

ALKALI EXTRACTION AND PHYSICOCHEMICAL CHARACTERIZATION OF HEMICELLULOSES FROM YOUNG BAMBOO (*PHYLLOSTACHYS PUBESCENS MAZEL*)

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Two hemicellulose fractions were obtained by extraction of one-month-old young bamboo (*Phyllostachys pubescens* Mazel). The fractionation procedure employed 2% NaOH as extractant, followed by filtration, acidification, precipitation, and washing with 70% ethanol solution. The total yield was 26.2%, based on the pentosan content in bamboo. The physicochemical properties were determined and sugar composition analysis showed that the hemicelluloses consisted mainly of xylose, arabinose, galactose, and a small amount of uronic acid. Furthermore, based on FT-IR and NMR spectra analyses, the structure of hemicelluloses was determined to be mainly arabinoxylans linked via (1→4)-β-glycosidic bonds with branches of arabinose and 4-O-methyl-D-glucuronic acid. The molecular weights were 6387 Da and 4076 Da, corresponding to the hemicelluloses HA and HB. Finally, the thermal stability was elucidated using the TG-DTG method. The obtained results can provide important information for understanding young bamboo and the hemicelluloses in it.

Keywords: Young bamboo; Hemicelluloses; Physicochemical characterization; Arabinoxylans

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INTRODUCTION

In recent centuries, humans have exploited fossil and mineral resources, resulting in a global energy crisis and a multitude of environmental problems. Therefore, new and renewable sources of energy, materials, and fine chemicals have attracted attention as a substitute for fossil resources. Lignocellulosic biomass from cereal straws, trees, and bamboo, *etc.* represent an abundant renewable energy source for bioconversion processes, as well as raw material for the chemicals industry and other uses in manufacturing. Bamboo can be described as a large woody grass of a particular taxonomic group (family Poaceae, subfamily Bambusoideae) that is widely distributed in Asian countries, including China and other Southeast Asian countries (Wen *et al.* 2011). So far, bamboo has mainly been used in construction and as reinforcing fibers in paper, textiles, and construction boards, as well as in the food industry (Scurlock *et al.* 2000). However, with the progress of the bamboo processing technology, many new approaches for utilization have been explored in recent years (Walter 2001), such as bioethanol production (Sun *et al.* 2011), activated carbon production (Mui *et al.* 2010), and polyurethane foam (Gao *et*

al. 2010). In Latin America, Africa, as well as in Europe and North America, people are paying more and more attention to bamboo research and utilization (Walter 2001).

Hemicelluloses are considered to be the second most abundant polysaccharides in nature, after cellulose. According to Scurlock *et al.* (2000), bamboo contains cellulose (40-48%), hemicelluloses (25-30%), and lignin (11-27%). Thus, it is potentially beneficial to explore further utilization of hemicelluloses from bamboo. However, in order to do this, it is necessary to know more about the chemical and physical characteristics of bamboo hemicelluloses. Although many studies have been carried out to investigate the physicochemical characteristics of hemicelluloses from different bamboo (Peng *et al.* 2012a, 2012b, 2012c; Peng *et al.* 2011a, 2011b; Wen *et al.* 2011; Yang *et al.* 2013), little information about hemicelluloses from young bamboo (*Phyllostachys pubescens* Mazel) aged one month has been reported. Some compounds from the young bamboo aged one month may be used as a functional food ingredient, such as xylooligosaccharides from hemicelluloses. Therefore, characterization of hemicelluloses from one-month-old bamboo (*Phyllostachys pubescens* Mazel) is of great importance for exploring and promoting the potential utilization of this kind of biomass. This research is one of part of our series concerning extraction and characterization of hemicelluloses from bamboo (*Phyllostachys pubescens* Mazel) of different ages.

In the present study, hemicelluloses were extracted from one-month-old bamboo (*Phyllostachys pubescens* Mazel) with an alkaline solution. The obtained hemicelluloses were characterized by sugar analysis, gel permeation chromatography (GPC), UV-vis absorption spectra, Fourier transform infrared (FT-IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and thermal stability analysis.

EXPERIMENTAL

Materials

One-month-old bamboo was obtained from a farm (Guanxi Village, Meiling Town, Nanchang City, China). After being fully dried in sunlight, the bamboo was cut into small pieces and automatically ground into powder without addition of any liquid. The outer and inner layers were not removed before grinding. After having passed through a 40-mesh screen followed by a 100-mesh screen, the bamboo powder was dried in an oven at 50 °C for 12 h prior to use. The content of pentosans in the fully dried young bamboo powder was determined to be 36.3% according to the standard methods (GB 2677.9-94, China). All chemicals used were of analytical grade and purchased from Tianjin Chemical Reagent Factory (Tianjin, China).

Methods

Isolation of hemicelluloses from bamboo

The method for hemicellulose fractionation from young bamboo was adapted from our earlier works (Peng *et al.* 2012a, 2012b) with partial modification. The scheme for hemicellulose isolation from young bamboo is shown in Fig. 1. The bamboo powder was first extracted with toluene-ethanol (2:1, v/v) for 6 h in a Soxhlet apparatus. The dewaxed sample was treated with sodium chlorite in an acidic solution (pH 4.2 to 4.7, adjusted with acetic acid) for 6 h at 75 °C. The obtained holocellulose was subsequently

extracted with hot distilled water and 2% NaOH aqueous solution. Finally, two alkali-soluble hemicellulosic fractions, HA and HB, were obtained.

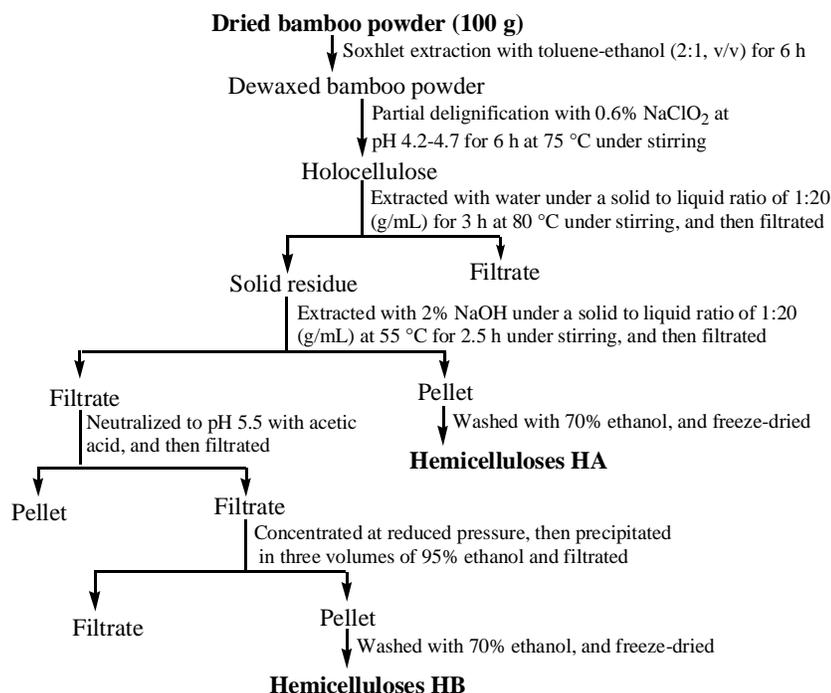


Fig. 1. Scheme for alkali extraction of hemicelluloses from one-month-old bamboo

Physicochemical characterization

Physicochemical characterization of the isolated hemicellulosic fractions, including the total uronic acid content, the neutral sugar compositions, FT-IR analysis, NMR analysis, molecular weight measurement, and thermal stability analysis were carried out according to the methods given in earlier works (Peng *et al.* 2012b, 2012c).

The total uronic acid content was analyzed by the *m*-hydroxybiphenyl method (Blumenkrantz and Asboe-Hansen 1973). For the determination of neutral sugar compositions of the five hemicellulosic fractions, 20 mg samples were hydrolysed using 3.0 M trifluoro acetic acid (TFA) at 120 °C for 3 h in a 30 mL pressure vessel. The hydrolysates were then diluted with anhydrous ethanol, and the TFA was removed using a rotary vacuum evaporator at 45 °C. Anhydrous ethanol was then added to the solids and this was followed by a re-evaporation. This procedure was repeated several times until the hydrolysates obtained were neutral. The dry hydrolysate solids were finally converted into their TMS derivatives and analyzed by gas chromatography-mass spectrometry (GC-MS) (Peng *et al.* 2010). GC-MS analyses were performed on an Agilent 5973 GC-MS device, equipped with a HP-5MS column (30.0 m×0.25 mm×0.25 mm), and an ICIS data system, with He as the carrier gas, and a gas flow rate of 1.0 mL/min. Mass spectra were obtained by electron impact (EI) ionization at 70 eV. The oven temperature was programmed for: 2 min at 70 °C, 10 °C /min to 100 °C, 6 °C /min to 260 °C, and then held for 5 min at 260 °C.

For FT-IR measurements, hemicellulose samples were blended with KBr to form pellets, and the spectra were obtained on a Nicolet 7500 FT-IR spectrophotometer (Thermo Nicolet Corporation, USA) in the range of 4000 to 400 cm^{-1} at 4 cm^{-1} resolution.

The solution-state ^1H NMR, and ^{13}C NMR spectra were recorded at 30 °C on a Bruker MSL-600 MHz spectrometer (Bruker Corporation, Germany) with a 5 mm-PABBO probe head. The ^1H NMR spectra were recorded at 600.5 MHz using 20 mg of fully freeze-dried hemicelluloses in 1.0 mL of DMSO- d_6 using tetramethylsilane (TMS) as internal standard. The ^{13}C NMR spectra were recorded at 100.6 MHz, and TMS was used as internal standard ($\delta=0$ ppm) after 45,000 scans. Before the ^{13}C NMR analysis, the hemicellulose sample was dissolved in 1.0 mL DMSO- d_6 to saturated state. The spectral widths were 9615.385 Hz for ^1H NMR and 36057.691 Hz for ^{13}C NMR, respectively.

Thermal analysis of the hemicelluloses was evaluated by thermogravimetric analysis (TG) and differential thermal analysis (DTA) carried out on a TG/DTA PYRIS DIAMOND instrument (PE Corporation, USA). The measurements were recorded under nitrogen atmosphere at a flow rate of 50 mL/min and at a heating rate of 10 °C /min from room temperature up to a final temperature of 700 °C. Approximately 2.9 to 11.1 mg of hemicelluloses were used. Calcined Al_2O_3 was used as a reference material in all experiments.

The molecular weight (M_w) measurements of hemicelluloses were determined by gel permeation chromatography (GPC). This analysis was conducted on an Agilent HPLC system (Agilent 1200 series, USA) with a PL aquagel-OH 40 column (7.5 × 300 mm). The samples were dissolved with 0.02 M NaCl in 0.005 M sodium phosphate buffer, pH 7.5, at a sample concentration of 0.1%, and 100 μL of this solution was injected. Before injection, the sample solution was filtered through a 0.45 μm microporous cellulose membrane filter. The column was operated at 30 °C and eluted with 0.02 M NaCl in 0.005 M sodium phosphate buffer, pH 7.5, at a flow rate of 0.5 mL/min. The eluent was monitored with a refractive index detector (BI-dn dc /GPC RID). The M_w distribution was determined using dextran (Sigma-Aldrich) with M_w of 1270, 5220, 11600, 23800, 48600 Da as M_w standards. The five dextran standards were eluted, resulting in a log-linear correlation between molecular weight and elution time. The obtained linear regression equation was $Y=-1.0834X+26.5415$ ($R^2=0.9626$). Therefore, rough molecular weights of hemicelluloses could be estimated according to this relation.

UV-vis absorption spectra were obtained using a LabTech 300563 UV-visible spectrophotometer within the range of 200 to 800 nm. Before UV-vis measurements, the hemicellulose samples were dissolved in distilled water.

RESULTS AND DISCUSSION

Yield and Sugar Composition of Hemicelluloses

The yields of the hemicellulosic fractions are given in Table 1. The yield of the hemicellulosic fraction HA (17.7%) was higher than that of the fraction HB (8.5%). The total hemicelluloses yield amounted to 26.2%, based on the content of pentosan in the fully dried one-month-old bamboo raw material. As shown in Table 1, xylose was the predominant sugar constituent in both HA (82.9%) and HB (56.6%). Xylose probably originated from the backbone of the xylans. The hemicellulosic fraction HA contained a much higher arabinose content (14.9%) than HB (7.8%), but the fraction HB contained a higher content of galactose (23.6%). Glucose was also detected in the HB hemicelluloses,

amounting to 12.1%. For both fractions HA and HB, uronic acids were determined to be a small amount. Those uronic acids possibly originated from 4-O-methyl glucuronic acids as side chains. Based on the calculated ratio of xylose to arabinose (xyl/ara), the ratio for the HA hemicelluloses was 5.6, which was smaller than the ratio for the HB hemicelluloses (7.3). It suggested that HA had more branches than HB.

Table 1. Yield of Isolated Hemicellulosic Fractions and the Contents of Neutral Sugars (Relative %) and Uronic Acids (% Dry Sample, w/w)

Samples	Yield (%) ^a	Sugar composition (%)				Uronic acid (%)	Xyl/ara
		Xylose	Arabinose	Glucose	Galactose		
HA	17.7	82.9	14.9	n.d.	2.2	2.0	5.6
HB	8.5	56.6	7.8	12.1	23.6	3.0	7.3
Total	26.2						

^a Based on the content of pentosan in the fully dried raw material young bamboo sample
n.d.- not determined

UV-vis Spectra Analysis

The UV-vis spectra of bamboo hemicelluloses are given in Fig. 2. The two spectra were similar. Both the spectra showed a maximum at 232 nm, and a shoulder at 280 nm. The absorption at 280 nm was attributed to the $\pi \rightarrow \pi^*$ electronic transition in the aromatic ring of the un-conjugated phenolic units, indicating that free and etherified OH groups resulting from the relatively high proportion of guaiacy units in bamboo lignin were determined (Li *et al.* 2010, 2012). The absorption at 312 nm originated from the $n \rightarrow \pi^*$ transition in lignin units containing $C_{\alpha}=O$ groups and $\pi \rightarrow \pi^*$ transition in lignin units with $C_{\alpha}=C_{\beta}$ linkages conjugated to the aromatic ring (Li *et al.* 2010, 2012) was absent. This result was not in agreement with that reported by Li *et al.* (2010), in which bamboo lignin was extracted directly with sodium hydroxide /urea. A possible reason was that the *p*-coumaric and ferulic acids type structures were destroyed during partial delignification with 0.6% NaClO₂ at pH 4.2 to 4.7 for 6 h at 75 °C under stirring.

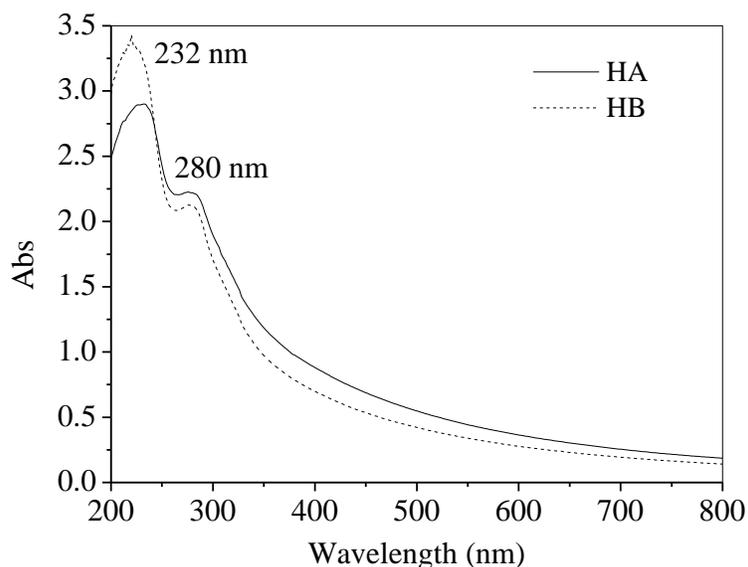


Fig. 2. UV-vis spectra of bamboo hemicelluloses separated

FT-IR Spectra Analysis

Figure 3 shows the FT-IR spectra of the alkali hemicellulosic fractions HA and HB. The two fractions exhibited similar FT-IR characteristics. The absorbance at about 3400 cm^{-1} was attributed to the stretching of $-\text{OH}$ groups. The $\text{C}-\text{H}$ stretching vibration gave a signal at 2932 cm^{-1} . The absorbance at 1571 cm^{-1} possibly originated from aromatic skeletal vibrations in associated lignin (Vazquez *et al.* 1997). The absorbances at 1414 , 1327 , 1255 , 1164 , 1044 , 984 , and 897 cm^{-1} were associated with hemicelluloses. The bands at 1414 , 1327 , and 1255 cm^{-1} represented the $\text{C}-\text{H}$, $\text{C}-\text{C}$, and $\text{C}-\text{O}$ bending or stretching frequencies. The bands between 1130 cm^{-1} and 1000 cm^{-1} were typical of xylans (Xiao *et al.* 2001). The presence of arabinosyl side chains was indicated by low-intensity shoulders at 1164 and 984 cm^{-1} (Kačuráková *et al.* 1998). The predominant absorbance at 1044 cm^{-1} was attributed to $\text{C}-\text{O}-\text{H}$ bending modes (Kačuráková *et al.* 2000). Moreover, the most important band observed at about 897 cm^{-1} , corresponded to the glycosidic $\text{C}1-\text{H}$ deformation mode with ring vibration contribution for the β -anomer form of the pyranoid ring, which is characteristic of β -glycosidic linkages between the sugar units (Peng *et al.* 2011a).

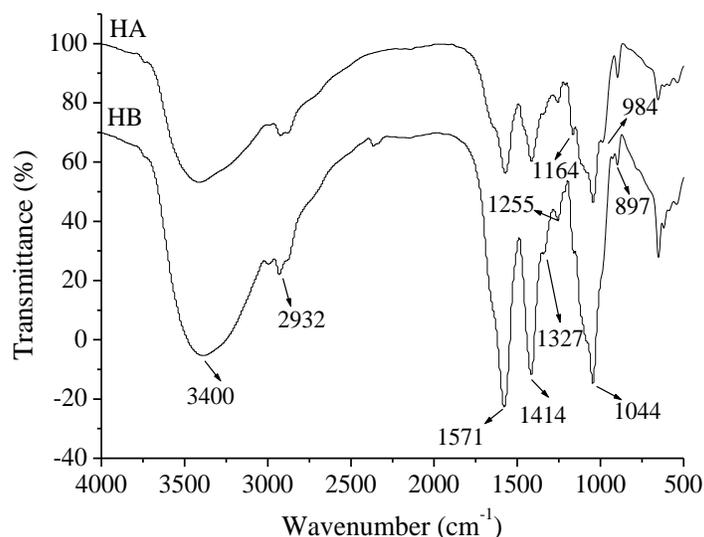


Fig. 3. FT-IR spectra of the hemicellulosic fractions HA and HB

NMR Spectra

^1H NMR spectra of the hemicellulosic fractions HA and HB are shown in Fig. 4. The two spectra were almost similar, suggesting that the fraction HA had a similar backbone to the hemicellulosic fraction HB. The anomeric protons of the terminal arabinose linked to O-3 of xylans were confirmed by resonances at about 5.33 ppm (Cyran *et al.* 2003; Wen *et al.* 2011). The signal at about 4.27 ppm corresponded to the β anomeric $\text{C}1$ protons of the anhydroxylose units of hemicelluloses. The chemical shifts ranging from 3.00 ppm to 4.00 ppm were assigned to the protons of xylose and arabinose units of the hemicelluloses. The signals at about 3.97 (H-5eq), 3.83 (H-4), 3.27 (H-5ax), and 3.17 ppm (H-2) were associated with non-substituted $(1\rightarrow4)\text{-}\beta\text{-D}$ -xylose residues. The signals at about 4.27 (H-5), 3.87 (H-3), and 3.56 ppm (H-2), originated from 4-O-methyl- $\alpha\text{-D}$ -glucuronic acid residues (Ebringerová *et al.* 2000). The strong signal due to $-\text{CH}_3$ from the acetate contaminant presented at about 1.67 ppm . The signal at about 2.52 ppm was ascribed to the DMSO solvent (Wang *et al.* 2012).

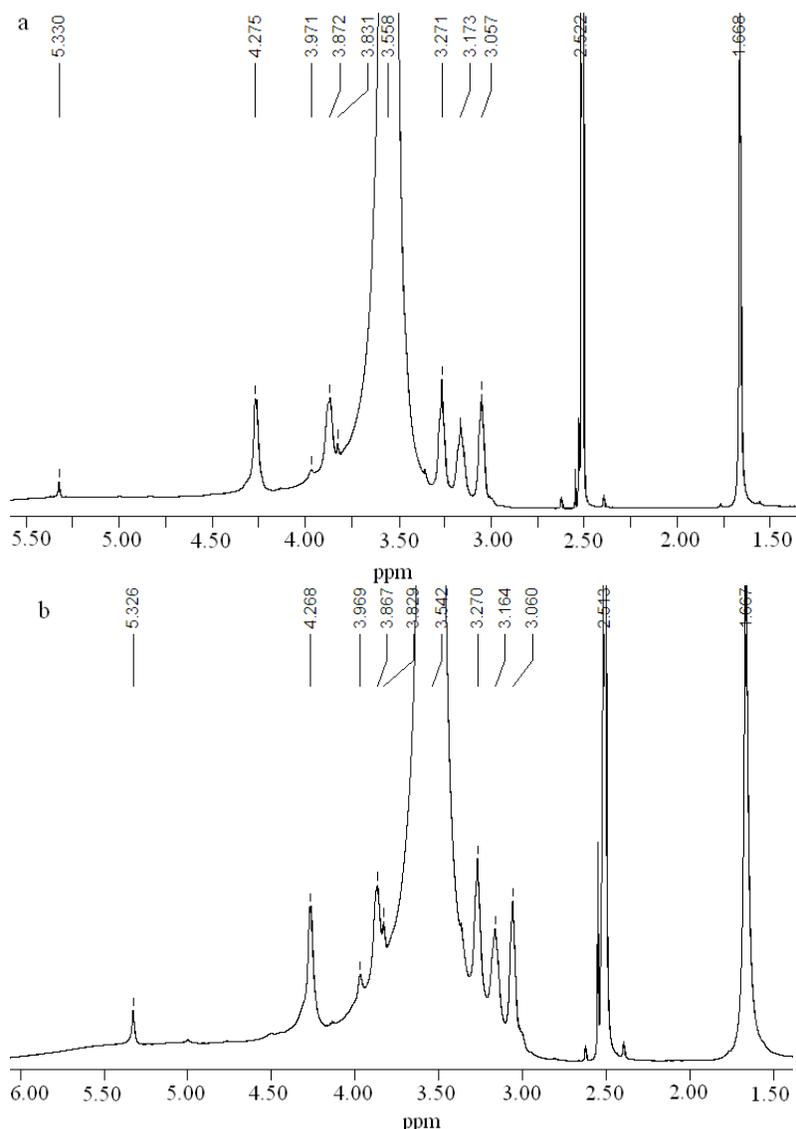


Fig. 4. ^1H NMR spectra of the hemicellulosic fractions HA (a) and HB (b)

The ^{13}C NMR spectra of the hemicellulosic fractions HA and HB are given in Fig. 5. Both fractions HA and HB (Fig. 5) showed a signal at about 175 ppm, which was characteristic of $-\text{COOH}$ at C-6 (Peng *et al.* 2011a, b). The five main observed signals at about 102.21, 76.82, 74.46, 73.43, and 63.75 ppm corresponded to the C-1, C-4, C-3, C-2, and C-5 of (1 \rightarrow 4)-linked- β -D-xylopyranosyl units, respectively (Fig. 5a) (Xu *et al.* 2007; Chaa *et al.* 2008). The signals at about 107.64 (C-1), 80.97 (C-2), 78.34 (C-3), 86.48 (C-4), and 61.75 ppm (C-5) were attributed to α -L-arabinofuranosyl units linked to β -D-xylans (Roubroeks *et al.* 2000; Sun *et al.* 2005; Chaa *et al.* 2008; Peng *et al.* 2011a). The strongest signal at about 39 ppm was assigned to the solvent DMSO-d_6 (Wang *et al.* 2012; Yang *et al.* 2013). At the same time, the ^{13}C NMR spectrum of the fraction HB (Fig. 5b) showed similar NMR signals as fraction HA. According to FT-IR, ^1H NMR, and ^{13}C NMR results, the hemicellulosic fractions HA and HB presented similar chemical structures, consisting mainly of arabinoxylans.

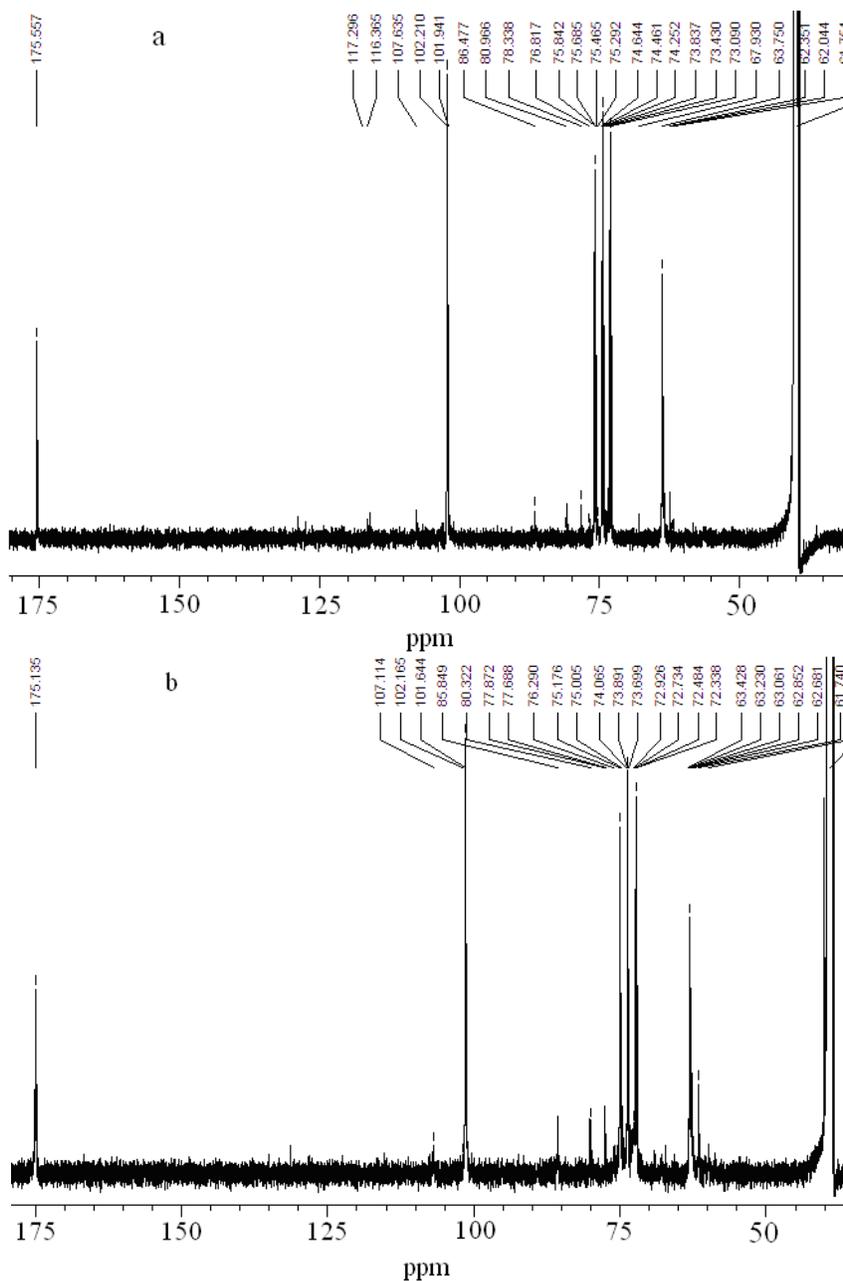


Fig. 5. ^{13}C NMR spectra of the hemicellulosic fractions HA (a) and HB (b)

Average Molecular Weight

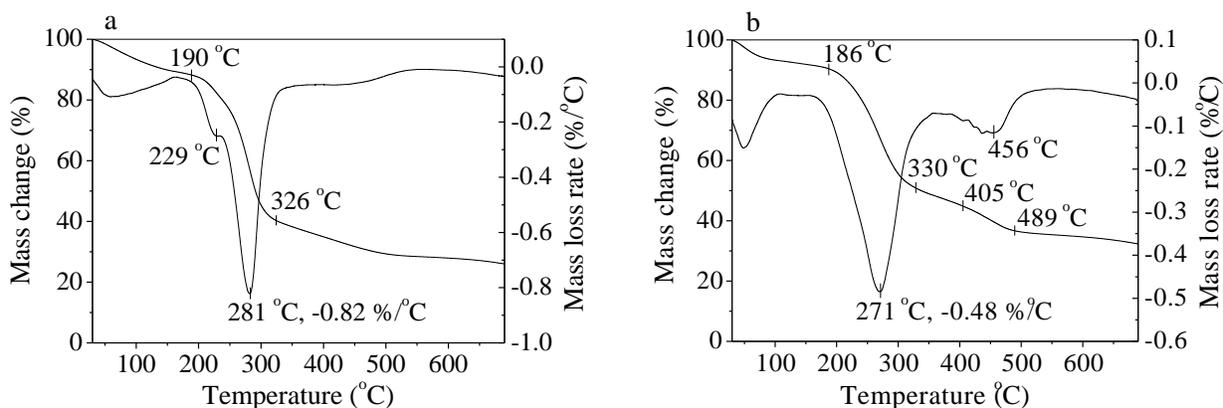
In order to determine the average molecular weights of the alkali-extracted hemicelluloses, the gel permeation chromatography (GPC) method was employed. The GPC results are listed in Table 2. It shows that the molecular weight of the HA hemicelluloses (6387 Da) was higher than that of the HB hemicelluloses (4076 Da). This demonstrated that the fraction HA, which was soluble only at pH values higher than 5.5, presented a higher average molecular weight than the fraction remaining soluble in an alkaline solution up to pH 5.5.

Table 2. Average Molecular Weight

	Standard (glucan)					Samples	
	1	2	3	4	5	HA	HB
Elution volume (mL)	21.499	21.189	20.866	20.483	20.082	20.986	21.166
IgM	3.1038	3.7177	4.0645	4.3766	4.6866	3.8053	3.6103
Average molecular weight (Da)	1270	5220	11600	23800	48600	6387	4076

Thermal Properties

The thermal properties of the hemicelluloses were investigated using the thermogravimetric analysis and derivative thermogravimetry (TG-DTG) technique in the temperature range of 30 °C to 690 °C. The TG-DTG curves are shown in Fig. 6, and the results are reported in Table 3 and Table 4. The pyrolysis of hemicelluloses fractions HA and HB could be divided into three stages: loss of water stage (< 190 °C), main pyrolysis stage (186 to 330 °C), and charring (> 330 °C). The maximum decomposition rate of HA occurred at 281 °C, whereas that of HB was at 271 °C. The weight losses observed within the fast pyrolysis temperature range were 48.04% for the HA hemicelluloses and 39.94% for the HB hemicelluloses, respectively. The corresponding maximum pyrolysis rates were 0.82%/°C and 0.48%/°C, as shown in Table 4. According to the TG-DTG curves of the HB hemicelluloses, there was another weight loss stage between 405 °C and 489 °C, with a corresponding maximum weight loss rate at 456 °C. This weight loss could be attributed to the further decomposition of carbon residues produced at lower temperatures. The total weight losses of 74.01% and 67.68% were observed at the final temperature of 690 °C for HA and HB, respectively (Table 3). The difference in the number of branches on the backbone chains of hemicelluloses possibly resulted in the difference in total weight losses and thermal properties.

**Fig. 6.** TG-DTG curves of hemicellulosic fractions HA (a) and HB (b)**Table 3.** Temperature Ranges and Weight Losses of Hemicellulosic Fractions

Samples	Stage 1		Stage 2		Stage 3		Total weight loss (%)
	Temperature range (°C)	Weight loss (%)	Temperature range (°C)	Weight loss (%)	Temperature range (°C)	Weight loss (%)	
HA	< 190	11.74	190-326	48.04	326-690	14.23	74.01
HB	< 186	9.37	186-330	39.94	330-690	18.41	67.68

Table 4. Characteristics of Hemicellulosic Fractions in the Temperature Range for Fast Pyrolysis

Samples	Temperature range for fast pyrolysis (°C)	T_{peak} (°C)	Weight loss (%)	Maximum weight rate (%/°C)
HA	190-326	281	48.04	0.82
HB	186-330	271	39.94	0.48

CONCLUSIONS

1. Two alkali-soluble hemicellulose fractions labeled HA and HB with a total yield of 26.2% were isolated from one-month-old young bamboo (*P. pubescens* Mazel). After a sequence of dewaxing (toluene-ethanol solution), partial delignification (NaClO₂ solution), and water extraction, the solid residue with a little amount of lignin and without water-soluble hemicelluloses was obtained. Then the solid residue was extracted with 2% NaOH. After filtration, the pellet was washed with 70% ethanol solution, and the HA fraction was obtained. The HB fraction was obtained by acidification of the filtrate with acetic acid solution, followed by precipitation with 95% ethanol.
2. Xylose was the main neutral sugar in the two hemicellulosic portions; in the fraction HA, arabinose came next, while for HB hemicelluloses, it was galactose. Neutral sugar composition measurement, FT-IR, and NMR spectra analyses indicated that arabinoxylans were the major polysaccharides in the young bamboo hemicelluloses. The HA hemicelluloses exhibited more branches than the HB hemicelluloses, according to neutral sugar analysis.
3. GPC analysis showed that HA hemicelluloses (6387 Da) had a higher molecular weight than HB hemicelluloses (4076 Da). Thermal stability showed that the main weight loss occurred between 186 °C and 330 °C. The temperature peaks of maximum weight loss rate were at 281 °C and 271 °C for HA and HB, respectively.
4. The hemicelluloses from bamboo can be utilized for many fields, such as hydrogels based on hemicelluloses, papermaking additives, and the food industry. The results obtained in this study can provide important information for exploring further utilization of young bamboo and the hemicelluloses in it.

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