

# 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.

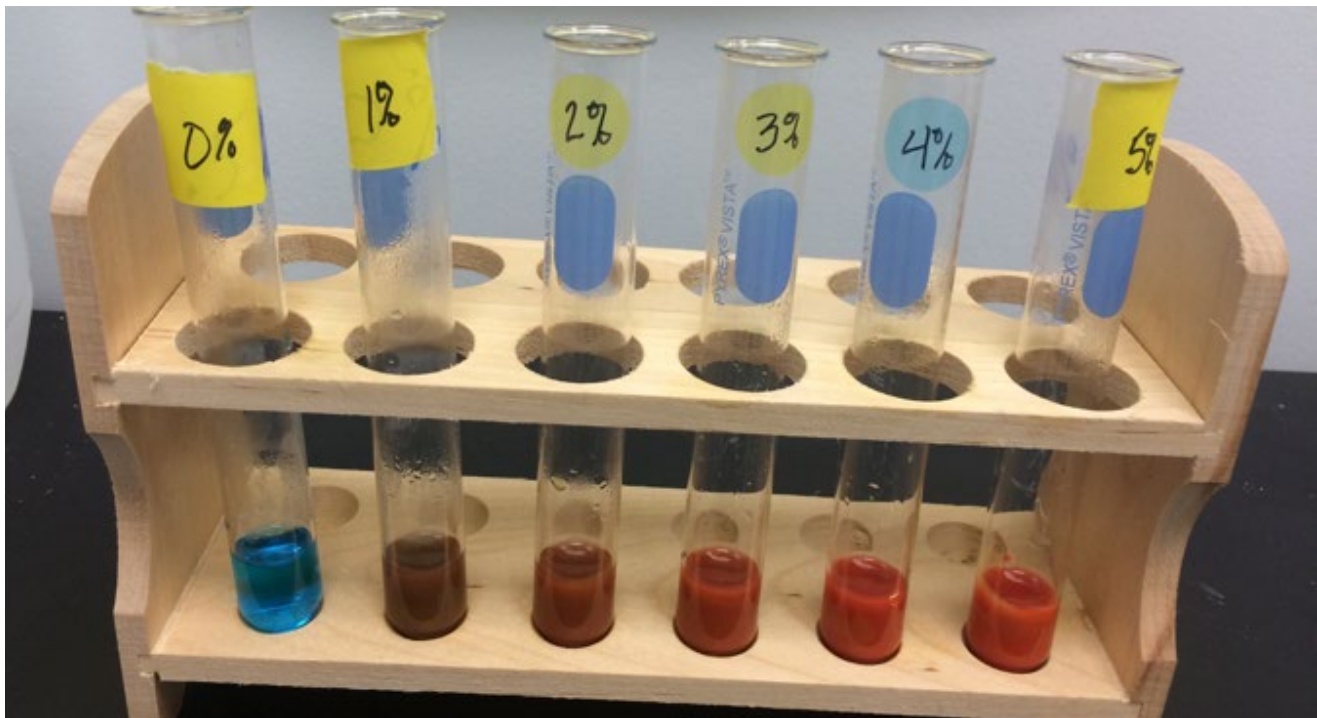


Figure 1

## 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*.

## 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?