The Effect Of Different Concentrations Of Ethanol On The Plasma Membrane Of Beetroot Cells

Introduction

The membranes of cells are made up of a fluid imitating, semi-permeable plasma membrane. This is made up of a phospholipid bilayer. A phospholipid bilayer is comprised of phospholipids (see figure 1) and various membrane proteins. The lipid tails of the phospholipids are hydrophobic (repel water) and the phosphate heads are hydrophilic (attract water). In order to ensure the hydrophobic tails remain dry, these phospholipids form bilayers. An emergent property of this is the solution on the inside of the cell and the cytoplasm of the cell are kept separate and ions such as potassium (K) and sodium (Na) and molecules including water (H2O) are diffused across the membrane when they are required (Dictionary, 2019). This can be used to create concentration gradients which is beneficial to the cell. For example, a concentration gradient is used to produce adenosine triphosphate (ATP) from adenosine diphosphate (ADP) in the electron transport chain, which used to produce the energy for numerous functions such as movement, active transport (endo/exocytosis), emission of light in bioluminescent organisms, biosynthesis of macromolecules (polymer assembly)and nerve transmission (BioNinja, 2016). Figure 1- Phospholipid

Beetroot (Beta vulgaris) (International, 2017) is a root vegetable that contains betacyanin ($C_{24}H_{26}N_2O_{13}$) (Information, n.d), a water-soluble dye in the central vacuoles, the tonoplast, of its cells. This dye is released when the cell membrane ruptures due to stress and the contents of the vacuoles are released, creating the signature purple colour. The level of stress the beetroot is under can be measured by the intensity of the dye released when the sample is placed in an alcohol solution. This is because the betacyanin dye is water-soluble, but not lipid-soluble and the dye released shows the amount of damage caused to the membrane as no dye can be released when the membrane is intact (Birmingham, 2019).

Alcohols are substances made up of alkanes (made up of carbon and hydrogen), with a hydroxyl functional group, represented by -O-H. These are organic molecules and have similar chemical properties and are commonly, highly reactive. The alcohol used in the following experiment was ethanol. Ethanol is commonly used in perfumes and other cosmetics, is used to sterilize (hand sanitizers), and in biofuels (ChemicalSafetyFacts.org, 2019). Alcohols are able to disrupt membranes due to their amphipathic properties. Phospholipids are generally soluble in non-polar solutions, such as alcohols. The polar heads of the phospholipids will react with the hydrophobic part of an alcohol (see figure 3). The larger the hydrophobic region of the alcohol is, the more damage it causes (Hirrel, n.d). Figure 2- Diagram showing the hydrophobic and hydrophilic regions of 1-Butanol

Butanol

The amount of dye released was measured by a colorimeter. Colorimeters are used to measure the absorbance of light waves by a solution and can determine the concentration of the desired solution. This is determined by a photocell which can deduct the amount of light able to pass

through the solution in comparison to the amount able to pass through a pure solution (Choudhury, 2014). The pure solution used in the experiment conducted was water.

In the experiment conducted, samples of beetroot were placed in solutions of ethanol with various percent concentrations (0%, 20%, 40%, 60%, 80%, and 100%) in order to determine the concentration at which the greatest amount of damage is caused to the membranes of the beetroot cells. This will be concluded by the comparison of the absorbance of light of the dye-ethanol solutions.

Hypothesis

- HO- There is no statistical difference between the amount of betacyanin dye released and the concentration of ethanol
- HA- There is a statistical difference between the amount of betacyanin dye released and the concentration of ethanol

It has been hypothesised 100% ethanol will do the most damage to beetroot membranes, causing a larger amount of betacyanin dye to be released as that the higher quantity of ethanol molecules will maximise the disruption of the membrane.

This hypothesis is supported as, increasing the concentration of the reactant in a experiment consequently increases the rate of reaction. This is due to the increased number of particles, such as ions or molecules, to form the products (Markgraf, 2018). It can be inferred the increase rate of reaction

Discussion

The aim of the experiment conducted was to determine the effect of ethanol on biological membranes. This was assessed by measuring the amount of betacyanin released from the tonoplast (main central vacuole) of beetroots. After conducting the experiment, obvious trends were identified in the data. The percent concentration of ethanol that caused the most betacyanin to be released from the beetroot cells was 60%, contrary to the predicted 100%

A students t-test was conducted to determine if there is a significant statistical difference between the absorbance of betacyanin in the various concentrations of ethanol (0%, 20%, 40%, 60%, 80% and 100%). This test revealed there was a significant statistical difference between all the concentrations assessed, excluding between 0% and 100% as the P value was greater than 0.05. This then gave enough evidence to reject the null hypothesis and accept the alternate hypothesis. Which stated there was a significant statistical difference between the amount of betacyanin released and the concentration of ethanol. The variation in the values of the standard error of the mean (SEM) suggests that there is a larger variation in data collected from some of the concentrations tested. Consequently, there is doubt as to the accuracy of the data and in order to ensure the reliability of the data, replication of the experiment would need to be conducted.

Throughout the experiment conducted, various precautions were taken to limit the amount of error introduced it the experiment. Firstly, pipettes were used to measure the amount of each solution of ethanol. This was done deliberately to avoid the large absolute uncertainty of

beakers and measuring cylinders due to the large distance between each increment, making it harder to measure a precise value. Secondly, the pipette was rinsed with distilled water to remove any excess ethanol still in it. This ensures the concentrations of the various solutions are accurate. Lastly, the colourimeter was zeroed with a blank of distilled water, which ensured the value read and recorded was correct.

(Vernier, n.d)The amount of dye released from beetroot when placed in ethanol solution can be linked to multiple factors such as the impact of ethanol on betacyanin. At a concentration higher than 60%, based on the data collected, it can be concluded that the ethanol begins to affect the dye as at 60% the mean absorbance was 0.07. Whereas, 80% was 0.51 and 100% was 0.42. There are many factors that impact the stability of betacyanin including pH, light, temperature, metal ion presence and enzymatic activity (Esquivel, 2016). Enzymatic activity may have impacted the dye in the experiment conducted as ethanol is known as a temporary 'enzyme inducer' then an 'enzyme inhibitor' (Lowery, 2004). In this instance, it can be concluded that ethanol acted as an enzyme inhibitor of betacyanin decolourising enzymes for a short period of time while it dissolved the plasma membrane to release the dye from the tonoplast (Gabriela Casique-Arroyo, 2014). Once it was released, the ethanol became an enzyme inducer and sped up the process of degradation of the betacyanin molecules (Lowery, 2004). The higher concentrations of ethanol (80%, 100%) would have released the dye faster as there was a higher number of ethanol molecules. This would also suggest more betacyanin molecules would've been degraded as there was more molecules of ethanol to induce the enzymes. This supports the decrease in the absorbance seen in the graph. Furthermore, this would imply that 100% ethanol did cause the most damage to the membrane of the cells, which supports the initial hypothesis, despite the lower levels of absorbance seen in the graph. In summary, it can be concluded that 100% ethanol caused the most damage to the membrane of beetroot cells, contrary to a deduction based on the levels of absorbance seen on the graph which indicates that 60% ethanol caused the most damage.

Limitations

Throughout the course of the experiment, various ways to improve the methodology were identified. Firstly, the samples of beetroot were not blotted dry before they were placed into the test tube. This did not remove any dye still on the sample from the cutting of the sample, despite rinsing it with distilled water. This created systematic error in the results as all the samples would've had slightly more dye than what was released due to the ethanol, as a result of this. This could be rectified by modifying the methodology to ensure the samples are blotted dry before placing them in the ethanol solutions. Secondly, when the test tubes were mixed every minute after the beetroot was added, the force used, and the exact time they were mixed for and, therefore the extent to which it was stirred was varied throughout the trials. This may have caused some dye to not be mixed throughout the solution properly, meaning the solution may have been read by the colorimeter to have less absorbance than it should have. This could be reduced by using centrifuges to spin the test tube as it would spin at a constant speed and for a designated time, reducing the random error introduced into the experiment by mixing it by hand.

Conclusion

In conclusion, based on the data collected, a 100% ethanol solution caused the most amount of damage to the cells as the dye was released but degraded due to enzymes being induced by

the increased amount of ethanol. This was supported by the research presented.

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