Nadia Mohamad Rom

AT1 Practical investigation

An investigation in to the effect of temperature on the release of pigment from beet root 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 completely pass through the lipid layers. They have a variety of functions, though many are 'carrier' proteins and channels that assist with transporting molecules through the membrane. These proteins have both an extra and intracellular part. Extrinsic membrane proteins are embedded in the outer phospholipid layer. They are fixed to one side of the bilayer or one depth of the bilayer. 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 tertiary 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. The most common temperature for proteins to denature in eukaryotes are at around 40 degrees Celsius. However 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 it is no longer able to maintain its precise shape and carry out its function. This will mean that the denatured proteins can possibly and most likely cause holes and ruptures in the membranes.

I will also take into account that lipids can also liquify 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). In this particualar investigation I will be looking at, especially these two functions of the membrane :-

- to act as a selective permeable barrier
- to keep the contents of the cell together (in this case, the red pigment).

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. The proteins inside the cytoplasm are more commonly found in the ribosomes, rough endoplasmic reticulum and golgi bod y vesicles. 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.

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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 in which the rate the red pigment leaves the cells through the membranes. As the input of kinetic energy increases so does the particles move even faster, hence the particles of the red pigment will move faster out of the cells.

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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 dif fusion rate of the red pigment, hence more release of red pigment out of cells will occur. 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. At first, after hitting 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 above the lipids will also cause ruptures in the membranes . This will further 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.

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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 take care when using the scalpel. I will only touch the handle of the scalpel and never touch the blade of the scalpel.**

- **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.**

- **Whilst working with a sixth form group and having to share water baths, I will not perform tests on particular water baths, where there are already two people doing investigations. This is to prevent any accidents from occurring.**

Apparatus

- 10cm white tile
- 21 test tubes
- Colorimeter
- Scalpel
- Ruler
- Measuring syringes
- Thermometers
- Stop Clocks
- Water baths
- Red beet root
- Curettes
- Test tube holder
- Beakers
- Paper towel
- Elastic bands
- Water
- Safety goggles
- Cork borer
- Tweezers
- Large beaker/ glass tub

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 pigment. I will be using a colorimeter to measure the amount of light absorptio n from test fluids - test fluids (substances) taken from which beetroot pieces have been left under controlled conditions in the water baths of tested temperatures to submerge in water contained by test tubes.

In this experiment the temperature is the independent variable (IV) and the amount of the colour that diffuses out from the beetroot cells into a test fluid is the dependant variable (DV).

Before planning the investigation in greater detail I would like to note down some points to make the proposed 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 :

- I will use both the same beetroot sizes for every test that I will perform.
- I will put the same amount of beetroot pieces for each test I will perform.
- I will 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 as an independent variable. It will only simply be a fluid to help test the IV- the increase of temperature.
- The same rationale also 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 neutral (pH 7), it will not be made an independent variable as it will not affect the membrane structure, hence the susceptibility of the release of red pigment. What the affects of pH, whether being too acidic or alkaline is that it can break bonds of the tertiary structure 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. 3 min) by using a stop clock to measure the time.
- I will keep the distance between transferring the test fluid from the test tubes that have been in the water baths and onto the curettes as short as possible. I will also 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 there particles (of red pigment) to diffuse either further out of cells or within the fluid more completely than others due to the kinetic energy may likely be present.

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Method (in greater detail)

I am going to use 7 different temperature ranges. I have chosen to us 20 C, 30 C, 40 C, 50 C, 60 C, 70 C and 80 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 eq uipment available. At 20 C, this is normal beetroot temperature, so this will give me a good basis to work from. This meaning, to analyse the result of the release of red pigment at 'normal' temperature conditions, and, in comparison with results from higher temperature being applied to beetroot cells.

I did not choose O C or any lower than 20 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 10 C difference between each temperature range mainly because it will enable me to investigate the effects of higher temperatures more which I have proposed to do with my highest tempe rature being at 80 C. This is at all times considering the limited time and equipment available. I especially needed the temperatures ranges to be at least over 40 C - 60 C, to conclude whether the denaturing of proteins and the liquification or lipids h ad 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 21 beetroot pieces and 21 test tubes all together. 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 the external factor, and not achieved solely due to my IV – 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 21 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 cylinders. 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 more 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, hence the tests to be performed. I will prepare 21 test tubes with 10cm 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. I will of course use a measuring syringe to measure the amount of water needed to go into the test tubes. 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 20 degrees Celsius 'water bath', where I will simply be putting test tubes in a large beaker containing tap water – as the temperature of tap water is at around 20 degrees Celsius. 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 ½ centimetres in length of beetroot pieces in each test I will conduct. This will mean that I will have to cut 42 pieces of beetroot altogether of ½ centimetres sized 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, hence diffusion rates of the red pigment will occur more effectively in the limited time available.

After I have cut the beetroot pieces I will need to rinse them well using tap water contained in beaker(s) along with the beetroot pieces inside. The beaker(s) will need to be sealed with a paper towel using elastic band(s). I will have to shake the beaker(s) and let the water pour through the paper towel(s)' sealing. The paper towel and elastic band are used so I can rinse the pieces by shaking the contents of the beaker well without the beetroot pieces falling out.

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 40 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 to put only 10cm and not any more so there would be no osmotic effect, where membrane may get damaged in the process. I have chosen no less than 10 cm because I believe that to get enough diffusion rate 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 2 minutes. I feel 2 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. 80) could possibly exceed the colorimeter readings due to the possible high intensity of light absorption. I will use a stop clock to time the 2 minutes. I will also use a colorimeter to carefull monitor the temperature of the water bath, making the required temperature ranges remain constant. 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 contol. I will first face the 'blank' standard curette inside the colorimeter, with the clear side facing the direction of the light. 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 ths same procedure for all of my 'to be' 21 test fluids. I will of course note my results (the readings of the light abrorption) from each test fluid. I will use blue filter with the colorimeter to measure the amount of light absorption from the test fluids because this is the complimentary colour of red pigment, hence the measurement of the light absorption will be extremely accurate.

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 will 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 will 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 temperauture range.

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

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 20 C temperature range, I have used 24 C, with 40 C, I have used 39 C, with 50 C, I have used 56 C, with 70 C, I have used 72 C, and with 80 C I have used 78 C. This was mostly due to the basic water baths. With the 24 degrees Celsius temperature range, I did use tap wate r. However the temperature range is 4 degrees Celsius above my temperature which meant that I will only have a 6 degrees difference between the next temperature above which was 30 degrees Celsius. I could have gone round the problem by possibly putting ice onto the large beaker containing the tap water. However this meant that I would run the risk of getting a temperature that would have been lower than 20 degrees Celsius. Anything lower than what is considered the normal beetroot temperature could have dam aging effects to the membranes. I would be testing on the effects of how the decrease of temperature would effect the release of red pigment, instead of the increase of temperature, so I had to settle for 24 degrees Celsius.

I will take note that the 24 degrees Celsius temperature range was the 1st temperature range that i had conducted tests on. This was mainly because I could get the approximate required temperature the most quickly, it was not necessary for me to wait for it to heat up first. Unfortunately there were further problems encountered with the testing of this particular temperature range. The problem was that whilst I was testing the test fluids onto the colorimeter, there was a member of the sixth form group that wanted to use the same colorimeter. For the third reading of the test fluid, I had about approx. 10 –13 seconds difference from the first and second readings taken from the test fluids of the same temperature range.

What I have also done during the preparation of the beetroot pieces is to cut an extra 6 pieces as a control giving me the choice of the reliable looking, same sized beetroot pieces. I was able to do this because of the extra space from the beetroot that I was able to gain more uniform cylinders.

Whilst actually cutting the beetroot pieces, I discovered that it was more easier for me to cut out the pieces into the required length after having cut each uniform cylinder. I had first cut the pieces into 1cm in length pieces, then I had cut the 1cm in length pieces into 2 ½ cm pieces using a ruler to measure it. I felt that this was much easier and a more accurate way to cut the ½ pieces like I had done. I had cut all of my ½ pieces using this way and hat managed to cut all the pieces to the correct sizes the 'first –time round'.

What I had also done is to perform the tests at a very high temperature range around the beginning of the investigation (i.e 72 degrees Celsius for my second testing of temperaure 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 78 degrees Celcius) 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 72 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 56 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 occured 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. However as you can imagine, with this external factor effecting the test fluids of temperature ranges of 72 degrees Celsius in comparison with temperature ranges at only 30 degrees Celsius – the increase and more time for the kinetic energy to work from will most likely be at 72 degrees Celsius, affecting the results of the temperature range more than that of a 30 degrees Celsius

temperaure. Anyhow this still is not completely fair with the overall 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 temperautre.

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.

<u>Analysis</u>

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 can not miss the other free standing results (readings) of testd from each of the different temperature ranges. This is the reasons 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 plottings of the mean averages. To also see even clearly the effect in which the other test results had made in the investigation, I have drawn lines of deviation of the plottings (the readings of the 3 tests for each of the temperature range).

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 match with my hypothesis. It could possibly be correct to say that my first recordings of each temperature range was 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 o f red pigment increases. The higher the temperature increase, the increase of red pigment out of beetroot cells occur. 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 24 degrees Celsius to 39 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 40 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 steepnees of the curve of best fit, where it is over 40 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 te 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) off 56 degrees Celsius and 60 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 have 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 56 degrees Celsius and 60 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 liquified at 56- 60 degrees Celsius. The lipids of the membrane(s) of beetroot cell(s) may still holding the structure of the membrane well.

At the plottings of the readings of the temperature range 72 degrees Celsius, and in comparison with the plotting of the readings of 60 degrees Celsius, there is an extremely high steep in the curve of best fit between them. And also in comparison with the increase of steepness in the curve of plottings of readings between 56 degrees Celsius and 60 degrees Celcius, the steeper curve beween the plottings of readings of 60 degrees Celsius and 72 degrees Celsius, shows the intensity of the enormous change of steepness. This suggests there is a sudden rush of red pigment flowing out of the beetroot cells at the temperature range 72 degrees Celsius, hence suggesting the membrane structure has been deeply disrupted. This is most likely due to the lipids having been liquified at this high temperature. Furthermore I can also note that there has been a further increase of release of red pigment out of cells from the readings taken from the 78 degrees Celsius range.

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

There were anomalous results recorded. The anomalous results that did not completely match my theory were the; 0.15 reading for the 24 degrees Celsius temperature range which did also cause an anomalous average and made my curve of best fit constructed using the mean average go of to the 'wrong' start, and the 0.09 reading for the 39 degrees Celsius temperature range. The reasons for these two anomalous results are what I believe to have been caused by external factors as I have partly explained under the 'modifications' section and will later discuss in the evaluation section.

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.

One other thing to note is that when I had cut the beetroot pieces and had rinsed them dry to clean the leakage of the red pigment using water and then dabbing the pieces dry. The problem is that although the red pigment has been dried off and rinsed, cutting the beetroot pieces would have inevitably cause damaged to the membranes of the beetroot cells. I was unable to measure the amount of the red pigment that had leaked out of the cells of the cut pieces. It maybe that some of the pieces had already leaked out the majority of their red sap, whilst other had little damage being still able to maintain the rich red sap in their cells. What I truly believe was the case (reason) for one of my anomalous results (i.e. the 0.09 reading fo r 39 degrees Celsius) was that it had contained beetroot pieces that were extremely dried out and had lost the majority of the red sap to do the membranes being cut.

What I believe was the case with the reading of 0.15 for 24 degrees Celcius is that it could have been caused due to several of external factors. It had been noted that whilst testing the test fluids for the light absorption, someone else in the sixth form group had gone in between the testings of test fluids.This will have meant extra time would have been given for the red pigment to diffuse even more within the fluid itself. I could take this into account as the reading had been recorded to be the third and last reading to be taken. However this was only at what is considered to be at just above the normal beetroot temperature, hence the increase of input of kinetic energy would not have really been an external factor, so there may have also been another

factor that may have caused this anomalous result. This could have been because the beetroot pieces that I had used for the test could have been less dried out than others as it was one of the first tests to be conducted, and it could have also been the ones that were not very much effected by the cutting of the pieces, hence the membanes have not been greatly cut. It may have been that the odds were that those particular pieces had the least effect in losing the red pigment and had maintained quite a lot of red pigment and was on the virge of releasing it an opportunity where it was given in the test. It could possibly be that the pieces that I had used had a greater mass, and was much more richer in cells than that of the others.

Although the 0.15 reading did bring make my mean average, being 0.12, anomalous for the temperature range 24 degrees Celsius in comparison to the mean average of 30 degrees Celsius 0.11, which also made my curve of best fit go off to the wrong start in my hypothesis's view. However as I had explained in my analysis, the free standing results included through the lines of deviation in the graph does not make the investigation's results overall anomalous. The first reading obtained from the test of the 24 degrees Celsius was 0.10, and in comparison with the first reading of the next temperature range above - which was 0.11, which technically does prove my hypothesis to be correct. And taking note of the overall investigation, although they were problems encountered, I believe I have obtained results and presented my results in a way that does make my results valid enough for analysis and drawing conclusions. The problems in which I did encounter were not problems that weighed a great significance in making the experiment unfair. The problems are what I would call minor due to my circumstances.

The different temperature ranges that I had used was due to the limited apparatus I had available. Myself and the rest of my classmates had only 6 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 10 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. The lines of deviation helps to draw accurate and reliable results from which I able to 'choose' or see from.

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 woul d 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.