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DETERMINATION OF VITAMIN C (ASCORBIC ACID) FROM *Annona squamossa* AND *Valvet tamarind* FRUITS

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ABSTRACT

This research was designed to compare two methods for determination of vitamin C (Ascorbic acid) content in *Annona squamosa* peel and pulp as well as in Velvet tamarind pulp. Vitamin C content of fresh fruit parts were determined by titrimetric and spectrophotometric methods using potassium permanganate as chromogenic reagent. Absorbance was measured spectrophotometrically at 530nm. The titration method was carried out using iodometric back-titration. The results obtained from the study revealed that there was significant difference between the two methods and the higher vitamin C content was recorded in A*nnona squamosa*. Both samples could therefore be used to supplement vitamin C daily intake requirements.

Keywords: Annona squamossa, Valvettamarind, Vitamin C, Ascorbic acid, Spectrophotometer.

INTRODUCTION

Vitamin C (Ascorbic acid) is an important water–soluble vitamin which is already implicated in most processes of life and principally function as an antioxidant. It is abundantly present in fruits and vegetables where the common man in developing countries receives most of their daily intake (Falade *et al*., 2004; Abitogun *et al*., 2010). Evidence of vitamin C playing a key role in decreasing the incidence of degenerative diseases is considered to be strong (Adepoju, 2009). Low ascorbic acid levels have been associated with fatigue and increased severity of respiratory tract infections, while high intake of vitamin C from food had been shown to raise serum HDL–cholesterol and lowers serum triglyceride concentration (Adepoju, 2009). Recommended daily allowance of vitamin C intake for infants, children, adult males and adult females, pregnant women and lactating mothers are 30, 45, 60, 60, 70, 90 mg/day respectively (Ganong, 2003). Excessive intake of vitamin C show no evidence that neither confers any benefits nor prevents any hazards, but doses as high as 2 g/day can increase oxalate excretion and hence increase the risk of developing oxalate renal stones (Geissler & Power, 2005). Deficiency of vitamin C is known as scurvy, which is due to impaired collagen synthesis and this is associated with listlessness and general malaise, and sometimes bring about changes in personality and psychomotor performance (Geissler & Power, 2005). Most animals can synthesize their own vitamin C, except human and other primates (Kumar *et al.,* 2013; Elgailani *et al*., 2017)). To maintain a good and sound health and prevention of colds and a healthy body, the human species must remain saturated with vitamin C (Hassan *et al*. 2016).

Several analytical methods have been reported for determination of vitamin C using spectrornetry and amperometry (Arya *et al,* 1998; Arya *et al,* 2000; Isam *et al*., 2017)). The development of rapid, simple and inexpensive analytical methods is one of the areas of growing interest, more especially since quick decisions are needed in environmental, medical and industrial fields

(Shephard *et al,* 1999). Many analytical methods have been used for ascorbic acid determination, including titrimetric, spectrophotometry and chromatography (Selirnovic *et al.,* 2011; Fadhel, 2012). Spectrophotometry is one of the most frequently used simple methods, because Vitamin C is able to absorb UV ray (Wonsawat, 2006). The method is suitable for use with vitamin C tablets, fresh or packaged fruit juices and solid fruits and vegetables (Anal *et al*., 2019)). Due to the importance of vitamin C in human beings, quantitative analysis of vitamin C has gained increased significance in several areas of analytical chemistry such as pharmaceutical and food applications. The purpose of the study is to compare titrimetric and spectrophotometric methods in determining the concentration of vitamin C in pulp and peel of *Annona squamosa* and *Valvet termarind* pulp fruits grown in the study area. *Annona squamosa* fruit is called sugar-apple or sweetsop in English. The *Valvet tamarind* is known as '*tsamiyar birai*' in Hausa; '*Awin*' or '*Igbaru*' in Yoruba and '*Icheku*' in Igbo.

MATERIALS AND METHODS

Sample Collection and Treatment

Fresh ripped fruits of *A. squamosal* and *V. tamarind* were purchased from the local market for analysis. The pulp, peel and seed were separated manually and were allowed to dry for two weeks. The samples were then ground into fine powder using mechanical blender and then stored in labeled plastic bottles for analysis.

Method

Preparation of Fruit Samples

Aqueous extracts for each of the two samples were prepared by accurately weighing 100g of the freshly prepared fruit sample in a 500 cm^3 beaker and blended vigorously to obtain the fruit juice by adding 50 cm^3 of oxalic acid (0.5% w/v) in order to prevent the oxidation of ascorbic acid (vitamin C). Each of the mixtures were filtered through a precleaned cloth andthe filtrate collected in a 50 cm^3 Erlenmeyer flask. The aliquot of each sample was transferred to a 100 cm^3 volumetric flask and then made up to the mark with oxalic acid solution (0.5%). A blank was prepared by the same manner except for the addition of fruit samples. Each of the two fruit samples was treated separately as described under the general procedure (lsam *et al*., 2017).

Preparation of Standard Solutions of Ascorbic Acid

A standard solution of ascorbic acid was prepared by dissolving an accurate weight of 0.0lg of standard ascorbic acid in a small amount of oxalic acid solution (0.5 %) and then completed to 100 cm^3 with the same solution to obtain a concentration of 100 mg/cm^3. A series of dilutions 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mg/cm^3 were prepared from the stock of ascorbic acid solution.

Preparation of Calibration Curve of Ascorbic Acid

A standard calibration curve of ascorbic acid was prepared by plotting a graph of concentrations versus absorbance of ascorbic standard solutions by taking 10 cm^3 of each of the standard solutions and putting them in a test tube, then 1cm^3 of KM_nO_4 solution (100 mg/cm^3) added. These solutions were let to stand for 5 minutes. The absorbance of these standard solutions was read at 530 nm against the blank.

Preparation of Samples for Analysis by Method UV-Visible Spectrophotometer

Each of the five fruit samples were accurately taken at 10 cm^3 for each sample, and then transferred into a test tube and 1.0 cm^3 of KM_nO_4 (100 mg/cm^3) added to each. The contents of each test tube were mixed well

and allowed to stand for 5 minutes. The absorbance of the prepared solutions were read using a spectrophotometer.

Determination of Ascorbic Acid in the Samples by Method Titration

An accurate measure of 1cm^3 of each of the freshly prepared fruit sample solutions was transferred into a test tube and diluted to 200 cm^3 with distilled water. Then 10 cm^3 of each of these solutions were put into a conical flask. To this flask 5.0 cm^3 of KI solution (0.2 M), 2.5cm^{3}of hydrochloric acid HC1 (1.0 M) and a few drops of starch solution were added. Each of the five solutions was then titrated against KIO_3 (0.015 M) until the appearance of blue -black colour which indicated the end point of the reaction. The titration was repeated three times for each of the fruit samples. The results were recorded, tabulated and calculated for ascorbic acid determination for each sample.

Statistical Analysis

The data obtained were analyzed and presented as mean ± standard deviation of triplicate.

RESULTS AND DISCUSSION

The results of Vitamin C content in the fruits samples (pulp and peels), calibration curve for determination of Vitamin C, effect of sample concentration on absorbance and effect of sample concentration on Vitamin C content, were summarized in tables 1, 2, 3, 4 and also in figures 1, 2, 3 and 4 respectively.

The calibration curve for the determination of ascorbic acid in the prepared samples and KM_nO_4 solution were made by plotting absorbance as a function of corresponding concentrationat 4 levels (figure 1). The absorbance over the concentration range was linear with a regression coefficient of 1.00.

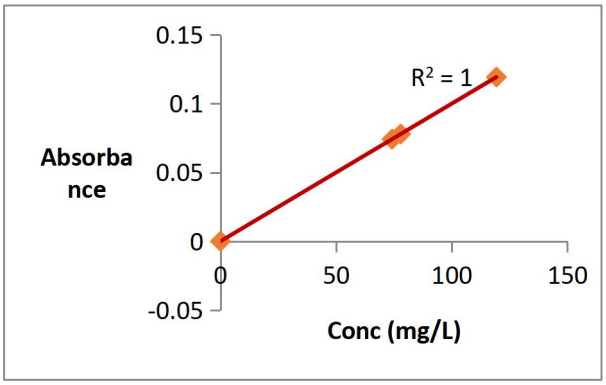

Figure 1: Calibration Curve for Determination of Ascorbic Acid (Vitamin C)

The quantitative relation between the concentration of titration and the decrease in absorbance of the $KMnO_4$ solution was studied by preparing serial dilution of standard solutions including 5 cm^3, 10 cm^3, 20 cm^3, 30 cm^3 and 40 cm^3, then the absorbance of the background solution in the absence of acid solution was recorded at 5 cm^3 after 5 minutes of incubation time.

Vitamin C Content of the Fruits as Determined by Spectrophotometric Method

Vitamin C content of *A. squamosa* pulp, peel and *V. tamarind* fruits were presented in Table 1, 2 and 3 respectively. The value obtained in *A. squamosa* pulp ranged from 95.7 to 141.9 mg/L, with a mean of 119.34 ± 17.27. This value was found to be higher when compared with that of *A. squamosa* peel and *V. tamarind* having vitamin C content ranged from 72.2 to 84.1 mg/L, with a mean of 77.92 ± 4.95 mg/L and 72.9 to 77.7 mg/L with a mean of 74.2 ± 2.024 respectively. There is sufficient evidence that the means of vitamin C content in all the samples are significantly different (One-way ANOVA, p-value = 0.000) at 95% confidence limit.

Table 1: Determination of Vitamin C Concentration in *A. squamosa* Pulp Using Spectrophotometric Method.

Sample / Concentration	Absorbance	Vitamin C (mg/L)	Vitamin C (g/L)*
5	0.0957	95.7	47.85
10	0.1135	113.5	56.75
20	0.1170	117.0	58.50
30	0.1286	128.6	64.30
40	0.1419	141.9	70.95

*Equivalent conc. After multiplying by dilution factor (500)

Table 2: Determination of Vitamin C Concentration in *A. Squamosa* Peel Using Spectrophotometric Method

Sample / concentration	Absorbance	Vitamin C (mg/L)	Vitamin C (g/L)*
5	0.0727	72.70	36.35
10	0.0748	74.80	37.40
20	0.0758	75.80	37.90
30	0.0822	82.20	41.10
40	0.0841	84.10	42.05

*Equivalent conc. After multiplying by dilution factor (500)

Table 3. Determination of Vitamin C Concentration in *V. Tamarind* Pulp Using Spectrophotometric Method.

Sample / concentration	Absorbance	Vitamin C (mg/L)	Vitamin C (g/L)*
5	0.0729	72.90	36.45
10	0.0730	73.00	36.50
20	0.0732	73.20	36.60
30	0.0742	74.20	37.10
40	0.0777	77.70	38.85

*Equivalent conc. After multiplying by dilution factor (500)

The result of absorbance ahainst sample concentration is presented in Fig. 2. The figure shows increase in absorbance with correresponding increase in samples concentration. *A. squamosa* pulp shows higher absorbance than its peel and *V. tamarind* pulp. A similar trend (in the concentration of vitamin C content) was also observed in all samples (figure 3). As evident from the results, a very good amount of vitamin C content was recorded in all samples, implying that they could supplement daily requirement of vitamin C.

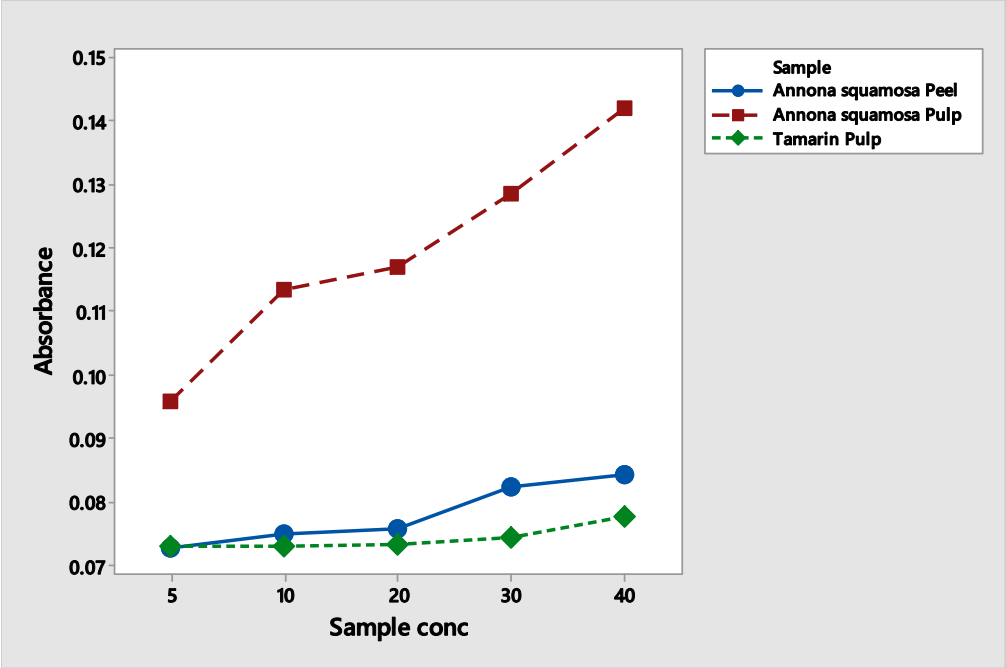

Figure 2: Effect of Sample Concentration on Absorbance

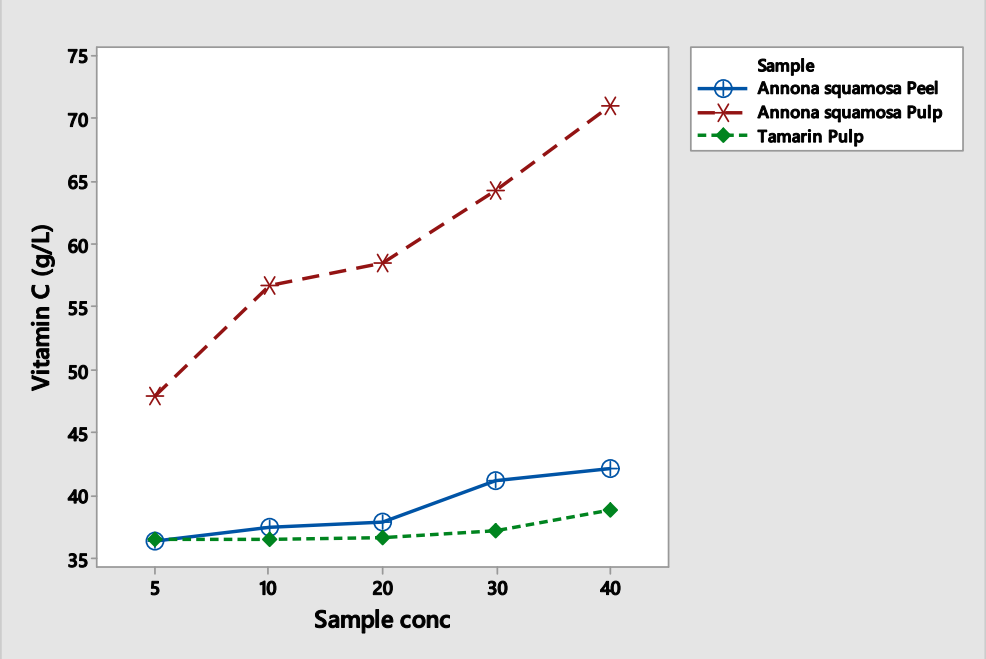

Figure 3: Effect of Sample Concentration on Vitamin C Content

Vitamin C content of the Fruits as Determined by Titrimetic Method

Table 4: Amount of Vitamin C in Fruit Samples Part Determined by Titrimetric Method.

Fruit Sample	Volume of KIO_3required for titration with vitamin C (cm^3)				Amount of Vitamin C (mg/L)	Amount of Vitamin C (g/L)
	1	2	3	Average volume		
Annona Squamosa peel	6.50	6.60	6.70	6.60	0.0299	5.2638
Annona Squamosa Pulp	8.20	8.10	8.10	8.13	0.0368	6.4828
Valvet Tamarind Pulp	4.60	4.70	4.80	4.67	0.0212	3.7249

Equal molar concentration multiplied by 176.12 (Molecular weight).

Different types of foods exists that contained vitamin C. fruits, vegetables and organ meats are known to be the best sources of vitamin C (Combs, 1992). Table 4 shows vitamin C content in *A. squamosa* pulp, peel and *V. tamarind* pulp. The vitamin C contents in investigated fruits by titrimetric method, were found to be 5.26 mg/L, 6.48 and 3.72 mg/L for *A. squamosa* peel, pulp and *V. tamarind* pulp respectively. The differences in the contents might result from the variations in species, variety, ecological factors and harvest time (Clik *et al*., 2006).

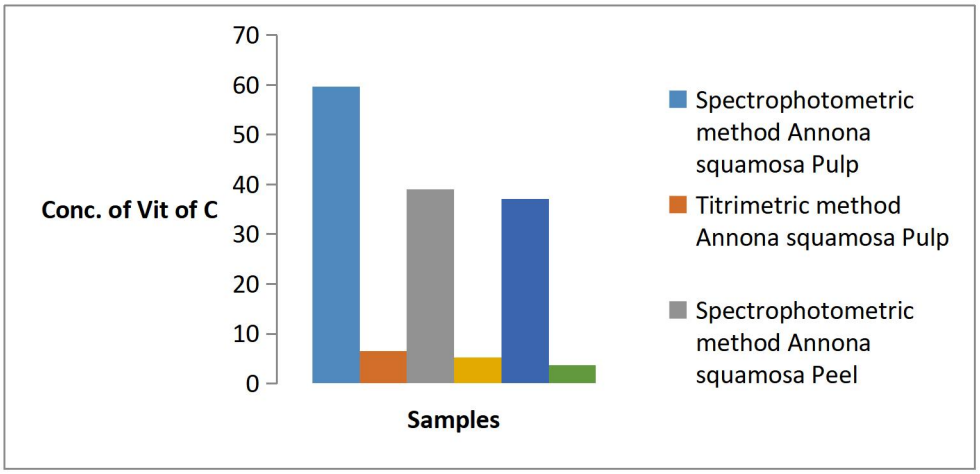

Figure 4. Comparison of Spectrophotometric and Titrimetric Methods.

It is clear from figure 4 that, spectrophotometric method used to measure the amount of vitamin C concents gave a better results than the titrimetric method in all the samples. In both methods, high vitamin C contents were recorded in *A. squamosa* pulp and peel with least vitamin C content been observed in *V. tamarind* pulp..

CONCLUSION

Vitamin C is a necessary dietary source for a good human health. The results revealed that A. squamosa pulp contained high concentration of vitamin C via both spectrophotometric and titrimetric methods when compared with that of *V. tamarind*.

Significant amount of vitamin C contents were recorded in all the samples. Spectrophotometric method demonstrated to be a better method in determination of vitamin C content, even though appreciable concentrations was obtained from the titrimetric method.

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