Oxidation rates of apple slices under different pH conditions

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Introduction:

When an apple is cut, oxygen enters the tissue of the apple. The oxygen, now present in plant cells, reacts with an enzyme called polyphenol oxidase (PPO), which is present in the chloroplasts and mitochondria of the apple. When PPO begins to oxidize phenols, the production of the melanin pigment occurs. This newly formed melanin is the same pigment that exists in the human body and is responsible for attributing color to the skin. PPO typically performs an antioxidant protective function for the apple, but when apple cells are broken down and their contents, including polyphenol oxidase, are released, the reagents required for apple oxidation mix, turning the apple brown where it was sliced.

As mentioned before, polyphenol oxidase (PPO) is an enzyme, and, thus a protein whose function requires specific conditions. The enzyme's shape determines its function: changes in temperature or pH lead to the protein denaturing or loss of its shape. In other words, outside of their optimal range enzymes will not function properly. Thus, this experiment looks at the impact of pH level on apples , which have a natural pH of 3.5 to 4.0. By putting in apples in solutions outside of that range, PPO activity, and thus browning rate of apples, will be affected. Acidic solutions such as lemon juice are known to slow the browning/oxidation rate of apples, implying that these acidic solutions denature the PPO enzyme. Thus, pH is known to affect the browning rate of apples, and this experiment will show how different pH solutions from acidic to basic impact oxidation. A solution of pH 2 was chosen to reflect an acidic solution, a solution of pH 6 to reflect a mildly acidic solution, and a solution of pH 10 to reflect a basic solution.

Hypothesis and Prediction:

The hypothesis is that pH will have an impact on apple oxidation. The more acidic the solution that the apple is placed into, the less browning will be observed due to the pH of the solution leading to the denaturing of the enzyme PPO. Lemon juice, an acid with a pH of about 2, is commonly used to prevent apple oxidation, providing support for the hypothesis that an acidic solution will slow browning more than a basic solution. The solution of pH 2 acted as the acidic solution that should reduce PPO activity to the greatest extent, the solution of pH 6 was mildly acidic, and the pH 10 solution was the basic solution, which will most likely not slow apple oxidation. Bases like baking soda, for example, are not typical used to slow apple browning like lemon juice is. Suppose apple slices are placed into three different experimental conditions, a solution of pH 2, pH 6, and pH 10, and two control conditions of air and dH2O. In that case, the apples placed into the acidic solution of pH 2 will exhibit a slower rate of oxidation over the course of 180 minutes by appearing less brown. Apples placed in a solution of pH 6 will also display a slower oxidation rate than the basic pH 10 solution due to it being mildly acidic. The solution of pH 2 will denature the enzymes to the greatest extent, while pH 6 will denature them slightly, and pH 10 will not be able to denature the enzymes. Finally, the browning rates of apples exposed to air will be higher than all the other apples placed in solutions because oxygen will be able to enter the damaged apple cells more easily. However, apples placed in water will display similar oxidation rater to the apples in pH 10. dH2O is neutral, but the water will help keep oxygen from entering the apple cells.

Materials and Methods:

A single apple was cut into fifteen even slices. The apples were then lightly scored with a fork to break down some cells. Three slices were placed into each of the five cups corresponding to five experiment conditions. For the four cups that contained the solution, enough of the condition's respective solution was added to cover the apple slices. In Control 1, three apple slices were placed into an empty cup for Air Control. Control 2 had three apple slices and enough deionized water to cover the apples. Condition 3 had three apple slices and enough pH 2 solution to cover the apples. Condition 4 had three apple slices and enough pH 6 solution to cover the apples. Finally, Condition 5 had three apple slices and enough pH 10 solution to cover the apples. For each condition, about 5mL of solution was enough to cover the apples. In order to observe oxidation, the apples were kept in the cups and allowed to soak in their respective solutions for five minutes before being taken off and placed onto the cutting board to let dry for twenty-five minutes. After thirty minutes, a picture was taken of the slices, and they were put back in the solution for five minutes immediately after. This process of five minutes on and twenty-five minutes off was repeated six times until 180 minutes had passed. The only exception to this process was at 0 minutes and 5 minutes. At 0 minutes, the apples had not yet soaked, while at 5 minutes, the apples had soaked for five minutes. Finally, following the total 180 minutes, the pictures taken of the apples after 0, 5, 30, 60, 90, 120, 150, and 180 minutes were analyzed according to the color palette shown below. The color of the apple was matched to the closest corresponding value, and the average color of the condition at every time point was calculated to examine the oxidation rate of apples (Figure 1).

Figure 1. Color scaling palette for oxidation of apple slices. A higher number correlates to a deeper brown color and, thus, a higher rate of melanin production and apple oxidation. In other words, the darker the color, the more the apple slice has oxidized. This color palette, thus, was used in order to quantify apple browning data.

Written Results:

Initially, the average color value for all the apple slices across all conditions was 1. After 5 minutes, however, the different conditions displayed different rates of browning. The Air Control showed the highest average color value of 1.7, while the dH2O, pH 6, and pH 10 solutions displayed an average color value of 1.3. The pH 2 solution, however, stayed at a color value of 1, with no browning being observed. At the 30-minute mark, the conditions had differentiated even further, with the Air Control again having the highest color value of 3.3 and pH 2 having the lowest of 1.3. The dH2O displayed an average color of 2.7, the pH 6 solution of 2.3, and the pH 10 solution of 1.7. After 60 minutes, the same trend of browning was observed with the Air Control being the highest and the pH 2 being the lowest. The average color value of the dH2O, and pH 2 and pH 10 solutions all seem to have fallen, however. But what seems to be a reverse of browning can be explained by issues with the pictures that made the apples appear lighter than they were. After 90 minutes, though, all average color values had increased, with most notably the average color value of pH 2 increasing to 2. For the rest of the conditions, the Air Control was 3.7, dH2O and pH 6 was 3.0, and pH 10 was 2.3. At the 120-minute mark, the average color values of pH 2 and 6 remained the same: 2 and 3, respectively. All others have increased: the value for the Air Control being 4.7, dH2O Control 3.3, and pH 10 3.0. After 150 minutes, pH 6 and pH 10 had the same average color value of 3.3, while pH 2 has increased slightly to 2.3. The Air Control has an average color value of 5.7, a significant increase, and the dH2O an average value of 4.3, another significant increase. Finally, after 180 minutes, the Air Control ended at the highest average color value of 6.3; the pH 2 solution ended at the lowest of 2.7. The dH2O control was the second highest at 5.0, and pH 6 ended at 4.7, while pH 10 had a similar value of 4.3. Overall, the Air Control showed the highest melanin production, while pH 2 showed the lowest. The rates of oxidation also appeared to stay relatively consistent throughout the entire experiment, meaning that the color value for each increased by about the same amount every time. The apples in the Air Control displayed the fastest rate of oxidation, whereas apples in pH 2 displayed the slowest. Overall, the Air Control had the highest average color value, and, thus highest PPO activity along with the fastest oxidation rate. pH 2, on the other hand, had the lowest average color value and, thus, lowest PPO activity along with the slowest rate of oxidation.

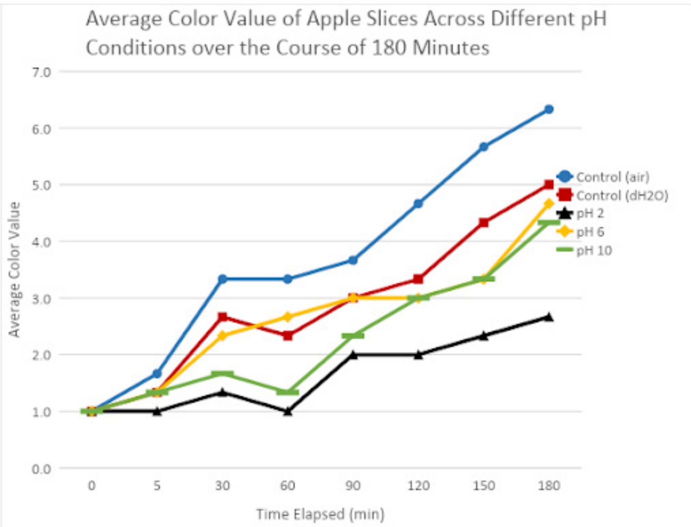

Figure 2. Color Scaling of Apple Slices over the course of 180 Minutes under Different pH and Control Conditions. The graph shows the Air Control displaying the highest average color value, and, thus, the greatest polyphenol oxidase activity; the pH 2 solution, on the other hand, displayed the lowest. Finally, the average color value of the dH2O Control, pH 6 solution, and pH 10 solution were all relatively similar, so polyphenol oxidase activity was similar in these conditions. Elapsed time served as the x-axis, or independent variable, and average color value, or the average "brownness" of the apple, was the y-axis and the dependent variable.

Conclusion:

The data found in this experiment supported the hypothesis that the oxidation and, thus, melanin production of apples is affected by the pH of the solution the apple is in. It was previously predicted that a lower pH would lead to less melanin production or a slower oxidation rate because the acid would denature the enzyme polyphenol oxidase. The prediction was partly correct, with the apples in a pH 2 solution having the lowest average color value at the end of the 180 minutes, but pH 6 had a higher average color value than the solution of pH 10. However, compared to pH 2, the average color values of pH 6 and pH 10 were relatively close. The scoring on the apples immersed in pH 10 solution also did not appear to be as deep as the others, which may reveal? affected melanin production because oxygen could not react with PPO. Only acidic solutions may be able to slow the rate of oxidation in comparison to mildly acidic solutions like pH 6. pH 6 is relatively close to the pH of dH2O, 7, possibly explaining the average color values being somewhat close to each other. Thus, the hypothesis is still supported due to similar melanin production by pH 6, pH 10, and dH2O. Regarding the two controls, the apples were in neutral conditions in both cases. However, in the case of dH2O, the water may have acted as a "buffer" for the PPO and the oxygen by preventing the oxygen from entering some of the damaged apple cells. The apples in the air have no protection from oxygen, in other words.

Looking at the experiment from a broader perspective, pH 2, pH 6, and pH 10 solutions were chosen to have a spectrum of acidic to neutral to basic. The solution of pH 2 represented an acidic solution, pH 6 a mildly acidic solution, and pH 10 a basic solution. As mentioned before, acidic solutions like lemon juice have been observed to slow melanin production in apples, and so that knowledge went into forming the hypothesis. As evidenced by the melanin production, PPO activity was most likely affected; if PPO activity was not impacted, at least all the apple slices in the solution, if not all apple slices, would all have shown the same average color value. The data, however, shows a wide range of color values, with a higher color value indicating higher PPO activity and, thus, greater melanin production. Initially, the intensity of the scoring of the apples may have also affected color rate. Differences in apple scoring were thus a limitation. If one apple has more damaged cells than another, then the apple with the more damaged cells will display greater browning. More PPO free from the apple's mitochondria and chloroplasts to react with oxygen. Keeping the scoring of the apples constant would help the results to be more reliable. Finally, given how pH 10 displayed a lower average color value than pH 6, a different experiment could be performed to see the impact of bases on apple browning and PPO activity. In this experiment, an acidic solution, a mildly acidic solution, and a basic solution were tested, but such future study could include apples being placed into a mildly basic solution. If the average color values of the mildly basic, mildly acidic, and basic solutions are all similar, then it could be concluded that only acidic solutions can slow the rate of oxidation and limit PPO activity. Right now, the data suggests that both extremes of the pH scale, basic and acid solutions, slow apple oxidation.

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