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

Endosperm

Bran

Germ

mixed-linkage β-glucan

Protein

Lignin

Xylan

Cellulose microfibril

Molecular Architecture of Cereal Cell Walls

Cellulose

Proteins

DiFA

Lignin

Pectins

β-glucans

Xyl, Ara, Glc
Extraction of Arabinoxylan from Cereal Cell Walls

- Antioxidant
- Health benefits

Ferulic Acid

- Alkali

\[
\text{Ferulic Acid} = \text{C}_{14} \text{H}_{10} \text{O}_5
\]

- Antioxidant
- Health benefits
Chemo-enzymatic Valorization of Dietary Fibres from Cereal Brans

Phenolics/lignin (12.2%)
Fats (4.6%)
Proteins (19.7%)
Starch (8.8%)
Total Carbohydrates (55.5%)
AX (39.5%)

Subcritical Water (100-160°C, pH 5-9, 100bar)

Cereal bran

Enzymatic treatment

Membrane filtration

Polysaccharides

Oligosaccharides Low M_w Biomolecules

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

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

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

\[ \text{AcXyn10A was most active on all substrates: produces X2 and small oligosaccharides} \]
\[ \text{TiXyn11 was more restricted than the GH10: produces X3 and small oligosaccharides} \]
\[ \text{BXyn8 was most restricted by Araf substitution: produced long linear XOS} \]
\[ \text{GpXyn5_34 required Araf substitution for hydrolysis: produced complex long (A)XOS} \]
Integrated bioprocess: maximisation and diversification of AX extraction

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

F-AX polymers (films, gels)

Enzymatic tailoring (α-Arafase)

Enzymatic crosslinking (peroxidase)

Chemical crosslinking (citric acid)

F-AX oligomers (plasticizers)

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

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

Wheat Bran FAX

Enzymatic Crosslinking

FAX-CL

Regeneration

FAX-CL-pH2

FAX-CL-pH5

FAX-CL-pH7

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

X_c = 16%

X_c = 27%

X_c = 29%

\[ I(q) = \frac{A}{q^n} + \frac{C}{1 + (q\xi)^m} + B \]

n: power-law exponent
m: Lorentz exponent
\( \xi \): correlation length

\( \xi = 113 \text{ Å} \)
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.
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Questions?

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