



# NMR-based plant metabolomics at Bordeaux Metabolome Facility: 3 short stories

Catherine Deborde

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# NMR-based plant metabolomics at Bordeaux Metabolome Facility: 3 short stories

Catherine Deborde

*Metabolome Facility, MetaboHUB-Bordeaux, Centre INRAE Nouvelle Aquitaine-Bordeaux,  
INRAE, UMR 1332 Biologie du Fruit et Pathologie, Centre INRAE Nouvelle Aquitaine-Bordeaux,  
F-33140 Villenave d'Ornon, France*



# > Metabolomic pipeline

## Metabolomic profiling & fingerprinting

### -1. Design

0. Sampling (dozens or hundreds of samples per experiment)

1. Extraction(s)



2. Targeted or Untargeted biochemical analyses

3. Data pre-treatment

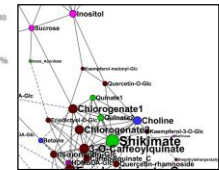
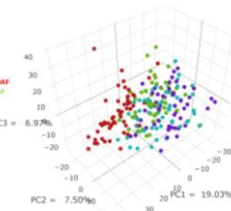
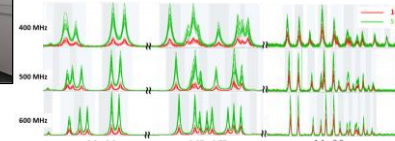
4. Data visualisation and mining

5. Data integration and fusion

6. Data & metadata sharing

0-5. Metadata

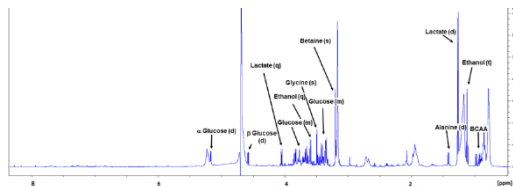
Sample	Metabolite1	Metabolite2	Metabolite3	...
S1	81024	25109	64349	
S2	83868	23798	62737	
S3	75038	19700	53531	
S4	32355	14219	22843	
S5	34559	11126	26513	
S6	24466	8831	19438	
S7	59931	13250	57774	
S8	63519	12885	55208	
...				



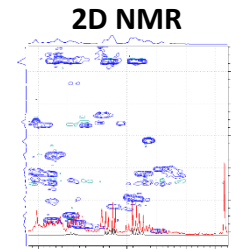
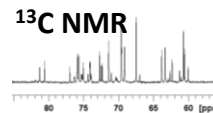
MetaboLights  
Data INRAE



## Structural analyses of selected samples



Metabolite identification



Databases (BMRB, SDBS, HMDB, ChemoX NMR Suite library...)

Commercial compounds

COSY, TOCSY, J-RES, HSQC, HMBC

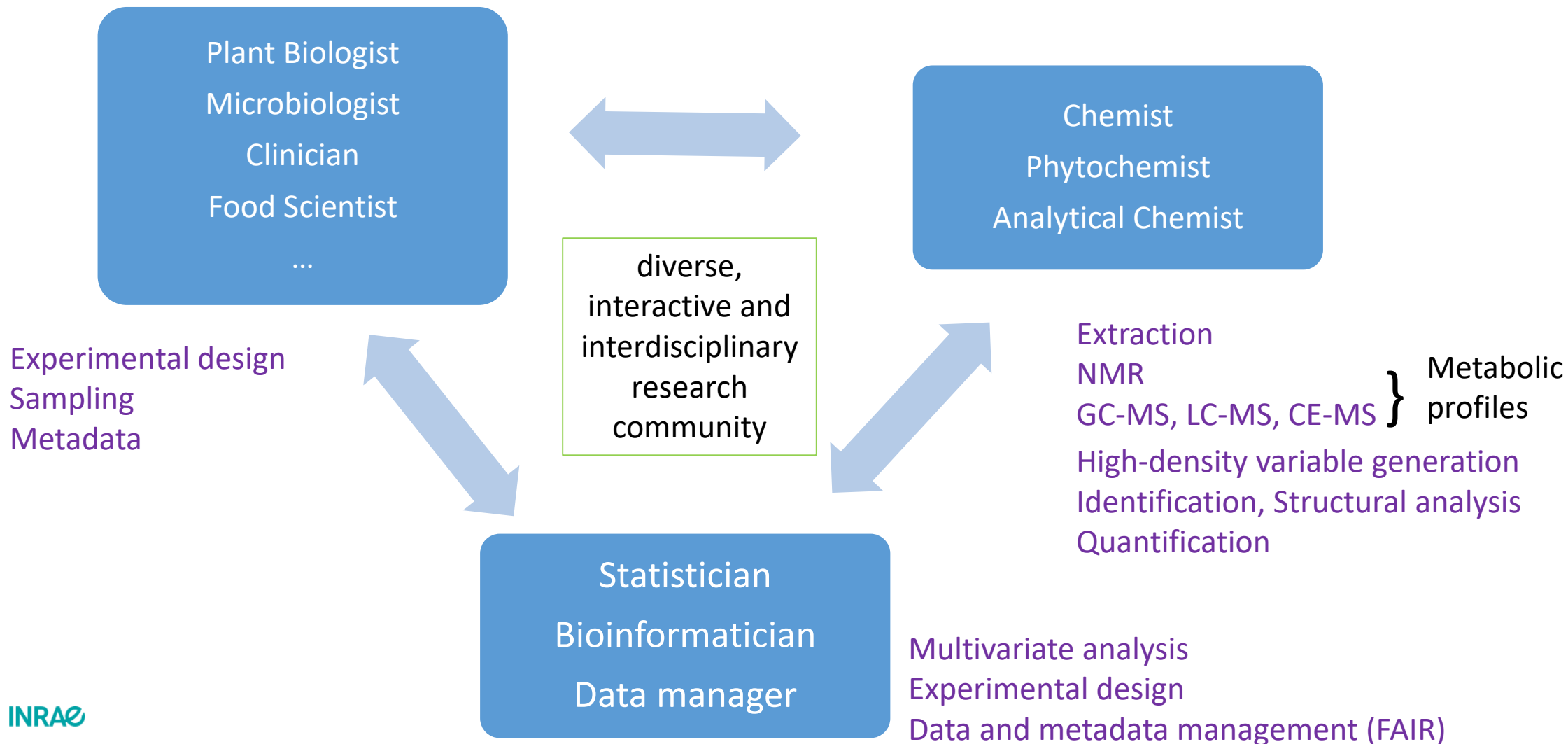
INRAE

CliMetabolomics Bordeaux

23-24/06/2022 / A. Moing

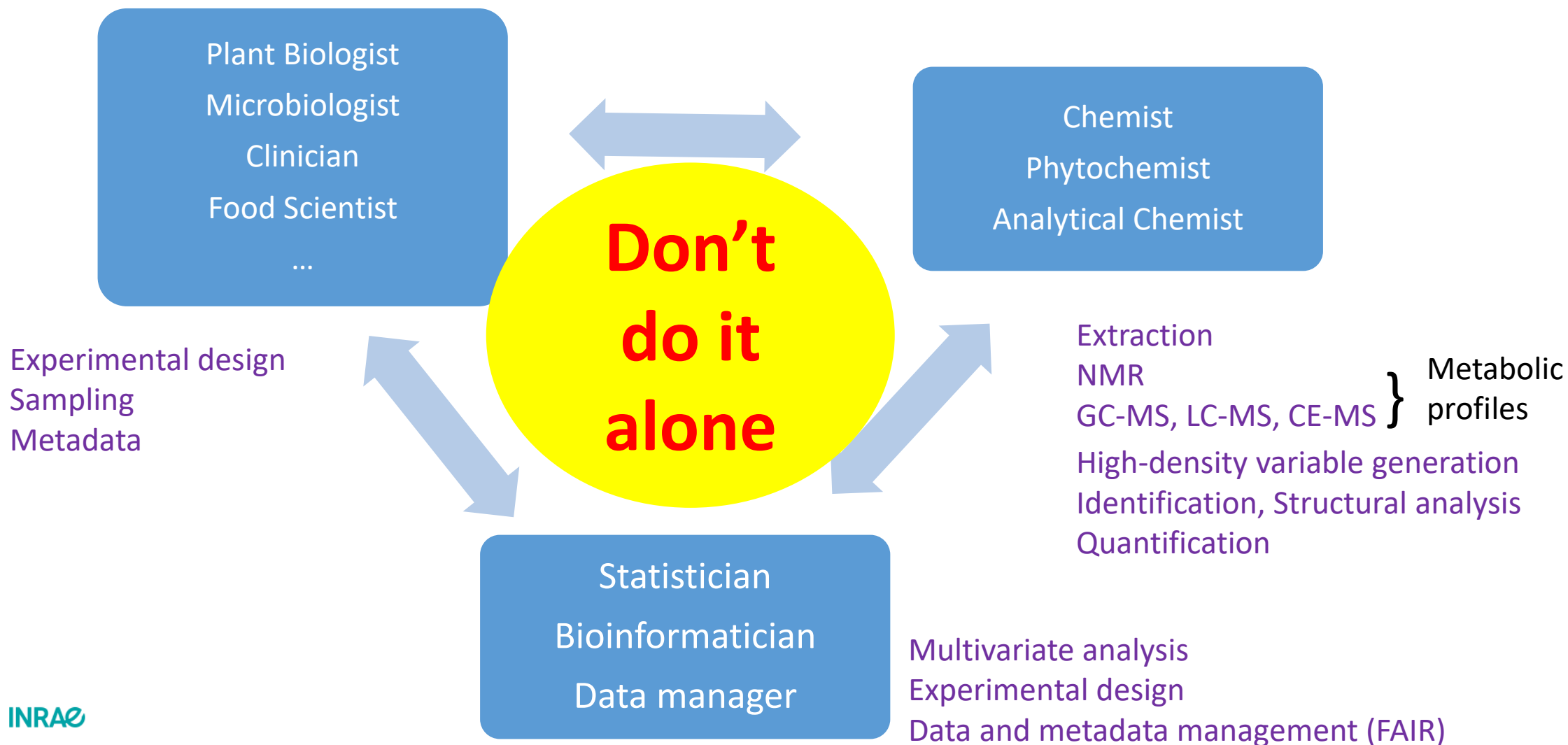
# Metabolomics ecosystem

## Expertise and Savoir-faire



# Metabolomics ecosystem

## Expertise and Savoir-faire



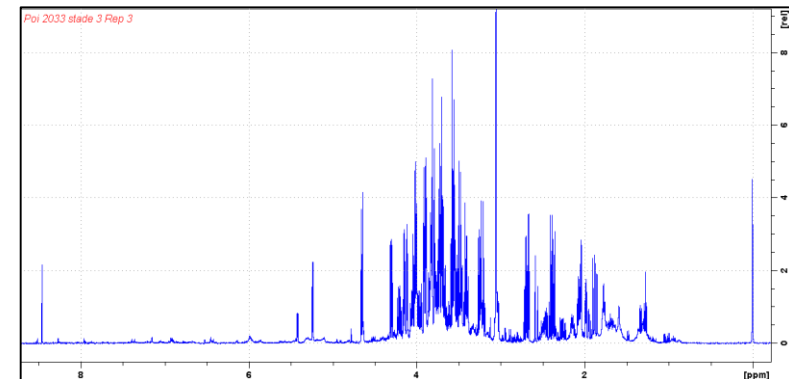
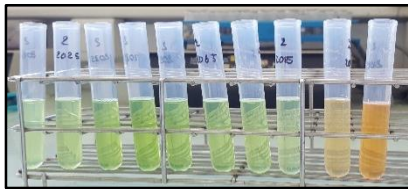
# Developments and applications in NMR-based plant metabolite profiling

**A selection of several developments and applications  
in NMR-based metabolite profiling of small molecules in plant extract  
performed with the 500 MHz spectrometer.**

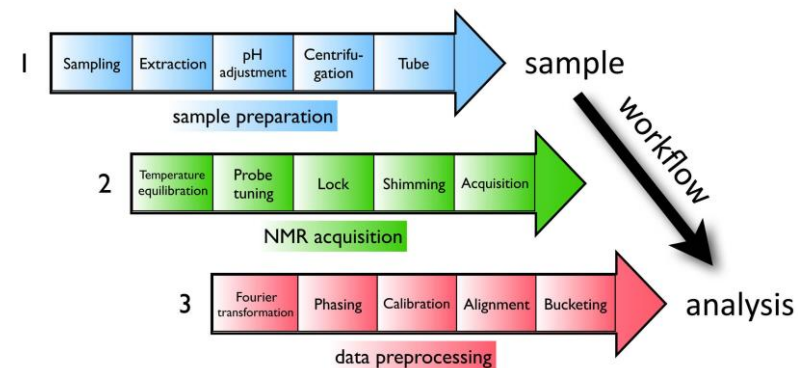


- 1 - Optimizing 1D  $^1\text{H}$ -NMR profiling of plant samples
- 2- Optimizing 1D NMR-based metabolomics processing
- 3- Case study in plant

## 1 - Optimizing 1D $^1\text{H}$ -NMR profiling of plant samples: extract preparation, standardization



# Optimizing 1D $^1\text{H}$ -NMR profiling of plant samples



- increasing the through-put by using deuterated solvents to avoid extract lyophilisation step (1 week to 0.5 day)
- minimizing uncontrolled variability in plant  $^1\text{H}$ -NMR profiling, by taking into account plant extract sample composition: pH and paramagnetic ion concentrations

## Plant solvent extraction:

50/50 (v/v) MeOD – 90 mM  $\text{K}_2\text{DPO}_4/\text{KD}_2\text{PO}_4$ / 11 mM EDTA- $d_{12}$ ,  $\text{pH}_{\text{apparent}}$  6.0

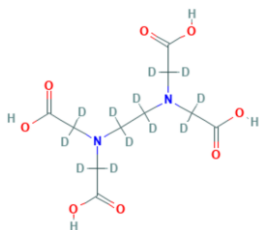
## Plant extract pH adjustment:

adjustment to  $\text{pH}_{\text{apparent}}$  6.0 with NaOD by means of BTpH Unit (Bruker)



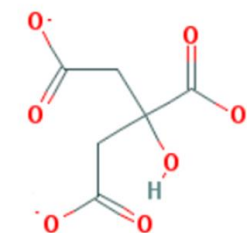
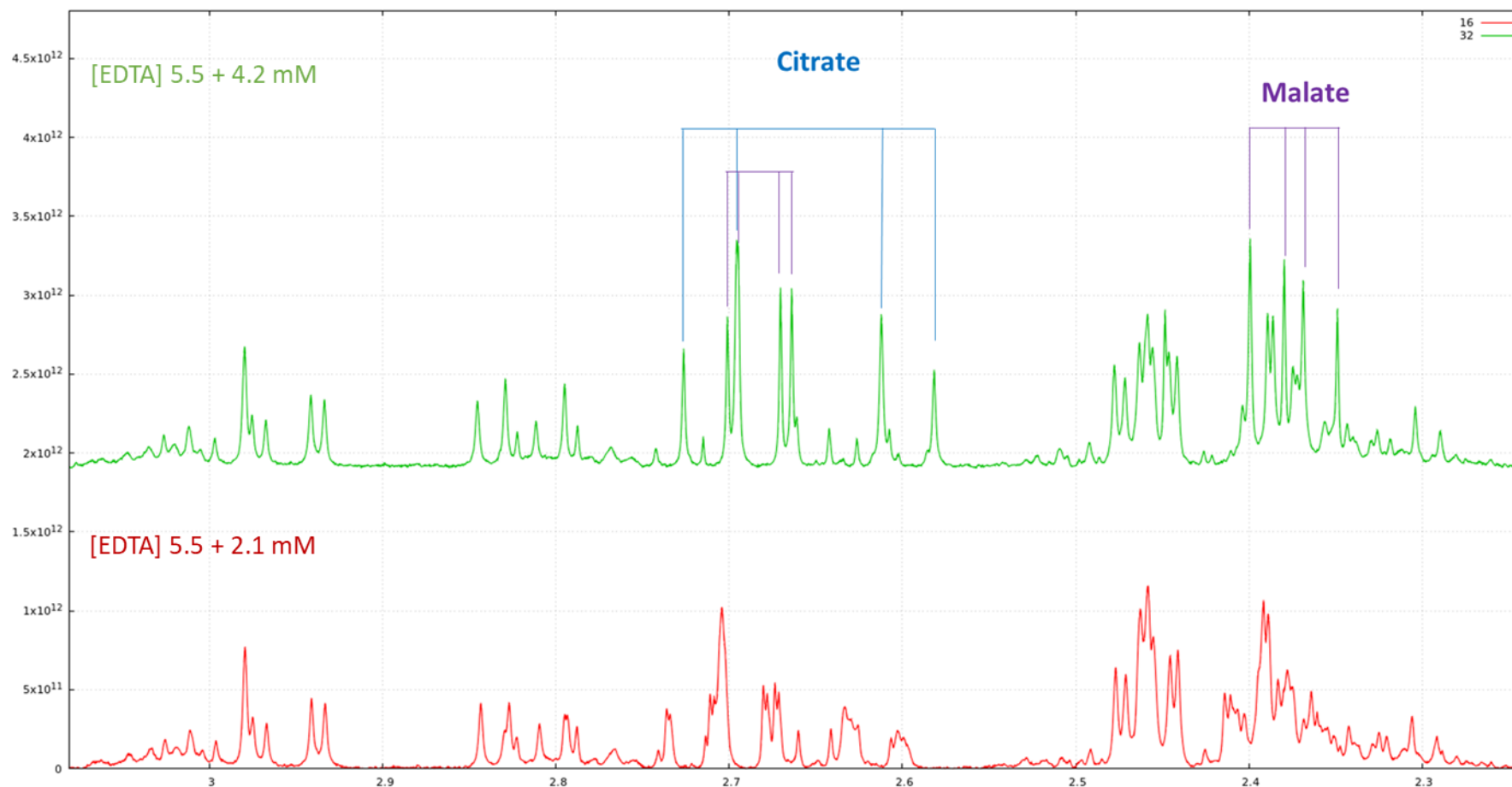


Effect of control of paramagnetic ions on  $^1\text{H}$  NMR spectra at 500 MHz – Wheat spikelet extract  
Citrate and Malate: two major organic acids in plant kingdom

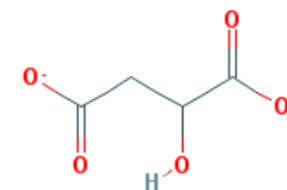


EDTA-*d*12

Chelation  
of  $\text{Mn}^{2+}$ ,  
 $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$



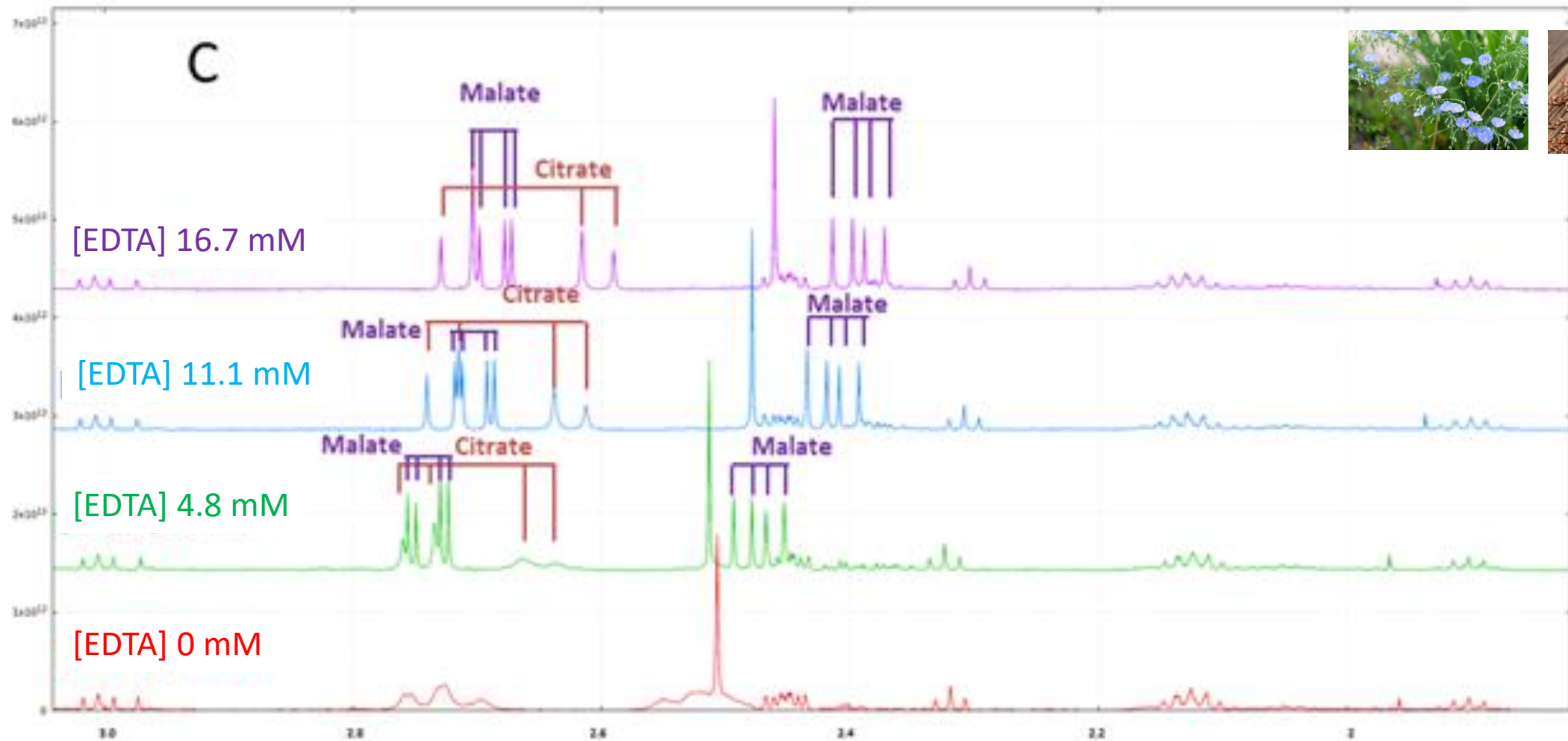
Citrate



Malate

## Effect of control of paramagnetic ions on $^1\text{H}$ NMR spectra at 600 MHz – Flax root extracts

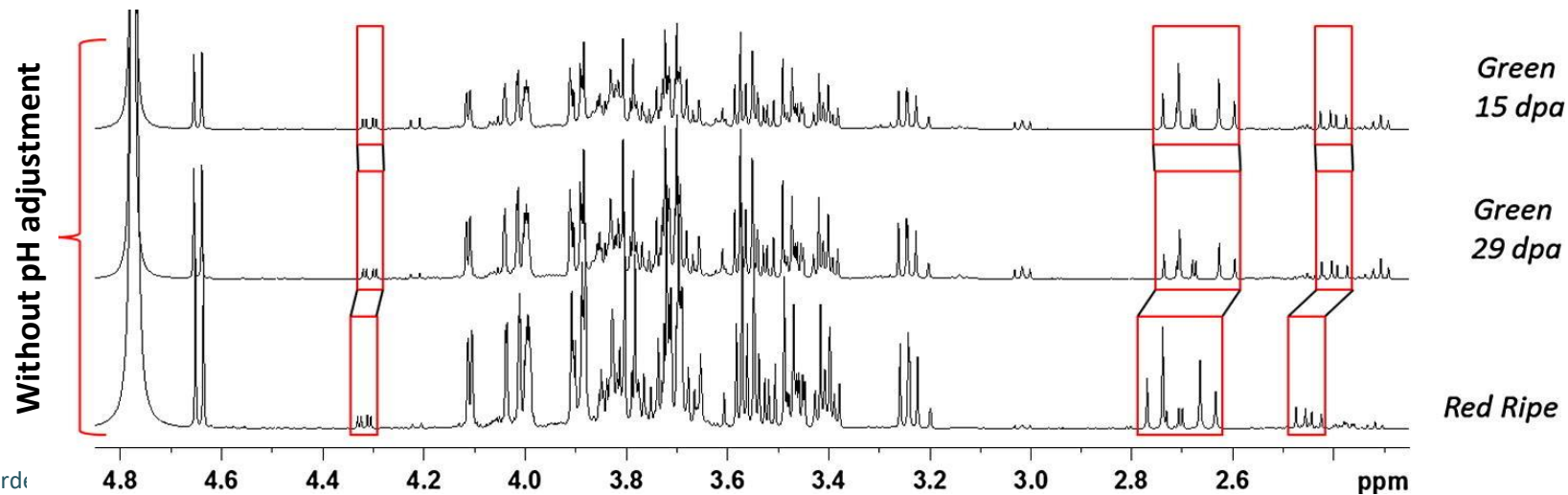
Without pH adjustment



JX Fontaine  
R Molinié  
F Mesnard

# Influence of pH adjustment step on $\delta$ of major organic acids in tomato fruit extracts

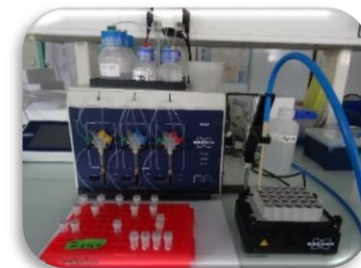
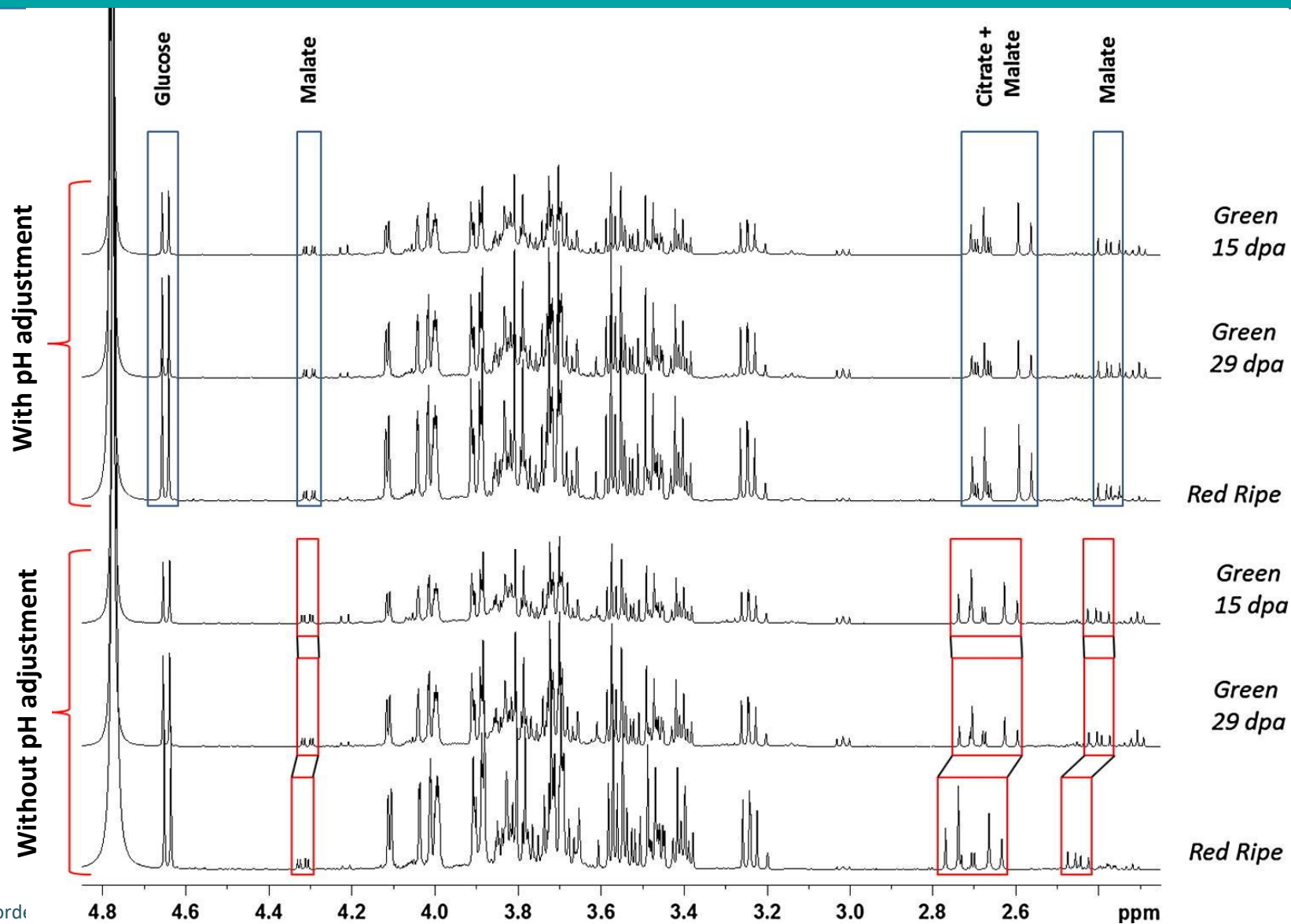
$^1\text{H}$  NMR spectra  
@ 500 MHz  
Tomato fruit  
extracts



dpa: day post anthesis







# Influence of pH adjustment step on $\delta$ of major organic acids in tomato fruit extracts

$^1\text{H}$  NMR spectra  
@ 500 MHz  
Tomato fruit  
extracts



dpa: day post anthesis

# pH range observed and EDTA-*d*12 needed for plant tissue extracts

Tissue	Number of plant samples (biological replicates)	Plant powder weight (mg DW)	Plant extract pH <sub>apparent</sub> before adjustment		pH <sub>apparent</sub> after adjustment	Volume of NaOD 1M added (μl)	Volume of DCl 1 M added (μl)	NMR tube EDTA- <i>d</i> 12 concentration (mM)
			Min	Max				
 Grape berry skin (veraison)	16	25 to 40	5.64	6.12	6.00 ±0.02	0.02 to 6.70	0.00 to 5.54	11.1
 White oak leaf	20		4.86	5.96				10.2
 Wheat spikelet	60		6.39	6.63				9.4
 Tomato pericarp (unripe or ripe)	36		5.08	6.16				11.8
 Wild tomato ripe fruit pericarp	24		4.82	5.81				11.5
 Sweet pepper ripe fruit	24		5.83	6.36				11.5
Flax root	80		5.70	6.79				16.0

- > Variability of plant extract pH<sub>apparent</sub> among species, tissues and intra experiment.
- > No universal EDTA concentration established for all plant tissues
- > EDTA optimal concentration should be determined experimentally for each specific plant tissues from an organ at a given developmental stage.

Adapted from Deborde *et al.*, **Optimizing 1D <sup>1</sup>H-NMR profiling of plant samples for high-throughput analysis: extract preparation, standardization, automation and spectra processing.** *Metabolomics* 2019, 15:28



# Stability of plant tissue extracts

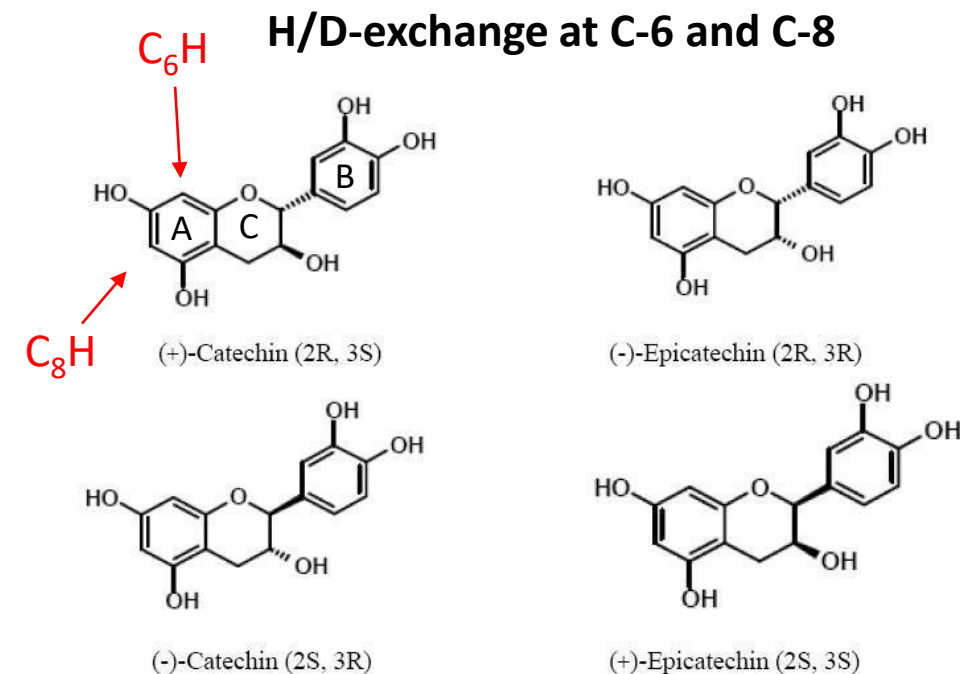
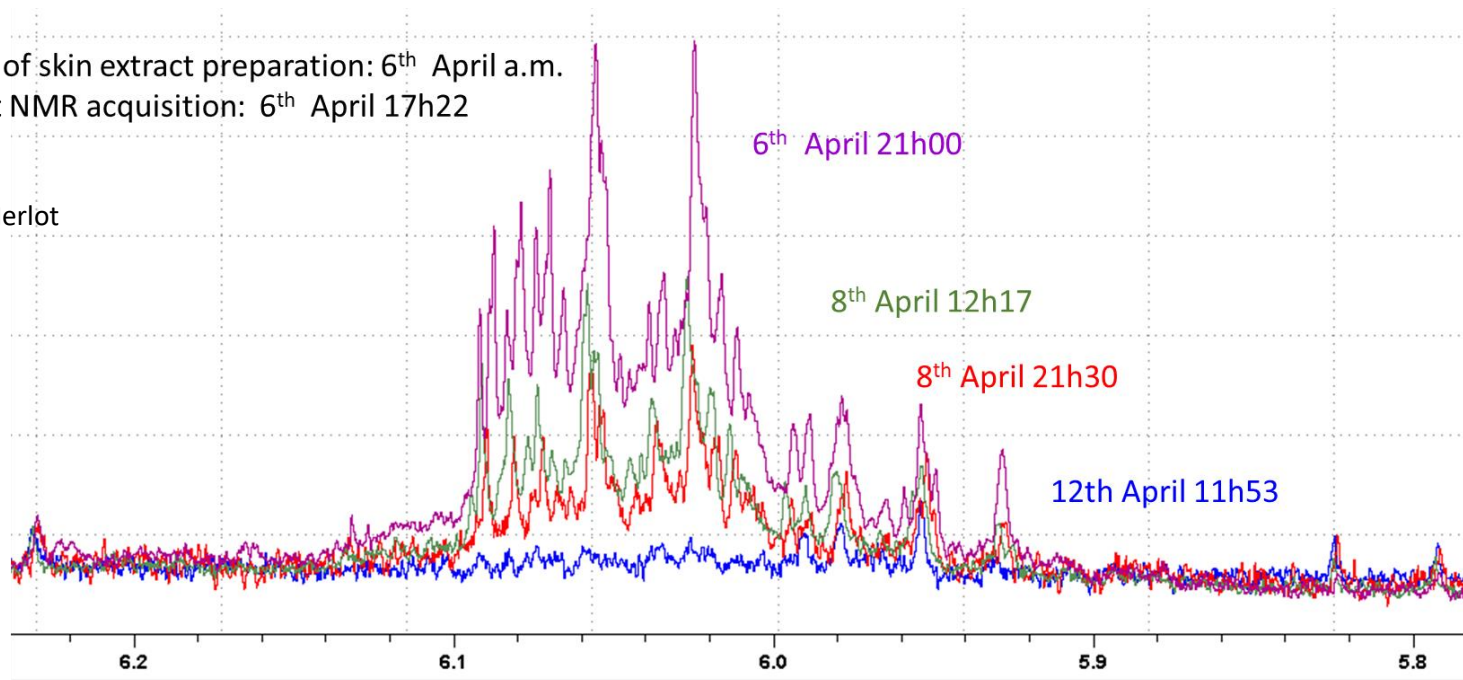
## ex: Grape Berry skin extract

On-going project on Grape Berry under heat stress

M. Cariou, M2 student

Day of skin extract preparation: 6<sup>th</sup> April a.m.  
First NMR acquisition: 6<sup>th</sup> April 17h22

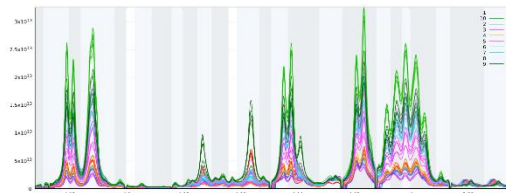
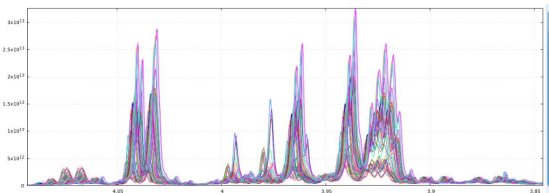
Merlot



-> catechin and epicatechin exhibit competitive deprotonation on B and A ring leading to a mixture of different monophenolates\*.

-> Plant extract stability should be checked experimentally for each specific plant tissues and at a given developmental stage – **impact on bucketing for untargeted approach.**

## 2- Optimizing 1D NMR-based metabolomics processing



$$M = \begin{matrix} \begin{matrix} \text{Variables} \\ \text{(spectral region area)} \end{matrix} \xrightarrow{\hspace{1cm}} \\ \begin{pmatrix} a_{1,1} & a_{1,2} & \cdots & a_{1,n} \\ a_{2,1} & a_{2,2} & \cdots & a_{2,n} \\ \vdots & \vdots & \ddots & \vdots \\ a_{m,1} & a_{m,2} & \cdots & a_{m,n} \end{pmatrix} \begin{matrix} \downarrow \\ \text{Samples} \end{matrix} \end{matrix}$$

Data matrix

**Spectra Visualisation** according to factors, *i.e.* tissue, organ, time, stress...  
**and Processing** Interactive processing  
 Variable Size and Intelligent Bucketing

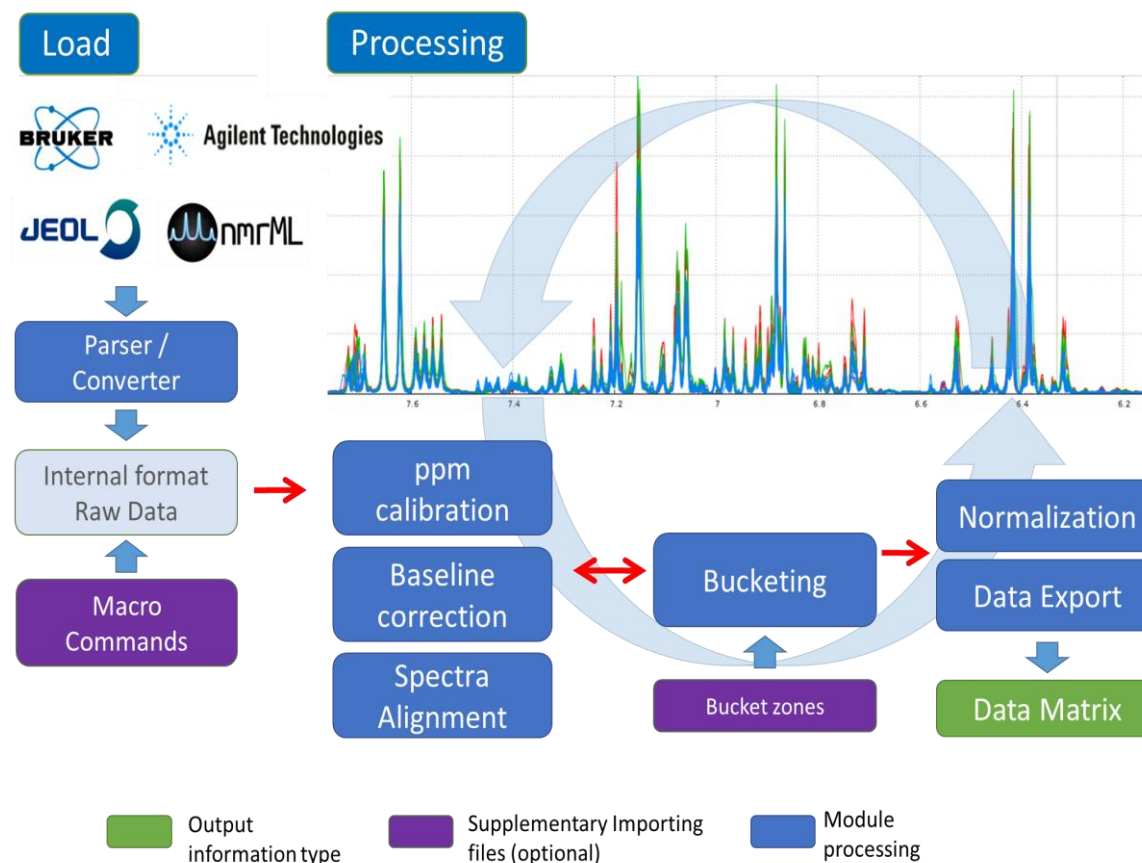
**Sample spectral series:**

Small < 50  
 Average 50 – 200  
 Large > 200



Jacob *et al.*, Metabolomics, 2017

[doi:10.1007/s11306-017-1178-y](https://doi.org/10.1007/s11306-017-1178-y)



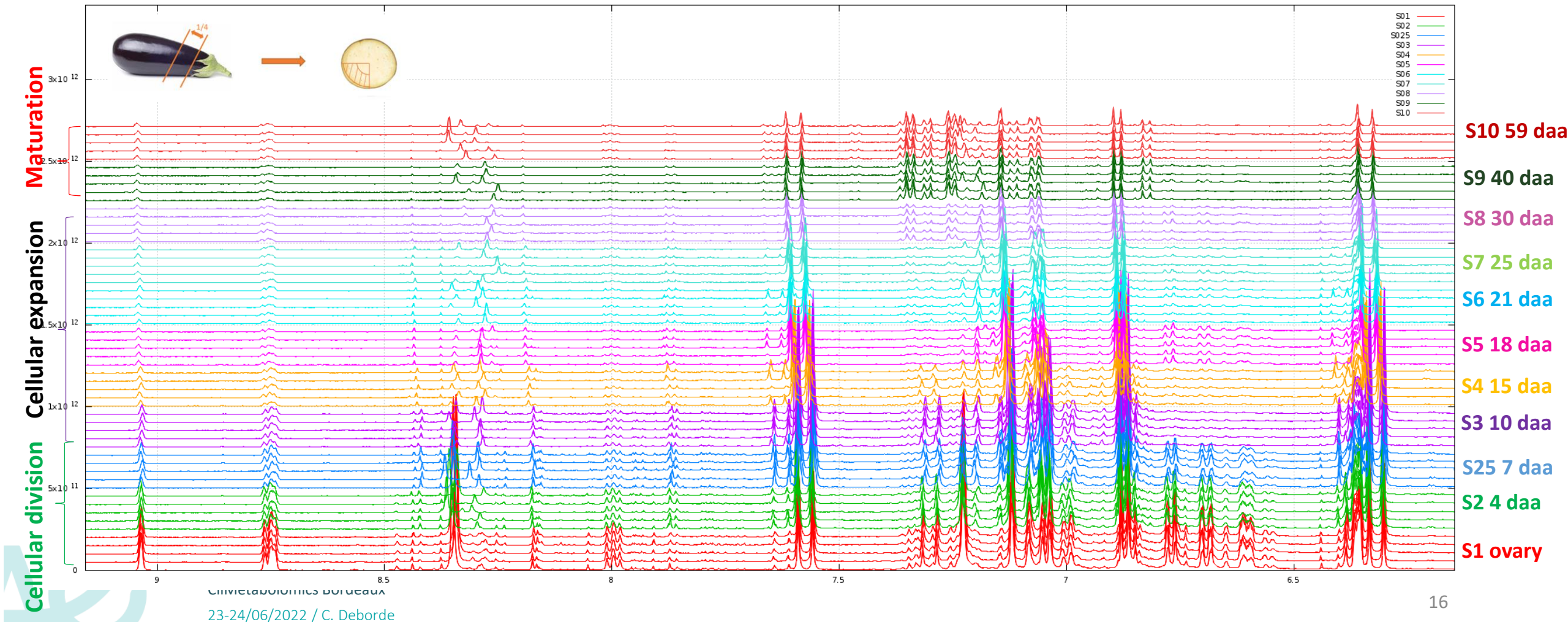
- baseline correction
- chemical shift calibration
- resonance alignment



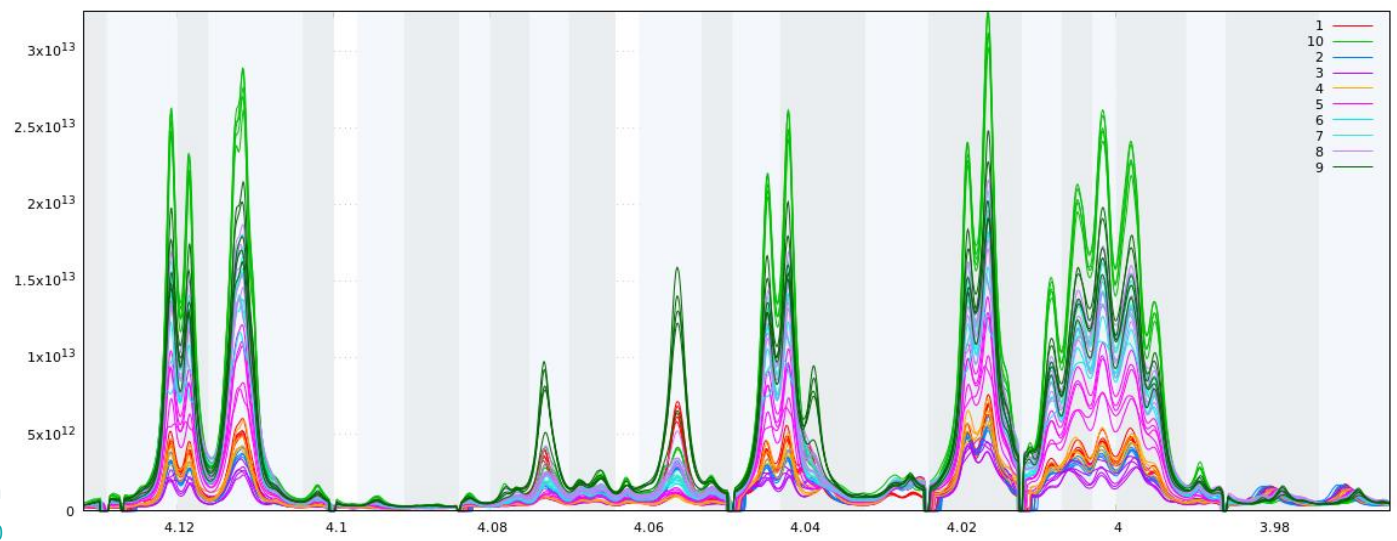
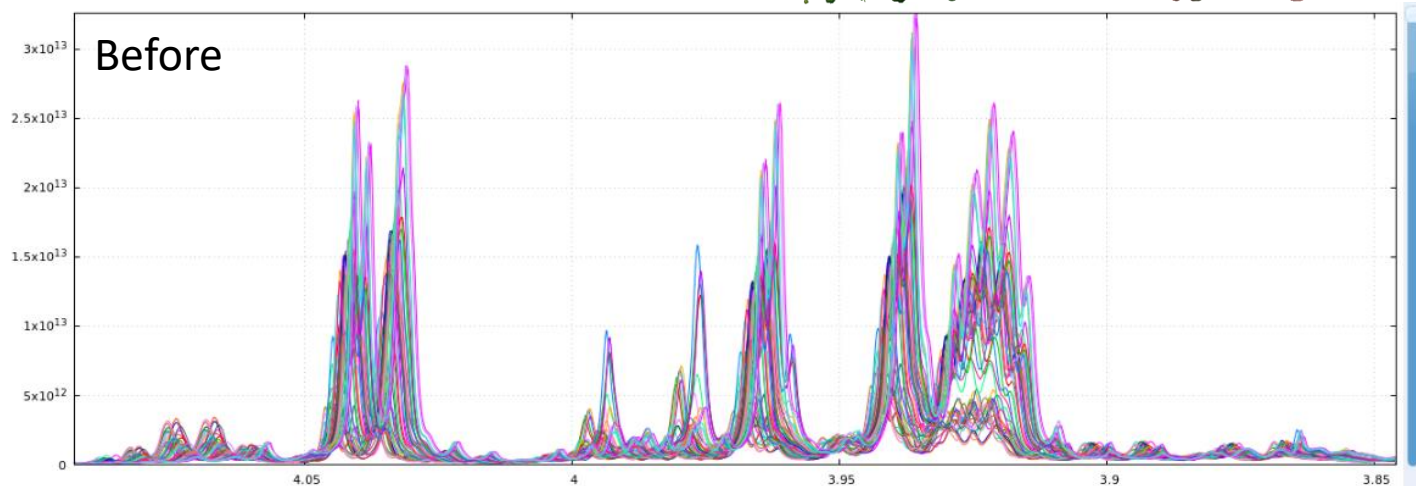
51 samples of Eggplant pericarp extracts  
11 developmental stages

according to developmental stages

A. Clavé M1 student



51 samples of pepper pericarp extracts  
10 developmental stages



Fingerprinting

Spectra preprocessing

PPM calibration

Global and local baseline  
corrections

Spectra alignment

Non-uniform bucketing

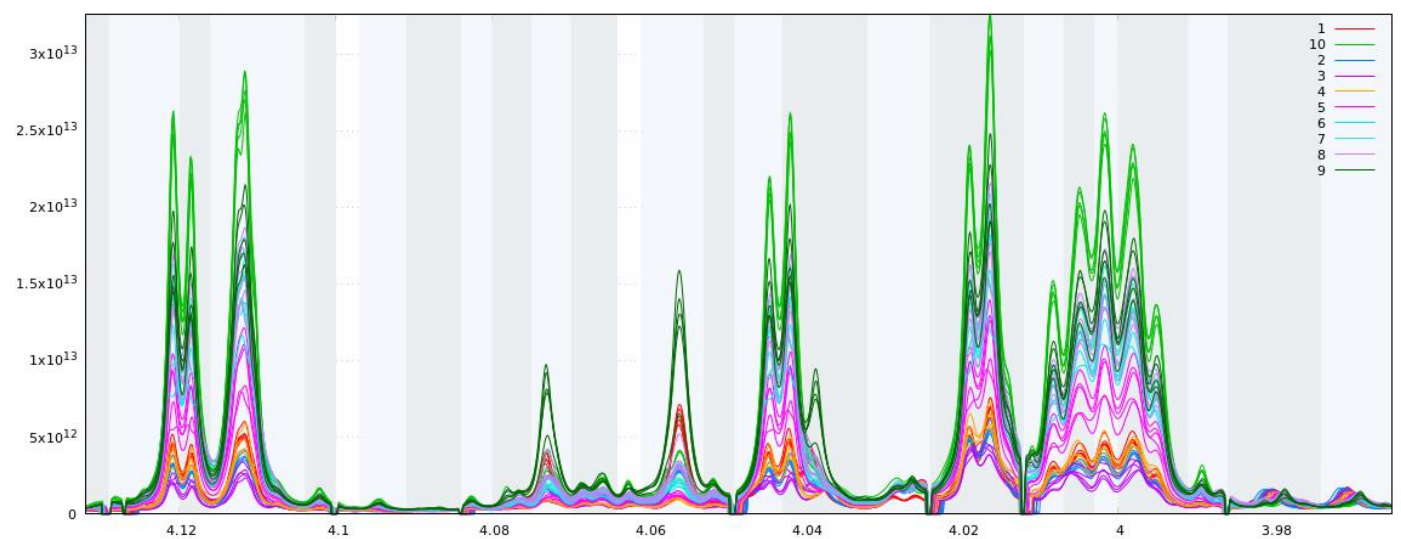
Signal-to-Noise ratio  
determination

Total Sum Normalization

51 samples of pepper pericarp extracts  
10 developmental stages



M. Batsale, M1 student



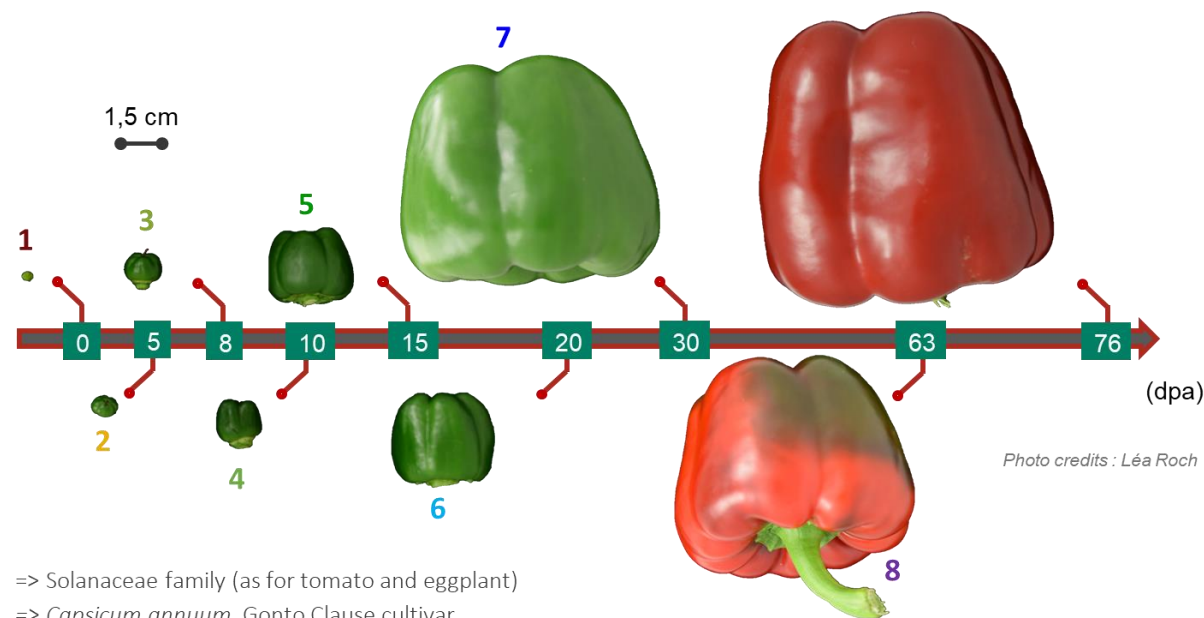
Variables  
(spectral region area or bucket)

$$M = \begin{pmatrix} a_{1,1} & a_{1,2} & \cdots & a_{1,n} \\ a_{2,1} & a_{2,2} & \cdots & a_{2,n} \\ \vdots & \vdots & \ddots & \vdots \\ a_{m,1} & a_{m,2} & \cdots & a_{m,n} \end{pmatrix}$$

Samples

Data matrix

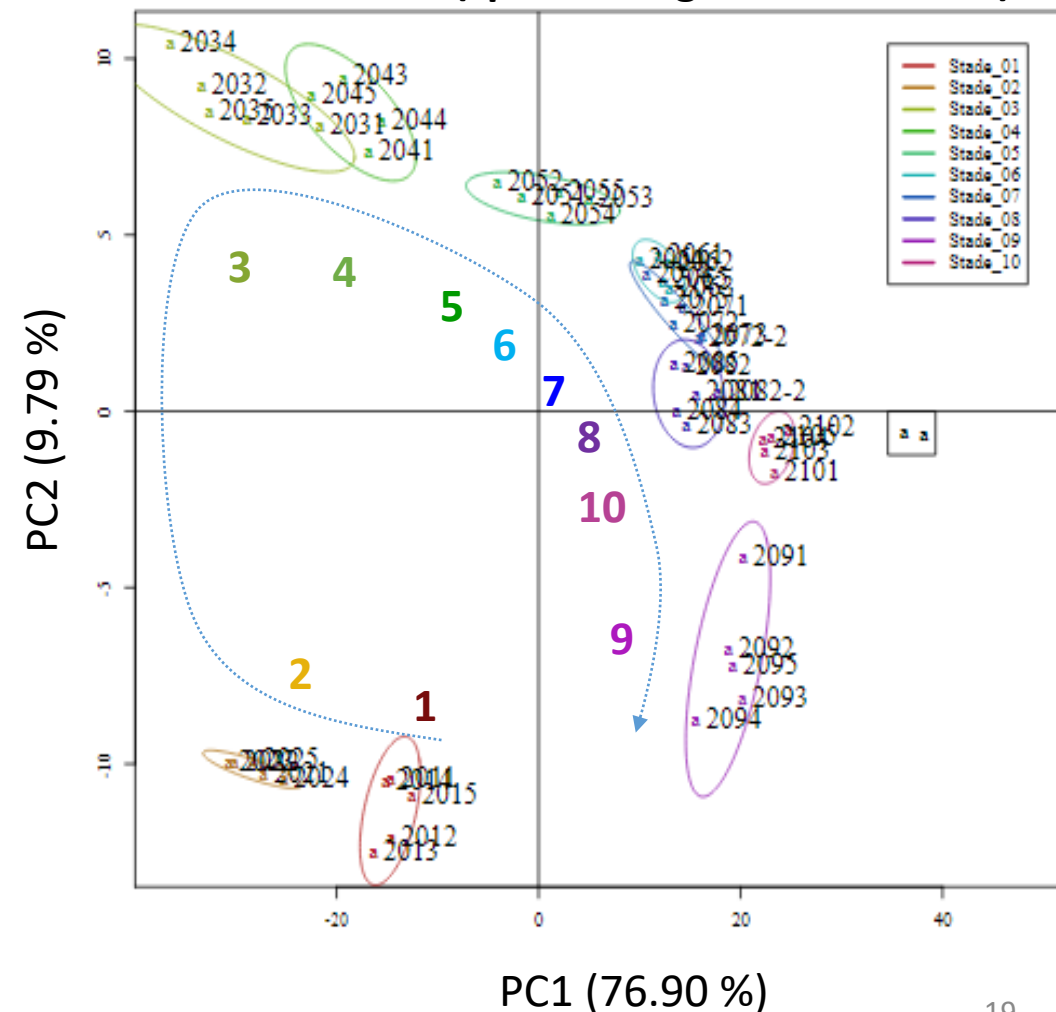
51 samples of pepper pericarp extracts  
10 developmental stages



=> Solanaceae family (as for tomato and eggplant)  
=> *Capsicum annuum*, Gonto Clause cultivar  
=> 10 development stages, at least 5 replicates for each stage 51 samples of pool of pericarp

⇒ Trajectory  
⇒ Earliest stages isolated





PCA analysis (CSN, UV scaling)  
with 496 variables (spectral regions or buckets)









## 3- Case study in plant



Species	Tissue or Organ	Biological question	Fingerprinting	Profiling	Collaboration
Grapevine <i>Vitis vinifera</i> 	Shoot Root Leaf Berry (skin, pulp)	Grafting Drought and Grafting Pathogen infection Heat Stress	X X X X	X X	International National Regional National
	Fruit Pericarp Leaf	Fruit metabolism Drought		X	National
<i>Buxus sempervirens</i> 	Leaf Root	Insect Herbivory	X	X	National
<i>Citrus</i>	Leaf Root	Pathogen infection Ploidy and grafting	X		International
Crops Wheat, Maize, Sunflower 	Spikelet Leaf Plantlets	Pathogen infection Stress & genotype selection	X	X	National

Species	Tissue or Organ	Biological question	Fingerprinting	Profiling	Collaboration
Grapevine <i>Vitis vinifera</i> 	Shoot	Grafting	X		International
	Root	Drought and Grafting	X	X	National
	Leaf	Pathogen infection	X		Regional
	Berry (skin, pulp)	Heat Stress	X	X	National
	Fruit Pericarp Leaf	Fruit metabolism Drought		X	National
<i>Buxus sempervirens</i> 	Leaf Root	Insect Herbivory	X	X	National
<i>Citrus</i>	Leaf Root	Pathogen infection Ploidy and grafting	X		International
Crops Wheat, Maize, Sunflower 	Spikelet Leaf Plantlets	Pathogen infection Stress & genotype selection	X	X	National

# NMR-based metabolite identification: major metabolites of kiwi (*Actinidia deliciosa*) fruit pericarp

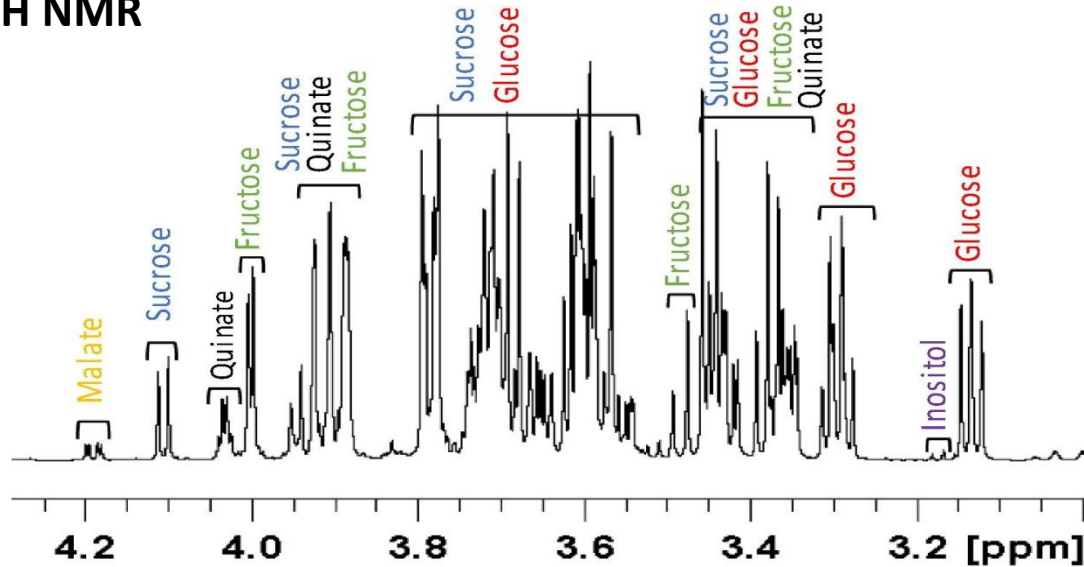


Léa Roch  
Thesis 2018

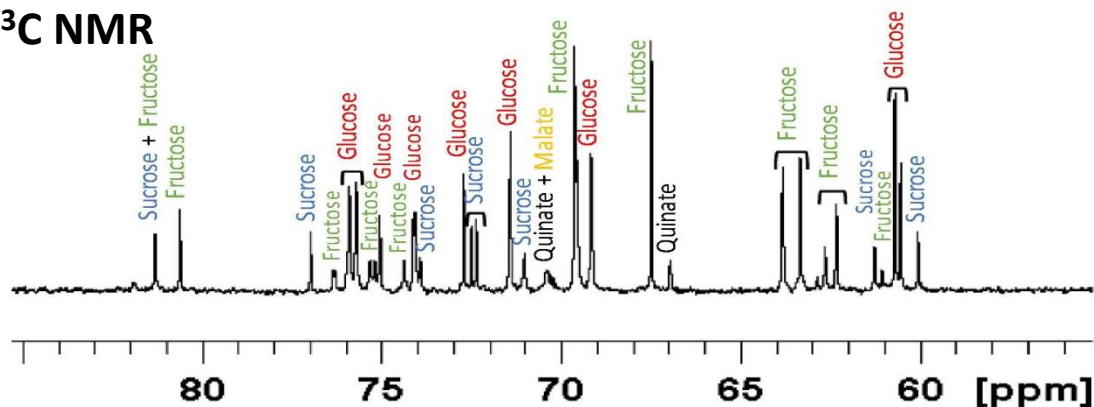
## NMR spectra of kiwi polar extracts



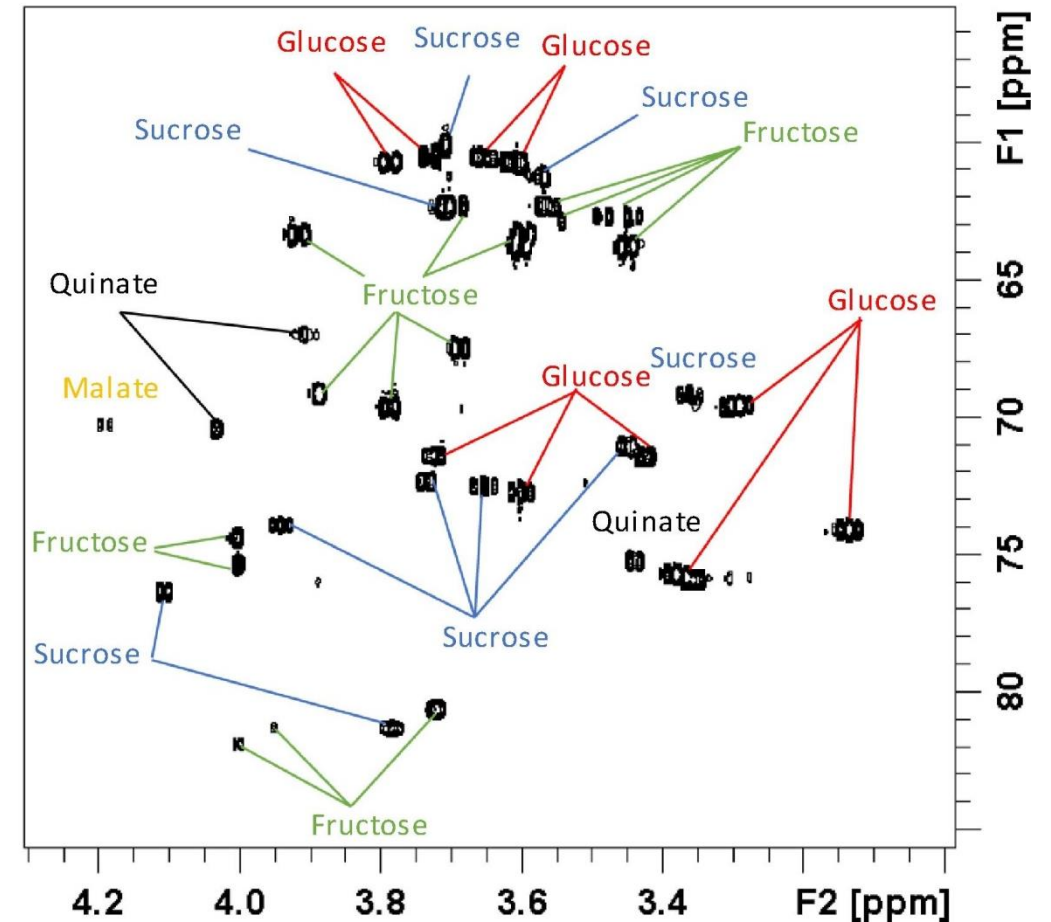
### $^1\text{H}$ NMR



### $^{13}\text{C}$ NMR



## 2D $^1\text{H}$ - $^{13}\text{C}$ HSQC @ 700 MHz

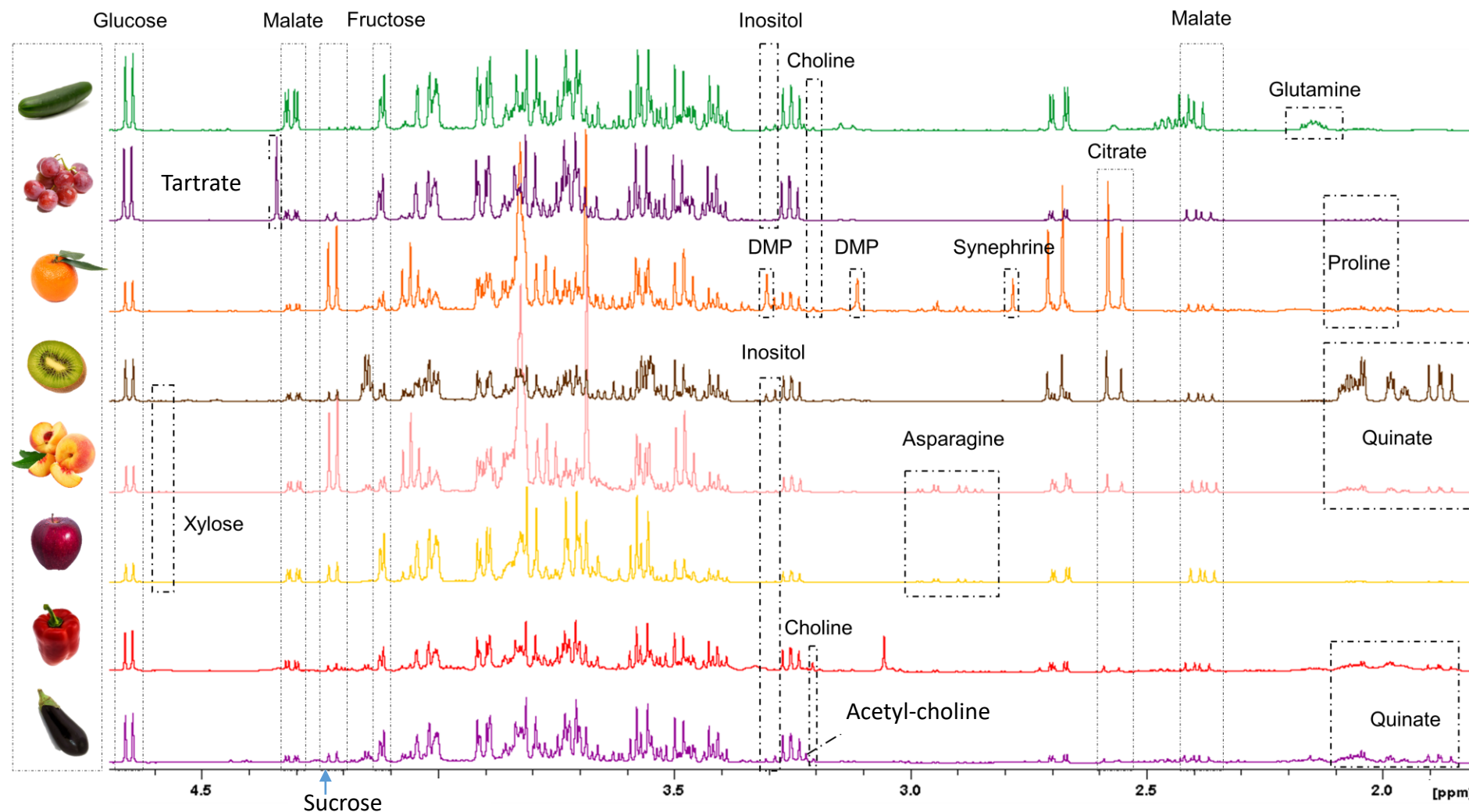




# $^1\text{H}$ NMR-based metabolic profiling of fruit: major metabolites common or specific to 8 species



$^1\text{H}$ -NMR spectra of polar extracts + dedicated NMR experiments for spectra annotation...



## Sugars and cyclitol

glucose, fructose, sucrose & inositol

*Kiwi* : raffinose

## Organic acids

citrate or malate

*Kiwi* : isocitrate

*grapevine* : tartarate





## Amino acids and N-containing compounds

alanine, valine & aspartate

*Eggplant* : histamine

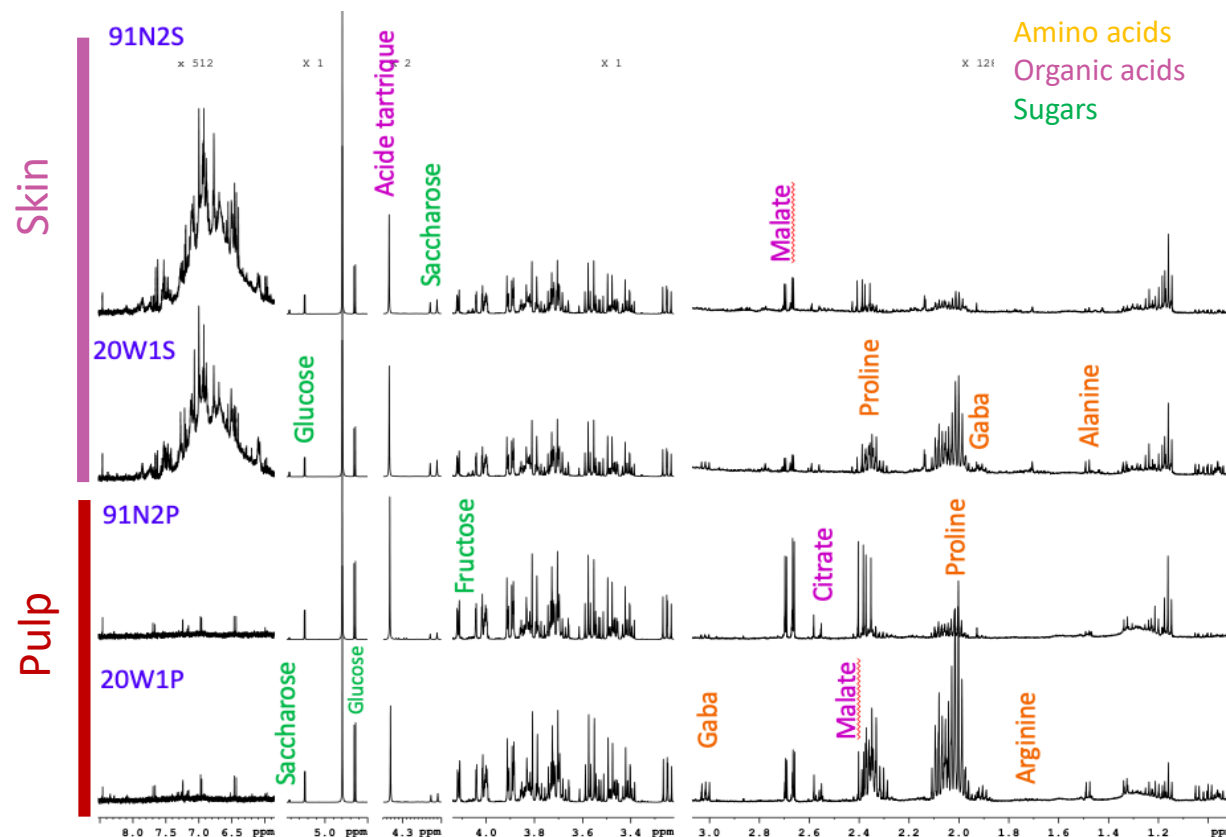
*Cucumber* : citrulline

*Clementine* : synephrine & dimethyl-proline

Species	Tissue or Organ	Biological question	Fingerprinting	Profiling	Collaboration
Grapevine <i>Vitis vinifera</i> 	Shoot	Grafting	X		International
	Root	Drought and Grafting	X	X	National
	Leaf	Pathogen infection	X		Regional
	Berry (skin, pulp)	Heat Stress	X	X	National
	Fruit Pericarp	Fruit metabolism		X	National
	Leaf	Drought			
<i>Buxus sempervirens</i> 	Leaf	Insect Herbivory	X	X	National
	Root				
<i>Citrus</i>	Leaf	Pathogen infection	X		International
	Root	Ploidy and grafting			
Crops Wheat, Maize, Sunflower 	Spikelet	Pathogen infection	X		National
	Leaf	Stress & genotype selection		X	
	Plantlets				



## Two examples of hot plots in the Saint-Emilion vineyard



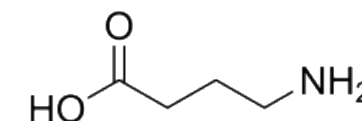
Fingerprinting with annotation of polar extract

-> Multivariate and univariate analyses

Hot plots :







+ Amino acids (Proline ; GABA)

- Organic acids (Malate ; Citrate)



GABA



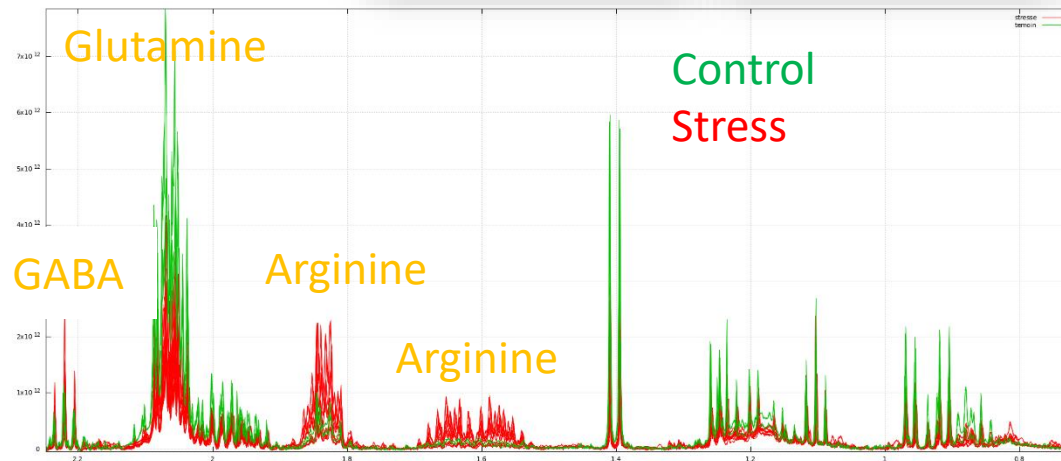
Species	Tissue or Organ	Biological question	Fingerprinting	Profiling	Collaboration
Grapevine	Shoot	Grafting	X		International
<i>Vitis vinifera</i>	Root	Drought and Grafting	X	X	National
 	Leaf	Pathogen infection	X		Regional
	Berry (skin, pulp)	Heat Stress	X	X	National
 	Fruit Pericarp	Fruit metabolism		X	National
	Leaf	Drought			
<i>Buxus sempervirens</i> 	Leaf	Insect Herbivory	X	X	National
	Root				
<i>Citrus</i>	Leaf	Pathogen infection	X		International
	Root	Ploidy and grafting			
Crops  Wheat, Maize, Sunflower	Spikelet	Pathogen infection	X	X	National
	Leaf	Stress & genotype selection			
	Plantlets				

# $^1\text{H}$ NMR-based metabolic profiling of vitis root under water stress in greenhouse



Profiling of root tip polar extract

-> Multivariate and univariate analyses



Water stress on root composition :

Amino acids :  
+ Arginine, GABA  
- Glutamine



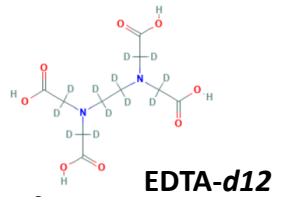
- NMR remains a useful tool for plant metabolomics approach


**Characterization of Organ, Tissue or whole organism composition & Metabolism,**



**Biomarkers of plant performance, of biotic or abiotic effect**

- real need to take into account sample chemical diversity  
for plant tissues specificity: pH and paramagnetic ions concentrations



-  **NMRProcFlow** fulfils the need for 1D NMR profiling processing of small to large sample series

- Don't do Metabolomics alone

# Thank you for your attention



BORDEAUX METABOLOME

## Bordeaux Metabolome Platform

A Moing (2006-2018 Facility leader)

D Jacob

M Lefebvre

M Maucourt

P Pétriacq (Facility leader since Nov 2018 )



D Rolin (2013-2020 Director)

F Jourdan (Director since 2021)

ML Lombard



## Financial Supports:

ANR CaDON, ANR FRIMOUS, ANR PARASOL  
MetaboHUB-ANR-11-INBS-0010 and INRAE



## Fruit Biology and Pathology Bordeaux

A Moing

L Roch (PhD Bordeaux 2018)

A Clavé (M1 Nantes)

M Batsale (M1 Bordeaux)

M Cariou (M2 Rennes 2022)

S Bernillon

Y Gibon (Metabolism Team leader)



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**INRAE**

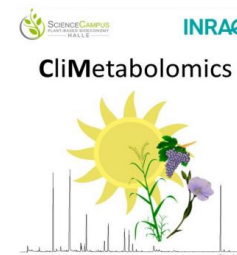


## BIOPI Amiens

JX Fontaine

R Molinié

F Mesnard (Director)



The organizing committee  
of CliMetabolomics

A-L Tissier



## Ecophysiology et Génomique Fonctionnelle de la Vigne

Bordeaux

N Ollat (Director)

G Hilbert

C Renaud

C Storme (L3 Montpellier)

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23-24/06/2022 / C. Deborde