Biological Chemistry: Testing for Proteins

Proteins are important biological molecules. Their roles in cells include serving as structural molecules, hormones, biocatalysts and in transport. Identifying proteins and quantifying the amount of protein in foods and biological fluids is important to nutrition and human health. The biuret reagent is used to test for the presence of proteins. It is the most commonly used reagent in laboratories worldwide to quantify the amount of protein in foods and biological fluids. Biuret reagent is used in this activity to identify the presence of proteins in foods.

The Biuret reagent is made of sodium hydroxide (NaOH) a strong base and copper sulfate ($CuSO_4$). Sodium hydroxide raises the pH and makes the solution alkaline. Under alkaline conditions copper sulfate ionizes, freeing the copper ion. The copper ion interacts with nitrogen atoms participating in peptide bonds to produce a color complex. In the absence of proteins, the Biuret reagent is blue in color. In the presence of protein the reagent turns purple/violet. The amount of color change that occurs depends on the number of peptide bonds in the solution. The greater the number of peptide bonds the deeper or more violet the color becomes.

Materials

Test tubes - 17
Test tube brush
Glass rod, scoopula
Large beaker (500 mL)
Test tube rack
Biuret reagent
Hotplate or Bunsen burner (if using a Bunsen burner you will also need a ring stand, wire gauze and ring clamp)
Distilled water
Pipettes
Various foods (cereal, bread, egg (separated), apple, orange, 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.

Write your hypothesis for this activity here. _____

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Procedure

1. Use a sharpie or permanent marker to label one set of test tubes: Control, cereal, bread, egg white, egg yolk, apple, ground beef, orange juice, potato, milk, chopped nuts, and vegetable oil. Mark these tubes 4 cm from the bottom of the tube.

2. Use a sharpie or permanent marker to label a second set of test tubes: cereal, bread, apple, ground beef, potato, chopped nuts. Mark this set of tubes at 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 apple, potato and nuts before adding them to the test tubes. Finely chopping these foods will facilitate nutrient extraction.
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 empty labeled tubes. Add the Biuret reagent to each tube to the 4 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 orange juice to the tube marked 'orange juice'.
12. Use a new pipette to add 1 mL or 20 drops of milk to the tube marked 'milk'.
13. Use a new pipette to add 1 mL or 20 drops of vegetable oil to the tube marked 'vegetable oil'.
14. If it has been at least 5 minutes and the ground food has settled, you can now pipette 1 mL or 20 drops of the supernatant (liquid above the solids). Remove only the liquid, do not removes solids from the tubes.
15. Use a new pipette to add 1 mL or 20 drops of cereal extract to the tube marked 'cereal'.
16. Use a new pipette to add 1 mL or 20 drops of bread extract to the tube marked 'bread'.
17. Use a new pipette to add 1 mL or 20 drops of apple extract to the tube marked 'apple'.
18. Use a new pipette to add 1 mL or 20 drops of ground beef extract to the tube marked 'ground beef'.
19. Use a new pipette to add 1 mL or 20 drops of potato extract to the tube marked 'potato'.
20. Use a new pipette to add 1 mL or 20 drops of chopped nuts extract to the tube marked 'chopped nuts'.
21. Mix the tubes by rolling the upright test tube between your hands.
22. Compare the food tubes to the control tube.
23. 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. Once you have filled in the first 3 columns, rank the samples from least (1) to greatest protein content.

Sample	Color	Is protein present?	Ranking
Control	Blue	No	1
Cereal			
Bread			
Egg white			
Egg yolk			
Apple			
Ground beef			
Orange juice			
Potato			
Milk			
Chopped nuts			
Vegetable oil			

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

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

Would a single amino acid produce a positive Biuret result? Why or why not?

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.