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Determination of ascorbic acid levels in oranges under different storage conditions using Redox Iodometric method

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Abstract

Ascorbic acid is one of the most important vitamins to the human body due to its potentially protective role as an antioxidant. Oranges by far the most important citrus species are an excellent source of ascorbic acid. The ascorbic acid in three varieties of Ghanaian local oranges was estimated after storage under four different storage conditions. The storage conditions were deep freezing, dark cupboard, open shelf and in the sun. The redox titrimetric method using potassium iodide as the titrant and starch as an indicator was employed to evaluate the ascorbic acid levels in the orange samples after two, four and six days. The result shows a decrease in the ascorbic acid content of oranges over the storage periods. The decrease was expressed as a percentage loss of ascorbic acid originally in oranges. Less ascorbic acid was lost with the refrigerated orange samples compared to the other storage conditions. A huge loss of ascorbic acid was however lost with the samples that were exposed to the sun.

Keywords: Ascorbic acid; Local orange; Titration; Storage conditions; Time

1. Introduction

Ascorbic acid; commonly known as vitamin C; is a vital nutrient that can be acquired from various sources such as fruits; vegetables; and multivitamin supplements [1-4]. It plays a crucial role in numerous physiological functions within the human body. Ascorbic acid is a six-carbon lactone; as depicted in Figure 1; and is naturally synthesized from glucose by many animal species [5-7].

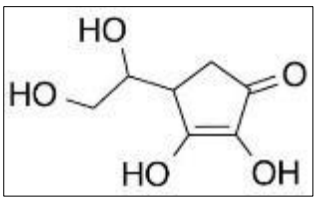

Figure 1 Ascorbic Acid

While certain mammals can synthesize ascorbic acid in their liver and birds and reptiles produce it in their kidneys; there are several species; including humans; non-human primates; guinea pigs; Indian fruit bats; and Nepalese red-vented bulbuls; that cannot produce ascorbic acid internally [8-9]. In humans and primates; the inability to synthesize ascorbic acid stems from a significant mutation in the gene responsible for encoding the terminal enzyme in the biosynthetic pathway; called l-gulonolactone oxidase [10-12]. This mutation results in the absence of the enzyme;

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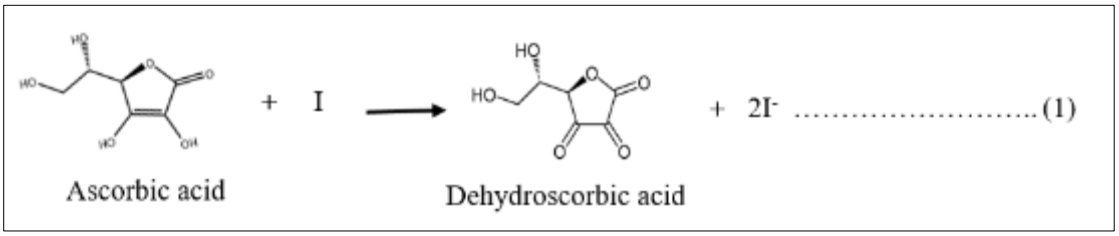
leading to the complete inability to produce the protein. When there is an inadequate intake of ascorbic acid through the diet; humans experience a severe deficiency condition known as scurvy; which can be life-threatening [13-15]. Ascorbic acid plays a pivotal role in preventing scurvy. Scurvy is a disease characterized by symptoms such as muscle weakness; swollen and bleeding gums; tooth loss; bleeding under the skin; fatigue; and depression. Adequate intake of vitamin C is crucial to prevent and treat scurvy. Ascorbic acid serves as an electron donor; functioning as a reducing agent or antioxidant [16-19]. All of its biochemical and molecular roles can likely be attributed to this particular function. Ascorbic acid; acting as an antioxidant; has the potential to protect against various health complications. It plays a role in regulating blood pressure and reducing cholesterol levels [20-22]. Furthermore; it acts as a scavenger of free radicals; which are associated with accelerated ageing and various adverse effects on health [23-24]. Ascorbic acid intake can be particularly beneficial for smokers; as they are more susceptible to oxidative stress and cellular damage; which can deplete vitamin C levels [25-27]. Overall; ascorbic acid's antioxidant properties; its ability to regulate blood pressure; its role in reducing cholesterol levels; and its involvement in scavenging free radicals make it a valuable nutrient for maintaining overall health and preventing various diseases and complications.

Oranges; which are rich in ascorbic acid; are considered an exceptional source of this essential nutrient [28-30]. They are widely recognized as the most significant citrus species; encompassing both popular dessert oranges and oranges used for juice extraction. In Ghana; there is a diverse range of local orange varieties; each unique to the specific towns where they are cultivated. Some of these varieties include Obuasi; Asians; Achiasi; Shama; Nkwanta; Anomabu; and Kwesi Nyarko oranges. The local oranges mentioned are commonly found in local markets and are often sold in large piles on the ground or in baskets. These oranges are highly valued for their deliciously sweet and juicy nature; making them popular for consuming fresh. However; it is important to note that the ascorbic acid potency of citrus products can diminish over time due to two significant factors: temperature and storage duration.

Various analytical methods have been documented for quantifying the concentration of ascorbic acid in fruits. These methods include titrimetry; spectrometry; high-performance liquid chromatography (HPLC); and amperometry [31-34]. Among these techniques; titration is a commonly employed method for the quantitative determination of various nutrients. In the case of ascorbic acid; a redox iodometric titration is often utilized [35-38]. This method involves the reaction between iodine and ascorbic acid; resulting in the oxidation of ascorbic acid to dehydroascorbic acid; which is a colourless product. Simultaneously; iodine is reduced to iodide ions according to Equation (1). Through this titration; the amount of ascorbic acid present in an unknown sample can be determined.



When iodine is introduced in the presence of both ascorbic acid and starch; it has a higher affinity for reacting with ascorbic acid rather than starch. This preference results in the formation of colourless products; namely dehydroascorbic acid and iodide ions. As a consequence; the iodine does not react with the starch to produce its characteristic dark blue colour. This phenomenon allows for the selective determination of ascorbic acid in the presence of starch by observing the absence of a colour change. As the titration progresses; the ascorbic acid present in the sample is gradually oxidized by iodine. Once all the ascorbic acid has been completely consumed; any excess iodine remaining in the solution reacts with the starch indicator. This reaction forms a dark blue product known as the starch-iodine complex. The appearance of this dark blue colour indicates the endpoint of the titration. Thus; by adding iodine drop by drop during the titration process; the ascorbic acid is consumed first until it is fully utilized; resulting in a colourless solution. Finally; when all the ascorbic acid has reacted with iodine; the remaining excess iodine reacts with the starch indicator; leading to the formation of the dark blue starch-iodine complex; marking the endpoint of the titration.

Investigating the optimal storage methods for preserving the ascorbic acid content of oranges over extended periods is crucial. This research is particularly relevant for various forms of oranges; including ascorbic acid tablets; fresh or packaged fruit juices; as well as solid fruits and vegetables. By studying the most effective ways to store oranges; it becomes possible to maintain the fruit's ascorbic acid content for longer durations; ensuring its nutritional value is preserved. The main objective of this study is to investigate how different storage environments and ageing factors affect the concentration of ascorbic acid in three groups of local orange samples. The study aims to provide insight into the preservation of ascorbic acid levels in oranges over time with on focus on investigating the impact of various storage

environments and ageing factors on the ascorbic acid levels. The purpose of selecting these different storage environments is to simulate real-life scenarios and evaluate their impact on ascorbic acid preservation.

2. Materials and Methods

Potassium Iodide (99 %) Central Drug house LtD; India; iodine (99.5 %); Potato starch (moisture Ld 20%); Shanghai Bichain Industrial Chemical; China; Iodine crystals (99.8%); Chem Lab Supplies; South Africa; distilled water (Resistivity: >10MΩ.cm); Source Chemical; UK.

2.1. Sampling of Fruits

The researchers employed a purposive sampling method to gather the necessary samples for the study [39-41]. This particular sampling technique was chosen because the research focused specifically on local orange samples that shared similarities in terms of colour and size. By using purposive sampling; the researchers were able to selectively select samples that met the specific criteria related to the local oranges under investigation. For the study; three types of orange fruits were chosen: those grown in Obuasi; Ayensudo; and Nkuanta. These particular orange varieties were selected due to their availability during the research period. The selection of fruits was based on their similarity in terms of colour and size. To ensure a comprehensive analysis; four groups of samples were collected for each type of orange. Each group comprised nine fruits; resulting in a total of four sets of samples for each orange variety. In total; thirty- six (36) oranges were collected for each type; resulting in a combined sample size of one hundred and eight (108) oranges. To assess the impact of storage conditions on the ascorbic acid levels; four different environments were established for the four groups of orange samples. These storage environments included a refrigerator; a cupboard; an open shelf; and exposure to direct sunlight. The orange sample groups were subjected to these respective storage conditions for six days; with measurements taken at two-day intervals during the storage period. By monitoring the ascorbic acid levels over time in each storage condition; the study aims to evaluate the effect of different environments on the preservation of ascorbic acid in the oranges.

2.2. Preparation of Potassium Iodide and Starch Solution

To determine the ascorbic acid content in the fruits; a titration process was employed using a 0.005 mol/L iodine solution and a 0.5% starch solution as an indicator [42]. The preparation of the iodine solution involved weighing 2 g of potassium iodide and 0.3 g of iodine crystals. These substances were then transferred into a 100 mL conical flask that already contained 50 mL of distilled water. The dissolved mixture was transferred into a 1L volumetric flask and then diluted with additional solvent until the volume reached the mark on the flask. The resulting solution served as the 0.005 mol/L iodine solution for the titration process. Similarly; for the preparation of the 0.5% starch solution; 0.25 g of starch powder was accurately weighed and placed in a 100 mL beaker. To this; 50 mL of distilled water was added. The beaker containing the mixture was then heated on a hot plate at 80°C for 5 minutes. This heating process helped to obtain a clear starch solution; ensuring the starch was fully dissolved in the water.

2.3. Standardization of the Iodine solution with the Ascorbic Acid Standard Solution

To establish a standard for measuring the ascorbic acid levels in the orange samples; a 0.250g quantity of ascorbic acid was precisely weighed and transferred into a 500 mL volumetric flask. Distilled water was then added to the flask to reach the desired volume; resulting in a standardized ascorbic acid solution. To standardize the iodine solution; 20 mL of the standardized ascorbic acid solution was transferred using a 25 mL pipette into an Erlenmeyer flask. Next; 1 mL of the 0.5% starch solution was added to the flask in the form of drops. A burette was filled with the iodine solution up to the 50 mL mark. During the titration process; the flask containing the standard ascorbic acid solution was placed on a hot plate magnetic stirrer to ensure proper mixing. The iodine solution was slowly added from the burette to the flask; with constant stirring. The endpoint of the titration was indicated by the appearance of a blue colour; signifying the completion of the reaction between iodine and ascorbic acid. To ensure accuracy; the titration process was repeated twice more; resulting in a total of three good measurements of the standardized ascorbic acid solution.

2.4. The Titration Process

To analyze the ascorbic acid levels in the various orange samples; the juice from each sample was extracted and filtered into a 500 mL beaker to prevent any clogging of the pipette. The filtered juice was then transferred into a 250 mL conical flask for subsequent titration analysis. For each titration analysis; a 20 mL portion of the orange juice was transferred using a pipette into an Erlenmeyer flask. 1 mL of the 0.5% starch solution was added to the flask in the form of drops. The titration process involved adding the potassium iodide solution from a burette; while continuously stirring the mixture. To ensure accuracy; the titration process was repeated twice more; resulting in a total of three measurements for each orange sample. The endpoint of the titration was indicated by the appearance of a blue colour; similar to the

previous standardization process. The titration analysis was performed on orange samples without storage and on those under storage conditions for two; four; and six days to assess the effect of storage duration on the ascorbic acid levels. The ascorbic acid level was calculated using the following equations (2); equation (3) and equation (4)

$$\text{mg of ascorbic acid in1mL of solution} = \frac{\text{mg of ascorbic acid (standard value)}}{100\text{mL}} \text{x 1m} \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

$$\text{mg of ascorbic acid oxidized by 1mL iodine solution} = \frac{\text{mg of ascorbic acid in flask}}{1\text{Average volume (mL) of iodine solution00mL}} \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

$$\text{mg of ascorbic acid orange juice} = \frac{20\text{mL x mg of ascorbic acid}}{1\text{mL of orange juice}} \text{x 1m} \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

3. Results and Discussion

Table 1 presents the findings concerning the levels of ascorbic acid in standard ascorbic acid; as well as the ascorbic acid levels in local orange samples before being subjected to different storage conditions. The ascorbic acid content of Ayensudo oranges and Nkwanta oranges was slightly higher than that found in the standard ascorbic acid. On the other hand; Obuasi oranges exhibited a lower ascorbic acid level compared to the standard.

Table 1 Comparison of experimental and standard ascorbic acid values

Types of Local Oranges	**Observed Standard for Ascorbic acid (mg/100mL)**	**Experimental Ascorbic acid (mg/100mL) in oranges under no storage condition**
Obuasi	74.67	72.43
Ayensudo	74.67	74.71
Nkwanta	74.67	74.32

Table 2 displays the findings regarding the ascorbic acid levels of Oboasi orange samples following exposure to different storage conditions for durations of two; four; and six days. It was observed that the ascorbic acid level generally decreased as the number of days increased for all storage conditions. The reduction in ascorbic acid content was minimal for the orange samples stored in the refrigerator; while it was substantial for the orange samples exposed to sunlight after six days.

Table 2 Oboasi Oranges under different storage conditions with increasing time

Oboasi Oranges Storage condition	**Days**	**Amount of Ascorbic acid (mg/100mL)**	**Ascorbic acid loss per storage period (mg/100mL)**
Shelf	0	72.43	**-**
	2	70.61	1.82
	4	68.24	4.19
	6	65.12	7.31
Cupboard	0	72.43	**-**
	2	69.17	3.26
	4	66.21	6.22
	6	62.14	10.29
Refrigerator	0	72.43	**-**
	2	71.01	1.42

	4	69.32	3.11
	6	68.67	3.76
Sunlight	0	72.43	-
	2	64.13	8.30
	4	57.27	15.16
	6	55.14	17.29

These trends can be seen in Figure 2. The orange samples stored in the refrigerator and on the shelf exhibited a close loss in ascorbic acid of 1.42 mg/100mL for refrigerator storage and 1.82 mg/100mL for shelf storage after 2 days. However; on the fourth and sixth day; the oranges stored on the shelf showed a lower ascorbic acid level compared to those stored in the refrigerator. The ascorbic acid level in oranges stored in the cupboard was slightly lower than; that of oranges stored on the shelf. A large decrease in ascorbic acid content was observed for oranges exposed to sunlight.

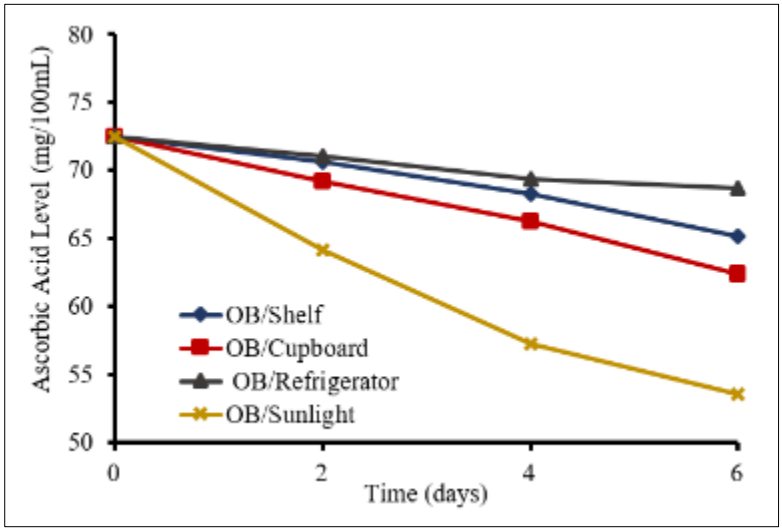


Figure 2 Assessment of ascorbic acid level in Oboasi (OB) oranges under different storage conditions with increasing time

Table 3 presents the findings regarding the ascorbic acid levels of Ayensudo orange samples following exposure to different storage conditions for durations of two; four; and six days. It was observed that the ascorbic acid level generally decreased as the number of days increased for all storage conditions. The orange samples stored in the refrigerator exhibited the least reduction in ascorbic acid content; while the orange samples exposed to sunlight showed the highest reduction in ascorbic acid after six days.

Table 3 Ayensodo Oranges under different storage conditions with increasing time

Ayensudo Oranges Storage condition	**Days**	**Amount of Ascorbic acid (mg/100mL)**	**Ascorbic acid loss per storage period**
Shelf	0	74.71	-
	2	72.62	2.09
	4	69.14	5.57
	6	66.82	7.89
Cupboard	0	74.71	-
	2	71.27	3.44

	4	67.75	6.96
	6	64.84	9.87
Refrigerator	0	74.71	-
	2	73.17	1.54
	4	71.16	3.55
	6	69.82	4.89
Sunlight	0	74.71	-
	2	70.13	4.58
	4	64.37	10.58
	6	57.52	17.19

These observations are depicted in Figure 3. The orange samples exposed to different storage conditions exhibited a close decrease in ascorbic acid level; with the highest reduction observed for those exposed to sunlight after 2 days. The oranges stored in the refrigerator showed the least loss in ascorbic acid content. Oranges stored on the shelf had a lower reduction in ascorbic acid level compared to those stored in the cupboard. Notably; there was a noticeable decline in ascorbic acid content for oranges exposed to sunlight on the fourth and sixth days.

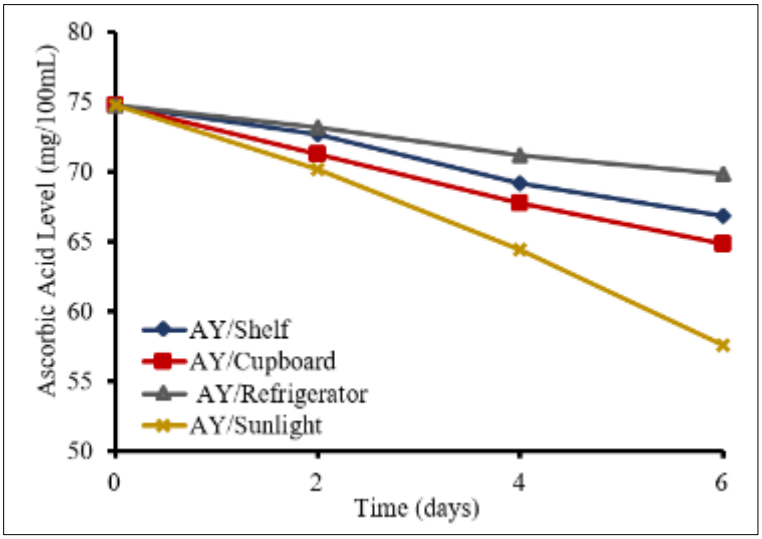


Figure 3 Assessment of ascorbic acid level in Ayensudo (AY) oranges under different storage conditions with increasing time

Table 4 Nkwanta Oranges under different storage conditions with increasing time

Nkwanta Oranges Storage condition	Days	Amount of Ascorbic acid (mg/100mL)	Ascorbic acid loss per storage period
Shelf	0	74.32	-
	2	71.21	3.11
	4	69.54	4.78
	6	66.83	7.49
Cupboard	0	74.32	-
	2	69.17	5.15
	4	67.01	7.31

	6	65.21	9.11
Refrigerator	0	74.32	-
	2	73.26	1.06
	4	71.61	2.71
	6	70.01	4.31
Sunlight	0	74.32	-
	2	70.23	4.09
	4	64.82	9.50
	6	59.21	15.11

Table 4 presents the findings concerning the ascorbic acid levels of Nkwanta orange samples following exposure to different storage conditions for six days. It was observed that the ascorbic acid level generally decreased as the number of days increased for all storage conditions. The least reduction in ascorbic acid content was observed for orange samples stored in the refrigerator while the highest reduction in ascorbic acid was exhibited by the orange samples exposed to sunlight after the six days.

These trends can be observed in Figure 4. The orange samples exposed to different storage conditions exhibited a similar decrease in ascorbic acid level; with the storage in the cupboard showing the highest reduction after 2 days. After 4 days; a larger loss of ascorbic acid was observed for the orange samples exposed to various storage conditions; with exposure to sunlight resulting in the highest reduction. A noticeable decline in ascorbic acid content was observed for oranges exposed to sunlight on day six.

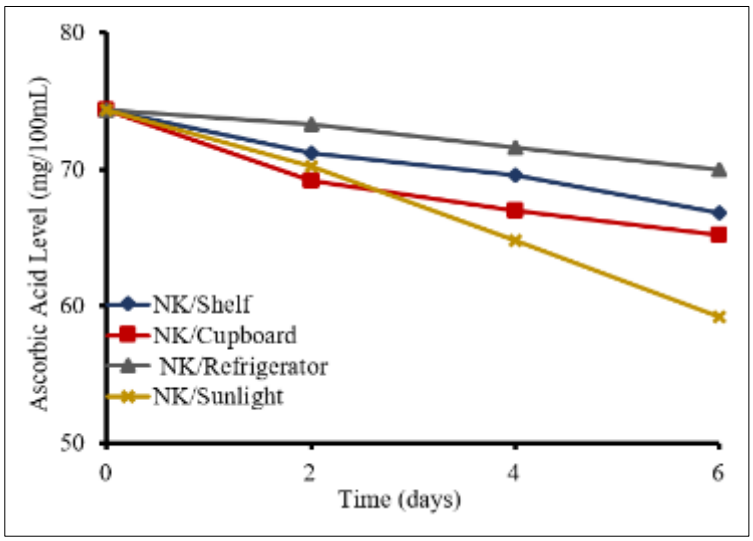


Figure 4 Assessment of ascorbic acid level in Nkwanta (NK) oranges under different storage conditions with increasing time

Figure 5 provides a comparative analysis of the ascorbic acid loss during different storage periods. The Ayensudo and Nkwanta orange samples exhibited a similar pattern of increasing ascorbic acid loss when exposed to sunlight; with Ayensudo oranges experiencing a higher loss. Oboasi oranges showed a slight loss of ascorbic acid on day two and day four when exposed to sunlight; and on day six; the loss value was similar to that of Ayensudo oranges. There were close ascorbic acid values observed for Oboasi; Ayensudo and Nkwanta oranges stored on the shelf after six days; with values of 7.31 mg/100mL; 7.89 mg/100mL; and 7.49 mg/100mL. Similarly; the values for oranges stored in the cupboard were 10.29 mg/100mL; 9.87 mg/100mL; and 9.11 mg/100mL; while for oranges stored in the refrigerator; the values were 3.76 mg/100mL; 4.89 mg/100mL; and 4.31 mg/100mL.

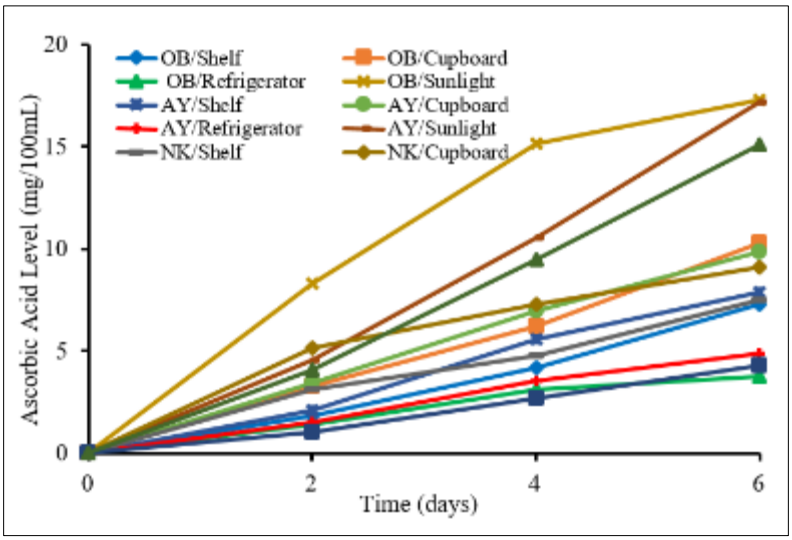


Figure 5 Comparative analysis on the loss of ascorbic acid in Oboasi; Ayensudo and Nkwanta oranges under different storage conditions with increasing time

The degradation of ascorbic acid in oranges is primarily attributed to the presence of oxygen; which is considered the most destructive component [43-44]. Furthermore; the major sugar found in fruits; namely fructose; can contribute to the depletion of ascorbic acid [45]. As the fructose content increases; the loss of ascorbic acid becomes more significant. Oboasi oranges; known for their sweeter taste compared to Ayensudo and Nkwanta oranges; may exhibit lower levels of ascorbic acid due to their higher fructose content. It should be noted that production practices; including the use of fertilizers; can also impact the levels of ascorbic acid in oranges. The retention of ascorbic acid in fruits is influenced by adequate potassium levels [46-47]. Additionally; the climate and total available heat also impact the levels of ascorbic acid in oranges [48-49]. Cool-temperature regions tend to produce oranges with higher levels of ascorbic acid; while hot tropical areas tend to yield oranges with lower levels of ascorbic acid. The selected orange samples displayed high levels of ascorbic acid; indicating that they may have had proper potassium levels for ascorbic acid retention. The cool climatic conditions during the growth period may have also positively influenced the ascorbic acid levels in these oranges. To maintain optimal levels of ascorbic acid; it is important to store oranges at a suitable cool temperature [50-51]. For all types of oranges; the reduction in ascorbic acid was lower in samples that were refrigerated. The low loss in ascorbic acid content may be due to the storage of the orange in the cool environment which helped retain the ascorbic acid content. On the other hand; exposing the orange samples to sunlight resulted in a high loss of ascorbic acid. High temperatures can inactivate oxidizing enzymes and consequently destroy ascorbic acid [52]. Therefore; exposing fruits to sunlight at a temperature range for extended periods can be highly detrimental to ascorbic acid content. Sunlight also can decrease the jellying power of pectin; which may contribute to the degradation of pectin found in the walls of oranges.

4. Conclusion

The ascorbic acid levels of the selected orange types were successfully measured using redox titration under different storage conditions and time durations. Overall; it was observed that the ascorbic acid level decreased in the various orange samples over time and under various storage conditions. Oranges stored in the refrigerator exhibited the least reduction in ascorbic acid content; while the highest reduction was observed in oranges exposed to sunlight. In conclusion; maintaining oranges in cool-temperature environments is optimal for retaining higher concentrations of ascorbic acid.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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