Q&A Session from the April 18, 2019 Webinar

Understanding Hop Enzymes and Their Actions on Dry-Hopped Beers

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1. **Did you remove the hops from the beer you tested at 40 days, or was it on the hops the entire time?**

   In this lab scale dry-hopping trial, beer was in contact with hops for the entire duration of 40 days.

2. **Do you get the same enzymatic activity and maltose production when doing a single dry-hop addition or doing 2 or more smaller additions? Or does it get worse when doing several additions?**

   That's an interesting thought, and certainly using these different dry-hopping strategies creates more complexity and therefore, challenges in predicting hop enzymatic potential. We did not test combinations of different hop varieties, nor multiple hop addition points. However, based on the differences observed when screening hop varieties, it is expected that by combining multiple dry-hop varieties enzymic potential could express differently than when using single varietals for dry-hopping.

3. **I also wonder how common it is to encounter Amyloglucosidase (typically a fungal enzyme) into hops?**

   Yes, and this is certainly a question that remains to be answered. One important distinction to make is that we used an assay to detect the *activity of* amyloglucosidase, not the presence of amyloglucosiase itself. The Megazyme assay essentially measures end products (sugar attached to a color indicator) using a substrate specific to the action of an enzyme. We did not isolate for particular enzymes, working with a rather crude hop extract, and it is possible that we were detecting activity of a similar enzyme that has the same function (such as cleaving maltose to release glucose). For example, a disproportioning enzyme found in Arabidopsis chloroplast has been shown to also release glucose from linear maltooligosaccharides (Streb & Zeeman, 2012).

4. **Regarding the DH temperature data: do you have data points to show that the sugar profiles started out the same at day zero?**

   For the lab scale dry-hop treatments at different temperatures, each treatment was prepared in an identical fashion; that is, same commercial base beer, same hop variety, same hop concentration, and sodium azide addition. Each treatment was prepared in triplicate to account for variation. Sugar profiles were therefore expected to be the same at day zero, however we did not start measuring sugar profiles until day one. We did measure the untreated, commercial base beer which started off with 0.23 ± 0.05 g/100 mL of maltose and 0.02 ± 0.00 g/100 mL glucose.

5. **Did you look at different yeast strains and impacts with the hop enzymes? Or packaging conditions like TPO, etc?**

No, we did not dry-hop using different yeast strains, nor did we investigate the impact of yeast strain on hop enzyme activity. More research is needed to understand the influence of different yeast strains on hops during fermentation. As for packaging conditions, TPO was not measured or taken into account for commercial beers.

6. **What about change in sugar profile with a temperature of 50C? That would be at a temperature where yeast activity would be stopped.**

   We did not look at temperatures greater than 30° Celsius in this study. If dry-hopping at higher temperatures, such as 50° Celsius, one would need to consider the effect on a number of things important to beer quality. Heat and oxidation are generally very detrimental to beer quality post fermentation as they accelerate staling reactions. It is possible that by raising the temperature of a dry-hop reaction, hop enzymes could express a higher rate of activity and different pattern of dextrin degradation (For instance, malt enzymes have an optimal temperature between 60-70° C). In the paper published by Janicki et al., enzyme experiments were carried out at 50 ° C, with some hops reported to produce up to 700 mg of maltose/g of starch after only 5 hours of incubation! If the point is to render yeast inactive, remember, hop enzymes will act on beer dextrins regardless of the presence of yeast. The action of hop enzymes on beer directly results in a change in the composition of dextrins and sugars in beer, which can impact flavor and stability. The presence of yeast in combination with hop enzyme activity is what causes "hop creep." It's also important to consider that a higher temperature as you have suggested could result in undesirable flavor compounds produced by yeast, as well as loss of or change in hop aroma compounds. If the goal is to stabilize beer by heat treatment, pasteurization can achieve this in a way that minimizes loss of flavor by applying high heat over short duration (for example, 72° C for 30 seconds). I think it would be really interesting to look at the impact of higher heat applications on hop enzymes; the question is whether there is a sweet spot that renders enzymes inactive but preserves flavor and quality of beer. Perhaps an easier solution for brewers could be to heat treat hops rather than beer, and this is what Dr. Shellhammer's group is currently investigating.

7. **pH has effects on the enzymatic activity. Has it correlated with the maltose production?**

   We did not investigate pH in this study, however Janicki et al. did not find that saccharifying activity (the ability of hop enzymes to produce maltose) of starch substrate varied across pH values from 4.1 – 4.8, which is a typical range for dry-hopped beer.

8. **Wondering if there have been any accounts of gushing or dangerous package pressure in unfiltered package.**

   Yes, there have been accounts of this occurring in unfiltered dry-hopped beers, although it remains challenging to attribute the cause of gushing or package overpressure from the end result alone. While "hop creep" can occur in package, and we have confirmed this with a number of brewers, there are other things that could cause "refermentation" in package. One of these being *Saccharomyces cerevisae* var. *diastaticus* which contains the gene for amyloglucosidase, an enzyme able to break down beer dextrins into fermentable sugars.

9. **Are there food safety concerns with increased pH's identified with hop creep and is there a correlation between HOP control of micro with the increased pH?**

   Absolutely, and this is an important question. As you may already be aware, the practice of dry-hopping has been shown to increase the pH of beer; while traditionally beer may have finished around 4.2, we are seeing dry-hopped beers reach a pH of 4.8. Alcohol strength and pH are critical in protecting beer from pathogenic bacteria, with hop acids having weak bacteriostatic activity against gram-negative bacteria, such as *Salmonella* and *E. Coli*. While there has been recent research around the microflora of hops, there is little information on whether the modern practice of dry-hopping can harbor food borne pathogens. We are currently undergoing a study with Dr. Randy Worobo at Cornell to evaluate the microbiological risks associated with dry-hopped beer. A series of conditions (pH, alcohol, pathogen type) are being investigated to determine whether common food borne pathogens can grow and survive in dry-hopped beer, in particular low and no alcohol products. If you'd like to see what we found, I will be presenting a poster on this topic at the American Society of Brewing Chemists conference in New Orleans!

10. **In your analysis of hop varieties, were there any other varieties that showed significant difference in activity between harvest years like Amarillo did?**

    No, but we only looked at Amarillo from two harvest years. This is something that needs to be further investigated; while we saw differences between varieties of hops, there are very likely other variables that influence enzyme activity; the question is, what are they and then, which ones can be controlled?

11. **How do you know that this creep is not from Saccharomyces with STA1 genes? Does creep occur at temperatures that inhibit yeast? (50C)?**

    The dry-hopping experiments carried out in our study included the use of an antimicrobial to prevent the growth of microbes such as yeast that might interfere with the assay. In our case, sodium azide was used but researchers in the past (Brown and Janicki) used chloroform and toluene to inhibit yeast growth, which allowed them to focus only on the activity of hop enzymes and not that of microorganisms. And to your point of increased temperature, those same researchers could still observe the degradation of soluble starch by hops at 50 ° C even after ruling out yeast activity. While hops certainly do harbor microbes, the growth and development of wild yeasts capable of hydrolyzing beer dextrins is quite slow in comparison, and this was ruled out as a possible explanation by Brown and Morris in their original investigation of this issue in the Trans. Inst. Brew. in 1893.

12. **Given the difference in your trial with the lager and IPA, the IPA produced more maltose because there were more dextrins present in the wort. Could the timing of dry hopping - earlier rather than later in primary fermentation, produce more maltose?**

    Possibly, however the dextrin profile will be more influenced by the mash than fermentation, since brewing yeast are unable to break down beer dextrins. If hops are added earlier in fermentation, it is possible that they would produce more maltose and glucose in part due to the extended duration of dry-hopping, or at least produce it earlier in the fermentation/dry-hop process. There is going to be a point where no more maltose can be produced after hop enzymes have exhausted the source of dextrins. We

saw that after several days of dry-hopping, maltose reached peak production, and then either leveled out or slowly tapered off; however, this depends on the beer dextrin profile, and dry-hopping conditions. Hop enzymes have also been observed to hydrolyze maltose, which would be present in higher concentrations at the start of fermentation. In this case, hop enzymes could change the ratio of glucose to maltose as a fermentation source for yeast, potentially impacting flavor metabolism. I can speculate, but the answer is, it's complicated and we really don't know!

13. **Did you measure the residual enzyme activities in beer? It would be interesting to see what's the enzyme levels leftover in dry-hopping beer, thanks!**

    Very interesting question! We did not measure this, having moved away from targeting specific enzymes and towards looking at the holistic effect on beer. We did measure the fermentable sugar produced in heat-treated finished dry-hopped beers, and observed an increase in fermentable sugars, notably maltose. This could indicate that there are dextrin hydrolyzing enzymes present in the finished dry-hop beers surveyed but does not specify their type or origin.

14. **Have there been any observed difference in enzyme potential between T90 and T45 hop pellets?**

    Our study only monitored hop enzymic potential in T90 pellet hops. I am not aware of a similar study comparing T90 and T45.

15. **Have you considered measuring alpha-glucosidase? That would explain why the Maltose is decreasing and Glucose is increasing.**

    Thank you for this question! Yes, this is something we noticed as well. Using the Megazyme amyloglucosidase assay reagent (R-AMGR3), we were able to detect low levels of amylglucosidase activity (0.02 U/g for Cascade hops), which is a 1,4-alpha-glucosidase, and could help explain the reduction of maltose concurrent with increase in glucose concentration. As you know, amyloglucosidase removes glucose from non-reducing ends of α-1,4 and branching α- 1,6 linkages, with a preference for α-1,4 linkages and longer chain oligosaccharides (EC 3.2.1.20). However, it's possible that we detected another enzyme having similar functions to amyloglucosidase, since we used only crude hop extracts.