Effect of Temperature on Beetroot Membrane Proteins

Planning

A – Independent Variable

The independent variable in this experiment will be the temperature. I will control this factor using thermostatically controlled water baths, and a thermometer. The temperatures at which the beetroot cubes will be tested will be 0° (ice), 20°C (room temperature), 40°C, 60°C and 80°C.

B – Dependent Variable

The dependant variable is the result – this experiment will initially yield qualitative results. This is not desirable as it is only approximate and as it is not viable to use it to plot graphs of the results. Therefore, the results will be converted from qualitative to quantitative using a colorimeter, which will measure the percentage light transmission through the different temperatures after the beetroot has been left in the boiling tubes for 5 minutes. A blue filter will be used to measure the redness of the solutions, as it is a complimentary colour.

The experiment will be repeated 3 times. This will make it possible to clearly identify errors or anomalous results by comparison, and to understand whether or not a mistake was made when carrying out the experiment. From the results of the original and repeat experiments, an average can be calculated for the percentage transmission of each temperature, giving more accurate results overall and providing values with which a graph can be plotted.

Controlled Variables

The surface area and mass of the beetroot cylinders may also affect the rate at which red pigment leaks out. This means that the cylinders must be as similar as possible in size and shape. They will all be as close as possible to 2.5cm long and will be extracted using the same cork borer.

Any pigment that is on the surface of the beetroot before it is put into the water will affect the results. To avoid this, the beetroot cylinders should all be rinsed and lightly blotted on tissue paper

During the experiment, the lids on the water baths will be removed and replaced. This may have an effect on the actual temperature inside them, and could mean a deviation from the originally planned temperatures. To get one of the boiling tubes to a temperature of 0°C I will be using a basin containing a test tube rack in which the boiling tubes will be placed and ice beneath which the water and beetroot will be submerged. Due to the fact that the ice will melt in the classroom, the actual temperature will rise very slowly. Similarly the boiling tube at room temperature will be placed on a test tube rack inside a basin filled with water at 20°C, although the temperature could move towards the actual temperature of the room.

D Collection and Presentation of Raw Data

Temperature/°C	% Transmission										
	1	2	3	4	5	6	7	8	9	10	Average
3.5	90	99	98	90	88	97	94	94	93	96	93.9
19	86	93	87	88	87	97	90	84	88	93	89.3
40	86	90	84	84	87	85	88	83	75	92	85.4
60	73	80	69	43	58	66	79	74	69	81	69.2
79.9	49	37	36	26	29	32	58	36	20	32	32

G Conclusion

The graph shows an imperfect negative correlation between temperature and percentage transmission. The higher the temperature of the water in the boiling tube surrounding the beetroot sample, the more pigment leaks out, resulting in a lower percentage transmission.

This trend may be a result of damage to the cell membranes of the beetroot. Denaturation of the carrier proteins within the phospholipid bi-layer would be a result of high temperatures and would allow substances (red pigment) to leak from inside the cell. Damage could also have been done to the phospholipids due to high temperatures, allowing pigment to escape into the water. This is not as likely as it would be had the beetroot been heated in ethanol, in which phospholipids are soluble.

At lower temperatures (3.5°C – 40°C), less damage is done to components of the fluid-mosaic model. This is shown by smaller amounts of pigment having entered the water, allowing more transmission of light through the solution. At 40°C to 60°C however, the graph shows a larger decrease in percentage transmission. There is more pigment in the water at these temperatures. This could be because a temperature higher than the optimum has denatured some of the proteins in the cell membranes of the beetroot. Over temperatures 60°C – 80°C the graph shows more of a decrease in percentage transmission. This indicates that most of the proteins in the cell membrane were destroyed at these temperatures, causing the most pigment to leak out. The graph implies that as the temperature rises, more pigment can leak out of the beetroot cell, and the lower the percentage transmission will be.

As the temperature increases, the water expands – this will have a disruptive effect on the membrane. Heat energy in the water is converted into kinetic energy in the beetroot. This would cause red pigment molecules to vibrate faster, and may have had the same effect on the phospholipids in the partially permeable cell membrane. As phospholipids can move freely within the ‘fluid mosaic’, heat caused them to move around faster, making the membrane more permeable and allowing more red betalain pigment out. Denaturation of proteins within the membrane forms holes in the

membrane, which destroys its delicate structure. With carrier proteins gone, more substances within the cell can spill out.

H Evaluation

The temperature of the water baths was inconstant and varied. An exact temperature could not be maintained due to the removal and replacement of the lids. While the cylinders of beetroot were cut as accurately as possible, some ends may have been slanted and they would therefore not have been identical in size.

The beetroot samples were pushed out of the cork borer using the rubber end of a pencil. This may have destroyed some of the cell membranes at one end of the cylinder, and it is possible that the plasmolysed cells will release red pigment faster than a healthy cell would, or could be obstacles for the red pigment being released from cells beneath then.

Due to the fluctuation in temperature in the water baths, one way to come to a more informed conclusion may be to widen the range of values tested – the water and beetroot could also have been boiled at 100°C for five minutes. This would help to either confirm or possibly disprove the theory that the percentage transmission decreases as the temperature increases. It is possible that diffusion of red pigment across the partially permeable membrane will stop at a higher temperature – perhaps if there came to be a higher concentration of the pigment in the surrounding water than in the cells or outer cells of the beetroot. There were other inaccuracies in temperature – the ice in the basin did melt during the experiment, giving only enough information in the outcome to speculate at what may have happened had the water been at freezing point.

Samples were taken from different parts of the beetroot, due to the assumption that the beetroot was the same the whole way through. It is possible that there was more red pigment in a certain part, or that cells in one part were damaged more easily by high temperatures than those in another. The outer cells may be more resilient in order to prevent damage to the main part of the beetroot.

The boiling tubes were stirred using a stirring rod when they were removed from the water baths, but there could still have been some more concentrated red pigment left at the bottom of some the tubes when the solution was being poured into the cuvettes.

The boiling tubes at lower temperatures also had more time than the higher as they were put in first. The time taken to remove lids of water baths, place boiling tubes into the test tube racks and replace the lids again was approximately 20 seconds, and timing was started once the last boiling tube was in place. The extra 20 seconds may have altered the results for the Ice (3.5°C) and Room (19°C) temperature results.The water levels in the water baths varied – meaning that some of the water in the test tubes may not have been fully submerged.