Looking Into Lactase

Understanding Enzyme Specificity and Activity

LEARNING UNDEFEATED
Educate | Elevate | Empower

Name:

Enzymes are proteins that catalyze chemical reactions by lowering the reaction's activation energy. Every enzyme has an active site that binds to another molecule called the substrate. Once bound to the substrate, an enzyme can catalyze a reaction up to 10 billion times faster than the comparable, non-catalyzed reaction. In other words, a reaction that only occurs once every ten billion seconds (316 years) will occur once every second if catalyzed by an enzyme. Enzymes are not consumed in the reaction and can bind to an infinite amount of substrates.

TABLE 1: Carbohydrate Breakdown in Cow's and Soy Milk

Milk	Carbohydrate in the Milk	Monosaccharide Products
Cow	Lactose (disaccharide)	Galactose & Glucose
Rice	Glucose (monosaccharide)	N/A
Soy	Sucrose (disaccharide)	Glucose & Fructose

Lactase deficiency, also known as lactose intolerance, is a condition caused by an absence of the enzyme lactase, a digestive enzyme found in the human body. Lactase binds to the carbohydrate lactose, a disaccharide sugar which consists of two joined monosaccharides. In particular, lactase breaks down lactose into galactose and glucose. Because the human body can't absorb disaccharides, it needs the help of enzymes like lactase to break them down into usable, monosaccharide products.

You are working in the quality control department of a local bioscience company that produces a lactase enzyme product used to treat lactose intolerance. It is your job to determine the optimal pH of the lactase product by testing the enzyme's activity in cow's milk at different pH levels. Unfortunately, due to a mistake in shipping, the labels were removed from the research lab's milk supply. You know that the lab carries cow, rice, and soy milk, but you don't know which is which. Before you can test the lactase activity at different pH levels, you must first identify which sample is cow's milk. To do this, you will use a special property of enzymes called "specificity." Lactase specificity describes the fact that the lactase enzyme will break down lactose but no other disaccharide substrate.

You know that each type of milk contains a unique carbohydrate. Cow's milk and soy milk both contain disaccharides which have glucose as one of their monosaccharide products (table 1). Rice milk, on the other hand, simply contains the monosaccharide glucose. You will determine which of the unidentified samples is cow's milk by testing the milk samples for glucose both prior to and following the addition of the lactase enzyme. After you identify which sample is cow's milk, you can determine the optimal pH of the lactase enzyme product.

What type of milk should test positive for the monosaccharide glucose (prior to adding lactase)?

MATERIALS

- Glucose test strips
- Micropipette & tips
- pH buffer solutions
- Lactase solution
- Test tubes (x5) Unidentified milk samples (x3)

PART I — Identify the cow milk

☐ 1. Locate the three test tubes that contain the unidentified milk samples, and predict which milk is in each tube using qualitative observations.

TABLE 2: Qualitative Milk Analysis	
A	
B	
C	

☐ 2. Locate the glucose test strips. Glucose test strips are used to test for glucose levels and will change color in the presence of glucose.

☐ 3. Label the three glucose test strips A, B, and C. Dip each strip into its corresponding test tube and immediately place each strip on your paper towel. In the table below, record any color change and the relative amount of glucose present.

TABLE 3: Quantitative Milk Analysis

	Test strip color	Relative amount of Glucose (mg/dl)
A		
B		
C		

☐ 4. Mix the lactase enzyme by inversion. Add 250uL to each test tube.

☐ 5. Locate your mini-vortex and set the dial to eight. Vortex each tube for two seconds.

QUICK CHECK:

? Predict which type(s) of milk will test positive for glucose after adding the enzyme. ___________________________

☐ 6. Label three new glucose test strips A, B, and C. Dip each strip into its corresponding test tube and immediately place each strip on your paper towel. Record any color change, the relative amount of glucose present, and whether or not the enzyme was active in the sample.

TABLE 4: Quantitative Milk Analysis – Enzyme Activity

		Post-Lactase Glucose Test Strip	
	Test strip color	Relative amount of Glucose (mg/dl)	Enzyme Activity
A			☐
B			☐
C			☐

Based upon your analysis what type(s) of milk did the enzyme effect and why is it used to treat lactose intolerance?

PART II — Determine the optimal pH condition for the enzyme lactase

- ☐ 7. Locate the five (5) empty test tubes. Label the test tubes **2, 4, 7, 10** and **12**.
- ☐ 8. Locate the pH buffer solutions labeled 2, 4, 7, 10 and 12. Add 500 µL of each pH buffer to the appropriate test tube.
- ☐ 9. Locate the lactase enzyme and mix by inversion.
- ☐ 10. Add 250 µL of the lactase enzyme to each test tube.
- ☐ 11. Add 500 µL of cow's milk to each of the experimental samples. Vortex each tube for two (2) seconds.
- ☐ 12. Label five new glucose strips and dip the labeled strips into the appropriate test tube. Immediately lay the test strip on the paper towel. Record your observations in Table 5.

TABLE 5: Enzyme Activity at Different pH Levels

	Test strip color	Relative amount of Glucose (mg/dl) after adding lactase
pH 2		
pH 4		
pH 7		
pH 10		
pH 12		

PART III — Data analysis

Graph the relative amount of glucose to the corresponding pH on the graph. Be sure to give your graph a title and label the axes.

❓ What is the independent variable?____________________

❓ What is the dependent variable?____________________

❓ What effect (if any) does pH have on lactase activity?

PART IV — Conclusion

Label each section of the digestive tract with its specific pH. Compare the results found in PART III to the pH's found in the digestive tract. Does the enzyme work where it needs to? What temperature do you think is optimum for the enzyme lactase?

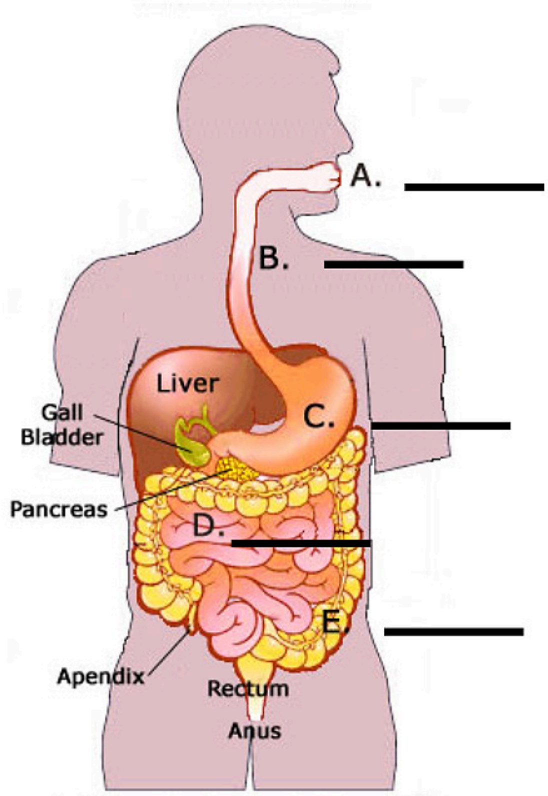

Figure 1