An Investigation into the effect of Temperature on the release of Betalain from Beetroot Tissue

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Problem

The aim of this investigation is to see what if any affect temperature has on the release of Betalain from beetroot tissue. To carry out this investigation I am going to need the following equipment and materials.

Apparatus

Electric water bath - This will be needed to keep the water temperature consistent throughout the experiment at the various required temperatures.

Thermometer - This will be used to check that the water bath is heating accurately at the required temperatures throughout the investigation.

Colorimeter - This is what will measure the affect that the heat has on the membrane by measuring how much light passes through the solution.

These are the apparatus that will be used to heat and record the data but in order to use these other apparatus must be used too;

Test tubes
Syringe (to accurately measure the fluid amounts)
Cork borer (to shape the beetroot equally)
Curettes
Measuring cylinder
Scalpel

Materials

Beetroot
Distilled Water

Method

Cut out three pieces of beetroot about 2cms long using a cork borer.

Place the cylinders of beetroot on a tile or board and using the scapulae cut into discs 5mm thick.

Label 3 test tubes, A B & C for each of the temperatures to be tested. The temperatures required are 20, 30, 40, 50, 60, 70 and 80°c

Put 10cm³ of distilled water in each test tube

Place the three test tubes for the required temperature in the water bath and heat to the required temp if needed

Check the required temp has been reached using the thermometer to measure both the water bath and the test tubes temps

Place the three pieces of beetroot in the three test tubes and leave for two minuets

After the time is over remove the test tubes from the water bath and using the syringe which should be clean, extract 5cl from each solution to fill up a curette for each which should also be labelled, check no pieces of beetroot are in the curette

Set the Colorimeter to 0 % transmission with water

Make quantitative measurements using the colorimeter and record for each

Repeat method for each of the temperatures

Variables

INPUT – Temperatures, 20, 30, 40, 50, 60, 70 & 80

CONTROL – Beetroot size/shape, beetroot type (use same beetroot), pH & amount of the water, temperature consistency, time in waterbath

OUTPUT – Rate of diffusion measured using colorimeter to measure concentration of dye (Betalain) in solution

Explanation
My input variable will be the temperature. This will be held at constant temperatures by the water bath and the temperatures changed consistently. The water temperature needs to be held consistently while the diffusing is taking place so that the rate isn't affected and it is a fair test.

My control variables will be controlled in the following ways. The size/shape of the beetroot will be controlled by the cork borer and by measuring its length. This has to be done and it is important that it is done accurately because the volume to surface area needs to be the same. This is needed so the rate of diffusion is the same for each piece of beetroot before the temperature is changed.

Beetroot type will be the same because I intend to use the same Beetroot - unless I run out! The Beetroot will be left covered while not being used and the only pieces to be cut from it will be the ones for the temperature, which will be measured next. This will prevent any of the beetroot drying up as if the membranes dry up they will release less Betalain. It will also prevent any individual differences between the beetroots affecting the results.

Distilled water will be used so as to keep the pH of the water the same. The pH needs to be consistent because it will affect the rate of diffusion, for instance a high acidic pH would denature the proteins in the membranes and completely compromise the results.

The temperatures will be kept constant by the water bath as explained in the input.

Output

Data will be recorded by the rate of diffusion. This will be the rate at which the Betalain will have diffused from the beetroot to the solution over the given amount of time. This will be measured by the transmission of the water as read by the colorimeter. This will give an accurate reading of how great the concentration of the dye in the water will be. This can be used to work out the rate of diffusion by dividing the transmission % of the solution by the time given for the diffusion to take place.

% / Time = Rate of Diffusion

This is assuming the pigment release is constant

Equipment Details

Colorimeter, device used to compare or measure colours and their intensities. A simple colorimeter uses an optical system to place an unknown colour, such as of a chemical sample, next to a well-established colour. In more advanced devices this comparison field can be adjusted in various quantifiable ways. In some, photoelectric cells may be used to measure the transmitted light. Colorimeters are used in chemical research and in various industries, such as the manufacture of dye and paint.

The Colorimeter is the best way to measure the diffusion rate with the equipment, which we have available to us. There are not many other alternatives and using eye site to measure colour would be very in accurate. The Colorimeter is very accurate providing it is set first for water having 100% transmission. It is a reliable piece of equipment, which is well suited to this investigation.

Electric Water Bath, heats water to a required temperature and then maintains this temperature for as long as required.

This is the best piece of equipment to use to get reliable, constant temperatures throughout the investigation. It can heat to exactly the required temperature and hold it whist the beetroot is placed in the test tubes. This would not be possible with a Bunsen Burner.

The **Thermometer** will be used to check the reliability of the water bath.
The syringe will be used to get an accurate amount of distilled water in the test tubes and then will be used to distract the solution afterwards without beetroot and placing it in a curette. The cork borer will be used to shape the beetroot consistently as explained in the method and variables. The scapulae will be used to cut the shaped beetroot into the right size and the curettes are what the colorimeter uses to read the transmission of the solution.

Method Details

I am going to use the following temperature ranges to collect my data; 20, 30, 40, 50, 60, 70 & 80°C. I have decided to use these to give me a valid and reliable set of results to analyse and draw graphs and conclusions from. I intend to start at 20°C because this is the normal temperature of Beetroot and will give me a good basis to work from. Not only will this give me readings for the investigation at normal temperatures but with the equipment available to me it is the lowest temperature I am willing to go to. Going lower would mean having to use ice, which I don't intend to use as it would be very hard to keep constant and may impeded the results.

I that two minuets should be sufficient for the diffusion to take place as Beetroot releases a large amount of betalain under normal conditions when cut. I feel that once in heated water or even in water at room temperature within two minuets enough betalain should have diffused for relevant data to be collected. I am also worried that if the Beetroot is left to long the rate of diffusion will slow and that the time taken to reach this point will decrease as the temperature increases. This would not help as my formula for working out the rate of diffusion, (transmission / time) is dependent on the pigment release being constant. I feel that allowing only two minuets for the diffusion will avoid this happening.

The data collected will be taken from the solutions after the two minuets is up. The syringe, which should be clean so as not to affect the solution, will be used to extract 5cl from the solution. This will then be put into a curette, and its transmission measured by the colorimeter. No bits of Beetroot should be in the solution as this could show up on the readings. The transmissions will be recorded in a results table. These will be recorded as percentages as that is how the colorimeter reads them. They then need to be recorded as their rate of diffusion using the formulae.

Each of the three rates for each temperature need then to be added up and given as an average. This is done to avoid anomalous results. if there are any outstanding anomalies then they should be removed before the averages are worked out.

After the averages have been recorded graphs can be drawn up and then analysed for correlation or anomalous results. Scientific theories can then be used to explain the results and then conclude the investigation.

Changes in Method

There were some problems whilst collecting the results which may have an affect on the findings from them. Firstly was with the temperatures of the water baths which we heated the beetroot in; these were less reliable than I had hopped as far as keeping the water at a consistent temperature. They could not hold the water at exactly 35°C, 45°C, 55° or 65°C etc so temperatures were recorded from around the right temperature, and that temperature recorded with them. I would also have liked the water baths to be as consistent as possible but I'm not sure they were as once they reached the required temperature they turned off. This may mean that the temperatures varied slightly over the five minuet period the beetroot was left to diffuse, however I still feel that the temperatures recorded are varied enough and close enough to the original aims to still be used to analyse and solve the problem. The Colorimeter's readings may also have an affect on the results. This is because they did not always read consistently. This could be because of smudges on either on the curettes or on the lens or perhaps due to the particles moving around in the solution. I feel, however that the data collected has been accurate enough and varied enough to analyse and solve the problem fairly. I also found that two minuets did not prove a sufficient amount of time for the diffusion to take place, and so I extended the time to 10 minuets. This is because I found that I had underestimated the rate of diffusion from the beetroot and that after just 2 minuets not very much dye had diffused at all and comparisons would be small. By leaving the Beetroot longer it allowed more Betalain to diffuse and a wider range of results to work with.

Analysis of original Results

The original set of results look quite promising. I have used the colorimeter to obtain data for; absorption, transmission and the rate of reaction. The absorption and transmission are readings given by the colorimeter and can be used to work out each other. The formula to use to work out the transmission from the absorption is to take the absorption from 100 to get a percentage for the transmission of the colorimeter reading. And this is the formula I have used in my results table (100-a) which was created using Microsoft Excel. The next figure in the table is the Rate of Diffusion per Minuet, which is the figure I intend to use to analyse my findings with. This is worked out by dividing the absorption by 5, (a/5) the amount of minuets the beetroot was left for (this is presuming the diffusion rate was consistent). This then relates directly back to the problem which asked how temperature affected the rate of diffusion of betalain from beetroot to water over a given amount of time. I have recorded all three of these in the table for each of the temperatures implemented and for all five repetitions I have then added them and divided by five to give an average.

I have also included the size of the beetroot in mm (length multiplied by diameter), the weight of the beetroot in grams, the volume of water from the test tube and the time in minuets. These are all control variables but I have included them in the table so all relevant stats are visible and they can be shown as consistent.

There are however some anomalous looking results, I have highlighted these results red but have not removed them yet. I will draw up a graph first and analyse the results further before deciding if these results are having to much of an effect on the averages to be included in the findings.

Analysis

The graph has used the rate of diffusion per minuet results from the results table. The calculation for this is the absorption rate divided by five; the amount of minuets the beetroot was left for. This gives you the amount a figure for the amount of diffusion taking place every minuet presuming the diffusion is consistent.

The graph shows a clear positive correlation for greater heat, greater release of Betalain. This would be because the hotter the Betalain gets the more energy its molecules will get and the more motion they will make and the more will diffuse through the membrane of the beetroot and into the water. However there is a large range in the error bars on most of the results and some overlap. I feel this could be because of the results I highlighted in the table I am therefore going to redo the table without these anomalies and see if I can improve the quality of the graph and findings.

Edited Results

Temp (ºC)	Figure	Repeat 1	Repeat 2	Repeat 3	Repeat 4	Repeat 5	Average
25	Rate %min^{-1}	3.2	2.2	3.8	3.2	3.2	3.12
35	Rate %min^{-1}	4.2	4.4	4	4.6	4.4	4.32
45	Rate %min^{-1}	4.6	5.2	5	6.4	6	5.44
55	Rate %min^{-1}	10.8	13	11.2	10	12.4	11.48
65	Rate %min^{-1}	14	14.2	14.6	15.4	15	14.64

I have removed the anomalies and used the average of the other four readings for that temperature to fit the Excel formula. This has given me more consistent results and should help to get a better correlation on the graph for my final readings. I have also removed the Absorption and Transmission readings from the table to make it more condensed and easier to read and evaluate. I decided that in this table only the essential figures should be kept in, the ones that I will be using to create my final graph with. Therefore I have gotten rid of the size, weight, water volume and time as these are all consistent and do not need to be present on the graph.

Analysis

Removing the main anomalies from the results has made the graph look more accurate and more relevant. There are smaller error bars and the results are in a better correlation. The only Results not closely corallined are those for 55°C but because these results were so varied that picking out anomalies would not work here.

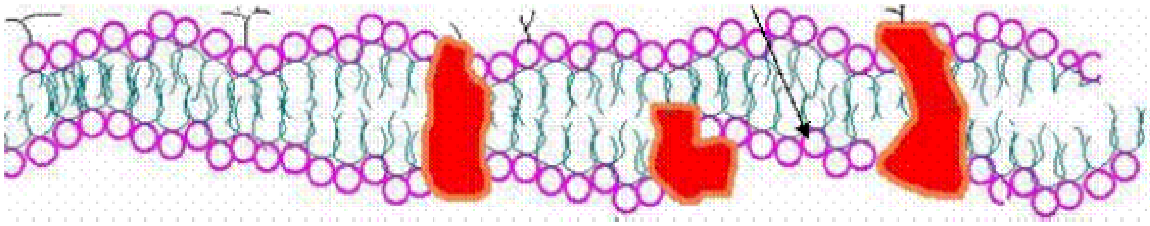
The line on the graph is more of a steeper gradient and would form an ‘S’ shape if I were to draw a line of best fit on it.

Conclusion and Background Information

I can now conclude that the relationship between heat and diffusion on a beetroots membrane is that the greater the heat, the greater the rate of diffusion. This is as I expected and of no great surprise. The main reason for this would be because the greater the heat, the greater the energy the Betalain molecules would have and the more motion they would have. This would lead to more diffusing in a shorter amount of time.

The cell membranes main function is to serve as a boundary between the cell and its environment. It is just like other organelles in the cell in that it serves the cell by having its own specialised jobs.

In terms of beetroot the Betalain is contained within the cell membrane, if this membrane is broken or disrupted the pigment will be released.



Temperature may be the cause of the disruption. High temperatures could distort the "active site" of the carrier, channel of gated proteins, therefore affecting the shape of the fluid mosaic model membrane which may release the betalian or other molecules held inside the beetroot. Temperature can also affect the rate at which the diffusion takes place by giving the particles more energy. I feel that this is more likely to be what caused the higher rate of diffusion rather than the disruption which was caused by cutting the beetroot up.

This has been shown on the graph and in the results and I can now conclude that the higher the temperature of the water and Beetroot the higher the rate of diffusion will be over the semi permeable membrane.

Evaluation

The Problem has been solved and even though there were anomalies and some of the equipment was perhaps not as accurate as would have been preferred the experiment has been a success and there can be no doubt of the effect on heat on the rate of diffusion of Betalain between the membrane of a beetroot and water.

There were limitations with the amount of equipment we could use and on methods we used as we only had the schools supply of equipment and only two lessons in which to collect data. The first of which and perhaps the most important of which was the water bathes.

Water Bathes
These were supposed to keep a level and consistent temperature throughout the duration of the experiment. This however they did not do, they did not reach the required temperatures very well and there gages often read differently to the thermometers used to back them up. Also once the required temperature or at least what the water bathes considered as the required temperature was reached, the water bathes shut themselves off. There would then be no heat or buffer to keep the temperature constant.

Whilst this is a much more effective and accurate way of reaching the temperatures and conducting the experiment than using Bunsen burners or any of the other equipment the school could have provided, it was a bit disappointing that it couldn't hold its temperature. The poor precision of the water bathes could have had an effect on the data recorded. The experiments were supposed to be conducted at 25, 35, 45, 55 and 65°C but the real temperatures were from around these temperatures. This could have led to variation in the in the in the data collected as some of the error bars were quite large, for instance the changing temperatures could mean that once you returned to repeat the experiment the water bath would be at a different temperature to when you first recorded the results. Another factor affecting the difference in results could the position in the water bathe, if two different thermometers (the water bathes thermostat and the separate thermometer) are reading different temperatures then maybe the temperature isn't consistent throughout the water bathe at the same time. If one test tube was placed directly above the heater and another away from it they would have different temperatures leading to a deviance in the results.

This lack of reliability may have had an effect on the conclusions as well as the results. On the first graph the error bars were clearly to large and needed editing to remove the anomalies and redo a more consistent line. The figures used for the graphs were suppose to be for the rate of reaction and to work this out the diffusion should have been constant, but if the temperatures weren't constant then its probable that the diffusion wasn't either. This could not be helped though and differences – although there were some anomalies were fairly consistent and showed enough reliability to be analysed, concluded and explained using Biological Knowledge.