UNIVERSITÉ DE REIMS CHAMPAGNE-ARDENNE

FARE

Fractionation of Agro-Ressources and Environment

INRAE

Chaire AFERE

Agro-ressources FERmentation Enzymes

Biocatalysis to produce a large portfolio of biomolecules from agro-industrial co-products

Pr Caroline Rémond

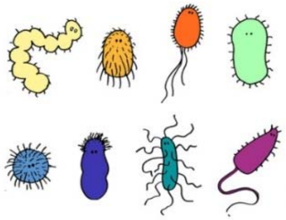
Agrosciences, Environnement Biotechnologies, Bioéconomie

UNIVERSITÉ DE REIMS CHAMPAGNE-ARDENNE

Webinar LUKE-URCA May 30th

AFERE: a team of 12 scientists and students with skills in molecular biology, microbiology, biocatalysis, analytic chemistry, bioinformatic

Agro-industrial co-products, biomasses from biorefineries :
brans, straws, bagasses, cobs, pulps, pomaces, oil-cakes, pretreated wood, …



Bacteria-fungi

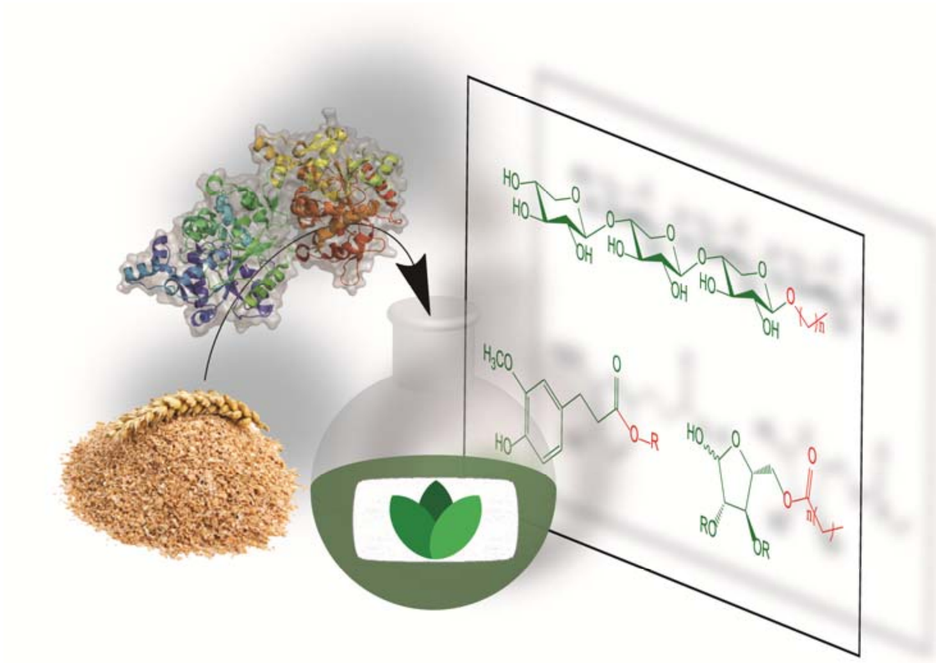
Fermentation-bioproduction

Enzymes

Biocatalytic fractionation-extraction functionalization

- Cosmetics : anti-oxidants, anti-aging, pigments and dies, sugar esters, alkyl glycosides, polyfonctional molecules
- Food/feed: colorants and dies, prebiotic oligosaccharides, sugar esters, anti-oxidants
- Biofuels : enzymes (cellulases-hemicellulases), 2G bioethanol

Interest of biocatalysis to fractionate biomass, to extract and to functionalize biomolecules

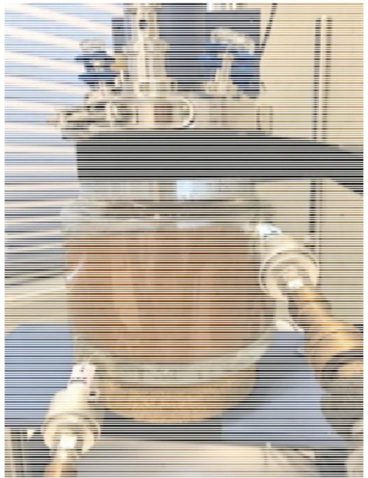


- **Soft** reaction conditions
- **1-step** reactions
- **High selectivity of enzymes**: well defined molecules
- **Limitation** of the production of undesirable compounds

Biocatalytic process = naturality of the products

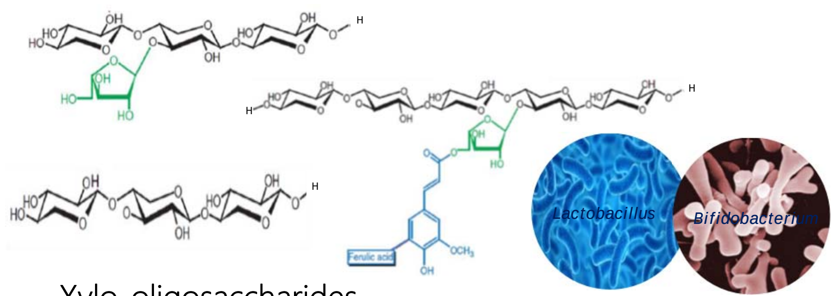
Production of bioactive molecules from wheat bran

- **Wheat bran**, agricultural co-product from milling industries and 1G bioethanol industries
- Main use: **food** (fibers) and **feed**



Enzymatic hydrolysis →

Prebiotic oligosaccharides



Xylo-oligosaccharides

↑ PROBIOTICS
(food-feed)

Rios-Rios *et al*. Journal of Agricultural and Food Chemistry 2021; Rios-Rios *et al*. Biochemical Engineering Journal. 2022

The prebiotics

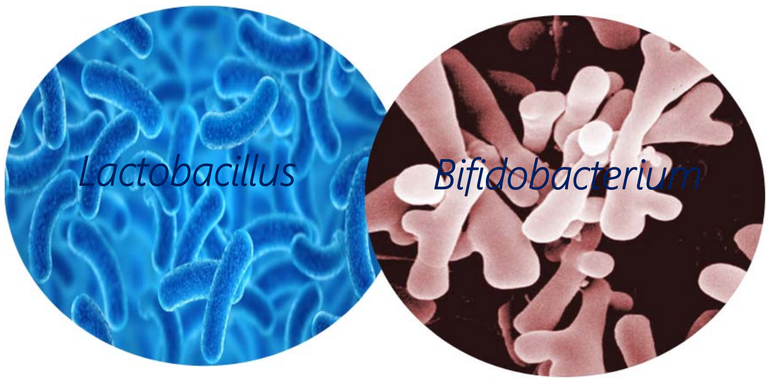
Prebiotic : "a substrate that is selectively used by host microorganisms conferring a health benefit"

Some **oligosaccharides** are **prebiotics of interest:** fructo-oligosaccharides, galacto-oligosaccharides,

Emerging prebiotics: xylo-oligosaccharides

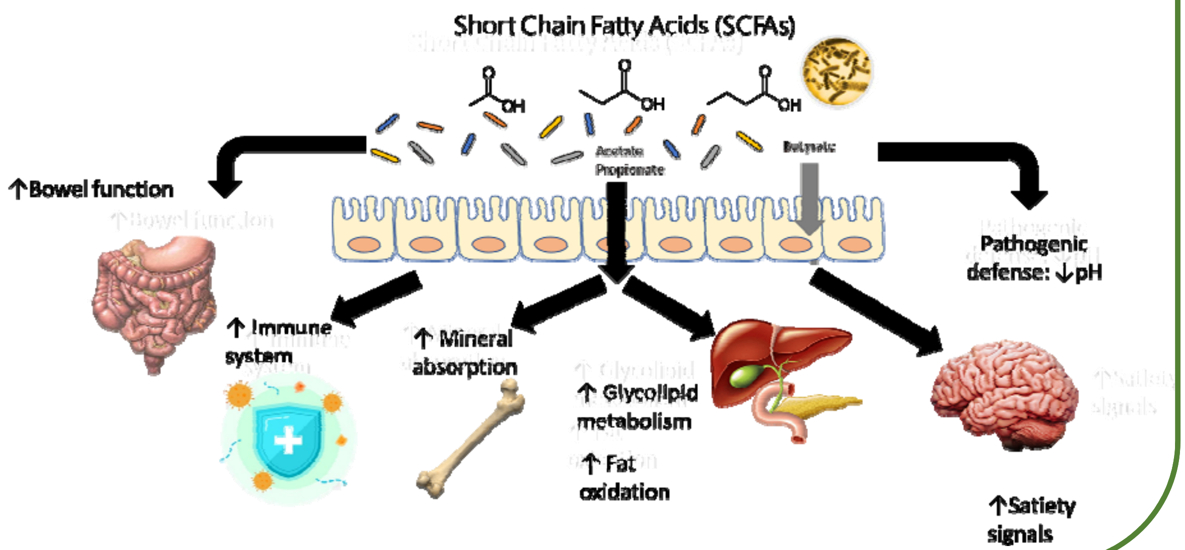
Roles of prebiotics in the gut

Probiotic boost



↑PROBIOTICS

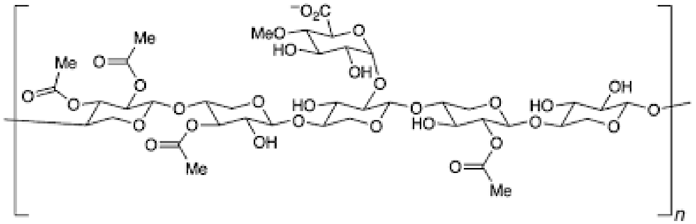
Health benefits



Mathew et al. 2018; Falck et al. 2018, 2013; Gong et al. (2019); Ravn et al. 2017; Chen et al. 2015, Kondepudi et al. 2012; Sanders et al. 2019

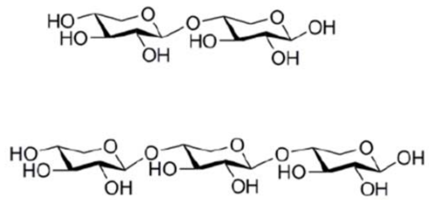
Xylo-oligosaccharides (XOs) as emerging prebiotics

Xylans = 20-40% DM of some lignocellulosic biomasses



Extraction → Hydrolysis →

XOs



Bottleneck:
Large chemical diversity of xylans → lack of knowledge about structure-functions of XOs as prebiotics

Our objective
- → Producing diverse XOs
- → Developing an environmentally-friendly process to produce XOs (Enzyme-membrane reactor)

vito vision on technology

Karina Rios-Rios' PhD thesis (2018-2022)

XOs production

Screening of **various agro-industrial co-products**

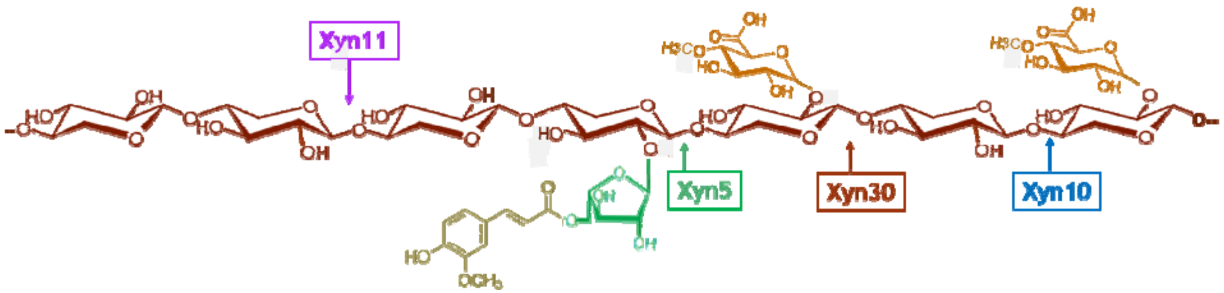
Wheat straw

Corn cob

Maize bran

Wheat bran

Assays of hydrolysis with **different xylanases**



Selection of **wheat bran** and a **GH11 xylanase**

After 1h of hydrolysis:

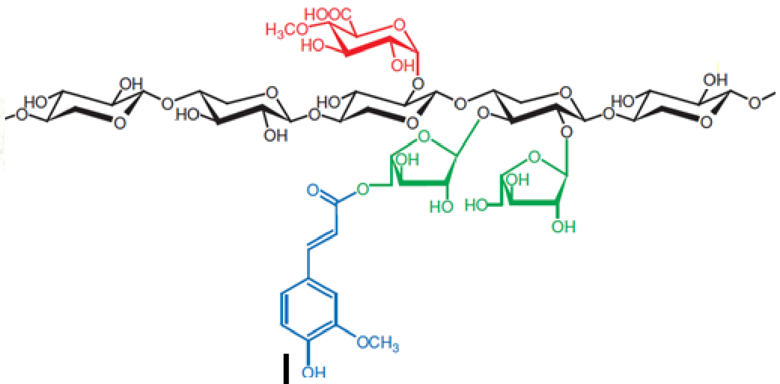
- ✓ 70% of xylans hydrolysis
- ✓ Mixture of XOs with DP 2 to 6

Rios-Rios et al. J. Agri. Food Chem. 2021. 69(44):13217-13226.

XOs production

Produced by enzymatic hydrolysis of xylans from lignocellulosic plant cell walls

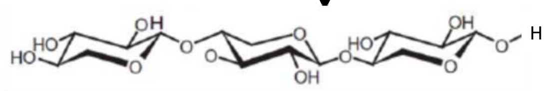
Wheat bran
Rich in xylans (20-25% DM)



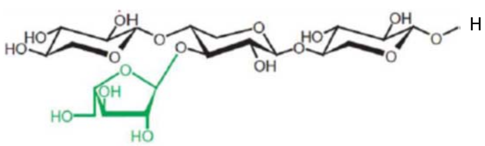
Xylans

Xylanase GH11
50°C

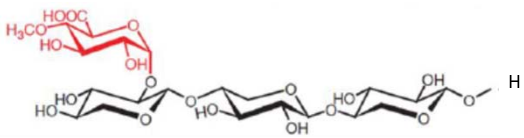
Examples of obtained XOs



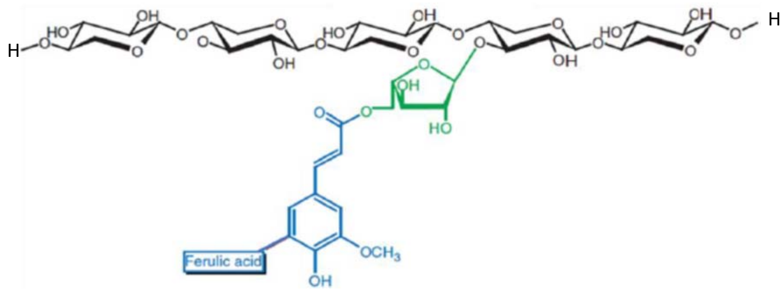
X3



X3A



X3GlcUA



X5AFe

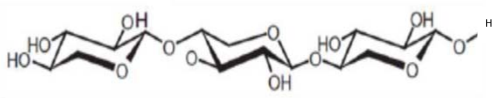
XOs production

Wheat bran hydrolysis with the xylanase in a 2 L reactor

XOs mixture:
XOs separation onto an anionic resin

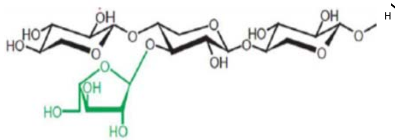
H_2O

EtOH 50%

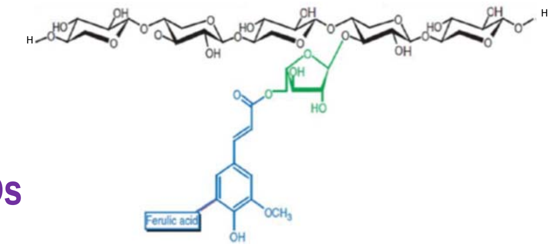


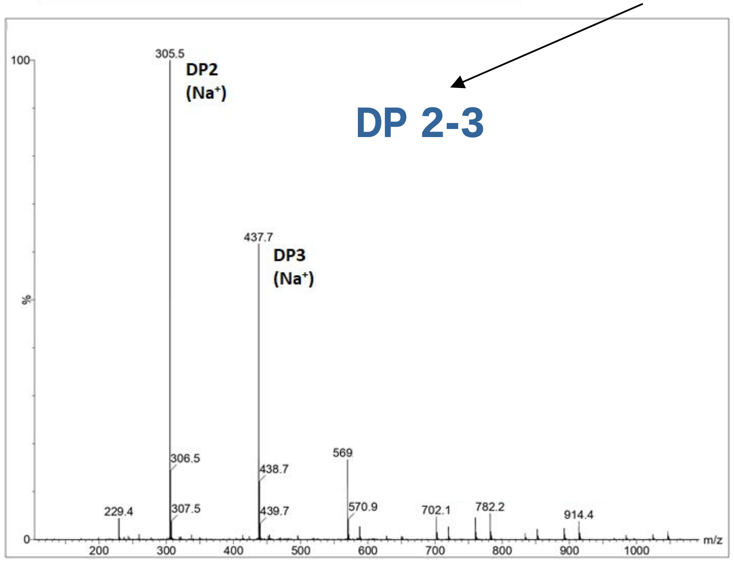
XOs (linear)

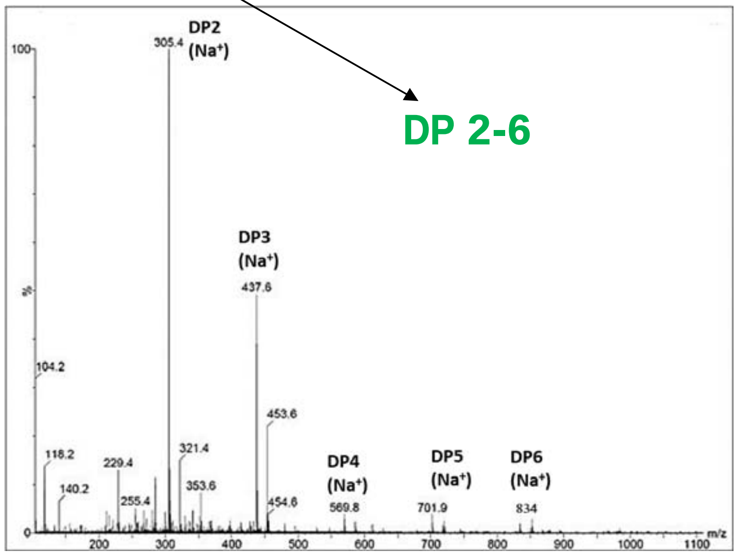
XOs (linear) **+ AXOs**

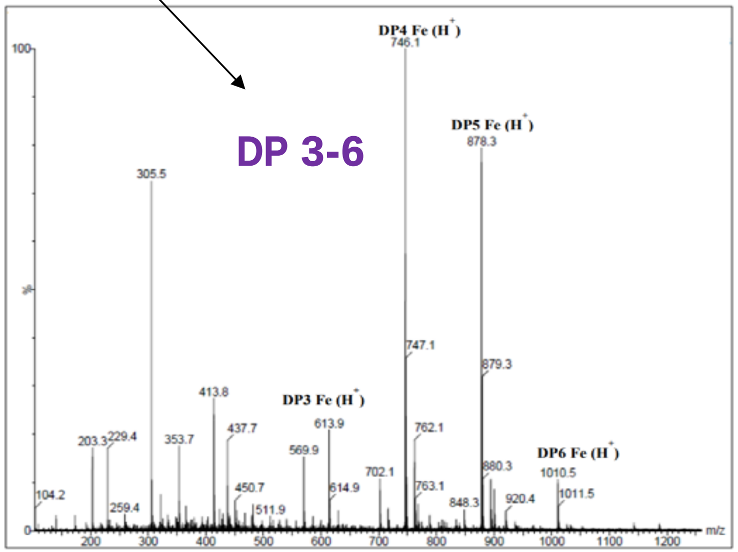


FAXOs







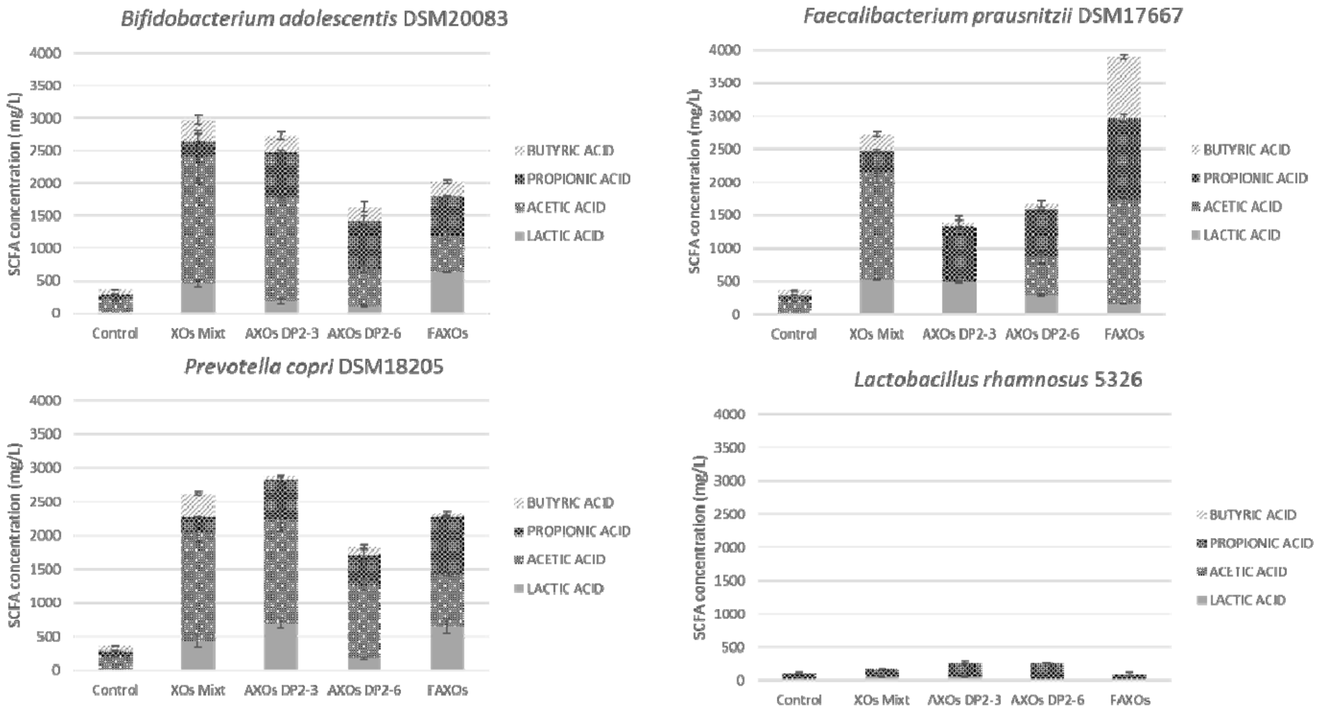


Rios-Rios et al. J. Agri. Food Chem. 2021. 69(44):13217-13226

Prebiotic properties of XOs

In vitro fermentation tests of various bacteria from gut in presence of XOs:
Bifidobacterium adolescentis, Lactobacillus rhamnosus, Faecalibacterium prausnitzii, Prevotella copri

Bacterial growth, XOs use, short chain fatty acids quantification

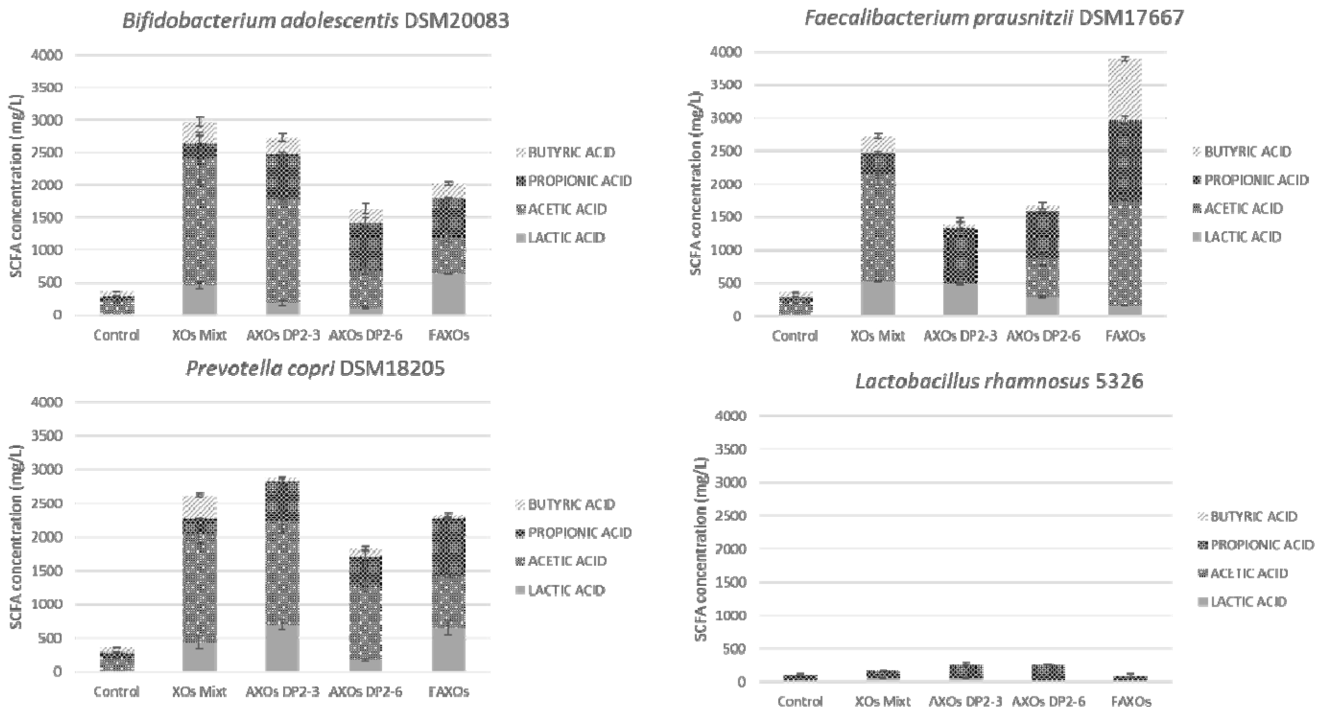


Rios-Rios et al. J. Agri. Food Chem. 2021. 69(44):13217-13226

Prebiotic properties of XOs

↑OD, ↓ pH, ↑ SCFA production for *B. adolescentis, F. prausnitzii and P. copri* but not by *L. rhamnosus* during *in vitro* fermentation tests with XOs mixture and its fractions, XOs, XOs+AXOs and FAXOs

→ These XOs can be considered as prebiotics



Rios-Rios et al. J. Agri. Food Chem. 2021. 69(44):13217-13226

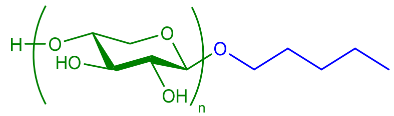
Production of bioactive molecules from wheat bran

- **Wheat bran**, agricultural co-product from milling industries and 1G bioethanol industries
- Main use: **food** (fibers) and **feed**

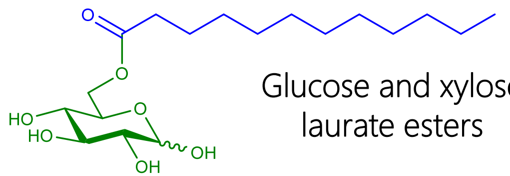
Enzymatic glycosylation & acylation

Non ionic biosurfactants

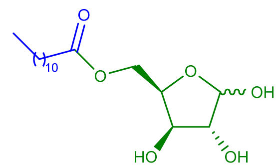
Alkyl polyxylosides



n = 1 to 3



Glucose and xylose laurate esters



Alkyl glycosides and sugar esters, non-ionic biosurfactants of interest

Alkyl (poly)glycosides (APG)

World market: 100 000 T/year
Emulsifiers, foaming agents, wetting agents for cosmetics, detergents, phytosanitary

Appyclean™ (Wheatoleo), Oramix™, Montanov™ (Seppic), …

Sugar esters

World market: 10 000 T/year
Emulsifiers for cosmetics and food

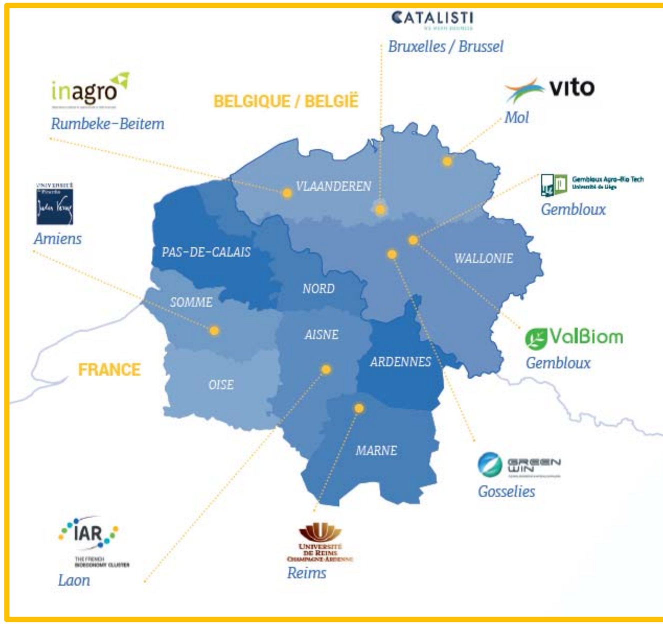
Crodesta™ (Croda), products from Sisterna, …

Produced with chemical routes



The ValBran project

ValBran



Wheat bran valorization into biosurfactants

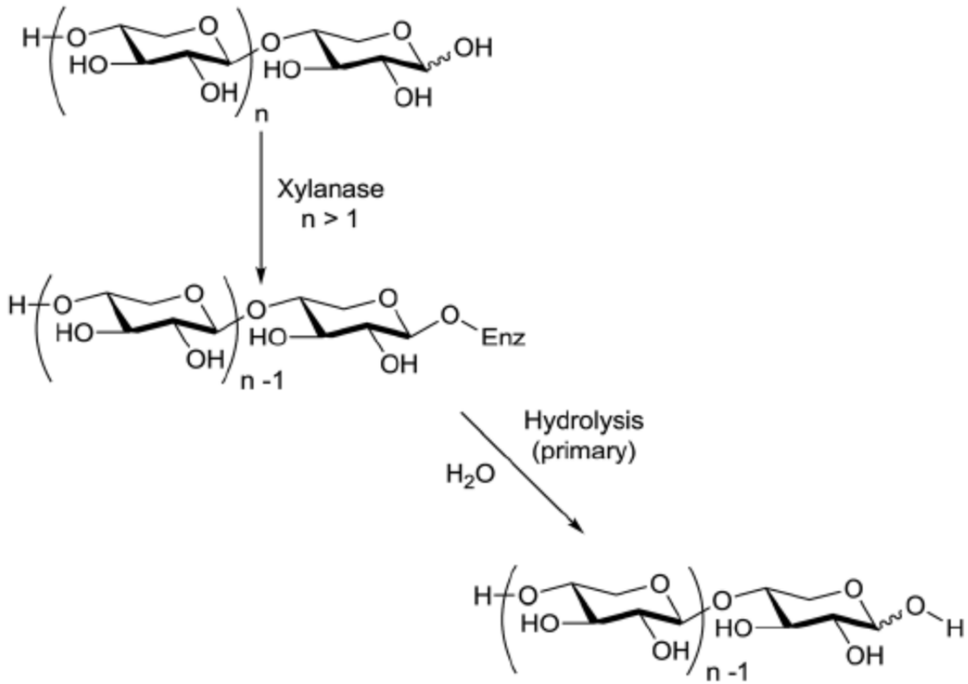
Enzymatic processes environmentally friendly :

- Cellulose and xylans **hydrolysis:** monosaccharides (Glc, Xyl)
- **Glycosylation** and/or **acylation reactions** with alcohols or fatty acids

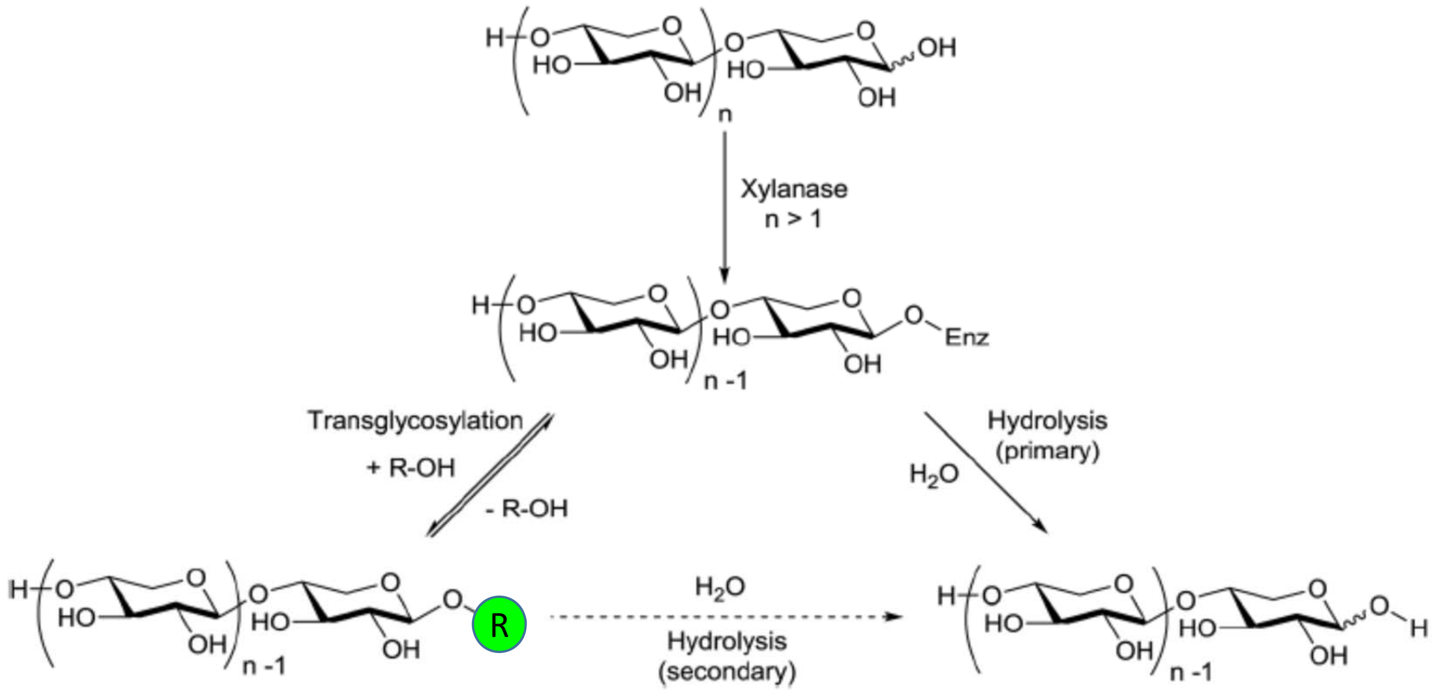
Alkyl polyglycosides and sugar esters

Wheat bran residues: feed

Enzymatic transglycosylation to produce alkyl (poly)xylosides



Enzymatic transglycosylation to produce alkyl (poly)xylosides



Regioselectivity

Muzard *et al.* 2009. J. Mol. Cat. B., Patent FRA 2967164 (2010), Ochs *et al.* 2011. Green Chemistry, Ochs *et al*.. 2013. J. Mol. Cat. B, Brusa *et al*. 2015. RCS Adv.

RSC Advances

REVIEW

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Cite this: *RSC Adv.*, 2015, 5, 91026

β-Xylopyranosides: synthesis and applications

Charlotte Brusa,[abc] Murielle Muzard,[a] Caroline Rémond[bc] and Richard Plantier-Royon*[a]

Enzymatic synthesis of alkyl xylosides

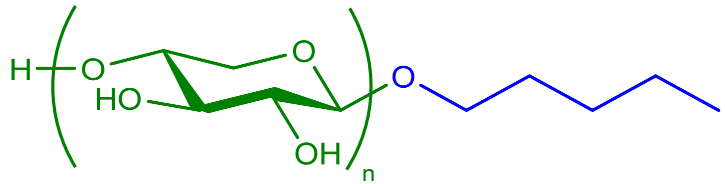
Synthesis of **pentyl (poly)xylosides** directly **from a lignocellulosic biomass**

Wheat bran
(10%)

Transglycosylation

Xylanase

pentan-1-ol / H_2O
(20/80)
48h, 50°C



n = 1 to 3

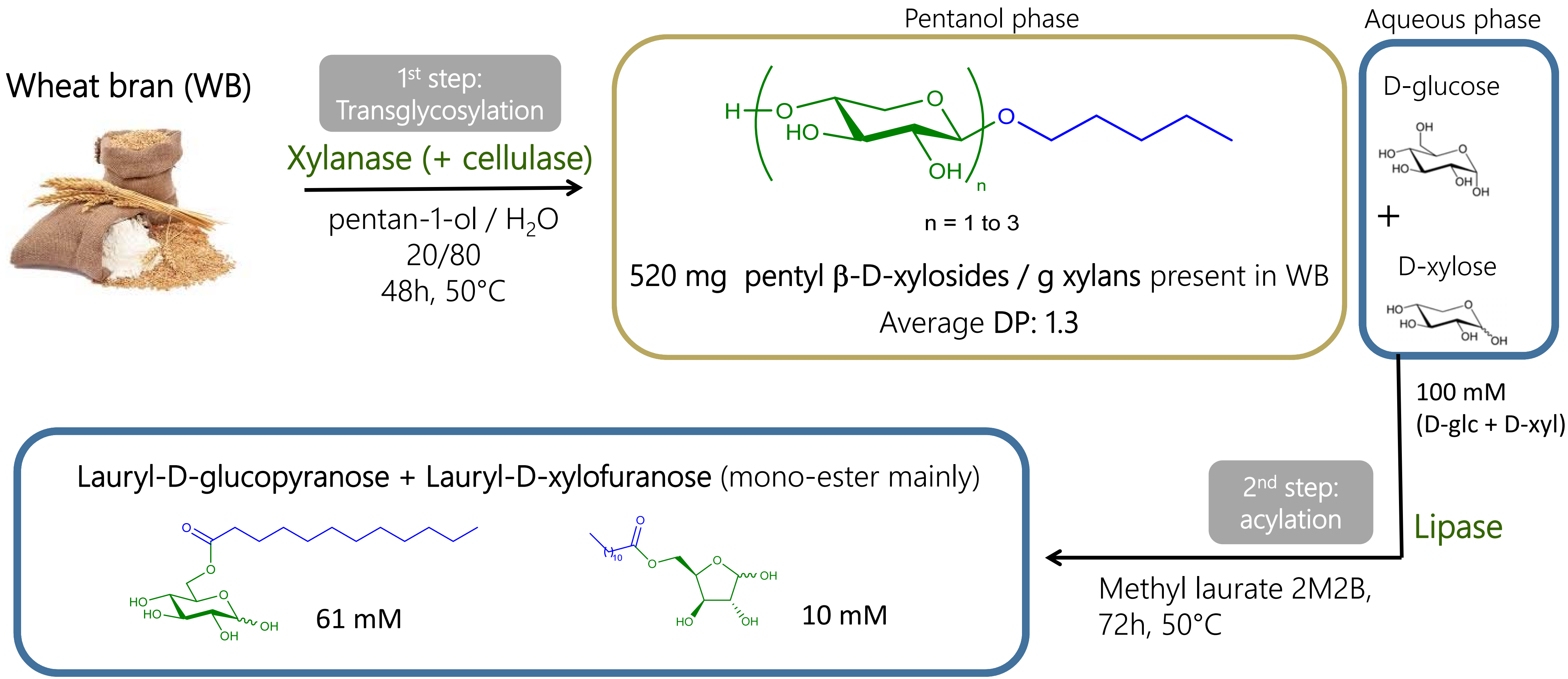
520 mg pentyl β-D-xylosides / g xylans present in WB

Average **DP: 1.3**

Optimization of the reaction parameters: enzyme and substrates loading, duration, agitation speed

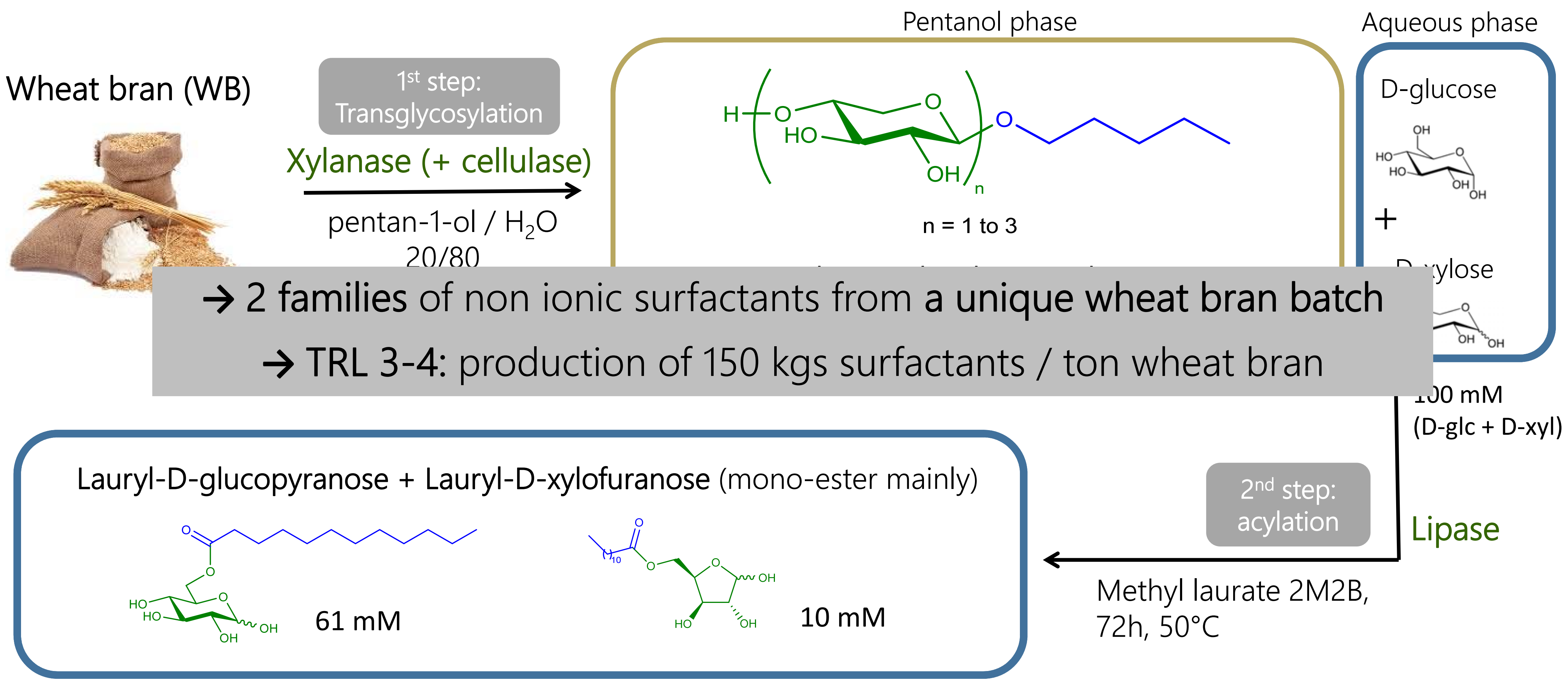
Approach developed with alcohols from C5 to C8

An **integrated approach** for the synthesis of **pentyl (poly)xylosides** and **laurate glucose and xylose esters**



Jocquel *et al.* 2021. Frontiers in Biotechnology and Bioengineering

An **integrated approach** for the synthesis of **pentyl (poly)xylosides** and **laurate glucose and xylose esters**



→ **2 families** of non ionic surfactants from **a unique wheat bran batch**

→ TRL 3-4: production of 150 kgs surfactants / ton wheat bran

Jocquel *et al.* 2021. Frontiers in Biotechnology and Bioengineering

Natural dies and pigments

- **Sourcing:** plants, minerals, animals, microorganisms

- An increasing demand: **naturality**
  5 à 10% of the global market of dies
  Annual growth rate : 7 - 10%

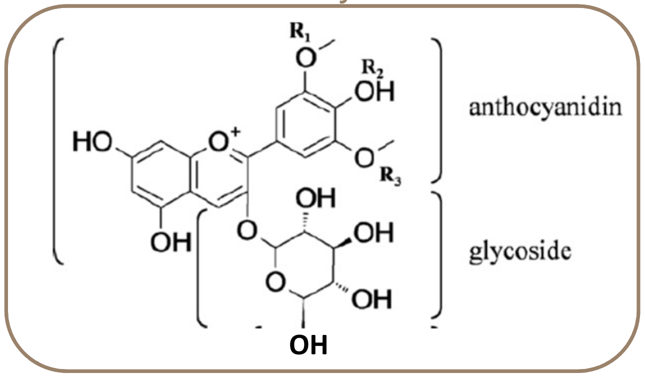
- **Various applications:** food, textile, cosmetics, paints …

Venil et al. RCS Advances. 2014 ; Rao et al. Frontiers in Microbiology. 2017

Production of bioactive molecules from grape pomace

- **Grape pomace**, an abundant and cheap co-product from distilleries
- Main use: **extraction of biomolecules** (polyphenols, anthocyanins), **composting**

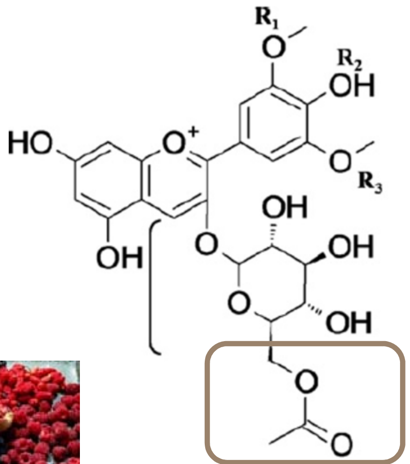
Anthocyanins



Anthocyanins: colored polyphenols

- More than **700 natural anthocyanins** (fruits, vegetables, flowers)
- **Dies for food** (E163); on the list of colorants allowed in cosmetics
- Some other properties: anti-oxidant, anti-microbial, anti-angiogenic

Anthocyanidin	R_1	R_2	R_3	Glycoside
Delphinidin	OH	OH	OH	
Cyanidin	OH	OH	H	Galactose;
Petunidin	OMe	OH	OH	sambubiose;
Pelargonidin	H	OH	H	glucose; arabinose;
Peonidin	OMe	OH	H	rutinose; xylose
Malvidin	OMe	OH	OMe	



- **Low color stability** when extrated from their natural environment
- **Acylated** anthocyanins are **more stable**

Barnes et al. J. Chromatography A. 2009; Wallace and Giusti. Adv. Nutr. 2015; Khoo et al. Food & Nutr. Res. 2017

Current extraction process of anthocyanins in distilleries

Grape pomace

Extraction with solvents and/or sulfite

Chemical input to be reduced : regulations, consumers demand, undesirable health effects

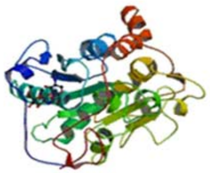
Chaire AFERE   FARE   Lbe   SQPOV UMR408

CARNOT 3BCAR
CARNOT Qualiment Réseau de recherche pour l'innovation alimentaire

ColorANTH project

Grape pomace

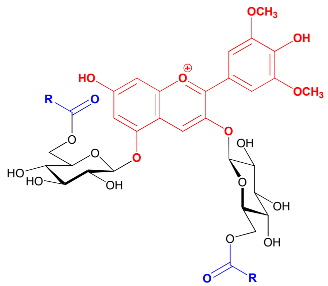
Enzymatic and micro-waves extraction of anthocyanins



Efficient process, environmentally-friendly, keeping the functionalities

Enzymatic acylation

Stable color
Anti-oxidant properties
Better biodisponibility



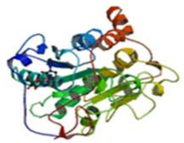
Grape pomace analysis

Main anthocyanins (UPLC-DAD analysis)

- malvidin-3-*O*-glucoside (60%)
- peonidin-3-*O*-glucoside (20%)
- delphinidin-, cyanidin- and petunidin-3-*O*-glucosides (5% each)

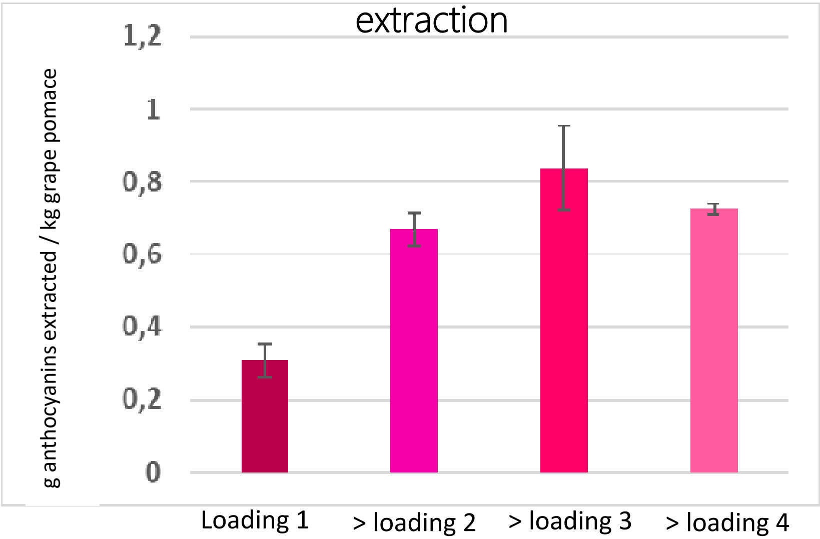
ColorANTH

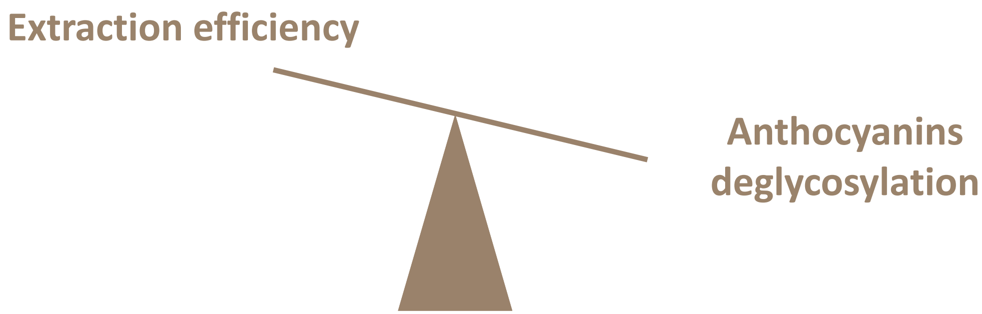
1st step : enzymatic extraction



Assays with diverse enzymes and different reaction conditions : enzyme loading, ratios pomace/enzyme, duration, …

Effect of the enzyme loading onto the anthocyanins extraction

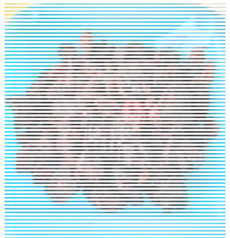




Importance of the enzyme choice, of its loading and of the extraction duration

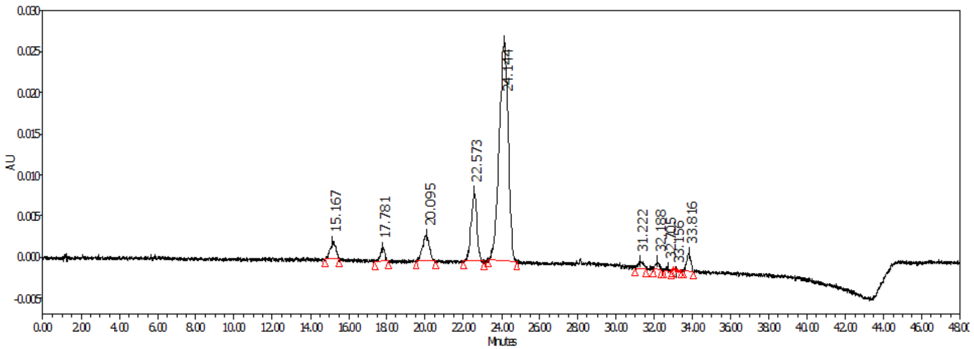
ColorANTH

Enzymatic extraction with the optimal conditions:



Enzymatic extraction

UPLC-DAD analysis



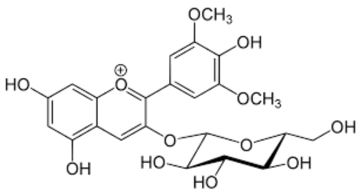
Major anthocyanin: malvidine 3-*O*-glucoside

1.44 g anthocyanins extracted / kg pomace *(1.00 g/kg for extraction with solvent)*

The enzymatic extraction of anthocyanins is rapid and efficient

ColorANTH

2nd step: enzymatic acylation

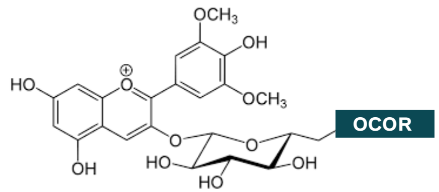


+

R-COOH

Lipase

Various conditions tested



Anthocyanins extract from grape pomace
(malvidine 3-*O*-glucoside mainly)

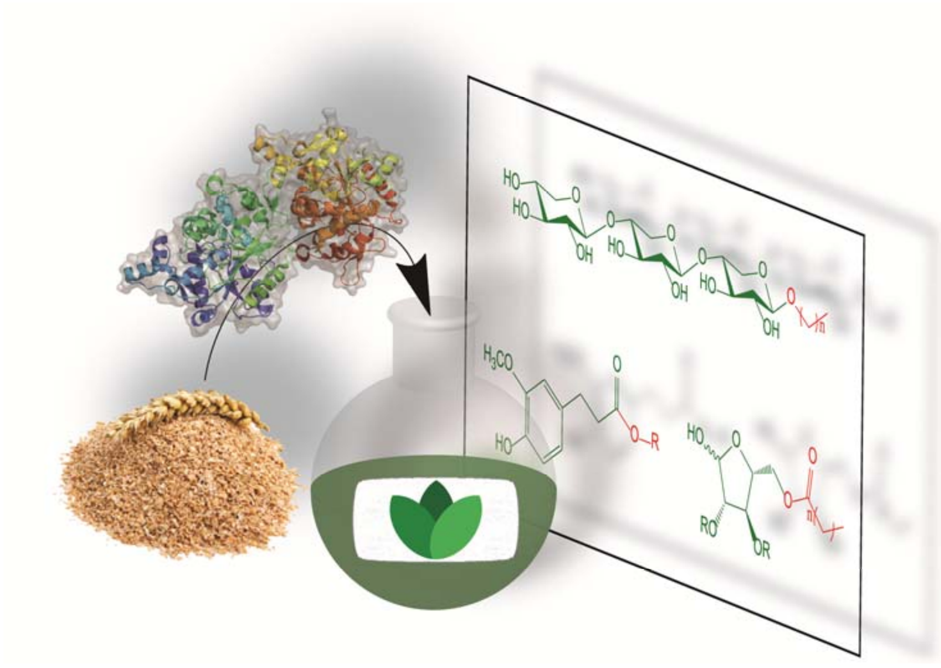
Fatty acids
Phenolic acids

Mixture of acylated anthocyanins

In the best conditions, the acylation yield is > 60% for some acyl donors

Under progress: evaluation of the properties of acylated anthocyanins

Interest of biocatalysis to fractionate biomass, to extract and to functionalize biomolecules



Our biocatalytic approaches can be applied to different biomasses to target a large panel of biomolecules

Thanks to all students, colleagues and collaborators

Thank you for your attention