Enzymatic fingerprinting of Isomalto/Malto-polysaccharides

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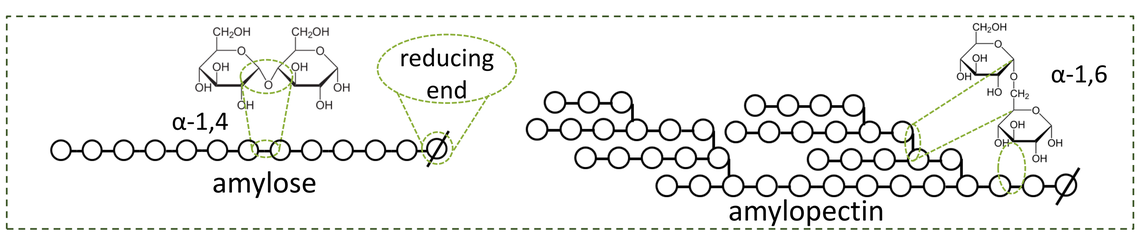
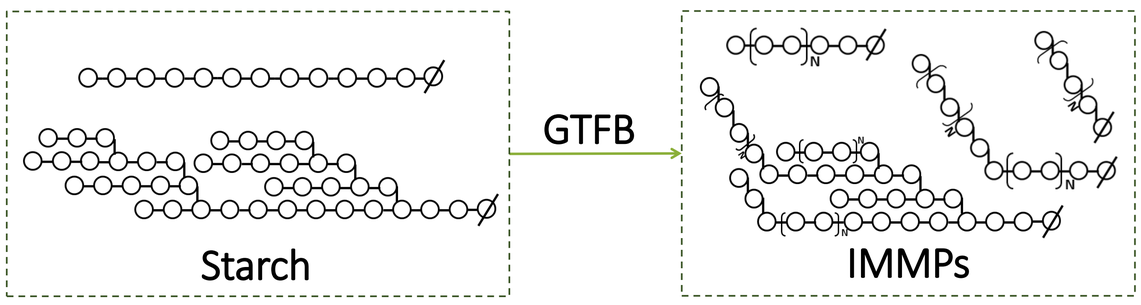
Introduction

Starch consists of amylose & amylopectin, α-linked glucose polymers



Starch can be modified in different ways to increase its techno-functional properties. The enzymatic modification of starch with 4,6-α-glucanotransferase-ΔN (GTFB) results in Isomalto/Malto-polysaccharides (IMMPs). IMMPs are molecules built of α-1,4 linked glucose chains, connected to α-1,6 linked glucose chains (Leemhuis et al., 2014).

Graphical representation of enzymatic modification of starch by 4,6-α-glucanotransferase-ΔN, producing Isomalto/Malto-polysaccharides



Objective

"To develop an enzymatic fingerprinting method, capable to characterise linear Isomalto/Malto-polysaccharides"

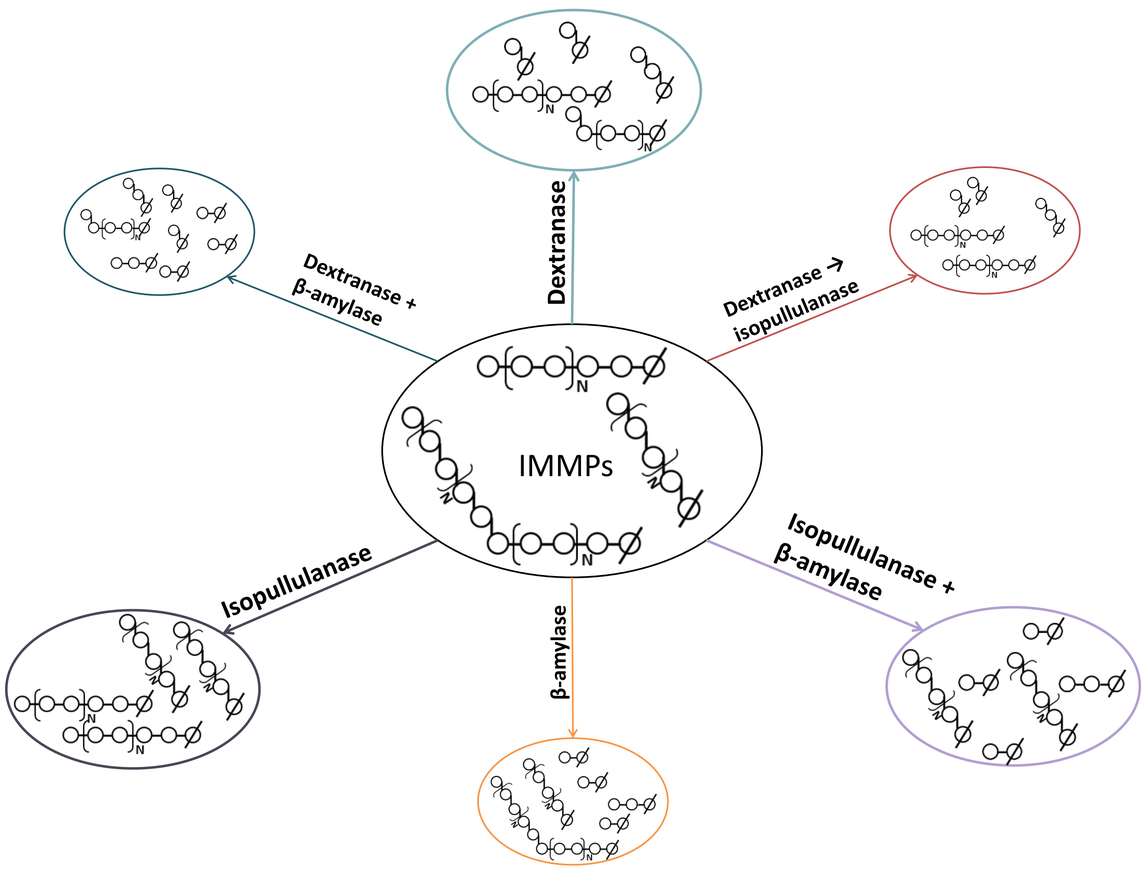
Approach

In order to characterise linear IMMPs three enzymes were used:

- β-amylase for hydrolysis of all α-1,4 linkages from the non-reducing end
- Dextranase for the endo-hydrolysis of all α-1,6 linkages
- Isopullulanase to split hybrid α-1,4/α-1,6 linked molecules

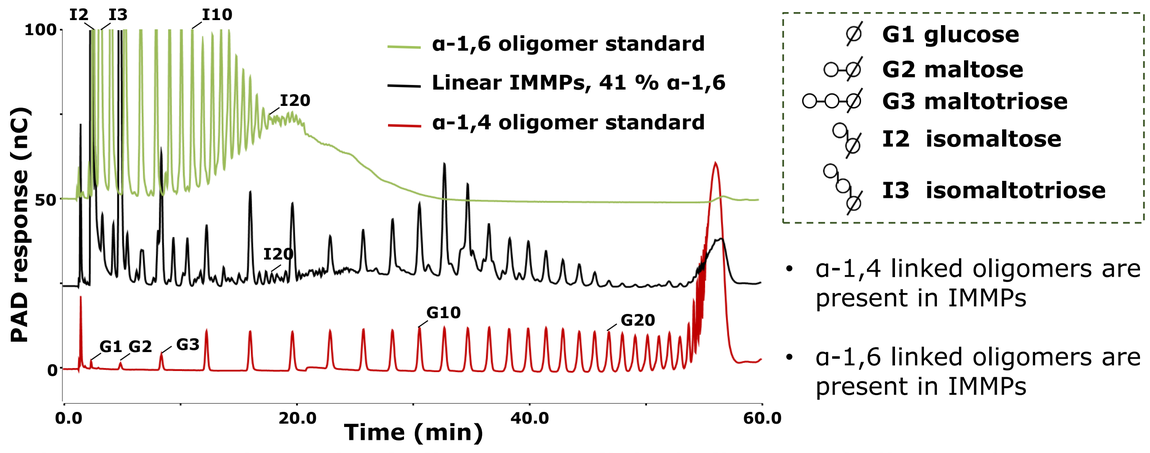
When using these enzymes in specific orders structural information can be obtained, as shown in the figure below.

Characterisation of linear IMMPs with β-amylase, dextranase and Isopullulanase. Enzymes with a "+" are incubated simultaneously, enzymes with a "→" are incubated after each other.



Results

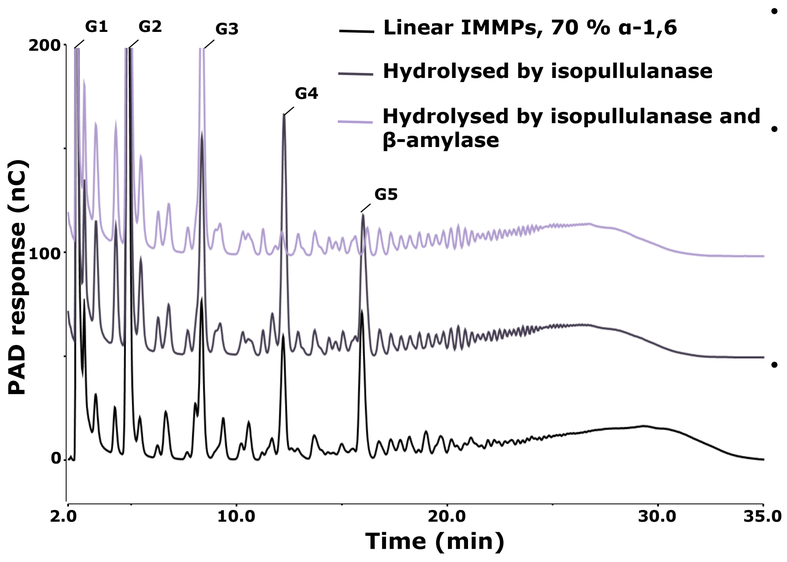
In order to determine the presence of linear α-1,4 or linear α-1,6 linked chains in the IMMP mixture. HPAEC was used to compare α-1,4 and α-1,6 linked oligomer standards to an IMMP mixture containing 41 % α-1,6 linkages.



- α-1,4 linked oligomers are present in IMMPs
- α-1,6 linked oligomers are present in IMMPs

The presence of completely linear α-1,6 linked oligomers indicates that GTFB is able to use glucose as an acceptor.

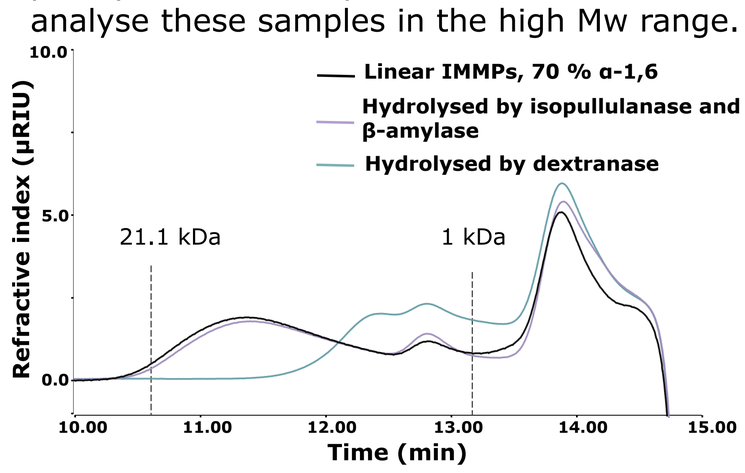
In order to determine the length of the α-1,4 linked chains within the IMMPs, a linear IMMP (70 % α-1,6) was incubated with isopullulanase or β-amylase and isopullulanase simultaneously.



- No α-1,4 linked oligomers larger than DP 5 can be observed in linear IMMPs.
- The increase in maltose, maltotriose, maltotetraose and maltopentaose concentration after hydrolysis by isopullulanase indicates the presence of DP2-5 α-1,4 linked segments within IMMPs.
- α-1,4 linked segments larger than DP 5 are not present within IMMPs, since there was no increase in concentration of α-1,4 linked DP≥6 molecules after hydrolysis with isopullulanase.

GTFB uses α-1,4 linked material ≥ DP 6 as donor substrate and it uses α-1,4 linked material < DP 6 as acceptor substrate.

In order to determine the length of the α-1,6 linked chains within IMMPs, a linear IMMP (70 % α-1,6) was incubated with dextranase or β-amylase and isopullulanase simultaneously. HPSEC was used to analyse these samples in the high Mw range.

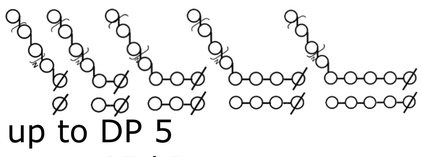


- After hydrolysis by isopullulanase and β-amylase a very slight change in Mw distribution can be observed, indicating that no long linear α-1,4 linked chains were present
- After hydrolysis by dextranase a decrease in Mw distribution is shown, indicating that many linear α-1,6 linked chains were present of around 15 kDa

GTFB is able to produce IMMPs with α-1,6 linked glucose chains up to 15 kDa.

Conclusions

The enzymatic fingerprinting method is able to characterise IMMPs.



GTFB produces:

- Fully linear α-1,6 linked chains
- IMMPs with α-1,4 linked glucose segments up to DP 5
- IMMPs with α-1,6 linked glucose segments up to 15 kDa

Acknowledgement

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