Journal of Agricultural, Food and Environmental Sciences Vol 78 No 1 (2024) 40-46
DOI: https://doi.org/10.55302/JAFES24781040bo

Original scientific paper

TITRIMETRIC AND SPECTROPHOTOMETRIC ANALYSIS OF VITAMIN C CONTENT IN FIVE CITRUS FRUITS IN BENIN CITY, NIGERIA

Oluwaseyi Bukky Ovonramwen*, Ruth Osayamen Imuentiyan

Department of Chemistry, Faculty of Physical Sciences, University of Benin, P.M.B. 1154, Benin City, Edo State, Nigeria;
*corresponding author: adebuloyeoluwaseyi@yahoo.com

ABSTRACT

Vitamins C are organic compounds which have to be obtained from the diet because of the inability to synthesise them. Vitamin C is a common household name. Vitamin C (ascorbic acid) is known for the role it plays in many physiological processes in humans. The present research determines vitamin C contents in five citrus fruits namely tangerine (*C. reticulata*), grape fruit (*C. paradisi*), orange (*C. sinensis*), lemon (*C. limon),* lime (*C. aurantiifolia).* The fruits were purchased from the New Benin market in Benin City, Nigeria. Vitamin C content of fresh fruit juices was determined by iodo-titrimetric (KI and KIO_3) and spectrophotometric methods (potassium permanganate as a chromogenic reagent at 521 nm absorbance). The results obtained by spectrophotometric method lime had the highest vitamin C concentration (29.27 mg/100 mL) and the lowest found in lemon (13.94 mg/100 mL) as lime > tangerine > sweet orange > grapefruit > lemon. In titrimetric method sweet orange had the highest vitamin C content (49.06 mg/100 mL) and the lowest vitamin C content was found in lemon (27.46 mg/100 mL), in this order sweet orange > lime > tangerine ≈ grapefruit > lemon. Therefore, adequate intake of these citrus fruits help in the maintenance of overall health and wellness.

Key words: citric fruits, vitamin C, iodometriy, UV-Vis spectrophotometriy.

INTRODUCTION

Vitamin C, an organic molecule, is an essential micronutrient needed in minute quantities in many multi-cellular organisms, especially in humans for proper functioning of its metabolism. Most animals are able to synthesise ascorbic acid, except humans, primates, guinea pigs, and some fruit bats which have lost the ability to synthesise it. The inability to synthesise vitamin C is due to mutations in the *L*-gulono-gamma-lactone oxidase (GULO) gene which codes for the enzyme responsible for catalysing the last step of vitamin C biosynthesis (Drouin *et al.*, 2011; Yang, 2013). Vitamin C otherwise known as ascorbic acid is a water-soluble vitamin (Zanini *et al.,* 2018) with molecular formula $C_6H_8O_6$ and the structural formula resembling a monosaccharide (Figure 1).

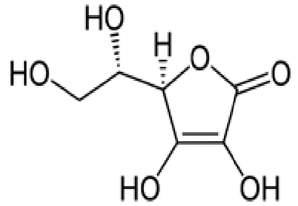

Figure 1. Chemical structure of *L*-vitamin C

Ascorbic acid is naturally present in some food, added to others and taken as dietary supplements. Vitamin C is readily available in many natural sources including fresh fruits and vegetables. The richest sources include papaya, Indian gooseberry, cantaloupes, kiwifruits, citrus fruits such as oranges, limes, lemons, tangerines and grapefruit and vegetables including tomatoes, green and red peppers, broccoli, potatoes and many others. Fortified cereals and its' juices are also rich sources of vitamin C. Vitamin C is also available as an oral supplement, mostly in the form of capsules and chewable tablets. Vitamin C has antioxidant properties that protects living organisms against oxidative stress. It plays important role in physiological processes in humans. It is needed for the repair of tissues in all parts of the body. Vitamin C is vital to the formation of protein used to make skin, tendons, ligaments and blood vessels. It is required for healing wounds and forming scar tissue, for repairing and maintaining cartilage, bones and teeth. Ascorbic acid aids in iron metabolism. Vitamin C also helps the body use carbohydrate, fat and protein. Therefore, its vital for the development, normal growth, and functioning of the human body. Its deficiency causes muscle weakness, swollen and bleeding gums, loss of teeth, bleeding under the skin and pain in joints and later results in a condition called scurvy, once common among sailors.

Aside, its catalytic properties, Chemist, use it as a reagent for the preparation of fine chemicals, enzymatic reagent and nano-materials (Bedhiafi *et al.,* 2023). The detection and quantification of ascorbic acid in food samples, products and nutraceuticals is gaining compelling significance among researchers, medical practitioners and also in the Food and Pharmaceutical industry. It occurs as two enantiomers, commonly denoted *'L'*- Levo and *'D'*- Dextro. The *L*-isomer is most often encountered: occurs naturally in many foods. *L*-ascorbic acid acts as a coenzyme, a food antioxidant, a flour treatment, a plant metabolite, a cofactor, a skin lightening and a geroprotector (National Center for Biotechnology Information, 2022). The *'D'*-ascorbic acid does not occur in nature, can be made *via* chemical synthesis but has no important biological role.

The ascorbic acid content in plants varies, depending on factors such as variety, weather, maturity, position of tree, handling storage and type of container. But the most significant determinant of vitamin C content in foods is the food storage and preparation as its easily destroyed by light, heat and oxygen (Dosedˇel *et al.,* 2021; Giannakourou and Taoukis, 2021). Meanwhile, storage temperature, pH, dissolved oxygen level, residual hydrogen peroxide after the sterilisation of packaging material and trace metal ions causes breakdown of vitamin C in packaged fruit juices. Several analytical methods have been used for quantification of vitamin C in food and drug samples. Examples of these analytical methods are titrimetric, spectrophotometry, chromatography, voltammetry, electrophoresis, amperometry, fluorometry, electrochemical and potentiometry (Najwa and Azrina, 2017; Desai and Desai, 2019).

MATERIALS AND METHODS

All reagents (distilled water, potassium iodide (KI), potassium iodate (KIO_3), 3 M Sulphuric acid (H_2SO_4), 100 µg/mL standard ascorbic acid, $KMnO_4$ solution, Iodine and fresh starch indicator solution 1%) were of analytical grade. The UV-Vis Spectrophotometer – Jenway 6715 was used. The fresh ripe oranges, lemons, limes, grapefruits and tangerines (Figure 3) were purchased from New Benin market in Benin City, on postal code 300102, 6º21′3″N, 5º37′53″E, Edo State, Nigeria in February 2022. The citrus fruits were identified by Dr. H. N. Akinnibosun of the Department Plant Biology and Biotechnology, University of Benin, Benin City. Fresh juice collected from the fruits were used in this study. The fruit samples were thoroughly washed with water to remove dust and unwanted particles. The fruits were peeled and cut into two halves and then squeezed to discharge their juice. The juice obtained was filtered with a pre-cleaned cloth. Each fruit sample have been dealt with a manner to prevent them from contamination by chemicals or any other pollutants and stored in containers at room temperature for instant analysis.

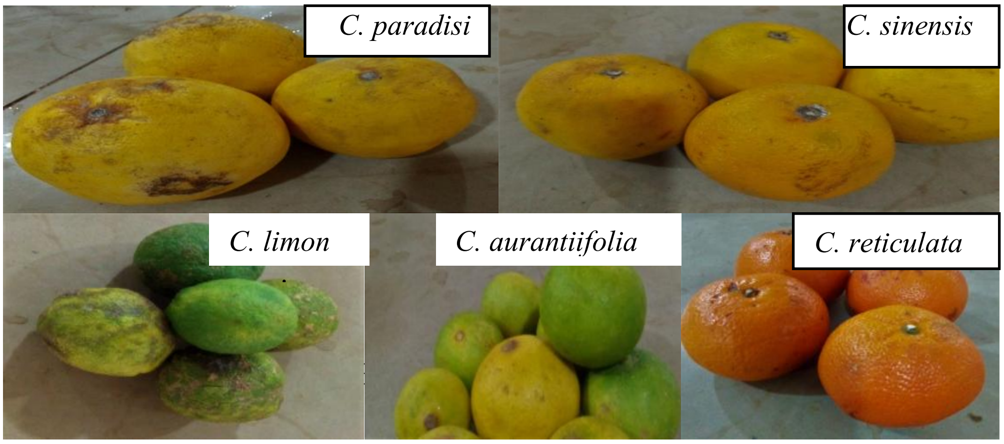

Figure 2. Analyzed samples

Preparation of fruit samples for analysis by UV-Visible spectrophotometry

Aliquots of 10.0 mL of each fruit sample was transferred into a test tube, mixed and homogenised with 1.0 mL $KMnO_4$ (100 µg/mL) and allowed to stand for 5 min. The absorbance of the samples was read in the spectrophotometer in a glass cuvette at 521 nm (Elgailani *et al.,* 2017). In each analysis, the same reaction analysis system was utilised to calculate blank by the substitution of the sample containing vitamin C by one containing the same amount of distilled water.

Determination of ascorbic acid in the fruit samples by Iodometric titration method

Vitamin C concentration was determined by Iodometric titration method (Elgailani *et al.,* 2017). The method was carried out with potassium iodate in the presence of potassium iodide. 10 mL each of the freshly prepared juice sample was pipetted into a 250 mL conical flask and diluted with 5 mL distilled water. 1 mL starch indicator solution (1%) was added. The sample was titrated against iodine solution (5.00 g KI and 0.268 g KIO_3 dissolved in 200 mL and 30 mL 3 M H_2SO_4 was added) until the appearance of permanent blue-black colour due to the starch-iodine complex indicating endpoint. Titration was carried out in triplicate until concordant results were obtained. (Titres agreeing within 0.1 mL). The result for each fruit samples was reported as mean and standard deviation. All statistical analysis was carried out using Microsoft Excel 2016.

RESULTS AND DISCUSSION

The concentration of vitamin C as contained in the samples *via* Iodometric titration and UV-Visible Spectrophotometric methods were given in Table 1.

Table 1. Vitamin C Content of the Fruit Samples using different methods

Sample	Botanical name	Vitamin C Concentration (mg/ 100 mL)	
		Iodometric	Spectrophotometric
Lemon	*C. limon* (L.) Burm UBH-C230	27.46 ± 0.26	13.94 ± 0.13

Lime	*C. aurantiifolia* Christm UBH-C195	42.13 ± 0.26	29.72 ± 0.00
Grapefruit	*C. paradisi* Macfad UBH-C152	31.2 ± 0.46	18.51 ± 0.055
Sweet orange	*C. sinensis (L.)* Osbeck UBH-C441	49.06 ± 0.26	23.05 ± 0.055
Tangerine	*C. reticulata* Blanco UBH-390	31.2 ± 0.46	27.27 ± 0.086

The vitamin C content in the fruit juices examined *via* titrimetric method were comparable as follows: sweet orange having the highest content (49.06 mg/100 mL ± 0.26) > lime (42.13 mg/100 mL ± 0.26) > tangerine and grapefruit having the same values (31.2 mg/100 mL ± 0.46). The lowest vitamin C content was found in lemon (27.46 mg/100 mL ± 0.26). In the determination of ascorbic acid of fruit samples by iodometric titration. Vitamin C is a weak acid and a good reducing agent. Iodine is a weak oxidising agent. It will only oxidise vitamin C in the fruit juice sample to dehydroascorbic acid and the iodine is reduced to iodide by vitamin C as shown in the equation below. The excess iodine reacts with the starch indicator to form a blue-black starch-iodine complex indicating endpoint. IO_3^- (iodate) oxidises I^- (iodide) to I_2

$IO_3^- + 5I^- + 6H^+ \rightarrow 3I_2 + 3H_2O$ ---------- ------------------------------ 1

Liberated I_2 (iodine), oxidises $C_6H_8O_6$

$C_6H_8O_6 + I_2 \rightarrow C_6H_6O_6 + 2I^- + 6H$ ------- ------------------------------ 2

The calibration curve was found by plotting the values of concentration of ascorbic acid against recorded absorbance. It was observed that as concentration increased there was an increase in the absorbance (Figure 3).

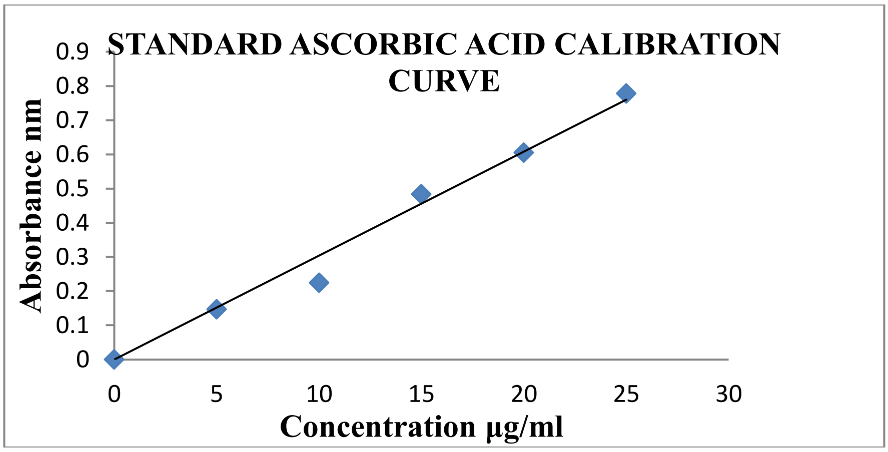

Figure 43. Standard Ascorbic Acid Calibration Curve Source (UV-Vis Spectrophotometric analysis)

The highest vitamin C content obtained by spectrophotometric method was found in samples of: lime > tangerine > sweet orange > grapefruit and the lowest found in lemon (13.94 mg/100 mL).

Manuha et al. reported lime juice having more vitamin C than lemon (Manuha *et al.,* 2019). This was in line with our report. In similar work carried out by Okungbowa *et al.* (2022) using titrimetric method orange has the highest concentration of vitamin C and grape with the lowest in this order sweet orange > lemon > lime > grape. This has similarity with our work. Okiei *et al.* (2009) reported that the ascorbic acid content of freshly prepared lemon juice is

48.61 mg/100 mL which is higher than values from this work. The vitamin C content *via* titrimetric method was similar to that reported by Dioha *et al*. (2011) who reported that the vitamin C content of freshly prepared orange juice was 41.84 mg/cm^3. Another study reported a vitamin C content of 33-50 mg/100 mL for orange juice by squeezing the fruits. In another similar work, Najwa and Azrina, (2017) using titrimetric and HPLC method had higher vitamin C in titrimetric method than in HPLC method which correlate with our titrimetric and spectrophotometric method. The observed varying results in the content of the vitamin C studied in the two methods and with those reported by some other studies may be explained on the basis of differences in maturity stage, regional varieties, different techniques of measuring and the environmental factors (Okungbowa *et al.,* 2022). Ascorbic acid concentrations vary with conditions such as temperature, climate, light and the storage period (Figure 5).

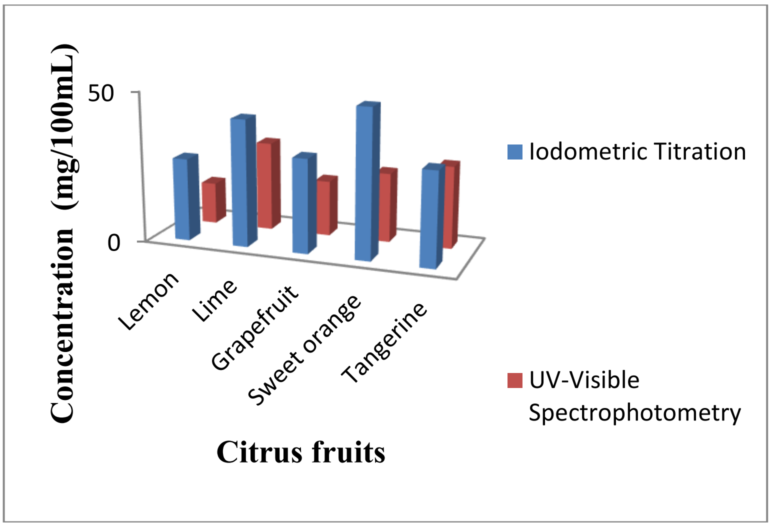

Figure 5. Concentration of ascorbic acid in different citrus fruits

F-test analysis (0.6626, 0.0012, 0.7319, 0.5991 and 0.4271 are lemon, lime, grape, orange and tangerine respectively at $p < 0.05$) reveal that the null hypothesis is true and the variance of the two groups are the same. Hence, no variation from the methods. The daily contributions of the citrus fruits from the two methods in relation to the standard (Food and Nutrition Board, Institute of Medicine. 2000) are reported in the Table 2 with the lower values represent the contribution from spectrophotometric analysis whereas the higher from iodometric titration.

Table 2. Daily Value of Ascorbic Acid in Citrus Fruits %

				Lemon		Lime		Grape fruit		Sweet orange		Tangerine	
Life Stage	Age	Males (mg/day)	Females (mg/day)	Male (%)	Female (%)	Male (%)	Female (%)	Male (%)	Female (%)	Male (%)	Female (%)	Male (%)	Female (%)
Infants	0-6 months	40 (AI)	40 (AI)	34.85-68.65	34.85-68.65	74.30-105.33	74.30-105.33	46.28-78.00	46.28-78.00	57.63-122.65	57.63-122.65	68.18-78.00	68.18-78.00
	7-12 months	50 (AI)	50 (AI)	27.88-54.92	27.88-54.92	59.44-84.26	59.44-84.26	37.02-62.40	37.02-62.40	46.10-98.12	46.10-98.12	54.54-62.40	54.54-62.40
Children	1-3 years	15	15	92.93-183.07	92.93-183.1	198.13-280.87	198.13-280.87	123.40-208.00	123.40-208.00	153.67-327.07	153.67-327.07	181.80-208.00	181.80-208.00

	4-8 years	25	25	55.76-109.84	55.76-109.8	118.88-168.52	118.88-168.52	74.04-124.80	74.04-124.80	92.20-196.24	92.20-196.24	109.08-124.80	109.08-124.80
	9-13 years	45	45	30.98-61.02	30.98-61.02	60.04-93.62	60.04-93.62	41.13-69.33	41.13-69.33	51.22-109.02	51.22-109.02	60.60-69.33	60.60-69.33
Adolescents	14-18 years	75	65	18.59-36.61	21.45-42.25	39.63-56.17	45.72-64.82	24.68-41.60	28.48-48.00	30.73-65.41	35.46-75.48	36.36-41.60	41.95-48.00
Adults	≥ 19 years	90	75	15.59-30.51	18.59-36.61	33.02-46.81	39.63-56.17	20.57-34.67	24.68-41.60	25.61-54.51	30.73-65.41	30.30-34.67	36.36-41.60
Smokers	≥ 19 years	125	110	11.15-21.97	12.67-24.96	23.78-33.70	27.02-38.30	14.81-24.96	16.83-28.36	18.44-39.25	20.95-44.60	21.82-24.96	24.79-28.36
Pregnancy	≤ 18 years	-	80	-	17.43-34.33	-	37.15-52.66	-	23.14-39.00	-	28.81-61.33	-	34.09-39.00
	≥ 19 years	-	85	-	16.40-32.30	-	34.96-49.56	-	21.78-36.71	-	27.12-57.72	-	32.08-36.71
Breastfeeding	≤ 18 years	-	115	-	12.12-23.88		25.84-36.63	-	16.10-27.13	-	20.04-42.66	-	23.71-27.13
	≥ 19 years	-	120	-	11.62-22.88		24.77-35.11	-	15.43-26.00	-	19.21-40.88	-	22.73-26.00

CONCLUSION

Vitamin C or ascorbic acid is vital for the human body. It is important to note that inadequate intake will result in the symptoms of scurvy and too much will lead to stomach cramps, kidney stones, diarrhea and interference with certain tests. Consequently, the studies on vitamin C content in foods are necessary in relation to the control of food databases and the establishment of dietary reference intakes. Existing literature support the relationship between diet, well stocked with sufficient vitamin C and the maintenance of overall health and wellness.

REFERENCES

Bedhiafi, F., Idoudi, S., Fernandes, Q., Al-Zaidan, L., Uddin, S., Dermime, S., Billa, N. & Merhi, M. (2023). Nano-vitamin C: A promising candidate for therapeutic applications. *Biomed. Pharma*. 158 114093. https://doi.org/10.1016/j.biopha.2022.114093.

Desai, A. P. & Desai, S. (2019). UV spectroscopic method for determination of vitamin C (Ascorbic acid) content in different fruits in South Gujarat Region. *Int. J. Environ. Sci. Nat. Res.* 21 (1), 556055. https://doi.org/10.19080/IJESNR.2019.21.556056.

Dioha, I. J., Olugbemi, O., Onuegbu, T. U. & Shahru, Z. (2011). Determination of ascorbic acid content of some tropical fruits by iodometric titration. *Int. J. Biol. Chem. Sci*. 5 (5), 2180-2184. https://doi.org/10.4314/ijbcs.v5i5.37.

Dosedˇel, M., Jirkovský, E., Macáková, K., Krˇcmová, L. K., Javorská, L. Pourová, J., Mercolini, L., Remião, F., Nováková, L. & Mladˇenka, P. (2021). Vitamin C—Sources, Physiological Role, Kinetics, Deficiency, Use, Toxicity, and Determination. *Nutrients*,13: 615-649.

Drouin, G., Godin, J. R. & Pagé, B. (2011). *The genetics of vitamin C loss in vertebrates. Curr Genom*, 12 (5), 371–378. https://doi.org/10.2174/138920211796429736.

Elgailani, I. E., Gad-Elkareem, M. A. M, Noh, E. A. A., Adam, O. E. A. & Alghamdi, A. A. (2017). Comparison of Two Methods for The Determination of Vitamin C (Ascorbic Acid) in Some Fruits. *American Journal of Chemistry.* 2. 1-7. https://doi.org/10.20448/812.2.1.1.7.

Food and Nutrition Board, Institute of Medicine. (2000): *Vitamin C. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids.* Washington, D.C. National Academy Press. 95-185.

Giannakourou, M. C. & Taoukis, P. S. (2021). Effect of alternative preservation steps and storage on vitamin C stability in fruit and vegetable products: critical review and kinetic modelling approaches. *Foods*. 10 (11), 2630. https://doi.org/10.3390/foods10112630.

Manuha, M. I., Paranagama, P. A. & Nageeb, B. M. (2019). Quantitative analysis of vitamin C in lime and lemon in vitro: verification of vitamin C on the impairment of obesity. *Int J. Adv. Sci. Res. Eng*. 5 (10). https://doi.org/10.31695/IJASRE 2019.33549.

National Center for Biotechnology Information (2022). *PubChem Compound Summary for CID 54670067, Ascorbic Acid*. Retrieved November 12, 2022 from https://pubchem.ncbi.nlm.nih.gov/compound/Ascorbic-Acid.

Najwa, F. R. & Azrina, A. (2017). Comparison of vitamin C content in citrus fruits by titration and high performance liquid chromatography (HPLC) methods. *Int. Food Res. J.* 24 (2), 726-733.

Okiei, W., Ogunlesi M., Azeez, L., Obakachi, V., Osunsanmi, M. & Nkenchor G. (2009). The Voltametric and Titrimetric Determination of Ascorbic Acid Levels in Tropical Fruit Samples, *Int. J. Electrochem. Sci.,* 4, 276-287

Okungbowa, A.L., Chukuemeke-Nwani, P.O., Mwesigwa, L.S., Omoarelojie, F.O. & Adesina-Ojobaro, A.G. (2022). Comparative Determination of Vitamin C Content of Fresh Fruit Juices. *BIU Journal of Basic and Applied Sciences* 7 (1): 53-61.

Yang, H. (2013). Conserved or lost: molecular evolution of the key gene GULO in vertebrate vitamin C biosynthesis. *Biochemical Genetics*. 51 (5–6), 413–25. https://doi.org/10.1007/s10528-013-9574-0.

Zanini, D., Silva, M., Aguiar O., Elizama., Mazalli, M. M., Kamimura, E., & Maldonado, R. R. (2018). Spectrophotometric Analysis of Vitamin C In Different Matrices Utilizing Potassium Permanganate. *European international journal of science and technology*. 7 (1), 70-84.