Name:

Looking into Lactase

Understanding Enzyme Specificity and Activity

Lactose intolerance is a medical condition that is due to little or no activity of the enzyme lactase. Normally the lactase enzyme breaks down lactose (a sugar) into glucose and galactose, simple sugars that are absorbed by the intestines. However, with low lactase activity in the small intestine, undigested lactose is passed into the colon where bacteria ferment the sugar to hydrogen gas and organic acids. Symptoms of lactose intolerance can include bloating, abdominal pain, cramps, gas, flatulence, and sometimes diarrhea.

http://www.freedigitalphotos.net/images/Livestock_g109-Cow_p1250.html

The symptoms usually are noticed anywhere from 30 minutes to two hours after the ingestion of dairy products containing lactose. The severity of symptoms varies by individuals depending upon how much lactose is consumed and whether it is consumed alone or with other foods; and on the type of dairy products, because some dairy products contain more or less lactose. Because the causation of lactose intolerance is well studied, biotechnology can be used to develop new products to treat the symptoms of this medical condition.

http://www.healthinlife.com/wp-content/uploads/2009/07/milk-300x290.jpg

Lactase

Lactase is an enzyme that is used by the body to hydrolyze lactose, a disaccharide (two sugars) unique to mammalian milk, into the monosaccharides glucose and galactose. Lactose has been shown to aid in the absorption of several minerals, including calcium, magnesium, and zinc.

Enzymes

Enzymes are organic catalysts that control the rate of chemical reactions in cells while not being permanently changed. The enzymes are not consumed in the reaction. In general, enzymes speed up the rate of reaction by lowering the activation energy required to start reactions. Enzymes are extremely efficient. They can catalyze reactions at rates up to 10 billion times higher than comparable non-catalyzed reactions. By their specific configuration, they hold the reactant molecules in close proximity and in the correct orientation for the reaction to occur. Each enzyme has a specific site (the “active site”) where the substrate and enzyme combines. Enzymes also show specificity in that each specific type of enzyme acts on a particular substrate or on a certain kind of chemical bond. The specificity of enzymes is due to their structures. Each enzyme has a characteristic three- dimensional shape. The analogy of a “lock and key” is often used to illustrate the temporary joining of a specific enzyme to a specific substrate in an enzyme-substrate complex.

High temperatures can cause an enzyme to stop functioning (There are exceptions such as the enzymes in thermophilic bacteria). This is due to the denaturing (unfolding of the 3-D structure) of the enzyme. At lower temperatures, the 3-D structure is in one piece, but there may not be enough energy for a reaction to occur. In this case, the enzyme is inactive, but not denatured. In addition to temperature, pH levels also affect enzymes. Different enzymes have different optimal pH ranges. Exposure to pH levels outside the optimal range can denature the enzyme protein.

You are working part-time at “Biome” Restaurant as a waiter/waitress during the breakfast rush. You have a table with a customer who is lactose intolerant and orders a glass of soymilk. You head back to the service window to find three glasses of milk. You ask the prep helper which glass is yours, and she is unable to remember. She tells you that she poured one glass of cow’s milk, one glass of rice milk, and one glass of soymilk. You decide to test the milk with glucose strips and Lactaid Ultra®, a lactase supplement pill, to identify the cow’s milk to ensure you do not give it to your customer. You know that cow’s milk has the sugar lactose, which in the presence of lactase breaks down into glucose and galactose. Rice milk has maltose, which breaks down into glucose and glucose. Soymilk has sucrose, which breaks down into fructose and glucose. Now, get to work!


http://3.bp.blogspot.com/_HnYemNi0lds/SGDzL5_lzpI/AAAAAAAAHPE/AC3Unr1YXjQ/s400/6+WAITRESS.jpg

IDENTIFY THE PROBLEM

What is the problem you are trying to solve?

What enzyme is in the Lactaid Ultra pill that helps to identify cow’s milk?

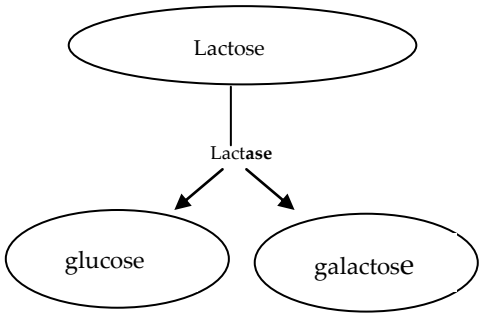
How will you use the Lactaid Ultra® pill and glucose test strips to identify the cow’s milk? What type of experiment could you set-up?

EXPERIMENT

You will need the following materials and equipment:

Marker	Glucose test strips	Micropipette & tips (100 – 1000 μL)
Lactase solution	Test tubes	Milk (cow, rice, soy)
pH Buffer solutions	Pipette tips	Test tube rack

PART ONE: IDENTIFY THE COW MILK



☐ 1. Each milk type has a unique sugar.

Milk	Disaccharide Sugar	2 Monosaccharide Sugars
Cow		
Soy		
Rice		

☐ 2. Locate the packet of Glucose test strips at your station. Glucose test strips are very common and can be found in drugstores such as CVS and Walgreens. These strips are frequently used to test for blood glucose levels. Test strips for lactose and other sugars are less common.

QUICK CHECK: How could you use the glucose test strips in your experiment? _____

_____

_____

☐ 3. Locate the three test tubes that contain the different milk samples.

QUICK CHECK: Why are the three different tubes of milk not labeled? _____

_____

☐ 4. Identify the three unknown milk samples labeled 1, 2, and 3 at your station using qualitative observations (i.e. your 5 senses). **Record your qualitative observations in Table 1 below.**

QUICK CHECK: How could you <u>quantitatively</u> identify the milk types? _____

_____

_____

☐ 5. Identify the unknown milk samples using the glucose test strips. Label three glucose test strips as 1, 2 and 3. Dip the labeled strips into the appropriate test tube. Immediately lay the test strip on the paper towel. **Record your quantitative observations in Table 1 below in the *pre-lactase* column.**

TABLE 1: Qualitative Milk Analysis

		Pre-Lactase Glucose Test Strip	
Sample Number	**Qualitative Observation** (Describe what you see in each tube)	**Color of Test Strip**	**Relative amount of Glucose** (mg/dl)
1			
2			
3			

Can you identify one of your samples? Yes / No (circle one)

If yes, how, which sample, and what type of milk do you think it is? _____

_____

_____

What can you do now with your samples? _____

_____

_____

PART TWO: VERIFY THE DIFFERENT TYPES OF MILK

Perform the following steps to continue your experiment to determine the different types of milk.

☐ 6. Locate the lactase enzyme supplement. (Note: The pill has been crushed and added to distilled water for you.) Locate your mini-vortex. Make sure the speed dial is at eight (8). *You do not want milk flying out of your tube!* Vortex each tube for two (2) **seconds**.

☐ 7. Add 250uL of the lactase enzyme to each milk type.

☐ 8. Vortex each tube again for two (2) seconds.

QUICK CHECK: What is the lactase enzyme expected to do to each type of milk?

Cow's Milk: _____

Soymilk: _____

Rice Milk: _____

Record two (2) observations regarding the milk samples after the second vortex step (step 8):

1. _____

_____

2. _____

_____

☐ 9. Label three new glucose test strips "1, 2 and 3." Dip the labeled strips into the appropriate test tube. Immediately lay the test strip on the paper towel. **Record your quantitative observations in Table 2 in the *post*-lactase columns below.**

TABLE 2: Milk Identification

		Post-Lactase Glucose Test Strip	
Sample Number	**Color of Test Strip**	**Relative amount of Glucose** (mg/dl)	**Milk Type** (after tests complete)
1			
2			
3			

What can you conclude about the milk samples based on your observations of the lactase enzyme? _____

_____

_____

Identify each sample as the following milk type: cow, soy, rice and record in the Table 2 under Milk Type above.

There are two different variables in part 2 of this experiment, a dependent variable (what you are actually measuring) and an independent variable (the variable that changes and is used to measure the dependent variable). Identify the different variables in part 2 of this experiment.

Independent variable: _____

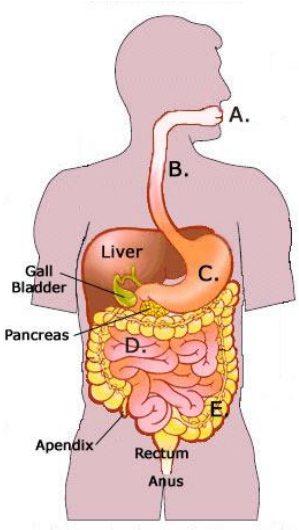
Dependent variable: _____

Holy cow! Your co-worker mistakenly took the cow's milk instead of the rice milk and delivered it to a high profile customer who is lactose intolerant. You have Lactaid Ultra®, but the directions on the label are unreadable and you cannot remember if the pill can be swallowed. You remember learning about the pH levels in the digestive system in school. At what pH levels will the lactase supplement work? Where should it work? Will it be safe to swallow? If you can determine at what pH the supplement is most effective, you may be able to help.

PART THREE: MEASURE THE EFFECT of pH on LACTASE ACTIVITY

http://www.i-can-drink-milk-again.com/images/Human-Digestive-System-Picture.jpg

Your digestive system has different pH levels throughout. Label the pH at points A-E.



A.

B.

C.

D.

E.

Perform the following steps to continue your experiment to determine the effects pH may have on lactase activity.

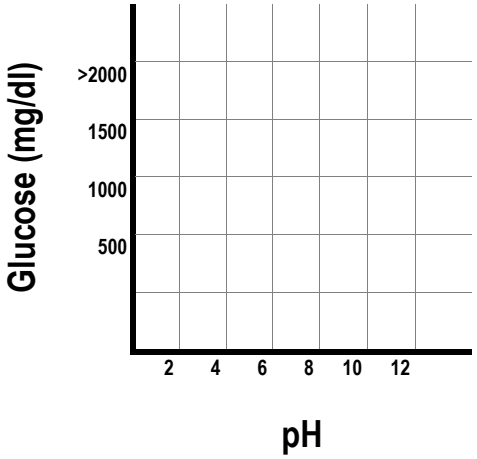
- ☐ 10. Locate five (5) empty test tubes. You will need these tubes to mimic the wide range of pH values our tablet will encounter in the digestive tract.
- ☐ 11. Label your test tubes pH 2, 4, 7, 10 and 12.
- ☐ 12. Locate the pH buffer solutions pH 2, 4, 7, 10 and 12. Add 500uL of each pH buffer to the appropriate test tube.
- ☐ 13. Locate the lactase enzyme product "Lactase." Gently mix the "Lactase" by inversion.
- ☐ 14. Add 250uL of the lactase enzyme to each pH tube and the water control. Vortex each tube for two (2) seconds.
- ☐ 15. Incubate all experimental samples at room temperature for three (3) minutes.
- ☐ 16. Add 500uL of cow milk to the control and all experimental samples. Vortex each tube for two (2) seconds.
- ☐ 17. Incubate all experimental samples and the control for three (3) minutes.
- ☐ 18. Prepare to test if your lactase enzyme is still active. Get six new glucose test strips. Label strips appropriately. Dip the labeled strips into the appropriate test tube. Immediately lay the test strip on the paper towel. **Record your observations in Table 3.**

TABLE 3: Effects of pH on lactase activity

pH	**Color of Test Strip**	**Relative amount of Glucose** (mg/dl) after adding lactase
2		
4		
7		
10		
12		

Based on your results, what is the best pH range for lactase activity? _____

- ☐ 19. Confirm the optimal pH range for lactase activity by graphing the relative amount of glucose to the corresponding pH below on the graph.



Identify the different variables in part three of the experiment:

Independent variable: _____

Dependent variable: _____

DATA ANALYSIS

It is time to analyze the results of your experiment. Think through what your results indicate. Use the following questions to help you along:

What were the problems you identified and used this experiment to solve?

Why did you use glucose test strips?

Lactase breaks lactose into which two sugars?

What is the optimum pH range for Lactase? How do you know?

Does lactase enzyme work on all the milk types? Why or why not?

Why do you think enzyme specificity is important?

Explain the effect changing the environment from an alkaline (basic) environment to an acidic environment has on the activity of the lactase enzyme.

CONCLUSION

Make your own conclusion. Based on the results of your experiment, would Lactaid Ultra® be safe to take as a pill by mouth? What other conditions might have an affect on enzyme activity? Can you conduct further experiments to test these conditions? What factors would you need to consider?