Journal of the Serbian Chemical Society
JSCS@tmf.bg.ac.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 74 (8–9) 885–892 (2009) UDC 633.31:547.992+54–74:661.185.6–32
JSCS–3884 *Original scientific paper*

Changes in the infrared attenuated total reflectance (ATR) spectra of lignins from alfalfa stem with growth and development

JORDAN P. MARKOVIĆ[1]*, JASMINA B. RADOVIĆ[1], RATIBOR T. ŠTRBANOVIĆ[1], DANICA S. BAJIĆ[2#] and MIROSLAV M. VRVIĆ[2,3#]

[1]*Institute for Forage Crops, 37000 Kruševac, Trg Kosturnica 50, 37000,* [2]*Department of Chemistry, Institute of Chemistry, Technology and Metallurgy,11001 Belgrade, Njegoševa 12, P.O. Box 473, Belgrade and* [3]*Faculty of Chemistry, University of Belgrade, 11158 Belgrade, Studentski trg 16, P.O. Box 51, Belgrade, Serbia*

(Received 24 February, revised 30 March 2009)

Abstract: Lignin is a poorly characterized polymer and its exact properties vary depending on both the species of the plant and its location within the plant. Three classes of lignins taken from alfalfa stem were examined. The investigation was concentrated on the determination of chemical changes in the lignins during growth and development by the attenuated total reflectance (ATR) infrared (IR) spectrometric technique. The spectrum of permanganate lignin was comparable to that of acid detergent lignin. The main differences were in the different relative absorbance of the peaks. The predominant component of acid detergent lignin and permanganate lignin was guaiacyl-type lignin. The predominant component of Klason lignin was syringyl-type lignin. A comparison between the signals from lignin in different development stages revealed the appearance of new peaks, which are indications of new bonds and changes in the structure of the lignins.

Keywords: alfalfa; acid detergent; permanganate and Klason lignin; ATR infrared spectra.

INTRODUCTION

Lignin is a complex polymer of high carbon content, which is distinct from carbohydrates, that impregnates the plant cell wall. Thus, lignin confers compressive strength and bending stiffness necessary for mechanical support; it also provides a hydrophobic surface, essential for longitudinal water transport, and provides a barrier against pathogens. Lignins are composed of three main units, named *p*-hydroxyphenyl, guaiacyl and syringyl units. These components originate from

* Corresponding author. E-mail: jordan.markovic@ikbks.com
# Serbian Chemical Society member.
doi: 10.2298/JSC0909885M

the polymerization of the three monolignols, *p*-coumaryl, coniferyl and sinapyl alcohols (Fig. 1).[1] The proportions of these three units in the cell wall vary according to plant species and tissue type.[2] During the early stages, coniferyl alcohol with small amounts of *p*-coumaryl alcohol is copolymerized into the primary wall to form mixed guaiacyl and *p*-hydroxyphenyl lignins (Fig. 2).[1,2] Later, during secondary wall development, coniferyl alcohol and increasing amounts of sinapyl alcohol are copolymerized to form mixed guaiacyl and syringyl lignins.[3]

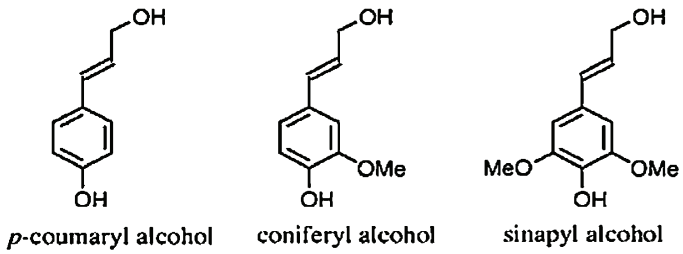

Fig. 1. The monolignols.

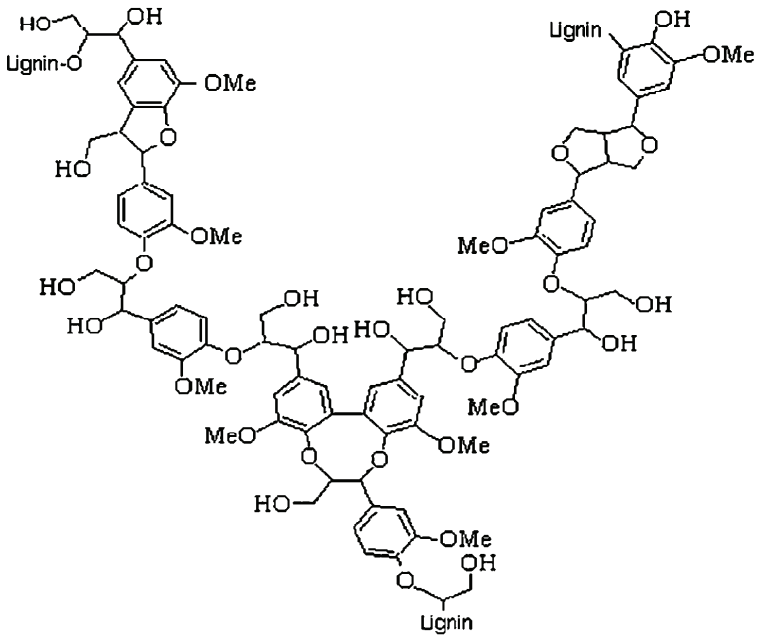

Fig. 2. Fragment of the hypothetic structural formula of lignin.

Many methods of lignin analysis have been developed because of the negative association of lignin with digestibility. In this study, the composition and structure of the lignin isolated by different methods from alfalfa stem at different stage of growth and development were investigated by the ATR-IR spectroscopic method.

EXPERIMENTAL

Material

A new cultivar K-22 of alfalfa (*Medicago sativa* L.) was created in the Institute for Forage Crops, Kruševac, Serbia. Three maturities of alfalfa after the first cut were chosen for this study. The first sample of forage crop was harvested on June 17th, 2008. The alfalfa was 0.38 m tall and at the mid-bud stage. The second was harvested on June 24th, 2008, when the plants were 0.68 m tall and at about 55 % bloom. The third harvest was harvested on July 1st, 2008, when the alfalfa was 0.91 m tall and in full bloom. All values are averages.

Methods

The acid detergent lignin (ADL) was determined as the lignin insoluble in 72 % (w/w) sulfuric acid, applying the method of Van Soest and Robertson.[4] The permanganate lignin (PerL) was determined as the residue remaining after oxidation with potassium permanganate by the method of Van Soest and Wine.[5] The Klason lignin (KL) was determined as the residue remaining after total hydrolysis of the cell wall polysaccharides by the method of Theander and Westerlund.[6]

The ATR spectra (1700–500 cm^{-1}) were obtained using a Nicolet Model 6700 FT-IR spectrometer. The crystal-diamond spectra were obtained with 4 cm^{-1} resolution and 32 scans for each sample spectrum were performed. The spectral values are in cm^{-1}.

RESULTS AND DISCUSSION

ATR-IR spectrometry was used as a structural, non-destructive and simple tool for the qualitative analysis of the chemical composition of lignins isolated by different methods and in different stages of herbal development.

The spectrum of ADL (the first stage of development) is presented in Fig. 3a. Peaks were observed at (cm^{-1}): 1727.7 (C=O unconjugated groups in lignin and carboxylic acid ester[7]); 1602.8 and 1490.7 (aromatic skeletal vibrations[8,9]); 1455.4 (benzene ring vibration in lignin, CH_3 and CH_2 substituted[8]); 1166.7 (C–O–C bonds of allyl ether[10]); 1031.3 (primary alcoholic and aliphatic ether groups[9]) and 960.0 (aromatic C–H out-of-plane deformations[11]). At the second stage of development (Fig. 3b), the signal at 1031.5 arises from the C–O bond of primary alcohols[9]). At the third stage of alfalfa development (Fig. 3c), the assigned peaks are: 1568.0 (vibrations of the aromatic rings present in lignin[8]); 1506.6 (C=C in plane aromatic vibrations from lignin[10]); 1420.5 (C–H deformation in lignin and carbohydrates[11]); 1316.9 (C–H vibrations in cellulose and the C_1–O vibration in syringyl derivatives[12]); 1244.4 (syringyl ring and C–O stretching in lignin and xylan[13]); 1027.3 (C–O–C vibration in cellulose and hemicelluloses[10]) and 896.4 (C–H deformation).

The spectrum of PerL (Fig. 4) is comparable to that of ADL. Peaks were observed at (cm^{-1}): 1731.7 (carbonyl stretching – unconjugated ketones and carboxyl groups[14]); 1316.3 (syringyl ring breathing with C–O stretching[8]); 1244.8 (guaiacyl ring breathing with C–O stretching[8]); an intensive signal at: 1159.1 (may represent aromatic C–H in plane deformation of the guaiacyl-type) and 897.3 (aromatic C–H out of plane deformation[8]). This spectrum of PerL could be

explained by alkylation, which protects the phenolic aromatic rings from degradation, while all the other aromatic rings are degraded in the permanganate oxidation. Some biphenyls and biphenyl ethers also survived the oxidative degradation.[10] On the other hand, the signals at (cm^{-1}): 1427.5 at the second stage of development and 1592.8 and at 1417.0 at the third stage of development (aromatic skeletal vibrations combined with C–H in plane deformation[11]) and 1245.7 (syringyl ring and C–O stretching in lignin and xylan[12]) disappeared.

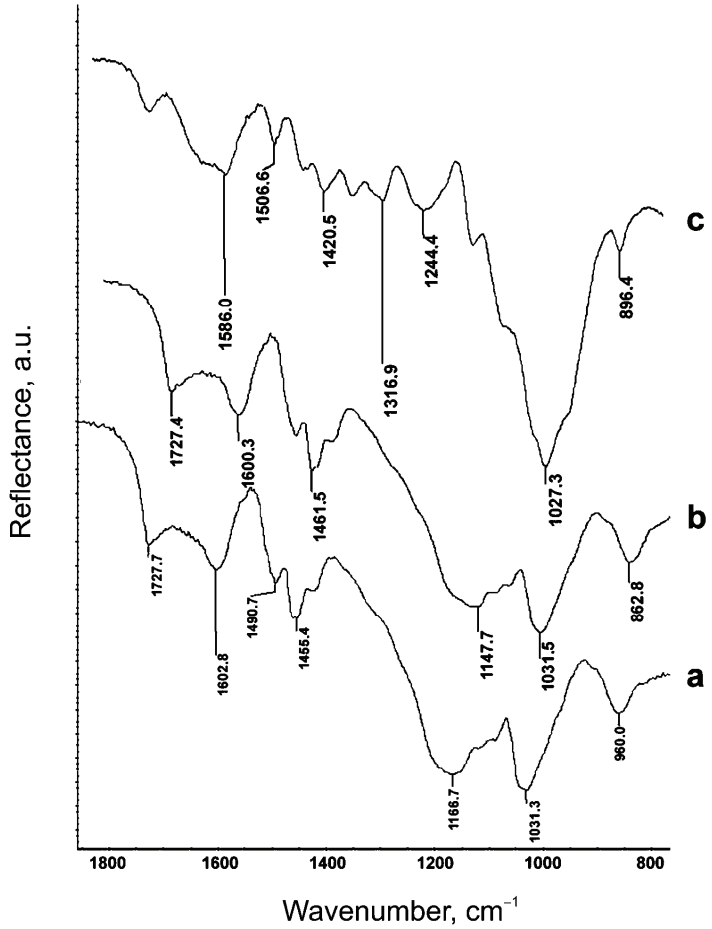

Fig. 3. IR spectrum of ADL from alfalfa stem by ATR spectrometry, a) the first stage, b) the second stage and c) the third stage of development.

At the first development stage, in the spectrum of KL (Fig. 5a), intensive signals at (cm^{-1}): 1090.1 and 1028.6 (syringyl units or condensed guaiacyl units[9]) and signals 1455.2, 1495.9 and 1613.5 (aromatic skeletal vibration as well as CH deformations, guaiacyl–syringyl-type[10]) were observed. At the second

development stage (Fig. 5b), signal at (cm^{-1}): 1651.0 (carbonyl groups of oxycelluloses, found in degraded materials[11]); 1423.2 (C–H vibrations and aromatic ring vibrations[7,15]) and 1210.1 (vibrations of guaiacyl rings and stretching vibrations of C–O bonds[9]) were registered. The spectrum of KL in the third development stage of alfalfa (Fig. 5c) showed syringyl ring and C–O stretching at 1232.8 in lignin and xylan.[10,15]

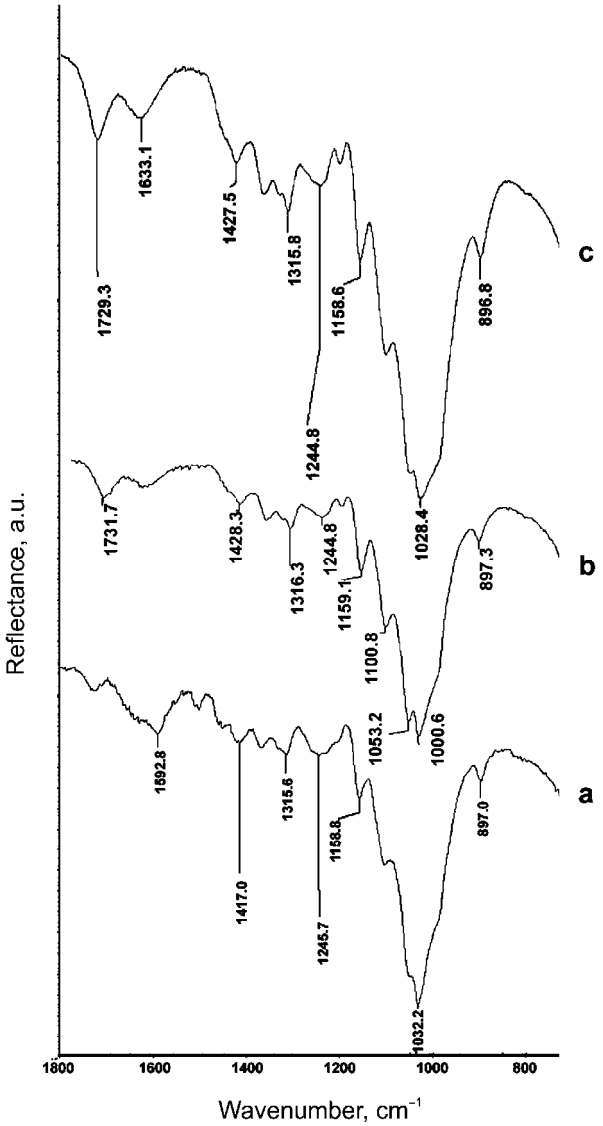

Fig. 4. IR spectrum of PerL from alfalfa stem by ATR spectrometry, a) the first stage, b) the second stage and c) the third stage of development.

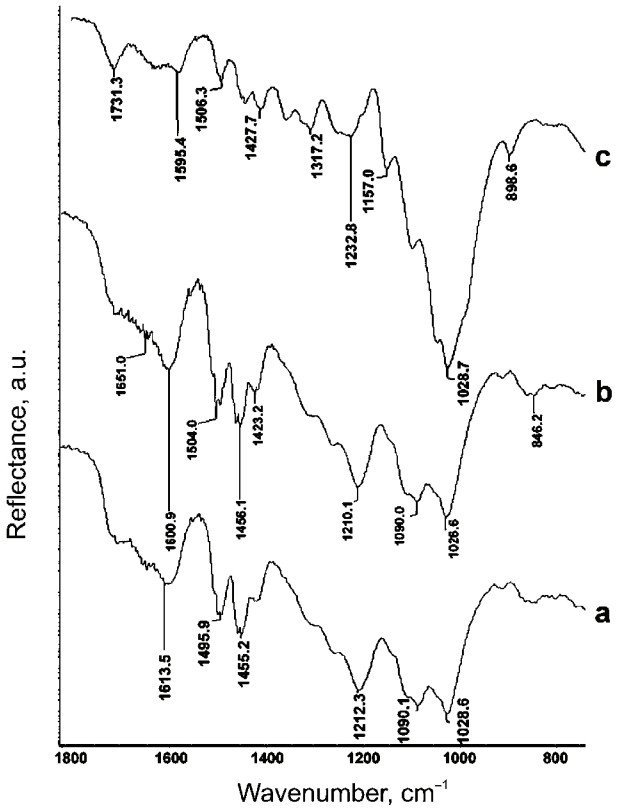

Fig. 5. IR spectrum of KL from alfalfa stem by ATR spectrometry, a) the first stage, b) the second stage and c) the third stage of development.

In other detailed structural study,[16–18] contemporary comparison of the composition of lignin from alfalfa stem with growth and development were made. The spectra of ADL and PerL were similar. The main difference was that in the PerL spectrum, guaiacyl and syringyl ring breathing signals were registered, whereas in the ADL spectrum, only a guaiacyl ring breathing signal was observed. The spectra of KL showed syringyl and condensed guaiacyl units from the first to the third stage of alfalfa stem development. The spectral data of lignins from alfalfa stem indicate changes of peaks in fingerprint region at different stages of growth. Comparison between the peaks from different development stages revealed the appearance of new signals after 7-day intervals, which are indications of new bonds. The increases of the new bands indicate that new bonds were formed owing to the breakdown of lignin and hemicellulose polymers. During this period, many chemical changes occurred in these constituents, primarily in the structure and amount of lignins.

Acknowledgments. This work was supported in part by the Ministry of Science and Technological Development of the Republic of Serbia (Grants: 142018B and 20131B).

И З В О Д

ПРОМЕНЕ У ИНФРАЦРВЕНИМ ATR СПЕКТРИМА ЛИГНИНА ИЗ СТАБЛА ЛУЦЕРКЕ У ЗАВИСНОСТИ ОД ФАЗЕ РАЗВИЋА

ЈОРДАН П. МАРКОВИЋ[1], ЈАСМИНА Б. РАДОВИЋ[1], РАТИБОР Т. ШТРБАНОВИЋ[1], ДАНИЦА С. БАЈИЋ[2] и МИРОСЛАВ М. ВРВИЋ[2,3]

[1]Институт за крмно биље, Трг Косturница 50, 37000 Крушевац, [2]Институт за хемију, технологију и металургију, Центар за хемију, Његошева 12, п. пр. 473, 11001 Београд и [3]Хемијски факултет, Универзитет у Београду, Студентски трг 16, п.пр. 51, 11158 Београд

Лигнини су врло мало проучавани, и њихова структура се разликује у зависности од врсте биљака и његове заступљености у биљци. Испитивања су обављена на три типа лигнина изолованих из стабла нове крушевачке сорте луцерке, К-22. Ово истраживање је обављено да би се боље упознале хемијске промене различитих типова лигнина, са напредовањем фазе развића, применом ATR спектрометрије. Спектар перманганатног лигнина је сличан спектру киселог детерџентног лигнина. Основна разлика је у интензитету сигнала. Главне компоненте киселог детерџентног лигнина и перманганатног лигнина јесу јединице „гвајацил“ типа, док су код Класон лигнина основне структурне јединице „сирингил“ типа. Поређењем спектара у различитим фазама развића бележи се појава нових сигнала, што указује на нове хемијске везе – промене у структури лигнина.

(Примљено 24. фебруара, ревидирано 30. марта 2009)

REFERENCES

1. O. Derkacheva, D. Sukhov, *Macromol. Symp.* **265** (2008) 61
2. W. Boerjan, J. Ralph, M. Boucher, *Annu. Rev. Plant Biol*. **54** (2003) 519R
3. R. Sibout, A. Eudes, G. Moulle, B. Pollet, C. Lapierre, L. Jounanin, A. Seguin, *Plant Cell* **17** (2005) 2059
4. P. J. Van Soest, J. B. Robertson, in *Standardization of analytical methodology in feeds*, W. J. Pigden, C. C. Balch, M. Graham, Eds., International Research Development Center, Ottawa, 1980, p. 49
5. P. J. Van Soest, R. H. Wine, *J. AOAC* **50** (1967) 50
6. O. Theander, E. A. Westerlund, *J. Agric. Food Chem.* **34** (1986) 330
7. H. L. Hergert, in *Lignins, occurrence, formation, structure and reaction*, K. V. Sarkanen, C. H. Ludwig, Eds., Wiley-Interscience, New York, 1971, p. 267
8. G. Brunow, in *Lignin, humic substances and coal*, M. Hofrichter, A. Steinbüchel, Eds., Wiley-VCH, Weinheim, 2001, p. 89
9. I. Bykov, *M.Sc. Thesis*, University of Technology, Luleå, 2008, p. 9
10. K. K. Pandey, *J. Appl. Polym. Sci.* **71** (1999) 1969
11. M. Behbood, *Int. Biodeterior Biodegrad*. **55** (2005) 247
12. J. Rodrigues, O. Faix, H. Pereira, *Holzforschung* **52** (1998) 46
13. P. D. Evans, A. J. Micehll, K. J. Schmalzl, *Wood Sci. Technol*. **26** (1992) 151
14. P. Garside, P. Wyeth, *Polym. Prepr*. **41** (2000) 1792
15. S. Raiskila, M. Pulkkinen, T. Laakso, K. Fagerstedt, M. Löija, R. Mahlberg, L. Paajanen, A. C. Ritschkoff, P. Saranpää, *Silva Fennica* **41** (2007) 351

16. V. Nikolić, Lj. Nikolić, M. Stanković, A. Kapor, M. Popsavin, D. Cvetković, *J. Serb. Chem. Soc.* **72** (2007) 737
17. D. Godjevac, B. Pejin, G. Zdunić, K. Šavikin, D. Stešević, V. Vajs, S. Milosavljević, *J. Serb. Chem. Soc.* **73** (2008) 525
18. B. M. Mandić, D. N. Godjevac, V. P. Beškoski, M. R. Simić, S. S. Trifunović, V. V. Tešević, V. V. Vajs, S. M. Milosavljević, *J. Serb. Chem. Soc.* **74** (2009) 27.