NMR Techniques in Lignocellulose Characterisation

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VALREO
VALORISATION OF RESIDUES FROM
THE VEGETABLE OIL INDUSTRY

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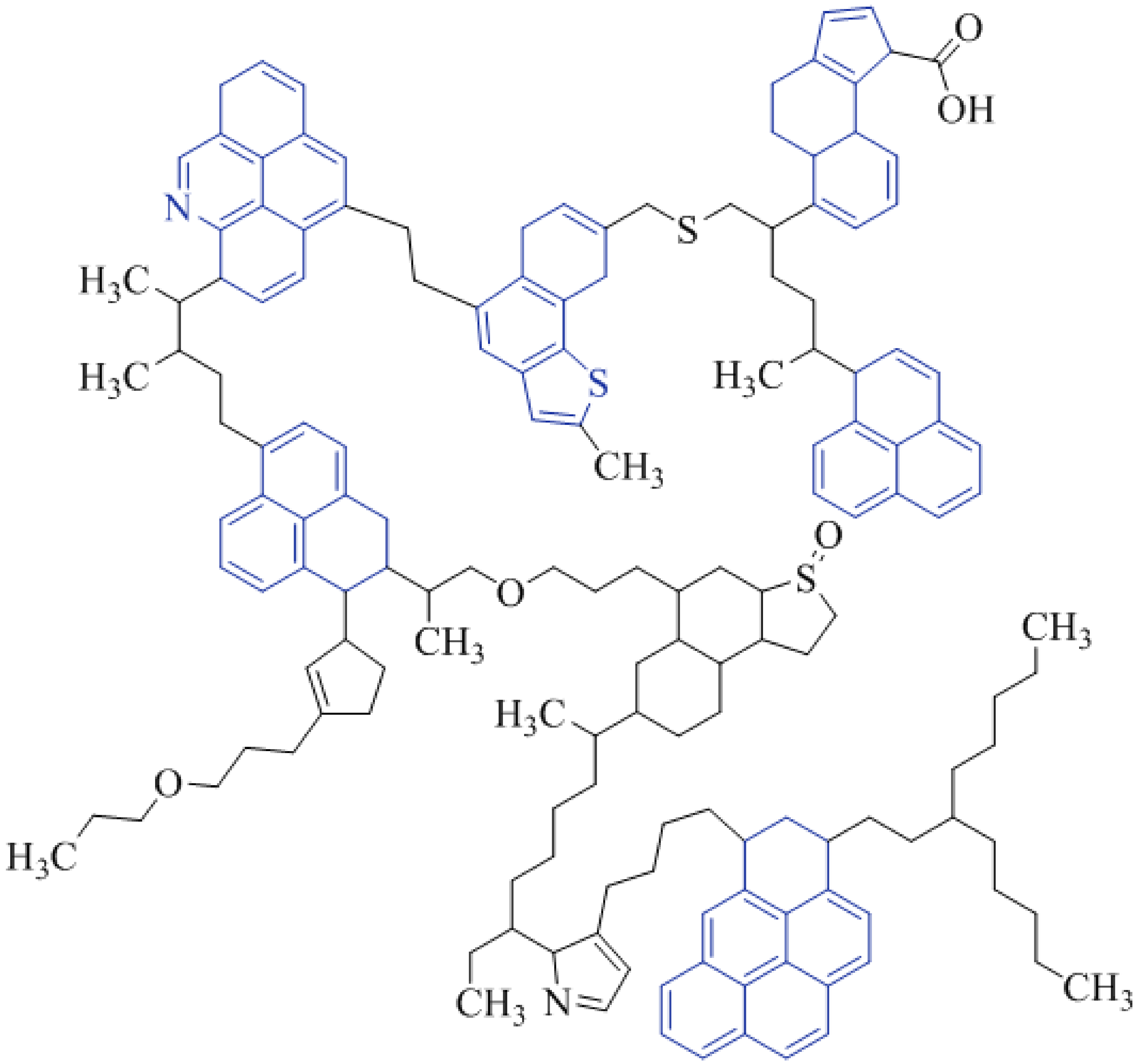
Presentation topics

- *possibilities of using NMR techniques in the analysis of lignocellulose biomass samples*
- *adventages, main drawbacks and future perspectives*

NMR spectroscopy

- ✓ one of the most powerful tools for identification and structure elucidation of compounds
- ✓ almost every atom has NMR active nucleus, but the most frequently observed are ^{1}H, ^{13}C, ^{15}N and ^{31}P
- ✓ today NMR spectroscopy is highly developed and technologically advanced method and one of the principal structural techniques for chemists to study organic and inorganic molecules as well as biomolecules
- ✓ it is employed in different scientific fields such as physics, chemistry, material science, biology, biochemistry, medicine for identification of physical and chemical properties of atoms and molecules, determination of composition and purity of various types of samples, monitoring of reactions and study metabolism in cells
- ✓ it has found a widespread use in industrial applications such as pharmaceutical, food, oil or biorefinery
- ✓ one of the bigest advantages of NMR is the fact that it is non- destructive technique that can operate both, in the liquid and the solid-state.

Advantages of NMR spectroscopy in analysis of complex organic samples



- *identification and structural analysis of compounds and compound mixtures without physical separation*
- *simple sample preparation, no external standards*
- *fast analysis, plenty of information in one spectum: hydrocarbon distribution, content of aromatics, olefins, naphtenes, paraffins, oxygenates...*

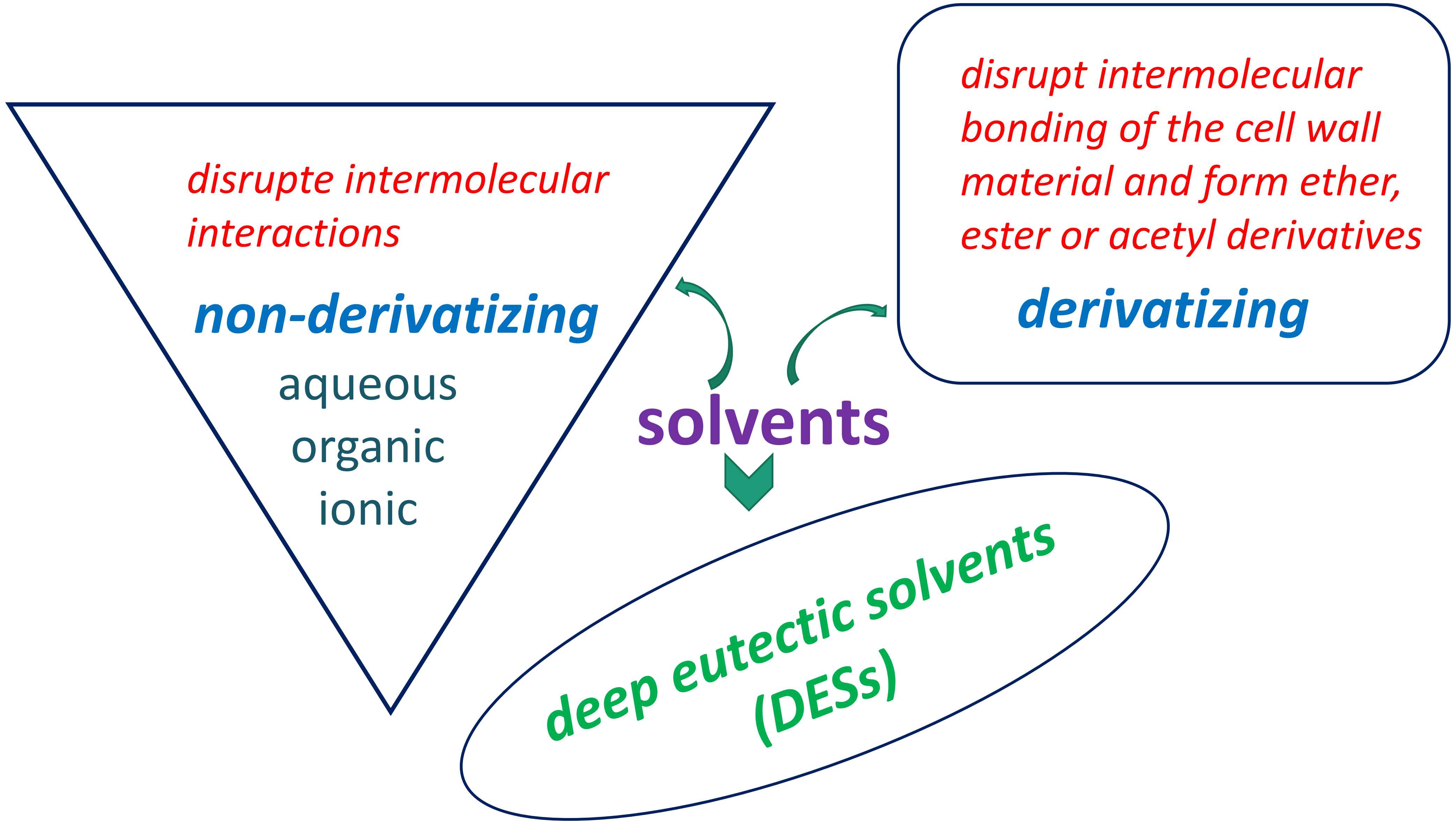
Lignocellulosic biomass - NMR spectroscopy

- promising renewable raw material for the production of various bio-based products, very complex organic mixture composed of many different molecules
- necessary to know its structural composition in order to optimize pretreatment process and further (bio)conversion into bio-based products
- NMR lignocellulose chemistry for more than 30 years:

- fast and reliable method that can provide advanced data on the molecular architecture and composition of whole lignocellulosic biomass and its structural units (lignin, cellulose, hemicellulose)
- chemical structure, molecular weight and polydispersity have been determined and reported in the literature
- analysis of the solution and solid state, one- and two-dimensional NMR methods
- spectra can provide qualitative and quantitative information on different types of lignocellulose and their constituents

Sample preparation

- one of the most important steps to achieve reliable results with NMR spectroscopy
- solution state NMR requires a sufficiently dissolved biomass sample
- solid state NMR does not require solubilization of the lignocellulose biomass
- NMR rotors are filled with dried and ground lignocellulosic biomass samples or previously separated lignocellulosic biopolymers by pretreatment processes



disrupte intermolecular interactions

non-derivatizing

aqueous
organic
ionic

disrupt intermolecular bonding of the cell wall material and form ether, ester or acetyl derivatives

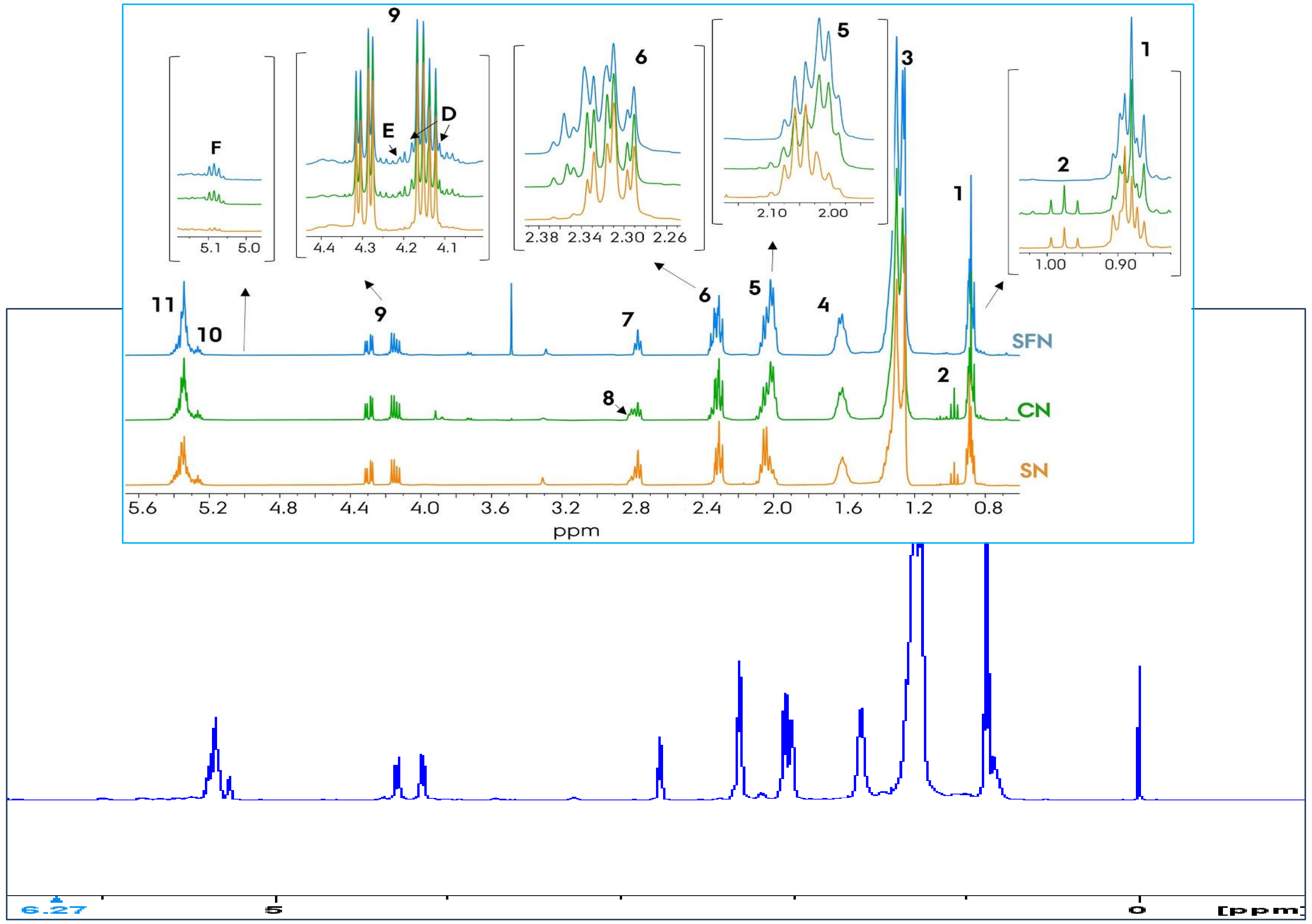
derivatizing

solvents

deep eutectic solvents (DESs)

- ○ *the most frequently used NMR techniques in the solution state for structural elucidation of LB (as well as for cellulose, hemicellulose and lignin):*
- ✓ *one-dimensional ^{1}H, ^{13}C and ^{31}P*
- ✓ *two-dimensional heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond correlation (HMBC)*
- ○ *solid state: ^{13}C CP MAS*

Lipid extracts



Typical ^{1}H NMR spectra of lipid extract of oilseed cake

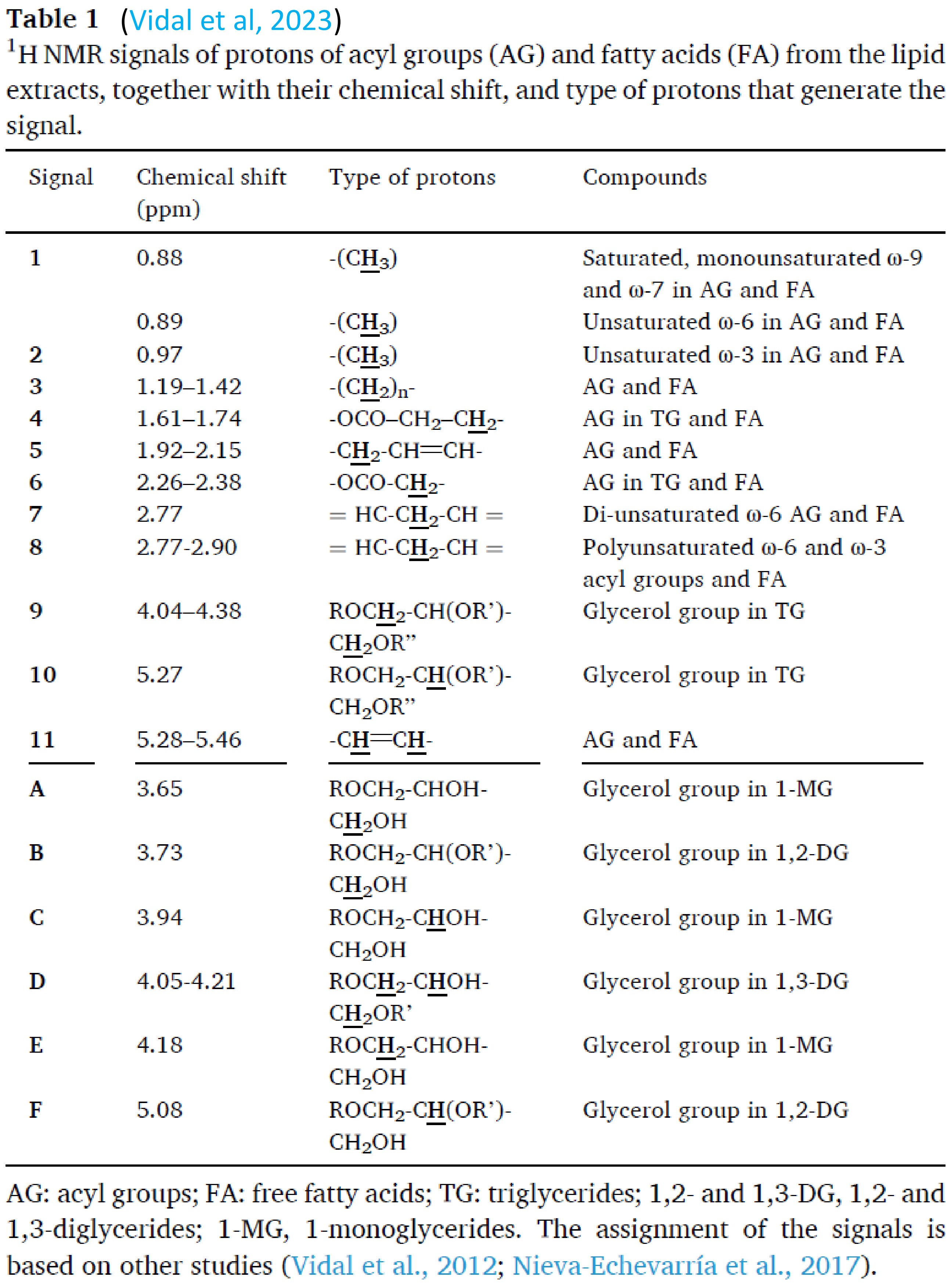


Table 1 (Vidal et al, 2023)

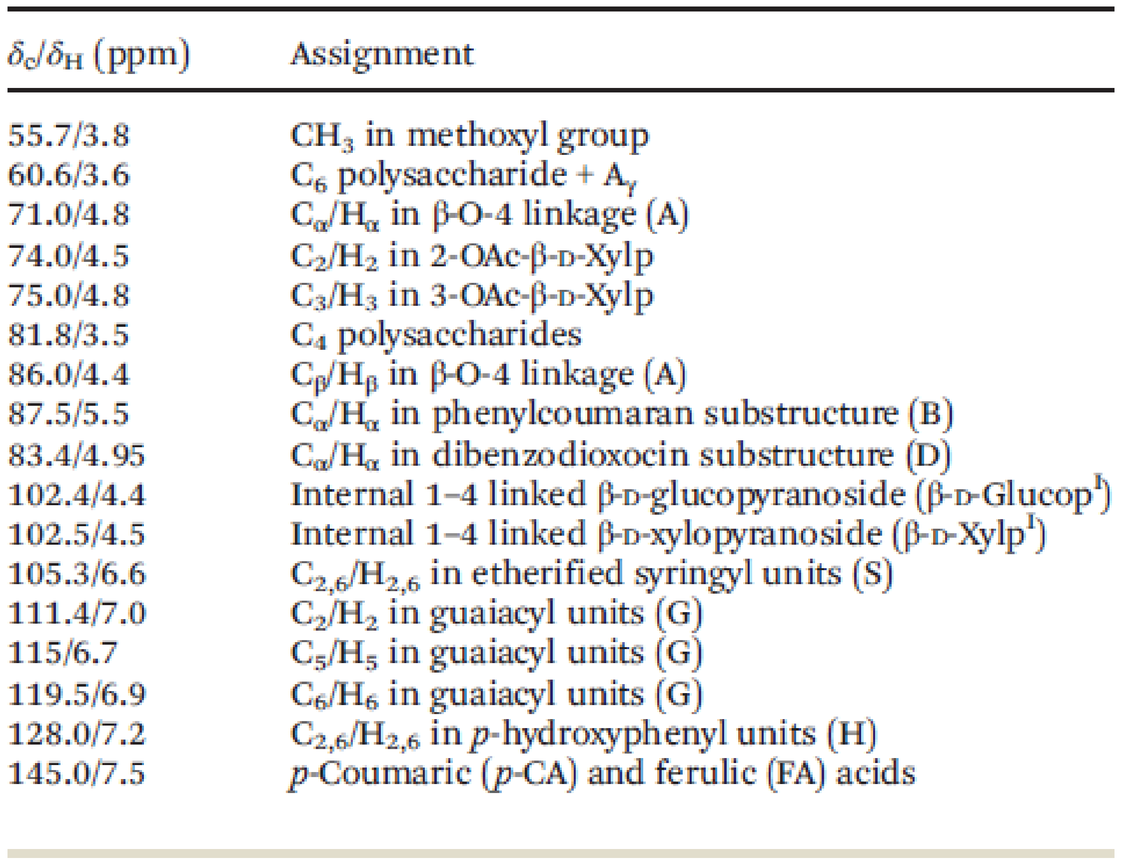
^{1}H NMR signals of protons of acyl groups (AG) and fatty acids (FA) from the lipid extracts, together with their chemical shift, and type of protons that generate the signal.

Signal	Chemical shift (ppm)	Type of protons	Compounds
1	0.88	$-(C\underline{H}_3)$	Saturated, monounsaturated ω-9 and ω-7 in AG and FA
	0.89	$-(C\underline{H}_3)$	Unsaturated ω-6 in AG and FA
2	0.97	$-(C\underline{H}_3)$	Unsaturated ω-3 in AG and FA
3	1.19–1.42	$-(C\underline{H}_2)_n-$	AG and FA
4	1.61–1.74	$-OCO{-}CH_2{-}C\underline{H}_2-$	AG in TG and FA
5	1.92–2.15	$-C\underline{H}_2-CH{=}CH-$	AG and FA
6	2.26–2.38	$-OCO-C\underline{H}_2-$	AG in TG and FA
7	2.77	$= HC-C\underline{H}_2-CH =$	Di-unsaturated ω-6 AG and FA
8	2.77-2.90	$= HC-C\underline{H}_2-CH =$	Polyunsaturated ω-6 and ω-3 acyl groups and FA
9	4.04–4.38	$ROC\underline{H}_2-CH(OR')-C\underline{H}_2OR''$	Glycerol group in TG
10	5.27	$ROCH_2-C\underline{H}(OR')-CH_2OR''$	Glycerol group in TG
11	5.28–5.46	$-C\underline{H}{=}C\underline{H}-$	AG and FA
A	3.65	$ROCH_2-CHOH-C\underline{H}_2OH$	Glycerol group in 1-MG
B	3.73	$ROCH_2-CH(OR')-C\underline{H}_2OH$	Glycerol group in 1,2-DG
C	3.94	$ROCH_2-C\underline{H}OH-CH_2OH$	Glycerol group in 1-MG
D	4.05-4.21	$ROC\underline{H}_2-CHOH-C\underline{H}_2OR'$	Glycerol group in 1,3-DG
E	4.18	$ROC\underline{H}_2-CHOH-CH_2OH$	Glycerol group in 1-MG
F	5.08	$ROCH_2-C\underline{H}(OR')-CH_2OH$	Glycerol group in 1,2-DG

AG: acyl groups; FA: free fatty acids; TG: triglycerides; 1,2- and 1,3-DG, 1,2- and 1,3-diglycerides; 1-MG, 1-monoglycerides. The assignment of the signals is based on other studies (Vidal et al., 2012; Nieva-Echevarría et al., 2017).

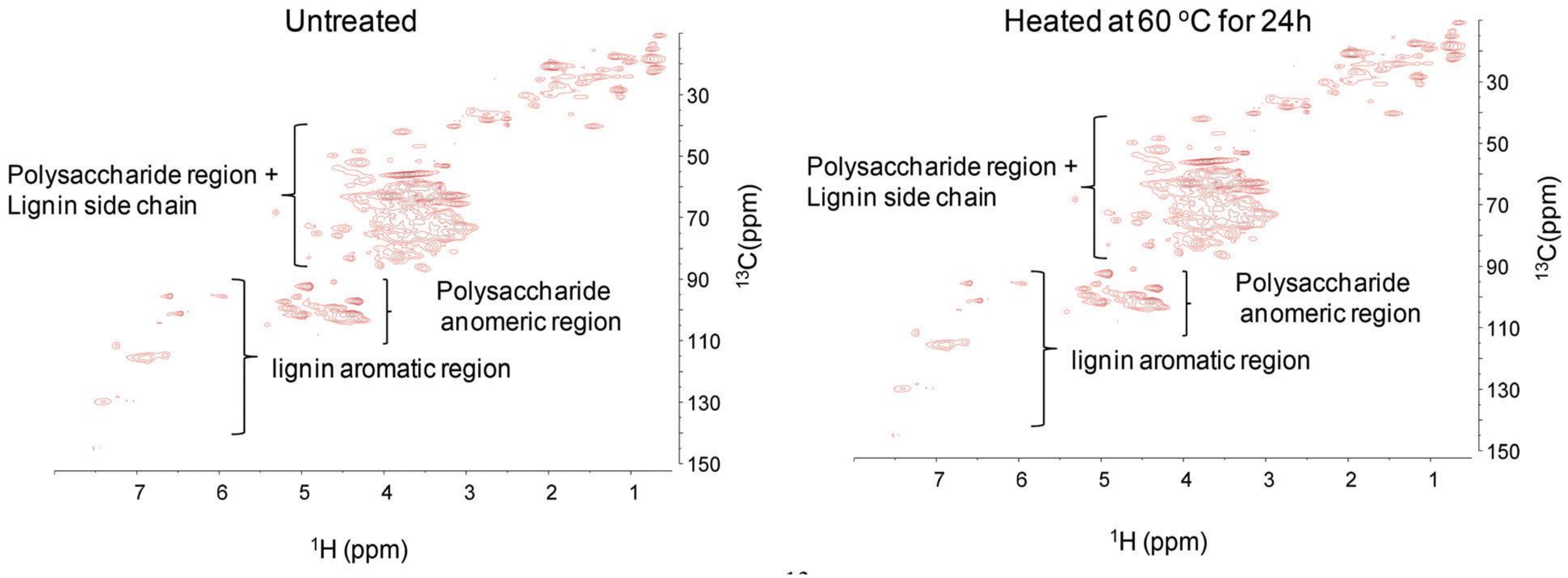
2D NMR

- due to severe signal overlapping, a complete assignment of 1H and 13C NMR spectra of lignocellulosic samples is almost impossible
- nowadays, the application of 2D NMR techniques provides more detailed signal assignment of natural polymers
- solution-state 2D HSQC NMR technique provide information on polysaccharide components profile, lignin composition and interunit linkage distribution, acetylated and native call walls



δ_C/δ_H (ppm)	Assignment
55.7/3.8	CH_3 in methoxyl group
60.6/3.6	C_6 polysaccharide + A_γ
71.0/4.8	C_α/H_α in β-O-4 linkage (A)
74.0/4.5	C_2/H_2 in 2-OAc-β-D-Xylp
75.0/4.8	C_3/H_3 in 3-OAc-β-D-Xylp
81.8/3.5	C_4 polysaccharides
86.0/4.4	C_β/H_β in β-O-4 linkage (A)
87.5/5.5	C_α/H_α in phenylcoumaran substructure (B)
83.4/4.95	C_α/H_α in dibenzodioxocin substructure (D)
102.4/4.4	Internal 1–4 linked β-D-glucopyranoside (β-D-Glucop^I)
102.5/4.5	Internal 1–4 linked β-D-xylopyranoside (β-D-Xylp^I)
105.3/6.6	$C_{2,6}/H_{2,6}$ in etherified syringyl units (S)
111.4/7.0	C_2/H_2 in guaiacyl units (G)
115/6.7	C_5/H_5 in guaiacyl units (G)
119.5/6.9	C_6/H_6 in guaiacyl units (G)
128.0/7.2	$C_{2,6}/H_{2,6}$ in *p*-hydroxyphenyl units (H)
145.0/7.5	*p*-Coumaric (*p*-CA) and ferulic (FA) acids

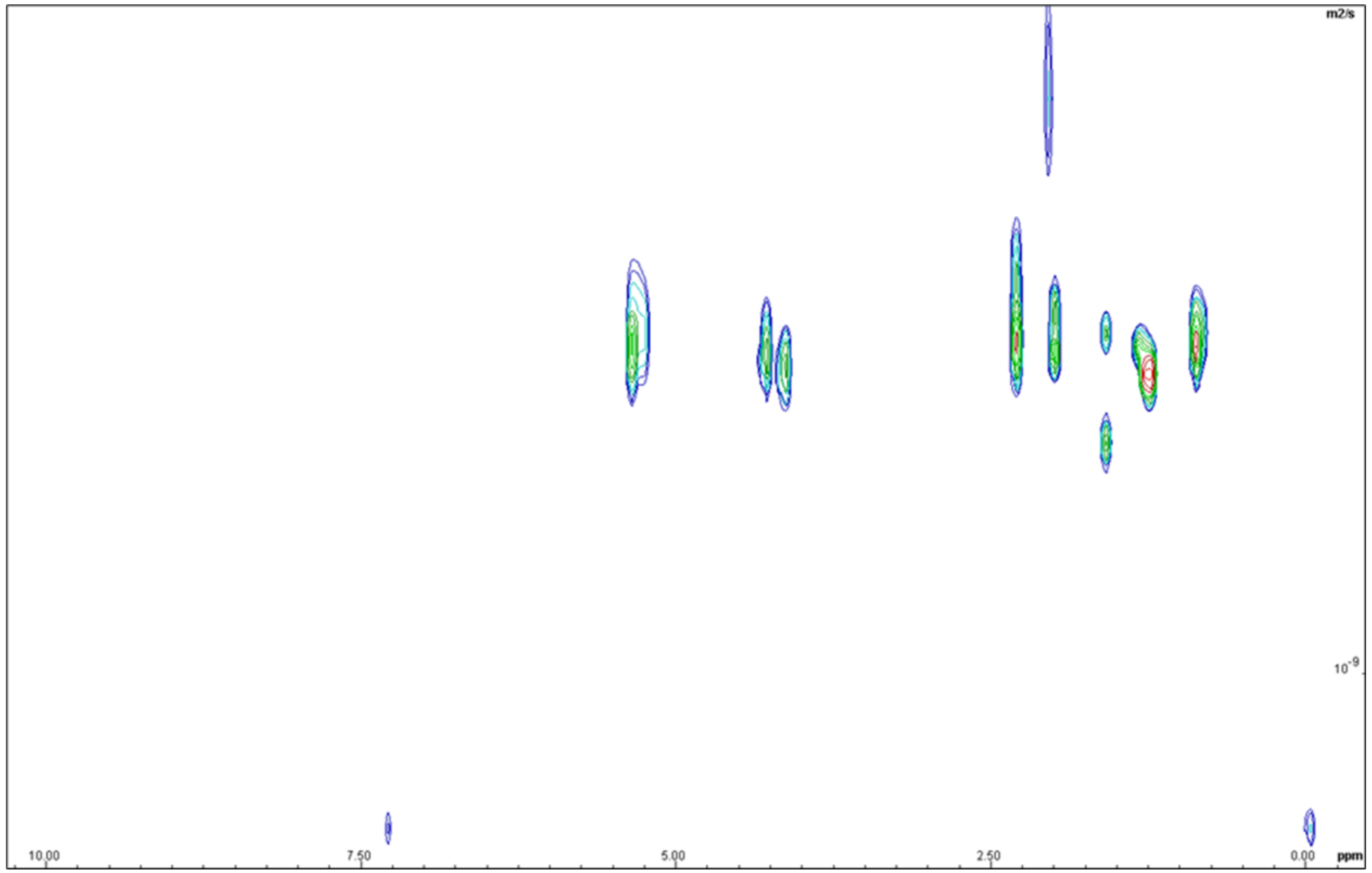
The typical chemical shift assignments of 1H–13C HSQC NMR spectra of biomass in PyCl-d6 : DMSO-d6 system (*Kim & Ralph, 2010*)



1H–13C 2D HSQC NMR spectra of corn stover in PyCl-d6 - DMSO-d6 solvent system (*Foston et al, 2016*).

DOSY, *diffusion ordered spectroscopy*

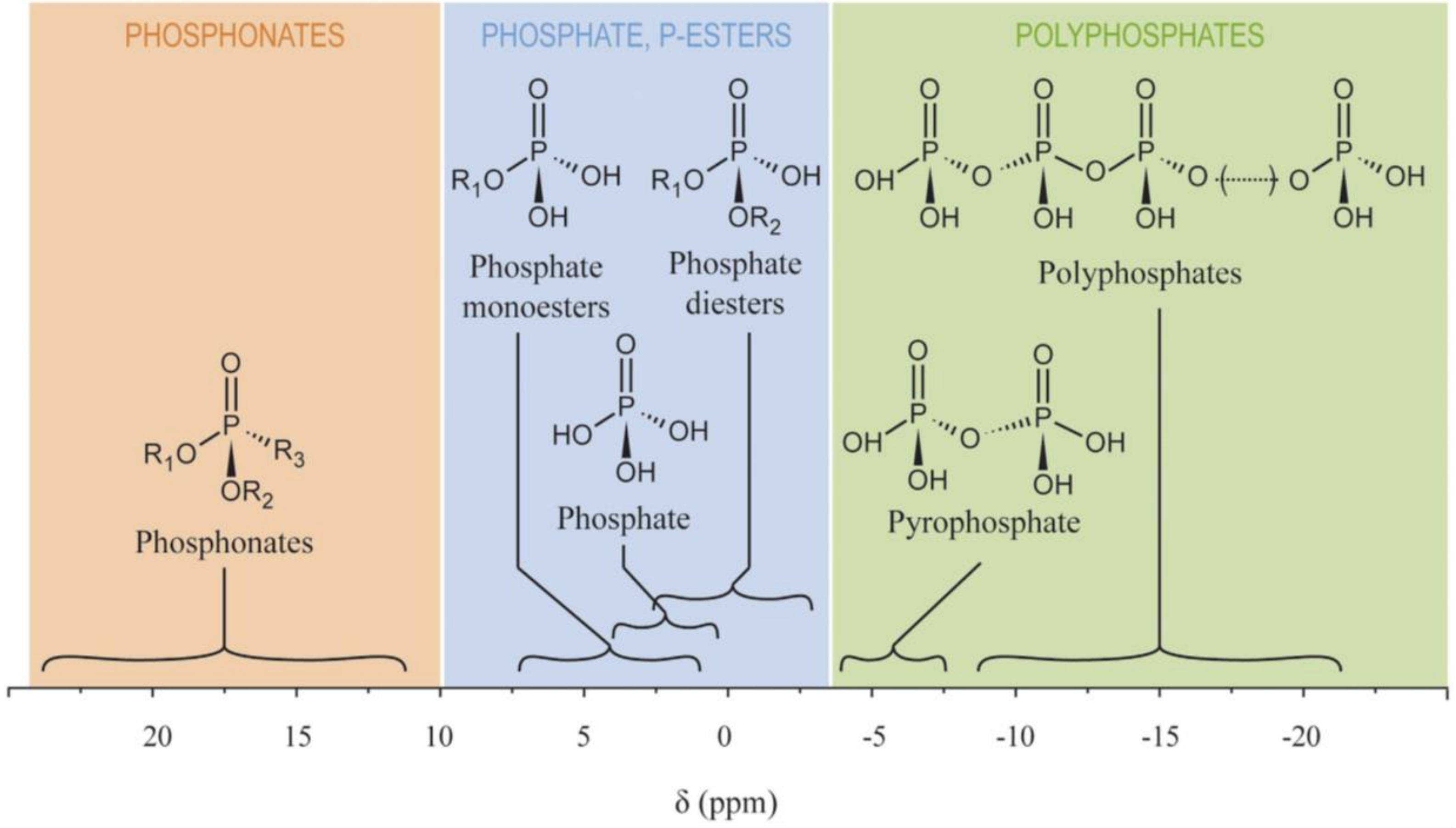
- *a pseudo-two-dimensional technique in which proton chemical shifts represent one dimension and translational diffusion coefficients the other*
- *signals of different types separated according to their diffusion coefficients*
- *analysis of complex and heterogeneous mixtures such as crude oil samples or food and drinks*
- *it is used in the research of proton-ligand molecular interactions and the characterization of reactive intermediates*
- *information on the mass, size and composition of the mixture*

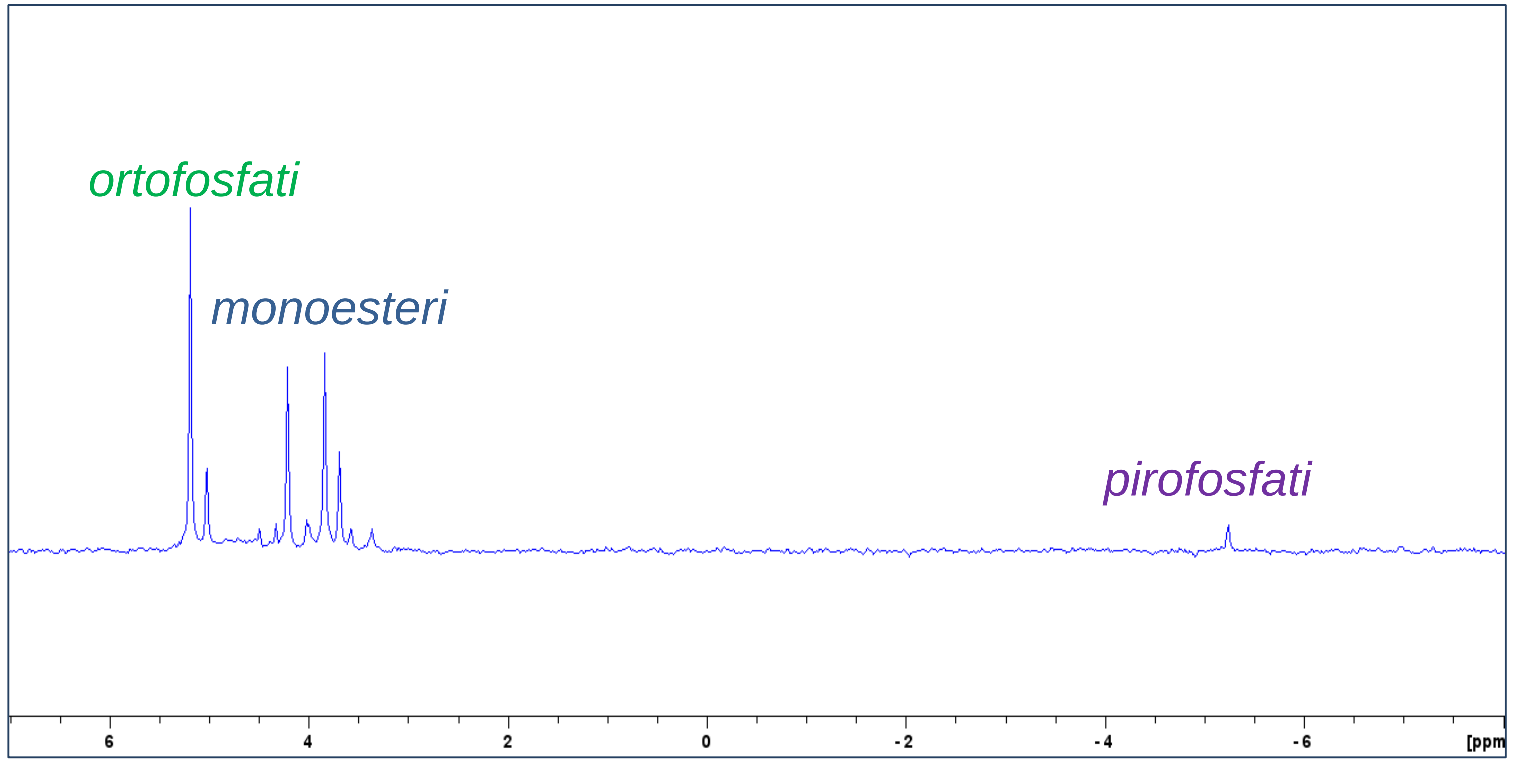


Typical DOSY NMR spectrum of lipid extract of oilseed cake sample

Solution ^{31}P NMR spectroscopy

- phosphorus is one of the most important essential macronutrient for plant
- it is important for appropriate physiological and biochemical conditions in plants
- inorganic phosphorus is present in plants in the form of orthophosphates, pyrophosphates and polyphosphates
- organic phosphorus in plants is usually in the form of phosphate monoesters and diesters, α- and β-glycerophosphates and organic polyphosphates
- the ^{31}P NMR can identify and quantify most of the organic and inorganic forms of phosphorus
- ^{31}P NMR spectroscopy offers major advances in the determination of hydroxyl groups in a single spectrum with high signal resolution compared to conventional wet chemical methods
- the phenolic hydroxyl groups attached to syringyl, guaiacyl and p-hydroxyphenyl units can be distinguished in a short experimental time with small sample amounts
- this method has some limitations, such as the presence of amine groups, which can interfere with the quantification of hydroxyl groups in some cases.



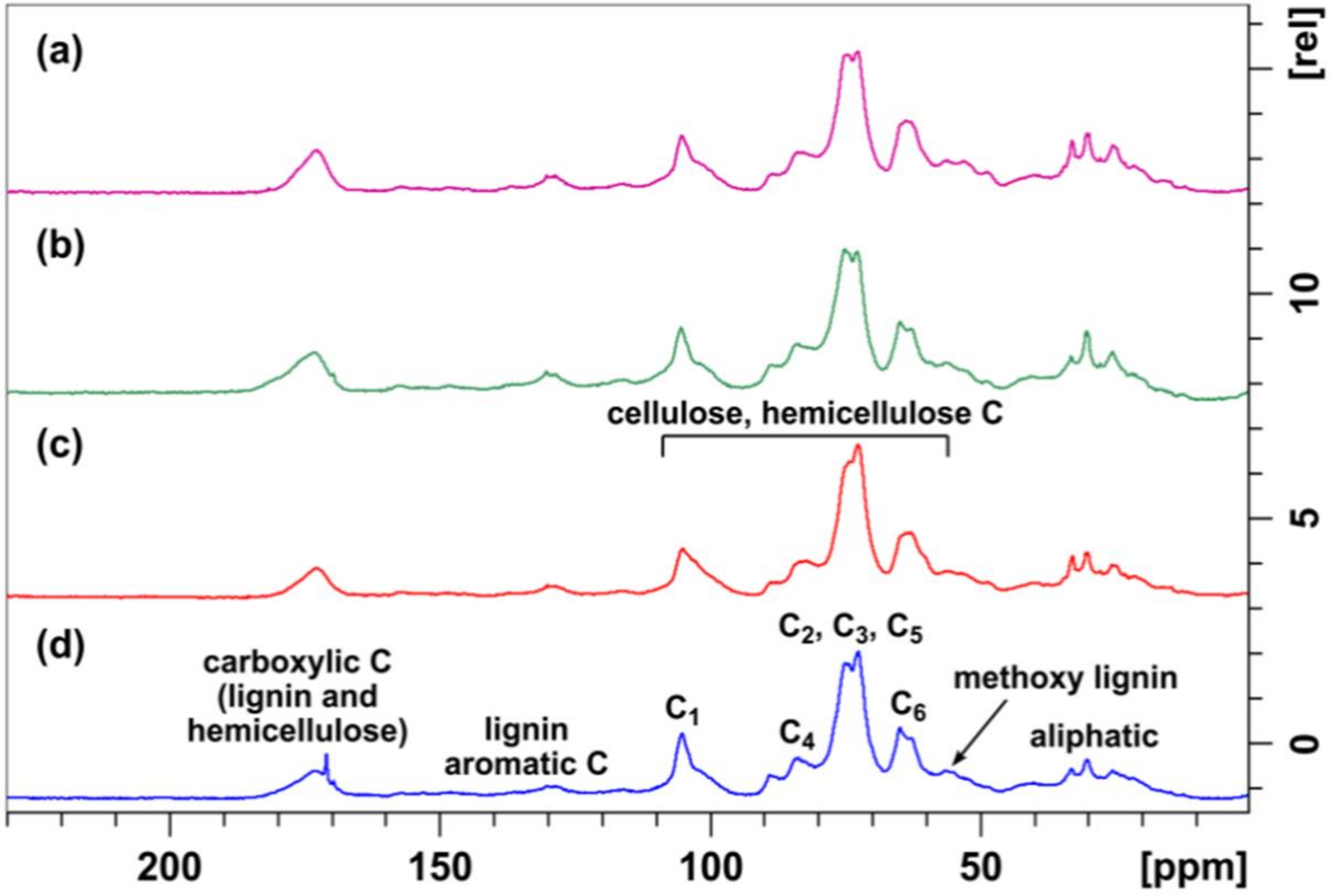


Typical ^{31}P NMR spectrum of NaOH–EDTA extract of lignocellulose

Solid state nmr

- the main advantage of solid state NMR is that it measures polymers in its native state (without destroying its native structure) compared to liquid state NMR analysis
- various solid state NMR techniques are employed in structural and mechanistic studies of lignocellulose material
- according to the literature, it has been evidenced that solid state NMR has become a promising non-destructive technique that provides a wealth of information on the structure, conformation, packing and polymorphism of lignocellulosic biomass and thus can serve as reliable technique for lignocellulose characterization

^{13}C CP MAS NMR spectra of brewery spent grain



Alkyl-C (0-45 ppm)/%	NCH/OCH_3 (45-65 ppm)/%	O-Alkyl C (65-90 ppm)/%	O-C-O (90-110 ppm)/%	Aromatic-C (110-145 ppm)/%	Aromatic C-O (145-165 ppm)/%	COO/N-C=O (165-190ppm)/%	Total Al-C (0-110 ppm)/%	Total Ar-C (110-165 ppm)/%

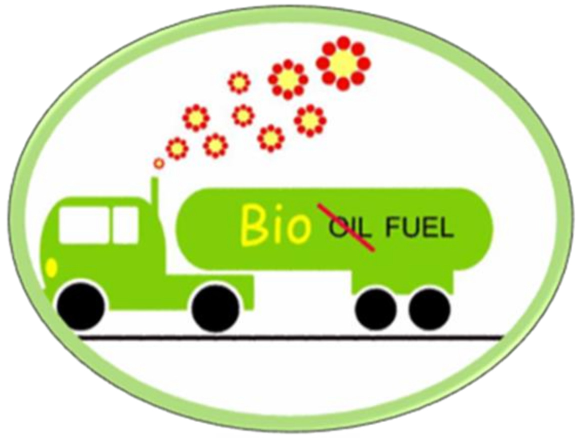
Biodiesel

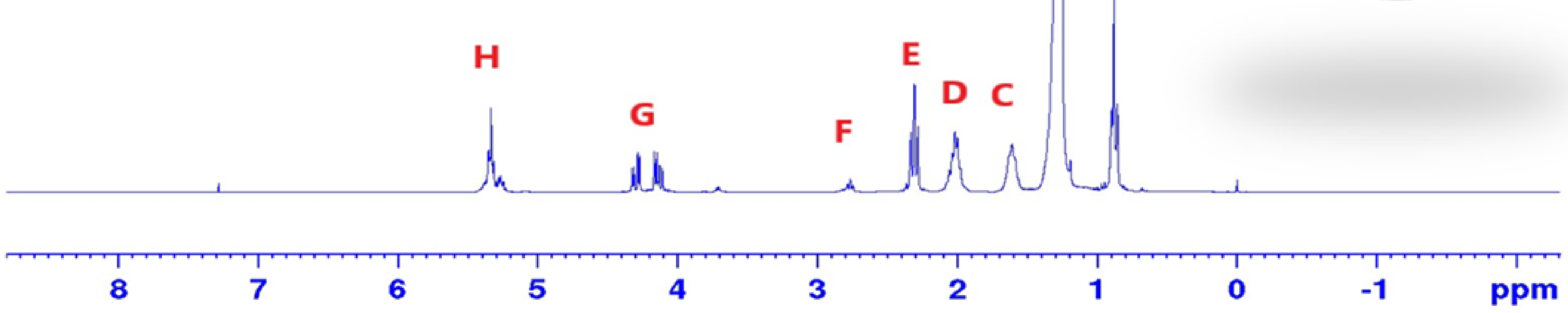
- biodiesel is now the most commonly used renewable energy source
- by definition a mixture of mono-alkyl esters of long chain fatty acids derived from different types of oils or animal fats
- commercially, it is produced by chemical transesterification of oil with alcohol in the presence of a catalyst
- previous studies showed that the combustion of biodiesel greatly reduced emissions
- based on the type of feedstock biodiesel can be categorised as first-, second- and third- generation

- feedstock analysis, monitoring the transesterification process, content of fatty acid methyl esters (FAME), distribution of saturated and nonsaturated fatty acids, triglycerides, monoglycerides and diglycerides , content of free fatty acids, iodine number, study biodiesel degradation

- A. Sander, M.A.Košćak, D.Kosir, N.Milosavljević,J.Parlov Vuković, L.Magić ; *Renewable Energy* **118** (2018) 752-760
- A. Sander, A. Petračić, J. Parlov Vuković, L. Husinec, *Separations* **7** (2020) 2; 22, 18.

δ/ppm	H-ATOM	OZNAKA
0,88	-CH3	A
1,29	-(CH2)n	B
1,65	(CH2)-β do -COO	C
2,01	(CH2)-α do –HC=CH-	D
2,31	(CH2)-α do -COO	E
2,78	HC=CH-CH2HC=CH	F
4,1-4,3	gliceridi	G
5,35	HC=CH	H

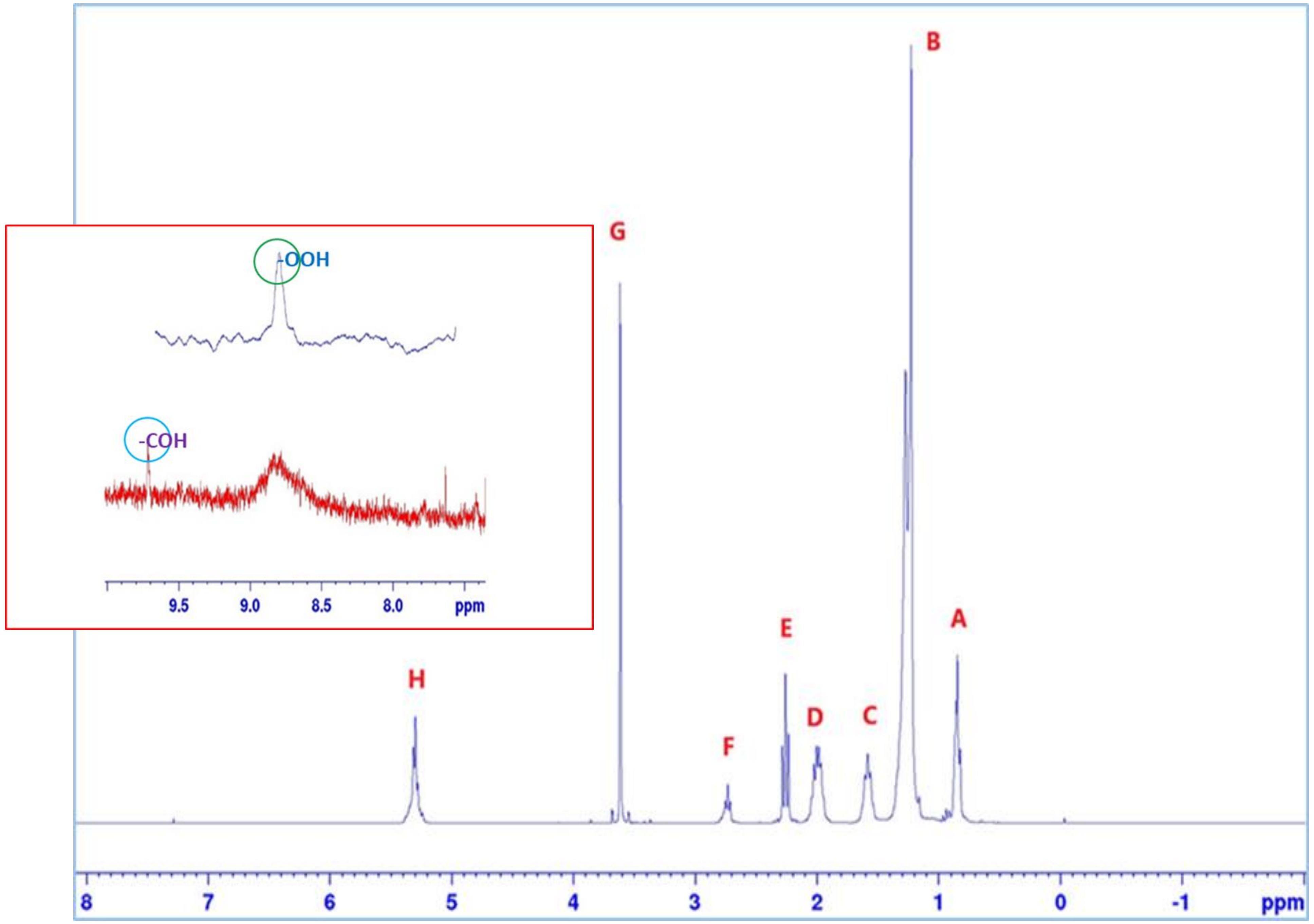




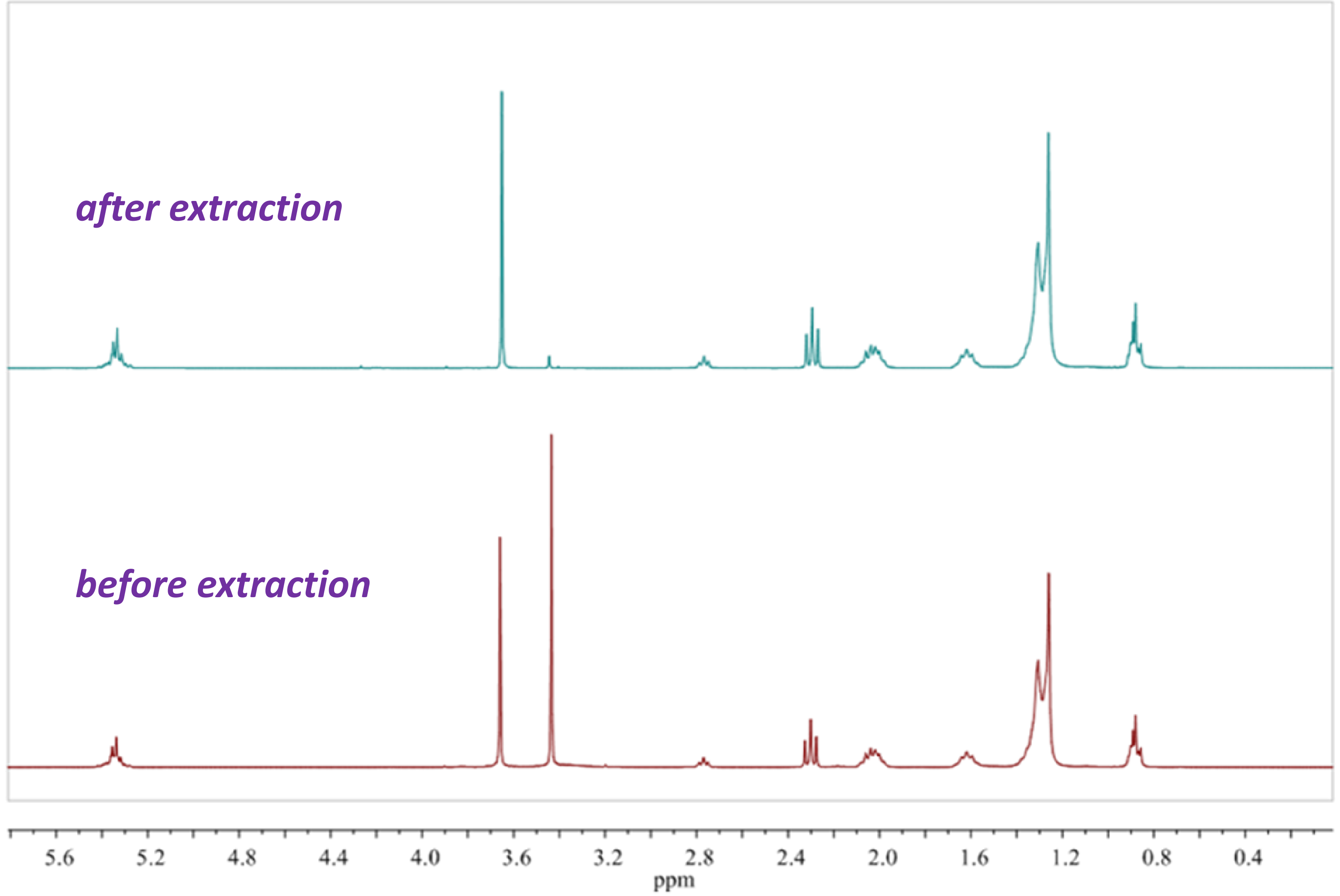
Typical ^{1}H NMR spectraum of UCO

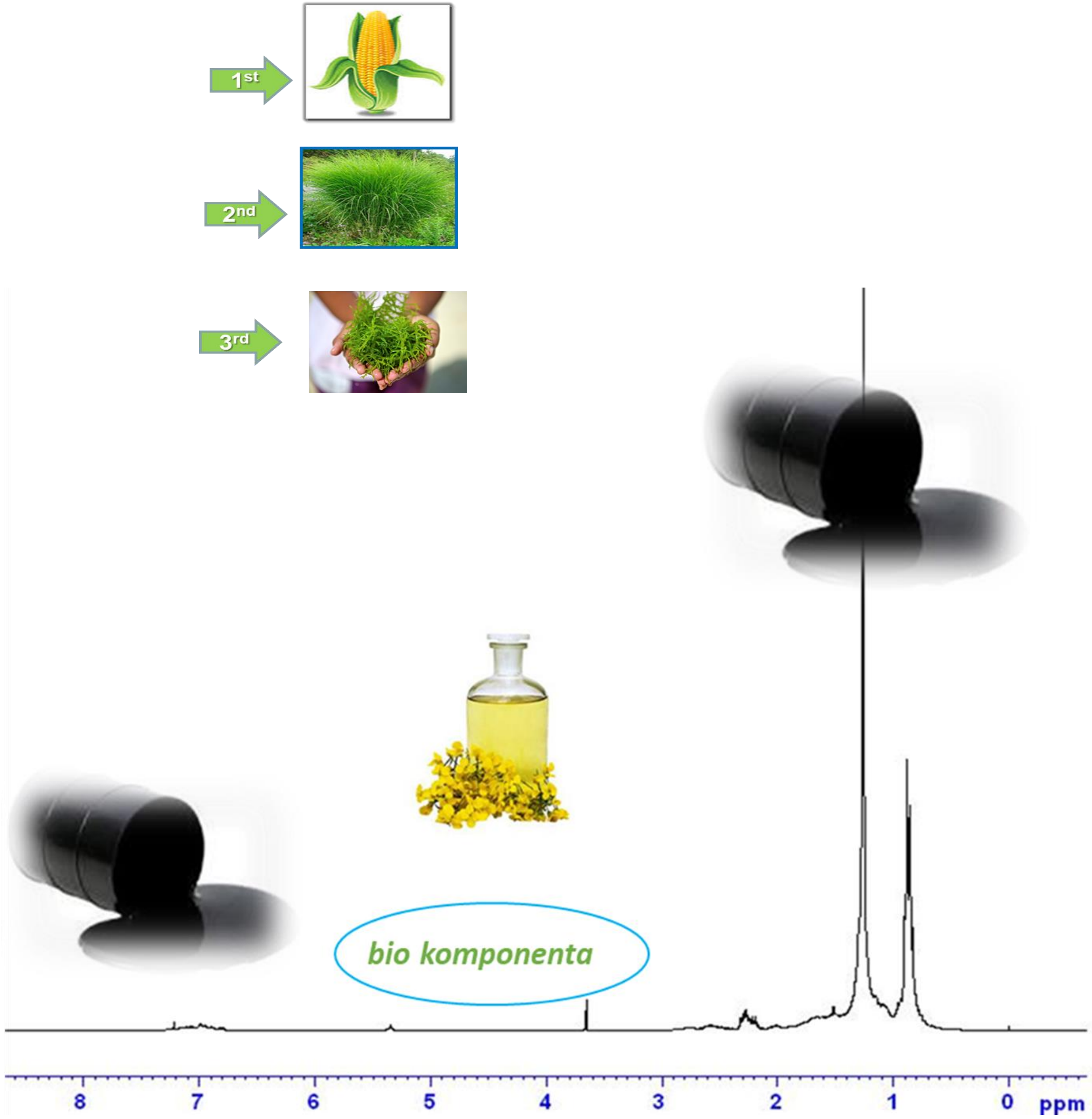
Biodiesel oxidation stability

- stability is one of the important criteria concerning fuel properties
- lower than common diesel fuel
- biodiesel obtained from different oil sources has different physico-chemical characteristics and chemical compositions
- during storage in the presence of air, heat, light and pro-oxidants oxidative degradation can occur, which leads to the formation of insoluble sediments that can negatively affect engine operability and reduces the quality of lubrication
- result of the oxidation: acids, aldehydes, ketones, peroxides and alcohols



- ^{1}H NMR spectra of biodiesel synthesized from coffee grounds before and after purification
- 10-15% w/w of coffee beans is oil
- coffee waste represents a great potential for biodiesel production

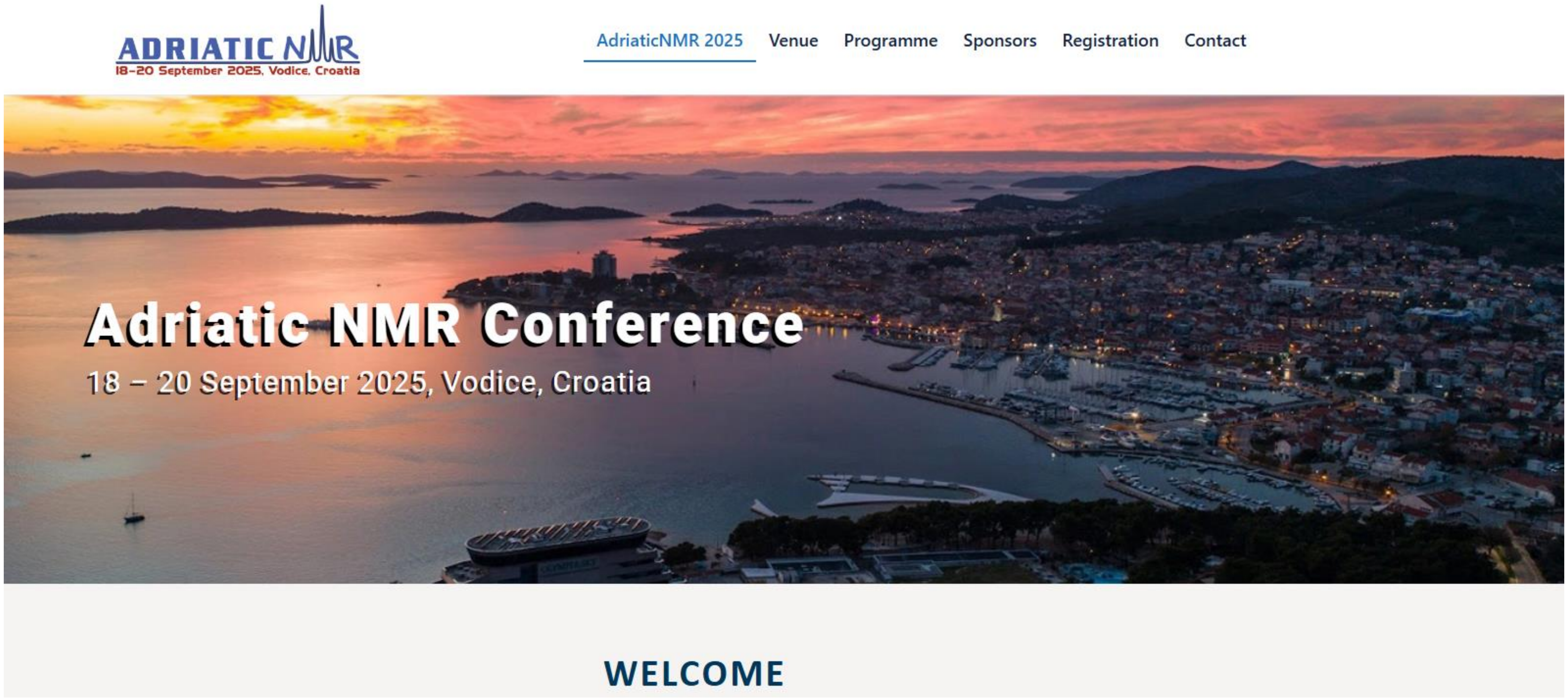




Typical ^{1}H NMR spectrum of diesel with biocomponent

Conclution

- ***many open questions in lignocellulose samples of NMR analysis still remain***
- ***due to a complex, insoluble and disordered lignocellulose biomass structure, further improvements in lignocellulosic sample preparation and characterization are expected***
- ***advancements in modern liquid- and solid-state NMR methods will enable further steps to clarify and elucidate unresolved structural data, polymorphism architecture, water-matrix interactions and packing of lignocellulosic material***
- ***since lignocellulosic biomass represents the most promising feedstock for the future biofuels and value-added products production, their detailed structural characterisation will be even more important***



Adriatic NMR Conference

18 – 20 September 2025, Vodice, Croatia

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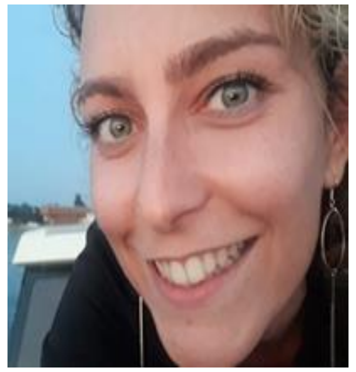
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Sunčica Roca


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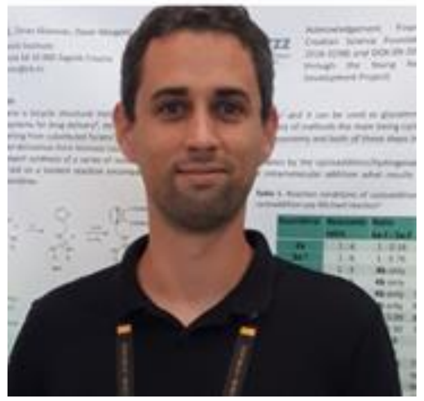
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K. Zangger
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CIL INA
centralni
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Thank you!!!