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Effect of Sugars on the Rate of Ethanol Production During Anaerobic Respiration of Yeast

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Fermentation is a form of anaerobic cellular respiration where yeast breaks down sugar into alcohol and carbon dioxide. This experiment tests why some cells respire at different rates than others. We hypothesized that disaccharides will produce less ethanol because it takes longer to break down the bond structure during anaerobic respiration. To test this hypothesis, we measured the amount of ethanol production, a byproduct, when yeast was combined with sucrose which is a disaccharide and glucose which is a monosaccharide, and raw honey, a mix of monosaccharides glucose and fructose. Our experiment found that there was not a significant statistical difference between the rate of ethanol production during fermentation of yeast and the tested sugars. We think our findings could be used in the alcohol industry to make production of ethanol more efficient by using different sugars during the fermentation process.

Introduction

The alcohol industry is reliant on the process of fermentation in order to produce alcohol, the main ingredient of products like beer and wine. Alcohol fermentation is a form of cellular respiration which occurs in the absence of oxygen and can be divided into two parts (Hoefnagels, 2017). The first part known as glycolysis, occurs by sugars such as glucose, sucrose, or fructose being broken down by the yeast into two pyruvate molecules in order to produce ATP. In the second part, two pyruvate molecules are converted in to two molecules carbon dioxide and two molecules of ethanol. The byproducts of this metabolic process are ethanol and carbon dioxide; breweries are concerned with both of these products as they affect alcohol concentration and how 'fizzy' the product will be. Some of the more common sugars that are used for fermentation are glucose, fructose, and sucrose in a number of different concentrations which affect different characteristics of the alcohol being produced such as: alcohol concentration, bubbliness from CO_2, and taste (Bauer et. al 2016). However, the process is very volatile based off of a number of factors that can inhibit or optimize the yeast fermentation (Verstrepen et. al 2004). One of the main ways to influence fermentation is by using a different type of sugar. Glucose and Fructose are common monomers of sugar, meaning that they are the smallest structure of sugar that are unbonded with other monosaccharides. The chemical structure of a monosaccharide is displayed in Figure 1.1. In comparison, refined sucrose is a disaccharide which is a solution that has 50/50 bonded glucose and fructose. The chemical structure of a disaccharide is displayed in Figure 1.1. Raw honey is a mix of

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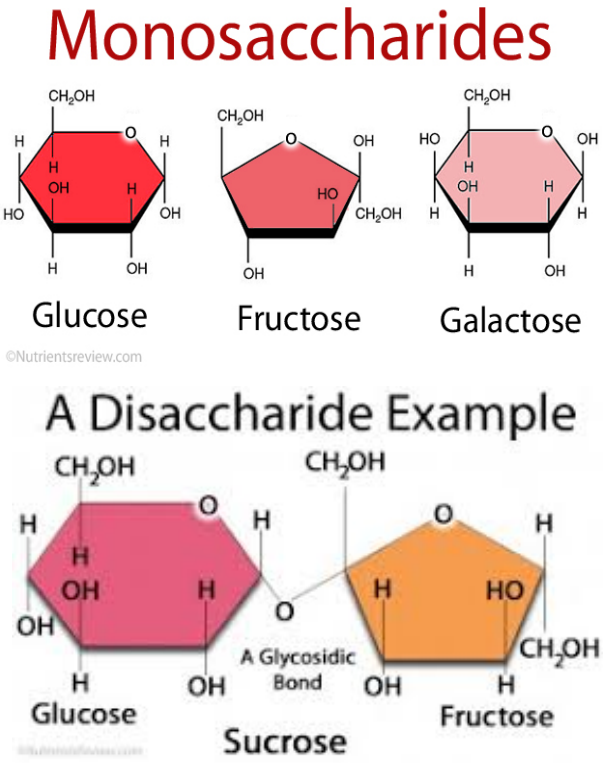
monomers glucose and fructose but in variable percentages based on a number of factors such as type of plant used for pollination and temperature at which the honey was formed (Missio de Silva et al. 2016). One of the main questions influencing research into this process is: How do different types of sugars impact the rate of ethanol production during yeast fermentation? Our hypothesis is that disaccharides will lead to fermentation producing ethanol at a slower rate because it takes longer to break the bonds down during anaerobic respiration. If our hypothesis is supported, the data will display that the anaerobic respiration of sucrose yields less average ethanol production in comparison to glucose or raw honey because there are no bonds to be broken before the monomers are utilized in fermentation. Alternatively, if our hypothesis is unsupported the data will display that there is more



ethanol produced during anaerobic respiration of sucrose in comparison to glucose or honey. **Figure 1.1** Displays the chemical structures of common monosaccharides and the disaccharide sucrose (Benscheidt 2017).

Methods

In order to test the effect of different sugars structures on rate of ethanol production, we created a solution with active dry yeast which we could perform fermentation. Three different sugars were tested: glucose, a monosaccharide, raw honey, a mix of monosaccharides glucose and fructose, and refined sucrose, a disaccharide composed of glucose and fructose. We chose these three sugars because they each represent differently bonded structures. Since glucose is the main sugar used during the process of glycolysis, it serves as a standard that we can compare the other two sugar structures to. Raw honey is composed of unbonded monosaccharides glucose and fructose which is compared to sucrose which is a disaccharide formed of bonded glucose and fructose. In order to evaluate the concentration of alcohol produced we measured the amount of ethanol emitted over the course of 10 minutes of yeast fermentation.

The ratios of each substance used to create each solution can be found in table 1.1. The mole ratio of each sugar was standardized with glucose and raw honey having a mole ratio of 0.3 M and refined sucrose having a mole ratio of 0.15 M.

Before beginning fermentation the ethanol probe was warmed for 5 minutes and we replaced the teflon tape on the end of the ethanol sensor. While we waited for the probe to warm up we made our yeast by following the process outlined in previous experiments (Shaw and French 2018). Then the sugar was added to begin the process of fermentation. Immediately upon adding the sugar the warmed up ethanol probe and a stopper was used to close the respiration chamber. The ethanol probe collected the output of ethanol in parts per mole (PPM) at an interval of 6 samples per minute over the course of 10 minutes, which was gathered by logger pro. The experiment was performed 5 times for each sugar and the rate of ethanol production was gathered and is displayed in Figure 1.1 by a box and whisker plot to clearly see the trends in data, and compare the tests to each other. To calculate the values used for Figure 1.1, using Logger Pro, calculated the slope of each run of each type of sugar, imported that data into Excel and generated a box and whisker plot. With the aggregated data we tested for normality and found that our data had no outliers. Because of this, we

chose to perform a one way ANOVA statistical test which is recorded in the results section.

Substance	Solution 1	Solution 2	Solution 3
Water	10.0 +/- 0.05 mL	10.0 +/- 0.05 mL	10.0 +/- 0.05mL
Yeast	0.60 +/- 0.05 g	0.60 +/- 0.05 g	0.60 +/- 0.05 g
Glucose	10.0 +/- .5 mL	0 mL	0 mL
Raw Honey	0 mL	10.0 +/- .5 mL	0 mL
Sucrose	0 mL	0 mL	10.0 +/- .5 mL

Table 1.1 Displays the amount of each substances used for the creation of three solutions for fermentation.

Results

Our trends and data display that glucose, compared to honey, has a lower variability and on average produced less ethanol over the time interval. When comparing sucrose to glucose however, the data for sucrose was more precise and produced more ethanol on average over the time interval. A One-Way ANOVA was conducted to compare the effect of sugar types on the production of ethanol in monosaccharides, disaccharides, and 50/50 mixture of the two. There was not a significant effect of sugar types on the production of ethanol between the three conditions; [$F(2,12) = 0.2687$; $p = 0.7688$].

Discussion

Our data displays that over the 10 minute interval, the refined sucrose produced slightly more ethanol than both the raw honey and glucose, however, not enough to produce a statistical difference. This data does not support our hypothesis and indicates the opposite of our

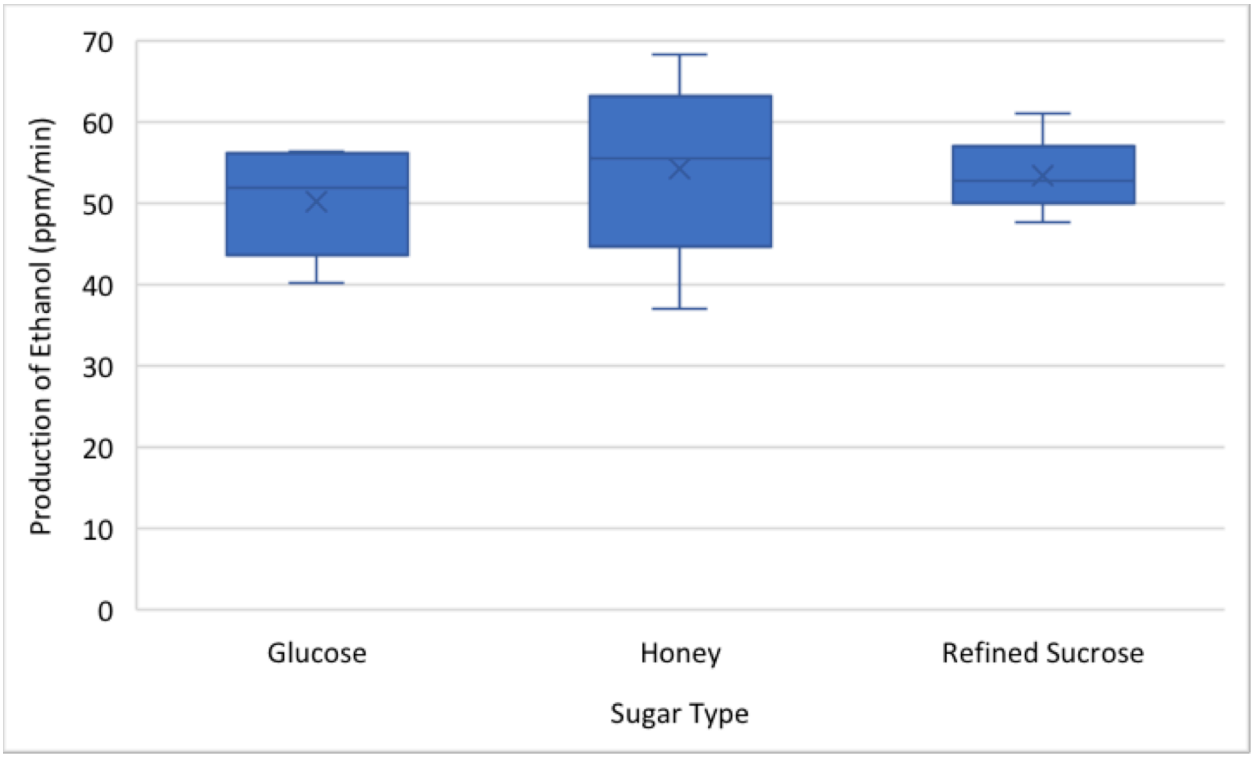


Figure 2.1 displays the production of Ethanol over the interval of 10 minutes for the three sugar types we tested. Our data displays a slight difference between the outputs of glucose and sucrose. However, the raw

predictions of rate of ethanol production.

When evaluating how this data influences the larger question of using different types of sugar during anaerobic fermentation of yeast, we can posit a few suggestions for breweries as well as questions for future research. Breweries looking to make drinks that have a higher alcohol content would be better served by using sugars that are composed of bonded disaccharides of sugar such as sucrose instead of a substance composed of monosaccharides like glucose. This is because the solutions composed of a mix of monosaccharides produce a lower amount of ethanol over the same time interval during fermentation when compared to sucrose. Our data brings into question the idea that there is a glucose concentration threshold, limiting the amount of glucose that should be used during yeast fermentation, and after that point excess glucose will not participate in yeast fermentation, or may even block further fermentation. This is consistent with current research that delves into the idea of 'osmotic stress' which occurs when the cell membrane blocks the transport of glucose into the cell when high concentrations of glucose are used during fermentation (Gomar et al. 2015). Sucrose, when broken down, is a combination of glucose and fructose, and although the molarity of the full solutions is the same, there is only 50% of the amount of glucose in sucrose when compared to the pure glucose solution.

Because that glucose is also in a bonded form, it would take the yeast longer to break down the bonds meaning respiration would occur for a longer period of time (Miller, 1957). We also know that in order for the cell to use fructose, it must first be converted into glucose, which is a process that normally occurs in the liver in humans. Obviously, the yeast we used does not have a liver and as a result this is a process that the yeast is unable to participate in. Instead, the yeast secretes an enzyme called invertase in order to break down the sucrose into its constituent monomers (Koschwanez, 2011). This could provide an explanation as to why the sucrose was also able to produce more ethanol, because after hitting the glucose threshold, the yeast then switched to using the fructose.

When evaluating our honey, which was a mix of unbonded monosaccharides fructose and glucose, there was variability in the data we recorded. This may have occurred because the makeup of honey can vary based off of a number of different factors. For example: the type of plant the bee grabbed pollen from, the environmental temperature the honey was produced in, any kind of refining that the honey was subject to (Missio de Silva et al. 2016). This means that the mixture is never a set 50/50 between glucose and fructose but could be shifted in either direction. Some honey also contain up to 10% disaccharides in their makeup depending on where it was sourced from. Applying our knowledge about glucose threshold, this means that depending on the honey, it could produces ethanol at different rates depending on its makeup.

If performing this experiment again, one could easily evaluate different types of disaccharides to compare if there is a difference in the rate of ethanol production and if there is a particular disaccharide which is more efficient for the alcohol industry to utilize. An experiment could also test if it was possible to reach the glucose threshold, experienced in our testing, when using a disaccharide. Two examples of definitive experiments that could be run are as follows: a. Comparing dextrin, a disaccharide of only glucose, to sucrose to see if the glucose threshold can be reached by disaccharides, or b. an experiment testing .6 M sucrose compared .3 M sucrose to see if one can reach the glucose threshold. One of the questions that could be evaluated by future research is how the differing types of disaccharides affect the rate of carbon dioxide production during yeast fermentation.This data in tandem with the ethanol studies would help better define and predict trends for how 'bubbly' or 'fizzy' a product would become when using different disaccharides for brewing.

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