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PLEASE DON'T BE SALTY: INVESTIGATING THE EFFECTS OF SALT CONCENTRATION ON YEAST CO_2 PRODUCTION DURING FERMENTATION

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In the absence of oxygen, microorganisms have evolved to metabolize salt through the process of fermentation. We hypothesized that a high salt concentration would result in a higher carbon dioxide production in yeast because the yeast will create more metabolites to handle the stress of additional salt. We found that the hypothesis was not supported by the results of our experiment. We found that the rate of fermentation failed to follow a trend and was inconsistent at best, first increasing with the addition of the salt solution then decreasing as the concentration increased.

Introduction

Fermentation causes yeast to produce carbon dioxide in metabolic reactions, and sugar compounds have the capability to cause different outcomes in that change the production of carbon dioxide (Jones and Greenfield 1982). This source goes into depth about the effects of carbon dioxide in correlation with metabolic reactions, including alcoholic fermentation. The sugar components are broken down in glycolysis of fermentation and turned into energy, carbon dioxide, and ethanol (Hoefnagels 2018). This is why glucose levels are important to fermentation in our research. Glucose is the most efficient sugar to use because it does not need energy to break down a six-carbon molecule (Piškur 2006). This could change how concentrations of glucose impact the amount of energy created. Because glucose itself has a significant impact on fermentation, we chose not to compare it to other sugar compounds for the sake of finding more specific information.

Fermentation is very common among organisms, especially in yeast *Saccharomyces cerevisiae.* We looked at a study that used this same type of yeast to test the differences in glucose and fructose in alcoholic fermentation (Liccioli et al. 2011). We wanted to recreate this study, but more specifically, find how dilutions of glucose effected fermentation. This study was helpful in our investigation because it showed that sugars, regardless of composition, have some effects on fermentation. In addition, we looked at another study involving carbon dioxide in fermentation. It was noted that with higher levels of carbon dioxide produced, there was stronger fermentation activity (Chen and Gutmanis 1976). These studies relate because we want to look at glucose dilations and

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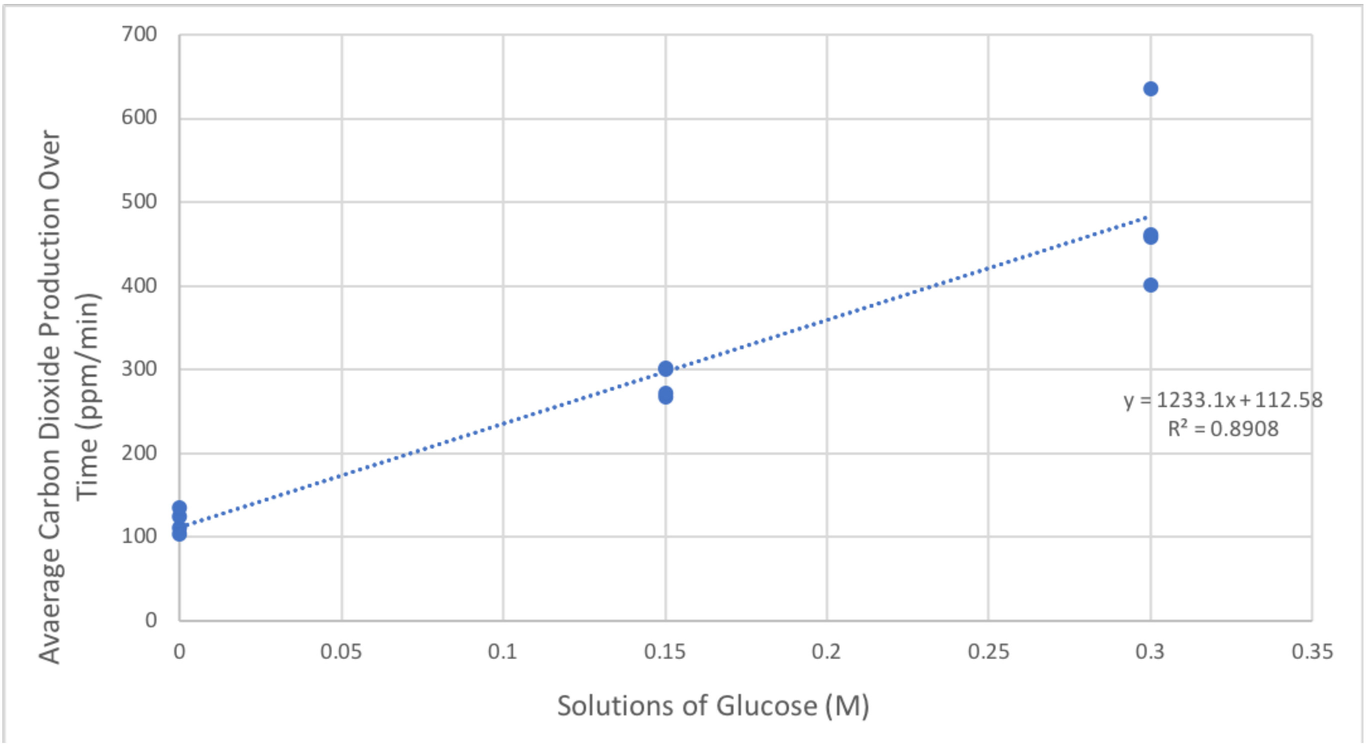

Figure 1. There is significant positive correlation between increased glucose molarity and carbon dioxide production over time in fermentation.

how they affect fermentation, which causes carbon dioxide levels to either increase or decrease. We were curious as to how different dilutions of glucose effected the production of carbon dioxide. Wine production was a real-life example that could be drawn from because we know that wine creates bubbles, or carbon dioxide. This production causes more bubbles to be created in the wine. We asked the question: What effects do glucose solutions have on yeast's production of carbon dioxide? We hypothesized that higher concentrations of glucose will cause the yeast to produce more carbon dioxide because, in fermentation, the more glucose available for consumption by the yeast will cause a higher metabolism and higher carbon dioxide production. We predict that the production of carbon dioxide will increase with increased levels of glucose in the yeast solutions. Because of this, we believe that the strongest glucose concentrations will produce more carbon dioxide.

Methods

We manipulated the different solutions of glucose to find carbon dioxide production over time. Our experimental group consisted of two different types of solutions: one with .3 M glucose and one with .15 M glucose. We compared this to our negative control group that did not contain glucose and only consisted of water because it had not been manipulated.

To conduct this experiment, we started by plugging in the Carbon dioxide sensor into the computer and left it for five minutes to warm up. While the sensor warmed up, we began to measure 0.6 grams of yeast in a weigh boat. We also put 10 mL of water into a graduated cylinder. After weighing the yeast and measuring the solution, we

turned on the mini mag stir plate and placed a beaker on top of it. We put the 10 mL of water inside of the beaker, then we added the stir bar to begin mixing the yeast solution. We then placed the different glucose solutions into the yeast solution. For our first trial, we added 10 mL of the glucose solution which had 0.0 Molarity, or just water. Once we put a glucose solution into the yeast solution, we put the carbon dioxide sensor on top of the beaker and logger pro collected the data in ppm (parts per million). We collected data every 15 seconds, and the data was collected for eight minutes for each trial. We repeated our experiment for glucose with

10 mL solutions of .3 Molarity and .15 Molarity. We ran four trials for each solution, creating a total of twelve trials. To share the results of this experiment, we transferred the data onto a spreadsheet. We then used a scatter plot graph that shows a line of best fit. We used the differences of carbon dioxide production as our points on the graph. Because we have all measurement data, we chose a correlational statistical test.

Results

The production of carbon dioxide over time steadily increased as the amount of glucose within the solution of yeast also increased. The carbon dioxide levels increased faster for the solutions with the highest molarity of glucose as well (see Table 1). A scatter plot graph is used to accurately represent this because it can show the line of best fit through all points and trials for each concentration of glucose (Figure 1). Trends are shown through the line of best fit on the graph.

Table 1. *This table displays the average production of carbon dioxide per minute for four trials of each solution that contained different molarities of glucose.*

Molarity of Glucose (M)	Average Carbon Dioxide Production (ppm/min)
118.625	0.0
285.46875	.15
493.0625	.3

The groups' glucose levels and carbon dioxide production over time were strongly correlated, r(11)=.94383, p=.0000040042.

Discussion

As we hypothesized, our data was consistent with our hypothesis that the presence of higher concentrations of glucose had a positive effect on carbon dioxide production because there was a noticeable increase of carbon dioxide in fermentation. As shown in Table 1, our data suggested that increased glucose molarity in solutions of yeast and carbon dioxide production over time have a significant positive correlation. There is a noticeable outlier in our data for our glucose solution with .3 M. This could be because of the rate of fermentation for the yeast in that particular solution that could be caused by the fluctuating temperature of the glucose as it was warming to room temperature despite waiting an appropriate amount of time for testing. Alternate results to our research could have been caused by differences in the initial carbon dioxide levels of each trial. However, the data follows our hypothesis despite difference because we took averages. Furthermore, our hypothesis was supported because the p-value was less than .05, as shown in our correlational statistical analysis.

In future research, the data can be compared to more trials of different dilutions of other sugars like raw and refined sugar. Other studies can look at the duration of data collected for fermentation. As Holcberg and Margalith suggest in a study they have done, the durations of fermentation also affect the production of carbon dioxide (Holcberg and Margalith 1981). This is a significant way to look at further research because one can look at the same experiment over that longer period of time and compare it to the data already collected in this study. This could possibly mean that the different solutions of glucose can have different outcomes of carbon dioxide production if the study looked at it over a period of weeks.

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