



## Invitation to PhD-defense

Monday, November 23. 2009 at 14.00  
in Auditorium 3-03, Building 2-83, Thorvaldsensvej 40,  
Frederiksberg C

### Molecular Structure and Functionality of Health Promoting Molecules in Food - with emphasis on $\beta$ -glucan

PhD thesis by:

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Supervisors:

- Professor Søren Balling Engelsen, Department of Food Science, LIFE-KU
- Associate Professor Lars Hemmingsen, Department of Basic Sciences and Environment, LIFE-KU

Opponents:

- Professor Igor Tvaroska, Department of Structure and Function of Saccharides, Institute of Chemistry, Center for Glycomics, Slovak Academy of Sciences
- Mark Lawther, PhD, Director, TLA-Development I/S
- Associate professor Frans W.J. van den Berg, Department of Food Science, LIFE-KU

List of publications included in the thesis

**Paper I:** Niels Johan Christensen, Peter Ibsen Hansen, Flemming Hofmann Larsen, Tore Folkerman, Mohammed Saddik Motawia, and Søren Balling Engelsen. *A combined nuclear magnetic resonance and molecular dynamics study of the two structural motifs for mixed linkage  $\beta$ -glucans*. Carbohydrate Research. Submitted.

**Paper II:** Niels Johan Christensen, Susana Murtinheira da Trindade Leitão, Mikael Agerlin Petersen, Birthe Møller Jespersen, and Søren Balling Engelsen. *A quantitative structure-property relationship study of the release of some esters and alcohols from barley and oat  $\beta$ -glucan matrices*. Journal of Agricultural and Food Chemistry, 2009, 57(11), 4924-4930. Published.

**Paper III:** Henrik Toft Simonsen, Mette Skau Nielsen, Niels Johan Christensen, Ulla Christensen, Thomas V. La Cour, Mohammed Saddik Motawia, Birthe Pontoppidan Jespersen, Søren Balling Engelsen, and Birger Lindberg Møller. *Molecular interactions between barley and oat  $\beta$ -glucans and phenolic derivatives*. Journal of Agricultural and Food Chemistry, 2009, 11, 57(5), 2056-2064. Published.

**Paper IV:** Åsmund Rinnan, Niels Johan Christensen, and Søren Balling Engelsen. *How the energy evaluation method used in the geometry optimization step affects the quality of the subsequent QSAR/QSPR models*. Journal of Computer-Aided Molecular Design. Accepted.

**Paper V:** Niels H. Andersen, Niels Johan Christensen, Peter Rygaard Lassen, Teresa B. Freedman, Laurence A. Nafie, Kristian Strømgaard, and Lars Hemmingsen. *Structure and absolute configuration of ginkgolide B characterized by IR- and VCD spectroscopy*. Chirality, 2009. Published (web).



## Summary

Numerous epidemiological and experimental studies have shown beyond doubt that dietary fibre from vegetables, fruit and cereals protects against a range of diseases. Mixed linkage (1→3),(1→4)- $\beta$ -D-glucan (referred to as BG in the following) is an example of a dietary fibre from oat and barley. This polysaccharide combines unique texture qualities with a range of health promoting factors such as the ability to lower postprandial blood glucose and insulin levels. Yet the mechanisms responsible for aspects such as immunopotential and the hypocholesterolemic effect pertaining to this dietary fiber are unknown. Similarly, a fundamental understanding of the influence of BG on the release of aroma compounds is lacking.

The main purpose of this PhD project was to apply computer based models and methods in the exploration and understanding of measured data from BG model systems. Aspects of particular importance were (1) the understanding of the structural and dynamical properties in solution of the fundamental BG motifs and (2) explanation of measurements of specific molecular interactions between aroma compounds, bile salts and BG.

The disaccharides methyl  $\beta$ -cellobioside and methyl  $\beta$ -laminarabioside are the fundamental structural motifs of BG, since they represent respectively the  $\beta$ (1→4) and the  $\beta$ (1→3) glycosidic linkage of the polymer. After a thorough molecular mechanics investigation of their conformational preferences, explicit solvent (water) molecular dynamics simulations were carried out for these disaccharides (Paper I) using a second generation carbohydrate force field. The study was supported by NMR (nuclear magnetic resonance) spectroscopy, and the combined computational and experimental approach showed that the conformational preferences of the two sugars in solution are dictated by a combination of intramolecular hydrogen bonds and hydration. The average values of the glycosidic dihedral angles  $\Phi$  and  $\Psi$  calculated from the most relevant molecular dynamics trajectories were employed in the construction of plausible three-dimensional models of BG polymers. These models revealed symmetries that were indicative of polymer packing.

Carbohydrate-aromatic interactions may play a role in e.g. the binding of flavor compounds to BG. The interactions in solution between the smallest BG motif methyl  $\beta$ -glucopyranoside and the important flavor compound vanillin and a reference molecule (phenol) were probed with 1D  $^1\text{H}$  NMR experiments. Comparison of the  $^1\text{H}$  spectra obtained for the sugar with and without aromatic compound indicated deshielding of all sugar protons in the presence of vanillin and shielding in the presence of phenol. A set of very pragmatic quantum chemical calculations were employed in an attempt to explain the differences. Despite their qualitative nature, the results were compatible with a previously postulated stacked complex of the sugar and the aromatic ring in case of phenol. For vanillin, calculations on the stacked model system did not agree with the measured shifts, which may indicate that the stacking interaction does not occur for vanillin.

QSAR (in general QSPR - quantitative structure property relationships) is a well-established method in the pharmaceutical sciences. In Paper II it was employed for the first time in the study of interactions between BG matrices and aroma-related compounds. Robust QSPR models for the flavour release behavior of alcohols and esters from BG matrices were constructed, demonstrating the pertinence and potential of the method in this novel food relevant context. The application of QSPR for the analysis of equilibrium dialysis of vanillin-related aromatic compounds in BG matrices (Paper III) turned out to be problematic. The fact that no reliable QSPR relationships could be established may be an indication of the problematic nature of the employed compounds, since detection problems for vanillin have been noted in other contexts. Nonetheless, the example may serve as inspiration for the investigation of the dialysis behavior of compounds with less structural resemblance to vanillin.

Investigations of a technical aspect of QSAR were undertaken in a study designed to reveal the influence of geometry optimization of molecular sets on the final QSAR/QSPR model (Paper IV). The results from the analysis of three diverse datasets showed that the QSAR models were not particularly sensitive to the method used for geometry optimization, even when based on molecular descriptors strongly influenced by the molecular geometry. This suggests that the computational time requirements in connection with certain types of QSAR can be reduced by the application of pragmatic geometry optimization methods.

Combined vibrational circular dichroism spectroscopy (VCD) and density functional theory (DFT) studies of the natural product ginkgolide B, which is an active constituent of widely used dietary supplements, allowed for the assignment of the absolute configuration of the compound (Paper V). This reinforces the view of the VCD/DFT combination as a powerful method for the determination of the absolute configuration of natural products with several chiral centers.

Together, the projects in this thesis emphasize the significant potential that methods from molecular modeling and chemometrics offer in the interpretation of advanced measurements of structure and functionality in food relevant model systems. It is expected that the employed computational methods will assume increasingly important roles in meeting the future demands for characterization of foods at the molecular level.