

WHY DOES THE COLOUR LEAK OUT OF COOKED BEETROOT?

Purpose

- To investigate the effect of temperature or alcohol concentration on membrane structure.
- To develop practical skills.

SAFETY

Wear eye protection and lab coats.

Take care using a cork borer, a knife and water baths at 60 and 70 °C.

Alcohol is highly flammable. Keep away from naked flames and ignition sources.



You need

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| <ul style="list-style-type: none"> • Raw beetroot • Size 4 cork borer • White tile • Knife • Ruler • Water baths at 0, 10, 20, 30, 40, 50, 60, 70 °C, or alcohol • Plastic beaker, about 250 cm³ • 8 boiling tubes | <ul style="list-style-type: none"> • 2 boiling tube racks • Crushed ice • Thermometers (one per water bath) • Colorimeter • Cuvettes • Stopclock • Distilled water • Pipettes for measuring 2 cm³ and 5 cm³ • Small measuring cylinders |
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If alcohol concentration is investigated several water baths and ice will not be required. Pipettes and alcohol will be needed instead.

Beetroot pigments

If you read a recipe for cooked beetroot it will usually recommend that you do not remove the outer skin of the beetroot and do not cut off all the stalk and root if you want to avoid getting lots of red dye in the cooking water. Beetroot contains red pigments called betalains, located within the cell vacuole. What happens to the membranes and pigments when beetroot is cooked or put in alcohol?

The aim of this practical is to use beetroot to examine the effect of temperature or alcohol concentration on cell membranes and relate the effects observed to membrane structure. To function correctly a cell needs to be able to control transport across the partially permeable cell membrane.

1 Scientific questions and information research

Before you start the experiment you should:

Research relevant information and state what you are going to investigate – decide what you think will be the effect of temperature or alcohol on beetroot cell surface membranes and how this will affect their permeability. Write down your idea as a hypothesis that you can test and support your idea with biological knowledge. To help you decide on what you are going to investigate and how you will carry out the practical work, you might need to research the background science and methods people have used to investigate similar problems.

2 Planning and experimental design

- a** Go through the procedure provided for the factor you are investigating and decide if:
- all the variables have been identified and, where possible, controlled or allowed for
 - the apparatus is suitable and will provide appropriate measurements that will allow you to test your hypothesis validly
 - the measurement will be precise and repeatable
 - there are likely to be any systematic or random errors
 - there are likely to be any safety issues and how you would minimise any risks
- b** Write up your decisions on each of the points above and describe any alterations to the procedure that may be needed and any detail that might need to be added.
- c** Write a risk assessment for the procedure including the safety precautions you will take.

Procedure to investigate the effect of temperature

- 1 Cut cylindrical samples from a single beetroot using a size 4 cork borer. Cut eight 1 cm length sections from these samples. Be careful not to spill beetroot juice on your skin or clothing as it will stain very badly.
- 2 Place the sections in a beaker of distilled water. Leave overnight to wash away excess dye.
- 3 Next day, place eight labelled boiling tubes, each containing 5 cm³ distilled water, into water baths at 0 °C, 10 °C, 20 °C, 30 °C, 40 °C, 50 °C, 60 °C and 70 °C. Leave for 5 minutes until the water reaches the required temperature. Place one of the beetroot sections into each of the boiling tubes. Leave for 30 minutes in the water baths.
- 4 Decant the liquid into a second boiling tube or remove beetroot sections using a technique that does not squeeze the slice. Shake the water/solution to disperse the dye.
- 5 Switch on the colorimeter and set it to read percentage absorbance.
- 6 Set the filter dial to the blue/green filter.
- 7 Using a pipette, accurately measure 2 cm³ distilled water into a cuvette. Place the cuvette into the colorimeter, making sure that the light is shining through the smooth sides.
- 8 Adjust the colorimeter to read 0 absorbance for clear water. Do not alter the setting again during the experiment.
- 9 Place 2 cm³ of the dye solution into a colorimeter cuvette and take a reading for absorbency. Repeat the readings for all the temperatures.

In ICT Support 3 there is a datalogging sheet on monitoring diffusion of pigment across beetroot cell membranes.

3 Carrying out practical work safely and ethically

Use your modified plan to carry out the practical work correctly and with appropriate safety precautions. If unexpected safety issues arise, deal with them sensibly, taking advice where needed, and make a note of them. Record all measurements, including repeated ones, as soon as they are taken, with appropriate precision (i.e. a suitable number of significant figures) and units. Note any possible errors.

4 Analysis and interpretation of data

- Present your results in an appropriate way.
- Identify any trends or patterns in your results.

5 Conclusion and evaluation

- Explain any trends or patterns, supporting your statements with evidence from your data, using biological knowledge.
- State a clear conclusion, summarising what you found out and comment on the validity of your conclusion.
- Evaluate your experimental apparatus and methods, commenting on the accuracy and precision of your results.
- Describe how you could have improved this experiment.

Procedure to investigate the effect of alcohol

- 1 Cut cylindrical samples from a single beetroot using a size 4 cork borer. Cut eight 1 cm length sections from these samples. Be careful not to spill beetroot juice on your skin or clothing as it will stain very badly.
- 2 Place the sections in a beaker of distilled water. Leave overnight to wash away excess dye.
- 3 Next day, place one of the beetroot sections into a boiling tube containing 5 cm³ distilled water. This is 0% alcohol concentration. Repeat with seven test tubes containing 10%, 20%, 30%, 40%, 50%, 60% and 70% alcohol. Leave boiling tubes for 30 minutes.
- 4 Decant the liquid into a second boiling tube or remove beetroot sections using a technique that does not squeeze the slice. Shake the water/solution to disperse the dye.
- 5 Switch on the colorimeter and set it to read percentage absorbance.
- 6 Set the filter dial to the blue/green filter.
- 7 Using a pipette accurately, measure 2 cm³ distilled water into a cuvette. Place the cuvette into the colorimeter, making sure that the light is shining through the smooth sides.
- 8 Adjust the colorimeter to read 0 absorbance for clear water. Do not alter the setting again during the experiment.
- 9 Place 2 cm³ of the dye solution into a colorimeter cuvette and take a reading for absorbency. Repeat the readings for all the alcohol concentrations.

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Notes on the procedure

The Planning and experimental design section of the activity sheet asks students to produce a hypothesis they can test. They then have to assess whether or not the procedure provided will validly test their hypothesis and produce appropriate results. They may wish to alter the procedure in the light of their thoughts. The procedure is reasonably comprehensive: the only thing that might require their consideration is whether electric water baths are available, and if not, how they will control temperature. The technique for removing the beetroot section from the boiling tube without squeezing needs additional detail: spearing with a pointed seeker or straining through a tea strainer are both suitable methods. The number of repeat measurements is not given in the procedure.

Students could work individually or in pairs. To save time, it might be a good idea to suggest that the number of temperatures or alcohol concentrations used is reduced and students combine results to provide repeats at each temperature or alcohol concentration. A class set of data can then be analysed.

Warn students that although beetroot juice is harmless, it will stain skin and clothes very badly.

- 1** Beetroot sections can be prepared for students in advance. For convenience, a bread slicer can be used to produce slices that are then cut into chips and then cubes. Beetroot must be raw, not cooked and pickled. If you cannot get beetroot that is not pickled, discs of red cabbage should work.
- 2** If the beetroot slices cannot be left overnight, wash beetroot and blot dry.
- 3** Students could share beakers and water baths.
- 4–9** A practical requirement is that students use appropriate instrumentation to record quantitative measurements: the colorimeter gives them this opportunity.

For help with presentation of data refer students to notes on tables and graphs in the Maths and Stats Support.

On completion of the practical investigation students could use the Developing Practical Skills Self-evaluation Sheet to reflect on what they have done in this practical. This can be found in the Practical Skills Support section.

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Take care using the bread slicer and cork borer.

Ensure water baths are calibrated to prevent overheating and thermometers inserted to provide readings.

Alcohol is highly flammable. Keep away from naked flames and ignition sources.



Requirements per student or group of students	Notes
Raw beetroot (enough to make eight 1 cm lengths with a cork borer)	Beetroot must be raw, not cooked. If beetroot is not available, discs of red cabbage can be used. Ten or more will be needed for each tube. The beetroot can be cut with a bread slicer to make even-sized slices, and the slices can then be cut into chips and then cubes. If it is not possible to have the beetroot slices soaking overnight, the students can cut them at the start of the lesson and wash them in distilled water before blotting dry and placing them in the water baths or alcohol.
Size 4 cork borer	The coring can be done in advance. This will save time and beetroot.
White tile	
Knife	
Ruler	
Seeker, plastic or blunt forceps, or a tea strainer	To remove the beetroot sections from the boiling tube without squeezing.
Plastic beaker about 250 cm ³	
8 or 16 boiling tubes	The additional eight boiling tubes are required if the solution is decanted from the beetroot. Alternatively, forceps or a seeker can be used to remove beetroot slices, but this is more difficult and likely to release additional dye from slices.
2 boiling tube racks	
Colorimeter	
Cuvettes	
Stopclock	
Distilled water	
Pipette for measuring 2 cm ³	
Small measuring cylinder or pipette	To measure 5 cm ³ .
Waterproof marking pens	For labelling boiling tubes.
If temperature investigated:	
Access to water baths at 0, 10, 20, 30, 40, 50, 60, 70 °C	Eight water baths will be required. These can be beakers with each group of students maintaining the temperature of one bath.
Crushed ice	Add salt to the ice to lower the temperature if needed.
Thermometer (one per water bath)	
If alcohol concentration investigated:	
Alcohol	
2 pipettes 1–5 cm ³	

Safety checked, but not trialled by CLEAPSS. Users may need to adapt the risk assessment information to local circumstances.

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This sheet may have been altered from the original.