Effect of hopping regime, cultivar, and yeast on terpene alcohol content in beer.

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Basic Yeast/Hop Interactions

- Stripping of volatiles
  - CO$_2$ production
  - Adsorption
  - Partitioning (e.g. foam)

- Solubility changes
  - Ethanol increase
  - Aroma masking
  - Direct Biotransformation

Sesquiterpene loss during fermentation (King and Dickinson, 2003)

caryophyllene  humulene
Yeast modification of hop derived compounds

- Carbonyls reduced to hydroxyls (Mielgard 1986)
- Ester hydrolysis and trans-esterification (Peacock 1981)
- Hop degradation products to fruity esters (Nielsen 2009)
- Cysteine conjugates are transformed into thiols (Nizet 2013)
- Monoterpene alcohols are isomerized (King 2003)
- Glycosidically bound aroma precursors are hydrolyzed (Kollmannsberger 2006)
Glycosides

- Sugar bound molecules
- Water soluble
- Non-volatile
- Used for storage and transport in plants

- Important source of aroma in wine
- Found in hops

Linalyl Glycoside (non-volatile)
Glycoside Hydrolysis

β-Glucosidase optimal pH

Other terpenoid aglycones
- Geraniol
- Nerol
- β-citronellol
- α-terpineol
- Terpin-4-ol
- Z-3-hexanol
- 1-octanol

(Kanauchi and Bamforth, 2012)
Objectives

1. Determine range of β-glucosidase activity in brewing yeast
2. Monitor hydrolysis throughout fermentation
3. Determine effect of yeast β-glucosidase activity on aglycone content in beer
4. Determine effect of hopping regime on glycoside extraction
β-Glucosidase Activity: Yeast Screening
Yeast β-Glucosidase Activity Analysis

\[
\text{4-MUG} \quad + \quad \text{Yeast} \quad \rightarrow \quad \text{Exλ = 365nm Emλ = 445nm}
\]

Yeast Types

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<thead>
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<th>Type</th>
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<td>Ale</td>
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<td>Other</td>
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Yeast Screening: β-Glucosidase Activity Results

Brewing Yeasts

- **Cell Associated**
- **Extracellular**
- **Total**

Specific Activity (U L^{-1} O.D_{605}^{-1})

Ale
Brett
Lager
β-Glucosidase Activity: Bench top trials
When does hydrolysis occur during fermentation?

Bench Top Trials
- 1 L wort (12P) @ 18 C, 25 ppm iso
- Octyl-glucopyranoside → 100ppb 1-octanol

Treatments
- Low enzyme(-) and high activity(+) ale yeast ferments
- Excess (>250 U/L) purified Bgase (calzyme)
- Control (no enzyme)

Monitor 1-octanol over time via SPME-GC-MS
When does hydrolysis occur during fermentation?

Octyl-glycoside → Hydrolysis → 1-Octanol

Hydrolysis of octyl-glucopyranoside

Percent

0 20 40 60 80 100

Hours

0 24 48 72 96 120 144 168 192 216 240

- Enzyme
- ale(low)
- control
- ale(high)
Yeast β-glucosidase activity and terpene alcohol content
Does increased yeast β-glucosidase activity increase aglycone content in beer?

- Hopping
  - Simcoe Whirlpool (25 min)
- Treatments
  - 12 different brewing yeasts
  - Excess enzyme
  - Control-no enzyme

Bench Scale Ferment: 1L, 12°P wort @ 18°C

SPME GC-MS Volatile Analysis (n=2)
- Linalool, Geraniol, Nerol, β-citronellol, α-terpineol, Terpin-4-ol, Z-3-hexanol, 1-octanol* (octyl glycoside)
Does increased yeast β-glucosidase activity increase aglycone content in beer?

Mean geraniol concentration by SPME

Mean β-citronellol concentration by SPME

Mean linalool concentration by SPME

Mean nerol concentration by SPME
Glycoside Extraction by Different Hopping Regimes
Does hopping regime influence glycoside extraction?

- Hopping
  - Kettle boil (60 min)
  - Whirlpool (25 min)
  - Dry hop (72 hours @ 18°C)
- Cultivar
  - Simcoe, CTZ, HHA, Centennial
- Enzyme
  - β-glucosidase 72 hours
  - Control-No Enzyme

**Benchtop boils:** 2L, 12°P wort (n=3, N=72)

**SPME GC-MS Volatile Analysis (n=2, N=144)**
- Linalool, Geraniol, Nerol, β-citronellol, α-terpineol, Terpin-4-ol, Z-3-hexanol, 1-octanol* (octyl glycoside)
Does hopping regime influence glycoside extraction?

- **Hopping**
  - Kettle boil (60 min)
  - Whirlpool (25 min)
  - Dry hop (72 hours @ 18C)
- **Cultivar**
  - Simcoe, CTZ, HHA, Centennial
- **Enzyme**
  - β-glucosidase 72 hours
  - Control-No Enzyme

**SPME GC-MS Volatile Analysis (n=2)**
- Linalool, Geraniol, Nerol, β-citronellol, α-terpineol, Terpin-4-ol, Z-3-hexanol, 1-octanol* (octyl glycoside)
Does hopping regime influence glycoside extraction?

Concentration Linalool by SPME

No significant difference between enzyme (E) treatments and no enzyme (NE) treatments.
Summary

• Brewing yeast exhibit wide range of glycosidase hydrolysis activity.
• Maximum hydrolysis occurs within 3 days of primary fermentation.
• Aglycone content did not increase in enzyme treated beers.
• No strong relationship between activity and increased aglycone content.
Glycoside content varies by cultivar

Total [terpene alcohol aglycone] from spent hop extracts

Cultivar

Vollmer and Shellhammer
Isomerization of terpenoids during fermentation

King and Dickinson, 2003
Thank You

Photo credits: Lynn Ketchum and John Castle
References


