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

Flavor changes in strongly hopped beers
**Agenda**

- current market situation - Why is the flavor stability of hop forward ales of interest?
- defining stability - What are typical indicators used to monitor beer ageing?
- Lager vs. Ales - What is (chemically) special about hop forward ales?
- project design - How do we try to understand flavor stability in hop forward ales?
- results - What do we know so far and what to do next?
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Current market state

+ increasing number of breweries

<table>
<thead>
<tr>
<th>Germany</th>
<th>USA</th>
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<tr>
<td>2016</td>
<td>2017</td>
</tr>
<tr>
<td>1 410</td>
<td>1 492</td>
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</table>

+ increasing number of German breweries that produce hoppy ales

- Brewery Beck und Co. (Beck’s Pale Ale)
- Radeberger Brewery (Braufactum Beers)

+ Singha Corporation Co., LTD. (EST. 33 Copper and Snowy Weizen)

+ hop forward beer styles are interesting for every brewery

source: Deutscher Brauer-Bund / Brewers Association / Statistisches Bundesamt
Current market state

searching: “Bangkok, craft beer” at Google Maps results in 20 hits, that sell special beer types

source: Google Maps
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What is „ageing stability“?

+ no fixed definition exists
+ basically 3 categories of “stability”
+ physical and flavor stability are partly connected

flavor stability

microbiological stability
physical stability

transportation, distribution, service

brewery site

source: https://www.mein-buntes-leben.de/artikel/craftbeer-massvoller-biergenuss-bei-diabetes
What is „ageing stability“?

+ aroma and taste are complex
  - they are an interaction of thousands of compounds from raw materials and technological aspects

+ molecule formation → new aroma impressions can arise
+ degradation / modification of existing substances → loss of flavor

source: Deutscher Brauer-Bund
Current state of research

- raw materials (pale / dark malt, adjuncts...)
- optimized filling conditions (O$_2$-uptake < 20ppb, pasteurization)
- brewing technology (LOX-activity, boiling, hopping regime...)
- storage conditions (temperature, light)
- brewing technique (mashing, boiling / stripping...)
- bottles, caps, cans

flavor stability
Agenda

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Taste influences - Lager vs. Ales

+ Maillard products can be positive in dark (bottom fermented) beers → malt aroma might cover carbonyl off-flavors

+ precursors of carbonyl compounds:
  - Maillard reaction / Strecker degradation products (e.g. can result in 2-Methylbutanal, 3-Methylbutanal, Methional, Phenylacetaldehyd…)
  - thermal wort stress (results in increase of 2-Furfural, 5-Hydroxymethyl-2-furfural)
  - fatty acid degradation / oxidation of lipids (trans-2-Nonenal → cardboard flavor)

+ increase of sweet, sherry like notes with ageing (sometimes wanted for special beers → barrel-aged or vintage beer)
Taste influences - Lager vs. Ales

+ fermenting by-products
  ➢ top fermented beers (Ales) are normally fermented at higher temperature
    → more higher alcohols and esters → fruity, ester like notes are wanted

+ amounts of pro- und antioxidant compounds vary in Ales and bottom fermented beers
  ➢ pale / dark malt, rise of polyphenols from hops e.g. by dry hopping, O₂-amount in craft beer, SO₂-amounts in Ales are lower by using top fermenting yeast

+ often high IBU‘s in Ales are wanted

+ hop dosage in varying stages of Ale production

bitterness and hop aroma are crucial for flavor impressions in hoppy Ales
Hop characteristics in Ales

+ hop compounds in high concentrations (compared with lager style)

+ hop substances are present, that are absent in lager beers

+ positive influence of (oxidative) stability by antioxidative constituents like polyphenols or bitter substances
Project
Flavor stability of hoppy, top fermented beers
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Pre-project procedure

"Consumers are very aware"

"Much shorter would be logistically impractical. Much longer would be unfair to consumers"

"We’d like it to be shorter than 6 months…."

"Slight oxidative qualities can appear as early as after 1 month."

brewery questionnaire on flavor stability and best by date
Stage 1: Check the behavior of known ageing parameters in highly hopped beers.

Stage 2: Specify data from stage one by further screening trials for unknown substances.

Stage 3: Verify data in brewing trials targeting hopped beers with improved shelf life.
Stage 1: Check the behavior of known ageing parameters in highly hopped beers.

Stage 2: Specify data from stage one by further screening trials for unknown substances.

Stage 3: Verify data in brewing trials targeting hopped beers with improved shelf life.
Experimental design - Stage one

Design of Experiment (DoE) based on Brewers Association „Beer Style Guidelines“ for Styles:
→ Blonde Ale / Pale Ale / Red Ale / India Pale Ale / Brown Ale / Double India Pale Ale

- Original gravity [°P]: 10.0 - 23.7
- Alcohol content [ABV]: 4.1 - 10.6
- Color [EBC]: 6 - 52
- IBU [EBC]: 15 - 100

Start with beers from 6 national breweries:

1. Room temperature storage (dark, 20 °C):
   - Starting point + forced ageing
   - 2 weeks, 4 weeks, 8 weeks, 12 weeks, 18 weeks, 24 weeks

2. Cold storage (dark, 5 °C):
### Experimental design - Stage one

<table>
<thead>
<tr>
<th>beerstyle</th>
<th>bitterness [EBC]</th>
<th>color [EBC]</th>
<th>original gravity [% mas]</th>
<th>ABV</th>
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<tbody>
<tr>
<td>brown Ale</td>
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<td>12.70</td>
<td>5.44</td>
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<td>17</td>
<td>14.97</td>
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<td>IPA #2</td>
<td>60</td>
<td>26</td>
<td>16.15</td>
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<td>170</td>
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<td>18</td>
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<tr>
<td>imperial IPA</td>
<td>65</td>
<td>27</td>
<td>21.63</td>
<td>9.94</td>
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# Analysis in stage one

<table>
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<th>Parameter</th>
<th>Method</th>
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<tbody>
<tr>
<td>sulfur dioxide</td>
<td>EBC 9.25.1</td>
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<tr>
<td>metal content (Fe, Cu, Mn)</td>
<td>ICP-MS (DIN EN ISO 17294-2:2005-02, mod.)</td>
</tr>
<tr>
<td>density, original gravity, alcohol content, apparent (and real extract)</td>
<td>EBC 9.43.2 und EBC 9.4</td>
</tr>
<tr>
<td>beer bitter acids (ratio of trans-cis iso-α-acid used for PCA)</td>
<td>UPLC-ToF-MS</td>
</tr>
<tr>
<td>pH-Value, color</td>
<td>EBC 9.35, EBC 9.6</td>
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<td>bitterness of beer (IBU)</td>
<td>EBC 9.8</td>
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<tr>
<td>DMS</td>
<td>HS-GC-PFPD (EBC 9.39, mod.)</td>
</tr>
<tr>
<td>fermentation by products (higher aliphatic alcohols and esters)</td>
<td>HS-GC-FID (EBC 9.39, mod.)</td>
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<tr>
<td>hop aroma compounds</td>
<td>HS-SPME-GC-MS/MS</td>
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<td>carbonyl compounds</td>
<td>HS-SPME-GC-MS/MS</td>
</tr>
<tr>
<td>short chain fatty acids (C4 – C12)</td>
<td>HS-SPME-GC-FID</td>
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<tr>
<td>sensory analysis</td>
<td>descriptive sensory trials with default attributes</td>
</tr>
</tbody>
</table>

point of measurement used for PCA:  

*fresh, forced aged, room temperature 12 und 24 weeks, cold storage 12 und 24 weeks*
Sensory trials stage one

+ 9 trained VLB testers on average

+ 14 descriptors
  - intensity of odor
  - quality of bitterness
  - malt character
  - duration of aftertaste
  - taste
  - intensity of hop aroma
  - sweetness
  - palatefulness
  - harmony
  - general quality
  - intensity of bitterness
  - acidity
  - oxidation
  - odor

+ additional free text option for comments
Experimental results stage one

+ in phase one we analyzed:

11 beers resulting in
88 samples, by using
14 methods (measured in duplets), leading to
1 056 chromatograms and
60 192 peaks …

→ correspondingly large dataset

+ we will focus on three major aspects here:

- primary hop aroma compounds (terpenes)
- staling aldehydes
- bitter acids
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Hop aroma compounds (Biplot)

PC 1 (46%)

PC 2 (20%)

- Lager
- dry hopped (VLB routine samples)
- brown Ale
- non-alcoholic IPA
- dark rye IPA
- imperial IPA
- Pale Ale
- IPA

α-pinene
β-pinene
α-humulene
β-caryophyllene
myrcene
caryophyllene oxide
geranyl acetate
limonene
2-methylbutyl isobutyrate
linalool oxide
cis-linalool oxide
trans-linalool oxide
α-terpineol
nerol
geraniol
linalool
Hop aroma compounds (Biplot)

- α-pinene
- β-pinene
- myrcene
- β-caryophyllene
- α-humulene
- citronellol
- trans-linalool oxide
- α-terpineol
- nerol
- geraniol
- linalool
- limonene
- 2-methylbutyl isobutyrate
- geranyl acetate
- caryophyllene oxide

Flavors:
- hoppy, grassy, herbal, earthy, resinous
- floral, citrusy, tropical

Samples:
- Lager
- dry hopped (VLB routine samples)
- brown Ale
- non-alcoholic beer
- dark rye IPA
- imperial IPA
- Pale Ale
- IPA

PC 1 (46%)
PC 2 (20%)
Summary hop aroma compounds

+ regarding hop aroma, beers split into two big groups

1. earthy, resinous styles
2. fruity, citrus styles

- Lager style isn’t any of them
  (but they are extremely similar in a cluster)

- in strong hopped beers, differences in beer styles are noticeable (IPA’s can be highly different in hop aroma)
Hoppy, top fermented samples (grouping)
Hoppy, top fermented samples (grouping)

PC 1 shows the beer style
Hoppy, top fermented samples (storage conditions trend)
Hoppy, top fermented samples (storage conditions trend)

Scores

PC-2 (14%) vs. PC-1 (20%) beer style

- 6798 (dark rye IPA)
- 6804 (IPA #6)
- 6802 (Pale Ale)

Legend:
- ▲ 12 W cold
- ▼ 12 W Rt
- △ 24 W cold
- ● 24 W Rt
- ◀ forced aged
- ▲ fresh

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Hoppy, top fermented samples (storage conditions trend)

PC 2 represents the storage conditions

6798 (dark rye IPA)
6802 (Pale Ale)
6799 (nab)
6804 (IPA #6)

PC 1 (20%) beer style

PC 2 (14%)

Scores

aged

fresh

12 W cold  12 W Rt  24 W cold  24 W Rt  forced aged  fresh
Hoppy, top fermented samples (Loadingsplot)
Principal component analysis (PCA)

PC-1 (20%) beer style

PC-2 (14%) storage conditions

sensory findings

staling aldehydes

hop aroma compounds

12 W RT
24 W RT
12 W cold
24 W cold
forced aged

© VLB e.V., Berlin, Germany
Summary storage trials

+ beer styles are clustering (similar beer styles) or distinguish

+ staling aldehydes correlate with storage conditions but sensory trials are not able to fully describe them

+ beers react as expected under storage conditions
  ➢ forced ageing test describes in a good way the behavior of ageing under room temperature conditions (12 – 24 weeks)
  → time savings for subsequent project steps
Staling Aldehydes

- **PC2 (19%)**
- **PC1 (47%)**

- ▲ hoppy Ale fresh
- ■ hoppy Ale forced aged
- + hoppy Ale room temperature 12 weeks
- ■ hoppy Ale room temperature 24 weeks
- x hoppy Ale cold storage 12 weeks
- □ hoppy Ale cold storage 24 weeks
- ◊ Lager fresh
- ◆ Lager room temperature / cold storage 12 weeks
- ◆ Lager room temperature 24 weeks
- ◆ Lager cold storage 24 weeks
Staling Aldehydes

Initial concentration of hoppy Ales

PC2 (19%)

Initial concentration of Lager

PC1 (47%)

Legend:
- ▲ hoppy Ale fresh
- ■ hoppy Ale forced aged
- + hoppy Ale room temperature 12 weeks
- ▼ hoppy Ale room temperature 24 weeks
- ■ hoppy Ale room temperature 12 weeks
- □ hoppy Ale room temperature 24 weeks
- w hoppy Ale cold storage 12 weeks
- □ hoppy Ale cold storage 24 weeks
- ◊ Lager fresh
- ◊ Lager room temperature / cold storage 12 weeks
- ♦ Lager room temperature 24 weeks
- ♦ Lager cold storage 24 weeks

Initial concentration of Lager:

- ▲ Lager fresh
- ■ Lager forced aged
- + Lager room temperature 12 weeks
- ▼ Lager room temperature 24 weeks
- ■ Lager cold storage 12 weeks
- □ Lager cold storage 24 weeks
Summary staling aldehydes

+ concentration of carbonyl compounds increase less in Lager style (compared to highly hopped beers)
  ➢ nevertheless stale taste is noticeable early in Lager beer

+ staling aldehydes in highly hopped beers started at a higher range than Lager reach after storage
  ➢ no “ageing” characteristic was recognized in sensory trials of fresh products
  ➢ they where noticed only after a high increase during storage
  ➢ mainly as “oxidized” → harmony, odor, taste and general quality seem less affected

staling aldehydes are of interest in hop forward beers but they are not that crucial as they are in Lager style
### trans/cis-iso-α-acids ratio

<table>
<thead>
<tr>
<th>Beer Type</th>
<th>Sample Status</th>
<th>trans/cis-iso-α-acids Ratio</th>
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<tbody>
<tr>
<td>brown Ale 1</td>
<td>Fresh</td>
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</tr>
<tr>
<td>brown Ale 1</td>
<td>Aged</td>
<td>0.27</td>
</tr>
<tr>
<td>brown Ale 1</td>
<td>Control</td>
<td>0.23</td>
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<tr>
<td>IPA #1</td>
<td>Fresh</td>
<td>0.20</td>
</tr>
<tr>
<td>IPA #1</td>
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<td>0.23</td>
</tr>
<tr>
<td>IPA #1</td>
<td>Control</td>
<td>0.27</td>
</tr>
<tr>
<td>IPA #2</td>
<td>Fresh</td>
<td>0.20</td>
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<tr>
<td>IPA #2</td>
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<td>dark Pye IPA</td>
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<tr>
<td>IPA #3</td>
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<td>IPA #3</td>
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<tr>
<td>IPA #4</td>
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<tr>
<td>imperial IPA</td>
<td>Control</td>
<td>0.23</td>
</tr>
</tbody>
</table>

**Notes:**
- Aged = 24 weeks and 20 °C
- Control = 24 weeks and 5 °C

### Sum of iso-α-acids [mg/L]

<table>
<thead>
<tr>
<th>Beer Type</th>
<th>Sum of iso-α-acids [mg/L]</th>
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<tbody>
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<td>dark Pye IPA</td>
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<td>brown Ale</td>
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<td>38</td>
</tr>
<tr>
<td>IPA #6</td>
<td>40</td>
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</tbody>
</table>
Summary bitterness

- *trans/cis*-iso-α-acids ratio decreases during storage

- *trans/cis*-iso-α-acids ratio does not have the expected (high) influence on sensory attribute “oxidation”
  - sensory attribute “oxidation” is negative correlated to “quality of bitterness” and “harmony”
  - “oxidation” is only correlated to staling aldehydes
Summary stage one

+ more restricted beer samples are needed
  - for example only use IPA or Pale Ale
  - differences in one category of products are adequate

+ sampling times of fresh, forced aged, 12 weeks and 24 weeks storage parameters are sufficient

+ carbonyl compounds are not elementary off-flavors for hoppy, top fermented beers
  - focus more on hop aroma compounds (e.g. linalool or esters from hops)

+ combine oxidative processes analysis into one (e.g. electron paramagnetic resonance)

+ sensory trials are good but not satisfactory at all
  - use other descriptors + ongoing training of panelists
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Dr. Jörg Maxminner
Dr. – Ing. Nils Rettberg

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