Hop Quality Group Research Proposal

Creature Comforts Brewing
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Project:

pH increase in beer from dry-hopping

Hypothesis:

Nitrogen and Amino Acid content, specifically the presence of arginine, drives the increase in pH observed from the dry-hopping of beer.

Objective:

Test the pH increase from hops with variable nitrogen application in the field. Subsequently, test the amino acid content of these hops and the resultant beers.

Rationale:

The pH of beer is known to increase from dry-hopping at a rate reported in literature roughly equivalent to 0.1 increase in pH for every pound per bbl of dry-hops (Bruner et al., 2021; Maye et al., 2018; Schmick, 2014). The cause of this pH increase is unknown, but other research has elucidated an increase in amino acid content from the addition of dry-hops (*Figure 1*, unpublished Bruner et al., 2021). The amino acid arginine was one of the largest gains associated from dry-hopping, and with its physio-chemical properties (polar with a pKa of 12.5) (Borman et al., 1946), it is believed to be the source of pH increase seen from dry-hopping. Additional research has found the seeds of most plants have arginine accounting for 40-50% of the nitrogen content of those seeds (Winter et al., 2015) and is a large percentage of the amino acid content of hops as well hops (*Figure 2*, unpublished Bruner et al., 2021). The source of this amino acid and nitrogen increase is also believed to be associated with the fertilizers utilized in the field during growth of hop bines (Cook, 1974; Näsholm and Ericsson, 1990; Nordin et al., 2001). Both the content and source of nitrogen and amino acids will be explored and hopefully correlated to the increase in pH observed during dry-hopping with this research.

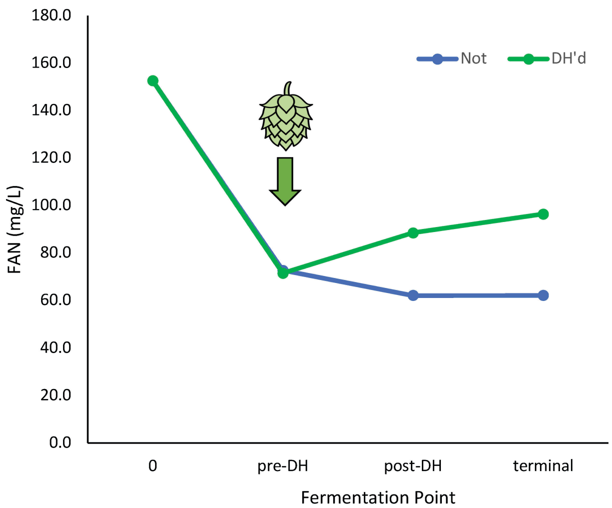

Figure 1. Averaged amino acid content for fermentations of twelve different yeast in mg/L. Measured via ion exchange chromatography for the both the non-hopped and dry-hopped treatments at the start of fermentation, on the day of dry-hopping, twenty-four hours after dry-hopping, and at terminal gravity.

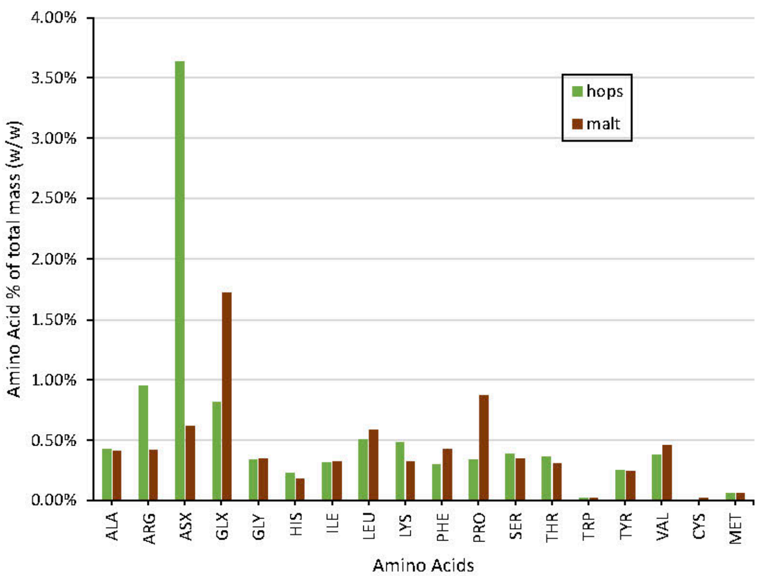

Figure 2. Amino acid content as percentage of total mass (w/w) wet basis in the Centennial hops and malt blend used for this study. ASX and GLX are a combination measurement of asparagine/aspartic acid and glutamate/glutamine respectively, accounting for their much higher relative concentrations.

Materials Needed:

- Hops: (delivered from OSU experimental plots) – no cost
  - Seven replicate samples of Citra cones from each of the following treatments:
    - 145 lbs/Acre nitrogen as calcium nitrate (all nitrate N source)
    - 145 lbs/Acre nitrogen as ammonium nitrate (high ammonium N source)
    - 195 lbs/Acre nitrogen as calcium nitrate
    - 195 lbs/Acre nitrogen as ammonium nitrate
- Seeds: *Humulus lupulus* cultivar unknown – already purchased
  - Ordered 10 g from website
- Experimental Beer – no cost
  - Side stream Tropicalia after pitch
  - Associated brewing supplies
- Bottles – around $21 each (possibly borrowing from Fox lab)
  - 31 1 L pyrex bottles for bench top fermentations
    - 28x hopped samples, one control, 2 seed treatments
  - Total potential cost = $650
- 50 mL falcon tubes for table-top centrifuge and sample transfer for amino acid analysis
  - 366 total samples
  - 500 pack for $121

Equipment Used:

- Anton Paar Alcolyzer with built-in pH analysis
- Table top centrifuge
- Autoclave
- Hop grinder
- Ion-Exchange Chromatography with Ninhydrin capabilities for amino acid analysis
    - Outside lab, Molecular Structure facility at UC Davis
    - $50 per sample = $18,300
    - We will also be getting the services of a new graduate student in Glen Fox's lab, with possible additional analysis via the ThermoFisher Gallery Plus Beermaster and possibly NMR for no cost.

Timeline:

1. Hops arrived from OSU
2. Bench trials at Creature Comforts – can start within 3 weeks of funding approval
    a. Need to source glassware for bench fermentations
3. Wort, beer, and hop samples to UC Davis within 2 months
4. Analysis 30-60 days, depending on availability at UC Davis Molecular Structure Facility
    a. Glen Fox is coordinating university pricing and collaboration
    b. We will also be getting the services of a new graduate student in his lab, with possible additional analysis via the ThermoFisher Gallery Plus Beermaster and possibly NMR for no cost.
5. Data analysis and write-up to follow
    a. Also possibly with the grad student involved so they can get a publication under their belt

Methods:

- Bench-top fermentations with 10g/L ground whole cone hops
    - Sort seeds from whole cone samples
    - Grind hops for bench trials Freeze with dry ice or liquid nitrogen first.
    - Frozen with LN2 or dry ice. Blended in iso-cleaned coffee grinder. Freeze until use
    - Pull Tropicalia wort off fermenter Where/how is this stored prior to day 4?
    - Pre-dry hop (Day 4). 900mL into 1L flask. No airlock, just loose parafilm and caps Are the hops added this day?
    - Dry-hop with 10 g/L per fermentation
    - Swirl to mix and then never mix again What are our 3 time points for sampling?
- Anton Paar analysis with pH - Confirm pH increase from dry-hopping
    - Sample 100 mL into two 50 mL conical centrifuge tubes
    - Spin down with tabletop centrifuge
    - Decant to Anton Paar carousel tubes for analysis
    - Save ~40 mL of 3 time points for amino acid analysis (AA analysis requires less than 1 mL per run, additional for other analysis)

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        - Pre-DH: directly before the addition of dry-hops (Down to 800mL)
        - Post-DH: 24 hours post the addition of dry-hops (Down to 700mL)
        - Terminal: when yeast activity has finished (Down to 600mL)
        - No mixing before pulling samples
        - Freeze until shipped for analysis (dry ice shipping preferred but not 100% necessary). Potential shipping cost: $250

- Ninhydrin amino acid analysis
    - Amino acid content to be determined using an L-8800a (Hitachi High-Tech America; Santa Clara, CA, USA) amino acid analyzer at the Molecular Structure Facility at UC Davis (Davis, CA, USA). 200 µL of centrifuged beer is diluted with 50 µL of 10% (w/v) aqueous solution of salicylic acid, frozen overnight, thawed, vortexed then centrifuged, and diluted with a norleucine standard before injection into the analyzer. Solid hop samples are hydrolyzed in both base (4.5N NaOH at 110 °C) then acid (6N HCl at 110 °C) for twenty-four hours, then diluted in a lithium citrate buffer before injection. The analyzer utilizes ion-exchange chromatography to separate amino acids followed by a post-column ninhydrin reaction detection system (Le Boucher et al., 1997).
- Next Steps:
    - Confirm which amino acids are causing the spikes with purified compound additions instead of dry-hops to beer.
        - Initial analysis (*Figure 2*) points to asparagine, arginine, glutamine, and leucine as possible candidates
        - Cost variable based on sample size
        - Will confirm with this work to dial in which are necessary

Total Samples:

- For Amino Acid Analysis
    - 7 replicates × 4 treatments = 28 hop samples
    - Baseline wort sample
    - 31 bench top fermentations × 3 time points = 93 beer samples
        - 1 control (Will probably actually do 2 or 3)
        - 2 "seeded" samples (high and low dosage)
            - What % of seed equivalent should we aim for?
            - Do we care that we don't know the variety?
        - 28 hops × 3 time points (pre-DH, post-DH, terminal)
    - **Total Samples = 122**
        - In triplicate = 366 samples

Total Cost: $19,321

- Possible Price Reductions:
    - Only run samples in duplicate (–$6100)
        - Less significant results
    - Cut terminal amino analysis (–$4200)
        - Freeze now and come back later
    - Borrow Pyrex Bottles from Fox Lab (–$650)

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Bibliography

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