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?
Probiotics and prebiotics

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

Selective enzymatic hydrolysis of arabinoxylan

\[
\begin{align*}
\rightarrow & \quad 4)-\beta-D-Xylp-(1\rightarrow 4)-\beta-D-Xylp-(1\rightarrow 4)-\beta-D-Xylp-(1\rightarrow 4)-\beta-D-Xylp-(1\rightarrow \\
& \quad 3 \quad 2 \quad 2 \quad 2
\end{align*}
\]

\[\alpha-L-araf \quad \alpha-L-araf\]

\[\text{\textit{\textalpha-L-arabinofuranosidase}}\]

\[\text{\textit{\textend- \textbeta-1,4-xylanase}}\]
Xylanase hydrolysis of rye flour arabinoxylan

Falck et al (2013)
J. Agric. Food Chem. 61, 7333
Processing of cereal residues

Products

- Barley1
- Oat1
- Rye1

- Rye2
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

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

PCA plot

Oat products stimulate *Lactobacilli*

Rye and guar products stimulate *Bifidobacteria*

Why are bifidobacteria stimulated by the oligosaccharide-rich product?
Bifidobacterium adolescentis consumes AXOS from rye flour

Falck et al (2013)
J. Agric. Food Chem. 61, 7333
Growth of *Weissella strains* on hydrolysed birchwood xylan

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

Patel et al (2013)
FEMS Microbiol. Lett. 346, 20
GH43 β-xylosidase from *Weissella* sp. strain 92

GH43 \(\beta\)-xylosidase from *Weissella* sp. strain 92

Hydrolysis of XOS by *Weissella* β-xylosidase

Kinetics of hydrolysis by *Weissella* β-xylosidase

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

Weissella β-xylosidase. Activity on arabinose substrates

- Low activity on \( p \)-nitrophenyl-\( \alpha \)-L-arabinofuranoside
- No activity on AXOS
- The bacteria do not grow on AXOS
Further development of (A)XOS production processes
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)
Process schemes

# Products

<table>
<thead>
<tr>
<th></th>
<th>Mass</th>
<th>AX</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Yield, % (w/w)</td>
<td>Yield, % (w/w)</td>
<td>Content, % (w/w)</td>
<td>A/X</td>
<td></td>
<td></td>
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<tr>
<td><strong>Products based on supernatants isolated before heat pretreatment</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2S-A</td>
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<td>11</td>
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<td>2S-B</td>
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<td>11</td>
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<td></td>
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<td><strong>Products based on supernatants isolated after heat pretreatment</strong></td>
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<td>41</td>
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<tr>
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<td>21</td>
<td>58</td>
<td>0.39</td>
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Falck et al (2014) Bioresource Technol. 174,
Product composition

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)
Hydrolysis by *R. marinus* xylanase (GH10)

[Graph showing HPAEC-PAD analysis of xylan samples before and after substitution removal, with labels for each sample]

Hydrolysis by Pentopan Mono BG (GH11)

Time course of xylanase catalysed hydrolysis

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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