WOOD RESEARCH
53 (1): 2008
55-68

A SUPPLEMENT TO THE RESEARCH OF NATIVE LIGNIN OF BEECH SAPWOOD (*FAGUS SYLVATICA* L.)

Alan Antonović, Vladimir Jambreković, Stjepan Pervan, Josip Ištvanić,
Krešimir Greger, Andrej Bublić
Faculty of Forestry, Wood Technology Department, University of Zagreb,
Zagreb, Republic of Croatia

ABSTRACT

Native or Brauns lignin (NL) isolated with ethanol extraction from beech sapwood (*Fagus sylvatica* L.) was studied with the purpose of researching its structure and chemical properties. Modified Brauns method was applied for native lignin isolation, which is different from original in disregarding purification processes with successive and repetitive precipitation of the dioxane solution into water and into ether. Furthermore, native lignin (NL) was studied using ultraviolet (UV) and Fourier transform infrared (FTIR) spectroscopic techniques of analysis. Availability for structural studies of GS lignins with spectral characterisation of modified NL isolation method was proved. Differences in spectroscopic behaviour between in this work obtained native lignins (NL) and milled wood lignins (MWL) from previous studies were determined using both spectroscopic technics.

KEY WORDS: beech (*Fagus sylvatica* L.), sapwood, native lignin (NL), UV spectrophotometry, FTIR spectrophotometry

INTRODUCTION

Lignin (20-35% of total wood) represents amorphous three-dimensional network polymers of phenylpropane units with many different chemical linkages between monomers leading to complex structure that can only be defined by the frequency of occurrence of the various linkages (Goldstein, 1991). Due to the properties of lignin resulting from its molecular structure and its localisation within the cell wall, the isolation of lignin in an unchanged form and its exact determination have not yet proved possible. All methods of isolation have a disadvantage of either fundamentally changing the native structure of lignin or releasing only parts of it, relatively unchanged (Fengel and Wegener 1989). Only three isolation methods are known which give partial chemical unchanged lignin. The first method is wood extraction with ethanol, followed by precipitation with ether (Brauns or native lignin), the second is using toluene after intensively ball milling (Björkmans or MWL – milled wood lignin), and third is from ground wood, using enzymatic hydrolysis (CEL – cellulolytic-enzyme lignin) (Sakakibara and Sano 2001). One experimental difficulty in studying the macromolecular properties of lignin is the fact that lignin has a very low solubility in most solvents. Instead of native

lignin, much of the research has been concentrated on its soluble reaction products or derivatives, such as lignosulfonates and kraft lignins obtainable after the pulping processes (Sjöström 1993).

Many researchers attempted to isolate lignin from its native structure with various solvents, but Brauns was the first who isolate a purified fraction and show that its properties were essentially identical to those of the major portion of the lignin in the wood (Brauns 1952, Melcer et al. 1976). Although many modifications of the original Brauns method have been used till today, the essential features of the method have usually been retained. The wood is pre-extracted with ether and cold water, and then it is exhaustively extracted with ethanol at room temperature. The ethanol extract is concentrated and the dissolved lignin is purified by successive and repeated precipitations of the dioxane solution into ether and into water. In all other methods of isolation lignin preparations were obtained, which no longer represent lignin in its native form but which have undergone more or less drastic changes such as condensation or polymerisation (Brauns 1952a). Native lignin, when compared with MWL, is obtained in very low yield, it has a low molecular weight and a high phenol content, and it is sometimes contaminated with extractives (Lai and Sarkanen 1971).

Despite many unresolved problems in lignin chemistry, basic elements and bond types are today well known. Improved spectroscopic techniques in the last decades resulted in much more reliable and improved quality data taking into consideration both the frequency and different types of bonds between basic phenylpropane units of lignin molecule, as well as the nature and location of different functional groups joined to these units. However, for the preparation of useful samples and for the selective isolation of native (natural) lignins from different morphologic parts of xylem much better methods are still required. The aim of this work is giving a supplement to the research of structure and chemistry of native lignin (NL) using ultraviolet (UV) and Fourier transform infrared (FTIR) spectroscopic techniques. NL of beech sapwood was isolated by alcoholic extraction using 96% ethanol according to the modified Brauns method. Therefore, also one of aims was to prove the usability of modified Brauns method for lignin structural studies. Based on this, spectral comparison was made between milled wood lignins (MWL) from previous studies and native lignins (NL) of beechwood obtained in this research.

Ultraviolet (UV) spectrophotometry of lignin

UV spectrophotometry is the most convenient and useful method for the quantitative and qualitative analysis of lignin in solution. Information relating to the different interpretations of UV light absorption spectra of lignin and its derivatives is described extensively in numerous scientific studies (Brauns 1952, Brauns 1952a, Goldschmid 1971, Fengel and Wegener 1989). The UV portion of the electromagnetic spectrum suitable for lignin spectroscopy extends from 200-380 nm (Lin 1992). The lignin spectrum of typical softwood has two maximums at 205 and 280 nm, shoulders at 230 and 330-340 nm, and a minimum at 260 nm. In general, softwood lignin shows a maximum at 280-285 nm, and hardwood lignin at 274-276 nm (Sakakibara and Sano 2001). This fact contributes to the higher symmetry of the phenylpropane units in hardwood lignins, caused by the higher amounts of syringyl units. Additionally the absorptiveness of hardwood lignins is generally somewhat lower than those of softwood lignins, with decreasing values at increasing OCH_3/C_9-ratios (Goldschmid 1971, Fengel et al. 1981).

Infrared (IR) spectrophotometry of lignin

Although considerable work has been carried out on the vibration analysis of wood and its constituents by infrared (IR) spectroscopy over the last two decades, knowledge about the molecular structure of wood constituents polymers (lignin, cellulose, wood polyoses) and their interaction in the polymer matrix is still not complete. Fourier transform infrared (FTIR)

spectroscopy is a very useful tool for obtaining rapid information about the structure of wood components and chemical changes taking place in wood due to various treatments (Pandey, 1999). While the infrared spectrum is a characteristic property of compounds with exactly known structures, there are several uncertainties with the interpretation of lignin IR spectra. This is mainly caused by two factors. Firstly, there are large variations in lignin structures and compositions, depending on the origin of the sample and the special isolation procedures; secondly variations are caused by different techniques of measuring lignins in suitable solvents, in the form of films, or in the most frequently applied form of potassium bromide (KBr) pellets. The assignment of a band cannot be deduced from a single spectrum, but must be proved by measuring derivatives of lignin model compounds and lignin samples, thus shifting the band position of structural elements or eliminating their bands. Suitable derivation methods are methylation, acetylation, reduction, sulphonation or conversion to salts, enabling the determination of functional groups, e.g. hydroxyl and carbonyl groups (Hergert 1971). The FTIR spectrum of a lignin sample provides an overall view of its structure (Hergert 1971, Faix 1991). The use of IR spectroscopy for the determination of functional groups in lignin has been described in literature (Hergert 1971, Faix 1986, Schultz and Glasser 1986). Qualitatively, the occurrence of carbonyl (C=O) groups, aromatic structures, C_{aliph} – O and C_{arom} – O bonds and also the substitution pattern in the benzene ring are detected using this technique (Hortling et al. 1997).

MATERIALS AND METHODS

Preparation of beech sapwood meal: TAPPI test method T257 cm-02 (Sampling and preparing wood for analysis) was used as sampling procedure. As the method requires, samples were taken promptly after tree cutting, and the criterion for taking samples was cutting off a ring on first log from trunk, approximately at 2 m height. Thickness of hatched rings was between 5-15 cm. All samples have been fresh and without any wood defects. Since the isolation analysis was carried out on beech sapwood, before grinding each ring was submitted to mechanical operation where sapwood was separated from other anatomical parts of wood (heartwood and bark).

Furthermore, TAPPI test method T264 cm-97 (Preparation of wood for chemical analysis) was used for grinding and screening procedure. Sapwood samples were grinded in cutting mill Fritsch – Pulverisette 19 (input power 2 kW, rotor speed 2800 rpm, particle granulations 0.09-6 mm) on specific wood particle size. Laboratory electromagnetic sieve shaker Cisa type RP.08 (shaking frequency 6 kHz – medium power, vibration amplitude 1.5 mm, shaking time τ = 30 min) was used for sample screening. Samples were screened through standard sieve (ISO – 3310.1) with 0.25 mm mash dimension (100–150 mash/cm^2).

Preparation of native lignin (NL) samples: Modified isolation method of native lignin was performed according to original Brauns' method (Brauns 1952, Melcer et al. 1976), but without performing purification procedures by successive and repeated precipitations of the dioxane solution into water and into ether, making this the starting material for its characterisation. Performance of modified isolation method for native lignin and its characterisation are presented in Fig. 1, and consists of a series standard methods related to isolation.

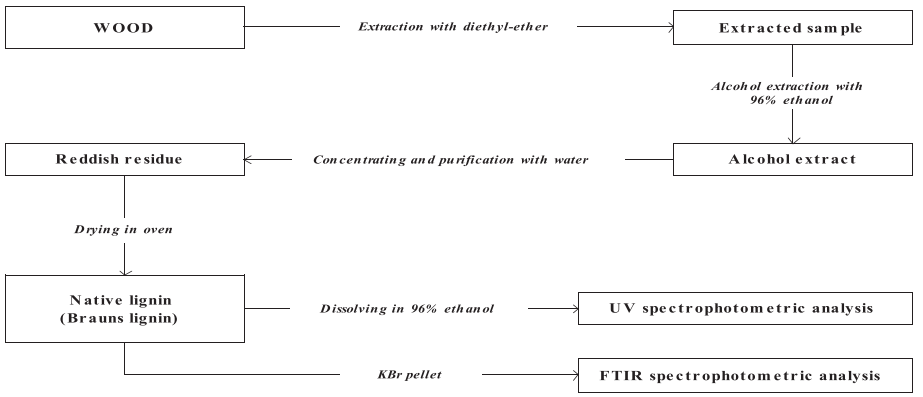

Fig 1: A schematic view of native lignin modified isolation method from beech sapwood

This modified isolation procedure of native lignin is presented for beech as hardwood (guaiacyl-syringyl GS lignins). Each ring represents one beech tree. For the performing of chemical isolation analysis of native lignin, after grinding and screening, were took three samples from each ring/tree (altogether 171 samples). Native lignin (NL) isolation procedure is comprises of:

- water content determination – by using thermogravimetric method in electronic moisture analyser Sartorius MA150 under temperature of 105±2°C,
- extraction using diethyl-ether (DEE extract) – 15-25 g of prepared beech sapwood sample was pre-extracted using diethyl-ether (p.a .) in Soxhlet apparatus (500 ml flask, 250 ml extractor) for 8 hours. Some 250–300 mL of diethyl-ether was used per sample, depending on sample amount. Flask content after completed extraction of each sample was placed in an oven at 80°C temperature to dry until constant weight was obtained, whereas dried residue represented accessory materials (DEE extract). Percentage of accessory materials (DEE extract) was calculated from obtained data:

$$\text{DEE extract} = \frac{b - a}{c} . 100 \qquad [\%]$$

where is: a – weight of empty flask (g), b – weight of flask with absolute dry accessory materials (g) and c – weight of absolute dry sample (g). After extraction was completed, the pre-extracted sample was left to dry at room temperature for 48 hours, with the aim of the remaining diethyl-ether evaporating from sample,

- alcohol extraction with ethanol – after desiccation, all obtained pre-extracted sample was placed in a previously weighted flask, which was weighed again together with the sample. The flask with sample then had 200 mL of 96% ethanol (p.a) added and left to remain for 48 hours with occasionally stirring the flask content. After 48 hours the dissolved lignin in ethanol was decanted into a dry and clean bottle, in the way that the bottle was filled only with liquid phase, while the crude sample remaining in the flask. After decanting, the sample in the flask had ethanol added again, but now in the volume of 100 ml, and was left to stay for another 48 hours in order to accomplish alcohol extraction of lignin. This procedure was repeated until the ethanol extract in the flask become

colourless. All that time lignin extract was collected in dark bottles, because it cannot be exposed to daylight or ultraviolet light. The entire procedure of sample extraction in ethanol had taken about 25 days, during which about 1200 ml of lignin alcohol extract was collected,

- evaporation (concentrating), purification and drying – for lignin isolation as solid residue from liquid ethanol with a relatively lower ebullition point evaporation (concentrating) was conducted using a vacuum rotation evaporator with a water bath. Some 1200 ml of alcohol extract evaporated ethanol was used with remaining residue at the end of the evaporation process was purified three times using 5 ml of water. Finally, evaporation took hold a reddish concentrated extract remained in the mixture with water, which was removed by drying in an oven. The drying of extract was performed in an oven at 30°C temperature for 24 hours. The dried sample was a powdery-solid reddish substance representing native or Brauns lignin,
- determination of native lignin content (NL) – native lignin content as an absolute dry substance was calculated according to following equation:

$$NL = \frac{a}{b} \cdot 100 \qquad [\%]$$

where is: a – native lignin weight (g) and b – absolute dry sample before alcohol extraction weight (g).

UV spectrophotometric analysis: UV spectra of NL were recorded using UV-Vis spectrophotometer Varian Cary 50 with double beam (start scanning: 400 nm, end scanning: 220 nm, speed scanning: medium). A sample in the form of spectrophotometric solution was prepared after the following: 10 mg of NL was first dissolved in 5 ml of 96% ethanol (p.a.) through 24 hours, after that 200 μL of obtained NL-ethanol solution was diluted with new 3 ml of ethanol. The same 96% ethanol was used for standard curve recording. Obtained UV spectra were handled using computer software Varian Cary WinUV. It should be mentioned that for spectra quality improving mathematical techniques were not utilised. For each prepared sample of NL from beech sapwood five scannings were conducted on these spectral technique.

FTIR spectrophotometric analysis: IR spectra of NL were recorded using FTIR spectrophotometer Perkin Elmer Spectrum One with double beam (detector: MIR TGS, apodization: strong, beam splitter: OptKBr, resolution: 4.00 cm^{-1}, start scanning: 4 000 cm^{-1}, end scanning: 450 cm^{-1}, speed scanning: medium). KBr pellets for FTIR spectrophotometry were prepared using the macro technique. Dry (120°C in oven for at least 1 h, followed by cooling in a desiccator over P_2O_5), spectroscopy-grade potassium bromide (KBr) was used as a suitable alkali halide in which was mixed the NL sample. A standard Ø13 mm diameter pellet was prepared by pressing a 2 mg sample in 300 mg of KBr. Dry and spectroscopy-grade KBr was also used for standard curve recording. Obtained FTIR spectra were handled using computer software Spectrum One (ver. 5.0.1). As well as for UV analysis, mathematical techniques for improving a quality of spectra were not utilised. Likewise, for each prepared sample five scannings were conducted on these spectral technique.

RESULTS AND DISCUSSION

Group chemical composition of beech sapwood

According to recent studies related to group chemical composition analysis of beech sapwood, the obtained results are presented in Table 1 (Antonović 2004). It should be mentioned that samples used in group chemical composition analysis are in every way absolutely identical to those used in this researches. Studies were made on beechwood samples (*Fagus sylvatica* L.) from seven sampling locations in Republic of Croatia, which differ among themselves according to specific phytocoenological criteria (soil type, altitude and phytocoenoses).

Tab. 1: Average chemical composition values of beech sapwood from different sampling locations

Sampling locations	*Ash (%)*	*MB extract (%)*	*Lignin (%)*	*Cellulose (%)*	*Wood polyoses (%)*
a	0.54	2.31	26.43	44.67	26.05
b	0.55	2.17	29.22	42.03	26.03
c	0.47	1.59	30.03	43.66	24.25
d	0.52	1.52	31.30	42.72	23.94
e	0.53	1.33	30.12	43.76	24.26
f	0.45	1.79	28.21	43.98	25.57
g	0.47	2.07	28.02	45.78	23.64
$\bar{x}$	0.50	1.83	29.05	43.80	24.82

The performed modifications made to the original Brauns method were: in time reduction of long-lasting sample extraction using diethyl-ether (reducing extraction time from 48 to 8 hours); lignin alcohol dissolving procedure in 95% ethanol, had due to the extension of ethanol staining with lignin been extended from the recommended time of 8–10 days for spruce (according to Brauns) to 25 days for beechwood, which proved a higher lignin content in beech in comparison to spruce; and avoiding of successive and repeated purification procedures of lignin residues, which was reduced only to purification with water during evaporation procedure in the rotation evaporator.

In order to obtain a clearer comparison of obtained results of accessory materials (DEE extract) and native lignin (NL) isolation of beech sapwood from all sampling locations, given values of all samples from the same sampling locations (number of rings/trees x three samples) was consolidated, while presented results were the average values of each determined components, which is shown in Tab. 2. All results are calculated in regard to absolute dry wood.

Obtained accessory materials content, with diethyl-ether pre-extraction of wood (DEE extract), had an average value of 0.25%, and native lignin (NL) content, with ethanol extraction, with an average value of 1.82% in comparison to absolute dry wood weight. This difference is somewhat lower if compared with the results of previously studies, where native lignin is represented with 2–3% compared to the starting wood sample (Lai and Sarkanen, 1971).

With mentioned lignin isolation methods, higher sulfuric acid lignin content (29.05%) was obtained in comparison to native lignin (1.82%), which was about fifteen times greater. With the isolation of sulfuric acid lignin (SAL), as result of polysaccharides hydrolysis with low concentrated acids and condensation reactions, lignin was obtained which contained considerable amount of sulphur, and that is the reason in the great differences in content of SAL and native lignin. Therefore, preparations like this are not useful for structure research, but they can mostly be applied in lignin content estimation, and in this case of being compared with native lignin (Fig. 2).

Tab. 2: Average values of beech sapwood accessory material (DEE extract) and native lignin (NL) content from different sampling locations

Sampling locations	*Rings (trees) number*	*Water content*^{A} *(%)*	*DEE extract (%)*	m_{U1} *(g)*	*Water content*^{B} *(%)*	m_{U2} *(g)*	m_{NL} *(g)*	*NL (%)*
a	10	7.69	0.25	23.33	8.66	21.31	0.4122	1.91
b	11	8.11	0.24	22.19	8.96	20.20	0.4538	2.23
c	11	8.10	0.19	24.05	9.04	21.88	0.3027	1.41
d	4	8.67	0.28	18.60	8.93	16.93	0.3135	1.86
e	6	8.70	0.29	17.79	9.08	16.17	0.3087	1.91
f	5	7.86	0.25	17.67	8.28	16.20	0.2811	1.72
g	10	8.01	0.23	21.11	8.84	19.23	0.3214	1.69
$\overline{x}$			0.25					1.82

^{A} ⇒ water content in samples before extraction, ^{B} ⇒ water content in samples after extraction with diethyl-ether, m_{U1} ⇒ airdry sample weight, m_{U2} ⇒ absolute dry sample weight, m_{BL} ⇒ absolute dry native lignin weight

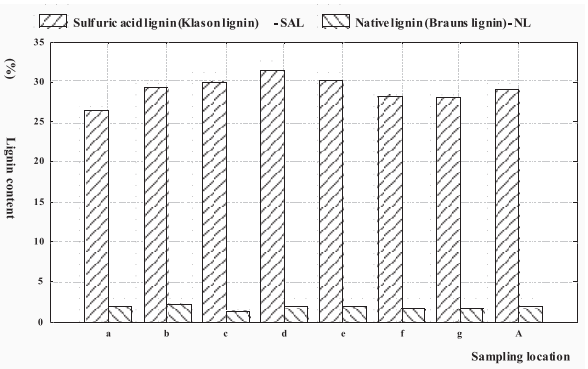

Fig. 2: Average content of sulfuric acid lignin (SAL) and native lignin (NL) depending on sampling locations

Comparing values of native lignin (NL) content with sulfuric acid lignin (SAL) depending on sampling locations, gain relation NL/SAL within the limit from 4.70–7.63%. This means that native lignin is on average represented with 6.28% regarding sulfuric acid lignin. These differences are on average somewhat lower if compared with reference data with the NL/SAL relations represented by 8–10%.

UV spectrophotometric characterisation of native lignin

Average UV spectra of native lignin isolated from beech sapwood in respect to all sampling locations is present in Fig. 3. Unfortunately, availability of information and description for UV spectra of native lignins for various wood species is exceptionally limited, so that obtained results are in many ways unique. Based on obtained UV spectra of NL from beech sapwood, comparison with available in literature for analytical MWL and technical sulphonate and organosolv beech lignins were made (Fengel and Wegener 1989). In Figure 3 is shown that the typical UV spectra

of NL from beech sapwood has maximum at around 281 nm followed by a slope to lower wave lengths, with a more or less pronounced shoulder in the region of 223–238 nm. Therefore, NL has maximum between 279–282 nm depending on sampling location, and shows absorptiveness values at somewhat higher wave length than beech MWL and technical lignins, which reach maximum at 274–276 nm according to Sakakibara and Sano (2001).

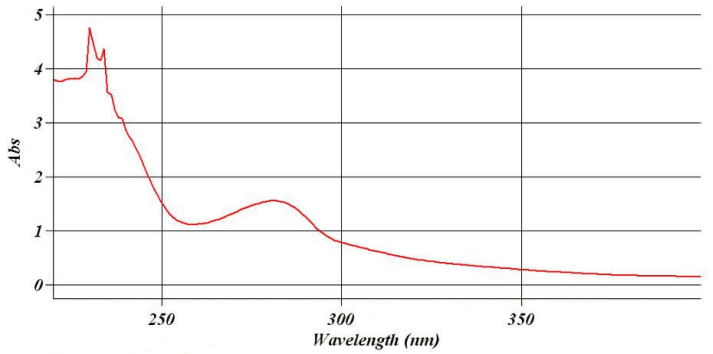

Fig. 3: UV spectra of NL from beech sapwood as a average of all sampling locations

FTIR spectrophotometric characterisation of native lignin

FTIR spectra of NL from beech sapwood show 17 major IR bands at defined wavelengths (Fig. 4), based on which were determined their characteristics, as a supplement to the spectroscopic research of native lignin.

Tab. 3 represents the position of bands at spectrum wavelengths, their intensity and interpretation for FTIR spectra of beech MWL from previous studies (Hergert 1971, Feckl 1981, Faix 1991, Faix and Böttcher 1992, Pandey 1999) including NL from beech sapwood recorded in this study.

FTIR spectra of beech MWL show the broad or first absorption band of the hydroxyl stretching (O–H) in the region of 3400 cm^{-1} (Tab. 2), which is considerably lower in obtained NL spectra and can be found in the region between 3328–3340 cm^{-1} (1). Spectral differences between beech MWL and NL were observed in the fingerprint region at around 1800–900 cm^{-1}. In these region we proved in general the existence of three most intensive and characteristic IR bands of NL (Tab. 4), which are found at around 1608–1611 cm^{-1} (5) and 1516–1517 cm^{-1} (6), whereas refer to aromatic ring (skeletal) vibrations (this region is poor in additional bands and can therefore be used to prove the existence of lignin in unknown preparations), and at around 1447–1451 cm^{-1} (7), which beside aromatic ring (skeletal) vibrations, refer to C–H deformities plus contain absorptions resulting from vibrations typical for functional groups that are present in lignin molecules, such as $–CH_2–$ and $–CH_3$ bending (according to Feckl 1981). For beech MWL position of this bands are at slightly lower or higher wavelengths, so there isn't any spectral difference between observed lignins.

With the observing of obtained beech NL bands and MWL bands from previous studies (Fengel and Wegener 1989) at defined wavelengths, it can be noted that NL band intensity at 1608–1611 cm^{-1} is significantly higher from that at 1516–1517 cm^{-1}, while MWL band intensity at 1608–1611 cm^{-1} is similar or somewhat lower than 1516–1517 cm^{-1} band. It is necessary to additionally interpretate this phenomenon by conducting further studies.

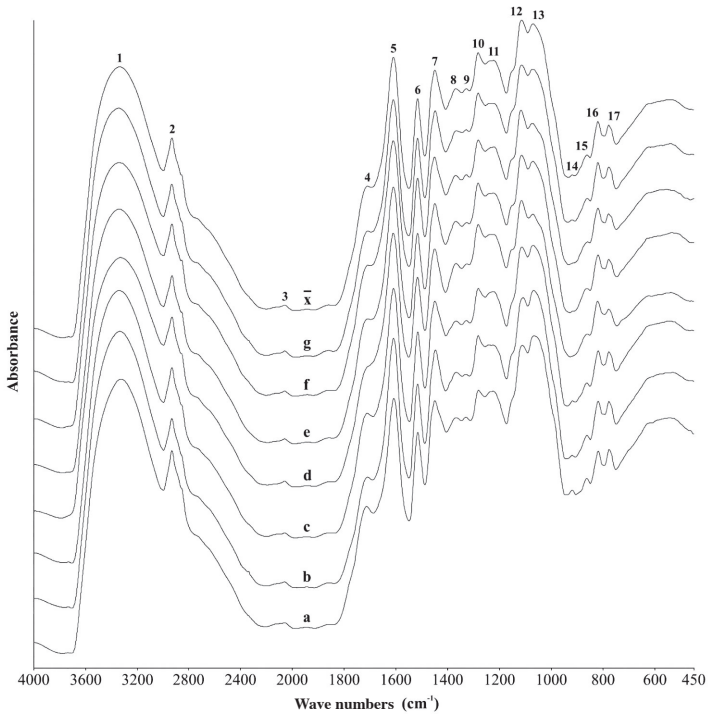

Fig. 4: FTIR spectra of NL samples from beech sapwood in respect to different sampling locations

Typical guaiacyl and syringyl bands of beech NL, which are also referred to as ring breathing of lignin were recorded between 1282–1283 cm^{-1} (10) for guaiacyl (G ring + C=O stretching) and 1327–1331 cm^{-1} (9) for syringyl (S ring) bands, and they are approximately located in the same position as in MWL.

Furthermore, as well as at MWL, C–C + C–O + C=O stretching was proved located at wavelength between 1222–1226 cm^{-1} (11), which shows the most pronounced methoxyl character, and aromatic C–H in-plane deformations which are characteristic of syringyl units located at wavelength between 1108–1117 cm^{-1} (12), whereas this band is attributed also to secondary alcohols and C=O stretching.

The greatest difference between beech MWL and NL is manifested in the appearance of a weak intensity band between 2058–2061 cm^{-1} (3) and a medium intensity band between 776–780 cm^{-1} (17) on the FTIR spectrum. Unfortunately, the interpretation of these two bands is impossible due to a lack of information about lignin behaviour at these wavelengths.

Tab. 3: Assignments of infrared absorption bands in MWL and in obtained NL of beech

Band No.[A]	Band intensity	Maxima[B]		Band assignment
		MWL	NL	
1	s	3460	3328-3340	O–H stretch (H–bonded)
2	s	2940	2930-2932	C–H stretch in CH_3 and CH_2 groups
3	w	–	2058-2061	–
4	w	1735	1708-1712	C=O stretch in unconjugated ketones, carbonyls and in ester groups (frequently of carbohydrate origin); conjugated aldehydes and carboxylic acids absorb around and below 1700 cm^{-1}
5	s	1593	1608-1611	aromatic skeletal vibrations + C=O stretch; S > G; G condensed > G etherified
6	s	1505	1516-1517	aromatic skeletal vibrations; G > S
7	s	1462	1447-1451	C–H deformations; asymetric in $–CH_3$ and $–CH_2–$
8	w	1367	1364-1368	aliphatic C–H stretch in CH_3, not in OMe; phenolic OH
9	w	1329	1327-1331	S ring + G ring
10	m	1266	1282-1283	G ring + C=O stretch
11	w	1227	1222-1226	C–C + C–O + C=O stretch; G condensed > G etherified
12	m	1126	1108-1117	aromatic C–H in-plane deformation (typical for S units) + secondary alcohols + C=O stretch
13	m	1033	1064-1074	aromatic C–H in-plane deformation, G > S + C–O deformation in primary alcohols + C=O stretch (unconjugated)
14	w	925	918-921	C–H out-of-plane; aromatic
15	w	835	859-864	C–H out-of-plane in positions 2 and 6 of S units
16	m	817	818-822	C–H out-of-plane in positions 2, 5 and 6 of G units
17	m	–	776-780	–

[A] – band number in Fig. 2; [B] – wave numbers in cm^{-1}; w – weak; m – medium; s – strong;; G – guaiacyl; S – syringyl;

CONCLUSION

The modified isolation method, in comparison with the original Brauns method isolation of native lignin, has proved to be justified by the obtained results of somewhat lower values of native lignin content then was mentioned in previous studies. Compared to previous studies of group chemical composition of beech sapwood, it shows great difference in the content of isolated accessory materials depending on type of applied solvent, as well as lignin content depending on isolation method.

Although the UV and FTIR spectroscopy in previous studies has been used for different lignins, the availability of information and descriptions for UV and IR spectra of native lignins for various wood species is exceptionally limited or even does not exist, so that the interpretation of obtained native lignin bands in this work was made based on previous research of UV and FTIR spectra of other beech analytical and technical lignins. UV and FTIR spectroscopic characterisation has proved the usability of modified Brauns isolation method of NL in the structural research of GS lignins, which is different from original in that it omits the purification processes using successive and repetitive precipitation of the dioxane solution into water and into ether. Based on obtained UV and FTIR spectra of NL from beech sapwood, were explained the differences in spectral behaviour between NL and MWL. UV spectrum of NL from beech sapwood, which has a maximum of between 279–282 nm depending on the sampling location, showing absorptiveness values

at somewhat higher wavelengths than MWL, which has a maximum at 275–277 nm. FTIR spectrum differences are manifested in a considerably higher absorptiveness of bands at around 1608–1611 cm^{-1} compared to that at around 1516–1517 cm^{-1} with NL, while with MWL band intensity at around 1608–1611 cm^{-1} is similar or slightly lower than in the band at around 1516–1517 cm^{-1}, as well as in the appearance of two new bands on NL spectra, which need to be additionally interpreted in further studies.

Since the main problem in lignin chemistry is the fact that so far not a single method had been found that would isolate the overall lignin in its native state, obtained results presented in this paper will give small impetus to its further research. In studies of UV and IR spectra of beech NL, with the use of these spectroscopic techniques and with the help of mathematical resolution techniques, such as derivative spectroscopy, deconvolution and band characteristics i.e. the analysis of the state of the band, it is necessary to determine the ratio of basic structural units of lignin polymer matrix (hydroxyphenyl, guaiacyl and syringyl units) as well as the ratio of functional groups (methoxyl, hydroxyl and carbonyl, and ratio phenolic OH and aliphatic OH groups), which will be the subject of the next paper.

REFERENCES

1. Antonović, A., 2004: Spectrophotometric analysis of beechwood lignin (*Fagus sylvatica* L.). Master thesis. University of Zagreb, Faculty of Forestry, 214 pp.
2. Brauns, F.E., 1952: The chemistry of lignin. Pp. 51-54, Academic Press Inc., New York
3. Brauns, F.E., 1952a: The chemistry of lignin. In: Wood chemistry (eds. L.E. Wise and E.C. Jahn). Pp. 424-425, Reinhold Publishing Corporation, New York
4. Faix, O., 1986: Investigations on lignin polymer models (DHP's) by FTIR spectroscopy. Holzforschung 40: 273-280
5. Faix, O., 1991: Classification of lignins from different botanical origins by FT-IR spectroscopy. Holzforschung 45 (Suppl.): 21-27
6. Faix, O., Böttcher, J.H., 1992: The influence of particle size and concentration in transmission and diffuse reflectance spectroscopy of wood. Holz als Roh- und Werkstoff 50: 221-226
7. Feckl, J., 1981: Gewinnung und Fraktionierung von lignin-polysaccharid-komplexen. Doctor thesis, Universität München
8. Fengel, D., Wegener, G., 1989: Wood – Chemistry, ultrastructure, reactions. Pp. 49-59, Walter de Gruyter, New York
9. Fengel, D., Wegener, G., Feckl, J., 1981: Contribution to characterization of analytical and technical lignins. Holzforschung 35: 51-57
10. Goldschmid, O., 1971: Ultraviolet spectra. In: Lignins – Occurence, formation, structure and reactions (eds. K.V. Sarkanen and C.H. Ludwig). Pp. 165-266, Wiley-Interscience, New York
11. Goldstein, I.S., 1991: Overview of the chemical composition of wood. In: Wood structure and composition (eds. M. Lewin and I.S. Goldstein). Pp. 1-5, Marcel Dekker Inc., New York
12. Hergert, H.L., 1971: Infrared spectra. In: Lignins. Occurrence, formation, structure and reactions (eds. K.V. Sarkanen and C.H. Ludwig). Pp. 267-293, Wiley-Interscience, New York

13. Hortling, B., Tamminen, T., Kenttä, E., 1997: Determination of carboxyl and non-conjugated carbonyl groups in dissolved and residual lignins by IR spectroscopy. Holzforschung 51: 405-410
14. Lai, Y.Z., Sarkanen, K.V., 1971: Isolation and structural studies. In: Lignins – occurence, formation, structure and reactions (eds. K.V. Sarkanen and C.H. Ludwig). Pp. 165-240, Wiley-Interscience, New York
15. Lin, S.Y., 1992: Ultraviolet spectrophotometry. In: Methods in lignin chemistry (eds. S.Y. Lin and C.W. Dence). Pp. 217-232, Springer-Verlag, Berlin
16. Melcer, I., Blažej, A., Šutý, L., 1976: Analytická chémia dreva. Pp. 273, Alfa, Bratislava
17. Pandey, K.K., 1999: A study of chemical structure of soft and hardwood and wood polymers by FTIR spectroscopy. Journal of Applied Polymer Science 71: 1969-1975
18. Sakakibara, A., Sano, Y., 2001: Chemistry of lignin. In: Wood and cellulosic chemistry (second edition, revised and expanded) (eds. D.N.-S. Hon and N. Shiraishi). Pp. 109-173, Marcel Dekker Inc., New York
19. Shultz, T.P., Glasser, W., 1986: Quantitative structural analysis of lignin by diffuse reflectance Fourier transform infrared spectrometry. Holzforschung 40 (Suppl.): 37-44
20. Sjöström, E., 1993: Wood chemistry – Fundamentals and applications (second edition). Pp. 71-89, Academic Press Inc., San Diego
21. TAPPI, 1997: Test method T 264 cm-97 – Preparation of wood for chemical analysis. TAPPI
22. TAPPI, 2002: Test method T 257 cm-02 – Sampling and preparing wood for analysis. TAPPI

Assistant Alan Antonović, MSc
Wood Technology Department – Institute for Material Technologies
Faculty of Forestry
University of Zagreb
Svetošimunska cesta 25
P.O. Box 422
10002 Zagreb
Croatia
Phone: 00385 1 235 2504
Fax: 00385 1 235 2544
E-mail: alan.antonovic@zg.htnet.hr

Assoc. Prof. Vladimir Jambreković, PhD
Wood Technology Department – Institute for Material Technologies
Faculty of Forestry
University of Zagreb
Svetošimunska cesta 25
P.O. Box 422
10002 Zagreb
Croatia
Phone: 00385 1 235 2504
Fax: 00385 1 235 2544

Assoc. Prof. Stjepan Pervan, PhD
Wood Technology Department – Institute for Material Technologies
Faculty of Forestry
University of Zagreb
Svetošimunska cesta 25
P.O. Box 422
10002 Zagreb
Croatia
Phone: 00385 1 235 2504
Fax: 00385 1 235 2544

Senior assistant Josip Ištvanić, PhD
Wood Technology Department – Institute for Material Technologies
Faculty of Forestry
University of Zagreb
Svetošimunska cesta 25
P.O. Box 422
10002 Zagreb
Croatia
Phone: 00385 1 235 2504
Fax: 00385 1 235 2544

Senior assistant Krešimir Greger, PhD
Wood Technology Department – Institute for Material Technologies
Faculty of Forestry
University of Zagreb
Svetošimunska cesta 25
P.O. Box 422
10002 Zagreb
Croatia
Phone: 00385 1 235 2504
Fax: 00385 1 235 2544

Assistant Andrej Bublić, BSc
Wood Technology Department – Institute for Material Technologies
Faculty of Forestry
University of Zagreb
Svetošimunska cesta 25
P.O. Box 422
10002 Zagreb
Croatia
Phone: 00385 1 235 2504
Fax: 00385 1 235 2544