International Journal of Food Nutrition and Safety, 2023, 14(1): 1-8

Modern Scientific Press

International Journal of Food Nutrition and Safety

Journal homepage:www.ModernScientificPress.com/Journals/IJFNS.aspx

ISSN: 2165-896X
Florida, USA

Article

Effect of Temperature, Storage Condition and Addition of Acid and Base on Vitamin C in Orange Juice

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Article history: Received 17 April 2023, Revised 14 July 2023, Accepted 15 July 2023, Published 21 July 2023.

Abstract: Vitamin C is an essential water soluble vitamin found mainly in fruits, vegetables and their derivatives. Orange juice is a popular thirst quencher and a convenient way to reach the daily recommended intake of vitamin C. In this study, iodometric titration was used to determine the Vitamin C content in orange fruit juices and also how the vitamin C content in the juice is affected by temperature, storage condition, system type and the addition of acid and base. The orange fruits were purchased randomly from local market in Aliero town, Kebbi State of Nigeria. The juices from the samples were extracted by mechanical pressing of the sliced fruits followed by filtration to remove pulp and vitamin C content in the juices were analysed at 5^{o}C, 27^{o}C, 40^{o}C, and 60^{o}C. Four of the samples were stored for five days where two are open systems while another two are closed systems from which a set of open and closed systems is refrigerated while another set is not refrigerated. The results revealed that high temperature caused an appreciable loss in vitamin C content from 40.4 mg/100mL at 27^{o}C to 34.52 mg/100mL at 40^{o}C and then to 30.92 mg/100mL at 60^{o}C respectively. The refrigerated open and closed systems had the highest vitamin C contents than their non-refrigerated counterparts. The addition of HCl did not significantly cause much change in the Ascorbic acid content of the juice samples. With NaOH, there was no end point which suggests that the base could have neutralized the Ascorbic Acid in the juice samples completely prior to titration analysis. The outcome of this study shows that the stability of vitamin C content in orange juice depends on the type of storage methods employed as seen

that the refrigerated systems had the highest value of the Vitamin over the 5-day storage period.

Keywords: storage, temperature, vitamin c, acid, juice.

1. Introduction

Ascorbic acid (Vitamin C) is an important vitamin which is vital for human and animal life. This water soluble vitamin contributes too many health benefits such as prevention of scurvy and cancer, relief from common cold, stimulate collagen synthesis and play a significant role in wound healing process (Iqbal *et al*,., 2004). According to Teucher *et al*,. (2004), vitamin C is used to enhance availability and absorption of iron from non-heme sources. Ascorbic acid also has antioxidant properties since it can easily lose the electron to neutralize and inhibit free radicals from being oxidized in preventing cell damage. It is also commonly used as food additive which acts as antioxidant (Whitney and Rolfes, 2008). Vitamin C is an organic compound consists of carbon, hydrogen and oxygen (Chinnici *et al*,., 2005). The terms vitamin C is not only used for ascorbic acids, but it includes all compounds exhibiting biological activity such as oxidized, ester and synthetic form. The main biological form of vitamin C is L-ascorbic acid, and it can reversibly change to oxidized form called dehydroascorbic acid (Fenoll and Martinez, 2010). Many factors can cause oxidation of vitamin C such as pH, light, temperature, presence of oxygen and metal ion (Wantz *et al*,., 2005).

Human is unable to synthesize their own vitamin C supply as human cells cannot perform the crucial last step in vitamin C biosynthesis,the conversion of L-gulono-g-lactone into ascorbic acid which is catalyzed by gulonolactoneoxidase enzyme (Linster and Van Schaftingen, 2007). Therefore, they require vitamin C for maintaining the physiological functions. To meet the requirement, vitamin C must be consumed from diet. The recommended nutrient intake of vitamin C for Malaysian adult is 70 mg per day. An intake of 45 mg/day will ensure measurable amount of ascorbate be present in the plasma of most people and available to supply tissue requirements for metabolism or repair at sites of depletion or damage (MOH, 2005). Many methods can be used for determination of vitamin C such as spectrophotometry, electrophoresis, titration, and high performance liquid chromatography (HPLC) (Tang and Wu, 2005; Dong *et al*,., 2007). The most commonly used method is oxidation-reduction titration method where ascorbic acids are oxidized to dehydroascorbic acid and the indophenol dye is reduced to a colorless compound or iodine. The end point of the titration can be easily detected when an excess of the unreduced dye give a rose pink color in an acid solution (Tee *et al*,., 1996). It is a simple and easy method to determine vitamin C in fruits and fruit juices. However, the method is not suitable for fruits that have reddish-purplish color. The titration method also is time-consuming and lack of

specificity due to interference of reducing substances in the food such as ferrous iron, stannous tin, cuprous copper, sulphur dioxide, sulphite or thiosulphate (Eitenmiller *et al*,., 2008).

Ascorbate and ascorbic acid are both naturally present in the body when either of these is introduced into cells, since the forms interconvert according to pH. Vitamin C is a cofactor in at least eight enzymatic reactions, including several collagen synthesis reactions that, when dysfunctional, cause the most severe symptoms of scurvy. In animals, these reactions are especially important in wound healing and in preventing bleeding from capillaries. Ascorbate may also act as an antioxidant against oxidative stress. However, the fact that the enantiomer D-ascorbate (not found in nature) has identical antioxidant activity to L-ascorbate, yet far less vitamin activity underscores the fact that most of the function of L-ascorbate as a vitamin relies not on its antioxidant properties, but upon enzymic reactions that are stereospecific. "Ascorbate" without the letter for the enantiomeric form is always presumed to be the chemical L-ascorbate. Ascorbate is required for a range of essential metabolic reactions in all animals and plants. It is made internally by almost all organisms; the main exceptions are most bats, all guinea pigs, capybaras, and the Anthropoidea (i.e., Haplorrhini, one of the two major primate suborders, consisting of tarsiers, monkeys, and humans and other apes). Ascorbate is also not synthesized by some species of birds and fish. All species that do not synthesize ascorbate require it in the diet. Deficiency in this vitamin causes the disease scurvy in humans. Ascorbic acid is also widely used as a food additive, to prevent oxidation (Nweze *et al*,., 2015).

Several other methods to determine vitamin C content like spectrometric, spectrofluorimetric, and electrophorhesis, still some of them are not practical and need re-evaluation due to insufficient of sensitivity and selectivity (Agar, 1995). It was also stated that the most preferred method of determining vitamin C content in foods is a chromatographic method using HPLC due to the rapid, high accuracy and consistency.

Vitamin C is water soluble vitamin which is the one of the most important vitamins and essential for human life is vital for human and animal life. Human is unable to synthesize their own vitamin C supply as human cells cannot perform the crucial last step in vitamin C biosynthesis. Therefore, they require vitamin C for maintaining the physiological functions. To meet the requirement, vitamin C must be consumed from vegetable and fruit. As orange is one of the most abundant sources of vitamin C, however, it very cheap in our society. Orange citrus (sinensis) is the cheapest and most excellence source of vitamin C and powerful natural antioxidant that build the immune system. Therefore; they require vitamin C for maintaining the physiological functions. Hence, analysis of vitamin c in oranges is important.

The primary aim of this research work is to analyze the amount of vitamin c in fresh orange juice and study some conditions that could cause loss of vitamin c during storage or processing of the fruit or juice.

2. Materials and Methods

2.1. Sample Collection and Preparation

The sample of Orange fruit was purchased randomly from local market in Aliero town, Kebbi State of Nigeria. The orange juice was extracted from slice of orange fruit by squeezing followed by filtration using filter paper to remove pulps and then transfer to volumetric flask.

2.2. Iodometric Titration

In this method, the iodine solution was prepared from potassium iodide (KI), potassium iodate (KIO_3), and sulfuric acid (H_2SO_4) and then standardized by using a standard ascorbic acid with starch solution as indicator. This method also determines the vitamin c concentration in a solution by a redox titration using iodine. As the iodine is added during the titration, the ascorbic acid is oxidized to dehydroascorbic acid, while the iodine is reduced to iodide ions which gives the end point as shown below:

$$IO_3^- + 8I^- + 6H+ \rightleftharpoons 3I_3 - + 3H_2O$$

$$C_6H_8O_6 + I_3^- \longrightarrow C_6H_6O_6 + 3I^- + 2H^+$$

2.3. Preparatoin of Reagent

2.3.1. Preparation of 1% starch solution

100 mL of distilled water was placed in a 250 ml beaker and brought to boiling on a hot plate. A smooth paste was made with 1 g of soluble starch and a small volume (several ml or so) of distilled water. Once the water is boiling, the beaker containing the boiling water was carefully removed from the hot plate. The starch paste was poured into the boiling water and stirred until all of the starch is dissolved. The starch solution was allowed to cool to room temperature before use. Note: This is especially important if the starch solution is to be used in a kinetics experiment where temperature is a factor

2.3.2. Preparation of standard ascorbic acid solution (0.00114mol/L)

0.2 g of ascorbic acid was weighed and transferred into a 250 mL beaker. A few mL of distilled water was added and swirled for a few minutes until the ascorbic acid is dissolved. The ascorbic acid solution was transferred to a 1 L volumetric flask, making sure that all traces of solution was rinsed into the volumetric flask using distilled water. The solution was made up to the 1 L mark with distilled water. The concentration of the standard ascorbic acid solution was calculated from the formula:

$$\text{Concentration of ascorbic acid} = \frac{\text{Amount (in moles)}}{\text{Volume (in litre or } dm^3)}$$

Where amount = $\frac{mass}{molar\ mass}$

2.3.3. Preparation of iodine solution

The solution was prepared by mixing 5.00 g potassium iodide (KI) and 0.268 g potassium iodate (KIO_3) and then dissolved into 250 mL beaker with 200 mL of distilled water. 30 mL of 3M H_2SO_4 was added into the beaker and then diluted with distilled water until 500 mL solution (Nweze *et al*,., 2015).

2.3.4. Standardization of iodine solution

The prepared iodine solution was added into a 50 mL burette and filled up to the zero mark. 25 cm3 of the prepared standard ascorbic acid was measured using a 25 mL pipette and transferred into a 250 mL Erlenmeyer or conical flask. 10 drops of 1% starch solution was added to the flask as indicator. The end point of the titration was marked by the first permanent blue-black colour of the starch-iodine complex. The concentration of the iodine solution was determined from the stoichiometry of the reaction of iodine and ascorbic acid using the known concentration of the ascorbic acid.

3. Results and Discussion

In table 3.1, the result obtained from the research work shows differences in values of vitamin C at different temperature 40.4 mg/100mL 34.52 mg/100mL and 30.92 mg/100mL for normal, and boiled respectively. The results show the degradation of vitamin C by heat. Effects of temperature on the ascorbic acid content of the different sources are shown in the Table. The high temperature cause significant loss of vitamin C in the orange juice the difference in vitamin C content of fresh orange sample and boiled orange sample shows that High temperature has effect on vitamin C content in orange fruits and cause an appreciable loss in vitamin C that is thermally labile. It can be inferred from results that the lower the temperature, the higher the availability of vitamin C in orange fruit.

Table 3.1: Effect of Temperature

	Conc. in mol/L	**Conc. in g/L**	**Conc. in mg/100mL**
ROOM TEMP. 27^{O}C	0.02269	0.404	40.4
TEMPERATURE OF 40^{O}C	0.001968	0.3455	34.52
TEMPERATURE OF 60^{O}C	0.00175	0.3009	30.92

In table 3.2, the results for storage condition at room temperature (open and closed system) are presented. Results were obtained at differences in values of vitamin C at different storage conditions of system type (open and close system) of normal temperature 33.74 mg/100mL, and 27.68 mg/100mL

respectively. Storage of orange juice cause an appreciable loss in vitamin C as a result of many chemical reactions which contribute to the loss of storage life of vitamin C. The majority of these reactions are enzymatically driven while others are chemical reactions that occur because of the senescence. This involves color, flavor, and odour changes that result from a chemical reaction between the constituents of the orange juice.

Table 3.2: Effect of storage condition and system type (5 days)

AT 27ºC	**Conc. in mol/L**	**Conc. in g/L**	**Conc. in mg/100mL**
OPEN SYSTEM	0,0019	0.3374	33.74
CLOSE SYTEM	0.00157	0.2768	27.68

In table 3.3, effect of refrigeration storage at 5°C is shown. Results were obtained at differences in values of vitamin C at different storage condition of system type (open and close system) at 5°C: 38.89 mg/100mL and 35.48 mg/100mL respectively. The results show the degradation of vitamin C by storage at low temperature in the open system than in the closed system.

Table 3.3: Effect of refrigeration storage condition and system type at 5ºC

REFRIGERATION	**Conc. in mol/L**	**Conc. in g/L**	**Conc. in mg/100mL**
OPEN SYSTEM	0.002208	0.3889	35.48
CLOSE SYTEM	0.0020	0.3548	38.89

In table 3.4, effect of addition of acid is shown. The result shows that the addition of acid have less effect on the stability of the ascorbic acid in the orange juice samples giving 35.48 mg/100mL, 35.952 mg/100 mL 36.7 mg/100 mL respectively.

Table 3.4: Addition of acid

HCl	**Conc. in mol/L**	**Conc. in g/L**	**Conc. in mg/100mL**
25ml into 25 ml of sample	0.0020148	0.3548	35.48
25ml of dilute HCl (50ml into 50ml of distilled water)	0.00204	0.3595	35.952
25ml of dilute HCl (5ml into 100ml of distilled water)	0.002083	0.3669	36.7

With NaOH, as in table 3.5, there was no end point which suggest that the base could have neutralized the Ascorbic Acid

3.5. Addtion of base (NaOH)

5g of NaOH in to 25mL of sample	No end point
25mL of NaOH solution in to 25mL of sample	No end point

4. Conclusions

It is verified that vitamin C is an easily oxidizable and unstable compound whose content in a fruit juice sample gets lowered at a higher temperature environment. High temperature has effects on vitamin C content of fruits, which cause an appreciable loss in vitamin C. It can be seen from results that the lower the temperature, the higher the availability of vitamin C in orange juice. It is better to maintain or store vitamin C in a place below the room temperature. Many chemical reactions contribute to the loss of vitamin C at storage life and hence the chemical deterioration of fruits. The refrigerated open and closed systems had highest vitamin C than their non-refrigerated counterparts. The addition of HCl did not significantly cause much change in the Ascorbic acid content the of the juice sample. With NaOH, there was no end point which suggests that the base could have neutralized the Ascorbic Acid even before the titration analysis.

Conflicts of Interest

The authors declare no conflict of interest

Acknowledgments

The authors are very grateful to the staff in the Department of Pure and Applied Chemistry, Kebbi State University of Science and Technology, Aliero, for providing all the chemicals, glass ware apparatus and other facilities used in this work. Special thanks go to Dr A. Muhammad, Head of the Department of Chemistry, KSUST, Aliero for his help regarding the chemicals and materials in this work.

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