Metabolomics a useful tool to study the quality of fermented foods

Andrea Gianotti

Department of Agricultural and Food Science and Technology (DISTAL), Interdepartmental Centre for Agri-Food Industrial Research

Alma Mater Studiorum University of Bologna, Bologna Italy

andrea.gianotti@unibo.it
**Foodomics and Metabolomics**

**Foodomics**
New discipline that studies the Food and Nutrition domains through the application of advanced omics technologies (Cifuentes, 2009).

**Metabolomics**
An emerging field within “omics” sciences that is also known as metabolome analysis, metabonomics (Nicholson, Lindon, & Holmes, 1999) or metabolic profiling (Niwa, 1986) dealing with the simultaneous determination and quantitative analysis of intracellular metabolites, which have been defined as low-molecular-mass compounds (<1500 Da) that are produced and modified by the metabolism of living organisms.

**Metabolites**
Endogenous and exogenous small molecules such as peptides, amino acids, nucleic acids, carbohydrates, organic acids, vitamins, polyphenols, alkaloids and minerals.

**Application in Food Science**
To monitor the quality, processing, safety, and microbiology of both raw materials and final products to improve the consumer's health and confidence (Cevallos-Cevallos, Reyes-De-Corcuera, Etxeberria, Danyluk, & Rodrick, 2009)

Metabolic footprinting or exometabolomics focuses on what the cell excretes under defined conditions and intends to define a pattern of extracellular metabolites.
Metabolomics in Fermented Foods

Metabolomics may be applied to study the metabolite profile in fermented foods to identify and measure their biochemical profiling and metabolite modifications during fermentation.

Possibility to predict, among others, the sensory and nutritional quality of the final fermented product.

Metabolomic data analysis generally integrate information collected through many different separation and detection techniques (HPLC, UPLC, GC, MS and NMR):

- identification and quantification, generally classified as targeted (specific), focused on a specific group of metabolites that require the identification and quantification within the group.

- untargeted (nonselective or integral) or comprehensive metabolomics focuses on the detection of as many groups of metabolites as possible to get patterns or fingerprints without identifying or quantifying a specific compound.

Metabolomics have been successfully used in some food science to evaluate the molecular fingerprints of fermented foods, such as soy foods, cheeses, and wines. (Mozzi et al., 2013) and consequently to improve the consumer's health and confidence (Cevallos-Cevallos et al., 2009).
Exploitation of Metabolomics approach

- In fermented foods metabolomics was applied to record metabolite modifications during fermentation and, possibly, to predict, the sensory, nutritional and healthy quality of the fermented final product (Mozzi, Ortiz, Bleckwedel, De Vuyst, & Pescuma, 2013).

- The kinetics of physicochemical parameters, microbiota population and their metabolites using NMR in cheese and probiotic yoghurt were investigated (Piras et al. 2013; Settachaimongkon, 2015).

- Metabolomics approach was also applied to study volatile metabolites in fermented bakery products in order to understand the role of flour and fermentation process in increasing rheological and volatile metabolites (Balestra et al., 2015; Makhoul et al., 2015).

- Quality shift in crab paste fermentation (Chen, Ye, Chen, & Yan, 2016).

- Pogacic et al. (2015) adopted this approach to screen various LAB strains cheese-related bacteria for their ability to produce aroma compounds.

- To increase the diversity of aroma properties by non commercial strains (Alves et al., 2015).
Aim

To use a metabolomic approach to exploit the effects of formulation and process on the metabolic shift in fermented foods.

5 examples are overviewed:

• Increase wine diversity;
• potential of non starter cheese related bacteria;
• chemically acidified fermented dough mimicking sourdough;
• sourdough bread;
• *L. plantarum* strains selection to improve healthy and sensorial properties.
Metabolic diversity of wine microflora aroma properties
(Alves, et al. (2015). PloS one, 10(11), e0143641.)

- Saccharomyces cerevisiae isolated strains BT2453 and BT2652
- Sacch. cer. commercial wine yeast strains CSc1 and CSc2

Minimal grow medium

Isolated vs commercial variability was observed by Heat maps.
PCA accounted mainly to esters and terpenic compounds (C10 and C15), metabolites of particular relevance to wine aroma,
BT2453, produced the higher terpenic content.

Metabolomics to increase peculiarity of wine
Analysis & Metabolomic Processing of GC-MS data

- After the GC-MS analysis the raw spectra has been used for the data processing on XCMS package (an R package for the processing of mass spectrometry data).

- The XCMS report automatically generates extracted ion chromatograms for each metabolites. The molecules are identified by comparing the characteristic ion of each mass spectra with those of pure compounds contained in the NIST library.

- Identification the ANOVA analysis on the whole data set and only the metabolites statistically significant is chosen for the multivariate analysis (PCA or CAP).
Volatile compounds in cheese-related bacteria

(Pogacic et al. / Food Microbiology 46 (2015) 145-153)

PCA of volatiles abundance in cultures of cheese-related bacteria: score plot of the first two components

PCA differentiated species by their ability to produce ethyl esters (Brachybacteria), sulfur compounds and branched-chain alcohols (H. alvei), branched-chain acids (H. alvei, P. freudenreichii and L. paracasei), diacetyl and related carbonyl compounds (M. gubbeenense and L. paracasei)

Metabolomics to screen the potential of non-starter strains
Metabolomics to study the effect of formulation and process of bakery products: a) acidified fermented dough

<table>
<thead>
<tr>
<th></th>
<th>Flour (g)</th>
<th>Deionised Water (g)</th>
<th>Salt (g)</th>
<th>Compressed Yeast (g)</th>
<th>Lactic Acid (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kamut® (K)</strong> Durum wheat (W)</td>
<td><strong>Control (C)</strong></td>
<td>500</td>
<td>352.5</td>
<td>314</td>
<td>10</td>
</tr>
<tr>
<td><strong>Acidified (A)</strong></td>
<td>500</td>
<td>349.5</td>
<td>311</td>
<td>10</td>
<td>7.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>KAMUT CONTROL</th>
<th>DURUM WHEAT CONTROL</th>
<th>KAMUT ACIDIFIED</th>
<th>DURUM WHEAT ACIDIFIED</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0h</strong></td>
<td>KC0</td>
<td>WC0</td>
<td>KA0</td>
<td>WA0</td>
</tr>
<tr>
<td><strong>1h30min</strong></td>
<td>KC1</td>
<td>WC1</td>
<td>KA1</td>
<td>WA1</td>
</tr>
<tr>
<td><strong>6h</strong></td>
<td>KC2</td>
<td>WC2</td>
<td>KA2</td>
<td>WA2</td>
</tr>
</tbody>
</table>
**Acidified dough:** Electronic nose (PCA plots and loading plots)

Good separation of acidified samples of K dough:

Effective arrays: 
- **S 7** (sulfur comp.),
- **S 9** (sulfur organic comp.),
- **S 10** (methane-aliphatic).

In fermented products W and K doughs are separated mainly due to sensor 6 and 8 (broad methane and broad alcohol respectively).

Ferm. time influenced enose results especially for W

Acidification may reduce the powerful of flour separation.
Acidified dough: GC-MS/SPME

Separation between the WA and KA clusters (F1), not in C (not acid.) but there is an overlapping between them depends on non acidified fermented samples.

The load coeff. of significant volatile compounds showed that 1-octen-3-ol, 2-methyl-propanol, 1-hexanol have the highest influence in the current pattern file.

The potential ability of SPME to discriminate in K matrix the two fermented type accounted for specific volatile compounds.

The chemical acidification induced an increase of detection aptitude that allow a better discrimination between groups.
Metabolomics to study the effect of formulation and process of bakery products: a) sourdough bread

Genetic variables
Kamut (K)
Durum wheat (W)

Agronomic variables
- Milky maturation (MM)
- Full ripe maturation (RM)

Fermentation
- Yeast fermentation (YF)
- Sourdough fermentation (SF)

Baking
- Hard thermal cycle (HT)
- Light thermal cycle (LT)

C: Yeast fermentation (1h 30’)
- *Saccharomyces cerevisiae* LBS

S: Sourdough (24 hours)
- *Lb. brevis* 3BHI
- *Lb. plantarum* 98A
- *Lb. sanfranciscensis*

M: Milky stage maturation
R: Fully ripe stage maturation

HT: High temperature (250°, 20’)
LT: Low temperature (210°, 10’)
Sourdough: Electronic nose

LEGEND:
- CLUSTER 1: RK3, RK1
- CLUSTER 2: MK4, MK2, MW1, MW3, MW2, MW4
- CLUSTER 3: RW1, RW3, RW2, RW4
- CLUSTER 4: MK3, MK1, RK4, RK2

(1-2 and 3-4 beginning and end of industrial and sourdough fermentation respectively)
Sourdough bread: GC-MS/SPME

Fig. 5. CAP loading plot of industrial fermentation vs. sourdough breads using SPME-GC-MS.
Fig. 2 CAP loadings plot of KAMUT vs. durum wheat dough samples using SPME-GC-MS
**L. plantarum** strain selection by combination of antioxidant and volatile compounds profiles: heat maps to visualize hierarchical clustering

**W**: durum wheat

W fermentation resulted to provide a high metabolic strain diversity according to specific cluster correlations:

- **WA**: 10 alcohols, 5 carbonyls, 3 acids and 3 hydrocarbons are highly correlated to EGC, EGCG, Flav and total Polyp (pred.?)

- **WB**: 2 alcohols, 4 carbonyls, 5 acids and 8 hydrocarbons highly correlated to GA), Fer ac and ABTS

- **WC**: 2-methyl-2-buten-ol, 8 hydrocarbons and one n.i. ester, correlated to Flav, GA, Fer ac, Van ac, CAT, Prot and ABTS
Antioxidant and volatile profile correlation in KAMUT wheat

- **KA**: Polyp, Flav, EC, Prot ac, Fer ac and EGC with 7-methyl-4-octanol, 3 carbonyls, octanoic and heptanoic acids and 2 hydrocarbons.

- **KB**: 5 alcohols, 6 carbonyls, dodecanoic acid and 1,3-hexadiene and GA, EGCG, EGC, Flav, Prot ac and Polyp.

- **KC**: Phenethyl alcohol, 1 ester (not identified), 2-nonanone, 4 acids and 8 hydrocarbons highly correlated to CAT, Fer ac, Van ac and ABTS.

K fermentation resulted to provide a certain metabolic strain diversity according to specific cluster correlations.
**L. plantarum** ability to produce antioxidant compounds

- Control 0 h and Control 16 h higher content of Fer ac
- 133 high correlation with between EGC content
- CAT highly correlated to all the other strains.

- highest correlation controls 0 h and 16 h) and EGCG.
- strains 133 and 6BHI were separated due to the negative correlation with EC
Durum wheat flour fermentation resulted to provide a higher metabolic diversity according to strain specificity.

KAMUT® khorasan wheat seemed to play itself a very important role in metabolic traits of fermented dough probably due to the higher qualitative profile of flour compounds.

*L. plantarum* fermentation was significantly effective on sensorial and healthy compounds in both the considered flours.

According to the clustering of correlations, wheat flour fermented by 98A, and 6BHI strains provided to good sensorial and excellent healthy characteristics.

On the other hand, strain 94A and 206 seemed to collect the best sensorial and healthy performances in KAMUT® khorasan wheat dough.

---

Metabolomics to select *L. plantarum* strains to increase sensorial and healthy properties
Impact of Kamut® Khorasan on gut microbiota and metabolome in healthy volunteers

Taneyo Saa et al., 2014 FRI

- 30 adults aged between 25 to 53 years
- 3 month balanced but free diet
- 2 source of cereal-based foods (baked goods and pasta): a) durum wheat, b) Kamut® Khorasan

Network clustering correlated taxa ○ and metabolites urine △ and faeces ◇ in co-abundance groups (CAGs).

Kamut® Khorasan
Less pronounced effect of reduction in Bacteroides/Prevotella and an increase in Clostridium cluster XIVa (butyrate producers) after Kamut® Khorasan intake compared to whole wheat

- Higher health-promoting SCFA-producing members of the gut microbiota in Kamut® Khorasan-based diet group
**Conclusions**

Fermentation processes are a complex system of interactive changes. Metabolite pattern (fermented dough) resulted most suitable than substrate (flour) to assess and predict the potential diversity of flour itself.

E-nose discriminated the presence of different type of flours in order to control and monitor the bread making process. Moreover, most of the volatile compounds including SCFA, identified by SPME-GC-MS on the sourdough were mainly from the Kamut® khorasan bread.

Metabolomics may represent: i) a tool to describe the effects of ecological modifications; ii) an innovative strategy to explain biodiversity of fermented foods; iii) a valuable tool to optimize formulation and fermentation process.
Acknowledgments

Special thanks to the colleagues contributing to this work:
Danielle Taneyo Saa, Annalisa Tassoni, Maura Ferri, Diana I. Serrazanetti, Luca Laghi, Federica Balestra

Companies partially funding:
KAMUT ® Enterprises of Europe

New!!!

FoodMicrobiOomics

Thank you!!!

andrea.gianotti@unibo.it