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Publication date:
2012

Document Version
Early version, also known as pre-print

Citation for published version (APA):
Schmidt, J. S., Daykin, C. A., & Stærk, D. (2012). Identification of -glucosidase inhibitors in apple peel - a chemometric approach. Poster session presented at The 33rd Danish NMR Meeting, Aarhus, Denmark.

Identification of α -glucosidase inhibitors in apple peel - A chemometric approach

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Scope

To identify α -glucosidase inhibitors in apple peel by multivariate data analysis of the combined ¹H NMR spectra and IC₅₀ values from α -glucosidase assay of raw apple peel extract.

Hypothesis

Compounds that are correlated with the α -glucosidase inhibitory activity can be identified by applying partial least squares (PLS) regression on ¹H NMR spectra and IC₅₀ values of raw extract.

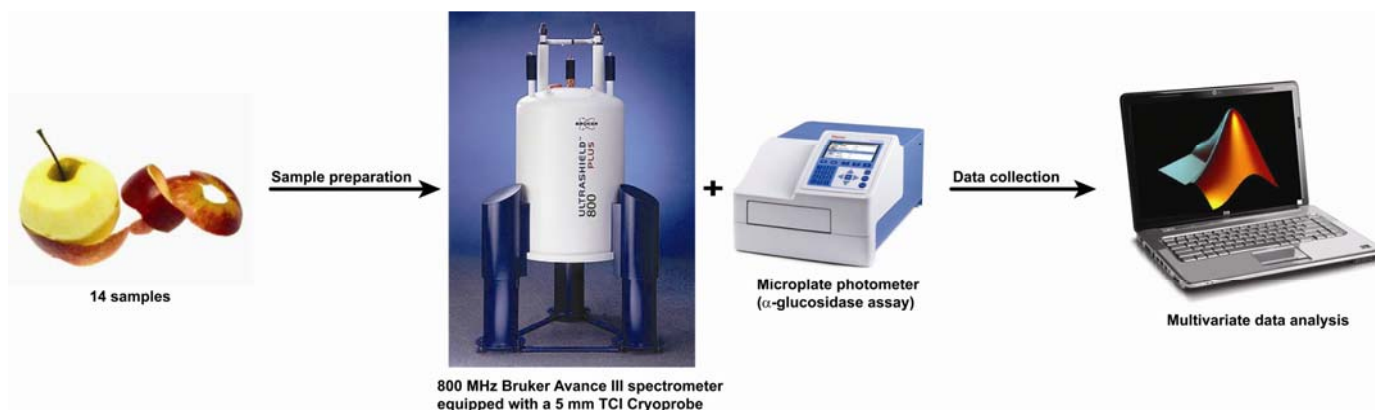
Introduction

There is an increasing interest in bioactive compounds responsible for the health-promoting properties of fruits and vegetables. However, plant extracts are very complex matrices containing hundreds of compounds, and identification of the individual constituents responsible for the bioactivity is a time-consuming task. The use of conventional (bio)analytical tools like *in vitro* bioassay and NMR spectroscopy in combination with multivariate data analysis might be an efficient way to speed up this process.

The enzyme α -glucosidase is involved in the hydrolysis of di-, tri- and oligo-saccharides to mono-saccharides in the small intestine. This makes α -glucosidase inhibitors potential leads for the management of blood glucose level in patients with type 2 diabetes.

Conclusion

PLS regression of ¹H NMR spectra and IC₅₀ values of apple peel extract identified chlorogenic acid, (-)-epicatechin and phlorizin to correlate with the α -glucosidase inhibitory activity.



Materials and Methods

Materials

A total of 14 different apple cultivars were obtained from "Pometet" in Tåstrup, Denmark.

Sample preparation

The peel from each apple cultivar was extracted with boiling methanol (70%). Sugars that might interfere with the assay was removed by pre-purification on an Optimized Polymer Technology (OPT) solid-phase column. This procedure was done in duplicate resulting in a total of 28 samples.

Bioassay

IC₅₀ values were determined for each of the 28 samples using a microplate-based α -glucosidase assay.

NMR spectroscopy

The samples were dissolved in 0.9 mM phosphate buffer (pH

6) and ¹H NMR spectra were acquired on a 800 MHz Bruker Avance III spectrometer equipped with a 5-mm TCI Cryoprobe. All samples were acquired with the same receiver gain, and a total of 128 transients were collected for each sample.

Data processing

IC₅₀ values were calculated in GraFit 7. The 28 ¹H NMR spectra were manually phase- and baseline corrected in MestReNova 6.1.1 and aligned using the *icoshift* algorithm [Savorani et al., Journal of Magnetic Resonance 202 (2010) 190-202] in MATLAB 7.10.0.

The data was Pareto scaled and PLS models were calculated using the PLS Toolbox. The models were cross-validated leaving each pair of duplicates out at a time. The Variable Importance for Projection (VIP) algorithm was used in an iterative process to select the variables correlating with the IC₅₀ values. Consecutive PLS models were calculated until

model performance didn't improve by further removal of variables.

Results

The PLS regression of ¹H NMR spectra and IC₅₀ values of raw extracts resulted in a fairly well-predicting, cross-validated model. Figure 1 shows the measured vs. predicted plot and model statistics of the final model. The chemical shift intervals used in this model are marked in Figure 2. The main peaks in the marked chemical shift intervals lead to the identification of chlorogenic acid, (-)-epicatechin and phlorizin. These three compounds are correlated with the α -glucosidase inhibitory activity, but correlation does however not imply causation. Therefore, it still needs to be determined whether these three compounds are responsible for the observed activity or not.

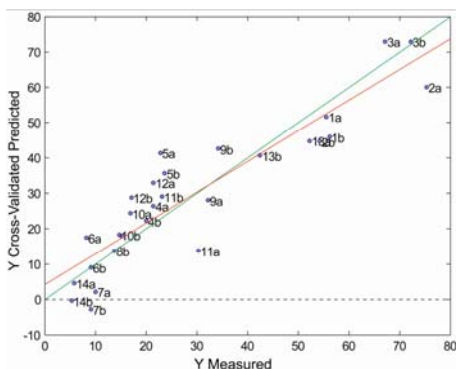


Figure 1. Measured vs. predicted plot of the final PLS model using 7 latent variables. The green line indicates a perfect regression. The red line indicates the regression of the PLS model. R² = 0.828, RMSEC = 2.5597 and RMSECV = 8.7027.

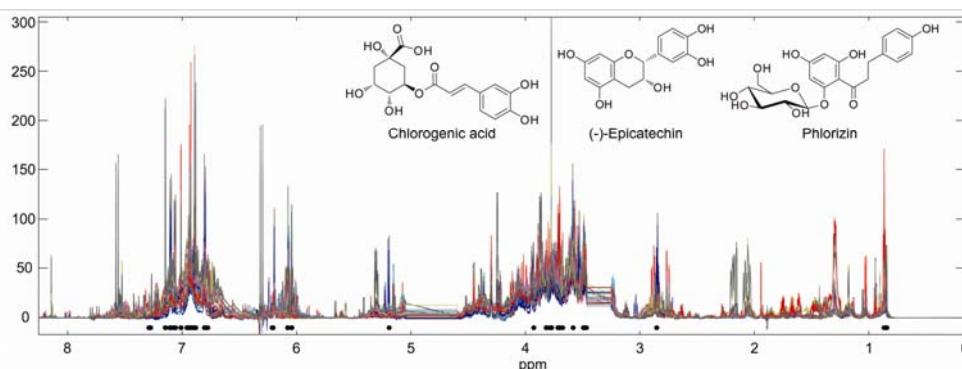


Figure 2. Overlaid ¹H NMR spectra of all 28 samples. The chemical shift intervals used in the final PLS model are indicated with black dots. Compounds identified from these chemical shift intervals are shown.