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Minerals and Nutrient Variations as Influenced by Harvesting Stages in selected African Leafy Vegetables Grown in Busia, Kenya

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Authors' contributions

This work was carried out in collaboration between all authors. Author MG designed the study, performed the statistical analysis, wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. Authors OO and OD managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Proper nutrition contributes to declines in under-five mortality rates and improves the productivity of adults. Addressing nutritional problems requires adequate information on the diets of individuals and populations. African leafy vegetables (ALVs) are widely consumed and often harvested at different stages after planting. Four ALVs namely *Vigna unguiculata*, *Amaranthus hybridus*, *Cleome gynandra* and *Solanum scabrum* are commonly grown in western Kenya, their potentials have not been evaluated for supply of nutrients. However, nutritional values may vary depending on the species and harvesting stage. The effects of harvesting stages on nutritional value of selected ALVs were evaluated. The trials were laid out in a randomized complete block design in three replicates. Leaves were sampled at different harvesting stages and analyzed for N, P, K, Na, Ca, Mn, Mg, Fe and Zn levels. The levels of nutrients significantly ($P \leq 0.05$) varied between species and harvesting stages. The Fe, Mg and Zn levels were above the Recommended Dietary Allowance. *Amaranthus hybridus* had higher ($P \leq 0.05$) levels of P, Ca, Zn, Mn and Na. The N, P, K, Ca and Zn levels significantly ($P \leq 0.05$) increased from 4 to 6 weeks after seed emergence (WAE) then

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decreased from 6 to 10 WAE. The Fe levels increased from 4 to 6 WAE while the increase from 6 to 10 WAE was not significant. Magnesium levels significantly ($P \leq 0.05$) increased from 4 to 8 WAE then decreased, while Mn and Na levels did not vary with harvesting stage. Harvesting the ALVs from 4 to 6 WAE for the supply of P, K, Ca and Zn, 4 to 8 WAE for Mg and 4 to 10 WAE for the supply of Na and Mn is recommended.

Keywords: African leafy vegetables; harvesting stage; nutrients; recommended dietary allowances; Busia Kenya.

1. INTRODUCTION

Essential elements in humans can be classified into macro and micro nutrients. Each element in both classes is essential and has indispensable functions in the human body [1]. Fruits and vegetables are the important sources of these nutrients. Proper nutrition contributes significantly to declines in under-five mortality rates [2] and also improves the productivity of adults. Approximately 30-40% of Kenyan children under-five years of age are stunted, 10-20% are underweight while 6-10% are wasted [2] possibly due to lack of some of these nutrients. Many people living in western Kenya suffer from nutrient deficiency diseases [3]. The alarming rates of child malnutrition and nutrient deficiency among households in western Kenya emphasize the urgent need to address nutritional issues [4,5].

Kenya is endowed with many varieties of indigenous food plants [6] like ALVs with potential to supply Recommended Dietary Allowances (RDAs) of the micronutrients [7]. The integration of foods rich in macro and micronutrients into the diet is a sustainable way to improve nutrient status in the human body [8]. Poor communities that cannot afford expensive nutrient dense animal source foods consume a lot of ALVs which are easily available, relatively cheap and a rich source of major macro and micronutrients [9]. However, the levels of beneficial nutrients in the ALVs grown in western Kenya have not been documented to guide consumption levels to supply RDAs for relevant nutrients.

Several food crops are grown within Busia County, with almost every household owning a small garden, often serving as a source of vegetables for household consumption. Specifically, residents of Busia County are known to produce *Vigna unguiculata*, *Amaranthus hybridus*, *Cleome gynandra* and *Solanum scabrum* almost throughout the year due to favorable soils and climatic conditions [10]. Affluent adults in Kenya are now resorting to indigenous foods [11] as they are believed to be nutritious. However, the nutritional value of the locally grown vegetables (ALVs) is unkown. Nevertheless there is a belief among the locals that vegetables are important sources of vitamins and minerals especially Fe and Ca [12,13].

Plants exhibit variations in nutrient levels due to several factors like season, geographical location and husbandry [14]. Up to-date information is required for all types of foods including ALVs. Kenya is yet to establish a comprehensive food composition table for Kenyan foods. The ALVs composition table developed by Sehmi [15] for Kenyan ALVs is questionable as the samples used in its development were bought from different markets and the causes of the variations could not be established.

Nutrient content in vegetables vary according to their availability in the soil at different collection sites and plant uptake [16]. Intra-species variations have been reported in Iringa and Morogoro districts in Tanzania [17]. The nutrient contents of *Cleome gynandra*, *Solanum scabrum* and *Amaranthus hybridus* leaves from the same location of production varied significantly in Tanzania [18], leading to the conclusion that variations may have been due to differences in genetic abilities to extract soil available nutrients. Another study on the nutritional value of different amaranths, African eggplant and African nightshade [19], showed that they too differed according to species, variety and geographical area of production. It is necessary to evaluate variations in different ALVs commonly consumed in Busia County.

Exotic vegetables are normally harvested and consumed at a known stage of plant development. However, ALVs have no documented information on the stage of plant development to define harvest maturity. Data on their nutritional value varies widely [20], due to influences of plant age. Plant age significantly affected yields and nutritive quality of four variants of black nightshades (*Solanum nigrum* L.) [21]. It is however, not known how harvesting stage affects the distribution of nutritive elements in ALVs. This study therefore sought to assess the total amounts of nutrients (nitrogen (N) and minerals (phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn), and sodium (Na)) in selected ALVs (*Vigna unguiculata*, *Amaranthus hybridus*, *Cleome gynandra* and *Solanum scabrum*) at different harvesting stages in Busia County, western Kenya.

2. METHODOLOGY

2.1 Planting of ALVs

The selected ALVS were grown at Bulanda (altitude: 1350 m above sea level, 0º28´N 34º6´E), Busia located in the former western province of Kenya. The trial was conducted from April to September, 2009. The experiment was laid down in a randomized complete block design (RCBD) replicated three times. Each replicate (4 m^2) was divided into four plots (each 1 m^2) to cater for the four ALVs species namely: *Cleome gynandra,* variety PS*, Solanum scabrum,* variety SS49*, Amaranthus hybridus,* amfune variety and *Vigna unguiculata,* fahari variety which were distributed randomly. Land preparation was done by clearing the weeds followed by deep ploughing. Harrowing was done using a folk and leveled with a rake. Certified seeds (from Lagrotech, Kisumu) were mixed with the soil in the ratio 1:10 and sawn directly 2 cm deep in rows with an inter-row spacing of 30 cm (planting density 11 plants m^{-2}, i.e. 30 cm by 30 cm) [22], and covered with a thin layer of soil. Weeding was carried out three times; the 1st on the 3rd, 2nd on the 5th and 3rd on the 7th week, while thinning was done with an inter-plant spacing of 30 cm on the 3rd week just before weeding and spraying done using Ortus 5 SC (fenpyroximate as the active ingredient) for insect and pathogen control after weeding.

2.2 Preparation of Leaf Samples

The upper palatable aerial parts of individual plants (2nd and 3rd leaves) were sampled fortnightly four times, after 4 WAE. The Association of Official Analytical Chemists [23] method of sample preparation was used. Leaf samples were washed in deionised water three times to remove soil particles, oven-dried at 60ºC and crushed into fine powder using a mill. Dried and ground leaves (0.5 g) in porcelain crucible were ashed in a muffle furnace at 450ºC until greyish white ash was obtained. The samples were cooled on top of asbestos sheet and 5 mL analytical grade 1 N HNO_3 solution added to each sample for digestion. This was evaporated to dryness on a steam bath to ensure complete digestion. The samples were returned to the furnace for a further 15 minutes until a perfect grey ash was obtained. The grey ash was cooled on asbestos sheet and 10 mL of analytical grade 1 N HCl added for mineral extraction then filtered into 50 mL volumetric flask. The crucible and filter paper were rinsed three times and the aliquots added to the flask before making to the mark with 0.1 N HCl. The extract was analyzed for K, Na and Ca using flame photometer (Jenway Model, PFP 7) and Mg, Mn, Fe, and Zn using atomic absorption spectrometer, AAS (Model AA-6200, Shimadzu, Corp., Kyoto, Japan). Analytical grade salts of potassium chloride, sodium chloride, calcium nitrate, potassium permanganate and metals: iron, magnesium, and zinc were used as standards. Total N was determined using a method described by AOAC [24] with slight modifications.

2.3 Data Analysis

The data for ALVs at different harvesting stages was analyzed using RCBD in a 2-factorial arrangement, with species as the main treatment and harvesting stage as sub-treatment, using MSTAT-C statistical package (Michigan State University, MI) for ANOVA. Fisher's least significant difference (LSD) test was used to identify significant differences among treatment means ($p \leq 0.05$).

2.4 Method Validation

Method validation for AAS was performed by assessing several analytical parameters namely; linearity, precision and accuracy [25]. Limit of Detection (LoD) and Limit of Quantification (LoQ) were not evaluated as the levels of Mg, Mn, Fe and Zn in leaf samples were high.

3. RESULTS AND DISCUSSION

3.1 Variation in Levels of Nutrients in Selected ALVs in Busia County

The levels of nutrients significantly ($P \leq 0.05$) varied in the four species studied (Tables 1 and 2). *Amaranthus hybridus* had significantly

Table 1. Levels of N, P, K, Ca and Mg (DW) in selected ALVs at different harvesting stages, RDAs and recommended intake amounts at Busia site

Nutrient	Vegetable species					M. stage
	H. stage (wks)	***V. unguiculata***	***A. hybridus***	***C. gynandra***	***S. scabrum***	
N (%)	4	2.241	1.735	1.641	2.019	1.909
	6	2.570	1.743	1.644	2.125	2.020
	8	2.546	1.749	1.659	2.227	2.045
	10	2.290	1.757	1.664	2.365	2.019
	Mean species	2.412	1.746	1.652	2.184	
	CV (%)			4.98		
	LSD (P≤0.05)			0.129	0.129	
	Interaction			0.184		
P (mg/100 g)	4	18.679	48.370	26.726	37.363	32.784
	6	23.097	52.128	29.583	42.010	36.704
	8	19.642	48.340	25.293	36.798	32.518
	10	16.988	42.790	21.215	35.432	29.107
	Mean species	19.602	47.907	25.704	37.901	
	RDA (mg)			800.000		
	AVC*	6.221*	2.517*	4.737*	3.178 *	
	CV (%)			4.12		
	LSD (P≤0.05)			1.756	1.756	
K (mg/100 g)	4	69.932	80.402	69.337	65.480	71.288
	6	76.472	83.000	79.916	75.475	78.715
	8	65.550	80.747	76.190	70.085	73.143
	10	62.472	71.944	68.808	58.302	65.381
	Mean species	68.607	79.023	73.563	67.335	
	RDA (mg)			2000.000		
	AVC*	2.932*	2.537 *	2.714 *	2.996*	
	CV (%)			9.48		
	LSD (P≤0.05)			8.771	8.771	
Ca (mg/100 g)	4	178.370	239.350	180.327	185.643	195.923
	6	190.890	254.447	254.293	192.570	223.050
	8	155.707	208.777	170.740	175.203	177.607
	10	149.420	187.367	156.337	145.423	159.637
	Mean species	168.597	222.485	190.424	174.710	
	RDA (mg)			1000.000		
	AVC*	0.784*	0.592*	0.705*	0.752*	
	CV (%)			0.83		
	LSD (P≤0.05)			2.032		
	Interaction			2.886		
Mg (mg/100 g)	4	28.823	17.807	34.527	366.743	111.975
	6	31.523	19.990	37.397	390.283	119.798
	8	34.103	22.380	29.270	424.707	127.615
	10	32.920	22.690	26.747	434.347	129.176
	Mean species	31.842	20.717	31.985	404.020	
	RDA (mg)			350.000		
	AVC*	1.103*	1.705*	1.112*	0.087*	
	CV (%)			1.44		
	LSD (P≤0.05)			2.287	2.287	
	Interaction			3.251		

*AVC *, Amount of vegetables (kg) to be consumed daily by a healthy adult to supply RDA*
NS, Not Significant

(P≤0.05) higher levels of P, K, Ca, Zn, Mn and Na. Higher levels of N were reported in *Vigna unguiculata*, Mg in *Solanum scabrum* and Fe in *Cleome gynandra*. Similar variations had also been reported in Maseno [26], Nairobi (Mwajumwa et al. [27]), ALVs sampled from different Kenyan markets, Ghana [28] and Swaziland [16]. The P, K, Ca and Na levels reported in this study were lower than the levels reported in different Kenyan markets [15] but Fe (in the four species) and Mg levels in *Solanum scabrum* were similar to the findings in Kenya [15] and East African ALVs [29]. However, the P, K, Zn and Fe levels in *Amaranthus hybridus* were similar to the levels reported in Nigeria [30]. Similarly, the Fe levels in amaranths reported by

Table 2. Levels of Fe, Zn, Mn and Na (mg/100 g DW) in selected ALVs at different harvesting stages, RDAs and recommended intake amounts at Busia site

Nutrient	**Vegetable species**					**M. stage**
	H. stage (wks)	***V. unguiculata***	***A. hybridus***	***C. gynandra***	***S. scabrum***	
Fe	4	9.413	9.079	15.304	9.100	10.724
	6	10.022	10.562	15.894	9.248	11.432
	8	10.206	10.929	16.556	9.346	11.759
	10	10.454	11.406	15.499	9.436	11.699
	Mean species	10.024	10.494	15.813	9.282	
	RDA (mg)			18.000		
	AVC *	0.179 *	0.172 *	0.114*	0.193 *	
	CV (%)			5.40		
	LSD (P≤0.05)			0.799	0.799	
Zn	4	1.554	2.065	1.531	1.795	1.736
	6	1.715	2.266	1.800	1.963	1.936
	8	1.615	2.045	1.382	1.675	1.679
	10	1.368	1.922	1.032	1.624	1.486
	Mean species	1.563	2.075	1.436	1.764	
	RDA (mg)			9.000		
	AVC*	0.514 *	0.386*	0.579 *	0.455 *	
	CV (%)			3.78		
	LSD (P≤0.05)			0.084	0.084	
	Interaction			0.119		
Mn	4	0.018	0.055	0.026	0.028	0.032
	6	0.029	0.044	0.021	0.026	0.030
	8	0.038	0.045	0.031	0.016	0.032
	10	0.041	0.039	0.024	0.025	0.032
	Mean species	0.031	0.046	0.025	0.024	
	RDA (mg)			1.800		
	AVC *	4.573 *	2.885 *	5.199 *	5.742 *	
	CV (%)			22.91		
	LSD (P≤0.05)			0.009	NS	
	Interaction			0.013		
Na	4	25.621	195.150	13.483	64.047	74.621
	6	25.593	152.557	13.593	63.673	63.854
	8	24.697	151.447	13.463	64.757	63.591
	10	25.783	156.537	13.290	64.470	65.020
	Mean species	25.470	163.923	13.457	64.237	
	RDA (mg)			2300.000		
	AVC *	9.048 *	1.433 *	1.709 *	3.580 *	
	CV (%)			26.93		
	LSD (P≤0.05)			16.606	NS	

AVC, Amount of vegetables (kg) to be consumed daily by a healthy adult to supply RDA*

FAO [29] were similar to the findings of this study but much lower in *Cleome gynandra*, *Solanum scabrum* and *Vigna unguiculata*, while the Fe levels in *Solanum scabrum*, *Vigna unguiculata* and *Amaranths* were lower than the levels reported in Maseno [26] and Ghana [31]. The leaf N content in *Cleome gynandra* [32] and Mn levels in Ghana [33] were higher than the levels reported in this study.

The observed differences could be associated with fertilizer application in the previous studies and the amount of nutrients which was already in the soil before planting [34]. Fertilizers such as NPK can increase the level of N, P and K in the soil and consequently the level of nutrients in plant tissues. The observed differences may also be due to agronomic practices, climatic conditions during growth and genetic factors such as differences in cultivars [35].

The ALVs are significant contributors of Fe, Mg and Zn. The Fe (in the four species), Mg in *Solanum scabrum* and Zn levels in *Amaranthus hybridus* were above the RDA. Consumption of 179 g, 172 g, 114 g and 193 g DW of *Vigna unguiculata*, *Amaranthus hybridus*, *Cleome gynandra* and *Solanum scabrum*, respectively is recommended to supply RDAs of Fe, while consumption of 172 g, 386 g and 592 g DW of *Amaranthus hybridus* is recommended to supply RDAs of Fe, Zn and Ca respectively and 87 g of

Solanum scabrum to supply RDA of Mg. The mineral composition of the leaves unravels a high concentration of Fe, Mg and Zn. Children, women of reproductive age and pregnant women are most vulnerable to micronutrient deficiency and anemia [36]. Hence, they need food with high iron content. When these green leafy vegetables with enough iron content are taken in dishes, there is no need for iron supplements. There is a risk of iron toxicity when iron supplements are over-dosed which results in damage to liver and pancreas, and even sudden death in young children [37] Consumption of *Amaranthus hybridus*, *Vigna unguiculata*, *Cleome gynandra* and *Solanum scabrum* is recommended to alleviate Fe deficiency.

People with HIV have the highest levels of zinc deficiency [38]. Although the rate of new HIV infection has decreased, the total number of people living with HIV continues to rise [39], and sub-Saharan Africa still bears an inordinate share of the global HIV burden. Promoting production and consumption of *Amaranthus hybridus* high in Zn will help to alleviate health problems associated with Zn. Magnesium plays an important role in the regulation of blood sugar levels and blood pressure. It is necessary for the transmission of nerve impulses, which affects contraction and relaxation of muscles [40]. Spontaneous deficiency of Mg in humans is unlikely. However, its prolonged deficiency causes neurological disturbances [41]. Adequate consumption of *Solanum scabrum* will reduce Mg deficiency.

The ALVs are not significant contributors of P, K, Mn and Na as consumption of more than half a kilogram is recommended to supply RDAs of such nutrients. There is need to find alternative ALVs for the supply of P, K and Mn. The low levels of sodium in the ALVs lends support to earlier findings that vegetables can help prevent or control hypertension and reduce the subsequent risk of stroke and heart diseases [42].

3.2 Variation in Levels of Nutrients in Selected ALVs in Busia with Respect to Stage of Harvesting

Different nutrients reached their highest levels (except P and Zn) at different harvesting stages in all species. The levels of N, P, K, Ca, and Zn in ALVs significantly ($P \leq 0.05$) increased from 4 to 6 WAE then decreased from 6 to 10 WAE, Fe levels increased (from 4 to 6 WAE) while Mg levels increased (from 4 to 8 WAE) then decreased from 8 to 10 WAE. The Na and Mn levels did not vary significantly ($P \leq 0.05$) with harvesting stage (Tables 1 and 2).

Similar variations had been reported in Nairobi, Kenya [32], Zimbabwe [43] and South Africa [44]. Highest levels of Ca and Zn in *Amaranthus cruentus* were attained at 3 WAE, K at 6 WAE and P at 4 WAE in Zimbabwe [43] and South Africa [44]. However, highest levels of Na were reported at 6 WAE in Zimbabwe [43] and 7 WAE in South Africa [44]. The levels of N significantly ($P \leq 0.05$) increased from 4 to 6 WAE contrary to the results reported in spider plant in Nairobi, Kenya [32]. Differences at the stage at which highest levels of nutrients were attained in the current study and the previous studies could be attributed to variations in the initial soil nutrient levels, agronomic practices, and environmental conditions of the study sites.

The uptake rate of many nutrients depend on the nutrients demand for growth [45,46] determined by the role the nutrients play in plant growth. Most of the functions of Ca and P are as structural components of macromolecules, K is required for cell expansion, protein synthesis and in stomatal regulation, while Mg is the central atom in the chlorophyll molecule.

The uptake of N, P, K, Ca, Mg and Zn was highest during the vegetative stage when roots were actively growing than in reproductive stages [47]. This explains the significant increase in the levels of N, P, K, Ca, Mg, and Zn from 4 to 6 WAE. During the reproductive stage, nutrients are partitioned towards the reproductive organs [48]. For example, developing fruits are stronger sinks for photoassimilates [49] and the relative increase in the proportion of structural material (cell walls and lignin) and of storage compounds (e.g starch) in the dry matter. In addition, retranslocation of nutrients N, P, K and in the form of amino acids from shoots to roots in the absence of soil nutrient replenishment and a decrease in demand for nutrients for new growth as plants age accounts for the decrease in the levels of nutrients in ALVs with increasing plant age. The results of this study are in agreement with findings of Marschner [50].

The non-significant increase in levels of Mn and Na in ALVs with increasing plant age may partly be attributed to the role the nutrients play in plant growth. Manganese plays an important role in redox processes and is required in very small amounts while Na is a beneficial element required for osmotic adjustment [51]. In addition, its uptake is concentration dependent with no specific binding sites in the plasma membrane [52].

Harvesting of ALVs to meet the requirement of a particular nutrient should be done at the stage they attain their highest levels for that particular nutrient. If they are grown to meet the nutritional requirement for different nutrients (N, P, K, Ca, Mg, Fe, Zn, Mn and Na) the average harvesting stage from 4 to 7 WAE for *Vigna unguiculata* and from 4 to 5 WAE for *Amaranthus hybridus, Cleome gynandra* and *Solanum scabrum* should be considered as the most appropriate. However, harvesting the four species from 4 to 6 WAE is recommended for the supply of P and Zn.

There were significant (P≤0.05) interactions between species and harvesting stages for N, Ca, Mg, Zn and Mn, suggesting the response patterns were different in each species and harvesting stages. This implies that variations in the levels of these nutrients at different harvesting stages did not follow similar patterns in different species. This can be attributed to the differences in genetic constitution of different species and thus in the demand for each nutrient [49].

3.3 Method Validation Parameters

3.3.1 Linearity

Linearity was evaluated through graphical representation of the measured absorbance at wavelengths of 285.2 nm, 279.5 nm 248.3 nm, and 213.9 nm of Mg, Mn, Fe, and Zn, respectively versus concentration in mg/L of standard solutions. The regression line was linear in the prediction interval for each of the above metals.

3.3.2 Precision

3.3.2.1 Repeatability (intra-day)

Repeatability was studied by calculating the relative standard deviation (RSD) for four determinations of the concentration of Mg, Mn, Fe, and Zn in mg/L, performed on the same day with an interval of an hour under the same experimental and laboratory conditions. The experimental data with mean RSD of 3.7, 15.0, 5.1 and 7.2 for Mg, Mn, Fe and Zn respectively showed that the method is precise.

3.3.2.2 Intermediate precision (inter-day)

The intermediate precision was assessed by analyzing two working standard solutions on three different days. The obtained RSD values were 3.6, 14.8, 5.3 and 7.0 % for Mg, Mn, Fe and Zn respectively which showed that the method is precise.

3.3.3 Accuracy

Accuracy studies were done using the spiking technique [25] as certified reference materials (CRM) were not available. The known concentration of standard solutions of Mg, Mn, Fe and Zn were added to leaf samples and the resulting spiked samples were measured, calculated and compared to the known values of standard solutions of Mg, Mn, Fe and Zn added. All measurements were performed in three replicates with three different concentrations. The recovery values ranged from 75.5-109.3%. A recovery range of 75.0-110.0% is acceptable for concentrations of 1-100 mg/L [25].

4. CONCLUSION

The ALVs are significant contributors of Fe, Mg and Zn. These vegetables can be consumed together with starchy staples and help to alleviate some nutrient deficiencies. Different nutrients reach their highest levels at different harvesting stages (except P and Zn). Harvesting of ALVs should be done at the stage they reach their highest levels for that particular nutrient and if ALVs are grown to meet the nutritional requirement for different minerals (P, K, Ca, Mg, Fe, Zn, Mn and Na) the average harvesting stage should be considered as the most appropriate.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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