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Effect of Heat Treatments on Vitamin A Content of Ten Selected Leafy Vegetables Using UV Spectrophotometric Method of Analysis

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Abstract

Although researchers have established the fact that vegetables play a vital role in the contribution of essential nutrients to the body and thus help to maintain a healthy life style, prevent diseases, and reduce mortality from infectious diseases such as measles and HIV; the traditional practice of boiling vegetables before consuming has been going on for ages in Uyo, without questioning their nutritional values such as vitamin components before and after heat treatments. Prompted by this, this research work was carried out to analyze vitamin A content of ten selected leafy vegetables. The concentration of vitamin A was determined for both raw and boiled vegetables samples at 60 and 80 °C (i.e., before and after heat treatments) for 15 min using UV-Spectrophotometry. The results of the analysis showed that all the vegetables contain vitamin A in this order: (*Gnetum africana*) > *Telfairia occidentalis* > *Piper guineense* > *Ocimum gratissimum* > *Vernonia amygdalina* > *Talinum fruticosum* > *Heinsia crinita* > *Justicea flava* > *Gongronema latifolium* > *Lasianthera africana*) both in raw and boiled samples at 60 °C and at 80 °C for 15 min. It was further observed that as heating temperature increased, the vitamin A content of the vegetables also increased at constant heating time. It can be concluded that the heating temperature affected the vitamin A content of all the vegetables tested.

Keywords: *Vegetables, Vitamin A, Heat treatment, Retinol, UV spectrophotometry, Fat Soluble*

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1. Introduction

Vegetables are plants that are consumed by humans or other animals as food. Vegetables can also collectively refer to all edible plant matter including the flowers, fruits, stems, leaves, roots, and seeds. Vegetables are important for human health because of their vitamins, minerals, phytochemical compounds and dietary fiber content which the human body needs to maintain good health. Report has shown that consumption of diets rich in vegetables and fruits protect the body from chronic degenerative disease (Heber, 2004). Some vegetables can be taken raw but most are commonly cooked before consuming. Generally, preparations of vegetables at home are based on taste preference and convenience rather than retention of nutrient and health promoting compounds (Masrizal *et al.*, 1997).

Vitamin A also known as Retinol is a member of the class of fat-soluble vitamins. It plays an important role in ocular function as it is involved in cell differentiation, in maintenance of eye integrity and in the prevention of xerophthalmia. Its deficiency is the main cause of preventable blindness worldwide. Vitamin A is also associated with bone development, has a protective effect on the skin and mucosa, plays a vital role in the functional capacity of reproductive organs, participates in strengthening the immune system, and is related to the development of normal teeth and hair. In addition to its important role in various body tissue, Vitamin A is essential for the normal development of the embryo (D'Ambrosio *et al.*, 2011).

Retinol can be obtained from food either as preformed retinol in animal products such as eggs and dairy products, or as provitamin A carotenoids, mainly beta carotene in plants products. Preformed Vitamin A, which is found in animal products and supplements is efficiently absorbed and epidemiological studies suggest that chronic high intakes of preformed Vitamin A are associated with hip fractures. Carotenoids however provide a safer form of vitamin A due to regulated bioconversion in the body.

Micronutrient malnutrition especially deficiency of vitamin A is globally affecting over 3 billion people. According to World Health Organization (WHO, 2017; 2023). Vitamin A Deficiency (VAD) has affected about 190 million preschool age children and 19 million pregnant women, mostly in Africa and South East Asia (Zhao *et al.*, 2022). Retinol deficiency is the leading cause of preventable blindness in children and increases the risk of disease and death from severe infections such as diarrhoea and measles according to WHO. Retinol deficiency may also occur in women during the last trimester of pregnancy in high-risk areas. A food-based approach is best to combat vitamin A deficiency among groups at risk of deficiency. Green leafy vegetables are grown abundantly in Akwa Ibom State, Nigeria and are relatively inexpensive.

Many vegetables contain Vitamin A (Retinol) and are not destroyed by cooking conditions rather readily oxidized. Preparations must therefore be protected from oxidation and prepared in an inert atmosphere of carbon dioxide or nitrogen. In the absence of atmospheric air, Vitamin A is unaltered at moderate temperatures. Many researchers have reported that Beta carotene is bioavailable from carrots and that processing via cooking or pureeing improves/increases its bioavailability. Brown *et al.* (1989) reported 21% bioavailability by the intake of 29 mg of beta carotene from cooked carrots in healthy men. Micozzi *et al.* (1992) provided 12 mg beta carotene as cooked carrots to healthy men for six weeks and reported 18% bioavailability.

Green leafy vegetables play a vital role in the food culture of Nigerians and Africans as a whole (Mensah *et al.*, 2008). A great variety of nutrients are found in vegetables consumed everyday through leaves, spinach, cabbage, carrot, onions, tomatoes and many others. In fact, vegetables are the cheapest and most available sources of important nutrients and they contribute substantially to protein, mineral, salts, vitamins, fibers, essential amino acids and other nutrients which are usually in inadequate supply in daily diets (Mosha and Gaga, 1999). The nutritional value of some of these vegetables lies in some chemical substances that produce a definite nutritional benefit on the body (Edeoga *et al.*, 2005). The most important features of some fresh vegetable is that they contain the nutritional value of economic importance and they are also sources of food (Okafor and Okoro, 2004). Vegetables are sources of many nutrients including potassium, fibre, folate (folic acid), and vitamin A, C and E. Eating a diet rich in vegetables may reduce risk of stroke, cancer, heart diseases and type-2-diabetes and ease the weight management according to the Centre of Disease Control and Prevention (*Premium Times* Online Newspaper, 2023).

Conversely, scarcity of vegetables in the diet is a major cause of vitamin A deficiency, which causes blindness, measles, xerolphthalmia and even death from preventable infections/diseases in young children throughout the arid and semi-arid areas of Africa and South Eastern Asia (Tang *et al.*, 2000).

Globally, vitamin A deficiency is an important public health problem. The World Health Organization (WHO) estimates that 3 million children and pregnant women develop clinical vitamin A deficiency annually compared to an estimated 200 cases of vitamin A toxicity diagnosed annually (Huiming *et al.*, 2005).

Deprivation of vitamin A replaces normal epithelium with stratified, keratinizing epithelium in the eyes, periocular glands, respiratory tract, alimentary tract, and genitourinary tract.

Supplementation is considered a key intervention to substantially reduce the rate of child morbidity and mortality due to preventable diseases in countries with high under-five mortality. It is recognized as one of the most cost-effective interventions to improve childhood survival rates.

Vitamin A supplementation is offered for the treatment of measles, xerophthalmia, severe malnutrition and to prevent deficiency in pregnant women living in areas endemic to vitamin A deficiency. Treatment of xerophthalmia is of special interest because it is one of the only diseases due to a vitamin deficiency to have reached epidemic levels. Its response to vitamin A supplementation to the eyes has been well established and can prevent night blindness, a major problem in developing countries.

Vitamin A also modulates a broad range of immune processes. It is involved in helping T cell and B cell development, which is important for adaptive immunity. Its contribution to mucosal epithelial regeneration and neutrophil, macrophage and natural killer cell functioning makes it important for innate immunity as well. Vitamin A deficiency and infectious diseases that transiently suppress serum retinol concentrations impair normal immune function. In particular, vitamin A deficiency is recognized as a risk factor for the measles virus, a major cause of childhood morbidity and mortality. Megadose (200,000 IU for two days) of vitamin A have been shown to lower the overall incidence of death related to measles (Huiming *et al.*, 2005).

Vitamin supplementation can be obtained from leafy vegetables cooked and consumed, thus the need to determine the effect of heat treatments on those leafy vegetables.

It should be noted indigenous vegetables have been consumed by the people for their desire, for aroma and taste without finding out their nutritional values such as vitamin components before and after cooking those vegetables. Therefore, this research work aimed at carrying out quantitative determination of vitamin A content of ten (10) species of leafy vegetables obtainable from local communities in Nigeria before and after heat treatment experiments.

2. Materials and Methods

2.1. Equipment and Reagents

All the reagents used in this research were of analar grade reagents and these included: Ethanol (Absolute alcohol, 99.86% v/v, Fisher scientific UK limited, Bishop Meadow Road, Loughborough Leicestershire, LEIIORG United Kingdom. Tel: 01509231166.). Potassium hydroxide (Loba Chemie Laboratory reagents and Fine chemicals CAS: 1310-58-3), Xylene (Fisher scientific UK limited, Bishop Meadow road, Longhorough Leicestersire, LEIIORG United Kingdom. Tel: 01509231166), Distilled water (Pharmaceutical Chemistry Laboratory, University of Uyo).

2.2. Biological Materials

Ten green selected leafy vegetables locally grown and consumed in Uyo, Akwa Ibom State were obtained from the Itam Market, Uyo, Akwa Ibom State and identified by Dr Imoh I. Johnny of the Department of Pharmcognosy and natural Medicine, Faculty of Pharmacy, University of Uyo. Taxonomic serial numbers were assigned to the individual plants. The vegetable samples were: Mmeme (*Justicea flava* UUPH 1(c)). Utasi (*Gongronema latifolium* UUPH 9(a)). Odusa (*Piper guineense* UUPH 61(b)). Atama (*Heinsia crinita* UUPH 67(c)). Bitter leaf (*Vernonia amygdalina UUPH 10(j)*), Editan (*Lasianthera Africana* UUPH 36(b)). Ikong-Ubong (*Telfairia occidentalis* UUPH 28(d)). Afang (*Gnetum africanum* UUPH 32(a)). Water leaf (*Talinum fruticosum* (UUPH 54 (b)), and Scent leaf (*Ocimum gratissimum,* UUPH 38(a)).

2.3. *Methods*

The methods of Rutkowski and Grzegorczyk (2007) were adopted.

2.3.1 Extraction of Vitamin A from Raw Leafy Vegetable Samples

The leafy part of each vegetable species (30 g) was weighed and thoroughly washed with water to remove sands and dirt from them after which it was placed in a sieve to drain out the water. The leaves were then squeezed to obtain the sample's aqueous extract into the beaker. 5 mL of the aqueous extract was measured into the test tube I with a tight stopper, and 5 mL of 1M ethanol KOH solution initially prepared was added. The test tube was plugged and shaken vigorously for 1 min. 5 mL of Xylene was added to the tube, plugged and shaken vigorously again for 1 min. The tube was then centrifuged at 1500 rev/min for 10 min. The whole of the separated extract (upper layer, i.e., vitamin A extract) was collected and transferred to another test tube (test tube II - made of soft (sodium) glass). The absorbance A1 of the extract in test tube II was measured at 335 nm against xylene. This extract (test II) was irradiated to the UV light for 30 min and the absorbance A2 was recorded. The absorbance readings were recorded in triplicates and the mean taken.

Concentration (Cx) of vitamin A (μM/30 g) in the analysed samples was calculated using the formula:

$$Cx = (\text{Mean of } A1 - \text{Mean of } A2) \times 22.23$$

where

22.23 – Multiplier received on basis of the absorption coefficient of 1% solution of vitamin A (as the retinol form) in xylene at 335 nm in a measuring cuvette about thickness = 1 cm.

This procedure was repeated for the other nine raw vegetable samples.

2.3.2 Extraction of Vitamin A from Boiled Leafy Vegetable Samples at 60 °C for 15 min

The procedure in 2.2.1 was repeated but test tube I was heated in a water bath at 60 °C for 15 min, after which it was cooled down in cold water before xylene was added.

2.3.3 Extraction of Vitamin A from Various Boiled Leafy Vegetable Samples at 80 °C for 15 min

The procedure in 2.2.1 was repeated but test tube I was heated in a water bath at 80 °C for 15 min, after which it was cooled down in cold water before xylene was added

2.4. *Statistical Analysis*

The mean and standard deviation of the Absorbance and concentration of vitamin A content for each species of vegetable (raw and boiled) was reported. All data were analyzed using Microsoft excel 2013 software.

3. Results

Xylene Absorbance at 335 nm = 0.117, 0.118, 0.120

Mean ± S.D = 0.1183±0.0012

Xylene Absorbance after irradiation to UV lamp at 335 nm = 0.069, 0.070, 0.072

Mean ± S.D = 0.0703±0.0012

3.1. *Results of Vitamin A Content in the Leafy Vegetable Samples at Different Levels of Heat Treatment*

Table 1: Concentrations of Vitamin A in the Leafy Vegetable Samples at Different Levels of Heat Treatment

S/N	Vegetables		Concentration (µM/30 g) Leafy Vegetable Samples		
	Local Name	Scientific Name	Raw	Boiled at 60 °C	Boiled at 80 °C
1	Mmeme	*Justicea flava*	0.889	1.044	1.089
2	Utasi	*Gongronema latifolium*	0.467	0.622	0.8
3	Odusa	*Piper guineense*	2.112	2.245	2.889
4	Atama	*Heinsia crinita*	0.133	0.689	1.356
5	Bitter leaf	*Vernonia amygdalina*	0.734	1.134	1.467
6	Editan	*Lasianthera africana*	0.022	0.4	0.667
7	Nkong-ubong	*Telfairia occidentalis*	3.668	3.934	4.313
8	Afang	*Gnetum africanum*	7.047	7.358	7.847
9	Water leaf	*Talinum fruticosum*	0.067	0.6	1.4
10	Scent leaf	*Occimum gratissimum*	0.111	2.156	2.401

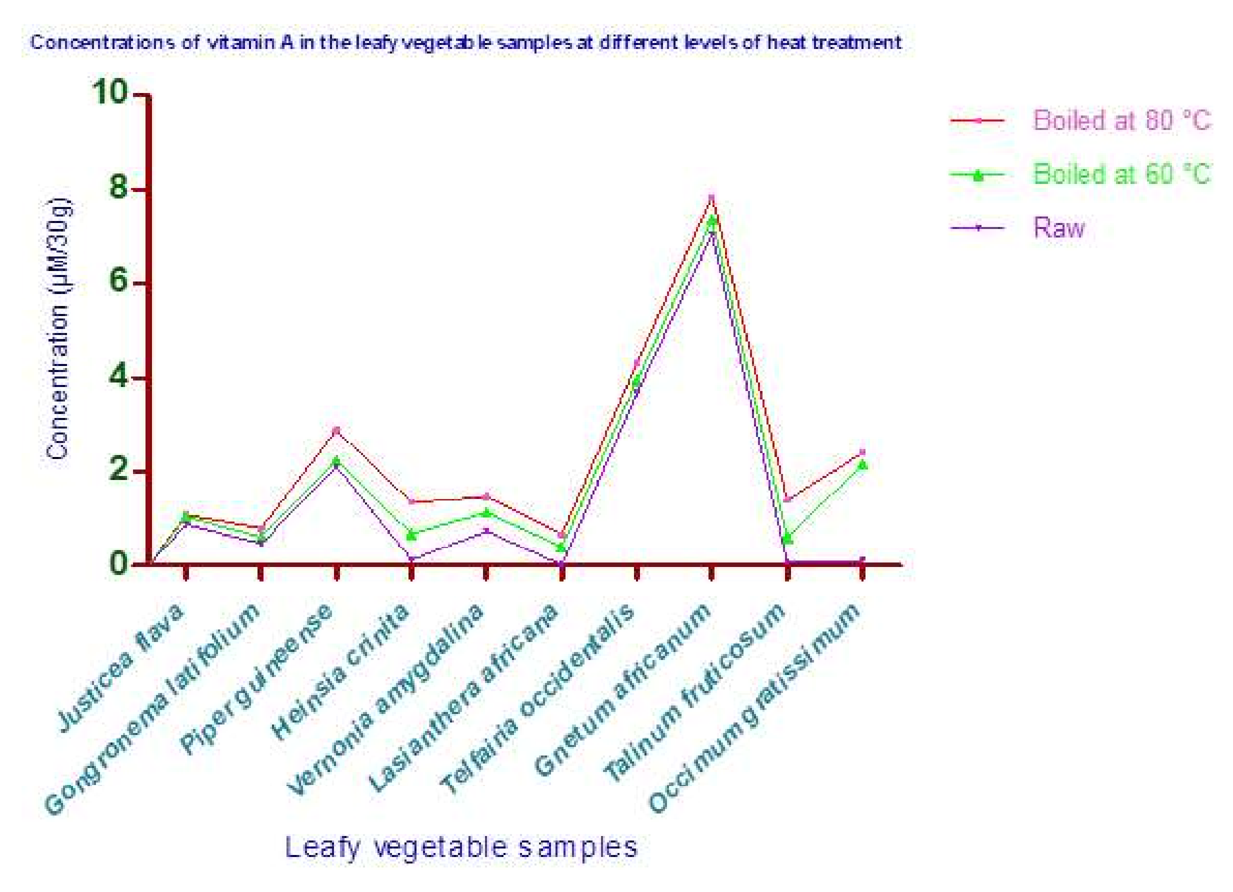

Figure 1: Vitamin A Concentrations of Vegetable Samples (µM/30 g) in Raw, Boiled at 60 °C and at 80 °C

4. Discussion

The concentration (µM/30 g) of vitamin A of the vegetable samples as affected by heat treatments are shown in Table 1 and Figure 1 above respectively. The results revealed that all the vegetables contained vitamin A.

Afang (*Gnetum africanum*) had the highest concentration implying it is a good source of vitamin A, while Editan (*Lasianthera africana*) had the least concentration. The vitamin A contents of the boiled vegetables at 60 °C and 80 °C respectively was generally high when compared with the raw vegetables' samples. It was also observed that heating temperature affected the vitamin A content of all the vegetables; as the heating temperature increased, the vitamin A content of the vegetables also increased, while the heating time was kept constant.

The vitamin A content of the vegetables analyzed were found to be in the order: Afang (*Gnetum africana*) > Ikong-ubong (*Telfairia occidentalis*) > Odusa (*Piper guineense*) > Scent leaf (*Ocimum gratissimum*) > Bitter leaf (*Vernonia amygdalina*) > Water leaf (*Talinum fruticosum*) > Atama (*Heinsia crinita*) > Mmeme (*Justicea flava*) > Utasi (*Gongronema latifolium*) > Editan (*Lasianthera africana*) for both raw and boiled vegetables' samples at 60 °C and at 80 °C for 15 min respectively.

It is interesting to note that the results obtained showed that heating at higher temperatures caused the concentration of vitamin A to increase (this is because vitamin A is a fat-soluble vitamin and as such it is not destroyed or degraded by heat). This reveals that heat is facilitating the release of carotenoids from the vegetable matrix which later results in increased release of vitamin A. This result however, is consistent with other studies on the effect of processing on carotenoid content for example, Graziani *et al*. (2003) showed that extractable carotenoid content significantly increased when vegetables were heated in an oil bath at 100 °C for 2 h. Haskell (2012) suggested that thermal processes might break down cell walls and weaken the bonding forces between carotenoids and the tissue matrix in vegetables. Such disruptions in the cell wall fraction may enhance the release of phytochemicals from the matrix.

This also knocks of the perception that carotenoids are destroyed or degraded by the heat process involved in cooking of vegetables. In fact, carotenoid loss is minimal with moderate cooking, and in many cases, carotenoids become more bioavailable after cooking, probably because heat processing liberates them from cell matrices. These heat treatments disrupt cell walls and may cleave carotenoids from proteins, facilitating their liberation and thus the absorption of carotenoids and consequently the liberation and absorption of vitamin A (Rock *et al.*, 1998). Heat processing converts some of the naturally occurring trans isomers of beta carotene to the cis-configuration. This does not appear to negate the increased bioavailability of beta carotene from moderate heat processed foods relative to their raw counterparts. In the practical situation of changing diet to improve health, fat intake is lowered, and fruits and vegetables intake or consumption is encouraged. For children, women or pregnant women making these dietary changes, the resulting decrease in calories from fat and increase in fruits and vegetables results in significant increase in vitamin content. Accumulating evidence strongly indicates that realistic increase in fruits and vegetables consumption can appreciably increase plasma carotenoid concentrations of the populace which in turn is supported by epidemiological studies suggestion that small but habitual increases in consumption of carotenoid-rich vegetables reduce the risk of chronic diseases, mortality from viral infections (such as measles and HIV), night blindness, xerolphthalmia, cancer and cardio-vascular diseases etc. (Clagett-Dame and DeLuca, 2002; WHO, 2023).

5. Conclusion

Wide variation was observed in the values of vitamin A contents of the 10 selected green leafy vegetables locally grown and consumed in Uyo, Akwa Ibom state, Nigeria. Among the raw green leafy vegetables, boiled green leafy vegetables at 60 °C for 15 min, and cooked green leafy vegetables at 80 °C for 15 min, the amount of vitamin A (µM/30 g) was found to be in the descending order; Afang (*Gnetum africana*) > Ikong-ubong (*Telfairia occidentalis*) > Odusa (*Piper guineense*) > Scent leaf (*Ocimum gratissimum*) > Bitter leaf (*Vernonia amygdalina*) > Water leaf (*Talinum fruticosum*) > Atama (*Heinsia crinita*) > Mmeme (*Justicea flava*) > Utasi (*Gongronema latifolium*) > Editan (*Lasianthera africana*).

The study has revealed that vegetables are good sources of vitamin A.

Significance of the Study

Knowing that young children and pregnant women are prone to vitamin A deficiency, the information from this research study can be used by mothers to plan their meals by including the minimum amount of beta carotene rich vegetables such as the ones studied in this research in meals. These can be incorporated in their meals in forms acceptable to them.

Authors' Contributions

Conceptualization - ECJ; Methodology - ECJ and AKO; Software - ECJ; Formal analysis - RAU and AKO; Investigation - ECJ and AKO; Resources - ECJ; Writing/original draft preparation - ECJ; writing-review and editing - RAU; Supervision - ECJ; Project administration - ECJ.

Conflicts of Interest

The authors declare no conflict of interest.

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