### Introduction

Vitamin C has been a crucial part of diets since vitamin C deficiency caused scurvy to plague sailors in the 19th century (Sardi). Vitamin C is an essential aspect of modern day diets as well. If people drink a glass of orange juice or consume a few green peppers in the course of their diet, it may not be enough to prevent them from the common cold; but studies have found that symptoms' severity and their time span can be lessened by the consumption of vitamin C (Douglas, Hemila, et ad). Therefore, who wouldn't buy a whole crate of oranges and eat them each and every day? The only complication with this home remedy of the common cold, is how and when to consume the highest intake of vitamin C as possible.

There have been multiple cases of vitamin C deficiencies, specifically in Africa. In the country of Zimbabwe, most of the adults have vitamin C deficiencies due to their genetic bloodline and poor diets. The adults have a protein called transferrin polymorphism, which overall causes the deficiency. This protein overturns the process of vitamin C in their bodies and causes them to not properly retain the nutrients of the vitamin ("Scurvy and Its Prevention and..."). Also, due to their poor diets throughout the country, they usually do not consume the vegetables directly following harvest; therefore, the Zimbabwean's usually do not have the chance to consume maximum nutrient intake available to them.

This research piqued the argument of which is better, fresh or frozen produce. This is due to the time span of when the different type of products are

packaged or consumed. Previous research conducted concluded that frozen produce yields a higher vitamin C concentration due to the lack of exposure to sunlight and air (Dominey). Through this experiment and analysis, the argument was settled.

To discover when to consume the highest intake of vitamin C as possible in fresh produce, the researchers tested the vitamin C concentration on the first, third and fifth days after harvest. The goal was to discover the peak of vitamin C concentration in green bell peppers over the course of the five days in addition to finding a clear trend of the vitamin C concentration decay over time. If a clear negative trend of vitamin C over time was discovered, it can be assumed that as time continued to progress past the five day interval, the vitamin C would continue to decay.

The researchers conducted this research on the trend of vitamin C concentration over time after harvest due to lack of specified research in the field. Green peppers were researched due no previous research similar to this was conducted and the pepper's higher yield of vitamin C concentration than oranges. On average, green bell peppers yields almost double the vitamin C as oranges at 120 mg compared to 69.7 mg per one cup (Mattheis).

To eradicate this issue, the researchers have decided to test what day locally grown green peppers contain the highest concentration levels of vitamin C. A set of 12 green peppers were harvested on a sunday from the same control home-grown garden. Four peppers were finely chopped and juiced on the first,

third and fifth days after harvest to attempt to prove the inverse relationship of time and vitamin C concentration. A consistent amount of green peppers were tested each day in addition to keeping conditions the same, all in an attempt to keep outliers and inconsistencies out of the data.

The researchers then titrated an iodine solution (Appendix B) with green peppers and soluble starch indicator solution (Appendix A). The endpoint of the titration between these two solutions is indicated by a blue-black complex (Figure 8). Testing and meticulous observations on these designated days allowed sufficient information about vitamin C levels in green peppers to be collected and analyzed.

The volume of iodine solution used to complete the titration was used as the quantifiable data that was compared. If an inverse relationship between time and vitamin C concentration is discovered, this would further prove that frozen produce is more beneficial to consume for nutritional purposes, due to the decay of vitamin C in fresh produce over time after harvest.

Through experimentation, it was analyzed that there is a significant trend to the vitamin C concentration levels throughout the week. It is evident that on the first day of harvesting the green peppers contain the most vitamin C. The days after the first day there is a prominent trend of vitamin C decay. In Figures 9 and 10 in Data and Observations, the graphs indicate an inverse relationship of the vitamin C levels over time.

It is now evident after completing this research that green bell peppers begin to oxidize and lose vitamin C concentration immediately following harvest. This research uncovered that an average of 42.12% of the original vitamin C concentration from the first day decayed by the fifth day after harvest. It can also be established that during the first week after harvest, there is nearly a 10% decrease in the vitamin C concentration per day.

This proves that frozen produce, which are frozen shortly after they are harvested, would be highly beneficial to consume when attempting to incorporate vitamin C in diets due to the lack of time exposed to air and sunlight. Frozen produce is allowed to ripen naturally and then frozen, "locking in" the nutrients. In contrast, fresh produce could have been harvested before their natural peak and forced to artificially ripen during transport (Sass). If maximum vitamin C consumption in one's diet is reached, symptoms from a common cold can be lessened along with the prevention of the 19th century deadly disease called scurvy ("Scurvy and Its Prevention and..."). Vitamin C deficiencies and nutritionists can benefit from this information that due to the rapid decay in vitamin C, the produce should be consumed as soon as possible.

#### **Review of Literature**

The nutritional difference between fresh and frozen fruits and vegetables varies with each. Produce containing high amounts of vitamin B and C are the best when fresh due to the vitamins being water-soluble, meaning that during the food process they will lose its water base, also losing the nutritional value. Green bell peppers are one vegetable that is suggested to be consumed fresh rather than frozen due to the water-solubility interfering with the food process fresh fruits and vegetables produce enzymes that ultimately cause loss of color, flavor, and nutrients just after harvesting. But the reaction can be stopped by defusing the enzyme - which freezing executes - leaving the frozen vegetables with more nutrients. When the freezing process is done correctly, certain storage processes can also cause some nutrient loss due to oxidation. The produce that are best frozen are those with high amounts of fat-soluble nutrients, like vitamin A and E, because they are more stable during the food processing and storage (Franklin).

In the United States the majority of food, with the exception of certain organic foods, contain pesticide residue. Although the pesticides present in food are at a very small trace level, their negative impact on health still remains present. Several organs in the body lose its ability to produce energy due to the pesticide exposure. Environmental Working Group's 2014 report "Shopper's Guide to Pesticides," conventionally grown bell peppers are one of the top 12 fruits and vegetables of which pesticide residues have been most frequently found. Therefore, people that prefer to avoid pesticide-induced health risks may

want to avoid consuming bell peppers unless they are grown organically without pesticides ("Bell Peppers...").

Two solutions are commonly used in iodometric titration: Iodine solution and starch indicator solution. The iodine solution is made up of three components: Iodine, I; Potassium Iodide, KI; and Water, H<sub>2</sub>O. Soluble starch solution is made up of two components: Potato Starch,  $C_{28}H_{48}O_{20}$ ; and near boiling water, H<sub>2</sub>O ("Determination of Vitamin C..."). Natural starch is usually made up of 10-20% amylose and 80-90% amylopectin. Amylose completely ionizes into hot water proving it soluble, whereas amylopectin is completely insoluble (Ophardt). This means if soluble starch is used, to ionize completely, the starch must be completely amylose. Starch is often added to titration mixtures with iodine because of its highly visible color change (Tucker).

The method of iodometric titration is utilized to find the concentration of vitamin C. University of Canterbury also used iodometric titration to determine the concentration of ascorbic acid, or vitamin C. Titration can be completed when two solutions are used: one with a known concentration, the titrant and one with an unknown concentration, the analyte. The concentration of the titrant is used to determine the concentration of the analyte, and in this case it is ascorbic acid. The titrant that is placed in the Burette to determine the concentration of vitamin C is a 0.005 M iodine solution. The analyte, in the 250 mL erlenmeyer flask contains 20 mL of pureed green pepper - the vitamin C solution - 150 mL of distilled water, and 1 mL of starch solution ("Determination of Vitamin C..."). The

concentration of vitamin C is then determined by the first permanent trace of blue-black trace in the erlenmeyer flask, indicating that concentration of ascorbic acid in the green pepper puree ("What Is a Titration?").

When the titrant, the molecular iodine, combines with the analyte, pureed green peppers, starch solution, and distilled water, it creates a blue-black complex. The color change displays an oxidation-reduction reaction between ascorbic acid and the iodine solution (Figure 2). The iodine formed by this reaction oxidizes the ascorbic acid to dehydroascorbic acid as the iodine is reduced to iodide ions (Figure 3).

 $\mathsf{A}_{\mathsf{ox}} + \mathsf{I}_2 \to \mathsf{A}_{\mathsf{red}} + 2\mathsf{I}^{\scriptscriptstyle -}$ 

Figure 2. Oxidation-Reduction Titration Base Equation

Figure 2 represents the base equation for oxidation-reduction titrations. In iodimetric titrations, the analyte (a reducing agent) reacts with iodine to produce iodide. It claims that an oxidizing agent,  $A_{ox}$ , reacts with the iodine to create a reducing agent,  $A_{red}$ , and iodide.

 $C_6H_8O_6 + I_2 \rightarrow 2 I^- + C_6H_6O_6 + 2H^+$ 

Ascorbic Acid + Molecular Iodine  $\rightarrow$  Iodide Ions + Dehydroascorbic Acid + Hydrogen Gas Figure 3. Oxidation-Reduction Titration Equation

The equation above indicates the reaction occurring when the ascorbic

acid reacts with the iodine solution.

Due to this process, the iodine formed is immediately reduced to iodide as long as there is any ascorbic acid present. Once all the ascorbic acid has been oxidized, the excess iodine is free to react with the starch indicator, forming the blue-black starch-iodine complex. This color change signifies the equilibrium point of the reaction ("Determination of Vitamin C...").



## Figure 1. Analyte and Titrant

The figure above displays the analyte and titrant in the new research. The analyte, or the solution with the unknown concentration, is the ascorbic acid concentration of the green peppers in the 250 mL erlenmeyer flask. The titrant in this case, or the solution with the known concentration, is the concentration of the iodine solution that is found in the burette.

In an experiment conducted by Dr. Raymond N Dominey at Richmond

University, executes a redox titration with iodine. This research explains that the

concentration of vitamin C tablets are unstable, due to the fact that they are slowly oxidized by air. This pertains to the current research in the aspect that the researchers believe that fresh produce could also be oxidized by hair, losing nutrients over time.

In comparison to the new research, an iodimetric reaction is taking place where the oxidizing agent would be ascorbic acid while the reducing agent is the dehydroascorbic acid (Dominey). Furthermore, Dominey elaborates on the fact that solid iodine is not likely to ionize in water alone. However, when dissolved with an iodide salt, like potassium iodide, the iodine becomes soluble. Although their execution of their experiment seems similar to the new researchers, Dominey also was attempting to find an analysis of hydrogen peroxide. This titration is still considered iodimetric because it is based on the reaction of an analyte with aqueous iodine. Additionally, contrasting titrant was used to cause the reaction. This proves the numerous types of ways that iodine titration can be applied (Dominey).

When the endpoint of the titration is complete, the concentration of vitamin C can be calculated using the volume of the iodine solution. This is done by calculating the number of moles of iodine reacting in the titrant. When finding this, the mole ratios from the balanced chemical equation of the titration are used to calculate the number of moles of ascorbic acid reacting (Figure 5). Overall, the quantifiable value from this titration that can be compared to one another would be the concentration of vitamin C, in mol L. The higher concentration of vitamin C

in a certain solution, the more of the nutrients it contains and the more it benefits your health by consuming it ("Determination of Vitamin C...").

 $\begin{array}{l} M \ of \ I_2 \ = \ 0.005 \ mol/L \\ mL \ of \ I_2 \ used \ = \ 13.5 \ mL \ = \ 0.0135 \ L \\ M \ = \ \frac{mol}{L} \\ 0.005 \ M \ = \ \frac{x \ mol}{0.0135 \ L} \\ Mol \ I_2 \ used \ = \ 6.75 \ \times \ 10^{-5} \\ \frac{6.75 \ x \ 10^{-5} \ mol \ I_2 \ \times \ 1 \ mol \ I_2 \ \times \ 1 \ mol \ I_2 \ M \ O_6 \\ = \ 6.75 \ \times \ 10^{-5} \ mol \ C_6 H_8 O_6 \\ \end{array}$ 

### Figure 5. Sample Calculation

Figure 5 displays a sample calculation from a trial that was completed. The molarity of the lodine solution is 0.005 due to the way the researchers prepared it. The mL ,converted to L, of I2 that was used was then recorded in the trial. The moles of I<sub>2</sub> was then calculated by using the equation for molarity,  $M = \frac{mol}{L}$ . Dimensional analysis was then completed using the balanced chemical equation. There is a one to one mol ratio, therefore the moles of ascorbic acid that is used is equal to the moles of iodine used from the burette.

Vitamin C is abundant in vegetables and fruits and allows the body to form and maintain connective tissue, including bones, blood vessels, and skin. Vitamin C is formally known as ascorbic acid ( $C_6H_8O_6$ ) and is considered a water-soluble vitamin and a powerful antioxidant. Vitamin C has useful attributes which protect against heart disease, aid in the absorption of iron, and decrease cholesterol. Also, some research indicates that vitamin C may aid the immune system to protect itself against a variety of cancers by helping neutralize the effects of nitrites, which are preservatives found in some packaged foods that may raise

the risk of these forms of cancer. Supplemental vitamin C may also lessen the duration and symptoms of a common cold, help delay or prevent cataracts, and support healthy immune function. Therefore, vitamin C, or ascorbic acid, contains many beneficial effects and solutions (Weil).

Vitamin C deficiency is a substantial problem in the world today. The signs of vitamin C deficiency include fatigue, muscle weakness, joint and muscle aches, bleeding gums, and leg rashes. It is crucial that each person consumes enough vitamin C so the possible signs of deficiency are not experienced. According to the National Institutes of Health (NIH), the recommended daily intake for an adult male is 90 mg per day. An adult woman should have 75 mg per day, while a pregnant woman would need 85 mg and a breastfeeding woman would need 120 mg. If a person is a smoker, the doctor suggests there is much benefit to having a higher intake for children between 4 to 13 should have about 35 mg per day, whereas teens ages 14 to 18 should have about 70 mg per day (Weil).

Due to people having vitamin C deficiencies and previous research conducted, the new researchers wanted to conduct research to see what day exactly, after harvesting the green bell peppers from a locally grown farm, contains the highest amount of ascorbic acid.

## Problem Statement

### Problem:

To determine on what day, in a trial period of five days, of locally grown green bell peppers, will yield the greatest vitamin C (ascorbic acid) concentration. This will prove if there is an inverse relationship between time and the vitamin C concentration in green bell peppers.

### Hypothesis:

The first day after the green bell peppers are harvested will yield the highest concentration of vitamin C in comparison to day three and day five after the pepper is harvested. There will be an inverse relationship between the concentration of vitamin C and time.

#### Data Measured:

The independent variable was the day the green pepper concentration was calculated on the harvested peppers after it was picked: day one, day three, and day five. The dependent variable in the experiment was the vitamin C concentration of the green bell peppers in moles per liter. Two sets of twelve trials were conducted throughout the course of the experiment, each week with a different batch of sweet bell peppers from the same local home garden. Therefore, four trials were completed on each of the three days during the week. Each one of the two sets were averaged and was used to statistically compare the concentration of the peppers on day one, day three, and day five after being picked. An ANOVA test was conducted, along with descriptive analysis, to compare the concentrations on these three days to calculate if there was a significant difference. These quantifiable results were also analyzed in attempt to discover an inverse relationship between time and the vitamin C concentration of green bell peppers.

# **Experimental Design**

Materials:

- (9) Day one homegrown green peppers, picked from a local garden
- (9) Day three homegrown green peppers, picked from a local garden
- (9) Day five homegrown green peppers, picked from a local garden
- 10 mL Graduated cylinder 25 mL Graduated cylinder (2) 100 mL Graduated cylinder 100 mL beaker 250 mL Erlenmeyer flask 500 mL beaker Scale (0.0001 precision)

5 g of potato starch,  $C_{27}H_{48}O_{20}$ 10 g of potassium iodide, KI 7 g of iodine,  $I_2$ Magnetic Stirrer 1.5 Cup food processor Burette <sup>1</sup>/<sub>2</sub>" Burette clamp Ring stand 1 mL pipette Pipette bulb (10) 10" by 10" Cheesecloth (3) Weigh Boats (3) Scupulas Stir plate Hot plate Ti-nspire calculator

# Procedure:

- 1. Finely chop one and a half cups of green peppers.
- 2. Purée the chopped green peppers in food processor until there is a visible watery consistency to the green peppers.
- 3. Measure 20 mL of pureed green pepper into a 250 mL erlenmeyer flask.
- 4. Measure and add 150 mL of distilled water and 1 mL of starch indicator solution (Appendix A) into the 250 mL erlenmeyer flask.
- 5. Pour 50 mL of 0.005 mol  $L^{-1}$  iodine solution into the burette.
- Titrate the sample in the 250 mL erlenmeyer flask with 0.005 mol L<sup>-1</sup> iodine solution (Appendix B) through the burette, while a magnetic stirrer and a stir plate mix the solutions together.
- 7. Record observations of the green pepper and soluble starch solution in the 250 mL erlenmeyer flask as titration is conducted.
- 8. Identify the end of the titration when the green bell pepper solution becomes a dark blue-black color due to the starch-iodine complex.

- 9. Record the amount of iodine solution used to titrate the green pepper and soluble starch solution, in milliliters.
- 10. Repeat the titration with further aliquots of sweet green bell pepper puree each day until data collection has concluded.

## Diagram:



Figure 6. Titration Diagram ("Determination of Vitamin C...") Above is a diagram containing the titration setup for this experiment. The

Burette stand holding the Burrett containing the iodine solution. The burrett is

placed over the Erlenmeyer Flask containing the vitamin C sample solution - the

homegrown green peppers - and the starch indicator solution.





## Data and Observations

Trial	lodine Solution (mL)	Concentration of Ascorbic Acid (mol/L)
1	33.00	1.65 x 10 <sup>-₄</sup>
2	34.00	1.70 x 10 <sup>-4</sup>
3	33.75	1.69 x 10⁻⁴
4	35.40	1.77 x 10 <sup>-4</sup>
		Average = 1.70 x 10 <sup>-4</sup>

Table 1Week One-Day One After Harvest Trails (October 17th)

This table shows the first week of trials on the first day of harvesting the

green bell peppers from the garden. The temperature on the Sunday the peppers were harvested, October 16th, had a high of 69°F low of 63°F (Appendix C).

Table 2Week One-Day Three/Four After Harvest Trials (October 19th/October 20th)

Trial	lodine Solution (mL)	Concentration of Ascorbic Acid (mol/L)
1	35.50	1.78 x 10⁻⁴
2	32.25	1.61 x 10 <sup>-4</sup>
3	30.00	1.50 x 10⁻⁴
4	29.50	1.48 x 10 <sup>-4</sup>
		Average = 1.59 x 10 <sup>-4</sup>

This table shows the first week of trials on the third and fourth day of picking the green bell peppers. The first two trials were done on the third day, but the reason the iodine solution used was so high because the starch solution was expired and was not completely dissolving. The last two trials were done on the fourth day using a new starch solution which dissolved completely. This is due to the fact that the trials on day three were not running normally, the researchers took all precautions to have the most reliable data possible. The amount of iodine solution, in mL, that was collected was converted to the concentration of ascorbic acid in the green peppers, in mol/L (Table 5). The temperature on the Sunday the peppers were harvested, October 16th, had a high of 69°F low of 63°F (Appendix C).

Trial	lodine Solution (mL)	Concentration of Ascorbic Acid (mol/L)
1	13.50	6.80 x 10 <sup>-5</sup>
2	14.25	7.13 x 10⁻⁵
3	14.20	7.10 x 10⁻⁵
4	15.00	7.50 x 10 <sup>-5</sup>
		Average = 7.13 x 10 <sup>-5</sup>

Table 3Week One-Day Five After Harvest Trials (October 21st)

This table shows the first week of trials and on the fifth day of picking the green bell peppers from a locally grown garden. The amount of iodine solution, in mL, that was collected was converted to the concentration of ascorbic acid in the green peppers, in mol/L. The temperature on the Sunday the peppers were harvested, October 16th, had a high of 69°F low of 63°F (Appendix C).

Table 4	
Week One-Day One Observations (October 17th)	

Trial	Observations	
1	The color change for the iodine-starch complex occurred quicker than other trials; Reached equilibrium point quicker than others. Conducted on Day 1.	
2	This trial ran normally; the dark blue-black color was reached at a moderate rate. Conducted on Day 1.	
3	This trial ran normally; the dark blue-black color was reached at a moderate rate. Conducted on Day 1.	
4	Frothy foam was formed on top of the green pepper solution. Therefore, when 20 mL of the green pepper solution was measured, the measurement may not have been exact. This trial ran normally for the most part, but the color change for the iodine-starch complex occurred quicker than others. Conducted on Day 1.	

In the table above, the observations taken during the first week of trials on

day 1 are stated. The researchers observed the trials meticulously and recorded

what was seen. The temperature on the Sunday the peppers were harvested,

October 16th, had a high of 69°F low of 63°F (Appendix C).

Table 5

Week One-Day Three/Four Observations (October 19th/October 20th)

Trial	Observations	
1	The green pepper solution was a lighter green than usual. The iodine solution that was made on Monday was used. A small amount of frothy foam was formed on top of the green pepper solution. The color change for the iodine-starch complex occurred after a longer period of time than the first day after harvest, but when comparing the data for the third day after harvest, it is constant. Conducted on Day 3.	
2	Used lodine solution from monday. The color change happened suddenly and was not uniform to the other trials. When mixing, the blue-black of the iodine solution would linger for long	

Trial	Observations	
2	Points at a time and did not have a sudden endpoint where the permanent black-blue trace was evident. It was concluded that fresh iodine solution must be made everyday. Day 3.	
3	Made new iodine and starch solution. Conducted on Day 4. This trial ran normally; the dark blue-black color was reached at a moderate rate.	
4	Made new iodine and starch solution. Conducted on Day 4. This trial ran normally; the dark blue-black color was reached at a moderate rate.	

In the table above, the observations taken during the first week of trials on

day 1 are stated. The researchers observed the trials meticulously and recorded

what was seen. The temperature on the Sunday the peppers were harvested,

October 16th, had a high of 69°F low of 63°F (Appendix C).

## Table 6

## Week One-Day Five Observations (October 21st)

Trial	Observations
1	Made new iodine solution and starch solution. Conducted on Day 5. The color of the green pepper solution was a more vibrant green. This trial ran normally; but the dark-blue-black color was reached faster than the other trials on this day.
2	This trial's green bell pepper solution contained some frothy solution. Conducted on Day 5. This trail ran normally and the blue-black color was reached at a moderate rate.
3	This trial contained a frothy substance on the green pepper solution. Conducted on Day 5. Since the green pepper solution had more bubbles than usual, so it used more iodine solution to titrate it.
4	This trial had a vibrant green pepper solution. Conducted on Day 5. This trial normally titrated and at a normal rate.

In the table above, the observations taken during the first week of trials on day 1 are stated. The researchers observed the trials meticulously and recorded what was observed. The temperature on the Sunday the peppers were harvested, October 16th, had a high of 69°F low of 63°F (Appendix C).

Table 7 Week Two-Day One After Harvest Trials (October 24th)

Trial	lodine Solution (mL)	Concentration of Ascorbic Acid (mol/L)
1	33.00	1.65 x 10⁻⁴
2	34.50	1.73 x 10⁻⁴
3	32.00	1.60 x 10 <sup>-4</sup>
4	35.00	1.75 x 10 <sup>-4</sup>
		Average = 1.68 x 10 <sup>-4</sup>

This table shows the second week of trials and on the first day of picking

the green bell peppers from a locally grown garden. The amount of iodine solution, in mL, that was collected was converted to the concentration of ascorbic acid in the green peppers, in mol/L. The temperature on the Sunday the peppers were harvested, October 23rd, had a high of 65°F low of 45°F (Appendix C).

 
 Trial
 Iodine Solution (mL)
 Concentration of Ascorbic Acid (mol/L)

 1
 18.25
 9.10 x 10<sup>-5</sup>

 2
 21.00
 1.05 x 10<sup>-4</sup>

 3
 19.75
 9.90 x 10<sup>-5</sup>

Table 8Week Two-Day Three After Harvest Trials (October 26th)

Trial	lodine Solution (mL)	Concentration of Ascorbic Acid (mol/L)
4	20.50	1.03 x 10 <sup>-4</sup>
		Average = 9.95 x 10 <sup>-5</sup>

This table shows the first week of trials and on the first day of picking the

green bell peppers from a locally grown garden. The amount of iodine solution, in mL, that was collected was converted to the concentration of ascorbic acid in the green peppers, in mol/L. The temperature on the Sunday the peppers were harvested, October 23rd, had a high of 65°F low of 45°F (Appendix C).

Table 9Week Two-Day Five After Harvest Trials (October 28th)

Trial	lodine Solution (mL)	Concentration of Ascorbic Acid (mol/L)
1	9.500	4.80 x 10 <sup>-5</sup>
2	10.00	5.00 x 10 <sup>-5</sup>
3	12.25	6.10 x 10 <sup>-5</sup>
4	10.50	7.50 x 10 <sup>-5</sup>
		Average = 5 85 x 10 <sup>-5</sup>

This table shows the first week of trials and on the fifth day of picking the green bell peppers from a locally grown garden. The amount of iodine solution, in mL, that was collected was converted to the concentration of ascorbic acid in the green peppers, in mol/L. The temperature on the Sunday the peppers were harvested, October 23rd, had a high of 65°F low of 45°F (Appendix C).

Table 10Week Two-Day One Observations (October 24th)

Trial	Observations	
1	This trial reached the blue-black complex quicker than usual and used less of the iodine solution than the other trials this day. Conducted on Day 1.	
2	This trial ran normally; the dark blue-black color was reached at a moderate rate. Conducted on Day 1.	
3	This trial took longer to titrate and it used up more iodine solution than the other trials this day. This green pepper solution had a lot of the frothy solution on top. Conducted on Day 1.	
4	The green pepper solution had a few bubbles but it did not seem to affect the data. This trial ran normally and at a moderate rate. Conducted on Day 1.	

In the table above, the observations taken during the first week of trials on

day 1 are stated. The researchers observed the trials meticulously and recorded

what was seen. The temperature on the Sunday the peppers were harvested,

October 23rd, had a high of 65°F low of 45°F (Appendix C).

Table 11

Week Two-Day Three Observations (October 26th)

Trial	Observations				
1	The green pepper solution was a normal color but with some bubbles. Made a new iodine solution and starch for the day. The color change for the iodine-starch complex occurred at a normal rate. Conducted on Day 3.				
2	The green pepper solution contained a lot of the bubbles and frothy solution on top. The final complex contained a purple tone. Additionally, the color change happened slower than accustomed for. Conducted on Day 3.				
3	The green pepper solution was exactly 20 mL with no bubbles this trial. The trial ran normally with the dark blue-black complex reaching at a moderate rate. Conducted on Day 3.				

Trial	Observations			
4	This green pepper solution contained a little bubbles, but did not seem to affect the data. The blue-black complex was reached at a moderate rate. Conducted on Day 3.			

In the table above, the observations taken during the first week of trials on

day 1 are stated. The researchers observed the trials meticulously and recorded

what was seen. The temperature on the Sunday the peppers were harvested,

October 23rd, had a high of 65°F low of 45°F (Appendix C).

Table 12Week Two-Day Five Observations (October 28th)

Trial	Observations
1	Made new iodine solution and starch solution. The color of the green pepper solution was a normal green. This trial ran normally and the dark blue-black color was reached at a moderate rate. Conducted on Day 5.
2	This trial's green bell pepper solution contained some frothy solution on top. This trail ran normally, but at a slower rate than usual to reach the blue-black complex. Conducted on Day 5.
3	The green pepper solution was a normal green color. The trial ran normally and the blue-black complex was reached at a normal rate. Conducted on Day 5.
4	The green pepper solution contained more bubbles than usual and the color was more vibrant. The titration process took longer to reach than normal. Conducted on Day 5.

In the table above, the observations taken during the first week of trials on

day 1 are stated. The researchers observed the trials meticulously and recorded

what was seen. The temperature on the Sunday the peppers were harvested,

October 23rd, had a high of  $65^{\circ F}$  low of  $45^{\circ F}$  (Appendix C).

Level One	Level Two	Level Three
Concentration of Ascorbic Acid (mol/L)	Concentration of Ascorbic Acid (mol/L)	Concentration of Ascorbic Acid (mol/L)
χ>	5.85 x 10 <sup>-5</sup> - 1.70 x 10 <sup>-4</sup>	< X



Level Two









Figure 8. Level of Color Changes Figure 8 above displays the three levels of titrated ascorbic acid and soluble starch. The image on the left is the color of the green pepper solution

before beginning the titration process. The middle image displays the end of the titration process, when there is the first trace of blue-black complex. The last

image is when the process is too titrated and the iodine solution overbearing the

ascorbic acid.

### Data Analysis

Descriptive analysis was completed to display clear patterns in our data. The average of each day was used in each line graph, therefore the four trials per day was averaged and used as a point in Figure 9 and Figure 10. The concentration of green pepper solution - in moles per liter- over time - days after harvest - was collected using a burette and converting the volume of iodine solution used (Figure 5). These two factors are then displayed in the two figures below, to display the inverse relationship in the concentration of green bell pepper solution over time.



### Figure 9. Graph of Week One Data

Week one's data displayed an inverse relationship in the time after harvesting - in days - and the concentration - in moles per liter - of the green pepper solution. As the days after harvest increased, the concentration of the green pepper solution decreased. The difference in the concentration of green pepper solution decreased 1.1 x 10<sup>-5</sup> moles per liter from day one after harvest to day three after harvest. In addition, the concentration of the green pepper solution decreased 8.8 x 10<sup>-5</sup> moles per liter from day three after harvest to day five after harvest. 49.41% of the original Vitamin C from the first day after harvest is lost over the five day span. This graph supports the hypothesis that as the time after harvest increases, the concentration - or amount of ascorbic acid (vitamin C) - of the green peppers decreases.



### Figure 10. Graph of Week Two Data

Week two's data displayed an inverse relationship in the time after harvesting - in days - and the concentration - in moles per liter- of the green pepper solution. As the days after harvest increased, the concentration of the green pepper solution decreased. The difference in the concentration of green pepper solution decreased 6.85 x  $10^{-5}$  moles per liter from day one after harvest to day three after harvest. In addition, the concentration of the green pepper

solution decreased 4.1 x 10<sup>-5</sup> moles per liter from day three after harvest to day five after harvest. 34.82% of the original Vitamin C from the first day after harvest is lost over the five day span. This graph supports the hypothesis that as the time after harvest increases, the concentration - or amount of ascorbic acid (vitamin C) - of the green peppers decreases.

Due to the fact that this research is comparing three sample means from three independent populations, it is appropriate to complete an ANOVA or Analysis of Variance test. This test was conducted to determine if there is a significant difference between at least one of the population means. The data collected during the experiment was quantitative data due to the fact that a measured value of the concentration of ascorbic acid in moles per liter was collected using titration. A comparative experiment was conducted to determine if there is a significant difference between at least one of the values of ascorbic acid concentration from the first day after harvest, third day after harvest, or fifth day after harvest. Two sets of 12 trials were completed for each week of harvest. The 24 data trials were completed for each treatment due to the fact that it is proven that the more data trials conducted, the less varied data becomes. Reliable data was collected with the utilization of a control of the same garden when harvesting the green peppers, replication to keep conditions constant, and randomization in choosing which peppers from what was harvested to puree and test on that certain day.

When developing the trial process, it was decided to keep a control by setting one garden to harvest from. By setting one garden to harvest all of the green peppers from, all produce tested was exposed to the same weather and growing conditions. This kept all the produce tested as similar as possible, therefore no outliers could be caused from the green peppers being harvested from different gardens with different growing conditions. As a result, any differences in the data calculated are not caused by lurking variables.

The replication to keep conditions constant was used to keep data as accurate as possible was mostly included in each researcher's responsibility during the trial. For example, at the beginning of each day of trials researcher one chopped and pureed the green peppers while researcher two made the iodine and starch indicator solutions. This was to keep the process constant on a daily basis, in addition to time management. Therefore, any data inconsistencies are not due to contrasting researchers completing tasks differently. When collecting data, researcher one handled the titration process and researcher two recorded all the observations and data for each trial. Randomization could be considered to reduce bias in this process; however, to create less variability between trials, it was concluded that constant responsibilities per researcher would create more accurate data.

Along with the repetition of the same researcher, the uniform equipment was used throughout the trials. This removes bias in the fact that different equipment could function differently than another, which may affect data trials.

Overall, the equipment was held at a constant to prevent any calculation variations. Randomization was also utilized for which green peppers were used for each trial. The researchers numbered the 12 green peppers harvested each week and utilized the TI-nspire to ensure equal chance to each locally grown pepper harvested.

The data that was collected was normal, even though the central limit theorem of 30 data points per trial was not met. The 30 data points could not be gathered due to a limited time and supply of the green peppers. The green peppers were harvested from a locally grown garden with limited supply and the testing was conducted in the month of October, so the weather permitted only two weeks of trials in the time allotted. By examining the box plots, it is shown that the data is normal and contains no outliers, therefore allowing the ANOVA statistical test to be conducted on the normally distributed populations.

To conduct the ANOVA statistical test, there are assumptions that need to be met. The first assumption is that the data collected needs to be randomized in as a Simple Random Sample, this was done on the TI-nspire software to reduce bias. The second assumption is normality and this was met by the evaluation of the graphical data through box plots and line graphs. There are no major skews in any box plots and no outliers, therefore determining that the data is normal. The last assumption was that the largest standard deviation was not more than twice the smallest standard deviation. This was met when comparing the smallest standard deviation of the 5.73 x  $10^{-6}$  to the largest standard deviation which was the 1.08 x  $10^{-5}$ .

$$\begin{split} &H_{o}: \mu_{\text{Day 1}} = \mu_{\text{Day 3}} = \mu_{\text{Day 5}} \\ &H_{a}: \text{Not all } \mu_{\text{Day 1}}, \mu_{\text{Day 3}}, \text{ and } \mu_{\text{Day 5}} \text{ are equal} \\ &\text{Figure 11. Hypotheses for ANOVA test} \end{split}$$

The figure above displays the hypotheses above were made for the ANOVA test conducted.  $\mu_{Day 1}$  represents the population mean of ascorbic acid concentration from the trials of green peppers harvested after one day,  $\mu_{Day 3}$  represents the population mean of ascorbic acid concentration from the trials of green peppers harvested after the third day, and  $\mu_{Day 5}$  represents the population mean of ascorbic acid concentration from the trials of green peppers harvested after the third day, and  $\mu_{Day 5}$  represents the population mean of ascorbic acid concentration from the trials of green peppers harvested after the fifth day. The null hypothesis, H<sub>o</sub>, states that there is no significant difference in the mean ascorbic acid concentration throughout a five day shelf life. This means that the population means of ascorbic acid concentration from day one, day three, and day five are all equal to each other, or do not prove a significant difference. The alternative hypothesis, H<sub>a</sub>, states that there is a significant difference between the mean ascorbic acid concentration throughout the five day shelf life.



## Figure 12. Week One Box Plots

This figure displays the three boxplots of the three days of trials on the first week. Day three displays the largest variability, due to the large range of  $3.0 \times 10^{-5}$ . This states that the data collected on day three after harvest had a wide range, therefore expressing the variability in the concentration of ascorbic acid during the third day after harvest. This possibly could have been caused by inconsistencies or lurking variables. In addition, the day three after harvest box plot is slightly skewed to the right, due to the fact that the mean is greater than the median. Day one after harvest expresses low variability in the data, due to its smaller range of  $1.2 \times 10^{-5}$ . This states that the concentration of ascorbic acid on the first day after harvest in comparison to the third day after harvest had less variability. Although the day one after harvest box plot appears to be

symmetrical, it is slightly skewed to the right because the mean of 0.0001700 is greater than the median 0.0001695. The day five box plot is practically normal, but holds a slight skew to the right due to the fact that the mean is slightly greater than the median. This holds the smallest range of 0.0000070, therefore holding the least variability of the ascorbic acid concentration in green peppers. None of the data of the three testing days contain outliers, which allows the ANOVA statistical test to be run normally. It can be concluded that there are no oddities in this set due to the fact that there are no outliers.

The box plots displaying the data for day one after harvest and day three after harvest seem to have overlapping data; whereas the box plot expressing the data for day five after harvest does not overlap any other data. It shows that all of day one after harvest's data is over the last 50% of the day three's data. The slight overlap of this data presents the fact that there was not as a significant amount of change in the concentration of ascorbic acid from day three to day five in comparison to from day one after harvest to day three after harvest. This could be due to the fact that only two trials were completed on the third day after harvest, in addition to two trials completed on the fourth day after harvest, due to experimental error. The large range of 0.000073 between the maximum concentration of ascorbic acid on day five after harvest proves that there was a significant change in the concentration of ascorbic acid in the green peppers as their shelf life increased. Overall, these three boxplots display evidence that the

concentration of ascorbic acid decreases in green bell peppers over time after

they are harvested.

Title	ANOVA
F	158.861
PVal	9.55638e <sup>-</sup> 8
df	2.
SS	2.35173e-8
MS	1.17586e <sup>-</sup> 8
dfError	9.
SSError	6.66168E <sup>-</sup> 10
MSError	7.40186E-11
sp	0.00009
CLowerL	{1.6051887069387e <sup>-</sup> 4,1.495188
CUpperL	{1.7998112930613E <sup>-</sup> 4,1.689811

Figure 13. ANOVA Statistical Test for Week One of Trials The MSE, or mean squared error, for week one of trials is equal to 7.40 x 10<sup>-11</sup>. This means that the weighted average of all sample variances was fairly small. The MSG, or the mean squared for groups, value is equal to 1.18 x 10<sup>-8</sup>; this means that the average squared deviations of the means of the samples from the population mean was relatively small. The F-statistic when this ANOVA test was ran was calculated to be very high at 158.86. This high F-statistic corresponded to a p-value of almost zero. The null hypothesis was rejected because the p-value of 9.55 x 10<sup>-8</sup> was less than the alpha level of 0.05. The p-value describes that there is a 9.55 x10<sup>-8</sup>% probability, almost no chance, of getting the results received by chance alone, assuming the null hypothesis to be true. This indicates that there was a significant difference between day one, day three, and day five trials to claim that at least one of the population means for the concentration of ascorbic acid was not equal. All of these values for the ANOVA statistical test were calculated in the TI-nspire software (Appendix D).





This figure displays three box plots that display each day of trials during the second week of trials. Week two's data is comparable to week one's data due to the fact that as the time after harvest increases, the concentration of ascorbic acid decreases. The boxplots that displays the data for day five after harvest displays the largest variability compared to the other two days, due to the large range of  $2.70 \times 10^{-5}$ . This displays the fact that during the second week of trials, say three had the highest variability in the concentration of ascorbic acid in green peppers than the other two days that were tested. Although the box plot looks symmetrical, the day five after harvest box plot is slightly skewed to the right due

to the mean of  $5.85 \times 10^{-5}$  being greater than the median of  $5.55 \times 10^{-5}$ . The day one after harvest box plot has has a range smaller than day five after harvest, but a range higher than day three after harvest. This shows that there is some variation in the concentration of ascorbic acid on day one after harvest, but not anything significant. Day one after harvest's box plot slightly skewed to the left due to the mean being smaller than the median, but could be considered mostly normal because of the small range of  $1.00 \times 10^{-6}$  between the mean and the median. The day three after harvest box plot is also slightly skewed to the left because of the mean being slightly smaller than the median; day three after harvest also contains the least variability in week two's data due to the smallest range of  $1.40 \times 10^{-5}$ . It can be concluded that there are no oddities in this set due to the fact that there are no outliers.

In week two's data there is no overlap between any of the days of trial. This represents that it can be concurred that there is a clear decline in the concentration of ascorbic acid in green peppers as the time after harvest increases. Due to no overlap of any of the days, it is clear that on day one proceeding the of harvesting the green bell peppers contains the highest concentration of ascorbic acid, therefore holding the most nutrients.

Title	ANOVA
F	153.187
PVal	1.12041e-7
df	2.
SS	2.46035e-8
MS	1.23018e-8
dfError	9.
SSError	7.2275e-10
MSError	8.03056e-11
sp	0.00009
CLowerL.	{1.5811402401183e <sup>-</sup> 4,8.936402
CUpperl	{1.7838597598817E-4.1.096359

Figure 15. ANOVA Statistical Test for Week Two of Trials

MSE, or the mean square error, for week two of trials is equal to  $8.03 \times 10^{-11}$ . This means that the weighted average of all sample variances was fairly small. The MSG value of  $1.23 \times 10^{-8}$ . corresponds with the fact that the average squared deviations of the means of the samples from the population mean was relatively small. The F-statistic calculated for the second week was very high at 153.19; the F-statistic corresponds to a p-value of nearly zero. The null hypothesis was rejected because the p-value of  $1.12 \times 10^{-7}$  was less than the alpha level of 0.05. The p-value describes that there is a  $1.12 \times 10^{-7}$ % probability, almost no chance, of getting the results received by chance alone, assuming the null hypothesis to be true. This indicates that there was a significant difference between day one, day three, and day five trials for week two to claim that at least one of the population means for the concentration of ascorbic acid was not equal. All of

This figure displays the ANOVA statistical test for week two of trials. The

these values for the ANOVA statistical test were calculated in the TI-nspire software (Appendix D).





This figure displays the three box plots of the total data. The overall data seems to have the same conclusion that as the shelf life of green bell peppers increases, the concentration of ascorbic acid decreases. The day three after harvest box plot displays a larger variability than the other days, due to the large range of  $8.7 \times 10^{-5}$ . This wide range of the concentration of ascorbic acid indicates a large variability in data that was collected. This could be due to inconsistencies or experimental errors. This box plot, representing day three after harvest, is mostly normal but can be considered slightly skewed to the right because of the value of the mean is greater than that of the median. The day one box plot is evenly distributed, due to the mean and median having the same

value of 1.69 x10<sup>-4</sup>. In addition, the box plot representing day one after harvest has the smallest range, therefore having the least variability within the three different days that were tested. The day five box plot is skewed to the left because the value of the mean is smaller than that of the median. There is overlap in the overall set of data. 100% of the day one data overlaps with the top 25% of the day three data. Due to the third day containing much variability, it is safe to say that the highest ascorbic acid concentration is present on the first day of harvest. The mean from the third day after harvest is still significantly lower than the mean from the fifth day after harvest, proving the drop in ascorbic acid over time.

Title	ANOVA
F	52.5252
PVal	6.72336E-9
df	2.
SS	4.43513 <b>∈</b> -8
MS	2.21757e-8
dfError	21.
SSError	8.866=9
MSError	4.22191 <b></b> ₌-10
sp	0.000021
CLowerL	{1.5414252052468E <sup>-</sup> 4,1.142675
CUpperL	{1.8435747947532e-4,1.444824

Figure 17. ANOVA Statistical Test for the Total Data

This figure displays the ANOVA statistical test conducted on the two

weeks worth of data completely. The data from all the day one, day three, and

day five trials were added together to create this data set and the outcome of the

ANOVA test is displayed. This data is considered more normal due to having more data points than the two weeks conducted separately. The MSE for the total data is 4.22\*10<sup>-10</sup>, meaning that the weighted average of all the sample variances was small. The MSG value 2.22 \* 10<sup>-8</sup>; this means that the average squared deviations of the means of the samples from the population means was relatively small. The F-statistic is fairly high at 52.52, which corresponded to a p-value of nearly zero. Overall, the null hypothesis was rejected because the p-value of 6.72\*10<sup>-9</sup> was less than the alpha level of 0.05. The p-value describes that there is a 6.72\*10<sup>-9</sup> % probability, almost no chance, of getting the results received by chance alone, assuming the null hypothesis to be true. This indicates that there was a significant difference between day one, day three, and day five trials throughout the whole data to claim that at least one of the population means for the concentration of ascorbic acid was not equal. All of these values for the ANOVA statistical test were calculated in the TI-nspire software (Appendix D).

A 95% confidence interval was also conducted for each day of trials. Due to the fact that an ANOVA test deals with sample means, a confidence interval must be calculated to estimate the population mean of the ascorbic acid concentration levels.

Title	t Interval		
CLower	0.000164		
CUpper	0.000174		
x	0.000169		
ME	0.000005		
df	7.		
sx := sn	0.000006		
n	8.		

Figure 18. Confidence Interval for Day One Data

This displays the confidence interval test conducted for the day one data.

It states that it is 95% confident that the interval will include the population

parameter of the concentration of ascorbic acid in green peppers. The interval for

the day one data is between 1.64 x  $10^{-4}$  and 1.74 x  $10^{-4}$ .

Title	t Interval
CLower	0.000101
CUpper	0.000157
x	0.000129
ME	0.000028
df	7.
sx := sn	0.000033
n	8.

Figure 19. Confidence Interval for Day Three Data

This displays the confidence interval test conducted for the day one data.

It states that it is 95% confident that the interval will include the population

parameter of the concentration of ascorbic acid in green peppers. The interval for

the day one data is between 1.01 x  $10^{-4}$  and 1.57 x  $10^{-4}$ .

Title	t Interval
CLower	0.000056
CUpper	0.000074
x	0.000065
ME	0.000009
df	7.
sx := sn	0.000011
n	8.

Figure 20. Confidence Interval for Day Five Data

This displays the confidence interval test conducted for the day one data. It states that it is 95% confident that the interval will include the population parameter of the concentration of ascorbic acid in green peppers. The interval for the day one data is between 5.60 x  $10^{-5}$  and 7.40 x  $10^{-5}$ .

After analyzing, an ANOVA test proved a significant difference between at least one of the population means. These calculations are reliable due to the researchers utilization of randomization, repetition, and control. The analyzed data justifies that the longer a green pepper is harvested the more the concentration of ascorbic acid decreases. Observed in figures 3, 5 and 7, the day one data has a significantly higher ascorbic acid concentration than day three and five. This means that according to this data, on the first day of harvesting green peppers is the day it provides the highest ascorbic acid concentration. These discoveries abide by what was stated in the background research conducted previously. This data is more evidence to prove that after the first day of harvest of green peppers that it contains the highest amount of ascorbic acid concentration.

### Conclusion

The purpose of this experiment was to observe a correlation between the amount of days after green peppers are harvested or the shelf life of the produce and the concentration of vitamin C. The goal was to determine the negative trend of vitamin C over time, or if the vitamin C begins to decay directly after harvest. The motivation that was taken into consideration for this research was vitamin C deficiencies in diets. If these cases of vitamin C deficiencies were able to pinpoint when the vital time to eat certain produce, they could utilize their vitamin C intake overall lessening their chance of vitamin C deficiencies. The researchers were confident from background research that there is a strong inverse relationship between time and vitamin C concentration as seen in Figures 9 and 10. It was hypothesized that the vitamin C concentration in green peppers would yield the highest concentration on the first day of harvest, in comparison to the fifth day of harvest.

After analyzing the ANOVA statistical test results, the researchers failed to reject their hypothesis that as time progresses, the vitamin C concentration decreases; In addition, the first day after harvest produced the highest concentration of vitamin C. After analyzing the figures displaying the trends in the two weeks of trial, an average of 42.12% of the vitamin C concentration was lost throughout the five day period after the produce was harvested. Additionally, when the ANOVA statistical test was conducted, the p-value of nearly zero, 6.72 x  $10^{-9}$ , strongly supported the hypothesis that as the time after green bell peppers

were harvested increases, the concentration of vitamin C decreases. This conclusion is explained by an experiment conducted by Dr. Raymond N. Dominey at Richmond University.

A redox titration with iodine conducted in this research explains that Vitamin C tablets' concentration are unstable, due to the fact that they are slowly oxidized by air. The inverse relationship between vitamin C concentration in green peppers as soon as they are harvested and time is also explained by the fact that vitamin C is water soluble and heat labile; Because it is an unstable compound, the vitamin C breakdowns as it is exposed to heat and air (Dominey). Due to this rapid start in decay, the faster the vegetable is consumed after harvest, the more nutrients it will provide. Therefore because the first day after harvest was not exposed to heat and air as long as the fifth day after harvest, the first day after harvest held a higher vitamin C concentration.

The exact result ranges may not be found to be consistent year round, due to different growing seasons. This is believed due to an article published by Chet Townsend on the different growing seasons of Florida oranges and their vitamin C concentrations. It was stated that late season oranges yielded a higher vitamin C concentration (Townsend). Both this article and this research on green peppers pertain to the vitamin C concentration in produce, therefore justifying the researchers reasoning. Since this research tested green peppers near the end of the growing season, the vitamin C concentration could be collected as much higher if harvested earlier in the growing season. In addition, this article also

stated that the longer the Florida oranges remained on the tree, the lower the concentration of vitamin C becomes (Townsend). This could also pertain to this research due to the fact that even though both plants from both weeks of data trials were planted at the same time, but harvested one week apart. This can be slightly displayed in figure 9 and 10 that convey the line graphs of each weeks data. The average data points from day one and day three after harvest in week one yield a slightly higher vitamin C concentration than the average data points from day one and day three after harvest in week two.

A substantial concern that occurred throughout the research process is the time period trials were conducted in mid to late October. This could be considered an issue because the known peak harvesting season for green peppers falls between July and September (Amidor). Although this research was not conducted between the peak season, the results are still conclusive with background research and previous experiments. Another issue the researchers faced was colder temperatures that affected the green pepper's sustainability. As told in an earlier section, the temperatures the day of harvest was approximately 65°F. Even though the temperatures were not significantly low, there was a great deal of wind and low water supply for the green peppers that were harvested. Even in these conditions however, the inverse relationship between vitamin C concentration and shelf life remained the same; The normal standards for green pepper development were not ideal, but precise enough to not significantly change the relationship.

Even though the research results confirm the prior hypothesis that the highest vitamin C concentration is recorded on the first day after harvest, further research can be conducted. This experiment can be conducted during the peak season of green peppers to give the most accurate measure of vitamin C concentration. In addition, a long spanned experiment testing the decline in vitamin C concentration over the harvesting season of green peppers could be conducted. This would be done to discover the exact relationship in the time in the harvesting season the peppers are harvested and the vitamin C concentration. Other research that can be conducted in this field is to measure which month between the peak season of green peppers (July - September) produces the highest concentration of vitamin C. This would further aid in cases of vitamin C deficiencies and how these people could utilize their produce intake to benefit them the best.

There are multiple cases of vitamin C deficiencies that are due to the origin of their blood line and the inability to process vitamin C. Vitamin C deficiency can be caused by low dietary intakes that increase the vitamin's turnover in the body ("Scurvy and Its Prevention and..."). One study concluded that nearly half of Zimbabwean adults have an ongoing issue with the transferrin polymorphism protein affecting how the body processes vitamin C . Along with the fact that the average vitamin C intake of a Zimbabwe adult is only 75% of the RDA recommendations, transferrin polymorphism - which causes a vitamin C deficiency - is raging across the country. Transferrin is the major iron binding

protein in human plasma, therefore the mass amount of Iron from beer consumption utilizes most of the protein's energy. This overconsumption overturns the process of the vitamin C, affecting how the Zimbabwean adult's bodies process vitamin C that is consumed (Kasvosve, Delanghe, et. ad.).

This cause of this vitamin C deficiency stems from a poor diet, but can be benefitted and the symptoms can be lessened by maximizing vitamin C consumption. Due to the fact that the highest vitamin C concentration was discovered on the first day after harvest and that an inverse relationship between the vitamin C concentration and time, Zimbabwe adults should consume fresh vegetables as soon as they are harvested. Proper vitamin C consumption is difficult to achieve in Zimbabwe since severe droughts ruining crops since 2000, but is a more achievable goal with the knowledge of when to consume produce containing vitamin C (BBC News).

Research like that conducted by Kasvosve and others can be expanded for further applications. Vitamin C deficiency is a global issue. In the US, 15% or more americans hold this deficiency along with 1 in 7 non-smoking Canadian Adults ("Vitamin C Deficiency Worse..."). It is evident that is not only a concern of third world countries, but territories that have the proper resources to prevent such deficiency. Due to the fact that recent studies suggest that vitamin C benefits early brain development and chronic disease prevention, vitamin C deficiencies need to be properly treated in each individual case ("Vitamin C Deficiency Worse). Research could be conducted comparing the vitamin C deficiencies in regions around the world and discovering what countries are experiencing similar severities of vitamin C deficiencies. Similarities in regions could be grouped together could be further analyzed to attempt to create a common solution to this global issue.

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# Appendix A: Preparing Starch Indicator Solution (0.5%)

<u>Materials (Figure 7)</u>: 5 g of potato starch,  $C_{27}H_{48}O_{20}$ 500 mL of distilled water,  $H_2O$ Weigh boat Glass rod Scupula

100 mL graduated cylinder 100 mL beaker 500 L beaker Scale (.0001 precision)

## Procedure:

- 1. Measure 0.25 g of potato starch  $(C_{27}H_{48}O_{20})$  in weigh boat.
- 2. Measure 50 mL of distilled water into a 100 mL beaker.
- 3. Heat the 100 mL beaker on a hot plate until the 50 mL of distilled water is near boiling.
- 4. Add approximately half of the 0.25 g of potato starch  $(C_{27}H_{48}O_{20})$  into the 100 mL beaker.
- 5. Remove the 100 mL beaker from the hot plate.
- 6. Swirl the 100 mL beaker for approximately one minute or until all particles of the potato starch is dissolved.
- 7. Add the remaining potato starch  $(C_{27}H_{48}O_{20})$  that is located in the weigh boat to the 100 mL beaker.
- 8. Swirl the beaker for an additional one minute or until all particles of the potato starch is dissolved.
- 9. Fill 500 mL beaker with 350 mL of cold water.
- 10. Place the 100 mL beaker with starch indicator solution in the cold water bath to cool the solution quicker.
- 11. Stir the solution with a glass rod to dissolve any excess solution before each use.



Figure 22. Final Starch Indicator Solution ("Aggie Curse") Figure 22 displays what the 100 mL of iodine solution resembles after it is

made. The solution holds a cloudy to clear white color after it is completed.

# Appendix B: Preparing Iodine Solution (0.005 mol L<sup>-1</sup>)

Materials (Figure 7):10 g of potassium iodide, KI500 m7 g of iodine,  $I_2$ (2) We5 L of distilled water,  $H_2O$ (2) Sc100 mL graduated cylinderScale

500 mL beaker(2) Weigh boats(2) ScupulasScale (.0001 precision)

## Procedure:

- 1. Measure 0.65 g of iodine  $(I_2)$  in weigh boat.
- 2. Measure 1 g of potassium iodide (KI) in weigh boat.
- 3. Break large particles of iodine into smaller portions for easier ionization.
- 4. Add the 0.65 g of iodine  $(I_2)$  and 1 g of potassium iodide (KI) into a 500 mL beaker.
- 5. Measure 50 mL of distilled water into a 100 mL graduated cylinder.
- 6. Add the 50 mL of distilled water into the 500 mL beaker.
- 7. Swirl the 500 mL beaker for approximately two minutes to dissolve the solution.
- 8. Measure an additional 50 mL of distilled water in a graduated cylinder and add to the 500 mL beaker.
- 9. Swirl the 500 mL beaker for approximately another two minutes or until all particles of potassium iodide and iodine are dissolved.
- 10. Measure and add 400 mL of distilled water to the 500 mL and continue to swirl with solution for an additional two minutes.



Figure 21. Final Iodine Solution ("Iodine Clock Reaction") Figure 21 displays what the 500 mL of iodine solution looks like after it is

made. The solution holds a dark copper-brown color after it is completed.

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SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
						1
2	3	4	5	6	7	8
9	10	11	12	13	14	15
16 Week One Peppers Harvested High of 65°F, Low of 63°F	17 4 Trials Conducted One Day after Harvest	18	19 * 2 Trials Conducted Three Days after Harvest	20 2 Trials Conducted Four Days after Harvest	21 4 Trials Conducted Five Days after Harvest	22
23 Week Two Peppers Harvested High of 65°F, Low of 45°F	24 4 Trials Conducted One Day after Harvest	25	26 4 Trials Conducted Three Days after Harvest	27	28 4 Trials Conducted Five Days after Harvest	29
30	31					1

# Appendix C: Trial Calendar

\* = Two Trials were completed each day due to inconsistancies in observations that were found during day three after harvest. New iodine solution was made which fized this issue.

: Week One Peppers (Harvested on October 16th) were used for this trial.

-Week Two Peppers (Harvested on October 23rd) were used for this trial.

## Figure 23. Trial Calendar

Figure 23 above displays the overall schedule that was followed

throughout research trials. The trials that used peppers harvested on October 16th for week one is indicated by blue, while trials that used peppers harvested on October 23rd for week two is indicated by purple. A small asterisk indicates two days that did not follow the experimental design due to experimental errors. In addition, the number of trials conducted per day was recorded and displayed in the figure above. The highs and lows for the day that the peppers were harvested on are indicated on the two harvest dates for trials, October 16th and October 23rd.

## Appendix D: Data Analysis ANOVA Calculations

To determine if at least one of the data groups had a significantly different population mean of static friction the following calculations were made. ANOVA tests calculate an F statistic that correlates with a p-value on table D. The F statistic in an ANOVA calculation is calculated by dividing the mean square group by the mean square error.

Table 13 Variables for ANOVA Test

	n	X	S	S <sup>2</sup>
Day One	8	1.69*10 <sup>-4</sup>	5.73*10 <sup>-6</sup>	2.30*10 <sup>-10</sup>
Day Three	8	1.30*10-4	3.34*10 <sup>-5</sup>	7.82*10 <sup>-9</sup>
Day Five	8	6.49*10 <sup>-5</sup>	1.08*10 <sup>-5</sup>	8.15*10 <sup>-10</sup>

Table 13 displays all the variables needed to conduct the ANOVA

statistical test.

$$SST = \sum n1(x1 - \bar{x})^{2} + n2(x2 - \bar{x})^{2} + n3(x3 - \bar{x})^{2}$$

 $SST = 8(1.69 * 10^{-4} - 4.75 * 10^{-13})^{2} + 8(1.30 * 10^{-4} - 4.75 * 10^{-13})^{2} + 8(6.49 * 10^{-5} - 4.75 * 10^{-13})^{2} = 3.97 * 10^{-7}$ 

$$MSG = \frac{SST}{I-1} = \frac{3.97 \times 10^{-7}}{3-1} = 1.98 \times 10^{-7}$$
  

$$SSE = \sum (n1-1) \times S1^{2} + (n2-1) \times S2^{2} + (n3-1) \times S3^{2}$$

$$SSE = (7 * 2.30 * 10^{-10}) + (7 * 7.82 * 10^{-9}) + (7 * 8.15 * 10^{-10}) = 6.21 * 10^{-8}$$

$$MSE = \frac{SSE}{N-I} = \frac{6.21 \times 10^{-8}}{24-3} = 2.96 \times 10^{-9}$$
$$F = \frac{MSG}{MSE} = \frac{1.98 \times 10^{-7}}{2.96 \times 10^{-9}} = 52.52$$

Degrees of Freedom = 
$$\frac{I-1}{N-I} = \frac{3-1}{24-3} = \frac{2}{21} \implies table D \implies p-value = 0.001$$

Figure 24. ANOVA Statistical Calculation

In the figure above, the steps to calculate the ANOVA statistical test are shown. The formula is given before inserting the actual numbers from this research. The final number found was the F statistic and that is used to find the p-value from Table D in the statistics book. The coordinating p-value is 0.001. This is a rounded p-value due to the fact that table D cannot include numbers as small as our exact p-value. The exact p-value, found on TI-nspire software, is  $6.72*10^{-9}$ . In the equations above, *I* stands for the number of population that are compared, N represents for all the data points in the entire experiment, n is the size of the sample distributions, x is the the mean of the sample distribution, and F is the ANOVA coefficient. SST is the sum of squares due to treatment, MSG is the mean sum of squares due to treatment, SSE is the sum of squares due to error.

### Appendix E: Professional Contact

# Professional Contact Information:

Name: Dr. Dawn Anderson

Title: Associate Professor

Organization: Bowling Green State University

Phone: (419) 372-8090

Email: dawna@bgsu.edu

# Proof of Contact:

*	Dawn Lynnette Anderson <u>via</u> falconbgsu.onmicrosoft.com to me	Nov 6 🔆 🔸 🔻	
	Hello, Paige,		
	This is a good draft of your Review of Literature. Can you send me the document as a Word file, so I can make comments?		
	Here are some general suggestions that you may want to make before sending me back the docur	nent.	
	<ol> <li>Move the two paragraphs near the end about vitamin C deficiencies and daily vitamin C requirement to the beginning. Your paper should be like an inverted triangle with more general information first then becoming more focused. Start with what vitamin C is, it's functions in the body, deficiencies, daily requirements, amount in produce, how the vitamin C content is affected postharvest, then a description of the method of analysis.</li> </ol>		
	2. Expand the first paragraph comparing the nutritional value of fresh vs. frozen produce. Check Google.doc for more research articles.	the USDA website and	
Pote	3. Eliminate the last paragraph on the first page. Discussing pesticide presence on produce is irre	elevant to your study.	
oto			
	Paige Redlin Hello Professor Anderson, My name is Paige Redlin and I attend the Macomb Mat	Sep 17 ☆	
	21 older messages		
	Paige Redlin Hello Dr. Anderson! Thank you so much for the feedback. My partner and I plan	edlin Dec 1 (4 days ago) 📩	
	Dawn Lynnette Anderson <u>via</u> falconbgsu.onmicrosoft.com € to me €	Dec 1 (4 days ago) 📩 🔺 🔹	
	Paige,		
	You are correct that t-tests are used to find the difference between two means. When there are more than ANOVA can be considered a series of t-tests, but it doesn't indicate which means are different.	two means, ANOVA is used.	

Figure 25. Email from Professional Contact

Above is a screen capture of an email thread from Dr. Dawn Anderson. She helped the researchers throughout different sections of the research paper by inputting suggestions due to her vast knowledge in food sciences (Anderson).

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