# Brewing Process Optimization: Mash Efficiency

A Major Qualifying Project Report
Submitted to the Faculty of
Worcester Polytechnic Institute
In partial fulfillment of the requirements for the
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# Abstract:

With microbreweries popping up just about everywhere in the world, innovation and efficiency is key to stay competitive. Working in conjunction with Purgatory Beer Company, this project seeks to investigate areas of improvement for the mash phase of the brewing process. The team established a variety of parameters that literature suggests affect the overall yield of the mash and began replicating these scenarios with a homebrew kit. Additionally, the team planned on cross referencing hydrometer data and HPLC data to detect changes in both the overall sugar yield and the specific sugar yields of relevant fermentable sugars, such as maltose, sucrose, glucose, and fructose. However, due to the unfortunate circumstances of the COVID-19 outbreak, these goals were not fulfilled. Therefore, the focal point of the paper falls upon the findings in the Background section. The team suggests that the full methodology as described in this paper be conducted, followed by a results-guided additional literature review, before any claims can be made on which method(s) increase mash yield.

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# 1. Introduction:

In the United States, the brewing industry has been shifting from major beer companies to local craft breweries, which offer a wider array of beer flavors and brew types. Those local breweries have been increasing exponentially throughout the country. As of June 2019, there were 7,480 local breweries, where the number was 6,464 a year before.*

Our team worked with Purgatory Beer Company, which is a small microbrewery, located in Whitinsville, MA. It was founded in 2017 by Brian DiStefano and Kevin Mulvehill, who wanted to share their love for brewed beer. Even though they have a great understanding of the brewing process, they wanted to gain more knowledge from a chemical engineer’s perspective of the brewing process. The co-owners focused on understanding how to improve their sugar yield in their mashing process to be able to craft a new kind of beer and a better tasting beer.

The project for our Major Qualifying Project is to improve the sugar yield in their mashing process. To achieve this goal, the team replicated the mash process of Purgatory Beer Co, and varied parameters to understand how they affect the sugar yield. Also, the team decided to try different stirring techniques such as Vorlauf and continuous stirring. We then analyzed the mash of each parameter using specific gravity tests and HPLC. Finally, we used these results and compared them to the control. From this data, we tried to find which varied parameters if any would increase the sugar yield in the mashing process.

Note that the experimentation and data collection for this MQP ceased on March 6, 2020 due to disruptions from the rapid spread of pandemic caused by the COVID-19 virus. WPI and the Commonwealth of Massachusetts mandated a stop to all nonessential activities. This paper includes data obtained up to that date.

# 2. Background:

Beer is interwoven into just about every culture. Germany, one of the pioneers of modern brewing, cites beer as its citizen's favorite beverage, with about 80% of the population drinking regularly (German Culture, 2015). Many early cultures concentrated their diets around beer due to its sanitary advantage over water. As its foundation in various cultures solidified, so too did its complexity. Every decade, thousands of microbreweries are emerging with exciting and new brews. This growth demands a complimentary amount of research and understanding. This section attempts to encapsulate the various complexities that go into the brewing process by breaking it down to a more understandable level.

## *2.1 The Brewing Process*

The four basic ingredients in the brewing process are malt, water, yeast and hops. The central process is to extract the sugars from the malt grain so that the yeast can catalyze the decomposition reaction of sugars into alcohol (ethanol) and $CO_2$, which create the essentials of a beer. Even though every brewer has their own variations and preferred conditions within their process, they all follow a reliable framework for the brewing process as shown and described in Figure 1.

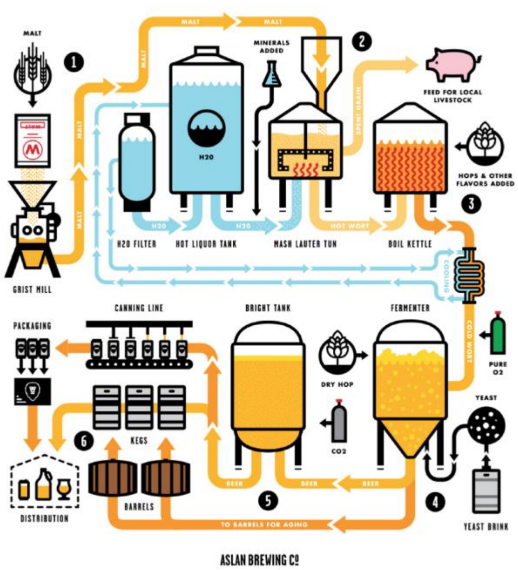


Figure 1: Flow Diagram of the Brewing Process (Trosset, 2018)

The first step of the process is to crush the whole grain malt with a mill. The purpose behind milling the malt is to create enough surface area on the part of the seed which acts as a food store for the developing plant embryo commonly called endosperm. Once the milling is complete, the grains go onto the second step, which is mashing. Here, the crushed malt is mixed with hot water, ranging between 144 - 158°F. Since the grains have been processed to increase the surface area on the grain's endosperm, the temperature of the water activates the enzymes in the endosperm. When active, the available starches are converted into sugars (maltose) and dextrins. The mashing process will usually take between 30 to 120 minutes depending on the temperature and the type of enzymes that are being focused on. Once the mash is complete, the liquid, commonly called the wort, is separated from the spent grain and transferred to a boil kettle.

Once the wort is fully transferred, it is brought to a boil. Boiling the wort is important because it becomes pasteurized. Also in this phase, hops and other flavors are added. Brewers will adjust and experiment with what they add to the boil to find the specific taste they are searching for. Boiling the wort usually lasts for 60 to 90 minutes. After the wort is boiled, it is transported into a fermenter. Since the functionality of yeast is highly dependent on temperature, the wort is sent through a heat exchanger system to achieve proper temperature conditions. Once the transfer from the boil is complete, the yeast is added into the fermenter. Over a period of 4 to 6 days, the yeast consumes all the fermentable sugars converted from the mashing process and turns it into alcohol and carbon dioxide. After the enzymatic process is complete, the yeast sinks to the bottom of the tank. Since the process is exothermic, the temperature of the tank is closely monitored by the brewers. When the fermentation is complete, the beer is cooled to 30 °F for conditioning. As the beer is transferred out of the tank, the brewers collect the yeast and recycle them into the next batch. At the end of the conditioning phase the beer is either filtered or directly transferred into a tank. In those tanks, the beer is carbonated and kept for either kegging, bottling or canning.

## *2.2 Sugar Chemistry*

Sugar is a major building block of the food and beverage industry. It can help increase the palatability of certain foods, act as a natural preservative, as well as a general flavor and color enhancer (SRAS, 2016). In the context of brewing, it dictates flavor, body, color, and alcohol content. This section will go over the importance of sugar in brewing, its complex interactions with yeast, and the takeaway for our specific project.

### *Sugars in Brewing*

Understanding the sugars in your brew and how they operate is the first step towards a successful, reproducible product. In a typical barley base malt, the most prevalent sugar extracted from the mash process is maltose, which is a glucose-glucose disaccharide. Maltose accounts for about half of the sugar in the wort. The following sugar by prevalence is maltotriose, a glucose trisaccharide, at around 14% (Peros, 2009). This sugar goes through the same enzyme as maltose (maltase), but maltose maintains preferential treatment in this arena. The remaining fermentable sugars are glucose (8%), sucrose (6%), and fructose (2%). The remaining 25% are dextrins, which are polysaccharides that are too complex for beer yeast to break down (Peros, 2009).

### *Yeast and Sugar*

The end goal of yeasts enzymatic process is to break sugars down to glucose and fructose monosaccharides that can then be fermented. This is achieved through two enzymes produced by the yeast: invertase and maltase. Typical yeast tackles these sugars in a very specific order, targeting sucrose first, followed by glucose, fructose, maltose, and maltotriose. Invertase hydrolyzes sucrose outside the yeast cell into its two components - glucose and fructose (Palmer, 2012). The components then pass through the cell wall and are metabolized by the yeast. For maltase, the full process occurs inside the cell. The enzyme targets both maltose and maltotriose, as mentioned before, but breaks down the maltose disaccharide before the maltotriose (Palmer, 2012). The pathways that these sugars take are integral to the final character of the beer. Manipulating either the sugar and/or enzyme profile of the fermentation process can yield a substantially different final product. Within the scope of the project, we are investigating the impact of pulling more fermentable sugars from the grain in the mash process, which is prior to the addition of yeast.

## *2.3 The Mash Process: A Chemical Analysis*

The mash sets the stage for any brew. It hydrates the malt, activates enzymes found within the grain, and most importantly converts starches into fermentable and unfermentable sugars. In order for these enzymes to do their magic, the grain starches must be soluble and reactable. In the mash, the grain must be subjected to water at around 130°F to be 90% soluble and 149°F to be fully soluble (Palmer, 2015). When the starches are hydrated via water, they can be gelatinized through a heat process. Any form of agitation during the mash, such as rolling the grain or crushing it, can help to improve the hydration of the malt and yield a readily usable wort. At this point, the enzymes can begin breaking down the grain.

## *Mash Enzymes*

Generic malts consist of seven main enzyme groups, each with their own preferred temperature range, pH range, and specific function (Refer to Table 1). It is important to note that when the mash temperature exceeds an enzyme's range, the enzyme will no longer be active and it will be denatured. Figure 2 provides an interpretable visual of the "sweet spot" of mash conditions that brewers aim for.

Table 1: Enzyme Groups (Palmer, 2015)

| **Enzyme Group** | **Preferred Temperature Range (°F)*** | **Functional pH Range** | **Purpose** |
|---|---|---|---|
| Phytase | 86-126 | 5.0-5.5 | Lowers mash pH, no longer used. |
| Debranching | 95-113 | 5.0-5.8 | Solubilization of starches |
| Beta Glucanase | 95-113 | 4.5-5.5 | Best gum breaking rest |
| Peptidase | 113-131 | 4.6-5.3 | Produces nutrients for the yeast |
| Protease | 113-131 | 4.6-5.3 | Reduces haze through breaking large proteins |
| Beta Amylase | 131-150 | 5.0-5.5 | Produces maltose |
| Alpha Amylase | 154-162 | 5.3-5.7 | Produces variety of malt sugars |

In ascending order of temperature range, phytase operates between 86 to 126°F. The enzyme breaks down organic phosphates and effectively lowers the pH of the mash. Before water chemistry was fully understood, early brewers would utilize an acid rest at these conditions to help reel in the proper pH of their mash. Today, this problem is alleviated prior to the mash by treating and testing the inlet water.

Next in line comes the debranching enzymes, such as limit dextrinase. This enzyme ranges from 95 to 113°F and creates linear polysaccharides through a hydrolytic process that are more feasible for beta and alpha amylase to break down (Izydorczyk, 2003). This enzyme can be overlooked and underutilized. However, many sources suggest using a rest called the dough-in that lasts around 20 minutes at 104°F (Palmer, 2015). This can increase yield by mitigating the bottlenecking of the amylase enzymes, as discussed later.

The function of beta glucanase is to break up gumming in the wort caused by beta glucans. This prevents the mash from becoming a solid gummy load and allows for easier physical filtration at the end of the process. As such, this enzyme has a subsidiary role in the context of our project and will not be investigated further (Bamforth).

The next range encompasses the protein-focused phase of the mash at 113-131°F, which sets the stage for growth and development of the yeast later on. It involves proteolytic enzymes like peptidase and protease. Peptidase yields amino acid nutrients into the wort that will eventually be consumed by the yeast during fermentation. Lack of peptidase can place a ceiling on the maximum productivity of the yeast. Protease works on large proteins and breaks them into manageable and consumable sizes. As a positive side effect, this reduces the haziness of the beer and improves head retention (Palmer, 2015). This phase can be taken advantage of with a protein rest, which will help to shape the character of the beer. However, the protein rest works in tandem with the degree of modification of the mash. Modification in the mash is the extent of breakdown that the protein-starch matrix, or endosperms, experience. A moderately-modified malt can utilize a protein rest to complete the breakdown. However, a fully-modified malt should not use a protein rest, as this will destroy the body of the beer.

### *Starch Conversion Enzymes*

The final enzyme group (diastatic) of beta and alpha amylase operates at the highest temperature ranges: 131-150°F and 154-162°F, respectively. Both of these enzymes work to convert the starches in the malt into both fermentable and unfermentable sugars in a process called saccharification (Palmer, 2015). At this point in the mash, the starches can be placed into two categories: amylopectin and amylose. Amyloses are single/straight chain starches while amylopectin are multiple amyloses branched together. Amylopectin are highly resistant to diastatic enzyme action at their branch point. Fortunately, beta amylase works around this by attacking the ends of the chains, sequentially lopping off glucose molecules one at a time. Because of this sequence, beta amylase is considerably more effective at amylopectin breakdown than amylose breakdown.

The alpha amylase, generated in the protein modification phase, works at random and can reduce large amylopectins into viable chains for the beta amylase to separate. Due to their differing temperature ranges, most mashes hold at 153°F to make use of both these enzyme's qualities. Operating below this temperature favors a beta-optimum wort and results in a plethora of large amylopectins alongside a reserve of fermentable glucose. Operating above this temperature favors an alpha-optimum wort that denatures the beta amylase and creates a large distribution of smaller amylopectins that aren't fermentable. Finding a healthy temperature balance can exploit the best qualities of each enzyme, resulting in a fermentable, wide variety wort. This same balance act is true for the pH of each enzyme. Beta amylase favors a lower pH around 5.0 and alpha amylase favors the higher 5.7 wort pH (Palmer, 2015). A typical compromise falls around 5.3-5.5 pH level.

## *Adjustable Parameters for Starch Conversion*

Two remaining factors in the starch conversion are the grain/water ratio and the total mashing time. A thinner mash of greater than two quarts of water per pound of grain produces a slower starch conversion process (Palmer, 2015). However, this can increase fermentability due to the lower concentration of starches, allowing the enzymatic processes to run effectively uninhibited. Conversely, a stiff mash of less than 1.25 quarts per pound of grain results in a faster starch conversion and a distribution of less fermentable sugars. This will end up being a sweeter, maltier beer. Another benefit of the stiff mash is that the enzymes are well protected. Because of the density of the grain, the relative heat capacity of the mash increases and the enzymes take longer to be denatured.

Finally, time changes everything. An alpha-optimum wort can convert to full extent in around 30 minutes where a beta-optimum wort reaches its extent at around 90 minutes (Palmer, 2015). The remaining time spent in the mash after this phase allows the brewer to shape the profile of the converted sugars. Comprehension of how all these enzymes work together can yield the maximum conversion of the starches in the grain into usable sugars, which encompasses the scope of our project. Based on the literature reviewed in this section, the team will develop experiments that feasibly address potential areas of improvement in a given mash process.

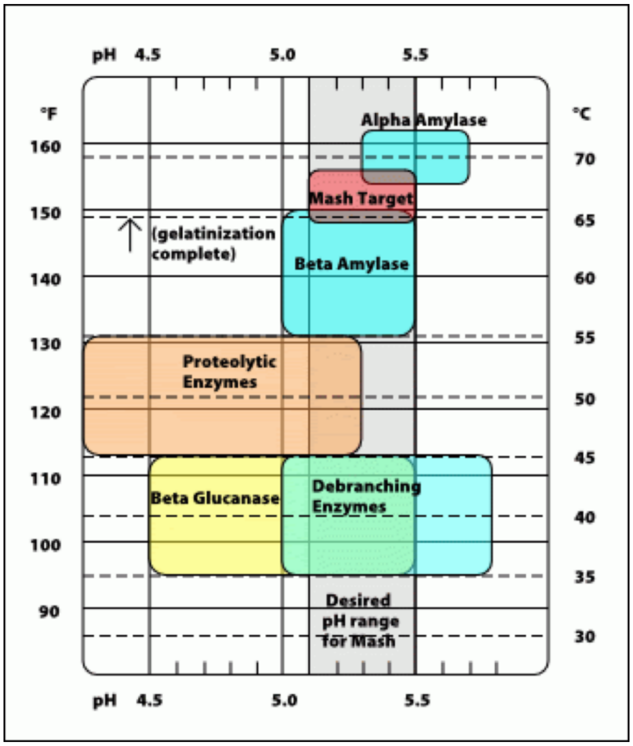


*Figure 2:* Ideal Mash Conditions of Each Enzyme (Palmer, 2015)

## *2.4 Factors Influencing Mash Sugar Yield*

There are several factors that have an effect on the amount of sugar produced in the mash that will be further converted into alcohol. These parameters will be experimentally tested and compared to theory along with the original recipe and control used for each experiment. When varied, each parameter has an altering effect on how much sugar can be extracted. Additionally, some depend on the types of malt used and the recipe style. The following parameters are detailed in how they affect sugar yield in the mash process.

### *Mash Temperatures*

Controlling desired mash temperatures is one of the most important factors in brewing a great beer. During the mash, starches are taken from the grain and converted into sugars for the wort, in which an ideal mash temperature (discussed in the Mash Process section above) is needed in order to maximize sugar output into the wort (Giovanisci, 2020). The mash-in temperature, or the water temperature entering the mash tun, is generally higher than what the mash temperature is held at. This accounts for heat loss and easier maintenance of the mash itself. Specific temperatures will be better at activating enzymes that break down complex sugars to simple sugars in the system (Giovanisci, 2020). For example, the enzyme alpha-amylase, which produces simple sugars like maltose, functions more effectively at around 60-70°C (Jackson, 2016). Other enzymes are activated at similar or slightly lower temperatures as explained in Section 2.3. Effective conversion from these enzymes will help to increase the body, flavor, and head retention of the beer, as well as provide higher alcohol content. The ideal mash temperature detailed in Table 1 will be attempted to be utilized within our experiments.

### *Sparge Temperature*

Sparging occurs in the lautering process, which describes the separation of the wort and the spent grain in the mash (Palmer, 2015). Sparging is necessary in brewing in order to rinse out all of the remaining sugars in the grain bed and achieve more sugar yield. The temperature of the sparging water is important to maintain, as only hot water will be effective in dissolving the sugars and transferring them to the wort. However, if the water is too hot, it will additionally dissolve tannins from the grain husks that can cause undesired taste in the finished beer (Colby, 2013). The ideal sparge temperature is said to be at approximately 170°F, so that enzyme activity is discontinued and the wort viscosity is decreased in order to increase lautering efficiency (Found, 2017). Any temperature slightly higher would cause there to be an excess of tannins in the wort, whereas lower temperatures are susceptible to inefficient dissolution of sugars. With respect to our experiments, the act of sparging will not be performed due to lack of equipment and is substituted with testing the vorlauf approach.

### *Grain Size*

Grain size has different effects on extraction and lautering efficiencies via the mass transfer that exists in the mash process. There are tradeoffs between a coarse crushed grain versus a finer grain. Coarse grain tends to allow for sufficient flow and lautering, however isn't converted as well partially due to decreased total surface area (Huolihan, 2018). On the other hand, fine crushed grain is converted easily by enzymes with better extraction, but make it more difficult to lauter (Palmer, 2015). It has been hypothesized that a wider range of coarse and fine particles in the crush can contribute to a compromise of better extraction and lautering efficiency. At Purgatory Beer Company, Brian purchases pre-milled grain at an expected uniform size. It is possible that his grain size does not yield an extraction efficiency achievable with another variety of grain size. The variable of grain size will not be tested due to lack of noticeably different grain size in comparison to Purgatory Brewery pre-milled grains.

## *Vorlauf Technique*

The term "vorlauf" is the german word for recirculation, in which the wort is recirculated through the system until it is rid of particulates and can be drained into a kettle. The brewing technique aims at reducing grain particles in suspension under the vessel's false bottom (CraftBeerBrew, 2015). If left in suspension, these particles would be run into the kettle to boil, likely causing undesired flavors in the completed beer. Additionally, the particles would not have been subject to the same enzyme activity that the previous grain experienced. During the vorlauf process, the wort is drained via gravity underneath the vessel's false bottom into a collection container called a grant (CraftBeerBrew, 2015). It is then pumped above the grain bed that acts as a filter to trap unwanted particles that could reenter the wort. This process is depicted in the figure below:

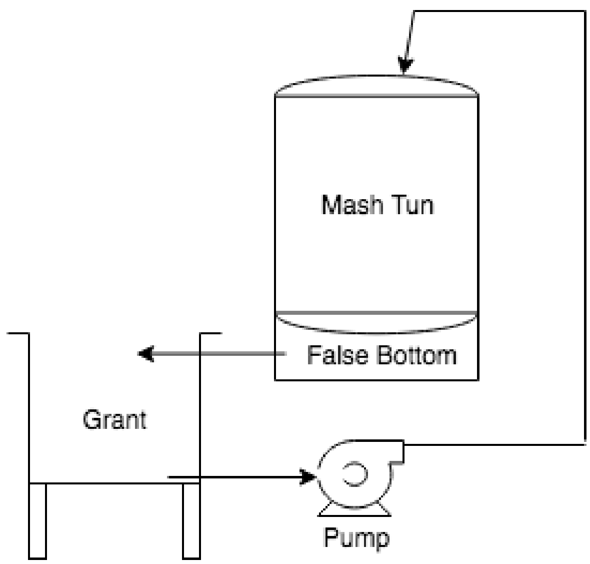


*Figure 3: Basic Mash Tun Set Up*

This procedure is continued until the wort runs clear or after a specific time period (usually a range of 10-20 minutes (CraftBeerBrew, 2016). Afterwards, the grain bed is ideally set up for the sparging process (CraftBeerBrew, 2016).

Within our experiments, we compare the use of the vorlauf technique to conventional brewing to determine the effect on sugar production. It is also important to note that some brewers believe the vorlauf technique can hinder run-off by reintroducing particles into the mash grain bed (CraftBeerBrew, 2015). This would ultimately defeat the purpose of the technique and prove more efficient to leave the wort not fully cleared of grain particles.

## *Continuous Mixing*

Continuous mixing during the mash is a technique used by some brewers in which the grain is mixed throughout the mash time. This can be done either with a mechanical mixer or manually by hand. Many brewers believe that there is a benefit to mixing the mash, however some believe it is counter-intuitive to mix outside of the first 15-20 minutes of mash time. These brewers believe that mixing after the initial mash-in can cause problems such as clogging the false bottom of the mash tun. Mixing the mash in the beginning ensures that the grain is wetted and set into the grain bed so that water can move more easily throughout the mash. Mixing helps to remove air pockets in the mash that would otherwise oxygenate the mash. Agitating the mash can also help to distribute heat evenly throughout the tank and reduce the risk of burning the malt when it comes in contact with the heated surface. Moving the malt/wort around helps to decrease the temperature gradient within the tank.

## *Continuous Whirlpool*

Whirlpooling, or recirculation, is the process of pumping the wort from the bottom of the mash tun to the top creating a "whirlpool" effect within the tank. Continuous whirlpool is a widely used technique used by home brewers and commercial breweries alike. Recirculating the wort helps to ensure that all the grain is in even contact with the water and that the wort is evenly mixed. Whirlpooling can help to make a clearer wort product as most of the proteins and particles get trapped within the grain bed. This process can also help to increase the overall mash efficiency by pulling more sugar out of the grain during the mash. Along with all these other benefits, whirlpooling also helps to improve the aroma that the wort gets from the grain and the overall body of the beer. There are many benefits to continuously whirlpooling and it can be done with little to no equipment, making it a very effective method of increasing mash efficiency and quality.

## *Mash Time*

Mash time is an important factor in the mash efficiency. The higher the mash time, the more time there is for the starches in the grain to break down into sugars. Most brewers use a mash time of about 60 minutes, however some people use longer mash times and will even mash overnight. This ensures that all of the starches in the grain are converted to sugars in the wort. Doing an overnight or extended mash is risky because it allows time for the temperature of the mash to drop or rise if not monitored closely. This could lead to both off flavors if the mash gets too hot and poor efficiency if the mash is allowed to cool too much. Mash time goes hand-in-hand with mash temperature to give the best possible mash efficiency for the specific beer being made. It is important to work out a good combination of time and temperature for each beer being brewed.

# 3. Methodology:

The goal of this MQP was to investigate methods by which Purgatory Beer Company could increase its mash efficiency. In order to do so, the sugar content and profiles generated by each method had to be analyzed and explored to understand the impact on the overall brewing process. The team broke down this goal into the following objectives:

1. Determine which parameters of the mash can be manipulated and perform homebrew experiments, isolating one parameter at a time.
2. Analyze empirical data using GC-MS and density analysis to discern sugar content and profile changes from each experiment.
3. Based on results from Objective 2, recommend various methods to Purgatory Beer Company to employ in their mashing process.

## *3.1 Mash Parameters*

Specific parameters were utilized to vary the mash process and determine the most productive and efficient way to maximize sugar extraction into the wort. A control experiment was established based on using Pilsner malt and fixed parameters. The control was varied according to each parameter that was individually tested. Due to the COVID19 pandemic outbreak, there was time for only a few parameters to be tested.

Table 2: Experimental Setup

| Experiment | Parameter Adaptations | Tested? |
|---|---|---|
| Control | Based on Pilsner malt and fixed parameters | Yes |
| Preheat | Water immediately enters mash tun at desired temperature of 150°F | Yes |
| Vorlauf | Wort is drained and added above grain bed for around 5 minutes until wort is purified | No |
| Continuous Mixing | Mash is slowly mixed for majority of mash process | Yes |
| Continuous Whirlpool | Wort is pumped from bottom to top to create a whirlpool | No |
| Increased Mash Time | Mash process time increased by 10 minutes | No |

## *3.2 Mash Experiment*

Based on Table 2, the team ran mash experiments using a five-gallon steel kettle and a propane burner. First, the team weighed out two pounds of grain using a small scale and measured three liters of water. The team then connected the propane burner to a propane tank and tested the burner to ensure that it worked properly. The kettle was then placed on top of the burner and the grain and water were added and mixed so that all of the grain was wetted. The temperature of this mixture was closely monitored via thermometer to make sure that the target temperature was not under or overshot by more than 7-8 °F. The propane burner was turned on and the time was recorded when the mash reached the target temperature of 150 °F. The pH was measured before and after the mash process to ensure sufficient pH levels of around 5.5. The propane burner was adjusted throughout the experiment to keep the mash temperature within a reasonable range around 150 °F. The experiment was performed for 60 minutes after the target temperature was reached, once the time hit 60 minutes the propane burner was shut off and the kettle was removed from the burner. This was allowed to cool and then the wort was strained through a fine screen strainer and cheesecloth into a collection vessel. The remaining wort was then disposed of and the spent grain was composted.  A hydrometer would have been used to measure the specific gravity of the samples. Below is the procedure for the homebrew experiments:

***Step-by-step Control Procedure:***

1. Connect propane tank to burner and carefully test to ensure secure connection.
2. Use scale to weigh out 2 lbs of malt.
3. Measure out 3L of hot tap water (approximately 110°F)
4. Add grain, then water to kettle and light gas burner using grill lighter. (Be careful to not turn gas too high as to not cause an explosion)
5. Stir grain/water mixture periodically to ensure grain moisture and top/bottom kettle temperature distribution.
6. Monitor temperature of mixture throughout the mash time and adjust gas level on burner to keep temperature steady around 150°F
7. Measure pH near beginning and at end of mash process
8. Mash for 50 minutes total time (from initial reading of 150°F)
9. Turn burner off, then remove kettle from burner and let cool for approximately 20 minutes.
10. Strain wort from kettle using strainer and cheesecloth into a collection vessel.
11. Put the sample into the refrigerator.

***Equipment:***

- Stainless steel mash kettle (5 Gal)
- Propane burner
- Propane tank
- Stirring rod
- Thermometer
- Scale
- Collection vessel
- Small strainer

- Cheesecloth
- pH strip
- Hydrometer

*Figure 4: Homebrew Set Up*

## *3.3 Calibration Curve of Fermentable Sugars*

To be able to determine the unknown concentration of the different fermentable sugars in each sample, the team planned to make a calibration curve using a HPLC. The team would have ordered standard concentrations of glucose, sucrose, fructose and maltose from Sigma Aldrich. For each calibration curve, the team aimed to diluted the standard into 7 different concentrations. Afterwards, each diluted sample would have been loaded into the HPLC. After each sample is run through the HPLC, the data would be shown as a graph plotted absorbance against retention time. The absorbance values of each peak was going to be recorded. With Excel, all the recorded values were going to be plotted against their concentration. Finally, the team would have used a best-line fit to find the equation for the calibration curve.

## *3.4 Analyzing Wort using HPLC*

Our team decided to use HPLC to test our wort since it has the ability to identify the sugar composition within a test sample. Prior to loading our sample into the HPLC, our group decided to use two separation methods to ensure that no impurities would be run through the HPLC. The first method is the centrifugation to ensure that the non-dissolved solid goes to the bottom. Afterwards, the wort will be filtered through a syringe filter to confirm that there is no impurities. Then the samples are run through the HPLC. With the data collected from the HPLC, our group will be able to identify the unknown sugar concentration using the calibration curve.

## *3.5 Total Sugars Calculation*

To verify that our calculations in the section above are correct, our group decided to calculate the total amount of sugar in the wort collected using a hydrometer. To do so, we planned to put a hydrometer in our sample,which would give us its special gravity. The value found can then be converted into degrees of Brix or degrees of Plato (Equation X & X).

Degrees of Brix = Grams of sucrose/100 grams of liquid

Degrees of Plato = Grams of sugar and of solubles/100 grams of wort

Degrees of Brix = [182.4601*SG3]-[775.6821*SG2]+[1262.7794*SG] -669.5622
Degrees of Plato =[135.997*SG3]-[630.272*SG2]+[1111.14*SG] -616.868

By calculating the wort collected, the team could have calculated precisely the total amount of sucrose (Brix) and the total amount of sugar in the wort (Plato). This would have been a confirmation of the data collected using the HPLC. Below is the protocol for proper sample preparation for the HPLC, as it is a very expensive piece of equipment:

***<u>HPLC Sample Preparation:</u>***

1. Transfer 10-20 mL of your raw samples into a 50 mL centrifuge tube.

2. Use the centrifuge to separate the solid particle from the liquid. I normally use Legend RT-Centrifuge located in 221. The parameters are: accelerate/decelerate rate: 9; speed: 3000 rpm; time: 20 minutes temp.: @RT-22C.

3. After the centrifuge, transfer the top clear liquid to a glass bottle for later analysis.

4. use pipette to draw 0.5 mL of the clear liquid from your bottle, then dilute that 0.5 mL liquid 5 times with DI water.

5. use syringe to draw 1-2 mL of your diluted sample, then put on the 0.22um filter and then inject the samples into HPLC analyzing vials for later HPLC analysis.

Syringe: 3 mL syringe is typically used.

0.22um filter: we use Polytetrafluoroethylene (PTFE) 0.22 um filter-Nalgene.

# 4. Results & Discussion:

Due to the outbreak of COVID-19, the team was unable to compile data and generate results from the wort samples. The laboratory on campus with an HPLC apparatus is necessary to gather data on the amounts of sugar within the samples. This apparatus could not be used by the team because the campus has since closed due to the virus.

The data that the team were looking to collect was the amounts of each different sugar present in the wort samples. Each parameter, such as continuous mixing and increased mash time, was expected to have a certain effect on the amounts of sugar in each sample. These amounts would have been compared and analyzed based on their effectiveness in sugar production. The team believes that after all the experiments were performed and the data were analyzed, that the continuous whirlpool method would have yielded the highest sugar content. This is because whirlpooling circulates the water throughout the mash tun ensuring proper mixing of the grain and water. This would also help to decrease the temperature gradient within the mash tun because of the constant motion of the water. We believe that the amount of total sugars would increase due to continuous whirlpooling, however the exact amount of this increase is uncertain. It is generally accepted in the brewing community and discussed in our background that recirculation of the wort increases the active surface area by which sugars can be drawn from. Other factors such as flavor and body were not taken into account in order to narrow down and only evaluate sugar production during the mash. Although we do not have results to support the underlying theory behind mash sugar production discussed in the background section, we did make some interesting observations during our experimentation of the mash brewing process.

After conducting multiple trials of the control experiment, it was easier to notice changes in the mash process when introducing other parameters such as continuous mixing, which was not performed in the control experiment itself. When the mash was continuously mixed, the temperature gradient across the 3 liters of water was smaller. Without mixing, the water at the bottom was consistently hotter than the water at the surface because of its proximity to the gas burner. The water temperature was more difficult to stabilize because of the gradient between top and bottom of the mash tun. For example, in some of the control experiments, the temperature difference between the top and bottom of the water was as much as 20°F. The improved temperature distribution with mixing allows for more of the enzymes to become active and successfully extract and break down complex sugars in the malt. Therefore, the team believes that continuously mixing the wort during the mash process would be successful in producing more usable sugars, and consequently provide the opportunity to create a beer with a higher alcohol content.

Another observation was the comparison between mashing in at a higher temperature of about 150-155°F and adding the water at a lower temp around 110°F. Preheating the water allows for easier stabilization of the mash water temperature, however some of the enzymes that help extract and break down sugars are activated at lower temperatures. Therefore, some enzyme activity may have not been maximized because the water was never at a temperature lower than 150°F. In turn, the main alpha amylase and beta amylase enzymes are more productive at around 150°F. Without preheating, enzymes that function better at lower temperatures are given a chance to act as the water is heated up by the gas burner. All enzymes are given an allotted time to act, however the temperature becomes more difficult to stabilize without control systems and less time is provided for alpha amylase and beta amylase enzymes.

# 5. Conclusions & Recommendations:

Due to the outbreak of COVID-19, there are limited conclusions that we can draw from our experimentation. However, observations from our experiments and underlying theory helped generate certain conclusions. We suspected that the continuous whirlpool method would have generated the highest sugar yield within the mash. However, we believe that this increase would have been marginal and that ultimately, it would be most beneficial to add specific sugars substrates like corn syrup to the wort after the mash if higher ABV is desired. The effect of continuous whirlpool is supported by theory, but raw data from our experiments isn't available to suggest that it would have a positive or negative outcome on Brian's mixes at Purgatory Brewery. Parameters that were tested to improve mash efficiency included varying mash temperatures to ensure maximum enzymatic activity, as well as providing continuous mixing in order to generate a more balanced temperature distribution within the mash. It was observed during our experiments that continuous mixing had a significant effect on reducing the temperature gradient in the system, leading us to conclude that the mixing is necessary to achieve better mash efficiency. The scope of this conclusion in terms of how much mash efficiency would increase is unknown due to lack of raw data from experiments. Parameters that were not tested include the Vorlauf technique and increased mash time. The Vorlauf method recirculates the wort through the system until it is rid of particulates and can be drained into a kettle to gather more leftover sugars in the grain. Vorlauf or a sparging related method would have been expected to slightly increase mash efficiency by extracting remaining sugars. An increase of mash time, although not experimentally tested, would theoretically allow the enzymes more time to extract and break down sugars in the system, thus increasing mash efficiency. However, the 50 minutes allocated to the mash in the control experiment could be enough time for the enzymes to run their course. These concepts are outlined in more detail in the background section of the paper.

We recommend that the experiments outlined by our team be completed in the future to further investigate our research and prove or disprove our hypotheses. We also recommend that the experiments in the future be performed with a more reasonably sized homebrew kit, as ours was oversized for the scale of the experiments we performed.

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# Appendix:

## Additional Background: Water Treatment

Most craft brewers use the water that is already available to them. This approach can result in a good beer; however, it is not the best way to produce a great beer consistently. The reason is that the composition and quality of the local water accentuates with each batch. Only a few changes to the mineral content can create a medium that will bring out the flavors you are looking for. Any discussion of water treatment must come with an understanding of pH, hardness and alkalinity. In short, pH is the measure of relative acidity or basicity of a water solution; hardness is the measure of the total magnesium and calcium ions in a solution; and alkalinity is a measure of the water's buffering capacity.

### *Potential of Hydrogen (pH)*

As mentioned above, pH is the measure of the relative acidity or basicity of a liquid. The pH of a liquid is determined by the combination of dissociated salts, undissociated salts and organic compounds that are contained in the liquid. A pure, distilled water is a mixture of hydrogen or hydronium (H+) and hydroxide (OH-) ions, which have an identical concentration of 10^-7 mol/L. The relative concentration of these two ions determines the water's pH. Thus, when they are in equilibrium, the water has a neutral pH. When a strong acidic or basic substance is introduced, it upsets the equilibrium and changes the characteristic of the water. It becomes basic when there is an excess of hydroxide ions, and becomes acidic when there is an excess of hydrogen ions.

Usually, the pH can be measured by an electronic pH meter or by lower-tech pH strips. Throughout the brewing process, different parts need to be kept at a different pH to optimize the product. For example, the brewing water is best kept at a pH range of 6 to 7. Ideally, the mash should be kept within a pH range of 5.2 and 5.5 to optimize the enzymatic action.

### *Hardness*

Hardness describes water that is hard to generate a lather from sodium-based soap. As the hardness increases, it is harder to start lathering. Hardness is defined as the total concentration of calcium and magnesium dissolved in solution. Alkali metals that are less electronegative, for example potassium and sodium, are more stable in water and have little effect on mash pH. Hardness is quantitatively measured by titration to endpoint with a chelating agent that binds magnesium and calcium ions. The results are listed in a water analysis report as particle per million (ppm) hardness. This reading also takes into account the other ions that make up the hardness. Each mineral has their own ppm range.

## *Alkalinity*

Alkalinity is a measure of the buffering capacity of the anions (negatively charged ions). A buffer prevents changes in the pH by maintaining a relatively constant concentration of hydrogen and hydroxide ions within a specific pH range. For example, bicarbonate is a strong buffer and the major component of the alkalinity of brewing water.

Similar to hardness, alkalinity is quantitatively measured by titration with a strong mineral acid until the buffering capacity of the anions within the solution is neutralized. Compare to hardness, it looks into the anions, and how each affects different parts of the brewing process.

Resources:

https://www.morebeer.com/articles/treating_homebrew_water https://beerandbrewing.com/brewing-water/