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EFFECT OF COOKING METHODS AND DURATION ON ASCORBIC ACID CONCENTRATION OF SELECTED VEGETABLES

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ABSTRACT: Cooking vegetables before they are consumed helps in making them more palatable in addition to improving taste and texture. On the other hand, cooking causes a substantial change in the composition of chemicals thus affecting concentration and availability of nutrients. There are some cooking methods that cause oxidization of the antioxidants therefore affecting the retention of nutrients of the vegetables. Therefore, it is vital to opt for a cooking method which will lead to optimal retention of nutrients. This study was designed to determine the reduction in Vitamin C concentration associated with different cooking methods of green leafy vegetables. Vitamin C content of green leafy vegetables namely, African nightshade (*Solanum nigrum*) and amaranth (*Amaranthus viridis*) were selected for the study. Vitamin C content of the above sample vegetables were estimated using spectrophotometric method at a wavelength of 245nm. Processing methods which were employed were boiling and microwave heating which were performed at a timely interval. The findings showed that microwaving had the highest vitamin C loss. The results also showed that long duration of cooking led to massive loss from the selected vegetables. It was concluded that the water used for cooking vegetables should not be discarded for maximum retention of vitamin C in vegetables. Based on the study, it is recommended that the best duration for cooking *Solanum nigrum* and *Amaranthus viridis* should be less than 10 minutes (dependent on consumer preference) so as to retain maximum ascorbic acid.

Keywords: Vitamin C, Boiling, Microwaving, Spectrophotometric method.

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1. INTRODUCTION

Green leafy vegetables constitute a major component of the human diet in Africa [1]. Most sub-communities in Africa continent rely on traditional leafy vegetables with a primary aim of dietary supplementation [2]. Different varieties of these vegetables are prepared whereby they are cooked and eaten as a relish together with starchy staple food, mostly in the form of thick porridge [3]. Green leafy vegetables dishes are quite often prepared with mono-plant species or in combination with other different species to upgrade the flavor, taste and color appeal to such diet [4, 5]. Ingredients such as salt, oil, butter, groundnuts, tomato and onion are usually added while cooking African leafy vegetables to enhance flavor, taste and color appeal [1]. These added ingredients also depend on availability and preference of the consumers [6]. Several studies have revealed that cooking or preparation methods and period of cooking may affect the nutritional value as well as the bioavailability of many nutrients in leafy vegetables [7]. Green leafy vegetables are primarily protective foods and are highly beneficial for the maintenance of health and prevention of certain infections due to the fact that they contain some valuable food ingredients which are vital in boosting the immune system and repairing body tissues [8]. Various epidemiological studies have indicated that increased dietary intake of green leafy vegetables is associated with decreased risk of cancer infections, cardiovascular diseases, cataracts and other age-related diseases [9]. Indeed, these plants are valuable sources of nutrients especially in rural areas where they contribute substantially to protein, minerals, vitamins, fiber and other nutrients to our daily dietary intake [10]. Among other vitamins, green leafy vegetables are a good source of Vitamin C [6,11]. Research findings have shown that the frequency of traditional green leafy vegetables consumption has decreased over the years. This have been contributed by consumers consideration based on their taste and nutritional value comparison with most currently grown exotic vegetables such as spinach (*Spinacea oleracea*) and cabbage (*Brassica oleracea*) [12]. Besides, preference of African green leafy vegetables species depends mostly upon gender and age of consumers, social background and geographical location [13]. However, comparative studies have indicated that green leafy vegetables consumed in the whole continent of Africa contain high level of micronutrients than the exotic ones [14]. Moreover, Jansen Van-Rensberg *et al.* [13] and Nesamvuni *et al.* [15] have proven experimentally that traditional and indigenous leafy vegetables have high nutritional quality. Despite nutritional contribution of leafy vegetables to the local dietary requirement and their health functions and protective properties, there has been very little effort towards exploiting these valuable and healthy resources for improved nutritional requirement by a greater population of sub-Saharan Africa [16]. This study aimed at determining the effects of different cooking methods and duration on ascorbic acid in green leafy vegetables (African nightshade and Amaranth). Vitamin C is a water-soluble vitamin [17]. It was first isolated in 1928 by the Hungarian biochemist and Nobel Prize winner Szent-Gyorgyi. Vitamin C exists in three redox states which are ascorbate, the fully reduced form;

semi-dehydroascorbate, the mono oxidized form and dehydroascorbate, the fully oxidized form [17]. Vitamin C is an essential dietary nutrient for a variety of biological functions. Under physiological conditions, it is important in the biosynthesis of collagen through facilitating the hydroxylation of lysine and proline residues, thus allowing proper intracellular folding of pro-collagen for export and deposition as mature collagen [18]. It serves also as a co-factor in several important hydroxylation reaction, such as the biosynthesis of catecholamine through the conversion of dopamine to norepinephrine, L-carnitine, cholesterol, amino acid and other peptide hormones [18]. Vitamin C is also associated with decreased incidence of cancer, blood pressure, drug metabolism, urinary hydroxyproline excretion and tissue regeneration [19]. The revised recommended dietary allowance (RDA) for vitamin C is 75 mg for women and 90 mg for men because the vitamin is linked to antioxidant activities and protective action against scurvy [19]. During preparations, vegetables are usually subjected to various cooking methods which include boiling, microwaving and blanching. These cooking methods alters the nutritional content of vegetables based on the cooking duration [20]. Additionally, cooking can have a significant impact on the content of vitamins and leads to an inaccurate estimation of nutrient content [21]. Cooking is that process which aims in producing safe and more edible food by preparing and combining ingredients and in most cases heat subjection [22]. This improves the bioavailability of some nutrients but at the same time some nutrients are lost. Therefore, this aspect provided an insight to this study which was based on the effect of different cooking methods and duration of cooking on ascorbic acid concentration in African nightshade (S*olanum nigrum*) and amaranth (*Amaranthus viridis)*.

2. MATERIALS AND METHODS

2.1 Chemicals

HPLC grade methanol and pure ascorbic acid were purchased from Sigma-Aldrich.

2.2 Preparation of Calibration Equations of Ascorbic Acid RS Solutions

10mg of ascorbic acid RS (reference standards) was weighed into 100ml flask and about 70ml of methanol was added into the flask and then stirred for 5 minutes. Methanol was added to 100ml mark of the conical flask before mixing the solution uniformly. Ascorbic acid RS solutions of 5 different concentrations (2, 5, 10, 20 and 40 ppm respectively) were prepared using the stock solution in 10ml volumetric flasks. The absorbance of each ascorbic acid RS solution was measured with the UV/Vis spectrophotometer, taking methanol as a blank, at the wavelength of 245nm. A calibration equation was obtained using Excel; abs= **a** × conc [ppm] +**b**, in which **a** is the slope and **b** is the y-intercept and this was used to subsequently calculate the concentration of our test sample

2.3 Sample Collection

The two selected vegetable samples i.e. African nightshade and *Amaranthus viridis* were purchased at Chuka open air market very early in the morning. During sample selection, these vegetables were sampled from different individuals in the market. A quantity of 2kg of the whole sample was purchased, 1kg of *Amaranthus viridis* and 1kg of African nightshade respectively.

2.4 Sample Treatment

On arrival from the market, using tap water the vegetables were cleaned separately to ensure there was no any solid contaminant such as soil particles. The vegetables were left to air dry for about 30 minutes before they were chopped into smaller pieces using a clean knife on a chopping board.

2.5 Cooking Treatments Employed

Different cooking methods that were employed are boiling and microwaving.

2.5.1 Boiling

500ml of distilled water was poured into a 1000ml beaker, and then covered with a lid before heating to boil using a hot plate. 100grams of homogenized *Amaranthus viridis* was added into the boiling water and then cooked for different time intervals of 5, 10, 15 and 20 minutes respectively. After every 5 minutes interval, 5ml of the cooking water was being collected into 50ml different beakers before thorough filtration was done into 4 different beakers. The same procedure was done to African nightshade at the same time intervals.

2.5.2 Microwaving

A microwave oven at full power [1000 W] was used for microwaving. 100g of *Amaranthus viridis* was again submerged into 500ml of distilled water in the microwave and microwaved for every 5 minutes interval as in the case of boiling. 5ml of microwaved water was collected into 4 various 50ml beakers. Again, the same procedure was repeated for the African nightshade at the same time interval. In addition to these cooking methods, same quantities (100g) of these two vegetables were prepared raw for Vitamin C concentration determination.

2.6 Preparation and Measurement of Testing Sample Solutions

5ml of each sample collected at each cooking time point in the two different methods was poured in a 25ml volumetric flask and 15ml of the HPLC grade methanol added into the flask. Methanol was added to the 25ml mark of the flask. Re-filtration was done to each sample solutions before immediate record of their absorbance taken using UV/Vis spectrophotometer at a wavelength of 245nm. The solution was diluted where necessary to ensure that its concentration was within the range of the RS solutions.

2.7 Determination of Vitamin C Concentration

The vitamin C concentration of each sample was determined within an hour after sample preparation. The content of vitamin C of each sample was determined using UV/vis spectrophotometer analytical method as outlined by Zeng *et al.* [23]. A UV/vis spectrophotometer was used to measure the absorbance of different concentrations in reference standard [RS] and testing sample solutions. The principle of the UV/vis spectrophotometer is based on the fact that vitamin C is soluble and stable in methanol and that its solution in methanol has a maximum absorption peak at the wavelength of 245nm [23]. Different vitamin C concentrations have different absorbance, and there is a linear relationship between absorbance and vitamin C concentration as per Beer- Lambert's law. The calibration equation of RS solutions was used to quantify the vitamin C concentration of test sample solutions.

3. RESULTS AND DISCUSSION

3.1 Preparation of Calibration Equations of Ascorbic Acid RS Solutions

Commercial ascorbic acid diluted to different concentrations with HPLC grade methanol was used to make the linear regression. The absorbance was obtained using UV/vis spectrophotometry at 245nm. The calibration equation was Y=0.02776x+0.19925 as plotted in Figure 1. The r^2 value was 0.87262 and the correlation coefficient (r) of 0.937 was obtained. The calibration equation was applied in calculating the concentration of the test samples. The concentration was then converted into amount (micrograms) in 25 ml of the sample.

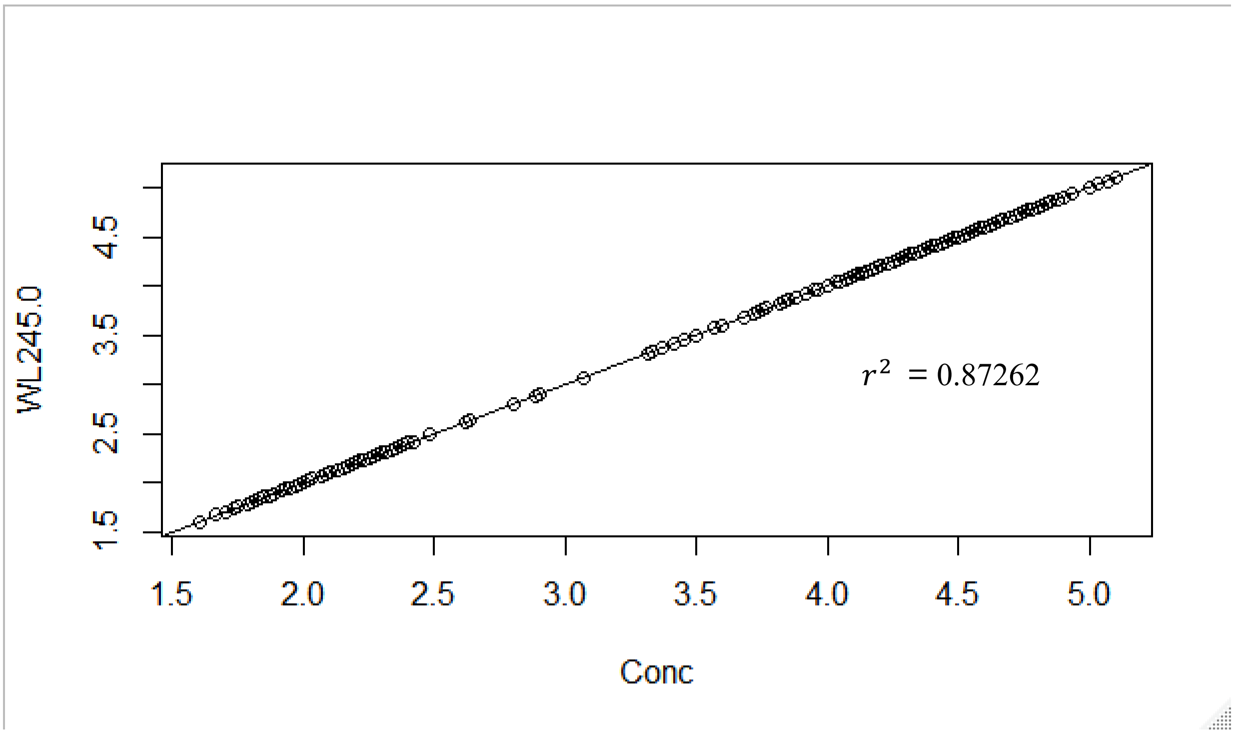

Figure 1: Standard curve of absorbance against concentration

3.2. Reduction of Vitamin C during Boiling and Microwaving under Different Time Durations of the Selected Green Leafy Vegetables

The analysis of the concentrations of ascorbic acid and amount of Vitamin C in the cooking water for amaranth are represented in Table 1, Figure 2 and 3. The results in Table 1 show that the concentration of ascorbic acid and amount of Vitamin C in the cooking water increased with increase in cooking time for both methods that is microwaving and boiling. However, the concentration of ascorbic acid and the amount of Vitamin C in the cooking water was higher in microwaving when compared to the boiling method for different cooking times. Figure 2 shows high concentration of ascorbic acid in the cooking water when using microwaving method as compared to boiling. Figure 3 presents an increasing trend in the concentration of ascorbic acid with increase in cooking time for the two cooking methods. This showed that for different cooking times, more vitamin C was lost when using the microwaving method as compared to the boiling method.

Table 1: Concentration of ascorbic acid and amounts of vitamin C lost for amaranth

Cooking method	Time (minutes)	Conc of ascorbic acid in mg/L	Vitamin C content in 25ml (micrograms)
Boiled	5	26.079	651.975
Boiled	10	77.64	1941
Boiled	15	133.36	3334
Boiled	20	187.58	4689.5
Raw	0	19.165	479.125
Microwaved	5	188.06	4701.5
Microwaved	10	412.5	10312.5
Microwaved	15	512.8	12820
Microwaved	20	627.7	15692.5

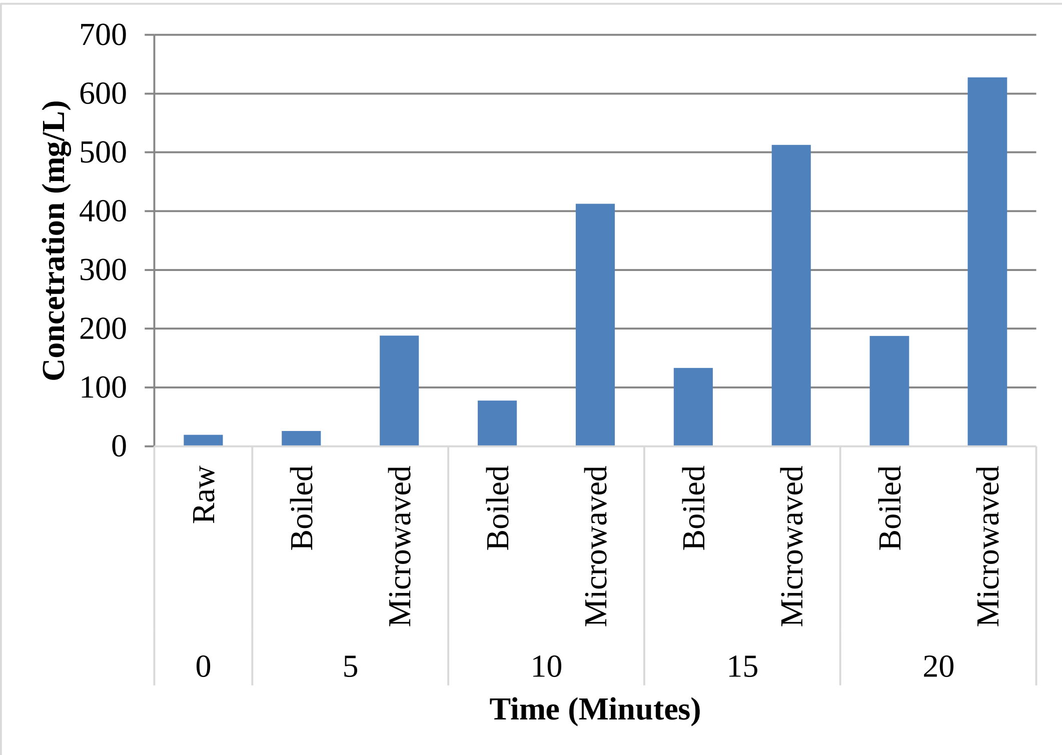

Figure 2: Concentration of ascorbic acid (mg/L) against cooking time for boiling and microwave methods for amaranth

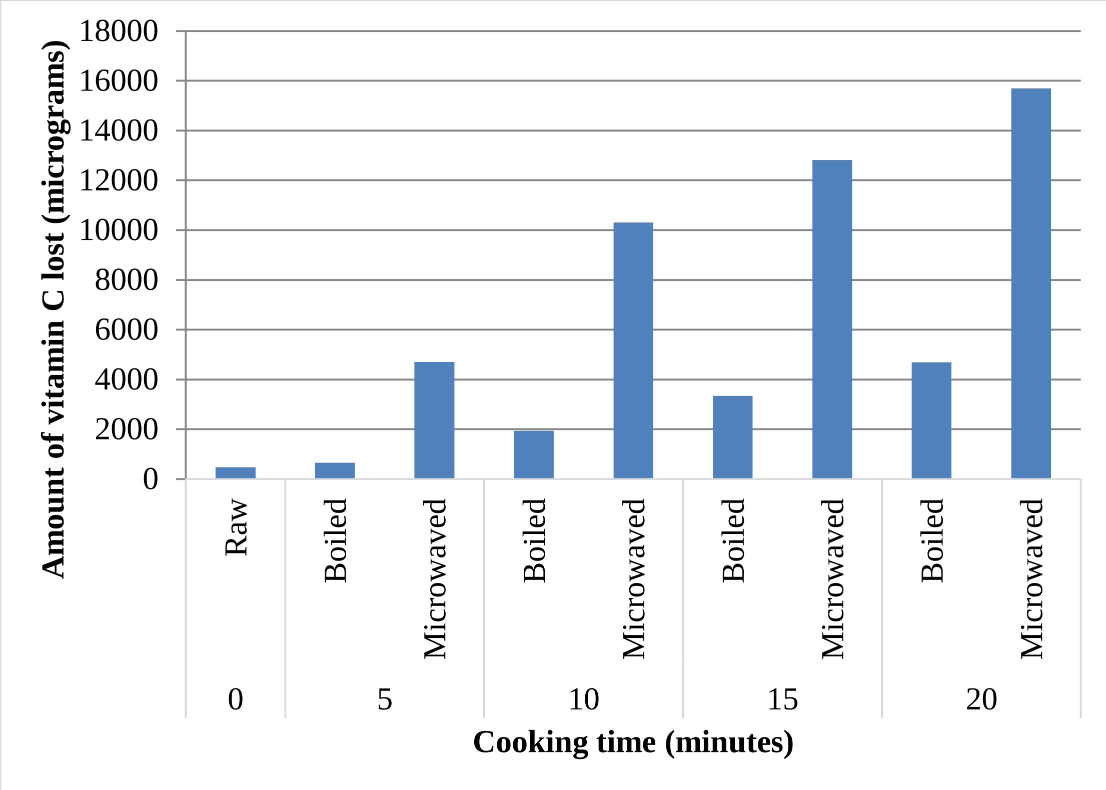

Figure 3: Amount of Vitamin C lost against cooking time for amaranth

The analysis of the concentration of ascorbic acid and amount of Vitamin C in the cooking water for African nightshade are represented in Table 2, Figure 4 and 5. The results in Table 2 show that the concentration of ascorbic acid and amount of Vitamin C in the cooking water increased with increase in cooking time for both methods. However, the concentration of ascorbic acid and the amount of Vitamin C in the cooking water was higher in microwaving than boiling for different cooking times. Figure 4 shows high concentration of ascorbic acid in the cooking water when using microwaving method as compared to boiling. The graph also presents the increasing trend in the concentration of ascorbic acid with increase in cooking time for the two cooking methods. Figure 5 showed that for different cooking times, more Vitamin C was lost when using the microwaving method as compared to the boiling method.

Table 2: Concentration of ascorbic acid and amounts of vitamin C lost for African nightshade

Cooking method	Time (minutes)	Ascorbic acid concentration in mg/L	Vitamin C content in 25ml (micrograms)
Boiled	5	245.34	6383.5
Boiled	10	276.33	6908.25
Boiled	15	401	10025
Boiled	20	276.22	6905.5
Raw	0	110.82	2770.5
Microwaved	5	301.86	7546.5
Microwaved	10	348.76	8719
Microwaved	15	440.18	11004.5
Microwaved	20	557.86	13946.5

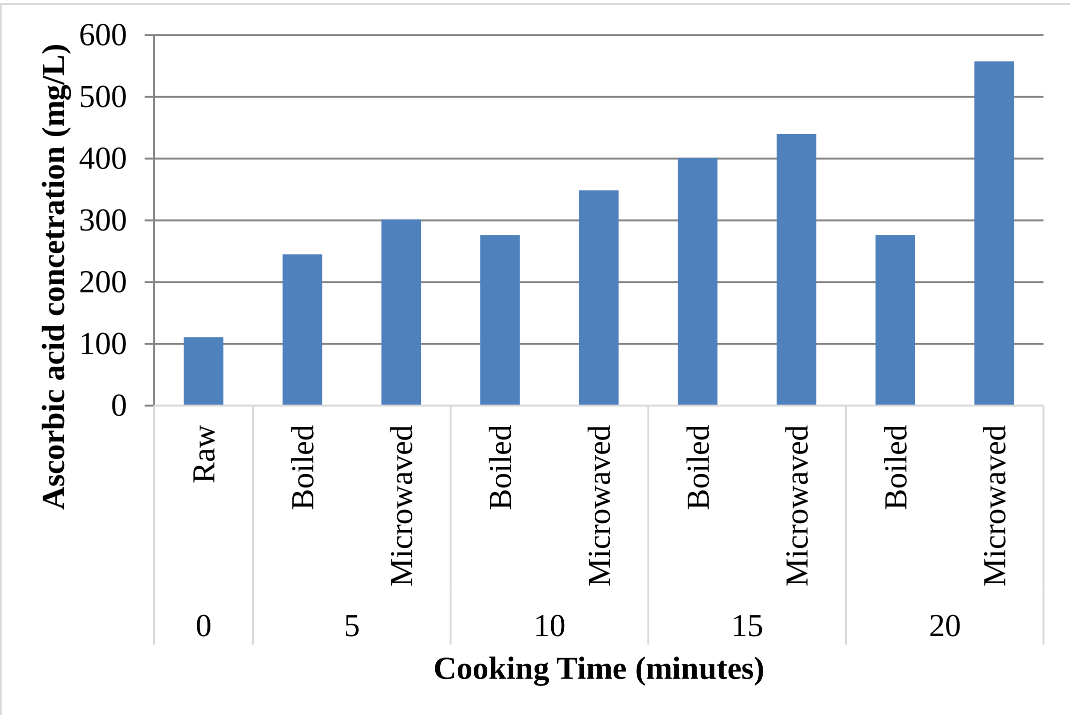

Figure 4: Concentration against cooking duration for boiling and microwave methods for African nightshade

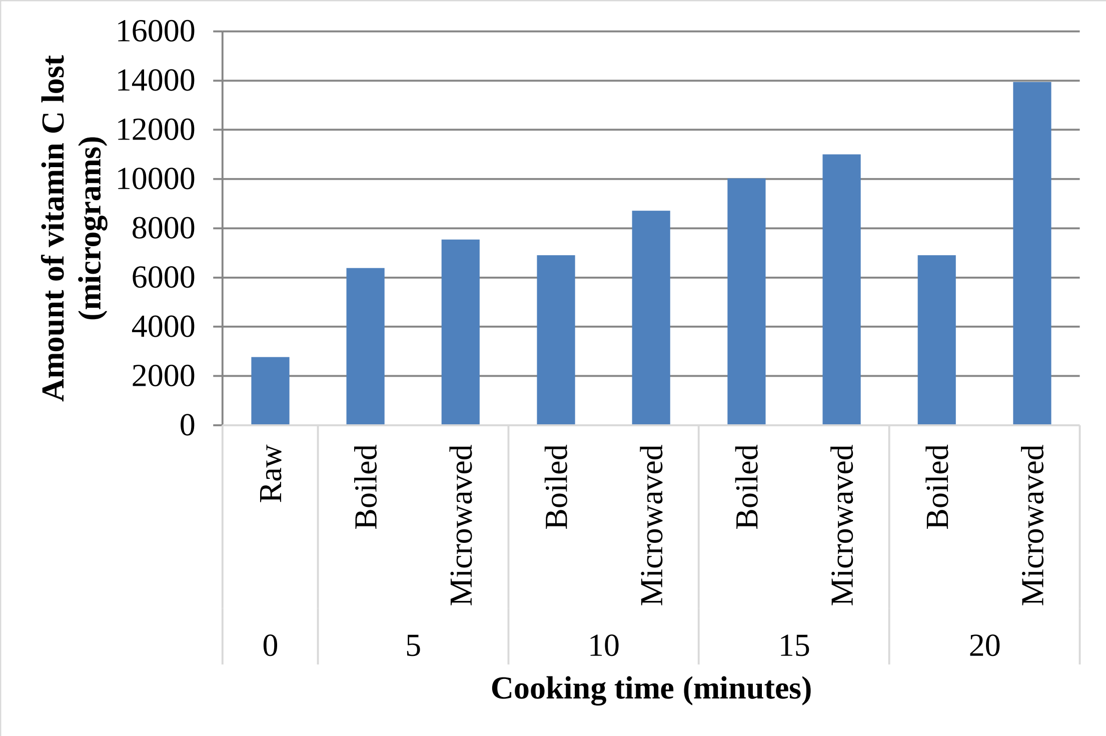

Figure 5: Amount of vitamin C lost against cooking time for African nightshade

The analysis of variance for the amount of concentration of ascorbic acid in the cooking water for both African nightshade and amaranth is presented in Table 3 and Table 4. The results were obtained by regressing concentration against cooking time and cooking methods. The results from the analysis showed that cooking time and cooking method had a significant effect on the amount ascorbic acid in the cooking water. This confirmed the results from the descriptive analysis which showed that the amount of vitamin C lost when cooking vegetable could be accounted for by the cooking method and cooking time. Similar results were obtained by other researchers such as Zeng [7] on broccoli and spinach, Adefegha and Oboh [24] and Fafunso *et al*. [25]. The results are also in agreement with the work of Babalola *et al*. [26] who confirmed that ascorbic acid levels in green leafy vegetables are method dependent.

Table 3: Analysis of variance for concentration of ascorbic acid when cooking African nightshade

	df	SS	MS	F	Significance F
Regression	2	54270.32	27135.16	5.766141	0.050303
Residual	5	23529.74	4705.948		
Total	7	77800.06			

Table 4: Analysis of variance for concentration of ascorbic acid when cooking African nightshade

	df	SS	MS	F	Significance F
Regression	2	312599.4	156299.7	33.56742	0.001265
Residual	5	23281.45	4656.291		
Total	7	335880.8			

The diagnostics for the regression of ascorbic acid concentration against cooking times and cooking method for both African nightshade and amaranth is presented in Table 5. The results showed that 69.7561% and 93.0685% of concentration of ascorbic acid in the cooking water was accounted for by cooking time and cooking method for African nightshade and amaranth respectively. This showed that the method of cooking and the time of cooking vegetables was responsible for the loss of vitamin C when cooking vegetables

Table 5: Diagnostics for regression for African nightshade and amaranth

Regression	Statistics (African nightshade)	Statistics (amaranth)
Multiple R	0.835201	0.96472
R Square	0.697561	0.930685
Adjusted R Square	0.576586	0.90296
Standard Error	68.59991	68.23702
Observations	8	8

The mean separation was done using the least significant differences technique. The aim was to do mean separation for average vitamin C concentrations in the cooking water at different cooking times for both boiling and microwaving methods as shown in table 6 and table 7. The results showed that the average amounts of vitamin C lost under different cooking times were significantly different. However, where means were not significantly different at different times, they were denoted using same letters.

Table 8: Mean separation for cooking times for African nightshade

Cooking method	Time (minutes)	Average Ascorbic acid concentration in mg/L	Mean separation
Boiled	0	110.82	a
	5	245.34	ab
	10	276.33	ab
	15	401	bc
	20	476.22	c
Microwaved	0	110.82	a
	5	301.86	bcd
	10	348.76	cd
	15	440.18	de
	20	557.86	e

[a]Means followed by same letter are not significantly different from each other at 5% probability level.

Table 9: Mean separation for cooking times for amaranth

Cooking method	Time (minutes)	Conc of ascorbic acid in mg/L	Mean separation
Boiled	0	19.165	a
	5	26.079	a
	10	77.64	a
	15	133.36	a
	20	187.58	b
Microwaved	0	19.165	a
	5	188.06	b
	10	412.5	cd
	15	512.8	de
	20	627.7	e

[a]Means followed by same letter are not significantly different from each other at 5% probability level.

4. CONCLUSION

Based on the study findings, it was shown that increasing cooking duration led to increased loss in Vitamin C from the selected study vegetables. Since boiling of vegetables is inevitable, precautionary measures such as boiling and microwaving for the very minimal period as possible should be taken into consideration to avoid nutrient losses. In addition, the traditional pre-boiling whereby the water used for cooking vegetable is discarded should be avoided for maximum retention of vitamin C.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The author confirms that the data supporting the findings of this research are available within the article.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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