"Effect of Storage Time on *Citrus sinensis* Ascorbic Acid Content"

My research question for this exploration was: What is the effect of storage time on *Citrus sinensis* ascorbic acid content? This relates to the IB Biology curriculum, as it falls under topic two (molecular biology) and under subtopic D.1 (human nutrition). I gravitated to this topic because vitamin C was brought up in my biology class, and it caught my attention. Since I want pursue a career in the medical field, I figured that it would be crucial to study human nutrition and elements that go along with that topic (for example, the different vitamins and minerals necessary for a human's diet). In general, I am curious about the science behind human nutrition, which is why I leaned to this topic for my internal assessment.

Vitamin C is a water-soluble type of vitamin that's also known as ascorbic acid. This vitamin is required for collagen (protein that is crucial for forming connective tissue and for wound healing) synthesis (The Editors of Encyclopedia Britannica). It also plays a significant role in the immune system, and it increases the absorption level of non-heme iron from plant foods. Multiple foods contain vitamin C, such as citrus fruits, tomatoes, and potatoes ("Vitamin C").

Oranges can be stored in a variety of ways. Whole oranges can be placed in a room temperature setting, and they will stay fresh for up to a week before they start degrading (when mold starts to appear). Oranges can also be stored in the refrigerator, which will prolong their lifespan due to the fact that the fridge's lower temperature slows down the process of molding. They can stay fresh for about two to four weeks. Freezing the oranges keeps them fresh for an even longer period of time (Snart). However, the method for keeping oranges fresh the longest is to leave them on the tree (the source of the fruit). The best humidity level for storing oranges is typically between 90-95% ("How Long").

A medium-sized orange, weighing about 130 grams, has about 70 milligrams of vitamin C when it's fresh ("How Many Mgs"). In order to measure the amount of vitamin C in a fruit (such as an orange), one can use the vitamin C titration method. In this particular method, iodine functions as the vitamin C indicator, as it reacts to vitamin C and starch (which will also be used in the experiment). A reference sample is necessary in order to make comparisons of the vitamin C contents. Essentially, the process of titration involves observing how much juice is produced from the fruit and how many iodine drops are put into the juice to make the juice sustain the iodine's color. These values are used to determine the amount of vitamin C per 29.57 mL (equivalent to one ounce) of fruit juice (Anderson).

I hypothesized that if the storage time of an orange is increased, then the vitamin C content in the orange will decrease. This is because vitamins have a very low ability to not be destructed by factors such as light, heat, water, acidity changes, and radiation when it's stored and exposed to air. Therefore, over time, vitamin C levels can decrease in fruits (Corleone). The null hypothesis states that there is no impact of storage time of an orange on that orange's vitamin C content level. On the other hand, the alternative hypothesis states that there is a significant impact of storage time of an orange on that orange's vitamin C content level. Below is the list of materials I used in the experiment:

- Iodine
- Water
- Corn starch (4.93 mL)
- Nature Made 500 mg vitamin C tablet (1)
- Stove (for boiling)
- Eye dropper
- Measuring cup
- Measuring spoons
- Clear glass jar
- 2 clear glass drinking glasses
- Spoon
- Small mixing bowl
- Juicer
- 25 paper cups
- Oranges (Enough to produce 739.338 mL of orange juice)
- Cardboard tray

Figure 1: Experiment Materials

In my experiment, the manipulated variable was the storage time. In order to manipulate the variable, I measured the vitamin C content per 29.57 mL of orange juice, repeated the trial five times, and took measurements over a period of five days. This means that the manipulated variable was measured in terms of days. Essentially, these levels were chosen in order to have a total of five trials within the experiment. Overall, I conducted a total of 25 measurements. Uncertainties may have arisen as a result of not conducting the experiment at the exact same time everyday. To figure out how to measure vitamin C content in an orange, I used an article from

LIVESTRONG.COM. This article provided information about how to determine vitamin C content using iodine as an indicator. Typically, vitamin C content calculation experiments use standardized iodine and starch solutions. However, I decided to use a reference sample, as standardized solutions were not available to me.

To make the vitamin C reference sample, I filled the glass jar with 473.12 mL of water, crushed the 500 mg vitamin C tablet, and poured the crushed tablet into the water. I then stirred to dissolve the vitamin C. After that, I measured 29.57 mL of the vitamin C solution and put it into another glass. I added 118.28 mL of water to this glass as well. This test glass contained approximately 31 mg of vitamin C. Next, I dissolved 4.93 mL of cornstarch in 29.58 mL of water and mixed the paste until the dry powder was not visible. Then, I poured 118.28 mL of boiling water into a measuring cup and added the cornstarch paste. I stirred this until the paste dissolved completely. Then, I let the starch solution cool and set it aside. Finally, I added 4.93 mL of the starch solution to the vitamin C test solution and stirred well, creating the reference sample.

To check the legitimacy of my reference sample, I needed to test it. Using the eyedropper, I added iodine by drops into the vitamin C reference sample. After each drop, I stirred the solution for 15 seconds. The iodine's reddish-brown color initially disappeared, but eventually, the color stayed even though I was stirring. I recorded the number of iodine drops it took for the color to persist. This was the number of drops of iodine required to oxidize 31 mg of vitamin C. Next, I calculated the number of iodine drops required to oxidize 1 mg of vitamin C by dividing the total drops by 31 (the amount of vitamin C that was in the reference sample). In the end, I recorded the number of drops required to oxidize 1 mg using the iodine solution (See Table 1 for reference sample data).

Table 1: Vitamin C Reference Sample Data

Amount of Vitamin C in Sample (mg) ±0.5 mg	Amount of Iodine Drops Necessary to Oxidize Vitamin C Sample (drops) ±0.5 drops	Amount of Iodine Drops Required to Oxidize 1 mg of Vitamin C (drops) ±0.5 drops
31.0	62.0	2.0

To test the fruit (orange), I juiced oranges using a juicer to produce 739.338 mL of orange juice (see Figure 2). Then, I divided the amount of juice evenly into 25 cups (about 29.57 mL of orange juice per cup). I set the 25 cups of orange juice on a cardboard tray to store them

Figure 2: Creating Freshly-Squeezed Orange Juice

(see Figure 3). Next, I took one of the cups and added the orange juice from the cup to 118.28 mL of water in a separate glass and stirred. Then, I added 4.93 mL of the starch solution I created to the juice and water mixture and stirred. This serves as an unknown vitamin C orange juice sample.

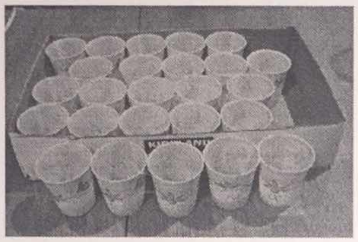


Figure 3: Storage Method

My next step was to drop iodine in the juice sample (see Figure 4) until the iodine's color persists in the sample (see Figure 5), stirring 15 times between drops. I used the same iodine solution and same eyedropper for this portion of the experiment. After stirring, I recorded the total number of drops of iodine required for the color to persist (see Appendix A for data). This was a relative measurement, as it was based off my perception of whether or not the iodine's color was persisting. Therefore, this method of measurement increases the amount of uncertainty in the measurement. Then, I calculated the amount of vitamin C per 29.57 mL of fruit juice. This was done by dividing the total number of iodine drops by the number of drops per mg of vitamin C from the reference sample. The amount of vitamin C is measured in milligrams.

Figure 4: Dropping Iodine into Orange Juice

This process was repeated for each 29.57 mL cup of orange juice, meaning that it was repeated a total of twenty-five times (see Appendix B for data). As for qualitative observations, I took notes on the physical appearance of the orange juice, and I also took notes on the scent (see Table 2). These observations were noted down for personal reference, in case any drastic physical change may have contributed to the vitamin C content measurement. Variables I controlled included the amount of stirring I did between each iodine drop (which I controlled by having a fixed amount of stirring rotations that I conducted each time). This was controlled so that the color wouldn't change based on how many times I stirred. I also used the same iodine solution and eyedropper when finding vitamin C content in the oranges. This was controlled to omit any preventable change in iodine drop size. I had to dispose the iodine with precaution, meaning that I had to try my best at avoiding any contact with my eyes and skin. This is because contact with the skin could lead to

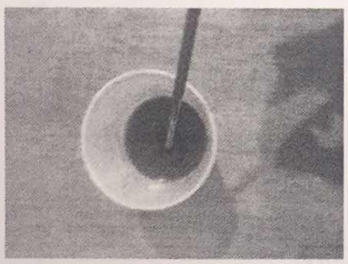


Figure 5: Iodine Color Persistence

validity

source of uncertainty

no reference to manipulating the age of the juice or fruit sample

these are validity measures.

CV would be things that could destroy vit. C
- light
- heat
- acidity

so, did you wear googles or gloves? How were the solutions disposed?

lesions, and iodine is also very irritating to the eyes. The oranges were disposed in the compost bin.

I'd like data to be in the body of the paper

Table 2: Qualitative Data	
Storage Time (days)	Description
Day 0	When the juice was freshly squeezed from the oranges, it had a bright, yellow-orange color to it. There was some remaining pulp left in the juice. Along with that, there was a slight orange scent coming from the orange juice. When the iodine was dropped into the orange juice, it was easily dissolved into the juice. At first, the color of the iodine completely disappeared, but over time, the color started to persist.
Day 1	The juice in the five trials still seemed to have a bright, yellow-orange color to it, and there still was a slight orange scent and some pulp in the juice. Overall, the juice did not have any major changes. The iodine still dissolves pretty easily.
Day 2	The smell of oranges started to become a little bit more evident. Little white dots are appearing on the surface of the orange juice (might be mold). It's a bit difficult to see whether the color of the iodine is fully dissolved or not.
Day 3	Mold is starting to grow in the orange juice in the form of white dots on the surface. The smell is becoming a bit more pungent. The iodine adds to the strength of the smell. It's getting more difficult to see the iodine color in terms of whether it persists or not.
Day 4	There is a layer of white-colored mold in the top of the orange juice in each trial (the mold is solid, and underneath it is the liquid form of orange juice). In some cups, there was a tiny bit of green mold. There is a much stronger orange scent to the orange juice. When paired with iodine, the pungency of the smell increased dramatically. It was a bit harder to see whether the iodine color persisted or not, as some of the iodine got on to the mold covering instead of in the actual orange juice.

very clear explanation of the analysis

My next step was to calculate the amount of vitamin C per 29.57 mL of fruit juice. This was done by dividing the total number of iodine drops by the number of drops per mg of vitamin C from the reference sample. Below is a sample calculation:

$$16\ iodine\ drops \div 2\ drops\ per\ mg\ of\ vitamin\ C\ from\ reference\ sample$$
$$= 8mg\ of\ vitamin\ C\ per\ 29.57\ mL\ of\ orange\ juice$$

I calculated mean to serve as a representation my data set. By finding the mean of each day that I conduct the experiment, I got an overall "summary" of each day. This allowed me to track the trend of how vitamin C content level changes in a much simpler way, as I was able to use less data points. Below is a sample calculation:

$$(8mg + 9mg + 8mg + 9mg + 8.5mg) \div 5 = 8.5mg$$

I calculated standard deviation in order to determine the amount of variation in my data (how spread out it is). This will give me an idea on how valid my results are. If the data is spread out, then I may have sources of experimental error. Figure 6 (below) shows a sample calculation of standard deviation:

Figure 6: Standard Deviation Online Calculator Results

cite source?

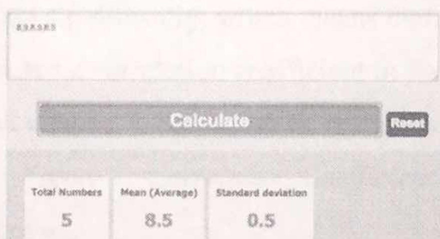


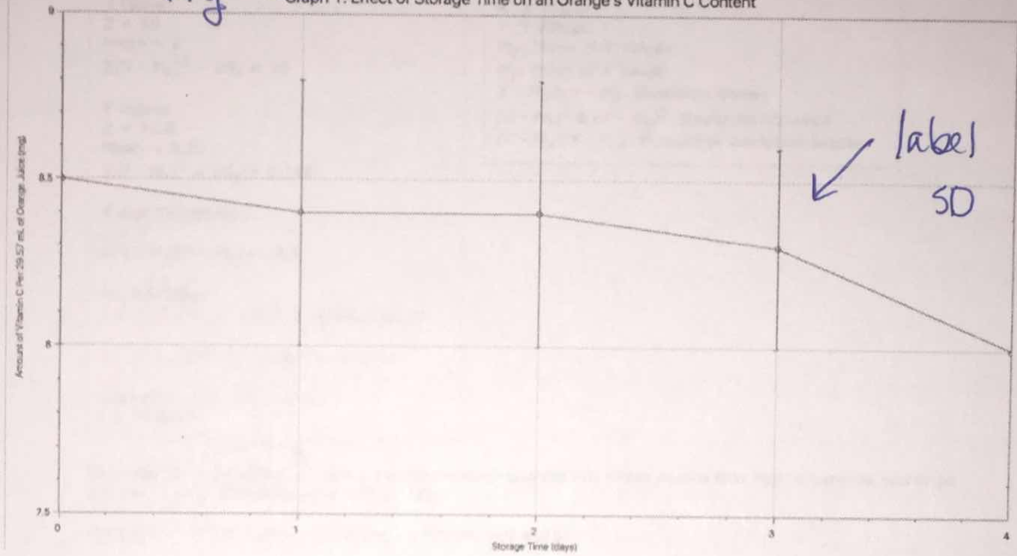
Table 3 (below) compiles the calculated averages, as well as the standard deviation values for each day I collected data.

Table 3: Effect of Storage Time on Average Amount of Vitamin C Per 29.57 mL of Orange Juice		
Storage Time (days) ±0.5 days	Average Amount of Vitamin C (mg) ±0.5 mg	Standard Deviation (mg)
0.0	8.5	0.5
1.0	8.4	0.4
2.0	8.4	0.4
3.0	8.3	0.3
4.0	8.0	0.4

I chose to visually display the data using a line graph because it depicts an overall trend of the data, and both the x-values and the y-values are quantitative (see Graph 1).

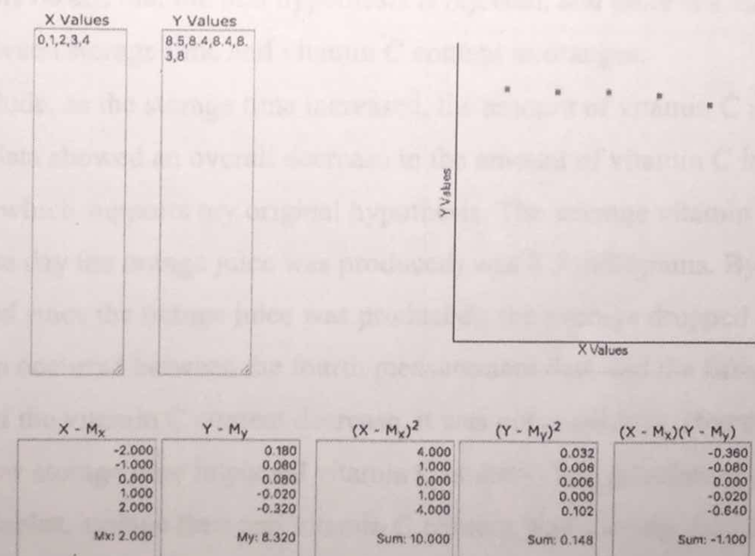
Fig ~~Graph~~ 1: Effect of Storage Time on an Orange's Vitamin C Content

slightly too small for legibility



This graph shows the effect of storage time on an orange's vitamin C content. The trend that is portrayed demonstrates that as storage time increased, the amount of vitamin C content decreased. The error bars overlap significantly, which means that the difference is not (Likely) statistically significant. I calculated a correlation coefficient to find out what kind of relationship exists between storage time and vitamin C content in an orange (see Figure 7).

Figure 7: Correlation Coefficient



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Result Details & Calculation

X Values
$\Sigma = 10$
Mean = 2
$\Sigma(X - M_x)^2 = SS_x = 10$

Y Values
$\Sigma = 41.6$
Mean = 8.32
$\Sigma(Y - M_y)^2 = SS_y = 0.148$

X and Y Combined
$N = 5$
$\Sigma(X - M_x)(Y - M_y) = -1.1$

R Calculation
$r = \Sigma((X - M_y)(Y - M_x)) / \sqrt{((SS_x)(SS_y))}$

$r = -1.1 / \sqrt{((10)(0.148))} = -0.9042$

Meta Numerics (cross-check)
$r = -0.9042$

Key

X: X Values
Y: Y Values
M_x: Mean of X Values
M_y: Mean of Y Values
$X - M_x$ & $Y - M_y$: Deviation scores
$(X - M_x)^2$ & $(Y - M_y)^2$: Deviation Squared
$(X - M_x)(Y - M_y)$: Product of Deviation Scores

The value of R is -0.9042. This is a strong negative correlation, which means that high X variable scores go with low Y variable scores (and vice versa).

The value of R^2, the coefficient of determination, is 0.8176.

In order to determine the significance of the relationship, I had to use a table of significance (see Appendix C). To do so, I found the degrees of freedom, which happened to be 3, as degrees of freedom is calculated by subtracting 2 from the amount of data pairs there are. In this case, there are five data pairs. The critical value that was associated with the degrees of freedom was 0.878. Since the absolute value of my calculated "R" value is higher than the critical value, this means that the null hypothesis is rejected, and there is a statistically significant relationship between storage time and vitamin C content in oranges.

To conclude, as the storage time increased, the amount of vitamin C in an orange decreased. My data showed an overall decrease in the amount of vitamin C in the orange juice as time passed by, which supports my original hypothesis. The average vitamin C amount on the first trial day (the day the orange juice was produced) was 8.5 milligrams. By the fifth trial day (four days passed since the orange juice was produced), the average dropped to 8.0 milligrams. The biggest drop occurred between the fourth measurement date and the fifth measurement date. As for the rate of the vitamin C content decrease, it was not consistent. However, the general trend showed how storage time impacted vitamin C content. The correlation coefficient showed that the two variables, storage time and vitamin C content, had a strong negative correlation. This means that an indirect relationship exists between the two variables. Along with that, it showed that the relationship was statistically significant.

conclusion is justified and supported

The results do match with the information that my background research gave me. In terms of the effect of storage time on vitamin C, my sources did say that prolonged storage time would decrease the amount of vitamin C in fruits. This pattern was evident in my experiment as well, since the trials on the last day of testing showed a smaller amount of vitamin C than when the experiment started. The published data that can be used to explain my results is found in an investigation done by Abubakar El-Ishaq and Simon Obirinakem. They tested the effect of temperature and storage time on various fruits and found that over time, vitamin C content decreases (El-Ishaq and Obirinakem). Their results mirror my results, which supports the reliability of my experimental outcome.

Some comparison to accepted context

There were multiple limitations associated with the experiment. For example, procedural elements such as judging how many iodine drops were necessary to make the color persist in the orange juice made an impact on my results. There was room for possible errors in terms of deciding whether or not color was persisting, especially during the final trials. The mold that formed in the orange juices toward the end of the experiment made it hard to decipher whether or not the color was being dissolved. In terms of irregularities, there wasn't a major outlier throughout the experiment. For the most part, the data seemed to be pretty consistent. This means that there was precision, but the accuracy may have been faulty. The vitamin C amount testing method was effective, but it did include some room for error. For instance, with the formation of the reference sample, I was not able to check if the solution had exactly 31 milligrams of vitamin C. If the amount of vitamin C varied in the reference sample, it would have altered my data. This is another example of a limitation I faced when conducting the experiment.

how? explain the effect on the raw data

-example of impact?

The amount of data collection was enough to portray the effect storage time had on the vitamin C content in an orange. However, more data collection would have helped with portraying whether or not the effect was significant. One change that could have been made is to extend the duration of the experiment by collecting data every week for five weeks as opposed to collecting daily. The measurement tools I used, such as the measuring spoons and measuring cup, have an uncertainty level that could have impacted my results. Once again, this falls into the topic of creating the reference sample. The uncertainty might have thrown off the ratio of ingredients within the sample, which impacts the amount of vitamin C within the sample. This could have altered my results, as the calculation of vitamin C content within the orange juice involves the amount of vitamin C in the reference sample.

suggestion

extension

vague. How?

Additional data that could have been collected include measuring temperature of the storage environment. This could be done to see if temperature may have had a bigger effect on the change in vitamin C amount in the orange juice. Some specific improvements that could be made to this experiment include controlling the variability within the environment. In other words, factors such as the temperature of the environment and air quality and composition could be controlled. An additional research question that arose from my results was about how storage type affects the vitamin C content in an orange. Essentially, this additional research question involves testing different storage environments (refrigerator, open air, etc.) on vitamin C content. This is related to the results of my experiment because it involves the two main factors: storage and vitamin C content.

Works Cited

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Appendix

Appendix A:

Table 4: Effect of Storage Time on Amount of Iodine Drops Necessary For Color Persistence in Orange Juice					
Storage Time (days) ±0.5 days	Amount of Iodine Drops (drops) ±0.5 drops				
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
0.0	16.0	18.0	16.0	18.0	17.0
1.0	18.0	16.0	17.0	17.0	16.0
2.0	17.0	17.0	16.0	16.0	18.0
3.0	17.0	16.0	17.0	17.0	16.0
4.0	16	16	17	16	15

should have consistant precision to tenths place

Appendix B:

Table 5: Effect of Storage Time on Amount of Vitamin C Per 29.57 mL of Orange Juice					
Storage Time (days) ±0.5 days	Amount of Vitamin C (mg) ±0.5 mg				
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
0.0	8.0	9.0	8.0	9.0	8.5
1.0	9.0	8.0	8.5	8.5	8.0
2.0	8.5	8.5	8.0	8.0	9.0
3.0	8.5	8.0	8.5	8.5	8.0
4.0	8.0	8.0	8.5	8.0	7.5

PE = 2, 1, 2 ⇒ 2
EX = 4, 3, 3, 3 ⇒ 3
AN = 5, 6, 3, 5 ⇒ 5
EV = 5, 4, 4, 4 ⇒ 4
CO = 4, 3, 4, 3 ⇒ 4
18

Appendix C:

Values of r for the .05 and .01 Levels of Significance

$df(N-2)$	.05	.01	$df(N-2)$	.05	.01
1	.997	1.000	31	.344	.442
2	.950	.990	32	.339	.436
3	.878	.959	33	.334	.430
4	.812	.917	34	.329	.424
5	.755	.875	35	.325	.418
6	.707	.834	36	.320	.413
7	.666	.798	37	.316	.408
8	.632	.765	38	.312	.403
9	.602	.735	39	.308	.398
10	.576	.708	40	.304	.393
11	.553	.684	41	.301	.389
12	.533	.661	42	.297	.384