

Background Correction Method for Determination of Ascorbic Acid in Baobab Fruit Pulp Using Direct UV Spectrophotometry

Abstract

Herein a background correction method has been utilized for the estimation of ascorbic acid in baobab (*Adansonia digitata* L.) fruit pulp using direct UV spectrophotometry. The background correction based on catalytic oxidation of the acid by copper (II). EDTA was used to correct the absorption due to copper (II). The absorbance and λ_{max} were remained essentially constant at 267 nm at pH range from 6 to 8, with highest absorption at pH 6. The calibration graph was linear up to $20 \mu\text{g ml}^{-1}$ of ascorbic acid, correlation coefficient ($R^2 = 0.9999$) and the molar absorptivity was $4 \text{ l mol}^{-1} \text{ cm}^{-1}$. The mean value of ascorbic acid was 236.66 ± 18.31 mg/100g using the proposed method and 250 ± 14.62 mg/100g using indophenol official method. The statistical analysis of data revealed no significant difference in precisions of the two methods and there is no evidence that the two sets of results are significantly different at 95% confidence interval. The proposed method is selective, accurate and valid for determination of ascorbic acid in baobab fruit pulp.

Keywords: *Adansonia digitata* L., Baobab fruit pulp, Ascorbic acid, Background correction

1. Introduction

Baobab (*Adansonia digitata* L.) belongs to the *Bombacaceae* family and *Malvaceae* sub-family^[1, 2] and is well distributed throughout the Africa's semi-arid and arid regions to the south of the Sahara and sub humid regions as well as in western Madagascar^[3, 4]. It has been introduced to many parts of the tropics although it does not grow at high altitudes or in moist tropical forests^[5]. Baobab leaves, bark and fruit are used as food and for medicinal purposes in many parts of Africa^[6]. Baobab fruits are differ in shape among the varieties from baloney

and cylinder to ovoid, measuring 12 – 40 cm length, 7 – 17 cm in diameter, filled with a white to roseate, floury acidic pulp^[7]. Baobab fruit pulp has been used as food ingredients in a variety of food formulations and could also be eaten raw or with milk or as a refreshing drink^[8] also can be made into light porridge (nesha)^[9].

Proximate analysis of ripe fruit points to an average of 8.7% moisture with 2.7% protein, 0.2% fat, 73.7% carbohydrate, 8.9% fibres and 5.8% ash^[10]. Concerning minerals, Baobab Fruit Company (2002)^[11] reported high Ca, Mg and P contents (4310, 2090 and 733 mg/100g, respectively) and moderate Fe, Zn and Na contents (17, 10.4 and 54.6 mg/100g, respectively). Fruit pulp proved to be rich in pectin, most of it being water soluble with a low content of propectin, low degree of esterification and intrinsic viscosity values of about one fifth of those of commercial apple pectin^[12]. However, the major interest on baobab product relies in its ascorbic acid (Vitamin C), a powerful antioxidant and dietary fibers content. In particular baobab fruit pulp represents the most important natural sources of ascorbic acid, in this regards, the baobab fruit pulp can be considered a highly valuable source containing levels of vitamin C ranging from 2.8 to 3 g/kg, in comparison to the fruits that are generally considered the best source of ascorbic acid approximately six times more than the content of an orange^[12, 13]. Ighodalo *et al.*, (1991)^[14] recorded 337 mg ascorbic acid/100g pulp for fruits in Nigeria, the Baobab Fruit Company (2002)^[11] recorded 34 - 200mg/100g. Joint efforts between the Malian Agronomic Research Institute and the Novartis Foundation for Sustainable Development reveal a range from 1505 to 4991 mg/kg^[5]. Sidibe and Williams (2002)^[3] stated that the daily recommended dose of vitamin C (65 mg per day) can be obtained from 23 g of baobab powder, the daily saturation of vitamin C pool in the body (140 mg per day) requires 50 g of baobab powder; the special dosage for convalescents is 90 g. Although *A. digitata* is mostly regarded as a fruit-bearing forest tree, it is a multipurpose, widely-used species with medicinal properties, numerous food uses of various plant parts,

53 and bark fibres that used for a variety of purposes^[3]. In traditional medicine baobab fruit
54 pulp is used in the treatment of fevers, diarrhea and malaria. Due to ample natural vitamin C
55 content, baobab fruit pulp has a well-documented antioxidant capability^[15].
56 A number of chemical methods have been used for the determination of ascorbic acid, based
57 mainly upon the reducing properties of the ascorbic acid. These methods include titration of
58 an acid extract with iodine, methylene blue, ferricyanide and 2,6-dichloroindophenol^[16, 17].
59 Oxidation of ascorbic acid with the dye 2,6-dichloroindophenol is the most satisfactory and
60 extensively used method. Ascorbic acid can also be determined by a microfluorimetric
61 method^[17], which is a very time consuming. Differential pulse polarography has been
62 used to determine ascorbic acid at the $\mu\text{g/ml}$ level but the method suffers from interferences
63 from electro-active impurities present in the sample. Ascorbic acid can also be determined
64 using HPLC method^[18]. Though several methods have been reported for the
65 spectrophotometric determination of ascorbic acid on the basis of the oxidation-reduction
66 reaction between L-ascorbic acid and the coloring agents^[19], they seem to be either
67 complicated or unreliable because the absorptivity of the colored solution varies with time.
68 Ascorbic acid absorbs strongly from 243 to 267 nm depending on the acidity of the medium.
69 Before background correction techniques were introduced, the ultraviolet spectrum of
70 ascorbic acid was seldom use for the direct determination of the acid in real samples with
71 complex matrices because of the very high absorption in the UV region. There are various
72 methods available in the literature to correct for background absorption in the UV region for
73 the determination of the ascorbic acid, such as thermal decomposition, an enzymatic method,
74 direct ultraviolet irradiation, alkaline treatment and the catalytic decomposition of the acid by
75 metal ion^[20]. It has long been known that copper (II) catalyses the oxidation of ascorbic acid
76^[19]. Onish and Harva^[21], measured the oxidation rate of ascorbic acid in the presence of
77 various kind of metal ions, and the catalytic effect of copper (II) was found to be much better

78 than exhibited by other ions. Also they reported that EDTA is the best chelating agent for the
79 correction of the absorption due to the copper (II).

80 In this work a direct UV spectrophotometric method using background correction technique
81 ~~was to develop~~, based on the metal-catalyzed oxidation of ascorbic acid was ~~to developed used~~
82 to
83 estimate the content of ascorbic acid (vitamin C) in baobab fruit pulp, collected from western
84 Sudan (western kordofan state).

84 2. Experimental

85 2.1. Materials

86 All chemicals used in this study were of analytical grade, metaphosphoric acid; acetic acid;
87 copper (II) sulphate; EDTA and sodium bicarbonate were obtained from CDH (central drug
88 house P Ltd.) and NICE India. Pure L-ascorbic acid and 2,6-dichloroindophenol (DCIP) were
89 purchased from PARK scientific limited, Northampton, U. K. A water bath shaker (Model
90 1008, G.F.L.). Whatman filter paper No.1, qualitative, UV/Visible Spectrophotometer
91 (Model CE7200, Aquarius) and pH meter (Model pH 211, HANNA instruments) were used.

92 2.2. Indophenols Titrimetric method

93 2.2.1. Reagents

94 *Extracting Solution:* 15 g of freshly pulverized stick of metaphosphoric acid was dissolved in
95 40 ml of acetic acid and 200 ml water, then diluted to 500 ml and stored in refrigerator.

96 *Ascorbic acid standard solution:* 100 mg of ascorbic acid was accurately weighed and
97 transferred to a 100 ml volumetric flask, and diluted to volume immediately before used.

98 *Indophenol standard solution:* 50 mg of 2,6-dichloroindophenol Na salt was dissolved in 50
99 ml water to which 42 mg of sodium bicarbonate has been added, and diluted to 200 ml with
100 water, and stored in refrigerator. Then the indophenol solution was standardized using
101 ascorbic acid standard solution, and the concentration of indophenol solution was expressed
102 as mg ascorbic acid equivalent to 1.0 ml reagent.

2.2.2. Ascorbic acid determination

Three sample aliquots each containing 5 ml were titrated against the dye (DCIP). And the content of ascorbic acid (vitamin C) in the samples was determined according to the indophenols method^[17]. The amount of ascorbic acid in mg/100 g of the sample can be determined using the following expression:

$$\text{mg ascorbic acid/ 100 g} = (X - B)(F/E)(V/Y) \times 100 \quad (2.1)$$

Where: X is the volume of dye in ml for the sample titration; B is the volume of dye in ml for the blank titration; F is the mg ascorbic acid equivalent to 1.0 ml indophenol standard solution; E is the number of grams assayed; V is the volume of initial assay solution; Y is the volume of sample aliquot titrated.

2.3. Spectrophotometric method

The ascorbic acid contents was determined using previous reported method.^[20]

2.3.1. Selection of wavelength and pH

The absorption maximum (λ_{max}) for ascorbic acid at different values of pH were determined using 1.0 ml of 10 ppm ascorbic acid in the presence of 5.0 ml of Cu (II)-EDTA complex.

2.3.2. Preparation of calibration curve

A series of ascorbic acid standard solutions in the range of 1.0 to 20.0 ppm were prepared by mixing 1.0 ml of the appropriate ascorbic acid solution with 5.0 ml of Cu (II)-EDTA solution.

2.3.3. Real Sample Analysis

Baobab fruit was obtained from western kordofan state, Sudan. The fruit capsules were broken, and then the pulp was scraped and pounded with a pestle. The pulp was separated from the seed and fibre, the extracted powder per fruit ranged from 30 to 118 g, with an average of 42 g. Then the pulp powder was sealed in black polyethylene sachets and stored in desiccators.

1.0 g of baobab pulp powder were pulverized by gentle grinding, and triturated with distilled water, then diluted to 100 ml. The solution was then centrifuged at 750 rev min^{-1} for 15 min, and the supernatant liquid was taken out. Then 5.0 ml of Cu (II)-EDTA solution were added to 1.0 ml of diluted sample solution, and the absorbance of the solution at 267 nm was measured (designated as A_1). Then 4.0 ml. of $5 \mu\text{g ml}^{-1}$ CuSO_4 solution were added to another 1.0 ml aliquot of the dilute sample and heated in a water bath at 50°C for 15 min. then 1.0 ml of $5 \times 10^{-4} \text{ M}$ EDTA solution was added. The absorbance of the resulting solution at 267 nm was measured (labeled as A_2). The ascorbic acid content was calculated using the following equation:

$$\text{Ascorbic acid content} = 6(f\Delta A)/S \quad (2.2)$$

Where: f is the dilution factor; ΔA calibration curve; and the factor of 6 included to account for the dilution by the buffer solution [17].^{the}

~~141~~ **3. Results and Discussion**

The ascorbic acid content of ten baobab fruit pulp samples were determined using the two methods. Firstly, the ascorbic acid content were obtained using the indophenol titration as a reference, and the results were shown in table (1).

Result and Discussion

The mean value of ascorbic acid was found to be $250 \text{ mg/ } 100 \text{ g}$, the 95% confidence interval (μ) for the ascorbic acid content was calculated using the formula [22]:

$$\mu = \bar{x} \pm \frac{t s}{\sqrt{n}} \quad (3.1)$$

Where: the mean; t is Student's t ; s is the sample standard deviation and n is number of data, and was found to be $250.00 \pm 14.62 \text{ mg/ } 100\text{g}$. Although the method is rapid, but the reagent itself is unstable and must be standardized before use~~d~~. Moreover, the method is not suitable for colored samples and limited by the presence of other reducing substances, which

gave rise to errors, particularly in food products. In addition, inorganic ferrous and ferric compounds also interfere with the detection by 2,6-dichloroindophenol^[23].

Table 1: Ascorbic acid (A.A.) content of baobab fruit pulp using indophenol method.

Sample No.	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇	S ₈	S ₉	S ₁₀
A.A. content mg/100g	268.9	288.9	249.0	244.0	234.1	263.9	239.1	234.1	219.1	258.9
Mean value	250.00 ± 14.62									

Secondly, the contents were determined using spectrophotometric method. In order to select suitable pH value, the were tested at values of pH and the results were shown in table (2&3). As shown in table (2), the are meanly affected by the change in the pH. At pH 2 where ascorbic acid is expected to exist entirely in the non-ionized form, the maximum absorption occurred at 244 nm. At pH 12, where the acid is expected to dissociate completely, the maximum absorption occurred at 298 nm. The λ_{max} remain essentially constant at 267 nm at pH range from 6 to 8, where the predominate species is the mono-anion of ascorbic acid. From the results in table (3), the highest absorbance was obtained at pH value of 6. Thus, we conclude that the optimum pH should be 6, and the absorbance would then be measured at 267 nm. λ_{max} values differ

Table 2: Effect of pH on the absorption and λ_{max} for a ascorbic acid in the presence of Cu (II)-EDTA complex

pH	2.0	4.0	5.0	6.0	7.0	8.0	10.0	12.0
λ_{max} / nm	244	254	265	267	267	267	270	298
Abs.	0.612	0.524	0.752	0.863	0.841	0.854	0.825	0.346

Table 3: Effect of pH on the absorption of ascorbic acid in the presence of Cu (II)-EDTA complex at 267 nm.

pH	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0
Abs.	0.120	0.362	0.459	0.749	0.863	0.841	0.854	0.812	0.805	0.351	0.144

3.1. Calibration curve

The calibration curve was obtained by plotting the concentration of the ascorbic acid standards vs their corresponding absorbance. The method was linear up to $20 \mu\text{g ml}^{-1}$ of ascorbic acid with correlation coefficient (R^2) of 0.9999. The molar absorptivity of ascorbic acid at 267 nm was calculated to be $1.52 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$.

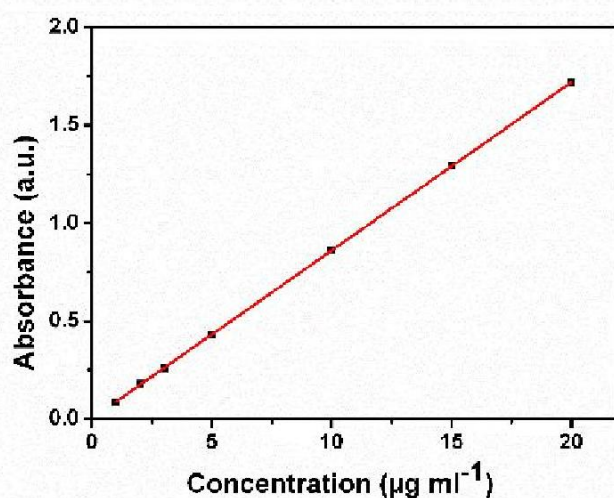


Figure 1: Calibration curve for ascorbic acid (Abs. versus concentration, at λ_{max} 267 nm)

The ascorbic acid contents of baobab fruit pulp were determined by the background correction method, the results obtained were shown in table 4, the mean value was found to be $236.66 \pm 18. \text{ mg/ 100g}$. In order to decide whether the standard deviations of the two methods are significantly different from each other or not, F test was performed using equation (3.2)^[22],

$$F_{calculated} = \frac{s_1^2}{s_2^2} \quad (3.2,$$

The results revealed that the value of $F_{calculated}$ (= 1.57) is less than F_{table} (= 3.18)^[24], therefore, it is obviously indicated that the standard deviations of the two data sets are not significantly different from each other at 95% confident level. Suggesting that the two results were agreed with each other within experimental error. The comparison between the means of the two methods was carried out using t-test, equation (3.3).

$$t_{calculated} = \frac{|\bar{x}_1 - \bar{x}_2|}{s_{pooled}} \sqrt{\frac{n_1 n_2}{n_1 + n_2}} \quad (3.3)$$

The $t_{calculated}$ was found to be 1.29, where the critical value for t_{table} (0.05, 18) is 2.10^[24], since $t_{calculated}$ is less than t_{table} (= 2.10) then it was concluded there is no evidence that the two sets of results are significantly different at the chosen confidence level (95%).

Table 4: Ascorbic acid (A.A.) content of baobab fruit pulp using background correction method.

Sample No.	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇	S ₈	S ₉	S ₁₀
A.A. content mg/100g	249.8	281.8	241.4	232.3	215.6	259.5	228.1	210.9	195.3	251.9
Mean value	236.66 ± 18.31									

Using the average vitamin C content of baobab fruit, the daily recommended dose of vitamin C, 120 mg per day, can be obtained from about 50.7 g of baobab powder. And the daily saturation of vitamin C pool in the body, 140 mg per day, requires about 60 g of baobab powder.

4. Conclusions

In this study a simple, accurate and precise UV spectrometric method was developed for the determination of the ascorbic acid in baobab fruit. Sudanese

baobab fruit pulp from Western Kordofan State proved to be rich source of ascorbic acid (vitamin C), ranged from 195.3 to 288.9 mg/ 100 g, thus about 51g of baobab fruit pulp provided the daily recommended dose of vitamin C. The proposed method is suitable and valid for the determination of ascorbic acid in baobab fruit pulp. The high concentration of vitamin C in baobab fruit, given the wide-spread distribution of the tree in western Sudan, offers exciting opportunities for more research.

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