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Evaluating a benchtop fermentation method for estimating dextrin degradation by hop diastatic enzymes during dry-hopping

Dry-hop creep is a gradual reduction in beer gravity after dry-hopping in the presence of yeast due to generation of fermentable sugars from nonfermentable dextrins by hop-associated enzymes. A benchtop forced fermentation-based method for estimating the dextrin reducing potential of hops during dry-hopping is proposed and evaluated for feasibility. In this paper, forced fermentations are compared to paired 2 hL pilot full-scale fermentations in order to evaluate how well a benchtop method will reflect the changes observed due to hop creep in a full-scale fermentation. The forced fermentations proceeded much more rapidly than the full-scale pilot fermentations, and apparent extract and apparent degree of fermentation correlated well between the dry hopped forced fermentations as measured at 72 hours and the terminal values of the full-scale fermentations. Results indicate that a small-scale dry-hopped forced fermentation is a promising tool for assessing the potential magnitude of hop creep in a given lot of hops, and that differences in apparent extract and/or apparent degree of fermentation of forced fermentations can be used to estimate the terminal gravity post-hop creep of full-scale fermentations.

Descriptors: hop enzymes, refermentation, dry-hopping, over attenuation, hop creep, *Humulus lupulus*

1 Introduction

With the rise in popularity of the IPA, and brewer's experimentation in the realm of ever-hoppier beers, hopping rates are on the rise. Hops are increasingly added later in the brewing process and are frequently added in the cellar using a technique called "dry-hopping" or in German, "hopfenstopfen" or "kalthopfen". Dry-hopping, generally speaking, is the practice of adding hops directly to the beer at some point in time after the beer has been chilled and moved to a fermentation or maturation vessel [1]. Additions of hops to the fermentation vessel or to casks is not a new technology, there is evidence from 17th century Germany and 19th century England indicating that hop additions in the cellar or even at the publican's were not uncommon [2, 3, 4]. The addition of these hops late in fermentation allows their volatile aromatic chemicals to be extracted without losses that would be encountered during the boiling process [5, 6]. Furthermore, because the addition occurs in cold fermenting wort or beer, there is no isomerization of alpha acids and thus the dosage rates can be considerably higher than kettle or late/whirlpool hopping. This is useful as the dry-hop dosage rates need to be quite high in order to extract the desirable volatile aromatic compounds, which generally have low transfer rates into beer [7]. These volatile aromatic compounds are desirable, as they can lend a tropical, lemon, herbal, pine, or any one of a variety of other aromatic characters to a beer [6, 8]. Consumers like these hop-forward beers, and the growth and diversification of the IPA segment does not appear to be slowing [9].

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Dry-hopped aroma in beer has been shown to increase with the amount of hops used to dry-hop [10]. This dry-hopped aroma in beer has proven to be desirable, as evidenced by the increasing popularity of hazy and New England-style IPA, which can have dry-hop loads of 1kg/hL or more [11]. Furthermore, brewers have begun dry-hopping non-pale ale beer styles such as Pilsners, Belgian-style Dubbels, and many more [12]. As dry-hop loads increase in size and a diverse array of beers are dry-hopped, brewers are increasingly encountering the issue of dry-hop induced refermentation, which is colloquially referred to by American brewers as "hop creep".

Hop creep is the slow but gradual reduction of gravity or apparent extract in a dry-hopped beer below what was originally considered to be the final gravity [13]. Hop creep is driven by hop-associated amylolytic enzymes introduced to the beer through dry-hopping [14, 15, 16]. These enzymes break down dextrins in the beer, converting the dextrins to shorter-chain oligosaccharides, including maltotriose, maltose, and glucose. While dextrins are unfermentable, maltose and glucose are both fermentable, and any yeast remaining in solution in the beer will consume these during refermentation, producing alcohol, CO_2, and secondary metabolites [17]. Among these secondary metabolites is diacetyl, which is an off-flavor defect that can be generated during hop-induced refermentation. For some brewers, the hop creep-induced diacetyl spike is more problematic than the greater attenuation level. Interestingly, issues related to unwanted diacetyl levels

following dry-hopping appears to be less ubiquitous than over attenuation.

Hop creep refermentation has been observed to result in significant amounts of alcohol and CO_2 production in some cases [13]. Consequences of hop creep can range from minor, like flavor changes, reduced residual sweetness, and less mouthfeel, to major, like potential consumer safety risk due to package overpressurization or legally non-compliant beer labels due to higher than expected alcohol levels [13]. However, the extent of hop creep can be unpredictable, as the enzymatic power of hops is difficult to measure directly and lot to lot variation within and between different hop varieties is known to exist. *Kirkpatrick* and *Shellhammer* developed a method for evaluating the enzymatic power of hops via HPLC by measuring the change in sugar concentration over two days after a base beer with high dextrin content has been dry-hopped in the presence of a strong antimicrobial agent, sodium azide [14, 15]. This method allows for independent confirmation via correlation of sugars produced with observed hop creep for a single hop sample, demonstrating that the sugars produced by the hops lead to the observation of hop creep.

Since the magnitude of hop creep is often unpredictable, the brewing industry seeks to have a method that can assess the hop creep potential of specific hops in a desired beer matrix and to be able to do that easily and quickly. Brewers already have established methods in their toolkit for determining the terminal gravity of a beer from the initial wort [18]. For example ASBC Method Wort-5, Yeast Fermentable Extract, is a method whereby a sample of wort is dosed with a high concentration of yeast and then fermented with agitation so that the beer achieves terminal gravity in a short period of time, generally 24 or 48 hours [18]. By performing these forced fermentations consistently on specific brands within their lineup, brewers can evaluate relatively quickly whether or not a particular batch of beer will meet specifications, troubleshoot wort production issues, and estimate a wort's terminal gravity with sufficient advance notice for the brewer to prepare and react, if necessary.

The benchtop forced fermentation experiment presented here was carried out in conjunction with a study by *Rubottom* et al. (2020) that examined the influence of commercial kiln temperatures on the enzymatic activity

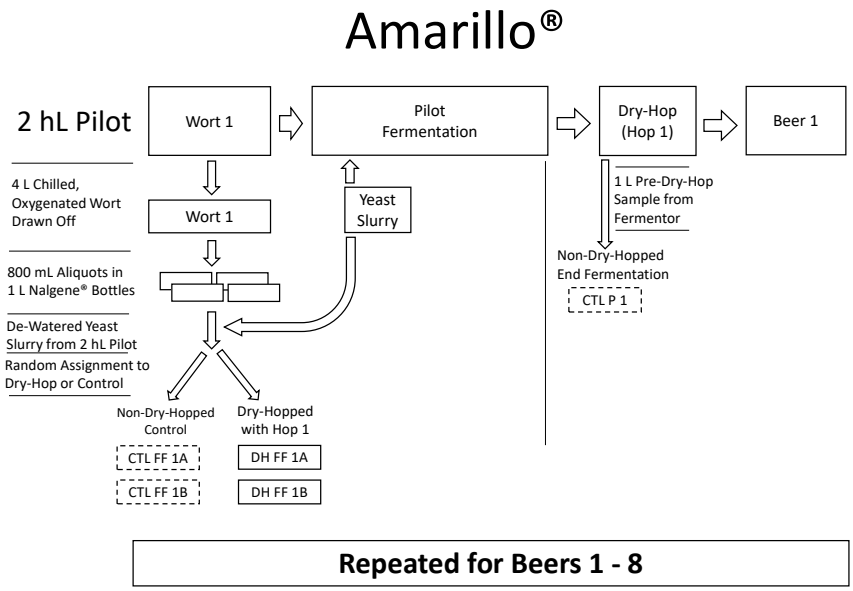

Fig. 1 Process flow chart for the eight beers in the Amarillo® set

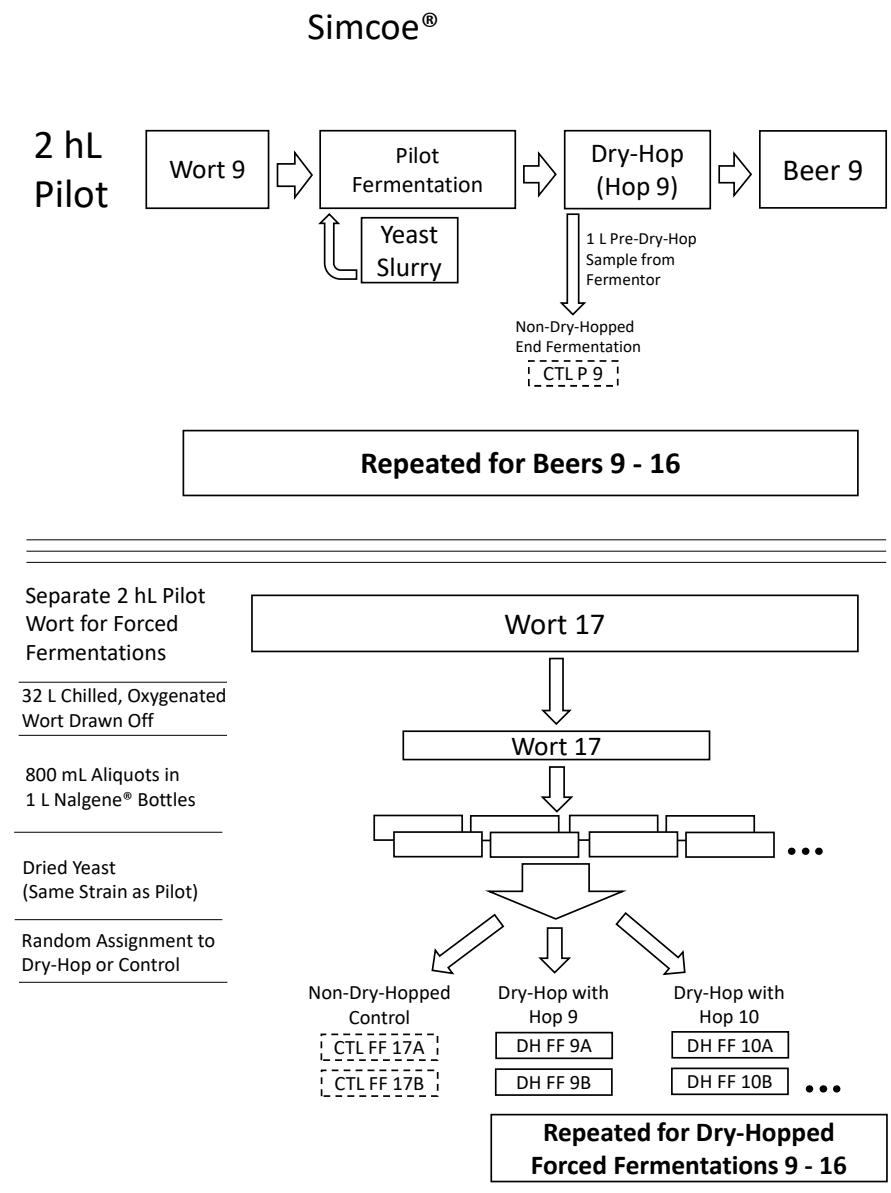

Fig. 2 Process flow chart for the eight beers in the Simcoe® set

of two popular aroma cultivars (Amarillo® and Simcoe®) by drying the hops at different air-on temperatures (120, 140, and 160 °F/ 49, 60, and 71 °C). Kilning trials took place over three years at two farms per variety and each temperature treatment was performed in duplicate [19]. This project produced hops that displayed a range of levels of enzymatic power within a given variety.

There is as yet no standard method for evaluating the potential for a given lot of hops to induce hop creep during dry-hopping. Due to the variability of hop enzymatic power between harvest year, hop variety, hop lot, and even within a lot of hops from the same grower and harvest year, each lot of hops intended for dry-hopping must be evaluated individually by the brewer [15, 19]. The extent of hop creep is further dependent on the beer matrix that is being dry-hopped, as the extent of hop creep can be influenced by yeast selection, wort fermentability, and dry-hop timing [13]. To evaluate the feasibility of the proposed method, two sets of eight dry-hopped pilot scale beers were brewed. From the first set of eight, dry-hopped forced fermentations were prepared using wort from each pilot-scale brew (Fig. 1). From the second set of eight, a separate batch of wort was brewed and dry-hopped forced fermentations were prepared using wort from the separate batch. Hops from the same lots used for the set of eight pilot fermetations were used to prepare the dry-hopped benchtop forced fermentations (Fig. 2). Fermentation performance and dry-hop creep was compared between the forced fermentations as measured at various time points and the terminal values of pilot scale brews. The feasibility study presented in this paper involves a three-day-long benchtop scale forced fermentation that will allow brewers to assess and understand how their dry-hopping regimen will impact the final gravity of their product, and will afford them enough time to make adjustments to their recipe or process before the beer is dry-hopped.

2 Materials and Methods

2.1 Enzymatic Activity Assay

2.1.2 Chemicals

Analytical grade maltose monohydrate 99.0 %, glucose > 99.5 %, fructose > 99 %, and maltotriose > 90 % from Sigma Aldrich (St. Louis, MO, USA) 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, USA).

2.1.2 Hops

Simcoe® and Amarillo® hops from separate 2019 harvest year hop kilning trials were used in this experiment. These hops were known to have different enzyme activities based on an HPLC method described below. Eight distinct lots of each variety were used in this study.

2.2 Enzymatic Activity Sample Preparation

In order to determine enzymatic activity of a hop sample, the HPLC-based benchtop dry-hopping method developed by Kirkpatrick and Shellhammer method was used [15]. This measurement of a hop's enzymatic power was performed using a non-dry-hopped commercial beer with high residual dextrin content (Ninkasi Total Domination, Ninkasi Brewing Company, Eugene, OR), dry-hopped with the hops in question at a rate of 10 g/L along with sodium azide (0.02 w/v %) to prevent microbiological growth. Samples were incubated at 30 °C for 48 hours after which a 10 mL portion was removed and centrifuged at 3180 x g RCF for 15 minutes. After centrifugation, a 1 mL aliquot was diluted 1:1 with a 10 % Tris-Base Buffer to quench the enzyme reaction and then filtered through a 0.45 µm nylon filter before high performance liquid chromatography (HPLC) analysis, as described below.

2.3 Sugar quantification method

The method used for carbohydrate analysis was adapted from ASBC Methods of Analysis, Sugars and Syrups 18 [18]. Carbohydrate quantification (fructose, glucose, maltose, and maltotriose) was performed using an Agilent 1200 series with a refractive index detector and a Rezex RSO Oligosaccharide Ag+ column (Phenomenex, Torrance, CA, USA) operating at 80 °C. The mobile phase was Milli-Q water with a flow rate of 0.3 mL/minute and the injection volume was 10 µL.

2.4 Enzymatic power determination

In order to evaluate the reduction of dextrins to fermentable sugars as a basis for enzyme activity, the inherent sugar content of the hops in question had to be determined. This was accomplished by performing a hot water extraction for each hop followed by HPLC measurement [10]. A 0.5 g sample of hop grist was added to 50 ml of hot (80 °C) sodium acetate buffer (0.02 M, pH 4.2, and 5 % EtOH) and extracted for 15 minutes followed by filtration through a 0.45 µm filter and freezing until HPLC analysis. The enzymatic power of each hop sample was determined by quantifying the increase in maltose and glucose produced by the hops during the 48 hours of 30 °C benchtop dry-hopping the commercial beer minus the amount of sugars coming from the hops themselves and what was in the base beer before dry-hopping (Eq. 1). Fructose was not considered, as the change in fructose content was so low that it was not reliably detected, and its contribution to hop creep would be negligible.

$$[Glucose + Maltose] = (2 * [Sample]) - ([HWE] + [Base\ beer]) \quad \text{(Eq. 1)}$$

where [Glucose + Maltose] = glucose + maltose concentration (g/100 ml) increase resulting from hop-derived enzymes after 48 hours contact
[Sample] = glucose + maltose concentration (g/100 ml) in solution after 48 hours (note the multiplier 2 is used to account for the 1:1 dilution of the sample with Tris buffer)
[HWE] = glucose concentration in hop sample based on hot water extract (g/100 ml)
[Base beer] = glucose + maltose concentration (g/100 ml) in the base beer

2.5 Pilot-scale (2 hL) Brewing:

Single-hop India pale ales were brewed using 100 % pale lager malt (Rahr Premium Pilsner, Rahr Shakopee, MN) to yield approximately 2 hL of wort with 14.8 °Plato original gravity. Hops were added in three stages – at the beginning of a 60-minute boil (weight determined by targeting a final beer bitterness of 40 IBU), at whirlpool (300 g/hL), and post-fermentation dry-hop (800 g/hL). Primary fermentation was carried out at 20 °C (68 °F) using American ale yeast (Wyeast 1056, Wyeast Hood River, OR). Acetolactate decarboxylase enzyme (Maturex™ from Novozymes, Denmark) was added at the beginning of fermentation to speed diacetyl reduction.

Each fermentation was dry-hopped with coarsely-ground whole-cone hops with 72 hours of contact time. To evaluate the final attenuation level of the non-dry-hopped beer, a 1 L sample was taken from each fermentor via a sample port situated in the lower third of the fermentor prior to dry-hopping, placed on a shaker table at room temperature and allowed to ferment to completion. This approach, called an end fermentation measurement, is modelled after the ASBC method Beer – 16 without extra yeast addition. The end fermentation sampling and subsequent hop addition occurred when the apparent extract was at approximately 3 – 3.2 °P, which was approximately 72 hours after the start of the fermentation. After 72 hours of dry-hopping had elapsed, the hop material was removed from the fermentor cone, and diacetyl was monitored daily by consensus sensory analysis. Once diacetyl dropped below detectable levels, beers were cooled to 1 °C (34 °F) and rough filtered (Pall HS6000) to remove any remaining hop material. The target specification for the finished beer was 6.8 % alcohol by volume, 2.1 %w/w apparent extract, 4.4 %w/w real extract, and a 71 % real degree of fermentation. During fermentation the extract and pH were measured every 8 – 12 hours until beers were chilled to 1 °C. After filtration (or centrifugation in the case of the shaken non-dry-hopped end fermentations), an Anton Paar Alcolyzer DMA4500 (Anton Paar, Graz, Austria) was used to determine final beer alcohol content, apparent extract, apparent degree of fermentation (ADF), real extract and real degree of fermentation (RDF). Sixteen brews were carried out in this fashion, eight using Amarillo® hops and eight using Simcoe® hops.

2.6 Benchtop Forced Fermentations

From each pilot-scale brew with the Amarillo® hops, approximately 4 L of chilled, oxygenated wort was drawn off an in-line port as the wort was being pumped into the fermentor. Forced fermentations of this wort were prepared in accordance with ASBC method Wort-5 but with the inclusion of hops [18]. The same yeast was used for the pilot scale and bench scale fermentations, which was a liquid slurry provided by a local commercial brewery. Sufficient slurry was collected to provide each forced ferment with 16 g of de-watered yeast cake. The yeast cake was prepared by first diluting the yeast slurry 1:1 with cool water, then pouring the mixture into a Büchner vacuum filtration apparatus with Whatman No. 1 filter paper. The filtration was run until the surface of the yeast cake began to show cracks, indicating dryness. An 800 mL sample of wort was decanted into a 1 L Nalgene® sample bottle, to which 16 g of de-watered yeast cake was added. Four forced fermentations were prepared per brew/hop treatment. Two of the bottles were randomly selected to be dry-hopped for hop creep evaluation, and to each of those bottles 8 g of ground whole-cone hops from the same lot used in the pilot scale brew were added to achieve a dry-hop rate of 10 g/L. The other two fermentations were non-dry-hopped but otherwise treated the same. To each sample, a stir bar was added, and the samples were placed on a stir plate set at 150 RPM at room temperature (20 °C). Each sample bottle was loosely covered with foil and manually swirled daily, as some of the hops rose to the top of the ferment to form a cap. The cap formation is more evident with ground whole cone hops than hop pellets.

The forced fermentations proceeded for 160 hours and were sampled daily. Samples were removed by uncovering the mouth of the sample bottle and decanting 15 mL into a 50 mL centrifuge tube. The centrifuge tubes were capped tightly and degassed by vigorous shaking for 2 minutes, then centrifuged for 15 minutes at 3180 x g RCF. A sample of the supernant was filtered through a 0.45 um PTFE filter, analyzed using an Anton Paar EasyDens densitometer (Anton Paar, Graz, Austria), and the apparent extract was recorded. The benchtop trials for the Simcoe® hop samples were carried out slightly differently – using one single lot of hopped wort from a pilot scale brew and fermented with dried yeast of the same strain (SafAle American Ale US-05) instead of dewatered yeast slurry.

2.7 Statistics

All figures and statistics (Pearson correlation coefficients; coefficients of determination) were generated in RStudio version 1.3.1056 using R version 4.0.2 “Taking off Again”.

3 Results

3.1 Fermentation performance comparisons with Amarillo® hops

Quantifying the extent of refermentation due to dry-hopping requires understanding the changes in wort fermentability relative to its non-dry-hopped counterpart. There are several approaches to assessing fermentability, but the most commonly used ones are the real degree of fermentation (RDF) and the apparent degree of fermentation (ADF) as outlined in ASBC method Beer – 6B and 6C, respectively. The RDF requires the ability to measure and quantify the ethanol concentration in the finished beer while the ADF includes ethanol in the gravity measurements (i.e. the apparent extract), hence it is the “apparent” degree of fermentation. For a smaller lab without access to ethanol measuring capabilities, the ADF is the only option of the two. And in fact, the ADF may be a preferable estimate since both the residual extract and ethanol levels are taken into account. That is, the reduction of beer gravity due to the decrease in residual extract by the enzymes associated with dry-hopping is further amplified by the reduction in gravity due to the added ethanol from the fermentation of these new fermentable sugars, which makes the ADF a potentially more sensitive estimate than the RDF. The potential shortcoming of both the RDF and ADF calculations is that the enzymatic degradation of the nonfermentable extract to simple, fermentable sugars can only be quantified

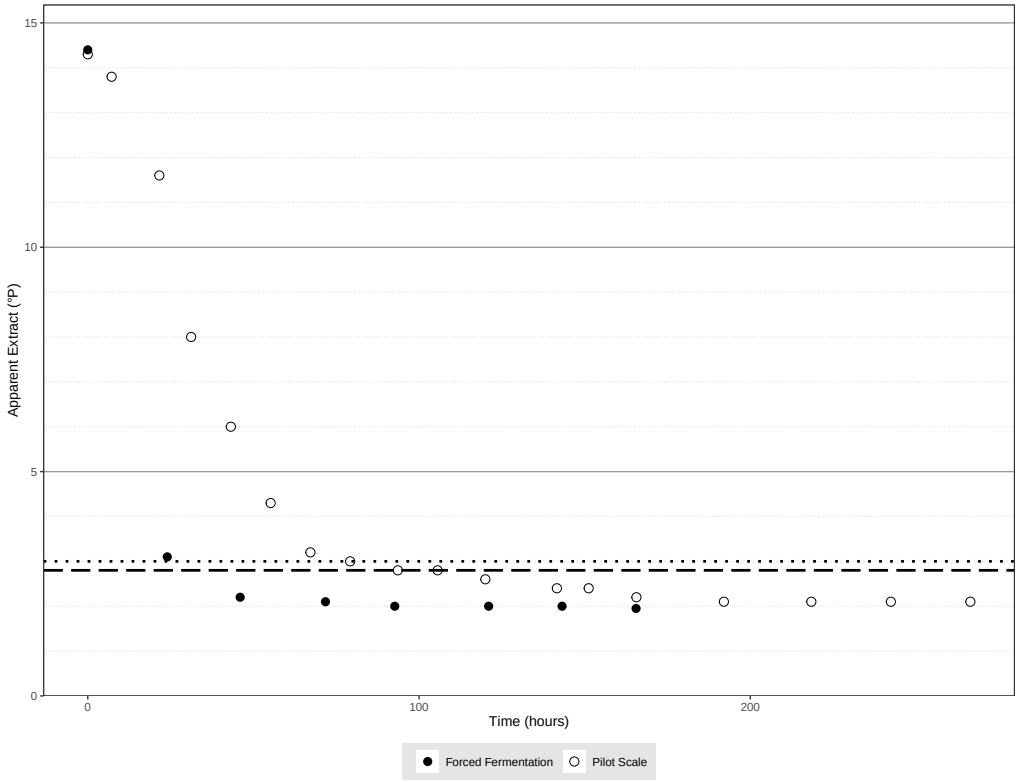

Fig. 3 **Progress of fermentation comparing a dry-hopped forced fermentation (closed circles) to a pilot scale fermentation (open circles). Terminal gravity of the non-dry-hopped forced fermentation (dashed line) is compared to the non-dry-hopped end fermentation (dotted line). Forced fermentation values represent the average of duplicate fermentation trials (average CV: 1 %)**

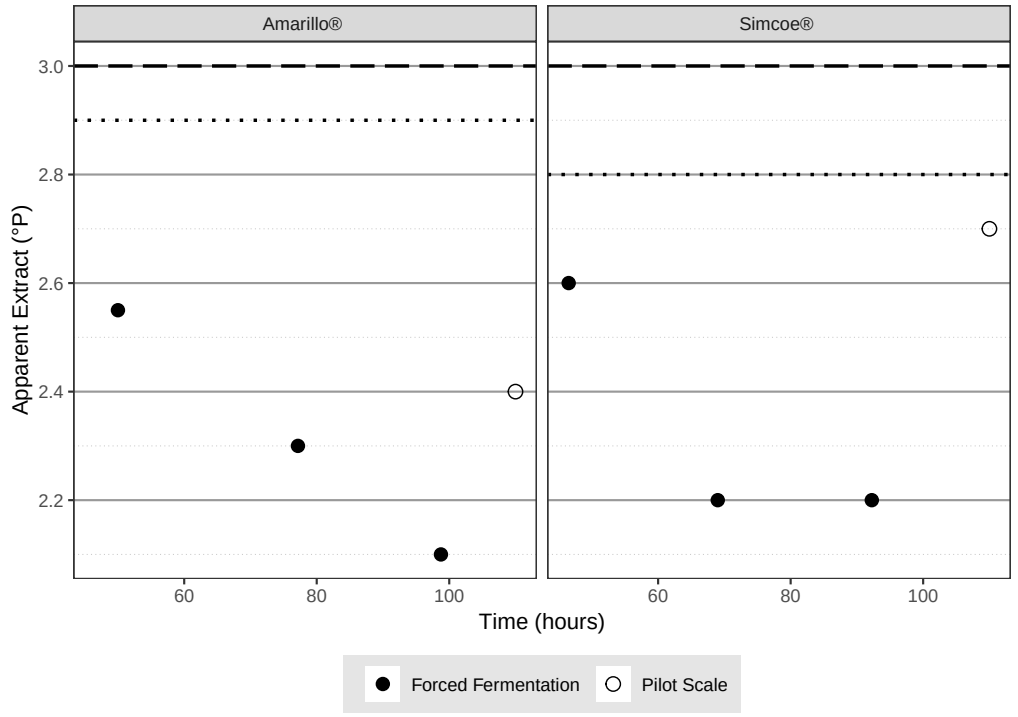

Fig. 4 **Comparison of dry-hopped forced fermentations as measured at 48, 72, and 96 hours (closed circles) to terminal gravity of pilot scale fermentation (~300 hours, x-axis truncated; open circle) between two varieties. Terminal gravity of the non-dry-hopped forced fermentation (dashed line) is compared to the non-dry-hopped end fermentation (dotted line). Forced fermentation values represent the average of duplicate fermentation trials (average CV: 1 %)**

if those sugars are consumed by yeast in solution thereby producing more ethanol. Without a loss of residual extract, the refermentation cannot be measured. Depending on the yeast in question, for instance with poorly attenuating yeast, it is possible that the residual extract may be degraded but not fully fermented. In this case, the only solution to measuring the changes in the dextrin/sugar profile is an HPLC analysis. An advantage to using ADF is that it is blind to the starting original gravity (OG) of the fermentation and therefore quite useful in comparing different treatments where the OG may slightly differ. Finally, if one is examining changes to different worts that start with the same original gravity, comparing apparent extracts (AE) at the end of fermentation may also provide insight to the extent of hop creep. Afterall, the term hop creep comes from a slow but steady drop in the AE of a dry-hopped fermentation.

Dry-hop induced refermentation in the pilot scale trials persisted for a long time, with some fermentations requiring more than 300 hours (12+ days) from yeast pitch to final attenuation (Fig. 3). Considering that the pilot scale trials were dry-hopped after three days of fermentation and the hops were subsequently removed three days later, the hop creep phenomenon continued for four to six days post-dry-hop-removal. This is not atypical for hop creep in a full-size, commercial brewery. By comparison, the dry-hopped forced fermentations proceeded much more rapidly than the pilot scale ferments, achieving terminal gravity in 24–48 hours and attenuating beyond the terminal gravity of the pilot scale fermentations in 72–96 hours (Fig. 4). For the forced fermentations, the non-dry-hopped control fermentations were considered complete after 48 hours, as per ASBC Wort-5 [18]. It is important to note that ASBC Wort-5 specifies 48 hours as the terminal point for a forced fermentation, as forced fermentations have been observed to continue attenuating

past the 48 hour mark, and can overestimate the observed attenuation in a full-scale fermentation if allowed to ferment to a plateau. Dry-hopped benchtop forced fermentations have been observed to continue attenuating for up to 30 days, and to levels well beyond where hop creep finishes in a brewery-scale beer. It is therefore more practical to find the time point at which the dry-hopped forced fermentation closely reflects the gravity at which the brewery-scale beer terminated.

The final attenuation of the non-dry-hopped forced fermentation, although close (within 0.2 °P), did not always match the final attenuation of the non-dry-hopped end fermentation of the pilot scale fermentations, even though both were agitated (Fig. 4). It is unclear why these two might be different and why one was not always greater or less than the other.

To gauge the effectiveness of the benchtop forced fermentation as a predictor of hop creep potential in a pilot scale fermentation, correlations were performed on a range of fermentation indices such as AE, change in AE between dry-hopped and non-dry-hopped treatments (labelled as "Delta AE"), ADF, and change in ADF (labelled as "Delta ADF") comparing the two fermentation approaches. Additionally, increases in maltose, glucose, and maltose+glucose production from the HPLC method were included in the correlations in order to compare with the HPLC based method. Given that the benchtop forced fermentations used to estimate hop creep potential attenuated beyond their pilot scale counterparts within 72–96 hours, three daily timepoints (48, 72 and 96 hours) were used for the forced fermentation in the correlations as opposed to all daily timepoints to 160 hours. A correlation matrix from these 19 comparisons is included in the appendix.

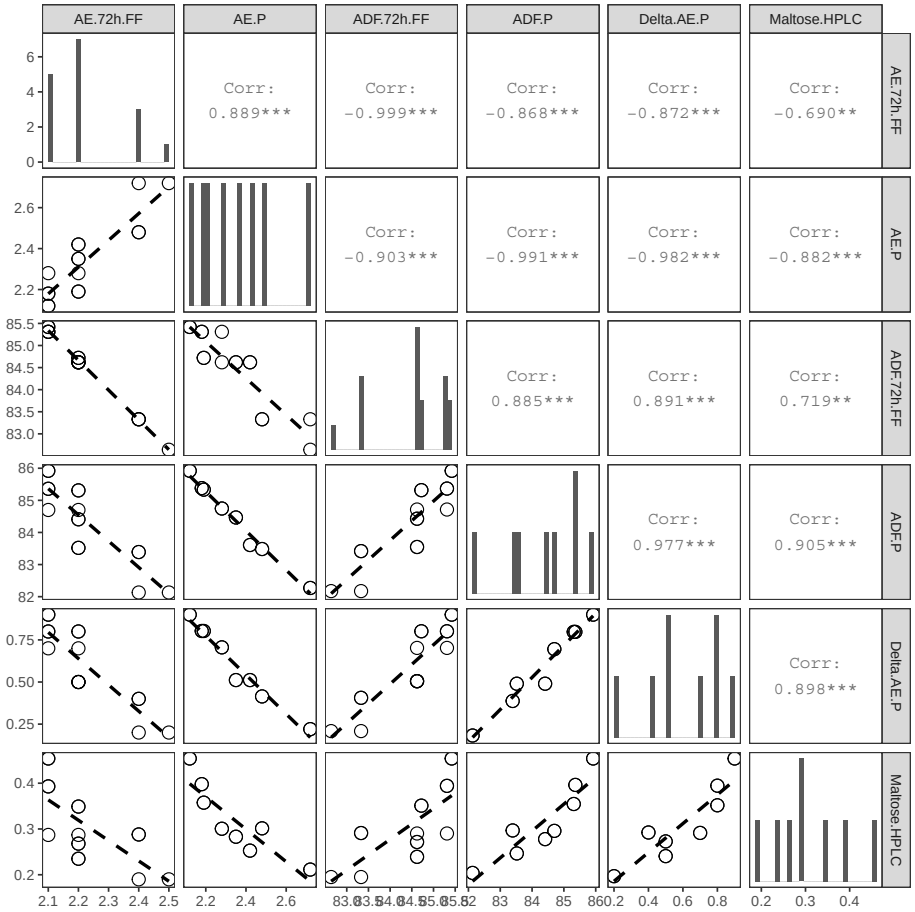

Fig. 5 **Correlation matrix comparing various dry-hopped fermentation metrics as measured at 72 hours to pilot scale fermentation terminal conditions for Amarillo® hops, where .FF refers to forced fermentations and .P refers to pilot scale fermentations. Significance of correlations indicated by asterisks (* = p < 0.05; ** = p < 0.01; *** = p < 0.001)**

The strongest correlations occurred at the 72-hour timepoint for the forced fermentations (Fig. 5). For example, the ADF correlation between the forced fermentation and the pilot fermentation was 0.885 (r^2 = 0.783, p < 0.001), and the AE correlation between the forced fermentation and the pilot fermentation was 0.889 (r^2 = 0.790, p < 0.001) The strength of these correlations, combined with the high r-squared values, indicate that the forced fermentations as measured at 72 hours were predictive of the terminal gravity of the pilot scale fermentations. At 72 hours with the forced fermentation there was sufficient enzyme degradation of the residual extract by the dry-hop addition to distinguish the relative enzymatic activity among the various hop treatments. As the forced ferments were left on the hops and stirred for longer periods of time, they continued to attenuate to quite low levels thereby diminishing these differences among the various hop treatments. This overattenuation phenomenon in stirred fermentations has been observed by others studying hop creep [13]. Time points past 96 hours were not considered, for the reasons stated above. Time points earlier than 72 hours did not display sufficient attenuation to serve as a suitable predictor for the pilot scale fermentations.

It should be noted, the final gravity measurements for the pilot scale fermentations were generated by an Anton Paar Alcolyzer with a DMA4500, which has a digital resolution to 0.00001 g/cm³, while the forced fermentations were measured using a handheld Anton Paar EasyDens which has a resolution to 0.1 °P. Thus, some of the differences in the strength of the correlations between these two data sets may be due simply to the differences in precision of the instruments. The rationale for using the Anton Paar EasyDens unit for the forced fermentations was the small volume required for sampling – 15 mL compared to 100 mL for the Alcolyzer with DMA4500 – and the low cost of the instrument, both of which are well suited for the needs of the method by a brewing lab that has limited access to advanced instrumentation.

The enzymatic activity assay based on the HPLC sugar measurements correlated with the AE and ADF of both the dry-hopped forced fermentation at all three time points and the pilot scale brews. At the 72 hour time point, the correlations between ADF of the dry-hopped forced fermentations with the HPLC assay (0.719, r^2 = 0.517, p < 0.01) were slightly lower than those with the pilot scale fermentations (0.905, r^2 = 0.819, p < 0.001), but that could be influenced by the aforementioned difference in precision between

the Anton Paar Alcolyzer and the EasyDens (Fig. 5). Nevertheless, both correlations were strong and highly significant and as such were reflecting similar behavior of hop enzymes degrading nonfermentable dextrins that were subsequently consumed by yeast. Furthermore, it confirms the relevance of the HPLC assay as a strong predictor of a hop's enzymatic strength.

3.2 Fermentation performance comparisons with Simcoe® hops

In a similar but separate trial, a set of Simcoe® hops from the kilning trials were brewed on a pilot scale, while a single separate batch of wort was prepared for the forced fermentations. Forced fermentations were carried out using direct-pitch, active dried yeast (same strain as wet yeast in Amarillo® evaluations). Both sets of fermentations proceeded similarly to the Amarillo® trials, although the terminal gravity of a number of the pilot scale treatments did not attenuate as much at their Amarillo® counterparts (for example, Fig. 4, right panel). This may have been due to differences in the inherent enzymatic power of the hops, yeast differences and/or issues related to poor fermenter mixing during dry hopping that were specific to the Simcoe® trials.

Whole-cone hops were used in all dry-hopping trials and they were coarsely ground with a meat grinder prior to dry-hopping the fermenter. For the Amarillo® trials only, after dry-hopping the 2 hL tanks were roused daily by bubbling CO_2 gently through the bottom port on the fermentation vessel using 5 pulses of approximately 10 seconds each. The Simcoe® fermentations were not roused and in some, but not all, cases a cap of hop grist floating on the surface of the dry-hopped beer could be observed. No cap of hop grist was observed in the roused Amarillo® fermentations. The variable degree of mixing in the pilot scale may have influenced hop extraction and the extent of hop creep. Such a mixing issue is specific to the case of using ground, whole-cone hops for dry-hopping, and would be unlikely to occur if pelletized hops are used.

Using the same comparison times and fermentation indices as Amarillo®, correlations were performed among the three sets of Simcoe® data (forced fermentations, pilot scale fermentation and HPLC sugar data). The correlations between the pilot scale and forced fermentations were close to zero in most cases, while the correlations with the HPLC-based enzyme activity remained strong (data not shown). This result indicated that both the forced ferments and the pilot scale brews displayed sufficient hop creep that could be predicted by following the production of sugars released by hops during dry-hopping but that there was a misalignment between the dry-hopped forced fermentations and the pilot scale fermentations. The poor correlations may have been due to the dextrin profile of the single batch of wort used to carry out the forced fermentation, yeast form, and/or potential dry-hopping mixing issues/variability.

4 Conclusions

Assessing the extent of refermentation following dry-hopping, a phenomenon colloquially known as hop creep, is easily quantified by looking at changes in final beer fermentability between a non-dry-hopped end fermentation versus a dry-hopped fermentation. The apparent degree of fermentation is a useful index in that changes to both the residual extract and production of additional ethanol resulting from the refermentation are captured in this measurement. This paper proposes a method developed from ASBC Method of Analysis Wort-5 whereby a room temperature, benchtop, forced fermentation of a brewery's core brand is performed using a consistent dosage rate for dry-hopping and assessing the AE and ADF after 72 hours. This approach offers the brewer the potential of estimating the enzymatic power, or hop creep potential, of the hops being evaluated. Furthermore, the method relies only on measuring the apparent extract of the fermentation and as such does not require specialized instrumentation.

This technique was developed using a set of Amarillo® hops with known differences in enzymatic power based on an HPLC based enzyme assay. Difficulties in the method arose when the form of yeast (dried vs wet) along with variations in pilot scale fermenter hydrodynamics/mixing. This suggests that using a forced fermentation assay to evaluate how much hop creep can be expected in a full-scale fermentation is likely to be sensitive to the medium on which that forced fermentation is performed. In other words, it is important to carry out the forced fermentations on the same wort, using the same yeast type and form, as the full-scale fermentation. One should also keep in mind that fermenter dynamics and the method of dry-hopping can have a significant influence on the extent of dry-hop induced refermentation. Further studies should look at varying wort composition for higher/lower dextrin load, different yeast strains, and impact of yeast health (count, viability, vitality) on hop creep.

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Appendix

Proposed Methodology:

Procedure:

To take place simultaneously with the pilot scale or full scale fermentation:

- Prepare 20 g de-watered yeast by mixing sufficient yeast slurry 1:1 with cool water, and filtering through a Büchner funnel and Whatman No. 1 or equivalent filter paper until the yeast cake shows cracks, indicating dryness.
  - Alternatively, use dried yeast. If using dried yeast, reduce the mass used by 2/10 to achieve same pitch rate as above.
  - Take care to use the same yeast that will be used in the full-scale fermentation.
- Prepare 5 g of hops from the same lot that will be used for dry-hopping.
  - Blend any hops of different varieties in the proportion that they will be added to the beer.
- Label two clean flasks, one 'Control,' and the other 'Treatment.'
- Collect 1000 mL of cool, oxygenated wort from the fermenter.
- To the Control flask, add:
  - 500 mL wort
  - 10 g de-watered yeast (or 2 g dried yeast)
- To the Treatment flask, add:
  - 500 mL wort
  - 10 g de-watered yeast (or 2 g dried yeast)
  - 5 g hops
- Place flasks on a shaker table or stir plate, shake/stir at 150 RPM at 20 °C.
- At 48 hours, remove a 50 mL sample from the 'Control' flask.
  - Degas, and centrifuge or filter to clarify.
  - Analyze for AE and ADF on a benchtop or handheld densitometer.
- After 72 hours, remove a 50 mL sample from the 'Treatment' flask.
  - Degas, and centrifuge or filter to clarify.
  - Analyze for AE and ADF on a benchtop or handheld densitometer
- Compare the apparent extract of the control sample and the treatment sample.
  - The apparent gravity of the treatment sample will reflect the estimated terminal gravity of the full-scale brew after hop creep.

The difference between the control and the treatment estimates the magnitude of hop creep, or how much additional alcohol and extract will be generated by hop creep.

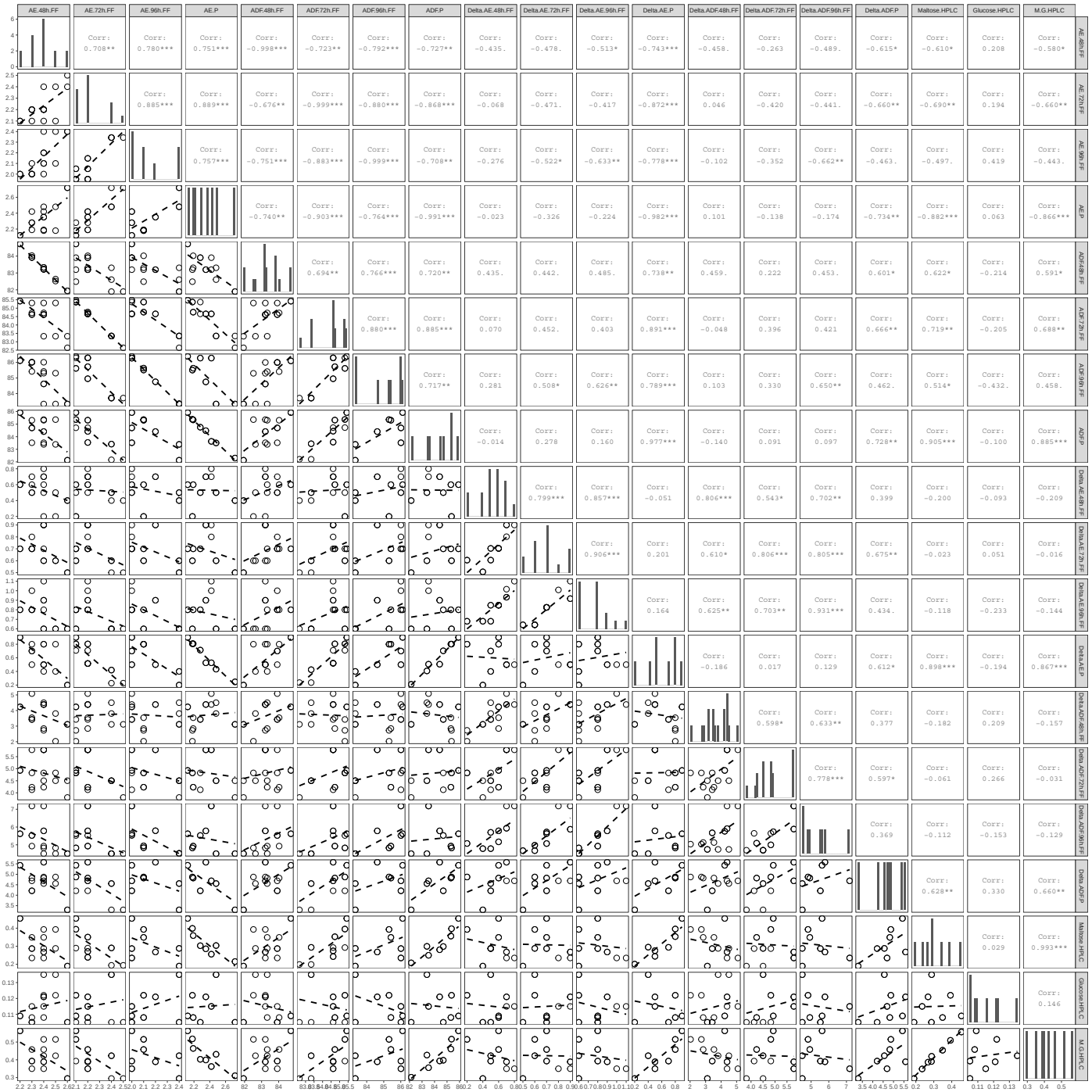

Fig. 6 **Correlation matrix comparing fermentation indices and sugar measurements for Amarillo® dry-hopped forced fermentations metrics as measured at 48, 72, and 96 hours to pilot scale fermentation terminal condition, where .FF refers to forced fermentations and .P refers to pilot scale fermentations. Significance of correlations indicated by asterisks (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$)**