Graded Ethanol Fractionation and Structural Characterization of Alkali-Extractable Hemicelluloses from *Olea europaea* L.

Xiaopeng Peng, [a,b] Jing Bian, [c,*] Mingfei Li,[c] Xiao Xiao, [d] Xinli Xia,[a] Weilun Yin,[a] and Runcang Sun [c,e,*]

Dewaxed *Olea europaea* L. was subjected to delignification followed by alkali extraction with 10% KOH containing 1% H_3BO_3. The released hemicelluloses were fractionated by precipitation through acidification and ethanol solutions with increasing concentrations from 20 to 30, 40, 50, 60, 70, and 90%. The structure of the subfractions obtained was comparatively characterized by sugar analysis, molecular weight, FT-IR, and NMR spectroscopy. Results indicated that 23.5% of hemicelluloses (% of the dewaxed material) were isolated by the alkaline extraction. An increase of ethanol concentration resulted in the precipitation of the more branched hemicelluloses as indicated by the increase of the ratios of arabinose to xylose and uronic acids to xylose. The hemicellulose subfractions Ha (precipitated by acidification to pH 5.5) and H30 (precipitated by 30% ethanol solution) had a similar structure, which was assumed to be glucuroxylan together with a small amount of α-glucan, whereas the hemicellulose subfraction H70 (precipitated by 70% ethanol solution) had a more complicated structure, which was mainly composed of a (1→4)-linked β-D-xylopyranosyl backbone with various side chains. The comprehensive structural characterization of the hemicelluloses of this species provides fundamental information for their potential applications in the fields of materials, chemicals, and energy production.

Keywords: Olea europaea L.; Ethanol precipitation; Hemicellulosic subfraction; Xylan

*Contact information: a: College of Biological Sciences and Biotechnology, Beijing Forestry University, Beijing 100083, China; b: State Key Laboratory of Tree Genetics and Breeding, Chinese Academy of Forestry, Beijing 100091, China; c: Beijing key laboratory of lignocellulosic chemistry, Beijing Forestry University, Beijing 100083, China; d: College of Science, Northwest A&F University, Yangling 712100, China; e: State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, China;*Corresponding author: bianjing31@yahoo.com.cn, rcsun3@bjfu.edu.cn*

INTRODUCTION

Olea europaea L. is an evergreen tree mainly distributed between 30 and 45° north latitude in both hemispheres. It has a cultivation area of approximately 9.2 million ha around the world (FAO 2011), which has tripled in the past 50 years. This species has great economic and ecologic potential, including nutritional and pharmacological value of the oil and the leaf extracts, extended plant adaption to drought, salinity, and temperature, and protection erosion function against desertification. In olive cultivation, old branches are pruned every 2 years after olive harvesting, generating large amounts of lignocellulose residues with an average annual production of 3000 kg ha^{-1} (Cara *et al*. 2007). In order to clean the field and prevent the propagation of diseases of the tree, these residues are usually disposed of by either burning or grinding and scattering on fields,

resulting in external handling costs and environmental pollution. The wood residue sometimes is used as firewood; however, to the best of our knowledge, there are no applications for preparing high-value products to achieve a full utilization.

Recently, in response to the depletion of fossil fuel resources, fractionation of lignocelluloses into constituent biopolymers – cellulose, hemicelluloses, and lignin, *etc*., and their applications represent great potential for providing natural chemicals and materials as alternative sources to fossil fuel-based products (Agbor *et al*. 2011; Deutschmann and Dekker 2012; Doherty *et al*. 2011). Hemicelluloses are heterogeneous polysaccharides composed of glucose, xylose, mannose, galactose, arabinose, and rhamnose together with uronic acids, which are specially combined according to the plant species and the living conditions. Four major groups of hemicelluloses are defined according to their primary structural monosaccharides: xylans, mannans, *β*-glucans, and xyloglucans (Ebringerova *et al*. 2005). Hemicelluloses have found numerous applications such as biomaterials (hydrogels, films, membranes, fibers, biocomposites), special chemicals (food additives, cosmetics, drug carriers, plant growth regulators), and pharmaceuticals (wound management aids), *etc*. (Ebringerova 2012; Ebringerova and Hromadkova 1999; Ebringerova *et al*. 2005; Hansen and Plackett 2008; Li and Pan 2010).

Hemicelluloses are thought to bind both covalently and non-covalently with cellulose, lignin, and proteins in the primary and secondary cell walls; thus, complex procedures are required to achieve effective separation of them from the plant material. Hemicellulose isolation involves the cleavage of the linkages between hemicelluloses and lignin in the lignocellulose matrix and solubilization into extraction solution. Due to the recalcitrant structure of the lignocellulosic material, the raw material is usually subjected to an initial delignification for the preparation of holocellulose before hemicellulose extraction (Cohen 2009; Naran *et al*. 2009; Peng *et al*. 2010a). Currently, alkaline extraction, alkaline peroxide extraction, liquid hot water extraction, and steam explosion-based extraction technologies are widely used to isolate hemicelluloses from lignocelluloses (Peng *et al*. 2012). Hemicelluloses from most agricultural residues, such as wheat straw, sugarcane bagasse, and rice straw, have been extracted for the purpose of structural characterization (Lawther *et al*. 1995; Sun *et al*. 2004, 1996). After the initial separation, the hemicelluloses obtained are subjected to further fractionation such as successive solvent precipitation (*e.g*., aqueous ethanol and aqueous ammonium sulfate), chromatograph separation (*e.g*., ion-exchange and gel-permeation), and membrane separation (*e.g*., ultrafiltration and nanofiltration) (Ebringerova 2012; Egues *et al*. 2012; Schlesinger *et al*. 2006).

Up to now there has been little information available on the structure of hemicelluloses from *Olea europaea* L.; thus, it is worthwhile to investigate this issue in detail. The aim of the present study was to isolate hemicelluloses from *Olea europaea* L. and characterize their structural features. The raw material was submitted to delignification followed by alkaline extraction, and the hemicelluloses dissolved in the alkaline solution were further fractionated into different subfractions through precipitation with graded ethanol. The structure of the subfractions was comparatively characterized with a set of analytical techniques, including acid hydrolysis, gel permeation chromatography (GPC), Fourier transform infrared spectroscopy (FT-IR), 1D nuclear magnetic resonance spectroscopy (^{1}H and ^{13}C NMR), and hetero nuclear signal quantum coherence spectroscopy (HSQC).

EXPERIMENTAL

Materials

Olea europaea L. was obtained from Gansu province, China. The stalks were first cut into sticks (approximately 30 × 0.5 × 0.3 mm), and then milled to pass a 0.8 mm screen. The dried sample was extracted with 2:1 (v/v) toluene-ethanol in a Soxhlet apparatus for 6 h and air-dried before use. The chemical composition of the dewaxed material was cellulose 45.8%, hemicelluloses 25.4%, and lignin 22.9%.

Extraction and Fractionation of Hemicelluloses

The scheme for alkaline extraction and fractionation of hemicelluloses from *Olea europaea* L. is shown in Fig. 1. The delignification of the sample was performed using sodium chlorite under acidic conditions (pH 3.8 to 4.0, adjusted by acetic acid) at 75 ^{o}C for 2 h. The solid residue was filtered and washed with water to neutralization and dried at 60 °C to obtain holocellulose. The holocellulose was extracted with 10% KOH containing 1% H_3BO_3 for 10 h at room temperature with a solid to liquor ratio of 1:25 (g mL^{-1}). The filtrate was neutralized with 6 M acetic acid solution to pH 5.5, and then was concentrated under reduced pressure. The precipitated hemicellulosic fraction was recovered by centrifugation and labeled as Ha. The supernatant was further subfractionated by graded precipitations at ethanol concentrations of 20, 30, 40, 50, 60, 70, and 90% sequentially, leading to seven subfractions, which were designated as H20, H30, H40, H50, H60, H70, and H90, respectively. All the subfractions were dialyzed against distilled water, then freeze-dried before analysis.

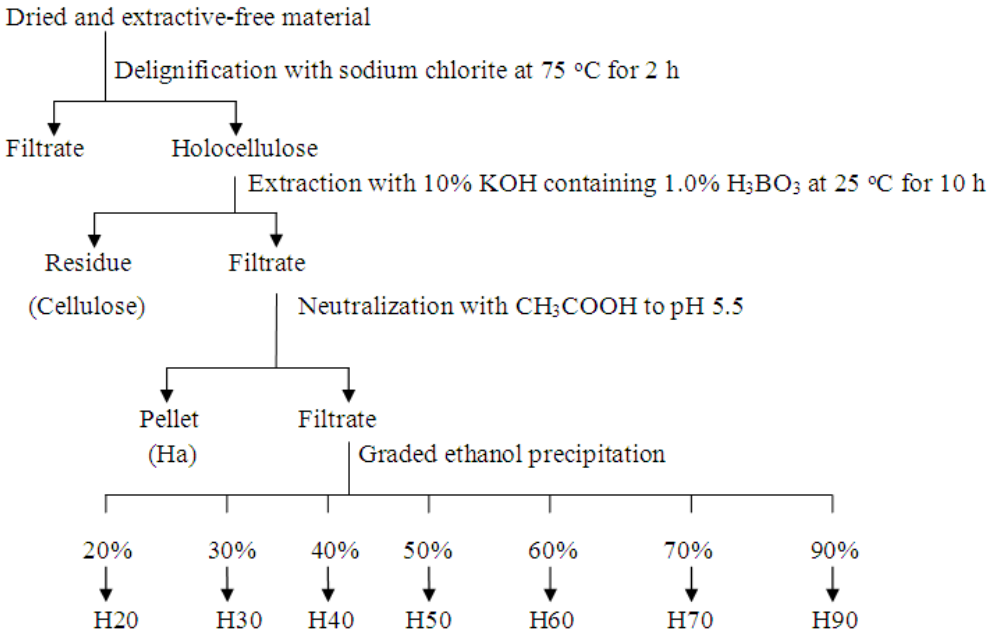

Fig. 1. Scheme for fractionation of hemicelluloses from *Olea europaea* L.

Structural Characterization

The neutral sugars and uronic acids of the eight hemicellulose subfractions were determined by high performance anion exchange chromatography (HPAEC) according to a previous report (Sun *et al*. 2012) after hydrolysis with 10% sulfuric acid. The weight-

average (M_w) and number-average (M_n) molecular weights of the hemicellulose subfractions were determined by gel permeation chromatography (GPC) on a PL aquagel-OH 50 column (300×7.7 mm, Polymer Laboratories Ltd.). Elution was conducted at 30 °C with 0.02 N NaCl in 0.005 M sodium phosphate buffer (pH 7.5) at 0.5 mL min^{-1}. Calibration was performed by using PL pullulans (M_w of 738, 12200, 100000, 1600000, Polymer Laboratories Ltd.). Fourier transform infrared (FT-IR) spectra of the hemicellulose subfractions were taken using a Nicolet iN 10 FT-IR Microscope (Thermo Nicolet Corporation, Madison, WI, USA) equipped with a liquid nitrogen cooled MCT detector. The spectra were recorded in the 4000 to 650 cm^{-1} range with a resolution of 8 cm^{-1}. Solution-state ^1H NMR spectra were obtained on a Bruker AV III NMR spectrometer at 400 MHz using 20 mg hemicellulose sample dissolved in 1.0 mL D_2O. ^{13}C NMR spectra were recorded on the same NMR spectrometer at 100 MHz using an 80 mg sample dissolved in 1.0 mL D_2O. HSQC spectra were acquired by HSQCETGP experiment mode and the parameters for data acquisition were set according to the literature (Bian *et al*. 2012).

RESULTS AND DISCUSSION

Yield and Chemical Composition

In the present study, *Olea europaea* L. was subjected to delignification followed by alkaline extraction to release hemicelluloses. The yield and monosaccharide composition of the hemicellulose subfractions are presented in Table 1. After acidification of the KOH-soluble liquid to pH 5.5, Ha was precipitated from the solution with a yield of 1.7% of the dewaxed material, corresponding to 6.7 % of the original hemicelluloses. Xylose (54.5%) was the major sugar in this fraction followed by glucose (21.0%) and glucuronic acid (18.5%), whereas rhamnose, arabinose, and galactose were observed as minor constituents. This phenomenon implied that Ha may be mainly composed of glucuro-xylan and a small amount of α-glucan since typical violet color was observed after the addition of iodine (Peng *et al*. 2010b). The hemicelluloses in the solution were then precipitated by 20, 30, 40, 50, 60, 70, and 90% ethanol, giving hemicellulose subfractions H20, H30, H40, H50, H60, H70, and H90, which accounted for 2.8, 10.8, 2.1, 2.1, 2.2, 1.1, and 0.7% of the dewaxed material, corresponding to 11.0, 42.5, 8.3, 8.3, 8.7, 4.3, and 2.8% of the original hemicelluloses, respectively. Altogether, the extraction and fractionation resulted in a total yield of 92.5% of the original hemicelluloses. Obviously, the major hemicellulose subfraction was obtained by precipitation at 30% ethanol concentration while the minor subfraction was obtained at ethanol concentration of 90%. H30 was enriched in xylose (57.7%), glucose (20.4%), and glucuronic acid (18.5%), and had a minor amount of rhamnose (1.0%), arabinose (3.0%), and galactose (1.0%). With increasing ethanol concentration from 30% to 90%, the contents of rhamnose, arabinose, and galactose increased, but the contents of xylose and glucose decreased. The contents of arabinose, galactose, xylose, and glucose in hemicellulose subfraction H90 were 30.5, 8.5, 25.2, and 7.5%, respectively. This data suggested that the hemicelluloses precipitated by low ethanol concentrations from *Olea europaea* L. were also composed of glucurono-xylan, whereas those obtained by high ethanol concentrations were composed of arabino-glucuronoxylan. Apart from these groups of hemicelluloses, small amounts of α-glucan were also detected. In addition, the ratio of uronic acid to xylose, and the ratio of arabinose to xylose, being indicative of the degree of linearity or branching of hemicelluloses, increased from 0.26 (H20) to 0.61

(H90), and from 0.04 (H20) to 1.21 (H90), respectively. This indicated that the increase of ethanol concentrations resulted in the precipitation of the more branched hemicelluloses. In light of this, hemicelluloses rich in backbone structure could be precipitated at low ethanol concentrations, while with increasing ethanol concentrations, hemicelluloses with some side chains and more complex structure were obtained. This observation also revealed that the higher the degree of substitution of the backbone, the higher the solubility of hemicelluloses in aqueous solution, which required a much higher ethanol concentration to precipitate them from the solution (Peng *et al*. 2011). In addition, analysis of the residue after the hemicellulose extraction showed that small amounts of xylose (6.5%), mannose (5.5%), arabinose (1.3%), galactose (1.1%), and uronic acids (1.4%) were presented in the solid residue. This suggested that most of the hemicelluloses were solubilized in the 10% KOH under the conditions used but still there was a small part of resistant hemicelluloses strongly bound with cellulose in the solid residue.

Table 1. Yield and Sugar Composition of Hemicellulose Subfractions from *Olea europaea* L.

Fraction [a]	Yield [b] (%)	Rha [c]	Ara [c]	Gal [c]	Glc [c]	Xyl [c]	Man [c]	GlcA [c]	GalA [c]
Ha	1.7	1.0	3.0	1.0	21.0	54.4	5.7	13.9	ND
H20	2.8	1.1	2.4	0.6	17.3	60.2	ND	18.5	ND
H30	10.8	1.0	2.1	0.3	20.4	57.7	ND	18.5	0.1
H40	2.1	1.5	3.7	0.5	26.1	42.2	ND	25.9	0.1
H50	2.1	1.7	6.7	1.7	26.1	37.1	1.8	23.7	0.1
H60	2.2	3.3	11.3	5.9	14.1	32.1	11.2	21.7	0.2
H70	1.1	5.2	17.0	8.5	10.3	29.4	9.5	19.8	0.3
H90	0.7	6.4	30.5	8.5	7.5	25.2	6.5	14.3	1.0
Residue		0.4	0.7	1.1	83.7	6.5	5.5	1.2	0.2

[a] H_a was precipitated from the KOH extraction solution by acidifying to pH 5.5 with 6 M acetic acid; H20, H30, H40, H50, H60, H70, and H90 represent the hemicellulose subfractions obtained by precipitation in 20, 30, 40, 50, 60, 70, and 90% ethanol, respectively; [b] % dry matter of the initial amount of dewaxed material; [c] Abbreviation: Rha, rhamnose; Ara, arabinose; Gal, galactose; Glc, glucose; Xyl, xylose; Man, mannose; GlcA, glucuronic acid; GalA, galacturonic acid; [d] ND, not detected.

Average Molecular Weight

The gel permeation chromatography (GPC) technique was conducted to determine the molecular weight distribution of the subfractions, aiming at evaluating the effectiveness of graded ethanol precipitation for fractionation in relation to their molecular weights. Figure 2 depicts the molecular weight distribution curves of all the hemicellulose subfractions. As can be seen, the hemicelluloses obtained by precipitating in ethanol concentrations of 20% and 30% had similar molecular weight regions. With the ethanol concentration increasing, the main peak of molecular weight distribution curves shifted to a higher molecular weight region first, then to a lower molecular weight region. Table 2 displays the weight-average (M_w) and number-average ($M_{n)}$ molecular weights and the polydispersity (M_w/M_n) of these subfractions. Ha had a moderate M_w of 44530 g mol^{-1}, comparable to those hemicelluloses obtained with lower concentrations of ethanol. An increase in ethanol concentration from 20% to 40% resulted in a growth of M_w from 50830 to 83610 g mol^{-1}, whereas a further increase in ethanol concentration to 90% led to a decrease in M_w to 16640 g mol^{-1}. The lowest molecular weight in these

subfractions was obtained in 90% ethanol concentration. The current results indicate that the ethanol concentrations of 40% and 50% favored the precipitation of the hemicelluloses with larger molecular weight, whereas with the ethanol concentration increasing, the average molecular weights of the precipitated hemicelluloses decreased. The polydispersity of the hemicellulose subfractions ranged from 1.35 to 2.24, which revealed the structural heterogeneity of the subfractions. This is also consistent with the narrow peaks of the curves in Fig. 2. It has been reported that the polysaccharides with polydispersity value below 3 are molecularly uniform polymers with potential commercial utility (Glasser *et al*. 2000). The hemicellulose fractions with narrow molecular weight distribution in the present study would be commercially utilized in many fields, such as conversion to xylose, xylitol, and furfural in an industrial scale, modification through esterification, etherification, and graft polymerization for adhesives, thickeners, stabilizers, film formers, *etc*. (Doner and Hicks 1997; Ebringerová and Heinze 2000).

Table 2. Weight-average (M_w) and Number-average (M_n) Molecular Weights (g mol^{-1}) and Polydispersity of Hemicellulose Subfractions from *Olea europaea* L.

	Hemicellulose Subfraction [a]							
	Ha	H20	H30	H40	H50	H60	H70	H90
M_w	44530	50830	52590	83610	54980	35850	26500	16640
M_n	19880	23220	24910	45260	31420	21340	16900	12370
M_w/M_n	2.24	2.19	2.11	1.85	1.75	1.68	1.57	1.35

[a] Corresponding to the hemicellulose subfractions in Table 1.

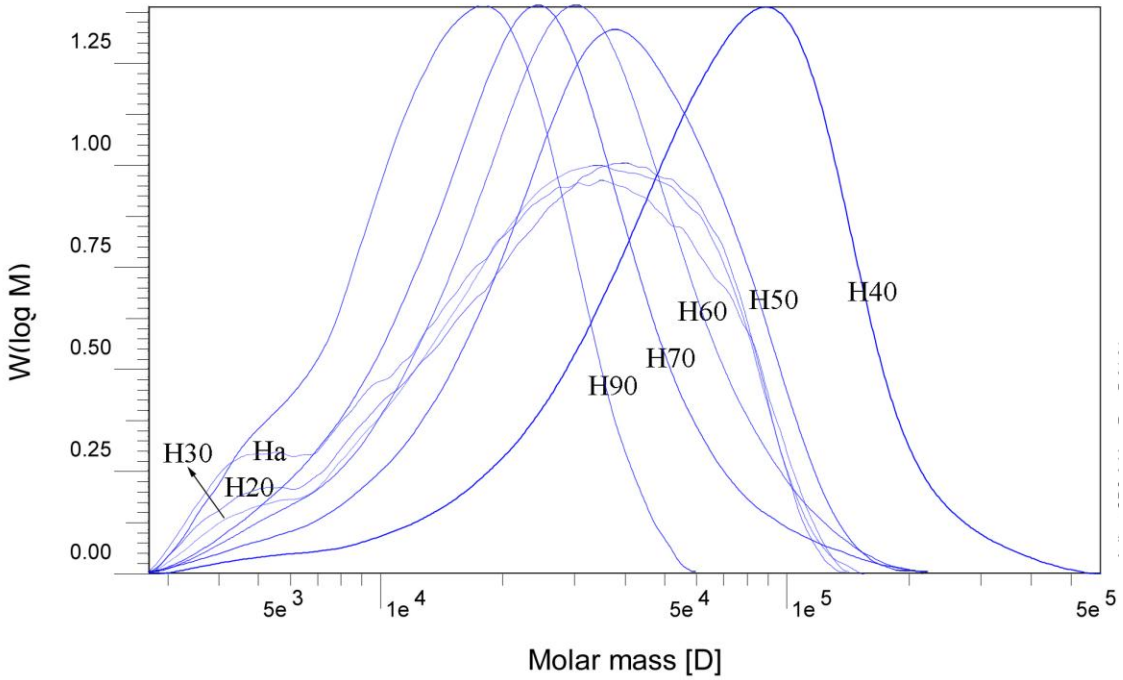

Fig. 2. Molecular weight distribution of the hemicellulose subfractions from *Olea europaea* L.

FT-IR Spectra

The FT-IR spectra of the hemicellulose subfractions Ha, H20, H30, and H40 are illustrated in Fig. 3. No significant difference in the main absorption was observed among these hemicellulose subfractions, indicating a similar chemical structure of these hemicel-

lulose fractions. The prominent absorption at 1045 cm^{-1} is originated from C–O, C–C stretching, and C–OH bending typical of xylans. This band showed variation of spectral shape depending on the branching at O-2 and O-3 positions (Kačuráková *et al*. 1994). The presence of arabinosyl side chains is indicated by the signal at 1157 cm^{-1}. The low-intensity shoulder at 986 cm^{-1} also indicates the presence of arabinose attached at the xylopyransyl constituents (Ebringerova *et al*. 1992). The bands at 1601 and 1416 cm^{-1} are indicative of the asymmetric and symmetric (C=O) stretching vibrations of the carboxylate group (Buslov *et al*. 2009). This indicated that arabinoglucuronoxylans are the dominant polysaccharides in these hemicellulose subfractions. The peaks at 1458, 1385, and 1246 cm^{-1} represent the CH_2, OH, and C–O bending. In the anomeric region (950 to 700 cm^{-1}), a small sharp band at 897 cm^{-1} is due to the C–1 group frequency or ring frequency, which is characteristic of *β*-glycosidic linkages between the sugar units. The prominent band around 3443 cm^{-1} is due to the OH stretching vibrations and hydrogen bonding. The intensive band for the C-H stretching vibrations was observed at 2926 cm^{-1}. No signal was visible at 1730 cm^{-1}, indicating that the ester groups were saponified when the polysaccharides were isolated by alkaline solution.

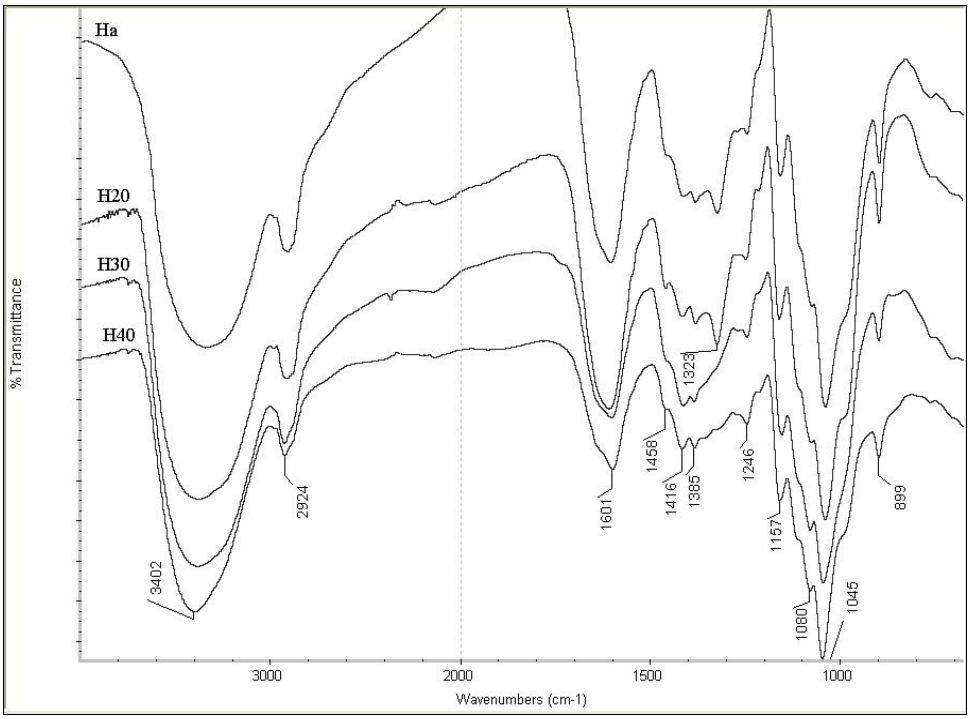

Fig. 3. FTIR spectra of the hemicellulose subfractions Ha, H20, H30, and H40

The FT-IR spectra of the hemicellulose subfractions H50, H60, H70, and H90 are illustrated in Fig. 4. As can be seen from the figure, the spectra of the hemicelluloses H50, H60, and H70 appeared to be rather similar. The absorbances at 1601, 1446, 1415, 1384, 1250, 1149, 1045, and 899 cm^{-1} were associated with hemicellulose moiety. However, the spectrum of H90 was clearly distinguished from the other spectra of hemicellulose subfractions.

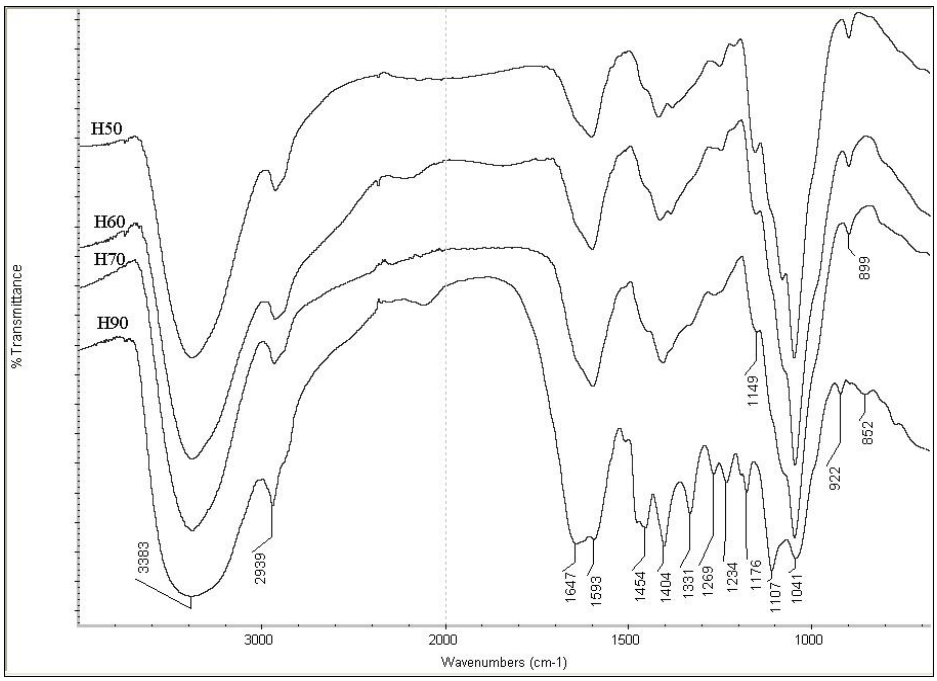

Fig. 4. FTIR spectra of the hemicellulose subfractions Ha, H20, H30, and H40

The largest differences in the spectra were the signals at 1647, 1454, 1331, 1269, 1176, 1107, and 1041 cm^{-1}. The appearance of signals at 1647 cm^{-1} is principally associated with absorbed water. The index of xylose and arabinose were recognized by the presence of the bands at 1045 and 1176 cm^{-1}, respectively. A sharply decreasing intensity at 1045 cm^{-1} and an increasing intensity at 1176 cm^{-1} were in agreement with the results of the sugar analysis. The intensity of the absorptions at 1454, 1331, 1269, and 1107 cm^{-1}, which were indicative of CH_2, –OH, CH_2, and CO bending, respectively, were also weakened. These phenomena were paralleled to the composition of the hemicellulose subfraction H90 that large amounts of multiple monosaccharides were presented in this fraction as compared to other subfractions.

NMR Spectra

To further characterize the structural features of the hemicelluloses, the three subfractions Ha, H30, and H70 were comparatively investigated by ^{1}H NMR (Fig. 5), ^{13}C NMR (Fig. 6), and 2 D-HSQC NMR (Fig. 7) spectroscopy. As can be seen from Figs. 5 and 6, the hemicellulose subfractions Ha and H30 showed analogous ^{1}H and ^{13}C NMR spectra while H70 appeared more complicated. The hemicellulose subfraction H30 exhibited anomeric protons at 4.31, 4.50, and 5.16 ppm, which are assigned to non-substituted backbone of D-xyl*p* units, 4-*O*-methyl-D-Glc*p*A attached via α-(1→2) linkage to xylose, and 4-*O*-methyl-D-Glc*p*A residues, respectively (Lisboa *et al*. 2005).

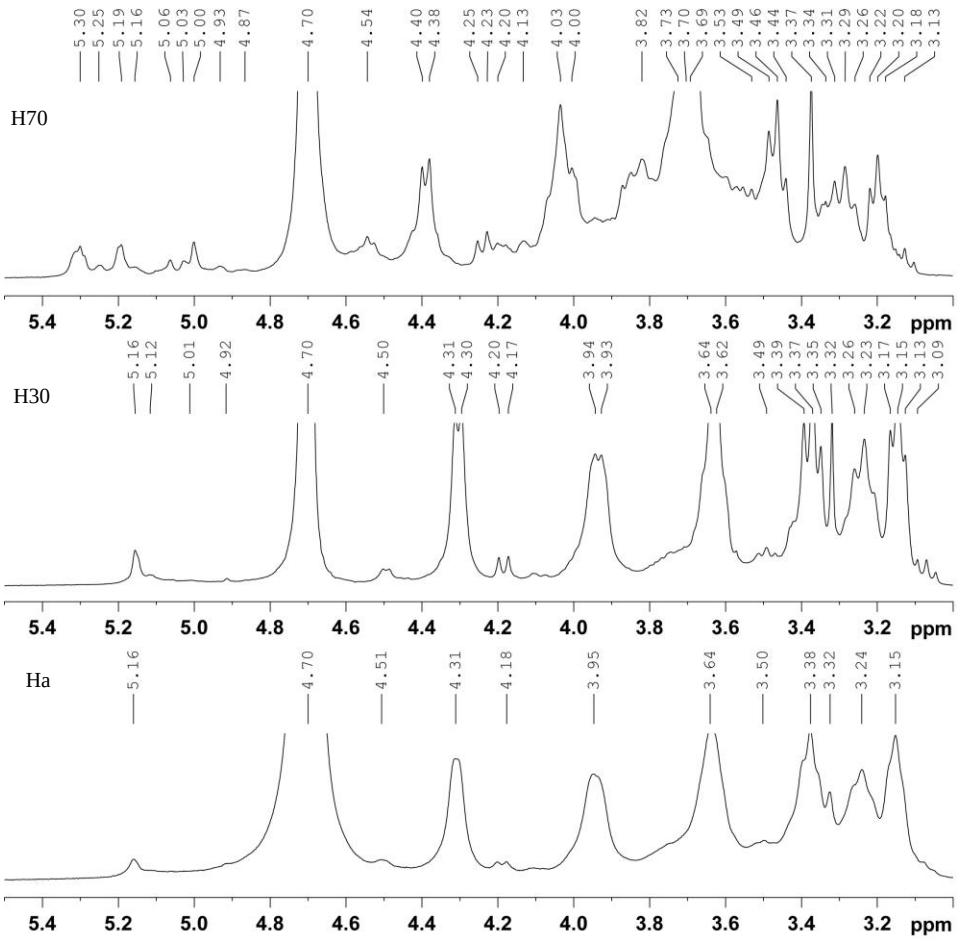

Fig. 5. ^{1}H NMR spectra of the hemicellulose subfractions Ha, H30, and H70

The relevant signals at 4.90 to 5.60 ppm are assigned to α-anomeric protons, and 4.30 to 4.90 ppm to *β*-anomers (Chiarini *et al*. 2004). This confirmed that the xylose unit is linked *β*-glycosidically, which was in agreement with the presence of a small sharp peak at 899 cm^{-1} in the FT-IR spectra. A strong signal at 4.7 ppm originated from the residual solvent (HDO). The spectra of Ha presented the same general features as was already observed in the case of H30, which indicated that the hemicellulose subfraction Ha had a similar backbone to that of H30. Apart from these units, two signals at 5.30 and 5.25 ppm in H70 belong to arabinose residues doubly bound to xylose (Debyser *et al*. 1997). There are extra groups of protons exhibited in the hemicellulose subfraction H70, which can be better interpreted on the basis of HSQC results afterwards.

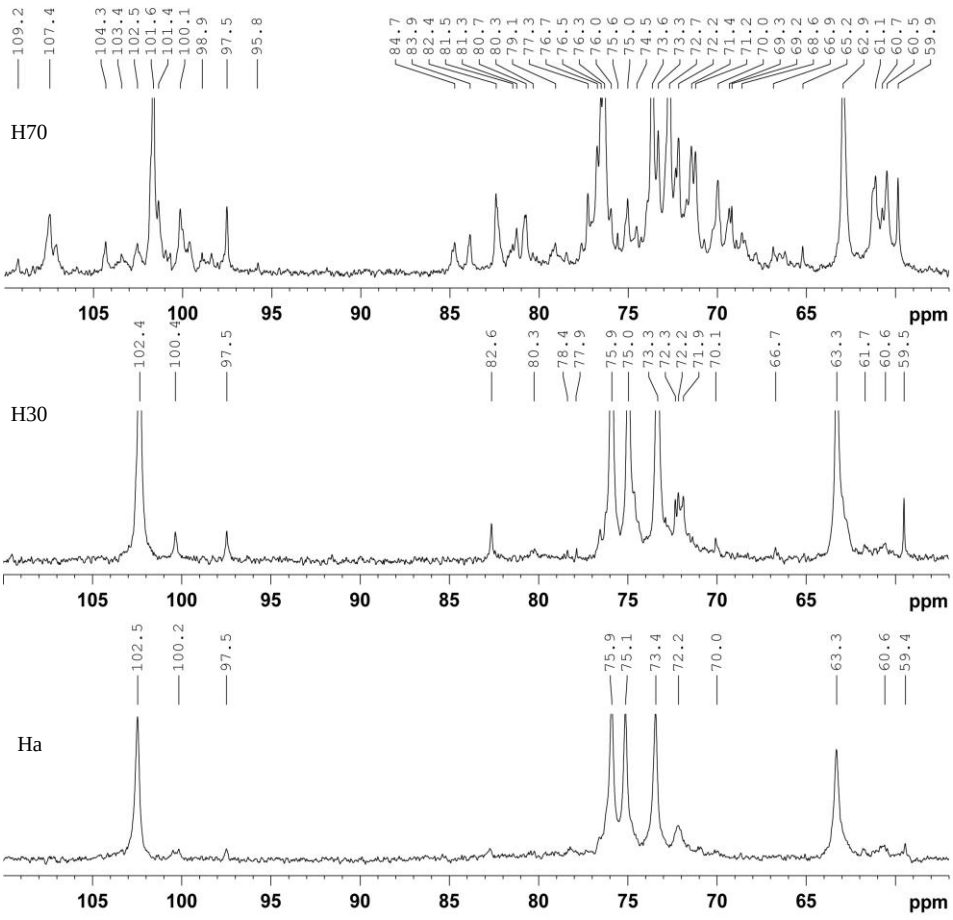

Fig. 6. ^{1}H NMR spectra of the hemicellulose subfractions Ha, H30, and H70

The ^{13}C NMR spectrum of Ha showed five dominating signals corresponding to (1→4)-linked-*β*-D-xylan. The signal at 102.5 ppm is ascribed to the anomeric region in *β*-configuration, as confirmed by the ^{1}H NMR spectra, whereas the signals at 75.9, 75.1, 73.4, and 63.3 ppm correspond to C-4, C-3, C-2, and C-5 of (1→4)-linked-D-xylan, respectively (Teleman *et al*. 2000). The small signals at 177.0 (data not shown), 97.5, 82.6, 72.2, and 59.4 ppm, are characteristic of COOH, C-1, C-4, C-5, and OCH_3 of 4-*O*-methyl-α-D-GlcA residues, respectively (Bendahou *et al*. 2007). Obviously, the small signals presented in Ha were different in intensity from those in H30 and H70. This was in agreement with the results of the sugar analysis, in which the relative content of glucuronic acid increased from Ha (13.9%) to H30 (18.5%), and to H70 (19.8%).

More specific information about the structural variation of the hemicellulose subfractions was investigated by HSQC spectra. The marked ^{1}H/^{13}C cross-peaks in all the spectra further confirmed the structural element of (1→4)-*β*-D-xylan, 4-*O*-methyl-α-D-GlcA, and (1→4)-*β*-D-Xyl*p*-2-*O*-(4-*O*-methy-α-D-GlcA) units. The spectra of the hemicellulose subfraction H70 showed similarities to those of Ha and H30, but much more complicated by the presence of extra signals of glucose, galacose, mannose, and arabinose. The signals at 107.4, 84.7, 80.7, and 61.6 ppm are indicative of C-1, C-4, C-2, and C5 of arabinose, correlated to the proton signals at 5.00, 4.23, 4.13, and 3.82 ppm, respectively (Kim and Ralph 2010). Other multiple signals probably originated from the

noticeable amounts of overlapped glucose, mannose, and galactose as seen in Fig. 7 (Kim and Ralph 2010).

From the results of NMR and sugar analysis, the hemicellulose subfractions were assumed to be mainly composed of (1→4)-linked *β*-D-xylan attached with various monosaccharides and a small amount of α-D-glucan. Linear hemicelluloses were precipitated in low concentration ethanol, whereas more branched hemicelluloses were precipitated in a high concentration of ethanol.

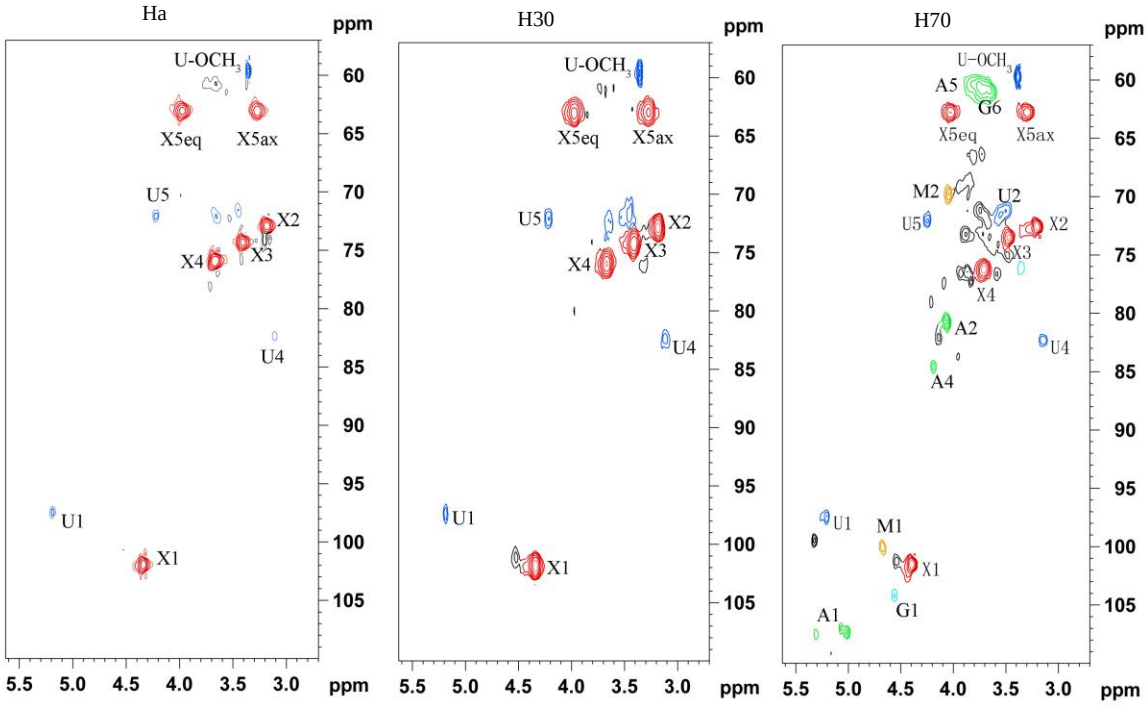

Fig. 7. HSQC spectra of the hemicellulose subfractions Ha, H30, and H70. Designations are as follows: X, Xyl*p*; U, 4-*O*-Me-GlcpA; A, Ara*f*; M, mannose; G, glucose

CONCLUSIONS

1. Hemicelluloses of *Olea europaea* L. were extracted with KOH containing H_3BO_3 and further precipitation by acidification and fractionation with graded ethanol, obtaining eight subfractions with a total yield of 23.5% based on the dewaxed material, which corresponded to 92.5 % of the original hemicelluloses.
2. Linear hemicelluloses were preferentially precipitated in lower ethanol concentrations, while more branched hemicelluloses were achieved in higher ethanol concentrations. The ethanol concentrations of 40% and 50% favored the precipitation of the hemicelluloses with large molecular weight.
3. The subfractions obtained by graded ethanol precipitation showed a similar structure, which was assumed to be mainly composed of (1→4)-linked *β*-D-xylan attached with minor amounts of uronic acids, arabinose, and galactose, as well as small amounts of α-D-glucan.

ACKNOWLEDGMENTS

The authors are grateful for grants from the Natural Science Foundation of China (No. 30930073), the Ministry of Science and Technology (973, 2010CB732204), and State Forestry Administration (201204803).

REFERENCES CITED

Agbor, V. B., Cicek, N., Sparling, R., Berlin, A., and Levin, D. B. (2011). "Biomass pretreatment: fundamentals toward application," *Biotechnol. Adv.* 29(6), 675-685.

Bendahou, A., Dufresne, A., Kaddami, H., and Habibi, Y. (2007). "Isolation and structural characterization of hemicelluloses from palm of *Phoenix dactylifera* L.," *Carbohydr. Polym.* 68(3), 601-608.

Bian, J., Peng, F., Peng, X. P., Xu, F., Sun, R. C., and Kennedy, J. F. (2012). "Isolation of hemicelluloses from sugarcane bagasse at different temperatures: Structure and properties," *Carbohydr. Polym.* 88(2), 638-645.

Buslov, D. K., Kaputski, F. N., Sushko, N. I., Torgashev, V. I., Solov'eva, L. V., Tsarenkov, V. M., Zubets, O. V., and Larchenko, L. V. (2009). "Infrared spectroscopic analysis of the structure of xylans," *J. Appl. Spectrosc.* 76(6), 801-805.

Cara, C., Romero, I., Oliva, J. M., Saez, F., and Castro, E. (2007). "Liquid hot water pretreatment of olive tree pruning residues," *Appl. Biochem. Biotech.* 137-140(1-12), 379-394.

Chiarini, L., Cescutti, P., Drigo, L., Impallomeni, G., Herasimenka, Y., Bevivino, A., Dalmastri, C., Tabacchioni, S., Manno, G., Zanetti, F., and Rizzo, R. (2004). "Exopolysaccharides produced by *Burkholderia cenocepacia* recA lineages IIIA and IIIB," *J. Cyst. Fibros.* 3(3), 165-172.

Cohen, W. (2009). "A study of "chlorite" and "chlorine" holocelluloses prepared from *Eucalyptus regnans* F. v. M," *Holzforschung* 7(4), 102-110.

Debyser, W., Schooneveld-Bergmans, M. E. F., Derdelinckx, G., Grobet, P. J., and Delcour, J. A. (1997). " Nuclear magnetic resonance and methylation analysis-derived structural features of water-extractable arabinoxylans from barley *(Hordeum vulgare* L.) malts," *J. Agric. Food Chem.* 45(8), 2914-2918.

Deutschmann, R., and Dekker, R. F. (2012). "From plant biomass to bio-based chemicals: latest developments in xylan research," *Biotechnol. Adv.*, http://dx.doi.org/10.1016/j.biotechadv.2012.1007.1001.

Doherty, W. O. S., Mousavioun, P., and Fellows, C. M. (2011). "Value-adding to cellulosic ethanol: Lignin polymers," *Ind. Crop. Prod.* 33(2), 259-276.

Doner, L. W., and Hicks, K. B. (1997). "Isolation of hemicellulose from corn fiber by alkaline hydrogen peroxide extraction," *Cereal Chem.* 74(2), 176–181.

Ebringerová, A., and Heinze, T. (2000). "Xylan and xylan derivatives - biopolymers with valuable properties, 1 - Naturally occurring xylans structures, procedures and properties," *Macromol. Rapid. Comm.* 21(9), 542–556.

Ebringerova, A. (2012). "The potential of xylans as biomaterial resources," *Polysaccharide Building Blocks*, John Wiley & Sons, Inc., 331-365.

Ebringerova, A., and Hromadkova, Z. (1999). "Xylans of industrial and biomedical importance," *Biotechnology and Genetic Engineering Reviews*, S. E. Harding, ed., 325-346.

Ebringerova, A., Hromadkova, Z., Alfoldi, J., and Berth, G. (1992). "Structural and solution properties of corn cob heteroxylans," *Carbohydr. Polym.* 19(2), 99-105.

Ebringerova, A., Hromadkova, Z., and Heinze, T. (2005). "Hemicellulose," *Polysaccharides 1: Structure, Characterization and Use* 186, 1-67.

Egues, I., Sanchez, C., Mondragon, I., and Labidi, J. (2012). "Separation and purification of hemicellulose by ultrafiltration," *Ind. Eng. Chem. Res.* 51(1), 523-530.

FAO (2011). "FAOSTAT online database, available at link http://faostat.fao.org/. Accessed on December 2011."

Glasser, W. G., Kaar, W. E., Jain, R. K., and Sealey, J. E. (2000). "Isolation options for non-cellulosic heteropolysaccharides (HetPS)," *Cellulose* 7(3), 299-317.

Hansen, N. M. L., and Plackett, D. (2008). "Sustainable films and coatings from hemicelluloses: A review," *Biomacromolecules* 9(6), 1493-1505.

Kačuráková, M., Ebringerová, A., Hirsch, J., and Hromádková, Z. (1994). "Infrared study of arabinoxylans," *J. Sci. Food Agric.* 66(3), 423-427.

Kim, H., and Ralph, J. (2010). "Solution-state 2D NMR of ball-milled plant cell wall gels in DMSO-d_6/pyridine-d_5," *Org. Biomol. Chem.* 8(3), 576–591.

Lawther, J. M., Sun, R., and Banks, W. B. (1995). "Extraction, fractionation, and characterization of structural polysaccharides from wheat straw," *J. Agric. Food Chem.* 43(3), 667-675.

Li, X. M., and Pan, X. J. (2010). " Hydrogels based on hemicellulose and lignin from lignocellulose biorefinery: A mini-review," *J. Biobased Mater. Bioenergy* 4(4), 289-297.

Lisboa, S. A., Evtuguin, D. V., Neto, C. P., and Goodfellow, B. J. (2005). "Isolation and structural characterization of polysaccharides dissolved in *Eucalyptus globulus* kraft black liquors," *Carbohydr. Polym.* 60(1), 77-85.

Naran, R., Black, S., Decker, S. R., and Azadi, P. (2009). "Extraction and characterization of native heteroxylans from delignified corn stover and aspen," *Cellulose* 16(4), 661-675.

Peng, F., Peng, P., Xu, F., and Sun, R. C. (2012). "Fractional purification and bioconversion of hemicelluloses," *Biotechnol. Adv.* 30(4), 879-903.

Peng, F., Ren, J. L., Xu, F., Bian, J., Peng, P., and Sun, R. C. (2010). "Comparative studies on the physico-chemical properties of hemicelluloses obtained by DEAE-cellulose-52 chromatography from sugarcane bagasse," *Food Res. Int.* 43(3), 683-693.

Peng, F., Ren, J. L., Xu, F., Bian, J., Peng, P., and Sun, R. C. (2010). "Fractionation of alkali-solubilized hemicelluloses from delignified *Populus gansuensis*: Structure and properties," *J. Agric. Food Chem.* 58(9), 5743-5750.

Peng, P., Peng, F., Bian, J., Xu, F., and Sun, R. (2011). "Studies on the starch and hemicelluloses fractionated by graded ethanol precipitation from bamboo *Phyllostachys bambusoides f. shouzhu Yi*," *J. Agric. Food Chem.* 59(6), 2680-2688.

Schlesinger, R., Gotzinger, G., Sixta, H., Friedl, A., and Harasek, M. (2006). "Evaluation of alkali resistant nanofiltration membranes for the separation of hemicellulose from concentrated alkaline process liquors," *Desalination.* 192(1-3), 303-314.

Sun, J. X., Sun, X. F., Sun, R. C., and Su, Y. Q. (2004). "Fractional extraction and structural characterization of sugarcane bagasse hemicelluloses," *Carbohydr. Polym.* 56(2), 195-204.

Sun, R., Lawther, J. M., and Banks, W. (1996). "Fractional and structural characterization of wheat straw hemicelluloses," *Carbohydr. Polym.* 29(4), 325-331.

Sun, S., Yuan, T., Li, M., Cao, X., Xu, F., and Liu, Q. (2012). "Structural characterization of hemicelluloses from bamboo culms (*Neosinocalamus affinis*)," *Cell Chem. Technol.* 46(3), 165.

Teleman, A., Lundqvist, J., Tjerneld, F., Stålbrand, H., and Dahlman, O. (2000). "Characterization of acetylated 4-*O*-methylglucuronoxylan isolated from aspen employing ^{1}H and ^{13}C NMR spectroscopy," *Carbohydr. Res.* 329(4), 807-815.

Article submitted: October 15, 2012; Peer review completed: January 10, 2013; Revised version received and accepted: January 14, 2013; Published: January 16, 2013.