

The Biochemical Components of Peanuts

Experiment #9

Objective: To isolate and identify several different components of peanuts, including carbohydrates, proteins and lipids (fats) and to determine the percentage of oil in a peanut.

Introduction

Peanuts are an important food item around the world. The peanut plant is a legume that bears its seeds in a pod underground, rather than on the aerial parts of the plant. For this reason, it is often called a groundnut. About 90 percent of the world's peanuts are grown in Asia and Africa. Peanuts are usually roasted, either in the shell (or pod) or after removing the peanut from the shell. Salt is often added to give salted peanuts. About half of the peanuts consumed in the United States are consumed as peanut butter and about one-fourth as roasted peanuts. The peanut seeds or kernels are nearly 50 percent oil (triglycerides). Peanuts are widely used in Asia and Africa to produce peanut oil, which forms less smoke when frying than some other types of oils used for frying. Low grade peanut oil can be used for making soap, shampoo, cosmetics, paint and explosives (nitroglycerin). After the oil is extracted from the peanut, the remaining meal is often used as a valuable feed for livestock because of its high protein content and may be used for human consumption. The peanut shell is also used for a wide range of applications after it is ground into a powder, and is an ingredient for synthetic composites, wallboard and cork substitutes.

George Washington Carver was an African-American who achieved international fame as an agricultural researcher in the early 20th century through his extensive work with peanuts. He produced more than 300 products from peanuts, including a milk substitute, printer's ink and soap. Carver received his bachelor's and master's degrees from Iowa State Agricultural College (now Iowa State University) and began his academic career at Tuskegee Institute (now Tuskegee University) in Alabama in 1896. Carver sought to teach Southern farmers, particularly African-American farmers, more productive farming and soil conservation practices. Carver became the head of the newly created Department of Research at Tuskegee in 1910 and was a strong advocate for civil rights throughout his career. He donated his life savings to Tuskegee Institute, which was used to establish the George Washington Carver Research Foundation. He died in 1943.

In this laboratory, you will be extracting oil from peanut kernels and showing that it has a high degree of unsaturation (polyunsaturated oil). You will test the peanut meal for several types of carbohydrates, including starches and reducing sugars, as well as testing for protein. The peanut shell will be analyzed for the presence of reducing sugars after hydrolysis of the polysaccharide.

Materials

Peanuts in the shell, mortar and pestle, long stem funnel, Erlenmeyer flask, filter paper, balances, hot plates, methylene chloride, 95% ethanol, Fehling's reagents A and B, concentrated nitric acid, 6 M hydrochloric acid, 10% sodium hydroxide, copper sulfate solution, iodine solution.

Procedure

Part A. Isolation of peanut oil.

1. Separate the kernels and shells of two or three peanuts (4 or 5 kernels). Save the shells for later analysis.
2. Remove any fine skins from the peanut kernels, weigh them on a balance and record the total mass on the Report Sheet.
3. Place the peanut kernels in a mortar and grind them with the pestle and note the consistency of the ground peanuts. It should be a fine meal or powder.
4. Add 10 mL of methylene chloride to the mortar and continue grinding the peanuts with the pestle until the pieces are like a powder.
5. Accurately weigh a clean and dry 125 mL Erlenmeyer flask. Place a piece of **fluted filter paper** in a long stem funnel and place the Erlenmeyer flask under the funnel to collect the filtrate. Use an iron ring or clamp to hold the funnel on the iron rack at the lab bench.
6. Loosen the crushed peanuts in the mortar and transfer the solvent and crushed peanuts from the mortar to the fluted filter paper in the funnel. Add a few more mL of methylene chloride to the mortar to rinse it and add this to the filter in the long stem funnel. It may take a few minutes for the solvent to pass through the funnel.
7. While you are waiting for the solution to pass through the filter, take half of a peanut shell and crush it with your fingers, placing the small pieces of the crushed shell in a large test tube. Add about 5 mL of 6 M hydrochloric acid (HCl) solution to the tube and make sure the acid solution covers the pieces of shell. Heat this on the hot water bath for about 20 min, mixing the shell with a stirring rod or thin spatula occasionally. This **hydrolyzed shell** will be analyzed for carbohydrates later in part C. Label it.
8. When all of the methylene chloride solvent has passed through the filter paper, remove the filter paper and let it air dry on a piece of paper towel. Allow the solvent to evaporate from the peanut meal and save the peanut meal for analysis of carbohydrate and protein in part C.
9. Evaporate the methylene chloride from the Erlenmeyer flask by placing it on a hot plate. When it stops boiling (after a few minutes at a medium heat setting), remove the flask from the hot plate, allow it to cool and weigh the flask. Record the weight of flask and oil on the Report Sheet and determine the percent of oil in peanuts.

Part B. Measuring Unsaturation of Peanut Oil

1. After weighing the oil in the flask and recording the weight on the report sheet, add about 5 mL of ethanol to the flask and swirl it to dissolve the oil. Mix it well.

2. Warm the ethanol solution gently, do not boil.
3. Add three to five drops of potassium permanganate (KMnO_4) solution to the warmed ethanol/peanut oil solution and observe whether the purple solution turns to a brown precipitate, which is indicative of a reaction with an unsaturated hydrocarbon (or unsaturated fat). See Experiment 2 for a description of this reaction with alkenes. Record your observations on the Report Sheet.

Part C. Analysis of Peanut Meal and Shell for Carbohydrates.

1. Place about one-fourth (equivalent to 1 kernel) of the peanut meal in a large test tube and add about 2 mL of hot water (from the hot water bath) to the meal. Use a glass stirring rod to mash or grind the meal in the bottom of the tube to get some of the material dissolved in the hot water. After grinding the meal for about a minute, add about 5 mL of deionized water to the tube and mix it well. This is **unhydrolyzed** peanut meal suspension used in the following analyses. Label it.
2. Allow the meal to settle in the tube and transfer about 2 mL of the upper solution to a clean test tube and add about 4 mL of 6 M hydrochloric acid (HCl) solution. Mix it well and place this tube in a boiling water bath for about 15 minutes to hydrolyze the starch and protein. This will be **hydrolyzed** peanut meal solution. Label it.
3. While the acidified solution is being heated for hydrolysis, transfer about 1 mL of the unhydrolyzed peanut meal suspension (step C-1) to a small clean test tube and add one or two drops of iodine solution to test for starch. What color change would you expect if starch is present? Record your observations on the Report Sheet. [Note: Starches from different sources give colors ranging from blue to green or brown].
4. Add 3 mL of Fehling's reagent A and 3 mL of Fehling's reagent B to a test tube and mix the solution well. Notice the color of the solution. Add equal amounts of this solution (about 2 mL) to three clean test tubes. Label one tube U (unhydrolyzed meal), one H (hydrolyzed meal), and the third S (hydrolyzed shell).
5. Add about 1 mL of the peanut meal suspension (unhydrolyzed) to tube U containing Fehling's reagent and heat this in the boiling hot water bath for about 5 min. Notice whether there is any change in color of the solution or formation of a red precipitate, which would indicate reducing sugars. See Experiment 4 for discussion of Fehling's reaction. Record your observations on the Report Sheet.
6. After the acidified solution of hydrolyzed peanut meal (step C-2) has heated for 15 min, remove about 1 mL of the solution with a dropper and place it in a clean test tube. Add about 3 mL of 10% sodium hydroxide solution to the acidified hydrolysate, mix well and transfer to the tube labeled H with Fehling's reagent. The solution should remain dark blue. If it changes to a light green color, you did not add enough 10% sodium hydroxide solution, so add more. Mix well and heat this on the hot water bath for 5 min. Notice whether there is any change in color or precipitate formation that would indicate the

presence of a reducing sugar. Report your observations on the report sheet.

7. If the acid solution containing shell has been heated for at least 15 min, transfer about 1 mL of this shell hydrolysate to a small test tube and add about 3 mL of 10% sodium hydroxide (NaOH) solution. Mix it well and add this solution to the Fehling's solution in tube S. The color should remain dark blue as the one in step 6 above. If it changes to light blue, add more sodium hydroxide solution. Heat this solution on the hot water bath for 5 min and record any change in color or precipitate formation on the Report Sheet.
8. Indicate what conclusions you can make regarding the carbohydrates that may be in the peanut kernel and in the peanut shell.

Part D. Analysis of Peanut Meal for Protein.

1. Add about 2 mL of 10% sodium hydroxide solution to a clean test tube and add 5 drops of copper sulfate solution. Mix the solution well and divide it equally into two test tubes (*i.e.*, pour half of this solution in another test tube). Transfer about 1 mL of the **unhydrolyzed** peanut meal suspension (from step C-1) to the test tube and allow to stand for about 5 min, noticing whether there is any color change. Add about 1 mL of deionized water to the other test tube as a control so you can better compare the color change. See Experiment 8 for a description of the biuret test for protein. Record your observations regarding color change on the Report Sheet.
2. Add about 0.5 mL of the peanut meal suspension to a clean test tube and add about 0.5 mL (about 8 drops) of concentrated nitric acid (in the hood) to this test tube. Mix the solution well being careful not to splash any solution out of the tube. Place the tube in the hot water bath for a few minutes noting any changes in color.
3. Remove the tube from the hot water bath after a few minutes and allow to cool before adding about 4 or 5 mL of 10% sodium hydroxide solution to make the solution alkaline. You should see the yellowish color become deeper yellow when it is alkaline. Test the solution with red litmus paper or universal pH paper. Record any color change on the Report Sheet. See Experiment 8 for a description of the xanthoproteic test for protein. The yellow color indicates the presence of aromatic amino acids in the protein.

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Pre-Lab Exercise

The three major categories of macronutrients in the diet are carbohydrates, proteins and lipids (or fats).

1. Indicate what kind of storage carbohydrate (that humans could metabolize for energy) you would expect to find in a natural food such as a peanut and a chemical test you could do to identify this storage carbohydrate. Check Experiment 5 if you need to refresh your memory.
2. What is the product of hydrolysis of storage carbohydrates and indicate a simple chemical test that would indicate the presence of this hydrolysis product.
3. Describe the differences between saturated fatty acids and unsaturated fatty acids in terms of their chemical structures. What is a polyunsaturated fatty acid?

4. What kinds of chemical reactions might be observed with unsaturated fatty acids that would not be observed with saturated fatty acids? Show a chemical reaction that could take place with an unsaturated fatty acid. You may want to check Experiment 2 where chemical reactions were used to distinguish saturated from unsaturated hydrocarbons or check the text book.
5. Describe (you need not show the chemical reaction) at least two tests that could be used to show that proteins are present in peanuts. You may want to check Experiment 8, where tests were used for protein.

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Data & Report Sheet

A. Extraction of Storage Fats from Peanuts

Mass of Peanut Kernels _____ g

Mass of Flask and Extracted Lipid _____ g

Mass of Empty Erlenmeyer Flask _____ g

Mass of Lipid Extracted _____ g
(Subtract mass of empty flask from mass of flask + extracted lipid)

What is the percent of fats in these peanuts: _____ percent
($100\% \times \text{mass of lipid extracted} / \text{mass of peanut kernels used for extraction}$)

Determine the number of kilocalories (kcal or Calories with a capital C) of energy derived from fat in 100 g of peanuts by multiplying 9 kcal/g fat times the answer for percent of fats obtained above. How does this compare with nutritional information for foods (peanuts)? You may need to check a nutrition book or the internet for this information.

B. Measuring Unsaturation of Fats in Peanuts

What was the result when testing peanut oil with potassium permanganate? Was there any color change in the permanganate solution when added to the oil? Describe it.

Would you say that peanuts contain unsaturated fatty acids?

C. Carbohydrates in Peanuts

What happened to the color of the iodine when it was added to the unhydrolyzed peanut meal suspension?

Describe the result after testing the peanut meal suspension (tube U) with Fehling's reagent. Was there any color change or precipitation? What does this indicate with respect to carbohydrates in peanuts?

Describe the result of testing the peanut meal suspension with Fehling's reagent after acid hydrolysis (tube H). Was there a color change or precipitate? What carbohydrates may be present?

What kind of carbohydrate is in a peanut, from the result of the iodine test and the two Fehling's tests on unhydrolyzed and hydrolyzed peanut meal suspension?

Describe the result of testing the peanut shell hydrolysate with Fehling's reagent after acid hydrolysis. Was there a color change or precipitate? What kind of carbohydrate would you expect to be in the peanut shell?

D. Protein in Peanuts

Describe the color of the alkaline copper solution after adding the peanut meal suspension to it, compared to alkaline copper solution in water. This is the biuret test. Would this indicate that protein is present in peanuts?

Describe the colors observed in the peanut meal suspension after heating it with nitric acid.

Describe the color after making the nitric acid solution alkaline with sodium hydroxide.

Does this indicate there is protein in the peanut kernels? Are there aromatic amino acids in the protein?