An investigation in to the effect of temperature on the release of pigment from beetroot tissue

Introduction and Hypothesis

I think that the increase of the temperature on the beetroot will affect the diffusion of the colour dye in the beetroot. The colour dye is held together by the membrane structure and this maintains the red rich colour in the beetroot. I believe that with the increase of temperature applied onto the plasma membrane, the structure of the membrane will become damaged and the membranes of the protein will eventually denature. Scientists know that cell membrane have the following general characteristics: -

- 40% Lipid
- 0 – 10% carbohydrate (as prosthetic groups)
- 50 – 60% protein.

You can see from above that proteins are major constituents of membranes. In membranes there are intrinsic and extrinsic proteins.

Intrinsic membrane proteins pass through the lipid layers. They have a variety of functions; one of them is assisting the transportation of molecules through the membrane. These proteins have both an extra and intracellular part. Extrinsic membrane proteins are embedded in the outer layer. They can often act as chemical receptors for the cells.

The majority of the proteins in the membranes are globular. This will mean that the 3 dimensional shape of the structured protein held together by the hydrogen bonds can eventually be broken if high enough temperatures are applied onto them – this is called denaturisation of proteins. I am working specifically on beetroot, it is known that proteins in plants are more likely to withstand higher temperatures and therefore denature at a higher temperature. So I would expect for the proteins in beetroot to denature at about around 50 degrees Celsius. Once the proteins are denatured they are no longer able to maintain the precise shape and carry out the functions. This will mean that the denatured proteins can possibly cause holes in the membranes.

I will also take into account that lipids can also become liquids if high temperatures are applied onto them. This will also have an effect on the overall function of the plasma membrane and cause ruptures in the membrane(s).

Having explained the effects temperature may have on the membrane proteins and lipids, I must also take into account the proteins that are present in the cytoplasm of the beetroot cells, where the red sap is also held together in the cytoplasm. These proteins can also be denatured when high enough temperatures are applied onto them. This could also have an effect on the release of red pigment. If part of the cell's cytoplasm is disrupted (the proteins being denatured), it can possibly contribute to further release of the red pigment out of cells. This is because the red sap (pigment) will not be held together as well inside the cytoplasm as it otherwise will be if higher temperatures are not applied and the proteins are not affected.

I have considered the structural components of the membranes that may be affected due to the increase of temperature; there is an external factor to consider. This factor is the increase of kinetic energy that will certainly be present where there is an increase of temperature. The increase of kinetic energy is going to effect the rate the red pigment leaves the cells through the membranes. As the input of kinetic energy increases, the particles move even faster, so the particles of the red pigment will move faster out of the cells.

I predict that as the increase of temperature is applied, there will also be an increase in the release of red pigment out of the beetroot cells. I predict this because the increase of kinetic energy will inevitably speed up the diffusion rate of the red pigment, therefore there will be more release of red pigment out of cells. I predict this to be the main cause of any increase of release of red pigment at the start of the application of temperatures (i.e. from 10 to about 40 degrees Celsius). From above about 40 -50 degrees Celsius, I believe that the damaged structure of the membrane and the denatured proteins of the cytoplasm will also have a part on the increase of the release of red pigment out of the cells. Soon as it hits the approximate 40 -50 degrees Celsius mark, I believe that the denaturing proteins will cause the membrane to be more permeable. Later on as the temperature increases to about 60 -70 degrees Celsius the lipids will also cause ruptures in the membranes. This will cause the membrane to be more permeable and the release of pigment will increase, along with the additional increase of kinetic energy also contributing to the cause of the further release of red pigment. And presumably at the higher temperatures the completely denatured proteins will be causing bigger 'holes' will also contribute to the further release of red pigment due to the membranes' increasing permeability.

Safety Aspects of the investigation

Before I start the experiment I am going to note down the safety precautions that I will take.

- **I will use safety goggles throughout the experiment so that any hot fluids or beetroot juice will not go into my eyes, as this may cause harm or distraction especially whilst handling apparatus.**

- **I will take extreme care whilst handling with glass and hot substances by using tweezers whilst transferring test tubes to and from water baths. I will bring test tube holders towards the area near the water bath to provide a short distance between the hot test tubes and the test tube holder for safe transfers.**

Having to share water baths, I will not perform tests on particular water baths, where there are already seven people doing investigations. This is to prevent any accidents from occurring.

Apparatus

- 15 test tubes
- Colorimeter

- Ruler
- Thermometers
- Stop Clocks
- Water baths
- Beetroot
- Test tube holder
- Small beakers
- Water
- Safety goggles
- Cork borer
- Tweezers
- White tile
- Curette

Method (Introduction)

I will be applying different temperature ranges on beetroot pieces to test if the increase of temperature does have an effect on the release of anthocyanin. I will be using a colorimeter to measure the amount of light absorption from substances taken from which beetroot pieces have been left under controlled conditions in the water baths.

In this experiment the temperature is the independent variable and the amount of the colour that diffuses out from the beetroot cells into a test fluid is the dependent variable.

Before planning the investigation in greater detail I would like to note down some points to make the idea behind the experiment as fair as possible. The points are written below in the 'Fair Test' section.

Fair test

To make the investigation as fair as possible I will:

- Use both the same beetroot sizes for every test that I will perform.
- Put the same amount of beetroot pieces for each test I will perform.
- Use the same amount of water for each test, where the beetroot pieces will be submerging in.

This will mean that I will not be testing, ' how the increase of water effects the diffusion rate of red pigment', and will not be making the water an independent variable. It will only simply be a fluid to help test the independent variable that is the increase of temperature.

- The same applies with using the same fluid (water) for the beetroot(s) to submerge in. As scientists have discovered, temperature is not the only factor that can affect membrane structure, it can also be solvents and their pH. With water the substance is neither acidic nor alkali, it is a neutral pH 7, it will not

be made an independent variable as it will not affect the membrane structure, and the release of red pigment. What the affects of pH, whether being too acidic or alkaline is that it can break bonds of proteins and denature them.

- I will make sure that each test I will perform will have the same amount of time spent in the water bath (e.g. 5 min) by using a stop clock to measure the time.

I will keep the distance when testing each test fluid using a colorimeter as short as possible. These points are to ensure that substantial amount of extra time are not given to other test fluids other that the 'first' of the same temperature range. This is because, if extra is given, the 'hot' test fluids are given a chance for their particles (of red pigment) to diffuse either further out of cells or within the fluid more than others due to the kinetic energy likely be present.

Method (in greater detail)

I am going to use 5 different temperature ranges. I have chosen to use 10 C, 30, 50 C, 70 C and 90 C. I have decided to use these temperature ranges to give me valid and reliable enough results to analyse and draw conclusions from, using the time available and equipment available.

I did not choose O C or any lower than 10 C because I will have to use ice which may lead to damaging effects. I say this because the cold temperatures can lead to the burst of the membranes of the beetroot cells, which will destroy the idea of the investigation.

I chose the 20 C difference between each temperature range mainly because it will enable me to investigate the effects of higher temperatures that I have proposed to do with my highest temperature being at 90 C. This is at all times considering the limited time and equipment available. I especially needed the temperatures ranges to be at least over 50 C – 80 C, to conclude whether the denaturing of proteins and the liquification or lipids had an effect of an increase of release of red pigment or not.

I will need to set the water baths at my set temperature range. I will perform three tests for each temperature range; therefore I will need 15 beetroot pieces and 3 test tubes. I have decided to conduct three tests for each temperature range to enable me to get a mean of 3 readings. I feel this will help me get a more accurate result for each temperature range. This is because, with only one reading or two readings, the readings could have been achieved or affected by other factors, and not achieved solely due to my independent variable – the increase of temperature. With three readings and getting a mean, it will be more believable to conclude that the results are evened out, and can also possibly prevent from getting anomalous results.

Before I can actually start my investigation, I will obviously need to cut all my 15 beetroot pieces at the beginning of the practical investigation being the organised and less distracting way to do so. I will use a cork borer to cut uniform c ylinders. I have decided to cut the beetroot pieces from sideways on. This will enable me to gain more pieces than using other ways. I will cut the beetroot pieces by placing the beetroot onto 10 cm white tile, whilst holding the beetroot down and cutting uniform cylinders

using the cork borer. This is much safer, as it holds the beetroot firm into position. It also helps to ensure that the cut pieces are cut accurately and precisely.

I will also need to set the test tubes containing water to be at the correct temperatures using the water baths for the beetroot pieces to submerge in. I will prepare 15 test tubes with 10cm water in each of them to make it fair. I will leave the test tubes onto test tube holders, whilst filling different test tubes with water. Once I have filled the test tubes with the correct amount water I will distribute them onto the inside of the water baths, 3 test tubes in each water bath of a proposed particular range. This is with the exception of the 30 degrees Celsius tube, where I will simply be putting test tubes in a rack – as room temperature is at around 30 degrees Celsius (27 C). I will have to wait for almost all of my temperature ranges to reach to its required temperature to enable me to perform the tests.

I have decided to put two 1/2 cm pieces of beetroot in each test tube. This will mean that I will have to cut 30 pieces of beetroot altogether of 1/2 cm size in length. The reason behind putting two beetroot pieces in each test is that I feel it would increase the surface area that it will come in contact with the heat water, so diffusion rates of the red pigment will occur more effectively in the limited time available.

When the water baths meet the required temperature ranges, measured using thermometers, I will start to perform the tests. However due to the fact that I am working alone, I am only able to perform tests for a particular temperature range at a time, for example I am not able to let tests carry on for both 30 degrees Celsius and 50 degrees Celsius, although they would be at different water baths. This is because I want to make the investigation as fair as possible to ensure careful monitoring of the stop clocks so the tests will not exceed the set and allowed time for them to be inside the water baths.

To perform the tests I will need to submerge the beetroot pieces by using 10 cm of water contained in each test tube for each test. I have chosen no less than 10 cm because I believe that to get enough diffusion rates happening, that particular amount of water is suited for the limited time available to perform the investigation. I will time the test tubes to be in the water baths for the beetroot pieces to submerge in for 5 minutes. I feel 5 minutes is valid enough for sufficient diffusion to occur. And that if any more time is given, especially with the higher temperature ranges (i.e. 90) could possibly exceed the colorimeter readings due to the possible high intensity of light absorption. I will use a stop clock to time the 5 minutes. After performing the tests of a particular temperature range, I will use a colorimeter to measure the amount of light absorption obtained from test fluids of each test (liquid substance in the test tube).

To measure the amount of red pigment that has been released out of the cells and onto the water, I will need to use a colorimeter. I will actually not be directly testing the amount of release of red pigment; I will really be testing the amount of light absorption obtained through the test fluids. I will actually need to produce a blank standard where water is placed into a curette as a control. I will first put the 'blank' standard curette inside the colorimeter. It should set the colorimeter at '0' light absorption. This will ensure that the colorimeter machine functioning correctly. After finishing the control test, I will start measuring the amount of light absorption from the real test fluids. I will have to follow the same procedure as I had done with my

blank standard control test, except of course that I will be expecting the readings of my real test fluids to be more than '0' light absorption as they will be real test fluids. I will have to follow exactly the same procedure for all of my 'to be' 15 test fluids. I will of course note my results (the readings of the light absorption) from each test fluid.

Note: There will be only be a limited amount of curettes available, so it essential for myself to rinse the curettes as clean as possible if they have been used for other tests so that they will not affect the readings of other test fluids. I will do this if necessary, during the time I leave the test tubes with the beetroot pieces inside of the water baths. I will do this using the sink nearest to the particular water bath, as this way the stop clock will be of close distance. This way I will be able to monitor the amount of time the test tubes have been in the water baths as well as clean the curettes - this saves valuable time that is limited.

N.B. When I mentioned that I would do a blank standard curette as a control for the colorimeter before starting to test the real test fluids. I will do this each time before testing each set of test fluids for each particular temperature range. This is to ensure that the colorimeter has not been affected in any way, possibly from too much testing, and my planned procedure will ensure that the colorimeter is functioning properly, ready to obtain accurate results for each of my particular temperature range.

Results

Results table	**Colorimeter Readings**			
Temperature	**1st**	**2nd**	**3rd**	**Mean Average**
10 C				
27 C	0.11	0.11	0.12	0.11
50 C				
72 C	1.18	1.28	1.68	1.38
90 C				

N.B. The colorimeter readings have all been rounded up to decimal places including the mean averages. This is fair and I can get enough accurate results by this.

Modifications that I have made in my investigation

Unfortunately it was not entirely possible for me to use all of my proposed temperature ranges for my tests. I have had to use the closest I could get of the planned temperature ranges in the time available for my investigation. For my proposed 10 C temperature range, I have used, with 30 C, I have used, with 50 C, I

have used, with 70 C, and with 90 C I have used. This was mostly due to the basic water baths.

Whilst actually cutting the beetroot pieces, I discovered that it was easier for me to cut out the pieces into the required length after having cut each cylinder. I had first cut the pieces into 1cm pieces, and then I cut the 1cm pieces into 1/2 cm pieces using a ruler to measure it.

What I had also done is to perform the tests at a very high temperature range around the beginning of the investigation (i.e. 70 degrees Celsius for my second testing of temperature ranges). This was to check if it would exceed any colorimeter readings (too much of light absorption), if it had done so, there would be not point in carrying on with higher temperatures (i.e. such as 90 degrees Celsius) However the results did not exceed the colorimeter readings, I was able to carry out tests at the higher temperature range(s) as well as having valid results from the tests of the 70 degrees Celsius temperature range.

There were other problems encountered, these were keeping the distances between the testing the curettes with the hot fluids onto the colorimeter as short as possible. This meant that the test fluid, say for example for 50 degrees Celsius, may have had 10 seconds of difference between the other test fluid of the same temperature range. This will mean extra diffusion may have occurred whilst being left with kinetic energy from the temperature increase, having further effect than the other test fluids both for the same temperature range and for the whole of the tests of the investigation.

For the actual graph that I will plot for my analysis from the results I have obtained, it will be a graph plotted showing the amount of light absorption against temperature.

I will probably most likely need to draw lines of deviation of my results for each temperature range. As I have said from above that there were problems that I had encountered which made the investigation to be not entirely fair. However to get round analysing results that should be considered reliable, I will not dismiss free – standing results (other than the mean) to help draw accurate conclusions. The lines of deviation will furthermore be discussed in the ‘analysis’ section.

Analysis

Overall the graph and table of results does show a positive correlation between the increase of temperature and the increase of release of red pigment (increase in light absorption). Therefore I believe my hypothesis is very correct. The crosses or dots of the graph can actually create the effect of a scatter graph. By this you can clearly see that the overall result to state of what would be a correlation, is that it is of a positive correlation. This is in terms of where the increase of heat (temperature is applied) onto the beetroot pieces, there is an increase in the release of red pigment.

Although the start of the curve of best fit does not completely my theory given in the hypothesis. However I cannot miss the other freestanding results (readings) of tested from each of the different temperature ranges. This is the reason why I have plotted all of my readings onto the graph, also along with calculated mean averages of the

readings. The curve of the best fit has been constructed through the plotting of the mean averages.

To explain further of the importance in showing the deviation, I will draw combinations of what could have been the 'main' collection of results, that are possibly reliable enough for analysis and drawing conclusions. Below is the table of results copied from the original table of results.

Results table	Colorimeter Readings			
Temperature	1st	2nd	3rd	**Mean Average**
24 C	0.10	0.11	0.15	0.12
30 C	0.11	0.11	0.12	0.11
39 C	0.19	0.09	0.13	0.14
56 C	0.22	0.27	0.34	0.27
60 C	0.24	0.34	0.51	0.36
72 C	1.18	1.28	1.68	1.38
78 C	1.50	1.70	1.72	1.64

Imagine if I had only taken one reading of each of the temperature range, the 1st of the colorimeter readings for all the 7 different temperature ranges are: 0.10, 0.11, 0.19, 0.22, 0.24, 1.18 and 1.50. This would have perfectly matched with my hypothesis. It could possibly be correct to say that my first recordings of each temperature range were actually the most accurate group of results due to the problems encountered.

Analysis (the scientific background)

I have agreed and the results seem to overall agree with my hypothesis, saying that as the increase of temperature has been applied, the release of red pigment increases. The higher the temperature increase, the increase occurs of red pigment out of beetroot cells. Although I have anomalous results, I still believe that the strength of my other results can back me up.

At first as the increase of temperature is applied, from 10 degrees Celsius to 30 degrees Celsius I would not expect any of the components of the membranes (lipids and proteins) to have become damaged or denature. This is because I believe that these temperatures are not high enough to damage or denature the proteins and/ or lipids. What I believe has caused the increase of red pigment is the increase of input of kinetic energy due to the increase of temperature(s) applied. This theory having already been explained in the hypothesis, where scientist know that there is an increase in kinetic energy there will also be an increase in diffusion rate of particles as they move faster (in this case with the particles of red pigment). As the red pigment particles move faster they are able to diffuse out of the membrane at a faster rate, increasing more as the temperature increase due to more kinetic energy.

However once the temperature reaches over 50 degrees Celsius I will need to take into account the factor in which proteins may be denaturing. There does seem to be a

slight increase in the steepness of the curve of best fit, where it is over 50 degrees Celsius, which concludes that the increase in the input of kinetic energy is not the only factor behind the increase of release of red pigment. The proteins in the cytoplasm of the cells may also be denaturing. So far from looking at the graph there has been a steady positive increase in the 'correlation', however when it does start to reach the recordings (readings) of 70 degrees Celsius and 90 degrees Celsius, the curve of best fit starts to have a more steeper curve. This actually does show that membrane structure is certainly affected, and the increase of release of red pigment is not only due to the increase of input of kinetic energy. Again not forgetting the proteins of the cytoplasm should likely been affected too. I had not expected a steeper rise in the curve than what I had got off my graph of results at this particular stage (between 70 degrees Celsius and 90 degrees Celsius). This is because I strongly think that the components of the cell membrane have not been completely damaged. Although I do feel that the majority of the proteins have been denatured, there will still be the components of the proteins that are at or close to the membranes, which may still cause blockages of the 'holes'. There is also the factor that lipids may not have been made into liquids at 70- 90 degrees Celsius. The lipids of the membrane(s) of beetroot cell(s) may still holding the structure of the membrane well.

From this I can conclude at this high level of temperature that the membrane lipids and proteins (or the components that are left of them) are especially sensitive to any increase of temperature, as it has already collapsed and deteriorated, they (or the components) are able to deteriorate even more.

There is one thing that I have not mentioned, this is one of the components of cell structure or wall to be precise is cellulose. I do not think cellulose had been affected because it takes over 90 degrees Celsius for it to have any effect on dismembering the structure and hence the increase of release of red pigment.

Evaluation

There were problems encountered, these problems was especially due to the hardship of keeping the variables under control. It was extremely hard to get the correct temperature range and to keep the distances from testing the test fluids using the colorimeter as short as possible. It was also quite difficult to find out whether the beetroot pieces were of the same mass or not. The problems are what I would call minor due to my circumstances.

The different temperature ranges that I had used were due to the limited apparatus I had available. My classmates and myself had only 5 water baths to share from. We had to all agree on using the temperature ranges that I had original planned for our investigations. Although the proposed temperatures did have a 20 degrees Celsius difference between them, I did manage to get a substantial amount of temperature difference between each temperature range.

The main problems were not able to achieve the correct temperature ranges, however, tests were conducted under the same conditions. And with the problem of keeping the distances apart from testing the test fluids of the same particular temperature range, the actual test tubes were taken out of the water baths as prompt as possible between one another- with hardly any amount of extra time given. Although they may have

been more delay whilst transferring and testing test fluids using the colorimeter, the actual test fluids are out of the heat of the water baths. To take note if extra time may have been given in between testing the test fluids using the colorimeter could have possibly been helpful. However it was recorded whether which ones were taken either, first, second or third, so comparisons and assessing reliability of the results can be drawn from there.

Although, I did say that I did not entirely know if the mass of the beetroot pieces were entirely the same. It was of the same size, although not exactly of the same weight, the same amount of surface area did come into contact with the same amount of water and at the same amount of time given. Although I did not weigh the pieces to ensure that the mass were the same, the beetroot pieces were all cut using the same way – through cutting sideways of the beetroot to obtain uniform cylinders- it was likely to have the same composition of cells. Though it was possible, altough rare that they would be exceptions, hence causing anomalous results. If I was to repeat the experiment again and start to consider weighing the pieces, I would have to use specialised weighing equipment. There would also be the problem of what to do if the pieces' weights did not match one another – could it be that I would have to cut a bit of the piece, hence making the surface area contact with the water to later be different and unfair during the tests.

If I were to make further improvements of the investigation I would have more than one person working in my group for the investigation and would also give my myself more time. I would have also like more water baths so I could have more temperature ranges to work from. This will enable me to analyse more precisely what would have happened between the temperatures that I had done. It could have been that between 60 degrees Celsius and 65 Celsius, the liquification of lipids had actually blocked the route of the release of red pigment a little, and a decrease of light absorption would have been evident.

I would have also have liked to perform more than 3 tests – say about 5 at least, with enough colorimeters and 'hands' to make it fair, and would get a very accurate mean of results from. As this would prove that the results obtained are most likely not obtained due to an external factor.