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Analytical Assessment of Vitamin C Stability in Citrus Fruit Juices Under Different Storage Conditions

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ABSTRACT: Vitamin C (ascorbic acid) is an essential water-soluble antioxidant widely distributed in citrus fruits and fruit-derived products. Due to its liability to degradation during storage, the preservation of vitamin C content is an important factor affecting the nutritional quality of fruit juices. The present study aimed to determine the vitamin C content in different citrus fruit juices and to evaluate the influence of storage conditions on its stability over time. Fresh juices obtained from Albanian and imported lemons, oranges, mandarins, and imported citron were analysed using iodometric titration method. Vitamin C concentrations were determined immediately after juice extraction and after storage under ambient conditions (22°C) and refrigerated conditions (4°C) for 0, 24 and 48 hours (the 1st, 2nd and 3rd day). The results revealed considerable variation in the initial vitamin C content among the analysed fruits. Imported citron exhibited the highest vitamin C concentration (62.77 mg), followed by Albanian orange (54.03 mg) and imported orange (46.64 mg), whereas imported mandarin presented the lowest concentration (3.97 mg). A gradual decrease in vitamin C content was observed in all samples during storage, with significantly greater losses occurring at ambient temperature than under refrigeration. After 48 hours of storage, Albanian lemon juice showed the greatest reduction in vitamin C concentration at room temperature, while Albanian orange juice demonstrated the highest stability. Refrigerated storage consistently preserved higher vitamin C levels in all fruit juices compared with ambient storage. These findings confirm that both storage duration and temperature significantly influence the retention of ascorbic acid in citrus juices. The study highlights the importance of refrigerated storage as an effective strategy for minimizing vitamin C degradation and maintaining the nutritional quality of fruit juices. Furthermore, the results support the applicability of iodometric titration as a simple, reliable, and cost-effective method for routine determination of vitamin C in food samples.

KEYWORDS: Ascorbic acid; Citrus fruits; Titration; Storage conditions

INTRODUCTION

Vitamin C (ascorbic acid) is a water-soluble vitamin and one of the most important dietary antioxidants required for maintaining normal physiological functions in humans. Since humans are unable to synthesize ascorbic acid due to the absence of L-gulonolactone oxidase, it must be obtained through dietary sources, primarily fruits and vegetables (Ali et al., 2024; Mittu et al., 2022). Citrus fruits, berries, kiwifruit, and several leafy vegetables are recognized as major sources of vitamin C and contribute significantly to daily nutritional requirements (Offor et al., 2015). Structurally, ascorbic acid is a six-carbon lactone derived from glucose metabolism and possesses strong reducing properties that underlie its biological activity (Kükürt & Gelen, 2024; Smirnoff, 2018).

The physiological significance of vitamin C extends far beyond its classical role in preventing scurvy. Ascorbic acid serves as an essential cofactor in numerous enzymatic reactions, including collagen biosynthesis, carnitine production, neurotransmitter synthesis, and iron absorption. Vitamin C deficiency can lead to impaired connective tissue formation, delayed wound healing, weakened immune function, and the development of scurvy, a disease still reported in vulnerable populations worldwide (Khalife et al., 2019; Bhattacharyya et al., 2019). Furthermore, growing evidence suggests that vitamin C contributes to immune regulation, epigenetic modulation, and cellular homeostasis, highlighting its importance in maintaining overall health (Yue & Rao, 2020; Murererehe et al., 2022).

The biological functions of ascorbic acid are largely attributed to its ability to act as a potent antioxidant. By donating electrons, vitamin C scavenges reactive oxygen species (ROS) and reactive nitrogen species, thereby protecting biomolecules from oxidative damage (Kaźmierczak-Barańska et al., 2020; Atta et al., 2017). This antioxidant activity has been associated with a reduced risk of oxidative stress-related disorders, including cardiovascular diseases, inflammatory conditions, and premature cellular aging (Ali et al., 2024). However, under certain conditions, particularly in the presence of transition metal ions, ascorbic acid may also exhibit pro-oxidant behavior, emphasizing the

complexity of its biochemical activity (Kaźmierczak-Barańska et al., 2020).

Despite its nutritional importance, vitamin C is among the most unstable vitamins naturally present in foods. Its concentration can be significantly affected by environmental factors such as temperature, oxygen exposure, pH, light, and storage duration. During storage and processing, ascorbic acid is readily oxidized to dehydroascorbic acid and subsequently degraded into biologically inactive compounds, leading to a gradual decline in nutritional quality (Offor et al., 2015; Smirnoff, 2018). Previous studies have reported substantial losses of vitamin C in fruit juices and fruit-derived products stored under unfavorable conditions, particularly at room temperature and during extended storage periods (Ali et al., 2024; Offor et al., 2015). Therefore, understanding the influence of storage conditions on vitamin C stability is essential for ensuring the nutritional value of fruit juices available to consumers.

Various analytical methods have been developed for the determination of vitamin C, including high-performance liquid chromatography (HPLC), spectrophotometric techniques, electrochemical methods, and volumetric titration procedures (Tadesse & Sirgawie, 2017). Among these, iodometric titration remains one of the most widely used methods because of its simplicity, low cost, and satisfactory accuracy for routine laboratory analysis. The method is based on the redox reaction between iodine and ascorbic acid, in which ascorbic acid is oxidized to dehydroascorbic acid while iodine is reduced to iodide ions. In the presence of starch indicator, excess iodine forms a characteristic blue starch-iodine complex that signals the endpoint of the titration, enabling the quantitative determination of vitamin C concentration.

Given the nutritional significance of vitamin C and its susceptibility to degradation, evaluating its stability in fruit juices under different storage conditions is of considerable scientific and practical interest. Fruit juices are widely consumed worldwide and are often regarded as convenient sources of dietary antioxidants. However, inappropriate storage conditions may substantially reduce their vitamin C content before consumption. Therefore, the present study aims to determine the vitamin C content of different fruit juices using an iodometric titration method and to investigate the effect of storage conditions, specifically room temperature and refrigerated storage, on the retention of vitamin C over time. The findings are expected to provide valuable information regarding optimal storage practices for preserving the nutritional quality and antioxidant potential of fruit juice products.

2. MATERIALS AND METHODS

2.1. Sampling of Fruit and Juice Samples

The determination of vitamin C was performed on citrus fruit samples collected between October and December 2025 from various local markets in Tirana, Albania. The analyzed samples included Albanian and imported lemons, oranges, mandarins, and citron fruits. Fresh fruits were selected based on their commercial maturity and absence of visible defects or signs of deterioration.

Fruit juices were prepared immediately prior to analysis by manually extracting the juice from each fruit sample. The extracted juice was filtered through a fine filter paper to remove pulp and suspended solids before analysis. Vitamin C quantification was carried out using an iodometric titration method based on the principles described in ISO 6557-2:1984, which relies on the redox reaction between ascorbic acid and iodine solution. This method is widely applied for the determination of relatively high concentrations of vitamin C in food matrices due to its simplicity, reliability, and satisfactory analytical performance.

To evaluate the effect of storage conditions on vitamin C stability, aliquots of freshly prepared fruit juices were stored under two different conditions: at ambient temperature (22 ± 2°C) and under refrigerated conditions (4 ± 1°C). Analyses were performed immediately after juice extraction (fresh samples) and subsequently after 24 and 48 hours of storage (the 1st, 2nd and 3rd day).

2.2. Preparation of Iodine and Starch Solutions

The determination of ascorbic acid was carried out using a 0.005 mol L^{-1} iodine solution as titrant and a 0.5% (w/v) starch solution as the indicator.

The iodine solution was prepared by accurately weighing 2.0 g of potassium iodide (KI) and 0.30 g of iodine crystals (I_2). Both reagents were dissolved in approximately 50 mL of distilled water in a 100 mL conical flask. After complete dissolution, the solution was quantitatively transferred into a 1 L volumetric flask and diluted to volume with distilled water to obtain the working iodine solution.

For the preparation of the starch indicator solution, 0.25 g of soluble starch was dispersed in 50 mL of distilled water in a 100 mL beaker. The mixture was heated on a hot plate at approximately 80°C for 5 minutes while stirring continuously until a clear homogeneous solution was obtained. The starch solution was allowed to cool to room temperature before use.

2.3. Storage Conditions

Freshly prepared juice samples were divided into separate containers and subjected to two storage regimes. One set of samples was stored at ambient laboratory temperature (22 ± 2°C), while a second set was stored under refrigeration (4 ± 1°C). Vitamin C content was determined immediately after juice extraction and subsequently after 24 and 48 hours of storage. These storage conditions were selected to simulate typical household storage practices and to assess their influence on vitamin C degradation.

2.4. Titration Procedure

The vitamin C content of the citrus juices was determined by iodometric titration. Freshly extracted juice from each sample was filtered and transferred to a clean container. A 20 mL aliquot of filtered juice was pipetted into a 250 mL

Erlenmeyer flask, followed by the addition of 1 mL of freshly prepared 0.5% starch solution.

Titration was performed by adding the standardized 0.005 mol L^{-1} iodine solution from a burette while continuously swirling the flask. As long as ascorbic acid was present, the added iodine reacted immediately with the vitamin C in the sample and no permanent color change was observed. The endpoint was reached when a persistent dark-blue coloration, characteristic of the starch-iodine complex, remained visible for at least 30 seconds.

Each sample was analyzed in triplicate, and the average value was used for data interpretation. The titration procedure was applied to fresh juice samples as well as to samples stored for 24 and 48 hours under ambient and refrigerated conditions, allowing the evaluation of vitamin C degradation as a function of storage time and temperature. The redox reaction involved in the determination of ascorbic acid is represented by the following equation:

$$C_6H_8O_6 + I_2 \rightarrow C_6H_6O_6 + 2I^- + 2H^+$$

where ascorbic acid is oxidized to dehydroascorbic acid while iodine is reduced to iodide ions.

2.5. Calculation of Vitamin C Content

The concentration of vitamin C was calculated from the volume of iodine solution consumed during titration according to the stoichiometric relationship between iodine and ascorbic acid (1:1 molar ratio). The amount of ascorbic acid present in the analyzed sample was calculated using the following equation:

$$m_{AA} = V_{I2} \times C_{I2} \times M_{AA}$$

where:

m_{AA} = mass of ascorbic acid (mg)

V_{I2} = volume of iodine solution used in the titration (L)

C_{I2} = concentration of iodine solution mol L^{-1})

M_{AA} = molar mass of ascorbic acid (176.12 g mol^{-1})

The vitamin C content was subsequently expressed as milligrams of ascorbic acid per analyzed juice aliquot. Results are presented as mean values obtained from three independent determinations.

2.6. Statistical Analysis

All measurements were performed in triplicate, and the results were expressed as mean values. Percentage losses of vitamin C during storage were calculated relative to the concentration measured in the fresh juice samples. Comparative evaluation of vitamin C retention at ambient and refrigerated conditions was performed to assess the influence of storage temperature and duration on ascorbic acid stability.

RESULTS AND DISCUSSION

The vitamin C content determined by iodometric titration showed considerable variation among the analyzed citrus fruits, reflecting differences associated with fruit type, cultivar, and origin. The highest initial vitamin C concentration was observed in imported citron (Citrus medica) (62.77 mg), followed by Albanian oranges (54.03 mg) and imported oranges (46.64 mg). In contrast, mandarins exhibited substantially lower vitamin C concentrations, ranging from 3.97 mg in imported samples to 9.62 mg in Albanian samples. The vitamin C content of lemons ranged from 13.93 mg in Albanian lemons to 18.03 mg in imported lemons. These findings demonstrate the natural variability of ascorbic acid concentrations among citrus fruits and are generally consistent with literature reports indicating that oranges and citrons are among the richest citrus sources of vitamin C.

The results presented in Table 1 revealed a progressive decrease in vitamin C concentration during storage under both ambient (22°C) and refrigerated (4°C) conditions. However, the rate of degradation was significantly lower in refrigerated samples, confirming the well-established influence of temperature on ascorbic acid stability.

After 24 h of storage at ambient temperature, all fruit juices exhibited measurable vitamin C losses. The most pronounced decrease was observed in imported citron juice, where vitamin C concentration declined from 62.77 mg to 55.06 mg, corresponding to a reduction of approximately 12.3%. Albanian lemons also showed substantial degradation, with vitamin C content decreasing from 13.93 mg to 11.20 mg (19.6% loss). In contrast, Albanian oranges exhibited a relatively small reduction of approximately 4.1%, decreasing from 54.03 mg to 51.81 mg.

Extending storage to 48 h further accelerated vitamin C degradation in all samples stored at room temperature. Citron juice retained only 51.76 mg of vitamin C, representing a total loss of approximately 17.5% from its original concentration. Albanian lemons showed a decrease of approximately 25.5% after 48 h, while imported lemons retained approximately 90.3% of their initial vitamin C content. Similarly, Albanian and imported oranges lost approximately 5.5% and 7.5%, respectively, of their original vitamin C concentrations during the same period.

Storage at 4°C markedly improved vitamin C preservation across all analyzed samples. After 24 h of refrigeration, losses remained limited, ranging from approximately 1.4% in Albanian oranges to 8.8% in imported citron. Even after 48 h, refrigerated samples consistently exhibited higher vitamin C concentrations than their counterparts stored at ambient temperature.

For example, Albanian lemon juice retained 12.56 mg of vitamin C after 48 h under refrigeration compared with only 10.38 mg when stored at room temperature. Similarly, imported citron retained 55.06 mg after 48 h at 4°C, whereas only 51.76 mg remained after storage at ambient temperature. Albanian orange juice showed remarkable stability, maintaining 52.54 mg after 48 h of refrigerated storage, corresponding to a loss of only 2.8% relative to the fresh sample.

The superior retention observed under refrigeration can be attributed to the reduced oxidation rate of ascorbic acid at lower temperatures. Since vitamin C degradation occurs

primarily through oxidation to dehydroascorbic acid and subsequent irreversible decomposition, lowering storage temperature slows reaction kinetics and limits nutrient loss. These observations agree with previous studies demonstrating enhanced vitamin C stability in refrigerated fruit products.

Figures 3-9 further illustrate the degradation profiles of vitamin C under different storage conditions. In all fruits examined, the decline in vitamin C concentration followed a clear time-dependent pattern, with the greatest losses occurring under ambient storage conditions.

Among the investigated fruits, Albanian oranges exhibited the highest stability, maintaining more than 94% of their initial vitamin C content after 48 h at room temperature and more than 97% during refrigerated storage. Conversely, Albanian lemons demonstrated the greatest susceptibility to degradation, particularly under ambient conditions, where approximately one-quarter of the original vitamin C content was lost within 48 h.

Imported citron showed the highest absolute vitamin C concentration throughout the experiment but also experienced significant degradation, suggesting that fruits with higher initial ascorbic acid content may undergo substantial absolute losses during storage. Mandarins contained the lowest vitamin C concentrations overall; however, their relative retention patterns were similar to those observed in the other citrus fruits, with refrigeration providing consistently greater protection against degradation.

Table 1. Mean concentrations of Vitamin C on the 1st, 2nd and 3rd day (fresh, after 24 and 48 hours)

	Vitamin C 1st day, fresh (mg)	Vitamin C Ambient temp 22ºC) 2nd day, after 24 hours (mg)	Vitamin C Ambient temp (22ºC) 3rd day, after 48 hours (mg)	Vitamin C Refrigerated temp (4ºC) 2nd day, after 24 hours (mg)	Vitamin C Refrigerated temp (4ºC) 3rd day, after 48 hours (mg)
Lemon (Albanian)	13.93 ± 2.15	11.20 ± 2.18	10.38 ± 4.15	13.11 ± 2.58	12.56 ± 3.62
Lemon (Imported)	18.03 ± 4.11	16.86 ± 4.52	16.28 ± 3.29	17.44 ± 4.21	16.92 ± 4.27
Orange (Albanian)	54.03 ± 11.22	51.81 ± 9.28	51.06 ± 13.37	53.28 ± 14.23	52.54 ± 15.22
Orange (Imported)	46.64 ± 10.36	43.85 ± 10.13	43.15 ± 11.37	45.24 ± 11.05	44.54 ± 10.04
Qitro (Imported)	62.77 ± 14.48	55.06 ± 14.71	51.76 ± 12.28	57.26 ± 16.24	55.06 ± 12.74
Mandarina (Albanian)	9.62 ± 2.51	8.93 ± 2.07	8.70 ± 3.11	9.16 ± 2.54	8.93 ± 1.95
Mandarina (Imported)	3.97 ± 0.58	3.68 ± 0.42	3.59 ± 0.51	3.88 ± 0.33	3.78 ± 0.48

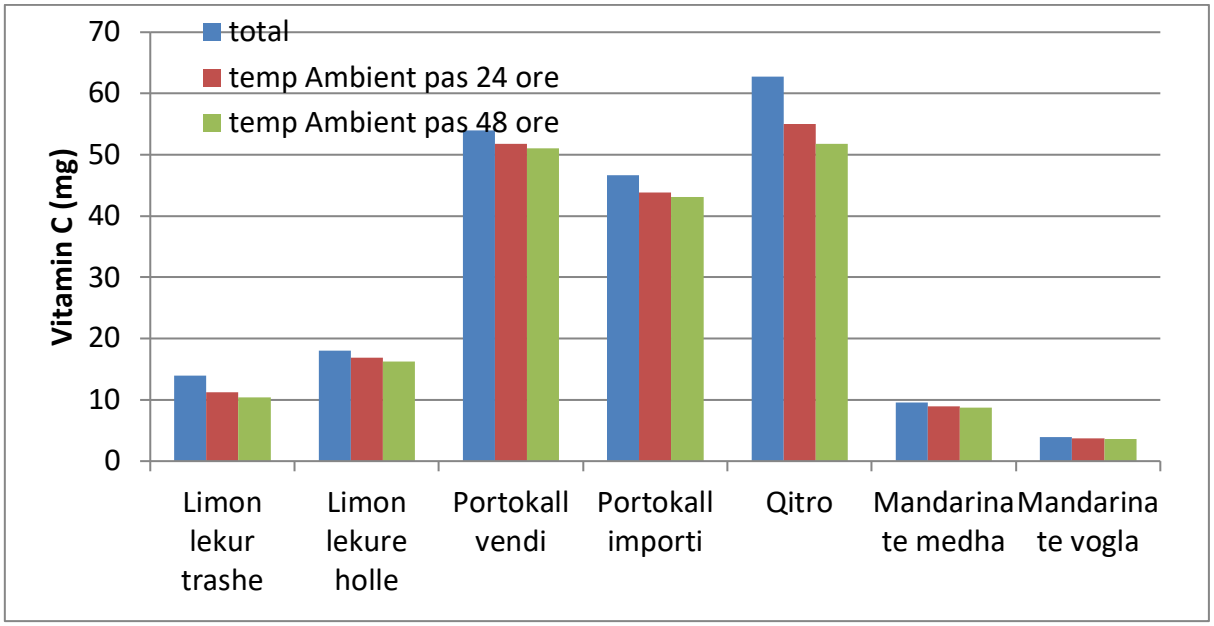

Figure 1. Concentrations of Vitamin C on the 1st, 2nd and 3rd day for ambient temperature

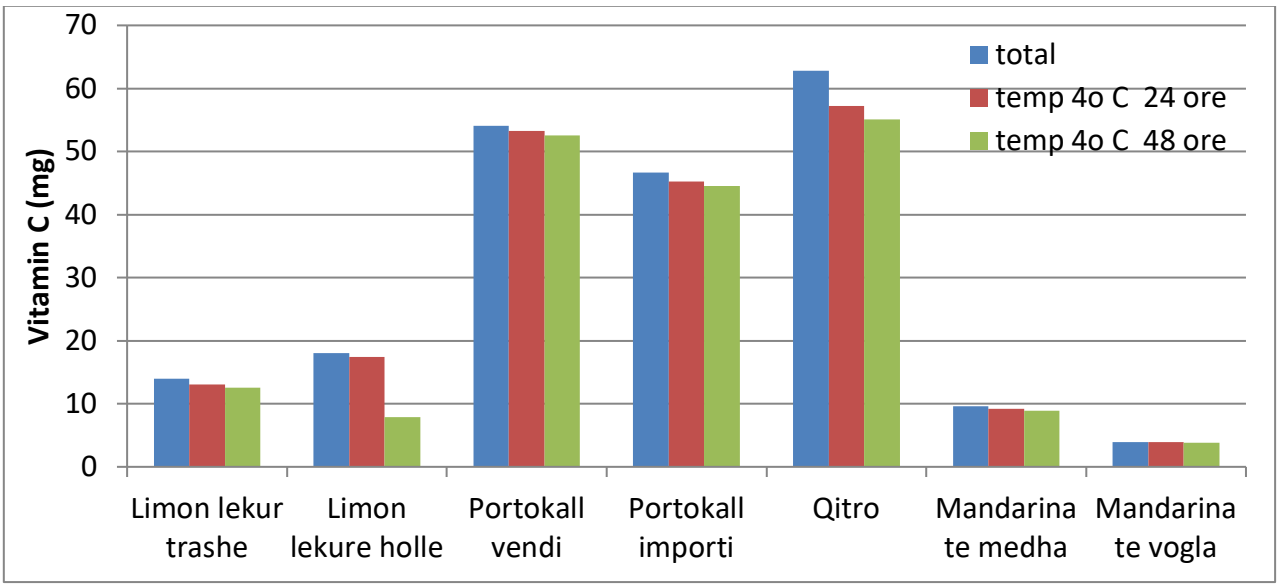

Figure 2. Concentrations of Vitamin C on the 1st, 2nd and 3rd day for refrigerated temperature

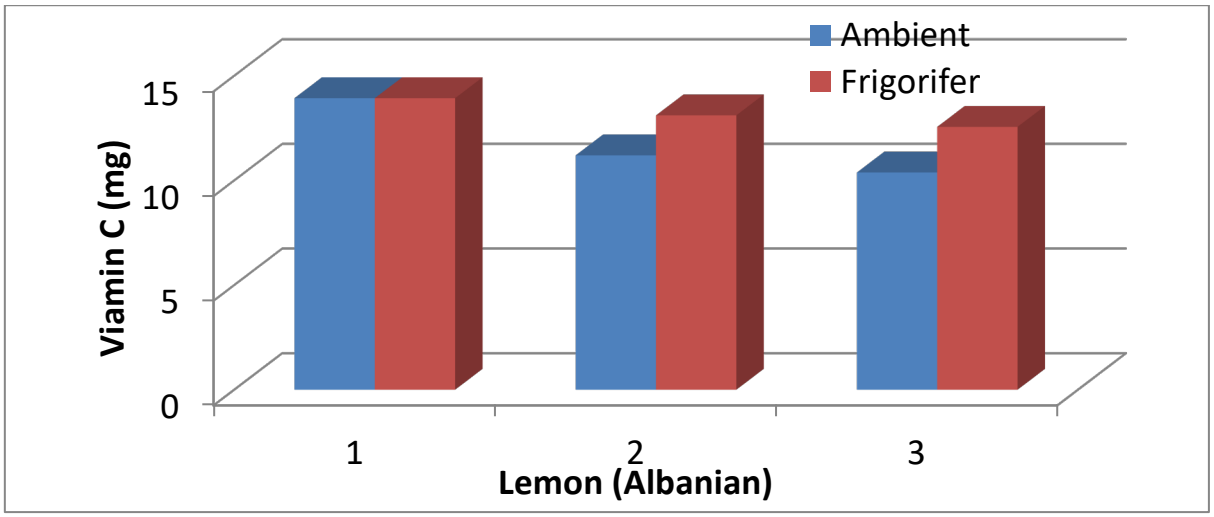

Figure 3. Concentrations of Vitamin C on the 1st, 2nd and 3rd day for lemon with Albanian origin

Figure 4. Concentrations of Vitamin C on the 1st, 2nd and 3rd day for lemon with imported origin

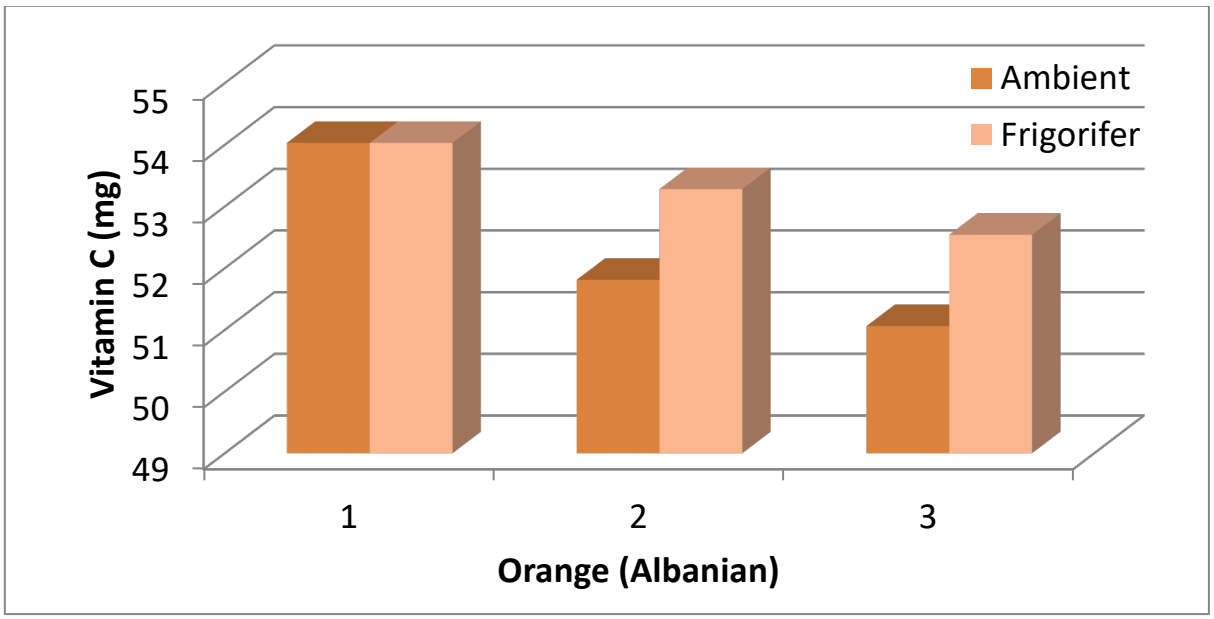

Figure 5. Concentrations of Vitamin C on the 1st, 2nd and 3rd day for orange with Albanian origin

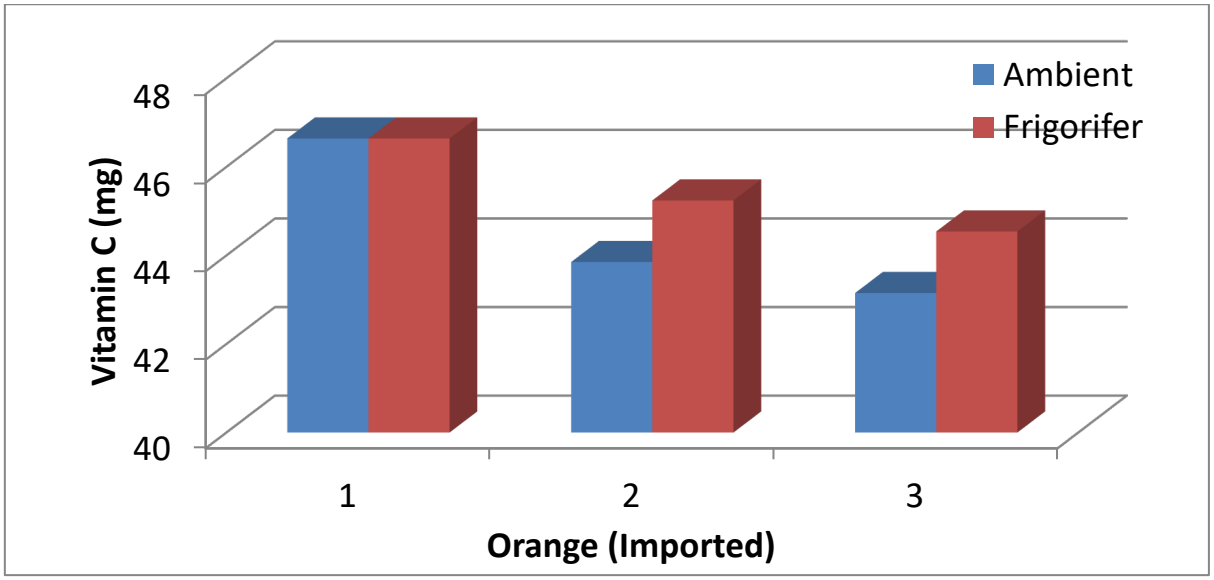

Figure 6. Concentrations of Vitamin C on the 1st, 2nd and 3rd day for imported orange

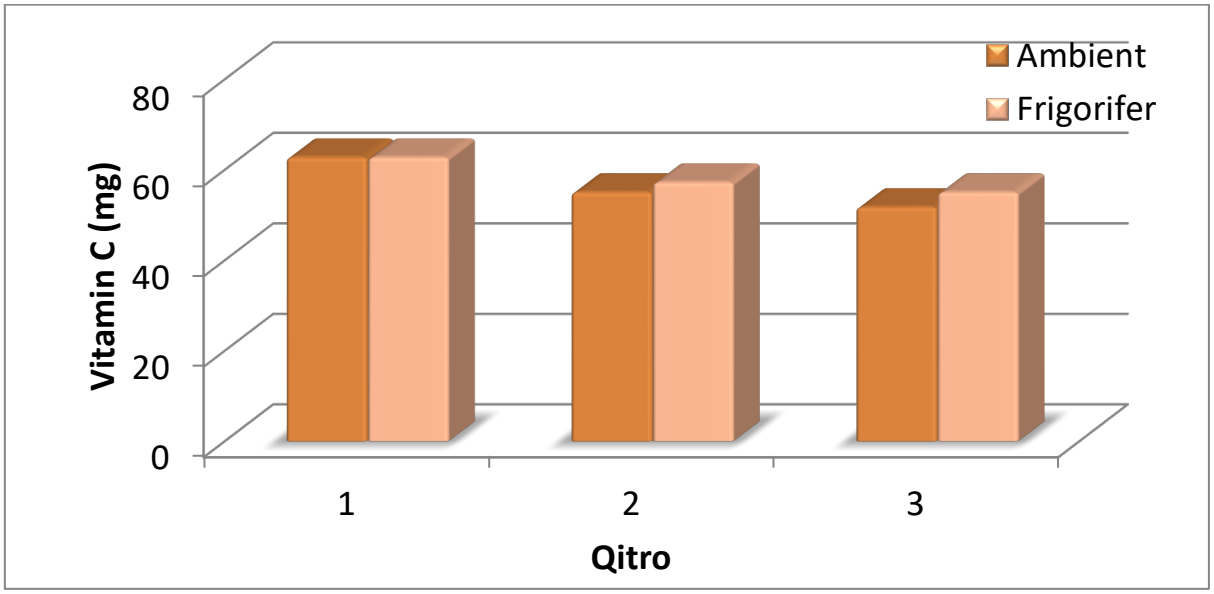

Figure 7. Concentrations of Vitamin C on the 1st, 2nd and 3rd day for imported citrus

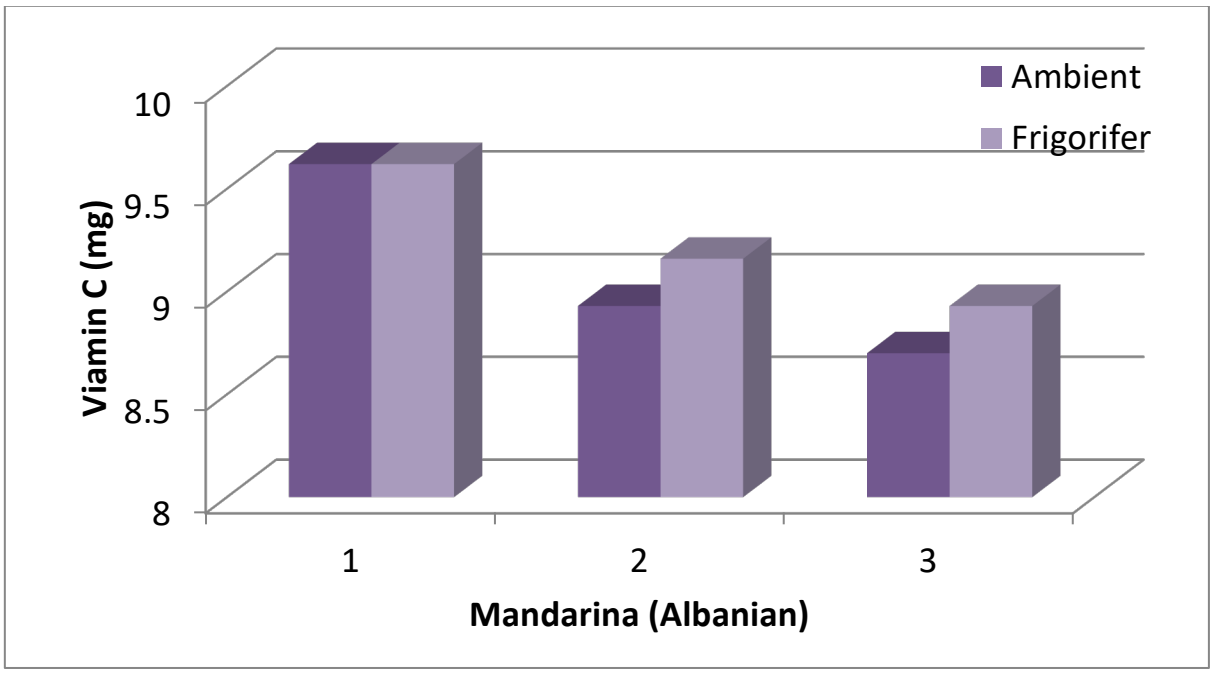

Figure 8. Concentrations of Vitamin C on the 1st, 2nd and 3rd day for Albanian mandarin

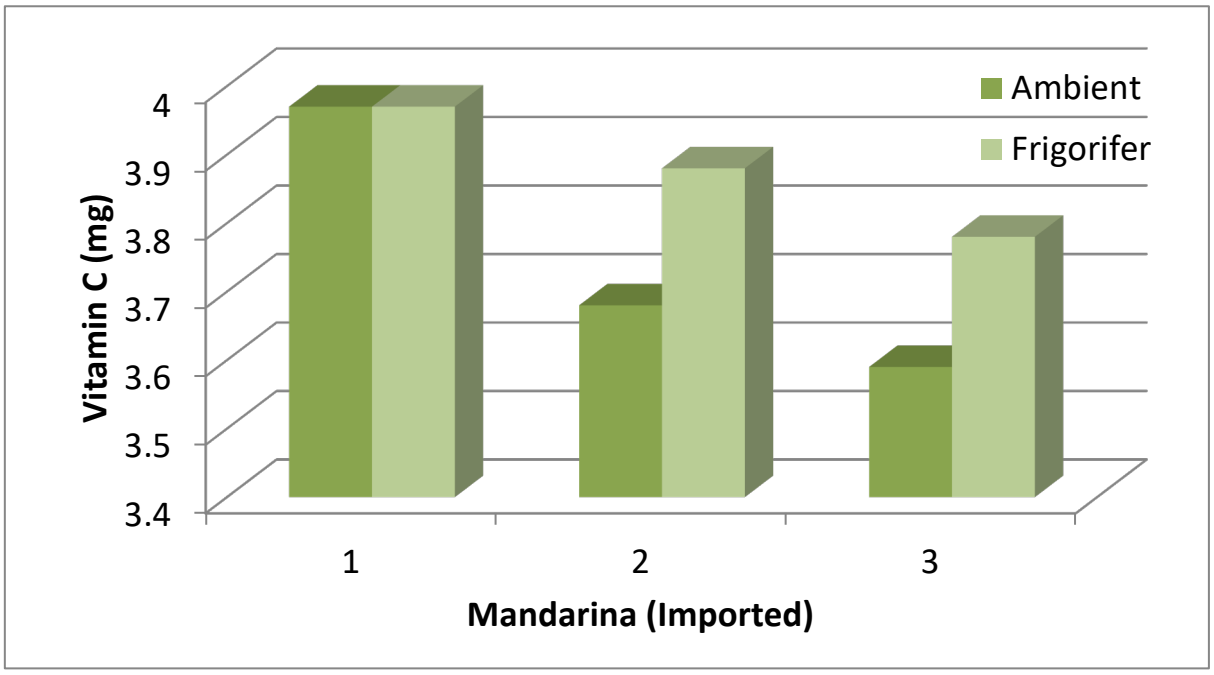

Figure 9. Concentrations of Vitamin C on the 1st, 2nd and 3rd day for imported mandarin

CONCLUSIONS

The present study demonstrated that iodometric titration is a simple, reliable, and cost-effective analytical method for the determination of vitamin C in citrus fruit juices. Significant differences in the initial vitamin C content were observed among the analyzed fruits, with imported citron exhibiting the highest concentration (62.77 mg), followed by Albanian orange (54.03 mg), imported orange (46.64 mg), imported lemon (18.03 mg), Albanian lemon (13.93 mg), Albanian mandarin (9.62 mg), and imported mandarin (3.97 mg). These results confirm the natural variability of ascorbic acid content among citrus species and fruit origins.

The study further demonstrated that both storage temperature and storage duration significantly influence vitamin C stability. A progressive reduction in ascorbic acid concentration was observed in all samples during the 48-hour storage period, with substantially greater losses occurring under ambient conditions (22°C) than under refrigerated storage (4°C). The degradation of vitamin C was evident after the first 24 hours and continued throughout the storage period, highlighting the sensitivity of ascorbic acid to environmental conditions.

Refrigerated storage proved to be considerably more effective in preserving vitamin C content, resulting in lower degradation rates and higher retention percentages across all

investigated fruit juices. Among the analyzed samples, Albanian orange juice showed the greatest stability during storage, whereas Albanian lemon juice exhibited the highest susceptibility to vitamin C degradation under ambient conditions.

The findings indicate that refrigeration is an effective strategy for minimizing vitamin C losses and maintaining the nutritional quality of citrus fruit juices during short-term storage. From a practical perspective, consumers should be encouraged to store freshly squeezed juices at refrigeration temperatures and consume them as soon as possible after preparation to maximize vitamin C intake. Furthermore, the results provide useful information for the food industry regarding the preservation of nutritional quality in fruit-based beverages and emphasize the importance of temperature control during storage and distribution.

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