Fun Microbiology

Making Root Beer & Other Naturally Carbonated Beverages

James K. Mitchell

Everyone likes an ice-cold soft drink or soda pop, especially on a hot summer day. Almost every gas station, convenience and/or grocery store will carry some commercial brand of soft drink on its shelves. These beverages are prepared by adding a flavor concentrate (syrup) and sugar to water, which is then mixed and carbonated under pressure with CO_2 gas. Soft drinks have been made by this same general procedure for the past 100 years (Dietz 1973; Riley 1972; Simmons 1983).

Only a limited number of flavors (brands) are commercially available, based primarily on production costs and consumer demand. Someone interested in preparing a soft drink to suit one's own taste might try combining a flavor concentrate (commercial or homemade) and sugar with soda water (club soda) for the carbonation (Johnson 1992). A more interesting (and original) method would be to allow yeast to carbonate a soft drink through the natural fermentation process.

Many people (including myself) prefer the "richer" flavor associated with the naturally carbonated homemade soft drink over the commercial store brands. An unlimited number of flavors are possible by mixing varied combinations/types of syrups, herbs and sugar sources. Prior to large-scale commercial production, people would collect locally available ingredients to prepare naturally carbonated soft drinks including spruce, birch, nettle, sassafras (sarsaparilla), honey, and molasses plus ginger (switchel) to name a few (Riley 1972).

James K. Mitchell is Assistant Professor of Biology, Ball State University, Muncie, IN 47306-0440.

The yeast *Saccharomyces cerevisiae* (baker's yeast) was the most commonly used microbe and is still used today to produce many naturally fermented products including bread, certain beers and wines, and single-cell protein (Jay 1992; Prescott, Harley & Klein 1993; Prescott & Dunn 1983). In the absence of oxygen (anaerobic), this yeast ferments simple sugars to carbon dioxide (CO_2) and ethanol. The CO_2 is produced as small gas bubbles which "carbonate" liquids within sealed containers, or lighten (leaven) the texture of dough causing it to rise. The ethanol produced remains in liquids (unless distilled-off), but evaporates during the baking process in breads. This yeast also imparts a characteristic flavor to the fermented product with a taste we also enjoy.

In this experiment, the yeast is provided with the disaccharide sucrose (table sugar) as a source of energy. This sugar is first broken down into a molecule each of glucose and fructose by the yeast enzyme invertase. These simple sugars then enter into the central metabolic pathway glycolysis, which is one of several pathways known generically as anaerobic fermentations, by which many organisms extract chemical energy from various organic fuels in the absence of oxygen (Lehninger 1975).

In animals, glycolysis serves as an important emergency mechanism capable of yielding energy for short periods when oxygen is not available (e.g. strenuous exercise). In this situation, glycolysis' end-product pyruvic acid is converted to lactic acid which is the molecule that causes the aching of muscles. Under normal living conditions (aerobic), animals convert the pyruvic acid from glycolysis to acetyl-CoA which then enters the Krebs or tricarboxylic acid cycle to eventually produce energy in the oxidative phosphorylation process. Yeast fermentation is limited to the same enzymatic pathway as glycolysis by forming pyruvic acid, but requires two different enzymatic steps at the end. A summary of the yeast fermentation pathway is shown in Figure 1. For each mole of sucrose metabolized by the yeast, potentially four moles each of CO_2 and ethanol are formed as products.

Within the time frame allowed for in the following experiments (8-48 hours) and the cocktail mixtures tested, very little ethanol is produced in homemade fermentations. Final alcohol (ethanol) content will range from trace amounts to 0.5% (v/v), depending upon how nutritious the cocktail mixture may be (e.g. root beer concentrate plus sugar is less nutritious than honey). Commercial American beer (e.g. pilsner) has an ethanol content of 5.1% (v/v).

Objectives of this study were to:

1. Determine which commercial syrups produce the best-flavored naturally fermented soft drink(s).
2. Test varying amounts of selected commercial syrups to optimize flavor.
3. Produce a low-calorie, naturally fermented soft drink.
4. Attempt to develop a new flavored soft drink.

Root Beer & Other Flavor Syrups

Commercial flavor concentrates of McCormick® (root beer) and Homebrew® (root beer, birch root beer,

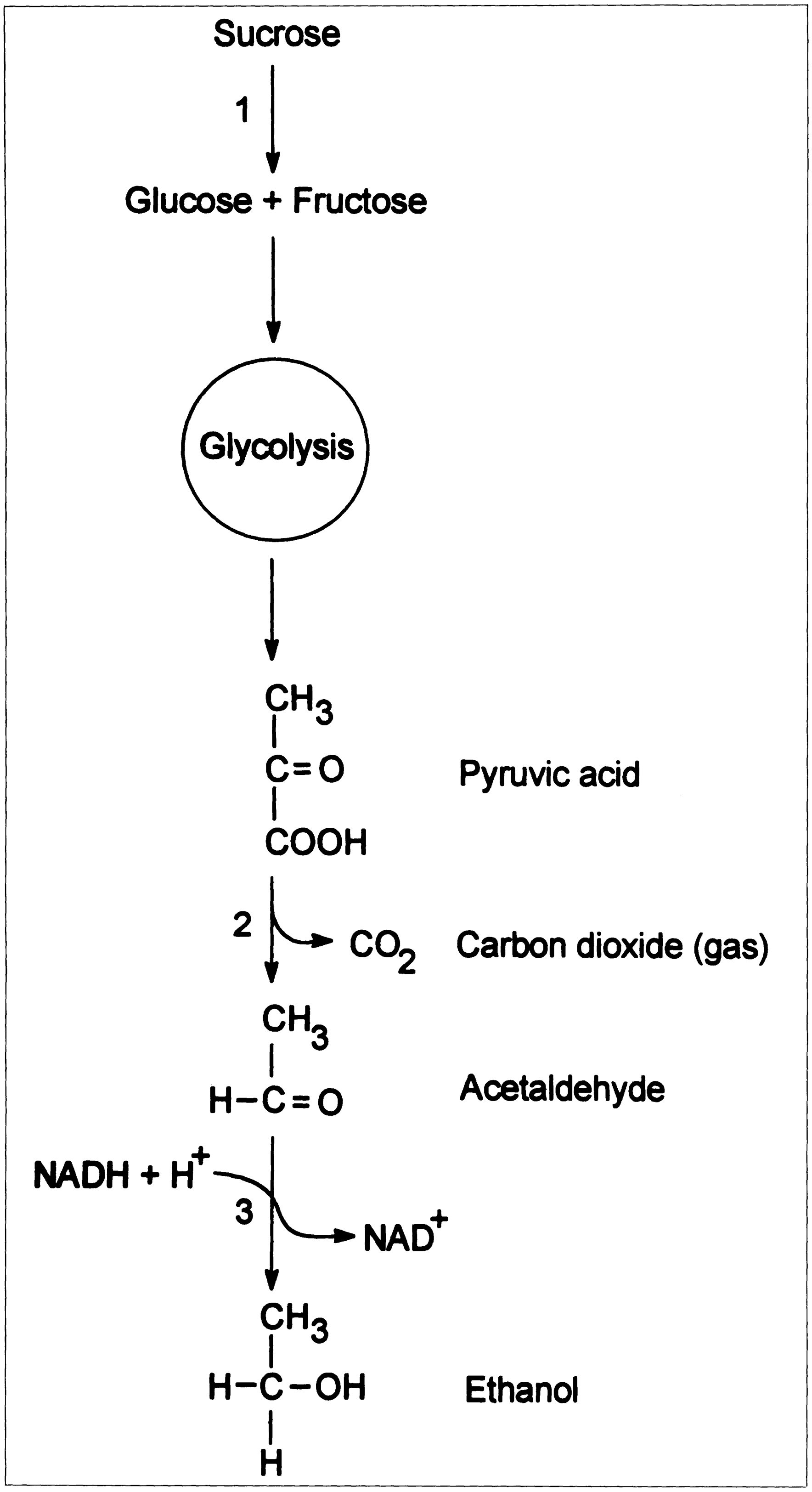

Figure 1. Yeast fermentation pathway. Unique enzymes labeled include: (1) invertase, (2) pyruvate decarboxylase, and (3) alcohol dehydrogenase.

sarsaparilla, cola, strawberry and ginger ale) brands were diluted according to label directions in 1 gallon of water. Table sugar (2.5 cups) and dried Fleischmann's® yeast (1 teaspoon) were added and the different cocktails thoroughly mixed and poured into clean 20-ounce plastic screw cap bottles (four bottles per cocktail mixture). Lids were tightly sealed and bottles were incubated in an upright position at room temperature (23-27° C) for 48 hours for sufficient carbonation to occur. When using plastic bottles, one may easily check for carbonation (without having to open the bottle) by squeezing. If the sides of the bottle do not give way, sufficient carbonation has occurred. Following carbonation, bottles were placed into a refrigerator for one week after which the different soft drinks were compared for pH, culture purity, and flavor qualities.

All soft drink cocktails where fermentation (carbonation) occurred exhibited a pH of 5.0 or less and contained only yeast cells, as determined by pH paper and microscopic examination, respectively. Homebrew "sarsaparilla" had no carbonation (even after allowing bottles to ferment an additional 24 hours) which suggests it contained ingredients that inhibited natural yeast carbonation. All other Homebrew syrups tested produced soft drinks that exhibited an unpleasant aftertaste which students disliked. McCormick "root beer" produced a smooth, good-flavored soft drink with no lingering aftertaste.

The McCormick "root beer" soft drink produced by following label directions (2 oz concentrate/5 gal water = 0.87 Tbl/gal) resulted in a weaker-than-desired root beer flavor. Different amounts of syrup concentrate (1-4 Tbl/gal) were diluted and carbonated as described above to determine which concentration(s) would produce the best-flavored soft drink. Results of student taste tests were compiled and the best root beer recipe is presented in Table 1.

To optimize conditions for successful carbonation, one should adhere to the following precautions:

1. Use dried yeast prior to expiration date.
2. Mix cocktail containing yeast well before pouring into bottles.
3. All containers and utensils should be clean and composed of nonmetal material.
4. Fill bottles to within 2 inches below the rim to exclude oxygen which will lessen or inhibit fermentation and possibly encourage growth of spoilage microbes.
5. Tightly seal bottles.
6. Use nonchlorinated water.
7. Incubate bottles at 23-27° C (74-80° F) for 41-48 hours.
8. Drink immediately after chilling or store no longer than 7-8 days in a refrigerator.

It is important to remember that the yeast are still alive and therefore continue metabolizing the sugar (even within the refrigerator). Storage longer than one week may result in root beer with a "dry" or "unsweetened" flavor. One may also add lemon juice when preparing the cocktail mixture to lower the initial pH to 5.0 or below. This additional precaution will inhibit growth of potential spoilage microbes (e.g. bacteria) if present, but still allow the yeast to carbonate.

The root beer recipe listed in Table 1 is just one variation that students developed in class. In your class, you may want to start with this recipe and try substituting table sugar with other types of sugars such as fructose or honey. My personal favorite is to sweeten the root beer cocktail with honey and to fortify flavor even more with vanilla extract. Feel free to experiment with your own sugar and flavor syrup measurements; you may come up with a better-flavored soft drink!

Table 1: Root beer recipe (makes almost 1 gallon).

14 cups (3316 ml) nonchlorinated water[a]
2–3 cups table sugar (sucrose)[b]
2–3 Tbl (27–41 ml) root beer concentrate[c]
1 tsp dried yeast[d]

[a] May use distilled water or tap water that has been allowed to stand 2–3 days.
[b] May substitute equal amount of honey for different flavor! For low-calorie root beer, use 0.5 cups sugar + 4.8 Tbl of Equal® sweetener.
[c] McCormick & Co., Consumer Affairs Department, 211 Schilling Circle, Hunt Valley, MD 21031.
[d] Add 1 tsp dried yeast (Fleischmann's®) to 40 ml nonchlorinated water and allow to stand 5–10 minutes prior to adding to above mixture. Incubate in sealed bottles 41–48 hours at room temperature (23–27° C).

Table 2: Combinations of table sugar (sucrose) and Equal (NutraSweet®) added to 14 cups water.

Treatment	*Table Sugar (cups)*	*Equal[a] Tbl (g)*	*# Calories per 20 oz.*
1	0	6.0 (60.0)	0
2	0.25	5.4 (54.2)	44
3	0.50	4.8 (48.4)	88
4	1.00	3.6 (35.9)	176
5	1.50	7.25 tsp (24.2)	264
6	2.00	3.50 tsp (11.7)	352
7	2.50	0	440

[a] Tbl = tablespoon, tsp = teaspoon, g = gram, oz = fluid ounces

Low-Calorie Root Beer

Several combinations of table sugar amended with the sugar substitute Equal® (NutraSweet®) were prepared so that the total sugar content (sweetness) of 2.5 cups/14 cups water resulted (Table 2). A sweetness equivalent chart is presented in Table 3. This experiment was conducted to determine the minimum amount of metabolizable sugar (e.g. sucrose) required by yeast for sufficient carbonation of a low-calorie (diet) soft drink. Equal sweetener was chosen because:

1. Its flavor is superior to other sugar substitutes.
2. It is commonly used in commercial diet soft drinks.
3. Yeast do not sufficiently metabolize (use or break down) this amino acid derivative.
4. No heating is required for natural yeast carbonation, a process which would destroy NutraSweet and result in a nondesirable, unsweetened flavored soft drink. As the reader may know, NutraSweet or aspartame is a sweet-tasting amino acid derivative used as a "non-calorie" sweetener because humans cannot metabolize this compound.

Sugar solutions (treatments) from Table 2 were each amended with 2.5 tablespoon McCormick "root beer" concentrate and 1 teaspoon dried yeast (Fleischmann's), bottled and tested as described previously. Carbonation occurred after 48 hours only within cocktail mixtures containing table sugar (0.25-2.5 cups). The least amount of table sugar (amended with Equal) which resulted in a sufficiently carbonated soft drink of good flavor was 0.5 cups (88 cal/20-oz bottle). This is one-fifth of the recommended sugar rate of 2.5 cups (440 cal/20-oz bottle), and indeed, a low-calorie soft drink.

Sweet-Tart Soft Drink

Students were also challenged to develop a new soft drink flavor from easily accessible ingredients. Creative endeavors focused on various combinations of honey and citrus fruits and resulted in a product that exhibited a sweet-tart flavor (Table 4). Using lemons and oranges not only added the citrus tart flavor, but also adjusted (lowered) the pH of the cocktail to below 4.0, a value known to inhibit bacterial spoilage while allowing yeast fermentation. Honey is more nutritious to yeast than many commercial flavor concentrates (most of which include poorly nutritious artificial ingredients). Therefore, sufficient carbonation occurs within 18-24 hours incubation at room temperature with the sweet-tart recipe listed in Table 4. Care should be taken to not allow longer fermentation, or bottles might

Table 3: Equivalent sweetness chart.

For this much sugar	*Use this much Equal[a]*
2 tsp	0.25 tsp[b]
1 Tbl	0.50 tsp
0.25 cup	1.75 tsp
0.33 cup	2.50 tsp
0.50 cup	3.50 tsp
1.0 cup = 232 g = 870 cal	7.25 tsp = 24.3 g
1 lb (~2.33 cups)	5 Tbl + 2 tsp = 2 oz = 56.7 g = 1 pouch

[a] Information taken from label on Equal package
[b] Tbl = tablespoon, tsp = teaspoon, g = gram, cal = calories, oz = ounces (mass)

Table 4: Sweet-Tart soft drink recipe.

1 gallon (3.8 L) nonchlorinated water[a]
4 oranges (juiced)
4 lemons (juiced)
3.8 lbs honey[b]
1 tsp dried yeast[c]

[a] May use distilled water or tap water allowed to stand 2–3 days; L = liters.
[b] May use fresh hive honey or commercial bottled honey.
[c] Add 1 tsp dried yeast to 40 ml nonchlorinated water and allow to stand 5–10 minutes prior to adding to above mixture. Incubate in sealed bottles 18–24 hours at room temperature (23–27° C).

explode or foam excessively when opened.

Several recipes for producing naturally carbonated soft drinks have been presented. Ingredients for preparing these are inexpensive and may easily be found in a local grocery store. To optimize a successful soft drink carbonation by natural yeast fermentation in your class (using commercial or homemade ingredients), two factors are of utmost importance:

1. Always use sanitary conditions when following the above procedures.
2. When producing your own cocktail recipe, conduct a trial test to determine the proper incubation period for carbonation.

Nutritionally rich fruit juices (e.g. grapes, apples, peaches, etc.) will carbonate sooner than a cocktail composed of a commercial flavor syrup. For example, yeast will carbonate apple juice (containing no chemical preservatives) within 6-12 hours when following the above procedures. The root beer recipe described in this paper will require at least 41-48 hours incubation for sufficient carbonation. Prior to drinking, it would also be advisable to ascertain that the product has a pH of 5.0 or less and only yeast cells are observed under the microscope.

This paper also summarizes results of the first laboratory exercise I conducted in my applied microbiology class. A major portion of the lab section challenges students to both design and conduct experiments to solve specific problems associated with microbiological production. In this exercise, students are asked to produce a naturally carbonated soft drink of comparable flavor/quality to a commercial brand. I use this exercise to introduce students to both the cooperative learning process and the use of the scientific method of question solving. With root beer as a model system, students rapidly learn the practical importance of replication, data collection and proper control treatments when conducting experiments. As with many scientific endeavors, more questions than answers are developed. Upon completing the root beer recipe, questions invariably arise as to whether a diet soft drink can be produced by natural fermentation. Freethinking and creativity are taking hold, and the class has interesting discussions on microbe metabolism and how to approach a low-calorie soft drink. By this time, students are more comfortable in experimental design and by the next lab period want to try to develop their own soft drink flavor!

This exercise is not just limited to the field of biology. Other science content areas, including chemistry and physics, play an important role in successful root beer fermentation. A potential chemistry project might involve the student trying to demonstrate how many moles of ethanol and CO_2 are produced when the yeast is presented with either a monosaccharide (e.g. glucose) or a disaccharide (e.g. sucrose) as an energy source. Fermentation flavor qualities based upon the type of sugar or flavor syrup used might also be investigated by individuals interested in food science. A physics project might investigate the effect of CO_2 concentration (pressure) on growth rate of yeast in the root beer cocktail. Dependent upon incubation temperature and ingredients of the cocktail mixture, yeast growth is inhibited at high CO_2 concentrations. Sugar fermentations are carried out by yeast cells at CO_2 pressures ranging from 0.48-14 atmospheres (ATM) or 7.0-205.8 pounds per square inch (psi) (Prescott & Dunn 1983). Whichever field of science you investigate with your class, the root beer experiment is a fun, problem-solving and knowledge-gathering experiment with an underlying applied aspect. Good luck with your own fermentation project!

Acknowledgment

Gratitude is extended to Lesslie Coffin for typing this manuscript.

References

Dietz, L. (1973). *Soda pop*. New York: Simon & Schuster.

Jay, J.M. (1992). *Modern food microbiology*. New York: Van Nostrand Reinhold.

Johnson, P.E. (1992, June-July). Root beer: Making tradition at home. *Mother Earth News, 132*, 18-19.

Lehninger, A.L. (1975). *Biochemistry, 2nd ed*. New York, NY: Worth Publishers, Inc.

Prescott, S.C. & Dunn, C.G. (1983). *Prescott & Dunn's industrial microbiology*. Saybrook, CT: Saybrook Press.

Prescott, L.M., Harley, J.P. & Klein, D.A. (1993). *Microbiology*. Dubuque, IA: Wm. C. Brown Publishers.

Riley, J.J. (1972). *A history of the soft drink industry*; reprint of the 1958 edition. New York: Arno Press, Inc.

Simmons, D. (1983). *Schweppes: The first 200 years*. Washington, DC: Acropolis Books LTD.