Letters in
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Flocculation of Yeast Based on Sugar Content During the Brewing Process of Ale

Jenn Metzger and Julia O'Hara

Department of Biology, Rutgers University, Camden NJ 08102

Abstract

The brewing of alcoholic beverages is a very simple and popular practice.; as well as, one inevitable part of this process is not well understood, flocculation. It is not well understood how flocculation occurs, and there are several hypotheses why flocculation occurs. This paper will describe one aspect of the flocculation of yeast cells and variables that could alter the flocculation process. More specifically, in the current study we tested our hypothesis that less flocculation occurs with more sugar incorporated in the solution due to a lack of necessity for yeast cells to utilize membrane sugars. To test if the lack of sugar content causes flocculation, several batches of hard apple cider were fermented with varying amounts of sugar. To test the possibility that yeasts are aggregating in an attempt to get sugars from the other yeast cell, a batch of hard apple cider with a lyophilized and powdered yeast cells. Flocculation, clarity, and alcohol content if each fermentation batch were measured. Our results supported our prediction that more sugar would lead to less flocculation, more flocculation would lead to more clarity of solution, but consequently less sugar would lead to lower alcohol content.

Introduction

The brewing process of ale requires fermentation of sugar in the mixture by yeast. During this process, aggregation, or flocculation, of the yeast cells occur. It is believed that flocculation occurs when sugar is no longer present in the solution, so the yeast cells begin attacking each other and end up sticking together. The actual mechanism of the flocculation remains unknown (Verstrepen 2003). Moreover, it is uncertain whether or not the amount of sugar added to the product has an effect on the amount of flocculation. When there is plenty of sugar available in the solution, the yeast will not need to flocculate to use the sugars from their own membranes. Conversely, if there is not much sugar present in the solution, the yeast will form flocs very quickly. Ultimately, the yeast will always somewhat flocculate when the sugar runs out, but it will happen much more quickly and in a higher quantity when there is less sugar from the beginning. Flocculation during the brewing process increases the clarity of the final product. This is because the yeast cells are pulled out of solution and sink to the bottom once they aggregate together. This, in turn, makes the final product more attractive appearing to consumers. However, if there is no usable sugar in the solution, there will not be sufficient fermentation to produce alcohol. There are many different kinds of brewing yeast, but a common one is the WPL002 yeast. This yeast a bottom aggregating yeast, meaning this yeast will flocculate on the bottom of the container versus top aggregating yeast, which makes decanting the liquid product much easier. One belief on how flocculation occurs is that when the sugar in the solution is used up, the yeast cells seek an additional source of sugar. In doing so, they actually attack each other so that they can use the sugars in their own cell membranes (Miki 1982). Based on this theory, we have designed an experiment to test. We will test several batches of product, each with varying concentrations of sugar and ground up yeast cells. From these we will measure the weight of the total flocculation, the clarity of the solution, and the alcohol content of each product. We hypothesize that more sugar in the solution will lead to less flocculation, since the yeast cells will not have to attack each other to use the membrane sugars. More flocculation will lead to a lower final alcohol content, but a product with more clarity.

Materials & Methods

Several batches of hard apple cider were prepared using 1L of Mott's no sugar added apple juice. To each of these batches, 1mL of living WPL002 yeast was added. The amount of sugar and ground yeast varied per batch as shown in Table 1. Batches were prepared in 1L autoclaved Erlenmeyer flasks in sterile operating conditions. The original yeast was bought and then more yeast was subsequently grown using a liquid YPD medium. The YPD medium consisted of 500mL distilled water, 5g Bacto yeast extract, 10g Bacto peptone, 10g Glucose, and our WPL002 yeast. This yeast was poured into centrifuged tubes and centrifuged for 10 minutes at 4C and 4000rpm. The liquid was decanted, distilled water was added, and the tube was mixed on a Vortex. The six tubes were concentrated into two tubes and centrifuged again for 10 minutes. The liquid was again decanted, distilled water was added, the tubes were mixed on a Vortex, and concentrated into one tube. One more 10 minute centrifuge session was run and the last of the liquid was decanted. The yeast was then freeze-dried for several hours using liquid nitrogen. Once the yeast was sufficiently dry, the yeast cells were ground with a pestle and mortar in liquid nitrogen. We then weighed the ground yeast and added 1g to batches four and five. Once complete, the batches were left to sit undisturbed in a room temperature closet until flocculation was complete.

Flocculation Harvesting

To harvest the flocculation from each batch, most of the liquid was decanted into a separate container and saved for later. The remaining liquid was then swirled with the flocculation on the bottom and this solution was poured into centrifuge tubes. The tubes were then centrifuged at 4 degrees Celsius for ten minutes at 4000rpm. The liquid was decanted and the tubes with the remaining flocculated yeast were weighed.

Spectrophotometry

To determine the clarity of the batches, the percent of light absorbed was measured for each sample using a spectrophotometer. Using the liquid that was saved, a small sample from each batch was put into a cuvette. First, a cuvette with distilled water was run in the spectrophotometer. Once this blank was run, each sample could be run.

Alcohol Content

To measure the alcohol content, the specific gravity was taken using a hydrometer. The specific gravity of each sample was compared to the specific gravity of plain apple juice to calculate the percent of alcohol in each sample using the following equation:

$$(((1.05)\times(OG-TG)\div TG)\div 0.79)\times 100$$

In this equation, OG is the specific gravity of plain apple juice and of plain apple juice and TG is the specific gravity of the sample.

Batch #	Amount of apple juice	Amount of viable yeast	Amount of sugar	Amount of ground yeast cells
1	1L	1 mL	½ cup	None
2	1L	1mL	1 cup	None
3	1L	1mL	None	None
4	1L	1mL	½ cup	1g
5	1L	1mL	None	1g

Table 1: Ingredients per batch

Results

To test the role of the amount of sugar in flocculation, we fermented apple cider with different amounts of sugar (Figure 1). For reference, each of the batches are described in table one indicating amount of yeast cells and sugar content. We found the batch with the most flocculation was batch four (Figure 1). This was the batch only with ground up yeast cells, and no added sugar. The other batches from heaviest to lightest were: batch five, batch three, batch one, and batch two (Figure 1). We were then able to test for clarity of our final product by using the spectrophotometer (Materials and Methods). After using distilled water for the blank reading, each sample was tested. We found the batch with the highest amount of light absorption was batch five. This batch had ½ cup sugar and 1g of ground up yeast cells. The batch with the least amount of light absorption was batch three. Overall, both batches four and five were high. These were the batches with the ground up yeast cells. This measurement could be higher due to the debris from the cell parts. The rest of these readings are summarized in Figure 2.

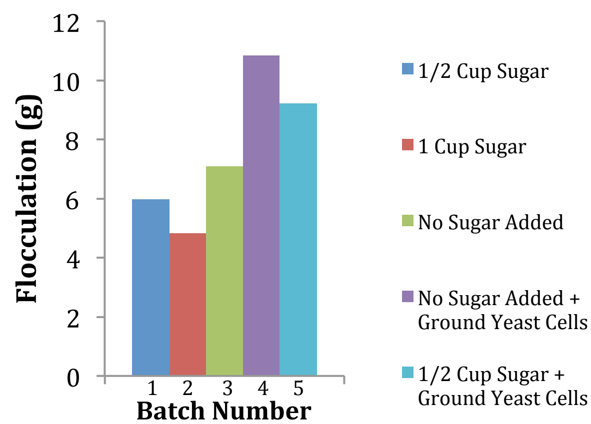

Figure 1. Grams of Flocculation per Batch The weights of each batch's flocculation are displayed.

As for alcohol content, we used a hydrometer to measure the specific gravity of each sample. The specific gravity readings are listed in Table 2 below.

Batch Number	Specific Gravity
1	1.014
2	1.026
3	1.011
4	1.024
5	1.016
Plain Apple Juice	1.052

Table 2: Specific Gravity of Batches

After substituting these specific gravity values into the previous equation we were able to find the percentage of alcohol present in each sample. We found batch two to have the highest alcohol content at 5.39%. This batch had the most sugar added to it. The lowest alcohol content was in batch three at 3.37%. This batch had no added sugar and no ground yeast cells. This data is shown in Figure 3.

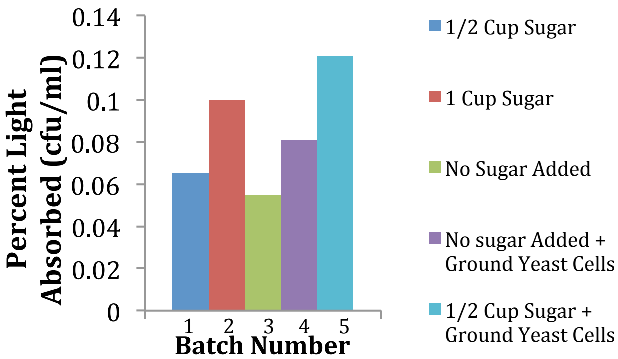

Figure 2: Percent of Light Absorption. The light absorption for each batch is displayed

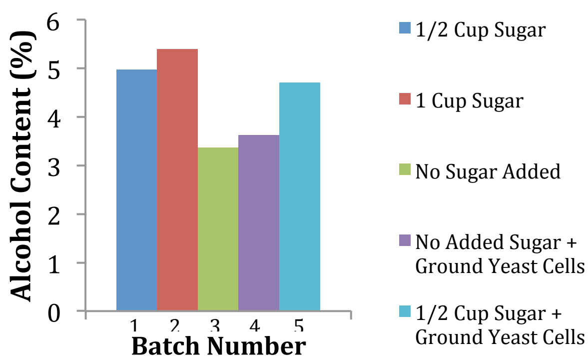

Figure 3: Percent of Alcohol in Each Batch
The alcohol contents for each batch are displayed

Discussion

Our experimental results are overall consistent with our hypothesized outcome. As we expected, the more sugar in solution led to less flocculation. This in turn led to higher alcohol percentage and less clarity.

Flocculation

The batches of hard apple cider with the ground yeast cells added to them both had more flocculation than all of the other batches. This is most likely due to the extra yeast that was added to these. Out of these two batches the batch with sugar added had less flocculation. This proves that since there was an abundance of sugar, the yeast cells did not need to use each other as a sugar source.

Clarity

As described earlier, the batches with more sugar absorbed more light in the spectrophotometer. This supports the fact that there was less flocculation in these batches. Since flocculation pulls the yeast cells in solution to the bottom of the container, there were more free yeast cells in the batches with less flocculation. This leads to a cloudier final product. The two batches with the ground up yeast cells had slightly higher light absorption than their counterparts. This could be due to cell debris from the ground up cells.

Alcohol Content

The alcohol content of our samples correlated with the idea the more sugar would lead to higher alcohol percentages. This is because sugar is necessary for fermentation to take place. Without fermentation, alcohol cannot be created.

Future Direction

Although we used only a very small sample size, our results prove to be very interesting in regards to understanding flocculation in the brewing process. For future studies, it would be a good idea to increase the sample size. We recommend at least five trials of each batch to determine the true significance of the results. Unfortunately, our lack of sample size led to graphs that could not have error bars, but we still believe our results deserve further research and experimental trials.

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