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The Voltammetric and Titrimetric Determination of Ascorbic Acid Levels in Tropical Fruit Samples

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The levels of ascorbic acid in 50 tropical fruit samples were determined by titrimetric method using N-bromosuccinimide and also by cyclic voltammetry using a glassy carbon as working electrode, Ag/AgCl as reference and platinum as the auxiliary electrode in 0.1 M phosphate buffer, pH 2.0 containing 1mM Na_2EDTA. The measurements were made in a potential range of 200 mV to 1000 mV in relation to reference electrode using a scan rate of 50 mV/s. The anodic peak currents for the electrochemical oxidation of ascorbic acid to dehydroascorbic acid were recorded at 580 mV. No cathodic peak current was observed in the potential range studied. Fruits found to have high levels of ascorbic acid include green pepper (182.34 mg/100g), long pepper (138.54 mg/100g), red pepper (125.59 mg/100g), tangelo (68.82 mg/100 cm^3), grape (68.82 mg/100 cm^3), orange (64 mg/100 cm^3), lime (56.57 mg/100 cm^3), pawpaw (55.8 mg/100 cm^3), cherry (54.86 mg/100g), and guava (51.02 mg/100g). The results obtained by cyclic voltammetric method and titration with N-bromosuccinimide are generally comparable but large differences are obtained in some fruits. The extensive data in this report serves as a database of the vitamin C levels in fifty tropical fruits which hitherto is not available in literature. The report will serve as a guide in the selection of fruits with appreciable concentration of vitamin C

Keywords: Vitamin C, cyclic voltammetry, tropical fruit samples, electrochemical oxidation

1. INTRODUCTION

Vitamin C, also known as ascorbic acid, is a valuable food component because of its antioxidant and therapeutic properties. It helps the body in forming connective tissues, bones, teeth, blood vessels and plays a major role as an antioxidant that forms part of the body defense system against reactive oxygen species and free radicals, thereby preventing tissue damage [1,2,3]. It is widely

used in the treatment of certain diseases such as scurvy, common cold, anemia, hemorrhagic disorders, wound healing as well as infertility [4].

Most plants and animals have the ability to synthesize vitamin C. The only mammals that are unable to synthesize vitamin C are primates, including man, and guinea pigs. Therefore humans depend on exogenous sources of the vitamin which include fruits and vegetables as well as food supplements and pharmaceutical preparations [3]. The increasing use of pharmaceuticals and other natural samples containing vitamin C has necessitated the development of an accurate and specific procedure for its determination.

Numerous analytical techniques have been reported in the literature for the determination of vitamin C in different matrices. These include titrimetric [5], fluorometric [6], complexometric methods [7], liquid chromatography [8], high-performance liquid chromatography [9], spectrophotometric [10, 11, 12], amperometric [13] and enzymatic [14]. Most of these methods overestimate the levels of vitamin C in different matrices due to the presence of oxidizable species other than vitamin C. The drawbacks in these methods have been reviewed previously [15].

The vitamin C levels in some tropical food samples have been reported by several investigators. The methods commonly employed in these determinations were titrimetric [16,17,18] or spectrophotometric [19]. Although titrimetric methods are simple to use in the determination of vitamin C, difficulties are encountered with commonly used titrants and interferences often occur with coloured samples. Direct spectrophotometric determination of ascorbic acid in the UV region is prone to matrix effect since many organic compounds in complex samples may also exhibit ultraviolet absorbance. Thus there is a need to adopt a procedure that will accurately determine the levels of vitamin C in tropical food samples. Electrochemical methods can be useful in the determination of vitamin C levels in foods because ascorbic acid is easily oxidized to dehydroascorbic acid. Electrochemical methods traditionally have found important applications in sample analysis and in organic and inorganic synthesis. The electrode surface employed in such determinations can be a powerful tool in such applications [20]. In this investigation we report on the use of cyclic voltammetry in the determination of ascorbic acid levels of fifty tropical fruit samples. This method is fast, sensitive, selective and gives linear response at low concentration range.

2. EXPERIMENTAL PART

2.1. Reagents and Materials

N-Bromosuccinimide, L-ascorbic acid, sodium dihydrogen phosphate and oxalic acid were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). The sample and reference solutions were prepared daily and stored in amber bottles to avoid oxidation. 0.1 M phosphate buffer solution was made up from NaH_2PO_4 and adjusted to pH 2.0 with phosphoric acid. pH measurements were made with a Metrohm pH meter model 780. De-ionized water was used for the preparation of all solutions throughout the period of the experiment.

2.2. Samples Analysed

50 samples of tropical fruits were obtained from Nigerian Institute of Horticulture (NIHORT), Ibadan, Nigeria, as well as from markets in Lagos environs. The samples obtained from the open markets were identified in NIHORT. The fruit samples analyzed are, African bush mango, apple (green), apple (red), apple (yellow), avocado pear, banana (big), banana (small), beans (brown, drum), beans (green), bitter cola, corn (yellow), cherry, cocoa, coconut, cucumber, garden egg (green), garden egg (white), garlic, grapefruit (sweet), grapefruit (unsweet), green peas, guava, guinea corn (red), kolanut (brown), kolanut (red), kolanut (white), lemon, lime, melon, okra, onion (brown), onion (white), orange (Agege), orange (king), pawpaw, pepper (fresh and long, Sombo tutu (Yoruba), pepper (green), pepper (red), pineapple (Badagry), pineapple (local), plantain, Rose apple, Sorghum (Falafara, (hausa)), spring onions, tangelo (Nogatee), tangerine, tomato (from Northern Nigeria), tomatoe (from Southern Nigeria), water melon (dark green) and water melon (dark green).

2.3. Determination of Ascorbic Acid with N-Bromosuccinimide

Stock solution of ascorbic acid was prepared freshly by dissolving 0.05g in 100 cm^3 of 0.5% oxalic acid solution. Serial dilutions were made from the stock solution (50 mg ascorbic acid/100 cm^3) to give the working solutions of 40 mg, 30 mg, 20 mg and 10 mg of ascorbic acid in 100 cm^3 of 0.5% oxalic acid solution.

10 cm^3 each of the standard ascorbic acid solutions was transferred to a standard flask and the volume made up to 100 cm^3 with de-ionized water. 10 cm^3 of this solution was then titrated against 0.01% N-bromosuccinimide solution according to the method earlier reported [21]. All titrations were performed in triplicate and the results used to construct a calibration curve for ascorbic acid.

2.4. Extraction Procedures for Fruits and Analysis of samples

Two categories of fruits were investigated. These include the juicy fruits and the non-juicy fruits. In the case of the juicy fruits, samples were washed with water, peeled and sliced into two. The juice from each fruit was squeezed out and filtered with a glass wool. The volume of juice obtained was recorded and made up to 100 cm^3 with 0.5% oxalic acid solution.

For the non-juicy fruits, a weighed amount of the edible portion of the fruit was minced and blended with 0.5% oxalic acid solution for approximately one minute. The homogenized sample was then filtered through a glass wool. The filtrate was transferred to a 100 cm^3 volumetric flask and the volume made up to the mark with 0.5% oxalic acid solution.

10 cm^3 of the sample solution was titrated against 0.01% N-bromosuccinimide as earlier described for standard ascorbic acid solutions. All analyses were done using triplicate samples and the mean values recorded.

The levels of ascorbic acid in the samples were then extrapolated from the calibration curves and calculated using the weight or volume measured and expressed in per 100 cm^3 or 100 g of sample.

2.5. Voltammetric measurements

A BASI-Epsilon potentiostat/galvanostat, obtained from Bioanalytical Systems Inc. (West Lafayette, IN, USA) was used in the study. The conventional three electrode configuration was used in the measurements. The working electrode (3 mm) was made of glassy carbon while a platinum electrode (1.6 mm) served as the auxiliary electrode. The working electrode was polished with alumina powder to obtain a mirror-like image, washed with de-ionized water and placed in a pyrana solution for ten minutes and washed with de-ionized water and dried.

For the voltammetric studies, 5mM ascorbic acid stock solution was prepared by dissolving 0.088g of ascorbic acid in 100 cm^3 of 0.1M phosphate buffer pH 2.0 containing 0.1mM Na_2EDTA. From the stock solution serial dilutions were made with 0.1M phosphate buffer pH 2.0 to obtain 4 mM, 3 mM, 2 mM and 1 mM ascorbic acid solutions. These solutions were used for calibration.

15 cm^3 each of the standard ascorbic acid solutions prepared in 0.1M phosphate buffer pH 2.0 containing 0.1mM Na_2EDTA was transferred to the electrochemical cell and purged with nitrogen for 10 minutes. The potential of each solution was scanned between 200 mV and 1000 mV using a scan rate of 50 mV/s to obtain the cyclic voltammogram. All measurements were carried out at room temperature. The anodic peak currents for the electrochemical oxidation of ascorbic acid were recorded at 580 mV. Calibration curves were prepared from plots of the anodic peak currents against ascorbic acid concentrations.

For real sample measurements, the juice from each juicy fruit was squeezed out and filtered with glass wool. Suitable aliquots (1-2 cm^3) were immediately added to the 0.1 M phosphate buffer, pH 2.0 containing 0.1mM Na_2EDTA in the cell to make a final volume of 15cm^3. The potential of the solution was scanned as described for the standard ascorbic acid solutions. For the non-juicy fruits, a weighed amount (5-6 g) of the edible portion of the fruit was minced and blended with 30 cm^3 of 0.1M phosphate buffer pH 2.0 containing 0.1mM Na_2EDTA for approximately one minute. The homogenized sample was then filtered through a glass wool. 15 cm^3 of this solution was transferred to the electrochemical cell, purged with nitrogen for 10 minutes before scanning the potential between 200 mV and 1000 mV. The results of the anodic peak current obtained at 580 mV were used to extrapolate the concentration of the ascorbic acid in the sample.

3. RESULTS AND DISCUSSION

The voltammograms of the phosphate buffer as well as 5mM ascorbic acid in the buffer are shown as I and II in figure 1. No anodic peak current was observed in the voltammogram of the buffer but was observed at 580 mV in the 5 mM ascorbic acid solution in the buffer as shown in II of figure 1. No cathodic peak current was found indicating an irreversible heterogenous charge transfer in this system [21]. The voltammograms of 1mM, 3mM, and 5mM ascorbic acid in the phosphate buffer pH 2.0 are shown in figure 2. These results show that ascorbic acid concentration can be measured quantitatively by cyclic voltammetry. The anodic peak currents at 580 mV were found to vary linearly with ascorbic acid concentration over 1-5 mmol. dm^{-3} as shown in figure 3. The voltammogram for the

determination of ascorbic acid content of pawpaw (*Carica papaya*) is shown in figure 4. The anodic peak current was found to increase with the addition of 1 and 2 $mmol^{-1}$ ascorbic acid thus confirming that the current obtained for the juice sample alone was that of ascorbic acid.

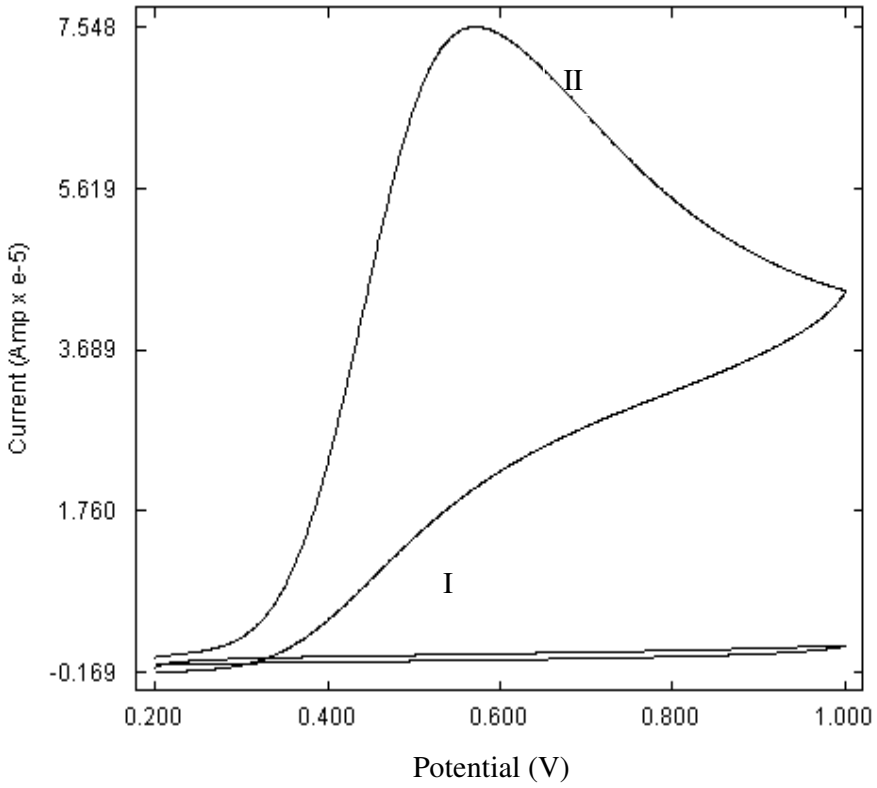

Figure 1. Voltammograms of (I) 0.1 M phosphate buffer, pH 2.0 and (II) 5mM ascorbic acid in phosphate buffer, pH 2.0 containing 1mM Na_2EDTA.

The results of the determination of ascorbic acid contents of tropical fruits by the voltammetric method and titration with N-bromosuccinimide are shown in Table I. The results obtained by the two methods are generally comparable within 10% in several samples such as African bush mango, avocado pear, bitter cola, corn (yellow), cherry, garlic, guava, guinea corn (red), kolanut (white), lemon, orange (Agege), orange (king), pepper (fresh and long), pepper (red), pineapple (local), plantain, spring onions, tangerine, tomato (from both Northern and Southern Nigeria). However, large differences of 11-30% in ascorbic acid contents were found in many fruits such as banana (big), coconut (flesh), garden egg (green), grapefruit (sweet), grapefruit (unsweet), kolanut (brown), lime, okra, onion (brown), onion (white), pawpaw, pineapple (Badagry), rose apple, sorghum (falafera) and tangelo (nogatee). Much larger differences of 31-50% were found in samples such as apple (green, red and yellow), beans (brown), beans (green), cocoa, garden egg (white), green pea, kolanut (red), melon, pepper (green) and water melon (dark green and light green) while a difference of about 70% is

observed in cucumber. All these calculations are based on using the values obtained by cyclic voltammetry as accurate. Higher contents of ascorbic acid were obtained with the voltammetric method for apples (red, green and yellow), avocado pear, banana (big and small), beans (green), corn (yellow), cocoa, coconut (flesh), garden egg (white and green), garlic, grape (sweet and unsweet), green peas, kolanut (red), lime, okra, onion (brown and white), orange (Agege and king), pawpaw, pepper (green), pineapple (local), plantain, rose apple, sorghum, spring onions, tangelo, tangerine, water melon (dark and green) when compared with the titrimetric method. This discrepancy between the ascorbic acid contents of some fruits and seeds obtained by the two methods may be due to poor detection of end point or the presence of substances that may interfere with the reagent in the titrimetric method.

Generally where there are significant percentage differences, the values obtained by cyclic voltammetry are higher except for beans (brown), cucumber and melon. It is to be observed that in samples with low vitamin C content, the percentage difference in values by the two methods may be large but the absolute differences are within limits of 10-12 mg which is about 0.1mg/g or 0.1mg/ml as in apple (green, red and yellow), banana (small), coconut (flesh), cucumber, garden egg (green and white), melon, okra, onion (brown and white) and rose apple. However, in six samples namely beans (brown), grape (unsweet), green peas, kolanut (red), pepper (green) and tangelo that this limit is exceeded. It is also to be noted that some food samples may contain enzymes that may interfere with the N-bromosuccininimide or the working electrode and this may result in the disparity in the values.

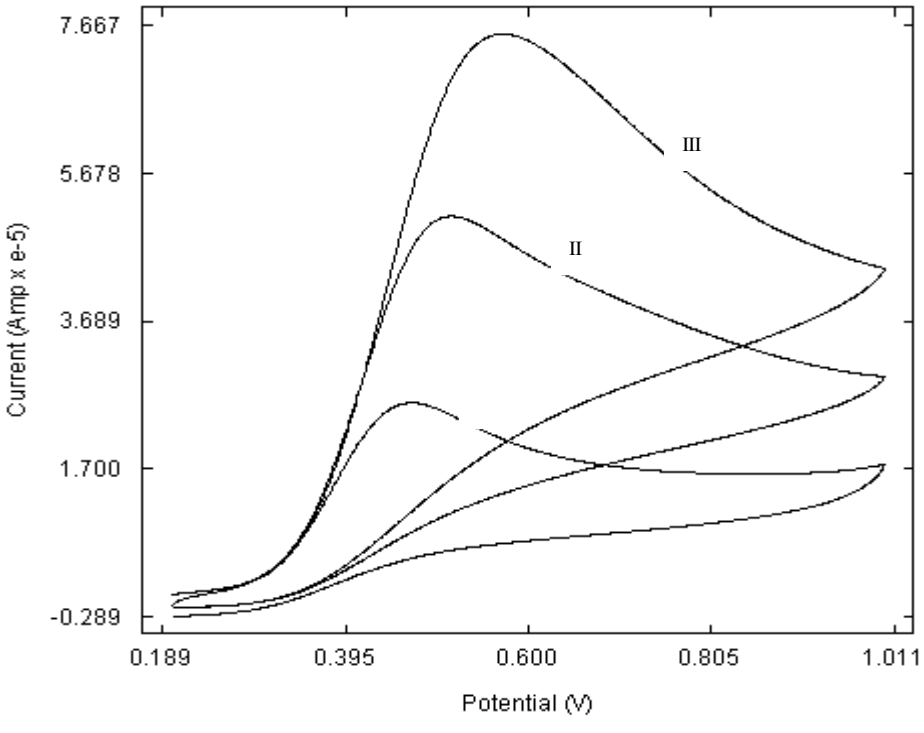

Figure 2. Cyclic voltammograms of different ascorbic acid concentrations. I = 1mM; II = 3 mM and III = 5 mM ascorbic acid in 0.1 M phosphate buffer pH 2.0

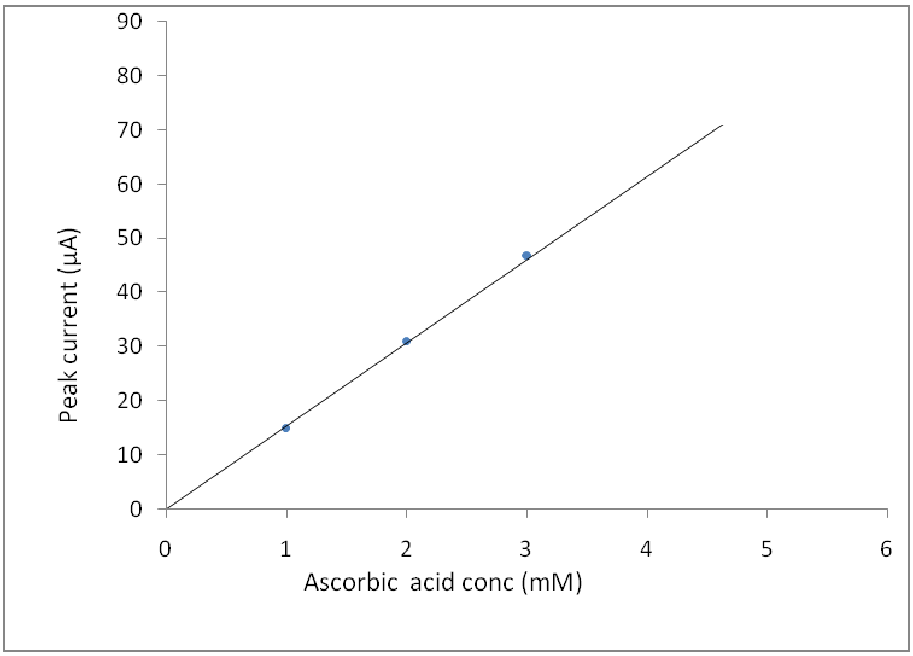

Figure 3. Variation of peak current with ascorbic acid concentration in 0.1 M phosphate buffer pH 2.0 containing 1mM Na_2EDTA.

Table 1. Comparison of ascorbic acid content of tropical fruit samples obtained by cyclic voltammetry (CV) and titration with N-bromosuccinimide (NBS)

S/No	Sample		Amount of ascorbic acid		Percentage Difference	Absolute Difference
	Common Name	Botanical Name	CV mg/100g	NBS mg/100g		
1.	African Bush Mango	*Irvingia gabonesis*	33.03	36.13	10	3.10
2.	Apple (green)	*Malus sylvestris*	11.73	6.00	50	5.73
3.	Apple (red)	*Malus sylvestris*	12.72	6.50	50	6.22
4.	Apple (yellow)	*Malus sylvestris spp*	11.73	6.00	50	5.73
5.	Avocado pear	*Persea americana*	30.00	28.24	5	5.73
6.	Banana (big)	*Musa sapientum*	15.00	12.72	15	2.28
7.	Banana (small)	*Musa sapientum*	15.23	3.09	80	12.14
8.	Beans (brown) drum	*Phaseolus vulgaris*	43.35	62.19	50	18.84
9.	Beans (green)	*Phaseolus spp*	35.16	24.52	33	10.64
10.	Bitter cola	*Garcina cola*	39.29	42.75	7.5	3.46
11.	Corn (yellow)	*Zea mays*	14.20	13.70	3.5	0.50
12.	Cherry (agbalumo, Yor)	*Pyrunus avium*	54.86	55.63	2	0.77
13.	Cocoa	*Theobroma cacao*	6.10	3.29	45	2.81
14.	Coconut (flesh)	*Cocos nucifera*	10.97	7.50	30	3.47
15.	Cucumber	*Cucumis sativus*	6.99	12.00	70	5.01
16.	Garden egg (green)	*Lagenaria siceraria*	12.40	8.70	30	3.70
17.	Garden egg (white)	*Solanum aethiopicum*	11.03	7.60	31	3.43
18.	Garlic	*Allium sativum*	38.68	35.24	5	3.44

19.	Grapefruit (sweet)	*Citrus paradisi*	68.75 mg/100cm^3	60.00 mg/100cm^3	12.5	8.75
20.	Grapefruit (unsweet)	*Citrus paradisi*	68.82 mg/100cm^3	46.58 mg/100cm^3	30	22.24
21.	Green peas	*Pisum sativum*	24.97	11.40	50	13.57
22.	Guava	*Psidium guajava*	51.02	50.3	2	0.72
23.	Guinea corn (red)	*Sorgum bicolor*	15.13	14.72	2.5	0.41
24.	Kolanut (brown)	*Cola nitida*	35.02	45.49	28	10.47
25.	Kolanut (red)	*Cola nitida*	29.93	14.50	50	15.43
26.	Kolanut (white)	*Cola nitida*	43.93	45.19	3	1.26
27.	Lemon	*Citrus limon*	49.00 mg/100cm^3	49.53 mg/100cm^3	1	0.53
28.	Lime	*Citrus aurantifolia*	56.57 mg/100cm^3	48.61 mg/100cm^3	14	7.96
29.	Melon	*Cucumis melo*	20.06	26.42	33	6.36
30.	Okra	*Abelmuschus esculentus*	25.22	19.65	12	5.57
31.	Onion (brown)	*Allium cepa*	13.19	10.57	25	2.62
32.	Onion (white)	*Allium cepa*	16.72	14.31	12.5	2.41
33.	Orange (Agege)	*Citrus sinensis*	64.00 mg/100cm^3	63.33 mg/100cm^3	2	0.67
34.	Orange (king)	*Citrus nobilis*	64.00 mg/100cm^3	61.36 mg/100cm^3	3	2.64
35.	Pawpaw	*Carica papaya*	55.24	43.33	20	11.91
36.	Pepper (fresh and Long, Sombo tutu (Yoruba))	*Capsium spp*	138.54	136.42	1.5	2.12
37.	Pepper (green)	*Capsium annum*	182.34	91.32	50	91.02
38.	Pepper (red)	*Capsicum frutesceus*	125.59	135.63	8	10.04
39.	Pineapple (Badadry)	*Ananas comosus*	35.20	40.64	14	5.44
40.	Pineapple (local)	*Ananas comosus*	24.93	24.81	1	0.12
41.	Plantain	*Musa parasidiaca*	10.45	9.07	10	1.38
42.	Rose apple	*Syzygium jambos*	15.12	10.29	30	4.83
43.	Sorghum (Falafara, (Hausa))	*Sorghum vulgare spp*	8.50	6.00	29	2.50
44.	Spring onions	*Allium ascalonicum*	17.90	17.43	5	0.47
45.	Tangelo (Nogatee)	*Citrus spp*	68.82 mg/100cm^3	45.42 mg/100cm^3	30	23.40
46.	Tangerine	*Citrus reticulata*	45.13 mg/100cm^3	43.37 mg/100cm^3	4	1.76
47.	Tomato(from Northern Nigeria)	*Lycopersicon esculentum*	19.36	21.00	8.5	1.64
48.	Tomato (from Southern Nigeria)	*Lycopersicon esculentum*	27.63	27.70	1	0.07
49.	Water melon (dark green)	*Citrullus lanatus*	12.84	7.77	35	5.07
50.	Water melon (light green)	*Citrullus lanatus*	12.80	6.80	45	6.00

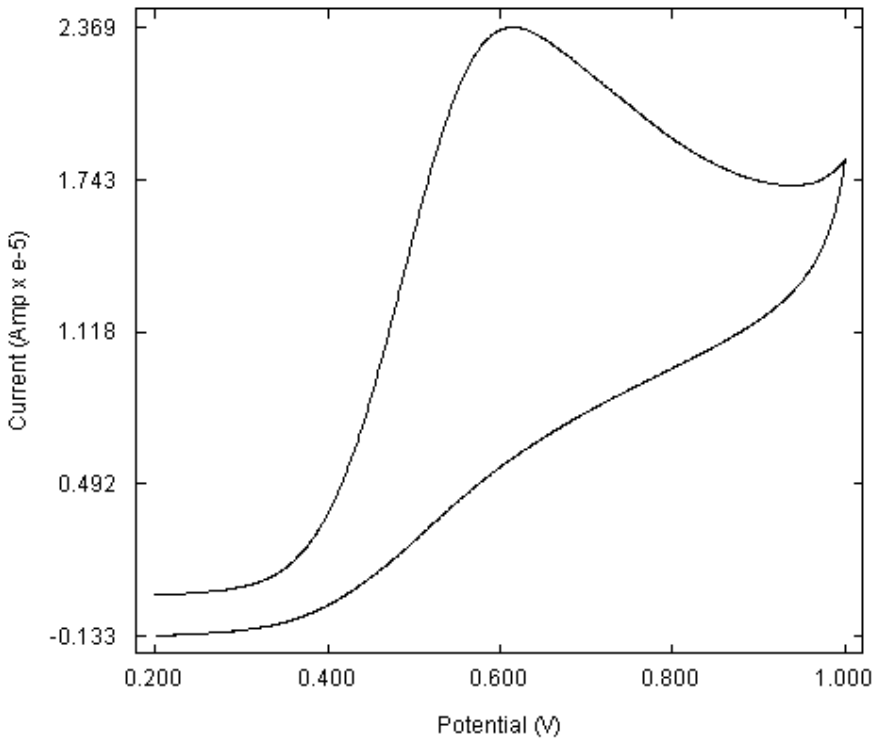

Figure 4. Voltammogram of *Carica papaya* in 0.1M phosphate buffer, pH 2.

The ascorbic acid value obtained for orange in this study compare favorably with those reported by Hernandez et al [8] for orange cv. "Navelino" (66 mg/100g); Vanderslice et al. [24] for orange cv. "Florida" (63 mg/100g) and Khorasani-Motlagh & Noroozifar [25] for orange samples obtained from Iranshahr city in Iran (64.5 mg/100g). Also the values we obtained for lemon and grape are in agreement with the range reported by Khorasani-Motlagh & Noroozifar [25].

However, there are significant differences in the values of ascorbic acid obtained in this study and those reported by other investigators for several fruit samples. Razmi and Harasi [22] using a cadmium pentacyanonitrosylferrate film modified glassy carbon electrode obtained ascorbic acid values of 49.24 mg/cm^3 and 8.80 mg/cm^3 for orange and grape respectively. Lim et al [17] using the reversed phase –HPLC technique reported the ascorbic acid content of 144 mg/100g for guava. Vanderslice et al. [24] reported ascorbic acid levels of 23.6 mg/100g and 9.7 mg/100g for grape and water melon respectively. Melo et al [26] using 2,6-dichlorophenolindophenol (DCIP) in the titrimetric method, reported the following values of ascorbic acid in mg/100g for orange (37.34), guava (89), green bean (3.55), papaya cv 'Hawaii'(141.97), cucumber (1.49), banana cv 'pacovan' (4.63), melon cv 'japones'(1.52). Although titrimetric determination of ascorbic acid using DCIP is the official method, it is not applicable to solutions containing Fe(II), Sn(II), Cu(I), which may be present in natural samples. Also, substances naturally present in fruits or biological materials such as tannins, betannins and sulfhydryl compounds are oxidized by the dye. The method is applicable only when the concentration of dehydroascorbic acid is negligible. The alkalinity of the sample also hinders the

determination of ascorbic acid with the DCIP method. The applicability of the method is restricted to only those samples of fruits which do not contain minerals. Materials that are coloured render the end point difficult to judge accurately thereby overestimating or underestimating the level of ascorbic acid in such samples [13].

The differences between our results and those reported by other investigators for many of the fruit samples analyzed may also be explained on the basis of the factors that affect ascorbic acid levels in fruits. These factors include climate, temperature and amount of nitrogen fertilizers used in growing the plant. Climatic conditions such as light and temperature have been reported to affect the chemical composition of horticultural crops [27]. Ascorbic acid is synthesized from sugars which are produced during photosynthesis in plants. Photosynthesis is well known to be affected by light. Fruits that are exposed to maximum sunlight have been shown to contain higher amount of vitamin C than those shaded on the same plant [28]. The composition of plant tissues during growth and development is also determined by temperature which varies from region to region It has been shown that grapefruits grown in coastal areas of California generally contain more vitamin C than those grown in desert areas of California and Arizona [29].

The ascorbic acid content of a fruit is also determined by the level of nitrogen fertilizer used in growing the plant. Lisiewska and Kmiecik [30] reported that increasing the amount of nitrogen fertilizer from 80 to 120 kg ha^{-1} decreased the vitamin C content by 7% in cauliflower.

Wawrzyniak et al [31] examined the Vitamin C content of three apple juice samples using the voltammetric method and the titrimetric method and found that the mean Vitamin C content was slightly higher for the voltammetric method than the titrimetric method. A similar regularity was found by Esteve et al [32] using voltammetric method and HPLC. These results along with the present work show that the voltammetric method is preferable to other very expensive methods in the assessment of juice quality. The voltammetric method of vitamin C determinations does not require expensive equipment and can be used directly without special sample preparations.

4. CONCLUSIONS

This report serves as a database for the vitamin C levels in tropical foods. There is a deficiency in such database in the literature. Fruits are generally available in tropical Africa, but problems related to vitamin C deficiency are common. These results will provide a suitable guide to the population in their choice of fruits with high levels of vitamin C. Adequate consumption of the fruits with high vitamin C content can result in improved health thereby reducing diseases such as diabetes, cataract, glaucoma, macular degeneration, atherosclerosis, osteoarthritis, stroke, heart diseases and cancer that are prevalent in Africa.

The cyclic voltammetric method can be used by Quality Control laboratories to identify and quantify Vitamin C in fruit samples as well as in pharmaceutical preparations.

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