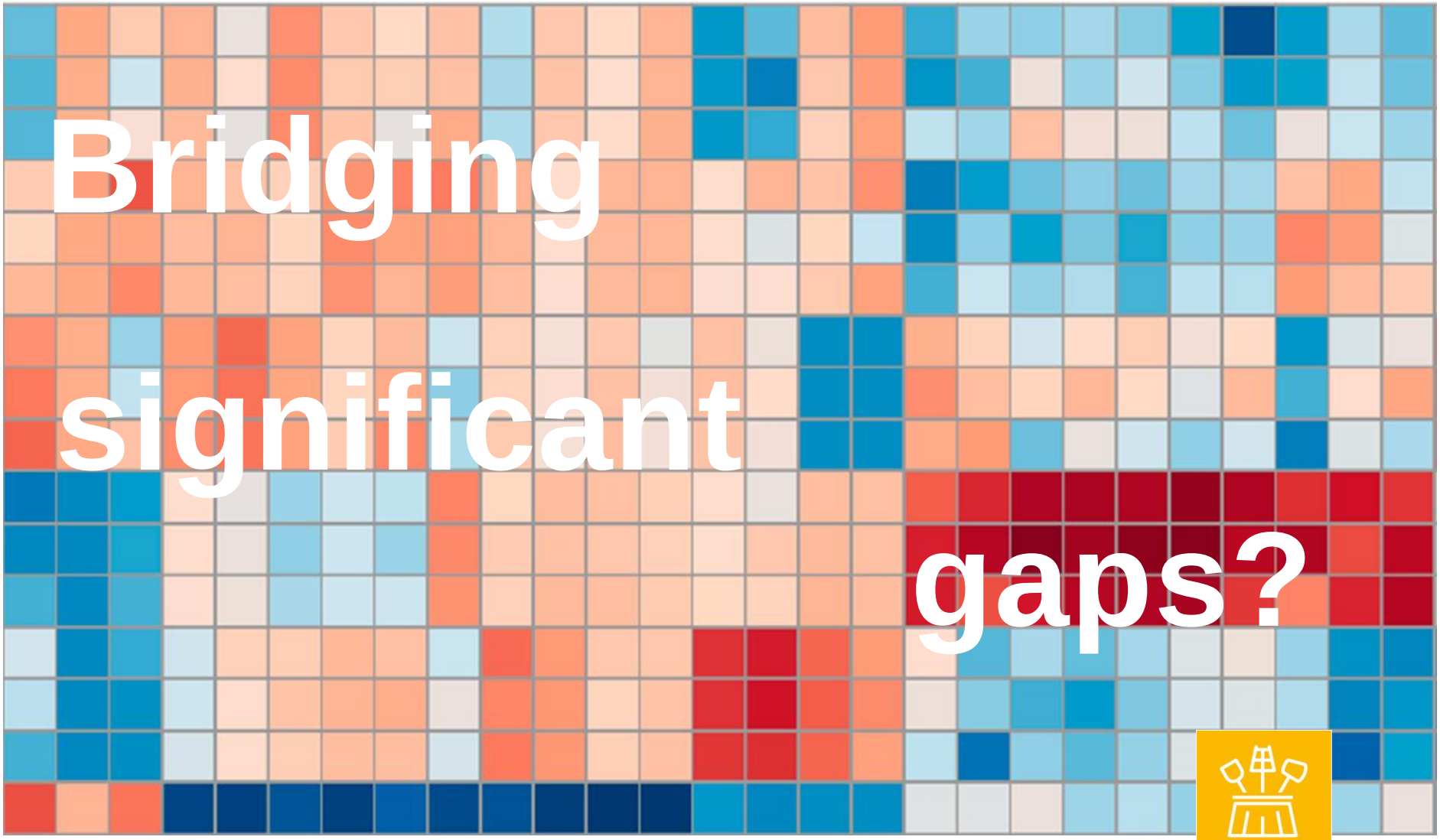


Dr. Nils Rettberg

Versuchs- und Lehranstalt für Brauerei in Berlin (VLB) e.V.

VLB BERLIN

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

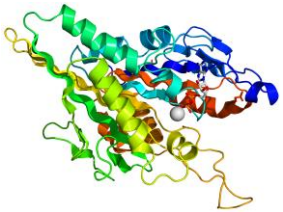
(Significant) gaps in quality control?

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.

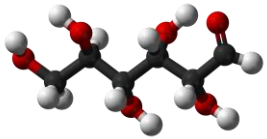
OMICS

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

genomics



proteomics

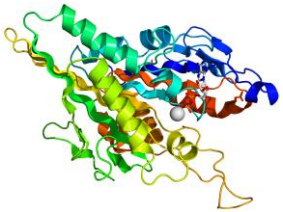


metabolomics

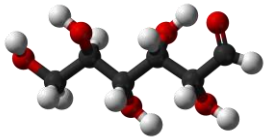
METABOLOMICS

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

genomics

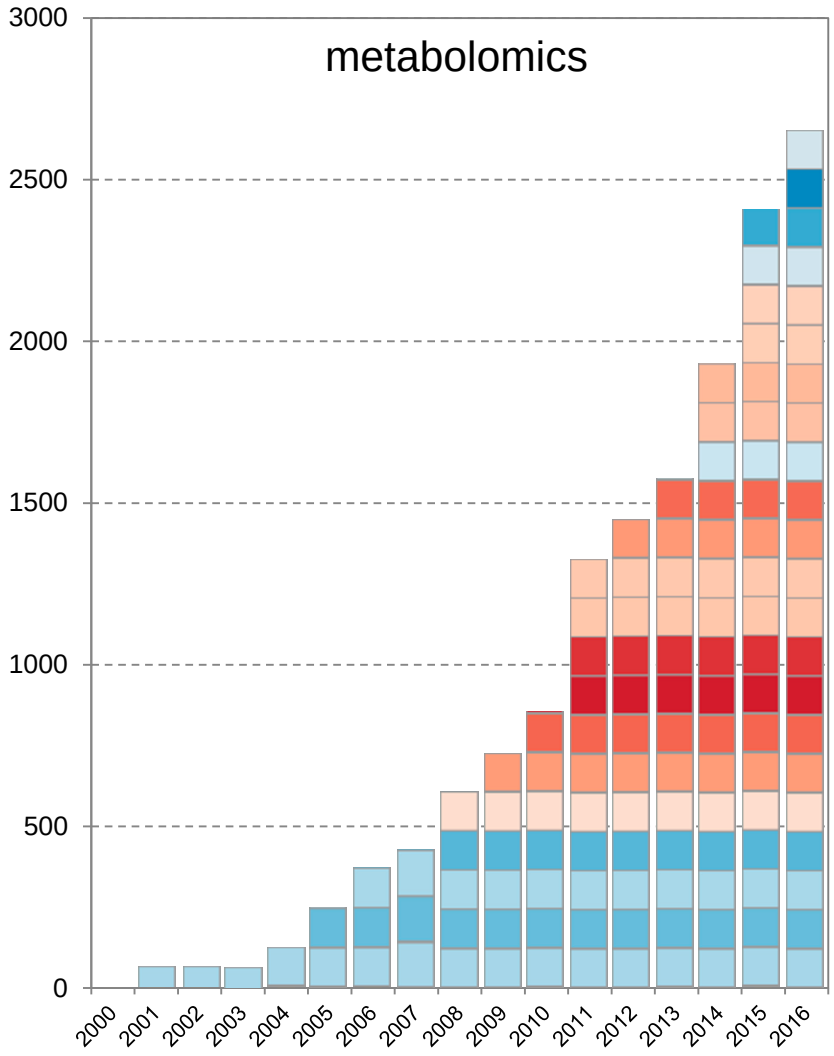


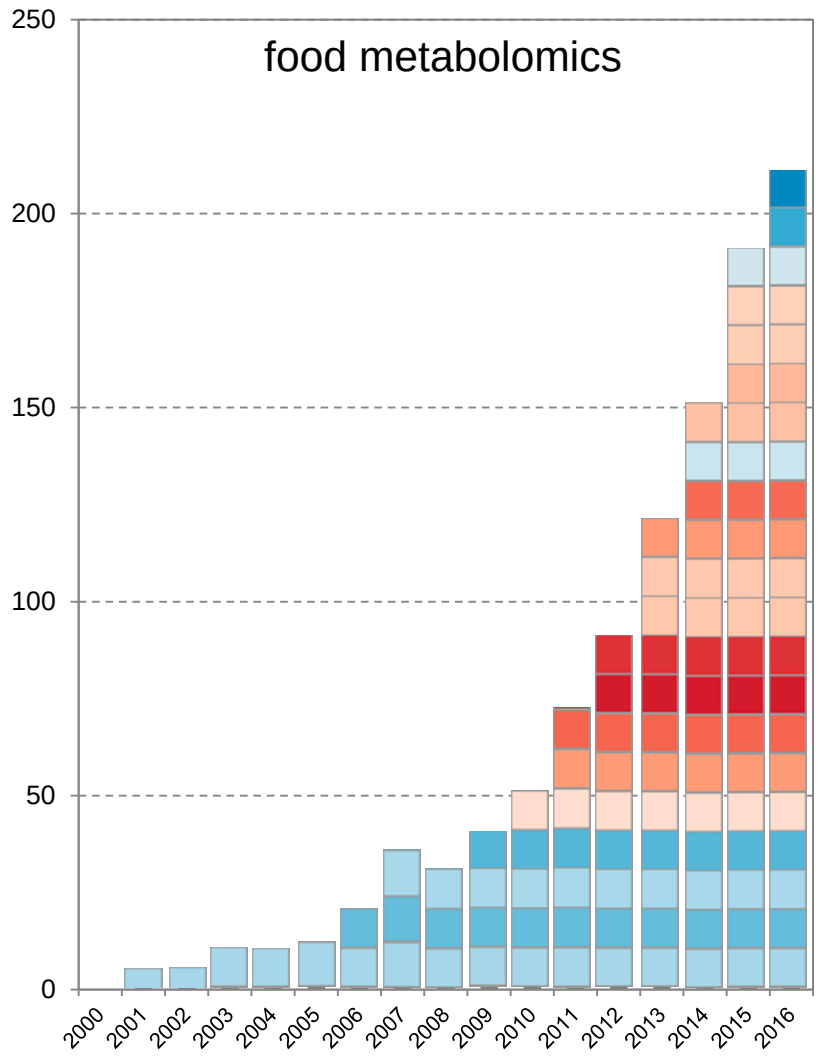
proteomics



metabolomics

physiological and environmental influence

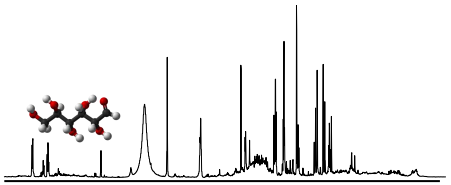




webofknowledge.com, Search „metabolmics“ + „food metabolomics“

1) instrumental analysis

2) data collection



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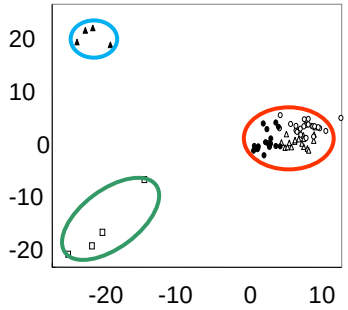
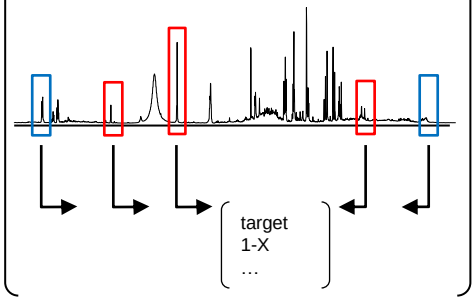
use metabolite profiles to distinguish between samples

3) data reduction and statistics

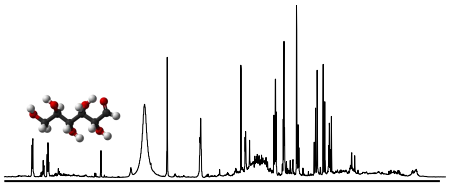


4) identification



1) Instrumental analysis

2) data collection



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use metabolite profiles to distinguish between samples

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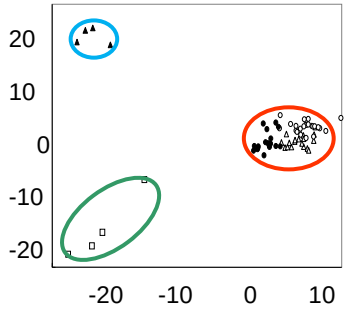

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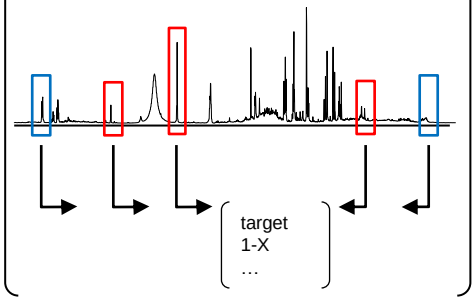
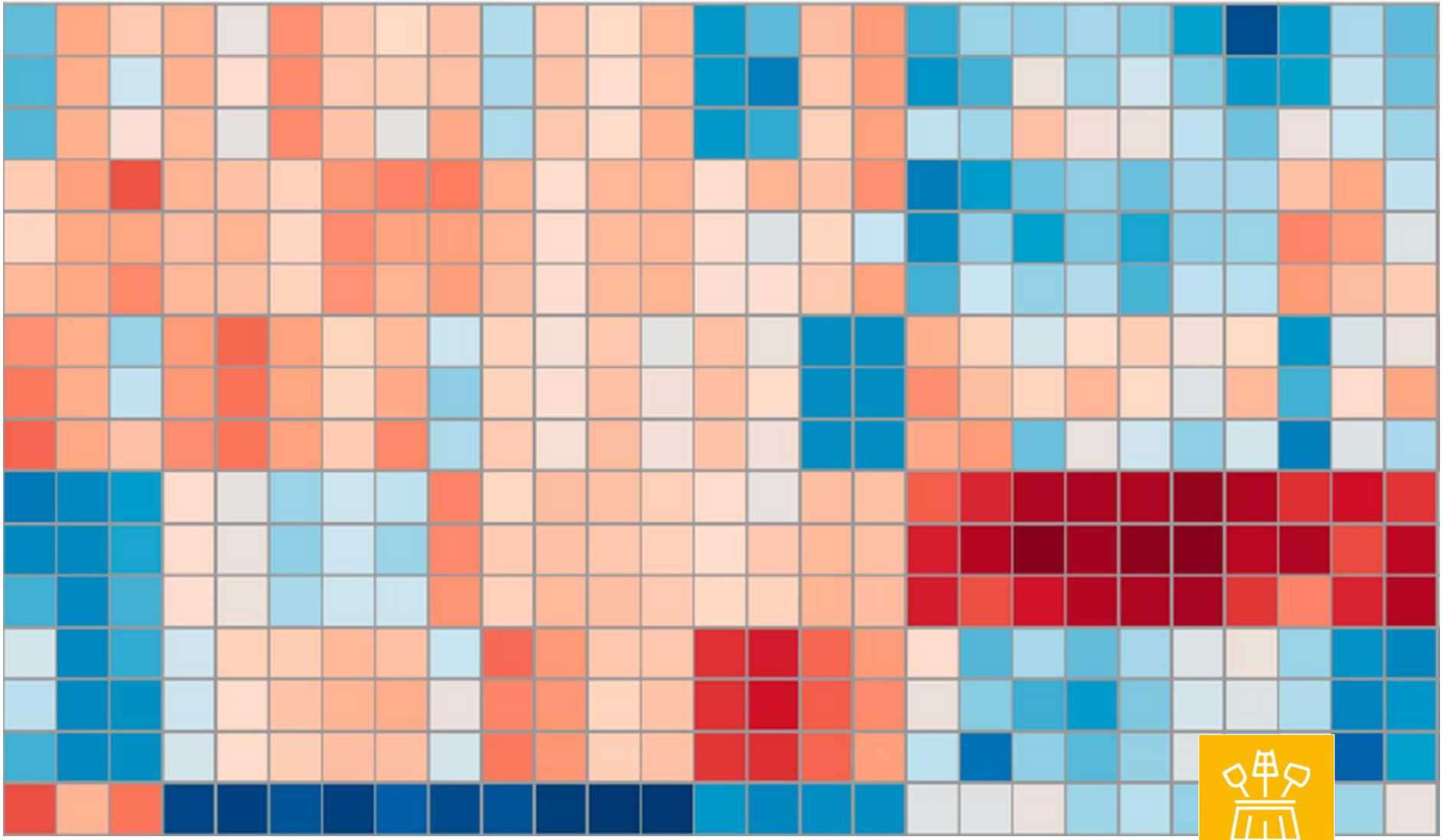


select significant and metabolites as markers for targeted approaches (optional)

beer metabolomics

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



Metabolomics project

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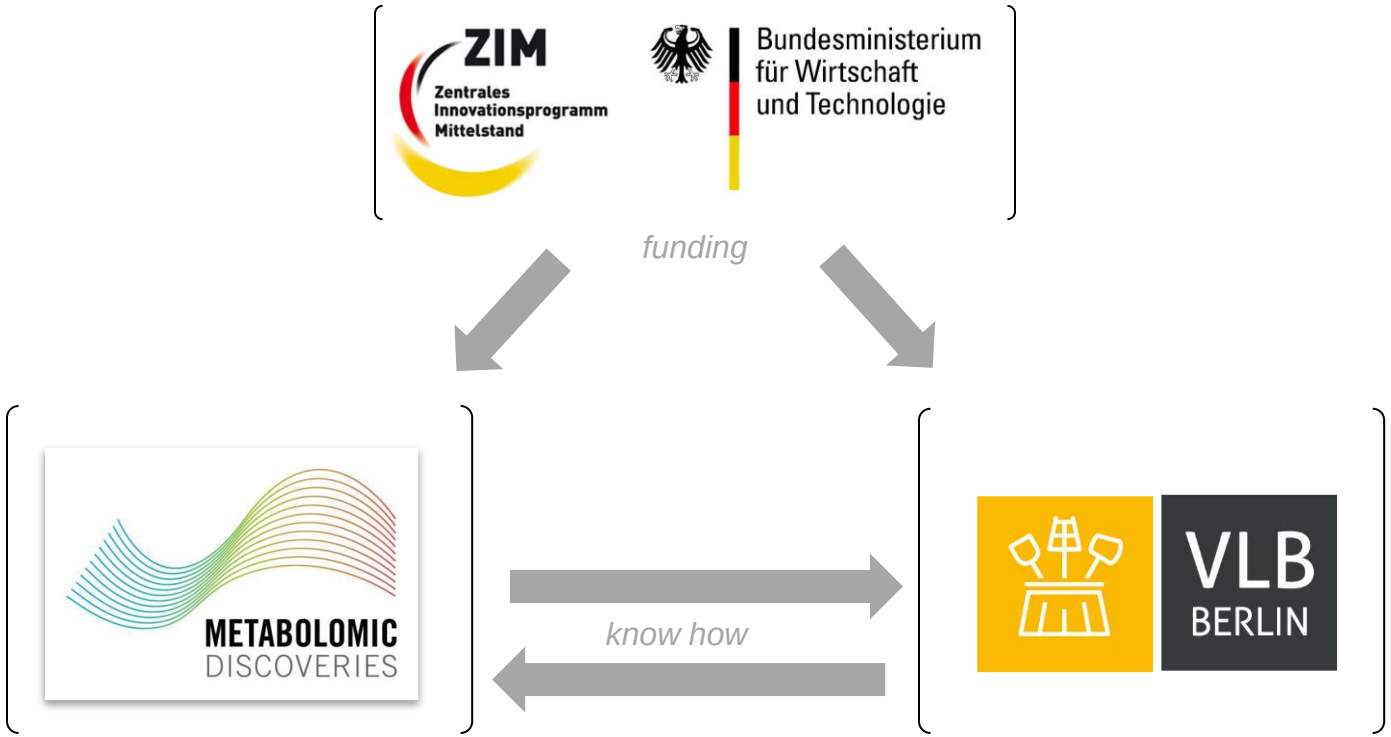
beer metabolomics project

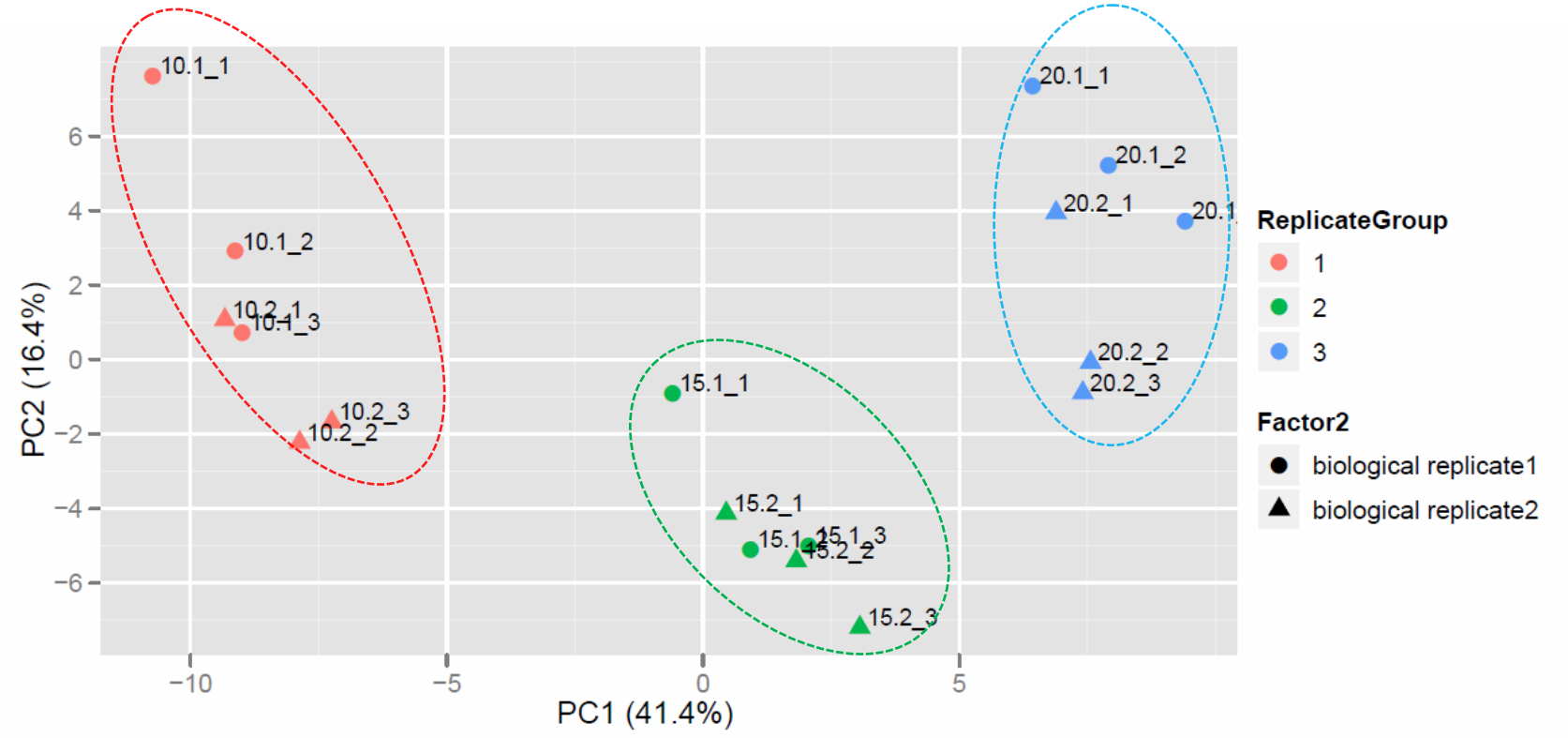


- the German “Federal Ministry for Economic Affairs and Energy” funds innovative, market oriented research carried out by networks of small/medium sized companies and research institutes
- targets improvement of competitiveness + economic growth

metabolic footprint

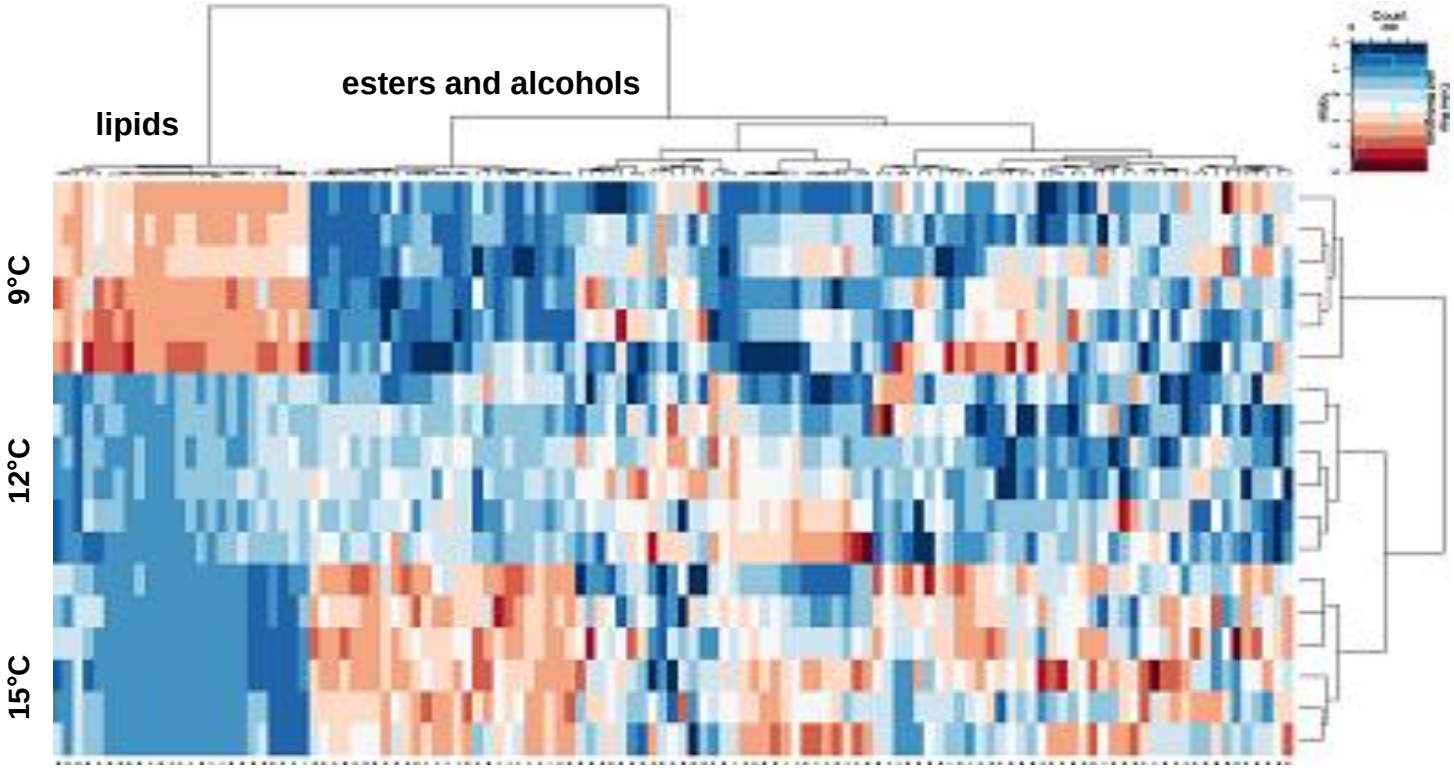
Are changes in the brewing process (here fermentation regime) properly reflected by a non-target metabolomic approach?

PCA discrimination

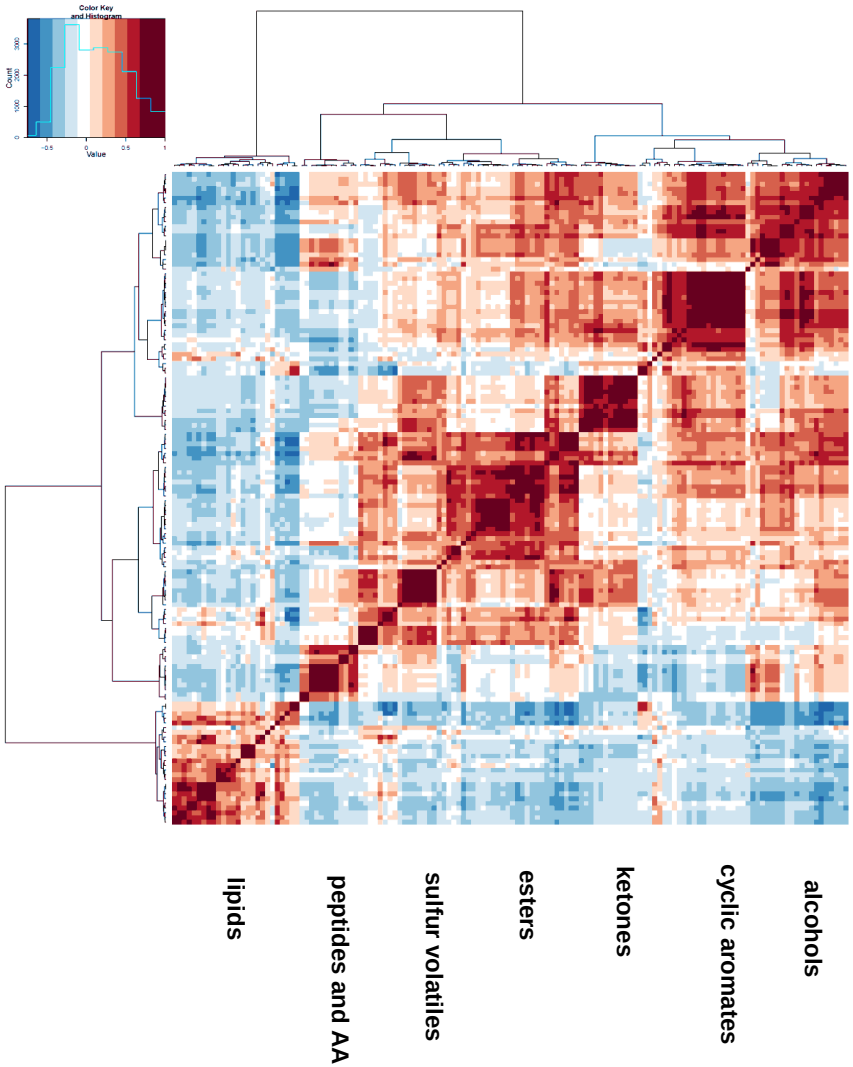


- based on metabolite data sets different fermentation regimes cluster
- rather high variance in biological replicates (pilot scale brewing?)

cluster heatmap



pairwise correlation heatmap



beer consistency

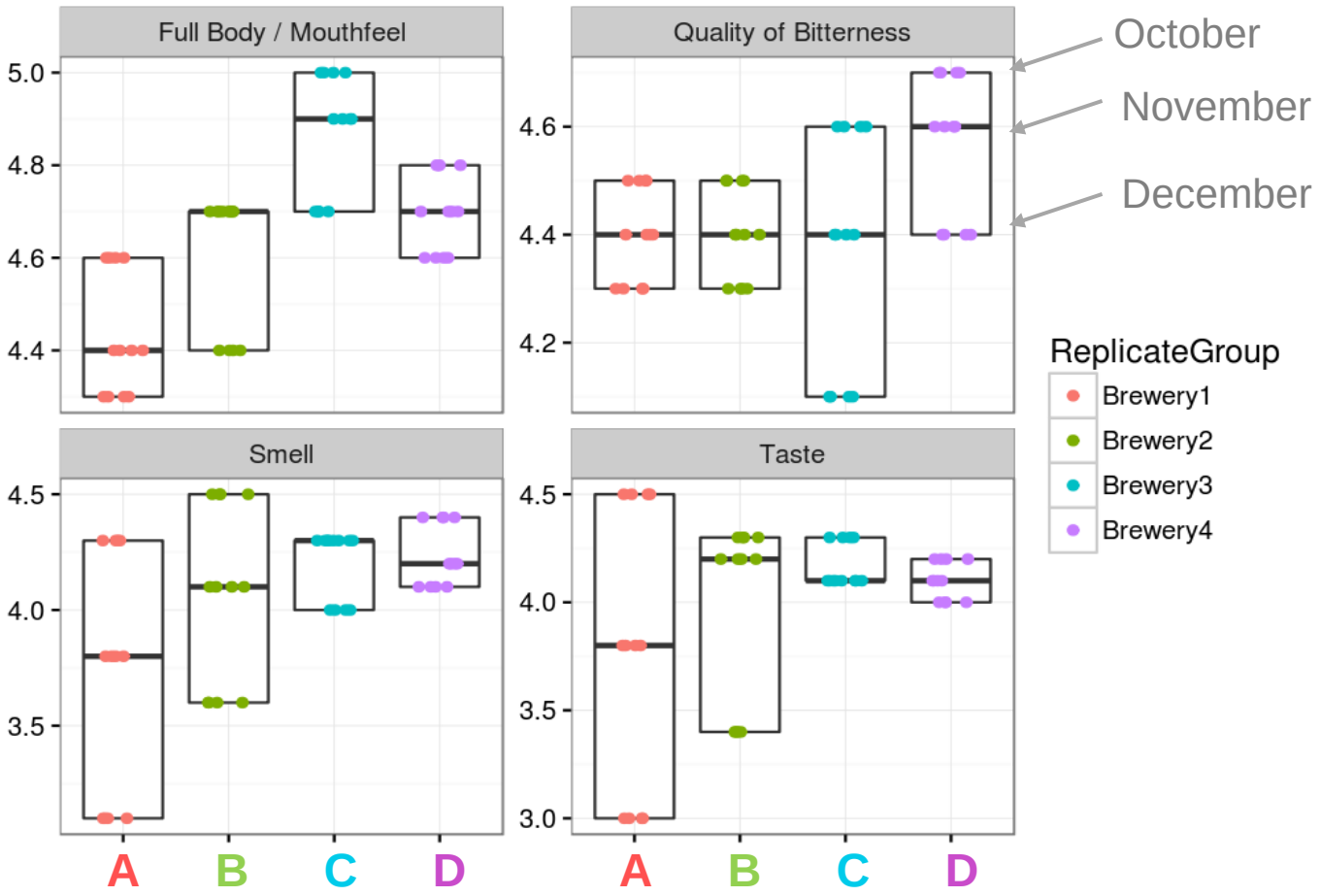
Which differences between (similar) beers can be identified by a global metabolite profiling approach?

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,…)

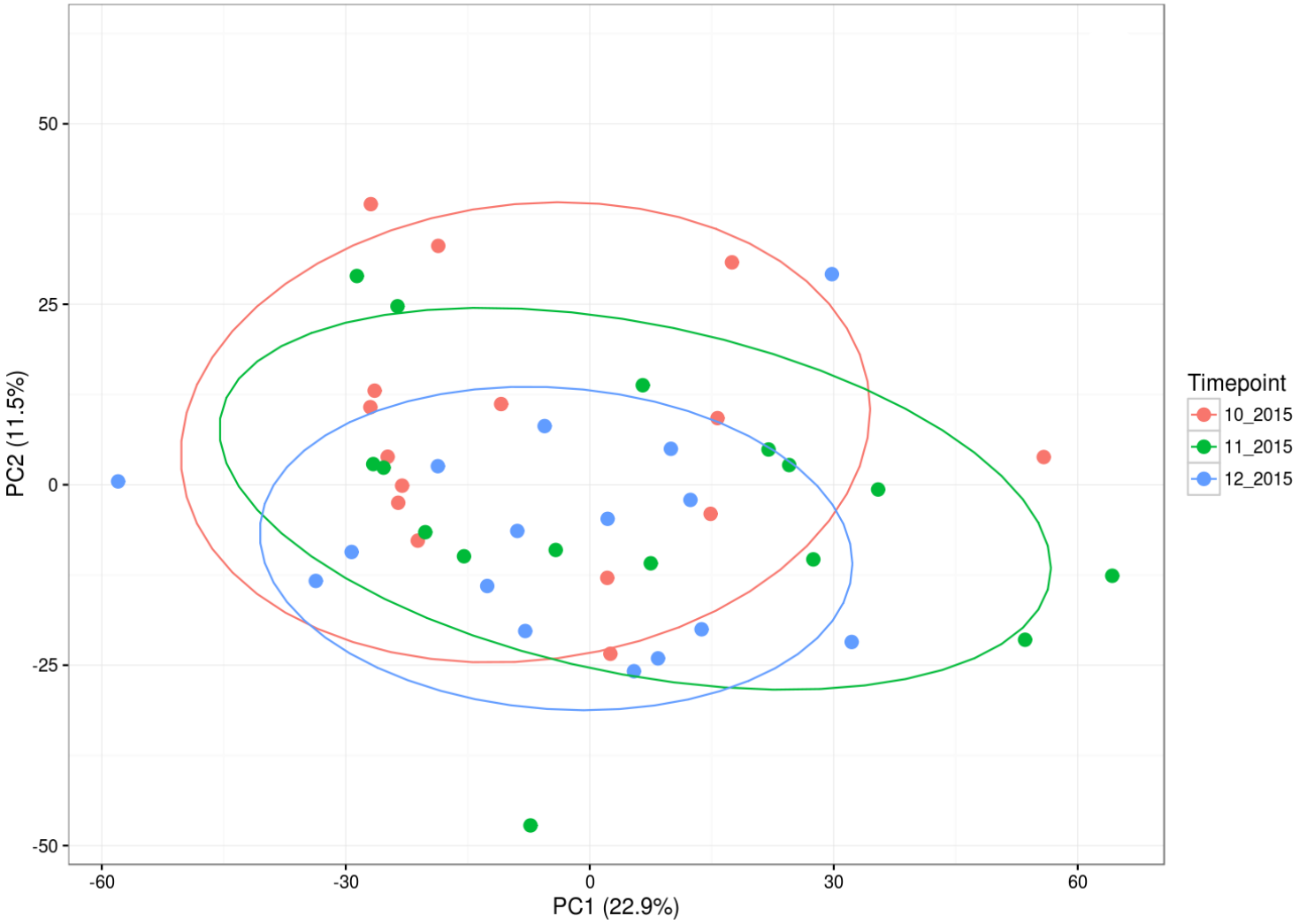
3. data analysis and visualization by PCA, identification of significant metabolites,…

sensory data



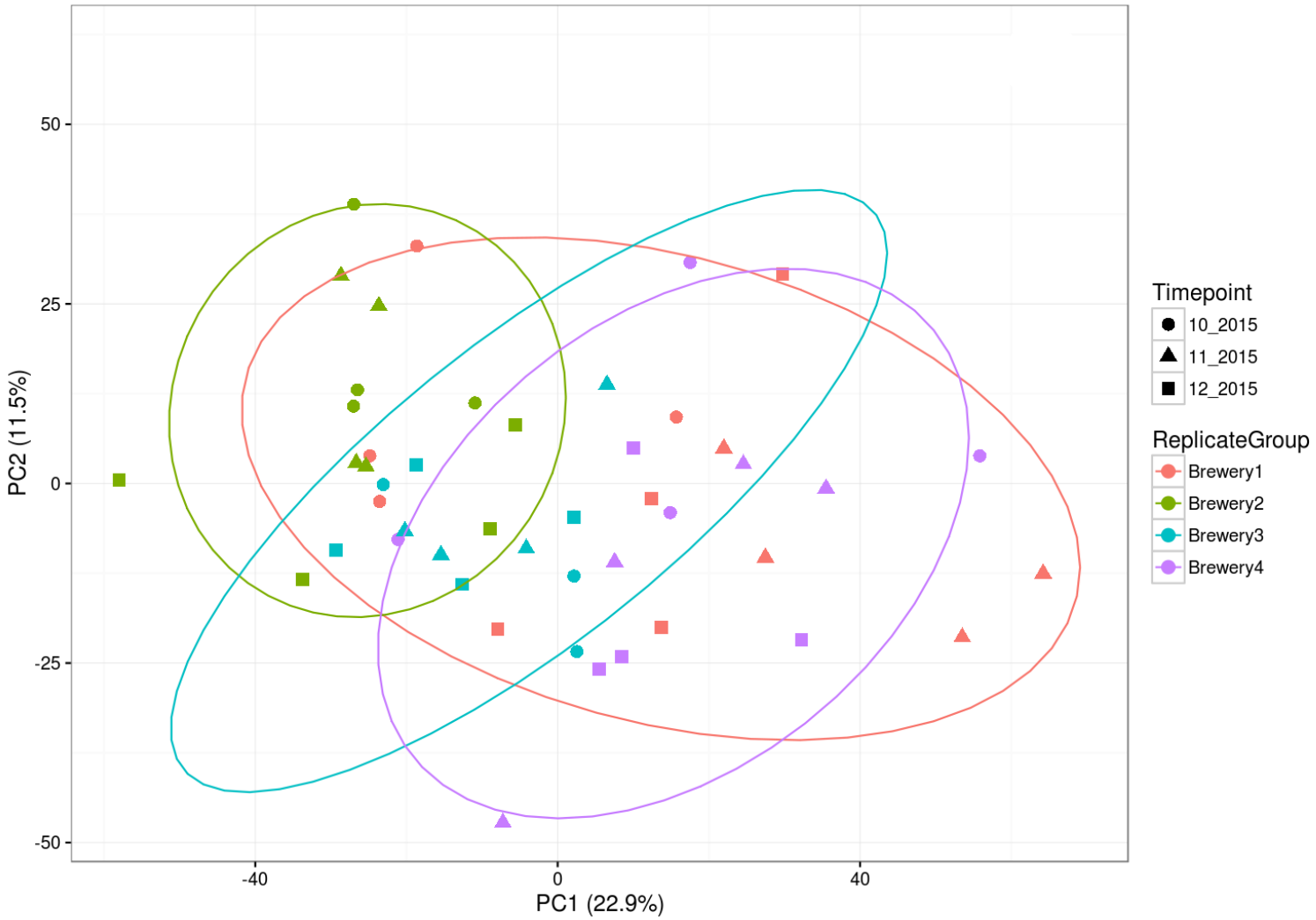
- Comparison of sensory data (5 Point scheme) applied to a single brand pilsner type beer from different production plants

PCA



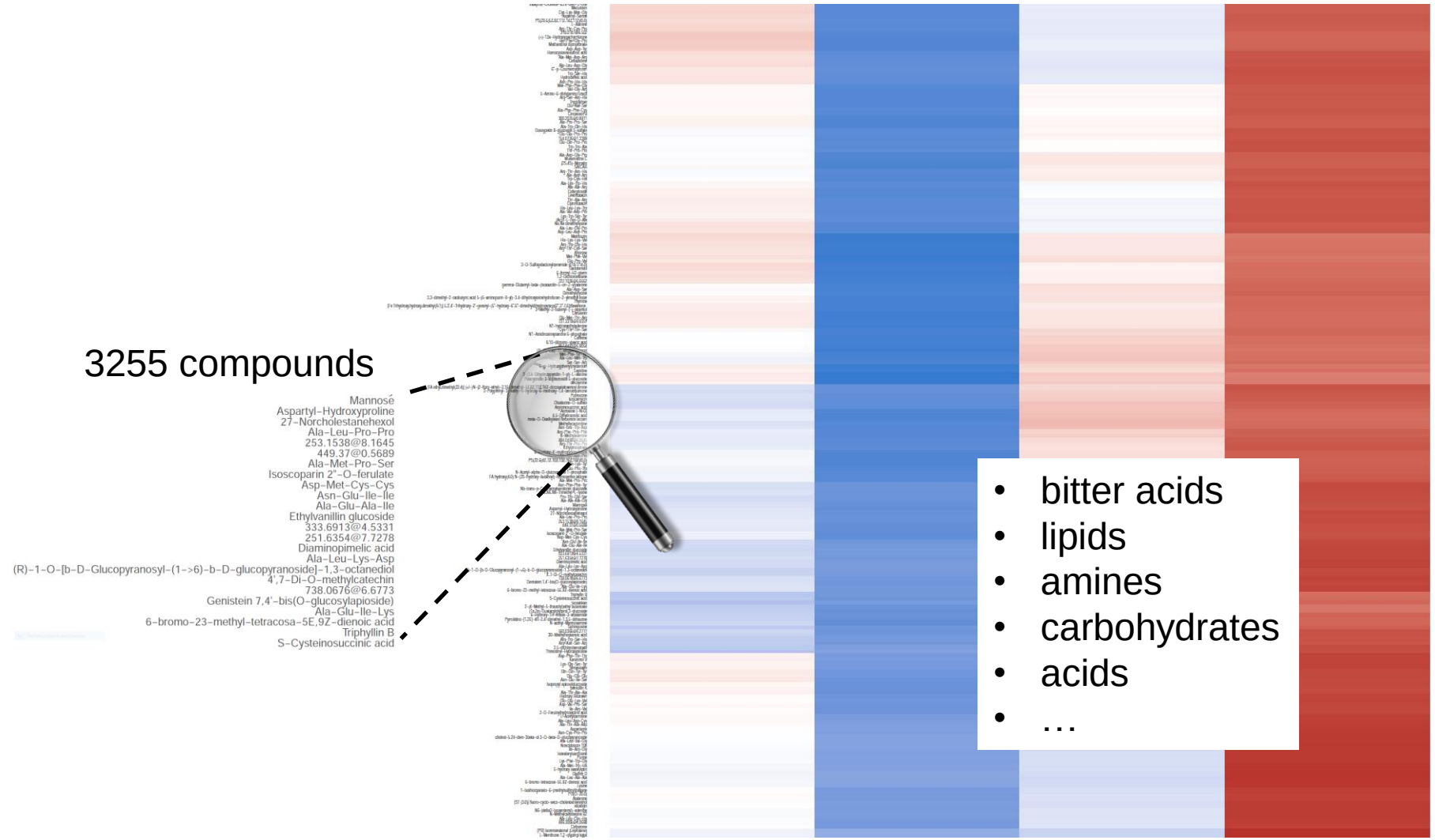
- metabolite data clustered in respect to production date (month), colored ellipses describe the 50% confidence interval of the normal distribution for each group in the two-dimensional space of the PCA

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, …



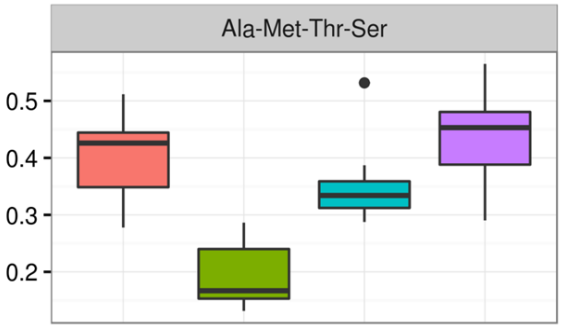
- bitter acids
- lipids
- amines
- carbohydrates
- acids
- …

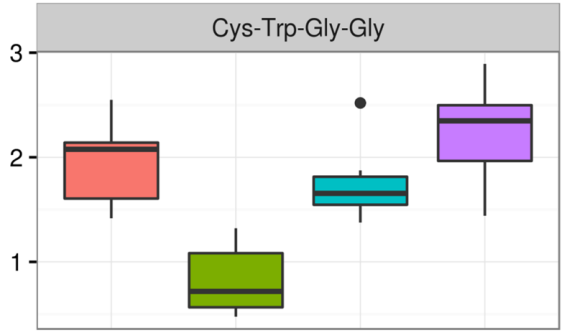
variable importance score

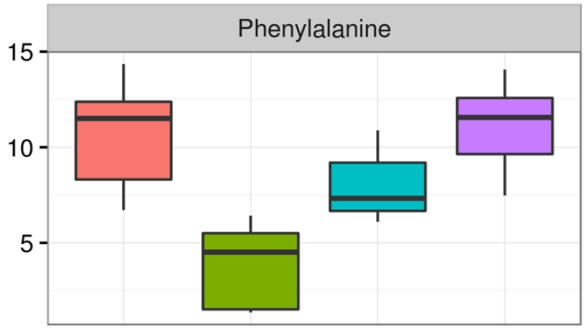
metabolite	VIP Score	Classification
549.12711@1.5813	100.00	unknown
Glu-Ser-Val	81.54978	Peptide(tri-)
421.2424@8.9307	76.57584	unknown
Asp-His-His-His	75.63124	Peptide(tetra-)
485.1783@4.1378	72.55315	unknown
2-Hydroxyethanesulfonate	71.22231	Sulfonic Acids and Derivatives
Pro-Pro-Ser	69.76658	Peptide(tri-)
352.1742@6.9477	69.15368	unknown
Asn-Asn-Asn-Asn	68.39585	Peptide(tetra-)
Asp-Val-Gly-Pro	67.09589	Peptide(tetra-)
Dimethyl succinate	60.76791	Carboxylic Acids and Derivatives
5-Amino-6-ribitylamino uracil	60.64853	Monosaccharides
Tyr-Lys-Arg-Tyr	60.34157	Peptide(tetra-)
Arg-Met-Phe-Asp	56.21668	Peptide(tetra-)
Methyl 2-aminobenzoate	55.70696	Benzoic Acid and Derivatives
8-Hydroxyguanosine	54.64307	Purine Nucleosides and Analogues
Gln-Trp-Gln-Gln	51.28974	Peptide(tetra-)
Piperitoside	50.8607	Flavonoids
Phe-Tyr-Phe-Lys-Ile	50.8044	Peptide(penta-)
Phenylalanine	50.36089	
Met-Ala-His	50.08508	
Cys-Trp-Gly-Gly	48.88984	
Glycerylphosphorylethanolamine	47.65788	
Gly-Pro-Trp	47.23474	
N6-(delta2-Isopentenyl)-adenine	45.93661	
Procyanidin B3	44.99882	

- 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

peptides

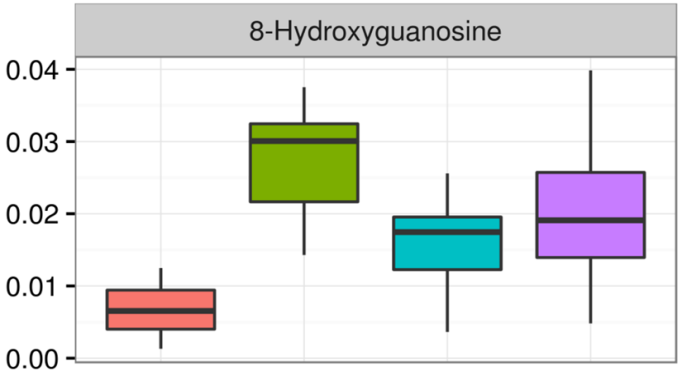


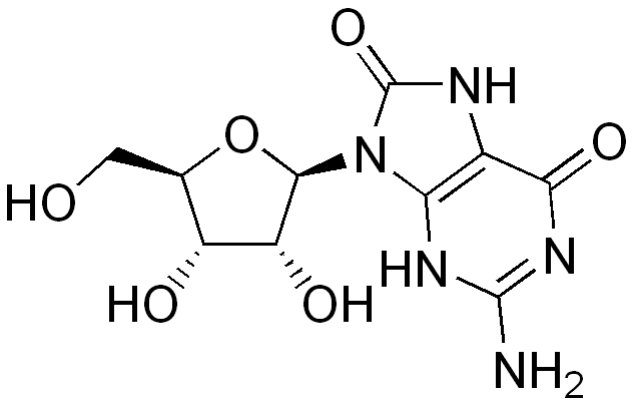




- 22 out of the 50 significant metabolites were peptides
- 13 peptides out of these 22 metabolites are showing the trends similar to box plots shown above
- peptides (and amino acids) were reported as significant metabolites in previous LC-MS based studies, direct correlation with beer quality are hard to draw

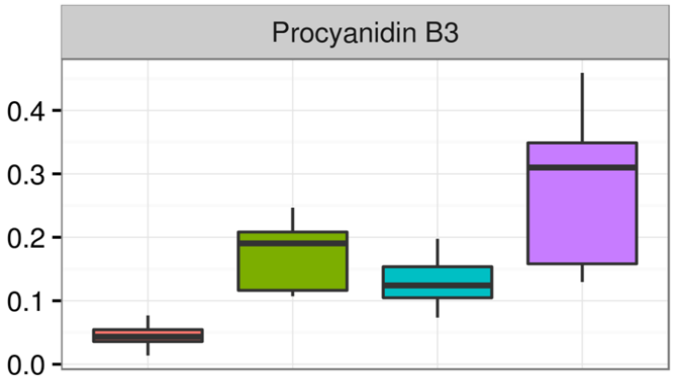
8-hydroxyguanosine

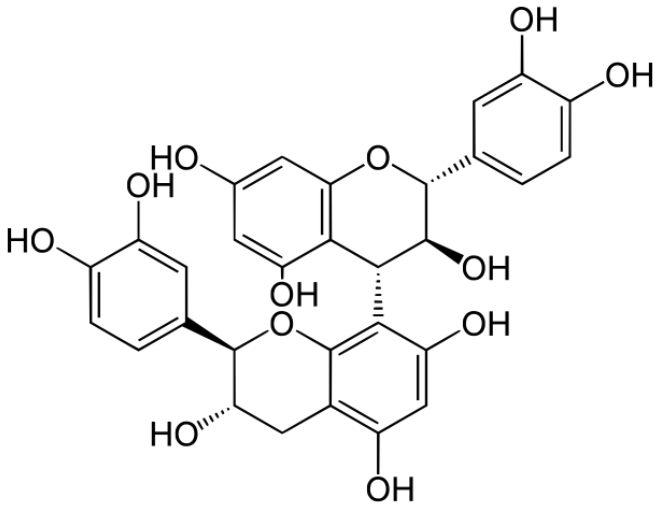




- 8-Hydroxyguanosine is a nucleoside formed by guanosine oxidation
- purines were reported to correlate with beer oxidation
- 5-MTA, Deoxyadenosine, and Guanine did not significantly contribute to differences between the breweries (fresh beer)

polyphenols

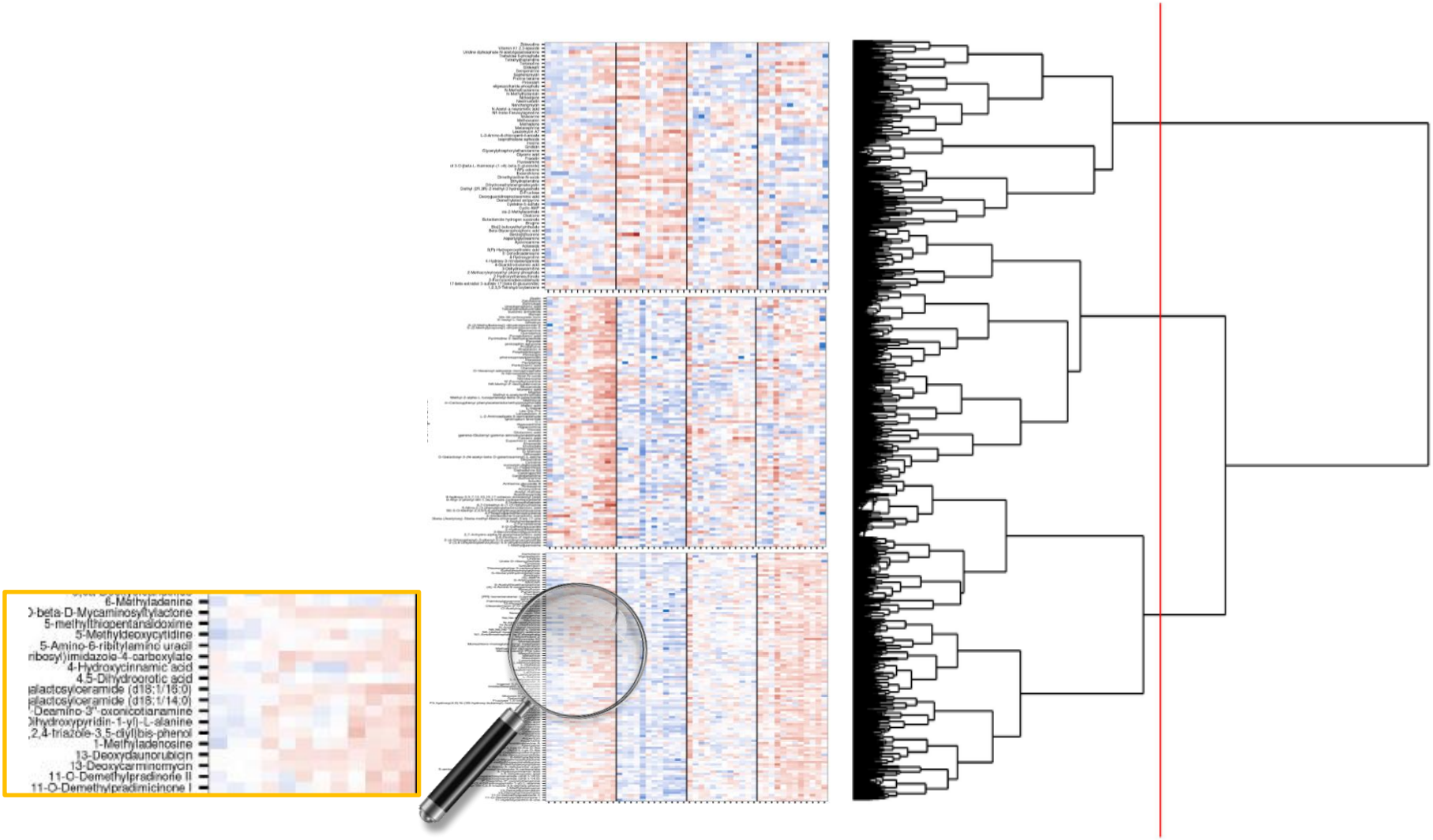


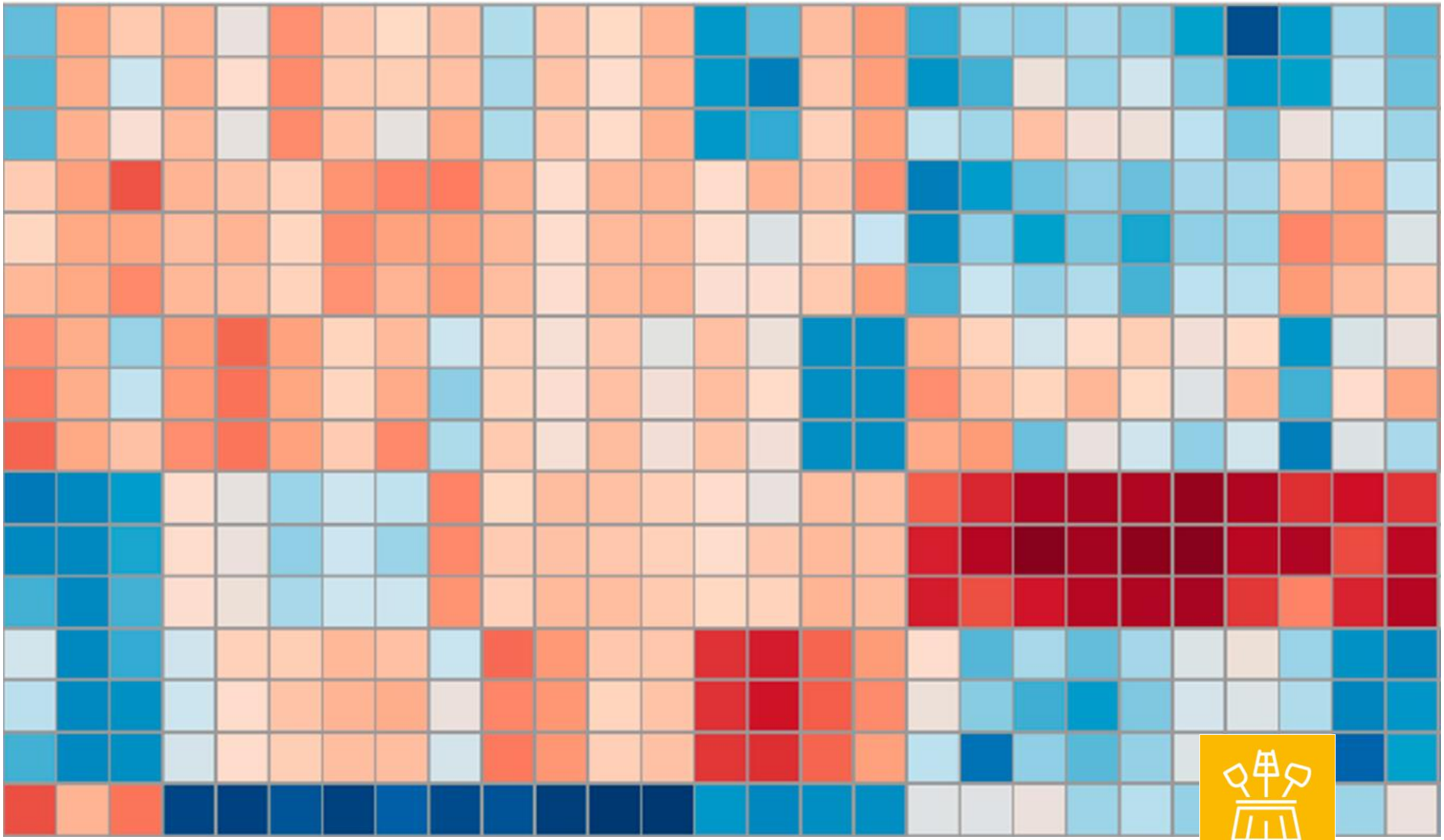


- Procyanidin B3 ((+)-catechin-(4α-8)-(+)-catechin) has been reported in malt, hops, and beer

- physical beer stabilty, flavor stability…

cluster analysis





Odorants?

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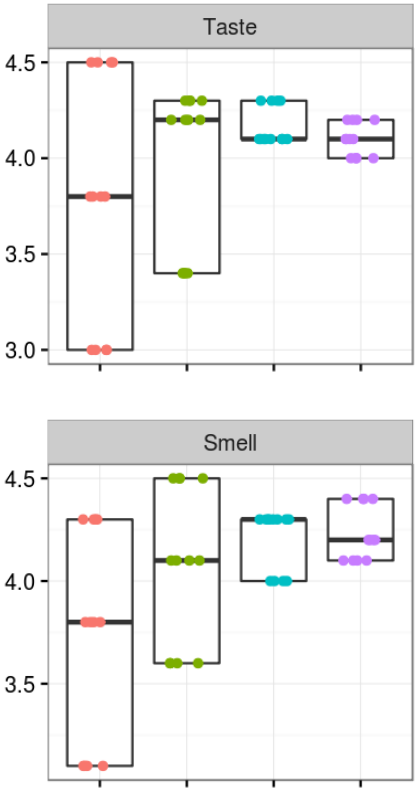
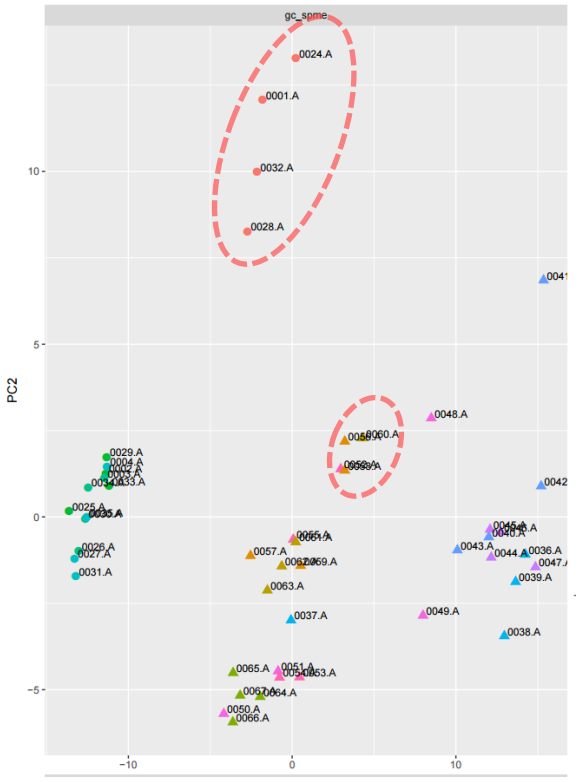
differences attributed to GC analysis?

- 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



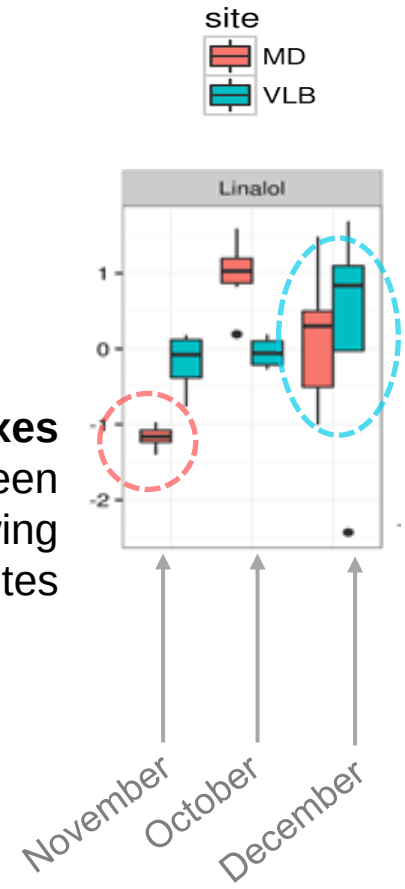


- 157.0835@5.0885 (31,75)
- 204.058@1.1643 (31,75)
- […]

✓ ethyl pyruvate
✓ ethyl lactate
✓ isobutyl isobutyrate
✓ linalool
✓ linalool oxide
✓ isoamyl acetate
✓ phenylethylethanol
✓ […]

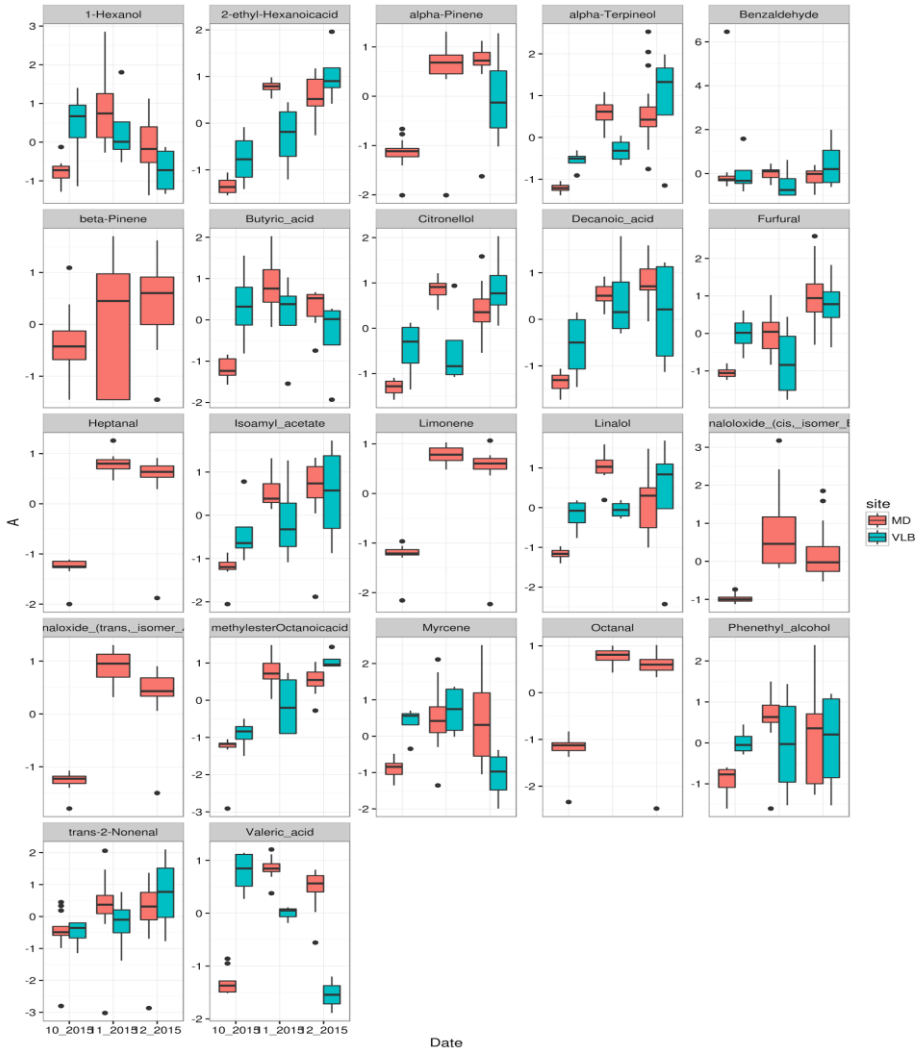
targeted vs. untargeted GC analysis

small boxes
minimal differences between samples from different brewing sites



large boxes
differences between samples from different brewing

targeted vs. untargeted analysis



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

<u>Assumption: targeted approach more accurate</u>

- 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…

METABOLOMIC DISCOVERIES

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

VLB BERLIN

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

ZIM Zentrales Innovationsprogramm Mittelstand

Bundesministerium für Wirtschaft und Technologie

- ZIM KF2132338

Thank you!

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