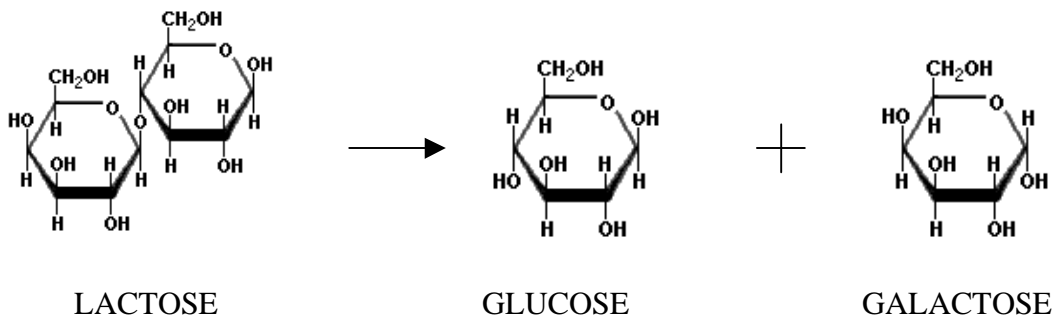


The Breakdown of Complex Sugars by Beano™ and Lactaid™

This lab will use two commercially available enzyme preparations, Lactaid™ and Beano™, to break down disaccharides and oligosaccharides found in foods. The presence of the end product of these reactions, glucose, will then be assayed using another enzyme complex: glucose trinder (a combination of glucose oxidase and peroxidase). The amount of glucose present will be determined by means of a glucose standard curve.

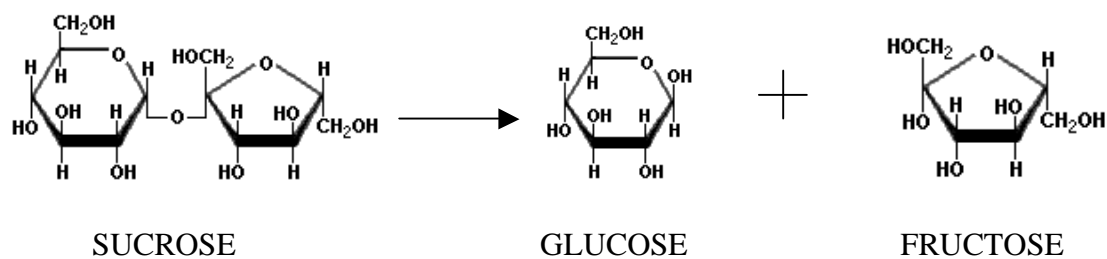
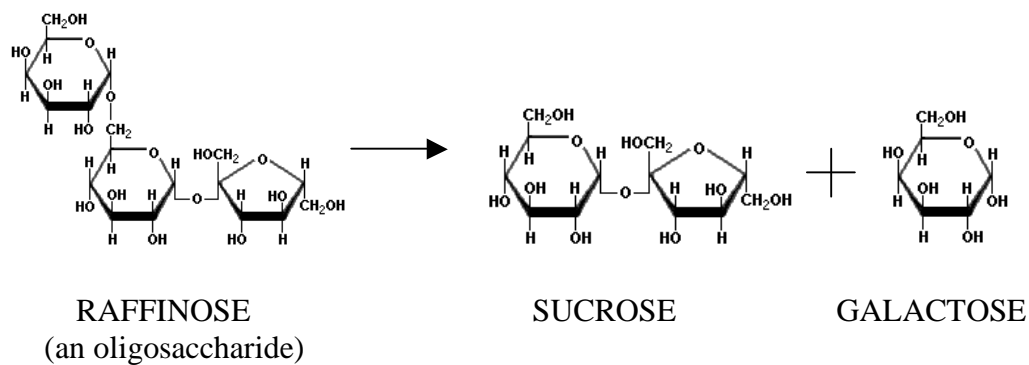
Lactaid™

Lactaid™, a supplement taken by lactose intolerant individuals, contains the enzyme β -galactosidase, also referred to as lactase. This enzyme breaks down the disaccharide lactose, a sugar unique to mammalian milk, to the monosaccharides glucose and galactose. People that are lactose intolerant produce little or none of this enzyme so lactose passes through the small intestine undigested. Bacteria in the colon are able to digest lactose, but in doing so gas is produced which causes the GI symptoms often associated with lactose intolerance – bloating, abdominal pain, cramps, and flatulence. These symptoms often occur 30 minutes to 2 hours after the ingestion of dairy products. Taking Lactaid™ with dairy products prevents these symptoms because the lactose is broken down to monosaccharides before it reaches the colon.



Beano™

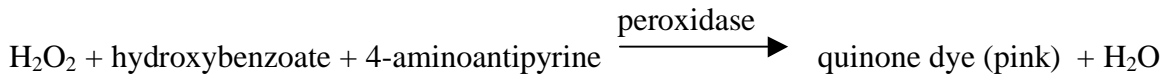
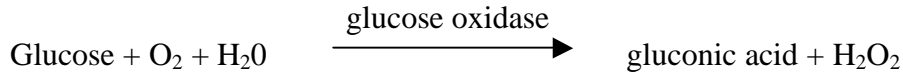
Legumes (e.g. beans and peas) and cruciferous vegetables (e.g. Brussels sprouts, broccoli, and cauliflower) contain complex sugars known as oligosaccharides. Some of these carbohydrates can be digested in the small intestine, but those that aren't pass into the colon where they are broken down by bacteria which produces gas. Beano™ is a digestive aid that contains two enzymes, α -galactosidase (produced by the fungus *Aspergillus niger*) and sucrase. First, α -galactosidase breaks down oligosaccharides to galactose and sucrose. Then sucrase breaks down the sucrose to glucose and fructose, which can be absorbed by the blood stream.



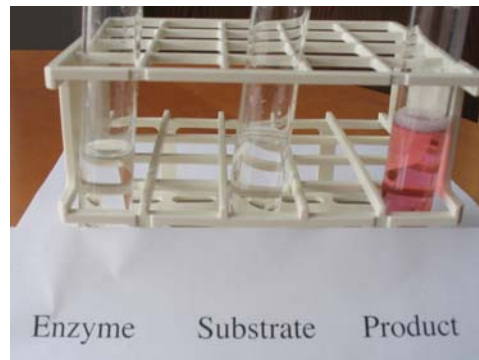
GLUCOSE TRINDER

Glucose trinder is a mixture of two enzymes, glucose oxidase and peroxidase. Glucose oxidase, isolated from the cell wall of the fungus *Aspergillus niger*, has been used extensively in clinical chemistry to determine blood glucose levels. Most glucose tests used by individuals with diabetes contain glucose oxidase because it is so specific for glucose. Glucose oxidase catalyzes the reaction of glucose to gluconic acid and hydrogen

peroxide. Peroxidase then catalyzes the reaction of hydrogen peroxide to quinone dye, which is pink in color.



Because the product of the overall reaction is colored (pink) we can use a spectrophotometer to get an absorbance reading for the reaction. An absorbance reading greater than zero indicates the pink product is present, thus glucose must have been present in the original sample. The more glucose present, the more pink product is formed.



A. Preparation

Materials:

- Glucose (0.1 mg/mL)
- Lactaid Fast Act Chewable Tablet - 1 Lactaid tablet ground up and dissolved in 50 ml dH₂O, filter, and put into a container
- Beano Tablet - 1 Beano tablet ground up and dissolved in 50 ml dH₂O, filtered, and put into a container
- Enzyme- glucose trinder from Diagnostics Ltd (reconstituted to 100 ml with dH₂O)
- Skim milk
- Pea Juice (filtered liquid from split peas soaked in water overnight)

B. Procedure

1. Glucose standard curve and unknowns

We will add glucose trinder to known amounts of glucose and then obtain an absorbance reading for the product produced in each reaction. The more glucose present, the more pink product is formed. From these data we can make our standard curve by graphing mg glucose vs. absorbance of product @510nm.

We can then add glucose trinder to our unknown, get an absorbance reading, and then use the standard curve to find the amount of glucose present in the unknown.

a. Make the glucose standard curve

1. Set up six test tubes with the volumes of glucose (0.1mg/mL), water, and enzyme listed in the table below. Mix.
2. Wait 10 minutes
3. Read tubes @510nm. TT 1 will be used as the blank.
4. Plot the glucose standard curve (mg glucose on the x-axis, absorbance@510nm on y-axis)

Test Tube	ml Glucose (0.1mg/mL)	ml dH ₂ O	ml Enzyme (glucose trinder)	mg glucose	Absorbance @510 nm
1	0.0	4.0	1.0	0	
2	0.1	3.9	1.0	0.01	
3	0.3	3.7	1.0	0.03	
4	0.5	3.5	1.0	0.05	
5	0.7	3.3	1.0	0.07	
6	1.0	3.0	1.0	0.1	

b. Assaying an unknown

Before we assay for glucose in our foods treated with Lactaid™ and Beano™, we'll do a trial run with a solution of glucose of unknown concentration.

1. Put 1.0 ml of the unknown into test tube #1 and 0.1 ml of the unknown into test tube #2. Why are we using two different volumes?
2. Bring the volume in each tube up to 4.0ml with dH₂O.
3. Add 1 ml of enzyme to each tube. Mix. Wait 10 minutes
4. Read at 510nm. Use TT1 from the standard curve for the blank.
5. Using the standard curve (equation of the line) determine the amount of glucose in your unknown and then the glucose concentration of the sample.

Test Tube	ml of unknown	ml dH ₂ O	ml Enzyme (glucose trinder)	Absorbance @510 nm	mg glucose	glucose concentration mg/ml
1	0.1	3.9	1.0			
2	1.0	3.0	1.0			

C. Determining the amount of glucose in foods treated with Lactaid™ and Beano

To assay for the amount of glucose produced after adding Lactaid™ and Beano™ to foods:

1. Set up test tubes A-F as described in table below. Mix. Wait 5 minutes
2. Using tubes A-F set up tubes A2-F2. Mix. Wait 10 minutes
3. Read at 510nm. Use TT1 from the standard curve as your blank.
4. Using the standard curve (equation of the line) determine the amount of glucose in your unknowns and then the concentration of glucose in your sample.

2. Extensions

Both Lactaid™ and Beano™ may be used to look at the effect temperature, pH, substrate concentration, or enzyme concentration may have on an enzyme reaction. Testing for the presence of glucose using either the glucose trinder or diastrix will tell whether or not the reaction has occurred after a change of any of the above-mentioned variables.

3. Preparation Tips

- a. Some Lactaid tablets have a coating that contains glucose so a positive result will occur in the control. To remedy this, use Lactaid Fast Act Chewable Tablets™ or try to find drops. Lacteeze™, a liquid form of the enzyme may be ordered from digestmilk.com or fitmart.com
- b. Glucose-testing strips such as Diastrix™ (cut into narrower strips) may be used to quickly test for the presence of glucose in solutions.

Test Tube	Contents	Test Tube	Contents	Glucose present?	Absorbance @510 nm	mg glucose	glucose concentration mg/ml
A	1.9 ml dH ₂ O 0.1 ml skim milk	A2	0.1 ml A 3.9 ml dH ₂ O 1 ml Enzyme				
B	1.9 ml Lactaid tablet soln. 0.1 ml skim milk	B2	0.1 ml B 3.9 ml dH ₂ O 1 ml Enzyme				
C	1.9 ml Lactaid tablet soln. 0.1 ml dH ₂ O	C2	0.1 ml C 3.9 ml dH ₂ O 1 ml Enzyme				
D	1 ml pea juice 1 ml dH ₂ O	D2	0.1 ml D 3.9 ml dH ₂ O 1 ml Enzyme				
E	1 ml pea juice 1 ml Beano soln.	E2	0.1 ml E 3.9 ml dH ₂ O 1 ml Enzyme				
F	1 ml Beano soln 1ml dH ₂ O	F2	0.1 ml F 3.9 ml dH ₂ O 1 ml Enzyme				

