HEALTH·GRAIN

The Grain "Biotechnology Toolkit"

Clare Mills, Craig Faulds (IFR), Gilles Charmet,
Luc Saulnier (INRA), Kurt Gebruers (Leuvan)
András Salgó (BUTE)

MŰEGYETEM 1782

Objective

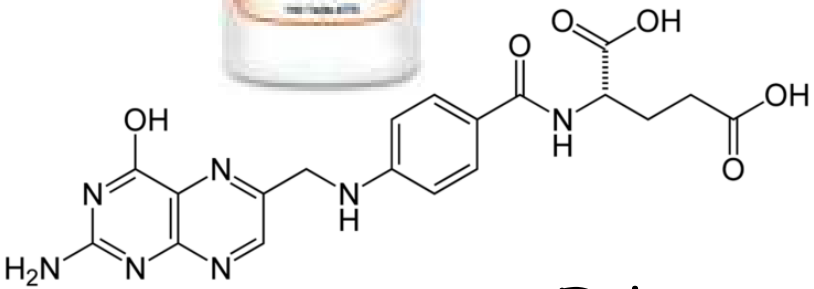
Develop high throughput methods for selection of wheats with enhanced nutritional quality accessible to plant breeders

Which nutritional quality?

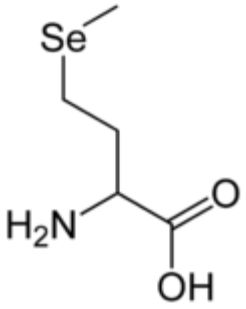
Nutritional Components in Wheat

Cell walls and fibre

Genotypic variation makes this of interest to breeders



Folates



15	16	17
P	S	Cl
33	34	35
As	Se	Br
51	52	53
Sb	Te	I

Selenium

SNP Marker Systems for fibre

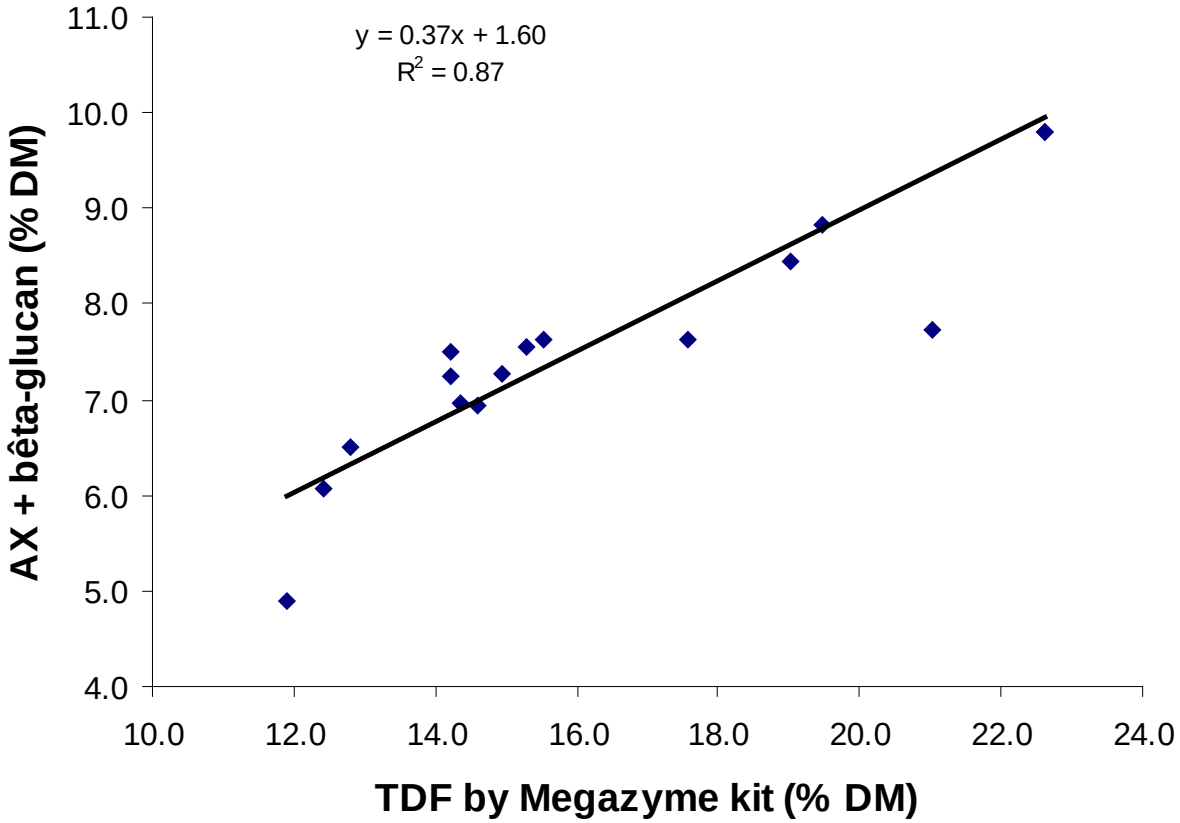
Gilles Charmet, INRA

Objective: transfer of SNP marker technology from experimental use to practical application in plant breeding programmes

Screening for fibre using kits?

Evaluation of current state-of-the art. A comparison of commercial kits for the measurement of total, soluble and insoluble dietary fibre using AOAC and AACC approved methods was undertaken.

Kurt Gebruers (Leuvan)



Kit measurements of AX + β-glucan content correlated well with TDF content using HG whole meal samples

BUT whilst giving a good analytical performance the methods were not suitable for high throughput screening

Two approaches have been explored both of which could be adapted for high-throughput screening

- NIR spectroscopy

- Biological analyses exploiting inactive enzymes able to bind feruloylated AX and Mab probes for AX in a microtitre plate format

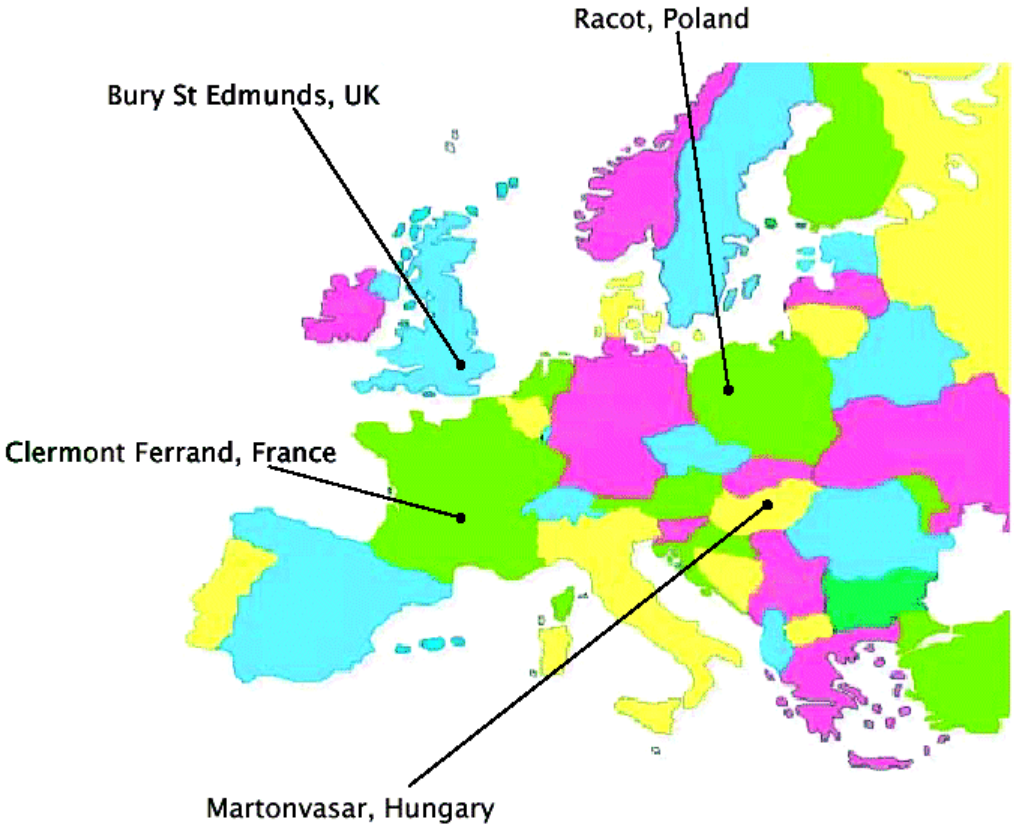
NIR Calibrations

NIRS technology is highly effective for discriminate analysis, exploiting the variation in bioactive compounds in grain of wheat and rye, both in whole grain, individual tissues and milling fractions for a streamlining of breeding programmes for development of improved cultivars for European production.

Szilveszter Gergely, András Salgó (BUTE)

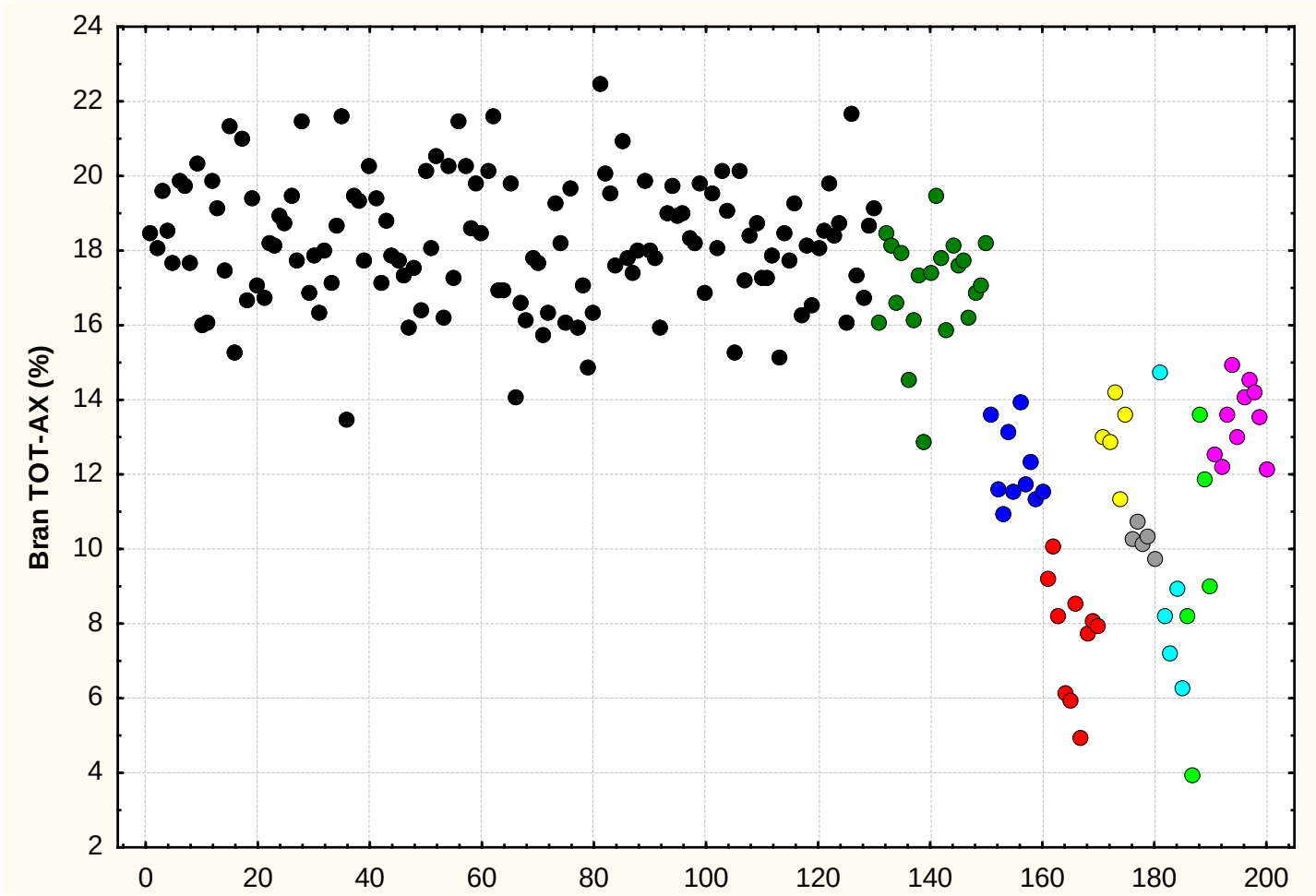
Field trials (G E)

- **FRANCE** · Clermont-Ferrand · crop year: **2007**
- **HUNGARY** · Martonvásár · crop years: **2005, 2006, 2007**
- **POLAND** · Dankow/Choryn · crop year: **2007**
- **UNITED KINGDOM** · Bury St. Edmunds · crop year: **2007**



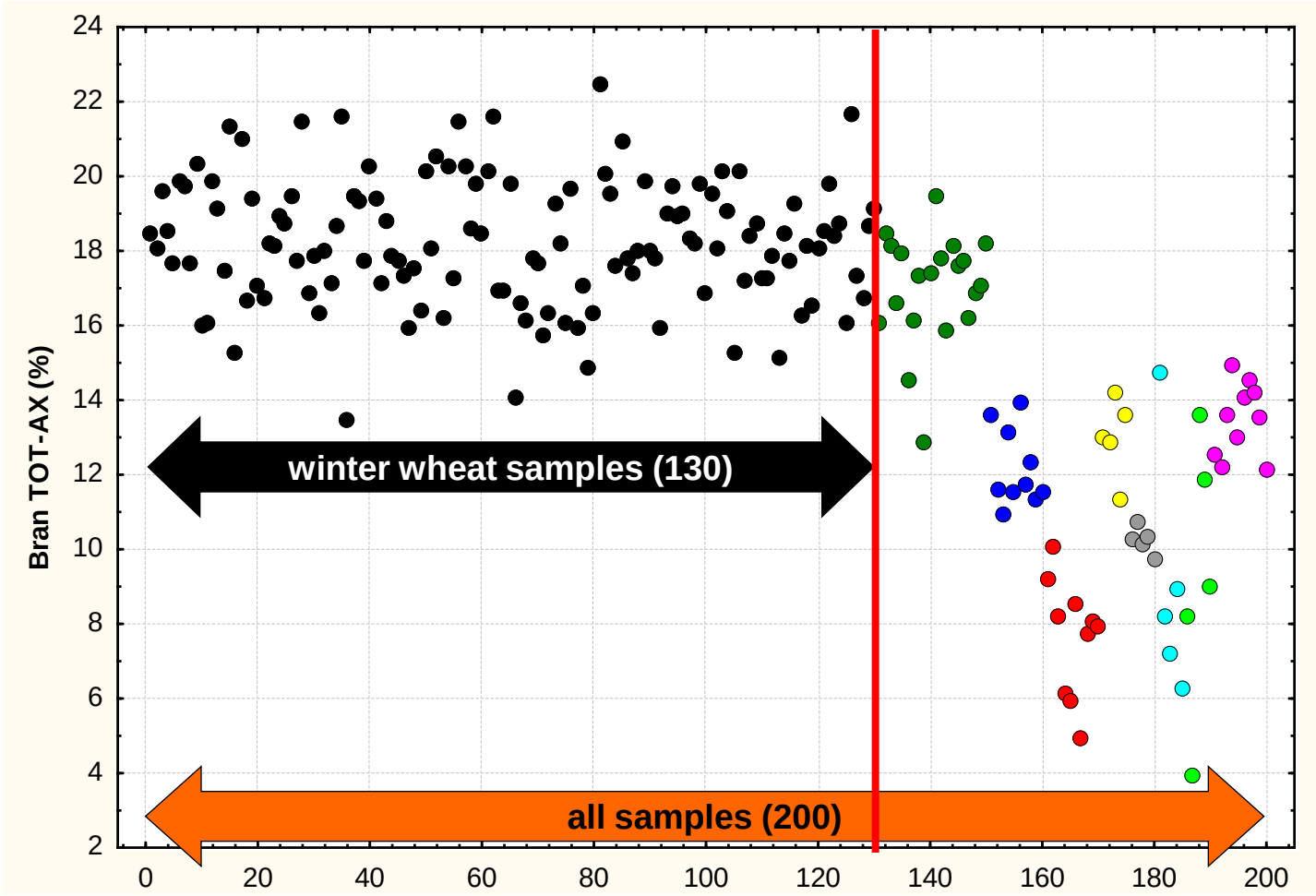
Diversity of HEALTHGRAIN collection - TOT-AX (2005) in bran fraction

Also done in white flour



winter wheat · spring wheat · durum wheat · barley · spelt · mon/dic · oat · r

Segregation of population for NIR of TOT-AX (2005) in bran fraction

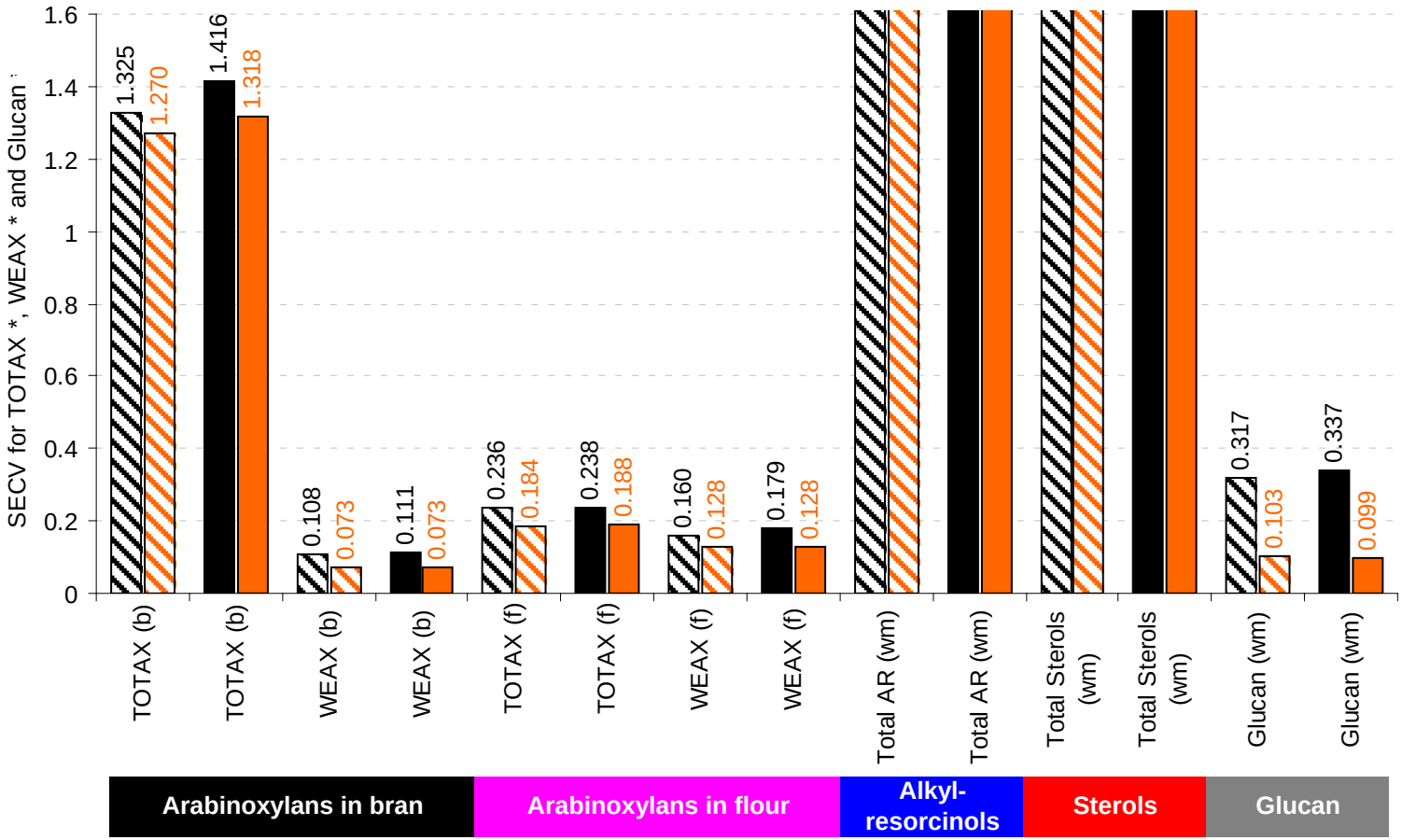


winter wheat • spring wheat • durum wheat • barley • spelt • mon/dic • oat • rye

HEALTHGRAIN

Comparison of calibrations using all and wheat only samples

- **SECV of mPLS calibrations calculated with REP file**



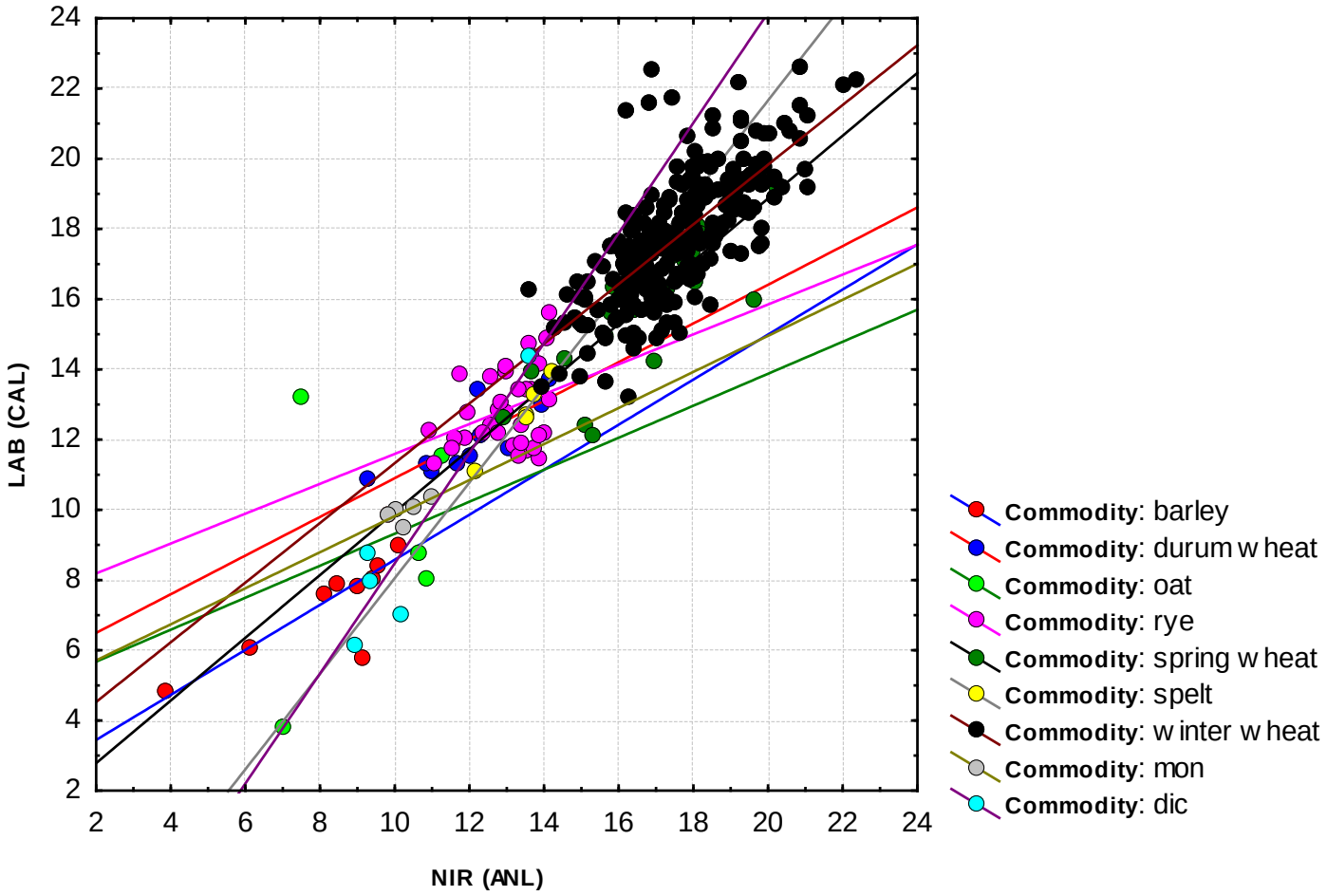
: STM : RCA

* : % of dry weight, except † : µg/g of dry weight, ‡ : µg/g of fresh weight

HEALTH·GRAIN

Checking the quality of calibration models with using repeatability (REP) files in case of all samples

Scatter plots of completed reference data vs. NIR predicted data based on average spectra – mPLS regression with REP file for TOTAX in bran



Conclusions

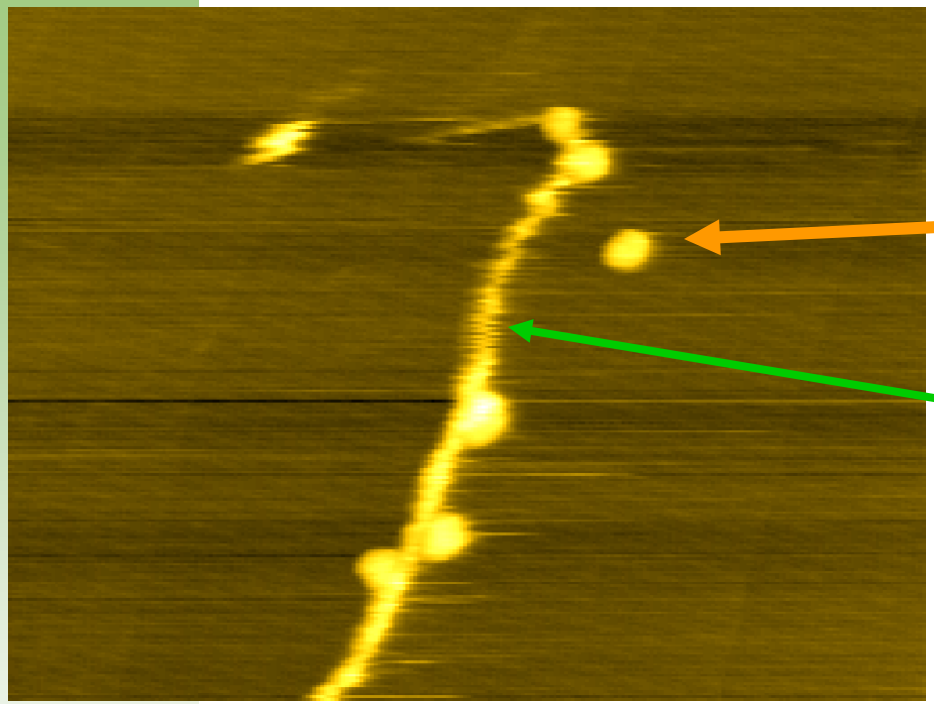
- NIR is already proven technology for grain screening
- Healthgrain has show it is possible to build calibrations for components such as AX in wheat
- Future work is required to demonstrate the robustness of such calibrations across
  - Different makes of NIR machines
  - Further harvest years and calibration sets.
  - Wholemeal flours
- Proof-of-concept applications in plant breeding underway with Martonvásár (ARIHAS) in order to select high dietary fibre lines (341) and developing with RRes and INRA

New kit development:

Using the combination of analytical and genetic data from module 2 activities candidate markers will be identified (e.g. proteins or compounds of interest) related to nutritional quality and **used to develop new antibody-based kits**

Feruloyl esterases as probes for feruloylated AX and FAXOS

(Craig Faulds, Ana Sancho, IFR)



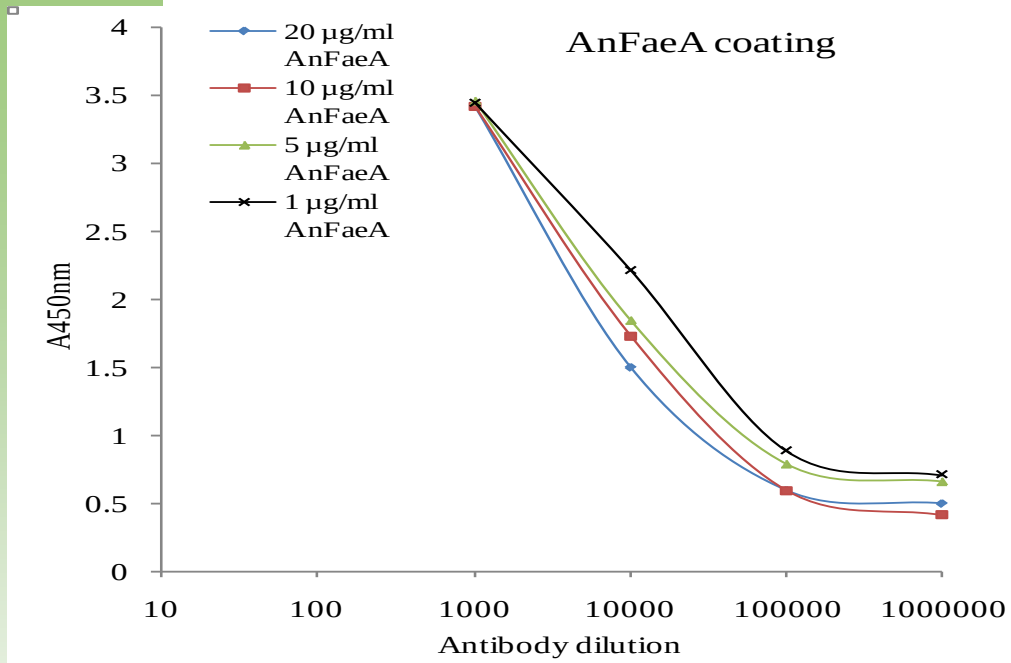
Atomic force microscopy (AFM) of *Ps fluorescens* XynA bound to wheat endosperm water-soluble arabinoxylan

Adams et al (2004) Carbohydr Res

Other researchers have shown carbohydrases such as xylanases can be used as probes for polysaccharides. We have extended this approach to enzymes able to bind phenolics bound to AX

HEALTH·GRAIN

Development of a competitive ELISA

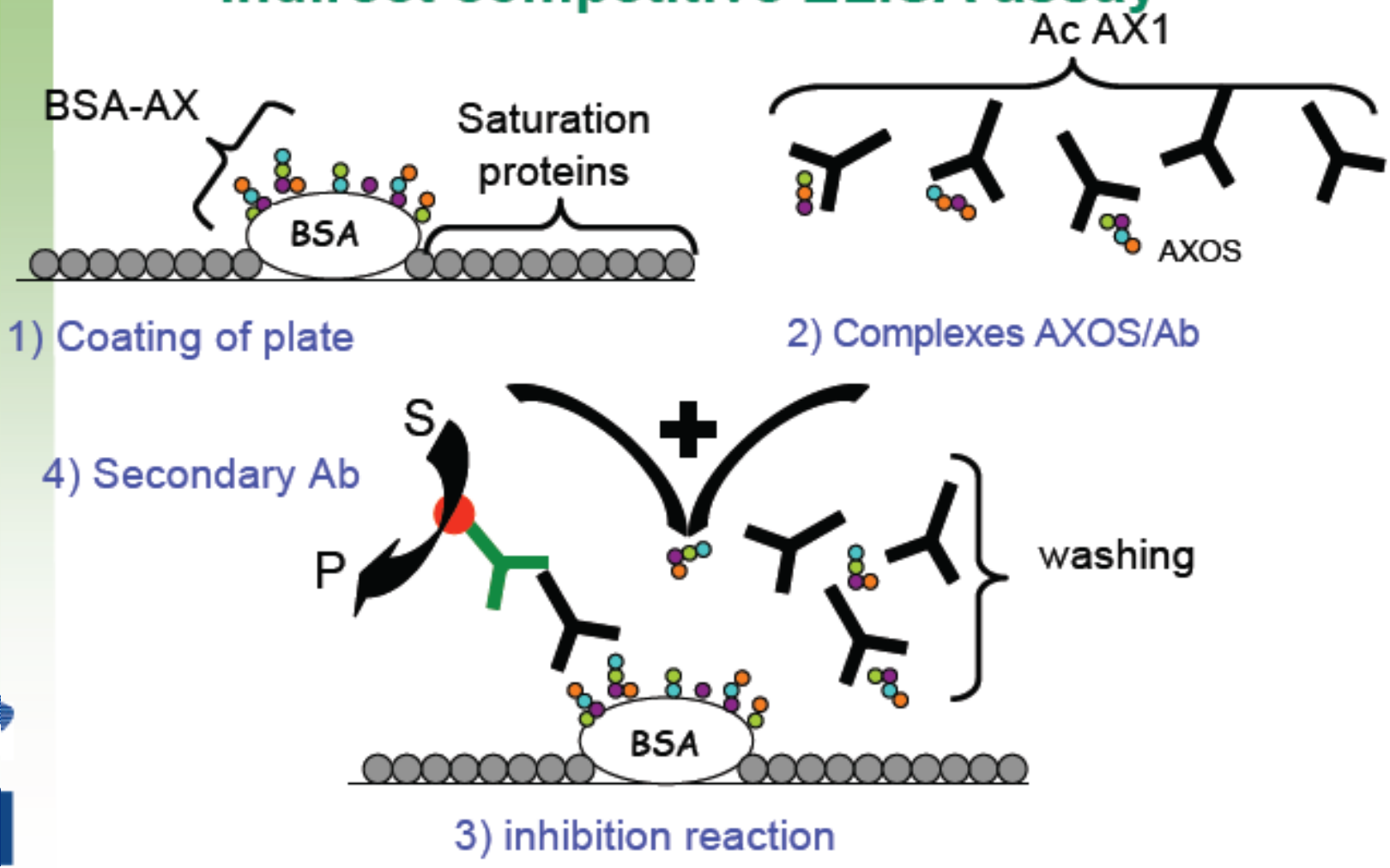


Inactive feruloyl esterase AnFaeA bound to a BSA-Ferulic acid conjugate adsorbed to microtitre plates but not inhibition curve could be developed.

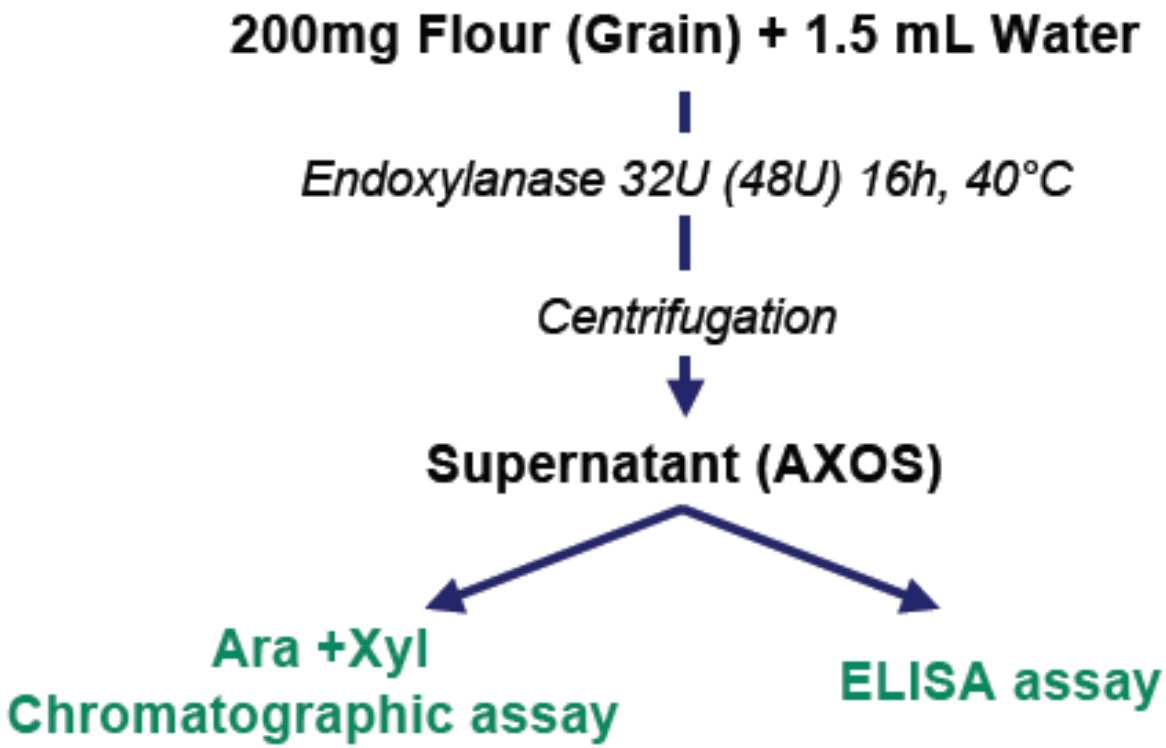
Conclusion: inactive esterase to detect feruloylated arabinoxylans requires further work, including further engineering of the enzyme to enhance interactions and development of better haptens

Anti-arabinoxylan antibodies as probes for the detection of arabinoxylan and AXOS

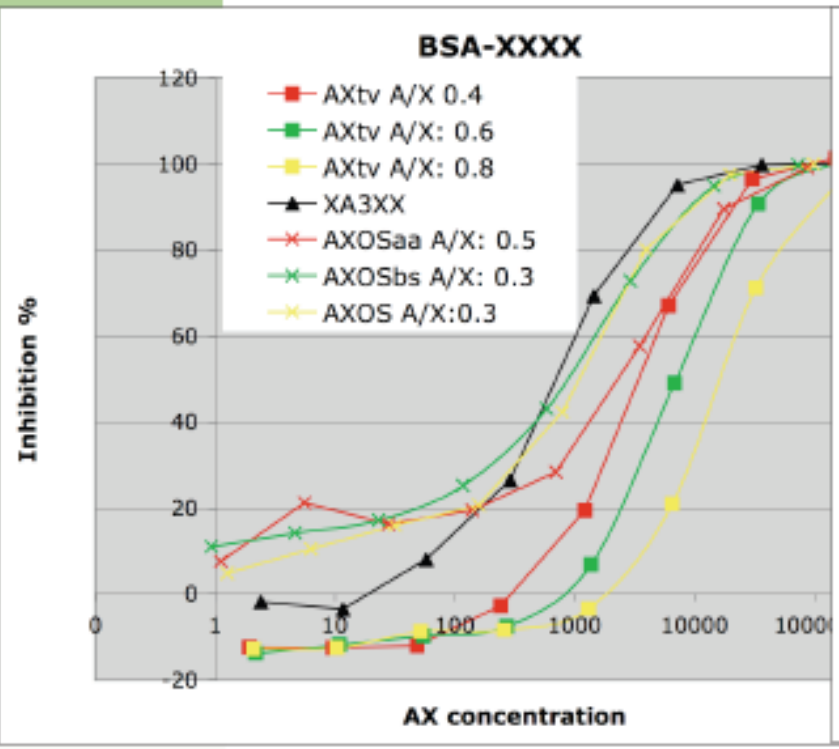
(Luc Saulnier, Olivier Tranquet, Rachelle Looten, INRA)

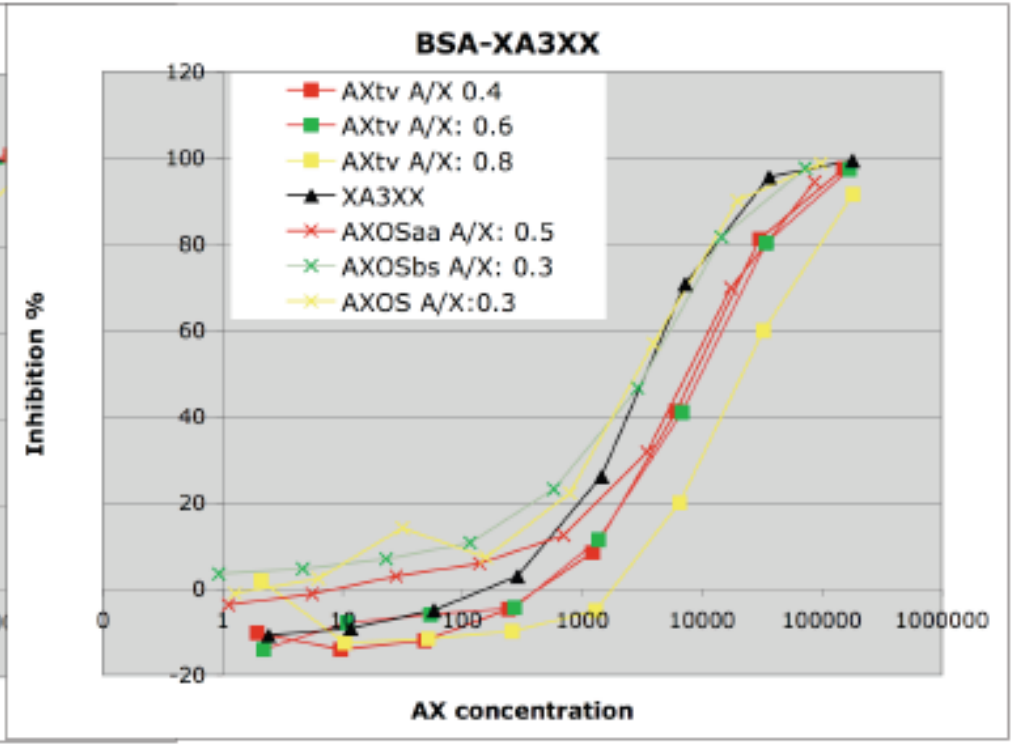


Principle of the assay on flour or grain



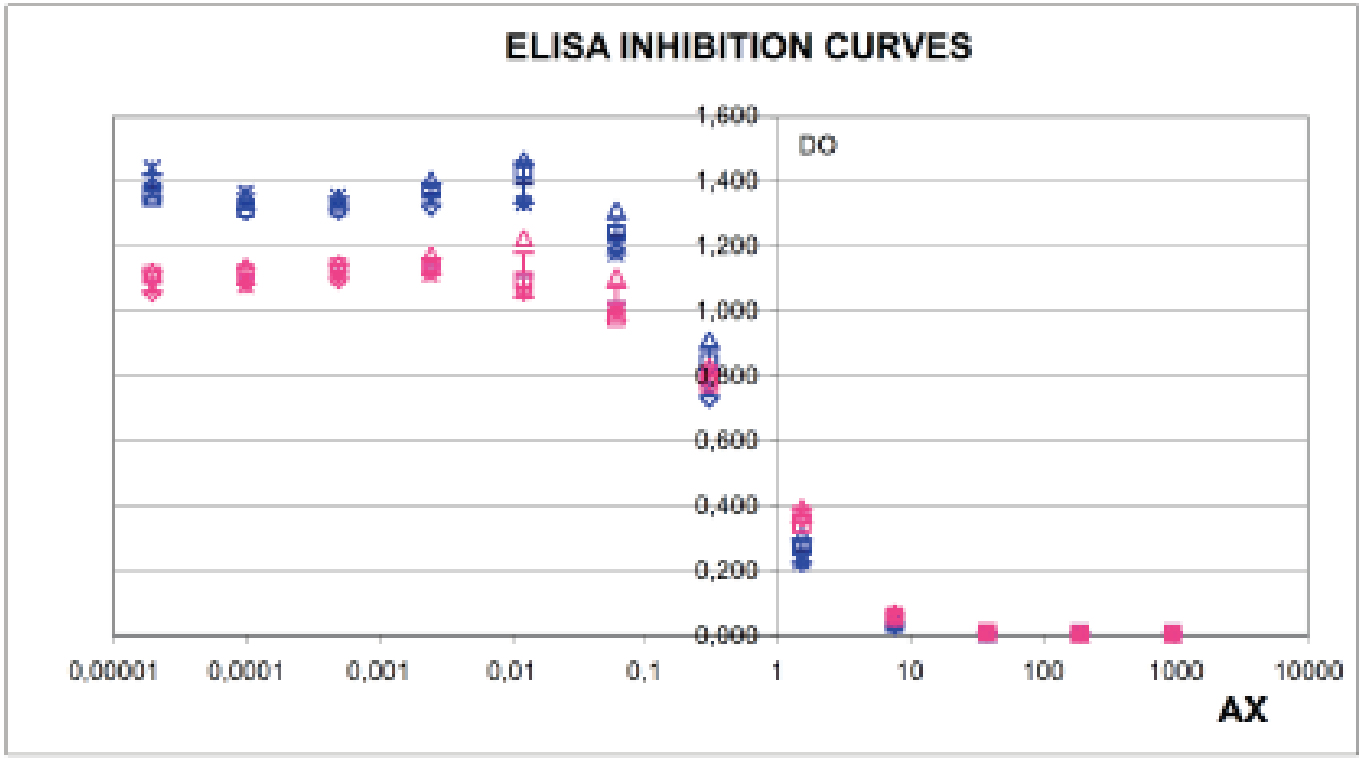
Competitive ELISA for purified AXOs and AX





AX from wheat flour with different A/X ratio (0.4 – 0.6 - 0.8) were obtained from INRA collection.
AXOS were generated using a GH11 endoxylanase from *Trichoderma viride*.

AXOS obtained with *Aspergillus aculeatus* (GH10) and *Bacillus subtilis* (GH11) endoxylanases were provided by KUL.



Precision of ELISA too low/ range of variation of AX

- ✓ Partly Linked to AX1 antibody

Making groups "high/low content" may be possible if reproducibility improved

- ✓ Need automation (robot) or turn to sandwich format

Conclusions

- Ab technology has real potential to offer an alternative means for quantifying AX and AXOs in grains and foods/ingredients
- Assay formats require further refinement and standardisation to improve assay reproducibility
- Market research assessment required to help develop Knowledge-exchange; potential for this to be undertaken with SME diagnostic company

HEALTH·GRAIN

Thank You!

To all WP2.4 partners and other Module contributors

- Luc Saulnier, Olivier Tranquet, Rachelle Looten, Gille Charmet, INRA
- Craig Faulds, Ana Sancho (IFR),
- Kurt Gebruers (Leuvan)
- András Salgó (BUTE)
- Szilveszter Gergely, András Salgó (BUTE)
- Mariann Rakszegi, Zoltan Bedo (Martonvásár)
- Peter Shewry (RRes)