Lloyd James (13.3)
16th March 2001
Biology A-Level Coursework
Planning Experimental Procedures

Design an Experiment to show that an increase in temperature influences the release of red pigment from beetroot

1. Design an experiment
2. Analyse
3. Produce a hypothesis that would enable a cell biologist to account for the result

Equipment needed:

Water tank, Test tubes, Test tube rack, Cork Borer, Beetroot, Colorimeter, Stop Clock, Scalpel, Forceps, Tile, Spatula, Thermometer, Ruler, Sharp Knife

Investigating the effects of temperature on the release of red pigment from beetroot, will consequently distinguish the effects that temperature has on the permeability of a cells membrane. The cells inside beetroot tissue contain in their vacuoles a water-soluble and a radiant red pigment which is an anthocyanin derivative, surrounded by a specialised vacuole membrane called a tonoplast. This red pigment can be detected easily by the eye and its concentration measured using a colorimeter (Clegg and Mackean, 1995). Using this acquired knowledge, and my sound basic understanding of a cell and membrane structure (learnt in Module 1 of my A–Level syllabus), I can plan a detailed investigation to distinguish the exact effect that an increase in temperature has on the release of red pigment from beetroot.

To start with in this investigation the beetroot will be cut into cylinders 4cm long and 1cm in diameter, by using a cork borer and then a ruler to firstly measure 4cm along the cylinder of beetroot, and then a sharp knife to cut the cylinders at this length. The beetroot will then be washed with distilled water and allowed to dry.

I will then align three test tubes in a test tube rack, with each one containing 25ml's of distilled water, ready before each of my experiments. When the desired water temperature in the water bath has been reached, at exactly the same time as the rack containing the tubes enter the water bath, I will get a partner to insert the pieces of beetroot into the test tubes. Then after exactly five minutes the rack and test tubes will be removed and the beetroot must then be immediately extracted using the forceps. Once the pieces of beetroot have been extracted from the test tubes, I will then stir the solution with twenty rotational movements using a spatula. Only then can the solution be tested using the colorimeter.

The colorimeter measures the absorbency of the solution, with the higher the absorbency, the higher the concentration of the red pigment that it contains released from the beetroot. The type of the filter used for this determination, which will be number 550 in this experiment, is complementary to the pigment colour. The readings taken from the colorimeter used in this investigation will provide me with qualitative data. The data acquired will them enable me to draw up graphs plotting temperature against the absorbency of the red pigment solution produced by the beetroot.

The independent variable in this experiment will be the temperature. It will be changed at intervals of 5 degrees Celsius, starting at 25 degrees Celsius and ending at 80 degrees Celsius. Three test tubes will be monitored at each five-degree interval with the beetroot inside. This will consequently give three sets of results, which can then be averaged at the end to hopefully eradicate any anomalies and give optimum accuracy.

Inhibiting the fluctuation of variables in this investigation is paramount if I am to obtain accurate and fair results, to allow me to make valid conclusions. The time to elapse with the cylinders of beetroot in the distilled water, prior to being extracted, must be kept constant. Too much or too little time to elapse with the beetroot inside the distilled water, may result in too much or too little of the red pigment being allowed to flow out into the distilled water, hence giving inaccurate results. I will also need to keep the amount of distilled water in the test tube constant by measuring out 25ml into each one, using a measuring cylinder. Too much or too little distilled water in a test tube, would hence result in a more dilute or more concentrated solution of red pigment acquired in the distilled water. It is also essential that I use distilled water and not normal tap water inside the test tubes. Using a spatula I will also conduct a constant 20 stirs in each test tube. This will be carried out after the beetroot has been extracted from the distilled water, to remove any concentration gradients that may be present in the solution (i.e. mix in the denser red pigment that falls to the bottom of the test tube with the rest of the solution).

The reason for washing the cut pieces of beetroot is to remove any of the excess red pigment located on the outside of the cylinder, caused by the knife breaching the tonoplasts and hence releasing pigment from the vacuoles inside the cells. If the beetroot were not washed, then this excess pigment would immediately be washed straight into the distilled water, leading to inaccurate results. Also, the advantage of using the water bath is that once the desired temperature is acquired, then this can be maintained for as long as is needed for the experiment to be carried out.

My prediction in this investigation is that an increase in temperature will cause a slow increase in the red pigment released by the beetroot, due to the kinetic theory. The increase in temperature will cause an increase in the movement of molecules leaving the cells via diffusion, from a higher red pigment solution inside the cells vacuoles, to a lower concentration of red pigment surrounding the beetroot cells in the distilled water. This diffusion will take place through both the membrane of the vacuole called the tonoplast, and the membrane of the beetroot cell itself. Then at high temperatures this rate will increase even further, due to the denaturation of proteins in the cell membrane and tonoplast, leading to the breaching of these membranes. This breaching will allow the red pigment to move out more rapidly from the vacuoles inside the beetroot cells, out into the surrounding distilled water.

Conclusion

The one major anomaly on the graph is at 35 degrees celcius. Apart from this, most the other points seem to fit to the trend. Between 25 and 50 degrees celcius there is a slow increase in absorbance, but between 50 and 70 degres celcius there is a far more rapid rise. However, between 70 and 80 degrees this increase does seem to stop.

An increase in temperature between 25 and 50 degrees celcius shown on the graph, undoubtedly causes a slow increase in absorbance of the solution produced. This slow increase in absorbance hence exemplifies the slow rise in the amount of red pigment released by the beetroot into the surrounding distilled water in the test tube. The reason for this slow increase in release of red pigment, as the temperature of the water in the water bath surrounding the test tube is increased, can be explained by the kinetic theory. The increase in temperature causes an increase in the movement of molecules inside the cell. This causes a significant rise in the number of molecules leaving the cells in the beetroot via diffusion. Hence, there is a movement from a higher red pigment solution inside the cells, to a lower concentration of red pigment surrounding the cells, down the concentration gradient. This diffusion of red pigment molecules from the cells in the beetroot, takes place through the partially permeable tonoplast membrane of the vacuole, and also the cell membranes of the beetroot cells.

However, it is visible from the graph that an increase in temperature above 50 degrees, triggers a much higher absorbance of the solution produced in the test tube, hence meaning a more rapid release of the red pigment from the beetroot. This temperature reached, whereby a more rapid release of red pigment is triggered, can be explained. The cells inside beetroot tissue contain in their vacuoles the water soluble, radiant red pigment, which is an anthocyanin derivative. High temperatures damage the tonoplast membranes of the vacuoles and also the cell membranes. This is caused by an increase in the kinetic energy of the molecules that surround it, breaking down the membrane proteins both in the tonoplast and surrounding cell membrane. Above this temperature the kinetic energy of the molecules in the cells and their membranes is at such a fast vibrant rate, that this causes proteins that the cell membrane and tonoplast contains to become denatured. It is the effect of heat on membrane proteins, as with most proteins, to denature them irreversibly (meaning they cannot be restored back to their original shape). Therefore, at the 55-degree celcius temperature mark, it can be stated that the beetroot cells I was investigating had reached their 'thermal death point.' The breaking down of the proteins causes the cell membrane and tonoplast to be breached, allowing the red pigment to escape from the cells. This consequently allows even more red pigment to pass via diffusion from the higher concentration of pigment inside the cell, to the lower concentration of red pigment outside the cell in the distilled water, through these breached membranes.

However, it is also visible on the graph how at 80 degrees the rate of increase in absorbance and therefore concentration of the red pigment released from the beetroot, actually stops increasing. The explanation for this is that the concentration gradient of the red pigment from the inside of the cells in the beetroot, compared to the distilled water outside containing red pigment, has been dramatically lowered. This gradient is eventually cancelled out, with the same amount of red pigment in the cell, compared to the outside of the cell. The reprucusions of this, is a stop in increase of the red pigment released from the beetroot at these high temperatures, hence meaning that the amount of red pigment released from the beetroot and therefore absorbance of the solution stops increasing. Hence the absorbance will stay at this constant rate, and will not rise or fall at all even if the temperature is changed. This is because no further pigment can pass from the inside of the beetroot cells to the distilled water outside, due to their no longer being a concentration gradient, hence meaning diffusion can no longer operate.

Therefore, from the results that I have obtained I can make some extremely interesting, valid conclusions. Firstly I can conclude that at low temperatures, between 25 and 50 degrees celcius, that an increase in temperature does undoubtedly cause a slow increase in the amount of red pigment released from beetroot. A further increase in temperature between 50 and 70 degrees celcius, causes an even more rapid increase in the release of red pigment from the beetroot. However, over 70 degrees celcius, an increase in temperature has no further effect on the release of the red pigment from the beetroot.

Evaluation

On the whole I am delighted with the data obtained in this investigation, as the graph and line of best fit do undoubtedly portray an excellent trend, despite their being a few anomalies. The fact that I conducted three experiments at each individual temperature, and then took an average from these to plot the graph, means that some anomalies visible in my table of results are actually masked on the graph. The only major anomaly on the graph is the average absorbance plotted at 35 degrees celcius. Apart from this most the other points seem to fit to the trend. Also at 50 degrees celcius there is no increase in average absorbance at all from 45 degrees celcius, which certainly does not seem to fit totally with the trend that increases. Apart from this, most the other points seem to fit to the trend. However, a number of anomalies are visible in the table of results that I have produced. There are hugely contrasting values obtained from the results at 55 degrees celcius, where test tube 1 had a reading of 0.85A and test tube 2 had a reading of only 0.29A. Also as 50 degrees celcius the reading from test tube 2 of 0.33A, contrasts considerably with the results from test tubes 1 and 3, which were both 0.59A and 0.57A respectively.

There are some main sources of experimental error, which may account for some of the anomalies that were acquired in the results of this experiment. One main source of experimental error, which I did not mention at all in my plan, is the essential need for gentle use of the forceps when extracting the beetroot from the distilled water. Rough and hard grabbing of the beetroot inside the distilled water when it is time to be extracted, could automatically pierce cell membranes and the tonoplast membranes surrounding the vacuoles containing the red pigment, hence causing the red pigment to be instantaneously squeezed out into the distilled water. The reprucusions of this experimental error would be far more red pigment entering the distilled water than should do, hence leading to a far higher absorbance reading on the colorimeter and therefore totally inaccurate results. The consequences of this particular source of error are most likely to result in more red pigment entering the distilled water, hence meaning a higher absorbance recorded. This could therefore account for the high anomaly of 0.85A at 55 degrees celcius where the other two values from the other two test tubes, were only 0.29A and 0.47A.

Also, another main source of experimental error is the fluctuation of time to elapse with the cylinders of beetroot in the distilled water, prior to being totally extracted. Occasionally the time it took to withdraw the beetroot from the test tubes, immediately after they had been taken out of the test tube rack, would differ due to the beetroot being slightly difficult to grab hold of with the forceps. This small fluctuation in time could certainly have affected the results slightly. If it took slightly longer to withdraw the beetroot then this would result in more red pigment being allowed to enter the distilled water, and less time inevitably having the opposite effect of their being less pigment.

There was one particular source of experimental error, which I found in the middle of conducting my investigation. Because the investigation was carried out over two separate lessons, in the second lesson I started off using a different colorimeter to the one we had used previously. The immediate results I got when testing to see that it gave similar to results to the other colorimeter were staggering. The values obtained were quite different. Therefore, the fact that I changed over the colorimeter half way through the experiment, could justify some of the anomalies that I acquired when filling in the results for temperatures that I had missed out previously.

The other major source of experimental error, which did not occur to me in the plan, was the decision to use 25ml's of distilled water in each test tube. The problem with using this large amount of distilled water, was that to use my limited time more efficiently I used different water baths around the room, due to many of them being at different required temperatures.

These water baths had varying water levels due to other groups conducting the investigation adding and removing water to speed up the rate that the baths reached their desired temperatures. Therefore by using such a lot of distilled water in the test tubes in some shallow water baths, only a small part of the distilled water at the bottom of the test tube was submerged by the hot water. This means that the distilled water at the top is surrounded by only room temperature making it more susceptible to heat loss. Contrary to this, in deeper water baths all of the distilled water was surrounded by water, meaning it is heated up much more. Therefore, this easy error in experiment to make would have resulted in slightly fluctuating temperatures, which hence effects the amount of the red pigment released and therefore the results obtained. This major error in experiment is undoubtedly a very likely cause of the anomalous results. This point is exemplified even further, due to the fact that quite a few results were far too low in absorbance. A prime example of this is visible on the graph at 35 degrees Celsius, where there was a huge average decrease in the absorbance of the solution produced, compared to the results at 25 and 30 degrees celcius. This may mean that the temperatures were not high enough in the distilled water, hence possibly caused by the distilled water in the test tubes not being covered totally by the surrounding warm water in the water bath.

Therefore, if I had more time to improve the reliability of this data I certainly would have conducted each experiment at every five degree interval five or even six times to hopefully eradicate altogether any anomalies on my final graph. I would also extend the investigation by seeing the effects of even higher temperatures at 85 and 90 degrees celcius, to see if my assumption that there would be no more increase in the release of red pigment from the beetroot and therefore the absorbance is correct. With the results that I have obtained, I only have the one average result at 80 degrees celcius to justify this assumption, which is not enough. This is undoubtedly a limitation in my data, as this point could well have been an anomaly, and the rate of red pigment loss from the beetroot could actually still increase with temperature. Conducting experiments above 80 degrees celcius in an extension to this investigation, would certainly rectify this limitation and allow me to have more concrete evidence to support this part of my conclusion. I would also conduct the experiment at 75 degrees celcius, which is one particular temperature that I missed out in this investigation due to limited time. Hopefully the results of this would reinforce the fact that the rate does actually stop increasing.

Hypothesis

At low temperatures, between 25 and 50°C, increased temperature causes a slow rise in the amount of red pigment released from beetroot, due to the pigment molecules having increasing energy to move and therefore diffuse out of the cells more quickly. A further increase in temperature between 50 and 70°C, causes an even more rapid release of red pigment from the beetroot, due to tonoplast and cell membranes being breached after protein denaturation. However, over 70°C, an increase in temperature has no further effect on the release of the red pigment from the beetroot, due to diffusion gradients being cancelled out.