Spring 4-12-2018

Sugar Analysis of Mashing Process in Beer Brewing

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Introduction

In beer brewing, the mashing process produces the simple sugars that eventually become ethanol. Two important enzymes, α- and β-amylase, are most active at specific temperatures, and the mashing process tries to maximize the their efficacy. The most common sugars hydrolyzed during this process are maltose, glucose, sucrose, and fructose. Different grains require different mashing temperatures and will produce a unique amount of each sugar. Many studies have focused on the enzymatic activity to observe the mashing profile of common beer grains, such as barley or wheat. The focus of this study has been observing the enzymatic activity of barley. Sufferers of celiac disease require gluten free beer, from grains such as quinoa. We would like to know if a gluten-free grain can substitute for barley in the mashing process and produce a sufficient amount of sugars for fermentation.

The Beer Brewing Process

Malting
- Grains germinate & enzymes are produced

Roasting
- Malted grains are roasted, adding flavor and color to the beer

Mashing
- Roasted grains are steeped at specific temperatures to activate enzymes, which hydrolyze starches into simple sugars.

Fermenting
- Yeasts convert simple sugars to ethanol

Filtering, carbonation, packaging, etc.
- After this process the product can be consumed

Mashing Kinetics Results

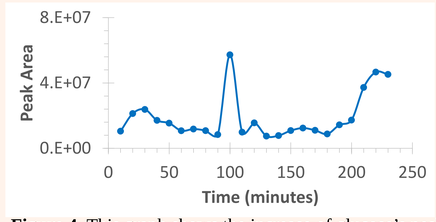


Figure 4. This graph shows the increase of glucose's peak area over the course of extraction time, where every 10 minutes a sample was extracted and every 30 min the temperature increased.

Distribio

A PCR thermocycler was used to dry the aqueous samples and to heat derivatized mixture

Thermo Fisher

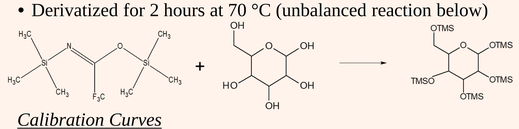
Samples and standards were analyzed via GC-MS

Materials and Methods

GC-MS analysis

In order to analyze the sugars within the limit of the GC-MS they had to be derivatized using N,O-Bis(trimethylsilyl)trifluoro-acetamide (BSTFA). The method for this is as followed:

- 20 µL dried sugar solution, 10 µL pyridine, and 40 µL BSTFA
- Derivatized for 2 hours at 70 °C (unbalanced reaction below)



Calibration Curves

The method of external standards was used to quantitate the sugars, with methyl p-anisate as an internal standard.

Mashing

- In a beaker 0.3 mL of H_2SO_4 (0.5 M) and 75 mg of $CaCl_2$ was added to 200mL of deionized water
- The solution was warmed to initial temperature before 50 g of malted grain (quinoa or barley) was added
- Samples were extracted at different times and temperatures and were immediately placed in a -20 °C freezer to stop enzymatic activity
- Samples were then defrosted, centrifuged (4000 rpm for 15 minutes) and filtered

Results

Table 1. Important retention times.

Sugar	Retention time (min)
α-Maltose	13.8
β-Maltose	13.9
Sucrose	13.6
α-Glucose	10.8
β-Glucose	10.4
Fructose	9.9

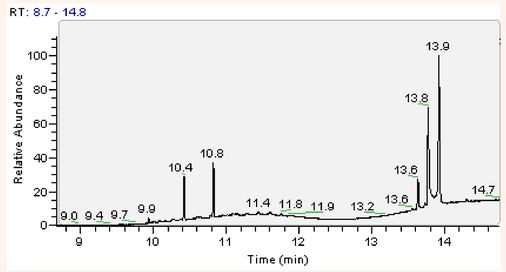


Figure 1. The retention times of each sugar were determined by GC-MS analysis. These retention times were used to identify the peaks in calibration mixtures and unknown samples taken during the mashing process. They were the basis of determining a sugar profile for the mashing mixture.

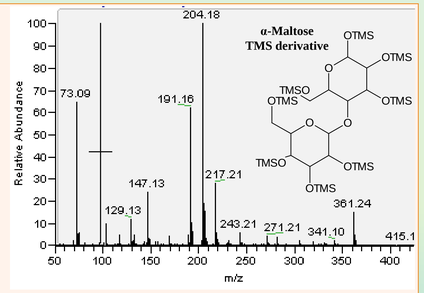


Figure 2. The derivatized sugars were identified using their mass spectra. Above is the mass spectrum for peak 13.9 min, which his the α-maltose TMS derivative

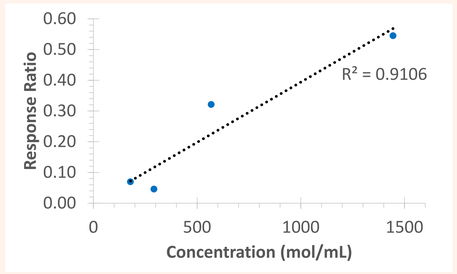


Figure 3. Calibration curve made for Glucose with a concentrated internal standard.

Dilution of internal standard

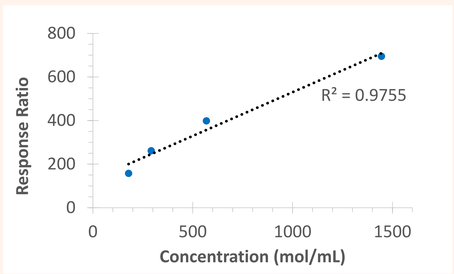


Figure 4. Calibration curve made for Glucose when internal standard was diluted by a factor of 10.

Calibration curves were made for a mixture of the 4 sugars. In the first calibration curve made (not shown) the response was too low to create a calibration curve. In order to ensure the calibration curves were able to be reproducible an internal standard had to be used, methyl p-anisate. Since, there are many steps that the sugar could be lost the internal standard was used to help correct this in the calibration curves. This can be seen in Figures 3 and 4.

Conclusions

As seen in Figure 4, there is an increase of sugar response over time, specifically shown for glucose. This is similar to the data found in the literature from Brandam. That as the temperature increases to about 50 °C starch concentration decreases, and thus the sugar content of the wort will increase.

In the future the mashing samples will be fit to a calibration curve to better understand the kinetics of the process. Then as the mashing profile of barley is determined quinoa will be analyzed for sugar content during mashing. This can lead to a better knowledge of the mashing process, and if this gluten free grain can be effectively used to make beer.

Literature cited

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Schummer, C.; Delhomme, O.; Appenzeller, B. M. R.; Wennig, R.; Millet, M. Comparison of MTBSTFA and BSTFA in derivatization reactions of polar compounds prior to GC/MS analysis. *Talanta* **2009,** *77*, 1473-1482.

Acknowledgments

I would like to thank the Chemistry department, and especially Dr. Overway, for their help and support on this project.

For further information

Please contact hgibson@eagles.bridgewater.edu *for more information.*