Biological Chemistry: Testing for Lipids

The lipids or fats have one common characteristic; they are all water insoluble. Lipids assume one of two forms, a steroidal lipids or fatty acid lipids (glycerides). The steroidal lipids are large molecules composed of two rings with various functional groups attached. The steroidal molecules include cholesterol, estrogen and testosterone. The backbone of fatty acid lipids, glycerol, is composed of three-carbon alcohol. If three fatty acids are attached to glycerol (one fatty acid per carbon in the backbone) then the lipid is considered a triglyceride. The most common lipid found in cell membranes is the phospholipid. Phospholipids are diglycerides. Two of the carbons in the glycerol backbone have attached fatty acids while the third carbon in the glycerol backbone has an attached phosphate functional group. This phosphate group is polar and imparts unique characteristics to the glycerol backbone allowing the lipid head to dissolve in water. The fatty acid tails of phospholipids are hydrophobic or water-fearing.

There are 4 common tests for the presence of fats; Sudan III (IV), water emulsion and the paper bag test.

Activity 1: Sudan III or IV Indicator

Materials

15x125mm test tubes - 11
Test tube brush
Glass rod, scoopula
Large beaker (500 mL)
Test tube rack
Sudan III or Sudan IV
Distilled water
Pipettes
Various foods (egg (separated), potato, ground beef, vegetable oil, whole milk, nuts)

Note on food preparation: If you are performing tests for lipids, proteins and carbohydrates you only need to prepare the solid foods once. There should be enough extract to perform all tests from one food preparation. If you have already prepared the solid foods extracts, start on step 7.

Procedure

1. Use a sharpie or permanent marker to label one set of test tubes: Control, egg white, egg yolk, ground beef, potato, milk, raw chopped nuts, and vegetable oil. Mark these tubes 1.5 cm from the bottom of the tube.
2. Use a sharpie or permanent marker to label a second set of test tubes: ground beef, potato, chopped nuts. Mark this set of tubes a 1 and 6 cms from the bottom of the tube. These tubes will be used to grind and suspend the solid foods.
3. To this second set of tubes add the appropriate solid materials to the 1 cm mark. Finely chop the potato and nuts before adding them to the test tubes.
4. Add water to approximately the 6 cm mark.
5. Use the glass rod or scoopula to crush the solid foods.

6. Allow the solid foods to settle for 5 minutes. Continue to step 7.
7. Retrieve the first set of labeled tubes. Add Sudan III (IV) reagent to each tube to the 1.5 cm line.
8. Use a pipette to add 1 mL or 20 drops of water to the control tube.
9. Use a pipette to add 1 mL or 20 drops of egg white to the tube marked 'egg white'.
10. Use a new pipette to add 1 mL or 20 drops of egg yolk to the tube marked 'egg yolk'.
11. Use a new pipette to add 1 mL or 20 drops of milk to the tube marked 'milk'.
12. Use a new pipette to add 1 mL or 20 drops of vegetable oil to the tube marked 'vegetable oil'.
13. If it has been at least 5 minutes and the ground food has settled, you can now pipette the supernatant (liquid above the solids). Remove only the liquid, do not removes solids from the tubes.
14. Use a new pipette to add 1 mL or 20 drops of ground beef extract to the tube marked 'ground beef'.
15. Use a new pipette to add 1 mL or 20 drops of potato extract to the tube marked 'potato'.
16. Use a new pipette to add 1 mL or 20 drops of chopped nut extract to the tube marked 'chopped nuts'.
17. Before proceeding to the next step write a hypothesis for this experiment below.
    Hypothesis:_____

    _____

    _____
18. Roll each tube between your palms to mix the indicator and extract.
19. Place the tubes back in the test tube rack. If a red oily layer develops on the surface of the liquid in the tubes then fats are present.
20. Clean up: Tap out the solid foods into the trash can. Use the test tube brush to clean all of the test tubes. Return materials to the appropriate area.

Was your hypothesis supported? Why or Why not?

What was the control? What was the dependent variable? What was/were the independent variables?

Record your results below.

Sample	Red oily layer (yes or no)	Is fat present?
Control	No	No
Egg white		
Egg yolk		
Ground beef		
Potato		
Milk		
Chopped nuts		
Vegetable oil		

Which food had the highest amount of fat as indicated by your results?

Which food had the least amount of fat as indicated by you results?

Design your own experiment with food and fat detection and summarize it below. For example, maybe you want to determine which snack food has the highest fat content. Include a hypothesis for your experiment.

Activity 2: Fat Emulsion

This activity will require the crushing of food stuff in ethanol, an alcohol. Fats are soluble in ethanol. Fats in the food will dissolve or be extracted into the ethanol.

Materials

15x125 mm test tubes - 16
Test tube rack
Test tube brush
Ethanol
Glass rod, scoopula
Distilled water
Large beaker (500 mL)
Pipettes
Various foods (egg (separated), potato, ground beef, vegetable oil, whole milk, nuts)

Before proceeding, write a hypothesis for this experiment below.

Hypothesis:_____

_____

_____

Procedure

1. Use a sharpie or permanent marker to label **<u>two sets</u>** of test tubes: Control, egg white, egg yolk, ground beef, potato, milk, chopped nuts, and vegetable oil.
2. Mark one set of tubes 1 cm from the bottom of the tube. These will be the EXPERIMENT tubes. Mark one set of these tubes at 1 cm and 4 cm from the bottom of the tube. This set of tubes is the EXTRACT tubes.
3. To this last set of extract tubes (tubes marked at 1 cm) add the test material to the 1 cm mark. Add ethanol to the control tube to the 1 cm mark.
4. Add twice as much ethanol as food, i.e., fill the test tube to approximately the 2 cm mark.
5. Use the glass rod or scoopula to crush or mix the foods.
6. Allow the solid foods to settle for 5 minutes.
7. Retrieve the first set of labeled tubes marked only at the 1 cm mark.
8. Use a pipette to transfer 1 mL or 20 drops of ethanol from the EXTRACT control tube to the control tube.
9. Use a new pipette to add 1 mL or 20 drops of egg white extract to the EXPERIMENT tube marked 'egg white'.
10. Use a new pipette to add 1 mL or 20 drops of egg yolk extract to the EXPERIMENT tube marked 'egg yolk'.
11. Use a new pipette to add 1 mL or 20 drops of milk to the EXPERIMENT tube marked 'milk'.
12. Use a new pipette to add 1 mL or 20 drops of vegetable oil to the EXPERIMENT tube marked 'vegetable oil'.
13. Use a new pipette to add 1 mL or 20 drops of ground beef to the EXPERIMENT tube marked 'ground beef'.
14. Use a new pipette to add 1 mL or 20 drops of potato to the EXPERIMENT tube marked 'potato'.

15. Use a new pipette to add 1 mL or 20 drops of chopped nuts to the EXPERIMENT tube marked 'chopped nuts'.
16. Add 20 drops or 1 mL of water to each tube. Mix the tubes vigorously.
17. Compare the food tubes to the control tube and record your results below.

Fats are not soluble in water. If fats are present in the ethanol extract when water is added and the tube shaken an emulsion will form, the tube will appear cloudy. The cloudier the water the more fat is present. Water is miscible in alcohol. If fats are not present, when the tube is shaken it will remain clear.

Clean up: Tap out the solid foods into the trash can. Use the test tube brush to clean all of the test tubes. Return materials to the appropriate area.

Record your results below. Once you have filled in the first 3 columns, rank the samples from least (1) to greatest fat content (8).

Sample	Clear or turbid	Are fats present?	Ranking
Control	clear	No	1
Egg white			
Egg yolk			
Ground beef			
Potato			
Milk			
Chopped nuts			
Vegetable oil			

Which food had the highest amount of fat as indicated by your results?

Which food had the least amount of fat as indicated by you results?

Was your hypothesis supported? Why or Why not?

What was the control? What was the dependent variable? What was/were the independent variables?

Design your own experiment with food and protein detection and summarize it below. For example, maybe you want to determine which bread has the highest protein content. Include a hypothesis for your experiment.

Activity 3: Grease Spot Test

The grease spot test is an easy test to identify the presence of fats. Fats are non-volatile, they have a high boiling point. When applied to paper, they will leave a translucent (light passes through) spot on the paper.

Materials

Brown paper bag
Distilled water
Pipettes
Various foods (orange juice, vegetable oil or salad dressing, whole milk, nuts)

Before proceeding, write a hypothesis for this experiment below.

Hypothesis:_____

_____

_____

Procedure

1. Pick up a piece of brown paper bag from the supply table.
2. With a pencil write distilled water, orange juice, vegetable oil, milk and nuts. Space the labels 3 cm apart.
3. Write a hypothesis for this activity.
Hypothesis: _____

_____

4. Pick up a nut from the supply table. Rub the nut on the brown paper bag above the label. You may need to use some pressure.
5. Use a new pipette to add 1 drop of distilled water to the paper bag above the paper bag label 'water'.
6. Use a new pipette to add 1 drop of orange juice to the paper bag above the paper bag label 'orange juice'.
7. Use a new pipette to add 1 drop of vegetable oil to the paper bag above the paper bag label 'vegetable oil'.
8. Use a new pipette to add 1 drop of milk to the paper bag above the paper bag label 'milk'.
9. Wait ~20 minutes and examine the brown paper bag. Water soluble compounds, non-fats will evaporate. Fats are non-volatile at room temperature. They will not evaporate. The paper where they are applied will have a translucent appearance.

Record your observations below.

Sample	Translucent (yes or no)	Is fat present?
Orange juice		
Milk		
Chopped nuts		
Vegetable oil		
Water		

Which food had the highest amount of fat as indicated by your results?

Which food had the least amount of fat as indicated by you results?

There is no designated control in this activity. Which sample could be used as a control? Is this a positive or negative control?

What other control is present in this activity? (If you identified a negative control in the previous question, is there a positive control also? If you identified a positive control in the previous question, is there a negative control also?)

Design your own experiment with food and fat detection and summarize it below. For example, maybe you want to determine which salad has the highest fat content. Include a hypothesis for your experiment.