



Hemicellulose structural changes during steam pretreatment and biogradation of *Lentinus edodes*



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Abstract To disclosed the internal factors for the growth of mycelium and *Lentinus edodes*, *Quercus* Linn wood, which was biotransformed during the artificial cultivation of *Lentinus edodes*, were synergistically characterized by TGA/DTG, FT-IR and NMR. The results showed that the different ingredients of hemicellulose decreased during steam explosion and biodegradation of *Lentinus edodes*, however hemicellulose content continued to increased. FT-IR showed that the transmittance of the characteristic peaks in hemicellose gradually increased after decreased after steam explosion and biodegradation of *Lentinus edodes*. TGA/DTG curves that thermal stability and maximum thermal degradation rates of hemicelloses were contiguous after steam explosion and biodegradation of *Lentinus edodes*. Structural determination based on FT-IR and ¹H, and 2D-HSQC NMR analyses showed that the alkali-extractable hemicelluloses shared the structure composed of (1 → 4)-linked β-D-xylopyranosyl backbone with 4-O-methyl-R-D-glucuronic acid attached to O-2 of the xylose residues and L-arabinose attached to O-3 of the xylose residues. And it revealed that the extractable hemicelluloses retained original structure without cleaving chemical linkages. Furthermore, a small

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amount of other minor hemicelluloses (β -glucans) including xylans in the extractable hemicelluloses could be identified by NMR and other approaches.

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1. Introduction

With the increase of global energy requirements and greater environmental awareness, alternatives to fossil fuels as energy sources have been widely followed on. Photosynthesis conversion solar energy into the storage of energy in the form of plant cell-wall polymers. The energy stored in these polymers could be accessed in many ways, ranging from simple burning to complex bioconversion processes. Currently plant cell walls were considerable biomass resources of biofuels and other chemicals. What's more, lignocellulosic biomass, which contained the agricultural residues, forestry waste and municipal solid waste, presented a sustainable and renewable source for the production of liquid biofuels such as bioethanol (Taherzadeh and Karimi, 2008; Razali and Said, 2017). That was, lignocellulose feedstock was considered to be an attractive raw material for liquid fuel and the production of chemicals and materials. As most often being a by-product from food and feed production, lignocellulosic biomass did not compete with the production of edible crops (Chen and Qiu, 2010; Petersson et al., 2007) and had the potential to be the feedstock for the production of a considerable proportion of transport fuels if cost effective conversion processes were available (Kristensen et al., 2008; Halim and Phang, 2017). The major components in lignocellulosic biomass were cellulose, hemicellulose and lignin. Hemicellulose sugars are the second most abundant carbohydrates in nature and its conversion to ethanol could provide an alternative liquid fuel source for the future (Jeffries, 2006; Shamsudin et al., 2017). However, hemicelluloses, which were restricted to poales and a few other groups, composed of different five and six-carbon monosaccharide units (Rubin, 2008; Ghafar et al., 2017). The detailed structure of the hemicelluloses and their abundance varied widely between different species and cell types. Especially, the biosynthesis of xylans and β -D-(1 \rightarrow 3, 1 \rightarrow 4)-glucans remains very elusive, and recent studies have led to more questions than answers. Hence, the effects of hemicelluloses on biomass digestibility need to be revealed.

In the plant cell wall, hemicelluloses were bound to lignin and cellulose, and detailed isolation procedures were required to separate these components from plant raw material (Hansen and Plackett, 2008; Khan et al., 2017). Removal of hemicelluloses in a pure form from plant cell wall involved hydrolysis of ester and ether bonds, which linked the hemicelluloses to lignin (Aziz and Hanafiah, 2017). A number of methods were used to isolate and richen hemicelluloses from plant sources, including extraction with alkali, dimethyl sulfoxide, water, methanol, steam or microwave treatment (Hansen and Plackett, 2008; Wen et al., 2010, 2011). These methods were developed for characterization purposes, but it could also be used as a preparative method. Besides, hot water could be used as a tool to separate water-soluble hemicelluloses from plant cell wall (Sun et al., 2004, 1998). Unfortunately, only a few papers about the comparison of hemicellulosic fractions obtained by hot water and biopretreatments had been published

(Xu et al., 2007; Ismail and Hanafiah, 2017). With the depletion of fossil fuel and the concern of environmental protection, the utilization of biomass resources has attracted increasing worldwide interest.

Steam explosion was the most commonly used method for pretreatment of lignocellulosic materials (Fernandez-Bolanos et al., 2001). Steam explosion, which was invented in 1925, was introduced to treat aspen wood in early 1980s. In the method, the factors of steam explosion pretreatment are residence time, temperature, chip size and moisture content (Duff and Murray, 1996). Optimal hemicellulose solubilization and hydrolysis could be achieved by different temperature and residence time (Duff and Murray, 1996; Lee et al., 2009). Steam explosion, whose advantages included the low energy requirement and no recycling or environmental costs, was recognized as one of the most cost-effective pretreatment processes for hardwoods and agricultural residues, but it is less effective for softwoods (Clark and Mackie, 1987; Basheer et al., 2017). However, Hemicellulose recovery in the range of 55–84%, together with low levels of inhibitory by-products, has been obtained through autohydrolysis (Ulbricht et al., 1984; Mes-Hartree et al., 1988).

The most promising method for enzymatic hydrolysis of the lignocellulosic biomass was preceded by a pretreatment process in which the lignin component was separated from the cellulose and hemicellulose to make it amenable to the enzymatic hydrolysis. In biopretreatment processes, microorganisms such as white-, brown-, and soft-rot fungi are used to degrade lignin and hydrolyse hemicellulose in waste materials (Njoku et al., 2013; Sassner et al., 2008). Brown rots mainly attack cellulose, while white and soft rots attack both cellulose and lignin. White-rot fungi are the most effective *Basidiomycetes* for biological pretreatment of lignocellulosic materials (Sukor et al., 2017; Hassan et al., 2017). Using varieties of white-rot fungi, 35% of the straw was converted to reducing sugars by *Pleurotus ostreatus* in five weeks (Hatakka, 1983). In order to prevent the loss of cellulose, a cellulase-less mutant of *Sporotrichum pulverulentum* was developed for the degradation of lignin in wood chips (Akin et al., 1995), also reported the delignification of Bermuda grass by white-rot fungi. The white-rot fungus *P. chrysosporium* produces lignin-degrading enzymes, lignin peroxidases and manganese-dependent peroxidases, during secondary metabolism in response to carbon or nitrogen limitation (Boominathan and Reddy, 1992; Halim et al., 2017). Many white-rot fungi could degraded wood cell walls, and other enzymes including laccases, polyphenol oxidases, H_2O_2 producing enzymes and quinone-reducing enzymes can also degrade lignin (Blanchette, 1991). The advantages of biopretreatment include low energy requirement and mild environmental conditions. However, the rate of hydrolysis in most biopretreatment processes was very low (Jeffries, 2006; Njoku et al., 2013; Zhu et al., 2009; Singh et al., 2011).

Lentinula edodes was first typically cultivated in the dead logs of hardwood. Before 1982, the Japanese variety of Lentinula edodes could only be grown in traditional locations using

ancient methods. And a 1982 report on the budding and growth of the Japan Islands variety revealed opportunities for commercial cultivation in the United States (Leatham, 1982). *Lentinula edodes* has been now widely cultivated all over the world (Chang and Hayes, 2013). Commercially, *Lentinula edodes* was typically grown in conditions similar to their natural environment on either artificial substrate or hardwood logs, such as oak. Now, *Lentinus edodes* was commercially cultivated on oak wood particles. Although *Lentinula edodes* had the wide growth, researches mainly concentrated in the pharmacodynamics, cultivation conditions and culinary uses (Ramasamy and Kandaswamy, 1976; Dhillon and Chahal, 1979; Miller and Jong, 1987; Bhatti et al., 1987; Royse et al., 1990; Palomo et al., 1998; Chang, 1999; Zhang et al., 2002; Obodai et al., 2003; Permana et al., 2009; Jiang et al., 2013; Kim et al., 2014), but little attention to wood biodegradation and reuse. Profiling of wood chips may help growers optimize their production media and reduce production costs (Royse et al., 1990). The extractives of *Lentinula edodes* contained the antibacterial substances (Njoku et al., 2013; Yamamoto et al., 1997; Hirasawa et al., 1999). However, oak wood extractives could be inhibitory to growth of *Lentinus edodes* (Leatham, 1985), and oak wood need be pretreated before cultivation of *Lentinula edodes*. *Lentinus edodes* had their ability to produce lignocellulolytic enzyme in solid-state and submerged fermentation of various plant raw material (Elisashvili et al., 2008; Rahman et al., 2017). They cellulolytic system enzymes, hemicellulases, the ligninolytic system, (gluco-)amylase, pectinase, acid protease, cell wall lytic enzymes (laminarinase, 1,4- β -D-glucosidase, β -N-acetyl-D-glucosaminidase, α -D-galactosidase, β -D-mannosidase), acid phosphatase, and laccase (Leatham, 1985). *Lentinus edodes* was the important wood lignin-degrading fungi (Leatham, 1986). Degradation of the lignin fraction of the wood cell wall by compounds of lower molecular weight than enzymes is implicated (Goodell et al., 1998). The overall effect of *L. edodes* on oak is similar to that of many white-rot fungi, which simultaneously degrade all cell wall components (Vane, 2003; Vane et al., 2003). Unfortunately, shiitake dermatitis were recorded by French Poison Control Centers – New case series with clinical observations (Hérault et al., 2010; Boels et al., 2014). The inexplicable cases become more and more from the vegetable growers in China. Merely, it

was easily found that the biodegradation of wood by *L. edodes* was not deeply understand to reuse. To date, the physicochemical features and structural characteristic of hemicelluloses from wood biodegraded by *L. edodes* had been poorly characterized (Aoyama et al., 1999). Therefore, the aim of this study was to firstly prepare hemicellulosic polymers isolated from *Quercus* Linn wood after steam pretreatment and biodegradation of *L. edodes*, and their chemical structure would be examined and analyzed by Fourier-Transform Infrared Spectroscopy (FT-IR), thermogravimetric analysis (TGA), nuclear magnetic resonance (NMR) in order to recognize hemicelluloses richen. And it was particularly important to elucidate the physicochemical features and structural characterization in this study.

2. Material and methods

The scheme of structural analysis on hemicellulosic polymers isolated from *Quercus* Linn wood during the artificial cultivation of *Lentinus edodes* were illustrated in Fig. 1.

2.1. Materials

The *Quercus Linn* was collected from Tongbaishan Forest Region China. Biomass was crushed into particles (GM1). The mycelium of *Lentinus edodes* were clonal and Industrial grade. Ethanol, benzene, acetic acid, and KOH were all analytic reagent for the following tests.

2.2. Experiment methods

2.2.1. Biotorefaction process

The above 1.0 t particles were steam pretreated for 60 h in order to ensure further decomposition, and then each 2 kg were packed in plastic bag tied by rope. These bags were drilled and put into by the *Lentinus edodes* mycelium. Then further storage were done under the confined space, high humidity for 140 days. *Lentinus edodes* mycelium could live in some samples (GM3), and died in others (GM2). After small plant of *Lentinus edodes*, grown, GM3 samples were placed in plastic shed, ventilation and sunlight during the daytime, airproof at night. The small plants could live in some samples (GM5), and died in others (GM4). After GM5 samples raised *Lentinus edodes* five times, these samples were the waste wood (GM6). The samples were crushed into 40–60 μ m powder, dried to moisture content of 0%, and extracted in ethanol-benzene solution ($V_{\text{ethanol}}:V_{\text{benzene}} = 2:1$) at 85–90 °C for 6 h. After ethanol-benzene extraction, the extracted flour was dried to moisture content of 0%.

2.2.2. Isolation and purification of hemicelluloses

The above extracted flour was treated in 10% KOH solution at 10 °C for 24 h ($V_{\text{extracted flour}}:V_{\text{KOH solution}} = 1:5$). The KOH extracted flour was filtrated, washed five times with 0.5% acetic acid solution, dried to a moisture content of 0%, then weighed. The filtrate was neutralized with 6 M hydrochloric acid or acetic acid solution to pH 5.5 ~ 6.0 and then concentrated under reduced pressure to about 50 mL. Three volumes of ethanol were added to each concentrated solution with continuous stirring, and then the flocculent precipitate appeared. After filtration with filter paper on a Buchner funnel, the iso-

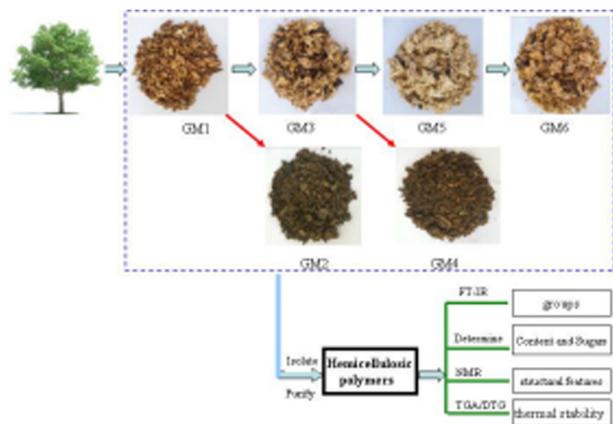


Fig. 1 Scheme of hemicelluloses isolated from natural, steam pretreatment and biodegraded wood.

lated hemicelluloses were purified by thorough washing with 70% ethanol and then freeze-dried (Wen et al., 2010, 2011).

2.2.3. Determination of chemical composition

To determine the composition of isolated hemicelluloses, the neutral sugars and uronic acids in isolated hemicellulosic fractions were determined by HPAEC (high-performance anion exchange chromatography). The neutral sugars and uronic acids in the hemicellulosic preparations were obtained by hydrolyzing 5 mg of sample with 10% H₂SO₄ for 2.5 h at 105 °C. After hydrolysis, the hydrolysate was diluted 50-fold, filtered, and injected into the HPAEC system (Dionex ICS3000) with pulsed amperometric detector and an ion exchange CarboPac PA-1 column (4 × 250 mm). The neutral sugars were separated in 18 mM NaOH (carbonate free and purged with nitrogen) with post-column addition of 0.3 M NaOH at a rate of 0.5 mL/min. Run time was 45 min, followed by a 10 min elution with 0.2 M NaOH to wash the column and then a 15 min elution with 18 mM NaOH to reequilibrate the column. Calibration was performed with a standard solution of L-rhamnose, L-arabinose, L-glucose, L-galactose, D-mannose, D-xylose, glucuronic acid, and galacturonic acids. Measurements were conducted with two parallels, and reproducibility of the values was found within the range of 5% (Wen et al., 2010, 2011).

2.2.4. TG analysis

Each sample was analyzed using less than 10 mg of hemicellulosic polymers. TG spectra were measured from room temperature to 805 °C on a TG20 thermogravimetric analyzer (209-F1 TG, Netzsch, Germany) using a carrier gas (N₂) velocity of 40 mL/min and a heating rate of 20 °C/min.

2.2.5. FT-IR analysis

The FT-IR spectra of the samples were obtained on a FT-IR spectrophotometer (Bruker Tensor 27) using KBr discs containing 1% finely ground sample. Thirty-two scans were taken for each sample recorded from 4000 to 400 cm⁻¹ at a resolution of 2 cm⁻¹ in the transmission mode.

2.2.6. NMR analysis

The solution-state ¹H NMR spectra were recorded on a Bruker AV III NMR spectrometer at 400.13 MHz using 15 mg of hemicelluloses in 1.0 mL of D₂O. In addition, to increase solubility of alkali-soluble hemicelluloses in D₂O, a few drops of sodium deuteroxide (7.5 M NaOD) were added. The chemical shifts were calibrated relative to the signals from D₂O, used as an internal standard, at 4.70 ppm for the ¹H NMR spectra. A semiquantitative analysis of the HSQC cross-signal

intensities was performed. The spectral widths for the HSQC were 5000 and 20,000 Hz for the ¹H and ¹³C dimensions, respectively. The number of collected complex points was 1024 for the 1H dimension with a recycle delay of 5 s. The number of transients for the HSQC spectra was 128, and 256 time increments were always recorded in the ¹³C dimension. The 1JC-H used was 146 Hz. Prior to Fourier transformation, the data matrices were zero filled to 1024 points in the ¹³C dimension. Data processing was performed using standard Bruker Topspin-NMR software. Because the cross-signal intensity depended on the particular 1JC-H value, as well as on the relaxation time, a direct analysis of the intensities was indeed impossible. Therefore, the integration of the cross-signals was performed separately for the different regions of the HSQC spectra, which contain signals that correspond to chemically analogous carbon-proton pairs (in similar samples). For these signals, the 1JC-H coupling value was relatively similar and was used semiquantitatively to estimate the relative abundance of the different constituents (Wen et al., 2010, 2011).

3. Results and discussion

3.1. Yield and chemical composition

Hemicelluloses, which were the second most abundant natural polymers and the main ingredient of plant cell wall, played a vital role in the lifetime of wood cell. It composed of different five and six-carbon monosaccharide units (Rubin, 2008), and included xyloglucans, xylans, mannans and glucomannans, and beta-(1 → 3, 1 → 4)-glucans. The hemicellulose content varied roughly from 20 to 35% in the wood. Although the test results were different by the different methods, they could assess the chemical changes of *Quercus* Linn wood during the growth of *Lentinus edodes*. The extractives, cellulose, hemicellulose and lignin content of *Quercus* Linn wood were 3.09%, 20.40%, 22.86%, 53.64%, respectively. After steam explosion and biodegradation of *Lentinus edodes*, the test results of hemicellulose content were 23.80%, 25.30%, 47.50%, 26.20%, and 56.10% in GM2, GM3, GM4, GM5, and GM6, respectively. And the measurement yield and relative content of sugars and uronic acids of hemicellulose isolated from natural, steamed and biodegraded *Quercus* Linn wood. Table 1 showed that hemicellulose of *Quercus* Linn wood regularly changed during steam explosion and biodegradation of *Lentinus edodes*. During steam explosion in 100 °C water vapor for 60 h, xylose of hemicellulose was hydrolyzed and degraded, and decreased from 70.37% to 48.70% or 40.47%, resulting that other ingredients increased relatively (Table 1). Though xylose decreased after steam explosion,

Table 1 Yield and relative content of sugars and uronic acids of the isolated hemicellulosic preparations and the residue.

| Samples | Arabinose | Galactose | Glucose | Xylose | Glucose acid |
|---------|-----------|-----------|---------|--------|--------------|
| GM1 | 2.37 | 2.21 | 15.14 | 70.37 | 9.92 |
| GM2 | 10.37 | 3.66 | 32.63 | 48.70 | 4.64 |
| GM3 | 8.84 | 2.36 | 43.99 | 40.47 | 4.34 |
| GM4 | 6.31 | 2.79 | 26.58 | 58.14 | 6.18 |
| GM5 | 7.99 | 2.86 | 37.65 | 47.09 | 4.40 |
| GM6 | 7.02 | 2.08 | 26.08 | 56.87 | 7.95 |

hemicellulose content increased because cellulose hydrolyzed and extractives were volatilized. Moreover, if xylose of hemicellulose was deficiently degraded during steam explosion, *Lentinus edodes* mycelium would not live until die in *Quercus* Linn wood. During the mycelium growth, arabinose and glucose of hemicellulose biodegraded, and other ingredients increased relatively. Furtherly, if arabinose and glucose of hemicellulose was over degradation during the mycelium growth, *Lentinus edodes* would not live until die in *Quercus* Linn wood. During *Lentinus edodes* growth, arabinose, galactose and glucose of hemicellulose biodegraded and continued to decreased, and other ingredients increased relatively. The above results suggested that the different ingredients of hemicellulose decreased during steam explosion and biodegradation of *Lentinus edodes*, however hemicellulose content continued to increased because cellulose, lignin and extractives produced the great changes.

3.2. FT-IR analysis

Infrared spectroscopy (FT-IR) had been proved to be useful for analysis and identification on functional groups of polysaccharides in plant materials. In addition, it could be applied to explore structural features when combined with chemical methods as well as other spectrum approaches, such as NMR spectroscopy. After steam explosion and biodegradation of *Lentinus edodes*, hemicellulose of *Quercus* Linn wood had the larger transmittance in the peaks. FT-IR spectra could be used to investigate the structural groups of *Quercus* Linn wood and biodegraded. For comparison, the spectra of the above six samples were demonstrated Fig. 2. For wood, the peaks at 3388 cm^{-1} , 2924 cm^{-1} , 1596 cm^{-1} , 1408 cm^{-1} , and 1042 cm^{-1} were O—H stretch, —C—H stretch stretch, C—C stretch, CH_2 bend, and C—O stretch respectively (Wen et al., 2010,

2011; Kwon et al., 2013; Aggarwal et al., 2003). After steam explosion, the transmittance of the characteristic peaks in hemicellulose increased, and hemicellulose of *Quercus* Linn wood had the larger transmittance and were fit to grow for *Lentinus edodes* mycelium. After the mycelium growth, the transmittance of the characteristic peaks in hemicellulose continued to increase, and hemicellulose of *Quercus* Linn wood had the larger transmittance and were fit to grow for *Lentinus edodes*. After *Lentinus edodes* growth, the transmittance of the characteristic peaks in hemicellulose continued to increase. Moreover, the different ingredients of hemicellulose decreased during steam explosion and biodegradation of *Lentinus edodes*, it was resulted that the transmittance of the characteristic peaks in hemicellulose gradually increased after decreased after steam explosion and biodegradation of *Lentinus edodes*.

3.3. TG analysis

In the present study, the pyrolysis of cellulose, hemicellulose, and lignin in TGA and packed bed, together with the energy consumption and gas product releasing behaviors were investigated in detail. The objective of this study is to gain a comprehensive understanding to hemicelluloses pyrolysis, thus facilitate to establish an universe model to simulate hemicelluloses pyrolysis. It was favorable for the development of advanced bioethanol feedstocks pyrolysis process. Moreover, the regular pattern of hemicelluloses changes was revealed. During steam explosion and biodegradation of *Lentinus edodes*, hemicelluloses of *Quercus* Linn wood was degraded by steam and mycelium. And their thermal stability was a kind of effective method of characterization by TG. The thermogravimetric analyzer (TGA) was an essential laboratory tool used for material characterization. TGA was used as a technique to characterize materials used in various environmental.

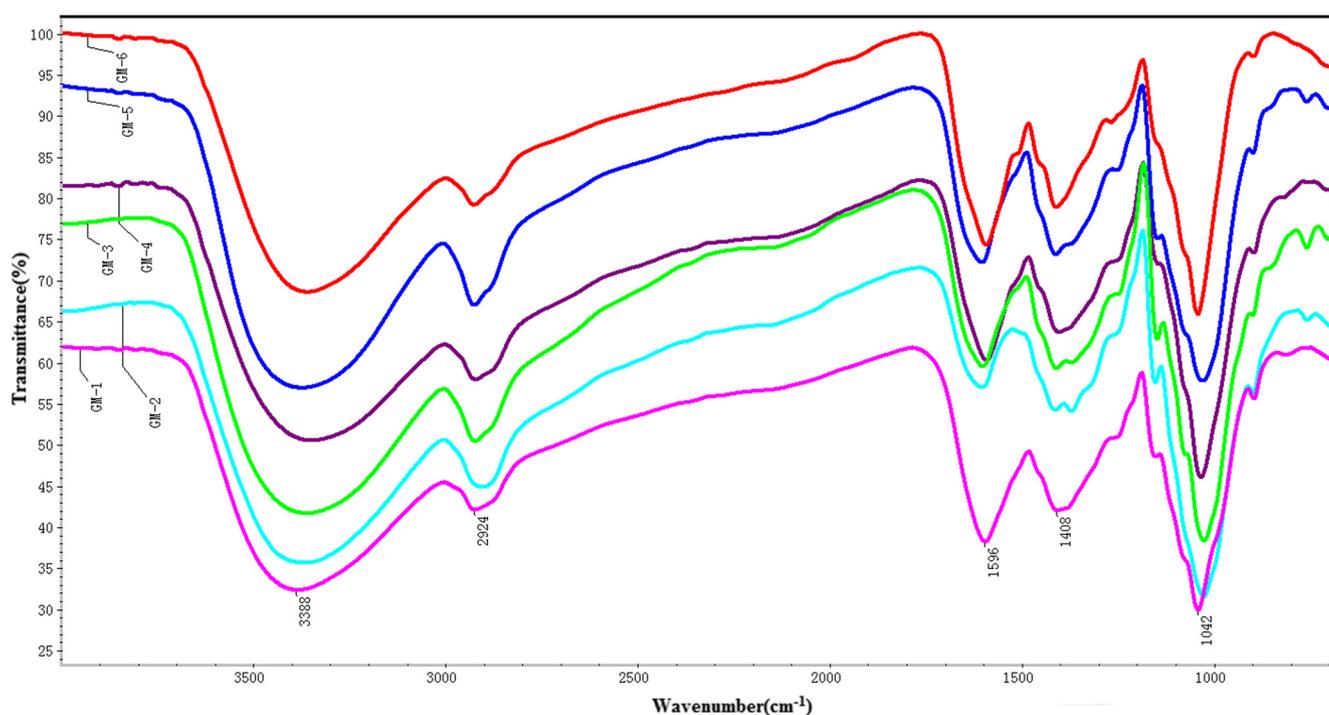


Fig. 2 FT-IR spectra of hemicelluloses isolated natural, steamed and biodegraded wood.

That was to say, TGA could measure weight changes in a controlled atmosphere with the temperature variation. In the controlled hot N_2 , hemicelluloses of *Quercus* Linn wood could react oxidation, reduction, hydration, dehydration, decomposition, and so on to cause weight loss. The GM1, GM2, GM3, GM4, GM5, and GM6 samples were contrastively investigated by TGA between room temperature and 804 °C. The TGA and DTG curves were illustrated in Fig. 4. As could be seen from Fig. 3, the thermal degradation of three samples proceeded over the wide temperature range (100–805 °C). At 10%, 30%, and 50% weight loss, the decomposition temperature were determined to be 118.9 °C, 272 °C, and 647.5 °C for GM1 sample; 114.1 °C, 276.8 °C, and 805.5 °C for GM2 sample; 116.1 °C, 269 °C, and 508 °C for GM3 sample; 130.7 °C, 275.8 °C, and 666.91 °C for GM4 sample; 118.3 °C, 275.5 °C, and 727 °C for GM5 sample; 168.4 °C, 285.62 °C, and 694.8 °C for GM6 sample; respectively. It was suggested that their thermal stability of hemicelluloses were adjacent at 10% and 30% weight loss, whereas these were variant at 50% weight loss. At 100 °C, the weight loss of GM1, GM2, GM3, GM4, GM5, and GM6 samples were 8.79%, 8.26%, 8.83%, 8.20%, 7.70%, and 6.06%, respectively. At 200 °C, the weight loss of GM1, GM2, GM3, GM4, GM5, and GM6 samples were 17.06%, 18.43%, 16.16%, 15.86%, 16.66%, and 13.11, respectively. At 300 °C, the weight loss of GM1, GM2, GM3, GM4, GM5, and GM6 samples were 37.16%, 33.44%, 37.52%, 34.81%, 34.39%, and 32.63%, respectively. At 803.3 °C, the weight loss of GM1, GM2, GM3, GM4, GM5, and GM6 samples were 58.97, 49.29%, 63.28%, 56.71%, 52.97%, and 58.21%, respectively. It was resulted that their thermal stability of hemicelluloses had tiny difference at 100 °C, 200 °C, 300 °C and 803.3 °C.

Furtherly, the DTG curves presented the weight loss rates, and DTG_{max} was the maximum thermal degradation rate, which could estimate degree of thermal degradation (Gedemer, 1974). The DTG curves of six samples had three turning points with the increase of temperature. The first DTG_{max} values, which ethanol evaporated, were found to be 70.67 °C, 68.16 °C, 73.21 °C, 69.52 °C, 70.53 °C and 69.24 °C for GM1, GM2, GM3, GM4, GM5 and GM6 sample, respectively. The second DTG_{max} values, which hemicelluloses produced the thermal cracking reaction, were found to be 184

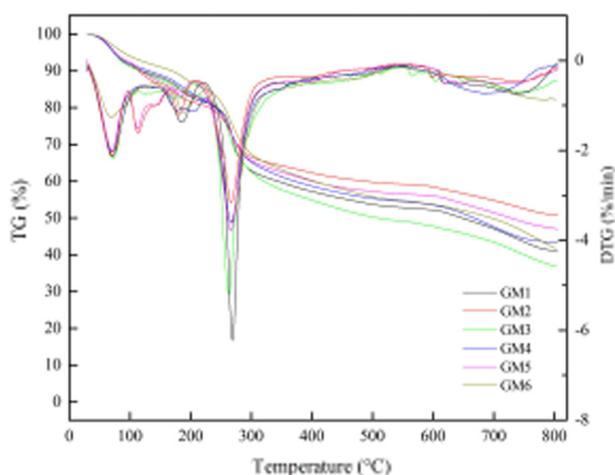


Fig. 3 TGA/DTG Curves of hemicelluloses isolated from natural, steamed and biodegraded wood.

°C, 182 °C, 182 °C, 178 °C, 185 °C and 184 °C for GM1, GM2, GM3, GM4, GM5 and GM6 sample, respectively. The third DTG_{max} values, which hemicelluloses produced the carbonation reaction, were found to be 269.90 °C, 264.85 °C, 262.97 °C, 266.61 °C, 267.16 °C and 265.93 °C for GM1, GM2, GM3, GM4, GM5 and GM6 sample, respectively. Whereas the other turning point of DTG_{max} values of GM2 and GM5 were 113.58 °C and 112.93 °C. It induced that the maximum thermal degradation rates of six samples were contiguous after steam explosion and biodegradation of *Lentimus edodes*.

3.4. NMR spectra

The most powerful tool for biopolymers analysis was NMR spectroscopy. For the majority of these biopolymers, well-resolved 1D and 2D NMR Spectra (^{13}C , 1H , and HSQC spectra) could be acquired from the intact polymers in D_2O , DMSO d_6 , or other solvents. To further investigate the structural characteristics of polymers extracted with different samples, the hemicellulosic preparations of GM1, GM3, GM5 and GM6 were studied using 1D and 2D NMR spectroscopy. NMR spectras were supposed to identify and assay the type of sidechain branching along the backbone and the polymer backbone. Hemicelluloses fractions GM1, GM3, GM5 and GM6 were isolated from wood samples during the growth of *Lentimus edodes*. The 1H and HSQC spectra of hemicelluloses fractions GM1, GM3, GM5 and GM6 were shown in Figs. 4 and 5, respectively. The signals for 1H NMR were assigned on the basis of the HSQC spectra and previous literature (Wen et al., 2010, 2011; Chaikumpollert et al., 2004; Vignon et al., 1998; Xiao et al., 2013).

As could be seen from Fig. 4, the two hemicellulosic preparations showed that analogous 1H NMR spectra indicated a similar structure of hemicelluloses. The anomeric signals in the 1H NMR spectra of GM1, GM3, GM5 and GM6 were assigned according to sugar analysis and the literature data, and listed in the spectral region of 4.3–5.6 ppm. The relevant signals distinguished at two regions, namely, the ring proton region (δ 4.5–3.0) and the anomeric region (δ 5.6–4.9 for α -anomers and δ 4.9–4.3 for β -anomers). The anomeric protons were well occurred in δ 4.28 and δ 4.30, which were assigned as (1 \rightarrow 4)- β -D-Xylp of GM1, GM3, GM5 and GM6, respectively. It confirmed that (1 \rightarrow 4)- β -D-Xylp was linked β -glycosidically, which was consistent with the presence of the small sharp peak at about 890 cm^{-1} in the IR spectra of GM1, GM3, GM5 and GM6.

Fig. 5 showed the HSQC spectras of GM1, GM3, GM5 and GM6. And the dominant four cross-peaks could be expressly identified at 63.3/3.94, 102.7/4.30, 76.0/3.63, 75.2/3.36, 73.3/3.15 ppm, which were assigned to C5-H5, C4-H4, C3-H3, C2-H2, and C1-H1 of the (1 \rightarrow 4)-linked- β -D-Xylp units, respectively. And the presence of the methyl group (OCH_3) of 4-O-methyl-D-glucuronic acid was found by a corresponding small but distinguishable cross-peak at 60.7/3.33 ppm. What's more, some weak cross-peaks, which represented 4-O-methyl-D-glucuronic acid and L-arabinose, were also found. But the weak signals were probably due to the low abundance of these components. HSQC contour volume integral was a promising method to estimate the relative quantity of components in hemicelluloses. Based on HSQC, the relative ratios of

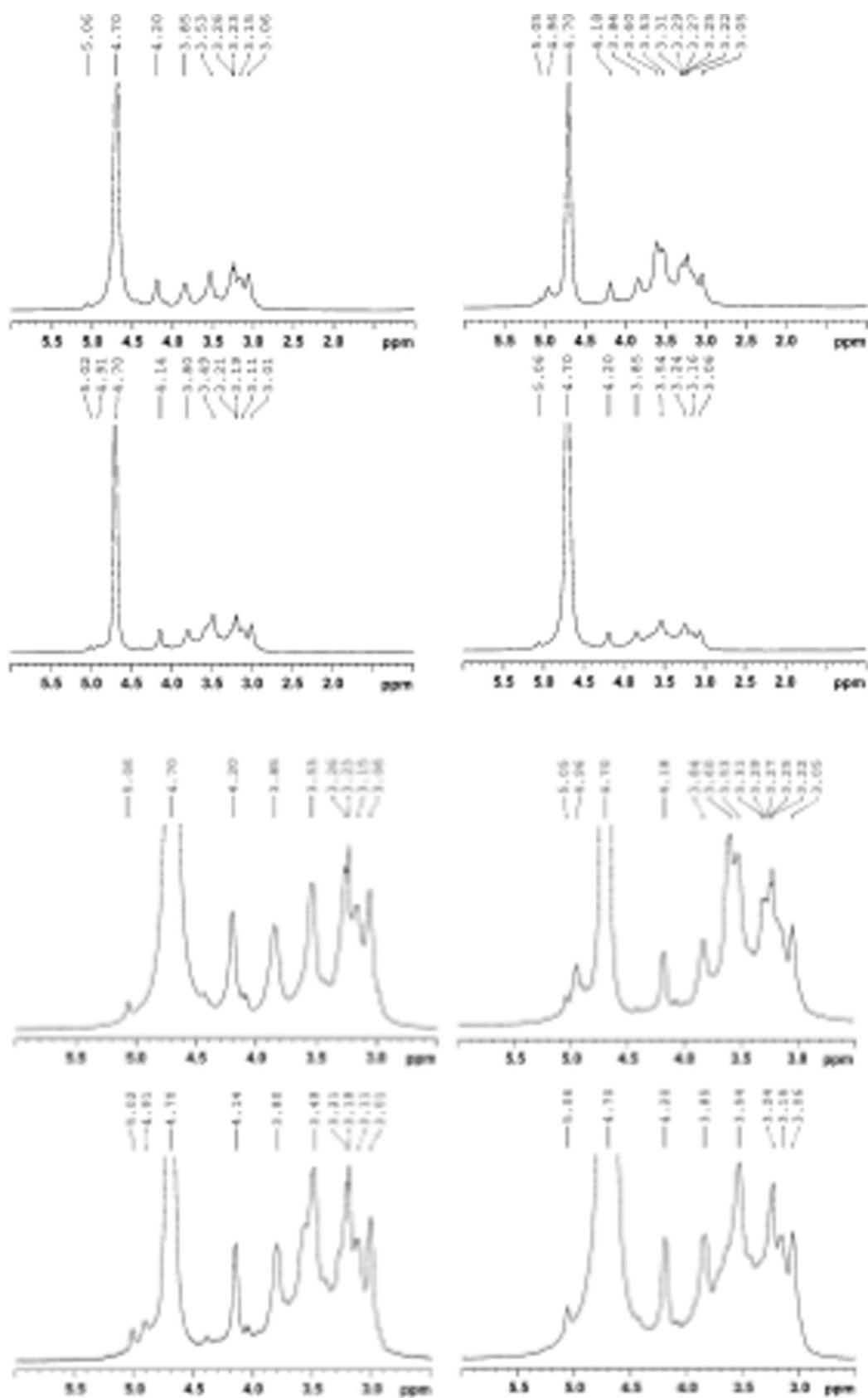


Fig. 4 ^1H NMR spectra of hemicellulosic fractions GM1, GM3, GM5 and GM6.

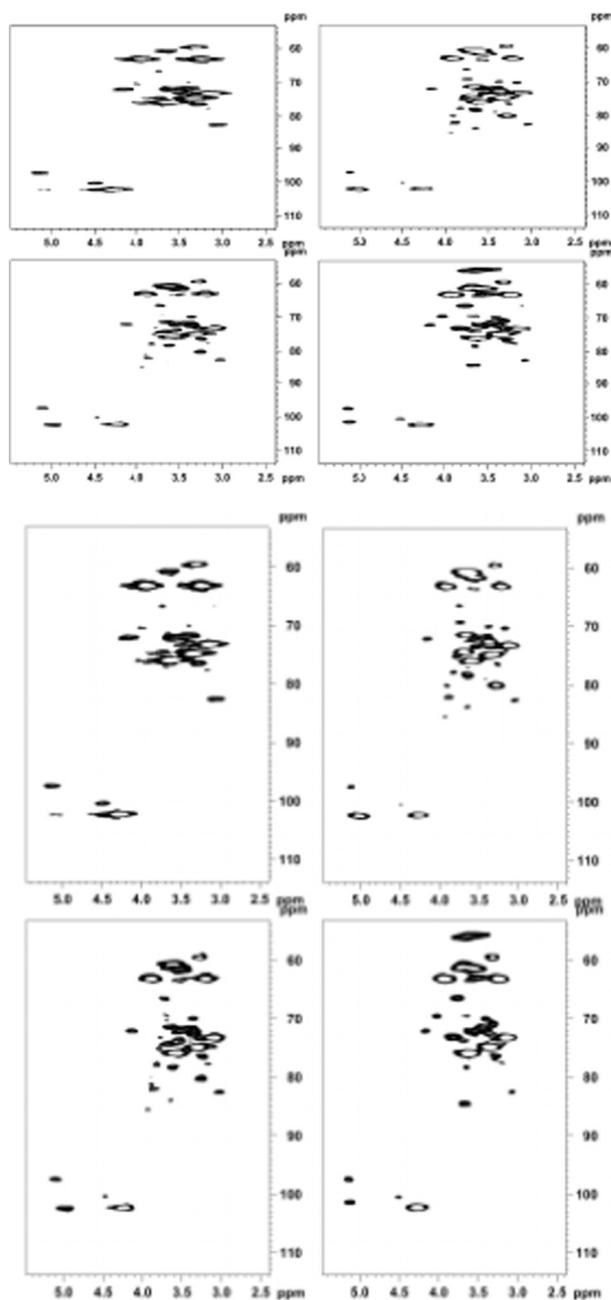


Fig. 5 HSQC spectra of hemicellulosic fractions GM1, GM3, GM5 and GM6.

compositions in hemicelluloses could be estimated by integrating contour volumes corresponding to carbohydrates' anomeric C1-H1 correlations. The relative ratio of xylose/arabinose in GM1, GM3, GM5 and GM6 was determined based on the integration of corresponding anomeric cross-peaks, and the ratio of xylose/arabinose was subsequently calculated. However, the relatively lower xylose/arabinose ratio obtained by HSQC integration was probably because the low content of hemicelluloses could not be detected in HSQC in Fig. 5. Compared with GM1, GM3, GM5 and GM6 fraction presented a similar molar ratio of xylose/arabinose estimated by contour volume integral. The discrepancy of ratios xylose/arabinose obtained from sugar analysis and NMR contour

volume integral was similarly attributed to the above-mentioned reason. However, in comparison with the relative sugar molar ratio obtained by sugar analysis, the HSQC contour volume integral could be still considered as a methodology of semiquantitative estimates. And then, as a novel and speedy approach to achieve both structural information and quantitative information, 2D-HSQC-NMR spectroscopy demonstrated the powerful functionalities in quantitative and qualitative estimation of polysaccharides.

Simultaneously, hemicellulosic structural analysis using ^1H NMR spectroscopy was limited because of the structural diversity of the different protons along the chain and their coupling patterns. HSQC together with the ^1H NMR spectrum clearly showed the typical signal patterns allowed accurate quantitative analysis and expected for a hemicellulosic moiety. The direct correlations between the hydrogen and carbon atom of the glycosyl residues of GM1, GM3, GM5 and GM6 could also be observed through the crosspeaks in the HSQC spectrum. The signals at $\delta 3.1$ – 5.4 were caused by the protons of the arabinose and xylose residues except for the strong signal at $\delta 4.7$. The resonance at $\delta 1.29$ was the methyl protons of ethanol retained from the ethanol precipitation of the hemicelluloses. Another resonance at $\delta 1.82$ was to the methyl protons of a few acetyl groups on the hemicelluloses. One point should be explained, which was probably due to degradation of ester bonds in the acetyl groups. While the ^1H NMR spectrum was conducted the sample was dissolved in D_2O , therefore, the occurrence of a signal at $\delta 1.82$ represented the protons of the CH_3 from the acetyl groups. The chemical shifts of $\delta 3.1$ – 4.3 were assigned to the protons of the anhydroxylose units of the hemicelluloses. The signal at $\delta 8.42$ was assigned to the proton of the carboxy group from 4-O-Me- α -D-GlcP. The signals at $\delta 5.25$ in the ^1H NMR spectrum have been assigned to protons at C-1 of 4-O-methyl-D-glucuronic acid residues. The relevant signals occurred in two regions, namely, the anomeric region ($\delta 4.9$ – 4.3 for the β anomers and $\delta 5.6$ – 4.9 for the α anomers) and the ring proton region ($\delta 4.5$ – 3.0). Another signal at $\delta 5.36$ in the ^1H NMR spectrum was attributed to protons at C-1 of the α -L-Araf residues. It confirmed that 4-O-methyl-D-glucuronic acid and L-arabinose were of the α -configuration, which was in agreement with the presence of a small peak at about 830 cm^{-1} in the FTIR spectrum of GM1, GM3, GM5 and GM6.

Based on the 2D-HSQC and ^1H NMR spectra, it was deduced that the anhydroxylose units of H3 were indicated by signals at $\delta_{\text{C}}/\delta_{\text{H}}$ 63.6/4.06 (H-5 eq), 102.3/4.45 (H-1), and 63.6/3.34 (H-5ax), 74.7/3.54 (H-3), 76.4/ 3.75 (H-4), and 73.4/3.28 (H-2), in which chemical shifts of $\delta 3.34$ and $\delta 4.06$ were assigned to the equatorial and axial protons linked at C-5, respectively. A very weak signal at $\delta 6.36$ and $\delta 6.32$ corresponds to the C-2 of ferulic acid and residual *p*-coumaric acid (Hallac et al., 2009). In addition, some very weak signals at 6.87, 6.89, 7.29, 7.33, 7.48, 7.50 ppm were assigned to the fractions of the associated lignin. The relative molar ratio of xylan and 4-O-Me- α -D-GlcA in the hemicelluloses GM1, GM3, GM5 and GM6 could be obtained by the integrated values for the H-1 in (1 \rightarrow 4)- β -D-Xylp-2-O-(4-O-Me- α -D-GlcP) and (1 \rightarrow 4)- β -D-Xylp to H-1 in 4-O-Me- α -D-GlcP (anomeric protons) in the ^1H NMR spectrum. It indicated that GM1, GM3, GM5 and GM6 contained 15 xylose units for every one 4-O-Me- α -D-GlcP unit in the hemicelluloses extracted from GM1, GM3, GM5 and GM6. The discrepancies could

be easily explained by the fact that aldouronic acids were difficult to hydrolyze under acidic conditions. Thus the 4-OMe- α -D-GlcpA was obtained in relatively low proportions in hemicelluloses. And one detail that should not be ignored was that the peak was more intense at δ 5.36 than at δ 5.25. GM1, GM3, GM5 and GM6 contained more arabinose than glucuronic acid, which was confirmed by the sugar analysis. To be more precise, it was obtained the relative molecular ratio of arabinose and 4-O-Me- α -D-GlcpA for the H-1 in L-Araf and 4-O-Me- α -D-GlcpA (anomeric protons) in the ^1H NMR spectrum. It further quantitatively described the side-chain structure of hemicelluloses extracted by aqueous alkali. Besides some weak signals at 59.8/3.43, 97.9/5.25 ppm, corresponding to OCH₃, and H-6 of the 4-O-methyl-D-glucuronic acid residues were also found.

Based on NMR spectra and FT-IR spectra concerning the linkages between the monosaccharides of GM1, GM3, GM5 and GM6, it could be concluded that hemicelluloses (GM1, GM3, GM5 and GM6) were formed by a linear backbone of (β -1 \rightarrow 4)-Xylp residues and the xylose residues was substituted at C-3 by arabinose, whereas the xylose residue was substituted at C-2 by 4-O-Me-GlcA. Furthermore, the (β -1 \rightarrow 4)-Xylp residues in the backbone might contain substituted phenolic acids (*p*-coumaric acid and ferulic acid).

The NMR spectra of GM1, GM3, GM5 and GM6 presented the straightforward structure as revealed by four major signals corresponding to (1 \rightarrow 4)-linked β -DXyl residues and other less intense signals characteristic of a 4-Omethyl- α -D-glucuronic acid residue as well as an L-Araf residue and β -D-Xyl units substituted with 4-O-methyl- α -D-GlcpA. However, the signals of the glucans couldn't be found in the NMR spectrum. And various analytical techniques, such as FTIR, sugar analysis, and NMR data, a possible chemical structure of hemicelluloses extracted with alkalis from GM1, GM3, GM5 and GM6 was L-*arabino*-4-O-methyl-D-glucurono-D-xylan.

4. Conclusions

The different ingredients of hemicellulose decreased during steam explosion and biodegradation of *Lentinus edodes*, however hemicellulose content continued to increase. During steam explosion in 100 °C water vapor for 60 h, xylose of hemicellulose was decreased from 70.37% to 40.47%, and other ingredients increased relatively. During the mycelium growth, arabinose and glucose of hemicellulose biodegraded, and other ingredients increased relatively. During *Lentinus edodes* growth, arabinose, galactose and glucose of hemicellulose biodegraded and continued to decrease, and other ingredients increased relatively.

The FTIR spectroscopy shows that hemicellulose of *Quercus* Linn wood groups firstly began to fracture and degrade after wood was steam explosion and biodegradation of *Lentinus edodes*. The six hemicellulose samples of natural, steamed and biodegraded wood had the similar spectral patterns. And the transmittance of the characteristic peaks in hemicellulose gradually increased after decreased after steam explosion and biodegradation of *Lentinus edodes*. TGA and DTG curves that thermal stability and maximum thermal degradation rates of six samples were contiguous after steam explosion and biodegradation of *Lentinus edodes*. According to the weight loss, the thermal stability of hemicelluloses had tiny difference

at 100 °C, 200 °C, 300 °C and 803.3 °C. According to the DTG_{max} values, the maximum thermal degradation rates of six samples were contiguous after steam explosion and biodegradation of *Lentinus edodes*.

By combining NMR with FTIR spectroscopy, The NMR spectra of GM1, GM3, GM5 and GM6 presented the straightforward structure as revealed by four major signals corresponding to (1 \rightarrow 4)-linked β -D-Xyl residues and other less intense signals characteristic of a 4-Omethyl- α -D-glucuronic acid residue as well as an L-Araf residue and β -D-Xyl units substituted with 4-O-methyl- α -D-GlcpA. Furthermore, a small amount of other minor hemicelluloses (β -glucans) including xylans in the extractable hemicelluloses could be identified by NMR and other approaches.

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