Use of beta-glucosidase activity for flavour enhancement in specialty beers

Luk Daenen
Chair De Clerck 2012
Flavour complexity

Malt, hops, spices, fruit

flavour

Yeast

Flavour precursors

Specific enzymes

Flavour of the final beer

Process

addition
Flavour modification

Here, focus on biological approach (no flavour addition):
- Use of plant parts/extracts, enzymes, microorganisms

Preferred by consumers:
- Natural product, traditional process, authenticity, health, environment

Advantage for producer:
- “clean label”, often cheaper, development of complex and subtle flavours

Malt, hops, spices, fruit

flavour

Yeast

Flavour of the final beer

flavour

Specific enzymes

addition

Process
Interest for wine-making

- Some grape varieties have a significant pool of glycosidically bound flavour compounds → hidden flavour potential
- Hydrolysis by yeast, enzymes,...
Interest for brewing industry

Hops
- Flavour precursors in “green” part of hops (flowers, leaves, ...)
- Enhance “hoppy” aroma by precursor hydrolysis
- Interest for lager, ale, geuze, specialty beers, ...

Cherry
- Flavour precursors in juice, kernel, skin
- Enhance cherry flavour by precursor hydrolysis
- Interest for fruit beers
Release of hidden flavour potential

Glycosidically bound flavour compounds = glycosides

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<td>Side activities possible:</td>
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<tr>
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<td>- HCD activity → 4-VG</td>
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<td>- Anthocyanidin breakdown (loss of colour)</td>
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→ Different treatments lead to different flavour profiles
Selection of yeast strains

**Saccharomyces sp.**
- Mostly **no** β-glucosidase
- All have exo-1,3- β-glucanase activity → Low to moderate activity
- Some commercial wine strains with inducible β-glucosidase

**Brettanomyces sp.**
- Some with **high** β-glucosidase
- Mainly cell associated (low extracellular)
- Also minor glycosidase activities

Diagram showing enzyme activity levels for different strains and glycosides.
Isolation of hop glycosides

Hop pellets (Saaz)

Supercritical CO₂ extraction

Essential oils
Soft resins

spent hops / hop residue / hop solids

1) ethanol / water
2) ethanol evaporation

Watery extract

Solid Phase Extraction (XAD2)
1) wash with water
2) elution with ethanol
3) evaporation
4) PVPP → remove polyphenols

Purification

Hop glycoside extract
Aglycones from hop glycosides

SPME GC/MS

1-octen-3-ol
benzyl alcohol
linalool
methyl salicylate
alpha-terpineol
nerol
dihydroedulan I
theaspirane A
theaspirane B
4-hydroxy-7,8-dihydro-beta-ionol

control pH 5.0

AR2000 enzyme prep.
YPD fermentation with added hop glycosides

Linalool:
- citrus, floral, aniseed
- important contributor to hop aroma

Methyl salicylate:
- wintergreen, minty, spicy

## Concentration (mg/l)

### Control at pH 5.0 without yeast

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<thead>
<tr>
<th>Yeast Strain</th>
<th>Concentration (mg/l)</th>
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<tbody>
<tr>
<td>Sc BY4742</td>
<td>0.000</td>
</tr>
<tr>
<td>Sc LD40</td>
<td>0.000</td>
</tr>
<tr>
<td>Sc LD25</td>
<td>0.000</td>
</tr>
<tr>
<td>Sc UVA228</td>
<td>0.000</td>
</tr>
<tr>
<td>Bc LD72</td>
<td>0.014</td>
</tr>
<tr>
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<td>0.014</td>
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### LD72

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<td>0.000</td>
</tr>
<tr>
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<td>0.000</td>
</tr>
<tr>
<td>Bc LD72</td>
<td>0.016</td>
</tr>
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<td>0.016</td>
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Wort fermentation with *S. cerevisiae* brewing strains

- **Wort** + Saaz hop glycosides, or post-fermentation addition of a pre-hydrolyzed extract
- **Fermentation** with *S. cerevisiae* LD25 and LD40 (low and high exo-1,3-β-glucanase)

**Results:**
- Linalool: significant ↑ in extract; similar for 2 yeasts
- Dihydroedulan & theaspiranes: clear ≠ for 2 yeasts
- Sensory: significant increase; other compounds seem to be involved

**Graphs:**
- Linalool
- Theaspirane B
Refermentation of a dry-hopped beer by different yeasts

- **S. cerevisiae**
- **S. cerevisiae + B. bruxellensis**
- **S. cerevisiae + B. Custersii**

### Graphs

**Theaspirane B**
- β-glucosidase activity

**4-ethyl guaiacol**
- Vinylphenol reductase activity

**Isoamyl acetate**
- Esterase activity

**β-ionone**
- Unknown activity
‘Kriek’-beer

Traditionally:
- intact sour cherries are added to wooden casks filled with young lambic
- leaving it for 5 to 6 months
- the sugar from the sour cherries triggers a second fermentation

Currently:
- also cherry pulp, cherry juice and cherry stones
- fruit extracts and essences
- flavours
Aglycones from sour cherry glycosides

Schmid and Grosch (1986): benzaldehyde, linalool and eugenol are some of the important contributors to cherry aroma

SPME GC/MS

Control pH 5.0

AR2000
Cherry stones contain the cyanogenic glycoside amygdalin

\[
\text{Amygdalin} + 2 \text{H}_2\text{O} \xrightarrow{\text{beta-glucosidase}} 2 \text{HCN} + \text{(R)-Mandelonitrile} + \text{Benzaldehyde}
\]

hydroxynitrile lyase or spontaneous

\[
\text{Benzyl alcohol} \xrightarrow{\text{chemical or enzymatic esterification}} \text{Benzyl acetate}
\]

\[
\text{Benzyl-beta-D-glucoside} \xrightarrow{\text{beta-glucosidase}} \text{Amygdalin}
\]
Development of cherry flavour

- Refermentation of a base beer, with addition of **200g sour cherries** per liter
- Yeast: **Brettanomyces custersii** strain, selected for its high beta-glucosidase activity

**Results:**
- Increase in benzyl compounds (especially benzaldehyde reduced to benzylalcohol)
- also other compounds important for cherry flavour: geraniol, eugenol, linalool

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**Concentration [mg/L]**

**eugenol**
- clove, spicy, medicinal flavour
- released from eugenyl-glycoside
- flavour threshold: 0.040 mg/l

**geraniol**
- floral, rose-like
- released from geranyl-glycoside
- flavour threshold: 0.010 mg/l
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<td><em>Hop</em> flowers, pellets or hop solids</td>
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<td><em>Cherries</em> (whole, juice, pulp and/or kernels)</td>
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<tr>
<td>Other fruits, spices, ...</td>
</tr>
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</table>
~ Thank you for your attention ~
Back-up slides
Hop glycoside flavour potential

- Concentrated hop glycoside extract in buffered medium
- Control at pH 5.0
- Acid hydrolysis at pH 3.0
- Enzyme hydrolysis (almond β-gluc.)
- Enzyme hydrolysis (AR2000)

- Release of sensory active aglycones
- Possible impact on the overall beer flavor?
**Brettanomyces LD72 identification**

1. **1st identification → physiological tests**
2. **2nd → PCR fingerprinting (primers M13 and OPA09)**

- *Brettanomyces custersii CMBS LD72*
- *Dekkera anomala CBS 8139*

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### Table 8: Adaptation-resistant yeasts in lambic fermentation (Verschuere and De Man, 1990)

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<thead>
<tr>
<th>Brettanomyces species</th>
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<th>Kloeckera</th>
</tr>
</thead>
<tbody>
<tr>
<td>abstinens</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>anomalous</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>bruxellensis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>custersianus</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>custersii</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>intermedius</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>lambicus</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>naardenensis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>nanonanus</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Number of species: 2 → 1

% of total: 0 → 6

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*a*: indicated by italic type
Experimental set-up of dry-hopping trial

- lagering tank ale
- dry hopping
  - normal (3.0 g l⁻¹)
  - concentrated (15.0 g l⁻¹)
  - spent hops (15.0 g l⁻¹)
  - no dry hopping
- S. cerevisiae + B. custersii + glucose
  - samples withdrawn after 1, 8, 18 and 32 days
  - glucoside hydrolase activity against model substrate pNPG
  - analysis of volatile compounds by GC-MS

Sc, Sc+Bb, Sc+Bc, Sc+AR
## HCN potential in sour cherry stones

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<th>Regulation of HCN in foodstuffs</th>
<th>EU-directive 88/388/EEC (1988)</th>
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<td>1 mg HCN per (v/v)% alcohol</td>
</tr>
</tbody>
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| Example: cherry beer            | 4 (v/v)% alcohol                 |
|                                 | maximum 4 mg HCN / litre         |

### 200 g cherries / litre beer

| Released benzaldehyde:          | 5.1 – 7.1 mg / litre             |
|                                 | threshold: 2 mg / litre          |

| 10 - 14 g stones / litre beer   | amygdalin in stones: 0.22 (w/w)% |
|                                 | (Chandra et al. 1993)            |

| 22.0 – 30.8 mg amygdalin / litre beer | MW$_{HCN}$ = 27 g/mol  |
|                                       | MW$_{amyg}$ = 457 g/mol |

| 1.30 – 1.82 mg HCN / litre beer    | < 4 mg HCN / litre !          |

### Considering:

- maximum release
- no evaporation

| Lethal dose | 42 mg HCN / 70 kg body weight |