OSJ | OPEN SCIENCE JOURNAL

REVIEW

Studying the Rate of Polyphenol Oxidase Activity in Apples Using a Vernier Colorimeter

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OPEN ACCESS

Citation: Chandrasekhar S. (2021) Studing the Rate of Polyphenol Oxidase Activity in Apples Using a Vernier Colorimeter. Open Science Journal 6(1)

Received: 6th July 2020

Accepted: 5th January 2021

Published: 15th March 2021

Funding: The author(s) received no specific funding for this work

Competing Interests: The author has declared that no competing interests exists.

Abstract:

Salt is one of the oldest methods used to preserve food and help it stay fresh by preventing rotting due to its antibacterial properties. This study was therefore conducted to understand how the increase in salt concentration affects the rate of change of polyphenol oxidase activity in apples using a colorimeter. A colorimeter is a device used to measure absorbance of particular wavelengths of light by specific solutions, in this case, Apple juice. The results correlated with the hypothesis which is predicted that as the salt concentration of the dilutions increase, there will be a decrease in the rate of change of absorbance because the chlorine ions from the salt will inhibit the polyphenol oxidase activity and prevent further oxidation of phenols to quinones, limiting the polymerization and formation of melanin. The results confirm that salt is a noncompetitive inhibitor of polyphenol oxidase because it slows down the catabolic processes in apples- mainly the breakdown of glucose. However, salt is a non-competitive inhibitor of PPO but as for lower concentrations, such as 0.25M and 0.50M, the higher concentration of substrates still mitigates the effect of salt as an inhibitor to a significant extent.

Keywords: Polyphenol oxidase, Apple, Vernier Colormeter

Introduction

Since the Food wastage is an important issue in today's generation. More people are dying from obesity than malnutrition and therefore it is important to learn to maximize food storage and quality. Approximately 16.91 pounds of

apples were consumed in 2018 in the United States and it takes approximately only five to 10 minutes for apples to brown. It is important for the public to know what they are eating; how nutritious it is and how to ensure they are eating the healthiest version of the food they consume. Browning is the process by which there is a color change seen in food during prepreparation, processing or storage and it can either be non-enzymatic or enzymatic. [4] Polyphenol oxidase catalyzes enzymatic browning reactions in most fruits. Polyphenol oxidase catalyzes the oxidation of o-diphenols and o-quinones followed by non-enzymatic formation of melanin through polymerization. [3] Apples and potatoes are rich in these polyphenols and therefore, browning takes place quickly. Apples, especially apple slices, are a very popular and nutritious snack, especially for school. However, when the skin of the apple is broken, the pressure causes the cells beneath the surface to rupture and exposes all the contents inside as juice to oxygen present in the air. Oxidation leads to enzymatic browning which changes the chemical and physical properties of the fruit.[4] When an apple is cut, oxygen comes into contact with the injured plant tissue. When oxygen is present in the cells, PPO enzymes in the chloroplast rapidly oxidize phenolic compounds naturally present in the apple tissues to o-quinones. O-quinones then produce brown color by reaction to form compounds with amino acids. There are a couple of methods to measure enzymatic browning. [8] Traditionally, enzymatic browning is measured using biochemical indices like polyphenol oxidase or physical indicators like surface color in the CIE L*a*b* co-ordinates.

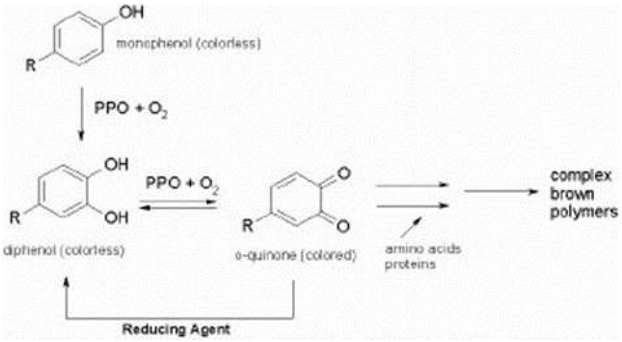

Figure 1- Oxidation reaction

Enzymes, also known as biological catalysts, are proteins that lower the activation energy of many complex, metabolic reactions in the body. [1] However, in certain reactions, enzymes are inhibited through competitive or non-competitive inhibition. Non-competitive inhibition takes place when a molecule binds to the allosteric site of the enzyme. Due to this reaction, the substrate can no longer bind to the enzyme. However, as the inhibitor is non-competitive in nature, higher substrate concentrations can overcome the effect of the inhibitor. [3]

Salt solution is used as it reduces the amount of water on the surface of the apple through osmosis as the salt has a lower concentration of water, hence water in the apple would move out of the apple, thus slowing down oxidation. [5] It is predicted that an increase in the salt concentration will result in a decrease in the rate of change of absorbance at 470nm because the chlorine ions from the salt will inhibit the polyphenol oxidase activity and prevent further oxidation of phenols to quinones, limiting the formation of melanin and polymerization. [3]

Risk assessment and ethical considerations

It is ideal to wear latex gloves and a lab coat while dealing with substances as direct contact with fingers can contaminate solutions. [2] Scalpels, syringes, needles, and other sharp objects must be handled with risk. Furthermore, All the waste from the apples must be composted or disposed for biodegradable waste.

Materials and method

Prepare $100cm^!$ of each salt solution (0.25, 0.50, 0.75 and 1.00M). Purchase 6 Granny Smith Apples were bought from the local supermarket. Peel an apple using a peeler and dice them into small pieces using a scalpel. Add a few pieces into the juicer one at a time making sure the juice is extracted into the beaker. Pour the juice concentrate into the test tubes and place it in the Vernier Force Apparatus Centrifuge. Set the timer to 5 minutes using a stopwatch. Fold the funnel paper, place it into the funnel and add 2-3 drops of distilled water to wet the paper. After the centrifuge has stopped, take out the test tubes. If the concentrate separates into the solid layer on top and apple juice at the bottom, use a skewer to poke a hole through the solid layer to allow the juice to flow out. Pour the separated mixture through the funnel, into a zip lock bag and seal out as much air as possible. Calibrate the Vernier Colorimeter by setting the absorbance to 0. Using a micropipette, drop $1cm^!$ of apple juice into the $10cm^!$ measuring cylinder and dilute it with $5cm^!$ of water. Fill 2/3 of the cuvette with the solution and insert into the colorimeter. Simultaneously, start the stopwatch and take the readings every 30 seconds. Repeat the last 2 steps for all the other solutions.

Results and conclusion

Table showing the raw data obtained when testing how different salt concentrations (0.25, 0.50, 0.75 and 1.00M) affect the rate of polyphenol oxidase activity in apples using a colorimeter?

Table 1- Raw data

	Absorbance at 470nm					Average
	Time (seconds)					
Concentration of solution	0	30	60	90	120	
0	0.05	0.06	0.08	0.10	0.10	0.078
0.25	0..04	0.05	0.06	0.06	0.07	0.056
0.50	0.02	0.02	0.03	0.04	0.05	0.032
0.75	0.01	0.01	0.02	0.02	0.02	0.016
1.00	0.01	0.01	0.01	0.01	0.01	0.01

Graph showing the effect of change in salt concentrations (0.25, 0.50, 0.75 and 1.00M) on the rate of polyphenol oxidase activity in apples using a colorimeter?

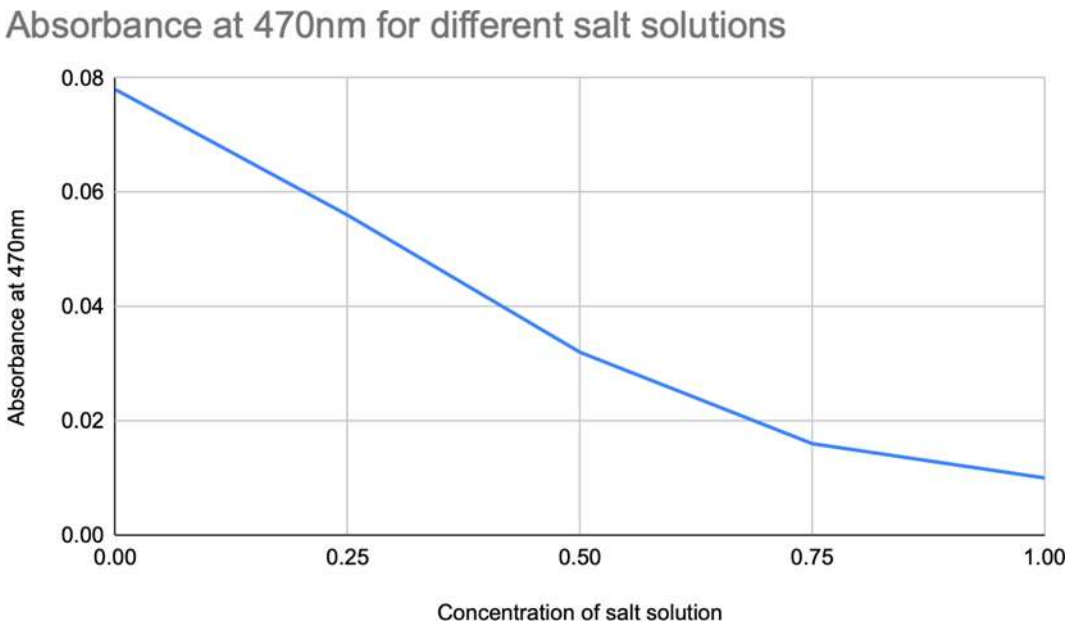

Figure 2-Average absorbance of solutions at 470nm

Analysis and evaluation of results

The rate of polyphenol oxidase activity in apples was measured at 470nm using a Vernier Colorimeter because apples appear green because they absorb red and blue so when they start the browning process, they absorb less red which lie within the wavelength band of 580-700nm.The graph shows a general decrease in the mean values of absorbance at 470nm as the salt concentrations increase from 0M to 1.0M. Therefore, it is evident that salt does act as a non-competitive

inhibitor of polyphenol oxidase because it slows down the catabolic reactions in the apples. Oxidation is the main cause of browning in fruits, and the one-two punch of submerging apple slices in cold water and salt interferes with oxygen reaching the fruit's surface and turning it brown. [9] The graph shows a trend where the salt concentration is directly proportional to the rate of enzymatic browning of polyphenol oxidase. As the time increases, the solutions change color and become darker due to the oxidation reactions and the absorbance increases. Therefore, as the concentration of salt increases, the rate of change of absorbance decreases. Salt is a non- competitive inhibitor of PPO and for the lower concentrations, a high concentration of substrate can mitigate the effect of salt as an inhibitor.

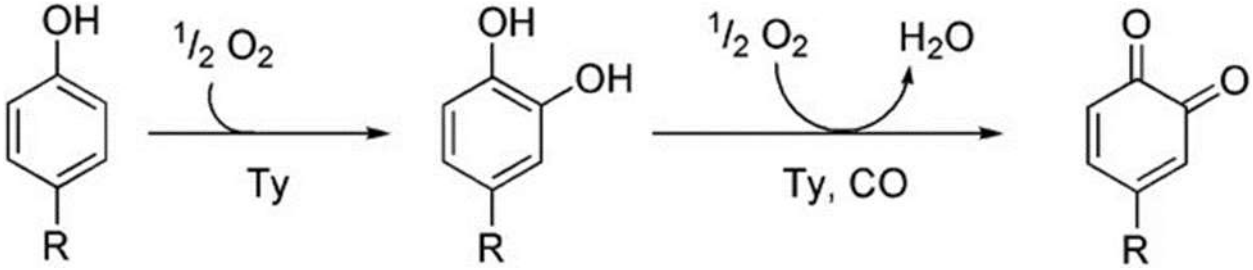

Figure 3- Oxidation reaction in apples

Acknowledgements

I would like to thank my teachers, seniors and counselor for guiding and supporting me while writing this report. I would also like to thank my parents and sister for being so encouraging and helping me every step through the way.

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