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Yeast Fermentation: The Effect of Sugar Type on Ethanol Production

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The process of yeast fermentation has been used for alcoholic drinks for many years, and investigations have been conducted to determine how sugar type can affect the rate of ethanol production in the yeast. We suggested that using a natural sugar, such as glucose, and an artificial sugar, such as saccharine, would help us to determine how rates of ethanol production differ amongst this specific variable of sugar type. The water is the negative control variable because it contains no sugar so it is used as a good comparison. We conducted five trials using the yeast with glucose, saccharine, and water and compared the rates of ethanol production between the three groups. Our findings showed that glucose produced the most ethanol, and saccharine and water produces little to no ethanol. We believe that our research will be interesting to people that are involved in the making of fermented beverages because it offers new information that can add variety to the beverage-making industry.

Introduction

In cellular respiration, organic molecules are broken down and used as fuel for producing ATP, carbon dioxide, and water (Cain et al., 2016). Cellular respiration uses glucose to create the ATP, which is energy for the cell (Brown and Schwartz, 2009). This process can happen in different ways, including anaerobically, without oxygen, and aerobically, with oxygen. Specifically, fermentation is an anaerobic process that occurs during cellular respiration and breaks down sugars (Cain et al., 2016). Our goal was to determine why cellular respiration rates differ among cells. This leads to the question of why different types of sugars have different fermentation rates. To do this, we used glucose and saccharine to create mixtures with a *S. cerevisiae* yeast solution to test the rate of fermentation. Since ethanol is a byproduct of fermentation, we decided to focus on its production to determine the fermentation rate. The amount of ethanol produced within yeast can be manipulated by the type of sugar that is used to make it (Bauer et al., 2016). We decided to attempt our own take on this idea and use a natural sugar (glucose) and compare the results to that of an artificial sugar (saccharine). Glucose is a natural sugar because it is a product of photosynthesis and an input for glycolysis in cellular respiration. It is also a requirement for the production of ATP energy,

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which is necessary for life processes (Dienel et al., 2013). Saccharine is artificial because it is a sugar substitute that has similarities comparable to monosaccharides (Shaw and French, 2018). Subsequently, it has even been used by humans that do not want much sugar or calories in their diets, which reveals that it does not provide a significant amount of food energy (Bebbington, 1977). These factors are important to understand because differences in the variables are necessary for variety when fermented drinks are made (Shaw and French, 2018). When yeast is combined with both artificial and natural sugars, glucose will have a higher rate of ethanol production than saccharine because the structure of saccharine does not serve as a sufficient food source for the yeast.

If our expectations are supported, glucose will have the highest rate of ethanol production when mixed with the yeast, water will have no ethanol production, and saccharine will have some, but little ethanol production.

Methods

We tested the effect of natural vs. artificial sugar types on the rate of ethanol production in a *S. Cerevisiae* yeast solution because it is commonly used in the alcohol industry. Refer to Table 1 for information regarding specific sugar types and amounts used. Our negative control group was the yeast mixed with the water, because it is establishing a standard to test if saccharine is a more nutritious food source than water by itself. Our positive control group was the yeast and glucose because glucose is the input for glycolysis in cellular respiration, which proves that it is a good food source. Our experimental group was the yeast and saccharine because we are testing how the artificial sugar will affect the rate of ethanol production vs. that of the natural sugar.

To conduct our experiment, we used a turntable, a respiration chamber (250ml beaker), a graduated cylinder, and an ethanol probe. Refer to Table 1 for the solutions used combined with yeast. Refer to the standard protocol of *S. cerevisiae* yeast preparation on Canvas (Shaw and French, 2018). We warmed up the ethanol probe for 5 minutes before we placed it into the respiration chamber to collect the amount of ethanol given off of the mixture (ethanol production is in units of PPM). We conducted 5 trials of 7 minutes for each of the three groups (glucose, water, and saccharine). Our data was collected every 10 seconds. We then used Logger Pro to calculate the rate of change, which is represented in ppm/minute, of each test. The data collected was displayed by using a box and whisker plot to make it easier to see the difference in ethanol production among the solutions. To complete our experiment and our understanding of our results, we ran a One-way ANOVA stats test, using Past 3. We chose this test because we had nominal and measurement data categories represented within 3 different groups. This was followed by a Tukey's pairwise test to reveal more detail about our experiment.

	Control (+)	Control (-)	Experimental
Yeast (*S. Cerevisiae)*	10ml + 0.6g	10ml + 0.6g	10ml + 0.6g
Solution	10ml Glucose (0.3M)	10ml D.I H20	10ml Saccharine (0.15M)

Table 1. Materials used for our experiment and the amounts of each solution used.

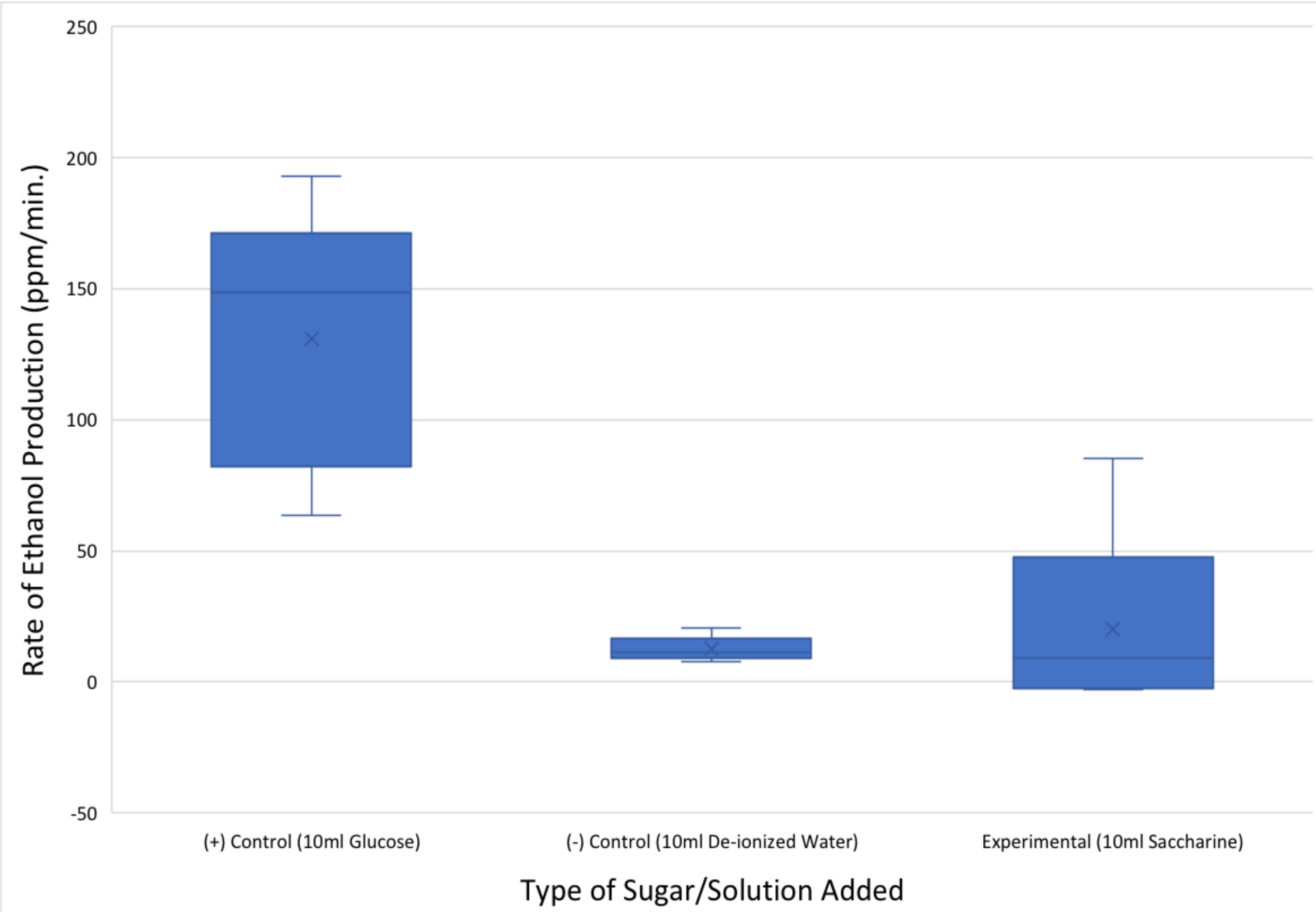

Figure 1. Effect of sugar/solution added (refer to Table 1 for more information about solutions) on the rate of ethanol produced by yeast. The water and the saccharine had the lowest rate of ethanol production, while the glucose had the highest (N = 5).

Results

Our results showed that glucose yielded a much higher production of ethanol than saccharine and water. The average rate of ethanol production when glucose was added was 6 times greater than when saccharine was added and was 10 times greater when water was added. The average rate of ethanol production after glucose was added was 131.026. It was 12.3276 after water was added and 19.8766 after saccharine was added. Saccharine and water's average rates of ethanol production were extremely close to one another, but saccharine had more variation than water did. Refer to Figure 1 for more information about the variation and trends we observed in our experiment.

A One-Way ANOVA was conducted to compare the effect of type of sugar added on the rate of ethanol production in artificial sugar, glucose, and water conditions. There was a significant effect of sugar type on the rate of ethanol production between the three conditions; [$F(2,12) = 17.01$; $p = 0.000314$]. A Tukey's pairwise test revealed that the rate of ethanol production was significantly greater in the positive control than the negative control [0.0005915], and in the positive control than the experimental group [0.001022]. There was no statistical difference between the negative control than the experimental group [0.9416].

Discussion

Yeast respires at different rates when certain variables are manipulated, such as the sugar type added to the yeast. We found that the glucose had the highest rate of ethanol production compared to both water and saccharine, which supports our hypothesis. Our statistics test also supported our hypothesis, showing that there was a significant difference in ethanol production between glucose and both water and saccharine. However, there was not a significant difference of ethanol production between the water and the saccharine. This is because neither water or saccharine serve as fermentable food sources for the yeast and cannot be metabolized as quickly as glucose since it is the most natural, raw sugar. A similar investigation found comparable results with glucose, stating that glucose had the highest rate of ethanol production between other sugars, fructose and sucrose, when added to *S. cerevisiae* yeast (Hu et al., 2004). This adds more support and credibility to our team's investigation because glucose still served as the quickest metabolised sugar. One variation that our team observed while performing our experiment is that on our first day, we conducted one trial with saccharine and found out the next week that our results varied extremely. The reason for this variation is because the first saccharine we used was found to have quite a bit of glucose in it. Therefore, our professor changed the saccharine to a type that does not contain glucose, and the variations in our experimental design diminished. Our results could also be used to answer the question of which sugars are most beneficial for consumption because we observed that the natural sugar was found to be fermented while the artificial one was not.

For future research, different sugar types and amounts could be tested to further knowledge on variations that may occur during the production of alcoholic beverages. For example, the fermentation of fructose could be observed in different amounts. This is important because fructose is known to cause many different, odd tastes in wine, and not many studies have been conducted on this issue (Hu et al., 2004). It could be possible that different amounts of fructose could lessen these odd tastes, and this research could improve wine-making because new, better-tasting beverages could be created with fructose for consumers to enjoy.

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