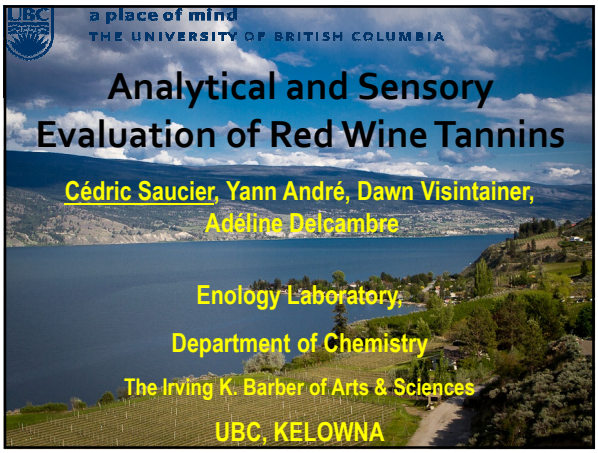


Introduction

Phenolic Compounds main properties:

- Color
- Taste
- Antioxidant (Enology and Medical effects)

Taste

- **Red wines:**

(+): Structure , backbone, body, flesh, persistance.

(-): Astringency, biterness, lack of structure.

Taste

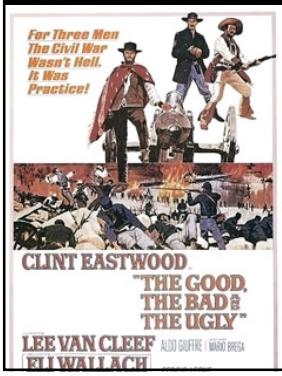
- **White wines, rosé wines:**

- Astringency, bitterness
- Oxidation or antioxidant effect on aromas (O_2, SO_2, Laccase,…)

DIAP (BCWGC/AAFC) Project

- **Astringency: Tannin/Protein interactions**
- Grape aspect: How do the tannin ripe ?
- Wine aspect: Could we have a fast/objective measure of astringency ?

DIAP (BCWGC/AAFC) Project



- **Astringency: Tannin/Protein interactions**

Good and bad tannins ?

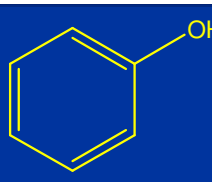
I) Phenolic compounds localisation and properties

II) First results on Tannin analysis by HPLC/MS

III) Perspectives

I) Phenolic compounds localisation and properties

Structures an properties of phenolic compounds

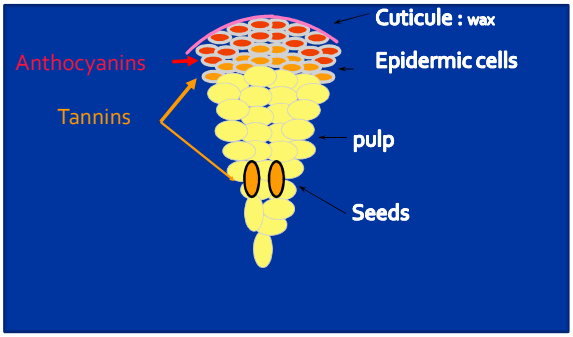


PHENOL

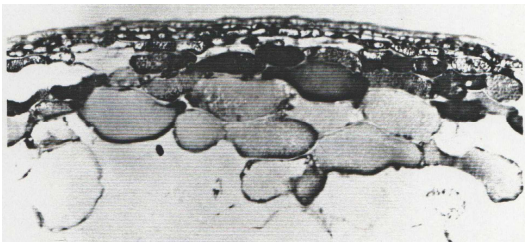
- 280 nm absorbance

-> TPI: Total Polyphenol Index (40 to 80)

Grape

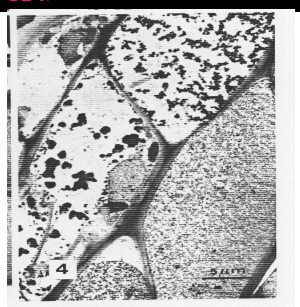


Skin tannins



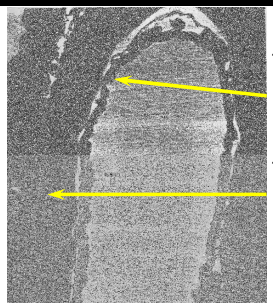
Skin tannins

(Amrani 1994)



Free vacuolar tannins

Slin tannins
(Amrani 1994)



Tannins linked to vacuole membrane

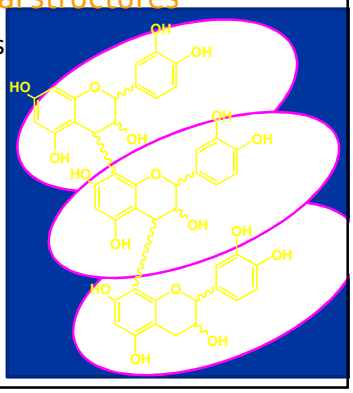
Tanins linked to cell wall

Chemical structures

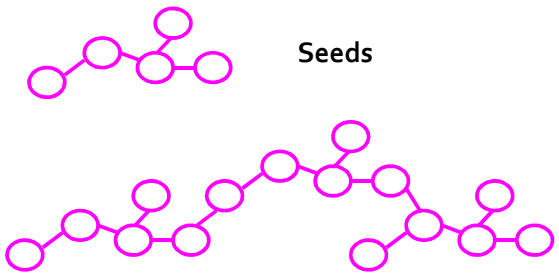
Proanthocyanidins

Flavanol units

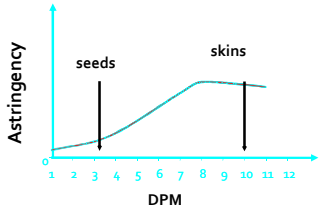
- **Seeds, skins differences : Units , Size**



Skin/size

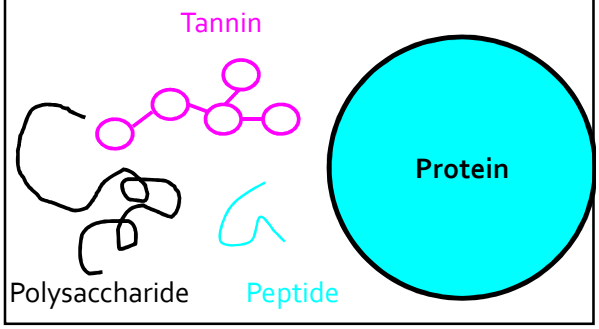


Size and astringency



Grape tannin Paradox
(d'après M.Mirabel 2000)

Tanins-Macromolecule Interactions



II) First results on Tannin analysis by HPLC/MS

Global Strategy

- Grape: monitor tannin structural modifications
- Wine and grape extracts understand tannin/Protein interaction to have a fast method that correlate with sensory (Astringency)

First results: Optimization of tannins detection By LC/QTOF/MS

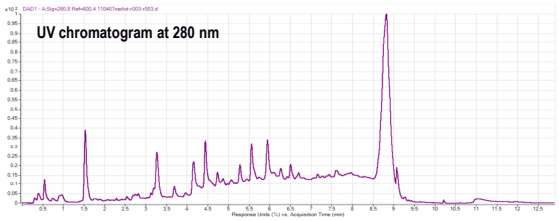
Tannin crude extract (TCE) dissolved in model wine solution 0.45µm filtration

Red wine (direct injection) After 0.45µm filtration

Merlot and Pinot Noir grapes sampled from the 2010 harvest (Okanagan Valley, BC).
The samples (grape seed and red wine) were analyzed using UHPLC-ESI-Q-TOF (Agilent 6530) in negative ion mode.

RESULTS I:Optimization

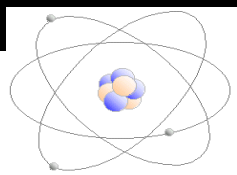
UHPLC Q-TOF MS



Thousands of tannins structures present in wine !
Impossible to analyze them all but could we at least analyze some of them including oligomers and poymers ?

-> Use of high resolution mass spectrometry

ISOTOPIC MASS



Isotopes
same number of protons (Z) but different number of neutrons (N)

	^{12}C	^{13}C
Protons (Z)	6	6
Protons + Neutrons (A)	12	13
Neutrons (A – Z)	6	7

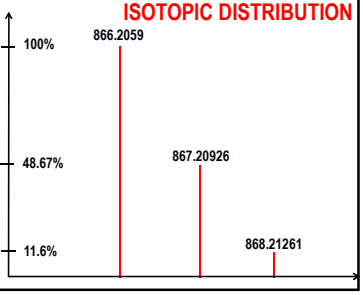
ISOTOPIC MASS Example of trimer

	Isoptopic Mass	Abundance %
^{1}H	1.0078	99.985
^{2}H	2.0140	0.015
^{12}C	12.000	98.93
^{13}C	13.0034	1.07
^{16}O	15.9949	99.76
^{17}O	16.9991	0.04
^{18}O	17.9991	0.2

Because of isotopic distribution,

single tannin species generate Multiple peaks.
Three main peaks for the trimer.

Trimer $C_{45}H_{38}O_{18}$ (monocharged)



RESULTS II: Identification

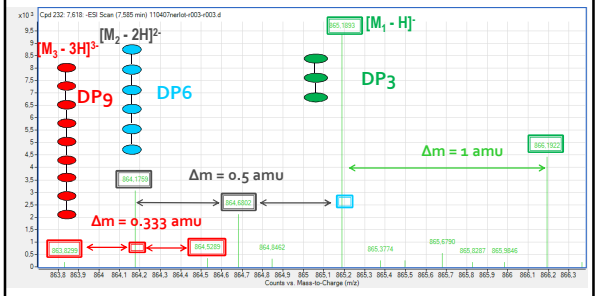
Example: **Discrimination of isotopic distribution for trimer, hexamer and nonamer (retention time is the same)**

Trimer (monocharged)		Hexamer (di-charged)		Nonamer (triply-charged)	
m/z	(% largest)	m/z	(% largest)	m/z	(% largest)
				863.8548	66.98%
				864.1892	100%
		864.1907	100%	864.5236	81.55%
		864.6924	99.55%	864.858	47.46%
865.1985	100%	865.1939	56.41%	865.1923	21.91%

RESULTS II: Identification

Sensitivity and high resolution:

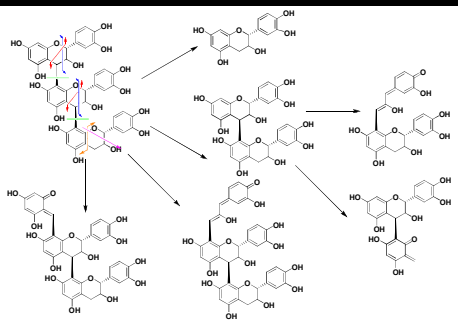
High resolution of the Q-TOF can be used to clearly distinguish several proanthocyanidins with different degrees of polymerization in red wine by direct injection of red wine and grape fractrions



Negative ion mode, fragmentor voltage, 150V, resolution of triply, doubly and singly charged proanthocyanidins by ESI-Q-TOF

RESULTS III: Identification 2

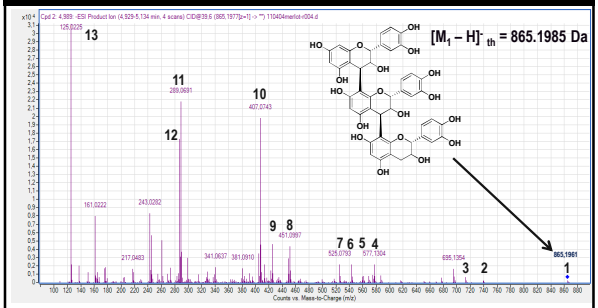
Example: **Trimers of Type B**



14 potential fragments (plus water molecules)

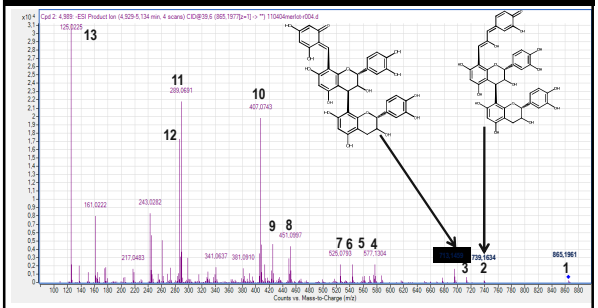
RESULTS III: Identification 2

Example: **Trimers of Type B**
Fragments foundby Targeted MS/MS



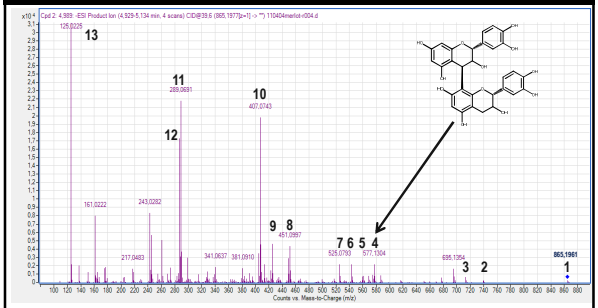
RESULTS III: Identification 2

Example: **Trimers of Type B**
Fragments foundby Targeted MS/MS



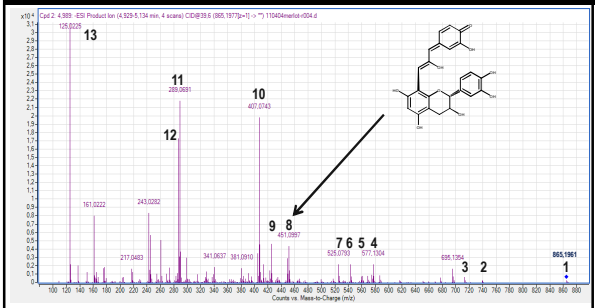
RESULTS III: Identification 2

Example: **Trimers of Type B**
Fragments foundby Targeted MS/MS



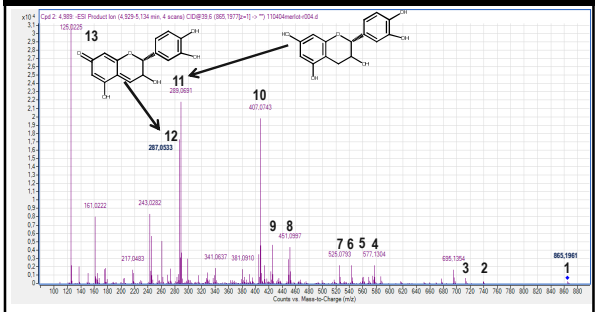
RESULTS III: Identification 2

Example: **Trimers of Type B**
Fragments foundby Targeted MS/MS



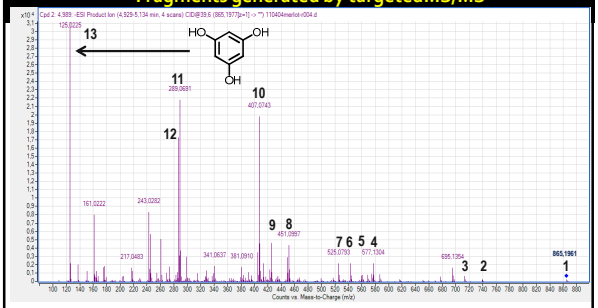
RESULTS III: Identification 2

Example: **Trimers of Type B**
Fragments foundby Targeted MS/MS



RESULTS III: Identification 2

Example: **Dimers of Type B**
Fragments generated by targetedMS/MS



Conclusion: We obtained the specific signature (fingerprint) of a trimeric proanthocyanidin

CONCLUSIONS

The **high resolution of the TOF in MS mode** :
We can discriminat the multiply charged ions of different sizes of tannins.

MS/MS Targeted mode allowed us to obtain a **specific signature for each tannin**

PERSPECTIVES

- **Quantify the proanthocyanidins with high polymerization degree in red wine and grape seeds based on molecular ions and fragments**
- **Discover new type of tannin in grape and wine ("native" and oxidized forms)**
- **Study Interaction grape fractions and wines with model proteins and peptides (BSA, Polyproline,…)**

PERSPECTIVES

- **TANNIN Fingerprinting**

Applications: Grape: tannin ripening, terroir, irrigation, soil,clones …
wine: winemaking, ageing , fining, …

THANKS: Funding

Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada

a place of mind
THE UNIVERSITY OF BRITISH COLUMBIA

Canada Foundation for Innovation
Fondation canadienne pour l'innovation

NSERC CRSNG

THANKS: The tannin Team !!!

Dr Adéline Delcambre, Yann André, Dawn Visintainer

The Enology Lab at UBCO: www.enologylab.ok.ubc.ca