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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.



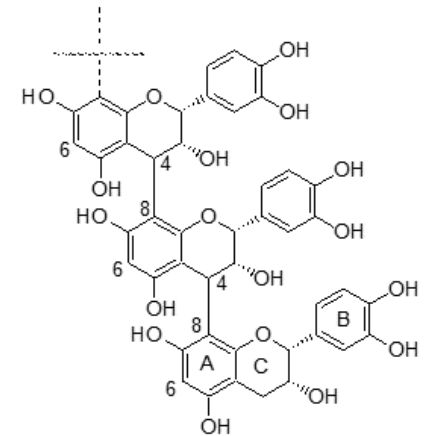
Sylvain Guyot

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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

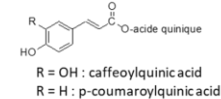
Food sciences and Analytical Chemistry
related to fruit processing...in particular
apple and **ciders** making

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

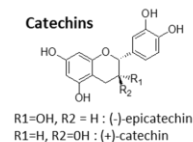
...with a specific focus on **the oxidation**
reactions involving polyphenols



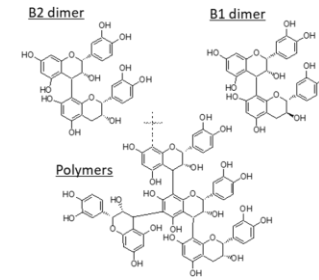
Hydroxycinnamic acids



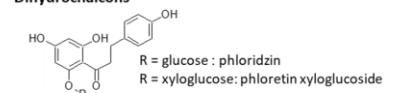
Catechins



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

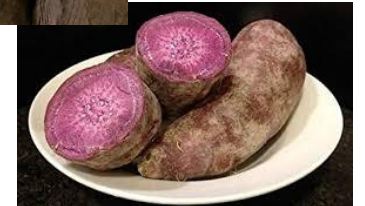
In nuts, beans, peas & lentils



In rapeseed



In roots and tubers (ex: Yam)



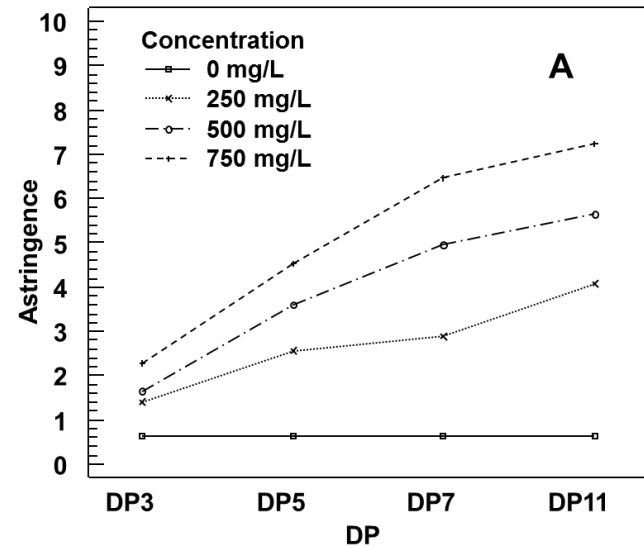
They largely contribute to polyphenol intake

Procyanidin and their contribution to the taste

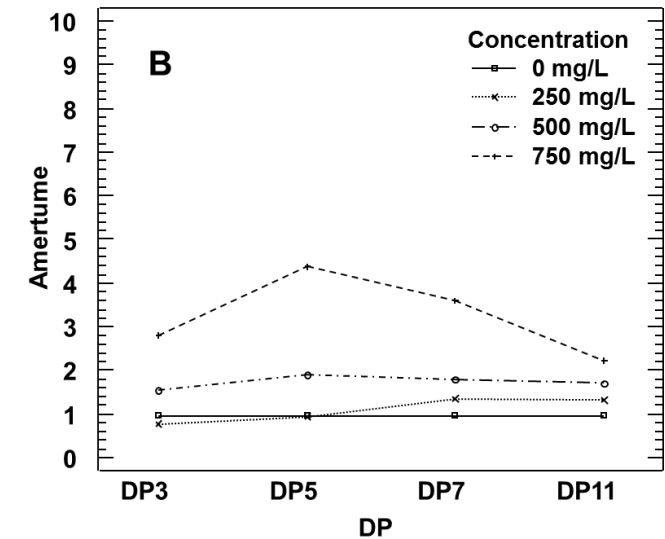
Example : in French ciders model solutions



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

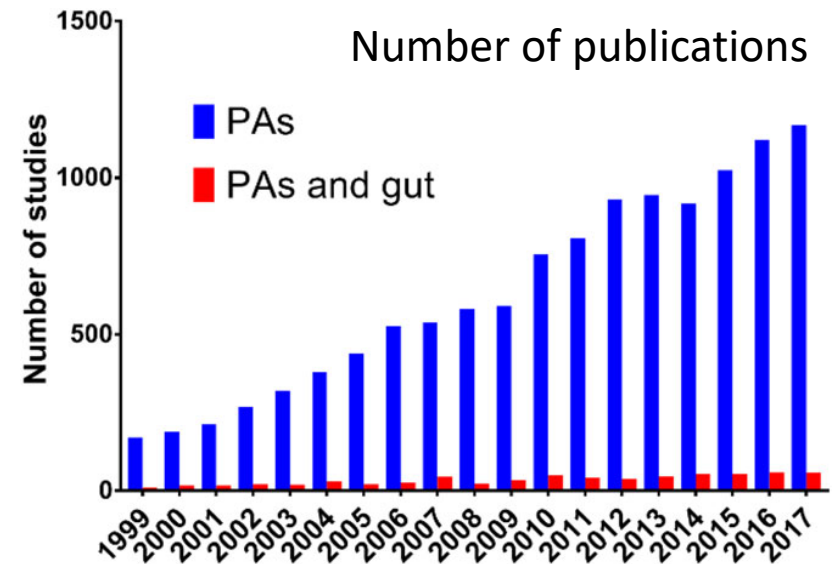
- **Antioxidant** in food and in the tractus (protection against lipid & protein oxidation)
- **Antimicrobial** (ex antibacterial activity of cranberry procyanidins)
- **Cardioprotective, antiinflammatory and neuroprotective** effects

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

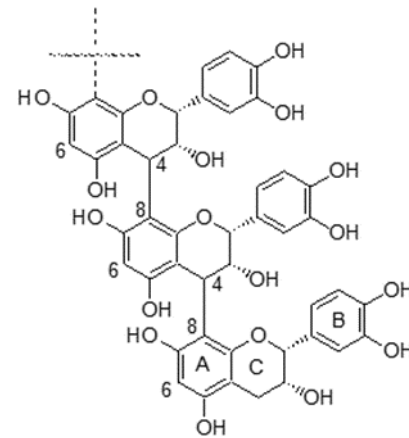
Wenyang Tao , Yu Zhang, Xuemin Shen, Yanping Cao, John Shi, Xingqian Ye, and Shiguo Chen 

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

ComprehensiveReviewsinFoodScienceandFoodSafety



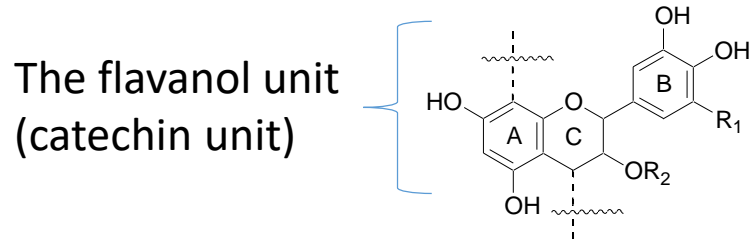
The understanding of their specific role (as bioactive compounds) in food is still limited



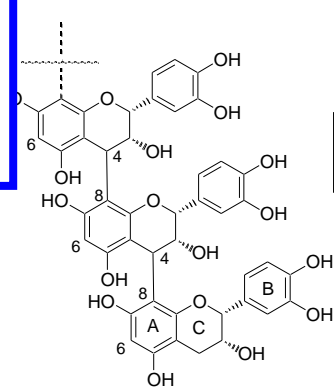
.....because of the difficulty for their extraction, structural analysis and quantification.

Diversity of molecular structures of proanthocyanidins

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



Colourless and highly water soluble

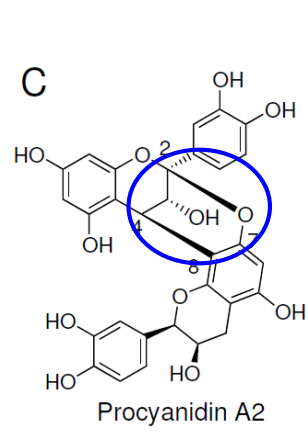
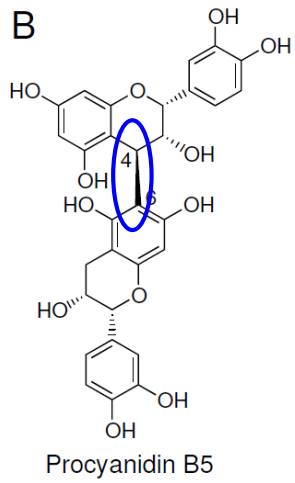
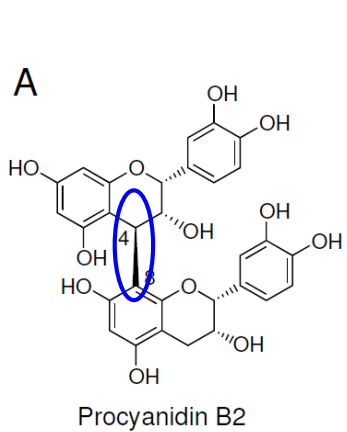


DP : degree of polymerisation

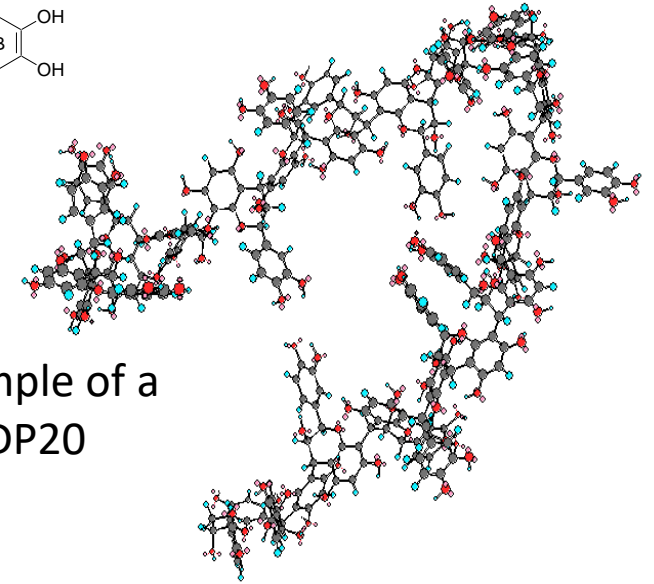
oligomers

B-type (one Interflavan Linkage)

A-type (two Interflavan Linkage)



Polymers



Example of a DP20

DPn > 40-60 kDa



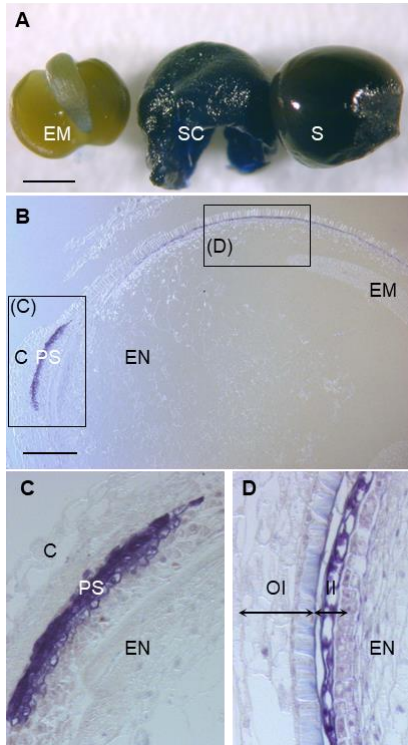
Their direct observation in plant tissues

Photonic microscopy....

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



In Rapeseed during the growth of the seeds

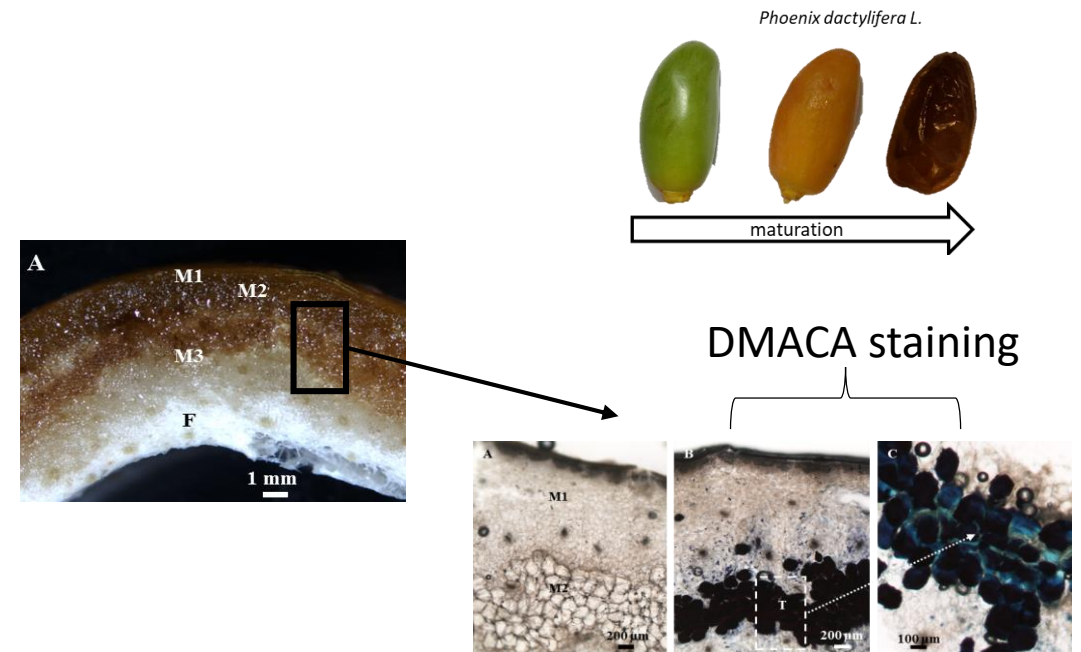


Evidence of condensed tannins in rapeseed tegument

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

(Dimethylcinnamaldehyde)

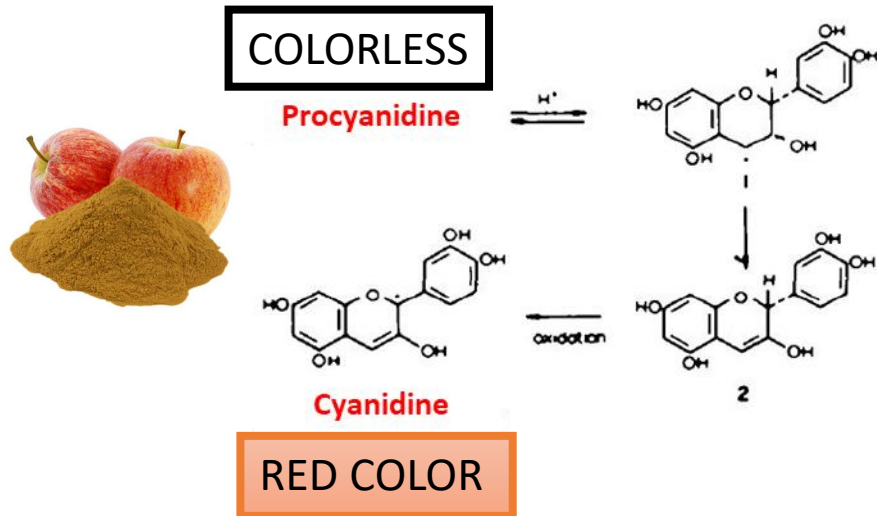
In dates (Phoenix dactylifera)



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

Their direct detection in (food) samples

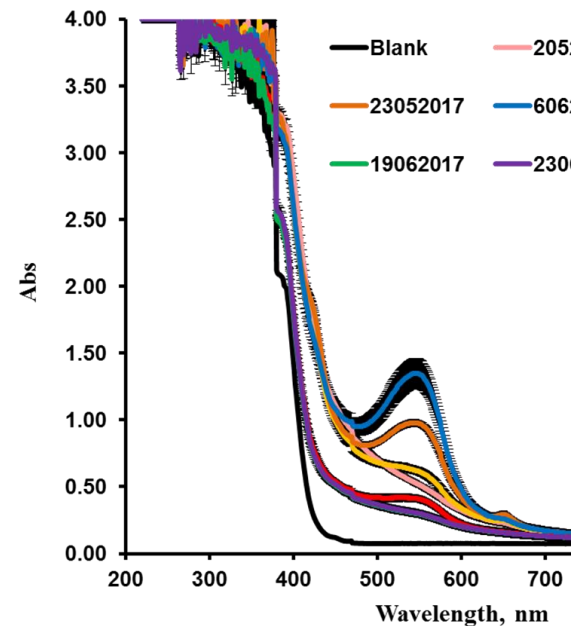
Strong acid treatment in organic solvent at elevated temperature



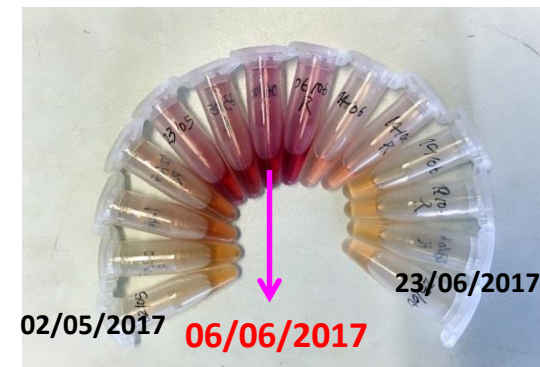
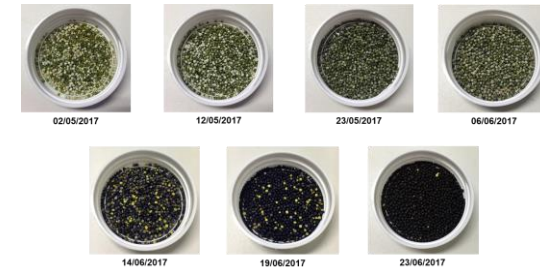
Example of Rapeseed meals



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 19-20th 2019



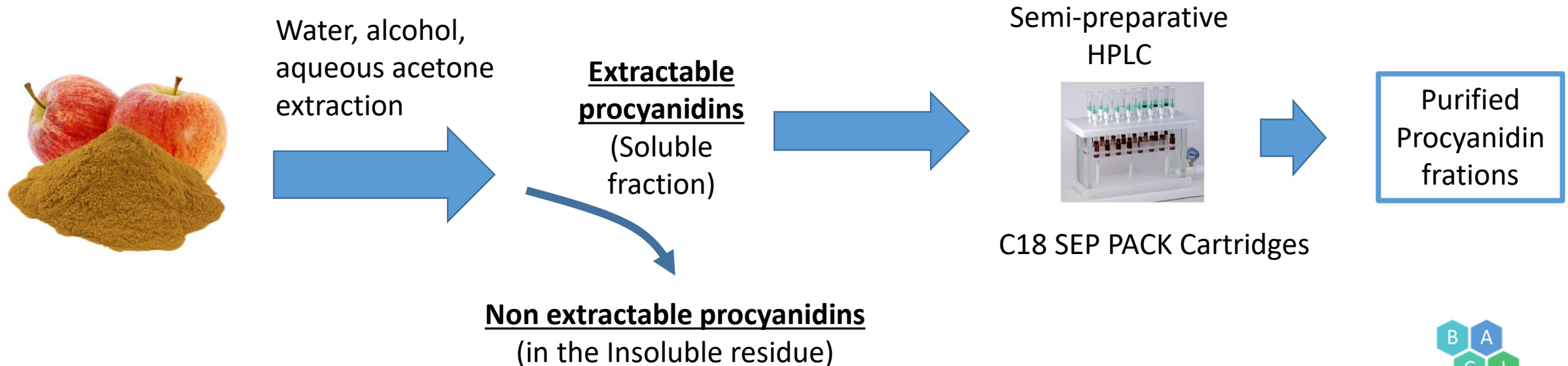
...a few words about their EXTRACTION ...

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

Aqueous methanol for oligomers

Aqueous-acetone for polymers

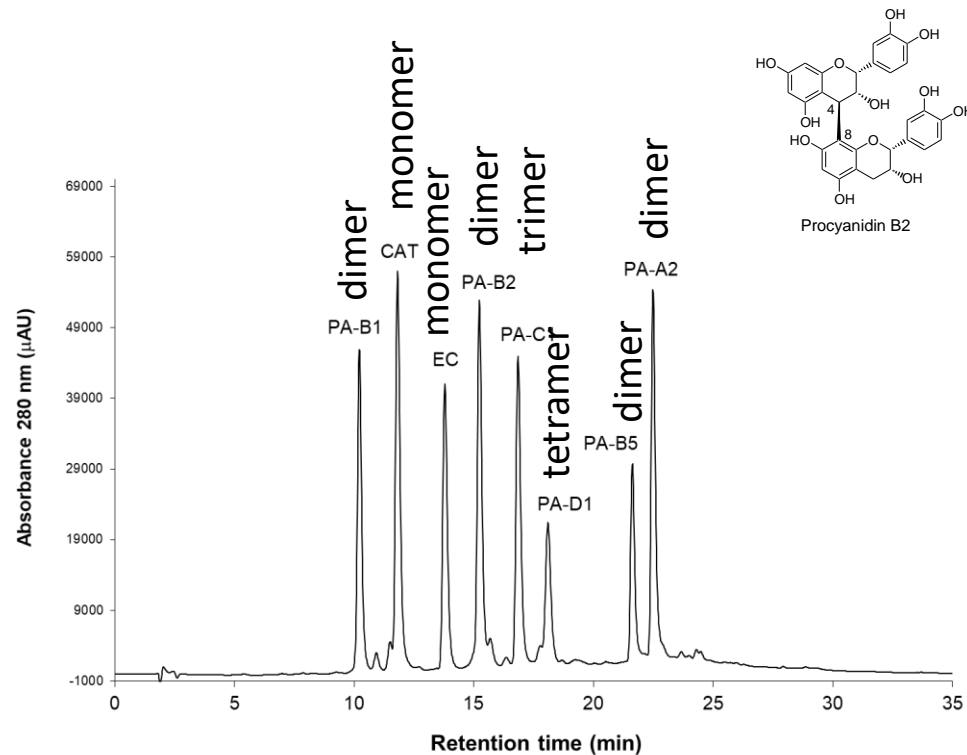
..in slightly acidic conditions



Direct HPLC is not adapted to PA polymers analysis

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

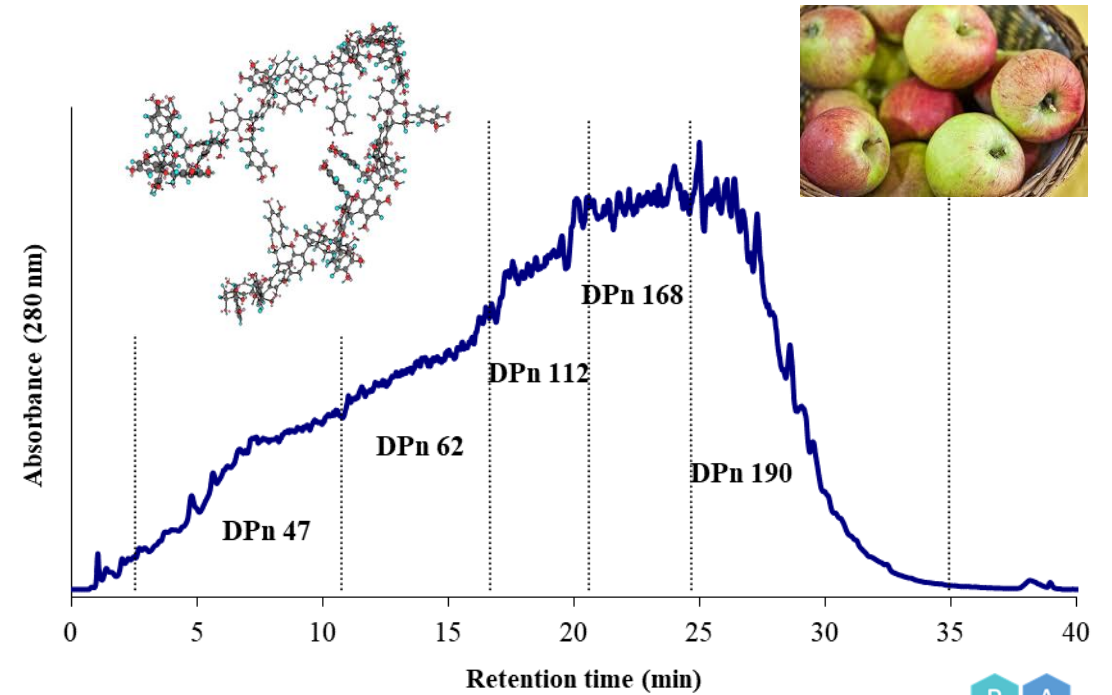
A mixture of purified or commercial standards



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

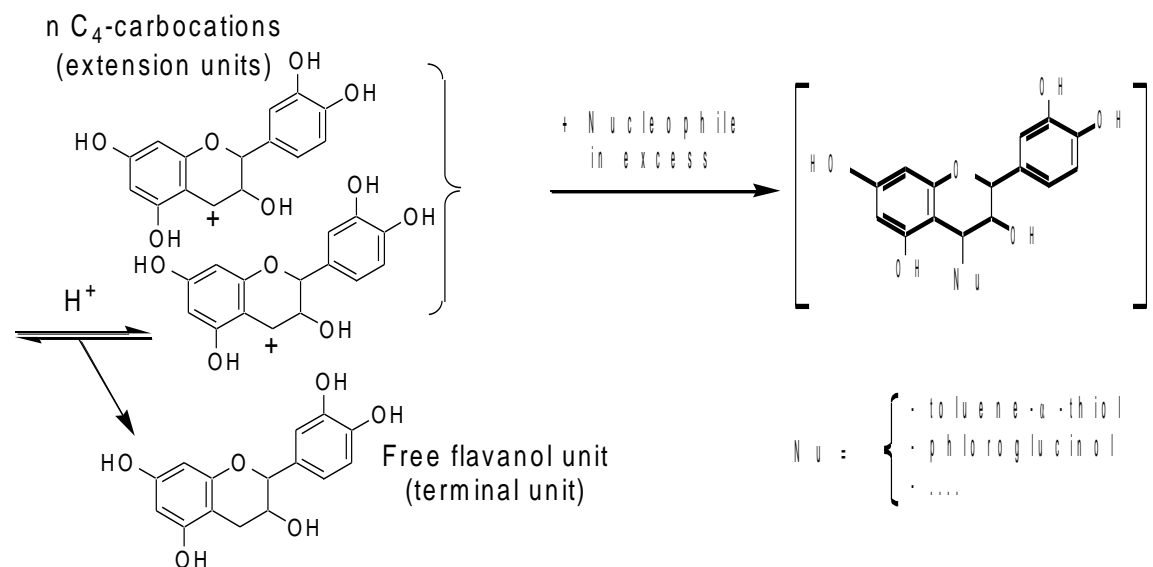
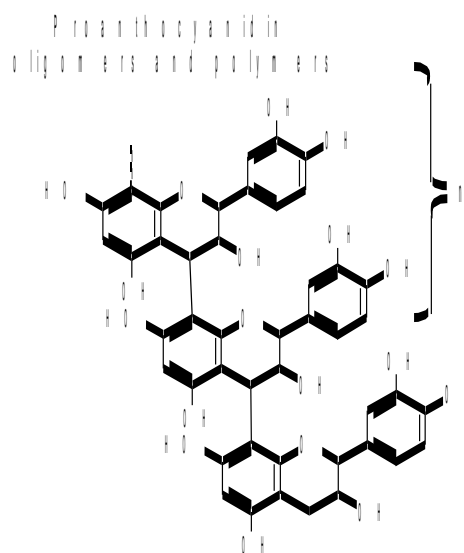
Biologically Active Compounds in Foods,
Lodz, September 19-20th 2019



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



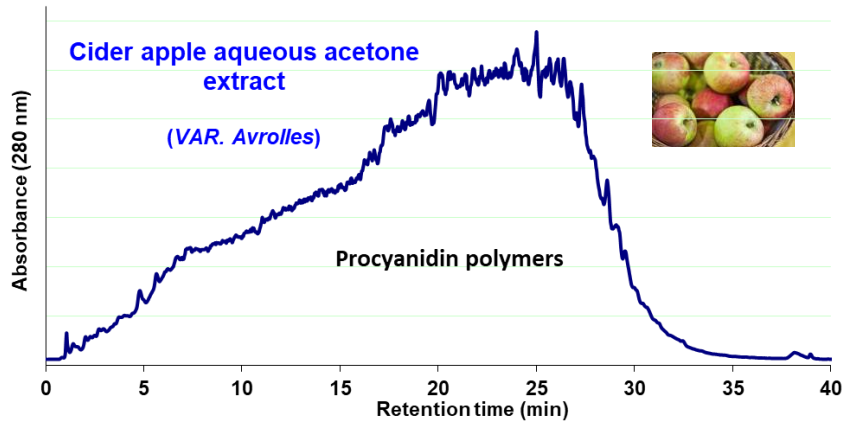
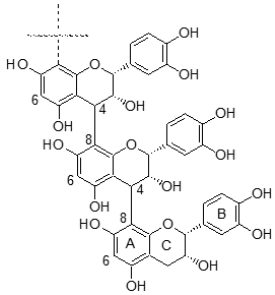
Polymers are converted into monomer derivatives

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

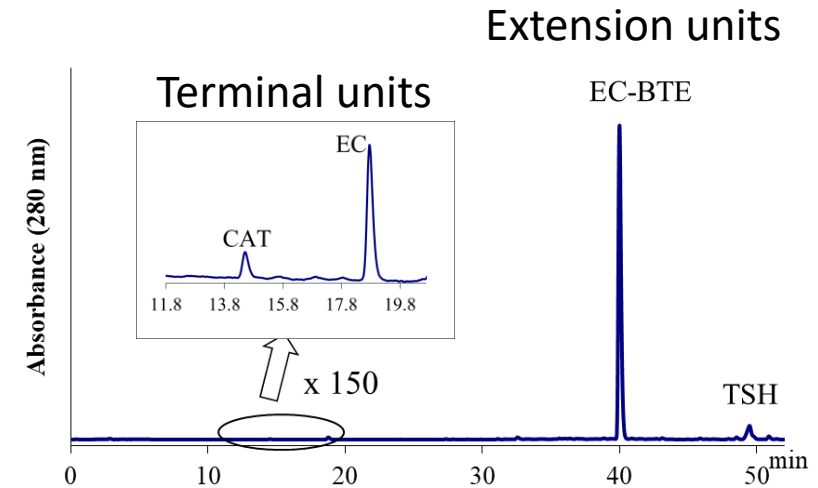


HPLC analysis of the Thiolysis or Phloroglucinolysis reaction media

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



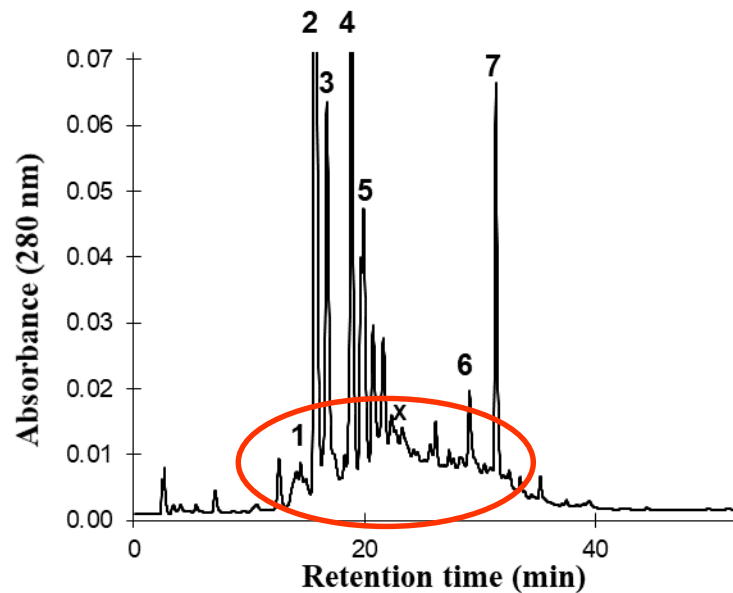
HPLC analysis of the Thiolytic or Phloroglucinolysis reaction media

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

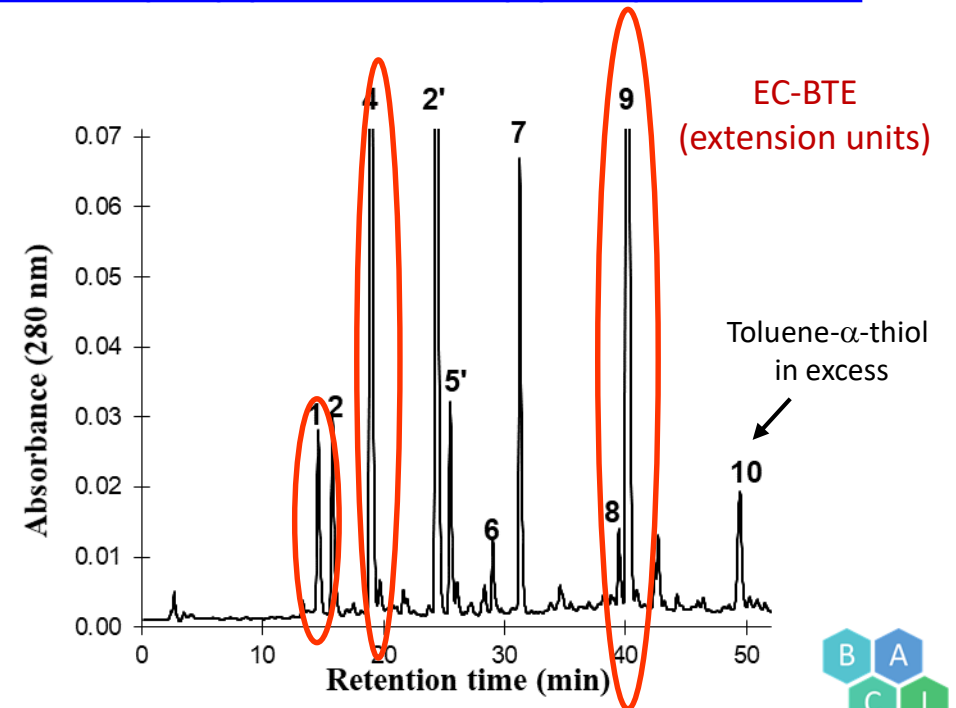
Cider apple (*Var. Kermerrien*)



Direct analysis (crude methanol extract)

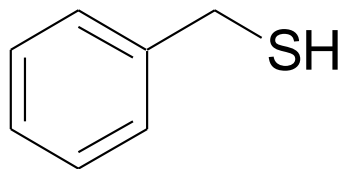


After thiolytic
(directly applied on apple powder)

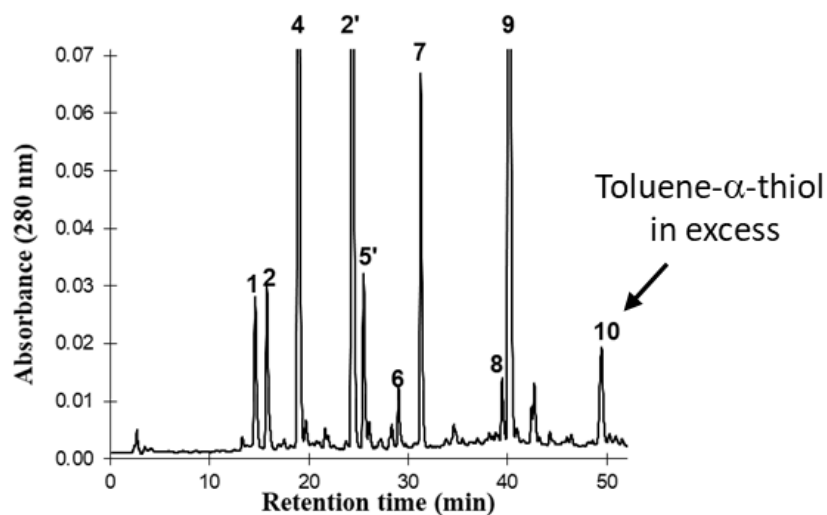


From Thiolyis...to Phloroglucinolysis

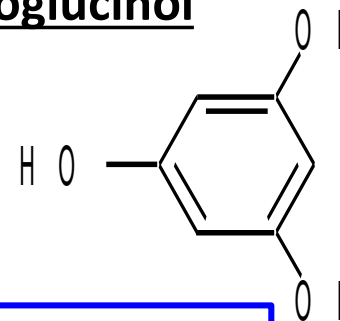
Toluene- α -thiol



- Volatile
- Highly nauseous
- Toxic

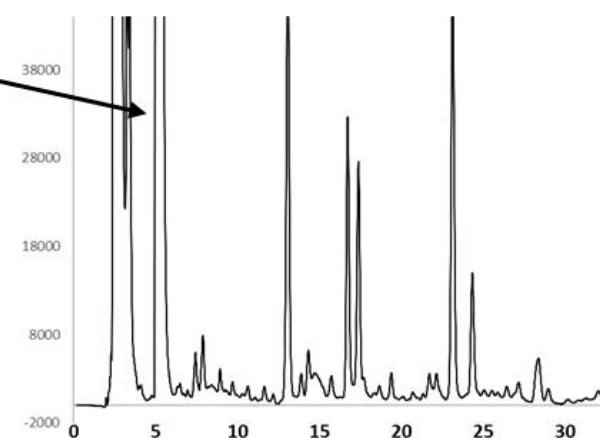


Phloroglucinol

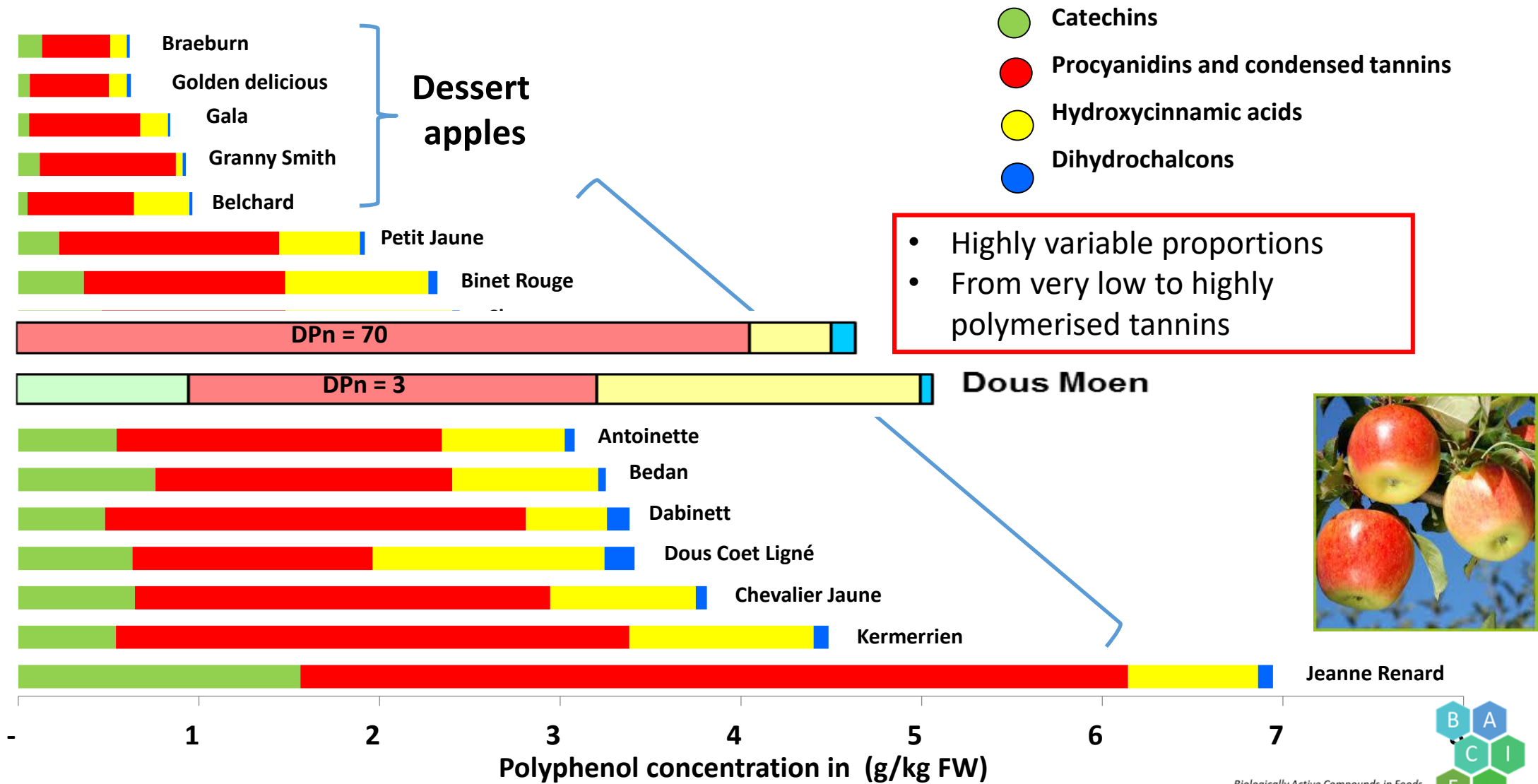


- Non volatile
- Odourless
- Safe

Phloroglucinol
in excess



Application to detailed procyanidin analysis in dessert and ciders varieties



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



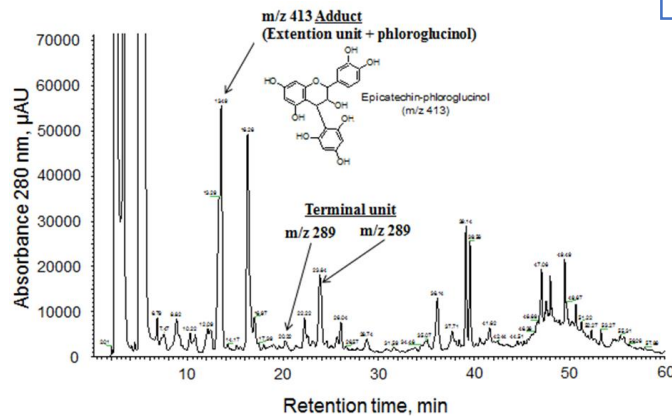
Acidolysis conditions should be optimized according to sample type

Example of condensed tannins in **Rapeseed hulls**

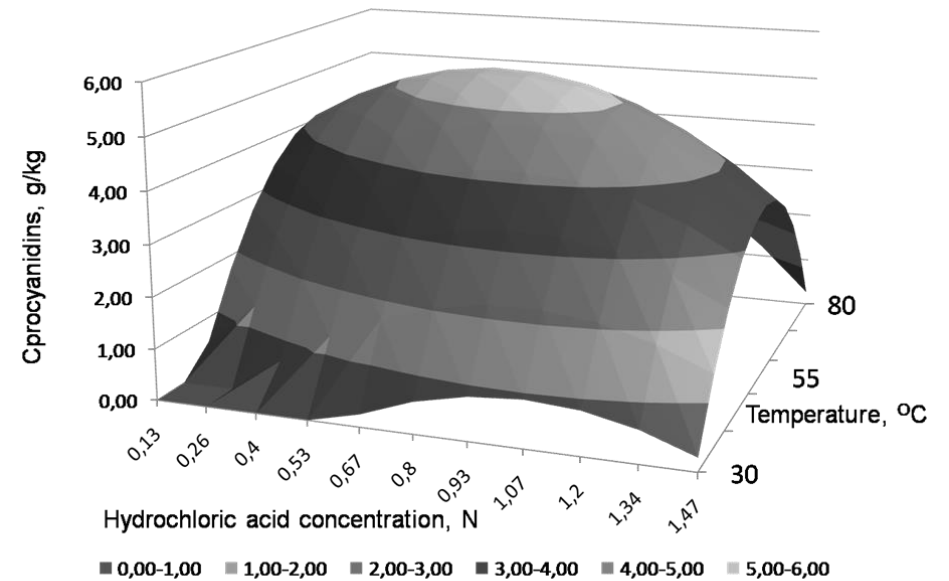


Experimental design

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



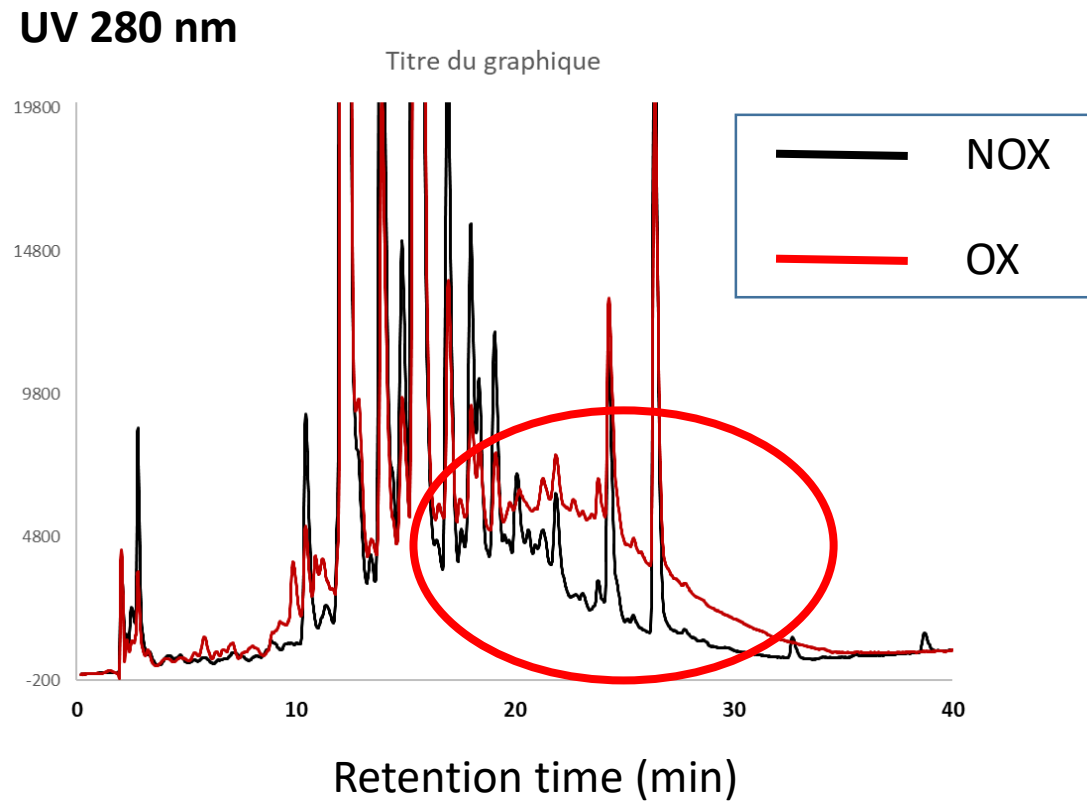
Storage and ageing



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

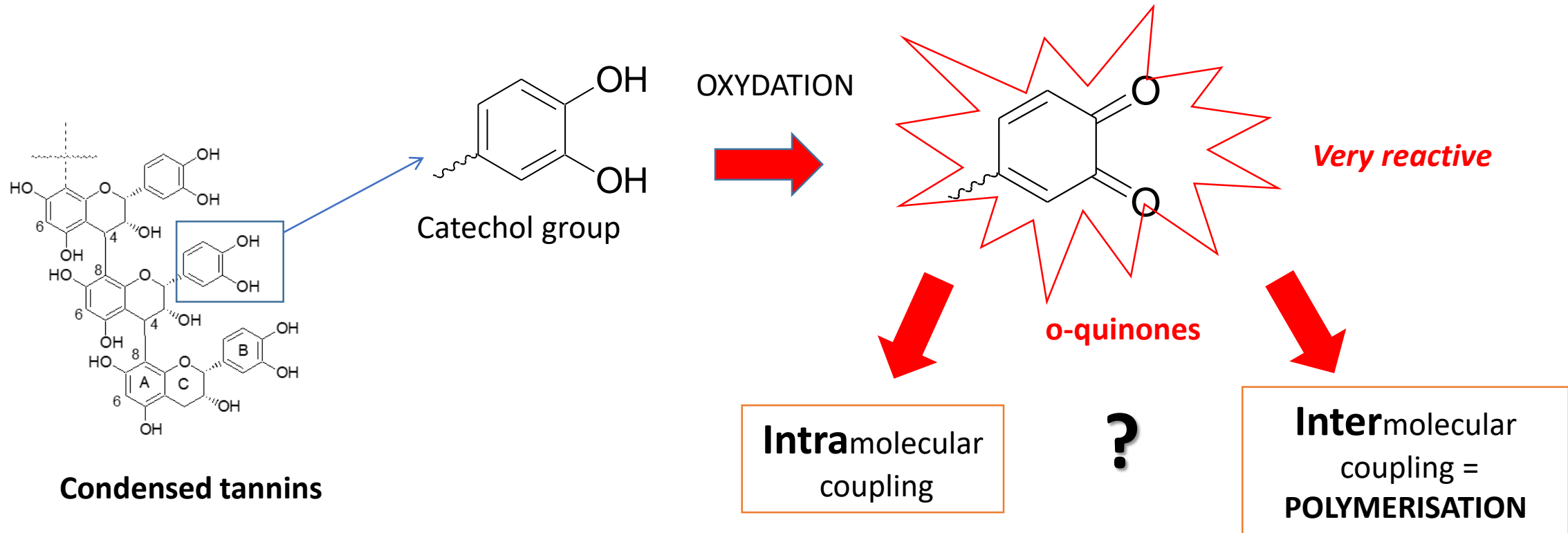
The limit of UV detection for LC analysis of oxidized polyphenols

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..)



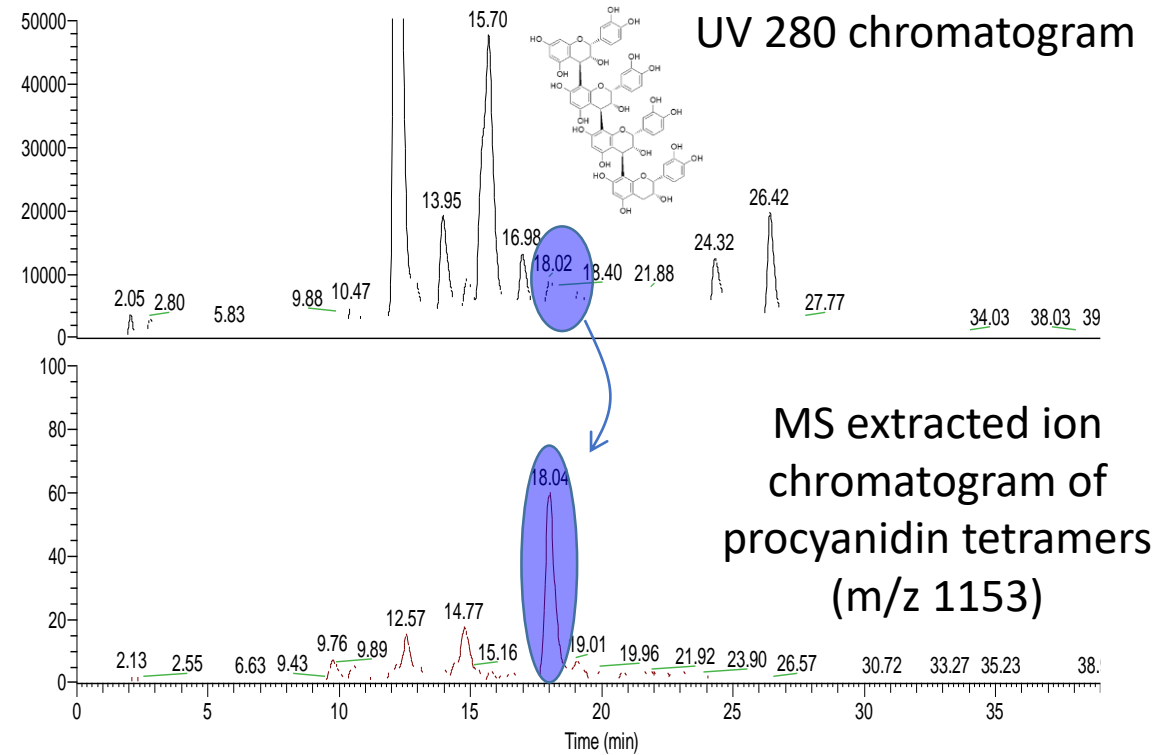
...A great diversity of oxidation products...

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

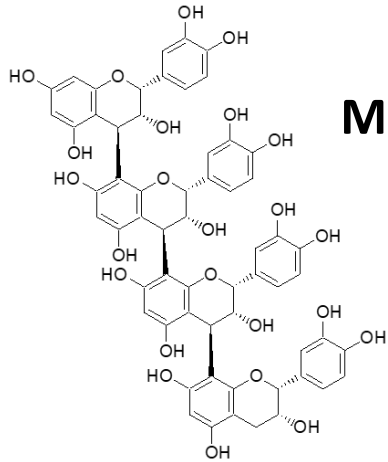


Oxidized apple juice

RT: 0.00 - 40.00 SM: 7B



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

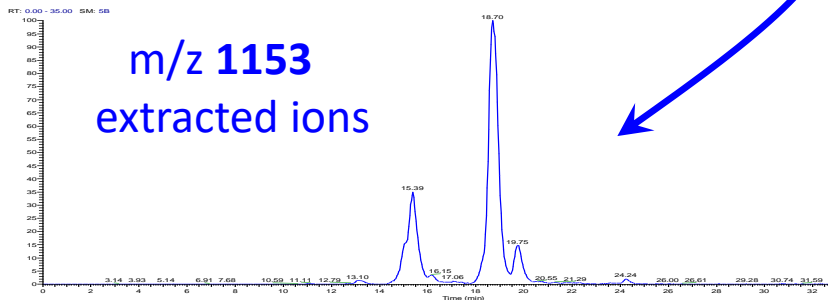
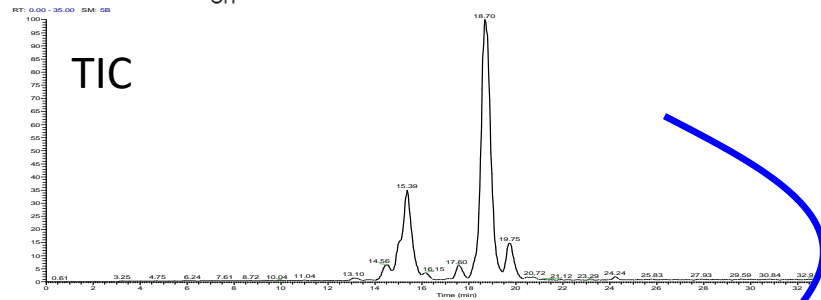


Mw = 1154 da

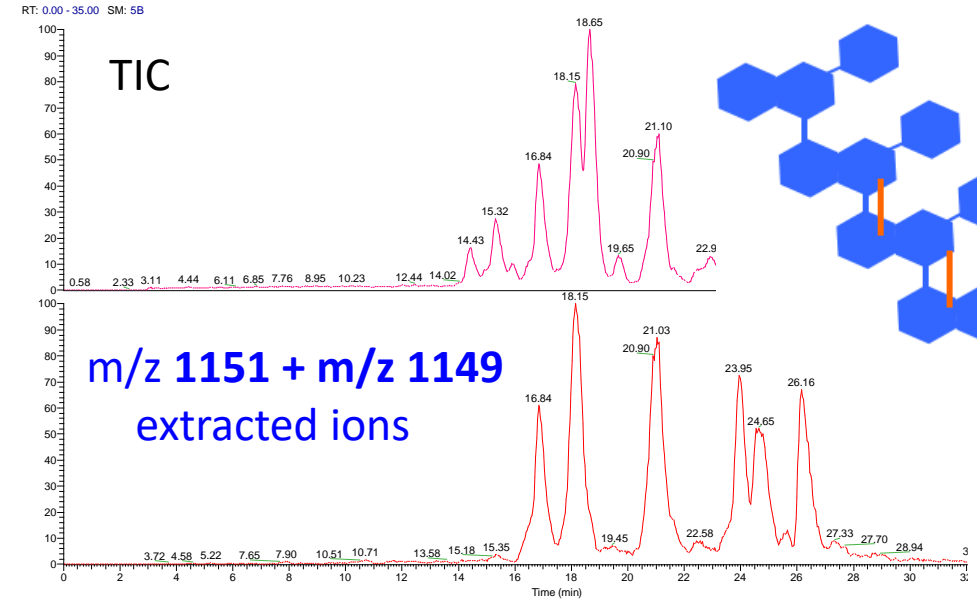
Native (non oxidized)
Tetramer
(DP4) fraction

OXIDATION
(IO4²⁻ fixed on resin)

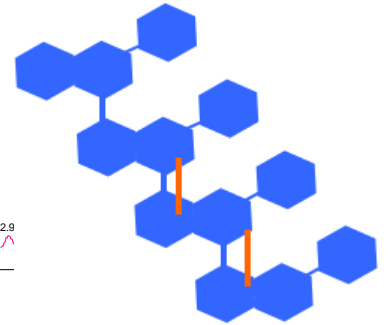
Many more peaks corresponding to oxidation products



Almost all
molecular
species are
native
tetramers



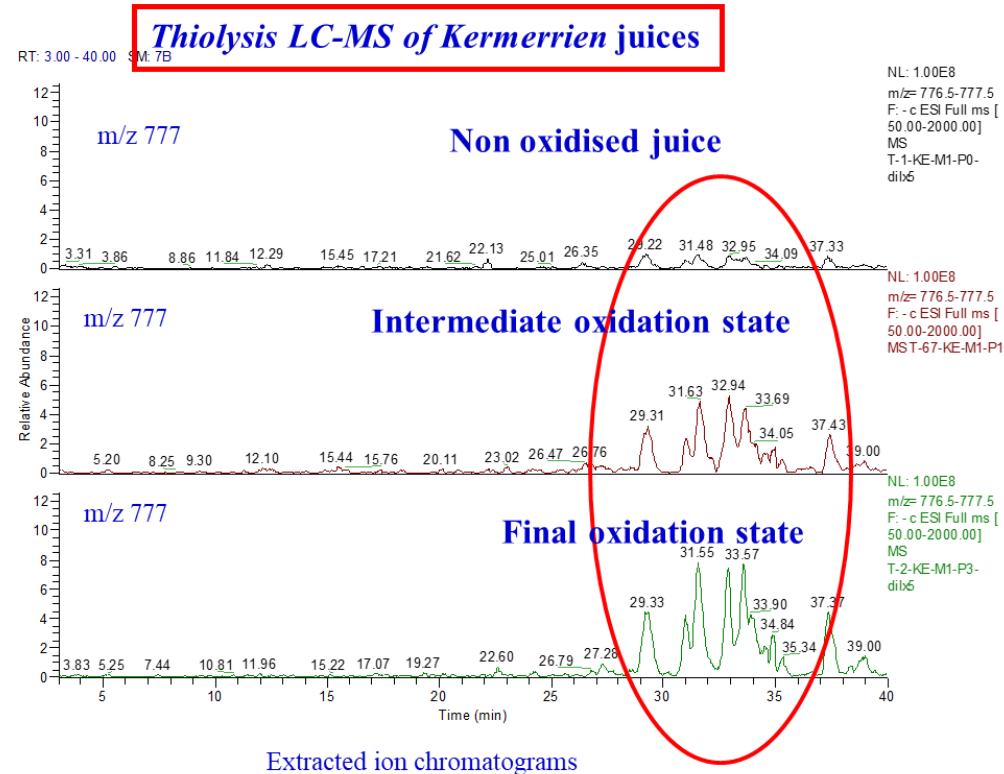
m/z 1151 + m/z 1149
extracted ions



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

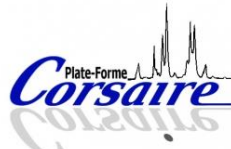


Xiaoxi Xu



Mariana-Castillo Fraire

Thank you for your attention !



Biologically Active Compounds in Foods,
Lodz, November 9-10th 2017