Effect of Temperature on Glucose Concentration in the Enzymatic Activity of Lactase in Lactose

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Abstract

This project was conducted to see the change in enzymatic activity of lactase in the presence of lactose in different temperatures. Our question is: How does the change in temperature impact the glucose concentration as a result of the enzymatic activity of lactase in the presence of lactose? Glucose concentrations were found using OneTouch Ultra 2® blood glucose meter to check the enzymatic activity of lactase. We utilized ANOVA, also known as Analysis of Variance, to check if there were any significant differences in the glucose concentrations between the four different temperature groups of 20°C, 40°C, 60°C, and 80°C. The Lactaid® Extra Strength pills were used as source lactase. At the end, we found there was a significant difference of enzymatic activity between the temperatures. The optimal temperature for the enzymatic activity of lactase was found to be 60°C, and the reactions were too slow to be recorded at lower temperatures. In the future, we can use different brands of lactase, concentration of lactose, and temperatures. This research can help inform our understanding of lactase activity *in vivo,* the use of lactase in the food industry, including lactose-free products, and in medical applications in treating lactose intolerance.

Introduction

Lactase is an enzyme that catalyzes the hydrolysis of lactose, a disaccharide found in milk, into glucose and galactose which is a process that is essential to the digestion of milk. Lactose intolerance is a common condition caused by a deficiency in lactase activity in the small intestine, leading to symptoms such as abdominal pain, bloating, and diarrhea(Lomer et. al 2007). Various industries such as the food and beverage industry or the pharmaceutical industry have turned their attention to the enzymatic activity of

lactase and its application in milk products. Products such as Lactaid Extra Strength® tablets containing the lactase enzyme have sought to relieve lactose intolerance by helping individuals digest lactose in dairy.

Previous literature reveals that lactase activity is influenced by pH, substrate concentration, and temperature. Changes in temperature can impact the rate of hydrolysis of lactose into glucose, as enzymes have an optimal temperature that they work at best. As dairy may be heated or cooled for a variety of purposes such as pasteurization, storage, cooking, previous studies have been conducted, giving a range of temperatures from 45-60°C where the lactase enzyme activity is highest (Yang & Okos, 1989; Voget, 2022, Panesar, 2010, Mirsalimi, 2021) . However, there is evidence that the optimal temperature differs depending on total time spent in the reaction and whether the lactase is immobilized (Popescu, 2021). A paper by Yang & Okos states that when operating time is short, the optimal operating temperature is 60°C but is close to 35°C for a long operating time. Glucose is important for ATP production, making it an essential source of energy for many organisms. In addition to its impact on lactase activity, temperature can also affect the stability of lactose in solution. At higher temperatures (30°C and 40°C), lactose can form brown compounds and a reduction in available substrate for lactase. At lower temperatures (0°C and 20°C), no changes were observed (Bottiroli et. al, 2021).

In this paper, we aim to investigate the impact of temperature changes on the enzymatic activity of lactase and the resulting glucose concentration in the presence of lactose. To accomplish this, we performed glucose tests at different temperatures before and after Lactaid Extra Strength® solution and analyzed the glucose concentration. We predicted that an increased temperature would induce an increased rate of lactase hydrolyzing lactose reaction, and by extension, a higher glucose concentration. Our findings may have implications for the development of treatments for lactose intolerance, and optimization of lactose hydrolysis in the food and beverage industry.

Methods

Firstly, to ensure that the OneTouch Ultra 2® blood glucose meter gave accurate glucose measurements, solutions with concentration of 0 mmol/L, 12.5 mmol/L, 25 mmol/L, and 50 mmol/L were tested to make sure that the readings were accurate. The dilutions were made from a 50 mmol stock glucose solution and were mixed with a trisaminomethane (Tris) buffer to allow the glucose solution to mimic the viscosity of human blood. The glucose concentrations were measured by the meter by micropipetting 7uL of the different glucose solution onto a Parafilm. To test the drop of solution a glucose test strip was placed into the OneTouch Ultra 2® then the measurements were taken for each concentration. From the readings small discrepancies were noticed from the observed glucose concentration and the actual glucose concentration of the solution. From the calibration it was determined that the OneTouch Ultra 2® blood glucose meter can only give measurements between 1.1 mM/L and 33.3 mM/L according to the meter's instruction manual. Since the difference between observed and actual glucose concentration were so small they were negligible in the reading for the experiment.

After the glucose meter had been calibrated for accuracy, the OneTouch Ultra 2® could now be used to measure the glucose concentration from the enzymatic activity of lactase in the presence of lactose. The stock lactase solution was made by grinding two Lactaid® Extra Strength pills in a mortar and pestle and dissolving the pill powder into 180mL of Tris Buffer. Then, 6mL of 50 mmol/L lactose stock solution was measured using a graduated cylinder and transferred into 12 large test tubes with caps. After transferring the measured lactose solution into the test tube, 7uL of the lactose solution in the was micropipette on to a Parafilm and the glucose concentration was then tested for each test tube and recorded. Then, three test tubes were placed into a 20ºC incubator to allow the lactose solution to increase to a temperature of 20ºC, three test tubes were placed into a 40ºC incubator to allow the lactose solution to increase to a temperature of 40ºC, three test tubes were placed into a 60ºC water bath to allow the lactose solution to increase to a temperature of 60ºC, and three test tubes were placed into a 80ºC water bath to allow the lactose solution to increase to a temperature of 80ºC.

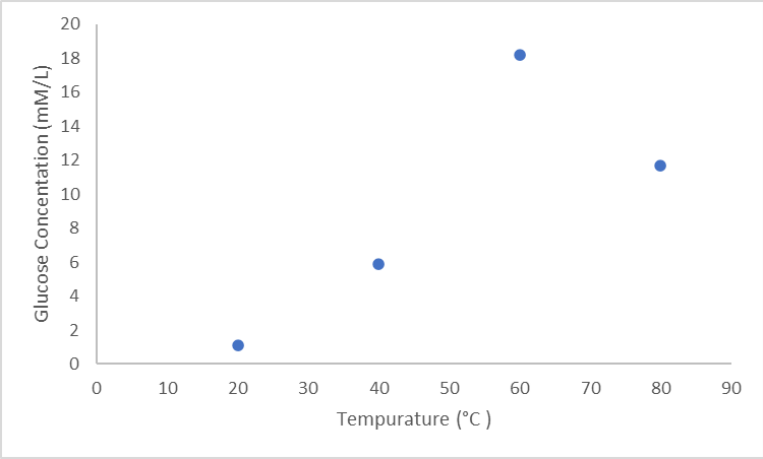
When the lactose solution reached the desired temperature when checked with a thermometer, 6mL of the lactase stock solution was measured using a graduated cylinder and added to the test tube and immediately inverted to ensure mixing of lactose and lactase. After the inversion a 15 minute timer was started and the mixtures were left in the apparatus they were coming up to temperature in to ensure the temperature was consistent throughout the entire reaction. At the 7 minute and 30 second mark the test tubes were inverted again to make sure that the lactase solution did not settle at the bottom of the test tube. After the 15 minute timer had completed 7uL of the mixture was pipetted onto a Parafilm. Then, the glucose concentration of the mixture was tested using the OneTouch Ultra 2® blood glucose meter that had been readied with a testing strip. Once the glucose measurement had been taken it was recorded and the process was repeated for each test tube, resulting in three trials occurring for each measurement of 20ºC, 40ºC, 60ºC, and 80ºC. For each of the trials the final glucose concentration was subtracted from the initial glucose concentration for each of the 12 trials and the change in glucose concentration was recorded.

The average change in glucose concentration for each temperature was then plotted in Excel to conduct a one way ANOVA on the mean change in glucose concentration over change in temperatures, to find the relationship between temperature and the change in glucose concentration.

Results

The aim of this study was to investigate the enzymatic activity of lactase based on glucose concentration (mM/L) at different temperatures. An analysis of variance (ANOVA) was conducted to determine if there were any statistically significant differences in the outcomes between the four different temperature groups: 20°C, 40°C, 60°C, and 80°C.

Figure 1
The mean glucose concentration from the temperature intervals



*Note.*The scatter plot graph shows the mean glucose concentration across the four different temperatures using the data collected).

The results of the single-factor ANOVA), revealed a significant difference in the lactase activity between the groups exposed to different temperatures due to the F-statistic(51.63208) being greater than the F-critical (4.006181) . The average glucose concentration for the 20°C group (M = 1.1, = 0) was significantly lower compared to the 40°C (M = 5.9, SD = 0.52), 60°C (M = 18.166, SD = 0.32), and 80°C (M = 11.667, SD = 3.50) groups The group exposed to 80°C had the highest variance (12.253), while the 60°C group had a variance of 0.103, the 40°C group had 0.27 variance while the 20°C group had 0 variance., indicating that the enzymatic activity was more consistent at the optimal temperature of 60°C. The between-group variance (488.96) was significantly higher than the within-group variance (25.25), suggesting that temperature had a significant effect on lactase activity.

According to the single- factor ANOVA the p-value (0.000014) we are able to reject the null hypothesis meaning that there is significant difference between treatment groups .

Data Collection and Analysis

Manipulated Variable : Temperature in intervals of 20, 40, 60, 80 (°C Celsius)

Responding Variable : Glucose reading (mM/L)

Controlled Variable: Time of reaction (min) , type of lactase, type of lactose, concentration of initial lactose solution for reaction (mM/L), concentration of initial lactase solution for reaction (mM/L)

Discussion

In summary, the results of this study showed that the enzymatic activity of lactase was significantly affected by temperature. The trend observed in the data suggests that the enzymatic activity of lactase increased with an increase in temperature, up to 60℃, beyond which there was a decrease in the enzymatic activity at 80℃. These results are consistent with previous studies on the effects of temperature on enzymatic activity (Arcus, 2016, Yang & Okos, 1989; Voget, 2022, Panesar, 2010, Mirsalimi, 2021).

At a temperature of 20°C, the change in glucose concentration was below 1.1 mM/L, indicating that there was little to no enzymatic activity taking place. As the temperature increased to 40°C, the average change in glucose concentration increased to 5.9 mM/L, indicating that the enzymatic activity of lactase had increased significantly. Further increases in temperature up to 60°C resulted in a further increase in the average change in glucose concentration to 18.2 mM/L, indicating that the enzymatic activity of lactase was at its peak. However, at a temperature of 80°C, the average change in glucose concentration decreased to 11.7 mM/L, indicating that the enzymatic activity of lactase had decreased significantly.

Outside of this optimal temperature range, the enzymatic activity decreases due to denaturation. This is consistent with the results demonstrating that the lactase protein denatures around 60 degrees. One source of error could have been introduced through temperature variations. The distribution of temperature in the water bath when opening and closing the water bath could have varied, making it difficult to make sure that the bath was constant. This was mitigated by making sure that the lactose was at the target temperature before adding the lactase and placing it in the water bath from start to finish.

Another source of error that may have impacted our results includes inconsistencies in timing of the reaction. This was mitigated by starting the timing of the reaction just as the lactase is added.

Furthermore, another source of error that may have impacted our results is the mixing of the reagents, leading to an inaccurate reaction. Lactase behaves differently depending on whether it is soluble / immobilized (Panesar, 2010). In our experiments, our lactase was ground up and rendered soluble by being put into the solution, but the ground up pill may not have been mixed adequately, potentially leaving immobilized lactase in our solution. We mitigated this by quickly vortexing the test tube after adding the lactase, in order to ensure that the lactase and lactose are mixing.

The other product of lactose hydrolysis, galactose, has a similar affinity to lactose. The products of lactose hydrolysis, galactose and glucose also have inhibitory effects on lactase activity. (Jurado, 2002). The inhibitory effects of galactose and glucose have implications for our results, as the presence of glucose and galactose may have inhibited the lactase activity, so it may not be a true representation of lactase activity. Measuring the concentration of both glucose and galactose may prove a better measure of lactase activity.

The results of this study demonstrate that changes in temperature have a significant impact on the enzymatic activity of lactase and subsequently on the concentration of glucose produced in the presence of lactose. The results indicate that lactase activity is optimal at temperatures between 40°C and 60°C, and that at temperatures outside of this range, the enzymatic activity decreases. However, the limitations of the study, such as the small sample size and the limited measures of lactase activity, suggest that further research is needed to confirm and extend these findings. Future research should look into measuring smaller intervals between temperatures, different brands of lactase, different concentrations of lactose and lactase, pH, as well as measuring galactose. These findings will help us understand lactase activity *in vivo,* and have important implications for the use of lactase in the food industry, including lactose-free products, and in medical applications in treating lactose intolerance.

Conclusion:

Based on previous literature and our scientific knowledge, we thought the increase of temperature would lead to higher enzymatic activity of lactase. This assumption turned out to be true, since there was an increase of glucose concentration as there was an increase in temperature. Therefore, we accept our hypothesis and reject our null hypothesis, which is that the change in temperature would not lead to any change in enzymatic activity of lactase in lactose solution. However, we noticed that the lactase activity decreases at 80°C. This could be due to protein denaturation, but the specific temperature the protein starts to denature is yet unknown. This should be further studied since a lot of people suffering from lactose intolerance take the Lactaid pill before drinking a hot drink, which would be around 70 degrees or higher.

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