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ANALYSIS OF VITAMIN C CONTENT IN LEMON

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ABSTRACT

Ascorbic acid is one of the essential vitamins necessary for the human body. The general recommended amount of vitamin C intake is around 60 - 95 milligrams per day. Although excess may result in a disease condition called, hemochromatosis. It is vital to consume vitamin C in an appropriate amount. Therefore, this research was carried out in order to determine the amount and concentration of ascorbic acid in lemon juice sample which is readily available without any economic cost and highly rich in ascorbic acid and belongs to hesperidia family. Through titration it is convenient to determine the ascorbic acid concentration along stages after it is ripped and collected, and the lemon juice is used for the process of titration. Titration is a volumetric analysis of concentration of a known analyte. Likewise, standard vitamin C tablet with a known mass was taken and its volume was found. At meantime the lemon juice volume also found through titration via the weight / volume ratio of the lemon juice was calculated and the concentration was also calculated. Thus it was decided that the consumption of the fruit in order to obtain vitamin C should be done in sufficient amount according to the type of fruit. The results were obtained as follow: beginning of ripening lemon fruit consisted the higher amount of ascorbic acid (1.8 mL) and the less volume (0.67 mL) was detected in the sample which was after 3 weeks since plucking. Further, fully green lemon consisted 1.4 mL, after 1 week since plucking fruit consisted of 0.93 mL, and lemon kept in refrigerator for 1 week was detected with 1.3 mL of ascorbic acid. Therefore, changes of ascorbic acid concentration along with the time period was detected through this project. To know the exact correlation, the project suggests to do the study with more number of samples to produce a clear idea about this correlation.

KEYWORDS: Ascorbic acid, Lemon, Anti-oxidant, Vitamin C.

1. INTRODUCTION

Fruits and vegetables are highly rich in nutrients and are readily available to most of the people. Less economic, fresh and natural. Fruits can be classified in to two types based on the pattern of ripening; they are classified as climacteric and non-climacteric. Avocados, bananas and pears fall in to climacteric category while orange, lemon, pine apple and strawberries fall in to non-climacteric category (Thurlor and Lumley, 1989).

Most of these non-climacteric fruits are rich in Vitamin C. This Vitamin C also referred as Xiloascorbic acid, hexuronic acid, ascorbic acid and cevitamin acid. However, it is widely known as ascorbic acid. Grapefruit, orange, lime and lemon are rich in ascorbic acid and they are also called as citrus fruits in general. More than 90% of the vitamin C requirement is fulfilled by fruits and vegetables in general. Although it could be synthesized in some organisms, humans and some primates need to consume through diet (Tareen *et al.*, 2015).

It plays a vital role in several metabolic pathways as it functions as co-enzyme. It is also a major reducing and antioxidant agent that enhances the immune responses against the bacterial invasions (Pisochi *et al*., 2008). Its activities are as follows; the ascorbate ion present in the ascorbic acid is responsible to react with reactive oxygen species such as hydroxyl radicals as these radicals are capable of initiating chain reactions, these ascorbate ion takes charge and terminates this chain through electron transfer reaction. Because this ascorbic ion can transfer electron to its own radical ions. The net reaction is as follow;

$RO^- + C_6H_7O_6^- \rightarrow RO^- + C_6H_7O_6^* \rightarrow ROH + C_6H_6O_6$

Further oxidation result in the formation of various acids such as xylonic acid, oxaclic acid diketogulonicacid which does not cause any cellular damage but in other hand enhances the immunity via reactive oxygen species (ROS) method (Hagelstam, 2014).

Ascorbic acid also take part in the detoxification reaction and the biological formation of collagen in places such as teeth, connective tissue, bone, fibrous tissue, capillaries and the skin. It also protects the body cells from damage (Grober, 2009).

Ascorbic acid sometimes also consists of sodium, potassium and calcium salt, which are commonly used as the antioxidant and food additives (Pisochi *et al*., 2008). Some other uses are, it is used to remove stain from metal, used in fluorescence microscope as an antioxidant

in order to enhance the fluorescence signal and for the processing of plastic, it is used to assemble molecular chains quickly along with minimal waste (Grober, 2009).

Apart from the common uses, biologically continuous supply of vitamin C to the body is vital as it is important in the synthesis of proteins and absorption of ions.

Once the body lacks vitamin C, it leads to signs and symptoms of deficiencies such as anemia, bleeding gums, decreased level of immunity, lack of wound healing ability, gingivitis swollen joints, weakened enamel of the tooth and so on (Weil, 2009). But major lack of vitamin C leads to a complicated deficiency called scurvy. Once it is not treated properly it can lead to death as well (Grober, 2009). For these conditions clinicians prescribe vitamin C for daily consumption. Some such branded clinical names of this are, ascot, C-500 (chewable tablet), C econ drops, centrum (single vitamin C), cevi- bid and Sunkist (Weil, 2009).

Always it is appropriate to consume this in a proper prescribed amount. As in rare cases vitamin C toxicity also happens and leads to a condition called hemochromatosis too. The symptoms of this condition is diarrhea, nausea, stomach cramps and renal problems. All this is due to the higher dosage consumption of vitamin C via the supplement (Grober, 2009).

In order to determine the level of vitamin C in the supplements, there are some laboratory based procedures which has no ethical issues. There by the chemical analytic method called titration plays a key factor in the production of these supplements for this process. Titration method of quantitative analysis can be used to determine the concentration of a known analyte (Weil, 2009).

A titrant of known concentration used to react with the solution the analyte of an unknown concentration. Using a calibrated burette, it is possible to determine the exact amount of titrant that has been consumed when the endpoint is reached (Pisochi *et al*., 2008).

An endpoint is a point in which the titrant is complete, as determined by the color change of an indicator (Michel, 2013). Vitamin C is known as scavenger of oxidants in the body it does this by reducing them to less reactive compounds. Ability of iodine to reduce the oxidants will result in a change of color. It can be used to analyze the vitamin C (Mequaninti, 2012).

In order to determine the level of vitamin C in the supplements as the first step iodine(I^-) ions and iodate (IO_3^-) ions are added to the vitamin C containing solution, both react among

themselves and forms elemental iodine (I_2). The vitamin C then quickly reacts with iodine to reduce iodine to iodine ions, through breaking and creating a conformational change in the structure of vitamin C.

Once all the vitamin C has reached, the remaining iodine formed reacts with a small amount of starch, which has been added in the process as an indicator, which produces dark blue complexes (Michel, 2013). The iodate as KIO_3 solution is added with burette. This blue black complex of the starch iodine product, illustrates the endpoint of the titration (Pisochi *et al*., 2008).

2. OBJECTIVE

To determine the amount of ascorbic acid in the lemon fruit with different times.

3. MATERIALS AND METHODOLOGY

3.1 Materials

Different lemons with appropriately same size and with each stage of ripening was used for this study. Three fruits from the same tree were used as the samples for this study.

Reagents used were vitamin C tablets, KIO_3 solution (0.05 M), KI solution (0.60 M), HCl solution (1.0 M), starch indicator, and distilled water.

Instrumentation - Conical flask (250 mL), beakers (400 ml, 100 ml), burette 50ml with stand, watch glass, glass stirring rod, analytical balance, and pipette with tips.

3.2 Methodology

To determine the vitamin C content in lemon titration method was carried out. A 500mg of vitamin C tablet was dissolved in 150 mL of distilled water. The tablet was carefully crushed with a string rod to help it dissolve faster. In to the conical flask 5 mL of 1.0M Hydrochloric acid (HCl), 10ml of 0.6M potassium iodide (KI) solution and four drops of freshly prepared starch indicator solution were added.

A burette was filled with 0.05M KIO_3 solution and the initial burette reading was taken. The conical flask was placed with reagent solution under the burette tip and the potassium iodate (KIO_3) solution was carefully added from burette, drop by drop until the solution took a permanent bluish color. When the solution in the flask showed color change permanently, at that point the titration was stopped immediately. The final burette reading was recorded and

the total volume of KIO_3 was noted. This analysis was repeated twice with another tablet of the same volume and the average volume was calculated.

Similarly, different three lemons with appropriately same size and with each stage of ripening was obtained. Each stage of the titrant process was done for separately for these three fruits. As a first step fruits were taken and the outer skin was peeled off gently. Fruit juice was collected into the conical flask by straining through cheese cloth. 10 mL of 0.6M KI and 5 mL of 1.0 M HCl were added into the conical flask. Then 4 drops of freshly prepared starch indicator were added. Then the solution was titrated using of KIO_3 solution.

The initial and final reading were recorded for each fruit. As each of the lemons ripening consisted with three values finally the average reading was calculated as the result.

4. RESULTS AND DISCUSSION

Table 1: Below shows the titrant volume in different stages of the lemon.

Samples of lemon at different stages of ripening		Titrant Volume (ml)			
		Trial 1	Trial 2	Trial 3	Average titrant volume
1	Fully green lemon	1.4	1.7	1.1	1.4
2	Beginning of ripening	1.4	2.2	1.2	1.8
3	At one week since plucking	0.9	1.0	0.9	0.93
4	Kept in refrigerator for one week	0.9	1.2	0.9	1.0
5	After 3 weeks since plucking	0.6	0.8	0.6	0.67

Table 2: The average weigh of ascorbic acid in lemon fruit.

Samples	Average weigh of ascorbic acid in lemon fruit.
Sample 1 -Fully green lemon	0.135g
Sample 2- Beginning of ripening	0.174g
Sample 3- After a week since plucking	0.090g
Sample 4-Fruit kept in refrigerator for a week	0.097g
Sample 5- After 3 weeks since plucking	0.065g

The weight of ascorbic acid in various stage of lemon fruit was determined based on the below ratio;

$$\frac{\text{Weight of Vitamin C (mg)}}{\text{Titrant volume of tablet (mL)}} = \frac{\text{Weight of juice (mg)}}{\text{Titrant volume of juice (mL)}}$$

5. DISCUSSION

Vitamin C or ascorbic acid can be determined using the process of acid base reactions or through a titration process. Although in this experiment KIO_3 solution was used as an indicator, DCPIP solution is more appropriate to use as an indicator. Because ascorbic acid has two proteins which can be donated to DCPIP. If vitamin C is a good reducing agent in the meantime DCPIP can act as a good oxidizing agent (Mequaninti, 2012).

For the estimation of ascorbic acid concentration, dilutions of the volume of the lemon juice sample were made. Lemon juice is selected for this process as it is hesperidium which is in the first choice of the citrus fruit by most of the commercial industries as it is a good source of dietary of fiber and which contains 0% of saturated cholesterol or fat along with 8% of citric acid and consists of heparin, naringin and naringenin. Even it is recommended by the clinicians for the daily consumption as it contains 29 calories per 100g, 88% of ascorbic acid in it. It highly take responsible and aid in smooth digestion, and to dissolve kidney stones.

As it also contains mineral ions Ca, K and Cu is obvious as it maintains heart rate and blood pressure. This project mainly localized around lemon juice for the analysis of the ascorbic acid (Pisochi *et al*., 2008). These samples were acidified in order to remove the protein components and ferric ions in it even these juices can be oxidized or acidity of these juices can be enhanced with the contact of air.

According to the many journal articles and scholarly sites, through the titration it was found out that different types of fruits consist of different amount of vitamin C. such as vitamin C in an orange is around 44.14mg/100ml, an apple 42.76mg/100ml however among all these fruits orange fruit has the highest concentration of vitamin C . It was also mentioned compared to all those concentrations of ascorbic acid in the commercial fruits are lower in amount.

As all the types of fruits juices are liquid in nature naturally. Even though many commercial juices are prepared from the natural fruits due to the process of this juice preparation. Its natural architecture is being lost along with addition of preservatives and chemicals the

reason for the majority attraction for these manufactured juices are the public doesn't have enough knowledge of this denaturation of real architectural components of fruit in these juice items.

The axiom is it is not the same concentration of vitamin C in the commercial fruit items and the real fruits. Therefore it is always advisable to consume more fruits for healthy existence. Thus this project analysis is carried out the highest and lowest concentration of ascorbic acid and the importance of consuming the natural fruits which are highly rich in vitamin C concentration (Pisochi *et al*., 2008).

Even the observation and results of this project also clearly emphasize that these commercial juices appeal to the taste preferences based on favor packs and chemicals.Based on theory it was mentioned content and texture of vitamin C rarely changes while it is preserved under cool temperature. But these content and texture is lost while it is preserved under higher temperature.

In this project while during the titration when it nears the endpoint, a blue purple should be seen around the KIO_3 drops and it should disappear with swirling of the flask. When the colour stays for about two seconds. It should be continued by adding the solution gradually drop by drop. When the solution in the conical flask turns dark bluish permanently the titration should be stopped immediately. It is the point at which the moles of iodine added are equal to the moles of vitamin C contained in the juice (Vancleave, 2014).

Air bubbles should be removed by turning the stopcock several times to force the air bubbles out of the tip. This colour should persist for at least 20 seconds before reading the final burette reading (helmeantine, 2014).

The starch indicator is bio degradable. There for fresh starch indicator needs to be prepared at least less than one week earlier. This is because after a week of storage, iodine becomes very weekly solvable in the water. This cause it to be easily lost from the solution due to it being volatile. However I_3^- ions are created in the presence of excess iodides (Glaser at al., 2007). This leads to the lowering of the free iodine concentration and these solution are stable enough to be used in lab practice nevertheless it should be kept in mind that this process is very short shelf lite. Therefore it should be stored inside dark bottles with lids tightly closed and they should be standardized every few weeks. If the analysis of the unknown takes more

than two weeks form when the solution was prepared the iodine solution should be standardized with the ascorbic acid again (Vancleave, 2014).

Beakers containing solution should never be held above eye level, since spilling could cause it to get solution outward and may even lead to severe damage HCl speeds up the reaction between I^- and IO_3^- (Mequaninti, 2012).

Even though the titration method was used in this study some of the unavoidable errors in the reading and endpoints were also happened which might affect the calculation. Through titration method the ascorbic acid concentration was estimated and minimize the errors each sample was done thrice and average mean value was taken as the volume.

Even form this project it was clearly illustrated the importance of ascorbic acid in humans as it plays an important role in the immune system and helps as antioxidant furthermore the over dosage also may turn as an allergy. Therefore the required amount of ascorbic acid to the body also analyzed clearly (Vancleave, 2014).

Among all the varies types of fruits lemon was selected for this project as it is leading fruit, widely used citrus family which consists of higher percentage of ascorbic acid and these juice contained zero saturated cholesterol and it is the fruit which clinicians advise and recommend for daily intake for the people who lacks vitamin C. this fruit can also be assessed easily.

6. CONCLUSION

This project illustrates different stages of lemon juice and their ascorbic acid concentration. This also gives a clear image about the importance of vitamin C and various natural fruits and vegetables which contains vitamin C. Compare to different stages of the lemon fruit, beginning stage of the ripping consisted higher amount of ascorbic acid and, after 2 weeks plucked fruit contained minimum concentrate of ascorbic acid. Therefore, this project results shows, with the timeline the amount of ascorbic acid of the fruit is being reduced. Hence the projects suggest to do the study with more number of samples to produce an appropriate correlation among the ascorbic concentration and the timeline. Finally the ascorbic acid concentration in each stage of the lemon was successfully calculated with minimum errors.

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