



Influence of sampling location on content and chemical composition of the beech native lignin (*Fagus sylvatica* L.)

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Abstract

Background and Purpose: The chemical composition of wood depends on a number of different factors, such as wood species, anatomical parts of wood, geographic location, habitation, growth, climatic characteristics, and degree of fungal and insect attacks. Recent studies report that the content of certain wood chemical components differed significantly in dependence on different sampling locations. As an extension of the mentioned studies, this study was aimed at investigating the influence of sampling locations which differ among themselves according to specific ecological factors (soil type, altitude and phytocoenoses) on the chemical composition of a wood native lignin (NL) model by Fourier-transform infrared (FT-IR) spectroscopy.

Materials and Methods: The beech (*Fagus sylvatica* L.) sapwood NL samples from seven sampling locations were used that differ among themselves according to specific ecological factors. NL was isolated according to a modified Brauns method described in previous research, and the FT-IR transmission spectroscopic technique was used for its characterization. The content of guaiacyl (G) and syringyl units (S), as well as syringyl/guaiacyl (S/G) ratio was estimated for different sampling locations. The observed FT-IR peaks were assigned and quantified as percentages of the peak spectral heights, and the differences between NL were discussed in terms of lignin composition.

Results: The results of statistical analysis of the NL contents showed significant differences between some sampling locations regarding phytocoenoses, soil type and altitude, as well as within some locations with the same sign of the mentioned ecological factors. The obtained FT-IR spectral results revealed that the observed NL composition differs in S/G ratios linked to different sampling locations, as evidenced by relative heights of FT-IR peaks at 1282–1283 cm^{-1} (represent G units) and 1327–1331 cm^{-1} (represent S units). The estimated relationship between the content of guaiacyl and syringyl units of the NL model structure was assigned from 63.06–36.94% and 71.62–28.38%. For different sampling locations the S/G ratios were from 0.40–0.59.

Conclusions: Statistical analysis and comparison between the results of NL content for all sampling locations showed significant differences between some sampling locations with regard to phytocoenoses, soil type and altitude, as well as within some locations with the same sign of the mentioned ecological factors. Furthermore, the results of this study, such as the obtained FT-IR spectras of NL, different S/G ratios and the content of guaiacyl and syringyl units, showed the significant influence of sampling locations on the chemical composition of the wood native lignin model in terms of the content of the observed phenylpropane units.

INTRODUCTION

Wood lignin represents amorphous three-dimensional network polymer formed by the oxidative polymerisation of hydroxycinnamyl alcohol derivatives termed monolignols (hydroxylated and methoxylated phenylpropane units linked via oxidative coupling that is probably catalyzed by both peroxidases and laccases), leading to a complex structure that can only be defined by the frequency of occurrence of the various linkages (1). It is a major structural component of secondarily thickened wood cell walls (20–35% of total wood), and it is generally distributed as a matrix component with hemicelluloses in the spaces of intercellulose microfibrils in primary and secondary walls and in middle lamellae, and functions to connect cells to one another and to harden the cell walls of xylem tissues (2).

Based on extensive research on dehydrogenative polymerisation of respective monolignols by peroxidase/H₂O₂ (3) and analyses of native lignins (4), it has been established that hardwood (angiosperm) lignin, also called guaiacyl-syringyl (GS) lignin is composed of coniferyl and sinapyl alcohol derived units in varying proportions.

The major monolignols in dicotyledonous angiosperm lignin are monomethylated guaiacyl (G) units derived from coniferyl alcohol (3-methoxy-4-hydroxycinnamyl alcohol), and dimethylated syringyl (S) units derived from sinapyl alcohol (3,5-dimethoxy-4-hydroxycinnamyl alcohol) (Figure 1).

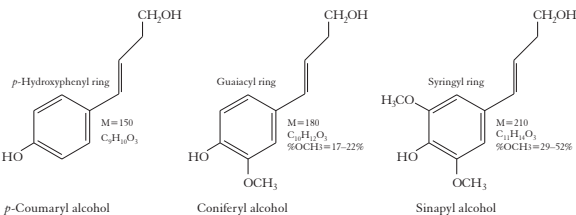


Figure 1. The building phenylpropanoid units of dicotyledonous angiosperm GS lignin.

The biochemical pathways leading to the formation of monolignols feature successive hydroxylation and *O*-methylation of the aromatic ring and conversion of the side chain carboxyl to an alcohol function (3).

It is known that GS lignin structural organization is a consequence of the type of combination involving phenoxy radicals formed from coniferyl (G) and sinapyl (S) alcohol precursors (Figure 2).

Coniferyl alcohol has four possibilities of linking on C-1, C-5 (aromatic ring), HO-Phe, and C-β, while sinapyl alcohol has only three free sites, located on C-1 (aromatic ring), Ho-Phe, and C-β. This means that lignin from coniferyl alcohol supposedly has the highest number of cross links, so that a high degree of complexity may be found in it, while lignin from a sinapyl alcohol precursor has a low number of cross links. The maximum number of combinations among phenoxy radicals at a (1:1) ratio is called TNIB (Theoretical Number of Intermolecular Bonds). The estimated TNIB for GS lignins is 46 (theoretical OCH₃ = 19.40%), compared to G lignins, with 78 (theoretical OCH₃ = 15.90%), and HGS lignins with 138 (theoretical OCH₃ = 11.48%) (5).

The chemical composition of wood is complex. It depends on a number of different factors, such as wood spe-

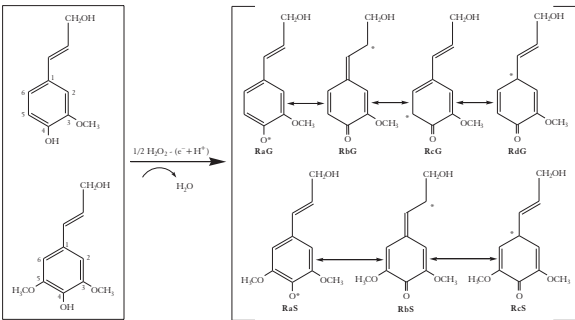


Figure 2. Enzymatic dehydrogenation of coniferyl (G) and sinapyl (S) alcohols yielding phenoxy radicals.

TABLE 1

Average values of beech group chemical composition depending on some ecological factors.

Ecological criteria	Ash (%)	Accessory material (%)	Lignin (%)	Cellulose (%)	Wood polyoses (%)
Soil type					
Luvic soil	0.51	1.95	28.23	44.17	25.15
Dystric cambisol	0.51	2.12	28.62	43.91	24.84
Rendzic leptosol	0.52	1.52	31.30	42.72	23.94
Mollic leptosol	0.49	1.56	29.17	43.87	24.92
Phytocoenoses					
Sub-mountain beech forest with sedge	0.51	1.95	28.23	44.16	25.15
Beech forest with woodrush	0.51	2.12	28.62	43.91	24.84
Dinaric beech-fir forest	0.53	1.43	30.71	43.23	24.10
Pre-mountain beech forest with holly fern	0.45	1.79	28.21	43.98	25.57

cies, anatomical parts of wood (heartwood, sapwood, stem, root, branches, leaves, etc.), geographic location, habitation, growth, climatic characteristics, degree of fungal and insect attacks, as well as the applied chemical analytical isolation methods and calculation techniques (6, 7). Recent quantitative studies of the chemical composition of beech wood revealed the most significant differences in the content of certain components (accessory material and cellulose) from different sampling locations, which differ among themselves according to specific ecological factors (Table 1) (8, 9).

In this study, the lignin was isolated with sulfuric acid method, and the final product was so called sulfuric acid lignin (SAL) or Klasons lignin. SAL content was obtained as a result of polysaccharide hydrolysis with low concentrated acids and condensation reaction, and therefore contained considerable amounts of sulphur, and such preparations are not useful for lignin structural research; nevertheless, they can generally be applied to lignin content estimation. Although the results of SAL studies did not show statistically significant differences in lignin content, based on its complex structure and behavior, with respect to content of methoxyl (OCH₃ or OMe) and hydroxyl groups (OH), significant differences within ratio of lignin alcoholic units in dependence on the sampling locations can be expected. Because of that, the Brauns or native lignin NL were isolated for chemical structural studies, which is according to its chemical properties nearest to the structure that can found in its natural state in the forest. Therefore, the aim of this pa-

per was to prove the influence of sampling locations on the content and chemical composition of NL model using FT-IR spectroscopic characterization.

MATERIAL AND METHODS

Native lignin (NL) isolation

To perform a FT-IR spectroscopy study of NL, beech sapwood (*Fagus sylvatica* L.) samples from seven sampling locations in Croatia were used that differ among themselves according to specific ecological factors (phytocoenoses, soil type, and altitude) (Table 2, Table 3).

As seen from the tables, as reference ecological factors of sampling locations, based on which comparison was made, four groups were taken for phytocoenoses: sub-mountain beech forest with sedge, beech forest with woodrush, dinaric beech-fir forest and pre-mountain beech forest with holly fern; four groups for soil type: luvic soil, dystic cambisol, rendzic leptosol and mollic leptosol; and three characteristic groups for altitude: 0–300 m, 300–700 m i 700–1100 m.

The samples were taken promptly after a tree was cut down. The criterion for taking samples was the cutting off of a ring on the first log from the stem, approximately at 2 m height. Since isolation analysis was carried out on wood sapwood, each ring was submitted to mechanical operation before grinding, where sapwood was separated from other anatomical parts of wood (heartwood and bark). After grinding (cutting mill Fritsch Pulverisette 19) and screening (100–150 mash), three smaller sam-

TABLE 2

Locations in which sampling and numberering of rings were performed.

Sampling location	Forestry authority	Forest areal	Section	Number of rings
A	Pleternica	Piramida	36A	10
B	Nova Gradiška	Gorica	50A	11
C	Bjelovar	Draga – rijeka	160A	11
D	Perušić	Javornik	5A	4
E	Novi Vinodolski	Jelovica	17A	6
F	Gerovo	Glajz	68A	5
G	Sljeme	Bistarska gora	20B	10

TABLE 3

Ecological criterias of sampling locations.

Sampling location	Phytocoenoses	Soil type	Altitude (m)
A	Sub-mountain beech forest with sedge	luvic soil	320–470
B	Beech forest with woodrush	distric cambisol	0–300
C	Sub-mountain beech forest with sedge	luvic soil	0–190
D	Dinaric beech-fir forest	rendzic leptosol	760–940
E	Dinaric beech-fir forest	mollic leptosol	900–995
F	Pre-mountain beech forest with holly fern	mollic leptosol	1000–1080
G	Beech forest with woodrush	distric cambisol	900–1100

ples (15–25 g of dry wood meal) were taken from each sample (a total of 171 samples). NL was isolated from them after the following procedures: pre-extraction with diethyl-ether (DEE extract) in Soxhlets apparatus for 8 hours (in order to remove accessory materials), and then alcoholic extraction using 96% ethanol for 25 days according to a modified Brauns NL isolation method described in previous research (8, 9). It differs from the original by disregarding purification processes with successive and repetitive precipitation of the dioxane solution into water and into ether. The NL content as an absolute dry substance was calculated according to the following equation:

$$NL = \frac{a}{b} \times 100 \quad [\%] \quad (1)$$

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

The results of isolation of the NL content in percentage were statistically analyzed in the Statistica program.

FT-IR spectroscopy

Changes in the chemical structure of NL from different sampling locations were analyzed by FT-IR spectroscopy. The FT-IR spectra of samples were obtained by direct transmission (KBr pellet technique) using Perkin Elmer Spectrum One spectrometer at a wavenumber range of 4000–450 cm^{-1} . KBr pellets for FT-IR spectroscopy were prepared using the macro technique. Dry (120°C in the oven for at least 1 h, followed by cooling in a desiccator over P_2O_5) and spectroscopy-grade potassium bromide (KBr) was used as a suitable alkali halide for the preparation of samples, as well as for recording standard curves. A standard Ø13 mm diameter pellet was prepared by pressing a 2 mg sample in 300 mg of KBr for 5 min under pressure of 200 bar. Each spectrum was taken as a mean value of 64 scans at a resolution of 4 cm^{-1} (8, 9).

Peak heights of IR peaks were measured using Spectrum One (ver. 5.0.1) software according to previously published methods (10). Heights of peaks were measured from the baseline, constructed by connecting the

lowest data points on either side of the peak. A vertical line is then drawn from the top of the peak to the X-axis. The portion of the line between the top of the peak and the baseline represents the corrected peak height in percentage.

RESULTS AND DISCUSSION

In order to obtain a clearer comparison of the obtained results of native lignin (NL) isolation of beech sapwood from all sampling locations, given values of all samples from the same sampling locations was consolidated, while the presented results were the average values of each determined components, which is shown in Table 4. All results are calculated with regard to absolute dry wood.

Statistical analysis of the obtained NL isolation results showed the existence of significant statistical differences between some sampling locations in relation to ecological factors (Figure 3). If we compare the results of NL content between specific locations, statistical analysis showed significant differences between location A

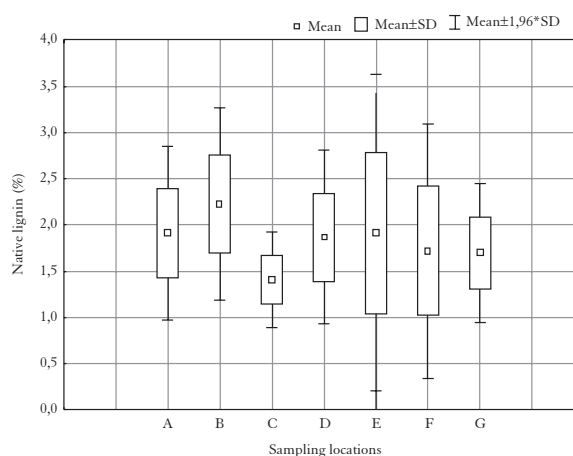


Figure 3. Statistical analysis of the NL isolation results depending on the sampling locations.

TABLE 4

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

Sampling locations	Water content ^A (%)	DEE extract (%)	m_{U1} (g)	Water content ^B (%)	m_{U2} (g)	m_{NL} (g)	NL (%)
A	7.69	0.25	23.33	8.66	21.31	0.4122	1.91
B	8.11	0.24	22.19	8.96	20.20	0.4538	2.23
C	8.10	0.19	24.05	9.04	21.88	0.3027	1.41
D	8.67	0.28	18.60	8.93	16.93	0.3135	1.86
E	8.70	0.29	17.79	9.08	16.17	0.3087	1.91
F	7.86	0.25	17.67	8.28	16.20	0.2811	1.72
G	8.01	0.23	21.11	8.84	19.23	0.3214	1.69

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

and location C, location B and locations C and G, and between location C and location D.

Some locations with the same phytocoenoses and soil type also showed statistically significant differences, such as locations A and C (sub-mountain beech forest with sedge on luvic soil) and locations B and G (beech forest with woodrush on dystric cambisol), although they differed among themselves according to altitude. These results could indicate the effect of altitude on NL content and their chemical compositions.

Based on these ecological factors for each location, the obtained results of NL isolation can be integrated, so that each location join to factor with the same sign as shown in Table 3. Statistical analysis of the obtained results for phytocoenoses showed that statistically significant difference exists only between the locations with sub-mountain beech forest with sedge (locations A and C) and beech forest with woodrush (locations B and G). When comparing the obtained results of NL for soil types, significant differences were determined only between locations on luvic soil (locations A and C) and locations on dystric cambisol (locations B and G). Furthermore, statistically significant difference exists also between the locations with an altitude of 0–300 m (locations B and C) and locations with an altitude of 700–1100 m (locations D, E, F and G).

FT-IR spectroscopy is a very useful tool for obtaining rapid information about the structure of a lignin model and chemical changes taking place in lignin due to various treatments. It provides an overall view of its structure (11, 12, 13).

FT-IR spectra of native lignins show 14 major IR bands in fingerprint region (Figure 4), on the basis of which bands were assigned.

The location of the mentioned bands on the FT-IR spectrum of NL at defined wavelengths in the fingerprint region is approximately the same if observed through sampling locations. However, taking into consideration the intensity of these very bands, some significant differences can be noticed. In general, the intensity of all bands is significantly lower in NL samples from the sampling locations A and B as opposed to other locations. The most pronounced spectral difference between native lignins from different sampling locations is manifested when the intensity band at 1108–1117 cm^{-1} (aromatic in-plane C–H bending typical for S units) is compared with the band at 1064–1074 cm^{-1} (aromatic in-plane C–H bending typical for G units). With regard to the sampling locations A and B, it can be noted that the intensity of the

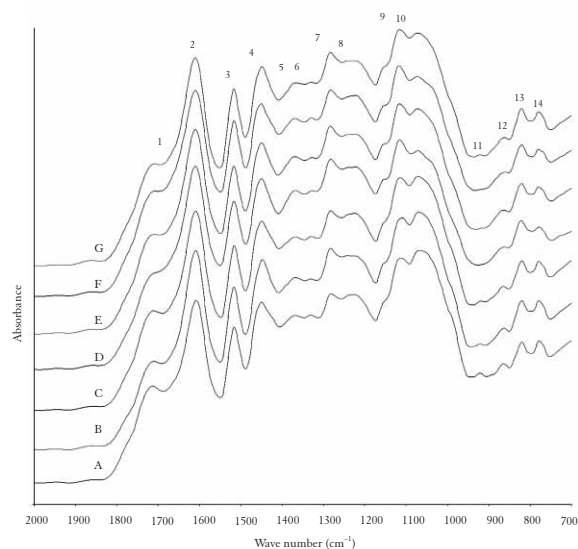


Figure 4. FT-IR spectra of NL samples from beech sapwood with respect to different sampling locations.

band at 1108–1117 cm^{-1} is lower than the band 1064–1074 cm^{-1} ; in location C it is equal, and in other locations it is higher.

Typical guaiacyl and syringyl bands of NL, which are also referred to as ring breathing of lignin, were recorded between 1282–1283 cm^{-1} for guaiacyl (G units ring breathing) and 1327–1331 cm^{-1} for syringyl (S units ring breathing) bands. Spectral differences between the mentioned native lignins, based on a band area relationship at 1327–1331 cm^{-1} and 1282–1283 cm^{-1} , can be expressed with the estimated syringyl-to-guaiacyl units (S/G) ratio, based on estimation method according to Pandey and Pitman (14). For different sampling locations the S/G ratios were from 0.40–0.59 (Table 3).

The *p*-hydroxyphenyl units (H) content, with band appearance approximately at 835 cm^{-1} , were not taken in calculation, because the mentioned band was not found on the FT-IR spectrum of NL, although according to some authors their representation may be up to 10% (15).

If we compare the estimated contents of syringyl and guaiacyl units, as well as their S/G ratio with statistical analysis of NL content results of each location for specific ecological factor, some similarities can be noticed. Based on the S/G ratio at phytocoenoses we can see the most significant differences between locations with sub-mountain beech forest with sedge (locations A and C) and locations with beech forest with woodrush (locations

TABLE 5

Estimated content of syringyl and guaiacyl units, and S/G ratio of NL from different sampling locations.

Sampling locations	A	B	C	D	E	F	G	
% S	36.94	29.56	33.83	30.78	29.29	32.65	28.38	31.63
% G	63.06	70.44	66.17	69.22	70.71	67.35	71.62	68.37
S/G ratio	0.59	0.42	0.51	0.45	0.41	0.49	0.40	0.46

B and G), as in the case of statistical analysis of the NL contents of each location. Consequently, the S/G ratio at soil types showed the most significant differences between locations on luvic soil and locations on dystic cambisol, which is equivalent to the phytocoenoses mentioned above. These results could indicate the influence of phytocoenoses and soil types on different NL contents and their chemical compositions.

Furthermore, from Table 5 we can see that locations at lower altitude (0–300 m), but with the same phytocoenoses and soil type, such as locations A and C (sub-mountain beech forest with sedge on luvic soil) and locations B and G (beech forest with woodrush on dystic cambisol), show a higher S/G ratio. We assume that for the same phytocoenoses on the same soil type with increasing altitude there is a increasing content of guaiacyl units, apropos to decreasing content of syringyl units.

According to Faix (14), in terms of intensity relationship between the specific bands and the estimated contents of guaiacyl and syringyl units, the obtained NL as represented by the GS lignins can be classified in the GS2 spectral type, which conforms to the G units content from 55–75% and the S units content from 25–35% based on 100 phenylpropane units (the content of methoxyl units OMe based on 100 C₉ units is between 110–130). The estimated relationship between the content of guaiacyl and syringyl units of the NL model structure was assigned from 63.06–36.94% and 71.62–28.38%.

CONCLUSION

Statistical analysis and comparison between the results of NL content for all sampling locations, which differ among themselves according to specific ecological factors, showed significant differences between some sampling locations with regard to phytocoenoses, soil type and altitude, as well as within some locations with the same sign of the mentioned ecological factors.

Furthermore, the results of this study, such as the obtained FT-IR spectras of NL, different S/G ratios and the content of guaiacyl and syringyl units, showed the significant influence of sampling locations on the chemical composition of the wood native lignin model in terms of the content of the observed phenylpropane units.

Further research is necessary in order to determine the ratio of functional groups (methoxyl, hydroxyl and carbonyl), and the ratio between phenolic OH and

aliphatic OH groups, which will give us more reliable information and answers on how ecological factors affect the chemical composition of the NL.

The main problem in lignin chemistry is that not one single method of isolating overall lignin in its native state has been found so far. We hope that the results presented in this paper will provide impetus for further research.

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