JUBLI
Journal of Undergraduate Biology Laboratory Investigations

How increasing glucose concentration in honey affects fermentation rate in *Saccharomyces cerevisiae*

Alexandra Hopkins, Chance Holloway, Krysta Colahan, Rodet Silva*
University of Oklahoma, Department of Biology, 730 Van Vleet Oval, Room 314
Norman, OK 73019

In this experiment, we aimed to determine the effect of glucose concentration in honey solutions on respiration rate in *Saccharomyces cerevisiae* yeast. Based on our research regarding the Crabtree Effect and the prevention of uptake of other sugars by glucose in high concentrations, we predicted that as the concentration of glucose in the solution increased, the change in CO_2 during each trial would decrease because the fructose in the solution would not be metabolized. To test this we conducted 9 trials with varying concentrations of glucose and fructose in honey, between 50-90% glucose. We recorded the change in CO_2 for each trial and glucose concentration. We then performed a correlational analysis that found a slight but not significant negative correlation between our dependent and independent variables. The results failed to support our hypothesis, and we cannot say that the Crabtree Effect plays a role in yeast's metabolism of fructose.

Introduction

The yeast, *Saccharomyces cerevisiae,* is used in varying capacities in our society, including in the production of soy sauce, alcoholic beverages, and even in many of the most common varieties of bread (Verstrepen et al., 2004). It is used because of its ability to metabolize naturally-occurring or added sugars and produce carbon dioxide and ethanol as byproducts of that process, which provides alcohol content and helps bread rise (Verstrepen et al., 2004). These all constitute industries where the effectiveness of yeast metabolism can impact how quickly the products are produced and how much it costs to get them to the market, and there are several processes which affect the rate of yeast metabolism which manufacturers of these products have a vested interest in.

One such process is the PasteurEffect, which describes the blocking of the fermentation pathway by an end-

product of aerobic respiration in studies conducted on suspended organisms (De Deken, 1966). However, in cases where there is growth of organisms occurring in an environment where glucose is abundant, this process is blocked through the Crabtree Effect, in which glucose is broken down and metabolized through fermentation only (De Deken, 1966). This occurs because high concentrations of glucose trigger yeast cells to deactivate the proteins that are involved in the uptake of other sugars, such as mannose and galactose (Verstrepen et al., 2004). This prevents the metabolism of these other sugars, which can provide very similar amounts of energy to the cell (Harden & Young, 1909). Even though this slows the metabolism of yeast and is not particularly energy efficient, this can be beneficial to the organism and to the alcohol industries that use it (D'Amore, 1992). Because forcing itself to ferment glucose produces ethanol, and because yeast strains have more tolerance for ethanol in their environment than other organisms, fermentation as the primary energy source makes the yeast more competitively successful (Goddard, 2008). The proteins which remain activated after this process have a strong preference for glucose, further stifling the metabolism, even fermentation, of sugars other than glucose (Verstrepen et al., 2004).

There have been many studies involving the metabolism of glucose by yeast cells, and the effects of high glucose concentrations on sugars such as mannose and galactose, but few researching the effects of glucose concentration on metabolism of fructose. Honey is a naturally-occurring substance which contains approximately equal concentrations of glucose and fructose, which makes it a potentially viable option for yeast metabolism in industrial and other settings. However, before that could become a reality, there would need to be more information available about honey metabolism in yeast cells, and how glucose concentrations affect the metabolism of fructose in a solution of honey, especially if there was a concentration of glucose which prevented the fructose from being metabolized at all. The current study aimed to investigate these questions by measuring changes in carbon dioxide levels in yeast solutions of honey with varying concentrations of glucose.

In this experiment, because high concentrations of glucose have been shown to prevent the uptake and metabolism of other sugars as described by the Crabtree Effect and related processes, we predicted that fermentation rate of yeast would decrease as the proportion of glucose to fructose in a honey solution increases. This would demonstrate that honey solutions with high concentrations of glucose would not be fully metabolized by yeast, and honey solutions with low glucose concentrations would be better options for industrial uses. We would know that our hypothesis was supported if the difference between the final and initial carbon dioxide levels was greater for honey solutions with low concentrations of glucose, while we would know the hypothesis was not supported if the difference in carbon dioxide levels was greater for honey solutions which contained high concentrations of glucose or if there was no difference in carbon dioxide levels between the solutions with different concentrations.

Methods

In order to test the hypothesis that increasing proportions of glucose in honey results in lower fermentation rates in yeast we conducted a series of experiments using prepared yeast solutions and differing honey solutions with varying concentrations of glucose. We measured the change in CO_2 levels over 5 minutes to determine whether high glucose concentrations resulted in reduced CO_2 production and therefore whether the rate of metabolism was decreased as we predicted it would be. Nine trials with different glucose concentrations were conducted for this experiment.

We prepared yeast solutions according to the procedure described in the lab manual by Shaw and French (2018). We then created nine different 10mL 0.3M honey solutions to add to the yeast with varying concentrations of glucose. The total amount of sugar available to the yeast and the amount of yeast was held constant, even as the proportions of glucose and fructose in the solutions were varied as our independent variable. The control solution used pure honey, which is 50% fructose and 50% glucose, while the experimental trials used glucose concentrations between 55% and 90% as we replaced 1 mL of 0.3M honey solution with 1 mL of 0.3M glucose solution at a time. This increased the proportional concentration of glucose in the honey solution, starting by adding 1 mL of glucose solution to 9 mL of honey solution, and continuing to increase the concentration of glucose until we only put 2mL of the honey solution in the last experimental trial solution.

To measure the rate of metabolism of the yeast in each of the nine trials we measured the change in CO_2 levels in parts per million (PPM) between the beginning and end of these 5-minute trials as our dependent variable using the CO_2 probe and Logger Pro software provided (LoggerPro 3.15). This allowed us to determine the difference in rate of fermentation of honey in yeast between solutions with varying concentrations of glucose in honey. To analyze the results of our experiment we conducted a correlational analysis to determine if there was a significant relationship between the measured values of concentration of glucose in honey and the CO_2 production after 5 minutes.

Results

We found that there was not a significant negative correlational relationship between glucose concentration and CO_2 production, $r(8) = -0.19$, $p = 0.63$. The CO_2 production of each 5-minute trial is recorded in Table 1. The analysis indicates that low concentrations of glucose in honey resulted in slightly higher CO_2 production, while high concentrations of glucose resulted in slightly lower CO_2 production, as shown in Figure 1, although the trend was not immediately apparent upon inspection and the correlation was in fact not statistically significant.

Discussion

This experiment failed to support our hypothesis that higher concentrations of glucose in honey would result in decreased CO_2 production. This means that the

Concentration of Glucose in 0.3M sugar solution (%)	CO_2 Produced in 5 minutes (PPM)
50	9,148
55	6,623
60	5,391
65	7,265
70	8,523
75	6,720
80	8,977
85	5,769
90	6,741

Table 1. The amount of CO_2 produced (PPM) in 5 minutes for each concentration of glucose (%) in the 0.3M sugar solution.

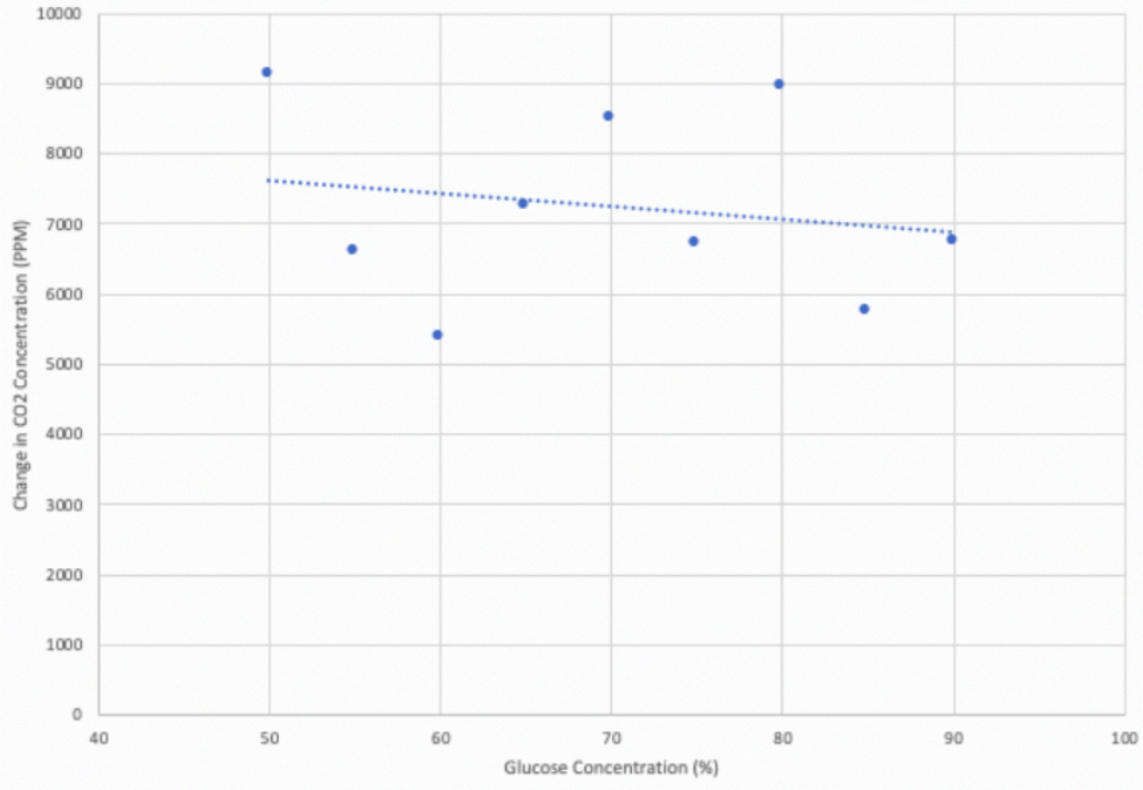

Figure 1. There was an insignificant negative correlational relationship between the change in CO_2 level (PPM) and glucose concentration in honey (%).

metabolism of fructose by yeast cannot be shown to be affected by the concentration of glucose around it, and there is no reason to assume that honey which contains high concentrations of glucose would be any more or less effective in industrial uses of yeast metabolism than honey with low concentrations.

Although the Crabtree Effect has been shown to affect the metabolism of mannose and galactose, it is possible that fructose is not affected in the same way, because it may still be able to enter the cell through the protein pathways that allow glucose and sucrose into the cell, even though most strains of *Saccharomyces cerevisiae* that were studied by Bisson and Fraenkel had the highest affinity for glucose, because they also had a high affinity for fructose that was not nearly as far behind as the yeast's affinity for other sugars (1983). This could mean that both sugars were being metabolized for any given trial, which could explain why there was not a significant difference in CO_2 production between the trials as we had predicted that there would be. Fructose can also be fermented, and so the competitive advantage fermentation gives the yeast in relation to other organisms may be present for either sugar, and there are some studies which say fructose fermentation proceeds and produces CO_2 more quickly than that of glucose, which could counteract the Crabtree Effect in terms of total CO_2 produced within the trial time even if the effect is at play in this scenario (Harden & Young, 1909).

There is also the possibility that clearer trends would have appeared if we had been able to run more trials and increase the sample size of each honey concentration to account for random variation in the yeast solution composition or in the amount of time the sugar and yeast were in contact before we inserted the CO_2 probe, which is something that could be used in future studies. To test whether or not the Crabtree Effect plays a role in fructose uptake at all, it could also be beneficial to study concentrations of glucose and fructose spanning the whole range of proportions, as our experiment only studied glucose concentrations of 50% or more, which may have been beyond the range of concentrations that would see the effects of the prevention of fructose uptake. To avoid the confounding effects of fructoses rapid metabolism by the yeast, shorter trial times may also work well to isolate the possible effects of the Crabtree Effect in fructose fermentation. Until more information is available, it seems as though honey of any glucose/fructose composition may be available for industrial yeast metabolism.

Literature Cited

Bisson, L., & Fraenkel, D. (1983). Involvement of Kinases in Glucose and Fructose Uptake by Saccharomyces cerevisiae. *Proceedings of the National Academy of Sciences of the United States of America, 80*(6), 1730-1734.

D'Amore, T. (1992, Fall). Cambridge Prize Lecture Improving Yeast Fermentation Performance. *Journal of the Institute of Brewing*, *98*, 375-382.

De Deken, R. H. (1966). The Crabtree Effect: A Regulatory System in Yeast . *Journal of General Microbiology*, *44*, 149-156.

Goddard, M. (2008). Quantifying the Complexities of Saccharomyces

cerevisiae Ecosystem Engineering via Fermentation. *Ecology, 89*(8), 2077-2082.

Harden, A., & Young, W. (1909). The Alcoholic Ferment of Yeast-Juice. Part IV-The Fermentation of Glucose, Mannose, and Fructose by Yeast-Juice. *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character,81*(549), 336-347.

Logger Pro 3.15 (Version 3.15) [Computer Software]. (2018). Beaverton, OR: Vernier Software & Technology.

Shaw, T. J., & French, D. P. (2018). *Authentic Research in Introductory Biology: A Laboratory Resource Manual*. United States of America: Fountainhead Press.

Verstrepen, K. J., Iserentant, D., Malcorps, P., Derdelinckx, G., Van Dijck, P., Winderickx, J., Pretorius, I. ,Thevelein, J., & Delvaux, F. (2004, October). Glucose and sucrose: hazardous fast food for industrial yeast? *Trends in Biotechnology*, *22*(10), 531-537.