to 1.92%. No significant differences were observed in the arabinose and uronic acid content. The small amount of galactose present may be due to galactoarabinoxylans and arabinogalactans.[24,25] The xylose to arabinose (Xyl/Ara) ratio can be used as an indication of the degree of linearity or branching of hemicelluloses.[26] A low Xyl/Ara ratio suggests a short-chain polymer with a large amount of branching with other monosaccharide constituents. Increasing the alkali concentration from 0.2 M to 2.0 M resulted in a gradual increment of the Xyl/Ara ratio from 11.0 to 22.9. These results indicate that the hemicellulosic fractions released in the alkali solution with a higher concentration had more linear structures.

Table 1
The contents of neutral sugars and uronic acids (relative %, w/w) of hemicellulosic fractions obtained from *Neosinocalamus affinis*

Sugars	Hemicellulosic fractions[a]					
	H_1	H_3	H_4	H_5	H_6	H_7
Arabinose	5.44	6.69	4.70	4.74	4.45	4.05
Galactose	2.45	0.82	0.22	0.33	0.13	0.09
Glucose	82.49	17.87	10.80	6.74	3.19	1.92
Xylose	8.14	73.53	83.06	87.29	91.40	92.65
Uronic acids	1.48	1.09	1.20	0.90	0.84	1.29
Xyl/Ara[b]	1.5	11.0	17.7	18.4	20.5	22.9

[a]H_1, H_3, H_4, H_5, H_6 and H_7 represent the hemicellulosic fractions isolated by successive extractions with water and 70% ethanol at 80 ºC, 0.2 and 0.5 M NaOH aqueous solution, 70% ethanol aqueous solution containing 0.6 M NaOH, and 1.0 and 2.0 M NaOH aqueous solution at 50 ºC for 3 h; [b]Represents xylose to arabinose ratio

Table 2
Weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) of hemicellulosic fractions isolated from *Neosinocalamus affinis*

	Hemicellulosic fractions[a]					
	H_1	H_3	H_4	H_5	H_6	H_7
M_w (g/mol)	9000	68770	65750	41200	45190	42790
M_n (g/mol)	7150	29030	32510	26620	30650	26610
M_w/M_n	1.26	2.37	1.67	1.55	1.47	1.61

[a] Corresponding to the hemicellulosic fractions in Table 1

Molecular weight analysis

The molecular weight parameters of the hemicellulosic polymers were assessed using GPC in aqueous medium. The weight-average (M_w) and number-average (M_n) molecular weights, as well as polydispersity (M_w/M_n) of the hemicellulosic fractions obtained, are shown in Table 2. As expected, the water-soluble hemicellulosic fraction H_1 showed a relatively low M_w value (9000 g/mol), compared to the

alkali-soluble hemicellulosic fractions (H_3-H_7) (68770 to 41200 g/mol). This indicates that the hot water dissolved only the small molecules in the hemicelluloses, such as galactoarabinoxylans and pectic substances, as well as *β*-glucans.[26] Furthermore, increasing NaOH concentration from 0.2 M to 2.0 M resulted in an obvious decrease of weight-average molecules from 68770 to 42790 g/mol (with the exception of H_5 extracted with 0.6 M NaOH in 70% aqueous ethanol solution). This indicates that a substantial cleavage of glycosidic linkages in hemicelluloses occurred in highly alkaline solutions, resulting in a significant degradation of the polymers. The results also suggest that the molecular weights of polymers vary depending on the extraction method, solvent quality and chain aggregation for their estimation.[24]

The molecular weight distributions are illustrated in Figure 2, and are in agreement with the results of polydispersity calculated by M_w/M_n. The hemicellulosic fraction H_1 gives a narrower molecular weight distribution curve corresponding to an M_w/M_n value of 1.26, compared to the alkali-soluble hemicellulosic fractions H_3-H_7 having M_w/M_n values from 1.47 to 2.37. The occurrence of a shoulder around 10000 g/mol in H_3-H_7 molecular weight distribution curves is thought to be due to the fragmentation of hemicelluloses induced by the degradation of the hemicellulosic macromolecules during alkaline extraction.[18]

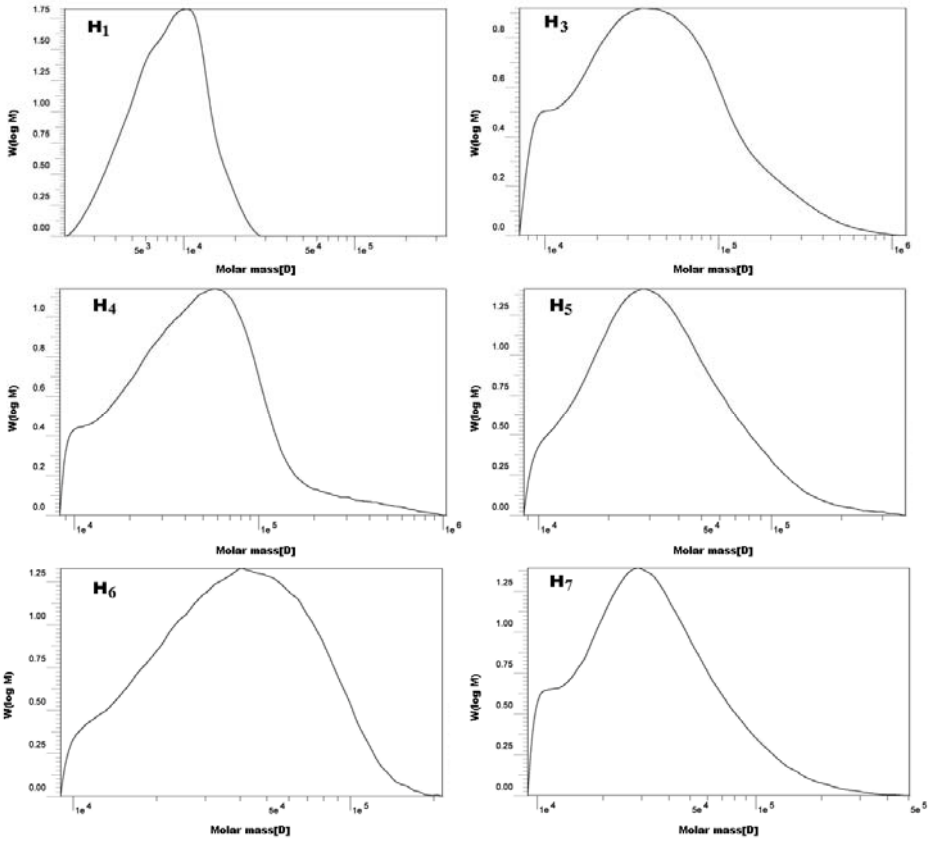

Figure 2: Molecular weight distributions of hemicellulosic fractions isolated from *Neosinocalamus affinis*

FT-IR spectra analysis

The FT-IR spectra of water-soluble (H_1) and two alkali-soluble hemicellulosic fractions (H_3, H_4) are illustrated in Figure 3.[26-33] The absorption at 3384 cm^{-1} is attributed to the hydroxyl stretching vibrations, and the band at 2930 cm^{-1} is due to the C-H stretching of methyl groups. The band around 1642 cm^{-1} is probably due to the bending

mode of water, since the hemicelluloses have a strong affinity for water, and these macromolecules in the solid state may have disordered structures that can be easily hydrated.[28,29] Furthermore, the presence of a shoulder at 1506 cm^{-1} in the H_3 spectrum is assigned to the aromatic skeletal vibrations, implying the occurrence of a small amount of the associated lignin. The bands at 1296, 1327 and 1431 cm^{-1} represent C-H bending; C-H wagging and OH bending; and C-H, OH bending, respectively.[30] The absorptions in the 1200-800 cm^{-1} region may give information about the polysaccharide types present.[31] The bands between 1152 and 995 cm^{-1} are typical of arabinoxylans,[32] attributed to the stretching and bending vibrations of C-O, C-C and C-OH. The absorption intensity in this region is highly related to the degree of branching at the *O*-2 and/or *O*-3 positions.[28]

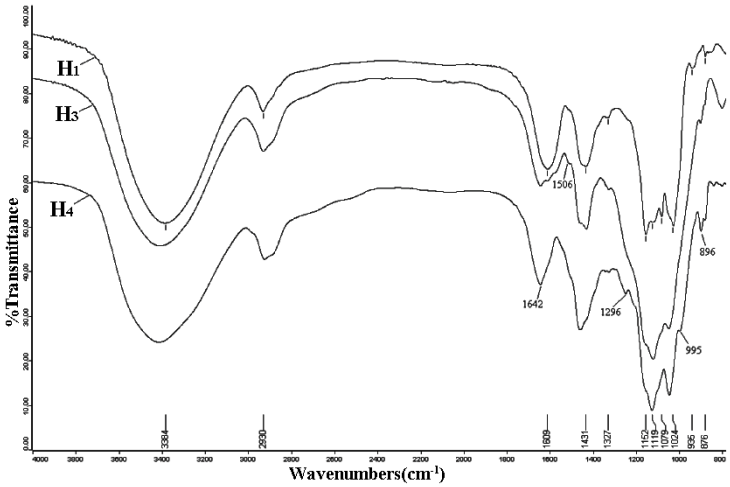

Figure 3: FT-IR spectra of water-soluble (H_1), 0.2 M (H_3) and 0.5 M (H_4) NaOH-soluble hemicellulosic fractions

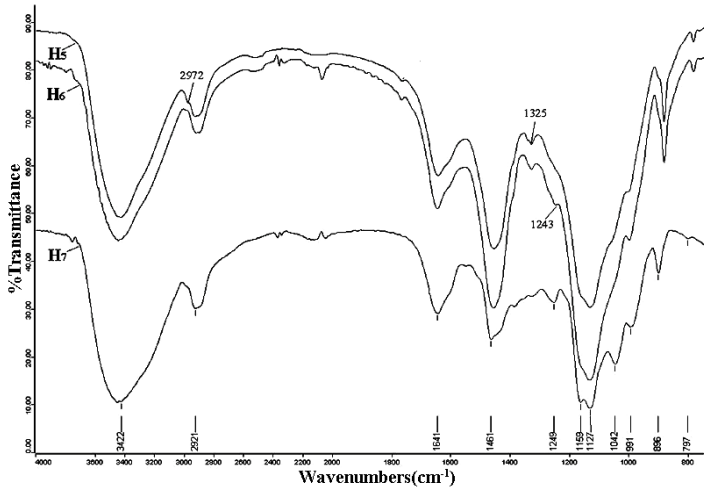

Figure 4: FT-IR spectra of hemicellulosic fractions isolated with 70% ethanol aqueous solution containing 0.6 M NaOH (H_5), 1.0 (H_6) and 2.0 (H_7) M NaOH aqueous solutions at 50 ºC for 3 h

The spectra of the other three alkali-soluble hemicellulosic fractions (H_5, H_6, and H_7) are shown in Figure 4. Clearly, a similar absorption of the peaks in these spectra indicates a similar

structure of the hemicellulosic fractions. All spectra are typical of arabinoxylans, characterized by the absorption peaks at 3422, 2921, 1461, 1325, 1159, 1127 and 1042 cm^{-1}. Furthermore, all of the hemicellulosic fractions give an intense absorbance at 896 cm^{-1}, assigned to ring frequency or the C-1 group frequency, indicating the presence of *β*-glycosidic linkages between xylose units in the hemicelluloses.[33]

NMR analysis

NMR spectroscopy was used to obtain more structural information on high molecular weight polysaccharides and their building blocks. The spectra were interpreted on the basis of reported data for hemicelluloses, which were assigned as arabinoxylan-type, glucoronoxylan-type and L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan-type, respectively, as well as hemicelluloses extracted from wheat straw before delignification.[34-36] Figure 5 shows the ^{1}H and ^{13}C NMR spectra of hemicellulosic fraction H_4 isolated with 0.5 M NaOH at 50 ºC for 3 h. The ^{1}H NMR data of oligomer fragments can be used in identifying specific structural domains present in the polymeric arabinoxylans, since the characteristic positions of the H-1 resonances are relative to the branching patterns in arabinoxylans.[24] The relevant signals occurred in two regions, namely: the anomeric region (δ 5.60-4.90 ppm for *α*-anomers and δ 4.90-4.30 ppm for *β*-anomers); and the ring proton region (δ 4.50-3.00 ppm), where resonance peaks of most protons appeared, and some of the signals were overlapping and hard to discern.[37] These data confirmed the *β*-glycosidic linkages of the D-xylopyranosyl units in the backbone, in agreement with the results of the FT-IR spectra. The signal at 5.24 ppm was assigned to anomeric protons of a terminal *α*-D-arabinofuranosyl residue, indicating a significant amount of arabinose substitution at C-3 and/or C-2 of the xylan backbone.[17] A strong signal at 4.70 ppm corresponded to the residual solvent (D_2O). The non-reducing terminal groups gave a signal at 5.22 ppm.[35] The broad peak around 7.00 ppm (data not shown) was assigned to the aromatic compounds, indicating the presence of small amounts of associated lignin in the hemicellulosic fraction (H_4), as illustrated by the FT-IR analysis.

To better understand the branched structure of hemicelluloses, a ^{13}C NMR spectrum of the hemicellulosic fraction H_4 was recorded. ^{13}C NMR spectroscopy, a non-destructive detection of molecular structure, has become a method for structure elucidation of native hemicelluloses, e.g., arabinoxylans. This spectroscopy allows the fast determination of the nature, configuration and relative content of monosaccharide residues constituting the hemicelluloses, as well as the type and amount of specific linkages.[24] However, it offers no information about the residue sequence in the chain. Most of the major resonances were assigned by references to data in literatures.[10,35-38] The ^{13}C NMR spectrum of the hemicellulosic fraction H_4 shows five strong signals at 102.2, 76.0, 74.7, 73.2, and 63.3 ppm, which are attributed to C-1, C-4, C-3, C-2 and C-5 positions, respectively, of the (1→4)-linked *β*-D-Xylp units. The weak signals at 109.5, 86.4, 80.3, 78.4 and 61.7 ppm correspond to C-1, C-4, C-2, C-3 and C-5 positions of *α*-L-arabinofuranosyl residues linked to *β*-D-Xylans, respectively. Two signals at 72.2 and 71.6 ppm originated from the C-3 and C-2 positions of glucuronic acid residues, respectively. The presence of signal at 60.7 ppm was probably due to *β*-glucans (C-6).[38] The signal at 168.5 ppm represented the carbonyl signal of the esterified ferulic or *p*-coumaric acid in lignin. In conjunction with the ^{1}H NMR spectrum analyses, these data confirm the existence of associated lignin in the isolated hemicellulosic fraction, which coincides with the findings of Kato *et al*.[36]

More structural information of the hemicellulosic fraction H_6 was provided by the investigation using 1D and 2D NMR techniques (Figures 6 and 7). Most of the major resonances were assigned by references to data in the literature.[10,35,39,40] From the correlation between anomeric hydrogens and carbons of the glycosyl residues for anomeric resonances, some primary structural characteristics for the hemicellulosic fraction H_6 could be elucidated. The five dominant signals gave HSQC $^{13}C/^{1}H$ cross-peaks at 102.4/4.31, 73.4/3.15, 75.0/3.36, 75.9/3.62 and 63.3/(3.93+3.24) ppm, which were assigned to C-1, C-2, C-3, C-4 and C-5 of the (1→4)-linked *β*-D-Xylp units, respectively. In addition, the presence of the methyl group of 4-*O*-methyl-D-GlcpA was confirmed by a corresponding small signal at 59.5/3.32 ppm. The cross-peaks at 109.6/5.15 (C-1), 80.4/3.93 (C-2), 78.4/3.66 (data not shown) (C-3), 86.4/4.10, 61.8/(3.57+3.51) (data not shown) (C-5) ppm indicate the presence of *α*-L-Araf residues. In summary, the assignments of all signals in the HSQC NMR spectrum are given in Table 3. For a

quantitative analysis, a molar ratio of Xyl/Ara of 21.0 was found by integrating the corresponding anomeric protons, in agreement with the Xyl/Ara ratio of 20.5 determined by the sugar analysis in Table 1, in which the content of xylose and arabinose was of 91.40% and 4.45%, respectively, in the hemicellulosic fraction H_6.

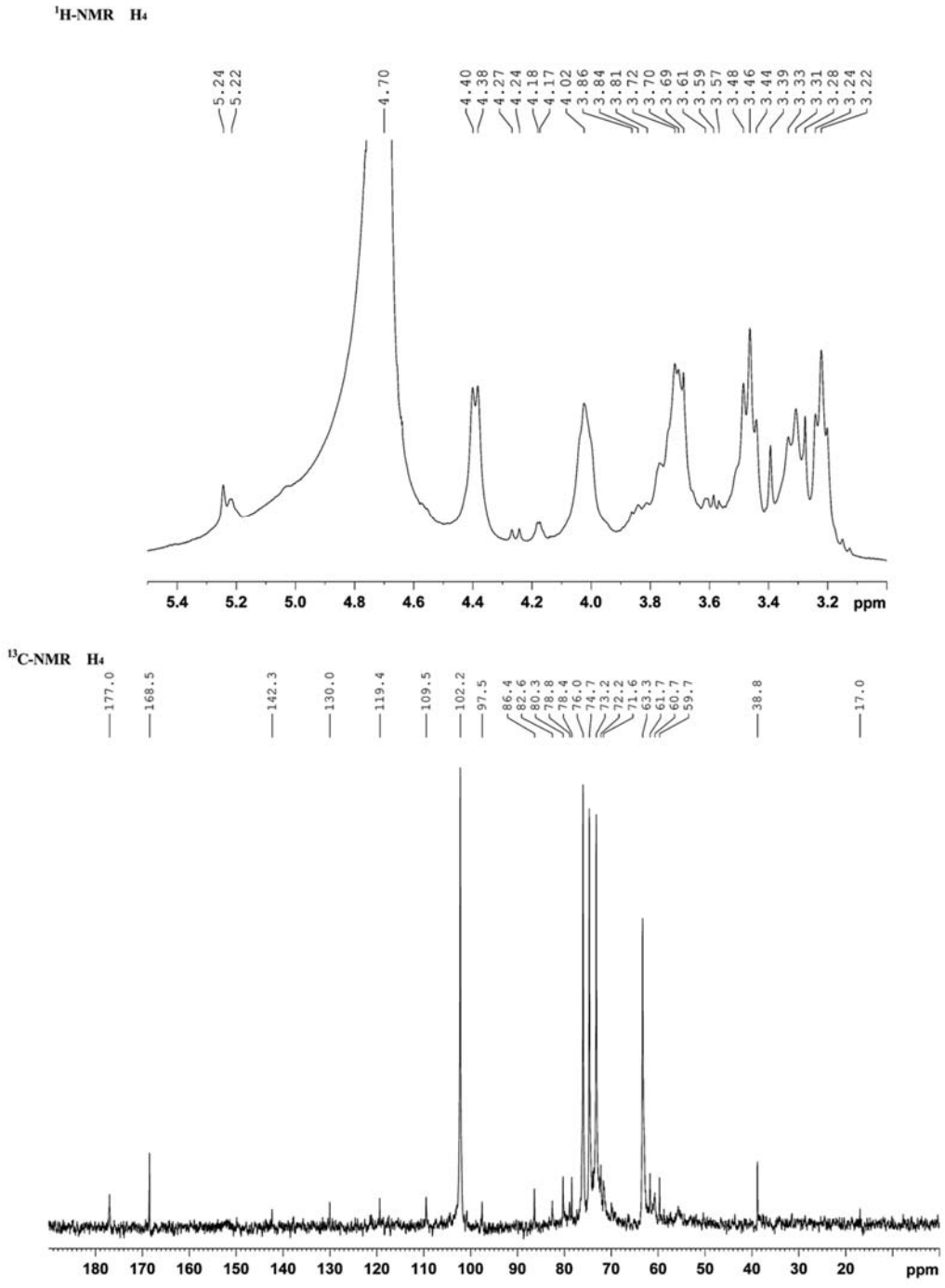

Figure 5: ^{1}H and ^{13}C NMR spectra of hemicellulosic fraction H_4 isolated with 0.5 M NaOH at 50 ºC for 3 h

Table 3

The chemical shifts in ^{1}H and ^{13}C NMR spectra of hemicellulosic fraction H_6 (δ/ppm)

Glycosyl		Assignments							
		1	2	3	4	5eq[e]	5ax[f]	6	-OCH_3
X[a]	^{13}C	102.4	73.4	75.0	75.9	63.3	63.3		
	^{1}H	4.31[g]	3.15	3.36	3.62[g]	3.93	3.24		
U[b]	^{13}C	97.5	72.0[g]	72.0[g]	82.7	72.0[g,i]		177.1	59.5
	^{1}H	na[d]	3.50	3.72[h]	na	4.31[g]			3.28
A[c]	^{13}C	109.6	80.4	78.4	86.4	61.8	61.8		
	^{1}H	5.15	3.93	3.62[g]	4.10	na	3.51		

[a]X – (1→4)-β-D-Xylp, [b]U – 4-*O*-Methy-α-D-GlcpA, [c]A – α-L-Araf residues, [d]na – not assigned, [e]eq – equatorial, [f]ax – axial, [g]Represents overlap peaks, [h]Represents data not shown, [i]C_5-H_5 in U[b] has one cross peak because only one proton is linked to C-5

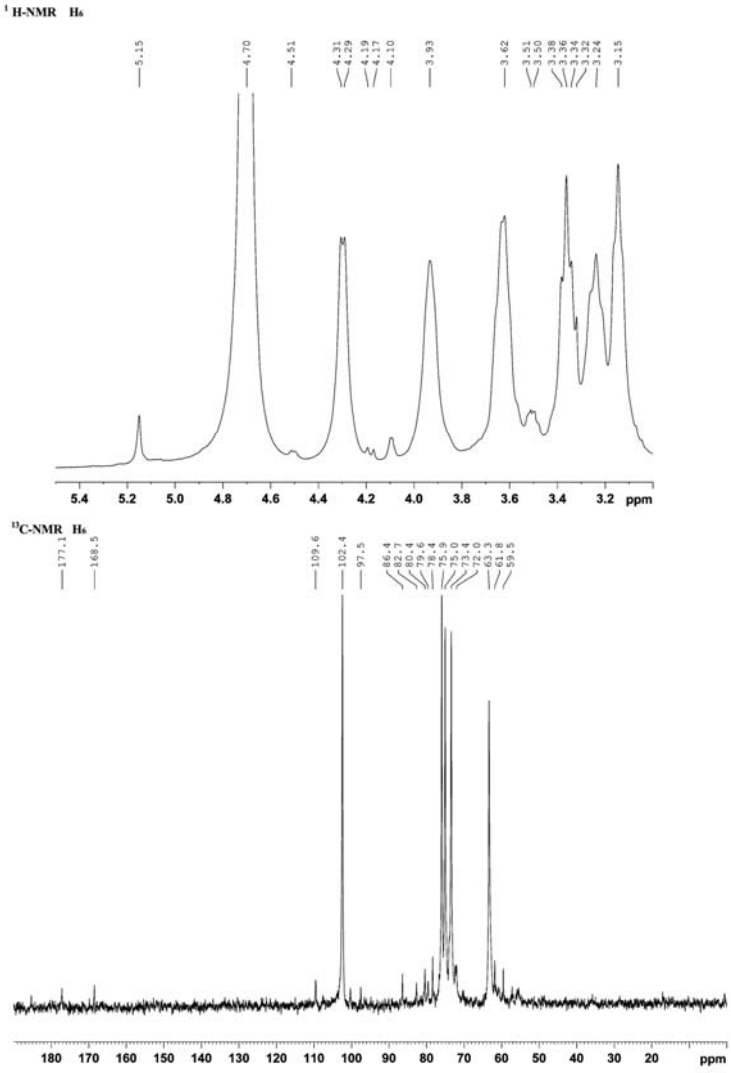

Figure 6: ^{1}H and ^{13}C NMR spectra of hemicellulosic fraction H_6 isolated with 1.0 M NaOH at 50 ºC for 3 h

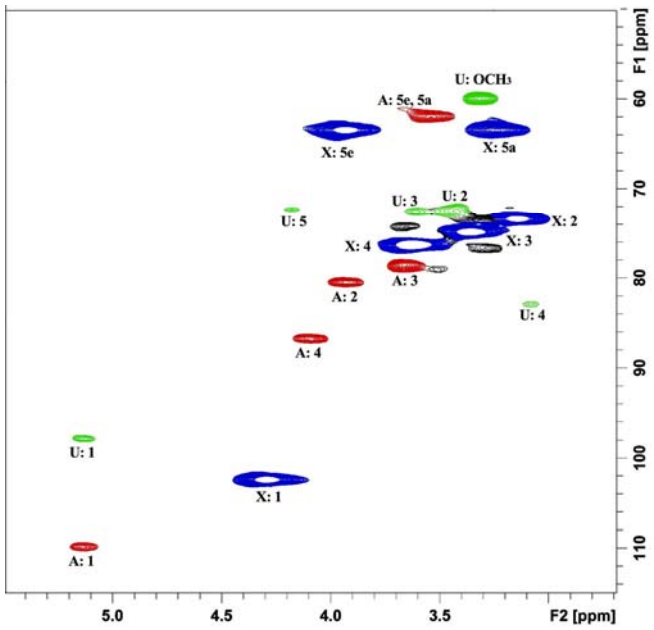

Figure 7: HSQC spectrum of hemicellulosic fraction H_6 (X – (1→4)-β-D-Xylp; U – 4-*O*-Methy-α-D-GlcpA; A – α-L-Araf residues)

CONCLUSIONS

The results obtained by sequential alkali extractions under the conditions given prove that this is an effective process for the fractionation of hemicellulosic polymers from bamboo culms (*Neosinocalamus affinis*). Glucose (82.49%) was found to be the dominant sugar component in the water-soluble hemicellulosic fraction, with a low molecular weight (9000 g/mol) and polydispersity (1.26), probably originating from undigested starch, pectic substances, or *β*-glucan. In comparison, the hemicellulosic fractions isolated under alkaline conditions mainly consisted of xylose (73.53-92.65%). The Xyl/Ara (from 11.0 to 22.9) ratios in the isolated hemicellulosic fractions increased with alkali concentrations, indicating an increasingly linear structure of the hemicelluloses obtained. In short, the alkali-soluble hemicellulosic polymers from bamboo culms (*Neosinocalamus affinis*) had a structure composed of (1→4)-linked *β*-D-xylopranosyl backbone with L-arabinofuranosyl attached to C-2 and/or C-3 and 4-*O*-methylglucuronic acid linked to C-2.

ACKNOWLEDGMENTS: The authors are grateful for the financial support from State Forestry Administration (200804015, 948-2010-4-16), Major State Basic Research Projects of China (973-2010CB732204), National Natural Science Foundation of China (30930073, 30710103906), and China Ministry of Education (111).

REFERENCES

[1] F. M. Squina, A. J. Mort, S. R. Decker and R. A. Prade, *Protein Expres. Purif.*, **68**, 65 (2009).
[2] E. Sjöström, in "Wood chemistry, fundamentals and applications", edited by E. Sjöström, Academic Press, New York, USA, 1981, p. 7.
[3] W. G. Glasser, W. E. Kaar, R. K. Jain and J. E. Sealey, *Cellulose*, **7**, 299 (2000).
[4] U. Edlund and A. C. Albertsson, *J. Bioact. Compat. Pol.*, **23**, 171 (2008).
[5] M. S. Lindblad, J. Sjberg and A. C. Albertsson, in "Materials, Chemicals, and Energy from Forest Biomass", edited by D. S. Argyropoulos and J. Hartman, American Chemical Society, Washing DC, USA, 2007, p. 153.
[6] S. A. Watson, in "Industrial Gums", edited by R. L. Whistler and J. N. BeMiller, Academic Press, New York, USA, 1959, p. 299.
[7] A. Ebringerová and T. Heinze, *Macromol. Rapid Commun.*, **21**, 542 (2000).
[8] R. Brunecky, T. B. Vinzant, S. Porter, B. Donohoe, D. K. Johnson and M. E. Himmel, *Biotechnol. Bioeng.*, **102**, 1537 (2009).
[9] S. G. Allen, D. Schulman, J. Lichwa, M. J. Antal Jr, M. Laser and L. R. Lynd, *Ind. Eng. Chem. Res.*, **40**, 2934 (2001).
[10] I. Gabrielli, P. Gatenholm, W. G. Glasser, R. K. Jain and L. Kenne, *Carbohydr. Polym.*, **43**, 367 (2000).
[11] C. Cara, E. Ruiz, I. Balesteros, M. J. Negro and E. Castro, *Process Biochem.*, **41**, 423 (2006).
[12] A. P. Deshpande, M. Bhaskar Rao and C. Lakshmana

Rao, *J*. *Appl*. *Polym*. *Sci*., **76**, 83 (2000).
[13] M. O. Scurlock, D. C. Dayton and B. Hames, *Biomass Bioenerg*., **19**, 229 (2000).
[14] J. L. Ren and R. C. Sun, in "Cereal straw as a resource for sustainable biomaterials and biofuels; chemistry, extractives, lignins, hemicelluloses and celluloses", edited by R. C. Sun, Elsevier, Oxford, UK, 2010, p. 73.
[15] F. Xu, C. F. Liu, Z. C. Geng *et al*., *Polym*. *Degrad*. *Stabil*., **91**, 1880 (2006).
[16] R. C. Sun, J. M. Fang, J. Tomkinson, Z. C. Geng and J. C. Liu, *Carbohydr*. *Polym*., **44**, 29 (2001).
[17] F. Peng, J. L. Ren, F. Xu, J. Bian, P. Peng and R. C. Sun, *J*. *Agric*. *Food Chem*., **57**, 6305 (2009).
[18] R. C. Sun, J. Tomkinson, P. L. Ma and S. F. Liang, *Carbohydr*. *Polym*., **42**, 111 (2000).
[19] F. M. Gírio, C. Fonseca, F. Carvalheiro *et al*., *Bioresource Technol*., **101**, 4775 (2010).
[20] S. DuPont and R. R. Selvendran, *Carbohydr*. *Res*., **163**, 99 (1987).
[21] R. C. Sun, J. M. Lawther and W. B. Banks, *J*. *Polym*. *Anal*. *Charact*., **4**, 345 (1998).
[22] K. C. B. Wilkie and S. L. Woo, *Carbohydr*. *Res*., **57**, 145 (1977).
[23] C. Maes and J. A. Delcour, *J*. *Cereal Sci*., **34**, 29 (2001).
[24] S. Izydorczyk and C. G. Biliaderis, *Carbohydr*. *Polym*., **28**, 33 (1995).
[25] C. D. Nandini and P. V. Salimath, *Food Chem*., **73**, 197 (2001).
[26] F. Xu, J. X. Sun, Z. C. Geng, C. F. Liu, R. C. Sun, P. Fowler and M. S. Baird, *Carbohydr*. *Polym*., **67**, 56 (2007).
[27] A. U. Buranov and G. Mazza, *Carbohydr*. *Polym*., **79**, 17 (2010).
[28] M. Kačuráková, A. Ebringerová, J. Hirsch and Z. Hromádková, *J*. *Sci*. *Food Agric*., **66**, 423 (1994).
[29] A. Chaikumpollert, P. Methacanon and K. Suchiva, *Carbohydr*. *Polym*., **57**, 191 (2004).
[30] M. Kačuráková and M. Mathlouthi, *Carbohydr*. *Res*., **284**, 145 (1996).
[31] Z. Hromádková and A. Ebringerová, *Ultrason*. *Sonochem*., **10**, 127 (2003).
[32] M. Kačuráková, P. S. Belton, R. H. Wilson, J. Hirsch and A. Ebringerová, *J*. *Sci*. *Food Agric*., **77**, 38 (1998).
[33] P. Robert, M. Marquis, C. Barron, F. Guillon and L. Saulnier, *J*. *Agric*. *Food Chem*., **53**, 7014 (2005).
[34] A. Ebringerová, Z. Hromádková, J. Alföldi and G. Berth, *Carbohydr*. *Polym*., **19**, 99 (1992).
[35] T. Imamura, T. Watanabe, M. Kuwahara and T. Koshijima, *Phytochemistry*, **37**, 1165 (1994).
[36] A. Kato, J. Azuma and T. Koshuima, *Agric*. *Biol*. *Chem*., **51**, 1691 (1987).
[37] L. Chiarini, P. Cescutti, L. Drigo *et al*., *J*. *Cyst*. *Fibros*., **3**, 165 (2004).
[38] J. P. Roubroeks, R. Andersson and P. Aman, *Carbohydr*. *Polym*., **42**, 3 (2000).
[39] I. Egües, M. G. Alriols, Z. Herseczki, G. Marton and J. Labidi, *J*. *Ind*. *Eng*. *Chem*., **16**, 293 (2010).
[40] M. R. Vignon and C. Gey, *Carbohydr*. *Res*., **307**, 107 (1998).