Investigation of bioactive components from apple, grape and pear agricultural residues
Tchoumtchoua Job, project leader CELABOR

Kick-off event of the AgriWasteValue project
December 11, 2019
17 varieties collected

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

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

**Pear trees**
- Adams
- Conference
- Doyenne

Fields located in this area
Interactive map online
Extractions & Bio-Activities

By-products
Mechanical Grinding
Extraction processes
Freeze-dried extracts
Cosmetic & nutraceutical actives

XX different biomasses

Interreg
North-West Europe
AgriWasteValue

AgroParisTech
Innovation

Université de Reims
Champagne-Ardenne
Safety characterisation of Agriwastes

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

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 aflatoxins lower than the authorized 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 authorized 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 authorized 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
- 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%, H₂O

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
Antioxidant activities of pears

- Significant levels of polyphenols (> 100mg EGA/g) in H₂O & 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

EtOH 70% & EtOAc extracts are the most interesting in term of phenolic contains
Anti-oxidant activities of apples

- Anti-oxidant activity of EtOH 70% extracts seems to be the most significant.
Determination of bioactive components

- 25 standard polyphenols from Celabor library
- Quantification by LC-MS/MS
  - Rapid
  - Sensible
  - Robust
## Bioactive components

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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

Percentage of polyphenols in EtOH70% extract of grapes

- Resveratrol:
Bioactive polyphenols of apples

Percentage of polyphenols in EtOH70% extract 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.
Thank you!