An experiment to investigate the effect of temperature on the leakage of Anthocyanin from beetroot tissue

My aim for this experiment to determine the effect of temperature on the leakage of Anthocyanin from the plasma membrane of a beetroot.

I think that as the temperature increases the beetroot will start leaking out more Anthocyanin because the cell membrane which consists of proteins and lipids is affected by heat. So I predict that as the temperature increases so the amount of Anthocyanin leakage will also increase.

To do my experiment I have chosen to use these temperatures : 30°, 40°, 50°, 60°, 70°C. I have chosen these temperatures because I estimate that at around 50°C the proteins tertiary structure will begin to denature and change, causing the Anthocyanin to leak out of the cells vacuole. So by using these temperatures I have a wide scope to see what happens around this temperature.

I have also decided to use 10cc of water because I think it will provide a fair base for my experiment.

Another choice was to put the beetroot solution into the waterbaths for five minutes each time because I estimate this will be long enough for the reaction to take place.

For my experiment I am going to use the following equipment; -

- Waterbaths- to heat up the solution. I am using water baths because they keep the temperature more constant the using a Bunsen burner.
- Test-tubes -to put the water and the pieces of beetroot into to heat up.
- A Colorimeter- I am using this because it will be able to tell how much Anthocyanin has leaked out of the beetroot. It does this by sending a beam of light through a curvet containing a solution and it then reads the amount of light which has passed through the solution.
- Curvets- I will use these to put my solution into and then put them into the colorimeter.
- Cork borer- This will be used to cut my pieces of beetroot; I will use this because it guarantees that the bits of beetroot are the same size.
- Digital Micrometer- This will be used to check that all the pieces of beetroot have the same size and surface area.

- Thermometer -to check the water in the test tube is at the right temperature
- Stopwatch- I will use this to make sure each beetroot solution is only in the waterbath for five minutes.
- Pipette- this will be used to take a sample from the beetroot solution and place it into a curvet.
- Measuring cylinder- I will use this to measure out 10cc water each time.
- Digital scales- to check the mass of the beetroot piece.

I will now outline my method for my experiment:-

1. I will put the test tubes with 10cc of water (which I measured with a measuring cylinder) and a thermometer into the waterbaths so that they are at the right temperature.
2. Then I will use the cork borer and digital micrometer to cut, check that all my beetroot pieces are exactly the same size and I will also weigh the beetroot on the digital scales to make sure they all have the same mass.
3. I will then wash the beetroot
4. I will then check the thermometers to make sure the temperatures are right.
5. I will then (starting with 30° and working up to 70°) place the test-tube which contains a piece of beetroot and water into the waterbath and time it for five minutes.
6. When the five minutes are up I will remove the test-tube and using a pipette and take a sample and place it into a curvet.
7. I will then take the curvet and place it into the colorimeter and then write down the result.
8. By repeating the experiment three times it will ensure a fair test.
9. Repeat steps one to seven for the other temperatures.

I have decided to use this method because I think it will give me a fair and just test with a limited chance of anything going wrong.

Here is a list the different variables that could affect my experiment and how I will try to control them.

Different Variable: - Time
How I will control this variable: - I will use a stopwatch to make sure all the tests start and finish exactly five minutes.
Effect it could have on the experiment: - If I left one test for five minutes and another for ten minutes they are bound to have very different results so the times must be exact.

Different Variable: - Consistent temperature
How I will control this variable: - I am using a waterbath to keep the temperature constant and checking it with a thermometer.
Effect it could have on the experiment: - The different temperatures would mean I would have varying results all the time. Thus ruining the experiment.

Different Variable: - Same amount of liquid used each time.
How I will control this variable: - I will use a measuring cylinder to make sure all the amounts are the same.
Effect it could have on the experiment: - If I put 9cc of water into a test-tube the result would be very different because more concentrated colour would be seen.

Different Variable: - Volume, mass and surface area of the beetroot.
How I will control this variable: - I will use a cork borer and digital micrometer to make sure all the pieces are identical.
Effect it could have on the experiment: - If one piece has a bigger surface area it would release much more Anthocyanin then a smaller surface area. The volume and mass would also affect the experiment because if I put a big bit of beetroot into the test-tube and a small bit into a different test-tube I would get very different results.

Different Variable: - Beetroot genetics
How I will control this variable: - I will use the same beetroot for all my experiments.
Effect it could have on the experiment: - Different beetroots have different genetic makeup so different beetroot might have slightly different results.

Results

Fig 1 is a table of my results.

Fig 2 is a graph of my results.

Analysis

After analysing my results I can see that as the temperature increases the amount of anthocyanin leaked out of the membrane is also increased. I am now going to look at the difference in the leakage of Anthocyanin at 30°C and 40°C, at 30°C there is an average of 72% of light being able to pass through the solution but at 40° C it is only 62% so from this you can see that as the temperature goes up so does the leakage of Anthocyanin. At 40 to 50°C the change is 12% from 61% at 40° C and 49 % at 50°C. So I can prove that as the temperature increases so does the percentage of Anthocyanin leaked.

Conclusion

The purpose of a cell membrane is to control the transport of substance moving into and out of the cell. A membrane is a very small layer which is partly permeable. The membrane consists of lipids which is a fatty substance and proteins which are made up of many amino acids. The lipids present in this specific membrane are called triglycerides and specifically phospiliphids. In this case they have one molecule of glycerol which is linked to three molecules of fatty acids.

All cells have a membrane, in the beetroot cells a substance called anthocyanin is contained within the plasma membrane . It is the anthocyanin which gives the beetroot its purple colour so if the membrane breaks down, the anthocyanin will leak out of the beetroot and if anything is near the beetroot it will be stained by the leaking anthocyanin.

Now I will use this knowledge to test whether heat affects the beetroot's membrane. From my results I can conclude that as the temperature increases so does the amount of pigment leaking out of the beetroot. As the temperature increases the membrane has become more and more permeable. By looking at my graph and result table I can see that this is

correct. From my graph I can predict that if I went to 80° C the leakage of Anthocyanin would be even higher then at 70° C. This is because the proteins which make up part of the membrane can become broken by heat. If the protein and its amino acids have become denatured, the hydrogen bonds can break causing the protein to lose its shape thus allowing the pigments to leak out. The lipids can also cause holes in the membrane to appear because they also break down under higher temperatures. The darker the colour in the test tube means that more of the pigment has been released by the increasing temperature.

At 30°C the proteins are starting to denature but as the temperature increases so does the amount of pigment released and by 70 °C most of the proteins have been denatured so more pigments have leaked out of the cell.

Evaluation

I think that the results from my experiment were good results as they did show the outcome that I had predicated in my plan. I think I performed my test accurately but if I repeated it again I would have used distilled water instead of just normal tap water. As distilled water is much purer then normal water the colorimeter may pick up slightly more accurate results when using distilled water. Because I was only using tap water it is possible that it all ready contain some pollution so my results could be slightly off by 1 or 2% but by using distilled water (which is 100% pure) I might have got slightly different results.

Another limitation was the fact that all beetroots are different by having different genetic make-ups; if I were to repeat this experiment it might have different results because the new beetroot would have a different genetic make-up to the one I had just used so it would have a different amount of leakage. A possible limitation is that the colorimeter isn't always 100% accurate so sometimes you can results which fluctuate 1 or 2%.

A few problems I did have with my experiment were making sure all the beetroot pieces were the same mass, surface area etc, I did try to keep the beetroot pieces identical to each other but it was impossible to get it exact. Another problem was that the water baths were constantly changing 1-2°C so a few of my results may have been performed in slightly changing temperatures. This was a bit of a problem because a few of my experiments could have been preformed at 38°C and another might have been at 40° C which could cause different results.

If I was to repeat this experiment again I might expand it slightly by using different plants as well to get a wider view of how heat affects cell membranes.

Bibliography

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