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Ultrasonics Sonochemistry 9 (2002) 85–93

Ultrasonics SONOCHEMISTRY

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Comparative study of lignins isolated by alkali and ultrasound-assisted alkali extractions from wheat straw

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Received 19 March 2001; accepted 21 May 2001

Abstract

Treatment of the dewaxed wheat straw with 0.5 M KOH at 35°C for 2.5 h without ultrasonic irradiation and with ultrasound assistance for 5, 10, 15, 20, 25, 30, and 35 min resulted in a dissolution of 43.9%, 43.9%, 43.9%, 44.4%, 44.4%, 45.6%, 46.8%, and 49.1% of the original lignin, respectively. Much better results were achieved under the ultrasonic irradiation time for 35 min where nearly 50% of the original lignin was solubilized at 35°C for 2.5 h. The purity of the lignin preparations isolated by alkali with ultrasound assistance was higher than that of the lignin fraction obtained by alkali without ultrasonic irradiation, and their purity increased with an increment of irradiation time between 5 and 35 min, in which the content of associated polysaccharides in the former lignin preparations (0.87–1.06%) was lower than that of the latter lignin fraction (1.16%). In addition, the lignins isolated by alkali with ultrasonic irradiation time between 5 and 30 min showed a slightly higher molecular weight and thermal stability than the lignin obtained by alkali without ultrasound assistance. No substantial differences in the main structure features between the lignin preparations isolated by alkali and ultrasound-assisted alkali extractions were found.

Keywords: Ultrasound; Irradiation time; Lignin; Phenolics; Wheat straw

1. Introduction

In the past decades, an increase in the world population and economy has resulted in a raising demand for various raw materials for industries such as raw materials for pulp and papermaking. At an average population annual rate of 3.2%, the global demand for pulp and paper is expected to reach 420 million metric tons by year 2010 [1]. To reach this demand, various resources of the pulp and paper need to be concerned. Among these, agricultural residues such as cereal straws may represent a potential material for pulp and paper industry since they contain comparable amounts of the major constituents common to wood species. In particular, wheat straw is extensively used as raw material for pulp and paper. More than 9 million tons of straw pulp are produced annual in China, which account for about 90% of the world's total straw pulp [2,3].

Lignin in wood or straw can be described as a three-dimensional macromolecule with high molecular weight in the range of 100 KD. It originates from phenylpropanoid precursors such as coumaryl, coniferyl, and sinapyl alcohol C_6–C_3 and is present in vascular plants [4]. Also it acts as an adhensive that binds together the cellulosic fibre structure and imparts stiffness to the wood matrix [5]. However, unlike hardwood lignin, straw lignin additionally contains small but significant amounts of structural elements derived from *p*-coumaryl alcohol. Straw lignin also contains *p*-coumaric and ferulic acids attached to the core lignin through ester linkages [6]. In spite of technology's progress at the end of last century, it has not been possible to determine exactly the inter- and intra-molecular bonds involving lignin and other polymers in the cell wall. In addition, its heterogeneity has been a struggle for all wood researchers worldwide. Delignification in pulping processes consists of the degradation and dissolution of lignin macromolecule into smaller fragments. In the case of alkaline pulping, the important reactions include the cleavage of phenolic α-O-4 linkages, cleavage of

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1350-4177/02/$ - see front matter
PII: S1350-4177(01)00106-7

non-phenolic β-O-4 linkages, and removal of residual lignin fractions, either by cleavage of carbon–carbon linkages or by carbohydrate degradation, releasing lignin-carbohydrate fractions, which are mainly oxidised into aliphatic carboxylic acids [7]. These degraded lignins in principle can be used either as fuel or as feedstock for chemical conversions.

Our previous studies have shown that high yield of lignin from herbaceous plants such as straw and grass can be obtained using mild alkaline treatments, even at room temperature, in which over 60% of the lignin from wheat straw was extracted with 1.5% NaOH at 40°C for 6 h [8]. It was found that the considerable proportion of free phenolic groups present in the guaiacyl units seems to play an important role in the solubility properties of Gramineae lignins [9]. Meanwhile, the participation of ester- or ether-linked cinnamic acids, particularly ferulic acid, as linked bridges between lignin and hemicelluloses in the cell walls of herbaceous plants is considered to play a role in the high extraction yields of alkaline lignin [10–12]. This high solubility of the lignins from Gramineae in alkali can be considered as a suitable method for fractional isolation and structural study of the lignins [13].

More recently, applications of ultrasound techniques in food industry result in more attentions, to depolymerise macromolecules, make emulsions, disrupt biological cells, and deflocculate droplets [14,15]. Sonication has been reported to improve pectin technology from apple pressings [16] and isolation of pharmaceutically active compounds from *Salvia officinalis* [17], and increase of the yield of xylans from corn hulls [18] and corn cobs [19]. In the case of polysaccharide depolymerisation, studies by ultrasound have been preliminary investigated on various polysaccharides [14,20,21]. It has been found that the extraction of organic compounds contained within the body of plants and seeds by a solvent is significantly improved by the use of power ultrasound. The mechanical effects of ultrasound provide a greater penetration of solvent into cellular materials and improves mass transfer [15]. However, the use of ultrasound for direct extraction of lignin polymer from straw or wood has not been reported. The aim of this study was to compare the yield, composition, physico-chemical properties, and structural features of lignins extractable from wheat straw, isolated with 0.5 M KOH aqueous solution by conventional and ultrasound-assisted alkali extractions.

2. Experimental

2.1. Materials

Wheat straw (*Variety Riband*) was kindly supplied by B Lloyd Co., Llangefni. The composition (%, w/w) of the straw is cellulose 38.8%, hemicelluloses 39.5%, lignin 17.1%, ash 1.8%, and wax 2.2% on a dry weight basis. After being dried at 60°C in an oven for 16 h, the straw was ground to pass through a 0.7 mm screen and stored at 5°C until use.

2.2. Ultrasound-assisted extraction and isolation of lignins

A scheme for alkali and ultrasound-assisted alkali extractions and isolation of lignins is shown in Fig. 1. The dried straw was first extracted with toluene–ethanol (2:1, v/v) in a Soxhlet extractor for 6 h. To 9.78 g dewaxed sample in a 500 ml beaker, 300 ml 0.5 M KOH aqueous solution was added. The irradiation was carried using the Sonic system SOMERSET (England, 20 kHz) provided with a horn at sonic power of 100 W and sonication time for 0, 5, 10, 15, 20, 25, 30, and 35 min in 0.5 M KOH aqueous solution at 35°C, respectively. The mixture was then successively mixed at 35°C for a total period of 2.5 h. After filtration on a nylon cloth, the hemicelluloses were isolated from the hydrolysates by precipitation of the acidified hydrolysate (pH 5.5 adjusted with 6 M acetic acid) with three volumes of 95% ethanol. The pellets rich in the hemicelluloses were filtered, washed with 70% ethanol and air-dried. After evaporation of ethanol, the alkali soluble lignins were obtained by precipitation at pH 1.5 adjusted by 6 M HCl from the corresponding supernatants. The isolated acid-insoluble lignin preparations were washed with acidified water (pH 1.5–2.0) and freeze-dried. The residues rich in cellulose were washed with water and ethanol,

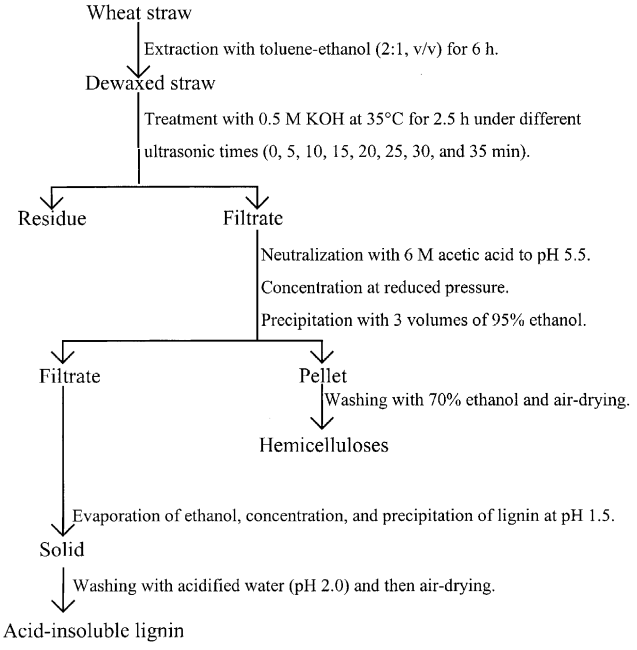

Fig. 1. Scheme for isolation of acid-insoluble lignins from wheat straw at different ultrasonic irradiation times.

then dried at 60°C for 16 h. All experiments were performed at least in duplicate. Yield of lignin fractions is given on a dry weight basis related to the wheat straw.

2.3. Physico-chemical and thermal analysis of the isolated acid-insoluble lignins

Both qualitative identification and quantitative determination of phenolic compounds released by alkaline nitrobenzene oxidation of the non-condensed monomeric units of the acid-insoluble lignin preparations [7] were carried out on a Hichrom H5ODS HPLC column of dimensions 250 mm × 4.6 mm. All nitrobenzene oxidation results represent the mean of at least triplicate samples and each oxidation mixture was chromatographed twice. The carbohydrate moieties associated with acid-insoluble lignin preparations were determined by hydrolysis of the lignin samples with 2 M trifluoroacetic acid for 2 h at 120°C. Liberated neutral sugars were analyzed as their alditol acetate derivatives by gas chromatography (GC) [22]. Methods for recording UV spectra and determination of molecular-average weights of the acid-insoluble lignin fractions are described in previous papers [23,24].

FT-IR spectra were obtained on an FT-IR spectrophotometer (Nicolet 750) using a KBr disc containing 1% finely ground lignin samples. The ^{13}C-NMR spectrum was obtained on a Bruker 250 AC spectrometer operating in the FT mode at 62.4 MHz under total proton decoupled conditions. It was recorded at 25°C from 180 mg of sample dissolved in 1.0 ml DMSO-d_6 after 20,000 scans. A 60° pulse flipping angle, a 3.9 μs pulse width and a 0.85 s acquisition time were used.

Thermal analysis of acid-insoluble lignin preparations was performed using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) on a simultaneous thermal analyzer (STA 625). The apparatus was continually flushed with nitrogen. The sample weighed between 8 and 12 mg. Each sample was heated from room temperature to 600°C at a rate of 10°C min^{-1}.

3. Results and discussion

3.1. Yield, purity, and molecular properties

In this study, eight alkaline lignin preparations were isolated with 0.5 M KOH at 35°C for 2.5 h with and without application of ultrasound and their yields are summarized in Table 1. As expected, treatment of the dewaxed wheat straw with 0.5 M KOH without application of ultrasound at 35°C for 2.5 h and with ultrasonic irradiation for 5, 10, 15, 20, 25, 30, and 35 min resulted in dissolution of 43.9%, 43.9%, 43.9%, 44.4%, 44.4%, 45.6%, 46.8%, and 49.1% of the original lignin and 62.8%, 63.0%, 62.8%, 62.8%, 63.5%, 63.5%, 64.3%, and 64.5% of the original hemicelluloses (data not shown in Table 1), respectively. Obviously, between the irradiation time 15 and 35 min the release of the lignin components from the cell walls was increased from 7.6% to 8.4%. Approximately half of the total lignin in wheat straw was released during the alkali extraction at sonication time of 35 min. Thus, application of sonication for 35 min resulted in raising solubility of lignin by 0.9% in comparison to the alkaline extraction procedure without ultrasound assistance. However, the differences between ultrasound-assisted extractions for 5–10 min and the alkali treatment without ultrasonic irradiation were found to be negligible. This implied that ultrasonic irradiation time was a main parameter affecting the lignin yields under the conditions used. The higher efficiency of the ultrasound-assisted extractions can be explained by the mechanical action of ultrasound on the cell walls resulting in an increased accessibility and extractibility of the lignin component. Such effects of sonication were known to improve the isolation of extractives from various plant materials [15] and xylans from corn cob [19]. More importantly, the yield of acid-insoluble lignin preparation representing the major fraction (Table 1), comprised 65.3–72.6% of the total solubilized lignins, while the yield of the lignin fraction associated in the solubilized hemicelluloses, accounted only for 13.1–19.0% of the total released lignin. The last was particularly lower when the extraction was

Table 1
The yield of lignin fractions (% dry matter) obtained by alkaline and ultrasonic-assisted alkaline extractions of wheat straw with 0.5 M KOH at 35°C for 2.5 h under different ultrasonic times

Lignin fractions	Ultrasonic time (min)							
	0	5	10	15	20	25	30	35
Total solubilized lignins	7.5	7.5	7.5	7.6	7.6	7.8	8.0	8.4
Acid-insoluble lignins[a]	4.9	5.0	5.1	5.1	5.3	5.2	5.8	6.1
Acid-soluble lignins[b]	1.0	0.9	1.0	1.1	0.9	1.2	1.1	1.2
Lignin associated in isolated hemicelluloses	1.6	1.4	1.4	1.4	1.4	1.4	1.1	1.1

[a] Represent the lignin fractions obtained by precipitation of the supernatant solution at pH 1.5 after isolation of the solubilized hemicelluloses.
[b] Represent the lignin fractions which are still solubilized in the pH 1.5 supernatant after precipitation of the acid-insoluble lignin fractions and obtained by difference (total solubilized lignin – acid-insoluble lignin – lignin associated in the solubilized hemicelluloses).

performed with ultrasound assistance for 30 and 35 min. These results indicated that alkaline extractions with ultrasonic irradiation under the conditions used had a more effect on the cleavage of the ether bonds between lignin and hemicelluloses from the cell walls of wheat straw than the alkaline treatment without ultrasound assistance. The current observations were consistent with the studies of the cleavage of interunitary bonds in lignin induced by ultrasonic irradiation of milled wood lignin by Yoshioka et al. [25]. The authors stated that the major ether linkages, i.e., β-O-4 bonds between lignin interunitary linkages and α-O-4 ether linkages between lignin and hemicelluloses can be homolytically ruptured or cleaved to some extent by the ultrasonic irradiation.

The isolated acid-insoluble lignin fractions were characterized by UV spectroscopy at λ 250–390 nm. Fig. 2 illustrates the four lignin preparations isolated by 0.5 M KOH at 35°C for 2.5 h without ultrasonic irradiation (spectrum a) and with ultrasound assistance for 5 (spectrum b), 10 (spectrum c), and 15 min (spectrum d). Evidently, the four lignin fractions showed the basic UV spectrum typical of lignins with two maxima at 280 and 320 nm. The first absorption maximum corresponds to nonconjugated phenolic groups in the lignin [11] and the second one is assigned to bound hydroxycinnamic acid, such as *p*-coumaric and ferulic acids. A lowest absorption coefficient of the lignin preparation, isolated by 0.5 M KOH at 35°C for 2.5 h without ultrasonic irradiation (spectrum a), was undoubtedly due to the more co-precipitated non-lignin materials such as carbohydrate degradation products, ash and salts.

Comparison of the sugar content between the eight acid-insoluble lignin fractions given in Table 2 indicated that all the lignin preparations obtained with ultrasound assistance contained lower amounts of bound hemicelluloses as shown by 0.87–1.06% in comparison to that of the lignin fraction obtained by alkali without ultrasonic irradiation. Obviously, an increase in sonication time from 5 to 35 min resulted in a decrease in the level of associated hemicelluloses from 1.06% to 0.87% in the acid-insoluble lignin fractions. These data suggested that treatment of the straw with alkali under the ultrasound-assisted conditions improved the cleavage of α-ether bonds between lignin and hemicelluloses from the cell walls of wheat straw. Xylose (0.45–0.53%) was identified as the major sugar component. Arabinose (0.16–0.25%), glucose (0.17–0.23%), and galactose (0.09–0.13%) were present in noticeable amounts.

Table 2
The content of neutral sugars (% of lignin sample, w/w) in isolated acid-insoluble lignin preparations obtained at different ultrasonic times from wheat straw

Neutral sugars	Ultrasonic time (min)							
	0	5	10	15	20	25	30	35
Arabinose	0.25	0.22	0.22	0.22	0.20	0.18	0.16	0.16
Xylose	0.53	0.50	0.49	0.46	0.47	0.47	0.48	0.45
Glucose	0.23	0.21	0.21	0.21	0.22	0.20	0.18	0.17
Galactose	0.13	0.13	0.12	0.11	0.12	0.10	0.11	0.09
Total	1.14	1.06	1.04	1.00	1.03	0.95	0.93	0.87

3.2. Lignin composition

To verify the lignin composition, the eight acid-insoluble lignin fractions were oxidized by alkaline nitrobenzene at 170°C for 2.5 h. In this case, the three constitutive monomeric lignin units, i.e. *p*-hydroxyphenyl, guaiacyl, and syringyl, produce the corresponding *p*-hydroxybenzaldehyde, vanillin, and syringaldehyde, respectively. Table 3 summarizes the yield of phenolic acids and aldehydes and the molar proportions of S (relative total moles of syringaldehyde and syringic

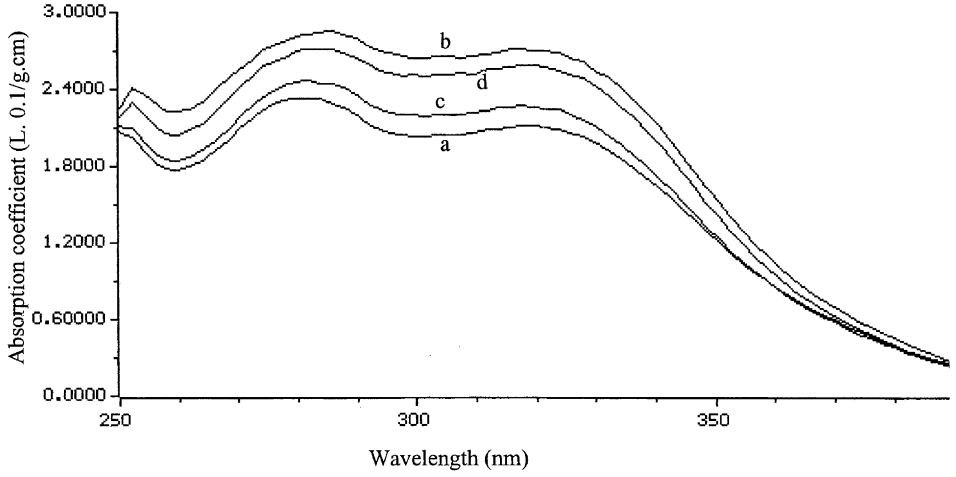

Fig. 2. UV spectra of acid-insoluble lignin preparations isolated with 0.5 M KOH at 55°C for 2.5 h without ultrasonic irradiation (spectrum a) and with sonication time for 5 min (spectrum b), 10 min (spectrum c), and 15 min (spectrum d).

Table 3
The content (% of lignin sample, w/w) of phenolic acids and aldehydes from nitrobenzene oxidation of the acid-insoluble lignin preparations obtained at different ultrasonic times from wheat straw

Phenolic acids and aldehydes	Ultrasonic time (min)							
	0	5	10	15	20	25	30	35
p-Hydroxybenzoic acid	1.84	1.84	1.64	1.32	1.20	0.96	0.88	0.52
p-Hydroxybenzaldehyde	2.92	2.85	2.48	1.88	1.44	1.56	1.54	1.44
Vanillic acid	1.87	1.38	1.34	0.76	0.63	0.58	0.58	0.40
Syringic acid	2.53	2.95	2.30	2.42	2.05	1.84	1.86	1.84
Vanillin	20.32	19.56	20.30	18.26	15.90	15.29	15.78	15.52
Syringaldehyde	18.04	18.01	18.20	15.00	10.65	10.44	10.37	10.30
p-Coumaric acid	1.08	1.00	0.92	0.73	0.78	0.74	0.50	0.48
Ferulic acid	1.67	1.56	1.48	1.39	1.48	1.44	0.98	0.96
Total	50.27	49.15	48.66	41.76	34.13	32.85	32.49	31.46
Molar ratio (S:V:H)[a]	3:4:1	3:4:1	4:5:1	4:5:1	3:5:1	3:5:1	3:5:1	3:5:1

[a] S represents the sum of total moles of syringaldehyde and syringic acid; V represents the sum of total moles of vanillin and vanillic acid; and H represents the sum of total moles of *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid.

acid):V (relative total moles of vanillin and vanillic acid):H (relative total moles of *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid) from nitrobenzene oxidation of the acid-insoluble lignin fractions. As can be seen, the predominant oxidation products were vanillin and syringaldehyde, which together represented 76.3%, 76.4%, 79.1%, 79.6%, 77.8%, 78.3%, 80.5%, and 82.1% of the total phenolic compounds in the lignin fractions isolated by alkali without ultrasonic irradiation and with ultrasound assistance for 5, 10, 15, 20, 25, 30, and 35 min, respectively. The yield of vanillin was higher than that of the syringaldehyde in all the lignin preparations. The relatively higher amounts of vanillin was particularly significant in the lignin fractions obtained by alkali with ultrasonic irradiation time between 20 and 35 min as shown by 3:5:1 molar ratios of S:V:H. Extension of ultrasonic irradiation time between 20 and 35 min enhanced the release of guaiacyl lignin. The high ratio of V/S units in the lignin fractions revealed that these lignins were released mainly from the primary cell wall since a larger amount of guaiacyl lignin is formed in the early stage of xylem differentiation than in the later stages, where the lignin is rich in syringyl units. The presence of fewer *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid was considered most probably to be indicative of non-condensed *p*-hydroxyphenyl units, indicating the incorporation of *p*-hydroxycinnamoyl alcohol in the wheat straw alkali lignins. In addition, except of partial conversion of *p*-coumaric acid to *p*-hydroxybenzaldehyde and ferulic acid to vanillin during the nitrobenzene oxidation process, the remaining occurrence of noticeable amounts of ferulic acid (0.96–1.67%) and small quantities of *p*-coumaric acid (0.48–1.08%) in all the lignin fractions indicated that these two phenolic acids are closely linked with lignins in the cell walls of wheat straw, in accord with our previous study [18]. Furthermore, the yield of nitrobenzene oxidation is considered to be related to the degree of lignin condensation [26]. It is higher in the lignin fraction obtained by alkali without ultrasonic irradiation than in the lignin preparations isolated by alkali with ultrasound assistance, and it decreased substantially from 49.15% to 31.46% with an increase of ultrasonic irradiation time from 5 to 35 min. This result demonstrated that ultrasound-assisted extractions under the alkaline conditions used resulted in significant condensation of the solubilized lignin molecules.

The values of the weight-average ($\overline{M}_w$) and number-average ($\overline{M}_n$) molecular weights and the polydispersity ($\overline{M}_w/\overline{M}_n$) of the eight acid-insoluble lignin fractions are given in Table 4. It should be noted that the data shown in this table can be used only in a comparative study since the calibration was carried out using polystyrene standards. As shown in Table 4, the six lignin preparations isolated by alkali with ultrasound assistance for 5–30 min showed a slightly higher molecular-average weights, ranging $\overline{M}_w$ from 3010 to 3570 $g\,mol^{-1}$ than that of the lignin fraction obtained by alkali without ultrasonic irradiation ($\overline{M}_w = 2890\ g\,mol^{-1}$). An increase in sonication time from 5 to 20 min led to an increment of $\overline{M}_w$ from 3010 to 3570 $g\,mol^{-1}$, indicating an increasing in solubilization of large molecular size lignins under the ultrasound conditions used. The reason for this increase in $\overline{M}_w$ is probably due to the condensation reactions between the lignin structures under ultrasound

Table 4
Weight-average ($\overline{M}_w$) and number-average ($\overline{M}_n$) molecular weights and polydispersity ($\overline{M}_w/\overline{M}_n$) of the isolated acid-insoluble lignin preparations obtained at different ultrasonic times from wheat straw

	Ultrasonic time (min)							
	0	5	10	15	20	25	30	35
$\overline{M}_w$	2890	3010	3130	3470	3570	3300	3070	2760
$\overline{M}_n$	1490	1540	1550	1620	1620	1340	1150	1040
$\overline{M}_w/\overline{M}_n$	1.94	1.95	2.02	2.14	2.20	2.46	2.66	2.65

irradiation conditions given. In contrast, as the irradiation time was further increased from 20 to 25, to 30, and to 35 min, the $\overline{M}_w$ decreased from 3570 to 3300, to 3070, and to 2760 g mol^{-1}, respectively. This decrease in $\overline{M}_w$ is assigned to the cleavage of the β-O-4 linkages between the lignin precursors under a relatively longer sonication period. Similar phenomenon was observed by Yoshioka et al. [25] in the study of homolytic scission of interunitary bond in wood lignins induced by ultrasonic irradiation. The authors stated that the alkyl phenyl ether bonds, ∼CH–O–phenyl, known as interunitary bonds in lignins were homolytically cleaved by the ultrasonic irradiation. On the other hand, the polydispersity of the molecular distribution of seven lignin preparations isolated by alkali under ultrasonic irradiation was higher than that of the lignin fraction obtained by alkali without ultrasound assistance, and it increased with increasing the irradiation time from 5 to 30 min. This implied that an increase in ultrasonic irradiation time between 5 and 30 min under the alkaline condition used also led to lignin fractions solubilized having a more broad molecular weight distribution.

3.3. Spectroscopic characterization

FT-IR spectra of some the acid-insoluble lignin preparations are illustrated in Fig. 3. All the lignins showed the spectral features of GSH type lignins, namely, the bands at 1126 and 845 cm^{-1} and a shoulder at 1158 cm^{-1} [27]. The band at 1702 cm^{-1} corresponds to the unconjugated ketone and carboxyl group stretching, while the band at 1657 cm^{-1} is attribute to conjugated carbonyl stretching in lignin. Aromatic skeleton vibrations in the lignin preparations are assigned at 1600, 1510, and 1424 cm^{-1}. Absorption at 1464 cm^{-1} relates to C–H deformations and aromatic ring vibrations. The aliphatic C–H stretching in CH_3 gives a small band at 1362 cm^{-1}. The bands at 1331, 1265, and 1230 cm^{-1} are indicative of ring breathing with C–O stretching. The 1126 and 1032 cm^{-1} bands arise from the aromatic CH in-plain deformation for syringyl type and guaiacyl type, respectively. Aromatic C–H out of bending appears at 845 cm^{-1}. The spectral profiles and the relative intensities of the absorption bands were rather similar, indicating that application of ultrasound under the alkaline extraction conditions given did not affect the structure of alkali lignin from wheat straw.

To further investigate the structural changes of the acid-insoluble lignin individually by ultrasonic irradiation, the lignin fraction isolated with 0.5 M KOH under sonication time for 35 min was investigated by ^{13}C-NMR spectroscopy (Fig. 4). Most of the observed signals have been previously assigned in wood and straw lignin spectra [7,11,28,29]. As shown in Fig. 4, the lignin fraction is the almost absence of typical polysaccharide signals between 57 and 103 ppm. The spectrum does show two signals at 79.6 and 62.5 ppm, which are attributed to C-α etherified to carbohydrates and C-5 in Xyl internal unit, respectively. However, the peak intensities are rather weak, indicating a trace amount of associated carbohydrates in the lignin fraction. This result corresponded to the lower carbohydrate content. Similar observation of C-α etherified to polysaccharides in lignin side chain has been reported by Xie and coworkers [30] from the study of ginkgo lignin–carbohydrate complexes (LCC). The authors demonstrated that

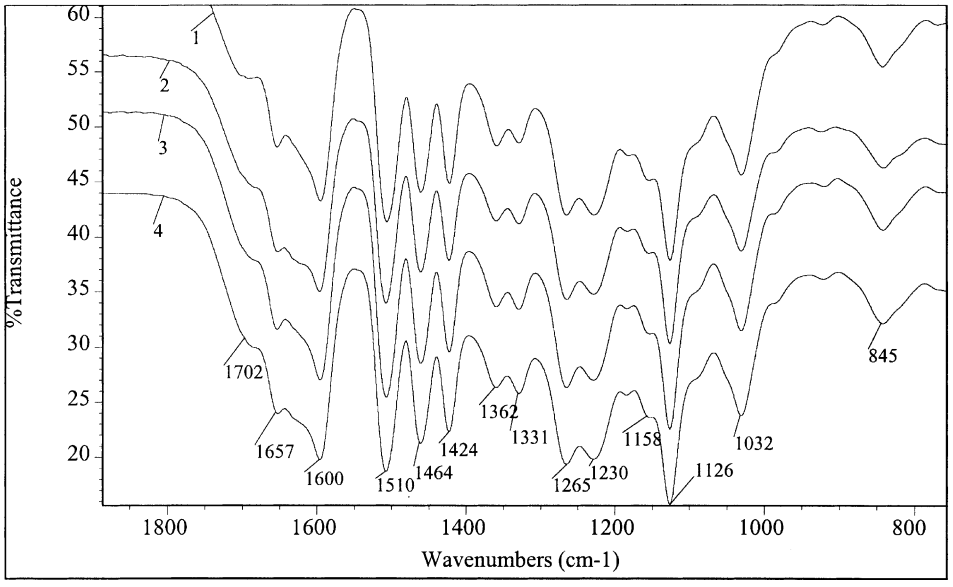

Fig. 3. FT-IR spectra of wheat straw acid-insoluble lignin preparations obtained by treatment with 0.5 M KOH (35°C, 2.5 h) without ultrasonic-assistance (spectrum 1) and with ultrasonic assistance for 5 min (spectrum 2), 15 min (spectrum 3), and 35 min (spectrum 4).

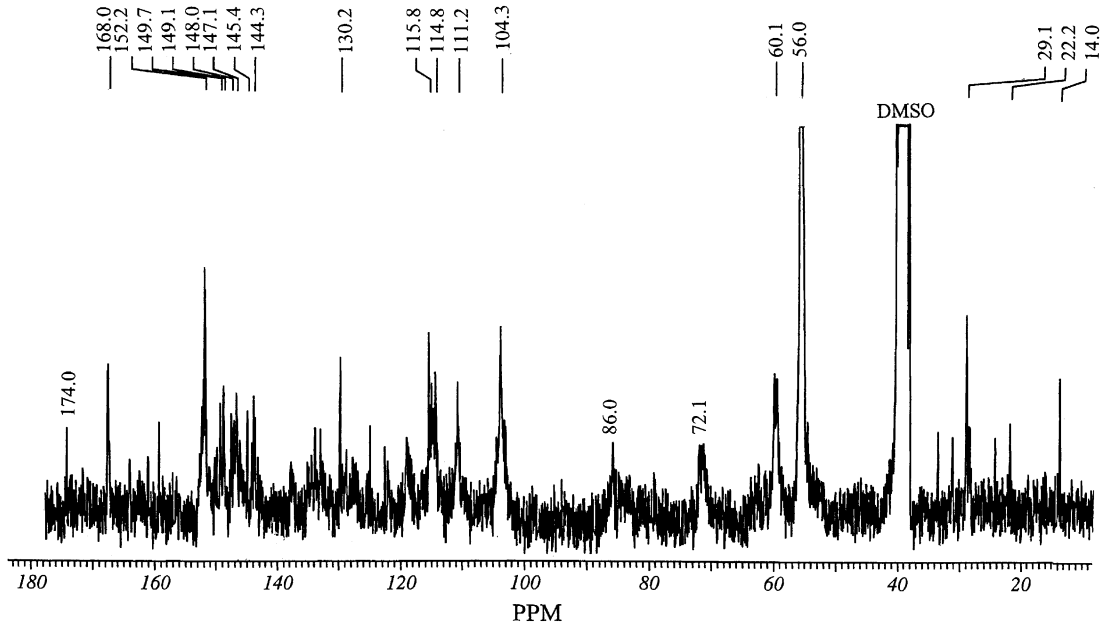

Fig. 4. ^{13}C-NMR spectrum of acid-insoluble lignin fraction isolated with 0.5 M KOH with ultrasonic irradiation time for 35 min from wheat straw.

three lignin–carbohydrate linkages (i.e., ether type, ester type, ketal type) were found at the C-α position of the side chain of phenylpropane units in ginkgo LCC, and no lignin–carbohydrate bond at the C-β or C-γ position of the lignin side chain was observed in the ^{13}C-NMR spectra of the ^{13}C-enriched LCC.

The signals for aromatic part of the lignin units can be observed in the region between 104.3 ppm and 152.2 ppm. The syringyl residues were identified by signals at 152.2 (C-3/C-5 in syringyl units), 147.1 (C-3/C-5 in non-etherified syringyl units), 138.2 (C-4 in etherified syringyl units, data not shown), 134.2 (C-1 in etherified syringyl units, data not shown), 133.0 (C-1 in non-etherified syringyl units, data not shown), and 104.3 ppm (C-2/C-6 in syringyl units). The guaiacyl units give signals at 147.1 (C-4 in etherified guaiacyl units), 145.4 (C-4 in non-etherified guaiacyl units), 134.2 (C-1 in etherified guaiacyl units), 133.0 (C-1 in non-etherified guaiacyl units), 119.0 (C-6 in guaiacyl units, data not shown), 114.8 ppm (C-5 in guaiacyl units). The signals at 159.8, (C-4 in esterified *p*-coumaric acid), 130.2 ppm (C-2/C-6 in esterified *p*-coumaric acid), 125.4 (C-1 in esterified *p*-coumaric acid), and 115.8 ppm (C-3/C-5 in esterified *p*-coumaric acid) represent the esterified *p*-coumaric acid. Etherified ferulic acid gives signals at 168.0 ppm (C-γ in etherified ferulic acid), 144.3 (C-α in etherified ferulic acid), and 122.5 ppm (C-6 in etherified ferulic acid), while the esterified ferulic acid exhibits a signal at 122.9 ppm (C-6 in esterified ferulic acid). These observations strongly supported our previous findings that *p*-coumaric acid is linked to lignin by ester bonds, whereas ferulic acid is linked to lignin by both ether and ester bonds [24].

In Fig. 4 three resonances at 86.0, 72.1, and 60.1 ppm are assigned to C-β in β-O-4, C-α in β-O-4, and C-γ in β-O-4, respectively. These signals revealed that the treatment with 0.5 M KOH at 35°C under ultrasound assistance for 35 min did not attack the β-aryl ether structure to a significant extent. A very strong signal at 56.0 ppm is due to the OCH_3 in syringyl and guaiacyl units. The signals for the γ-methyl, α- and β-methylene groups in n-propyl side chains of the lignin fraction occurred in the spectrum between 14.0 and 33.8 ppm.

3.4. Thermal analysis

The thermal stability of the lignin preparations was determined by TGA and DSC. Fig. 5 illustrates the thermograms of the lignin preparation obtained by 0.5 M KOH without ultrasound assistance (Fig. 5a) and the two lignin fractions isolated by alkali with sonication time for 10 (Fig. 5b) and 30 min (Fig. 5c). As can be seen from the Fig. 5, the decomposition temperature of the three lignin fractions in Fig. 5a–c started at 176°C, 208°C, and 205°C, respectively. Similarly, at 10% weight loss the decomposition temperature of the three lignins appeared at 238°C in Fig. 5a, 254°C in Fig. 5b, and 248°C in Fig. 5c. This trend of the initial weight loss correlated well with the lignin molecular weights given in Table 4, and showed that the thermal stability of the lignins increased with the molecular weight even though they showed a similar maximum decomposition temperature ranged between 300°C and 500°C and gave similar thermal stability when the temperature raised to 500°C. At this temperature the weight loss amounted to 50% for all the three lignin preparations. The current

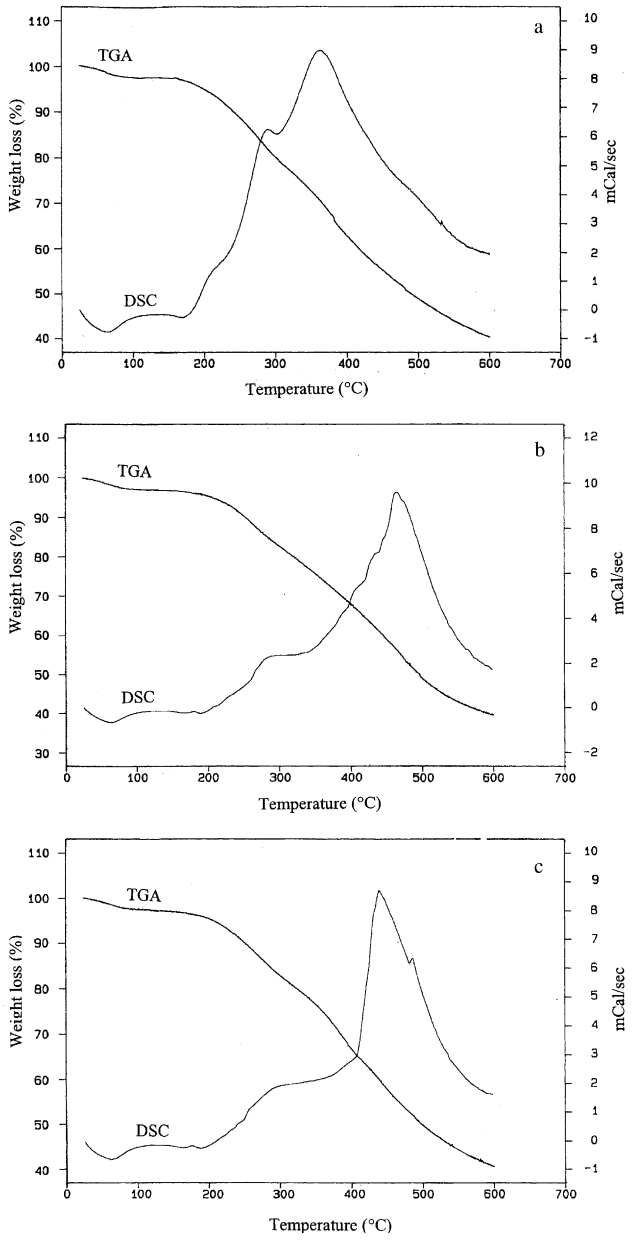

Fig. 5. TGA/DSC curves of acid-insoluble lignin fractions isolated with 0.5 M KOH (35°C, 2.5 h) (a) without ultrasonic-assistance, (b) with ultrasonic assistance for 10 and (c) 30 min.

results were in good agreement with the thermal stability of lignins from wood samples, in which the thermal stability increased with the molecular weight [31,32]. In addition, the DSC curve of the lignin fraction, isolated with 0.5 M KOH without ultrasound assistance (Fig. 5a), showed a big exothermic peak between 200°C and 600°C, whereas in the curves of the two lignin fractions, isolated by alkali with sonication time for 10 (Fig. 5b) and 30 min (Fig. 5c), this exothermic peak shifted to a higher temperature of 400–600°C, due to exothermic reactions of the lignin [33].

4. Conclusions

The above results indicated that ultrasonic irradiation in alkaline medium promoted the extraction of lignins from wheat straw, and their yield and purity increased with sonication time from 5 to 35 min under the conditions given. Except for the lignin preparation isolated with 0.5 M KOH with ultrasonic irradiation time for 35 min, all other lignin fractions obtained by the alkali under ultrasound-assisted extractions showed a higher molecular weight than that of the lignin sample extracted with alkali without ultrasonic irradiation. The content of associated carbohydrates in the lignin preparations obtained by alkali with ultrasonic irradiation was lower than that of the lignin fraction isolated by alkali without ultrasound assistance, and it decreased with an increase in sonication time between 5 and 35 min. However, ultrasonication under the alkaline conditions used did not cause significant changes in lignin composition and its structure. This is of major importance from industrial point of view and makes the ultrasound-assisted extraction process very advantageous.

Acknowledgements

We are grateful for the financial support of this research from the LINK program (Fractionation of wheat straw for industrial utilizations) of the UK Ministry of Agriculture, Fisheries, and Food.

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