

INFLUENCE OF STEAM PRESSURE ON THE PHYSICO-CHEMICAL PROPERTIES OF DEGRADED HEMICELLOSES OBTAINED FROM STEAM-EXPLODED LESPEDEZA STALKS

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Steam explosion pretreatment was used to release hemicelluloses from the stalks of *Lespedeza crytobotrya*, a potential woody biomass crop. Hemicelluloses from *Lespedeza crytobotrya* subjected to five different pretreatment severities were extracted with 60% aqueous ethanol solution containing 1% NaOH, characterized by component analysis, gel permeation chromatography (GPC), FT-IR, NMR spectroscopy, and thermal analysis, and compared with hemicelluloses obtained from untreated stalks. It was found that the hemicellulosic fractions mainly consisted of arabinoxylans and β -glucans or xyloglucans. Steam explosion pretreatment yielded noticeable degradation and debranching reactions, illustrated by a linear decrease of molecular weight and Ara/Xyl ratio with increasing severity. For further high-value utilization of the hemicellulosic polymers, steam explosion at 20 or 22.5 kg/m² for 4 min is promising because of improved extraction efficiency and avoidance of over-drastic degradation of the polymers.

Keywords: Steam Explosion; Fractionation of polymers; GPC; Polysaccharides

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INTRODUCTION

High worldwide demand for energy, unstable and uncertain petroleum sources, and concern over global climate change has led to resurgence in the development of alternative energy sources that can displace fossil transportation fuel. Lignocellulosic biomass has long been recognized as a potential sustainable source of mixed sugars for fermentation to biofuels and other biomaterials (Himmel et al. 2007). The recalcitrant nature of lignocellulosic biomass makes its cellulosic components difficult to hydrolyze into its component saccharides. Hence, the biorefinery concept has been brought forward. The ultimate goal of a biorefinery is the efficient fractionation of lignocellulosic biomass into multiple streams that contain value-added compounds so that the overall economics of a biofuel production facility can be significantly improved. Hemicelluloses are a large group of well-characterized polysaccharides found in the primary and secondary cell walls of all land and fresh water plants and in some seaweeds. They consist of mixed-linkage compounds (1 \rightarrow 3/1 \rightarrow 4)- β -glucans, arabinoxylans, arabinogalactans, arabino-

galactan-proteins, and arabinogalactan-peptides (Salimath and Tharanathan 1982). They have a backbone of β -1,4-D-pyranosyl residues in which O-4 is in the equatorial orientation (e.g., Glc, Man, and Xyl) (Shallom and Shoham 2003). Hemicellulosic polysaccharides also have the ability to bind non-covalently to cellulose through hydrogen bonding and to bind covalently to lignin. They therefore serve to interconnect the cellulose fibrils and the lignin in the cell walls (Windham et al. 1987). Due to their various functional properties, the potential applications of hemicelluloses are immense and span diverse fields. They are applicable as gels, films, coatings, adhesives, and gelling, stabilizing, and viscosity-enhancing additives in food, pharmaceutical, and other industries (Ebringerová 2006). Recently, oligosaccharides attracted more interests as new functional additives, not only for modifying food flavor and physicochemical characteristics, but possessing properties beneficial to health (Pazur 1970). In comparison, the utilization of xylose to produce chemicals, such as furfural, can be regarded as less valuable and less economically attractive.

For a long time, thermochemical pretreatment of biomass has been considered as a critical technology to produce material with acceptable enzymatic digestibilities. However, existing pretreatment approaches have not made efficient use of the hemicellulosic component. More recently, with the recognition that complete utilization of the entire substrate will be required for sustainable process economics, fractionation strategies have focused on the recovery of hemicelluloses and lignin. This has been accomplished through the use of less severe, single-stage pretreatment and/or two-stage pretreatment processes, which facilitate efforts to optimize both hemicelluloses and lignin recovery, as well as cellulose treatment (Robinson et al. 2003). The combination of untreated and steam-exploded biomass and various solvents and delignification agents has been studied (Glasser et al. 2000; Höije et al. 2005). Although the hemicellulosic fraction was comprehensively hydrolyzed in the cited work due to the acidic conditions generated by acetic acid derived from the acetylated hemicelluloses, the macromolecular structure was changed and yielded low molecular weight components that are apt to be dissolved in the solvents.

Lespedeza crytobotrya, a perennial shrub species of the leguminous genus *Lespedeza*, has the excellent properties of substantial biomass, anti-sterility, drought-resistance and frost-hardiness, a well-developed root system, and fast growth. Besides, it contains considerable biomass in terms of energy usage, 39.6 t/(hm²·a), and the quantity has been found to dramatically increase by about 99-2026% after the first stumpage (Kang 2007). All of these distinguishing features make it a favorite choice as an energy tree species for biofuel production.

In this investigation, we first explored the structure of hemicelluloses obtained from *Lespedeza crytobotrya* stalks by applying the steam explosion technique and sequential mild alkaline-ethanol post-extraction. Any notable structural differences induced by increasing steam pressure in the pretreatment could help to optimize the pretreatment process and obtain an intermediate molecular weight (around 10,000 g/mol) hemicellulosic product for high-value utilization.

EXPERIMENTAL

Materials

Lespedeza stalks (*Lespedeza crytobotrya*) were obtained from the experimental farm of Beijing Forest University, air-dried, and cut into an average size of 50 mm × 30 mm × 20 mm before being subjected to steam explosion. The analysis of the chemical composition, including cellulose (44.6%), hemicelluloses (29.3%), lignin (17.0%), wax and other extractives (4.5%), and ash (4.3%), were performed on debarked material according to the Chinese standard methods as used in the papermaking industry (GB/T 2677). The deviations of these contents from their respective means were all less than 5%. All chemicals used were of analytical grade. L-Rhamnose (98%), L-arabinose (99%), D-glucose (98%), D-galactose (97%), D-mannose (99%), and D-xylose (98%) were purchased from Sigma-Aldrich Company (Beijing).

Pretreatment and Isolation

The steam explosion pretreatments were studied in a batch pilot unit by varying the steam pressure (from 15 to 25 kg/m², accordingly the temperature range from 201.3 to 226.7 °C) for a fixed time (4 min). Because of the high heterogeneity of the feedstock, the entire exploded samples at the same severity were mixed together and ground to 40 mesh before the further isolation. Figure 1 gives the isolation sequence of the degraded hemicellulosic fractions. Briefly, the steam-exploded samples were post-treated with 60% aqueous ethanol solution containing 1% NaOH at 78 °C for 3 h with a biomass to liquid ratio of 1:20 (g/ml). The solution phase was separated, neutralized to pH 5-6 with 6 M HCl, and further concentrated under reduced vacuum.

Cut Lespedeza stalks (300g)

Steam exploded at 15, 17.5, 20, 22.5, and 25 kg/m² for 4 min, respectively, then collected the solid fractions, oven-dried at 50 °C for 12 h.

The steam-exploded stalk

Treated with 60% aqueous ethanol containing 1% NaOH at 78 °C for 3 h with a biomass to water ratio of 1:20 (g/ml), then washed with water and 70% ethanol.

Cellulose rich fraction

Filtrate

Adjusted to pH 5-6 with 6 M HCl, concentrated at reduced pressure, and then precipitation in 3 volumes of 95% ethanol.

ethanol.

Lignin rich filtrate

Pellet

Degraded hemicelluloses fractions (H₁, H₂, H₃, H₄, and H₅)

Fig. 1. Scheme for isolation of hemicelluloses fractions from steam-exploded Lespedeza stalks

The hemicellulosic fractions were precipitated by the addition of three equivalent volumes of 95% ethanol, freeze-dried, and labeled H₁, H₂, H₃, H₄, and H₅, corresponding to the steam pressures of 15, 17.5, 20, 22.5, and 25 kg/m², respectively. H₀, which represents the degraded hemicellulosic fraction released by direct alkaline ethanol treatment of the raw material without steam explosion, was taken as a comparison. All the samples were kept in a desiccator at room temperature before analysis.

Characterization of the Degraded Hemicellulosic Fractions

The analysis of neutral sugars and associated lignin was described in a previous paper (Wang et al. 2009). The measurement of hemicellulosic molecular weights was determined by gel chromatography on a PL Aquagel-OH Mixed column (300×7.5 mm, Agilent). The eluent was 0.02 M NaCl in 0.005 M sodium phosphate buffer, pH 7.5 with a flow rate of 0.5 ml/min. Detection was achieved using a Knauer differential refractometer. The column oven was maintained at 30 °C. The samples were dissolved in 0.005 M sodium phosphate buffer with 0.02 NaCl added to a concentration of 0.1%. To calibrate the column, a monodisperse polysaccharide of known molecular weight was used as a standard.

Fourier transform infrared (FT-IR) spectra of the hemicellulosic fractions were recorded in the transmission mode with a KBr disc containing 1% finely ground samples. The infrared spectra were collected on a Tensor 27 spectrophotometer (Bruker, Germany) equipped with a DTGS detector. The solution-state ¹H and ¹³C NMR measurement was obtained on a Bruker DRX-400 spectrometer (Germany) at 75.5 MHz. Sample concentrations of approximately 25 mg in 1.0 ml D₂O for ¹H NMR and 80 mg in 1.0 ml DMSO-*d*₆ for ¹³C NMR were placed in 5 mm i.d. glass tubes and run at a temperature of 25 °C for 0.5 and 18 h, respectively. A 60° pulse flipping angle, a 3.9 μs pulse width and a 0.85 s delay time between scans were used. Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) were performed with a simultaneous thermal analyzer (DTG-60, Shimadzu, Japan) at a range of temperatures between ambient and 600 °C at a heating rate of 10 °C/min with nitrogen as the purge gas.

RESULTS AND DISCUSSION

Fractional Yield of the Degraded Hemicellulosic Fractions

Information related to the yields of hemicelluloses is given in Table 1. It is well known that alkaline treatment can completely or partially cleave ester bonds, such as linkages between ferulic acid and arabinan or galactan in the cell walls, resulting in the dissolution of hemicelluloses (Lawther et al. 1996). Meanwhile, ethanol acts as a swelling solution to increase the polarity of the stalk structure and extract lignin (Oliet et al. 2002). In this study, the direct alkali-ethanol treatment of the *Lespedeza crytobotrya* stalks released hemicelluloses amounting to 8.2% of the initial *Lespedeza* stalks, which corresponds to 27.9% of the hemicelluloses initially contained in the raw material. In most cases, after the steam explosion pretreatment, an increasing trend of yields was observed (Table 1), and it is undoubtedly due to the enhanced digestibility with higher severity. At more severe conditions, the saturated steam penetrated into the depth of the

stalk structure, resulting in an increase in the hydrolysis of hemicelluloses and the cleavage of the bonds between hemicelluloses and lignin. The degraded hemicelluloses-derived products, mainly oligosaccharides, were prone to being extracted in the alkaline environment. A similar result has been reported by Sun et al. (2005) in a study of the fractionation of hemicellulosic polymers from wheat straw, using a two-step process based on steam explosion pretreatment followed by alkaline peroxide post-treatment (8.4 to 13.3%). Meanwhile, the depolymerized monosaccharide and oligosaccharides could probably dissolve in the water present after depressurizing, which inevitably results in the loss of some part of the original hemicelluloses. As more polysaccharides dissolved in the condensing water than released by post-treatment, a lower yield of the hemicellulosic fraction at the lowest steam pressure (H_1) than that of the fraction H_0 was observed.

Table 1. The Yield (Weight %), Weight Average (\bar{M}_w), Number Average (\bar{M}_n) Molecular Weight and the Polydispersity (\bar{M}_w/\bar{M}_n) of the Hemicellulosic Fractions

	Hemicellulosic fractions*					
	H_0	H_1	H_2	H_3	H_4	H_5
Yield (%)**	8.2	6.5	8.7	9.9	10.2	11.1
\bar{M}_w	47,960	15,720	13,420	9,550	9,440	6,380
\bar{M}_n	11,420	6,680	6,860	5,210	5,050	4,060
\bar{M}_w/\bar{M}_n	4.2	2.4	2.0	1.8	1.9	1.6

* H_0 represents the hemicellulosic fraction released by post-treatment from raw material, while H_1 , H_2 , H_3 , H_4 , and H_5 represent the hemicellulosic fractions from the steam-exploded stalks performed at 15, 17.5, 20, 22.5, and 25 kg/m² for 4 min, respectively.
 **Based on initial whole raw biomass.

Distribution of Molecular Weight

The molecular weights of the degraded hemicellulosic fractions were analyzed by gel permeation chromatography (GPC). The weight-average molecular weight (\bar{M}_w) of the hemicellulosic fraction obtained from the raw material (H_0) was 47,960 g mol⁻¹. Steam explosion with higher steam pressure obviously decreased the value of \bar{M}_w (Table 1), demonstrating a significant degradation of the hemicellulosic polymers during the pretreatment process. A similar trend of the calculated values of polydispersity (\bar{M}_w/\bar{M}_n) (Table 1) was also observed. A high ratio (4.2) of the alkali-organosolv-extractable *Lespedeza crytobotrya* hemicelluloses revealed its inherent polydispersity. The polydispersity value suddenly dropped to 2.4 at the condition of 15 kg/m² for 4 min, and then linearly declined to 1.6 at the greatest severity (25 kg/m² for 4 min), clearly manifesting a significant structural modification of the macromolecule into the smaller polymer induced by steam explosion. These noticeable changes were further intuitively reflected by the distribution profiles (Fig. 2). The principal hemicellulosic fraction H_0 consisted predominantly of a population in the high molecular weight (HMW) (>10,000 g mol⁻¹) region, and a minute quantity in the low molecular weight (LMW) (<5,000 g mol⁻¹) proportion, corresponding to a high \bar{M}_w value. After steam explosion at 15 kg/m² for 4 min (H_1), the molecular distribution curve was significantly divided into three parts: a considerable number of HMW portion, and comparatively two small peaks in the middle molecular weight (MMW) (5,000-10,000 g mol⁻¹) and LMW regions. When the

steam pressure was elevated to 17.5 kg/m² (H₂), the intensity of LMW region obviously increased as the intensities of MMW and HMW regions decreased to a similar level. It is interesting to observe that, at 20 and 22.5 kg/m² (H₃ and H₄), both of the intensities of LMW and MMW regions were increased. It is suggested that the depolymerization of hemicelluloses was greater for higher molecular weight polysaccharides at these two conditions, and, therefore, it is the right pressure for the production of middle molecular weight polysaccharides. At the most severe condition (H₅, 25 kg/m²), the LMW fraction appeared as a sharp and symmetric peak, and the HMW region fraction appeared as a lump.

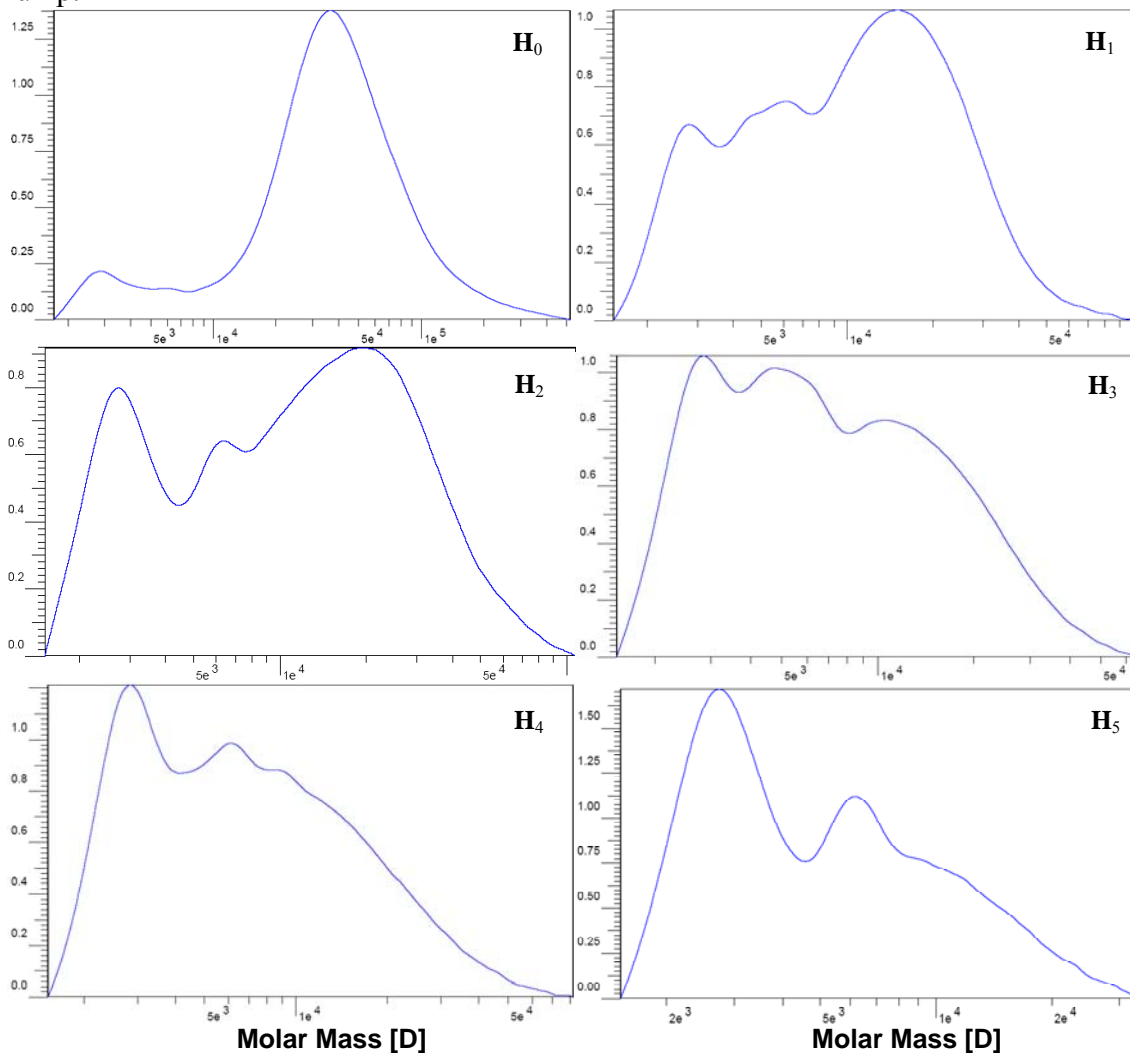


Fig. 2. Molecular weight distribution curves of the degraded hemicellulosic fractions H₀, H₁, H₂, H₃, H₄, and H₅

Neutral Sugar and Uronic Acids Composition

The compositions of neutral sugars and uronic acids in the hemicellulosic fractions are given in Table 2. Obviously, xylose (44.4%) and glucose (44.0%), nearly equal parts of neutral sugars, were the predominant sugars constituents in the extracted

hemicelluloses from the raw *Lespedeza crytobotrya* stalks (H₀). The noticeable amounts of arabinose (3.3%) and galactose (2.5%), and minor amount of mannose (1.7%) and rhamnose (0.8%) were also identified as constituents. From these data, it can be speculated that the hemicellulosic fraction, isolated by 60% ethanol under the alkaline condition from the raw material, is a mixture of arabinoxylans and β -glucans or xyloglucans. Arabinose to xylose (Ara/Xyl) ratios is indicative of the degree of linearity or branching of the arabinoxylans (Wedig et al. 1987). The low arabinose to xylose ratio (0.08) of the original hemicelluloses fraction indicated a high degree of polymerization with few linkages to other monosaccharide constituents. Meanwhile, arabinosyl residues have been also occasionally found linked to the xylosyl residues of some xyloglucans (McNeil et al. 1975; Mori et al. 1980). As can be seen, the content of xylose was first increased and then gradually decreased with elevating steam pressure. However, the content of glucose manifested a wholly opposite trend. This phenomenon could probably be explained by differences between low and high severity conditions. At lower severity conditions ($\leq 17.5 \text{ kg/m}^2$), the degradation of hemicelluloses resulted in more oligosaccharides being able to be extracted by alkaline ethanol solution. However, β -glucans, as one of the main components in the *Lespedeza crytobotrya* stalks hemicelluloses, were cleaved and formed insoluble glucans (Eda et al. 1983). At higher severities ($\geq 20 \text{ kg/m}^2$), xylose could be easily further degraded into furfural and other furfural derivatives as reported by Suzuki et al. (1998). Under the highest severity condition (25 kg/m^2), the obviously decreased yield of xylose indicated the comprehensive degradation of hemicelluloses, and further pointed out that it is not a suitable condition for obtaining hemicelluloses. Besides, the degradation of amorphous cellulose to glucose by acidic hydrolysis caused a prevalence of glucose associated in the isolated hemicellulosic fractions. It is also worthy of notice that debranching reactions occurred during the steam explosion. As shown in Table 2, the Ara/Xyl ratio linearly decreased from 0.05 to 0. This result also partially led to a decreasing content of xylose in the hemicellulosic fractions, because xylans with infrequent side chains are less water soluble and bind more tightly to cellulose (Woodward et al. 1983).

Table 2. The Content of Neutral Sugars (Relative Weight %) and Uronic Acid (Weight %) in the Degraded Hemicellulosic Fractions

Sugars	Degraded hemicellulosic fractions					
	H ₀	H ₁	H ₂	H ₃	H ₄	H ₅
Rhamnose	0.8	0.3	0.3	ND*	ND	ND
Arabinose	3.3	2.7	2.1	1.1	1.2	ND
Galactose	2.5	4.1	3.6	2.7	2.8	1.0
Glucose	44.4	28.5	31.4	48.7	53.1	86.6
Mannose	1.7	4.4	2.8	4.3	4.5	1.4
Xylose	44.0	59.2	58.4	40.0	36.7	10.6
Ara/Xyl	0.08	0.05	0.04	0.03	0.03	0
Galacturonic acid	2.8	0.1	0.1	ND	ND	ND

* ND = Not detectable.

Composition of the Associated Lignin

Alkaline nitrobenzene oxidation provides both an estimate of the total amount of lignin and an indication of the composition of phenolic units. As can be seen in Table 3, the major products were identified as vanillin and syringaldehyde, indicating that the associated lignin in the degraded hemicellulosic fractions was composed mainly of non-condensed guaiacyl and syringyl units. Minor amounts of *p*-hydroxybenzoic acid, vanillic acid, and syringic acid as well as traces of *p*-hydroxybenzaldehyde, acetovanillone, acetosyringone, *p*-coumaric acid, and ferulic acid were also detected. Slightly higher yields (4.3-5.7%) of total phenolic acids and aldehydes were obtained in the steam exploded samples (except for H₅, 25 kg/m²) than the raw material (3.9%). This indicated that steam explosion pretreatment cleaved or weakened the linkages between lignin and hemicelluloses, and resulted in the improved co-extraction of lignin in the alkaline ethanol solvent. However, at the most severe condition (H₅, 25 kg/m²), the lowest yield (1.9%) of total phenolic acids and aldehydes was probably due to the formation of so-called pseudolignin (Garrote et al. 1999; Hetiz et al. 1991). Chua and Wayman (1979) reported that under dramatic conditions of steam pretreatment, the reactive hemicelluloses degradation products, such as furfural and its precursors, can react with lignin during auto-hydrolysis to form polymer. Another probable explanation could be condensation reactions involving the formation of new carbon-carbon bonds within lignin and only to a minor extent to the modification of the side chains of the lignin, resulting in a lignin structure that is much less amenable to oxidation to phenolic aldehydes (Wayman and Chua 1979).

Table 3. The Yield of Phenolic Acids and Aldehydes (Weight %) Obtained by Alkaline Nitrobenzene Oxidation of the Associated Lignin

Phenolic acids and aldehydes	Degraded hemicellulosic fractions					
	H ₀	H ₁	H ₂	H ₃	H ₄	H ₅
<i>p</i> -Hydroxybenzoic acid	0.2	0.1	0.4	0.1	0.1	0.2
<i>p</i> -Hydroxybenzaldehyde	T*	T	0.1	T	T	T
Vanillic acid	0.1	0.1	0.1	0.1	0.1	0.1
Vanillin	1.5	1.9	1.9	1.8	1.8	0.7
Acetovanillone	T	T	T	T	T	T
Syringic acid	0.2	0.2	0.3	0.1	0.1	0.1
Syringaldehyde	1.8	2.4	2.6	2.3	2.0	0.7
Acetosyringone	0.1	T	0.2	0.1	0.1	0.1
<i>p</i> -Coumaric acid	ND	0.1	0.1	T	0.1	0.1
Ferulic acid	T	ND	0.1	ND	T	ND
Total	3.9	4.8	5.7	4.5	4.3	1.9

* T = Trace

FT-IR Spectral Analysis

The FT-IR spectra of all the six hemicellulosic fractions are illustrated in Figs. 3 and 4. Clearly, the spectra showed typical signal patterns expected for the hemicellulosic moiety. In particular, all the spectra exhibited specific band maxima in the 1250 and 950 cm⁻¹ regions, which are dominated by ring vibrations overlapped with stretching vibrations of C-OH side groups and by C-O-C glycosidic bond vibration (Kauráková et al. 1994; Kauráková et al. 2000). It is worth noting the absence of a signal at 1745 cm⁻¹ for

the acetyl and uronic ester groups of the hemicelluloses or from the ester linkage of carboxylic group of ferulic acid (Sun and Sun 2002) in all the spectra. This implied that alkali-ethanol post-treatment under the given conditions completely saponified these ester bonds in hemicelluloses. The occurrence of obvious aromatic skeleton vibration bands at 1616 (1612), 1511, 1425 (1426), and 1464 (1460) cm^{-1} and the syringyl ring breathing absorption at 1324 (1322) cm^{-1} further confirmed the presence of a noticeable amount of associated lignin in the hemicellulosic fractions. This indicates that, not only steam explosion pretreatment under the conditions in present study, but also the mild alkali-ethanol solution post-treatment could not completely destroy the lignin-carbohydrate complex structure. The wavenumber characteristic of the hemicellulosic fraction is the distinct absorption of typical xylans at 1059 cm^{-1} , which is assigned to the C-O and C-C stretching and the glycosidic linkage ν (C-O-C) contributions. This typical feature of xylan in all the spectra suggested again that the treatment under the conditions given did not result in any significant changes in the “core” structure of hemicelluloses. Meanwhile, the presence of the β -(1 \rightarrow 4)-glucan is indicated by the obvious intensity shoulder at 1163 (1162) cm^{-1} in all of the hemicellulosic fractions, corresponding to the C-O-C vibrations in the anomeric region of hemicelluloses (Ebringerova et al. 1992). The in-plane ring stretching gives a shoulder at 1113 cm^{-1} , which is the normal FT-IR absorption of cellulosic materials (Kauráková et al. 2002; Kauráková and Wilson 2001), and the all-equatorial -OH group position in glucose shows in the IR spectra the lowest frequency maximum at 1035 (1034) cm^{-1} in the IR spectra (Kauráková et al. 2002). The intensities of these two signals were slightly increased in the pretreated samples, indicating that a minimum amount of low-molecular cellulose-degraded products was present in the isolated hemicellulosic fractions. As a function of increasing the incubation time, no significant changes of the curves were observed, except that the absorptions belonging to the associated lignin in the spectrum of H₅ (25 kg/m^2) were obviously decreased. The characteristic “anomeric region” absorption bands for β -linkage (898 cm^{-1}) was observed as a small sharp peak in all the spectra, which was consistent with the β -glycosidic between sugar units in the backbone structure of the hemicelluloses.

Thermal Analysis

The thermal degradability is affected by the chemical composition of the material because different components of lignocellulosic materials have different thermal behaviors. The results showed that all of the hemicelluloses began to decompose at around 220 °C, and their maximum rate of weight loss was observed between 260 and 320 °C (Fig. 5). Similar results have been obtained for the alkali-dioxane-soluble hemicelluloses from sugarcane bagasse (Xu et al. 2006) and the hemicellulosic preparation released during the alkaline peroxide post-treatment after steam explosion from wheat straw (Sun et al. 2005). From the TGA curves, it can be observed that the rate of weight loss during 260 and 320 °C decreased as the increasing fraction number from H₀ to H₂, and to H₅. Similarly, at the end of pyrolysis process, the weight loss reached 93%, 91%, and 77% for hemicellulosic preparations H₀, H₂, and H₅, respectively. This phenomenon implied that slightly higher thermal stability occurred as the result of steam explosion and higher pressure. The probable reason was the generation of condensation substances between the

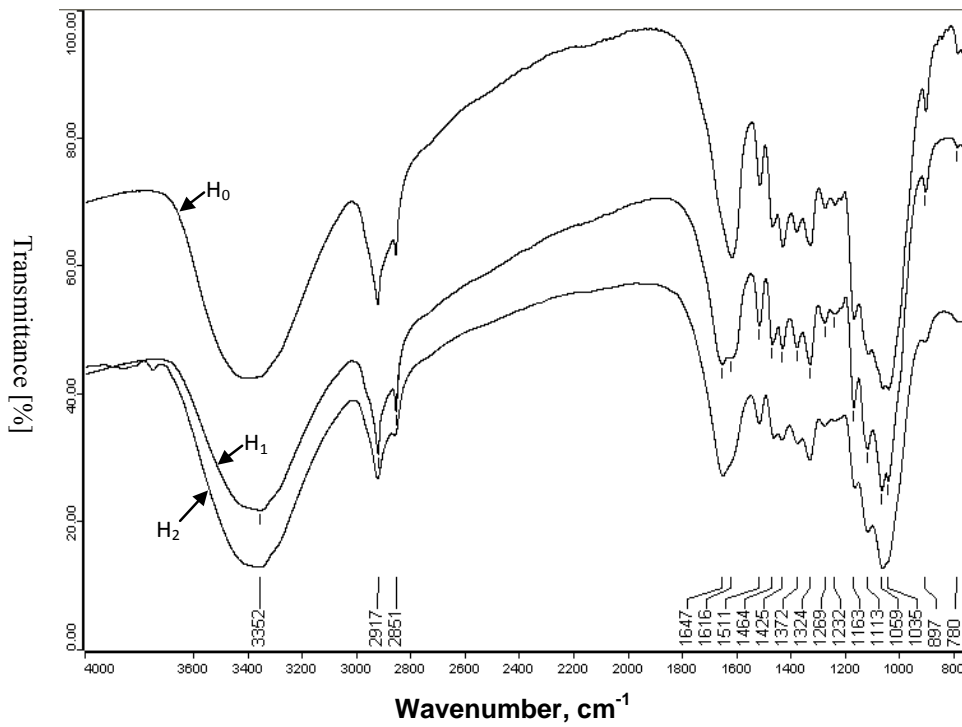


Fig. 3. FT-IR spectra of hemicellulosic fractions H₀, H₁ and H₂

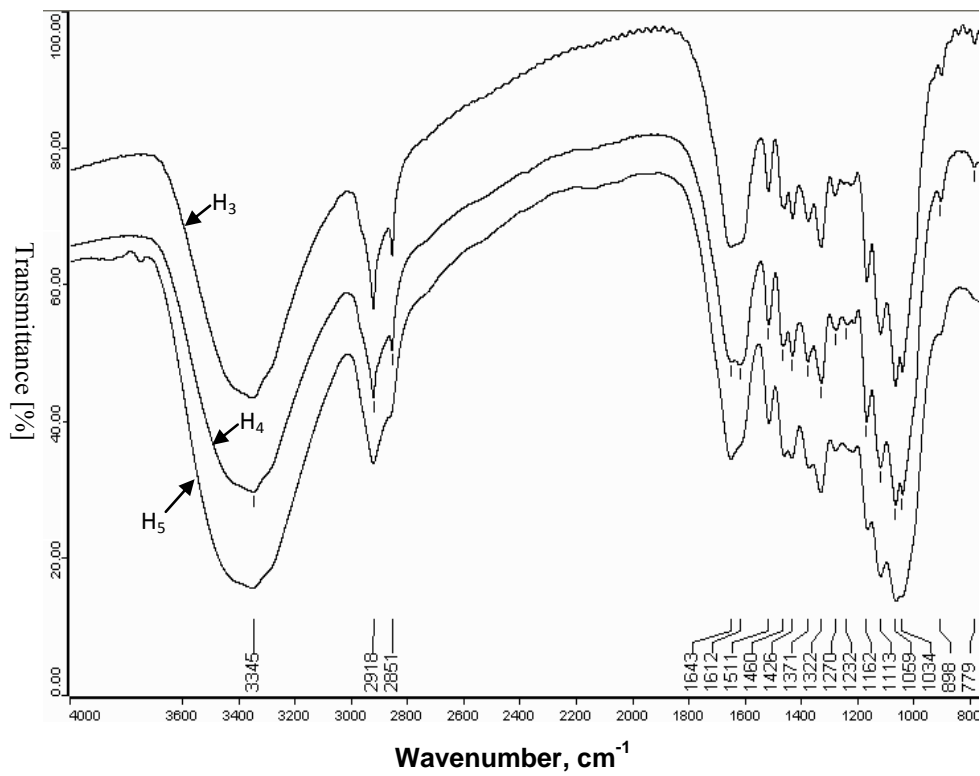


Fig. 4. FT-IR spectra of hemicellulosic fractions H₃, H₄ and H₅

hemicellulosic products or the lignin degradation products, leading to a more recalcitrant residue that requires higher temperature ranges for complete decomposition. The DTA profile of H₀ displayed a distinct peak at 320 °C, which was attributed to release CO₂ (mainly caused by the cracking and reforming of functional groups of carboxyl C=O and COOH), CO (mainly released out with the cracking of carbonyl C-O-C and carboxyl C=O), and CH₄ (caused by the cracking of methoxyl -OCH₃) (Yang et al. 2007). In comparison, the DTA patterns of H₂ illustrated the comparatively greater intensity of the effects at 320 °C and the highly obvious exothermic peak of the effects at 460 °C. This result was also represented in the TGA curve of H₂ (17.5 kg/m²), which seems to be a two-step degradation process. At a comparatively higher temperature around 460 °C, the sudden weight losses (18%) and increment of weight loss rate were attributed to the destruction of the more resistant macromolecular moieties, such as lignin. Lignin was the most difficult to decompose among the three main components in the lignocellulosic material and released the highest yield of CH₄ at around 500 °C because of its highest -OCH₃ content (Yang et al. 2007). We need to mention that the lignin sample used in Yang's report (2007) was a commercial product, kraft lignin. It is well known that a lot of condensation reactions occur during the kraft pulping process, and the exothermic peak around 500 °C is probably related to the condensed lignin structure. Thereby, the probable reason for the absent peak around 500 °C on the DTA curve of H₀ could be the uncondensed structure of lignin in the unpretreated sample. In contrast, the DTA curve of H₅ (25 kg/m²) had a much smaller feature for the whole pyrolysis process. It is no wonder that the exothermic peak around 500 °C was absent, probably due to the lowest content (1.9%) of the associated lignin. However, it was a surprise to find that the distinct exothermic peak around 320 °C was changed into a small bulge. Yang et al. (2007) had pointed out that the hemicelluloses and lignin pyrolysis was a charring process due to the observed exothermic peaks. Thereby, we can speculate that obvious charring reactions occurred between the polysaccharides at the most severe condition (25 kg/m² for 4 min). The almost complete change in the thermal characteristics also revealed that steam explosion at 25 kg/m² for 4 min is not a suitable condition for isolating hemicelluloses component. In summary, the thermal stability of the isolated hemicellulosic fractions was improved by the steam explosion pretreatment.

¹³C and ¹H NMR Spectroscopy Analysis

In the ¹³C NMR spectrum (not shown) of the hemicellulosic fraction H₁ obtained by post-treatment with alkaline ethanol from the steam exploded (15 kg/m² for 4 min) stalks, the main 1,4-linked β-D-Xylp units were obviously observed by five distinct signals at 102.2, 75.9, 74.4, 73.1, and 65.5 ppm, which were assigned to C-1, C-4, C-3, C-2, and C-5 of the β-D-Xylp units, respectively (Bendahou et al. 2007). In light of this, it is confirmed that xylan is one of the main components in the degraded hemicellulosic fraction. On the other hand, the occurrence of β-glucans is difficult to recognize, because the resonances are probably overlapped with those of other hemicelluloses.

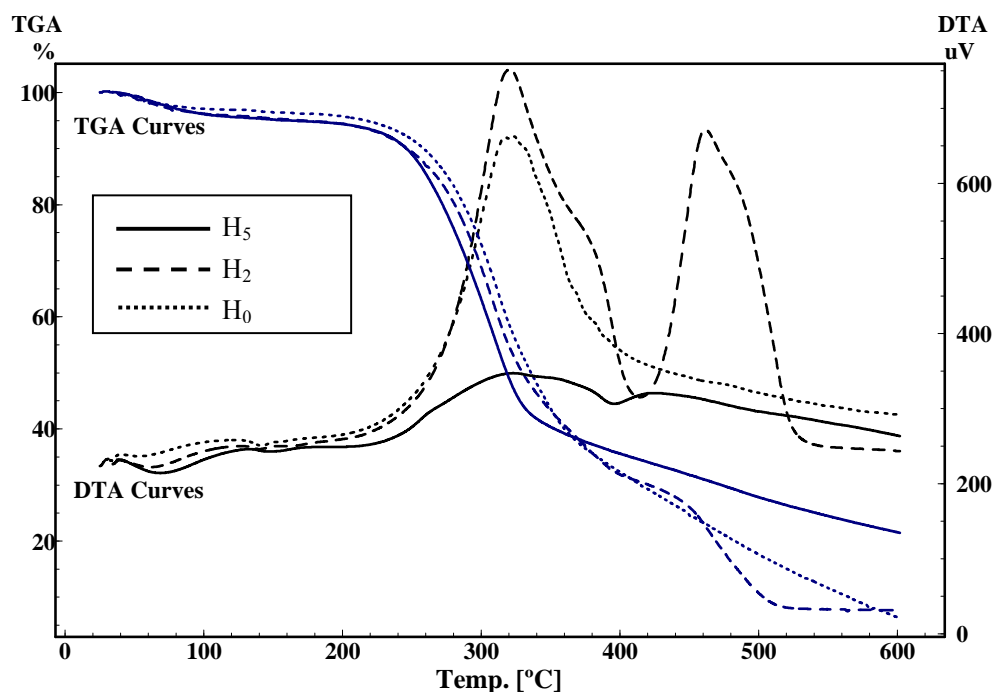


Fig. 5. TGA/DTA curves of the hemicellulosic fractions H₀, H₂ and H₅

The strong signal at 29.4 ppm is assigned to the γ -methyl and β - and α -methylene groups in lignins associated in the hemicelluloses. In order to obtain further information about the influence of steam pressure on the structure of hemicelluloses, the ^1H spectrum of the degraded hemicellulosic fraction, released from the samples steam exploded at 15 kg/m² for 4 min, was recorded and assigned (Fig. 6).

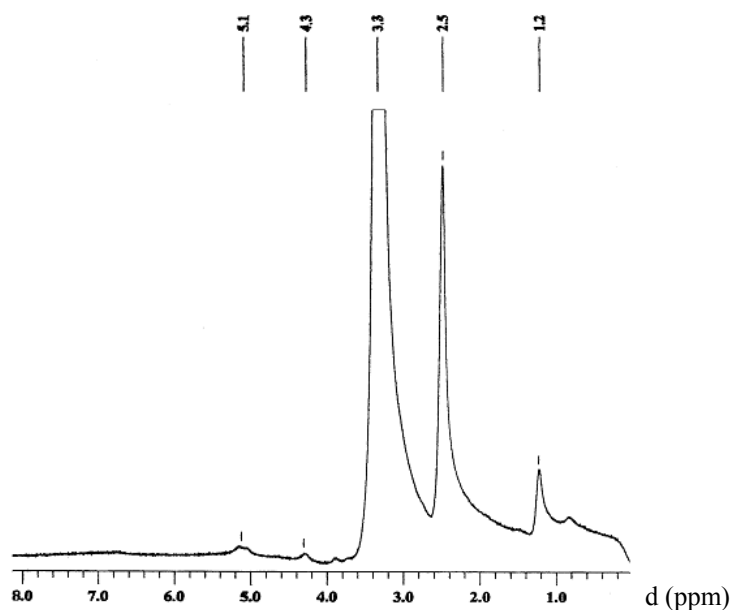


Fig. 6. ^1H NMR spectrum of the degraded hemicellulosic fractions H₁

Anomeric protons of terminal α -D-arabinofuranosyl residues gave a shoulder at 5.1 ppm (Teleman et al. 2000) in the spectrum of H₁, corresponding to the 2.7% arabinose content from sugar analysis. The absence of this signal in the spectrum of H₅ (25 kg/m², spectrum not shown) suggested that elevating steam pressure could significantly cleave the linkage between the backbone chain (xylose) and branch (α -L-arabinofuranose). A signal at 4.3 ppm was due to the anomeric protons of β -D-xylose substituted at C-3 (monosubstituted) residues, since the region between 4.1 and 4.5 ppm corresponds to the β -configuration and the region 4.9-5.6 ppm corresponds to the α -configuration (Kawagishi et al. 1990). The signals for other protons of glucose, arabinose and xylose were overlapped in the region of 3.2-4.3 ppm and centered at 3.3-3.4 ppm.

CONCLUSIONS

1. Steam explosion pretreatment under the conditions given resulted in a significant improvement of the extraction efficiency of alkaline ethanol as solvent to isolate hemicellulosic fractions from *Lespedeza crytobotrya* stalks.
2. The native hemicellulose in *Lespedeza crytobotrya* stalks consists of almost equal contents of xylose (44.4%) and glucose (44.0%), from which the structural characteristics could be speculated as a mixture of arabinoxylans and β -glucans or xyloglucans.
3. Steam explosion pretreatment induced obvious depolymerization and debranching reactions in hemicelluloses, which were clearly revealed by the lowered average molecular weights (from 47,960 to 6,380 g mol⁻¹), together with a linear decrease of the Ara/Xyl ratio (from 0.08 to 0). On the other hand, it should be noted that the hemicelluloses were also condensed to form more thermostable substrates during this process.
4. A slightly higher amount of the associated lignin was co-extracted by post-treatment, due to the cleavage of linkages between hemicelluloses and lignin. That also partially leads to the thermostable characteristics of the isolated hemicellulosic fraction.
5. Steam explosion pretreatment at 20 or 22.5 kg/m² for 4 min was considered to be an optimum condition for isolating the relative high molecular weight hemicelluloses to be further utilized as oligosaccharides.

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