Hop and Yeast Interactions

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Lallemand’s Core Activity

Development, production and marketing of yeast and bacteria... and their derivatives
Overview

- A brief review of studies regarding hop derived compounds and how they are modified by yeast during fermentation
- Starting off simple: Hop composition & yeast factors
- Known Interactions
- Hop Derived Compounds:
  - Carbonyl Compounds
  - Esters
  - Monoterpenes Alcohols
  - Glycosidically bound precursors
  - Hop Degradation Acids
  - Thiol precursors
- Trials
Background
What is “Biotransformation’?”

- Biotransformation = “alteration of organic compounds by organisms or enzymes”
- “an array of complex chemical processes during which yeast cells transform hop components into (new), often boldly aromatic compounds”
- “oil components that yeast have modified”
- “Yeast derived impact or modification of hoppy aroma”
Why “Biotransformation”? 

It’s a small (but important!) part of hoppy aroma in beer 

We know that fresh/processed hop aromas are different then that of the final beer aroma

• Disproportionate extraction
• Losses by e.g. evaporation or adsorption
• Modification of hop derived volatiles by yeast enzymes
Hop Composition

POLYPHENOLS
RESINS
ESSENTIAL OILS
Hop Composition

ESSENTIAL OILS

- Hydrocarbon fraction (50-80%)
  - Myrcene, Caryophellene, Farnesene, Humulene, ...
- Oxygenated fraction (20-50%)
  - Linalool, Geraniol, Nerol, ...
- Sulphur fraction (<1%)
Hop oil fractions

Hydrocarbons
  - Aliphatic
  - Monoterpenes
  - Sesquiterpenes

HOP OILS
  - Oxygenated
    - Alcohols
    - Aldehydes
    - Acids
    - Ketones
      - Esters
      - Epoxides
      - Miscellaneous
  - Sulfur
    - Thioesters
    - Straight Chain Sulfides
    - Cyclic Terpenoid Sulfides
    - Miscellaneous

Myrcene
Estimated threshold: ~30 ppb in beer (Hieronymus, 2012)

3-sulphanylhexan-1-ol
Estimated threshold: ~60 ng/L (Takoi, 2006)

Linalool
Estimated threshold: ~10 ppb in beer (Almaguer, 2014)
Yeast Factors

- Yeast are complex
- Many factors influence how yeast behaves
Yeast Factors: DH during late primary / early secondary

Reasons to dry hop with yeast:

• dissolved oxygen protection
• natural mixing by convection
• biotransformation of hop oil compounds

Things to consider:

• stripping of aroma
• loss of volatiles due to adsorption onto biomass
Commonly Known Interactions

• Stripping compounds during fermentation\(^{(1,2)}\)
  • CO2 production/Stripping
  • Adsorption
• Masking?
• Biotransformation
Volatile compounds are lost by stripping

**carbon dioxide evolution rate**

**release curves of aroma compounds**

Haefliger and Jeckelmann, Anal. Methods, 2013, 5, 4409-4418
Influencing Factors (not discussed here)

- Yeast strain
- Cell count
- Temperature
- Point of hop addition
- Contact time
Various Studies Review
Hop Derived Compounds

- Carbonyl compounds (also from derived from malt, mashing, boiling)
- Esters (branched chain esters, not found in unhopped beer)
- Monoterpenene alcohols
- Glycosidically bound precursors
- Acids
- Polyfunctional thiols
Carbonyl Compounds

Carbonyl $\rightarrow$ Alcohols

Dehydrogenases and reductases

Example: methyl ketones are partially reduced to the corresponding secondary alcohols$^{(3)}$
Esters can be hydrolyzed or trans-esterified

- Geranyl and citronellyl acetate esters are formed by lager yeast, but not by ale yeast \(^4\)
- Esterase activity?
- Chemical esterification during beer ageing alters flavor
Monoterpenic alcohols

- Responsible for spicy/floral/hoppy aromas
- Do not transform spontaneously, but are catalyzed by yeast (or low pH)
  - *Saccharomyces cerevisiae* can reduce, translocate, and isomerize monoterpenic alcohols

![Chemical structures of monoterpenic alcohols](image)
Monoterpene alcohols - Geraniol

- Research from e.g. Takoi et al. classified hops into two categories:
  - Free geraniol dominant hops
  - Geraniol precursor dominant hops

- Sources of geraniol: free geraniol, geraniol precursors, and geranyl acetate (5)

- Geraniol decreases during first 3 days of fermentation – is used for ergosterol biosynthesis (5)
Glycosidically bound geraniol precursors

Geranyl esters (geranyl acetate, geranyl isobutyrate, etc.)

Ergosterol biosynthesis

geraniol

citronellol

nerol

Linalool

\( \alpha \)-terpineol

(5) K. Takoi et al. 2017
Glycosidically bound precursors

What are glycosides?

• Sugar bound molecules
• Non-volatile
• Found in hops\(^{(2)}\)

Research has shown that glycosidically bound aroma precursors are hydrolyzed.\(^{(2)}\)

• Non-aromatic compounds are hydrolyzed: releasing one aromatic compound, one glucose, and one molecule of water
• \(\beta\)-glucosidase enzyme
Glycoside Hydrolysis

\[
\text{ENZYME (}\beta\text{-glycosidase)}
\]

\[
\text{LINALYL GLYCOSIDE (Non-aromatic)}
\]

\[
\text{CARBOHYDRATE (Glucose)} \quad \text{HOP OIL (Linalool)} + \text{H}_2\text{O}
\]
Researchers from OSU have done significant research in this area:

- Found evidence that hops contain substantial amounts of glycosidically bound volatiles
- Brewing yeast exhibit wide range of glycosidase hydrolysis activity
- Maximum hydrolysis occurs within 3 days of primary fermentation
- Since glycoside concentration in wort are low, yeast B-glucosidase activity makes small contributions to hop aroma
### Sensory impacts

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<tr>
<th>Enzymatic hydrolysis</th>
<th>Addition of enzyme</th>
<th>Without enzyme</th>
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<tbody>
<tr>
<td>β-Glucosidase, pH 5, 24 h, 40 °C</td>
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<tr>
<td>3(Z)-Hexenol</td>
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<td>1,5-Octadien-3-ol</td>
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<td>α - Terpineol</td>
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<td>8-Hydroxy-linalool II</td>
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<td>Benzylalcohol</td>
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<td>3-Hydroxy-7,8-dihydro-β-ionol</td>
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<table>
<thead>
<tr>
<th>Aroma</th>
<th>Mostly grapefruit and pine with some tropical pineapple, orange and caramel backin’ it up</th>
<th>Mostly pine with some citrus, orange and grapefruit, followed by tropical pineapple aroma as well, slight caramel and isoamyl acetate</th>
</tr>
</thead>
</table>

Kollmannsberger et al, 2006
Hop degradation acids

Esterification of short chain hop derived acids

Hop degradation products → ethyl esters (fruity esters)\(^{(6)}\)

Researchers found that ester concentration in beer can increase by the use of aged hops\(^{(6)}\)

This conversion is not particularly clear, but there are probably many factors that play into this.
Polyfunctional thiols

What are Thiols?

• Sulfur containing compounds – highly potent

Free and bound forms available

Bound: Cysteinylated and Glutathionylated thiol precursors are found in hops
Non-volatile

Possible β-lyase activity catalyzing a release of aromatic thiols

(7)
Trials
Lallemand Trials - NaparBier

- Commercial production of a Brut IPA at Naparbier
- Wort split into two separate fermentations: BGl enzyme 1 addition vs. control
- First dry hopping was at day 0, with addition of Chinook and Centennial hops; fermentation held at 18°C
- A 2nd dry hop was added at day 12 using the same hop regime with Chinook and Centennial; temperatures lowered to 16°C.
Lallemand Trials – Garage Beer Co.

- Brewed their house IPA, 100g/hL dried yeast pitch
- Control vs Addition of BGI enzyme
Glen Affric Beers – tasting Preference

A: Enzyme added at yeast pitch
B: No enzyme added
C: Enzyme added at yeast pitch + 25% of the dry hop
D: Enzyme added with the dry hop on day 4

### Mascoma

<table>
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<tr>
<th>Beer</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>Total</th>
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<tbody>
<tr>
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Preference

NRC
Summary

- Aroma of fresh/processed hops ≠ aroma of final beer
- Changes of fermentation parameters or hopping regime can effect beer flavor
- Biotransformation is complex and there is still a lot unknown
- Room for exploration, creativity and research!
References


& more:

Special thanks....... 

Nils Rettberg  
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Sylvie Van Zandycke  
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Thank you!!