Novel Arabinoxylanase for Processing of Oat Carbohydrates into Prebiotics

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Introduction

- Dietary fiber consumption needs to be increased globally to prevent diet related diseases
- Fibers with prebiotic potential can be solubilized from the hemicellulose arabinoxylan (AX) in cereal side-streams using different carbohydrate active enzymes [1]
- Food industry needs specific arabinoxylanases, with limited side activity, for production of taylored fibers
- We have identified, produced and characterized a novel GH5_34 arabinoxylanase, *Hh*Xyl, for intended use in oat processing to increase soluble prebiotic fiber content
- Comercially avaliable homologue *Ct*Xyl5A (nzytech) was used for comparison[2]

Materials and Methods

- Standard *E. coli* expression system
- Affinity chromatography purification using ÄKTA
- DNS reducing end assay to evaluate enzymatic activity
- MODDE software to design experiment and create model
- HPAEC-PAD for identification of enzymatic products

Substrates used

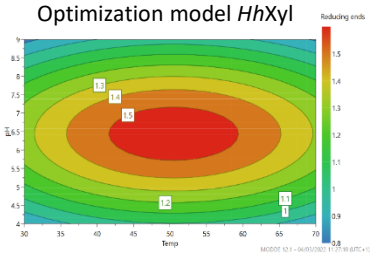
- Commercial AX from various biomass
- Industrial oat bran fraction
- In-house alkali extracted oat AX fiber products

Results

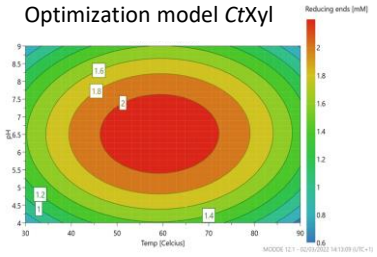
- Substrate preference determined using DNS assay show *Hh*Xyl activity on commercial rye and wheat AX as well as on extracted oat bran fibers, without showing cleavage of β-glucan
- Optimal temperature and pH conditions for a 10 min reaction on wheat AX was determined to 50 °C and pH 6.5 for *Hh*Xyl. The enzyme reaction rate was stable at these conditions for at least 36 h
- Maximal reactions rates obtained using optimized reaction conditions were determined to 0.21 mM/min for *Hh*Xyl (50 °C) and 0.32 mM/min for *Ct*Xyl (60 °C) at 20 mg/L enzyme concentration

Substrate	*Hh*Xyl	*Ct*Xyl
Rye AX	✓✓	✓✓
Wheat AX	✓✓	✓✓
Beechwood xylan	×	×
Oat β-glucan	×	×
Insoluble oat bran fibers	×	×
Soluble oat bran fibers	✓	✓
Desalted soluble oat bran fibers	✓✓	✓✓

Optimization model *Hh*Xyl



Optimization model *Ct*Xyl

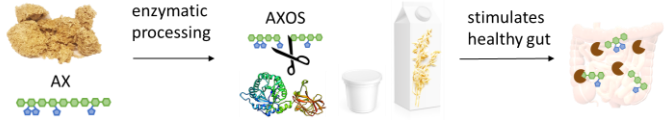


Conclusions

- Our findings show the great potential of using *Hh*Xyl for fiber solubilization and modification in oat grain processing, without compromising the beneficial β-glucan content in novel oat products

Future studies and experiments

- Product profile analysis using HPAEC-PAD-MS
- Docking simulations
- Domain organization influence on activity and stability
- Optimization for prebiotic and high-fiber oat products



References

[1] https://doi.org/10.1016/j.biortech.2021.126114

[2] https://doi.org/10.1107/S1744309111020823

Acknowledgements

This work was supported by Lund University, ScanOats and SSF

Stiftelsen för Strategisk Forskning

ScanOats

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