

A Comparative Analysis of Variation in Vitamin C Content Of Kinnow Mandarin (*Citrus Reticulata*) and Guava (*Psidium Guajava*) as a Function Of Days Of Storage at Room Temperature

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Abstract - This study depicts an analysis of Vitamin C content of two fruits, namely a citrus fruit Kinnow Mandarin and a non-citrus fruit Guava grown at Sophia Girls' College (Autonomous), Ajmer. Vitamin C or ascorbic acid is a very essential nutrient for human body which cannot be synthesized but can be obtained only from the food we eat. Thus, estimation of Vitamin C content and the effect of storage duration becomes significant. In this investigation, ascorbic acid content was estimated in juices extracted from freshly plucked fruits and in the stored fruits after an interval of 7 and 14 days. The analysis of the data shows that kinnow fruits show a decrease in Vitamin C content from 36.45 mg/100mL to 34.87 mg/100 mL after storage of 7 days which reduces to 24.83 mg/100 mL after 14 days of storage while guava on the other hand shows an increase in Vitamin C content from 100.91 mg/100 mL in fresh fruit sample to 127.86 mg/100 mL in after 7 days followed by a decrease to 119.40 mg/100 mL after 14 days of storage due to ripening effect, but it reached near the point of rotting decreasing its shelf life. Thus, it can be concluded that consuming fruits when fresh is best.

Key Words: Vitamin C, Guava, Kinnow, Redox titration, Ascorbic acid

1. INTRODUCTION

Fruits and Vegetables constitute a very important part of human diet. They are rich sources of dietary fiber, vitamins and minerals including Vitamin C, A and E, Mg, Zn, Folic acid as well as phytochemicals especially the antioxidants. A diet containing a high number of fruits and vegetables helps in lowering blood pressure, minimizes the risk of heart disease and stroke, assists in prevention of some types of cancer, decreases the risk of various problems concerning with eyes and digestive system and show a positive effect on the blood sugar level [1].

Vitamins are one of the most important constituents of fruits and vegetables that are vital for humans to maintain a healthy diet and form a necessary part of various biochemical and physiological processes taking place in the body. Some of the vitamins like Vitamin D, K, Niacin, Biotin can be synthesized within human body while some others cannot be synthesized, one of which is Vitamin C. This is because of the mutation in the gene which codes for the

enzyme L- gulono lactone oxidase required for the biosynthesis of Vitamin C [2]. As a result of this, human cells cannot perform the last step of synthesis of Vitamin C i.e., formation of ascorbic acid from L- gulono g- lactone catalyzed by enzyme L- gulono lactone oxidase [3]. Thus, the only source of Vitamin C in humans is the food we eat. According to Office of Dietary Supplements, the Recommended Daily Intake (RDI) of vitamin C is around 90 mg/day for an adult male and 75 mg/day for an adult woman which increases to 85 mg/day for a pregnant woman and to 120 mg/day for a woman during lactation [4]. The daily intake of Vitamin C increases by around 35 mg/day for smokers as vitamin C gets oxidized by free radicals and oxygen species produced by cigarette smoking [5,6].

Vitamin C, chemically known as L- Ascorbic Acid (C₆H₈O₆) is a white or slightly yellow odourless crystalline powder which is highly soluble in water and performs numerous biochemical and physiological functions in human body. It functions as an antioxidant by scavenging and neutralizing the most of the biologically active and harmful radicals such as superoxide, hydroperoxyl, aqueous peroxy radicals, NO₂, nitroxide radicals etc. [7] thus preventing humans from a condition known as oxidative stress [8] which is caused by increased number of free radicals. Moreover, ascorbic acid is a cofactor involved in the synthesis of Carnitine, Collagen and Neurotransmitters catalysed by hydroxylases and monooxygenase enzymes [8 -11]. It also behaves as a catalyst in amidation reactions of peptide hormones [12] oxytocin, cholecystokinin, α - melanotropin and vasopressin at their maximum efficiency [11].

Recently many Investigations have proved that Vitamin C reduces the risk of breast, lungs, rectum, colon as well as mouth cancers [2, 13 - 14]. In addition to this, ascorbic acid helps prevention of Hypercholesterolemia, enhances dietary absorption of non-heme iron [10-12, 15-17], improves immunity during cold infections, advantageous in cardiovascular diseases [12, 18] and cataracts [12].

Deficiency of Vitamin C causes anaemia, bleeding gums, poor wound healing, capillary haemorrhage, atherosclerotic plaques, muscle degeneration, and neurotic disturbances [8], tooth loss, joint pains, bone and connective tissue disorders, fatigue and lethargy, depression and mood changes [9]. Its

deficiency can also cause a pathologically fatal disease known as Scurvy, [19] the symptoms of which include fatigue, weakening of connective tissues and capillary fragility [10, 20] thus it is also known as “Anti- scorbic factor”, as it helps to prevent the disease known as scurvy [21].

Vitamin C content of fruits and vegetables can be used as an indicator of nutrient quality since it is highly susceptible to degradation. Various factors such as climatic conditions, culture practices, pH, Temperature and days of storage affect the amount of Vitamin C.

Due to its importance for the various biochemical and physiological functions of human body, the effects of its deficiency and the degradation factors which influences the nutrient quality of fruits and vegetables, the quantitative estimation of Vitamin C and the factors affecting it assumes significance.

This study involves the determination and analysis of effect of duration of storage on Vitamin C content of two fruits viz. Kinnow (*Citrus reticulata*) and Guava (*Psidium guajava*) grown at Sophia Girls' College (Autonomous), Ajmer by redox titration using potassium iodate as the titrant.

Kinnow (*Citrus reticulata*) is a citrus fruit which is a hybrid of *Citrus nobilis* (King) and *Citrus deliciosa* (Willow Leaf) mandarins. It contains a large amount of ascorbic acid, provitamin A, Folate, Limonoids, Phenolics, Carotenoids, Flavones and Flavonoids [22]. These metabolites boost the immune system against various infections, heart diseases as well as cancer [23]. It also contains minerals such as Na, K, Ca, Mg, P, Fe, Zn, Cu and Mn which are necessary for healthy bones and proper functioning of mitochondrial enzymes [22].

Guava (*Psidium guajava*) is a highly nutritive and a delicious fruit containing proteins, carbohydrates, lipids, minerals (calcium, iron, phosphorus, potassium), water, vitamins (A, B1 & C) [24-25]. It can also be used to treat various diseases by being a part of drugs [25].

2. RESEARCH METHODOLOGY

2.1 Sample Collection

Fresh Fruits of Kinnow Mandarin (*Citrus reticulata*) and Guava (*Psidium guajava*) were plucked from the trees grown in Sophia Girls' College (Autonomous), Ajmer campus and were analysed for Vitamin C content on the day of plucking and consecutively after 7 days and 14 days of storage at room temperature.

2.2 Sample Preparation

The Collected fruit samples were washed with distilled water to remove any adhering dust particles and impurities.

100 g of both the fruit samples were weighed and blended with a minimal amount of distilled water. After blending, the pulp was filtered using a sieve and a muslin cloth and then washed using few 10 mL portions of distilled water to extract the juice. The extracted juice was taken in a 100 mL volumetric flask and the flask was filled up to the mark using distilled water. Similarly, the sample were prepared from fruits after storage of 7 and 14 Days.

2.3 Preparation of Reagents

- **0.002 M Potassium Iodate**

0.428 g of Potassium iodate was weighed and dissolved in minimum amount of distilled water in a one-liter volumetric flask and then the flask was filled up to the mark using distilled water.

- **0.6 M Potassium Iodide**

0.6 M solution of Potassium Iodide was prepared by dissolving 9.96 g of KI in distilled water in a 100 mL volumetric flask.

- **1 M Hydrochloric Acid**

8.33 mL of HCl was taken in 100 mL volumetric flask and the solution was made up to the mark by distilled water.

- **0.5% Starch Indicator**

0.25 g of starch was dissolved in 50 mL of boiling distilled water by stirring. The solution was cooled before using.

2.4 Procedure

In this study, Vitamin C content in fruit juices was determined by oxidation-reduction titration using potassium iodate as the titrant in presence of KI and starch as the indicator [26]. 20 mL of fresh fruit juice was taken using a pipette and poured in a 250 mL conical flask to which 150 mL of distilled water, 5 mL each of 0.6 M KI and 1M HCl and 1 mL starch indicator was added. Then the sample was titrated with 0.002 M Potassium iodate solution till a permanent blue black colour appears due to formation of starch iodine complex at the equivalence or the end point of titration. A colour variation may be observed due to colour of fruit juice sample. The final volume of the iodate solution used to neutralize the ascorbic acid present in the sample was recorded and the titration was repeated at least two more times to get the concordant readings. An identical process was repeated to analyze the Ascorbic acid (Vit. C) content in juices extracted from stored fruits. The change in colour observed due to titration for the various fruit juices of both guava and kinnow are shown from figure 1 to 6.

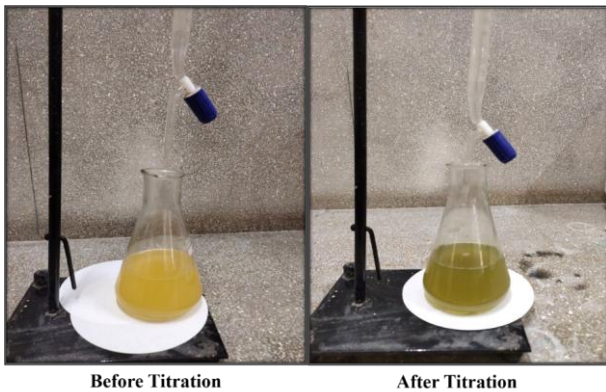


Figure 1- Vitamin C Analysis in Freshly plucked Kinnow fruits

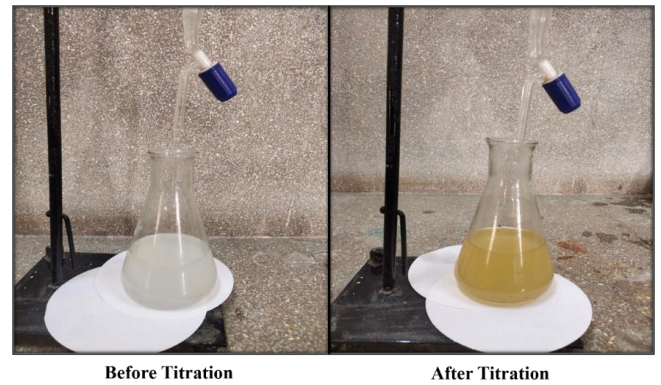


Figure 4- Vitamin C Analysis in Freshly plucked Guava fruits

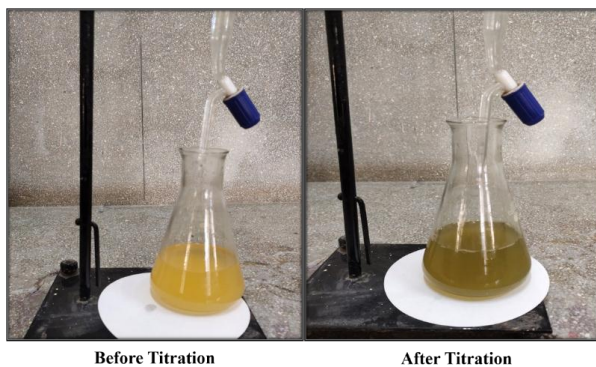


Figure 2- Vitamin C Analysis in Kinnow fruits after 7 Days of storage

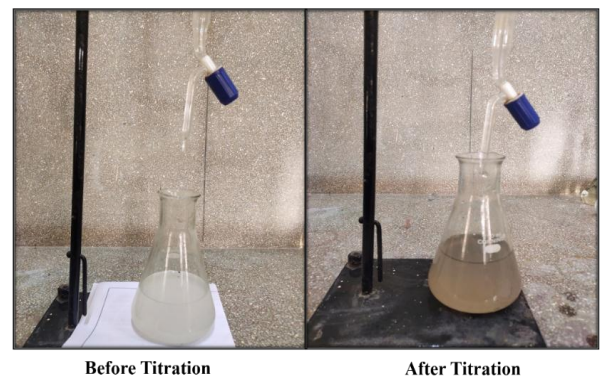


Figure 5- Vitamin C Analysis in Guava fruits after 7 Days of storage

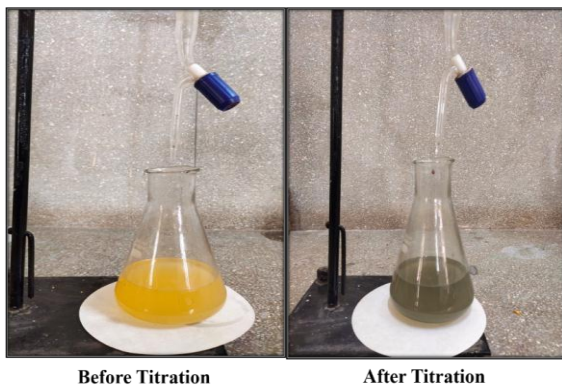


Figure 3- Vitamin C Analysis in Kinnow fruits after 14 Days of storage

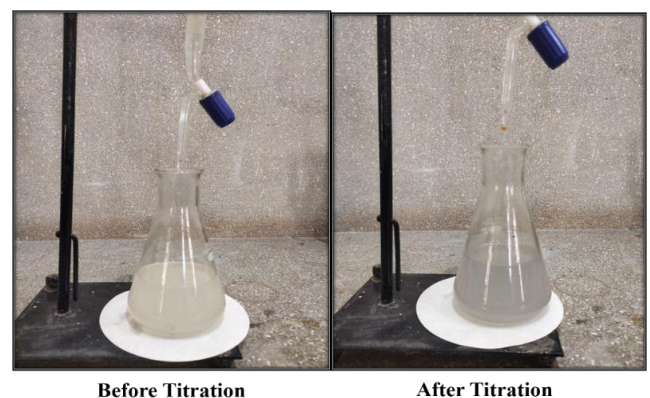
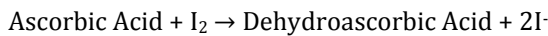
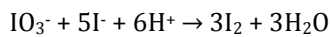


Figure 6- Vitamin C Analysis in Guava fruits after 14 Days of storage

During this titration, iodate ions are reduced and the iodide ions are oxidized to form iodine which oxidizes the ascorbic acid present in the sample to dehydroascorbic acid and is itself reduced back to iodine. The excess iodine after the end point reacts with starch to form a blue black coloured complex. The vitamin C content of the fruit juices was calculated on the basis of the stoichiometry of the following reactions-



According to the above reactions,

No. of moles of iodate used = 3 x No. of moles of Iodine formed = 3 x No. of moles of ascorbic acid

This shows that,

1 mole of iodate used = 3 moles of ascorbic acid present

That is, the molar ratio of iodate and ascorbic acid is **1:3**

Mass of ascorbic acid (mg) can be computed by-

Mass of Ascorbic acid present (in mg) = Moles of ascorbic acid x Molecular weight of ascorbic acid x 1000

This gives the concentration in mg/ 20 mL of sample as the initial volume of sample taken was 20 ml. Multiplying this value by 5 gives the concentration in mg/100 mL of sample.

Percentage (%) loss in concentration of Vitamin C is estimated by the formula:

$$\% \text{ loss} = \frac{\text{Ascorbic acid content in Fresh fruit juices} - \text{Ascorbic acid content in juices extracted from stored fruits}}{\text{Ascorbic acid content in Fresh fruit juices}} \times 100$$

2. RESULT AND DISCUSSION

This investigation involves the estimation of Vitamin C or Ascorbic acid content of freshly plucked fruits of Guava and Kinnow Mandarin by redox titration using potassium iodate as the titrant and its variation due to storage at ambient temperature. Average volume of Potassium iodate required by the juices extracted from freshly plucked and stored fruits of Guava and Kinnow Mandarin and the corresponding concentration of ascorbic acid in samples is given in table 1 and 2. The graph showing the variation of Vitamin C content as a function of days of storage is shown in figure 7.

Table -1: Average Volume of Titrant used and the concentration of Vitamin C in Various Fruit Juice Samples of Kinnow Mandarin (*Citrus reticulata*)

Fruit Juice Samples of Kinnow Mandarin	Average Volume of Iodate Solution Used (mL)	Concentration (mg/20 mL)	Concentration (mg/100 mL)
Freshly Plucked	6.9	7.29	36.45
After 7 Days of Storage	6.6	6.97	34.87
After 14 Days of Storage	4.7	4.966	24.83

Table 2 -Average Volume of Titrant used and the concentration of Vitamin C in Various Fruit Juice Samples of Guava (*Psidium guajava*)

Fruit Juice Samples of Guava	Average Volume of Iodate Solution Used (mL)	Concentration (mg/20 mL)	Concentration (mg/100 mL)
Freshly Plucked	19.1	20.18	100.91
After 7 Days of Storage	24.2	25.57	127.86
After 14 Days of Storage	22.6	127.86	119.40

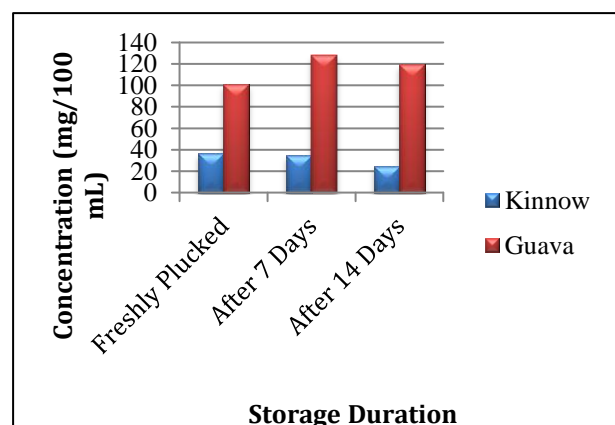


Figure 7 - Vitamin C content (mg/100mL) in Juices extracted from freshly plucked fruits and from stored fruits

The obtained results show that fresh as well as stored guava fruits have a higher Vitamin C as compared to kinnow fruits. Moreover, it shows that kinnow fruits show a gradual decrease in the ascorbic acid content from 36.45 mg/100mL to 34.87 mg/100 mL after a storage period of 7 days which reduces to 24.83 mg/100 mL after 14 days of storage. This is consistent with the various investigations which reveal that fruits and vegetables show a gradual decrease in the concentration of Vitamin C as the time of storage increases [27-29]. Percentage loss in the concentration of Vitamin C as computed from equation 1 is illustrated in Figure 8.

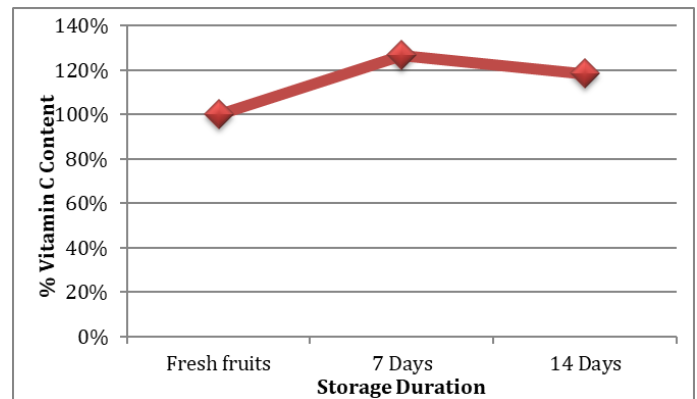


Figure 9 – Variation in % Vitamin C Content of Guava over time

The figure shows that guavas do not exhibit any % loss in Vitamin C content over time, else it shows 26.70% gain in the amount of Vitamin C when stored for 7 days which then show 8.38% loss in Vitamin C content as compared to the amount of Vitamin C in fruits stored for 7 days but still it has 18.32% more Vitamin C in comparison to the freshly plucked fruits which were not completely ripened.

It was also observed that storage of fruits at room temperature brought about colour and textural changes as shown in figure 10 and 11.

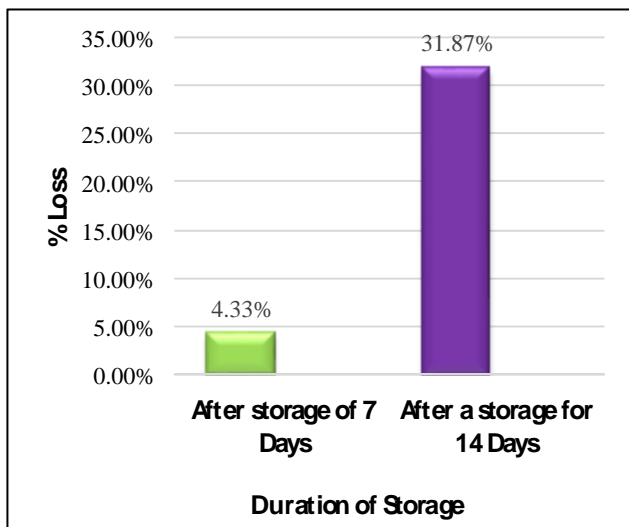


Figure 8 – Percentage (%) loss in concentration of Vitamin C as a function of duration of storage in Kinnow Mandarin

Figure 8 demonstrates that kinnow fruit displays a 4.33% loss in Vitamin C content after a period of storage of 7 days and on subsequent storage for 7 more days a sharp loss in Vitamin C content of 31.87% was observed.

Guava (*Psidium guajava*), however follows a different trend of variation in ascorbic acid content as a function of storage period. It shows an initial increase in Vitamin C content from 100.91 mg/100 mL in fresh fruit sample to 127.86 mg/100 mL in juice extracted from fruits stored for a period of 7 days followed by a decrease to 119.40 mg/100 mL as determined after 14 days of storage.

This may be because the guavas were plucked when they were not completely ripened and storage at ambient temperature brought about the ripening of fruits which increases the amount of Vitamin C as proved in an earlier investigation [30].

Percentage variation in the concentration of Vitamin C in guava is represented in figure 9.



Figure 10 – Colour and Textural changes observed in *Citrus reticulata* (Kinnow Mandarin) on storage



Figure 11 – Colour and Textural changes observed in *Psidium guajava* (Guava) on storage

The figures above display that softness of peels of both kinnow as well as guava fruits decreased with time. Although Guava showed a gain in the amount of vitamin C on storage but in a period of two weeks the fruit reached very near to the point of rotting. This may be because of its perishable nature [31].

Along with this, it was observed that guava shows browning of the skin as the time of storage increases. This is because of the enzymatic browning reactions carried out by polyphenol oxidase and polyphenyl ammonia lyase [32].

Thus, this study shows that even though guava has a higher Vitamin C content but its shelf life is less than that of kinnow fruits as it ripens very rapidly after harvest and its storage for a longer time is difficult to chances of its spoilage.

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