Ethanol: nutrient testing

What are the nutrient values of corn before and after ethanol production?

Materials

- Hot plate
- Funnel
- Filter paper
- Parafilm or vortex for mixing
- Graduated cylinders (10, 100 mL)
- Benedict's solution
- Lugol's iodine solution
- Beakers (100, 250, 600 mL)
- Biuret solution
- Distilled water
- Test tubes and rack
- Cracked corn (ground up)
- Scale or triple beam balance
- Pipette pump
- 50 mL pipettes
- Mortar and pestle

Background

Commercial production of fuel ethanol in the United States involves breaking down the starch present in corn into simple sugars (glucose), feeding these sugars to yeast (fermentation), and then recovering the main product (ethanol) and byproducts (animal feed, corn oil, and carbon dioxide).

All the remaining nutrients: protein, fat, minerals, and vitamins, are concentrated into distiller's dried grain (DDGs), a valuable feed for livestock, and carbon dioxide. Some ethanol plants also remove the corn oil from DDGs to create biodiesel. In this simple experiment, students will be able to determine the nutrient analysis of corn before, during, and after ethanol production.

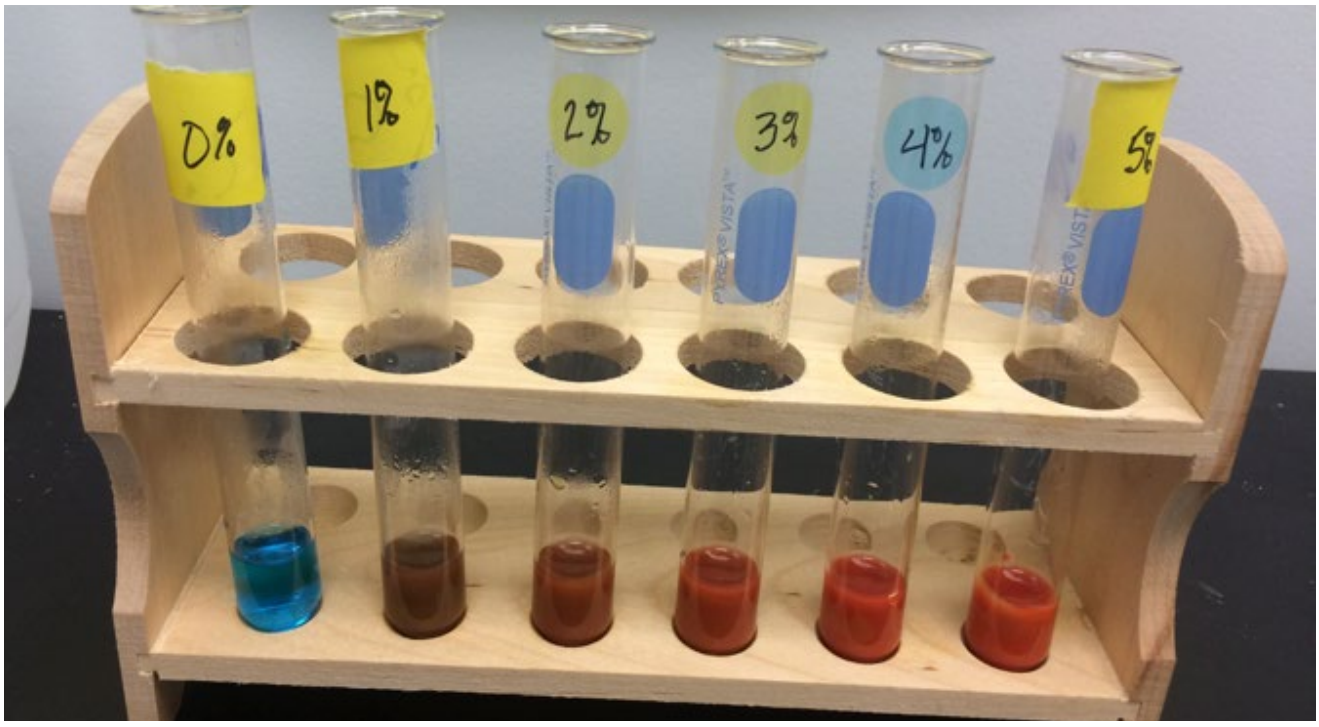

Figure 1

Resources adapted with permission from the Ohio Corn and Wheat Growers Association.

Directions

The following tests should be conducted at the beginning on raw product day 1, day 2 after fermentation, and again after the distillation process.

Day 1: Nutrient testing and corn mash

PREP OF SOLID SAMPLE FOR TESTING BEFORE AMYLASE

1. Weigh out 5g of corn sample using electronic balance. Place sample into a mortar.
2. Add 20mL of distilled water to food sample in mortar. Grind sample with a pestle into a slurry.
3. Filter slurry using filter paper and funnel and collect liquid food sample into a small graduated cylinder or beaker.
4. Use the filtrate to complete the Carbohydrate Indicator Tests and Protein Indicator Test.

CARBOHYDRATE INDICATOR TESTS

Monosaccharide indicator standard test (glucose):

1. Add 2 mL of food sample solution with 2 mL of Benedict's solution in a test tube.
2. Use Vortex to give sample a quick mix (or cover with parafilm and invert test tube). Record sample color in data chart.
3. Place test tube containing food sample and Benedict's solution in a boiling water bath and heat for 2 minutes. Record sample color in data chart.
4. The glucose present in the solution reacts with the copper sulfate in the Benedict's reagent creating copper oxide, which results in an orange to red-brick precipitate. The intensity of the color depends on the concentration of glucose present in the sample.
5. Rate the precipitate color change: *0: no color change/negative, 1: weak/positive, 2: strong/positive, 3: very strong/positive*. Record sample data in chart.

Complex carbohydrate indicator standard test (starch):

1. Add 1mL of food sample solution with 1 drop of Lugol's Iodine Solution in a test tube.
2. Use Vortex to give the sample a quick mix (or cover with parafilm and invert test tube). *Do not heat!*
3. A bluish black color indicates a positive test for starch.
4. Rate the precipitate color change as *0: no color change/negative, 1: weak/positive, 2: strong/positive, 3: very strong/positive*. Record sample data in chart.
5. Keep sample to observe until day 2 of the lab.

Protein indicator standard test:

1. Add 1 mL of food sample solution with 2 mL of Biuret Solution in a test tube.
2. Gently mix using a Vortex (or cover with parafilm and invert test tube).
3. After 30 seconds, the filtrate solution will result in a color change to purple if proteins are present in the sample.
4. Rate the precipitate color change as *0: no color change/negative, 1: weak/positive, 2: strong/positive, 3: very strong/positive*.

PREPARATION OF CORN MASH

1. On a scale or triple-beam balance, tare (set the weight to zero) a 250 mL beaker then add 100 g of corn meal. Record the exact weight in your lab.
2. Transfer the corn to a 600 mL beaker. Add 300 mL of warm distilled water. Record observations

in Data section. Stir well. Place the beaker on a hotplate and set the temperature to 100°C, cover it with watch glass, boil gently and stir often for 15 minutes. (If the mixture becomes too dry, more water may be added).

3. After boiling is completed, remove the beaker from the hotplate and allow it to cool to touch. Record observations on consistency, color and smell in Data section. Measure 100 mL of deionized water and pour into a 250 mL beaker. Measure 10 mL of the amylase solution into a small graduated cylinder and add to the 250 mL beaker of water. Be sure to shake the amylase solution before using. Stir the resulting mixture and add it to the corn mash. Stir the resulting mixture occasionally with a glass rod during the next 10 minutes.
4. At the end of the 10-minute period, add 35 mL of the buffer solution (to maintain a slightly acidic pH), 10 mL of amyloglucosidase solution, and 10 mL of the yeast solution together in a 100 mL beaker. Be sure to shake the buffer and amyloglucosidase solutions before mixing and pour the mixture into the corn mash. Stir the entire mixture after mixing.
5. Place a piece of plastic wrap over the mouth of beaker and secure it with a rubber band or use parafilm.
6. Weigh the beaker (make sure it is not too heavy for your electric balance) and record its weight. You will be reweighing this beaker again so be careful and consistent (with rubber band and plastic wrap or parafilm each time).
7. Place your beaker on the counter and allow it to sit overnight (next lab period) so that the enzymes have time to work.

Day 2: Nutrient testing after fermentation

Perform both the carbohydrate Indicator Tests as well as the Protein Indicator Test (as completed in Day 1) on the corn mash after fermentation and again after ethanol distillation is complete. Please see Ethanol: corn mash & distillation lesson to create the filtrates that will be necessary to finish the nutrient testing for this lab.

Nutrient testing

Sample	Benedict's test	Starch test	Protein
Cracked corn slurry			
Corn after fermentation			

Reflection

1. How did the corn's nutrient content change from its original state to fermentation to ethanol?
2. What affect does the yeast have on the nutrient profile of the corn mash before distillation? How is this related to fermentation?