

# Polyphenol-cell wall interactions: mechanisms and consequences

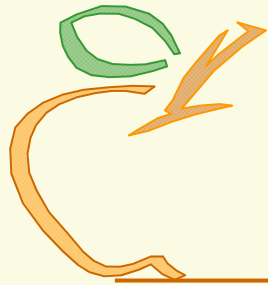
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Carine Le Bourvellec



UMR408 Sécurité et Qualité des Produits d'Origine Végétale,  
INRA, Université d'Avignon et des Pays du Vaucluse

*Biologically Active Compounds in Food,  
15-16 oct 2015, Łódź (Poland)*





# Why attempt to understand these interactions?

## ↳ Polyphenols

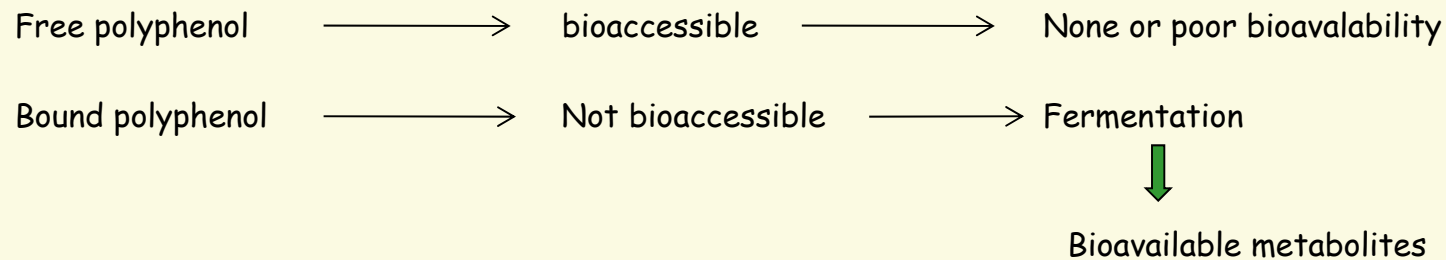
- FOOD Ubiquitous in plant foods; roles in taste and colour
- HEALTH Effects on degenerative diseases

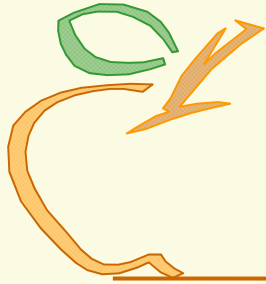
## ↳ Extraction of polyphenols

- Understand the mechanisms for retention

## ↳ Health effects: two mechanisms

- Antioxidants: prevention of oxidation in the stomach
- Specific actions - interaction with receptors
  - **Must be bioavailable - phenols or metabolites**
  - **Relevance of colonic fermentation metabolites**





## ↖ Introduction

- Occurrence of polyphenol and polysaccharide adducts
- Proanthocyanidins
- Cell wall preparation

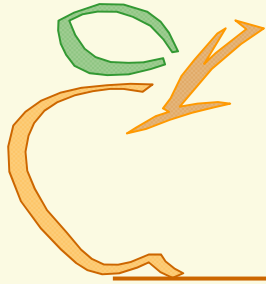
## ↖ Non-covalent interactions

- Binding isotherms
- Isothermal titration calorimetry
- Modification of cider composition

## ↖ Formation of procyanidin cell wall adducts

- Preparation by oxidation
- Effect on extractability of pectins
- Formation during heat treatment

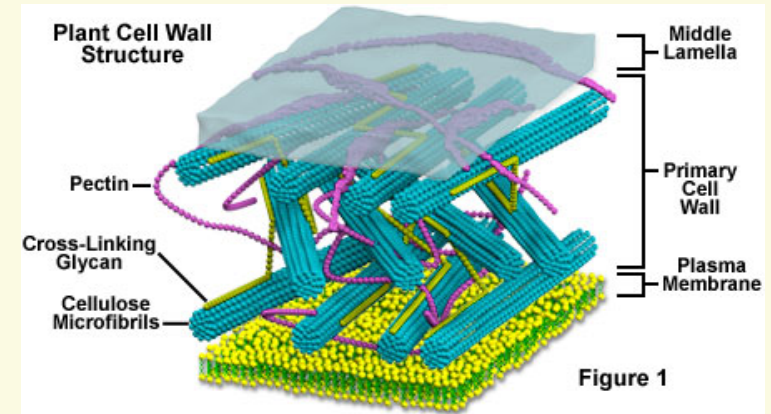




# Polyphenols in the cell walls

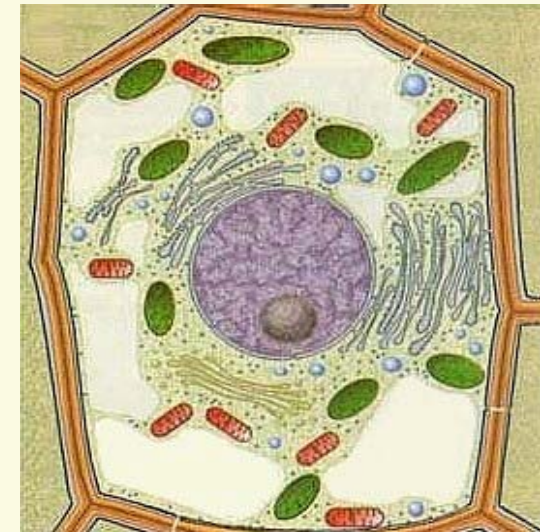
## ↳ Structural in the cell walls

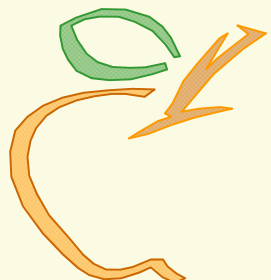
- Ferulic acid - xylans and pectins
- Biosynthesis of lignin
- « Polyphenol - polysaccharide complexes »
  - Detected after extraction of cell walls
  - Very poorly defined structures



## ↳ Some intracellular polyphenols (tannins) bind spontaneously to macromolecules

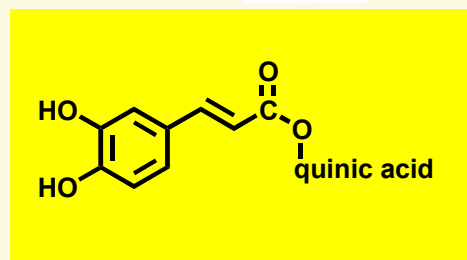
- Well known for proteins
- What about cell-wall polysaccharides?
- What happens during tissue disruption?



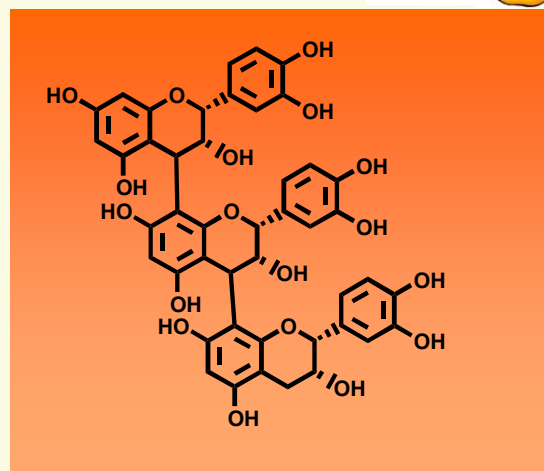


# Polyphenols of apples and pears

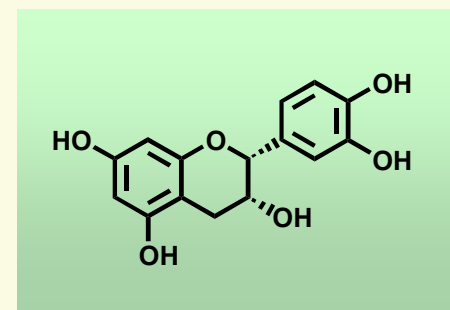
OH-cinnamic acids



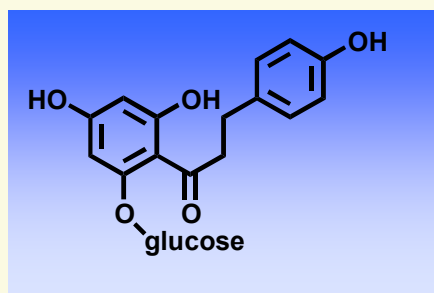
Procyanidins



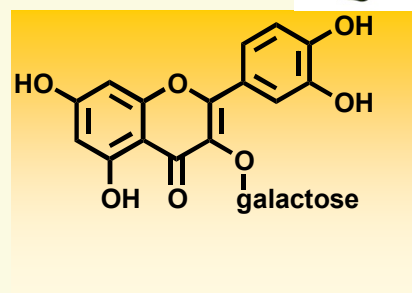
Catechins



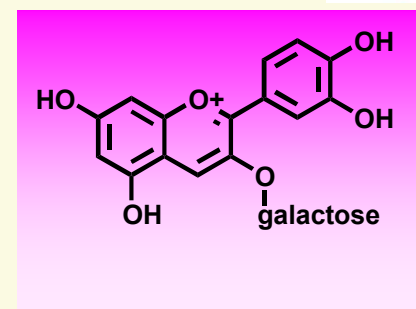
Dihydro-chalcones

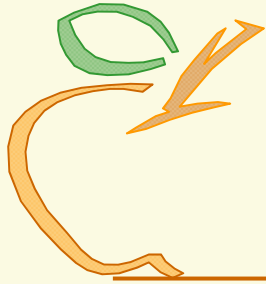


Flavonols



Anthocyanes

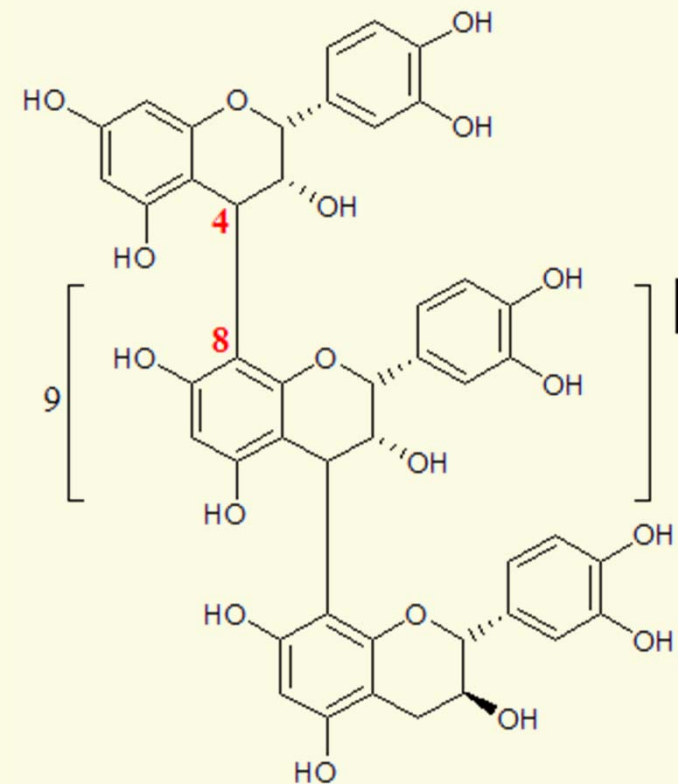


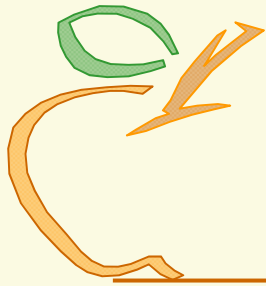


# Proanthocyanidins

## ↳ Proanthocyanidins = « condensed tannins »

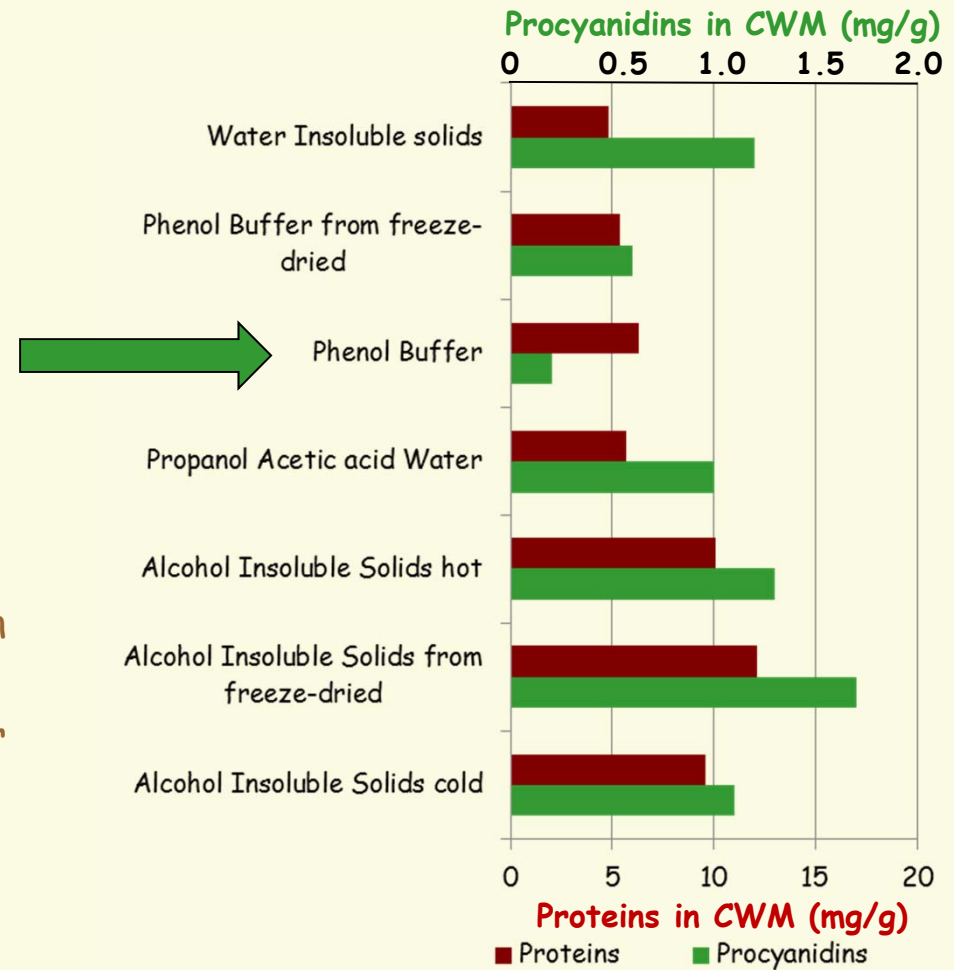
- Flavanol oligo and polymers
- Intracellular
- Characterized by:
  - Constitutive unit
  - Interflavanol binding
  - Degree of polymerization
- Abundant in apple and pear:  
procyanidins
  - Epicatechin (catechin)
  - B-type, primarily 4-8 binding
  - DP usually 3-8, up to a 100
  - Concentrations up to 5 g/ kg

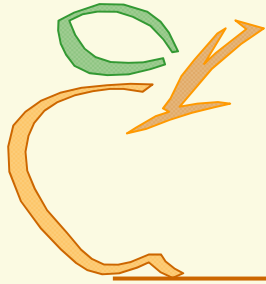




# Preparing cell walls without procyanidins

- ↳ Tests were carried out on apple to modulate CWM composition
- ↳ The « best » procedure was Phenol - Buffer
- ↳ It included
  - Extensive washing at 4°C in apple-like buffer
  - an acetone-water step for procyanidins extraction





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## ↖ Non-covalent interactions

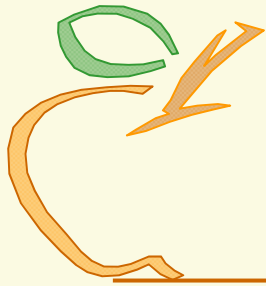
- Binding isotherms
- Isothermal titration calorimetry
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## ↖ Formation of procyanidin cell wall adducts

- Preparation by oxidation
- Effect on extractability of pectins
- Formation during heat treatment







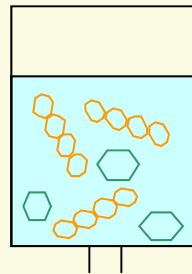
# Quantifying interactions by binding isotherms

## Model suspensions

Purified procyanidins

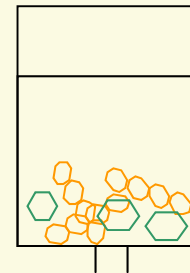


Cell walls  
Devoid of phenolics



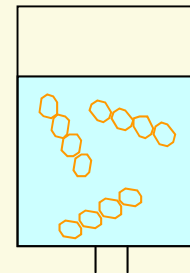
1-Incubation

2-Filtration



Bound procyanidins

- Freeze-dry and thiolysis

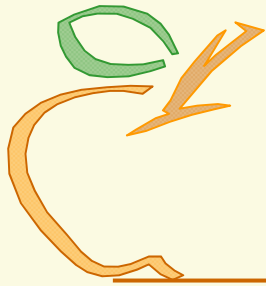


Free procyanidins

- OD 280 nm
- Freeze-dry and thiolysis

## What can be compared:

- Physico-chemical conditions (pH, T°, ionic strength, solvent...)
- Proanthocyanidins structure and size
- Cell walls: origin and pretreatment, drying...



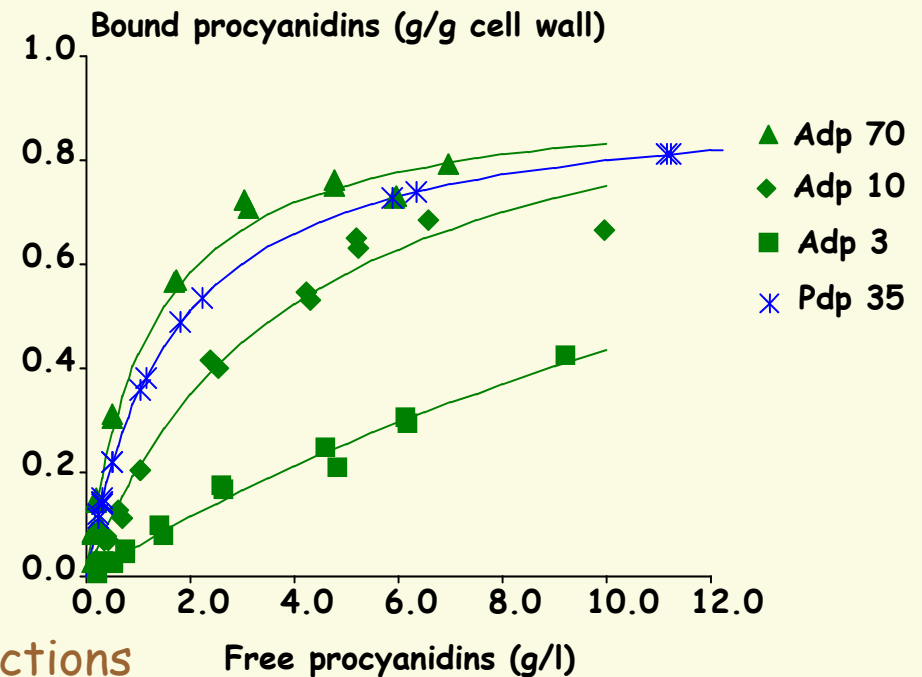
# Procyanidins adsorb spontaneously to cell walls

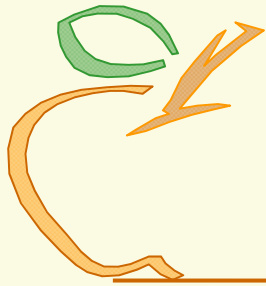
## Binding isotherms

$$PPf = \frac{N_{\max} \cdot K_L \cdot [PP_L]}{1 + K_L \cdot [PP_L]}$$

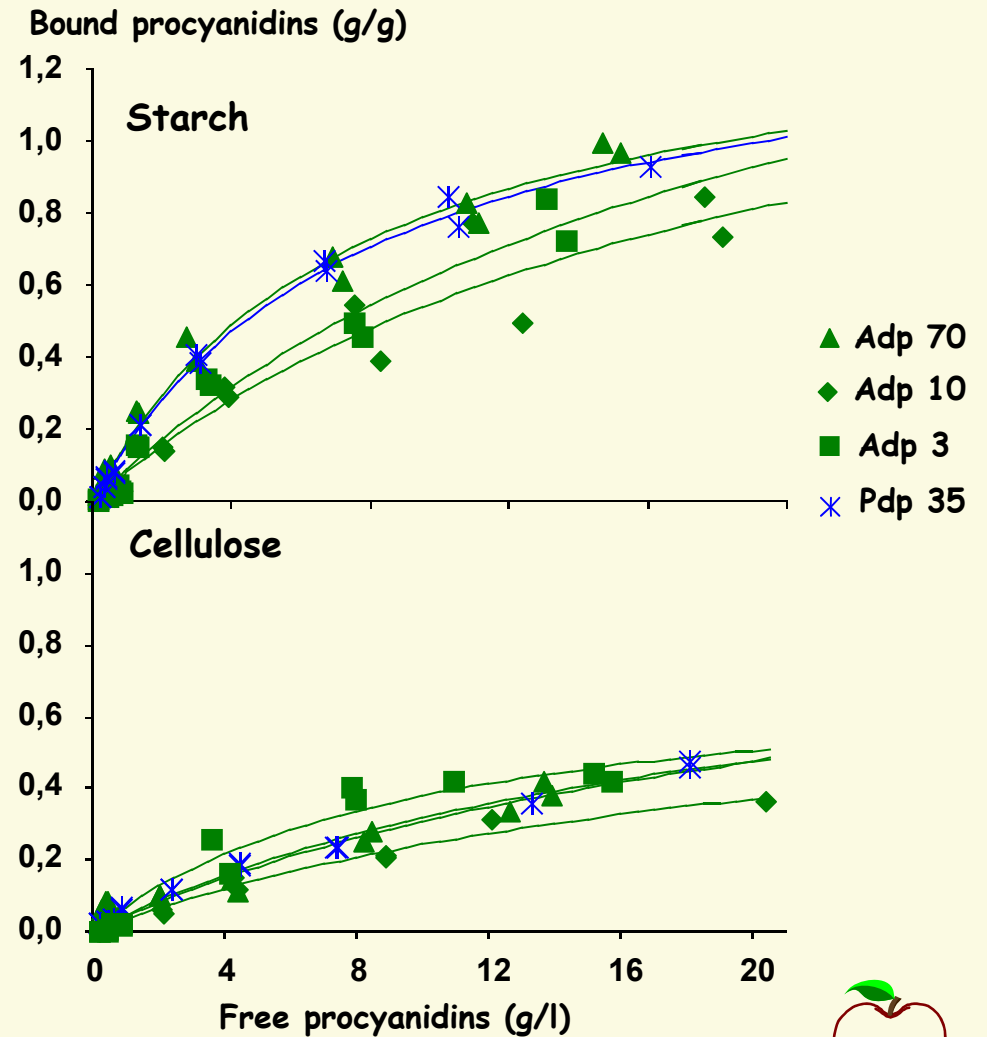
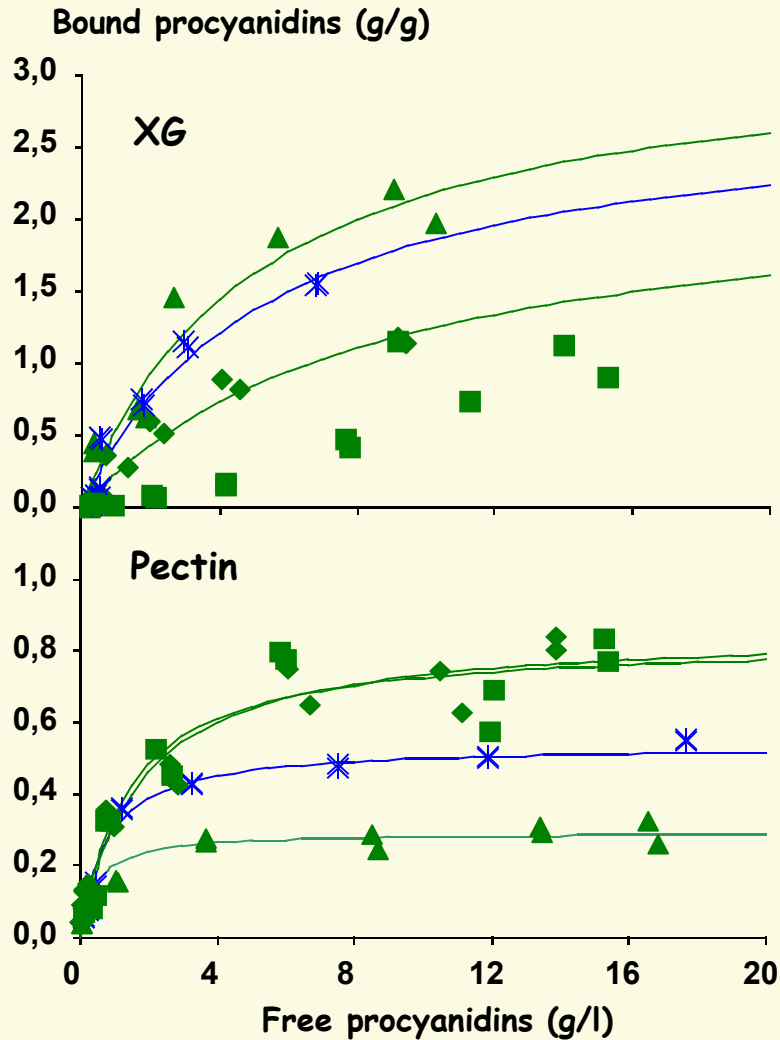
## Binding

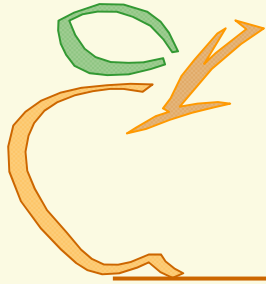
- H-bonds and hydrophobic interactions
- Totally reversible using appropriate solvent (not water!)
- Binding increases with DP of procyanidins
- Amounts adsorbed vary with initial concentrations, cell wall mode of production and physico-chemical conditions
- High affinity for pectins, no impact of protein





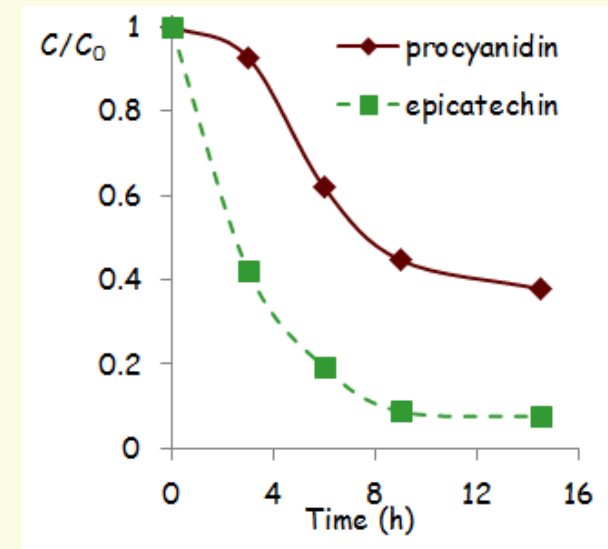
# Comparison of various polysaccharides

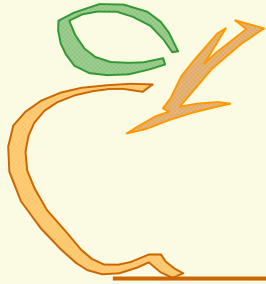




# Diffusion of polyphenols from apple pieces

- ↖ Apples were treated 70°C 30 min then leaching in distilled water
  - Water intake and free diffusion of solubles
- ↖ Procyanidins are partially retained in the solid particles
  - The cell walls retain their ability to bind procyanidins
  - Procyanidins do not diffuse totally from fruit pieces





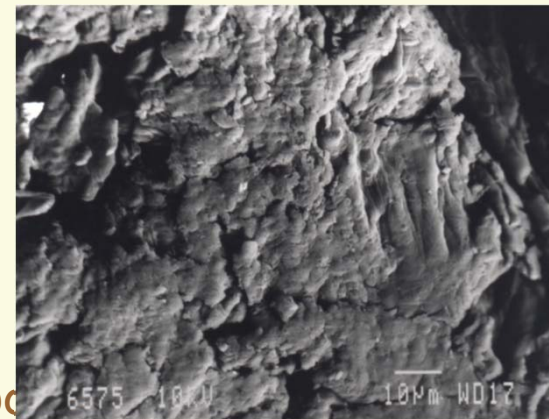
# Modifying cell wall affinity for procyanidins

## ↳ Extraction of pectins

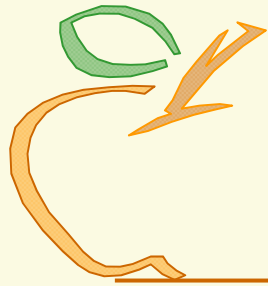
- Chemical extraction or heat-treatment
- Decreased affinity

## ↳ Modifying surface properties by drying

- Mild drying (solvent-exchange) vs harsh drying



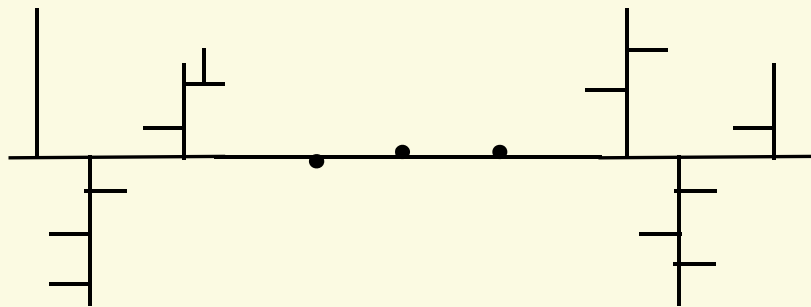
- Increase specific area and decrease porosity
- Decrease affinity and increase max absorption



# Quantification of affinity for soluble pectins and fractions

- Quantification by ITC on commercial pectins, pectins extracted from cell walls or pectin fractions

Polysaccharides from middle lamella and primary cell wall of plant cells

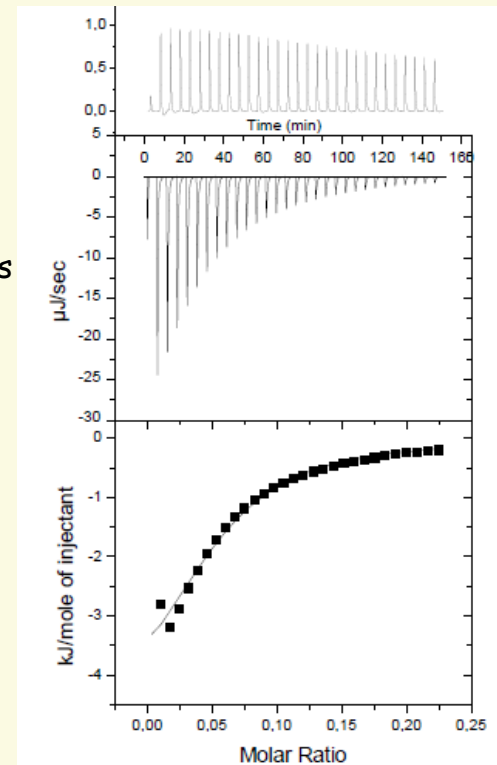
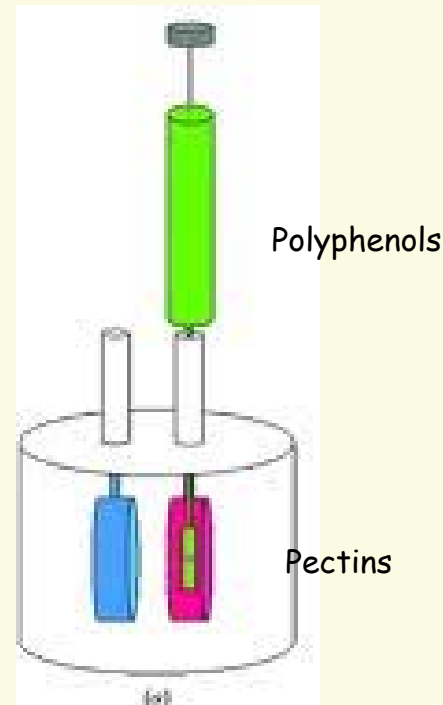


Smooth regions = Homogalacturonans (HGs)

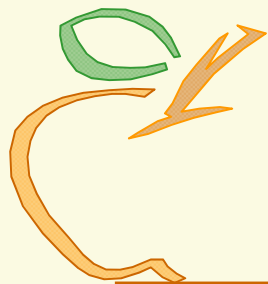
- Galacturonic acid chains
- Degree of methylation (DM)

Hairy regions = Rhamnogalacturonans I (RGs)

- Galacturonic acid and rhamnose chains
- Neutral sugar side chains (on rhamnose units)



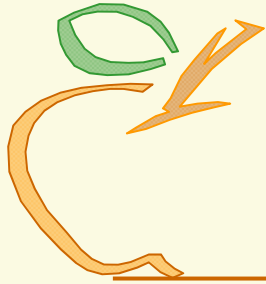
Thermodynamic parameters



# Structure / affinity and mechanisms

	Pectins		HGs			RGs			nd: not determined -: no titration +: $K_a$ of $10^2 \text{ M}^{-1}$ ++: $K_a < 2 \times 10^3 \text{ M}^{-1}$ +++: $K_a > 2 \times 10^3 \text{ M}^{-1}$
	Apple	Citrus	0%	30%	70%	RG	AR A	HR- H	
EPI	-	-	nd	nd	nd	nd	nd	nd	
DP2	-	-	nd	nd	nd	nd	nd	nd	
DP9	++	++	-	-	-	+++	++	++	
DP30	++	++	-	+++	+	+++	+	+++	

- ↖ Affinity ↗ with DM (pectin) and DP (procyanidin)
- ↖ Impact of neutral sugar side-chains
- ↖ Predominance of hydrophobic interactions

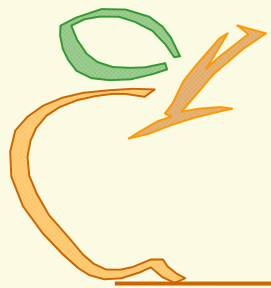


# Polyphenols and cider

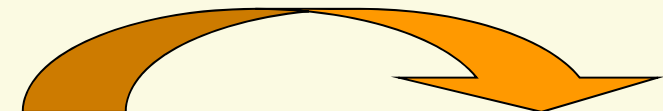
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- ↳ **Organoleptic characters**
  - Colour, bitterness and astringency
- ↳ **Evolution of phenolic composition during processing**
  - Polyphenol extraction from the fruit:
  - *Modification of structures (oxidation)*
- ↳ **Experiments with isolated procyanidins indicate an effect of temperature**
  - Extraction at different temperatures
  - Apples with different average degrees of polymerisation
  - Different levels of oxidation :
    - No oxidation for impact of non-covalent interaction





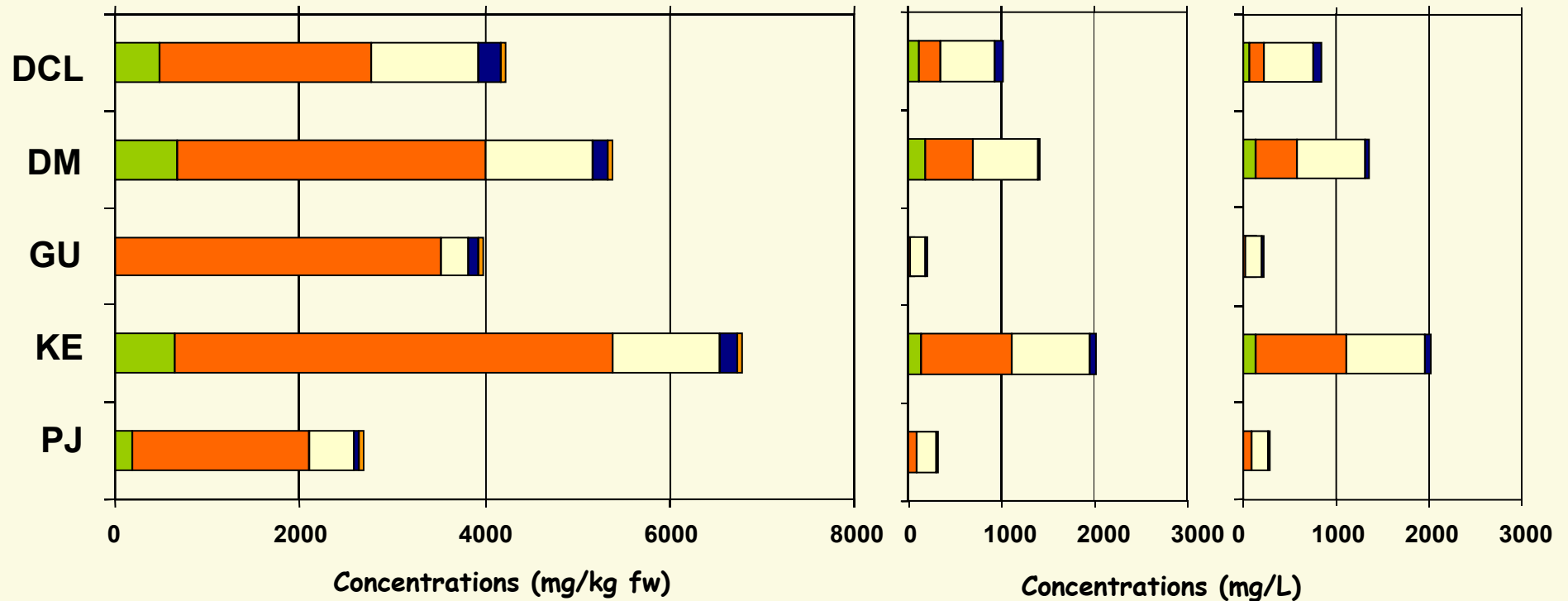
# Phenolic composition changes from apple to juice



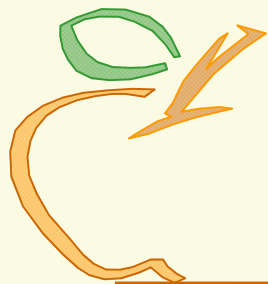
Apples

Juice

Cider



■ Monomeric catechins    ■ Procyanidins    □ Hydroxycinnamic acids  
■ Dihydrochalcones    ■ Flavonols



# Extraction of procyanidins does vary with pressing temperature

## Experiment:

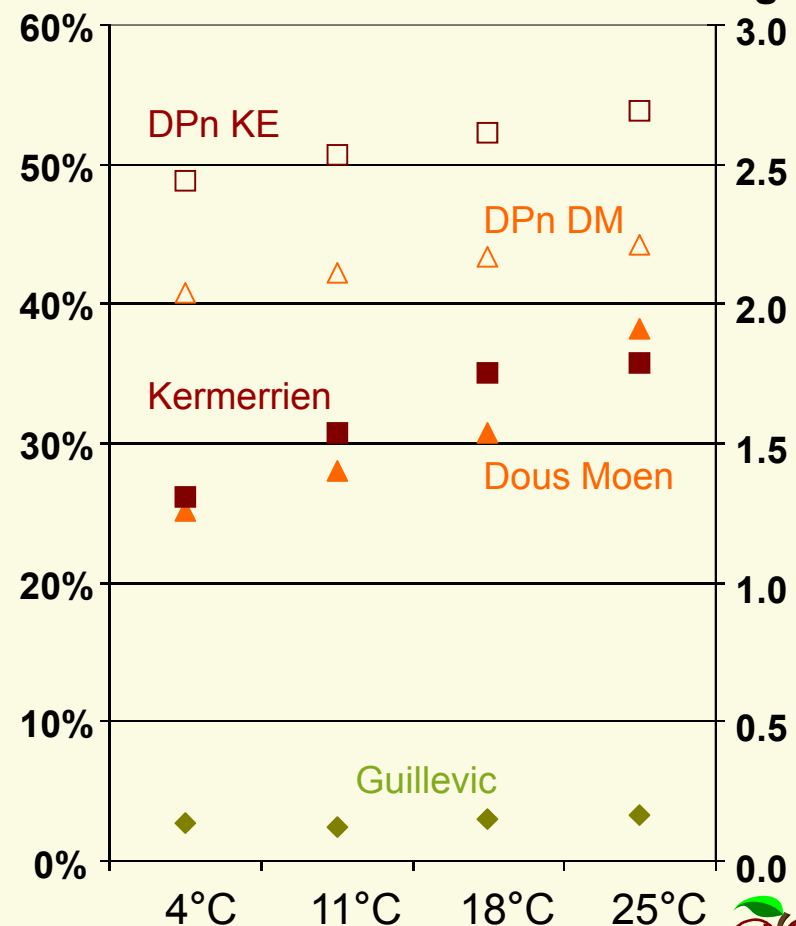
- Three varieties
  - DM: DP = 3, GU: DP = 63, KE: DP = 3.5
- Four temperatures
  - 4°C, 11°C, 18°C, 25°C
- Inerted press and juice with NaF



Extraction variation with temperature is validated

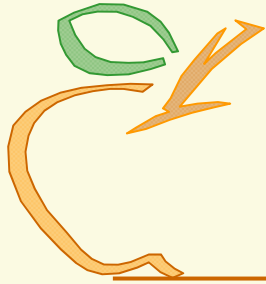
% concentration  
in theoretical juice

Average DP



Extraction temperature

18



## ↖ Introduction

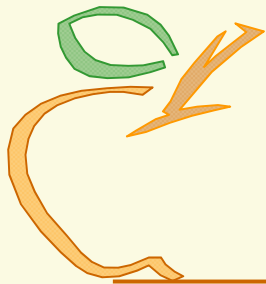
- Occurrence of polyphenol and polysaccharide adducts
- Proanthocyanidins
- Cell wall preparation

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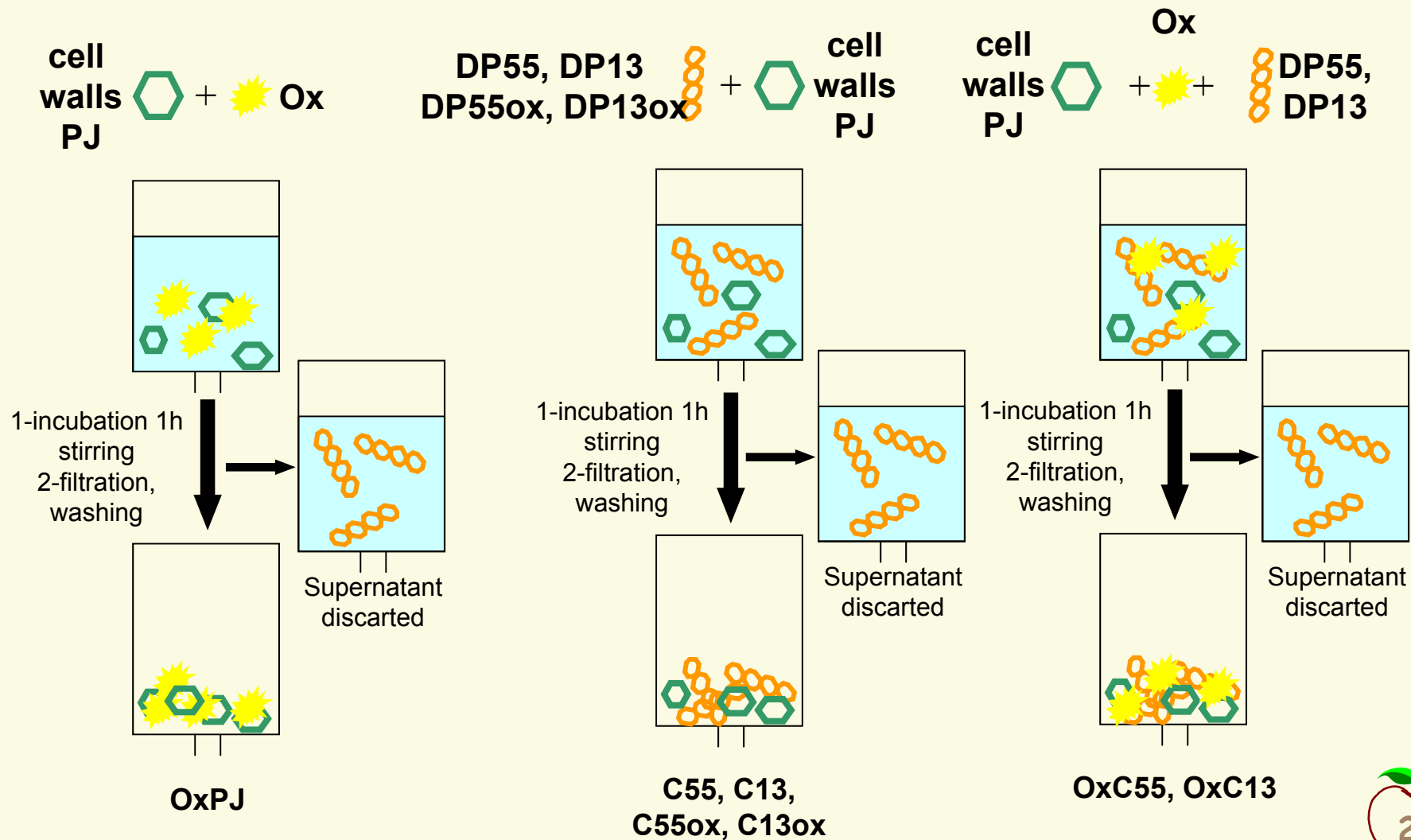
- Binding isotherms
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- Modification of cider composition

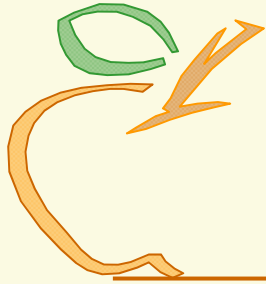
## ↖ Formation of procyanidin cell wall adducts

- Preparation by oxidation
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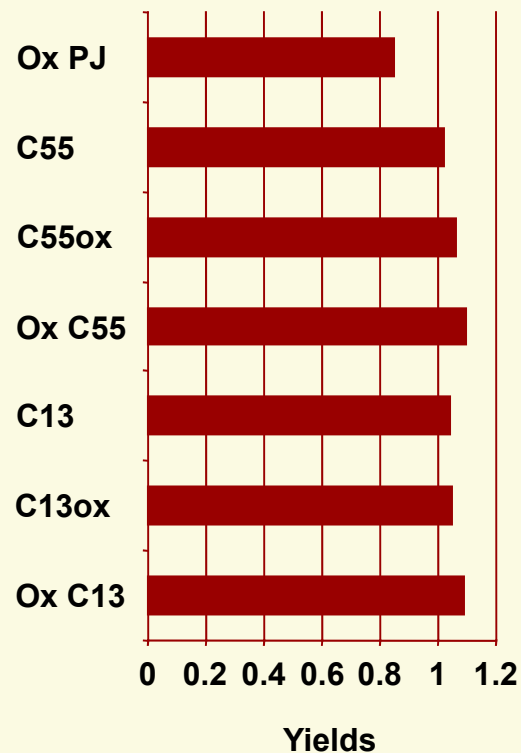
# What happens if we add oxidation?





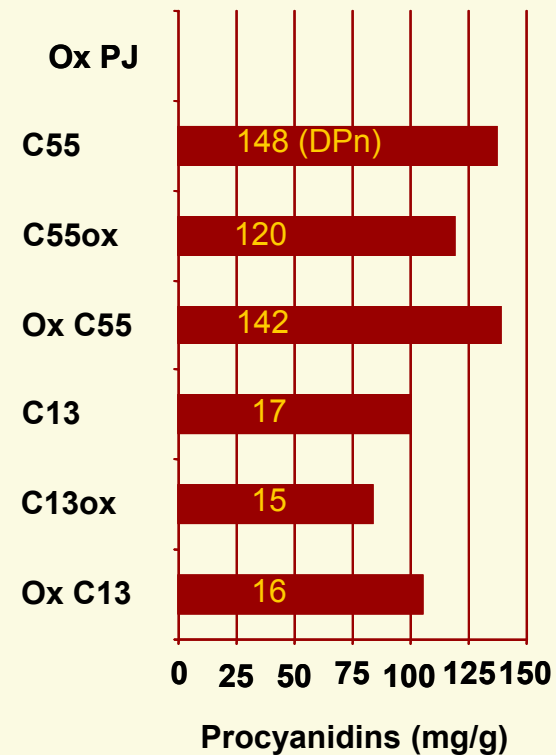
# Increased, non reversible binding

## •Yields

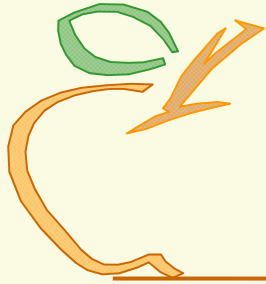


–Weight gain:  
Native < Oxydized < Oxydised with  
CW

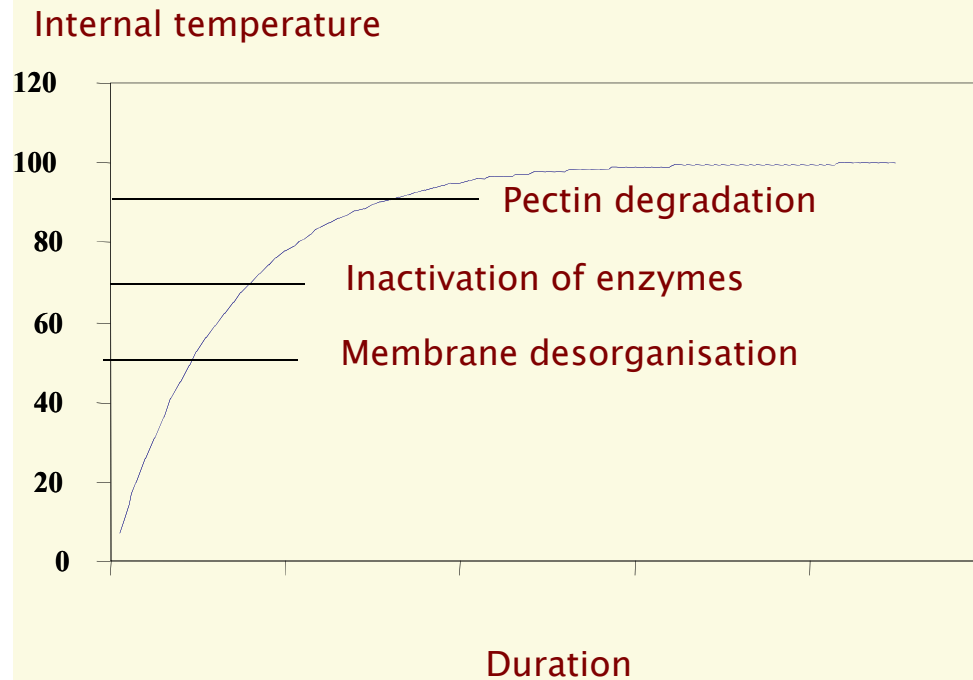
## •Composition (mg/g)



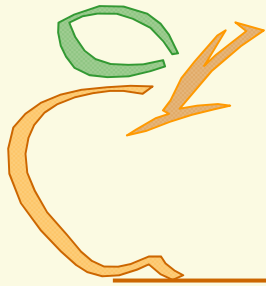
– Amounts bound increase with DP and oxidation  
- Higher DP for bound procyanidins (washings?)



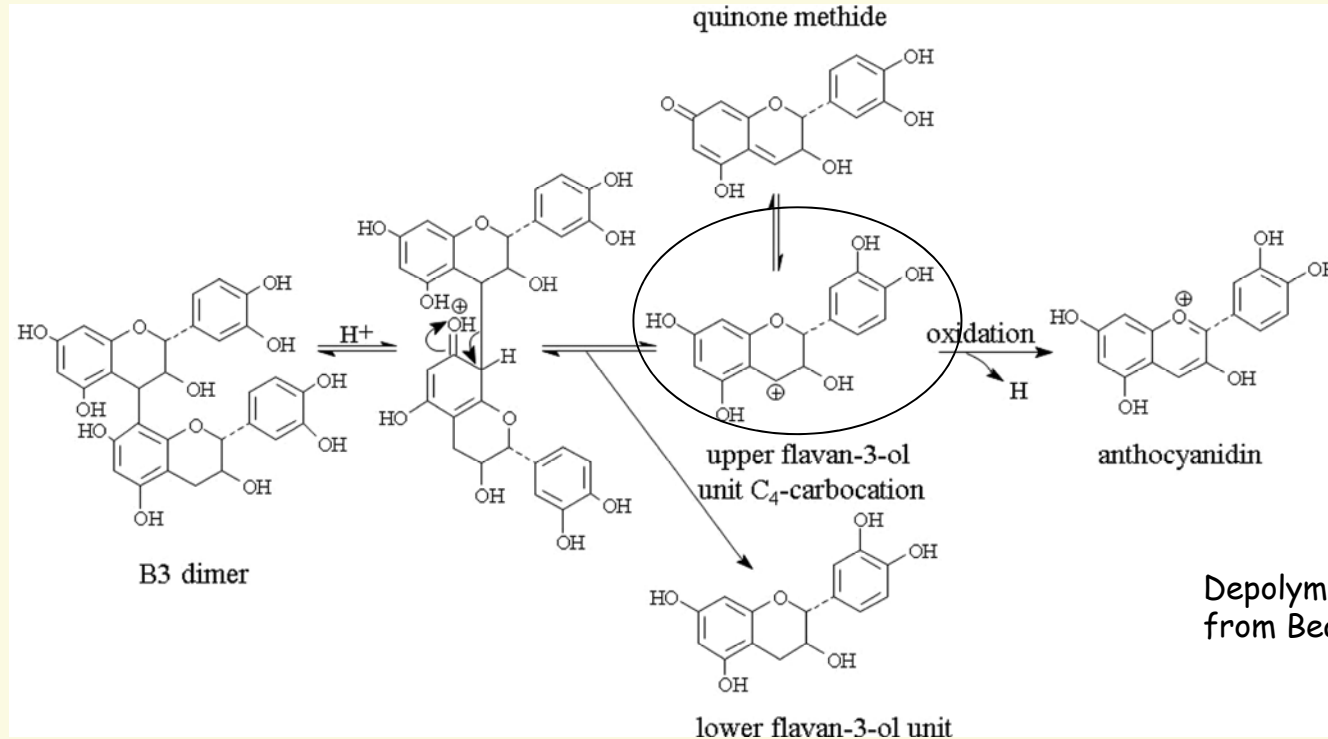
# What about thermal treatments?



- ↳ Loss of membranes enables contact between polyphenols, cell walls, enzymes, oxygen
- ↳ Enzyme inactivation, including PPO, PME...
  - Do they have time to act?
- ↳ Cell wall degradation and procyanidin acidolysis
- ↳ Autooxydation of polyphenols
  - But [O<sub>2</sub>] decrease
  - Influence of pH, catalysts...



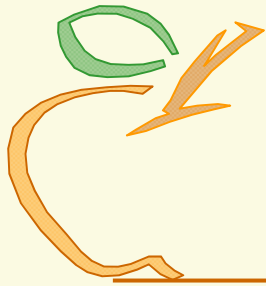
# Acidolysis of procyanidins



Depolymerisation of B3 dimer, adapted from Beart et al 1985

## ↳ Generation of a reactive carbocation

- Reaction with other polyphenols, with thiols, with proteins
- Polysaccharides?



# Structural modification of procyanidins in pear

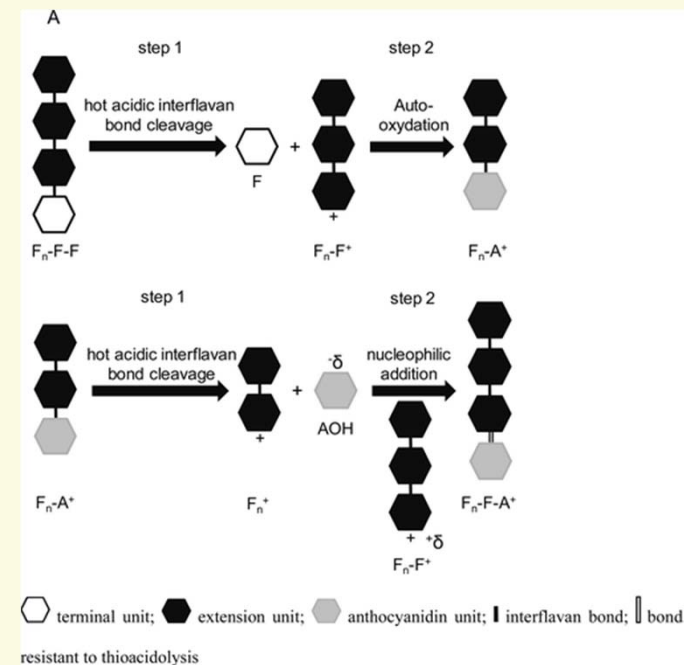
## Formation of (coloured) adducts

- Upon prolonged heat treatment
- Can be mimicked in model suspension
- Retained after solvent extraction and enzyme degradation

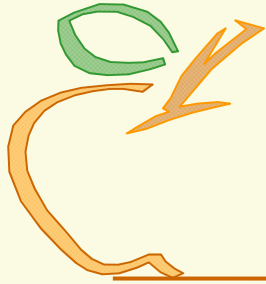


## Molecules identified in solution

- Formation of cyanidin and epicatechin-cyanidin
- Acidolysis + oxidation







# Conclusions and perspectives

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## ↖ **Procyanidins bind spontaneously to cell walls**

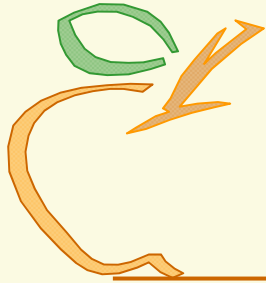
- This is a very common artefact:
  - **Procyanidins in all Rosaceae...**
- Requires specific extraction techniques
- Oxidation makes the interactions non reversible
- Acidolysis may also lead to irreversible binding

## ↖ **Consequences**

- In food processing and for procyanidins health impact

## ↖ **Perspectives**

- Non covalent interaction: impact of cell wall fine structure
- Covalent interaction: degradation of cell wall-polyphenol adducts?
- Fate of non-covalent and covalent adducts in the gut?



# Thank you for your attention!

## ↳ Unité de Recherches Cidricoles - Rennes

- A. Baron,
- S. Guyot,
- JM Le Queré,
- C. Le Bourvellec

## ↳ UMR SQPOV - Avignon

- C. Le Bourvellec
- A. Watrelot
- M. Kebe
- JF Maingonnat

## ↳ ESA Ronan Symoneaux

## ↳ CERMAV Anne Imberty

## ↳ U. Bordeaux S. Quideaux

## ↳ Pôle Agronomique Ouest

« Cidre et Polyphénols » 2000-2004

## ↳ FP6 FLAVO and ISAFRUIT



## ↳ ANR TEMPANTIOX



## ↳ FP7 DREAM



Thank you for your attention  
Looking forward to welcoming you soon at:



## 2nd Euro-Mediterranean Symposium on Fruit and Vegetable Processing

An integrated view on sustainability and how it can interact with quality, safety and the consumers

Avignon, 4-6 April 2016

**For more info:**

<https://colloque.inra.fr/fruitvegprocessing2016>

