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Khaled Younes[a,b*], Laurent Grasset[b]

[a]*College of Engineering and Technology, American University of the Middle East (AUM), Kuwait*

[b]*Université de Poitiers, Institut de Chimie des Milieux et Matériaux de Poitiers IC2MP CNRS UMR 7285, Equipe Eaux, Biomarqueurs, Contaminants Organiques, Milieux, B27, 4 rue Michel Brunet, 86073 Poitiers Cedex 09, France*

Abstract

Traditional acid hydrolysis is the main method used for carbohydrates characterization in soils and sediments. It has been used to cleave the glycosidic bond, yielding monomers that compose polysaccharides of the non-cellulosic pool (free, ligno-cellulosic and hemicellulosic carbohydrates). In this study, the widely used chemical and thermochemical degradation procedures have been compared with the derivatisation followed by reductive cleavage (DFRC) method. This method presented efficiency in specifically cleaving the aryl ether bonds, yielding for sugar moieties linked to aromatic structures (ligno-cellulosic fraction). Once applied for peat samples, some discrepancies were observed. DFRC method revealed some information about the origin of carbohydrates that was hidden from the traditional chemical and thermochemical degradation methods. Several patterns of carbohydrates have been obtained with DFRC indicating difference in the origin and/or degradation rates between monosaccharides. For xylose, it showed an increase in the catotelm (deepest layer of the peatland), indicating an angiosperm vegetation input at the early stage of the peatland formation. Ribose, commonly known as a microbial indicator, showed the same profile as for hexoses and deoxyhexoses, with acid hydrolysis. The difference in the profile yielded in the case of DFRC method, revealed its microbial input. These information were hindered from acid hydrolysis and thermochemolysis, as the first showed nearly the same profile for all monosaccharides and the second one didn't have the capacity to analyze arabinose and ribose.

Generally, higher similarity was obtained between DFRC and acid hydrolysis than between DFRC and thermochemolysis. This is due to the difference of mechanisms involved in chemolytic and thermochemolytic methods. For the first group, both methods shared acid conditions and milder temperature conditions if compared to the second group. In addition thermochemolysis is performed under alkaline conditions.

1. Introduction

Northern peatlands are estimated to store up to a third of the terrestrial carbon as peat (Vitt et al., 2000); the continental C as peat is estimated for 20% while covering up only 3% of the Earth's surface (Gorham, 1991). These very complex ecosystems impact both carbon cycle and water at local as well as global scale. Since peat is progenitor of coal, a better understanding

of the molecular fingerprint of different pools in peats will improve our insight in the formation of coal.

Carbohydrates are the major pool of terrestrial vegetation and represent the main form of photosynthetically assimilated organic matter (OM) in the biosphere. Understanding carbohydrates dynamics in peatlands will allow a better understanding of coal formation. Given their ubiquitous character, carbohydrates potentially represents a powerful tool for elucidating processes, sources and diagenetic pathways of biologically important OM in sediments (Jia el al., 2008). Although in most settings (soils and sediments) carbohydrates composition have shown high sensitivity to diagenetic alterations, the OM-rich character of peat deposits and the potential for preservation below the acrotelm makes *ombrotrophic* peatlands a suitable environment for also exploring the potential of carbohydrates as paleovegetation proxies (Bourdon et al., 2000; Comont et al., 2006; Delarue et al., 2011; Estournel-Pelardy et al., 2013; Younes et al., 2017).

The analysis of OM at the molecular level commonly requires several techniques and each technique targets all patterns of one of the three major pools. Most studies dealing with lignin analysis were conducted using cupric oxide under alkaline conditions (CuO-NaOH) (Hedges and Ertel, 1982). For lipid fraction analysis, various organic solvents have been used (Bligh and Dyer, 1959). For carbohydrates analysis in soils and sediments, it is mostly performed with acid hydrolysis (Bourdon et al., 2000). The high heat and concentration of acid used, contributes to the cleavage of all glycosidic bonds (relative to non-cellulosic/cellulosic fractions) (Younes and Grasset, 2018). Derivatisation Followed by Reductive Cleavage (DFRC) method showed a high efficiency for cleaving α and β-aryl ethers and is therefore suitable for ligno-cellulose materials. Therefore, all studies conducted for the DFRC method were done for lignin subunits analysis (Del Río et al., 2007; Grasset et al., 2010; Lu and Ralph, 1998; Matsui and Ohira, 2013; Peng et al., 1999).

The only attempt to use this technique in a geochemistry study was done by Grasset el al. (2010). The authors applied this technique to characterize lignin in gymnosperm wood, and in soils and sediments formed from degraded gymnosperm wood, in order to assess the supply of terrestrial OM to marine sediments. The purpose of applying this method is a need for finding efficient and more selective method for lignin α- and β-aryl ether cleavage. It was evident that the overall lignin yield and the compound ratios used as plant source proxies have been found to be considerably different from those yielded from the mostly used lignin degradation techniques.

For our knowledge, this is the first attempt to analyze the carbohydrate pool in peat samples with the DFRC method. One of the advantages of using peatland samples to conduct this comparison is the bilayer character of this ecosystem. The upper half is the “living” part where fresh OM input and microbial activity occur. The bottom half is the “dead” part where decomposed OM is preserved making the peats a reference for paleoenvironmental interpretations (see Younes et al. (2017)). In this study, the DFRC method was applied for characterizing carbohydrates in a French peatland. In order to reveal the extent of DFRC method, carbohydrates yielded moieties were compared with lignin input from CuO-NaOH oxidation and the thermally assisted hydrolysis and methylation (THM) method. In case of discrepancy in carbohydrates profile, this will give more insight into the molecular aspect of OM and the

occurrence of a carbohydrate fraction that was hindered from the acid hydrolysis and THM method. PCA was used to compare between the employed chemical and thermochemical degradation methods.

2. Materials and methods

2.1. Sampling

Samples were collected from a peatland site in the Limousin, France. Settings of the studied site was developed in a previous study by Younes et al. (2017). Samples of a 1m peat core were were divided into 4 cm thick slices and then freeze-dried.

2.2. Derivatization followed by reductive cleavage (DFRC)

The samples were subjected to DFRC analysis according to the procedure of Lu and Ralph, (1998), as dried peat samples were treated with 5 mL of acetyl bromide/acetic acid (8% v/v), stirred at 50 °C for 2 h, then evaporated under reduced pressure until dry. Zinc powder (500 mg) and 25 mL of dioxane/acetic acid/water (5 :4 :1, v/v/v) were added and stirred for 30 min at 50 °C. Saturated aqueous ammonium chloride (50 mL) were added, whereupon the solution was acidified to pH 3 with HCl. The mixture was then extracted with $CHCl_3$ 2 times (total 50 mL). The chloroform layer was dried over magnesium sulfate, then evaporated. Degradation products were acetylated with acetic anhydride (2 mL), acetic acid (0.1 mL) and 1-methylimidazole (0.1 mL) at room temperature for 10 min and analyzed by GC (Shimadzu GC-2010) with an FID and by GC-MS and 50 µL of nanodecanoic acid methyl ester was added as an internal standard.

2.3. Methods used for validation and comparison

2.3.1. CuO- NaOH oxidation for lignin characterization

The release of lignin monomers was performed by alkaline CuO-NaOH oxidation. This is the classical chemical degradation method used for lignin characterization. About 10 mg of the dried peat samples was oxidized for 2 h at 170 °C in sealed Teflon vials, following the procedure developed by Hedges and Ertel, (1982). CuO-NaOH oxidation yields a suite of one ring phenols (H, *p*-hydroxyphenol; V, vanillyl; S, syringyl and C, cinnamyl; Table. 1) with their aldehyde, ketone and acid counterparts (for further information see Younes et al. (2017) and Younes and Grasset, (2018)).

2.3.2. Acid hydrolysis

Acid hydrolysis was carried out with HCl for 200-300 mg of the dried peat samples at 100 °C for 3 h, the obtained monosaccharides (check Table. 1 for symbols) was derivatized into alditol acetates following the procedure of Rumpel and Dignac, (2006) and well developed in a previous study on the same site by Younes et al. (2017).

2.3.3. Thermally Assisted Hydrolysis and Methylation (THM) method

The THM method is based on the offline procedure developed by Grasset and Amblès, (1998). About 50 mg of the dried peat samples were mixed with 4 ml of tetramethylammonium hydroxide (TMAH) and heated at 400 °C (for further information see Younes and Grasset, (2018)). THM method yields a suite of monosaccharides and one ring phenols (check Table. 1 for symbols).

2.4.Gas Chromatography (GC) analysis

GC measurements were performed with a SHIMADZU GC-2010 Plus gas chromatograph equipped with a flame ionization detector at 300 °C. Separation of the compounds was achieved with a 30 m fused silica capillary column (Supelco Equity 5%, 0.25 mm internal diameter, 0.25 µm film thickness), and helium as carrier gas. The oven temperature was programmed from 60 to 300 °C at 5 °C min^{-1} and held for 15 min.

2.5. Data normalization

The relative contribution of monosaccharide with depth were scaled and centered following the formula below:

$$Normalized\ P_i(\%) = \frac{P_i - P_{min}(\%)}{P_{max}(\%) - P_{min}(\%)}$$

Where:

P_{min} (%) = smallest Pi (%) for each monosaccharide along peatland depth records,

P_{max} (%) = biggest Pi (%) for each monosaccharide along peatland depth records.

2.6. PCA

PCA is used in order to reduce dimensionality of dataset by producing linear combinations of original variables. PCA breaks down the matrix of initial data, X, to express them as a least-square model (Geladi et al., 2003):

$$X = A \times F + U$$

Where, X is the original data matrix, A is the matrix of loadings of the original variables in the new reduced space, F is the matrix of scores of objects or samples and U is the matrix of residuals. PCA aims to reduce the dimensionality of datasets containing many interrelated variables, while retaining as much as possible of the systematic variation in the original data (Joliffe and Morgan, 1992). In the analysis, the original variables are replaced by a few principal components (PCs) variables. The PCs are totally independent with each other and ordered so that PC_1 contains most of the original variation and PC_2 contains the second largest amounts, etc. PCA was carried out using XLSTAT 2014.5 software. The peat depth samples were considered as the observations and normalized data of monosaccharide and lignin as the variables.

3. Results and Discussion

3.1. Validation of the DFRC method for carbohydrates molecular characterization :

In order to validate the DFRC method as an efficient technique for the molecular characterization of carbohydrates, the relative abundance of total carbohydrates yielded with DFRC has been compared to lignin content yielded by CuO-NaOH oxidation and THM method

in one hand and to the total carbohydrates yielded with HCl hydrolysis and THM method in another hand (Fig. 1).

3.1.1. Carboydrates vs. Lignin input

Contrary to monosaccharides, lignin biomarkers provide more reliable proxies for vascular plant input (Filley et al., 2006; Wysocki et al., 2008; Mason et al., 2009; Abbott et al., 2013; Younes and Grasset, 2018). The relative abundance of carbohydrates can be examined using the ratio of carbohydrates yield (mg carbohydrate / 1 g OC; Jia et al., 2008) at a given depth in the peat core. Fig. 1 presents bi-dimensional plots of total carbohydrates yielded with DFRC, HCl hydrolysis and THM method as a function of "Λ" proxies, yielded by CuO-NaOH oxidation and THM method. Lignin proxies were used in Younes and Grasset, (2018) as described by Filley et al. (2006) with all phenols normalized to 1 g OC. Lambda $\Lambda_{THM} = G_4 + G_5 + G_6 + S_4 + S_5 + S_6 + G_{18} + P_{18}$ and $\Lambda_{CuO\text{-}NaOH} = S + V + C$, indicates the relative lignin yielded by THM method and CuO-NaOH oxidation, respectively.

Because THM primarily cleaves β-O-4 bonds and CuO-NaOH oxidation presumably cleaves β-O-4 as well as some C-C bond, the latter accesses a larger part of the unaltered non-oxidized lignin structure (Filley et al., 2006; Wysocki et al., 2008; Younes and Grasset, 2018).

Following Fig. 1, high correlation were obtained between input of carbohydrates yielded by DFRC method and HCl hydrolysis, from one side, and lignin yielded by CuO-NaOH oxidation from the other side (67.45% and 69.57% for DFRC method and HCl hydrolysis, respectively). These trends indicate the high correlation of hemicellulosic and ligno-cellulosic fraction along the peat core. The low correlation between the input of carbohydrates yielded by DFRC and HCl hydrolysis from one side, and lignin yielded by THM from another side (4.56% and 2.43% for DFRC and HCl hydrolysis, respectively) indicates that the ligno-cellulosic fraction of the investigated peat core is bonded to the intact non-oxidized lignin that is most likely revealed by CuO-NaOH oxidation. Surprisingly, even the total carbohydrates yielded with THM method showed a low correlation with Λ_{THM}. Its higher correlation with carbohydrates yielded with DFRC indicates that the DFRC method is a more reliable technique for carbohydrates analysis as it reveals both the free and ligno-cellulosic fraction rather than only the free and terminal carbohydrates, in the case of THM method. This is due to a higher efficiency of ether cleavage under acidic conditions (Acetic acid used) in case of DFRC than basic conditions (pk_b of TMAH = 4.2) in case of THM (Wade, 2012).

3.1.2. Relative carbohydrate abundance: DFRC vs. Acid Hydrolysis & THM

The total non-cellulose sugar content varied from 6 mg/g at the top to 1 mg/g at the bottom of the peat core, in the case of DFRC method. For HCl hydrolysis and THM method, it varied from 26 mg/g at the top to 4 mg/g at the bottom of the peat core and from 227 mg/g in the acrotelm to 29 mg/g at the bottom of the peat core, respectively (see Supplementary Information). The difference in the yielded amounts shows the capacity of HCl hydrolysis to target the whole non-cellulose fraction (free sugars, sugars bonded to polyphenols and hemicellulose); as for DFRC method, it targets only a specific pool of free sugars and sugars bonded to polyphenols. For THM method, it has showed the capacity of targeting free and terminal carbohydrates (Younes and

Grasset, 2018; Estournel-Pelardy et al., 2011) which is more likely a smaller pool compared to hemicellulose and ligno-cellulose. The higher abundance yielded for THM method is related to the semi-quantitative approach used in this case. This was not the case of DFRC method and HCl hydrolysis, as full quantitative approach is used (response factor of each monosaccharide is identified with calibration curves).

Fig. 2 shows that depth amounts of the nine investigated monosaccharides was higher in the acrotelm (0 to -20 cm) than the mesotelm (-20 to -50 cm) and catotelm (-50 to -100 cm), for all three methods, indicating degradation of carbohydrates with depth (Jia et al., 2008). High correlation between DFRC method and HCl hydrolysis has been obtained (between 65% and 87%, Fig. 2). This shows a high consistency between the two methods and proves the findings of the comparison between carbohydrates amounts and lignin input (Fig. 1) as hemicellulosic and ligno-cellulosic pools occurs in a synchronous fashion in the investigated peat core. Lower correlation has been obtained between DFRC and THM methods (between 32% and 41%, Fig. 2). Even know that these methods share the free sugar pools, but this pool is more likely less frequent in a terrigenous sediment as peatland, if compared with the occurrence of ligno-cellulose and hemicellulose (Clymo, 1991).

More detailed investigations revealed several patterns. For acid hydrolysis, two main patterns of variation in concentration of each group of monosaccharides were revealed. The first composed of Ara)$_{HCl}$ and Xyl)$_{HCl}$, showed a rapid decrease from the first depth of the acrotelm (0 to -20 cm), and concentration was approximatively constant down core. The second pattern composed of Deoxyhexoses (Rha + Fuc)$_{HCl}$), Rib)$_{HCl}$ and hexoses (Glu + Man)$_{HCl}$ and Gal)$_{HCl}$) showed a comparatively slower decline down core (for further details see Younes et al. (2017)). For THM method, the highest input was observed at -20 cm, indicating a free and/or terminal carbohydrate occurrence. The interface between the mesotelm and the catotelm (around -50 cm) showed an increase in non-cellulosic carbohydrates. This indicates free carbohydrates input at the interface of these ecological layers and a probable microbial deposition during peatland accumulation. In the catotelm, the higher input of Xyl)$_{THM}$ indicates a past vegetation changes and/or past humification processes (Cowie and Hedges, 1984), due to the alkali attack of TMAH (for further details see Younes and Grasset, 2018).

For DFRC method, different monosaccharides showed a significant difference and the decreasing trend was skewed by small increases at particular positions. For pentose pattern (Ara)$_{DFRC}$ and Xyl)$_{DFRC}$), it showed a different decreasing profile, Ara significantly decreases along depth as for Xyl it showed a more stable input in the acrotelm and an increase at the interface between mesotelm and catotelm and at -70 cm in the catotelm. These trends indicate some difference in the origin between pentoses and/or degradation rate that was hindered by HCl hydrolysis (same profile for Xyl)$_{HCl}$ and Ara)$_{HCl}$; Fig. 2) and THM (no detection of Ara)$_{THM}$; Fig. 2). For the acrotelm and mesotelm, the higher decrease of Ara)$_{DFRC}$ from the very first depth records indicates a higher degradation rate, once compared with Xyl)$_{DFRC}$. In the catotelm, the increase of Xyl)$_{DFRC}$, at particular depths, indicates the preservation of a ligno-cellulosic fraction downcore and an input of vascular vegetation. Several studies have shown a higher input of Xyl in angiosperms than gymnosperms (Wicks et al., 1991). In our case, the angiosperm input in the

first stages of the Sagnes peatland formation was shown by an S/V value of 1 by lignin analysis (Younes et al., 2017). These trends indicate that differential degradation between monosaccharides occurred and suggests that Ara and Xyl are components of polysaccharides that are more degradable than other carbohydrates. Differences in degradation among carbohydrates have been observed in several investigations (Jia el al., 2008). Thus the preferential degradation of Ara and Xyl, compared to deoxyhexoses and hexoses, suggests that these monosaccharides were in a less refractory form. The difference of depth profile, in the case of DFRC method, and the absence of increases in the case of Ara indicates that this monosaccharide presented a higher degradation rate, in comparison with Xyl.

For Deoxyhexoses $(Rha)_{DFRC}$ and $Fuc)_{DFRC}$) and hexoses $(Gal)_{DFRC}$ and $Glu + Man)_{DFRC}$), it showed approximatively the same profile (Fig. 2). The highest input was around -20 cm at the interface between acrotelm and mesotelm. Unlike the decreasing trend in the case of HCl hydrolysis, monosaccharides of this pattern showed their highest input in the mesotelm. This indicates most likely their belonging to the same origin.

3.2.Geochemistry of Carbohydrates

3.2.1. Source identification

Carbohydrate moieties can come from different plant and microbial sources. Therefore, their compositional patterns can be useful to differentiate between terrestrial and microbial origin, as well as some specific tissue or type of organisms within each of these categories (Cowie and Hedges, 1984; Popper and Fry, 2003; Comont et al., 2006; Jia et al., 2008; Delarue et al., 2011; Younes et al., 2017; Younes and Grasset, 2018). Carbohydrate monomers have been investigated to a lesser extent than lignin moieties. This is due to the rapid degradation of carbohydrates and their incorporation in the so called “humic fraction” (Estournel-Pelardy et al., 2013; Younes and Grasset, 2017; Younes and Grasset, 2018).

Despite the limited number of combinations that can be done with carbohydrate moieties, the variation in the compositions of hemicelluloses from one species to another can provide a plant source signature (Comont et al., 2006). Xyl and Ara are indicators of vascular plant input (Cowie and Hedges, 1984). Popper and Fry (2003) mentioned the occurrence of high proportions of Gal, Rha and Man in the hemicelluloses of bryophyte primary cell walls. Commont et al. (2006) showed that these monosaccharides are indicators of moss in ombrotrophic peat bogs. Furthermore, Fuc and Rha could originate from *Sphagnum* species, given the work of Jia el al. (2008). Other studies have attributed the contribution of these monosaccharides to microbial biomass (Cowie and Hedges, 1984). In our case, deoxyhexoses $(Rha + Fuc)_{DFRC}$ and hexoses $(Gal)_{DFRC}$ and $Glu + Man)_{DFRC}$) showed a similar pattern between them and a different one with $Rib)_{DFRC}$ (Fig. 2). The latter is considered to have a microbial origin (Jia el al., 2008). Delarue et al. (2011) identified Rib as marker of bacterial and/or fungal activity in peatlands. Following the present data and given the previous studies, hexoses and deoxyhexoses are considered as a *Sphagnum* indicator and Rib a microbial indicator.

3.2.2. Carbohydrate proxies :

In order to assess the origin of carbohydrates, two ratios were commonly employed for monosaccharides (Amelung el al., 1996; Nierop et al., 2001; Rumpel and Dignac, 2006), the C_6/C_5 (Gal + Man / Xyl + Ara) and the Deoxy/C_5 (Rha + Fuc / Xyl + Ara).

Bi-dimensional plots of carbohydrates ratios obtained with DFRC method as a function of their HCl hydrolysis and THM method homologues are presented in Fig. 3. For HCl hydrolysis, both ratios showed roughly the same profile with depth (Fig. 3), as their values increased from 0 to -20 cm and decreased slightly at the remainder of the core. This indicates a probable microbial input occurred in the mesotelm (Younes et al., 2017). In the case of DFRC method, smaller values of both ratios were obtained (between 0.96 and 0.20 for DFRC against 0.85 and 3.21 for HCl hydrolysis, in the case of C_6/C_5; and between 0.03 and 0.18 for DFRC against 0.08 and 0.47 for HCl hydrolysis, in the case of Deoxy/C_5; Fig. 3). This difference of magnitude between the two methods, could be explained by the higher concentration of Ara and Xyl yielded with DFRC method, as for the HCl hydrolysis both pentoses and hexoses yielded concentrations of the same magnitude. Both ratios showed two increases, one located in the mesotelm and the other one in the catotelm. For the mesotelm, it could be an indicator of microbial and/or *Sphagnum* dominance in the upper half of the peat core. The latter is more probable as a microbial dominance is discarded in this case. For the catotelm, it could be an indicator of an old microbial deposit that was hidden from the mostly used acid hydrolysis method. Once DFRC and acid hydrolysis were compared, high correlation were obtained for Deoxy/C_5 (67.88 %; Fig. 3) and a low correlation for C_6/C5 (15.2 %; Fig. 3). This indicates the occurrence of a hemicellulosic fraction of hexoses (Gal and Man) that is inaccessible to the DFRC method. Although small correlation was obtained between $C_6/C_5)_{HCl}$ and $Gal/C_5)_{DFRC}$, this comparison have allowed to split the peat core into the three ecological layers. The acrotelm is characterized by small values of both ratios, indicating the input of Xyl and Ara from the Sedges vegetation growing the uppermost depth (Younes et al., 2017). The mesotelm is characterized by low values of $Gal/C_5)_{DFRC}$ and high values of $C_6/C_5)_{HCl}$, indicating the occurrence of a hemicellulosic fraction mainly composed of Gal and Man that was hindered from the DFRC method. The catotelm characterized by high values of both ratios due to the higher rate of degradation of pentoses compared to hexoses (Jia et al., 2008; Younes et al., 2017).

$Xyl/Glu)_{THM}$ has been used to distinguish between angiosperm and gymnosperm occurrence (Grasset el al., 2009; Younes and Grasset, 2018). Xyl is an indicator of plant input and it is produced in a more significant way by angiosperms than gymnosperms vegetation (Cowie and Hedges, 1984). Glu is characterized by its ubiquity in the living kingdom and is commonly present at high levels in vascular vegetation (Cowie and Hedges, 1984). Comparison of this ratio with it's DFRC homologue showed a low correlation (5.7 %; Fig. 3), even know both methods showed a decreasing profile of Xyl is the upper half (acrotelm and mesotelm; Fig. 2) and an increase in the bottom half (catotelm; Fig. 2). Minor correlations between DFRC and THM methods ratios is due to the difference of mechanisms occurred between these two methods. DFRC is a chemolytic method in acid conditions yet THM is a high temperature thermochemolysis under alkaline conditions. This allow the latter method to access the so-called "Humic Fraction" that will be hindered from molecular degradation methods under acid conditions.

3.3. Mechanistic and technical implications

The DFRC degradation method includes two key steps: (a) solubilization of lignin by bromination and acetylation with AcBr and (b) reductive cleavage catalyzed by solid zinc (Zn; Lu and Ralph, 1998). Since ligno-cellulosic fraction is the matter of interest of this study, adjacent phenolic structures at the α- and β- positions were substituted by monosaccharides (highlighted in blue) (Fig. 4).

For the free monosaccharides, the lone pairs of electrons of the hydroxyl groups will attack the carbonyl function of the acetyl bromide. This attack is enhanced by the fact that bromide is considered as a good leaving group. For the ligno-cellulosic bond, the bromide will perform a nucleophilic substitution (SN) reaction, releasing monosaccharides directly bonded to the aromatic structure. The substitution is of SN_2 since the monosaccharide is bonded to the aryl group by the primary alcohol (Wade, 2012). Once the monosaccharide is released, it will undergo acetylation. The Zn is well known as a reducing agent (Wade, 2012), its function in this case is to reduce the α,β-Carbon bond. This reduction will enhance the elimination of the β-glyosidic bond and the yielded monosaccharide will attack the carbonyl group of an acetyl bromide, thereby performing acetylation.

Following this mechanism, several assumptions can be made about the efficiency of DFRC as a degradation technique, thereby confirming or infirming its efficiency in the molecular characterization of carbohydrates in sediments:

(1) An exclusive cleavage to lignin moieties as proposed by Lu and Ralph, (1998).
(2) Higher yield for DFRC due to the higher corrosive effect and higher affinity of acetic acid and acetyl bromide compared with a mineral acid the HCl (since the investigated matrix is OM).
(3) Lower yield but higher selectivity for DFRC due the milder reaction conditions once compared with the conditions of HCl hydrolysis (See Materials and Methods).

Assumption (1) is directly discarded since the carbohydrates has been analyzed via DFRC. Assumption (2) can also be discarded as the DFRC yielded lower amounts for all monosaccharides. Assumption (3) can be accepted, since a very good correlation is obtained for the relative amount of monosaccharides between the two methods ($52\% < R^2 < 87\%$; Fig. 2). HCl is considered as a strong acid, its capacity to cleave ether and ester type bonds has been proven (Wade, 2012), especially if manipulated at high temperatures (> 100 °C). Therefore, we consider that all non-cellulosic bonds are cleaved with the followed HCl procedure. The highest correlation between DFRC and HCl hydrolysis were yielded for Xyl and Ara (Fig. 2). These monosaccharides are considered as a vascular vegetation indicator. The obtained trends can show the efficiency of AcBr in cleaving the ligno-cellulosic bonds and that pentoses are the major carbohydrate components of ligno-cellulose. Lower correlations for the other monosaccharides can be related to the occurrence of hexoses and deoxyhexoses in the free, hemicellulose and ligno-cellulose compartments.

At the technical level, DFRC method is an all-in-one method for the characterization of various classes of soil OM, such as carbohydrates, lignin and lipids. Several studies have used

this procedure or similar ones in order to analyse lignins (Lu and Ralph, 1998; Matsui and Ohira, 2013) and aromatic compounds in fossilized sediments (Grasset el al., 2010). In this study, although we were interested in revealing the capacity of this degradation technique to analyze carbohydrates, it yielded aromatic compounds, fatty acids and ω-hydroxy fatty acids. These families of molecules will be investigated in upcoming studies.

If one considers the time and temperature it takes for the different methods (2 h and 50 °C for DFRC method, 3 h and 100 °C, for acid hydrolysis and 30 min and 400 °C for THM method), DFRC is faster and/or requires milder conditions.

Beyond the yielded molecules, DFRC extract varied in weight from 300 mg/g to 500 mg/g of the freeze-dried weighted peat samples. This reveals, not only the degradation capacity, yet the extraction capacity of this method. A consideration of a double-shot procedure, like DFRC treatment followed by acid/alkaline hydrolysis could be interesting (*study under progress*).

In its mechanistic behavior, the acetyl bromide and acetic acid combination employed in DFRC, has the capacity to degrade, in a specific way, ligno-cellulose fraction and/or free carbohydrates entrapped in aromatic macromolecular structures. The "softer" acidic conditions for DFRC method, compared to acid hydrolysis, and the difference of the temperatures used, will make the cleavage of the strong glycosidic bond less probable, in the case of DFRC method.

3.4.Statistical analysis of method comparison

The application of PCA in geochemistry falls into three categories. (1) Determine OM sources based on biomarker specifity (terrigenous or marine) and trace the fate of OM in the oceanic environment (Hu el al., 2006). (2) Revealing similarities and dissimilarities among samples (Pearson et al., 2008). (3) Uncovering relationships among variables by developping summary statistics (i.e. "degradation indices") (Boheme et al., 2004; Younes et al., 2017). Other studies have, specifically, focused on the application of multidimensional statistical analysis for interpreting molecular fingerprints of peat. Schellekens et al. (2009, 2011, 2012, 2015 a and b) used the factor analysis method to extract parameters that might be used to interpret the vegetation change, anaerobic and aerobic decomposition and fire incidence of several peat bogs. In our case, PCA was used in order to compare between samples and between two methods of degradation.

Some of the strengths of PCA is its ability to analyze variables with interactive relationships as they vary with respect to other variables. This method removes intercorrelation between variables by creating independent ones (PC_1, PC_2 …). It also presents an "all in one" data visualization method as one can assess geochemical relationships among different compound classes (Younes and Grasset, 2017). In our analysis, two PCAs has been performed in order to statistically validate the comparison between DFRC and HCl hydrolysis and CuO-NaOH oxidation, from one side, and DFRC and THM methods from another side (Fig. 5). This division is done following the difference of mechanisms between the employed methods. As DFRC, CuO-NaOH oxidation and HCl hydrolysis are qualified as "Chemical Degradation Methods" and THM is qualified as a "Thermochemical Method".

PC_1 and PC_2 of the chemical methods (left plot; Fig. 5) accounted for 86.70% of the total variance. Lower but a high variance was obtained once DFRC and THM methods data was investigated (PC_1 and PC_2 accounted for 76.31% of the total variance; Fig.5, right plot). These

values indicate that the comparison of the employed parameters is statistically meaningful for all methods and reliable trends can be concluded from this study.

For the chemical methods (left plot, Fig. 5), PCA analysis showed all four ratios (Deoxy/C_5 and C_6/C_5) plotted together, indicating a stronger relationship than suggested by simple correlation. Ara and Xyl in DFRC and HCl hydrolysis were closer to $\Lambda_{CuO\text{-}NaOH}$ than other monosaccharides. This indicates that the ligno-cellulosic fraction is more likely composed of pentoses than hexoses proving the higher Xyl contribution in the case of DFRC method (Fig. 2). For the PCA of DFRC and THM methods (right plot, Fig. 5), lower clustered were yielded than the case of chemical methods. Although carbohydrate moieties of the two methods were clustered together, Λ_{THM} was clustered by itself in the negative side of both PCs.

Conclusion

This study presents the application of a recently developed and less used molecular analytical technique for the characterization of the carbohydrate pool in peat samples, the DFRC method. Acetyl bromide method is previously used to digest aromatic macromolecular structures, for analytical and industrial purposes. Following this principle, an application of this degradation technique to the OM matrix of a peatland will allow the access of a specific carbohydrate pool linked to lignin/polyphenols and/or entrapped into macromolecular aromatic structures.

In order to validate the DFRC method, a comparison have been held with lignin input. The high correlation between total carbohydrates yielded with DFRC method and acid hydrolysis from one side and lignin input yielded with CuO-NaOH oxidation, from another side, indicates that hemi-cellulosic and ligno-cellulosic pools of carbohydrates occurs simultaneously along the investigated peat core. It also shows that the ligno-cellulosic fraction is more likely linked to the intact non-oxidized lignin (yielded with CuO-NaOH oxidation).

Lower carbohydrates abundance, in the case of DFRC method, indicates its capacity to only target a portion of the carbohydrate fraction which is the free and ligno-cellulosic pools. For carbohydrates amounts, all three methods (acid hydrolysis and DFRC and THM methods) showed a decreasing trend from acrotelm, mesotelm and catotelm indicating the degradation of carbohydrates with depth.

Unlike acid hydrolysis, the DFRC method showed several patterns. This indicates some difference in the origin of the yielded monosaccharides. DFRC showed some difference in the origin and/or degradation rate between Xyl and Ara. It also allowed the identification of Rib as a microbial indicator. These information were hindered from acid hydrolysis and THM method, as the first showed nearly the same profile for all monosaccharides and the second one didn't have the capacity to analyze Ara and Rib.

Briefly, higher correlation (therefore higher similarity) was obtained between DFRC and acid hydrolysis than between DFRC and THM methods. This is due to the difference of mechanisms involved in chemolytic and thermochemolytic methods. For the first group, both methods shared acid conditions and milder temperature conditions if compared to the second group. In addition thermochemolysis is performed under alkaline conditions. These differences allow the application of chemical degradation methods to more recent less oxidized sediments and

thermochemical methods to ancient more oxidized sediments. In order to confirm this scope of applications, additional analyses should be performed on mutiple soils and sediments samples. Therefore, we caution against making direct comparison of carbohydrates data yielded with the three methods because of different mechanisms involved in each process. Yet DFRC shows as a prominent method for the molecular characterization of carbohydrates in soils and sediments.

Acknowledgments

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Fig.1. Total Carbohydrates (normalized per 1 g OC) yielded by DFRC method compared to total lignin yielded by CuO-NaOH oxidation and THM method.

Fig. 2. Depth record of relative carbohydrate abundance (mg carbohydrate / 1 g OC) with DFRC method, acid hydrolysis and THM method (mg carbohydrate / 1 g OC). Small boxes in HCl hydrolysis and THM method charts present the relationship between relative carbohydrate abundance of these methods from one side and DFRC method from other side (R^2 is the coefficient of determination).

Fig. 3. DFRC carbohydrate proxies compared to their homologues yielded by acid hydrolysis (HCl) and THM method.

Fig. 4. Comparison of the Lu and Ralph mechanism with the proposed mechanism

Fig. 5. PCA of carbohydrate and lignin parameters analyzed using DFRC and CuO-NaOH and HCl (left plot) and DFRC and THM (right plot).

Fig.1

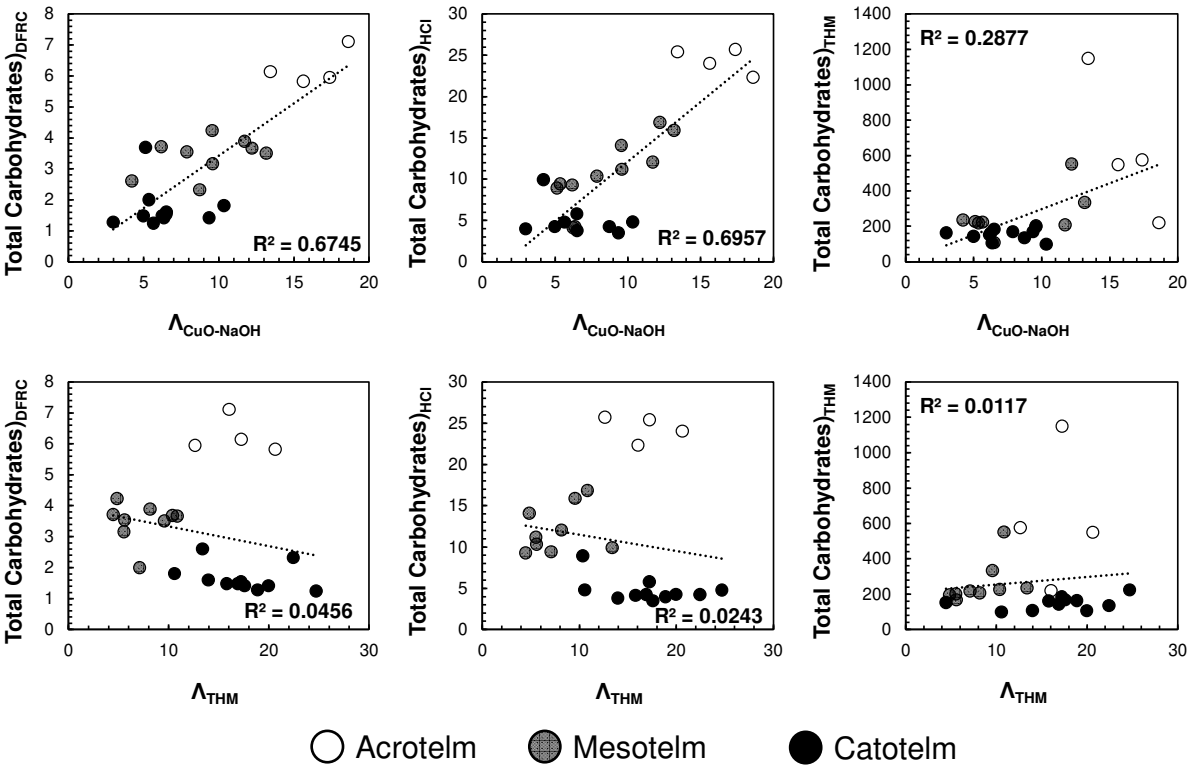

Total Carbohydrates)DFRC
Total Carbohydrates)HCl
Total Carbohydrates)THM
R² = 0.6745
R² = 0.6957
R² = 0.2877
R² = 0.0456
R² = 0.0243
R² = 0.0117
ΛCuO-NaOH
ΛTHM
Acrotelm
Mesotelm
Catotelm

Fig. 2

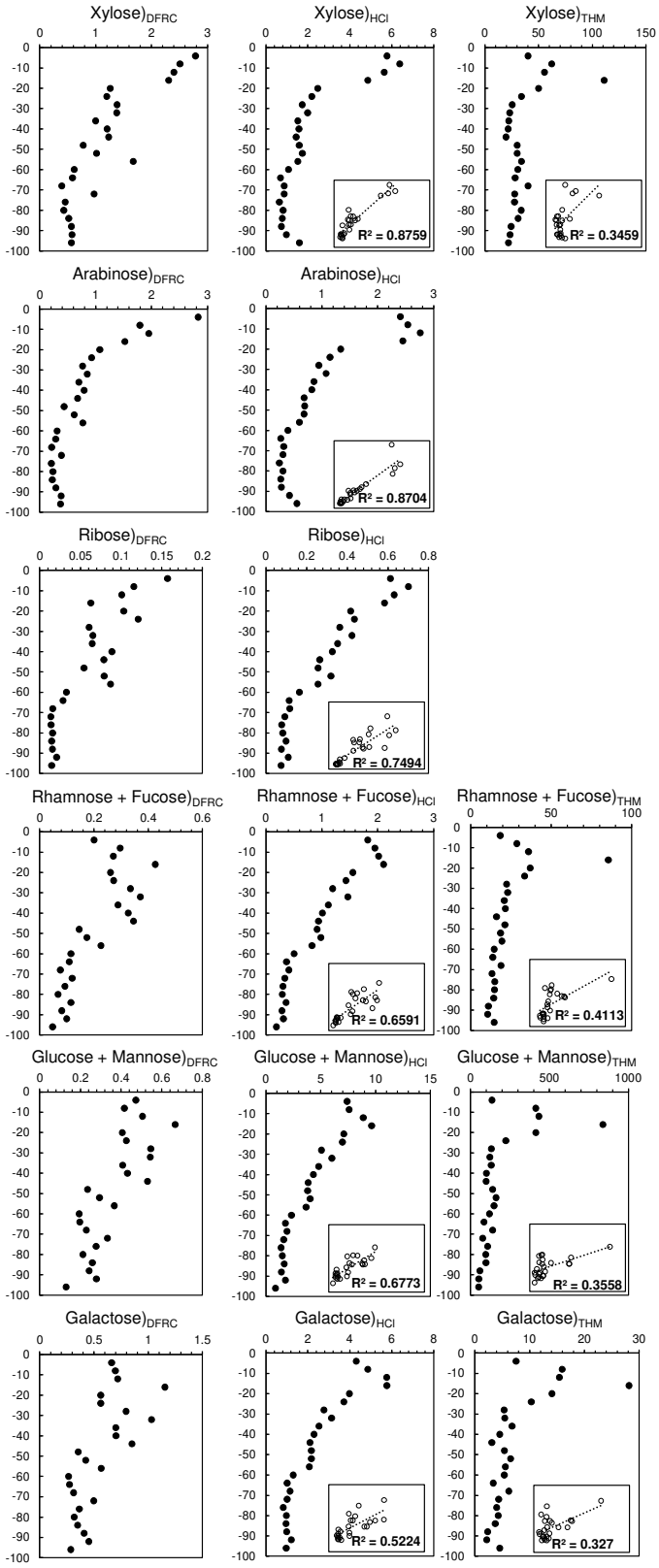

Xylose)DFRC
Xylose)HCl
R² = 0.8759
Xylose)THM
R² = 0.3459
Arabinose)DFRC
Arabinose)HCl
R² = 0.8704
Ribose)DFRC
Ribose)HCl
R² = 0.7494
Rhamnose + Fucose)DFRC
Rhamnose + Fucose)HCl
R² = 0.6591
Rhamnose + Fucose)THM
R² = 0.4113
Glucose + Mannose)DFRC
Glucose + Mannose)HCl
R² = 0.6773
Glucose + Mannose)THM
R² = 0.3558
Galactose)DFRC
Galactose)HCl
R² = 0.5224
Galactose)THM
R² = 0.327

Fig. 3

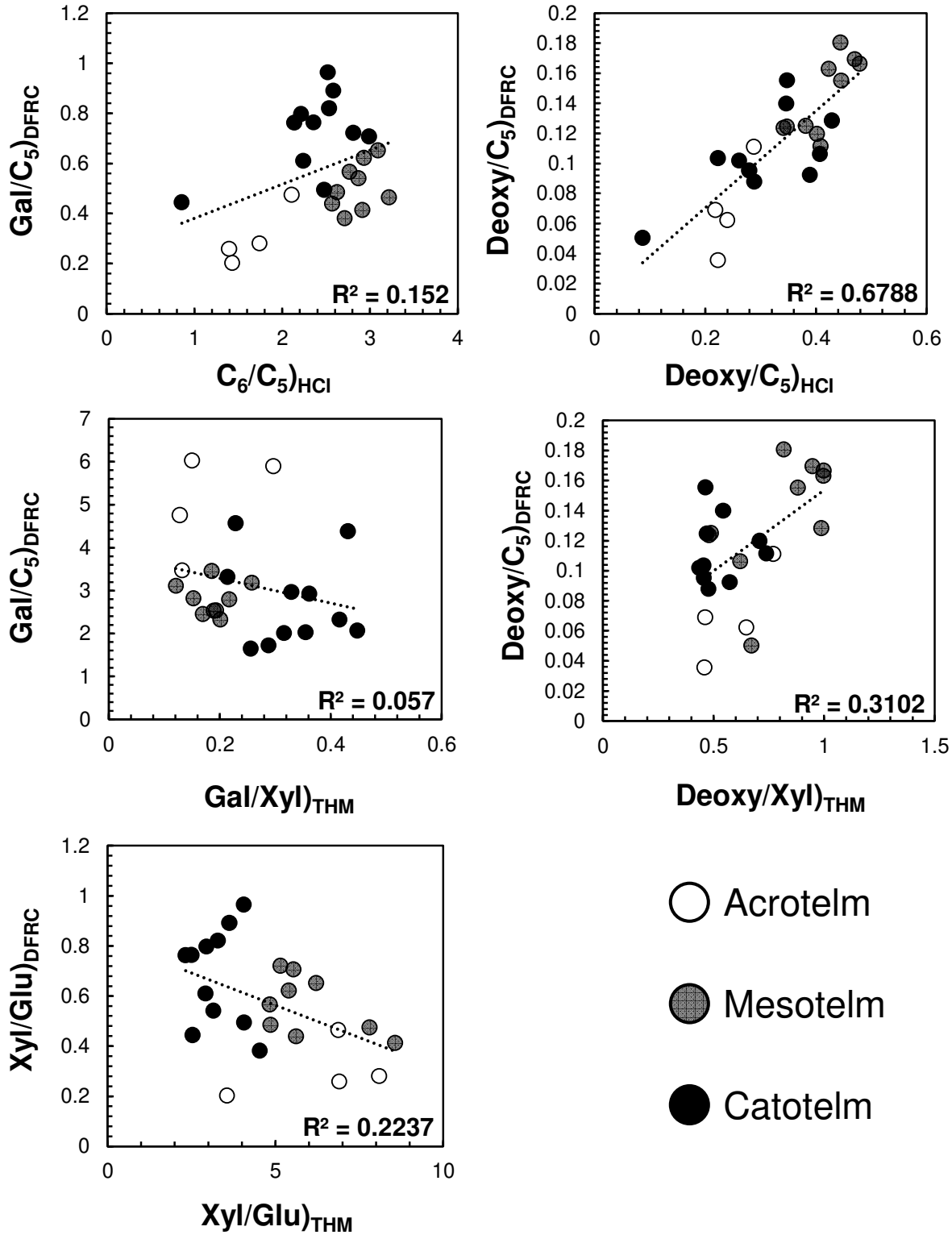

Gal/C5)DFRC
C6/C5)HCl
R² = 0.152
Deoxy/C5)DFRC
Deoxy/C5)HCl
R² = 0.6788
Gal/C5)DFRC
Gal/Xyl)THM
R² = 0.057
Deoxy/C5)DFRC
Deoxy/Xyl)THM
R² = 0.3102
Xyl/Glu)DFRC
Xyl/Glu)THM
R² = 0.2237
Acrotelm
Mesotelm
Catotelm

Fig 4

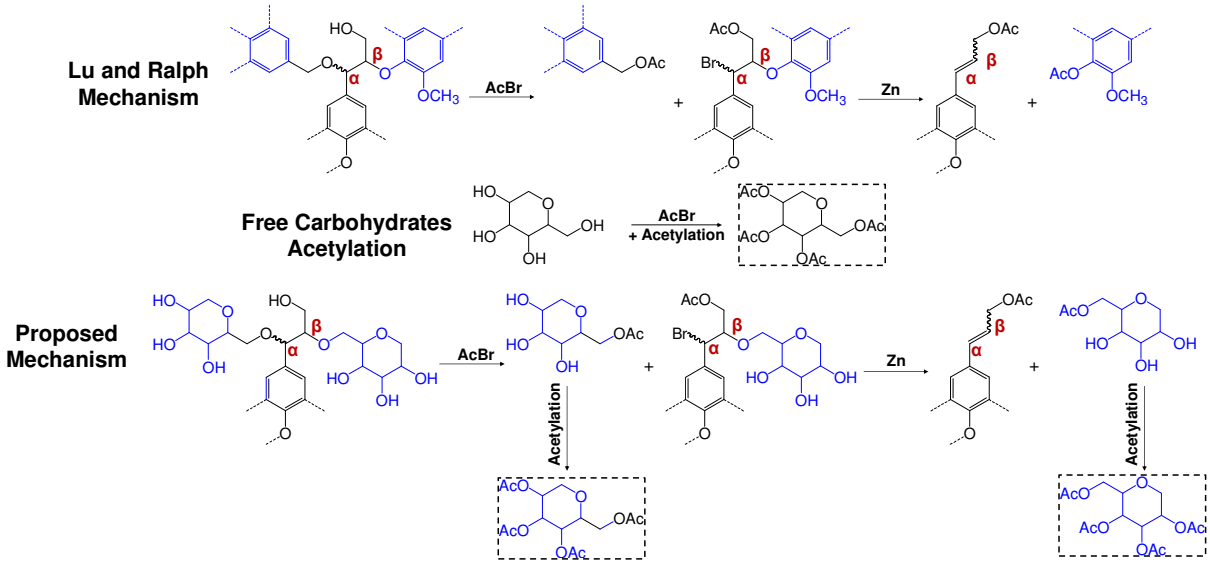
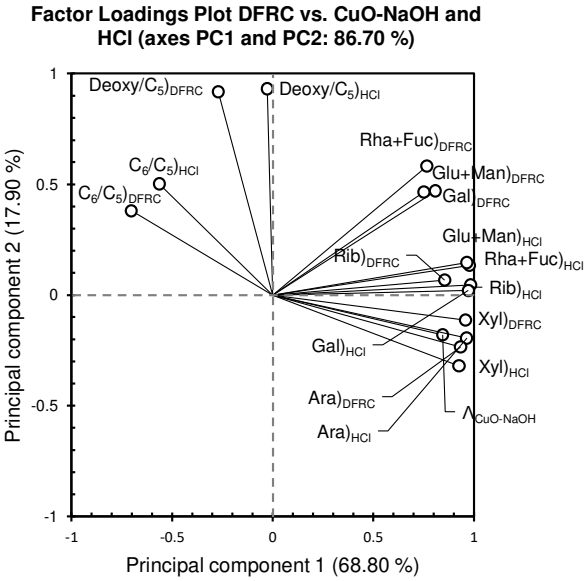

Lu and Ralph Mechanism
AcBr
Zn
Free Carbohydrates Acetylation
AcBr + Acetylation
Proposed Mechanism
AcBr
Acetylation
Zn
Acetylation

Fig. 5


Factor Loadings Plot DFRC vs. CuO-NaOH and HCl (axes PC1 and PC2: 86.70 %)
Deoxy/C5)DFRC
Deoxy/C5)HCl
Rha+Fuc)DFRC
C6/C5)HCl
Glu+Man)DFRC
C6/C5)DFRC
Gal)DFRC
Glu+Man)HCl
Rib)DFRC
Rha+Fuc)HCl
Rib)HCl
Xyl)DFRC
Gal)HCl
Xyl)HCl
Ara)DFRC
ΛCuO-NaOH
Ara)HCl
Principal component 2 (17.90 %)
Principal component 1 (68.80 %)
1
0.5
0
-0.5
-1
-1
-0.5
0
0.5
1

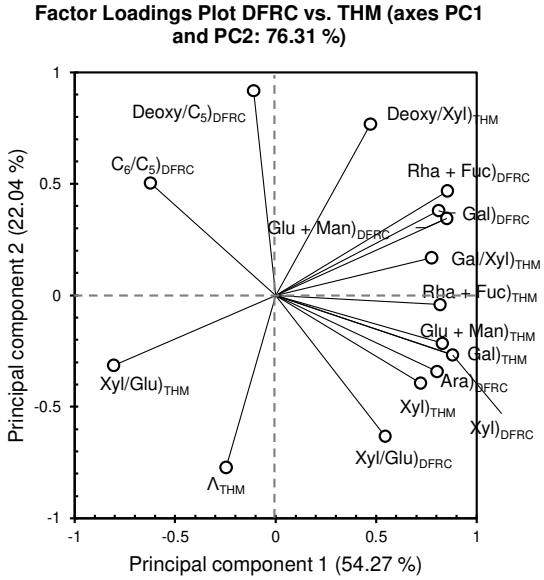

Factor Loadings Plot DFRC vs. THM (axes PC1 and PC2: 76.31 %)
Deoxy/C5)DFRC
Deoxy/Xyl)THM
C6/C5)DFRC
Rha + Fuc)DFRC
Gal)DFRC
Glu + Man)DFRC
Gal/Xyl)THM
Rha + Fuc)THM
Glu + Man)THM
Gal)THM
Xyl/Glu)THM
Ara)DFRC
Xyl)THM
Xyl)DFRC
Xyl/Glu)DFRC
ΛTHM
Principal component 2 (22.04 %)
Principal component 1 (54.27 %)
1
0.5
0
-0.5
-1
-1
-0.5
0
0.5
1

Table. 1. Symbols of the investigated lignin and carbohydrate moieties.

Lignin monomers			Monosaccharides[b]	
THM[a]		CuO-NaOH oxidation		
G_4:	3,4-dimethoxybenzaldehyde	S: Syringyl	Xyl:	Xylose
G_5:	1-(3,4-dimethoxyphenyl)ethanone	V: Vanillyl	Ara:	Arabinose
G_6:	3,4-dimethoxybenzoic acid methyl ester	C: Cinnamyl	Rib:	Ribose
G_{18}:	3-(3,4-dimethoxyphenyl)-2-propenoic acid methyl ester		Rha:	Rhamnose
P_{18}:	3-(4-hydroxyphenyl)-2-propenoic acid methyl ester		Fuc:	Fucose
S_4:	3,4,5-trimethoxybenzaldehyde		Man:	Mannose
S_5:	1-(3,4,5-trimethoxypehnyl)ethanone		Gal:	Galactose
S_6:	3,4,5-trimethoxybenzoic acid methyl ester		Glu:	Glucose

[a] The symbols used are the same as the ones used in Filley et al. (2006) and Younes and Grasset, (2018)

[b] Symbols are followed by a subscript indicating the method the monosaccharides have been analyzed with (DFRC, THM or HCl).

Filley, T. R., Nierop, K. G., Wang, Y., 2006. The contribution of polyhydroxyl aromatic compounds to tetramethylammonium hydroxide lignin-based proxies. Org. Geochem. 37(6), 711-727.

Younes, K., Grasset, L., 2018. Comparison of thermochemolysis and classical chemical degradation and extraction methods for the analysis of carbohydrates, lignin and lipids in a peat bog. J. Anal. Appl. Pyrol. 134, 61-72.