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*Original article*

# Determination of Vitamin C Concentration in Various Fresh Orange and Lemon Samples from Janzour Region Using Volumetric Titration

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## ARTICLE INFO

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## ABSTRACT

*Vitamin C is an essential nutrient crucial for human health, playing a vital role in numerous physiological processes, including immune system support and collagen synthesis. Citrus fruits, such as oranges and lemons, are recognized as rich sources of this vitamin. The primary purpose of this study is to determine the vitamin C concentration in various fresh orange and lemon juices. The samples were randomly collected from the local market in Janzour. The study included 18 samples representing 6 different citrus varieties (3 samples per variety). Vitamin C concentration was statistically estimated using a titrimetric method with a standardized solution of 2,6-dichlorophenolindophenol (DCPIP) as the titrant. The obtained results revealed some kind of variations in vitamin C content among different citrus varieties and even within samples of the same variety. Abu Surah orange samples exhibited the highest vitamin C concentration (mean range: 38.9-41.6 mg/dl), while sweet orange samples had the lowest (mean range: 5.1- 8.5 mg/dl). Vitamin C concentrations in other varieties ranged as follows: Sour orange: 9.22-24.9 mg/dl, Mandarin: 9.0 -16.2 mg/dl, Tarocco: 28.2-33.3 mg/dl, and Lemon: 14.5-15.8 mg/dl. These results showed evidently that vitamin C levels were varied even within the same sample type. Such variations would draw attention to the importance of choosing varieties when searching for food sources of this vital nutrient.*

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## INTRODUCTION

Despite their relatively minute required quantities, vitamins are organic compounds essential for various physiological functions. There has been a common belief that insufficient intake of these micronutrients may cause a myriad of diseases. On average, an adult human being requires at least thirteen types of vitamins broadly categorized into two groups based on their solubility. (i) Water-soluble vitamins including C and the B-complex vitamins. (ii) Fat-soluble consisting of A, D, E, and K vitamins. It has been evidenced that each vitamin plays a distinct role in supporting vital biological processes such as growth, metabolism, and immune functionality. However, inadequate vitamin intakes may bring about various negative health outcomes, with severity depending on the specific vitamin deficiency and its extent [1]. Indeed, Ascorbic acid, discovered by Albert Szent-Gyorgyi in (1928) is commonly known as vitamin C. This vitamin is biologically essential for humans and exists in two primary forms, namely; ascorbic acid (the reduced form) and dehydroascorbic acid (the oxidized form) [2]. It is a crucial nutrient for human health due to its potent physiological antioxidant properties. Besides, it is thought that vitamin C contributes significantly to body's defense against oxidative stress by protecting cellular components from damage caused by reactive oxygen species (ROS) [3]. This protection is attributed to its exceptional ability to donate electrons, effectively neutralizing ROS and halting the chain reaction of lipid peroxidation. Furthermore, a large and growing body of literature has showed that ascorbic acid exhibits a unique regenerative capacity because it reduces oxidative stress, and effectively restores the antioxidant activity of other crucial antioxidants within the body, such as alpha-tocopherol (vitamin E) [4,5]. Of course, such a synergistic interaction significantly enhances the body's overall antioxidant defense system, contributing to the mitigation of oxidative damage and promoting overall cellular health [6].

In addition to its diverse biological functions, vitamin C is an essential nutrient for humans due to their inability to synthesize it endogenously. Indeed, such an inability is attributed to the lack of the enzyme L-gulonolactone oxidase (GULO) [7] which catalyzes the final step in vitamin C biosynthesis in most other mammals. Resultantly, humans rely on dietary sources to meet their vitamin C requirements. In the same way, Vitamin C plays a critical role in various metabolic processes because it functions as a co-factor for numerous enzymatic reactions. Notably, it functions as an electron donor for enzymes involved in collagen synthesis, carnitine biosynthesis, and neurotransmitter production [8]. Fresh fruits and vegetables, especially citrus fruits, serve as significant sources of vitamin C. Dietary supplements also contribute to vitamin C intake [9]. The bioavailability of vitamin C from food and supplements is estimated to be approximately 19%. Absorption primarily occurs in the small intestine, followed by transportation through the bloodstream to various tissues and organs. Furthermore, it can be incorporated into fruit-containing products to fortify their vitamin content and function as an antioxidant, mitigating enzymatic browning, nutrient degradation, and flavor deterioration [10].

Chemically speaking, vitamin C, with a molecular formula of $C_6H_8O_6$ and a molar mass of 176.12 g/mole , has a density of 1.65 g/cm$^3$ [11]. In its pure form, vitamin C appears as an odorless, white crystalline powder with a melting point ranging from 190-192°C.

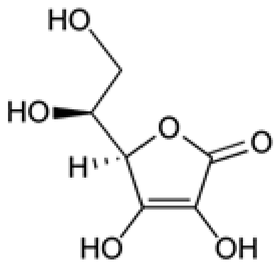


***Figure 1. The Structural formula of vitamin C***

However, it is insoluble in non-polar solvents such as benzene, chloroform, and ether [12]. While lacking a carboxyl group (-COOH) in its structure, ascorbic acid derives its acidic nature from the presence of a dienol group [13]. Although dehydroascorbic acid lacks the "enediol" group responsible for the antioxidant activity of ascorbic acid, it exhibits notable biological activity. Ascorbic acid (vitamin C) is categorized as a compound with limited stability, rendering it highly susceptible to degradation during processing and storage. While relatively stable in its solid state, it demonstrates instability in aqueous solutions. The degradation of ascorbic acid is significantly influenced by environmental factors such as pH. It exhibits higher stability within a pH range 2-5. While dehydroascorbic acid lacks the "enediol" group responsible for the antioxidant activity of ascorbic acid, it exhibits significant biological activity [14]. Additionally, ascorbic acid is highly sensitive to temperature variations, with heating and cooling processes, including cooking, leading to its decomposition. Therefore, it is not advisable to rely on cooked foods as a primary source of vitamin C [15].

Undoubtedly, Vitamin C is an important constituent in numerous vital biological processes for its diverse biochemical and biological effects. This significance stems from its unique properties, which can be categorized as metabolic and cellular properties. Previous investigations have illustrated that vitamin C is an essential factor in various metabolic and enzymatic processes within cells. In other words, evidence suggests that vitamin C functions as a co-factor for peptidyl glycine alpha-amidating monooxygenase, an enzyme crucial for the biosynthesis of vasopressin [16]. Similarly, it plays a critical role in synthesizing essential neurotransmitters like dopamine, norepinephrine, and epinephrine in the central nervous system and adrenal glands [17]. Equally important, Vitamin C is a potent antioxidant, contributing to protecting cells from free radical damage. It enhances the activity of immune cells such as neutrophils, stimulating their phagocytosis and chemotaxis abilities. Complementary to the above stated functions, it contributes to eliminating microbes and exhibits antiviral, anti-inflammatory effects [18]. In brief, Vitamin C is a vital element for various bodily functions, from its role in metabolic processes to its support of the immune system. This study aims to estimate the amount of vitamin C in samples of fresh oranges and lemons from local sources, in order to evaluate their quality. The results will be compared with the results of previous studies and standard specifications.

## MATERIAL AND METHODS

### *Equipment*

Analytical balance, Centrifuge.

### *Materials*

2, 6-dichlorophenol indophenol, glacial acetic acid, distilled water.

### *Sample Collection and Preparation*

The chosen citrus samples, including: Sour orange, sweet orange, Abu surah orange, Mandarin orange, Tarocco orange, and Lemon, were collected from the local market in Janzour. Each type was individually juiced, filtered, and then centrifuged to obtain a clear supernatant containing vitamin C.

### *Preparation of Standard Solution*

A standard solution of 2, 6-dichlorophenolindophenol (DCPIP) dye was prepared by dissolving 0.005 mg in 100 ml of distilled water to obtain a solution with a concentration of 0.005 mg/dl for use in titration.

### *Titration for Vitamin C Quantification*

Vitamin C content was determined by titration using 2,6-dichlorophenolindophenol (DCPIP) as the titrant. A 5 mL aliquot of the liquid sample (fresh juice) was pipetted into a conical flask. 1 mL of glacial acetic acid was added immediately prior to titration to ensure an acidic environment (pH ≈ 4). Maintaining a pH of approximately 4 ensures optimal stability of ascorbic acid, facilitates complete reduction of the dye, and ensures a quantitative reaction [14, 19]. The sample was titrated against a standard dye solution via a slow titration process. The standard solution was added incrementally to the sample until a faint pink color persisted, indicating the endpoint of the titration. The burette reading was recorded to determine the volume of standard solution consumed. To ensure accuracy, the titration was performed in triplicate for each sample, and the volume of standard solution consumed was recorded for each replicate.

## RESULTS

The concentration of ascorbic acid (vitamin C) in all samples was determined by titration against a 0.005 mg/dl solution of 2, 6-dichlorophenolindophenol (DCPIP) and was expressed in mg/dl. The results, as shown in Figure 2 indicate some kind of inconsistency regarding the vitamin C concentration based on the samples variation. In other words, the obtained statistics would make it apparent that the average vitamin C concentration in the analyzed samples ranged from (5.1 to 41.6 mg/ dl). In a more obvious sense, the highest concentration of vitamin C was observed in the Abu Surah orange sample (41.6 mg/ dl). Conversely, the lowest vitamin C concentration was found in the Sweet orange sample (5.1 mg/ dl).

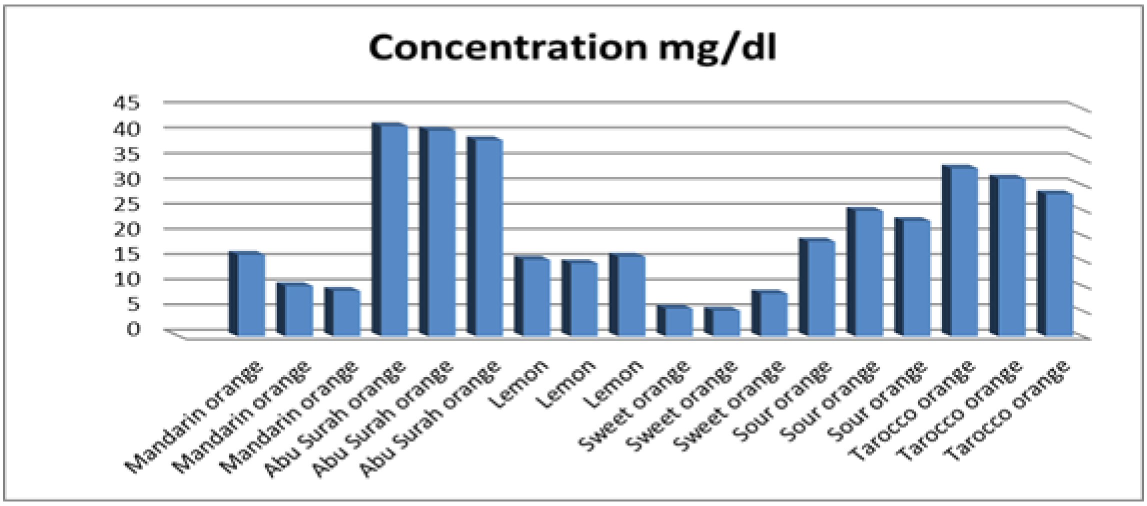


***Figure 2. The Results of Vitamin C estimation in the Selected Samples***

## DISCUSSION

There has been a common hypothesis that oranges are renowned for their significant vitamin C content, a potent natural antioxidant, which grants them immense nutritional importance. However, few comprehensive studies were conducted to investigate the vitamin C content in locally-grown orange species. This study produced scientific evidence which corroborate the undeniable belief that vitamin C levels might be different with regard to orange species. Hence, contrary to expectations, this study found a significant difference in terms of vitamin C concentration in orange species.

From the data in Figure 2, it is apparent that the comparative analysis presents undeniable evidence that vitamin C concentrations were varied among the local orange species. A comparison of the results reveals that vitamin C concentration in Abu surah orange was the highest. On the contrary, the sweet orange exhibited the lowest concentration. The present findings seem to be consistent with a study carried out in the northern coast of Syria. The final measurements of that study offered evidenced data that vitamin C content in various orange and lemon were varied. The reported vitamin C concentration range was between 18 and 45 mg/ dl [2]. Surprisingly, the results indicated differences in vitamin C concentration even within samples of the same variety of orange fruit. These differences can be attributed to certain agronomic conditions such as fruit development stage, farming practices, soil management, and sample sources. Besides, more possible influences related to growth stage, irrigation, fertilization, pest management, pH, nutrient availability, storage temperature, and source differences might play influential roles in determining vitamin C content. Yet, understanding these agronomic factors is critical to maintaining and improving the nutritional quality of citrus fruits for consumers. In this study, the vitamin C concentration in Abu Surah orange samples ranged from 38.9 to 41.6 mg/dl. These values are notably higher than those reported in a 2022 study conducted in Tripoli (35.2 mg/dl) [20]. Though, the findings might be more comparable to a 2021 study in Tajoura, which found a range of 33 to 64 mg/dl [21]. Nonetheless, the aforementioned values are considerably lower than the 120 mg/dl reported for Abu surah oranges in a study conducted in Al Khums, which investigated vitamin C levels in various orange and lemon varieties [1].

Regarding the sweet orange samples, the vitamin C concentration ranged from 5.1 to 8.5 mg/ dl. These results are lower than those reported in a 2021 Tajoura study (28 to 40 mg/dl) .[21], and significantly lower compared to studies conducted in Al khums in 2017 (90 mg/ dl) [22], and Misrata (93.35 - 117.13 mg/ dl) [10].

Returning to the aim posed at the beginning of this study, it is now possible to state that current study compared the quantities of vitamin C (in milligrams) present in 100 ml of fresh juice samples and revealed that all samples contained adequate levels of vitamin C. One of the more significant findings to emerge from this study is that the obtained results align with the recommended dietary allowances (RDAs) for vitamin C established by the Food and Nutrition Board, demonstrating the reliability and accuracy of the employed methodology [6].

## CONCLUSION

This study revealed variations in vitamin C content among different orange and lemon varieties from Janzour region. Using titration method using 2,6-dichlorophenolindophenol (DCPIP) solution proved effective in quantifying these differences., it was evidently found that Abu surah orange samples recorded the highest vitamin C concentration (38.9 to 41.6 mg/dl), followed by Tarocco, Sour, Mandarin, and Lemon varieties. In contrast, Sweet orange samples contained the lowest vitamin C content (5.1 to 8.5 mg/dl). These findings highlight the variability in vitamin C levels even within the same fruit type, emphasizing the importance of varietal selection when seeking dietary sources of this vital nutrient.

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# تقدير فيتامين C في عينات مختلفة من البرتقال و الليمون الطازج بمنطقة جنزور باستخدام طريقة المعايرة الحجمية

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**المستخلص**

يُعتبر فيتامين C عنصرًا غذائيًا أساسيًا بالغ الأهمية لصحة الإنسان، حيث يلعب دورًا حيويًا في العديد من العمليات الفسيولوجية، بما في ذلك دعم جهاز المناعة وتخليق الكولاجين. تشتهر الحمضيات، مثل البرتقال والليمون، بكونها مصادر غنية بهذا. لذا، هدفت هذه الدراسة إلى تقدير تركيز فيتامين C في عينات مختلفة من عصائر البرتقال والليمون الطازجة والتي تم جمعها عشوائيًا من السوق المحلي بمنطقة جنزور. شملت الدراسة 18 عينة من 6 أنواع مختلفة من الحمضيات (3 عينات من كل نوع). تم تقدير تركيز فيتامين C باستخدام طريقة المعايرة الحجمية بمحلول قياسي من صبغة 2,6- ثنائي كلورو فينول إندوفينول .أظهرت النتائج التي تم الحصول عليها من خلال هذه الدراسة تباينًا في تركيز فيتامين C بين أنواع الحمضيات المختلفة، بل وحتى بين عينات من نفس النوع. سجلت عينات برتقال أبو سرة أعلى تركيز لفيتامين C بمعدل (38.9-41.6 ملجم/ دل)، بينما سجلت عينات البرتقال الحلو أقل تركيز بمعدل (5.1-8.5 ملجم/ دل). تراوحت تراكيز فيتامين C في الأنواع الأخرى كالآتي: البرتقال الحامض بمعدل (9.22-24.9 ملجم/ دل)، اليوسفي بمعدل ( 9-16.2 ملجم/ دل)، التاروكي بمعدل (28.2-33.3 ملجم/ دل)،و الليمون بمعدل (14.5-15.8 ملجم/ دل) و من خلال هذه النتائج تم تسليط الضوء على التباين في مستويات فيتامين C حتى داخل نفس نوع العينة، مما يؤكد أهمية اختيار الأصناف عند البحث عن مصادر غذائية لهذه المغذيات الحيوية .

**الكلمات المفتاحية:** الحمضيات, البرتقال,الليمون, فيتامين C, 2,6-ثنائي كلوروفينولاندوفينول, المعايرة الحجمية.