Determination of vitamins in five selected West African green leafy vegetables

Adetutu Adewale[1*] Adesokan A. Ayoade[1] and Ezekiel Ayinde Alani[2]

[1]Department of Biochemistry, Ladoke Akintola University of Technology, Faculty of Basic Medical Sciences, PMB 4000, Ogbomoso, Nigeria; aadetutu@lautech.edu.ng
[2]Department of Agricultural Economics, Faculty of Agricultural Sciences, Ladoke Akintola University of Technology, PMB 4000, Ogbomoso, Nigeria.

Abstract: Increased awareness of the usefulness of vegetable inclusions in human food has enhanced their consumption as part of the daily diet. The therapeutic potential of the vegetables is usually attributed to the high content of vital vitamins. The aim of this study is to detect the presence of vitamins A, B_2, C and E in *Launaea taraxacifolia, Solanum aethiopicum, Telfairia occidentalis, Amaranthus caudatum* and *Amaranthus hybridus* leaf extracts. The vitamins were extracted using chemical methods and reversed-phase high performance liquid chromatography system was employed for detection and quantification of the vitamins. These findings show that all the assayed vitamins were present in the selected vegetables in different amounts. The results show that among the green leafy vegetables studied, the level of β-carotene (1957.26 µg/mL) and riboflavin (224.67 µg/mL) was higher in *S. aethopicum* than the other leafy vegetables. In addition, *A. caudatus* had the highest level of ascorbic acid and α-tocopherol which are 5298.94 µg/mL and 1375.46 µg/mL respectively. Based on the widely reported health benefits of the selected vitamins and the quantity detected in this study, these vegetables might prevent the occurrence of the various diseases. Thus, the level of the various vitamins in these vegetables is of great importance for the generation of a database on nutritional value which is important for future research.
[Adetutu A. **Determination of vitamins in five selected green leafy vegetables.** *J Am Sci* 2013;9(2s):40-43]. (ISSN: 1545-1003). http://www.jofamericanscience.org.

Keywords: Vegetables; Vitamins; Antioxidants; Protective effect

1. Introduction

Leafy vegetables are those plants whose leaves or aerial parts have been integrated into a community's culture for use as food over a large span of time (Ogoye-Ndegwa, 2003). Vegetables are highly recommended because of the relatively high nutritional value and their consumption gives diversity to daily food intake, adding flavour and taste to the diet (Asfaw, 1997). Vegetables constitute essential components of the diet, by contributing protein, vitamins, iron, calcium and other nutrients which are usually in short supply (Thomson and Kelly, 1990). Besides these biochemicals, the moisture, fiber and ash contents, and the energy values of individual vegetables have also been regarded of importance to human health (Jiang *et al.*, 2001).

Taking a step back from modern times, it is important to note that many leafy vegetables have been used for medicinal purposes in addition to their use as food for thousands of years (Packer , 1993). In some cases, it is possible that leafy vegetables were domesticated for this reason (Rubatzky 0and Yamaguchi, 1997). The recognition that certain diets are associated with reduced risk of diseases has been very well established in the medical literature in both experimental and epidemiological studies since the 20th century (Heber and Bowerman, 2001).
In many of these studies, diets with higher intake of vegetables, fruits, whole grains, and plant-based proteins are clearly favored for disease prevention. This led nutritional scientists to suspect that the effect was due primarily to diets low in fat or high in fiber, or to the action of one particular phytonutrient (Heber and Bowerman, 2001).

Epidemiological studies have shown a relationship between intake of vitamin-rich foods such as fruits and vegetables and protection against chronic diseases such as cancer and have led to dietary recommendations by prestigious government and scientific organizations. (Block *et al*., 1992). Vitamins are biologically active organic compounds that are essential micronutrients involved in metabolic and physiological functions in the human body. (Santos *et al.,* 2012). These vitamins greatly differ in their chemical composition, physiological action and nutritional importance in the human diet, even within the same group (Finglas *et al.*, 1993). Some vitamins are involved in complex metabolic reactions related to important biological functions, such as vision (vitamin A), calcium absorption (vitamin D), antioxidant protection of cell membranes (vitamin E) and blood coagulation (vitamin K), among other functions (Herrero *et al,* 2012). Several vitamins of the B-group act mainly as coenzymes in the catabolism of foodstuffs to produce energy (Ball, 2006).

Based on the widely noted medicinal values derived from the consumption of vegetables, it is imperative to document the standards and quantities of these useful vitamins. Therefore, the aim of this study is to detect the presence of vitamins in five selected vegetables and if present the benefit of their consumption.

2.0 Material and Methods:

2.1 Plant materials, chemicals and equipments

Launaea taraxicofolia, *Solanum aethopicum*, *Telfairia occidentalis*, *Amaranthus caudatus* and *Amaranthus hybridus* were selected for this study based on their popular use as food supplements. The vegetables were obtained from LAUTECH Research farm, Ogbomosho, Oyo state. The leaves were identified by a qualified taxonomist, Dr Ogunkunle, in the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomosho, Oyo state and were stored in the LAUTECH herbarium. The vegetables were assigned the voucher numbers as follows: *L. taraxicofolia* – LHO 231; *S. aethopicum* – LHO 230; *T. occidentalis* – LHO 229; *A. caudatus* – LHO 233; *A. hybridus* – LHO 232. All the chemical used were of analytical or HPLC grade and obtained from BDH, UK. The Agilent Technology 1200series High Performance Liquid Chromatography (HPLC) was used for the detection of the vitamins. The vitamins were separated on HPLC columns, Hypersil® 150 x 4.6 mm i.d C18 column (Phenomenex®, Torrence, CA, USA).

2.2 Methodology

2.2.1 Extraction of vitamins

Ascorbic acid was extracted according to the modified method of Abdulnabi *et al*., (1997). Riboflavin was extracted according to the method of AOAC International, 1990. β-carotene was extracted according to the modified method of Tee *et al*., (1996). The method described by Abdulnabi *et al*., (1996) was used for alpha tocopherol extraction. This is then subjected to HPLC for chromatographic determination of vitamins.

2.2.2 HPLC analysis

The vitamin contents of the leafy vegetables were analyzed using Agilent Technology 1200series High Performance Liquid Chromatography apparatus. In brief, the various vitamins were separated on column equipped with an injector. Column effluents were monitored at 254nm with a detector. Peak areas were determined. The mobile phase was composed of 0.015% formic acid buffer and Methanol/Acetonitrile in ratio 17:83. The flow rate was 0.2 mL/min.

Table1: HPLC chromatographic conditions for the separation of the vitamins in the selected vegetable

Column Size	Hypersil® 150 x 4.6 mm i.d C_{18} column		
Mobile Phase	A: 0.015% Formic acid B: 17:83 Methanol/Acetonitrate		
Gradient	Time (mins)	% A	%B
	0.1	-	45 – 100
	3	100	-
	5	-	0 – 45
	16.9	-	100
Flow Rate	0.21 mL/min		
Injection volume	20 µL		
Detection	UV (DAD) at 254nm		

3.0 Result:

Table 2 shows the composition of the vitamins (µg/mL) in the selected vegetables. The vitamins identified are β-carotene, ascorbic acid, riboflavin, and α-tocopherol. In this study, the vitamins were eluted within a minute in order of vitamin A, B_2, E and C with their various standards (Table 3). The chromatograms of the standard samples had almost the same retention time as the leaf extracts of vegetables.

Table 2: Composition (µg/mL) of selected green leafy vegetables as compared with the standard

Leafy vegetables	βcarotene	Ascorbic acid	Riboflavin	αtocopherol
Solanum aethopicum	1957.26	84.15	224.67	905.76
Amaranthus caudatus	1611.60	5298.95	102.07	1375.46
Launaea taraxicofolia	1508.22	61.60	8.71	68.81
Telfairia occidentalis	864.97	82.66	36.74	675.00
Amaranthus hybridus	106.59	4487.08	30.70	646.85
Standard	160.88	198.64	84.26	60.77

Table 2 shows the composition of assayed vitamins in selected vegetables. All the vitamins were confirmed to be present in all the selected vegetables.

Table 3: Retention time (mins) of selected green leafy vegetables as compared with the standard

Leafy vegetables	β-carotene	Ascorbic acid	Riboflavin	α-tocopherol
Solanum aethopicum	0.922	0.919	0.872	0.857
Amaranthus caudatus	0.920	0.910	0.867	0.862
Launaea taraxicofolia	0.920	0.910	0.870	0.859
Telfairia occidentalis	0.923	0.917	0.869	0.871
Amaranthus hybridus	0.921	0.922	0.870	0.870
Standard	0.923	0.910	0.852	0.861

Table 3 shows the retention time of all the vitamins as confirmed from the various standards. The retention time is between 0.852mins and 0.923 mins.

4. Discussion:

In this study, the HPLC profiles of all the selected green leafy vegetables were found to be similar and the major difference appears to be in their compositions. The presence of the vitamins in the selected vegetables had credit to their widely use as food supplements and medicine. Interestingly, *L. taraxicofolia* has been used for centuries as a remedy for various ailments such as skin and eye diseases (Obi *et al.,* 2006). This is probably due to the presence of vitamin A and B_2 in this vegetable as confirmed by this study and previously by Mackenzie (2001) and Sobana (2008).

S. aethopicum is also used in the treatment of high blood pressure (Lester, 1986). Vitamin C is known to help protect the body against Low Density Lipoprotein (LDL) and cholesterol (Frei, 1991). Atherosclerosis is a major contributor to heart diseases but vitamin C may prevent this plaque formation by inhibiting the oxidative modification of LDLs (Jialal, 1990). Vitamin C also helps to prevent atherosclerosis by strengthening the artery walls through its participation in the synthesis of collagen and by preventing the undesirable adhesion of white blood cells to damaged arteries (Weber, 1996). Vitamin C supplementation from *S. aethopicum* also effectively reverses the vasomotor dysfunction often found in patients with atherosclerosis (Levine, 1996). In addition, vitamin C may increase the beneficial High Density Lipoprotein (HDL) cholesterol (Gaby and Singh, 1991; Sobana, 2008).

T. occidentalisis is cultivated in Southern Nigeria for its leaves and seeds which are eaten mainly for their high content of vitamins and minerals. It has also been reported for its antioxidant and antimicrobial activities. The intake of *T. occidentalis* reduces the deficiency symptom of riboflavin which are lesions of the eyes and lips, congestion of conjuctival blood vessels and desquamation of the skin. (Okwu, 2004). These pharmacological uses might be related to the presence of vitamin A and E detected in *T. occidentalis*. The results of this study are in conformity with that of Donatus and Nneka, 2007 which detected ascorbic acid, riboflavin, thiamine and niacin in ***T.*** *occidentalis* leaf extracts**.** The traditional use of *A. caudatus* in the treatment of eye diseases has been previously reported by Yoganarasimhan (2000) and this might be related to the presence of vitamin A as confirmed in this study and that of Evarando (2005), as vitamin A is known to be vital for good functioning of the eye.

In Nigerian traditional medicine, *A. hybridus* is used for the treatment of inflamed or ulcerated tissue (Akinpelu and Onakoya, 2006) and for the treatment of intestinal bleeding and menstruation (He *et al.,* 2003). The choice of *A. hybridus* for these treatments might be due to the presence of Vitamins B_2, E and especially vitamin C because of the role of these vitamins as scavengers of free radicals and making the them effective as a protector of the integrity of lipids and phospholipids membranes (Sobana, 2008). This study thus shows that the selected leafy vegetables with medicinal value, but less commonly used for nutritional purpose contain substantial levels of β-carotene, ascorbic acid, α-tocopherol and riboflavin. The data on the composition and medical uses of these four vitamins could be helpful to create nutritional awareness among various Nigerians on the importance of these green leafy vegetables and thus increase the consumption of these vegetables.

Corresponding Author:
*Dr. Adetutu Adewale
Department of Biochemistry
Faculty of Basic Medical Sciences
Ladoke Akintola University of Technology
Ogbomoso, Nigeria
E-mail: wale_836@hotmail.com

References

1. Ogoye-Ndegwa C. and Aagaard-Hansen, (2003). Studies on the proximate analysis of Solanum aethiopicum, Lactuca taraxacifolia. *J. Ecol. Food and Nutri*. 69.
2. Asfaw, Z., (1997). Conservation and Use of Traditional Vegetables in Ethiopia. *J. Nutr*. P67.
3. Thomson, H.C., and Kelly WC, (1990). Vegetable Crops (5th edition). McGraw Hill Publishing Co. Ltd., New Delhi.
4. Jiang, Q.; Christen, S.; Shigenaga, M. K.; Ames, B. N. 2001. gamma-tocopherol, the major form of vitamin E in the US diet, deserves more attention. *Am. J. Clin. Nutr.,* 74, 714-722.
5. Packer, L.; Fuchs, J. (1993)In *Vitamin E in health and disease;* Dekker: New York, pp 1000.
6. Rubatzky, V.E. and Yamaguchi, M., (1997). World vegetables. (2nd ed). Chapman and Hall, New York.
7. Heber, D. and S. Bowerman, (2001). Applying science to changing dietary patterns. *J. Nutr.* 131. 3078s–3081s.
8. Block G, Patterson B, Subar (1992). Fruit, vegetables, and cancer prevention: A review of the epidemiological evidence. *Nutr Cancer* 18 .1-29.
9. Santos, J., J.A. Mendiolab, M.B.P.P. Oliveiraa, E. Ibá˜ nezb, M. Herrerob, (2012). Sequential determination of fat- and water-soluble vitamins in green leafy vegetables during storage. *Journal of Chromatograghy A*. 1261, 179-188.
10. Finglas P.M., U. Faure, P.J., (1993). *J Anal Chem*. Wagstaffe, Fresenius 345 (180), 171–176.

11. Herrero, M., A. Cifuentes, E. Ibáñez, (2012). Comprehensive sampling and sample preparation, *Elsevier*, J. Pawliszyn Editor, Amsterdam. 3.
12. Ball, G.M.F., (2006). Vitamins in Food Analysis, Bioavailability, and Stability. CRC Press, Boca Raton. 3.
13. Abdulnabi A. Abushita, Emhemed A. Hebshi, Husssein G. Daood and Peter A. Biacs., (1996). Determination of antioxidant vitamins in tomatoes. *Food Chemisty* 60. 207-212.
14. AOAC International, (1990). Official methods of analysis. (15th ed.) Arlington, VA, USA.
15. Tee E.S, Kuladevan R., Young S.I., Khor S.C. and Zakiyah H.O. (1996). Nutrient analysis of foods.
16. Kuala Lumpur Institute of Medical Research. Obi R.K., Umeh .SC, Okurede O.H and Iroagba I.I, (2006). Prevalence of Hepatitis B Virus among Pregnant Women attending an antenatal clinic in Port-Harcourt, Nigeria. *Afr. J. of Clin. Exp. Microbiol*.7. 2-6.
17. Lester, R.N**,** (1986). Taxonomy of scarlet eggplant, *Solanum aethiopicum*. *Acta Horta* 182. 125–132.
18. Frei, B (1991). Ascorbic acid protects lipids in human plasma and Low-Density Lipoprotein against oxidative damage. *Am. J. Clin. Nutr*., 54. 1113S-1118S.
19. Jialal, I.,. Vega, Gloria Lena Grundy, Scott.M., (1990). Physiologic levels of ascorbate inhibit the oxidative modifications of Low Density Lipoproteins.*Atherosclerosis*, 82, 185.
20. Weber, C., (1996). The increased adhesiveness of isolated monocytes to endothelium is prevented by vitamin c intake in smokers. *Circulation*, 93. 1488-1492.
21. Levine, N.G., (1996). Ascorbic acid reverses endothelial vasomotor dysfunction in patients with coronary artery disease. *Circulation*, 93, 1107-1113.
22. Gaby, S.K. and V.N. Singh, (1991). Vitamin C – Vitamin intake and health: A Scientific review.
23. Okwu D.E., (2004). Phytochemical and vitamin content of indigenous species of South Eastern Nigeria. *Journal of Sustainable Agric and Environ* 6. 30-37.
24. Donatus E. O. and Nneka S. U., (2007). Nutritive value and phytochemcal contents of fluted pumpkin (*Telfairia occidentalis Hook f)* vegetable grown with different levels of turkey droppings. *Africa crop science proceedings.* .8, 1759-1764
25. Yoganarasimhan, S.N, (2000). Medicinal plants of India. Inline Publishing private Ltd. p38.
26. 26. Evarando L.S, Oliveira L.E, Freire L.K.R, Sousa P.C. (2005). Inhibitory action of some essential oils and phytochemicals on growth of various moulds isolated from foods. *Braz. Arch. Biol. Technol*. 48234-48241.
27. Akinpelu D.A, Onakoya TM (2006). Antimicrobial activities of medicinal plants used in folklore remedies in south-western Africa. *Afr. J. Trad*. 112-115.
28. He H.P., Corke H., Cai J.G., (2003). Supercritical carbon dioxide extraction of oil and squalene from Amaranthus Grain. *J. Agric. Food Chem* 51. 7921-7925.
29. He H.P, Cai Y, Sun M, Corke H (2002). Extraction and purification of squalene from *Amaranthus* grain. *J. Agric. Food Chem* 50, 368-372.

1/25/2013