Titration of 2,6-Dichloroindophenol and Ascorbic Acid to Determine the Difference In Vitamin C Content of Fresh Substances and Canned Substances

I. Introduction

Vitamin C, ascorbic acid, is nutritionally important with many naturally occurring sources such as citrus fruits and green vegetables/plants. Because all plants and animals except humans, other primates, and guinea pigs, produce Vitamin C naturally, it is an important part of the human diet.

The Recommended Dietary Allowance (RDA) of Vitamin C for teenagers is 60 mg per day.[1] Some medical and dietary professionals believe that higher daily doses of Vitamin C can potentially have a positive effect on the immune system. A deficiency in Vitamin C can result in scurvy, a disease that causes bleeding, spongy gums, and bruising.[2] One of the most important roles of Vitamin C is its role in the formation of collagen, the ubiquitous structural protein component found in connective tissues, skin, bones, cartilage, ligaments and elsewhere.[3]

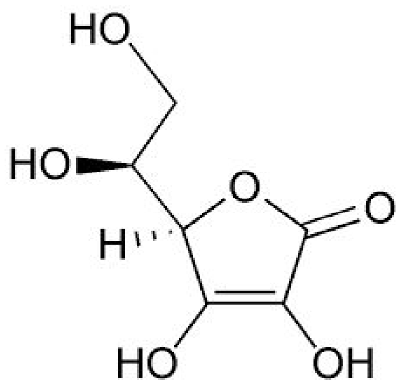

Figure 1 - Chemical structure of ascorbic acid[4]

A. Personal Engagement/Rationale for Study

During the COVID-19 pandemic, many people will not have direct access to fresh produce and will have to include more canned foods in their diet. This study aims to investigate the Vitamin C content in canned produce compared to fresh produce. In doing so, it can be used to help determine whether consumption of canned products can lead to Vitamin C deficiencies, and which canned products are the best sources of Vitamin C compared to their fresh counterparts. As it is important to consume Vitamin C on a regular basis, when fresh produce is scarce or hard to obtain (such as in the case of a pandemic) many people turn to canned produce for

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[1] Anitra C Carr, Balz Frei, "Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans", The American Journal of Clinical Nutrition, Volume 69, Issue 6, June 1999, Pages 1086–1107, https://doi.org/10.1093/ajcn/69.6.1086
[2] Maxfield, Luke. "Vitamin C Deficiency (Scurvy)." StatPearls Publishing, U.S. National Library of Medicine, 19 Nov. 2019, www.ncbi.nlm.nih.gov/books/NBK493187/.
[3] "Vitamin C and Skin Health." Linus Pauling Institute, Oregon State University , 2 Jan. 2020, lpi.oregonstate.edu/mic/health-disease/skin-health/vitamin-C.
[4] "Chemical Structure of Ascorbic Acid, (Aka Vitamin C)." File: Ascorbic Acid Structure, Wikimedia Commons, 22 Feb. 2007.

consumption.This investigation compared the Vitamin C content in canned produce to that of fresh produce: Do people need to consume more canned products to get the same amount of Vitamin C? Is there a noticable difference?

B. Research Question: How can titration be used to compare the concentration of Vitamin C in canned produce to the concentration of Vitamin C in fresh produce?

C. Hypothesis: Vitamin C is sensitive to heat (and highly water-soluble) so it is affected by the thermal processing that precedes canning.[5] After being vacuum sealed, the produce is either pasteurised (80°C and above) or sterilised (100°C and above. Thus, the hypothesis for this experiment is that the canned produce will have a lower concentration of Vitamin C. Past research also shows that a "decrease in ascorbic acid during commercial thermal processing conditions" was reported for produce such as tomatoes, asparagus, green beans, etc. and loss of ascorbic acid on a wet and dry weight basis was common in many types of produce as well.[6]

II.Investigation

A. Background Knowledge:

Vitamin C (ascorbic acid) is water soluble and is a strong reducing agent. In this experiment, ascorbic acid reduces an indicator, 2,6-dichloroindophenol (DCPIP). The indicator solution will first change from a blue color to an intermediate pink or purple color. After that it will turn colorless, or the color will become a very faint amber endpoint. The decolorization of the DCPIP solution indicates the presence of Vitamin C.

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[5] Rickman, Joy C, et al. "Nutritional Comparison of Fresh, Frozen and Canned Fruits and Vegetables. Part 1. Vitamins C and B and Phenolic Compounds." Journal of the Science of Food and Agriculture, vol. 87, no. 6, 2007, pp. 930–944., doi:10.1002/jsfa.2825.

[6] Rickman, Joy C, et al. "Nutritional Comparison of Fresh, Frozen and Canned Fruits and Vegetables"

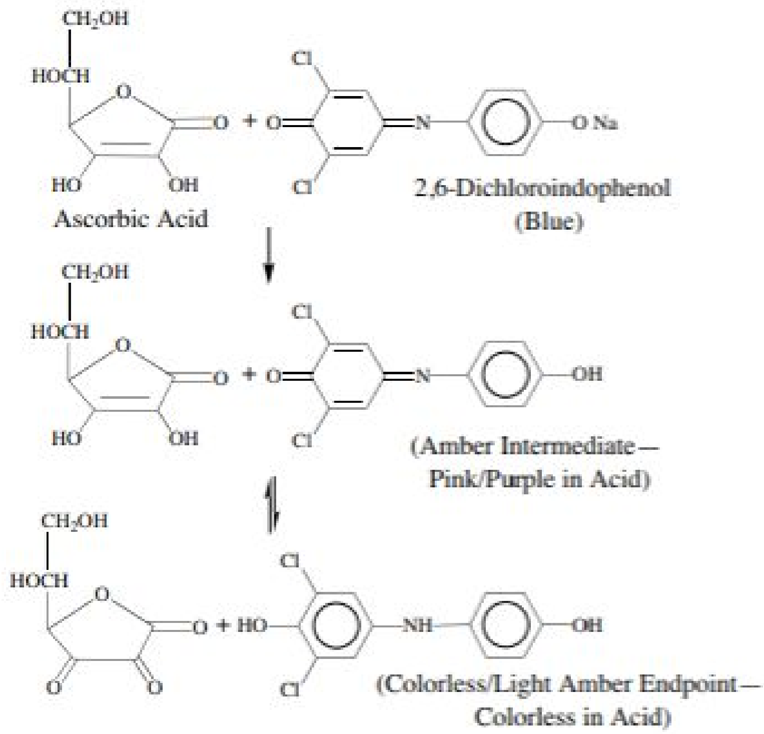

Figure 2 - reaction mechanism between DCPIP and ascorbic acid[7]

This figure shows the reaction that occurs when ascorbic acid is added to the dichloroindophenol indicator solution. Using the Vitamin C/ascorbic acid solution as a standard, the amount of Vitamin C in produce can be calculated[8]. This can be done using a Concentration-Volume equation and solving for the unknown concentration, because the number of drops is equal to the volume of the solution.

(Volume standard)(Concentration standard) = (Volume unknown)(Concentration unknown)

An example calculation would be:

(93.33µL) (100mg Vitamin C/100 mL Vitamin C) = (600µL) (x mg Vitamin C/100 mL pineapple)

x = 93.33*100/600 =

15.55 mg of Vitamin C in pineapple sample

Titration is a common laboratory method of quantitative analysis that can be used to determine the concentration of a known substance. This titration will be done first to determine the amount of Vitamin C in the standard ascorbic acid solution, and then to determine the amount of vitamin

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[7] "Vitamin C Testing." Vitamin C Testing Chemistry of Food, Flinn Scientific, Inc, 2016, www.flinnsci.com/api/library/Download/6dd645b70d0349579bfeef04796c29a4.

[8] Ugbe, Fabian Audu, et al. "Determination of Ascorbic Acid Concentration of Some Commercial Fruits Juices Sold in Ugbokolo Benue State, Nigeria." International Annals of Science, vol. 3, no. 1, Nov. 2017, pp. 19–22., doi:10.21467/ias.3.1.19-22.

C in the prepared fruit/vegetable samples. Vitamin C content can also be determined through the iodine titration method, which uses starch as an indicator, as well as through spectroscopic determination.[9]

Materials:

l-Ascorbic acid, 0.1 g
2,6-Dichloroindophenol (DCPIP) 0.05 g
Eppendorf pipette, 1000 µL
Pipette tips
Beakers, 250 mL (2)
Conical tubes, 15 mL(25)
Conical tubes, 50 mL (50)
Tube rack
Flask, 250 mL (2)
Stirring rod
Fruit/vegetable samples, canned and fresh (orange, mango, pineapple, pear, beet, tomato, green bean, asparagus, carrot)

Safety:

Ascorbic acid (Vitamin C) and 2,6-dichloroindophenol are considered non hazardous, but hands should be washed thoroughly after handling.[10] [11] Food items in the lab are considered chemicals and, as such, should not be ingested. Chemical-splash goggles and gloves should be worn as PPE. There are no major environmental/ethical issues with this procedure.

Variables:

Independent Variable	Dependent Variable
Type of produce, fresh or canned	Concentration of Vitamin C (mg)

III. Methodology

A. Preparation of solutions:

Dichloroindophenol Solution:

1. Solutions are to be prepared no more than one day in advance and refrigerated until use.
2. Prepare the 0.025% dichloroindophenol solution by first dissolving 0.05 g of dichloroindophenol powder in 100 mL of distilled water in a 250 mL beaker, then pour out 50 mL of the solution into 100 mL beaker and add another 50 mL of distilled water to the solution.
3. Mix solution thoroughly with stirrer.

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[9] Shrestha, Nishanta & Shrestha, Sudan & Bhattarai, Ajaya. “Determination of Ascorbic Acid in Different Citrus Fruits of Kathmandu Valley.” Brazilian Journal of Medical and Biological Research. 2. 9-14. 2015
[10] “Material Safety Data Sheet L-Ascorbic Acid.” Fisher Scientific, ACC# 12385.
[11] “Material Safety Data Sheet 2-6 Dichlorophenolindophenol (DCPIP).” Scientific & Chemical Supplies Limited. 2008.

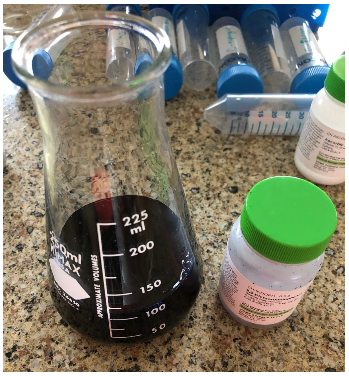

Figure 3 - Prepared DCPIP solution

Ascorbic Acid Solution:

1. To prepare vitamin C solution measure 0.1 g of L-ascorbic acid.
2. Place 0.1 g of L-ascorbic acid into a 250-mL beaker and dilute with 100 mL of distilled water.
3. Mix solution thoroughly using a stirrer.

B. Preparation of samples:

Fresh Produce

1. Measure out 50.0g of the fresh produce sample
2. Blend the 50.0 g sample of the fruit or vegetable with 50.0 mL of distilled water
3. Strain the mixture through a filter into a 250 mL beaker
4. Wash the filter with a few milliliters of distilled water if the sample is not passing through the filter to make a final solution of 50 mL in the beaker

Canned Produce

1. Measure out 50.0g of the fresh produce sample
2. Blend the 50.0 g sample of the fruit or vegetable with 50.0 mL of distilled water
3. Strain the mixture through a filter into a 250 mL beaker
4. Wash the filter with a few milliliters of distilled water if the sample is not passing through the filter to make a final solution of 50 mL (±10mL) in the beaker.

C. Determination of Standard by Titration of l-Ascorbic Acid:

1. Measure out 10000 µL of 0.025% dichloroindophenol solution. Transfer to one of the 15mL conical tubes.
2. Set the pipette to 10µL, add the 0.1% ascorbic acid solution, counting each 10 µL added to the 15 mL conical tube until the color changes from blue to the colorless/very light amber endpoint. Make sure to stir or swirl the solution after each drop is added
3. Repeat the procedure for two more trials to obtain accurate results.

D. Determination of Concentration of Vitamin C in Prepared Samples:

1. Measure out 1000 µL of 0.025% dichloroindophenol solution. Transfer to one of the test tubes.
2. Using the pipette, add the prepared produce samples, counting each of the 100 or 250 increments (µL) added to the 15 mL conical tube. Make sure to stir or swirl the solution after each drop is added.
3. When the color changes from blue to the original color of the produce sample, it is the endpoint color. Compare the 15 mL tube to the 50 mL tube containing the sample solution using a white piece of paper.
4. Repeat steps 1-3 for additional trial, and average out the volume.
5. Repeat steps 1-4 for each produce sample

Figure 4.1 - Mandarin orange

Figure 4.2 - Orange-water solution

Figure 4.3 - Filter

Figure 5.1 - Green beans (15mL)

Figure 5.2 - Fresh green bean solution (50mL) + two titrated samples

Figure 6 - Canned carrot solution + titrated samples

Figure 7 - Canned orange solution + titrated samples

In these examples, the color of the titrated samples closely resembles that of the original solution, made of the 50g produce and 50 mL water blended. A white background was used for maximum color accuracy.

IV. Data Analysis

A. Calculations:

C1V1=C2V2

C2 = C1V1/V2

93.33*100/V2 = 9333/V2 (Use this formula to calculate amount of Vitamin C in samples)

Volume of 0.1% ascorbic acid solution needed to titrate 1000µL of 0.025% dichloroindophenol solution

Trial 1	100 µL (±0.3)
Trial 2	90 µL (±0.3)
Trial 3	90 µL (±0.3)
Average	93.33 µL (±0.3)

Raw Data for Produce Sample Titrations

Produce	Trial 1 (µL)	Trial 2 (µL)	Average Volume (µL)	Vitamin C Content (mg)
Fresh Pineapple	600	600	600	15.55
Canned Pineapple	2500	2300	2400	3.889
Fresh Orange	1600	1750	1675	5.572
Canned Orange	2050	2100	2075	4.498
Fresh Beet	2000	2000	2000	4.667

Canned Beet	2600	2750	2675	3.489
Fresh Carrot	3300	3100	3200	2.917
Canned Carrot	9000	9250	9125	1.023
Fresh Green Bean	6000	6250	6125	1.524
Canned Green Bean	12500	12300	12400	0.7527
Fresh Tomato	1500	1600	1550	6.021
Canned Tomato	1600	1800	1700	5.490
Fresh Asparagus	1900	1750	1825	5.114
Canned Asparagus	2350	2400	2375	3.930
Fresh Potato	50000	N/A	N/A	N/A
Canned Potato	4000	4150	4075	2.290
Fresh Mango	1500	1450	1475	6.327
Canned Mango	2500	2600	2550	3.660

Comparing Vitamin C Content in Canned vs. Fresh Produce

Type of Produce	Difference in Vitamin C Content (mg)
Pineapple	11.67
Orange	1.074
Beet	1.178
Carrot	1.894
Green Bean	0.7711
Tomato	0.5313
Asparagus	1.184
Potato	N/A
Mango	2.668

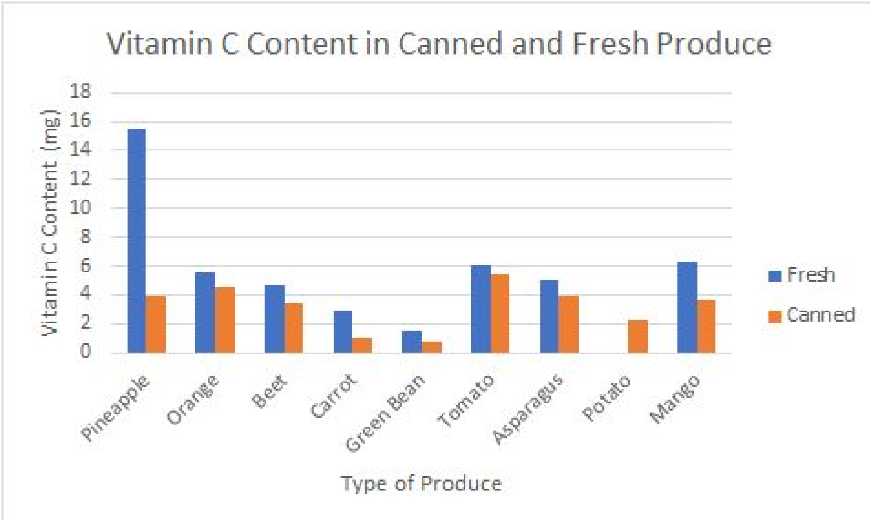

Figure 8 - Graph comparing canned and fresh produce

B. Error Analysis

According to the eppendorf pipette manufacturers, the pipette has a systematic error reading of ±0,8 µL for a volume of 100 µL, as well as a ±1,7 µL reading for a volume of 250 µL.[12] When averaged out, this gives a final systematic error reading of ±1,25 µL. For volumes of 10 µL there is a systematic error of ±0.3 µL.

One large error that occurred during the experiment was the titration of the fresh potato sample, in which all of the sample was put into the DCPIP solution but the blue color from the DCPIPv did not disappear even after 50 mL of the potato solution was put into it. I chose not to include this experiment in the final data because it was not an accurate titration. This error could have been due to the DCPIP being saturated due to uneven mixing (some solid DCPIP could have been deposited on the bottom), or I could have forgotten to shake/invert the original potato-water solution before pipetting it into the DCPIP solution.

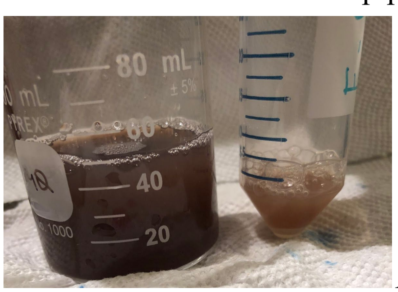

Figure 9.1 - Left, 1mL DCPIP + 50 mL fresh potato solution. Right, original fresh potato solution

[12] "Eppendorf Research Plus Operating Manual." Operating-Manual-Research-Plus.pdf, Eppendorf Research Plus, 2013, www.pipette.com/Manuals/Operating-manual-Research-plus.pdf.

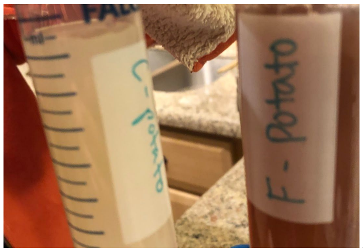

Figure 9.2 - Interesting color difference between fresh (F) and canned (C) potato; color of fresh potato could have interfered with DCPIP titration

Other possible errors include the acidity of the produce, which could affect the indicator readings.[13] The method of titration itself is also flawed due to human error, because the endpoint is hard to determine by color alone; the human eye has difficulty distinguishing between miniscule changes in color. In the future, a colorimeter or spectrophotometer could be used to measure changes in color because they are instruments designed to track those changes.

V. Conclusion

The amount of Vitamin C found in the canned food (for this experiment specifically) was on average 2.62 mg lower than the amount of Vitamin C in the fresh produce counterparts. Pineapple had the highest difference between canned and fresh samples, with a 11.67 mg difference. These values were expected, because as I expanded on in the hypothesis section, the canning process destroys some of the Vitamin C in the produce. However, this experiment did not take an important factor into consideration which is the presence of liquid. In calculating the Vitamin C content of produce, I did not consider the effect water had on the dilution of the produce, and also the oxidation effects. This did not affect my experiment overall because all of the samples were diluted in water, but could be noted for future experiments. Liquid is also important for canned vs. fresh produce because there is liquid in the canned food: I did not account for liquid when I measured out 50g of the canned food, but in reality any remaining Vitamin C in the canning liquid would most likely be insignificant because it is easily oxidised. USDA nutrient data supports this, with minimal differences in Vitamin C content when the fruit or vegetable is analyzed with the canning liquid.[14]

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[14] Rickman, Joy C, et al. "Nutritional Comparison of Fresh, Frozen and Canned Fruits and Vegetables"

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