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## Methods for analysis of procyanidins (condensed tannins) and their oxidation products in foods

Sylvain Guyot

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# Methods for analysis of procyanidins (condensed tannins) and their oxidation products in foods.



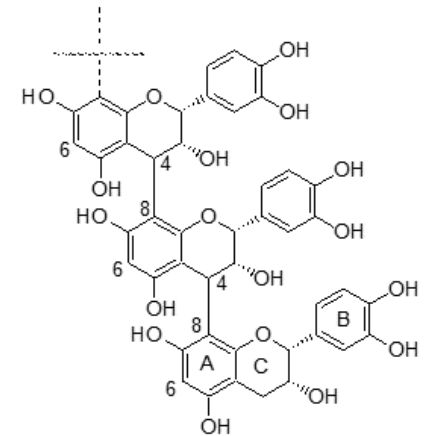
**Sylvain Guyot**

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Lab. BIA (Biopolymers, Interactions & Assemblies)

PRP Group (Polyphenols, Reactivity & Process), 35653 Le Rheu

(sylvain.guyot@inra.fr)



# Our research group

PRP Group  
(Polyphenols, Reactivity & Process)



Rennes INRA Center



Near the city of  
RENNES in  
Brittany, in France

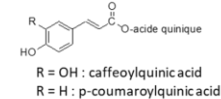
Food sciences and Analytical Chemistry  
related to fruit processing...in particular  
**apple** and **ciders** making

...the contribution of **polyphenols**  
regarding their (bio)**reactivity** during  
processing

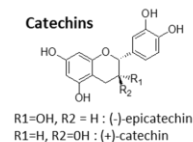
...with a specific focus on **the oxidation**  
**reactions** involving polyphenols



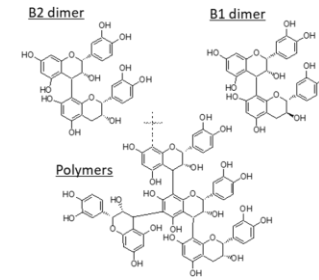
### Hydroxycinnamic acids



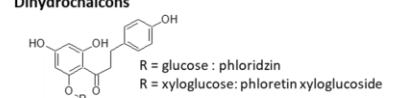
### Catechins



### Procyanidins and condensed tannins



### Dihydrochalcones



Apple polyphenols



# *Procyanidins and condensed tannins are very widespread in edible plant*

## Fruits, berries and derived products

Grapes, juices & wine



Apples, juices & ciders



Dates, berries,



chocolate

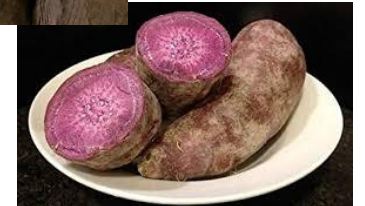
## In nuts, beans, peas & lentils



## In rapeseed



## In roots and tubers (ex: Yam)



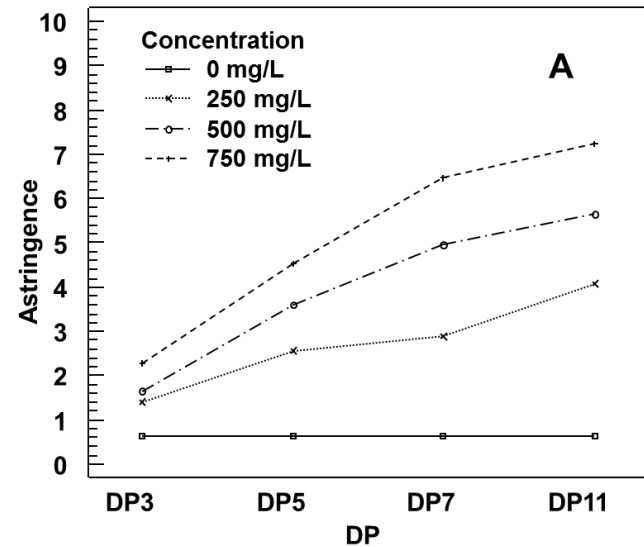
They largely contribute to polyphenol intake

# Procyanidin and their contribution to the taste

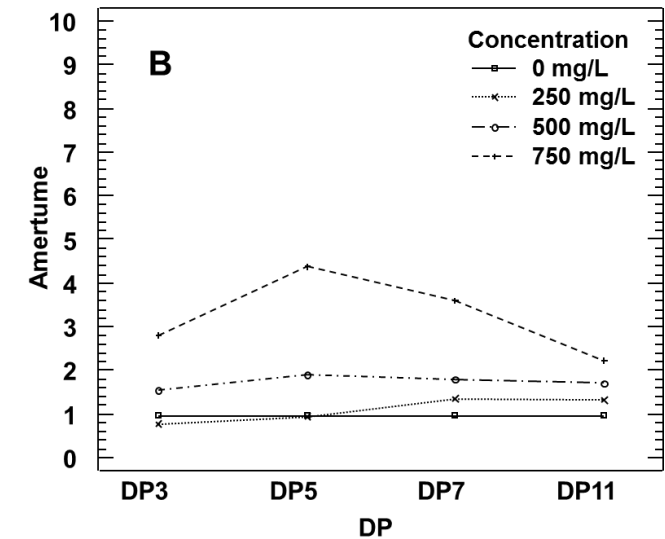
Example : in French ciders model solutions



**Astringency**



**Bitterness**



DP = Degree of polymerisation of tannins  
(related to the size of the molecules)



From Symoneaux et al., 2014

*Their structures and concentrations influence the balance between bitterness & astringency*

# Procyanidins and condensed tannins are contributors to “polyphenols health effects” in foods

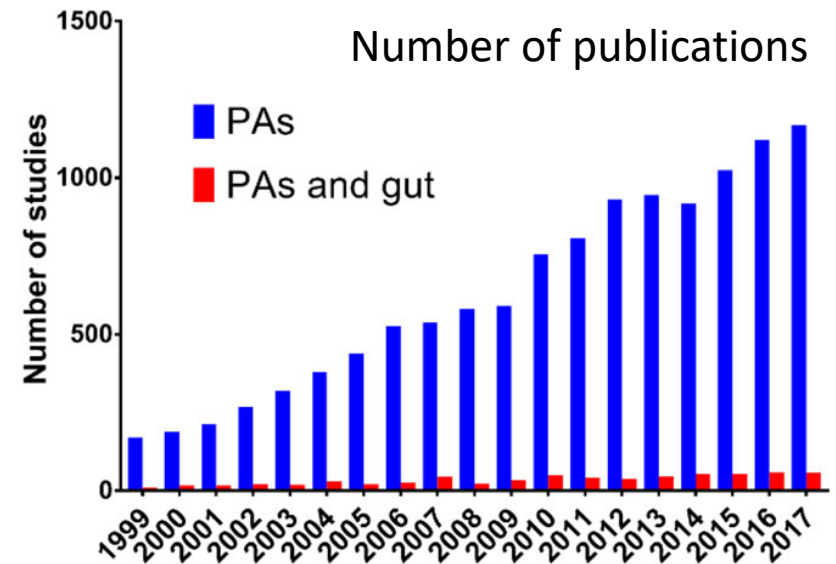
- **Antioxidant** in food and in the tractus (protection against lipid & protein oxidation)
- **Antimicrobial** (ex antibacterial activity of cranberry procyanidins)
- **Cardioprotective, antiinflammatory and neuroprotective** effects

## Rethinking the Mechanism of the Health Benefits of Proanthocyanidins: Absorption, Metabolism, and Interaction with Gut Microbiota

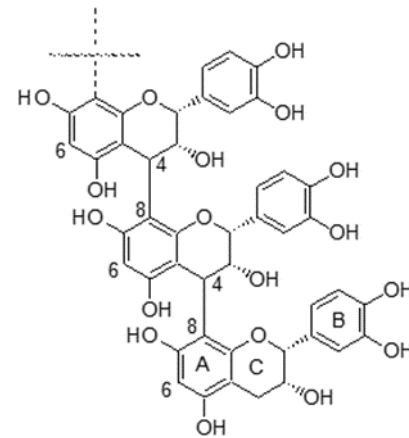
Wenyang Tao , Yu Zhang, Xuemin Shen, Yanping Cao, John Shi, Xingqian Ye, and Shiguo Chen 

Vol.18,2019, doi: 10.1111/1541-

ComprehensiveReviewsinFoodScienceandFoodSafety



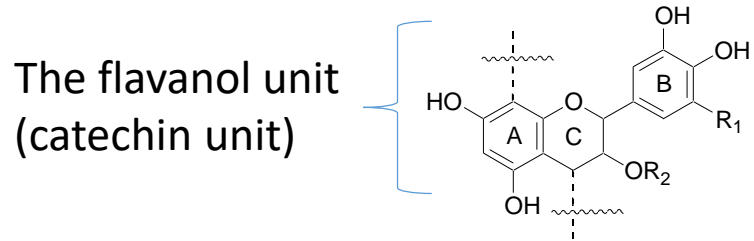
The understanding of their specific role (as bioactive compounds) in food is still limited .....



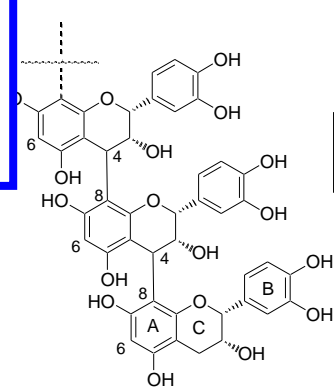
.....because of the difficulty for their extraction, structural analysis and quantification.

# Diversity of molecular structures of proanthocyanidins

Flavanol oligomers, from dimers.....to very large polymers



**Colourless and highly water soluble**

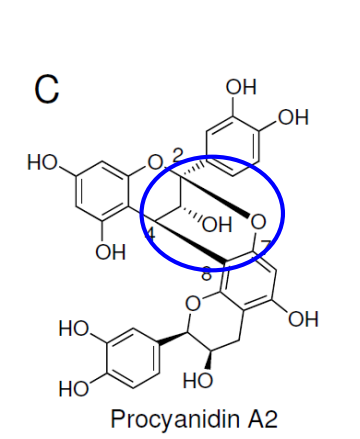
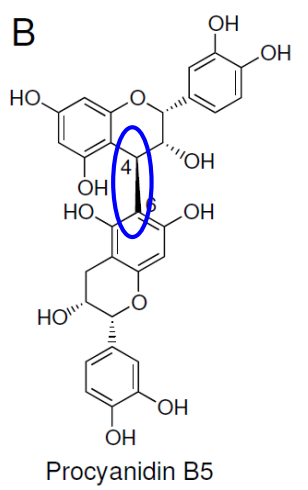
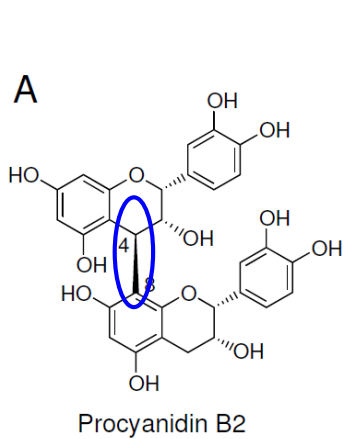


DP : degree of polymerisation

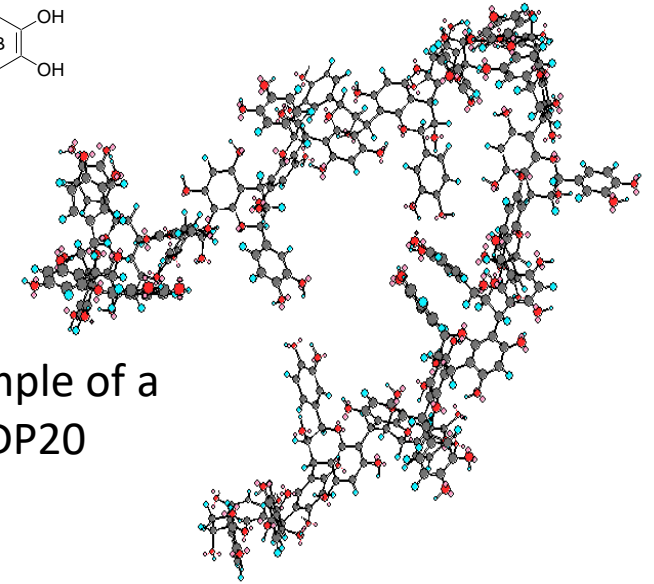
**oligomers**

**B-type** (one Interflavan Linkage)

**A-type** (two Interflavan Linkage)



**Polymers**



Example of a DP20

DPn > 40-60 kDa





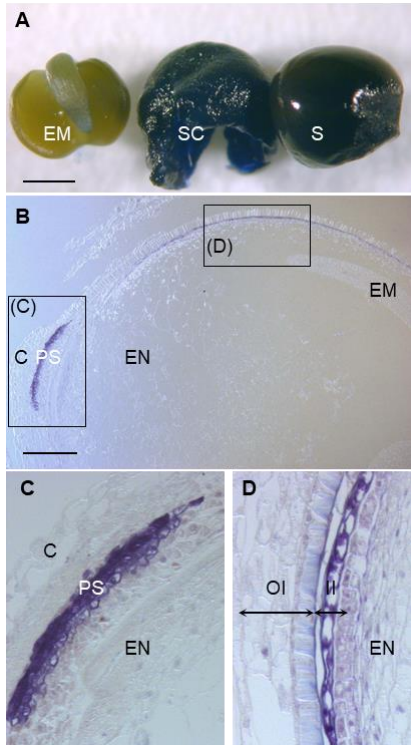
# Their direct observation in plant tissues

Photonic microscopy....

....coupled to **DMCA staining : specific reaction of Flavanols with aldehydes**



In Rapeseed during the growth of the seeds

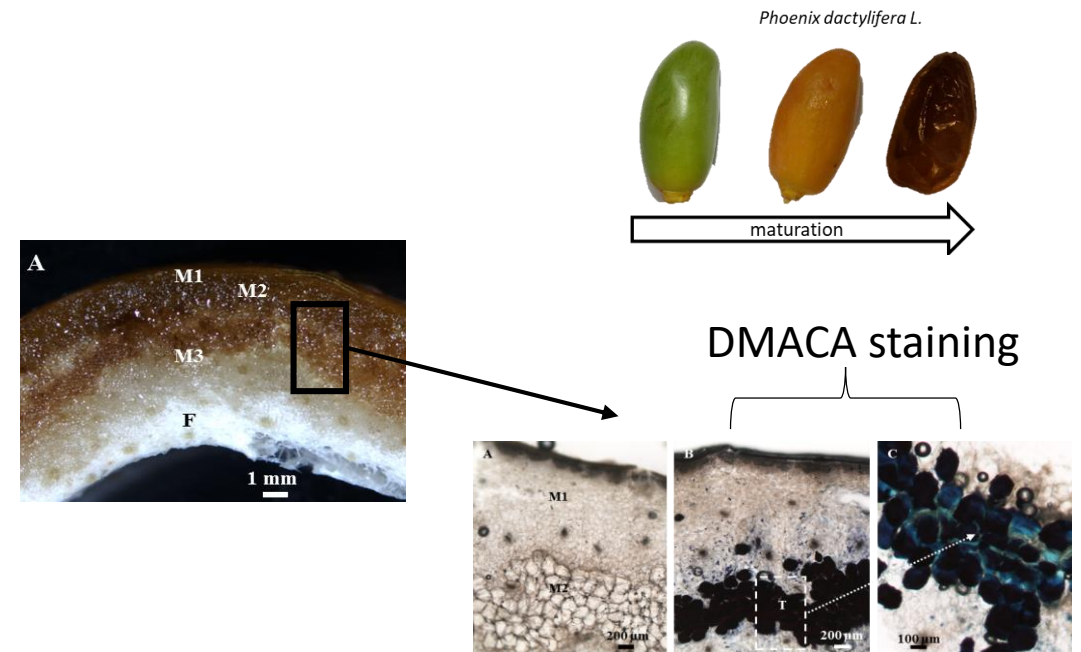


Evidence of condensed tannins in rapeseed tegument

*Auger et al., J. Agric. Food Chem., 2010*

(Dimethylcinnamaldehyde)

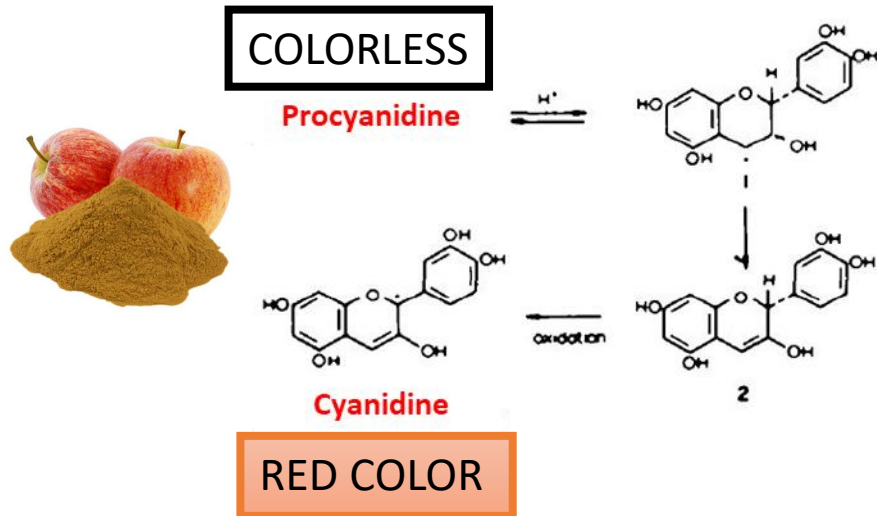
In dates (Phoenix dactylifera)



*Hammouda et al., J. Agric. Food Chem., 2014*

# Their direct detection in (food) samples

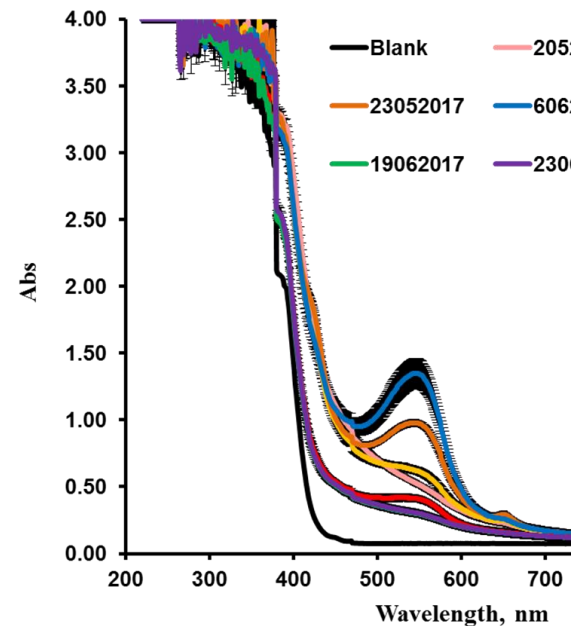
Strong acid treatment in organic solvent at elevated temperature



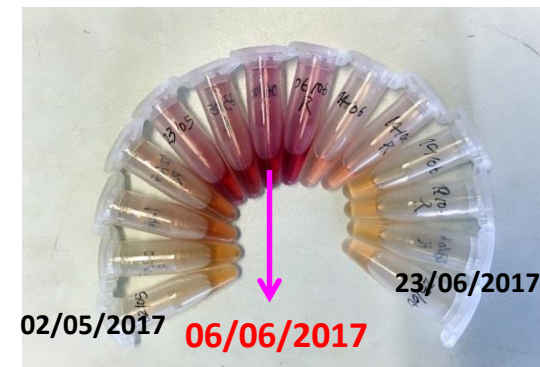
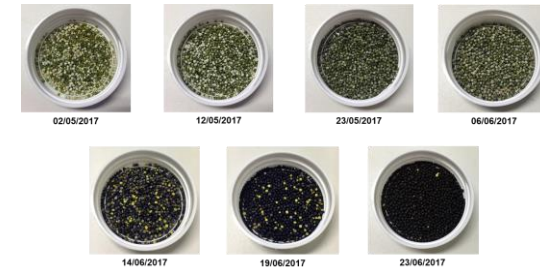
Example of Rapeseed meals



UV visible spectrum



Maturation of the seeds



The **butanol-HCL Assay** (Bate-Smith reaction, Porter's reagent, PRO-cyanidin)

*Bate-Smith, Biochem. J., 1954*

*Porter et al., Phytochemistry, 1986*

*Yu et al., publication ongoing*

Biologically Active Compounds in Foods,  
Lodz, September 19-20<sup>th</sup> 2019



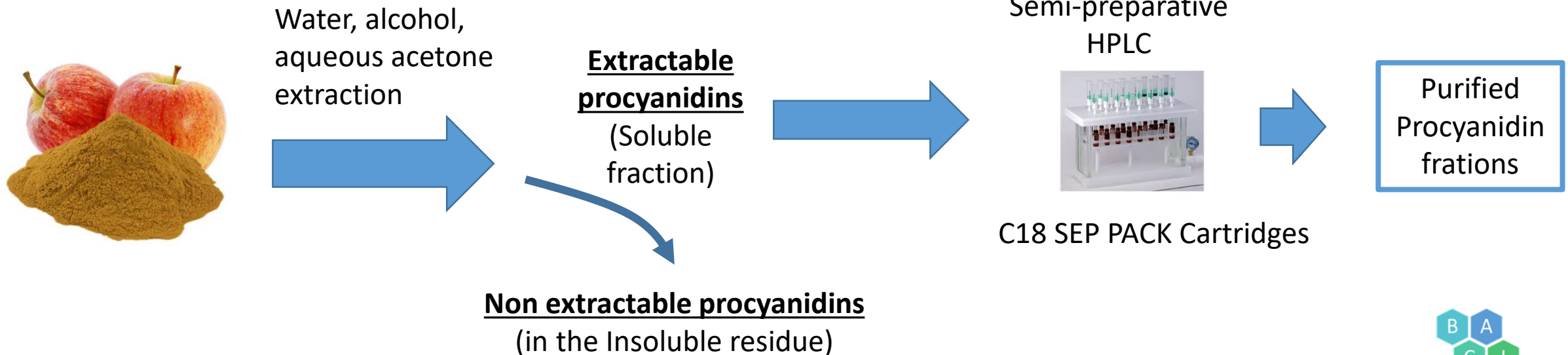
# ...a few words about their EXTRACTION ...

Difficulty regarding their adsorption on cell-wall materials (tannin properties)

Aqueous methanol for oligomers

Aqueous-acetone for polymers

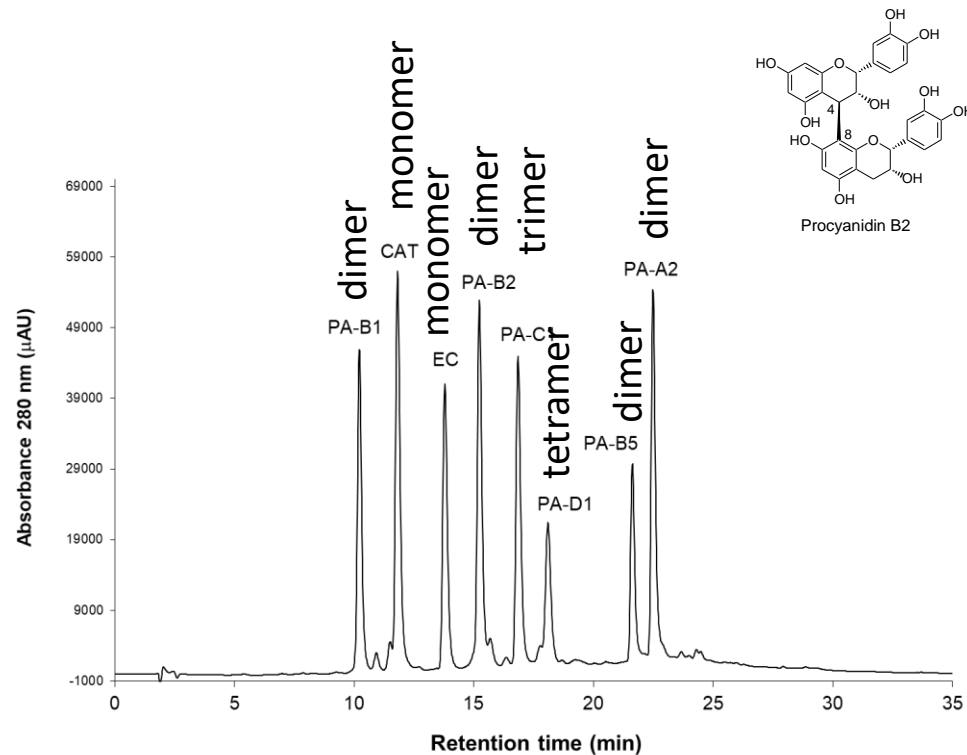
..in slightly acidic conditions



# Direct HPLC is not adapted to PA polymers analysis

## C18-HPLC of a series of procyanidin **OLIGOMERS**

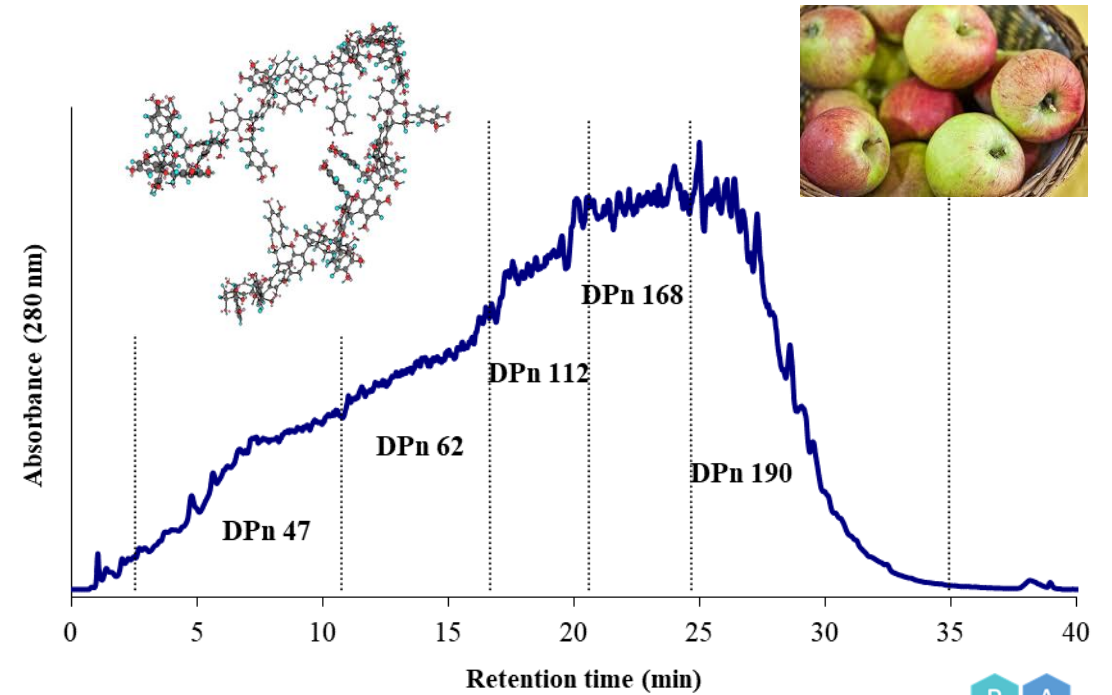
A mixture of purified or commercial standards



From Guyot S., Handbook of analysis of active compounds in functional foods, CRC Press, 2012

## Direct Reversed phase HPLC of procyanidin **POLYMERS**

An aqueous-acetone fraction from *Avrolles* cider apple



*DPn = Average degree of polymerisation*

From Guyot et al., J. Agric. Food Chem., 2001

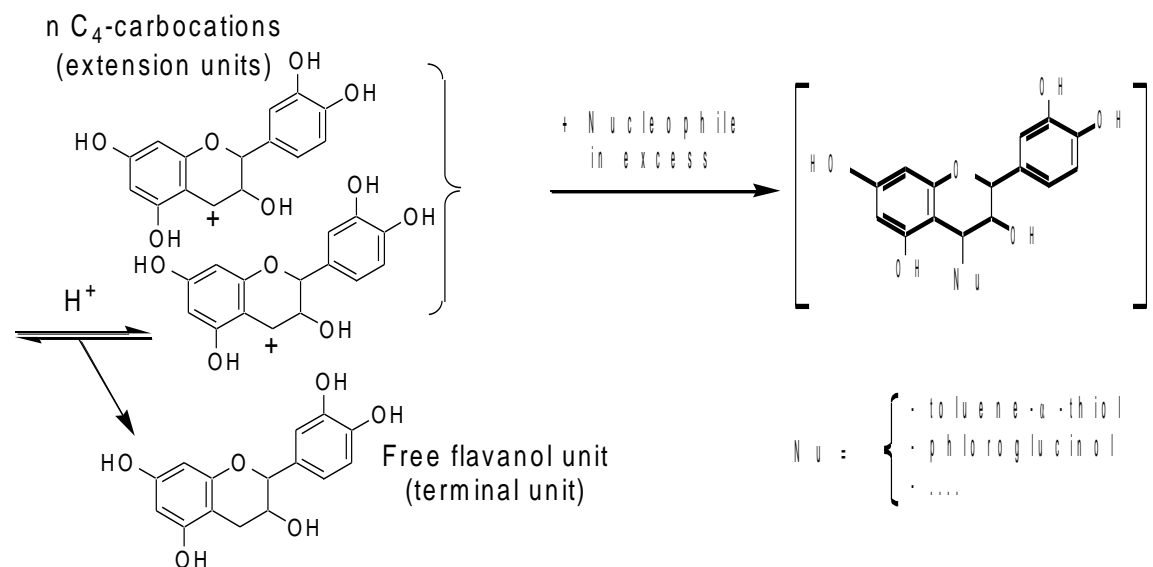
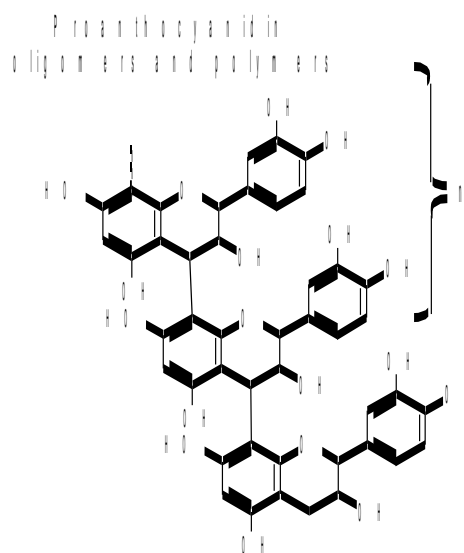
Biologically Active Compounds in Foods,  
Lodz, September 19-20<sup>th</sup> 2019



# The acidic lability of the interflavan linkage: a chance for analysis of proanthocyanidin polymers

In methanol,  
nucleophile in excess  
(<20 mM),  
40°C, HCl 0.2N, 30 min

## Thiolysis & Phloroglucinolysis reactions



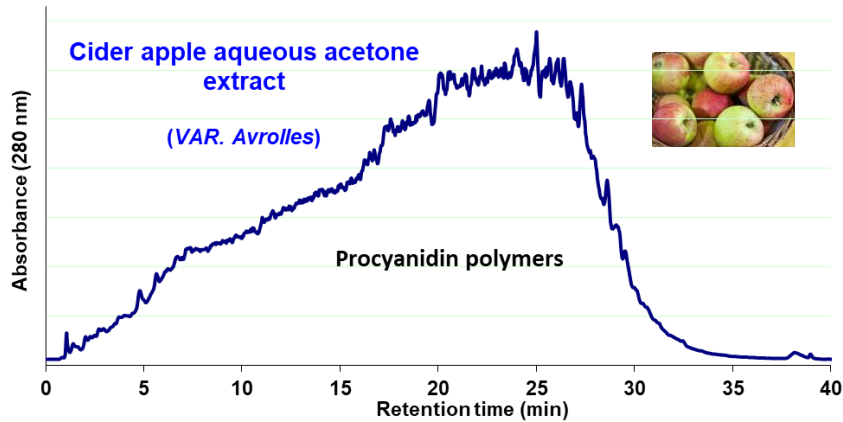
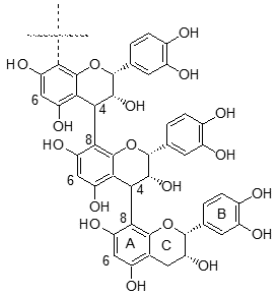
Polymers are converted into monomer derivatives

The products in the reaction medium can be efficiently analysed by Reversed phase HPLC/UPLC with UV detection

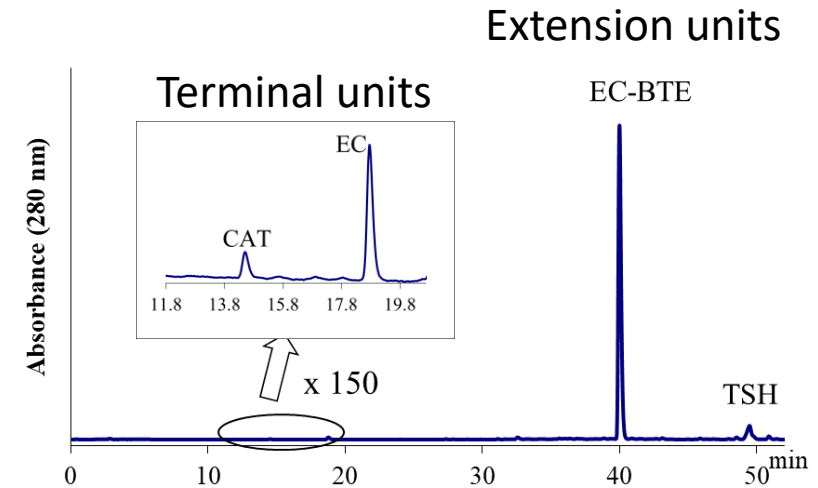


# HPLC analysis of the Thiolysis or Phloroglucinolysis reaction media

Application on **purified apple procyanidin fractions**



Thiolysis reaction



From Guyot et al., J. Agric. Food Chem., 2001

- Nature of the constitutive units
- Average degree of polymerisation ( $DP_n = \text{all units} / \text{term. units ratio}$ )
- Concentration (sum of the concentrations of all units)

For instance,  
 $DP_n = 70$  for this  
aqueous-acetone  
extract from Avrolles



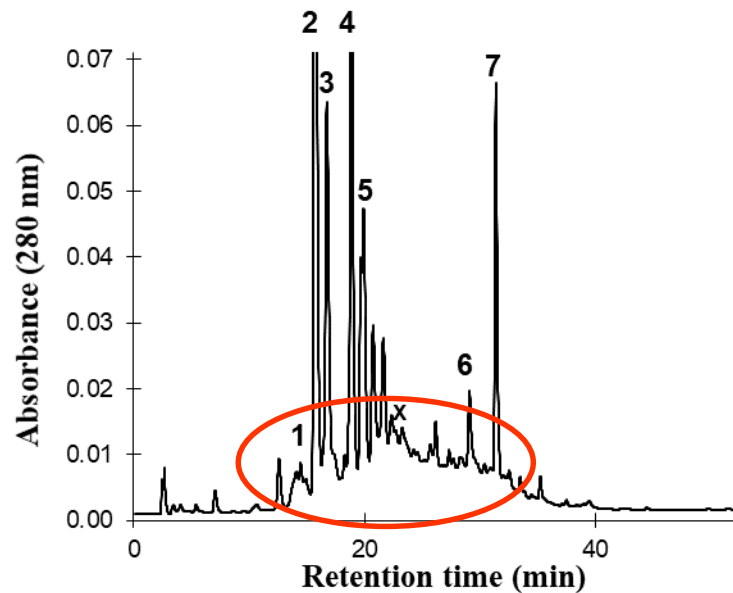
# HPLC analysis of the Thiolytic or Phloroglucinolysis reaction media

Direct application on **crude apple samples (powders, freeze-dried juices or ciders)**

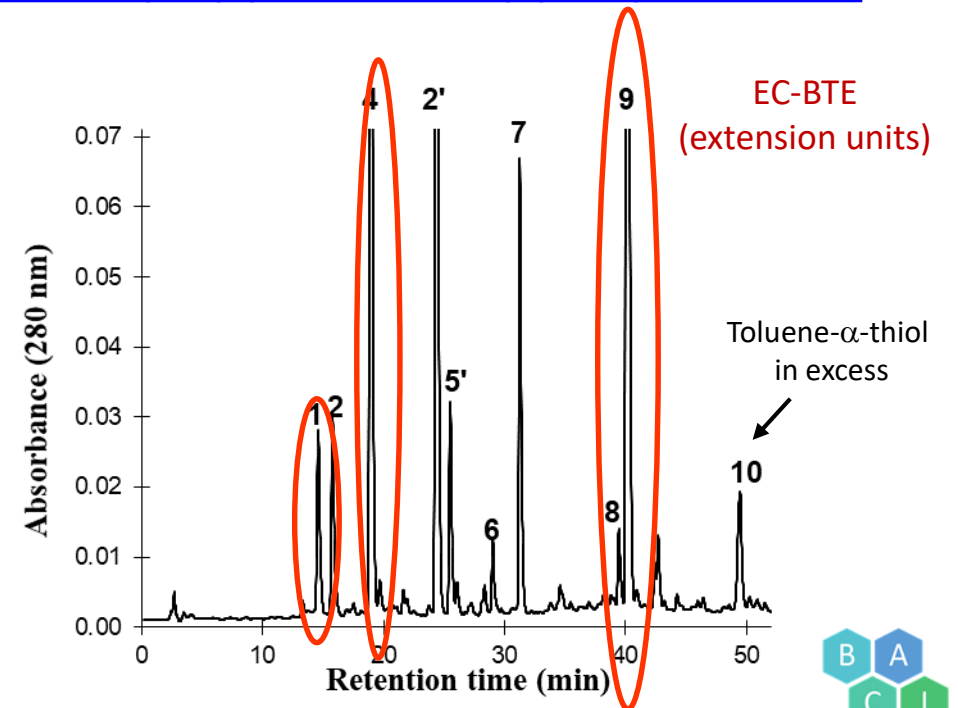
Cider apple (*Var. Kermerrien*)



Direct analysis (crude methanol extract)

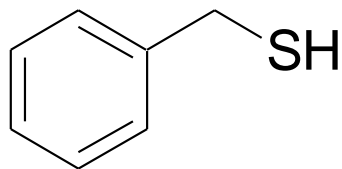


After thiolytic  
(directly applied on apple powder)

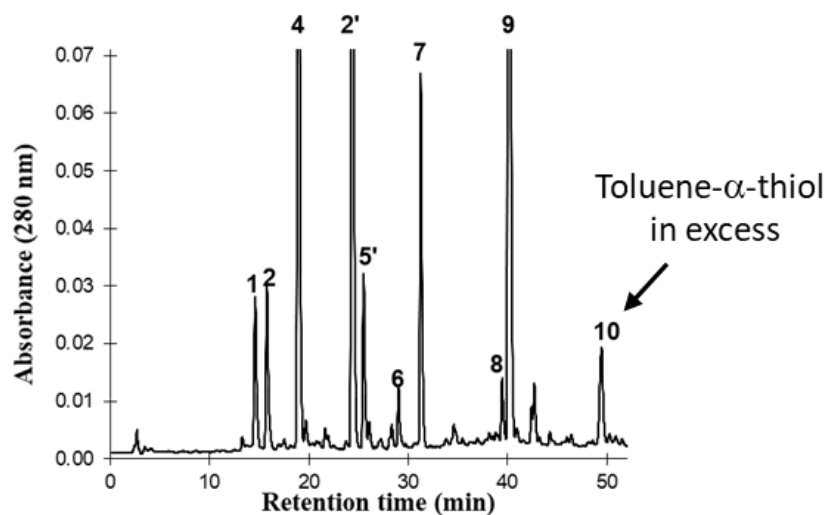


# From Thiolysis...to Phloroglucinolysis

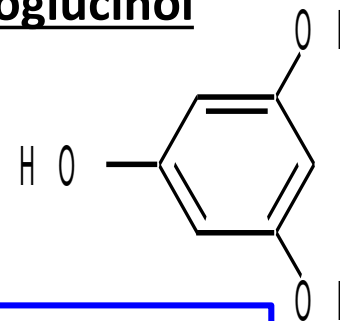
## Toluene- $\alpha$ -thiol



- Volatile
- Highly nauseous
- Toxic

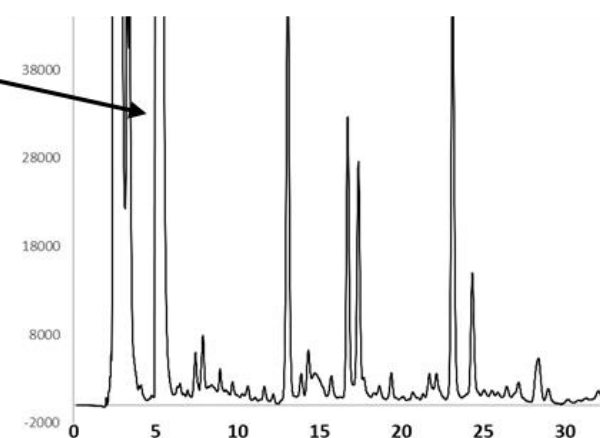


## Phloroglucinol



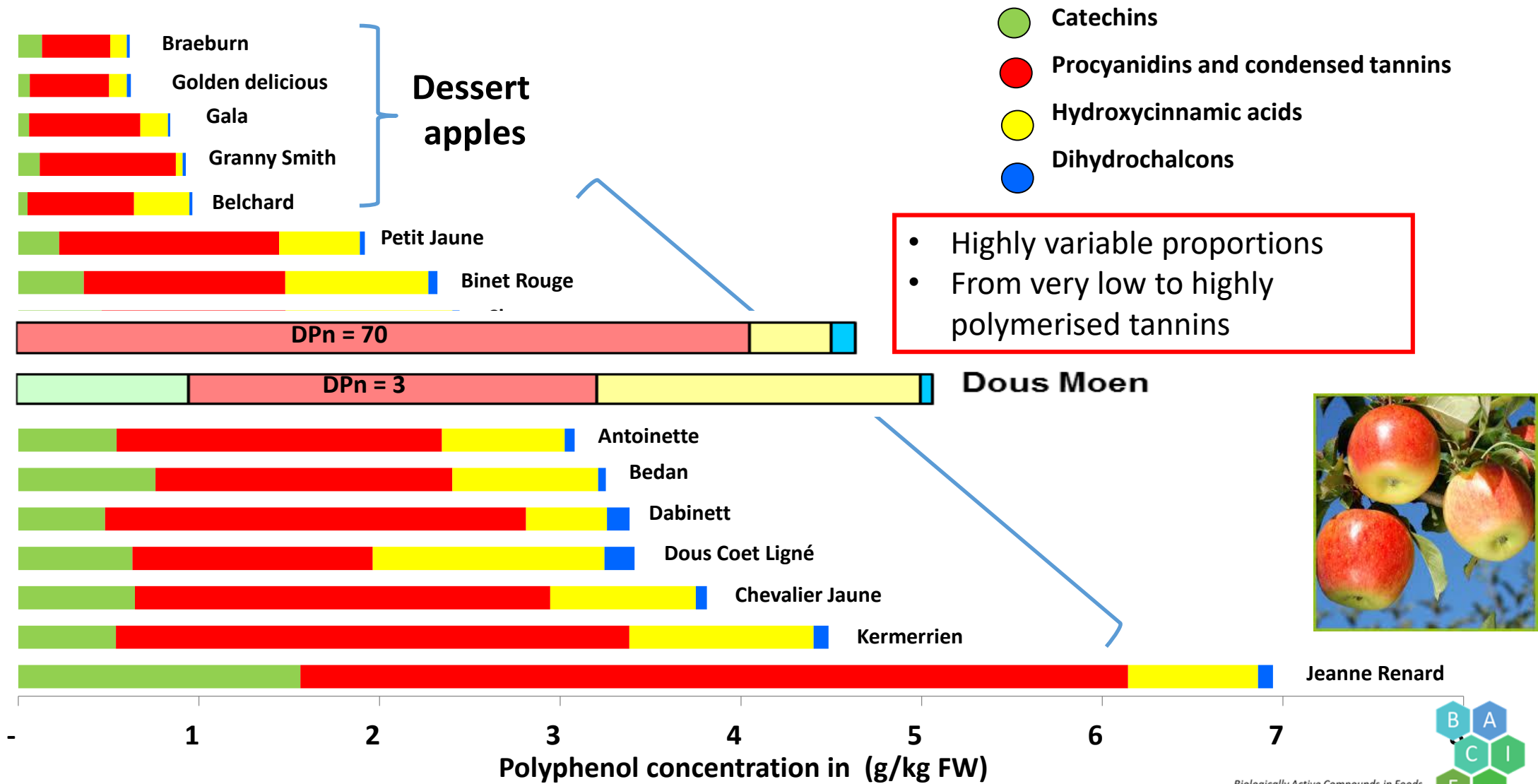
- Non volatile
- Odourless
- Safe

Phloroglucinol  
in excess





# Application to detailed procyanidin analysis in dessert and ciders varieties



Sanoner et al., J. Agric Food Chem., 1999



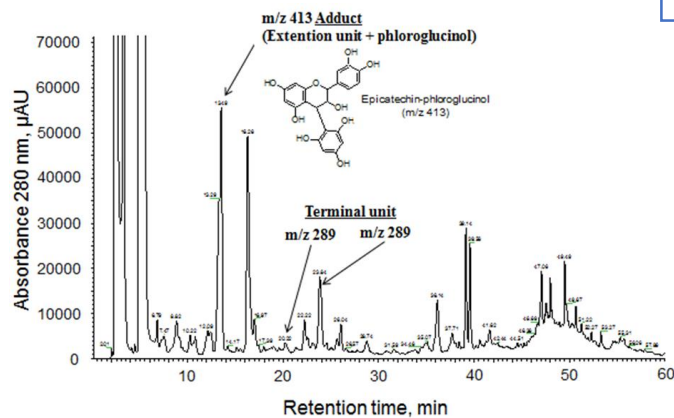
# Acidolysis conditions should be optimized according to sample type

Example of condensed tannins in **Rapeseed hulls**

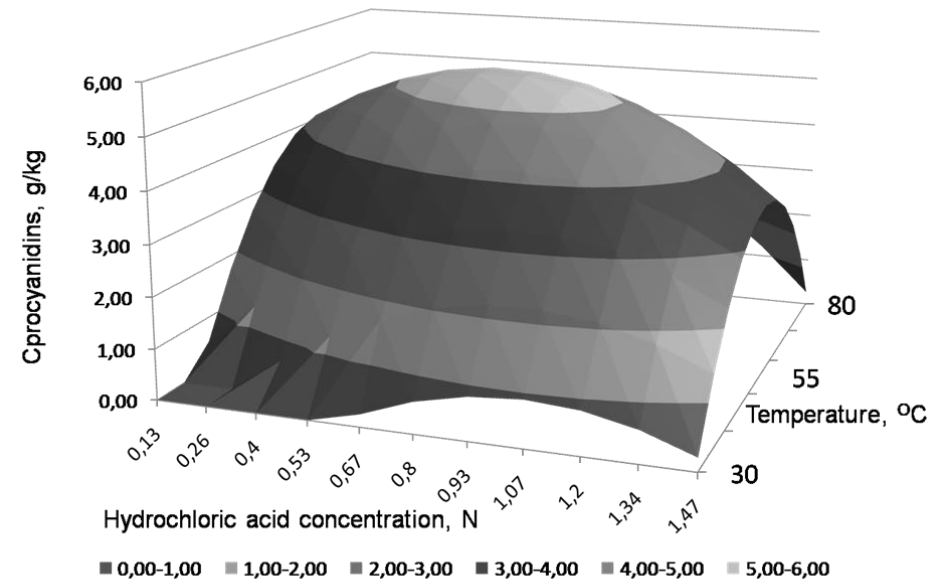


Experimental design

Phloroglucinolysis-HPLC



[Hydrochloric acid] & Temperature



Yield of acidolysis is strongly influenced by the reaction conditions

....Are analytical methods for “native tannins” also adapted to analysis of “oxidized tannins” ?

# Oxidation : widespread reactions that may deeply modify the structure of proanthocyanidins/condensed tannins

Fruit maturation and drying

*Phoenix dactylifera L.*



Processing



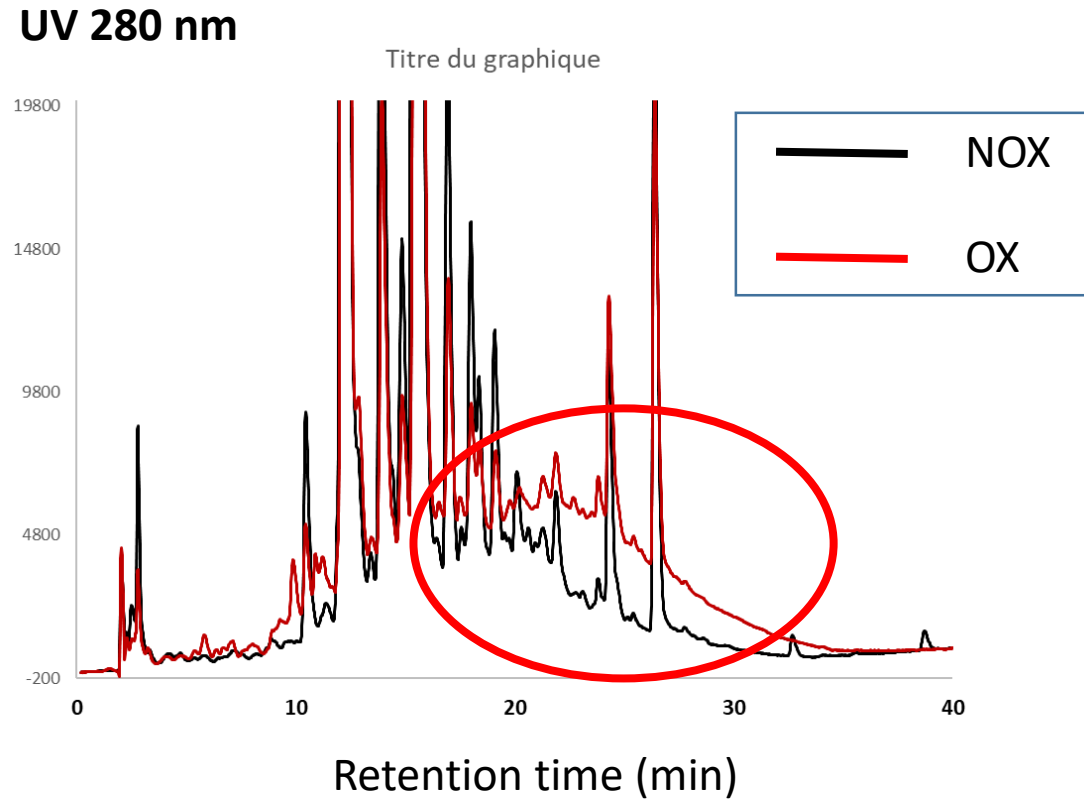
Storage and ageing



Most of the polyphenols are concerned by oxidation...in particular tannins  
(...many catechol groups)

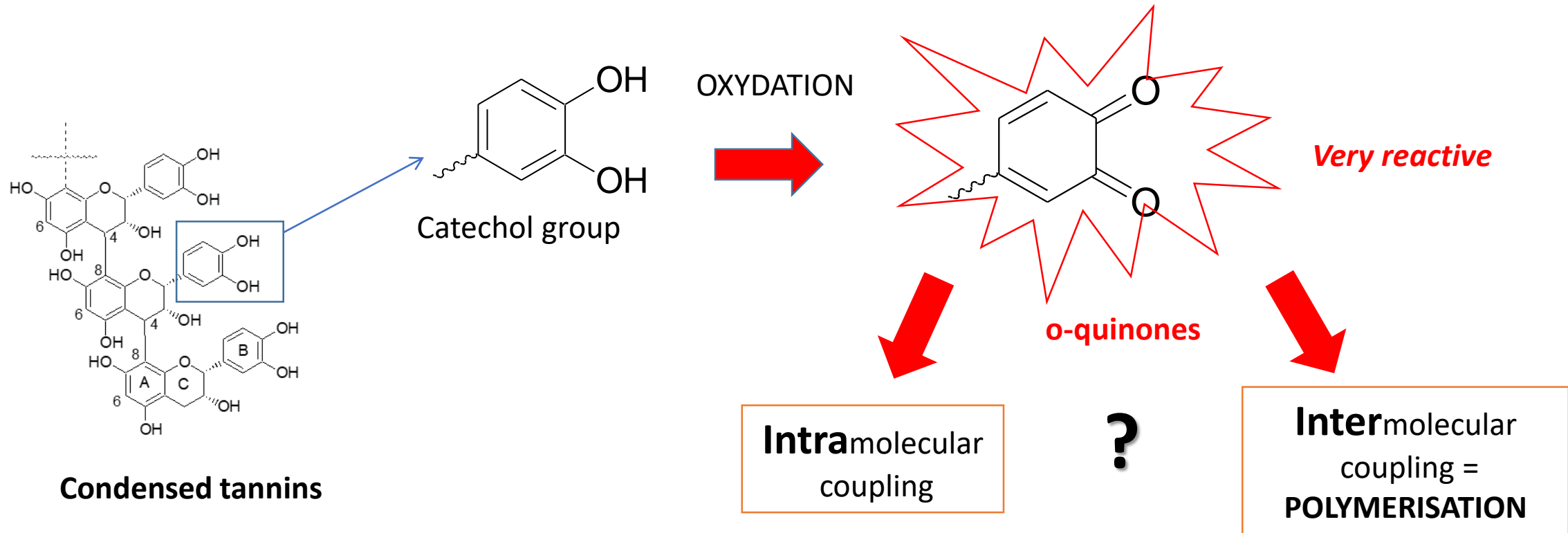
# The limit of UV detection for LC analysis of oxidized polyphenols

UV chromatogram of a **Oxidized versus Non Oxidized** cider apple juice



Oxidized polyphenols do not give well-resolved peaks on UV Chromatograms

Overall oxidative reactivity: phenol, catechol groups are first converted to highly reactive intermediates (quinones, semiquinones, phenoxy radicals..)



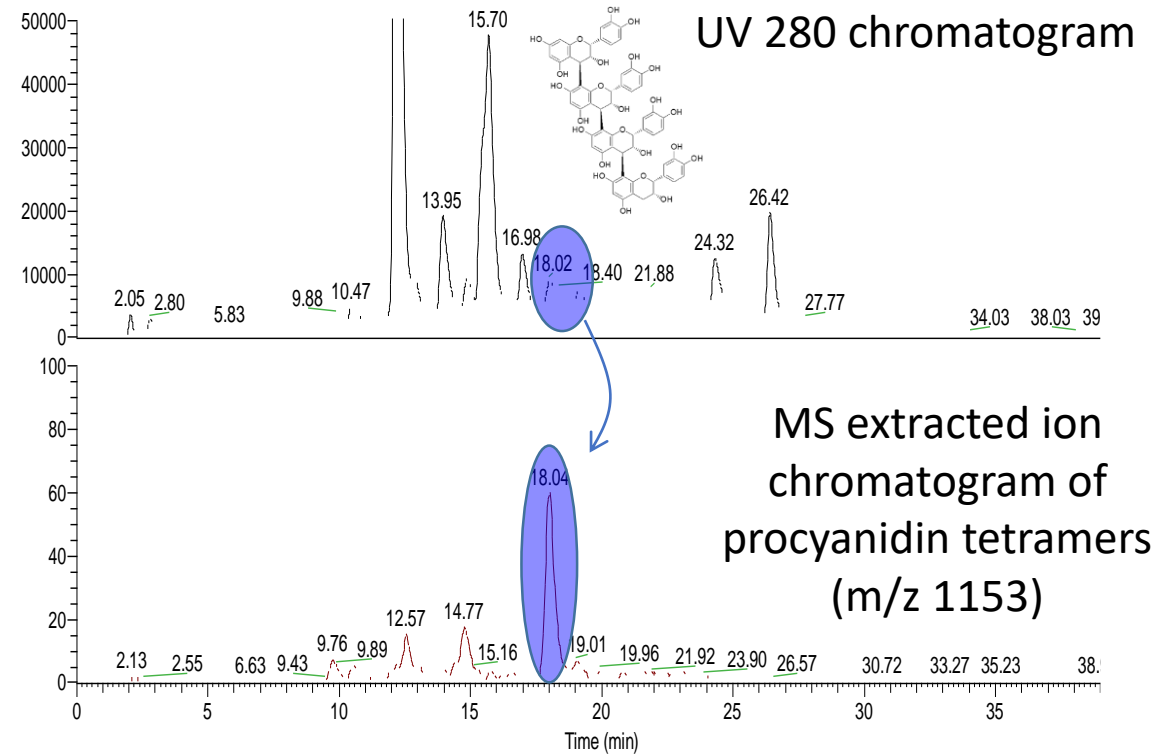
*...A great diversity of oxidation products...*

# Selectivity of Mass Spectrometry coupled to LC (HPLC/UPLC) is of great help for analysis oxidized tannins

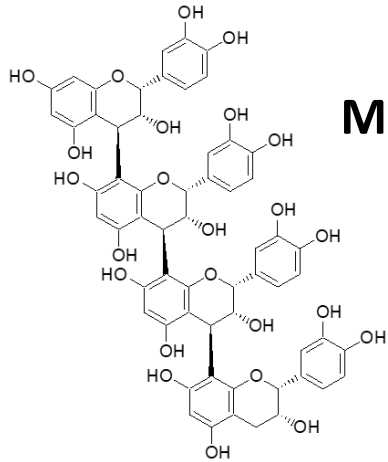


Oxidized apple juice

RT: 0.00 - 40.00 SM: 7B



# Mass spectrometry coupled to LC is of great help for oxidized tannins analysis

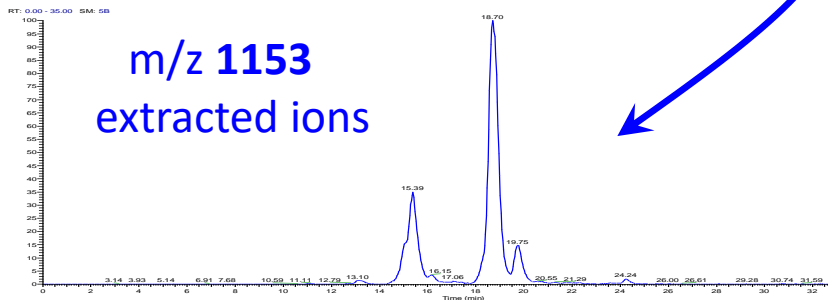
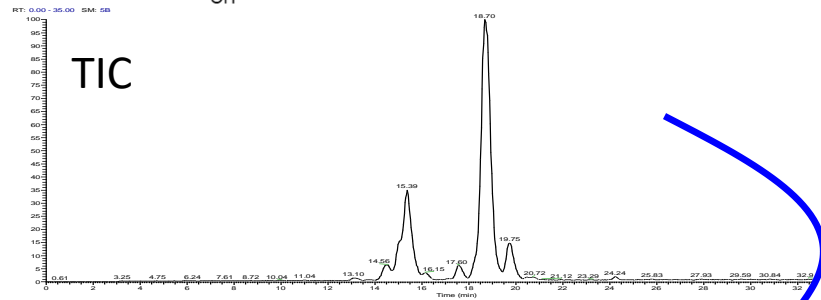


Mw = 1154 da

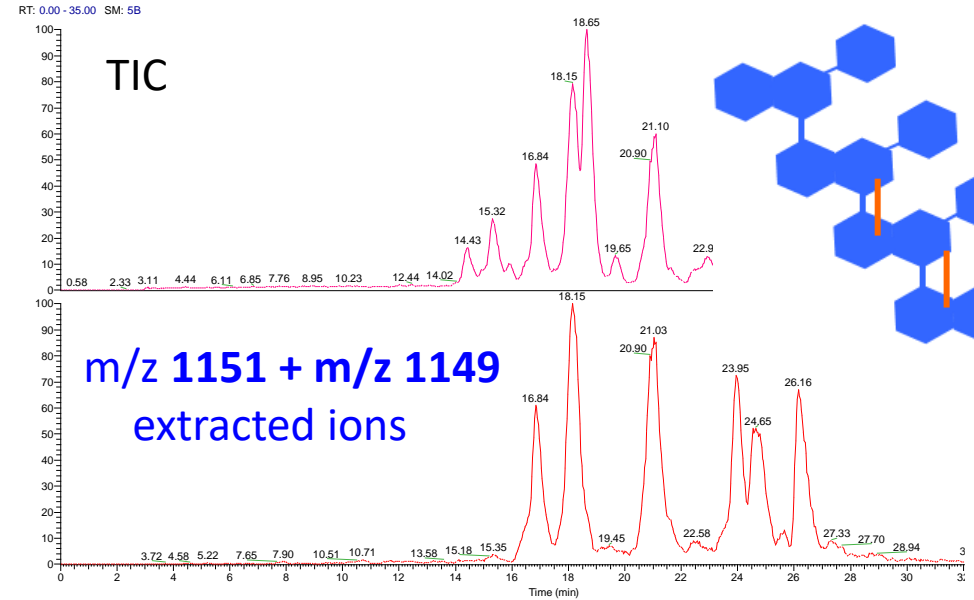
Native (non oxidized)  
Tetramer  
(DP4) fraction

**OXIDATION**  
(IO4<sup>2-</sup> fixed on resin)

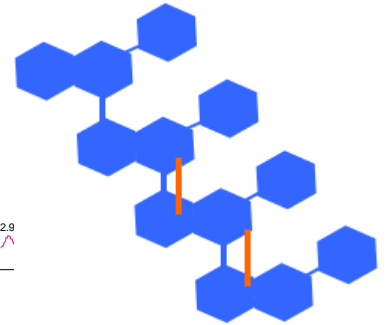
Many more peaks corresponding to oxidation products



Almost all  
molecular  
species are  
native  
tetramers



m/z 1151 + m/z 1149  
extracted ions

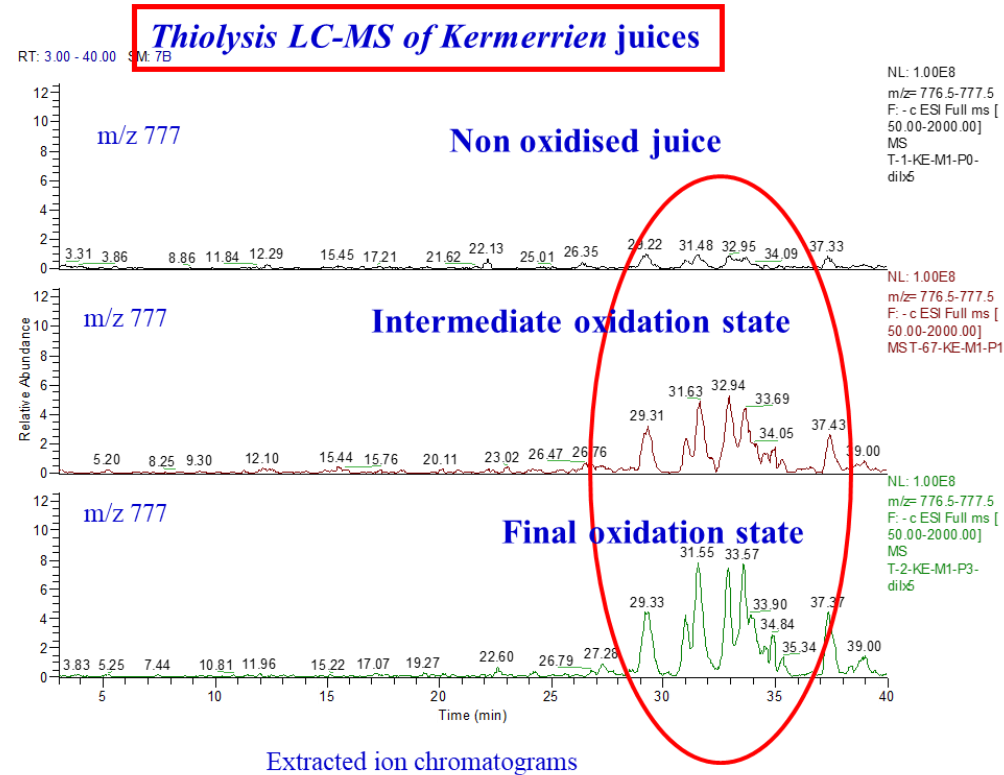


Loss of 2 or 4 hydrogen atoms : a signature of INTRA molecular coupling



# And what about acidolysis associated to LC-MS for the exploration of procyanidin oxidation products ?

## Oxidation kinetic of a cider apple juices



Specific markers of oxidative coupling products between tannins and other polyphenols



# Conclusion

- Procyanidins/condensed tannins ) show a great diversity of molecular structure, in particular the large size distribution, the nature of the constitutive units, the branching...
- Important consequences in terms of “functional properties” : solubility, interactions with macromolecules (proteins, polysaccharides..), bio-accessibility, etc...
- Analytical method still need to be developed in particular for the exploration of the oxidized compounds



Jean-Michel  
Le Quéré



Hélène Sotin



Mélanie Millet



Sophie Guilois



Pascal Poupard

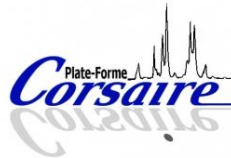


Xiaoxi Xu



Mariana-Castillo Fraire

# Thank you for your attention !



Biologically Active Compounds in Foods,  
Lodz, November 9-10<sup>th</sup> 2017