Nigerian Journal of Applied Science, Vol. 39, pp96-103 (2021)

COMPARATIVE STUDIES ON THE DETERMINATION OF ASCORBIC ACID CONTENT IN SOME SELECTED FRUITS IN BENIN CITYUSING TITRIMETRIC AND SPECTROPHOTOMETRIC METHODS

U. Omoruyi and H.O. Ashamah
Department of Chemistry, University of Benin, Benin City, Edo State, Nigeria.

Abstract
Ascorbic acid is an essential water-soluble vitamin that is only available to humans through dietary supplements such as the intake of fruits, vegetables, and drugs. In this study, the quantification of ascorbic acid in five different fruits – apple, guava, orange, pineapple, and watermelon were carried out using titrimetric and spectrophotometric method. Irrespective of the method of analysis employed, guava contained the highest amount of ascorbic acid 215.600mg/100 g for the titrimetric method. This was followed by orange with an ascorbic acid content of95.500mg/100 g. Pineapple, watermelon, and Apple gave values of 77.80, 11.800 and 8.500mg per 100 g of samples respectively. Spectrophotometric analysis of the ascorbic acid of the fruit samples also followed the same order (guava > orange > pineapple > watermelon > apple) with values of 223.000, 96.741, 78.411, 12.220 and 8.147 mg per 100 g of the respective fruit samples obtained. Although spectrophotometric method gave higher values of ascorbic acid in respective fruit extractsrelative to the titrimetric method, a p-value of 0.97 obtained from the statistical analysis (one-way ANOVA), revealed that there was no significant difference between the mean values, thus, either method is efficient in quantifying ascorbic acid in fruits. The findings shows that the consumption of 100 g of either pineapple, guava or orange sourced from our local market meets the prescribed US recommended dietary allowance (RDA) of 90 and 75mg/day for an adult male and female and a combination of these fruits can meet the requirement of ascorbic acid for others based on their health conditions.

Keywords: Ascorbic acid or vitamin C, fruits, standard methods, recommended dietary allowance (RDA).

Introduction
Vitamins C with the trivial name L- ascorbic acid belongs to the lactone family with the IUPAC name (2R)-2-[(1S)-1,2-dihydroxyethyl]-3,4-di- hydrox-2H-furan-5-one. It is a special class of organic compounds which is essential for normal human metabolism and regulation of cell functions. Based on solubility, vitamins are either classified as water-soluble or fat-soluble vitamins. Amongst the water-soluble vitamins, vitamin C is arguably the most important because of its antioxidantproperties which helps fight against diseases and in most cases, prevent ailment. It is known to mop up free radicals and reactive oxygen species(ROS) generated from normal body functions that can potentially damage important body macromolecules such as nucleic acid (DNA and RNA), proteins, lipids, and carbohydrates[1], thus forming a protective shield for the human body. In addition, vitamin C is required in the synthesis of collagen, which is needed for proper healing

of wounds, maintenance of a healthy skin, teeth, cartilage, tendons, blood vessels, heart valves, inter vertebral and bones [2, 3]. It is also reported to be effective in the treatment of certain types of cancer and cardiovascular diseases [4-7]. Most plants and animals synthesize ascorbic acid naturally. Unfortunately, humans are among the few primates that are unable to synthesize it due to the absence of gulonolactone oxidase enzyme required to catalyze the last stage of vitamin C biosynthesis [8,9]. Therefore, it can only be made available to us through dietary supplements such as the intake of fruits, vegetables and drugs with aUS recommended dietary allowance (RDA) of 90 and 75mg/day for an adult male and female respectively [10].

Until recently, titrimetric redox reaction method based on the reduction of iodine with ascorbic acid is the most common and widely reported method employed in the quantification of ascorbic acid in different media. More recent research is now focused on validating the results obtain from titrimetric method by either developing new method in addition to titrimetric method or employing more than one known method for ascorbic acid estimation [11-14]. Some of these methods require sophisticated equipment like HPLC which may not be readily available in laboratories in Nigeria. Iodometric titration or spectrophotometric methods are the most employed in the estimation of ascorbic acid in Nigeria. There exists no literature report on method comparison between these two methods despite its simplicity and ready availability of equipment in Nigeria. To the best of our knowledge, the only reported study on the comparison of these two methods was carried out recently by Elgailani*et al*. [15]. In their work, they estimated ascorbic acid in fresh fruits obtained in India employing both iodometric titration and spectrophotometric methods using potassium permanganate as a chromogenic reagent. In this present study, we aimed at quantifying the ascorbic acid content of some locally sourced fruits in Benin City, Nigeria, employing both redox iodometric titration method and redox typespectrophoto-metric method using potassium dichromate and diphenylcarbazide (DPC). The analytical methods were compared with a view of establishing if variation exists between them. In addition to this, the ascorbic acid content in each of the fruitselected for this study were compared with the recommended dietary allowance (RDA) to ascertain if they meet the required daily amount for healthy living.

Materialand Method

Apple, guava, orange, pineapple, and watermelon were purchased from a local market in Benin City, Edo State, Nigeria and taken to the laboratory in well labeled clean sac bags for analysis.

Sample Extraction

All the samples were thoroughly cleaned with water to remove any adhering contaminants present, after which the samples were pealed and then subjected to extraction procedure.100g of sample, was accurately weighed and ground into paste with the aid of a mortar and pestle. The paste obtained was sieved through a clean cotton fabric to extract the juice. The juice obtained was further filtered through a Whatman filter paper (pore size 2.5µm). All the fruit samples were treated in like manner and the ascorbic acid content was then determined from the fruit extracts.

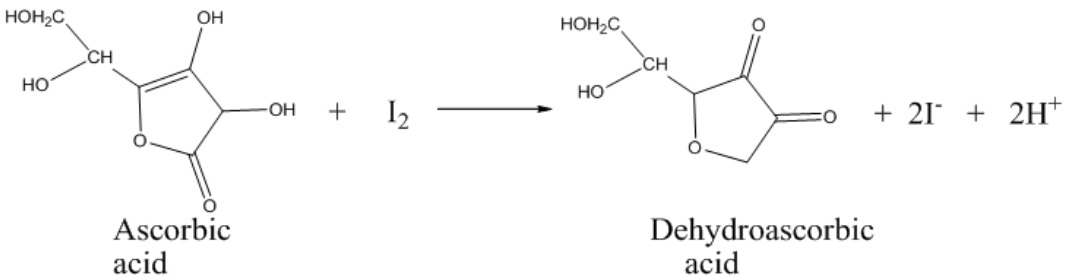
Ascorbic Acid Assay

The ascorbic acid content in the five fruits selected were determined following reported redox iodometric titration method [16-18] also by spectrophotometric method following a slightly modified procedure reported byNoroozifar and Motlagh in 2003 [19].

Iodometric Titration Method

In this method, the concentration of vitamin C generally referred to as ascorbic acid was determined via a redox titration of ascorbic acid using iodine as titrant in the presence of 1 ml starch indicator (0.5% in H_2O). Ideally, iodine reacts with starch solution to form the dark blue-black starch tri-iodide complex. However, in the presence of ascorbic acid, this process is hindered due to the reaction of iodine with the acid. The ascorbic acid undergoes oxidation via the loss of acidic protons on addition of the iodine titrant to form dehydroascorbic acid with a concomitant reduction of iodine to the iodide ion as shown in Scheme 1. Upon the complete oxidation of the ascorbic acid present, the excess iodine titrant then reacts with the starch indicator resulting in the formation of a blue-black colour which is indicative of the endpoint of the titration.

The iodine was prepared by dissolving 10g of Potassiumiodide (KI)and 3.15 g iodine (I_2) in a 200 ml beaker.The solution was transferred into a 500 ml volumetric flask and made to the mark with distilled water. The iodine solution was standardized by titrating against 0.1M sodium thiosulphate ($Na_2S_2O_3$) solution in the presence of 1ml starch indicator (0.5% in H_2O).



Scheme 1: Redox Reaction of Ascorbic Acid (Vitamin C) and Iodine

10 ml of the different (five) fruit extracts were pipetted into five - 250 ml conical flasks. 1 ml of starch indicator (0.5% in H_2O) was added to each flask and the solutions were gradually acidified with 1M H_2SO_4using a graduated pipette. The content in each flask was then titrated against the standardized iodine solution. The ascorbic acid content in each fruitwas estimatedin mgper 100 g (≡ mgl^{-1}) of fruit sample using the equations 1 and 2:

Mass of ascorbic acid in mg/100g fruit sample (m)= n × mm x $\frac{1000mg}{1g}$ (1)

$n = M \times V$ (2)

Where:

n = number of moles of ascorbic acid

mm = molar mass of ascorbic acid (176.12g/mol)

M = Molarity of iodine used ($0.048molL^{-1}$)

V = average titre of iodine used (L) as estimated in Table 1

From the equation for the reaction:
$C_6H_8O_6 + I_2 \rightarrow C_6H_6O_6 + 2H$
1mole of I_2 reacts with 1mole of $C_6H_8O_6$

∴ Number of moles (n) of iodine reacted during each titration = Number of moles (n) of ascorbic acid present in the respective fruit extracts.

Table 1: Redox- Titration Values of Ascorbic Acid Content in 10 ml of Fruit Extracts obtained from 100g Fruits Samples against Standardized 0.048M Iodine Solution

Fruit extract	V_1(ml) = volume of iodine consumed	V_2 (ml) = volume of iodine consumed	V_3 (ml)= volume of iodine consumed	V (ml) = average titre of iodine consumed. [a]
Apple	1.00	0.90	1.10	1.00
Guava [b]	25.70	25.30	25.50	25.60
Orange	11.30	11.30	11.30	11.30
Pineapple	9.30	9.10	9.20	9.20
Watermelon [b]	1.20	1.40	1.60	1.00

[a] $= \frac{V_1+V_2+V_3}{3}$, [b] $= \frac{V_1+V_3}{2}$.

Spectrophotometric Method

10 ppm of standard ascorbic acid was prepared from a standard stock solution (0.1 g/100ml), from which the different concentrations of the working standard were prepared. 1 ml from each working standard were pipetted into the 50 ml volumetric flask. 0.8 ml of $K_2Cr_2O_7$ was then added to each flask, thereafter, 1ml of 1M H_2SO_4 was added. After 10 minutes, 1ml of diphenylcarbazide (DPC; 25% in acetone) was added, the solutions made to mark (50ml) with distilled water. A blank solution was prepared in like manner without the introduction of ascorbic acid working standard. The fruit extracts were treated in like manner as the working standards. The absorbance was measured at 550nm using JENWAY 6715 uv/vis spectrophotometer.

The concentration of ascorbic acid in the extracts was estimated (mg/L) from the standard calibration curve (Fig. 1) using the equation of a straight line:

$Y = MX + C$ (3)

$X = \frac{Y-C}{M}$ (4)

Where:
Y = Absorbance of fruit extract
M= Slope (0.0242)
X= Concentration
C = intercept (0.0000)

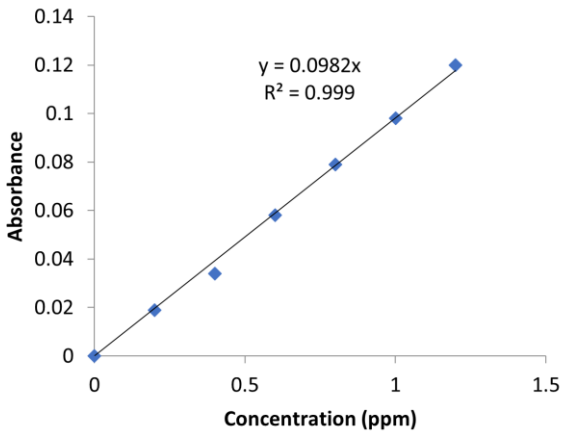


Fig. 1: Plot of Absorbance vs Concentration of standard Ascorbic Acid Solution

Statistical Analysis

The mean value was obtained for each triplicate analysis and data were summarized using mean and standard deviation. Comparison between the two methods on the mean values obtained was

carried out using One-way Analysis of Variance (One-way ANOVA).The analysiswas performed on SPSS13 software Package.

Results and Discussion

The ascorbic acid content of the fruits determined by both titrimetric and spectrophotometric methods is shown in Table 2. Apple was found to contain 8.500 $\pm$0.058 and 8.147 $\pm$0.000 mg per 100g for titrimetric and spectrophotometric methods of analysis respectively.

Values of 215.600 $\pm$ 0.115, 95.500 $\pm$ 0.000, 77.800 $\pm$ 0.058, and 11.800 $\pm$ 0.100 mg per 100 g of fruit extracts were obtained for guava, orange, pineapple, and watermelon respectively via redox titration method.

Table 2: Mean Values and Standard Deviations of Ascorbic acid Content (mg /100g $\equiv$ mgL^{-1}) in Fruit Extract

Sample	Titrimetric Method	Spectrophotometric Method
Apple	8.500 $\pm$ 0.058	8.147 $\pm$0.000
Guava	215.600 $\pm$ 0.115	223.000$\pm$0.034
Orange	95.500 $\pm$ 0.000	96.741 $\pm$ 0.000
Pineapple	77.800 $\pm$ 0.058	78.411 $\pm$0.588
Watermelon	11.800 $\pm$ 0.115	12.220 $\pm$ 0.588

Employing the spectrophotometric method, 223.000 $\pm$0.034, 96.741 $\pm$0.000, 78.411 $\pm$ 0.588 and 12.220 $\pm$0.588 mg were obtained for guava, orange, pineapple, and apple fruit extracts (100 mg) respectively. Irrespective of the method of analysis employed, it was observed that pineapple, guava, and orange were rich in ascorbic acid while watermelon and apple contained reasonable amount of ascorbic acid.

Our findings as depicted graphically in Fig. 2, shows that the consumption of 100 g of either pineapple, guava or orange sourced from our local market meets the recently prescribed Recommended Dietary Allowance (RDA) of 90 mg/day and 75 mg/day for an adult male and female respectively by United States Department of Health and Human Services [10]. In addition, we found the ascorbic acid content in these fruits to be higher than the nutrient reference value (NRV) prescribed by the National Agency for Food and Drug Administration and Control (NAFDAC) for all fortified food sold in Nigeria [20]. Furthermore, the ascorbic acid content in the pineapple extract (77.8 mg/ 100g) was found to be on par with literature value of 78.02 mg/100g reported by Abdulrazak*et al*. [21] for pineapple obtained in Northern Nigeria. However, a higher ascorbic acid level was found in orange than previously reported in Northern Nigeria.

The ascorbic acid content in watermelon (~12 mg/100g) and apple (~9 mg/100g) were very low. However, they were found to be in good agreement with USDA standard reference of 12.31 mgl^{-1} and 8.37 mgl^{-1} for watermelon and apple respectively [22]. Interestingly, it was observed that the level of ascorbic acid in watermelon depleted rapidly on exposure to air. This observation is currently being investigated to find out the rate of degradation as most watermelon consumed in Benin City are sold in the exposed form. This may account for the low ascorbic acid content recorded for watermelon during our investigations.

Although the ascorbic acid in apple fell within the USDA approved standard, higher values of 27 mg/100 mL have been reported for Danish apples [23]. Perhaps, a plausible explanation for the observed low content of ascorbic acid despite its sour taste, may stem from the fact that the apples investigated were imported into the country (not locally grown in Nigeria). Therefore, these fruits may have undergone prolonged storage which has been widely reported to deplete ascorbic acid content [24-26]. Of all the fruits studied, guava was found to contain the highest amount of vitamin C with an ascorbic acid content of 215.60 and 223.00 mg/100 g recorded for titrimetric and spectro-photometric method respectively as previously mentioned (Table 2). The high level of vitamin C in guava may account for its sour taste on consumption.

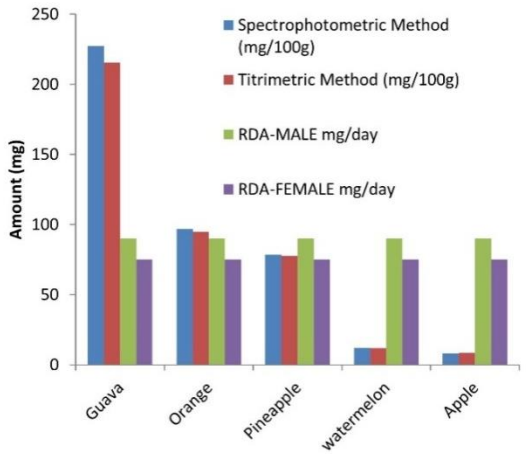


Fig. 2. Comparison of Ascorbic Acid Levels in different Fruit Extract obtained from both Titrimetric and Spectrophotometric Methods of Analysis with US Recommended Dietary Allowance (RDA).

Comparing both methods of analysis, it was observed that a higher value of ascorbic acid content was recorded for all fruit extracts using the spectrophotometric method relative to the titrimetric method. However, comparing the mean obtained from the analysis of the fruit extracts using both methods; there was no significant difference in the levels of ascorbic acid in each fruit suggesting that either method is efficient. Furthermore, subjecting the mean values to a One-way Analysis of Variance (one-way ANOVA), a a P-value of 0.97 was obtained, confirming that no significant difference exists between the two methods of analysis employed in this study.

Conclusion

In conclusion, the determination of ascorbic acid in some locally sourced fruits in Benin city employing both titrimetric and spectrophotometric methods, has provided significant insight into the various level of ascorbic acid present in these fruits. Among the fruits investigated, guava contained the highest of level ascorbic acid, while orange and pineapple were found to be equally rich in ascorbic acid. In addition, our findings reveal that the consumption of reasonable amount of any of these fruits (pineapple, guava, or orange) meets the Recommended Dietary Allowance (RDA) of 90 mg/day and 75mg/day prescribed for an adult male and female respectively by United States Department of Health and Human Services. However, the consumption of apple or watermelon in this regard does not meet with the recommended daily requirement due to their low ascorbic acid content. Also, our finding showed that both titrimetric and spectrophotometric methods of analysis are cheap, easy, and efficient methods of estimating the ascorbic acid content in fruits. Statistically, the one-way analysis of variance revealed that no significant difference exists between these two methods.

References

1. A.C.Ross, B.H.Caballero, R.J. Cousins, K.L. Tuckerand T.R. Ziegler,*Modern nutrition in health and disease,* Lippincott Williams and Wilkins publishers, Baltimore US (2014).
2. N.Boyera, I.Galey, and B A. Bernard,Effect of vitamin C and its derivatives on collagen synthesis and cross-linking by normal human fibroblasts. *International Journal of Cosmetic Science*, **20**: 151–158 (1998.).
3. A.J. Michels, M.H.Tory, and F.Balz, Human genetic variation influences vitamin C homeostasis by altering vitamin C transport and antioxidant enzyme function. *Annual review of nutrition* **33**: 45-70 (2013).
4. M.Levine, K.R.Dhariwal, R.W. Welch, Y.Wang and J.B. Park, Determination of optimal vitamin C requirements in humans. *The American journal of clinical nutrition*, ***62***(6): 1347S-1356S(1995).
5. S. Uddin, and S. Ahmad, Antioxidant protection against cancer and other human diseases. *Comprehen Therap.,***21**:41-45 (1995.
6. C.Rekha, G.Poornim, M.Manasa, V. Devi, J.Abhipsa, H.Kumar and T. Kekuda, Ascorbic acid, total phenol content and antioxidant activity of fresh juices of four ripe and unripe citrus fruits. *Chemical Science Transactions*, **1(2)**: 303–310 (2012).
7. M.M.Heinen, B.A.Verhage, R.A. Goldbohmand P.A. Van den Brandt, Intake of vegetables, fruits, carotenoids and vitamins C and E and pancreatic cancer risk in The Netherlands Cohort Study. *International journal of cancer*, **130(1)**:147-158, (2012.).
8. H. Siesand S. Wilhelm, Vitamin E.C. Beta -Carotene and other carotenoides as Antioxidants, *The American journal of clinical nutrition*,**62**:315S-321S, (1995).
9. Y.Li, and H.E. Schellhorn, New developments and novel therapeutic perspectives for vitamin C. *J. Nutr*., **137**:2171-84 (2007).
10. Institute of Medicine, Food and Nutrition Board,*Dietary Reference In takes for Vitamin C, Vitamin E, Selenium and Carotenoids*. Washington, DC: National Academy Press, US (2000).
11. M.J.Esteve, R.Farré, A.Frigola, J.C.López, J.M.Romera, M.Ramirez and A.Gil, Comparison of voltammetric and high-performance liquid chromatographic methods for ascorbic acid determination in infant formulas. *Food Chemistry*, **52(1)**: 99-102 (1995).
12. V.Raghu, K. Plateland K, Srinivasan, Comparison of ascorbic acid content of *Emblica officinalis* fruits determined by different analytical methods. *Journal of Food Composition and Analysis*, **20(6)**: 529-533 (2007).
13. I. Odriozola-Serrano, T. Hernández-Jover and O.Martín-Belloso, Comparative evaluation of UV-HPLC methods and reducing agents to determine vitamin C in fruits. *Food chemistry*, **105(3)**: 1151-1158 (2007).
14. T. Balogh and A.Szarka, A comparative study: Methods for the determination of ascorbic acid in small and middle-sized food analytic laboratories. *Acta Alimentaria*, **45(3)**: 354-362.2016.
15. I. Elgailani, M.Elkareem, E.Noh, O. Adamand A.Alghamdi, Comparison of two methods for the determination of vitamin C (ascorbic acid) in some fruits. *American Journal of Chemistry*, **2(1)**:1-7 (2017).
16. C.R. Silva, J.A.Simoni, C.H. Collins and P.L. Volpe, Ascorbic acid as a standard

for iodometric titrations. An analytical experiment for general chemistry. *Journal of chemical education*, **76(10)**:1421, (1999).
17. C.J. Ikewuchi and C.C. Ikewuchi Iodometric determination of the ascorbic acid (Vitamin C) content of some fruits consumed in a university community in Nigeria. *Global Journal of Pure and Applied Sciences*, **17(1)**: 47-49 (2011).
18. R.T. Varsha and N.P.Padma, Comparative Studies on Ascorbic acid content in Various Fruits, Vegetables and Leafy Vegetables. *Int. J. of Life Sciences*, **5(4)**: 667-671, (2017).
19. M. Noroozifar, and M.K. Motlagh, Application of potassium chromate-diphenylcarbazide in the quantitative determination of ascorbic acid by spectrophotometry. *Turkish Journal of Chemistry*, **27(6)**: 717-722 (2003).
20 National Agency for Food and Drug Administration and Control (NAFDAC) (2018), Food Fortification regulation pp 1-14.
21. S. Abdulrazak, Y.A. Oniwapele, D. Otie and Y. Ishola, Comparative Determination of Ascorbic Acid in Some Selected Fruits and Vegetables Commonly Consumed in Northern Nigeria. *J. of Global Bio-sciences*, **4(1)**:1867-1870 (2015).
22. B Frei, and M. Traber, The new U.S. dietary reference for Vitamin C and E *Redox rep*., **6**:5-9 (2001).
23. C. Varming, M.A. Petersen, and T.B. Toldam-Adersen, Ascorbic acid contented in Dannish Apples cultivars and commercial apple juice. LWT-Food Science and Technology, **54(2)**: 597-599 (2013).
24. L. Vermeiren, F. Devlieghere, N. de Kruijf, and J. Debevere, Development in the active packaging of foods. *Journal of Food Technology in Africa*, **5(1)**: 6-13 (2000).
25. M. Weinstein, P. Babyn and S. Zlotkin, An orange a day keeps the doctor away: scurvy in the year 2000. *Pediatrics*, **108(3)**:e55-e55 (2001).
26. V.O. Ajibola, O.A. Babatunde, and S. Suleiman, The effect of storage method on the vitamin C content in some tropical fruit juices. *Trends Appl. Sci. Res*, **4(2)**:79-84 (2009).