#### The Effect of Acidity Levels on Eggshells

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#### Abstract

Daily consumption of acidic foods such as citrus fruits, soft drinks, and vinaigrettes may be more severe than many believe; overconsumption can cause detrimental effects on tooth enamel. Previous research has mainly focused on the negative health effects from consuming acidic foods and drinks; however, the effect of different acidity levels on tooth enamel is still unclear. Accordingly, the objective of our study is to explore the effects of varying dilutions of vinegar on eggshells. Vinegar was used due to the low pH value and popularity in food, and eggshells were used to simulate tooth enamel since they have a similar chemical composition. We predicted that the higher the acidic level, the more dissolved the eggshell would be. To test this prediction, we submerged eggs in different dilutions of vinegar: 100%, 75%, 50%, and 25%. Our data was analyzed using a one-way ANOVA test and post-hoc Tukey-Kramer test. It was found that there was a statistically significant difference in the acidity levels of vinegar on eggshell dissolution and the difference between the levels (p-value =  $3.46 \times 10^{-17}$ ). Overall, the study concludes that the consumption of acidic foods and drinks should be done with caution.

## I. Introduction

Carbonated drinks are consumed for their flavorful taste, however, the consumption of these drinks come with negative effects (Richards 40). Studies have been conducted on the negative health effects from consuming carbonated drinks ranging from obesity to teeth erosion (James 1237). The following study looked specifically into the connection between carbonated drinks and teeth erosion. Teeth erosion is the loss of tooth enamel caused by acid without any bacterial involvement (Li et al., 1). This topic is relevant because the consumption of acidic drinks has been found to be one of the major causes of dental erosion (Li et al., 2).

The enamel is the outermost layer of the teeth, and is vital to oral health by protecting from decay and damage. An issue concerning oral health is the consumption of acidic foods and beverages, such as sodas, citrus fruits, pickles, and vinegar dressing. The low pH in these food and drinks cause the demineralization on the surface of teeth, leading to weaker and brittle enamel due to the acidic oral environment (Moynihan et al., 162). The frequency and duration of eating acidic food, as well as the type of strength of acid determines the amount of erosion (Zero et al., 202).

To begin, egg shells were used to simulate tooth enamel, and the effects of Sprite and Coca Cola, carbonated water, and non-carbonated water on eggshells were observed. Tooth enamel is made of calcium carbonate and calcium phosphate, which is a similar chemical composition to eggshells (Xu et al., 8035). Therefore, the effects observed on the egg shells could be later extrapolated as results that would be similar to using teeth enamel with further research. The variables initially tested were the different acidity levels of the sodas and sugar content. However, the preliminary tests of this experiment showed little to no changes in the composition of eggshells submerged in Sprite and in Coca Cola. Due to the lack of results, this approach was abandoned and vinegar was used instead (a stronger acid with a similar pH value to Coca Cola ~2.5) to dissolve the egg shells. Using vinegar at various concentrations to observe how vinegar can dissolve the eggshell; more immediate and easily identifiable changes occurred.

We hypothesized that if vinegar dissolves egg shells, then acidic food and drinks lead to tooth decay because the enamel can be eroded by exposure to an acidic environment.

### II. Methods

To determine the effects of acidic drinks on eggshells, the initial setup of our experiment started with placing six egg shells into separate cups. Large white eggs were used and submerged in 355mL of each drink as follows: two cups with Coca Cola, two with Sprite, one with

carbonated water and one with non-carbonated water. Carbonated water was used to control for acidity whereas the non-carbonated water was used to control for sugar effects. These cups were covered with plastic wrap and secured with elastic bands or a lid to prevent the escape of  $CO_2$  gas (setup shown in Figure 1). The carbonation is the dissolved  $CO_2$  that is responsible for the acidity of the soda, so the closed system limits the dissolution of  $CO_2$ . Observations of any physical changes to the egg shells were recorded every eight hours up until the eggshells were dissolved. The egg shells in the Coca Cola and Sprite were compared to the control groups. Changes in colour, shape, cracks, or evidence of a reaction occurring were looked for.

In addition to looking for the effects of soda on eggshells, the effect of different dilutions of white vinegar on eggshells were also observed. This next experiment bridged the knowledge gap by studying the effects of using vinegar - a stronger acid - as the treatment groups instead. Four whole, white eggs were placed into separate cups. Whole eggs were used instead of egg shells so that the membrane would be visible once the shell had fully dissolved. Next, white vinegar was added to each cup at concentrations of 100%, 75%, 50%, 25%. Various amounts of water was used to dilute the white vinegar to ensure that each cup had a total liquid volume of 200 mL. Once the proper dilutions were completed, the cups were covered with plastic wrap or lid to avoid any potential contaminants (Fig. 2). The reaction of white vinegar with the eggshells was expected to be quicker than the soda reactions, so changes were recorded every hour until the eggshells fully dissolved. The main observation noted was the time it took for the eggshell to completely dissolve and leave only the membrane behind. Non-carbonated water was used as a control for acidity. A recording device was used to continue watching the eggs that had not

dissolved by the end of the day. Each member of the group conducted both experiments for a total of four replicates.

For the statistical analysis, a one-way ANOVA test was used to analyze the time needed to dissolve the eggshell in the white vinegar. This could test for any statistically significant differences between the four independent groups of data. The null hypothesis is that the mean time dissolved for each dilution of white vinegar is not statistically different for all of the groups, while the alternative hypothesis is that there are significant differences between the means of each group. The ANOVA was conducted using an **a** value of 0.05 to determine the statistical significance. In addition, a Tukey-Kramer post-hoc test was performed to determine if there were any significant differences between each treatment group.



Figure 1: Setup of Soda effects on eggshells experiment, labels on each cup correspond with the soda being tested. Note, this experiment was abandoned due to lack of effects on the eggshell.



Figure 2: Setup of Vinegar effects on eggshells experiment, labels correspond to percentage of vinegar in solution.

# III. Results



Figure 3: The egg with the shells fully dissolved from the vinegar solutions.



Figure 5: Boxplot of average time to dissolve egg shell, plot's y-axis begins at 60 hours elapsed. Average time to dissolve: 25% = 0 hours, 50% = 132.75 hours, 75% = 83.75 hours, 100% = 70.5 hours

According to Fig. 5, the time to fully dissolve egg shells took 132.75, 83.75, and 70.5 hours at vinegar concentrations of 50%, 75%, and 100%, respectively. Four replicates of each vinegar concentration level were made to calculate the average. The 95% confidence interval for each of the vinegar concentrations are [127.49 - 138.01] for 50%, [81.03-86.47] for 75%, and [67.19-73.81] for 100% vinegar concentration. There are no data points for 25% vinegar concentration because the egg did not dissolve in the given time frame. As such, it is not included in the ANOVA test and is mentioned in Figure 5 as 0.

In the one-way ANOVA test, an alpha value of 0.05 was used to determine whether there was a statistical difference between the vinegar concentrations and the time it took to dissolve the egg. The calculated p-value was  $3.46 \times 10^{-17}$ , giving support that the treatments are significantly different. As well, the Q critical value from the Tukey-Kramer test was calculated to be 4.475. When comparing the Q critical value to the absolute mean values of each treatment group, the value confirms that all treatments were individually significantly different from each other.

#### IV. Discussion

The preliminary tests in this experiment used Coca Cola, Sprite, carbonated water, and non-carbonated water to determine any effects that acidic beverages may have on egg shells (Wongkhantee,. 215). The carbonated water was a control for sugar levels, while the non-carbonated water was a control for acidity (carbonation leads to lower pH). Unfortunately, the results from this experiment showed little to no changes in the egg shell composition because the erosive effects from different drinks can vary widely (Zimmer et al., 4). Only the Coca Cola dissolved some of the eggs slightly, leading to cracks in the shell around the 48 hour mark.

Since Coca Cola was not able to fully dissolve the egg shell, the experiment was modified to apply a different acidic food that is also commonly consumed. White vinegar has a similar pH value as Coca Cola, around 2.3 - 2.5, and fully dissolved the egg around three days later in the 100% vinegar solution (Fukae et al., 384). A note on acid strength is that the

phosphoric acid found in Coca Cola is a stronger acid than the acetic acid in white vinegar (Balbay et al., 243). However, the amount of phosphoric acid in Coca Cola is low, while the amount of acetic acid in vinegar is relatively high. This may explain why vinegar was able to dissolve more of the egg shell than Coca Cola, despite containing a weaker acid.

Based upon our results, the null hypothesis that acidity level has no effect on the egg shell was rejected. The one-way ANOVA test revealed that there is a statistically significant difference in white vinegar concentration and the egg shell dissolution time. Our p-value was found to be  $3.46 \times 10^{-17}$ , which is smaller than 0.05. This indicates that various concentrations and contact times of vinegar can dissolve egg shells at different rates. The 100% vinegar solution dissolved the fastest, then the 75%, and finally the 50%. The Tukey test also indicated that each of the treatment groups are significantly different as the absolute mean differences were all greater than the Q critical value (4.475), showing that the results of this experiment are credible.

Applying our results to tooth enamel, higher intake of acidic foods and drinks may lead to more damage to the enamel layer (Lussi et al., 390). Since there were no dissolving effects in the 25% vinegar concentration, a threshold might exist. This brings implications to our teeth, where in small concentrations of acids, tooth enamel may not be affected in the short term. Furthermore, a study by Hughes suggests that moderation might be the key to protecting our enamel while still enjoying acidic foods (Hughes et al., 345). This could be further studied with experiments that test the effects of low acidity on enamel by decreasing frequency, contact time, or concentrations of acidic foods, mimicking the lower concentration of the 25% solution of white vinegar.

### **Sources of Error**

This study has many limitations such as limited access to laboratory equipment - scales, sterile environment, and controlled temperatures. Egg shells were used in place of teeth to perform the experiment due to similar chemical composition. Tooth enamel is composed of calcium phosphate, while egg shells are made of calcium carbonate, but this difference could affect the reaction observed in acidic environments. Also, the 25% vinegar concentration seemingly showed no effects, but it's effects may be on the strontium, magnesium, lead, and fluoride content of tooth enamel, which may not be observable on the macroscopic level (Wang et al., 40). Thus, the experiment can only represent the effects vinegar has on calcium phosphate, and does not represent the other possible ways vinegar could impact the integrity of enamel.

## **Future Research**

Although we were able to reject the null hypothesis, there still needs to be future research that is performed before it can be confirmed that there is a statistical difference between the effects of vinegar and tooth enamel damage. Firstly, it is unlikely for an individual to consume vinegar at such a high acidic concentration. The proposed methodology for a future study would be to observe lower acidic concentrations, such as the 25% dilution vinegar or less, over a longer time period, or perhaps using different acids that are more commonly consumed in high concentrations (Zheng et al., 933). The use of scanning electron microscopy can also help determine the effects on a microscopic level (Wang et al., 40). Furthermore, conducting an experiment in a proper lab environment would have yielded more accurate results since all the materials used would be uniform. Using real teeth with enamel layers would address the biggest

limitation of this study, since we are unable to correlate our results from egg shells towards tooth enamel. This study showed that acidity may lead to teeth corrosion, but other factors may play a role that needs to be further tested, such as temperature, exposure time, and type of food or drink (Barbour et al., 209).

### V. Conclusion

In conclusion, our research has found evidence in the relationship between high acidic levels and egg shell dissolution. The trend of egg shell dissolution at higher acidic levels is consistent with previous research and our prediction, and rejects our null hypothesis that acidity has no effect on egg shells. As a result, if consuming food or drinks with relatively high acid concentration, one should carefully monitor the duration of high acidic exposure to prevent damage to our tooth enamel.

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# Appendix

| Raw data | of egg | dissolving | times, | average time, | and 95% CI |
|----------|--------|------------|--------|---------------|------------|
|----------|--------|------------|--------|---------------|------------|

| Vinegar Content | Leanne      | Kelin             | Andrew         | Martin      |
|-----------------|-------------|-------------------|----------------|-------------|
| 25%             | 0           | 0                 | 0              | 0           |
| 50%             | 128         | 135               | 133            | 135         |
| 75%             | 82          | 86                | 84             | 83          |
| 100%            | 68          | 73                | 71             | 70          |
|                 | Time to dis | ssolve in hours f | or each person |             |
| Percentage:     | 25          | 50                | 75             | 100         |
| Average:        | 0           | 132.75            | 83.75          | 70.5        |
|                 | 25          | 50                | 75             | 100         |
| STD dev =       | 0           | 3.304037934       | 1.707825128    | 2.081665999 |
| Sample Size     | 4           | 4                 | 4              | 4           |
| 95% CI          | 0           | 5.257461658       | 2.717530884    | 3.312395134 |
| add             |             | 138.0074617       | 86.46753088    | 73.81239513 |
| minus           |             | 127.4925383       | 81.03246912    | 67.18760487 |

| Anova: Single Factor |         |     |          |          |                 |           |               |
|----------------------|---------|-----|----------|----------|-----------------|-----------|---------------|
| SUMMARY              |         |     |          |          |                 |           |               |
| Groups               | Count   | Sum | Average  | Variance | Pooled Variance | Q Value ( | df = 12, k =4 |
| 25%                  | 4       | 0   | 0        | 0        | 4.541666667     | 4.2       |               |
| 50%                  | 4       | 531 | 132.75   | 10.91667 |                 |           |               |
| 75%                  | 4       | 335 | 83.75    | 2.916667 |                 |           |               |
| 100%                 | 4       | 282 | 70.5     | 4.333333 |                 |           |               |
| ANOVA                |         |     |          |          |                 |           |               |
| Source of Variation  | SS      | df  | MS       | F        | P-value         | F crit    |               |
| Between Groups       | 36058.5 | 3   | 12019.5  | 2646.495 | 3.46236E-17     | 3.490295  |               |
| Within Groups        | 54.5    | 12  | 4.541667 |          |                 |           |               |
| Total                | 36113   | 15  |          |          |                 |           |               |

One way ANOVA test calculation results, p-value of  $3.46 \times 10^{-17}$ 

| Comparison   | Abs mean diff | Q critical Value | Significant |
|--------------|---------------|------------------|-------------|
| 25% vs 50%   | 132.75        | 4.475349148      | yes         |
| 50% vs 75%   | 49            | 4.475349148      | yes         |
| 75% vs 100%  | 13.25         | 4.475349148      | yes         |
| 25% vs 100 % | 70.5          | 4.475349148      | yes         |
| 25% vs 75%   | 83.75         | 4.475349148      | yes         |
| 50% vs 100%  | 62.25         | 4.475349148      | yes         |

Tukey-Kramer Post Hoc Calculation results, all data sets were individually significantly different from one another by comparing the absolute mean value differences to the Q critical value, where all the absolute mean value differences were greater than the Q critical value.

# **Serial Dilution Calculations**

Dilution for 100% vinegar solution  $C_1V_1 = C_2V_2$ (x)(V<sub>1</sub>) = (x)(200 mL)  $V_1 = 200 mL$ 

Dilution for 75% vinegar solution  $C_1V_1 = C_2V_2$   $(x)(V_1) = (0.75x)(200 mL)$  $V_1 = 150 mL$ 

Dilution for 50% vinegar solution  $C_1V_1 = C_2V_2$ (x)(V<sub>1</sub>) = (0.5x)(200 mL)  $V_1 = 100 mL$ 

# **Dilution for 25% vinegar solution** $C_1V_1 = C_2V_2$ (x)(V<sub>1</sub>) = (0.25x)(200 mL)

 $V_1 = 50 \, mL$