Effect of temperature on the enzymatic activity of lactase in breaking down lactose

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Abstract

This study was conducted to test glucose production in the first order reaction of lactose hydrolysis with the help of the enzyme β-galactosidase (lactase). Lactose is a disaccharide naturally found in most mammalian milk and is composed of the two monosaccharides glucose and galactose (Buller & Grand, 1990). This experiment tested the effect of temperature on the enzymatic activity of lactase by measuring the glucose concentration of lactose over time. A standardized curve was created to account for discrepancies between the actual glucose concentrations and the readings from the OneTouch Ultra 2® blood glucose meter. Lactaid® Extra Strength pills containing lactase were dissolved in solution and added to 50 mmol/L lactose. Glucose concentrations were measured every 5 minutes across a 45 minute interval to calculate the reaction rates of lactose hydrolysis at 22ºC and 37.5ºC with enzyme catalysis. Reaction rate graphs were created to compare the breakdown of lactose at the two temperatures, and rate laws for the reactions with the two treatments were determined. From the rate laws, the reaction of lactose hydrolysis at 22ºC produced a rate constant of $k = 0.0109s^{-1}$, and the group that received a treatment of 37.5ºC produced a rate constant of $k = 0.0150s^{-1}$. Ultimately it was shown that at a higher temperature, reaction kinetics were higher as expected. From this it was concluded that the human body is at an optimal temperature for the breakdown of lactose.

Introduction

Lactose is the protein found within milk that is broken down into glucose and galactose by the enzyme β-galactosidase, also known as lactase (Buller & Grand, 1990; Lomer et al., 2007). Glucose is primarily used by cells for ATP production (Buller & Grand, 1990). At birth, infants express the gene for lactase in order to gain energy from their mother's milk while feeding, which is crucial during this critical developmental period (Lomer et al., 2007). Lactose intolerance is a digestive problem in which the body is unable to digest the carbohydrate lactose. The inability to digest lactose is the dominant and basal level trait, and the ability to digest lactose after infancy is typically only found in those with European heritage. Therefore, lactose intolerance is one of the most common deficits in the world since most ethnicities lack the ability to digest lactose (Lomer et al., 2007). Those with lactose intolerance are incapable of using dairy as an extra source of vitamins and nutrients. In order to combat this intolerance, medications such as Lactaid® Extra Strength tablets have been invented to help individuals digest lactose in dairy foods (Lactaid Brand, 2016). These pills are dietary supplements containing the enzyme lactase that digest lactose.

Multiple experiments have been conducted to gain insight on the reaction kinetics of lactose digestion by lactase (Ring, 1998). This is a first order reaction producing glucose and galactose that occurs within the temperature range of the human body (Ring, 1998). These experiments can help determine the implications of increased body temperature on energy production within an individual, as certain temperatures are known to catalyze the reaction (Gekas & Lopez-Leiva, 1985). Ultimately, this experiment is important as it is known that lactose consumption and digestion are required during infancy to produce energy crucial for infantile development. Thus, without lactose digestion an individual's development lacks the necessary energy to produce glucose, an essential carbohydrate for biochemical reactions in the body. The temperatures in lactose experimentation must not cause the denaturation of either lactose or lactase (Das et al., 2015).

Consequently, this experiment is set up to test whether temperature will affect the reaction rate for the hydrolysis of lactose into glucose and galactose. Literature reviews have stated that the ideal temperature range for the human body is between 37ºC - 37.5ºC (Ring, 1998). This knowledge is critical since the reaction naturally takes place within the human body. Additionally, the temperature range for the interaction between lactase and lactose was determined to be between 15ºC to 45ºC, as it would prevent the enzyme from denaturing (Das et al., 2015). Differences in temperature will affect the reaction rate. We predicted that the reaction rate will increase with temperature as long as the temperature is within the optimal temperature range of lactase activity in the functioning human body. Ultimately, the temperatures 22ºC and 37.5ºC were tested in this experiment, as 22ºC was close to the lower bound of the functioning enzymatic temperature range, while 37.5ºC was close to the denaturing temperature but still functioning (Jasewicz & Wasserman, 1961). Consequently, we predicted that the reaction rate will be higher at 37.5ºC compared to 22ºC.

Methods

Firstly, in order to calibrate the OneTouch Ultra 2® blood glucose meter's measurements of glucose concentration, standard solutions of glucose (0 mmol/L, 5 mmol/L, 10 mmol/L, 15 mmol/L, 20 mmol/L, 25 mmol/L, 30 mmol/L, and 35 mmol/L) were created to plot a standardized curve for glucose concentration measurements. Serial dilutions were done using a 50 mmol/L stock glucose solution and

trisaminomethane (Tris) buffer. The concentrations of glucose were measured by pipetting 7 µL of each dilution onto a labeled Parafilm and loading them into the OneTouch Ultra® test strips for the glucose meter to take measurements (Figure 1). The glucose measurements were taken three times for each concentration, and an averaged glucose concentration was calculated for each. A standardized glucose curve was then created by plotting the actual glucose concentrations against the measured average glucose concentrations from the glucose meter using Microsoft Excel. Minor differences between the glucose meter readings and the actual concentrations were observed and considered for the following experimental measurements of glucose from lactose.

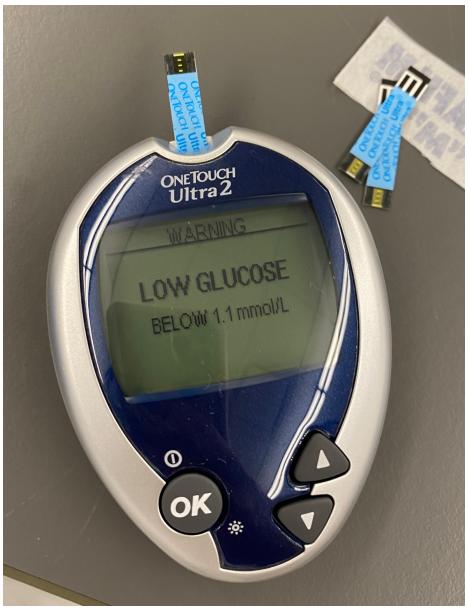

Figure 1. The OneTouch Ultra 2® blood glucose meter and test strips.

Next, to measure the glucose concentrations produced from the breakdown of lactose, ten Lactaid® Extra Strength pills were ground into fine powder and dissolved in 40 mL of Tris buffer. Then, 12 mL of 50 mM lactose was pipetted into 6 different test tubes. 3 samples were placed into a 40ºC water bath (Figure 2) to account for the fact that every five minutes, the solutions would be taken out for vortexing and measuring. Cooling occurred each time the solutions were taken out of the water bath, and resultantly the solutions remained constantly around the desired temperature of 37.5ºC. The remaining 3 samples were left at room temperature (22ºC). Once the samples in the water bath reached the target temperature, 5 mL of lactase were added to each test tube using a 10 mL graduated cylinder. Some

undissolved Lactaid® also remained inside the graduated cylinder, so the lactose/lactase solution was transferred between the graduated cylinder and the test tube twice to ensure quantitative transfer for each test tube. Subsequently, 7 µL of solution was immediately transferred to a piece of Parafilm to take a measurement of the initial concentration with the glucose monitor. The lactose/lactase solution had an opaque, solid layer of undissolved lactase at the bottom of the test tubes while the rest of the solution was a cloudy, white liquid. Every five minutes, the samples were vortexed for 3 seconds to distribute the solid lactase particles. 7 µL of solution was then immediately withdrawn from each sample onto labeled sheets of Parafilm. The glucose measurements were taken and logged into Microsoft Excel. This was repeated for each test tube up to a total of 45 minutes.

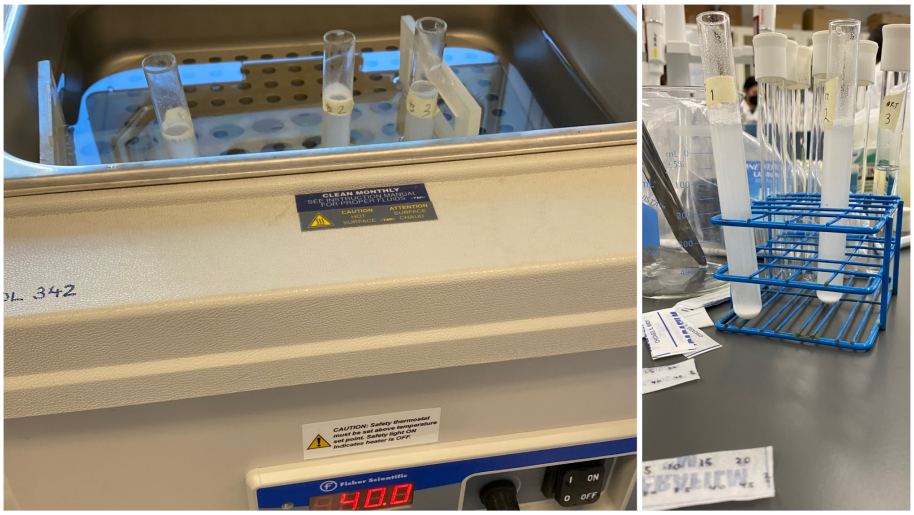

Figure 2. Experimental set-up of the samples in a 37.5ºC (left) and 22ºC (right) environment.

For each of the two temperatures, the calculated glucose concentrations were subtracted from the initial lactose concentrations (50 mmol/L) to determine the amounts of lactose that were in solution at each timepoint. A first order reaction rate was determined, and the natural logarithm of the lactose concentration values were plotted against time to obtain a linear graph of the reaction rates at 22ºC and 37.5ºC using Microsoft Excel. The three trials for each temperature were plotted against time to determine the reaction rate graphs and their rate constants (see Appendix). Using the rate constants from each trial, an unpaired t-test was conducted using GraphPad to compare the means of the reaction rates for the two temperature treatments.

Results

Proceeding with the methods, Figure 2 shows qualitative data. During the experiment, the lactase pills were not fully dissolved in the buffer solution as desired during the initial preparation stages. Therefore, white bubbles with bits of pill powder were floating at the top of each test tube. White residue that appeared to be more undissolved lactase pills was also shown to have sank to the bottom of each solution. These unmixed precipitates of solution stayed constant throughout the duration of the treatments for each trial and temperature treatment. All other liquids were transparent and clear in color with low viscosity.

The reaction of lactose hydrolysis is in first order reaction kinetics, which has an integrated rate law:

$$\ln[A] = -kt + \ln[A_0]$$

where [A] = substrate concentration, k = rate constant, and $[A_0]$ = initial substrate concentration.

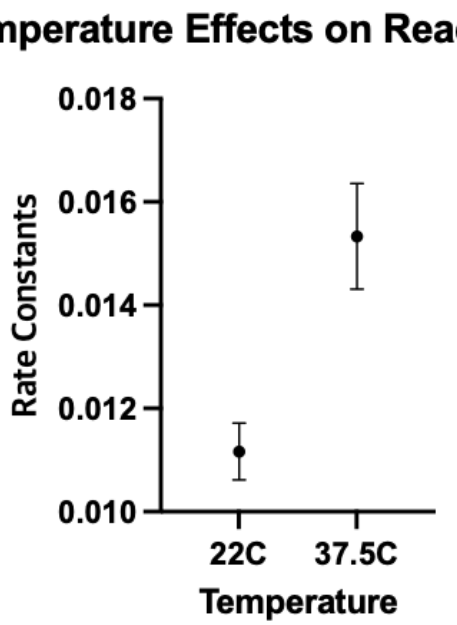

Figure 3. Statistical analysis for the effects of temperature on reaction rates.

The collected lactose concentrations determined the reaction rate law for the breakdown of lactose at the two different temperatures, 22ºC and 37.5ºC. From the rate laws, the rate constants of each

reaction that took place were determined by finding the slope of the linear plot. The treatment group that underwent lactose hydrolysis at 22ºC produced a mean rate constant of k = 0.0112s^{-1}, and the group that received a treatment of 37.5ºC produced a mean rate constant of k = 0.0153s^{-1}.

Figure 3 shows the results of the statistical analysis conducted using an unpaired t-test, illustrating the significance in the differences in the rate constants for each temperature. The p-value was determined to be 0.0034, which was evidently under the boundary for the testing significance of 0.05. Furthermore, the rate constants produced for the 22ºC temperature treatment resulted in a 95% confidence interval of 0.01117 ±0.000623 (±5.58%), and the data from the 37.5ºC temperature treatment groups resulted in a 95% confidence interval of 0.01533 ±0.00116 (±7.57%).

Discussion

It is well understood that the rates of all chemical reactions are dependent on the temperatures at which the reactions are performed (Arcus et al., 2016). Since milk is commonly heated for a variety of reasons in the dairy industry, multiple studies have been conducted on lactose-rich milk to examine the impact of heat on the lactose breakdown rate. Most of these studies demonstrated that heating milk would increase the lactase activity rate (Guzman et al., 2002; Wendorff et al., 1970; Van Dam et al., 1950). Based on the conclusions of these studies, we defined our null and alternative hypotheses as follows: the null hypothesis was that the change in temperature does not significantly affect the reaction rate of lactase, and the alternative hypothesis was that the reaction rate of the lactose breakdown is faster at higher temperatures as long as the experimental temperature was below the lactase denaturation temperature. To test these hypotheses, experiments were performed and the reaction rates at 22ºC and 37.5ºC were plotted (see Appendix). Based on the results of our experiment, lactose breaks down to form glucose and galactose with a rate constant of 0.0109s^{-1} at 22ºC and a rate constant of 0.0150s^{-1} at 37.5ºC. The rate constant quantifies the rate and direction of a chemical reaction (McMurry et al., 2014). A higher rate constant indicates a faster reaction rate, therefore we can infer that the breakdown rate of lactose is higher at 37.5ºC compared to 22ºC (McMurry et al., 2014). The unpaired t-test performed on the results demonstrated a p-value below 0.05 and therefore the reaction rate was found to be statistically significantly higher at 37.5ºC than it is at 22ºC. The analysis of data demonstrated that the reaction rates

for both treatment groups fell under the 95% confidence interval. The results support the predicted outcomes and based on these results, we can reject the null hypothesis and support the alternative hypothesis. By extrapolating the outcome of this study to other temperatures, we can conclude that lactose breakdown rate in the presence of lactase is faster at higher temperatures. There were two main sources of error in this experiment: the lactase pills dissolved in the solution at first but slowly precipitated by the time that we started measuring the glucose content. We vortexed all the solutions immediately before glucose measurements but the lactase powder did not fully dissolve in the solution even after mixing. The second source of error was the glucose meter's internal error which led to lower or higher glucose readings compared to the actual glucose concentration (see Appendix). One limitation of this study was the lack of pH measurements at all time points. Changes in the pH might be an important factor affecting the reaction rate and must be considered in the future studies. Despite the errors and limitations, previous studies that analyzed the effect of increased temperature on lactase activity rate had a similar outcome, which further supports the conclusion of our study (Guzman et al., 2002; Wendorff et al., 1970; Van Dam et al., 1950). These results could indicate that the human body breaks down lactose faster at a higher temperature; however, this study was conducted in experimental conditions that did not replicate the human body environment. Therefore, to confirm the impact of increasing temperature on lactase activity levels in the human body, additional studies must be performed in cultures and environments that are more similar to that of the human body such as mammalian models. Furthermore, studies could be performed to analyze the impact of fevers on lactose intolerance in mammalian or human bodies. The outcomes of such studies could then be used for the development of new treatments for lactose intolerance in humans.

Conclusion

Based on the conclusions of the previously published literature, it was hypothesized that lactase reaction rate is faster at higher temperatures. The results of this study were sufficient to support this hypothesis and to reject the null hypothesis, which suggested that the change in temperature does not significantly affect the reaction rate of lactase. Furthermore, the results supported the prediction that in the presence of lactase, lactose will be broken down into glucose and galactose at a faster rate at 37.5ºC

compared to 22ºC. The results may infer that the human body could break down lactose at a faster rate at higher temperatures. This phenomenon should be further studied as it could potentially lead to the development of new treatments for lactose intolerance.

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Appendix

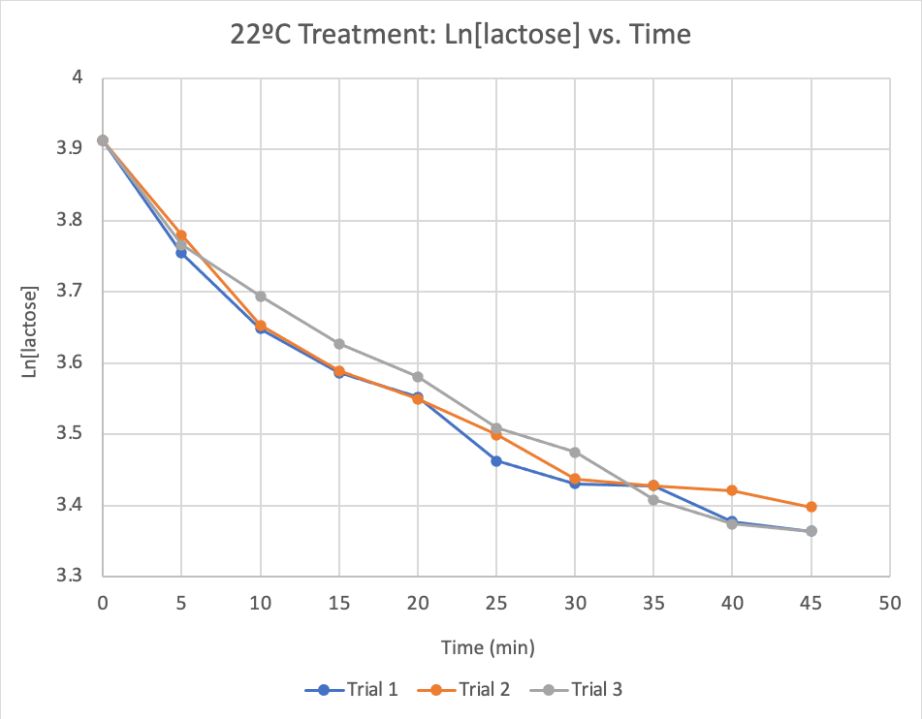

Figure 1. Reaction rates of lactose hydrolysis at 22ºC treatment.

Trial 1: Ln[lactose(mM)] = -0.0112(s^{-1})x + 3.9120(mM)

Rate Constant = k = 0.0112s^{-1}

Trial 2: Ln[lactose(mM)] = -0.0106(s^{-1})x + 3.9120(mM)

Rate Constant = k = 0.0106s^{-1}

Trial 3: Ln[lactose(mM)] = -0.0117(s^{-1})x + 3.9120(mM)

Rate Constant = k = 0.0117s^{-1}

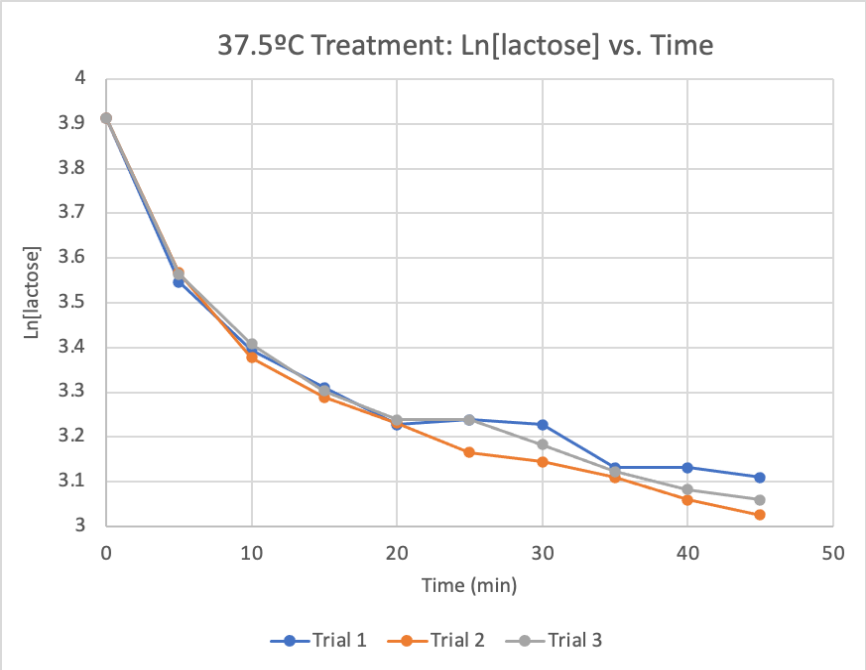

Figure 2. Reaction rates of lactose hydrolysis at 37.5ºC treatment.

Trial 1: Ln[lactose(mM)] = -0.0142(s^{-1})x + 3.9120(mM)

Rate Constant = k = 0.0142s^{-1}

Trial 2: Ln[lactose(mM)] = -0.0162(s^{-1})x + 3.9120(mM)

Rate Constant = k = 0.0162s^{-1}

Trial 3: Ln[lactose(mM)] = -0.0156(s^{-1})x + 3.9120(mM)

Rate Constant = k = 0.0156s^{-1}

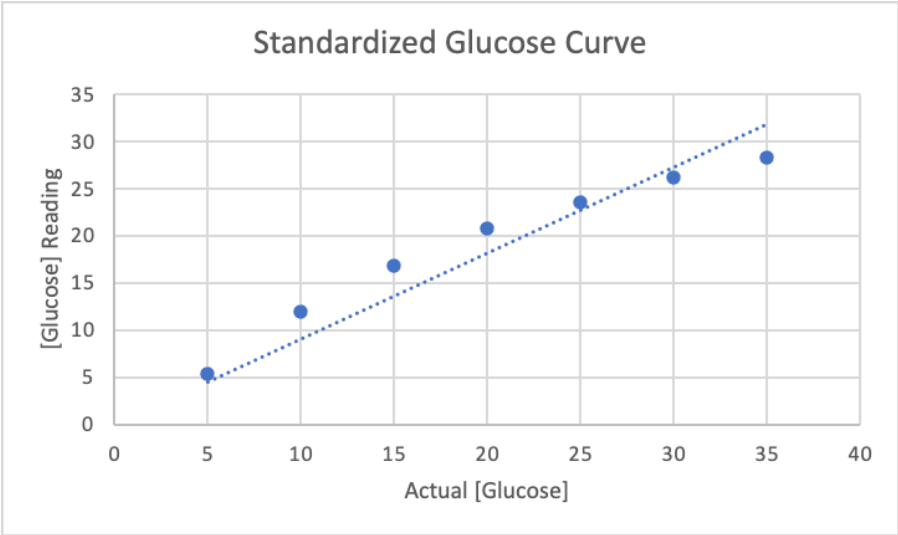

Figure 3. Standardized glucose curve of the OneTouch Ultra 2® blood glucose meter.