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Yeast's Sweet Tooth: Natural Sugars Result in Greater Ethanol Production in Saccharomyces Cerevisiae than Processed Sugars

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Fermentation is a form of anaerobic respiration that breaks down carbohydrates to produce energy and makes ethanol as a byproduct. The aim of this study was to determine how sugar type , natural vs. artificially processed, impacts the rate of ethanol production in the fermentation of yeast. This particular study addressed this in a unique way by comparing the rate of ethanol production between glucose and saccharine in search of a correlation in the data. In this experiment, a stir station was used to promote fermentation in a beaker containing yeast, water, and a sugar solution. Results from this study showed that the average rate of change in ethanol production when glucose was used was greater than the average rate of change in ethanol production when saccharine was used. This may be beneficial to producers of alcoholic beverages who are aiming to maximize efficiency in fermentation.

Introduction

A farmer is interested in how manipulating raw and processed sugar to produce more ethanol may potentially help her quest to ferment wine and soy sauce. This has led to the exploration of how the use of raw sugar may impact fermentation and the rate of ethanol production when compared to the use of processed sugar. Fermentation, which is a type of anaerobic respiration, refers to the process by which ATP is produced through the break down of carbohydrates in an environment lacking oxygen (D'Amore, 1992). During this process, ethanol is produced as a waste product (Bauer, 2016). Ethanol levels are a good indication of the rate of fermentation and thus the metabolic rate. With regards to sugar types, raw sugar is typically harvested and then boiled once in production, so it has a higher molasses concentration than processed sugar, which is boiled multiple times and has a low molasses concentration (Baikow 2013). For example, saccharine has no caloric value while glucose has a caloric content (Carper, 1953). The higher molasses contents in raw sugars result in

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increased ethanol production in fermentation because molasses increases the caloric content of the sugar. In a study of similar nature, the use of corn sugar yielded the highest rate of ethanol production while the use of Stevia resulted in the lowest rate of ethanol production. In this study, corn sugar had the highest caloric content while Stevia had the lowest, suggesting sugars with higher caloric content increase the rate of fermentation and overall ethanol production (Bauer, 2016). Based on this research, it was hypothesized that glucose will produce more ethanol in fermentation than saccharine because natural sugars, like glucose, have a higher caloric content allowing for more efficient metabolism. The hypothesis would be supported if glucose does in fact result in a greater rate of ethanol production (ppm/min) than saccharine.

Methods

In this experiment, the independent variable was the type of sugar, either the processed sugar saccharine or the raw sugar glucose, and the dependent variable was the rate of ethanol production (ppm/min). There was no control group because it was a correlational study comparing the rate of ethanol production between the raw or processed sugar. The experimental group included both the glucose and saccharine trials because both types of sugar were being tested for the amount of ethanol produced.

Prior to testing, the tape on the ethanol probe was replaced to prevent moisture from getting into the sensor. The probe was then plugged in and given 5 minutes to warm up. During this waiting period, 10mL of deionized water was measured using a dropper and graduated cylinder. The water was then poured into a 250mL beaker and placed on the stir station. A stir magnet was dropped into the beaker, and the stir station was adjusted so the magnet began spinning at a speed that did not splash the solution onto the sides of the beaker. Once the stir station was set up, a weigh boat was placed on a scale, and the scale was zeroed. 0.6 grams of yeast was measured. The yeast was then poured into the beaker and was left on the stir station to stir for 5 minutes in order to activate the yeast. After 5 minutes had passed, 20 mL of a sugar solution was added to the beaker (glucose for the control group, saccharine for the experimental group). The ethanol probe was then placed into the opening of the beaker. The tip of the sensor was kept a safe distance away from the surface of the yeast, water, and sugar solution to prevent moisture from shorting out the sensor. The *LoggerPro* software was used to record ethanol levels in the chamber throughout the duration of the 8 minute trials (LoggerPro3). After each trial was completed, the solution was dumped out and the beaker was rinsed prior to beginning the next trial. A total of four trials were completed.

The rate of change of ethanol in the chamber was recorded for each individual trial and was then averaged for each sugar (raw or processed). An unpaired t-test was then performed to test the significance of the data.

Results

Results show a positive trend in ethanol production when sugar was added. However, glucose produced ethanol at a quicker rate than saccharine. The average rate of change in ethanol levels when glucose was used was 58.52ppm/min, while the average rate of change in ethanol levels when saccharine was used was 5.64ppm/min. This is seen in *Figure 1* below.

An Unpaired t-Test was conducted to compare the type of sugar being tested to the amount of ethanol produced. The t-test produced a p-value of 1.2247e-5, indicating a highly significant difference between the two conditions.

Discussion

In our investigation, we explored the effect that raw and processed sugars had on the rate of ethanol production via fermentation in yeast. It was hypothesized that glucose will produce more ethanol in fermentation than saccharine because natural sugars, like glucose, have a higher caloric content allowing for more efficient metabolism. The results supported this hypothesis because there was a significant difference between the average rate of ethanol production between the two different types of sugars. Glucose produced ethanol at a greater rate than saccharine.

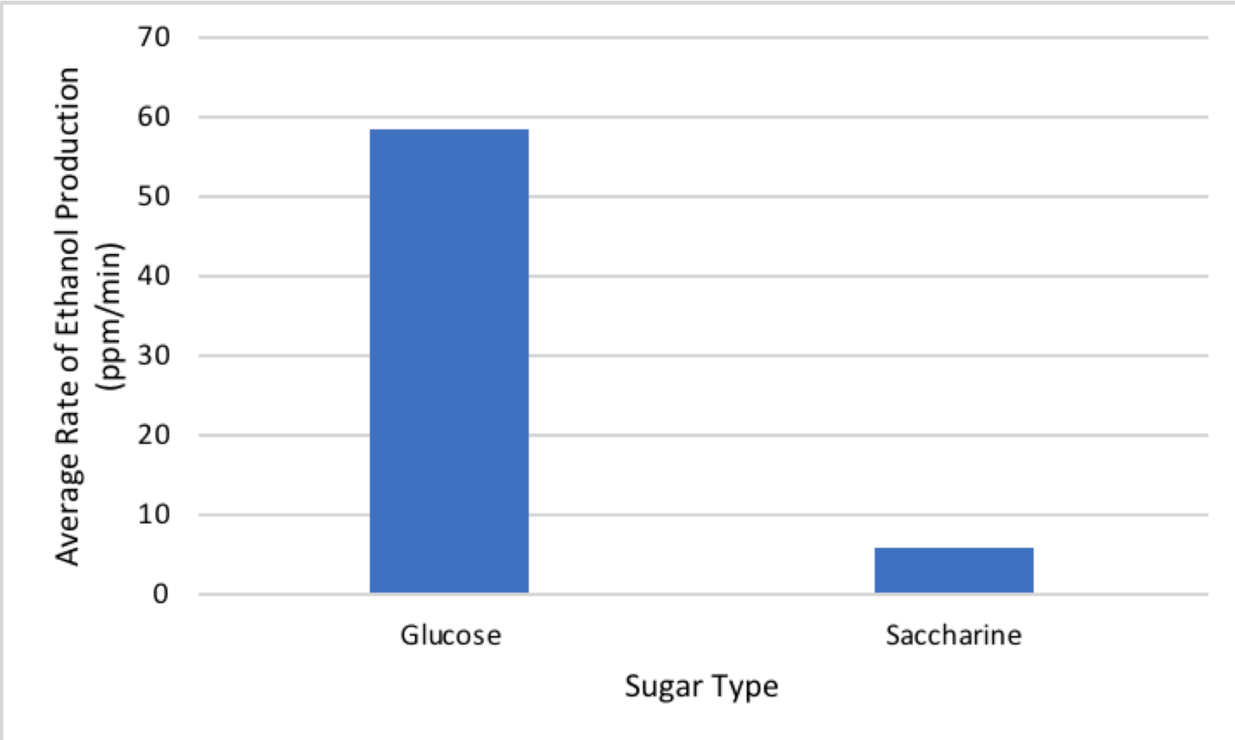

Figure 1: Glucose, a natural sugar, resulted in a greater average rate of ethanol production (58.52ppm/min) than saccharine (5.64ppm/min), a processed sugar.

Research has shown that sugars which closely resemble glucose are metabolized the quickest because they are more easily broken down. Sugars that have had refinements, such as those seen in artificial sugars, are harder to break down and thus metabolism occurs at a slower rate (Bhardi, 2016). As previously discussed, saccharine is refined. The refining of this sugar may make it harder to break down, aligning with previous research. Another study supports similar findings, indicating that saccharine significantly inhibits fermentation of glucose when the two carbohydrates interact (Pfeffer, 1985). This explains why the rate of ethanol production was higher in glucose than in saccharine in this specific study.

As previously alluded to, the results of this experiment were expected. However, there were slight variances in the results between the trials for each sugar. These variances may be directly related to the sample of yeast selected for that specific trial. Some of the trials may have had a greater proportion of active yeast than others. This would have impacted the rate of ethanol production directly. Additionally, yeast may have mutations in certain genes. One study found that yeast with one specific type of mutation increased the fermentation rate by close to 25% (Ramirez, 1998). This also could potentially reflect the small variances in results from trial to trial.

The results from this study may be useful to individuals working in both the bread and alcohol industries as well as individuals working with pharmaceuticals. The knowledge provided by this study may help individuals to select the optimal carbohydrate sources to promote maximum efficiency in fermentation and overall respiration. Since our hypothesis that raw sugars would be more efficient in ethanol production than processed sugars was supported, future research could be done exploring which specific raw sugars are most efficient for fermentation of yeast.

Literature Cited

Baikow V.E. (2013). Manufacturing and Refining of Raw Cane Sugar. Elsevier Scientific Publishing Company.

Bauer, J., Burton, J., Christopher, K., Bauer, B., & Ritchie, R. (2016). Ethanol production in

yeast according to sugar type. Journal of Introductory Biology Investigations. 5(2).
Bhardi, S. (2016). The sweet job of yeast: an investigation of the metabolism of different sugars using yeast microorganisms. http://www.youngscientist.com.au/wp-content/uploads/2017/02/35-Inv-Sabena-Bhadri-R eport-FINAL.pdf
Carper, J., & Polliard, F. (1953). A comparison of the intake of glucose and saccharin solutions under conditions of caloric need. The American Journal of Psychology. 66(3): 479-482.
D'Amore, T. (1992). Improving Yeast Fermentation Performance. Cambridge Prize Lecture Vol. 38: 375-382
LoggerPro3 (Version 3) [Computer Software]. (2016). Beaverton, OR: Vernier Software & Technology
Pfeffer, M., Ziesenitz, S.C., & Siebert, G. (1985). Acesulfame K, cyclamate and saccharin inhibit the anaerobic fermentation of glucose by intestinal bacteria. Z Ernährungswiss.
Ramirez, J., Ramirez, O., Saldana, C., Coria, R., & Peña, A. (1998). A Saccharomyces cerevisiae mutant lacking a K+/H+ exchanger. Journal of Bacteriology. 180(22): 5860-5865.
Glucose Concentration Rather Than the Glucose Flux. The Journal of Biological Chemistry. 273: 24102-24107
Roy, A. et al. (2015). Assessing Glucose Uptake through the Yeast Hexose Transporter 1 (Hxt1). PLOS One. 10(3)
Shashkova, S. et al. (2017). The yeast Mig1 transcriptional repressor is dephosphorylated by glucose-dependent and -independent mechanisms. FEMS Microbiology Letters. 364(14)
Verstrepen, K. et al. (2004). Glucose and sucrose: hazardous fast-food for industrial yeast? Trends Biotechnol. 22(10): 531-7