PyroWood

A holistic approach to assess phenotype-dependent variations in chemical composition of softwoods on the tree-ring level

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Final Report

8 – Detailed description of activities

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1 Summary

The main achievements of the project, listed not in terms of importance, but in relation with the task order were:

The successful development of a semi-micro drilling procedure that enhance the speed of the sample collection at a sub year level and the development of a scale-up procedure for medium throughput extraction of the samples (Task1).

The development of a MS library for the pyrolysis of the pyrolysis products of the extractive-free wood, while the library of the extractives is still ongoing (Task 2).

The separation of species and tissues types according to the polysaccharide pyrolysis products (Task 3).

The challenging identification and quantification of the oxidized and dehydrated resinic acids from *Pinus sylvestris* trees, and the comparison of the quantification between GC/MS and NMR (Task 4).

Confirmation of the trend for lignin and new insights from the polysaccharide composition (Task 5) and content (Task 6) for altitude, latitude and age. As well as the analysis and quantification of lignin amount and composition at sub year level (early- and late-wood) and the quantitative analysis of sugars based on pyrograms (Task 6).

The development of PLS-R models based on NIR and ATR-FTIR for solid state analysis of *Picea abies*. The development of PLS-R models based on NIR for the extractives content of *P. sylvestris* and *P. halepensis*. The development of PLS-R models based on NIR for sugars analyzed by HPLC and cellulose diglyme. The detailed analysis of the cellulose diglyme yield and the correction of the yield for residual lignin determined by analytical pyrolysis.

The main weak point is the record of publication since the core results achieved were not published yet.

In association with the project one paper was published in the application of analytical pyrolysis to the characterization and identification of the wood in a wood-plastic composite. Also a paper under revisison for publication in the area of the characterization of wood by analytical pyrolysis in a collaboration with Universidad Politecnica de Madrid. Recently a paper was submitted to Holzforschung (Task 7). Two manuscripts are prepared in article format for submission (Task 4). Previewed at least two more publications with the main results from tasks 5 and 6.

2 Introduction

This project was a IC&DT Project for Consolidation of Competences and Research Resources (Consolidação de Competências e Recursos em Investigação), aiming to develop competitive competencies for the participation in International Programs, so the budget was mainly for hiring scholarships and the acquisition of dedicated equipment. However, there was no budget for travel, so it was not possible to collect samples.

The first accomplishment was the selection and purchase of the automated analytical pyrolysis equipment required for the implementation of the project. It was selected and acquired a micro furnace pyrolyzer from Frontier-Laboratories Ltd (Fukushima, Japan), model Single-Shot Pyrolyzer Py-3030 S equipped with a 48 position auto-sampler AS 1020E. The equipment was installed on October 2013.

The overall objective of the project was 1) increase the throughput of the analytical pyrolysis, that alone would justify the acquisition of the equipment for the quantitative analysis of lignin amount and composition, parameters already proved to work by analytical pyrolysis, and the acquisition of an equipment would in principle quintuplicate the throughput n a week basis, and 2) develop procedures to allow the qualitative and quantitative assessment of the extractives and the polysaccharide wood components from the pyrograms. The result of this achievement is a single, simple technique capable of providing the complete chemical analysis of the wood samples in a 50 min pyrolysis run.

The final goal and the main objectives were to use this tool for:

a) confirmation of the trend found for latitude- and altitude-dependent lignin composition obtained in the preliminary work by analyzing additional samples.

b) develop a protocol for sample preparation down to a sub-tree-ring level (earlywood-latewood) for analytical pyrolysis,

c) Identification of the extractives fragments not known up-to-date

d) and knowledge about possible interdependencies between the main wood components and the extractives during pyrolysis

e) qualitative and quantitative phenotypic discriminations of latitude-, site-, species-, age-, genetic-, and drought-dependent variations in lignin-, polysaccharide-, and extractives content and composition based on the pyrograms are obtain.

The objective attained fully attained were a, b and d while c and e were only partially attained. The identification of a few important extractives fragments is still going one (e)

that are also needed to fully fulfil objective e regarding the extractives since lignin and polysaccharide were fully attained.

The project suffered however one major drawback the death of Dr. Manfred Schwanninger in December 2013 a good friend and an enthusiastic researcher that accepted to join the project despite the fact that the project could not support financially its activities, namely the activities related to the interpretation and acquisition of the spectra in solid state by infrared microscopy using an ATR-objective (Task 7). This part of the work was not performed, instead we assessed a single reflection ATR-FTIR equipment and also the FT-NIR using a fiber probe to partly replace the information expected form the Microscope-FTIR.

However, this was not the only problem because the new samples MS was preparing to send us for the confirmation of latitude-, site-, species-, age-studies suffered a delay and were received in the beginning of 2015. Although enough for the fulfillment of the project requirements, more interesting results could be attained if more samples could be received to further validate the new findings.

The project suffered other delays some due to the way the budget is delivered from FCT, even in a project like this were expensive equipment were to be purchased we received only 12.5% of the budget, not even enough to buy the equipment let alone buying the equipment and hiring the scholarships. Also due to a problem in the FCT accounting system the second tranche was only received one year after the initial payment.

Finally, the contract was initially signed by IICT were PI worked, that was extinct in 31 July 2015 and the PI and the project integrated in the Instituto Superior de Agronomia, Universidade de Lisboa.

Although all efforts were done to the fulfillment of the objectives of the project the publication of the results suffered the most, this activity will be continually pursued in the coming months.

3.1 Task 1 Sample preparation

This task, besides the preparation of the samples for the remaining tasks it included the establishment of a protocol for the sample preparation down to a sub-tree-ring level (earlywood-latewood) for analytical pyrolysis. The samples prepared in this task were not mentioned here to avoid duplicating the information. Here we will only mention major changes in the sample preparation of the protocols both related to particle size reduction as well as extraction. Extraction is an important step for preparation of samples for further chemical analysis

Particle size reduction

The main requirement for analytical pyrolysis is to obtain a fine powder (sub-micron to 10 microns), that is usually achieved by using of a vibratory ball mill as the last milling step. But an essential step before ball-milling is the size reduction of the sample by a coarser (200 to 2000 microns) grinding with a knife mill followed by a fine grinding (< 500 microns) using an ultra-centrifugal mill. One of the main constrains of the multistep size reduction is the loss of sample at each step. Even if for the sampling at a sub-tree-ring level (earlywood-latewood) annual cross rings the coarser mill (200 to 2000 microns) could be avoided the loss of material at the ultra-centrifugal grinding make it more difficult the sampling at the sub ring level especially so in the smaller rings. One way to circumvent the loss of material due to grinding was to obtain microtome sections as a sampling procedure for the sub-tree-ring level (earlywood-latewood). However, this procedure that was foreseen in the beginning of the project soon revealed its limitations. The main problem was that it was almost impossible to use with core samples that constituted the sole type of samples available for some species. The second limitation was that after steaming, required for microtome, it become increasingly difficult to distinguish earlywood and latewood, especially so at the microtome cuttings.

Semimicro-drilling

The main scientific achievement of this task was the successful establishment of a new protocol for wood sampling for analytical pyrolysis analysis (Py analysis). This protocol was not foreseen in the project proposal. The new procedure consists of sample collection by means of a semimicro-drill with a nominal diameter equal or below 0.5 mm, similar to the ones used in watchcraft (Figure 1.1), a very simple idea quite easy to implement and with several advantages over the proposed microtome cuttings or also milling:

i) time saving by overriding the necessity of the time consuming wood steaming step (minimum 4 hours) required for microtoming let alone the cutting process; instead, drilling will typically take less than 5 sec; ii) saves samples: to obtain a microtome slice, a small block is required to be clamped in the microtome, to obtain each set of slices a large part of the block is discarded due to the steaming discoloration issues. The material removed by drilling is more than enough for the Py-analysis and the remaining material can still be kept for future analysis, this is a minimally invasive procedure; iii) the particle size which results from the microdrill sampling is very similar to the obtained using the "normal Py-analysis procedure" (in use for more than 15 years) that requires a first reduction of the material using a two-step milling, first with a knife mill followed by an ultra-centrifugal mill (the normal procedure for chemical analysis) and finally a fine milling required for Py-analysis with a ball mill for at least 15 to 30 min, depending on the sample size. The resulting pyrogram is not only very similar but also the quantitative results derived from it are not statistically different from the "normal procedure" results; iv) semimicro-drilling also allows a precise sampling down to a subtree-ring level (earlywood/latewood) even when the annual increment growth is very small. This surpasses the sampling problems with the microtoming procedure. Due to the darkening of the sample after steaming it was very difficult, in some cases almost impossible, to recognize the earlywood/latewood boundaries, in some cases even in two adjacent growth rings after the steam treatment; v) the attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra of the microdrill samples were not only easier to obtain but also of better quality than the spectra obtained with microtome slices. The additional advantage is that the acquisition of transmission spectra using the KBr pellet technique is now possible which is, by far, the technique used on most reference spectra available. The quality of the ATR-FTIR spectra depends largely on the intimate adherence of the material to the diamond window of the ATR accessory, the disruption of the thin microtome slices during clamping forced the use of thicker slices that in turn make it more difficult to obtain a proper adherence to the window hence a poor quality spectrum. So far the smallest drill we found is a 500 micron, which allows a very detailed subsampling in some cases a transept in earlywood can also be performed.

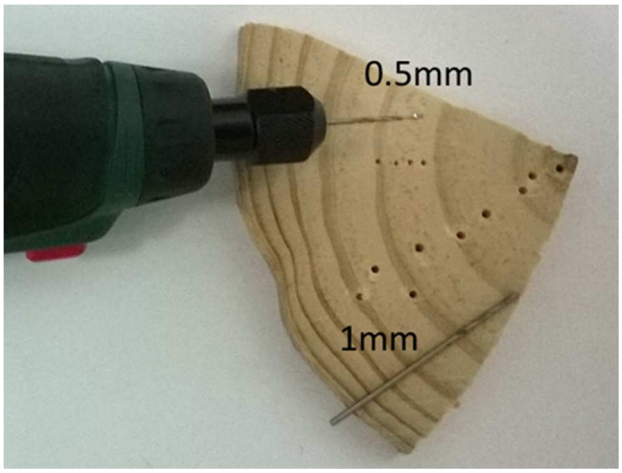


Figure 1.1 Semi-micron drilling with 1mm and 500 micron drills

Scale up extraction with filter bags

Wood extractives are substances that can be removed by solvents which are not considered a structural part of the cell wall or the middle lamella. A sequence of solvents such as toluene, dichloromethane, ethanol, methanol and water with increasing polarity are used in accordance with standard methods using Soxhlet extractors. The removal of extractives from the wood meal is generally the first, very time consuming step for the wet chemical analysis of wood meal. For analytical pyrolysis if the goal is to assess the wood chemical composition it is also necessary to remove the extractives. The standard procedure using thimbles and especially so using a single thimble per Soxhlet extractor was at very least cumbersome and really not efficient. A new approach was developed that allow scaling up the number of samples analyzed per Soxhlet extractor and at the same time make it more practical and with lower probability of losing material, so important not only for the correct assessment of the extractives content but also due to the low amount of material for the sampling to a subtree-ring level (earlywood/latewood). The procedure uses F57 Ankon (https://www.ankom.com) filter bags instead of the traditional cellulose thimbles, each F57 filter bag can accommodate about 1200 mg of wood powder, so for the low sample amount of the early- latewood sampling, the bags could be further divided in 4 sections. The identification of the sample is also simplified by marking the filter bag with a pencil, the filter bag is also a convenient storage of the sample while waiting for analysis. The sample filled filter bags were sealed with a heat sealer. An analysis of the influence of several steps was conducted in order to assess for instance the influence of the heat in the tare that was find negligible but previous extraction of the filter bags with organic solvent were deemed necessary for quantification

purposes or for the further analysis of the extractives, despite the manufacturer early claiming that the F57 filter bags were extractive-free. By using the samples in heated closed filter bags allowed a variable number of filter-bags/samples, depending on the sample amount in each filter-bag or the size of the filter-bag, to be extracted together in the same Soxhlet (Fig. 2.1). The quantification using this procedure is based in the weight loss at each solvent-extraction step. A protocol for the extraction was setup.

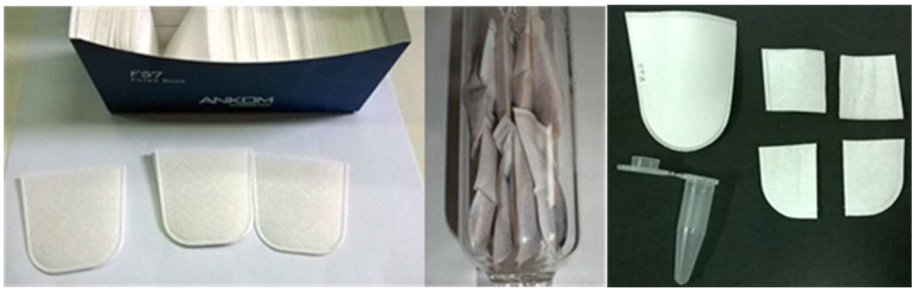


Figure 2.1 F57 filter bags (Ankon), Soxhlet extractor with 8 samples ready for extraction, and a filter bag sized to fit 4 samples.

3.2 Task 2 Setup of the automatic pyrolysis analysis

The main objective of this task was the development of a protocol for the pyrolysis (Py) analysis using the micro-furnace Single-Shot Pyrolyzer Py-3030S equipped with a 48 position auto-sampler AS 1020E (Frontier-Laboratories Ltd), hereafter referred to as MF-Py. The first step was the identification of the pyrolysis products in the MF-Py pyrogram (chromatogram of pyrolysis products). This step took considerably more time than expected due to the fact that at least 30 % of the pyrolysis products were not found in the existent mass spectra library of the pyrolysis products obtained with a resistively heated filament pyrolyser (CDS), hereafter referred to as RH-Py. While the pyrolysis products with known mass- spectra was trivial, the identification of the new py-products was challenging. One major achievement of this task was the creation of a dedicated mass spectra library of the wood MF-Py pyrolysis products with the identification of the most important pyrolysis products (about 70) required for the characterization of the softwoods. The quantitative analysis of the pyrolysis results obtained by the MF-PY versus RH-Py was based only in the well proved quantification results for lignin content (py-lignin) and composition (H/G) based on RH-Py. For this analysis twenty selected samples of *Pinus sylvestris* were analyzed by both pyrolysers. The comparison of the py-

lignin and H/G between the two pyrolysers considering all identified peaks was pretty bad, but did not improved by selecting only the pyrolysis products present in both pyrograms. The new pyrolysis products obtained with MF-Py were removed from these pyrograms as well as the pyrolysis products that were only present in the RH-Py were also removed (Figure 2.1). The correlation between MF-Py/RH-Py was even worst.

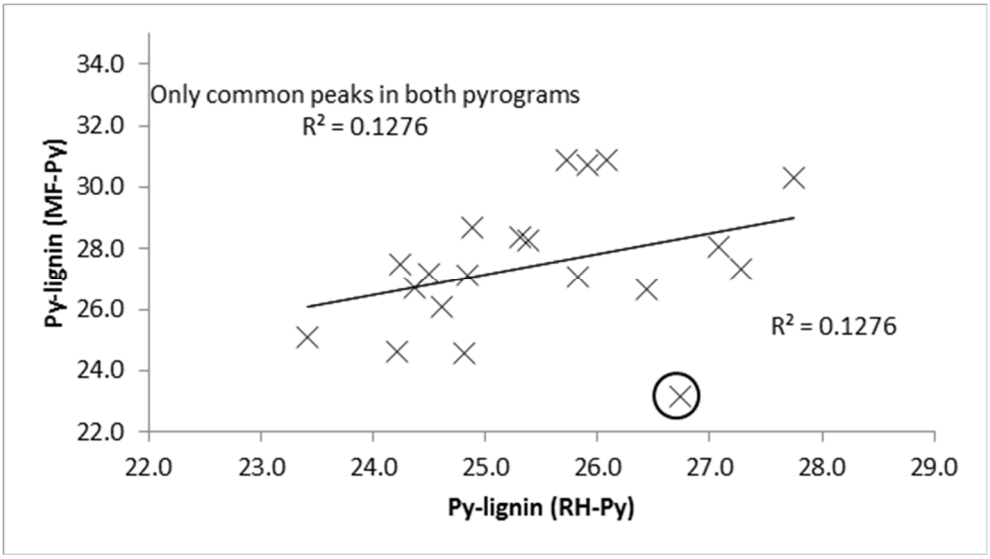


Figure 2.1 Py-lignin based on MF-Py vs RH-Py

By the way the correlation between the RH-Py quantification using all identified peaks vs using only the common peaks are almost identical with a coefficient of determination close to one. This was indeed an interesting finding, meaning that a simplification of the calculations by using less peaks is possible and giving identical results. This also showed that the inconsistencies were mainly attributable to MF-Py, indeed replicates of the same sample gave inconsistent results. A further analysis of the results in Figure 2.1 call the attention for the sample marked with a circle, clearly the most deviant results. A study was setup to access the influence of the conditions of the capillary column (J&W Scientific, DB-1701, 60 m × 0.25 mm, 0.25 µm film thickness) in the pyrolysis results. It was suspected and confirmed that the enhanced degradation of the column was responsible for the inconsistency of the pyrolysis results that, in the worst cases, consisted in the disappearance of pyrolysis peaks from the pyrogram. The most affected pyrolysis products were 36cP, 69cH and 73cH, that almost disappeared from the pyrogram, and the 41cP that was much smaller than usual. The degradation of the column in principle is due to the higher number of samples analyzed with the auto sampler, 10 times more samples

per week than using the manual system and also possibly due to the required higher interface temperature maintained at 300 ºC. A maintenance protocol was established and scheduled to once a week, that involved the usual maintenance of the pyrolyser as well as the chromatograph but also by cutting the first 25 cm of the capillary column attached to the injector. Since the time was running very fast and due to the uncertainty regarding a major improvement of the repeatability of the data, also associated to the fact that the results, no matter what, would not be similar to the RH-Py, it was decided to keep the MF-Py with the MS detector for qualitative work and circumscribed analysis, and maintain all the quantification by the RH-Py with the FID detector.

However, since the GC of the CDS system was also changed from an Agilent 6890 to an Agilent 7820 a test with a sample was performed in order to compare the results of both GCs. The reproducibility of the new GC was generally better than the old GC, for all parameters assessed.

New GC 5 aliquots x 3 replicates							**Old GC** 2 replicates
Different aliquots	**1**	**2**	**3**	**4**	**5**	**AV Ali**	**AV**
Py-lignin	**8.9 ± 0.11**	**9.2 ± 0.02**	**9.0 ± 0.18**	**9.0 ± 0.14**	**9.0 ± 0.23**	**9.0 ± 0.15**	**9.2 ± 0.26**
H/G	**0.15 ± 0.002**	**0.15 ± 0.004**	**0.16 ± 0.003**	**0.16 ± 0.006**	**0.16 ± 0.011**	**0.15 ± 0.005**	**0.16 ± 0.012**
cP/cH	**6.1 ± 0.01**	**6.3 ± 0.13**	**6.4 ± 0.13**	**6.2 ± 0.21**	**6.2 ± 0.17**	**6.3 ± 0.16**	**7.4 ± 0.30**

3.3 Task 3 Polysaccharide assessment

The objective of Task 3 was to study the volatile polysaccharide fragments to gain knowledge on the amount and composition of cellulose and hemicelluloses in order to allow the quantification of the main components. At least a quantification of the cellulose and hemicelluloses content was expected, but a finer detail was also thought possible at least for the C6/C5 ratio that could provide useful information for applications like the

saccharification potential. Wood, a composite of cellulose, hemicelluloses, lignin, and extractives, is the imprint of past ontogenic and environmental effects occurring during the life of the tree. The biosynthesis of wood formation and therefore its chemical composition depends on a number of factors, such as wood species, genetic, anatomical parts of wood, geographic location, habitation, growth, climatic characteristics, and degree of fungal and insect attacks. This has been exploited for more than 100 years as a record of climate and biological events among others, mainly based on growth patterns of the tree-rings. Variations in the lignin and polysaccharide content and composition can thus be used as indicators for changes in the environmental conditions recorded on the wood of the living tree. However, few studies have been focused on the genetic as well as environmental factors controlling the formation of tree-rings in terms of lignin amount and composition and none on the polysaccharides and extractives. The main limitations of the analysis by the traditional analytical methods used for wood chemistry is the necessity to combine several degradative techniques, with associated high costs, and the large amount of sample needed, which makes the analyses of inter-annual variation, especially within narrow rings, very difficult or impossible. Analytical pyrolysis, a cost-effective technique that needs below 100 microgram of a sample would allow overcoming these limitations. Analytical pyrolysis (Py-GC/MS and Py-GC/FID), provides a fingerprint of the whole chemical composition of the wood that can be used for the phenotypic variation at the tree-ring level. It is a micro-chemical technique that converts, by heat in the absence of oxygen, nonvolatile polymers into a volatile degradation mixture that are separated by gas chromatography (GC) identified by mass spectrometry (MS) and quantified with a flame ionization detector (FID).

The initial insight in the information of the carbohydrate pyrolysis products of the wood polysaccharide fraction was performed with the same samples already used to assess the information for the lignin derived pyrolysis products. The sampling included 74 (12–14-year-old) Maritime pine (*Pinus pinaster* Aiton) wood samples (48 from Blagon and 26 from Vacquey both France), 57 spruce (55 samples from about 19-year-old spruce trees (*Picea abies* [L.] Karst.) from Sweden, two samples from a 30-year-old spruce tree (*P. abies* [L.] Karst.) from Austria), 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.

Subjecting all samples to principal component analysis (PCA) using c-, cP and cH-carbohydrate-derived pyrolysis products (peaks from the pyrogram, hereafter referred as

variables) resulted in three cluster according to species, as shown in the first principal score plane (Fig. 3.1A). The variances explained are 36% by PC 1 and 23% by PC 2, respectively. The three species, pine, spruce, and larch were separated into three clusters with the two pine wood sites clustered in one group and separated along the first principal component form the other two species. (Fig. 1A). The three clusters were large showing a large variation among trees within species. The separation between spruce and larch is along PC 2.

The loadings plot (Fig. 1C) reveals that, Pyran-(4H)-4-one, 2-hydroxymethyl-5-hydroxy-2,3-dihydro (32cH) and 5-Hydroxymethyl-2-furaldehyde (30cH) on one hand and Hydroxypropanone (8cH), Butanone-(2) (5c) and (5H)-Furan-2-one (18c) on the other hand separate spruce and larch from pine. The separation of larch from spruce along PC 2 is mainly due to 1,6-Anhydro-glucopyranose (levoglucosan - 34cH) plus 1,4:3,6-Dianhydro-glucopyranose (28cH) in one hand and among others 6cH in the other hand.

The samples in the left upper corner are the ones with the higher levoglucosan content, according to its position in the loadings plot. Using only cH variables also produce the same pattern as well as using only c also produce a very similar pattern but not using the cP variables.

These findings have two main differences regarding the clustering of the samples in the first principal scores plane based on the lignin derived products, that showed four clusters since the two pine wood sites clustered in two separate clusters. Also the larch and especially so the spruce, clusters were tight in comparison with the pine cluster.

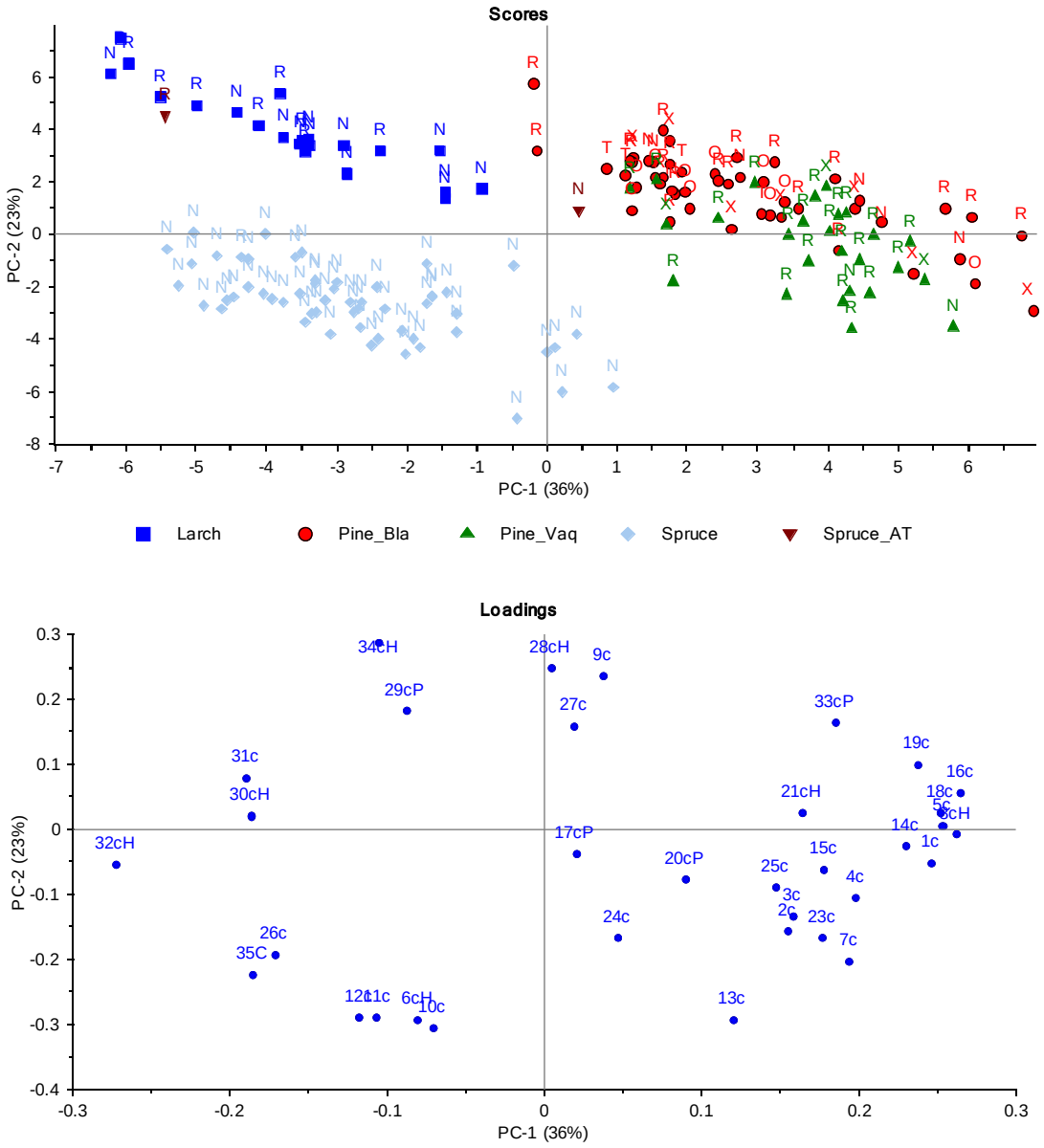


Figure 3.1 Results of the PCA using all samples and all carbohydrate pyrolysis variables. Upper score plot, lower loadings plot. Labels in the score plot indicates tissue type (N-normal, O-opposite, R-reaction, T-total) and color refer to species.

The labels referring to tissue types (N-normal-, R-reaction-, O-opposite- and T-total-wood) did not show the separation among them that was evident in the first scores plane of the lignin variables. The first main result from these analysis is that the reaction wood only affects the lignin composition but not the polysaccharide. Another point is that, as it is the case of the spruce wood, even if it is homogenous in terms of lignin content and composition does not imply that it will be homogeneous as well regarding the polysaccharide composition. No separation between pine from Vaquey and Blagon could

be reached, even using only pine wood samples or the individual set of carbohydrate groups alone (c, cH and cP). This shows that wood site-specific differences are only related to changes in the lignin composition, as revealed in the PCA of lignin products (not shown).

To avoid the influence of pine wood on the separation of spruce from larch wood, a PCA without pine using all carbohydrate variables was calculated (Figure 3.2).

A clearer separation was obtained in the principal scores plane, but more interesting is the respective loadings plot. It is levoglucosan, the main pyrolysis product of cellulose, that separates larch from spruce indicating that larch has higher cellulose content or its cellulose has a higher crystallinity degree, this is an interesting finding that should be further investigated. It seems that the variation among trees within species is related to Hydroxypropanone (8cH), 2-Hydroxy-1-methyl-cyclopenten-(1)-3-one (21cH) and a Gamma-lactone derivative (24c) in one hand and in the other hand 1,5-Anhydro-b-D-xylofuranose (33cP).

Also interesting are the Austrian spruce samples, they neither lie within the Sweden spruce cluster nor close to it. It should be noted that the Austrian spruce labeled as compression wood is actually only compression wood tissue, the remaining samples labeled as reaction wood refer to samples with important percent of compression wood but the compression wood tissue was not isolated as was the case of the Austrian spruce compression wood sample. This in principle could explain why it is so well separated from the Austrian spruce normal wood. However, this does not explain why the Austrian spruce normal wood also lie close to larch cluster than to the Swedish cluster. The fact that the Austrian samples (30 years-old) are close to larch (38 years-old) than to the Swedish spruce (19 years-old) in terms of age could be an explanation? More samples of the same and different age from Austria are needed to confirm/discard this supposition. It is certainly worth further investigation.

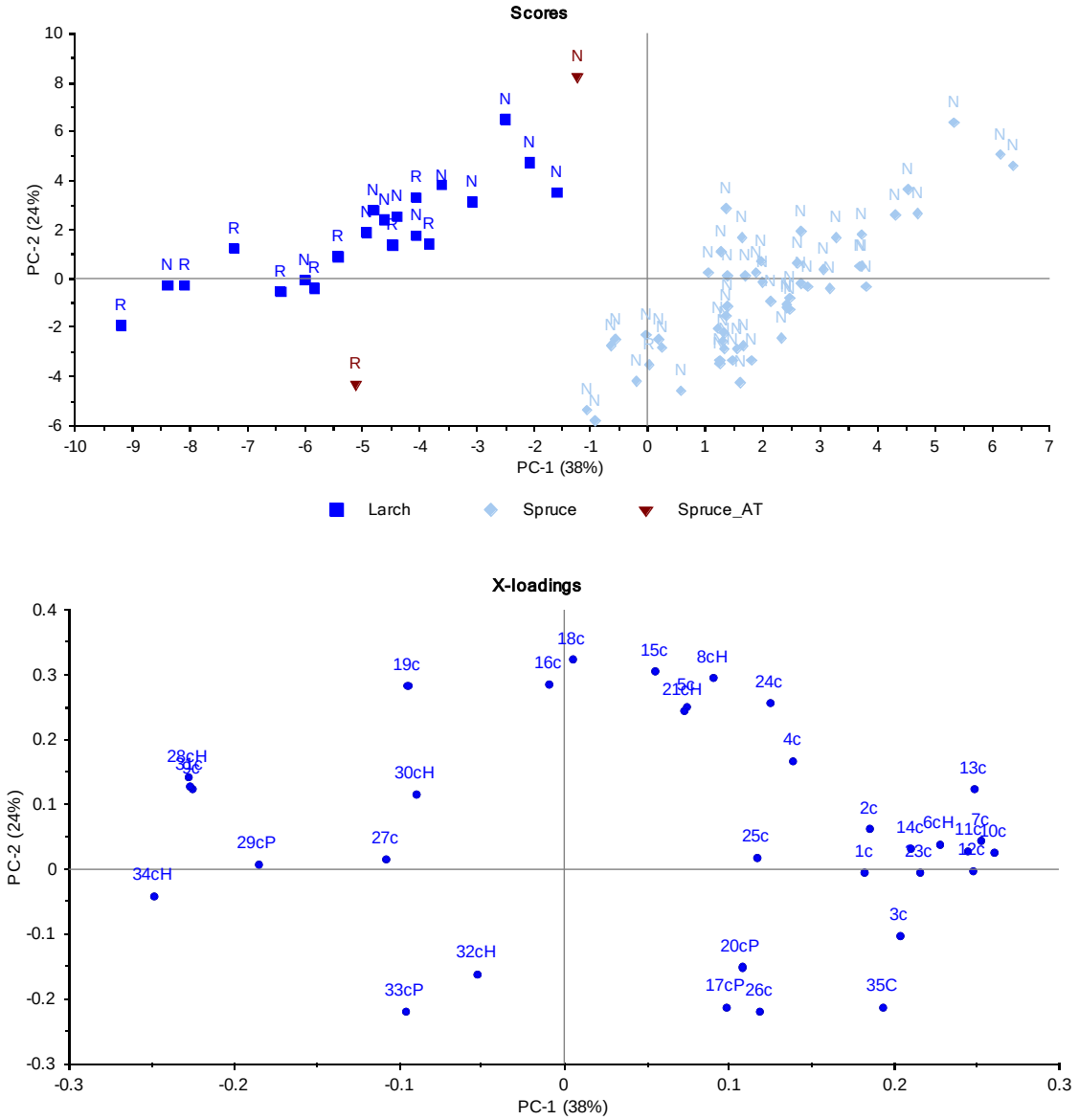


Figure 3.2 Results of the PCA using larch and spruce samples and all carbohydrate pyrolysis variables. Upper score plot, lower loadings plot. Labels in the score plot indicates tissue type (N-normal-, R-reaction-wood) and color refer to species.

3.4 Task 4 Wood extractives analysis

Task 4 was devoted to the identification of the extractives content and composition. Extractives have not only important industrial utilizations but their content and composition play important roles in many wood utilization and performance, such as natural durability. However, despite of their importance the information related with the

influence that the environment and genetic factors play in their formation is still scarce. The development of fast accurate methods especially in solid-state will allow to cover gaps in fundamental knowledge on extractives formation. Solid state analysis was performed by ATR-FTIR, FT-NIR and Py-GC/MS that was used for fast screening of the samples and selection of the interesting ones for detailed analysis.

Extracts of selected samples were analyzed in detail, the small molecules by GC/MS, bigger sized molecules, and structural confirmation and quantification of the main chemical families done by NMR. Additionally, the same extracts were also analyzed by Py-GC/MS.

Infrared spectroscopy, ATR-FTIR and FT-NIR

Fourier transform infrared spectroscopy with an attenuated total reflectance accessory (ATR-FTIR) is a solid state technique that provides a fingerprint of the chemical composition of the material. For qualitative analysis it excels in terms of high-throughput and the amount of information that can be retrieved form the spectra. It is a routine technique used for many purposes including quantitative and qualitative work.

Fourier transform near-infrared spectroscopy is a solid state technique that provides a fingerprint of the chemical composition of the material. For quantitative analysis it excels in terms of high-throughput although being an indirect technique requires calibration. In our laboratory it is a routine technique for many years now, used mainly for quantitative analysis, here we show that it can also be used for qualitative work providing a high-throughput screening tool.

The ATR-FTIR spectra of the *Pinus sylvestris* wood were acquired on the solid wood (strip), on the non-extracted meal, on the extractive-free meal and on the extracts.

The limitations of the ATR single reflection cell is evident for solid wood, in the spectra of the x-strip the spectra does not show the resin peaks in fact it is more similar to the spectra of the wood strip part without resin. On the other hand, the spectra collected in a thin slice taken from the strip could be squeezed against the ATR cell promoting the intimate contact, so the spectra clearly reflect the presence of resin with the most prominent carbonyl elongation peak from resinic acids at 1721 cm^{-1} and the aliphatic CH_2 elongation symmetric and asymmetric at 2925 cm^{-1} and 2869 cm^{-1}.

The problem is partly explained by the fact that the diamond cell is not levelled in the plate instead it is at a few microns below the surface, in the case of the strip wood this prevents an intimate contact between the sample and the cell window. As can be seen the

remaining of the spectra resembles the spectra of the wood, meaning that the other chemical components are less affected by this problem. This means that resin has a different refraction index from the remaining material and probably more close to the refraction index of the diamond cell so the evanescent ray is lost due the lack of contact. This means that at least for extractives (softwood) the equipment does not offer a real screening high-throughput tool to assess differences both in content or composition.

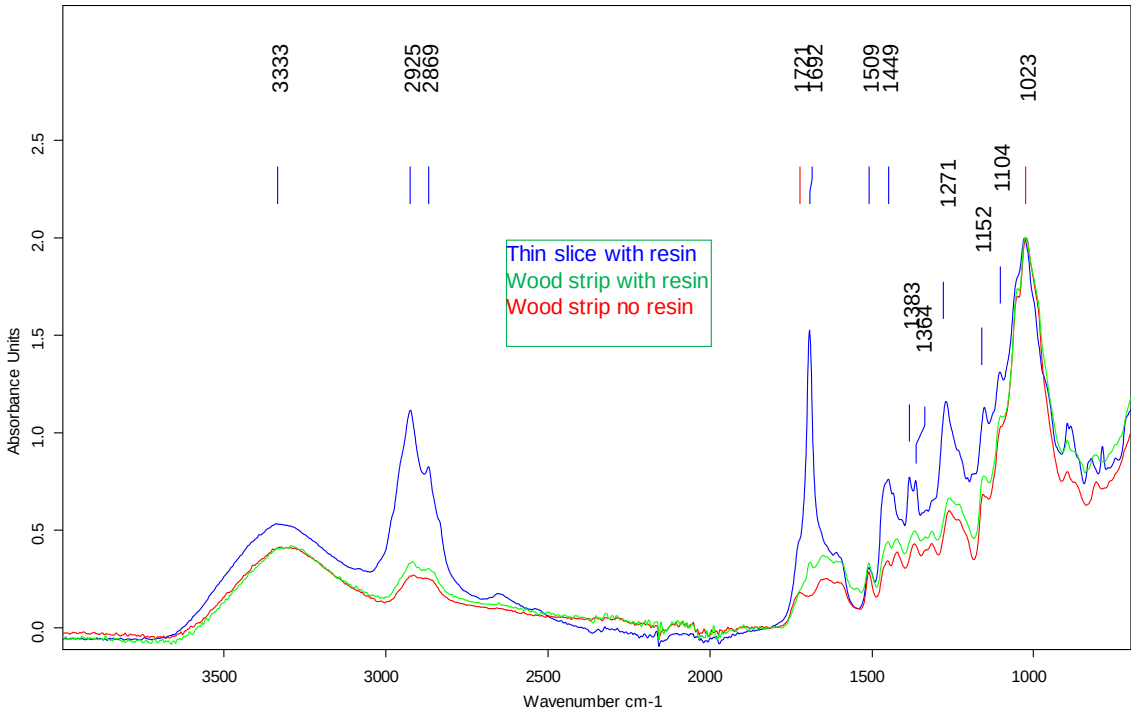


Figure 4.1 ATR-FTIR spectra of a thin slide with resin (blue), and from the x-ray strip with resin (green) and without resin (red).

In the same strip FT-NIR spectra were acquired to test the suitability of NIR for screening samples for extractives content. The spectra of the wood strip reveals clearly the presence of the CH_2 from the resinic acids at 4363 cm^{-1} form combination bands at 5824 cm^{-1} and 5708 cm^{-1} from the first overtone and from second overtones at 8364 cm^{-1}.

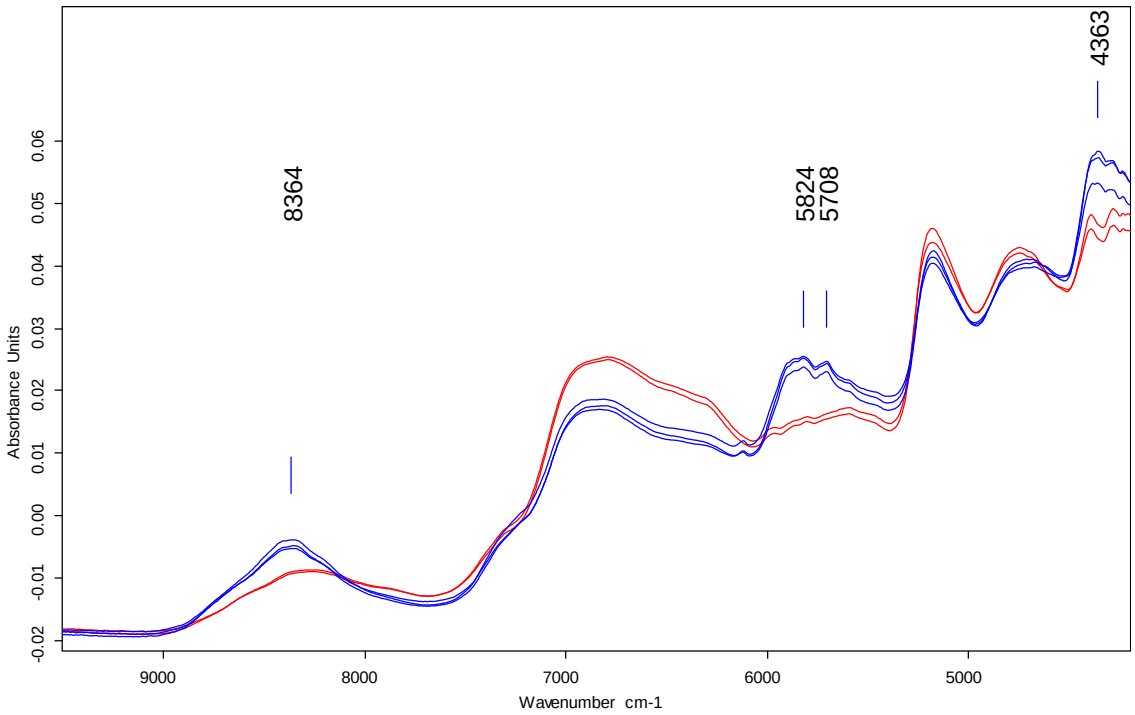


Figure 4.2 NIR spectra of from the wood strip with resin (blue) and without resin (red).

The PCA analysis of the spectra collected in the above strip from pith to bark at 1 mm interval reveal a clear separation along PC 1 of the spectra with resin and the spectra without resin.

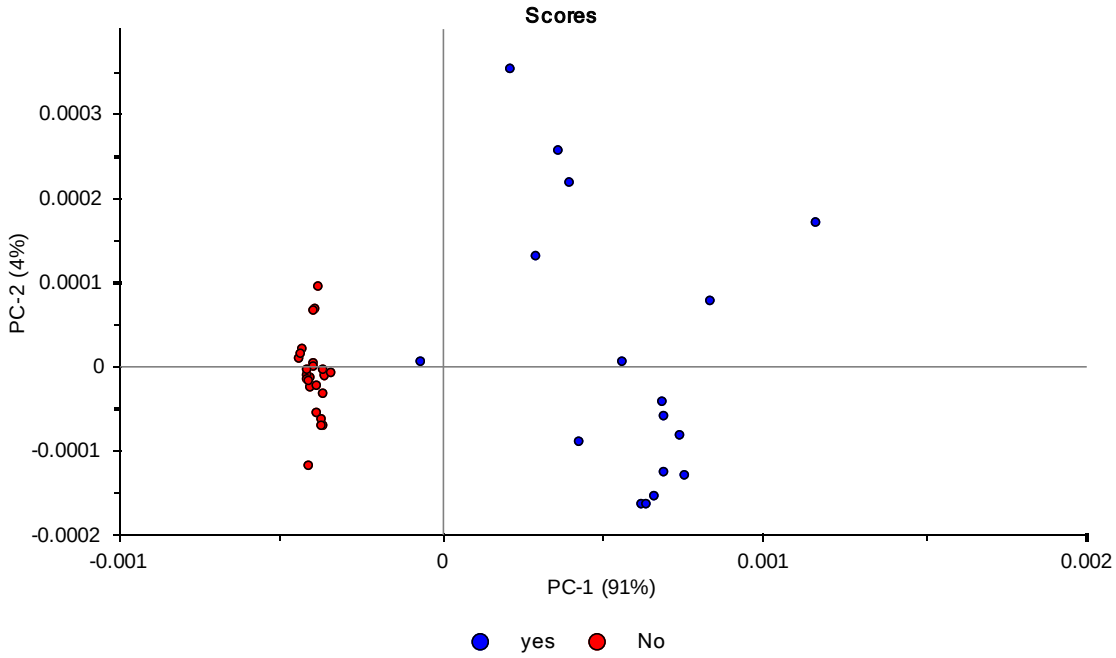


Figure 4.3 PCA scores plot of the NIR spectra from the wood strip collected at 1 mm interval with resin (blue) and without resin (red).

Besides the separation in two groups, it is clear that the non-resin wood part is more homogeneous than the part with resin, this opens an opportunity for fine sampling in predefined points for a deeper understanding of the distribution and composition of the resin in the wood.

The ATR-FTIR spectra of the dichloromethane, ethanol and water extracts show the potential of this technique for a fast screening of content and composition of the extracts

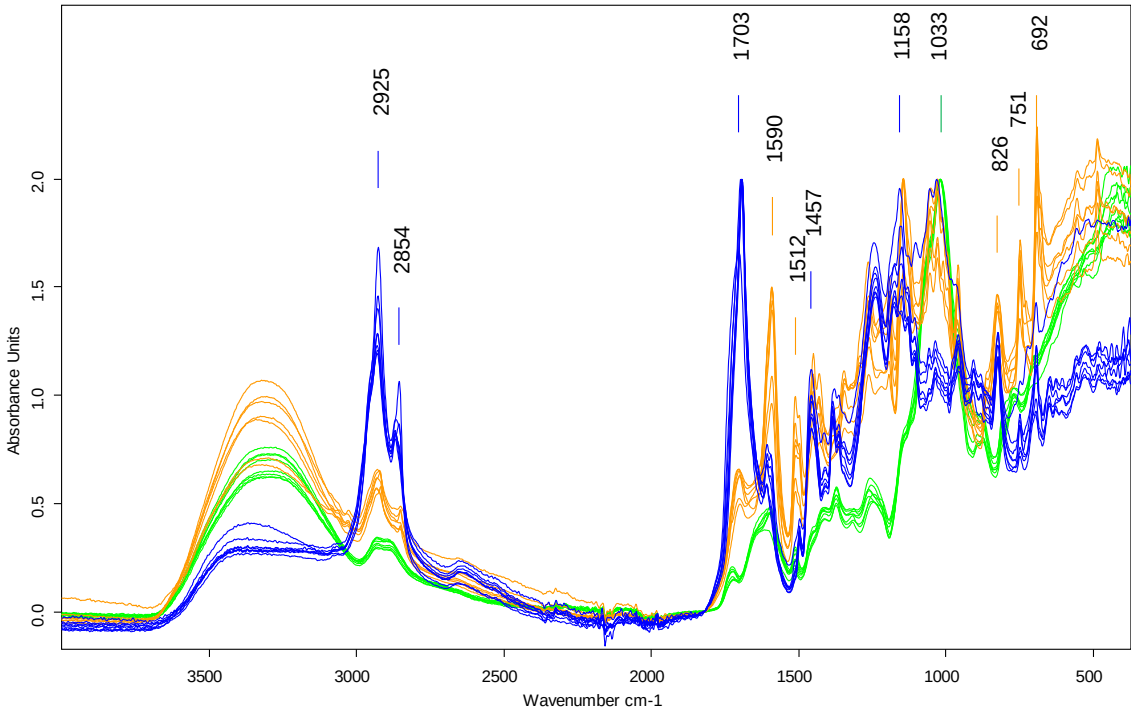


Figure 4.4 ATR-FTIR spectra of dichloromethane extract (blue); ethanol extract (orange) and water extract (green) from *Pinus sylvestris* wood trees.

In a glance it is possible to distinguish the spectra according to the solvent. The spectra of the dichloromethane extract is dominated by carbonyl elongation at 1703 cm^{-1} and the aliphatic CH_2 elongation at 2925 cm^{-1} and 2854 cm^{-1} from resin acids. The spectra of the ethanol extracts show the characteristic aromatic rings vibration at 1590 cm^{-1} and 1512 cm^{-1}. The spectra of the water extracts are dominated by the intense C-O-C band at 1033 a characteristic band of the polysaccharides. This shows also that the polyphenols were lixiviated almost quantitatively by the ethanol extraction, this in part could be explained by the fact that ethanol was only 98 % pure with also 2 % of water. This in addition to the fact that in the beginning of the extraction the samples also have a residual amount of water explains the almost complete removal of polyphenols with the ethanol extraction.

A detailed observation of the spectra of two contrasting samples regarding the dichloromethane content shows that more detailed information can be retrieved from the ATR-FTIR spectra.

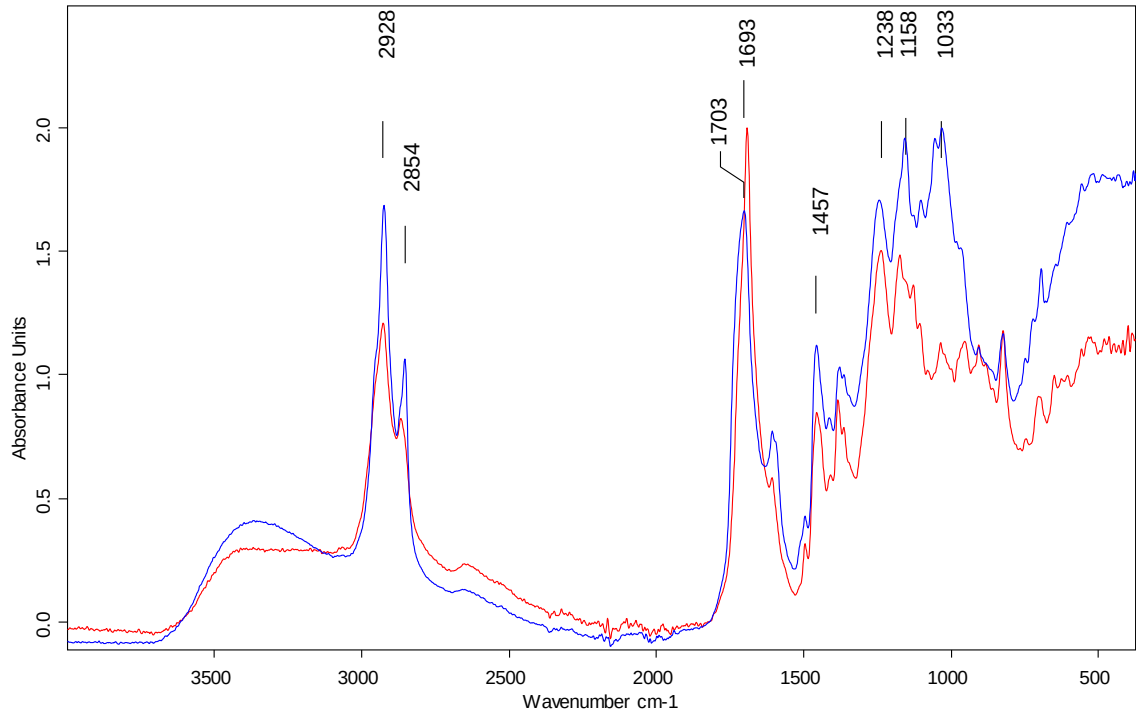


Figure 4.5 ATR-FTIR spectra of two contrasting *Pinus sylvestris* wood samples with high (red ~20 %) and low dichloromethane content (blue ~3 %).

The main differences are in the abundance of the carbonyl band, more intense in the red spectra (high), the maximum that is shifted to higher wavenumbers in blue spectra (low), as well as the broader band that goes up to 1730 cm^{-1}, with almost a shoulder, a further indication of the contribution of the esters of the fatty acids. While in the red spectra the carbonyl is mainly from resinic acids origin in the blue also as a contribution from the esters of fatty acids.

Also supporting this observation is the fact that the CH2 elongation bands at 2928 cm^{-1} and 2854 cm^{-1} are more intense in the blue spectra, also due to the aliphatic CH_2 chain from the fatty acids.

In addition, below 1500 cm^{-1} the intensity of the blue spectra is higher again due to the contribution of the esters of the fatty acids.

Conclusion

The ATR-FTIR excels in the detailed information regarding the composition of the extracts and in particular for the most important ones the dichloromethane. However the

FT_NIR spectra due to the high throughput and in combination with PCA it is particularly suitable for the detailed screening of the wood strips.

Characterization of Pinus sylvestris wood resin acids from trees with different provenances and contrasting resin content compared by GC-MS and PCA analysis

Pines trees produce resin in its wood in varying quantities, depending on species and environmental conditions. When injured or attacked by any means, pine trees can produce high quantities of resin. In *Pinus sylvestris* the wood resin is concentrated in the heartwood and, in normal conditions, in relatively low quantities, less than 5%. In the present work we analysed the resin acid content and composition of the wood resin of P. sylvestris trees from 4 provenances in north and central Portugal. The resin acids content found were very high, varying from 4 to 31% of the wood mass. The resin acid samples were pooled according to their resin acid content and provenance and analysed by GC-MS. The resin composition was dominated by dehydroabietic acid and its hydroxylated forms (80-90%), with much smaller quantities of abietic, pimaric and isopimaric acid. The GC-MS resin acid composition was analysed by PCA to detect eventual associations with resin acid content or tree provenance. The sample of lowest resin acid content, 4%, was discriminated mainly due to its exceptionally high dehydroabietic acid content; within the "16% resin acid" content group two provenances were separated due their very different phenolic acid content.

The detailed analysis is already in paper format for submission (Graça et al. 2017a).

A complex mixture of diterpenic acids and their oxidation products from Pinus sylvestris resin: a compared GC-MS and NMR analysis

The wood of Pine trees can have significant amounts of diterpenic (resin) acids, particularly when the trees are stressed or injured. Resin acids are an important industrial raw material used in a myriad of applications. The analysis of complex resin acid mixtures have been done more commonly by GC-MS but also by NMR. Here we analyzed a resin acid sample from a P. sylvestris wood with an abnormally high resin acid content of 30%, both by GC-MS and NMR to compare the results. The GC-MS analysis showed a mixture of dominantly abietane-type resin acids, namely dehydroabietic acid (50%) and their hydroxylated forms (35%), and much smaller quantities of pimarane type resin acids (8%). The NMR analysis showed a higher proportion of abietic acid (42% to 3%) and

pimarane type acids (95% to 15%) relative to dehydroabietic acid, comparatively to the GC-MS analysis. It is hypothesized that the conversion of abietadienoic acids (e.g. abietic) to dehydroabietic acid can be significant in high-temperature GC-MS and that pimarane (pimaric and isopimaric) acids are under-evaluated in GC-MS analysis relative to abietane acids comparatively to NMR analysis. It is concluded that the later can give more accurate results, minimizing resin acids degradation and avoiding GC-MS quantitation bias.
The detailed analysis is already in paper format for submission (Graça et al. 2017b).

Py-GC/MS of non-extracted samples

Selected samples non extracted and extracted of *Pinus sylvestris*, *Pinus halepensis* and *Pinus pinaster* were further analyzed by Py-GC/MS. So far the main problem has been the identification of the fragments of resin peaks, as found in the GC/MS and also partially also found in the NMR analysis was the fact that that the resinic acids were oxidized and dehydrated, so the identification of the main resinic acids fragments is still ongoing.

3.5 Task 5 Qualitative phenotypical evaluation

The objective of Task 5 was the qualitative discrimination of latitude-, site-, species-, age-dependent variations in lignin-, polysaccharide-, and extractives composition. The expected results were:

a) confirmation of the trend found for latitude- and altitude-dependent lignin content obtained in the preliminary work, by analyzing additional samples and to investigate the pattern of polysaccharide.

Altitude-dependent chemical composition of spruce trees investigated by analytical pyrolysis

Increment cores were taken from Norway spruce trees (*Picea abies* [L.] Karst.) along altitudinal gradients of (i) 500 m in the Achental valley and (ii) 900 m in the Zillertal valley, Austria. The first fifty tree rings were selected to investigate the lignin composition using analytical pyrolysis. Two additional trees/site/ altitude were assessed and also the single tree/site/altitude previously assessed in the preliminary work. These

samples were repeated to ensure comparability of the results due to a change in the GC attached to the pyrolysis system between preliminary work and these new results.

Analytical pyrolysis (Py-GC/FID) was performed with a CDS Pyroprobe 1000 with a coil filament probe connected to an Agilent 7820 by a heated interface (270 ºC). Each sample (75-80 µg) was pyrolyzed at 650 ºC for 10 s with a temperature rise time of approximately 20.0 ºC ms-1.

Principal component analysis (PCA) was performed using The Unscrambler® X version 10.4.1 (CAMO A/S, Trondheim, Norway). For PCA the percentage of the c-, cH-, cP-polysacharide derived peaks as well as 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 used. Py-lignin was calculated as [(G+H)/(c+cH+cP+G+H)]x100.

Main results

Subjecting lignin and polysaccharide-derived pyrolysis products of the samples to PCA led to a separation of the samples without showing a clear altitude-dependent pattern

The principal component analysis based on G-lignin show a separation of the samples according to site except for one Achenkirch sample labelled as 1250 (meaning from 1200 m altitude) that lie in the Zillertal cluster. This sample is one of the samples with the lower py-lignin as well as the lowest H/G ratio among Achenkirch samples which could in part explain this behavior.

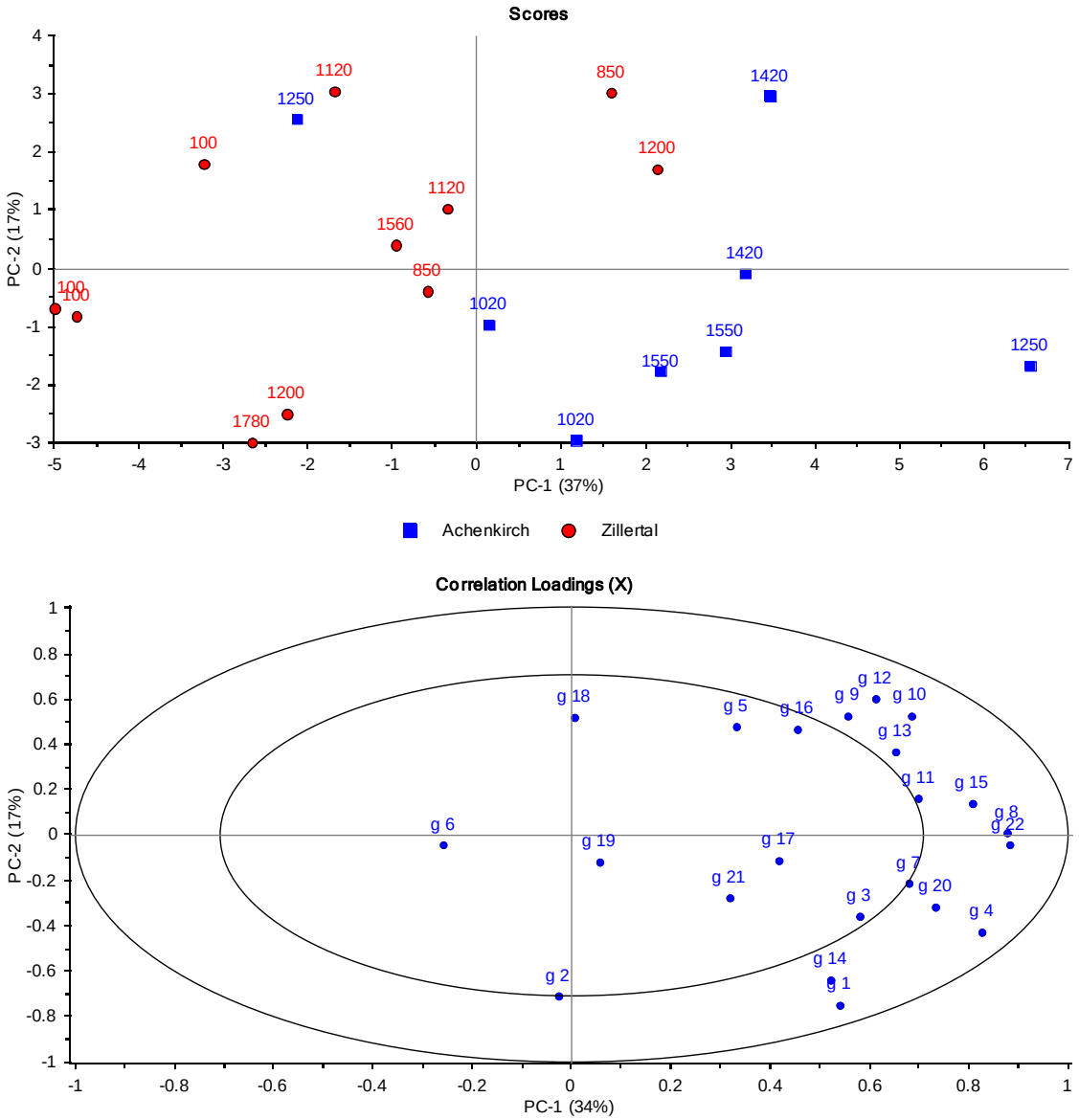


Figure 5.1: PC1 – PC2 scores plot using results for each altitude from Zillertal and Achenkirch sites and G lignin-derived pyrolysis products (upper) and the corresponding correlation loadings (lower).

The correlation plot shows that there are four peaks, labeled as g1, g4, g 14 and g20, accounting for the separation between the two sites.

These results also make clear that there is a separation among trees within sites besides the separation between sites but there is no separation according to the altitude. A further PCA analysis within each individual site also show a clear separation among trees but also no separation among altitudes.

The principal component analysis based on cH-polysaccharide peaks show the same separation and again the Achenkirch sample labelled as 1250 (altitude) do not lie within

nor close to the Achenkirch cluster (but not also within the Zillertal cluster as happen before.

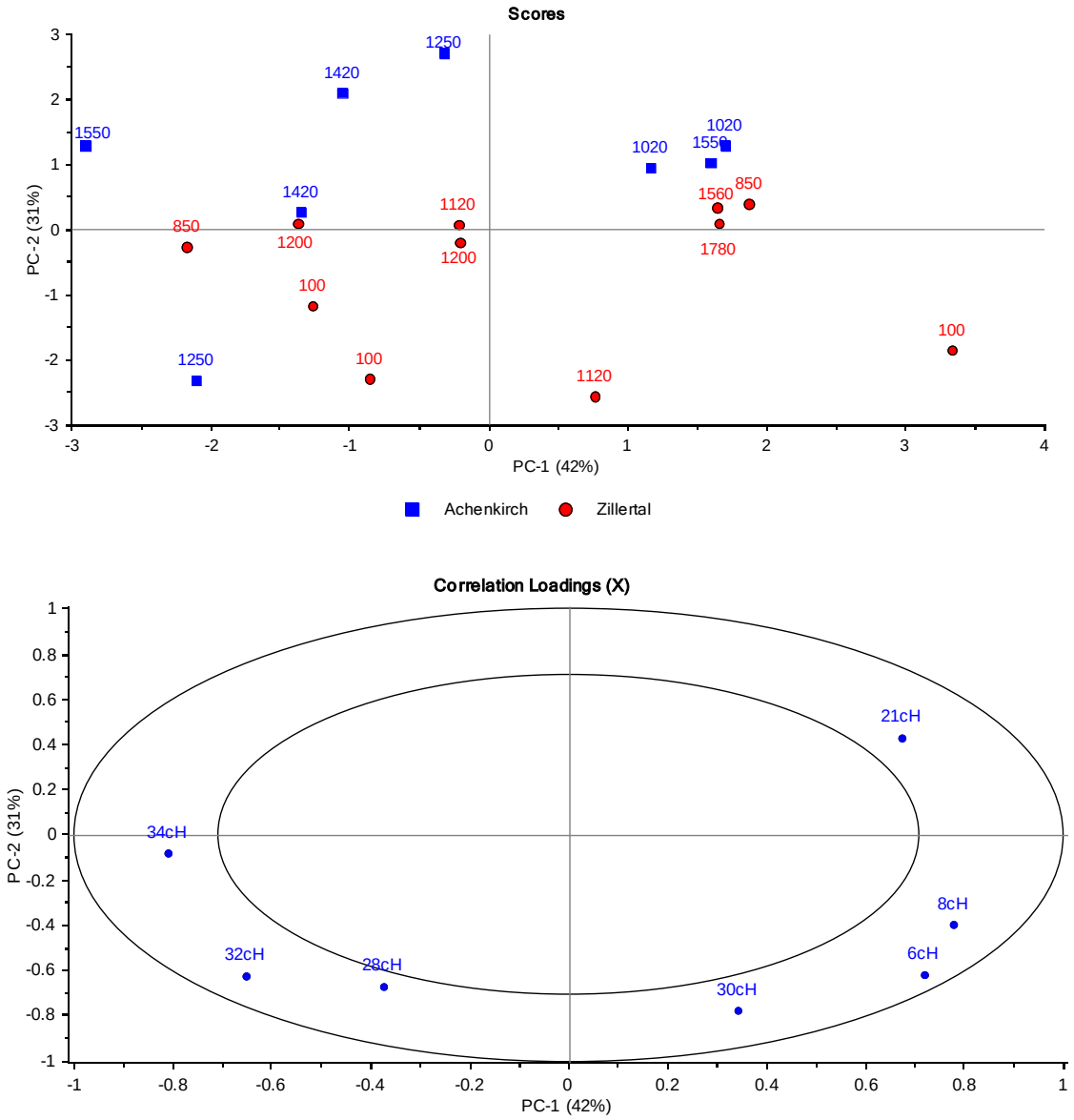


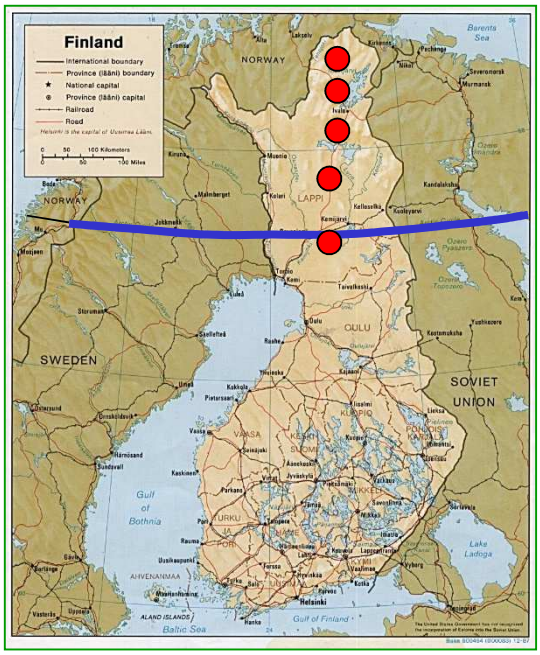
Figure 5.2: PC1 – PC2 scores plot using results for each altitude from Zillertal and Achenkirch sites and cH pyrolysis products (upper) and the corresponding correlation loadings plot (lower).

Interesting the left to right separation, along PC 1 is related to the amount of levoglucosan (34cH) the main cellulose pyrolysis peak accounting on average for about 15% on the total identified area, but the separation of the outlier 1250 (altitude) is more linked to the amount of Pyran-(4H)-4-one, 2-hydroxymethyl-5-hydroxy-2,3-dihydro (32cH).

These results show that even if the lignin content show an increase (concomitant cellulose decrease) with altitude, the composition of lignin . The results also revealed

polysaccharide- and lignin-specific differences between sites and additionally a clear separation among trees within sites

Latitude-dependent chemical composition of Pinus sylvestris trees investigated by analytical pyrolysis. From Artic Circle up to Timberline.



- **Site 5: Kenesjärvi (north)**
- **Site 4: Inari, Kaamanen**
- **Site 3: Inari, Laanila**
- **Site 2: Tähtelä**
- **Site 1: Rovaniemi (south)**

Scots pine (*Pinus sylvestris* L.) trees of well-defined sites north of the Arctic Circle in Finland (from south to north: site 1 Rovanieni; site 2 Tähtelä; site 3 Inari, Laanila; site 4 Inari, Kaamanen; site 5 Kenesjärvi) were investigated. Tree rings of the consecutive years 1989, 1990, and 1991 of each tree were analyzed. Due to the changes in pyrolysis system (different GC) all samples analyzed previously were analyzed again. Since in the preliminary work the main differences were between site 1 and 2, all the remaining available samples from site 1 (3) and 2 (4) were analyzed.

Analytical pyrolysis (Py-GC/FID) was performed with a CDS Pyroprobe 1000 with a coil filament probe connected to an Agilent 7820 by a heated interface (270 ºC). Each sample (75-80 µg) was pyrolyzed at 650 ºC for 10 s with a temperature rise time of approximately 20.0 ºC ms-1. The measurement was repeated two to four times. The identification table of all compounds (pyrolysis products) can be found elsewhere.

Principal component analysis (PCA) was performed using The The Unscrambler® X version 10.4.1 (CAMO A/S, Trondheim, Norway). For 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 %) was calculated. For 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 %) were calculated.

Main results

The scores plot based in all samples and all variables (peaks of the pyrolysis products) show a separation of the site 2 samples (left) from the other groups along PC 1 and a smaller separation with site 3 samples along PC 2. Additionally, four samples from site 1 were separated to the lower far right. Two samples from same tree of the site 2 were also clearly separated in the right upper corner. Unfortunately, the year 89 from the same tree was missing. A close look at the results show that these two samples have, most probably, compression wood since they had the highest py-lignin in combination with the highest H/G ratio. Apart from that, a separation among trees within sites was also noted and in general a small separation could be observed among consecutive rings of the same tree but not following a particular pattern. Notable exceptions were samples 2-6-91 and 3-3-89 (site-tree-year ring, respectively) that were clearly separated from the other two corresponding samples (89, 90 and 90, 91 respectively). Labelling the samples according to the variation of levoglucosan (not shown) shows a clear trend from left (about 5%) to lower right (from 12 to 16%). The two outliers (in the upper right corner) are the only ones that do not follow this trend since despite of high the lignin content and composition due to compression wood they also have a relatively high amount of levoglucosan.

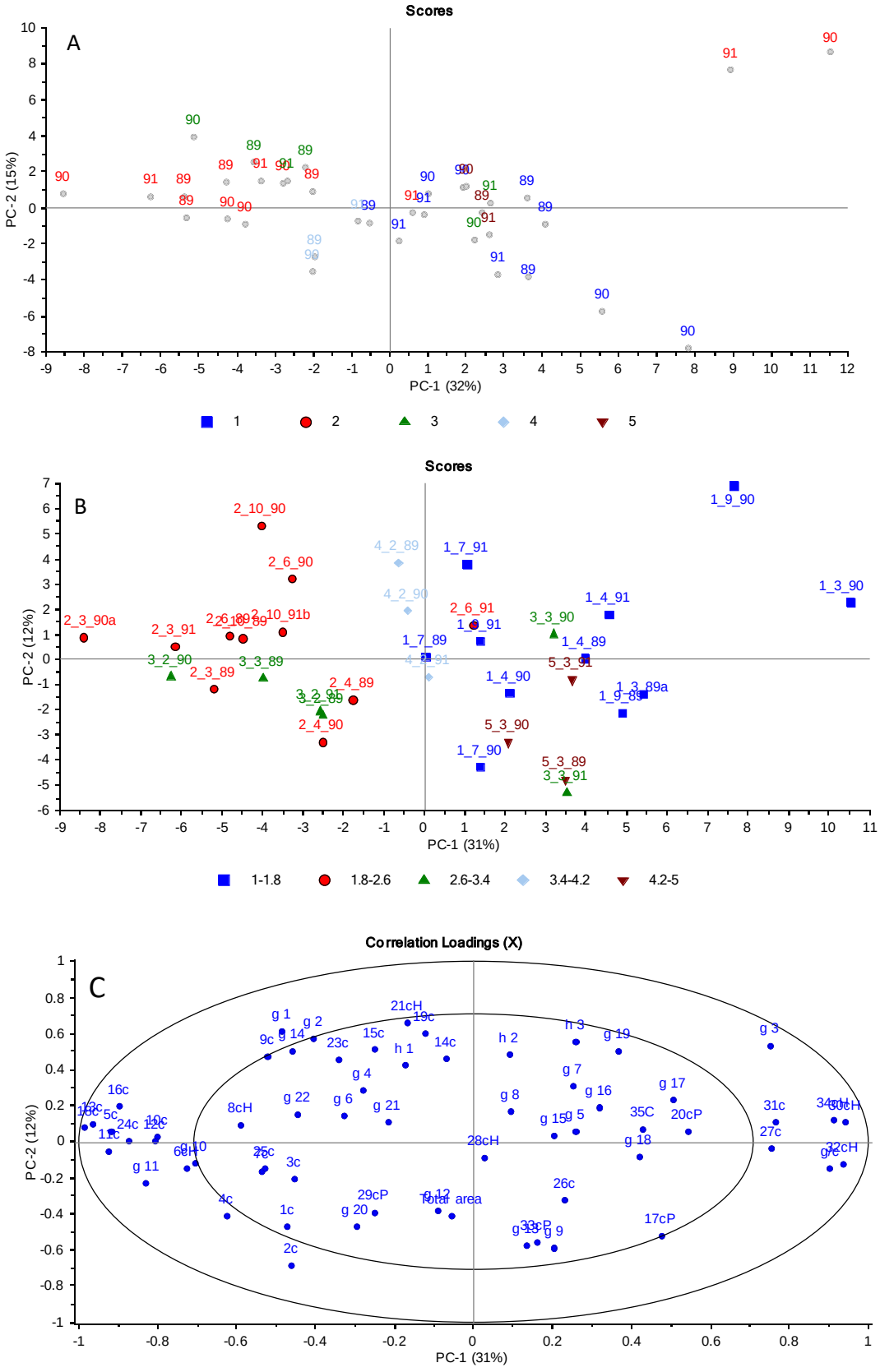


Figure 5.3 PC1 – PC2 scores plot using results for each site (3 trees/site) and the years 89, 90 and 91 and all pyrolysis products (A) with red labeled (90, 91) samples removed (B) and the corresponding correlation loadings plot (C).

The PCA analysis with the two outlier samples removed show a separation in two clusters with all the site one samples to the right and all the site 2 samples to the left the samples from site 4 fall in the frontier between the two. The samples from site 5 lie well inside of the XCuster of the site 1 as well as two samples from site 3. The remaining samples from site 3 lie in the middle of the site 2 cluster.

Interestingly the PCA analysis using lignin variables (all, G or H) did not showed a separation pattern regarding sites or year, only a larger separation among trees and among years within trees.

However, an interesting pattern resulted from the PCA analysis of the cH-polysaccharide derived peaks (Fiure 5.4).

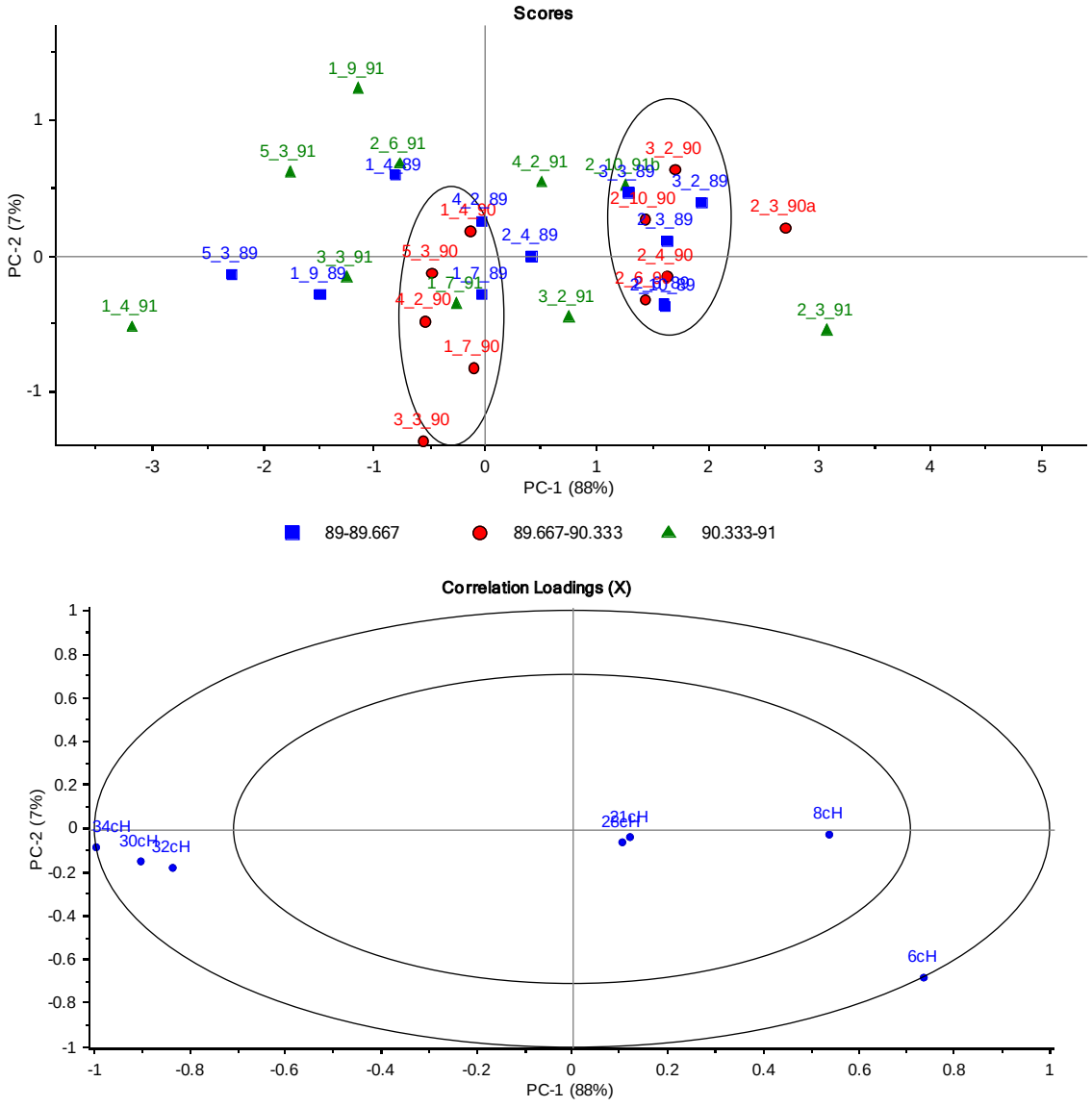


Figure 5.4 PC1 – PC2 scores plot using results for each site (3 trees/site) and the years 89, 90 and 91 and cH variables (A) and the corresponding correlation loadings plot (C).

The samples from year 90 lie in two tight groups (circled) one to the right including trees from site 2 and one from site 3, with the sample 2-3-90 close by, and the other near the center including trees from sites 1, 5, 4 the other site 3 sample 3 3-3-90. This year-specific separation of the polysaccharide products is indeed a very interesting finding, as far as we know, it is the sole registered influence of some sort of environmental condition on the composition of the polysaccharide but not on lignin composition which is knowingly influenced by environmental conditions. This point is more interesting than the latitudinal so further analysis of the remaining samples from the other sites will be analyzed to confirm this year-specific pattern.

Age-induced changes of wood chemical composition of Pinus sylvestris trees from Boreal sites assessed by analytical pyrolysis.

Scots pine (*Pinus sylvestris* L.) trees of well-defined sites north of the Arctic region in Finland (site 1 Rovanieni, site 4 Inari, Kaamanen and site 5 Kenesjärvi at the northern timberline) were investigated. Up to ten annual rings of the about 40- year-old trees were combined to a sample, one tree per site. The samples were labeled e.g. 5_90 meaning site 5 decade 90 (combined tree rings of the nineties).

Analytical pyrolysis (Py-GC/FID) was performed with a CDS Pyroprobe 1000 with a coil filament probe connected to an Agilent 7820 by a heated interface (270 ºC). Each sample (75-80 μg) was pyrolyzed at 650 ºC for 10 s with a temperature rise time of approximately 20.0 ºC ms-1. The measurement was repeated two to four times. The identification table of all compounds (pyrolysis products) can be found elsewhere.

Principal component analysis (PCA) was performed using The Unscrambler® X version 10.4.1 (CAMO A/S, Trondheim, Norway). For 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 %) was calculated. For 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 %) were calculated.

Main results

Consecutive decades of the same site (and tree) are well separated in sites 1 and 4 but not in site 5 for the 70, 80, 90 decades of the 20th century. Decade 60 cluster in a tight group well separated from all the other samples, it seems that in this decade some environmental conditions superimposed the different genetic background producing similar lignin and

polysaccharide composition. In the same way decade 90 are separated in two groups one including site 1 and 2 the other including site 4 and 5, these two groups are separated along PC 1 as well as PC 2 (Figure 5.5).

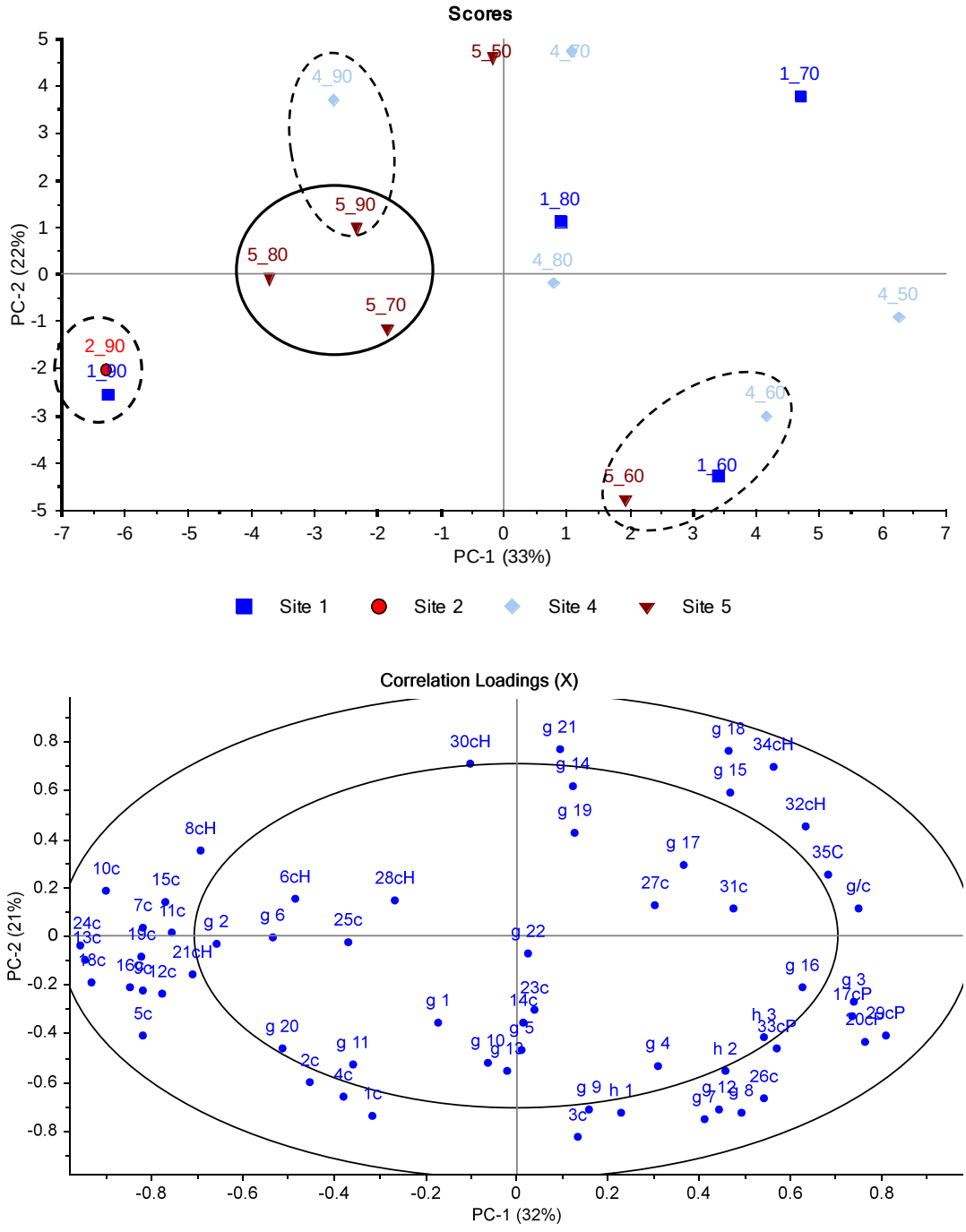


Figure 5.5 PC1 – PC2 scores plot using all variables (pyrolysis peaks) results for sites 1, 4 and 5 and decades 50, 60, 70, 80 and 90. Site 2 only the decade 90 is available also decade 50 from site 1 is missing (upper) and the corresponding loading plot (lower).

The loadings plot show that the site 1 decades 70,80, and 90 are aligned in a decreasing order according to the amount of levoglucosan (34cH) and Pyran-(4H)-4-one, 2-hydroxymethyl-5-hydroxy-2,3-dihydro (32cH). The loading plot also show that the separation of the 60 decade is mainly due to high G and low cH in principle with a concomitant higher amount of lignin.

The PCA of the lignin peaks alone show the same separation of the 60 decade samples, and the distribution of the site 1 samples now in reverse order.

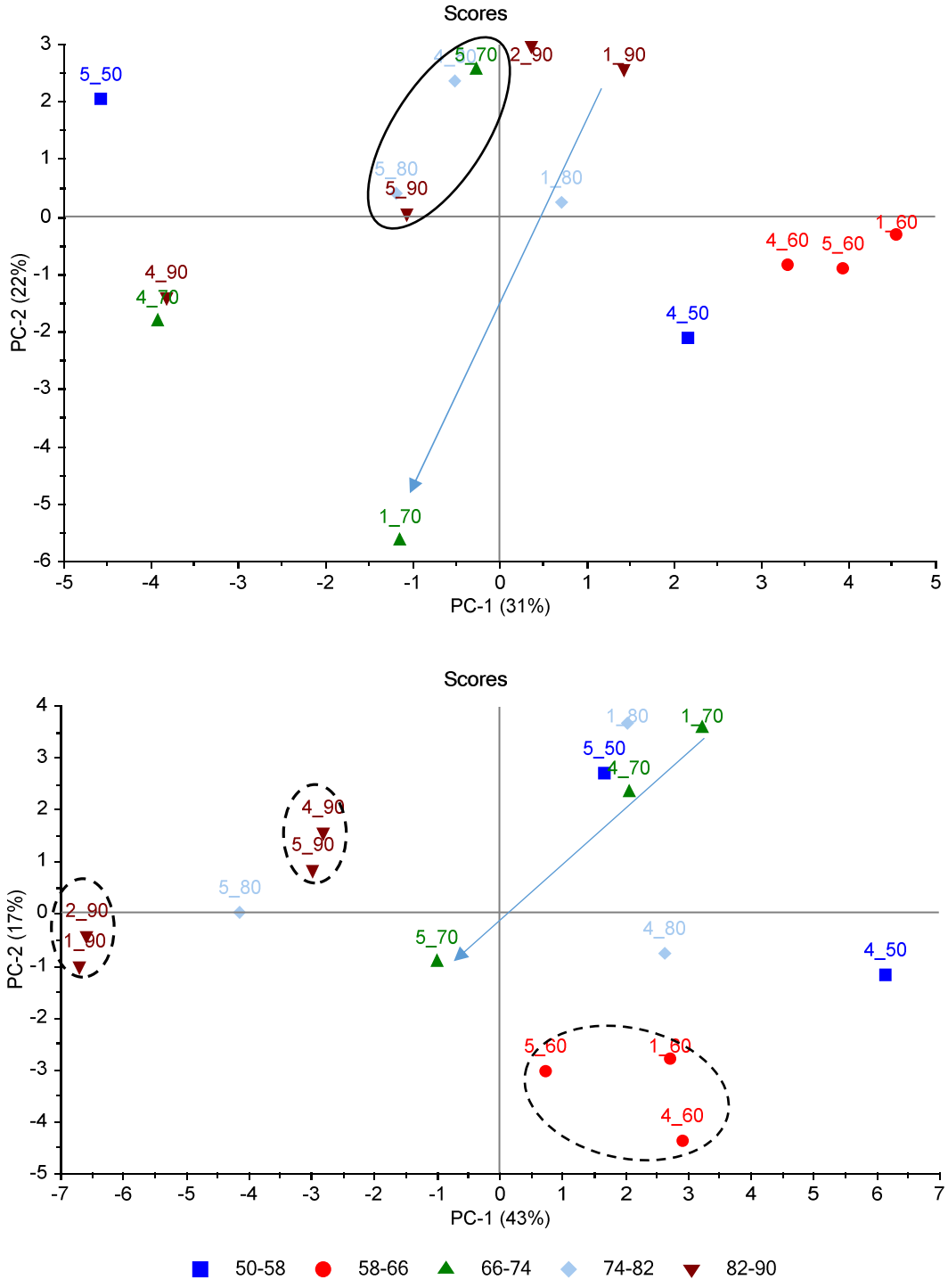


Figure 5.6 PC1 – PC2 scores plot using lignin variables (top) and polysaccharide variables (below).

The score plot of the polysaccharide-derived pyrolysis products shows the samples of site 1 scattered in the principal score plane and the familiar separation of the 60 decade samples (sites 1, 4 and 5), which shows that the 60 decade are clearly separated, both in the lignin as well as polysaccharide composition, from the other decades. The decade 70 also show a kind of a decreasing pattern of cellulose content from site 1 to 5 (arrow, Figure 5.6), although site 4 is more close to the site 1 than to the site 5. The two small clusters of the 90 decade are now even more tight especially so the one including site 4 and 5 that in the lignin score plot did not appear.

Conclusions

Due to the different pyrolysis products obtained it can be concluded that differences in the lignin and polysaccharide building blocks exist due to varying environmental conditions.

Analytical pyrolysis, provides a fingerprint of the whole chemical composition of the wood that can be used for the phenotypic variation at the tree-ring level. It is a micro-chemical technique that converts, by heat in the absence of oxygen, nonvolatile polymers into a volatile degradation mixture that are separated by gas chromatography (GC) identified by mass spectrometry (MS) and quantified with a flame ionization detector (FID). Analytical pyrolysis in combination with principal component analysis (PCA) to evaluate the pyrolysis results with respect to differences in lignin and carbohydrate composition allowed to reveal age- and latitude-specific differences in lignin content and composition of pine and altitude-specific differences for spruce lignin composition.

Although some hints on structural differences of lignin or polysaccharides can be obtained from several studies as summed up recently, the assignment of pyrolysis products to lignin or polysaccharide structures is still a challenge for the next decade.

3.6 Task 6 Quantitative phenotypical evaluation

The pyrograms obtained in Task 5 were subsequently used in task 6 to obtain the quantitative information. So only the results are shown in order not to duplicate the part referring to the material and methods.

Altitude-dependent chemical composition of spruce trees investigated by analytical pyrolysis

The lignin content increases from the valley up to the timberline (Table 6.1) in both sites. The evaluation of the pyrolysis results, with respect to differences in lignin composition using G- and H-lignin-derived peaks from the pyrograms, shows that the ratio of the lignin components varies along the altitudinal gradient. Moreover, it provides evidence for an altitude-dependent trend in the lignin composition. Both G and H increase with altitude but since G are the predominant peaks and showing also larger variation the net result is the increase of the H/G ratio. These results confirm previous results obtained with one tree/site/altitude. On the other hand, hexoses markers decreased with altitude, while pentose markers, like lignin, tend to increase with altitude (Table 6.1, Figure 6.1). Carbohydrate pyrolysis products that could not be unequivocally assigned to hexoses or pentosanes (c) show no particular trend on Achenkirch but in Zillertal show a clear pattern increasing with altitude (Table 6.1, Figure 6.1). The higher and lower H values were obtained in the upper and lower altitudes respectively in both sites (Table 6.1).

Table 6.1 Analytical pyrolysis results obtained for Achenkirch and Zillertal valleys at different altitudes, results are the average of three trees/site/altitude.

Local	Altitude	cP/cH	h/g	(h+g)/all	c	cH	cP	g	h
Achental	**1550**	10.7	0.047	24.0	34.6	34.6	3.7	22.0	1.05
	1420	10.5	0.047	24.4	34.2	34.9	3.7	22.4	1.05
	1250	10.1	0.046	23.7	34.7	35.0	3.5	21.7	1.02
	1020	10.2	0.045	23.2	34.4	35.7	3.6	21.3	0.96
Zillertal	**1780**	11.1	0.047	23.7	35.4	34.2	3.8	21.8	1.0
	1560	11.9	0.043	22.6	35.6	34.6	4.1	20.8	0.9
	1200	9.4	0.041	22.5	34.1	36.9	3.5	20.8	0.8
	1120	9.8	0.046	23.0	33.7	36.7	3.6	21.2	1.0
	850	9.6	0.041	23.0	32.7	37.7	3.6	21.2	0.9
	750	9.6	0.037	21.4	33.9	37.9	3.6	19.8	0.7

In order to see in a glance all the variations the results of table 6.1 were normalized and presented as graphs, the idea is only to look at the trends because not all variables have the same weight, it seems that cP has a larger variation in relative terms but since it's weight is smaller compared both with c and cH it's impact is smaller.

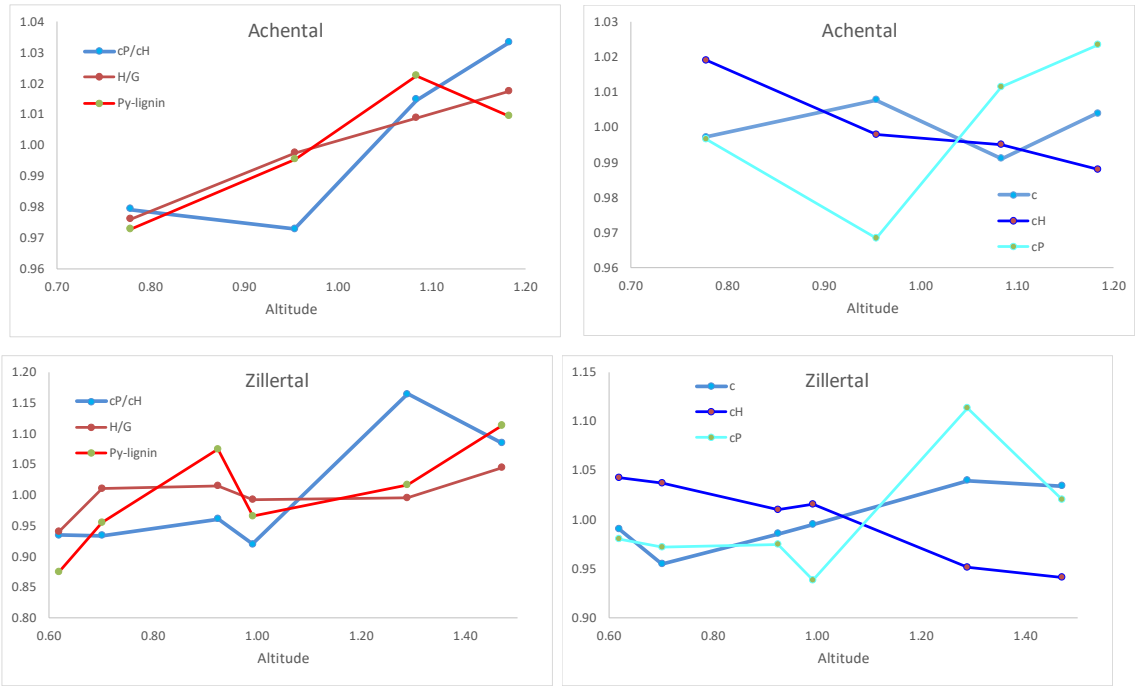


Figure 6.1 Plots of the normalized variables for Achental and Zillertall.

Latitude-dependent chemical composition of Pinus sylvestris trees investigated by analytical pyrolysis. From Artic Circle up to Timberline.

With the increased number of trees analyzed the increasing lignin pattern with latitude no longer applies. In fact, as can be seen only cP/cH shows an increasing pattern with latitude with the exception of the Northeastern site (Table 6.2).

Table 6.2 Analytical pyrolysis results obtained the latitudinal gradient from the Circle Artic (1) to Timberland (5).

Site	cP/cH	h/g	(h+g)/all	c	cH	cP	g	h
1	**11.2**	**0.038**	**22.9**	**37.0**	**34.2**	**3.8**	**21.5**	**0.81**
2	**11.8**	**0.041**	**23.6**	**39.6**	**31.3**	**3.7**	**22.2**	**0.92**
3	**11.9**	**0.035**	**23.2**	**39.6**	**31.7**	**3.8**	**21.9**	**0.76**
4	**14.2**	**0.035**	**22.6**	**39.1**	**31.7**	**4.5**	**21.3**	**0.75**
5	**12.7**	**0.040**	**23.0**	**38.0**	**32.6**	**4.1**	**21.4**	**0.85**

The lignin content is positively and moderately correlated with lignin composition as assessed by the H/G ratio (R=0.53), and weakly with cP/cH and cP, and negatively with cH, among years and sites. The generic polysaccharide pyrolysis product c is negatively correlated with cH (moderately) and cP (weakly) (Table 6.3).

Table 6.3 Cross-correlation among aggregate quantitative pyrolysis products.

	cP/cH	h/g	(h+g)/all	c	cH	cP	g	h
cP/cH	-	0.33	0.36	0.02	-0.48	0.88	0.32	0.38
h/g		-	0.53	-0.39	-0.07	0.31	0.40	0.97
(h+g)/all			-	-0.25	-0.44	0.16	0.99	0.72
c				-	-0.72	-0.36	-0.15	-0.38
cH					-	-0.01	-0.50	-0.19
cP						-	0.08	0.30
g							-	0.61
h								-

Age-induced changes of wood chemical composition of Pinus sylvestris trees from Boreal sites assessed by analytical pyrolysis.

The age induced effects only on polysaccharide pyrolysis products, cP/cH (decrease), cP (decrease) and c (increase) with age, but even in these cases it is clears that decade 60 does not follow the trends as it was in part expected according to the PCA results (Figure 5.5). Also interesting is the stable cH results of the decades 50, 70 and 80, the variation among them is within the error of determination and the decrease at 90 (Table 6.4).

Table 6.4 Analytical pyrolysis results obtained for decades 50 to 90, averages for site 1, 4 and 5.

Decade	cP/cH	h/g	(h+g)/all	c	cH	cP	g	h
50	14.5	0.039	22.4	36.1	33.7	4.9	20.7	0.81
60	16.2	0.042	24.1	37.4	30.8	5.0	22.3	0.93
70	12.8	0.038	22.8	36.6	33.7	4.3	21.2	0.80
80	12.8	0.040	21.8	37.5	33.8	4.3	20.3	0.81
90	12.0	0.038	22.5	38.9	32.5	3.9	21.1	0.80

The cross correlation results for the age induced changes is quite different from the previous cross correlation for latitude. The lack of correlation between lignin composition (H/G) and content (py-lignin) is noticeable, but it was not the only surprise, also the correlation of py-lignin with cH (moderately negative) and positive with cP.

The generic polysaccharide pyrolysis product c is negatively correlated with cH (moderately) and cP (weakly) in line with previous findings (Table 6.3). These suggests that the age induced changes can also be seen in the quantitative and aggregate pyrolysis results.

Table 6.3 Cross-correlation among aggregate quantitative pyrolysis products.

	h/g	py-lignin	c	cH	cP	g	h
cP/cH	-0.01	0.62	-0.17	-0.64	0.96	0.57	0.26
h/g	-	-0.22	-0.08	0.16	0.04	-0.33	0.91
py-lignin		-	-0.19	-0.64	0.49	0.99	0.21
c			-	-0.53	-0.40	-0.12	-0.14
cH				-	-0.40	-0.66	-0.13
cP					-	0.43	0.25
g						-	0.10

Longitudinal and radial variation of wood chemical properties.

It is well known the physical and anatomical of radial variation pattern form pith to bark, it is an important ontogenic regulator of wood formation that serve as criterion for distinction between juvenile- and mature-wood. This is well marked in the steep increase of fiber length (also density) in the first 15 years or so (juvenile wood) followed by a plateau (mature wood).

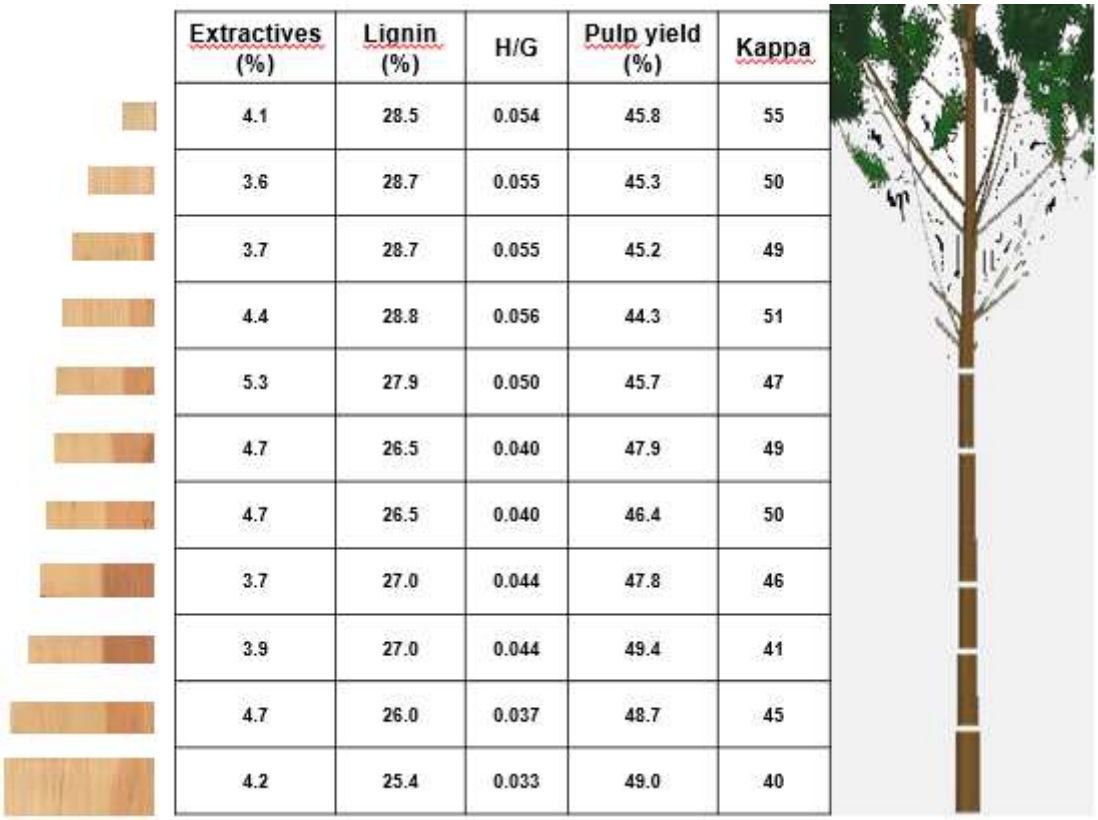
It is also readily described in wood chemistry textbooks that a similar pattern of variation for chemical composition exists consisting in a decrease of the lignin content with a concomitant increase of the cellulose content.

An adult *Pinus pinaster* tree from the famous Leiria National Forest was felled and disks were taken at several heights to study the longitudinal and radial variation of wood chemistry.

The disk closer to base was additionally sectioned in more or less a decade, except the sample closer to the pith that was sampled containing all heartwood including about 15 years, considered by many as the corresponding to the juvenile-wood.

The longitudinal pattern showed a trend of increasing lignin content and composition with height which implied a decrease in the pulp yield and increase in kappa number, no clear pattern was found for extractives.

The radial variation did show a higher lignin amount and composition for the juvenile wood and a leveled off after that, in line with the expected juvenile/mature wood dichotomy. (Figure 6.2). Regarding extractives content apart from heartwood it was noticeable a decreasing content to the bark. But the fact that these disk the most interesting years (below 15 years) were already heartwood make it not that interesting for a detailed observation of the sub-tree-ring level analysis.



Extractives (%)	Lignin (%)	H/G	Pulp yield (%)	Kappa
4.1	28.5	0.054	45.8	55
3.6	28.7	0.055	45.3	50
3.7	28.7	0.055	45.2	49
4.4	28.8	0.056	44.3	51
5.3	27.9	0.050	45.7	47
4.7	26.5	0.040	47.9	49
4.7	26.5	0.040	46.4	50
3.7	27.0	0.044	47.8	46
3.9	27.0	0.044	49.4	41
4.7	26.0	0.037	48.7	45
4.2	25.4	0.033	49.0	40

Extractives (%)	2.8	3.0	3.2	17.7	Heartwood Extracted
Lignin (%)	27.5	27.5	27.7	29.6	
H/G	0.047	0.047	0.048	0.061	
Pulp yield (%)	51.4	50.9	50.4	42.0	49.1
Kappa	40	40	46	68	52

Figure 6.2 Longitudinal and radial variation of extractives and lignin amount and composition and its impact in pulping yield and delignification

Sub-tree-ring level (earlywood-latewood) analysis by analytical pyrolysis

Opposite side

The detailed analysis of a *Pinus pinater* wood disk taken at DBH show a systematic variation at year-ring level between early-wood (EW) and late-wood (LW) both for lignin content (- 2 %) and composition (+ 0.005) at the opposite side (Figure 6.2, left). Moreover, there are no radial trend both for lignin content or H/G ratio with age, although the two first years close to the pith do show the higher lignin content. For instance, the lowest lignin content at year-ring (YR) age was at YR-6 (24.1 %) it seems likely that the within ring variation is more determined by yearly variations in the environmental conditions than determined by ontogenesis.

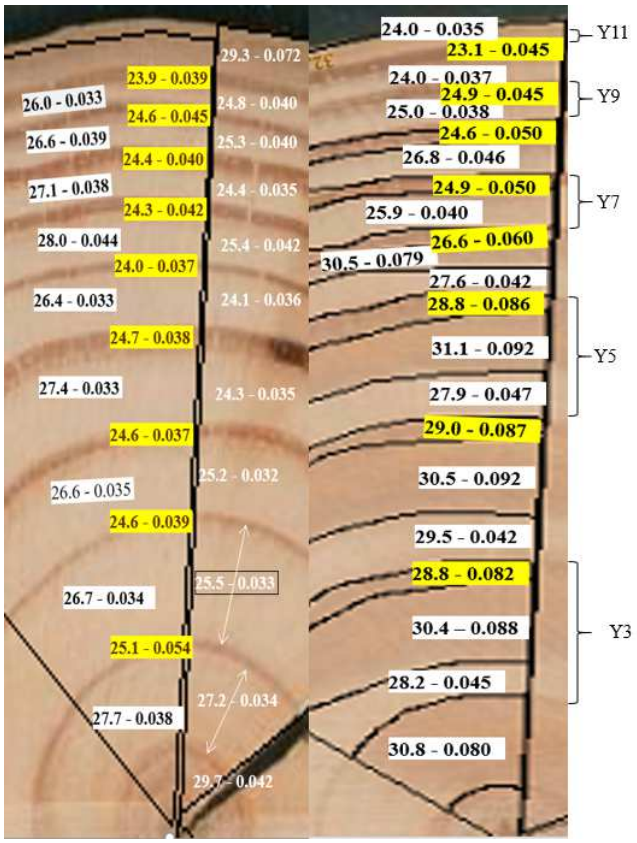


Figure 6.3 Radial variation of the lignin content and composition (H/G ratio) at sub ring level of a *Pinus pinaster* wood disk (12 years-old). Compression wood (CW) side (left) and opposite side (right) of the same disk. White boxes early-wood, yellow boxes late-wood.

Compression wood side

The compression wood side reveals very interesting patterns, apart from the year 2 that seems like that the entire wood ring is CW. In the following YR (3) it is visible a clear EW followed by a brownish CW that extends to the end of the season/beginning of the

following year. This CW was formed in the environment conditions related to the formation of EW (CW-EW) but since its growth clearly extends to the end of the season was also formed in the conditions were LW was usually developing (CW-LW). The lignin content and composition of the CW-LW is systematically different from the CW-EW, as can be seen in the year-ring 3 (YR-3) to YR-6. At YR-3 the lignin content was lower at EW (28.3 %) than increased to EW-CW (30.4 %) and decreased again to LW-CW (28.8 %). The lignin composition also varied from 0.045 (EW) to 0.088 (CW-EW) and 0.082 (CW-LW). All the values were higher that the YR-3 of the opposite wood 26.7 % – 0.034 (EW) and 24.6 % – 0.037 (LW) (lignin content and composition respectively). This pattern was kept through YR-4 to YR-6. At YR-7 however, coincident with the disappearance of the CW the lignin content and composition was lower in the EW (25.9 % - 0.040) and LW (24.9 %, 0.050) of the compression wood side in comparison with the opposite side (EW 28.0 – 0.044, and LW 24.3 % - 0.042). This pattern was maintained in the remaining YRs.

Sub-tree-ring level (earlywood-latewood) Pinus pinaster wood disks

In order to further confirm the EW-LW pattern previously found (Figure 6.3), additionally 6 different genotypes trees were assessed at three year-ring 2, 5 and 8 for lignin content and composition.

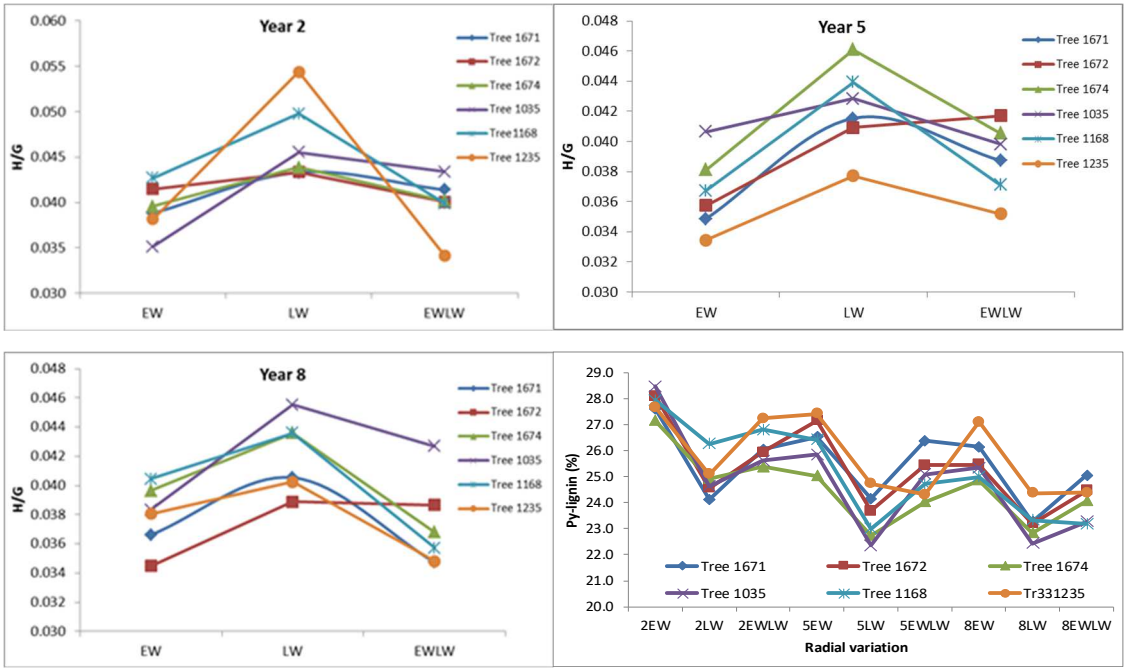


Figure 6.3 EW-LW and YR variation of the lignin composition (H/G ratio) and content at YR 2, 5 and 8 for 6 different *Pinus pinaster* genotypes.

The pattern of variation found earlier was convincingly confirmed. For each YR the lignin content was lower at EW (at least 2 % absolute values) and the lignin composition was higher in the LW (at least 0.03).

Sub-tree-ring level (earlywood-latewood) larch spp. wood disks

Twenty-two year-rings from 10 wood trees of larch spp. Form Austria were assessed for lignin content and composition.

The patter EW-LW previously found for *Pinus pinaster* was again observed in larch spp. Wood disks from 10 trees from Austria.

	Py-lignin		H/G	
	LW	EW	LW	EW
231_1	22.1	23.6	0.047	0.045
231_2	22.8	23.5	0.048	0.048
232_1	25.0	25.3	0.082	0.049
232_2	22.9	24.2	0.038	0.047
305_1	23.1	25.1	0.041	0.044
305_2	23.3	24.4	0.044	0.040
305_3	23.7	25.4	0.055	0.046
311_1	24.7	26.1	0.057	0.050
311_2	22.8	25.3	0.046	0.045
325_1	24.4	24.9	0.048	0.043
325_3	22.6	26.0	0.046	0.039
325_2	24.1	26.2	0.056	0.044
331_1	22.6	25.6	0.050	0.043
331_2	22.6	23.8	0.045	0.051
331_3	23.7	23.9	0.048	0.046
335_1	22.2	27.3	0.048	0.050
335_2	23.9	24.2	0.051	0.057
345_1	24.4	24.7	0.042	0.048
345_2	24.2	24.1	0.043	0.045
338-1	26.1	25.7	0.124	0.049
361_1	24.7	25.6	0.041	0.042
361_2	23.5	24.7	0.044	0.050
Average	**23.8**	**25.2**	**0.053**	**0.047**

On average the lignin content was higher in EW (+ 1.4) and the lignin composition was lower (-0.006).

Conclusions

It was found both for *Pinus pinaster* and *Larch spp*. that the lignin content is higher in the EW than in LW. This can be explained by the combine effect of two factors, in one hand the relative proportion of secondary cell wall (SCW) to the compose middle lamella (CML) is considerably lower in EW than in LW, this in combination with the known fact that the lignin is uneven distributed with a lower concentration in the SCW and higher, up to twice as much, in the LCML.

More interesting and so far a novelty is the pattern of the higher H/G ratio found in LW. This is even more interesting due to the fact that it constitutes an inverse correlation with lignin content which is exactly the opposite correlation between the two generally found.

Analysis of sugars by analytical pyrolysis

Pinus pinaster samples (31) were analyzed for lignin content and the hydrolysate was sent to Hamburg for sugar analysis. Prior de analysis analytical pyrolysis was performed in an aliquot of the sample used for hydrolysis.

Correlations between analytical pyrolysis data and the results of sugar analysis were correlated by partial least squares regression (PLS-R) using The Unscrambler® X version 10.4.1 (CAMO A/S, Trondheim, Norway). Several attempts were performed by varying the predictors variables, from all variables to specific groups like all carbohydrate, only c, only cP and only cH. Overall the best results were obtained using all variables including the polysaccharide and lignin variables.

The best overall model was obtained for glucose followed by galactose and mannose, the model for arabinose is useless. It was not possible to develop a model for xylose. These are promising results that show that in principle it would be possible to assess the wood polysaccharide composition by analytical pyrolysis. Nevertheless, they are only a bit inferior to the models obtained using near infrared spectra data.

Detailed discussion of this results will be published soon.

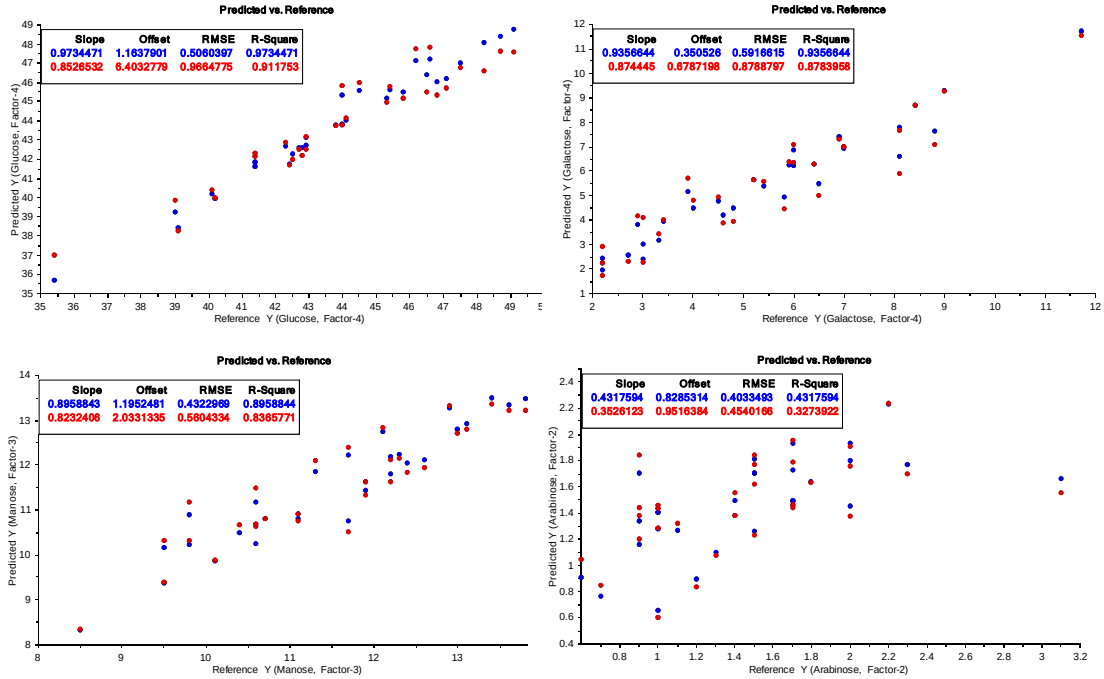


Figure 6.4 plots of predicted versus determined for glucose, galactose, mannose and arabinose.

3.7 Task 7 Infrared spectroscopy

The activities previewed in this task related to the acquisition and solid state analysis of infrared spectra collected with an infrared microscope using an ATR-objective, were not fulfilled. These activities were planned to be pursued in the University of Boku, Austria by Manfred Schwanninger that passed away in December 2013.

The main advantages of the infrared microscope using an ATR-objective were: i) the ATR cell is pressed from above against the wood so what is needed is just to have a surface well prepared, ii) additionally the system was automated, so apart form the time required to the set up put the sample in the microscope and set the coordinates the remaining part, spectra acquisition, proceeded unattended.

Since we did not possess a similar system and we could not find a researcher, in BOKU, interested in pursuing the work we were left with our only option a convention infrared with a single reflection ATR equipped with a diamond cell. The limitations of this equipment apart from the fact that it is not automatic is that this equipment was not designed for the acquisition of the spectra acquisition in solid state. The spectra of liquids and slurries are excellent in wood power are very good but in solid state are of limited

quality. The quality of the FTIR-ATR relies in the close adherence of the sample to the ATR window, this is difficult to do with solid wood.

ATR-FTIR and FT_NIR solid state calibrations for the wood of Picea abies.

The sampling consisted of 8 cores from +30 year-old *Picea abies* trees from Sweden. From the increment cores, 2 mm radial strip segments (from pith to cambium) were prepared. These strips were air-dried and conditioned at 22% relative humidity and 22 ºC. The spectra were obtained in the transverse section (axial direction).

In each core between 2 and 3 semi-micro drillings were obtained as described in Task 1. ATR-FTIR spectra were acquired in the closet position of the drilled hole. FTIR-ATR spectra (32 scans per sample, spectral resolution of 4 cm−1 and wavenumber range of 4000–400 cm−1), using a diamond single reflection attenuated total reflectance (ATR) device, were recorded with a Bruker FT-IR spectrometer (Alpha) and a zero filling of two was applied.

FT-NIR spectra were acquired with a fiber probe on a Bruker MPA instrument (100 scans per spectrum at 8 cm^{-1} resolution, and a zero filling of two, in the wavenumber range from 12000 cm^{-1} to 4000 cm^{-1}).

For the PLS-R modelling Opus Quant package was used to relate the spectra information with analytical pyrolysis data.

In general, better models were obtained for FT-NIR than for ATR-FTIR, except for H/G, this in part can be explained due to the already mentioned fact that using a strip of wood, even if it is thin, makes the intimate contact between the wood and the ATR cell more difficult which is detrimental for the quality of the spectra. Even dough the quality is enough to perform the screening of the material. Table 7.1. However, for H/G even with the constraints referred, the better model was obtained with ATR-FTIR. It will be to validate the models using an independent number of samples, covering the range of variation, to further confirm this results. If the same trend persist it could be related with the fact that in the middle infrared range (FTIR) the variation in H/G ratio also affects the position of the maximum of the lignin peak in the vicinity of 1510 cm^{-1} due to the ring vibration, that could have helped in the quality of the model. If confirmed it would open

a wider range of applications for the ATR-FTIR spectroscopy. One of the main advantages of ATR-FTIR is that it requires a lower number of samples for modeling.

For the pentosans/ hexosans ratio (cP/cH) it was only possible to develop a model for (FT-NIR) with modest statistics that only allow the separation of the samples in two extreme groups (high and low).

Table 7.1 Statistics of the PLS-R models based on ATR-FTIR and FT-NI spectra collected on *Picea abies* wood strips.

		PreProRange	Data set (no.of samples)	Cross-validation				
				r^2	RMSECV (%)	RPD	RK	Out
FT-NIR (Fiber probe)	cP/cH	2ndDer (7500-5450 cm^{-1})	CV (26)	54.3	1.67	1.5	3	2
	H/G	1stDer VN (6100-5450 cm^{-1})		85.4	0.008	2.6	3	2
	Py-lignin (%)	1stDer (6100-5450 cm^{-1})		93.6	0.73	4	3	3
FTIR-ATR	cP/cH	-		-	-	-	-	-
	H/G	1stDer VN (1463-1098 cm^{-1})	CV (26)	91.3	0.0053	3.4	5	3
	Py-lignin (%)	MSC (1824-1098 cm^{-1})		76.3	1.3	2.06	3	8

Although the best model was obtained with ATR-FTIR in fact it is quite easier and faster to acquire the spectra using the fiber probe, it is particularly more difficult to align the position of the wood strip in the wood strip, for instance for the 18 cm strip in Figure 7.1 it takes 180 min to acquire the spectra using the fiber probe in the conditions described, but with ATR-FTIR it is minimum is twice as much.

Figure 1 shows the predicted variation of one of the Zillertal samples collected at 750 m (Task 6) at 1 mm resolution using the PLS-R based on the FT-NIR spectra with a fiber probe.

The general pattern of higher H/G associated to latewood and lower at earlywood can be seen.

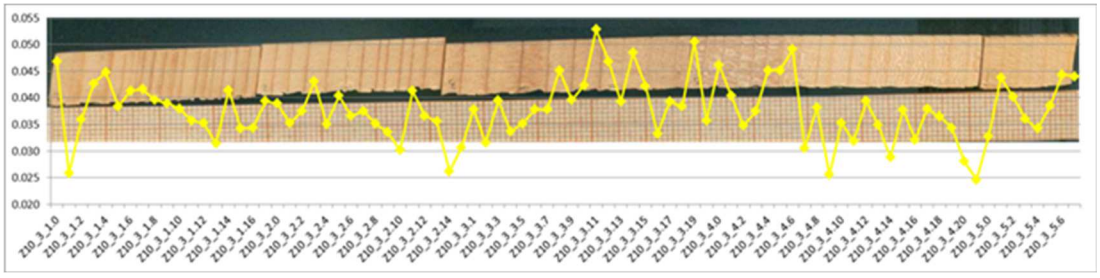


Figure 7.1 Predicted H/G ratio of a *Picea abies* solid wood radial strip with spectra collected with a fiber probe at a 1 mm resolution (Zillertal 750 m.).

Extractives calibration for Pinus sylvestris and Pinus halepensis

Although the ultimate goal was to obtain models for extractives content with spectra acquired in solid state the first step was to prove that it would be possible to obtain models in wood powder. The powder models can be further used to help in the development of the solid wood modelling.

Pinus sylvestris

The samples of *Pinus sylvestris* were available from the project PTDC/AGR-CFL/110988/2009 (coordinated by Lima Brito, UTAD, Vila Real), in this project it was previewed the analysis of 90 samples collected from five representative sites of P. sylvestris distribution area in Portugal. The wood samples were collected in adult trees at breast height (1.3 m) by extraction of one increment core of 12 mm per tree, from bark - bark. From the increment cores, 2 mm radial strip segments (from pith to cambium) were prepared. The material left from each increment core, after removing the radial strip for x-ray analysis, was preliminarily milled in a cutting mill Retsch SM 100 with a 6 mm sieve and further with a RETSCH Ultra Centrifugal Mill ZM 100 with a 1mm screen sieve. Samples were milled to pass 1 mm sieve, and aliquots of the milled wood were successively Soxhlet-extracted with dichloromethane, ethanol and water.

Fourier transform near infrared (FT-NIR) spectra were obtained in the wave number range from 12000 to 4000 cm^{-1} with a FT-NIR Bruker MPA spectrometer (Bruker Optics, Ettlingen, Germany) in diffuse reflectance mode using a spinning cup module for the wood powder and using the a fiber probe with a 1mm slit for the strips used for x-ray analysis. Each spectrum was obtained with 100 scans at a spectral resolution of 8 cm^{-1} and Zero-filling 2.

Instead of performing 270 extractions in total (90 samples times 3 solvents, dichloromethane, ethanol and water), as previewed in the project proposal, only 117 analyses were performed (39 samples times 3 solvents), the remaining ones were predicted after successful models development. From these the results of 27 trees were used for developing the models and 12 for validation.

These samples had an unusually high extractives content (Table 7.1) especially so in dichloromethane, (selected samples were analyzed for the analysis of extractives, c.f. Task 4).

Table 7.1 Descriptive statistics of the extractives content of Pinus sylvestris wood

Extractives content	Dichloromethane	Ethanol	Water	Total
No. of samples	39	39	39	39
Maximum (%)	28.5	3.0	2.0	32.4
Minimum (%)	2.9	1.8	0.3	5.9
Average (%)	10.7	2.2	1.0	13.9
SD (%)	5.9	0.2	0.5	5.9
CoeVar (%)	55	10	50	42

SD: standard deviation; CoeVar: coefficient of variation

Models with very good statistics were obtained for dichloromethane and total extractives content, but not for ethanol or water or even the combination of ethanol and water (Table 7.2), despite the CoeVar for water being similar to the CoeVar for dichloromethane (Table 7.1). This in part can be explained by the much lower range of variation for ethanol (1.2 %) and water (1.7 %) when compared to dichloromethane (25.6 %).

Table 7.2 Statistics of the PLS-R models for extractives content of Pinus sylvestris wood

Extractives (%)	'reProRang	Data set (no.of samples)	Cross-validation r^2	RMSECV (%)	RPD	RK	Out	Data set (no.of samples)	Test set r^2	RMSEP (%)	RPD	RK	Out
Dichloromethane	1 c	27	99.48	0.35	13.9	1	1	12	99.21	0.47	13.1	1	0
	2 c		99.35	0.38	12.4	1	1		99.41	0.45	13.4	1	0
	3 c		97.76	0.63	6.7	1	1		98.91	0.62	10.8	1	0
	4 c		99.01	0.43	10.1	1	0		99.24	0.51	11.5	1	0
Total	1 c	27	99.00	0.59	9.99	1	0	12	97.78	0.77	6.8	1	0
	2 c		98.72	0.65	8.8	1	0		98.32	0.71	7.8	1	0
	3 c		98.11	0.74	7.3	1	0		98.02	0.83	7.5	1	0
	4 c		98.28	0.72	7.6	1	0		98.56	0.69	8.6	1	0

c) 8000 to 4000 cm-1; 1. 1stDerVN; 2. 1stDerMSC; 3. 2ndDer; 4. 1stDer

The analysis of the spectra obtained in the x-ray strips showed that the high extractives content in dichloromethane were restricted to the year-rings close to the pith. Clearly the first YR close to the pith were impregnated with resin in such quantity the strip in this part become translucent, the light trespassed the strip during the spectra acquisition (Figure 7.2). This was confused with heartwood (Figure 7.3), it was only cleared the confusion during the acquisition of spectra for the solid wood models.

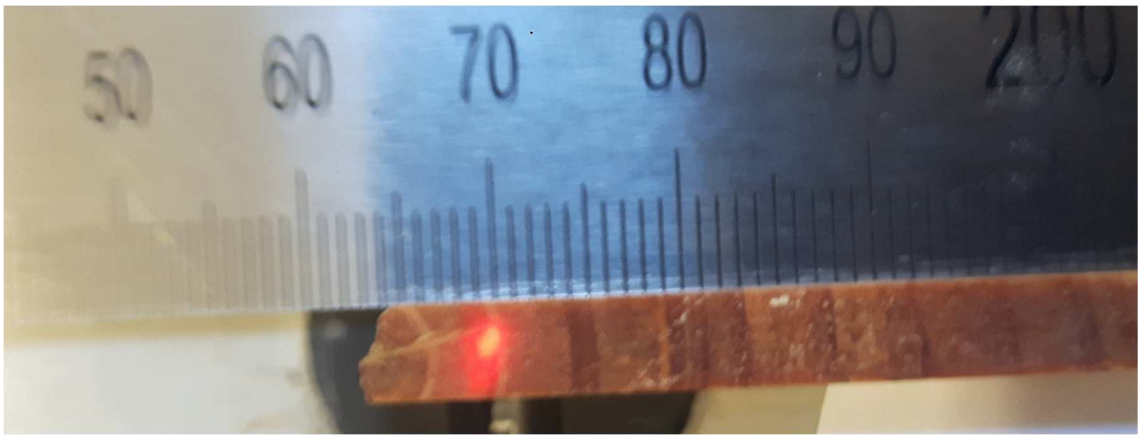


Figure 7.2 Translucent light in a strip (c.f. strip 49 Fig. 7.3) of wood impregnated with resin during the spectra acquisition.

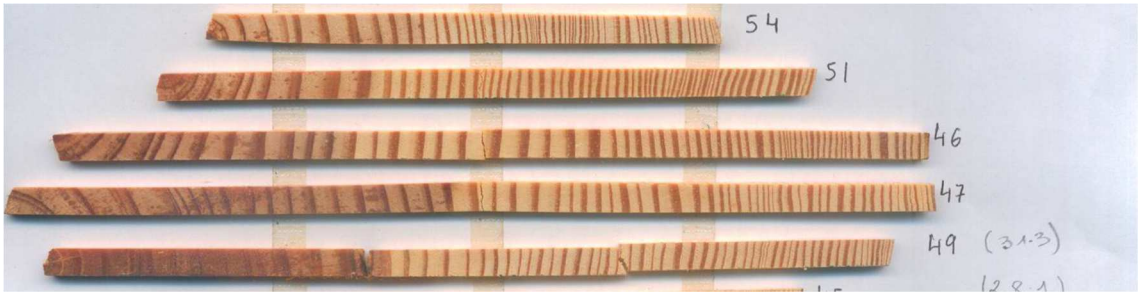


Figure 7.3 Wood strips prepared for x-ray analysis were the solid wood spectra were acquired. Strip 49 was the one with the highest resin content (c.f. Figure 7.2)

It is clear from Figures 7.2 and 7.3 that the average extractives content of the strip could not be used to develop models for extractives content. At the ring or sub-ring level, due to the uneven distribution of the resin, for instance since the strip 49 (Figure 7.3) the part with the resin is safely less than half of the total length of the strip, this means that the resin content in the first years could account for twice as much of the value found for the all strip.

Further analysis will be necessary to assess the extractives content along the radii to develop models at ring or sub-ring level.

Pinus halepensis

Pinus halepensis samples were available form a joint collaboration with INIA, Madrid, Spain, from two trial one in Valdeolmos, Madrid the other in Valencia. The samples were drilled in the tree with a 12 mm drill. For the analysis the small wood ships obtained were further milled in ultra-centrifugal mill Retcsh ZM100, with the 1 mm sieve in reverse order. For this samples it would not be possible to obtain solid wood spectra or models.

In total 93 samples were assessed for the extractives content.

Table 7.3 Descriptive statistics of the extractives content of *Pinus halepensis* wood

Extractives %	Dichloromethane	Ethanol	Water	Total
No. of samples	93	93	93	93
Maximun	6.7	3.2	2.7	11.4
Minimun	1.1	0.2	0.4	3.4
Average	2.6	1.7	1.6	5.9
SD	1.3	0.5	0.5	1.6
CV (%)	48.5	31.1	29.4	27.5
Range (%)	5.6	3	2.3	8

SD- Standard deviation; CV- Coefficient of variation

The range of variation, especially for dichloromethane although high it was by no means comparable to the *Pinus sylvestris*. As for the *Pinus sylvestris*, only for dichloromethane and total extractives content, it was possible to build models (excellent models indeed). The models for P halepensis were even better than the ones obtained for P. sylvestris with about half the error (RMSECV c.f. tables 7.3 and 7.4).

Figure 7.4 Box whiskers plot for the results of the extractives content in dichloromethane (CH_2Cl_2) ethanol (C_2H_5OH), water (H_2O) and total extractives content.

Extractives (%)	PreProRange	Data set (no.of samples)	Cross-validation r^2	RMSECV (%)	RPD	RK	Out
Dichloromethane	1st Der MSC 6100-5450 cm^{-1}	CV (93)	97.8	0.189	6.68	2	0
	1st Der VN 6100-5450 cm^{-1}		97.7	0.189	6.52	2	0
	1st Der MSC 6100-5450; 5000-4597 cm^{-1}		97.7	0.193	6.52	3	0
	1st DerVN 7500-5450; 5000-4250 cm^{-1}		96.5	0.235	5.37	5	0
Total	1st Der MSC 6100-5450 cm^{-1}	CV (93)	96.23	0.312	5.15	4	0
	1st Der VN 6100-5450 cm^{-1}		96.68	0.295	5.49	4	2
	1st Der MSC 6100-5450; 5000-4597 cm^{-1}		96.51	0.302	5.53	4	1
	1st DerVN 7500-5450; 5000-4250 cm^{-1}		95.63	0.325	4.93	3	0

In conclusion, it seems possible to obtain good models for extractive content in dichloromethane and total extractives content in solid wood but not for ethanol or water, houever more work is still to be done in order to obtain models at ring or sub-ring level.

Calibrations for cellulose and hemicellulose content in softwoods.

The determination of the polysaccharide fraction in wood is long recognized as a challenging task. The majority of cellulose methods require a two-step procedure starting with the isolation of holocellulose followed by isolation of alpha-cellulose. Once the

cellulose determined the hemicellulose can be obtained by difference. A simple method for cellulose determination is the diglyme method. Not only it is a one-step method but also the procedure is quite simple and easily scaled up for large scale screening.

Nevertheless, in terms of simplicity and throughput the diglyme method is not comparable to NIR spectroscopy coupled with multivariate analysis, even though it is a proven effective method for the establishment of partial least squares regression (PLS-R) models based on near infrared (NIR) spectroscopy for cellulose content of Eucalyptus species.

The diglyme method

A total of 27 samples of *Pinus pinaster* (4, Pnb), *Pinus sylvestris* (9, Psyl) and *Pinus halepensis* (14, Phal) were assessed by the diglyme method. The Psyl and Phal were selected between the samples previously extracted and covering the range for lignin content. The range of cellulose content of the samples were clearly complementary being the Phal the ones with the lower cellulose content, followed by the Psyl and finally the Pnb samples with the higher cellulose content. (Table 7.4). In total almost 10% absolute values were considered enough for modulation.

Table 7.4 Chemical characterization of the wood Pine samples.

	Phal			Psyl			Pnb		
	H/G	Klason	Celldig	H/G	Klason	Celldig	H/G	Klason	Celldig
Av	0.061	27.4	43.4	0.047	27.5	48.2	0.038	26.3	52.6
Stdev	0.009	2.1	1.9	0.007	1.3	1.8	0.004	0.56	1.4
Max	0.077	31.1	48.0	0.057	29.4	50.8	0.041	27.0	54.4
Min	0.048	24.4	41.1	0.035	25.5	45.5	0.033	25.8	50.9

However, in spite of the cellulose content range of the samples the models developed were not very good, the one with the best statistics (R^2, RMSECV and RPD), were not as good as expected considering the 10 % range (0.77, 1.9, 2.0 respectively).

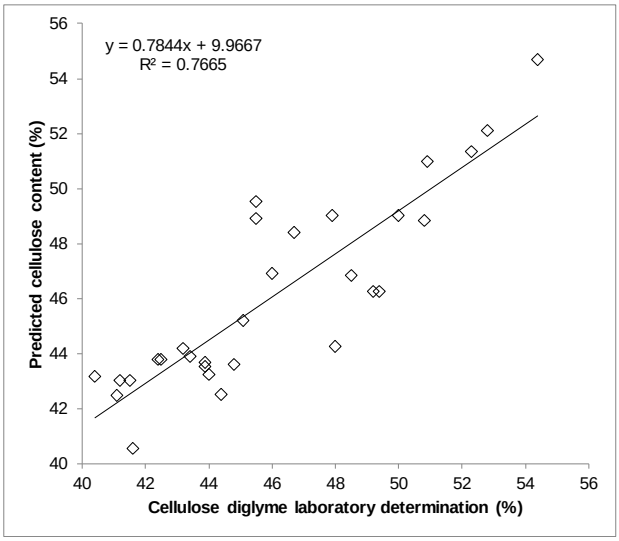


Figure 7.3 Plot of the predicted versus determined cellulose content for Pine samples.

The ATR-FTIR spectra of the cellulose extracted by the diglyme method showed clearly not only the presence of residual lignin, but also that the amount varied between the samples.

The analytical pyrolysis of all cellulose extracts allowed the quantification of the residual lignin amount and confirmed a large variation between samples. The cellulose yield was further corrected for residual lignin which allowed to improve the statistics of the model (R^2 = 0.82, RMSECV = 1.5, RPD = 2.4). These results and in particular the most important for models comparison, the RMCECV are closer to the ones obtained for *Eucalyptus* species. Nevertheless, an attempt to improve lignin removal by ball milling the samples did result primarily in the loss of the cellulose.

The full description of the analysis of the cellulose diglyme was submitted to Holzforschung (Alves et al. Holzforschung).

Pinus pinaster NIR based PLS-R models for sugar determined by HPLC

Another way to assess the polysaccharide content is to assess the sugar released upon acid hydrolysis for lignin content assay. To assess the hydrolysable sugars there are two methods one by GC the other by HPLC. The GC procedure is time consuming and tedious to perform since it requires the derivatization to alditol acetates. There are in fact different HPLC methods using various detector strategies.

In this experiment sugar were analyzed in Hamburg using quantitative borate-ion-exchange chromatography with two replicate hydrolyses per sample and three injections per hydrolysate according.

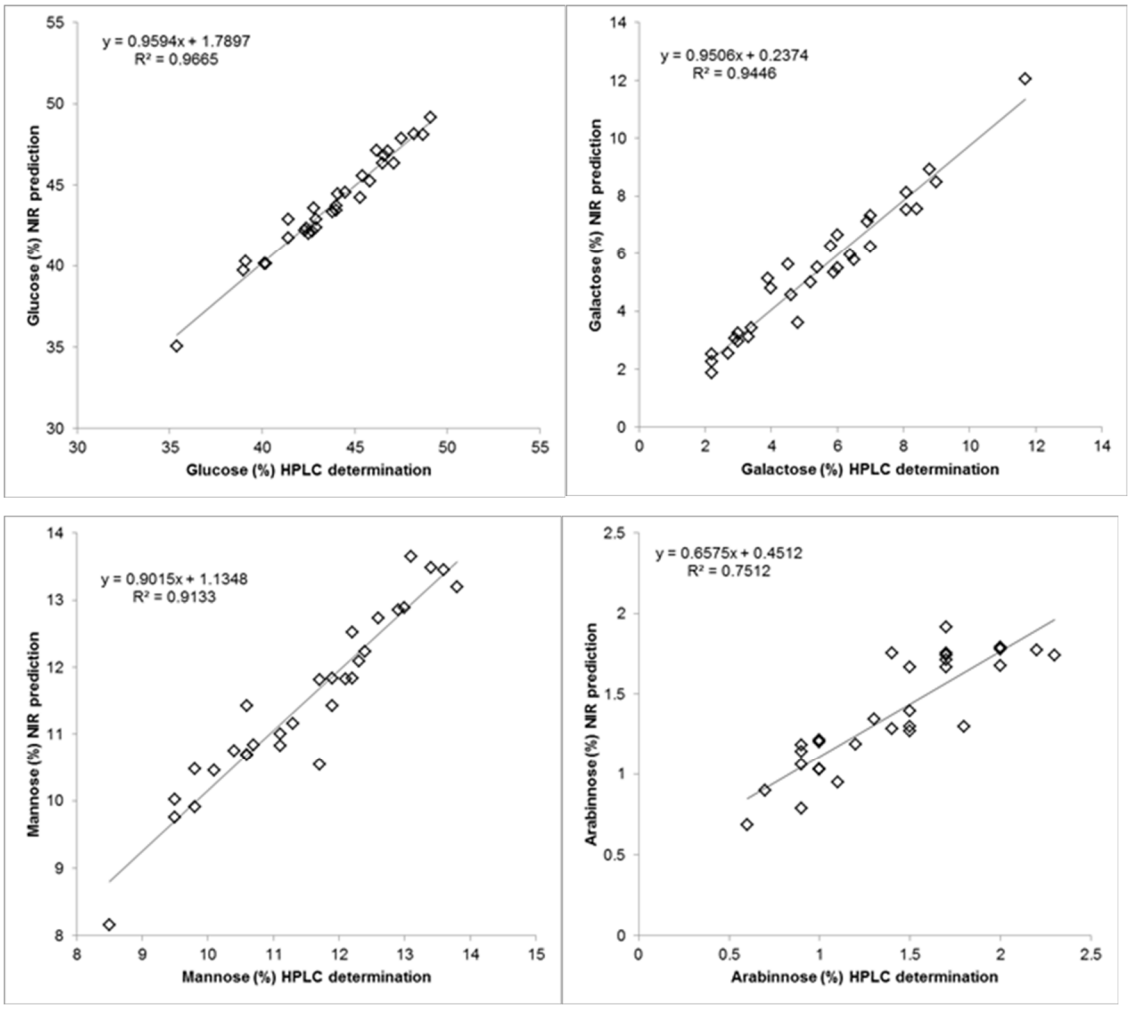
Pinus pinaster samples (31) were analyzed for lignin content and the hydrolysate was sent to Hamburg for sugar analysis. Prior de analysis FT-NIR spectra were obtained in exactly the same sample used for analysis.

Table 7.5 Statistics for sugar analysis of wood Pine samples by quantitative borate-ion-exchange chromatography.

	Arabinose	XYlose	Manose	Galactose	Glucose
Mean	1.5	7.0	11.5	5.4	43.8
Std Deviati	0.5	0.4	1.4	2.4	3.2
Max	3.1	8.1	13.8	11.7	49.1
Min	0.6	6.3	8.5	2.2	35.4
Range	2.5	1.8	5.3	9.5	13.7

The results obtained allowed the development of excellent models for glucose and galactose content and also a very good model for mannose. However, the results for the pentosane was not acceptable for xylose the most important hemicellulose sugar after mannose, and barely acceptable for arabinose mainly due to the very low range of variation found for both pentosans.

The main problem being that not all the glucose determined is from cellulose it is needed to make some assumptions in order to recalculate the cellulose content to correct for the glucose from galactoglucomanan. Nevertheless, the models so far are superior to the diglyme method.



Conclusion

Both methods, diglyme and sugar analysis, can be used for development models for the analysis of the polysaccharide wood fraction by FT-NIR. Further analysis is being performed notably the samples used for the diglyme method are now in due course to be analyzed by the HPLC method.