Towards scaling up of polysaccharide production with mixed α-1,4 & α-1,6 backbones

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Introduction

- Starch consists of α-1,4 and α-1,6 linked glucose units.
- α-1,6 linkage content varies depending on starch type (≤ ±8%).
- α-1,6 linkages are more flexible and resistant to α-amylase.

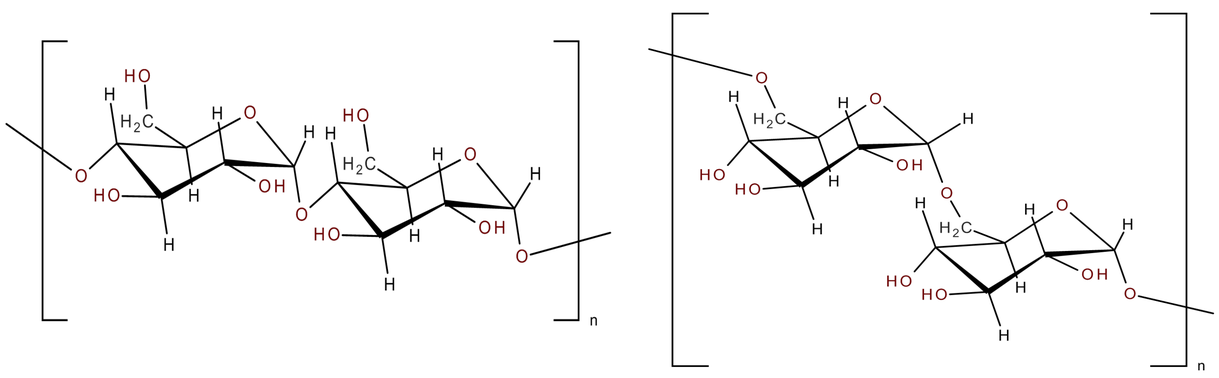

Figure 1. Molecular structure of an α-1,4 linked glucose backbone (left) and an α-1,6 linked glucose backbone (right).

Objective

To create novel polysaccharides by converting α-1,4 to α-1,6 linkages with added functionality for products in and outside the food industry.

Approach

- The discovery of 4,6-α-glucanotransferase (GtfB) enzyme opens up a new way to modify starch.
- GtfB is able to alter the intrinsic properties of starch by cleaving α-1,4 glycosidic linkages and introducing α-1,6 glycosidic linkages.

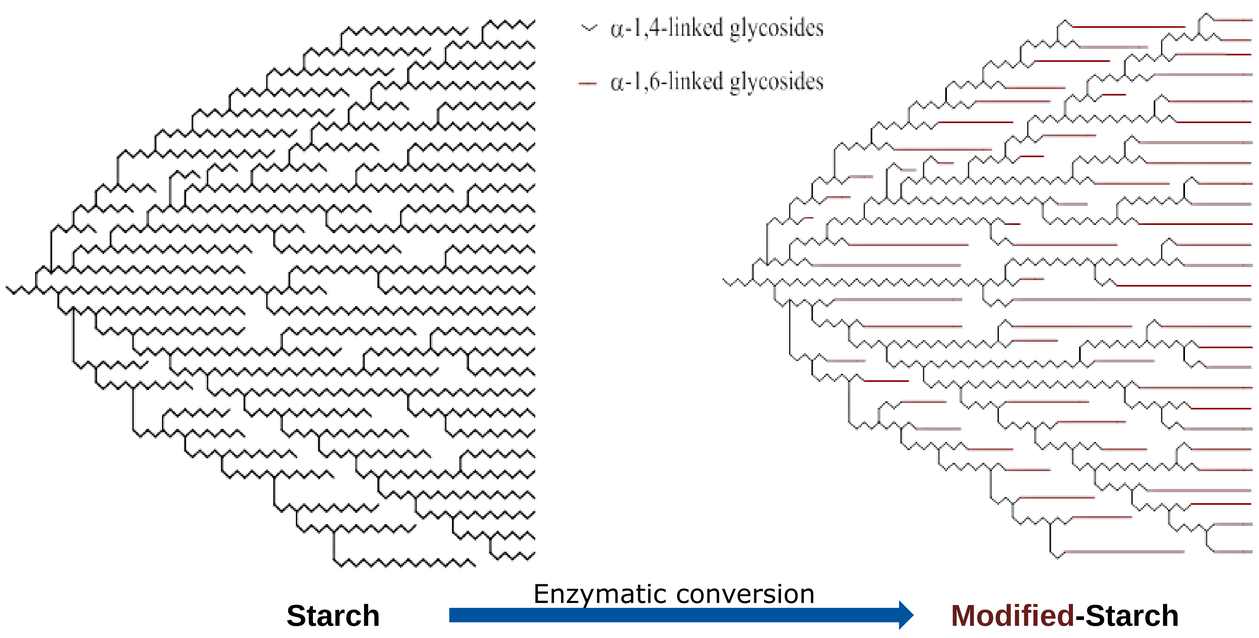

Figure 2. Theoretical representation of enzymatic starch amylopectin conversion with GtfB, adapted from Yuxiang Bay (University of Groningen).

- This change in the molecular building blocks of starch results in novel polysaccharides with new physico-chemical properties.
- GtfB conversion products are analysed to enable custom modification towards specific functionalities in the future.

Results

- Multiple starch types were incubated with GtfB, with a substrate concentration of 2.5% (w/v), T=37°C, t=24h and pH=5.
- An increase in α-1,6 linkages was observed after conversion, although higher conversion yields have been reported at lower substrate concentrations.

^{1}H 1D-NMR in D_2O	α-1,6(%)	α-1,6 conversion(%)
Potato starch	3.3	+4.6
Potato starch + GtfB	7.9	
Waxy potato starch	3.6	+0.9
Waxy potato starch + GtfB	4.5	
Highly branched starch	7.3	+1.9
Highly branched starch + GtfB	9.2	

Table 1. α-1,6 conversion in potato starch, waxy potato starch and highly branched starch incubated with sumo-dN-GtfB.

Future applications

- Pharmaceuticals
- Biomedical materials
- Slow energy release compounds
- Food fibres
- Texturizers
- Natural adhesives

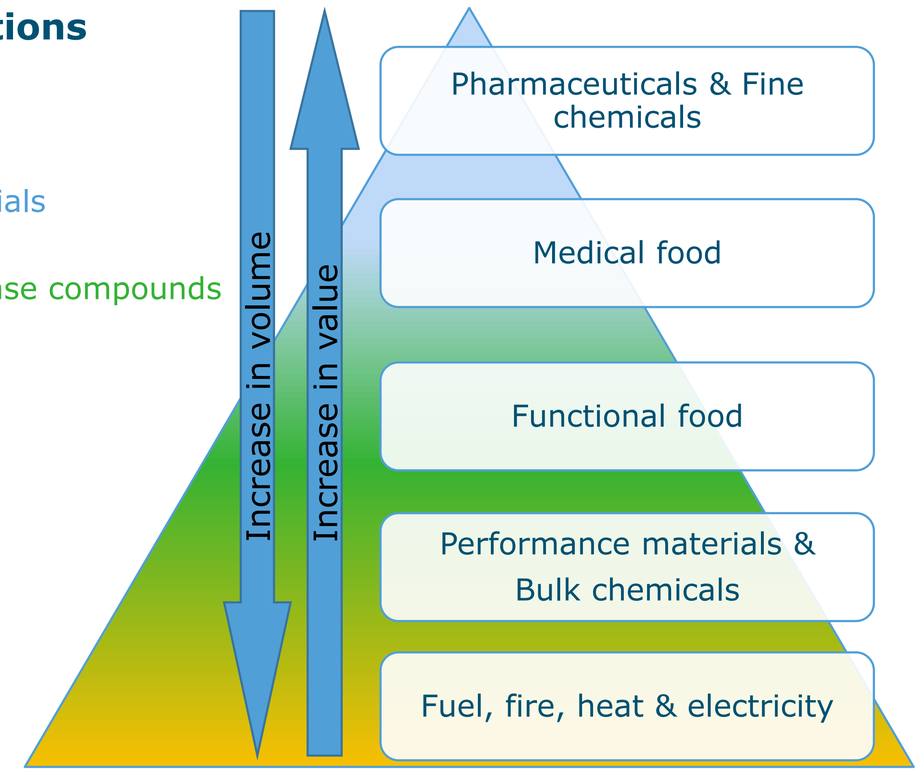

Figure 3. Possible applications of GtfB conversion products and their corresponding position on the value-pyramid.

Challenges & future work

- Optimization of 'high' substrate (2.5% (w/v)) conversions.
- Standardize NMR protocol for a wide variety of hard to dissolve starches and conversion products.
- Optimize and standardize a suitable AF4-MALLS protocol for starches and conversion products.
- Describe properties of conversion products (viscosity, gelling, etc.).

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