When is a Bug a Feature?

Enzymatic activity in the beer brewing process

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Abstract

The beer brewing industry has grown rapidly over the past several years. Because of this, the number of hop varieties has greatly expanded, making them a very valuable resource. To alleviate the financial strain, a beta-glucosidase enzyme product (X-zyme) has been created with the intention to reduce the amount of hops used in the brewing process while maintaining the levels of aroma and flavor. However, in previous studies, X-zyme has displayed potential esterase activity, producing undesirable phenolic off-flavors (POFs).[1] To study this esterase activity of X-zyme, a spectrophotometric assay was designed using *para*-nitrophenyl ferulate. Detected esterase activity could indicate that X-zyme has potential off-target effects that could lead to the dumping of an entire load of beer, a significant financial loss. The assay showed significant feruloyl esterase activity that may have an impact on the conditions X-zyme should and should not be used.

Background

Historically, beer has been composed of four main ingredients: barley, hops, yeast, and water. While this is no longer the exclusive list, the sources of most of the beer flavor still comes from the barley, hops, and yeast used in the brewing process. Hops are especially important, as they release the terpene alcohols that produce the most intense aromas and flavors.[1] Because of the importance and value of these hops, brewers have been looking for ways to maximize the yield of volatile flavor compounds from their hops. Thus, X-zyme was born, designed to be a β-glucosidase enzyme product and intended to increase the diversity and intensity of hop flavors and aroma through the release of terpene alcohols by hydrolysis of glycosidic bonds to terminal non-reducing residues in glucosides. Brewers have also identified compounds that produce phenolic off-flavors (POFs), which are undesirable in many cases and can be attributed to specific strains of yeast.

What X-zyme is supposed to do

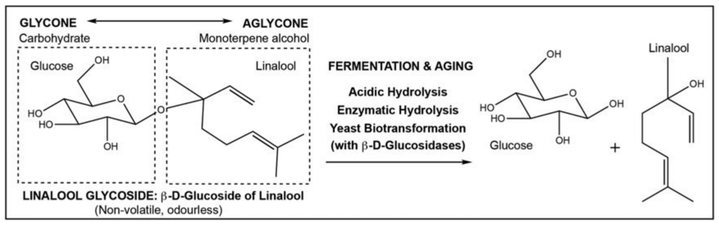

Fig. 1. Scheme for release of terpene alcohols from glycosides.[2]

What X-zyme REALLY Does

The effects of X-zyme on the chemical composition of beer, specifically the concentration of the terpene alcohol linalool, was investigated through GC-MS. The compounds were separated and detected using an Agilent Technologies gas chromatography (model 7820 A) with quad mass selective detector. While there was only a small increase in linalool concentration, there was a significant increase in a compound called 4-vinylguiacol (4-VG), a product considered a phenolic off-flavor (POF) and undesirable when not intended to be in the final product (Fig.2).

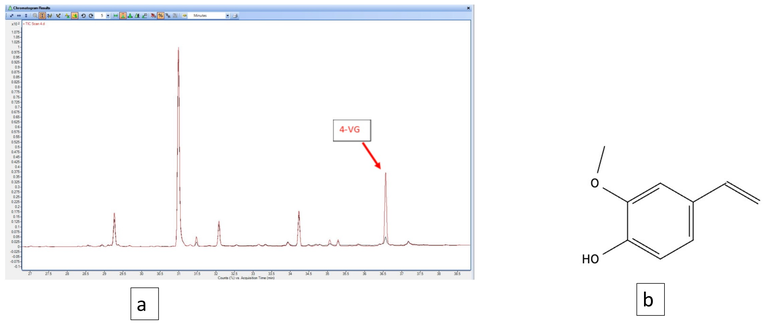

Fig. 2: (a)Gas Chromatography/Mass Spectrometry chromatogram of beer spiked with X-zyme (red) vs. control (black).[3] (b) Chemical structure of 4-vinylguiacol.

Where does this 4-VG come from?

It turns out that 4-VG can be produced through the processing of ferulic acid by some strains of yeast. This ferulic acid is a component in some plant cell walls that can be released by enzymes with esterase activity (Fig. 3).

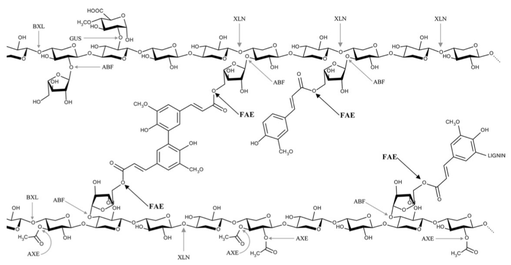

Fig. 3: Diagram of plant cell wall polysaccharide with linked ferulic acid.[4]

To investigate whether X-zyme could be responsible, an assay measuring esterase activity was performed using *para*-nitrophenyl ferulate as the substrate. Esterase activity releases the ferulic acid from the *para*-nitrophenyl moiety (Fig. 4) , which can be measured using colorimetric spectrophotometry. Samples were prepared by adding 1.5mL TX-100/1mM PNF solution to a test tube and placing it in a water bath at 37°C for two minutes to equilibrate. Tubes containing 1.5 mL of the 20X X-Zyme were placed in the water bath to equilibrate for this same amount of time. After these two minutes, 1.5 mL of the X-Zyme solution was added to the sample tube, diluting the substrate concentration 1:1. These samples were allowed to react for two hours. The absorbance at 405nm was measured after this period. This experiment was performed in triplicate. A control was prepared by addition of 1.5mL of deionized water to the sample tube in place of the X-Zyme solution while still containing 1.5mL of the 1mM PNF substrate solution. The control was used as the baseline during analysis with a Cary 60 spectrophotometer.

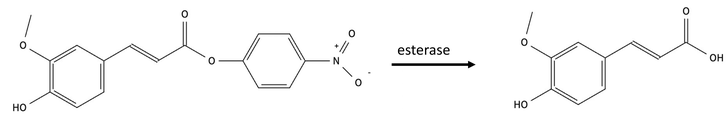

Fig. 4: Chemical structures of *para*-nitrophenyl ferulate and ferulic acid.

The results of Michaelis-Menten analysis of the esterase assay (Table 1) indicate that X-zyme does indeed possess esterase activity. A possible explanation for this could be that X-zyme is derived from the fugus *Aspergillus niger*, which is known to have significant esterase activity. This provides evidence for the final X-zyme product not being pure β-glucosidase.

Table 1: Calculated values from Michaelis-Menten Analysis

V_{max}	31.06 nmol/min
K_m	0.73 mM

When is the esterase activity a bug?

To an unsuspecting brewer, the presence of 4-VG due to the use of X-zyme could lead to the dumping of an entire load of beer, an extreme financial loss. Extra care would need to be taken while using X-zyme to ensure that the yeast strain being used does not produce undesired POF compounds.

When is the esterase activity a feature?

In today's industry, funky flavors in beer have become more desirable as brewers experiment with different styles and flavor combinations. In addition, some Belgian styles traditionally contained these flavors, and the use of X-zyme in these situations may help bring them out.

Acknowledgements

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References

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