# The Effects Of pH On The Plasma Membrane And Betacyanin Absorbance In Beet Roots

## Abstract

When looking at Beetroots the effects of pH can be seen through the pigment of Betacyanin stored within the cell membrane by testing absorbance of the pigment released in a solution due to effects pH has on the permeability of the membrane. The solutions applied to the cells roots leads the cells to become disrupted causing an open membrane which allows the leakage of betacyanin because it diffuses itself into the cell membrane creating distortion and leakage of pigment once stored inside. Upon research, the proposal that pH inside the optimal range for beetroots will be higher than those outside did show significant with experimentation.

## Introduction

The plasma membrane of the cell is semi-permeable, meaning it allows certain substances to pass through, but not all. The root cells of plants are a key regulator factor of water transportation when looking at the movement through the entire plant. Depending on the environmental conditions and the plant–water balance, plants can modify the relative contributions of apoplastic (the space outside of the plasma membrane which materials can diffuse freely) and cell-to-cell pathways of water flow across roots to adjust the overall hydraulic conductivity (ability of the material to transit fluid through pore spaces and fractures in the presence of an applied hydraulic gradient). Aquaporins are essential in the cell-to-cell pathway as their presence allows not only higher water permeability but also control and regulation of water flow producing a fine control of hydraulic conductivity, which are highly sensitive to pH.

Plasma membrane vesicles isolated by two-phase partitioning from the storage root of Beta vulgaris show atypically high-water permeability that is equivalent only to those reported for active aquaporins in tonoplast or animal red cells (Alleva et al. 2006).However, this method may be disrupted when a toxic substance attempts to diffuse itself into the cell, thus allowing other materials to leak out and overall, altering the permeability of the cell. A prime example of such substances would be ethanol, which can distort the plasma membrane, and consequently, make it more fluid. It has been proposed that alcohols are physiologically active because they can penetrate the phospholipids (class of lipids that are a major component of all cell membranes) of the membranes (Grunwald 1968). The addition of alcohol results in a dehydration of the membrane, which leads to an open membrane configuration and, in turn, to a betacyanin efflux (Grunwald 1968). In a previous tested experiment, we were able to test a toxic substance theory by placing a beet cube in 50% ethanol solution, and measuring the absorbance of betacyanin, which is a non-toxic food colorant found in beets. Betacyanin is a pigment belonging to the family of pigments known as betalains that are water soluble alkaloids found in beetroots and it withholds high concentrations of it within the plant (Dini 2019).

Through testing we learned a 50% ethanol solution is strong enough to make membranes leaky because absorbance reading was taken at 2-minute intervals and were able to observe the absorbance increase of betacyanin in 50% ethanol due to the readings on a spectrometer. We

were also able to observe the absorbance increase due to the color change of ethanol by way of the diffusion of the betacyanin. For our individual investigation, we decided to take it a step further and look at what effect would adding different pH solutions to 50% ethanol have on the absorbance readings of betacyanin found in beet roots? Our reason for being interested in the Ph effect is Water transport in purified plant membrane vesicles was also reversibly blocked by H+ in a study done on Arabidopsis (small flowering plant related to cabbage) (Gerbeau et al. 2002). The H+ contribute to a membrane?delimited switch from active to inactive water channels that may allow coupling of water transport to cell signaling and metabolism (Gerbeau et al. 2002). When looking at Beetroots the stability of betanin depends directly on its pH, which ranges from 3 to 7, with the optimum pH being between 4 and 5 (Antigo et al. 2017). In other research it was concluded that color intensity was strongly dependent on the pH (Cejundo et al. 2016). That being said, our hypothesis is that if we add various pH levels to a solution of 50% ethanol, then the absorbance readings will be higher for pH solutions in the optimal range of beetroots because values higher and lower of the optimal pH cause the membrane to become less effective until it reaches the point where it becomes denatured. At the point of denaturation, the membrane is no longer able to control what travels within the cell.

## Materials and Methods

We will be observing the effects of different Ph levels within the absorbance of betacyanin found in beet root by measuring different absorbance readings using a spectrometer. To test our hypothesis, we used a modified version of Dr. Michael Dini's of Lab 3: The Plasma Membrane. Before beginning the experiment, you will need to turn on the spec 20, set it to absorbance reading and calibrate it to a wavelength of 520 nm using the "up" or "down" arrows. The setting is because betacyanin produces a red pigment which can be read on the spectrometer around 520nm. While the spectrometer is warming up, you will need to obtain 5 small chunks of beet root for each test by using a metric ruler and scalpel to cut the meat of the beet root into 4-mm cubes. Once the 5 chunks of beet root have been cut to the proper size rinse them off with RO water and set them aside. You will set up and label 5 different test tubes according to the test being ran for the according Ph Level. The control test tube can be labeled "C" and will contain 10 ml of 50% ethanol. The following 4 test tubes will be labeled according to the Ph used and will contain 5 ml of 50% ethanol along with 5 ml of Ph 4, 6, 7, and 9. Once the spectrometer is set, calibrate the Spec 20 by inserting the control test tube in the sample port, closing the port, and adjusting the A/T/C setting until transmission is selected. Next push the 0 Abs 100% T button. Calibration is crucial because even without the reaction occurring the ethanol solution may have some absorbance reading of its own. After calibrating your Spec 20, begin by taking your 5 labelled test tubes, pieces of parafilm and the 5 (4-mm) cubes of beet root over to the Spec 20 where you will begin the experiment. To begin you will need to invert your control test tube twice by using a piece of parafilm and coving it with your thumb.

After inverting the test tube, you will need to clean the outside of the tube using a micro-wipe before putting it into the sample port and recording this reading to have as a baseline for the experiment. In the second step, you will proceed to lower the one beet root cube into the control test tube by using a dissecting needle to spear the cube. Once the beet root is in the solution begin a timer and begin continuously agitating the cube in the solution for a 2-minute interval. A simple way to perform agitation is by using your thumb and 2-3 other fingers to grip the rim of the cuvette and the initiate a gentle vibration in your wrist. At the two-minute mark you will take the beet root cube out, place a parafilm sheet over the opening of the test tube and invert it

twice, then use a micro-wipe to clean the test tube before inserting it in the sample port to take your second absorbance reading and recording it in Table 1 every two minutes for the control test tube. You will proceed to do the second step outlined taking a reading every two-minutes for a total of ten minutes. At the end of the first run make sure to have the readings of the control test tube, recorded in a labelled table, you should have 5 readings for the 10-minute trial. You will complete these first two steps for every trial of the labelled test tube with the different amounts of Ph solution. Tables 2-5 are given a specific title to correlate to the test being done at that time. Make sure to record the absorbance reading in the appropriate table. To be able to analyze data it is importance to consider the aspect of replication. Replication is essential to science because it allows data to become reliability when there is certainty that the similar data can be reproduced in replicate trials. In our experiment, we did a replication of two for each given solution: our control (1o ml of ethanol), Ph 4 solution, Ph 6 solution, Ph 7 solution, and Ph 9 solution.

With the data obtain from our experiment a t-test was preformed between the pH with the highest absorbance reading and the pH with the lowest absorbance reading. With the data provide in Table 1-5 the average absorbance was taken from the two replications. Table 6 provides the information needed to perform the test, the pH with highest absorbance was pH 6 with the final reading after 10 minutes being .882 and the pH with the lowest absorbance was pH 9 with the final reading after 10 minutes being .469.With the pool variance the t-test value was calculated out to be 1.515. Running a one-tail t-test the probability is set at .05and using the t-distribution chart, our value if between .01 and .05. With this information it can be said, that there is between a 10% probability and a 5% probability that our results could have been due to the operation of chance alone, which is moderately low. With the cut off at 5% the difference between pH 6 and pH 9 is not statistically significant. (t=1.5154, d.f. 8 p > .05).

## Discussion

After analyzing the current data, we fail to reject the null hypothesis that the absorbance readings of betacyanin will not be higher within the optimal pH range of 3-7. To accept that there is a significance in pH values the t-value needed to be in-between 1.860 and 2.306. In science the degree of certainty needs to be less than 5% and ours was not, in order to show a relationship between pH and absorbance of betacyanin’s optimal range level. The optimal range found in research was not back up by our data which was found to be 3-7 in previous research done by Antigo et al. Part of the possible problems of our experiment could have been due to using 50% ethanol which is found to be strong enough to make membranes leaky. In having every solution contain ethanol within the solution it takes away from the effects of different pH values alone being used because it doesn’t allow you to just see the effects alone. Rather we should have used various pH’s in solutions that do not cause the plasma membrane to leak such as RO water. Allowing you to see the effects of pH alone. Our gap of knowledge in making sure every variable is control is shown through the Ro water vs. ethanol. Procedural errors may have occurred within the agitation of the beetroot which can be seen in Table Threes two replications because human error is natural to occur within various people performing the experiment. Another potential to see if it results in having data that is significantly statistically is the ability to run more than two replications of the experiment to have more data to base the conclusion off of because it could be that there was too little to base research off of.

## Literature Cited

1. Alleva, K., Niemietz, C. M., Sutka, M., Maurel, C., Parisi, M., Tyerman, S. D., & Amodeo, G. (2006). Plasma membrane of Beta vulgaris storage root shows high water channel activity regulated by cytoplasmic pH and a dual range of calcium concentrations. Journal of Experimental Botany 57(3), 609–621 (https://academic.oup.com/jxb/article/57/3/609/510675)
2. ANTIGO, Jéssica Loraine Duenha, BERGAMASCO, Rita de Cássia, & MADRONA, Grasiele Scaramal. (2018). Effect of ph on the stability of red beet extract (Beta vulgaris l.) microcapsules produced by spray drying or freeze drying. Food Science and Technology, 38(1), 72-77. Epub May 15, 2017. (https://dx.doi.org/10.1590/1678-457x.34316)
3. Cejudo-Bastante, M. J., Hurtado, N., Delgado, A., & Heredia, F. J. (2016). Impact of pH and temperature on the colour and betalain content of Colombian yellow pitaya peel (Selenicereus megalanthus). Journal of food science and technology, 53(5), 2405–2413. doi:10.1007/s13197-016-2215-y (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4921092/)
4. Dini, M. L. (2019). Laboratory Manuel for Biology I (Biol 1403). STIPES PUBLISHING L.L.C.
5. Gerbeau, P., Amodeo, G., Henzler, T., Santoni, V., Ripoche, P., & Maurel, C. (2002). The water permeability of Arabidopsis plasma membrane is regulated by divalent cations and pH. The Plant Journal, 30(1), 71–81. doi: 10.1046/j.1365-313x.2002.01268.x (https://onlinelibrary.wiley.com/doi/full/10.1046/j.1365-313X.2002.01268.x)
6. Grunwald C. (1968). Effect of sterols on the permeability of alcohol-treated red beet tissue. Plant physiology, 43(4), 484–488. doi:10.1104/pp.43.4.484 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1086876/?page=1)