Bridging significant gaps?

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Beer quality
Chemical, physical, and organoleptic properties of a product, that aim to be consistent

Quality control and management
All measures carried out to assure product quality, mainly driven by diverse analytical tools

(Significant) gaps in quality control?
Beer is a complex mixture of 1000s of substances covering a wide range of molecular weight, polarity, concentration. Understanding variations of beer quality on molecular basis is an ongoing challenge of brewing science.
OMICS

- collective characterization and quantification of pools of biological molecules responsible for the structure, function, and dynamics of cells/organisms/biological systems

- genomics
- proteomics
- metabolomics
METABOLOMICS

- analysis of organic molecules detectable in an organism / plant / cell / sample with a molecular weight typically < 1500 Da

physiological and environmental influence
webofknowledge.com, Search "metabolomics" + "food metabolomics"
use metabolite profiles to distinguish between samples

1) instrumental analysis

2) data collection

3) data reduction and statistics

4) identification
2) data collection

use metabolite profiles to distinguish between samples

3) data reduction and statistics

4) identification

select significant and metabolites as markers for targeted approaches (optional)
# beer metabolomics

<table>
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<tr>
<th>Autor</th>
<th>Year</th>
<th>Analytics</th>
<th>Statistics</th>
<th>Findings</th>
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<tr>
<td>Duarte et al.</td>
<td>2002</td>
<td>$^1$H NMR</td>
<td>PCA</td>
<td><strong>Distinction</strong> between lager and ale</td>
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<tr>
<td>Duarte et al.</td>
<td>2004</td>
<td>FTIR-ATR und $^1$H NMR</td>
<td>PCA</td>
<td><strong>Distinction</strong> between lager, ale, and non alcoholic beer</td>
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<tr>
<td>Nord et al.</td>
<td>2004</td>
<td>$^1$H NMR</td>
<td>PLS</td>
<td>Identification and quantification of various metabolites in beer</td>
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<tr>
<td>Lachenmeier et al.</td>
<td>2005</td>
<td>$^1$H NMR</td>
<td>PCA, PLS</td>
<td><strong>Distinction</strong> of malt types and brewing sites</td>
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<td>Almeida et al.</td>
<td>2006</td>
<td>$^1$H NMR</td>
<td>PCA</td>
<td>Distinction between brewing site and date</td>
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<tr>
<td>Khatib et al.</td>
<td>2006</td>
<td>1D und 2D $^1$H NMR</td>
<td>PCA</td>
<td>Distinction of pilsner style beers from different brands</td>
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<td>Pope et al.</td>
<td>2007</td>
<td>DIMS/GC-ToF</td>
<td>PCA/CVA</td>
<td><strong>Comparison</strong> of metabolic footprinting of different yeast strains</td>
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<td>Cajka et al.</td>
<td>2010</td>
<td>SPME-GC-ToF</td>
<td>PLS-DA, LDA, ANN-MLP</td>
<td><strong>Distinction</strong> of trappist beers vs. belgian beers via volatile fingerprints</td>
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<tr>
<td>Zhu et al.</td>
<td>2010</td>
<td>(ESI)-MS</td>
<td>PCA</td>
<td><strong>Distinction</strong> between pale pilsner, wheat/white beer, and lager</td>
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<tr>
<td>Heuberger et al.</td>
<td>2012</td>
<td>UPLC-ESI-QToF</td>
<td>PCA</td>
<td>Effects of storage temperature on non-volatile beer components</td>
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<tr>
<td>Inui et al.</td>
<td>2013</td>
<td>GC×GC-ToF</td>
<td>PCA</td>
<td>Changes in concentrations of hop aroma components during production</td>
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<tr>
<td>Andrés-Iglesias et al.</td>
<td>2014</td>
<td>UPLC-ESI-QToF</td>
<td>PCA</td>
<td><strong>Distinction</strong> between alcoholic/non alcoholic beer</td>
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<td>Heuberger et al.</td>
<td>2016</td>
<td>UPLC-ESI-QToF</td>
<td>PCA</td>
<td>Possible biological markers for rapid testing of beer stability</td>
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<tr>
<td>Spevacek et al.</td>
<td>2016</td>
<td>NMR</td>
<td>PCA, LMM</td>
<td><strong>Comparison</strong> of metabolic profile during brewing process; Comparison purine metabolism of late and dry hopped beers</td>
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<tr>
<td>Hughey et al.</td>
<td>2016</td>
<td>UPLC-ESI-QToF</td>
<td>PCA</td>
<td>Identification of differentially expressed compounds in single hop IPAs</td>
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</tbody>
</table>
Metabolomics project
beer metabolomics project

- the German “Federal Ministry for Economic Affairs and Energy” funds innovative, market oriented research carried out by networks of small/medium sized companies and research institutes

- targets improvement of competitiveness + economic growth
metabolic footprint

Are changes in the brewing process (here fermentation regime) properly reflected by a non-target metabolomic approach?
PCA discrimination

- based on metabolite data sets different fermentation regimes cluster
- rather high variance in biological replicates (pilot scale brewing?)
cluster heatmap

- lipids
- esters and alcohols

9°C
12°C
15°C
pairwise correlation heatmap
beer consistency

Which differences between (similar) beers can be identified by a global metabolite profiling approach?

1. consistency trial (4 breweries, same product, 3 months)

2. beer analysis by GC-MS, SPME-GC-MS, and LC-TOF-MS (targeted beer analysis, sensory analysis,…)

3. data analysis and visualization by PCA, identification of significant metabolites,…
sensory data

- Comparison of sensory data (5 Point scheme) applied to a single brand pilsner type beer from different production plants
metabolite data clustered in respect to production date (month), colored ellipses describe the 50% confidence interval of the normal distribution for each group in the two-dimensional space of the PCA
• metabolite data clustered in respect to production date (month) and brewing site (1-4), colored ellipses describe the 50% confidence interval of the normal distribution for each group in the two-dimensional space of the PCA
metabolite identification, annotation, ...

- bitter acids
- lipids
- amines
- carbohydrates
- acids
- ...
variable importance score

<table>
<thead>
<tr>
<th>metabolite</th>
<th>VIP Score</th>
<th>Classification</th>
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<tbody>
<tr>
<td>549.12711@1.5813</td>
<td>100.00</td>
<td>unknown</td>
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<tr>
<td>Glu-Ser-Val</td>
<td>81.54978</td>
<td>Peptide(tri-)</td>
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<tr>
<td>421.2424@8.9307</td>
<td>76.57584</td>
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<tr>
<td>Asp-His-His-His</td>
<td>75.63124</td>
<td>Peptide(tetra-)</td>
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<tr>
<td>485.1783@4.1378</td>
<td>72.55315</td>
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<tr>
<td>2-Hydroxyethanesulfonate</td>
<td>71.22231</td>
<td>Sulfonic Acids and Derivatives</td>
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<td>Pro-Pro-Ser</td>
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<tr>
<td>352.1742@6.9477</td>
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<tr>
<td>Asn-Asn-Asn-Asn-Asn</td>
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<td>Peptide(tetra-)</td>
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<tr>
<td>Asp-Val-Gly-Pro</td>
<td>67.09589</td>
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<tr>
<td>Dimethyl succinate</td>
<td>60.76791</td>
<td>Carboxylic Acids and Derivatives</td>
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<tr>
<td>5-Amino-6-ribitylamino uracil</td>
<td>60.64853</td>
<td>Monosaccharides</td>
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<td>Tyr-Lys-Arg-Tyr</td>
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<td>Peptide(tetra-)</td>
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<td>Arg-Met-Phe-Asp</td>
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<td>Peptide(tetra-)</td>
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<td>Methyl 2-aminobenzoate</td>
<td>55.70696</td>
<td>Benzoic Acid and Derivatives</td>
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<td>8-Hydroxyguanosine</td>
<td>54.64307</td>
<td>Purine Nucleosides and Analogues</td>
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<td>Gln-Trp-Gln-Gln</td>
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<td>Piperitoside</td>
<td>50.8607</td>
<td>Flavonoids</td>
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<td>Phe-Tyr-Phe-Lys-Ile</td>
<td>50.8044</td>
<td>Peptide(penta-)</td>
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<td>Phenylalanine</td>
<td>50.36089</td>
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<td>Met-Ala-His</td>
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<tr>
<td>Cys-Trp-Gly-Gly</td>
<td>48.88984</td>
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<td>Glycerylphosphorylethanolamine</td>
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<td>Gly-Pro-Trp</td>
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<tr>
<td>N6-(delta2-isopentenyl)-adenine</td>
<td>45.93661</td>
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<tr>
<td>Procyanidin B3</td>
<td>44.99882</td>
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- metabolites showing significant variations between the four breweries calculated by “random forest technique”
- aim: rank the importance of variables in a regression or classification problem
22 out of the 50 significant metabolites were peptides.

13 peptides out of these 22 metabolites are showing the trends similar to box plots shown above.

Peptides (and amino acids) were reported as significant metabolites in previous LC-MS based studies, direct correlation with beer quality are hard to draw.
8-hydroxyguanosine

- 8-Hydroxyguanosine is a nucleoside formed by guanosine oxidation
- Purines were reported to correlate with beer oxidation
- 5-MTA, Deoxyadenosine, and Guanine did not significantly contribute to differences between the breweries (fresh beer)
polyphenols

- Procyanidin B3 ((+)-catechin-(4α-8)-(+)catechin) has been reported in malt, hops, and beer

- physical beer stability, flavor stability…
cluster analysis
Odorants?
differences attributed to GC analysis?

• global metabolite profiling yielded 3255 compounds
• approx. 1000 metabolites were ranked with a VIP score > 0
• approx. 70 metabolites from HS-SPME-GC-MS ranked with a VIP score >0

- 157.0835@5.0885  (31,75)
- 204.058@1.1643  (31,75)
- [...]  
  ✓ ethyl pyruvate
  ✓ ethyl lactate
  ✓ isobutyl isobutyrate
  ✓ linalool
  ✓ linalool oxide
  ✓ isoamyl acetate
  ✓ phenylethylethanol
  ✓ [...]
targeted vs. untargeted GC analysis

- **small boxes**: minimal differences between samples from different brewing sites
- **large boxes**: differences between samples from different brewing sites
targeted vs. untargeted analysis

- normalized (semi-) quantitative data of both assays match (similar trends)

Assumption: targeted approach more accurate

- untargeted assay is not sensitive enough to detect differences between samples (high standard deviation of technical replicates)

- some compounds (e.g. beta-pinene) were below LOQ in targeted assay but reported by the untargeted
bridging significant gaps?

- metabolomics are indeed applicable to practical issues (comparative studies)

- LC-MS based technologies (will) surely help to understand the complex polar and non-volatile beer metabolome

- profiling of the volatile fraction is crucial (sensitivity), the quality of targeted GC-MS assays is high

- metabolomics complement the toolbox of brewing analytics / research

- compound identification remains challenging

- there is plenty of work ahead…
Thank you!

- Dr. N. Schauer
- Dr. O. Gräbner
- M. Heiser

- Dr. S. Thörner
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- L. Knoke
- C. Schubert
- Prof. L.-A. Garbe

- ZIM KF2132338