**Title:** A complete quantification of fermentable sugar within hops: free, bound, and starch-held sources

**Summary:**

The goal of this project is to investigate the additional fermentation that occurs as a result of sugars within hops. There are potentially three forms of fermentable sugar contained within the plant: (1) Free, unbound, simple sugars that have been previously reported; (2) Starches, in small quantities, within seeds observed in hop samples, which are broken down by starch degrading enzymes during secondary fermentation; (3) Most interestingly, an unknown quantity of bound sugars are freed by yeast B-lyases during fermentation.

Plants use simple sugars such as glucose to hold molecules in inactive forms for later metabolic and chemical uses. Historically, brewers and hop researchers have only investigated certain glycosidcally bound molecules due to the aromatic compounds released. However, a multitude of other molecules are also bound to simple sugars, and subsequently release after reacting with enzymes such as B-lyase, from yeast, during fermentation. Researchers tend to focus on the aroma molecules and not the sugars bound to these compounds; what happens to that sugar moiety could have significant implications for brewing and scientific understanding of hops' – specifically dry hops' – effects upon the fermentation process.

**Duration:** 1 year: New Proposal

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Other Funding Sources and Support: None

Send Funding to: Delanee Bourland bftvacct@ucdavis.edu

# Body

**Title:** A complete quantification of fermentable sugar within hops: free, bound, and starch-held sources

**Statement of problem:**

Hop creep, a result of adding hops to fermentation vessels post-boil ("dry hopping"), results in unpredictable and prolonged fermentations as compounds within the hop cone contribute various forms of fermentable sugars. The total concentration of both bound (glycosidic and within starch) and unbound sugars within hops that contribute to secondary fermentations is currently unknown. Therefore, every brewery that dry hops their beer does so with marked uncertainty as to expected fermentation parameters, risking quality, revenue, and possible refermentation within the packaged product.

**Justification and Importance:**

Within the brewing industry, tank timelines and fermentation consistency are key to profits and reliable recipe development. Accurately forecasting tank residency allows breweries to remain profitable; failing to do so can incur thousands of dollars in costs per day. Fermentation inconsistency also threatens beer quality and creates legitimate safety issues for brewers and customers alike: refermentation in the packaged product can result in exploding cans or bottles.

This work aims to significantly reduce the complexity and uncertainty associated with secondary fermentation due to dry hopping. Hop creep occurs when dextrin degrading enzymes cleave dextrins within the wort, creating simple sugars which are then fermented by yeast. An additional, less researched step of secondary fermentation involves fermentable sugars contained in the hops themselves, and the variety of sources for those sugars within the plant.

Plants bind molecules to sugar to store them in an inactive form. When the plant needs to use the molecule, it enzymatically cleaves the bond, activating the molecule and releasing the sugar. These molecules have a variety of purposes that include defense from insects, attraction for pollinators, or even intracellular uses. Aroma in beer is just one small component of these molecules, but the only glycosidcally bound portion that has been explored in the existing body of hop research.

A complete quantification of unbound, bound, and starch-laden sugars could allow brewers to better understand the types and concentrations of sugars that dry hop additions introduce, thereby increasing predictability, and reducing the risks of secondary fermentation. Do hops contain a constant or variable quantity of fermentable sugar? How do hop terroir, product type, or variety impact this concentration? This research will also explore starch from seeds' prevalence within hops and its impact on secondary fermentation. It will confirm previous quantities of free fermentable sugars, but on a larger scale than existing research. Finally, and most interestingly, it will quantify the glycosidcally bound portion of sugars that has not been meaningfully researched up to this point.

**Objectives:**

1. Quantify unbound, free fermentable sugars. Confirm and expand upon previous works exploring fermentable sugars within hops by exploring a comprehensive cross-section of varieties, terroirs, and product types.
2. Quantify fermentable sugar created from starch within crushed seeds found in hop samples. These include the dextrins broken down by previously established “hop creep” enzymes during secondary fermentation.
3. Quantify bound fermentable sugars from glycosidcally bound inactive molecules formed within the hop plant.
    a. Objectives 1-3 will occur in stages over the next two-year period. Each hop will be evaluated for all three types of sugars, followed by the next hop. As a planning factor, it will take about one week to fully process 5 samples; each sample is tested in triplicate.
4. Compare hops across different terroirs in total sugar quantity within all three categories.
    b. Objective 4 will be completed in the final two months. This will require data management and coding within RStudio.

**Procedures and Methods to accomplish objectives:**

All research will be conducted at the Robert Mondavi Institute (South) at University of California, Davis in the Sierra Nevada Laboratory.

The experiments will be performed in triplicate on all hop samples provided. The samples will include whole cone, T90 pellets, Cryo hops, and enriched polyphenol pellets. In addition, the samples include varieties from the Pacific Northwest, Australia, New Zealand, Michigan, Germany, France, Czech Republic, England, and Slovenia growing regions. Proposed experimental method is as follows:

1. A spectrophotometric method of quantifying plant sugars via grinding, liquid extraction in a chloroform methanol water solution and then quantified using Megazyme starch kits and UV/Vis. This method will be effective in quantifying the baseline of free sugars within hop samples. This method is laid out in “Soluble Sugar and Starch Extraction and Quantification from Maize *(Zea mays*) Leaves” by Kristen A. Leach and David M. Braun. (Full citation below)
2. Samples will be treated with B-lyase and measured again to quantify bound sugars with the Megazyme kits and UV/Vis Leach and Braun method.
3. Comparison with a method from CDRBeerLab will be used in conjunction with these results. Beer will be used as the medium to extract sugars. Hops will be incubated with and without B-Lyase. The beer will be measured based on CDRBeerLab methods to quantify fermentable sugars. In addition to its application for this study, this represents a new method for brewers to employ utilizing technology within their grasp.

Results will be analyzed in Excel, R, and with RStudio. Data will be analyzed using statistical methods of ANOVA to determine whether significant differences exist between product types and terroirs. Tukey post-hoc analysis will statistically categorize results based on sugar quantities and types of sugar sources.

**Outcomes:**

This project will produce an actionable understanding of the hop sugars that drive secondary fermentation following dry hopping. Hop creep is just one aspect of the process that should also include

the different sources of sugars within hops. This project's scientific conclusions will empower brewers with more complete information and realistic expectations for secondary fermentations and hop additions. The starch portion of the study will inform hop farmers on the downstream brewing impacts of starch within hop seeds, and seeds' relative prominence within hop products. This study will further illuminate the complexities that arise from the addition of hops in fermentation.

**Extension and Outreach Activities:**

Information obtained from this study will be distributed in academic and industry publications with open access availability to brewers and farmers. Presentations on the results will occur at the Hop Research Council conference as well as other leading brewing events and conferences which will be determined upon research completion and publication.

**Literature Review**


1. Leach KA, Braun DM: **Soluble Sugar and Starch Extraction and Quantification from Maize (Zea mays) Leaves.***Curr. Protoc. Plant Biol.* 2016, **1**:139–161.
    a. This paper specifies the base method that will be used to quantify free sugars and starch.
    b. A modified version of this with the addition of B-lyase will be used to measure the bound sugars.
    c. A modified version of this with an acid hydrolysis step will be used to measure the bound sugars.
2. Mattiacci L, Dicke M, Posthumus MA: **beta-Glucosidase: an elicitor of herbivore-induced plant odor that attracts host-searching parasitic wasps.** *Proc Natl Acad Sci USA* 1995, **92**:2036–2040.
3. Singh G, Sharma S, Rawat S, Sharma RK: **Plant Specialised Glycosides (PSGs): their biosynthetic enzymatic machinery, physiological functions and commercial potential.** *Funct. Plant Biol.* 2022, doi:10.1071/FP21294.
4. Minic Z: **Physiological roles of plant glycoside hydrolases.** *Planta* 2008, **227**:723–740.
5. Mithöfer A, Boland W: **Plant defense against herbivores: chemical aspects.** *Annu. Rev. Plant Biol.* 2012, **63**:431–450.
6. Frandsen TP, Svensson B: **Plant alpha-glucosidases of the glycoside hydrolase family 31. Molecular properties, substrate specificity, reaction mechanism, and comparison with family members of different origin.** *Plant Mol. Biol.*1998, **37**:1–13.
7. Gholami M, Coombe BG, Robinson SP, Williams PJ: **Amounts of glycosides in grapevine organs during berry development**. *Aust. J. Grape Wine Res.* 1996, **2**:59–63.
8. Caffrey AJ, Lafontaine S, Dailey J, Varnum S, Lerno LA, Zweigenbaum J, Heymann H, Ebeler SE: **Characterization of Humulus lupulus glycosides with porous graphitic carbon and sequential high performance liquid chromatography quadrupole time-of-flight mass spectrometry and high performance liquid chromatography fractionation.** *J. Chromatogr. A* 2022, **1674**:463130.

    a. These articles spurred some of the thought process of sugar bound entities in plants and their uses. Where do the glycosides come from? How are they formed in plants and what are their purposes?

9. Morcol TB, Negrin A, Matthews PD, Kennelly EJ: **Hop (Humulus lupulus L.) terroir has large effect on a glycosylated green leaf volatile but not on other aroma glycosides.** *Food Chem.* 2020, **321**:126644.
    a. Difference of glycosylates, but again focused volatile/aroma glycosides.

**Attachments:**

1. Gannt style timeline attached

2. C.V. (JY and GPF)

3. Table with outline of funding