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Sugar, sugar, oh, honey, honey: Variable rates of ethanol gas production in honey, refined sucrose, and glucose when combined with yeast

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Abstract

Understanding the fermentation of yeast is critical to the production of alcoholic products. By analyzing the impact of sugar components in aerobic fermentation, differences in the rate of ethanol production can be observed. Specifically, the difference between monosaccharide and disaccharide solutions as well as the difference between monosaccharide concentrations of different stages in glycolysis is something that has not been fully established in ethanol production. In this study, the rate of ethanol gas production was compared between honey, refined sucrose, and glucose experimental conditions. It was found that there was not a significant difference in the rate of ethanol gas production as a result of glyosidic bonding between similar monosaccharide compositions in honey and refined sucrose. Additionally, there was not a significant difference in the glucose and honey group's rate of ethanol gas production.

Introduction

The production of alcoholic-based drinks has been an established practice of human culture before the beginning of written history (Guerra-Doce, 2015). Alcohol has primarily been produced through the fermentation of plants. Yeast, a fungus, is an example that has been commonly used to produce ethanol through fermentation. Fermentation is a metabolic pathway that occurs under anaerobic conditions that breaks down pyruvate into ethanol, adenosine triphosphate (ATP), and carbon dioxide (Bauer et al., 2016). Yeast undergoes fermentation and produces ethanol as a result of this pathway; however, it has also been shown that under aerobic conditions, yeast produces higher rates of ethanol which has been named the Crabtree effect (De Deken, 1966).

Current research in fermentation has studied the effect of temperature, salt concentration, and caloric density on the amount of ethanol production in yeast, yet the impact of different sugar types has not been fully developed (Kim et al., 2013; Lin et al., 2012; Bauer et al., 2016). It is believed that by using saccharide molecules that are

commonly found within glycolysis, it is possible to increase the rate of pyruvate production, and thus, ethanol production through fermentation (Oomuro et al., 2018).

Due to the fact that glucose is converted to fructose in glycolysis, we hypothesize that honey, which contains a mixture of monosaccharides glucose and fructose, will present a higher rate of ethanol gas production when compared to glucose and refined sucrose. This is because sucrose, which is a disaccharide of glucose and fructose, must be broken down into its monomer components whereas honey can bypass this process. Additionally, glucose must be converted into fructose, whereas honey can again partially bypass this to produce ethanol gas at a higher rate. Specifically, if the addition of honey to yeast yields a higher rate of ethanol gas production compared to the other sugar types, then it can be shown that our hypothesis is supported; however, if a lower rate of ethanol gas production is depicted due to honey, then the hypothesis should be rejected. By comparing the rates of ethanol gas production to a negative control of water and yeast, the impact of differences in sugar molecules can be quantified.

Methods

In a controlled laboratory setting, three sugar types were chosen to test the hypothesis: glucose (a monosaccharide), honey (a 50/50 mixture of monosaccharides glucose and fructose), and refined sucrose (a disaccharide of glucose and fructose). A controlled laboratory setting was used in order to stabilize the variables of changing temperature and rainfall. A negative control was also used that is composed of water and yeast without a sugar condition as yeast requires glucose molecules to undergo glycolysis and will not produce ethanol without sugar molecules. These groups were chosen as there are similarities of molecular structure, glucose and fructose, with different conformations of disaccharides and monosaccharides to compare the differences in metabolic pathway. Sucrose and honey were chosen specifically as the impact of the breakdown of the disaccharide bond in sucrose can be compared to a mixture of two monosaccharides as they are both composed of the same monomers. We chose to measure the rate of ethanol gas produced by each sugar solution after 7 minutes.

0.6 g of yeast was combined with 10 mL of DI water in a respiration chamber and was mixed using a magnetic stir rod for 5 minutes. After initial mixing, 5 mL of each sugar experimental condition was diluted with 5 mL of DI water and was added to the respiration chamber, and an ethanol sensor was inserted to measure the concentration of ethanol gas. To maintain constant monosaccharide concentrations, we used sugar concentrations of 0.075 M for the refined sucrose groups, and sugar concentrations of 0.15 M for fructose and honey groups since the disaccharide contains two monosaccharide groups per molecule. The ethanol sensor was warmed up for five minutes before the experiment and was set to measure in ppm. The concentration of ethanol gas in ppm was measured for 7 minutes and was repeated among each experimental group for 4 trials as a result of the changing sugar conditions. The data was displayed using box and whisker plots and the mean rates of ethanol gas production were displayed on the graph.

A one-way ANOVA was used to compare the experimental conditions to determine if there was a significant change as a result of sugar types. This test was chosen as there are three conditions, two experimental conditions and a negative control, being tested for each comparison group. These groups being the impact of fructose concentration in monosaccharides, and the impact of disaccharide compared to monosaccharide concentrations involving the same monomeric groups. Using a standard alpha value of 0.05, 4 trials (n=4) were recorded and compared for each condition. Since a one-way ANOVA does not show significance among specific conditions, a post-hoc Tukey test is conducted after a significant one-way ANOVA test to determine which groups were significant.

Results

Figure 1 shows that there was a larger difference in mean values between glucose and honey, with glucose having a higher average rate of ethanol gas production of 0.71 0.11 ppm/sec, while honey had a rate of 0.63 0.18 ppm/sec. The negative control water group was constant between Figure 1 and 2, which expressed a rate of 0.15 0.01 ppm/sec. Honey showed a larger range compared to glucose, and both were more spread out compared to the water group.

Figure 2 visually shows that there was not a much difference between honey and refined sucrose, but there was a large difference between the negative control, water. Specifically, the mean values were 0.63 0.18 ppm/sec for honey, 0.61 0.25 ppm/sec for refined sucrose, and 0.15 0.01 ppm/sec for the water group. The refined sucrose had a larger range than the honey experimental condition, but both groups were further spread compared to water, which did not have much variance

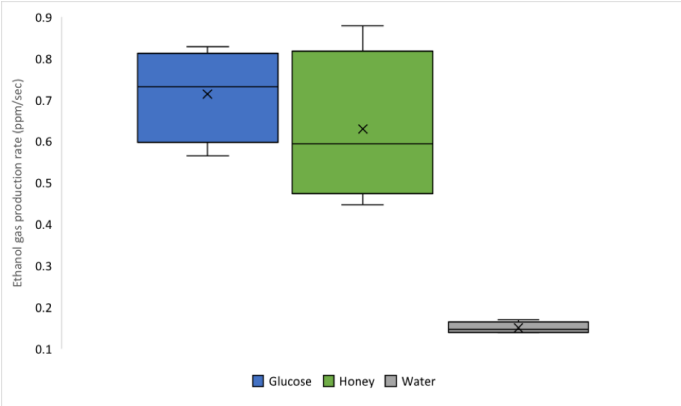

Fig 1. A box and whisker plot of varied fructose concentration groups in monosaccharide solutions. The means and standard deviation for the glucose, honey, and water groups were 0.71 0.11 ppm/sec, 0.63 0.18 ppm/sec, and 0.15 0.01 ppm/sec respectively.

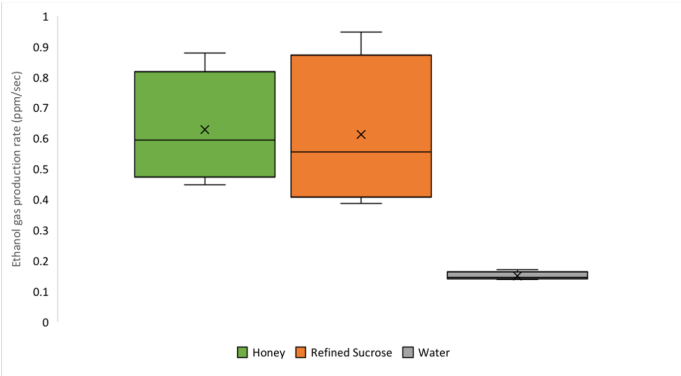

Fig 2. A box and whisker plot of monosaccharide and disaccharide groups involving similar monomeric components. The means and standard deviation for the honey, refined sucrose, and water groups were 0.63 0.18 ppm/sec, 0.61 0.25 ppm/sec, and 0.15 0.01 ppm/sec respectively.

between trials.

To show if there were significant differences between the two groups, two one-way ANOVA tests were conducted separately. The statistical test between honey, refined sucrose, and water was significant with a p value of 0.0065. Additionally, the statistical test between glucose, honey and water were shown to also be significant with a p value of 0.00026. Since these were both significant to the alpha value of 0.05, post-hoc Tukey tests were conducted to determine which group was significant. It was found that there was not a significant difference between the glucose and honey group as it displayed a p value of 0.61, but both groups were significant to the water control having p values of 0.001 for both comparisons. The post-hoc Tukey tests between the other testing group provided similar results. The comparison between honey and refined sucrose was not significant with a p value of 0.90, while the honey to water and refined sucrose to water provided significant p values of 0.011 and 0.013.

Discussion

It was observed that honey, refined sucrose, and glucose all produced variable rates of ethanol production, which does not support our original hypothesis. When comparing honey to glucose, the results were insignificant due to an unstable range of ethanol production. The same was recorded when comparing honey to sucrose. However, it was found that glucose produced more ethanol gas than refined sucrose and it is believed to be a result of differences in molecular structure. Since glucose is a monosaccharide and sucrose is a disaccharide of glucose and fructose, it was found that the breakdown of the glycosidic bond that holds the monosaccharides together was a slower process than the conversion of glucose into fructose during glycolysis (Oomuro et al., 2018). Additionally, the negative control without a sugar additive showed the lowest amount of ethanol production, which was expected due to a lack of energy source (Lin et al., 2012). Other interpretations of the data could conclude that fructose does not play a vital role in the rate of ethanol production during fermentation. This could be attributed to the fact that glucose showed a more stable rate of ethanol production when compared to solutions containing fructose.

One limitation was due to the fluctuating temperature of the room. Since yeast productional of ethanol is dependent on temperature, the temperature of the room decreased over the data collection period and it may have led to inaccuracies in trial comparison as a result. This was not due to researcher error as we had no control over the ambient temperature.

Our study can be directly related to the production of alcohol through the fermentation of yeast. In a real-life setting, brewers who want to increase the amount of ethanol produced during fermentation should use a monosaccharide solution similar to glucose in order to maximize the rate of ethanol production. Future researchers should explore the production of ethanol using other monosaccharide solutions or disaccharides such as high fructose corn syrup. The differences in glucose and fructose compositions as well as other types of monomers may influence the metabolic pathway and lead to differences in ethanol produced via fermentation.

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