Diastatic Activity in Citra® Hops as a Function of On-Bine Maturity

by

Aidan Trent Long

A THESIS

submitted to

Oregon State University

Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Food Science and Technology
(Honors Scholar)

Presented May 26, 2021
Commencement June 2021
Dry-hopping has grown from a novel to widespread technique in commercial craft beer production. It yields large returns in aroma that cannot be emulated with kettle hopping. However, it also leads to beer refermentation post-dry-hopping, a phenomenon colloquially referred to by U.S brewers as “hop creep.” This is a result of native hop enzymes hydrolysing unfermentable dextrins into fermentable sugars, causing refermentation in the presence of yeast. This leads to quality and even safety issues for the product. Previous studies have provided some evidence for the activity of these enzymes and their potential dependence on on-bine maturity as a key factor. By measuring changes in fermentable sugar production in a bench-top dry-hopping protocol using Citra® hops with a range of on-bine maturities, a relationship was observed. This relationship showed decreasing enzymatic power with increasing maturity, suggesting a number of potential biochemical factors as a rationale. These findings provide additional insight into agronomic factors that lead to different levels of hop diastatic power, while paving the way for future research and fuller realization of the topic.

Key Words: Dry-hopping; hop creep; enzymes; dextrins; hops; *Humulus lupulus*; on-bine maturity

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I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

__________________________
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CONTRIBUTION OF AUTHORS

The hop enzyme activity collection via HPLC, data analysis and thesis writing were carried out by Aidan T. Long. Extensive guidance and assistance with the analytical methods for measuring hop enzyme activity was provided by Lindsey N. Rubottom. Hop chemistry analyses was performed by Richard W. Molitor and the Shellhammer laboratory technical assistants. Study conception, design, assistance with data analysis, interpretation and editing is credited to Professor Dr. Thomas H. Shellhammer.
INTRODUCTION

*Humulus lupulus*, or the hop plant, is one of four key constituents in beer, making up the majority of bitter and aromatic compounds. Bitterness is derived from the isomerization of native alpha-acids in the hops, via thermal conversion of the compound during wort boiling. Iso-alpha acids are flavor active unlike their non-isomeric form. The aromatic element in hops is made up by essential oils; a grouping of organic compounds that are produced as secondary metabolites and contribute distinctive aroma to this plant. Within hops, these essential oils are mostly terpenes, terpene alcohols and esters (as well as a very small amount of highly potent thiols).¹ Both alpha acids and essential oils are derived from a lupulin gland found within the hop cone of this perennial plant.

Hops are used primarily during the wort boiling stage of the beer brewing process. To obtain wort prior to boiling, a process known as ‘mashing’ occurs. During this process, hot water is mixed with malted and milled barley to induce an enzymatic liquefaction and saccharification of natural barley-derived starches. Amylose and amylopectin are the primary starches present in the endosperm and are the primary substrates for enzymatic action, although there is a very small amount of fructose present in barley as well. These polysaccharides are liquefied and then saccharified into simple sugars which include glucose, maltose and maltotriose. Liquefaction and saccharification takes place through the course of 0.5-1 hours, primarily through the actions of endogenous and hydrolytic alpha- and beta-amylases. Alpha-amylase is an endoenzyme that randomly cleaves internal alpha—1,4 glycosidic bond linkages in a
Beta amylase is an exoenzyme that works from the non-reducing end of carbohydrate chains. This enzyme systematically cleaves alpha—1,4 glycosidic bonds along the chain, producing maltose molecules. The increase in number of chains, and non-reducing ends, by alpha amylase leads to increases in the ability for beta amylase action, resulting in a beneficial relationship. However, these enzymes cannot cleave the alpha—1,6 glycosidic bonds that are part of the branch points on amylopectin molecules. This results in certain branched carbohydrate chains, referred to as limit dextrins, to remain in the wort. These limit dextrins can be processed by the lesser common enzyme limit dextrinase. This enzyme cleaves alpha—1,6 glycosidic bonds and produces straight chain dextrins. However, due to its low concentration in most malted grain and its temperature sensitivity, limit dextrinase in malted barley is never able to act on all limit dextrins during the mash. Therefore, typically 20-25% of carbohydrates in the mash are not completed hydrolyzed and remain as branched limit dextrins. Limit dextrins comprise the portion of unfermentable carbohydrates in beer that form part of the primary dogma of study within this paper.

Following the mashing period is the separation phase. This phase of brewing involves various engineering techniques to separate the high sugar content solution, wort, from the solid matter. This typically takes place in a dual-purpose mash tun (with a false bottom), a lauter tun or a mash filter. Wort is then boiled, where hops are added. There are a number of methods for hop additions during boiling, known as kettle hopping. By adding hops at earlier or later periods in the boiling process, iso-alpha acid content is
manipulated by changing allotted time for thermal conversion. Moreover, aroma levels are controlled by hop addition timing since essential oils are volatilized over time during boiling. Following the period of boiling and alpha acid isomerization, wort is typically whirlpooled to facilitate separation of suspended solids and as a final opportunity to add further hops. Hops added at this point allow significant aroma contribution due to minimal volatilization, but offer relatively negligible iso-alpha acid formation. Therefore, most kettle hopping is typically focused on formation of bitterness (via iso-alpha acids) while late addition and whirlpool hops are primarily for aroma (via essential oils). Post whirlpool, the wort is cooled to fermentation temperature and yeast is added (known as pitching). Yeast facilitates fermentation, utilizing the simple sugars in the wort to produce carbon dioxide and ethanol. There is also a large matrix of biproducts produced during fermentation, which trigger their own cascade of reactions both directly and indirectly. Upon the completion of fermentation, beer is nearly at its completed stage. It is at this point that the beer is often clarified by various methods and eventually packaged into a range of container options. Many commercial breweries will pasteurize beers to prevent microbial activity persisting in the product. Most small to medium size breweries, however, do not have this ability or desire.

Modern beer production generally utilizes hops to a far greater extent than any previous times in history; and, in different ways. A contemporary technique, which is heavily utilized by the craft brewing industry, is called dry-hopping. Dry-hopping is the process of adding hops to a beer directly, after the beer has completed its primary fermentation
period. After primary fermentation, the vast majority of fermentable extract has been
acted upon by yeast, but most yeast cells are still in suspension and viable (remaining
active to act on residual fermentable extract and metabolize byproducts). Since there is
no heating of the hops, no alpha acids are isomerized and also no essential oils are lost
to steam volatilization. This retains essential oils to a far greater extent than kettle
hopping and even whirlpool hopping. And, without the formation of bitterness,
significantly greater amounts of hops can be added to beer during dry-hopping than via
kettle or whirlpool hopping. The strength in retaining essential oil aromatics makes this
process very popular for many modern beers. However, as the hops are not added to
boiling wort, there is no denaturation of any hop enzymes.

Dry-hopping causes a cascade of chemical changes in beers, both directly and
indirectly. A large function of these chemical changes is surmised in the process of
‘hop creep,’ as it is colloquially referred to by craft brewers. This is defined well by
Stokholm and Shellhammer as a function of enzymatic action that breaks down short
chain (unfermentable) carbohydrates into fermentable sugars.³ The unfermentable
carbohydrates being referenced are those of branched limit dextrins, which cannot be
hydrolyzed due to the limited ability of mash enzymes to cleave alpha—1,6 glycosidic
bonds, as previously discussed. The newly hydrolyzed fermentable sugars are
metabolized by active yeast, causing a refermentation. This means a decrease in limit
dextrins that result in a proportional increase of carbon dioxide and ethanol, with
roughly one molecule of each of these products for two molecules of each substrates.
In addition to these major products are a number of minor products, namely diacetyl,
that are also released into the beer. The conditions for hop creep are thus the presence of branched dextrins contained in the unfermentable ‘real’ extract, presence of un-boiled hops and live yeast for refermentation.³ ‘Real’ extract refers to the actual amount of dissolved solids without obscuration, while the ‘apparent’ extract is obscured by the presence of ethanol. The ‘real’ extract contains small amounts of minerals, protein and a range of carbohydrates. These carbohydrates are principally dextrins in beer that are produced during typical mashing regimes. The unexpected refermentation, caused by the above conditions, can cause a number of issues in the beer. One issue related to the increase in beer fermentability results in potentially decreased perceived body and increased dryness. This is due to the decrease in unfermentable limit dextrins that form the sensory aspect of a beer’s ‘body.’ This is likely the etymology of the term dry-hopping, whereby British brewers discovered that adding hops to beers post-fermentation during cask conditioning created a drier tasting beer.⁴ In addition to decreased body, the amount of ethanol is also increased. Not only can this also change the sensory profile of the beer, but it could also lead to legal issues in stating exact alcohol content of products. Another change in sensory profile experienced due to refermentation is the increased flavor issues such as diacetyl. Diacetyl, a vicinal diketone chemically known as 2,3-butane-dione, is a compound that is normally formed by yeast as a biproduct of amino acid syntheses. It has a characteristic ‘buttery’ or ‘butterscotch’ flavor considered undesirable at high concentrations. Without adequate time and temperature, this compound cannot be re-metabolized and reduced by yeast to the 2,3-butanediol.⁵ The reappearance and slow uptake of diacetyl in response to dry-hopping is particularly challenging for some brewers. Another potential off-flavor
produced during refermentation is acetaldehyde, which is an intermediate compound in the production of ethanol. This compound also needs time to be fully metabolized and dissipate from the beer. The presence of both of these compounds (among others) alter the flavor profile of a beer in a generally undesirable manner and would require an extended maturation time in order to be mitigated in the final product. This is obviously a logistical and cost issue for many breweries. The final issue caused by refermentation is the buildup of carbon dioxide post packaging. As yeast continue to ferment newly hydrolyzed simple sugars after dry-hopping, carbon dioxide is produced. In the cases where beer is packaged shortly after dry-hopping induced fermentation might appear to be complete, the slow refermentation can easily continue or start after packaging and result in carbon dioxide buildup to dangerous levels in the package. At a low level of refermentation, this could lead to a quality issue being over-foaming of a beer once the package is opened or the beer dispensed. At a high level of refermentation, this could lead to a serious safety issue whereby packaged beers could explode due to extreme pressure buildup. As beers are often packaged in glass bottles, this is an extremely dangerous prospect that can cause serious harm to consumers.

Hops, and the enzymes associated with them, are the direct cause of the dry-hop induced refermentation phenomenon. A degree of existing research has been performed on different aspects of this topic that create a current knowledge base for this study. The first records of the presence of hop-derived diastatic enzymes are back in 1893, by Brown and Morris, which were in specific reference to dry hopping. Their enzymatic effect on beer was referenced as the “‘freshening or condition’ power of the hops,”
causing a “cask fermentation” (post-packaging refermentation). One of the three potential conclusions drawn was that hops contain a ‘diastase’ that hydrolyses beer dextrins, thus creating the first reference of the hop enzyme phenomenon in beer.\(^4\) However, this was only one theory and it was not until 1941 that it was academically investigated. At this time, Janicki et al. reinvestigated the presence of diastatic activity in hops. Using scientific methods, Janicki et al. confirmed the presence of a ‘saccharifying enzyme’ which is manipulated in its activity by “additional factors, at present unknown.” \(^6\) In modern hop chemistry research, the presence of hop enzymes and their action on finished beer was confirmed with contemporary laboratory techniques by Kirkpatrick.\(^7\) Kirkendall et al. also studied the nature glycolytic enzymes in hops, factors involved in their action and their impact on final products.\(^8\) Additionally, Sharpe et al. and Lafontaine et al. provide strong evidence for the phenomenon of changing chemical composition of hops with changing maturity, while they offered no investigation into enzymatic changes.\(^9\) Further studies by Kirkpatrick and Shellhammer demonstrated variation in dextrin degrading enzyme levels of different hops due to factors of genetic differences, growing and processing techniques.\(^10\) And most recently, Rubottom measured the effect of kilning parameters on dextrin degrading enzyme potential.\(^11\) Kilning is a principal processing technique that has large potential in manipulating enzyme content of hops. By heating the hops at high temperatures, for a sufficient time, native hop enzymes would become denatured. This, along with Kirkpatrick and Shellhammer’s work regarding other factors influencing dextrin degrading enzymes, provide the most relatable and recent research for understanding the phenomenon that is hop enzyme activity.
However, there are still substantial gaps in the full realization of this phenomenon due to the plethora of different variables that could affect hop enzymes from farm to beer. Some of these potential variables include possible varietal differences, regional differences and other environmental differences. One of these relatively unstudied variables is that of on-bine maturity. On-bine maturity refers to the amount of time the hop cone remains part of the plant before being harvested, specifically within the harvesting period where the plant is considered fully developed. This maturity time effects a number of biochemical factors of the hop cone, including essential oil composition & quantity, alpha & beta acid content and presumably enzyme content and/or activity. The relationship between dextrin degrading enzymes and on-bine maturity is not greatly known, but would be very helpful for both growers and brewers. This is principally due to the fact that brewers who utilize dry-hopping may want hops with lower enzyme content to mitigate the effect of hop creep. And, ergo, hop growers would want to cater to this demand. It is an important part of the growth of hop usage and the hop industry. The research herein will lead to a conclusion regarding the important relationship between on-bine maturity and dextrin degrading enzymes, and reason potential causes that drive this phenomenon.
EXPERIMENTAL

Hop Harvesting and Selection

There are a large range of hops used for dry-hopping, with Citra® being a popular cultivar according to the USDA 2020 acreage report. For this experiment, Citra® hops grown on the Perrault Hop Farm in Toppenish, Washington were selectively hand harvested from the same location, on the same field over the period of approximately one month (from late August to late September). There were six samples of hops harvested in intervals of seven days (Table 1). Approximately one pound of wet hops were picked at each time interval and then dehydrated to reduce their moisture to approximately 10% w/w. The dried hops were vacuum packaged in high barrier pouches and sent to the Shellhammer Lab at OSU for chemical and biochemical analyses. Packaged hops were stored at -23 °C (-10 °F) until analysis.

Table 1- Summary of Basic Hop Chemistry Information by Sample

<table>
<thead>
<tr>
<th>Lab ID</th>
<th>Total oil (ml/100g)</th>
<th>Moisture Content</th>
<th>% Alpha</th>
<th>% Beta</th>
<th>H.S.I.</th>
<th>Alpha-HPLC</th>
<th>Beta-HPLC</th>
<th>%CoH-HPLC</th>
<th>%CoL-HPLC</th>
<th>Dry Matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>20/8/2020</td>
<td>0.3</td>
<td>16.61</td>
<td>5.56</td>
<td>3.56</td>
<td>0.26</td>
<td>5.68</td>
<td>3.41</td>
<td>18%</td>
<td>40%</td>
<td>20.0%</td>
</tr>
<tr>
<td>27/8/2020</td>
<td>1.5</td>
<td>13.45</td>
<td>11.00</td>
<td>3.47</td>
<td>0.26</td>
<td>9.89</td>
<td>3.23</td>
<td>23%</td>
<td>44%</td>
<td>20.5%</td>
</tr>
<tr>
<td>3/9/2020</td>
<td>1.2</td>
<td>13.02</td>
<td>13.60</td>
<td>4.02</td>
<td>0.25</td>
<td>12.55</td>
<td>3.92</td>
<td>23%</td>
<td>45%</td>
<td>21.4%</td>
</tr>
<tr>
<td>10/9/2020</td>
<td>3.2</td>
<td>13.52</td>
<td>12.72</td>
<td>3.17</td>
<td>0.27</td>
<td>10.70</td>
<td>3.10</td>
<td>23%</td>
<td>41%</td>
<td>21.9%</td>
</tr>
<tr>
<td>17/9/2020</td>
<td>3.0</td>
<td>14.10</td>
<td>13.51</td>
<td>3.48</td>
<td>0.26</td>
<td>11.96</td>
<td>3.63</td>
<td>23%</td>
<td>42%</td>
<td>22.1%</td>
</tr>
<tr>
<td>23/9/2020</td>
<td>2.5</td>
<td>10.80</td>
<td>12.06</td>
<td>2.90</td>
<td>0.28</td>
<td>10.33</td>
<td>3.05</td>
<td>24%</td>
<td>45%</td>
<td>23.0%</td>
</tr>
</tbody>
</table>

All analyses other than moisture content and dry matter are adjusted to 8% for consistency in reporting.
**Chemicals**

Analytical grade maltose monohydrate 99.0%, glucose >99.5%, fructose >99%, and maltotriose >90% from Sigma Aldrich (St. Louis, MO) were used for HPLC analysis. For enzyme and dry-hop extraction buffers, sodium azide 99.5%, sodium acetate, glacial acetic acid, and Tris-Base were used (Fisher Scientific Waltham, MA).\(^\text{11}\)

**Hop Chemical Analysis**

A 150 g sample of homogenized, whole cone hops was coarsely ground using a grinder (Cabela’s Inc. Carnivore model #541555 (1-1/2 HP)) to prepare for chemical analysis. Hops were analyzed following ASBC Methods for moisture content (Hops-4A), total hop acids by spectrophotometry (Hops-6A), hop storage index (Hops-12) and total hop acids by HPLC (Hops-14).\(^\text{13}\)

**Hot Water Extract**

In order to quantify the native sugar content in each hop sample, a hot water extraction was performed and subsequently analyzed by HPLC. This was a method based on Rubottom.\(^\text{11, 7}\) The Hot Water Extract (HWE) was obtained by adding 0.5 grams of hop grist to 50 ml of a sodium acetate buffer (0.02 M, pH 4.2, and 5% EtOH). The hop grist was extracted into the buffer mixture over a period of 15 minutes in a hot water bath at 80 °C. Afterwards, a 1.5 ml aliquot was filtered through a 0.45µm syringe filter and frozen until HPLC analysis.
Dry-hopped Sample Preparation

To quantify the magnitude of dextrin-reducing enzyme activity in each hop sample, a small-scale model dry-hopping system was utilized. This was based on Kirkpatrick and Shellhammer.\textsuperscript{10} A 0.55 gram sample of hops was added to a separate 668.2 grams of a commercially available high dextrin beer (Total Domination, Ninkasi Brewing Company, Eugene, OR) to achieve a 10 g/L hopping rate. A 0.02 w/v % aliquot of sodium azide was added to the dry-hopped mixture to inhibit any microbiological activity native to the hops. These samples were incubated at 30 °C for a period of 48 hours, after which a sample was centrifuged and the supernatant ‘quenched’ with a 10% Tris-Base buffer to stop any enzymatic activity from proceeding further. Afterwards, this solution was filtered through a 0.45µm syringe filter and a 1.5 ml sample was frozen until further analyses. This benchtop biochemical assay was performed in duplicate for each sample.

HPLC Analysis

High Performance Liquid Chromatography (or HPLC) is a method that separates compounds on the basis of its chemical properties, specifically its polarity and elution with the solvent in question. It is a common method for measuring individual sugar concentrations in liquid solutions and was used here to differentiate and quantify the sugars present in the HWE or dry-hopped samples. The method used was that of Rubottom,\textsuperscript{11,7} whereby fructose, glucose, maltose and maltotriose were quantified using an Agilent 1200 series HPLC with a refractive index detector, using a Rezex RSO Oligosaccharide Ag + column which was operated at 80 °C under isocratic conditions.
of 0.30 mL/min Milli-Q treated water for the mobile phase. The specific methodology used for carbohydrate analysis was an adaptation of the ASBC Methods of Analysis, Sugars and Syrups 18.14

The HPLC gave outputs in retention time and peak area. Retention time changes are based on the characteristics of each sugar and was used for qualitative identification. Peak area was a function of the concentration of the compound present in the sample, thus yielding quantity.

**Data Analysis**

Pre-data collection, a set of sugar calibration standards were run on the HPLC to obtain response factors for the four carbohydrates being analysed. This provided a relationship between each carbohydrate’s peak area and its real concentration. After all data collection was completed, raw data was analysed with a master spreadsheet in Microsoft Excel. Peak area values were compiled across both two trials undertaken, including both HWE and dry-hopped samples. Response factors were then used to convert peak area values into concentrations (in ppm). An additional set of correction factors was also created for each sample and each trial, which consisted of a ratio of actual hop weight to desired hop weight and actual beer weight to desired beer weight where applicable. These correction factors were applied to concentration values for both HWE and dry-hopped samples, correcting for small differences in hop or beer quantities measured. Data from the duplicate trials were averaged for the dry-hopped samples. Finally, the applicable HWE corrected concentration values were subtracted
from the averaged dry-hopped sample concentration values. This yielded a final set of values that represented solely the change in concentration of the pertinent carbohydrates in the beer sample, due to dry-hop enzyme exposure.

**Statistical Analysis**

Microsoft Excel was used for graphing purposes and to quantify Pearson correlation coefficients and linear regression coefficients.
RESULTS AND DISCUSSION

Summary of Principal Data

As discussed in the previous section, HWEs were measured to obtain the native sugar content in the hops. Since the biochemical assay utilized the change in individual sugar concentration as a result of the hop enzymes’ action on beer dextrins, it was important to know the amount of baseline sugars present in the hops. This was subtracted from the enzyme samples to yield the true value of change, induced by native hop enzymes. The change in sugar concentration (over 48 hours) data set represents enzymatic activity. Raw data were collected, corrected with correction factors and HWE amounts, then averaged. On-bine maturity had a significant impact on the level of hop enzymes with an earlier harvest date, resulting in greater activity (Figure 1). The Pearson’s correlation coefficient for this relationship was -0.830.

Figure 1- Harvest Date vs Enzyme Activity (change in Maltose + Glucose concentration)
Through the HPLC analysis, it was obvious that maltose and to a lesser extent, glucose, were the sugars experiencing the most significant change in concentrations (Figure 2). Maltotriose and fructose changes were negligible in comparison (Figure 3). This speaks to how significant an impact is made with the introduction of native hop enzymes, completely changing the beer’s sugar composition. With such high increases in maltose content, one can postulate the presence of beta-amylase in the hop material being a significant component of the hop’s enzyme activities. This same conclusion was drawn by Kirkpatrick and Shellhammer.\textsuperscript{15}

![Figure 2- Harvest Date vs Logarithmic Increase in Maltose and Glucose from Native Beer Content](image)
Figure 3- Harvest Date vs Logarithmic Increase in Maltotriose and Fructose from Native Beer Content

Evaluation of Principal Data

As shown through the principal data collected, both through the graphical representation and Pearson’s Correlation Coefficient, there is a significant inverse relationship between enzyme activity in Citra® hops and their on-bine maturity. Additionally, it has been shown that the largest segment of sugar production from beer dextrin hydrolysis is attributed to maltose and (to a lesser extent) glucose. While the relationship is generally linear, certain data points demonstrate a slight increase in sugar content from those previous, despite the hops being more mature. Each data point alternates from lower than previous, then slightly higher and so on. In terms of the data,
this is referring to lower or higher sugar concentration with an increasing on-bine maturity.

There are several implications from this data collected. There is the immediate confirmation of previous understanding that dry-hopping does in fact provide the enzymatic power necessary to break down unfermentable dextrins into fermentable sugars, maltose and glucose. Moreover, with the trend and data produced, there is a sufficient connection drawn to implicate that greater on-bine maturity results in reduced native enzyme content or potency. The presence of these fermentable sugars leads to the aforementioned phenomenon of hop creep whereby yeast metabolize these sugars, a situation which most brewers seek to avoid.

**Further Discussion**

The relationships evaluated lend themselves to an array of existing evidence and analysis that furthers the basis of this research. Findings from the data explored here suggest a change in either hop enzyme content, potency or type that leads to deterioration in ability to degrade unfermentable dextrins. There are a multitude of factors that could have effects on the change in enzyme content, potency or type. A comparison of on-bine maturity to key growth constituents has the potential to yield insights into possible affecting factors.

One of the principal constituents in hops is alpha acids, which have a relationship with on-bine maturity shown below (Figure 4). % Alpha acid increases and peaks in early
September (around the picking window), after which it plateaus. This relationship between on-bine maturity and alpha acid content is corroborated in works by Oladokun et al. demonstrating its compact nature.\textsuperscript{16} While this relationship is not exactly the inverse of the maturity vs enzyme activity relationship (Figure 1), it does represent a somewhat opposite phenomenon that has potential connections to the principal data set.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Harvest Date vs % Alpha Acid and Total Oil Content}
\end{figure}

Essential oils are also a key constituent in hops. The relationship between total oil content and on-bine maturity is also shown above (Figure 4). Excluding the outlier point (from the 3/9 harvest sample), there is a general increase in total oil content until early September (around the picking window). After this point there is an incremental decrease with ongoing on-bine maturity. The above relationship between total oil content and harvest date is nearly identical to those in works by Sharp et al. and
Lafontaine et al. These corroborating results help solidify the integrity of the hop enzyme data presented above. Much like the relationship of alpha acid % with maturity, this relationship has potential links to the principal data set. There are a number of potential biochemical effects within the matrix of the hop plant that could have direct or indirect links between either of these factors and enzymatic activity.

% Dry matter is often used by hop growers as an index for measuring on-bine maturity and is one of the factors used to determine when hops are ready for harvesting (ideally the intersecting peak of % alpha acid and total oil content). The relationship above (Figure 5) demonstrates the linear relationship between % dry matter and harvest date, which also indicates the linear relationship between on-bine maturity and harvest date. The data points in red represent the start and end of the typical harvest window, these are the typical range of % dry matter values for regular hops. Changes in dry matter
content could also be a factor in the decrease in enzymatic activity over time, either by indirect or direct biochemical effects through the hop matrix. The fact that the harvest date vs % dry matter relationship is almost opposite that of the harvest date vs enzymatic activity begs the need for further investigation.

Although there is no evidence that can be conclusively implicated in the relationship of hop enzymes deteriorating with greater on-bine maturity, there is an obvious range of aforementioned possibilities. For further examination of these possibilities and solidification of the primary data evidence, a number of future studies need to be conducted. First and foremost, as this is a single study within a limited study pool (a single harvest year from a single field), another study from another harvest year, location and potential varietal needs to be completed in order to corroborate the evidence presented within this study or point out any differences that may be experienced. Preferentially, there could be multiple studies completed that all have single changes in one variable such as growing location (and associated environmental conditions), varietals, etc. Individual studies could also be completed to examine the enzymatic change over time in relation to the comparison data points of dry matter, alpha acid content and total oil content. Finally, a further study needs to be undertaken to examine some of the extremely complex biochemical relationships within the beer/hop matrix that can expose the nature of how these hop enzymes interact or react with related constituents within a dry-hopped beer.
Considering the limited current evidence in hop enzyme development with maturity, there could also be connections drawn to existing studies of the same nature that involve different plants. One of these plants is tea, which has been chemically studied in relation to harvest maturity as well. While the breadth of this field is much greater than hops, yielding greater potential for explanation of this phenomenon, findings within this realm have focused on demonstrating the impact of processing or storage factors on enzyme activity in tea. Examples of this include the effect of oxygen presence on activity of certain enzymes, or the denaturing effect of heat on enzymes during drying. These are also areas for further study within hops.

Finally, it must be considered that the regular harvesting period for Citra® hops at Perrault Farms is between the 6th and 10th of September. However, specifically for the purposes of this study, the picking window was widened by more than a month, by being extended about two weeks before and after the regular picking time period listed above. This picking time period was extended to obtain a range of data, even outside the traditional window. This puts into context the nature of the data shown, as the typical period of harvesting demonstrates the usual values for enzyme activity and related data in comparison to the range of information examined in this study. While suggestions could be made for using non-traditional harvest windows to mitigate enzymatic activity, this would have unintended consequences. If picked too early, inadequate alpha acids or essential oils would have accumulated. If picked too late, the cones become too dry and can simply shatter. While alpha acid and essential oil content could also decrease with increasing maturity, evidence from Sharpe et al. also
demonstrates that essential oil composition changes with late harvest maturity. This leads to changes in aroma expression, potentially in a negative way. For example, sweaty/onion/garlic aromas increase in some varieties with later harvest date.\textsuperscript{9} The purpose for the harvest data extension was specifically to see the effects of harvest maturity in its more complete scope.
CONCLUSION

This study has a number of limitations, primarily that the experiments carried out can be considered a simple laboratory representation of dry-hopping. Naturally, there are many differences in dry-hopping between the laboratory setup and dry-hopping in a commercial brewing setting. These data are only a representation of certain biochemical actions that occur in the dry-hopping process. There are also limitations within the experimental procedure itself. One of these being that potential enzyme inhibitors or cofactors within the beer or hop matrixes have not been accounted for, potentially causing a manipulation in the results. Other uncontrolled factors also exist within the experimental process, including the effectiveness of arresting enzyme activity with the ‘quenching’ by use of the 10% Tris-Base buffer. There were also potential discrepancies on the growing and harvesting side of the experiment. Growing and harvesting was under the supervision of Perrault Farms completely and there is always natural potential for differences in harvesting, kilning or storage between the samples. Finally, there is also a gap in understanding the nature of enzymes present. Only dextrin hydrolyzing enzymes were studied, while other enzymes such as proteases could exist and also have some effect on the beer matrix and even hop creep. Therefore, this data can only refer to enzymes as those that are dextrin hydrolyzing.

Further limitations are also present within the nature of the data itself. As noted previously, this study pertains only to a single varietal, from a single harvest year and a single field location. This restricts the ability to draw wide ranging, grounded conclusions without further supporting studies. Additionally, it also increases the risk
of confounding factors that could arise as anomalies in relation to any one of the specific factors that are related to the hops used in this study.

Despite these potential limitations, this study does provide conclusive evidence, albeit from a single field trial, that on-bine maturity does affect enzymatic potential of hops used for dry-hopping. Moreover, it yields a reasonably strong negative correlation that as the on-bine maturity period increases, the enzymatic potential decreases. Comparison data, which is corroborated from other studies, also allows an insight into potential causes for this phenomenon. Support of the primary evidence and an examination of these causal factors would be the next steps to take for fully understanding this relationship, furthering the ability for hop growers to potentially mitigate it if needed. Additional research would also need to be undertaken in studying related enzymes or enzymatic interaction in the hop/beer matrix to allow this research to be all-encompassing in its nature. The feasibility of this data is dependent on these further studies, in hops and other plants. This current study continues the search for understanding what drives the dextrin reducing enzymatic power of hops. From the first publication nearly 150 years ago by Brown & Morris to more recent work by Kirkendall et al., Kirkpatrick and Shellhammer, and Rubottom et al.; all of which are steps towards fully unravelling the phenomenon that involves dextrin degrading enzymes in hops.

Although preliminary in nature, this study does provide a useful basis for hop growers and commercial brewers to understand the relationship between maturity and enzyme
activity in relation to dry-hopping. This could allow brewers to select hops for dry-hopping with some concern for on-bine maturity, while it could also yield growers the opportunity to cater to brewers by selecting low-enzyme hops for dry-hopping usage. In any case, it increases the understanding of how the activity of enzymes is related to on-bine maturity and also adds to existing evidence of dextrin hydrolyzing enzyme activity in Citra® hops. By initiating further research, more can be realized about further measures to take in these regards, but for now this study adds to a concrete basis of understanding going forward.
REFERENCES


4 Brown, H.T., & Morris, G.H. (1893). *The Brewers Gaurdian*


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