



A Proteomics Dive into Yeast-Dependent Colloidal Haze

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Keith Lacy
Omega Yeast Labs



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Keith Lacy

- Working with Omega since 2018
 - Propagation Technician 2018-2019
 - Assistant Production Manager 2019-2020
 - Research & Development Technician 2020- onward
- Graduate of the University of Illinois at Chicago



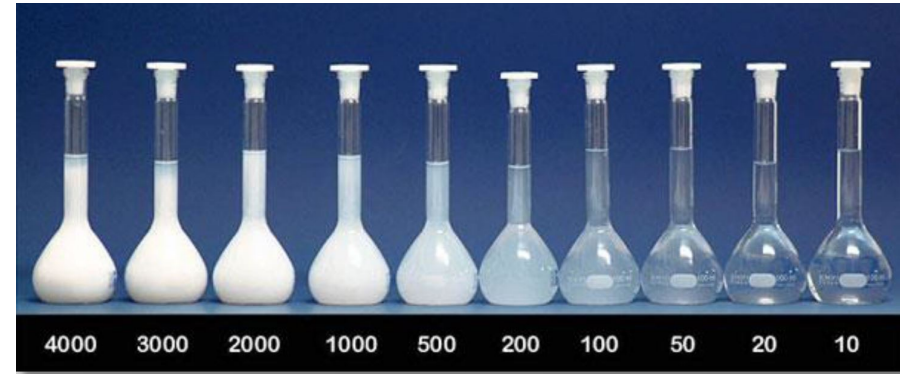
Talk Outline

- Introduction to Haze
- Haze Positive Yeast
- Proteomics Experiments
- Conclusions and Next Steps



What kind of haze are we talking about?

NEIPA levels



Sample	Turbidity Measurement
Drinking water	0.05-1.5 NTU
Lager	5-20 NTU
Porter	20-200 NTU
Hazy IPA	200-1000 NTU
Orange juice	300-900 NTU
Milk	>4000 NTU

Development of an Assay to Study Yeast-Dependent Haze

Wort: All barley malt (2-row) for target 15°P

Pitch Rate: 10 million/ml

Temperature: 70°F

Fermentation End Point: 14 days

Dry Hop Amount: 2 lb/bbl (8g/L)

Dry Hop Addition:

- Control (no dry hop)
- Knockout (in fermentor pre-pitch)
- Day 1
- Day 2
- Day 3
- Day 4
- Day 7
- DDH (½ Day 4, ½ Day 7)



Dry Hop Timing and Yeast Choice - Dramatically Impacts Degree of Haze

Haze Positive
OYL-011
British V, London III



Haze Neutral
OYL-004
West Coast Ale I, Chico



Pictures at 14 days from left to right:

- Control (no dry hop)
- Day 0 "Knockout"
- Day 1
- Day 2
- Day 3
- Day 4
- Day 7
- DDH (Day 1 and 7)

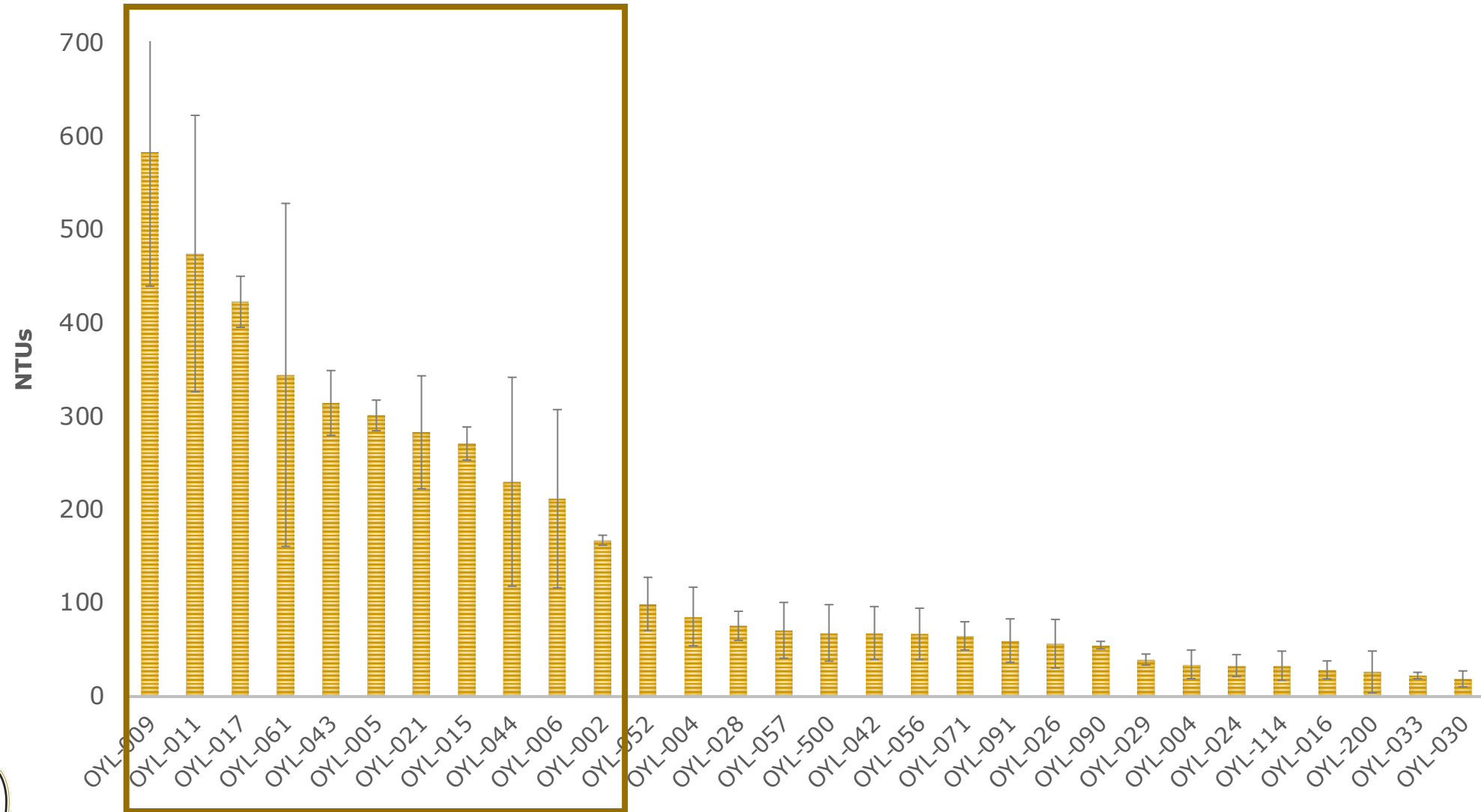


Dry Hop Timing



Dry Hop Timing

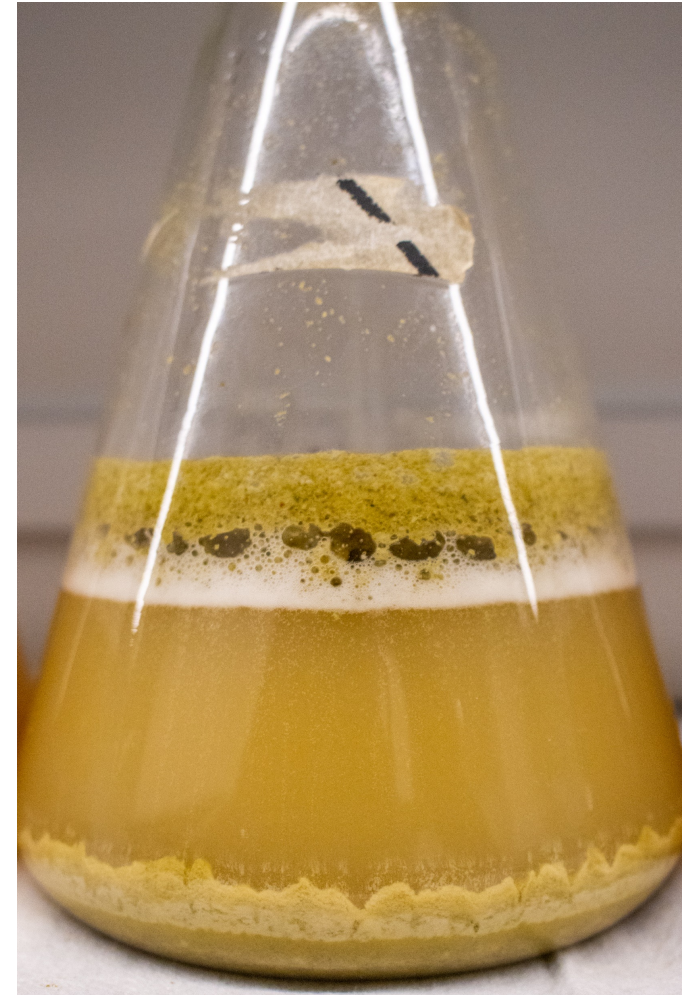
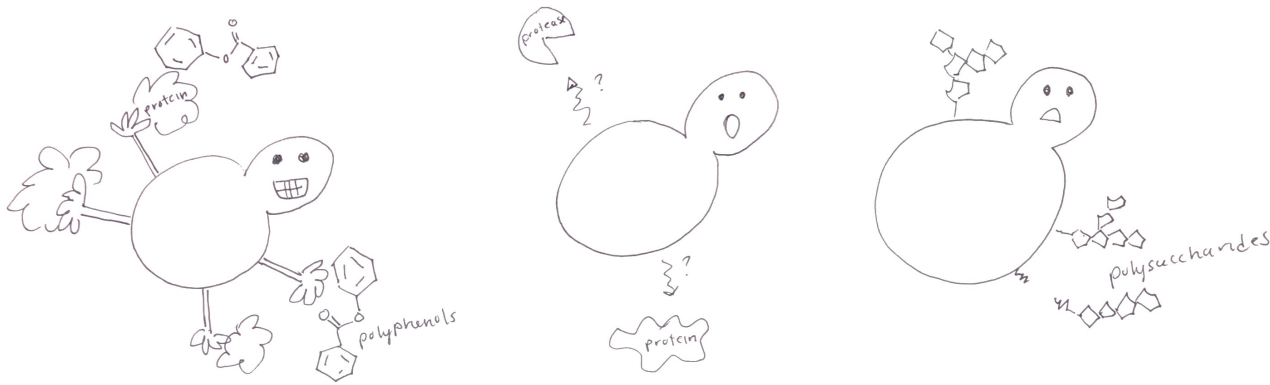
Yeast-Dependent Haze – “Haze Positive” strains



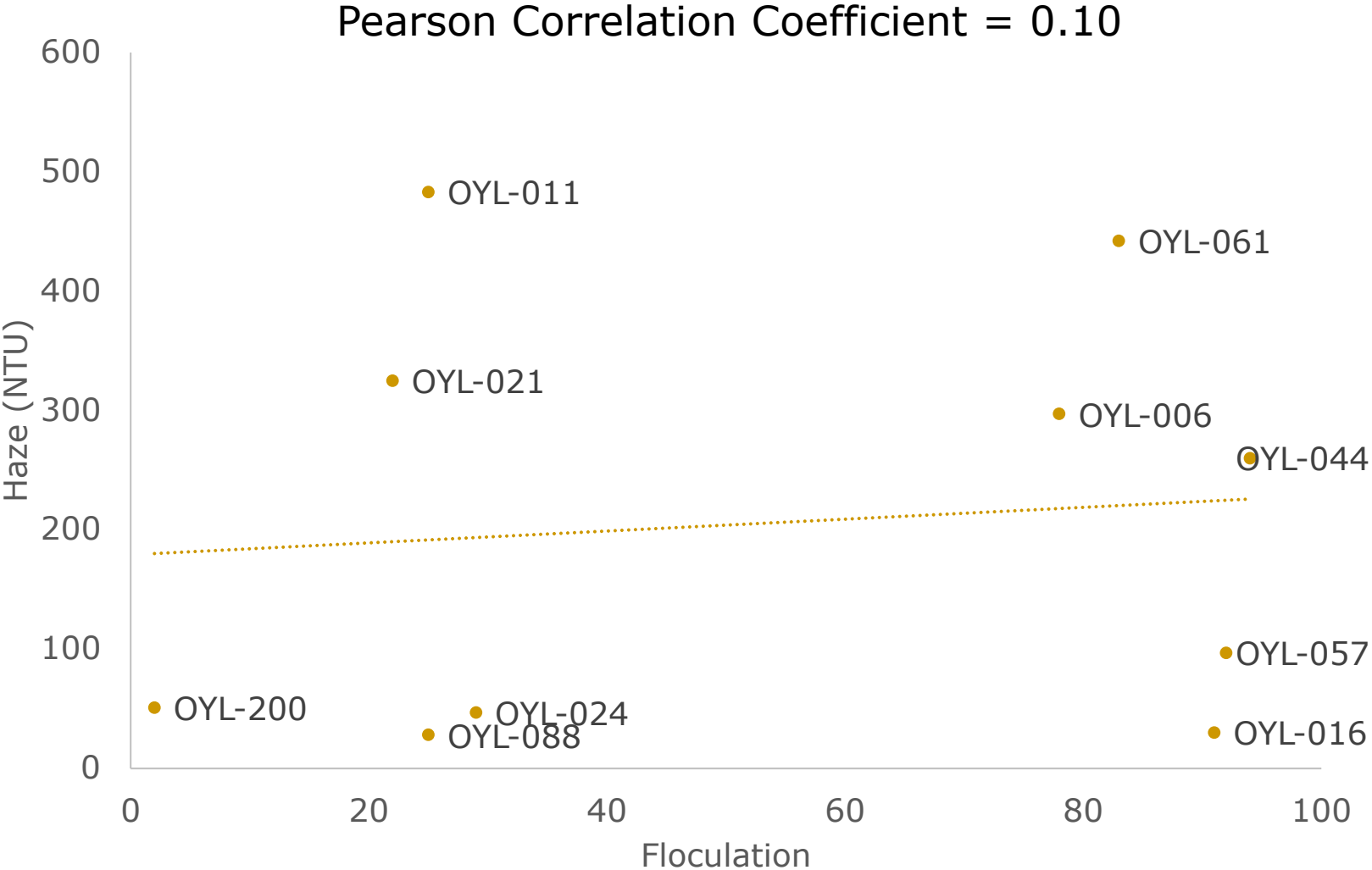
What makes a strain “Haze Positive” or “Haze Neutral”?

Potential Mechanisms:

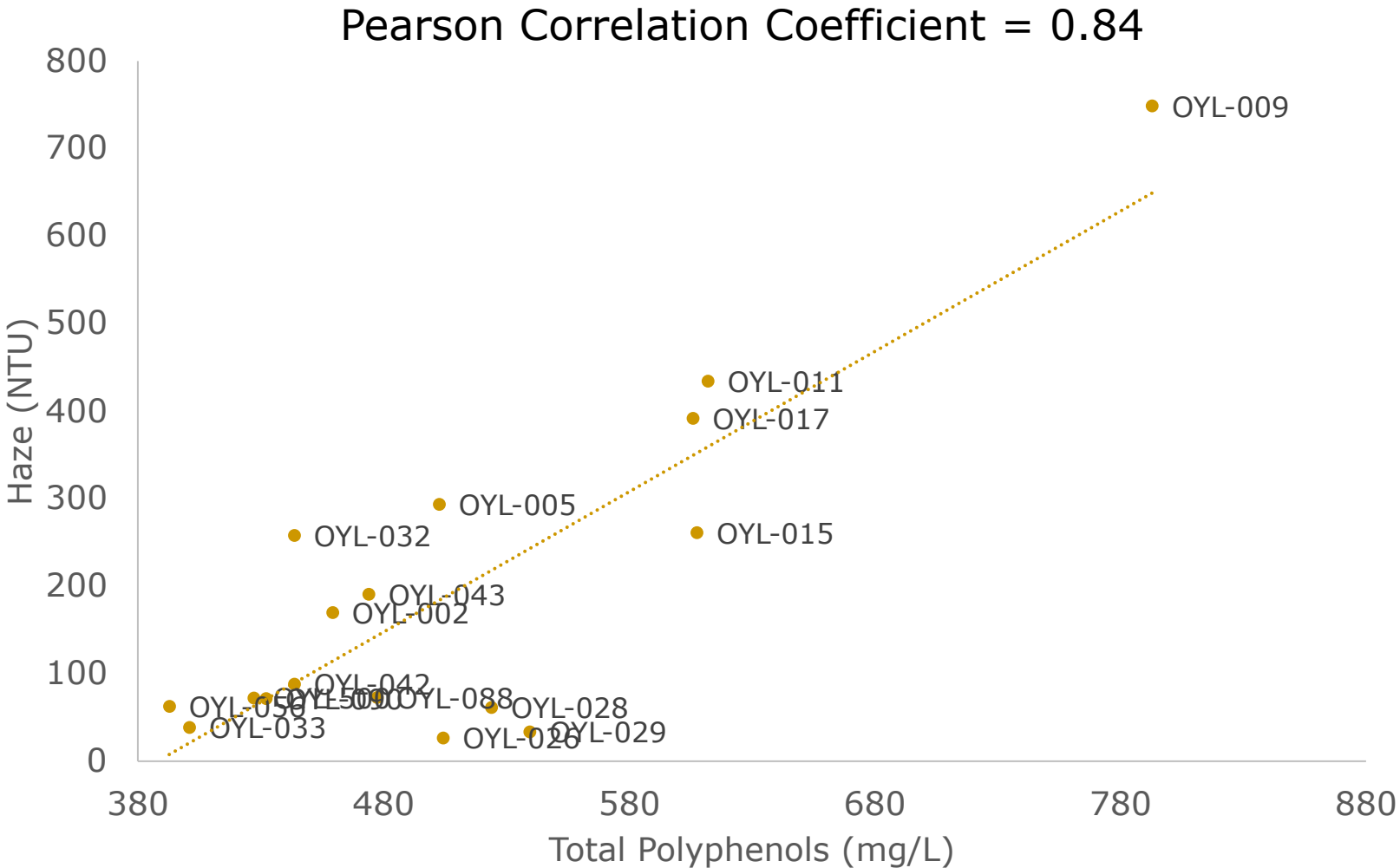
- Adsorption of polyphenols/proteins by yeast cell wall
- Yeast secreted protein (or secreted proteases)
- Cell wall polysaccharides (Mannan, β -glucan)
- Impact of yeast on pH and non-covalent interactions



Haze is Not Correlated to Flocculation

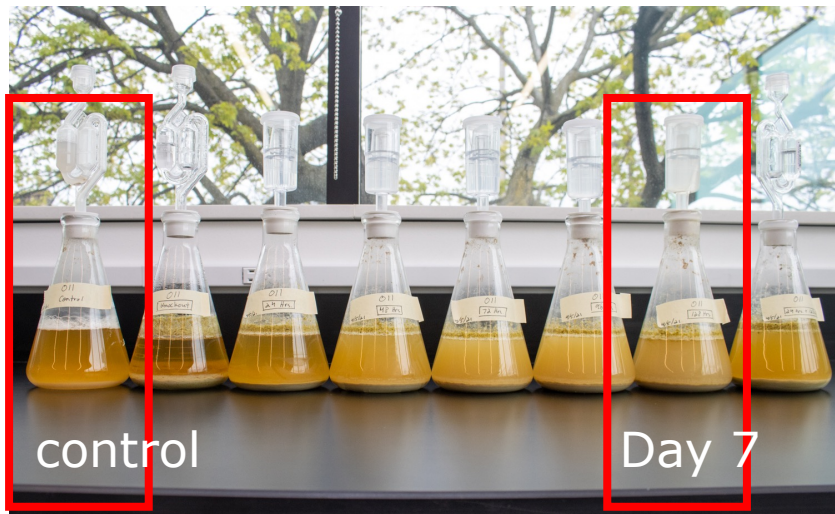


Haze is Correlated to Total Polyphenols

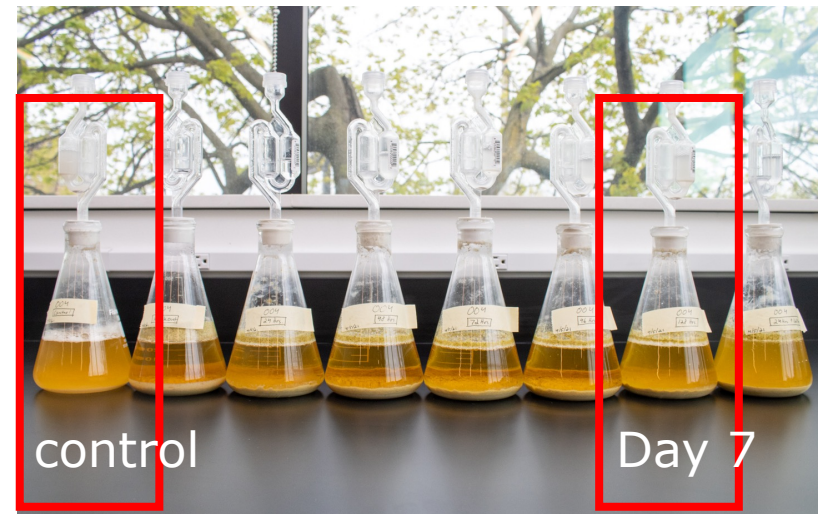


What proteins are changing in the haze samples?

- Are certain yeast proteins correlated to hazy or non-hazy samples?
- Proteins specific to haze positive or haze neutral yeast?

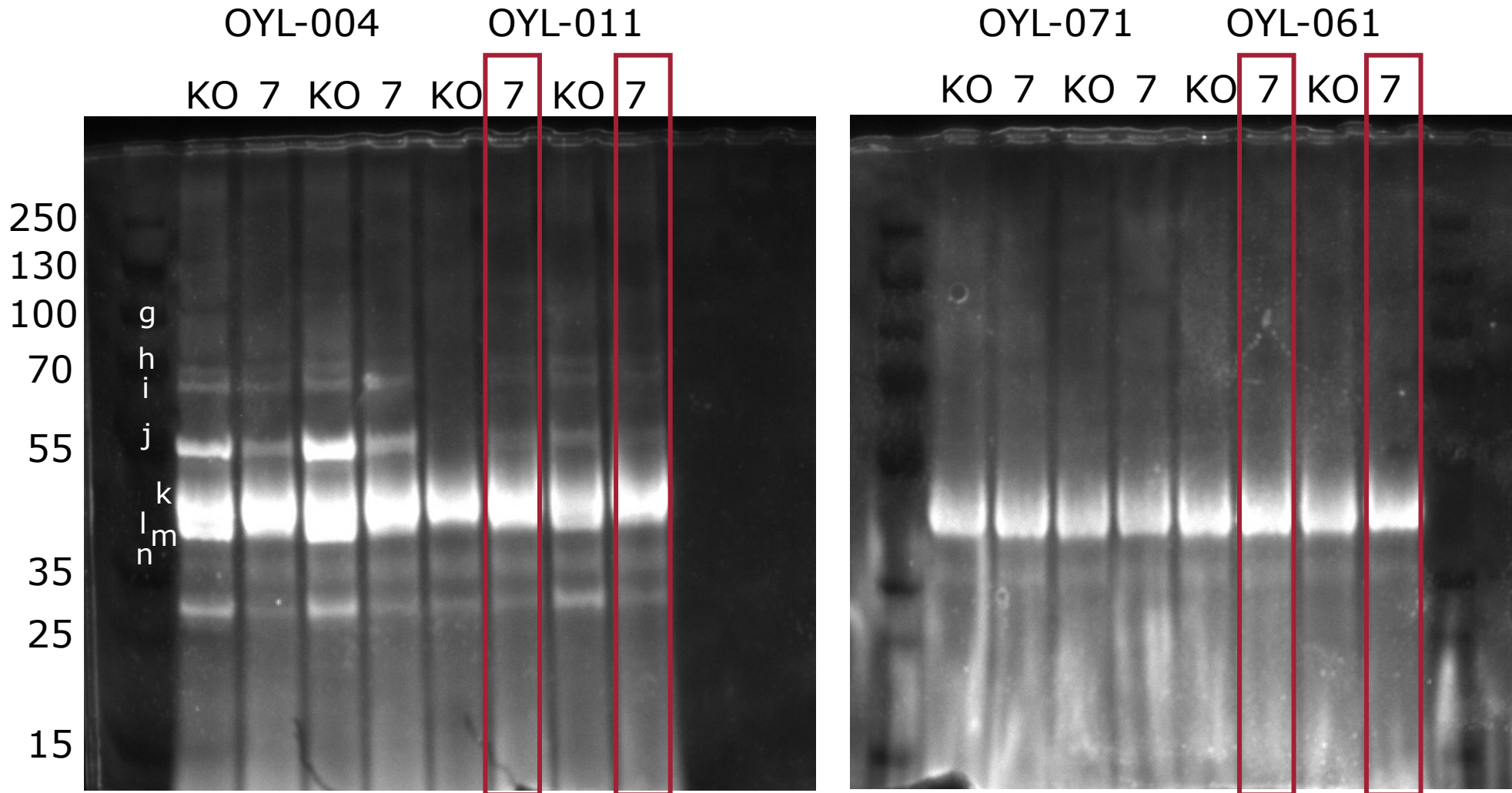


Haze positive yeast



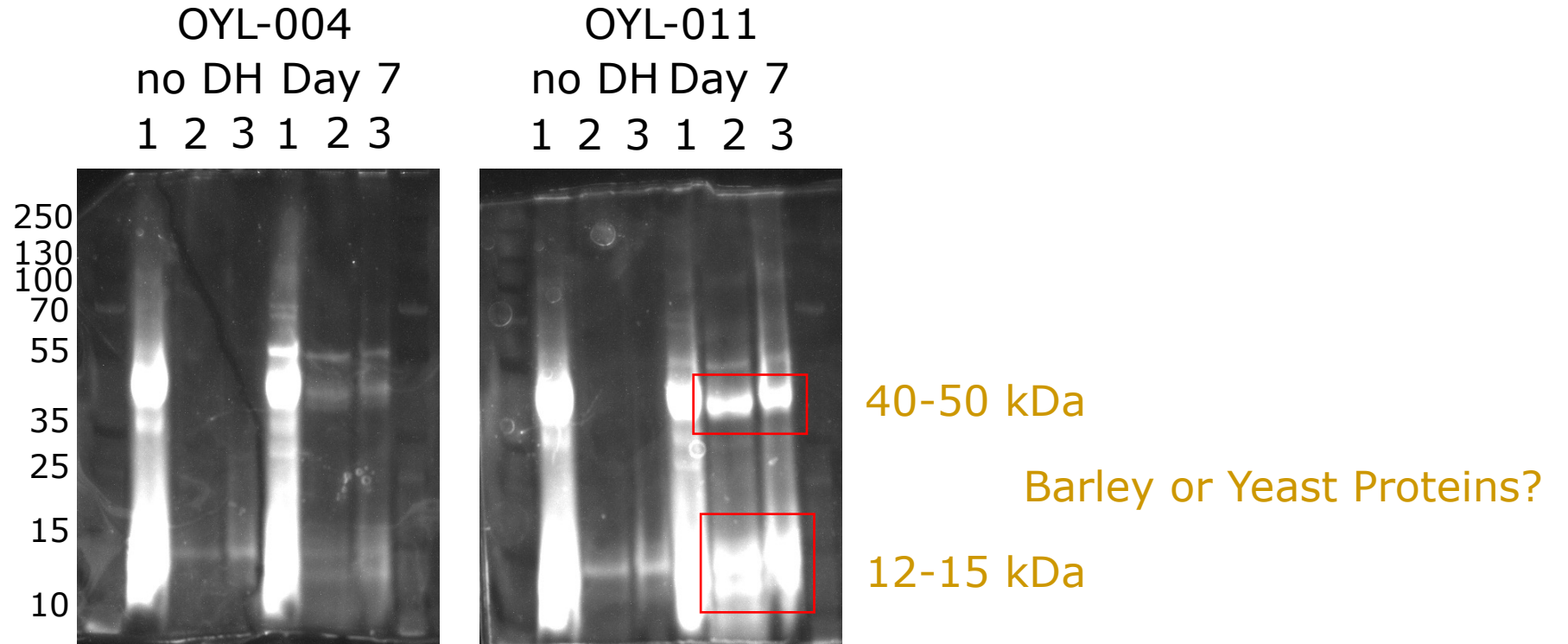
Haze neutral yeast

SDS page gels show no difference associated with haze



D-hordein (**g**, 93.9 kDa), C-hordeins (**h**, 70.5; **i**, 63.7; and **j**, 55.6 kDa), B-hordein (**k**, 47.8 kDa) partly obscuring γ 1-hordein (**l**, 45.0 kDa), γ 2-hordein (**m**, 40.0 kDa), γ 3-hordein (**n**, 38.0 kDa)

Centrifuged haze shows similar proteins found in beer



- 1 - 30ul beer
- 2 - centrifuged haze from 500 ul
- 3 - centrifuged haze from 500 ul + 8M urea

Proteomics Experiment

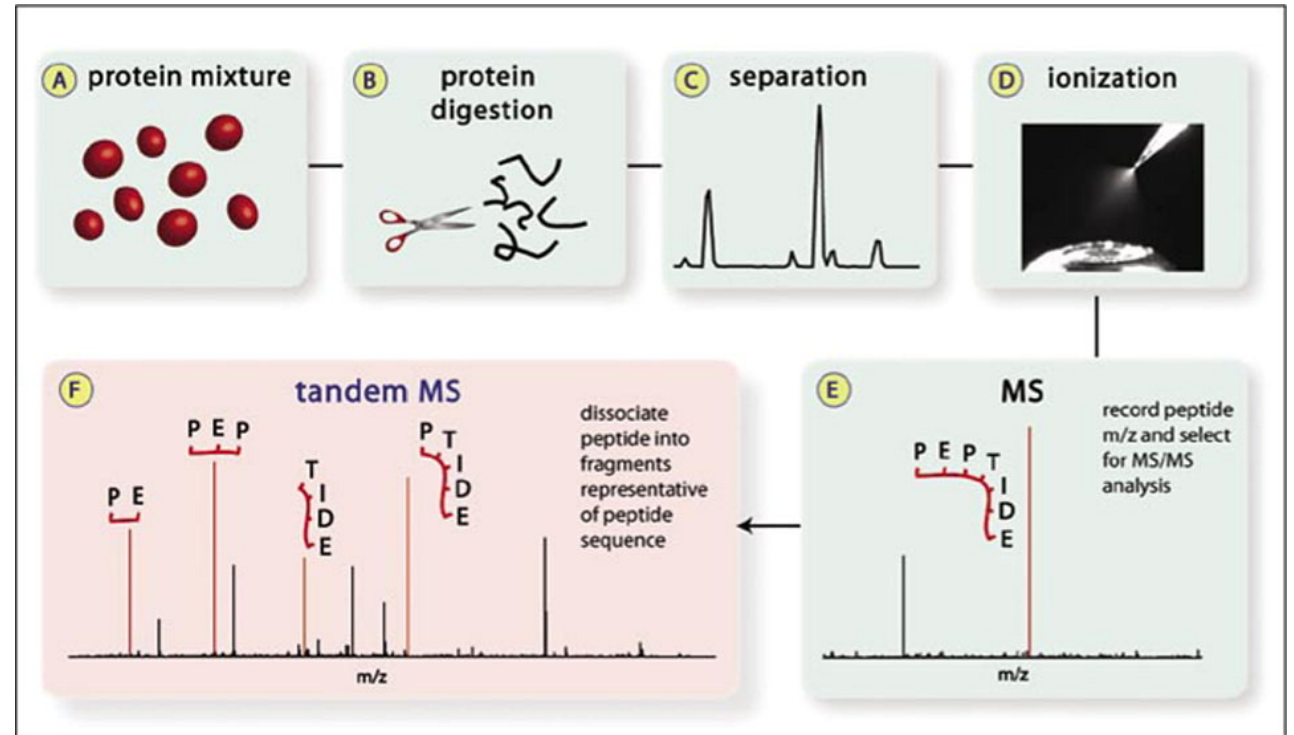
- 4 yeast strains
 - Haze positive: OYL-011, OYL-061
 - Haze neutral: OYL-004, OYL-071
- Two fermentation conditions
 - Control no dry hop
 - Day 7 dry hop
- Samples were centrifuged at 3000 rpms and transferred 3 times to remove yeast cells
- BSA was used to determine the total protein and samples were digested for LC/MS
- Each of the 8 samples were run in biological triplicate



Easy nLC 1200 system and an Eclipse Tribrid mass spectrometer

How Proteomics Works

- Total protein is extracted and quantified
- Proteins are digested into small peptides
- Peptides are separated by liquid chromatography
- Peptides are ionized and MS determines a mass/charge ratio of each peptide
- Peptides ions are fragmented and further analyzed by tandem MS
- This information is used to identify the peptide against a database of known peptides
- Our results were compared to the yeast and barley peptide databases
- Protein abundance is determined by the number of unique peptides and total peptides per protein

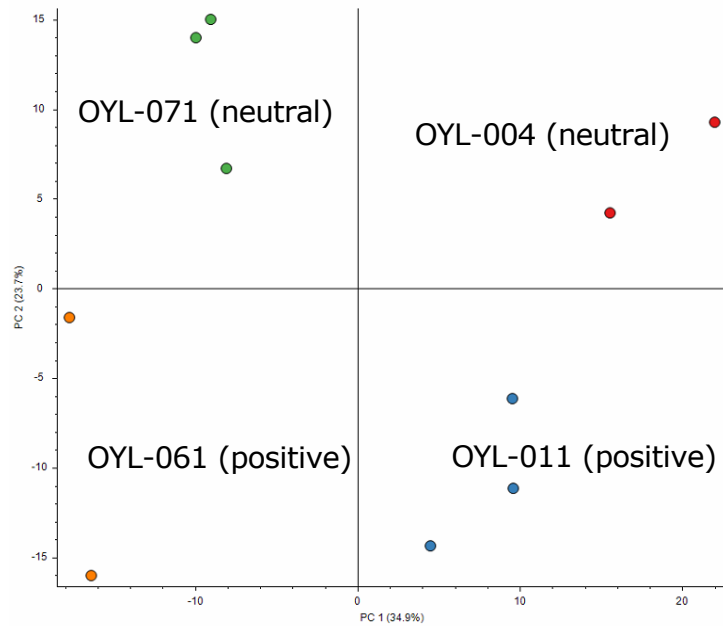


Coon *et al.* Biotechniques. 2018

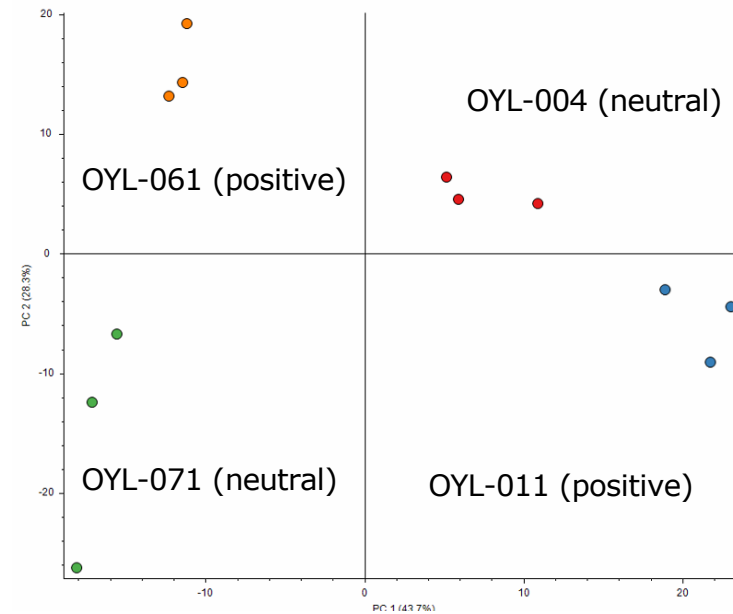
Quality data!

PCA plots show strong clustering of sample replicates

Control no dry hop



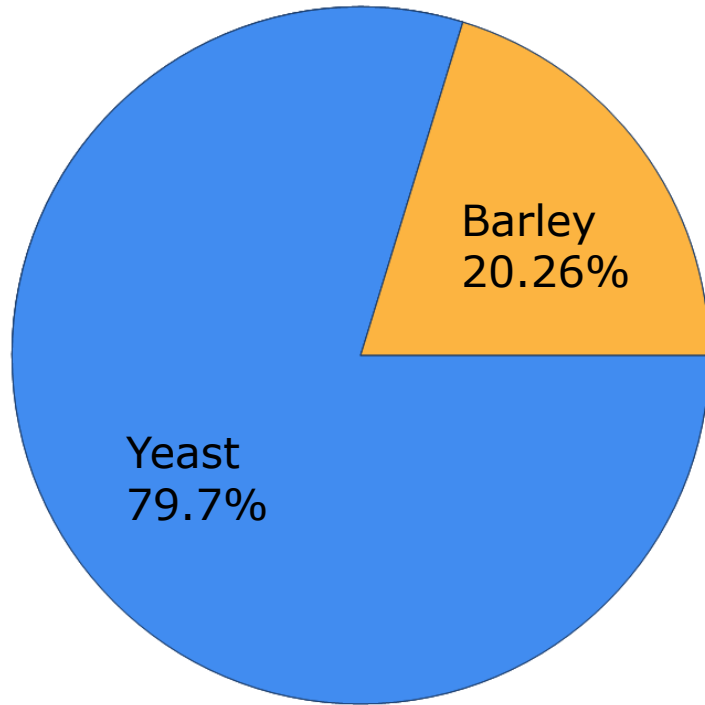
Day 7 dry hop



1. Sample replicates are consistent
2. Strain proteomes are distinct
3. Clear difference between control and dry hop samples



What types of proteins were identified across samples?



Surprising number of yeast proteins in the samples

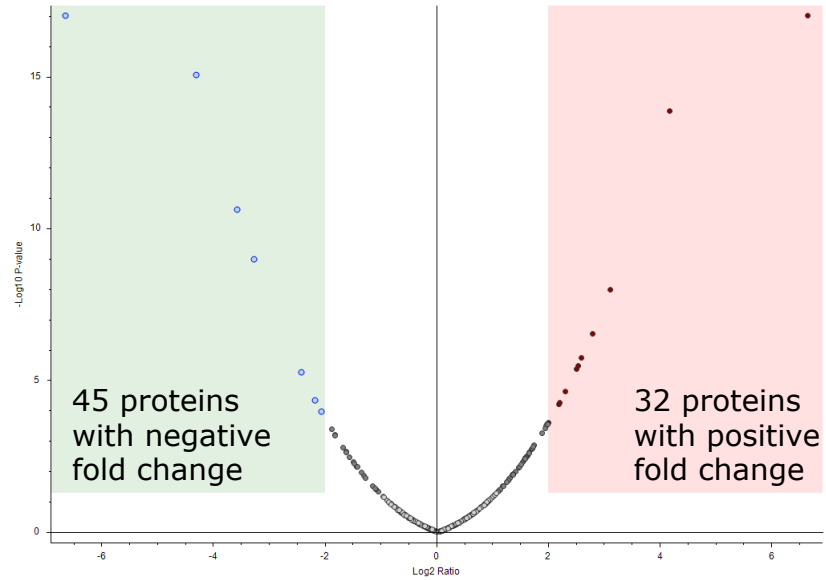
Total number of proteins in each sample set

	OYL-011 positive	OYL-061 positive	OYL-004 neutral	OYL-071 neutral
Control	509	481	551	483
Day 7	524	391	527	439

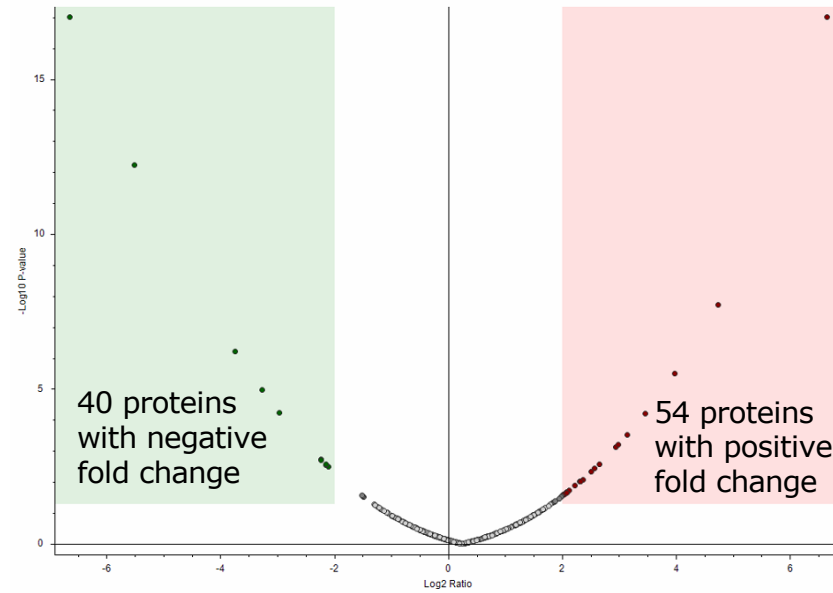


Identifying enriched and depleted proteins in haze positive (OYL-011) vs haze neutral yeast (OYL-004)

Control no dry hop



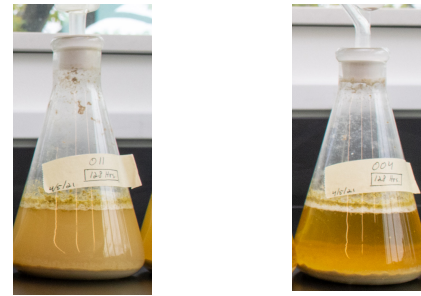
Day 7 dry hop



Ratio of OYL-011 : OYL-004



Ratio of OYL-011 : OYL-004



p value < 0.01



Specific Candidates Previously Associated with Haze in Beer Samples

Cell wall mannoproteins or secreted mannoproteins

Uth1 – cell wall protein, deletion results in thickening of cell wall

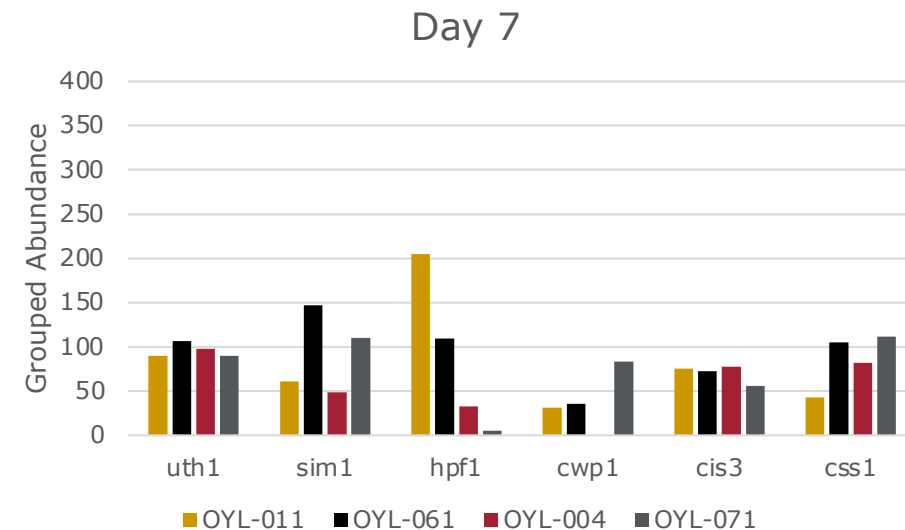
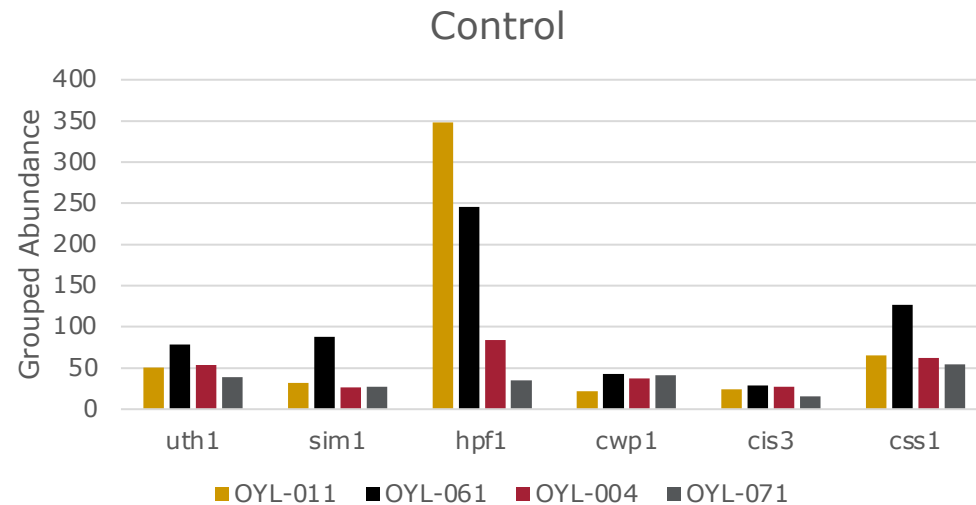
Sim1 – cell wall protein

Hpf1 – secreted protein, haze protective factor, overexpression reduces turbidity in wine

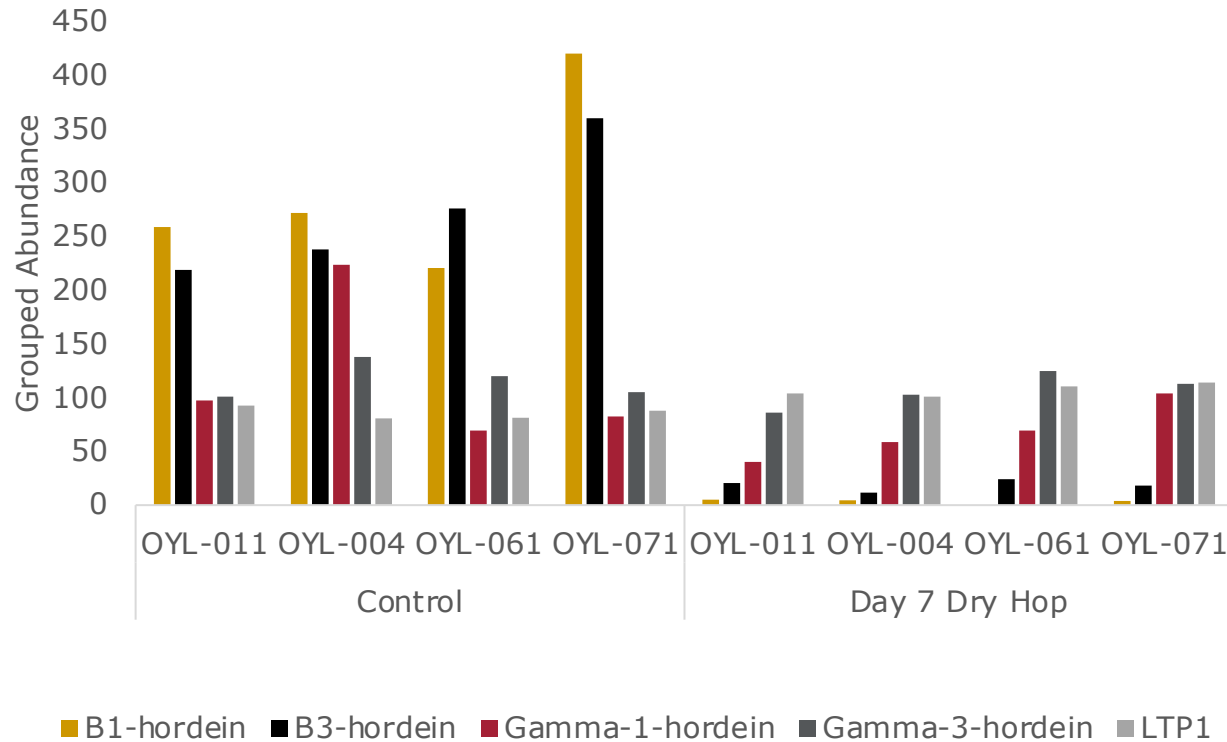
Cwp1 – cell wall protein, deletion results in thinning of cell wall

Cis3 – cell wall protein

Css1 – secreted protein, overexpression reduces turbidity in white wine



Interesting Patterns with Barley Proteins



B-hordeins

- Are these covalently bound by hop polyphenols and no longer detectable in dry hopped samples?
- Absent in all dry hopped samples, not specific to haze

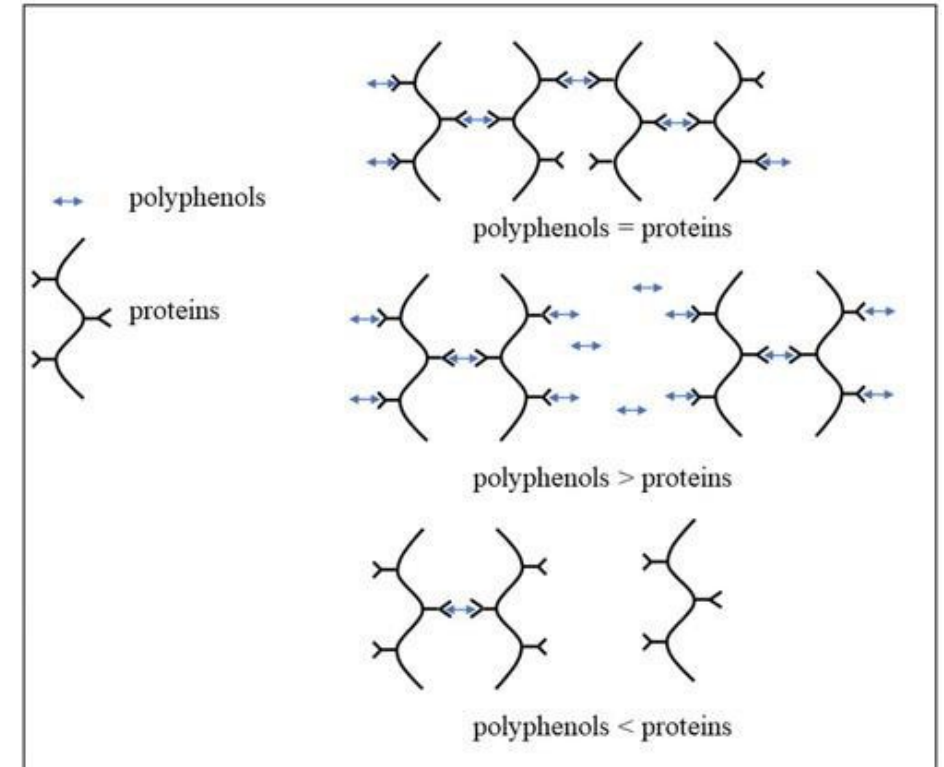
Gamma-hordeins/LTP1

- Other barley proteins appear to be unaffected by dry hop



Likely not one specific “haze” factor

- Haze positive strains are unrelated and may have distinct mechanisms of generating haze
 - English Ale, Kolsch, Kveik, American Ale, Hefeweizen
- Our proteomics experiments did not identify yeast proteins that were specifically enriched in haze positive, day 7 dry hopped samples
- Similar to the balance required for protein polyphenol interactions, subtle shifts in protein amounts/compositions may change the stability of haze generated



Siebert *et al.* 1996
Kahle *et al.* 2020

Limitations with a Proteomics Approach

- Sample quality
 - Beer samples are heavily oxidized, modified and degraded. These will not be seen as readily with proteomics.
- Insoluble proteins
 - Difficult to analyze by proteomics
- Not just protein
 - Missing information for other non-proteinaceous haze components (ie. carbohydrates, lipids, polyphenols)
- Proteome variation in brewing strains
 - Will not recognize mutated peptide sequence



Thank you!!

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Contact me:

Keith Lacy
keith@omegayeast.com

The Omega Yeast Crew



Resources

1. Kahle EM, Zarnkow M, Jacob F. Beer Turbidity Part 1: A Review of Factors and Solutions. JASBC 2021.
2. ASBC Methods of Analysis. Beer 27, Beer 35, Yeast 11.
3. Kahle EM, Zarnkow M and Jacob F. JASBC 2020.
4. Burns LC, Lacy K, Shaner L. Investigations into Yeast-Dependent Colloidal Haze. Master Brewers Conference 2021.
5. Burns LC, Preiss R. Dialing in Haze: Yeast Choice and Dry Hop Timing. CBC 2022.



A graphic consisting of two overlapping circles. The left circle is gold and contains a white letter 'Q'. The right circle is dark grey and contains a white letter 'A'. A gold ampersand '&' is positioned between the two circles, overlapping both.