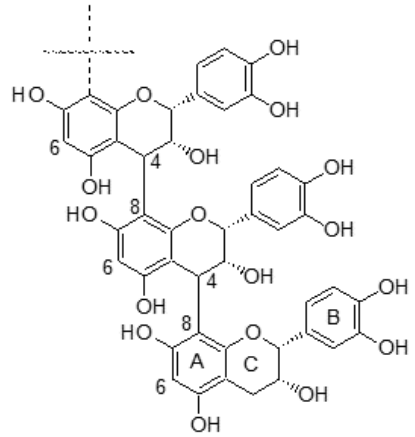
Methods for analysis of procyanidins (condensed tannins) and their oxidation products in foods.



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INRA SCIENCE & IMPACT

bia Biopolymères Interactions Assemblages

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

BACIF 2019

Our research group

PRP Group
(Polyphenols, Reactivity & Process)

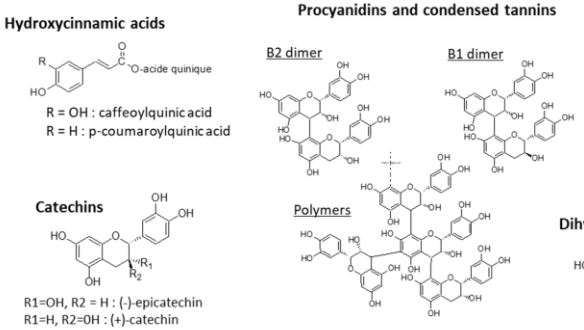
Rennes INRA Center

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



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

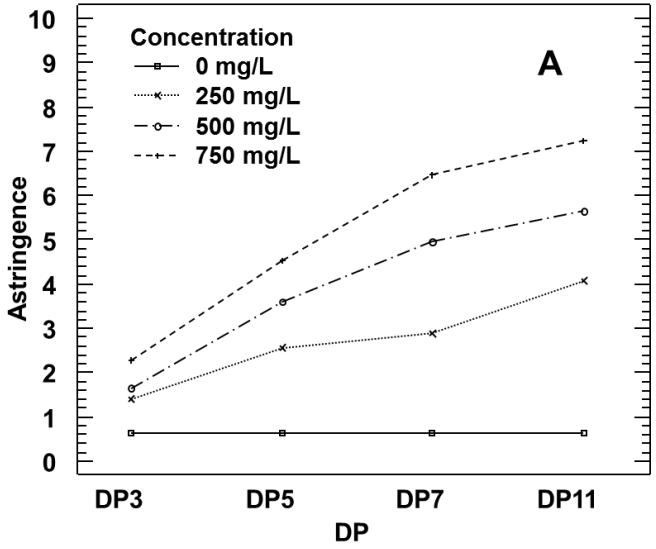
In roots and tubers (ex: Yam)

They largely contribute to polyphenol intake

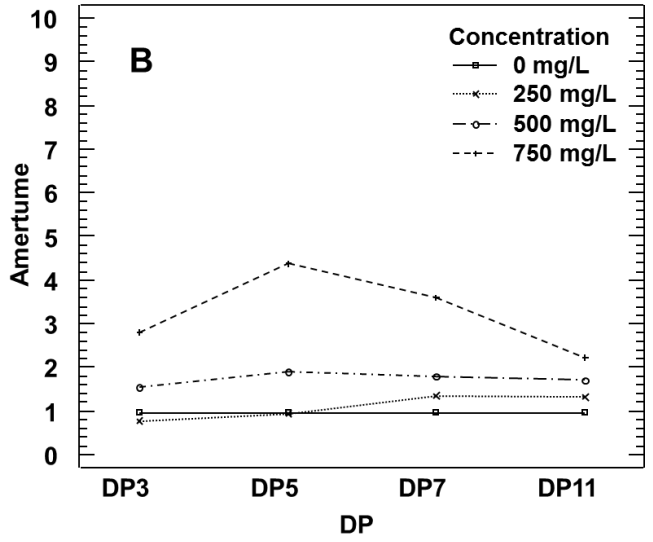
Procyanidin and their contribution to the taste

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

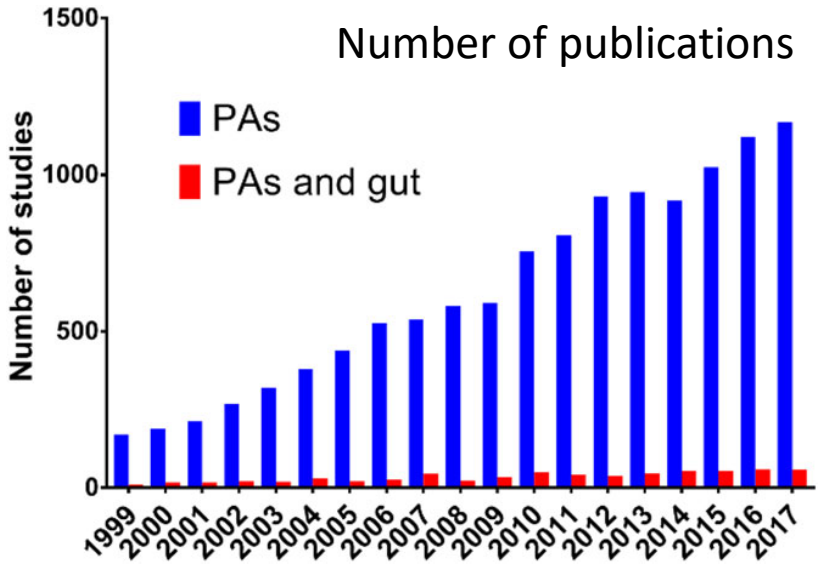
- **Antioxidant** in food and in the tractus (protection against lipid & protein oxidation)
- **Antimicrobial** (ex antibacterial activity of cranberry procyanidins)
- **Cardioprotective, antiinflammatory** and **neuroprotective** effects

Comprehensive REVIEWS in Food Science and Food Safety

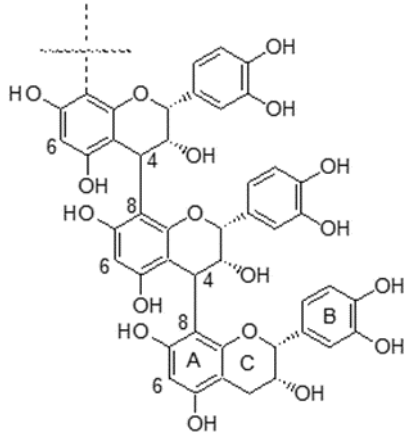
Rethinking the Mechanism of the Health Benefits of Proanthocyanidins: Absorption, Metabolism, and Interaction with Gut Microbiota

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 ……

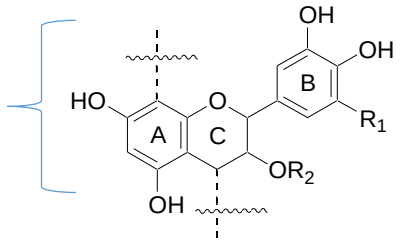


…..because of the difficulty for their extraction, structural analysis and quantification.

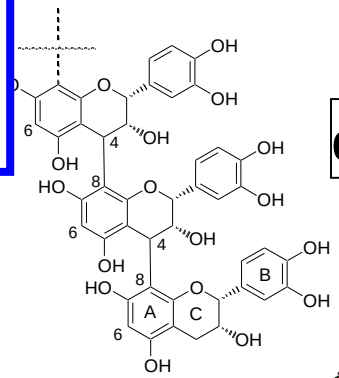
Diversity of molecular structures of proanthocyanidins

Flavanol oligomers, from dimers……to very large polymers

The flavanol unit (catechin unit)



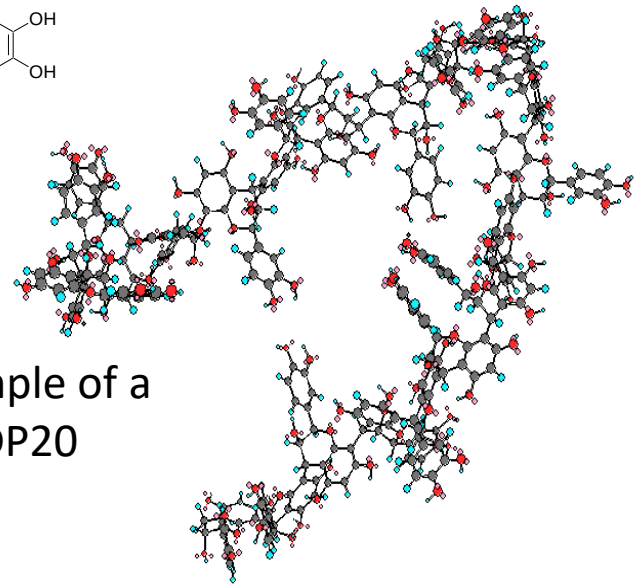
Colourless and highly water soluble



DP : degree of polymerisation

oligomers

Polymers

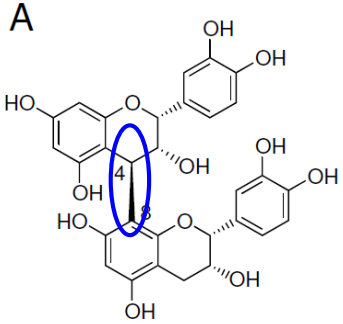


Example of a DP20

B-type (one Interflavan Linkage)

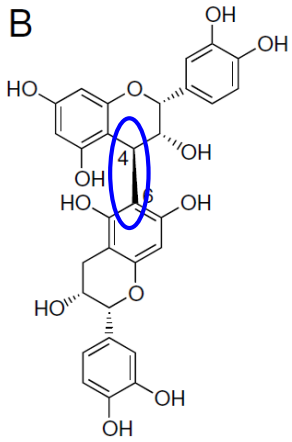
A-type (two Interflavan Linkage)

A



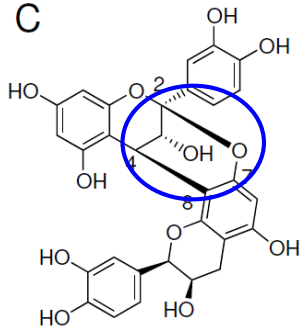
Procyanidin B2

B



Procyanidin B5

C



Procyanidin A2

DPn > 40-60 kDa

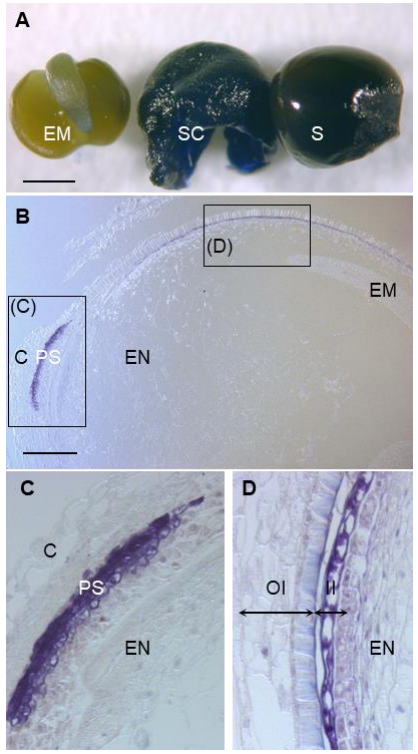
Their direct observation in plant tissues

Photonic microscopy….

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

(Dimethylcinnamaldehyde)

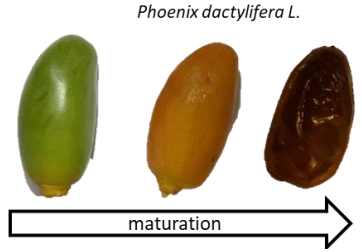
In Rapeseed during the growth of the seeds

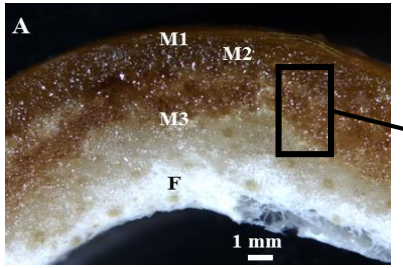


Evidence of condensed tannins in rapeseed tegument

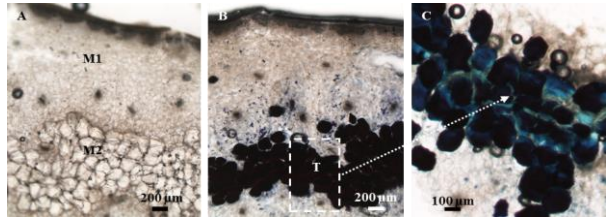
Auger et al., J. Agric. Food Chem., 2010

In dates (Phoenix dactylifera)





DMACA staining



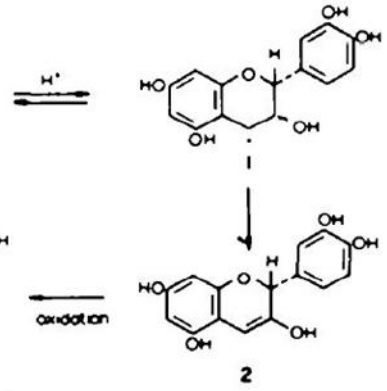
Hammouda et al., J. Agric. Food Chem., 2014

Their direct detection in (food) samples

Strong acid treatment in organic solvent at elevated temperature

COLORLESS

Procyanidine



Cyanidine

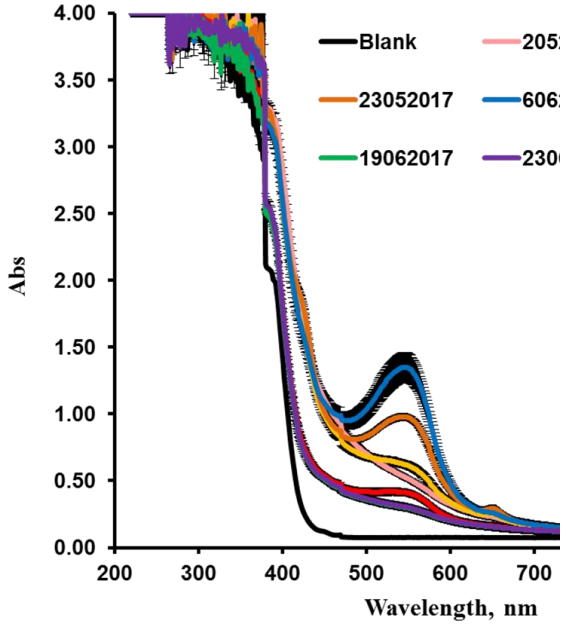
RED COLOR

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

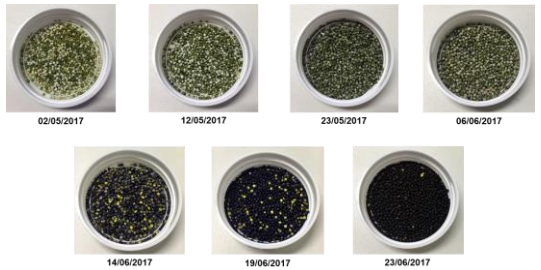
Bate-Smith, Biochem. J., 1954
Porter et al., Phytochemistry, 1986

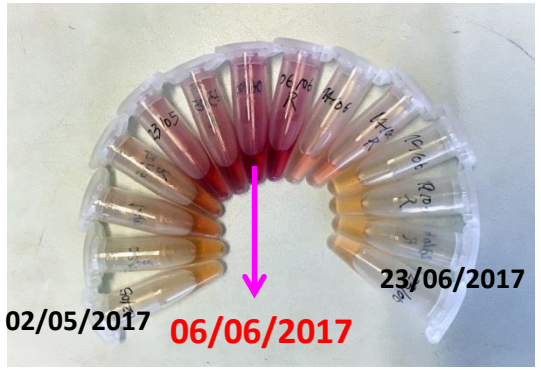
Example of Rapeseed meals

UV visible sprectrum



Maturation of the seeds





Yu et al., publication ongoing

….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

Water, alcohol, aqueous acetone extraction

<u>Extractable procyanidins</u> (Soluble fraction)

<u>Non extractable procyanidins</u> (in the Insoluble residue)

Semi-preparative HPLC

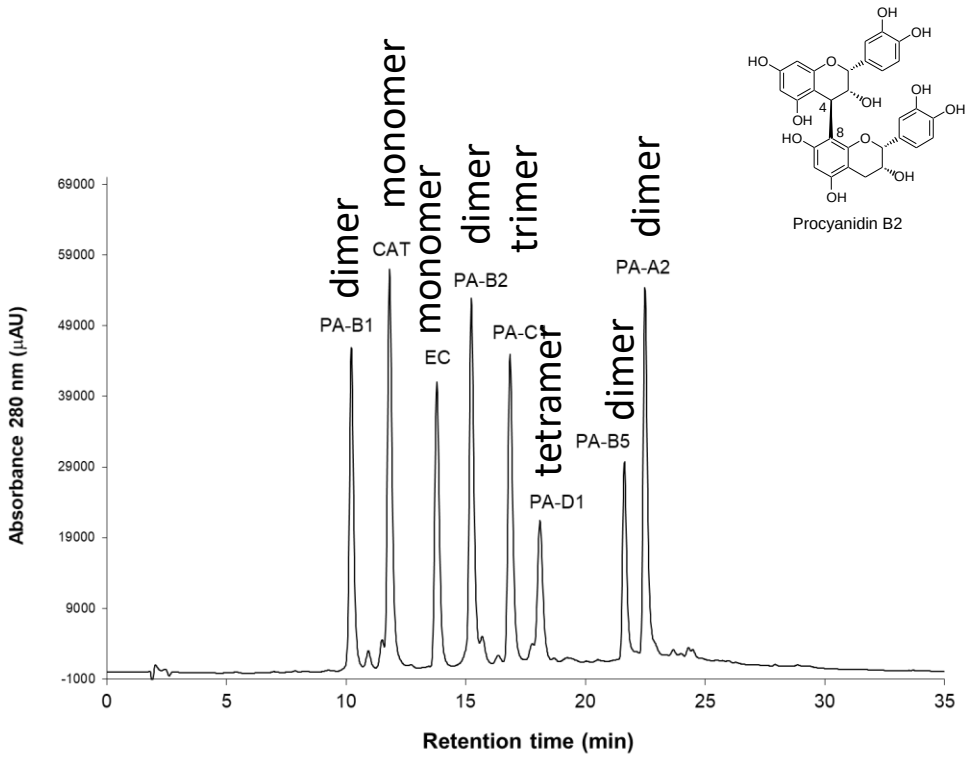
C18 SEP PACK Cartridges

Purified Procyanidin frations

Direct HPLC is not adapted to PA polymers analysis

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

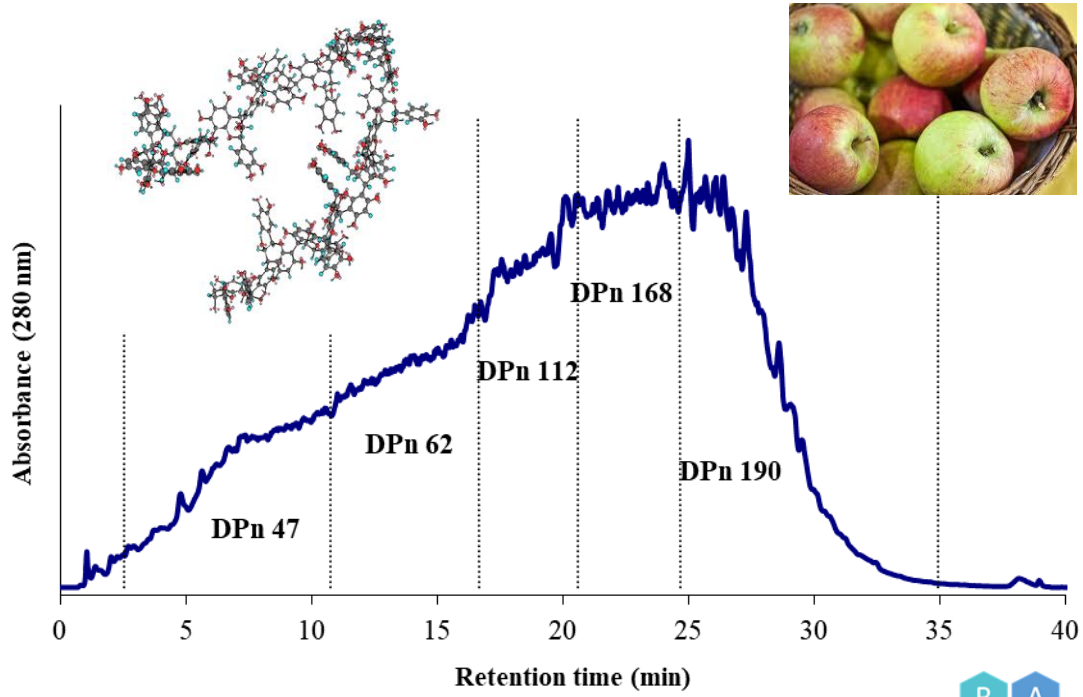
A mixture of purified or commercial standards



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



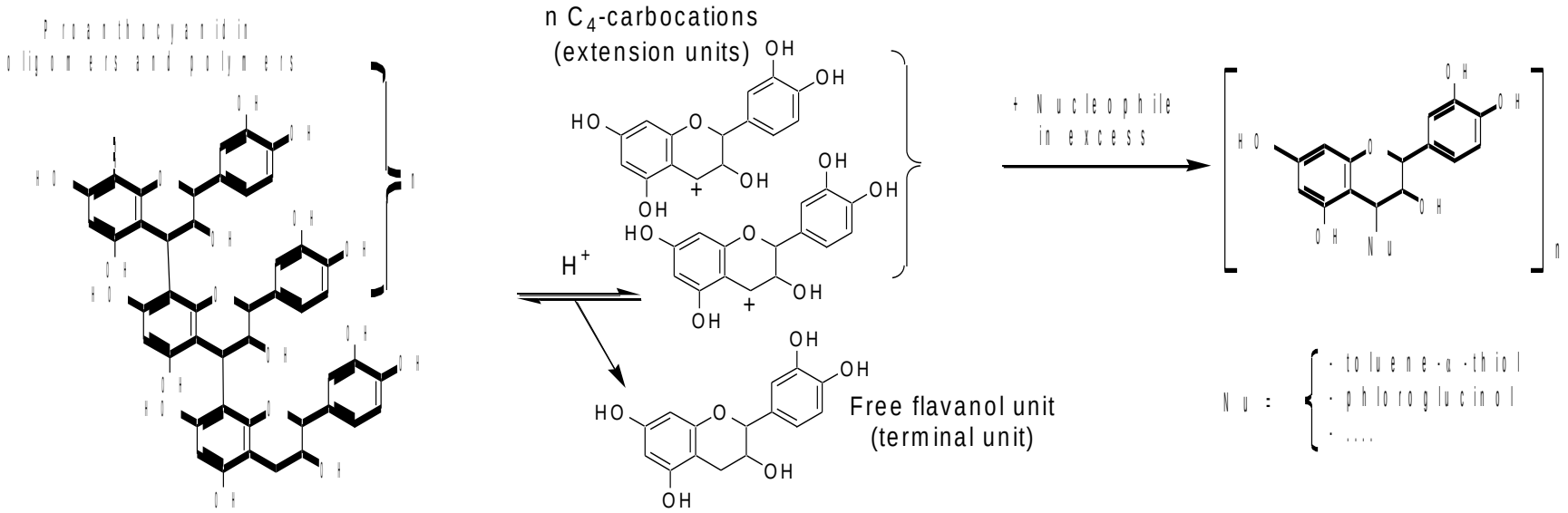
DPn = Average degree of polymerisation

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

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

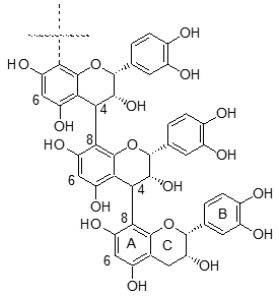
Thiolysis & Phloroglucinolysis reactions



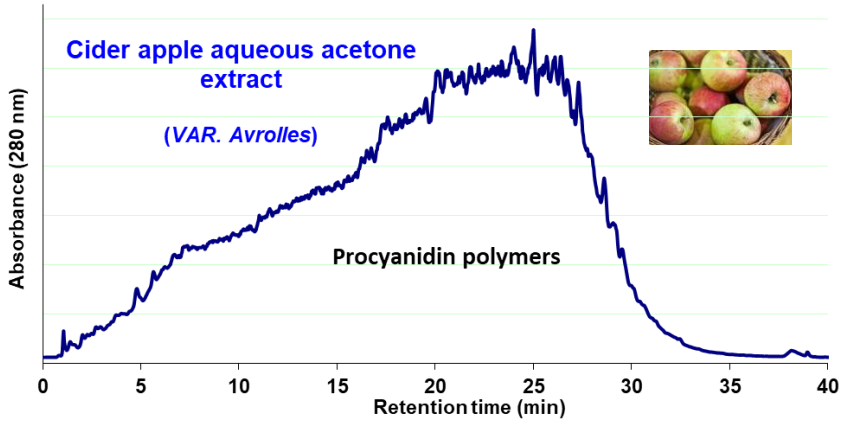
Polymers are converted into monomer derivatives

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

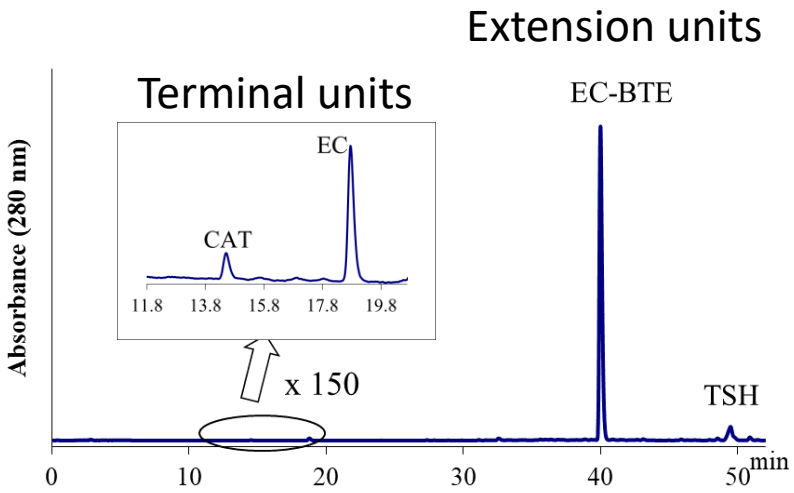
HPLC analysis of the Thiolysis or Phloroglucinolysis reaction media



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 (DPn = all units/ term. units ratio)
- Concentration (sum of the concentrations of all units)

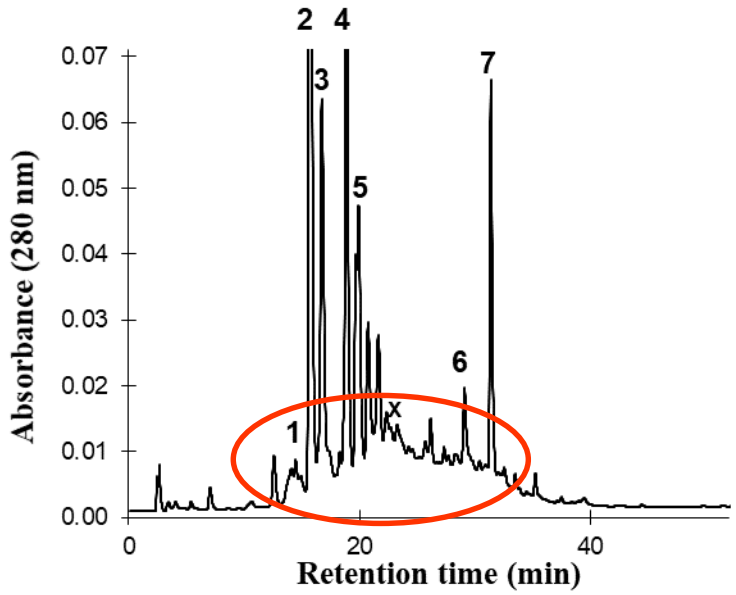
For instance,
DPn = 70 for this aqueous-acetone extract from Avrolles

HPLC analysis of the Thiolysis or Phloroglucinolysis reaction media

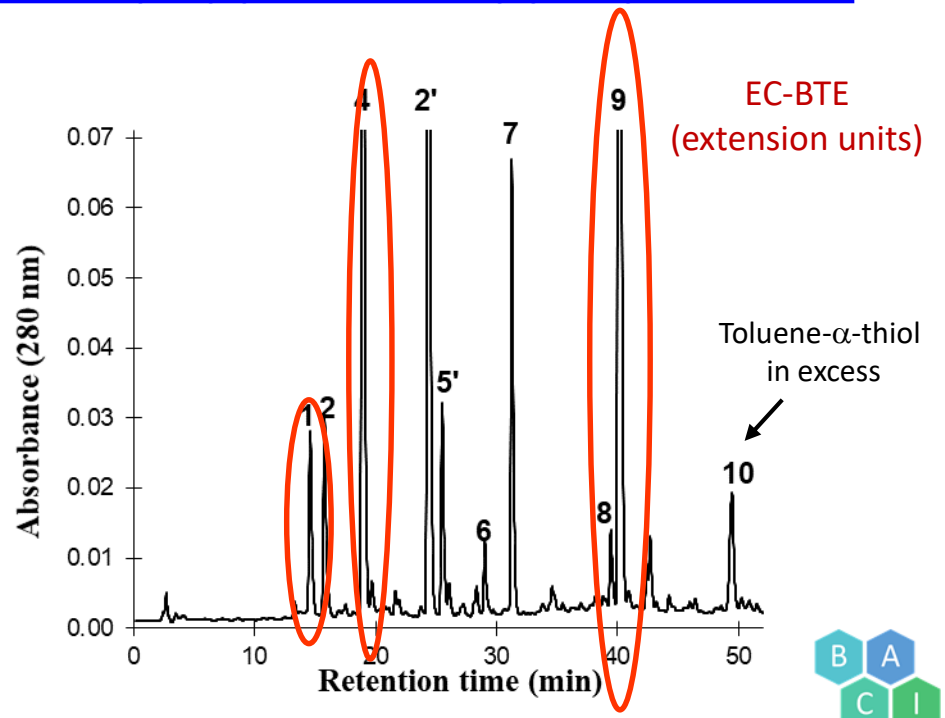
Direct application on **crude apple samples (powders, freeze-dried juices or ciders)**

Cider apple *(Var. Kermerrien)*

Direct analysis (crude methanol extract)



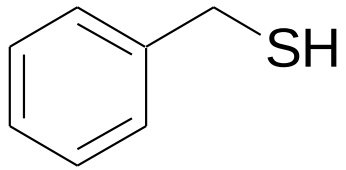
After thiolysis
(directly applied on apple powder)



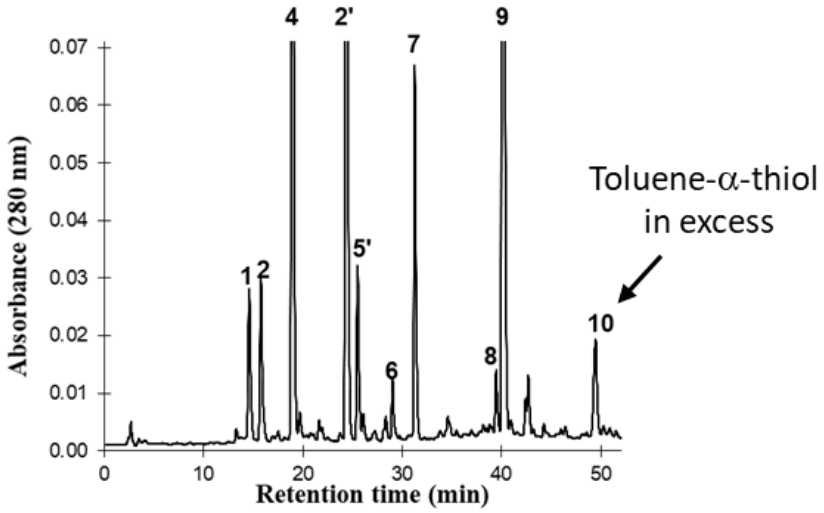
Guyot et al., Meth. Enzym, 2001

From Thiolysis….to Phloroglucinolysis

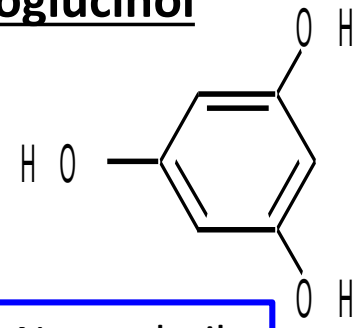
Toluene-α-thiol



- Volatile
- Highly nauseous
- Toxic

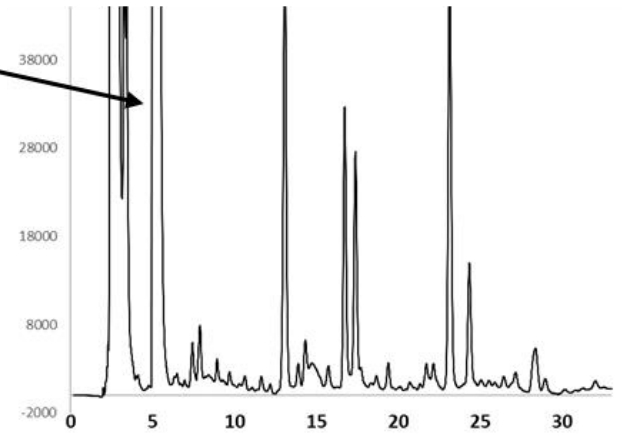


Phloroglucinol

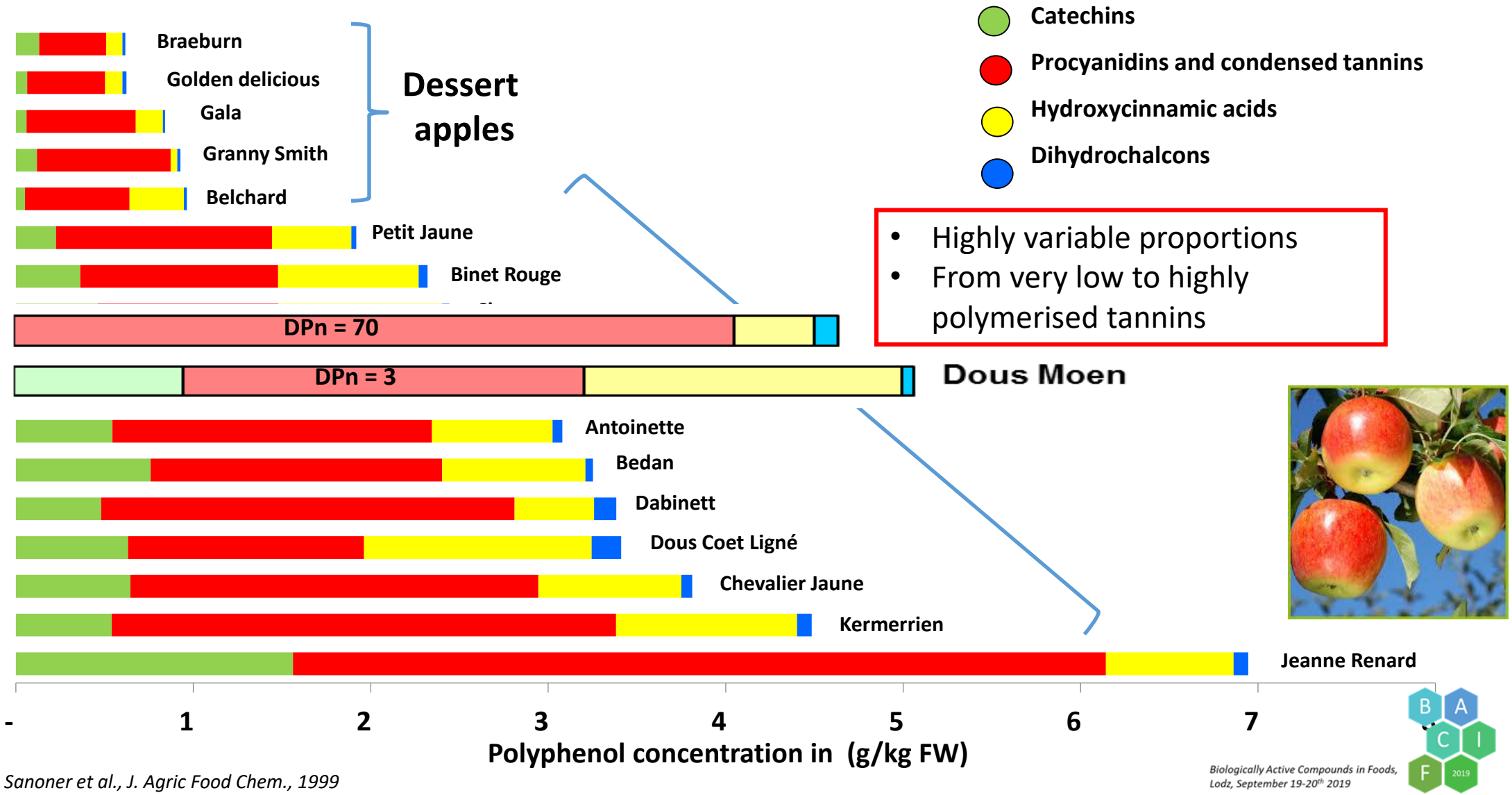


- Non volatile
- Odourless
- Safe

Phloroglucinol
in excess



Application to detailed procyanidin analysis in dessert and ciders varieties



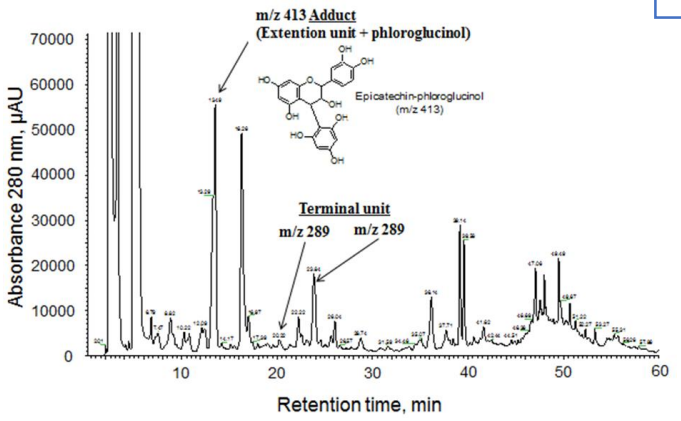
- Highly variable proportions
- From very low to highly polymerised tannins

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

Acidolysis conditions should be optimized according to sample type

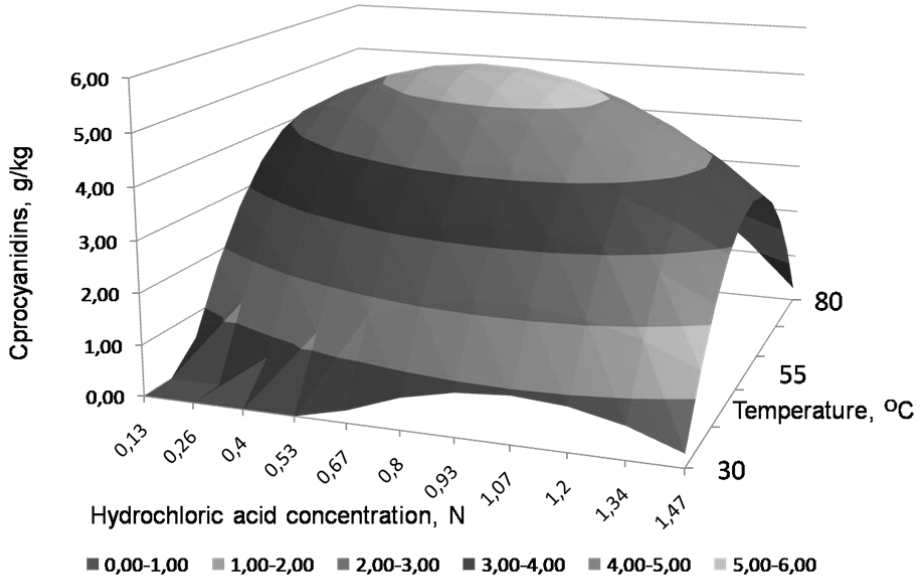
Example of condensed tannins in **Rapeseed hulls**

Phloroglucinolysis-HPLC



Experimental design

[Hydrochloric acid] & Temperature



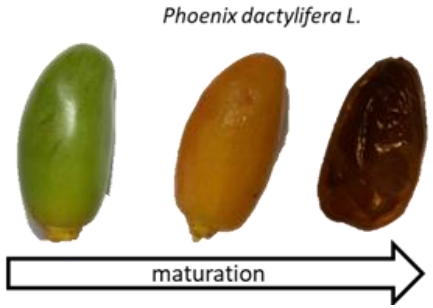
Yield of acidolysis is strongly influenced by the reaction conditions

Yu et al., to be submitted to J.Agric. Food Chem.

….Are analytical methods for “native tannins” also adapted to analysis of “<u>oxidized tannins</u>” ?

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

Fruit maturation and drying



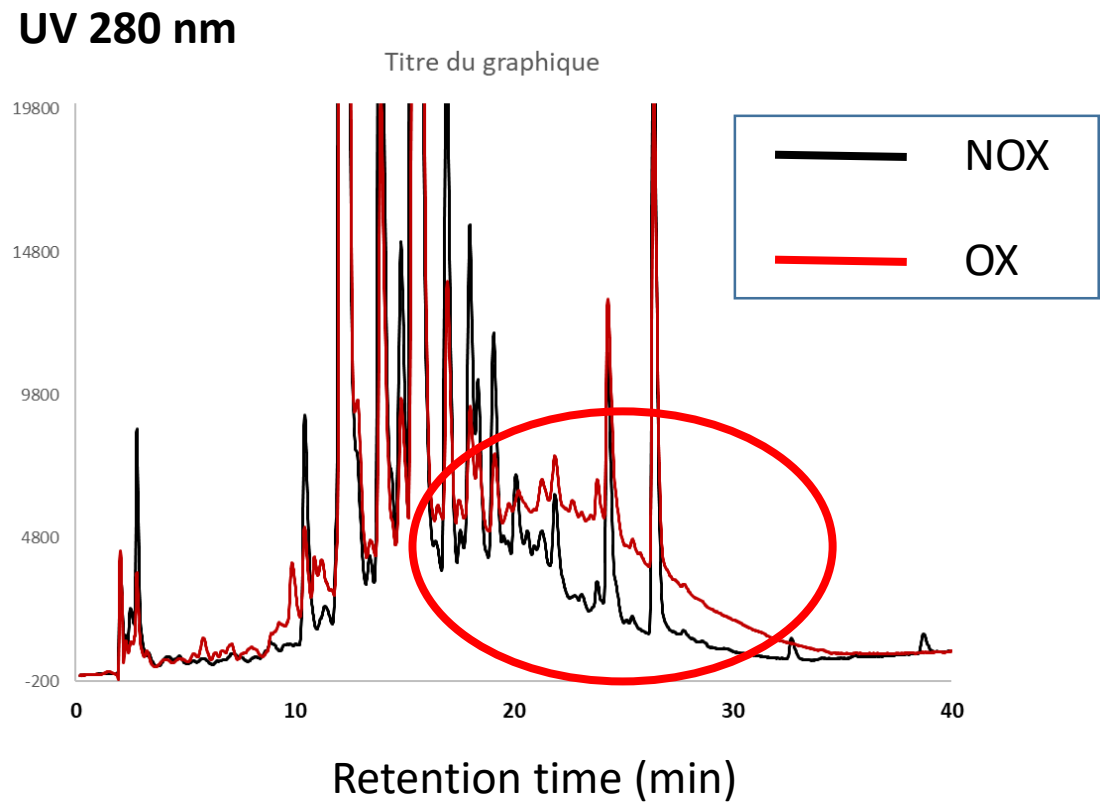
Processing

Storage and ageing

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

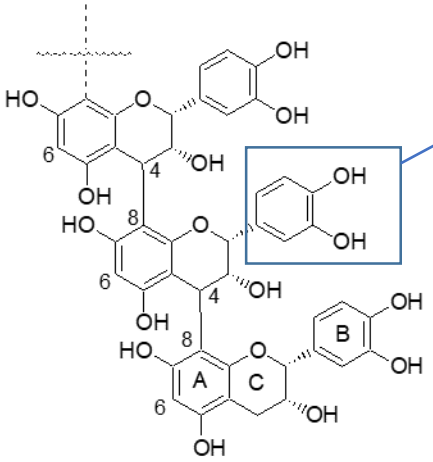
The limit of UV detection for LC analysis of oxidized polyphenols

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

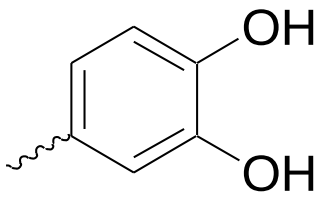


Oxidized polyphenols do not give well-resolved peaks on UV Chromatograms

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

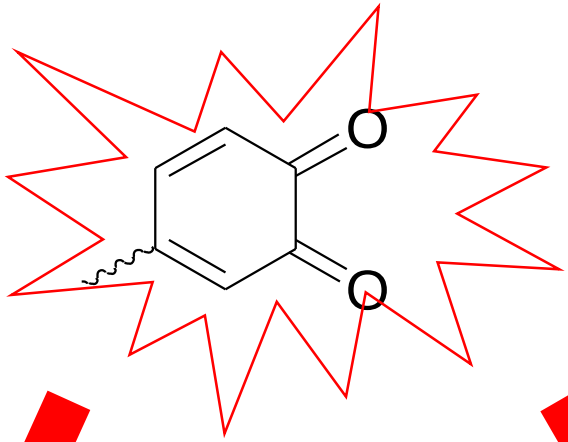


Condensed tannins



Catechol group

OXYDATION



Very reactive

o-quinones

Intramolecular coupling

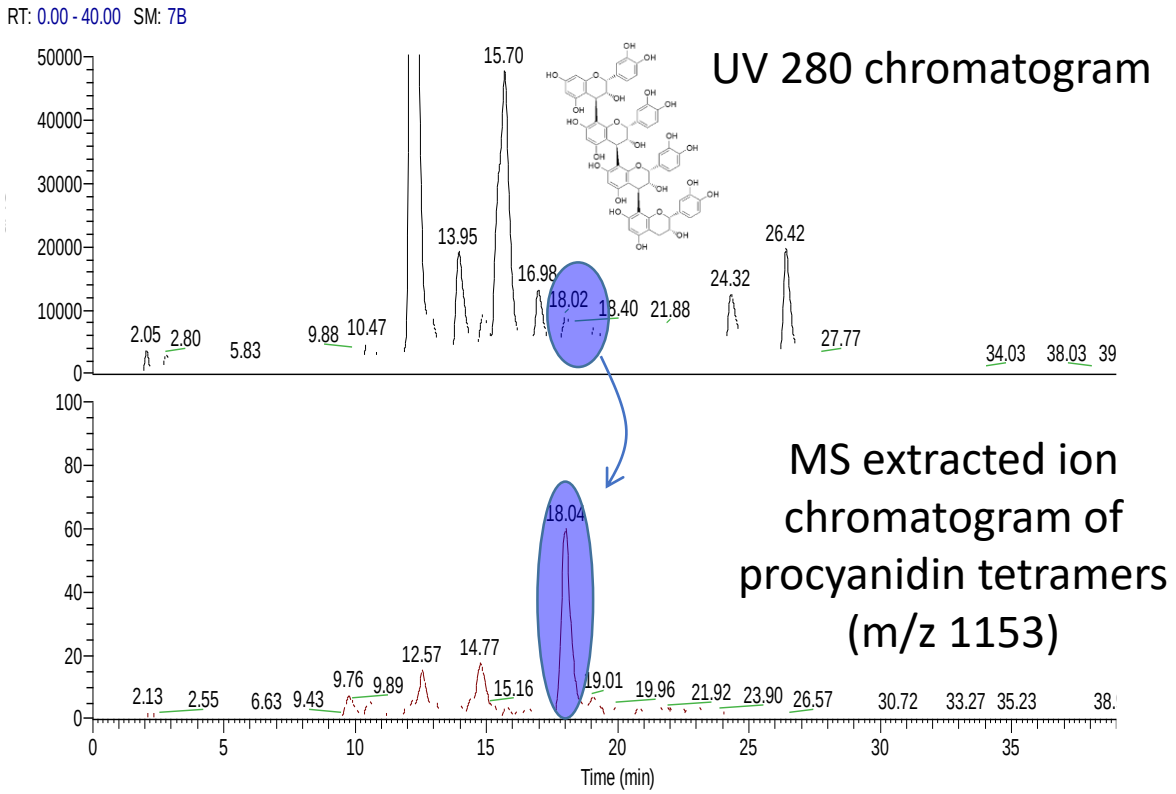
?

Intermolecular coupling = **POLYMERISATION**

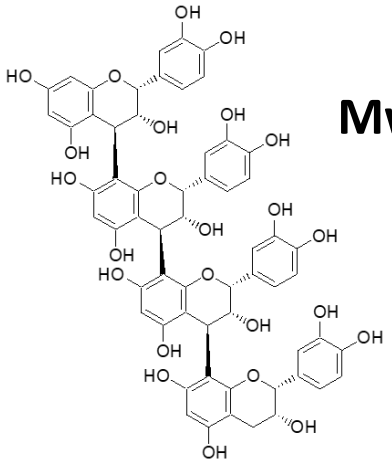
…A great diversity of oxidation products…

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

Oxidized apple juice



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

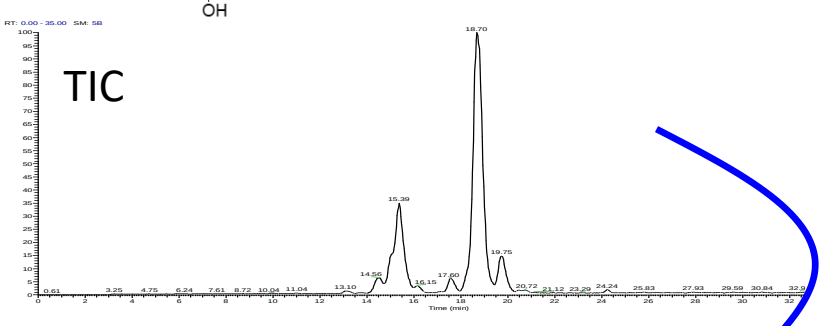


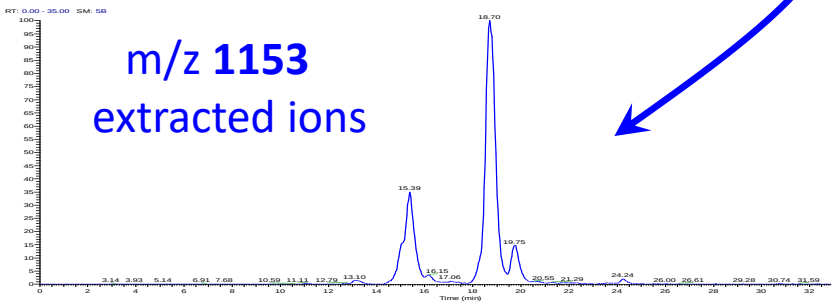
Mw = 1154 da

Native (non oxidized) Tetramer (DP4) fraction

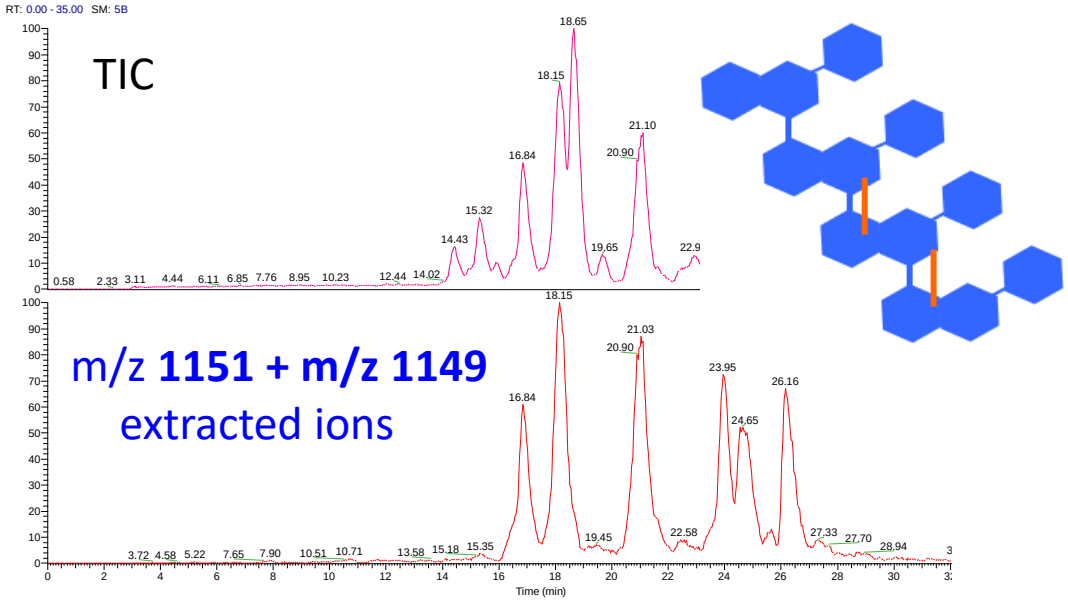
OXIDATION
(IO_4^{2-} fixed on resin)

Many more peaks corresponding to oxidation products





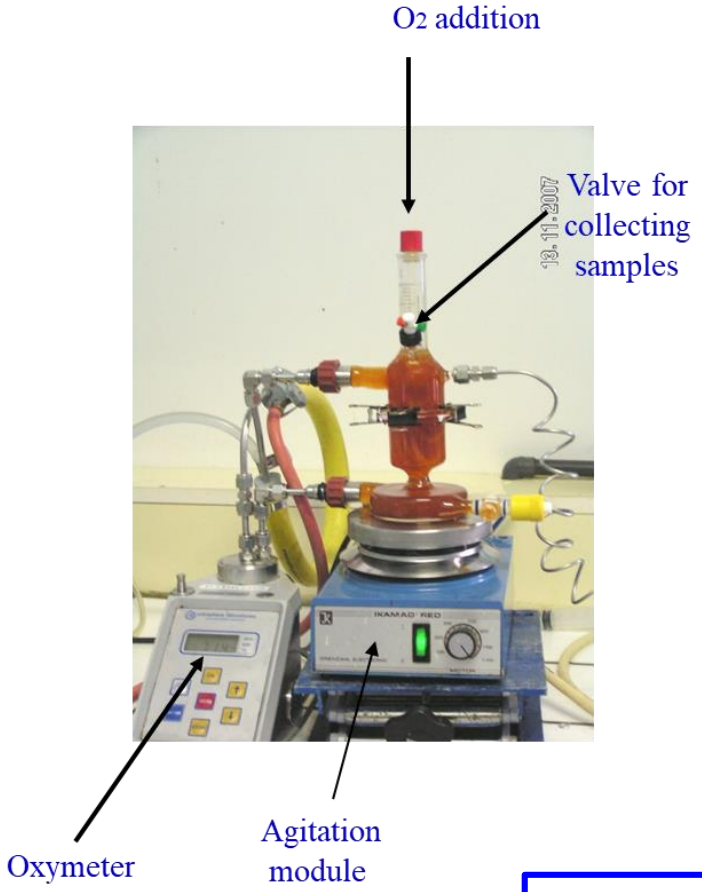
Almost all molecular species are native tetramers

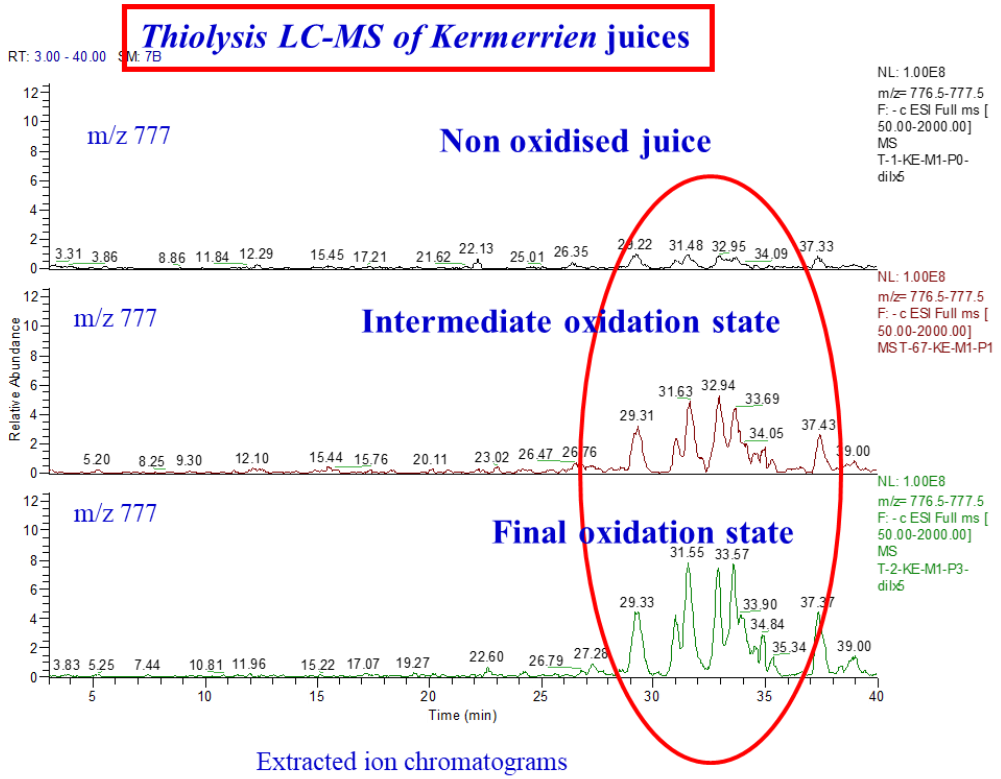


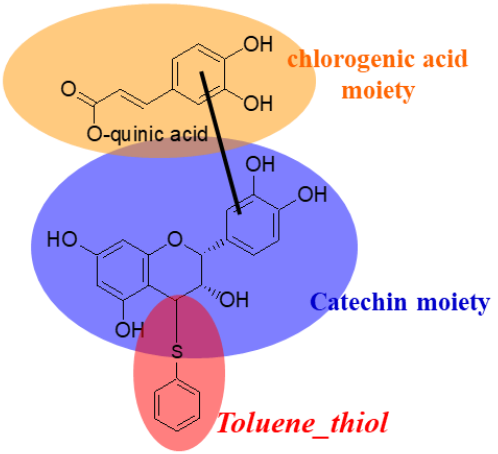
Loss of 2 or 4 hydrogen atoms : a signature of INTRA molecular coupling

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

Oxidation kinetic of a cider apple juices







m/z 777

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

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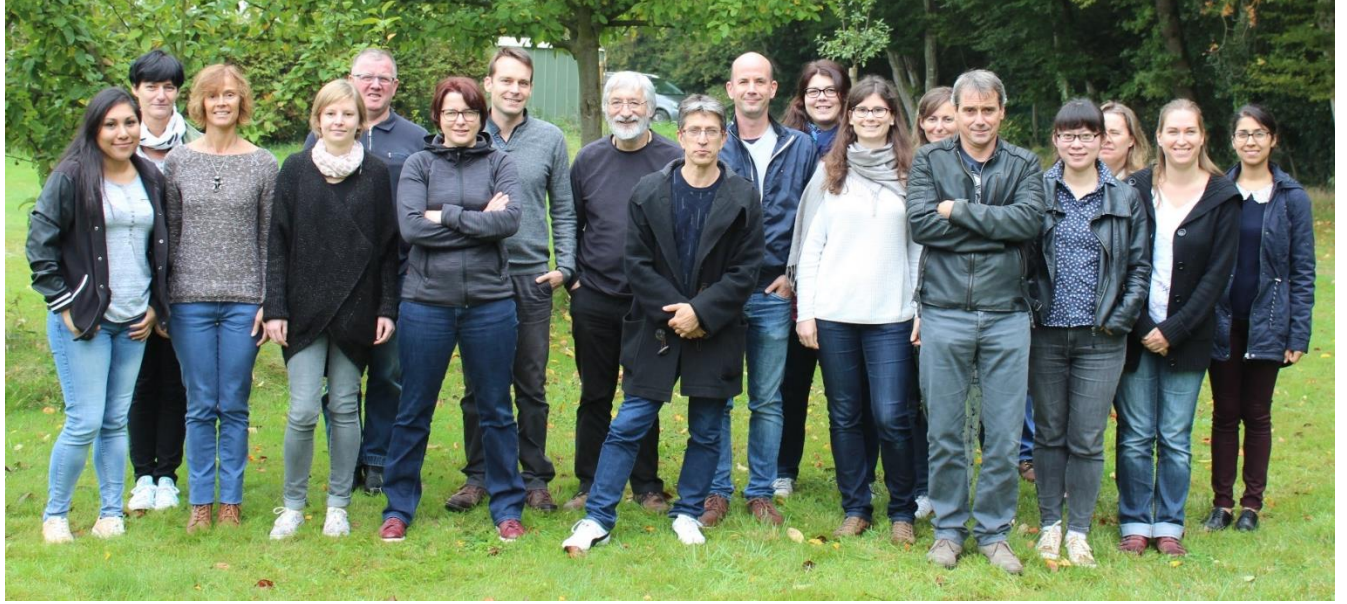
Thank you for your attention !



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