Conduct A Qualitative Test For Starch, Fat, A Reducing Sugar, A Protein

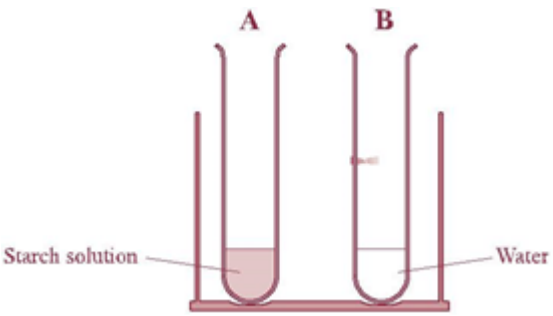
Biology – Leaving Cert

Experiments

Materials/Equipment

Starch solution (1%)	Pipettes/droppers
Iodine Solution	Thermometer
Glucose Solution (1%)	Hot water bath (80°C-100°C)
Benedict's reagent (qualitative)	Test-tube holder
Protein solution eg. Albumen (1°C) or Milk	3 Test-tube racks
Sodium hydroxide solution (10%), copper	Disposable gloves
Sulfate solution (1%) (or biuret reagent)	Brown Paper
Vegetable Oil	Scissors
6 Boiling Tubes	Labels
Beaker (250 cm^3)	Timer

(i) Test for Starch



Procedure

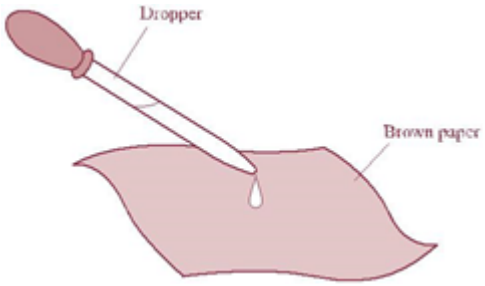
1. Familiarise yourself with all procedures before starting.
2. Label boiling tube A 'starch solution' and boiling tube B 'water'.
3. Place 2 cm^3 of the starch solution into tube A.
4. Place 2 cm^3 of water into tube B. This acts as the control.
5. Add 2–3 drops of iodine solution to each tube.
6. Swirl each tube.
7. Record result.

Result

Sample	Initial Colour	Final Colour
A – Starch Solution		
B - Water		

Conclusion/Comment

(ii) Test for Starch



Procedure

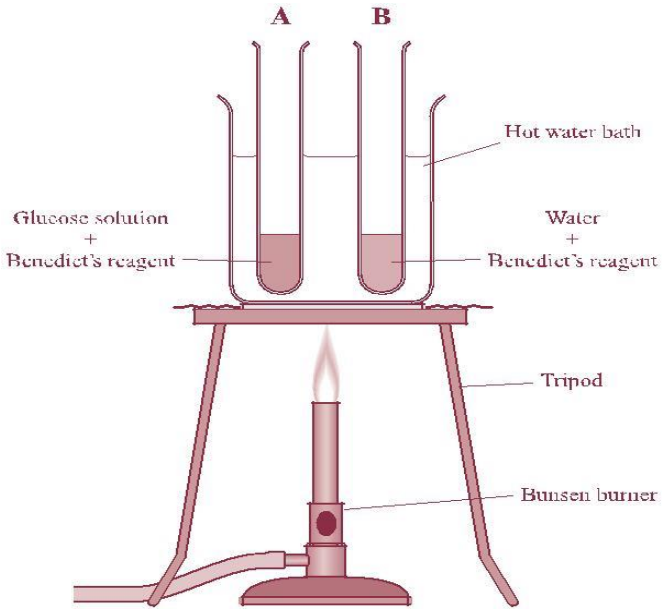
1. Familiarise yourself with all procedures before starting.
2. Cut two pieces of brown paper of similar size.
3. Place 2–3 drops of oil on one piece of paper and label it ‘oil’.
4. Place 2–3 drops of water on the other piece of paper and label it ‘water’. This acts as the control.
5. Leave both aside to dry.
6. Hold both pieces of paper up to the light.
7. Record result.

Result

Conclusion/Comment

Sample	Translucent spot	
	Before drying	After drying
Oil		
Water		

(iii) Test for a Reducing Sugar



Procedure

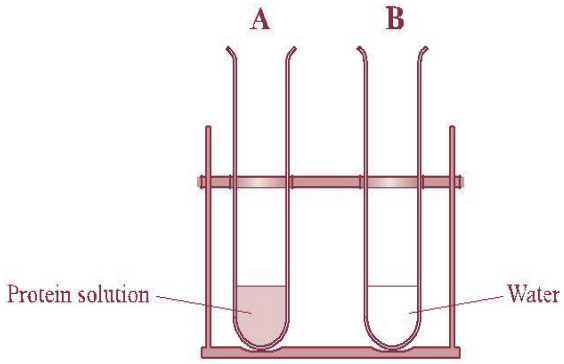
1. Familiarise yourself with all procedures before starting.
2. Label boiling tube A 'glucose solution' and boiling tube B 'water'.
3. Place 2 cm^3 of the glucose solution into tube A.
4. Place 2 cm^3 of water into tube B. This acts as the control.
5. Add 2 cm^3 of Benedict's reagent to each tube.
6. Swirl each tube.
7. Place both tubes in the hot water bath and heat for 5 minutes.
8. Using the test-tube holder, carefully remove both tubes from the water bath and place in the test-tube rack.
9. Record result.

Result

Sample	Initial Colour	Final Colour
A – Starch Solution		
B - Water		

Conclusion/Comment

(iv) Test for a Protein



Procedure

1. Familiarise yourself with all procedures before starting.
2. Label boiling tube A ‘protein solution’ and boiling tube B ‘water’.
3. Place 2 cm^3 of the protein solution into tube A.
4. Place 2 cm^3 of water into tube B. This acts as the control.
5. Add 2 cm^3 of sodium hydroxide solution to each tube followed by 2–3 drops of copper sulfate solution (or add 2 cm^3 of biuret reagent to each tube).
6. Swirl both tubes.
7. Record result.

Result

Sample	Initial Colour	Final Colour
A – Starch Solution		
B - Water		

Conclusion/Comment

SKILL ATTAINMENT

CONDUCT A QUALITATIVE TEST FOR: STARCH, FAT, A REDUCING SUGAR, A PROTEIN

Following instructions
Familiarise yourself with all procedures before starting
Follow instructions step by step
Listen to the teacher's instructions

Correct manipulation of apparatus
Fill the pipette
Set up the water bath
Use the test-tube holder
Use the timer
Label the tubes
Swirl the tubes

Observation
Observe a colour change
Note the appearance of the translucent spot
Appreciate the significance of heat
Notice the effect of swirling

Recording
Write up the procedure
Record colour change/translucent spot
Tabulate results
Compare with controls

Interpretation
Draw reasonable conclusions from your observations and results

Application
Become aware of any other application(s) of what you learned in this activity

Organisation
Exercise caution for your personal safety and for the safety of others
Work as part of a group or team
Label as appropriate
Work in an organised and efficient manner
Clean up after the practical activity

Background information

Starch

Starch is a complex, sparingly soluble, polysaccharide of plants. It consists of two main components: amylose and amylopectin. The amylose stains blue-black with iodine solution.

The solution of iodine used to test for starch contains potassium iodide and water in addition to iodine. The potassium iodide is necessary to dissolve iodine in water. This reagent changes from a brownish or yellowish colour to blue-black when starch is present, but there is no colour change in the presence of monosaccharides or disaccharides.

Fat

Fat does not evaporate from brown paper, but instead leaves a translucent spot.

Reducing sugar

Carbohydrates with a free or potentially free aldehyde (RCHO) or ketone (RCOR') group have reducing properties in alkaline solution. For monosaccharides, the aldo- and keto- groups carry out reducing sugar reactions. Common disaccharides such as lactose and maltose have at least one exposed aldo- or keto- group and can give a positive reducing sugar reaction also.

When a reducing sugar solution, such as glucose or maltose, is mixed with Benedict's reagent and heated, the reaction reduces the blue copper (II) ion to form a brick red precipitate of copper (I) ion. The colour of the reagent changes from blue to green to yellow to reddish-orange, depending on the amount of reducing sugar present. Orange and red indicate the highest proportion of these sugars. However, sucrose is not a reducing sugar because it is a particular kind of disaccharide in which both the aldo- and keto- groups of glucose and fructose (its contributory monomers) are locked into a covalent bond. As a result the aldo- and keto- groups are not free to react in a reducing sugar reaction. Therefore, when sucrose is tested in a reducing sugar reaction, a negative result is obtained.

Protein

Protein molecules are long chains of amino acids joined by peptide bonds. The biuret reagent reacts with any compound containing two or more peptide bonds to give a violet-coloured complex. Any compound containing two carbonyl groups (C=O) linked through either a nitrogen or a carbon atom will give a positive reaction. Therefore this test is not fully specific for proteins. However, the intensity of the reaction is an indication of the number of peptide bonds present in a protein.

The biuret reagent, which is blue in colour, contains a strong solution of sodium or potassium hydroxide (NaOH or KOH) and a smaller amount of dilute copper sulfate solution. The name of the test actually comes from another compound called biuret ($H_2NCONHCONH_2$), which also gives a positive reaction. The biuret compound itself is not part of the reagent, but just gives the test its name. The reagent changes colour in the presence of proteins or peptides because the amino group (H_2N-) of the protein or peptide chemically combines with the copper (II) ions in the biuret reagent. A purple/violet copper (II) complex indicates a positive result. This reagent changes to pink when combined with short-chain polypeptides. A negative result occurs with free amino acids because there are no peptide bonds present.

Advance preparation

- Set the water bath and check the temperature with the thermometer.
- Prepare the following solutions: starch solution (1% w/v), iodine solution, glucose solution (1% w/v), albumen solution (1% w/v), sodium hydroxide solution (10% w/v), copper sulfate solution (1% w/v).

Helpful hints

- Starch solution should always be freshly prepared to avoid fungal contamination.
- To quickly dry the brown paper in the fat test place it on a radiator for a minute or two.
- Filter paper may be used instead of brown paper in the fat test.
- When using Benedict's reagent it is essential to use the qualitative rather than quantitative type to obtain a brick-red colour with a reducing sugar.
- To reduce staining of boiling tubes they should be washed as soon as possible after a result is obtained, preferably using warm soapy water.
- Commercially available glucose test strips specifically test for glucose and not for other reducing sugars.
- When carrying tubes a test-tube holder tends to be better than a tongs.
- Biuret reagent tests for proteins in solution only