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Impact of Heat Treatment on Vitamin C Content in Native Leafy Vegetables of Uyo, Nigeria

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Abstract

Background: The native vegetables of Uyo, Nigeria, valued for aroma and taste, are often consumed without knowledge of nutritional content. This study evaluated vitamin C levels in ten leafy vegetables before and after boiling, highlighting their role in nutrition, disease prevention, and reducing mortality from infectious diseases. **Methods:** Vitamin C concentrations were analyzed using UV spectrophotometry. **Results:** Among the vegetables analyzed, Afang (Gnetum africanum) had the highest vitamin C levels across treatments: raw (2,066.95 µM / 30 g), boiled at 60 °C (1,492.14 µM / 30 g), and boiled at 80 °C (1,110.44 µM / 30 g), emphasizing its nutritional value. Conversely, the lowest concentrations were found in Atama (215.27 µM / 30 g raw), Waterleaf (285.70 µM / 30 g at 60 °C), and Bitter leaf (182.33 µM / 30 g at 80 °C). **Conclusion:** The study confirmed vitamin C presence in all the vegetables studied, showing varied responses to boiling, with both reductions and occasional increases under moderate heat. Most vegetables exhibited net vitamin C loss. Findings suggest unique release patterns and build on prior research on heat effects on vitamin A in these vegetables.

Keywords: *Vitamin C, Vegetables, Heat treatments, Gnetum africanum*

1. Introduction

In continuation of our study on vitamin contents of locally grown vegetables before and after heat treatments, the water-soluble vitamin content of the same set of vegetables was examined in this research work (Johnson *et al.*, 2023). Vegetables are defined as edible herbaceous plants or parts of plants consumed raw or after

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cooking and are rich in vitamins and minerals, low in calorific value and neutralize the acid substances produced during digestion of high energy foods (Gopalakrishnan, 2007). Research has shown that consumption of diets rich in vegetables and fruits protect the body from chronic degenerative disease (Heber, 2004). Green leafy vegetables play a vital role in the food culture of Nigerians and Africans as a whole (Mensah *et al*., 2008). 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 are that they contain the nutritional value of economic importance and they are also sources of food (Okafor and Okoro, 2004).

Vitamin C also known as Ascorbic acid is a water-soluble vitamin which occurs naturally in fruits and vegetables (Carr and Silvia, 2017). It is a potent antioxidant, protecting cells from oxidative stress, and supports the immune system by enhancing the function of white blood cells. It is essential for various bodily functions including collagen synthesis, which is vital for skin, blood vessels, and cartilage. Vitamin C supports epithelial barrier function against pathogens and promotes the oxidant-scavenging activity of the skin, thereby potentially protecting against environmental oxidative stress Vitamin C is an essential nutrient that must be consumed on a regular basis to prevent deficiency (Carr and Frei, 1999). Different tissues and organs have variable requirements for vitamin C, as reflected by their vitamin C concentrations (Hornig, 1975). Tissues with the highest concentrations of the vitamin include the brain, adrenals and pituitary gland. This reflects one of the major functions of vitamin C, which is to act as a cofactor for a family of biosynthetic and regulatory metalloenzymes, including those involved in the synthesis of catecholamine and peptide hormones (Englard and Seifter, 1986; Carr *et al*., 2015). Recent research has also indicated a role for vitamin C in genetic and epigenetic regulation via enzymes that regulate gene transcription and the methylation of DNA and histones (Vissers *et al*., 2014; Young *et al*., 2015). As a result, vitamin C has the potential to regulate thousands of genes in the body and thus play pleiotropic roles in human health and disease. Historically, recommended intakes of micronutrients have been based on daily intakes required to prevent disease secondary to deficiency. (Young *et al*., 2015; Lee *et al*., 2019).

A severe lack of vitamin C in diet for at least three months can cause scurvy. Reasons for this deficiency include not eating enough fresh fruits and vegetables. Certain conditions increase the body’s vitamin C requirement and risks deficiency, they include: pregnancy and breastfeeding, Disorders that cause high fever, Overactive thyroid gland (hyperthyroidism) and chronic diarrhea (Cleveland Clinic, 2025).

However, scurvy treatment is essential to avoid further complications. The condition is easily treatable by consuming more vitamin C which is achieved by maintaining a nutritious diet that includes one to two times the daily recommended amount of vitamin C. This can be by adding fresh fruits and vegetables to every meal but it has been seen in previous studies that heating leafy vegetables can reduce the Vitamin C content of those vegetables and this hypothesis will be proven in this study (Diengdoh *et al*., 2015).

Scurvy, resulting from prolonged vitamin C deficiency, manifests through various symptoms that develop over time. Initial signs include general weakness, fatigue, irritability, and joint pain. If untreated, more severe symptoms can emerge, such as anemia, swollen and bleeding gums that may become purple and spongy, and loosened teeth that may eventually fall out. In infants and children, scurvy may present as irritability, pain during movement, loss of appetite, failure to gain weight, and anemia (Cleveland Clinic, 2025).

Recent reports indicate a resurgence of scurvy in developed countries, attributed to poor dietary habits and economic challenges. For instance, a case in Australia involved a middle-aged man developing scurvy symptoms due to a diet lacking in fruits and vegetables, exacerbated by financial constraints (News.com.au, 2025; Nationwide News, 2025).

Similarly, in the United Kingdom, there has been an increase in scurvy cases, with 188 treatments recorded in English hospitals in 2022, compared to 61 in 2008. This rise is linked to diets high in ultra-processed foods and low in fresh produce, often influenced by the rising cost of living (The Sun US, 2020; News.com.au, 2025).

These cases underscore the importance of maintaining a diet rich in vitamin C to prevent scurvy, even in modern societies where the disease was once considered obsolete.

A food-based approach emphasizing the consumption of fruits and vegetables can be instrumental in addressing vitamin C deficiency, particularly among at-risk populations. Green leafy vegetables and fruits, which are cultivated abundantly in Akwa Ibom State, Nigeria, are not only nutrient-rich but also relatively inexpensive, making them accessible options for improving dietary vitamin C intake. Studies have shown that promoting local produce consumption is a sustainable strategy for combating micronutrient deficiencies in resource-limited settings (Oguntona and Akinyele, 2020; FAO, 2021).

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 that 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 C content of ten (10) species of leafy vegetables obtainable from local communities in Nigeria before and after heat treatment experiments. It should be recalled that a similar research work had been undertaken in this laboratory on the effect of treatments of same species of leafy vegetables on their vitamin A contents (Johnson *et al.*, 2023).

2. Materials and Methods

2.1. Materials

2.1.1. Biological Materials

All plant materials were obtained from Itam market, near Uyo, in Akwa Ibom state, Nigeria.

2.1.2. Equipment, Chemical and Reagents

Electronic balance JA3103 [Shimadzu-Japan], Centrifuge, VIS or UV spectrophotometer—L7 Double beam [UVS-2700), USA; Sodium tungstate molybdenium-free (Fisher scientific U.K.), Sodium hydrogen phosphate anhydrous, Deionized water, Concentrated sulphuric acid, Vitamin C (L-Ascorbic Acid), Fisher scientific U.K.

2.2. Experimental Methods

2.2.1. Collection and Identification of Plant Materials, Apparatus and Reagents

Ten pre-selected green leafy vegetables locally cultivated and consumed in Uyo, Akwa Ibom State were sourced from the Itam Market, one of the most popular markets in Uyo, Akwa Ibom State. This market is reputable for having fresh foods including fruits, vegetables and animal products. The vegetables collected were Mmeme (*Justicia 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)). The vegetable plants were authenticated by Taxonomist Mrs Emmanuella, G. Udoma of the Department of Pharmacognosy and Natural Medicine, University of Uyo. All the apparatus used were properly washed and rinsed with distilled water. Analar grade reagents were used in this research study.

2.2.2. Extraction of Vitamin C from Various raw Leafy Vegetable Samples

30 g of the leafy part of each vegetable species was weighed and thoroughly washed with clean water to remove sands and dirt 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. 2 mL of the aqueous extract was measured into a centrifugal test tube and 2 mL of Phosphotungstate Reagent (initially prepared) was added and mixed thoroughly 1 min and allowed to stand for 30 mins under room temperature. The tube was then centrifuged

at 7000 rev / min for 10 mins. The whole of the separated supernatant (vitamin C extract) was collected with a pipette and transferred to another test tube to be used as the test sample (Rutkowski and Grzegorczyk, 2007). This procedure was repeated for the other nine raw vegetable samples.

2.2.3. Extraction of Vitamin C from Various Leafy Vegetable Samples Boiled at 60 °C for 15 Minutes

30 g of the leafy part of each vegetable species was weighed and thoroughly washed with clean water to remove sand and dirt 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 a test tube and heated in a water bath at 60 °C for 15 mins and cooled using cold water. 2 mL of the boiled and cooled extract was measured into a centrifugal test tube and 2 mL of Phosphotungstate Reagent (initially prepared) was added and mixed thoroughly and allowed to stand for 30 mins under room temperature. The tube was then centrifuged at 7000 rev / min for 10 min. The whole of the separated supernatant (vitamin C extract) was collected with a pipette and transferred to another test tube to be used as the test sample (Rutkowski and Grzegorczyk, 2007). This procedure was repeated for the other nine raw vegetable samples.

2.2.4. Extraction of Vitamin C from Various Leafy Vegetable Samples Boiled at 80 °C for 15 Minutes

30 g of the leafy part of each vegetable species was weighed and thoroughly washed with clean water to remove sands and dirt 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 a test tube and heated in a water bath at 80 °C for 15 mins and cooled using cold water. 2 mL of the boiled and cooled extract was measured into a centrifugal test tube and 2 mL of Phosphotungstate Reagent (initially prepared) was added and mixed thoroughly and allowed to stand for 30 mins under room temperature. The tube was then centrifuged at 7000 rev / min for 10 min. The whole of the separated supernatant (vitamin C extract) was collected with a pipette and transferred to another test tube to be used as the test sample (Rutkowski and Grzegorczyk, 2007). This procedure was repeated for the other nine raw vegetable samples.

2.2.5. Preparation of Standard Sample for Determination of Absorbance

A standard solution of 56.8 µM vitamin C (L-ascorbic acid) made using 50 mM solution of oxalic acid as a solvent was prepared. 2 mL of standard solution was measured into a test tube and 2 mL of Phosphotungstate Reagent (initially prepared) was added. The solution was mixed thoroughly and allowed to stand for 30 mins under room temperature. The resulting solution is the standard sample for determination of absorbance (Rutkowski and Grzegorczyk, 2007).

2.2.6. Determination of Absorbance of Vitamin C Extract of the Various Raw Leafy Vegetable Samples

The absorbance A_x of the obtained vitamin C extracts of the various raw leafy vegetable samples and the absorbance A_s of the standard sample was measured at 700 nm against the mixture Phosphotungstate Reagent: 50 mM solution of oxalic acid = 1:1 (v / v) as a reference sample and recorded in triplicates.

The concentration of vitamin C (μM) in the analyzed liquid was then calculated using the formula:

$$c_x = \frac{A_x}{A_s} \cdot c_s$$

where: c_s – concentration of the standard solution (Rutkowski and Grzegorczyk, 2007).

2.2.7. Determination of Absorbance of Vitamin C Extract of the Various Boiled Leafy Vegetable Samples at 60 °C and 80 °C for 15 Minutes Respectively

The absorbance A_x of the obtained vitamin C extracts of the various boiled leafy vegetable samples at 60 °C and 80 °C for 15 minutes and the absorbance A_s of the standard sample was measured at 700 nm against the mixture Phosphotungstate Reagent: 50 mM solution of oxalic acid = 1:1 (v / v) as a reference sample and recorded in triplicates.

The concentration of vitamin C (μM) in the analyzed liquid was then calculated using the formula:

$$c_x = \frac{A_x}{A_s} \cdot c_s$$

where: c_s – concentration of the standard solution (Rutkowski and Grzegorczyk, 2007).

2.3. Statistical Analysis

The mean and standard deviation of the Absorbance and concentration of vitamin C content for each species of vegetable (raw and boiled) were reported. All data were analyzed using ANOVA and Post-hoc Tukey test from GraphPad Prism software.

3. Results

The concentrations of Raw, Boiled at 60 ºC, Boiled at 80 ºC are shown in Table 1.

Table 1: Concentration of Vitamin C in Ten Selected Vegetables Before and After Heating

S/No.	Vegetables	Scientific Name	Concentration (µM/30 g)		
			Raw	Boiled at 60 ℃	Boiled at 80 ℃
1	Waterleaf	*Talinum fruticosum*	323.76	285.7	218.68
2	Odusa	*Piper guineense*	275.48	306.15	342.5
3	Atama	*Heinsia crinita*	215.27	368.06	397.6
4	Bitter-leaf	*Vernonia amygdalina*	626.5	316.94	182.33
5	Mmemme	*Justicia flava*	383.97	411.23	502.68
6	Editan	*Lasianthera africana*	466.33	479.96	418.05
7	Scent-leaf	*Ocimum gratissimum*	435.66	585.04	509.5
8	Utasi	*Gongronema latifolium*	858.82	646.95	361.25
9	Ikong ubong	*Telfairia occidentalis*	1,194.50	569.7	449.29
10	Afang	*Gnetum africanum*	2,066.95	1,492.14	1,110.44

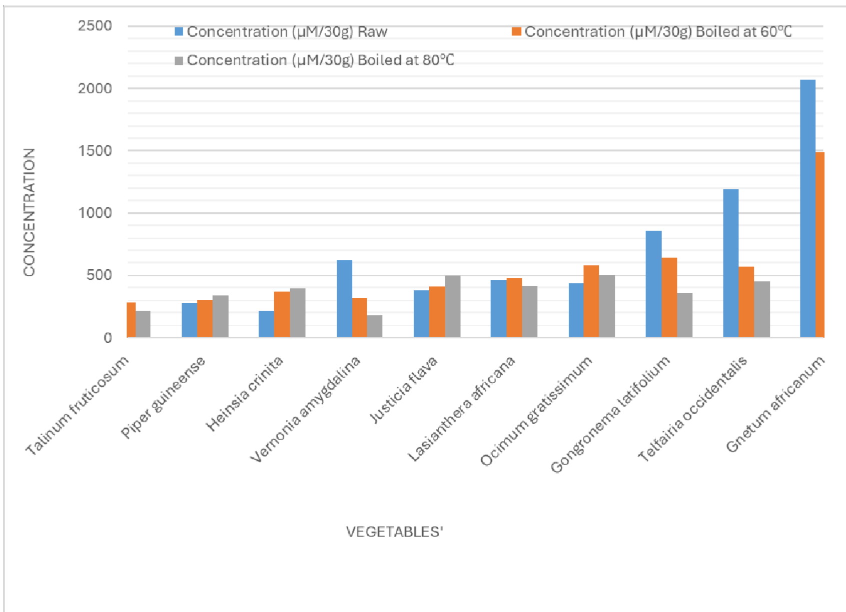

Figure 1: Vitamin C Concentration of Ten Selected Vegetables Before and After Heating

4. Discussion

The concentration (µM / 30 g) of vitamin C in the vegetable samples as affected by boiling are shown in the Table 1 above respectively. Figure 1 shows the relationship between the concentrations of the vegetable samples analyzed pre- and post-heating. The results revealed that all the vegetables contain vitamin C, but Afang (*Gnetum africanum*) gave the highest value of concentration implying it is a good source of vitamin C, while Atama (*Heinsia crinita*) gave the least value of concentration in their raw forms. It was also observed that the vitamin C content of the boiled vegetable samples at 60 °C and 80 °C experienced both an increase and decrease; some vegetables released more Vitamin C from their cell matrix when exposed to heat thereby increasing the concentration, whereas, others gradually lost their Vitamin C content under the same conditions. Out of 10 vegetable samples, 6 showed a reduction in Vitamin C content as expected, 3 showed a rather fascinating increase in Vitamin C content, while 1 displayed an initial increase when boiled at 60 °C before decreasing on an increase in temperature to 80 °C.

The vitamin C content of the vegetables analyzed were found to be in the order: Afang (*Gnetum africanum*) > Ikong-ubong (*Telfairia occidentalis*) > Utasi (*Gongronema latifolium*) > Bitter leaf (*Vernonia amygdalina*) > Editan (*Lasianthera africana*) > Scent leaf (*Ocimum gratissimum*) > Mmeme (*Justicia flava*) > Water leaf (*Talinum fruticosum*) > Odusa (*Piper guineense*) > Atama (*Heinsia crinita*) in terms of absorbance and concentration for raw vegetables samples.

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

The findings indicated both a reduction and increase in vitamin C concentration with low to moderate boiling, highlighting the unique release kinetics in each analyzed vegetable. The mechanism of vitamin C release from the cell matrix of vegetable leaves primarily involves physical and enzymatic processes, which allow the vitamin to become available for absorption during digestion. In raw vegetable leaves, vitamin C is stored within cellular compartments. When these vegetables are chopped, chewed, or mechanically processed, their cell walls break down, releasing intracellular contents, including vitamin C. This physical disruption is the initial step that makes vitamin C available for absorption by the body. Heat processing, such as blanching or boiling, can also disrupt the cell wall structure, although prolonged heat can degrade vitamin C, given its sensitivity to high temperatures (Howard *et al.*, 1999; Igwemmar *et al.*, 2013; Diengdoh *et al.*, 2015). Moreover blanching or steaming vegetables inactivates enzymes that could otherwise degrade vitamin C, preserving more of its bioavailability (Rickman *et al.*, 2007a).

The analysis of variance (ANOVA) used to compare the concentrations of vitamin C across the different vegetable treatments revealed that none of the pairwise comparisons (Raw vs. Boiled to 60 ºC; Raw vs. Boiled to 80 ºC; and Boiled to 60 ºC vs. Boiled to 80 ºC) showed statistically significant differences. This finding was confirmed by the post-hoc Tukey test, which indicated that the differences between individual treatment groups were not statistically significant.

These results suggest that the observed variability is primarily attributable to individual differences (as reflected by the high sum of squares for individuals) rather than the treatments themselves. Importantly, the findings imply that all the vegetables contained high levels of vitamin C, and boiling them up to a temperature of 80 ºC within 15 minutes did not significantly destroy their vitamin C content. Therefore within these conditions vitamin C content of these vegetables are still preserved and available for human consumption.

In the previous study of these same species of vegetables (Johnson *et al.*, 2023) it was discovered that heat facilitated the release of fat-soluble vitamin A and carotenoids from the vegetable matrix, which enhanced their bioavailability. However, in the present study most vegetables showed a net loss of vitamin C following heat treatment, as vitamin C is sensitive to heat and prone to degradation during cooking.

It is interesting that the results obtained showed that boiling at higher temperatures at a longer time, Vitamin C Concentration began to decrease (this is because Vitamin C is a water-soluble vitamin and as such it is destroyed or degraded by heat). This result is consistent with other studies on the effect of processing on ascorbic acid content (Rickman *et al.*, 2007b).

This research significantly contributed to the body of knowledge by addressing a critical gap in understanding how commonly consumed indigenous vegetables respond to heat treatment, shedding light on their nutritional dynamics. The perception that vitamin C is destroyed or degraded by the heat process involved in cooking of vegetables is subjective and dependent on factors such as the temperature of heating, time, and importantly, the release kinetics of the vegetable being heated (Favell, 1998; Lee and Kader, 2000). Ascorbic acid loss increases generally with an increase in boiling temperature and time. Still, at low temperatures, vitamin C becomes more bioavailable after boiling, probably because heat processing liberates them from cell matrices. These heat treatments disrupt cell walls and may cleave L-ascorbic acid from proteins, facilitating the liberation. It is believed that at higher temperatures and further heating, vegetables with such characteristics may exhibit a net loss of vitamin C since it is prone to oxidative alterations. L-ascorbic acid undergoes structural modification when exposed to heat to dehydroascorbic acid, an oxidized derivative of vitamin C (Davey *et al.*, 2000; Lee and Kader, 2000).

In the practical situation of changing diet to improve health, fat intake is lowered, and fruit and vegetable 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, there is a significant increase in vitamin content (Frei and Trabe, 2001). Accumulating evidence strongly indicates that a realistic increase in fruits and vegetables consumption can appreciably increase plasma ascorbic acid concentrations of the populace and in turn epidemiological studies suggest that small but habitual increases in consumption of ascorbic acid-rich vegetables can improve the skin (since it is involved in the synthesis of collagen), enhance iron absorption, improve cardiovascular health thereby reducing the risk of cardiovascular diseases amongst other benefits (Block *et al.*, 1992).

5. Conclusion

The study highlights that these vegetables are valuable sources of vitamin C, an essential nutrient, especially for young children and pregnant women who are at higher risk of deficiency. Findings from this research can help mothers make informed meal choices by incorporating a sufficient amount of ascorbic-acid-rich vegetables, like those studied in this research work, into their family's diet in forms that are both palatable and nutritious. To preserve vitamin C content, it is recommended that these vegetables be heated at lower temperatures, which minimizes the nutrient loss typically associated with cooking.

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Authors Contributions

ECJ conceptualized the study, performed the laboratory analyses, and drafted the manuscript. EOE carried out the sample collection and processing. SO contributed to manuscript writing and proofreading. All authors read and approved the final version of the manuscript.

Conflict of Interest

The authors declared no conflict of interests.

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