A Better-Tasting and More Digestible Soybean

Agricultural Genetics Resource Unit for Grades 9-12

E-SET
Biotechnology at Iowa State University
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Based on a graphic by Lisa Lorenzen, Biotechnology Industrial Liaison, ISU Office of Biotechnology

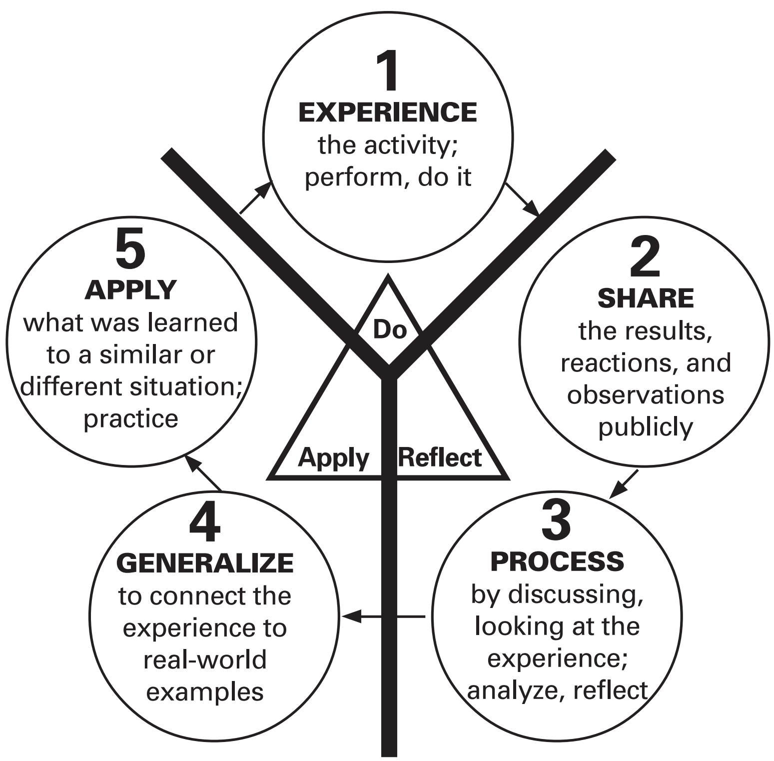
About This Resource Unit

A Better-Tasting and More Digestible Soybean is the second in a series of resource units that focus on new products developed through agricultural genetics. This unit introduces advanced middle school or high school students to a new high sucrose soybean that tastes sweeter and produces less intestinal gas than traditional varieties.

Over the past few years, an increasing amount of research has linked soybean products with possible health benefits. However, traditional soybean products often have a flavor described as "beany" or "grassy" and may produce embarrassing gas in the human digestive system. Several Iowa companies are involved in the value-added chain for high sucrose soybean varieties to reduce these undesirable characteristics.

This unit is intended for use with students and educators in science, nutrition, agriculture, or family and consumer sciences. Extension educators may find these materials useful for their youth and adult audiences. Participants learn why the new soybean was developed and the genetic science behind its development. Using an inquiry-based approach and the experiential learning model illustrated below, participants conduct "See for Yourself" activities that reinforce the science principles being taught. The Teacher/Leader information for each activity includes the science content and how it relates to the National Science Education Standards, as well as the science process skills.

Experiential Learning Model



National Science Education Standards and Associated Concepts and Principles

All activities in this curriculum relate to Content Standard A, Science as Inquiry, as developed by the National Research Council Standards. Some activities also relate to Content Standard B, Physical Science, and Content Standard C, Life Science. To help educators locate the standards and underlying concepts and principles cited for each activity, the page number and first sentence or two of the applicable principle are cited in the "Science Education Standards" section of the instruction pages for teachers and leaders. All page numbers refer to the seventh printing of the *National Science Education Standards*, November 1999. The *National Science Education Standards* are also available on the Internet at http://www.nas.edu.

Content Standard A – Science as Inquiry[1]

As a result of activities in this curriculum, students in grades 9-12 should develop

- abilities necessary to do scientific inquiry
  - identify questions and concepts that guide scientific investigations
  - design and conduct scientific investigations
  - use technology and mathematics to improve investigations and communications
  - formulate and revise scientific explanations and models using logic and evidence
  - recognize and analyze alternative explanations and models

- understanding about scientific inquiry
  - scientists conduct investigations to
    1. explain new discoveries
    2. test conclusions
    3. explain observed phenomena

Content Standard B – Physical Science[2]

As a result of activities in this curriculum, students in grades 9-12 should develop an understanding of chemical reactions.

Content Standard C – Life Science[3]

As a result of activities in this curriculum, students in grades 9-12 should develop an understanding of

- the cell
- the molecular basis of heredity
- matter, energy, and organization in living systems

[1,2,3] *National Science Education Standards*, ©National Academy of Sciences, 1996, pp. 175, 176, 181. Used with permission.

For Teachers/Leaders – Soybean Taste Test

During this activity, students learn how the absence of one or more lipoxygenase enzymes affects the flavor of soybeans.

Science Content

To teach students about the enzyme activity of lipoxygenase and how it affects soybean flavor

Science Education Standards

Life Science, Content Standard C, The Cell – Most cell functions involve chemical reactions. (p.184)

Life Science, Content Standard C, The Molecular Basis of Heredity – Changes in DNA (mutations) occur spontaneously at low rates. (p. 185)

Source: National Science Education Standards, *©National Academy of Sciences, 1996. Used with permission. Page numbers refer to the seventh printing, November 1999 – also available on the Internet at http://www.nas.edu.*

Science Process Skills

Comparing/measuring
Inferring

Life Skill

Decision making

Time

Preparation: 5-10 minutes

Iowa teachers can order without charge lipoxygenase and lipoxygenase-free soybeans from ISU's Office of Biotechnology by phoning 1-800-643-9504.

Duplicate optional student handouts 1 and 6 on pp. 31 and 41.

Activity: 20-30 minutes

Materials

Lipoxygenase and lipoxygenase-free soybeans
Water in paper cups for rinsing mouth between tastings
Optional student handouts 1 and 6, pp. 31 and 41.

Educator Background Information

The beany or grassy flavor associated with soybean products results from the action of an enzyme called lipoxygenase that oxidizes fats. Three forms or isozymes of lipoxygenase are referred to as lipoxygenase 1, 2, and 3.

At least one of the three lipoxygenase isozymes must be inactive in soybeans in order to produce a milder flavor. Only recently have scientists discovered how to combine the genes that control the three isozymes to suppress their activity.

Products made from soybeans that have one or more of the lipoxygenase isozymes suppressed are now on the market. This curriculum refers to two examples, high sucrose soybeans and a line of liquid soy drinks produced from high sucrose soybeans.

Lesson Plan

The day before the activity

1. Give students handout 1 and ask them to read it. Tell them that during the next class period, they will be doing a taste test of lipoxygenase and lipoxygenase-free soybeans. Ask students if anyone is allergic to consuming or handling soy products. **Allergic students should not participate in the Soybean Taste Test activity.**

2. Ask students not to eat or drink anything flavored such as candy, gum, mints, or pop, for an hour before the class period to avoid interfering with the taste test.

On the day of the activity

1. Give each student one of the lipoxygenase-free soybeans. It is important to have students taste the lipoxygenase-free soybean first. If the soybean that contains lipoxygenase enzymes is tasted first, the aftertaste will interfere with the taste of the lipoxygenase-free soybean.

2. Ask students to chew the bean and complete items A-1 and A-2 on handout 6. Caution students to be

careful not to injure their teeth when chewing the soybean.

3. Ask students to rinse their mouths with water before proceeding to step 4.

4. Give each student one of the soybeans that contain lipoxygenase enzymes. Ask students to chew the bean and complete items B-1 and B-2 on handout 6. Again, caution students to be careful not to injure their teeth when chewing the soybean.

5. Divide students into groups if class size allows. Ask groups to analyze their results by completing part C of handout 6. Groups should report their results to the class.

Reflect and Apply

1. What are the results of the taste test? What are the differences between the taste of the two soybeans?

   The answers will differ. Most students will probably say that the lipoxygenase-free soybean that they tasted first had a milder, less beany flavor than the traditional soybean that they tasted second.

2. Why could some students detect a greater difference in taste than others?

   Taste buds detect flavors differently. Some students may have chewed gum or eaten candy before the taste test.

3. Suppose that you manufactured a soy product that is marketed to your classmates. You currently use traditional soybeans in your product. Based on the answers of your group/class to questions 1-5, section C of handout 6, should you switch from using traditional soybeans in your product to using lipoxygenase-free soybeans? Why or why not?

   If the majority of the group/class members could detect a difference and liked the lipoxygenase-free soybeans better, students will probably recommend changing.

4. What other factors, besides taste, would a manufacturer of food products have to consider before deciding to change a product?

   Students might mention the costs of the new ingredients, revising product labels, and whether sales would increase enough to cover such costs.

For Teachers/Leaders – Soybean Drink Laboratory

During this activity, students will learn how the absence of one or more lipoxygenase enzymes affects the flavor of a product made from the soybeans.

Science Content

To teach students about the enzyme activity of lipoxygenase and how it affects soybean flavor

Science Education Standard

Life Science, Content Standard C, The Cell – Most cell functions involve chemical reactions. (p. 184)

Source: National Science Education Standards, ©*National Academy of Sciences, 1996. Used with permission. Page numbers refer to the seventh printing, November 1999 – also available on the Internet at http://www.nas.edu.*

Science Process Skills

Comparing/measuring
Inferring

Life Skill

Healthy lifestyle choices

Time

Preparation: Varies

Iowa teachers can order without charge lipoxygenase and lipoxygenase-free soybeans from Iowa State University's Office of Biotechnology by phoning 1-800-643-9504.

If using, duplicate optional student handouts 1 on p. 31 (unless already used in the soybean taste test activity) and 7 on p. 45.

Gather materials and supplies for the soybean drink activity.

Teachers may wish to save some of the soybean filtrate produced in this activity for use in the two "Comparing the Quantity of Sucrose..." activities on pp. 19 and 24.

Activities
Day One Activity: 15 minutes
Day Two Activity: 60 minutes

Materials

- Lipoxygenase and lipoxygenase-free soybeans
- Measuring cup
- Two large bowls or other containers
- Masking tape and marking pen
- Two kitchen strainers
- Two 1000 ml beakers
- Electric blender
- Cheesecloth or coffee filters
- Hot plate or stove
- Two 2-quart or larger saucepans or glass cookware safe for stove-top use
- Candy thermometer
- Sink or other source of hot, soapy water and water for rinsing
- Spoons for stirring
- Refrigerator or cooler with ice
- Sugar, chocolate syrup, vanilla, or other flavorings
- Labels from traditional cow's milk containers and commercial containers of soy milk.
- If using, optional student handouts 1 (unless already used in the soybean taste test activity) and 7 on pp. 31 and 45
- 2% milk
- Commercial soy milk products (optional)

Educator Background Information

The beany or grassy flavor associated with soybean products results from the action of an enzyme called lipoxygenase that oxidizes fats. The three forms or isozymes of lipoxygenase are referred to as lipoxygenase 1, 2, and 3.

At least one of the three lipoxygenase isozymes must be inactive in soybeans in order to produce a milder flavor. Only recently have scientists discovered how to combine the genes that control the three isozymes to suppress their activity.

Products made from soybeans that have one or more of the lipoxygenase isozymes suppressed are now on the market. This curriculum refers to two examples, high sucrose soybeans and a line of soy drinks produced from high sucrose soybeans. Soy drinks are an alternative for individuals who are lactose intolerant. They also have a longer shelf-life than cow's milk.

Lesson Plan

The day before the activity

Give students handout 1 and ask them to read it. Omit this step if students have already read the handout for the soybean taste test activity. Ask students if anyone is allergic to consuming or handling soy or milk products. **Allergic students should not participate in the Soybean Drink Laboratory activity.**

Day 1

1. Ask students to clean the measuring cup, large bowls or other containers, kitchen strainer, beakers, spoons, and blender container with hot soapy water before they begin. The teacher/leader may complete this step before class if preferred.

2. Tell students to measure one cup (150 grams) of soybeans that lack the lipoxygenase enzymes and place them in one of the large bowls/containers. Direct students to pour at least two cups of water at room temperature into the container so the soybeans are completely covered with water. Students should use the masking tape and marking pen to make a "Lipo-Free" label and attach it to the container.

3. Repeat step #2 using soybeans that contain the lipoxygenase enzymes. Label the bowl/container "Lipo."

4. The soybeans should soak in the water for 18-24 hours. The soybeans should stay covered with water for this entire time, so designated students or the teacher/leader should check the containers periodically. Before leaving for the day, add enough water to cover the soybeans overnight.

Day 2

Divide the class into half. One half of the class will process the soybeans in the "Lipo-Free" container and the other half will process the soybeans in the "Lipo" container. Distribute handout 7, if using it.

1. Tell students to drain the water from the soybeans and rinse them three times with clean water.

2. Add water to the soaked soybeans in a 7:1 (water:soybean) mixture. Do this by telling students to place 700 ml of water in a 1000 ml beaker and add only enough soaked soybeans to bring the water level in the beaker to 800 ml.

3. Tell students to pour the water/soybean mixture into a blender container and grind at high speed for three minutes.

4. While the mixture is grinding, ask students to place cheesecloth or a coffee filter in a kitchen strainer. Direct students to filter the mixture, now a whitish liquid, through the cheesecloth or coffee filter into the saucepan or glass cookware.

5. Students should cook the filtered liquid at 98-100^{o} C (208-212^{o} F) for 15 minutes. Students may take turns stirring the liquid continuously so it does not burn. Students should use a candy thermometer to monitor temperature and begin timing when the temperature reaches 98^{o} C or 208^{o} F.

6. After 15 minutes, tell students to pour the cooked liquid into a clean bowl/container. Refrigerate the liquid immediately after cooking.

7. After the soy milks are cold, students may taste the lipoxygenase-free (high sucrose) soy milk, the lipoxygenase (regular) soy milk, and 2% milk. They can compare the taste of the drink made from soybeans that lack the lipoxygenase enzymes with the drink made from soybeans with the enzymes. After their initial taste test, students may add sugar, chocolate syrup, or other ingredients to the soy drinks for flavor.

Adapted from a protocol developed by the ISU Department of Food Science and Human Nutrition.

Reflect and Apply

1. Based on your initial taste test, which of the drinks did you prefer? Why?

   Answers will vary.

2. How do soy milk and cow's milk compare nutritionally?

   Use the labels from traditional cow's milk containers and soy milk containers to compare fat, protein, and other nutrients. Answers will depend on the type of milk products from which labels were taken.

3. Some people cannot consume cow's milk because of lactose intolerance, allergies, or other health reasons. Besides health concerns, for what other reasons might people choose to use soy milk?

   Answers could include taste, nutritional value, vegetarian choice, or convenience.

4. What food products use soybeans? What other non-food uses can you think of for soybeans?

   Answers will vary. Some examples of food uses for soybeans are soy protein in meat substitutes, soy flour in baked products, and soybean oil in cooking oils and baked goods. Some examples of non-food uses are soy ink, soy plastics, crayons, high performance fuels and additives, candles, soaps, and industrial solvents and cleaners.

Extended Activity

Add a fourth drink, a commercially-made soy drink, to the taste test.

For Teachers/Leaders – A Walk Through the Digestive System

This activity was adapted from A Walk Through the Gut, an activity submitted to the Access Excellence Fellows Collection by VivianLee Ward, Sequoia High School, Redwood City, California. The Access Excellence site at http://www.accessexcellence.org/ is sponsored by the National Health Museum. This activity is used with the permission of VivianLee Ward and Access Excellence.

Science Content

To teach students what happens to food as it passes through the digestive system

Science Education Standards

Physical Science, Content Standard B, Chemical Reactions – Chemical reactions occur all around us, for example in health care, cooking, cosmetics, and automobiles. (p. 179)

Life Science, Content Standard C, The Cell – Most cell functions involve chemical reactions. (p. 184)

Cells can differentiate, and complex multicellular organisms are formed as a highly organized arrangement of differentiated cells. (p. 184)

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Science Process Skill

Sequencing

Life Skill

Disease prevention

Time

Preparation: Collection time varies. Assembling time is one hour for the teacher to assemble the digestive stations.

Collect materials for and assemble stations, including directions for each.

Activity: For a class of 30, the stations will take 20 minutes and the analysis questions require 30 minutes.

Materials

- 8 pieces of 8 1/2 x 11 in. poster board or other sign paper
- Several pieces of poster board
- Transparent tape
- Red and black felt tip markers
- 24 in. section (approximate length) of 4 in. diameter dryer exhaust vent hose
- Two large bowls or other containers
- Hot water bottle with ends removed
- Green balloon
- 12 inches of string
- Four pairs of scissors
- String bag used for potatoes or fruit (or basketball net)
- One large paper cylinder, at least two inches in diameter, either made from poster board or from an empty wrapping paper or paper towel roll
- Hospital specimen bowl or bed pan
- Shoe box
- Slinky wire-coil toy
- 3 x 5 in. index card for each student
- Optional student handout 8, p. 47.

Educator Background Information

Traditional soybeans are difficult for some people to digest because they contain the complex sugars raffinose and stachyose. These sugars pass into the large intestine where *E. coli* and other bacteria naturally live.

Some of the bacteria in the large intestine cannot break down sugars. However, *E. coli* bacteria can metabolize them into hydrogen and other gasses that have objectionable odors.

This simulation helps students understand what happens to food as it passes through the digestive system. Students have a 3x5 index card representing food. They carry the card to eight digestive stations around the room that have the following structures and functions:

1. Mouth – Digestion of food begins, primarily starches.

2. Esophagus – Food passes to the stomach.

3. Stomach – Digestion continues, especially protein.

4. Gallbladder – Fats are emulsified (suspended in liquid).

5. Small intestine – Continuing digestion and absorption of nutrients occurs.

6. Large intestine – Absorption of water and minerals happens.

7. Rectum – Remaining food passes to anus.

8. Anus – Undigested food exits from the body.

Each digestive station creatively represents one of these eight structures of the digestive system. At each station, students are given a specific instruction to "digest" the index card and then are instructed to proceed to the next station. At the end of the simulation, students form cooperative learning groups to analyze and apply the results of the simulation to the human digestive system.

This simulation works best after the students have been introduced to the structures and functions of the digestive system. It works well if students visit each station singly, then form groups of three to analyze the simulation. Or students can visit the stations in pairs and form learning groups of four.

If desired, students can design and assemble the digestive stations. A day or two before the simulation, students work in cooperative groups with one assigned digestive structure. Each group must research its functions, decide on a way to creatively represent that structure, and write the digestion directions. (The teacher must give the groups the requirement that reduction of the food can only take place by one cut per station.) The students' homework is to construct the structure. On the day of the simulation, each group must place its structure and directions at the correct sequence station. The simulation may not proceed smoothly, so each group must analyze what stations were in conflict and propose a set of directions that will allow consecutive digestions of the index card.

Extensions and Reinforcements

Extension: Students may research and list the specific digestive enzymes and their substrates for each digestive structure.

Reinforcement: Students select their favorite food, such as tacos, and trace it through the digestive system. They can write, graphically organize, or draw the process.

Lesson Plan

Before the activity

The teacher assembles the digestive stations and places them around the classroom. Here are some creative construction suggestions for each digestive structure, although teachers or students may devise other ways to represent each organ.

1. Mouth – Using the felt markers, draw a mouth shape on poster board. Cut out a tongue shape from red poster board or use the red felt marker to color it red. Cut a slit in the mouth drawing and insert and tape the tongue in the slit so it protrudes and can be raised.

2. Esophagus – Use a one or two-foot length of 4" diameter flexible clothes dryer exhaust vent hose.

3. Stomach – Remove the ends of a hot water bottle. Insert one pair of scissors inside the bottle.

4. Gallbladder – Use string to attach a pair of scissors to a green balloon.

5. Small intestine – Place a pair of scissors inside a string shopping bag (such as a mesh potato bag) or a basketball net that is open at both ends. Place the

bag or net, with the scissors inside it, inside a clear garbage bag or other clear plastic bag.

6. Large intestine – Place a large paper cylinder, either made from posterboard or an empty wrapping paper roll, in a specimen bowl or hospital bedpan. Put a pair of scissors inside the bowl or pan.

7. Rectum – Cut a circular hole slightly smaller than the diameter of the slinky toy in the lid of a shoebox. Position the slinky toy atop the shoebox over the hole. The slinky represents the rectum.

8. Anus – The hole in the shoebox lid represents the anus.

Make signs for each of the eight steps in the activity. The signs should read as follows:

1. Mouth – Digestion of food begins, primarily starches. Lift tongue. Digest by tearing food into two equal pieces.

2. Esophagus – Food passes to the stomach. Pass the two equal pieces of food through by peristaltic motion.

3. Stomach – Digestion continues, especially protein. Digest only one of the two pieces of food by cutting it into two equal pieces.

4. Gallbladder – Fats are emulsified. Cut the two smaller pieces of food in half to make four pieces.

5. Small intestine – Continuing digestion and absorption of nutrients occurs. Digest the four small pieces of food by cutting them in half so you have eight smaller nutrients. Place all eight nutrients in the mesh bag. Push through the mesh and into the clear garbage bag only those pieces that fit through the mesh without folding or bending. (The large piece of food moves to the next station.)

6. Large intestine – Absorption of water and minerals happens. Cut one corner from the large piece of food and leave the corner in the bowl (or pan).

7. Rectum – Remaining food passes to anus. Write your name on the remaining food and deposit it in the rectum.

8. Anus – Undigested food exits from the body. If the undigested food has not already dropped, guide it into the box.

Doing the activity

Give each student or pair of students a 3 x 5 card representing food. This food is carried to each digestive structure station where the students follow the digestion directions and take the food to the next sequential station. At the conclusion of the walkthrough, the students have deposited the "undigested" food with their name on it in the anus. If the students have followed the simulation directions correctly, the pieces of food in the anus box should be identical.

Reflect and Apply

Teachers/leaders may wish to use optional handout 8, p. 47.

1. What did you learn about the digestive system?

   Answers will vary.

2. What does every cut or tear represent? List the structures of digestion and whether the food is digested physically, chemically, or both in that structure.

   Each cut or tear represents digestion. The type of digestion that occurs is:
   Mouth – both physical and chemical
   Esophagus – no digestion, just physical movement
   Stomach – both physical and chemical
   Gallbladder – physical emulsification
   Small intestine – both physical and chemical absorption
   Large intestine –physical absorption
   Rectum – no digestion
   Anus – no digestion

3. What does each piece of the index card that is cut represent? For each digestive structure, list the nutrient(s) that are digested there.

   Each piece of the card represents partially digested food. The nutrients are:
   Mouth – starch
   Esophagus – no digestion
   Stomach – proteins
   Gallbladder – emulsification of fats
   Small intestine – sugars, proteins, fats
   Large intestine – absorption of water and minerals
   Rectum – no digestion
   Anus – no digestion

4. What does each piece of card that is left at the station represent? Where do these nutrients go?

For each digestive structure, list the structure and which nutrient(s) are absorbed there.

Each piece of card left at a station represents nutrients that are absorbed. The nutrients are:
Mouth – no nutrient absorption
Esophagus – no nutrient absorption
Stomach – no nutrient absorption, only water or drugs
Gallbladder – no nutrient absorption
Small intestine – sugars, proteins, fats
Large intestine –water and minerals
Rectum – no absorption
Anus – no absorption

5. Do you or anyone in your family have trouble digesting certain foods? Which food(s)? What factors cause digestive problems?

   Answers will vary. Spicy foods, pork, and raw vegetables are hard to digest for some people. The lack of certain enzymes, diseases such as diverticulitis, food allergies, and unwise food choices are some of the factors that students might mention.

6. Babies and elderly people often have more digestive problems than the general population. Why do you think this happens?

   Babies may not have matured enough to have a complete set of stomach enzymes. The elderly may not produce enough digestive enzymes.

7. What can people do to avoid digestive problems?

   Answers may include avoiding the offending food, taking acid blockers or other digestive medicines, and chewing food more thoroughly.

For Teachers/Leaders – Digesting Lactose

This activity was adapted from Enzyme Action, an activity submitted to the Access Excellence Fellows Collection by Jane Crumlish, retired from Neshaminy High School, Langhorne, Pennsylvania. The Access Excellence site at http://www.accessexcellence.org/ is sponsored by the National Health Museum. This activity is used with the permission of Jane Crumlish and Access Excellence.

Science Content

To teach students why lactose intolerant people can digest soy milk more easily than cow's milk

Science Education Standards

Physical Science, Content Standard B, Chemical Reactions – Chemical reactions occur all around us, for example in health care, cooking, cosmetics, and automobiles. (p. 179)

Catalysts, such as metal surfaces, accelerate chemical reactions. (p. 179)

A large number of important reactions involve the transfer of either electrons (oxidation/reduction reactions) or hydrogen ions (acid/base reactions) between reacting ions, molecules, or atoms. (p. 179)

Life Science, Content Standard C, The Molecular Basis of Heredity – Changes in DNA (mutations) occur spontaneously at low rates. (p. 185)

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Science Process Skills

Sequencing

Life Skill

Healthy lifestyle choices

Time

Preparation: Materials collection time varies.

Activity: One 40-50 minute class period

Materials

For each group of students:

- One spot plate or glass slide
- One marker pen
- Two toothpicks for mixing
- One forceps for handling the glucose test paper
- Milk – whole, skim, 1% or 2%, soy milk
- Six eyedroppers
- Lactaid® (lactase source) in liquid form (available from a pharmacy)
- Six glucose test papers such as Tes-Tape®
- Glucose solution
- Sucrose solution
- A clock or watch for timing
- Optional student handouts 2, 3, and 9 on pp. 33, 35, and 49

Lactaid® is a registered trademark of McNeil Consumer Products Company. Tes-Tape® is a registered trademark of Eli Lilly.

Educator Background Information

For a clear explanation of lactose intolerance, visit the Web site of The National Digestive Diseases Information Clearinghouse at http://www.niddk.nih.gov/health/digest/pubs/lactose/lactose.htm. The clearinghouse is a service of the National Institute of Diabetes and Digestive and Kidney Diseases, which is part of the National Institutes of Health under the U.S. Department of Health and Human Services. The e-text found at the Web site is not copyrighted and this statement appears, "The clearinghouse encourages users of this e-pub to duplicate and distribute as many copies as desired." "What Is Lactose Intolerance" is from the clearinghouse Web site.

What Is Lactose Intolerance?

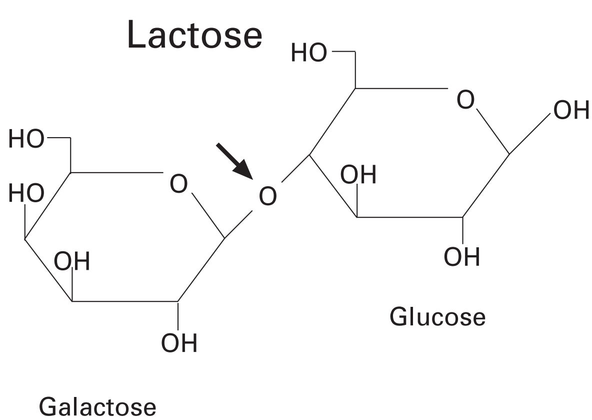
Lactose intolerance is the inability to digest significant amounts of lactose, the predominant sugar of milk. This inability results from a shortage of the enzyme lactase, which is normally produced by the cells that line the small intestine. Lactase breaks down milk sugar into simpler forms that can be absorbed into the bloodstream. When there is not enough lactase to digest the amount of lactose consumed, the results, although not usually dangerous, may be very distressing. While not all persons deficient in lactase have symptoms, those who do are considered to be lactose intolerant.

Common symptoms include nausea, cramps, bloating, gas, and diarrhea, which begin about 30 minutes to 2 hours after eating or drinking foods containing lactose. The severity of symptoms varies depending on the amount of lactose each individual can tolerate.

Some causes of lactose intolerance are well known. For instance, certain digestive diseases and injuries to the small intestine can reduce the amount of enzymes produced. In rare cases, children are born without the ability to produce lactase. For most people, lactase deficiency is a condition that develops naturally over time. After about the age of 2 years, the body begins to produce less lactase. However, many people may not experience symptoms until they are much older. Between 30 and 50 million Americans are lactose intolerant. Certain ethnic and racial populations are more widely affected than others. As many as 75 percent of all African-Americans and Native Americans and 90 percent of Asian-Americans are lactose intolerant. The condition is least common among persons of northern European descent.

The Biochemistry of Lactose

Lactose, commonly called milk sugar, is a carbohydrate represented by the chemical formula $C_{12}H_{22}0_{11}$. Lactose is an isomere of sucrose. Lactase is an enzyme that catalyzes the hydrolysis of lactose; that is, water hydrolyzes (splits) the lactose into two sugars, galactose and glucose. The split occurs at the location designated by the arrow below.



Lesson Plan

The day before the activity

Give students optional handouts 2 and 3, if using. Ask the students to read the handouts and complete as many of the questions as they can by the next class period. Ask students if anyone is allergic to handling soy or milk products. **Allergic students should not participate in the Digesting Lactose activity.**

The day of the activity

Discuss the answers for the handouts with the students.

1. Define:

   enzyme – *a protein molecule produced by living organisms that speeds ups (catalyzes) chemical reactions of other substances, without destroying or altering itself*

   hydrolysis – *a chemical process in which a chemical compound is broken down by reacting with water*

   substrate – t*he substance on which an enzyme acts*

   active site – *a specific area on an enzyme where a substrate binds and catalysis takes place*

2. How many sugar rings are there in lactose? 2 Based on this, classify lactose. *Lactose is a disaccharide, a sugar made up of two simple sugars, galactose and glucose.*

3. Complete the following:

$C_{12}H_{22}0_{11}$	Lactose	Decomposition
+ H_20	Add water	
$C_{12}H_{24}0_{12}$		
- $C_6H_20_6$	Subtract glucose formula	Hydrolysis
$C_6H_20_6$	Galactose formula	

When lactase is added to lactose, a chemical reaction called hydrolysis occurs. The disaccharide lactose is broken down into the monosaccharides glucose and galactose.

4. What would be the enzyme that activates this reaction?
   Lactase

5. Where does this enzyme act in your body?
   Small intestine

6. Where is this enzyme produced in your body?
   Lining of the intestine

7. Name the substrate.
   Lactose

Divide the students into groups of six and conduct the following experiment:

Digesting Lactose with Lactase

Tell students: T*oday we are going to conduct an experiment to digest the milk sugar lactose by using the enzyme lactase. We are going to determine whether the lactose has been digested by testing for the presence of glucose. Which of the following solutions do you predict will test positive for glucose?*

Circle 1: glucose –
Circle 2: milk –
Circle 3: lactase –
Circle 4: lactase plus milk –
Circle 5: soy milk –
Circle 6: lactase plus soy milk –

Ask students to record their predictions and complete the following procedure (can use optional handout 9):

1. Each student in the group of six should use a marker to draw a circle on a glass plate or slide and label the circles Glucose, Milk, Lactase, Lactase and Milk, Soy Milk, and Lactase and Soy Milk. Each student should be responsible for adding one of the solutions to the appropriate circle:

   Circle 1: Add two drops of glucose solution.
   Circle 2: Add two drops of milk.
   Circle 3: Add one drop of lactase.
   Circle 4: Add one drop of lactase plus two drops of milk and mix with a toothpick.
   Circle 5: Add two drops of soy milk.
   Circle 6: Add one drop of lactase plus two drops of soy milk and mix with a clean toothpick.

2. After three minutes, students should test each of the circles for the presence of glucose by placing one end of a piece of glucose test paper in each circle.

3. Two minutes after placing the glucose test paper in the circles, students should record in the data table on the handout whether the test paper changed color, indicating positive for the presence of glucose. Their tables should look like this:

Circle Contents		Test Paper
1	Glucose	Positive
2	Milk	Negative
3	Lactase	Negative
4	Lactase + Milk	Positive
5.	Soy Milk	Positive
6.	Lactase + Soy Milk	Positive

Reflect and Apply

1. In which circle(s) would you expect the hydrolysis of the milk sugar, lactose?

   In circle 4

2. What products would result after this hydrolysis? How did you know this?

   Glucose and galactose would result because lactase cleaves lactose into glucose and galactose.

3. Why did you test the glucose with the glucose test paper?

   Testing the glucose with the glucose test paper acted as a control and provided information about the color of the test paper when glucose is present.

4. Why did you test the lactase with the glucose test paper?

   Testing the lactase with the glucose test paper confirmed that lactase by itself is not a source of glucose.

5. Does milk contain glucose? Explain how you know this.

   No, because the circle with milk alone did not test positive for glucose.

6. Does soy milk contain glucose? Explain how you know this.

   Yes, because the circle with soy milk alone tested positive for glucose.

7. Does milk combined with lactase contain glucose? Explain how you know this.

   Yes, because the circle with lactose plus milk tested positive for glucose.

8. Some people cannot drink cow's milk because their digestive systems do not produce enough lactase. If milk is ingested, it cannot be digested. This results in cramping, bloating, gas, and diarrhea. Suggest a treatment that would allow such a person to drink cow's milk.

   Treat the milk with lactase before drinking it.

9. Why would a lactose intolerant person be able to drink soy milk?

   Soy milk does not contain lactose, so the lactase enzyme would not be needed to digest it.

Additional Ideas

After students have done the initial activity, have them design and execute labs to illustrate that enzymes are specific and are reusable. For example, sucrose solutions can be used to show that the lactase will not break down sucrose.

To show that enzymes are reusable, students can take a little of the milk plus lactase solution and add it to a new drop of milk. The added amount of glucose produced by the enzyme reaction should turn the test paper a more intense color.

For Teachers/Leaders – Comparing the Quantity of Sucrose in Normal and High Sucrose Soybeans by Using Invertase

This activity was developed by Mike Zeller for the ISU Office of Biotechnology. The activity is used with permission.

Science Content

- To help students discover genotypic evidence that normal soybeans have less sucrose than high sucrose soybeans
- To give students experience evaluating enzyme action

Science Education Standards

Physical Science, Content Standard B, Chemical Reactions – A large number of important reactions involve the transfer of either electrons (oxidation/reduction reactions) or hydrogen ions (acid/base reactions) between reacting ions, molecules, or atoms. (p. 179)

Catalysts, such as metal surfaces, accelerate chemical reactions. (p. 179)

Life Science, Content Standard C, The Cell – Most cell functions involve chemical reactions. (p. 184)

Plant cells contain chloroplasts, the site of photosynthesis. (p. 184)

Life Science, Content Standard C, The Molecular Basis of Heredity – Changes in DNA (mutations) occur spontaneously at low rates. (p. 185)

Life Science, Content Standard C, Matter, Energy, and Organization in Living Systems – The energy for life primarily derives from the sun. (p. 186)

Source: National Science Education Standards, *©National Academy of Sciences, 1996. Used with permission. Page numbers refer to the seventh printing, November 1999 – also available on the Internet at http://www.nas.edu.*

Science Process Skills

Comparing/measuring	Observing
Inferring	Reflecting

Life Skill

Healthy lifestyle choices	Science processing
Learning	

Time

Preparation: 30 minutes each day

Iowa teachers can order the following supplies without charge from the ISU Office of Biotechnology by phoning 1-800-643-9504:

- High sucrose soybeans
- Normal soybeans
- Invertase enzyme

Teachers may purchase glucose test strips at a local pharmacy. Bayer's Diastix® were used in the original development of this protocol. (For more precise results, teachers may want to purchase a glucometer and test strip system. See alternative procedure on p. 22.)

Optional: Make copies of student handouts 4 and 10, pp. 37 and 53.

Activity: Two or three 40-minute class periods

Materials

Note: If using soybean filtrate saved from the "Soybean Drink Laboratory" activity, omit steps 1-3 in the pre-lab prep and steps 1-8 in the Day 1 instructions.

Student Supplies

Students should work in groups of three to five students. Each group of students will need:

Pre-Lab Prep

- High sucrose soybeans*
- Normal soybeans*
- Two 400-600 ml beakers or containers
- Tap water
- Marker and two labels

*Iowa teachers can order without charge from the ISU Office of Biotechnology by phoning 1-800-643-9504.

Day 1
- High sucrose soybeans from pre-lab prep
- Normal soybeans from pre-lab prep
- Two blenders
- Distilled water
- Two large wire strainers
- Four #6 coffee filters
- One 600-1000 ml beaker or collection containers
- Four test tubes
- Aluminum foil or plastic wrap to cover container
- Marker and six labels
- Clock, watch, or timer
- Handout 4 on p. 37 (optional)

Day 2
- Four test tubes labeled "Normal," "High Sucrose," "C Normal," and "C High Sucrose" prepared on Day 1
- Test tube rack
- Four test tube corks
- Glucose test strips for urine (Bayer's Diastix® strips were used in the original lab tests)
- Four 1 ml transfer pipettes
- 0.01 grams of invertase in a 1.5 ml tube
- Distilled water
- 50-55°C water bath
- Forceps
- Clock, watch, or timer
- Paper towel and pen or marker
- Handout 10 on p. 53 (optional).

Educator Background Information
High sucrose soybeans offer improved flavor and digestibility for use in soy milk and other soy food products. These newly developed soybeans contain 90% less of the indigestible carbohydrate sugars raffinose and stachyose that can produce abdominal discomfort and gas. The beans also contain 40% more sucrose and lack the lipoxygenase-2 enzyme that is responsible for "beany" flavor. Because they taste better and produce less gas, they allow the manufacturers of soy products to increase the percentage of soy in beverages, bakery products, pasta, and other foods.

As suggested by their name, high sucrose soybeans are 40% higher in sucrose, a carbohydrate that is made up of two simpler sugars, glucose and fructose. In this lab you will produce evidence that high sucrose soybeans contain more sucrose than normal soybeans by using a sucrase enzyme called invertase. Invertase breaks down (hydrolyzes) sucrose into the monosaccharides glucose and fructose.

A commercial glucose test used by diabetics to monitor the sugar levels in their urine will be used to measure the glucose in the soybeans because the test is designed to be very specific for glucose. After adding invertase to normal and high sucrose soybeans, the quantities of sucrose in the two different types of soybeans should vary directly with the quantity of sucrose in the soybeans. The more sucrose that is available to the invertase enzyme, the more glucose will be produced.

Both normal and high sucrose soybeans contain sucrose naturally. However, the high sucrose soybeans contain 40% more sucrose for invertase to break down into glucose and fructose. For that reason, the commercial glucose test will detect more glucose in the hydrolyzed high sucrose soybeans than it will in the hydrolyzed normal soybeans.

Initially, you will note that both the normal and high sucrose soybeans show an increase in glucose levels, although the glucose in the high sucrose soybeans starts at a higher level. Teachers should explain to their students that a normal soybean plant has its own natural enzymes that convert sucrose to glucose as part of its metabolism.

Lesson Plan

Pre-Lab Preparation
The pre-lab preparation can be completed by the students or the teacher. Ask students if anyone is allergic to handling soy products. **Allergic students should not participate in the Invertase activity.**

Materials:
For each group:
- High sucrose soybeans*
- Normal soybeans*
- Two 400-600 ml beakers or containers
- Tap water
- Marker and two labels

*Order from the ISU Office of Biotechnology

Procedure:
Each class should prepare to soak the two different kinds of soybeans in water overnight by using the following procedure:

1. Add 75 grams (1/2 cup) of normal soybeans to a container and add at least twice the volume of regular tap water. Label the container "Normal."

2. Repeat step 1 with the high sucrose soybeans. Label the container “High Sucrose.”

3. Let the soybeans soak overnight until the next day’s lab time. The soybeans will begin to swell as they absorb the water. It is important that the soybeans stay covered with water for 24 hours. A student and/or teacher should check periodically to make sure the beans stay covered. Make sure to add a sufficient amount of water to cover the soybeans overnight. You cannot add too much water.

Doing the Laboratory

Day 1

Materials:
For each group:
- High sucrose soybeans from pre-lab prep
- Normal soybeans from pre-lab prep
- Two blenders
- Distilled water
- Two large wire strainers
- Four #6 coffee filters
- One 600-1000 ml beaker or collection containers
- Four test tubes
- Aluminum foil or plastic wrap to cover container
- Marker and six labels
- Clock, watch, or timer
- Handout 4 on p. 35 (optional)

Procedure:
1. Select two groups of students to make the soybean mixtures. Ask each group to follow steps 2 through 10. Optional: The teacher/leader can prepare ahead of time.

2. Drain the water from the normal and high sucrose soybeans.

3. Place the “Normal” soybeans in a blender and add an equal volume of distilled water.

4. Blend the mixture on high for 1 minute.

5. While the mixture is blending, place two #6 coffee filters in the strainer positioned over another container to collect the filtrate.

6. After a minute of blending, pour the mixture into the coffee filters and let filter. Label this container “Normal.”

7. Clean the blender and repeat steps 3-6 with the high sucrose soybeans. Label the collection container “High Sucrose.”

8. Let the soybean mixtures drain completely (15-20 minutes).

9. While the soybeans drain, each group should:

   a. Label two test tubes. Label one test tube “Normal” and the other test tube “High Sucrose.”

   b. Label two additional tubes as “C Normal” and “C High Sucrose.” One group of students can be the control group and will share their

Readings	Initial Trial (no invertase)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Normal (mg/ml)						
High Sucrose (mg/ml)						

results with the other groups.

c. Create a data table for the Day 2 lab. The data table should look similar to the table on the previous page. Ask each group to make predictions about which trials will have the most glucose.

10. Cover the containers and place in the refrigerator overnight.

Day 2

Materials:

For each group:

- Four test tubes labeled "Normal," "High Sucrose," "C Normal," and "C High Sucrose" prepared on Day 1
- Test tube rack
- Four test tube stoppers
- Glucose test strips for urine (Bayer's Diastix® strips were used in the original lab tests)*
- Four 1 ml transfer pipettes in size
- 0.01 grams of invertase in a 1.5 ml tube
- Distilled water
- 50-55°C water bath
- Forceps
- Clock, watch, or timer
- Paper towel and pen or marker*
- Handout 10 on p. 53 (optional)

*Not needed if using the alternative procedure for glucometers.

Procedure:

1. Fill each test tube 1/4 full with each mixture as it corresponds to the label on the tube. Use a different pipette for each mixture.

2. Using the same pipettes that were used to transfer each of the four mixtures in step 1, add enough distilled water to each test tube to bring the level to half full. Stopper each test tube and mix by inverting each test tube several times.

3. Test each mixture for glucose by dipping a glucose test strip into the mixture in each test tube. Use a different test strip for each mixture. Shake the excess mixture off each test strip and lay it on a paper towel. Write the name of the test tube mixture, "Normal," "High Sucrose," "C Normal," or "C High Sucrose" on the towel near the corresponding test strip.

4. Let the glucose test strips sit for 1 minute or the amount of time stated in the directions that came with the glucose strips.

5. Read the concentration of glucose by comparing the color of each strip with the chart on the glucose strip container.

6. Students should record their results on their data tables.

7. Students should get from their teacher a tube of the enzyme invertase. Add 1 ml (20 drops) of distilled water to the invertase tube and dissolve the enzyme. **The control group should skip steps 7-9. They will not add the enzyme to the control "C" tubes.**

8. Using a transfer pipette, add half of the contents of the invertase mixture (0.5 ml or 10 drops) to the "Normal" mixture and the other half to the "High Sucrose" mixture.

9. Mix the contents of each tube by placing a stopper in the mouth of the tube and inverting the tube several times.

10. Place the test tubes in the 50-55°C water bath.

11. After five minutes, test the mixtures with the glucose test strips and record the results on the data table. Read the test strip exactly 30 seconds after dipping.

12. Repeat the glucose test every five minutes. Record the results on the data table.

    NOTE: To save strips, instead of repeating the glucose test every five minutes, students can wait to test the mixture until just before the end of the lab period.

13. Use the last five minutes before the end of the lab period to record the results.

14. Students should clean up the lab area according to their teacher's directions.

Alternative Procedure for Glucometers:

For more precise results, teachers may want to purchase a glucometer and test strip system. Because of the increased sensitivity of glucometers, the soy filtrate produced on Day 1 should be diluted to a 4:1 mixture of distilled water to soy filtrate. Direct students to follow the alternative procedure below.

1. Fill each of the four labeled test tubes prepared on Day 1 with 4 ml of distilled water.

2. Add to each test tube 1 ml from each of the mixtures as it corresponds to the label on the tube. Use a different pipette for each mixture. Stopper the test tube and mix by inverting each test tube several times.

3. Calibrate your glucometer according to the manufacturer's specifications.

4. Place a glucose test strip recommended for use with your glucometer into the instrument and ready it for use.

5. Place a drop of the "Normal" mixture on the designated area of the test strip.

6. Wait for the glucometer to finish its test.

7. Record the reading from the glucometer on the data table under "Initial Trial."

8. Repeat steps 4-7 with the "High Sucrose" mixture.

9. Students should get from their teacher a tube of the enzyme invertase. Add 1 ml (20 drops) of distilled water to the invertase tube and dissolve the enzyme. **The control group should skip steps 9-11. They will not add the enzyme to the control "C" tubes.**

10. Using a transfer pipette, add half of the contents of the invertase mixture (0.5 ml or 10 drops) to the "Normal" mixture and the other half to the "High Sucrose" mixture.

11. Mix the contents of each tube by placing a stopper in the mouth of the tube and inverting the tube several times.

12. Place the test tubes in the 50-55°C water bath.

13. After five minutes, test the mixture with the glucometer and record the results on the data table.

14. Repeat the glucometer test every five minutes. Record the results on the data table.

    NOTE: To save strips, instead of repeating the glucose test every five minutes, students can wait to test the mixture until just before the end of the lab period.

15. Use the last five minutes before the end of the lab period to record the results.

16. Students should clean up the lab area according to their teacher's directions.

Reflect and Apply

1. Why is it important to read the test strip in 30 seconds? (Omit this question if using a glucometer.)

   The color will change on the strip. Invertase continues to react and glucose will oxidize.

2. Explain what is happening chemically in this activity.

   Invertase is breaking down sucrose into fructose and glucose. Higher amounts of sucrose yield higher amounts of glucose, which are detected by the test strip.

3. How do you explain the presence of glucose in both normal and high sucrose soybean samples?

   Both normal and high sucrose soybeans naturally contain sucrose. The plant's natural metabolism and enzymes break down the sucrose to glucose and fructose.

4. On a commercial scale, what is the importance of developing a high sucrose soybean?

   Students might mention benefits to farmers, manufacturers, and consumers. Because high sucrose soybeans taste better and produce less gas, the percentage of soy in manufactured foods can be increased.

5. As a consumer, what other traits would you consider valuable enough to want in soybeans, if the technology were available?

   Answers will vary depending on students' imaginations. Possible traits include increased protein, vitamins, minerals, or pharmaceuticals.

For Teachers/Leaders – Comparing the Quantity of Sucrose in Normal and High Sucrose Soybeans by Using Thin-Layer Chromatography

This activity was developed at Iowa State University by John Robyt, Professor, Biochemistry, Biophysics, and Molecular Biology Department, and Mike Zeller, Biotechnology Outreach Education Coordinator.

Science Content

- To teach students how the sugars found in soybeans can be altered genetically
- To give students experience evaluating sweetness and sugar content

Science Education Standards

Physical Science, Content Standard B, Chemical Reactions – A large number of important reactions involve the transfer of either electrons (oxidation/reduction reactions) or hydrogen ions (acid/base reactions) between reacting ions, molecules, or atoms. (p. 179)

Catalysts, such as metal surfaces, accelerate chemical reactions. (p. 179)

Life Science, Content Standard C, The Cell – Most cell functions involve chemical reactions (that are catalyzed by enzymes). (p. 184)

Plant cells contain chloroplasts, the site of photosynthesis. (p. 184)

Life Science, Content Standard C, The Molecular Basis of Heredity – Changes in DNA (mutations) occur spontaneously at low rates. (p. 185)

Life Science, Content Standard C, Matter, Energy, and Organization in Living Systems – The energy for life primarily derives from the sun. (p. 186)

Science Process Skills

Comparing/observing
Inferring
Reflecting

Life Skills

Learning
Science processing

Time

Preparation: One hour

Iowa educators can order the following supplies without charge from the ISU Office of Biotechnology by phoning 1-800-643-9504:

- High sucrose soybeans
- Normal soybeans
- Thin-layer chromatography (TLC) plates – 5 centimeter (cm) x 10 cm size
- N-(-1-naphthyl) ethylenediamine dihydrochloride
- Carbohydrate standards – 10 milligrams (mg) per milliliter (ml) mixture of stachyose, raffinose, and sucrose

In addition to the supplies listed in the Materials section, obtain at least one of the following alcohols if your school laboratory does not regularly stock it:

- 70% isopropyl alcohol – available from a pharmacy
- 91% isopropyl alcohol – available from a pharmacy or mix 90 ml isopropyl alcohol in 10 ml H_2O

Optional: Make copies of student handouts 4, 5, and 11, pp. 37, 39, and 57.

Activity: One 90-minute class period or two 40-minute class periods

Materials

Student Supplies

Students should work in pairs. For each pair, you will need:

- High sucrose soybeans*
- Normal soybeans*
- N-(1-naphthyl) ethylenediamine dihydrochloride*

- Measuring cup
- Kitchen strainer
- One 1-liter glass container with no metal or plastic parts
- Two pairs standard laboratory gloves and goggles
- One 5 cm x 10 cm Whatman K5 thin-layer chromatography plate*
- Carbohydrate standards*
- Three 10-microliter (µl) micropipettes
- One pencil (Do not substitute a pen.)
- One ruler marked in millimeters (mm) and cm
- One piece of plastic wrap (Saran wrap or similar) large enough to enclose the chromatography plate
- Two glass beakers, mixing bowls, or other containers each large enough to contain 1/2 cup of soybeans plus enough water to keep them covered overnight
- Three or four paper towels
- Three microscopic plates
- Masking tape and marker pen

Students can share some equipment. Two or more pairs of students can share:

- A blender
- Two wide-mouth (at least 5 cm wide) glass jars with lids. Wide-mouth mason jars such as those used for canning garden produce will work.
- A hairdryer that has low/high heat settings

Teacher Supplies

To prepare enough of the necessary solvent and dipping reagent for a class of 20, teachers will need the following chemical supplies:

<u>Solvent for One Chromatograpy Jar</u>
100 ml of 91% isopropyl alcohol **or**
100 ml of 70% isopropyl alchohol **or**

<u>Dipping Reagent</u>
3 grams N-(1-naphthyl) ethylenediamine dihydrochloride*
1 liter (L) methanol
50 ml concentrated H_2SO_4 (sulfuric acid)

**Available from the ISU Office of Biotechnology.* *See p. 24.*

Educator Background Information

As members of the legume family of plants, soybeans and other beans can produce embarrassing digestive gas. The reason has to do with the carbohydrates in soybeans, specifically the sugars. The digestive gas produced by normal soybeans is largely associated with two sugars, raffinose and stachyose, that are not easily digested by enzymes in the small intestine. Instead, these sugars pass into the large intestine where *E. coli* and other bacteria naturally live. As the bacteria eat the sugars, they begin dividing and produce gasses like hydrogen, nitrogen, and carbon dioxide that cannot be smelled. Unfortunately, the bacteria also produce sulfur compounds and the gas methane that have offensive odors. On average, humans release about one quart of gas a day or approximately 14 expulsions.

Raffinose and stachyose are reduced by 90% in high sucrose soybeans, greatly decreasing the digestive gas problem. In addition, the sucrose content is increased 40% to give the soybeans a sweeter taste.

Sucrose

Sucrose, one of the sugars found in soybeans, is easily digestible. The sucrose molecule is made up of two simpler sugars called glucose and fructose. One glucose molecule bonded to one fructose molecule makes one sucrose molecule. If we use Glc to represent glucose and Fru to represent fructose, then **Glc – Fru** represents the structure of sucrose. Because sucrose is made of two simpler sugars, sucrose is classified as a disaccharide. The prefix “di-” means two and saccharide means sugar.

Raffinose

Some sugars in soybeans are not easily digested. One of these is called raffinose. Raffinose is created when a sucrose molecule adds another sugar called galactose. One galactose bonded to one sucrose makes one raffinose molecule. Using Gal for galactose, then **Gal – Glc – Fru** represents the structure of raffinose. Raffinose is made of three simpler sugars, so it is classified as a trisaccharide. Because raffinose is difficult to digest, it passes into the large intestine where *E. coli* and other bacteria naturally live. The *E. coli* bacteria digest raffinose, producing gasses having objectionable odors.

Stachyose

Another sugar in soybeans that is difficult to digest is called stachyose (STACK e ose). When one more galactose bonds to a raffinose molecule, stachyose is formed. **Gal – Gal – Glc – Fru** represents the structure of stachyose. Because stachyose has four sugars, it is classified as a tetrasaccharide or oligosaccharide.

Thin-Layer Chromatography

Carbohydrates differ in the number of carbon atoms that they have and in the size of the molecules. If two carbohydrates have similar characteristics, they can be difficult to separate. Because carbohydrates play important roles in living systems and are the building blocks for a large number of important naturally-occurring products, methods for separating them were among the earliest chromatographic procedures developed. Today, thin-layer chromatography is the preferred method for separating carbohydrates. Using thin-layer chromatography (TLC) to separate and detect different carbohydrates requires a support material, a solvent, and a detection method.

Support material
The support material used is silica gel. The gel is a stable and inert substance that is usually spread a uniform 250 µm thick over a glass plate. Drops of the carbohydrate solution are placed on the silica gel plates.

Solvent
The separation of carbohydrates is accomplished by using a solvent. The particular solvent that is used depends on the kinds of carbohydrates that are to be separated, that is, whether they are mono-, di-, tri-, or oligo-saccharides. The TLC plate is vertically placed in a developing tank with the solvent. The solvent ascends up the plate and over the carbohydrate, separating out the different carbohydrate components.

In this activity, isopropyl alcohol is the solvent used to separate sucrose, raffinose, and stachyose. One end of the TLC plate with the drops of carbohydrate solution is placed into a container with the alcohol. The alcohol slowly rises up the plate and over the carbohydrate, separating out the different carbohydrate components. The next step is detecting the separations.

Detection method
A very sensitive detection system has been developed that can detect most carbohydrates in the nanogram range of 50-2,000 ng directly on the TLC plate. There are one billion nanograms in a gram. The method uses a detecting reagent that consists of N-(1-naphthyl) ethylenediamine dihydrochloride, methanol, and sulfuric acid.

The TLC plate is taken out of the alcohol solvent container, dried with a hairdryer, dipped quickly into the reagent, and dried with the hairdryer again. The carbohydrate separations will appear as blue-black spots on the white background of the TLC plate. By comparing the position and darkness of the spots with a previously processed TLC plate with sucrose, raffinose, and stachyose, students can identify the three carbohydrates on their own plates. The previously processed TLC plate is called a carbohydrate standard.

Pre-Lab Preparation

1-3 days before the activity:

Ask students if anyone is allergic to handling soy products. **Allergic students should not participate in the Thin-Layer Chromatography activity.**

Teachers will need to prepare the solvent and the dipping reagent. Both the solvent and the reagent should be mixed and stored in glass containers that can be capped. The reagent should not be capped with a metallic lid.

WEAR LABORATORY GLOVES, APRONS, AND GOGGLES WHEN PREPARING THE CHEMICALS AND USE STANDARD SAFE LABORATORY PRACTICES. THE SOLVENT AND THE DIPPING REAGENT ARE POISONOUS IF INGESTED AND THE DIPPING REAGENT CONTAINS A CORROSIVE ACID.

<u>Directions for Preparing the Solvent Jars</u>

1. Pour the alcohol concentration that you have chosen to use (91% or 70%) into wide-mouth (at least 5 cm wide) glass jars to a depth of 7 to 8 millimeters.

2. Tightly cap the jars. The solvent jars may be prepared up to three days before the activity and stored at room temperature.

<u>Directions for Preparing the Dipping Reagent</u>

1. In a glass container that can hold at least 1 liter, dissolve 3 g N-(1-naphthyl) ethylenediamine in 1 L methanol.

2. VERY CAREFULLY AND SLOWLY add 50 ml H_2SO_4 (sulfuric acid). Be sure to follow all safety precautions and recommendations that appear in bold caps above and on the chemical containers.

3. Mix by stirring with a **glass** rod. DO NOT stir with a metallic utensil.

4. The reagent may be stored indefinitely in a closed glass container at room temperature. Do not use a metal lid.

Doing the Activity

Note: If using soybean filtrate saved from the “Soybean Drink Laboratory” activity, omit steps 1-4 in Day 1 instructions and omit steps 1-3 in Day 2 instructions.

Day 1

1. Depending on the availability of blenders, teachers may wish to have the students work in pairs or in groups of four or more. Ask students to clean the bowls, measuring cups, beakers, strainers, and blender containers with hot soapy water before they begin. The teacher may complete this step before class if preferred.

2. Tell students to measure one-half cup (75 g) of normal soybeans into a container and add enough tap water (two cups) to keep the soybeans covered as they soak for 18-24 hours. The soybeans should stay covered with water for this entire time, so designated students or the teacher should check the containers periodically. Before leaving for the day, add a sufficient amount of water to cover the soybeans overnight. You cannot add too much water. Students should use the masking tape and marker to label the container “Normal.”

3. Repeat step two with the high sucrose soybeans. Label the container “High Sucrose.”

4. Each group or pair of students should prepare a thin-layer chromatography plate for the next day’s activity. Tell students to turn the plate white-side-up. Direct students to use a pencil and ruler to make a **very light** line 15 mm from one side of the plate, as shown in figure 1. It is important that students use a pencil to draw lightly on the plate. Alcohol dissolves ink which would migrate up the plate, staining it.

5. If students are making their own standard plate for pure sucrose, raffinose, and stachyose, as well as testing the two kinds of soybeans, ask students to make three dots along the length of the horizontal line 1.5 cm apart. Be sure that students do not make any dots closer than 1 cm to the edge of the plate. If the standard plate for pure sucrose, raffinose, and stachyose was made by the teacher, students need to make only two dots on the horizontal line. In this case, teachers may wish to have more than two lab pairs share a plate.

6. Label the left dot by writing “N” for normal, label the middle dot by writing “S” for sugar standards, and label the right dot “H” for high sucrose. Write the labels under each dot.

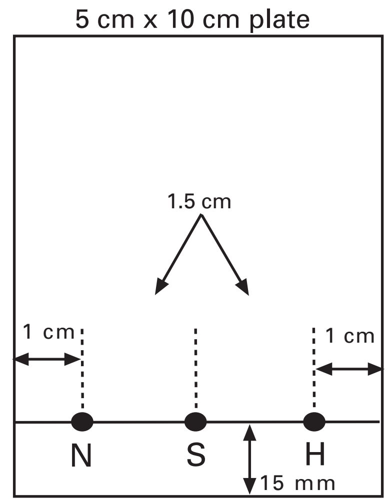


Figure 1

Day 2

1. Tell students to drain the water from the high sucrose and normal soybeans.

2. Measure the normal soybeans into a measuring cup. Put the soybeans and an equal amount of distilled water in a blender and blend on high for one minute.

3. While the mixture is blending, ask students to place a double layer of coffee filters in a kitchen strainer. Direct students to filter the mixture, now a whitish liquid, through the double layer of coffee filters into a glass beaker or container.

Note: At this point, teachers may wish to centrifuge the filtrate to obtain better results. If desired, centrifuge the filtrate with either a clinical or microcentrifuge for 10-20 minutes.

4. Prepare a 1/5 dilution by transferring a drop of the mixture to a 1.5 ml test tube. Add 4 drops of distilled water to make the 1/5 dilution.

5. Transfer 2 drops of the dilution liquid to a clear microscopic slide.

6. Label the slide “Normal.”

7. Repeat steps 2 through 5 with the high sucrose beans. Label the second microscopic slide “High Sucrose.”

8. Ask one student in each pair to place one end of a 10 µl micropipette into the liquid on the slide labeled “Normal.” The micropipette should fill through capillary action. The student should place the end of the micropipette on the TLC plate labeled “N” until a drop of liquid about 2.5 mm wide transfers to the plate. The smaller the dot, the better.

9. Using a new 10 µl micropipette, students should repeat step 8 with the standard mixture. This time, the drop should be placed on the pencil dot labeled “S.”

10. Using another new 10 µl micropipette, students should repeat step 8 with the high sucrose soybeans. This time, the drop should be placed on the pencil dot labeled “H.”

11. When all drops are on the plate and have been dried, students should put on lab gloves. The plate should be placed in the solvent jar so that it is standing vertically, leaning against the side of the jar with the pencil line at the bottom. See figure 2. Cap the jar tightly. The level of solvent should be about halfway between the bottom of the plate and the horizontal line.

12. The solvent will rise up the plate by capillary action, turning the plate a gray, damp color. When the solvent has risen to the top of the plate in about 1 hour, uncap the jar. A student (or the teacher) wearing lab gloves should carefully remove the plate and place it on several thicknesses of paper towels to dry. Leaning the plate upright is the best way to dry it. If the plate is dried horizontally, the coated side should face up. Re-cap the solvent jar tightly.

    At this point, the TLC plates can be left to air-dry and the experiment can be continued the next class period. The dry plates should be covered with plastic wrap if left overnight to prevent dust contamination. Be sure the TLC plates are dry before wrapping them with plastic wrap.

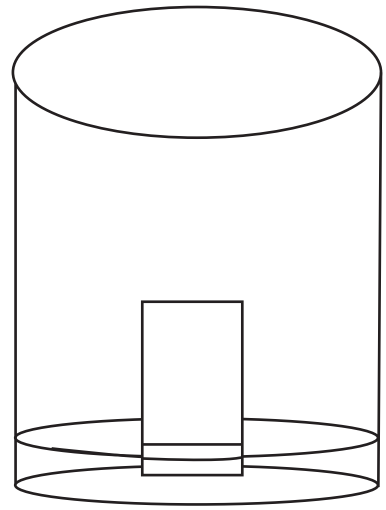


Figure 2

Day 3

1. The teacher should pour dipping reagent into a glass jar to a minimum depth of 15 cm. A mason jar works well.

2. Wearing lab gloves, students or the teacher should dip the TLC plate into the reagent to immerse it. The plate should be immersed for only a second. See figure 3.

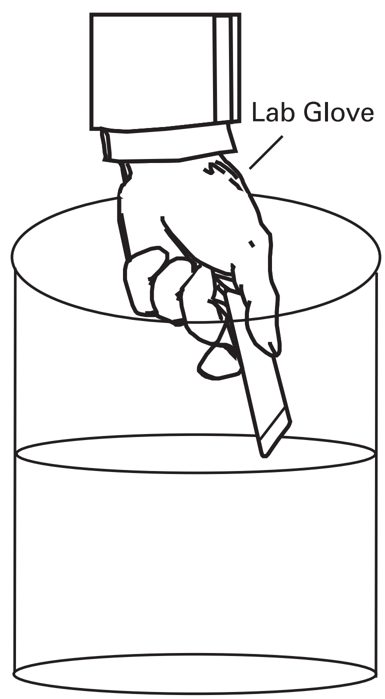
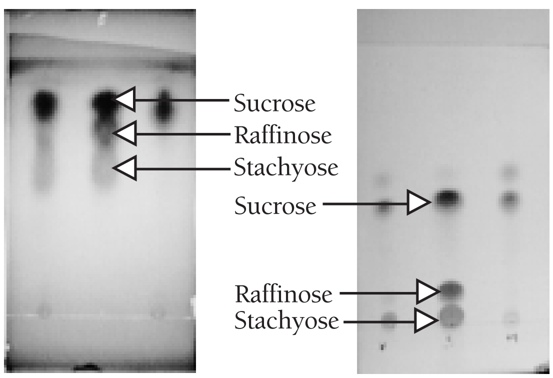


Figure 3

3. Place the plate on several thicknesses of paper towels to air-dry or use the hairdryer on low setting to dry. Leaning the plates upright is the best way to dry them. If dried horizontally, the coated side of the plate should face up. The plates are dry when the white color

returns. Continue to dry/heat the plates until the dark spots appear.

4. Varying sizes and densities of black spots will appear in a lane that stretches above each labeled pencil dot on the plate. Using figure 4 as a guide, help students locate the spots that correspond to sucrose, raffinose, and stachyose in the "S" lane. Compare the spots that appear in the lanes above the "N" for normal soybeans and "H" for high sucrose soybeans to the positions of the spots for pure sucrose, raffinose, and stachyose that appeared in the "S" lane. Use a pencil to mark the spots with an "S" for sucrose, "R" for raffinose, and "ST" for stachyose. (Note: Other spots that may appear in the lanes are other sugars.)



70% isopropyl alcohol	91% isopropyl alcohol
No centrifuge	No centrifuge
1/5 filtrate concentration	1/5 filtrate concentration

Figure 4

5. Compare the size and blackness of the sucrose, raffinose, and stachyose spots that appear in the normal soybean lane with those in the high sucrose soybean lane. The sucrose spot should be blacker and/or larger in the high sucrose soybean lane, reflecting the 40 percent increase in sucrose. The raffinose and stachyose spots should be lighter and/or smaller in the high sucrose soybean lane, reflecting the 90 percent reduction in these sugars.

6. The plates can be kept for up to two years before turning dark, if wrapped in plastic wrap and kept in a cabinet or cupboard away from light.

Safety and Clean-Up

1. Gloves and eye protection should be worn when mixing the dipping solution and handling the plates afterwards.

2. Be sure there are no open flames. Some materials used in this laboratory are highly flammable.

3. Finished plates should be wrapped with plastic wrap or placed into plastic bags to minimize exposure to the sulfuric acid residue on the plates.

4. Normal (local) laboratory precautions should be used for the handling, clean-up, and disposal of flammable (methanol, isopropyl alcohols) and corrosive (sulfuric acid) materials.

Reflect and Apply

1. What are the three carbohydrates that you are trying to identify in this lab activity?

   Sucrose, raffinose, stachyose

2. How do these carbohydrates differ molecularly?

   Sucrose is composed of two saccharides, glucose and fructose. Raffinose is composed of three saccharides, galactose, glucose, and fructose. Stachyose is composed of four saccharides – two galactose, one glucose, and one fructose.

3. Explain why the three carbohydrates found in soybeans move up the plate at different rates.

   The rate is an inverse relationship to a sugar's molecular size. The more saccharides there are in the sugar, the slower/less the rate.

4. Why is it important to test soybeans for the presence of the carbohydrates?

   Answers may vary. Two possible answers are that testing could be used to 1) measure the amount of commercially useful sucrose in the soybean or 2) check the soybean to confirm that it is a high sucrose variety.

5. Why could it be important socially and commercially to have a soybean high in sucrose?

   Students might mention benefits to farmers, manufacturers, and consumers. Because high sucrose soybeans taste better and produce less gas, the percentage of soy in manufactured foods can be increased.

Science Facts About . . .
Soybean Flavor

The soybean grain commonly produced by farmers has a grassy or beany flavor. This flavor is not a problem when the grain is used as animal feed, but it can be objectionable to some persons when soybeans are used in food products.

Lipoxygenase

The beany flavor is the result of the action of an enzyme called lipoxygenase (li POX i jen ace). The name of this enzyme reflects what it does. As the "lipo" part of its name implies, the enzyme is involved with **lipids**. Lipids are a class of molecules that includes fats and oils.

The middle part of lipoxygenase is "oxygen." Lipoxygenase adds oxygen to fats in a process called oxidation. It is this oxidation process that causes the beany flavor in soybeans.

The last three letters of lipoxygenase "ase" are a common suffix that scientists use to name enzymes. If you see a chemical-sounding word with "ase" on the end, the odds are that the word is the name of an enzyme.

There are three forms or isozymes of lipoxygenase, commonly referred to as lipoxygenase 1, 2, and 3.

"Sorry to break up the act, but at least one of you has to go!"

Isozymes are forms of an enzyme that affect the same characteristic of an organism. Lipoxygenase 1, 2, and 3 affect the flavor of soybeans. The three isozymes occur in common soybean varieties grown by farmers.

To eliminate the beany flavor, soybean scientists had to control one or more of the three lipoxygenase isozymes. Soybean scientists evaluated soybean varieties from throughout the world in an attempt to find those that did not have one or more of the lipoxygenase enzymes. They found a few varieties that lacked enzyme 1, enzyme 2, or enzyme 3, but no variety lacked all three.

Hybridization, Mutation, and Selection

By using the tools of hybridization, mutation, and selection, soybean scientists combined the forms (alleles) of the three lipoxygenase genes that resulted in absence of the lipoxygenase enzyme. Soybeans are now available that lack one, two, or all of the lipoxygenase isozymes. These soybeans can be used to produce soy drinks and other food products that no longer have the beany flavor of traditional soybeans.

Learn the Language

Hybridization – cross-breeding two distinct species types to combine their characteristics in their offspring

Isozyme – one of two or more forms of an enzyme that have different chemical structures but control the same process

Lipids – a class of almost non-soluble molecules that includes fats and oils

Lipoxygenase – enzyme that catalyzes oxidation of lipids

Mutations – genetic changes in DNA that result in changes within species of living organisms. Mutation is a natural process in nature, but it can be speeded up by using chemical treatments or other means.

Selection – choosing the offspring that have desired characteristics

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Science Facts About . . .
Lactose Intolerance

What Is Lactose Intolerance?

Lactose intolerance is the inability to digest significant amounts of lactose, the predominant sugar of milk. This inability results from a shortage of the enzyme lactase, which is normally produced by the cells that line the small intestine. Lactase breaks down milk sugar into simpler forms that can then be absorbed into the bloodstream. When there is not enough lactase to digest the amount of lactose consumed, the results, although not usually dangerous, may be very distressing. While not all persons deficient in lactase have symptoms, those who do are considered to be lactose intolerant.

Common symptoms include nausea, cramps, bloating, gas, and diarrhea, which begin about 30 minutes to two hours after eating or drinking foods containing lactose. The severity of symptoms varies depending on the amount of lactose each individual can tolerate.

Some causes of lactose intolerance are well known. For instance, certain digestive diseases and injuries to the small intestine can reduce the amount of enzymes produced. In rare cases, children are born without the ability to produce lactase. For most people, lactase deficiency is a condition that develops naturally over time. After about the age of 2 years, the body begins to produce less lactase. However, many people may not experience symptoms until they are much older.

Between 30 and 50 million Americans are lactose intolerant. Certain ethnic and racial populations are more widely affected than others. As many as 75 percent of all African-Americans and Native Americans and 90 percent of Asian-Americans are lactose intolerant. The condition is least common among persons of northern European descent.

How Is Lactose Intolerance Diagnosed?

The most common tests used to measure the absorption of lactose in the digestive system are the lactose tolerance test, the hydrogen breath test, and the stool acidity test. These tests are performed on an outpatient basis at a hospital, clinic, or doctor's office.

The Lactose Tolerance Test

The lactose tolerance test begins with the individual fasting (not eating) before the test and then drinking a liquid that contains lactose. Several blood samples are taken over a two-hour period to measure the person's blood glucose (blood sugar) level, which indicates how well the body is able to digest lactose.

Normally, when lactose reaches the digestive system, the lactase enzyme breaks down lactase into glucose and galactose. The liver changes the galactose into glucose, which enters the bloodstream and raises the person's blood glucose level. If lactose is incompletely broken down, the blood glucose level does not rise and a diagnosis of lactose intolerance is confirmed.

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The Hydrogen Breath Test

The hydrogen breath test measures the amount of hydrogen in the breath. Normally, very little hydrogen is detectable in the breath. However, undigested lactose in the colon is fermented by bacteria, and various gases, including hydrogen, are produced. The hydrogen is absorbed from the intestines, carried through the bloodstream to the lungs, and exhaled. In the test, the patient drinks a lactose-loaded beverage, and the breath is analyzed at regular intervals. Raised levels of hydrogen in the breath indicate improper digestion of lactose. Certain foods, medications, and cigarettes can affect the test's accuracy and should be avoided before taking the test. This test is available for children and adults.

Stool Acidity Test

The lactose tolerance and hydrogen breath tests are not given to infants and very young children who are suspected of having lactose intolerance. A large lactose load may be dangerous for very young individuals because they are more prone to dehydration that can result from diarrhea caused by the lactose. If a baby or young child is experiencing symptoms of lactose intolerance, many pediatricians simply recommend changing from cow's milk to soy formula and waiting for symptoms to abate.

If necessary, a stool acidity test, which measures the amount of acid in the stool, may be given to infants and young children. Undigested lactose fermented by bacteria in the colon creates lactic acid and other fats that can be detected in a stool sample. In addition, glucose may be present in the sample as a result of unabsorbed lactose in the colon.

How Is Lactose Intolerance Treated?

Intolerance is relatively easy to treat. No treatment exists to improve the body's ability to produce lactase, but symptoms can be controlled through diet. Young children with lactase deficiency should not eat any foods containing lactose.

Most older children and adults need not avoid lactose completely, but individuals differ in the amounts of lactose they can handle. For example, one person may suffer symptoms after drinking a small glass of milk, while another can drink one glass but not two. Others may be able to manage ice cream and aged cheeses, such as cheddar and Swiss but not other dairy products. Dietary control of lactose intolerance depends on each person's learning through trial and error how much lactose he or she can handle.

For those who react to very small amounts of lactose or have trouble limiting their intake of foods that contain lactose, lactase enzymes are available without a prescription. One form is a liquid for use with milk. A few drops are added to a quart of milk, and after 24 hours in the refrigerator, the lactose content is reduced by 70 percent. The process works faster if the milk is heated first, and adding a double amount of lactase liquid produces milk that is 90 percent lactose free.

A more recent development is a chewable lactase enzyme tablet that helps people digest solid foods that contain lactose. Three to six tablets are taken just before a meal or snack.

Lactose-reduced milk and other products are available at many supermarkets. The milk contains all of the nutrients found in regular milk and remains fresh for about the same length of time or longer if it is super-pasteurized.

The preceding information is from the Web site of the National Digestive Diseases Information Clearinghouse, 2 Information Way, Bethesda, MD 20892-3570, E-mail: nddic@info.niddk.nih.gov, http://www.niddk.nih.gov/health/digest/pubs/lactose/lactose.htm. Used with permission.

The National Digestive Diseases Information Clearinghouse is a service of the National Institute of Diabetes and Digestive and Kidney Diseases, which is part of the National Institutes of Health under the U.S. Department of Health and Human Services. NIH Publication No. 98-2751, April 1994, e-text updated: November 1998.

Learn the Language

Lactase – an enzyme produced in the small intestine that breaks down lactose into glucose and galactose

Lactose – a sugar found in dairy products such as milk and cheese; commonly called milk sugar

Lactose intolerance – the inability to digest significant amounts of lactose

Science Facts About . . .

Lactose Intolerance Biochemistry

The Biochemistry of Lactose Intolerance

Try to answer as many of the following questions that you can. The questions deal with the biochemistry of lactose intolerance. You may use a dictionary, your science textbook, or other sources to find the answers.

1. Define:

   enzyme –

   hydrolysis –

   substrate –

   active site –

2. How many simple sugars are there in lactose? Based on this, classify lactose.

3. Complete the following:

   $C_{12}H_{22}0_{11}$ Lactose

   \+ Add water

   ____

   $C_{12}H_{24}0_{12}$

   \- Subtract glucose formula

   ____

   Galactose formula

4. What would be the enzyme that activates this reaction?

5. Where does this enzyme act in your body?

6. Where is this enzyme produced in your body?

7. Name the substrate.

This activity was adapted from Enzyme Action, an activity submitted to the Access Excellence Fellows Collection by Jane Crumlish, retired from Neshaminy High School, Langhorne, Pennsylvania. The Access Excellence Web site at http://www.accessexcellence.org/ is sponsored by the National Health Museum. This activity is used with the permission of Jane Crumlish and Access Excellence.

Science Facts About . . .
Soybean Digestibility

As members of the legume family of plants, soybeans and other beans can produce embarrassing digestive gas. The new soybean products that you are studying in this unit are more digestible than traditional soybean foods. The reason has to do with the carbohydrates in soybeans, specifically the sugars.

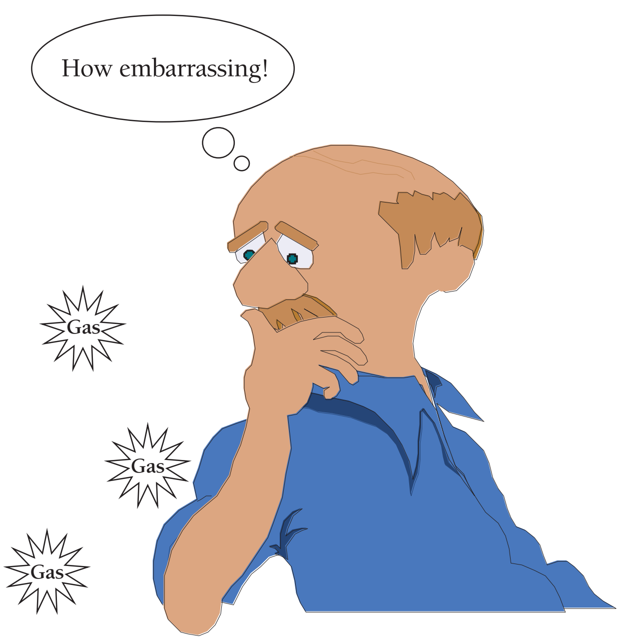
The Sugars in Soybeans

Sugars are not always as sweet as they seem. In fact, sometimes they can be downright stinky! Take the sugars in soybeans, for example.

Sucrose

Sucrose, one of the sugars found in soybeans, is easily digestible. The sucrose molecule is made up of two simpler sugars called glucose and fructose. One glucose bonded to one fructose makes one sucrose molecule. If we use Glc to represent glucose and Fru to represent fructose, then the structure of sucrose looks like this:

Glc – Fru



Because sucrose is made of two simpler sugars, sucrose is classified as a disaccharide. The prefix "di-" means two and saccharide means sugar.

Raffinose

Some sugars in soybeans are not easily digested. One of these is called raffinose. Raffinose is formed when another sugar called galactose is added to a sucrose molecule. One galactose bonded to one sucrose makes one raffinose molecule. Using Gal for galactose, the structure of raffinose can be written like this:

Gal – Glc – Fru

Raffinose is made of three simpler sugars, so it is classified as a trisaccharide. The prefix "tri-" means three.

Because raffinose is difficult to digest, it passes into the large intestine where *E. coli* and other bacteria naturally live. The *E. coli* bacteria convert raffinose into hydrogen and other gasses that smell awful.

Stachyose

Another sugar in soybeans that is difficult to digest is called stachyose (STACK e ose). When one more galactose bonds to a raffinose molecule, stachyose is formed. The structure of stachyose looks like this:

Gal – Gal – Glc – Fru

Because stachyose has four sugars, it is classified as a tetrasaccharide. You guessed it – the prefix "tetra-" means four.

Life raffinose, stachyose is difficult to digest so it passes into the large intestine where it is converted into smelly gasses.

A Sugar That Is Not in Soybeans

The sugar lactose, commonly called milk sugar, is found in dairy products. Some people have insufficient levels of lactase, the enzyme that digests milk sugar. These individuals, described as lactose intolerant, cannot digest milk sugar and experience discomfort such as abdominal pain, bloating, gas, or diarrhea when they eat dairy products. There are now dietary aids on the market that can help lactose intolerant people digest milk sugar.

Lactose is not found in soybeans or a drink commonly called soy milk that is made from soybeans. Advertisers promote the lactose-free characteristic of soybean beverages as an alternative for people who are lactose intolerant.

Learn the Language

Disaccharide – a sugar made up of two simple sugars

Lactose – a sugar found in dairy products such as milk and cheese; commonly called milk sugar

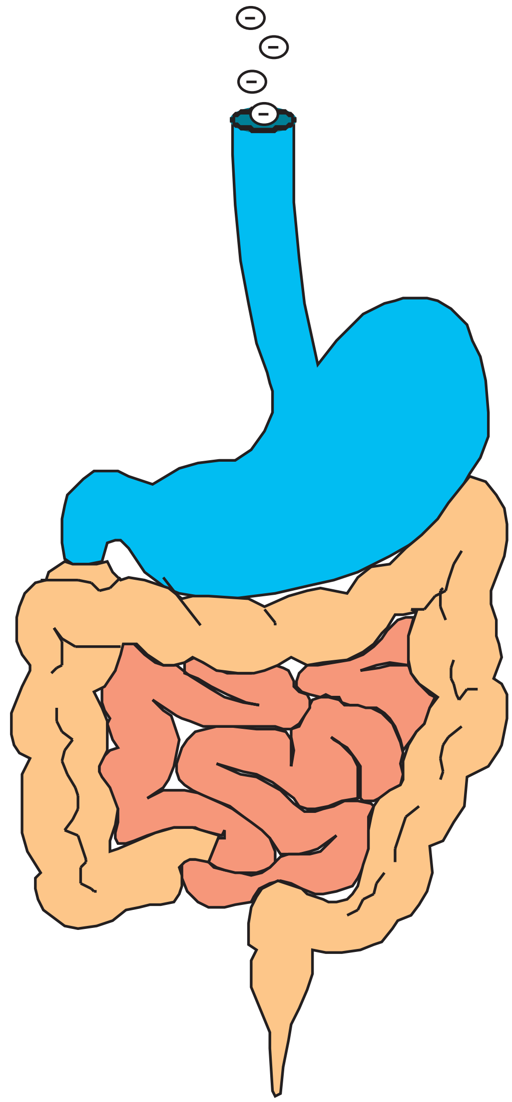
Raffinose – the sugar formed when sucrose links to one galactose

Sucrose – an easily digestible sugar made up of one glucose unit linked to one fructose unit

Stachyose – a sugar that consists of one raffinose linked to one galactose

Tetrasaccharide – a sugar made up of four simple sugars

Trisaccharide – a sugar made up of three simple sugars



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Science Facts About . . .
Thin-Layer Chromatography

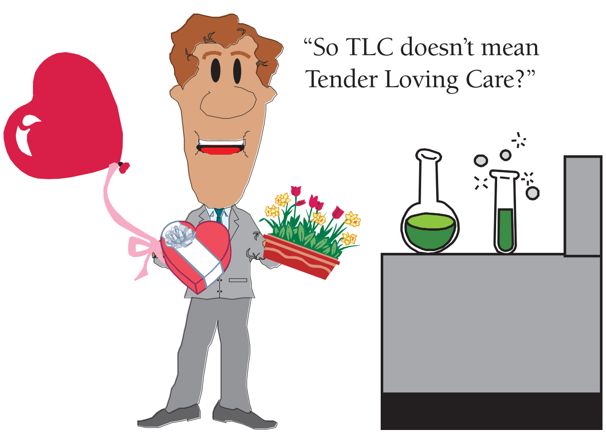
What Is Thin-Layer Chromatography (TLC)?

Chromatography is a laboratory method used to separate compounds so that they can be studied. The word chromatography means "color" (chromato) and "writing" (graphy). Chromatography was invented by Russian botanist Michael Tswett in the early 1900's while he was studying the colored pigments in plants.

There are several different types of chromatography, including liquid, gas, paper, and thin-layer. Thin-layer chromatography uses an absorbent material coating on glass or plastic plates.

Because carbohydrates play important roles in living systems and are the building blocks for a large number of important naturally-occurring products, methods for separating them were among the earliest chromatographic procedures developed. Today, thin-layer chromatography is the preferred method for separating carbohydrates. Using thin-layer chromatography to separate and detect different carbohydrates requires a support material, a solvent, and a detection method.

Support material
The support material used is silica gel. The gel is usually spread a uniform 250 µm thick over a glass plate. Drops of the carbohydrate solution are placed 15 mm from the bottom of the silica gel plate.



Solvent
The separation of carbohydrates is accomplished by using a solvent. The particular solvent that is used depends on the kinds of carbohydrates that are to be separated. The TLC plate is vertically placed in a developing tank with the solvent. The solvent ascends up the plate and over the carbohydrate, separating out the different carbohydrate components.

Detection method
A very sensitive detection system has been developed that can detect most carbohydrates directly on the TLC plate. The method uses a detecting reagent that consists of N-(1-naphthyl) ethylenediamine dihydrochloride, sulfuric acid, and methanol. The first two of these three are dehydrating agents. After the solvent separates the carbohydrates, the TLC plate is removed from the solvent container, dried, and quickly dipped into the reagent. The plate is dry-heated until blue-black spots of carbohydrates appear on the white background of the TLC plate.

Scientists compare the position and darkness of the spots on the TLC plate with spots on a previously processed TLC plate that had known carbohydrates on it. The previously processed TLC plate is called a carbohydrate standard. The comparison helps scientists determine which carbohydrates are present in the compound that they are testing.

Learn the Language

Chromatography – a laboratory method used to separate compounds and mixtures

Reagent – a substance that causes reactions

Solvent – a substance that dissolves another substance

Thin-layer chromatography – a laboratory technique that uses an absorbent material coating on glass or plastic plates, a solvent, and a reagent to separate compounds

Based on information provided by Professor John F. Robyt, Biochemistry, Biophysics, and Molecular Biology Department, Iowa State University.

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See for Yourself . . .
Soybean Flavor

Soybean Taste Test

In this demonstration, you will experience first-hand the effect that lipoxygenase enzymes have on soybean flavor. Record the results of your taste test below. Be careful when you are chewing on the soybean seeds to avoid damaging your teeth! **If you are allergic to consuming or handling soy products, you should not participate in this activity.**

A. Soybean without lipoxygenase enzymes

1. Describe the taste of this soybean using taste descriptive words such as bitter, nutty, sweet, salty, or others. Don't make value judgements at this point such as "good" or "bad," just describe the taste as accurately as you can.

2. Rate the way that the soybean tastes to you by circling your rating on the scale below.

1	2	3	4	5
My dog wouldn't eat this.	Not good, but I could choke it down.	OK	Good flavor	Give me more!

B. Soybean with lipoxygenase enzymes

1. Describe the taste of this soybean using taste descriptive words such as bitter, nutty, sweet, salty, or others. Don't make value judgements at this point such as "good" or "bad," just describe the taste as accurately as you can.

2. Rate the way that the soybean tastes to you by circling your rating on the scale below.

1	2	3	4	5
My dog wouldn't eat this.	Not good, but I could choke it down.	OK	Good flavor	Give me more!

C. Analyze the results

1. Which of the two soybeans that you tasted do you think tastes better?

2. Look at the ratings in parts A-2 and B-2 on the previous page. Subtract the lower number that you circled from the higher number that you circled and write the answer below. The number you wrote represents how much difference you could detect between the taste of the two soybeans.

   Explanation: The greater your answer, the greater the difference that you could detect in taste between the two soybeans. If you circled the same number on both scales, your answer was zero, meaning that you detected no taste difference between the two soybeans seeds.

3. What percentage of your group/class could detect a difference in taste between the two soybeans? Write your answer below.

4. Calculate the degree of difference between the taste of the two soybeans as detected by the students in your group/class by adding together everyone's answers for number 2 above. Write the sum of everyone's answers below.

5. The maximum possible taste difference that could be detected by each person would be 4 if he or she circled 1 on one of the scales and 5 on the other. Therefore, the maximum degree of difference for your group/class would be represented by the formula:

   no. of students x 4 =maximum degree of difference.

   Using the formula, what is the maximum degree of taste difference that is possible for your group/class?

   How does the maximum degree of difference compare to your answer for number 4 above (the actual degree of difference for your group/class)?

D. Reflect and Apply

1. What are the results of the taste test? What are the differences between the taste of the two soybeans?

2. Why could some students detect a greater difference in taste than others?

3. Suppose that you manufactured a soy product that is marketed to your classmates. You currently use traditional soybeans in your product. Based on the answers of your group/class to numbers 1 through 5 in section C, should you switch from using traditional soybeans in your product to using lipoxygenase-free soybeans? Why or why not?

4. What other factors, besides taste, would a manufacturer of food products have to consider before deciding to change a product?

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See for Yourself . . .
Soybean Flavor

Soybean Drink Laboratory

In this experiment, you will make soy drink (commonly called soy milk) from two varieties of soybeans. One variety contains lipoxygenase enzymes and one does not. This experiment illustrates the difference in the taste of soy products made from the two types of soybeans. **If you are allergic to consuming or handling soy or milk products, you should not participate in this activity.**

Materials

- Lipoxygenase and lipoxygenase-free soybeans
- Measuring cup
- Two large bowls or other container
- Masking tape and marking pen
- Kitchen strainer
- Two 1000 ml beakers
- Electric blender
- Cheesecloth or coffee filters
- Hot plate or stove
- Two 2-quart or larger saucepans or glass cookware safe for stove-top use
- Candy thermometer
- Sink or other source of hot, soapy water and water for rinsing
- Spoons for stirring
- Refrigerator or cooler with ice
- Sugar, chocolate syrup, vanilla, or other flavorings
- Labels from containers of cow's milk and soy milk
- 2% milk

Doing the experiment

Day 1

1. Clean the measuring cup, bowls or other containers, kitchen strainer, beakers, spoons, and blender container with hot soapy water before you begin, if your teacher has not already cleaned them.
2. Measure one cup (150 grams) of lipoxygenase-free soybeans and place them in one of the large bowls. Pour at least two cups of water into the container so that the soybeans are completely covered with water. Use the masking tape and marking pen to make a "Lipo-Free" label and attach it to the container.
3. Repeat step #2 using soybeans that contain the lipoxygenase enzymes. Mark the container "Lipo."
4. Let the soybeans soak for 18 to 24 hours. The soybeans should stay covered with water during this entire time, so check the soybeans periodically and add more water when necessary.

Day 2

One half of the class will process the soybeans in the "Lipo-Free" container and the other half will process the soybeans in the "Lipo" container.

1. Use a strainer to drain the water from the soybeans and rinse them three times with clean water.
2. Add water to the soaked soybeans in a 7:1 (water:soybean) mixture. Do this by pouring 700 ml of water into a 1000 ml beaker. Add only enough of the soaked soybeans until the water level in the beaker reaches 800 ml.
3. Grind the water/soybean mixture in a blender at high speed for three minutes.

4. Filter the mixture, now a whitish liquid, through cheesecloth or coffee filters placed in a kitchen strainer over a saucepan.

5. Cook the filtered liquid at 98-100° C (208-212° F) for 15 minutes. Stir the liquid continuously so it does not burn. Use a candy thermometer to monitor the temperature. Begin timing at 98° C or 208° F.

6. Refrigerate the liquid immediately after cooking.

7. After the soy milks are cold, compare the taste of lipoxygenase, lipoxygenase-free, and commercial soy milk. After your initial taste test, you can add sugar, chocolate syrup, or other ingredients to the soy drinks for flavor.

Adapted from a protocol developed by the ISU Department of Food Science and Human Nutrition.

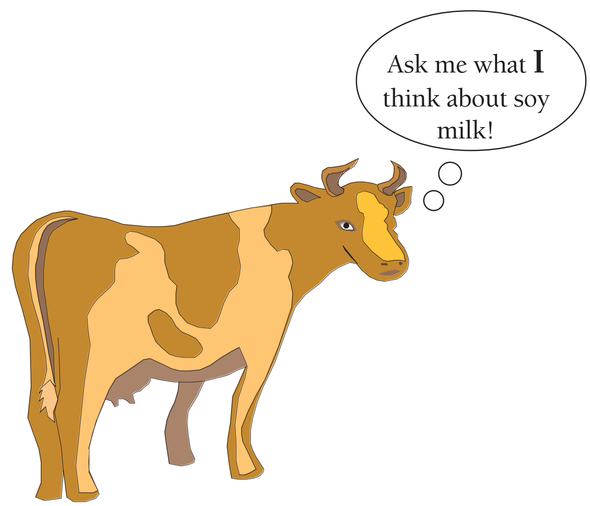
Reflect and Apply

1. Based on your initial taste test, which of the drinks did you prefer? Why?

2. How do soy milk and cow's milk compare nutritionally?

3. Some people cannot consume cow's milk because of lactose intolerance, allergies, or other health reasons. Besides health concerns, for what other reasons might people choose to use soy milk?

4. What food products use soybeans? What other non-food uses can you think of for soybeans?



Issued in furtherance of Cooperative Extension work, Acts of May 8 and June 30, 1914 in cooperation with the U.S. Department of Agriculture. Stanley R. Johnson, director, Cooperative Extension Service, Iowa State University of Science and Technology, Ames, Iowa.

See for Yourself . . .
Digestive System

A Walk Through the Digestive System

After you have completed the walkthrough of the digestive system, think about the following questions:

1. What did you learn about the digestive system?

2. What does every cut or tear represent? List the structures of digestion and whether the food is digested physically, chemically, or both in that structure.

3. What does each piece of the index card that is cut represent? For each digestive structure, list the nutrient(s) that are digested there.

4. What does each piece of card that is left at the station represent? Where do these nutrients go? For each digestive structure, list the structure and which nutrient(s) are absorbed there.

5. Do you or anyone in your family have trouble digesting certain foods? Which food(s)? What factors cause digestive problems?

6. Babies and elderly people often have more digestive problems than the general population. Why do you think this happens?

7. What can people do to avoid digestive problems?

This activity was adapted from A Walk Through the Gut, an activity submitted by VivanLee Ward, Sequoia High School, Redwood City, California, to the Access Excellence Fellows Collection. The Access Excellence site at http://www.accessexcellence.org/ is sponsored by the National Health Museum. This activity is used with the permission of VivianLee Ward and Access Excellence.

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See for Yourself . . .
Lactose Intolerance

Digesting Lactose with Lactase Experiment

Predictions

In this experiment, you will test six different solutions for the presence of glucose. Using what you have learned about how lactase catalyzes (speeds up) the digestion of the milk sugar lactose, in which solutions do you predict that the presence of glucose will be detected? Write yes or no under each circle.

1	2	3	4	5	6
Glucose	Milk	Lactase	Lactase and Milk	Soy Milk	Lactase and Soy Milk
________	________	________	________	________	________

The Experiment

Note: If you are allergic to handling soy or milk products, you should not participate in this experiment.

1. Your teacher will give your group a glass plate or slide. Use the marker to draw six circles on the glass plate or slide and label them like the circles above.

2. Take turns putting the following solutions in each of the circles that you drew on the glass plate or slide. To avoid cross-contamination of your solutions, use a different dropper for each solution and two different tooth-picks for mixing.

   Circle 1: Add two drops of glucose solution.

   Circle 2: Add two drops of milk.

   Circle 3: Add one drop of lactase.

   Circle 4: Add one drop of lactase plus two drops of milk and mix with a toothpick.

   Circle 5: Add two drops of soy milk.

   Circle 6: Add one drop of lactase plus two drops of soy milk and mix with a clean toothpick.

3. After three minutes, use the forceps to place one end of a piece of glucose test paper into the solution in each circle. You will need six different glucose test paper strips.

4. After the glucose test papers have been lying in the solutions for two minutes, observe the color of each test paper. If the test paper has changed color, the solution tested positive for glucose. Make a data table to record your results.

Data Table:

Reflect and Apply

1. In which circle(s) would you expect the hydrolysis of the milk sugar, lactose?

2. What products would result after this hydrolysis? How did you know this?

3. Why did you test the glucose with the glucose test paper?

4. Why did you test the lactase with the glucose test paper?

5. Does milk contain glucose? Explain how you know this.

6. Does soy milk contain glucose? Explain how you know this.

7. Does milk combined with lactase contain glucose? Explain how you know this.

8. Some people cannot drink cow's milk because their digestive systems do not produce enough lactase. If milk is ingested, it cannot be digested. This results in cramping, bloating, gas and diarrhea. Suggest a treatment that would allow such a person to drink cow's milk.

9. Why would a lactose intolerant person be able to drink soy milk?

This activity was adapted from Enzyme Action, an activity submitted to the Access Excellence Fellows Collection by Jane Crumlish, retired from Neshaminy High School, Langhorne, Pennsylvania. The Access Excellence Web site at http://www.accessexcellence.org/ is sponsored by the National Health Museum. This activity is used with the permission of Jane Crumlish and Access Excellence.

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See for Yourself . . .
Sucrose Comparison

Invertase Experiment

Background Information

High sucrose soybeans offer improved flavor and digestibility of soy milk and other soy food products. These newly developed soybeans contain 90% less of the indigestible carbohydrate sugars raffinose and stachyose that can produce abdominal discomfort and gas. The beans also contain 40% more sucrose and lack the lipoxygenase-2 enzyme that is responsible for "beany" flavor. Because they taste better and produce less gas, they allow the manufacturers of soy products to increase the percentage of soy in beverages, bakery products, pasta, and other foods.

As suggested by their name, high sucrose soybeans are 40% higher in sucrose, a carbohydrate sugar that is made up of two simpler sugars, glucose and fructose. In this lab you will produce evidence that high sucrose soybeans contain more sucrose than normal soybeans. You will use a sucrase enzyme called invertase. Invertase breaks down (hydrolyzes) sucrose into the monosaccharides glucose and fructose. A commercial glucose test used by diabetics to monitor the sugar levels in their urine will be used to measure the glucose in the soybeans because the test is designed to be very specific for glucose.

Note: **If you are allergic to handling soy products, you should not participate in this experiment.**

Doing the Pre-Lab

Materials

- High sucrose soybeans
- Normal soybeans
- Two 400-600 ml beakers or containers
- Tap water
- Marker and two labels

Procedure

1. Add 75 grams (1/2 cup) of normal soybeans to a container and add at least twice the volume of regular tap water. Label the container "Normal."

2. Repeat step 1 with the high sucrose soybeans. Label the container "High Sucrose."

3. Let the soybeans soak overnight until the next day's lab time. The soybeans will begin to swell as they absorb the water. The soybeans should stay covered with water for 24 hours. A student from your group and/or your teacher should check periodically to make sure the beans stay covered. Make sure to add a sufficient amount of water to cover the soybeans overnight. You cannot add too much water.

Doing the Laboratory

Day 1

Materials
- High sucrose soybeans from pre-lab prep
- Normal soybeans from pre-lab prep
- Two blenders
- Distilled water
- Two large wire strainers
- Four #6 coffee filters
- One 600-1000 mL beaker or collection containers
- Four test tubes
- Aluminum foil or plastic wrap to cover container
- Marker and six labels
- Clock, watch, or timer

Procedure
1. Your teacher may select two groups to do the following procedure.

2. Drain the water from the normal and high sucrose soybeans.

3. Place the "Normal" soybeans in a blender and add an equal volume of distilled water.

4. Blend the mixture on high for 1 minute.

5. While the mixture is blending, place two #6 coffee filters in the strainer positioned over another container to collect the liquid (filtrate).

6. After a minute of blending, pour the mixture into the coffee filters and let filter. Label this container "Normal."

7. Repeat steps 3-6 with the high sucrose soybeans. Label the collection container "High Sucrose."

8. Let the soybean mixtures drain completely (15-20 minutes).

9. While the soybeans are draining, make the following labels::

   a. Label two test tubes. Label one test tube "Normal" and the other test tube "High Sucrose."
   b. Label two additional tubes as "C Normal" and "C High Sucrose."
   c. Create a data table for the Day 2 lab. Your teacher will show you how.

10. Cover the containers and place in the refrigerator overnight.

Make Predictions
1. Which of the four mixtures do you think will test positive for glucose? Why?

2. Of the mixtures that text positive for glucose, which one(s) do you think will have the largest amount of glucose? Why?

Day 2

- Four test tubes labeled "Normal," "High Sucrose," "C Normal," and "C High Sucrose" prepared on Day 1
- Test tube rack
- Four test tube stoppers
- Glucose test strips for urine (Bayer's Diastix® strips were used in the original lab tests)
- Four 1 ml transfer pipettes or medicine droppers
- 0.01 grams of invertase in a 1.5 ml tube
- Distilled water
- 50-55°C water bath
- Forceps
- Clock, watch, or timer
- Paper towel and pen or marker

Procedure

1. Fill each test tube 1/4 full with each mixture as it corresponds to the label on the tube. Use a different pipette for each mixture.

2. Using the same pipettes that were used to transfer each of the four mixtures in step 1, add enough distilled water to each test tube to bring the level to half full. Stopper each test tube and mix by inverting each test tube several times.

3. Test each mixture for glucose by dipping a glucose test strip into the mixture in each test tube. Use a different test strip for each mixture. Shake the excess mixture off each test strip and lay it on a paper towel. Write the name of the test tube mixture, "Normal," "High Sucrose," "C Normal," or "C High Sucrose" on the towel near the corresponding test strip.

4. Let the glucose test strips sit for 1 minute or the amount of time stated in the directions that came with the glucose strips.

5. Read the concentration of glucose by comparing the color of each strip with the chart on the glucose strip container.

6. Record your results on your data table.

7. Get from your teacher a tube of the enzyme invertase. Add 1 ml (20 drops) of distilled water to the invertase tube and dissolve the enzyme. **If your group is the control group, you should skip steps 7-9. You will not add the enzyme to the control "C" tubes.**

8. Using a transfer pipette, add half of the invertase mixture to the "Normal" mixture and the other half to the "High Sucrose" mixture.

9. Mix the contents of each tube by placing a stopper in the mouth of the tube and inverting it several times.

10. Place the test tubes in the 50-55°C water bath.

11. After five minutes, test the mixture with the glucose test strips and record the results on the data table. Read each strip exactly 30 seconds after dipping.

12. Repeat the glucose test every five minutes or as often as your teacher directs.

13. Record your results on the data table.

14. Clean up the lab area according to your teacher's directions.

Reflect and Apply

1. Why is it important to read the test strip in 30 seconds?

2. Explain what is happening chemically in this activity.

3. Explain the presence of glucose in both normal and high sucrose soybean samples.

4. On a commercial scale, what is the importance of developing a high sucrose soybean?

5. As a consumer what other traits would you consider valuable enough to want in soybeans, if the technology were available?

This activity was developed by Mike Zeller for the ISU Office of Biotechnology. The activity is used with permission.

See for Yourself . . .
Sucrose Comparison

Thin-Layer Chromatography Experiment

Note: If you are allergic to handling soy products, you should not participate in this experiment.

Materials

For each pair of students:

- High sucrose soybeans
- Normal soybeans
- N-(1-naphthyl) ethylenediamine dihydrochloride
- Measuring cup
- Kitchen strainer
- One 1-liter glass container with no metal or plastic parts
- Two pairs standard laboratory gloves and goggles
- One 5 cm x 10 cm Whatman K5 thin-layer chromatography plate
- Carbohydrate standards
- Three 10-microliter (µl) micropipettes
- One pencil (Do not substitute a pen.)
- One ruler marked in millimeters (mm) and cm
- One piece of plastic wrap (Saran wrap or similar) large enough to enclose the chromatography plate
- Two glass beakers, mixing bowls, or other containers each large enough to contain 1/2 cup of soybeans plus enough water to keep them covered overnight
- Three or four paper towels
- Three microscopic plates
- Masking tape and marker pen

Two or more pairs of students can share:

- A blender
- Two wide-mouth (at least 5 cm wide) glass jars with lids. Wide-mouth mason jars such as those used for canning garden produce will work.
- A hairdryer that has low/high heat settings

Doing the Activity

Day 1

1. Depending on the availability of blenders, your teacher may wish to have you work in pairs or in groups of two or more pairs. Clean the bowls, measuring cups, beakers, strainers, and blender containers with hot soapy water before you begin, unless your teacher has already completed this step.

2. Measure one-half cup (75 g) of normal soybeans into a container and add enough tap water (about two cups) to keep the soybeans covered as they soak for 18-24 hours. The soybeans should stay covered with water for this entire time, so designated students or the teacher should check the containers periodically. Before leaving for the day, add a sufficient amount of water to cover the soybeans overnight. You cannot add too much water. Use the masking tape and marker to label the container "Normal."

3. Repeat step two with the high sucrose soybeans. Label the container "High Sucrose."

4. Each group or pair of students should prepare a thin layer chromatography plate for the next day's activity. Turn the plate white-side-up. Use the ruler to make a **very light** line 15 mm from one side of the plate, as shown in figure 1. It is important to use a pencil to draw lightly on the plate. Alcohol dissolves ink which would migrate up the plate, staining it.

5. Your teacher may want you to make your own standard plate for pure sucrose, raffinose, and stachyose, as well as testing the two kinds of soybeans. If so, use a pencil to make three dots along the length of the horizontal line 1.5 cm apart. Be sure that you do not make any dots closer than 1 cm to the edge of the plate.

   If the standard plate for pure sucrose, raffinose, and stachyose was made by the teacher, you need to make only two dots on the horizontal line.

6. Label the left dot by writing "N" for normal, label the middle dot by writing "S" for sugar standards, and label the right dot "H" for high sucrose. Write the labels under each dot.

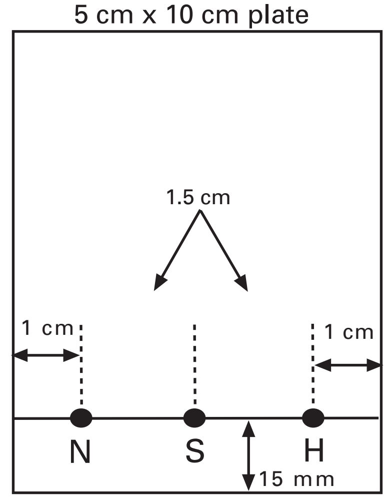

Figure 1

Day 2

1. Drain the water from the high sucrose and normal soybeans.

2. Measure the normal soybeans into a measuring cup. Put the soybeans and an equal amount of distilled water in a blender and blend on high for one minute.

3. While the mixture is blending, place a double layer of coffee filters in a kitchen strainer. Filter the mixture, now a whitish liquid, through the double layer of coffee filters into a glass beaker or container.
   Note: At this point, your teacher may wish to centrifuge the liquid (filtrate) to obtain better results. If desired, centrifuge the filtrate with either a clinical or microcentrifuge for 10-20 minutes. Continue to prepare the dilution in step 4 from the top layer of the filtrate.

4. Prepare a 1/5 dilution by transferring a drop of the mixture to a 1.5 ml test tube. Add 4 drops of distilled water to make the 1/5 dilution.

5. Transfer 2 drops of the dilution liquid to a clear microscopic slide.

6. Use the marking pen to label the slide "Normal."

7. Repeat steps 2 through 5 with the high sucrose beans. Label the second microscopic slide "High Sucrose."

8. One student in each pair should place one end of a 10 µl micropipette into the liquid on the slide labeled "Normal." The micropipette should fill through capillary action. Place the end of the micropipette on the TLC plate labeled "N" until a drop of liquid about 2.5 mm wide transfers to the plate. The smaller the dot, the better.

9. Using a new 10 µl micropipette, repeat step 8 with the standard mixture. This time, the drop should be placed on the pencil dot labeled "S."

10. Using another new 10 µl micropipette, repeat step 8 with the high sucrose soybeans. This time, the drop should be placed on the pencil dot labeled "H."

11. When all drops are on the plate and have been dried, put on lab gloves. Place the plate in the solvent jar so that it is standing vertically, leaning against the side of the jar with the pencil line at the bottom as shown in figure 2. Cap the jar tightly. The level of solvent should be about halfway between the bottom of the plate and the horizontal line.

12. The solvent will rise up the plate by capillary action, turning the plate a gray, damp color. When the solvent has risen to the top of the plate in about 1 hour, uncap the jar. A student wearing lab gloves (or the teacher) should carefully remove the plate and place it on several thicknesses of paper towels to dry. Leaning the plate upright is the best way to dry it. If the plate is dried horizontally, the coated side should face up. Re-cap the solvent jar tightly.

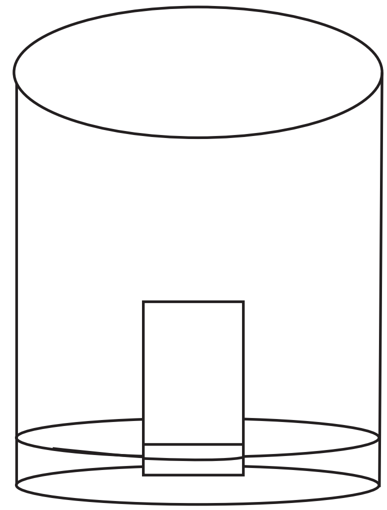

Figure 2

At this point, your teacher may wish to continue this experiment on the following day. If this is the case, the dry plates should be covered with plastic wrap if left overnight to prevent dust contamination. Be sure the TLC plates are dry before wrapping them with plastic wrap.

Day 3

1. Your teacher should pour dipping reagent into a glass jar to a minimum depth of 15 cm. A mason jar works well.

2. Wearing lab gloves, you or your teacher should dip the TLC plate into the reagent to immerse it. The plate should be immersed for only a second. See figure 3.

3. Place the plate on several thicknesses of paper towels to air-dry or use the hairdryer on low setting to dry. Leaning the plates upright is the best way to dry them. If dried horizontally, the coated side of the plate should face up. The plates are dry when the white color returns. Continue to dry/heat the plates until the dark spots appear.

4. Varying sizes and densities of black spots will appear in a lane that stretches above each labeled pencil dot on the plate. Locate the spots that correspond to sucrose, raffinose, and stachyose in the "S" lane. Compare the spots that appear in the lanes above the "N" for normal soybeans and "H" for high sucrose soybeans to the positions of the spots for pure sucrose, raffinose, and stachyose that appeared in the "S" lane. Use a pencil to mark the spots with an "S" for sucrose, "R" for raffinose, and "ST" for stachyose.

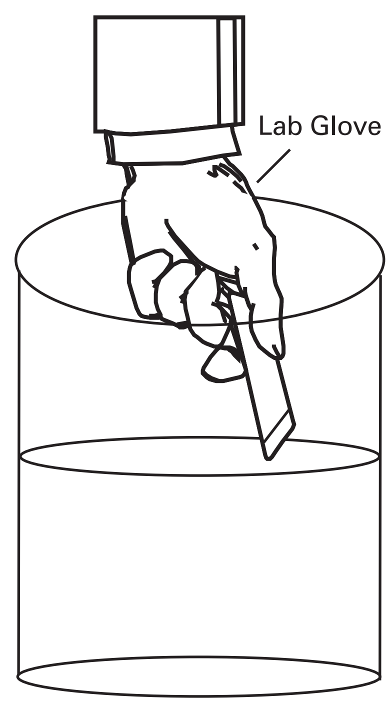

Figure 3

5. Compare the size and blackness of the sucrose, raffinose, and stachyose spots that appear in the normal soybean lane with those that appear in the high sucrose soybean lane.

Safety and Clean-Up

1. Wear gloves and eye protection when "dipping" the TLC plates and when handling the plates afterwards.
2. Be sure there are no open flames. Some materials used in this laboratory are highly flammable.
3. Wear gloves when handling the finished plates.
4. Follow the clean-up directions as provided by your teacher.

Reflect and Apply

1. What are the three carbohydrates that you are trying to identify in this lab activity?

2. How do these carbohydrates differ molecularly?

3. Explain why the three carbohydrates found in soybeans move up the plate at different rates.

4. Why is it important to test soybeans for the presence of the carbohydrates?

5. Why could it be important socially and commercially to have a soybean high in sucrose?

This activity was developed at Iowa State University by John Robyt, Professor, Biochemistry, Biophysics, and Molecular Biology Department, and Mike Zeller, Biotechnology Outreach Education Coordinator.

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Educator Evaluation

Please complete the following evaluation and return it by mail or fax to the Office of Biotechnology, 1210 Molecular Biology Building, Iowa State University, Ames, IA 50011-3260. Fax (515) 294-4629. If you prefer to send your comments by e-mail, please send them to biotech@iastate.edu. Thank you.

A. Which activities from this curriculum did you do with your class(es)? Please mark with an x or checkmark.

_____ Soybean Taste Test

_____ Soybean Drink Laboratory

_____ A Walk Through the Digestive System

_____ Digesting Lactose Experiment

_____ Comparing Sucrose Quantities using Invertase

_____ Comparing Sucrose Quantities Using Thin-Layer Chromatography

B. Please rate the curriculum on the following:

	Poor				Excellent
1. Ease of pre-class preparation	1	2	3	4	5
2. Time required for pre-class preparation	1	2	3	4	5
3. Ability to do activities in available class time	1	2	3	4	5
4. Content of activities	1	2	3	4	5
5. Helpfulness of background information	1	2	3	4	5
6. Organization/sequence	1	2	3	4	5
7. Creating interest in benefits and risks of biotechnology	1	2	3	4	5
8. Hands-on student involvement	1	2	3	4	5
9. Grade appropriate	1	2	3	4	5
10. Correlation to your curriculum	1	2	3	4	5

C. Please share the problems you encountered while doing the activities, the things you thought worked great, and any suggestions you may have for improving one or more activities. Use the back of this sheet if you need more space.

Glossary

Chromatography
A laboratory method used to separate compounds and mixtures

Disaccharide
A sugar made up of two simple sugars

Hybridization
The cross-breeding of two distinct types of a species to combine their characteristics in their offspring

Isozyme
One of two or more forms of an enzyme that have different chemical structures but control the same process

Lactase
An enzyme produced in the small intestine that breaks down lactose into glucose and galactose

Lactose
A sugar found in dairy products such as milk and cheese; commonly called milk sugar

Lactose intolerance
The inability to digest significant amounts of lactose

Lipids
A class of almost non-soluble molecules that includes fats and oils

Lipoxygenase
An enzyme that catalyzes the oxidation of lipids

Mutations
Genetic changes in DNA that result in changes within species of living organisms. Mutation is a natural process in nature, but it can be speeded up by using chemical treatments or other means.

Raffinose
The sugar formed when sucrose links to one galactose

Reagent
A substance that causes reactions

Selection
The process of choosing the offspring that have desired characteristics

Solvent
A substance that dissolves another substance

Sucrose
An easily digestible sugar made up of one glucose unit linked to one fructose unit

Stachyose
A sugar that consists of one raffinose linked to one galactose

Tetrasaccharide
A sugar made up of four simple sugars

Thin-layer chromatography
A laboratory technique that uses an absorbent material coating on glass or plastic plates, a solvent, and a reagent to separate compounds

Trisaccharide
A sugar made up of three simple sugars

People Resources for Educators

ISU Biotechnology Outreach Education Center

The Biotechnology Outreach Education Center is located in the Molecular Biology Building on the Iowa State University campus in Ames. The 2,300 square-foot center consists of two adjacent state-of-the-art laboratories with 40 lab stations and a prep room.

The center is fully stocked with the laboratory supplies needed for a number of biotechnology experiments. Equipment in the center includes centrifuges, incubators, electrophoresis gel boxes, and more. The center was funded by Iowa State University, contributions from private individuals, and:

- AgrEvo/Plant Genetic Systems
- Ajinomoto U.S.A., Inc./Heartland Lysine, Inc.
- Cargill
- Genencor International, Inc.
- Golden Harvest Research
- Growmark
- The Greater Cedar Rapids Foundation–Diamond V Mills Donor-Advised Fund
- The Iowa Farm Bureau Federation Agricultural Foundation
- The Iowa Soybean Promotion Board
- Kemin Industries, Inc.
- MBS, Inc.
- Novartis Seeds, Inc.
- Pioneer Hi-Bred International, Inc.
- The Roy J. Carver Charitable Trust
- West Central Cooperative

K-12 teachers, extension professionals, and other educators who want to teach about the science that leads to new biotechnology developments can find help at the Biotechnology Outreach Education Center. Not only can educators do the same experiments that they will be teaching, but they can practice the lab preparation needed to do the experiments.

Learn how to do DNA extractions, transformations, and fingerprinting; see if Bt corn really does stop corn borers; explore recombinant DNA techniques; or develop a scope and sequence plan for your school. ISU has been training teachers in summer workshops since 1994, and now is able to offer workshops during the summer or during the academic year. Workshops especially for educators include:

- Basic Biotechnology for Science Educators, Agriculture Education Instructors, and Family and Consumer Sciences Educators
- Advanced Biotechnology for Science, Agriculture, and Family and Consumer Sciences Educators or
- Customize a workshop for your educators

The Biotechnology Outreach Education Center offers a number of hands-on laboratory experiences for students. Laboratory experiments that can be done in the center include the activities from the list that follows. Teachers may consult with biotechnology outreach education coordinator Mike Zeller to develop a unique laboratory experience for their students.

For K-6 Grade Students:
- Build a DNA model
- DNA extraction
- Gene deletion simulation
- Infectious disease simulation
- Soybean flavor demonstration

For 6-12 Grade Students or Adults:
- Bt corn
- Chymosin demonstration
- DNA extraction from bacteria, kiwi, or onion
- DNA fingerprinting
- DNA transformation of bacteria – ampicillin or red colony
- Forensic science demonstration
- Micropropagation of plants
- Plasmid isolation and analysis – red colony
- Soybean carbohydrate demonstration
- Soy drink
- Soybean flavor demonstration

For the most recent additions to the list of experiments, free materials, and lab supplies provided to Iowa educators, see the Office of Biotechnology Web page at http://www.biotech.iastate.edu.

Contacts

Michael Zeller – Biotechnology Outreach Education Center Coordinator. Phone 515-294-5949 or toll-free in Iowa 1-800-643-9504. E-mail: mzeller@iastate.edu

Lori Miller – Program Secretary. Phone 515-294-9818 or toll-free in Iowa 1-800-643-9504. E-mail: lorimill@iastate.edu

Gary Comstock – Bioethics Program Coordinator. Phone 515-294-0054. E-mail: comstock@iastate.edu

ISU Extension Biotechnology Team

Iowa State University Extension biotechnology youth specialists are available throughout the state. These specialists provide consultation in presenting biotechnology educational programs, assistance in presenting biotechnology laboratory workshops, and assistance in borrowing biotechnology resources such as model kits, displays, and videotapes.

Each school district in Iowa has been provided with a kit of equipment to conduct DNA extraction from bacteria, DNA transformation of bacteria, and the red colony DNA transformation procedures. To conduct DNA fingerprinting, teachers may check out electrophoresis equipment by contacting their Area Education Agency.

If an AEA kit is unavailable, Iowa teachers can contact ISU or one of the following extension offices for the equipment. Check the map below to find the office in your area. For the most recent updates, visit the ISU Office of Biotechnology Web site at www.biotech.iastate.edu.

Iowa State University

Extension – Science, Engineering, and Technology (E-SET) Program
32 Curtiss Hall
Ames, IA 50011-1050
Phone: 515-294-8417
Fax: 515-294-4443
E-mail: jstaker@iastate.edu

Office of Biotechnology
1210 Molecular Biology Building
Ames, IA 50011-3260
Phone: 515-294-9818 or toll-free 1-800-643-9504
Fax: 515-294-4629
E-mail: biotech@iastate.edu

Central Area (C)

Polk County Extension
5201 NE 14th Street, Suite A
Des Moines, IA 50313-2005
Phone: 515-261-4204
Fax: 515-263-2704

North Central Area (NC)

Calhoun County Extension
521 4th Street, Box 23
Rockwell City, IA 50579
Phone: 712-297-8611
Fax: 712-297-7011

Humboldt County Extension
P.O. Box 158, 727 Sumner Avenue
Humboldt, IA 50548
Phone: 515-332-2201
Fax: 515-332-2211

East Central Area (EC)

Clinton County Extension
331 East 8th Street
De Witt, IA 52742
Phone: 563-659-5125
Fax: 563-659-5126

Northeast Area (NE)

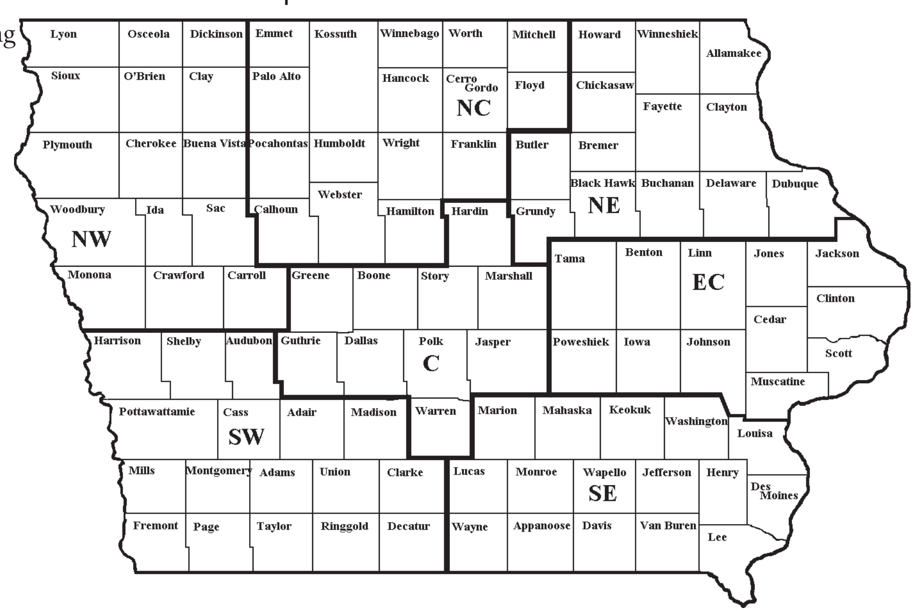
Dubuque County Extension
14742 Hwy 20 W., Suite 2
Dubuque, IA 52003
Phone: 563-583-6496
Fax: 563-583-4844

Southeast Area (SE)

Monroe County Extension
107 Benton Avenue E.
Albia, IA 52531
Phone: 641-932-5612
Fax: 641-932-5662

Northwest Area (NW)

Clay County Extension
110 W. 4th Street, Suite 100
Spencer, IA 51301
Phone: 712-262-2264
Fax: 712-262-8481



Dickinson County Extension
1600 15th Street
Spirit Lake, IA 51360
Phone: 712-336-3488
Fax: 712-336-3498

Southwest Area (SW)
Harrison County Extension
304 East 7th Street
Logan, IA 51546
Phone: 712-644-2105
Fax: 712-644-2100

Biotechnology Master Teachers in Iowa Schools

The Master Biotechnology Teachers are a group of specially trained Iowa teachers who are willing to help other educators incorporate biotechnology into the classroom. If you have a biotechnology question, feel free to contact one of the master teachers for help. If no one is listed for your AEA, please direct your questions to Mike Zeller, Biotechnology Outreach Education Coordinator at ISU, 515-294-5949, e-mail mzeller@iastate.edu, or to Jay Staker, E-SET Director, ISU Extension, 515-294-8417, e-mail jstaker@iastate.edu.

AEA 1
Dave Millis
Edgewood-Colesburg High School
403 West Union
Edgewood, IA 52042
School phone: 563-928-6412
School fax: 563-928-6414
E-mail: dmillis@edge-cole.k12.ia.us

AEA 3
Morris Green
Emmetsburg High School
2nd and King
Emmetsburg, IA 50536
School phone: 712-852-2966
School fax: 712-852-3317
E-mail: mgreen@emmetsburg.k12.ia.us

AEA 4
Kevin Brasser
South O'Brien School
216 South Rutledge, Box 638
Paullina, IA 51046
School phone: 712-448-3454
School fax: 712-448-3453
E-mail: kjbrasser@s-obrien.k12.ia.us

Diane Den Herder
Sioux Center Community High
550 9th Street NE
Sioux Center, IA 51250
School phone: 712-722-2981
School fax: 712-722-2986
E-mail: dndnhrdr@sioux-center.k12.ia.us

AEA 5
Cindy Reher
Humboldt Senior High
1500 Wildcat Road
Humboldt, IA 50548
School phone: 515-332-3574
School fax: 515-332-7150
E-mail: reher_c@humboldt.k12.ia.us
or creher@humboldt.k12.ia.us

AEA 6
Jerry Heying
Marshalltown High School
1602 South 2nd Ave.
Marshalltown, IA 50158
School phone: 641-754-1130
School fax: 641-754-1136
E-mail: biology@adiis.net or
gheying@marshalltown.k12.ia.us

AEA 9
Brian Cummings
Columbus Community High
1004 Colton Street
Columbus Junction, IA 52738
School phone: 319-728-2231
School fax: 319-728-8750
E-mail: bcummings@mail.columbus.k12.ia.us

Barb Jacobsen
Bettendorf High School
3333 18th Street
Bettendorf, IA 52722
School phone: 563-332-7001
School fax: 563-332-8761
E-mail: bjacobse@po-1.bettendorf.k12.ia.us

AEA 10
Gary Garton
Iowa City High
1900 Morningside Drive
Iowa City, IA 52245
School phone: 319-688-1040
School fax: 319-339-5705
E-mail: GLGarton@aol.com

Doug Herman
Biology Department
Iowa City West High School
2910 Melrose Avenue
Iowa City, IA 52246
School phone: 319-339-6817
School fax: 319-339-5738
E-mail: hermand@iowa-city.k12.ia.us

AEA 12
Carol Sadler
Battle Creek/Ida Grove High School
900 South Montgomery
Ida Grove, IA 51445
School phone: 712-364-3371
School fax: 712-364-4463
E-mail: csadler@bc-ig.k12.ia.us

Ronald Wilmot
Akron-Westfield School
850 Kerr Drive
Akron, IA 51001
School phone: 712-568-2020
School fax: 712-568-2997
E-mail: rwilmot@aw-frontier.akron-westfield.k12.ia.us

AEA 13
Pat Campbell
Lewis Central High School
1600 East South Omaha Bridge
Council Bluffs, IA 51503-7820
School phone: 712-366-8222
School fax: 712-366-8340

AEA 15
Steve Dent
Davis County High School
106 NE Street
Bloomfield, IA 52537-1432
School phone: 641-664-2200, Ext. 140
School fax: 641-664-2221
E-mail: dents@aea15.k12.ia.us

AEA 16
Ernest Schiller
Central Lee High School
Highway #218 South
Donnellson, IA 52625
School phone: 319-835-5121
School fax: 319-835-5709
E-mail: eschiller@central-lee.k12.ia.us

Laboratory Resources for Educators

Laboratory Supplies and Instructional Materials

Iowa State University's Office of Biotechnology offers Iowa teachers free equipment, supplies, and curriculum materials for biotechnology lab activities plus free access to biotech experts.

Free laboratory supplies and/or curriculum materials are available for these laboratory protocols. For the most recent updates, visit the Office of Biotechnology Web site at www.biotech.iastate.edu.

- Bt corn
- Chymosin demonstration
- DNA extraction from bacteria, kiwi, or onion
- DNA fingerprinting
- DNA transformation of bacteria - ampicillin or red colony
- Micropropagation of plants
- Plasmid isolation and analysis - red colony
- Soybean flavor demonstration
- Soy drink
- Using invertase to detect sucrose in soybeans
- Using thin-layer chromatography to detect sucrose in soybeans

Contact
To obtain free supplies and instructional materials, please contact:

Lori Miller
Office of Biotechnology
1210 Molecular Biology Building
Iowa State University
Ames, IA 50011-3260
Phone: 1-800-643-9504 or 515-294-9818
Fax: 515-294-4629
E-mail: lorimill@iastate.edu.

The supplies are delivered to Iowa teachers by a parcel service or by mail, postage paid.

Downloadable Illustrated Instructions for Lab Procedures

Downloadable, illustrated instructions for laboratory protocols can be found in the educational resources section of the ISU Office of Biotechnology Web site at http://www.biotech.iastate.edu. These presentations offer teachers graphic, step-by-step procedures in lab preparation and student instructions for protocols such as DNA extraction, DNA fingerprinting, and bacterial transformation. There also are instructions on how to properly handle and use the micropipettor.

The presentations are in two formats, PowerPoint® and html. The PowerPoint format allows educators to download the protocols so they can easily edit, print, and/or present information about the protocols to their students. The html format allows educators to view the presentations as Web pages. This requires a Web browser such as MS Explorer® or Netscape®. The Web browser option allows educators to view the presentations, even without the most recent version of PowerPoint software.

PowerPoint® and MS Explorer® are registered trademarks of Microsoft Corporation. Netscape® is a registered trademark of Netscape Communications Corporation.

Printed Resources for Educators

Iowa Biotech Educator Newsletter

The *Iowa Biotech Educator* newsletter is provided primarily for Iowa teachers and extension educators. Each issue describes biotechnology educational opportunities in Iowa, the content of educational programs, classroom resources for educators, and tips for teachers. Subscribers receive five free issues each year.

Contact
To receive a free subscription, please contact:
Lori Miller
Office of Biotechnology
1210 Molecular Biology Building
Iowa State University
Ames, IA 50011-3260
Phone: 1-800-643-9504 or 515-294-9818
Fax: 515-294-4629
E-mail: lorimill@iastate.edu.

ISU Biotechnology Curricula

ISU Extension and the Office of Biotechnology have collaborated on a number of curriculum materials that are available for free or for the cost of printing.

4H 955D	*Biotechnology School Enrichment* Grades 7-8
4H 955C	*Biotechnology School Enrichment* Grades 5-6
4H 955B	*Microscopes and Cells: Biotechnology School Enrichment* Grades 4-5
4H 950	*A Crime, a Clue, and Biotechnology* Ages 12-14
4H 956	*Cut the Fat – Keep the Flavor* Grades 6-12
4H 957	*Biotechnology Careers Brochure* Grades 9-12

Biotechnology Information Series

These easy-to-read bulletins produced by ISU Extension and the Office of Biotechnology were written for the non-scientist. The first copy of each bulletin ordered from an Iowa mailing address is free.

NCR 487	*Principles of Biotechnology*
NCR 483	*Careers in Biotechnology*
NCR 488	*Bovine Somatotropin (bST)*
NCR 529	*Porcine Somatotropin (pST)*
NCR 492	*Plant Disease Diagnostics*
NCR 550	*DNA Fingerprinting in Human Health and Society*
NCR 554	*DNA Fingerprinting in Agricultural Genetics Programs*
NCR 551	*Genetically Engineered Fruits and Vegetables*
NCR 553	*Insect-Resistant Crops Through Genetic Engineering*
NCR 552	*Pharmaceutical Production from Transgenic Animals*
NCR 557	*Regulation of Genetically Engineered Organisms and Products*

Contact
To order curricula or one or more titles of the Biotechnology Information Series, please contact:

Extension Distribution Center
119 Printing and Publications Building
Iowa State University

Ames, IA 50011
Phone: 515-294-5247
Fax: 515-294-2945
E-mail: pubdist@iastate.edu.

Online Resources for Educators

ISU Office of Biotechnology Web Site

The homepage of the ISU Office of Biotechnology at **http://www.biotech.iastate.edu** has education, bioethics, research, and industrial resources. Among the resources for educators are:

- Classroom lab activities
- Step-by-step illustrated tutorials for selected lab activities in html and PowerPoint®
- Information about the ISU Biotechnology Outreach Education Center
- Bioethics case studies
- Biotechnology-related ISU courses and seminars
- Educational funding resources
- Sources of equipment and supplies
- Resource people
- Current and archived copies of the *Iowa Biotech Educator* and other newsletters and news releases
- *Research in Biotechnology* faculty directory of ISU biotechnology researchers
- Careers in biotechnology
- Links to other Web sites

ISU Extension Web Sites

Found at **http://www.extension.iastate.edu**, the ISU Extension Web site has many resources that can be used by educators. Examples include:

- Extension Science, Engineering, and Technology (E-SET) at **http://www.extension.iastate.edu/e-set/**

    - Science activities
    - E-SET curriculum
    - Workshops
    - Issues investigations
    - Links to other sites

- Information about biotech crops can be found at the Iowa Grain Quality Initiative site at: **http://www.iowagrain.org**

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