Delaying Enzymatic Browning Reaction in Gala Apples

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Abstract

When an apple is injured (or cut into pieces), the plant tissue is exposed to oxygen. This triggers an enzyme known as polyphenol oxidase (PPO) to oxidize polyphenols in the apple's flesh (Deutch 80). This produces new chemicals (o-quinones), which then react with amino acids to produce brown-colored melanins. The objective of this work was to test what treatments were effective in slowing down the enzymatic reaction and browning in gala apple slices. The measured data was collected by comparing the color of the treated apple slices after 60 hours. To quantify the colouring and browning of the apples, each slice was compared to an apple browning scale by Van Cleve. The slices which take longer to turn brown were deemed a more effective treatment at slowing down the enzymatic browning reaction. This paper tested how some common household solutions, such as carbonated water, lemon juice, vinegar, saltwater, rubber bands, and plastic wrap, could slow down the enzymatic process and oxidation in apples. The results confirmed the hypothesis that if apple slices are soaked in saltwater, then there will be less browning in comparison to other treatments. There was less browning in the slices treated with saltwater due to the chloride ions which inhibit the polyphenol oxidase (PPO) enzymes (Zhang and Liang).

Introduction

Gala apples are a common variety of red apples produced in the United States but originated from New Zealand (FreshPoint, Inc). Gala apples are known for their mild and sweet crisp flavors which has now led this variety of apples to become the most popular apple grown today. Apples are commonly consumed fresh, dried, or processed as juices or candies but the fresh fruit pulp can easily lose its attractiveness and appeal due to its brownish color. This is a common problem mainly caused by an enzymatic reaction that leads to browning, which can cause undesirable changes in quality during the handling, processing, and storage of fruit.

Mechanical and physical stimuli like peeling, cutting, slicing, and dicing during food processing, and significant temperature changes during storage can cause physical tissue damages. This tissue damage ruptures the cellular compartment where polyphenol oxidase (PPO) is located initiating the enzymatic browning reaction. The active site of PPO consists of a group of copper-containing enzymes that catalyzes in the presence of oxygen. Two different reactions occur, the hydroxylation of monophenols (monophenolase activity) to o-diphenols, followed by the oxidation of o-diphenols to o-quinones (diphenolase activity) (Queiroz et al.

368). As seen in Figure 1, this reaction is followed by the polymerization of other quinones and amines to form brown pigments known as melanin, pigments of high molecular mass and dark brown colour (Queiroz et al. 368). The rate of enzymatic browning is determined by the enzymatic activity of PPOs.

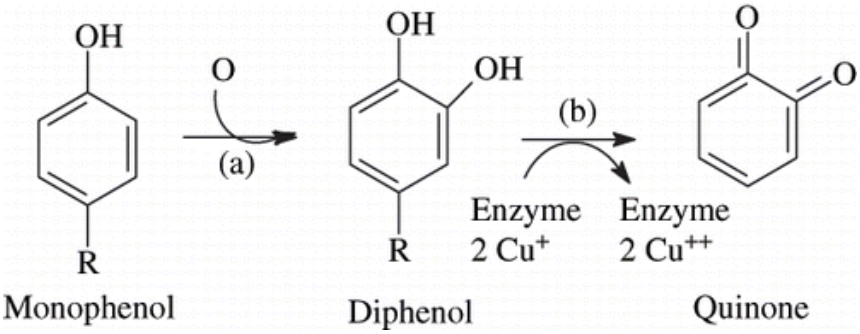

Figure 1. Reactions of (a) hydroxylation and (b) oxidation catalyzed by PPO (Queiroz et al. 368)

As the fresh-cut fruits and vegetable markets are rapidly growing in the food processing industry, understanding the characteristics of polyphenol oxidase could help to develop or choose more effective methods for controlling the browning of fruits and vegetable products. The challenge for food processors is controlling the enzymatic browning by maintaining quality and extending the shelf life of fresh-cut products. Several techniques and mechanisms have been developed to control and PPO activity. Some of these mechanisms act on the essential components that are a part of the reaction to occur, such as oxygen, enzymes, and copper of substrate. Heat treatments or antibrowning agents, like antioxidants, chelating agents, acidulants, or competitive inhibitors are commonly applied to inhibit the process of enzymatic browning (Moon et al. 2754).

The purpose of this paper was to explore six different at-home anti-browning activities and agents to decrease the rate of browning of gala apples over a 60 hour period. Two out of the anti-browning techniques included wrapping apple slices with plastic wrap and using rubber bands to reform and hold the slices together. The other four techniques used natural agents, such as soaking the apple slices in an acidic acid, vinegar, a carbonated liquid, and saltwater. These techniques and treatments were selected in order to determine which of these remedies could slow down the enzymatic browning the best. Understanding how these techniques work and which is the best option at delaying the browning process could be helpful not just personal use but would also be useful for hospitality industries, such as restaurant and food services. We hypothesize, based on Zhuang and Liang's (2017), if the apple slices are soaked in saltwater, then there will be less browning in comparison to other treatments due to the chloride ions which inhibit the polyphenol oxidase enzymes.

Methods

To conduct this experiment, we used four Gala apples that were cut into eight slices each. Six different treatment groups were studied. The control group were three apple slices that did not receive the experimental treatment and they were placed on the same tray as treatment groups 1-4. The first four treatment groups involved the use of various common household solutions including citric acid (lemon juice), carbonated drinks (e.g. carbonated water), acetic acid (vinegar) and saltwater. In each of these groups, three apple slices were soaked in each of their respective treatment baths for ten minutes. The apple slices were then spaced out on a tray and covered with clear plastic wrap. For the citric acid treatment bath, an equal amount of water and citric acid was used. 1 cup of both acetic acid (vinegar) and carbonic acid (carbonated water) were used, and lastly ½ tsp salt was thoroughly stirred in 2 cups of water. For the fifth treatment group, an entire apple that was cut up into slices was wrapped immediately with plastic wrap. The last treatment group involved an apple cut up into 8 slices that was wrapped in a rubber band and then covered in plastic wrap. We recorded observations in six intervals, starting immediately after the experiment started and then every 12 hours up until the 60th hour. Observations included comparing the apple slices' colour to Van Cleve's apple browning color scale so that the browning of apples could be measured quantitatively. Following the experiment, we conducted a two-way ANOVA (Type III) to explore the interaction between the time and treatment groups and a post hoc Tukey test to determine the significance between each treatment group. Both tests were conducted through R studio.

Results

By the end of the experiment, there were 9 apple slice samples in each sample group. These sample groups included the control, carbonated water, vinegar, lemon juice, and saltwater group. Because all apple slices were treated as a whole, we had three replicated samples for the plastic wrap group and the rubber band group. The browning effects in each of the seven treatment groups over 60 hours were recorded in Figure 2.

The start of the experiment was marked by time zero. According to Figure 2, most of the treatment groups showed no browning effects during preparation. However, by the time we finished preparation, two treatment groups already showed browning effects. The vinegar group showed slight browning effects (mean=0.67) with the largest error bars while the control group showed a mean of 2 on the browning colour scale. Additionally, this group had the highest browning effect at time zero.

After 12 hours, as seen in Figure 2, there was an increase in the browning effects on the y-axis, reflected in the slopes, from time zero in all of the treatment groups. In Figure 2, the control group showed lowest increase, from a mean of 2 to a mean of 3 on the browning colour scale. Though the control group showed the smallest increase from time zero to 12 hours after, the saltwater group had the lowest browning effects (mean of 1.11) and the lemon juice group also had a relatively low browning effect (mean of 1.56). However, the lemon juice group had a much larger error bar than the saltwater group. In the opposite to the treatment groups discussed at this time slot, the vinegar treatment group had the largest slope with the highest browning effects with a mean of 7. The other three treatment groups (carbonated water, plastic wrap, and rubber band) showed the similar browning effects as the control group from the range of 2.44 to 3.

From 12 hours to 24 hours, all treatment groups only had a slight increase in comparison to the change from 0 hour to 12 hours. Among all the groups, the carbonated water group and the saltwater group had the largest slope. Most of the groups had small error bars, except the vinegar group. The vinegar treatment group also had the largest number of outliers on both the high and low ends. In addition, the lemon juice group showed two lower end outliers in Figure 2. At this time period, the lemon juice group had the lowest browning effects with a mean of 2.06. Following the lemon juice group, the saltwater also had a relatively low browning effect with a mean of 2.13. On the side of high browning effects, the vinegar group showed a mean of 7.39 on browning colour scale.

At the time of 36 hours, the browning effects did not increase much from the 24 hours mark. Only the vinegar group, plastic wrap group, and the lemon juice group showed an obvious increase in the slope. Most of the outliers at this time period shown in Figure 2 were from the vinegar group. In addition, the vinegar group maintained to have the highest browning effects (mean=7.78) with the widest error bar among all treatment groups. Meanwhile, the saltwater group indicated the lowest mean at 2.28 for browning effects. Except the lemon juice group showed a slightly higher browning effect (mean= 2.72) than the saltwater group, all other groups ranged from 3.3 to 3.5 in terms of browning effects.

In the 12 increments from 36 hours to 48 hours, all groups showed a visible increase in their slopes except for the carbonated water group. The vinegar group maintained the highest browning effects at a mean of 8.22 with a smaller error bar than the one from 36 hours. Also, this group had both higher and lower outliers. Following the vinegar group, the control group (mean=4.94), the plastic wrap group (mean= 4.5), the rubber band group (mean= 4.17), and the

lemon juice group (mean=4.06) ranged from 4 to 5 in terms of browning effects. The carbonated water group and the saltwater group had the lowest browning effects, ranging from 3.2 to 3.5. Therefore, the saltwater group had the lowest browning effect among all groups at this time period. Besides outliers shown by the vinegar group, the control group had two outliers on the higher end, whereas the saltwater group and the lemon juice group each had one extremely low outlier.

At our final recorded time period, 60 hours after the experiment started, more increases in browning effects were shown in all treatment groups, except for the rubber band group. The vinegar group maintained the highest browning effects with a mean of 8.56. Although the vinegar group still showed a few outliers with one on the much lower end, the error bar of this treatment group decreased from previous time slots. The control group had the largest error bar at this time slot with the second highest mean browning effects (5.11). It also had two outliers on the much higher end. The lemon juice group and the plastic wrap group showed their mean browning effects slightly higher than 4.5. The browning effects from the carbonated water group and the rubber band group fell around 4.1. The saltwater group was the only group which had its mean browning effect lower than 4, landing at 3.39. The lemon group had both extremely higher and lower outliers, and the saltwater group had one extremely low outlier.

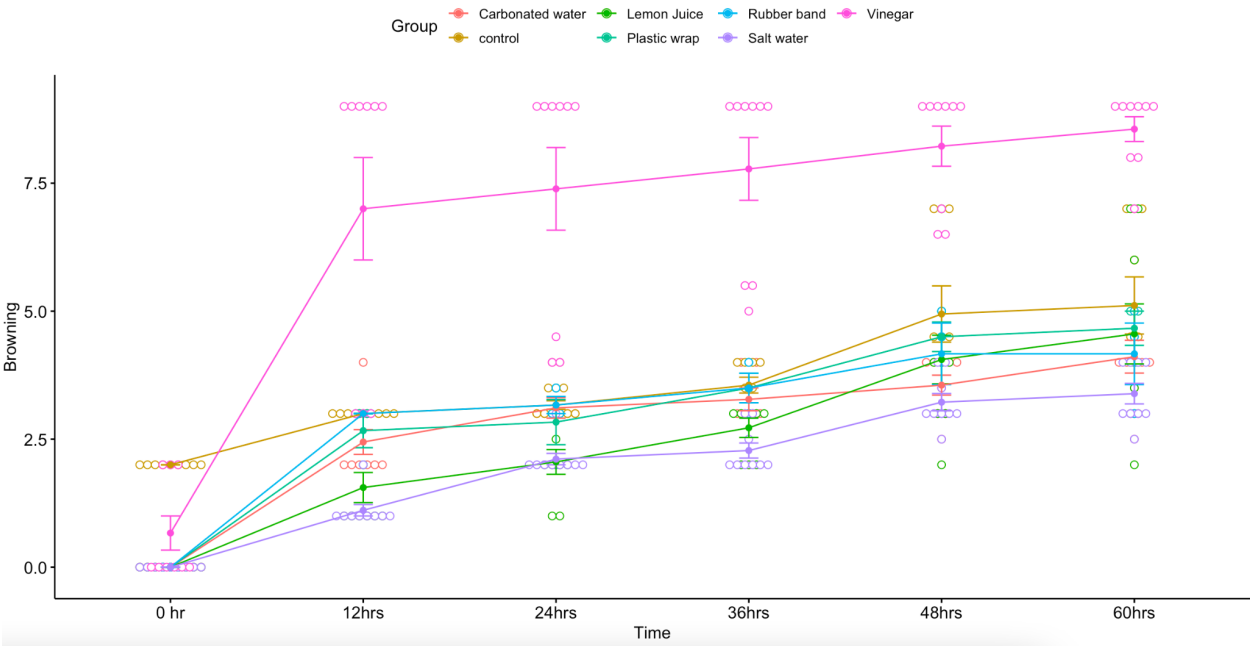

Figure 2: The line plot for the change in browning effects across 7 treatment groups and 6 time slots. The solid dots in the plot represented the mean of the browning effects for each treatment group at a given time. The hollow dots represented the outliers in each treatment group. Error bars represented the standard errors (SE).

Although Figure 2 showed many browning differences across treatment groups and across time, a statistical analysis would be required for the significance of all the differences discussed above. Because there were two dependent variables in this experiment, a two-way ANOVA (Type III) was conducted. Type III of the ANOVA test was conducted because we were interested in the interaction between the time and treatment groups. The p-value obtained for the difference across time alone was 4.707e-14, which was much lower than the chosen alpha value of 0.05. The p-value reported for the differences between treatment groups was 0.0005753, which was also much lower than the alpha value. Lastly, the p-value of the interaction between two dependent variables was determined to be 1.335e-10, which was again much less than 0.05.

A post hoc Tukey test was conducted following the ANOVA type III test to determine the significance between each treatment group. The adjusted p-values in the following 9 relationships out of 21 in total were greater than 0.05: lemon juice and carbonated water, lemon juice and plastic wrap, lemon juice and rubber band, lemon juice and salt water, plastic wrap and carbonated water, plastic wrap and control, rubber band and carbonated water, rubber band and control, and rubber band and plastic wrap. The remaining 12 relationships had adjusted p-values much smaller than 0.05.

Discussion

According to our results, the saltwater group treatment group had the lowest browning effects across all time periods which supported our initial hypothesis, and the vinegar treatment group had the highest browning effects across all time periods. However, other treatment groups showed various browning effects across time. At time zero, all treatment groups showed better delaying browning effects in comparison to the control group. Thus, we can suggest that all six treatment groups will be effective for delaying browning effects within a short period of time, such as an hour. From 12 hours to 60 hours, only the vinegar group had much severe browning effects than the control group. Within the 12 hours to 36 hours period, the rubber band group showed similar browning colour effects in terms of the colour scale. Meanwhile, all the other four treatment groups all had lower mean browning effects than the control group. This suggested that lemon juice, carbonated water, saltwater, and plastic wraps could all be potential ways to delay browning effects in the relatively long term, but some of the effects might be too small to be statistically significant.

According to the two way ANOVA (type III) test that we conducted, we obtained all p-values less than 0.05 which suggested that the differences in browning effects between

treatment groups alone, across time alone, and between treatment groups across time were all statistically significant. The results from the Tukey test provided us more information on the relationships between different treatment groups. According to the adjusted p-values from the post hoc test, the rubber band group and plastic wrap group were statistically insignificant from the control group. This implies that treating apple slices with the rubber band or plastic wraps does not significantly delay the browning effects in comparison to the control group overall. Although the vinegar group was statistically different from the control group, it had more rapid and intense browning effects which suggested that treating with vinegar is not effective. The treatment group of lemon juice and rubber band showed insignificant differences from many other treatment groups. This could be explained by the inconsistent effectiveness to display browning effects in each treatment group. From our earlier conclusion, the saltwater group was shown to have the lowest browning effects across time. The post hoc test also showed the significant difference between the saltwater group and all other groups, except the lemon juice group. In Figure 1, the lemon juice group and the saltwater group had similar mean browning effects from time zero to 36 hours. Though the difference in mean increased between these two groups after 36 hours, the insignificant adjusted p-value suggested that lemon juice would be a good supplement to saltwater, especially within the first two hours. On the other side, the vinegar treatment group was significantly different from all other groups by having extremely high browning effects across time. This implies that we should avoid treating apple slices with vinegar.

Many experiments run by different research groups and individuals showed the similar results of having saltwater as the best treatment group to delay browning effects (Liang and Zhang; Pinola; Splawn). Moon et al. (2754) suggested that salt solution is a method for apple slices to avoid from contacting Oxygen. This mechanism of salt would attribute mostly to the anion chloride (Pizzocaro et al., 26). Similarly, Liang and Zhang explained this mechanism by suggesting the chloride ion in the salt would inhibit the PPO enzymes, which then inhibits the enzyme's access from browning. Lemon juice was shown to be another effective treatment to delay browning effects from our experiment. Despite its low pH to inhibit PPO activities, lemon juice is also one of the copper-chelating agents that can suppress PPO activity by binding to metal cofactors in the PPO enzyme structure (Moon et al., 2754). Although carbonated water as a type of carbonic acid, and vinegar as a type of acetic acid are also potential methods to inhibit PPO activities by acidification, the pH of the carbonated water was higher than lemon juice and our vinegar solution was much more concentrated comparing to the lemon juice treatment group. Because we did not dilute the vinegar solution, osmosis might have occurred in apple slices which led to more browning effects than the control group. Nevertheless, the plastic wrap

group and the rubber band group would be characterized as the physical methods to inhibit browning effects. Our results showed that these two treatment groups were not as effective relative to the other treatment groups, especially in the short term. This could be explained by the limitations in preventing apple slices from contacting air through physical barriers.

Although most treatment groups in our experiment had nine replications in total conducted by three different individuals, there were still limitations in our studies. Room temperature and humidity could also lead to different browning effects. Due to COVID constraints, experiments were conducted in three different houses across Vancouver. In future studies, humidity, temperature, and other environmental factors should be controlled. Besides the chosen treatment groups in this study, future studies can examine the effects of other treatment groups such as edible Calcium Chloride. Past studies have also indicated the effective reduction of PPO activities after mixing salt with acid groups(Yousuf 201; Pizzocaro et al 27-28). This pattern was not examined in our research, but could be another future research direction on this topic.

Conclusion

In this study we tested the effectiveness of several at-home treatments in slowing down the enzymatic reaction and browning of Gala apple slices. In terms of the results, the saltwater treatment group had the lowest amount of browning while the vinegar treatment group had the highest amount of browning. Conducting the two way ANOVA (type III) test showed that statistically significant differences were present in browning effects between treatment groups. The Tukey test showed that the rubber band and plastic wrap treatments on the apple slices would not significantly delay the browning effects in comparison to the control group. With the exception of the vinegar treatment, all treatments were effective in slowing down the browning effects on the Gala apple slices after a short period of time.

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Works Cited

Deutch, Charles E. “Browning in Apples: Exploring the Biochemical Basis of an Easily-Observable Phenotype.” *Biochemistry and Molecular Biology Education*, vol. 46, no. 1, 2017, pp. 76–82. *Crossref*, doi:10.1002/bmb.21083.

FreshPoint, Inc. “Apples, Gala.” *FreshPoint*, 16 Mar. 2021, freshpoint.com/archive/commodity-apples-gala/#:%7E:text=The%20Gala%20apple%20is%20a,in%20most%20markets%20year%2Dround.

Moon, Kyoung Mi, et al. “Recent Trends in Controlling the Enzymatic Browning of Fruit and Vegetable Products.” *Molecules*, vol. 25, no. 12, 2020, p. 2754. *Crossref*, doi:10.3390/molecules25122754.

Pilona, Melanie. *Prevent Apples from Browning with a Quick Saltwater Soak*. 29 Sept. 2015, lifehacker.com/prevent-apples-from-browning-with-a-quick-saltwater-soa-1733562639.

PIZZOCARO, FRANCESCO, et al. “INHIBITION of APPLE POLYPHENOLOXIDASE (PPO) by Ascorbic ACID, Citric Acid and Sodium Chloride.” *Journal of Food Processing and Preservation*, vol. 17, no. 1, 1993, pp. 21–30., doi:10.1111/j.1745-4549.1993.tb00223.x.

Queiroz, Christiane, et al. “Polyphenol Oxidase: Characteristics and Mechanisms of Browning Control.” *Food Reviews International*, vol. 24, no. 4, 2008, pp. 361–75. *Crossref*, doi:10.1080/87559120802089332.

Splawn, Meghan. *This pro Trick Keeps Apples from Browning (Nope Not Lemons!)*. 1 May 2019, www.thekitchn.com/keep-sliced-apples-from-browning-260265.

Van Cleve, J. (2010) Apple browning scale. http://scienceprojectideasforkids.com/wp-content/uploads/2010/04/apple-color-scale3.jpg. Accessed March 24, 2021.

Yousuf, Basharat, et al. “Recent Developments in Shelf-Life Extension of Fresh-Cut Fruits and Vegetables by Application of Different Edible Coatings: A Review.” *LWT*, vol. 89, 2018, p. 201.

Zhang, Emily, and Christina Liang. “SLOWING THE OXIDATION OF PH’RUITS.” *Slowing the Oxidation of PH’ruits*, 2017, slowingtheoxidationoffruits.weebly.com.

Appendix

Group	0hr	12hr	24hr	36hr	48hr	60hr
Lemon Juice	0	1	1	2	4	6
Lemon Juice	0	1	2	3	7	7
Lemon Juice	0	1	1	2	5	7
Lemon Juice	0	1	2	3	3	3.5
Lemon Juice	0	1	2	3	4.5	4
Lemon Juice	0	1	2.5	3.5	4	4.5
Lemon Juice	0	2	2	2	2	2
Lemon Juice	0	3	3	3	3	3
Lemon Juice	0	3	3	3	4	4
Carbonated water	0	3	3	3	3	4
Carbonated water	0	4	4	4	4	5
Carbonated water	0	3	3	4	4	6
Carbonated water	0	2	3	3.5	4	4
Carbonated water	0	2	2	2	2.5	2.5
Carbonated water	0	2	3	3	3.5	3.5
Carbonated water	0	2	3	3	3	4
Carbonated water	0	2	4	4	4	4
Carbonated water	0	2	3	3	4	4
Vinegar	0	9	9	9	9	9
Vinegar	0	9	9	9	9	9
Vinegar	0	9	9	9	9	9
Vinegar	0	3	4.5	5.5	6.5	7
Vinegar	0	3	4	5.5	7	8
Vinegar	0	3	4	5	6.5	8
Vinegar	2	9	9	9	9	9
Vinegar	2	9	9	9	9	9
Vinegar	2	9	9	9	9	9
Salt water	0	1	2	2	3	3
Salt water	0	1	2	2	3	4
Salt water	0	1	2	2	4	4
Salt water	0	1	2	2	2.5	2.5
Salt water	0	1	2	3	3.5	4
Salt water	0	1	2	2.5	3	3

Salt water	0	2	3	3	4	4
Salt water	0	1	2	2	3	3
Salt water	0	1	2	2	3	3
Plastic wrap	0	2	2	3	4	4
Plastic wrap	0	3	3.5	3.5	4.5	5
Plastic wrap	0	3	3	4	5	5
Rubber band	0	3	3	3	3	3
Rubber band	0	3	3.5	3.5	4.5	4.5
Rubber band	0	3	3	4	5	5
control	2	3	3	4	7	7
control	2	3	3	4	7	7
control	2	3	3	4	7	7
control	2	3	3.5	3.5	4.5	6
control	2	3	3.5	4	4.5	4.5
control	2	3	3.5	3.5	4.5	4.5
control	2	3	3	3	3	3
control	2	3	3	3	4	4
control	2	3	3	3	3	3

Table 1. The summary table of browning effects across seven treatment groups.

Relationship	Adjusted p-values
control-Carbonated	0.0006140
Lemon Juice -Carbonated water	0.8740217
Plastic wrap-Carbonated water	0.9645251
Rubber band-Carbonated water	0.9790545
Salt water-Carbonated water	0.0089456
Vinegar-Carbonated water	0.0000000
Lemon Juice -control	0.0000020
Plastic wrap-control	0.3856014
Rubber band-control	0.3295049
Salt water-control	0.0000000

Vinegar-control	0.0000000
Plastic wrap-Lemon Juice	0.5292395
Rubber band-Lemon Juice	0.5931574
Salt water-Lemon Juice	0.2600178
Vinegar-Lemon Juice	0.0000000
Rubber band-Plastic wrap	1.0000000
Salt water-Plastic wrap	0.0119413
Vinegar-Plastic wrap	0.0000000
Salt water-Rubber band	0.0162700
Vinegar-Rubber band	0.0000000
Vinegar-Salt water	0.0000000

Table 2. The summary of Tukey test results. The adjusted p-values on the differences in browning effects between any of the two treatment groups were reported in this table.