

Current Biofuels

Activity 1F - Carbohydrate testing

Learning outcomes: By the end of the session students should be able to:

- Use a variety of chemical tests to identify carbohydrates in plant material.
- Evaluate the merits of the sugar content of different biofuel feedstocks.
- Suggest suitable crops for bioethanol production.

Background

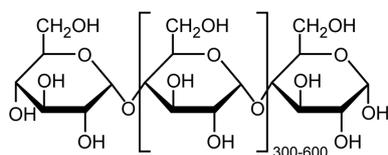
The ability of microorganisms to carry out fermentation is dependent upon the type of sugar substrate. The yeast *Saccharomyces cerevisiae* readily produces ethanol by fermentation of **sucrose** or **glucose** but is less efficient at fermenting other sugars and is unable to ferment pentose (C5) sugars. Current biofuels utilise monosaccharides in the form of sucrose from sugar beet and sugar cane or **polysaccharides** which must be broken down by hydrolytic enzymes prior to fermentation, in the form of starch from maize. Advanced biofuels utilise the polysaccharide cellulose, made up of glucose monomers, and hemicelluloses which are primarily made up of **pentose** sugars such as xylose. Both cellulose and hemicelluloses must be broken down into mono- and di-saccharide sugars before the sugar they contain is accessible for fermentation.

Testing plant material to identify the sugars and carbohydrates present is essential to determine suitable uses of a feedstock to produce biofuel and the enzymes needed to convert them to fermentable substrates.

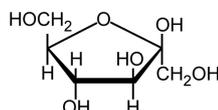
Plants produce a diverse range of sugars which provide energy stores in seeds and form the backbone of plant cell walls. Polysaccharides, such as glucomannans and glucuronoxylans, are of considerable structural complexity in ways that we are only beginning to understand. Recent efforts to unravel the biosynthetic machinery that constructs individual polysaccharides are beginning to provide fundamental insights into plant development, and could hold the key to optimising fermentation processes.

Understanding how sugars are locked into plant cell walls will enable researchers to select the right plants and the right enzymes to release the maximum amount of sugars for conversion to biofuels.

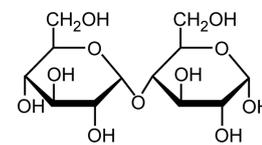
The following set of experiments enables identification of carbohydrates based on the properties of functional groups and the differences between structures.



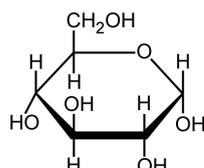
Starch $[C_6H_{12}O_6]_n$



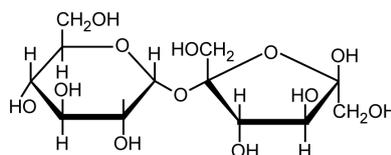
Fructose $C_6H_{12}O_6$



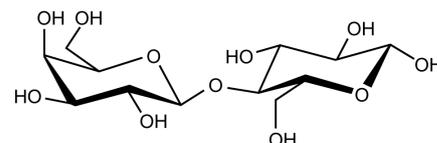
Maltose $C_{12}H_{22}O_{11}$



Glucose $C_6H_{12}O_6$



Sucrose $C_{12}H_{22}O_{11}$



Lactose $C_{12}H_{22}O_{11}$

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Test for starch

The starch content of a variety of biofuel feedstocks can be compared. This activity could be carried out at a science fair or similar event.

Age Range: This experiment is suitable for secondary and post-16 students.

Duration: 10-15 minutes.

Suggested prior knowledge: It is recommended that you elicit the existing student knowledge of properties of carbohydrates, chemical reactions and the carbon cycle.

What you will need

- Iodine solution
- Dropping pipette
- Spotting tile
- Plant material

Health and Safety

Ensure students are not allergic to any of the plant material being tested. Wash off any iodine that comes into contact with skin immediately.

CLEAPSS® laboratory handbook – section 20.3.1 Carbohydrate tests page 2006

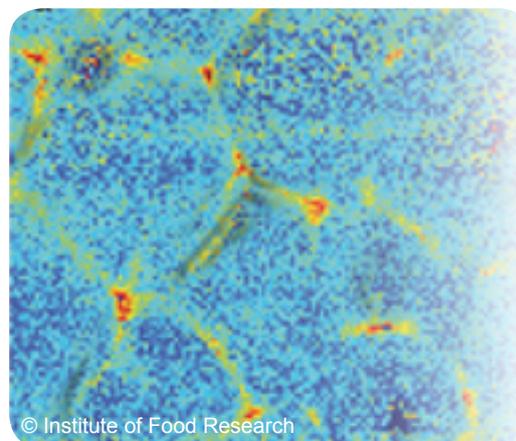
CLEAPSS® Recipe book RB50 (Iodine solution), RB93 (Stains for plant material).

CLEAPSS® Hazcards 40C (Carbohydrates), 54 (Iodine).

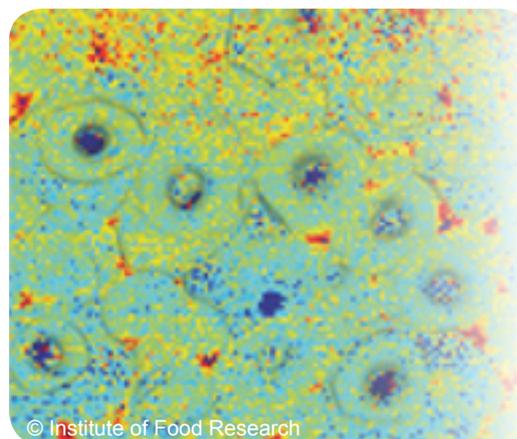
CLEAPSS® Model risk assessment 3.002 (Chemical testing of food).

Method

1. Grind or mash some of the plant material using a pestle and mortar.
2. Add a small amount of the plant material (about 1-2 g) to the spotting tile.
3. Add a drop of iodine solution.
4. A change in colour to blue/black indicates the presence of starch.



Starch branching in wild type and mutant maize kernels. Blue areas depict a normal ratio of linear amylose and branched amylopectin



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Test for reducing sugars including glucose

Reducing sugars include the monosaccharides as well as the disaccharides maltose and lactose. They are able to carry out the reduction of copper ions.

Benedict's reagent is a blue solution of copper sulphate containing copper(II) ions (Cu^{2+}), that produces an insoluble red-brown precipitate of copper(I) oxide on reaction with reducing sugars. The copper II ions are reduced to copper I ions by the aldehyde group that is formed on isomerisation of the cyclic to linear form of the sugar.

Non-reducing disaccharides such as sucrose can be broken down to monosaccharides by heating in an acidic solution. This hydrolysis reaction breaks the bond between the two sugars. The resulting monosaccharides will then be able to reduce the blue Benedict's reagent to produce a colour change.

Sucrose is the only common example of a non-reducing sugar, and starch is a poor reducing agent with only the end of the carbohydrate chains having aldehyde groups.

This activity requires a science laboratory.

An alternative method for testing for reducing sugars can be carried out with Fehling's solution. Fehling's solution does work faster but it is more corrosive and must be stored as two separate solutions and made up fresh.

Age Range: This experiment is suitable for GCSE and post-16 students.

Duration: 50-60 minutes.

Suggested prior knowledge: It is recommended that you elicit the existing student knowledge of properties of carbohydrates, chemical reactions and the carbon cycle.

What you will need

- Bunsen burner
- Heatproof mat
- Tripod
- Gauze
- Pestle and mortar
- Boiling tubes
- Boiling tube rack
- Glass beaker
- Graduated pipette or syringe
- Spatula
- Funnel
- Test tube holder
- Dropping pipette
- Benedict's solution (1.73 g of copper(II) sulfate pentahydrate, 10 g of anhydrous sodium carbonate and 17.3 g of sodium citrate made up to 100 ml)
- Selection of plant material or sugar beet extract from [activity 1E](#)
- Glucose solution
- Timer
- Eye protection

Optional

- A variety of known carbohydrate solutions including starch, fructose, sucrose, maltose and lactose

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Health and Safety

Eye protection must be worn. Ensure that a heatproof mat is used. Fill the beaker a third to half full with water to avoid boiling over and splashing hot water. Take care with hot liquids and glassware. Place the test tube in the water bath and remove it after heating using the test tube holder. Do not overheat the solution or heat the boiling tube directly with the Bunsen burner as this may lead to the ejection of hot liquids. Use Benedict's solution rather than Fehling's solution.

CLEAPSS® Laboratory handbook – section 20.3.1 Carbohydrate tests page 2006.

CLEAPSS® Recipe book, RB11 (Benedict's qualitative reagent), RB12 (Benedict's quantitative reagent).

CLEAPSS® Hazcards 27C (Copper salts), 40C (Carbohydrates), 95C (Sodium and Potassium salts).

CLEAPSS® Model risk assessment 3.002 (Chemical testing of food).

Method

1. Grind or mash some of the plant material using a pestle and mortar.
2. Add a small volume of distilled water (approx. 5 ml) and continue to grind the plant material for another couple of minutes.
3. Transfer approximately 5 g of the extract from the plant material into a boiling tube.
4. Carry out the following steps with a positive glucose control solution in addition to the test samples.
5. Add 10 drops or 3 ml of Benedict's reagent using a pipette or syringe.
6. Place the boiling tube into a beaker of warm water.
7. Heat the beaker until the water boils and maintain the heat for 8-10 minutes.
8. Carefully remove the boiling tube from the beaker using a test tube holder and transfer to a boiling tube rack.
9. The presence of glucose is indicated by a change in colour from blue to green, yellow, orange and brick-red colours depending on the amount of reducing sugar present.

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Extension activity: Testing glucose concentrations using Benedict's reagent

What you will need

- Bunsen burner
- Heatproof mat
- Gauze
- Tripod
- Boiling tubes
- Boiling tube rack
- Glass beaker
- Graduated pipette or syringe
- Spatula
- Test tube holder
- Dropping pipette
- Benedict's solution (1.73 g of copper(II) sulfate pentahydrate, 10 g of anhydrous sodium carbonate and 17.3 g of sodium citrate made up to 100 ml)
- 10% glucose solution
- Selection of solutions prepared for glucose testing (ensure the same volume of distilled water is added to each sample)
- Distilled water
- Timer
- Eye protection

Method

1. Make up a dilution series using the 10% glucose solution and the following dilutions
1 ml of 10% glucose + 9 ml of water = 1%
1 ml of 1% glucose + 9 ml of water = 0.1%
1 ml of 0.1% glucose + 9 ml of water = 0.01%
2. Test each dilution by adding ten drops of Benedict's reagent to 1 ml of the glucose solution.
3. Place the boiling tube into a beaker of warm water.
4. Heat the beaker until the water boils and maintain the heat for 8-10 minutes.
5. Carefully remove the boiling tube from the beaker using a test tube holder and transfer to a boiling tube rack.
6. The colour of the solutions from blue to green, yellow, orange and brick-red colours indicates the concentration of glucose present.

Repeat steps 2-5 with the test samples and compare with the colour of the standards to determine the concentration.

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Alternative Method: Testing glucose concentrations using potassium permanganate

This technique uses an acidified solution of potassium permanganate as the indicator. The purple pink solution of potassium permanganate (MnO_4^-) is reduced to a colourless solution of manganese ions (Mn^{2+}) by glucose.



The concentration of glucose can be determined by the time taken for the colour change. Using a standard solution of potassium permanganate and a set of standard glucose solutions, the rate of glucose oxidation can be calculated and compared to unknowns. The rate of reaction is directly related to the glucose concentration.

To ensure accurate results, concentrations and measurements need to be carefully made and clean glassware used.

See the Science and Plants for Schools (SAPS) Glucose concentration protocol: Estimating glucose concentration in solution. For further details: www.saps.org.uk/secondary/teaching-resources/103-estimating-glucose-concentration-in-solution

Age Range: This experiment is suitable for GCSE and post-16 students.

Duration: 50-60 minutes

Suggested prior knowledge: It is recommended that you elicit the existing student knowledge of properties of carbohydrates, chemical reactions and the carbon cycle.

What you will need

- Glass beakers
- Boiling tubes
- Boiling tube rack
- Graduated pipette or syringe
- Dropping pipette
- 1M sulfuric acid
- Potassium permanganate solution (0.4 g in 1 litre)
- 12% glucose solution
- Selection of solutions prepared for glucose testing (ensure the same volume of distilled water is added to each sample)
- Distilled water
- Timer
- Glass rod
- Eye protection
- Gloves

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Health and Safety

Eye protection must be worn and gloves are recommended. Potassium permanganate is harmful and oxidising, avoid contact with the skin. In the case of contact with skin wash off immediately. Potassium permanganate solution presents a low hazard but stains hands and clothing. Prepare the potassium permanganate and sulphuric acid solutions for the students and avoid contact of the solid with concentrated sulphuric acid.

CLEAPSS® Student Safety sheet 48, Recipe book RB73 and Hazcard 81 Potassium manganate(VII)

CLEAPSS® Hazcards 40C (Carbohydrates) and 98a (Sulfuric(VI) acid)

CLEAPSS® Laboratory handbook – section 20.3.1 Carbohydrate tests page 2006

CLEAPSS® Model risk assessment 3.002 (Chemical testing of food).

Method

1. Label the beakers and pipettes to avoid cross contamination of potassium permanganate, sulphuric acid and glucose.
2. Make up a series of glucose solutions (2%, 4%, 6%, 8%, 10%, and make up a 12% stock solution of glucose).
10 ml of 12% glucose + 2 ml of water = 10%
10 ml of 12% glucose + 5 ml of water = 8%
7.5 ml of 12% glucose + 7.5 ml of water = 6%
5 ml of 12% glucose + 10 ml of water = 4%
2.5 ml of 12% glucose + 12.5 ml of water = 2%
3. Make up a fresh stock indicator solution of equal volumes of sulphuric acid and potassium permanganate.
4. In the following order place 10 ml of the first glucose solution, 5 ml of sulphuric acid then 2 ml of potassium permanganate into the boiling tube.
5. Start the timer.
6. Stir with a stirring rod and stop the timer as soon as the pink colour disappears.
7. Record the time and the glucose solution used.
8. Rinse the pipette used for the glucose solution.
9. Repeat using the other glucose solutions of known concentration.
10. Plot a standard curve of glucose solution against time taken for complete colour change.
11. Repeat for a solution of unknown concentration and record the time.
12. Use the standard curve to estimate the concentration of the unknown solution.

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Testing for non-reducing sugars including sucrose

Age Range: This experiment is suitable for secondary and post-16 students.

Duration: 50-60 minutes.

Suggested prior knowledge: It is recommended that you elicit the existing student knowledge of properties of carbohydrates, chemical reactions and the carbon cycle.

What you will need

- Bunsen burner
- Heatproof mat
- Tripod
- Gauze
- Pestle and mortar
- Boiling tube
- Boiling tube rack
- Beaker
- Spatula
- Test tube holder
- Graduated pipette or syringe
- Dilute hydrochloric acid (1 mol per dm³)
- Sodium hydrogen carbonate solution
- Benedict's solution (1.73 g of copper(II) sulfate pentahydrate, 10 g of anhydrous sodium carbonate and 17.3 g of sodium citrate made up to 100 ml)
- Selection of plant material or sugar beet extract from [activity 1E](#)
- Glucose solution
- Sucrose solution
- Eye protection

Health and Safety

Eye protection must be worn. Ensure that a heatproof mat is used. Fill the beaker a third to half full with water to avoid boiling over and splashing hot water. Take care with hot liquids and glassware. Place the test tube in the water bath and remove it after heating using the test tube holder. Do not overheat the solution or heat the boiling tube directly with the Bunsen burner as this may lead to the ejection of hot liquids. Use Benedict's solution rather than Fehling's solution.

CLEAPSS® Student Safety sheet 48, Recipe book RB73 and Hazcard 81 Potassium manganate(VII).

CLEAPSS® Hazcards 40C (Carbohydrates) and 98a (Sulfuric(VI) acid).

CLEAPSS® Laboratory handbook – section 20.3.1 Carbohydrate tests page 2006.

CLEAPSS® Model risk assessment 3.002 (Chemical testing of food).

Current Biofuels

Method

1. Grind or mash some of the plant material using a pestle and mortar.
2. Add a small volume of distilled water (approx. 5 ml) and continue to grind the plant material for another couple of minutes.
3. Transfer the extract from the plant material into a boiling tube.
4. Carry out the following steps with a positive sucrose control solution and a negative glucose control solution in addition to the test samples.
5. Add about 2 ml of dilute hydrochloric acid using a pipette or syringe.
6. Place the boiling tube into a beaker of warm water.
7. Heat the beaker until the water boils and maintain the heat for 10 minutes.
8. Carefully remove the boiling tube from the beaker using a test tube holder and transfer to a boiling tube rack.
9. Allow the boiling tube to cool for 5 minutes before adding 2 ml of sodium hydrogen carbonate solution or slowly adding small amounts of solid sodium hydrogen carbonate until the fizzing stops.
10. Then follow steps 5–7 from the reducing sugar test.

Extension activity

How could you investigate whether a solution contains both sucrose *and* glucose?

Suppliers

Glucose, sucrose, as granulated sugar, and fructose can be obtained from most supermarkets. Maltose and lactose can be obtained from brewery suppliers.

Further reading and links

Benedict, S. R. (1 December 1908). [“A Reagent For the Detection of Reducing Sugars”](#). *J. Biol. Chem.* **5** (6): 485–487.

Goubet F., Barton C.J., Mortimer J.C., Yu X., Zhang Z., Miles G.P., Richens J., Liepman A.H., Seffen K., Dupree P., 2009. Cell wall glucomannan in Arabidopsis is synthesised by CSLA glycosyltransferases, and influences the progression of embryogenesis. *Plant J.* **60**(3), 527-38.

Mortimer J.C., Miles G.P., Brown D.M., Zhang Z., Segura M.P., Weimar T., Yu X., Seffen K.A., Stephens E., Turner S.R., Dupree P., 2010. Absence of branches from xylan in Arabidopsis gux mutants reveals potential for simplification of lignocellulosic biomass. *PNAS.* **107**(40), 17409-14.

Plant sugars provide clues to sustainable bioenergy production www.bbsrc.ac.uk/news/business-magazine/2010/winter/feature-sustainable-bioenergy-production.aspx

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Detecting starch in food, Practical chemistry www.practicalchemistry.org/experiments/detecting-starch-in-food.223.EX.html

Science and Plants for Schools (SAPS) Estimating glucose concentration in solution www.saps.org.uk/secondary/teaching-resources/103-estimating-glucose-concentration-in-solution

Novel imaging technique looks inside starch granules www.bbsrc.ac.uk/news/research-technologies/2011/110211-pr-novel-imaging-inside-starch-granules.aspx

The Royal Society, January 2008. *Sustainable biofuels: prospects and challenges*, ISBN 978 0 85403 662 2. <http://royalsociety.org/Sustainable-biofuels-prospects-and-challenges/>

Nuffield Council on Bioethics, April 2011, *Biofuels: ethical issues* www.nuffieldbioethics.org/biofuels-0

Research groups

Prof. Paul Dupree, BSBEC Cell Wall Sugars Programme, Department of Biochemistry, University of Cambridge www.bsbec.bbsrc.ac.uk/programmes/cell-wall-sugars.html www.bioenergy.cam.ac.uk