Proteomic and peptidomic tools combined with immuno-chemistry to analyze in vitro gastrointestinal digestibility of bread wheat

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Developping a strategy highlighting the links between wheat cultivars and the digestibility of gluten proteins in bread : combining proteomics, peptidomics and immunochemistry approaches

Wheat grain storage proteins consist in **gliadins and glutenins.** These proteins form the gluten, a network with remarkable cohesiveness and viscoelasticity properties.

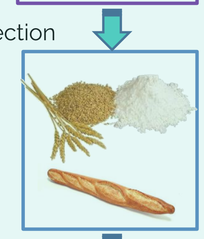
High molecular weight glutenins are able to form very large macropolymers driving **dough elasticity and tenacity**, while gliadins contribute to its viscous properties responsible for the dough extensibility. They are key determinants of the wheat end-use quality.

Resistance of gluten to gastrointestinal digestion is involved in adverse reactions to wheat.

Phenotyping

sélection

Processing

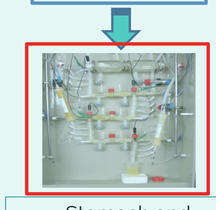


A set of sixteen cultivars illustrating the bread wheat (*Triticum aestivum*) diversity, including old and modern cultivars.

A standardized process, for baking. Each bread produced using flour from a single cultivar.

Finding cultivars with gluten proteins easier to proteolyze offers the possibility to use them as varieties, which may decrease adverse reactions.

***In vitro* digestion**



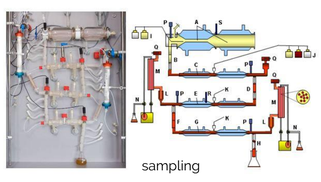
Stomach and Jejunum contents after 2-h digestion

The dynamic *in vitro* TIM-1 system (TNO Gastro-Intestinal Model) simulating the human digestion in the stomach and the small intestine was used to digest each bread.

Proteomics & Peptidomics

Peptides Extraction

The multi-compartmental Dynamic TIM-1 model of the gastrointestinal system



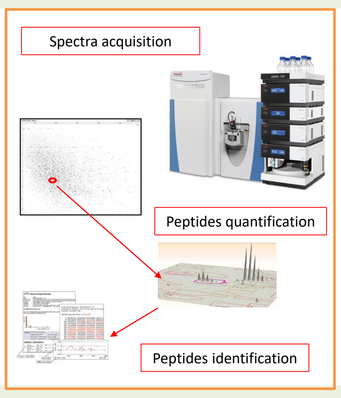
sampling

Peptides extraction

Precipitation — Solid phase sorbent

Mass Spectrometry Analysis

Spectra acquisition

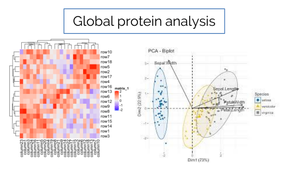


Peptides quantification

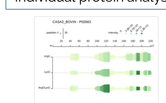
Peptides identification

Bioinformatics Analysis

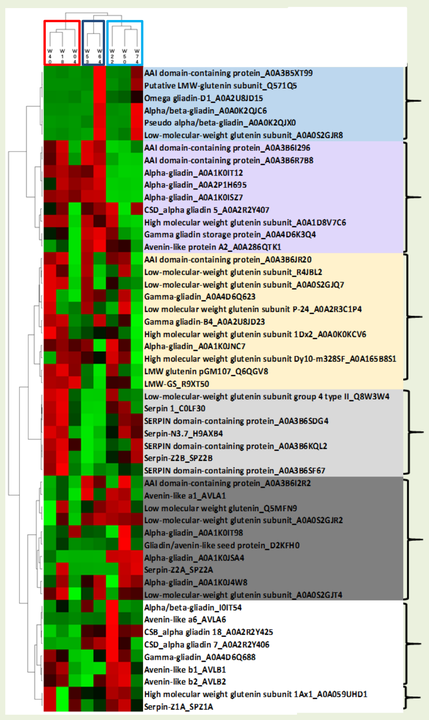
Global protein analysis



Individual protein analysis



Individual peptides analysis



473 unique peptides originating from **106 proteins** evidenced (52 associated to a family)

3 clusters for cultivars :

W53-W64
W22- W50-W74
and **W04-W18-W40**
7 clusters for proteins

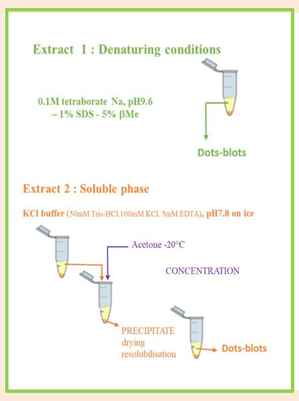
Gluten proteins (glutenins and **gliadins)** were identified as well as some **α-amylase inhibitors** (ATIs) or **serpins proteins**

Heatmap of **proteins hydrolyzed in gastric compartment after 2h**. A hierarchical clustering was performed to classify **cultivars** (column) and **proteins** (Line).The data were reduced for a double classification using Ward's method and Pearson distance.

High protein proteolysis
Low protein proteolysis

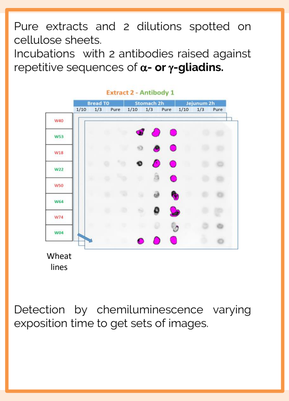
Immunochemistry

Proteins/Peptides Extraction



Dot Blot

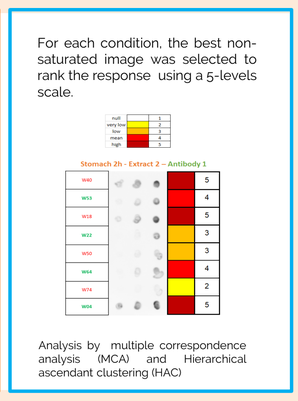
Pure extracts and 2 dilutions spotted on cellulose sheets.
Incubations with 2 antibodies raised against repetitive sequences of **α- or γ-gliadins.**



Detection by chemiluminescence varying exposition time to get sets of images.

To Semi Quantitative Variables

For each condition, the best non-saturated image was selected to rank the response using a 5-levels scale.

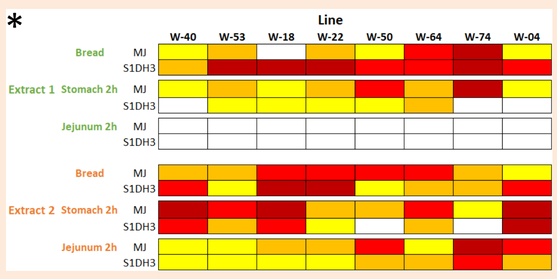


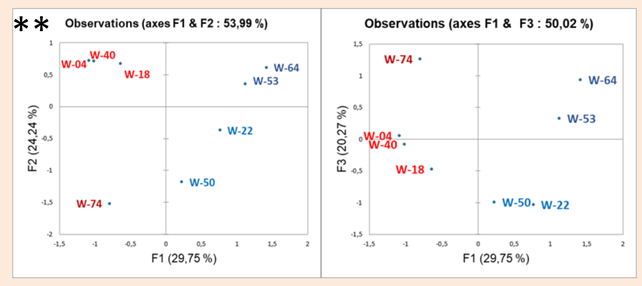
Analysis by multiple correspondence analysis (MCA) and Hierarchical ascendant clustering (HAC)

Evaluation of **gluten proteins proteolysis** by dot blot approach and immunochemistry using gluten-specific antibodies on a lines subset

Results with the two antibodies targeting gluten proteins (polyclonal MJ and monoclonal S1 DH3)

Using **global approach (*)** including results obtained for bread, stomach and jejunum contents or highlighting on **stomach content(**)** we show that lines differ in the course of proteolysis of gluten proteins.

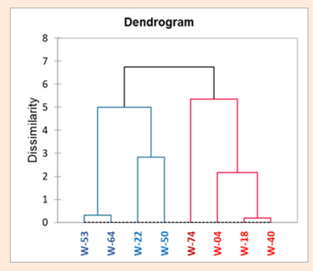




MCA plot : the three first dimensions capture 74 % of the variability

MCA separates cultivars in **3 clusters** :

W53-W64
W22- W50
and **W04-W18-W40** to which cultivar**W74**, the most different one, finally aggregates.



HAC on principal coordinates in MCA

Combining immunochemistry and peptidomic approaches

Combined Analysis

Peptidomics/proteomics:

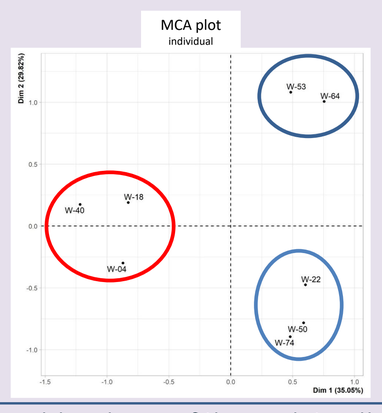
1. **Reduction of the dimensionality by** hierarchical cluster analysis
HCA analysis permitted to select **five clusters**

2. **Creation of latent variables** to synthetize the protein information
The five selected clusters are assimilated to latent variables **alpha/LMW_Clu1**, **AlphaGliad_AAI_Clu2**, **LMW_alpha_Clu3**, **Serpin_Clu4** and **LMW_Clu5**

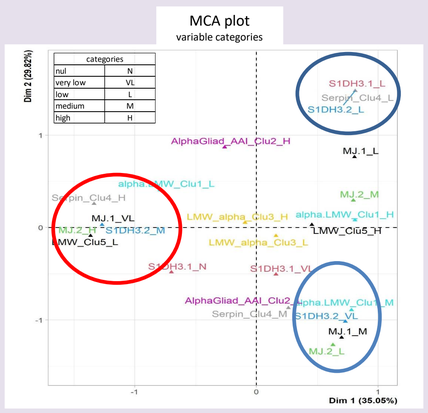
3. **Ranking** using K means on the latent variables
Using ranking, an intensity level to each latent variable was attributed to the cultivars: **Low, Medium or High**

Immunochemistry:
From results four latent variables were used named according the extract buffer and antibody used.
MJ-1, **S1DH3-1**, **MJ-2**, **and S1DH3-2**

→ **Multiple Correspondence Analysis** to associate latent variables (peptidomic/immunologic) to cultivars



The combined use of these data allows a grouping of cultivars into **3 distinct groups** :
W53 – W64
W22 – W50 – W74
W04 – W18 – W40



MCA graph shows **the associations between immunological and peptidomic variables.**

The intensity levels of the variables can be associated with the cultivars.

Examples:
Association of **low serpin** level to **low reactivity of Ac S1DH3-1.**
➢ Levels of these variables were associated to the cultivars **W53 – W64**
Association of **high serpin** level to **high reactivity of Ac MJ-2.**
➢ Levels of these variables were associated to the cultivars **W04–W18–W40**

In progress : link to digestibility index.

Such **quantitative and qualitative differences** between cultivars could help to identify varieties with an improved proteolysis of gluten proteins after two hours of digestion. **Peptidomic approach** appears to be helpful for characterizing how protein profiles change during proteolysis. **Wheat cultivars could be compared. Combined analysis is efficient** to integrate different types of data in order to extract/valid information.

Perspectives

Among other quantified proteins, **α-amylase/trypsin inhibitors (ATI)** involved in baker's asthma and recently proposed to play a role in Non Celiac Wheat Sensitivity (NCWS) will be studied.
The peptide list obtained will be screened for *in silico* **toxicity/immunogenicity risk assessment**, with the aid of bioinformatics tools for epitopes matching in order to tackle implications for celiac disease or wheat allergy.

References
Duval et al., Foods 2020, 9, 1499; doi:10.3390/foods9101499
Sayd et al. Food Chemistry 2016, 197, 1311: doi.org/10.1016/j.foodchem.2015.11.020 0308-8146

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