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The structural features of hemicelluloses dissolved out at different cooking stages of active oxygen cooking process

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ABSTRACT

This work described the morphologic changes of corn stalk and the structural characterization of its hemicelluloses dissolved in yellow liquor at different cooking stages. The results showed that active oxygen cooking process was an efficient method to depolymerize the corn stalk into cellulose, hemicelluloses, and lignin as a pretreatment of biomass conversion. This cooking process can also be divided into three phases: bulk delignification, extended delignification, and residual delignification. During the heating-up period 57.67% of hemicelluloses and 62.31% of lignin were removed from the raw material. However, only 15% of hemicelluloses and 23.21% of lignin were removed during at temperature' period. The hemicelluloses from the corn stalk and yellow liquor were composed of (1→4)-β-D-xylopyranose backbones substituted with α-L-arabinofuranosyl, 4-O-methyl-α-D-glucuronic acid, and some methoxyl residues. The backbones of hemicelluloses were gradually cleaved during the cooking process. The acetyl groups substituted with xylopyranosyl residues were completely cleaved during the cooking process.

1. Introduction

The energy demands of the transportation sector for the strong dependence on the petroleum-derived fuels are becoming increasingly difficult to fulfill the diminishing fossil fuel and concerns about the growing levels of CO_2 in the atmosphere (Alonso, Bond, & Dumesic, 2010). With the ever-growing energy demands and environmental concerns, together with the diminishing fossil fuel reserves, it is necessary to make alternative production chains to reduce the dependence on fossil fuel and offset greenhouse gas emissions (Foley, Beach, & Zimmerman, 2011). Lignocellulosic biomass, composed of cellulose (40–50%), hemicelluloses (25–35%), and lignin (15–20%) and produced 1.6×10^8 tons per year from agriculture and forest residue, is being considered as one of most promising feedstocks for the production of fuels and chemicals (Kaparaju, Serrano, Thomsen, Kongjan, & Angelidaki, 2009; Ramsay, Aly Hassan, & Ramsay, 1998). However, the intertwined components in a complex composite matrix is resistant to microbial and enzymatic deconstruction, known as biomass recalcitrance. Therefore, the pretreatment of lignocellulosic biomass to generate the three main feedstock streams is a pre-requisite "first-step" for production of biofuels and chemicals (Vom Stein et al., 2011). So far, many pretreatment procedure have been developed and can be classified into physical, chemical, physicochemical, and biolgical methods (Sun et al., 2014). Chemical method is considered to be the most efficient pretreatment process.

Pulping is one of industrial technologies on utilizing agriculture and forest residue. Industrialized conventional cooking processes mainly include soda, Kraft, and sulfite, in which NaOH, Na_2S, SO_2, and sulfite are applied as the cooking chemicals (Shi, Yang, & Lin, 2013). Those soluble acidic and basic chemicals can efficiently remove the lignin and hemicelluloses and high quality pulp is produced. However, if the cooking chemicals in the cooking process could not well be recycled, the serious pollution can be caused. The complicated chemicals recovery system increases the cost of construction and production, meanwhile, the lignin and hemicelluloses dissolved in the cooking liquor are burnt in the recovery process. Besides, mineral acids and ionic liquids (ILs) are used to hydrolyze lignocellulosic biomass, both of them can efficiently degrade the lignocellulosic biomass (El-Zawawy, Ibrahim, Abdel-Fattah, Soliman, & Mahmoud, 2011; Tadesse & Luque, 2011).

Abbreviations: X, (1→4)-β-D-xylopyranose; XU, (1→4)-β-D-xylopyranose-2-O-(4-O-methyl-α-D-glucuronic acid); U, 4-O-methyl-α-D-glucuronic acid; A, α-L-arabinofuranose; G, (1→4)-β-D-glucopyranose; L, lignin; ax, axial; eq, equatorial.

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However, drawbacks of acidic hydrolysis are cannot be ignored, such as, wasteful and energy inefficient, requiring separation, recycling and treatment of the acid waste residue, and equipment corrosion (Zhou et al., 2013). For ILs, they can modify the tight linkages in the cell-wall componets and make biomass easier to degrade (Tadesse & Luque, 2011). But the cost of them has been one of major concerns. Therefore, it is necessary to develop an efficient, low-cost, and environmental friendly preteatment process for the biomass conversion.

For soft agricultural residues, such as corn stalk, wheat straw, bagasse, a novel cooking process, active oxygen with solid alkali cooking process, was developed by our research group (Pang et al., 2012; Shi, Yang, Lin, Gong, et al., 2012; Shi, Yang, Lin, & Peng, 2013; Yang et al., 2012). In this cooking process, active oxygen (O_2 and H_2O_2) was added as cooking chemicals for the removal of lignin and hemicelluloses, and MgO was used as a protective agent of carbohydrate, especially for cellulose. The cooking chemicals in conventional cooking process were replaced by the clean chemicals. After cooking, most of MgO was dissolved because of the production of acids, and the pH of cooking liquor (yellow liquor we called) is about 8.0. Compared with the conventional cooking process, Mg^{2+} is the only ion introduced to the cooking system. The lignin and hemicelluloses can be easily recycled from the weak alkality yellow liquor, and pulp can be used for the feedstocks for the further biomass conversion and produce different high-added chemicals, and materials, such as biofuels (Alonso et al., 2010), 5-Hydroxymethylfurfural (HMF) and furfural (Rosatella, Simeonov, Frade & Afonso, 2011; Sahu & Dhepe, 2012), propionic acid (Ramsay, Aly Hassan & Ramsay, 1998), films (Goksu, Karamanlioglu, Bakir, Yilmaz & Yilmazer, 2007), surfactants (Bouquillon, 2011), and xylooligosaccharides (Parajó, Garrote, Cruz & Dominguez, 2004).

The hemicelluloses and lignin were removed from the raw material during the cooking process. In previous study, we explained the structural changes of lignin and hemicelluloses during the cooking process (Shi, Yang, Lin, Gong, et al., 2012; Shi, Yang, Lin & Peng, 2013; Yang et al., 2012), but morphologic changes of corn stalk and the structural characterization of its hemicelluloses dissolved in yellow liquor at different cooking stages is completely unknown. In this work, we have isolated the hemicelluloses from the corn stalk and precipitated the hemicelluloses dissolved in the yellow liquor at different cooking time of active oxygen cooking process. To clarify the structural changes of hemicelluloses at different cooking stages and comprehensively understand the cooking mechanism, the structure and composition of hemicelluloses from the raw material and yellow liquor were studied in detailed.

2. Materials and methods

2.1. Materials

Corn stalk was supplied by China BBCA Group Corp., China. It was first dried in the sunlight and cut into small pieces (less than 10 cm). Solid alkali was magnesium oxide powder with a purity of over 98.0%, obtained from Tianjin Kermel Reagents Co. Ltd., China. Deuterated water (D_2O) with a purity of over 99.9% was obtained from Sigma–Aldrich (Shanghai, China). Other reagents of analytical grade were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China).

2.2. Active oxygen cooking process

The cooking operations were described in our previous papers (Pang et al., 2012; Shi, Yang, Lin, Gong, et al., 2012; Shi, Yang, Lin, & Peng, 2013; Yang et al., 2012). The detailed operations are given as follows: 50 g corn stalk (oven dried weight), 15% by weight dosage of MgO (based on the oven dry weight of corn stalk) and 3% by weight dosage of hydrogen peroxide (based on the oven dry weight of raw material) were placed in a 2 L stainless, rotating autoclave at a solid-to-liquor ratio of 1:6 (w/v). After being sealed, the autoclave was filled with oxygen of 1.0 MPa. The cooking was performed at 165 °C for 2 h. Cooking was interrupted at 100 °C, 165 °C-0 h, 165 °C-0.5 h, 165 °C-1 h, and 165 °C-2 h to show the effect of time on the pulp. After cooking, the yellow liquor was extruded from the raw stock and stored in a refrigerator. The pulp was washed, air dried and weighted for pulp yield determination.

2.3. Characteristics of the raw material and pulp

The methods for characteristics lignin, cellulose, and holocellulose in the raw material and pulp were presented in our previous paper (Yang et al., 2012). The loss rates of celluloses, hemicelluloses and lignin were calculated according to the follow formula:

$$\text{loss rate of main component}\,(\%) = 100\% - \frac{YC_P}{C_R}$$

where Y is the yield of the pulp at different cooking stages (%), C_P is the content of celluloses, hemicelluloses, lignin in the pulp got from different cooking stages (%), C_R is the content of celluloses, hemicelluloses, lignin in the raw material (%).

2.4. Isolation of hemicelluloses from the raw material

Hemicelluloses were extracted with 1:1 dioxane: water. The experiment was carried out as follows: the 10 g ball-milled corn stalk directly suspended in 1:1 dioxane: water with a solid-to-liquid ratio of 1:20 (g/mL) and heated at 80 °C for 3 h. The mixture was centrifuged at 5000 rpm for 10 min. This step was performed twice and mixed the supernatant. The supernatant was concentrated to 20 mL under vacuum and poured into three volumes of 95% ethanol. After centrifugation at 5000 rpm for 10 min, the precipitated hemicelluloses was washed with 70% ethanol and freeze-dried to obtain hemicelluloses fraction HR.

2.5. Isolation of hemicelluloses from the yellow liquor

The 50 mL yellow liquor from the cooking process was centrifuged at 5000 rpm for 10 min, and the supernatant was poured into three volumes of 95% ethanol. After centrifugation at 5000 rpm for 10 min, the precipitated hemicelluloses were freeze-dried. The hemicelluloses precipitated from the yellow liquor of 100 °C, 165 °C-0 h, 165 °C-0.5 h, 165 °C-1 h, and 165 °C-2 h cooking process were named H-1, H-2, H-3, H-4, and H-5, respectively.

2.6. Scanning electron microscopy (SEM)

The pulp and raw material were washed with deionized water and dried using a vacuum drying oven for 72 h before testing. The dried samples were then coated with Au and visualized using a scanning electron microscope (S-3700N; Hitachi, Japan) at 10 kV.

2.7. Monosaccharides and uronic acids

The monosaccharides and uronic acids in the hemicelluloses were liberated by hydrolyzing approximately 10 mg sample using 5.5 mL of 6.5% H_2SO_4 for 2.5 h at 105 °C. The hydrolyzates were diluted to 50-fold (after adjusting the pH value to 7.0), filtered, and injected into the high-performance anion-exchange chromatography (HPAEC) (Dionex ICS-3000, USA) with pulsed amperometric detection, a CarboPac™ PA20 column (3 × 150 mm) and a CarboPac™ Guard column (3 × 30 mm). The operations were

run at 30 °C for 30 min, and the flow rate during the analysis process was 0.5 mL/min. When eluting the monosaccharide, the eluent was 2 mM NaOH. However, due to the weak eluting power of the NaOH, the eluent was 100 mM NaAc and 2 mM NaOH when eluting the uronic acids.

2.8. Molecular weight analysis

The molecular weights of the hemicelluloses were determined by gel permeation chromatography (GPC). The GPC system comprises a Waters 1525 binary HPLC pump, a Waters 717 plus Auto-sampler, a Waters 2414 refractive index detector, and a Breeze (V3.3) GPC work station (Waters, USA). The samples were dissolved in the eluent and injected into the TSk-GELG-5000PW xL column (7.8 × 300 mm) and TSk-GELG-3000 PW xL column (7.8 × 300 mm) (TOSOH, Japan) in a series. The eluent flow (0.02 M pH 6.0 KH_2PO_4) was 0.6 mL/min. The GPC columns and injection system were maintained at 35 °C during the analysis. Glucan was applied as a reference substance.

2.9. Fourier-transform infrared spectroscopy (FT-IR) analysis

FT-IR spectra of the hemicellulosic fractions were obtained on a FT-IR spectrophotometer (Bruker Tensor 27, Germany). The samples were combined with KBr in slices containing 1% hemicelluloses. The scan range was 4000–400 cm^{-1} in the transmission mode.

2.10. Nuclear magnetic resonance (NMR) analysis

The ^{1}H–^{13}C 2D hetero-nuclear single quantum coherence (HSQC) spectra of the hemicelluloses (80 mg in 1 mL D_2O) were obtained on Bruker AV 600 instrument in the HSQC experiment mode at 25 °C. The spectral widths were 6000 and 25,000 Hz for the ^{1}H- and ^{13}C-dimensions, respectively. The acquired time per scan was 0.0984 s, the relaxation delay time was 1.5 s, and the pulse width was 12.1 Hz.

3. Results and discussions

3.1. Morphologic changes and delignification of the corn stalk during the cooking process

Figs. 1 and 2 show the photographs and SEM pictures at multiple magnifications of corn stalk and pulp under different cooking time, respectively. The photographs showed that the morphology of corn stalk barely changed when the temperature reached 100 °C. However, the corn stalk appeared fibration when the temperature reached 165 °C, but the pulp was very rough and the fiber was not completely seperate. The corn stalk can also be easily identified, especially for the stem. After cooking for 1 h, the leaves in the raw material were completely turned into pulp, but the stem of corn stalk also can be still found in the pulp. Prolonging the cooking time to 2 h, the raw material almost changed into pulp and the crude pulp was very good. The similar results also were got in the SEM pictures. From the SEM images of the pulp under 165 °C for 2 h, it can be found that the fiber was smoother than other pulp fiber, and fiber was well isolated. The morphologic changes showed that the corn stalk were slowly become into pulp during the cooking process. Meantime, it indicated that active oxygen cooking process is moderate pretreatment procedure, compared with the chemical cooking process (Alaejos, Lopez, Eugenio, & Tapias, 2006). In the chemical process, the chemicals can easily break the linkages in the cell wall. But in the active oxygen cooking process, the gaseous oxygen can not directly react with raw material. First a little molecular oxygen must dissolve in the water, then they form different active ions under the cooking conditions. Then the active ions in the water can enter into the water-soaked cell wall and break the linkages between and among the main component. Therefor, the rate and quantity of mas transfer process determine the active oxygen cooking process.

The yields of pulp and the main components of raw material and pulp are listed in Table 1. As can be seen, the yields of pulp decreased from 104.2% at 100 °C to 52.2% at 165 °C for 2 h. Correspondingly, the yields of hemicelluloses precipitated from 50 mL yellow liquor increased with prolonging of cooking time. Ash contents in all pulp sample were higher than in raw material, which may have caused by the adsorption of fiber to the powdered MgO. It also can explain the phenomenon of yield of pulp at 100 °C reaching 104.2%. As can been seen from Table 1, the rates of removal of hemicelluloses and lignin after the temperature reaching 165 °C were 57.67% and 62.31%, respectively, which showed that most of lignin and hemicelluloses were removed during heating-up period. The heating-up period was about 2 h, but the time from 100 to 165 °C was about 1.5 h. Under this temperature and pressure, the water can permeate through the corn stalk cell wall and soften the fiber, which was in favor of removal of hemicelluloses and lignin. Besides, the heating rate became very low when the temperature reached 160 °C, which make most of lignin and hemicelluloses were removed during this period. During at temperature' period, the rates of removal of lignin and hemicelluloses were 23.31% and 15.67%, respectively, which were much lower than that in the heating-up perriod and meant that heating-up period was significant for the removal of lignin and hemicelluloses and can be called for bulk delignification phase in the active oxygen cooking process. After cooking for 1 h, 18.25% lignin and 15.4% hemicelluloses were removed from the raw material; nevertheless, only 4.96% lignin and 0.27% hemicelluloses were removed from the rest of cooking time, which can be regards as the two phase in the active cooking process. From the removal rates of lignin and hemicelluloses and pictures of pulp samples, we can call former as extended delignification phase and the latter as residual delignification phase, which were similar to the conventional cooking process (Lisboa, Evtuguin, Neto, & Goodfellow, 2005).

It is unavoidable to degrade the cellulose during the cooking process. And the loss rates of cellulose increased with cooking time prolonging. And it reached 8.08% after cooking for half an hour. However, in the rest time of cooking process, the loss rate of cellulose was less than 3.5%, which suggested that the degradation of cellulose mainly took place after half an hour of temperature reaching 165 °C. At end of cooking, the low loss rate (11.51%) of cellulose and high removal rates of hemicelluloses (73.34%) and lignin (85.52%) indicated that active oxygen cooking process was an efficient pretreatment method for the biomass conversion and the active oxygen showed high selectivity for the hemicelluloses and lignin.

3.2. Contents of neutral monosaccharides and uronic acids

The hemicelluloses are composed of different sugar residues, which differ with respect to their species, location in the cell wall and extraction process (Sun et al., 2014). In this study, to analyze the difference of hemicellulosic fractions obtained from the raw material and the yellow liquor at different cooking stages the content of neutral monosaccharides and uronic acids of are listed in Table 2. In hemicellulosic fraction H-1, which was extracted by the hot water for the short time, the dissolved carbohydrates were mainly composed of arabinose (18.73%), galactose (19.32%), glucose (17.36%), xylose (29.22%), and mannose (11.84%), which indicated that the corn stalk may release not only short chain xylan hemicellulosic polymers, but also arabinogalactans, and pectic substances during room temperature to 100 °C. However, xylose was the dominant

Fig. 1. The photographs of corn stalk and pulp in different cooking stages.

sugar component (52.76 – 65.16%) in the other hemicellulosic fractions, which were mainly originated from the backbone of xylans. Arabinose and galactose appeared as the second and third major sugars in the hemicellulosic fractions, comprising 14.81–21.37% and 7.61–11.73%, respectively. The glucose (5.83–14.20%) mainly came from the α-glucan and pectic polysaccharides (Peng et al., 2009), and the degraded cellulose during the cooking process. Mannose (1.97–3.00%) and uronic acids (mainly glucuronic acid or its 4-O-methyl derivative, 2.47–3.89%) were observed as minor constituents. The sugar residual components of hemicelluloses isolated from the raw material and precipitated from the yellow liquor cooking under 165 °C at different cooking time were similar, except for the content of glucose in the raw material. Generally speaking, there is no big different on the sugar residual component of hemicelluloses extracted under the low temperature (blow 50 °C) (Bian et al., 2012). In the active oxygen cooking process, the high temperature and active oxygen can break the linkage in the hemicellulosic chain, which effected the content of netural monosaccharides and

Table 1
Characteristics of corn stalk and pulp and the yields of hemicelluloses from the yellow liquor.

	Yield			Components of corn stalk and pulp/%		
	Y-1/%[a]	Y-2/g[b]	Ash	Lignin	Hemicelluloses	Cellulose
Raw material	–	–	7.95	21.73	23.96	39.12
100 °C	104.2	0.21	12.75	17.21	23.49	37.24
165 °C-0 h	70.0	1.41	8.08	11.7 (62.31)[c]	14.49 (57.67)	53.44 (4.38)
165 °C-0.5 h	61.0	1.72	11.06	8.69 (75.60)	12.62 (67.87)	58.95 (8.08)
165 °C-1 h	52.2	2.13	8.69	8.09 (80.56)	12.36 (73.07)	67.53 (9.89)
165 °C-2 h	50.2	2.14	9.02	6.27 (85.52)	12.72 (73.34)	68.96 (11.51)

[a] The yields of pulp.
[b] The yields of hemicelluloses precipitated from 50 mL yellow liquor.
[c] The data in the bracket showed the loss rates of cellulose, hemicelluloses and lignin during the cooking process.

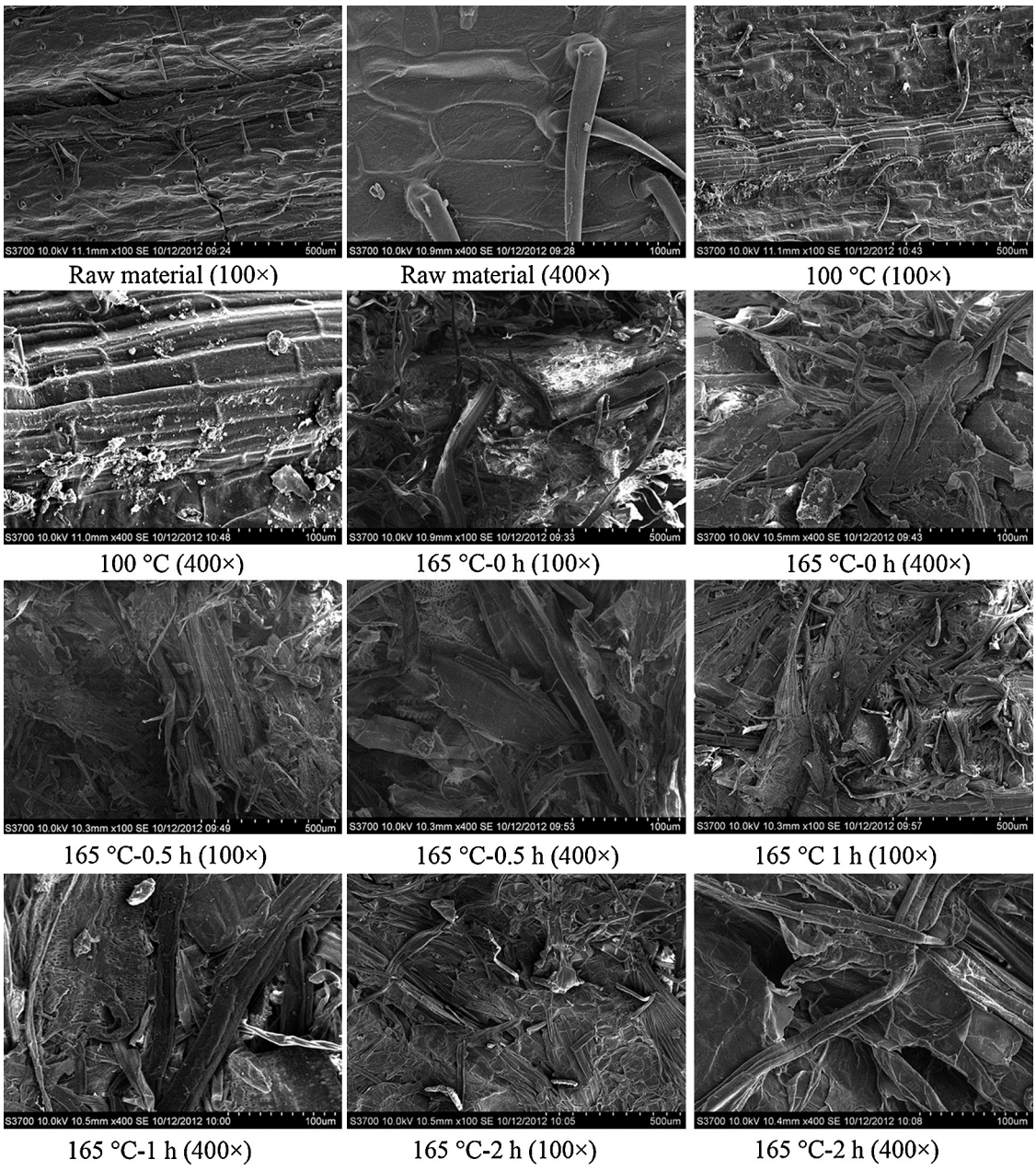

Fig. 2. The SEM pictures of stalk and the pulp.

uronic acid. The contents of arabinose and galactose decreased with the cooking time prolonging. Nevertheless, almost 80% hemicelluloses in the cooking process was came from heating-up period, which indicated the sugar residuals substituted to the backbone of xylans were removed during the cooking process.

Additonally, the ratio of xylose to arabinose (Xyl/Ara) is indicative of the degree of linearity or branching of hemicelluloses (Bian, Peng, Xu, Sun & Kennedy, 2010; Peng et al., 2012). The increased ratios of Xyl/Ara in H-2, H-3, H-4, and H-5 showed that the degree of branching of dissolved hemicelluloses during the cooking process

Table 2
The monosaccharide composition and uronic acid content (%, w/w) of the hemicelluloses from the corn stalk and the yellow liquor of different cooking stages.

Neutral sugars and uronic acids	Hemicellulosic fractions					
	HR	H-1	H-2	H-3	H-4	H-5
Arabinose	14.81	18.73	21.37	18.33	16.45	16.36
Galactose	9.03	19.32	11.73	8.30	7.61	8.51
Glucose	14.20	17.36	9.20	7.26	5.82	6.52
Xylose	57.14	29.22	52.76	60.63	65.16	62.94
Mannose	2.72	11.84	3.00	1.97	1.96	2.20
Uronic acid	2.09	3.52	1.95	3.51	3.00	3.46
Xyl/Ara	3.86	1.56	2.47	3.31	3.96	3.85

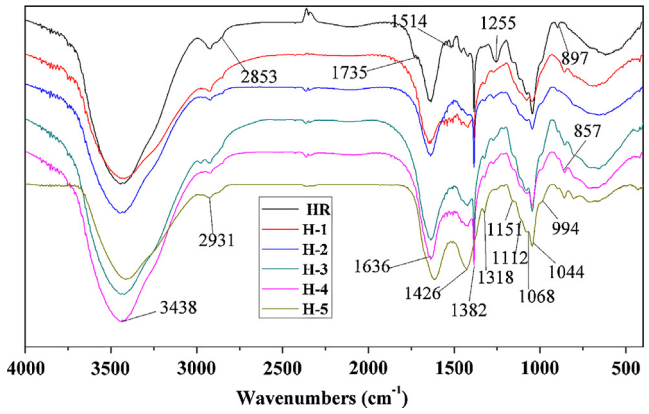

Fig. 3. The FT-IR spectra of the hemicelluloses obtained from the corn stalk and the yellow liquor of different cooking stages.

decreased with the cooking time prolonged, which may be caused by the fell off substituted sugar residues with backbone and the cleavage of glycosidic bonds of xylans. Compared with hemicelluloses extracted by NaOH (Peng et al., 2009; Sun, Wen, Ma, & Sun, 2013), the higher degree of branching was in favor of the removal of hemicelluloses from the cell wall matrix.

3.3. Average molecular weight

Average molecular weight is an important parameter to assess the hemicellulosic backbone changes during the active oxygen cooking process. The weight-average (M_W) molecular weights, number-average (M_N) molecular weights (g/mol) and polydispersities (M_W/M_N) of the hemicelluloses from the corn stalk and the yellow liquor at different cooking stages are listed in Table 3. Obviously, H-1 showed a much lower M_W value of 3788 g/mol than that other hemicellulosic fractions (5470–19640 g/mol), which indicated that the dissolved hemicelluloses blow 100 °C was small molecular size. When the temperature reached 165 °C, 57.67% of hemicelluloses was removed from the raw material. After that, the removal rates of hemicelluloses were 67.87%, 73.07% and 73.34% in 0.5, 1 and 2 h, respectively, which showed that the hemicelluloses mainly came from the heating-up period. During this phase, the M_W value of hemicelluloses decreased from 19640 g/mol to 5470 g/mol, which suggested that the hemicelluloses were first dissolved in the yellow liquor and then gradually degraded under cooking conditions. Besides, the molcular weighs of hemicelluloses dissloved during heating-up period is much higher than that we extracted by hot water and KOH (Shi, Yang, Lin, Gong, et al., 2012). It was noted that removal rate of hemicelluloses in 1 h were almost equal to that in 2 h (Table 1), but the M_W of hemicelluloses were reduced from 11560 to 5470 g/mol, which meant that the backbone of hemicelluloses were severed during this period. The main changes in the residual delignification phase was that the hemicelluloses, cellulose, and lignin dissolved in the water were further degraded, even into the small molecular substance, just like formic acid, acetic acid.

Generally speaking, the polysaccharides with molecular weights in excess of DPn 50 and the polydispersities below 3 are indicative of molecularly uniform polymers with potential commercial utility (Yang, Song, Yuan, Xu, & Sun, 2011). The polydispersity of hemicelluloses obtained from the final yellow liquor was 1.527, which illuminated the structural homogeneity of hemicelluloses in the yellow liquor. The hemicelluloses released during active oxygen cooking process possess potential commercial utility. In the other words, the active oxygen cooking process gives a novel and clean way to pretreat the corn stalk for the biomass conversion.

3.4. FT-IR spectra

The FT-IR spectra of hemicelluloses isolated from the raw material and yellow liquor of active oxygen cooking process are listed in Fig. 3. The profiles and relative intensities of the most bands are rather similar, indicating the similar chemical structures for the hemicelluloses. The absorbance for the —OH stretching vibration of hemicelluloses and water involved in hydrogen bonding band appeared at 3438 cm^{-1} (Peng et al., 2012c). Peaks at 2931 and 2853 cm^{-1} were assigned to C–H stretching from the methylene and methyl, respectively (Anugwom et al., 2012). The absorbance of absorbed water in the hemicelluloses fraction was at 1640 cm^{-1} (Fang, Sun, & Tomkinson, 2000). In the spectrum of HR, the small peaks at 1735 (C=O ester) and 1255 cm^{-1} (—C—O— in ester group) were originated from the acetyl, uronic, and ferulic ester groups of hemicelluloses between lignin (Jin et al., 2009). However, those signals nearly disappeared in the spectra of hemicelluloses isolated from the yellow liquor, which indicated that the subtotal ester bonds in the hemicelluloses were broken under the cooking conditions. In the cell wall, lignin and hemicelluloses can form lignin-carbohydrate complex (LCC) by the esters, glycoside bonds, and benzyl ethers (Yuan, Sun, Xu, & Sun, 2011), and the break of esters bonds is favor of the removal of lignin and hemicelluloses during the cooking process. Furthermore, the weak peaks at 1514 cm^{-1} were attributed to the aromatic skeletal vibration associated lignin (Jin et al., 2009; Peng et al., 2012c). However, this peak was almost absent in hemicelluloses obtained from the yellow

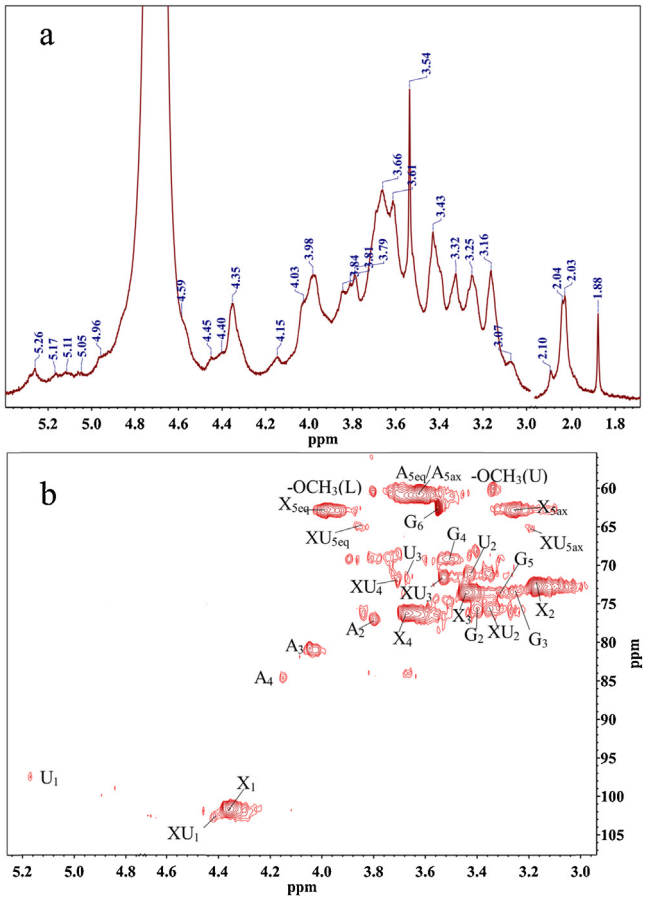

Fig. 4. The ^{1}H and ^{1}H–^{13}C HSQC spectra of hemicelluloses from the raw material. The designations are as follows: X, (1→4)-β-D-xylopyranose; XU, ((1→4)-β-D-xylopyranose-2-O-(4-O-methyl-α-D-glucuronic acid); U, 4-O-methyl-α-D-glucuronic acid; A, α-L-arabinofuranose; G, (1→4)-β-D-glucopyranose; L, lignin; ax, axial; eq, equatorial.

Table 3
Weight-average (M_W) molecular weights, number-average (M_N) molecular weights (g/mol) and polydispersities (M_W/M_N) of the hemicelluloses from the corn stalk and the yellow liquor of different cooking stages.

	Hemicellulosic fractions					
	HR	H-1	H-2	H-3	H-4	H-5
M_N	6010	2693	13572	5073	6165	3583
M_W	8655	3788	19643	11843	11565	5471
M_W/M_N	1.440	1.407	1.447	2.335	1.876	1.527

liquor, which verified that there is little of lignin in the hemicellulosic fractions, which caused by the break of linkages between lignin and hemicelluloses. The sharp peak at 1382 cm^{-1} came from the impurities in KBr.

The peaks in the 1120–1000 cm^{-1} were the typical characteristic of xylans substituted by the arabinose at C-3 of xylopyranose (Jin et al., 2009), and the C—O—C stretching vibrations of glycosidic linkages between xylose and arabinose were assigned to the bands at 1163 cm^{-1}. The absorbances at 897 cm^{-1}, the carbohydrate C_1—H deformation and ring-stretching frequency, were characteristic of β-glycosidic linkages between the sugar units in the hemicelluloses (Peng et al., 2010). The signals at 994 and 857 cm^{-1} were indicative of α-glycosidic linkages, which showed that the arabinose, glucose, galactose, and glucuronic acid probably linked by α-glycosidic linkages (Ma, Jia, Zhu, Li, Peng & Sun, 2012; Peng et al., 2012a). The intensities of bands at 897 cm^{-1} in the hemicelluloses precipitated from the yellow liquor nearly disappeared. However, the intensities of bands at 857 cm^{-1} increased with cooking time extented for the hemicellulosic fraction H-2 to H-5. Those phenomenons were observe in our previous study (Shi, Yang, Lin, Gong, et al., 2012; Shi, Yang, Lin, & Peng, 2013), and indicated that the struture of hemicelluloses in the yellow liquor is different with the hemicelluloses in the raw material, which may caused the cooking process. Additionally, another band at 1426 cm^{-1} related to the —COO^- symmetric stretching of uronic acid carboxylate (Zhang, Meng, Xu, & Sun, 2011), and the increasing intensity was observed from H-2 to H-5, corresponding to the increase trend of uronic acids.

3.5. NMR spectra analysis

The hemicelluloses obtained from the corn stalk were analyzed by NMR spectroscopy in order to characterize the structural features. Fig. 4 shows the ^1H and HSQC spectra of hemicelluloses extracted from the raw material. As can be seen from Fig. 4a, the signals at 3.0–5.3 ppm mainly came from the protons of arabinose and xylose residues except for the strong signals at 4.7 ppm, which was caused by the residual solvent of HDO. The relevant signals occurred in anomeric region (6.5–4.9 ppm for the α anomers and 4.9–4.3 ppm for the β anomers) and ring protons region (4.5–3.0 ppm) (Wen et al., 2011). The signal at 4.35 ppm was assigned to the anomeric protons of (1→4) linked β-D-xylopyranosyl residues. The ring protons of β-D-xylopyranosyl

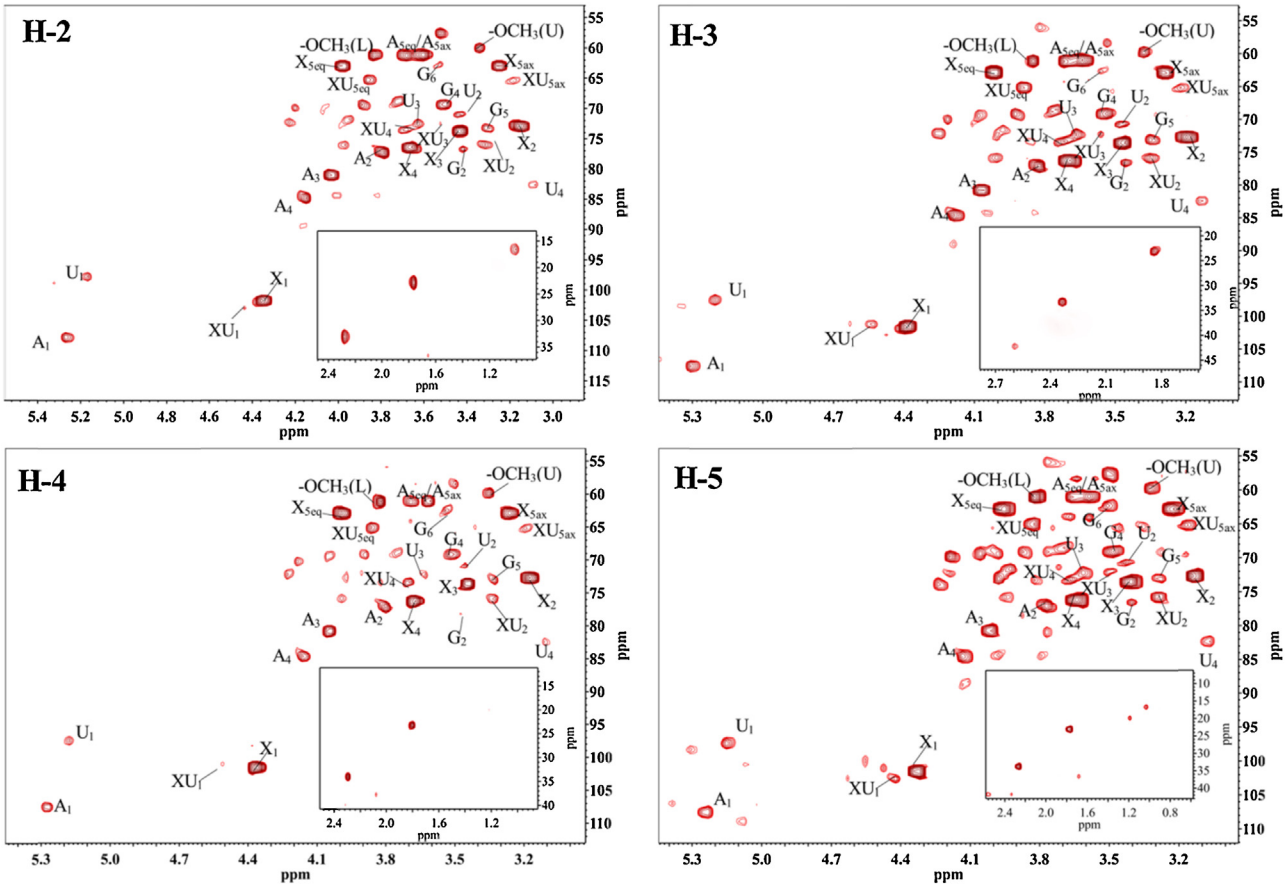

Fig. 5. The ^1H–^{13}C HSQC spectra of hemicelluloses from the yellow liquor of active oxygen cooking at different cooking stages. The designations are same as Fig. 4.

residues were attributed to 3.98 (H-5eq), 3.66 (H-4), 3.43 (H-3), 3.32 (H-5ax), and 3.16 (H-2) ppm. Meanwhile, the anomeric protons of β-D-xylopyranosyl residues substituted by L-arabinofuranose at C-3 (mono-substituted), C-2 and C-3 (disubstituted), and 4-O-methyl-α-D-glucuronic acid residues at C-2 were shown at 4.45, 4.59 and 4.40 ppm, respectively (Jin et al., 2009; Peng et al., 2012b; Vignon & Gey, 1998). Correspondingly, the anomeric protons of α-L-arabinofuranosyl residues corresponded to 5.26 ppm (mono-substituted), and 5.11 and 5.05 ppm (disubstituted); and the anomeric protons of 4-O-methyl-α-D-glucuronic acid residues were attributed at 5.17 ppm (Peng et al., 2012; Revanappa, Nandini, & Salimath, 2010). The other main chemical shifts at 3.81, 4.03, 4.15, 3.66, and 3.61 ppm were assigned to the H-2, H-3, H-4, H-5eq, and H-5ax of L-arabinofuranose, respectively (Shi, Yang, Lin, Zhuang, et al., 2012; Shi, Yang, Lin, Gong, et al., 2012). The methoxyl protons of 4-O-methyl-α-D-glucuronic acid residues produced peak at 3.32 ppm. The acetyl groups substituted at C-3 of xylopyranosyl residues was visible as a strong signal at 2.03 ppm (Peng et al., 2012c), which agreed with the presence of small sharp peaks at 1735 and 1255 cm^{-1} in the FT-IR spectrum of HR. The ^{1}H–^{13}C HSQC spectrum of hemicelluloses from raw material is shown in Fig. 4b. The xylopyranosyl residues were indicated by signals at 4.36/101.58 (H1–C1), 3.17/72.71 (H2–C2), 3.45/73.54 (H3–C3), 3.67/76.23 (H4–C4), and 3.99 and 3.25/62.77 (H5–C5) ppm in HSQC spectrum, in which the chemicals shifts of 3.99 and 3.25 were assigned to the equatorial and axial protons linked in C-5, respectively (Peng, Peng, Bian, Xu, & Sun, 2011). Concerning the arabinofuranosyl residues, which were decorated at C-2 and/or C-3 of β-D-xylopyranosyl units, the signals of H2–C2, H3–C3, H4–C4, H5ax–C5, and H5eq/C5 appeared at 3.80/77.06, 4.04/80.95, 4.15/84.53, 3.62/60.80, and 3.69/60.80 ppm, respectively. However, the anomeric proton and carbon of arabinofuranose residues were not observed in the spectrum. Moreover, weak signals at 5.17/97.53, 3.42/71.12, 3.67/71.94, and 3.34/60.05 ppm were characteristic of H1–C1, H2–C2, H3–C3, and –O–CH_3 of 4-O-methyl-α-D-glucuronic acid residues, respectivly. Additionally, the (1→4) linked β-D-xylopyranosyl residues related with 4-O-methyl-α-D-glucuronic acid at C-3 position were also determined in the spectrum (4.4/102.64 ppm). A strong contour at 60.48/3.80 ppm corresponded to the methoxyl groups in lignin, which indicated that there was slight amount of lignin in the hemicellulosic fractions. Besides the signals of sugar residues from hemicelluloses, weak signals corresponding to glucose were also observed. The NMR spectra demonstrated that the hemicelluloses from corn stalk were composed of (1→4) linked β-D-xylopyranose backbones substituted with α-L-arabinofuranosyl and 4-O-methyl-α-D-glucuronic acid residues, even some methoxyl groups.

The ^{1}H–^{13}C HSQC spectra of hemicelluloses isolated from yellow liquor the active oxygen cooking process at different cooking stages are listed in Fig. 5. All sugar units of hemicelluloses can be observed from the spectra, which indicated that the structures of hemicelluloses in the yellow liquor and raw material were very similar. The acetyl groups substituted with xylopyranosyl residues could not be found from the spectra of hemicelluloses precipitated from yellow liquor, which verified that acetyl groups were completely cleaved during the cooking process.

4. Conclusion

The active oxygen cooking process is an efficient method to depolymerize the corn stalk into cellulose, hemicelluloses, and lignin as a pretreatment of biomass conversion. The cooking process can also be divided into three phases: bulk delignification, extended delignification, and residual delignification. During the heating-up period 57.67% of hemicelluloses and 62.31% of lignin were removed from the raw material. However, only 15% of hemicelluloses and 23.21% of lignin were removed during at temperature' period. The hemicelluloses from the corn stalk and yellow liquor were composed of (1→4)-β-D-xylopyranosyl backbones substituted with α-L-arabinofuranosyl, 4-O-methyl-α-D-glucuronic acid residues, and some methoxyl residues. The backbones of hemicelluloses were gradually cleaved during the cooking process. The acetyl groups substituted with xylopyranosyl residues were completely cleaved during the cooking process.

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