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# EFFECT OF HEAT PROCESSING ON VITAMIN COMPOSITION OF SELECTED STAPLE GREEN LEAFY VEGETABLES IN NIGERIA

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**Abstract:** This study assessed the effect of the steamed and boiling cooking methods on the vitamin content of six staple green vegetables in Nigeria. Vitamin levels in fresh, steamed and boiled leafy vegetables were determined using spectrophotometric and titrimetric methods. The results of vitamin analysis showed that fresh vegetable of Amaranthus hybridus contained the largest amount of ascorbic acid, thiamin and niacin (358.40 mg/100 g, 0.09 mg/100 g and 0.08 mg/100 g), while Talinum triangulare had the highest level of riboflavin (0.18 mg/100 g). The steamed Amaranthus hybridus contained the highest levels of ascorbic acid, thiamin and niacin (322.56 mg/100 g, 0.08 mg/100 g and 0.73 mg/100 g), while steamed leaves of Talinum triangulare had the highest quantity of riboflavin (0.16 mg/100 g). The boiled Amaranthus hybridus had the highest amount of ascorbic acid, thiamin and niacin (197.12 mg/100 g, 0.05 mg/100 g and 0.41 mg/100 g), whereas the boiled Talinum triangulare had the highest level of riboflavin (0.09 mg/100 g). The percentage of vitamin loss in steamed leafy vegetables varies from 9 to 11 %, 9 to 17 %, 9 to 13 % and 9 to 12 % in ascorbic acid, thiamin, riboflavin and niacin respectively. Almost 50 % of the essential vitamins were lost in boiled leafy vegetables. The results revealed that vegetables could be improved source of some important vitamins that can help humans meet their nutritional needs.

Keywords: Leafy vegetables, Steaming/Boiling methods, Vitamin composition, % loss of vitamin

#### 1. Introduction

Generally, herbaceous plants' fresh and edible parts are known as green vegetables. They are a great source of important vitamins [1]. Leafy vegetables contain beneficial ingredients in food which serve as energy sources and also possible to be utilized as body building, regulatory and protection. Leafy vegetables have become regular ingredient in the daily diet of Nigerians in both urban and rural areas because they are readily available and affordable. Leafy vegetables can be consumed raw or cooked, and the majority

of their vitamins are water-soluble and heatsensitive. According to reports, when the leafy vegetables are soaked in hot water, these water-soluble vitamins might drain away [2].

Nevertheless, these leafy vegetables are commonly exposed to various cooking processes in order to increase their palatability [3]. They are extremely vulnerable vitamins loss to processing [4], hence the contents of watersoluble vitamins can be altered by most of these processing methods, particularly the thermolabile components, thereby reducing the amount of the water-soluble vitamins required for daily body metabolism [5]. Therefore, this study evaluates and compares the vitamin content and retention in six regularly consumed vegetable species, namely, *Amaranthus hybridus*, *Corchorus olitorius*, *Ocimum gratissimum*, *Talinum triangulare*, *Telfaria occidentalis* and *Vernonia amygdalina*, both fresh and processed, in order to acquire the necessary

vitamins, like, ascorbic acid (vitamin C), thiamin (vitamin  $B_1$ ), riboflavin (vitamin  $B_2$ ) and niacin (vitamin  $B_3$ ) that are important for human metabolism and protection of human body against diseases. Table 1 lists the local names, botanical names, common names and portions of the plant that were used.

Table 1.

Staple Leafy Vegetables, names and parts used

**Botanical name** Local name Common name Part used Tete Amaranthus hybridus African spinach Leaves Ewedu Corchorus olitorius Jute plant Leaves Efinrin-nla Ocimum gratissimum Basil Leaves Gbure Talinum triangulare Water lettuce Leaves Ugwu Telfaria occidentalis Fluted pumpkin Leaves Vernonia amygdalina Ewuro Bitter leaf Leaves

# 2. Materials and Methods Reagents

Chemicals and solvents of analytical grade were utilized without additional purification. Ethanol, and petroleum ether, concentrated sulphuric acid and hydrochloric acid were obtained from BDH Chemicals, England, while trichloroacetic acid was obtained from E. Merck, Germany.

#### **Plant Material**

Fresh leaves of the six vegetables were bought in the market at Okada, Edo State, Nigeria. The identification and authentication of the plant leaves were carried out at the Taxonomy section, Biological Sciences Department, Igbinedion University, Okada, Nigeria.

#### **Sample Preparation**

Each sample was completely mixed, the stalks were removed and de-ionized water was used to rinse the samples, which was evaporated at ambient temperature. The residual moisture was evaporated during air-drying process that took place for few weeks on a clean paper with regular turning over to avoid fungal growth. Using pestle and mortar, each air-dried sample was crushed into a fine powder and sieved through a 2.0 mm mesh sieve [6].

#### **Thermal Processing**

Also, thermal processing (steaming and boiling) of the samples fresh leaves was carried out following the procedure described by Soni and Brightwell [7] with minor modification.

#### Steaming

5 g each of fresh vegetable leaves were cooked in a clean stainless-steel pot using an electric cooker, fitted with temperature control for 10 minutes. After that, the sample was immersed in a water bath at 4°C for 30 seconds to stop cooking. Excess water was further removed with desiccant paper. The steamed leaves were air-dried for few weeks on a clean paper with regular turning over to avoid fungal growth. Pestle and mortar were used to crush each air-dried sample into a fine powder, sieved

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through a 2.0 mm mesh sieve and stored in an air tight container for subsequent use [7].

#### **Boiling**

5 g each of fresh vegetable leaves were cooked with distilled water that was brought to boiling for

10 minutes with the lid on. The vegetables were sieved and left to cool at ambient temperature after boiling. Pestle and mortar were used to crush each air-dried sample into a fine powder, sieved through a 2.0 mm mesh sieve and stored in an air tight container for subsequent use [7].

## Vitamin Analysis Ascorbic acid (Vitamin C)

In an extraction tube, 5.0 g of the sample was weighed, along with 100 mL of EDTA/TCA (2:1). The extracting solution was combined and shook for 30 minutes before being transferred to a centrifuge tube and centrifuged for roughly 20 minutes at 3000 rpm. The extracting solution was then transferred to a 100 mL volumetric flask and made up to 100 mL mark. 1% starch indicator was added to 20 mL of extract pipetted into a volumetric flask. To obtain a dark end point, these were added and titration was done using a 20% CuSO<sub>4</sub> solution [8].

#### Thiamin (Vitamin B<sub>1</sub>)

A total of 5.0 g of the sample was homogenized in 50 mL of ethanolic sodium hydroxide and filtered into a 100 mL flask. 10 mL of the filtrate was pipetted into a 100 mL flask, along with 10 mL of potassium dichromate to develop the colour and the results were read at 360 nm. At the same wavelength, a blank sample was prepared and the colour was established and read [9].

#### Riboflavin (Vitamin B<sub>2</sub>)

100 mL of 50% ethanol solution was used to extract 5.0 g of the sample, which was agitated for 1 hour. It was filtered into a 100

mL flask, and 10 mL of the extract was pipetted into a 50 mL volumetric flask. 10 mL of potassium permanganate (5%) and 10 mL of H<sub>2</sub>O<sub>2</sub> (30%) were added and allowed to stand for 30 minutes over a hot water bath. Thereafter, 2 mL of sodium sulphate (40%) was added, made up to 50 mL mark and the absorbance was measured in a spectrophotometer at 510 nm [9].

#### Niacin (Vitamin B<sub>3</sub>)

50 mL of 1 N sulphuric acid was added to 5.0 g of the sample and agitated for 30 minutes. The sample was filtered after 3 drops of ammonia solution was added. 10 mL of the filtrate were transferred into a 50 mL volumetric flask, then 5 mL potassium cyanide were added. This was acidified with 5 mL of 0.02 N H<sub>2</sub>SO<sub>4</sub> and the absorbance was measured with the spectrophotometer at 470 nm [9].

#### **Statistical analysis**

For each parameter, a triplicate study was performed on each independent replication. Each treatment had three independent replicates (n = 3), with the data displayed tables and expressed as means  $\pm$  standard deviation (SD). Data were analyzed by ANOVA (P < 0.05).

#### 3. Results and Discussion

Vitamin composition of fresh and processed leafy vegetables

Table 2a-2c summarizes the evaluated values of fresh, steamed and boiled vegetables. All fresh steamed and boiled green vegetables are particularly high in vitamins, according to the results of the vitamin analysis. For both fresh and steamed leafy vegetables, ascorbic acid (vitamin C) ranges from 358.40 mg/100 g to 215.63 mg/100 g and 322.56 mg/100 g to 191.91 mg/100 g in *A. hybridus* to *T. triangulare* respectively, but for boiled leafy vegetables, it ranges from 179.12

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mg/100 g in *A. hybridus* to 95.94 mg/100 g in *O. gratissimum*. For both fresh and steamed leafy vegetables, thiamin (vitamin B<sub>1</sub>) ranges from 0.09 mg/100 g to 0.03 mg/100 g in *A. hybridus* to *O. gratissimum*, whereas for boiled leafy vegetables, it ranges from 0.05 mg/100 g in *A. hybridus* to 0.02 mg/100 g in *C. olitorius*. For fresh, steamed and boiled leafy vegetables, riboflavin (vitamin B<sub>2</sub>) levels range from

0.18mg/100 g to 0.06mg/100 g, 0.16 mg/100 g to 0.05 mg/100 g and 0.09 mg/100 g to 0.03 mg/100 g in *T. triangulare* to *C. olitorius* respectively. Niacin (vitamin B<sub>3</sub>) levels in fresh, steamed and boiled leafy vegetables range from 0.80 mg/100 g to 0.29 mg/100 g, 0.73 mg/100 g to 0.26 mg/100 g and 0.40 mg/100 g to 0.15 mg/100 g in *A. hybridus* to *O. gratissimum* respectively.

Table 2a. Vitamin composition of fresh leafy vegetables on mg/100 g dry weight

Vegetable	Vitamin			
	Ascorbic acid	Thiamin	Riboflavin	Niacin
Amaranthus hybridus	$358.40 \pm 0.20$	$0.09 \pm 0.01$	$0.12 \pm 0.01$	$0.80 \pm 0.01$
Corchorus olitorius	$316.80 \pm 0.22$	$0.03 \pm 0.01$	$0.06 \pm 0.01$	$0.61 \pm 0.02$
Ocimum gratissimum	$241.06 \pm 0.21$	$0.03 \pm 0.02$	$0.08 \pm 0.02$	$0.29 \pm 0.01$
Talinum triangulare	$215.63 \pm 0.11$	$0.06 \pm 0.01$	$0.18 \pm 0.02$	$0.56 \pm 0.02$
Telfaria occidentalis	$356.11 \pm 0.20$	$0.07 \pm 0.01$	$0.09 \pm 0.01$	$0.74 \pm 0.01$
Vernonia amygdalina	$285.92 \pm 0.21$	$0.06 \pm 0.01$	$0.07 \pm 0.01$	$0.66 \pm 0.01$

Results are mean of triplicate determinations on a dry weight basis  $\pm$  standard deviation.

Table 2b. Vitamin composition of steamed leafy vegetables on mg/100 g dry weight

Vegetable	Vitamin			
_	Ascorbic acid	Thiamin	Riboflavin	Niacin
Amaranthus hybridus	$322.56 \pm 0.11$	$0.08 \pm 0.02$	$0.11 \pm 0.01$	$0.73 \pm 0.01$
Corchorus olitorius	$288.29 \pm 0.21$	$0.03 \pm 0.02$	$0.05 \pm 0.01$	$0.54 \pm 0.02$
Ocimum gratissimum	$216.95 \pm 0.18$	$0.02 \pm 0.01$	$0.07 \pm 0.02$	$0.26 \pm 0.01$
Talinum triangulare	$191.91 \pm 0.12$	$0.05 \pm 0.02$	$0.16 \pm 0.02$	$0.50 \pm 0.02$
Telfaria occidentalis	$316.94 \pm 0.21$	$0.06 \pm 0.02$	$0.08 \pm 0.01$	$0.65 \pm 0.01$
Vernonia amygdalina	$257.33 \pm 0.20$	$0.05 \pm 0.01$	$0.06 \pm 0.01$	$0.60 \pm 0.01$

Results are mean of triplicate determinations on a dry weight basis  $\pm$  standard deviation.

Table 2c. Vitamin composition of boiled leafy vegetables on mg/100 g dry weight

Vegetable	Vitamin			
_	Ascorbic acid	Thiamin	Riboflavin	Niacin
Amaranthus hybridus	$197.12 \pm 0.20$	$0.05 \pm 0.01$	$0.06 \pm 0.01$	$0.41 \pm 0.02$
Corchorus olitorius	$158.40 \pm 0.22$	$0.02 \pm 0.01$	$0.03 \pm 0.01$	$0.31 \pm 0.02$
Ocimum gratissimum	$95.94 \pm 0.21$	$0.02 \pm 0.01$	$0.04 \pm 0.01$	$0.15 \pm 0.01$
Talinum triangulare	$107.82 \pm 0.11$	$0.04 \pm 0.01$	$0.09 \pm 0.01$	$0.29 \pm 0.01$
Telfaria occidentalis	$178.06 \pm 0.20$	$0.04 \pm 0.01$	$0.05 \pm 0.01$	$0.37 \pm 0.01$
Vernonia amygdalina	$145.82 \pm 0.21$	$0.04 \pm 0.01$	$0.04 \pm 0.01$	$0.34 \pm 0.01$

Results are mean of triplicate determinations on a dry weight basis  $\pm$  standard deviation.

The results revealed that vegetables contain significant amounts of vital vitamins with

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vegetables higher fresh having followed concentrations. bv steamed vegetables and boiled vegetables having the lowest. The disparity in the vitamin levels in fresh leafy vegetables could be as a result of soil profiles in which the vegetables were planted, impacts of human activities and varied climatic factors, while that of steamed and boiled leafy vegetables could be credited to changes in analytical protocols, processing time and conditions [8, 10].

In general, leafy vegetables are high in vitamins, which are required for a variety of daily biological processes, including cell reproduction [11], growth and cellular energy processing [12]. The result of this study showed that the fresh, steamed and boiled A. hybridus had the highest quantity of ascorbic acid, thiamin and niacin, indicating that A. hybridus can be consumed on regular basis, as ascorbic acid is crucial for biosynthesizing collagen in human body protein metabolism [13],[14] increasing the body's resistance infections [15, 16]. Thiamin also allows the body to use carbohydrates as energy, which it does mostly in muscles, brain, liver and kidney. It also has an impact on glucose metabolism [17]. Niacin is also essential in cells for energy transfer and to repair DNA [18]. Meanwhile, the maximum amount of riboflavin was found in the fresh, steamed and boiled *T. triangulare*, which is crucial for growth and overall health. It also helps the human body in breaking down carbohydrates, proteins and fats to produce energy, in addition, allowing the body to use oxygen [19, 20].

Percentage loss of vitamin composition of fresh and processed leafy vegetables

The impact of heat processing on the vitamin contents of the vegetables was also

investigated. Table 3a and 3b show the estimated percentage loss of vitamin composition of fresh and processed vegetables. For steamed leafy vegetables, the percentage loss of ascorbic acid varies from 9 to 11 % in C. olitorius to T. triangulare and T. occidentalis, whereas the percentage loss of ascorbic acid in boiled leafy vegetables varies from 45 to 50 % in A. hybridus, C. olitorius, T. triangulare and For occidentalis. steamed *T*. vegetables, the percentage loss of thiamin varies from 9 to 17 % in T. occidentalis and O. gratissimum, whereas the percentage loss of thiamin in boiled leafy vegetables varies from 48 to 50 % in A. hybridus, O. gratissimum, C. olitorius, T. triangulare and V. amygdalina. For steamed leafy vegetables, the percentage loss of riboflavin varies from 9 to 13 % in T. triangulare and C. olitorius, whereas the percentage loss of riboflavin in boiled leafy vegetables varies from 49 to 50 % in C. olitorius and V. amygdalina, A. hybridus, T. triangulare, O. gratissimum and T. occidentalis. For steamed leafy vegetables, the percentage loss of niacin varies from 9 to 12 % in V. amygdalina, *A*. hybridus and occidentalis, whereas the percentage loss of niacin in boiled leafy vegetables varies from 48 to 50 % in V. amygdalina, C. Т. occidentalis olitorius. and 0. gratissimum.

According to the findings, boiling had the most adverse impact on the vitamin content of the vegetables, accounting for nearly 50 % loss, while steaming accounted for approximately 17 % loss. This is in agreement with Oladejo's findings [21]. To obtain the necessary daily intake of the aforementioned vitamins, we recommend that steamed vegetables rather than boiled vegetables be consumed on a regular basis.

Table 3a.

#### Percentage loss of vitamin composition of steamed leafy vegetables

Vegetable	% Loss of Vitamin			
	Ascorbic acid	Thiamin	Riboflavin	Niacin
Amaranthus hybridus	10	11	10	9
Corchorus olitorius	9	14	13	11
Ocimum gratissimum	10	17	11	10
Talinum triangulare	11	10	9	10
Telfaria occidentalis	11	9	10	12
Vernonia amygdalina	10	11	12	9

Table 3b.

Percentage loss of vitamin composition of boiled leafy vegetables

Vegetable	% Loss of Vitamin			
	Ascorbic acid	Thiamin	Riboflavin	Niacin
Amaranthus hybridus	45	48	50	49
Corchorus olitorius	50	50	49	50
Ocimum gratissimum	49	48	50	50
Talinum triangulare	50	50	50	49
Telfaria occidentalis	50	49	50	50
Vernonia amygdalina	49	50	49	48

#### 4. Conclusion

The findings of this study revealed that the leafy vegetables studied are high in vitamins that are required for cell growth reproduction, and energy utilization. The study found that A. hybridus had the highest quantity of ascorbic acid, thiamin and niacin in the fresh, steamed and boiled vegetables assessed, implying that regular consumption of A. hybridus will aid during the biosynthesis of the collagen in the body and increase the body's resistance to infections. Furthermore, the results of the impact of heat treatment on the vitamin content of the green vegetables revealed that boiling had the greatest detrimental effect on the vitamin content of the vegetables, accounting for nearly 50 % loss, while steaming accounted for about 17 % loss.

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#### 6. Declaration of interest

No disagreement of interests was declared by the authors.

#### 7. References

- [1] OPAZO-NAVARRETE, M., BURGOS-DÍAZ, C., SOTO-CERDA, B., BARAHONA, T., ANGUITA-BARRALES, F. & MOSI-ROA, Y. Assessment of the nutritional value of traditional vegetables from Southern Chile as potential sources of natural ingredients. *Plant Foods for Human Nutrition*, 76, 523–532, (2021).
- COELHO, S.C., ESTEVINHO, B.N. & ROCHA, F. Recent advances in water-soluble vitamins delivery systems prepared by mechanical processes (Electrospinning and Spray-Drying Techniques) for food and nutraceuticals applications—A review. *Foods.* 11(9), 1271, (2022). AZIMA, F., ASBEN, A., REFDI, C. W., SURYA AULIA, H. S. & SYUKRI, D. Effect of different cooking methods on the content of vitamin C, phenolics and minerals in several green leafy vegetables. Pakistan Journal of Nutrition, 19(4), 160-165, (2020).
- [4] NUPUR, M. Effects of thermal treatments on ascorbic acid content of leafy vegetables: An *in vitro* analysis. *Journal of Phytonanotechnology and Pharmaceutical Science*, 1(1), 10-15, (2021).
- [5] DAVEY, M. W., MONTAGU, M. V., INZE, D., SANMARTIN, M., KANELLIS, A., SMIMOFF, N., BENZIE, I. F. F., STRAIN, J. J.,

Sunday Adegoke ADENIYI, Peter Agbomire AGBUGUI, Saheed Olanrewaju Abiodun SOLOLA, Jacob Sunday OYEKU, **Effect of heat processing on vitamin composition of selected staple green leafy vegetables in Nigeria,** Food and Environment Safety, Volume XXII, Issue 4–2023, pag 248 – 254

- FAVELL, D. & FLETCHER, J. Plant L ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *Journal of the Science of Food and Agriculture*, 80, 825-860, (2000).
- [6] ADENIYI, S. A., MUHAMMAD, W. E. & SOLOLA, S. O. A. Comparative study of mineral composition of selected staple green leafy vegetables in Nigeria. *Asian Food Science Journal*, 20(12), 61-68, (2021).
- [7] SONI, A. & BRIGHTWELL, G. Effect of hurdle approaches using conventional and moderate thermal processing technologies for microbial inactivation in fruit and vegetable products. *Foods*, 11(1811), 1-17, (2022).
- [8] HAILEMARIAM, G. A. & WUDINEH, T. A. Effect of cooking methods on ascorbic acid destruction of green leafy vegetables. *Journal of Food Quality*, 2020, Article ID 8908670, 5 pages, (2020).
- [9] NASREEN, A., GHOSH, R. K., ALI, M., RAHMAN, T., AKHTER, T. & AKHTER, S. Estimation of thiamine, riboflavin and niacin content in jute leaves. *GSC Advanced Research and Review*, 13(01), 116–124, (2022).
- [10] KINYI, H. W., TIRWOMWE, M., NINSIIMA, H. I. & MIRUKA, C. O. Effect of cooking method on vitamin C loses and antioxidant activity of indigenous green leafy vegetables consumed in Western Uganda. *International Journal of Food Sci*ence, 2022, Article ID 2088034, 7 pages, (2022).
- [11] ZHOU, J., CHEN, C., CHEN, X., FEI, Y. & JIANG, L., WANG G. Vitamin C promotes apoptosis and cell cycle arrest in oral squamous cell carcinoma. *Frontiers in Oncology*, 10(976), 11 pages, (2020).
- [12] TARDY, A-L., POUTEAU, E., MARQUEZ, D., YILMAZ, C. & SCHOLEY, A. Vitamins and minerals for energy, fatigue and cognition: A narrative review of the biochemical and clinical evidence. *Nutrients*, 12(1), 228, (2020).
- [13] GREF, R., DELOMÉNIE, C., MAKSIMENKO, A., GOUADON, E., PERCOCO, G., LATI, E., DESMAËLE, D., ZOUHIRI, F. & COUVREUR, P. Vitamin C–squalene bioconjugate promotes epidermal

- thickening and collagen production in human skin. *Scientific Reports*, 10(16883), 12 pages, (2020).
- [14] JEONG, H. & VACANTI, N. M. Systemic vitamin intake impacting tissue proteomes. *Nutrition and Metabolism*, 17(73), 16 pages, (2020).
- [15] GOMBART, A. F., PIERRE, A. & MAGGINI, S. A review of micronutrients and the immune system—working in harmony to reduce the risk of infection. *Nutrients*, 12(236), 41 pages, (2020).
- [16] KUMAR, P., KUMAR, M., BEDI, O., GUPTA, M., KUMAR, S., JAISWAL, G., RAHI, V., YEDKE, N.G., BIJALWAN, A., SHARMA, S. & JAMWAL, S. Role of vitamins and minerals as immunity boosters in COVID-19. *Inflammopharmacology*, 29, 1001–1016, (2021).
- [17] GRALAK, M. A., DĘBSKI, B. & DRYWIEŃ, M. Thiamine deficiency affects glucose transport and β-oxidation in rats. *Journal of Animal Physiology and Animal Nutrition*, 103(5),1629–1635, (2019).
- [18] PIRINEN, E., AURANEN, M., KHAN, N. A., BRILHANTE, V., URHO, N., PESSIA, A., HAKKARAINEN, A., KUULA, J., HEINONEN, U., SCHMIDT, M. S. HAIMILAHTI, K., PIIRILÄ, P., LUNDBOM, N., TASKINEN, M-R., BRENNER, C., VELAGAPUDI, V., KIRSI, H., PIETILÄINEN, K. H. & SUOMALAINEN, A. Niacin cures systemic NAD+ deficiency and improves muscle performance in adult-onset mitochondrial myopathy. *Cell Metabolism*, 31(6), 1078-1090.e5, (2020).
- [19] MERRILL, A. H. & MCCORMICK, D. B. "Riboflavin". In Marriott B.P., Birt D.F., Stallings V.A., Yates A.A. (eds.). Present knowledge in nutrition, Eleventh Edition. London, United Kingdom: Academic Press (Elsevier), pp. 189–208, (2020).
- [20] SUWANNASOM, N., KAO, I., PRUß, A., GEORGIEVA, R. & BÄUMLER, H. Riboflavin: The health benefits of a forgotten natural vitamin. *International Journal of Molecular Sciences*, 21(3), 950, (2020).
- [21] OLADEJO, A. A. Comparative vitamin analysis of some selected Nigerian green leafy vegetables from two different zones. *Acta Scientific Nutritional Health*, 3(11), 15-18, (2019).