# *An investigation of how the volumes of lemon juice added would affect the rate of enzymatic browning reaction in apple juice*

***Research question:*** How do different volumes (0mL, 2mL, 4mL, 6mL, and 8mL) of lemon juice affect the degree of catechol oxidase's enzymatic browning reaction in ten minutes, measured by the transmittance of different wavelengths of light (at 430nm, 470nm, 565nm, and 635nm) and the hue and lightness values of the color of apple juice?

## *Introduction*

When we slice am apple, we can observe the cut surfaces of apple slices gradually turning brown. This process is called "enzymatic browning" (*"Why Do Sliced Apples Turn Brown? | Britannica,"* 2023).

In this reaction, polyphenol oxidase (PPO), a family of enzymes including catechol oxidase, catalyzes o-hydroxylation of monophenols to o-diphenols and oxidases o-diphenols to quinones (*Soha Ahmadzadeh Araji et al.*, 2014). O-quinones are then used to form melanin, a dark pigment which contributes to the brown color of oxidised apples (*Solano et al.*, 1999).

## *Background Information*

The chemical "catechol" is a benzenediol comprising of a benzene core carrying two hydroxy substituents (*PubChem*, 2023). O-quinone is produced from it and further oxidised into melanin, contributing to browning. The reaction pathway is shown in Fig. 1.

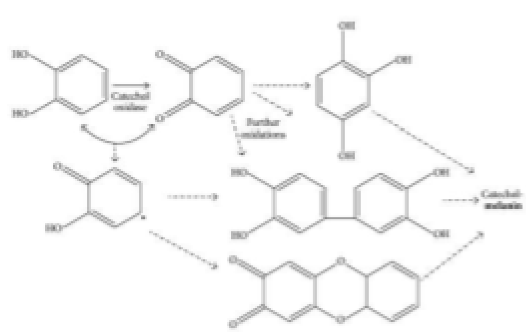

**Fig 1** The reaction pathway of the production of melanin from catechol (*Solano*, 2014)

Polyphenol oxidase I (PPO) is the enzyme that oxidases catechol into 1,2-benzoquinone. The formula of the reaction is:

$$2 \text{ catechol} + O_2 \rightarrow 2 \text{ 1,2-benzoquinone} + 2 H_2O$$ (*UniProt*, 2023)

The cofactor of PPO is copper (Cu) (*UniProt*, 2023). The loose binding of the copper to the PPO allows it to function properly (*5.2: Enzyme Cofactors*, 2020). When copper cannot bind to the enzyme, the enzyme would fail to function, and the reaction rate would decrease significantly.

Lemon juice has a low pH value (approximately 2), which means that it is an acid (*2.4: The PH Scale*, 2020). As an acid, lemon juice is a proton donor, capable of donating $H^+$ ions in a reaction. When lemon juice is added into the oxidation reaction catalyzed by PPO, the $H^+$ ions interfere with the binding of copper to PPO. When there is a sufficient amount of acid that interferes with the binding of copper to PPO, the reaction cannot be catalyzed because all the enzyme complexes are saturated by $H^+$ ions. When the enzymes cannot catalyze the reaction, reaction rate decreases drastically. In the case of oxidizing apple juice, the catechol in apple juice would not be oxidized by PPOs and there would be no color change.

The Vernier colorimeter can generate light in many different wavelengths: 430nm (violet), 470nm (blue), 565nm (yellow), and 635nm (red) (Discover the Wavelength, 2018). When set to a specific wavelength, the colorimeter can measure the transmittance. When an object shows a color, the wavelengths of the color are transmitted and the other wavelengths that are not shown are absorbed. After the apple juice undergoes oxidation, it would show a brown color. The more oxidation occurs, the browner the color would be. It is expected that the colorimeter would detect a higher transmittance of yellow and red wavelengths when the catechols in apple juice undergo more oxidation reaction.

There are other  methods to “quantify” a color, such as RGB, CMYK, and HSL. In this experiment, I also expressed the colors of apple juice in terms of hue and lightness values in HSL. The hue is a degree on the color wheel from 0 to 360, where 0 is red, 120 is green, and 240 is blue. The lightness is a percentage where 0% is black and 100% is white (HTML HSL and HSLA Colors, 2023). It is expected that the apple juice would have a hue closer to 0 and a smaller lightness value when it undergoes more oxidation.

## *Variables*

**Independent variable** - The volume of lemon juice added to the apple before juicing it (0mL, 2mL, 4mL, 6mL, 8mL)

**Dependent variable** - The transmittance of different wavelengths of light (at 430nm, 470nm, 565nm, and 635nm); the hue and lightness of apple juice

**Table 1 - Controlled variables**

| **Variable** | **Why it should be controlled** | **How to control** |
|---|---|---|
| The weight of the apple being juiced | The more apple is used, the more catechol would be in the juice. Since catechol is the substrate, the rate of catalysis would increase as it increases until a maximum. This is a factor other than the volume of lemon juice that would affect the rate of oxidation reaction. | The weight of apple pieces juice each time should be 70g |
| Time spent juicing the apple | When the time spent juicing the apple with the blender is not long enough, there would be less juice that is extracted and more pulp left. As a result of a low apple juice to lemon juice ratio, more catechol oxidases in the apple juice can be inhibited, decreasing oxidation rate significantly. | 20 seconds to prepare apple juice in each trial |
| The total volume of liquid added | When more liquid (i.e., water and lemon juice) is added to the apples, the final mixture of apple juice would be clearer, increasing the transmittance of all the colors. This variable directly impacts the dependent variable measured by colorimeter. | The total volume of water and lemon juice adds up to 50mL for each trial. |
| The time allowed for reaction to take place | I have limited time to conduct this lab, so it is impossible to wait until the reaction to finish to collect the results. But the time of reaction directly impacts how much oxidation reaction takes place. Therefore, a duration of time that is enough for results to be measured is required for the experiment. | Let apple juice sit for 10 minutes after being filtered so that it can undergo enzymatic browning reaction |

## *Methodology*

### Safety considerations

In general:

Tie hair up before participating in the experiment

Avoid breaking any instrument. If any intrument is broken, make sure to not touch the sharp edges when cleaning

Wear gloves during the experiment

When handling the blender:

Avoid using hands to touch the blades of the blender

Do not open the lid of the blender until the blades have completely stopped spinning

When preparing apple:

Do not touch the blade of the peeler

Avoid cutting fingers when using a knife to cut apple into pieces

No ethical consideration is required when conducting this lab

### Preliminary Trials

In the preliminary trials, I found out the suitable time that allows the catechol to undergo oxidation reaction and the suitable values for the independent variable (the volume of lemon juice). Initially, I juiced the apple adding no lemon juice and observed that the juice turns into a yellow-brown color after approximately 10 minutes. Hence, 10 minutes is an appropriate time span to allow reaction to occur and results to be successfully determined. I gradually increased the volume of lemon juice added to apple juice when juicing apples and discovered that there is no measurable oxidation that occurs after 10 minutes when about 8-10mL of lemon juice is added. Thus, the upper boundary for the values of independent variable is 8mL, with the rest being 0mL, 2mL, 4mL, and 6mL.

### Materials

| **Material/apparatus** | **Quantity** | **Material/apparatus** | **Quantity** |
|---|---|---|---|
| Apples | 13 | Cuvettes | 6 |
| Lemon juice | 110mL | Colorimeter | 1 |
| Adapters | 2 | Blender | 1 |
| Timer | 1 | Balance | 1 |
| 250mL measuring cup | 1 | Glass cups | 2 |
| Sieve | 1 | Knife | 1 |

| | | | |
|---|---|---|---|
| Peeler | 1 | | |

**Procedures**

Preparing the apple

1. Peel an apple
2. Cut the apple into two 70g halves and put half of the apple into water for later use
3. Cut half an apple into 9 small pieces suitable for juicing

Warming up the calorimeter

1. Open LoggerPro software on the laptop and connect the colorimeter to the laptop with adapters
2. Allow the colorimeter to warm up for a few minutes
3. Fill a clean curette with 3mL of filtered water and insert the cuvette into the calorimeter (smooth sides facing the light source), close the lid tight
4. Select the desired wavelengths from 430nm, 470nm, 565nm, and 635nm
5. Click “cal” (calibrate)
6. Calibration is complete when the red light on colorimeter stops flashing

Experiment procedures

1. Add 0 mL of lemon juice into the blender and add water until the total volume of liquid is 50mL
2. Add the apple into the blender and close the lid of blender, operate for 20 seconds
3. Open the lid of the blender and let the apple juice sit for 10 min so that oxidation reaction occurs
4. Filter the juice into a clear glass cup to leave out apple pulp
5. Take a photo of the apple against a white background
6. Fill a clean cuvette with 3mL of filtered apple juice
7. Calibrate the colorimeter with the wavelength set to 430nm
8. Take out the cuvette with water and replace it with the cuvette with apple juice
9. Record the transmittance value into the raw data table
10. Repeat steps 7-9 with other wavelengths (470nm, 565nm, and 635nm)
11. Repeat steps 1-10 with 5mL, 10am, 15mL, and 20mL of lemon juice added to the apple juice
12. Repeat steps 1-11 for 5 trials

Processing color into HSL values

1. Use the APP Snapseed to adjust the white balance of the photos of results taken at each trial
2. Use color dropper tool in Adobe Photoshop to identify the color and its HSL values
3. Record the hue and saturation values into the raw data table using laptop

## *Data Collection*

### Raw Data

| Volume of Lemon Juice (mL) (±0.5) | Color of Light | Transmittance (%) (±0.05%) | | | | |
|---|---|---|---|---|---|---|
| | | Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 5 |
| 0mL | Red | 21.75 | 23.18 | 23.41 | 20.27 | 22.97 |
| | Yellow | 18.19 | 18.24 | 19.02 | 18.08 | 21.57 |
| | Blue | 6.33 | 7.01 | 6.98 | 6.21 | 6.42 |
| | Violet | 6.30 | 6.54 | 7.04 | 6.01 | 6.39 |
| 2mL | Red | 18.57 | 18.89 | 16.51 | 15.74 | 18.62 |
| | Yellow | 16.37 | 17.09 | 15.77 | 14.92 | 17.89 |
| | Blue | 6.43 | 6.39 | 6.23 | 5.17 | 6.52 |
| | Violet | 6.43 | 6.42 | 6.19 | 5.06 | 6.37 |
| 4mL | Red | 15.3 | 17.06 | 17.67 | 16.26 | 16.71 |
| | Yellow | 12.39 | 14.80 | 15.40 | 14.88 | 16.22 |
| | Blue | 4.18 | 6.28 | 5.39 | 6.16 | 6.28 |
| | Violet | 4.01 | 6.36 | 5.71 | 6.19 | 6.21 |
| 6mL | Red | 10.56 | 13.51 | 12.65 | 11.85 | 11.06 |
| | Yellow | 10.23 | 12.99 | 12.07 | 10.92 | 10.47 |
| | Blue | 5.05 | 6.45 | 6.21 | 5.44 | 5.17 |
| | Violet | 5.40 | 6.37 | 6.27 | 5.06 | 5.12 |
| 8mL | Red | 10.46 | 12.24 | 11.97 | 10.99 | 11.60 |
| | Yellow | 10.61 | 11.31 | 11.17 | 11.03 | 11.34 |
| | Blue | 6.02 | 6.43 | 6.15 | 5.98 | 6.28 |
| | Violet | 6.40 | 6.33 | 6.21 | 6.04 | 6.13 |

**Table 1** Raw data of the transmittance of apple juice when 0mL, 2mL, 4mL, 6mL and 8mL of lemon juice are added

| Volume of Lemon Juice (mL) (±0.5) | Hue (°) (±2) and Lightness (%) (±0.5) | Hue and Lightness Values | | | | |
|---|---|---|---|---|---|---|
| | | Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 5 |
| 0mL | Hue | 36 | 38 | 36 | 37 | 37 |
| | Lightness | 44 | 44 | 46 | 45 | 46 |
| 2mL | Hue | 41 | 40 | 39 | 38 | 41 |
| | Lightness | 44 | 40 | 57 | 45 | 44 |
| 4mL | Hue | 39 | 42 | 48 | 43 | 43 |
| | Lightness | 50 | 67 | 60 | 61 | 60 |
| 6mL | Hue | 48 | 54 | 51 | 47 | 47 |
| | Lightness | 60 | 63 | 62 | 60 | 61 |
| 8mL | Hue | 54 | 57 | 51 | 55 | 53 |
| | Lightness | 62 | 62 | 68 | 67 | 65 |

**Table 2** Raw data of the hue and lightness of apple juice when 0mL, 2mL, 4mL, 6mL and 8mL of lemon juice are added

## Qualitative Observations

Overall, the visible trend it that the color of apple juice becomes less brown after oxidation when more lemon juice is added. The trend is show by Fig 2, where the first cup of apple juice has a color significantly darker than that of the last cup.

In some trials, I press the pulp against the sieve harder with a spoon, trying to filter out more apple juice. I observed that the clarity of the juice varies slightly as some pulp may have passed through. This may have affected the data of transmittance values.

**Fig 2** The color of apple juice after 10 minutes of oxidation in trial 4, arranged in an increasing order of the volume of lemon juice added

## Formulae and Sample Calculations

*Formulae*

$$Average = \frac{sum\ of\ data\ for\ one\ dependent\ variable}{number\ of\ trials}$$

$$Standard\ deviation = \sqrt{\frac{\Sigma(x_i - \mu)^2}{N}}$$ (calculated with Microsoft Excel STDEV.S formula)

*Sample Calculations*

The average transmittance of red light when the volume of lemon juice added is 0mL:

$$Average\ transmittance = \frac{transmittance_1 + transmittance_2 + transmittance_3 + transmittance_4 + transmittance_5}{5}$$

$$= \frac{21.75+23.18+23.41+20.27+22.97}{5}$$

$$\approx 22.31\ (\%)$$

## Processed Data

| | Color of Light | Volume of Lemon Juice (mL) (±0.5) | | | | |
|---|---|---|---|---|---|---|
| | | 0mL | 2mL | 4mL | 6mL | 8mL |
| Average Transmittance (%) (±0.05%) | Red | 22.31 (1.24) | 17.67 (1.36) | 16.60 (0.840) | 11.93 (1.12) | 11.45 (0.685) |
| | Yellow | 19.02 (1.39) | 16.41 (1.08) | 14.74 (1.35) | 11.34 (1.10) | 11.09 (0.279) |
| | Blue | 6.59 (0.356) | 6.15 (0.525) | 5.66 (0.854) | 5.66 (0.594) | 6.17 (0.175) |
| | Violet | 6.46 (0.358) | 6.09 (0.553) | 5.70 (0.918) | 5.64 (0.595) | 6.22 (0.138) |

**Table 3** Processed data of the transmittance of apple juice when 0mL, 2mL, 4mL, 6mL and 8mL of lemon juice are added

| | | Volume of Lemon Juice (mL) (±0.50) | | | | |
|---|---|---|---|---|---|---|
| | | 0mL | 2mL | 4mL | 6mL | 8mL |
| Hue (°) (±2) and Lightness (%) (±0.5) | Hue | 36.8 (0.714) | 39.8 (1.11) | 43 (2.76) | 49.4 (2.60) | 54 (1.90) |
| | Lightness | 45 (0.853) | 46 (5.50) | 59.6 (5.21) | 61.2 (1.11) | 64.8 (2.37) |

**Table 4** Processed data of the hue and lightness of apple juice when 0mL, 2mL, 4mL, 6mL and 8mL of lemon juice are added

## Graphs

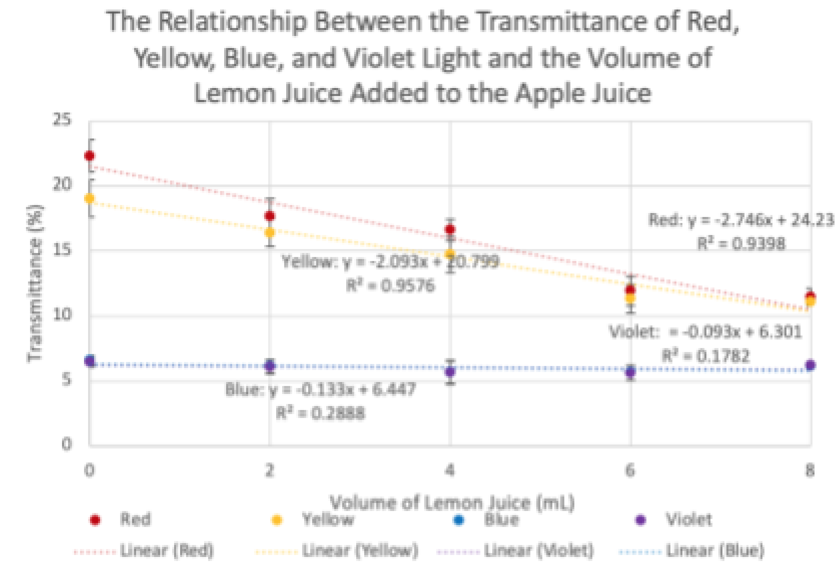


**Fig 3** The relationship between the transmittance of red, yellow, blue, and violet light and the volume of lemon juice (0mL, 2mL, 4mL, 6mL, 8mL) added to the apple juice

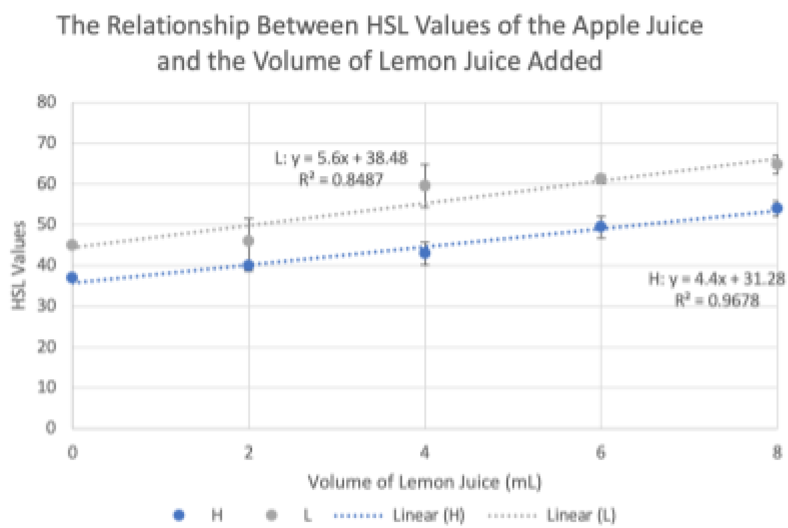


**Fig 4** The relationship between hue and lightness values of the apple juice and the volume of lemon juice added (0mL, 2mL, 4mL, 6mL, 8mL)

Vertical error bars represent standard deviations of transmittance, hue, and saturation values, indicating how scattered the raw data for these variables are.

## *Conclusion*

Linear trend lines are chosen for both graphs. As the volume of lemon juice increases linearly while its acidity remains constant, the number of $H^+$ ions increases linearly as well since it is proportional to the volume ($n = CV$). The number of PPOs (catechol oxidases) that are inhibited by the $H^+$ ions is also proportional to the number of ions present in the solution until it is saturated with the ions. Therefore, the degree of color change is proportional to the number of $H^+$ions. The transmittance of colors would decrease linearly; the hue and lightness values would increase linearly. The $R^2$ values represent how strongly the trend lines relate to the data points—the closer the $R^2$ value is to 1, the more strongly the trendline relates to the data. The $R^2$ value is 0.9398 for the transmittance of red light, 0.9576 for the transmittance of yellow light, 0.8487 for the lightness, and 0.9678 for the hue. These values show that the trend lines fit the data relatively well.

The first graph shows the relationship between the volume of lemon juice that is added when making apple juice and the transmittance of the apple juice after 10 minutes. The trend line for red light and yellow light shows a negative correlation with the volume of lemon juice. Meanwhile, the transmittance of blue and violet light remains unaffected by the volume of lemon juice added. The transmittance is positively proportional to how much of a light in a certain color is reflected, implying how saturated the specific color is. Therefore, the decreasing trend of the transmittance of red and yellow light reflects that the apple juice shows less red and yellow color as the volume of lemon juice increases while the saturation of blue and violet remains low. The trend shows as the volume of lemon juice increases, the catechol in apple juice would undergo less enzymatic browning reaction due to the inhibition of catechol oxidases.

The second graph shows the relationship between the volume of lemon juice that is added when making apple juice and the hue and lightness values of the color of apple juice after 10 minutes.The data points of both hue and lightness values show a positive correlation with the volume of lemon juice. The higher the volume of lemon juice is, the higher the hue and lightness values are. The hue value represents colors on a color wheel from 0 to 360 degrees (HTML HSL and HSLA Colors, 2023). Overall, the increasing trend of hue value reflects that the color of the apple juice turns yellower as the volume of lemon juice added increases. The lightness value tells us how dark a color is, where 0% is black and 100% is white (HTML HSL and HSLA Colors, 2023). The increasing trend of lightness values reflects that the color of apple juice becomes brighter as the volume of lemon juice increases. It can be deduced from Graph 2 that as the volume of lemon juice increases, more catechol oxidase would be inhibited, leading to a lower rate of oxidation reaction, so the color of lemon juice becomes yellower and brighter.

In conclusion, the trends shown by both graphs show that the color of apple juice after 10 minutes of enzymatic browning reaction becomes less brown as the volume of lemon juice added increases. This is due to the increase in the number of $H^+$ ions, which inhibits the catechol oxidase, decreasing the rate of oxidation reaction. When less catechol undergoes oxidation, less melanin would be formed and the color of the juice would be less brown.

## *Evaluation*

Before conducting the trials, I conducted a few preliminary trials to identify controlled variables and the values of the independent variables. Initially, I added the same volume (50mL) of water and different volumes of lemon juice. The difference in colors is not obvious. I realized that the difference in the total volume of liquid added to the apple may impact the clarity of the juice, diluting the color. For the following preliminary trials, I used 50mL of liquid (total volume of lemon juice and water). The difference in results became more detectable. I also determined the values for the independent variable produce a trend from the data. I gradually increased the volume of lemon juice added to the apples by 2mL each trial. Eventually, I determined that 8mL of lemon juice would be the maximum value for the independent variable, with the rest being 0mL, 2mL, 4mL, and 6mL. The 0mL scenario also acts as a control group because the catechol oxidases would not be inhibited at all.

There are some random and systematic errors in the experiment that may have affected the data collected. The standard deviation value represents how scattered the data set is – the higher the standard deviation is, the more scattered (less precise) the data set is.

Firstly, one systematic error that may lead to imprecise data occurs in the process of filtering apple juice to remove the pulp. When filtering the apple juice, I tried to get the most apple juice out of it by pressing the pulp against the sieve lightly with a spoon. This process may have caused extra pulp to pass through the sieve. As a result of having more opaque pulp mixed in the juice, the juice's transmittance may have decreased, which causes the data to vary from other trials, decreasing the preciseness of data. To improve, the amount of pulp mixed in the juice should be controlled. One solution is using a juicer instead of a blender, which filters out all the pulp. With no pulp in the juice, the transmittance of the juice would not be impacted by the amount of pulp. Another solution is to not press the pulp while filtering the juice. Without applying different pressures to the pulp, the amount of pulp that passes through the sieve would vary less, leading to a higher preciseness of the data.

The second systematic error occurs with the impreciseness of the apparatus. Because this experiment requires materials such as a knife and a blender, which are difficult to carry to school, I conducted the experiment at home. However, some apparatuses at home, such as balance and measuring cup, are less precise than apparatus in the school lab. Uncertainties in the measurements of materials (i.e., the mass of apple, the volume of water, etc.) would lead to fluctuations in the results. For instance, when the mass of apple is larger, there is more catechol and catechol oxidase present, so a smaller proportion of the enzyme would be inhibited by $H^+$ ions, leading to a browner color of the apple juice. When the volume of water increases, the apple juice would be less concentrated and clearer, showing a higher transmittance. These fluctuations in the data can lead to a larger standard deviation of data sets, which indicates impreciseness. The solution to this issue is to use more precise apparatuses. It is difficult to carry a heavy blender and a knife to the school labs, but I can purchase apparatuses that are more precise to decrease the variations in data caused by imprecise measurements.

The third systematic error is caused by the lack of control of reaction time. The longer the apple juice undergoes enzymatic browning reaction, the browner the color would be. Although I listed the time of reaction as a controlled variable, it is in fact very difficult to control. I may have spent extra time in these steps: opening the lid of the blender, filtering apple juice, and filling the cuvette with apple juice.

The first two steps are done before setting the 10-minute timer, while the last step is done after; the time spent completing all these steps was not considered into the reaction time. The time of reaction varies based on the time it takes to complete these reactions, which affects the color of the apple juice. The solution is to control the timing more precisely by including the time spent on all these steps into the 10-minute timeframe. By ensuring all these steps are well-controlled within 10 minutes, the result would not be impacted by varying total reaction time.

An extension of this experiment could be done by correcting the errors. Additionally, I can investigate the difference in the degree of enzymatic browning of apple slices when different volumes of lemon juice are rubbed against its surface by comparing their hue and lightness values. I expect the same trend in results to occur, but the rate of browning may differ from this experiment since the apples are in different forms.

The results of the experiment may apply to real life. When making apple juice at home, we rarely have the equipment that factories use to produce juice. But we also want to avoid the juice turning into a dark brown color since the color is less appetizing. The results of this lab show that we can make apple juice that still looks fresh after a period of time by adding lemon juice. Lemon juice inhibits catechol oxidases, so the production of melanin from catechol cannot be achieved, and there would be less color change.

References

*2.4: The pH Scale*. (2020, July 16). Biology LibreTexts. https://bio.libretexts.org/Courses/Lumen_Learning/Biology_for_Non-Majors_I_(Lumen)/02%3A_Chemistry_of_Life/2.04%3A_The_pH_Scale

*5.2: Enzyme Cofactors*. (2020, September 17). Chemistry LibreTexts. https://chem.libretexts.org/Courses/Brevard_College/CHE_301_Biochemistry/05%3A_Enzymes/5.02%3A_Enzyme_Cofactors

*Discover the Wavelength*. (2018). Vernier. https://www.vernier.com/vernier-ideas/discover-the-wavelength/

*HTML HSL and HSLA Colors*. (2023). W3schools.com. https://www.w3schools.com/html/html_colors_hsl.asp#:~:text=HSL%20Color%20Values&text=Hue%20is%20a%20degree%20on,100%25%20is%20the%20full%20color.

Soha Ahmadzadeh Araji, Grammer, T. A., Gertzen, R., Anderson, S. C., Maja Mikulič-Petkovšek, Veberič, R., Phu, M. L., Solar, A., Leslie, C. A., Dandekar, A. M., & Escobar, M. A. (2014). Novel Roles for the Polyphenol Oxidase Enzyme in Secondary Metabolism and the Regulation of Cell Death in Walnut . *Plant Physiology*, *164*(3), 1191–1203. https://doi.org/10.1104/pp.113.228593

Solano, F. (2014). Melanins: Skin Pigments and Much More—Types, Structural Models, Biological Functions, and Formation Routes. *New Journal of Science*, *2014*, 1–28. https://doi.org/10.1155/2014/498276

Solano, F., Hearing, V. J., & García‐Borrón, J. C. (1999). Neurotoxicity due to o-Quinones: Neuromelanin formation and possible mechanisms for o-Quinone detoxification. *Neurotoxicity Research*, *1*(3), 153–169. https://doi.org/10.1007/bf03033287

*UniProt*. (2023). Uniprot.org. https://www.uniprot.org/uniprotkb/Q9ZP19/entry#function

Why Do Sliced Apples Turn Brown? | Britannica. (2023). In *Encyclopædia Britannica*. https://www.britannica.com/story/why-do-sliced-apples-turn-brown