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The contribution of β-amylase from hops to the fermentability of dry hopped beer

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Abstract

Why was the work done: The addition of hops (*Humulus lupulus*) to beer during fermentation or conditioning is known as 'dry hopping'. This procedure introduces hop amylases, catalysing unintended fermentation known as 'hop creep'. The impact of hop creep includes extended attenuation, production of diacetyl off flavour, elevated alcohol formation, and potentially hazardous over carbonation of packaged beer. Enzyme assays and genetic analysis have indicated that hops produce four types of amylases, α-amylase, β-amylase, glucoamylase, and limit dextrinase. It is not known which amylase, alone or in combination, are responsible for the increase in fermentability produced by hops. The goal of this study was to identify the hop amylases responsible for hop creep.
How was the work done: Using forced attenuation assays, the different amylases found in hops were screened for increased fermentability similar to that produced by endogenous hop amylases.
What are the main findings: Barley β-amylase extended fermentability equivalent to that produced by the five hop cultivars examined. This suggests that hop β-amylase alone, potentially explains the increase in fermentability associated with hop creep in dry hopped beer.
Why is the work important: The identification of β-amylase as the enzyme reproducing the increase in fermentability through dry hopping advances understanding of the hop creep phenomenon. Greater knowledge of the mechanisms driving hop creep may lead to new approaches for controlling and avoiding the negative impacts on the quality of dry hopped beer.

Keywords

dry hopping, hop creep, β-amylase, super attenuation.

Introduction

The phenomenon known as 'hop creep' is driven by interaction between dry hop additions and yeast, resulting in increased fermentability and a lower final gravity than anticipated by the brewer. The first report that enzymes in hops were responsible for the additional fermentation observed after dry-hopping was made in the 19th century (Brown and Morris 1893). Hop creep may occur in the fermenter, during conditioning, or after packaging through the enzymatic degradation of non-fermentable dextrins, producing fermentable sugars that are consumed by yeast (Kirkpatrick and Shellhammer 2018a; 2018b; Stokholm et al. 2020; 2022; Rubottom et al. 2022; Jobe et al. 2025). Hop creep is also associated with the development of diacetyl and off flavours together with alcohol concentrations higher than intended (Kirkendall et al. 2018; Kirkpatrick and Shellhammer 2018b; Bruner et al. 2020). Additional fermentation of a finished dry hopped beer can produce dangerously high pressure in cans and bottles, leading to package failure (Kirkendall et al. 2018). Current approaches for managing hop creep rely on an empirical understanding of the phenomenon, allowing mitigation through altering the timing or extent of the process.

The behaviour of hop creep has led to different methods for reducing its negative impact. Methods can be grouped as either accelerating or slowing the process (Stokholm and Shellhammer 2020). Rapid hop creep, accomplished by dry hopping early in fermentation allows time for the process to finish before fermentation has completed. Slowing hop creep, by dry hopping at lower temperature or by reducing the concentration of viable yeast, can reduce but not eliminate the production of alcohol, carbon dioxide, and off flavours such as diacetyl. Utilising hop products with reduced green matter - containing associated hop amylase - may slow hop creep. Pasteurisation negates hop creep in packaged beer as residual yeast is killed.

Knowledge of the active components of hop creep provides an understanding of the mechanism and variability of hop creep with malt, hops and yeast, playing essential roles. Hops provide the amylase converting malt dextrins to sugars which yeast then metabolises to carbon dioxide, ethanol, and secondary metabolites. Whilst all three are required for hop creep, much remains unexplained about the enzymology of converting dextrins to sugars.

Enzyme assays suggest that hop cones possess amyloglucosidase, α-amylase, β-amylase, and limit dextrinase (Kirkpatrick and Shellhammer 2018b) and genes encoding α-amylase and β-amylase have been identified in hops (Cottrell 2023). It is unclear which of the enzymes identified in hops is responsible for hop creep. Accordingly, a better understanding of the initial enzymatic step of dextrin degradation is needed to better predict and manage hop creep and the fermentation of dry hopped beer.

This study explored the effect of various amylases on wort fermentability to identify the hop amylase producing the increased fermentability of dry hopped beer and understand how hop amylases interact with wort dextrins to initiate hop creep. The approach used forced attenuation assays to screen amylases for the ability to generate an increase in wort fermentability like that produced by endogenous hop amylases. The results advance our understanding of hop creep, producing a picture of dextrin degradation by a single type of hop amylase that appears responsible for the unplanned fermentation of dry hopped beer, known as hop creep.

Materials and methods

Hops and yeast

Amarillo, Cascade, Citra, Magnum, Mosaic, and Simcoe hop T-90 pellets were obtained from Yakima Chief Hops, Yakima, WA, USA. Dried yeast (WLP001) was obtained from White Labs, San Diego, CA, USA.

Wort preparation

Wort used in the forced fermentation/attenuation assays was composed of 107 g/L Pilsen Light dry malt extract (Briess Malt & Ingredients, Chilton, WI, USA), 53 g/L maltodextrin (Brewmaster Inc., Pittsburg, CA, USA), and 0.33 g/L Magnum hop. Using municipal water the mixture was boiled for 30 min, cooled to 20°C, and 150 mL dispensed to 250 mL Erlenmeyer flasks with vented caps.

Source and application of enzymes

The glucoamylase and high temperature α-amylase were obtained from North Georgia Still Company, Dahlonega, GA, USA. The β-amylase from barley (cat. no. E-BARBP-2G) was obtained from Neogen, Lansing, MI, USA, and the β-amylase from soybean (cat. no. A0448) was obtained from TCI America, Portland, OR, USA. According to the manufacturer, the barley beta-amylase was prepared from crystalline enzyme and appears as a single band on SDS-gel electrophoresis (MW = 58,300) and two major bands on isoelectric focusing (pi = 5.4 and 5.7) with a minor band at pi 5.0.

The high temperature α-amylase was added to wort maintained at 85°C for 30 min before cooling to 20°C and adding the yeast. The α-amylase was added at a concentration of 0.5 mL/L. The glucoamylase was added at a concentration of 0.25 mL/L and the β-amylase was added at concentrations ranging from 27 to 213 U/L. The glucoamylase and β-amylase were added with the pitching yeast at the start of the forced attenuation incubations.

Icubation conditions and sampling

Dried yeast was added at 7g/L. For each experimental treatment, two flasks were prepared with the dry hop pellets (20 g/L) and amylase additions. Control flasks received no hop or enzyme addition. The forced attenuation assays were performed in 250 mL Erlenmeyer flasks in a rotary shaker at 50 rpm and 28°C. Apparent extract (AE) was monitored daily, in samples clarified by centrifugation at 3210 x g for 3 min with present gravity determined using a DMA 35 densitometer (Anton Paar GmbH, Austria).

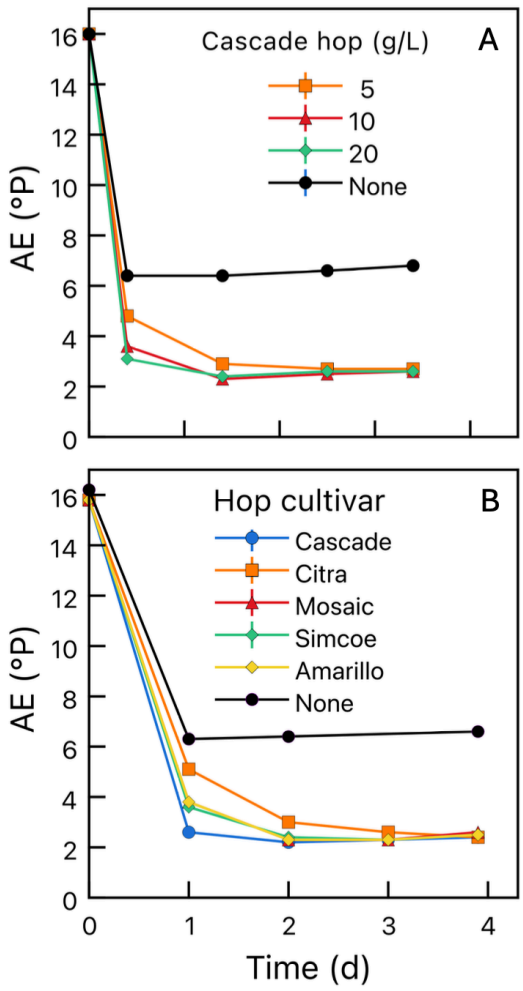
Results

Forced attenuation assays with Cascade hops attenuated to lower final gravity compared to the control assay without hop addition. The control fermentations terminated at 6.8°P residual extract. In contrast, addition of Cascade hops at 5, 10 and 20 g/L resulted in fermentations with a final gravity of 2.6°P residual extract (Figure 1a). The time required for apparent extract to plateau decreased with increasing Cascade hop addition. In the control the apparent extract plateaued in <1 day, with the assays with 10g/L of Cascade hop plateauing after 1.5 days and 2.5 days with 5 and 20 g/L.

The increased fermentability produced by dry hopping showed little variation among hop cultivars. Compared to the control with no hop addition, the increase in fermentability with different cultivars ranged from 4.0 to 4.2°P (Figure 1b). However, the initial rates of fermentation varied. After one day of fermentation, 2.6 ± 0.1 (Cascade) and 5.1 ± 0.0°P (Citra) apparent extract remained but was similar with Simcoe, Mosaic and Amarillo hop ranging from 3.6 ± 0.0°P to 3.8 ± 0.0°P. Despite the approximately two fold difference in initial rates, the time required for fermentations to finish ranged from three days with Cascade, Mosaic, Simcoe and Amarillo to four days with Citra hop.

Figure 1.

Time course of fermentation in forced attenuation assays with (a) different rates of addition of Cascade hops and (b) the addition at 20 g/L of various hop cultivars. The control (None) received no hop addition. Values of apparent extract (AE) are the average of duplicate incubations. Error bars are smaller than the symbols.



The impact of exogenous enzymes on attenuation is shown in Figure 2a. Compared to the control, the addition of amylases increased fermentability. The extent varied, with α-amylase increasing fermentability by 16% whereas glucoamylase achieved 110% The addition of β-amylase resulted in 66% greater fermentability, similar to that achieved with the addition of hops (n=5 cultivars).

Apparent extract in fermentations with Cascade hop and β-amylase and Cascade hop alone plateaued after four days of fermentation at 2.5 ± 0.0 and 2.6 ± 0.1°P residual extract, (Figure 2b). For both, attenuation was 63% greater compared to the control with no hop or enzyme addition. However, the time course differed with Cascade hop, the apparent extract decreased to 5.0 ± 0.1°P after one day of fermentation, with attenuation continuing for four days before reaching 2.6°P final gravity. In contrast, β-amylase and Cascade hop attenuated to 2.6°P after one day of fermentation remaining unchanged thereafter.

The rate of attenuation to achieve final gravity varied with the amount of exogenous β-amylase added. Addition of β-amylase from four to 32 U resulted in fermentations finishing after four days and one day (Figure 3a).

Figure 2.

Time course of fermentation in forced attenuation assays with (a) hops or different amylases added and (b) Cascade and β-amylase added. Values of apparent extract (AE) for the hop addition are the average of five cultivars added at 20 g/L. The control (None) received no hop or amylase addition. AE for the amylase treatments are the average of duplicate incubations. Error bars are smaller than the symbols.

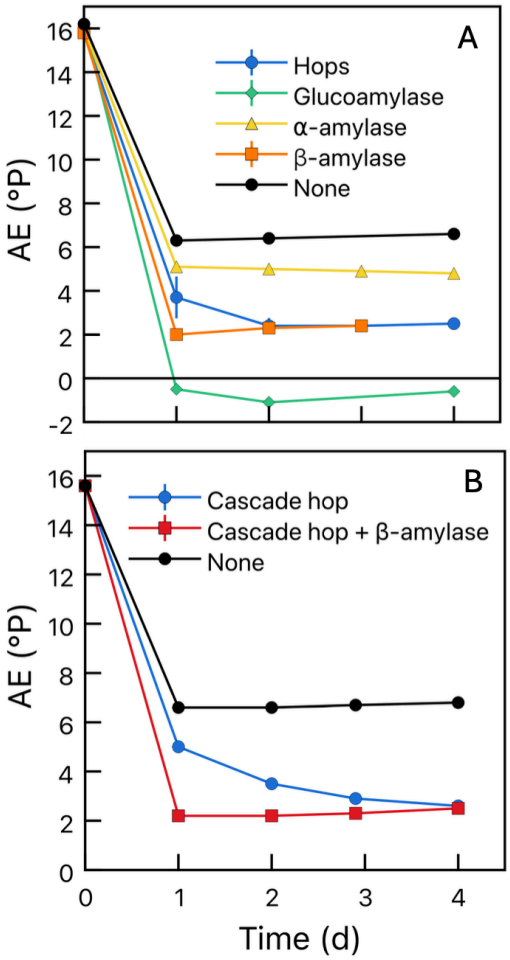
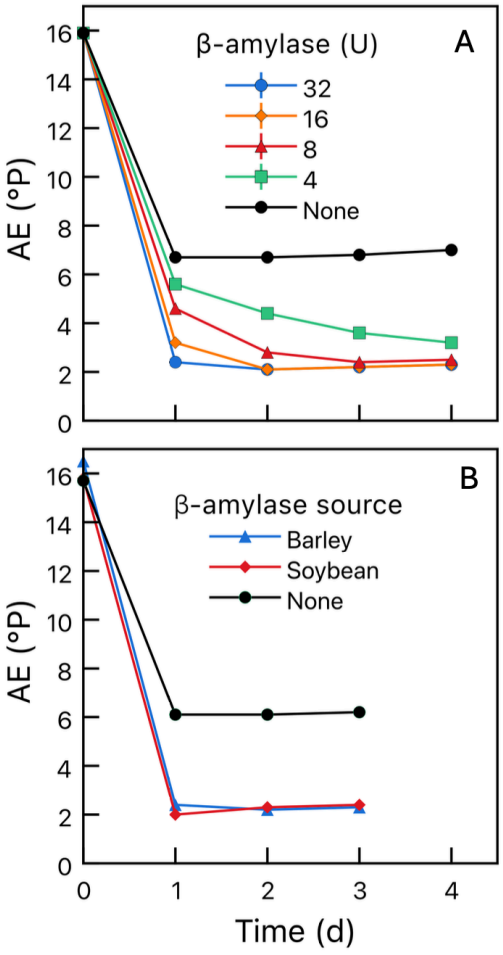


Figure 3.

Time course of fermentation in forced attenuation assays with (a) different concentrations of barley β-amylase and (b) additions of β-amylase from from different sources. The control (None) received no hop or amylase addition. Values of apparent extract (AE) for the amylase treatments are the average of duplicate incubations. Error bars are smaller than the symbols.



Apparent extract after one day of fermentation with 32, 16, 8, and 4 U of β-amylase was 2.4 ± 0.1, 3.2 ± 0.1, 4.6 ± 0.1, and 5.6 ± 0.0°P. The apparent extract with 32 U of β-amylase added was broadly stable after one day but continued to decline with lower levels of β-amylase. Assays with 16 and 8 U of β-amylase reached final gravities of 2.1 ± 0.0 and 2.4 ± 0.0°P after two and three days. With the addition of 4 U of β-amylase, attenuation continued to decrease and after four days had yet to reach a stable terminal gravity. β-amylase from barley and soybean were equally effective in the extent and rate of dextrin degradation, producing 61-63% greater fermentability compared to the control (Figure 3b).

Discussion

The increase in wort fermentability mediated by hops resembled that obtained with β-amylase but differed from that with glucoamylase and α-amylase. β-amylase is an exoamylase producing maltose by hydrolysis of α-1,4 glycosidic linkages of starch and dextrins from the nonreducing end (Kossmann and Lloyd 2000). In contrast, α-amylase is an endoamylase catalysing the hydrolysis of starch to shorter oligosaccharides through the cleavage of internal α-D-(1–4) glycosidic bonds. Accordingly, the main role of α-amylase in starch degradation is its degradation (Tangphatsornruang et al. 2005), while β-amylase converts oligosaccharides to fermentable sugars. In contrast, glucoamylase can convert starch and dextrins to fermentable sugars. Glucoamylase hydrolyses 1,4-α-glucosidic linkages in starch from the nonreducing end to produce glucose, and cleaves 1,6-α-glucosidic branch points successively releasing glucose from the non-reducing end (Mertens and Skory 2007; Kumar and Satyanarayana 2009).

The preparation and purity of the enzyme from soybean was less well documented. However, as soybean and barley β-amylases had a similar impact on fermentability, this suggests that other plant β-amylases, such as those from hops, may also increase fermentability. Hops are likely to produce a diversity of β-amylases, as the hop genome includes several genes encoding β-amylase (Cottrell 2023). It remains to be seen whether the expression of one or all of these genes are required for hop creep.

Further exploration of the hop proteome (Neugrodda et al. 2014; Champagne and Boutry 2017) could help in identifying the hop β-amylase genes encoding the enzymes responsible for hop creep.

Dry hopped beer that experiences hop creep provides insight into the composition of the wort used to produce the beer. It is proposed that an increase in fermentability driven by endogenous hop β-amylase, suggests that during wort production, β-amylase in the mash did not complete the conversion of starch and dextrins to maltose. Wort fermentability by diastatic enzymes (Evans et al. 2005; De Schepper et al. 2021; 2022) is limited by the thermal stability of β-amylase (Evans et al. 2005) or by limit dextrinase (MacGregor et al. 1999; Stenholm and Home 1999; Evans et al. 2010). Assuming there is sufficient malt β-amylase and mashing temperatures are within the tolerances of malt diastatic enzymes (Evans et al. 2003; De Schepper et al. 2021), the combined activities of malt α-amylase and β-amylase would be expected to reduce dextrins to 'limit' dextrins. These dextrins contain α (1-6) glycosidic bonds, which require the action of limit dextrinase before β-amylase can continue generating maltose. The work reported here supports the notion that dry hopping increases fermentability through the action of hop β-amylase and suggests the presence of dextrins that were not converted to fermentable sugar by barley β-amylase during mashing. Variability in the amount of dextrin susceptible to degradation by hop β-amylase may contribute to the variability of hop creep.

Brewers of dry hopped beer have benefited from improved understanding of the factors influencing hop creep. The variability contributed by hops has been the focus of many studies aiming to understand hop creep. These include assessment of the differences among hop cultivars, growing regions, and farm management (Kirkpatrick and Shellhammer 2018a; Stokholm et al. 2022; Jobe et al. 2025). The impact of kilning during hop processing has also provided insight into the variability of hop creep (Rubottom et al. 2022; Rubottom and Shellhammer 2024). Further, the role of yeast strain and contribution of other microorganisms has provided further insights into the variability of hop creep (Bruner et al. 2021; Young et al. 2023).

This study suggests a role for hop β-amylase in driving hop creep together with mash conditions controlling the extent of malt β-amylase activity during wort production. Brewers of dry hopped beer looking to minimise hop creep, would benefit from dry hopping during fermentation enabling hop β-amylase to degrade dextrins before packaging. In addition, with consideration of how mash conditions impact dextrin production, brewers would benefit by managing the composition of limit dextrins (Michiels et al. 2024).

There remains much uncertainty around hop creep and its impact on the quality and safe production of dry hopped beer. Even the words used to describe the phenomenon are unsettled, with 'after-fermentation', 'ABV creep', and 'the freshening power of hop' being used (Kirkendall et al. 2018). The identification of a dextrin hydrolysing enzymatic activity in hops was an important step towards establishing the current understanding of how hops increase the fermentability of dry hopped beer (Kirkpatrick and Shellhammer 2018b). However, identifying the activity of different amylases in hops, including amyloglucosidase, α-amylase, β-amylase, and limit dextrinase leaves questions to be answered about how the process operates, recognising the different substrates, products, and overall function of the various enzymes.

Conclusions

The results of this study support a picture of hop creep relying on the activity of β-amylase alone in degrading wort and beer dextrin. Participation by other hop amylases appears not to be required, although it is likely that the diverse hop amylases have roles which are yet to be described. Identifying β-amylase as the candidate enzyme replicating the increase in fermentability produced by dry hopping, advances the understanding of hop creep and better informs the production of dry hopped beer.

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Conflict of interest

The author declares no known competing financial interests or personal relationships that could appear to influence the work reported in this paper.

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