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**Budapest University of
Technology and Economics**
(BUTE)



Catholic University of Leuven
(KU Leuven)

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

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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 a 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.





Introduction and aims

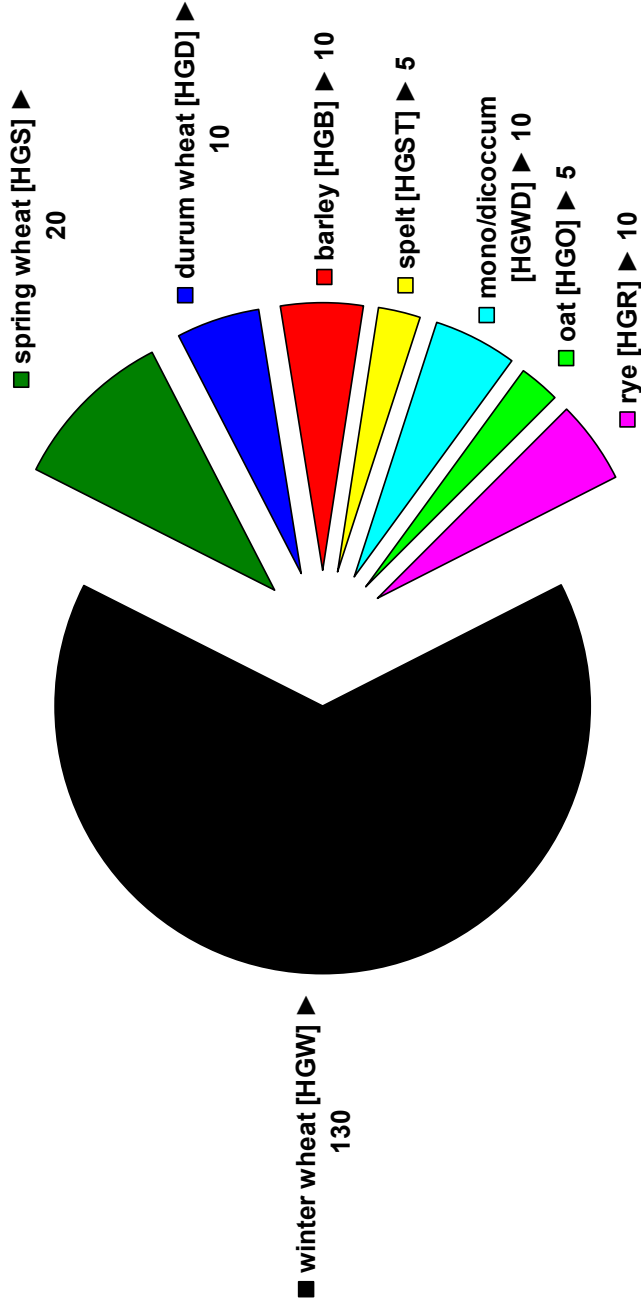
- **Definition, roles and analysis of β -glucans**
 - ✗ β -glucan is a **fibre-type polysaccharide** that comes **from the cell wall**.
 - ✗ It is primarily used to **enhance the immune system** and to **lower cholesterol levels**.
 - ✗ The mixed linkage β -glucan content is **determined enzymatically** 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

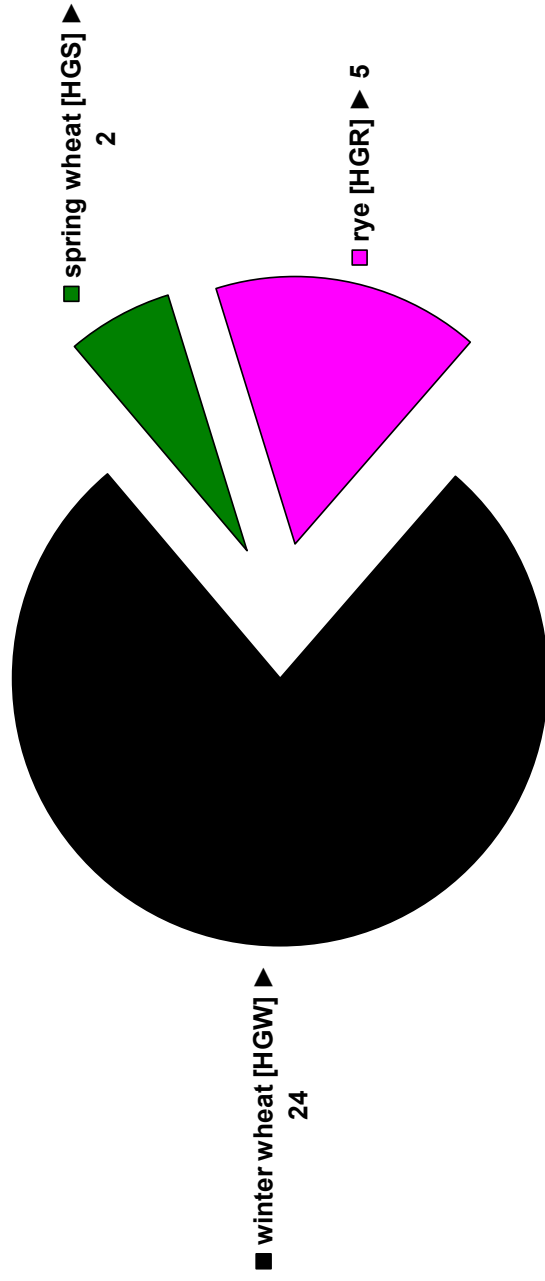
- **Sample group #1 • 200 samples from crop year 2005** **H**
- ✗ The "Diversity Screen": 200 cereals from crop year 2005





Materials and methods

- **Sample group #2 • 31 samples from crop year 2006** (H)
 - ✗ Selection: 31 cereals from the "Diversity Screen" from crop year 2006



- **Sample group #3 • 4 x 31* samples from crop year 2007** (F) (H) (PL) (UK)
 - ✗ G×E: 31 cereals harvested from 4 crop sites during crop year 2007
(* Note: HG 25 and HG 26 samples are missing from growing site PL)

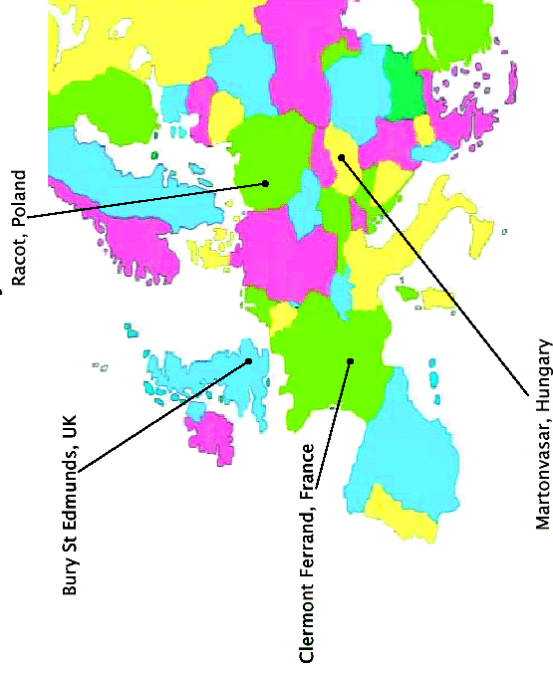




Materials and methods

- **Cereals from field trials**

- ✕ **(F) FRANCE** • Clermont-Ferrand • crop year: **2007**
- ✕ **(H) HUNGARY** • Martonvásár • crop years: **2005, 2006, 2007**
- ✕ **(PL) POLAND** • Dankow/Choryn • crop year: **2007**
- ✕ **(UK) UNITED KINGDOM** • Bury St. Edmunds • crop year: **2007**





Materials and methods

- **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 (ARIHAS)





Materials and methods

- **Determination of TOTAX and WEAX in bran and flour samples ***
 - ✗ * with 3-3 parallels (% 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 ► only 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 parallels (% 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/peroxidase reagent by mixed linkage β -glucan assay kit
 - ✗ Catholic University of Leuven (KU Leuven)





Materials and methods

- **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 dataprocessing
- ✗ Budapest University of Technology and Economics (BUTE)

FOSS NIRSystems 6500 [S/N 2327]
Sample Transport Module (STM) [S/N 241]
Amplifier (Reflectance) [S/N 665]



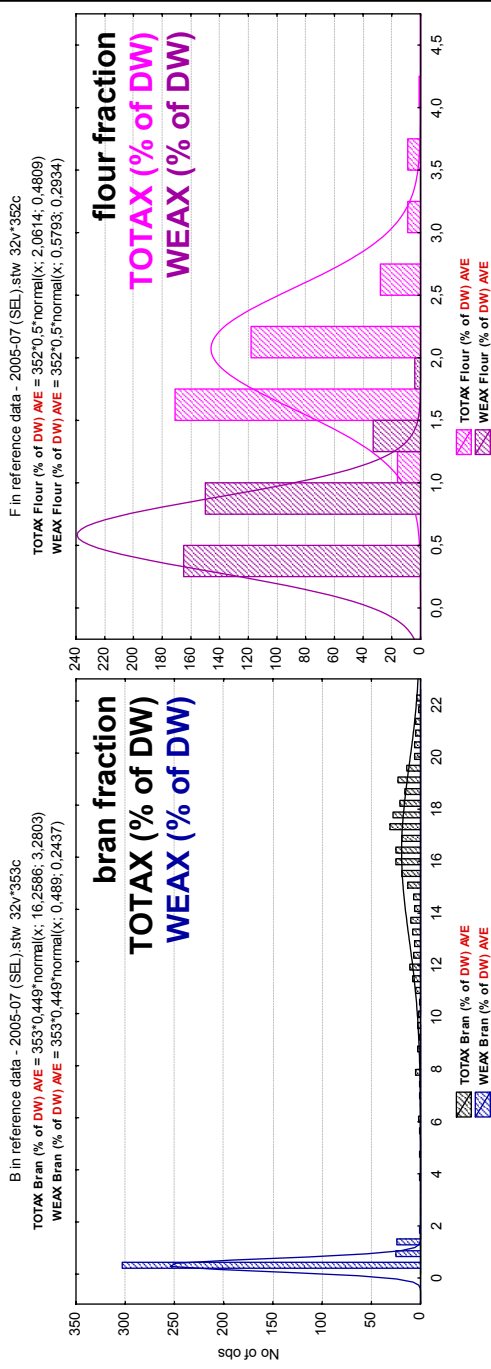
FOSS NIRSystems 6500 [S/N 7997]
Rapid Content Analyzer (RCA) [S/N 4544]
Amplifier (Reflectance) [S/N 4544]





Processing of reference data

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

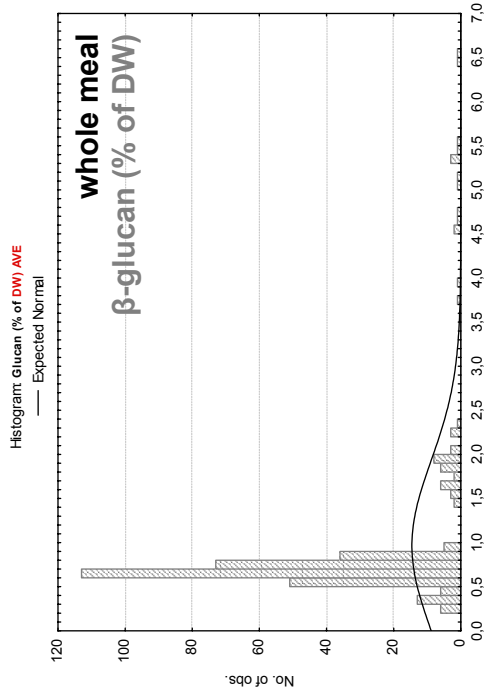




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Processing of reference data

- Histograms of β -glucan distribution in whole meal

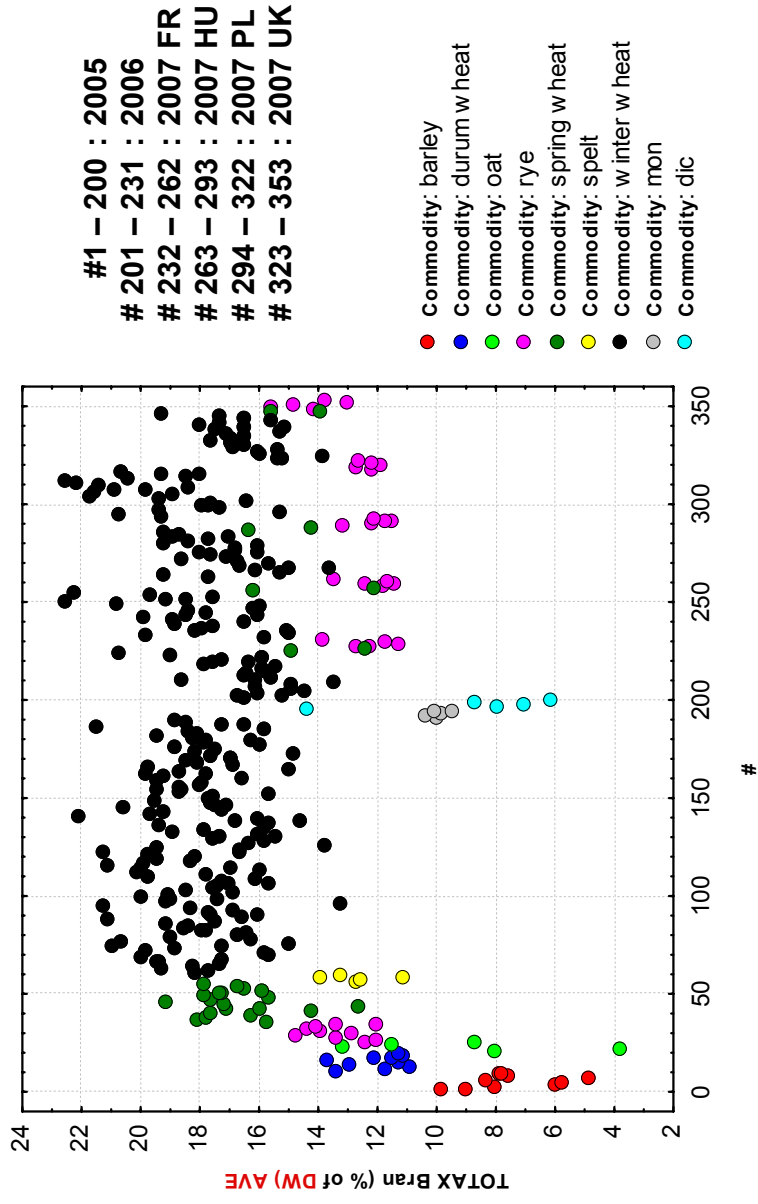




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Processing of reference data

Scatter plots of TOTAX in bran fraction





Processing of reference data

- **Further processing of reference data (results not shown)**
 - ✗ SEL (standard error of laboratory analysis) and R^2_{MAX}
 - ✗ Scatter plots of SD² data of parallels of reference data
 - ✗ Cross correlation matrices between reference data files

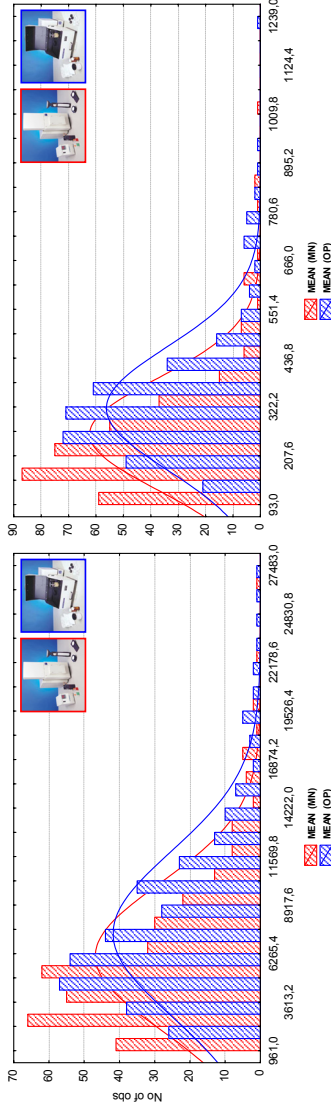


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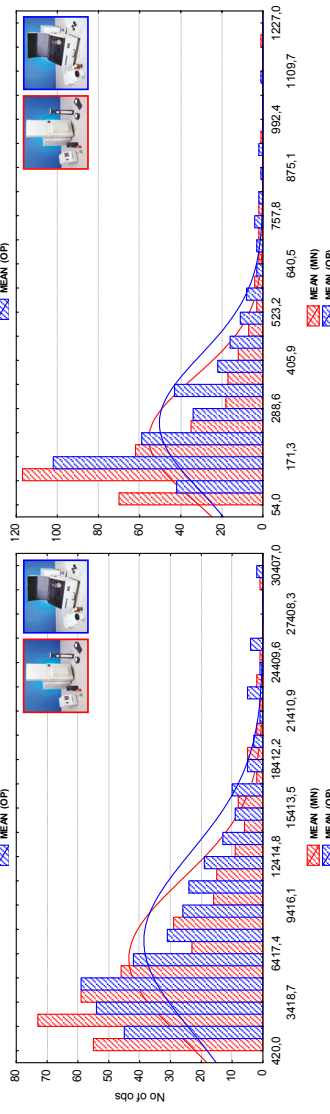
Processing of spectral data

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

Si + PbS detector



PbS detector



raw spectra (0-0-1-1)

first derivative spectra (1-4-4-1)

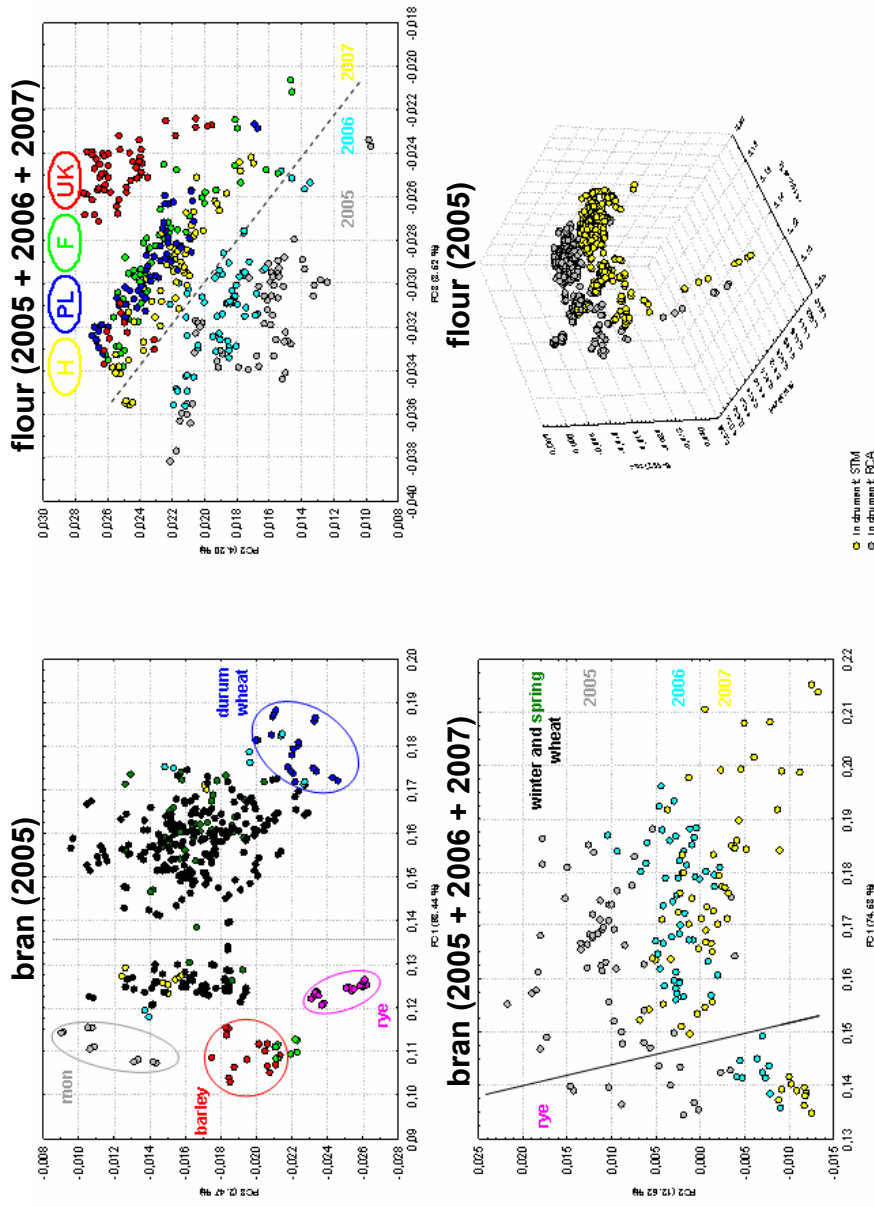




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Processing of spectral data

- 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 2: 1,8,4,1 • Maximum number of terms: 16 • Cross validation groups: 4 • Number of elimination passes: 0



Development of calibration models

- Fully independent validation of calibration equations for 5 sets of samples ~ the price of independence

Files for calibration “C” files	Files to 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



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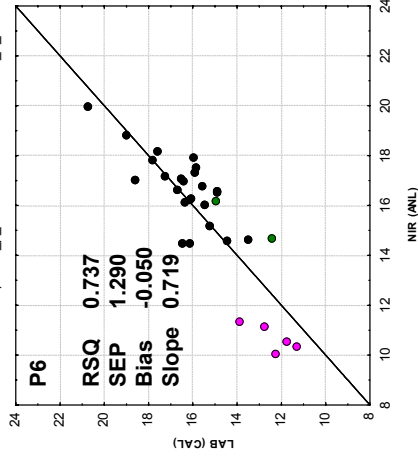
Development of calibration models

- The results:
TOTAX
in bran

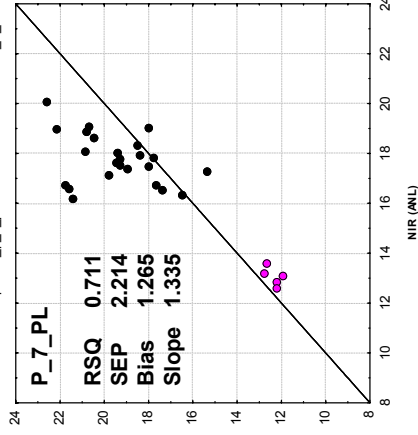


STM

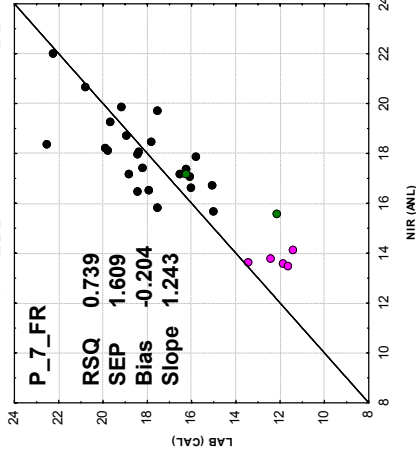
Scatterplot of LAB (CAL) against NIR (ANL); categorized by Commodity
A_B_MN(STM) in summary (ref. + cal. + pred.) + stw 15v*306c
Include condition: Output="b_p_6" AND Constituent="TOTAX_B_DW"



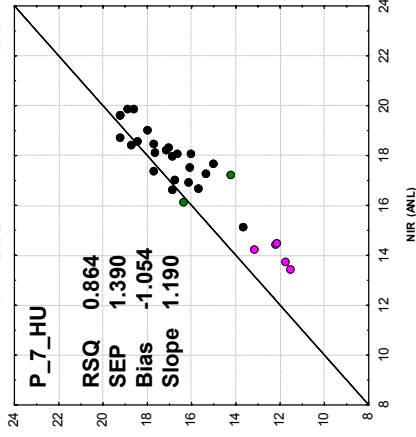
Scatterplot of LAB (CAL) against NIR (ANL); categorized by Commodity
A_B_MN(STM) in summary (ref. + cal. + pred.) + stw 15v*306c
Include condition: Output="b_p_7_pl" AND Constituent="TOTAX_B_DW"



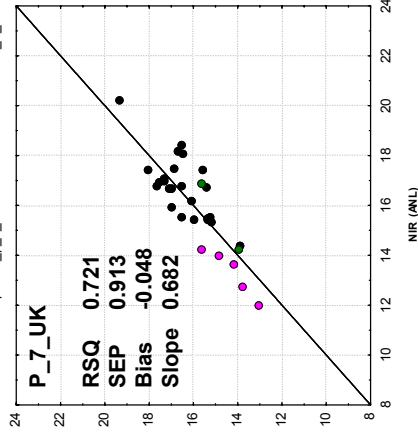
Scatterplot of LAB (CAL) against NIR (ANL); categorized by Commodity
A_B_MN(STM) in summary (ref. + cal. + pred.) + stw 15v*306c
Include condition: Output="b_p_7_fr" AND Constituent="TOTAX_B_DW"



Scatterplot of LAB (CAL) against NIR (ANL); categorized by Commodity
A_B_MN(STM) in summary (ref. + cal. + pred.) + stw 15v*306c
Include condition: Output="b_p_7_hu" AND Constituent="TOTAX_B_DW"



Scatterplot of LAB (CAL) against NIR (ANL); categorized by Commodity
A_B_MN(STM) in summary (ref. + cal. + pred.) + stw 15v*306c
Include condition: Output="b_p_7_uk" AND Constituent="TOTAX_B_DW"

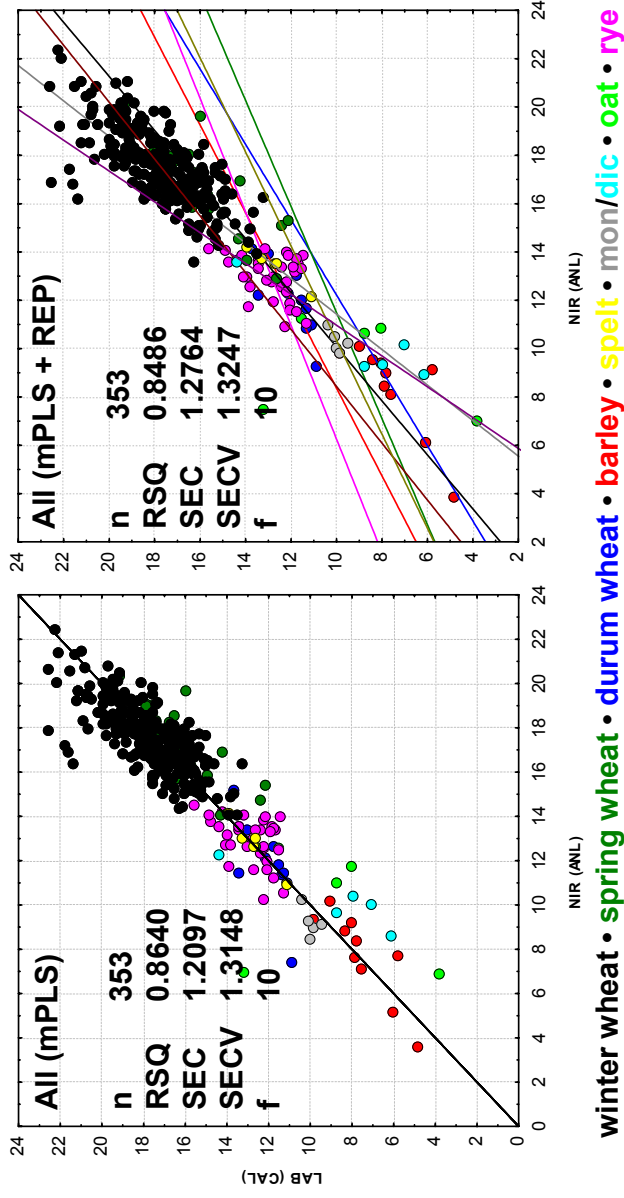




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Development of calibration models

- **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 (CRA-W)
 - ✗ All samples (commodities) included ~ screening

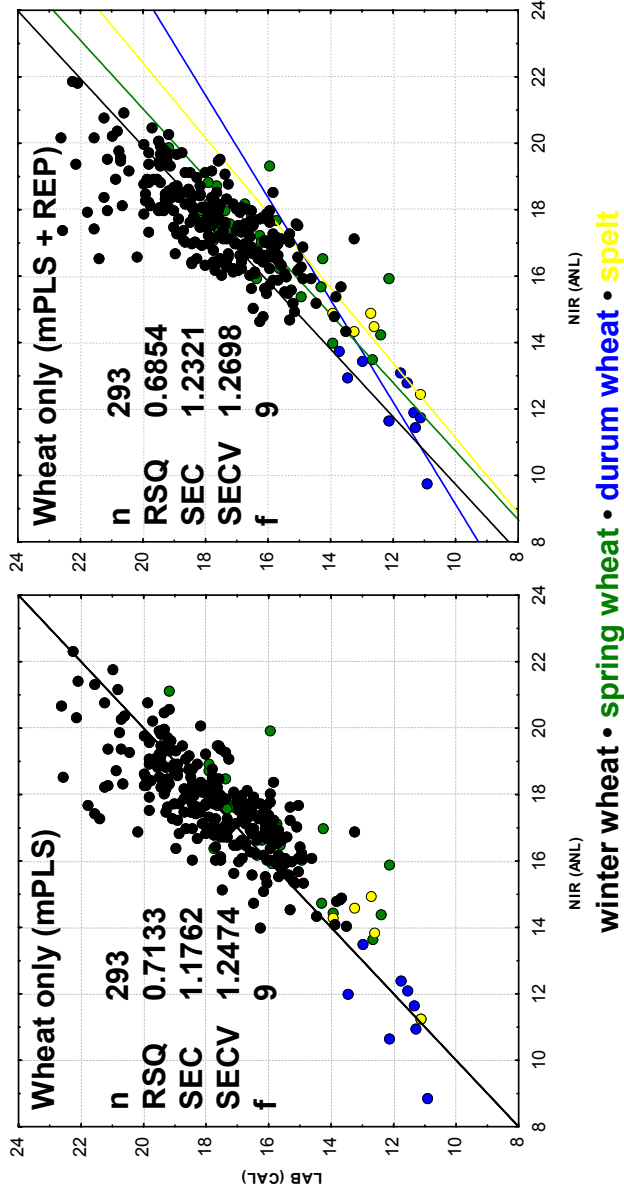




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Development of calibration models

✗ Only wheat samples included ~ practical aspect





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Development of calibration models

- **Comparison – mPLS calibrations calculated with and **without** REP file**
 - ✗ All samples (commodities) included





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Development of calibration models

Frac- tion	Bran				Flour				Whole meal	
	TOTAX (% of DW)		WEAX (% of DW)		TOTAX (% of DW)		WEAX (% of DW)		Glucan (% of DW)	
Con- stitu- ent	STM	RCA	STM	RCA	STM	RCA	STM	RCA	STM	RCA
Instru- ment	STM	RCA	STM	RCA	STM	RCA	STM	RCA	STM	RCA
n	353		353		352		352		352	
Range	3.83 – 22.60		0.15 – 1.53		1.05 – 4.31		0.15 – 1.94		0.24 – 6.54	
Mean	16.259		0.489		2.061		0.579		0.966	
Std. dev.	3.280		0.244		0.481		0.294		0.964	
RSQ	0.849 0.864	0.843 0.868	0.833 0.871	0.836 0.861	0.771 0.929	0.791 0.905	0.712 0.837	0.710 0.687	0.917 0.952	0.900 0.909
SEC	1.276 1.210	1.300 1.193	0.099 0.088	0.099 0.091	0.230 0.128	0.220 0.148	0.158 0.118	0.158 0.164	0.278 0.210	0.305 0.290
SECv	1.325 1.315	1.416 1.395	0.108 0.098	0.111 0.103	0.236 0.153	0.238 0.196	0.160 0.134	0.179 0.178	0.317 0.258	0.337 0.335
f	10 10	13 13	13 13	14 14	13 15	11 16	11 13	11 7	13 15	13 10





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Development of calibration models

✖ Only wheat samples included

Frac- tion	Bran				Flour				Whole meal	
	TOTAX (% of DW)		WEAX (% of DW)		TOTAX (% of DW)		WEAX (% of DW)		Glucan (% of DW)	
Instru- ment	STM	RCA	STM	RCA	STM	RCA	STM	RCA	STM	RCA
n	293		293		293		293		292	
Range	10.89 – 22.60		0.27 – 0.92		1.31 – 2.74		0.24 – 1.07		0.24 – 0.96	
Mean	17.317		0.427		1.952		0.514		0.673	
Std. dev.	2.197		0.092		0.270		0.141		0.117	
RSQ	0.685 0.713	0.679 0.731	0.433 0.515	0.430 0.462	0.558 0.821	0.573 0.721	0.173 0.452	0.206 0.459	0.267 0.552	0.349 0.507
SEC	1.232 1.176	1.244 1.139	0.069 0.064	0.069 0.067	0.180 0.114	0.176 0.143	0.128 0.104	0.125 0.103	0.100 0.078	0.094 0.082
SECV	1.270 1.247	1.318 1.257	0.073 0.071	0.073 0.072	0.184 0.132	0.188 0.163	0.128 0.115	0.128 0.120	0.103 0.091	0.099 0.093
f	9 9	11 11	11 10	10 9	11 13	9 10	7 8	6 9	7 12	8 10





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 mixing 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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Summary

**This work was supported by
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