Effect of Different Edible Solutions on Apple Enzymatic Browning

Nicholas Suzuki (nicholas0831.suzuki@gmail.com)

Abstract

The Importance of fruit and vegetable consumption has been known for generations as expressions like "an apple a day keeps the doctor away" continue to be passed down. However, new insights into the importance of proper food storage and preservation methods has been on the rise. Not only has it been shown to be important in terms of vitamin intake, but over 50% of certain subsets of produce go to waste due to their unappealing browning when exposed to air (Gritzer, 2019). This study investigates this concept by analyzing the rate at which apples undergo enzymatic browning when coated in different edible solutions. Apples contain an enzyme known as polyphenol oxidases, and when these enzymes come in contact with oxygen, they interact with different phenol groups and eventually form brown polymers (Moon et al., 2020). These are responsible for the unappetizing brown stains on exposed or bruised apples. In order to stop this process from happening, there are many routes of intervention and thus, 4 different treatments (water, vinegar, milk and lemon juice) have been selected to determine how common household solutions can be used to increase the shelf life of fruits and vegetables. Through this study, it is also intended to encourage future research on the topic, such that new innovative ideas for food preservation are explored. This study showed that the most acidic and antioxidant containing solution was the most successful at slowing down the progression of the enzymatic browning when the apples were exposed to air. Results were confirmed by performing a one-way analysis of variance statistical, and the findings indicated that lemon juice proved to be the superior treatment.

Introduction

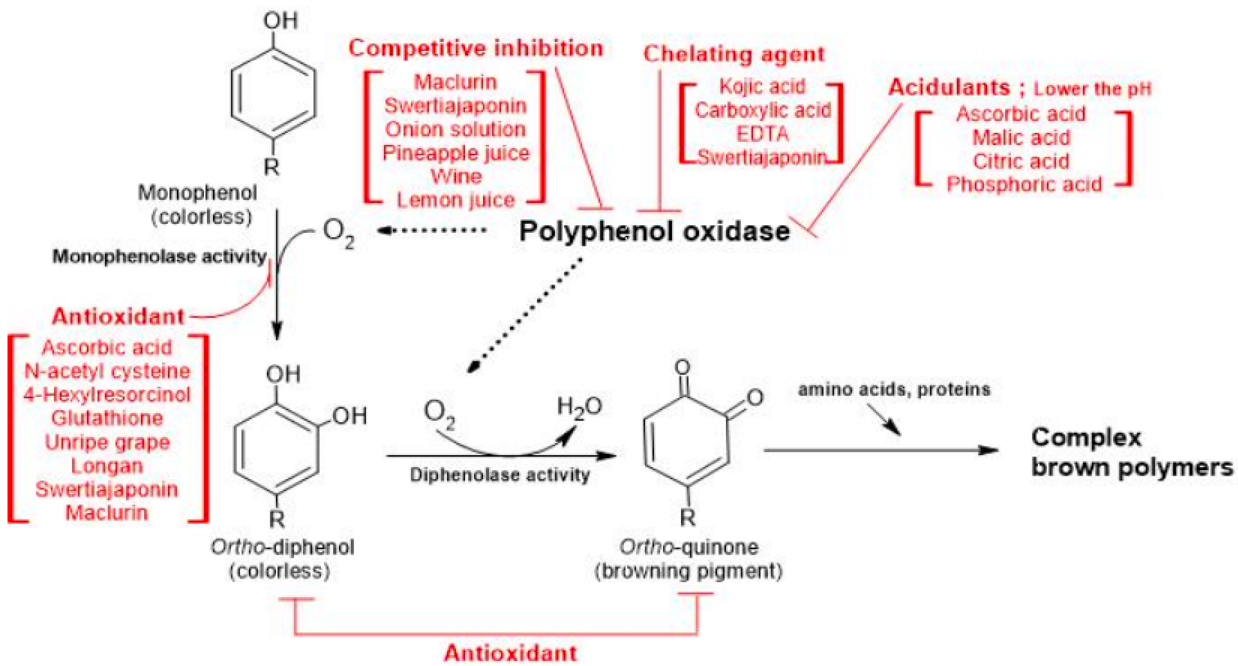
Have you ever left a freshly cut apple out, only to return to a browned and less flavourful apple within minutes. This issue is continuously being researched within the food industry to ensure the employment of the most effective methods of fruit and vegetable preservation. Not only does the oxidation of these foods serve as a flavour issue, but previous studies have indicated consumer choice of non-bruised apples is much higher than those with discolouration (Gritzer, 2019). Thus, this issue goes deeper than the taste of these items, but can ultimately change the trajectory of grocery stores, restaurants and food markets alike. Additionally, fruit and vegetable exposure to air can cause oxidation of pivotal vitamins like Vitamin C, which can affect the nutritional content of the consumable (Moon et al., 2020). When an apple is cut or bruised, the introduced air causes a process known as "enzymatic browning". Enzymatic browning occurs when apple flesh is exposed to oxygen, this causes polyphenol oxidase (PPO) enzymes within the chloroplasts of the apple to rapidly oxidize phenolic compounds into o-quinones. O-quinones react to form compounds with amino acids or proteins, or they self assemble to make polymers (Moon et al., 2020). The act of this compound formation is what is responsible for the formation of the brown-coloured melanin.

The process of enzymatic browning is not unique to apples and occurs in bananas, pears and eggplants, it is also responsible for the colour of coffee, black tea and cocoa (Zhou et al., 2020). However, the variations in the polyphenol oxidase enzymes as well as the variations in the amount of polyphenols is responsible for the differences of enzymatic browning rates. Apples are shown to have the quickest rates of enzymatic browning, as well as their light initial colour allowing for easy observation (Zhou et al., 2020). Because of these reasons, apples were chosen for analysis within this study.

Within this study, the enzymatic browning rates were measured to determine which solution caused the greatest delay.The first solution used was acetic acid more commonly known as vinegar (pH ≈ 2.7). The second solution utilized was lemon juice (pH ≈ 2.7) containing a commonly known citric acid and many antioxidants like vitamin C (Moon et al., 2020). The third solution used was milk (pH ≈ 6.7) containing lactic acid. The fourth solution utilized was water (pH ≈ 7). And lastly, a control of no solution was measured. Because vitamin C is a known antioxidant, meaning it has the ability to neutralize free radicals responsible for the enzymatic browning. Vitamin C can do this in a way such that it is able to donate electrons to the unstable free-radicals whilst remaining stable themselves (Moon et al., 2020). Additional studies have shown that Polyphenol Oxidase is pH dependent, and may be inactivated at lower pH levels (Nicolas et al., 1994). Because the lemon juice has the highest antioxidant concentration and the lowest pH, it is predicted that the apples coated in lemon juice will do the best job at stunting the enzymatic browning rate.

Methods

Two Fuji apples were exposed to oxygen by individually cutting each into five separate portions. Utilizing the known reactions responsible for enzymatic browning (Equation 1) solutions were selected to examine their ability to interfere with the below process. Apple slices were then coated with one of the predetermined treatment solutions. The four treatments consisted of: Vinegar (treatment 1), Lemon juice (treatment 2), Milk (treatment 3), water (treatment 4), and lastly a control group of no added solution. Tongs were used to prevent contamination of the apples, and slices were held in the designated solution for 30 seconds. Apple slices were then placed on appropriately labelled plates based upon selected treatment. After completion, each sample set consisted of 2 treatment coated apple slices (as demonstrated in Figure 1).



Equation 1: chemical reactions responsible for production of the brown polymers seen on apples after exposure to air. As well as potential points of intervention by which the treatments may be acting (Moon et al., 2020).

At a room temperature of 19 °C, enzymatic browning levels were observed. Quantitative results were determined through percentage covered. Reports were made at the 10 minute, 20 minute, 30 minute, 1 hour and 2 hour benchmarks. This process was completed on four different dates to verify accuracy of results. All apples were bought on the day that the first experimental trial took place (March 21st 2021), the following trials were completed after consecutive 24 hour periods. While trials were taking place, apples not being used were stored at the 19 °C room temperature in a disinfected bowl. A total of 20 sample sets were collected and analyzed at the five different timestamps. The percentage of browning observed at particular time intervals served as the dependent variable, and the five different treatment options made up the independent variables. With five different independent variables, mean values were collected as well as the standard deviations. After all data was collected, a one-way ANOVA test was performed as well as an F-test statistic to find the p-value of the experiment.

Results

Each treatment solution contained 4 representative samples for every time marker. Figure 1, demonstrates the mean values of the observed % of the apple covered by enzymatic browning at the observed time stamps. At the 10 minute time period the mean value of percent cover per treatment is as follows: control (10%, SD: 0), Lemon juice (1%, SD: 1.4), Water (5.8%, SD: 1.5), Vinegar (4%, SD: 1.2), Milk (5%, SD: 1). The mean value covered at the 20 minute period: control (20%, SD: 0.0), Lemon juice (3.5%, SD: 1.0), Water (10.5%, SD: 1.0), Vinegar (7.5%, SD: 2.1), Milk (9.5%, SD:1). The mean value covered at the 30 minute period: control (46.7%, SD: 5), Lemon juice (8.5%, SD: 1.26), Water (14.3%, SD: 1.5), Vinegar (10.5%, SD: 1), Milk (13.5%, SD: 1.7). The mean value covered at the 1 hour mark: control (68.8% SD: 8.54), Lemon juice (10.5% SD: 1), Water (19% SD: 1.2), Vinegar (14.3%, SD: 1.5), Milk (18.8%, SD:2.5). The mean value covered at the 2 hour mark control (93.8%, SD:4.8), Lemon juice (38.8%, SD: 2.5), Water (62.5%, SD: 5), Vinegar (48.75%, SD: 2.5), Milk (60%, SD: 4.1).

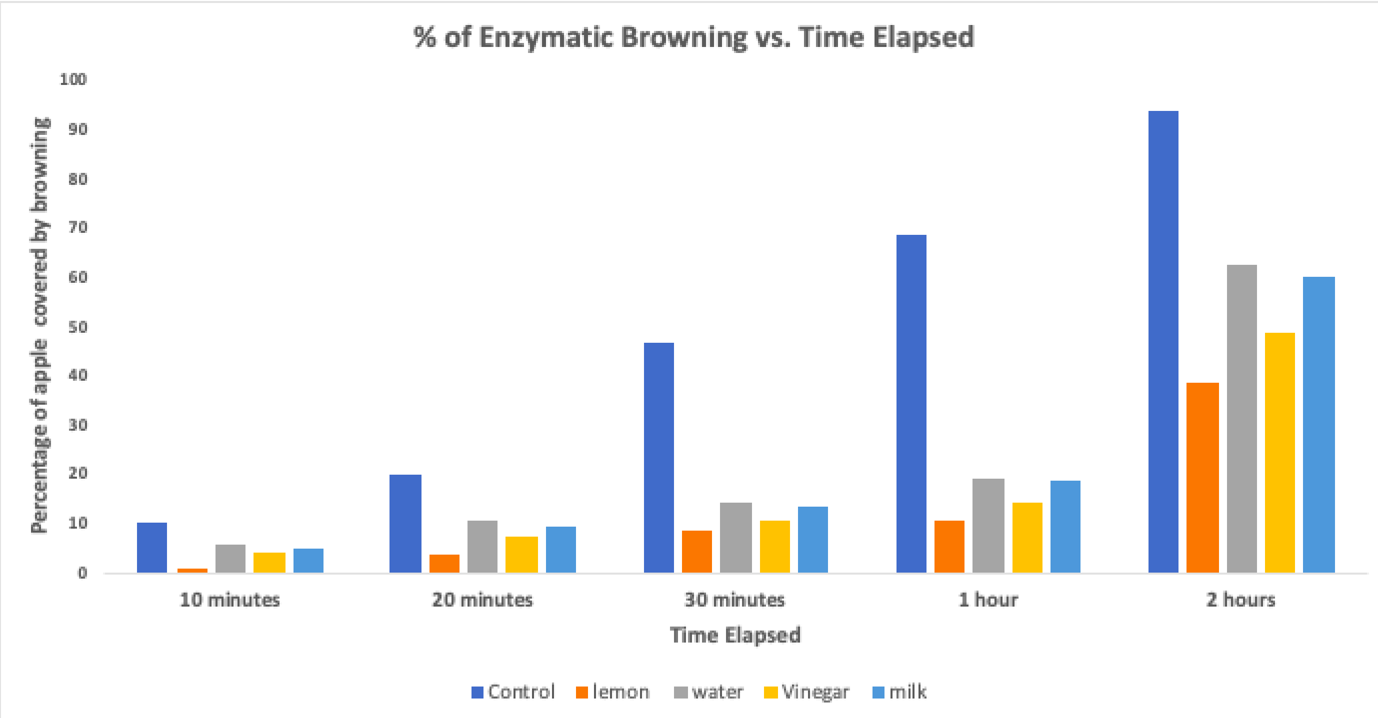


Figure 1: Mean values of observed % covered by enzymatic browning across different time points.

Variation between results is clearly seen through patterns, and distinctions between the different treatments resulted in differing results. This was backed up by the results of the one way analysis of variance. Five ANOVA calculations were completed (one for each time stamp) and it was shown that at a 0.05 significance level, p-values were all < 0.05, thus demonstrating statistical significance. p-values across all time frames were shown to be 0, and the F-statistic values are as follows: 10-minute time frame = 32.3, 20-minute time frame = 101.5, 20-minute time frame = 167.9, 1-hour time frame = 137.7, 2-hour time frame = 111.6.

Discussion

In analyzing the results, there is a clear indication of which treatment was the most effective at delaying the enzymatic browning of apples. At all of the observed times, lemon juice had the least amount of observable browning. Vinegar appeared to be the second most effective, followed by milk. Although water was the least effective of the treatments, it still proved to be more effective than the control. Thus, the hypothesis stating that lemon juice would be the most effective treatment was confirmed.

Additional analysis of results show that the control appeared to increase at a fairly constant rate, and a large majority of these apple slices had undergone enzymatic browning that almost fully covered them. This is vastly different to the apples that were coated with solution as it appears that the initial enzymatic browning appeared at a very slow rate, it wasn't until the later time stamps that large increases were noted. Although, this could be largely attributed to the larger gaps in time between checks in the second half of the experiment, the rate at which the browning increases suggests that the solutions have a stronger effect in the initial stages of oxygen exposure, but perhaps their ability wears off as time progresses.

It should also be noted that the effectiveness of each treatment showed correlation to the idea of lower pH treatments causing higher delay in the enzymatic browning. As well, the solution containing the most amount of antioxidants (lemon juice) resulted in the least amount of browning. Although, direct mechanisms cannot be pinpointed, previous evidence is indicative that a combination of both a low pH and high antioxidant content has the best outcome, as antioxidants prevent oxidation of the food item and acidic compounds seem to inactivate the polyphenol oxidase responsible for catalyzing the enzymatic browning reaction.

However, it is important to note that results may have been skewed due to experimental errors. Firstly, results were based on observed percent coverage. This method of measurement introduces the potential for human error and inconsistent data collection. Although, all data was collected by the same experimenter, because of a percentage based quantifier, estimation was required to form the results. These estimations are unlikely to accurately measure the precise amount of enzymatic browning that occured, and future studies with more accurate methodology should be employed. As well, data was collected over 5 different time frames. Because the experimenter was limited in supply, restraints on observation arose. If done with greater equipment, cameras recording the entirety of the 2 hours will be able to generate more data points and will thus allow for greater analysis on the topic.

As well, the likelihood of receiving data that has p-values of 0 on all calculated time frames is indicative of experimental bias. This could be in the form of unconscious bias, where the experimenter unknowingly confirmed previous results by estimating numbers that were similar to the previous day's findings. As well with only 4 samples of a treatment at each time frame, the amount of data is far too small to generalize results to a greater population. Future studies should focus on gaining more data through increased amounts of replicates.

Although many of these concepts are currently being employed, like McDonalds coating their sliced apples in antioxidant rich sulfites to prevent browning, there is much more to be learnt within the realm of food science and food preservation. Further examination into the factors affecting enzymatic browning should be explored, as well how concepts of genetic engineering can be implemented such that enzymes like PPO can be inhibited or not coded for entirely (Key et al., 2008).

Conclusion

The Hypothesis stated that due to its low pH and high antioxidant content, lemon juice would be the most effective wash for preventing enzymatic browning in apples. After collection of results at various time intervals, along with statistical analysis of these results, the hypothesis was confirmed. However, due to the introduction of many experimental errors that may have altered the results, further experimentation should be done. These types of studies serve great importance in ensuring that people are receiving the desired amount of nutritional benefits they expect when buying products prone to enzymatic browning, but it also is important from a marketing perspective as enzymatic browning can greatly stunt consumer choice. This experiment also opens the door for greater studies in optimal conditions for consumables as well as can be integrated into promising fields of study such as genetic modification that can lead to removal of unwanted enzymes.

Acknowledgments:

I would like to thank Dr. Celeste Leander and the teacher assistants (Jordan Hamden, Tessa Blanchard, and Sofya Langman) for providing me with the resources and support to complete this experiment and complete this paper. I would also like to share my thanks towards theUniversity of British Columbia staff for continuing to provide educational classes like Biology 342 Integrated Biology Laboratory course during the Covid-19 pandemic.

Citations:

1. Gritzer, D. (2019, October 10). *The Best Way to Prevent Cut Apples From Browning*. Serious Eats. https://www.seriouseats.com/2015/09/how-to-prevent-apple-pear-browning.html
2. Key, S., Ma, JK. Drake., PM. Genetically modified plants and human health. *J R Soc Med*. 2008;101(6):290-298. doi:10.1258/jrsm.2008.070372
3. Zhou, L., Liao, t., Liu, W., Zou, L., Liu, C., & Terefe, N. (2020) Inhibitory effects of organic acids on polyphenol oxidase: From model systems to food systems, Critical Reviews in Food Science and Nutrition, 60:21, 3594-3621, DOI: 10.1080/10408398.2019.1702500
4. Moon, K. M., Kwon, E. B., Lee, B., & Kim, C. Y. (2020). Recent Trends in Controlling the Enzymatic Browning of Fruit and Vegetable Products. *Molecules (Basel, Switzerland)*, *25*(12), 2754. https://doi.org/10.3390/molecules25122754
5. Nicolas, J. J., Richard-Forget, F. C., Goupy, P. M., Amiot, M. J., & Aubert, S. Y. (1994). Enzymatic browning reactions in apple and apple products. *Critical Reviews in Food Science and Nutrition*, *34*(2), 109-57.

APPENDIX

Raw Data: Data shows recorded percentages of perceived enzymatic browning across four different days.

1		10 minutes	20 minutes	30 minutes	1 hour	2 hours
	Control	10	20	40	60	90
	lemon	0	3	6	10	40
	water	8	10	12	18	60
	Vinegar	5	8	10	12	50
	milk	5	8	12	15	60
2		10 minutes	20 minutes	30 minutes	1 hour	2 hours
	Control	10	20	50	70	90
	lemon	0	3	6	10	40
	water	5	12	15	20	60
	Vinegar	3	5	10	15	50
	milk	5	10	12	20	65
3		10 minutes	20 minutes	30 minutes	1 hour	2 hours
	Control	10	20	50	65	95
	lemon	1	3	5	10	35
	water	5	10	15	20	60
	Vinegar	5	7	10	15	45
	milk	7	10	15	20	55
4		10 minutes	20 minutes	30 minutes	1 hour	2 hours
	Control	10	20	50	80	100
	lemon	3	5	8	12	40
	water	5	10	15	18	70
	Vinegar	3	10	12	15	50
	milk	5	10	15	20	60

Mean Data:

Mean		10 minutes	20 minutes	30 minutes	1 hour	2 hours
	Control	10	20	46.666667	68.75	93.75
	lemon	1	3.5	8.5	10.5	38.75
	water	5.75	10.5	14.25	19	62.5
	Vinegar	4	7.5	10.5	14.25	48.75
	milk	5	9.5	13.5	18.75	60

ANOVA calculations Results Using R-studio:

- 10 minute time period

F-statistic value = 32.3, P-value = 0.0,

- 20 minute time period

F-statistic value = 101.5, P-value = 0.0

- 30 minute time period

F-statistic value = 167.9, P-value = 0.0

- 1 hour time period

F-statistic value = 137.7, P-value = 0.0

- 2 hour time period

F-statistic value = 111.6, P-value = 0.0