

Squeezing More Tropical Aromas Out of Your Beer



with Thiolized yeast

Bill McFarland – Omega Yeast

Who are we?

Omega Yeast Labs
Chicago, IL / St. Louis, MO

High quality, pitch-ready liquid yeast. Handful of microbiologists, homebrewers, professional brewers and craft beer fans who made it our express purpose to make brewing easier and better for everyone.

- Be Helpful
- Be Creative
- Be Fresh

www.omegayeast.com



TALK OUTLINE

1. Introduction
 - What are Thiols?
 - Biotransformation
2. How thiols are made into beer
3. Thiol Releasing Yeast
 - What enzymes?
 - How brewing strains are engineered
4. Considerations for recipe design
 - Thiol precursors
 - Balancing hop intensity with thiol intensity

WHAT ARE THIOLS?

Tropical and citrus aromas

- Found in many tropical fruits

Very potent aroma compounds

- Threshold in the nanomolar concentrations (parts per trillion!)

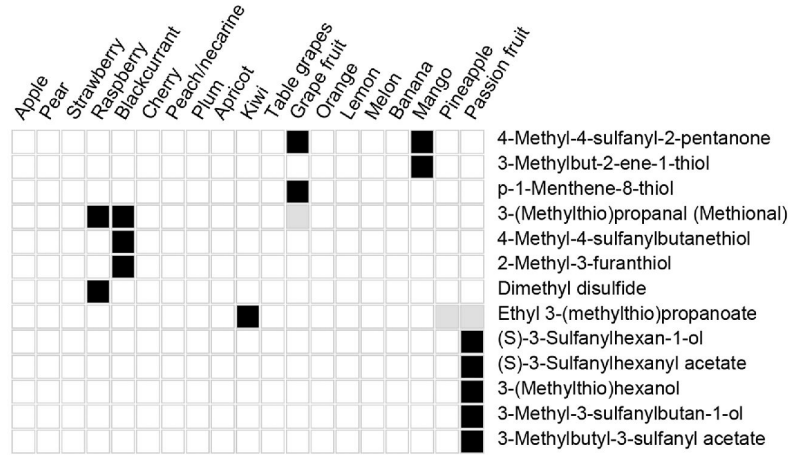
Characteristic flavor

- Sauvignon blanc wines (tropical fruit, citrus)
- Nelson Sauvin, Hallertau Blanc (exotic fruit-like, white wine-like)



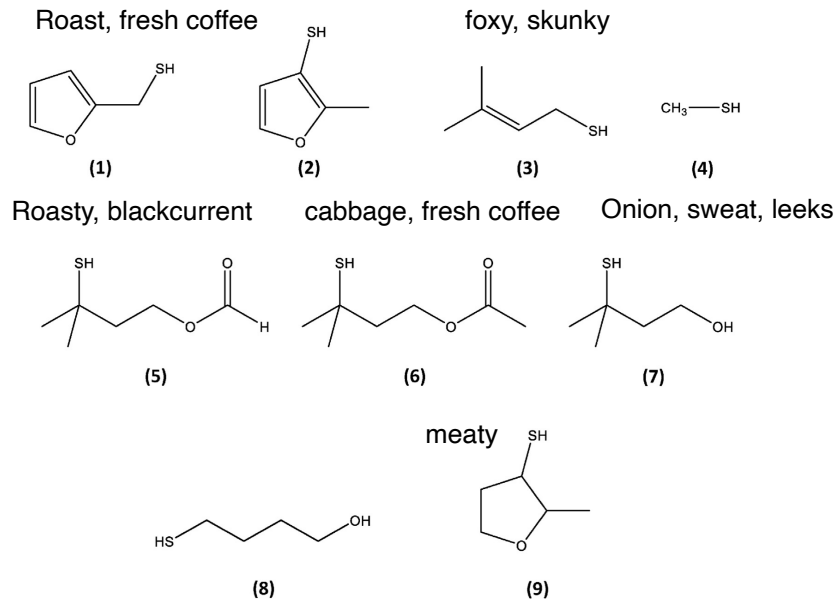
THIOLS CONTRIBUTE SIGNATURE AROMAS

Fruits "Tropical"

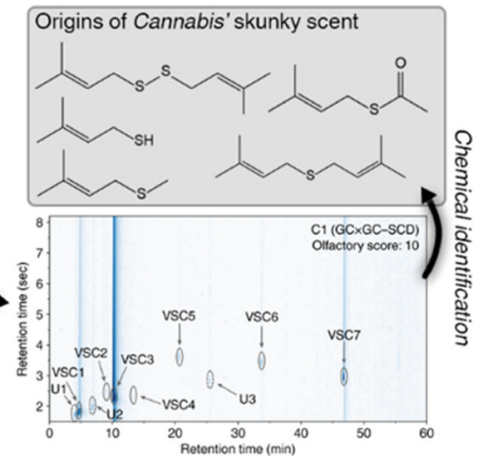


FEMS Microbiology Reviews 2019, 43,193-222

Coffee "Roasty"



Cannabis "Skunk"



THIOLS IN BEER

Most studied:

Polyfunctional Thiol	Sensory	Threshold (ng/L)
4MSP (4MMP)	Box Tree, Black Current	1.5
3SH (3MH)	Grapefruit, Passion Fruit	60
3SHA (3MHA)	Passion Fruit	4
3S4MPol	Grapefruit, Rhubarb	40
3S4MPA	Grapefruit, Rhubarb	120

Common Descriptors:

Tropical
Passion Fruit
Guava
Grapefruit
Skunky
Diesel
Vinyl/Rubber

THIOLS ARE VERY ODOR ACTIVE



21,000 bbls (660,000 gal)

Parts per million (mg/L)



5 beers – 21 bbl
Esters, aldehydes

Parts per billion (ug/L)



2.5 ml - 5 beers
Diacetyl

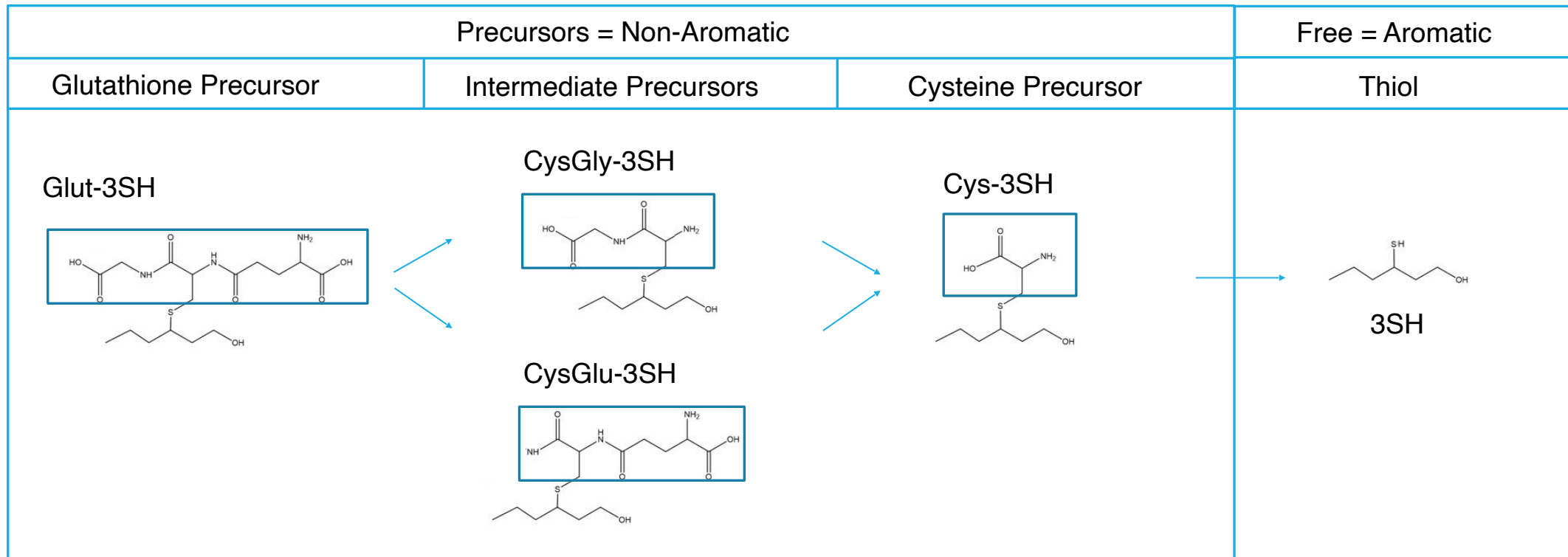
Parts per trillion (ng/L)



2.5 ul – 2.5 ml
Thiols



THIOLS AND THIOL PRECURSORS



HOW DO WE INCREASE THIOL LEVELS IN BEER?

1. Hops with lots of free thiol (tropical, dank)
2. Yeast that can release thiols from precursors

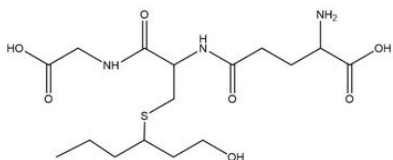


HOW DO WE INCREASE THIOL LEVELS IN BEER?

Plant Synthesis and Breakdown

Yeast Biotransformation

Glut-3SH

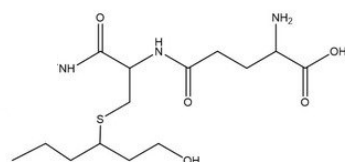


Fatty Acid Metabolism

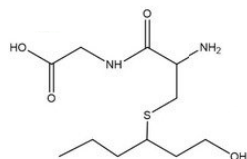
E-2-hexanol (C6) = forms under stress
Detox by conjugating to Glutathione
Stored in the Vacuole



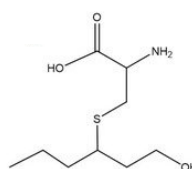
CysGlu-3SH



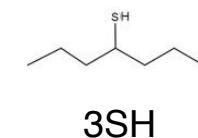
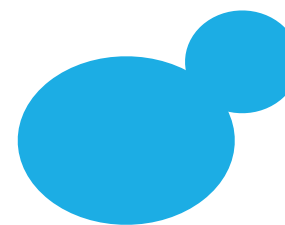
CysGly-3SH



Cys-3SH



3SH

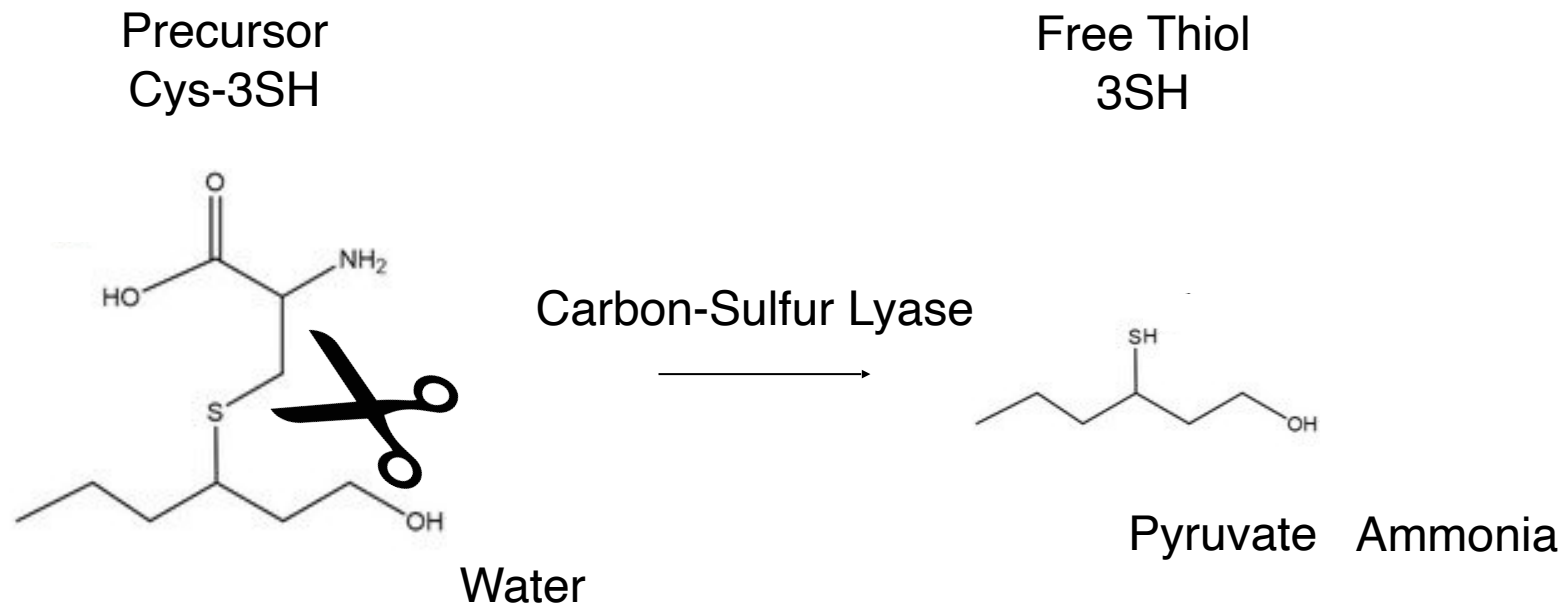


1. Uptake
Transporters
2. Processing to Cysteine Precursor
Gamma-glutamyl transferase
Carboxypeptidase
3. Release thiol
Carbon Sulfur Lyase

Certain Hop Varieties Release during Ripening



CARBON-SULFUR LYASE: RELEASES THE FREE THIOL



Known β -lyase Enzyme in Yeast:



Irc7

β -lyase enzyme that frees thiols from precursors



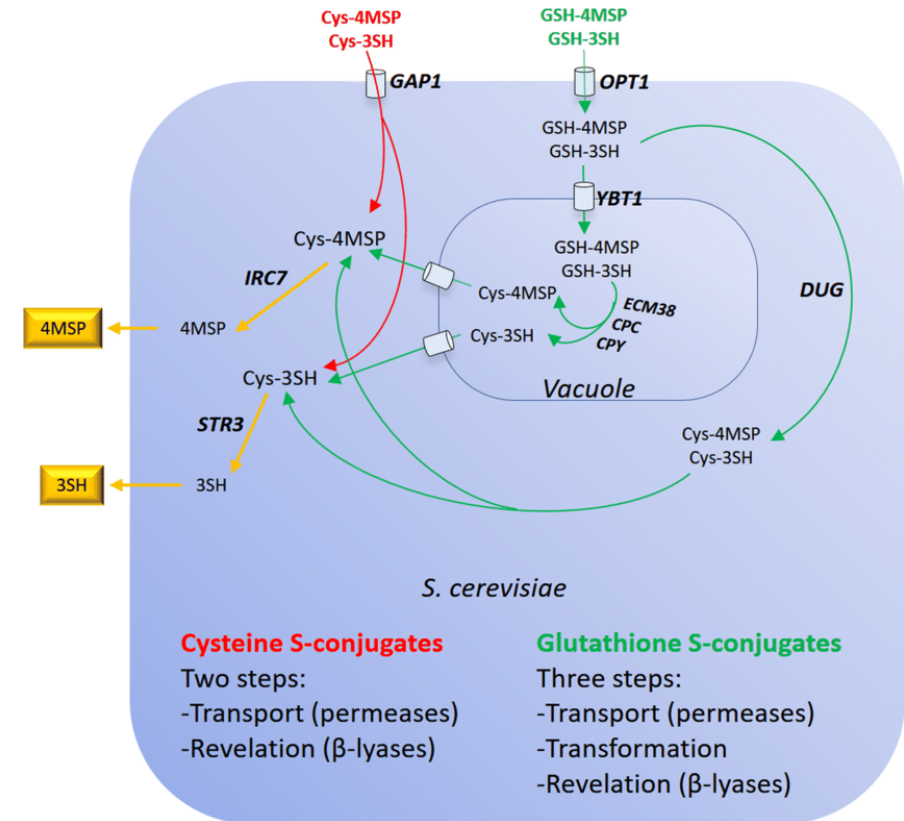
Wine Studies

Irc7 is activated when nutrients are limited
Many wine strains have inactive versions of Irc7



Beer Studies

Limited Irc7 activity in wort

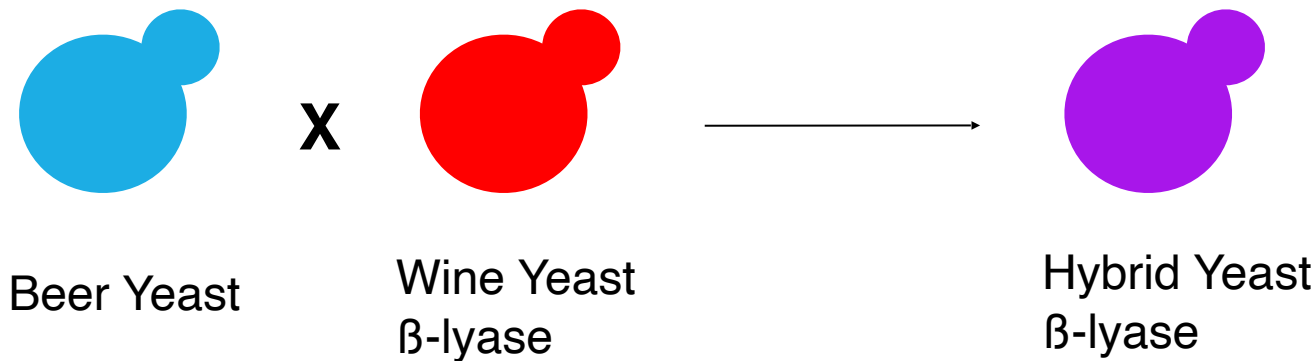


Ruiz et al. App. Micro. And Biotech. 2019



YEAST HYBRID APPROACH FAILED TO SIGNIFICANTLY INCREASE THIOLS IN BEER

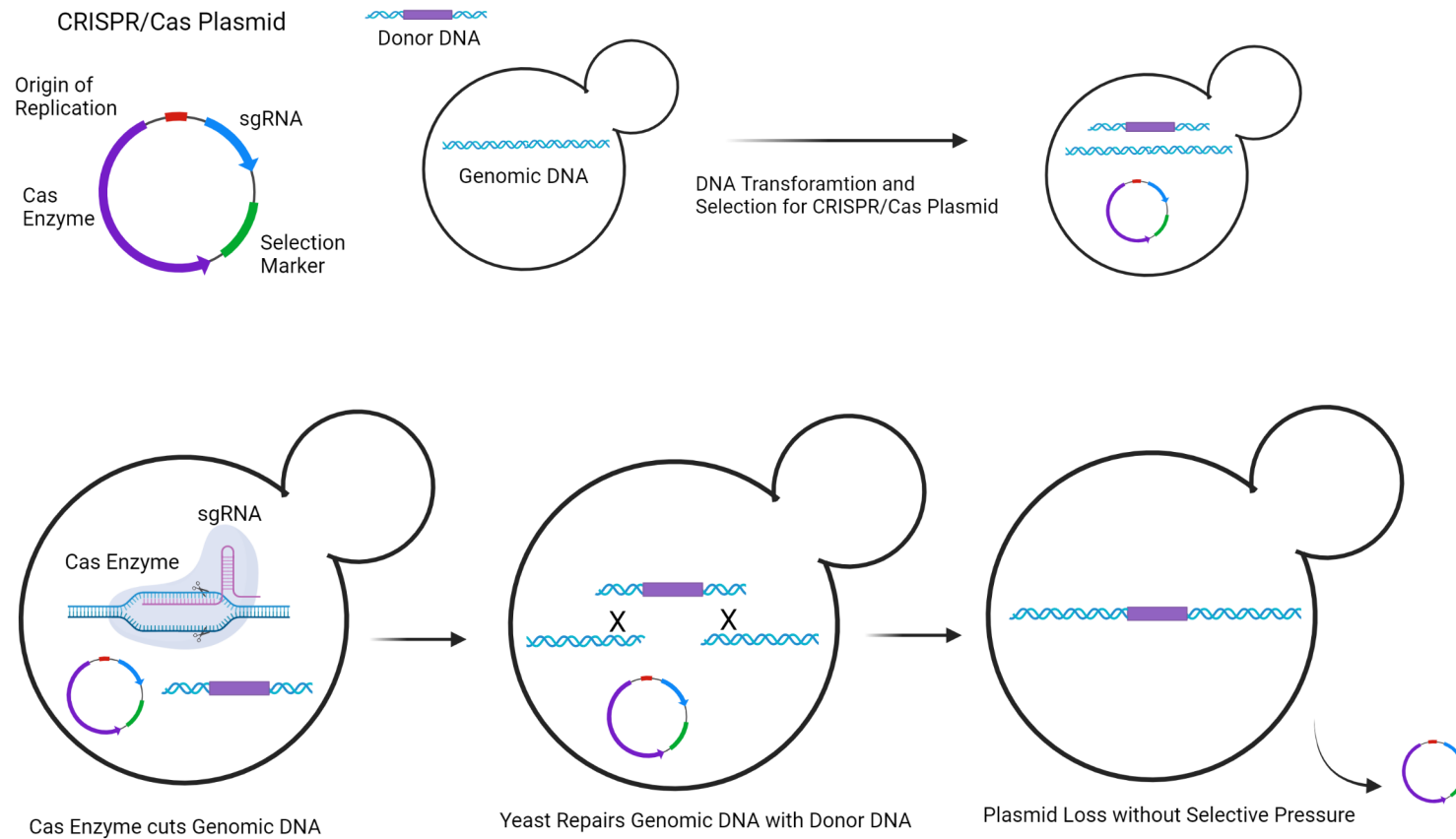
1. Wine strains with high β -lyase activity used to ferment beer.
2. Crossing wine strains with beer strains.
Vin13 x Saison
Maxithiol x Hazy IPA strain



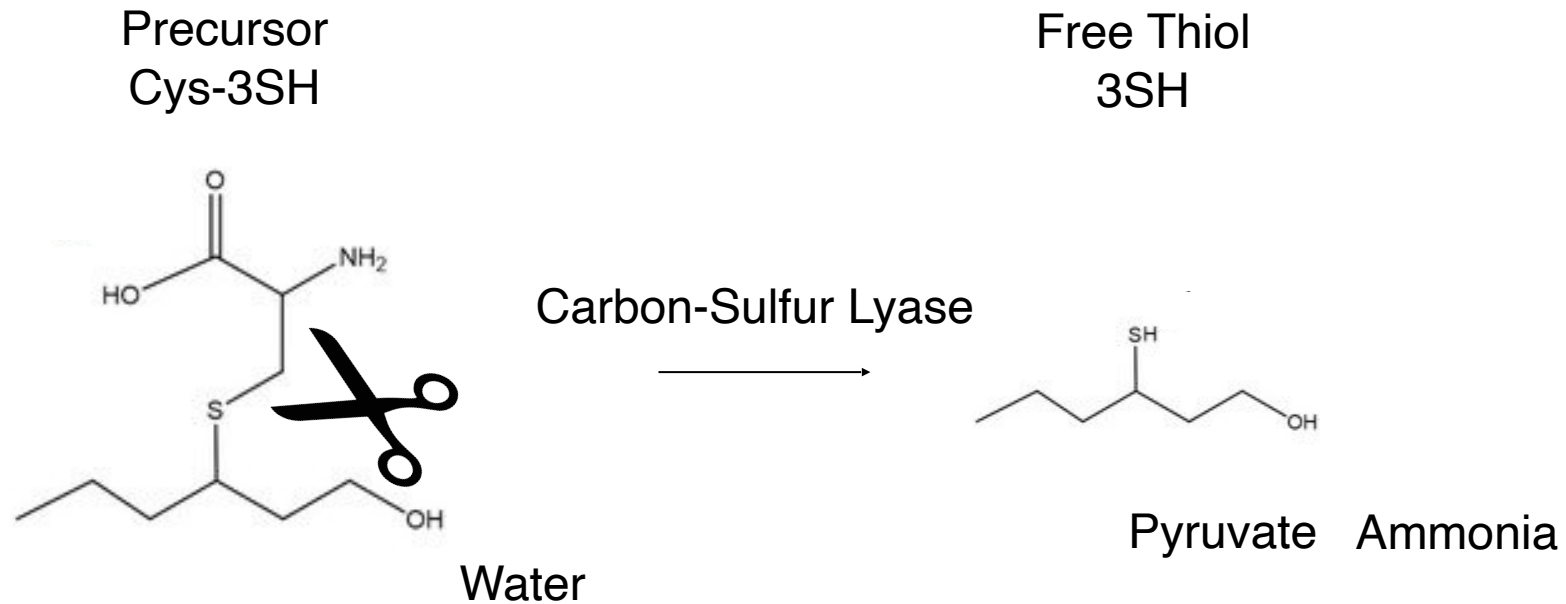
Thiols were not significantly elevated

- Nitrogen levels in beer are in excess
- Low/no IRC7 expression

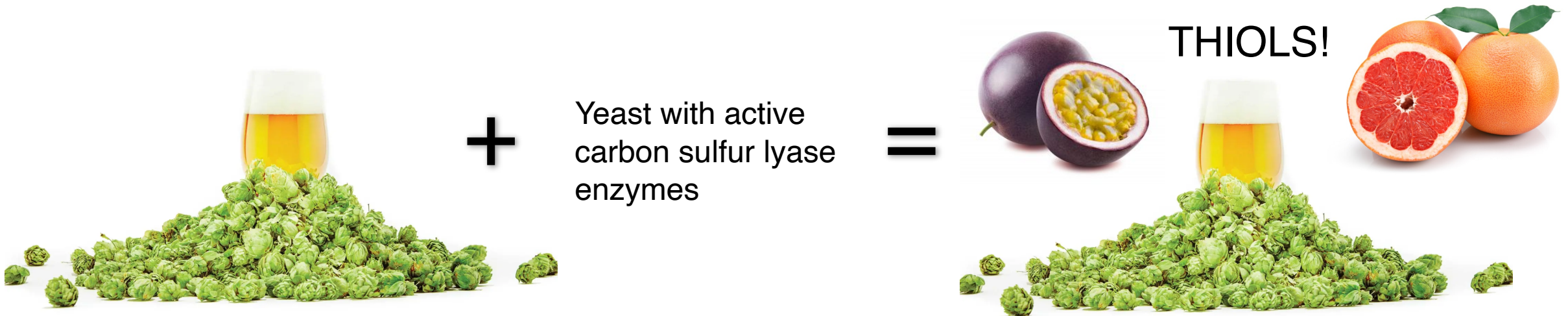
How we use CRISPR/Cas to engineer brewing yeast



CARBON-SULFUR LYASES: IRC7 AND PATB



YEAST ENGINEERED TO RELEASE THIOLS



1. Cosmic Punch (Irc7)
2. Star Party, Helio Gazer, Lunar Crush (PatB)



COSMIC PUNCH AND HELIO GAZER

Strain	Parent Strain	Carbon Sulfur Lyase	Thiol output	Best uses?
Cosmic Punch	OYL-011	Irc7	10x sensory threshold	Enhance thiol notes, NEIPA or hazy IPAs, house strain that can be versatile
Helio Gazer	OYL-011	PatB	300x sensory threshold	Intense thiol aromas, stands out in heavily hopped beer styles



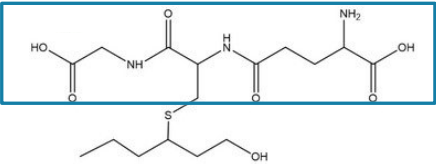
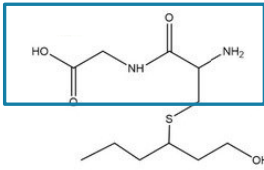
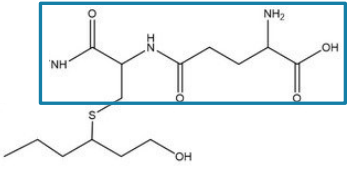
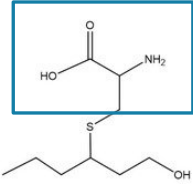
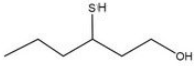
Cosmic Punch



Helio Gazer



WHAT ABOUT THIOL PRECURSORS?

Precursors = Non-Aromatic			Free = Aromatic
Glutathione Precursor	Intermediate Precursors	Cysteine Precursor	Thiol
<p>Glut-3SH</p> 	<p>CysGly-3SH</p>  <p>CysGlu-3SH</p> 	<p>Cys-3SH</p> 	 <p>3SH</p>

WHAT ABOUT THIOL PRECURSORS?



Glutathione Precursors In Malt

Loads of Glutathione-3SH in malt!

Corresponds to 100-1000 times the amount of free 3SH in beer

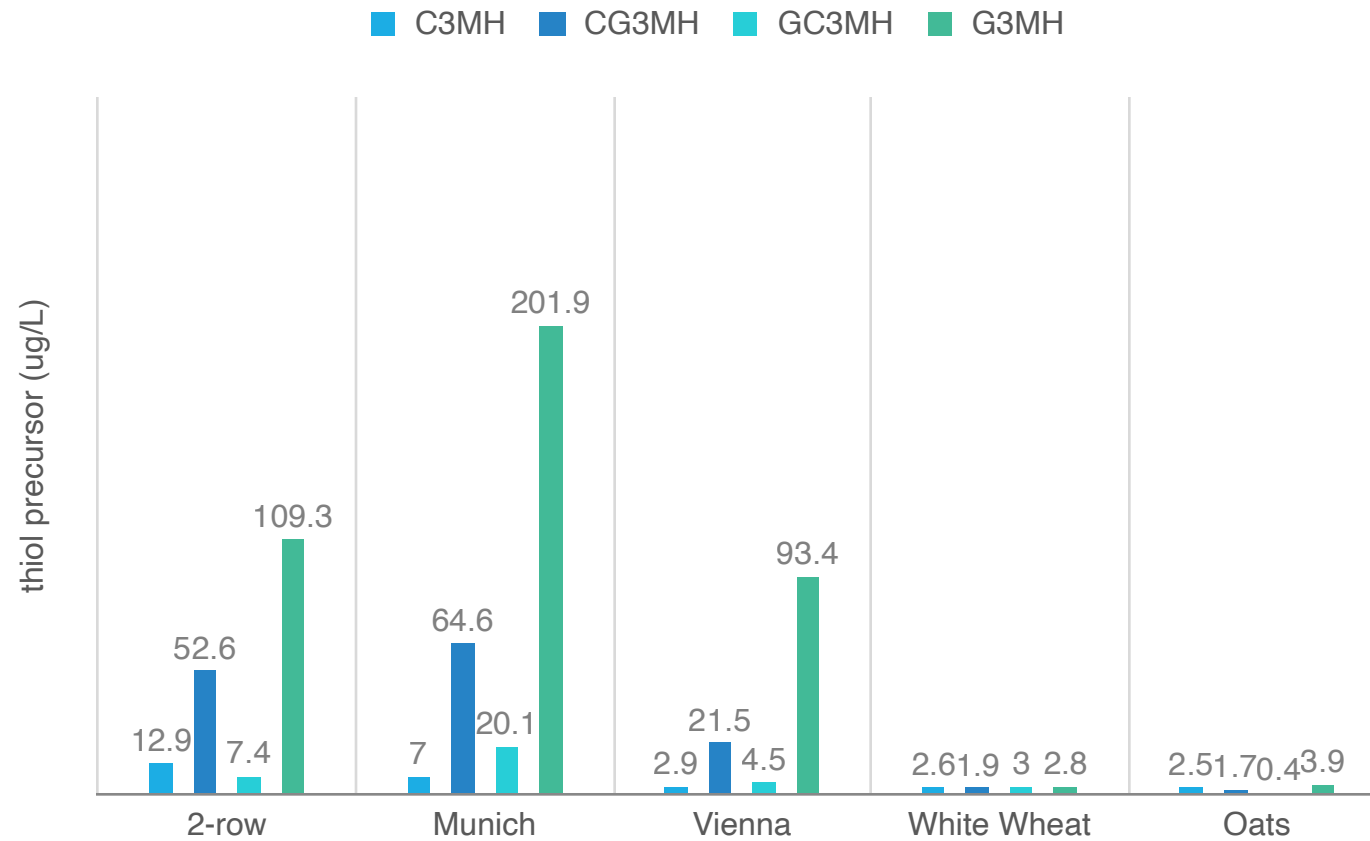
Variable across different barley varieties

Kilning destroys the precursors

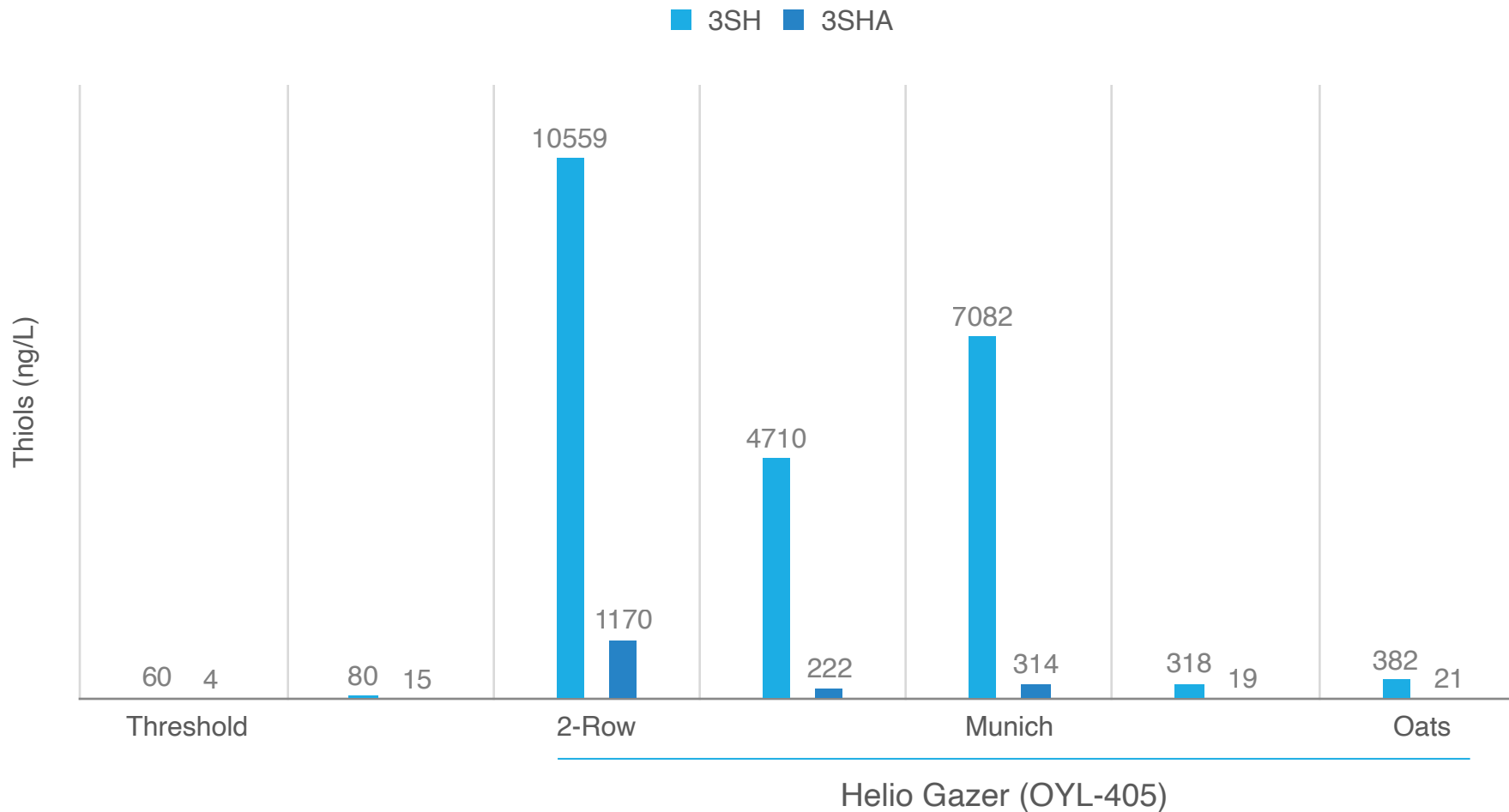
3SH precursor levels increase in the boil



THIOL PRECURSORS IN DIFFERENT MALTS



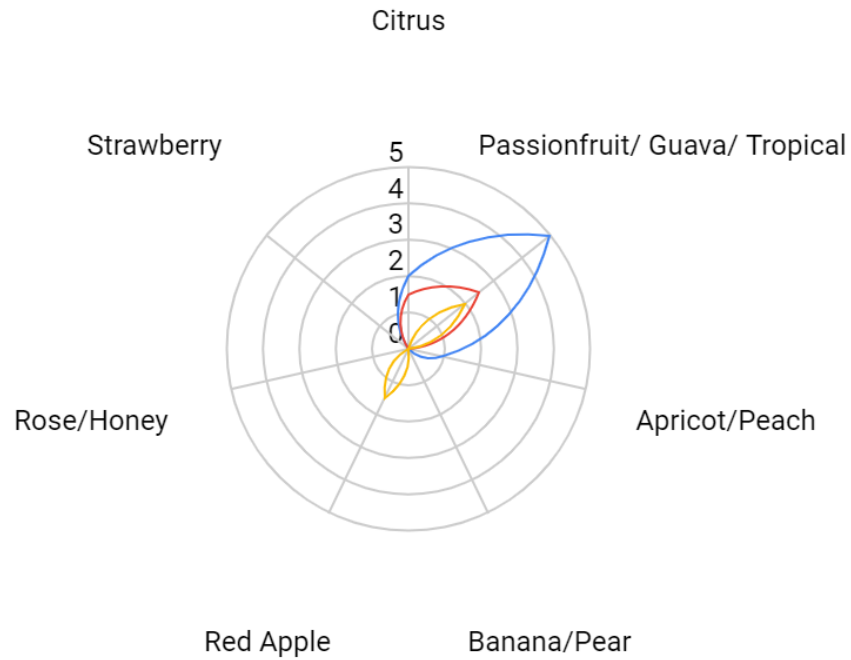
Thiol Levels in Wort Fermentations (No Hops, Just Precursor From Malt!)



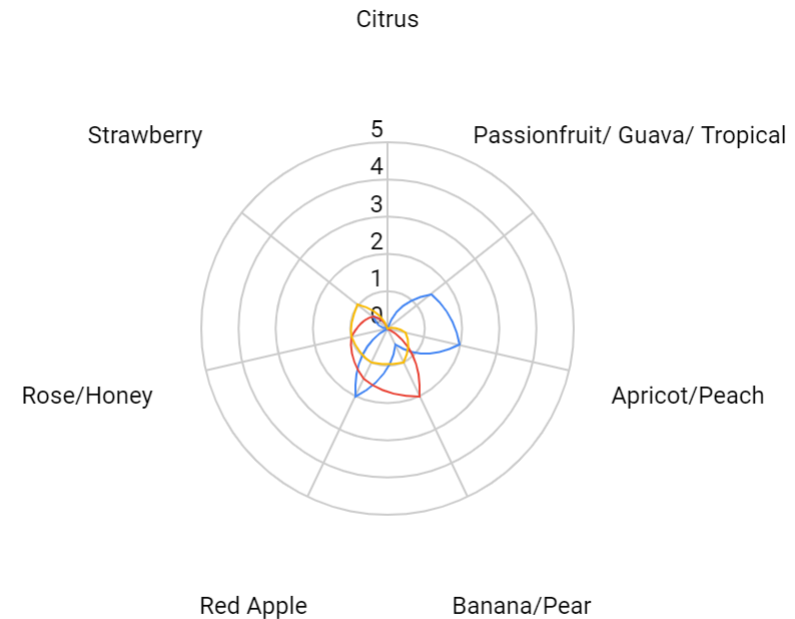
MALT PRODUCTS AND THIOL SENSORY

- 2-Row
- Vienna
- Munich

Helio Gazer



OYL-011



FREE THIOLS AND PRECURSORS IN HOPS

Thiol Precursors –

- Thiols bound to **glutathione**, cysteine and dipeptide intermediates
- Nobel hops and C-hops are high in bound
- Parts per billion to parts per million in finished beer

Free Thiols –

- Tropical, Grapefruit, Skunky, Diesel
- Typical “New World” aroma hops are high in free thiols
- Parts per trillion to parts per billion in finished beer

HOPS WITH HIGH THIOL PRECUSOR

Table 2 Analysis report of 17 hop samples (nd: not detected)

Hop cultivars	Thiols (ug/kg)			Thiol Precursors (ug/kg)			
	3MH	3MHA	4MMP	Cys-3MH	G3MH	Cys-4MMP	G4MMP
Apollo	11.1	nd	7.5	382	7340	0.02	0.06
Bravo	20.1	nd	0.5	240	5901	0.02	0.03
Calypso	15.5	nd	0.1	1905	14421	0.03	0.03
Cascade	10.5	2.8	2.4	456	13498	nd	nd
Citra 1	24.2	nd	28.4	394	4821	0.03	0.03
Citra 2	16.0	4.7	43.5	376	5209	nd	nd
Eureka	11.0	nd	17.5	326	7595	0.04	0.06
Hallertau Cascade	15.8	6.0	1.0	142	3418	nd	nd
Hallertau Hallertauer Tradition	2.3	1.5	0.2	444	10637	nd	nd
Hallertau Herkules	6.3	2.3	0.3	0	5993	nd	nd
Hallertau Nugget	nd	4.4	0.6	333	6252	nd	nd
Hallertau Perle	2.1	1.5	0.6	455	15467	nd	nd
LES-Nugget	2.2	3.4	0.3	488	8753	nd	nd
Saaz 1	4.7	1.5	0.5	431	9184	nd	nd
Saaz 2	2.3	0.9	0.3	532	20678	nd	nd
Saaz 3	4.4	1.0	0.2	890	19890	nd	nd
Simcoe	22.5	nd	13.5	646	8981	0.03	0.01

Traditional hops

Saaz, Hallertau Mittelfrüh, Perle

West Coast “C” hops

Cascade, Chinook, Calypso

NZ hops

Motueka

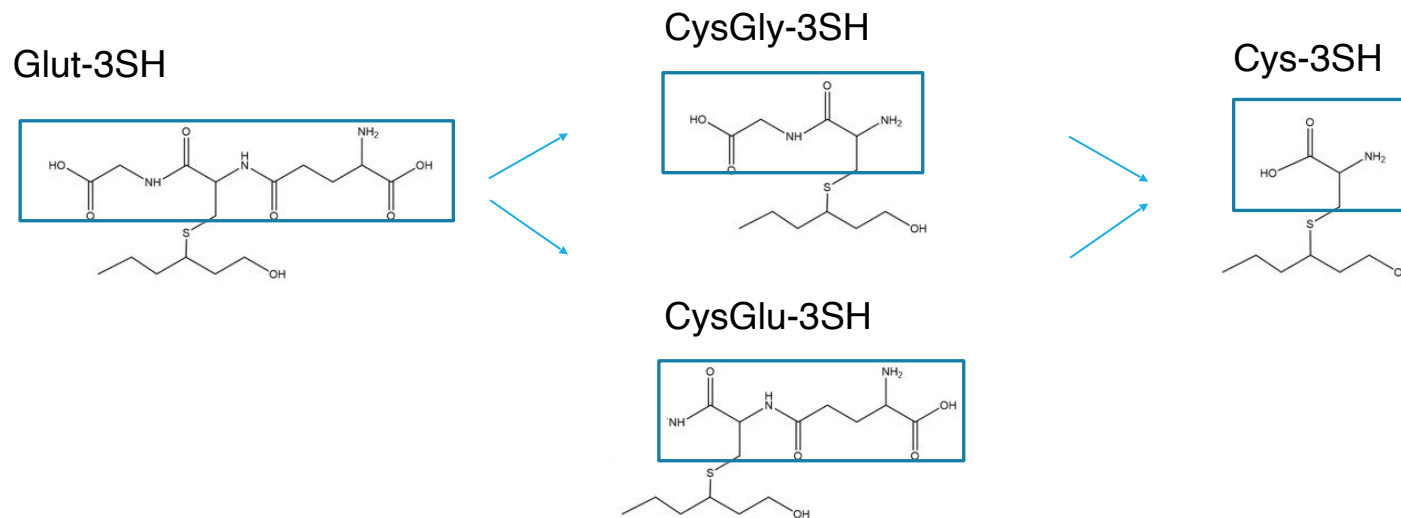
Roland *et al.* 2017 BrewingScience



MASH HOPPING

Hops have a lot of glutathione precursor, but β -lyase enzymes are more active on the cysteine precursor

Adding hop in the mash, promotes the conversion of glutathione precursors to cysteine precursors

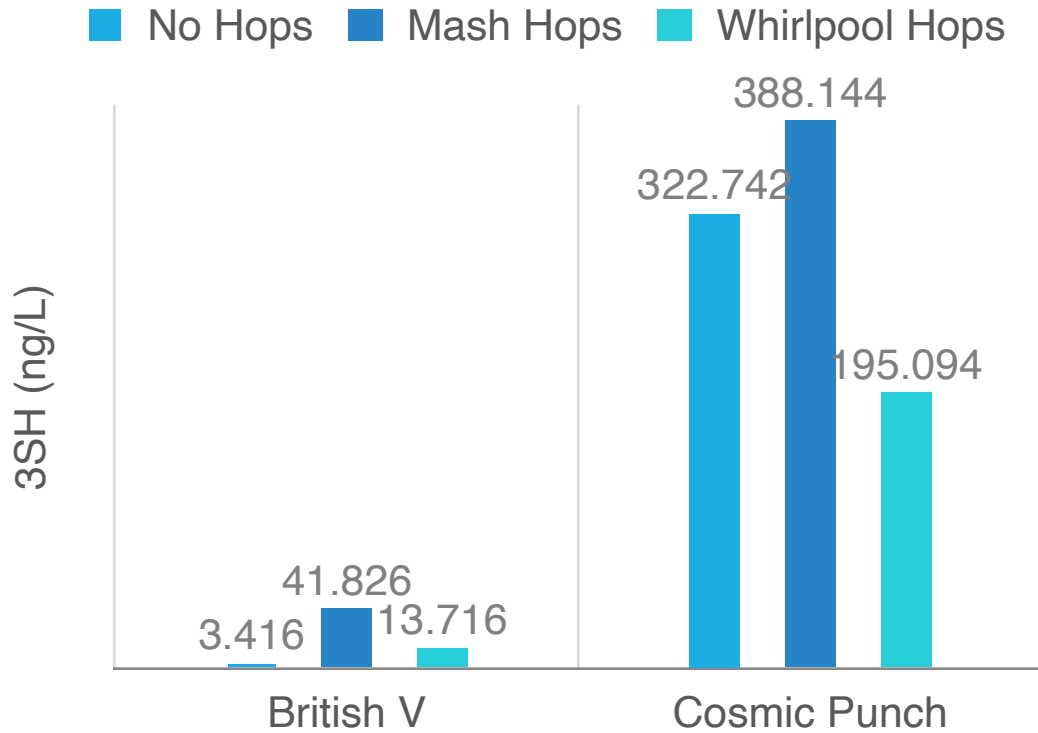


Mash hopping

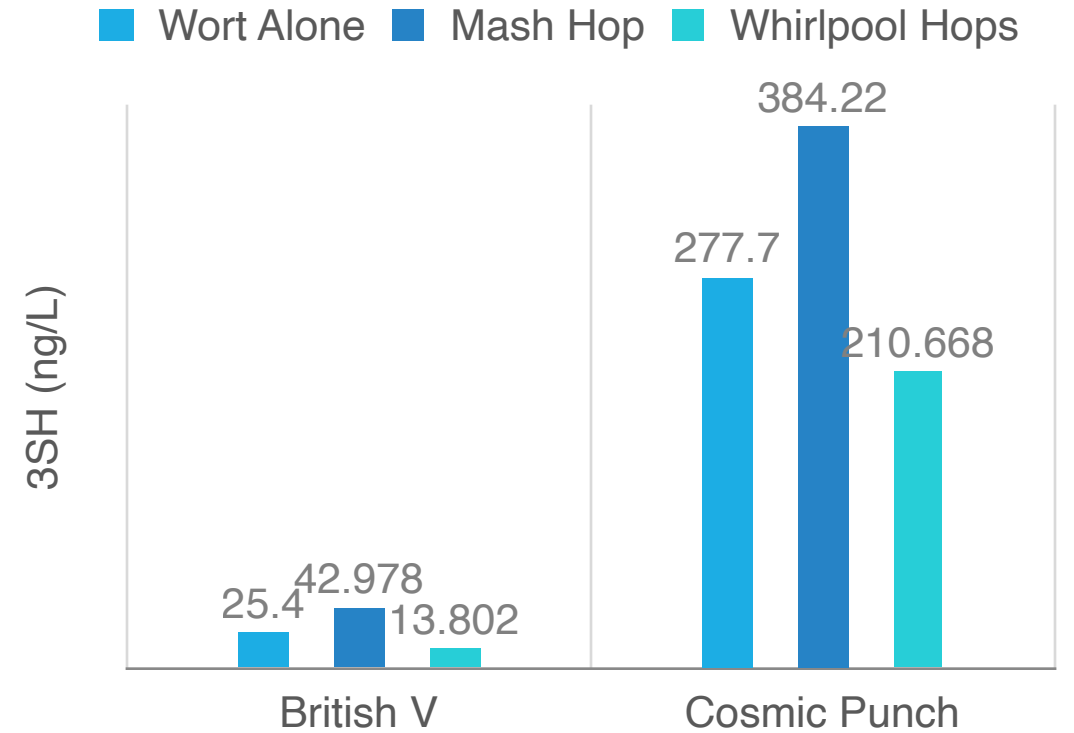


MASH HOPPING AS A METHOD TO ADD THIOL PRECURSORS

Chinook 1 lb/bbl



Motueka 1 lb/bbl



THINGS TO CONSIDER WHEN MASH HOPPING

Remember you will get bitterness from mash hopping

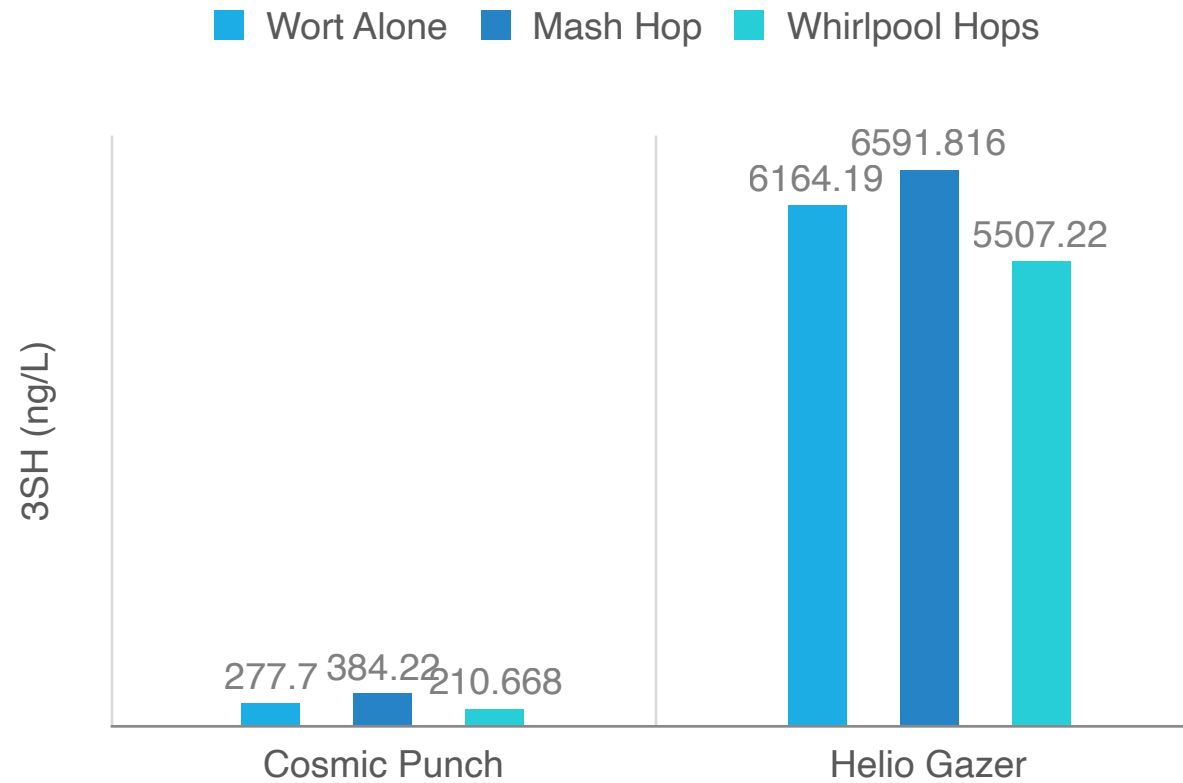
- 30% of the IBU levels that you would expect from a beginning of boil addition.
- Addition rates between 0.5 lb/bbl to 2 lb/bbl depending on the alpha acid content of the hop

Avoid expensive aroma hops

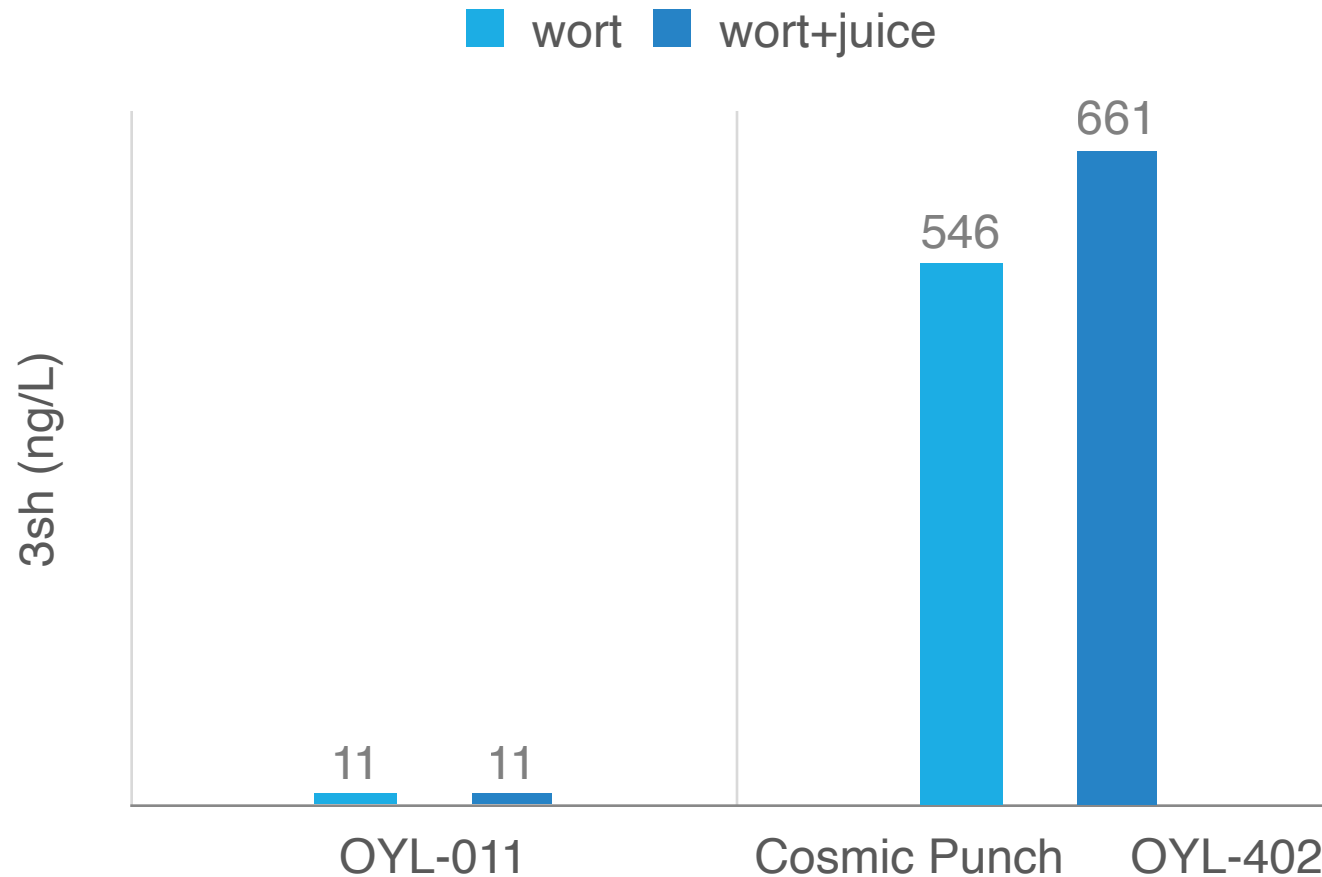
- Other volatile hop aroma compounds will be lost in the boil and beginning of fermentation.



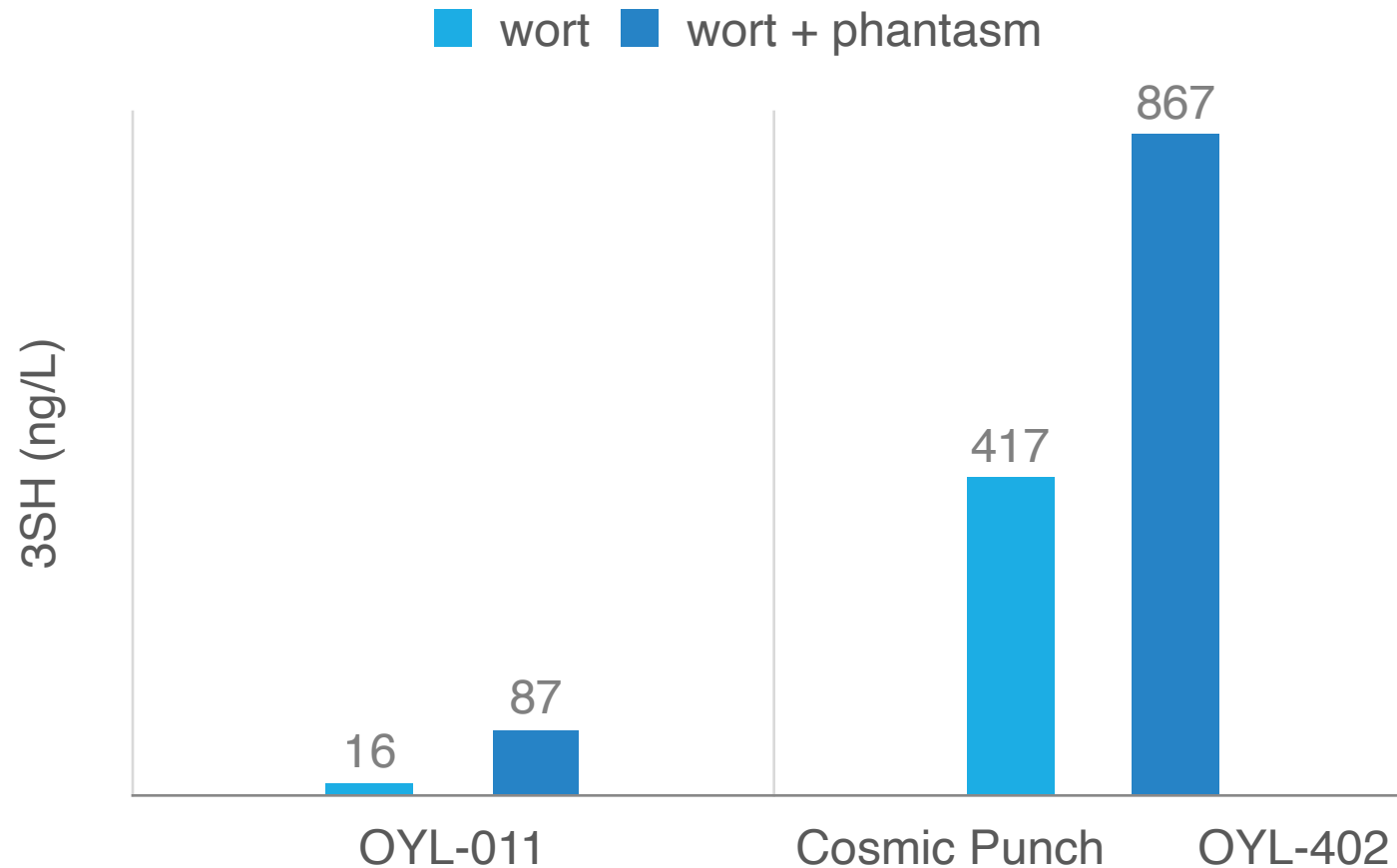
MASH HOPPING IS NOT NECESSARY WITH PATB STRAINS



Addition of NZ Sauvignon Blanc Juice: 20% Increase in 3SH With 5% Juice



Sauvignon Blanc grape skins rich in thiol precursors boosts biotransformation



MAKE THIOL-DOMINANT BEER



To emphasize a passion fruit aroma, pare down your recipe to a simplified grain bill with a majority barley base malt (wheat and oats have little, if any, thiol precursors).



For a thiol precursor boost, try mash hopping with precursor-rich varieties like Cascade, Saaz, Calypso or Motueka. Adding hops so early in the brewing process may seem counterintuitive, but it turns out that using hops in the mash is an efficient way to add thiol precursors from hops, and get even more tropical aromas from Thiolized yeast.



To avoid overpowering thiols with intense hop aromas, pull back on hopping rates in the whirlpool and dry hop.



Hops that have high amounts of free thiols provide complementary aroma profiles that work hand in hand with Thiolized yeast.

MAKE THIOL-COMPLEMENTARY BEER



Thiols produced in fermentation can be balanced out with a heavier dry hop dose, leading to an intensely hoppy, thiol forward beer.



Avoid adding extra thiol precursor via mash hopping or grape-derived products.



Utilize adjuncts in the mash bill such as oats, wheat and/or rice since they have little, if any, thiol precursor.

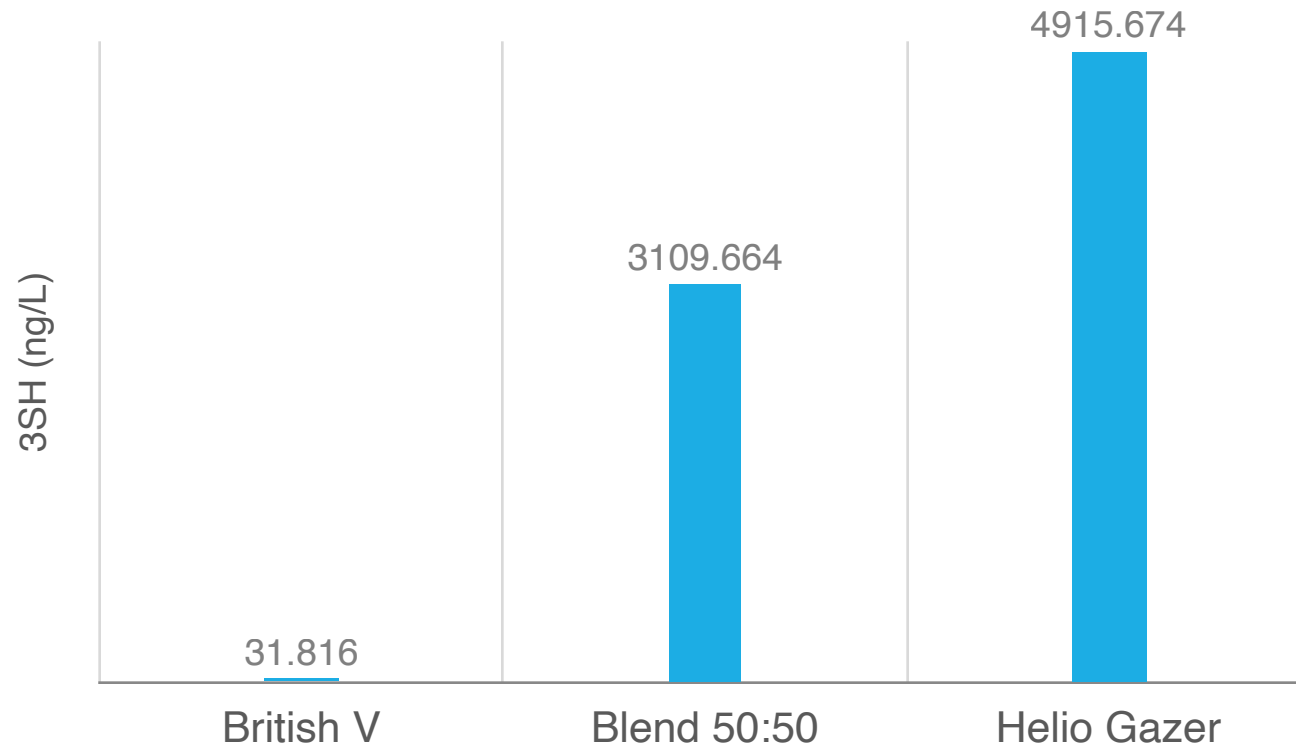


Blending Thiolized strains with the parent strain can be a great method for halving or even quartering thiol levels in the finished beer. This service is free.



If you're looking for a hazy house strain, Cosmic Punch adds nuanced thiol profile that elevates hop character.

YEAST BLENDING TO DIAL IN THIOL LEVELS



DIFFERENT STYLES TO TRY:

NEIPAs/Hop-forward styles

- Expect an overall juicier-tropical aroma
- Complex interactions between thiols and other hop aromas!
 - Think adding passionfruit to juice... more tropical
- Dry hop with combinations that play well with thiols (juicy + thiols!)

A broken down simple thiol-driven style

- The simpler the recipe, the more thiol– blonde, pale, lager
- Mostly barley grist, mash hopping, minimal late hopping
- Thiol bombs and defined passionfruit/guava aromas



WHY USE THIOLIZED YEAST? ... OUR FUTURE

More sustainable brewing practices

- Bringing out more aroma from hops and malt
- Reducing the cost and product losses associated with excessive hopping rates

Encouraging use of local hops and malt

- Uncover new potential for local ingredients
- Define and build a local terroir
- Support the people and resources of your region

FINAL THOUGHTS ON BIOTRANSFORMATION

BIG QUESTION: How do yeast and hops aroma compounds interplay?

Esters, monoterpene alcohols, thiols etc. do not exist on their own...

Important to experiment and play with different yeast and hop combinations, because there is a lot that we don't know!

THANK YOU!!

Acknowledgments:

Lance Shaner, PhD
Laura Burns, PhD
Keith Lacy
Chris Bernardo
Allison Lange
Rita Mormando
Nate Morton



The Omega Yeast Crew

Let's chat

Bill McFarland

bill@omegayeast.com