

Determination of arabinoxylans and beta-glucans in cereals and their fractions with NIR techniques

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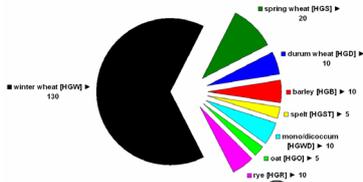
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Introduction and aims

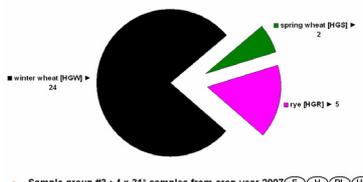
- Definition, roles and analysis of arabinoxylans**
 - Arabinoxylans (AX) are **non-starch polysaccharides** and the main **dietary fiber components** in cereal grains.
 - As a dietary fiber, AX have **positive effects on glucose/insulin response** and can **reduce the blood lipid level** significantly. In addition, they are partially fermentable in the large intestine, which **promotes bowel health**.
 - The quantitative analysis of total AX (TOTAX) content and their fractions (water-extractable AX (WEAX) and water-unextractable AX) can be carried out by **gas liquid chromatography** after complicated and time-consuming extraction, hydrolysis, reduction and derivatisation of the samples.
- Definition, roles and analysis of β-glucans**
 - β-glucan is a **fibre-type polysaccharide** that comes from the **cell wall**.
 - It is primarily used to **enhance the immune system** and to **lower cholesterol levels**.
 - The mixed linkage β-glucan content is **determined enzymatically** using Megazyme kit.
- Aims of present study**
 - This study is focusing on the development of **near infrared (NIR) analysis methods** for polysaccharide-type bioactive components in whole meal and milling fractions of cereals like **TOTAX, WEAX as well as β-glucans**.
 - The developed **calibration models** cover the **commodity** (winter wheat, spring wheat, rye, oat, barley etc.), **crop year** (three years), **GxE** (4 growing locations, 31 varieties) and **instrument variations** enhancing the robustness of models as big as possible.

Materials and methods

- Sample group #1 - 200 samples from crop year 2005**
 - The "Diversity Screen": 200 cereals from crop year 2005



- Sample group #2 - 31 samples from crop year 2006**
 - Selection: 31 cereals from the "Diversity Screen" from crop year 2006



- Sample group #3 - 4 × 31 samples from crop year 2007**
 - GxE: 31 cereals harvested from 4 crop sites during crop year 2007 (*Note: HG 25 and HG 26 samples are missing from growing site PL)



- Bran, flour and whole meal samples**
 - Bran and flour fraction are prepared by grinding cereals in a Chopin CD2 Mill equipped with an 0.9 mm and 0.25 mm sieves
 - Whole meal is prepared by grinding cereals in a Perten Falling Number Laboratory Mill 3100 equipped with an 0.5 mm sieve
 - Agricultural Research Institute of the Hungarian Academy of Sciences (AHRS)

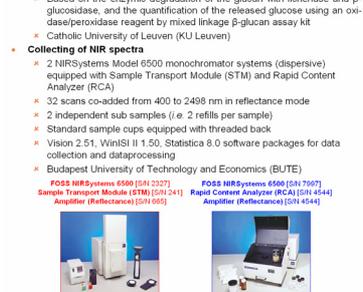
- Determination of TOTAX and WEAX in bran and flour samples**
 - with 3-parallel (% of DW)
 - Based on the acid hydrolysis of all sugars, derivatisation of the produced monosaccharides to alditol acetates and the quantification of these alditol acetates by gas liquid chromatography
 - TOTAX → the complete sample is subjected to hydrolysis
 - WEAX → the water extractable fraction of the sample is analysed
 - Catholic University of Leuven (KU Leuven)

- Determination of β-glucan in whole meal samples**
 - with 2 parallel (% of DW)
 - Based on the enzymic degradation of the glucan with lichenase and β-glucosidase, and the quantification of the released glucose using an oxidase/periodidase reagent by mixed linkage β-glucan assay kit
 - Catholic University of Leuven (KU Leuven)

- Collecting of NIR spectra**
 - 2 NIRSystems Model 6500 monochromator systems (dispersive) equipped with Sample Transport Module (STM) and Rapid Content Analyzer (RCA)
 - 32 scans co-added from 400 to 2498 nm in reflectance mode
 - 2 independent sub samples (i.e. 2 refills per sample)
 - Standard sample cups equipped with threaded back
 - Vision 2.51, WinISI II 1.50, Statistica 8.0 software packages for data collection and data processing
 - Budapest University of Technology and Economics (BUTE)

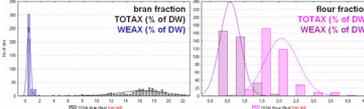
- POSS NIR systems 6500 (SN 2327)**
- POSS NIR systems 6500 (SN 7907)**
- Sample Transport Module (STM) (SN 241)**
- Rapid Content Analyzer (RCA) (SN 4544)**
- Amplifier (Reflections) (SN 4595)**
- Amplifier (Reflections) (SN 4546)**

- The results: TOTAX**

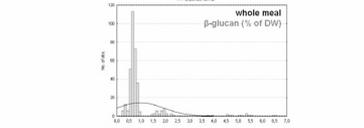


Processing of reference data

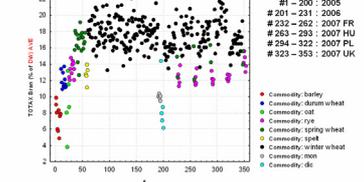
- Histograms of TOTAX and WEAX distribution in bran and flour fraction**



- Histograms of β-glucan distribution in whole meal**



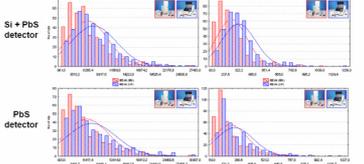
- Scatter plots of TOTAX in bran fraction**



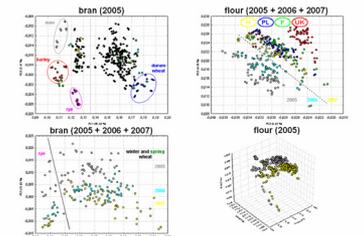
- Further processing of reference data (results not shown)**
 - SEL (standard error of laboratory analysis) and R²_{max}
 - Scatter plots of SD² data of parallels of reference data
 - Cross correlation matrices between reference data files

Processing of spectral data

- Standard error of difference between two scans collected from the same instrument (RMS test, bran spectra) - instrument drift**



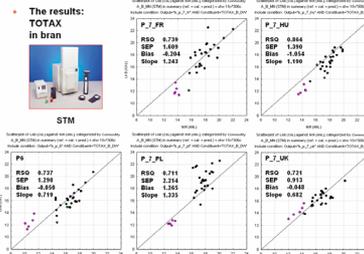
- PCA scatter plots of scores - outlier filtering, trends, patterns**



Development of calibration models

- Parameters of mPLS regressions**
 - Wavelengths: 348 • Segment: 1300-1420.2; 1600-1750.2; 2030-2450.2 • Scatter: SNV and Detrend • Derivative: Gap, Smooth, Smooth 2: 1,8,4,1 • Maximum number of terms: 16 • Cross validation groups: 4 • Number of elimination passes: 0
- Fully independent validation of calibration equations for 5 sets of samples - the price of independence**

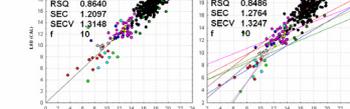
| Files for calibration "C" files | Files for prediction "P" files |
|---------------------------------|--------------------------------|
| C_6_cal = ALL - 2006 | P_6_cal = 2006 only |
| C_7_FR_cal = ALL - 2007_FR | P_7_FR_cal = 2007_FR only |
| C_7_HU_cal = ALL - 2007_HU | P_7_HU_cal = 2007_HU only |
| C_7_PL_cal = ALL - 2007_PL | P_7_PL_cal = 2007_PL only |
| C_7_UK_cal = ALL - 2007_UK | P_7_UK_cal = 2007_UK only |



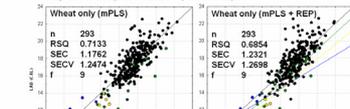
- Calculation of mPLS calibrations using average spectra in spectroscopic data files with limited outlier elimination**

- Checking the quality of calibration models with using repeatability (REP) files in cooperation with Walloon Agricultural Research Centre (CRAV)

- All samples (commodities) included - screening



- Only wheat samples included - practical aspect



- Comparison - mPLS calibrations calculated with and without REP file**

- All samples (commodities) included

| Fraction | Bran | | | | Flour | | | | Whole meal | |
|--------------------|-----------------|----------------|-----------------|----------------|-----------------|----------------|-----------------|----------------|------------|-----------|
| | TOTAX (% of DW) | WEAX (% of DW) | TOTAX (% of DW) | WEAX (% of DW) | TOTAX (% of DW) | WEAX (% of DW) | TOTAX (% of DW) | WEAX (% of DW) | | |
| Calibration method | STM | RCA | STM | RCA | STM | RCA | STM | RCA | STM | RCA |
| n | 353 | 353 | 353 | 353 | 352 | 352 | 352 | 352 | 352 | 352 |
| Range | 3.83-22.60 | 0.15-1.53 | 1.00-4.31 | 1.00-4.31 | 0.24-1.14 | 0.24-1.14 | 0.24-1.14 | 0.24-1.14 | 0.24-1.14 | 0.24-1.14 |
| Mean | 16.259 | 0.469 | 2.041 | 2.041 | 0.979 | 0.979 | 0.966 | 0.966 | 0.966 | 0.966 |
| Std. dev. | 3.280 | 0.244 | 0.481 | 0.481 | 0.294 | 0.294 | 0.294 | 0.294 | 0.294 | 0.294 |
| RSD | 0.849 | 0.843 | 0.833 | 0.836 | 0.771 | 0.771 | 0.710 | 0.710 | 0.710 | 0.710 |
| SEC | 0.904 | 0.908 | 0.871 | 0.881 | 0.790 | 0.790 | 0.707 | 0.707 | 0.707 | 0.707 |
| SECV | 1.276 | 1.300 | 0.899 | 0.899 | 0.230 | 0.230 | 0.158 | 0.158 | 0.158 | 0.158 |
| SEP | 1.210 | 1.300 | 0.899 | 0.899 | 0.230 | 0.230 | 0.158 | 0.158 | 0.158 | 0.158 |
| SECV | 1.220 | 1.416 | 0.899 | 0.899 | 0.230 | 0.230 | 0.158 | 0.158 | 0.158 | 0.158 |
| F | 10 | 13 | 13 | 13 | 13 | 13 | 11 | 11 | 11 | 11 |

- Only wheat samples included

| Fraction | Bran | | | | Flour | | | | Whole meal | |
|--------------------|-----------------|----------------|-----------------|----------------|-----------------|----------------|-----------------|----------------|------------|-----------|
| | TOTAX (% of DW) | WEAX (% of DW) | TOTAX (% of DW) | WEAX (% of DW) | TOTAX (% of DW) | WEAX (% of DW) | TOTAX (% of DW) | WEAX (% of DW) | | |
| Calibration method | STM | RCA | STM | RCA | STM | RCA | STM | RCA | STM | RCA |
| n | 293 | 293 | 293 | 293 | 292 | 292 | 292 | 292 | 292 | 292 |
| Range | 10.89-22.60 | 0.27-0.92 | 1.31-2.74 | 1.31-2.74 | 0.24-1.07 | 0.24-1.07 | 0.24-1.07 | 0.24-1.07 | 0.24-1.07 | 0.24-1.07 |
| Mean | 17.317 | 0.427 | 1.952 | 1.952 | 0.916 | 0.916 | 0.673 | 0.673 | 0.673 | 0.673 |
| Std. dev. | 2.197 | 0.092 | 0.279 | 0.279 | 0.141 | 0.141 | 0.117 | 0.117 | 0.117 | 0.117 |
| RSD | 0.685 | 0.679 | 0.431 | 0.430 | 0.580 | 0.573 | 0.171 | 0.171 | 0.206 | 0.207 |
| SEC | 0.710 | 0.710 | 0.442 | 0.442 | 0.251 | 0.251 | 0.140 | 0.140 | 0.140 | 0.140 |
| SECV | 1.232 | 1.244 | 0.899 | 0.899 | 0.190 | 0.190 | 0.125 | 0.125 | 0.125 | 0.125 |
| SEP | 1.176 | 1.139 | 0.899 | 0.899 | 0.145 | 0.145 | 0.105 | 0.105 | 0.105 | 0.105 |
| SECV | 1.270 | 1.318 | 0.871 | 0.871 | 0.188 | 0.188 | 0.128 | 0.128 | 0.128 | 0.128 |
| F | 9 | 11 | 11 | 11 | 10 | 10 | 7 | 7 | 7 | 7 |

Summary

- Conclusion**
 - The calibration models for total arabinoxylans (TOTAX) and water extractable arabinoxylans (WEAX) provide fast and reliable routine screening methods, providing either quantitative or semi-quantitative results.
 - The apparently good calibration model of glucan in whole meal samples is reflecting a strong commodity effect. In spite of acceptable statistical results this model does not measure the concentration of glucan properly, so the model is ranking only the different commodities based on their glucan level.
- Outlook**
 - These calibration models may be used by breeders, for assessment of the AX level and type in seeds; millers to evaluate the efficiency of separation and milling procedures; food technologists to select milling products or fractions for manufacturing products (with dedicated functional properties) with designed composition of bioactives.
 - The models are transferable between different instruments validation process needed following the calibration transfer.

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