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The Titrimetric and Spectrophotometric Determination of Ascorbic acid levels in Selected Nigerian Fruits

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Abstract: *In this study, the ascorbic acid levels in mango, pawpaw, pear, banana and plantain were determined by titrimetric and spectrophotometric methods using oxalic acid and orthophosphoric acid as extracting solvents. The yield of ascorbic acid by titrimetric method ranged from 0.20 ± 0.02 mg/100g to 13.20 ± 0.05 mg/100g while spectrophotometric method ranged from 7.76 ± 4.7 mg/100g to 87.1 ± 6.3 mg/100g. Regardless of the extracting solvents and the estimation methods, the results revealed that ascorbic acid content was very high in pawpaw and mango while it was very low in plantain, banana and pear. Comparison of the two estimation methods indicated that spectrophotometric method detected more ascorbic acid than titrimetric method in all the selected samples. While the results from both methods are generally comparable in all the samples, the spectrophotometric method is a preferred method because higher contents of ascorbic acid were obtained relative to the titrimetric method.*

Keywords: *Ascorbic acid, Titrimetric, Spectrophotometric, Oxalic acid, Orthophosphoric acid.*

I. Introduction

Ascorbic acid, otherwise known as Vitamin C is an essential nutrient. It is a water-soluble vitamin whose dietary sources include fruits and vegetables. Humans are unable to synthesize vitamin C endogenously, and as such, it is consumed as essential dietary component (Li Y and Schellhorn, 2007). L-Ascorbic acid is the main biologically active form of vitamin C. It is a valuable food component because of its antioxidant and therapeutic properties (Okiei *et al.,* 2009). As a potent antioxidant, it has the capacity to eliminate several different free radicals (Davey et. al., 2000). It also has important biological and metabolic functions, particularly with respect to its role in the biosynthesis of connective tissue (Jacques, 1992). Mega doses of vitamin C is used in the treatment and prevention of large number of disorders like diabetes, cataracts, glaucoma, macular degeneration, atherosclerosis, stroke, heart diseases and cancer (Iqbal *et. al.*, 2004). Vitamin C also functions in collagen formation, absorption of inorganic iron, reduction of plasma cholesterol level, inhibition of nitrosamine formation, enhancement of the immune system, and reaction with singlet oxygen and other free radicals (Rekha *et. al*., 2012). Vitamin C deficiency is known to cause scurvy in humans (Holley et. al., 2011). The symptoms of scurvy include fatigue or lassitude, widespread connective tissue weakness, and capillary fragility (Sani et. al., 2015) Scurvy can be prevented with as little as 10 mg vitamin C per day, an amount easily obtained through consumption of fresh fruits and vegetables. A layman generally believes that the best sources of vitamin C are citrus fruits and their juices. However, there exists a large number of tropical fruits and vegetables that contain vitamin C. For better utilization of fruits and vegetables as a human food, clear understanding of their nutrition value as well as the content of vitamin C estimation is essential (Rahman et al., 2005). The study was thus undertaken to ascertain the preferred method to estimate the levels of Vitamin C in some selected fruits.

II. Materials And Methods

The plant materials used for this study include mango, pawpaw, pear, banana and plantain. The fruits were purchased at Sabo market in Ile-Ife, South West Nigeria.

2.1 Preparation Of Ascorbic Acid Extracts

The fruits were washed with distilled water, peeled, cut into small bits and weighed. The extraction of ascorbic acid from the fruits was done using 0.4% (w/v) oxalic acid and 2% (v/v) orthophosphoric acid as solvents.

2.2 Extraction With 0.4% (W/V) Oxalic ACID

5 g of each sample was weighed in triplicate, cut into tiny bits followed by homogenization with 5 ml of oxalic acid solution using mortar and pestle. This was followed by the addition of 10 ml of oxalic acid. The homogenate was then transferred into clean dried centrifuge tubes, followed by rinsing of pestle with another 5 ml of oxalic acid. The homogenate was then transferred into clean dried centrifuge tubes, followed by rinsing of pestle with another 5 ml of oxalic acid. The homogenate was centrifuged at 4,000 rpm for 10 mins at room

temperature. The supernatant was collected into vials while the residue was discarded. The extracts were stored frozen until analyzed.

2.3 Extraction With 2% (V/V) Orthophosphoric ACID

Extraction of each sample was also carried out as described above using 2% (v/v) orthophosphoric acid as solvent.

2.4 Estimation Of Ascorbic ACID Level By Titrimetric Method

The ascorbic acid level was estimated by titrimetric method using 1, 2-dichloro-6-indophenol dye as indicator. Ascorbic acid standard solution was prepared by dissolving 50 mg L-ascorbic acid in 50 ml of oxalic acid in a beaker. The solution was poured into 250 ml standard flask and the volume adjusted to mark with oxalic acid solution. This procedure was repeated using orthophosphoric acid solution. 5 ml of the ascorbic acid standard solution was titrated against 1, 2-dichloro-6-indophenol reagent until a rose-pink colour that persisted for about 15 seconds was obtained. The titration was done in triplicate and the titre values were recorded. 5 ml of each extract was also titrated against 1, 2-dichloro-6-indophenol reagent in triplicate and titre values were equally recorded.

2.5 Estimation Of Ascorbic ACID Level By Spectrophotometric Method

Ascorbic acid level was estimated by the method of Roe and Kuether (1943) with some modifications. 1 ml of each extract was placed in a test tube in triplicate. 9 ml of 4% trichloro acetic acid (TCA) was added to give a 10-fold dilution. The suspension was thoroughly shaken and filtered. 0.5 ml of 10% thiourea solution was added to 1 ml of the filtrate placed into clean test tubes. This was followed by the addition of 0.5 ml of 2% 2, 4-dinitro phenyl hydrazine (DNP) reagent to the remaining test tubes apart from the blank. The test tubes were incubated in a water bath at 37^oC for 3 hours and cooled in ice. 2.5 ml of 85% H_2SO_4 was added to each of the test tubes through the burette. Finally, 0.5% of 2% 2, 4-dinitro phenyl hydrazine (DNP) reagent was added to the blank. The tubes were shaken thoroughly in ice and left for 30 min for colour formation. Absorbance was read at 540 nm using a Pharmacia LKB spectrophotometer against the reagent blank. A standard curve of ascorbic acid 0-100 mg per liter was established. The values of ascorbic acid were expressed as mg/100g of fresh sample.

2.6 Statistical Analysis

The results were expressed as mean ± standard error of mean of three determinations.

III. Results And Discussion

Table 3.1: Ascorbic acid contents of selected fruits by titrimetric method

Sample		Ascorbic acid content by titrimetric method	
Common name	Botanical name	Oxalic acid (mg/100g)	Orthophosphoric acid (mg/100g)
Banana	*Musa paradisiaca*	0.20 ± 0.02	0.30 ± 0.02
Mango	*Mangifera indica*	6.50 ± 0.20	4.60 ± 0.10
Pawpaw	*Carica papaya*	13.20 ± 0.05	9.98 ± 0.09
Pear	*Pyrus communis*	0.53 ± 0.03	0.29 ± 0.02
Plantain	*Musa sapientum*	0.30 ± 0.01	0.30 ± 0.02

Table 3.2: Ascorbic acid contents of selected fruits by spectrophotometric method

Sample		Ascorbic acid content by spectrophotometric method	
Common name	Botanical name	Oxalic acid (mg/100g)	Orthophosphoric acid (mg/100g)
Banana	*Musa paradisiaca*	39.2 ± 0.8	19.0 ± 4.6
Mango	*Mangifera indica*	45.8 ± 7.6	70.2 ± 3.9
Pawpaw	*Carica papaya*	51.5 ± 6.3	87.1 ± 6.3
Pear	*Pyrus communis*	13.8 ± 0.8	7.76 ± 4.7
Plantain	*Musa sapientum*	24.2 ± 6.0	15.3 ± 2.3

The results of the determination of the ascorbic acid content of selected fruits using oxalic acid and orthophosphoric acid as extracting solvents by titrimetric and spectrophotometric methods are shown in tables 1 and 2 respectively. The ascorbic acid content by titrimetric method ranged from 0.20 ± 0.02 mg/100g to 13.20 ± 0.05 mg/100g while spectrophotometric method ranged from 7.76 ± 4.7 mg/100g to 87.1 ± 6.3 mg/100g. The spectrophotometric method showed that the ascorbic acid content was highest in pawpaw and lowest in pear for both extracting solvents. However, there was a different outlook with the titrimetric method, which showed that ascorbic acid was highest in pawpaw and lowest in banana when oxalic acid was used as the extracting solvent, while it was highest in pawpaw and lowest in pear when orthophosphoric acid was used as the extracting

solvent. This shows that the titrimetric method could be inconsistent and unreliable, and thus cannot be preferred.

Higher contents of ascorbic acid were obtained with the spectrophotometric method in all the selected fruits when compared with the titrimetric method. This demonstrates clearly that the titrimetric method is less sensitive for the determination of ascorbic acid content of fruits. This disparity could be attributed to poor detection of end point or the presence of substances that may interfere with the reagent in the titrimetric method as suggested by Okiei *et. al.*, (2009).

In this study, the ascorbic acid levels detected in pawpaw was lower than what was obtained by other authors (74 ± 7 mg/100 g (Franke *et al.*, 2004), 68 ± 13 mg/100 g (Leong & Shui, 2002) and 147 ± 10 mg/100 g (Yurena *et. al*., 2006) using titrimetric method with oxalic acid as extracting solvent. Franke *et al.* (2004) and Leong and Shui (2002) established ascorbic acid content for mango as 13.2 ± 5.1 and 19.7 ± 9.1 mg/100 g respectively, both of which were lower compared to the values obtained for the spectrophotometric method used in this study.

IV. Conclusion

The study concludes that spectrophotometric method was a more sensitive method for a satisfactory, consistent and reliable quantitative analysis of ascorbic acid in the selected fruits.

References

[1]. Y. Li., and H.E. Schellhorn, New developments and novel therapeutic perspectives for vitamin C, *Journal of Nutrition 137(10),* 2007, 2171-84.

[2]. W. Okiei, M. Ogunlesi, L. Azeez, V. Obakachi, M. Osunsanmi, G. Nkenchor, The voltametric and titrimetric determination of ascorbic acid levels in tropical fruits samples, *International Journal of Electrochemical Science, 4(2),* 2009, 276 – 287.

[3]. M.W. Davey, M.V. Montagu, D.I.M. Sanmartin, A. Kanellis, N. Smirnoff, I.J.J. Benzie, Plant L-ascorbic acid: Chemistry, function, metabolism bioavailability and effects of processing, *Journal of the Science of Food and Agriculture, 80(7),* 2000, 825-860.

[4]. P.F. Jacques, A Cross Sectional Study of Vitamin C Intake and Blood Pressure in the Elderly, *International Journal of Vitamin and Nutritional Research, 62(3),* 1992, 252-255.

[5]. K. Iqbal, K. Alam and M.M.A.K. Khattak, Biological Significance of Ascorbic acid (Vitamin C) in Human Health- A Review, *Pakistan Journal of Nutrition 3(1),* 2004, 5-13.

[6]. C. Rekha, G. Poornima, M. Manasa, V. Abhipsa, J. Pavithra Devi, H.T. Vijay Kumar, T.R. Prashith Kekuda, Ascorbic acid, total phenol content and antioxidant activity of fresh juices of four ripe and unripe citrus fruits, *Chemical Science Transactions 1(2),* 2012, 303-310.

[7]. A.D. Holley, E. Osland, J. Barnes, A. Krishnan, J.F. Fraser, Scurvy: historically a plague of the sailor that remains a consideration in the modern intensive care unit, *International Medicine Journal 41(3)* 2011, 283-285.

[8]. Sani, A.O. Yusuf, O. Dennis and I.S. Yusuf, Comparative Determination of Ascorbic acid in some selected fruits and vegetables commonly consumed in Northern Nigeria, *Journal of Global Bioscience 4(1),* 2015, 1867-1870.

[9]. M.M. Rahman, M.M. Khan, A.T.M. Murad and S.A. Begum, Determination of vitamin C content in various fruits and vegetables by UV- spectrophotometric method at Sylhet area, Bangaladish, *Journal of Environmental Science, 11*, 2005, 190 – 193.

[10]. J.H. Roe and C.A. Kuether, The determination of ascorbic acid in whole blood and urine through 2, 4-dinitro phenyl hydrazine derivative of dehydroascorbic acid. *Journal of Biological Chemistry 147*, 1943, 399-410.

[11]. W. Okiei, M. Ogunlesi, L. Azeez, V. Obakachi, M. Osunsanmi, G. Nkenchor, The voltametric and titrimetric determination of ascorbic acid levels in tropical fruits samples. *International Journal of Electrochemical Science, 4(2),* 2009, 276 – 287.

[12]. A.A. Franke, L.J. Custer, C. Arakaki, & S.P. Murphy, Vitamin C and flavonoid levels of fruits and vegetables consumed in Hawaii. *Journal of Food Composition and Analysis, 17(1)* 2004, 1-35.

[13]. L.P. Leong, & G. Shui, An investigation of antioxidant capacity of fruits in Singapore markets, *Food Chemistry, 76(1),* 2002, 69-75.

[14]. H. Yurena, M.G. Lobo, M. González. Determination of vitamin C in tropical fruits: a comparative evaluation of methods. *Food Chemistry 96 (4),* 2006, 654-664.