### Enzyme-assisted extraction and ultrafiltration of value-aded compounds from sour cherry wine pomace

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FACULTY OF ENGINEERING



North Sea Region



European Regional Development Fund

**EUROPEAN UNION** 

## **BIOCAS, circular BIOmass CAScade to 100%**

- InterRegional project: The Netherlands, Belgium, Germany and Denmark ٠
- Vision of the project: To reach the complete utilization of biomass through an integral approach •
- Development of bio-cascades in which the waste from one process is the feed for the next •



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### Anthocyanins as food additives and nutraceuticals

- Water soluble pigments (red-orange-purple hues)
- Common in fruits, berries, vegetables and some flowers
- Can substitute synthetic food colorants
- Reported health promoting effects

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- Can be recovered from some industrial biowaste streams



Cyanidin-3-O-glucoside







## **Recovery of anthocyanins from plant material**

#### Extraction

- Conventional solvent extraction using methanol, ethanol or alcohol-water mixtures
- Extraction in aqueous solutions aided by:
  - Pressure (PLE)
  - Sonication (UAE)
  - Microwaves (MAE)
  - Enzymes (EAE)
- Supercritical solvent extraction

#### Purification

- Chromatography
- Membrane technology
- Co-precipitation with polymers





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### **Enzyme-assited extraction of anthocyanins**



- Degradation of the polysaccharides on the cell wall by a wide variety of enzymes:
  - Cellulases
  - Polygalacturonases
  - Beta-glucanases
  - Pectin lyases
- Effects reported
  - Increase in extraction efficiency
  - Decrease in the viscosity of the mash
  - Increase in filtration efficiency
  - Flavonoid glycosidase side activity: Anthocyanin degradation



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# Sour Cherry (Prunus Cerasus L.)



- It is mainly processed to juice, marmelade, liquour or wine due to its sour taste
- This results in the production of side streams that can potentially be valorised.
- Worlwide production: 1,199,139 tonnes (2017)
- Harvested area: 188,888 ha harvested (2017)





Source: Food and Agricultural Organization of the United Nations



ICheaP14, Bologna

27/05/2019

### Case study: Sour cherry wine pomace

We refer to pomace as the residue after fermentation and pressing

### **Objective of the study:**

- High extraction yield of anthocyanins and phenolic acids at a competitive cost
- Mild process to minimize degradation
- Compliance with current legislation, labelling, and market demands
  - Water-based process (avoiding, when possible, the use or organic solvents)
  - Purification strategy based in membrane filtration



#### Sour cherry wine pomace





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### **Bio-cascade for sour cherry wine biowaste**



### **Effect of the solvent on the extraction**

Extraction for 60 minutes at 50°C using:

- Water
- Citrate-phosphate buffer pH 3.0, 50 mM
- Citrate-phosphate buffer pH 3.0, 150 mM
- Citric acid solution 50 mM

Quantification by HPLC

Calibration using external standards

#### **Observations**

- The extraction of AC is favoured at lower pH values
- The pH of the extraction mixture increases when adding the pomace, even when using buffered solutions
- The highest extraction yield was obtained using citric acid 50 mM (final pH 2.9)





### Effect of the pomace dosage on the extraction

#### Methodology:

Extraction for 60 minutes at 50°C using:

- Citric acid solution 50 mM
- Dosages of 200 and 500 g/L



#### **Observations**

- A lower dosage of pomace results in increased amount of products extracted
- The dosage was not lowered further due to the already low concentration



## Effect of enzyme addition on the extraction

Two commercial enzyme preparations were added to the reaction mixture at a dosage of 200 mL ton<sup>-1</sup>

- Fructozym<sup>®</sup> Flash-C (Erbslöh) : blend of pectinases
- Celluclast<sup>®</sup> 1.5L (Novozymes) : single-enzyme product cellulase



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#### Observation

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The use of enzymes seems to be counter productive. Flavonoid glucosidase activity is suspected
SDU Image: SDU Image:



# **Enzyme side-activity essay**

Filtered samples containing anthocyanins and phenolic acids were incubated for 24 hours at 50 °C

- Control sample
- Sample spiked with Fructozym<sup>®</sup> Flash-C 200 mL ton<sup>-1</sup>
- Sample spiked with Celluclast<sup>®</sup> 1.5L 200 mL ton<sup>-1</sup>



#### **Observations**

No degradation observed



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### **Ultrafiltration set-up and parameters**



The e-MBR: enzyme - Membrane BioReactor

Membrane information and filtration parameters	
Membrane cut-off	25 kDa
Membrane material	ZrO <sub>2</sub>
Manufacturer	Atech Innovations GmbH
Membrane area	0.011 m <sup>2</sup>
Trans-membrane pressure	0.5 bar
Cross-flow velocity	3.0 m s <sup>-1</sup>



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## **Ultrafiltration in presence of enzymes**

Filtration performed on a Atech 25 kDa ZrO<sub>2</sub> tubular ceramic membrane



- The flux is relatively stable
- The use of enzymes results in higher permeate flux



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### **Conclusions and perspectives**

- Pomace from the sour cherry wine industry can be valorized to produce added-value extracts
- $82.1 \pm 2.9$  mg anthocyanins/kg and  $48.6 \pm 0.9$  mg phenolic acids/kg were reported.
- The use of pectinases and cellulases seemed to result in lower extraction yields, even though enzymatic degradation was not reported.
- The use of enzymes resulted in an increased permeate flux during ultrafiltration
- Enzymes can be used as a pre-treatment for the ultrafiltration step to increase the process throughput
- Other biowaste streams from the same process need to be investigated
- Different enzyme dosages need to be tested to find an optimum
- A technoeconomical evaluation is needed to assess the feasibility of this biocascade



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### Acknowledgements

- Frederiksdal Kirsebærvin (Lolland, Denmark) for supplying the raw material
- Ersblöh and Novozymes for providing the enzyme formulations tested
- European Regional Development Fund as part of the Interreg North Sea Region project 38-2-4-17 BIOCAS, circular BIOmass CAScade to 100%



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# Thank you





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