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Core practical - Why does the colour leak out of cooked beetroot?

Investigating the effect of temperature on a beetroot cell membrane.

Introduction:

It is recommended to leave the outer skin on beetroot and to leave the stalk and root when cooking it because otherwise red dye will leak into the cooking water. This red dye comes from pigments present in the beetroot called betalains, which are located within the cell vacuole. Due to the temperature when cooking beetroot it causes the pigments to leak through the cell membranes when usually it wouldn't when not cooking.

The purpose of a cell membrane is to control the transport of substances moving into and out of a cell. The membrane is an extremely thin layer (8 to 10 nanometers (nm) thick, which is semi-permeable. It consists mostly of lipids and proteins. The lipids found in cell membranes belong to a class known as triglycerides; they are called this because they have one molecule of glycerol chemically linked to three molecules of fatty acids. The majority belong to one subgroup of triglycerides known as phospholipids.

In the cells of a beetroot plant, a substance called anthocyanin is contained within the cell membrane. If a cell is damaged in a beetroot plant and the membrane is broken, the anthocyanin 'bleeds' from the cells like a dye.

As we are experimenting with the effects of temperature on the membrane, we will place the samples of beetroot into a water baths of varying temperatures and measure the colour change in the water. Temperature is just one of the possible variables. The dependant variable in this experiment is colour change in water caused by anthocyanin leakage. This will be recorded using a colorimeter and the results can be expressed in units of parts per million (ppm), milligrams per liter (mg/l), grains per gallon (gpg) or other useful and appropriate scales like the percentage used.

Aim:

This experiment aims to determine what effect an increase in the surrounding temperature has on the beetroot cell membrane structure. The cell needs to be able to control transport across a semi-permeable membrane to function correctly.

Independent variable:

The independent variable is the temperature of the water the beetroot pieces will be tested in varying from 10°C to 70°C using thermostatically controlled water baths.

Dependent variable:

The dependent variable is the abundance of the water after trial in a specific water bath. This is calculated using a calorimeter which will measure how much of the beetroot 'dye' has leaked out from the cells of the beetroot.

Risk assessment:

This is a harmless experiment as fresh beetroot is being used and also distilled water so there are no chemicals involved. However protective eye ware and gloves must be worn and any products must not be consumed as any apparatus used in contact may not be sterile.

Hypothesis:

An increase in temperature will damage the cell membrane and cause the cytoplasm and other substances contained within the membrane to leak out. So the greater the temperature the more anthocyanin will leak out. I will observe the solutions over the time period to see the varied color changes which will help with testing the idea and also to prove this hypothesis visually.

Method:

Apparatus used include:

- a size 4 cork borer
- white tile
- knife
- ruler
- water baths
- 250cm^3 plastic beaker
- 2X boiling tube racks
- crushed ice
- 8X boiling tubes
- thermometer (one for each water bath)
- colorimeter
- curvettes
- stop clock
- distilled water
- Pipette for measuring 2cm^3

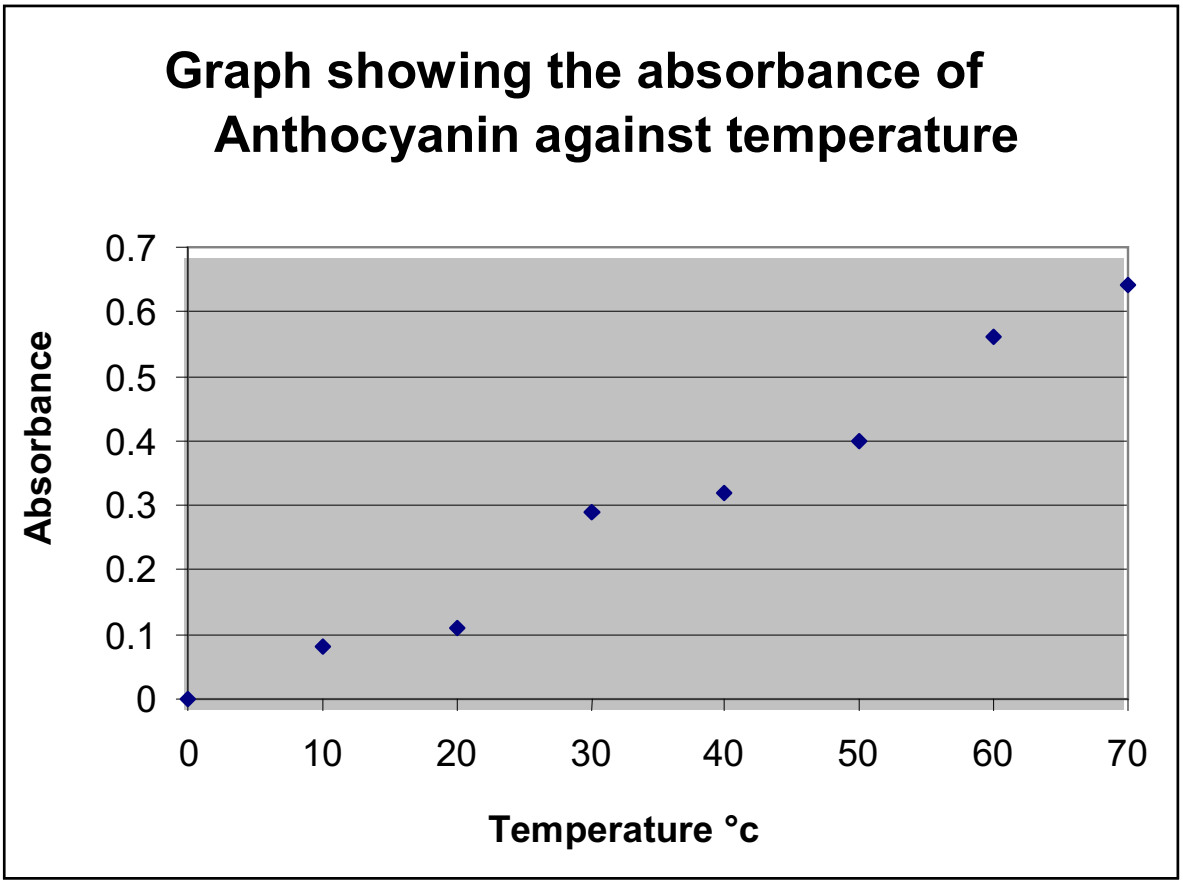
Some of the apparatus are used for more suitable circumstances like the colorimeter because it is more suitable than having just a visual judgement of the color differences. The colorimeter uses a method to measure the concentration/intensity of the color that develops when a specific reagent is introduced to a solution containing a parameter which is the substance or chemical quantity that is to be measured. The tests evaluate how intense the color becomes and so the greater the intensity of color, the higher the parameter concentration. Visually comparing intensity and color concentration can be highly inaccurate because of individuals' abilities to identify color and see variations in the color. Also, having thermostatically controlled water baths would have been to a much greater advantage than using manually controlled beaker water baths as they are more accurate. This is because manually controlling the temperature of water and altering it to a certain degree is very difficult. If the temperature raised too high ice cubes were added to reduce the temperature, which reduced it too much so a flame was needed to heat it up a bit more. This wasn't really sufficient enough and so the electronically controlled water baths are a lot better.
Firstly a beaker of distilled water is needed and 8 x 1cm3 of beetroot pieces cut using a size 4 cork borer. These pieces must now be left overnight so that any excess dye is washed away.
Now fill 8 boiling tubes with 5cm3 of distilled water and place them into water baths of temperatures 0°C, 10°C, 20°C, 30°C, 40°C, 50°C, 60°C and 70°C. Make sure that each

tube is labeled with the correct temperature. Leave these for 5mins and then add one beetroot piece per tube and again leave for 30mins.
After the time period, remove all beetroot pieces from the tubes and shake each solution. The fluid in each of the test tubes will be analysed using a colorimeter and compared against the control, which is distilled water to check for any variations in the colour of the water.
The colorimeter is now needed so must be set to read 0% absorbance with a green 550 filter. Measure 2cm3 of distilled water into a curvette and also measure 2cm3 of each solution of temperatures into separate curvette's.(be very careful not to mix up solutions). Adjust the colorimeter to read 0 for clear water and then take an absorbance reading for one beetroot solution and then set the colorimeter with the same clear water again. Repeat this until all solutions have an individual reading.

Results:

Table showing how readings from colorimeter vary with temperature.

Temperature °c	Absorbance %
0	0.00
10	0.08
20	0.11
30	0.29
40	0.32
50	0.40
60	0.56
70	0.64



Conclusions:

After collecting and correlating the results, I have come to the conclusion that the experimental hypothesis is correct in that an increase in temperature will damage the cell membrane and cause the cytoplasm and other substances contained within the membrane to leak out. This has been shown by a steady increase in anthocyanin leaked out of plant cells as the temperature increases.

Evaluation:

The results that were collected follow the same pattern as results collected by similar studies carried out within our class, so therefore it is safe to say that the results can be repeated reliably. However, there was just one result that was a slight inaccuracy in comparison to the rest. It was 0.29 the result for 30°C which was a considerable jump to the 0.11 before. This odd result indicates that the temperature of the water was not kept constantly the same so this made the result differ to the rest. This reason exactly showing the need of all thermostatically controlled water baths so all results can be accurate and valid to each other.

Many improvements can be made to make the experiment better and more accurate. It would have been beneficial to have repeated the experiment more times to make certain that the results were reliable and not gained through chance. The control experiment used was highly accurate, using distilled water, which is the clearest possible liquid, meant that even the slightest deviation in colour could be detected by the colorimeter.

Controlling the variables in the experiment is not an easy task. The first problem is the size of the beetroot piece. The pieces could have a different surface area to one another. This obviously alters the effect of the experiment. The other difficult variable to maintain was the temperature of the heated water. With only basic equipment, keeping the water at the correct temperature was made a complicated task. If the experiment was to be repeated, the use of a proper controlled water baths may be a consideration, and also a template made for cutting the beetroot pieces.

Using beetroot as the sample is not the only representation of cell membrane groups. Other cell membranes may have better or worse heat tolerance, some may not be affected at all, however, using a beetroot does give a good representation of the theories behind the cell membrane and how it behaves.