Lesson Plan Overview for 2014 – 2015 NATURE Sunday Academy Program

Project Title

The Importance and Analysis of Aflatoxin and Gluten in Corn

Developed by

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Summary

We intend to have 3 parts to this activity. After the cultural connection, presentation on aflatoxin in corn and related food safety concerns will be presented along with a discussion on gluten. We will bring several food containers for the students to review, including gluten free. During the discussion, we will ask students to indicate what main ingredients would be gluten free. We will end the presentation with a powerpoint highlighting some of the ingredients listed as gluten free. Activity I will focus on the virtual lab activity. We will need access to a computer lab. Each student should team up and run through the activity. Activity II will include the analysis of aflatoxin using a Neogen Aflatoxin test kit. Students will gain experience in using ELISA based test kits. The third activity will be to assess whether corn has gluten using a lateral flow test kit. In addition to corn, some students will have a wheat sample (positive control) to evaluate. After students have had the chance to evaluate the products, we will have a discussion about aflatoxin and gluten in corn. Students will complete the activities by indicating if a product is gluten free simply by looking at a food label.

Project Objectives – Students will learn the following

1. The importance of corn culturally and economically to North Dakota and the Upper Great Plains
2. What is aflatoxin and how it can negatively affect the quality and safety of corn.
3. Definition of gluten, the health consequence associated with gluten, and foods to avoid.
4. A basic test to determine the presence of aflatoxin and gluten in corn.

North Dakota Science Content and Achievement Standards Met

9-10.2.2.	Use appropriate safety equipment and precautions during investigations
9-10.2.3	Identify questions and concepts that guide scientific investigations
9-10.2.6.	Design and conduct a guided investigation
9-10.2.8.	Analyze data found in tables, charts, and graphs to formulate conclusions
9.10.7.2	Identify factors that affect populations (e.g. food webs, carrying capacity, overpopulation, disease, food supply, algal blooms, resources, conservation practices)
9-10.8.5.	Explain how views and attitudes have influenced the development of science (e.g., religion, previous knowledge, cultural tradition, superstition, folklore, legends)

Introduction

Background information on the importance of corn to the region and how certain molds can produce aflatoxin that cause the corn to unsuitable for food or feed uses. The concept of gluten and health implications will be covered. The difference between corn gluten and wheat gluten will be presented and why corn gluten is no really the gluten associated with Celiac disease.

Session Organization

11:00-11:30	General organization/Cultural connection
11:30-12:00	Background about corn, molds and aflatoxin and gluten in cereals
12:00-12:45	Lunch
12:45-1:30	"Virtual" Aflatoxin in Corn Laboratory Activity
1:30-2:30	"Hands-on" Aflatoxin and Gluten in Corn Laboratory Activities
2:30-3:00	Wrap-up and Evaluation

Cultural Activity

The Importance of Corn to the Native American People

Note: This activity will be completed by the Cultural Leader at Each Academy Location

Activity I

Students will work with a computer generated virtual laboratory program that will teach them some of the basic steps of aflatoxin determination in corn.

Activity II

Using some of the laboratory principles learned in the virtual laboratory, students will evaluate the presence of aflatoxin in samples of corn using appropriate laboratory procedures.

Activity III

Students will evaluate the presence of gluten in samples of corn and wheat using appropriate laboratory procedures.

Wrap-Up, Summary & Discussion

Lesson Plan for 2014 – 2015 NATURE Sunday Academy Program

The Importance and Analysis of Aflatoxin and Gluten in Corn

Project Description

The importance of corn to Native American peoples cannot be overstated. Corn is a native crop to the Western Hemisphere (North and South America) and a staple crop to the native peoples of the region. Today, corn is still a major crop in this part of the world. Corn is the number one crop produced in the United States and is used for a variety of different products used for food, feed and fuel. Therefore the importance of keeping the corn we produce safe cannot be overemphasized. Today you will learn about how a mold can produce a toxin that can make corn unsafe to eat and how we can detect this toxin in corn.

Objectives

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4. A basic test to determine the presence of aflatoxin and gluten in corn.

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Introduction

Corn is important to Native American peoples historically and today as well as the entire United States economy. For this reason many preservation methods are used to prevent the corn from being contaminated by microorganisms that can make corn unsafe to eat. One of these organisms is a mold call *Aspergillus flavus* that can produce a toxin mold called aflatoxin. Today we will learn how to detect this mold in corn using both a computer virtual laboratory and a hands-on laboratory exercise.

Activity I – Virtual Laboratory Exercise

Please get into groups of 2 persons (if necessary, 3-4 persons depending on the availability of computers) and enter into the Corn Mold Virtual Laboratory. Please follow the instructions as directed by the virtual laboratory instructor (http://virtuallab.nmsu.edu/)

a. You can choose to repeat the exercise again to learn the procedure.

1. Did you learn the key steps in analyzing for aflatoxin in corn?
2. How many times did you repeat the exercise before you felt comfortable with the procedure?

b. Remaining in your same group, please proceed to part two virtual laboratory exercise where you will be given an unknown sample to test for aflatoxin. Please follow the instructions as directed by the virtual laboratory instructor.

1. How many of the steps could you remember before having to use the hint button?
2. Did the sample that you chose have aflatoxin present? How did you know?

Activity II – Hands-On Exercise

Please get into groups of 2 persons (if necessary, 3-4 persons depending on the availability of supplies). Please find the ziplock bag labeled aflatoxin test kit located on your bench. Please read through the instructions provided at your table and in the lesson plan. You also have a sample of ground corn in the kit for you to use. This ground sample will be used specifically in part B of this activity.

Part A. Black Light Test

1. Remove the ground corn from the test kit.

2. Record the code number on the data sheet (provided by instructors).

3. Find the corn sample located by the black light box that matches your code. This sample will be the one that you will test in step 4.

4. Caution: Do not look directly at the black light. Place the corn sample (keep sample in bag) into the light box. View the corn sample for fluorescence. Note if fluorescence exists (bright yellow or green-yellow color).

Part B. Sample Analysis

1. Add 10 g ground grain sample to suitable screw cap container.

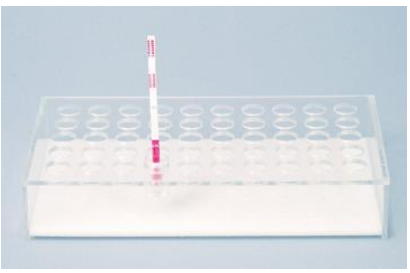
2. Add 20 ml of 70% Methanol Extraction Solution and shake vigorously for 1-3 minutes to extract aflatoxin from grain sample.

3. Allow sediment to settle for 5 minutes. It is important **not** to transfer sediment to the test strip since it can interfere with the flow of liquid and this may affect test results. It is important to allow the recommended settling time. Test line intensity may increase when testing very fine ground samples if sufficient settling has not occurred.

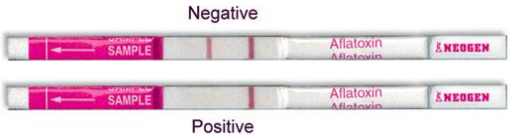
4. Add 200 µL of diluent (please obtain bottle from instructors) to the sample cup (small plastic cup) using a clean calibrated transfer pipette.

5. Transfer 200 µL of grain extract to a sample cup using the same calibrated transfer pipette. Mix the sample using the pipette by drawing and expelling the liquid in and out of the pipette 3 times (be careful not to push liquid from the sample cup during expelling of the liquid).

6. Insert test strip into liquid and allow test to develop.



7. Read results after 5 minutes and interpret according to the *Interpreting the Lateral Flow Strip* section.



<u>Interpreting the Lateral **Flow** Strip Test</u>
Check the result window at five (5) minutes after inserting the strip. At least one line, the Control Line, should always develop approximately one (1) cm down from the Top Pad. A red line in this position indicates that the device is functioning properly. A red line appearing below the Control Line is the Test Line and indicates a negative result. If the test strip displays two (2) red lines, the test is complete and the sample is negative for aflatoxin-contaminated com. If at 5 minutes the test strip only shows a clearly visible Control Line, then the sample is positive for aflatoxin contamination at or above a 20 ppb level. If no control line develops, the result is inconclusive and need to be repeated.

Question:

1. Did the black light test give positive results?

2. Did you find aflatoxin in your sample? If so, how did you know that you had aflatoxin present? If not, how did you know that aflatoxin was not present?

3. Did the black light test and ELISA test method agree?

4. Please check with your laboratory instructor to see if you were correct. If you did not get the correct answer, why do you think you did not get the correct answer? Please explain.

Activity III - Gluten Test

In this exercise you will be completing the Reveal 3D Gluten test. Please get into groups of 2 persons (if necessary, 3-4 persons depending on the availability of supplies). Please find the ziplock bag labeled gluten test kit located on your bench. Please read through the instructions provided at your table and in the lesson plan. In your packet, a test tube with either wheat or corn is present.

Sample Analysis

1. Add 5 mL of distilled water to the sample provided.

2. Shake the sample for 2 minutes.

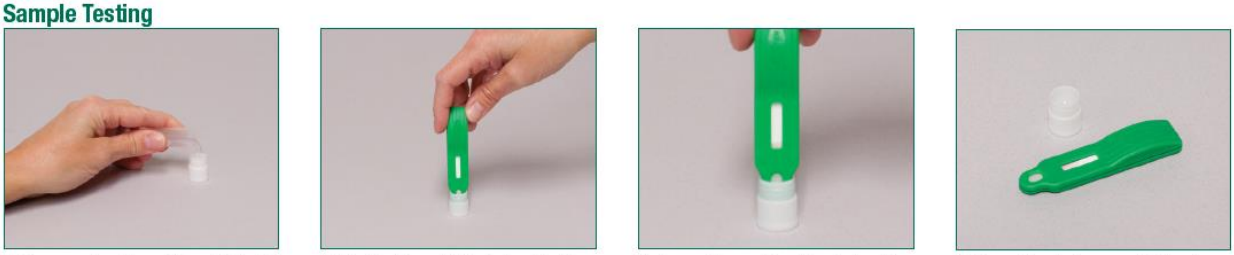
3. Allow the sample to sit 3 minutes for the large particles to settle. The liquid portion (i.e. sample) obtained will be used in step 2 below.

4. Follow the test as described below for the extraction of gluten.

1. Open Type 4 extraction buffer sachet and add to a sample tube.
2. Add 0.25 mL of sample to the sample tube.
3. Secure cap and shake for 1 minute.
4. Add contents of Type 5 extraction buffer sachet to tube and shake for 1 minute.

5. Follow the test as described below to determine the presence of gluten.

Sample Testing



1. Remove the lid and fill with liquid from the tube.
2. Dip the Reveal 3-D device into the liquid in the lid; ensure that the cavity is saturated with the liquid.
3. Leave the cavity saturated until the liquid is seen running into the test window.
4. Place the device on a flat surface and allow the test to develop for 5 minutes.

6. Interpret the results

One dark blue line

One dark blue line with two lighter blue lines

One dark blue and one light blue line

Evaluation and Wrap-up

Please answer the following questions:

1. What is the importance of corn to this region

2. What is *Aspergillus flavus* and why we so concerned about this organism?

3. What is aflatoxin and why is it harmful

4. Did you find the virtual laboratory exercise to be helpful in your aflatoxin analysis? Why or why not?

5. Did the corn samples produce a positive gluten test? How about wheat? Was this expected? Explain

6. Below are two food labels. Which product could I label gluten free?

Ingredients: Whole Grain Corn, Corn Meal, Sugar, Honey, Salt, Molasses, Brown Sugar Syrup, Canola and/or Rice Bran Oil, Yellow Corn Flour, Natural Flavor, Natural Almond Flavor. BHT Added to Preserve Freshness.

Product 391

Ingredients: Whole Grain Corn, Modified Wheat Starch, Corn Bran, Inulin, Sugar, Bleached Oat Fiber, Pea Fiber, Honey, Corn Starch, Salt, Gum Arabic, Color Added, Trisodium Phosphate, Natural and Artificial Flavor, Sucralose, Acesulfame Potassium, Vitamin E (mixed tocopherols) Added to Preserve Freshness.

Product 972

SUNDAY ACADEMY DATA SHEET

Activity	Circle the response for test outcome	
Black Light		
What was the code number for your sample?	_____	
Did sample give positive result?	Yes	No
Aflatoxin Test		
What was the code number for your sample?	_____	
Was your sample positive or negative for aflatoxin?	Positive	Negative
Did the black light and aflatoxin test results agree?	Yes	No
Gluten Test		
What was the code number for your sample?	_____	
Did sample give positive result?	Yes	No
Did your result match the expected result (see instructor)?	Yes	No

1. Obtain a representative sample. Grind and weigh out a 10 g sample.

2. Add 20 mL of 70% methanol to sample.

3. Shake sample vigorously for 1 minute, or blend for one minute.

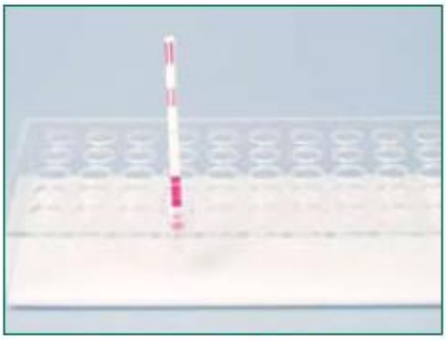
4. Allow the sample to settle. **OR**

4. Filter sample using a filter syringe. **OR**

4. Filter sample using a Whatman #1 filter paper.

5. Add 200 µL sample diluent to a sample cup.

6. Add 200 µL sample extract to the sample cup and mix 3 times.



7. Place a new Reveal Aflatoxin strip into a sample cup. Set timer for 3 minutes.

Source: http://www.neogen.com/FoodSafety/pdf/procedures/8015_Pro.pdf