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Abstract: 13C nuclear magnetic resonance and mid-infrared spectroscopies were used for characterizing changes in the chemical structure of wood polymers (cellulose, hemicellulose and lignin) in relation to the tree growth location. Samples of three provenances in Europe (Finland, Poland and Italy) were selected for studies. The requirement was to use untreated solid wood samples to minimize any manipulation to the nanostructure of native wood. The measurement reliability of the NMR experiment has been quantified by means of internal reference in form of HDPE strip rolled together with wooden sample.
The results confirm that the chemical and physical properties of samples belonging to the same wood species (Picea abies) differ due to the origin. Both FT-IR and dynamic NMR spectroscopies were able to correctly discriminate samples originating from three different provenances in Europe. Such methods might be very useful for both, research and understanding of wood microstructure and its variability due to the growth conditions.

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Dear Editor,

Please find attached our manuscript entitled "**Solid state NMR and IR characterization of wood polymer structure in relation to tree provenance**", describing the results of both nuclear magnetic resonance and infrared spectroscopies studies on wood from different European areas. The wood of different geographical origins differs in term of chemical, phenological and physical properties (as mentioned in the Introduction of our manuscript). However, it is a very hard task to define/describe such differences. The development of a reliable technique allowing classification of wood in regard to its provenance is therefore of great interest.
The work here proposed is a continuation of our previous research, where NIR spectroscopy has been successfully applied for the differentiation of wood samples. The previous results were convincing, but there were several questions regarding the link between spectrum differentiation and polymer structures. The joint use of different techniques and skills has been an important step toward better understanding of the factors that discriminate among the wood samples, at the molecular level (related to protons mobility and chemical functional groups). To our knowledge no such research was reported before.

We do sincerely believe that the content and way of presentation of our manuscript would be of interest for the Carbohydrates Polymer readers. Thus, we are submitting our work as an original full-length research paper.

Looking forward to have your kind evaluation,
With sincerely regards,

Jakub Sandak, on behalf of authors

Highlights

- FT-IR and NMR spectroscopies were used for the discrimination of samples with different geographical provenance
- $T_{1\rho(H)}$ values, obtained from Variable Contact Time NMR experiments highlighted the differences between wood groups
- Principle Component Analysis of NMR and IR data allowed the most effective discrimination of wood due to provenance

Solid state NMR and IR characterization of wood polymer structure in relation to tree provenance

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Abstract

^{13}C nuclear magnetic resonance and mid-infrared spectroscopies were used for characterizing changes in the chemical structure of wood polymers (cellulose, hemicellulose and lignin) in relation to the tree growth location. Samples of three provenances in Europe (Finland, Poland and Italy) were selected for studies. The requirement was to use untreated solid wood samples to minimize any manipulation to the nanostructure of native wood. The measurement reliability of the NMR experiment has been quantified by means of internal reference in form of HDPE strip rolled together with wooden sample.
The results confirm that the chemical and physical properties of samples belonging to the same wood species (*Picea abies*) differ due to the origin. Both FT-IR and dynamic NMR spectroscopies were able to correctly discriminate samples originating from three different provenances in Europe. Such methods might be very useful for both, research and understanding of wood microstructure and its variability due to the growth conditions.

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1. Introduction

The adaptation of species had been scientifically demonstrated by Charles Darwin already in 1868. Local climate, soil, slope, forest density, silvicultural practices, fungi, wildlife, disease and other factors can strongly influence the tree growth and consequently, wood formation. The timber in a given site carries a record of all the conditions listed above. As an adaptation mechanism of trees to different site conditions several variations in the tree-ring structure have been observed by Miina (2000), Park & Spicker (2005), Manetti & Cutini (2006) and Andreassen, Solberg, Tveito & Lystad (2006) among others. Norway spruce (*Picea abies*), often called whitewood, is a northern and central European species, living primarily in the mountainous areas. The vertical limit for the spruce presence in the Alps is at an altitude of ~2,200m. There are three main regions of spruce presence in Europe;

- Scandinavia, West Siberia (up to the Urals), Belarus and Northeast Poland
- mountainous areas of Central Europe – the Sudetes and the Carpathian Mountains
- Southern Europe including the Balkans and the Alps

According to recent forest inventories, the actual distribution of spruce is much wider than its natural ranging (Spicker, 2000).

The Norway spruce wood from northern provenances (Scandinavian countries) has the highest overall density and percentage of latewood comparing to trees growing in Central and Southern Europe (Skrøppa, Hylen & Dietrichson, 1999). Krzysik (1978) reported that different amounts of cellulose, hemicelluloses, lignin, and extractive components were measured in spruce wood from different provenances. Analogous observations were noticed for other wood species; *Eucalyptus globus* (Miranda & Pereira, 2002), *Pinus helepensis* (Tahar, Tayeb & Chaabane, 2007), *Pinus ponderosa* (Smith, Peloquin & Passoff, 1969). It is expected, therefore, that trees growing in various parts of the world would differ in such a way to discriminate their specific distinctive features.

The currently employed PEFC (Programme for the Endorsement of Forest Certification), used for tracking the wood flow from the forest to the mill system, is based only on written documentation. Several methods including chemical and genetic fingerprinting are currently employed for verification of product identity by examining its chemical or genetic composition (Dykstra *et al.* 2002). Chemical fingerprinting methods include: analysis of trace elements, pyrolysis, gas chromatography, as well as, near- and mid- infrared spectroscopies. Genetic fingerprinting methods consist of DNA marker analysis from one or more of the following genomes: nuclear, plastid or mitochondrial. Even if several successful applications of the above techniques have already been reported, (Nielsen & Kjær, 2008, Sandak, Sandak & Negri, 2010, 2011) none of these methodologies has reached a stage of development that would warrant its general use up to this point. Some techniques are too costly and/or time consuming for routinely use in timber tracking. These applications are limited to a small number of samples, to be investigated in the laboratory. In contrary, infrared spectroscopy is a fast and non-destructive method. Unfortunately, the detailed interpretation of spectra and the following correlation of acquired data in order to discriminate the provenance are still questionable. Therefore, an additional research supporting better understanding of the infrared relation with wood provenance is of great interest.

Nuclear magnetic resonance (NMR) is becoming a routine technique in agro-food fields for quality control and traceability of cheese and wine (Sacchi & Paolillo, 2007, Aghemo, Albertino, & Gobetto, 2011, Mazzei & Piccolo, 2012, Ritota *et al.* 2012, Ritota, Casciani, Failla, & Valentini, 2012). It is expected, therefore, that it might have also a great potential for wood characterization. Several researchers have proposed the use of NMR for detailed investigation of the chemical structure of wood (Gil & Neto, 1999, Maunu, 2002, Bardet *et al.*

2009, Mburu, Dumarcay, Huber, Petrissans, & Gerardin, 2007). Thus, many papers focus on the structural differences between natural and treated wood-based materials, the efficiency of chemical extraction methodologies, and characterization of its polymeric components. NMR characterization of wood was a subject in the archaeological research to test the wood aging and storage effects of historical materials (Bardet, Foray, & Tran, 2002, Bardet, Foray, Maron, Goncalves, & Tran, 2004, Crestini, Hadidi, & Palleschi, 2009). It has also been used for monitoring the decomposition level of forested areas (Preston, Trofymow, Niu & Fyfe, 1998).

Wood is, in its nature, a complex and heterogeneous material, considered as a matrix of three main polymers: cellulose, hemicellulose and lignin. The advantage of NMR technique is, therefore, in its ability to analyse a mixture of such polymers without extensive chemical modifications to obtain separated fractions. Several authors have reported NMR research results on lignin structures (Nimz, Robert, Faix, & Nemr, 1981, Malkavaara, Alén, & Kolehmainen, 2000, Capanema, Balakshin & Kadla, 2004). Other studies have been dedicated to woody carbohydrates, hemicelluloses (Gil & Neto, 1999, Maunu, 2002) and cellulose (Newmann, 1999, Larsson, Hult, Wickholm, Pettersson, & Iversen, 1999, Okushita, Komatsu, Chikayama, & Kikuchi, 2012). These have been a starting point for the following research on wood by solid state NMR. This has delt with the structural modifications induced directly by processes such as pyrolysis, densification, bleaching, pulping or biodegradation (Sivonen, Maunu, Sundholm, Jamsa, & Viitaniemi 2002, Delmotte, Ganne-Chédeville, Leban, Pizzi, & Pichelin, 2008, Popescu, Larsson, & Vasile, 2011). Over the past 30 years, the technical advances of NMR instrument hardware and the evolution of theoretical knowledge have made it possible to vary sequences and to apply high magnetic fields and spinning speeds in order to study wood, especially with 1H, 13C and 31P magic angle spinning (MAS) NMR. Recently, the 13C cross polarization magic angle spinning (CPMAS) is the most used NMR technique for wood characterization, due to the high signal-to-noise ratio obtainable in a relatively short experimental time, despite the loss of quantitative reliability. However, it must be noted here that physical manipulation of wood, such as scratching and grinding while preparing suitable samples lead to changes in its polymeric structure, i.e. the composition appears to be method dependent to some extent (Viel, Capitani, Proietti, Ziarelli, & Segre, 2004, Bardet, Foray, & Tran, 2002). This means that the results of liquid and solid state NMR analyses of isolated components are not comparable and, therefore, provide little information on the original structure of solid wood. Small cylindrical samples were thus proposed to get a fingerprint of the pristine material (Preston, Trofymow, Niu, & Fyfe, 1998, Bardet *et al.* 2009). The preparation process of such samples made of wood is rather complicated. Therefore, an alternative procedure should assure a proper representation of the complex wood structure while minimizing any chemo-physical manipulation of the wood during sample preparation.

Taking into account the above considerations, the aim of this research was to develop an original MAS NMR-based methodology (including experimental set-up and wooden sample preparation) for studies of chemical/physical wood properties.. The overall objective was to characterize cellulose, hemicellulose and lignin of selected wood samples possessing intra-species differences resulting from variation of geographical provenance. Both IR and NMR spectroscopies, assisted by modern multivariate analysis, were investigated.

2. Material and methods

2.1. Material selection

Sets of Norway spruce (*Picea abies* L. Karst.) wood samples collected in three different locations within Europe were used as experimental materials. The countries of origin included Finland, Poland and Italy, all differencing in geographical position, elevation, climate and silviculture. The summary of site characteristics is presented in Table 1. The procedure of samples harvesting, conditioning and preparation was described previously (Sandak, Sandak, & Negri, 2011). Because it was impossible to collect trees of exactly the same age, experimental samples were obtained from the adult wood zones assuring equal cambial age of the all investigated samples.

Table 1. General characteristics of the provenance sites

2.2. Chemical composition

The chemical content of main woody polymers was determined on milled samples from each provenance.

The concentration of cellulose was determined according to the Seifert procedure (by using acetylacetone-dioxane-hydrochloric acid). Holocellulose content was obtained by wood delignification with sodium chlorite with the addition of acetic acid (Browning, 1967). The content of hemicellulose was computed as the difference between holocellulose and cellulose. The quantities of other wood components were determined according to the following standards:

lignin (TAPPI T 222 om-06)
hot water extractives (TAPPI T 207 cm-08)
1% NaOH extractives (TAPPI T 212 om-07)
organic solvent extractives (TAPPI T 204 cm-07)
ash content (TAPPI T 211 om-07)

The chemical analyses were repeated three times and maximum standard deviation of results was considered as a measurement error indicator.

2.3. FT-IR-ATR analysis

The set of 30 samples (10 for each provenance) were analyzed by means of mid infrared spectroscopy using an FT-IR spectrometer (ALPHA produced by Bruker Optics GmBH) equipped with a ZnSe external module for attenuated total reflection (ATR) with the following settings: 24 scans per sample; spectral resolution: 4cm-1, wavenumber range: 4000 to 600 cm-1. The OPUS 7.0 (by Bruker Optics) software package was used for instrument control and data processing. An original procedure for selection of the representative samples was established on the base of samples homogeneity. Firstly, an average spectrum representing one provenance was calculated from the set of all spectra acquired within samples of same provenance. Three samples with spectra closest (in term of spectral distance) to the average value were selected for NMR analysis. The homogeneity was quantified on the base of cluster analysis. The selection was essential to diminish the natural variability of woody material and to assure a compromise between the generality of results and the relatively time consuming NMR analyses.

2.4. NMR analysis

The Nuclear Magnetic Resonance measurements were restricted to the nine representative samples (P1-P3, I1-I3, F1-F3) selected from the set 30 samples measured by FT-IR. MAS NMR analyses were carried out with a Bruker 300WB instrument operating at a proton

frequency of 300.13 MHz. NMR spectra were acquired with a cross-polarization (CP) pulse sequence under the following conditions: ^{13}C frequency: 100.07 MHz, $\pi/2$ pulse length: 3.5 µs, ^{1}H decoupling pulse power: 47 kHz, recycle delay: 3 s, contact time range: 0.2 – 9 ms, 2k scans. Samples in the form of rolled thin wood slices were packed in 4mm diameter zirconia rotors, which were spun at 8 kHz under air flow. Both the sample shape and size ensure the use of a representative sample.

In addition, the use of an internal standard was evaluated in order to ensure quantitative analysis and to estimate the measurement stability. For this purpose, a high density polyethylene (HDPE) film, with a mass of about 10% the corresponding wood weight, was rolled together with the wooden slice. The HDPE did not affected the NMR spectrum, its peak does not overlap with the wood signals. Bruker Topspin 1.3 software was used for data analysis and spectra deconvolution.

Basic CPMAS experiments were recorded to identify the main signals assigned to the wood and the HDPE. Since intrinsic parameters, like conformation and mobility depend both on homonuclear and heteronuclear interactions, variable contact time experiments (VCT) were performed in order to obtain complementary information. The CP spectrum intensity depends on two competing factors, the magnetization transfer by dipolar coupling and the spin-lattice relaxation times in the rotating frame. Thus, measuring spectrum intensity (M) as a function of CP contact time (t), it is possible to extract cross-relaxation parameters T_{CH} (cross-polarization rate constant) and $T_{1\rho(H)}$ (spin–lattice relaxation time) (Equation 1). Numerical estimation of T_{CH} and $T_{1\rho(H)}$ values are usually obtained by fitting the obtained experimental curve with single or multiple exponential laws according to homogeneity, segregation and/or domain size (Kolodziejski & Klinowski, 2002).

$$M(t) = M_0 \cdot e^{\frac{-t}{T_{1\rho(H)}}} \cdot (1 - e^{\frac{-t}{T_{CH}}}) \qquad \text{equation 1}$$

where; $M(t)$ is the peak intensity as a function of contact time t, M_0 is the normalization constant, $T_{1\rho(H)}$ is the proton spin-lattice relaxation time in the rotating frame, and T_{CH} is the cross-polarization time constant.

2.5. Multivariate data analysis

Principal Component Analysis (PCA) and Cluster Analysis (CA) were used for evaluation of spectral data obtained by FT-IR and NMR spectroscopies. Mid infrared spectra were post-processed and evaluated with OPUS 7.0 software (Bruker Optics GmBH). Ward's algorithm and the calculation of Euclidean distances was applied when creating dendrograms of the CA. Identity test (part of the Opus package) was used for development of the PCA models discriminating woods due to the provenance. Unscrambler 10.2 (Camo Software) was used for PCA analysis of NMR data.

3. Results and Discussions

The chemical composition of Norway spruce samples representing three different provenances is presented in Table 2. The apparent difference between quantities of cellulose and holocellulose within samples of all locations are negligible and smaller than the measurement error. It was not possible, however, to explain (by wet chemistry analysis) the possible qualitative differences between carbohydrates extracted from woods of different

origin. Some more evident differentiation was, however, noticeable for the lignin and extractives content.

Table 2. Contents of chemical components extracted from Norway spruce samples of different provenances

Selected physical properties, as summarized in Table 3, indicate clear morphological differentiation of spruce samples from Finland, Poland and Italy. The specific density and the late wood ratio of Italian wood samples were the lowest. The highest ring width was noticed for wood from Poland. The tendency followed the results of Franceschini et al. (2010) where the ring width is negatively correlated with the tree age. The yearly ring size was comparable for samples from Finland and Italy, even if the geographical locations were the most distant. However, the climatic conditions, especially the average yearly temperature and the length of vegetation season, were similar in both Finland and Italy.

Table 3. Physical characteristic of spruce samples.

Figure 1 presents results of FT-IR Principle Components Analysis (PCA) performed on spectra collected from wood samples of three different provenances. A clear separation between groups was achieved after plotting the three first PC scores on the graph. These results for mid-IR are in correspondence with previous studies using near –IR. They proved that wood of the same species (*Picea abies*) from different provenance can be separated through FT-NIR spectroscopy (Sandak; Sandak, & Negri, 2011). The question rises here: what are the chemical/physical bases for such distinction of different spruce woods.

Figure 1. FT-IR-based discrimination of the spruce woods due to provenance

It is clear that the infrared spectroscopy, due to its functioning principle, is only capable of detecting the presence of functional groups such as -OH, -CH or -CH_2. The three structural polymers (cellulose, hemicelluloses and lignin) have an extremely complex cross-configuration and make up 90 – 98% of the woody matter. This makes IR spectroscopy insufficient to fully characterize wood at the molecular level. The chemical structure of cellulose, hemicelluloses and lignin is schematically presented in Figure 2.

Figure 2. The chemical structures of the main wood components (according to Bardet et al. 2009): cellulose (a), hemicellulose (b) and lignin (c), and the numbering scheme corresponding to the resonance peaks summarized in Table 4

Cellulose is present in wood as microfibrils and as amorphous regions. The microfibril structure is crystalline and characterized by a high degree of polymerization. The quantity and quality of the amorphous regions of cellulose differ due to species and the growing conditions of the tree. The chemical structure of lignin is very complex and it is, in fact, impossible to determine its precise composition as it varies even between trees in the same forest. In softwoods, it is an amorphous polymer consisting of p-hydroxyphenyl -guaiacyl units (p-hydroxyphenyl -syringil-guaiacyl in case of hardwoods) inter-linked in non-regular structures.

Lignin is hydrophobic, where cellulose is highly hydrophilic. Hemicellulose connects lignin and cellulose together. Beside of genetic factors; the growing environment (including climate, soil, silviculture and the yearly variation of sun/rain), strongly affects wood physiology, xylogenesis, tree morphology and, in consequence, wood chemical composition.

FT-IR spectroscopy, through detection of the main functional groups, ensures a good separation among wood samples, but cannot give an overall picture of the materials. Therefore, solid state NMR was selected as an additional technique for providing supplementary/alternative set of information on the molecular structure at short and medium order.

As already mentioned, a good fingerprint of wood samples can be obtained with ^{13}C CPMAS NMR measurements. Representative NMR spectra of wood from Finland, Poland and Italy are shown in Figure 3. The spectra present the typical resonances of wood components that produce highly overlapped signals. Two key regions; from 160 to 110 ppm and from 110 to 15 ppm, can be distinguished in the NMR spectra, according to Bardet et al. (2009). The first is characterized by broad and low intensity resonances typical of the aromatic structures in lignin. The second region includes sharp intense peaks due to carbohydrate polymers (cellulose and hemicellulose) that are highly overlapping each other due to their chemical similarity.

Table 4 shows the resonance assignments (indicated by the numbering scheme in Figure 3 and corresponding to the carbon atoms labeled in Figure 2), It should be mentioned that ^{13}C CPMAS NMR spectroscopy allows for the distinction of chemically equivalent carbons in different chain packings or conformation, as it is in the case of amorphous and crystalline cellulose. The most intense peaks 11 and 12 are due to C-2, C-3 and C-5 carbons in glucose. The two peaks 9 and 10 are assigned to C-4, in crystalline and amorphous (or less ordered surface) cellulose, respectively. Peak 14 is related to C-6 in cellulose and Cγ in lignin. Peak 8 represents cellulose's C-1 with a high-field shoulder attributed to hemicellulose (103 ppm). Only two signals can be undoubtedly attributed to hemicellulose: the methyl carbon peak 17 and the carboxylic carbon signal 1, although these are of rather weak intensity. The three aromatic units constituting the lignin lattice are recognized from three groups of signals in the range 160-105 ppm. Finally, the small peak 15 is assigned to the lignin methoxyl group.

Figure 3 NMR spectra of spruce woods differing in provenance and the peaks assignments

Table 4. 13C CPMAS resonance assignments of wood and HDPE

The three representative NMR spectra of Figure 3 suggest that all the provenances (P, F and I) produce similar signals and only minor intensity differences can be noticed without a quantitative analysis, for example: lignin's peaks (3, 6, 7, 14) and carbohydrates' signals (1, 13, 17).

Chemically equivalent carbons produce the same response to cross-polarization and the resulting peak areas are comparable. For that reason, an internal standard has been used allowing for both quantitative spectra evaluation and probing the stability of the dynamic NMR measurements. The polyethylene standard produces a sharp peak at 32 ppm corresponding to the methylene carbon of the polymer backbone (16 in Figure 3). The peak does not overlap with wood signals and, in consequence, its intensity may be used for signal normalization and the estimation of the measurement error.

It has been reported (Teeäär, Serimaa, & Paakkarl, 1987 and Mansfield, & Meder, 2003) that the cellulose crystalline index *CrI* can be calculated on the basis of NMR spectra according to equation 2.

$$CrI = \frac{A_9}{A_9 + A_{10}} \qquad \text{equation 2}$$

where: A9 and A10 are the de-convoluted peak areas of signals 9 and 10 corresponding to the C-4 carbon atom.

The range of *CrI* values were computed for the investigated samples, as shown in Figure 4, and correspond to the reference literature data (Samuel, Pu, Faston, & Ragaukas, 2010, Park, 2010). It can be noticed that spruce samples originating from Poland have higher CrI than the other two series. Even if there is a clear trend, it has to be mentioned that nominal differences are too small to draw any conclusion.

Figure 4. Cellulose crystalline index CrI computed from the NMR spectra of spruce from different provenance

Additional NMR analyses were done because the straightforward comparison of raw NMR spectra was not sufficient for undoubtable discrimination of the wood provenances. Dynamic NMR measurements were, therefore, performed in order to provide supplementary information on the polymeric interconnections testing wider material domains. A series of CPMAS spectra were collected for each sample varying the contact time (*ct*) between 0.2 and 9.0 ms. The calculated signal intensity vs. contact time was compared after the normalization of the peak height. To neglect differences due to variations in sample weights, the intensity values of the selected peak y_n were extracted from the spectra and normalized with respect to the corresponding peak intensity at ct=1 ms (y_1) according to Equation 3:

$$y'_n = \frac{y_n}{y_1} \cdot 100 \qquad \text{equation 3}$$

The resulting y'_n values were then plotted against the contact time. The HDPE signal (16) was used to ensure the reproducibility of the measurement procedure. The obtained results are presented in Figure 5a. The HDPE VCT curves show similar profiles independently of the wood sample. The reproducibility of the measurements was good except for the shortest and the longest contact times, due to the relatively low peak intensity. Thus for the peak 16, the calculated measurement percentage error was ±6%.

Since *CP* spectral intensity is a measure of the efficiency of magnetization transfer from ^{1}H to ^{13}C by the dipolar coupling, the *CP* time constant (T_{CH}) can give a quantitative description of the cross-polarization and relaxation behaviour (Abelmann, Totsche, Knicker, & Kogel-Knabner, 2004, Cheng, Wartelle, & Klasson, 2010). The less mobile carbon groups exhibit both fast cross-polarization rate, i.e. short T_{CH}, because the cross-polarization is most efficient for the static ^{1}H-^{13}C dipolar interactions, and long $T_{1\rho(H)}$ values. As mobility increases (i.e., with increasing amorphous or homogeneous character of sample series) T_{CH} increases and $T_{1\rho(H)}$ decreases. It has been demonstrated that the measure of $T_{1\rho(H)}$ relaxation time can be used to estimate the domain size in polymers. (Conte, Spaccini, & Piccolo, 2006). Diffusion of ^{1}H magnetization within a hybrid system tends to average the ^{1}H relaxation time thus

leading to an averaging effect on the $T_{1\rho(H)}$ of the different components. In a homogeneous system, a single spin-lattice relaxation time is usually observed. For a heterogeneous system, more than one $T_{1\rho(H)}$ value are often found, because there is insufficient time for spin diffusion to equilibrate the magnetization in different phases (Kao, Chao, & Chang, 2006). Through measures of contact time variation, mobility and interactions of polymers can be studied (Bardet *et al.* 2009, Okushita, Komatsu, Chikayama, & Kikuchi, 2012). As a matter of fact, similar relaxation times of polymeric species inside the same matrix correspond to chemical interactions between chains (Liitia, Maunu, & Hortling, 2000, 2001, Bardet *et al.* 2009).

Figure 5. Normalized peak intensity vs ct of HDPE signal 16 (a) and wood signal 8 (b).

HDPE is a homogeneous polymer, and therefore, the magnetization behaviour follows the single exponential law (Equation 1). The calculated values of the two time constants were T_{CH} = 0.17 μs and $T_{1\rho(H)}$ = 21.1 μs, in agreement with the results reported for HDPE measurements in similar conditions (Nogueira, Tavares, Nogueira, 2004). It was possible to conclude therefore that polyethylene was a suitable reference and that all the experimental results from tests are comparable.

The wood spectra display intense sharp peaks together with weak and broad resonances. In order to keep the confidence level of 6%, the exponential trend of intensity vs. contact time was studied only for some selected peaks. These are the sharpest and fall in the range between 110 to 60 ppm. Accordingly, seven peaks were chosen: 8 C-1 from cellulose, 9 C-4 from crystalline cellulose, 10 C-4 from amorphous cellulose and Cβ from lignin, 11 C2,3,5 from cellulose and Cα from lignin, 12 C2,3,5 from cellulose, 13 CH_2OH C-6, and 14 Cγ from lignin. Examples of the VCT curves related to peak 8 at 105ppm are presented in Figure 5b. The above peak selection includes mainly the cellulose peaks, but the overall signal overlapping ensures also the contribution of other woody polymers. Assuming that all the carbons are in similar motional domains, the rising part of the exponential curve is dominated by the static dipolar interaction between carbon atom and nearest neighbor protons (13C-1H distance) described by the T_{CH} relaxation time. The T_{CH} values for the evaluated signals are shown in Figure 6. It is clear that variations of T_{CH} do not display particular trends due to sample provenance and any differences among the samples are not systematic.

Figure 6. TCH values obtained from the fitting of VCT curves of selected 13C signals with Equation 1.

The obtained average T_{CH} values, around 0.7ms, can be considered as the result of the presence of both rapidly and slowly cross-polarizing components, with a predominance of the latter. In literature it was reported that the determination of T_{CH} is inherently difficult because, in principle, there are as many T_{CH} values as there are chemical environments. In the studied case, the values were closer to those typical of aromatics, in particular for samples from Italy and Poland (Conte, Spaccini, & Piccolo, 2006). This could also be explained with the presence of small proton-rich hydrophobic domains or short polymeric chains randomly distributed within the wood cell walls.

In case of rigid and heterogeneous materials, such as wood, equation 1 is a good model because the assumption $T_{CH} << T_{1\rho(H)}$ is satisfied. The effect of relaxation due to spin diffusion

is therefore commonly investigated (Smernik, Baldock, & Oades, 2002, Nogueira, Tavares, & Nogueira, 2004). The *M(t)* curve fitting was performed by means of only one exponential factor, due to the high complexity of the system. Additionally, consideration of multiple exponential fitting factors did not significantly improve the result. The average extrapolated parameters $T_{1\rho(H)}$ for each peak are presented in Figure 7. It should be noticed that for most peaks the $T_{1\rho(H)}$ values can be clustered according to the geographical provenance of wood. In particular, samples from Finland, show uniform values that are clearly lower than those of samples from other series. High values of $T_{1\rho(H)}$ are related to slow motion and minimised variations among $T_{1\rho(H)}$ values for different peaks suggest an intimate connection among the structural components of wood. Quite homogeneous values are found for the sample from Poland, with the exception of peaks 10 and 12. The series of Italian woods posses more scattered values, especially for peaks 8, 9, and 10. In general, samples from Italy and Poland show rather analogous results with the exception of the resonance 8. The C-1 $T_{1\rho(H)}$ (peak 8) produces the highest discrimination among samples and suggests that mobility increases according to Italy<Poland<Finland trend.

Figure 7. $T_{1\rho(H)}$ values obtained from fitting VCT curves of selected ^{13}C signals with Eq. 1

As T1ρ(H) is derived from the molecular organization of polymers, it could be related to some physical features of wood presented in Table 3. The trend of $T_{1\rho(H)}$ of the peak 8 (cellulose C-1) appeared to be inversely related to the sample density (r^2=0.92), implying that smaller relaxation times correspond to higher density. Similarly, the $T_{1\rho(H)}$ of peak 9 (crystalline cellulose) can be linked to the quantity of latewood (r^2=0.85), i.e. samples with higher latewood ratio show shorter relaxation time.
The peculiar characteristics of wood from Finland might be interpreted by distinct chemical and physical properties of trees growing in Scandinavia, i.e. the highest wood density, high ratio of late wood, and the high extractives content. All these are clearly related to the climatic conditions that shorten the growth periods and stimulate the development of efficient defence systems against local pathogens.

Multivariate analyses, similar to those performed on the FT-IR spectra were applied as well to NMR data. The results obtained are summarized in Figure 8. The PCA discrimination of the NMR spectra (obtained with *ct*=1.0 ms) did not provide sufficient differences for undoubtable discrimination of wood samples from different provenances. As shown in Figure 8a, the values of the samples from Poland (P1 and P3) overlapped the values of samples from Italy (I3). Some improvement of the discrimination algorithm was achieved by computing the principal components from VCT curves of selected peaks (8, 9, 11, 12, 13, 14) and is presented in Figure 8b. Component PC1 alone (explaining 55% of the variance) was sufficient for separation of Finnish samples from other provenances. On the other hand, the clear discrimination of Polish samples from Italian samples on the basis of component PC3 was limited as both clusters were close to each other and even slightly overlapping.

A significant improvement of the origin determination capability of the software was achieved after performing PC analysis of the VCT experimental results, but only analysing $T_{1\rho(H)}$ coefficients. As a matter of fact, it was impossible to create any reliable PCA model for discrimination of wood origin based only on the T_{CH} coefficients. Figure 8c shows the overlapping of the clusters even if both principal components covered over 83% of the

variance. This is in agreement with the previous considerations on T_{CH} presented in the NMR section. The PCA scores from analysis of the $T_{1\rho(H)}$ coefficients are shown in Figure 8d. PC1 (explaining 81% of the variance within spectra) separates samples into two groups: Finland (with PC1 values of negative order) and Poland/Italy (with positive values of PC1). Moreover, PC2 (explaining 12% of the data variance) separates samples coming from Italy and those originating from Poland. The cluster distribution is slightly similar to that of Figure 8b, but the most important difference is the higher spectral distance of Italian and Polish samples assuring an apparent differentiation of the wood provenance.

Figure 8. Principal Components Analysis of; all NMR spectra (a), dataset of all the VCT curves of selected peaks (b) and T_{CH} (c) and $T_{1\rho(H)}$ (d) computed from VCT curves

4. Conclusions

The work presented here was devoted to the development of a novel methodology for characterization and discrimination of a wood in regard to its origin on the basis of infrared and nuclear magnetic resonance spectra. The requirement was to use untreated solid wood samples to minimize any manipulation to the nanostructure of native wood. The results confirm that the chemical and physical properties of samples belonging to the same wood species (*Picea abies*) differ due to the origin. Both FT-IR and dynamic NMR spectroscopies were able to correctly discriminate samples originating from three different provenances in Europe. The successful discrimination by means of infrared spectroscopy was mostly related to differences in the molecular configuration of the cellulose, hemicellulose and lignin (related to functional groups as -OH, -CH and -CH_2). Although the number of analysed samples in the present work cannot be considered statistically meaningful, the results clearly indicated that FTIR outcomes can be confirmed by means of solid state nuclear magnetic resonance. NMR, also permits to highlight additional differences both in the connections among constitutional polymers and in the homogeneity of molecular domains within wood samples of different provenances. The measurement reliability of the NMR experiment has been quantified by means of internal reference in form of HDPE strip rolled together with wooden sample. It was also possible to normalize the spectra according to the wood/HDPE mass ratio.

The most effective chemometric tool for discrimination of spectra due to provenance was principal components analysis. It provided reliable prediction models for infrared spectroscopy, but was also very useful for analysis of NMR spectra. In the second case the analysis of $T_{1\rho(H)}$ coefficients as computed from fitting the *M(t)* curve was the most efficient. Further interpretation of $T_{1\rho(H)}$ suggests a higher polymer mobility and a higher homogeneity in wood from Finland. On the other hand, equally less homogeneity was noticed in wood samples from both Italy and Poland. The highest crystalline index *CrI* was detected in Polish samples. Discrimination of provenance was achieved by means of $T_{1\rho(H)}$ PC analyses. It was expected, and followed both theoretical background and literature references.

Concluding, it was possible to apply presented methodologies for the characterization of wood according to its origin by means of both infrared and nuclear magnetic resonance spectroscopies. Such methods might be very useful for both, research and understanding of wood microstructure and its variations induced by growth conditions.

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Table 1. General characteristics of the provenance sites

Country of origin	Country code	Elevation (m.o.s.l.)	Geographical coordinates		Climatic condition		Silvicultural details
			latitude	longitude	Average temperature	Average precipitation	
Italy	**I**	1700-1800	46° 11’	11° 50’	+2.4°C	1260mm	natural stand
Finland	**F**	140	63° 22’	30° 42’	+2°C	650mm	artificial forest
Poland	**P**	600-810	50° 44’	16° 9’	+8.4°C	600mm	zone of industrial emissions

Table 2. Contents of chemical components extracted from Norway spruce samples of different provenances

Country code	Cellulose %	Lignin %	Holocellulose %	Hemicellulose %	Extractives %	Hot water soluble %	1% NaOH soluble %	Ash %
	(± 1.36)	(± 0.25)	(± 1.80)	(± 1.50)	(± 0.32)	(± 0.30)	(± 0.60)	(±0.26)
I	45,5	28,8	70,4	24,9	1,15	0,87	10,65	0,3
F	45,2	28,4	70,2	25	1,82	2,05	11,48	0,3
P	45,7	29,3	70,2	24,5	1,84	0,56	9,73	0,3

Table 3. Physical characteristic of spruce samples.

Sample code	Density (g/cm^3)	Late wood (%)	Age of tree (yr)	Average ring width (mm)
I1	0,42	0,27	127	1,08
I2	0,43	0,30	137	0,97
I3	0,39	0,17	131	1,60
F1	0,50	0,36	147	0,95
F2	0,56	0,38	141	1,14
F3	0,51	0,36	144	0,91
P1	0,46	0,30	100	1,59
P2	0,47	0,20	83	2,86
P3	0,45	0,28	99	1,80

Table 4. ^{13}C CPMAS resonance assignments of wood and HDPE

Signal Number*	Chemical shift (ppm)	Polymer assignments	Chemical species or carbon atom**
1	172.0	Carbohydrate;	-COO-R, CH3-COO-
2	152.6	Lignin;	S3 (e), S5 (e)
3	147.0	Lignin;	S3 (ne), S5 (ne), G1, G4
4	136.0	Lignin;	S1 (e), S4 (e), G1 (e)
5	134.3	Lignin;	S1 (ne), S4 (ne), G1 (e)
6	121.0	Lignin;	G6
7	114–106	Lignin;	G5, G6, S2, S6
8	104.8	Carbohydrates;	C1
9	88.7	Carbohydrates;	C4 crystalline cellulose
10	83.8	Carbohydrates;	C4 amorphous cellulose; lignin Cβ
11	74.8	Carbohydrates;	carbohydrates; C2,3,5; lignin Cα
12	72.2	Carbohydrates;	C2,3,5
13	64.7	Carbohydrates;	C6
14	61.6	Lignin;	Cγ, C6
15	55.7	Lignin;	OCH3
16	32.3	Polyethylene	CH2
17	21.0	Carbohydrates;	CH3-COO-

* reported in Figure 3
** according to labelling of Figure 2

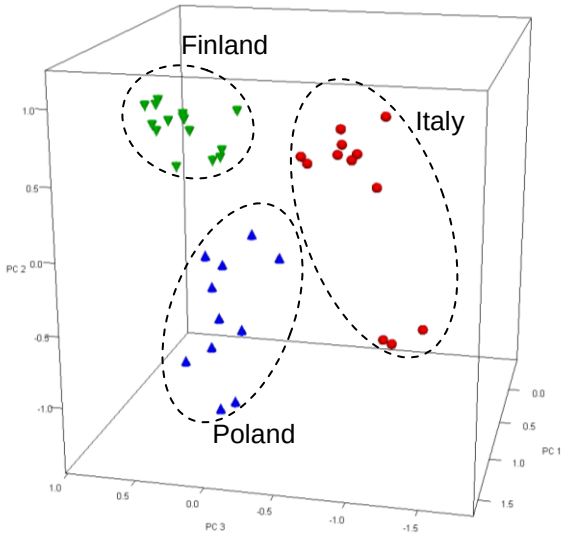

Figure 1. FT-IR-based discrimination of the spruce woods due to provenance

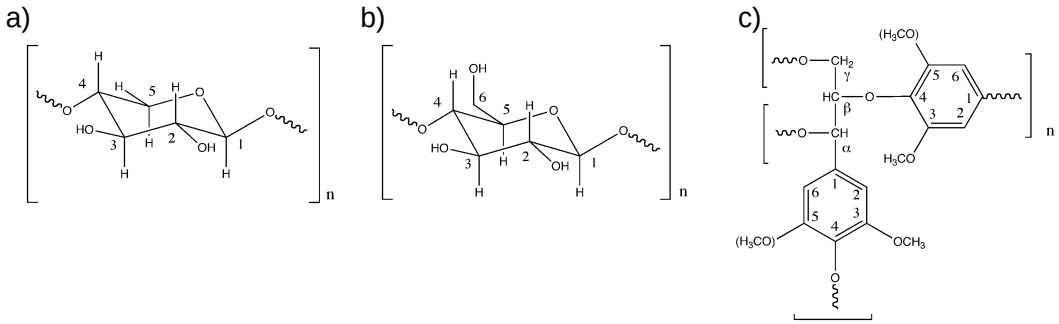

Figure 2. The chemical structures of the main wood components (according to Bardet et al. 2009): cellulose (a), hemicellulose (b) and lignin (c), and the numbering scheme corresponding to the resonance peaks summarized in Table 4

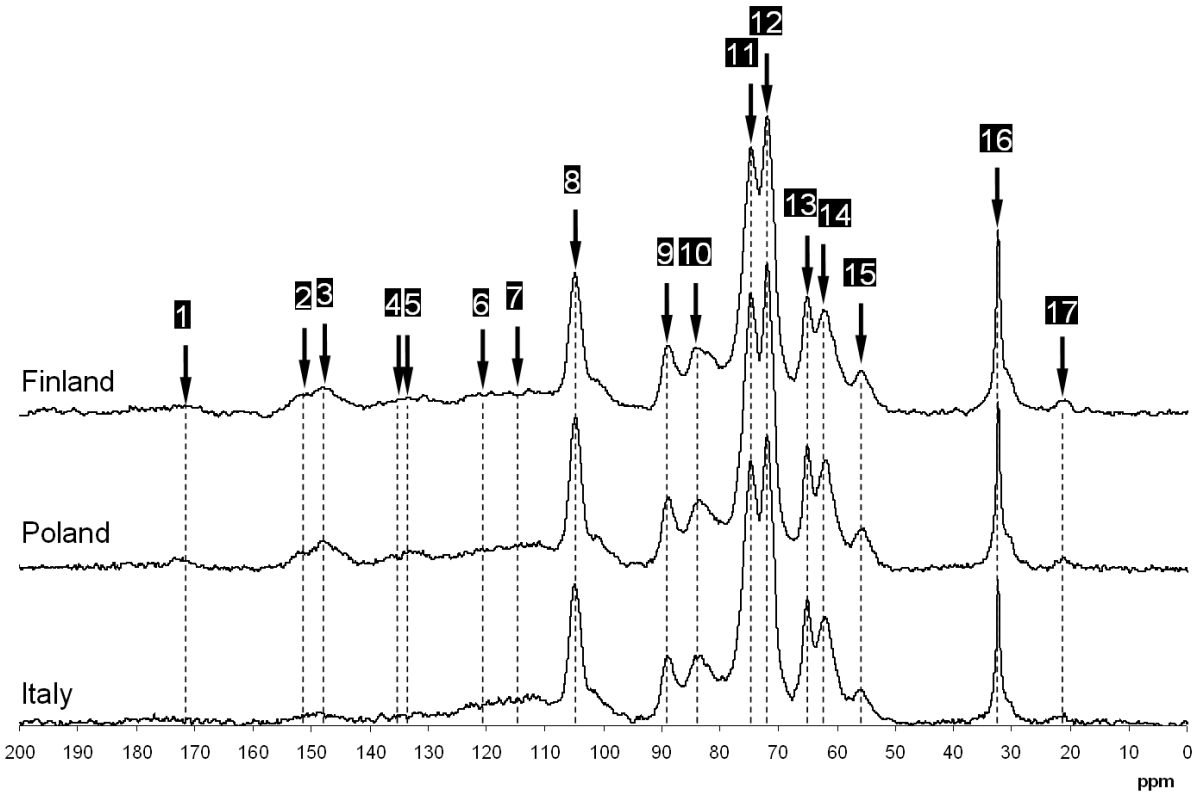

Figure 3 NMR spectra of spruce woods differing in provenance and the peaks assignments

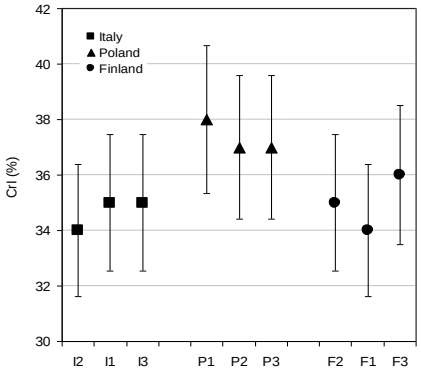

Figure 4. Cellulose crystalline index CrI computed from the NMR spectra of spruce from different provenance

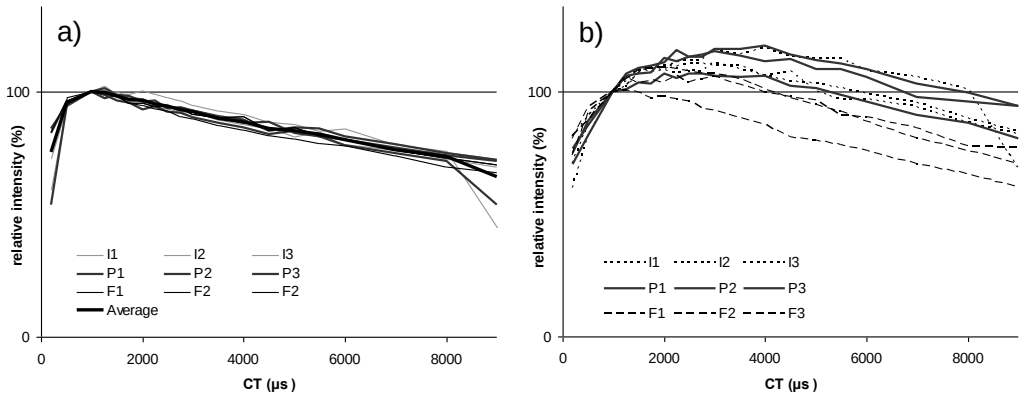

Figure 5. Normalized peak intensity vs ct of HDPE signal 16 (a) and wood signal 8 (b).

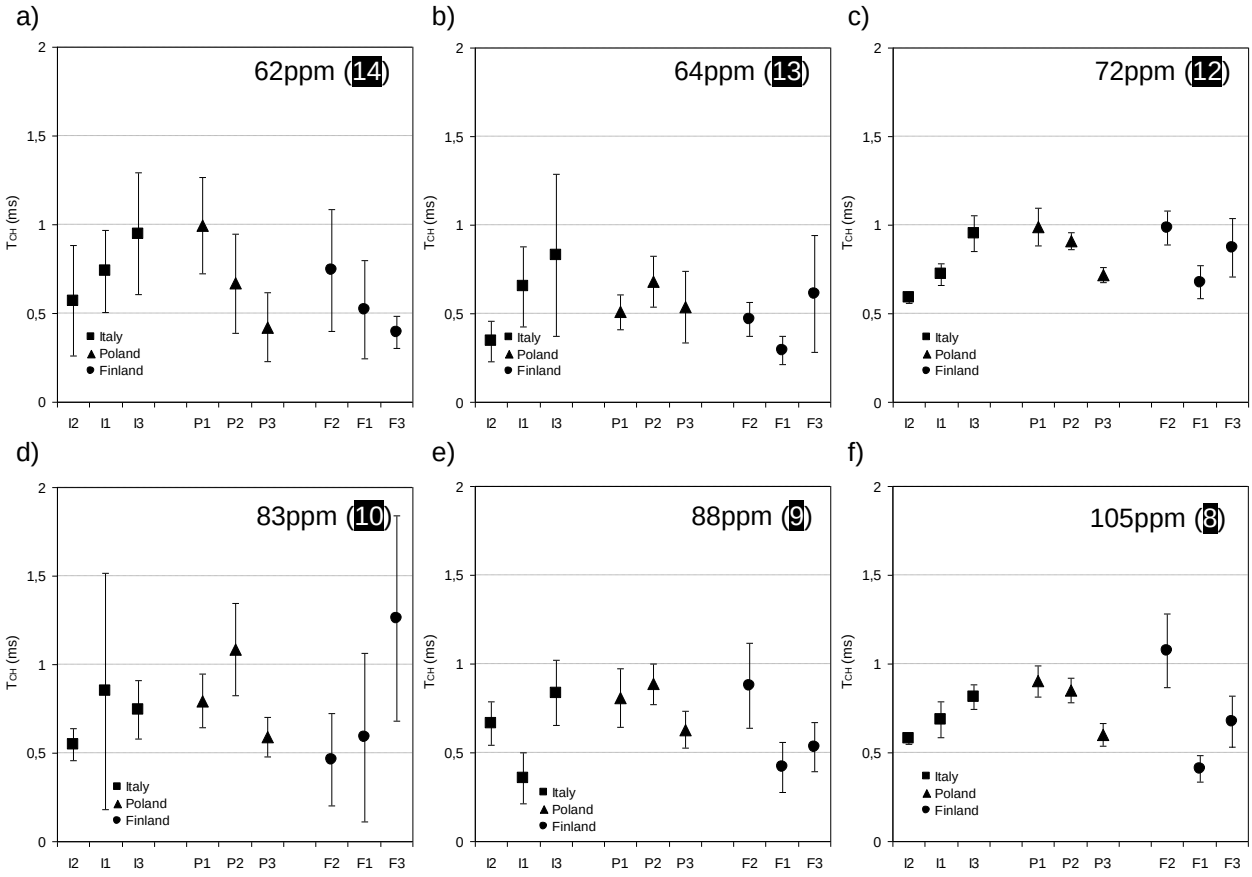

Figure 6. T_{CH} values obtained from the fitting of VCT curves of selected ^{13}C signals with Equation 1.

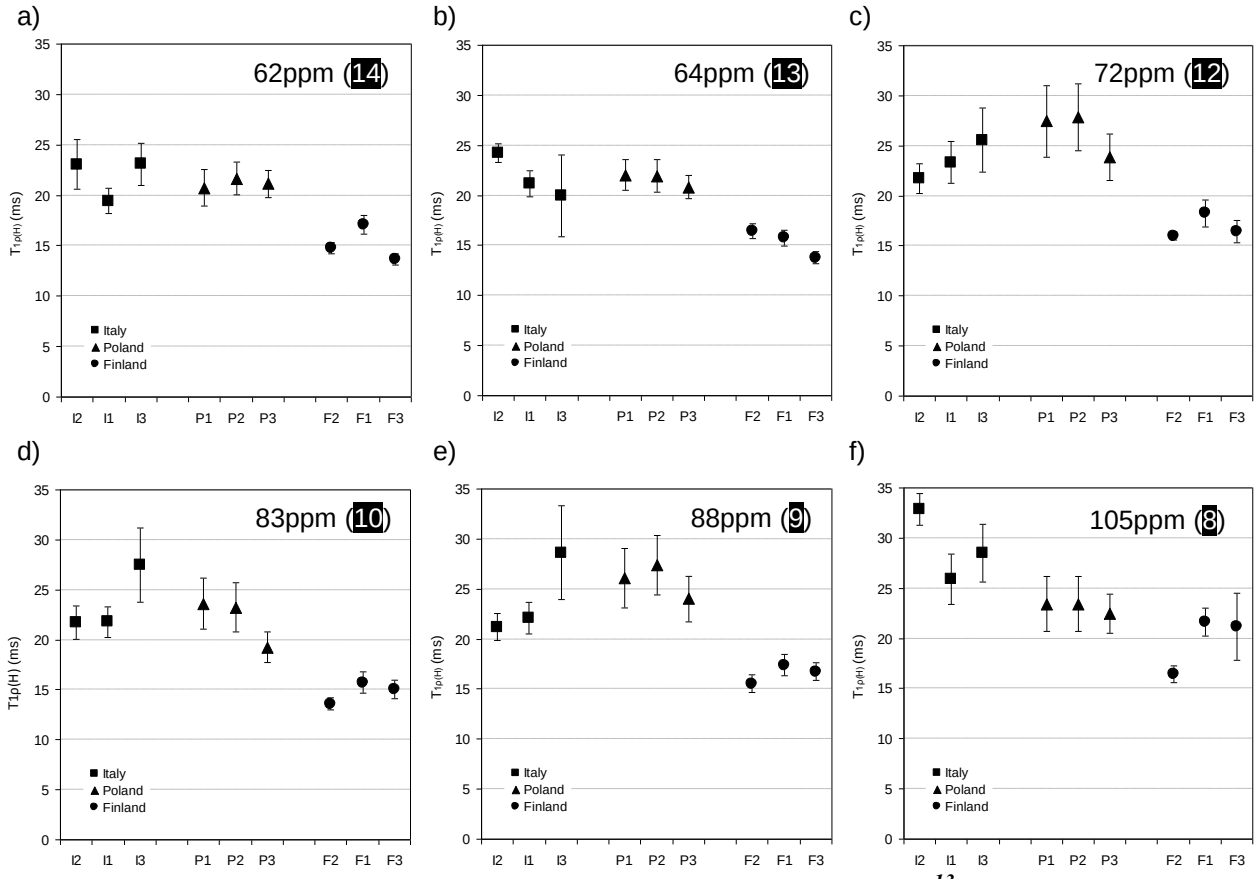

Figure 7. $T_{1\rho(H)}$ values obtained from fitting VCT curves of selected ^{13}C signals with Eq. 1

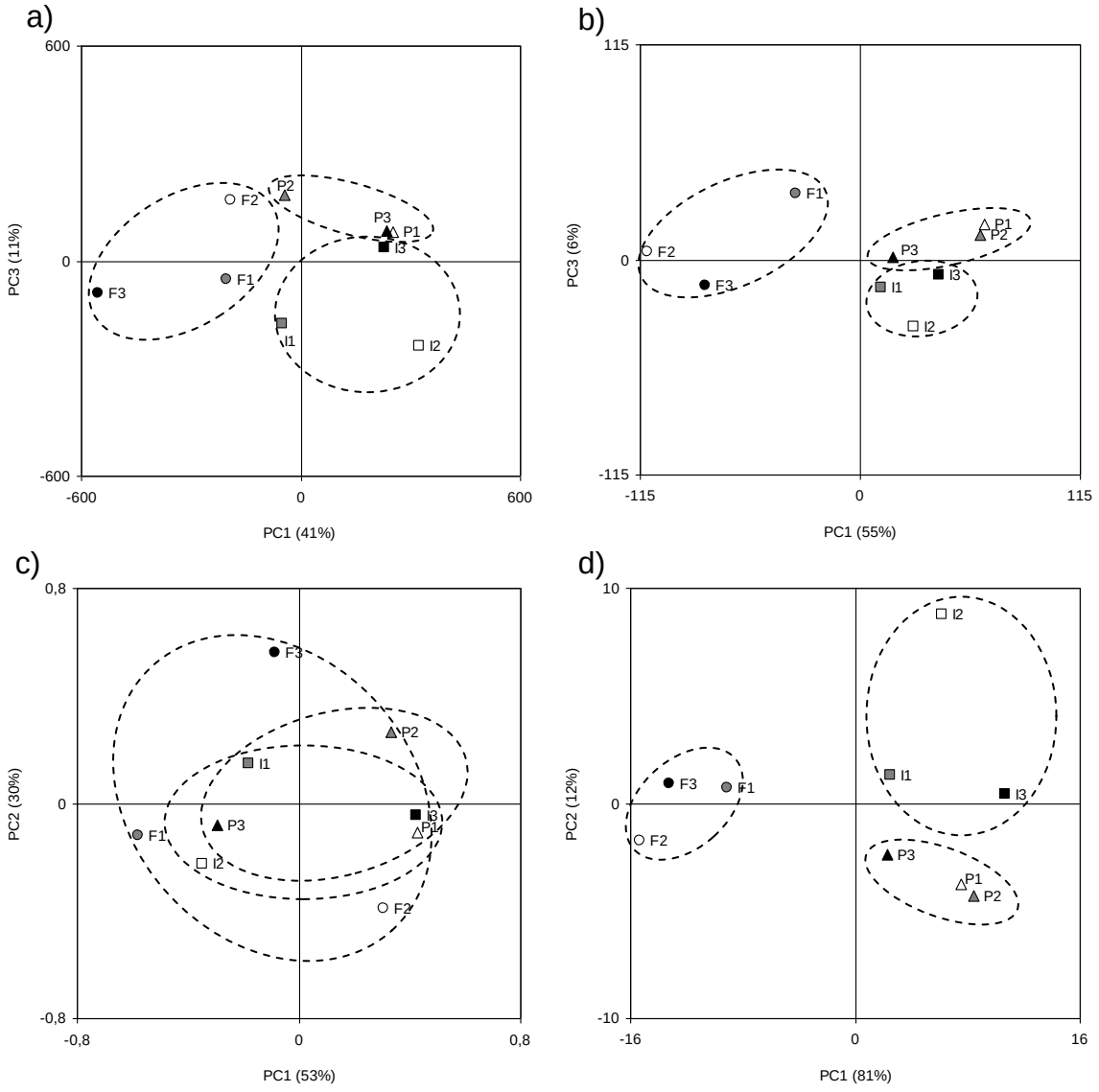

Figure 8. Principal Components Analysis of; all NMR spectra (a), dataset of all the VCT curves of selected peaks (b) and T_{CH} (c) and $T_{1\rho(H)}$ (d) computed from VCT curves