

The Fermentation Lab: Soda and Yogurt

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Abstract

Students enrolled in microbiology are paired with botany students to form fermentation teams with the assigned task of designing and producing their own unique soda. Microbiology students are responsible for teaching the botany students relevant biochemical paths of the *Saccharomyces cerevisiae* fermentation process. Botany students in turn teach microbiology students about plant secondary metabolites and their role in aesthetics. Together, teams choose all ingredients for their soda and collaborate to make the beverage. Microbiology students additionally produce yogurt to compare fermentative processes. The entire Biology division and guests join together to judge the sodas in several categories at a final taste testing.

Activity

Invitation for User Feedback. If you have used the activity and would like to provide feedback, please send an e-mail to MicrobeLibrary@asmusa.org. Feedback can include ideas which complement the activity and new approaches for implementing the activity. Your comments will be added to the activity under a separate section labeled "Feedback." Comments may be edited.

INTRODUCTION

Learning Objectives.

At the completion of this activity, students will:

- Describe the similarities and differences between lactic acid fermentation and ethanol fermentation.
- Explain the effect of plant secondary metabolites on product taste, consistency, and aesthetics.
- Identify possible sources of contamination.

Background.

Microbiology students should have a clear understanding of aseptic technique and be able to apply the concept to this nonlaboratory environment. Students should also have been exposed to the aerobic respiration and anaerobic fermentation pathways. Botany students should have been introduced to plant secondary metabolites in class.

PROCEDURE

Materials.

Special locations needed (See Instructor Version of procedure for notes)

- Space for food preparation (nonlaboratory location)
- Room temperature location for sodas to ferment and carbonate
- Taste test location

Special note: most of the materials are available in grocery stores. Some materials are available through brewer supply companies. If your community does not have a brewer supply store, please contact Jean Cardinale (cardinale@alfred.edu) or Cheryld Emmons (emmonsc@alfred.edu) for a list of suppliers available over the web.

Materials for Soda section

Materials needed per student group

- 2 tablespoons of sanitizer—ozone generator, available from brewing supply stores, for sterilizing utensils that will contact food

- Cheese cloth—unused, 1 foot by 1 foot
- 5% bleach solution, for sterilizing work surfaces and cutting boards
- Freezer paper or butcher paper to cover work surfaces
- Wine or champagne yeast—1/8 cup pitched culture, available from brewing supply stores
- 2 2-liter soda bottles with caps
- Plant materials, sugars as needed
- 1 gallon spring water
- Gloves (nonlatex, food service supply)

Materials needed per group, but may be shared by multiple groups by staggering start times

- Hydrometer, for specific gravity measurements, (or brix refractometer), available from brewing supply stores
- 1.5 gallon or larger pot with lid, stainless steel or enamel canning are best
- Cooler or plastic tub large enough for ice bath to surround stainless steel pot
- Funnel—6 inches or larger at mouth, must fit in lip of 2-liter soda bottle
- Sponges and/or paper towels for wiping surfaces and washing utensils and pots
- Food processing equipment: zester, cheese grater, knives
- Large stainless steel spoon
- Cutting board (sterilizable plastic or glass, or one-time use disposable mats)
- Stove or hot plate
- Ice for large ice bath
- Food thermometer
- Electronic balance
- pH paper or pH meter
- Small disposable cups (e.g., Dixie cups) (for tasting and pH measurements)
- Refrigerator for storing food products prior to taste testing
- Vacuum oven or vacuum line and side arm flask for degassing soda sample for final hydrometer reading (this item is not needed if using a brix refractometer)

Materials for taste testing

- Pitchers
- Small disposable cups

Materials for Yogurt section

Materials needed per student group

- 2 teaspoons unflavored yogurt with live active cultures (starter culture)(found in grocer's refrigerated section)
- 2 cups skim milk
- 4 gm nonfat dry milk powder
- Sterilized pint-sized Mason jar with lid

Materials needed per group, but may be shared by multiple groups by staggering start times

- Sauce pan—glass, enamel or stainless steel is best
- Stainless steel spoon
- Stove or hot plate
- Ice
- Food thermometer
- Electronic balance
- pH paper or pH meter
- Small disposable cups (e.g., Dixie cups) (for tasting and pH measurements)
- Refrigerator for storing food products prior to taste testing

Student Version.

We have long relied on microbes as a means of preserving food—many products for human consumption are products of fermentation. Can you think of some? In the food industry, fermentation is a means by which microbial growth is encouraged in order to induce desirable changes in product texture and/or flavor, or in stabilization of the product. One example is lactic acid fermentation, which is involved in the formation of yogurt, buttermilk, and cheeses. Production of lactic acid by bacteria such as *Lactobacilli* and *Leuconostoc* reduce the pH of the food, thereby preventing acid-sensitive organisms from growing and spoiling the food.

Fermentation is not limited to bacterial species. The yeast *Saccharomyces cerevisiae* is a unicellular facultative anaerobe that is also capable of fermentation to produce ethanol and carbon dioxide. The process of anaerobic respiration begins with glycolysis and the production of pyruvate from glucose. Pyruvate is converted to acetaldehyde by the enzyme pyruvate decarboxylase with the release of carbon dioxide. Acetaldehyde is reduced to ethanol by the enzyme alcohol dehydrogenase. Alcohol dehydrogenase needs NADH as a cofactor, and therefore regenerates NAD⁺ for glycolysis.

Fermented dairy products, breads, and fermented beverages have been staples of the human diet for thousands of years. *S. cerevisiae* has been cultivated for human use since the early Sumerians learned how to brew barley, as evidenced by a 3,800-year-old recipe found etched in clay (the Hymn to Ninkasi) from the Mesopotamian region. The finding raises the argument whether barley was first domesticated for baking or brewing! Since that time, many grains and other plant products such as grapes have been fermented for foods and beverages. Secondary metabolites in these fruits and grains provide flavor and nutritional value.

Throughout this exercise, we will use some language that is standard to brewers. Some terms you may be unfamiliar with are wort, hydrometer, and pitching yeast. Look these up before you begin the lab.

You will be assigned a partner from the Botany or Microbiology class—together you and your partner will need to decide on a plant material and an energy source. In the brewing process, you will allow *S. cerevisiae* to ferment the plant material in combination with a carbohydrate to produce "soda." In your team, the microbiologist will be the fermentation expert and the botanist will be the plant and secondary metabolite expert. The microbiologist will teach the botanist the metabolic contributions of the yeast to the finished product, while the botanist will explain the contributions of the plants. You both may need to look some information up! Put your brains together and use the information you have learned this semester to create a master soda! And yes, we will drink these concoctions.

Some examples of plant materials used in brewing include twigs from sweet birch (*Betula lenta*), boughs of Norway spruce (*Picea abies* (L.) Karsten), rhizomes of ginger (*Zingiber officinale*), or everyday fruits and vegetables. Some examples of carbohydrate sources include honey, malt, sucrose (common table sugar), glucose (corn sugar), maple syrup, or high fructose syrup.

Please read through the procedure very carefully and then decide what your ingredients will be. Please let us know of any weird or wild ingredients that may take more time to get. For safety reasons, both the microbiology and the Botany instructors must approve all ingredients used in the sodas. The sodas are "due" at the final tasting. Sodas will be made in the Biology student lounge during the week prior to the tasting. There will be a sign-up sheet posted on the door for your team to sign up for a brewing and bottling time—only one group may process their beverage at a time. On the day of processing, it will take about 1.5 hours to boil, cool, and bottle your soda. Time to process plant materials should be added on to that, depending on your ingredients. Plant materials need to be chopped by hand, blenders are not permitted due to the fact that there is too great a breakdown of plant material that leads to turbidity of the final soda. All preparation will take place in the Biology Student Lounge, without exception, and sodas will be stored at room temperature in the designated location until tasting. Soda bottles will need to be checked daily for "firmness," and any bottle deemed "ready to blow" will be chilled until the taste testing.

While you are waiting for the wort to cool, the microbiology students will also be required to make yogurt. Botany students may help out. It is an easy procedure and will allow you to compare ethanol fermentation by yeast with lactic acid fermentation.

For the final taste testing, we would like each team to design a display for their beverage. At a minimum, there should be an informational sheet to let taste testers know the name of the soda, the materials you used, any special steps you took, who the brewers are, and any other information you feel the testers should know in order to accurately judge your creation. On the day of the testing, your friends and colleagues will be invited to participate and judge the brews. The categories are: Best Overall Taste, Worst Overall Taste, Most Marketable, Most Holiday Spirit, Most Colorful, Most Surprising (thought it would be yuck, tasted great), Bubbiest (most explosive), Your Favorite, and Best Display.

**** Very Important Note!****

The items prepared in this lab will be consumed by you and your peers. Aseptic technique is absolutely required! If you are sick, you will be required to watch the proceedings from a safe distance or on another day (you will not be penalized.) Everyone should wash their hands thoroughly before handling any food product and should take care to ensure that all equipment and surfaces remain as clean as possible. Wear gloves when handling food products. Do not touch your face or hair and then touch food. Microbiologists, you are the experts here—please demonstrate good hand washing technique to the botanists and be vigilant that aseptic technique is followed throughout the preparation. Remember: cleanliness means good eats!

Prior to food preparation

1. Wipe down all surfaces with a 5% bleach solution and a clean sponge.
2. Cover work surfaces with supplied freezer paper. Tape edges as needed. Do not place paper under hot plate.
3. Thoroughly wash hands with soap and warm water.
4. Wear gloves to handle food.

Wort preparation and bottling

1. Add chopped plant material to 4 liters of spring water and bring to a boil. Add the carbohydrate, bring back to a boil, and boil vigorously for 10 minutes, stirring occasionally. Do not let the wort boil over.
 - How much to add? Here are some sample amounts for 4 liters of soda:
 - 1 liter of finely cut twigs or inner bark of sweet birch
 - 600 g of cut spruce boughs
 - 30 g of grated ginger plus 1 lemon
 - 0.9 kg of honey
 - 0.45 kg of sucrose
 - 0.45 kg of malt
 - Consider the strength of flavor of your plant products and the chemical composition of the carbohydrates and compare these to the above ingredients.
 - You can always sample some boiled wort—taste it and decide if you like the flavor! If the flavor is too weak, more material may be added, and the mixture reboiled.
2. While the wort is heating and boiling, wash the soda bottles with dishwasher detergent. Put a good squirt in each bottle, followed by a cup of very hot water. Shake vigorously, drain, and rinse extensively with tap water (minimum of 15 complete rinses.) Allow the bottles to completely drain. Don't forget to wash the bottle caps as well.
3. When the wort is done boiling, cool the wort. You may place the pot in an ice bath to speed the chilling process; stir the wort occasionally. Keep the top on the pot to prevent airborne dust, etc., from falling into the wort. Be sure to use a sterile spoon to stir, and be careful when you set the spoon down on the counter.
 - A spoon that has been used to stir the boiling wort is considered to be sterile.

4. While the wort is cooling, sterilize the bottles, funnel, and cheesecloth. Place 2 tablespoons of powdered sanitizer plus 1 cup of cold tap water into one bottle. Screw the cap tightly on the bottle and coat all surfaces of the bottle with the sanitizer solution. Keep the solution in contact with the bottle sides for 2 minutes. Pour the solution into the next 2-liter bottle and repeat. Finally, place the cheesecloth in a bundle at the bottom of the funnel and pour the sanitizer so that it coats all inner surfaces of the funnel and completely saturates the cheesecloth. Leave both bottle and cheesecloth-funnel filter to air dry on a fresh piece of freezer paper.

5. When the wort has cooled to 70 to 75°F, pour it into the bottles using the funnel and cheesecloth as a strainer. There should not be any plant materials in the final beverage, and your wort should not be turbid. Fill the bottles, leaving approximately 3 inches unfilled (for a 2-liter bottle).

6. Pour some of the wort into the hydrometer tube and take a hydrometer reading. Take a temperature reading and adjust your hydrometer reading as necessary. Pour a small amount of wort into a disposable cup and take a pH reading of the wort.

7. Add 1/8 cup (approximately 2 tablespoons) of yeast culture to each bottle and cap tightly. Store in a cool (room temperature) dry place.

8. Be sure to check back periodically to move your bottle to the refrigerator when it has built up enough pressure (when you are no longer able to squeeze the bottle.) If someone from your team can't check on the bottle, make arrangements with a classmate or your instructor.

Yogurt preparation

The following procedure may be done while the wort is cooling or at an alternate time.

1. Pour a small amount of milk into a disposable cup and take a pH reading of the milk.

2. Pour 2 cups of milk into the glass pot, add 4 g of powdered milk, and heat until almost boiling. Do not allow milk to boil.

3. Allow the milk to cool to approximately 60°C. To speed this step up, you may place the pot in a water bath after it has partially cooled.

4. Add 1 to 2 teaspoons of commercial yogurt. Mix well.

5. Carefully transfer the yogurt to a sterile container, cover with a lid, and incubate at least 6 hours at 45°C. Yogurt may be left overnight.

6. Refrigerate yogurt.

7. Remove a small amount of yogurt, place in a disposable cup, and take a pH reading of the finished product.

Taste testing

****Important note:** do not taste any sodas until both instructors have approved them. This is for your safety! We must ensure that you do not have any bacterial contamination of your soda and that it is safe to drink.

1. Hold bottle gently; do not shake or turn it upside down.

2. Observe the bottle—it should be firm, indicating an increase of the partial pressure of CO₂ within the bottle. The soda should have clarity. There should not be an increase in turbidity from when you bottled it. How much yeast is there as compared to when you made the soda and where is this yeast located?

3. Hold bottle over a container to catch any overflow; gently and slowly open the cap and allow the excess gas to escape the bottle. If you open the cap too quickly at this point, the release of pressure will allow gas bubbles to rapidly form in the soda. The rapid formation of these bubbles will displace the yeast from the bottom of the bottle and necessitate filtering of the soda before tasting.

4. Once the cap is completely removed, very carefully decant the soda from the bottle into a pitcher, so as not to disrupt the yeast at the bottom of the bottle. It is necessary to pour the soda in a single motion; starting and stopping will create fluid disturbances in the bottle which will cause the yeast to return to a suspended state in the soda. Your goal is to pour as much soda out of the bottle as you can while leaving all of the yeast in the original bottle.

5. Wait at your station for both faculty instructors to approve the soda.

6. If your soda is okay, set up your display. Set aside 1 cup of your finished product for hydrometer and pH readings. Label the cup with your name.

- Sodas must be degassed in order to accurately take a hydrometer reading. Your instructor will collect the samples from everyone and place the sodas in a vacuum oven at room temperature. At the end of the tasting, samples will be available in the lab, at which time you can take the hydrometer reading. Samples for hydrometer readings may not be consumed.

7. Pick up a ballot and start tasting everyone else's sodas!

Questions for Reflection

1. Describe the consistency and taste of starting and ending materials for each of your products. How did the pH change? Level of carbonation? Specific gravity?

2. How and/or why do fermentation products stabilize food? What would happen to the starting materials if fermentation was not encouraged?
3. How does the variety of starting materials affect the final outcome of the fermentations? Are each of the different sugars processed in the same manner?
4. What were the differences in the yeast before and after soda production? What might account for these differences?
5. Describe the similarities and differences between lactic acid fermentation and ethanol fermentation.
6. Explain the role of secondary metabolites in product taste, consistency, and aesthetics.
7. Identify possible sources of contamination.

Instructor Version.

We strongly recommend that this exercise not be done in a lab if the sodas will be consumed. Possible locations for where this lab can take place are: a student lounge with kitchens, a kitchen in a dorm, a kitchen in a student center, or a kitchen in a food science division. A meeting room can become a kitchen if you have a hot plate, although it is good to have access to running water for clean-up. The students enjoy getting out of the lab and "doing science" in a real world location.

We have a single location that we use to prepare the wort and bottle the soda—a student lounge that we equip with a hot plate for the exercise. The lounge has a sink, which is where washing takes place. Groups of two to four students sign-up for time slots and are supervised during the brewing process by one of the two faculty instructors.

We have found that smaller groups work best, two rather than four or five students, and that grouping students who are at the same academic level is most effective. For example, one year we teamed botany students with a nonmajors freshmen class in groups of four. While everyone still loved the taste testing, the freshmen felt "out of it" during the decision-making process. Additionally, there was less group work during the preparation process, with one or two students doing the bulk of the work. We believe larger groups would work fine for larger classes where students are already used to working in groups of three or four students, and where students are at the same academic level.

We make our sodas in 2-liter plastic soda bottles that we collect throughout the fall. Bottles are rinsed as they are collected, and then students wash the bottles as part of the lab exercise. A few drops of dishwasher detergent and about a cup of hot water are very effective at completely cleaning the bottle. The dishwasher detergent also rinses out much better than standard "bubbly" dish soap. Residual detergent in the bottle may inhibit yeast growth. Most brewer supply stores have one-step sanitizers that do not need a final rinsing step.

Bottles with fermenting sodas are stored in a 55-gallon garbage can (dedicated to soda brewing) until they are ready to be refrigerated. They are usually done in 1 to 3 days, depending on how heavy the yeast inoculation is. Sodas prepared close to the date of testing should receive more yeast than those that are prepared 5 days ahead of time. We have a cabinet with a door for overflow of less mature bottles. We use the garbage can with lid in the event that bottles explode before we can move them to a refrigerator. Plastic soda bottles have the advantage that they will stretch and expand quite a bit before they will break. Additionally, explosions are messy, but not dangerous. The first year we did this exercise, sodas were made 10 to 14 days prior to the tasting, and we did not check them over the weekend and lost several bottles due to explosions. We now brew 2 to 6 days prior to the tasting and check the bottles every morning and evening for bottles that are fully carbonated (firm to the touch, and cannot be squeezed).

The 55-gallon garbage pail also serves as a handy vessel to open the soda bottles in. One student holds the bottle down inside the pail and slowly opens the cap, while a second student shields the operation by holding the pail lid over the opening soda. In the event that the soda fizz is greater than expected or if the bottle is opened too fast, the lid will prevent the students and the area from being sprayed with the soda.

Many students will want to chop their fruits by blender, because it is easier than chopping with a knife. We do not recommend processing most plant products by blender, because the greater breakdown of the fruit increases the turbidity of the final product and makes visualization of contamination in the final product much more difficult. Overboiling the wort will also lead to greater breakdown of the plant material and may also increase turbidity of the final soda.

Food preparation gloves should be used rather than latex gloves, as students and tasters may have latex allergies.

We recommend using a gallon of spring water for each starting batch (available at your local grocery store). This should reduce or eliminate any interesting organisms or minerals that may be present in tap water. Additionally, the spring water will contain trace minerals that are not present in distilled water.

As a rule for this lab, if we can purchase items whose purpose is food handling or food contact, we will use those items rather than find an alternate. For example, we use freezer paper to cover work surfaces as freezer paper is prepared to come in contact with food. The new one-time use cutting mats may provide a good alternative to sterilizing a cutting board.

We do not have the luxury of dedicating an electronic balance for this once-a-year exercise. Instead we use a standard lab balance and prepare it in the following manner: the balance is wiped down several times with wet towels and dried. The cord is wiped down as well. The balance is then placed into a clear 1-gallon food storage bag with the electric cord extending out of the opening. The opening is twisted around the cord, and sealed with tape. The balance may then be used in the food prep area. Plastic drinking cups are used as weigh boats.

Yeast cultures are available from brewer supply companies. Any wine or champagne yeast that flocs and settles should be acceptable. We have successfully used *S. cerevisiae* EC1118 prise de mousse, which is a very nice champagne yeast that produces bubbly sodas and does not add significant flavor. We have a dehydrated supply of yeast, which we use to make a fresh liquid culture (in honey water) each year. Yeast cultures may also be purchased as fresh liquid cultures each year.

The New Complete Joy of Home Brewing by Charlie Papazian, or most home-brewing books will have information about maintaining your own yeast lines. The online book *How To Brew* (<http://www.howtobrew.com/>) is a good source of information. It has also been our experience that most home-brewers love to give advice about how to maintain and produce beverages. A quick call to your local home brew supply store may result in more information than you need to know!

Procedure for preparing yeast cultures:

- Honey is diluted 1:10 in spring water, ¼ cup is aliquoted in clean baby food jars and autoclaved.
- A few grains of dehydrated yeast (EC1118 prise de mousse) are added to the honey water in each jar.
- The yeast cultures are allowed to sit overnight, with shaking, and used the following day.

Bottles are capped tightly after the yeast is pitched, and stored with the caps tight. This prevents the carbon dioxide from escaping, carbonates the soda, and inhibits the pyruvate decarboxylase reaction. Most carbonation in the final product is probably due to aerobic respiration, as the yeast remove residual oxygen from the wort. The increase in partial pressure of carbon dioxide inhibits production of acetaldehyde, and hence the production of ethanol. The sodas may be consumed by students of all ages, as there is no ethanol in them.

Occasionally, we have had teams of students who are of legal drinking age, and who really want to let their bottles vent to produce ethanol. We permit them to do so, provided that the beverage was still made in the lounge under the same supervision as the other students. What this effectively does is ensure that this team's beverage is so disgusting that no one wants it! First, the time frame of 2 to 6 days is far too short to produce any significant percentage of ethanol. Second, the beverage is almost completely flat, as carbon dioxide is allowed to escape. Finally, the yeast does not floc, as it is still productive. The yeast remains suspended in the beverage making the beverage fairly disgusting—it tastes like yeast. The bottles are vented by loosening the caps twice a day to let the build-up of gas escape. The caps are then tightened to prevent contamination of the product.

How do you know if the final soda is contaminated? It will smell odd. Much like smelling old milk in your refrigerator and knowing that it has soured, contaminated sodas will have an odor of spoiled food. Additionally, the soda may appear turbid. In the recipe above, the sodas are initially pitched with a very large inoculum of yeast culture, allowing the yeast to quickly out-compete any volunteer bacteria that may have joined the wort.

The most likely contaminants are acetobacteria, lactic acid-producing bacteria, *Brettanomyces* and *Pediococcus*. So if your products have a strong acidic taste (other than the starting plant material), or smell like vinegar, horse sweat, or diacetyl (butter or butterscotch flavor), then you probably have a contaminated batch of soda. In the brewing world, however, the latter three contaminants are actually desired in Belgian lambic beers! Other off flavors that may indicate bacteria infection are sulfur (rotten eggs) and dimethyl sulfides (cooked vegetables, rancid cabbage). Mold contamination is recognized by smell, taste, and sight, however, in the very short time frame for this exercise, there typically is not enough time for molds to establish themselves.

The following flavors may be in your beverage, but do not necessarily indicate contamination. They are the result of ingredients or materials used. When you purchase a yeast strain, you should be able to find out the characteristics of that strain from the supplier, including any secondary flavors the yeast may be responsible for. This list is adapted from John Palmer's *How to Brew*:

Grassy: high chlorophyll in the initial ingredients will carry through in flavor

Husky or Grainy: astringent flavor from husk material

Estery or Fruity: banana or other fruit flavor—a secondary metabolite of many yeast strains

Cidery: green apples or freshly cut pumpkin taste due to acetaldehyde—wort should be more aggressively poured into bottles to fully aerate it

Medicinal: smells like band-aids—caused by phenolic compounds produced by the yeasts when chlorine-based sanitizers are not adequately rinsed from utensils

Metallic: due to leaching of metals from the pot during boiling. Stainless steel pots will not contribute to metallic flavors, whereas aluminum might, and enamel pots with cracks in the enamel will contribute an iron flavor.

Oxidized: wet cardboard or sherry-like flavor, due to exposure to oxygen at temperatures above 80°F

Yeasty: be careful pouring!

For the taste testing, we invite the entire division, administration, colleagues, friends, etc., anyone brave enough, to come! We are actually getting a good repeat crowd year after year. We combine the tasting with a holiday gathering and hold it on the last day of classes in the fall. Each taster is given one ballot sheet (the Soda Tasting Chadless Ballot) and several feedback sheets (one for each soda made that year). The feedback sheets allow the testers to take anonymous notes and are returned to the brewers. The ballots are tallied, winners announced, and certificates for each winner are hung in the Biology student lounge for a year.

We do not have a supply of pitchers in our department, but are able to borrow a large number of them each year from our on-campus catering service.

If you do not have any local brewing supply stores in your area, please contact J. Cardinale (cardinale@alfred.edu) or C. Emmons (emmonsc@alfred.edu) for a list of brewer supply companies available on the internet.

Safety Issues.

The product of this lab is meant to be consumed, which is a large safety issue. All stages of preparation should be supervised and good aseptic technique followed throughout the preparation. Nonmicrobiology students may not have the same understanding of the need for aseptic technique, therefore it is important to emphasize to the microbiology students

that part of their role as “microbiology experts” is to make sure the beverage is not contaminated in any way.

Faculty must approve all ingredients before the sodas are made. All plant materials must be appropriate for human consumption. This is less of an issue if the students choose a plant material that may be purchased from the grocery store. The use of interesting and less common plant materials is encouraged, but the safety of those materials must be investigated by the students and presented to the faculty instructors for approval. Materials harvested from the wild or even from a garden or lawn should come from an area that is not subjected to fertilizers, herbicides, or pesticides.

Each beverage must be smelled, tasted, and approved by faculty instructors to ensure that it was not contaminated during preparation before any student may try the beverage. Please see faculty notes for hints on how to tell if a soda is contaminated.

ASSESSMENT and OUTCOMES

Suggestions for Assessment.

We require students to turn in a written lab report that outlines their procedure and ingredients, an assessment of their beverage, and answers to the questions for reflection given in the student protocol.

Field Testing.

This exercise has been done for 3 years, with modifications (improvements) made each year. We have summarized changes from the above protocol and reasons for why changes were made.

Year One (Fall, 2001): Students who participated were upper-level biology majors enrolled in either microbiology or botany courses. Nine sodas were made with assigned ingredients—three plant materials (ginger/lemon, sweet birch, and Norway spruce) were combined with one of three carbohydrates (malt, table sugar, and honey). Wort preparation took place in 1 day, with students coming and going as they had time (most students voluntarily stuck around for the whole time). Sodas carbonated for 2 weeks, with some bottles exploding after 5 to 6 days. Many remaining bottles were overcarbonated and fizzed to such a great extent that the residual sodas were undrinkable with the suspended yeast. Everyone tried all sodas, even the disgusting ones.

Year Two (Fall, 2002): Students who participated were upper-level biology majors enrolled in either microbiology or botany courses. Students were assigned to teams of two to three students and allowed to choose their own ingredients. The idea of a contest was born. Carbonation time was reduced to 3 to 6 days, and initial yeast inoculum adjusted depending on the day of preparation. This arrangement of students and prep time has proved to be the optimal arrangement.

Year Three (Fall, 2003): Students who participated were upper-level biology majors enrolled in botany or freshmen nonmajors enrolled in a microbiology freshman year experience course. Students were assigned to teams of four, with two botanists and two freshmen per group. The remainder of the protocol remained the same. The freshmen were tentatively teamed up with upperclassmen, and less likely to participate in the decision-making process. Additionally, work was not as evenly divided among members of the student groups.

Student Data

Some soda combinations that have been made in our classes:

- Strawberry, banana, mint, honey
- Cherry, vanilla, high fructose corn syrup
- Strawberry, high fructose corn syrup
- Cilantro, raspberry, lime, high fructose corn syrup
- Peppermint, walnut, honey
- Ginger, lemon, honey
- Sweet birch, honey
- Sweet birch, malt
- Norway spruce, malt
- Apple, rosemary, high fructose corn syrup

SUPPLEMENTARY MATERIALS

Possible Modifications.

With larger classes, it may be more advantageous to standardize the recipe or have a limited number of ingredients for the students to choose from. For example, you could have a few complementary plant products (e.g., strawberries, mint, and cherries) and a few carbohydrates (e.g., table sugar and honey.) Student teams would use a combination of these limited ingredients. They would still be able to compare different tasting sodas, but the lab would be more manageable for the larger groups.

Not all schools have microbiology and botany classes—don't let this stop you! This exercise can easily be adapted for just microbiology students. Other suggestions depend on your school. A microbiology class could be teamed up with a chemistry class or a food science class. The procedure could also be modified for use in a majors or nonmajors introductory biology class.

References.

1. **Krause, S. A.** 1982. Wine from the wilds. Turning wild fruits and flowers into anytime table wine. Stackpole Books, Harrisburg, Pa.
2. **Palmer, J.** 1999. How to brew. [Online.] <http://www.howtobrew.com>.
3. **Papazian, C.** 1991. The new complete joy of home brewing. Avon Books, New York, N.Y.

Recipes.

We have provided two recipes that were part of the original inspiration for the lab. As you can see, there were modifications that we made. For example, we cut back on volume and placed more of an emphasis on sterile technique. Additionally, both of these recipes are for alcoholic brews, so they had to be adapted for soda production. We have included them here to give other educators a model for how to change traditional brewing recipes into undergraduate-appropriate recipes.

Birch Beer recipe

1. Place 4 quarts of finely cut sweet birch (*Betula lenta*) twigs or inner bark into a 5-gallon crock.
2. Add 4 gallons of water or birch sap and bring to a boil.
3. Stir in 1 gallon of honey and bring to a boil. Boil 10 minutes. Remove from heat.
4. Cool and strain to remove bark and twigs.
5. Keep the liquid in the crock and place one cake of yeast on a piece of toast to float on top.
6. Cover the pot and let ferment for 1 week until it begins to settle.
7. Bottle the birch beer and store in a cool dry place.

How to Make Old-Fashioned Spruce Beer (From your Halifax, Nova Scotia, Guide)

In the old days, Nova Scotians made their own refreshments. Spruce beer was a favorite in coastal villages, where spruce trees are plentiful.

1. To make Old-Fashioned Spruce Beer, gather 7 pounds of good spruce boughs and boil them in 5 gallons (about 22 liters) of water until the bark peels off.
2. Remove spruce boughs, and add 3 gallons (about 14 liters) of molasses.
3. Bring to a boil again, and continue boiling, skimming the foam from the top when necessary.
4. Put liquid into a cooler.
5. When lukewarm, add 1 pint of yeast and mix well.
6. Pour into a 10-gallon barrel, and let it work (ferment) for 2 or 3 days.
7. Keep filling the barrel with water as the mixture ferments.
8. When done, place a bung (wooden plug) in the barrel, and let it vent now and then.
9. Alternately, start making Modern-Day Spruce Beer with spruce extract instead of spruce boughs.
10. Dissolve 1 1/2 cups of sugar in 1 quart (or 1 liter) of boiling water.
11. Add 3 quarts (about 3 liters) of cold water and 1 teaspoon of vanilla. Sprinkle 4 to 6 packages of dry yeast over and combine gently until well blended.
12. Add 2 to 3 tablespoons of spruce extract.
13. Cover and set in a warm place for 12 to 16 hours.
14. Skim the foam from the top and bottle. Keep in a cool place.