J. Agric. Food. Tech., 4(5)16-26, 2014

ISSN 2090 – 424X
Journal of Agriculture and Food Technology
www.textroad.com

Nutritional Composition of Slenderleaf (*Crotalaria Ochroleuca and Crotalaria Brevidens*) Vegetable at Three Stages of Maturity

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Received: June 1, 2014
Accepted: October 12, 2014

ABSTRACT

African Leafy Vegetables (ALV) are commonly consumed in Kenya. They have a high potential of playing a significant role in alleviating the high rate of malnutrition in the country. Among the ALVs commonly consumed among many communities in Kenya is slenderleaf.

Despite the widespread use of these vegetables, relatively very few studies have been reported on their nutritional quality. Specifically, it is not clear at which stage of maturity the leaves, which are the main edible portion, give optimum nutrition value. Stage of maturity of leafy vegetables is known to affect the nutrient content.

The main objective of this study was therefore to determine the nutrient composition of slenderleaf vegetable at 3 stages of maturity (before, during and after flowering).

Fresh leaves of slenderleaf were harvested at weeks 8, 13 and 16 after planting. Moisture, protein, ash, iron, zinc, and calcium were determined in the samples.

Results indicated that stage of maturity did not have a significant effect on protein and calcium content ($p \leq 0.05$ %,). However, there was significant difference in the values of moisture, ash, iron, and zinc in the slenderleaf leaves at different stages of maturity ($p \leq 0.05$ %,).

These results indicate that optimum nutrition value for some of the nutrients in the slenderleaf leaves can be obtained by harvesting the leaves during flowering.

KEY WORDS: Slenderleaf, ALVs, Nutrients, Stage of growth.

INTRODUCTION

Leafy vegetables form a significant part of the traditional diets of agricultural communities [1, 2]. African leafy vegetables (ALVs) have been reported to be important because of their nutrition quality and health benefits [3]. Unfortunately, due to environmental, economic, and sociocultural changes, a good number of ALVs are neglected, under-exploited and threatened with extinction [4]. One of such neglected but important ALVs is Slenderleaf [5].

Slenderleaf is one of the ALVs that has been grown and consumed in Africa, especially in Kenya for a long time [6]. The plant is regarded as a multi-purpose crop in agricultural production and has a high germination percentage rate (83% after five days). It is mainly cultivated by local communities in Western and Coast provinces of Kenya for the purpose of food, and allegedly restoring fertility to soils and to combat weeds in areas where cultivable land is a problem [7]. It can be intercropped with regular food or cash crops, or it may be grown alone.

Currently, about 100 million people suffer from diseases, blindness and other problems associated with malnutrition [8]. Malnutrition is an underlying cause in 55% of deaths in Africa. Iron deficiency anaemia alone is a contributing factor in over 20% of post-birth maternal deaths in Africa [9]. About one third (35%) of children under five in Kenya are stunted, 7% are wasted and 16% are underweight [10].

Consumption of ALVs including slenderleaf can contribute towards alleviating some of these malnutrition problems. However research efforts have previously focused mainly on breeding and cultivation aspects of the ALVs. There is therefore relatively very little data available on its nutrients and antinutrient compounds. Slenderleaf has been reported to have health promoting attributes that are likely linked to its nutritional and non-nutrients components. As is the case with other ALVs, slenderleaf is a likely source of some of the key micronutrients whose deficiency is of public health concern [12, 13, 14, and 15]. But the content of these nutrients are likely to differ at different stages of maturity.

The overall objective of this study was therefore to determine the nutrient content of eight slenderleaf accessions at different stages of growth (8^{th}, 11^{th} and 15^{th} week).

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MATERIALS AND METHODS

Study site

Eight Slenderleaf accessions, collected from eight different geographical locations within western Kenya, were grown in 5x5 metre plots in an open field at Jomo Kenyatta University of Agriculture & Technology (JKUAT) farm. Poultry manure mixed with soil at a rate of 20 ton per hectare was applied to the plot. Seeds of each accession were selected, mixed with soil at the rate of 1:10 and drilled in the respective plots at a space of 30 cm between crops in a raw. The seeds germinated in 3–4 days. After two weeks, thinning was done to leave an inter-raw spacing of 15 cm to ensure optimum growing conditions. Harvesting was done by removing the leaves at three different stages of growth: before (8 weeks after seedling germination), during (11 weeks after seedling germination) and after flowering (15 weeks after seedling germination).

Study design

This was a laboratory based study where the eight samples of slenderleaf leaves, at three stages of maturity, were harvested from the farm, prepared and analyzed to determine the relevant nutritional component. Analyses were carried out in triplicate and results were presented in term of percentage (%) and/or mg/100g. The differences in means were analyzed using GenStat (software), and were considered significantly different at Significance level of 5 %.

Sample handling and preparation

Fresh leaves were harvested in the farm, brought to the laboratory, and destalked to separate leaves from the stems. The leaves were then weighed and washed to remove dirt. Some of the leaves were dried at 105°C for an hour then ground into a fine powder using a mortar and pestle, to await analysis for ash, minerals and protein. The other fresh leaves were kept in cold storage at 5-10°C, and analyzed within 24 hours (particularly for Vitamin C and beta-carotene).

Nutrition Composition

Some parameters of proximate composition including moisture, protein and ash were determined according to AOAC methods specification 950.46 [16].

Moisture determination

The moisture content was determined by oven drying method as per the AOAC Method [17]. About 2 g of fresh leaves of the sample was accurately weighed into a moisture dish and transferred to a Hot-air-oven previously heated to temperatures of 105°C and drying done until constant weight. The final weight (wt) of the sample was taken after cooling it in a desiccator. Loss in weight as moisture was given by the formulas given below.

$$\% \text{ Moisture} = \frac{\text{Wt of sample before drying} - \text{Wt of sample after drying}}{\text{Wt of sample before drying}} \times 100$$

Protein determination

The protein was determined using the Kjedahl Method [18]. About 1 g of the pre dried (at 105°C for an hour) sample was weighed into a digestion flask together with a catalyst composed of 5 g of K_2SO_4 and 0.5 g $CUSO_4$ and 15 ml of concentrated H_2SO_4. The mixture was heated in a fume hood till the digest colour turned blue. The digest was cooled, transferred to a volumetric flask and topped up with distilled water. 10 ml of diluted digest was transferred into the distilling flask and washed with about 2 ml distilled water. 15 ml of 40 % NaOH was added and washed with about 2 ml distilled water. Distillation was done to a volume of about 60 ml and titrated using 0.02 N-HCl. Appearance of an orange colour signified the end point of the titration[18].

$$\text{Nitrogen \%} = (V_1 - V_2) \times N \times f \times 0.014 \times 100 / V \times 100 / S$$

V_1 = Titer for sample (ml);
V_2 = Titer for blank (ml),
N = Normality of standard HCl solution (0.02);
f = Factor of standard HCl solution,
V = Volume of diluted digest taken for distillation (10 ml),
S = Weight of sample taken (g)

$$\text{Protein \%} = \text{Nitrogen} \times \text{protein factor (6.25)}$$

Ash determination

Ash was determined using the dry ashing method [19]. A sample weight of between 2- 5 g was measured in pre-weighed crucibles. The samples were first charred to eliminate smoking before being incinerated at 550°C in a muffle furnace to the point of white ash (about 18 hours). The residues were cooled in desiccators and the weights taken [19].

$$\text{Crude ash \%} = \frac{\text{Weight of Ash} \times 100}{\text{Weight of sample}}$$

Mineral determination

Atomic absorption spectrophotometer was used for the determination of iron, zinc, and calcium in the ash [20].

Vitamin C determination

The ascorbic acid content was determined by 2,6-dichlorophenolindophenol (DCPI) method [21]. A 5 g of fresh slenderleaf leaves was ground using a mortar and pestle with acid washed sand and 10 ml of TCA reagent. The content was transferred into a flask and topped up with TCA. The extract was mixed well and filtered immediately. Then 10 ml was titrated with indophenol solution until pink color appeared. For the blank, 10 ml of water was used and titrated with indophenol until rose pink color appears.

$$\text{Vitamins C content (mg/100g)} = \frac{(A - B) \times C \times 10 \times 100}{S}$$

A (ml) = volume of indophenols solution used for the sample,
B (ml) = volume of indophenols solution used for the blank,
C (mg) = mass of ascorbic acid equivalent to 1 ml of standard indophenols solution,
S (mg) = sample weight taken

β-carotene and total carotenoids

β-carotene and total carotenoids were determined as described by Rodriguez [22]. A sample of 5 g of fresh leaves was mixed with 3g of hyflosupercel and 50ml of acetone, ground and filtered. Then 25ml of petroleum ether was put with in a separatory funnel and a small portion of acetone extract was then added. Distilled water was slowly added, and the lower acetone phase discarded. Butylated hydroxytoluene and an equal volume of potassium hydroxide in methanol were added to the carotenoids solution in petroleum ether in the dark at room temperature. The mixture was washed with distilled water to remove the potassium hydroxide using a separatory funnel. The carotenoids phase was collected and dried with sodium sulphate.

The total carotenoids content of the extract was determinated by measuring the absorbance in a spectrophotometer using the extinction coefficient. β-carotene was read at 440 ŋm while total carotenoids was read at 450 ŋm [23].

$$\text{Total carotenoids content (µg/g)} = \frac{A \times \text{volume (ml)} \times 10^4}{A^{1\%}_{1cm} \times \text{sample weigh (g)}}$$

A = absorbance,
V = volume of extract,
$A^{1\%}_{1cm}$ = absorption coefficient of β-carotene in petroleum ether (40-60°C) of 2592

Data quality

Each sample analysis was carried out in triplicate. Relevant standards were used in the determination of the various parameters (minerals, vitamin C, β-carotene and total carotenoids).

Data management and analysis

Descriptive statistics were used to describe the results of the nutrient composition. Data obtained was subjected to analysis of variance (ANOVA) to establish means with significant differences [24].

RESULTS & DISCUSSION

Nutrient Composition

Moisture content

Moisture content for all the accessions before, during and after flowering varied significantly ($p \leq 0.05$) (Table 1). The mean moisture content was significantly ($p \leq 0.05$) higher in the samples before flowering than after flowering. There were significant ($p \leq 0.05$) differences in moisture content due to variety at each of the growth stages.

The result showed a reduction in moisture as the vegetables matured, decreasing from an average value of 88.4% (before flowering) to 78.2% (after flowering). This may reflect the fact that, generally as plants mature, the dry matter increases.

These results were also similar to those reported by Abukutsa-Onyango [25]. Under the same environmental and research conditions, Abuktusa-Onyango analyzed the Moisture Content of *Crotalaria Brevidens* and found out that as the crop grew the values were significantly different, higher before flowering while lower after flowering.

Table 1: Moisture Content of Slenderleaf at three stages of growth

Moisture (%)

Samples	**Before Flowering**	**During Flowering**	**After Flowering**
1	89.4 ± 0.8^{b}	85.1 ± 0.5^{c}	76.4 ± 0.4^{a}
2	91.6 ± 1.9^{b}	83.6 ± 0.7^{b}	79.8 ± 0.3^{d}
3	89.9 ± 2.5^{b}	82.4 ± 1.8^{a}	76.5 ± 0.5^{a}
4	83.5 ± 3.2^{a}	81.7 ± 1.4^{a}	77.8 ± 0.3^{b}
5	86.4 ± 2.2^{ab}	83.6 ± 1.3^{b}	79.3 ± 0.3^{cd}
6	88.9 ± 2.4^{b}	81.9 ± 1.2^{a}	79.5 ± 0.5^{d}
7	88.8 ± 2^{b}	82.0 ± 1.9^{a}	77.7 ± 0.7^{b}
8	88.8 ± 0.2^{b}	82.1 ± 1.6^{a}	78.5 ± 0.5^{c}
Means	88.4^{b}	82.8^{ab}	78.2^{a}

Value= Mean ± S.D on fresh weight basis. Each value is a mean of 3 replicates. Means of the same parameter followed by the same letter are not significantly different ($p \leq 5\%$). S.D= standard Deviation.

Ash content

Ash content before, during and after flowering was respectively of 0.6 %, 0.4 % and 0.3 %, respectively (Figure 1). The mean ash Content was significantly higher ($p \leq 0.05$) in the samples before flowering (0.6%) than during and after flowering.

The result showed a reduction in Ash as the vegetables matured, decreasing from an average value of 0.6% (before flowering) to 0.3% (after flowering). This may reflect the fact that, as plants matured, the Ash content decreased.

These results are in line with those observed by Mibei [26]. He did some research on some ALV and found similar results on ash content. The research was carried out under the same conditions and showed that the lowest Ash content was observed after flowering.

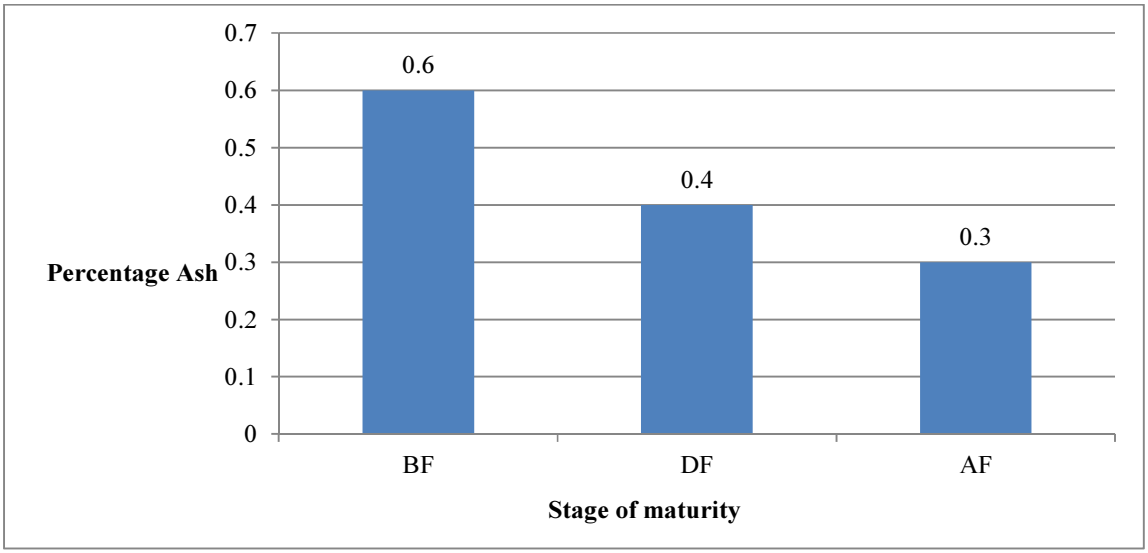

Figure 1: Ash content (mean) of Slenderleaf before, during and after flowering
BF: Before Flowering DF: During Flowering AF: After Flowering

Protein content

Proteins content before, during and after flowering in the slender leaf leaves varied from 1.5 to 2.6 %; 1.6 to 2.3 % and 1.3 to 2.1 %, respectively (Table 2). There was a significant ($p \leq 0.05$) difference in protein content due to variety between Accession 2 and others before flowering, accession 8 and others during flowering and accessions 5 and 6 and others after flowering. The mean protein content at the three maturity stages, was not significantly different ($p \geq 0.05$) and was relatively low, at 1.8 – 1.9%.

These results are similar to those reported by Mibei [26] in his research on phytochemical, antioxidant and in vitro antimicrobial screening of some African Leafy Vegetables who reported protein content of 2.6 ± 0.8 %. As slenderleaf leaves mature, protein level increases then starts reducing after flowering.

Table 2: Protein Content of Slenderleaf at three stages of growth

	Proteins (%)		
Samples	**Before Flowering**	**During Flowering**	**After Flowering**
1	1.5 ± 0.4^{a}	1.6 ± 0.5^{a}	1.3 ± 0.2^{a}
2	2.6 ± 0.5^{b}	2.0 ± 0.1^{ab}	1.9 ± 0.1^{a}
3	1.8 ± 0.3^{a}	1.7 ± 0.2^{a}	1.7 ± 0.2^{a}
4	1.6 ± 0.4^{a}	1.7 ± 0.2^{a}	1.6 ± 0.3^{a}
5	1.8 ± 0.2^{a}	1.7 ± 0.1^{a}	1.9 ± 0.3^{b}
6	1.9 ± 0.1^{a}	2.1 ± 0.3^{b}	2.1 ± 0.2^{b}
7	1.5 ± 0.3^{a}	2.1 ± 0.4^{b}	1.8 ± 0.1^{a}
8	1.9 ± 0.3^{a}	2.3 ± 0.2^{c}	1.9 ± 0.3^{a}
Means	1.8^{a}	1.9^{a}	1.8^{a}

Value= Mean ± S.D on fresh weight basis. Each value is a mean of 3 replicates. Means of the same parameter followed by the same letter are not significantly different ($p \leq 5\%$). S.D= standard Deviation.

Mineral Composition

Iron content

Iron content before, during and after flowering varied from 9.3 to 11.1 mg/100g; 12.3 to 14.5 mg/100g and 9.8 to 11.1 mg/100g, respectively (Table 3). The mean iron content was significantly ($p \leq 0.05$) higher in the samples during flowering than after and before flowering.

There was a significant ($p \leq 0.05$) difference in Iron content due to variety between Accession 3 and others before flowering, accession 4 and others during flowering and accessions 3 and others after flowering. The mean Iron content at the three maturity stages was significantly different ($p \leq 0.05$) between before flowering and during flowering.

These results are similar to those reported by Habwe [27], who reported a value of 13.6 mg / 100 g in a study of iron content of East African indigenous vegetable recipes. That research was surely done during flowering stage under similar research conditions.

Table 3: Iron content of Slenderleaf before, during and after flowering

	Iron (mg/100g)		
Samples	**Before Flowering**	**During Flowering**	**After Flowering**
1	10.1 ± 0.1^{a}	12.3 ± 0.6^{a}	10.6 ± 0.4^{b}
2	9.3 ± 0.3^{a}	12.7 ± 0.3^{a}	9.8 ± 0.3^{a}
3	11.1 ± 0.6^{b}	13.4 ± 0.1^{b}	11.1 ± 0.6^{b}
4	9.7 ± 0.6^{a}	14.5 ± 0.2^{b}	10.3 ± 0.4^{a}
5	10.1 ± 0.5^{a}	13.3 ± 0.7^{b}	10.3 ± 0.3^{b}
6	10.2 ± 0.2^{a}	13.4 ± 1.1^{b}	10.7 ± 0.4^{b}
7	10.1 ± 0.2^{a}	13.5 ± 1.6^{b}	10.4 ± 0.5^{a}
8	9.7 ± 0.7^{a}	13.4 ± 0.7^{b}	10.7 ± 0.3^{a}
Means	10.0^{a}	13.3^{b}	10.5^{a}

Value= Mean ± S.D on fresh weight basis. Each value is a mean of 3 replicates. Means of the same parameter followed by the same letter are not significantly different ($p \leq 5\%$). S.D= standard Deviation.

Zinc content

Zinc content before, during and after flowering varied from 29.4 to 34.2 mg/100g; 33.9 to 40.5 mg/100g and 28.9 to 29.6 mg/100g, respectively (Table 4). The mean zinc content was significantly ($p \leq 0.05$) higher in the samples during flowering than after and before flowering. After flowering there were no significant ($p \geq 0.05$) difference in zinc content among the slenderleaf accessions while before and during flowering some accessions were significantly ($p \leq 0.05$) different from each other.

There was a significant ($p \leq 0.05$) difference in Zinc content due to variety between Accession 7, accession 6 and accession 2 before flowering, during flowering accession 7 and others (accession 5 and accession 2) and after flowering there were no significant ($p \geq 0.05$) differences between accessions. The mean Zinc content at the three maturity stages was significantly different ($p \leq 0.05$) between before flowering, after flowering and during flowering.

The results in this study were similar to the results reported by Shackleton [28] in his book on African Indigenous Vegetables in Urban Agriculture in which he reported about his analyses did under similar conditions.

Table 4: Zinc Content of Slenderleaf at three stages of growth

	Zinc (mg/100g)		
Samples	**Before Flowering**	**During Flowering**	**After Flowering**
1	33.1 ± 1.6^{b}	33.9 ± 2.4^{a}	29.4 ± 0.4^{b}
2	29.4 ± 1.4^{a}	36.6 ± 2.1^{b}	28.9 ± 0.9^{a}
3	33.8 ± 1.3^{b}	40.5 ± 1.1^{c}	29.1 ± 0.5^{a}
4	30.9 ± 1.4^{a}	38.8 ± 1.3^{b}	28.9 ± 0.2^{a}
5	32.6 ± 1.9^{b}	35.2 ± 2.7^{a}	29.5 ± 0.4^{ab}
6	32.3 ± 1.9^{b}	38.0 ± 2^{b}	29.2 ± 0.3^{a}
7	34.2 ± 2.1^{c}	40.4 ± 0.6^{c}	29.4 ± 0.3^{a}
8	30.3 ± 1.3^{a}	35.1 ± 3.6^{b}	29.6 ± 0.4^{b}
Means	32.1^{b}	37.3^{c}	29.3^{a}

Value= Mean ± S.D on fresh weight basis. Each value is a mean of 3 replicates. Means of the same parameter followed by the same letter are not significantly different ($p \leq 5\%$). S.D= standard Deviation.

Calcium content

The results of calcium content are shown in Figure 2. Calcium content before, during and after flowering was 0.2 %. The mean calcium content was not significantly ($p \geq 0.05$) different due to growth. There was also no significant ($p \geq 0.05$) difference due to variety at the three stages of growth.

These results were similar to those observed by Leung [29] who found in his work on kales' nutritional composition that the Ca level has a constant trend throughout the development cycle of kales.

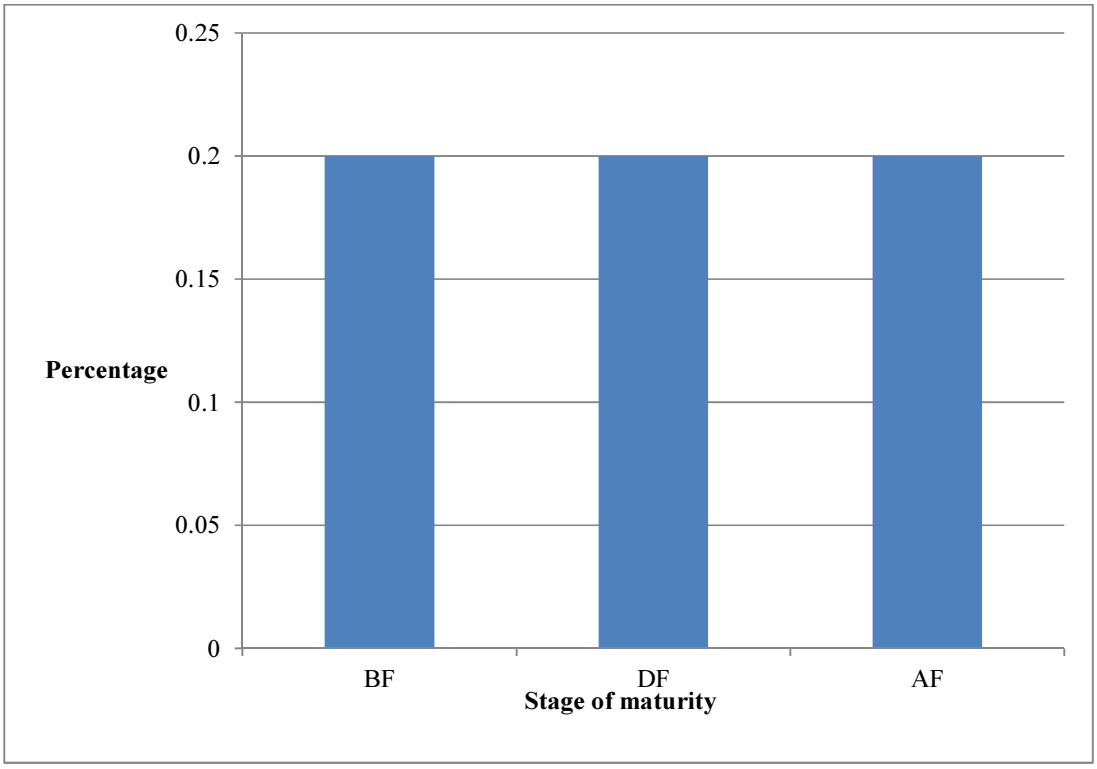

Figure 2: Calcium content (mean) of Slenderleaf before, during and after flowering
BF: Before Flowering DF: During Flowering AF: After Flowering

Vitamin C content

Vitamin C before, during and after flowering varied from 9.9 to 10.9 mg/100g, 11.4 to 14.3 mg/100g and 8.9 to 9.9 mg/100g, respectively (Table 5). The mean Vitamin C content was significantly ($p \leq 0.05$) higher in the samples during flowering than after and before flowering. There was significant ($p \leq 0.05$) difference in Vitamin C content due to variety during flowering. However, there was no significant ($p \geq 0.05$) difference in this respect before and after flowering.

The results were lower than those observed by Abukutsa-Onyango [25], who analyzed, under the same condition, Vitamin C content in Slenderleaf. She reported that Vitamin C content was 9.2 mg/100g in fresh slenderleaf leaves. That difference may be due to the variety or accession that was analyzed. It was probable that the accession of Slenderleaf analyzed here was not the same as the one in this research.

Table 5: Vitamin C Content of Slenderleaf leaves at three stages of growth

	Vitamin C (mg/100g)		
Samples	**Before Flowering**	**During Flowering**	**After Flowering**
1	9.9 ± 0.7^{a}	13.9 ± 0.5^{a}	8.9 ± 0.1^{a}
2	10.7 ± 0.4^{ab}	11.4 ± 2.2^{a}	8.9 ± 0.3^{a}
3	10.6 ± 0.7^{a}	13.2 ± 0.8^{a}	8.9 ± 0.2^{a}
4	10.9 ± 0.7^{b}	13.6 ± 0.4^{a}	9.2 ± 0.3^{a}
5	10.8 ± 0.3^{a}	11.9 ± 2.4^{a}	9.2 ± 0.2^{a}
6	10.1 ± 0.6^{a}	14.3 ± 0.3^{b}	9.9 ± 0.1^{c}
7	10.3 ± 0.8^{a}	12.4 ± 1.2^{a}	9.5 ± 0.2^{b}
8	10.5 ± 0.5^{a}	13.9 ± 0.1^{b}	9.7 ± 0.5^{d}
Means	10.5^{b}	13.1^{c}	9.3^{a}

Value= Mean ± S.D on fresh weight basis. Each value is a mean of 3 replicates. Means of the same parameter followed by the same letter are not significantly different ($p \leq 5\%$). S.D= standard Deviation.

β- Carotene content

β- Carotene content before, during and after flowering varied from 71.9 to 75.4 mg/100g, 85.7 to 88.3 mg/100g and 75.5 to 80.3 mg/100g, respectively. The mean β- Carotene Content was significantly ($p \leq 0.05$) higher in the

samples during flowering than before and after flowering (Figure 3). This probably is due to the fact that after maturity the sun and the light by their effect on the plant reduced the content.

These results are similar to those observed by Mibei [26] who reported under the same research conditions a value of 6.2 mg/100g of β- carotene content in slenderleaf leaves on dry matter basis.

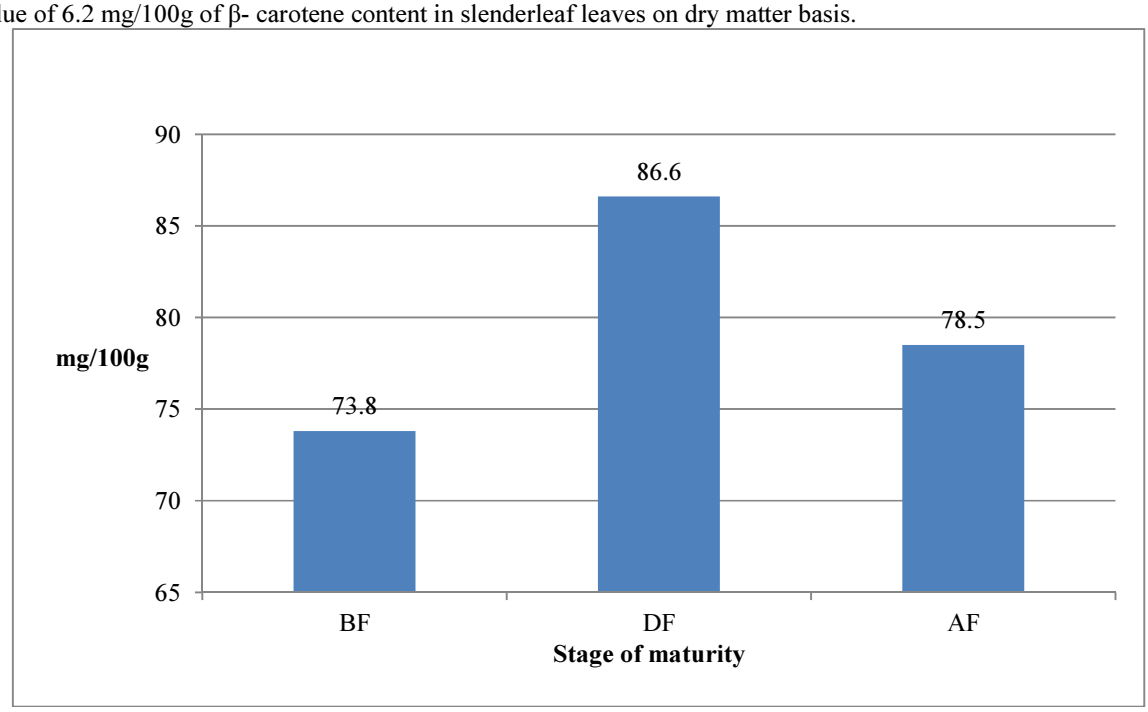

Figure 3: β-Carotene content (mean) of Slenderleaf before, during and after flowering
BF: Before Flowering DF: During Flowering AF: After Flowering

CONCLUSION

There were no significant differences ($p \leq 0.05$) in the levels of protein and calcium contents in the leaves of the slenderleaf accessions due to maturity stage. For zinc, and iron, Vitamin C, and β-carotene, the values were s significantly higher ($p \leq 0.05$) in the slenderleaf leaves at flowering in comparison to before and after flowering.

Before the flowering stage, nutrients rate was high compared to before and after flowering stage. We can therefore say that the best time of consumption of slenderleaf is during flowering. The plant needs to be harvested and consumed at that stage so that users could get full profit with most added values both for nutrition and health.

ACKNOWLEDGEMENT

Research Production Extension (RPE) Slenderleaf research project.

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