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Kinetics of Mashing in Beer Brewing Process

Ken Overway
Bridgewater College, koverway@bridgewater.edu

Austin Reed
Bridgewater College

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Austin Reed and Dr. Kenneth Overway

Department of Chemistry, Bridgewater College, 402 East College Street, Bridgewater, VA 22812-1599

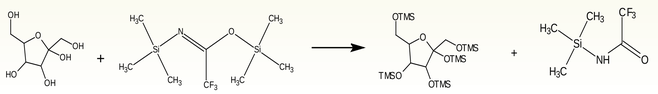
Introduction

The mashing process in beer brewing produces simple sugars that are later fermented into ethanol. These sugars are produced by the enzymatic activity of α- and β-amylase. Activity and sugar production are specific to temperature and vary from grain to grain. The most common sugars produced during this process are fructose, glucose, maltose, and sucrose. As most beer is brewed with barley or wheat, a lot is known about the optimal temperatures in their mashing process. The focus of this study has been to recreate barley's mashing temperature profile, and to use that method to create a mashing profile specific to quinoa. Those who suffer from celiac disease are allergic to gluten, and for now the majority of beers on the market are produced with gluten-containing grains. Quinoa, however, is gluten free and could be a good alternative to brewing with grains such as barley or wheat. This study hopes to observe the sugar production of quinoa and to determine if it produces enough sugar during the mashing process to ferment into ethanol.

Experimental

Derivatization

In order to analyze sugars via GC-MS sugars needed to be derivatized using N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA). The reaction mechanism is below.



Derivatization mechanism of Fructose and BSTFA

Balanced Reaction. $C_6H_{12}O_6 + 5C_8H_{18}F_3NOSi_2 \rightarrow C_{20}H_{52}O_6Si_5 + 5C_6H_{10}NOF_3Si$

Calibration Curves

Quantitation was achieved through the method of multi-point internal standard using a Thermo GC-MS (Fig. 2). Varying amounts of individual stock solutions for each sugar were dried along with 5 µL of internal standard (2-deoxy-D-glucose), then 10 µL of pyridine along with 40 µL of BSTFA were added to the dried sugar solutions. This solution was heated at 70 °C for two hours (see Fig. 1).

Mashing

A beaker was filled with 200 mL of deionized water, and to it 0.3 mL of 0.5 M H_2SO_4 was added along with 75mg $CaCl_2$. Three separate vials were filled with 6 mL of this solution and ~1.5 g of barley malt were added when solution reached 40 °C. Every 10 minutes 150 µL were extracted, and the temperature was raised 5 °C every 30 minutes until 75 °C was reached. Samples were immediately placed in a freezer to stop enzymatic activity. Samples were then thawed, filtered, and derivatized to be analyzed.

Figure 1. PCR Thermocycler was used to dry sugars out of aqueous solution, and then to heat the derivatized mixture.

Figure 2. Trace 1310 GC-MS was used to analyze derivatized samples and calibration standards.

Results

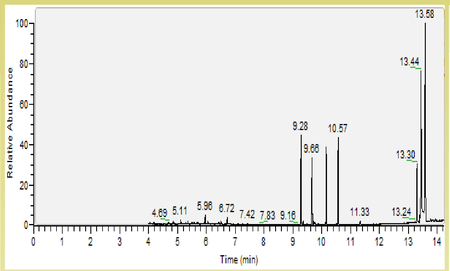


Figure 3. GC-MS was used to find retention times of derivatized sugars. These peaks were used to identify sugars in calibration standards and unknown samples. These retention times were the basis for quantitation, and ultimately for creating a mashing profile.

Sugar	Retention Time (min)
α-2-Deoxy-D-Glucose	9.28
β-2-Deoxy-D-Glucose	9.66
α-Glucose	10.16
β-Glucose	10.57
Sucrose	13.30
α-Maltose	13.44
β-Maltose	13.58
Fructose*	9.68

Table 1. The retention times for sugars being analyzed. *Fructose eluted with β-2-Deoxy-D-Glucose too closely for the GC to resolve. Changes to method didn't separate them so fructose was eventually disregarded in the analysis.

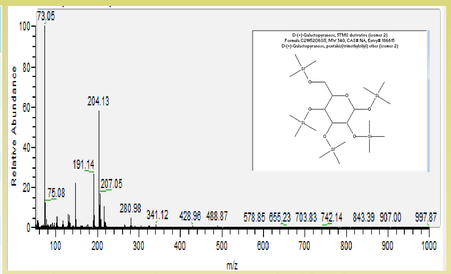
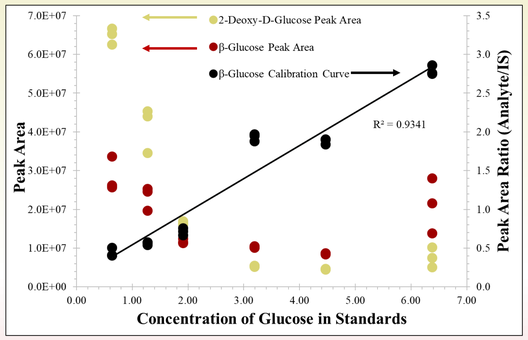
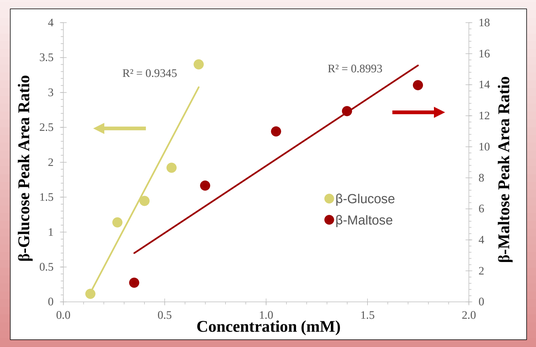
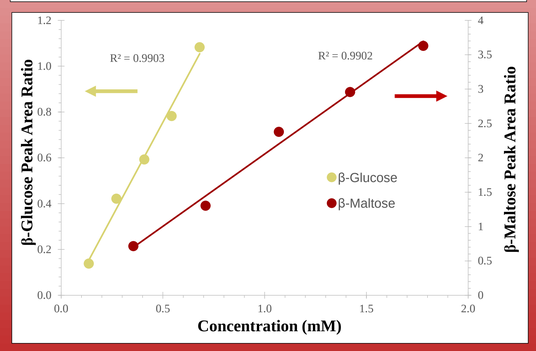


Figure 4. Peaks were identified by their mass spectrum. Above is the mass spectrum of the β-Glucose-TMS derivative.

Method Refinements







Figures 5 & 6. Figure 5 to the left was an initial attempt at a calibration curve in which a single stock solution was made for all four sugars. As pictured above in Fig. 6, the sugars ended up clumping during the derivatization process and not allowing for a full derivatization. This resulted in the calibration curve for β-Glucose in Fig. 5.

Figure 7. The calibration curves for β-Glucose and β-Maltose. These two sugars have the highest production during barley's mashing process. These calibration curves were produced after the sugar stock solution was diluted by 5X. This dilution resolved the sugar clumping and variation seen in Fig. 5 as there was less sugar to clump and to dissolve into solution. These calibration standards were also made using larger centrifuge tubes as the ones previously used had issues with the lids deforming, which forced a transfer to a new tube to derivatize. With both of those modifications, however, the resulting calibration curves weren't great.

Figure 8. To the left are the final calibration curves made for β-Glucose and β-Maltose. These calibration curves were obtained by making individual stock solutions for each sugar rather than having them all in a single solution. Five standards were made from each individual solution by pipetting 5, 10, 15, 20, and 25 µL into centrifuge tubes along with 10 µL of internal standard. They were then dried, derivatized, and analyzed according to the corrected method.

Future Work

Next the study transitions to examining the sugar production of quinoa during the same mashing process. Sugars produced will then be quantitated by use of the calibration curves to see if concentrations are comparable to that of barley.

For further information

Please contact areed@eagles.bridgewater.edu for more information.

Mashing Results For Barley

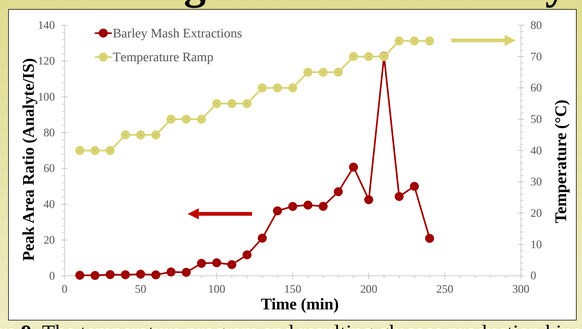


Figure 9. The temperature program and resulting glucose production kinetics for β-Glucose. This profile was produced by calculating the peak area ratio of β-Glucose with the internal standard for each extraction. The resulting profile closely follows that of the known barley mashing profile with optimal temperatures from 60-70 °C.

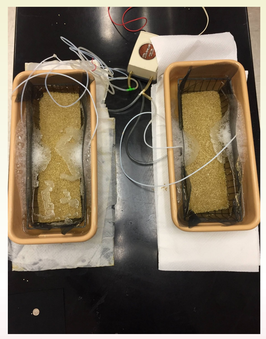


Figure 10. Germinating Quinoa

Malting Quinoa

To analyze quinoa's sugar production during the mashing process the grain was malted in order to produce the enzymes necessary for sugar production. This was done by first soaking the grains in tap water with a bubbler for two days (Fig. 10). They were pulled out of the water for three short periods during the two days to further assist in aerobic respiration. The grains were then allowed to germinate for four days in a humid environment with occasionally stirring so the rootlets wouldn't knot.

Mashing Results For Quinoa

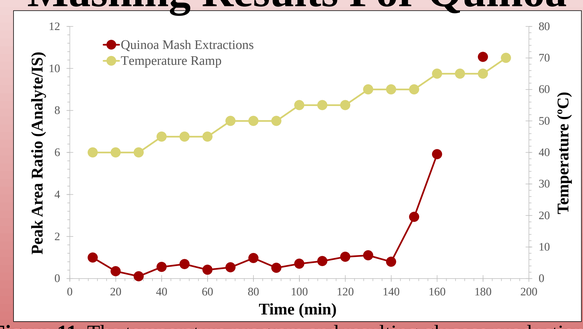


Figure 11. The temperature program and resulting glucose production kinetics for β-Glucose. This profile was produced using the same method as barley. Sugar production began steadily increasing at 55 °C and peaked around 65 °C similarly to barley. α- & β-Glucose were the only sugars really produced from the quinoa mash which could result in a less complex beer. Data points at 170 & 190 minutes were removed as they had extremely high peak area ratios which inconsistent with the others making it hard to see the trend.

Literature cited

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