

All About Thiols: Practical Ways to Maximize Thiol Potential

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Presentation Overview:

1. Overview of thiols
2. Thiol precursors
3. Biotransformation of precursors to free thiols
4. Brewer's perspective
5. Brewery trials with hops rich in free and bound thiols
6. Incorporating thiols into new and existing brands
7. Future of thiols



Thiols Overview

Thiols are found in many tropical fruits

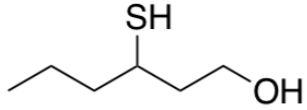
Also what distinguishes new school hops, Sauv Blanc from New Zealand, etc.

Grapefruit, Passion Fruit, Guava



Most commonly discussed thiols in beer

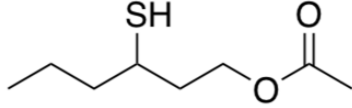
3MH



Passionfruit,
Guava

60 ng/L

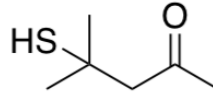
3MHA*



Passionfruit,
Grapefruit

4 ng/L

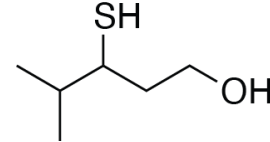
4MMP



Boxtree, Black
Currant

4 ng/L

3S4MP



Passionfruit,
Tropical

70 ng/L

*acetate ester of 3MH and a relevant player in tropical aromas



Let's talk units

ppm: parts per million

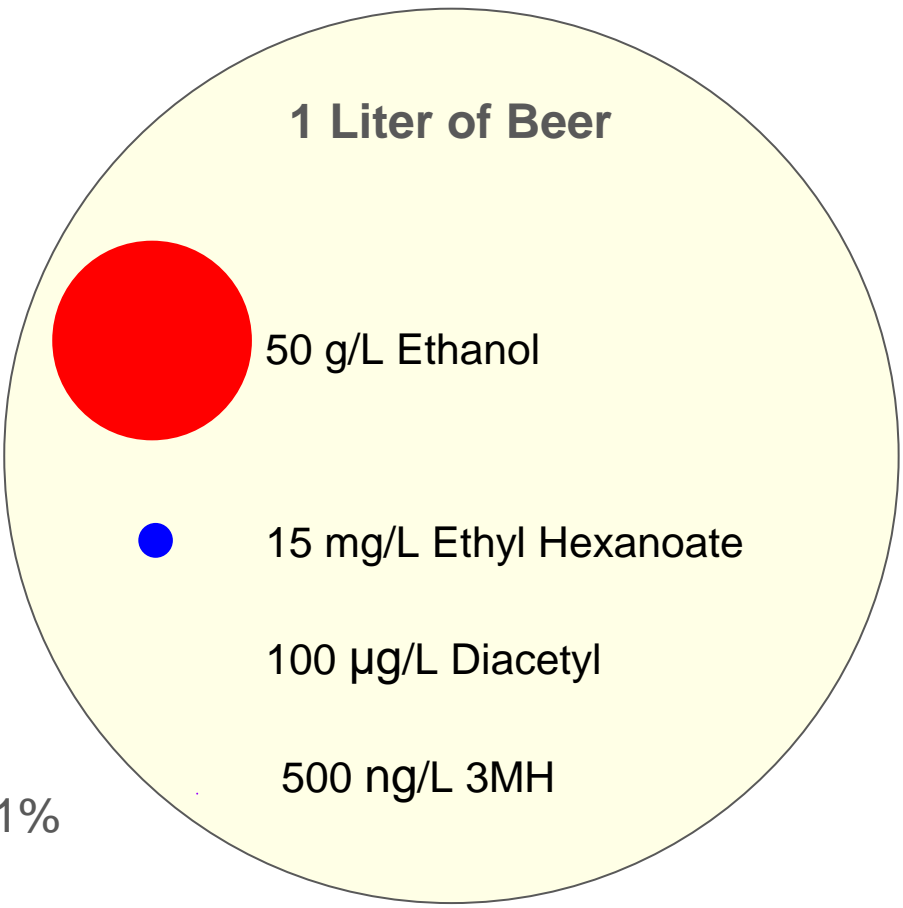
1 ppm = (1×10^{-6}) or 0.0001%
mg/L , mg/kg , $\mu\text{g/mL}$, $\mu\text{g/g}$

ppb: parts per billion

1 ppb = (1×10^{-9}) or 0.0000001%
 $\mu\text{g/L}$, $\mu\text{g/kg}$, ng/mL , ng/g

ppt: parts per trillion

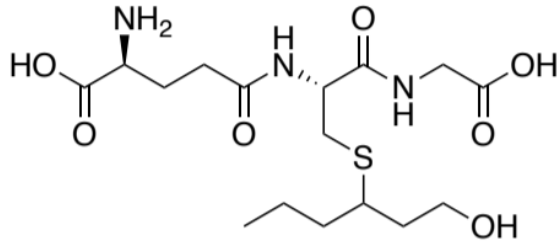
1 ppt = (1×10^{-12}) or 0.0000000001%
 ng/L , ng/kg , pg/mL , pg/g



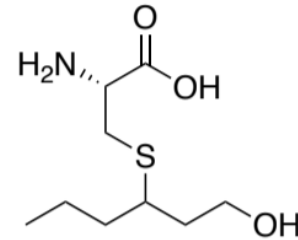
Where do thiols come from: Precursors

Barley and hops have thiol precursors

Most common 3MH precursors are: **Cysteinylated** and **Glutathionylated** thiol precursors (often written seen as Cys-3MH and Glut-3MH)



Glut-3MH

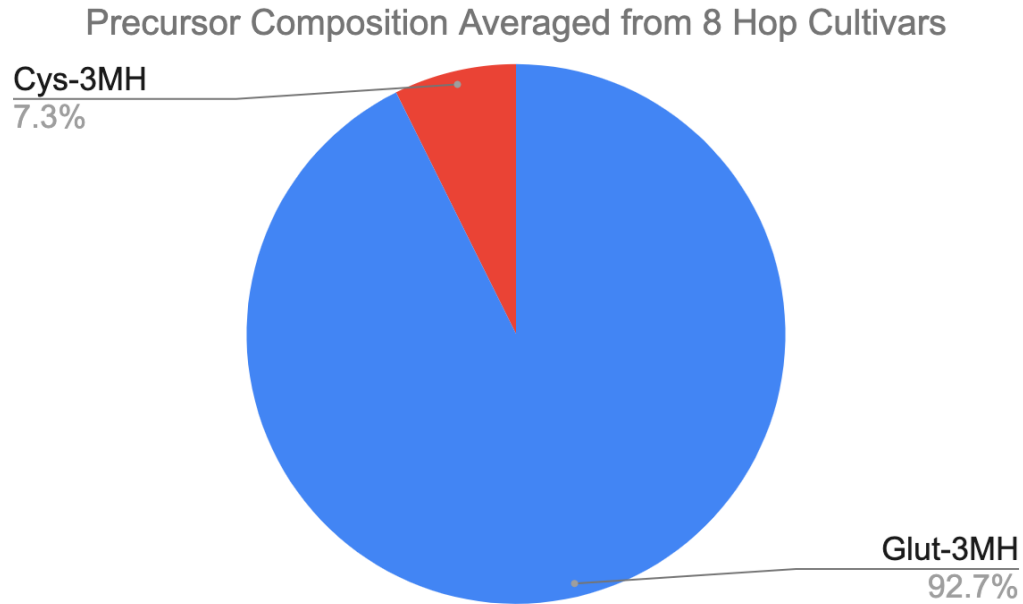


Cys-3MH



Precursor ratios

Glut-3MH is typically more abundant than Cys-3MH in both barley and hops



Adapted from: Roland et al, *Flavour Fragr J.*, 2016



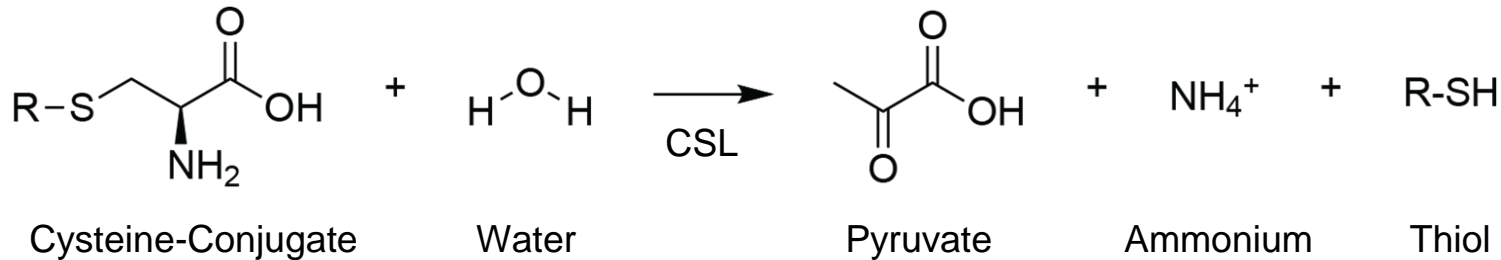
Carbon-Sulfur Lyase mediates the conversion of bound thiol to free thiol

Carbon-Sulfur Lyase (CSL)

-Describes the breaking of a carbon-sulfur bond

Cysteine-S-Conjugate β -Lyase

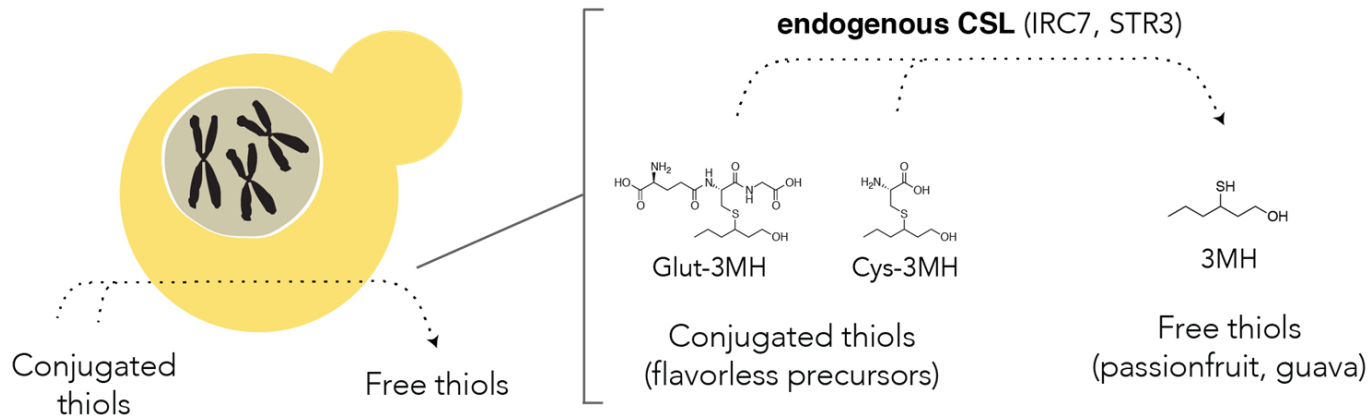
-Describes beta elimination of the substrate cysteine



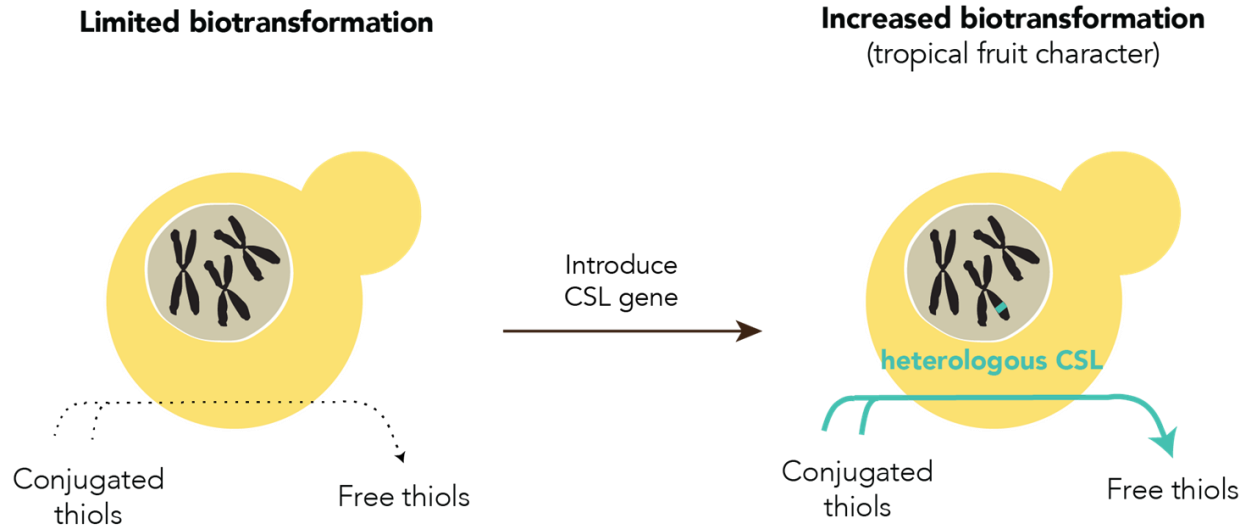
Yeast can biotransform conjugated thiols into free thiols

Carbon-Sulfur Lyase (CSL)

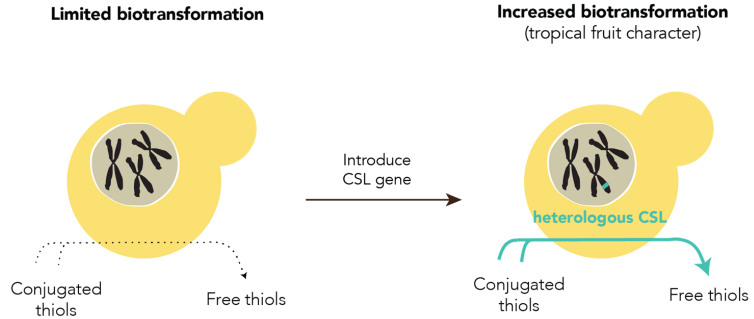
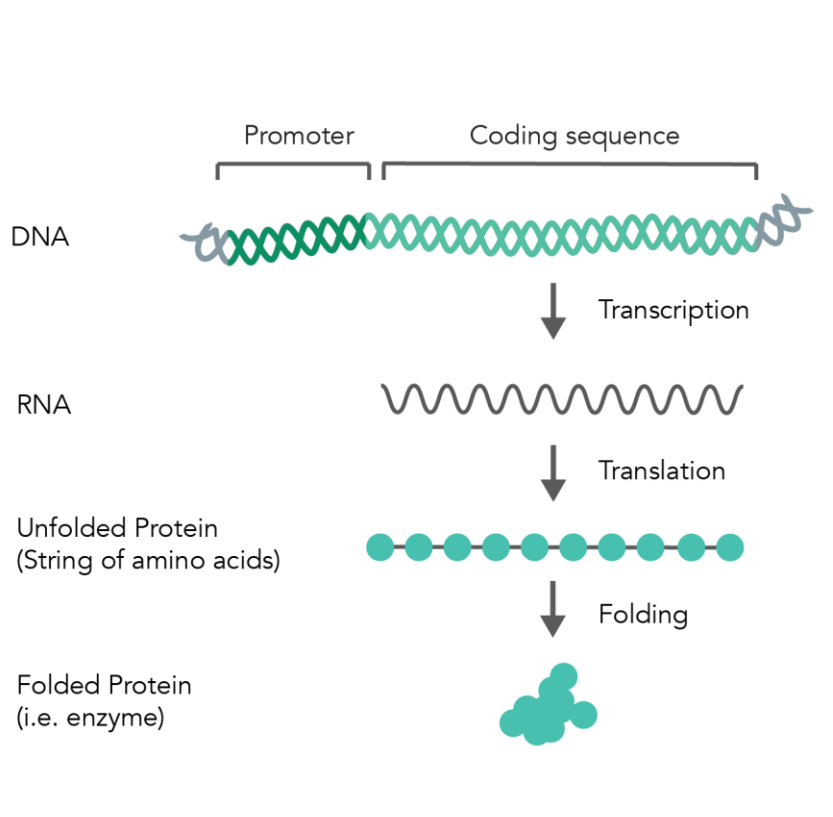
Breaks carbon-sulfur bond to release free thiol



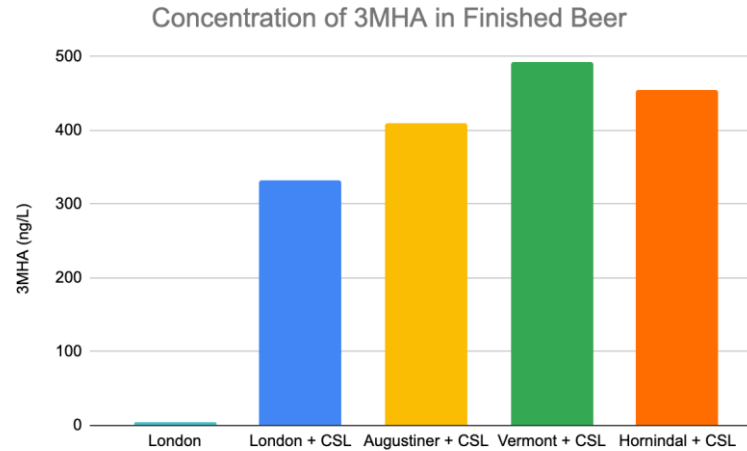
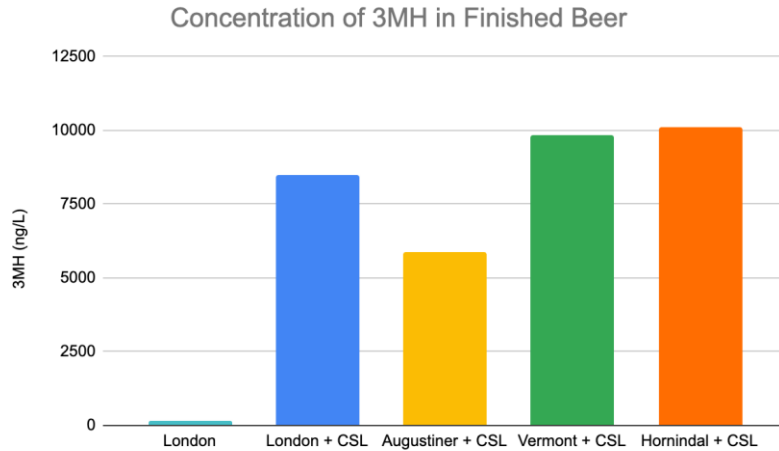
Inserting CSL from *C. freundii* increases biotransformation



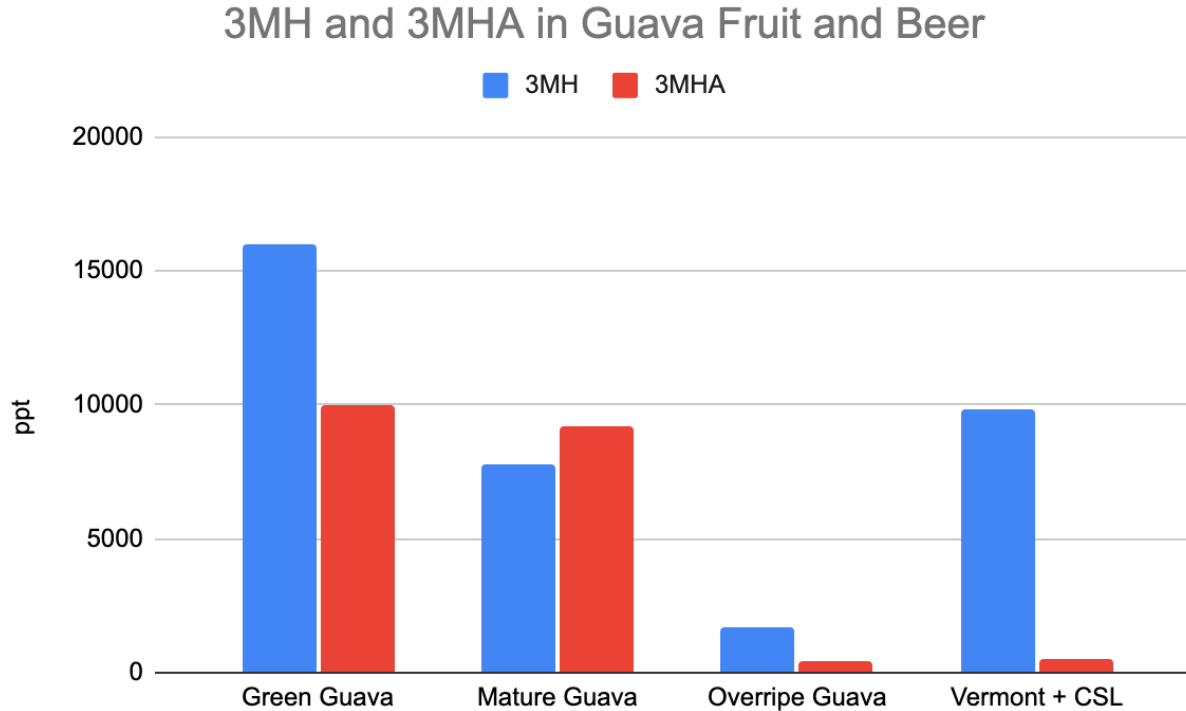
Central Dogma of Molecular Biology



3MH production varies between engineered strains expressing CSL



Comparison 3MH and 3MHA Concentrations in Guava Fruit and Beer

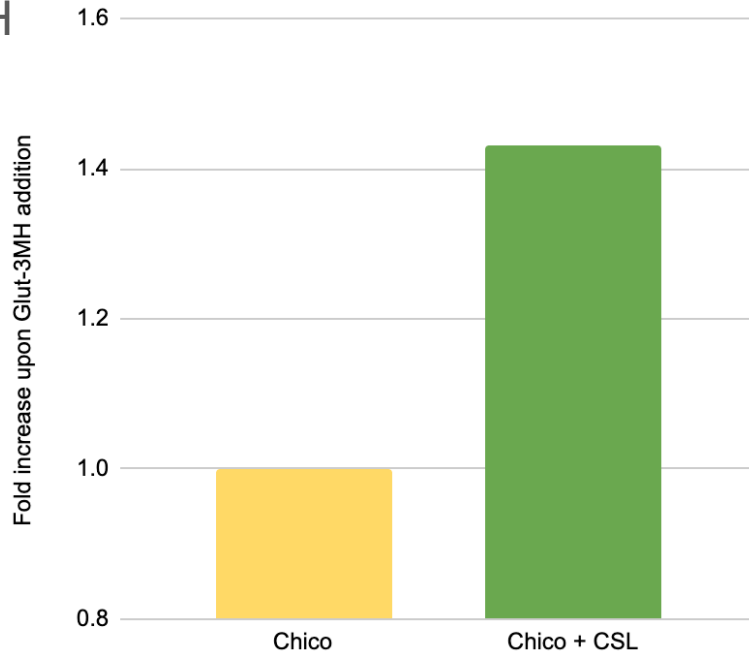


Guava data from: Cannon and Ho, *J Food Drug Anal*, 2018



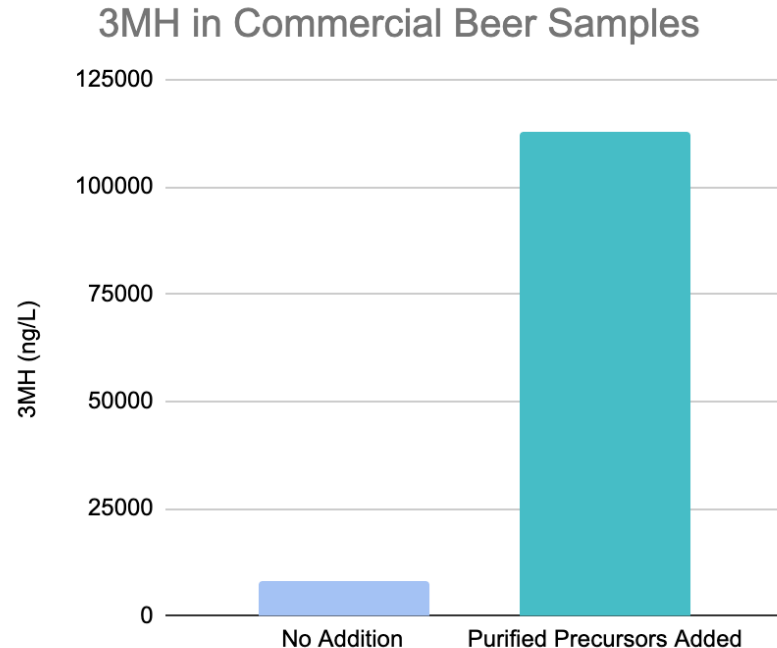
Addition of Glut-3MH increases 3MH production

- Experiment at BY using pure Glut-3MH added to the fermentor (20 L Scale)
- Requires an active CSL
- Unconfirmed if CSL acts on Glut-3MH
- Data indicates that whole-cell conversion of Glut-3MH to 3MH occurs.



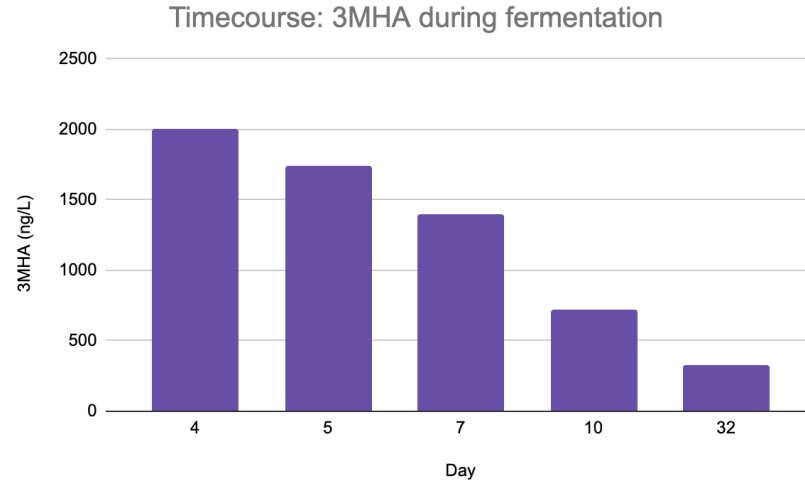
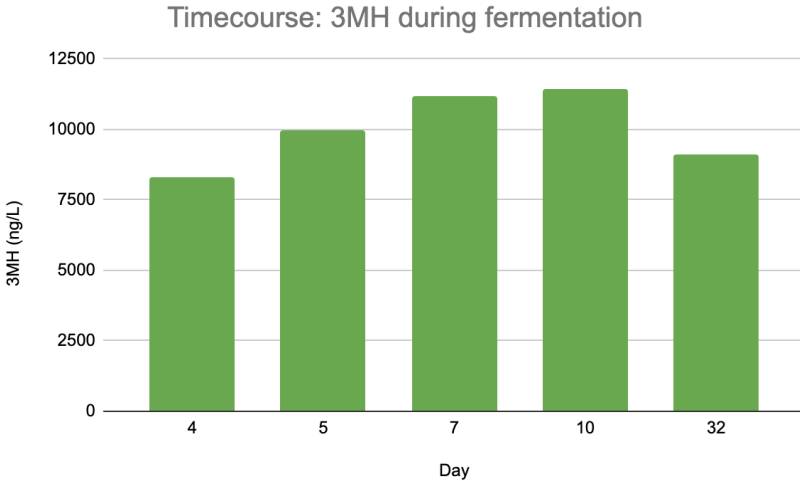
Addition of purified precursors to wort increases final 3MH concentration

- Purified precursors (derived from plants) added ~24 hrs into fermentation
- Requires and active CSL
- At this concentration, beer is tasty, but much higher and 3MH may begin to present as an off-flavor.



3MH is stable but 3MHA decreases over time

-Beer is still tropical/juicy after 32 days, but the flavor profile may change as the ratio of 3MH:3MHA changes



A Brewer's Perspective on Thiols & Precursors



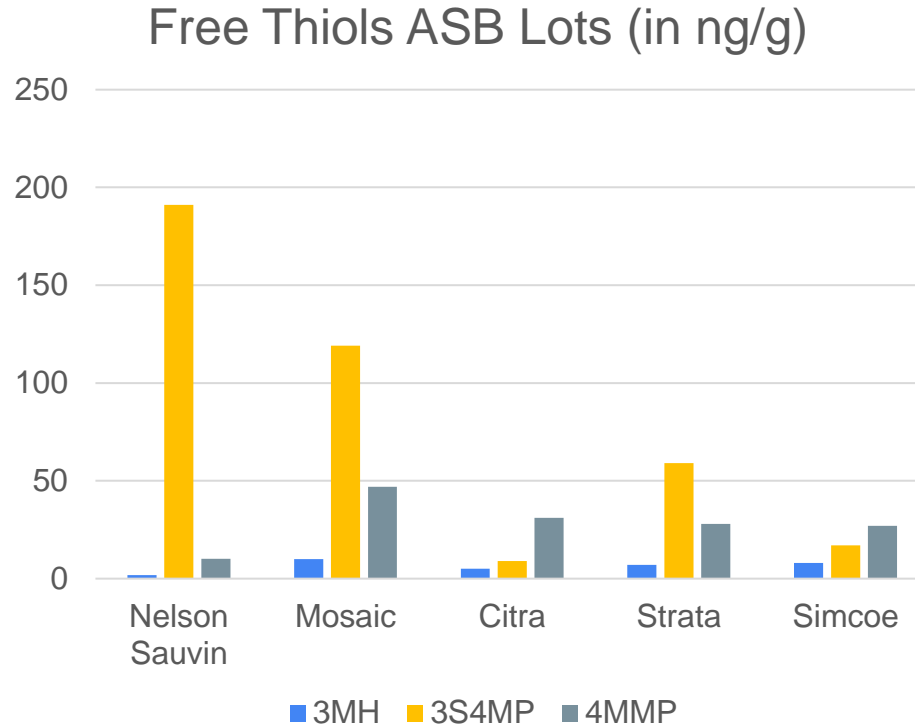
Our Path with Thiols

- First learned about Thiols while visiting Freestyle Farms in New Zealand in 2019
- Examine Thiols in their “Free” form in common hops
- Discuss Trials with Berkeley Tropics yeast
- Examine Common Precursors in Hops to “Front Load” Wort
- Through Trial & Error, look at two ASB ”core” beers focused on maximizing thiols.
- Takeaways, Brewing Implications and the Future of Thiols



Free Thiols in Hops

- 4MMP (threshold: 4.2 ppt)
 - Catty, grapefruit, sauv blanc
- 3MH (threshold: 60 ppt)
 - Passionfruit, tropical, juicy
- 3S4MP (threshold: 70 ppt)
 - Passionfruit, tropical, juicy
- 3S4MP is dominant thiol in Nelson Sauvignon (passionfruit, tropical, juicy)



Source: Hop Quality Group

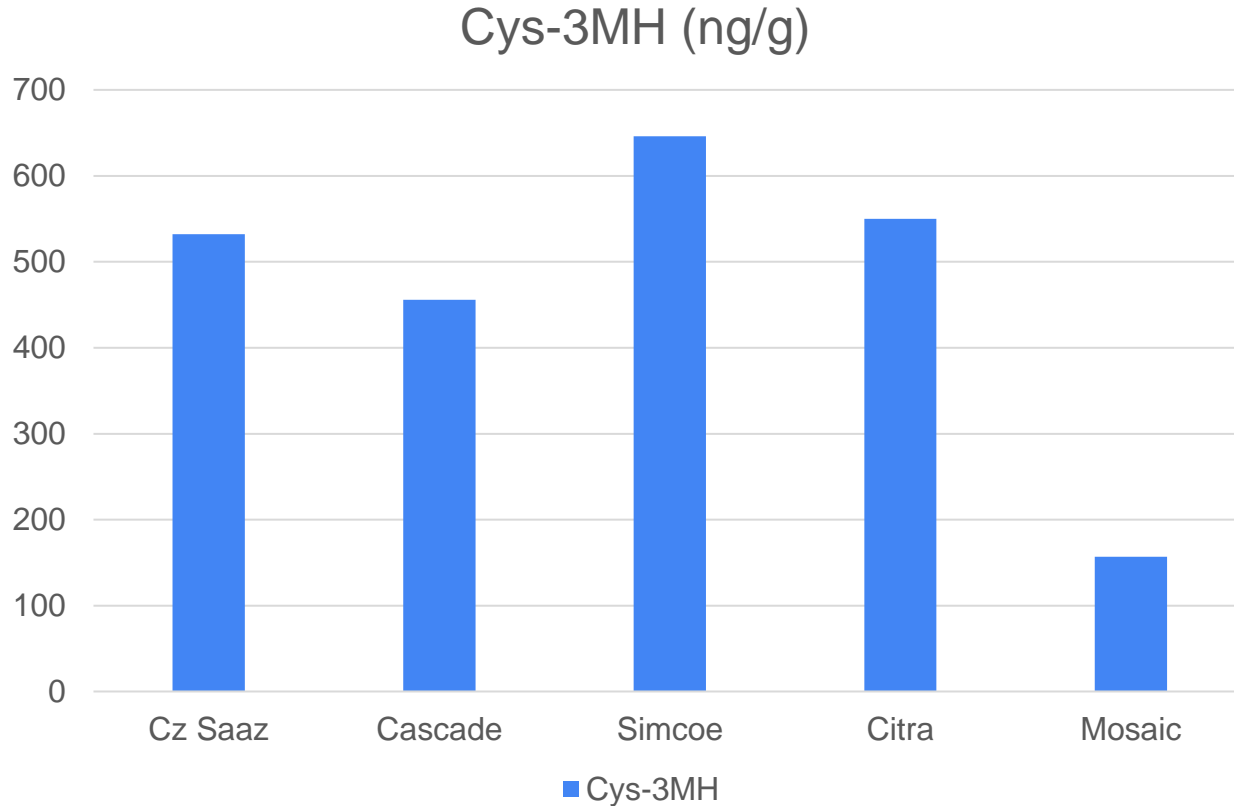


Trials with Thiol Yeast

- Brewed a series of 5 bbl pilot batches to understand implications of bound thiols.
 - First renditions to determine on sensory level the contribution of precursors from grain and minimal hopping.
 - Amazing aromatic properties but unbalanced, “hollow” profile and unstable haze.
- Tried a regular Hazy IPA recipe, not what we expected.
 - Thiols took a back seat in the aroma, dry hop dominated & had a scrubbing effect.
 - Complex flavor profile, could pick up the thiols on the palate.
- ***How do we front load wort with Thiol Precursors from hop products?***



Front Loading Wort with Cys-3MH Precursors

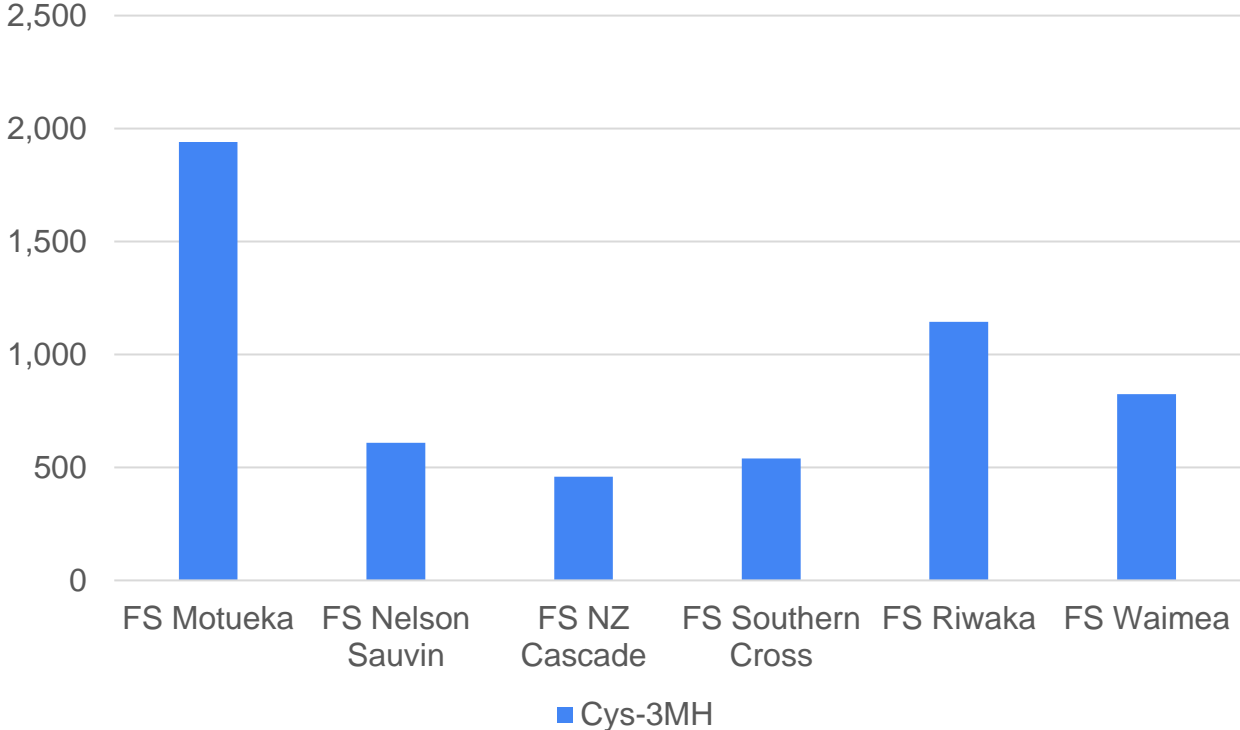


Source: Roland et.al., BarthHaas / Dagan



Front Loading Wort with Precursors – NZ Hops

Cys-3MH (ng/g)



Source: David Dunbar, Freestyle Hops via Nyseos CY2021 Testing



Cold Pressed Hazy IPA

- 2-Row Malt for Base, 25% malted wheat & oats make up the grist.
- Mash Hopping with High Precursor Motueka Lot from Freestyle Hops, just 0.25 lbs / bbl.
- Whirlpool at 175, 0.75 lbs / bbl same Motueka lot.
- Ferment with Tropics yeast at 68 deg.
- Dry Hop 2.75 lbs / bbl with 40% HBC 586, 40% El Dorado, 20% Sabro Cryo.
- Centrifuged to <200 EBC




**BERKELEY
YEAST**


**ALVARADO
STREET
BREWERY**

Contains No Juice Hazy IPA

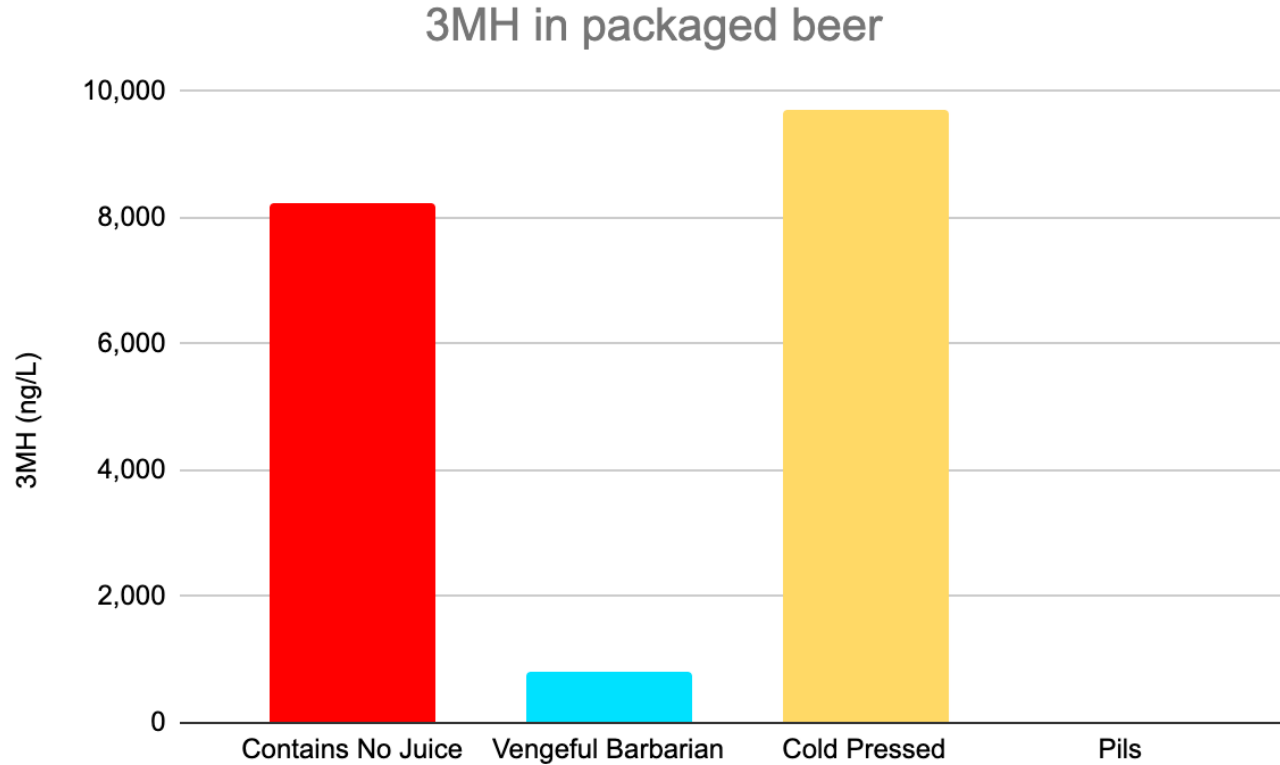
- 2-Row Malt for Base, 25% all wheat, incl. high protein red wheat from Mecca Grade.
- Mash Hopping with US Cascade Hops
- Whirlpool at 175, 2 Kg of Idaho 7 Full Spectrum Plasma & 0.5 lbs. / bbl Simcoe
- Ferment with Tropics yeast at 68 deg.
- Dry Hop 5 lbs / bbl with blend of Mosaic, Citra Cryo, Waimea & Nelson hops.




**BERKELEY
YEAST**


**ALVARADO
STREET
BREWERY**

Final 3MH Concentration in Packaged Beers



Takeaways – Brewing Process

- High-Precursor hops in Mash & Whirlpool (cooled to 175)
- Thiol Yeast for Fermentation (Tropics used in these examples).
- Moderate Dry Hop seems to prevent decrease of 3MH, although difference between 2.75 and 5 lbs was 2,000 ng/L – moderately perceptible. More efficiency with raw materials
- Comparing with Berkeley’s lab trials (unhopped): these beers were tested after packaging (3-4 weeks old), vs. Fresh and in the lab. Likely higher at packaging.
- At what point is the thiol threshold so far beyond perception that there’s little difference between x and y? (i.e. the “100 IBU” fatigue example). Odor Activity Values!
- Vengeful Barbarian shows importance / reliance of monoterpene alcohols to generate hop flavor. Thiols just part of the story.
- We know we’re going to get a TON of 3MH concentration from using thiol releasing yeast, focus on good hops to do the rest of the work.



Future of Thiols & Brewing Implications

- Further testing needed via Mass Spec for Alternative Hop Products
 - Plasma, Incognito, Cryo, Lupomax, Cryo Kief, Distilled Hop Oil, Essences, etc.
 - Phantasm – freeze dried sauv blanc grape powder
- Controls of Non-Thiol yeasts to understand concentration of free thiols in final beer from hops and adjuncts alone, provide a base to understand threshold.
- Understand reasons why dry hopping decreases levels of 3MH.
 - Oxidative effects? Interference with Vegetal Matter? Presence of copper in hops?
- Bound & Unbound Thiols vary from lot to lot of the same hop variety, picking window and harvest date will be critical to determining these levels.
- Understand how to sustain 3MHA over the course of a beer's lifetime.
- Establish sensory thresholds through tasting panels and cross reference with Odor Activity Values (OAV)
- OAV is the measurement of importance of a specific compound to the odor of a sample.



Incorporating Thiols & Monoterpenes in Recipes

- Results from VB show us that Thiols are only part of the story. Must take into account:
 - Presence of Monoterpene Alcohols: Geraniol, Linalool, Citronellol
 - Ester compounds found in hops like 2MIB
- Knowing Free Thiols & Monoterpene Alcohol Concentrations, a brewer can extrapolate when planning dry hops.
 - ***1 Kg T90 Nelson hops / bbl = 1300 ng/L of potential 3S4MP if 100% extraction occurs.***
 - ***1 Kg T90 Sabro Cryo hops / bbl = 497.84 ppm of potential Geraniol if 100% extraction occurs.***
- Thinking about how much "special sauce" could be going into your DH, rather than a blind lbs / bbl benchmark.
- Establish Odor Activity Values for various compounds, then determine how to get there with hops.



Skip the Math...Great Combos

Linalool	Geraniol	3S4MP	3MH	4MMP
Centennial	Sabro	Nelson Sauvignon	Citra	Citra
Zeus	Talus	Mosaic	Simcoe	Mosaic
Idaho 7	Simcoe	Riwaka	US Cascade	Simcoe
Citra Cryo	Idaho 7	Hallertau Blanc	Idaho 7	NZ Casc / Mot



Thanks for listening!

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Tom Nielsen, Sierra Nevada & Hop Quality Group

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