Interreg North-West Europe
AgriWasteValue
European Regional Development Fund

Investigation of bioactive components from apple, grape and pear agricultural residues

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Kick-off event of the AgriWasteValue project

December 11, 2019

17 varieties collected

Vine

5 varieties

- *Regent*
- *Cabernet noir*
- *Dornfelder*
- *Pinot noir*
- *Chardonnay*

Apple trees

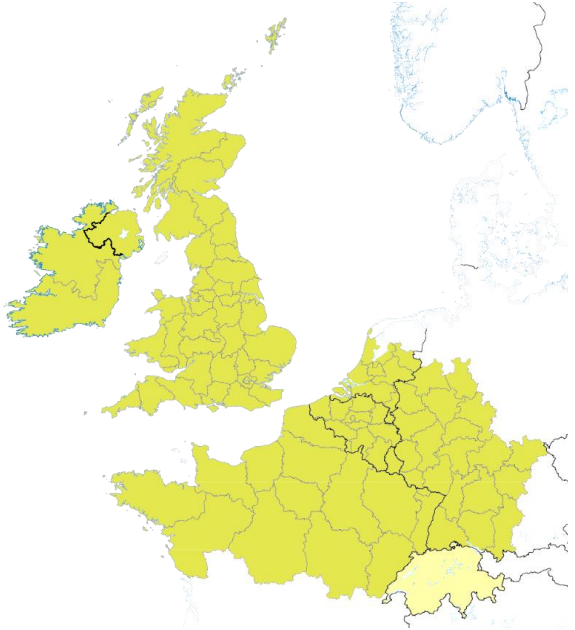
9 varieties

- *Jonagold*
- *Jonagored*
- *Braeburn*
- *Golden*
- *Gala*
- *Elster*
- *Novajo*

Pear trees

3 varieties

- *Adams*
- *Conference*
- *Doyenne*



Fields located in this area
Interactive map online

Extractions & Bio-Activities

By-products

Mechanical Grinding

Extraction processes

Freeze-dried extracts

Cosmetic & nutraceutic actives

XX different biomasses

Safety characterisation of Agriwastes

- ✓ Heavy metals and metalloids
- ✓ Mycotoxins: Aflatoxins
- ✓ Polycyclic aromatic hydrocarbons (PAHs)
- ✓ Pepticides residues

Regulation (EC) 1881/2006, 19.12.2006 modified by **Regulation (UE) 2018/290**, 26.02.2018

Determination of levels of contaminants

- 4 regulated regulated aflatoxins were determined by **HPLC fluorescence:** Aflatoxines **B1**, **B2**, **G1**, **G2**

All our samples contain a level of aflatoxines lower than the autorized limit!

- 4 regulated regulated PAHs were determined by **GC-MS:** Benzo[a]anthracene-**BaA**, Benzo[a]pyrene-**BaP**, Benzo[b]fluoranthene-**BbF**, Chrysene-**CHR**

All our samples contain a level of PAHs lower than the autorized limit!

- 6 regulated heavy metals and metalloids were determined by **ICP-MS**: Arsenic-**As**, Cadmium-**Cd**, Chromium-**Cr**, Nickel-**Ni**, Lead-**Pb**, Mercury-**Hg**

Most of our samples contains a level of Cd higher than the autorized limit for cosmetics in Germany!
But these values will be probably decreased after the extraction process.
Cd levels will be determined in further extracts.

Experimental procedure

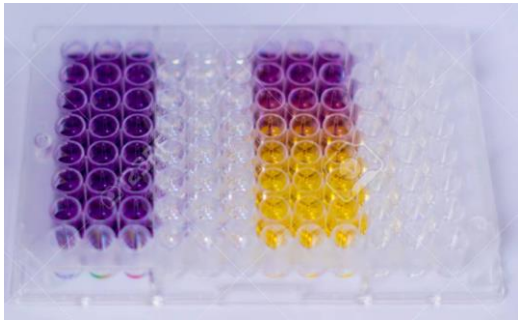
Collection

Collection
- Drying at 60°C
- Milling at 4mm

Eco-extraction

- Pressure :100 bars
- Temperature: 120°C
- Duration: 10 min x 2 cycles
- Solvents of large polarities: Hexane, EtOAc, EtOH70%, H_2O

Biological activities

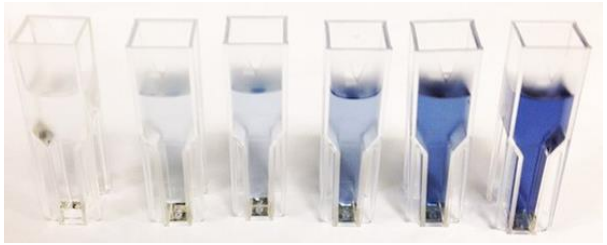


Anti-oxidant activities
- Folin assay → Phenolic compounds
- FRAP assay → Anti-oxidant capacity
- DPPH assay → Anti-oxidant capacity

Anti-oxidant assays

→ **Folin assay: total phenolic content**

The Folin–Ciocalteu reaction is based on electron transfer, which measures the reductive capacity of any substance. It is widely applied in determination of the total phenol/polyphenol content of plant-derived samples.

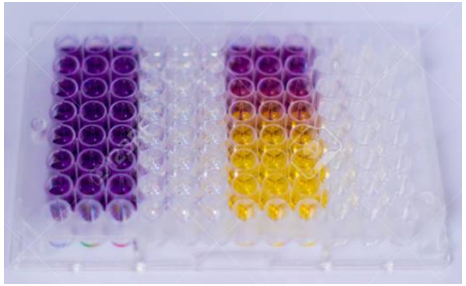


→ **FRAP assay : Ferric reducing anti-oxidant power**

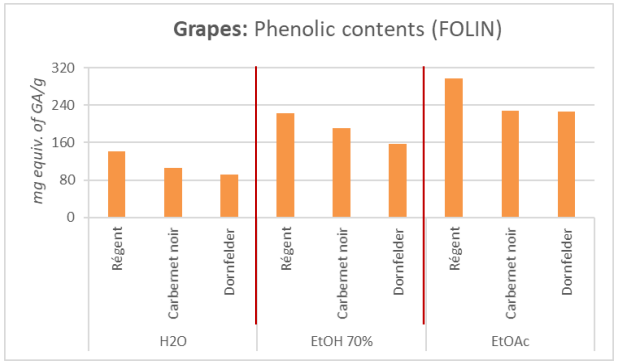
The colorless oxidized Fe^{3+} form of iron is converted to a blue-colored Fe^{2+} tri-pyridyl triazine (TPTZ)-reduced form, which is due to the action of the electron donation from antioxidants.
The change in the absorbance at a wavelength of 593 nm is measured with spectrophotometer.

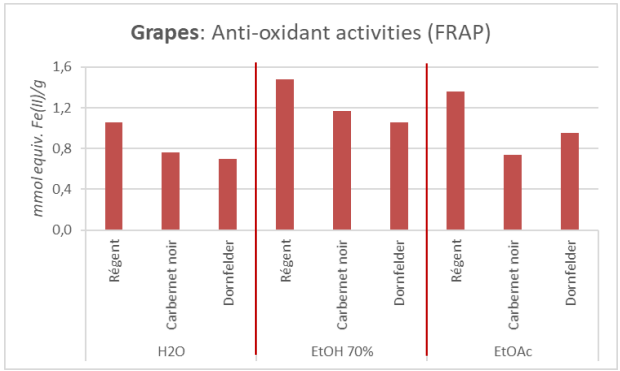
→ **DPPH assay: 2,2-Diphenyl-1-picrylhydrazyl radical scavenging**

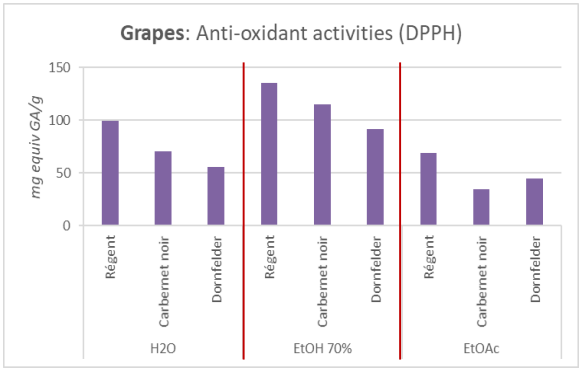
The DPPH radical has a deep violet color in solution, and it becomes colorless or pale yellow when neutralized.
Rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of that reaction.



Anti-oxidant activities of grapes

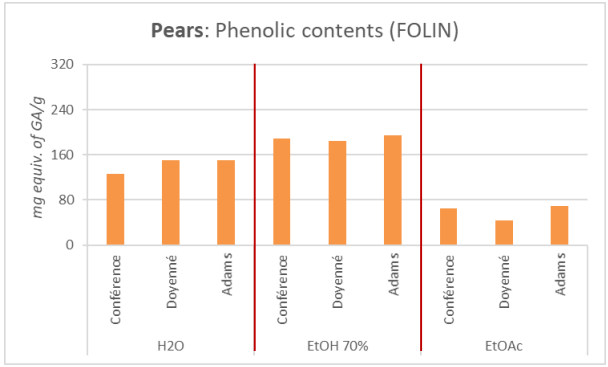


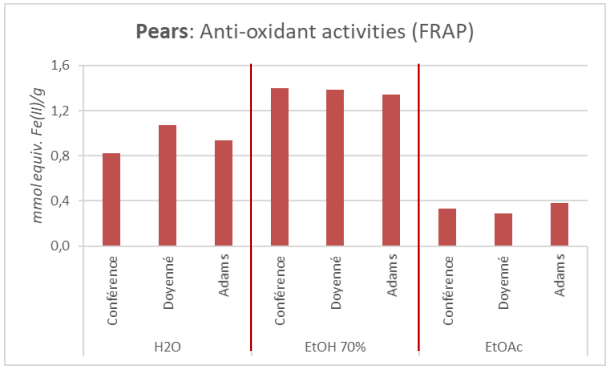


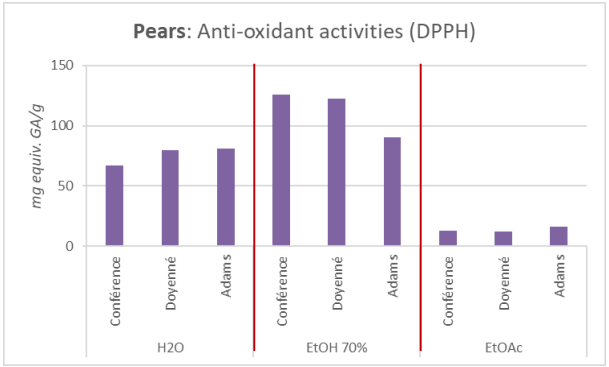


- Significant levels of polyphenols (> 100mg EGA/g) in EtOAc & EtOH70% extracts
- Important anti-oxidant activities of EtOH70% extracts in both test systems

Anti-oxidant activities of pears

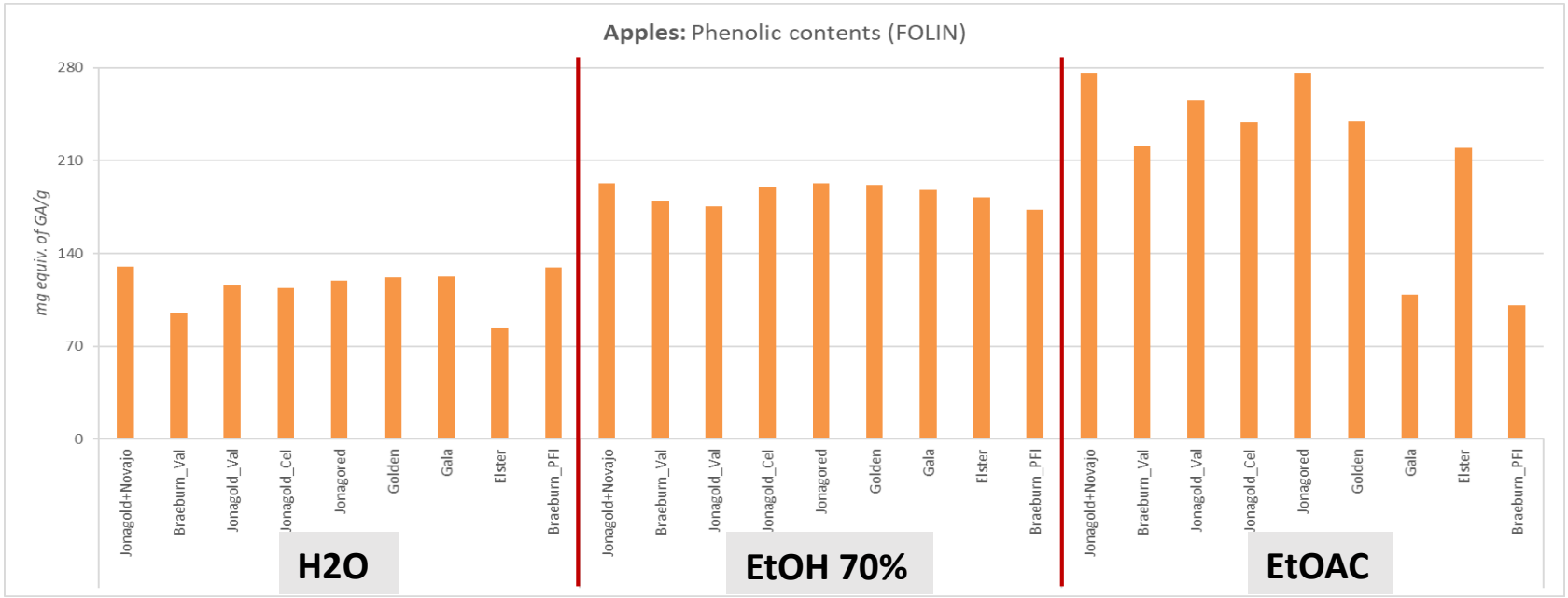






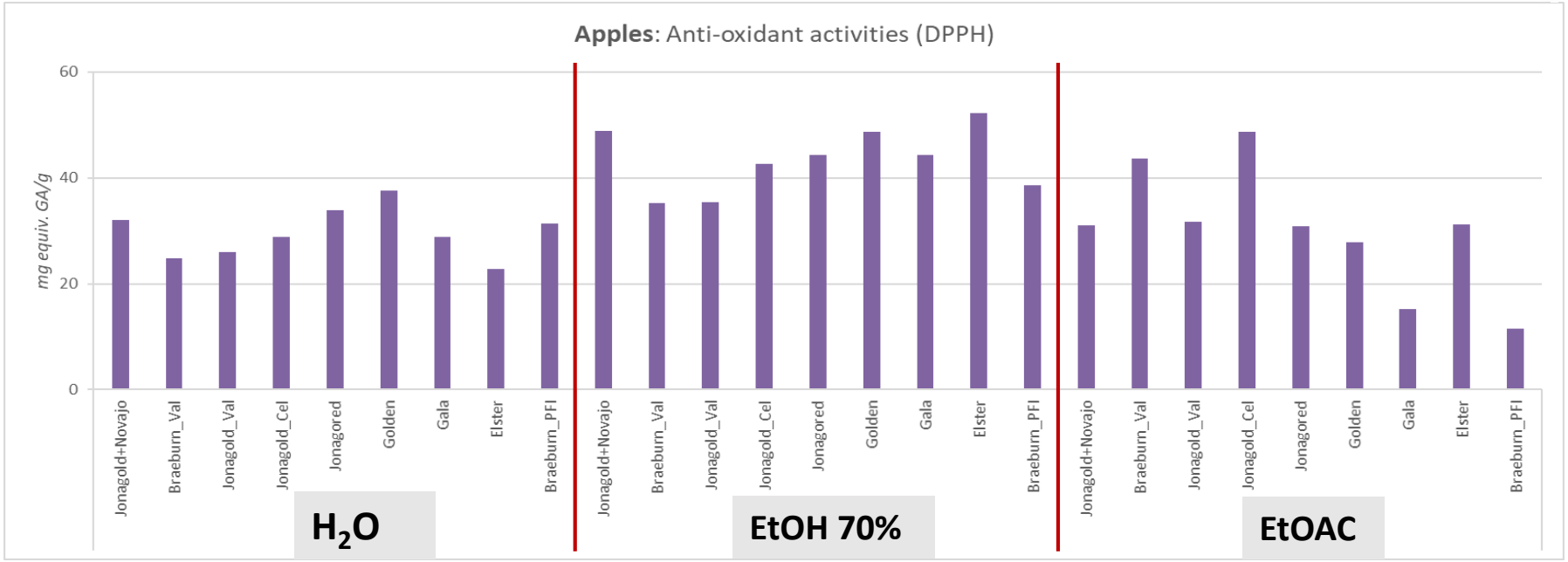
- Significant levels of polyphenols (> 100mg EGA/g) in H_2O & EtOH70% extracts
- Important anti-oxidant activities of EtOH70% extracts in both test systems
- Low polyphenol contents and low anti-oxidant activities for EtOAc extracts

Total phenolic contents of apples



- EtOH70% & EtOAc extracts are the most interesting in term of phenolic contains

Anti-oxidant activities of apples

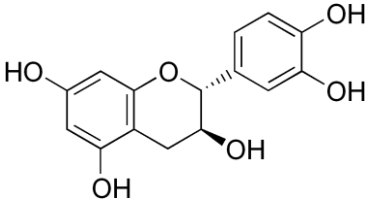


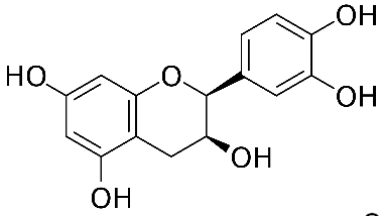
- Anti-oxidant activity of EtOH70% extracts seems to be the most significant

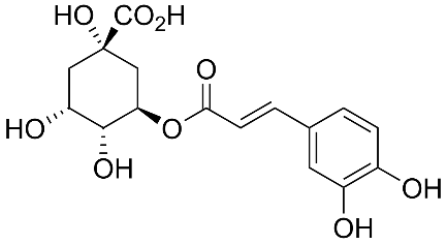
Determination of bioactive components

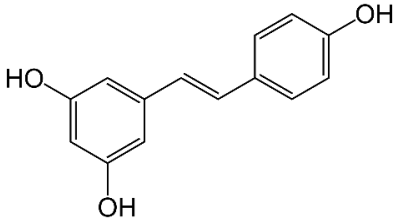
ACQUITY UPLC® **BEH Shield RP18** columns

- 25 standard polyphenols from Celabor library
- Quantification by LC-MS/MS
  - *Rapid*
  - *Sensible*
  - *Robust*





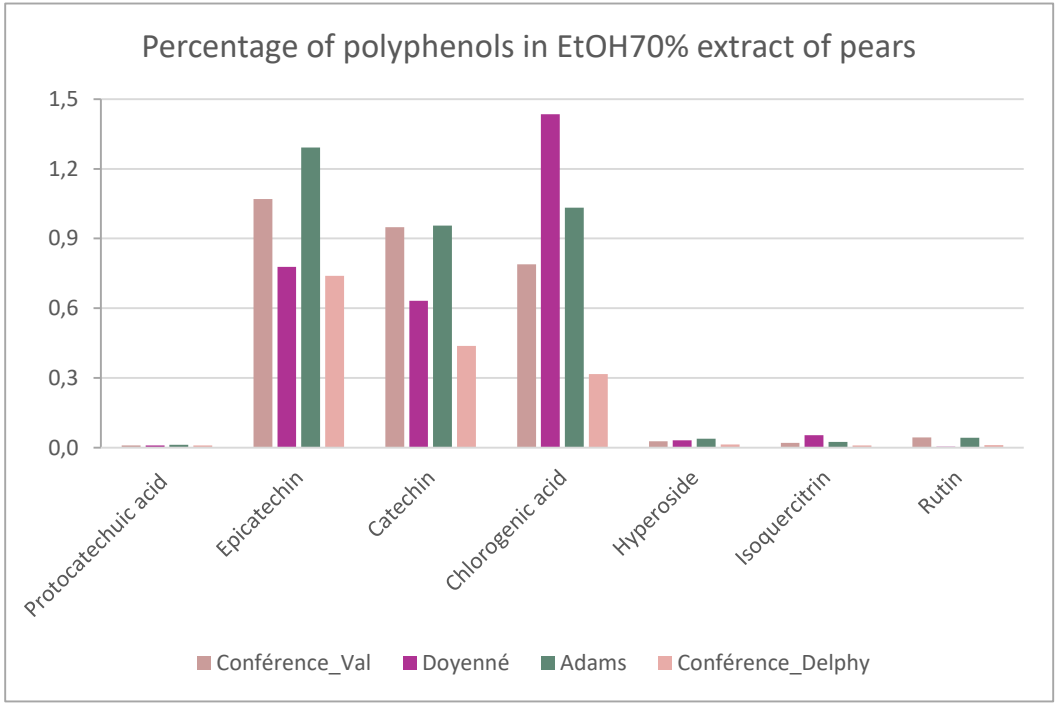




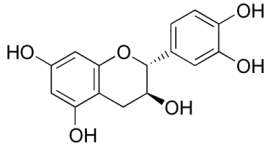
Bioactive components

	Grapes						Pears				Apples					
%	Regent	Carbernet noir	Dornfelder	Pinot noir buds	Pinot noir	Chardonnay	Conference Val	Doyenné	Adams	Conference Delphy	Jonagold Val	Braeburn Val	Jonagored	Golden	Gala	Elster
Hydroxybenzoic acid	LQ	LQ	LQ	0,007											0,010	0,008
Protocatechuic acid	0,014	0,013	0,015	0,004	LQ	0,016	0,010	0,010	0,012	0,010	0,013	0,012	0,011	0,013	0,023	0,019
Coumaric acid	LQ	LQ				LQ					LQ	LQ	LQ	LQ	0,023	0,009
Vanilic acid																
Gallic acid	0,061	0,080	0,054	0,178	0,175	0,060									0,021	LQ
Caffeic acid	LQ	LQ	LQ	0,020	0,023	LQ	LQ	LQ	LQ		LQ				LQ	
Ferrulic acid		LQ				LQ		LQ								
Resveratrol	0,648	0,391	0,312	LQ		0,797										
Apigenin																
Naringenin	LQ	LQ	LQ	LQ	LQ	LQ	LQ	LQ	LQ	LQ	LQ	LQ	LQ	LQ	LQ	LQ
Luteolin				LQ												
Kaempferol				0,017	LQ											
Epicatechin	0,658	0,610	0,586		LQ	0,210	1,070	0,778	1,292	0,739	0,276	0,126	0,214	0,185	0,117	0,110
Catechin	2,097	1,153	0,864	0,151	0,202	0,589	0,949	0,631	0,956	0,438	0,043	0,035	0,024	0,020	0,018	0,015
Quercetin	0,016	0,008	0,007	0,100	0,044	0,010					0,022	0,016	0,039	0,037	0,073	0,044
Myricetin	LQ			LQ												
Chlorogenic acid	LQ					LQ	0,789	1,434	1,033	0,317	0,453	0,411	0,157	0,192	0,069	0,059
Polydatin	0,058	0,018	0,018	LQ		0,120	LQ	LQ	LQ	LQ	LQ	LQ	LQ	LQ	LQ	0,035
Avicularin	LQ	LQ	LQ	LQ	LQ	LQ	LQ	LQ	LQ	LQ	1,033	0,928	1,024	1,041	0,696	0,500
Phloridzin	0,014	LQ	LQ	0,004	LQ	0,049		0,005			*15,748*	*15,416*	*16,194*	*15,541*	*12,777*	*8,156*
Epicatechin gallate	0,231	0,189	0,110	LQ	LQ	0,161	LQ	LQ								LQ
Quercitrin	LQ		LQ	0,006	LQ	0,006					0,513	0,542	0,909	1,127	0,712	0,549
Hyperoside	LQ	LQ	LQ	0,074	0,017	LQ	0,027	0,031	0,038	0,014	0,195	0,181	0,106	0,154	0,178	0,114
Isoquercitrin	0,010	0,009	0,006	0,171	0,051	0,010	0,020	0,054	0,024	0,009	0,060	0,064	0,063	0,079	0,065	0,035
Rutin	0,010	0,016	LQ	0,169	0,064	0,004	0,044	0,004	0,043	0,011	0,008	0,006	0,007	0,014	0,008	0,004

Bioactive polyphenols of pears

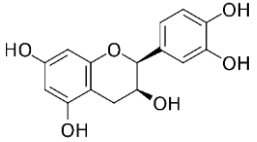


- Catechin:

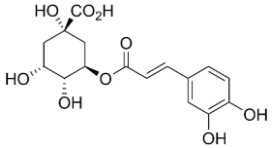


Polyphenol decreasing the oxidative stress

- Epicatechin:

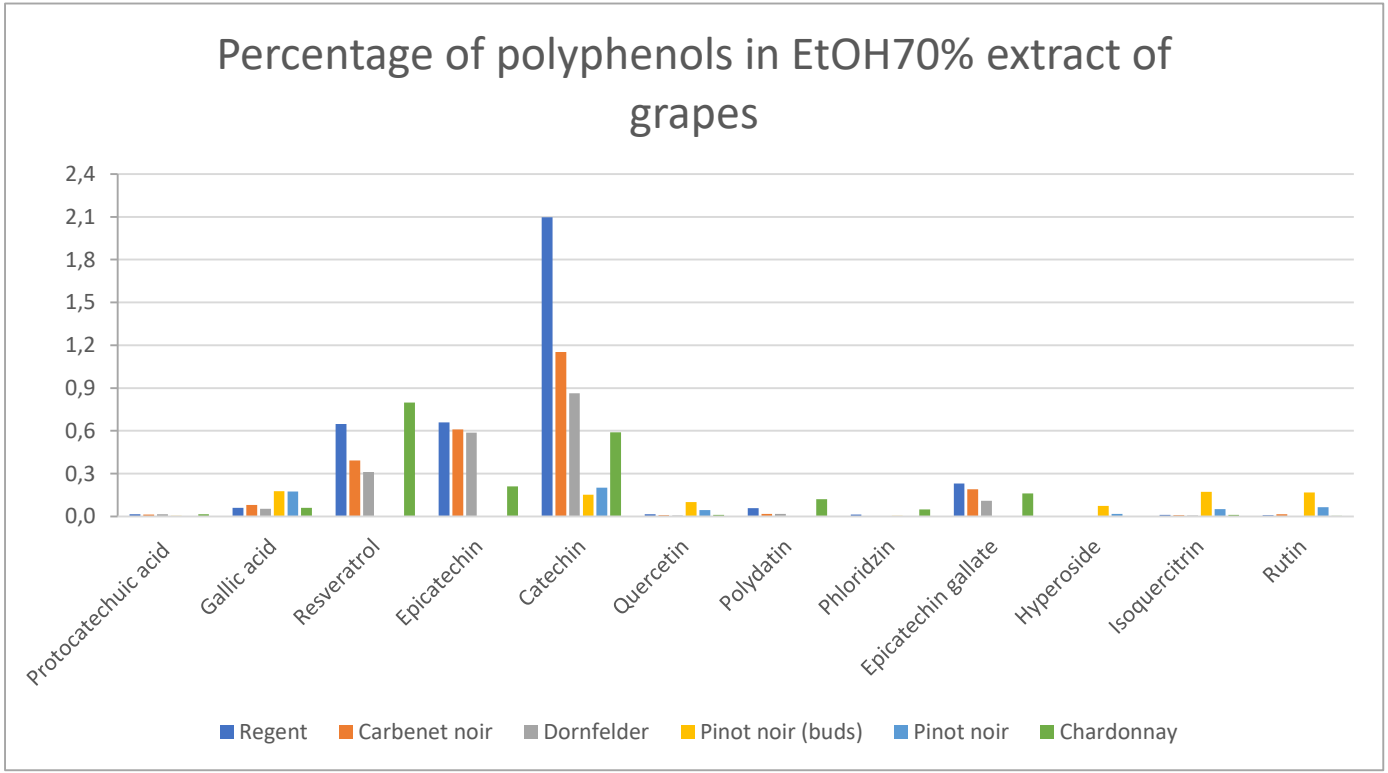


- Chlorogenic acid:

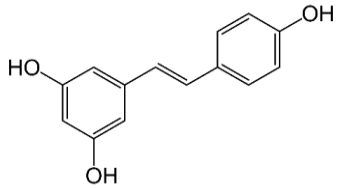


Anti-oxidant used in food industry to fight against obesity

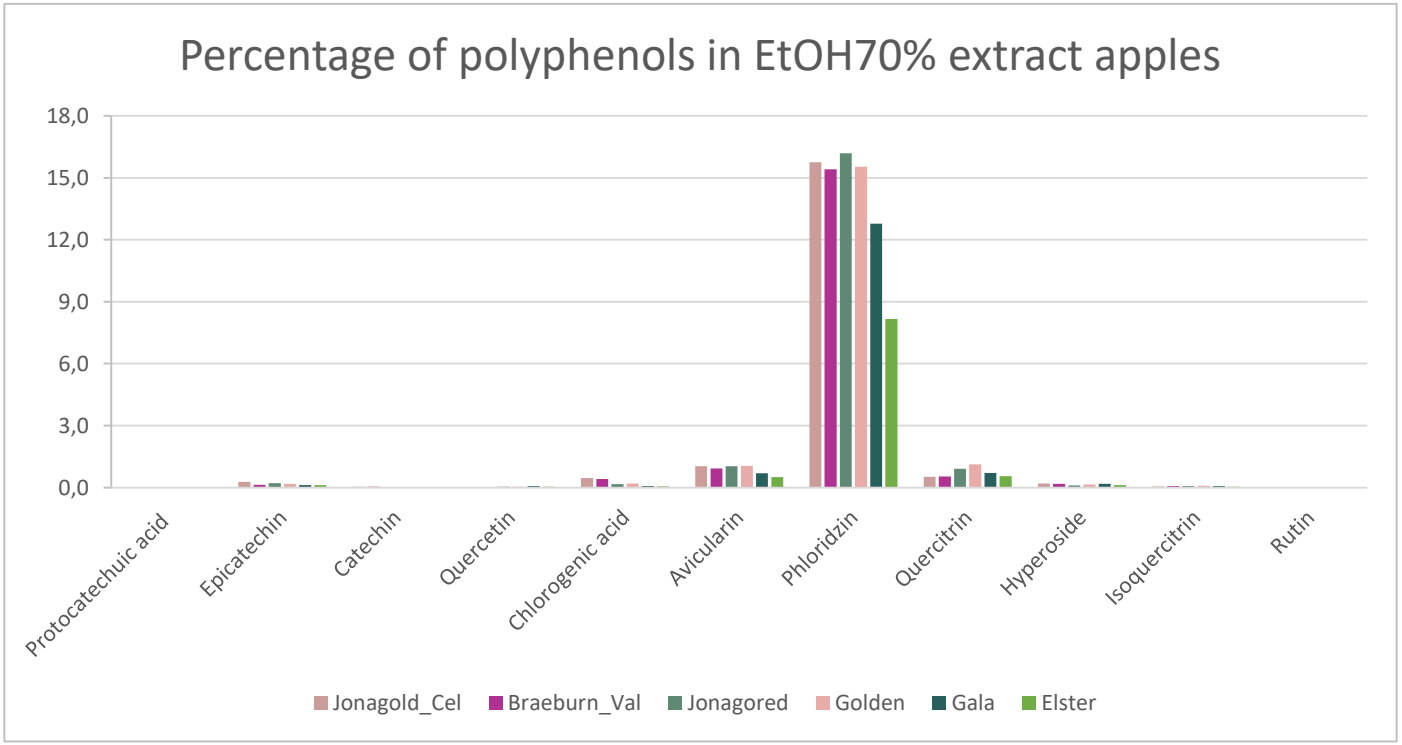
Bioactive polyphenols of grapes



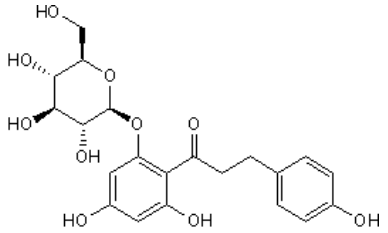
- Resvertol:



Bioactive polyphenols of apples



- Phloridzin:



Dihydrochalcone used in cosmetic and food industries as colourant

Conclusions and perspectives

- Determination of levels of contaminants in extracts
- Up-scaling green extraction of most interesting extracts
- Fractionation and isolation of hit compounds
- New collection & variability studies
- Pilot extraction & formulation with industrial partners
- Shipment of extraction residues to AgroParisTech, PFI & EPFL.

Supercritical CO2 extraction:
Kg bath production of unipolar extracts

Sub-critical water extraction:
Kg bath production of polar extracts

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Thank you !

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