Effect Of Small Intestine pH On The Enzymatic Activity Of Lactase In Breaking Down Lactose

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Abstract

This study aimed to evaluate the enzymatic activity of β-galactosidase (lactase) at different pHs, quantified by reaction rate constants of lactose hydrolysis. Lactose, composed of galactose and glucose, is a fundamental nutrient and primary ingredient in fermentative procedures (Adam et al., 2005). The experiment monitored the glucose levels at various time intervals during lactose hydrolysis reactions to investigate the impact of pH on lactase enzyme activity. Lactaid® Extra Strength pills containing lactase were mixed with 50 mmol/L lactose. We recorded glucose concentrations every 5 minutes for 15 minutes at ~37.5°C to determine lactose hydrolysis reaction rates with enzyme catalysis at pH 5, 6, 7, and 8. Reaction rate graphs were produced to assess the degradation of lactose under varying pH conditions, and rate laws were derived to describe the reactions. The mean rate constant for lactose hydrolysis at pHs 5, 6, 7 and 8 were respectively $k = 0.1619\ min^{-1}$, $k = 0.0975\ min^{-1}$, $k = 0.1037\ min^{-1}$ and $k = 0.1804\ min^{-1}$. The findings demonstrated that reaction kinetics were greater at pH 5 and 8, unlike what we expected. From this, we can conclude that pH does significantly impact lactase activity.

Introduction

The enzyme lactase-phlorizin hydrolase, more commonly known as lactase, is a β-galactosidase found within the brush border of the small intestine in mammals (Lomer et al., 2007). This enzyme is responsible for the hydrolysis of lactose, a unique disaccharide present only in mammalian milk. Lactose is broken down into two monosaccharides, glucose and galactose, that are eventually absorbed by intestinal enterocytes and enter the bloodstream (Heyman, 2017). Both monosaccharides are extremely crucial to the human body; glucose is a source of energy and galactose is a major component of glycolipids and glycoproteins (Lynch & Buckin, 2022).

Evidently, lactase is a critical enzyme for daily metabolic activities. However, 70% of the world's population has lactase deficiency, thus failing to digest lactose and causing lactose intolerance (Lomer et al., 2007). Individuals with lactose intolerance cannot digest milk

products, resulting in symptoms such as abdominal cramping, abdominal distention and diarrhea (Heyman, 2017).

Several studies have explored the relationship between lactase enzyme efficiency at varying temperature values (Wendorff et al., 1970). However, very limited studies have been conducted to investigate the effect of lactase enzyme efficiency at pH values outside of normal small intestine pH ranges. This study will aim to explore and understand how varying small intestine pH values (ranging from normal to abnormal) affect the enzymatic activity of lactase in breaking down lactose. We plan to test enzymatic activity of lactase of 4 different pH values; 5, 6, 7, 8.

The prevalence of lactose intolerance differs amongst different ethnic groups (Heyman, 2017) and small intestine pH is found to differ within various age groups (Dressman et al., 1993). In populations where the diet consists predominantly of dairy foods, such as in European people, only 2% of the population experiences lactase intolerance. However, in populations where dairy is not a major component of diet, such as Hispanic, Asian and Indian people, 50%-90% of people experience lactose intolerance (Heyman, 2017). Moreover, small intestine pH of elderly individuals statistically differs compared to younger individuals (Dressman et al., 1993); the pH of elderly individuals is lower following a meal, potentially limiting the enzyme activity of lactase. Thus, the goal of this research experiment is to draw relationships between ethnicity, age, small intestine pH and lactase performance.

The optimal pH for lactase performance is approximately 6, but lactase can function in environments ranging between pH values of 2 to 7, which corresponds to the typical pH of the human small intestine (Popescu et al., 2021). We predict that the enzymatic activity of lactase will be the greatest at normal small intestine pH. Trials using pH 6 will likely have optimal

lactase activity, which is more within the normal range of small intestine pH. Comparably, trials using pH 5, 7, and 8 will likely have lower lactase activity, which are more outside the normal range of small intestine pH.

Methods

We first prepared four solutions which would later be added to 12 mL of 0.05 M lactose solution in our test tubes to reach the final pHs of 5, 6, 7, and 8 (see Appendix for sample calculations). We used 12 test tubes in total, 3 replicates for each pH. For our pH 5 and 6 trials, we made $3.4*10^{-5}$ M HCl solution and $3.4*10^{-6}$ M HCl solution via dilutions of 0.01 M HCl and deionized water. For our pH 7 trials, we prepared deionized water. For our pH 8 trials, we made $6.208*10^{-5}$ M $NaHCO_3$ solution via a dilution of 0.01 M $NaHCO_3$ and deionized water.

As a source of lactase, 18 Lactaid® Extra Strength pills were ground into fine powder using mortar and pestle.

For our pH 5 trials, three test tubes each received 12 mL of 0.05 M lactose solution, 5 mL of $3.4*10^{-6}$ M HCl solution, and 0.537 g of Lactaid® Extra Strength pill powder then vortexed and placed in a 40°C water bath. After every five minutes until fifteen minutes had passed, each test tube was vortexed and a glucose measurement was taken. The vortexing was necessary since the pill powder would settle to the bottom of the test tubes between glucose measurements. After vortexing, test tube solutions were cloudy white. To take a glucose measurement, 7 μL of solution from each test tube was pipetted to a piece of Parafilm to have its glucose concentration measured using the OneTouch Ultra 2® blood glucose meter in mmol/L. Since the test tubes cooled each time they were taken out of the water bath, their temperature on average was around 37.5°C.

The pH 5 trial procedure above was repeated for our pH 6, 7, and 8 trials. Instead of $3.4*10^{-5}$ M HCl solution, we used $3.4*10^{-6}$ M HCl solution for our pH 6 trials, deionized water for our pH 7 trials, and $6.208*10^{-5}$ M $NaHCO_3$ solution for our pH 8 trials.

In the beginning of our pH 5 trials, an incubator was used instead of a water bath due to unavailability.

Data Analysis

From obtaining the glucose concentrations over time in each replicate, we were able to determine the reaction rate constant of the lactose to glucose and galactose reaction facilitated by lactase for each replicate. Lactose hydrolysis is a first order reaction which has the integrated rate law:

$$Ln[A] = -kt + Ln[A_0]$$

[A] = substrate (lactose) concentration (mM), k = rate constant (min^{-1}), t = time (min), and $[A_0]$ = initial substrate (lactose) concentration (mM)

By subtracting the glucose concentrations over time from the initial lactose concentration (35 mM), we obtained the concentration of lactose over time. Then via Microsoft Excel, we graphed the natural logarithm of lactose concentration over time and used a linear regression to find the slope of each replicate (see Appendix). The slope for each replicate represents their rate constant.

Different regression models (linear, logarithmic, and quadratic) were tested and compared via ANOVA in R Studio to see if any of those models were able to significantly predict our rate constant data from the pHs they were obtained at. We opted to test regression models instead of a one-way ANOVA since a regression model allowed us to find if pH significantly affected the rate constant and gave us an idea of how pHs intermediate to our treatment levels would affect the rate constant.

Results

We found that the rate constant of the lactose to glucose and galactose reaction facilitated by lactase was predicted by pH with the quadratic regression model equation:

$$\text{rate constant in } min^{-1} = -0.4524(pH) + 0.0353(pH^2) + 1.5420$$

The quadratic regression model that we found was significant with a P-value of 1.182e-06 ($< \alpha = 0.05$) and adjusted R-squared of 0.9411.

As shown in Figure 1, the rate constant was high at pH 5 (mean = 0.1619 min^{-1}, 95% CI [0.1498, 0.1740] min^{-1}), dropped at pH 6 (mean = 0.0975 min^{-1}, 95% CI [0.0959, 0.0990] min^{-1}), rose slightly at pH 7 (mean = 0.1037 min^{-1}, 95% CI [0.0992, 0.1085] min^{-1}), then rapidly increased at pH 8 (mean = 0.1804 min^{-1}, 95% CI [0.1623, 0.1985] min^{-1}).

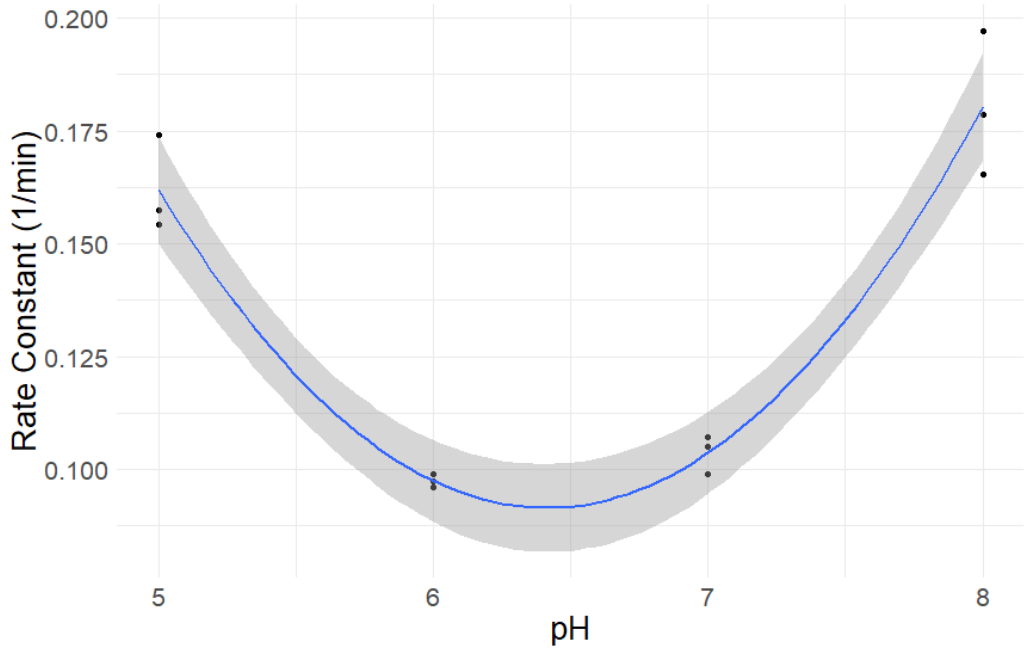

Figure 1. Effect of pH on reaction rate constant in min^{-1} of the lactose to glucose and galactose reaction facilitated by lactase with a quadratic regression line (blue) and standard error (shaded grey). P-value = 1.182e-06 < α = 0.05, adjusted R-squared = 0.9411, and N = 3.

Discussion

Lactase deficiency is a widespread complication causing digestive issues with people worldwide. Much research has been conducted in determining the cause, effect and treatment of lactose intolerance; however there have not been many studies tackling the potential effect of pH on the activity of lactase. Since small intestinal pH is a varying factor with people of different ages and diet (Dressman et al., 1993); our results will provide potential clues to the prominence of lactose intolerance in different people. Unfortunately, there are no studies that have investigated if small intestine pH varies significantly between ethnic groups. If those studies are conducted, we may be able to further understand why the prevalence of lactose intolerance varies based on ethnicity and if pH is a significant factor. Additionally, the regression model of our

study can be utilized to predict the lactose digestion of an individual given their small intestine pH.

The purpose of our study is to determine the optimal pH for lactase efficiency within the normal range of small intestine pH, by evaluating the breakdown of lactose at four different pHs ranging from 5 - 8 at the normal body temperature of 37.5°C. Despite the vast variation of small intestinal pH across various ages (Heyman, 2017; Dressman et al., 1993), we hypothesized that the enzymatic activity of lactase will be the greatest at a pH of 6 since this is approximately the typical small intestine pH in healthy individuals (Aburb et al., 2018; Dressman et al., 1990). Contrary to our prediction, our findings demonstrated that the reaction rate of lactase was highest at pH 5 and 8, and lowest at the pH of 6 as seen in Figure 1. We found that the mean rate constant for lactose hydrolysis at pHs 5, 6, 7 and 8 were respectively $k = 0.1619\ min^{-1}$, $k = 0.0975\ min^{-1}$, $k = 0.1037\ min^{-1}$ and $k = 0.1804\ min^{-1}$. This result was unexpected since the normal pH of the small intestine is around 6 (Aburb et al., 2018; Dressman et al., 1990), and lactase performs its function within this exact environment. Dressman et al., found in 1990 that healthy young individuals have duodenal pH of 5.4 to 6.1; in 1993, they found that elderly individuals have a duodenal pH of around 6.5. With our results, we can expect that elderly individuals may have more difficulty breaking down lactose compared to younger individuals since the enzymatic activity of lactase is reduced at pH 6-7 compared to pH 5-6.

Possible flaws in our experiment and sources of error may also contribute to our unexpected conclusion. Our source of lactase comes from grinding Lactaid® Extra Strength pills into a fine powder to dissolve into solution. There is a limit to how fine the powder could be ground based on the equipment available, so a portion of the powder remains in suspension. This increases the chance of settlement and also results in an uneven concentration of lactase being

sampled. The solutions were kept to their desired temperature through two different methods: water bath and incubator; both of which have their own flaws. The water bath was unable to submerge our test tubes entirely. The incubator was slow to warm up the solution to our desired temperature through convection and was frequently opened by others. The two methods were used interchangeably based on their availability during the lab, which produced inconsistencies since samples were heated for the same duration for either method. Setting up the glucose meter and vortexing each sample requires an abundance of time. Due to this, samples that have been taken out of the 37.5°C environment would spend a prolonged amount of time cooling down in room temperature, negatively impacting the accuracy of our results.

Future studies may be conducted to evaluate the effect of pH and temperature in conjunction on lactase efficiency. It has been established that a higher temperature would result in faster enzymatic reaction rates (Choi et al, 2022); which is why we have chosen a constant temperature of 37.5°C for all of our experimental trials in addition to 37.5°C being the normal internal temperature of humans. Further experimentation may be done under cooler conditions, or even conditions exceeding normal body temperature, along with varying pH to test how the two factors may interact and each play a distinct role in lactase efficiency. Since our results did not align with our prediction, small adjustments to the procedure could yield more useful results or confirm our findings. Such adjustments include decreasing the interval in between different pHs to precisely locate the optimal pH; and testing more acidic and basic environments since the pHs that yielded the highest reaction rates were on the ends of the spectrum. Investigations may be conducted to determine why the normal small intestine pH is not optimal for lactose breakdown; henceforth finding an approach to improve lactase efficiency in the small intestine.

Conclusion

The hypothesis that pH affects lactase activity was proved by the results of this study. The null hypothesis, which proposed that pH does not affect the breakdown of lactose was rejected. Moreover, the results did not confirm the prediction that lactose would be broken down into glucose and galactose at a faster rate at pH 6 which is the pH of the small intestine, compared to the other tested pH levels in the presence of lactase. It is possible that various sources of error in our procedure contributed to these results.

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Appendix

Sample Acid/Base and pH Calculations

Since HCl is a strong acid, all the H+ ions will dissociate.

To achieve pH 5 using HCl, we need:

$$[H_3O^+] = 10^{-pH} = \mathbf{10^{-5}\ M\ HCl}$$

HCO_3^- is a weak base and won't dissociate completely.

K_a of H_2CO_3 is at 40°C is 5.058×10^{-7} (Harned & Davis, 1943) and K_w at 40°C is $10^{-13.533}$ (Butler, 1982). Therefore, K_b of HCO_3^- at 40°C is:

$$K_b = \frac{K_w}{K_a} = \frac{10^{-13.533}}{5.058 \times 10^{-7}} = \mathbf{5.795 \times 10^{-8}}$$

	$HCO_3^-{}_{(aq)}$	+	$H_2O_{(l)}$	⇌	$H_2CO_{3(aq)}$	+	$OH^-{}_{(aq)}$
I	b		/		0		0
C	-x		/		+x		+x
E	b-x		/		x		x

$$K_b = \frac{[H_2CO_3][OH^-]}{[HCO_3^-]} = \frac{x^2}{b-x} = 5.795 \times 10^{-8}$$

To achieve pH 8, we need $[OH^-]$ to be:

$$[OH^-] = 10^{-(14-pH)} = \mathbf{10^{-6}\ M\ OH^-}$$

This means we need HCO_3^- to be:

$$K_b = 5.795 \times 10^{-8} = \frac{[H_2CO_3][OH^-]}{[HCO_3^-]} = \frac{x^2}{b-x} = \frac{(10^6)^2}{b-10^6}$$

$$[HCO_3^-] = \mathbf{1.82575 \times 10^{-5}\ M}$$

Sample Dilution Calculations

$$C_1V_1 = C_2V_2$$

Since 5 mL of our prepared HCl solution will be diluted with 17 mL lactose solution in each test tube, we need to know what concentration of HCl in our prepared solution is needed to reach 10^{-5} M to get pH 5 after this dilution.

$$C_1 = \frac{C_2V_2}{V_1} = \frac{(10^{-5}\ M)(17\ mL)}{(5\ mL)} = \mathbf{3.4 \times 10^{-5}\ M}$$

To have 25 mL our desired HCl concentration of 3.4×10^{-5} M, we need to dilute the following volume of stock HCl with a concentration of 0.001 M:

$$V_1 = \frac{C_2V_2}{C_1} = \frac{(3.4 \times 10^{-5}\ M)(25\ mL)}{(0.001\ M)} = \mathbf{0.85\ mL}$$

Rate Constant Determination

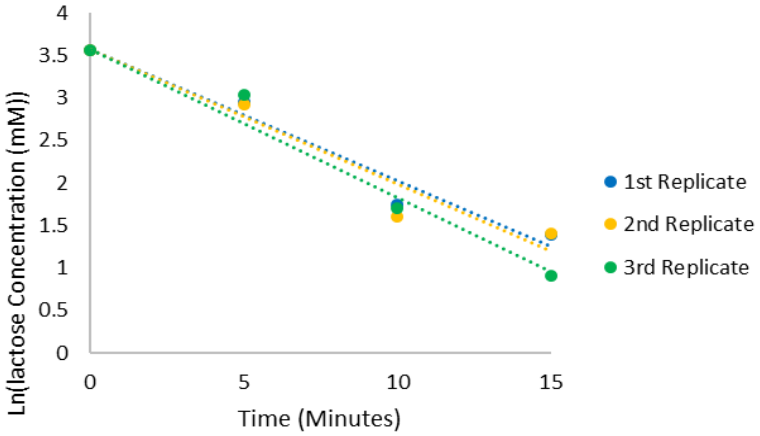

Figure 2. Ln(lactose concentration in mM) over time in minutes at pH 5 for three different replicates.

Replicate 1: Ln(lactose concentration(mM)) = -0.1542(min^{-1}) + 3.5637(Ln(mM))
Rate constant = 0.1542 min^{-1}
Replicate 2: Ln(lactose concentration(mM)) = -0.1573(min^{-1}) + 3.5637(Ln(mM))
Rate constant = 0.1573 min^{-1}
Replicate 3: Ln(lactose concentration(mM)) = -0.1741(min^{-1}) + 3.5637(Ln(mM))
Rate constant = 0.1741 min^{-1}
Mean rate constant at pH 5: 0.1619 min^{-1}

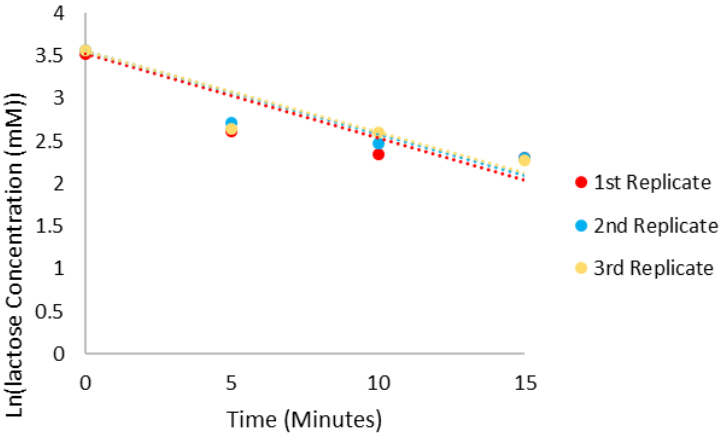

Figure 3. Ln(lactose concentration in mM) over time in minutes at pH 6 for three different replicates.

Replicate 1: Ln(lactose concentration(mM)) = -0.0989(min^{-1}) + 3.5232(Ln(mM))
Rate constant = 0.0989 min^{-1}
Replicate 2: Ln(lactose concentration(mM)) = -0.0974(min^{-1}) + 3.5637(Ln(mM))
Rate constant = 0.0974 min^{-1}

Replicate 3: Ln(lactose concentration(mM)) = -0.0962(min^{-1}) + 3.5637(Ln(mM))

Rate constant = 0.0962 min^{-1}

Mean rate constant at pH 6: 0.0975 min^{-1}

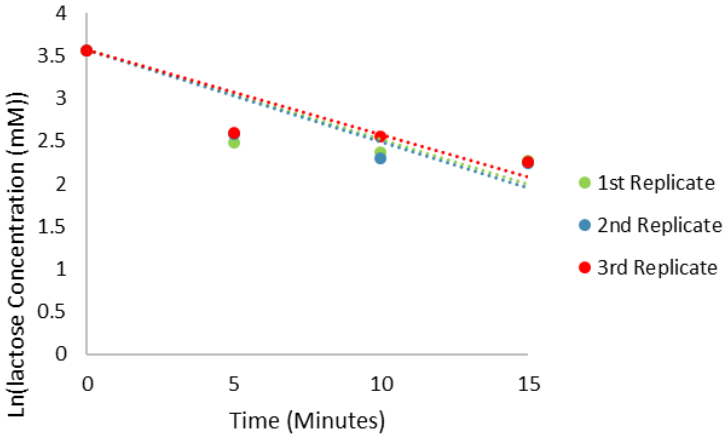

Figure 4. Ln(lactose concentration in mM) over time in minutes at pH 7 for three different replicates.

Replicate 1: Ln(lactose concentration(mM)) = -0.1050(min^{-1}) + 3.5637(Ln(mM))

Rate constant = 0.1050 min^{-1}

Replicate 2: Ln(lactose concentration(mM)) = -0.1071(min^{-1}) + 3.5637(Ln(mM))

Rate constant = 0.1071 min^{-1}

Replicate 3: Ln(lactose concentration(mM)) = -0.0989(min^{-1}) + 3.5637(Ln(mM))

Rate constant = 0.0989 min^{-1}

Mean rate constant at pH 7: 0.1037 min^{-1}

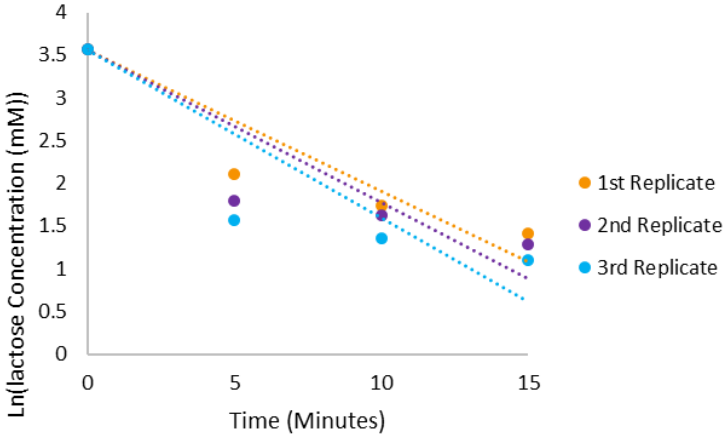

Figure 5. Ln(lactose concentration in mM) over time in minutes at pH 8 for three different replicates.

Replicate 1: Ln(lactose concentration(mM)) = -0.1653(min^{-1}) + 3.5637(Ln(mM))
Rate constant = 0.1653 min^{-1}
Replicate 2: Ln(lactose concentration(mM)) = -0.1785(min^{-1}) + 3.5637(Ln(mM))
Rate constant = 0.1785 min^{-1}
Replicate 3: Ln(lactose concentration(mM)) = -0.1972(min^{-1}) + 3.5637(Ln(mM))
Rate constant = 0.1972 min^{-1}
Mean rate constant at pH 8: 0.1804 min^{-1}

Statistical analysis of different regression models on the rate constant data from each trial

Regression Model	**P-value** (smaller is better)	**Adjusted R squared** (closer to 1 is better)
Linear	0.4218	-0.02787
Logarithmic	0.7106	-0.0842
Quadratic	1.182e-06*	0.9411

Comparisons between quadratic model and other models using anova()

Regression Model	**P-value**
Log vs. Quadratic	3.33e-7*
Quadratic vs. Linear	0.02277*