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Vitamin C Contents of Tropical Vegetables and Foods Determined by Voltammetric and Titrimetric Methods and Their Relevance to the Medicinal Uses of the Plants

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Vitamin C contents measured as ascorbic acid, in thirty-eight samples of tropical leafy vegetables and foods were determined by two methods. One was by cyclic voltammetry using glassy carbon, Ag/AgCl and platinum electrode system in 0.1M phosphate buffer, pH 2.0 containing 1mM Na_2EDTA in a potential range of 200 mV – 1000 mV using a scan rate of 50 mV/S. The anodic peak current for the electrochemical oxidation of ascorbic acid to dehydroascorbic acid was recorded at 580 mV. The other method involved titration of aqueous mixtures of the samples using N-bromosuccinimide. Samples identified to be rich in vitamin C include red pepper (123.73 mg/100 g) and the leaves of white camwood (211.20 mg/100 g), climbing black pepper (181.19 mg/100 g), curry plant (140.50 mg/100 g), fluted pumpkin (129.39 mg/100 g), amaranth globe (97.49 mg/100 g) and jute mallow (serrated edge, 89.94 mg/100 g). Boiling of aqueous mixtures of some vegetables reduced the vitamin C content by 20-43%. The results obtained by both methods were comparable for several samples but were appreciably different for some green leafy vegetables. The data in this report further enlarge the database of vitamin C contents in tropical fruits and vegetables which are sparse in literature and will serve as a useful guide in the selection of plants which are rich in vitamin C. The relevance of the vitamin C contents with medicinal uses of some of the plants is discussed.

Keywords: Vitamin C, Cyclic voltammetry, N-bromosuccinimide, Tropical leafy vegetables, Medicinal uses

1. INTRODUCTION

Most tropical countries have vegetation containing a diversity of leafy vegetables such as spinach, amaranth, lettuce that serve as indispensible constituents of the human diet. The use of green leafy vegetables for the preparation of soups cuts across different cultures in Nigeria and other parts of West Africa. Vegetables supply the body with minerals, vitamins, certain hormone precursors as well as proteins and energy [1-4]. Consumption of fruits and vegetables in diet has been reported to protect

the human body from degenerative diseases [5, 6]. In addition vegetables are important in the diets of postpartum women as they aid the contraction of the uterus. The main protective action of vegetables has been attributed to the antioxidants present in them. The oxidative stress experienced by a tissue, organelle or organ results from the balance between the production and removal of potentially damaging reactive oxygen species. Antioxidants can prevent the chemical damage caused by reactive oxygen species such as free radicals that are generated by a variety of sources including pesticides, tobacco smoke, exhaust fumes, certain pollutants and organic solvents [7-9]. The potential cancer-inducing oxidative damage might be prevented or limited by dietary antioxidants found in fruits and vegetables.

Vitamin C also known as ascorbic acid is a water soluble antioxidant which is found in variable quantities in fruits and vegetables [10, 11] and has been found to prevent tissue damage [12-14]. It has been identified to prevent sperm agglutination thus making them more motile with resultant improvement in male fertility. It also enhances sperm quality [15, 16]. Several doctors in Nigeria routinely prescribe vitamin C to aid recovery in several ailments and diseases including cold, cough, influenza, sores, wounds, gingivitis, skin diseases, diarrhoea, malaria and bacterial infections.

The increased knowledge of the role of vitamin C has necessitated the development of accurate and specific methods for its determination. Numerous methods based on instruments, titrations or colour-formation reactions are available in literature for the determination of vitamin C. Instrument-based methods which involve the use of specialized and expensive equipment have been reported [17-20].

Several workers who reported the contents of vitamin C in leafy vegetables grown in Nigeria and other tropical countries used titrimetric [21-25] and spectrophotometric methods [26, 27]. Direct spectrophotometric determination of vitamin C in the ultraviolet 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 determine the contents of vitamin C in tropical leafy vegetables accurately. Electrochemical methods such as cyclic voltammetry can be used because ascorbic acid is readily oxidized to dehydroascorbic acid, thus the reaction is electroactive [28-31]. In the cyclic voltammetric method there is no need to determine end point, thus no error arises from this and there is little interference by colour. The method is sensitive, fast and peak currents are recorded by the instrument hence no reading error. In a previous report, we presented values of vitamin C contents in fifty tropical fruits and seeds determined by cyclic voltammetry and compared to the values obtained by titrimetric method using N-bromosuccinimide (NBS) [32]. In order to enlarge the database, the values of vitamin C contents in several tropical leafy vegetables and foods are hereby presented. The medicinal uses of the plants in diseases where vitamin C can promote healing are discussed.

2. EXPERIMENTAL PART

2.1. Materials and Methods

N-Bromosuccinimide, L-ascorbic acid, sodium dihydrogen phosphate and oxalic acid were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). The reagents and reference solutions

were prepared daily and stored in amber bottles to avoid oxidation. 0.1 M phosphate buffer solution was made up from 0.1M 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.

2.2. Samples Selected For Analysis

38 samples of tropical leafy vegetables and foods commonly consumed in Nigeria were obtained from Nigerian Institute of Horticulture (NIHORT), Ibadan, Nigeria and Mushin and Oyingbo markets in Lagos and environs. The samples obtained from the open markets were identified in NIHORT. The vegetable and food samples analyzed include, African bush mango leaves (Oro, Yoruba) (*Irvingia gabonesis*), amaranth globe leaves (Utazi, Igbo), (*Gongronema latifolium*), bitter leaf (*Vernonia amygdalina*), cabbage leaves (*Brassica oleracea*), carrot (*Daucus carota*), climbing black pepper (leaves and seeds) (Uziza, Igbo) (*Piper guinenses*), cucumber unpeeled (*Cucumis sativus*), curry leaf (*Murraya koenigii*), fluted pumpkin leaves (Ugu, Igbo) (*Telferia occidentalis*), ginger (unpeeled) (*Zingiber officinale*), green (Amunututu, Yoruba) (*Basella alba*), green amaranth (tete, Yoruba) (*Amaranthus hybridus*), groundnut seeds (*Arachis hypogaea*), jute mallow leaves (serrated edge) (Ewedu awoyaya, Yoruba) (*Corchorus spp*), jute mallow leaves (smooth edge) (Ewedu angbadu, Yoruba) (*Corchorus olitorius*), koko vine leaves (Okazi, Igbo) (*Gnetum africanum*), lemon grass leaves (*Cymbopogon citratus*), lettuce (*Lactuca sativa*), locust bean (Igba, Yoruba) (*Parkia biglobosa*), mint leaf (Efinrin, Yoruba) (*Ocimum gratissimum*), miracle berry leaves (Ewe moinmoin, Yoruba) (*Thaumatococcus danielii*), myriantus leaves (Ujuju, Igbo) (*Myrianthus arboreus*), nut grass (Ofio, Yoruba) (*Cyperus escillentus*), Potato (Irish) (*Solanum tuberossum* L), potato (sweet) (*Ipomoea batatas*), raddish (red) (*Raphanus sativus*), raddish (white), (*Raphanus sativus acanthiformis*), roselle calyx [(Isapa,Yoruba) *Hibiscus sabdariffa*, *green variety*)], roselle calyx [(Zobo, Hausa) (*Hibiscus* sabdariffa, red *variety*)], sour sop (*Annona muricata*), spinach (*Spinacia oleracea*), water hyacinth (*Eichhornia crassipes*) which is used as animal feed, water leaf (Gbure, Yoruba) (*Talinum triangulare*), water lettuce (Oju-oro, Yoruba) (*Pistia stratioles*), white camwood leaves (Oha, Igbo) (*Pterocarpus mildbraedii*) and yam (*Dioscorea spp*). Honey though not a vegetable was included in the samples because it is added to some plant materials and juices in several dietary menu and herbal preparations. (*Yoruba and Igbo are some Nigerian Languages*).

2.3. Determination of ascorbic acid by titration with N-bromosuccinimide

The determination was essentially as described in a previous report [32]. A weighed amount of the sample (5 g) was washed, minced and blended with 0.5% oxalic acid solution for approximately one minute and filtered through 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. A mixture of 10 cm^3 of the sample solution, 2 cm^3 of 3 % ethanoic acid, 5 cm^3 of 4 % KI and 10 drops of starch indicator was titrated using 0.01% N-bromosuccinimide as titrant. The mean of three titre values was recorded. All

analyses were done in triplicate. The ascorbic acid contents in the samples were obtained from the calibration curves and were used to obtain the values in mg/100 g of sample.

2.4. Determination of ascorbic acid using cyclic voltammetry [32]

A BASI-Epsilon potentiostat/galvanostat was used in the study. A weighed amount (5-6 g) of the sample was minced and blended with 30 cm^3 of the phosphate buffer for approximately one minute. The homogenized sample was filtered through glass wool. 15 cm^3 of the filtrate was transferred to the electrochemical cell, purged with nitrogen for 10 min before scanning the potential between 200 mV and 1000 mV using a three electrode system consisting of glassy carbon (3 mm) as working electrode, Ag/AgCl as reference and platinum (1.6 mm) as the auxiliary electrode. The values of the anodic peak current obtained at 580 mV were used to calculate the concentration of the ascorbic acid in the vegetable samples using the calibration curve.

2.5. Effect of Heat on Vitamin C Contents of Some Vegetables

The vitamin C contents of jute mallow leaves (*C. olitorius*), bitter leaf (*V. amygdalina*) and fluted pumpkin leaves (*T. occidentalis*) were also determined by cyclic voltammetry after boiling them in water for 20 min to ascertain the effect of heat on the vitamin C contents of these leafy vegetables.

3. RESULTS AND DISCUSSION

The voltammograms of the calibration and the generated calibration curve were as reported previously [32]. The voltammograms for the determination of ascorbic acid content of jute mallow (*Corchorus olitorius*) before and after boiling are shown in Figures 1a and 1b.

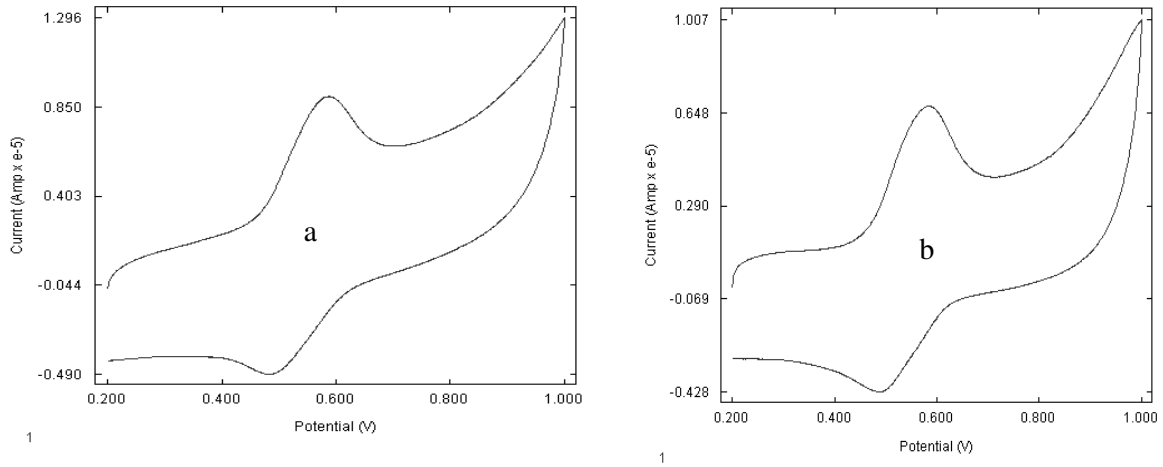

Figure 1. Voltammograms of *Corchorus olitorius* extract in 0.1M phosphate buffer, pH 2.0. a=before boiling; b=after boiling in water for 20 min.

Table 1 shows the ascorbic acid contents of the different vegetables and foods determined by the two methods. Vegetables found to have high concentrations of ascorbic acid (as measured by

cyclic voltammetry) in the range of 90-211 mg/100 g of sample include the leaves of amaranth globe, climbing black pepper, curry leaves, fluted pumpkin, jute mallow (serrated edge) red pepper and white camwood. Lower but significant levels of ascorbic acid determined by cyclic voltammetry in the range of 10-75 mg/100 g of sample were found in African bush mango leaves, bitter leaf, cabbage, climbing black pepper seeds, ginger (unpeeled), green leaves, green amaranth, groundnuts, jute mallow (smooth edge), koko vine leaves, lettuce, locust bean, mint leaves, miracle berry leaves, myrianthus leaves, nut grass, potato (Irish), raddish (red and white), roselle calyx (green and red), sour sop, spinach, water hyacinth, water leaf, water lettuce and honey. Thus when honey is added to menu or herbal preparations, there is enhancement of vitamin C content.

Low levels of ascorbic acid in the range of 6-10 mg/100 g (cyclic voltammetric determination) were found in carrot, cucumber, lemon grass, potato (sweet) and yam.

Comparing the values obtained by the two methods, it is observed that the values differ by not more than 10 mg/100 g that is, 0.1 mg/g in 28 samples, thus are comparable. In 6 samples, the values differ by 10-20 mg/100 g of sample. In three of these, the differences are 12.58, 10.62, 11.97 mg/100 g in carrot, green amaranth leaves and jute mallow leaves (serrated edge) respectively. These values are just above the 10 mg/100 g and can be considered reasonable. The remaining three samples are locust bean, water leaf and roselle calyx (red variety) where the differences are 19.47, 14.90 and 13.86 mg/100 g respectively. It is also observed that in all the six samples, except green amaranth, the values obtained by titrimetric are consistently higher. Thus the differences may be due to inaccurate detection of the end point especially in the coloured materials. However, in five samples, namely bitter leaf, climbing black pepper leaves, curry leaves, fluted pumpkin leaves and white camwood leaves, the differences are 93.32, 122.1, 39.66, 41.90 and 82.23 respectively. The values obtained by cyclic voltammetry are higher in climbing black pepper leaves, curry leaves and white camwood leaves. Interferences with the reagent or the working electrode by enzymes in the samples may account for the differences observed.

The effect of heat on the ascorbic acid content of vegetable samples is shown in Figures 1a and 1b for jute mallow leaves (*C. olitorius*). The vitamin C content of 40.21 mg/100 g for the fresh sample was found to decrease to 30.65 mg/100 g after boiling the vegetable in water for 20 min. The reductions of 21% and 47% in the vitamin C contents of bitter leaf (*V. amygdalina*) and fluted pumpkin leaves (*T. occidentalis*) were similarly obtained when these samples were boiled in water for 20 min.

Ejoh et al., 2005 reported ascorbic acid content of 166.5 mg/100 g for bitter leaf (*V. amygdalina*) by titrimetric method using the N-bromosuccinimide [22]. Chinma and Igyor, 2007 [25] reported 14.61 mg/100 g, 21.03 mg/100 g and 12.50 mg/100 g for climbing black pepper leaves, amaranth globe leaves and white camwood leaves respectively by titrimetric method using 2,6-dichloroindophenol reagent after the samples had been washed in water and dried in the oven at 60^{o}C for 24 h. The 2,6 dichloroindophenol reagent and the excessive exposure of the samples to heat make it difficult to draw any comparison between the values of Chinma and Igor [25] and those contained in this report.

Table 1. Vitamin C contents of tropical vegetables and foods obtained by cyclic voltammetry (CV) and titration with N-bromosuccinimide (NBS).

S/No	Sample		Amount of ascorbic acid		% Difference	Absolute Difference	Relevant medicinal uses [33]
	Common Name	Botanical Name	CV mg/100g	NBS mg/100g			
1.	African Bush mango leaves [Oro (Yor)]	*Irvingia gabonensis*	23.30	27.51	18	4.21	Spleen infections
2.	Amaranth globe leaves [Utazi (Igbo)]	*Gongronema latifolium*	97.49	100.00	3	2.51	Sore gums
3.	Bitter leaf [Ewuro (Yor)]	*Vernonia amygdalina*	32.15	125.47	290	93.32	Gingivitis, diabetes, malaria, anti-microbial.
4.	Cabbage leaves	*Brassica oleracea var capitata*	23.05	13.59	41	9.46	Skin diseases, antimicrobial
5.	Carrot	*Daucus carota*	9.28	21.86	136	12.58	
6.	Climbing black pepper leaves. Uziza (Igbo)	*Piper guinenses*	181.19	59.01	67	122.18	Impotence, hypertension, antimicrobial
7.	Climbing black pepper seeds. Uziza (Igbo)	*Piper guinenses*	14.59	15.48	6	0.89	Impotence, hypertension, antimicrobial
8.	Cucumber (unpeeled)	*Cucumis sativus*	6.99	14.35	105	7.36	Diuretic
9.	Curry leaves	*Murraya koenigii*	140.50	100.84	28	39.66	Herpes, fever
10.	Fluted Pumpkin leaves. Egusi iroko (Yor), Ugu (Igbo)	*Telfaria occidentalis*	129.39	171.29	32	41.90	Anti-inflamm-atory, arthritis, cancer, blood tonic
11.	Ginger (unpeeled)	*Zingiber officinale*	11.50	15.00	30	3.50	Cold, cough, malaria, fever.
12.	Green leaves. Amunututu, (Yor)	*Basella alba*	65.32	70.15	7	4.83	Laxative
13.	Green amaranth Tete abalaye, (Yor)	*Amaranthus viridis.*	60.12	49.50	18	10.62	Eye diseases, gonorrhea, diuretic, anti-inflammatory
14.	Groundnuts	*Arachis hypogaea*	43.74	42.95	2	0.79	Antimicrobial
15.	Jute mallow leaves (serrated	*Corchorus spp*	89.94	101.91	13	11.97	Abscesses, fever.

	edge). Ewedu awoyaya (Yor)						
16.	Jute mallow leaves (smooth edge) Ewedu angbadu(Yor)	*Corchorus olitorius*	40.21	44.74	11	4.26	Abscesses, fever
17.	Koko vine leaves Okazi, (Igbo)	*Gnetum africanum*	56.27	46.36	18	9.91	Wounds, colds and spleen
18.	Lemon grass leaves	*Cymbopogon citrates*	9.37	10.00	7	0.63	Malaria, coughs, cold, diuretic
19.	Lettuce leaves	*Lactuca sativa*	22.27	13.00	42	9.27	Diuretic, constipation
20.	Locust bean. [Igba (Yor)]	*Parkia biglobosa*	73.23	92.65	27	19.47	Wounds, malaria, high blood pressure, anti-tumour.
21.	Mint leaf. Efinrin-nla (Yor)	*Ocimum gratissimum*	52.75	46.00	13	6.75	Wounds, anti bacterial, cold, hypertension
22.	Miracle berry leaves. [Ewe moimoin (Yor)]	*Thaumatococcus danielii*	28.67	26.40	8	2.27	
23.	Myriantus leaves [Ujuju (Igbo)]	*Myrianthus arboreus*	15.93	10.55	34	5.38	Dysentery, cough,anti-tumour
24.	Nut grass [Ofio, (Yor)]	*Cyperus esculentus*	11.45	13.37	17	1.92	
25.	Potato (Irish)	*Solanum tuberosum* L	11.45	8.00	30	3.45	
26.	Potato (sweet)	*Ipomoea batatas*	6.15	4.28	30	1.87	Boils, wounds, antimicrobial
27.	Raddish (red)	*Raphanus sativus*	36.20	36.45	1	0.25	
28.	Raddish (white)	*Raphanus sativus acanthiformis*	39.19	40.82	4	1.63	
29.	Red pepper [Bawa (Hau)]	*Capsicum spp*	123.73	126.05	2	2.32	Colds. Anti-inflammatory
30.	Roselle calyx (green variety) [Isapa (Yor)]	*Hibiscus sabdariffa*	27.50	29. 20	6	1.70	Cough, dressing of wounds, diuretic
31.	Roselle calyx (red variety).	*Hibiscus sabdariffa*	32.14	46.00	43	13.86	Cough, diuretic,

	[Zobo (Hau)]						dressing of wounds
32.	Sour sop	*Annona muricata*	10.51	13.63	30	3.12	Fever
33.	Spinach	*Spinacia oleracea*	35.67	38.75	9	3.08	
34.	Water hyacinth	*Eichhornia crassipes*	10.19	16.34	60	6.15	Skin care, goiter
35.	Water leaf [Gbure (Yor)]	*Talinum triangulare*	21.82	36.72	68	14.90	Shistosomiasis, scabies, fresh cuts, high blood pressure, anaemia.
36.	Water lettuce [Oju oro (Yor)]	*Pistia stratiotes*	21.02	19.00	10	2.02	Skin rashes, boils, sores, colds.
37.	White camwood leaves [Oha (Igbo)]	*Pterocarus mudbraedii*	211.20	129	39	82.2	
38.	Yam tuber	*Dioscorea rotundata*	7.77	7.52	3	0.25	
39.	Honey		27.78*	33.47*	21	5.69	Cold, cough, wound healing

*Values as mg/ 100 cm^3

KEY: CV = cyclic voltammetric determination; NBS = N-Bromosuccinimide titrimetric determination; Yor = Yoruba; Hau = Hausa.

It is pertinent to relate the vitamin C content of these vegetables and food samples to the alleged curative uses in herbal medicine. Several of these samples are alleged to be useful in the treatment of sores, cuts, wounds, abscesses, malaria, skin diseases and cold [33]. These medicinal uses are stated in Table 1. Such samples include amaranth globe (97.49), bitter leaf (32.15), cabbage (23.05), ginger (11.50), honey (27.78), jute mallow (serrated, 89.94; smooth, 40.21), koko vine (56.27), lemon grass (9.37), locust bean (73.23), mint leaf (52.75), potato (sweet, 6.15), red pepper (123.73), roselle calyx (green variety) (27.50), roselle calyx (red variety) (32.14), water leaf (21.82), water lettuce (21.02). These plants, except ginger, lemon grass and potatoes (sweet) have medium-high vitamin C content. Potatoes (sweet) are often consumed in larger quantities than vegetables and thus significant quantities of vitamin C will be present in such menu.

Vitamin C has been reported to be protective and therapeutic in cardiovascular diseases [34-36] and some of these plants are alleged to be useful as diuretic or antihypertensive [33]. These include climbing black pepper [leaves (181.19), seeds (14.59)], cucumber (unpeeled, 6.99), lemon grass (9.37), locust bean (73.23), mint leaf (52.75) and roselle calyx [(green variety) (27.50), (red variety) (32.14)]. Several of these plants have high vitamin C content. Raised blood pressure is a major cause of stroke and studies have shown that high intake of fruits and vegetables is associated with reduced risk of

ischemic stroke [37, 38]. Thus, the plants listed have the potential to reduce high blood pressure and hence would have preventive activity against stroke.

Vitamin C has also been reported to be protective and therapeutic in cancer [39]. Fluted pumpkin (129.39) is alleged to be useful in the herbal treatment of cancer while myriantus (15.93) is alleged to be helpful as an antitumour herbal medicine [33]. While fluted pumpkin leaves are rich in vitamin C, myriantus leaves will not be considered rich. Thus myrianthus leaves probably contain other anti-cancer constituents.

Vitamin C is also reported to be protective and therapeutic in eye diseases [40]. Green amaranth is moderately rich in vitamin C (60.12). It is used as herbal treatment for eye diseases [33, 41].

Vitamin C, functioning as an antioxidant, has been reported to be relevant in the treatment of male infertility factor [15, 16]. Climbing black pepper (leaves, 181.19, seeds, 14.59) are used in herbal medicine to treat impotence which is synonymous with male infertility in local terminology.

Thus several plants which are rich in vitamin C appear to have therapeutic values although there may be no clinical trials to support this observation.

4. CONCLUSIONS

This report further enlarges the database for the vitamin C content in several tropical fruits, vegetables, seeds and foods. The results presented in this report will provide a suitable guide to the population in their choice of vegetables with high content of vitamin C. Adequate consumption of the vegetables with high vitamin C content will result in improved health thereby reducing cardiovascular diseases, diabetes, eye diseases, infertility and cancers that are prevalent in Africa.

The cyclic voltammetric method can be used in Quality Control laboratories for rapid and accurate determinations of quantitative values of vitamin C in agricultural samples and in pharmaceutical preparations.

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