Fractional Isolation and Physico-Chemical Characterization of Hemicelluloses by a Two-Stage Treatment from *Haloxylon ammodendron* and *Elaeagnus angustifolia*

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The cell wall material of Chinese shrubs *Haloxylon ammodendron* and *Elaeagnus angustifolia* was fractionated by successive extractions with ethanol/H_2O (60:40, v/v) under acidic conditions (0.2 N HCl) at 70 °C for 4 h, and 2% H_2O_2 at pH 11.5 for 16 h, respectively. The sequential two-step treatment resulted in the dissolution of 83.9% and 87.6% of the original hemicelluloses from dewaxed *H. ammodendron* and *E. angustifolia*, respectively. Xylose, glucose, and galactose were the major sugar constituents in the two acidic organosolv-soluble hemicellulosic preparations. The two alkaline peroxide-soluble hemicellulosic fractions were shown to be composed primarily of xylose, comprising over 80% of the total sugars. The results also showed that the two alkaline peroxide-soluble hemicellulosic fractions were more linear and acidic, and had higher molecular mass and thermal stability than the two acidic organosolv-soluble hemicellulosic preparations. The 2% H_2O_2 posttreatment did not result in any significant changes in the macromolecular structure of the isolated hemicelluloses. It is probable that lignin protects hemicelluloses and cellulose from being attacked by peroxide.

INTRODUCTION

About 10 000 ha of forests of *Haloxylon ammodendron* and *Elaeagnus angustifolia* have been planted in the desert region of Gansu Province, China, since 1960 to prevent wind erosion and control desertification (*1*). These shrubs not only have great importance in the reforestation of deserts and dry steppes, but they also provide wood, fuel, and fodder, etc (*2*). Studies on the utilization of these shrubs have shown the potential of this lignocellulosic raw material for a variety of applications. Particularly, hemicelluloses comprise about 40% of the cell walls of *H. ammodendron* and *E. angustifolia* (*3*). The preparation and properties of new polymers from hemicelluloses should be an important part of any research program aimed at utilizing renewable polymers as extenders and replacements for polymers prepared from petrochemicals.

The plant cell wall is a dynamic and highly ordered complex of biopolymers containing homoglycans and structurally related heteroglycans in various contents, which are dependent on the development, age, and type of the cell wall (*4*). Cellulose, hemicelluloses, pectins, and structural cell-wall proteins are the four principally independent, but interacting, networks that form local microdomains (*5, 6*). Hemicelluloses are located primarily in the secondary cell walls, and together with cellulose and lignin, build up the plants in a fashion that gives the best combination of mechanical support and transport properties (*7*). Cellulose, the main cell wall constituent, is a highly uniform 1→4-β-linked polyglucan, whereas the hemicelluloses represent polysaccharides of a different structure containing glucose, xylose, mannose, galactose, arabinose, fucose, glucuronic acid, and galacturonic acid in various amounts. These hemicellulosic polymers include xylans, arabinoxylans, glucuronoxylans, glucuronoarabinoxylans, xyloglucans, glucomannans, and galactoglucomannans, and can be isolated by extraction either with water, or, more frequently, aqueous alkali (*8*). Among these, xylans are the most common hemicelluloses. They are considered to be the second-most abundant biopolymer in the plant kingdom, and are estimated to account for one-third of all renewable organic carbon available on earth (*9*). Therefore, there is an increasing interest in the application of potential xylan polymers in the fields of papermaking, baking, and food additives. Moreover, the variability in sugar constituents, glycosidic linkages, and structure of glycosyl side chains, as well as two reactive hydroxyl groups at the xylose repeating

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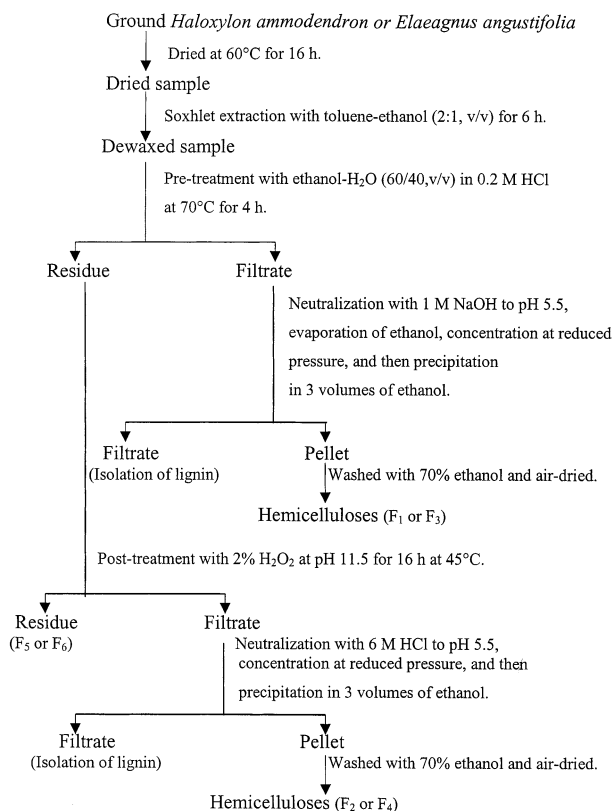 [§] Academy of Forestry.

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Ground *Haloxylon ammodendron* or *Elaeagnus angustifolia*

Dried at 60°C for 16 h.

Dried sample

Soxhlet extraction with toluene-ethanol (2:1, v/v) for 6 h.

Dewaxed sample

Pre-treatment with ethanol-H$_2$O (60/40,v/v) in 0.2 M HCl
at 70°C for 4 h.

Residue   Filtrate

Neutralization with 1 M NaOH to pH 5.5,
evaporation of ethanol, concentration at reduced
pressure, and then precipitation
in 3 volumes of ethanol.

Filtrate   Pellet
(Isolation of lignin)

Washed with 70% ethanol and air-dried.

Hemicelluloses (F$_1$ or F$_3$)

Post-treatment with 2% H$_2$O$_2$ at pH 11.5 for 16 h at 45°C.

Residue   Filtrate
(F$_5$ or F$_6$)

Neutralization with 6 M HCl to pH 5.5,
concentration at reduced pressure, and then
precipitation in 3 volumes of ethanol.

Filtrate   Pellet
(Isolation of lignin)

Washed with 70% ethanol and air-dried.

Hemicelluloses (F$_2$ or F$_4$)

Figure 1. Scheme for fractional isolation of hemicellulosic fractions from dewaxed *Haloxylon ammodendron* or *Elaeagnus angustifolia*.

unit of the main chain, offer various possibilities for chemical modifications. This functionalization creates novel opportunities to exploit the various valuable properties of xylans for industrial end uses (*10*).

Until now, no survey has been published in which carbohydrate-derived fractions from *Haloxylon ammodendron* and *Elaeagnus angustifolia* are characterized with respect to their hemicellulosic composition. The purpose of the present investigation was to isolate the cell wall hemicelluloses and cellulose from *H. ammodendron* and *E. angustifolia* using an environment-friendly method (a two-stage acidic organosolv pretreatment followed by alkaline peroxide posttreatment) and to characterize them in order to perform further structural investigations of the important polymers.

MATERIALS AND METHODS

Materials. Eight-year-old *Haloxylon ammodendron* and *Elaeagnus angustifolia* shrubs were harvested in July 1999, from the desert region of Gansu Province, China. The material was air-dried and then chipped into small pieces. The chips were then ground to pass a 1.0-mm screen. The contents of cellulose, lignin, and hemicelluloses in *Haloxylon ammodendron* and *Elaeagnus angustifolia* (%, w/w) are 44.6% and 41.2%, 12.1% and 11.7%, and 47.9% and 50.8%, on a dry weight basis, respectively. Waxes were removed by extraction with toluene/ethanol (2:1, v/v) in a Soxhlet apparatus for 6 h. The extractive-free sample was dried in a cabinet oven with air circulation at 60 °C for 16 h and then kept at 5 °C before treatment. All weights and calculations were made on an oven-dried (60 °C, 16 h) basis.

Two-Stage Acidic Organosolv Pretreatment and Alkaline Peroxide Posttreatments. The extractive-free dewaxed *H. ammodendron* and *E. angustifolia* were sequentially extracted with ethanol/H$_2$O under acidic condition and hydrogen peroxide under alkaline condition (**Figure 1**) using the method described previously (*3*). In short, 9.75 g of extractive-free powder was treated with ethanol/H$_2$O (195 mL, 60:40, v/v) under acid conditions (0.2 N HCl) at 70 °C for 4 h. After the

solution was filtered, the residue was subsequently washed with ethanol and distilled water, and then oven-dried at 60 °C for 16 h. Ethanol in the pooled supernatant was removed with a rotary vacuum evaporator at 40 °C. The supernatant was then neutralized to pH 5.5 with 1 M NaOH, concentrated on a rotary evaporator under reduced pressure to approximately 100 mL, and then mixed with 3 volumes of 95% ethanol (12 h, 25 °C) for isolation of hemicelluloses. The products were further purified by thoroughly washing with 70% aqueous ethanol acidified with 1% HCl (v/v) and then dried in air. To obtain alkaline peroxide-soluble hemicellulosic preparations, the above residues were successively extracted with 2% H$_2$O$_2$ (ratio of liquor to residue, 25, w/w) at pH 11.5 for 16 h at 45 °C (posttreatment). No further adjustments in pH were made during the course of the posttreatment. Under this condition, the reaction pH remained nearly constant for 2 h before slowly rising to a final value of approximately 12.9. Note that the fractions 1 (F$_1$) and 2 (F$_2$) represent the hemicellulosic fractions extracted successively with acidic organosolv and alkaline peroxide from *Haloxylon ammodendron*, and the fractions 3 (F$_3$) and 4 (F$_4$) represent the hemicellulosic preparations extracted successively with acidic organosolv and alkaline peroxide from *Elaeagnus angustifolia*. The two residues of *H. ammodendron* and *E. angustifolia* were considered to be crude cellulose fractions 5 (F$_5$) and 6 (F$_6$), respectively.

Physico-Chemical and Thermal Analysis. The neutral sugar composition of the isolated hemicelluloses and residues was determined by gas chromatography (GC) according to Blakeney and co-workers' method (*11*). The total uronic acids was determined colorometrically by the method of Blumenkrantz and Asboe-Hanson (*12*). The lignin content in hemicellulosic and residues was determined as Klason lignin (*13*). Methods for alkaline nitrobenzene oxidation of associated lignin in hemicellulosic fractions and residues, hemicellulosic molecular mass determination, recording FT-IR and ^{13}C NMR spectra, and thermal analysis have been described in a previous paper (*14*).

RESULTS AND DISCUSSION

Fractional Yield and Chemical Composition. During organosolv pulping a mixture of organic solvent and water is used as the cooking liquor. The primary solvent acts on the promotion of vegetal tissue impregnation, and the solubilization of the lignin and hemicellulosic fragments so-produced (*15*). For example, some processes that use alcohols for pulping are Kleinert (ethanol or methanol) (*16*), Alcell (ethanol−water) (*16, 17*), MD Organocell (ethanol−soda) (*18*), Organocell (methanol−soda−anthraquinone) (*19*), ASAM (alkali−sulfite−anthraquinone−methanol) (*20*), and ASAE (alkali−sulfite−anthraquinone−ethanol) (*21*). During organosolv acid delignification, lignin is dissolved essentially by acid-catalyzed cleavage of α-aryl ether and arylglycerol-β-aryl ether bonds in the lignin macromolecule (*22*). Meanwhile, the degradation of hemicelluloses is also promoted in these organosolv pulping processes. These processes can fractionate the lignocellulosic raw materials into hemicellulose-degradation products, lignin-degradation products, and cellulose, all of them potentially being utilizable for different end-product applications (*23*).

Table 1 shows yields of lignin and hemicelluloses solubilized during the pretreatment of dewaxed *H. ammodendron* and *E. angustifolia* with ethanol/H$_2$O (60:40, v/v) under acidic conditions. The results demonstrated that the major part of the material recovered is the organosolv-soluble hemicelluloses, and the acid organosolv pretreatment under these conditions given resulted in the dissolution of 34.4% of the original lignin and 15.5% hemicelluloses from dewaxed *H. ammodendron,* and 34.0% of the original lignin and 17.1% hemicelluloses from dewaxed *E. angustifolia*.

Recently, environmental concerns have heightened interest in chlorine-free bleaching sequences. Alkaline peroxide, oxygen−alkali, ozone, and peroxyacetic acid systems are of particular interest because their byproducts are environmentally benign

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Table 1. Yield of Hemicelluloses (% Dry Matter) Solubilized in Pretreatment of Dewaxed *Haloxylon Ammodendron* and *Elaeagnus Angustifolia* with Ethanol/H$_2$O (60:40, v/v) under Acidic Conditions (0.2 M HCl) at 70 °C for 4 h and Posttreatment with 2% H$_2$O$_2$ at pH 11.5 for 16 h

	yield (%)	
hemicelluloses/lignin/residue	*Haloxylon ammodendron*	*Elaeagnus angustifolia*
total solubilized hemicelluloses in pre- and posttreatment	40.2	44.5
solubilized hemicelluloses in pretreatment[a]	15.5	17.1
solubilized lignin in pretreatment[a]	4.1	3.9
solubilized hemicelluloses in posttreatment[b]	24.7	27.4
solubilized lignin in posttreatment[b]	6.1	5.7
residue (crude cellulose)	45.0	41.7

[a] Represents the hemicelluloses and lignin fractions obtained by pretreatment of dewaxed *Haloxylon ammodendron* and *Elaeagnus angustifolia* with ethanol/H$_2$O (60:40, v/v) under acidic conditions (0.2 N HCl) at 70 °C for 4 h. [b] Represents the hemicelluloses and lignin fractions obtained during the posttreatment with 2% H$_2$O$_2$ at pH 11.5 for 16 h.

(*24*). In general, chemical pulp-bleaching involves mainly electrophilic reactions of hydrogen peroxide or peroxyacids as delignifying agents, and mechanical pulp-bleaching involves nucleophilic reactions and lignin-retaining bleaching (*25*). The role of hydrogen peroxide in delignifying and bleaching to purify hemicelluloses from barley husks and yellow poplar wood chips before precipitation in methanol has been extensively investigated by Glasser and co-workers (*26*). The release of hemicelluloses was suggested to be due to direct attack of α-aryl ether bonds between lignin and hemicelluloses, although hydroxyl radicals attack lignin structures more rapidly than carbohydrate structures (*27*). As shown in **Table 1**, the posttreatment with 2% H$_2$O$_2$ at pH 11.5 for 16 h solubilized 24.7% and 27.4% of hemicelluloses (% dry starting material) from the acidic organosolv-treated *H. ammodendron* and *E. angustifolia*, except for the release of 50.0% and 48.1% of the original lignins, respectively. Remarkably, the two-stage treatments together solubilized 84.4% and 82.1% of the original lignin and 83.9% and 87.6% of the original hemicelluloses from dewaxed *H. ammodendron* and *E. angustifolia*, respectively. In addition, the yield of crude cellulose (% dried weight of the sample) obtained after sequential two-step treatments, was found to be 45.0% from *H. ammodendron* and 41.7% from *E. angustifolia*. The reason for this higher solubility of hemicelluloses is probably due to the fact that hemicelluloses are present mainly on the outer surface, from where they dissolve easily in the liquor during the two-stage treatments. On the other hand, the long cellulose chains are located in the inner parts of the fibers and therefore are not easily dissolved. Furthermore, cellulose is a semicrystalline biopolymer with ordered crystalline and disordered amorphous regions. This partly crystal structure reduces its solubility (*28*).

The neutral sugar composition and content of uronic acids of the four hemicellulosic preparations and two residues were determined (**Table 2**). The two acidic organosolv-soluble hemicelluloses consisted mainly of xylose (42.8−56.1%), glucose (18.5−23.4%), and galactose (14.6−17.1%). In contrast, uronic acids (5.3−6.2%), rhamnose (4.4−7.3%), and mannose (4.4−5.9%) were found to be present in noticeable amounts, and arabinose (1.9−3.4%) appeared in a minor quantity. On the other hand, the most abundant sugar of the two alkaline peroxide-soluble hemicellulosic preparations was xylose, which accounted for 86.0 and 81.6% of the total neutral sugars from *Haloxylon ammodendron* and *Elaeagnus angustifolia*, respec-

Table 2. Contents of Neutral Sugars (Relative % Hemicellulosic Sample, w/w) and Uronic Acids (% Hemicellulosic Sample, w/w) in Isolated Hemicellulosic Fractions and Residues Obtained in the Pretreatment of Dewaxed *Haloxylon ammodendron* and *Elaeagnus angustifolia* with Ethanol/H$_2$O (60:40, v/v) under Acidic Conditions (0.2 N HCl) at 70 °C for 4 h and Posttreatment with 2% H$_2$O$_2$ at pH 11.6 for 16 h

	hemicellulosic and crude cellulosic fractions					
sugars (%)	F$_1$[a]	F$_2$[a]	F$_3$[b]	F$_4$[b]	F$_5$[c]	F$_6$[c]
rhamnose	4.4	3.9	7.3	4.5	ND[d]	0.2
arabinose	1.9	1.2	3.4	1.3	0.2	0.4
xylose	56.1	86.0	42.8	81.6	16.0	13.2
mannose	4.4	0.6	5.9	1.0	0.9	1.4
glucose	18.5	2.9	23.4	7.8	82.8	84.8
galactose	14.6	5.3	17.1	3.7	Tr[e]	Tr
uronic acids	5.3	8.7	6.2	8.1	Tr	Tr

[a] Fractions 1 (F$_1$) and 2 (F$_2$) represent the hemicellulosic fractions extracted sequentially with ethanol/H$_2$O (60:40, v/v) under acidic conditions (0.2 N HCl) for 4 h at 70 °C and 2% H$_2$O$_2$ at pH 11.5 for 16 h at 45 °C from dewaxed *Haloxylon ammodendron*. [b] Fractions 3 (F$_3$) and 4 (F$_4$) represent the hemicellulosic fractions extracted sequentially with ethanol/H$_2$O (60:40, v/v) under acidic conditions (0.2 N HCl) for 4 h at 70 °C and 2% H$_2$O$_2$ at pH 11.5 for 16 h at 45 °C from dewaxed *Elaeagnus angustifolia*. [c] Fractions 5 and 6 represent the two-stage treated residues (crude cellulosic fractions) of *Haloxylon ammodendron* and *Elaeagnus angustifolia*, respectively. [d] ND, not detected. [e] Tr, trace.

tively. Most of the xylose residues probably originated from the xylan backbone. The presence of noticeable amounts of uronic acids (8.1−8.7%), mainly glucuronic acid, indicated that the two hemicelluloses are additionally substituted with glucuronic acid. Minor amounts of glucose (2.9−7.8%), galactose (3.7−5.3%), rhamnose (3.9−4.5%), arabinose (1.2−1.3%), and mannose (0.6−1.0%) indicated presence of short side chains of the hemicellulosic fractions. Higher contents of xylose and uronic acids, but lower contents of glucose, galactose, rhamnose, arabinose, and mannose of the two alkaline peroxide-soluble hemicellulosic preparations than the two acidic organosolv-soluble hemicellulosic fractions indicated that the former two hemicelluloses were more linear and acidic, and had a lower degree of substitution, than those of the two acidic organosolv-soluble hemicellulosic fractions.

Analysis of the two residue fractions showed that the two-step treatments were not able to solubilize all the hemicellulosic substances. About 55% of the dewaxed *Haloxylon ammodendron* and 58% of the dewaxed *Elaeagnus angustifolia* were extracted, leaving the residues, which consisted of 82.8% of glucose in F$_5$ and 84.8% in F$_6$, and 17.1% noncellulosic sugars in F$_5$ and 15.2% in F$_6$. The hemicellulose which remains in the residues is probably tightly bound to cellulose in the cell walls.

To compare the isolated lignin preparations with the lignin contaminated in the hemicellulosic preparations, and the residual lignin associated in the residues, the four hemicellulosic fractions and two crude cellulose samples were oxidized by alkaline nitrobenzene (**Table 3**). This method provided an estimate of the amount of associated lignin and an indication of its composition. The lignin associated in the hemicellulosic or cellulosic fractions was found to be quite different from the lignin preparations isolated from the acidic organosolv pretreatment process. The lignin associated with the two hemicellulosic and crude cellulosic fractions (**Table 3**) had lower guaiacyl units, but higher syringyl units, than the two acidic organosolv-soluble lignin fractions (*3*). This suggested that the lignin with more syringyl units were more difficult to extract by acidic organosolv, and were tightly linked to the polysaccharides in the cell

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Table 3. Yield (% Sample, w/w) of Phenolic Acids and Aldehydes from Alkaline Nitrobenzene Oxidation of Lignins in Isolated Hemicellulosic Fractions and the Residues

phenolic acids and aldehydes	hemicellulosic and crude cellulosic fractions[a]					
	F_1	F_2	F_3	F_4	F_5	F_6
p-hydroxybenzoic acid	0.50	0.28	1.01	0.55	0.43	0.27
p-hydroxybenzaldehyde	0.14	0.10	0.17	0.11	0.04	0.05
vanillic acid	0.12	0.14	0.13	0.13	0.10	0.11
syringic acid	0.28	0.40	0.31	0.25	0.23	0.24
vanillin	1.56	0.88	1.41	1.22	0.76	1.38
syringaldehyde	2.14	1.62	1.63	1.35	1.15	1.47
acetovanillin	0.11	0.27	0.22	0.32	0.10	0.13
p-coumaric acid	0.05	0.06	0.05	0.11	0.02	0.03
acetosyringone	0.15	0.31	0.19	0.33	0.12	0.20
ferulic acid	0.05	0.04	0.08	0.11	0.02	0.03
total	5.10	4.10	5.20	4.48	2.97	3.91
content of lignin	9.06	7.52	9.38	7.66	5.62	6.91

[a] Corresponding to hemicellulosic and crude cellulosic fractions in **Table 2**.

wall of *Haloxylon ammodendron* and *Elaeagnus angustifolia*, or that syringyl units were more condensed or cross-linked than guaiacyl units in the lignins associated to the four hemicellulosic and two cellulosic preparations. Conversely, the lignins with more guaiacyl units were more easily dissolved or degraded by pretreatment with ethanol/H$_2$O under acidic conditions. Moreover, as shown in **Table 3**, a higher amount of associated lignins in the two acidic organosolv-soluble hemicellulosic preparations (9.1% in F_1 and 9.4% in F_3) than in the two alkaline peroxide-soluble hemicellulosic fractions (7.5% in F_2 and 7.7% in F_4) and two residues (5.6% in F_5 and 6.9% in F_6) revealed that the linkages between lignin and hemicelluloses were more readily cleaved during the alkaline peroxide posttreatment conditions. However, the amount of residual lignin in the two-stage treated residues also implied that the polysaccharides in the cell walls of *Haloxylon ammodendron* and *Elaeagnus angustifolia* are tightly linked to lignin. Similar results have been reported from ginkgo wood by Xie and co-workers (*29*).

Despite the different content of Klason lignin in the four hemicellulosic and two cellulosic preparations, the major products obtained from the alkaline nitrobenzene oxidation of the associated lignins in the six polysaccharide fractions were identified to be syringaldehyde and vanillin, which together accounted for 72.6% of the total phenolic acids and aldehydes in F_1, 61.0% in F_2, 58.5% in F_3, 57.4% in F_4, 64.3% in F_5, and 72.9% in F_6. *p*-Hydroxybenzoic acid was found in noticeable amounts, ranging from 0.3% in F_6 to 1.0% in F_3. Syringic acid, vanillic acid, *p*-hydroxybenaldehyde, acetovanillin, and acetosyringone were also present in small amounts. The hydroxycinnamic acids, such as *p*-coumaric and ferulic acids, were detected only in trace quantities.

Molecular Mass of Hemicelluloses. The molecular mass of the four hemicellulosic preparations was characterized by gel permeation chromatography (GPC), and their weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) are shown in **Table 4**. It was apparent that the weight-average molecular masses of the two alkaline peroxide-soluble hemicellulosic fractions F_2 (31 800 Da) and F_4 (34 020 Da) were much higher than those of the two acidic organosolv-soluble hemicellulosic preparations F_1 (15 910 Da) and F_3 (17 780 Da). The elution patterns of the two organosolv-soluble hemicelluloses showed a significant shift to lower molecular mass material compared to the two alkaline peroxide-soluble hemicelluloses (elution chromatograms not shown). Furthermore, the two alkaline peroxide-soluble polymers demonstrated greater polydispersal. This was confirmed by their M_w to M_n ratio (**Table 4**), which is a measure of the heterodispersity.

Table 4. Weight-Average (M_w) and Number-Average (M_n) Molecular Weights and Polydispersity (M_w/M_n) of the Hemicellulosic Fractions Isolated Sequentially with Ethanol/H$_2$O (60:40, v/v) for 4 h at 70 °C and 2% H$_2$O$_2$ at pH 11.5 for 16 h at 45 °C from Dewaxed *Haloxylon Ammodendron* and *Elaeagnus Angustifolia*

	hemicellulosic fractions[a]			
	F_1	F_2	F_3	F_4
M_w	15 910	31 800	17 780	34 020
M_n	15 690	24 140	15 740	25 890
M_w/M_n	1.01	1.32	1.13	1.32

[a] Corresponding to hemicellulosic fractions in **Table 2**.

The two acidic organosolv-soluble hemicelluloses were more homogeneous than the two alkaline peroxide-soluble polymers; and the hemicellulosic fraction solubilized during the acidic organosolv pretreatment process from *Haloxylon ammodendron* was the most homogeneous extract. The higher degree of polymerization of the two alkaline peroxide-soluble hemicelluloses indicated that the posttreatment did not degrade the hemicellulosic polymers significantly.

Spectroscopic Characterization. FT-IR spectroscopy has been extensively applied in plant cell wall investigations and allows thorough monitoring of the functional groups in the polymers (*6*). In agreement with the chemical analysis, the FT-IR spectra of the four hemicellulosic fractions (**Figure 2**) clearly showed the typical signal pattern expected for a hemicellulosic moiety. In particular, all the spectra are dominant by signals in the region 3600−2800 cm^{-1}, which can be ascribed stretching vibrations of CH and OH, and by signals in the C−O stretching region (1200−950 cm^{-1}) (*30*). A sharp band at 1049 cm^{-1} in spectra b and d is indicative of xylans, indicating a predominant xylan of the two alkaline peroxide-soluble hemicelluloses, which corresponded to the results obtained by sugar analysis. In the anomeric region (950−700 cm^{-1}) two bands are present, including absorption at 903 cm^{-1} (typical for β-anomers in spectra b and d) and at 830 cm^{-1} (typical for α-anomers in spectra a and c). This observation illustrated the presence of dominant β-glycosidic linkages between the sugar units in the two alkaline peroxide-soluble hemicellulosic fractions, and major α-glycosidic linkages between the sugar units in the two acidic organosolv-soluble hemicellulosic preparations. In the carbonyl stretching region, in addition to the signal due to the absorbed water at 1632 cm^{-1}, a small signal at 1751−1745 cm^{-1} in the spectra (a and c) of two acidic organosolv-soluble hemicelluloses is unambiguously identified due to the acetyl and uronic ester groups of the hemicelluloses or from the ester
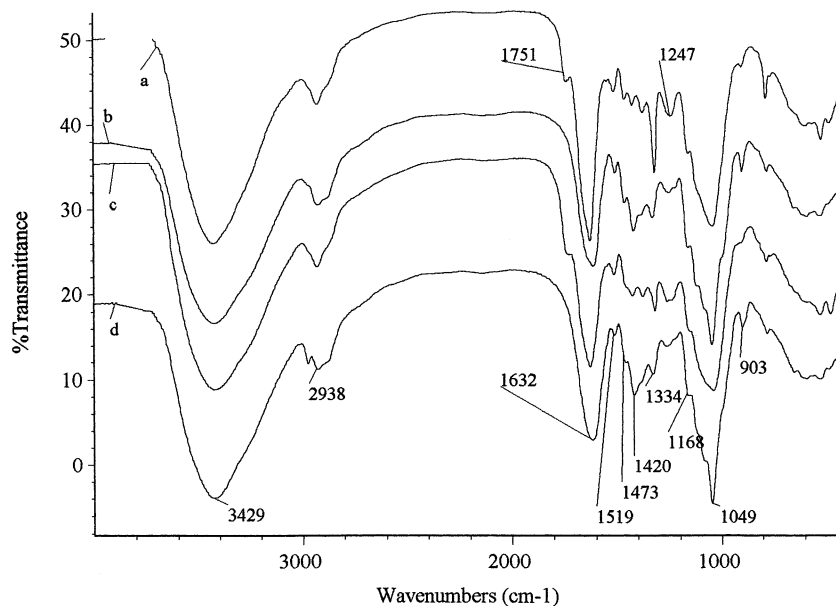
Figure 2. FT-IR Spectra of hemicellulosic fractions F_1 (spectrum a), F_2 (spectrum b), F_3 (spectrum c), and F_4 (spectrum d).
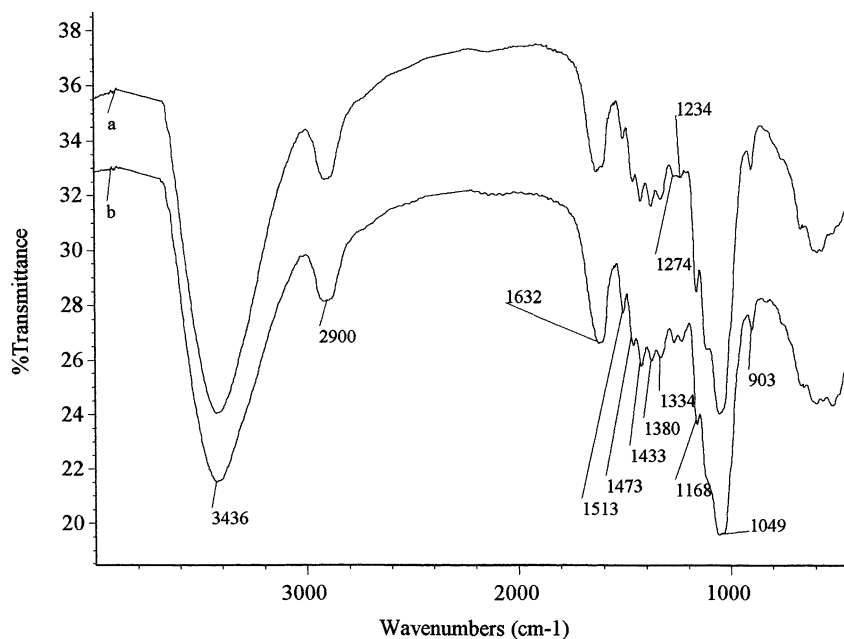

Figure 3. FT-IR Spectra of sequential ethanol/H$_2$O and sequentially alkaline peroxide treated residues of *Haloxylon ammodendron* (spectrum a) and *Elaeagnus angustifolia* (spectrum b).

linkage of carboxylic group of the ferulic acid, whereas the absence of this signal in the spectra (b and d) of alkaline peroxide-soluble hemicellulsic fractions revealed that the alkaline peroxide posttreatment under the condition used completely saponified this ester bond from the hemicelluloses. Interestingly, the absence of a signal at 1720 cm^{-1}, indicative of carbonyl stretching, in all four spectra revealed that both pre- and posttreatments under the conditions given did not significantly attack or oxidize the glycosidic linkages and hydroxyl groups of hemicelluloses. The fact that the glycosidic linkages and hydroxyl groups of hemicelluloses remained unattached during the two-stage treatments indicated a great difference in reaction rates between glycosidic and phenolic structures. Previous investigations on alkaline peroxide-soluble lignin from *Haloxylon ammodendron* and *Elaeagnus angustifolia* in our laboratory revealed that the degradation of phenolic structure in the lignin was very rapid, as shown by a noticeable band at 1720 cm^{-1} for carboxylic groups. However, the degradation of the carbo-

hydrates part was much slower (*3*). The absence of this band in the two spectra of alkaline peroxide-soluble hemicelluloses suggested that lignin protects hemicelluloses from being attacked by alkaline peroxide. In other words, hydroxyl radicals attack lignin structures more rapidly than they attack hemicellulosic structures (*27*).

The FT-IR spectra of two residues obtained by sequential treatments from dewaxed *Haloxylon ammodendron* (spectrum a) and *Elaeagnus angustifolia* (spectrum b) are shown in **Figure 3**. The most obvious feature is the absence of a absorption band at 1720 cm^{-1} for carbonyl or carboxyl groups, indicating once again that lignin also exhibited a protective effect on the oxidation of cellulose during the alkaline peroxide posttreatment. An intense band at 1632 cm^{-1} is related to the absorbed water. The prominent absorption at 1049 cm^{-1} is originated from the glycosidic linkage ν (C−O−C) contributions. The small sharp band at 903 cm^{-1} is attributed to β-glycosidic linkages between glucose units in cellulose. The fact that lignin provides some

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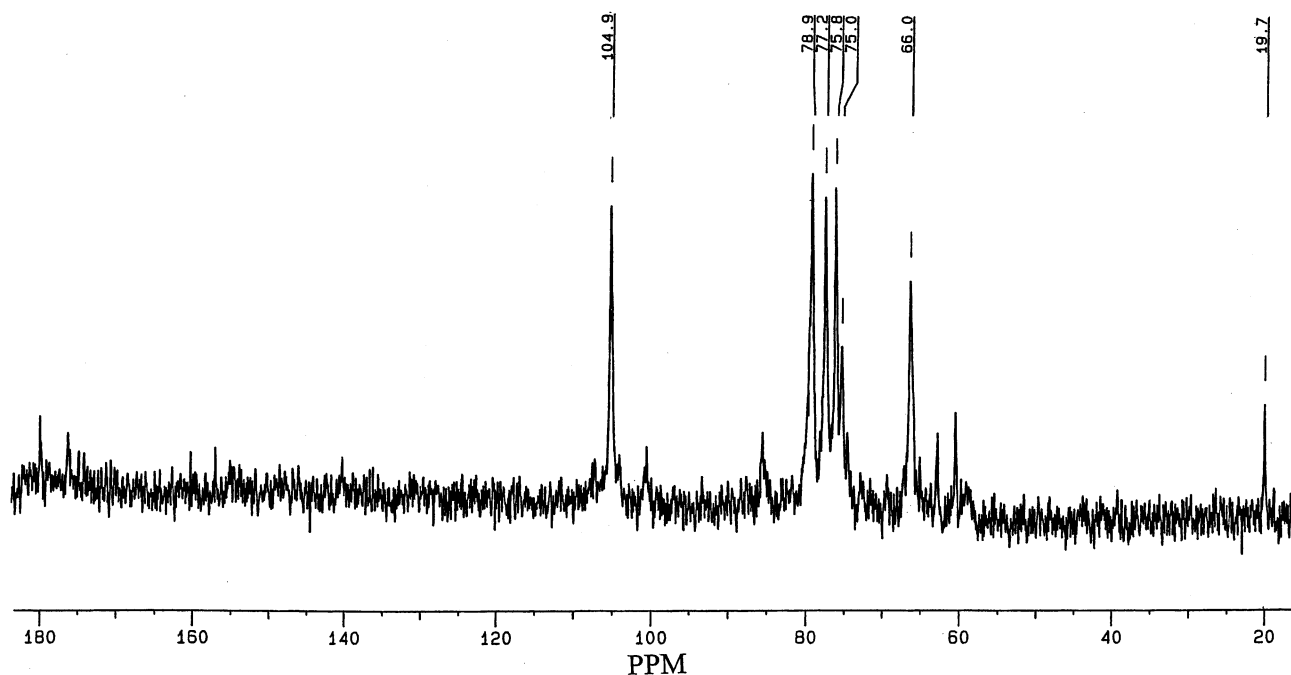*J. Agric. Food Chem.,* Vol. 50, No. 22, 2002 **6405**

Figure 4. ^{13}C NMR Spectrum of hemicellulosic fraction F_2 extracted with 2% H_2O_2 at 45 °C for 16 h at pH 11.5 from the pretreated *Haloxylon ammodendron*.

protection for the hemicelluloses and cellulose in lignin-containing materials against attack by ozone or alkaline peroxide has also been reported from the degradation of model compounds for cellulose and lignocellulosic pulp during ozonation in aqueous solution. The authors (*31*) stated that the degradation of phenolic structure in the lignin was very rapid, while the degradation of the carbohydrates part was slower.

Comparing the published ^{13}C NMR spectra of structurally defined arabinoxylan-type, glucuronoxylan-type, and L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan polysaccharides (*32*−*34*) to the spectrum of alkaline peroxide-soluble hemicellulosic fraction F_2 from *Haloxylon ammodendron* (**Figure 4**), it was apparent that the hemicellulosic fraction F_2 contained xylan as a major component. The main 1,4-linked β-D-Xylp units are obviously characterized by five strong signals at 104.9, 78.9, 77.2, 75.8, and 66.0 ppm, which are assigned respectively to C-1, C-4, C-3, C-2, and C-5 positions of the β-D-Xylp units. A signal at 60.2 ppm (data not shown) originates from the 4-*O*-methoxyl group of glucuronic acid residue in the xylan. The carbonyl resonances from uronic acids may contribute to a signal at 175.8 ppm which indicates C-6 in methyl uronates. The signals at 100.1 and 85.2 ppm correspond to C-1 and C-4 of the 4-*O*-methylglucuronic acid residue in the hemicelluloses. A signal at 62.5 ppm represents C-6 of D-Glcp units. The current results supported the data obtained by sugar analysis. This finding is of significance in the understanding of the structure of the primary plant cell wall from *Haloxylon ammodendron* in which 4-*O*-methylglucuronoxylan is a major component. A signal at 19.7 ppm is probably due to the methyl groups in acetyl group. The presence of minimal quantities of associated lignin was identified by very weak signals at 179.5, 160.2, 157.0, and 139.6 ppm (data not shown, *3*).

Thermal Analysis. The thermal properties between the four hemicellulosic preparations were investigated by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). **Figure 5** shows the thermograms of the acidic organosolv-soluble hemicellulosic preparation F_1 (**Figure 5a**) and alkaline peroxide-soluble hemicellulosic fraction F_2 (**Figure 5b**) from dewaxed *Haloxylon ammodendron*. It was apparent that

the two hemicelluloses F_1 and F_2 began to decompose at 230 and 200 °C, respectively, and their maximum rate of weight loss occurred between 235 °C and 340 °C. At 10% weight loss the degradation temperature was observed to be 258 °C for preparation F_1 and 235 °C for preparation F_2. In contrast, when the weight loss was 50%, the temperature raised to 357 °C for F_1 fraction and 402 °C for F_2 fraction. This phenomenon implied that, in general, the alkaline peroxide-soluble hemicelluloses had a slightly higher thermal stability than that of the acidic organosolv-soluble hemicelluloses, indicating that the thermal stability of the hemicelluloses increased with an increasing molecular weight. A similar phenomenon was found in the F_3 and F_4 hemicellulosic fractions. In comparison with the lignin fractions isolated from *Haloxylon ammodendron,* the hemicelluloses were degraded at a much faster rate between 200 °C and 300 °C. This observation was consistent with the thermogravimetric analysis of softwood lignin and hemicelluloses by Yoshida and co-workers (*35*). Also, the nonvolatile residue at 600 °C increased from acidic organosolv-soluble hemicellulosic fraction (∼30%) to alkaline peroxide-soluble hemicellulosic preparation (∼42%), indicating once again that the thermal stability of the hemicelluloses increased with increasing molecular weight. This effect may be explained in terms of differences in structures between the acidic organosolv-soluble and alkaline peroxide-soluble hemicellulosic fractions. Furthermore, owing to a relatively broad molecular weight distribution in the F_2 fraction, a lower starting decomposition temperature of the alkaline peroxide-soluble hemicelluloses may result from some lower molecular weight materials. It is therefore quite possible that a high-molecular-weight fraction with higher polydispersity would also show a lower starting decomposition temperature than the organosolv-soluble fraction having lower molecular weight and narrow distribution. In addition, the DSC curve of the acidic organosolv-soluble hemicellulosic fraction F_1 gave only one sharp exothermic peak centered at 310 °C (**Figure 5a**), whereas the alkaline peroxide-soluble hemicellulosic preparation F_2 exhibited three exothermic peaks at 237, 300, and 390 °C, due to exothermic reactions of the hemicelluloses.
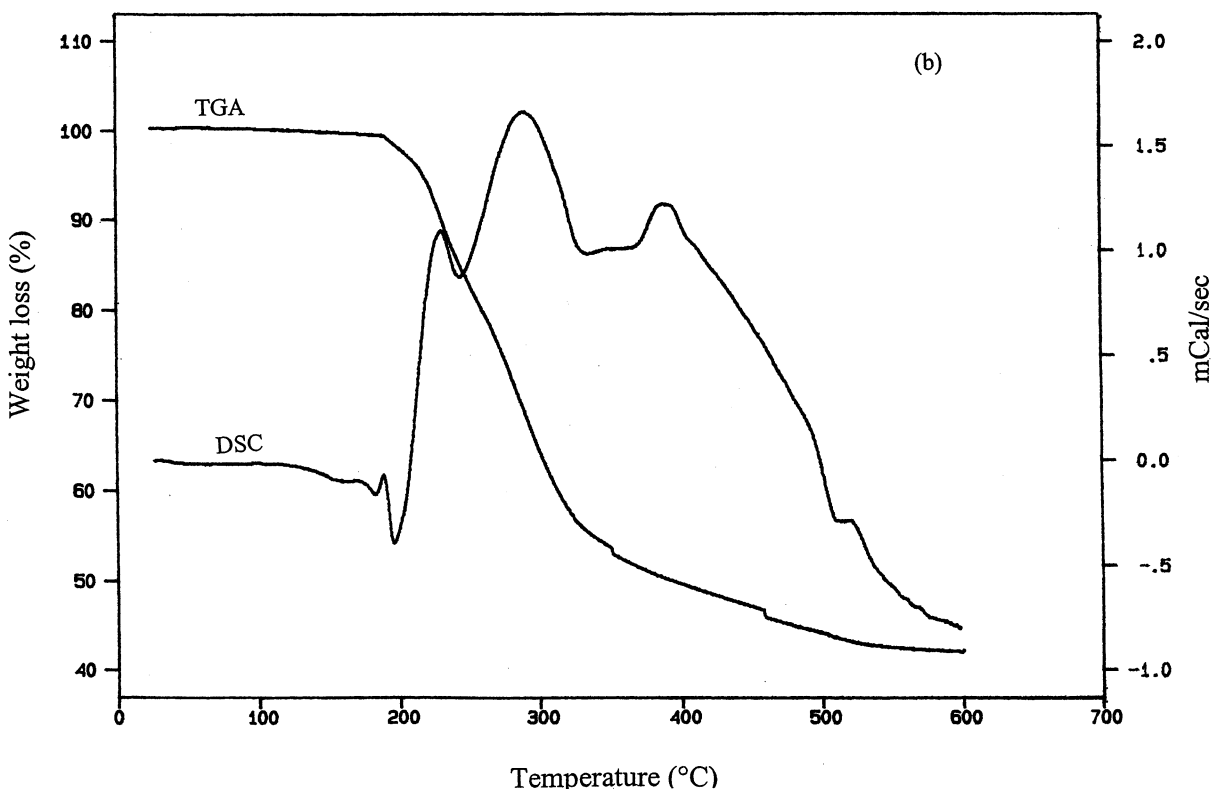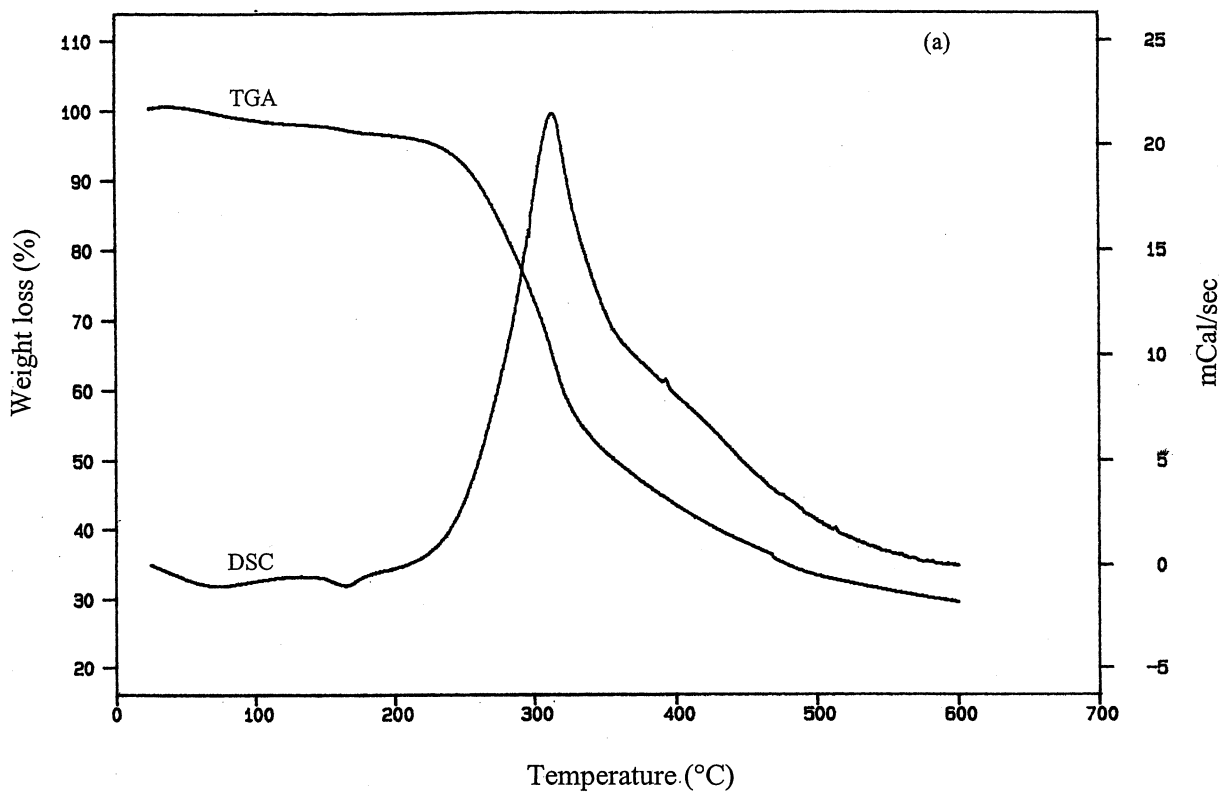
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Figure 5. Thermograms of hemicellulosic fraction F$_1$ (a) and F$_2$ (b) obtained from dewaxed *Haloxylon ammodendron.*

The results obtained showed that sequential treatments with ethanol/H$_2$O (60:40, v/v) under acidic conditions at 70 °C for 4 h and with 2% H$_2$O$_2$ at pH 11.5 for 16 h, together solubilized 84.4% and 82.1% of the original lignin and 83.9% and 87.6% of the original hemicelluloses from dewaxed *Haloxylon am-* *modendron* and *Elaeagnus angustifolia*, respectively. The two alkaline peroxide-soluble hemicellulosic preparations were more linear and acidic, and had a higher molecular mass than the two acidic organosolv-soluble hemicellulosic fractions. ^{13}C NMR spectrum of the alkaline peroxide-soluble hemicelluloses

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from *Haloxylon ammodendron* revealed that 4-*O*-methylglucu-ronoxylan was a major component of this fraction. Posttreatment with alkaline peroxide did not result in a significant degradation or oxidation of the macromolecular hemicelluloses, whereas degradation and oxidation of lignin were faster. It is very likely that the lignin part in lignin-containing materials may help to protect the hemicellulosic and cellulose matrix from degradation and oxidation.

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