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# 2014 ASBC Annual Meeting

## Analyzing the sugar and flavor profile of *Brettanomyes* wild yeast during primary versus secondary fermentation

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### Abstract

Brewing with *Brettanomyces* yeast has been rising in popularity as such yeast can create different flavors and aromas to increase the unique character of a particular beer. Recent research on *Brettanomyces* strains available in the brewing industry focused on strain-specific fermentations and have attempted to identify the major compounds produced during fermentation. This study attempts to investigate the differences in flavor compounds after experimenting in primary and secondary fermentation with various *Brettanomyces* strains. The concept of primary versus secondary fermentation relates to different sugars being available for *Brettanomyces* to metabolize since *Saccharomyces* yeast will readily consume most of the fermentable sugars. By analyzing the performance of various *Brettanomyces* strains during primary versus secondary fermentation, it will be essential to measure the carbohydrate sugar profile before and after fermentation, as well as look at attenuation. The focus of this research will be to explore the flavor compounds produced when *Brettanomyces* has fermentable sugars available in contrast to which flavor compounds are produced from dextrins after secondary fermentation. The *Brettanomyces* strains that will be able to metabolize different sugars resulting in differences on a sensory level will be evaluated.


### Introduction

*Brettanomyces* create different flavors and aromas that can contribute to the unique character of the finished beer. The type and quantity of sugars present will affect the fermentation flavors. For instance, the characteristic horsey aroma and flavor are by-products of the metabolism of some *Brettanomyces* strains. The object of this study is to quantify the types of sugars that remain after primary fermentation with a *Saccharomyces cerevisiae* strain and evaluate sugar utilization and resulting flavor compounds produced by *Brettanomyces* strains pitched in secondary, compared to pure *Brettanomyces* culture fermentation with all wort sugars available. The correlation between the sugars consumed and the detection of flavor compounds such as esters, fusel alcohols, and ketones between the four specific *Brettanomyces* strains in primary vs. secondary was explored.

### Methods

Four *Brettanomyces* strains were pitched in primary and secondary anaerobic fermentation trials at 24°C. These strains consisted of *Brettanomyces bruxellensis Trois*, *Brettanomyces claussenii*, *Brettanomyces bruxellensis*, and *Brettanomyces lambicus*. The original gravity (OG) wort was not heavily hopped at a specific gravity of 1.045. The pitch rate for all *Brettanomyces* strains undergoing primary fermentation was 500,000 cells/ml. The pitch rate for the *S. cerevisiae* ale yeast was 10 million cells/ml. After seven days of primary fermentation with this ale yeast strain, each *Brettanomyces* strain was pitched at a concentration of 200,000 cells/ml, which was calculated from the formula $C_1V_1=C_2V_2$. However, before the *Brettanomyces* strains were pitched in secondary, samples were taken and filtered by membrane filtration. These samples were then measured for carbohydrate sugar profile analysis by High-performance liquid chromatography (HPLC). These results are represented in Figures 1, 3, 5 and 7 as the difference of the sugar profile after *S. cerevisiae* fermentation to the end of *Brettanomyces* fermentation for secondary. For primary, the sugar profile of the unfermented wort is factored for the sugar utilization. Detection of esters, fusel alcohols, and ketones were analyzed by gas chromatography (GC). This same process of membrane filtration, carbohydrate sugar profile analysis by HPLC, and flavor compound analysis by GC were also performed at the end of a 15-day primary fermentation and 15-day secondary fermentation. The data includes the average of two trials.

### Results

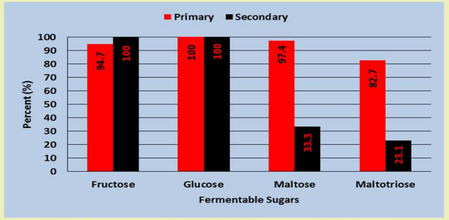


Figure 1. Sugar utilization for *B. bruxellensis Trois* in primary vs. secondary fermentation

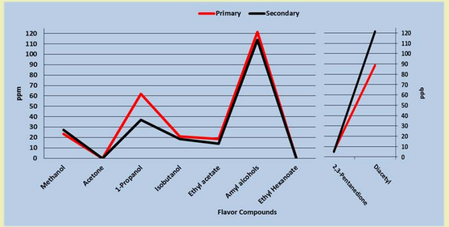


Figure 2. Flavor compound analysis for *B. bruxellensis Trois* at end of primary vs. secondary fermentation

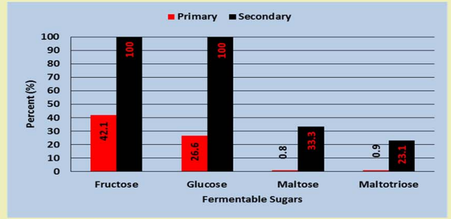


Figure 3. Sugar utilization for *B. claussenii* in primary vs. secondary fermentation

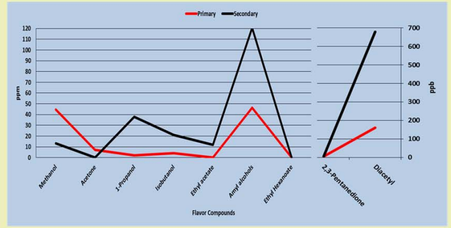


Figure 4. Flavor compound analysis for *B. claussenii* at end of primary vs. secondary fermentation

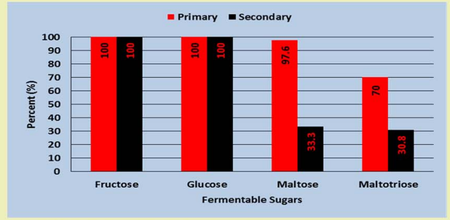


Figure 5. Sugar utilization for *B. bruxellensis* in primary vs. secondary fermentation

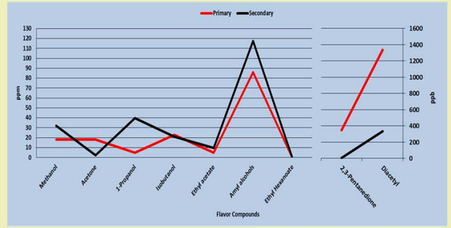


Figure 6. Flavor compound analysis for *B. bruxellensis* at end of primary vs. secondary fermentation

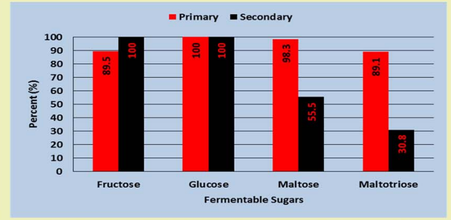


Figure 7. Sugar utilization for *B. lambicus* in primary vs. secondary fermentation

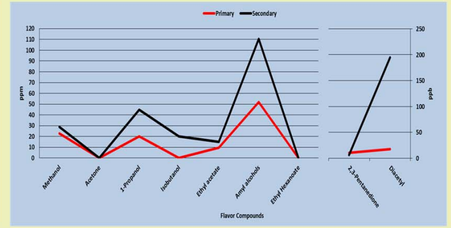


Figure 8. Flavor compound analysis for *B. lambicus* at end of primary vs. secondary fermentation

### Discussion & Conclusions

It is widely known that an important flavor compound in beer are esters. Ethyl hexanoate, responsible for the flavor described as “apple, fruit”, was only detectable in *B. bruxellensis Trois* among the *Brettanomyces* strains in primary fermentation. In addition, *B. bruxellensis Trois* in primary fermentation produced the largest amount of ethyl acetate, responsible for the flavor described as “fruity, solvent”, confirming that this *Brettanomyces* strain is most likely to produce the notable fruity characteristics among the strains analyzed, thereby proposing that *B. bruxellensis Trois* is most suitable for primary fermentation in comparison to the other *Brettanomyces* strains. This study suggests a correlation between the level of ester production to level of higher alcohol production, which is responsible for the flavor described as “pungent, solvent”, but it appears to be dependent on yeast strain. The slow consumption of simple sugars might suggest the inability to produce esters as represented by *B. claussenii* in Figure 4. The percentages of maltose and maltotriose consumed for the *Brettanomyces* strains in secondary are relatively similar, indicating that *Brettanomyces* more readily consume these two sugars at a faster rate when in primary. Ethyl butyrate and Isoamyl acetate were analyzed, but did not produce any detectable amounts in the *Brettanomyces* strains. The production of vicinal diketones, responsible for the flavor described as “buttery”, indicates to be primarily a by-product of *S. cerevisiae* fermentation. This study would benefit on further research including the analysis of aroma compounds. Also, the extension of fermentation time and its effect on the differences in sugar consumption with flavor compound production should be explored.

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