Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Phytochemistry 63 (2003) 617–623
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

The unmasking of lignin structures in wheat straw by alkali

Nathalie Durot, François Gaudard, Bernard Kurek*

Equipe Parois Végétales et Matériaux Fibreux, UMR de Fractionnement des Agroressources et Emballages, Institut National de la Recherche Agronomique (INRA), 2 Esplanade Roland Garros, F-51686 Reims Cedex 2, France

Received 7 October 2002; received in revised form 28 February 2003

Abstract

This study reports on the structural modifications of wheat straw cell wall promoted by potassium carbonate and sodium hydroxide that lead to the unmasking of some lignin structures. The first impact of the treatments was the extraction of a particular fraction of lignin enriched in C–C linked structures compared to the mean composition in reference wheat straw. Concomitantly, an apparent increase in the amount of lignin monomers released by the cleavage of alkyl–aryl ether bonds was observed in alkali-extracted samples. By summing the amount of ether linked monomers analyzed by thioacidolysis in the solubilized lignin to that found in the extracted wheat straw, an excess of up to 37% is apparent, relative to the corresponding amount in the reference wheat straw. Other modifications of the cell wall were also found. Indeed, a fraction of uronic acids was lost during the treatments and a new fractionation pattern of the lignin-carbohydrate complexes was evidenced. It can thus be concluded that a significant proportion of lignin within the cell wall was unmasked after (i) the selective removal of a particular lignin fraction, (ii) a partial saponification of the esterified fraction of lignin with uronic acids and (iii) a modification of the interactions between the cell wall constituents.

Keywords: Wheat straw; Lignin; Lignin carbohydrate complexes; Uronic acids; Alkaline extraction; Fractionation; Abiotic reaction

1. Introduction

The various covalent linkages and physico–chemical interactions between lignin, cellulose, hemicelluloses, phenolic acids and other polyphenolic or proteinaceous or mineral extractives all contribute structural integrity to the wheat straw cell wall composite (Iiyama et al., 1994). However, they are also controlling factors and obstacles for specific and efficient fractionation and bioconversion processes of wheat straw into valuable products, such as animal feed, ethanol, high value chemicals, fiber materials and composites (Bledzki and Gassan, 1999; Grabber et al., 1998; Lee, 1997; Zilliox and Debeire, 1998).

A particular aspect of wheat straw cell walls, that is particularly relevant for all fractionation and/or technological processes, is the existence of a non-core lignin easily soluble in alkali and representing up to 20% of the total lignin. The removal of this particular lignin generally increases the digestibility of the material and enhances further microbial or enzymatic bioconversion (Jackson, 1977; Zilliox and Debeire, 1998). This step also represents the first stage of delignification for fiber production, in several pulping processes (Fiala and Nardi, 1985; Hultom et al., 1997).

The release of lignin from the cell wall is mainly related to the hydrolysis by alkali of ester bonds between lignins and phenolic acids and/or acidic sugars from pectins or hemicelluloses associated with lignin (Jacquet et al., 1995; Lam et al., 2001; Scalbert et al., 1986b). Solubility in alkali is also related to the particular structure of the lignins. Indeed, they have been shown to be of relatively low molecular mass and to be enriched in phenolic structures (Lapierre et al., 1989).

However, there are as many structures of alkali soluble lignins described in the literature as processes used to isolate them (Scalbert et al., 1986a; Sun et al., 1997). Also, it is difficult to compare strictly the molecules isolated, as the characterization methods used differed from one published paper to the other. Nevertheless, the high structural variability of the polymers extracted by alkali leads to the concept that the alkali fractions of

* Corresponding author. Tel.: +33-3-2677 3593; fax: +33-3-2677 3599.
E-mail address: kurek@reims.inra.fr (B. Kurek).

0031-9422/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0031-9422(03)00187-0

lignin are encompassed in a set of different sub-structures, with specific behavior during the extraction. As a consequence, the properties of the residual extracted cell wall would be different, according to the nature of the lignin extracted.

Thus, in order to delineate certain mechanisms of the enzymatic fractionation processes (Lequart et al., 2000) and of the degradation of wheat straw during maceration in carbonate rich calcareous soils (Durot and Kurek, 2000), we examined the structural modifications of the cell wall components induced by a very mild NaOH and K_2CO_3 treatment at pH 10 and at room temperature. The chemical compositions of the wheat straw material soluble in alkali and of the alkali-extracted cell wall were determined, as well as the extraction pattern and structures of the lignin–carbohydrate complexes. It then became apparent that some lignin substructures in wheat straw were not accessible to analysis before the extraction of the alkali-soluble lignin, emphasizing the complexity of the relationship between core and non-core lignins in wheat straws.

2. Results and discussion

In this study, we investigated four aspects of the impact of very mild alkali treatments by NaOH and K_2CO_3 on wheat straw:

- the amount and structure of the lignin solubilized by alkali
- the structure of the remaining lignin in the alkali-extracted cell wall
- the content of bridging molecules like uronic and *p*-hydroxycinnamic acids
- the impact on the fractionation pattern of the lignin–carbohydrate complexes extracted by water.

2.1. Global composition of the starting wheat straw

The extractive free wheat straw (EFW) used throughout this study was composed of 21.8% (w/w) lignin and 65% (w/w) of neutral sugar (41% glucose, 24% xylose and 2.7% arabinose). Minor constituents such as uronic acid and *p*-hydroxycinnamic acids represented 0.7% and 0.9% (w/w), respectively.

2.2. Solubilization of specific lignin fractions by alkali

The incubation of wheat straw in potassium carbonate and sodium hydroxide at room temperature at pH 10 induced a loss of weight of less than 10% of the starting material in 24 h.

As expected, a significant fraction of lignin was solubilized in the reaction media. This was measured indirectly by the delignification extent of the wheat straw cell wall, relative to the reference sample (32.6 and 16.5%, for K_2CO_3 and NaOH treated samples, respectively; Table 1A).

These lignins (named hereafter OH–Lig and CO3–Lig, when extracted by NaOH and K_2CO_3, respectively) were purified. Their structures were analyzed by thioacidolysis (Table 1A) and compared to the whole lignin present in sound wheat straw (ref–Lig in ref–WS; see Table 1A and B). The CO3–Lig contained three times fewer ether-linked monomers than the OH–Lig and ref–Lig (Table 1A). They were also shown to be contaminated by some co-extracted xylose and glucose units, representing less than 5% of the total polysaccharides present in the cell wall (Table 2).

Thus, K_2CO_3 solubilized a greater amount of lignin that contained more condensed structures than NaOH at pH 10. The differences in amount and composition of lignins were not related to different co-extractions of polysaccharides, as qualitative and quantitative contamination by sugars of OH–Lig and CO3–Lig were similar (Table 2). However, the swelling of the polysaccharide matrix could have been different (see below) and the solubility parameters for lignins may also differ in hydroxydes and carbonates, allowing therefore a specific fractionation of the lignin from the cell wall.

2.3. Unmasking of lignin structures in wheat straw after alkali extraction

The wheat straw partially delignified by K_2CO_3 and NaOH (named hereafter CO3–WS and NaOH–WS, respectively) contained about 67–83% of the original lignins (see above and Table 1B). The structures of the remaining lignins in straw were analyzed both by the nitrobenzene oxidation (not shown in Tables) and by the thioacidolysis reaction methods (Table 1B).

Table 1B shows that the amount of lignin monomers released by thioacidolysis of CO3–WS and OH–WS was 1.5–1.6 higher compared to that of the water extracted reference straw (ref–WS). This phenomenon could be first related to the depletion through preferential extraction by alkali of a significant portion of condensed lignin fraction, particularly in the case of K_2CO_3 treatment (see CO3–Lig, Table 1A). However, this explanation cannot be valid for the NaOH treatment where lignin is extracted in relatively low quantities (16%) and with structural features close to the starting material (ref–WS; see Tables 1A and B).

Thus, by adding the amount of monomers released by thioacidolysis in OH–Lig to that measured in OH–WS, an excess of up to 37% in the frequency of alkyl aryl ether bonds is obtained, compared to the ref–WS (wheat straw incubated in water only, Table 1C). The same phenomenon, although less marked, is observed for the wheat straw treated by K_2CO_3 and yielding CO3–Lig

Table 1
Chemical analysis of lignins solubilized by alkali and remaining in the corresponding extracted wheat straws

Straw treatments[a]	H_2O	K_2CO_3	NaOH
A. Lignins extracted from straw (extd-Lig)		CO3–Lig	OH–Lig
Amount extracted (g/100 g original straw)	0	7.1±0.6	3.6±0.5
Relative amount extracted (% of original lignin)	0	32.6	16.5
Amount of ether linked monomers in lignins (μmol/g of lignin)[b]	0	234±8	701±20
Total amount of ether-linked monomers extracted (μmol/g of wheat straw)	0	16.6±0.6	25.2±0.7
B. Extracted wheat straw (extd-WS)	Ref–WS	CO3–WS	OH–WS
Lignin content (g/100 g of wheat straw)	21.8±0.3[c]	14.7±0.3	18.2±0.2
Amount of ether-linked monomers in lignins (μmol/g of lignin)	859±30[c]	1382±60	1269±97
Relative yields of thioacidolysis monomer released (% of ref–WS)	100	161	148
Total amount of ether-linked monomers remaining (μmol/g of straw)	187.3±6.5[c]	203.1±8.8	231.0±17.6
C. Balance of lignin structures analyzed by thioacidolysis			
Total amount of ether-linked monomers analyzed in extd-Lig plus in extd-WS (μmol/g of starting material)	187.3±6.5[c]	219.8±9.4	256.2±18.4
Relative amount of total ether-linked monomers measured (% of ref–WS)	100	117	137

[a] Refer to Section 4.
[b] Determined by thioacidolysis.
[c] Corresponds to ref–Lig.

Table 2
Type and amount of neutral sugars associated with alkali-soluble lignins[a]

	Ara	Gal	Gluc	Xyl	Man	Total
CO3–Lig (g/100 g of lignin)	0.92±0.16	0.83±0.08	7.9±0.4	5.0±0.1	0.80±0.01	15.4
OH-Lig (g/100 g of lignin)	1.56±0.07	0.99±0.02	10.0±0.1	5.3±0.3	1.10±0.06	18.9

[a] Ara, arabinose; Gal, galactose; Gluc, glucose; Xyl, xylose; Man, mannose.

and CO3–WS (Table 1C). Furthermore, the use of alkaline nitrobenzene oxidation for the characterization of the ether linked lignin fraction confirmed these qualitative results. Indeed, 1295 μmol aldehyde/g of lignin were recovered from ref–WS whereas 1806 and 1650 μmol aldehyde/g of lignin were recovered from CO3–WS and OH–WS, respectively. It can be then concluded that a fraction, not accessible to the thioacidolysis and nitrobenzene oxidation reactants, became available after the mild K_2CO_3 and NaOH treatments, leading to the increased recovery yield in thioethylated and aldehydic monomers from lignins during analysis.

2.4. Mechanisms of the unmasking of the lignin structures by alkali

2.4.1. Saponification of ester bonds

Concomitantly to lignin solubilization, the uronic acid content was modified in OH-WS and CO3–WS (Table 3). The composition in neutral sugars and in *p*-hydroxycinnamic acids varied however only for galactose (13% loss in weight) and *p*-coumaric acid (23% loss in weight) in OH–WS compared to the ref–WS (full data not shown).

The loss of uronic acids confirmed that a solubilization and/or degradation of a pectic and/or a hemicellulosic fraction occurred in each alkaline medium tested, probably through hydrolysis of ester linkages

Table 3
Content of uronic acids in alkali-extracted wheat straw

	Extracted wheat straw[a]		
	Ref–WS	CO3–WS	OH–WS
Galacturonic acid (g/100 g straw)	0.19±0.08	0.061±0.005	0.041±0.006
Glucuronic acid (g/100 g straw)	0.47±0.03	0.15±0.03	0.14±0.03

[a] Refer to Table 1 for abbreviations.

involving the lignin side chains (Imamura et al., 1994). On the other hand, only NaOH treatments had a significant impact on the final content of OH–WS in *p*-coumaric acid, known to be mainly ester-linked to lignin (Lam et al., 1990). Then, some breakdown at important reticulating points between polysaccharides and lignin occurred under our experimental conditions. Such a reaction may then release a free alcoholic group on the side chain of the lignin monomers, which in turn may enhance their reactivity during thioacidolysis, as exemplified by Grabber et al on *p*-coumaroylated syringyl units in maize (Grabber et al., 1996), or pectin–lignin models (Cathala et al., 2001).

From a quantitative point of view, the absolute amount of lignin structures unmasked in straw by NaOH is equal to 4400 μmol per 100 g of ref–WS (Table 1B); the corresponding loss of uronic acids is

equal to 2420 μmol per 100 g of ref–WS (calculated from data in Table 3); considering that model studies have shown that less than 10% of lignin may be esterified to uronic acids in α-position (Cathala et al., 2001; Imamura et al., 1994), the hydrolysis of ester bonds would only release around 240 μmol% of lignin units for thioacidolysis, that is only 1/7 of the total excess structures measured relative to ref–WS.

On the other hand, considering that the propensity of the lignin monomers to give oxidation products during nitrobenzene oxidation follows the electron density of the aromatic ring carbons, the removal of esters in α-or λ- position cannot be involved in the observed increase of the recovery yields of aldehydes in OH– or CO3–WS (Cathala et al., 2001; Wallis et al., 1995). Thus, other blocking structures than lignin esters of uronic and *p*-coumaric acid must also protect the polymer from chemical degradation during thioacidolysis and nitrobenzene oxidation, as discussed below.

Table 4
Extraction yields and composition of lignin carbohydrate complexes (LCC) isolated from wheat straw

Sample[a]	Ref–WS	CO3–WS	OH–WS
Extracted LCC	Ref–LCC	CO3–LCC	OH–LCC
LCC extraction yield (g/100 g of straw)	5.4±1.5	21.8±5.0	7.6±3.0
LCC composition (g/100 g LCC)			
Arabinose	5.4±0.3	1.2±0.2	5.1±0.5
Galactose	1.8±0.2	1.1±0.1	1.0±0.2
Glucose	7.3±0.3 (1)[b]	30.7±1.2 (14)[b]	8.9±0.2 (12)[b]
Xylose	31.0±4.9 (7)[b]	31.0±4.0 (27)[b]	41.0±4.0 (12)[b]
Galacturonic acid	0.18±0.01	0.11±0.02	0.13±0.03
Glucuronic acid	0.65±0.03	0.40±0.04	0.53±0.03
Lignin	48 (*459±55*)[c]	38 (*341±55*)[c]	53 (*652±32*)[c]

[a] Refer to Table 1 for abbreviations.
[b] In brackets:% weight of corresponding structure in ref–WS.
[c] Italicized, in brackets: release yields of alkyl–aryl ether-linked structures by nitrobenzene oxidation; in μmol/g of lignin.

2.4.2. Modifications of the chemical fractionation pattern of EFW

The pre-impregnation of wood by water and EtOH was shown recently to result in the increases of around 25% of the recovery yields of the thioacidolysis products (Önnerud and Gellerstedt, 2001). The same type of phenomenon was also reported for wheat straw treated with oxalic acid at pH 2.5, but at the level of the C–C linked dimers released by the thioacidolysis method (Lequart et al., 2000). In these two cases, however no explanation relative to the underlying mechanisms, which can be different, have been proposed so far.

Thus, another hypothesis for the unmasking of the lignin structure in wheat straw could be the modifications of the interactions between the cell wall polymers induced by the chaotropic alkali treatment (Ahvazi and Argyropoulos, 1999). As a consequence of this, the permeability and/or porosity of the cell walls would also be modified, thus enhancing the impregnation of lignin by the reactants and thus boosting the chemical depolymerization process.

To test this hypothesis, the fractionation patterns of the OH–WS and CO3–WS residues by hot and cold water after a solvent delignification was investigated. The extraction procedure used was that described for the isolation of lignin carbohydrate complexes (LCC) in wood (Imamura et al., 1994; Watanabe, 1989). The ref–WS, OH–WS and CO3–WS were ultra-milled, delignified with aqueous dioxan and extracted by water, as described in the experimental section.

The treatment of wheat straw by K_2CO_3 was shown to enhance by a factor 4 the amount of LCC extracted within CO3–WS, compared to the reference ref-WS and OH–WS (Table 4).

The global compositions of the two OH–LCC and CO3–LCC (=extracted from OH–WS and CO3–WS, respectively) were of a similar type, but different from that of ref–LCC (Table 4). The depletion in uronic acids as well as in hemicellulose minor sugars measured in OH– and CO3–LCC followed logically the depletion almost measured for OH– and CO3–WS.

The CO3–LCC was characterized by a very high content in glucose (30% in weight of the starting EFW; Table 4). This glucose represented as much as 14% (w/w) of the total glucose from EFW or ref–WS. In the same way, the amount of xylose present in CO3-LCC represented 27% of the total xylose in EFW or ref–WS. It can therefore not come from xyloglucan, which represents only 0.7% in weight of mature wheat stem (Buchala and Wilkie, 1973). Also, as no modification of the neutral sugar content in samples could be evidenced in CO3– or OH–WS, depolymerization of cellulose through peeling reactions is unlikely to occur under the conditions used. The reason for enhancement of glucose and xylose extractability in water is then not clear for the moment, and more investigations are required on this particular point.

Finally, confirming the differences between the extraction patterns according to the alkali treatment used on wheat straw, the lignin in CO3–LCC was more condensed than that of OH–LCC, as shown for CO3– and OH–Lig (Table 4; see also Table 1A).

Thus, the differences in the fractionation pattern between ref–WS, CO3–WS and OH–WS and the corresponding structural features of the LCC obtained, strongly suggested that modifications of the cell wall cohesion and interactions between the polymeric components was obtained after the alkali treatments. How this topochemical phenomenon superimposes to the unmasking of some lignin structures remains to be delineated.

3. Conclusions

This study has shown that the unmasking of hidden lignin structures in wheat straw cell wall can be obtained through mild alkali treatments. A specific delignification step, the swelling of cellulose and the hydrolysis of ester bonds by alkali were shown to be involved in this phenomenon, but their relative importance as such remain to be determined. The different fractionation patterns obtained during LCC isolation from hydroxide- and carbonate-treated wheat straw also indicated that several topochemical factors controlling (oligo)polymer interactions and swelling may also play an important role in the unmasking of lignin sub-structures to chemicals.

4. Experimental

4.1. Plant materials and substrates

The straw from wheat (*Triticum aestivum* L. cv. Scipion) was harvested at full maturity and air-dried. Internodes were separated, collected and reduced into 2-mm particles with a rotative knife mill. The powder obtained was then exhaustively extracted in a Soxhlet apparatus with toluene/EtOH (2/1 v/v) for 24 h, followed by EtOH 96% for 24 h and then water for 12 h. The resulting extractive-free wheat straw (EFW) was freeze-dried. Before treatment by alkali, EFW was homogenized by a short time (~3 min) ball milling yielding a final particle size of about 0.1 mm.

4.2. Treatments of wheat straw by alkali

Extractive free wheat straw (EFW; 50 mg) was incubated for 24 h with stirring at room temperature (20–25 °C) in alkali solution (10 ml). In order to avoid abiotic oxidations, the potassium carbonate, sodium hydroxide and hydrochloric acid used in the experiments contained less than 0.05 ppm of transition metal salt (Suprapur® quality from Merck).

Two reaction media were used: (1) potassium carbonate, 0.1 mol/l, pH = 10 (adjusted with hydrochloric acid); (2) sodium hydroxide, 0.1 mol/l, carefully titrated to pH = 10 with dilute HCl solution, and adjusted, if necessary, after addition of the wheat straw.

At the end of the reaction period, the straw was separated from the alkali medium by filtration. The reaction medium was kept for further purification of the lignins extracted from EFW, as described below; the straw was washed extensively with deionized water and freeze-dried.

The control experiments consisted of EFW incubated for 24 h at room temperature in 10 ml of deionized water. The EFW treated by water is named in the text and tables the "reference sample" or "ref–WS".

4.3. Lignin recovery from alkali soluble fraction

The reaction medium was first acidified with 6 M HCl to pH = 1 and kept at 4 °C during 1 h. The ppt. formed was recovered by centrifugation and dissolved in dioxane/water mixture (9/1v/v). Further purification was done by precipitation overnight at 4 °C in 1M acetic acid. The lignin was then recovered by centrifugation, washed twice with a minimum of cold water and freeze-dried.

4.4. Fractionation of cell wall for the extraction of lignin-carbohydrate complexes (LCC)

The procedure used to extract LCC was similar to that described by Watanabe et al. (Imamura et al., 1994; Watanabe, 1989). Wheat straw (20 g) was first ultra-milled with 1 kg of porcelain balls (5 mm diameter) in 2 l porcelain jars for 72 h (Vibratom, Siebtechnik). The ultra-milled straw was delignified for 48 h in dioxane/water mixture (8/2 v/v). The solubilized lignin was recovered by centrifugation. The solid residual pellet was first extracted with cold water (20 °C) for 24 h. The solubilized products (LCC fraction 1) were recovered by centrifugation. The remaining pellet was extracted again, but with hot water (80 °C) for 5 h. The solubilized products (LCC fraction 2) were recovered by centrifugation. LCC fraction 1 and LCC fraction 2 were combined and concentrated by evaporation under reduced pressure. The LCC were then precipitated by an excess of absolute EtOH recovered by centrifugation, washed with EtOH and freeze-dried.

4.5. Lignin content in wheat straw

The lignin content was estimated gravimetrically according to a procedure similar to that described by Effland (1977) and modified by Monties (1984).

4.6. Lignin characterization

Two methods were used to determine the amount of monomers linked by alkyl–aryl ether bonds in lignins: thioacidolysis and oxidation by nitrobenzene.

4.6.1. Thioacidolysis

Lignin was depolymerized by thioacidolysis according to the published procedure (Lapierre et al., 1988; Rolando et al., 1992). The analysis was performed with a ratio of reactant to EFW of 1.5 ml/mg. The main monomers released were separated by capillary gas chromatography as trimethylsilyl derivatives on a DB-5 column (Hewlett-Packard) and detected with FID, as described previously (Lequart et al., 1998).

4.6.2. Oxidation by alkaline nitrobenzene

The oxidation of lignin by nitrobenzene in alkaline conditions was performed as described by Billa et al. (1996). The reaction time and temperature used were 3 h and 170 °C, respectively. The aldehydes released were separated by HPLC on a Lichrospher RP-18 column (Merck) with a CH_3CN/water gradient as described previously (Billa et al., 1996). The products eluted were detected by UV absorption at 310 nm.

4.7. Polysaccharide characterization

4.7.1. Neutral sugars

Hydrolysis of wheat straw polysaccharides was performed by H_2SO_4 according to Blakeney et al. (1983). After hydrolysis, the reaction medium was diluted with water and the monosaccharides released were separated by high performance anion-exchange chromatography (HPAEC) on a PA-1 Column (Dionex) in NaOH as described previously (Lequart et al., 1998). The products were detected by pulsed amperometry (PAD), using a gold electrode coupled to an ED-40 device (Dionex).

4.7.2. Uronic acids content

Uronic acids were released from the cell wall after the combination of acid methanolysis and hydrolysis, according to de Ruiter (1992). The reactant for methanolysis (2 M HCl in anhydrous MeOH) was prepared with an Alltech® Kit (ref 18503) according to the described procedure in the Aldrich technical sheet. The HCl–MeOH reactant was then added to the cell wall residue and heated at 80 °C for 16 h. The samples were dried under N_2 gas flow and subjected to 4 M trifluoro acetic acid (TFA) (Cathala et al., 2001). TFA was eliminated by evaporation under vacuum. The solid residue obtained was dissolved in water, filtered and injected on a carboPac PA-1 anion exchange column (Dionex) for HPAEC analysis. Acidic sugars were separated with a gradient of NaOH and sodium acetate as described before (Lequart et al., 1998) The detection of uronic acids was performed by PAD, as for neutral monosaccharides—see above.

Acknowledgements

This work was supported by a grant from ''Europol'Agro'' #98A02. The authors also wish to thank C. Young, from the INRA translation service (Jouy en Josas), for revising the English.

References

Ahvazi, B.C., Argyropoulos, D.S., 1999. Proton spin–lattice relaxation time measurements of solid wood and its constituents as a function of pH: Part II. Solid State Nucl. Magn. Reson. 15, 49–57.

Billa, E., Tollier, M.T., Monties, B., 1996. Characterization of the monomeric composition of in situ wheat straw lignins by alkaline nitrobenzene oxidation; effect of temperature and reaction time. J. Sci. Food Agric. 72, 250–256.

Blakeney, A.B., Harris, P.J., Henry, R.J., Stone, B.A., 1983. A simple and rapid preparation of alditol acetates for monosaccharide analysis. Carbohyd. Res. 113, 291–299.

Bledzki, A.K., Gassan, J., 1999. Composites reinforced with cellulose based fibres [Review]. Prog. Polym. Sci. 24, 221–274.

Buchala, A.J., Wilkie, K.C.B., 1973. Total hemicelluloses from wheat at different stages of growth. Phytochemistry 12, 499–503.

Cathala, B., Chabbert, B., Joly, C., Dole, P., Monties, B., 2001. Synthesis, characterization and water sorption properties of pectin-dehydrogenation polymer (lignin model compound) complex. Phytochemistry 56, 195–202.

de Ruiter, G.A., Schols, H.A., Voragen, A.G.J., Rombouts, F.M., 1992. Carbohydrate analysis of water soluble uronic acid-containing polysaccharides with high performance anion exchange chromatography using methanolysis combined with TFA hydrolysis is superior to four other methods. Anal. Biochem. 207, 176–185.

Durot, N., Kurek, B., 2000. Wheat straw degradation by copper acetate in carbonate medium: a model for abiotic reactions in calcareous soils. Proc. Int. Meet. Int. Humic Subst. Soc. 1, 927–930.

Effland, M.J., 1977. Modified procedure to determine acid-insoluble lignin in wood and pulp. TAPPI J. 60, 143–144.

Fiala, W., Nardi, F., 1985. The NACO process. Paper Tech. Ind. 3, 75–79.

Grabber, J.H., Quideau, S., Ralph, J., 1996. *p*-Coumaroylated syringyl units in maize lignin: implications for β-ether cleavage by thioacidolysis. Phytochemistry 43, 1189–1194.

Grabber, J.H., Ralph, J., Hatfield, R.D., 1998. Ferulate cross-links limit the enzymatic degradation of synthetically lignified primary walls of maize. J. Agric. Food Chem. 46, 2609–2614.

Hultom, T., Lonnberg, B., Laxen, T., Elsakhawy, M., 1997. Alkaline pulping of cereal straw. Cellulose Chemistry and Technology 31, 65–75.

Iiyama, K., Lam, T.B.T., Stone, B.A., 1994. Covalent cross links in the cell wall. Plant Physiol. 104, 315–320.

Imamura, T., Watanabe, T., Kuwahara, M., Koshijima, T., 1994. Ester linkages between lignin and glucuronic acid in lignin–carbohydrate complexes from *Fagus crenata*. Phytochemistry 37, 1165–1173.

Jackson, M.G., 1977. The alkali treatment of straws. Anim. Feed Sci. Technol. 2, 105–130.

Jacquet, G., Pollet, B., Lapierre, C., Mhamdi, F., Rolando, C., 1995. New ether-linked ferulic acid-coniferyl alcohol dimers identified in grass straws. J. Agric. Food Chem. 43, 2746–2751.

Lam, T.B.T., IIyama, K., Stone, B.A., 1990. Distribution of free combined phenolic acids in wheat internodes. Phytochemistry 29, 429–433.

Lam, T.B.T., Kadoya, K., Iiyama, K., 2001. Bonding of hydroxycinnamic acids to lignin: ferulic and *p*-coumaric acids are predominantly linked at the benzyl position of lignin, not the β-position, in grass cell walls. Phytochemistry 57, 987. –992.

Lapierre, C., Jouin, D., Monties, B., 1989. On the origin of the alkali solubility of gramineae lignins. Phytochemistry 28, 1401–1403.

Lapierre, C., Monties, B., Rolando, C., 1988. Thioacidolysis of diazomethane-methylated pine compression wood and wheat straw in situ lignins. Holzforschung 42, 409–411.

Lee, J., 1997. Biological conversion of lignocellulosic biomass to ethanol. J. Biotechnol. 56, 1–24.

Lequart, C., Kurek, B., Debeire, P., Monties, B., 1998. MnO_2 and oxalate: an abiotic route for the oxidation of aromatic components in wheat straw. J. Agric. Food Chem. 46, 3868–3874.

Lequart, C., Ruel, K., Lapierre, C., Pollet, B., Kurek, B., 2000. Abiotic and enzymatic degradation of wheat straw cell wall: a biochemical and ultrastructural investigation. J. Biotechnol. 80, 249–259.

Monties, B., 1984. Dosage de la lignine insoluble en milieu acide: influence du prétraitement par hydrolyse acide sur la lignine Klason. Agronomie 4, 387–392.

Önnerud, H., Gellerstedt, G., 2001. Inhomogenities in the chemical structure of spruce wood. Proc. 11th Int. Symp. Wood Pulp Chem. 1, 59–62.

Rolando, C., Monties, B., Lapierre, C., 1992. Thioacidolysis. In: Lin, S.Y., Dence, C.W. (Eds.), Methods in Lignin Chemistry. Springer-Verlag, Berlin, Heidelberg, pp. 334–349.

Scalbert, A., Monties, B., Guittet, E., Lallemand, J.Y., 1986a. Comparison of wheat straw lignin preparations. I. Chemical and spectroscopic characterization. Holzforschung 40, 119–127.

Scalbert, A., Monties, B., Rolando, C., Sierra-Escudero, A., 1986b. Formation of ether linkage between phenolic acids and gramineae lignin: a possible mechanism involving quinone methides. Holzforschung 40, 191–195.

Sun, R.C., Lawther, J.M., Banks, W.B., 1997. Fractional isolation and physico-chemical characterization of alkali-soluble lignins from wheat straw. Holzforschung 51, 244–250.

Wallis, A., Chan, F., Ngyuen, K., 1995. Contribution of lignin substructures to nitrobenzene oxidation products. Proc. 8th Int. Symp. Wood Pulp Chem. 1, 549–556.

Watanabe, T., 1989. Structural studies on the covalent bonds between lignin and carbohydrate complexes by selective oxidation of the lignin with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone. Wood Research 76, 59–123.

Zilliox, C., Debeire, P., 1998. Hydrolysis of wheat straw by a thermostable endoxylanase: adsorption and kinetic studies. Enzyme Microb. Technol. 22, 58–63.