The Effect of Temperature on Membrane Permeability in Beetroot Cells.

Abstract

The cell membrane is a semi-permeable membrane with a phospholipid bilayer embedded with proteins. Small molecules and ions are able to pass through the membrane. At high temperatures, proteins within the membrane can become denatured. Beetroot contains pigments within the cell that when heated are released, causing the red colour. Discs of beetroot tissue were tested in this experiment. It was hypothesised that the higher the temperature of the water, the greater the amount of pigment released into the water. Discs of beetroot tissue were left in different temperatures of water and the solution after a certain amount of time was tested in a spectrophotometer. The solution from the higher temperature had a significantly higher absorbance than the other temperatures. The first three temperatures had a slightly higher absorbance than the fourth temperature. The results for the first three temperatures was unexpected but could be explained by factors such as the temperature of the water and the length of time the beetroot remained in the solution.

Introduction

Beetroot contains two classes of plant pigments called betacyanin and betaxanthin. These pigments cause the red colour of beetroot and are produced and stored within the cell. The cell membrane has a mosaic of protein molecules embedded and attached to it (Mystrica, 2012). Proteins within the membrane of the beetroot cell can be denatured by high temperatures. Vibrations in the phospholipids of the cell membrane are increased by heat energy so when heat is applied to the cell, the membrane is destabilised and the coloured pigments are released. The coloured pigment is released by phospholipids breaking down to produce holes in the membrane (123HelpMe.com, 2015).

The relationship between temperature and pigment release is the aim of the investigation. The amount of pigment released can be inferred using a spectrophotometer and looking at the absorbance. The higher the absorbance, the greater the amount of pigment released into the water. The hypothesis for the investigation is the higher the temperature of the water, the higher the absorbance.

The independent variable for the investigation is the temperature of the water. The independent variable was changed by heating up the water at different temperatures. The dependent variable was the absorbance. This was measured using a spectrophotometer.

There were some control variables in the experiment. The size of the beetroot discs needed to be kept the same, 3mm. The amount of beetroot discs on each stack needed to be kept the same. The amount of distilled water in each test tube, 10mL, needed to be kept the same. The beetroot stacks needed to stay in the water for the same amount of time. The amount of solution put into the cuvette also needed to be kept the same, 3mL.

Method

1. A cork borer was used to cut cylinders of fresh beetroot tissue. The cylinders of beetroot were taken from a similar area of tissue to minimise any errors. The cylinders were then placed on a tile and cut into 30 discs, 3mm thick.
2. Free red pigments were removed by placing all the discs in a small beaker and washed with distilled water for three minutes by agitating the beaker. The water was then drained and the step repeated a further two times, a total of 9 minutes, until the washing solution ran clear.
3. Five of the beetroot discs were taken and impaled onto a mounting needle. Even spaces were left between each disc of beetroot as shown by the image to the right. This step was repeated five more times to obtain a total of 6 beetroot stacks.
4. Six test tubes were then collected and each labelled with a different temperature (30°C, 40°C, 50°C, 60°C, 70°C, and 80°C). 10mL of distilled water was added to each of the labelled test tubes.
5. Six water baths were set up using a large beaker filled with water on a hot plate. The water was left until it reached the desired temperature. The temperature of the water was controlled using a thermometer. The labelled test tubes were placed in the different water baths of their labelled temperature and left until the distilled water reached the desired temperature. Once the test tube reached the desired temperature, it was removed from the water bath and placed in a test tube rack.
6. The temperature of the water was recorded and the beetroot stack was immediately immersed in the water for one minute. After the one minute, the test tube was taken, with the beetroot stacks still in them, and placed in a room temperature water bath for 30 minutes. This was repeated for each temperature.
7. After 30 minutes, the test tubes were taken out of the water bath and the beetroot stacks removed.
8. 3mL of the solution from each of the test tubes were transferred, using a plastic pipette, into a different spectrophotometer cuvette for each of the labelled test tubes.
9. A second spectrophotometer cuvette was filled with distilled water. The second cuvette was used to calibrate the spectrophotometer and ‘zero’ it at 530nm.
10. The first cuvette was then placed in the spectrophotometer and the absorbance was read and recorded in the table. This step was repeated for each cuvette.

**Results**

**Table 1- The Effect of Temperature on Membrane Permeability in Beetroot Cells.**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>0.630</td>
</tr>
<tr>
<td>37</td>
<td>0.581</td>
</tr>
<tr>
<td>46</td>
<td>0.535</td>
</tr>
<tr>
<td>56</td>
<td>0.556</td>
</tr>
<tr>
<td>65</td>
<td>0.728</td>
</tr>
<tr>
<td>76</td>
<td>1.120</td>
</tr>
</tbody>
</table>

**Graph 1**

The Effect of Temperature on Absorbance.
The absorbance decreases slightly before increasing dramatically from 0.556 to 1.120. The overall trend of the graph is that as the temperature increases, the absorbance also increases. The first three temperatures recorded on the graph do not follow this pattern. This may be because an error may have occurred when testing these temperatures.

**Discussion**

**Interpretation**

The absorbance increased as the temperature increased because as more heat is applied to the cells, the more the membranes are destabilised. Therefore, the more coloured pigments that are released hence the higher absorbance.

**Analysis**

Within the experiment, there were many possible sources of random error. When measuring the beetroot tissue to cut it into discs, it was possible for errors of parallax to occur. This error is random as the measurement may have been slightly higher or lower than the actual value because of the angle at which the measurement was read from the ruler.

When the distilled water was measured, a random error may have occurred because of the angle at which the measurement was read from the measuring cylinder. The same thing may have happened when the temperature was read from the thermometer. An improvement to this would be to use measuring equipment with a higher resolution.

Another possible source of random error may be the reaction time of the experimenter starting and stopping the stopwatch. The error is random as by the time the experimenter started and stopped the stopwatch, the beetroot may have been left in the water for longer than the specified time. However, a stopwatch is a very accurate measuring implement to use.

When the spectrophotometer was used, it first had to be calibrated. If the experimenter hadn’t calibrated it correctly or hadn’t calibrated it at all then a systematic error may have occurred. This would be a systematic error as the error would have effected each result in the same way. To ensure the spectrophotometer was calibrated, the task could be designated to one of the experimenters.

The results from the experiment are not very precise. The first three results fluctuate from the expected result. If the experiment were to be done again the results may not be the same because of factors like the temperature of the water and the beetroot tissue used.

**Evaluation**

The method for the experiment enables valid results to be collect even though some of the results do not follow the expected trend. This may have been because of some errors within the method.

The beetroot discs had to be 3mm in size. There may have been discs that were slightly larger or slightly smaller than 3mm. This difference in size may have impacted the results at there was less pigments to leak out of the cell affecting the accuracy of the results. A way to improve this would be to use a caliper to accurately measure the beetroot discs and to use a knife with a straight edge rather than a curved edge to cut the discs.

When the experiment was taking place, it was noted that not all 5 discs of beetroot were in the distilled water in a couple of the test tubes. This may have impacted the results as not all of the tests had the same amount of beetroot leaking pigments into the solution. This may mean that some of the results are less accurate as they have not been tested under the same conditions as the other results. A way to improve this would be to measure, using a ruler, how high the water fills the test tube, and to then only skewer the discs onto the mounting needle at that height and not any higher.

After the test tubes have been in the room temperature water bath, the beetroot needed to be removed from the test tube. However in our experiment, this was only done after the first solution had been tested in the spectrophotometer. This would have impacted the results as the other solutions had more time for beetroot
to leak pigments into it. This could be why our first result doesn’t follow the expected trend. A way to improve this would be to read the instructions more carefully and designate someone to remove the beetroot after the 30 minutes is up.

The temperature of the water in the test tube changed dramatically after it was taken out of the water bath. Whilst the temperature of the test tube was recorded straight away before the beetroot was emerged in it, it still wasn’t the desired temperature ±3°C. These temperatures may not have been hot enough for the cell membrane to be disturbed to its full potential. This may impact the results as the results may not be as accurate as they could be. An improvement to this could be to leave the test tube in the water bath for longer, constantly keeping it at the correct temperature, or by completing the experiment right next to the water bath rather than taking it back to the work bench. That way the test tube should stay at the correct temperature for longer.

There were only six temperatures involved in the experiment. This is not a very large sample size. If the sample size was increased and more temperatures were tested, then the results may be more accurate and reliable. Only one repeat of the experiment was done. If more repeats of the experiment were done then it would increase the reliability of the results and reduce the number of random errors.

The experiment was repeated by other students and a similar trend was concluded. This verifies the results in Table 1. However, the first three results that were unexpected may have been caused by errors that didn’t effect the other student’s results.

The validity of the experiment is good as it measures what wanted to be measured. However, the reliability is not very good as if the experiment were to be repeated then the same results would not be gathered because of errors that occurred within the experiment.

**Conclusion**

In conclusion, the results support the hypothesis that states, the higher the temperature of the water, the higher the absorbance.

**Reference List**
