

Comparative Analysis of Ascorbic Acid Content in Fruits: Impact of Temperature and Storage Conditions

Dhruv S. Prashant

Obero International School, JVL R, Jogeshwari East, Mumbai, 400060 India; dhruv.s.prashant@gmail.com

ABSTRACT: Ascorbic Acid (Vitamin C) is an indispensable nutrient for its crucial role in immune functions, as well as a multitude of other health benefits. This study aims to compare the ascorbic acid content of 3 fruits (orange, guava, lemon) using a redox titration method using Iodine. Among the fruits analyzed, guava contained the highest amount of Vitamin C (307 mg/ 100 g of fruit sample), followed by orange (142 mg/ 100 g) and lemon (103 mg/ 100 g). All three fruits exhibited a reduction in ascorbic acid content after boiling for 3 minutes. The observed reduction in ascorbic acid concentration was 8% for guava, 13% for orange, and 4.6% for lemon. Additionally, storing fruits for 3 days in an open container at an ambient temperature of 28 °C also led to degradation with a reduction of 8% for guava, 20.7% for orange, and 6.6% for lemon. The present study suggests that storage conditions and processing methods impact the retention of ascorbic acid, and the extent of reduction differs depending on the type of fruit.

KEYWORDS: Biochemistry, General Biochemistry, Ascorbic Acid, Fruits, Temperature.

■ Introduction

Vitamins are a group of organic compounds that are needed for the healthy functioning of the body. Vitamins are categorized as fat-soluble (Vitamins A, D, E, and K) and water-soluble (Vitamins B and C). L-Ascorbic acid (Vitamin C) is an important vitamin essential for various metabolic processes and is primarily considered a potent antioxidant. Its physiological role is extensive and includes various processes such as the absorption of iron as a key facilitator, hormone synthesis, and playing significant roles in wound healing and collagen production. Humans cannot synthesize Vitamin C and hence are fully dependent on its intake from diet.¹ Citrus fruits, which belong to the genus *Citrus* of the family Rutaceae, such as oranges, lemons, are well established sources of Vitamin C.² Apart from Citrus fruits, there are fruits like guava, which also have significant amounts of Vitamin C. Guava belongs to the genus *Psidium guajava* in the family Myrtaceae. There have been extensive studies on the Vitamin C content in guava, and it has been established as a potent source of Vitamin C.³⁻⁵ In addition, commercial fruit juices contain added Vitamin C to enhance nutritional value.

The stability of Vitamin C is dependent on several factors, including exposure to oxygen, temperature, light, and storage conditions. These parameters can affect Vitamin C concentration during processing and storage.

Given the vital role of this vitamin in metabolism and its exclusive dietary origin, this study aims to quantitatively compare the levels of ascorbic acid in commonly consumed fruits (oranges, lemons, and guavas). Further, the study estimates the impact of heat treatment and ambient storage duration on the degradation of ascorbic acid. This study will provide insight

into how processing and storage conditions influence nutrient conservation.

■ Methods

Preparation of fresh fruit samples:

Mandarin Oranges, Thai Pink Guava, and Indian Lemon were procured from an online vendor. Details of the procurement are as below.

Table 1: Details of the sourcing of fruit samples for the experiments.

Fruit Name	Name of Vendor	Variety/ Scientific Name	Extent of Ripeness
Thai Pink Guava	Bhagavati Stores Pvt Ltd, JVL R, Mumbai	<i>Psidium guajava</i> , Country of Origin: India	Fully ripe
Mandarin Orange	Bhagavati Stores Pvt Ltd, JVL R, Mumbai	<i>Citrus reticulata</i> , Country of Origin: India	Fully ripe
Indian Lemon	Bhagavati Stores Pvt Ltd, JVL R, Mumbai	<i>Citrus limon</i> , Country of Origin: India	Fully ripe

100 g sample of 3 fruits (Mandarin Oranges, Thai Guava, or Lemon) was cut into small pieces (without the peel for orange and lemon) and blended in an electric blender. 10 ml portions of distilled water were repeatedly added multiple times, and the liquid was subsequently carefully decanted into a conical flask. Finally, the blended fruit juice was strained through a cheesecloth, rinsing the pulp with 10 ml portions of water. The extracted solution was made up to 100 mL with distilled water.

Preparation of the standard iodine solution:

Analytical-grade Potassium Iodide was procured from Nice Chemicals (Pvt) Ltd. Analytical-grade Iodine crystals (I₂) were procured from Research-Lab Fine Chem Industries. 2 g of Potassium iodide was added to a 100 ml beaker. 1.3 g of iodine crystals were weighed on an electronic weighing scale and added to the same beaker, and swirled until the iodine crystals were fully dissolved. The solution was transferred to a 1-liter

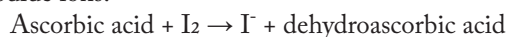
volumetric flask, and the resulting solution was made up to the 1-liter mark with distilled water.

Preparation of the starch indicator:

Soluble Starch was procured from Spectrum Reagents and Chemicals Pvt Ltd. 0.25g of soluble starch was added to 50 ml of near-boiling water in a 100 ml conical flask and stirred until it was fully dissolved.

Redox titration using iodine solution:

The method for determining the ascorbic acid (Vitamin C) content in solution uses redox titration using iodine solution (Redox titration method, University of Canterbury). Upon the addition of iodine during titration, the ascorbic acid is oxidized to dehydroascorbic acid, and the iodine is reduced to iodide ions.



Standard Vitamin C tablets were procured from Abbot Healthcare Limited (Brand Limcee® 500 mg, Batch No. HAJA4137, Expiry date, April 2026). 1 Limcee® (500 mg) Vitamin C Chewable tablet was dissolved in 200 ml of distilled water to make the standard solution. In the first experiment, a 20 ml aliquot of the standard solution (50 mg of Vitamin C from Limcee® 500 mg) was added into a 250 ml conical flask using a pipette, and 150 ml of distilled water was added. 1 ml of starch indicator solution was added. This was titrated with the prepared iodine solution (0.005 mol/ litre) and the endpoint was identified with the first permanent trace of the dark blue-black colour. The standard titer value (C_{standard}) for 50 mg of Vitamin C was determined.

In the second experiment, a 20 ml aliquot of the fruit sample solution was added to a 250 ml conical flask using a pipette, and 150 ml of distilled water was added. 1 ml of starch indicator solution was added. This was titrated with the prepared iodine solution (0.005 mol/ Litre), and the endpoint was identified as the first permanent trace of the dark blue-black colour. This was done for all three fruits (orange, guava, lemon) until the endpoint was reached.

Preparation of samples for high temperature and extended storage:

In the third experiment, the fruit samples were boiled for 3 minutes at 100 degrees, and the titration was carried out as mentioned previously with 20 ml aliquots. In the fourth experiment, the fruit samples were stored in open conical flasks for 3 days, at a temperature of 28 °C, to study the effects of storage on the concentration of ascorbic acid. The titration was carried out as mentioned previously with 20 ml aliquots.

Determination of ascorbic acid concentration:

The titer volume for each sample in all the above experiments was compared against the standard Vitamin C titer value (C_{standard}) for determining the concentration of ascorbic acid in the samples (C_{sample})

The calculation of the amount of ascorbic acid in the samples (C_{sample}) was calculated using the formula below.

$$C_{\text{sample}} = (\text{Observed titer value} / C_{\text{standard}}) \times (250) \text{ mg} / 100\text{g of the sample}$$

■ RESULTS

Ascorbic acid concentration varies in different freshly prepared fruit juices:

Ascorbic acid is a water-soluble micronutrient that is essential for the growth of healthy cells. First, the concentration of ascorbic acid in Limcee® Tablet was estimated, which served as a positive control. Titration results are shown in Figure 1. Titer value was determined to be 24.4 ml. This titer value represents the equivalent of 50 mg of ascorbic acid. Subsequently, the concentration of ascorbic acid in freshly prepared juices of different fruits was estimated by the redox titration method. Titration start and end point images for orange, guava, and lemon are presented in Figures 2, 3, and 4, respectively. The endpoint for the fresh orange sample (Figure 2) was achieved at a titer value of 13.8 mL, translating to ascorbic acid content of 142.07 mg/ 100 g. The endpoint for the fresh guava sample (Figure 3) was achieved at a titer value of 30 ml, translating to ascorbic acid content of 307.3 mg/ 100 g. The endpoint for the fresh lemon sample (Figure 4) was achieved at a titer value of 10.06 ml, translating to ascorbic acid content of 103.14 mg/ 100 g. Figure 5 represents the concentration of ascorbic acid in mg per 100 g of the fruit samples. Table 1 mentions the calculated values of the concentration of ascorbic acid. Among the 3 fruits, guava juice contained the highest levels of ascorbic acid (307.3 mg/ 100 g), followed by orange (142.07 mg/ 100 g) and lemon (103.14 mg/ 100g). This is in line with similar research done earlier.⁵

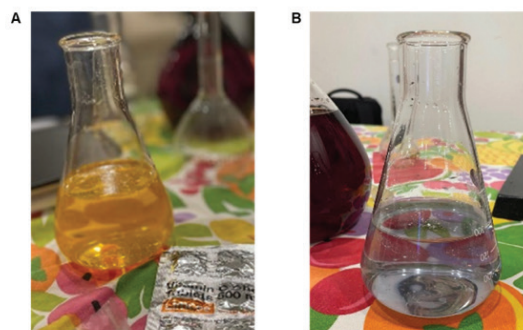


Figure 1: Redox titration results for ascorbic acid standard (Limcee® Tablet): A) Titration start point. B) Titration endpoint. The blue-black color indicates the endpoint of the titration.

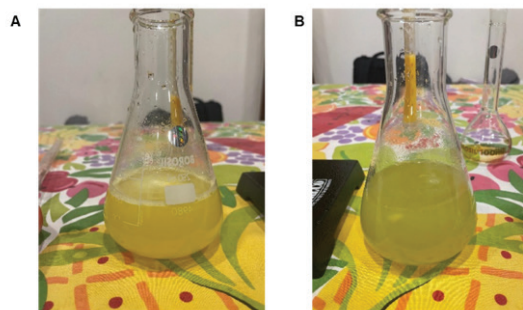


Figure 2: Redox titration results for a freshly prepared orange juice sample: A) Titration start point. B) Titration endpoint. The appearance of the blue-black complex indicates the endpoint of the titration.

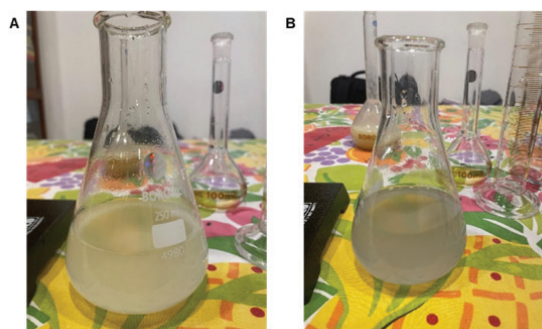


Figure 3: Redox titration results for freshly prepared guava juice sample: A) Titration start point. B) Titration endpoint. The appearance of a blue-black complex indicates the titration endpoint.

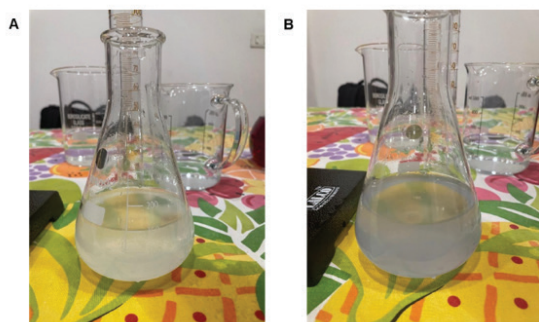


Figure 4: Redox titration results for a freshly prepared lemon juice sample: A) Titration start point. B) Titration endpoint. The appearance of a blue-black complex indicates the endpoint of the titration.

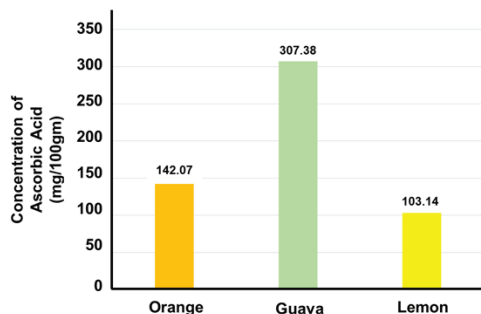


Figure 5: The concentration of ascorbic acid varies in different fruit juice samples. The bar graph represents the concentration of ascorbic acid (mg) per 100 g of fruit sample. The figure indicates the highest amount of Vitamin C in guava, followed by orange, and then lemon.

Table 2: The concentration of ascorbic acid (in mg) in 100 g of freshly prepared fruit samples indicates the highest concentration in guava, followed by orange and lemon.

Fruit Sample	Concentration of ascorbic acid in mg/ 100 g
Orange	142.07 mg/ 100g
Guava	307.38 mg/ 100 g
Lemon	103.14 mg/ 100 g

Boiling fruit samples reduces the ascorbic acid concentration:

Ascorbic acid is a temperature-sensitive vitamin, and an increase in temperature significantly reduces the concentration of ascorbic acid. To study the effect of temperature, separate fresh juice of orange, guava, and lemon, each followed by boiling for 3 minutes, was prepared. Redox titration of both freshly prepared juice and boiled juice was carried out. Boiling

of fruit juice samples reduced the concentration of ascorbic acid as compared to freshly prepared fruit juice samples (Figure 6 and Table 2). All the samples showed a degradation of ascorbic acid after boiling for 3 minutes. The concentration of ascorbic acid was estimated to be 123.63 mg/ 100 g for the boiled orange juice sample, 282.79 mg/ 100 g for the boiled guava juice sample, and 98.361 mg/ 100 g for the boiled lemon juice sample. These values indicate a reduction of 13%, 8%, and 4.6% of ascorbic acid content for orange, guava, and lemon, respectively, when subjected to boiling for 3 minutes.

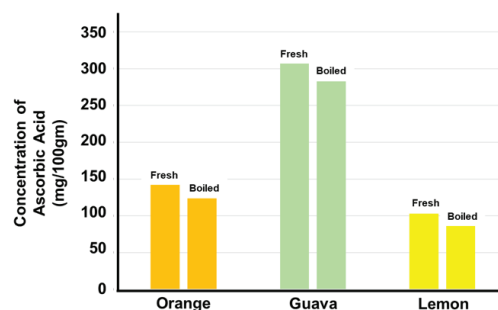


Figure 6: Boiling degrades ascorbic acid concentration in fruit juice samples. The bar graph represents the concentration of ascorbic acid (mg) per 100 g of fruit sample in freshly prepared and boiled fruit juices of orange, guava, and lemon. The figure indicates the reduction in Vitamin C content in the boiled samples across all 3 fruits.

Table 3: Concentration of ascorbic acid in 100 g of freshly prepared fruit juice and 100 g of fruit juice samples boiled at 100 °C for 3 minutes (orange, guava, lemon).

Fruit Sample	Concentration of Ascorbic Acid (mg) per 100 g of sample	
	Freshly prepared juice	Boiled for 3 minutes
Orange	142.07 mg/ 100 g	123.63 mg/ 100 g
Guava	307.38 mg/ 100 g	282.79 mg/ 100 g
Lemon	103.14 mg/ 100 g	98.361 mg/ 100 g

Storage at ambient temperature reduces the ascorbic acid concentration:

To retain the concentration of ascorbic acid in fruits, it is important to store fruits at an optimum temperature. To study the effect of storage conditions on ascorbic acid concentration, redox titration was carried out on two experimental groups. The first group consisted of freshly prepared juice of orange, guava, and lemon. The second group consisted of fruit juice samples, which were stored at 28°C for 3 days. It was observed that there was a reduction in ascorbic acid concentration in 3-day stored samples as compared to freshly prepared fruit juices (Figure 7 and Table 3). The concentration of ascorbic acid was estimated to be 112.7 mg/ 100 g for the orange juice sample, 282.79 mg/ 100 g for the guava juice sample, and 96.311 mg/ 100 g for the lemon juice sample. Exposure to the atmosphere in ambient conditions reflects a degradation of ascorbic acid, and the extent of degradation was 20.7% for orange, 8% for guava, and 6.6% for lemon.

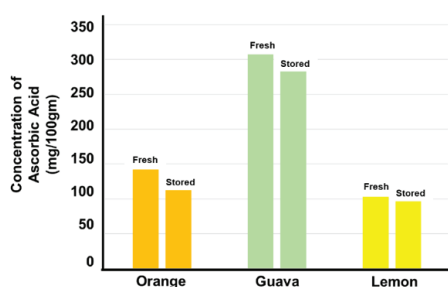


Figure 7: Storage at ambient temperature degrades ascorbic acid concentration in fruit juice samples. The bar graph represents the concentration of ascorbic acid (mg) per 100 g of fruit sample in freshly prepared and stored juices at ambient temperature, 28 °C, for 3 days of orange, guava, and lemon.

Table 4: Concentration of ascorbic acid in 100 g of freshly prepared fruit samples and 100 g of fruit samples stored at an ambient temperature of 28 °C for 3 days

Fruit Sample	Concentration of ascorbic acid (mg) per 100g of sample	
	Freshly prepared juice	Stored for 3 days at 28°C
Orange	142.07 mg/ 100 g	112.7 mg/ 100 g
Guava	307.38 mg/ 100 g	282.79 mg/ 100 g
Lemon	103.14 mg/ 100 g	96.311 mg/ 100 g

■ Discussion

Vitamin C plays a crucial role in forming collagen, supporting immune functions, and cyto-protection. Our body cannot produce Vitamin C; thus, we need to be dependent on different dietary sources of Vitamin C, like citrus fruits, vegetables, etc. Vitamin C is an extremely sensitive Vitamin, and thus maintaining optimum temperature and storage conditions is crucial to retain the concentration of this Vitamin. The present study aims to estimate the concentration of Vitamin C in different fruit samples and to study the effect of temperature and storage conditions on the concentration of Vitamin C.

The study shows that the concentration of ascorbic acid (Vitamin C) varies across different fruits. The study showed that guava has the highest concentration of Vitamin C (307.38 mg/ 100 g), followed by orange (142.07 mg/100 g) and lemon (103.14 mg/100 g). In prior studies, the Vitamin C content in orange was estimated to be (58.304 mg/ 100 g) by Najwa *et al.*,⁶ (42.7 mg/100 g) by Tee *et al.*,⁷ (56.020 mg/100 g) by Cioroi *et al.*,⁸ and (56.4 mg/100 g) by Bungau *et al.*⁹ The concentration of Vitamin C in orange is lower by 59%–70% across these aforementioned studies compared to the observed concentration in these experiments. Lack of standardization of fruit sourcing (species, extent of ripening, storage conditions) as well as different testing methodologies (High Performance Liquid Chromatography⁶, Microfluorometry⁷, dye-based titration⁶) are reasons for this variation compared to earlier studies. For guava, the Vitamin C content was estimated to be (206–334 mg/100 g) in prior studies by Ghani *et al.*,¹⁰ (330.77 mg/100 g) by Alok *et al.*,¹¹ and (181.6 mg/100 g) by Simran *et al.*³ in prior studies. The observed concentration of Vitamin C in guava is lower by 12% compared to the estimates by Ghani *et al.*¹⁰ and higher by 7% compared to the estimates by Alok *et al.*¹¹. For lemon, the Vitamin C content was estimated to be (43.956 mg/100 g) by Najwa *et al.*,⁵ (46.8 mg/ 100 g) by Tee *et al.*,⁷ (51.78 mg/100 g) by Cioroi *et al.*,⁸ and (49 mg/100

g) by Bungau *et al.*¹⁰ in earlier studies. The observed concentration of Vitamin C in lemon is lower by 57% compared to the estimates by Najwa *et al.*,⁵ lower by 55% compared to the estimates by Tee *et al.*,⁷ lower by 50% compared to estimates by Cioroi *et al.*,⁸ and lower by 52% compared to estimates by Bungau *et al.*¹⁰ These differences are due to the lack of standardization of the fruit sources, stage of ripening, and storage conditions of the different samples. The testing methods used across these studies are also varied (High Performance Liquid Chromatography,⁶ Microfluorometry,⁷ dye-based titration.⁶ However, guava has been shown to have the highest concentration of ascorbic acid across these three fruits, and this is consistent with prior studies.

All the citrus fruits show a degradation of ascorbic acid even when subjected to short durations of boiling temperatures. Prior studies also demonstrated similar results where boiling fruit juices resulted in the reduction of Vitamin C content. In an earlier study, a 10% reduction of ascorbic content was shown when orange samples were boiled/ exposed to heat.¹² Similarly, a degradation of 18% was reported for guava samples at boiling temperatures.¹³ In the case of lemon, earlier studies by Njoku *et al.*¹² have shown a reduction of 18%, while another study showed a reduction of 13% when exposed to a boiling temperature.¹⁴ The extent of degradation is directionally in line with other studies; however, other studies did pursue the extent of degradation for much larger time durations. Ascorbic acid is sensitive to temperature, both while cooking, food processing, as well as transport and storage conditions, and this is reflected in the varied levels of ascorbic acid when subjected to higher temperatures.

Storage conditions in the open also lead to degradation of ascorbic acid, resulting in reduced concentrations across fruits. In a prior study, reduction of ascorbic acid was shown to the extent of 16.27% when orange samples were stored.¹⁵ For guava, this was reported to be 28% in a prior study.¹⁶ For lemon, there was a reduction of 10% reported in an earlier study.¹⁷ It is to be noted that the duration of storage was varied in the prior studies, but all studies were consistent in the reduction of ascorbic acid over time. Thus, the present study indicates that the concentration of Vitamin C varies in different samples, and high temperature and adverse storage conditions reduce the concentration of this Vitamin.

The limitations of this study are the lack of experimental replicates and statistical analysis. This study does not have enough experimental data points for robust statistical analysis. It is recommended that, for more robust data and analysis, experiments should be performed in triplicate and should include basic statistical analysis (means, standard deviations, statistical significance).

■ Conclusion

Vitamin C (ascorbic acid) is an important micronutrient that plays a crucial role in maintaining human health. Dietary guidelines recommend the consumption of Vitamin C-rich fruits to meet daily dietary requirements. The present findings demonstrate that the concentration of Vitamin C is influenced by external factors such as the source, storage, and transport conditions of the fruit. Boiling for 3 minutes and incubation

of fresh juice for 3 days at an ambient temperature of 28 °C significantly reduced Vitamin C concentration. Optimum temperature and storage conditions are critical for preserving Vitamin C content in fruits since this vitamin is highly sensitive to heat, light, and oxygen. Thus, optimum temperature and storage conditions should be maintained to reduce degradation of Vitamin C, thereby reducing the nutritional value of the fruit.

The practical implications of this study can broadly be summarized into three broad areas: choice of fruits for Vitamin C supplementation, cooking and food processing implications, and storage implications. Firstly, from a nutritional supplementation perspective, guava, followed by orange, and subsequently lemon, provide the greatest amount of Vitamin C supplementation based on the results of the study. These fruits can therefore be the choice of Vitamin C supplementation based on patient/ consumer needs. Secondly, cooking and exposure of these fruits to boiling temperatures results in degradation and lesser availability of Vitamin C. High temperature food processing should be avoided on these fruits, to maximize nutritional availability of Vitamin C. Thirdly, the storage of these fruits for extended periods at 28 °C or higher results in degradation of Vitamin C. These fruits should ideally be kept in cold storage to maximize the concentration of Vitamin C in these fruits, and should be consumed immediately after being removed from cold storage.

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■ Author

Dhruv Prashant is a Grade 11 student researcher with a deep interest in biochemistry and molecular biology. He is committed to serving the underprivileged, leveraging the transformative power of science and medicine. He seeks to pursue a career in medicine that can uplift society by improving human health.