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Large-scale extraction and molecular analysis of cereal β-glucans

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Aim

To conduct thorough comparison of barley and oat β-glucan structure and functionality all the way from raw plant material to health effects

Background

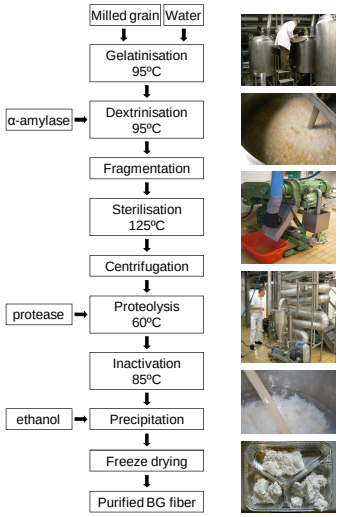
Health effects of cereal β-glucans are often related to dose, size and viscosity without taking the specific molecular structure into account[1].

We have studied the β-glucan molecular structure and physico-chemical functionalities of large-scale extracted barley and oat β-glucans prior to testing in a human intervention study.

This is the first time high performance anion exchange chromatography in combination with advanced spectroscopy (nuclear magnetic resonance, NMR and Raman) and multivariate data analysis has been used for studying complex relations contained in wide-ranging β-glucan data types[2].

Large-scale extraction

High β-glucan mutant barley, mother barley and oat β-glucans were extracted by comparable protocols using hot water, enzyme assisted hydrolysis and ethanol precipitation.

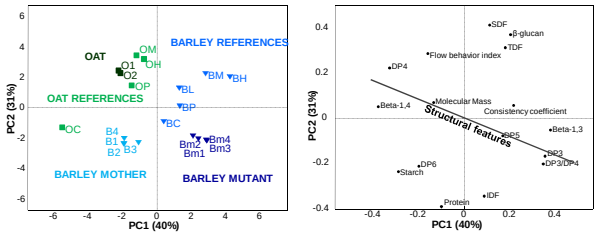


See movie on β-glucan precipitation in ethanol

Multivariate data analysis

Multivariate data analysis[3] on all compositional, structural and functional β-glucan features demonstrated that the main variance among samples was primarily explained by block structural differences

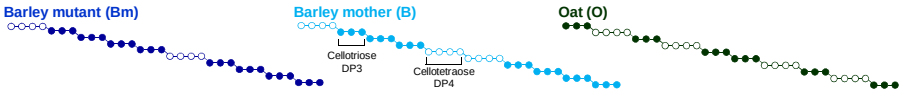
	BARLEY MUTANT	BARLEY MOTHER	OAT
β-glucan (%dm)	54-60	45-47	71-73
Insoluble fibres, IDF (%dm)	17-35	17-24	5-7
Molecular mass (kDa)	270-290	250-270	270-340
DP3/DP4 (molar ratio)	4.6-4.8	3.3-3.6	2.2-2.3
β-1,3 linkages (%)	30.2-30.9	29.1-29.5	28.7
Consistency coefficient (Pas)	0.1-0.4	0.01-0.05	0.05-0.06



Left Compositional, structural and rheological features of β-glucan samples. Data are presented in ranges.
Middle Principal Component Analysis (PCA) score plot of the above features. The first two principal components explain 71% of the data variation.
Right PCA loading plot of the above features.

Molecular structure

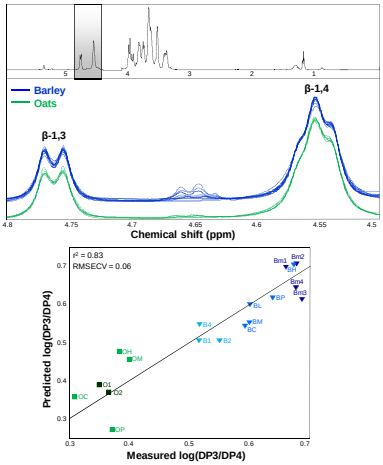
The barley mutant proved to exhibit a significantly changed β-glucan block structure as found by high performance anion exchange chromatography. Low solubility of barley samples was an effect of a high DP3/DP4 ratio possibly constituted by longer repetitive DP3 sequences.



Exaggerated molecular structures of extracted barley and oat β-glucans.

^{1}H NMR spectroscopy

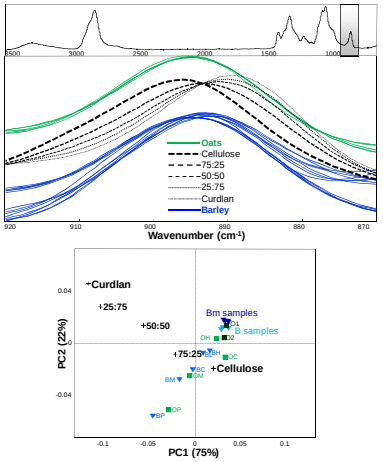
Signals of the β-1,4 and β-1,3 peaks can be used for building a prediction model of the DP3/DP4 ratio.



Top NMR spectra (0-6 and 4.5-4.8 ppm regions) of β-glucan samples. **Bottom** Partial least squares regression (PLSR) result for spectral (4.7-4.8 and 4.5-4.6 ppm) prediction of log(DP3/DP4 ratio) using a 4 latent variable model.

Raman spectroscopy

Systematic variation of the β-peak position is correlated to the β-1,4 and β-1,3 linkage ratio.



Top Raman spectra (full and β-peak region) of β-glucan and cellulose:curdlan blended samples. **Bottom** PCA score plot of spectral region 870-920 cm^{-1} with PC1 and PC2 explaining 97% of the data variation.

Conclusions

We succeeded in extracting fully comparable and reproducible high purity barley and oat β-glucan isolates with similar molecular masses and specific genotypic structural characteristics for the testing in a human intervention study on β-glucan health effects.

References [1] Wood, P.J. (2004), Trends Food Sci. Tech., 15, 313-320; [2] Mikkelsen, M.S. et al. (submitted), Food Chem.; [3] Hotelling, H (1933), J. Educ. Psychol., 24, 417-441