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Rate of Yeast Fermentation Production of Differing Carbohydrates: Monosaccharides vs Disaccharides

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Numerous experiments have been done to look at the rate of fermentation of glycolysis in Saccharomyces cerevisiae, which is a type of yeast, and different carbohydrates. This led us to wonder if the difference of monosaccharide or disaccharide solutions affect the process of fermentation. Our hypothesis stated, monosaccharides of glucose and fructose in honey will metabolize and produce CO_2 faster than a disaccharide solution of glucose and fructose in refined sucrose because monosaccharides are already broken down into smaller sugar units. We tested this by having a positive control group of glucose and a negative control group of water in the yeast recipe from the lab manual directions. We ran three trials comparing the rate of carbon dioxide production for the four solutions. We predicted the monosaccharide solution will have a higher metabolic rate; however, statistically our hypothesis and prediction were wrong. We found that statistically speaking, solutions of monosaccharides and disaccharides are about the same.

Introduction

The understanding of fermentation among yeast and different types of sugar begin with the process of anaerobic respiration and aerobic respiration. Anaerobic respiration allows glycolysis to perform without the input of oxygen, while aerobic respiration is with oxygen (Angustia, et al., 2013). Fermentation happens when yeast or other microorganisms metabolize in different types of carbohydrates. The waste products of glycolysis is carbon dioxide and alcohol ethanol production. The Crabtree Effect happens when a high input rate of sugar is added to yeast. The process of glycolysis significantly speeds up, making NADH+ rapidly. When this happens, glycolysis directly goes into fermentation instead of the Krebs and Electron Transport Chain to recycle the NADH+ back into the cycle. It does this so it can to continue to keep up with the input of sugar and respire at rapid speeds (De Deken, 1966). The ethanol, which is the second product, is produced as the result of the cells regenerating NAD+ in the attempt to maintain the glycolysis process and make enough ATP (Walker, 2004).

Saccharomyces cerevisiae, which is a type of strain of yeast fermentation found in several things such as bread, soy sauce, alcoholic beverages, African indigenous fermented foods, and has been found to have different probiotic effects (Van der Aa Kuhle, Skovgaard, and Jespersen, 2005). However, the process of fermentation can happen with different types of sugar. For example,

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the wine industry can manipulate the type of sugars that are mixed with yeast to hopefully produce a higher metabolic rate giving off higher amounts of alcohol ethanol and carbon dioxide (Shaw & French, 2018). In having a higher metabolic rate, the wine will essentially be more bubbly. You can manipulate the sugar content by looking at if it is made up of monosaccharides or disaccharides. Monosaccharides contain one unit of sugar and are building blocks for disaccharides, while disaccharides are two sugar units linked together. Disaccharides makes glycolysis take an extra step of breaking down the sugar into a simple monosaccharide to be transported to metabolize.

Although the fermentation is still rapid, adding an extra step for disaccharides takes more time than the already broken down monosaccharides with one sugar unit. Since it takes more time for disaccharides, the metabolic rate is slowed down slightly and makes the production of carbon dioxide decrease (Angustia, et al., 2013). A carbohydrate containing only monosaccharides is glucose. Honey is a 50/50 mix of glucose and fructose monosaccharides and refined sucrose is disaccharides of glucose and fructose. Wondering what type of sugar concentration fermented the best, made us ask ourselves which type of solution will lead higher carbon dioxide production. This question led us to hypothesize that the monosaccharide solution of honey will have a higher metabolic rate, giving off more carbon dioxide production from fermentation than a disaccharide solution of refined sucrose because monosaccharides are already broken down into smaller sugar units. We predict the monosaccharide solution of honey will have a higher rate of carbon dioxide production than the disaccharides of sucrose. However, we predict that our control group of pure glucose will have a highest rate of respiration out of all the solutions. Although, if our hypothesis and prediction are incorrect the results of the rate of carbon dioxide could come out to be equal between monosaccharide and disaccharide solutions, or refined sucrose of disaccharides could have the higher rate of carbon dioxide production.

Methods

For our experiment, we compared the fermentation rate of glycolysis and different types of carbohydrates in Saccharomyces cerevisiae, which is a specific type of yeast. We used this type of yeast because it is commonly known in commercial stores. It is also well studied, so the fermentation of this type of yeast is well known. We compared monosaccharides to disaccharides in the carbohydrates of honey and raw sucrose as our experimental groups. Honey contains a 50/50 mix of monosaccharides glucose and fructose, while 100% refined sucrose contains disaccharides of glucose and fructose. When comparing the fermentation rate of our two solutions, we will be looking at the product of carbon dioxide produced. By looking at how much carbon dioxide is produced, you can compare the metabolism for each carbohydrate solution. Therefore, our independent variable is the type of solutions used and our dependent variable is the fermentation rate of carbon dioxide.

We have a positive and negative control group for our experiment as well. The negative control group is the group containing the constant 10 mL of water and 0.6g yeast with the addition of another 10 mL water to make the volume a total of 20 mL. This is negative because it does not have any sugar solution added to it. On the other hand, our positive control group had 10 mL glucose added to the constant 10 mL water and 0.6g yeast to make up the total volume of 20 mL. This control is described as positive because it contains a simple sugar solution of glucose, which is a monosaccharide. Our experimental groups were honey and refined sucrose and the dependent variable we measured is carbon dioxide. We chose these because they both only contain glucose and fructose and the only difference is that honey is made of up monosaccharides and refined sucrose is made up of disaccharides. Therefore, it allows us to focus and compare the difference in monosaccharide and disaccharide carbon dioxide production from honey and refined sucrose in aerobic respiration. All of our control groups and experimental groups along with their amounts of solution added is shown below in table 1.

We designed our experiment by using a carbon dioxide probe to measure the amount of carbon dioxide produced from 0.6g yeast, 10 mL water, and 10 mL of an additional solution in a respiration chamber. First, we got out the carbon

	Negative Control	Positive Control	Exp. Group 1	Exp. Group 2
Yeast	10 mL H2O + 0.6g yeast	10 mL H2O + 0.6g yeast	10 mL H2O + 0.6g yeast	10 mL H2O + 0.6g yeast
Solution 1	10 mL H2O	10 mL glucose	10 mL honey	10 mL refined sucrose
Volume	20 mL	20 mL	20 mL	20 mL

Table 1. Shows the type of solution and amount of yeast used for each experimental and control group

dioxide sensor probe and plugged it into the logger pro system. The probe must be turned on and warmed up for five minutes before using it in your experiment. We then followed the exact instructions in the canvas protocol for making the yeast solution (Shaw & French, 2018). Then, we measured our yeast in a weigh boat and placed it on a balance to get precisely 0.6 grams. Following, we measured out exactly 10 milliliters of water in a graduated cylinder and poured both the water and yeast into the respiration chamber. Next, we placed a magnetic stir bar in the center of the respiration chamber and turned it on to start spinning the yeast and water solution for exactly 5 minutes. For each control or experimental group, we added the correct 10 mL of either water, glucose, honey, or refined sucrose into the respiration chamber. We then placed the carbon dioxide probe inside of the chamber and turned on our Logger Pro system for 8 minutes to measure the carbon dioxide production in ppm. We made sure to have the speed of the stir station on low, so the solution would not splash up on the side and get the sensor probe wet.

The Logger Pro system collected 33 total samples during the 8 minute time lapse and created a graph. From these samples and graph, we collected the rate of change in carbon dioxide production (ppm), the median, and minimum and maximum values. This allowed us to interpret our results correctly. We put this data into four different box and whisker plots in order to show the spread of our data for each type of control and experimental groups. In addition to the box and whisker plot, we used our data to run a statistical test in order to look for differences in the means between our different sugars. By testing for normality, we found that our data was normally distributed. We then decided to use a One-way ANOVA test, followed by a Tukey's Pairwise test.

Results

As the data is shown in Figure 1, the rate of carbon dioxide production for our positive control group, and two independent variables varied only slightly. The solution of glucose had the highest average slightly for the metabolic rate because it was pure monosaccharides. The mean from our three trials for glucose was 1174.3 ppm/min. Next, honey followed close behind with the next highest metabolic rate, giving off the most waste production of carbon dioxide of 1110 ppm/min. The solution of refined sucrose had the lowest rate of production from the carbohydrates, which was 1008.9 ppm/min. Finally, the production if carbon dioxide of water was 345.2 ppm/min. The production of the three different types of sugars were about 3 times higher than our negative control group, water.

Figure 1. also displays the interquartile range, which is the middle 50% of the data, and the minimum and maximum values for each solution. Honey had the widest spread of data shown by the interquartile range, which was 306.5 ppm/min. While, water had the lowest spread of 60.9 ppm/min. Glucose had a spread of 77.5 ppm/min, and refined sucrose 87.35 ppm/min. Therefore, honey had the biggest variation in its data. Honey also had the highest maximum value of 1443 ppm/min, while water had the lowest minimum value of 300.2 ppm/min.

The results of the box-whisker graph show that there is a slight relationship between different carbohydrates and fermentation production. However, the difference is not statistically strong

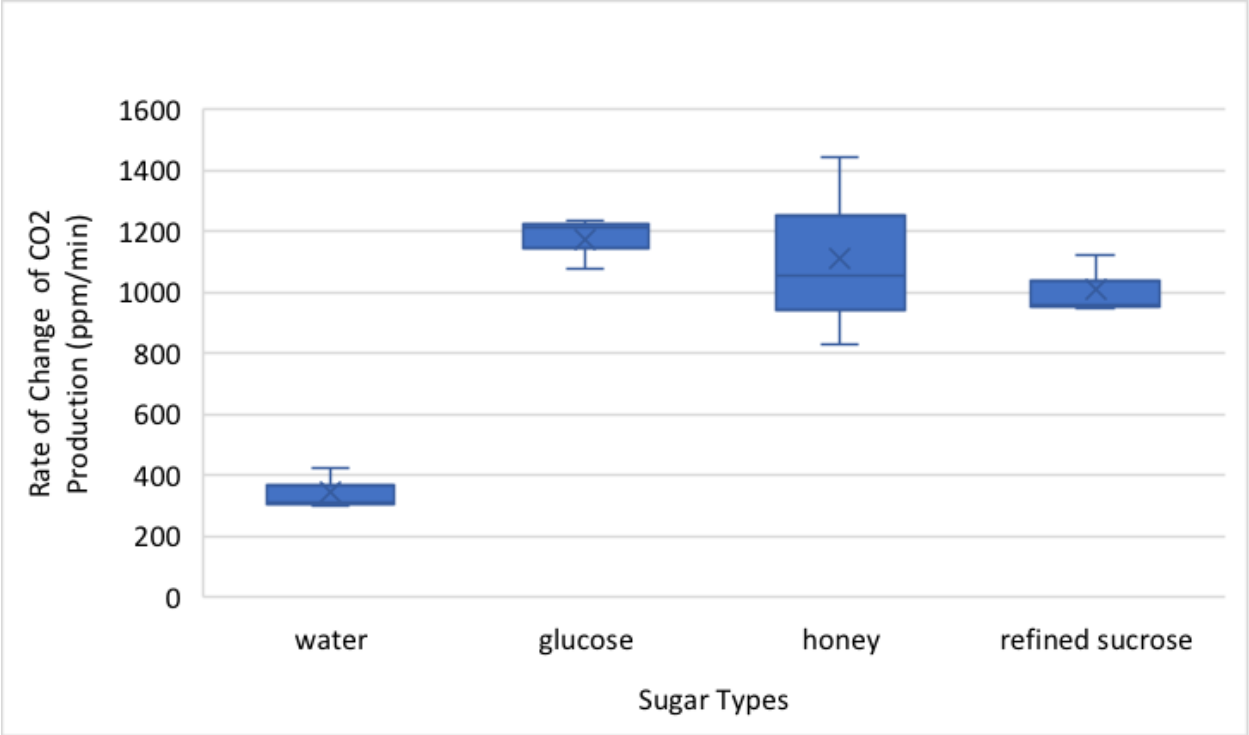

Figure 1. Shows the rate of carbon dioxide production of fermentation in each solution. Trends show there is hardly any difference in CO_2 production in carbohydrates. But they are almost 3 times more productive than water.

enough to prove monosaccharides and disaccharides make a difference in fermentation from yeast.

Along with our graph, we conducted a statistical test. A One-way ANOVA was conducted to compare the effect of different sugar types on the production of carbon dioxide in water, glucose, honey, and refined sucrose conditions. There was a significant effect of sugar types on carbon dioxide production between the four conditions; [F (3, 8) = 15; p=0.001199]. A Tukey pairwise test revealed that carbon dioxide production was statistically lower in water than in glucose (p=0.001558), honey (p=0.002623), and refined sucrose (p=0.006272). There was no statistically significant difference between glucose, honey, and refined sugar.

Discussion

Our hypothesis and prediction of monosaccharides having a high metabolic rate is incorrectly supported by the data from our results. Our experiment was conducted to find the rate of change in carbon dioxide production which answered our question about whether or not monosaccharides or disaccharides have a higher rate of fermentation. From the results of the One-way ANOVA test and Tukey's pairwise test, we found that the water was significantly different from the other sugars tested because it has less effect in yeast fermentation than a sugar does because it is a pure substance. Since the statistical tests also showed that there were no significant differences between each type of sugar, it disproved our hypothesis that monosaccharides have a higher metabolic rate than disaccharides.

A speculation we made about an experimental error is the amount of time we used to collect data. We originally collected data for 8 minutes. After looking at our results, we speculate that we should have collected the CO_2 longer because yeast fermentation is normally over a longer period of time. For example, yeast fermentation takes weeks for making alcoholic beverages. An alternate interpretation we speculated was that the glucose concentration was too high, therefore, the carbon dioxide production could have possibly maxed out. Our glucose began to max out; Furthermore, when the concentration of glucose is too highly concentrated for a longer period of time,

it can cause a significant decrease in the performance of fermentation (Verstrepen, et al., 2004). If we were to do this experiment again, we could dilute the glucose solution to see if it makes a difference in the rate of monosaccharides and disaccharides. During our experiment we realized that fermentation can be affected by many aspects. However, monosaccharides and disaccharides do not affect it that significantly. For future research, you could change variables such as, temperature, osmotic pressure, presence of oxygen, time, nutrient supplementation, and concentrations of solutions (D'Amore, T. 1992). We hypothesis for future research, Saccharomyces cerevisiae fermentation rate of different types of carbohydrates change when they have a longer time to go through fermentation because specific sugars have different effects on metabolic rates.

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