# Enzymatic upgrading of hemicelluloses for materials and nutrition

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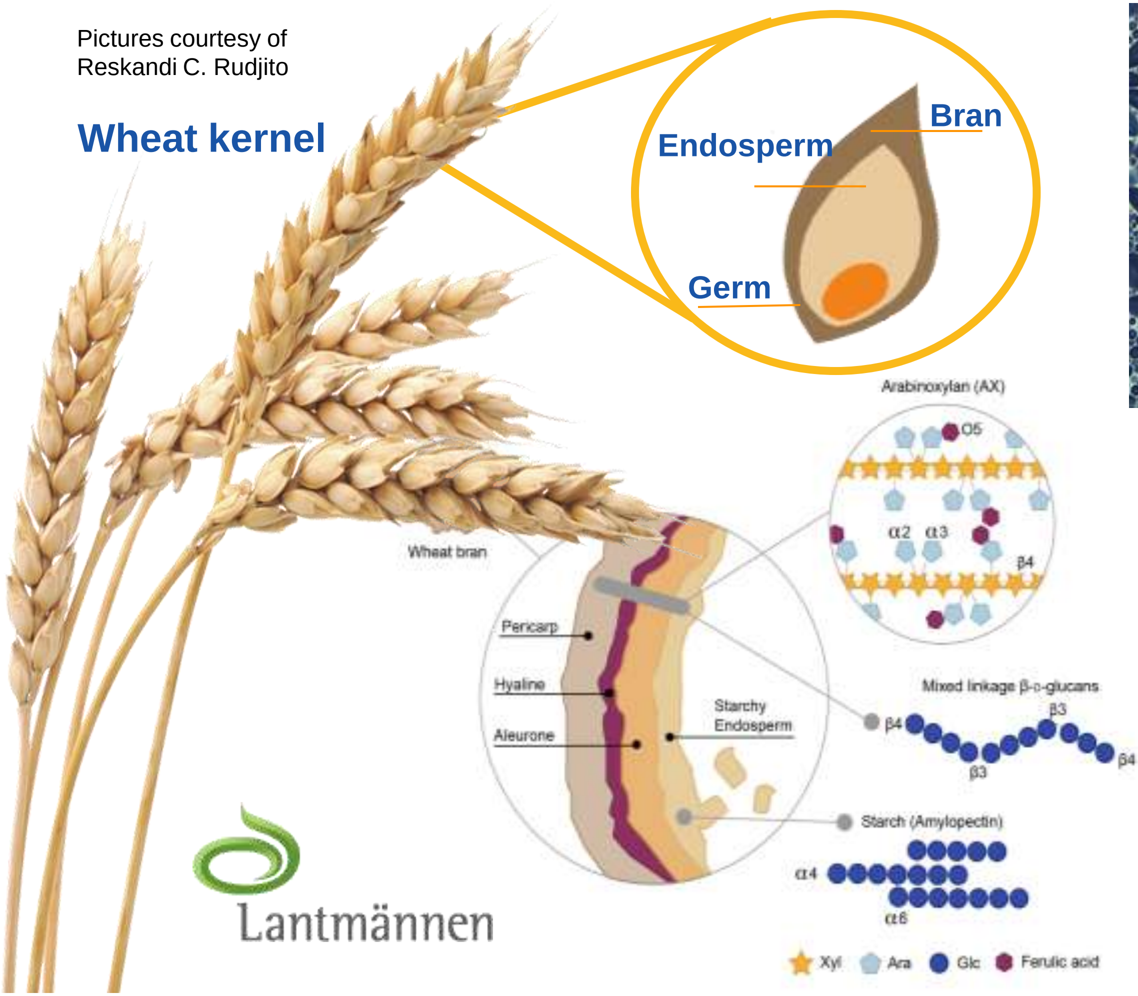
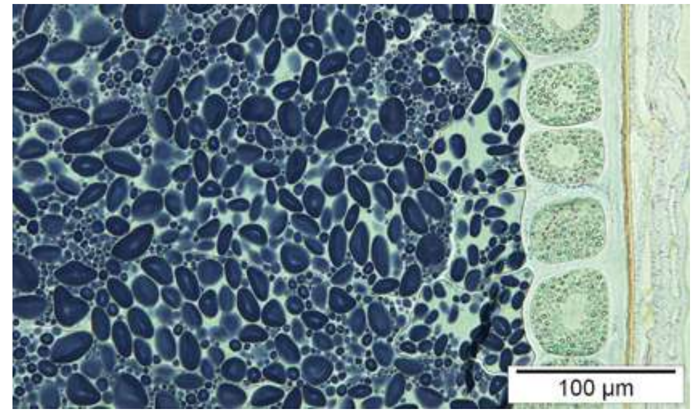
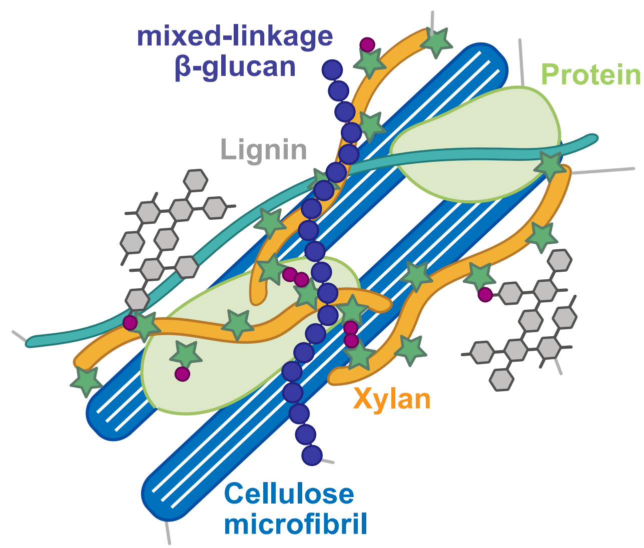
# Cereal Cell Walls as Source of Novel Materials and Food Ingredients

Pictures courtesy of
Reskandi C. Rudjito

## Wheat kernel



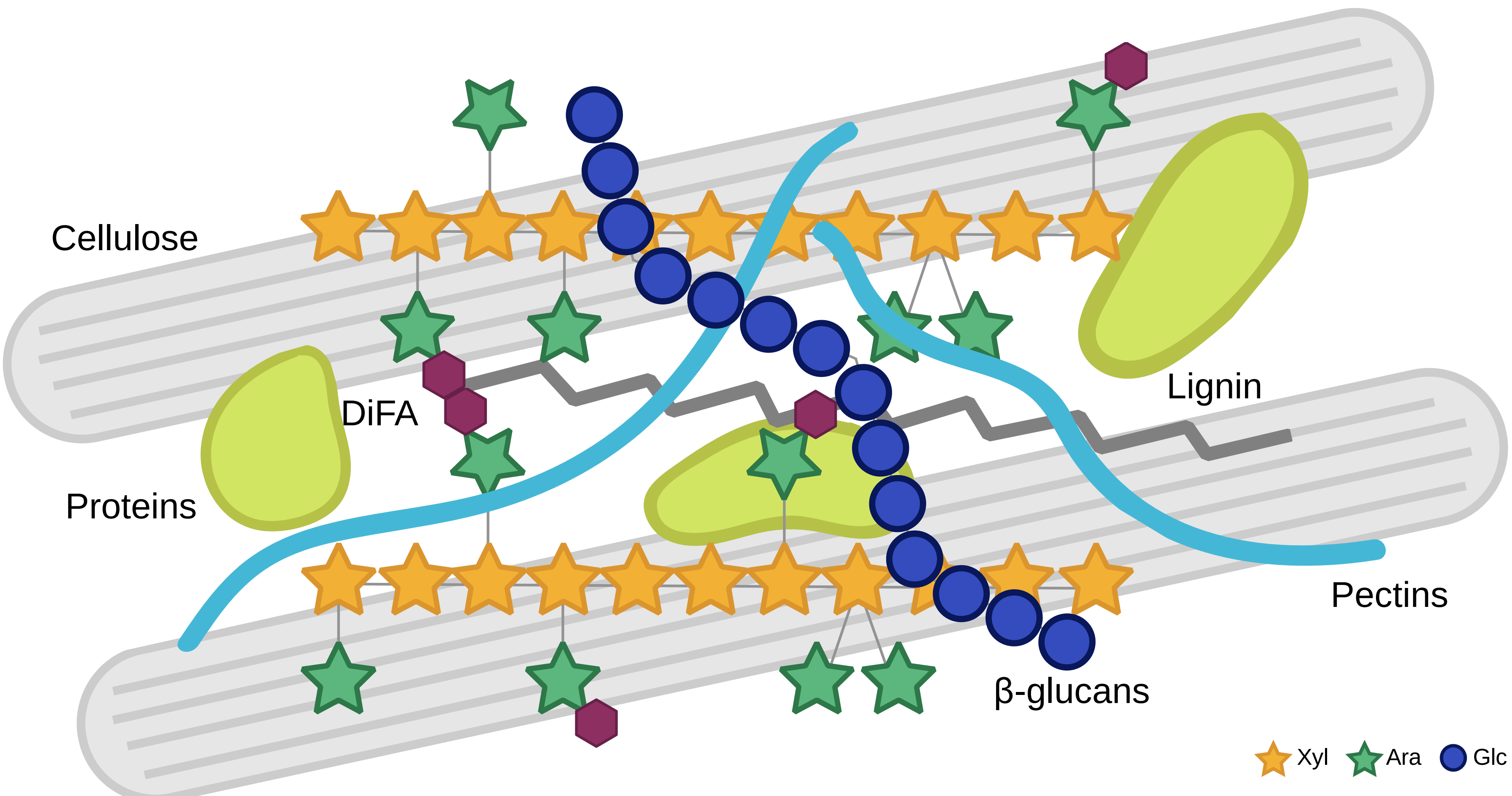



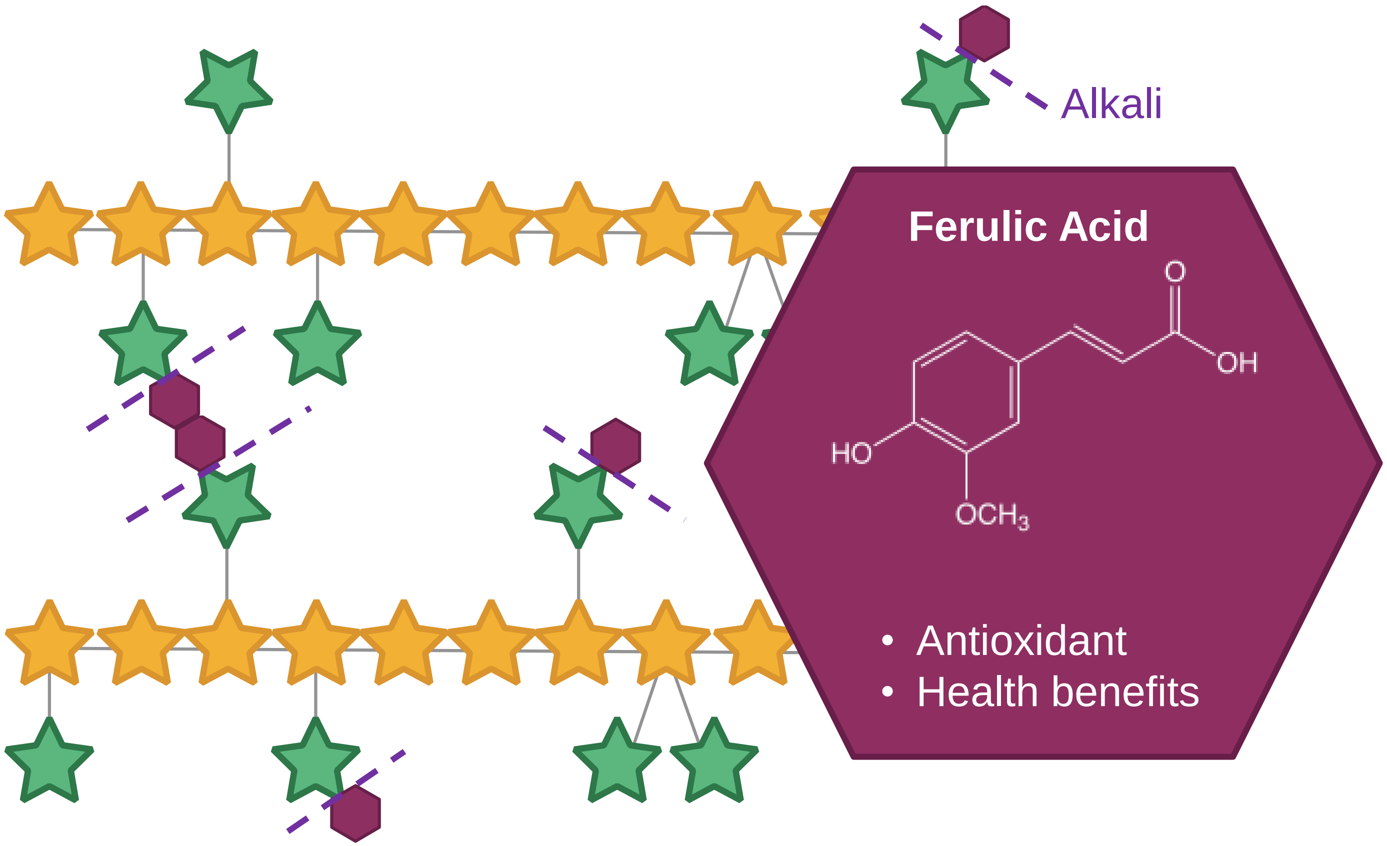

Lantmännen


RC Rudjito, AC Ruthes, A Jiménez-Quero, F Vilaplana. ACS Sustainable Chem & Eng (2019) 7, 13167
U Holopainen-Mantila, M Raulio. Imaging Technologies and Data Processing for Food Engineers, 1 (2016).

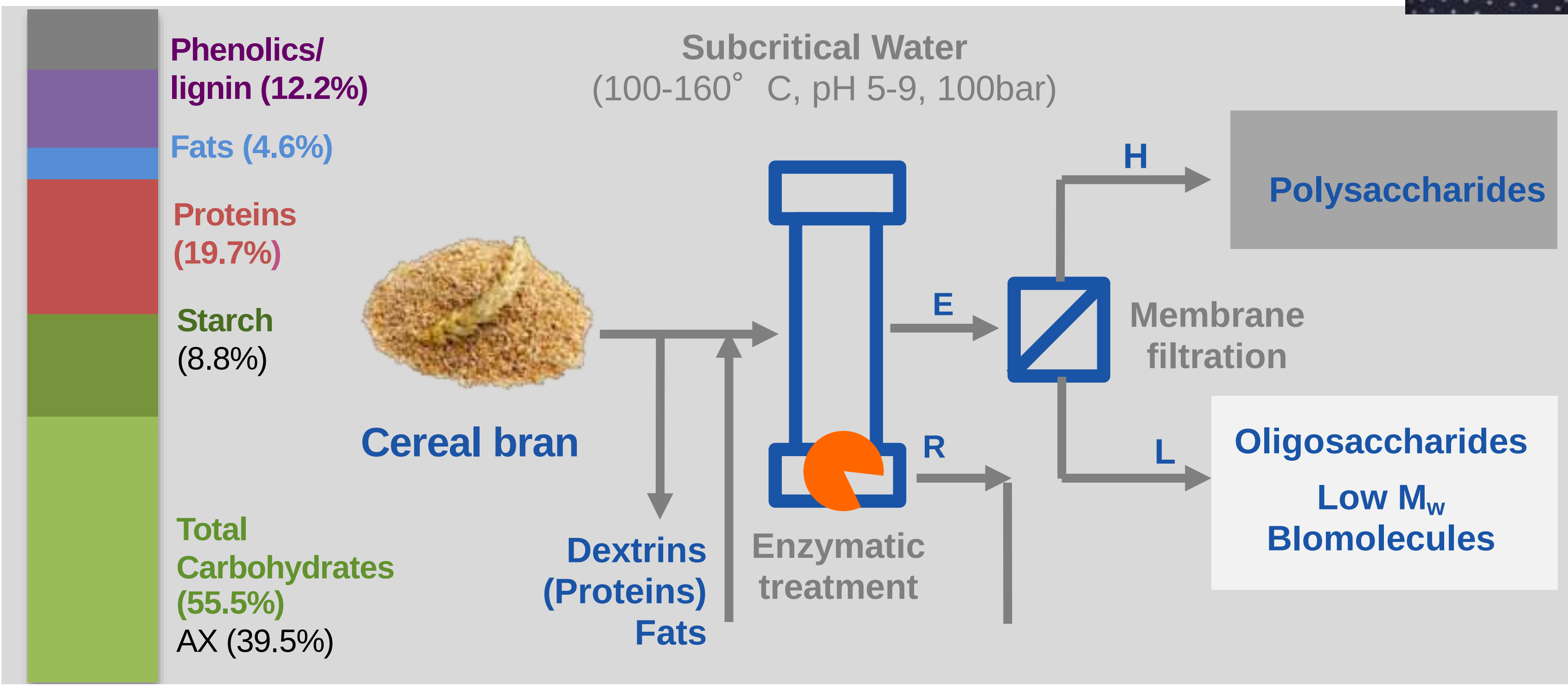
# Molecular Architecture of Cereal Cell Walls



# Extraction of Arabinoxylan from Cereal Cell Walls



# Chemo-enzymatic Valorization of Dietary Fibres from Cereal Brans

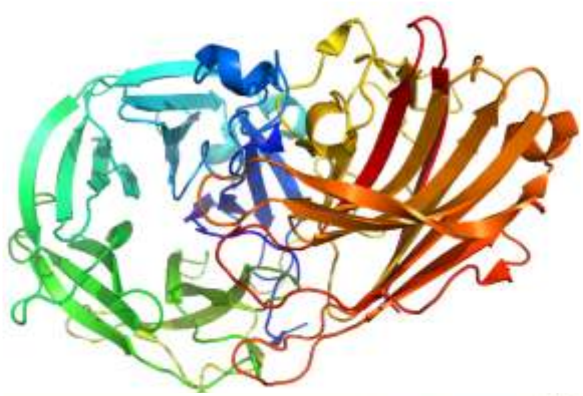



Lantmännen

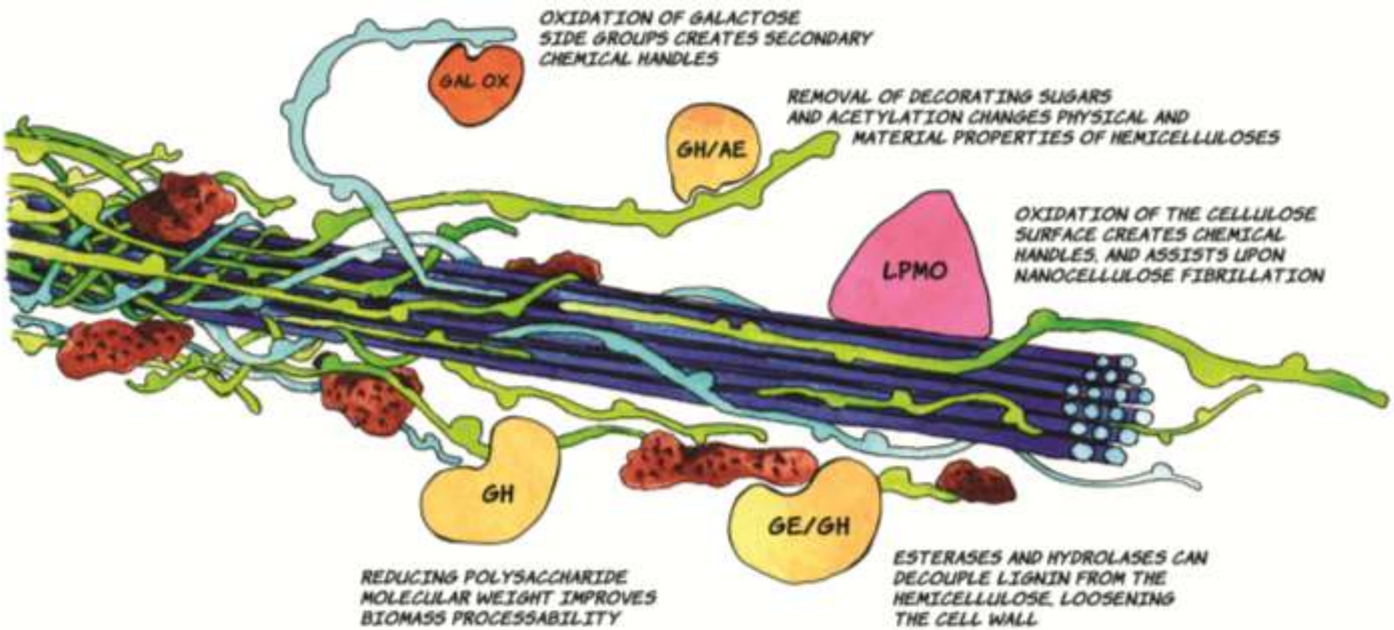
novozymes Rethink Tomorrow


AC Ruthes, AM Abad, HW Tan, V Bulone, F Vilaplana. Green Chemistry (2017) 19, 1919
RC Rudjito, AC Ruthes, A Jiménez-Quero, F Vilaplana. ACS Sustainable Chem & Eng (2019) 7, 13167
RC Rudjito, … F. Vilaplana. Green Chemistry (2020), 22, 8337-8352.

# Enzymes as versatile tools in bioprocess and material engineering



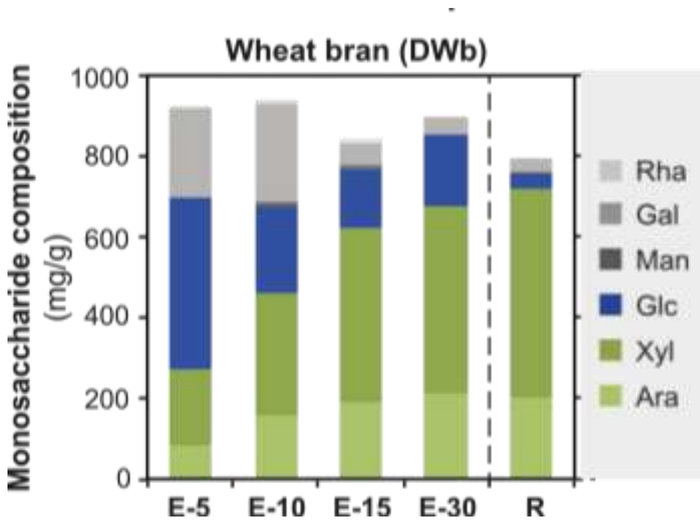
**Enzymes** are biological catalysts involved in both the formation or the cleavage of a chemical bond.




AM Abad, AC Ruthes, F Vilaplana. J Applied Polymer Science (2015), DOI: 10.1002/APP.42523

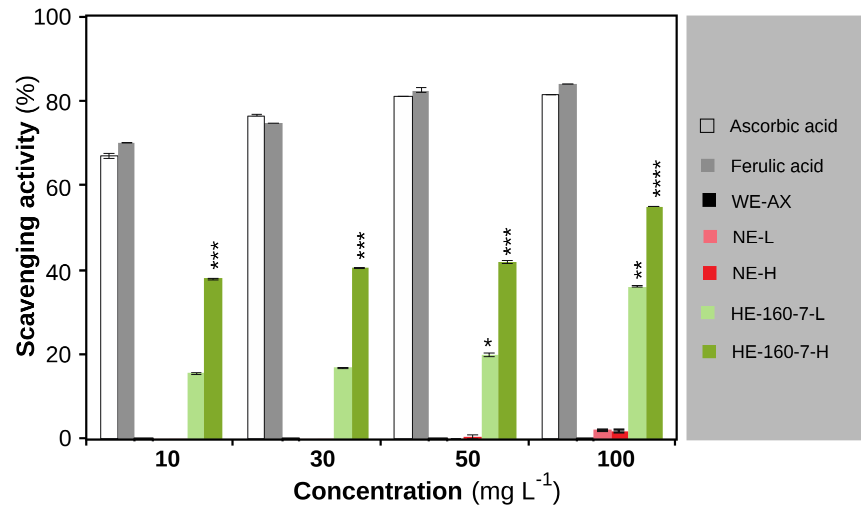
# Chemo-enzymatic Valorization of Dietary Fibres from Cereal Brans

## Enrichment of F-AX during time



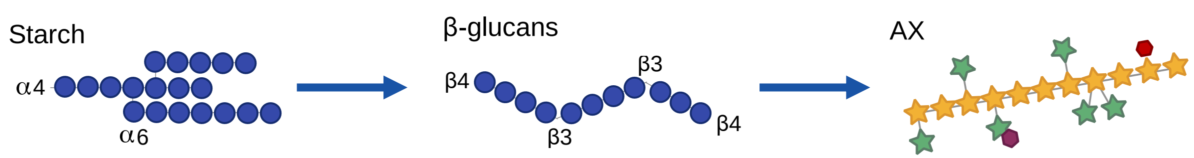

## Ferulic acid is preserved during SWE:

(FA content 4 – 12 mg/g in wheat bran)




## AX with low A/X ratio (0.3-0.5)

## Radical scavenging properties





AC Ruthes, AM Abad, HW Tan, V Bulone, F Vilaplana. Green Chemistry (2017) 19, 1919
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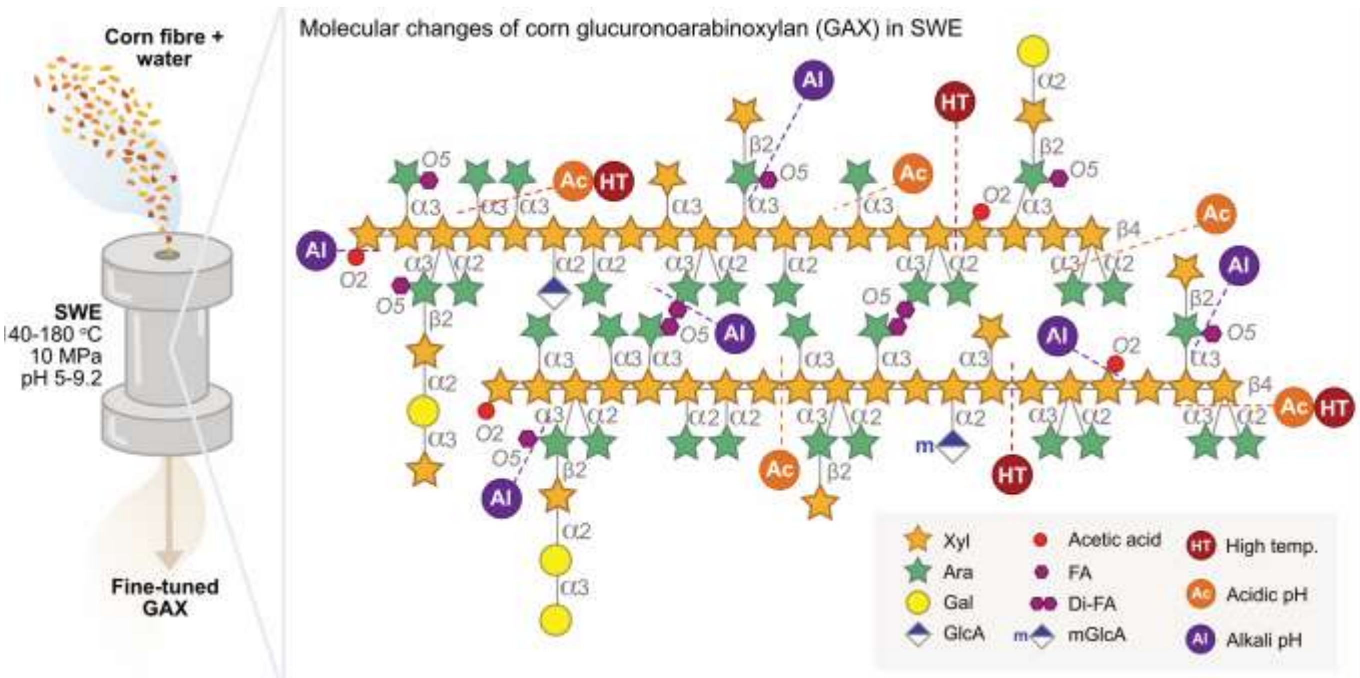
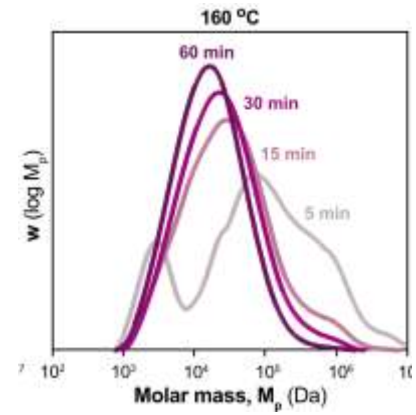
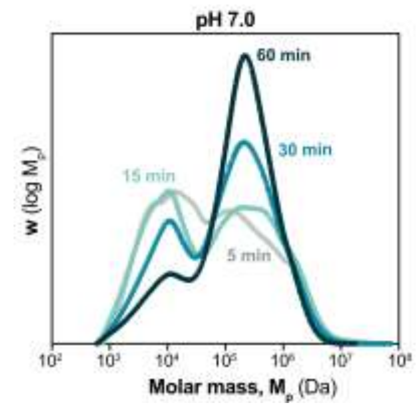
RC Rudjito, … F. Vilaplana. Green Chemistry (2020), 22, 8337-8352.

# Cereal source and processing conditions influence molecular structure of F-AX

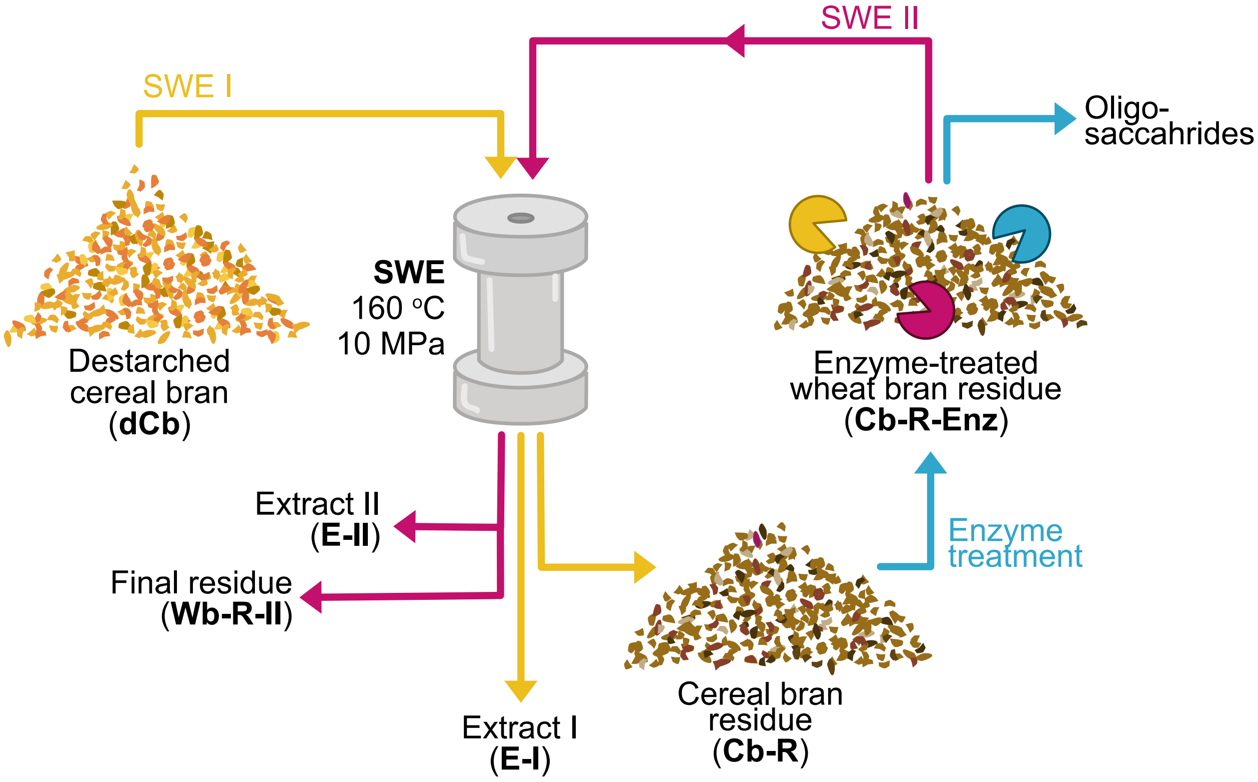




RC Rudjito, AC Ruthes, A Jiménez-Quero, F Vilaplana. ACS Sustainable Chem & Eng (2019) 7, 13167
RC Rudjito et al. Green Chemistry (2020), 22, 8337-8352.

# Integrated bioprocess to release remaining AX in residue

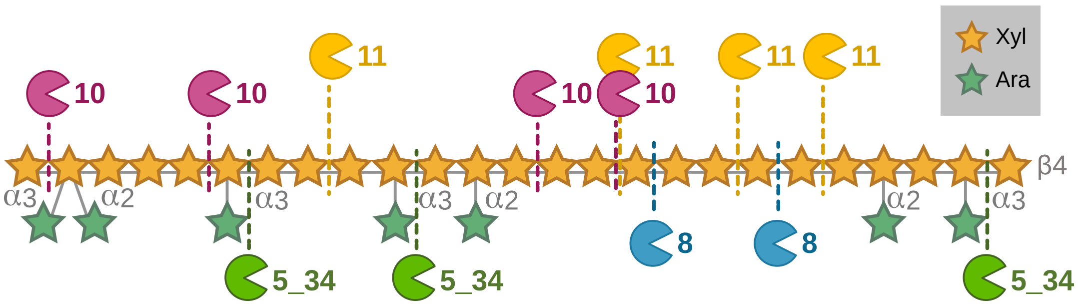
**Subcritical water extraction (SWE) and xylanolytic enzymes**




- Approx. 43.5 % of AX remain in the residue (R) after SWE I
- Xylanolytic enzymes (xylanases, arabinofuranosidases and FAEs) followed by SWE II

**Focus: Xylanases**

# Family-specific activity of xylanases

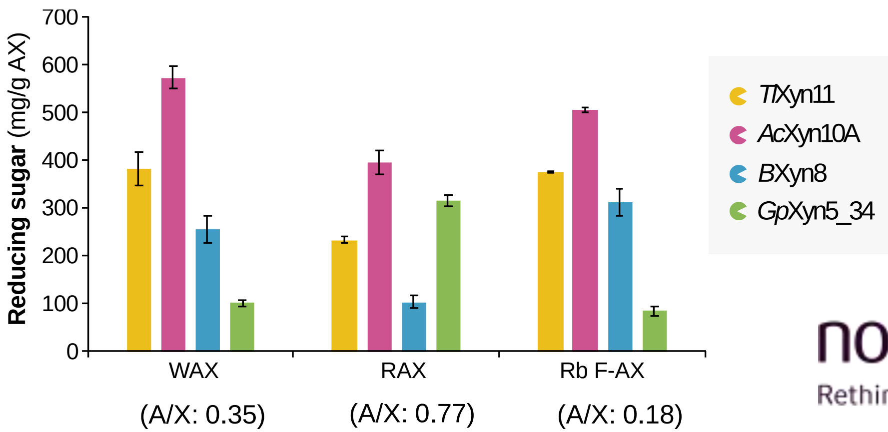



**WAX** (A/X: 0.35)

**RAX** (A/X: 0.77)

**Rb F-AX** (A/X: 0.18)

## Activity on 3 AX substrates with different A/X and FA content

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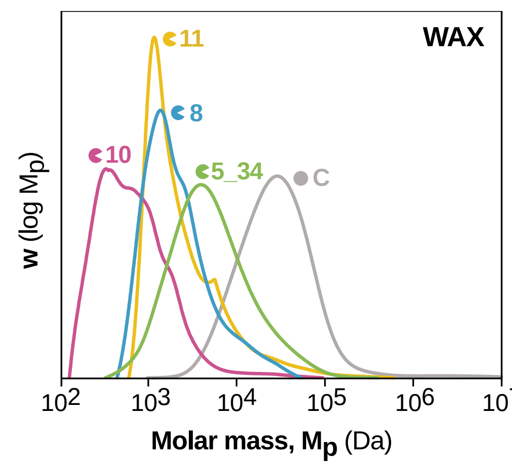


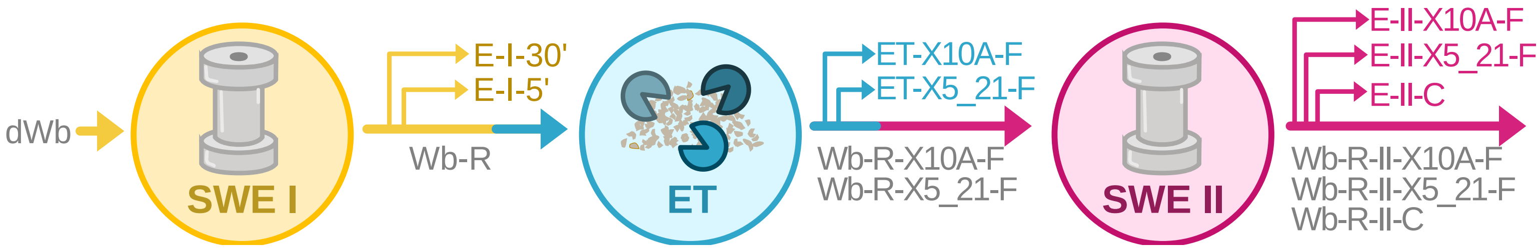

***Ac*Xyn10A** was most active on all substrates: produces X2 and small oligosaccharides

***Tl*Xyn11** was more restricted than the GH10: produces X3 and small oligosaccharides

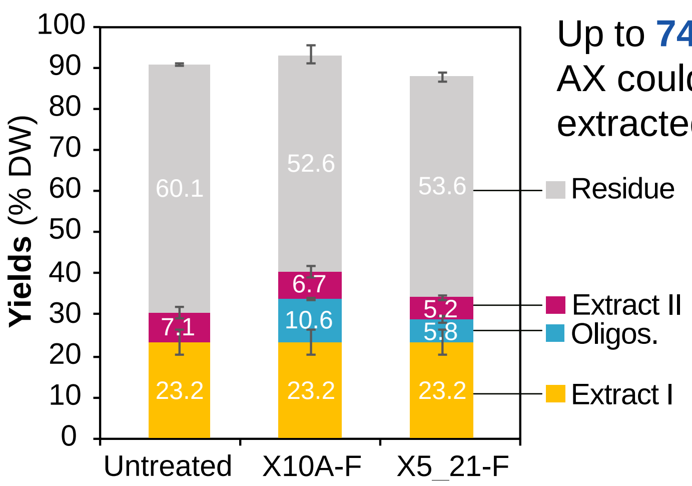
***B*Xyn8** was most restricted by Ara*f* substitution: produced long linear XOS

***Gp*Xyn5_34** required Ara*f* substitution for hydrolysis: produced complex long (A)XOS

# Integrated bioprocess: maximisation and diversification of AX extraction

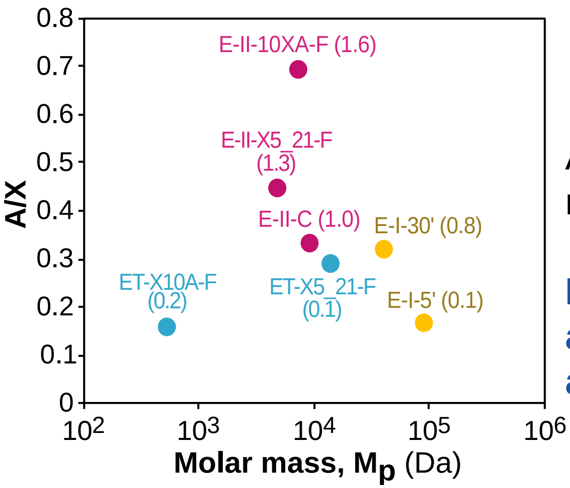



## Extraction yields




Up to **74.2%** AX could be extracted

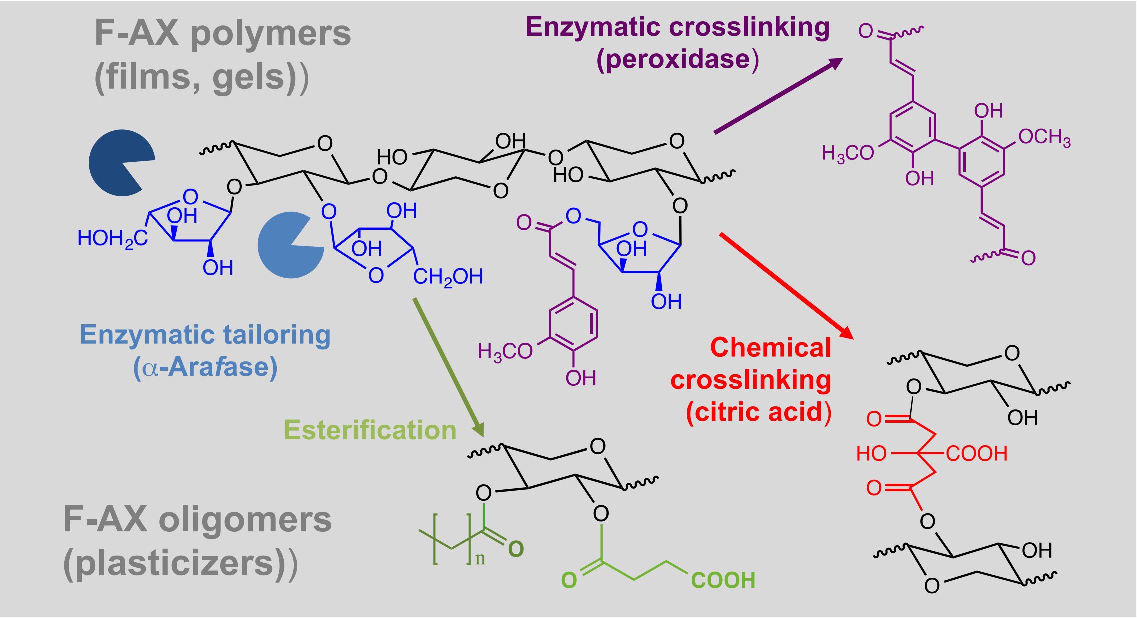
## Molecular features




AX fractions with differing molecular structures

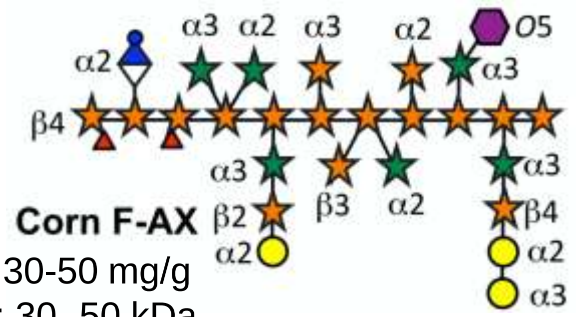
**Important for material and nutritional applications**

# Chemo-Enzymatic Valorization of Dietary Fibres from Cereal Brans



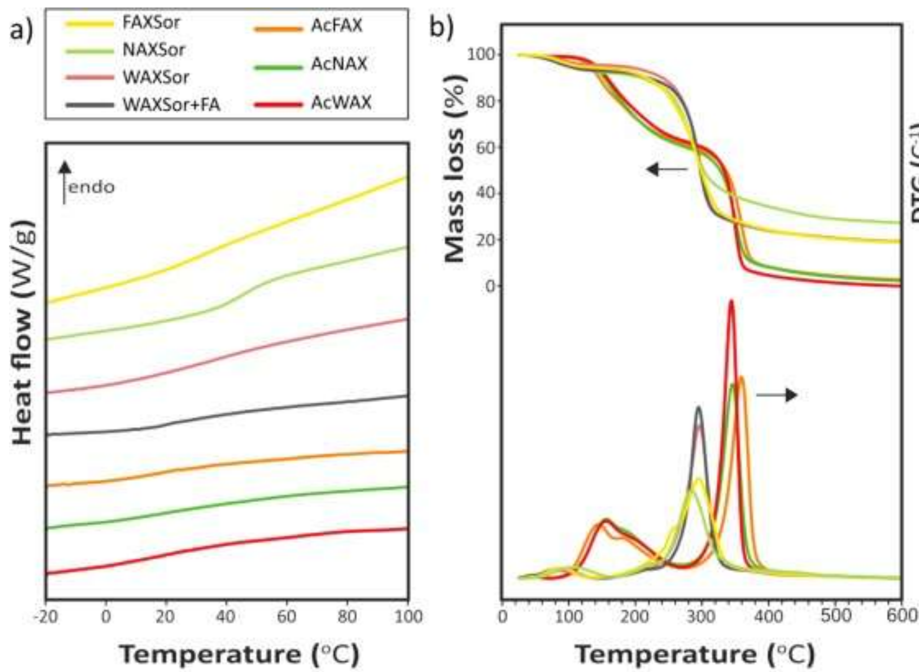


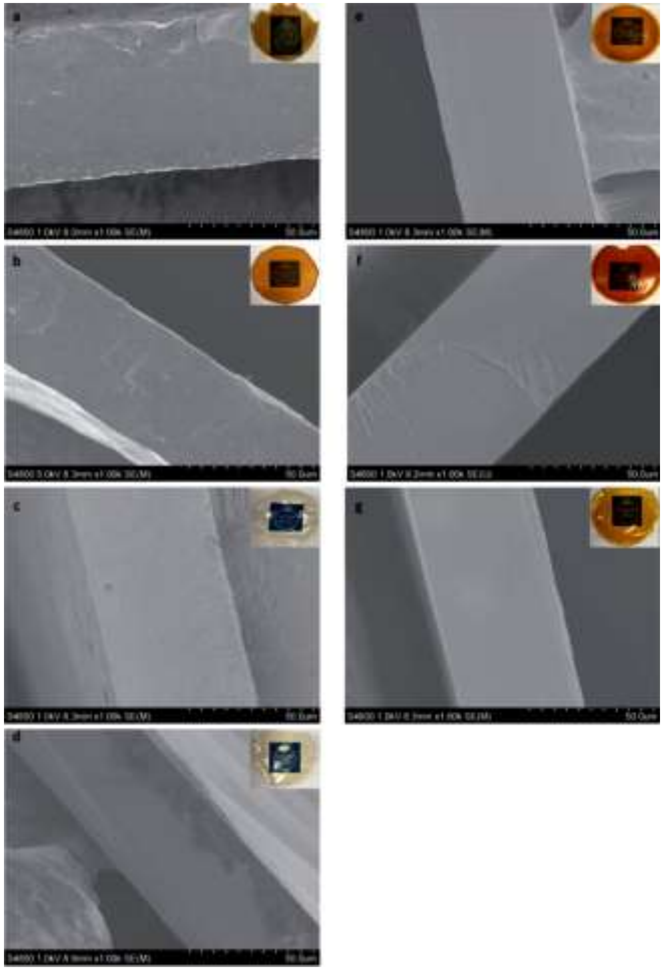
FA: 8-10 mg/g
Mw: 100 kDa




FA: 30-50 mg/g
Mw: 30- 50 kDa

# Bio-based films from wheat bran feruloylated arabinoxylan



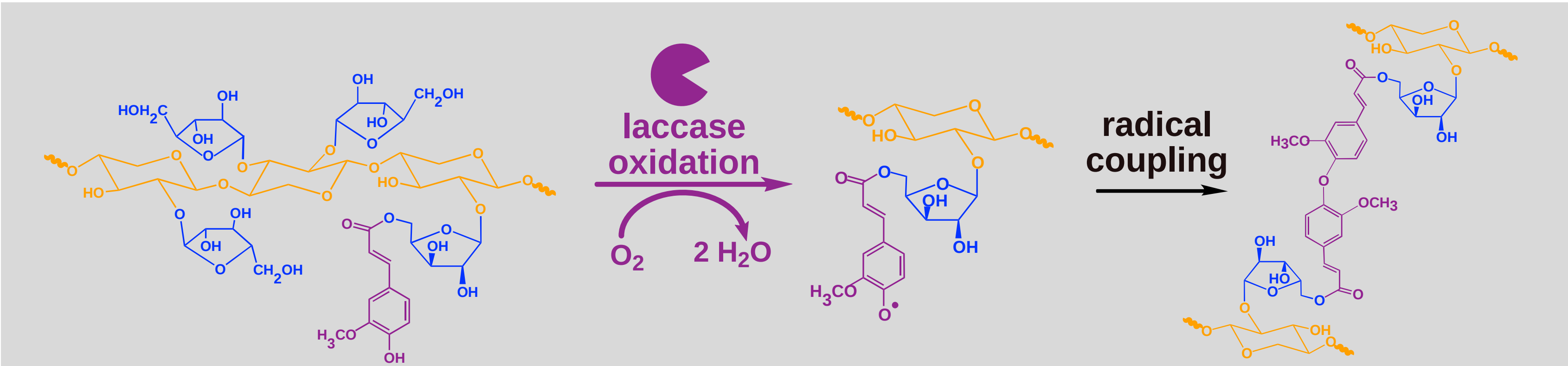



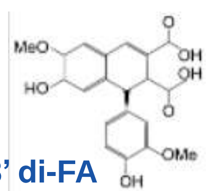
## Better bound than free!

- Higher degree of substitution and molecular weight favour **film properties**
- Bound FA has higher **antioxidant activity** than free FA
- Chemical acetylation improves **thermal stability**

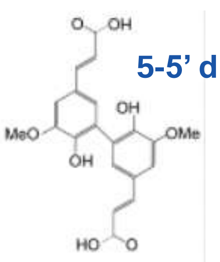
S Yilmaz-Turan et. al. Bio-based films from wheat bran feruloylated arabinoxylan: Effect of extraction technique, acetylation and feruloylation
Carbohydrate Polymers (2020) 250, 116916

# Enzymatic Engineering of F-AX gels from wheat bran



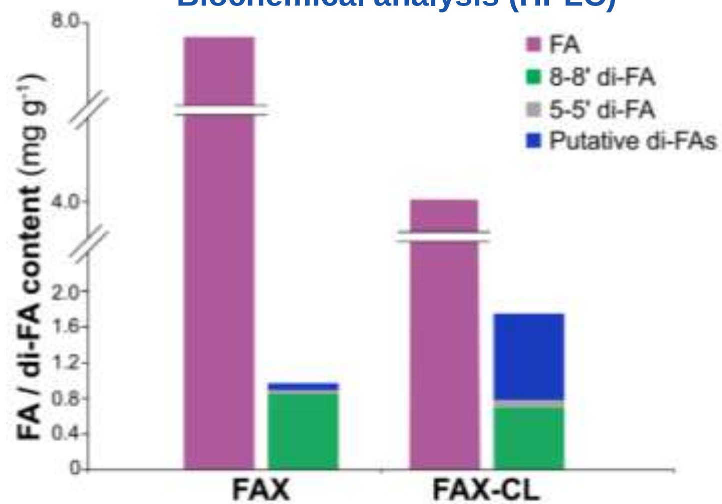



8-8’ di-FA

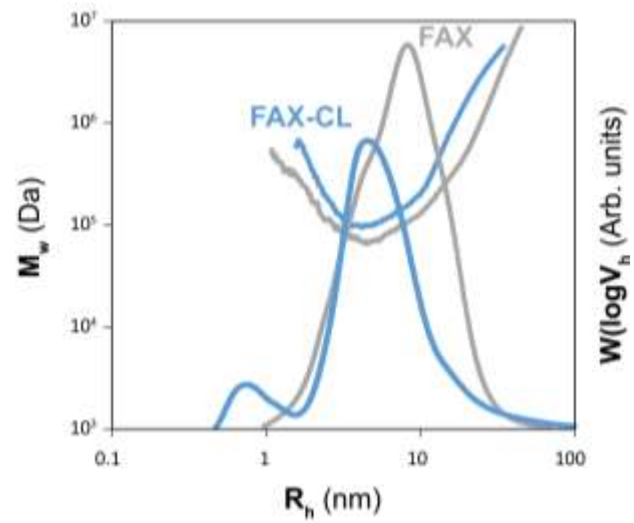


5-5’ di-FA

Biochemical analysis (HPLC)

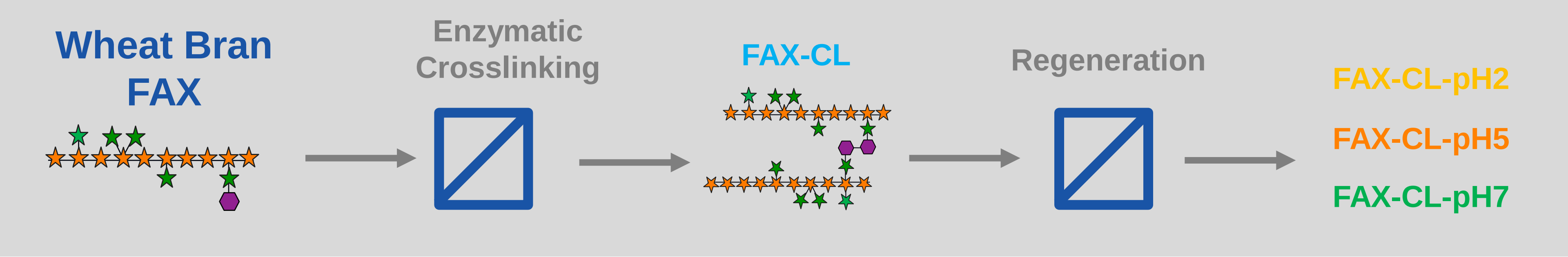


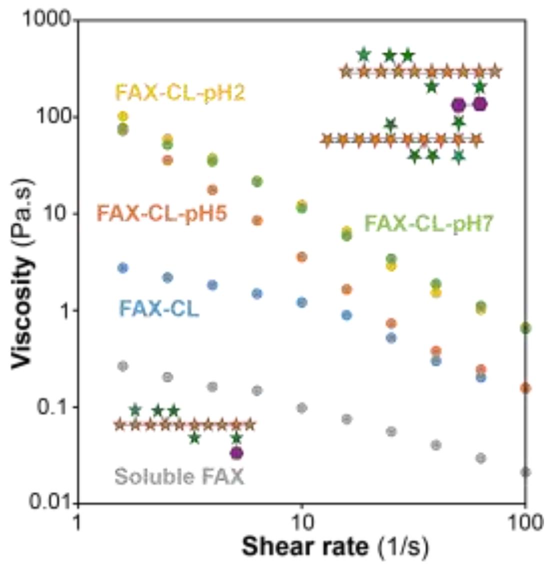

Molar mass distributions




S. Yilmaz-Turan, A. Jiménez-Quero, P. López-Sánchez, T. Plivelic, F. Vilaplana, Food Hydrocolloids (2022), 128, 107575

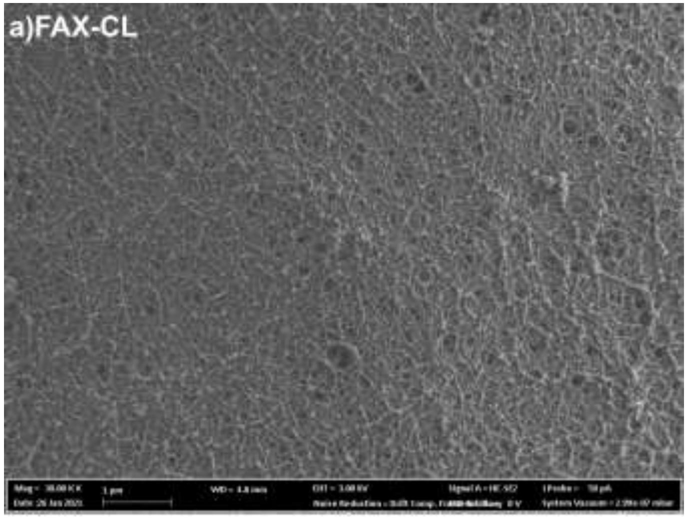
# Enzymatic Engineering of F-AX gels from wheat bran




FAX-CL-pH7

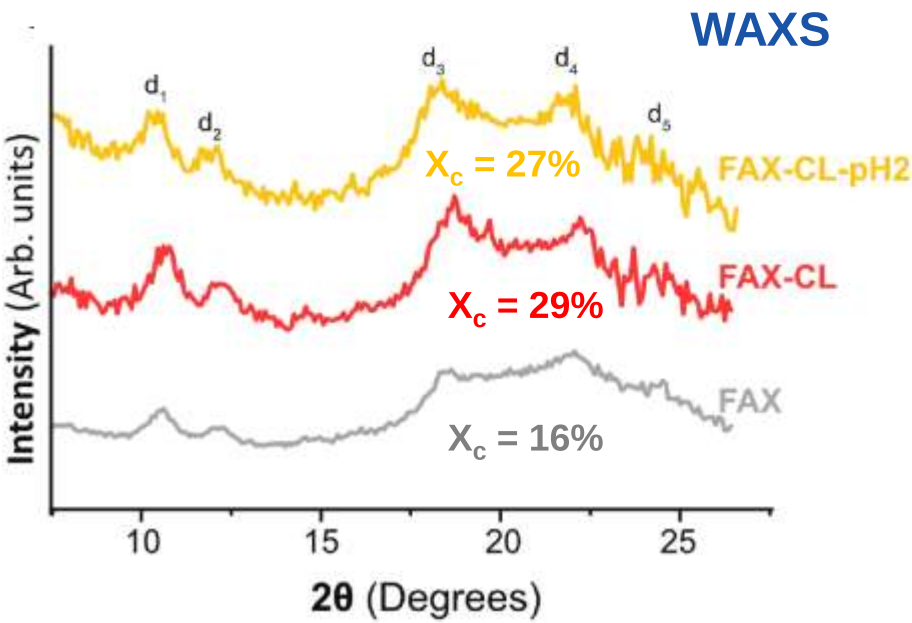
FAX-CL

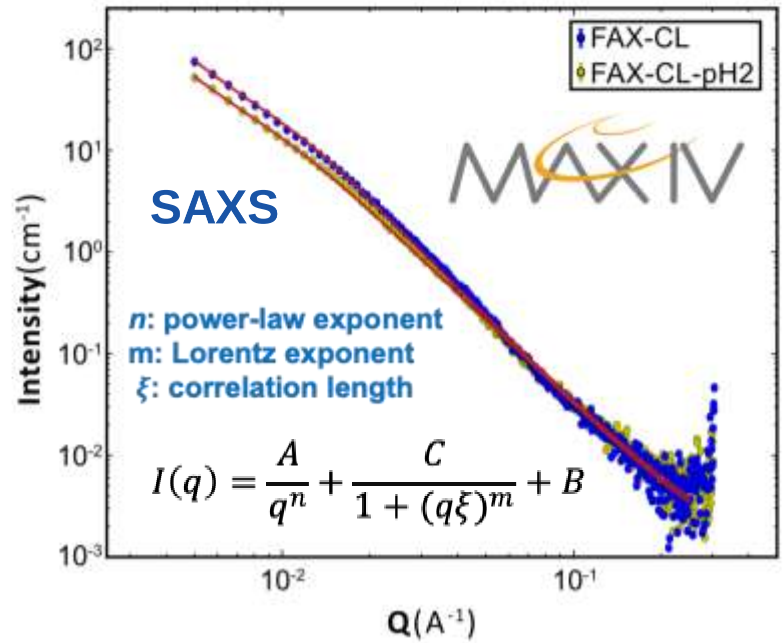


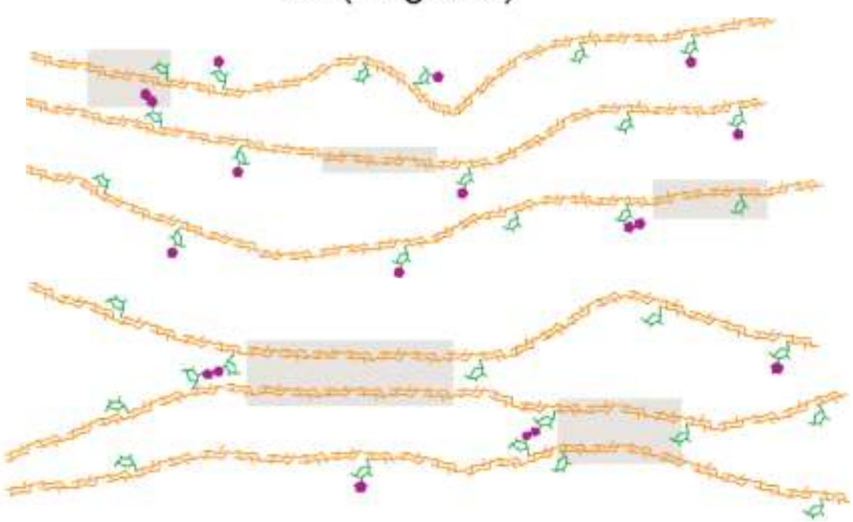

Cryo-SEM gel morphology

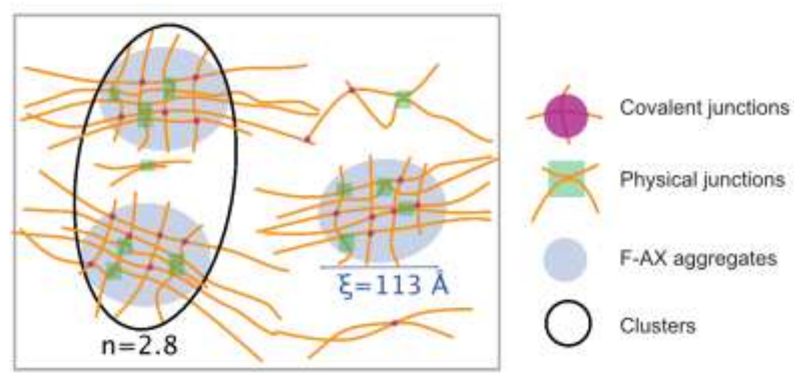

S. Yilmaz-Turan, A. Jiménez-Quero, P. López-Sánchez, T. Plivelic, F. Vilaplana, Food Hydrocolloids (2022), 128, 107575

# Chemical and physical processes influence network assembly

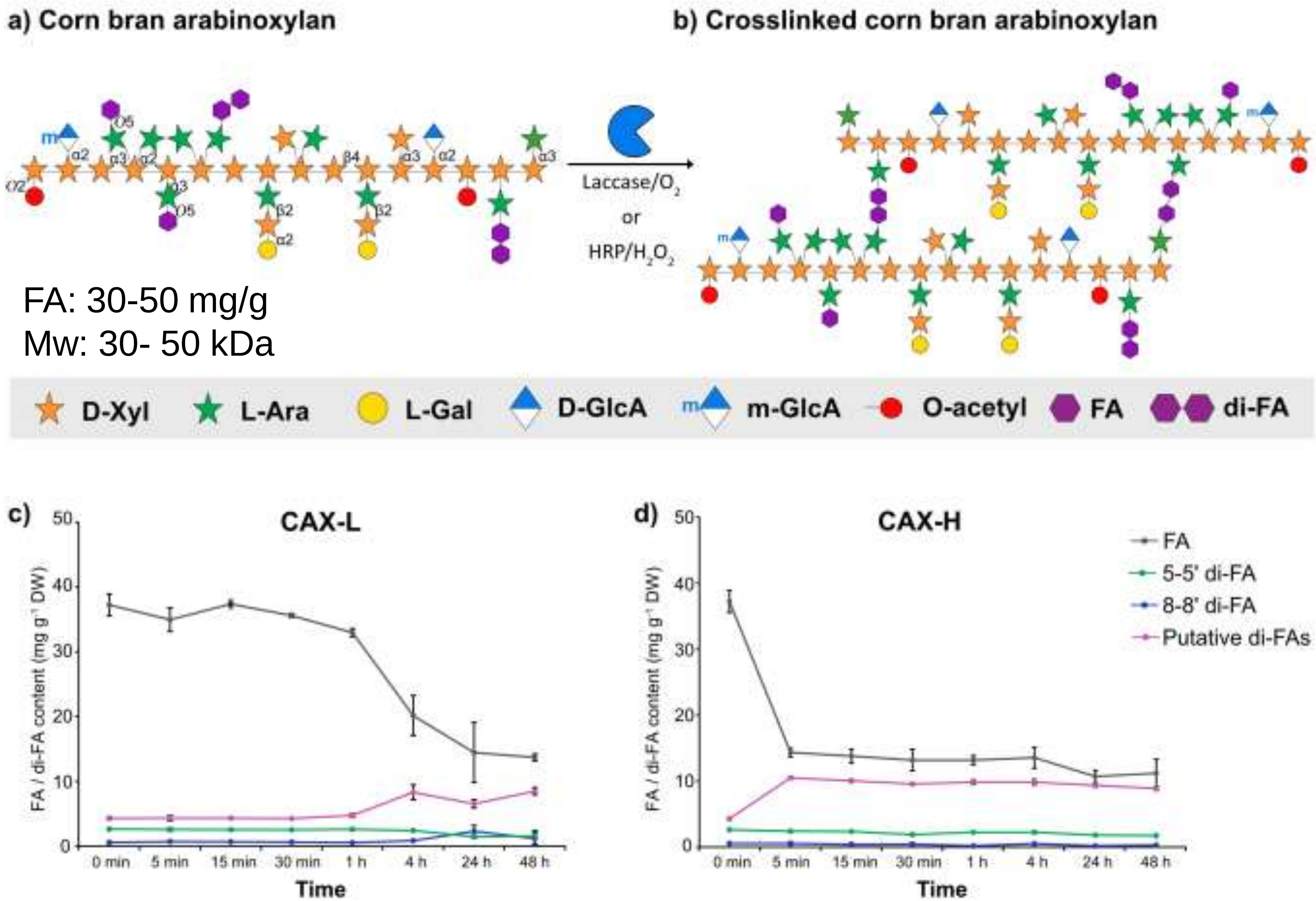




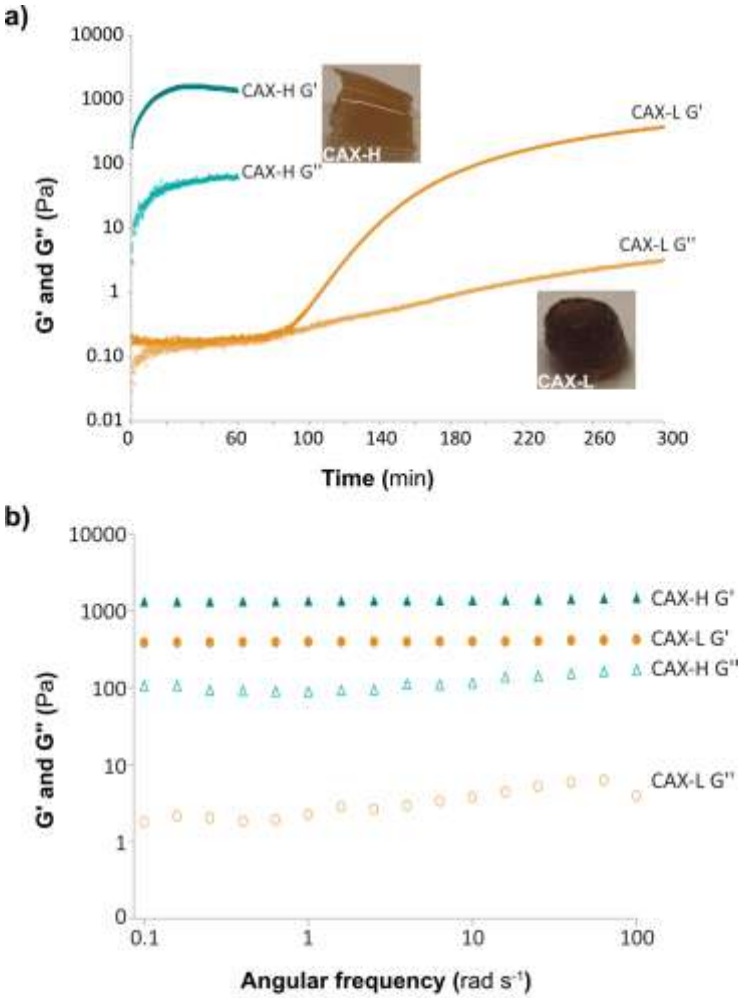
# Laccase and peroxidase for crosslinking of corn FAX

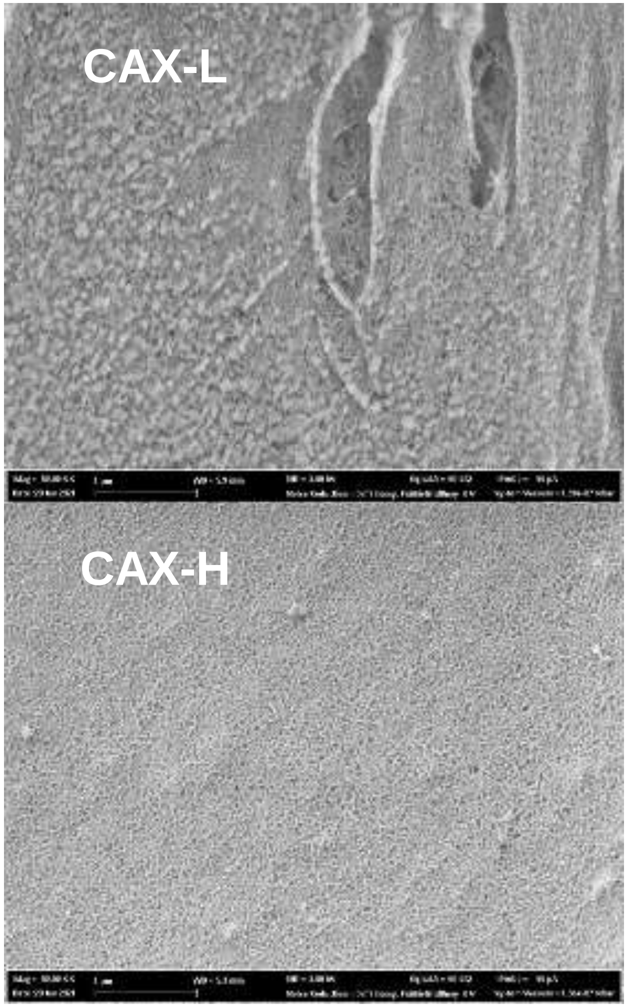



FA: 30-50 mg/g
Mw: 30- 50 kDa

Secil Yilmaz-Turan … Francisco Vilaplana. Hydrogels with protective effects against in vitro cellular oxidative stress via enzymatic crosslinking of corn bran arabinoxylan. Manuscript in preparation

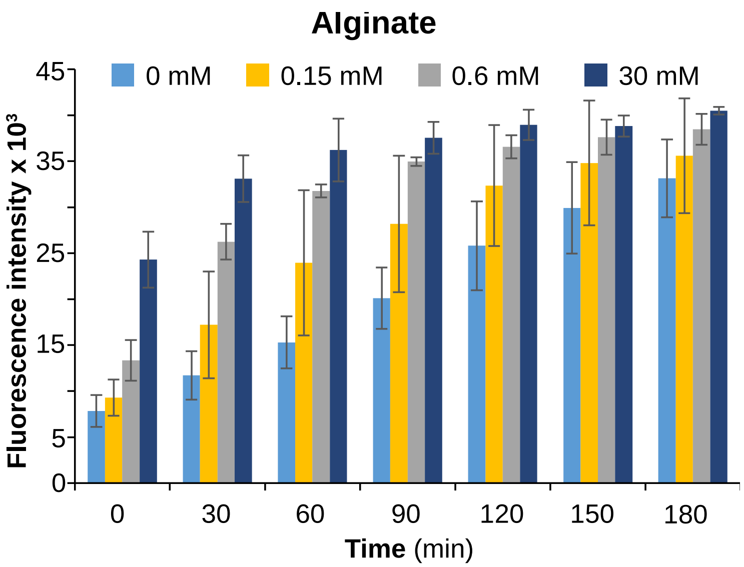
# Rheological properties and morphology

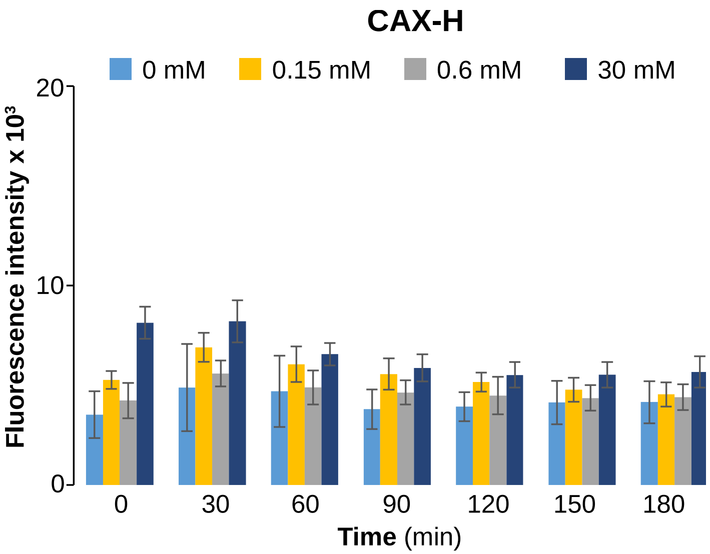


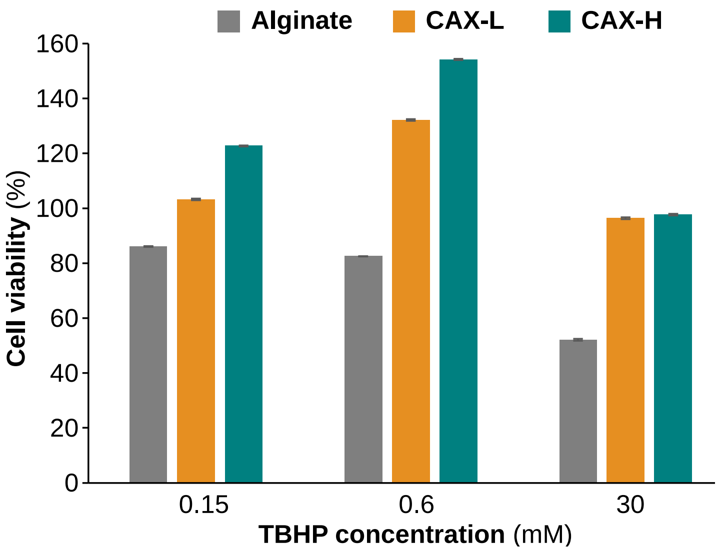




Secil Yilmaz-Turan … Francisco Vilaplana. Hydrogels with protective effects against in vitro cellular oxidative stress via enzymatic crosslinking of corn bran arabinoxylan. ACS Applied Materials and Interfaces *Under review*

# Scavenging Properties Against Reactive Oxygen Species



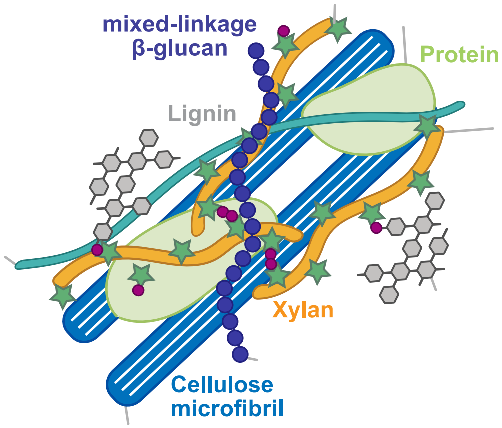



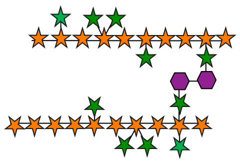



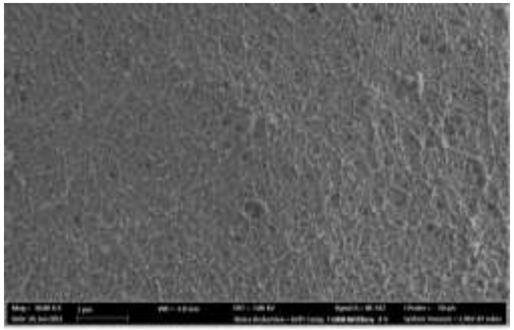

- Seeding **human epithelial cell line** (HT-29-MTX) on top of the CAX-L and CAX-H hydrogels
- **Cyto-compatibility** and **antioxidant activity** against TBHP-induced oxidative stress.
- Cells cultured on CAX-L and CAX-H **produced lower ROS** for all TBHP concentrations applied
- **Increased cell viability** compared to a reference alginate gel

# Take Home Messages

- Feruloylated AX from **cereal sources** as a polymeric matrix for the development of **functional hydrogels** with antioxidant properties
- **Enzymatic oxidative coupling** enables the formation of covalent bridges between the phenolic moieties.
- The **molecular structure** of AX (ferulic acid content, A/X ratio, molar mass) influence the morphology and rheological properties of the hydrogels.
- **Chemical and physical effects** control the mechanisms of hydrogel formation
- The presence of ferulic acid renders hydrogels with protective effects against **cellular oxidative stress**





# Acknowledgements



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Dr. Diego Rebaque


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Emiia Heinonen



## Questions?


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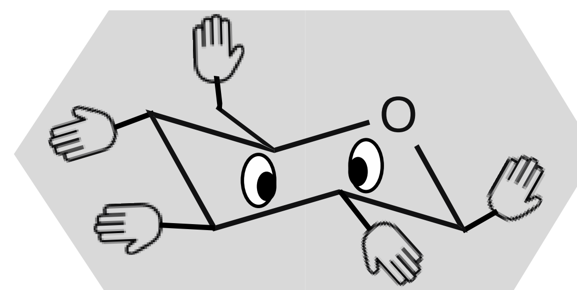



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