Production of prebiotics from hemicellulose

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Gut microbiota

- Gut microbiota – microorganisms in our gastrointestinal tract
- The gut microbiota has a large influence on our health and well-being
- How can we influence our gut microbiota?

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Probiotics and prebiotics

- Probiotics – beneficial microorganisms in the gut microbiota
- Prebiotics – substrates promoting beneficial microorganisms

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Arabinoxylan, AXOS and XOS

- Arabinoxylan (AX), and oligosaccharides derived from AX (AXOS and XOS) are prebiotics
- XOS and AXOS have positive effects on glucose metabolism, lipid metabolism and metabolic disorders
- The effects depend on the molecular size and structure

Broekaert et al. (2011) Crit. Rev. Food Sci. Nutr. 51, 178

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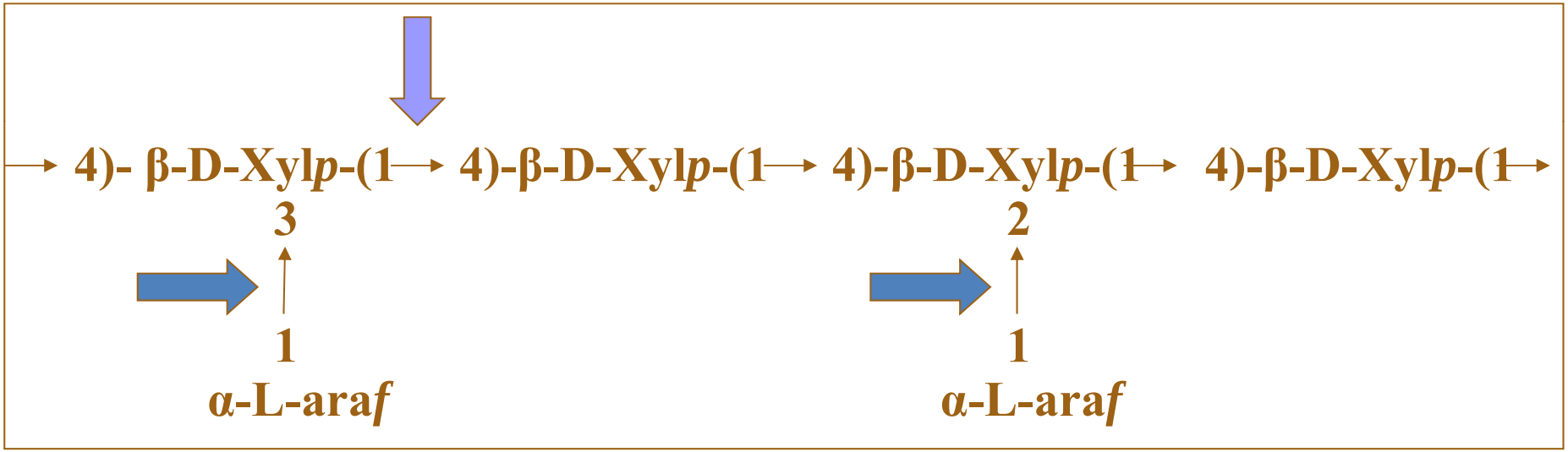
Selective enzymatic hydrolysis of arabinoxylan



α-L-arabinofuranosidase

endo- β-1,4-xylanase

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Xylanase hydrolysis of rye flour arabinoxylan

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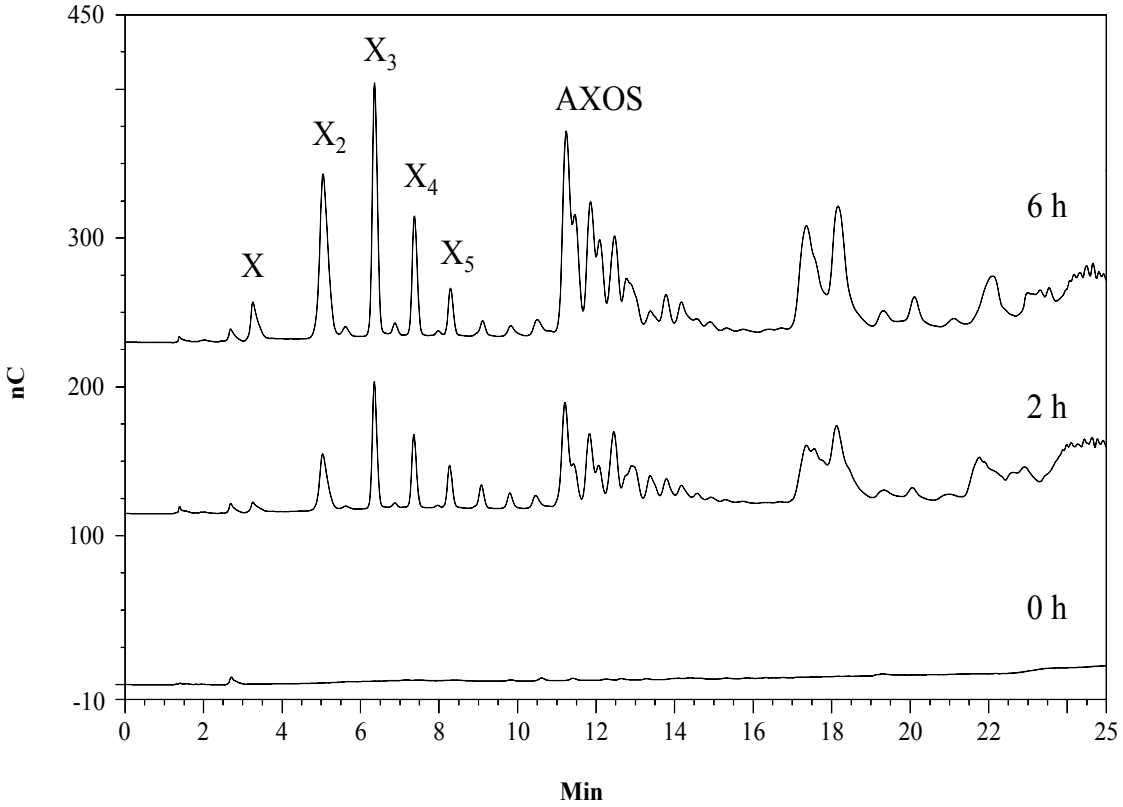

Falck et al (2013)
J. Agric. Food Chem. 61, 7333

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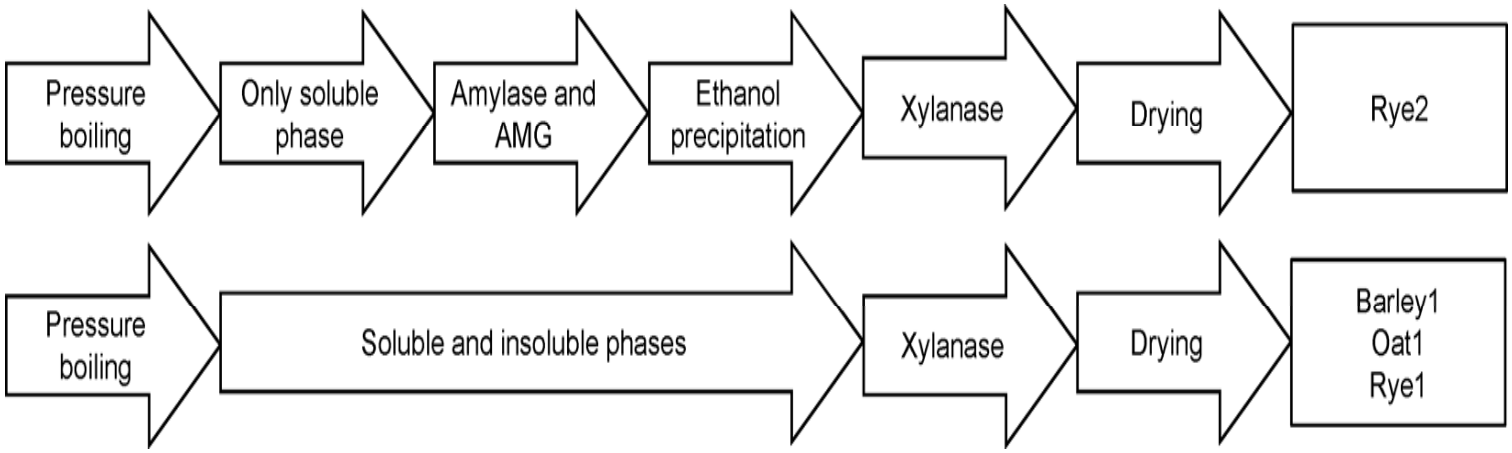
Processing of cereal residues



Products

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Lund University

ANTIDIABETIC FOOD CENTRE

A Centre of Excellence in Research and Innovation

- Duration 2007-2017
- Budget 34 000 000 USD
- Approx. 50 senior researchers from 4 faculties
- Mission: *Preventing type 2 diabetes with food.*

Effects on mice

	HFD	Oat0	Oat1	Rye0	Rye1	Rye2	Guar	LFD
Glucose (mM)	6.5 ± 0.3	6.1 ± 0.2	6.1 ± 0.3	7.2 ± 0.2	6.6 ± 0.3	6.0 ± 0.2	6.0 ± 0.3	5.4 ± 0.3
Fructosamine (mM)	505 ± 17	550 ± 9	515 ± 25	470 ± 16	523 ± 25	435 ± 18	497 ± 40	535 ± 14
ALT (U/L)	4.4 ± 0.4	4.3 ± 0.4	5.8 ± 0.9	3.1±0.4	3.4 ± 0.5	1.4 ± 0.2	2.3 ± 0.4	2.9 ± 0.2

K Berger, P Falck, C Linninge, U Nilsson, U Axling, C Grey, H Stålbrand, E Nordberg Karlsson, M Nyman, C Holm, P Adlercreutz (2014) J. Agric. Food Chem. 62, 8169

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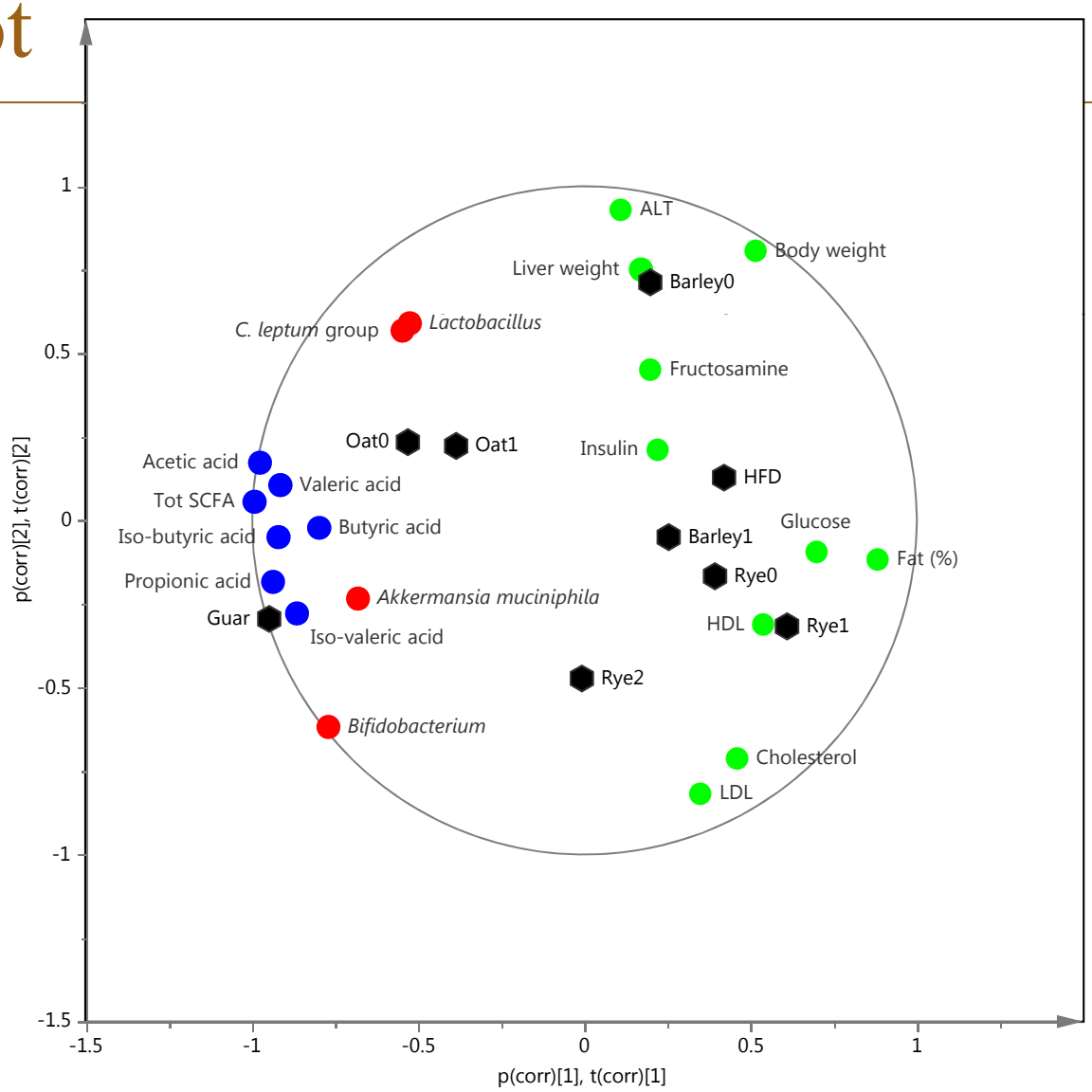
PCA plot



Berger et al (2014) J. Agric. Food Chem. 62, 8169

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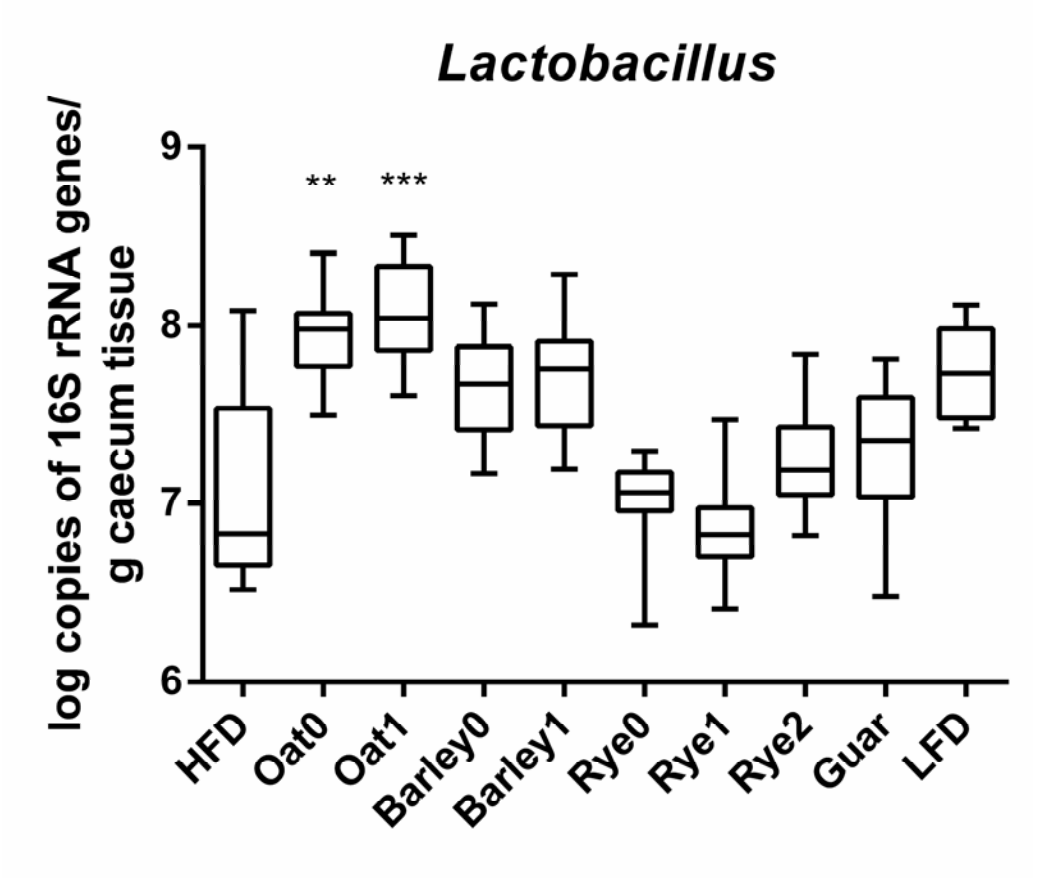
Oat products stimulate *Lactobacilli*



Berger et al (2014) J. Agric. Food Chem. 62, 8169

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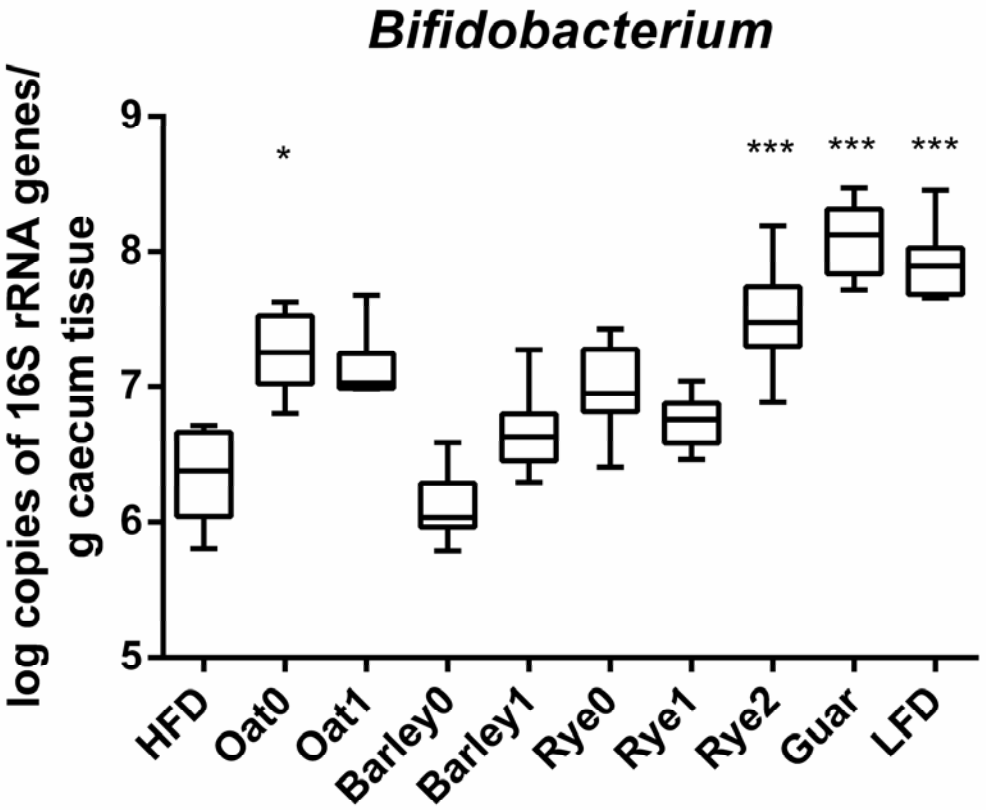
Rye and guar products stimulate *Bifidobacteria*



Berger et al (2014) J. Agric. Food Chem. 62, 8169

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Why are bifidobacteria stimulated by the oligosaccharide-rich product?

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Bifidobacterium adolescentis consumes AXOS from rye flour

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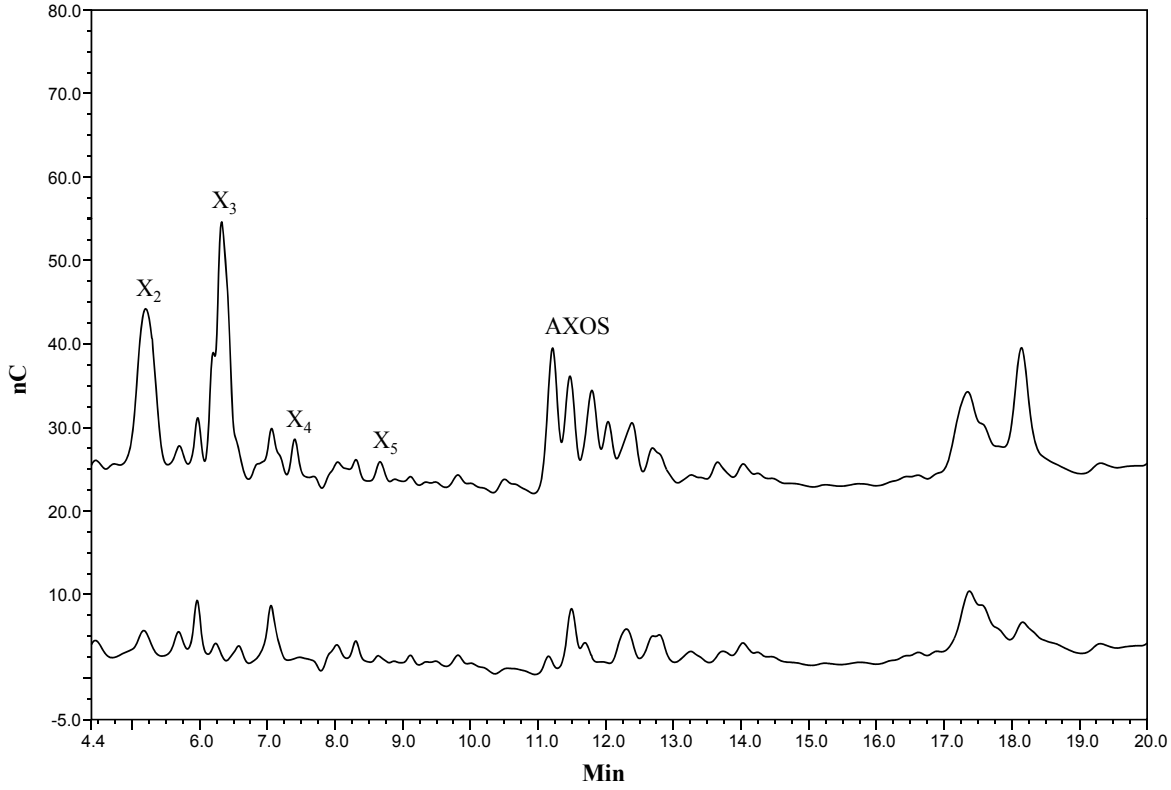

Before growth

After growth

Falck et al (2013)
J. Agric. Food Chem. 61, 7333

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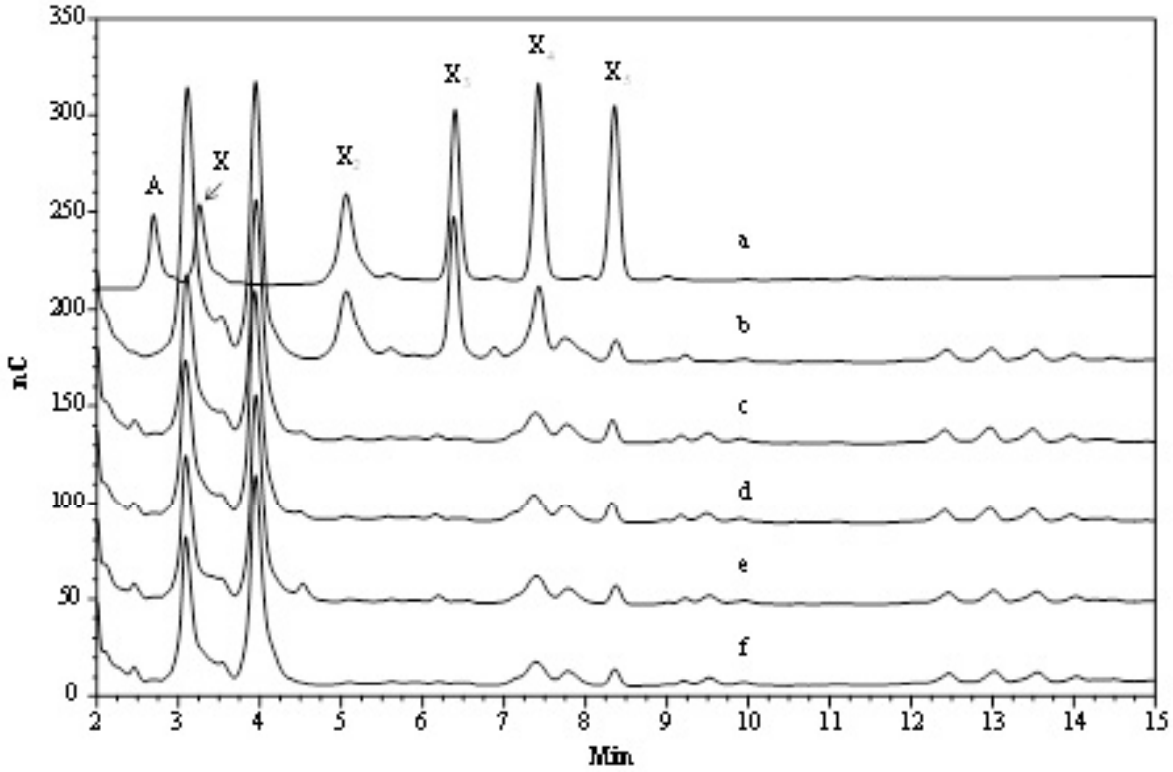
Growth of *Weissella strains** on hydrolysed birchwood xylan

*isolated from Indian food (by 16S rDNA either *W. cibaria* or *W. confusa*)



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a: Standards

b: Before growth

c-f: After growth
XOS consuming strains!

Patel et al (2013)
FEMS Microbiol. Lett. 346, 20

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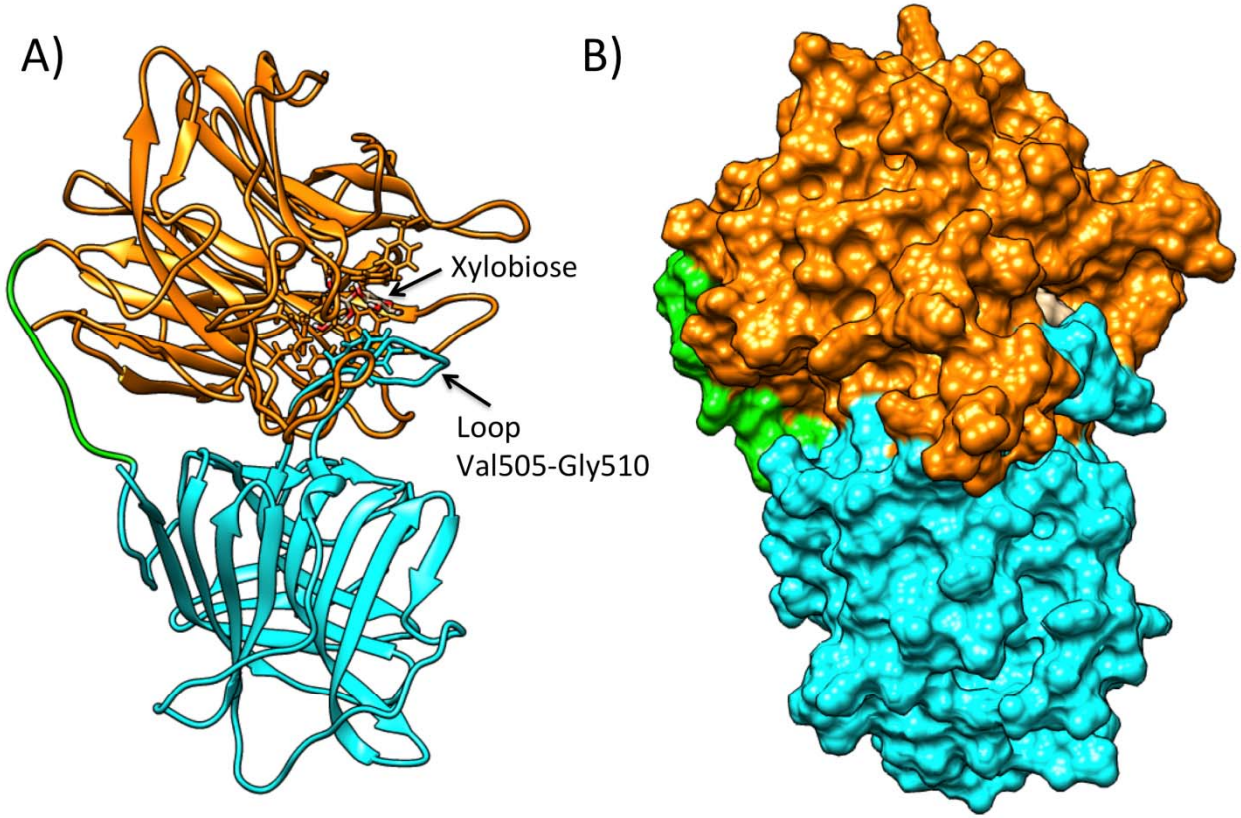
GH43 β-xylosidase from *Weissella* sp. strain 92



P Falck, J Linares-Pastén, P Adlercreutz, E Nordberg Karlsson (2015) Glycobiology

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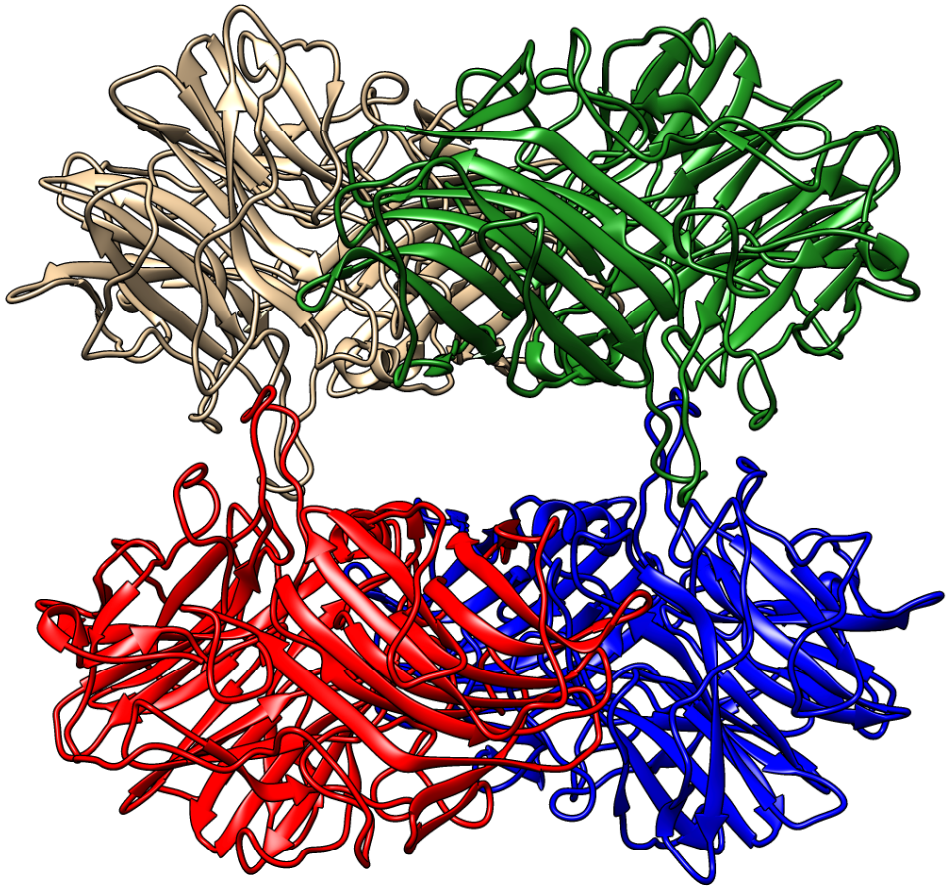

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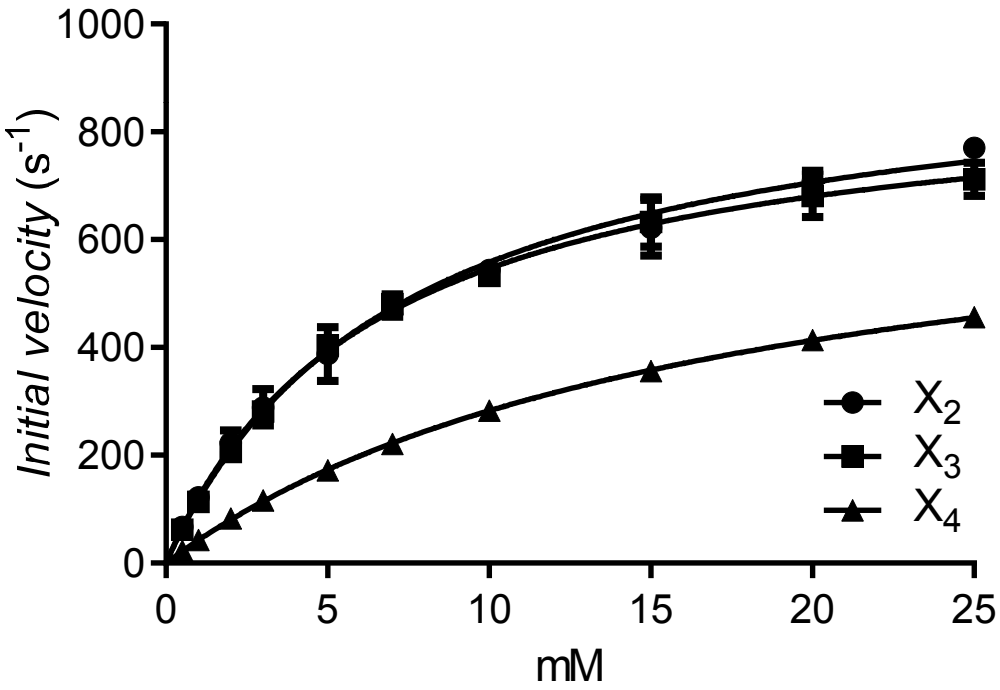
Hydrolysis of XOS by *Weissella* β-xylosidase



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Kinetics of hydrolysis by *Weissella* β-xylosidase

Substrate	k_{cat} (s^{-1})	K_M (mM)	k_{cat}/K_M ($s^{-1}mM^{-1}$)
p-Nitrophenyl-β-D-xylopyranoside	258 ± 11	7.4 ± 1.1	34.9 ± 5.4
(1→4)-β-D-xylobiose (X2)	961 ± 25	7.2 ± 0.5	134 ± 10
(1→4)-β-D-xylotriose (X3)	900 ± 13	6.5 ± 0.3	138 ± 7
(1→4)-β-D-xylotetraose (X4)	770 ± 7	17 ±0.3	54.3 ± 0.9

P Falck, J Linares-Pastén, P Adlercreutz, E Nordberg Karlsson (2015) Glycobiology

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Weissella β-xylosidase. Activity on arabinose substrates

- Low activity on *p*-nitrophenyl-α-L-arabinofuranoside
- No activity on AXOS
- The bacteria do not grow on AXOS

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Further development of (A)XOS production processes

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Process steps.
From rye bran to prebiotic products

- Heat pretreatment
- Starch degradation (amylase, amyloglucosidase)
- Protein degradation (protease)
- Separation steps to remove small molecules (ethanol precipitation)
- Xylan hydrolysis (xylanase)

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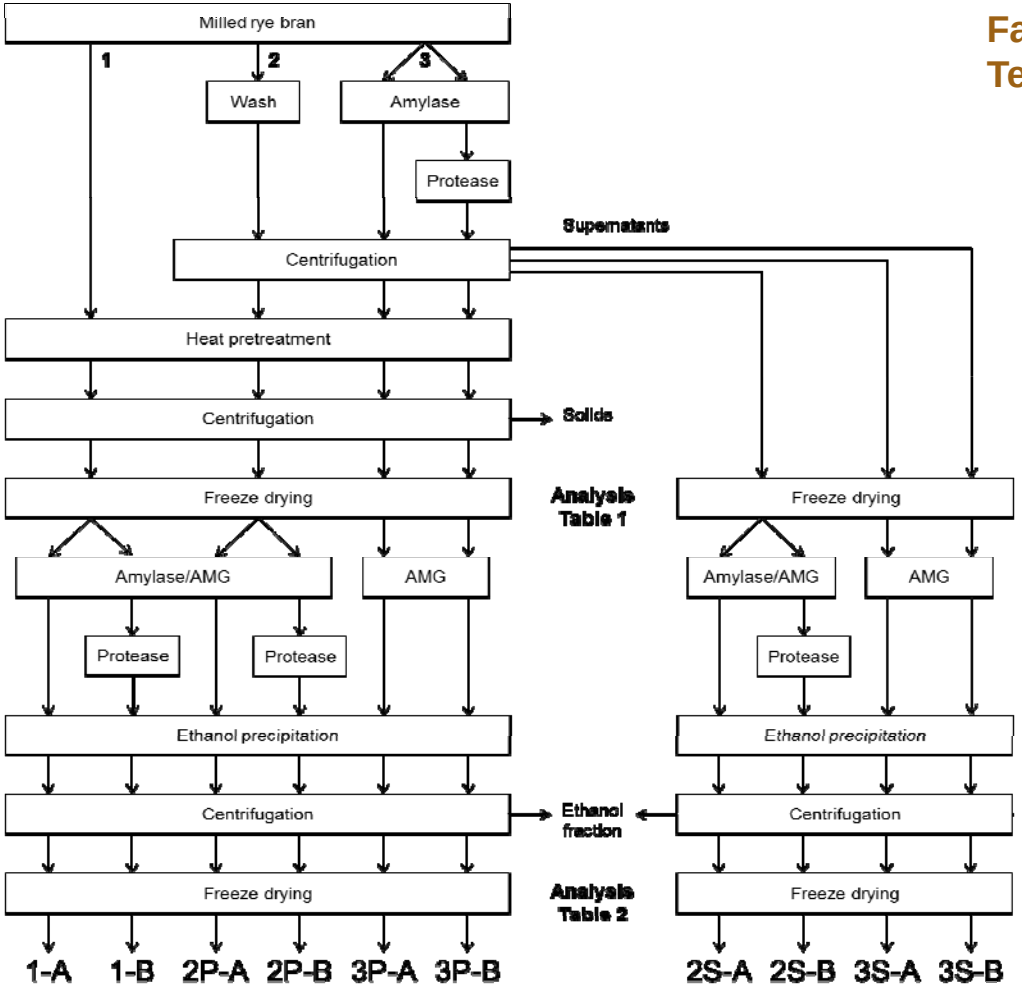
Process schemes

Falck et al (2014) Bioresource Technol. 174, 118



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Products

	Mass	AX		
	Yield, % (w/w)	Yield, % (w/w)	Content, % (w/w)	A/X
Products based on supernatants isolated before heat pretreatment				
2S-A	22	12	11	0.62
2S-B	20	10	11	0.47
3S-A	23	10	9	0.63
3S-B	23	11	10	0.52
Products based on supernatants isolated after heat pretreatment				
1-A	17	33	41	0.38
1-B	13	33	53	0.34
2P-A	11	23	45	0.38
2P-B	8	25	60	0.30
3P-A	11	26	48	0.30
3P-B	8	21	58	0.39

Falck et al (2014) Bioresource Technol. 174, 1

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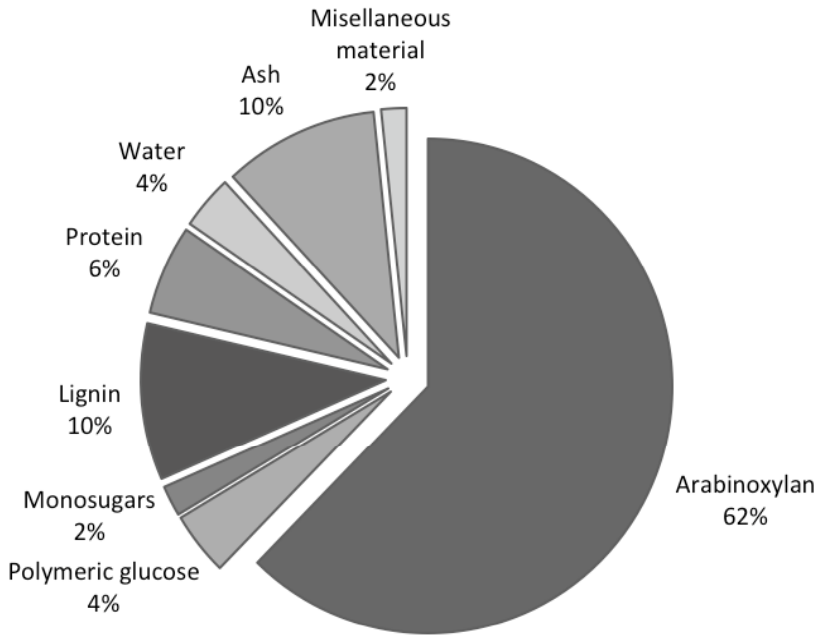
Product composition



Falck et al (2014) Bioresource Technol. 174, 118

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Process steps.
From rye bran to prebiotic products

- Heat pretreatment
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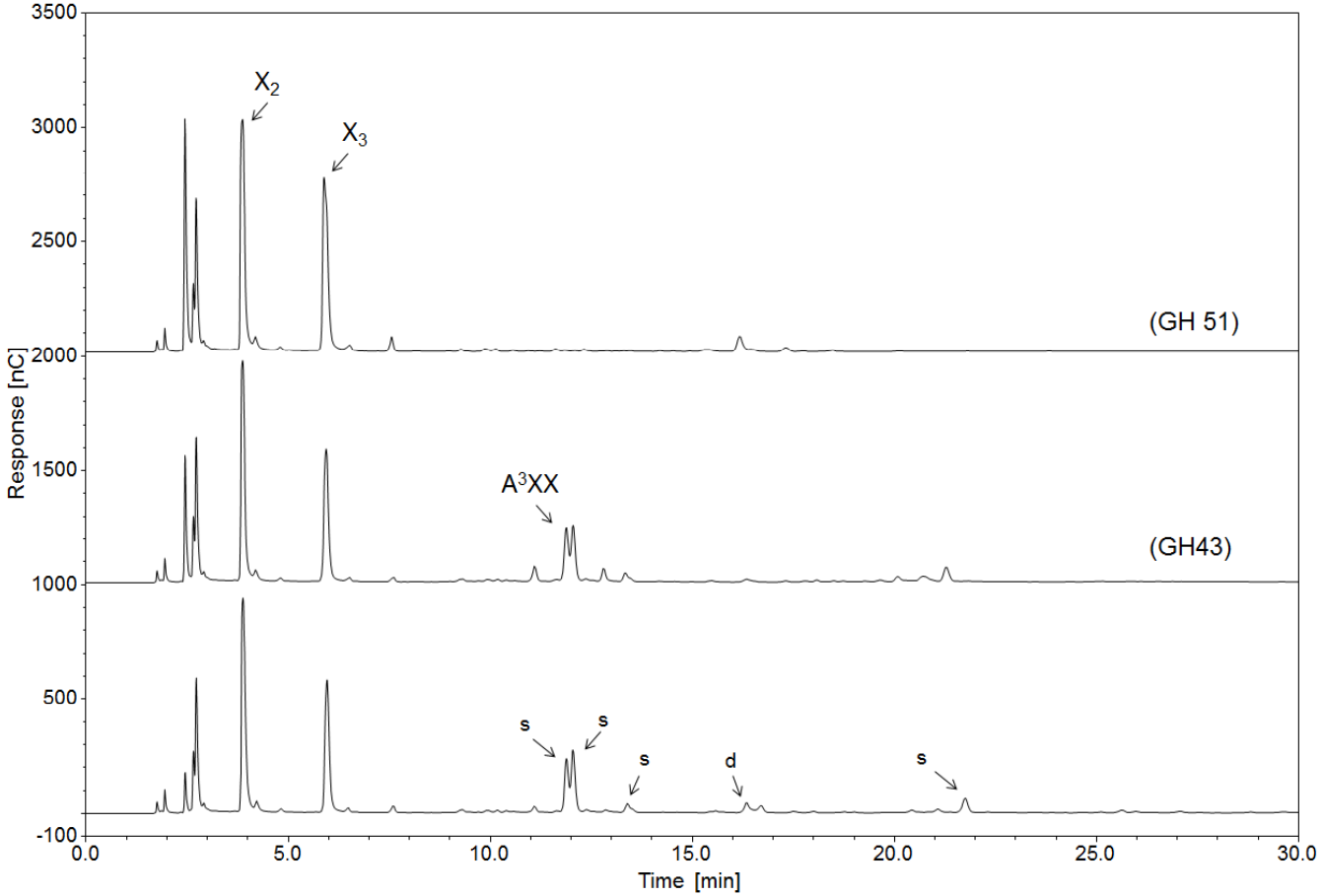
Hydrolysis by *R. marinus* xylanase (GH10)



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After removal of single Ara substituents

After removal of double Ara substituents

Original sample

Falck et al (2014) Bioresource Technol. 174, 118

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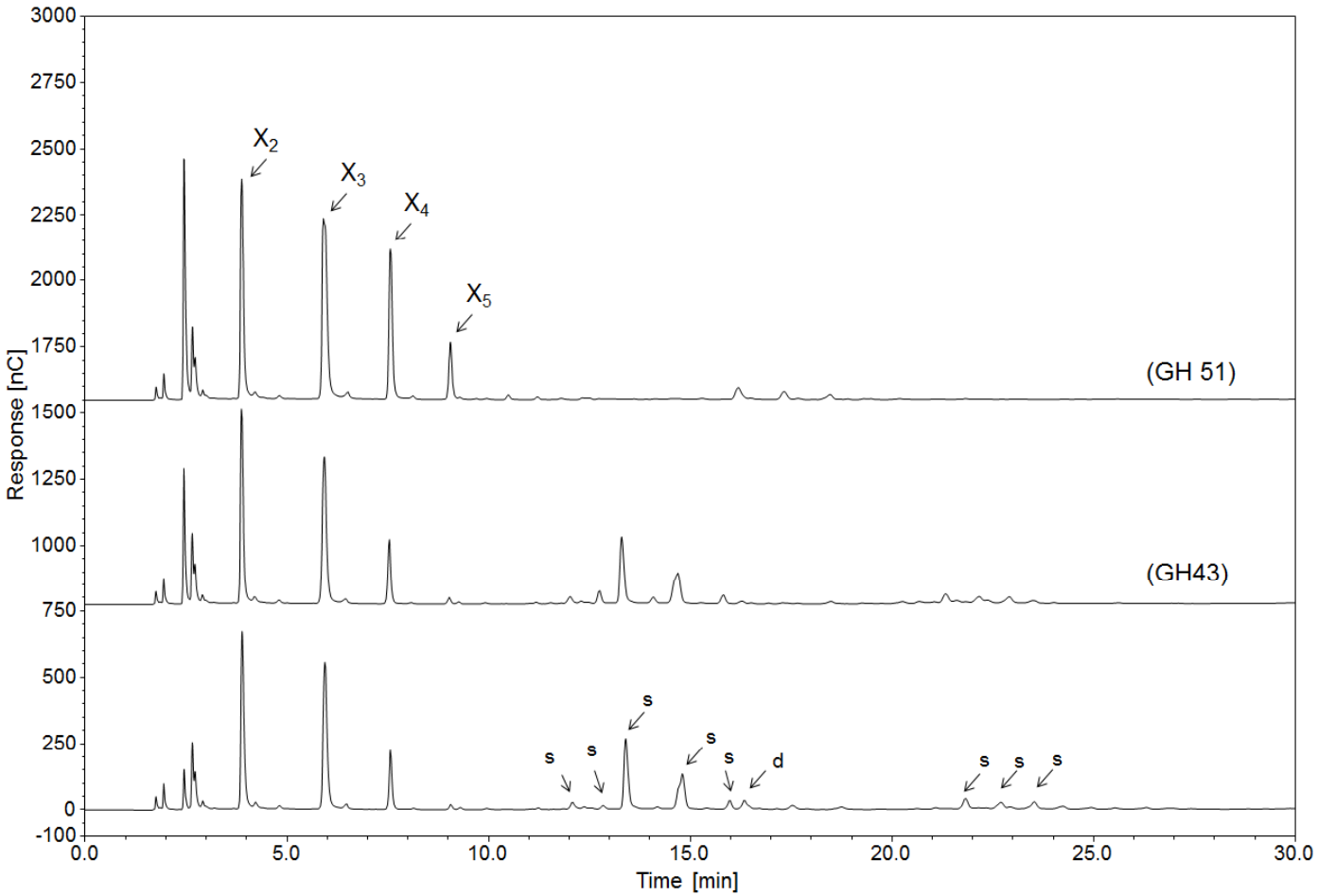
Hydrolysis by Pentopan Mono BG (GH11)



HPAEC-PAD

After removal of single Ara substituents

After removal of double Ara substituents

Original sample

Falck et al (2014) Bioresource Technol. 174, 118

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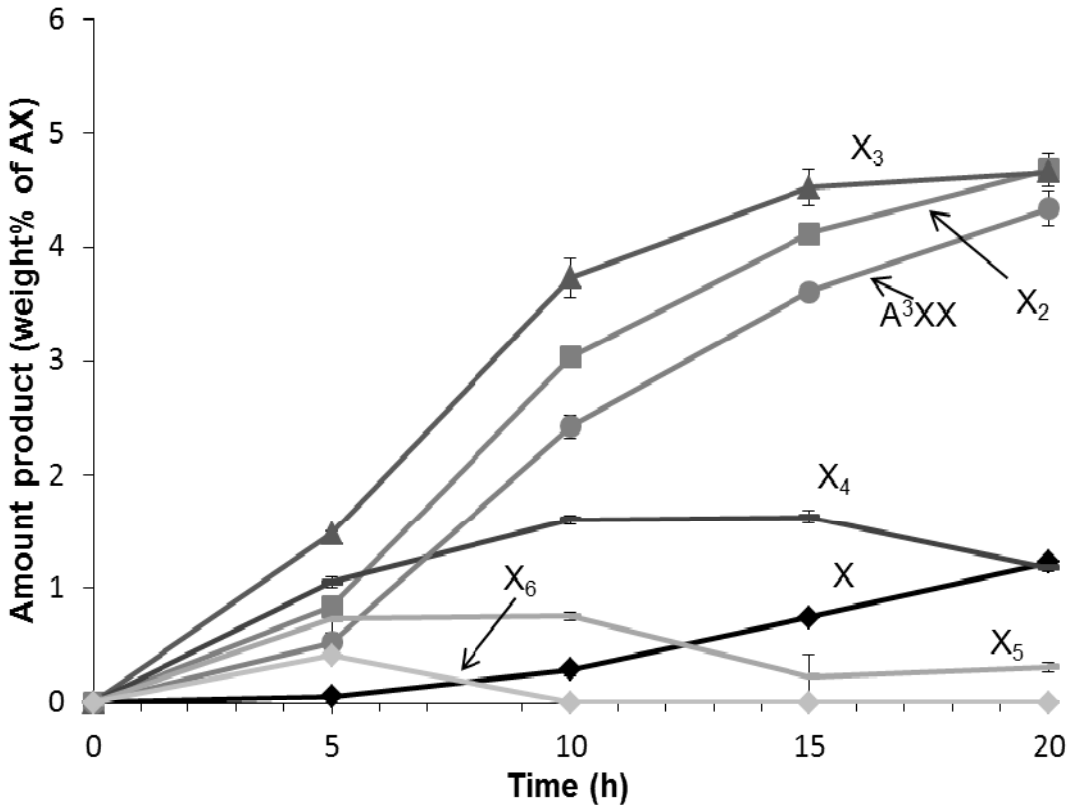
Time course of xylanase catalysed hydrolysis



Falck et al (2014) Bioresource Technol. 174, 118

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Conclusions

- Xylanases are useful for production of prebiotic oligosaccharides from arabinoxylan
- The products have positive health effects in mice on a high fat diet
- Different xylanases produce different AXOS
- Selective stimulation of beneficial gut bacteria (such as *Bifidobacteria*) is possible
- *Weissella* strains proven to use XOS. Putative probiotics.

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