Analytical pyrolysis as a direct method to determine the lignin content in wood Part 3. Evaluation of species-specific and tissue-specific differences in softwood lignin composition using principal component analysis

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ABSTRACT

Both the genetics and the environment determine the chemical composition of wood. To assess the chemical composition analytical pyrolysis is being increasingly used. Each single pyrogram is a fingerprint of the chemical composition that should reflect tissue, species, and site related information although hidden in an amount of data.

Principal component analysis was applied to evaluate the pyrolysis results with respect to differences in lignin composition using G- and H-lignin-derived peaks from the pyrograms. The three species: pine, spruce and larch were separated in the first principal scores plot and the corresponding loadings plot revealed that it is vanillin (G 9) and G-C=C (G 11) on one hand and isoeugenol (G 8) and dihydroconiferyl alcohol (G 19) on the other hand that separate spruce and larch from pine. Beside others G 9 plus G 11 and G 8 plus G 19 separate spruce from larch as well as Vaquey pine from Blagon pine. In addition an investigation of the different tissues – normal wood and reaction wood – and the discussion of these results together with the loadings helped to reveal the differences in lignin composition between the species, tissues, and two sites. It was shown that analytical pyrolysis combined with principal component analysis could be useful for the identification of species and their origin.

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1. Introduction

Analytical pyrolysis is being increasingly used as a quantitative method to assess chemical composition of lignocellulosic materials [1–5]. The main advantages of analytical pyrolysis over classical wet-chemical methods are an easy sample preparation (drying and milling), short analysis times and small sample sizes (μg range) [6]. Analytical pyrolysis data were related with classical wet chemical data by direct comparison of peak areas of characteristic pyrolysis products [7–10], using multivariate statistical techniques for qualitative analysis such as principal component analysis (PCA) [11] or for calibration [12,13], and

absolute quantification of pyrolysis products using internal standards [14–16].

Unfortunately less is known about the lignin structures from which the analytical pyrolysis products derive [17]. Methods such as thioacidolysis [18–20], derivatization followed by reductive cleavage (DFRC) [21,22], hydrogenolysis [23], acidolysis [24], ozonation [25], selective tosylation (T) of primary hydroxyl group, iodination (I), and zinc-metal treatment (Z) (TIZ) to cleave β -O-4 linkage [26] were used to characterize lignin structure/composition. DFRC was combined with ^1H – ^{13}C HMQC NMR spectroscopy [27] or ^{31}P NMR [28,29] and compared to thioacidolysis [27,28] to elucidate lignin structure. The two of the most effective methods for elucidating lignin structure have proven to be thioacidolysis and DFRC, whereas the latter method provides lower monomer yields from β -aryl ether bonds [28]. All these methods were developed to investigate the lignin composition of lignocellulosic materials in native as well as processed state, e.g. pulps.

In Part 1 of this series a method for the quantification of the lignin content (Py-lignin) of Maritime pine (*Pinus pinaster* Aiton)

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and spruce wood (*Picea abies* [L.] Karst.) samples directly from the pyrograms was presented [3]. The good correlation found between the Py-lignin and Klason lignin content gave a common model for both species. In Part 2 [5] five subspecies of larch wood (*Larix* sp.) were used to evaluate this common model, revealing only small differences between the measured and the predicted Klason lignin contents. Compression wood was included due to the difference in lignin composition and content compared to normal wood. As the influence of compression wood was small a so-called “softwood model” including all samples was calculated which can be used for pine, larch, and spruce wood with the limitation of the highest and lowest values were the species-specific models lead to better results. However, slightly different slopes of the linear regression lines of pine and larch, and especially the one of spruce suggested possible differences in lignin and/or carbohydrate composition that should be investigated.

In this Part 3 we investigated differences in lignin composition using analytical pyrolysis and PCA. The focus was on species-specific differences as well as tissue-specific differences including the influence of so-called compression wood, a type of reaction wood formed under mechanical stresses [30].

2. Experimental

2.1. Samples

The 74 (12–14-year-old) Maritime pine (*P. pinaster* Aiton) wood samples (48 from Blagon and 26 from Vaquey both France) [31], 57 spruce (55 samples from about 19-year-old spruce trees (*P. abies* [L.] Karst.) from Sweden [32], two samples from a 30-year-old spruce tree (*P. abies* [L.] Karst.) from Austria [33], and 18 larch wood samples (*Larix decidua*, *Larix eurolepis*, *Larix kaempferi*) harvested at an age of 38 years and three larch wood samples (*L. decidua*) harvested at an age of 160 years [34–37] were used. The range of Klason lignin and Py-lignin contents as well as the H/G ratios of the samples is compiled in Table 1. The determination of the Py-lignin content was described in Part 1 of this series [3]. Details about the samples, preparation, extraction, and Klason lignin determination have been published [3,5,32–37].

Except for the Austrian spruce compression wood sample where the compression wood was also investigated and identified by microscopy [33], the tissue type assignment was mainly done visually on large samples (mostly discs). The presence and the amount were confirmed by analytical pyrolysis using the H/G ratio [3,5], Klason lignin [3,5,32], and infrared spectroscopy [4,33,36,38].

The following labels for species/sites: spruce from Sweden (S), spruce from Austria (A), pine from France Blagon (B) and Vaquey (V), larch wood from Europe (E), and for types of wood tissues: normal wood (N), reaction (compression) wood (R), opposite wood (O), total wood (T) meaning the whole disc including compression wood, opposite wood, aside wood (wood aside compression wood and opposite wood), and normal wood and unknown amount of

compression wood (X) were used. To simplify matters the latter will be called tissue types further on.

2.2. Analytical pyrolysis

Analytical pyrolysis (Py-GC/FID) was performed with a CDS Pyroprobe 1000 with a coil filament connected to a HP 5890 series II by a heated interface (270 °C). Each sample (75–80 µg) was pyrolysed at 650 °C for 10 s with a temperature rise time of approximately 20.0 °C ms⁻¹ [3].

Details about the samples, their preparation and the analytical pyrolysis were previously described in Parts 1 and 2 [3,5]. Moreover, the identification table of all compounds (pyrolysis products) can be found in Part 1 [3]. The peaks labelled H 2,3 in the table in Part 2 were separated (recalculated) for data analysis in this paper and are now labelled H 2 and H 3, respectively.

2.3. Multivariate data analysis

PCA was performed using the Unscrambler™ Vsn. 9.7 (CAMO). Prior PCA the percentage of each peak from the pyrogram (the area of a peak divided by the sum of the area of all used peaks multiplied by 100% [3]) was standardized (weighted by the standard deviation of each variable – peak). To level off the influence of the carbohydrates and varying lignin contents (see Section 3.4) the percentage of the G- and H-lignin-derived peaks from the pyrogram (the area of a peak divided by the sum of the area of all used peaks multiplied by 100%) was standardized (weighted by the standard deviation of each variable – peak).

3. Results and discussion

3.1. G- and H-lignin of all samples

Subjecting all samples to PCA using G- and H-lignin-derived pyrolysis products (peaks from the pyrogram which will further be called variables) clustering according to species – as shown in the PC 1–PC 2 scores plot (Fig. 1A) – was obtained. The variances explained are 40% by PC 1 and 22% by PC 2, respectively. The three species, pine, spruce, and larch were separated into four clusters with a small overlapping region between pine and larch wood at about zero (the average) of PC 1 (Fig. 1A) as well as between pine wood sites. The Sweden spruce samples even in higher number showed a tighter cluster compared to the other species. This suggests a more homogeneous lignin composition for spruce normal wood, also confirmed by the smallest H/G ratio range (Table 1).

The loadings plot (Fig. 1C) reveals that vanillin (G 9) and G=C=C (G 11) on one hand and isoeugenol (G 8) and dihydroconiferyl alcohol (G 19) on the other hand separate spruce and larch from pine. Beside others G 9 plus G 11 and G 8 plus G 19 separate spruce from larch as well as Vaquey pine from Blagon pine. Using only these four variables (G 8, G 9, G 11, and G 19) the

Table 1
Range of Klason lignin contents and H/G ratios of the samples.

Site	Species	Klason lignin (%)			Py-lignin (%)		H/G ratio	
		Min	Max	Average	Min	Max	Min	Max
Blagon, France	Pine	23.0	29.3	25.6	23.0	27.8	0.041	0.093
Vaquey, France	Pine	28.2	35.3	31.4	24.6	29.6	0.047	0.113
Europe	Larch	26.6	32.0	29.1	23.6	27.2	0.037	0.084
Sweden	Spruce	24.9	32.1	27.7	21.6	28.3	0.038	0.062
Austria ^a	Spruce	26.0	37.2	31.6	25.1	31.7	0.034	0.118

^a Only two samples, one normal wood and one reaction (compression) wood.

Fig. 1. Results of the PCA using all samples and G- and H-lignin variables (peaks from the pyrogram). (A and B) Show the PC 1 (40% explained variance) versus PC 2 (22% explained variance) scores plots, whereas in (A) the label indicates site and specie and in (B) the tissue type. (C) Shows the corresponding loadings plot of the G- and H-lignin variables. For abbreviations, see Section 2.1.

same pattern (not shown) was obtained. Of course also other variables contribute to the clustering but only the most important are mentioned in the text as the others are shown in the figures and the identification of the labels is given in Table 2.

Both Austrian spruce samples (A) are written in larger letters because they neither lie within the Sweden spruce cluster nor close to it. The main reason why the Austrian samples do not fit into the spruce cluster is the different lignin composition and the extreme high Klason lignin content (37.2%) of the compression wood (R) sample. Beside the high H-lignin content (H 1: phenol and H 2: *m*-

Table 2

The identification of the lignin-derived pyrolysis products used for the principal component analyses to reveal species-, tissue-, and/or site-specific differences. Separation of spruce and larch wood along PC 1 (52% explained variance) due to differences in lignin composition using only G- and H-lignin variables is indicated by S (more in spruce), O (average for both) or L (more in larch).

Label	Compound	S–L, PC 1
H 1	Phenol	L
H 2	<i>m</i> -Cresol	L
H 3	<i>p</i> -Cresol	S
G 1	Guaiacol	L
G 2	3-Methylguaiacol	L
G 3	4-Methylguaiacol	L
G 4	4-Vinylguaiacol	L
G 5	Eugenol	O
G 6	4-Propylguaiacol	L
G 7	Isoeugenol (<i>cis</i>)	S
G 8	Isoeugenol (<i>trans</i>)	L
G 9	Vanillin	S
G 10	G–C=C	S
G 11	G–C=C	S
G 12	Homovanillin	L
G 13	Acetoguaiacone	S
G 14	Guaiacyl acetone	L
G 15	Propioguaiacone	S
G 16	Structure isomer of coniferyl alcohol	O
G 17	G–CO–CH=CH ₂	S
G 18	G–CO–CO–CH ₃	S
G 19	Dihydroconiferyl alcohol	L
G 20	Coniferyl alcohol (<i>cis</i>)	L
G 21	Coniferyl alcohol (<i>trans</i>)	L
G 22	Coniferyl aldehyde	O

cresol) and high homovanillin (G 12) and propioguaiacone (G 15) contents (Fig. 1C) this compression wood sample shows a high 5-hydroxymethyl-2-furaldehyde content and a lower hydroxyacetaldehyde, 3-hydroxypropanal, 3-butenal-2-one, (3H)-furan-2-one content. This is an extreme and unusual spruce sample that is hard to find, more samples like this would be interesting to analyse and necessary to draw better conclusions.

Besides the separation of the three species a kind of separation between pine from Vaquey and Blagon could be reached, although some of the Blagon samples look closer to Vaquey than to Blagon (Fig. 1A). This shows that besides species-specific differences analytical pyrolysis has the potential to reveal also site-specific differences. Labelling the samples according to tissue types in the PC 1–PC 2 scores plot shows some separation of the tissue types within the species clusters (Fig. 1B). Pine wood samples could be analysed in detail, because several different tissue types were available.

The PCA using only pine wood samples and only G- and H-lignin variables separates Blagon pine from Vaquey pine with the exception of one Vaquey and one Blagon sample in the PC 1–PC 2 scores plot (Fig. 2A). Labelling the samples according to tissue types shows a partial separation revealing a progression from normal wood, over opposite wood, total wood to reaction wood in the PC 1–PC 2 scores plot (Fig. 2B) within each site (Vaquey or Blagon) cluster. The loadings plot (Fig. 2C) presents which lignin-derived pyrolysis products are responsible for the separation, whereas on the rightmost of PC 1 the H-lignin products can be found.

To avoid the influence of pine wood on the separation of spruce from larch wood, a PCA without pine using only G- and H-lignin variables was calculated and the results are listed in Table 2.

3.2. G- and H-lignin of reaction wood

A PCA using all reaction wood samples and G- and H-lignin variables was calculated focusing on lignin composition

Fig. 2. Results of the PCA using only pine wood samples and G- and H-lignin variables (peaks from the pyrogram). (A and B) Show the PC 1 (38% explained variance) versus PC 2 (26% explained variance) scores plots, whereas in (A) the label indicates the site and in (B) the tissue type. (C) Shows the corresponding loadings plot of the G- and H-lignin variables. For abbreviations, see Section 2.1.

differences of the reaction wood samples. The PC 1 (33%)–PC 2 (22%) scores plot allows distinguishing between species and also between sites for pine (Fig. 3A). Additionally to G 9 and G 11 that allowed separating larch from pine (Fig. 1A) G 3 (4-methylguaiacol) and G 20 (*cis*-coniferyl alcohol) contribute to the reaction wood pattern being above average represented by larch. The H-lignin products are higher in pine with H 2 at the average, H 1 higher in Blagon pine and H 3 higher in Vaquey pine. The PC 1 (33%)–PC 3 (16%) scores plot shows positive values for the spruce samples (Fig. 3C) with the extreme one far

from all others, which is due to the high H-lignin (mainly H 2) content (Fig. 3D).

3.3. G- and H-lignin of normal wood

Subjecting the normal wood samples to PCA using G- and H-lignin variables shows a clear clustering in the PC 1 (42%)–PC 2 (20%) scores plot (Fig. 4A). The Austrian spruce sample is close to H 2 and larch wood contains the most of H 2 besides G 3, G 8, and G 19, whereas much of H 1 can be found in pine and H 3 besides G 9, G 11, G 13, and G 17 contribute mainly to spruce (Fig. 4B). The PC 2 (20%)–PC 3 (13%) scores plot (Fig. 4C) that shows some separation within pine along PC 3 and between pine, spruce and larch along PC 2, whereas spruce is closer to pine, which is interesting from the genetic point of view (see also the loadings plot Fig. 4D), because phylogenetically spruce is closer to pine than to larch [39]. However, as the trees were grown on different sites (France and Sweden) a possible site effect cannot be excluded. Moreover it should be kept in mind that the average lignin contents of the species are different (Table 1) which may partly contribute to the separation.

3.4. G- and H-lignin of pine wood from one site

The pine wood samples from Blagon were used to investigate differences in the lignin composition between the tissue types. The carbohydrates-derived pyrolysis products self-evident contribute to the total sum of all peaks, which has an impact on the percentage of the lignin-derived peaks. To level off the influence of the carbohydrates and varying lignin contents, only G- and H-lignin-derived peaks were used (the area of each lignin peak divided by the sum of the area of all lignin peaks multiplied by 100%) and standardized (weighted by the standard deviation of each variable – peak) prior to PCA.

A PCA calculated using the Blagon pine wood samples and G- and H-lignin variables gave a PC 1 (31%)–PC 2 (19%) scores plot that allows distinguishing between tissues (Fig. 5A). Along PC 1 reaction wood could be separated from normal wood and opposite wood, and along PC 2 normal wood could be partly separated from opposite wood. It was expected that the separation of normal wood from opposite wood is difficult because, e.g. Lohrasebi et al. [40] found for black spruce that the differences in the chemical composition between them are small. The contribution of each variable is shown in the loadings plot (Fig. 5B). Around the average of PC 1 several tissue types can be found. The assignment of tissue type was done visually on large samples (mostly discs), which is not always unambiguous. Even using fluorescence microscopy [41,42] or digital imaging [43] only three kinds of wood-types in the images: normal wood, mild, and severe compression wood [42] can be discriminated normally from small samples. Nanayakkara [42] stated that the lignin content of mild compression wood was 18% higher while severe compression wood lignin content was 30–38% higher than that of opposite wood, and found a good agreement between the anatomical classification of the wood samples and their lignin content [42].

As it is known that the lignin composition is different in tissue-types [28] or cell wall types [19,44,45] analytical pyrolysis was shown to have the potential to discriminate them. The samples with unknown compression wood amount can now be tentatively assigned, and also the ones found around the average of PC 1 should maybe (re)assigned to the same tissue-type. Interestingly H 3 (*p*-cresol), which was found to be higher in the pine samples from site Vaquey (Fig. 2C) and the normal wood spruce samples (Fig. 4B), is about the average in all tissue types of Blagon pine. However, to reduce the number of variables focusing on the most relevant, the

Fig. 3. Results of the PCA using all reaction wood samples and G- and H-lignin variables (peaks from the pyrogram). (A) Shows the PC 1 (33% explained variance) versus PC 2 (22% explained variance) scores plot and C the PC 1 (33% explained variance) versus PC 3 (16% explained variance) scores plot, whereas the label indicates the site. (B and D) Show the corresponding loadings plots of the G- and H-lignin variables. For abbreviations, see Section 2.1.

ones having loadings below ± 0.1 of the average of PC 1 (H 3 and G-lignin products written in light grey in Fig. 5B) were removed and the PCA recalculated without them. As expected a similar pattern was obtained for the PC 1 (45%) versus PC 2 (18%) scores plot (Fig. 5C). Reaction wood (R1, R2, and R3) and opposite wood (O1, O2, and O3) samples obtained from the same discs that are connected with arrows lie diametrically. Beside H-lignin (phenol (H 1) and *m*-cresol (H 2)) that is well known to be higher in compression wood, isoeugenol (G 7), homovanillin (G 12), propioguaiacone (G 15), and the coniferyl alcohols (*cis* G 20 and *trans* G 21) were higher compared to normal and opposite wood.

Vanillin (G 9) and G-C=C=C (G 11) that separated spruce from the other species (Fig. 1A) using all samples were also important when only normal wood was investigated (Fig. 4A and B). Also within pine these products were enriched in normal and opposite wood (Fig. 5B and D). Isoeugenol (G 8) and dihydroconiferyl alcohol (G 19) that separated larch from the other species (Fig. 1A) were always important for the separation of larch meaning for reaction wood (Fig. 3A–D) as well as normal wood (Fig. 4A–D). Besides the high amounts of 4-methylguaiacol (G 3) formed from larch wood from both compression wood (Fig. 3) and normal wood (Fig. 4) compared to the others, *cis*-coniferyl alcohol (G 20) was also always found in reaction wood (Fig. 3B and D, and Fig. 5 B and D).

3.5. Lignin-derived pyrolysis products and lignin structure

Methods such as thioacidolysis, DFRC, and analytical pyrolysis were developed to investigate the lignin composition of lignocellulosic materials in native as well as processed state, e.g. pulps. As the lignin composition is known to influence the pulping and the bleaching efficiency [46–48] several attempts were undertaken

to alter lignin content and composition by tree breeding [49] and genetic improvement [48,50,51], which by the way lead to new insights into the biosynthetic pathway [52].

Gellerstedt and Zhang [53] summarized some of the residual Kraft lignin features: a low remaining amount of β -O-4 structures [54], linkages between lignin and polysaccharides, the presence of reduced structures such as methylene and methyl groups [55], high degree in discoloration [56], successive increase of “condensed” structures with high degree of delignification [55], and an uneven distribution of lignin across the fibre wall. Although thioacidolysis and DFRC are the nowadays preferred methods to investigate lignin structure [27], analytical pyrolysis gives an overall picture of the sample [1,2,6,57–61] accounting for interactions between carbohydrates and lignin [62].

Pyrolytic degradation of lignin starts at about 200 and up 400 °C linkages between the lignin units are cleaved of which the α -O-4 ether bond is the weakest. Product yields increase with increasing temperatures but the decomposition chemistry becomes more complex as secondary decomposition also takes place resulting in, e.g. the conversion of guaiacols into catechols [16]. The various instrumental set-ups and different pyrolysis conditions, e.g. temperatures from 200 to 400 °C [63], 450 °C [64], 500 °C [65], 580 °C [57], 650 °C [3,5], and up to gasification temperature at 800 °C [62,66] used additionally complicate comparison of results obtained by several groups, which suggests a kind of standardisation. Moreover, care has to be taken by comparing the results with the literature, although most of them are from analytical pyrolysis works, some are from pyrolysis works for energy. Although in the past analytical pyrolysis was focused on the determination of G-, S-, and H-lignin contents and their alterations during processing, e.g. pulping [48], bleaching [67], and fungal treatment [68,69] based on

Fig. 4. Results of the PCA using normal wood samples and G- and H-lignin variables (peaks from the pyrogram). (A) Shows the PC 1 (42% explained variance) versus PC 2 (20% explained variance) scores plot and C the PC 2 (20% explained variance) versus PC 3 (13% explained variance) scores plot, whereas the label indicates the site. (B and D) Show the corresponding loadings plots of the G- and H-lignin variables. For abbreviations, see Section 2.1.

their pyrolysis products [6,59,67], the potential to provide more information is well known [11] even lacking an assignment of pyrolysis products to lignin structure [11]. Nevertheless several efforts were made to assign lignin-derived pyrolysis products using wood, lignin isolated from wood and/or lignin model compounds [17,57,58,63,70–74] especially in the last years [75–78]. Model compound studies have shown that the β -O-4 linkage is cleaved extensively in pyrolysis, but only partial cleavage of carbon–carbon bonds are reported to take place [57,73], and that the phenolic forms are in general much more reactive than the non-phenolic forms [75,76].

Unfortunately nothing could be found about the lignin structure of larch wood. The higher contents of 4-methylguaiacol (G 3) and dihydroconiferyl alcohol (G 19) may arise from higher amounts of β -5 structures as Kuroda and Nakagawa-izumi [72] found those products after pyrolysis of phenolic 2-aryl coumaran type lignin model compounds. Recently Kawamoto et al. [77] published that α -ether-type dimers and α,β -diether-type trimers give isoeugenol, *para*-substituted phenols, and guaiacol during pyrolysis which gives hint that possibly more of these structures are present in pine compression wood where higher contents of these pyrolysis products were found (Fig. 5B and D). According to Choi et al. [59] higher yields of isoeugenol found in spruce wood pulps can be interpreted as indication of the presence of β -O-4 linkages with OH groups in α -position from which water is split easily during pyrolysis. This reaction leads to degradation products with unsaturated side-chains. Therefore the β -O-4 linkages with OH groups in α -position can be a source of the high isoeugenol (G 8) contents found in larch wood (Figs. 3 and 4). The isoeugenol isomers were found to be of different importance for the differentiation

between larch and pinewood. In larch reaction wood and pine normal as well as opposite wood the *trans* isomer dominates and in pine compression wood the *cis* isomer is higher. The *cis* isomer was also found to be higher in spruce wood than in larch wood (Table 2). The higher amount of coniferyl alcohol found in pine compression wood could arise from the cleavage of β -O-4 linkages as Nakamura et al. [63] found that coniferyl alcohol is one of the major pyrolysis products from phenolic β -ether dimers especially formed at higher temperatures. In contrast a decrease of uncondensed arylglycerol- β -aryl ether linkages in compression wood compared to normal wood of southern pine was reported [79,80] but they found higher phenolic and aliphatic OH, a higher number of etherified 5–5' linkages, and the major difference in H-units were attributed to non-conjugated *p*-hydroxyphenyl moieties.

Using thioacidolysis and DFRC a much higher content of uncondensed β -O-4 linkages were found in southern pine normal wood compared to compression wood with both being higher than the ones found in spruce [28]. Yeh et al. [81] investigated loblolly pine compression wood of juvenile wood and mature wood with the main difference found was the higher content of aliphatic OH groups mature compression wood. Moreover, it is also reported that H-lignin pyrolysis products are formed not only from lignin but arise also from proteins [59] and from carbohydrates that can form aromatics during pulping [57]. Homovanillin – higher amounts obtained from compression wood – was found to increase during bleaching of softwood Kraft pulps [57]. As it is well known [40,80,82] that compression wood is more difficult to pulp and bleach, it is likely that lignin-structures giving more homovanillin during pyrolysis are enriched during compression wood formation.

Fig. 5. Results of the PCAs using pine wood samples from Blagon and G- and H-lignin variables (peaks from the pyrogram). (A) Shows the PC 1 (31% explained variance) versus PC 2 (19% explained variance) scores plot with labels indicating the tissue type. (B) Shows the corresponding loadings plot of the G- and H-lignin variables. (C) Shows the PC 1 (45% explained variance) versus PC 2 (18% explained variance) scores plot with labels indicating the tissue type. The arrows connect the reaction wood with the opposite wood obtained from the same disc. (D) Shows the corresponding loadings plot of the G- and H-lignin variables. For abbreviations, see Section 2.1.

Although speculative and no direct or definitive conclusions can be drawn from the cited works (different species, conditions, model compounds) they give hints to probable structures and/or structural differences of lignin between species and tissues.

Moreover, differences in lignin composition were found, but no explanation can be provided at this time why the slopes of the correlations found between Py-lignin and Klason lignin in Part 2 [5] are slightly different for the species.

4. Conclusions

PCA performed using G- and H-lignin-derived pyrolysis products allowed to separate pine, spruce, and larch wood in the scores plot according to species. The corresponding loadings plot revealed the substances responsible for the clustering. Besides these species-specific differences, in the case of pine site-specific as well as tissue-specific differences could be shown. Tissue types could be separated partially showing a progression from normal wood, over opposite wood, total wood to reaction wood.

However, further investigations will reveal the differences in the carbohydrate composition, their contribution to the Py-lignin determination as well as their influence on the separation according to species, tissues and sites.

Analytical pyrolysis has proved to be a good technique to reveal species- and tissue-specific differences with the potential to disclose site-specific differences in lignin composition.

Although some hints on structural differences of lignin could be obtained from several studies, the assignment of pyrolysis products to lignin structures is still a challenge for the next decade.

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