Journal of Undergraduate Biology Laboratory Investigations

2018

The Impact of Saccharin on *Saccharomyces Cerevisiae* Yeast Fermentation

Parker Brown, Megan Hanson, Edison Huo, Derica Smith, and Bianca Galletti *

[1] University of Oklahoma, Department of Biology, 730 Van Vleet Oval, Room 314 Norman, OK 73019

Previous research has shown that mammals struggle to metabolize sugar substitutes such as saccharin. But these results are less clear when it comes to microorganisms such as yeast. Because many animals cannot metabolize saccharin, we hypothesize that yeast growth will be less in saccharin dominate solutions as compared to glucose dominate solutions. To test this, differing solutions of glucose and saccharin were fed to *Saccharomyces cerevisiae*, a yeast type which uses the Crabtree Effect to carry out alcoholic fermentation in the presence of glucose. Like in animals, it was found that saccharin also negatively impacts the growth of yeast meaning that saccharin could not be used in industrial processes wishing to use yeast to make ethanol.

Introduction

In the last several decades, sugar substitutes have become more common in the food and beverage industry as they allow "healthier" low calorie foods to be produced and marketed to consumers. However, these sugar substitutes have not been introduced without controversy with past research voicing their harmful impacts and others revealing no deleterious impacts (Sharma et. al 2016). In the midst of this controversy, "diet food" sales have surged at the same time obesity continues to rise (Imes and Burke 2014). While the questions raised by sugar substitute critics cast doubt on whether artificial sugars should be used to replace sugar substitutes in general food items, they don't explore whether or not they can in fact replace real sugars in processes requiring yeast growth such as the alcoholic beverage industry.

Because yeast is a commonly used organism in the food industry, the process of yeast fermentation has been well established. In certain types of yeast, such as *Saccharomyces cerevisiae*, yeast can utilize glucose to undergo alcoholic fermentation in what is called the Crabtree Effect (De Deken 1965). Typically, sugars of varying types (glucose, honey, and corn syrups) are added to yeast yielding ethanol and carbon dioxide in differing amounts. Therefore, the amount of ethanol and carbon dioxide produced is positively correlated to yeast growth and can be used as a measure of yeast growth.

Previous studies have shown differing levels of ethanol production depending on the type of

* Research Mentor

sugar added to yeast. For example, Bauer et al (2016) found that brown sugar and corn sugar yielded far more ethanol than the sugar substitute Stevia. In addition, research by Verstrepen et. al (2004) showed that in industrial processes differing the mixture of sugars could trigger problems such as 'off flavors', incomplete yeast fermentation, and loss of yeast vitality. Thus, as sugar substitutes become more ubiquitous the need to understand how yeast is impacted by them is of growing importance.

Due to health concerns, past research has focused more on sugar substitutes' impact on the body rather than in industrial yeast cells. For example, research by Byard and Goldberg (1973) investigated the metabolism of saccharin in laboratory animals and found that it was difficult to metabolize with more sugar substitute molecules showing up in the animal's urine than that of regular sugar. When it comes to the metabolism of sugar substitutes, in contrast to glucose, saccharin may be more difficult for animals to metabolize because of its chemical structure (Bauer et. al 2016). Its chemical formula, CH_5NO_3S, contains more double bonds and constituents than that of glucose, $C_6H_{12}O_6$. Therefore, not only is saccharin more difficult to break apart but it also contains less carbon and hydrogen thus yielding less energy and a slower rate of glycolysis in yeast.

Based on the differing structures between the two sugars, we hypothesize that saccharin will reduce yeast growth due to its difficulty in being metabolized. Within our experimental design using *Saccharomyces cerevisiae* yeast, we predict our hypothesis will be supported if there is less yeast growth with a saccharin solution, and more yeast growth with a glucose solution.

Methods

To test whether or not saccharin can be utilized in real world applications such as industrial alcoholic fermentation and if saccharin impacts the growth of yeast, we set up three groups and subjected them to differing concentrations of saccharin. In our controlled yeast group, we added 10 mL of a 100% glucose solution so as to provide a baseline of yeast growth for a non-sugar substitute solution. The two experiment yeast groups included 10 mL of 50/50 glucose-saccharin solution and 10 mL of 100% saccharin solution that tested the effects of differing amounts of saccharin and to further compare our research to that of the 100% glucose. The control and each experimental group were provided 0.6g of yeast.

To start, 10 mL of distilled water was added to a glass respiration chamber. A magnetic stir bar was then added, and the chamber was then placed on the magnetic stir station. With the stir bar then spinning at a low rate, 0.6g of yeast was added to the chamber and left to sit for five minutes uncovered. After the five minutes, we added our control or experimental concentrated solutions of sugars. Once the solution was added, we capped the respiration chamber with a stopper containing an ethanol probe. Then, it was left to sit stirring in the respiration chamber for seven minutes, after the seven minutes, we used the Logger Pro software connected to the ethanol probe to measure the ethanol level in parts per million for a three-minute period as to measure the change in ethanol being produced by the yeast. This process was then completed for all groups 5 times. Lastly, to analyze the statistical significance of our data, we then conducted a One-Way ANOVA test using the software program PAST3.

Results

The results of our experimental trials are presented in figure 1 which shows the average ethanol produced during each trial for all experimental groups. Of note is the 100% saccharin solution which had little to no ethanol produced in comparison to the 50/50 saccharin/glucose solution and 100% glucose solution which showed

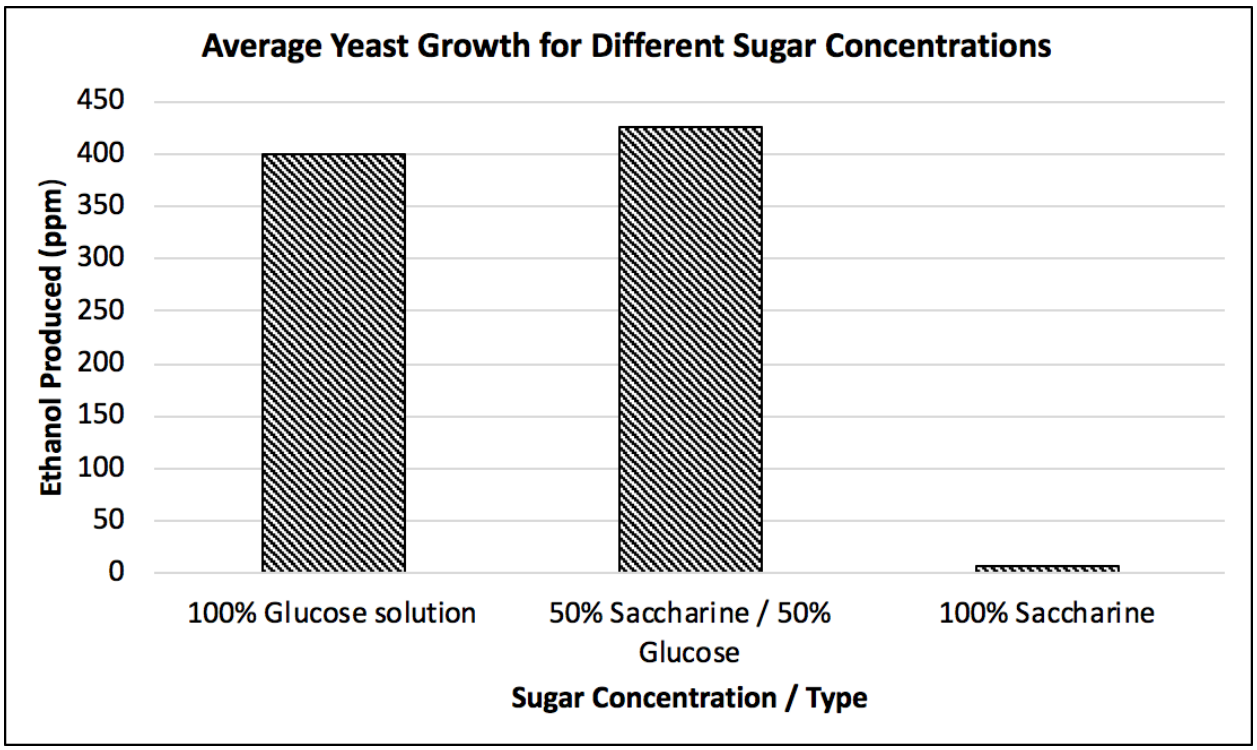

Figure 1: Shows the average ethanol (ppm) produced by the yeast during the 3-minute measurement period for all 5 trials of each solution tested. The 50/50 saccharin-glucose solution had the most ethanol production while the 100% saccharin solution had little to no ethanol production.

significantly more ethanol production. The 50/50 solution had the highest amount of ethanol produced.

We ran an One-Way ANOVA test to compare the effect of an artificial sugar saccharin on yeast growth comparing a 100% glucose solution, 100% saccharin solution, and a 50/50 glucose-saccharin solution. There was a significant effect of saccharin on yeast growth between three conditions; [$F(2, 12) = 86.6$; $p < 0.001$]. A Tukey's pairwise test revealed yeast growth was statistically lower in the 100% saccharin solution than in the 50/50 glucose-saccharin solution [$p<0.001$] as well as the 100% glucose solution, [$p<0.001$].

Discussion

Our results confirm our hypothesis to a high degree of statistical significance ($p <0.001$) that yeast growth would be less in saccharin dominate solutions due to its inability to be metabolized by yeast cells. This is in line with our initial thinking that the molecular structure of saccharin would prove to be difficult to breakdown and used during glycolysis. Overall, our findings concur with that of Bauer et. al (2016) suggesting that it would in fact be unwise to use sugar substitutes in industrial processes requiring yeast fermentation.

It is clear from Figure 1 which reveals the average yeast growth rates for the three experimental groups that the all saccharin solution had the lowest yeast growth rate while the 50/50 glucose-saccharin solution had the highest. While we initially expected yeast growth to be the highest in the 100% glucose solution given that it would have the most 'energy' to feed on, we were surprised to find that the 50/50 glucose-saccharin solution had the highest rate of growth. We suspect this may be due to osmotic concentration pressure as in the all glucose solution whereby water was forced out of the yeast cell and into the solution thus

hampering growth (Myrich, 1916). Further research could be done on other combinations of sugar substitutes to determine how yeast would behave given sugars with differing molecular structures.

Literature Cited

Bauer, Jacqueline. Burton, John. Christopher, Kyle. Bauer, Brianna. Ritchie, Rachel. (2016). Ethanol Production in Yeast According to Sugar Type. Journal of Introductory Biology Investigations. 5(2): 1-4.

Byard, J.L. and Goldberg, L. (1973). The Metabolism of saccharin in laboratory animals. Food and Cosmetics Toxicology. 11(3): 391-402.

De Deken, R. H. (1966). The Crabtree Effect: A regulatory System in Yeast. Institut de Recherches du C.E.R.I.A., Brussels, Belgium. 44(2): 149-156.

Hammer & Harper. (2013). PAST3 (3.2) [Computer Software]. Oslo, Norway: https://folk.uio.no/ohammer/past/

Imes, Christopher C., Burke, Lora E. (2014). The Obesity Epidemic: The USA as a Cautionary Tale for the Rest of the World. Current Epidemiology Reports. 1(2): 82-88.

LoggerPro3 (Version 3) [Computer Software]. (2016). Beaverton, OR: Vernier Software & Technology.

Myrich, R. (1908). A study of the osmotic pressures of concentrated solutions of sucrose with a resistance pressure gauge. Eschenbach Printing. 30(5): 668-683.

Sharma, Arun, Amarnath, S., Thulasimani, M., and Ramaswamy, S. (2016). Artificial sweeteners as a sugar substitute: Are they really safe? Indian J. Pharmacol. 48(3): 237-240.

Verstrepen, Kevin J. Iserentant, Dirk. Malcorps, Philippe. Derdelinckx, Guy. Van Dijck, Patrick. Winderickx, Joris. Pretorius, Isak S. Thevelein, Johan M. and Delvaux, Freddy R. (2004). Glucose and Sucrose: hazardous fast-food for industrial yeast? Trends in Biotechnology 22(10): 531-537.

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