TOWARDS A DIAGNOSIS OF NON-CELIAC GLUTEN SENSITIVITY: the contribution of metabolomics for monitoring metabolites produced by in vitro digestates of bread

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the contribution of metabolomics for monitoring metabolites produced by *in vitro* digestates of bread

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INTRODUCTION

- Over the past decade, the non-celiac wheat sensitivity (NCWS) is more and more self-diagnosed, which makes the gluten-free diet more frequent, without objective clinical criteria.
- NCWS is poorly understood and challenging to diagnose in contrast to celiac disease because of a lack of clinical indicators.
- One objective in this study was to monitor metabolites produced by *in vitro* digestates using an untargeted metabolomics approach.

  ➡ Finding biomarkers associated with this phenotype is critical for an accurate diagnosis and innovative patient management.

PREPARATION OF THE STOMACH AND INTESTINAL DIGESTATES

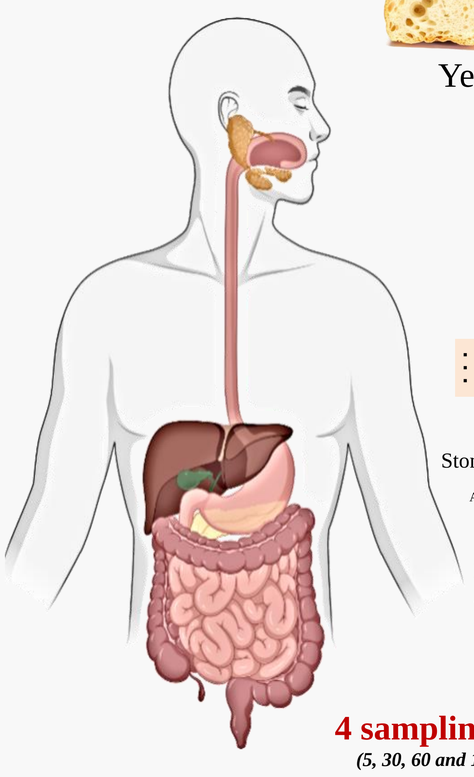
- A recent approach with *in vitro* investigation was applied to study the overall digestive process of different breads, combining tools from the oral step thanks to the AM2 masticator apparatus, until the end of digestion thanks to a dynamic digester (DIDGI©) mimicking the physiology of the adult gastrointestinal tract "GIT".

4 varieties

Yeast

Sourdough

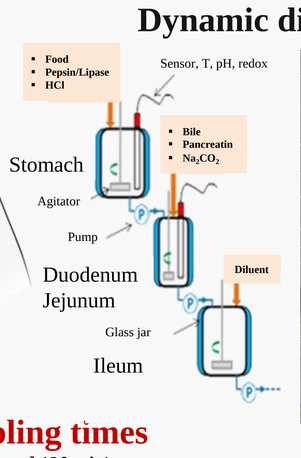
2 processes



AM2 masticator

Scan me

Dynamic digester (DIDGI©)



4 sampling times

(5, 30, 60 and 120 min)

METABOLOMIC ANALYSIS

Analysis **LC-MS**

Acquisition

ESI+ **ESI-**

LC

UHPLC: Thermo Scientific U3000

Chromatographic conditions: Reverse phase

- Pre-filter column Waters
- Column: HSS T3 150 x 2.1mm x 1.8µm Waters
- Column temp: 30 °C

➢ Mobile phase: Polar

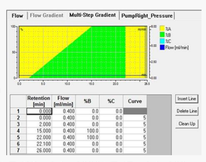
- Mobile phases: A: H2O + 0.1% formic acid

  B: ACN + 0.1 % formic acid

➢ Injection vol: 5µL

➢ Gradient

- Run time: 26 min
- Flow rate: 0.4 mL/min
- Step gradient:



- **0% B** (2 min), **0-100% B** (13 min), **100% B** (7 min), **100-0% B** (0.1 min), **0% B** (3.9 min)

MS

High Resolution QTOF, Bruker Impact II

➢ Source :

- ESI mode: positive (capillary 2500 V, end plate offset: 500 V) and negative (capillary 1500 V, end plate offset: 500 V)
- Nebulisation gaz flow as: 10 L/min
- Nebulizer: 40 psi
- Dry temp: 200°C

- Scan range: 50 to 1000 mass-to-charge ratio (m/z)
- The mass resolving of the mass spectrometer was 50,000 full width at half maximum (FWHM) at m/z 1,222.

Pre-processing

1895 ions | 644 ions

Normalisation

1619 ions | 642 ions

Quality control

1553 ions | 572 ions

Analytical redondance

1146 ions | 197 ions

4 Wm Workflow4metabolomics

SIMCA

Multivariate

PCA **PLS-DA** **OPLS-DA**

Annotation

ESI+ 43 30 109 88 680

In-house Bank HMDB Sirius

Univariate ➡ **Selection** ➡ **MS level**

RESULTS

Untargeted metabolomic analysis (ESI+) of gastric bread digests

➢ **Effect of the digestion time:**
Results reveal that metabolic profiles of gastric digestates allow discriminating the sampling time. Hence the effectiveness of our in vitro digestion system is shown.

Sampling Time

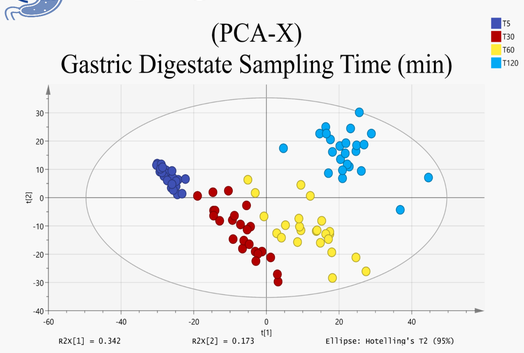
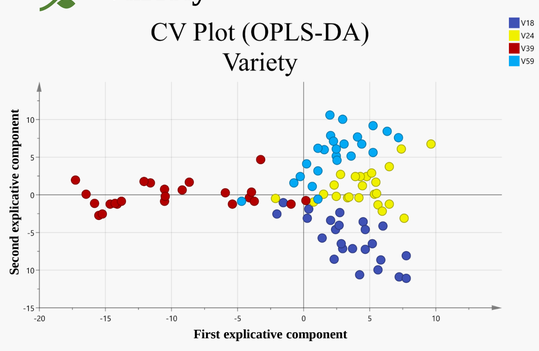


Fig.1 *Principal component analysis (PCA) of the metabolite - related features identified in gastric digestate taken after 5, 30, 60 and 120 min).*

➢ **OPLS-DA analyses can clearly discriminate between:**
the wheat varieties used, particularly the V39 variety, and the two processes (Yeast and Sourdough). This shows the influence of bread type on digestion and therefore on the production of metabolites.

Variety



Process

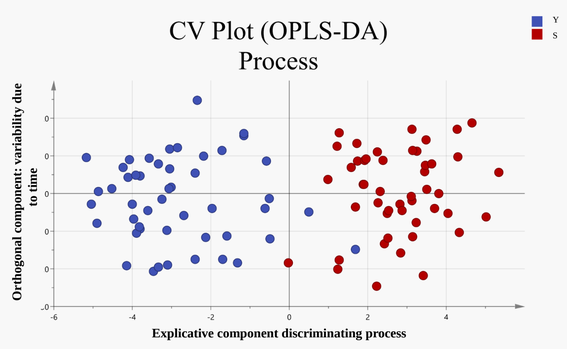
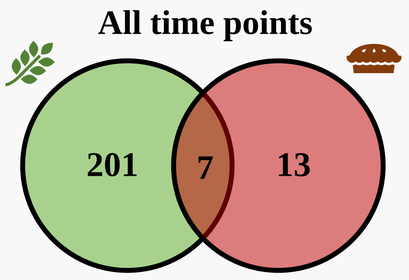


Fig.2 *Cross-validated scores plot of the OPLS-DA model built to discriminate between the four varieties (v18, v24, v39 and v59) and the two processes (Y: Yeast and S: Sourdough).*

➢ **Univariate statistical analysis whole dataset as well as per time point:**
Significantly different metabolites were identified depending on the variety and the process.

All time points



Per time point

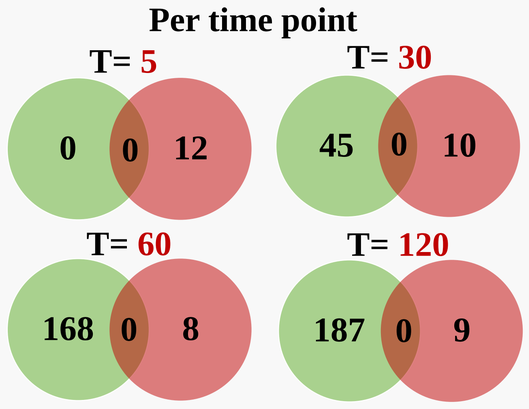


Fig.3 *Venn diagram depicting the number of unique and shared metabolites – related features observed in the gastric digestate. In the green/red circles the number indicates metabolites witch are differentially presented between varieties/processes. The metabolites were found modulated following univariate statistical analysis (P-value <0.05).*

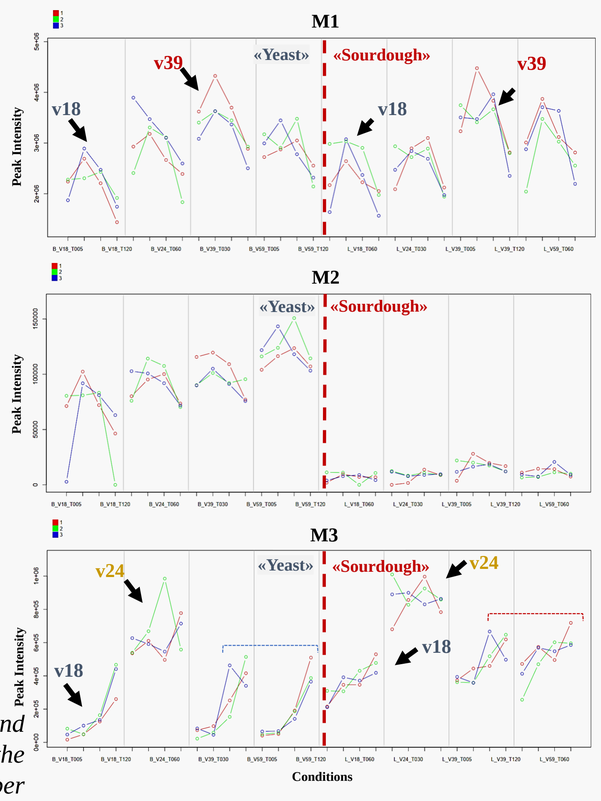


Fig 4. *Examples of metabolites (M1, M2 and M3) that significantly modulate three biological replicates denoted by the numbers 1, 2 and 3 colored red, green and blue respectively.*

➢ **Shared significant metabolites are present across varieties and processes, although no overlap could be highlighted when observing data time by time.**

CONCLUSION AND PERSPECTIVES

These first results show modulations in certain metabolites identified according to the type of bread digested. This reveals the impact of type of bread on the digestibility and allows us to emphasize the contribution of metabolomic approach for monitoring the metabolites produced by *in vitro* digestates.

The upcoming metabolomic analysis of intestinal digestates can help us understand the evolution and emergence of metabolites, and the biological interpretation of significant metabolites can contribute to identify metabolic pathways through *in vivo* experiments or clinical studies.

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METABOHUB Plate-Forme EXPLORATION du METABOLISME des gènes aux métabolites

METABOHUB
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