STRUCTURE OF HEMICELLULOSES UPON MATURATION OF BAMBOO (*Neosinocalamus affinis*) CULMS

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The DMSO-soluble hemicelluloses were obtained from the delignified one-, two-, and three-year-old bamboo (*Neosinocalamus affinis*) culms. Chemical composition analysis showed that the content of carbohydrates, lignin, extractives, and ash for differently aged bamboo culms had relatively small differences. The yields of hemicelluloses ranged from 6.53% to 9.78% of the holocellulose by the DMSO extraction. The GPC analysis revealed that the hemicelluloses had relatively high molecular weight and low polydispersity index, which indicated that they had a lower degree of degradation under the treatment with DMSO. The degree of acetylation values provided evidence indicating that DMSO-soluble hemicelluloses were highly acetylated. Based on the analysis of HPAEC, GPC, FT-IR, and NMR, the structure of bamboo hemicelluloses was barely changed during its maturation. The DMSO-soluble hemicelluloses mainly consisted of *O*-acetyl-arabino-4-*O*-methylglucurono-(1→4)-*β*-D-xylan, while relatively higher starch content was present in the DMSO-soluble fractions of the three-year-old bamboo culms.

Keywords: *Neosinocalamus affinis* bamboo, DMSO-soluble hemicelluloses, structural characterization, maturation, starch

INTRODUCTION

Cell walls in higher plants are kinds of composite materials, which consist of a large amount of polymers, including cellulose, non-cellulosic polysaccharides, and polyphenol lignin.[1,2] The polymer type, structure, and abundance can vary greatly depending on the plant species, tissue type, developmental stage, as well as the wall location within a single plant cell.[3] The non-cellulosic polysaccharides contain heterogeneous substituents or other linkages in their backbone, which render them amorphous and mostly soluble in aqueous solution.[4] There is evidence that these polysaccharides can be covalently linked to lignin,[5,6] and to some of the pectic polysaccharides.[7,8] It is also believed that the polysaccharides interact non-covalently with cellulose chains via H-bonds.[9] Some non-cellulosic polysaccharides have been historically grouped into the class of hemicelluloses.[4] In general, hemicelluloses represent around one-third of the dry mass of cell walls in the plant.[10] It is reported that hemicelluloses can be used for gelling agents, tablet binders, xylitol, and wet strength additives in industrial applications.[11] Recently, hemicelluloses have increasingly gained in importance as a basis for new biomaterials and functional polymers accessible by chemical modification.[12]

Bamboo is an important renewable resource, and it has attracted more attention due to its short life cycle.[13] Bamboo is one of the fastest growing plants, and most of the species can attain maturity within five years.[14] In general, the chemical composition of bamboo is similar to that of wood with cellulose, hemicelluloses and lignin accounting for more than 90% of the total mass.[13] The utilization of bamboo is very extensive, approximately 1500 commercial applications of bamboo have been identified, which may be

divided up into five categories: construction materials, paper or paper board, textiles, food, and combustion materials.[14]

Bamboo hemicelluloses are polysaccharides that consist of polymerized monosaccharides, such as arabinose, glucose, galactose, xylose, mannose, fucose, glucuronic acid, and galacturonic acid. Compared with lignin, the hemicelluloses have a lower heating value. Therefore, the conversion of the low heating value hemicelluloses into bioproducts such as polymers presents a great economic opportunity.[15] Researches on the life cycle of an individual culm from its immature stage towards maturation and death become more and more important.[16] The total amount of hemicelluloses was found to increase with increasing maturity of the plant in a previous report.[17] Bamboo hemicelluloses also appear to vary with age, and their structure might change as a function of the culm segment from which they were extracted. Suzuki *et al.*[18] studied the variation of hemicelluloses during tissue development by using immunohistochemical localization and found that the hemicelluloses varied at different stages of cell walls in the bamboo culms.

Neosinocalamus affinis is a bamboo species with large area and high economic value in Sichuan Province, China. It grows extremely fast, and its height can reach 10 meters within merely 100 days from unearthed bamboo shoots if the condition allows. However, few studies have been done on the formation and structure of *Neosinocalamus affinis* bamboo hemicelluloses during maturation. The purpose of this study is to determine the structural characteristics of hemicelluloses extracted by DMSO from one-, two-, and three-year-old *Neosinocalamus affinis* bamboo culms.

EXPERIMENTAL

Materials

Neosinocalamus affinis bamboo culms for this study were harvested in August 2013 from Sichuan Province, China. Several random bamboo culms were picked to represent three age groups (one-, two-, and three-year-old). After removal of leaves and epidermis, three sections (bottom, middle, and top) of the internodes were equally selected at the same height of each group. In other words, they were totally divided into nine sections, and the nine sections were designated as 1-1, 1-2, 1-3, 2-1, 2-2, 2-3, 3-1, 3-2, and 3-3, respectively. The marking scheme is shown in Fig. 1. The nine sections of the bamboo culms were dried at 60 ^{o}C for 16 h, then cut into small pieces (1-3 cm), and ground with a micro plant grinding machine. The 40-60 mesh powder was sifted, and dewaxed with toluene-ethanol (2:1 v/v) in a Soxhlet apparatus for 6 h. The dewaxed samples were further dried in a cabinet oven. All chemicals used were of analytical or reagent grade.

Extraction of hemicelluloses

To study the structural differences, the hemicelluloses were extracted by dimethyl sulfoxide (DMSO) in this study. DMSO is a common neutral solvent that has been applied to extract hemicelluloses from holocellulose without cleaving chemical linkages.[19] In other words, it can keep the original structure of hemicelluloses to a great extent. The extraction of hemicelluloses from *Neosinocalamus affinis* is illustrated in Fig. 2. The dewaxed powder (25.0 g) was delignified with 6% sodium chlorite at pH 3.6-3.8, adjusted with acetic acid, at 78 ^{o}C for 2 h. The residue, holocellulose, was subsequently washed with distilled water and ethanol, and dried at 60 ^{o}C for 16 h.

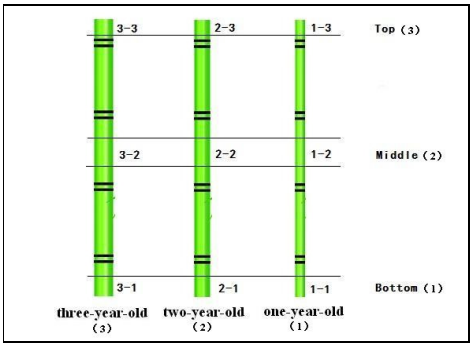

Figure 1: Scheme of marking *Neosinocalamus affinis* samples

Then the holocellulose (15.0 g) was extracted by DMSO with a solid to liquid ratio of 1/25 (g/mL) at 80 ^{o}C for 7 h under stirring. After treatment of DMSO, the insoluble residues were collected by filtration, washed

with distilled water, and then dried at 60 °C for 16 h. The filtrate was concentrated at reduced pressure, and then mixed with three volumes of 95% ethanol. After stirring and standing for 12 h, the precipitated hemicelluloses were obtained by centrifugation (3500 r.p.m, 10 min), and freeze-dried. The hemicelluloses were labeled as $H_{1\text{-}1}$, $H_{1\text{-}2}$, $H_{1\text{-}3}$, $H_{2\text{-}1}$, $H_{2\text{-}2}$, $H_{2\text{-}3}$, $H_{3\text{-}1}$, $H_{3\text{-}2}$, and $H_{3\text{-}3}$, respectively.

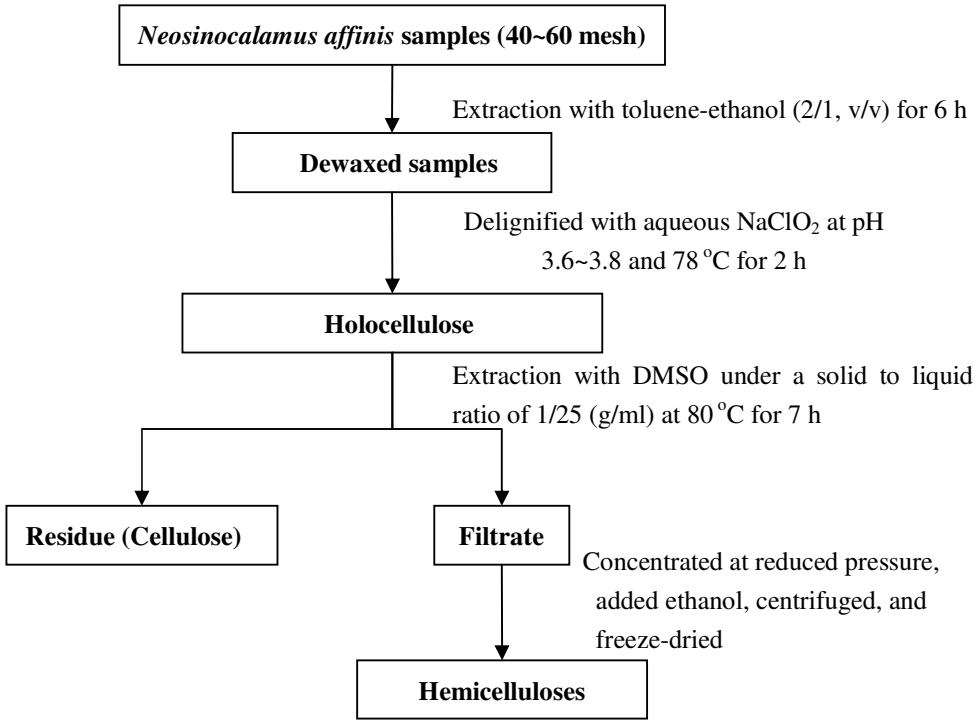

Figure 2: Scheme for the extraction of hemicelluloses from *Neosinocalamus affinis* bamboo culms

Analytical methods

The chemical components of the bamboo culms were determined according to the standard of National Renewable Energy Laboratory (NREL),[20] and the results were expressed on a percentage basis of the oven-dry raw material. The composition of neutral sugars and uronic acids and the molecular weight of the hemicelluloses were determined by high performance anion exchange chromatography (HPAEC) and gel permeation chromatography (GPC) according to a previous study.[21] According to the literature,[11] the physicochemical properties and structural features of the hemicelluloses were evaluated and characterized by Fourier transform infrared (FT-IR), ^{1}H and ^{13}C nuclear magnetic resonance (NMR), as well as by heteronuclear single quantum coherence (HSQC) NMR spectroscopy. The degree of acetylation (DS_{AC}) was determined from the relative intensities of signals of the acetyl group at 2.1 ppm and those of all carbohydrate signals in ^{1}H NMR spectra.[22] The equation was:

$$DS_{AC} = \frac{\frac{\text{Sum of integrals for acetyl groups at 2.1 ppm}}{3}}{\frac{\text{Sum of integrals for carbohydrate signals at 3.0 - 5.5 ppm}}{6}}$$

RESULTS AND DISCUSSION

Chemical composition analysis

The main chemical components of each section are listed in Table 1. As can be seen from Table 1, the relative proportions of the main chemical constituents (glucose 41.75-48.59%, xylose 17.05-20.88%, arabinose 0.71-1.92%, galactose 0.31-0.49%, glucuronic acid 0.51-0.66%, total lignin 24.48-27.14%, extractives 1.20-4.02%, and ash 1.43-5.25%) were similar to those observed in other species of bamboo.[13,22] The contents of ash and extractives decreased with age from one- to two-year-old bamboo culms, while increased to the highest level in the three-year-old bamboo culms. The content of glucose, which stands for cellulose to a certain degree, was accumulated to the highest level in two-year-old bamboo culms, suggesting that the cellulose content at first rose and then reduced upon maturation. The other four sugars (especially xylose and arabinose), partly representing hemicelluloses, showed relatively small differences. The xylose content of the top

section was slightly higher than in the other culm section for all three ages. The content of lignin exhibited an increasing trend with the increase of age roughly, and it was the highest in the bottom and the lowest in the middle sections of the culms of all three ages. The reason is that higher lignin content ensures good bearing capacity and resistance to bending to bottom bamboo sections.

Yield and sugar composition of hemicelluloses

Hemicelluloses are a mixture of a number of different polysaccharides, and the yield and sugar composition of hemicelluloses can vary depending on the method of isolation.[17] It has been proved that the hemicelluloses content of gramineous plants is between 21-28%.[23] DMSO was first used as a solvent for the isolation of hemicelluloses from plant materials by Hägglund *et al*.[24] The yields of hemicelluloses are relatively lower than those of alkali extraction.[11] However, the acetyl groups and the glycosidic linkages are destroyed under the alkaline treatment. Therefore, DMSO was chosen as a suitable solvent for investigating the original structure of hemicelluloses in this study.

Table 1
Main chemical components of bamboo culms from *Neosinocalamus affinis*

	Ash (%)	Extractives (%)	Carbohydrates (%)					Total lignin (%)
			Glucose	Xylose	Arabinose	Galactose	Glucuronic acid	
1-1	2.25	2.25	41.75	18.59	1.02	0.49	0.52	26.25
1-2	2.48	1.80	45.91	19.24	1.92	0.42	0.55	24.48
1-3	1.43	1.44	43.81	20.88	1.01	0.44	0.66	25.47
2-1	1.53	2.17	48.03	18.26	0.71	0.31	0.53	25.32
2-2	2.02	1.04	48.59	18.51	0.75	0.34	0.51	24.76
2-3	2.03	1.20	48.07	20.04	0.83	0.38	0.58	25.06
3-1	2.74	4.02	44.88	18.79	0.79	0.41	0.52	27.14
3-2	3.99	3.70	43.17	17.05	0.72	0.45	0.61	25.08
3-3	5.25	3.10	44.78	19.02	0.86	0.44	0.64	26.72

Table 2
Yields of hemicelluloses from *Neosinocalamus affinis* bamboo culms

Sample	H_{1-1}	H_{1-2}	H_{1-3}	H_{2-1}	H_{2-2}	H_{2-3}	H_{3-1}	H_{3-2}	H_{3-3}
DMSO-extraction Hemicelluloses (%)[a]	9.70	6.98	6.53	9.72	9.09	6.66	7.82	9.59	9.78

[a] Relative to the holocellulose

Table 3
Sugar composition of hemicelluloses from *Neosinocalamus affinis* bamboo culms

	Molar composition (relative%, mol/mol)					Molar ratio	
	Arabinose	Galactose	Glucose	Xylose	Glucuronic acid	Ara/Xyl[a]	GlcA/Xyl[b]
H_{1-1}	7.8	0.9	1.7	86.5	3.1	0.09	0.04
H_{1-2}	7.3	0.6	1.3	88.0	2.8	0.08	0.03
H_{1-3}	6.7	0.7	2.2	87.4	3.0	0.08	0.03
H_{2-1}	6.6	0.5	1.1	88.9	2.8	0.07	0.03
H_{2-2}	7.4	0.6	1.6	87.0	3.3	0.09	0.04
H_{2-3}	6.7	0.6	4.8	84.9	3.0	0.08	0.04
H_{3-1}	6.6	0.6	2.7	87.4	2.7	0.08	0.03
H_{3-2}	5.7	0.6	18.1	73.3	2.3	0.08	0.03
H_{3-3}	3.1	0.5	52.4	42.8	1.2	0.07	0.03

[a] Arabinose to xylose ratio; [b] Glucuronic acid to xylose ratio

As shown in Table 2, the yields of hemicelluloses ranged from 6.53% to 9.78% of the holocellulose. Interestingly, the yields of DMSO-soluble hemicelluloses decreased with the

height of the culms in one- and two-year-old bamboo culms, while increased in three-year-old bamboo culms.

The nine hemicelluloses were hydrolyzed to determine their sugar constituents, and the sugar composition is listed in Table 3. As can be seen from Table 3, xylose was the predominant sugar component (84.9-88.9%) among the five kind of sugars from the one-year-old and two-year-old bamboo hemicelluloses ($H_{1\text{-}1}$ to $H_{2\text{-}3}$), suggesting the presence of a high proportion of xylans. Arabinose appeared as the second major sugar, representing 6.6-7.8% of the total sugars. Glucose (1.1-4.8%), glucuronic acid (2.8-3.3%), and galactose (0.5-0.9%) were identified in an unnoticeable amount. These data implied that the hemicelluloses from *Neosinocalamus affinis* mainly consisted of glucuronoarabinoxylans, which were also found in other bamboo species.[25] Interestingly, significant changes appeared in the hemicelluloses from $H_{3\text{-}1}$ to $H_{3\text{-}3}$. The proportion of glucose increased dramatically from 2.7% to 52.4%, while the corresponding proportion of xylose decreased sharply from 87.4% to 42.8%, which indicated that in addition to xylans, a kind of glucans composed mainly of glucose probably existed in $H_{3\text{-}2}$ and $H_{3\text{-}3}$.

Arabinoxylan (AX) is a dominating member of the hemicelluloses in the primary cell wall of monocots, while less appears in dicotyledonous plants. The linear β-(1→4)-D-Xyl*p* backbone is substituted by α-Ara*f* units at the positions 3-*O* in AX. In addition, the AX is also substituted by α-D-glucopyranosyl uronic unit or its 4-*O*-methyl derivative in the position 2-*O*.[19] The ratios of arabinose to xylose (Ara/Xyl) and glucuronic acid to xylose (GlcA/Xyl) represent the degree of linearity or branching of hemicelluloses.[22] The substitution degree of arabinose on the main chain of the xylans, to a considerable extent determines the solubility of xylan and its ability to combine with cellulose.[26] As can be seen from Table 3, the ratios of Ara/Xyl (0.07-0.09) and GlcA/Xyl (0.03-0.04) were relatively low, indicating that DMSO-soluble hemicelluloses mainly consisted of lowly substituted xylans.

Molecular weight analysis

The weight-average (M_w) and number-average (M_n) molecular weights, as well as polydispersity (M_w/M_n) of the nine hemicelluloses are given in Table 4. All of the values were determined by GPC. The M_w values of the hemicelluloses ranged from 39550 to 47910 g/mol, and the M_w/M_n values of the hemicelluloses ranged from 5.61 to 6.88 in the one-year-old and two-year-old bamboo hemicelluloses. This result indicated that the hemicelluloses had a lower degree of degradation upon the treatment with DMSO. Interestingly, significant changes of M_w and M_w/M_n appeared in hemicelluloses from $H_{3\text{-}2}$ and $H_{3\text{-}3}$. The $H_{3\text{-}2}$ and $H_{3\text{-}3}$ had relatively low molecular weights and high polydispersity index. This result is consistent with the sugar analysis in Table 3, and was probably due to the presence of glucans.

FT-IR spectra analysis

The FT-IR spectra of $H_{1\text{-}1}$, $H_{1\text{-}2}$, and $H_{1\text{-}3}$ are shown in Fig. 3. All of the three spectra show strong absorption at 3436 cm^{-1}, which is attributed to the hydroxyl stretching vibrations, while the absorptions at 2924 cm^{-1} and 2866 cm^{-1} are produced by saturated C–H stretching vibrations. Evidently, the peak at 1321 cm^{-1} represents the C–H bending vibrations.[27] The sharp signal at 1737 cm^{-1} is attributed to –C=O stretching of acetyl groups of the hemicelluloses,[28] which indicates that the hemicelluloses can be extracted without cleaving the acetyl ester groups by the treatment with DMSO.

Table 4
Weight-average (M_w) and number-average (M_n) molecular weights (g/mol) and polydispersity (M_w/M_n) of hemicelluloses from *Neosinocalamus affinis* bamboo culms

	Hemicelluloses								
	$H_{1\text{-}1}$	$H_{1\text{-}2}$	$H_{1\text{-}3}$	$H_{2\text{-}1}$	$H_{2\text{-}2}$	$H_{2\text{-}3}$	$H_{3\text{-}1}$	$H_{3\text{-}2}$	$H_{3\text{-}3}$
M_w (g/mol)	47910	47650	39550	44150	44490	40680	45550	33410	20200
M_n (g/mol)	8180	7910	6680	7130	7930	5910	8870	4460	2505
M_w/M_n	5.85	6.02	5.92	6.19	5.61	6.88	5.14	7.49	8.06

In addition, the peak at 1641 cm^{-1} is assigned to the overlap of the bending mode of water and the $–COO^-$ antisymmetric stretching of glucuronic acid or glucuronic acid carboxylate, which indicates that

hemicelluloses have strong affinity for water and can be easily hydrated.[29,30,31] The signals at 1388 cm^{-1} and 1250 cm^{-1} are assigned to the $–COO^–$ symmetric stretching and the –OH in-plane bending vibrations, respectively.[27,32] Furthermore, the absence of a signal at 1506 cm^{-1} implies that the lignin was almost removed during the previous process.[33] The small band at 1164 cm^{-1} is assigned to the C–O stretching in C–O–C glycosidic linkages, and the contribution of C–OH bending from arabinoxylans and the variation of the signal intensity reflect the degree of substitution by arabinose residues.[28] The strong signal at 1046 cm^{-1} is attributed to the C–OH bending mode.[29] The weak absorption at 902 cm^{-1} is characteristic of β-glycosidic linkages, indicating that the xylose of xylans is linked by a β-glycosidic linkage.[22]

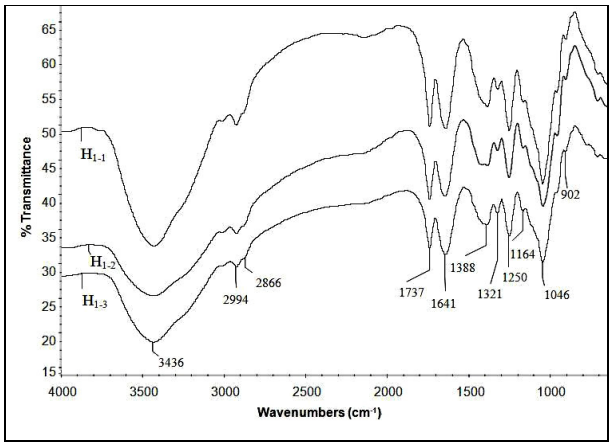

Figure 3: FT-IR spectra of $H_{1\text{-}1}$, $H_{1\text{-}2}$ and $H_{1\text{-}3}$ from one-year-old *Neosinocalamus affinis* bamboo culms

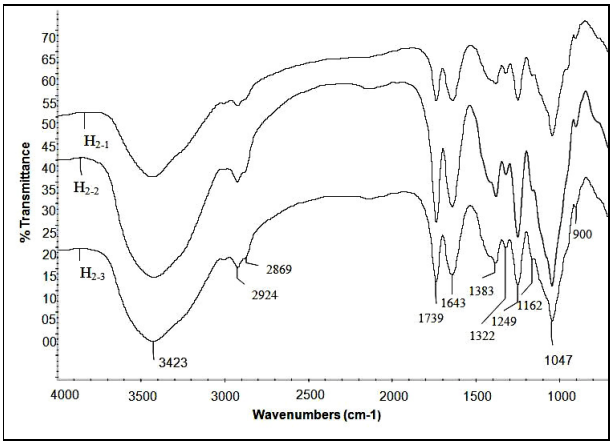

Figure 4: FT-IR spectra of $H_{2\text{-}1}$, $H_{2\text{-}2}$ and $H_{2\text{-}3}$ from two-year-old *Neosinocalamus affinis* bamboo culms

The spectra of the other six hemicelluloses are shown in Fig. 4 and Fig. 5, respectively. Typical signals of hemicelluloses are observed at 3423 cm^{-1}, 2924 cm^{-1}, 2869 cm^{-1}, 1739 cm^{-1}, 1643 cm^{-1}, 1383 cm^{-1}, 1322 cm^{-1}, 1249 cm^{-1}, 1162 cm^{-1}, 1047 cm^{-1}, and 900 cm^{-1} (Fig. 4), as well as 3414 cm^{-1}, 2927 cm^{-1}, 1736 cm^{-1}, 1640 cm^{-1}, 1380 cm^{-1}, 1322 cm^{-1}, 1249 cm^{-1}, 1156 cm^{-1}, 1037 cm^{-1}, and 900 cm^{-1} (Fig. 5). In comparison with other hemicelluloses, the spectrum of $H_{3\text{-}3}$ (seen in Fig. 5) shows a special absorption at 858 cm^{-1}. The signal at 858 cm^{-1} is indicative of α-glycosidic linkages,[34] suggesting that $H_{3\text{-}3}$ probably consisted of glucans, which were linked by α-glycosidic linkages. This result is in good agreement with the results of sugar and molecular weight analysis.

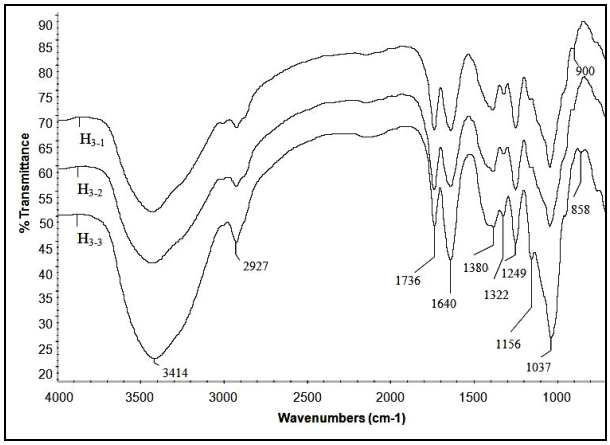

Figure 5: FT-IR spectra of $H_{3\text{-}1}$, $H_{3\text{-}2}$ and $H_{3\text{-}3}$ from three-year-old *Neosinocalamus affinis* bamboo culms

Table 5
Degree of acetylation (DS_{AC}) of hemicelluloses from *Neosinocalamus affinis* bamboo culms

	$H_{1\text{-}1}$	$H_{1\text{-}2}$	$H_{1\text{-}3}$	$H_{2\text{-}1}$	$H_{2\text{-}2}$	$H_{2\text{-}3}$	$H_{3\text{-}1}$	$H_{3\text{-}2}$	$H_{3\text{-}3}$
DS_{AC}	0.385	0.385	0.391	0.402	0.382	0.387	0.373	0.338	0.319

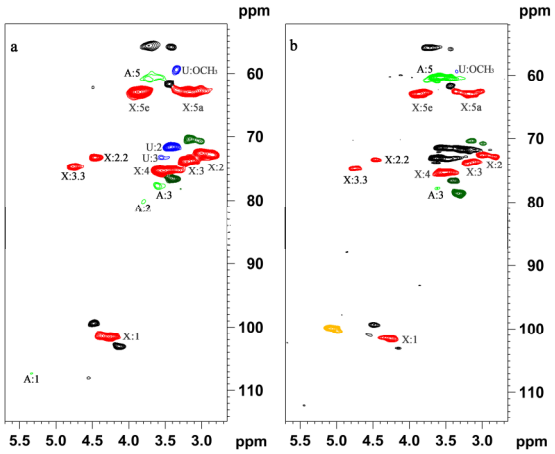

Figure 6: HSQC spectra of $H_{3\text{-}1}$ (spectrum a) and $H_{3\text{-}3}$ (spectrum b)

NMR spectra analysis

The ^{1}H NMR spectra can be used to calculate the degree of acetylation. The calculation method is dividing the relative intensities of signals at 2.1 ppm by those of all carbohydrate signals. The results of DS_{AC} are listed in Table 5. High values of DS_{AC} (0.319-0.402) indicated that the hemicelluloses extracted by DMSO were highly acetylated. However, the value of DS_{AC} in $H_{3\text{-}2}$ and $H_{3\text{-}3}$ were relatively lower, which was due to the presence of the low acetylated sugar in hemicelluloses of the three-year-old bamboo culms.

The HSQC NMR spectra of $H_{3\text{-}1}$ and $H_{3\text{-}3}$ are shown in Fig. 6, and the chemical shift assignments are given in Table 6. As can be seen from Fig. 6 and Table 6, the marked $^{1}H/^{13}C$ cross peaks at 4.26/101.6, 5.32/107.2, and 3.34/71.6 ppm confirmed the structural unit of the (1→4)-*β*-D-xylan backbone and Ara*f* residues at *O*-3, 4-*O*-Me-*α*-D-Glc*p*A units at position *O*-2. The cross peak at 4.42/73.1 ppm is attributed to H-3/C-3 atoms as a result of acetylation at position 3 of 1,4-linked *β*-Xyl*p* residues. Similarly, the cross peak at 4.75/74.5 ppm is due to H-2/C-2 atoms of acetyl 1,4-linked *β*-Xyl*p* units, which are substituted at position 2 by acetyl groups.[31] On the

basis of the NMR analysis, it can be concluded that $H_{3\text{-}1}$ and $H_{3\text{-}3}$ mainly consisted of *O*-acetyl arabino-4-*O*-methylglucurono-(1→4)-*β*-D-xylan, but a new signal at 5.09/99.7 ppm was present in the $H_{3\text{-}3}$ spectrum (Fig. 6b).

In order to further determine the new signal, $H_{3\text{-}3}$ was fractionated by dissolving in the distilled water. The scheme for the fractionation of $H_{3\text{-}3}$ is described in Fig. 7. The water-soluble subfraction and water-insoluble subfraction were obtained, labeled as $H_{3\text{-}3a}$ and $H_{3\text{-}3b}$, respectively. The sugar analysis results for $H_{3\text{-}3a}$ and $H_{3\text{-}3b}$ are shown in Table 7. As can be seen from Table 7, in $H_{3\text{-}3a}$, the glucose content was 3.54%, and the xylose content was 86.23%, which was similar to the results of $H_{3\text{-}1}$ in Table 3. However, in $H_{3\text{-}3b}$, the glucose content was 86.21%, and the xylose content was 11.48%, which was close to the results of $H_{3\text{-}3}$ in Table 3. Xylose was the major component of water-soluble $H_{3\text{-}3a}$, while glucose was the major component of water-insoluble $H_{3\text{-}3b}$. Based on the FT-IR and NMR results, the new signal originated from $H_{3\text{-}3b}$. In addition, the sample $H_{3\text{-}3b}$ turned to an intense blue-black color by adding the Iodine-KI reagent.

Table 6
^{1}H and ^{13}C chemical shift (ppm) assignments for $H_{3\text{-}1}$

Sugar residue	Chemical shift (ppm) H/C							
	1	2	3	4	5ax[a]	5eq[b]	6	OCH_3
→4)-*β*-Xyl*p*(1→	4.26 101.6	3.00 72.5	3.20 73.8	3.53 75.4	3.15 63.0	3.88 63.0		
α-GlcA*p*-(1→2		3.43 71.6	3.56 73.2					3.32 59.5
α-Ara*f*-(1→3	5.32 107.2	3.80 80.1	3.61 77.8		3.70 60.9	3.73 60.9		
→4)-*β*-Xyl*p*(1→, 3-*O*-Ac			4.42 73.1					
→4)-*β*-Xyl*p*(1→, 2-*O*-Ac		4.75 74.5						

[a] ax = axial; [b] eq = equatorial

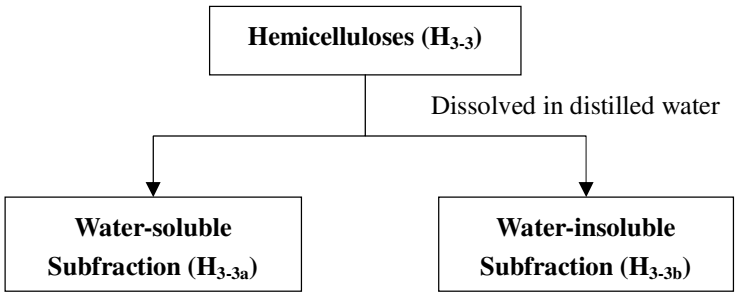

Figure 7: Scheme for fractionation of $H_{3\text{-}3}$

Table 7
Sugar composition of $H_{3\text{-}3a}$ and $H_{3\text{-}3b}$

	Molar composition (relative%, mol/ mol)					Molar ratio	
	Arabinose	Galactose	Glucose	Xylose	Glucuronic acid	Ara/Xyl	GlcA/Xyl
$H_{3\text{-}3a}$	6.56	0.92	3.54	86.23	2.75	0.076	0.032
$H_{3\text{-}3b}$	0.81	0.18	86.21	11.48	0.76	0.072	0.066

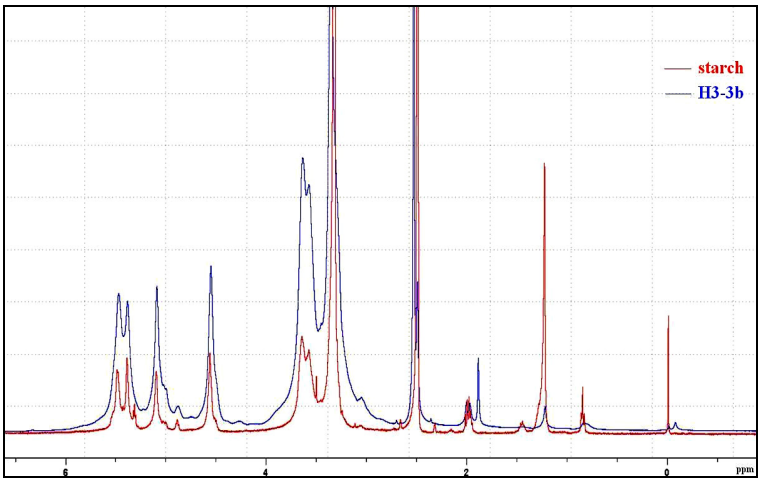

Figure 8: ^{1}H NMR spectra of $H_{3\text{-}3b}$ and starch

In order to better investigate the structure of $H_{3\text{-}3b}$, ^{1}H NMR was used to compare the structural difference between starch and $H_{3\text{-}3b}$. The ^{1}H NMR spectra of $H_{3\text{-}3b}$ and starch are shown in Fig. 8, and the two spectra were very similar, suggesting that insoluble amylose was the main component of $H_{3\text{-}3b}$, which proved that the $H_{3\text{-}3}$ obtained from the three-year-old bamboo contained xylans and starch. A possible reason was that with the increment in age, the interactions between starch and hemicelluloses are stronger. In addition, it was reported that a young culm may not contain starch during the growing phase, since all nutrients must be utilized immediately for metabolic processes.[16] Therefore, the starch content was relatively higher in older culms.

CONCLUSION

The present study was aimed at investigating the structural characteristics of hemicelluloses extracted by DMSO from one-, two-, and three-year-old *Neosinocalamus affinis* bamboo culms. The results of chemical composition analysis revealed that the main chemical components of the differently aged bamboo culms had relatively small differences. However, the contents of ash, extractives, and lignin were higher in older bamboo culms than in the others. The two-year-old bamboo culms had the highest content of glucose, representing the distribution of cellulose in a certain degree. Xylose, the main component of hemicelluloses, reached the highest level in the top culm sections, compared to the other parts of the culms for all three ages. The bottom section had the highest content of lignin, which offers high resistance to bending. The yields of hemicelluloses ranged from 6.53% to 9.78% of the holocellulose. The DMSO-soluble hemicelluloses were highly acetylated. GPC analysis results showed that the hemicelluloses from one- and two-year-old bamboo culms had a relatively higher molecular weight and lower polydispersity index, whereas $H_{3\text{-}2}$ and $H_{3\text{-}3}$ had a relatively lower molecular weight and higher polydispersity index. Comprehensive analysis of HPAEC, GPC, FT-IR, and NMR results proved that the DMSO-soluble hemicelluloses from bamboo culms of the three ages mainly consisted of *O*-acetyl-arabino-4-*O*-methylglucurono-(1→4)-*β*-D-xylan, while a relatively higher starch content was present in the DMSO-soluble fractions of the three-year-old bamboo culms. The structural changes of bamboo DMSO-soluble hemicelluloses during culm maturation are slight.

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