

Quantitative analysis of vitamin C content in three apple varieties using Tillman's method

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ABSTRACT

Vitamin C, also known as ascorbic acid, is a very important coenzyme in many functions in the body. Vitamin C is easily destroyed by cooking or canning foods and by exposure to air and light. This means the best way to get Vitamin C into our bodies is by consuming it raw. In this procedure the most popular apple types (gala, granny smith, and red delicious) were tested for vitamin C content. The experiment was done using Tillman's method with metaphosphoric acid. The apple extract was titrated against 2,6-dichlorophenol-indophenol diluted solution at pH 0.6, to a steady pink endpoint. The results suggest that granny smith apples produce the most Vitamin C compared to the other two varieties.

Keywords: *vitamin c, Tillman's method, apple varieties, titration, oxidation reduction reaction*

INTRODUCTION

Vitamin C, also known as ascorbic acid, is a very important coenzyme in many functions in the body. It functions as a coenzyme in iron consumption and in peptide hormone metabolism (Fenech 2019). Ascorbic acid occurs naturally in many fruits and vegetables, particularly in tomatoes, citrus fruits, cantaloupe, broccoli, spinach, green peppers, cabbage and potatoes (Njoku 2011). The highest of which being red raw bell peppers and orange juice.

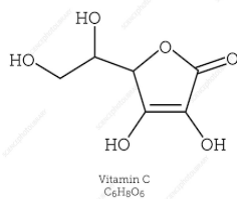


Figure 1. Vitamin C

Vitamin C is widely known to help cure scurvy, which is a skin disease caused by lack of vitamin C in the diet. Vitamin C helps build collagen in the skin which lacking this enzyme causes changes to the gums and skin. Vitamin C has also been found to boost antioxidant levels, lower blood pressure, protect structure against gout attacks, improve iron absorption, boost immunity, and reduce heart disease and dementia risk (Raman 2020).

Since ascorbic acid is a weak, dibasic acid which is water soluble at physiological pH, it has long been assumed that its antioxidant function is confined to the aqueous phase of the cell and circulatory system. However, the demonstration that vitamin C may interact with the alpha-tocopherol radical, regenerating the antioxidant form of vitamin E, alpha-tocopherol, provided evidence that vitamin C also serves indirectly in the protection of membrane components susceptible to free radical damage.

(Beyer 1994)

Vitamin C is easily destroyed by cooking or canning foods and by exposure to air and light. Vitamin C present in food is sensitive to heating above the temperature of 60°C (Njoku 2011). This means the best way to get vitamin C into your system is by consuming it raw. Which means eating fruits or vegetables that aren't cooked. This experiment emphasizes on vitamin C content in apples and deciding which apple variety will produce the most vitamin C.

My research was done with titration method, which I decided would show the best result of how much vitamin C content there is in the fruit. In the other articles I read they used three different methods; Tillman's Method, Iodometric titration and Spectrophotometric method (Kostecka 2017). These three methods are all effective in getting vitamin C content in different fruits. The Iodometric titration uses iodine and potassium iodine with a starch indicator to see the vitamin C content. It also uses Oxalic acid which stabilizes the vitamin C content in the solution, which is titrated against the iodine and potassium iodine solution. This same method is used in the Spectrophotometric method. However this method uses EDTA, 50% sulfuric acid, and 5% ammonium acid solution. The last one is Tillman's method, which uses a metaphosphoric acid titrated against 2,6-dichloroindophenol (DCIP) and sodium bicarbonate solution. I chose the Tillman's method for my research because metaphosphoric acid was shown to help protein precipitant agent (Benassi 1998). Both oxalic acid and metaphosphoric acid showed the same uses in titration and were fairly the same price, metaphosphoric acid has normally been chosen as a reagent due to the protein precipitation.

I tested gala, red delicious, and granny smith apples using the Tillman's titration. This showed me

the average vitamin C content in each variety of apple using the DCIP titrant. I then was able to see which out of the three varieties provides the highest vitamin C content in the raw form. I chose this research because you walk through a market or grocery store and see all these different apples and I wanted to know which variety out of the three I chose has the most vitamin C content.

MATERIALS AND METHODS

In this procedure the most popular apple types (gala, granny smith, and red delicious) were tested for vitamin C content. The experiment was done using six of each variety, a total of 18 apples, using this many apples will give good results in comparison of the varieties. The experiment was in a laboratory environment. This experiment was a modification to an experiment done by Nerdy(Nerdy, Nerdy 2018) however they used bell peppers, so the amount of vitamin C found was a lot higher than my research. I also had to dilute the titrant used due to the lower vitamin C levels in apples.

The materials needed in the experiment was apples(6-gala, 6-granny smith, and 6-red delicious), metaphosphoric acid (50g) , vitamin C (20g) 2,6-dichlorophenol-indophenol (10g), sodium bicarbonate (20g), distilled water, burette (1), volumetric flasks (5), analytical balance, Erlenmeyer flask (30), Microsoft excel, pipette-5ml, and a blender.

When doing the experiment the first steps were to create the solutions. There was four solutions needed first was the metaphosphoric acid solution. This was made by adding 30g of metaphosphoric acid into a 1L volumetric flask and adding 500mL of distilled water, shaken and then distilled water added to the marker line. The sodium bicarbonate solution is found by adding 8.4g of sodium bicarbonate, put into a 1L volumetric flask, added 500 mL of distilled water, shaken, dissolved, and distilled water added to the marker line. Next is the 2,6-dichloroindophenol solution. This was found by mixing .25 g 2,6-dichloroindophenol was weighed, put into a 1L volumetric flask, added 250 mL of sodium bicarbonate solution 0.84% (mass/volume). This however had to be diluted due to amount of vitamin C being less than in the original study. To dilute the solution I added 50mL of the original solution and put it into a 1L volumetric flask, then putting DI water to the marker line.

To then create the standard sample I used 10mg of vitamin C and placed it into a 100ml volumetric flask. Then adding 50mL of metaphosphoric acid solution to the flask and shaking until it was dissolved. Metaphosphoric acid solution was then added again to the marker line. Then 10mL of this solution was pipetted into another 100mL erlenmeyer flask with 5 mL of metaphosphoric acid solution. This was then

titrated using the 2,6-dichloroindophenol diluted solution. This was also repeated six times finding 19.11mL as the average volume of the titrant.

The apple extract was titrated against 2,6-dichloroindophenol diluted solution at pH 0.6, to a steady pink endpoint. All the solutions were homogenized mixtures. To add the components, the first step was cutting the apple. 20g of that apple was blended. All of the blended apple was placed into a 100mL volumetric flask. Then 50mL of metaphosphoric acid solution was added to the volumetric flask. This was then shaken, then filled with metaphosphoric acid to the line. This flask will then be the standard sample solution for that apple variety. Then 10mL of the standard sample solution was added to a new flask with 15mL of metaphosphoric acid solution. This was repeated two more times giving the three samples for each apple variety to be tested against the 2,6-dichloroindophenol diluted solution to a pink steady endpoint. This was done to each apple, making 18 titrations of the six apple types. This determined how much vitamin C content was in each apple with using the amount titrated in a formula. Last all the apples data was collected and put into a Microsoft Excel spreadsheet testing the amount of vitamin C depending on the different varieties. There was a total of 64 titrations including the standard titration.

To find the vitamin C content it was calculated by using this equation,

$$\text{Vitamin C content} = (V \cdot F \cdot 100) / A$$

Where V is volume of titration, F is Tillman's factor, and A is volume of sample after titrant is added. The Tillman's factor was found using this equation:

$$\text{Tillman's Factor} = (\text{Mass of vitamin C used in the titration}) / (\text{volume of titrant used in titration of standard})$$

Where the Mass of vitamin C was set at 10mg and volume of titrant used in the standard was 19.11ml.

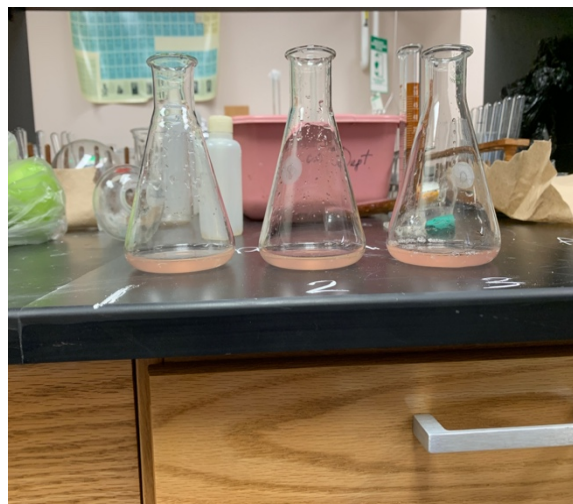


Figure 2. Comparison of three titrations after end point

RESULTS

As shown in Figure 2, the titrations gave off a pink color compared to the blank titration which showed a vibrant red. This was the expected color of the titrated sample after the experiment.

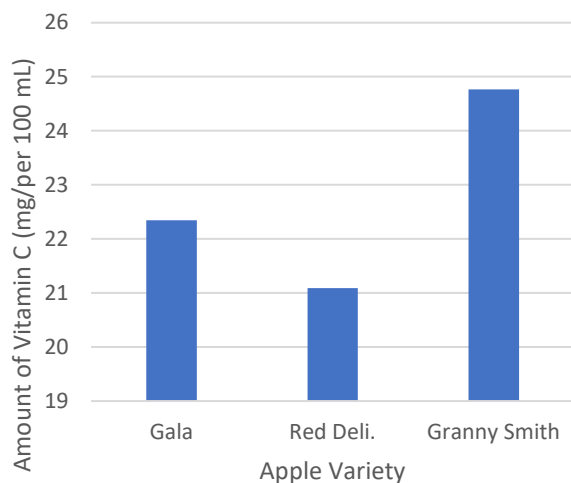
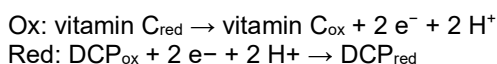


Figure 3. Graph of vitamin C content in the apple varieties

Figure 3 shows that granny smith apples have the highest vitamin C content compared to the other varieties. The apple had a vitamin C content of 24.77mg/ per 1 mL. Gala apple had a vitamin C content of 22.34mg/per 100mL. Red delicious had a vitamin C content of 21.09mg/per 100mL.

DISCUSSION

The chemistry as to why the solution is pink and not colorless, is because we are dealing with an acid solution which turns the solution pink and not colorless. This reaction is an oxidation reduction reaction, which the vitamin c is being oxidized by the 2,6-dichloroindophenol (Advanced etc. 2011).



It cannot be done as an acid-base titration because there are many acids and bases in foods, as well as other products that interfere with acid-base titration. Also using 2,6-dichloroindophenol titration or Tillman's method is it does not oxidize any other substances in the titration process other than vitamin C (Nerdy 2017). The solution is a pink color compared to colorless because of the acid involved in the oxidation of the vitamin C.

Looking at my results it shows that granny smith apples were the highest in vitamin C. Future research that can be done would be the same research but a

different method. This could show either which method is more accurate or comparing the results to the different apples. This would then provide more reliability on the calculation of vitamin C in either different apple varieties or the same three that I chose. Another research that this can be done is if color has effect on the vitamin C content.

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