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Comparative Determination of Ascorbic Acid in Apple (*Malus Pumila*), Lime (*Citrus Aurantiifolia*) andWatermelon (*Citrullu Lanatus*) Using Uv-Spectrophotometric and Titrimetric Methods

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Abstract

Ascorbic Acid, (Vitamin C), is an essential antioxidant needed by the human body. Ascorbic acid is of great importance in biochemical reactions as a reducing agent. This study was performed to compare UV-Spectrophotometry and Titrimetric quantitative methods of analysing ascorbic acid content of Apple (Malus pumila), Lime (Citrus aurantiifolia) and Watermelon (Citrullu lanatus). The identification tests for the standard Ascorbic acid and fruits samples were carried out using Standard Procedures. The melting point and absorbance of the standard Ascorbic acid were 190.3 ^{0}C-192.1 ^{0}C and 0.569 respectively. The chemical tests for the fruit samples were conducted and grey precipitates were observed, which confirmed the presence of Ascobic acid in the samples. The amount of Ascorbic Acid in Apple, Watermelon and Lime were 0.4209 g/L, 0.2906 g/L and 0.2639 g/L respectively using Titrimetric method. The amount of Ascorbic acid in Apple, Watermelon and Lime using UV-Spectrophotometry method, were 0.06207 g/L, 0.06982 g/L and 0.08382 g/L respectively.The difference between the two methods was large, as all the three fruits showed difference amounts. Although UV-Spectrophotometric method has been reported in other researches to be preferred, reliable, fast and accurate, this research concluded that Titrimetric method was more favourable for the determination of Ascorbic Acid in the fruits selected.

Key words: Ascorbic acid, UV- Spectrophotometry, Titrimetric, Fruits

Introduction

Vitamins are organic chemical compounds which are found in tiny amounts in natural foodstuffs. These vitamins are required by an organism as a vital nutrient to sustainto its life, because they play a major role in normal metabolism process, growth and vitality. Diet has been major source of Vitamin C or from vitamins supplements.Vitaminsare micronutrient; required in defined quantity by the body. The dietary requirement for a micronutrient is defined as an intake level which meets with specificcriteria for adequacy, thereby minimizing risk of nutrient deficit or excess. Most animals can synthesize their own vitamin C, except human and other primates (Kumar *et al.*, 2013). The body needs vitamin C to make a substance called collagen which is required for the health and repair of the skin, bones, teeth and cartilage (Rahman *et al*., 2006). Vitamin C was first

isolated in 1928, and in 1932 it was proved to be the agent, which prevents scurvy. The recommended daily amount of Vitamin C for adults is 65 – 90 mg/day and the upper limit is 2 g/day. Mega dose of Vitamin C supplements might be associated with any or some these; diarrhoea, nausea, headache, abdominal cramps, heartburn and, Insomnia (Pauling, L. (1976).The common sources of vitamin C are citrus fruits,Vitamins are generally classified into two main broad categories which are, water soluble vitamins (Vit B12, Vit C, folic acid, niacin Vit B3, Vit B1 and Vit B2) and fat soluble vitamins (Vit K, Vit E, Vit D and Vit A) (Wonsawat, W., 2006).The chemical formula of ascorbic acid is (R)-5-((S)-1,2-dihydroxyethyl)3,4-dihydroxyfuran2(5H)-one, molar mass 176.12 g mol−1, Density 1.65 g/cm3, melting point 190-192 °C, with solubility in water about 33 g/100 mL (Rahman *et a*l., 2006; Fadhel, 2012) L-enantiomer of ascorbic acid, it is a water-soluble vitamin used by the body for several purposes.

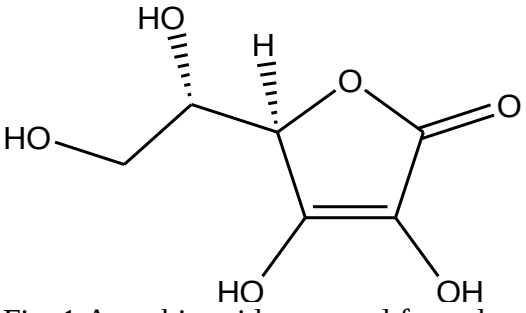

Fig. 1 Ascorbic acid structural formular

Several methods of analysis have been used to determine Ascorbic acid contents in Fruits,Spectrophotometry and Amperometry had been compared. (Arya et al., 1998; Arya *et al*., 2000). The development of rapid, simple and cheaper analytical method is one of the areas of growing interest especially in the fields of Environment, Medical and Industrial (Shephard *et al*., 1999). Other methods used for Ascorbic acid determination includes Titrimetric, Chromatography (Rahman *et al*., 2006) Voltammetry, Fluorometry and Potentiometric (Kabasakalis *et at*., 2000). But the most frequently used simple method is Spectrophotometric (Fadhel, 2012). In this research, Spectrophotometry and Titrimetry methods were compared for the Ascorbic acid analysis.

Materials and methods:

Fruits samples were collected from Gawon Nama area, Wamako Local Government of Sokoto, Sokoto State, Northwest of Nigeria. The samples used in this study include apple, watermelon and lime. They were peeled, wrapped with aluminium foil and kept in a clean, dry area before use.

Materials

All chemicals and reagents used in this study were of analytical grade. UV-Visible Spectrophotometer was also used for spectrophotometric measurements and Digimelt (MPA161 MSRS made in US) was used for checking the melting point of the standard Ascorbic Acid powder. The glassware and other apparatus used in this work were cleaned and dried before use.

Reagents/ apparatus

Oxalic acid (0.5%w/v) 100ml, Standard Ascorbic acid, Potassium Permanganate solution Potassium Iodide solution, Hydrochloric acid, Potassium Iodate, Volumetric flask (25 mL, 100 mL, 250 mL, 500 mL), Measuring cylinder (25 mL, 100 mL), Conical flask (250 mL), Graduated pipette (1 mL, 5 mL, 25 mL)

Methods

Identification Tests

Standard Ascorbic Acid: Standard Ascorbic acid powder 0.10 g was dissolved in distilled water and diluted immediately to 100 mL in a volumetric flask with the same solvent. 1.0 mL of this solution was added to 10.0 mL of 0.1M Hydrochloric acid and diluted to 100 mL with distilled water in a volumetric flask. The absorbance this resultant solution was taken and recorded at 243 nm.

Physical Test of Standard Ascorbic acid: A little quantity of the standard ascorbic acid powder was transferred into a capillary tube and the melting point was determined.

Chemical Test for Fruits Samples: To 1 mL of each of the samples (A, B and C) taken in a test tube, 0.2 mL of dilute nitric acid (HNO_3) and 0.2 mL of silver nitrate ($AgNO_3$) were added respectively and observed.

Preparation of Fruit Samples

The aqueous extracts for each of the three samples (A,B and C) were prepared by weighing accurately 100 g of the freshly prepared fruit sample in a 500 mL beaker and blended vigorously with 30 mL of oxalic acid (0.5 %w/v). Each of the mixtures were filtered through a pre-cleaned cloth into a 250 mL Erlenmeyer flask. The aliquot of each sample was transferred to a 100 mL volumetric flask and then completed to the mark with oxalic acid solution (0.5%w/v). Blank solution was prepared by the same manner without the fruit samples. Each of the three (3) fruit samples was treated separately as described above.

Preparation of Stock and Standard Solutions of Ascorbic Acid

Standard solution of ascorbic acid was prepared by dissolving an accurate weight of 0.01g of standard ascorbic acid in small amount of oxalic acid solution (0.5 %w/v) and then completed to 100 mL with the same solution to obtain a concentration of 100 µg/mL. A series of dilutions 1.0, 4.0, 8.0, 12 and 16 µg/mL were prepared from the stock ascorbic acid solution.

Preparation of Potassium Permanganate Solution

A solution of $KMnO_4$ of concentration of 100 µg/mL was prepared by dissolving an accurate weight of 0.01 g of $KMnO_4$ in H_2SO_4 solution (5.0M), then transferred into a 100 mL volumetric flask and completed to the mark with distilled water and thoroughly mixed. This solution was usedfor the determination of ascorbic acid (vitamin C) by a spectrophotometer.

Preparation of fruit samples for uv-visible Spectrophotometer

To each of the three fruit samples (A, Band C), 10.0 mL were accurately measured and then transferred into a test tube and 1.0mL of $KMnO_4$ (100µg/mL) was added for each. The contents of each test tube were mixed and allowed to stand for 5 minutes. The absorbance of the prepared solutions was read at 530 nm against blank by spectrophotometer.

Titration method

An accurately 1 mL of each of the freshly prepared fruit sample solution was transferred and then diluted to 200 mLwith distilled water. Then 10 mL of each of these solutions were transferred into a conical flask. To this flask, 5.0 mL of(0.2 M)KI

solution,2.5 mL of (1.0 M) hydrochloric acid (HCl)and three drops of starch solution were added. Each of the three solutions was then titrated against (0.015 M) KIO_3until the appearance of blue -black colour which indicates the end point of the reaction. The titration was repeated two times for each of the fruit samples. The results were recorded and tabulated for ascorbic acid determination for each samples.

Preparation of standard calibration curve of ascorbic acid

Standard calibration curve of ascorbic was established by plotting a graphof concentrations versus absorbance of ascorbic standard solutions at concentrations 1, 4, 8, 12 and 16 µg/mL by adding1 mL of $KMnO_4$ solution. These solutions were allowed to stand for 5 minutes. The absorbance of these standard solutions was read at 530 nm against blank.

Results

Identification Tests

Standard Ascorbic acid

Melting point = 190.3^0C-192.1 ^{0}C

Absorbance = 0.569

Chemical test for the fruit samples

Table 1: Result for chemical test on the fruits samples

SAMPLES	TEST	OBSERVATION	INFERENCE
Apple	1 mL of sample+ 0.2 mL of dilute HNO_3+ 0.2 mL of $AgNO_3$	Grey precipitate	Ascorbic acid was confirmed
Watermelon	1 mL of sample+ 0.2 mL of dilute HNO_3+ 0.2 mL of $AgNO_3$	Grey precipitate	Ascorbic acid was confirmed
Lime	1 mL of sample+ 0.2 mL of dilute HNO_3+ 0.2 mL of $AgNO_3$	Grey precipitate	Ascorbic acid was confirmed

Determination of ascorbic acid in the fruit samples by titrimetric method

Table-2: Amount of vitamin C in the fruit samples as determined by Titrimetric Method

Fruit samples	Vol. of $\boldsymbol{KIO_3}$ used (cm^3)				Vitamin C in mole/L	Vitamin C in g/L
	I.	II.	III.	Average volume		
Apple	0.600	0.500	0.500	0.533	0.00239	0.4209
Watermelon	0.400	0.300	0.400	0.367	0.00165	0.2906
Lime	0.400	0.300	0.300	0.333	0.00149	0.2639

Preparation of the calibration curve of Ascorbic acid (Vitamin C)

Calibration curve graph for the Ascorbic acid in the prepared fruit samples (Apple, Watermelon and Lime) were plotted by plotting the absorbance as a function of the corresponding concentrations. There was an observed proportional increase in absorbance at the respective λmax with increasing concentration of each ascorbic acid standard solutions.

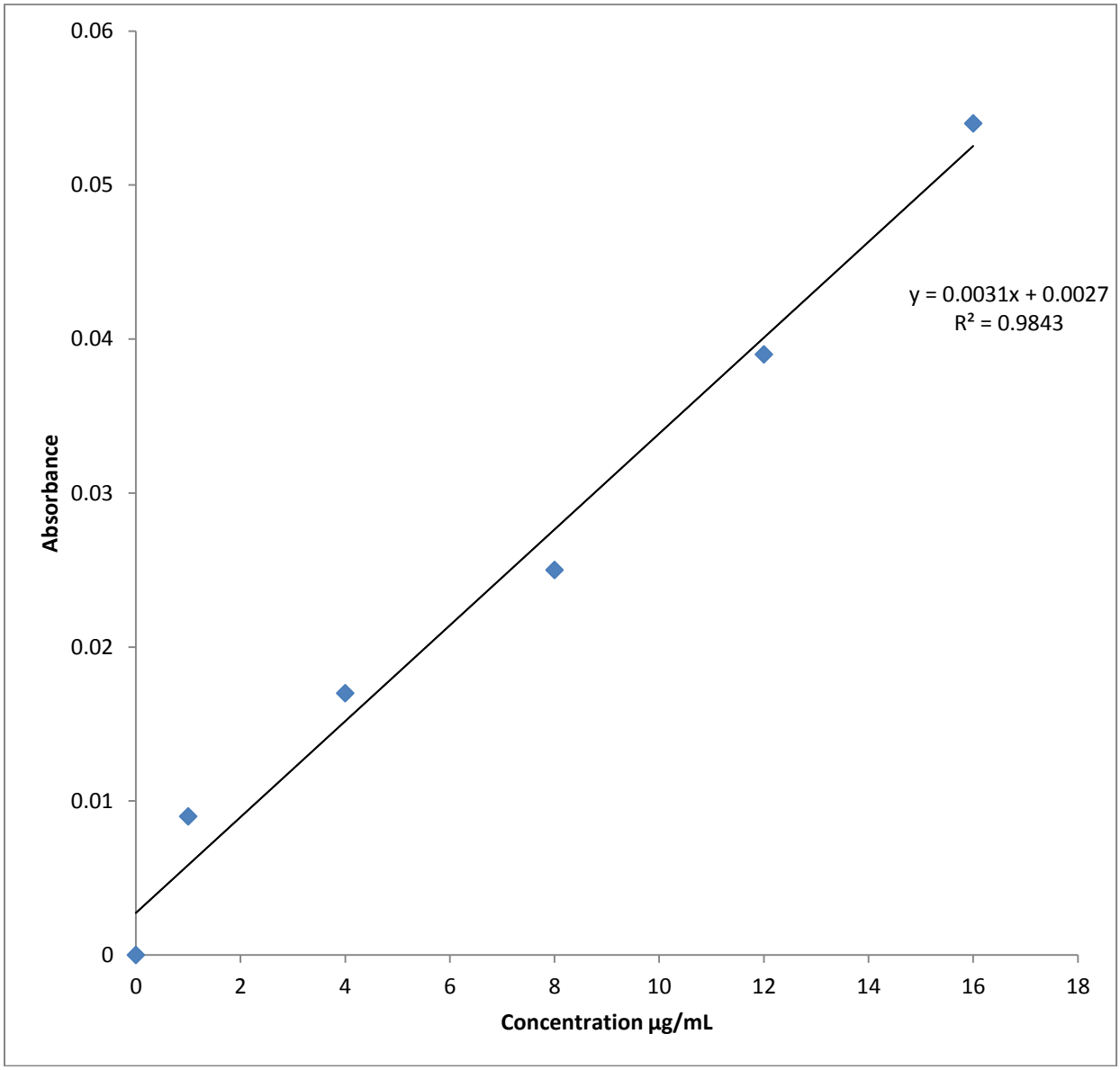

Figure-1.Calibration Curve for the determination of ascorbic acid (vitamin C) by UV-Visible Spectrophotometry

Determination of ascorbic acid (vitamin C) in the fruit samples by spectrophotometer

Each of fruit samples solutions were treated in the same way for ascorbic acid determination. The absorbance of the sample solutions was measured. These absorbance values were referred to their related concentrations in the calibration curves (figure 1). The results obtained by UV-Visible Spectrophotometer reveal varying amounts of vitamin C in fruit samples.

Table-3: Amount of vitamin C in the fruit samples as determined by UV-Spectrophotometry at 530nm

Fruits Samples	Absorbance	Vitamin C (µg/mL)	Vitamin C (g/L)
Apple	1.927	62.07	0.06207
Water melon	2.167	69.82	0.06982
Lime	2.601	83.82	0.08382

Comparing the results of uv-spectrophotometry and titrimetric analysis

The comparison of the two methods was carried out; it was found that there is quite a large difference between the two methods as all the three fruits (Apple, Water melon and Lime) showed differences in the amount of vitamin C as seen in (Figure 2).

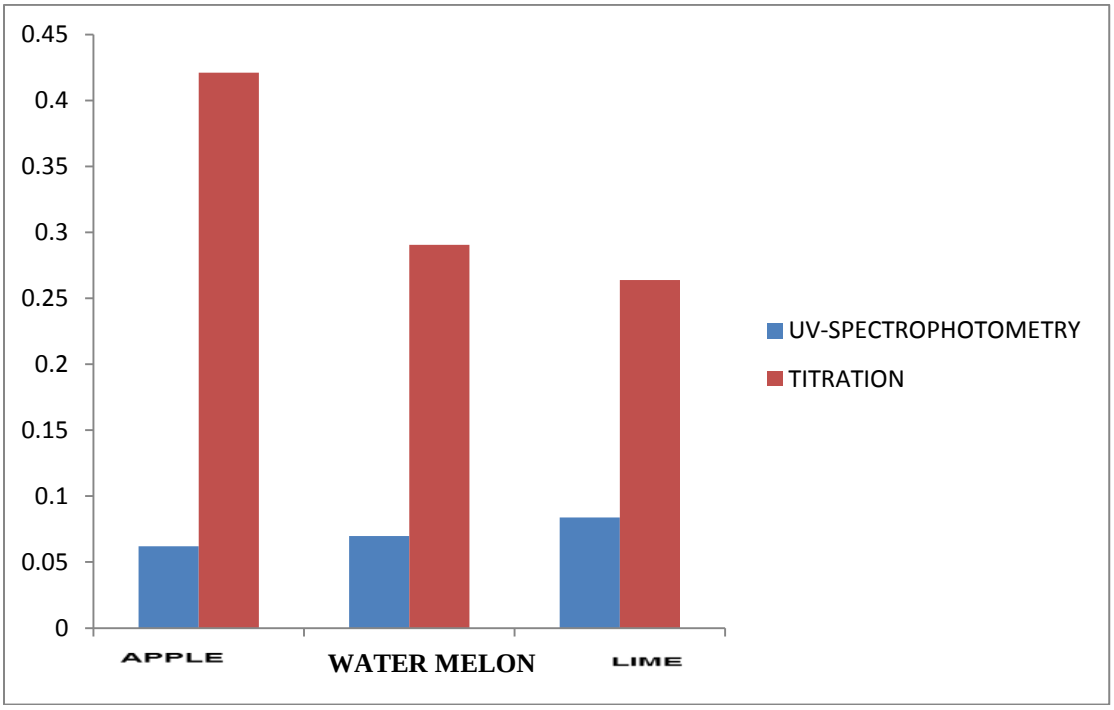

Figure 2: Comparing UV-Spectrophotometry and Titrimetric analyses results.

Discussion

Ascorbic acid (Vitamin C) is a strong reducing agent which reacts stoichiometrically with oxidizing agents such as iodineand 2, 6 dichloro phenolindophenol (Ajibade GA.1997). Similar research has shown that iodine and 2, 6 dichloro phenolindophenol was used in the titrimetric determination of ascorbic acid and the results of the analysis showed as followed; orange - 11.80 cm^3, pineapple - 1.60 cm^3 and apple - 0.80 cm^3 (Ajibade GA.1997). From thisresearch carried out iodine was used in the titrimetric determination of ascorbic acid and the results of the analysis are 0.533 cm^3, 0.367 cm^3, 0.333 cm^3 for Apple, Watermelon and Lime respectively.The values vary when compared to the one in the previous research. This indicated negligible amount of foreign matter.

Preliminary analysis was carried out on pure ascorbic acid sample and was used to determine its level of purity. The results showed that the sample was of analytical grade from its melting point = 190.3 ^{0}C-192.1 ^{0}C and absorbance = 0.569. The results obtained from analysis carried out confirmed the presence of ascorbic acid in the fruit samples analysed.

The high values of ascorbic acid content of the three fruit samples analysed proved that the use of Potassium Iodate solution in the titrimetric estimation of ascorbic acid in liquid diet as a satisfactory analytical method (Plummer, 1978).The highest quantity using titrimetric method was obtained from Apple, followed by Watermelon and Lime while the highest quantity using UV-Spectrophotometry method was obtained from Lime followed by Watermelon and then Apple. Low ascorbic acid value was obtained from Apple (*Malus pumila*) using UV-Spectrophotometry and this could be attributed to the presence of coloured pigments as its dilute solution was lightly coloured. Isam*et al*., 2017, carried out the same methods comparison of UV-Spectrophotometry and Titrimetric analysis of some citrus fruits. But his results showed a negligible difference in the two methods.

From the research conducted it can be concluded that the sample and standard ascorbic acid powder were identified using physical and chemical test. The ascorbic acid content of sample A, B and C using Titrimetric method and UV-Spectrophotometry method were determined.The comparison of the two methods was carried out; it was found that there is a large difference between the two methods as the three fruits (Apple, Watermelon and Lime) showed differences in the amount of vitamin C using Titrimetric and UV-Spectrophotometry methods. Although UV-Spectrophotometric method has been reported in other researches to be preferred, reliable, fast and accurate, this research concluded that Titrimetric method is more favourable for the determination of Ascorbic Acid in fruits from the results obtained.

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