

# Kinetic modelling of enzymatic hydrolysis of starch

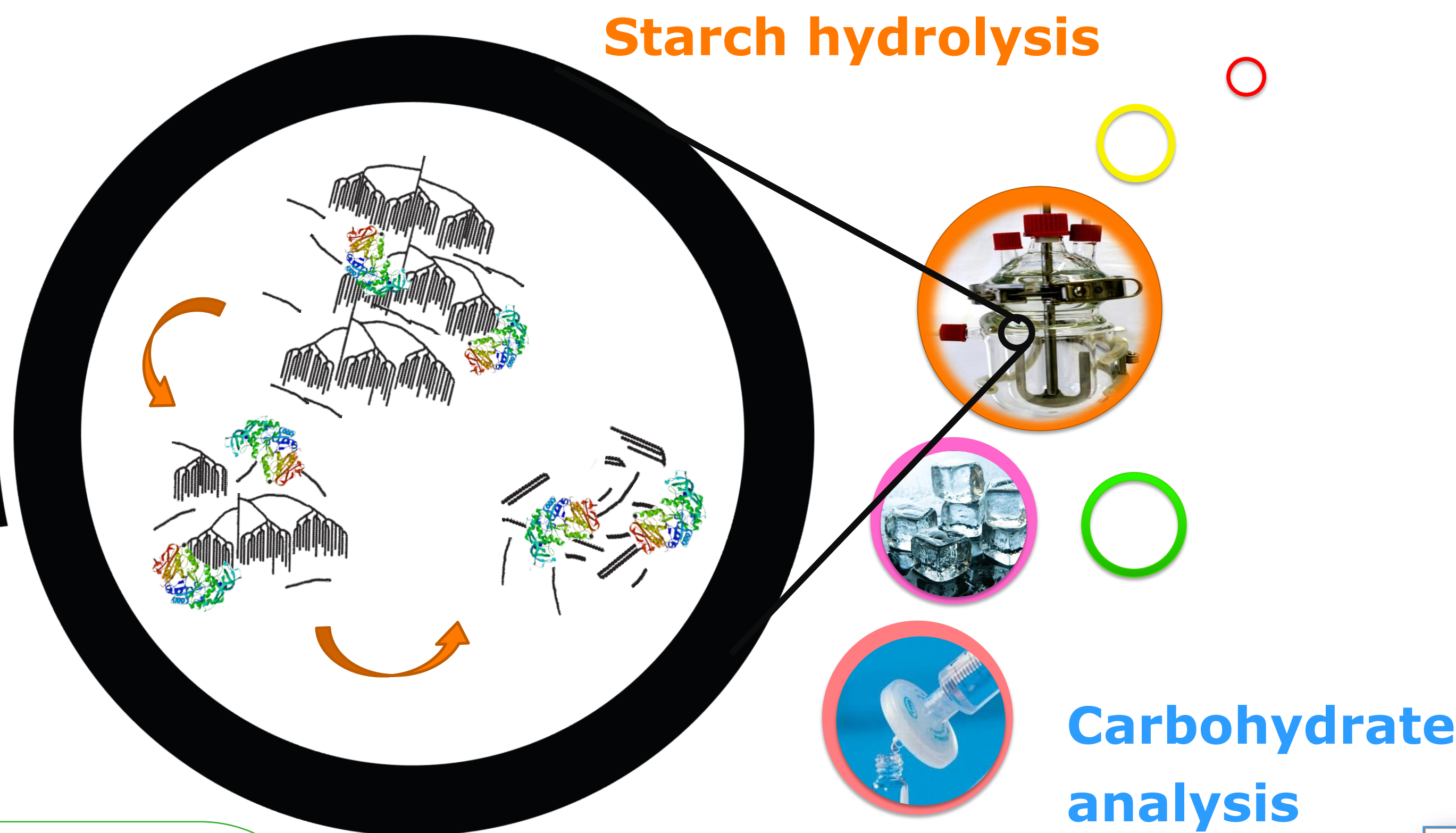
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## Objective

The aim of this research was to improve and extend the model of Besselink et al. (2008)<sup>[1]</sup> to predict the concentrations of carbohydrates up to degree of polymerization (DP) of 700. The key components of the model (e.g. subsite map) were re-evaluated to find the best fit between the model and the data.

### Figure 1. Starch hydrolysis.

The gelatinization of wheat starch at 90°C and subsequent liquefaction by BLA: *Bacillus licheniformis* alpha amylase at 50°C.

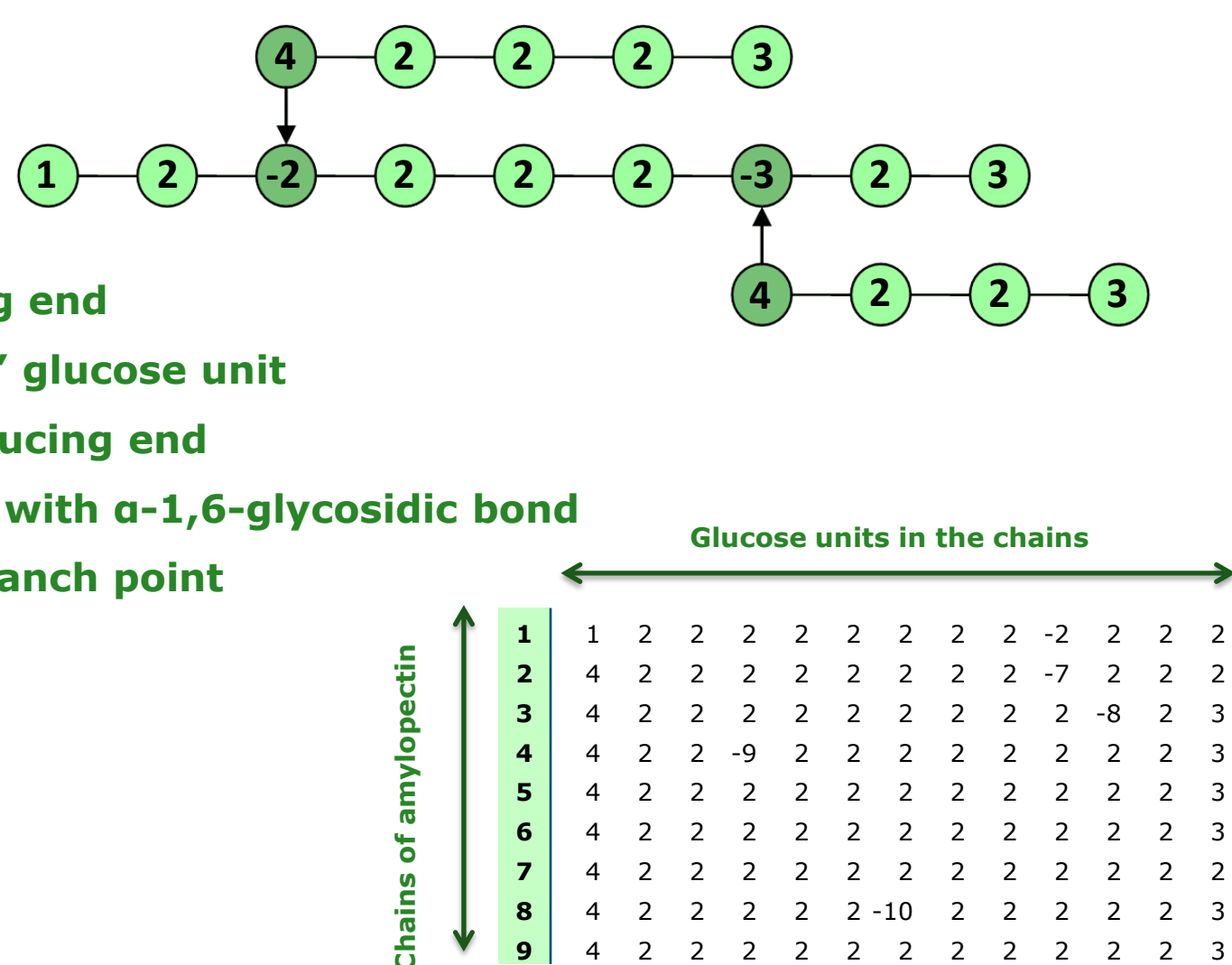


## Carbohydrate analysis results

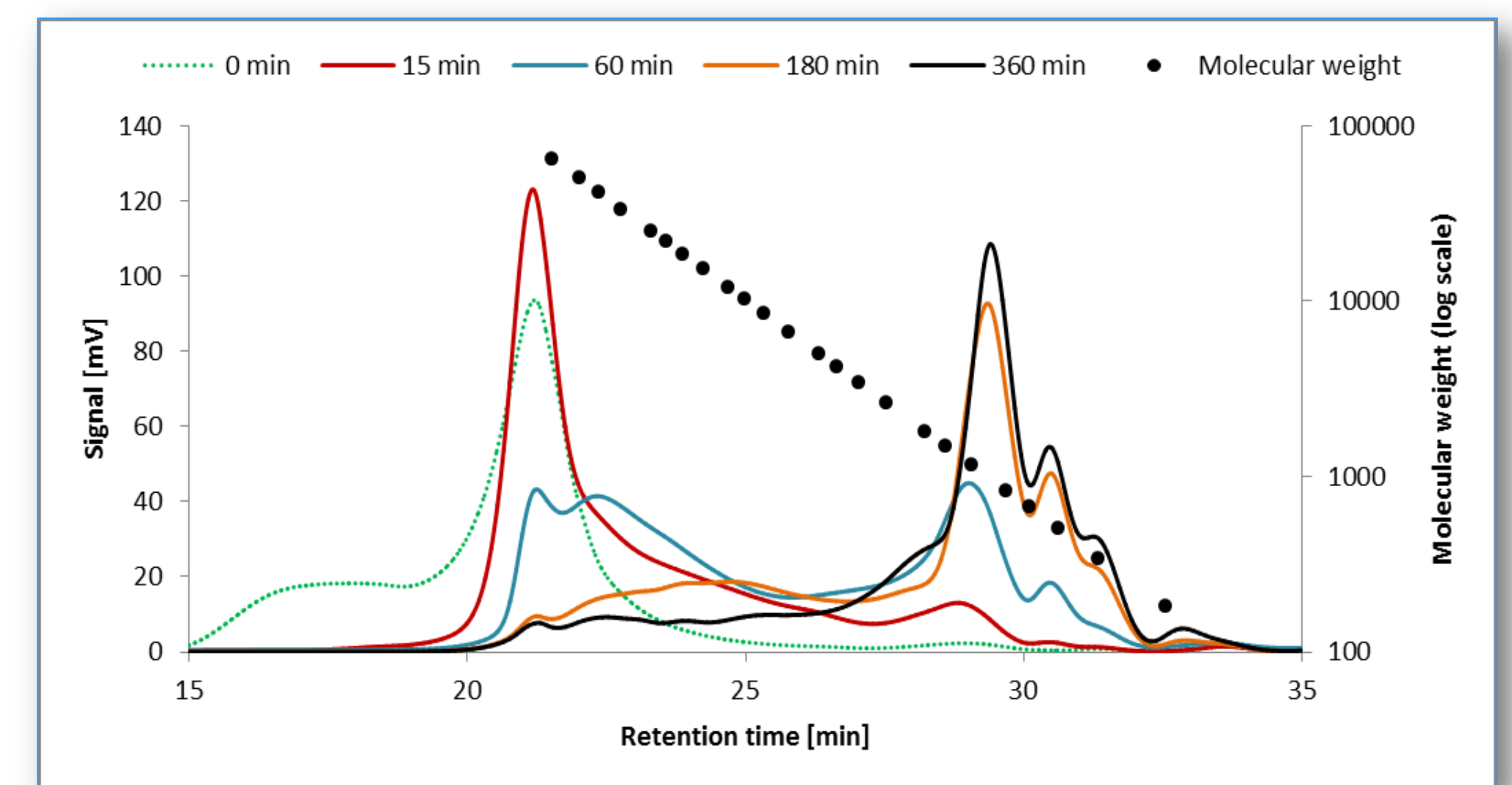
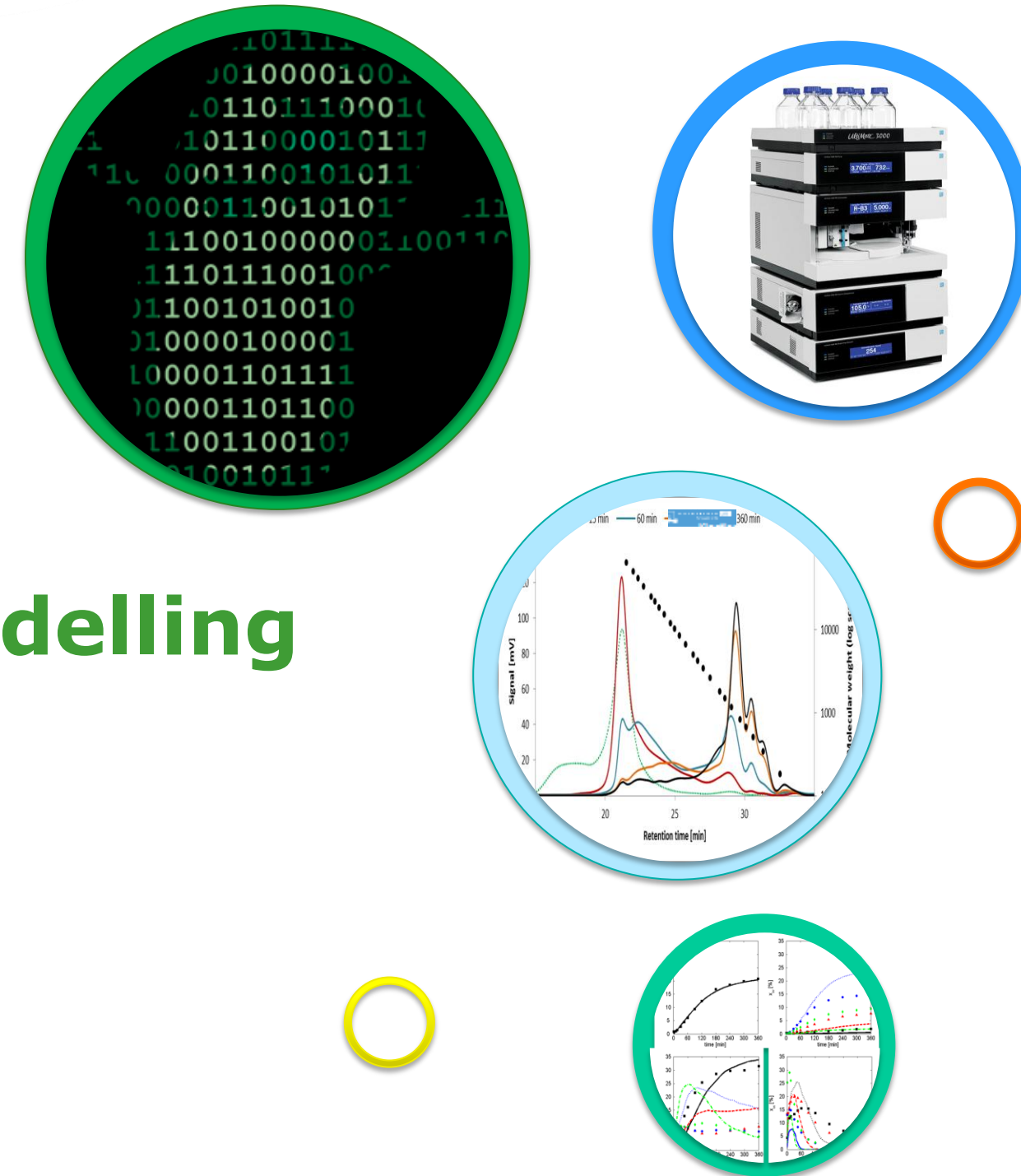
The composition of the hydrolysed samples was determined with size-exclusion chromatography HPLC-SEC. The concentrations of carbohydrates presented in Figure 2 show a trend. In the first stages of the reaction the clusters of amylopectin are separated from each other and the concentrations of only the large molecules increase. As the reaction proceeds, the individual clusters are being digested and the small oligosaccharides are being produced.

## Composition of the model

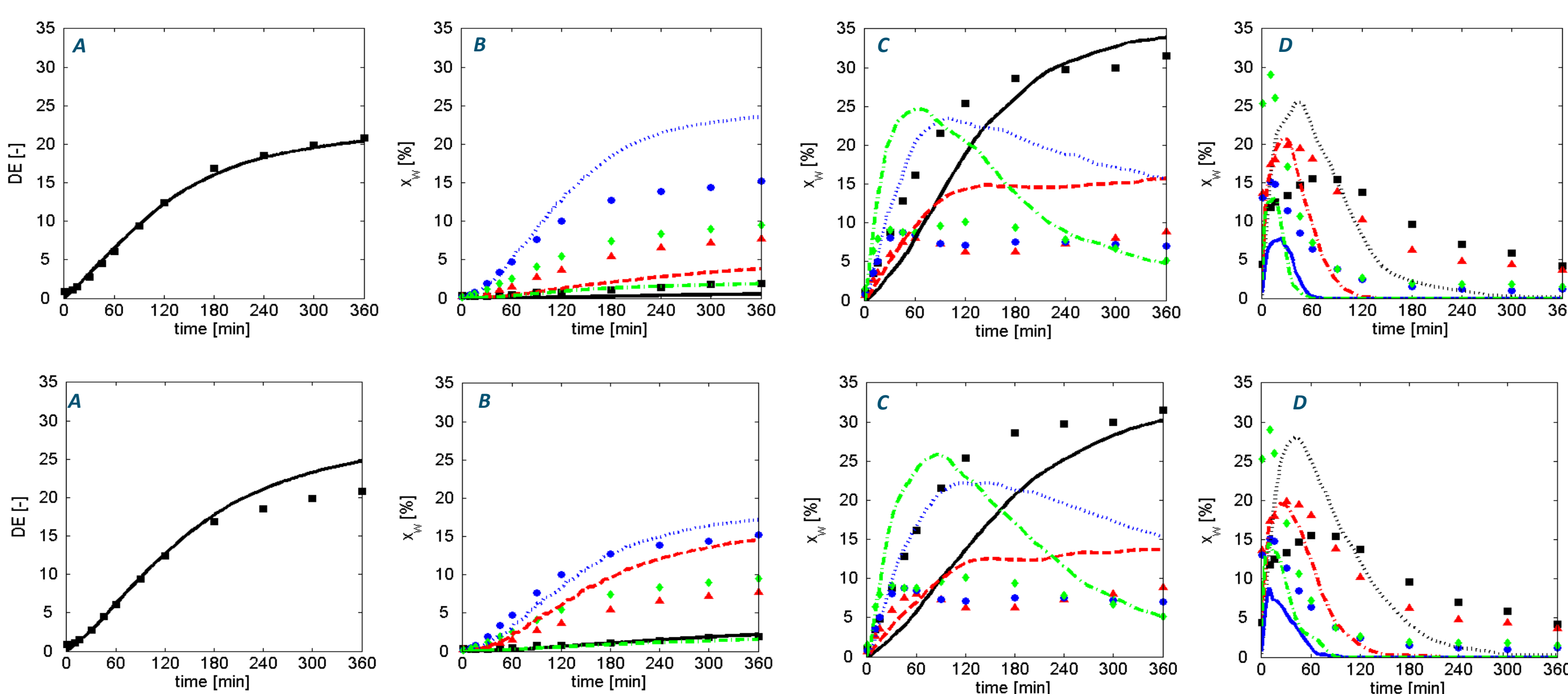
### Substrate matrix



## Modelling



**Figure 2. The chromatograms of all carbohydrates measured at different stages of enzymatic hydrolysis.**

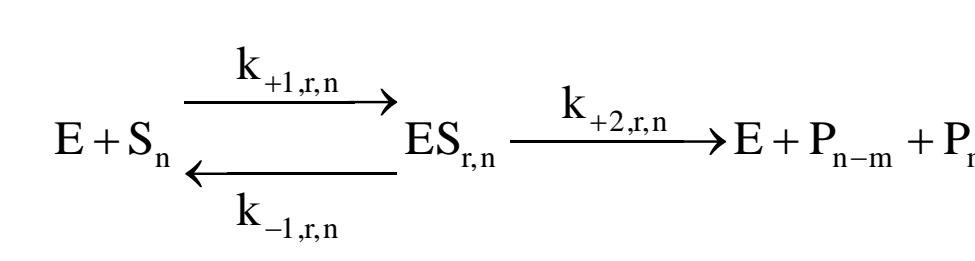


**Figure 3. Top line:** experimental values (points) fitted to the updated original model (lines). **Bottom line:** experimental values (points) fitted to the model (lines) in which the value of subsite +3 was changed to 0. The charts present the fits for:

- A) Dextrose equivalent
- B) Glucose (black), maltose (red), maltotriose (blue) and maltotetraose (green).
- C) Carbohydrate groups (by DP): 5-8 (black), 9-15 (red), 16-35 (blue) and 36-80 (green).
- D) Carbohydrate groups (by DP): 81-180 (black), 181-350 (red), 351-450 (blue) and 451-700 (green).

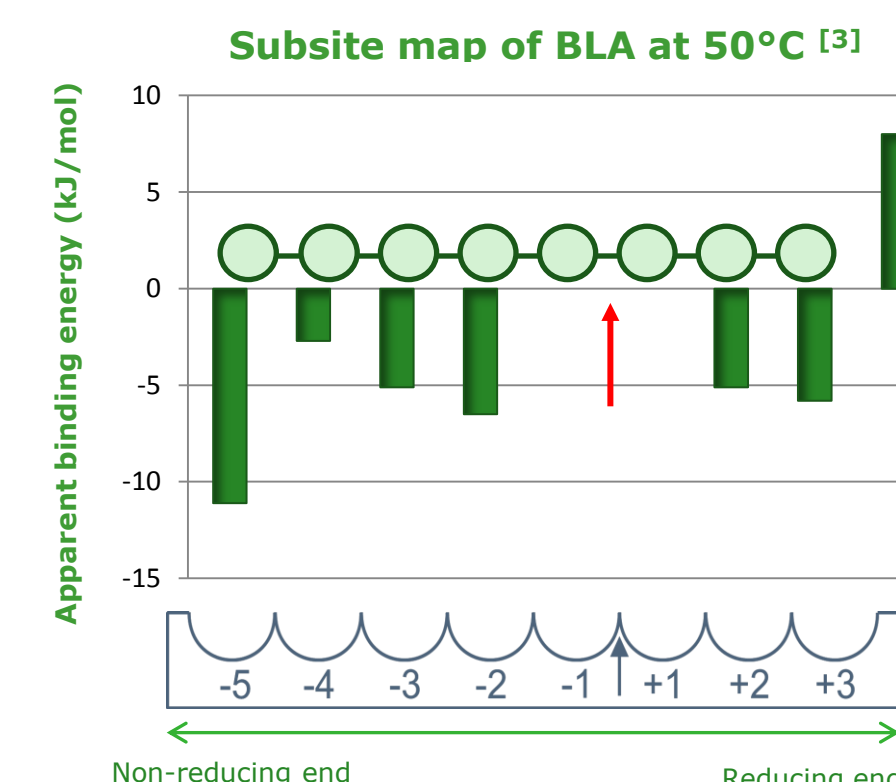
## Composition of the model

### Subsite theory<sup>[2]</sup>, subsite map<sup>[3]</sup> and binding energies



Subsite theory:

- $k_{+2,n} < k_{-1,n}$
- $K_{r,n} = k_{+1,n} / k_{-1,n}$
- Fastest reaction: highest value of  $k_{+2,n}$   $K_{r,n}$



$$EOB = \sum_{i=r-n+1}^r \Delta G_i + \Delta G_{mix}$$

$$K_{r,n} = \exp\left(\frac{-EOB}{R \cdot T}\right)$$

$$K_{max} = \exp\left(\frac{-EOB_{min}}{R \cdot T}\right)$$

## Conclusions

- The original subsite map does not provide an accurate fit.
- Changing the subsites values improves the fit, but not for all carbohydrates.
- The structure of the modelled amylopectin requires improvement.

## References

- [1] Besselink T, Baks T, Janssen AEM, Boom RM. 2008. Biotechnol Bioeng 100: 684-97
- [2] Allen JD, Thoma JA. 1976a. Biochem J 159:105-120.
- [3] Kandra L, Gyémánt G, Remenyik J, Hovánzski G, Lipták A. 2002. FEBS Lett 518:79-82.

## Acknowledgements

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