

Full Length Research Paper

Vitamin C, iron and zinc levels of selected African green leafy vegetables at different stages of maturity

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Nutrient level at harvest of vegetables is an important aspect in the efforts to combat food and nutrition insecurity in developing countries. However information on the nutrient levels at different maturity stages of most indigenous African green leafy vegetables is scanty. This study was undertaken to determine the levels of vitamin C, iron and zinc in *Amaranthus cruentus*, *Cleome gynandra* and *Solanum villosum* at different stages of maturity. The vegetables were planted on plots and harvested at 21, 28 and 35 days. At each stage, about 500 to 600 g of the edible part was harvested and standard chemical analyses procedures were followed to determine the levels of vitamin C, iron and zinc. Vitamin C increased significantly ($p < 0.05$) with maturity in all vegetables except *S. villosum* Nduruma BG 16 which had similar values for stage I and II. Vitamin C content was highest (163.4 ± 2.3 mg/100 g) in *C. gynandra* stage III and lowest (27.7 ± 3.9 mg/100 g) in *S. villosum* SS 49 stage I. Iron content increased significantly ($p < 0.05$) at all maturity stages. *Amaranthus cruentus* Madiira Ex zim had the highest iron concentration (99.9 ± 3.7 mg/100 g) while *S. villosum* Olevolosi SS 49 had the lowest value (23.1 ± 1.5 mg/100 g). Zinc content decreased with plant age, highest values (7.8 ± 0.4 mg/100 g) were observed in *C. gynandra* at the first stage of maturity which was reduced to about half at the third stage of maturity (3.9 ± 0.0 mg/100 g). There were marked differences in nutrient content among the various varieties and cultivars involved in this investigation. Iron and vitamin C concentrations increased with plant age whereas levels of zinc decreased with plant maturity. Consuming the different vegetables varieties would be the best approach to get all the essential nutrients in adequate amounts. Further studies are needed to investigate organoleptic acceptability of these vegetables at the different stages of maturity.

Key words: Iron, zinc, vitamin C, maturity stage, green leafy vegetables.

INTRODUCTION

Indigenous African Green Leafy Vegetables (GLVs) are valuable sources of nutrients especially minerals and vitamins (Uusiku et al., 2010). They also contain non-

nutrient bioactive phytochemicals that have been linked to protection against cardiovascular and other degenerative diseases and play an important role in

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income generation and subsistence for most households (Moyo et al., 2013; Schippers, 2000). In Tropical Africa where the daily diet is dominated by starchy staples, indigenous GLVs are the cheapest and most readily available sources of important nutrients. They are often easier to grow, resistant to pests and diseases and hence do not require intensive management (Dzomeku et al., 2011; Moyo et al., 2013; Cordeiro, 2013). In Tanzania and many other African countries, consumption of vegetables including indigenous ones, differ according to season. It was reported that consumption of vegetables among four regions in the rural households in Tanzania varied from once to several times per week (Kinabo et al., 2016; Weinberger and Msuya, 2004); and about 64% of the households consumed vegetables at least three times per week in Morogoro, Kilosa and Gairo districts (Kinabo et al., 2016).

Studies show that among other nutrients, GLVs are generally rich in vitamin C, iron and zinc (Uusiku et al., 2010; Weinberger and Msuya, 2004). Vitamin C has numerous metabolic functions that are largely dependent on its reducing properties (Sen et al., 2014). It is required for the maintenance of healthy skin, gums and blood vessels. It is also known to have many biological functions including collagen formation, absorption of inorganic iron, and it is an antioxidant, that reportedly reduces the risk of arteriosclerosis, cardiovascular diseases and some forms of cancers (Amanabo et al., 2011; Nyonje et al., 2014). Iron is a mineral found in every cell of the body. It is considered an essential mineral element because it is needed to make part of blood cells (Abbaspour et al., 2014). Iron deficiency is the main cause of anemia in developing world, and in Tanzania, it affects mostly pregnant women and children below five years of age (Gautam et al., 2008; TDHS-MIS, 2015-2016). Zinc is crucial for normal development and function of cells mediating innate immunity cells and therefore its deficiency can adversely affect the growth and function of immune cells leading to impaired immune system (Roth et al., 2008).

Micronutrient deficiencies can be reduced by educating communities to increase production and consumption of indigenous and traditional dark green leafy vegetables especially in resource poor communities (Aphane et al., 2002; Ismail and Fun, 2003). Green leafy vegetables are harvested from crop fields at different stages of plant growth. For most of green leafy vegetables there is a preferred stage of plant development when flavor and palatability are favorable for human consumption. Moreover, studies have indicated that levels of nutrients and toxic substances in vegetable are influenced by stages of plant development (Khader and Rama, 2003; Modi et al., 2006). Most studies on indigenous vegetables concentrated on nutrient content and authors reported variations in nutrient content of crops depending on cultivar (Singh et al., 2001). However, there is limited information on their nutrient contents when harvested at

various maturity stages. In view of this, the current study aimed to establish the content of vitamin C, iron and zinc at different maturity stages in amaranthus (*A. cruentus*), African nightshade (*S. villosum*) and spider plant (*C. gynandra*) so as to determine best harvesting time for optimal nutritional benefits to the consumer.

MATERIALS AND METHODS

Description of the vegetables under study

Five varieties of indigenous green leafy vegetables namely *A. cruentus* (Madiira 1 EX Zim and Madiira II AM 38), *S. villosum* (Nduruma BG 16 and Olevolosi SS49) and *C. gynandra* were used in the study. Seeds of these vegetables were purchased from the Horticulture Training Institute of Tengeru (HORTI - Tengeru), Arusha and from the Horticulture Unit of Sokoine University of Agriculture (SUA), Morogoro, Tanzania. Comparatively, these vegetables were chosen for this research because they are indigenous vegetables that are preferred and consumed by many communities in Tanzania.

Planting and management

The seeds were sown at the crop museum at SUA. Before sowing the seeds, the land was cleared, ploughed, harrowed and fertilized using poultry manure. Seeds of the five vegetables varieties were sown in three replications. The area was divided into three rows for the three replications and each row was divided into five beds, one for each vegetable variety. The seeds were sown in 30 cm inter and intra spacing. The density of the plants was 45 plants per bed. The vegetables were watered twice daily (mornings and evenings) and weeding was done weekly.

Harvesting, collection of samples and sample preparation

The three maturity stages were 21, 28 and 35 days from sowing the seeds (named as maturity stages I, II and III, respectively). At each stage, about 500 to 600 g of the edible parts (leaves and young stems) were harvested by uprooting the whole plant and picking the edible leaves and shoots. The picked leaves were placed in dark colored polythene bags and transported to the food laboratory for chemical analyses. The edible portions of the samples were washed with distilled water. Excess water was left to drain off before being homogenized by cutting into small pieces (about 2 mm) using domestic sharp knife and cutting board. About 2 g of each fresh sample was weighed for vitamin C analysis. The remaining portions were then oven dried for 24 h at 60°C. The dried samples were removed from the oven and immediately ground using motor and pestle into a fine powder. The powdered samples were placed in transparent polythene bags, labeled and stored for subsequent chemical analyses.

Analytical procedure

Vitamin C determination

The vitamin C concentration was determined using AOAC procedure (AOAC, 1995) where 2 g of homogenized samples was ground using motor and pestle and vitamin C compounds were extracted using metaphosphoric acid (AOAC, 1995). The pH of the extracts was adjusted to about 1.2. The reducing capacity was then

Table 1. Vitamin C contents (mg/100 g) in different vegetables at different stages of maturity.

Vegetable	Stage of Maturation		
	Stage I (21 Days)	Stage II (28 Days)	Stage III (35 Days)
<i>Solanum villosum</i> (Nduruma BG 16)	49.6±0.6 ^c	49.2±1.6 ^c	115.5±1.6 ^g
<i>Solanum villosum</i> (Olevolosi SS 49)	27.7±3.9 ^a	41.5±1.3 ^b	104.7±3.2 ^f
<i>Cleome gynandra</i> (Spider Plant)	32.4±2.9 ^a	63.0±4.5 ^{de}	163.4±2.3 ⁱ
<i>Amaranthus cruentus</i> (Madiira Ex Zim)	51.0±2.3 ^c	113.5±3.6 ^g	132.0±3.5 ^h
<i>Amaranthus cruentus</i> (Madiira AM 38)	69.2±4.6 ^e	57.1±2.0 ^{cd}	102.0±3.4 ^f

Data presented as arithmetic means ± SD (n = 3). Means within the vegetable variety in row/column with different superscript letters are significantly different at p<0.05.

filtered using No. 1 Whatman filter papers to obtain clear extract and then measured by titrating with standardized 2,6-dichlorophenolindophenol (DCIP). In this oxidation-reduction reaction, ascorbic acid in the extract was oxidized to L-dihydroascorbic acid (DHAA) and the indophenols dye was reduced to a colourless compound. End point of the titration was detected when excess of the unreduced dye gave a rose pink colour in acid solution. The L-dihydroascorbic acid was calculated using the formula below:

$$R = \frac{C \times (V_1 - B) \times V_2}{S_a \times V_3} \times 100$$

Where, R = Concentration of ascorbic acid in mg/100g of the sample; C = Concentration of 2, 6-dichlorophenolindophenol dye; V₁ = Volume of DCIP used for the sample; B = Volume of DCIP used for the blank; V₂ = Total extraction volume; S_a = Sample weight taken and V₃ = Sample extract analysed.

Determination of iron and zinc

Iron and zinc content were determined by following the procedure described by Eslami et al. (2007) where one gram of the sample was incinerated into ash, then the ash obtained was dissolved in 6 M HCl acid. The digest was then filtered using Ashless Whatman filter paper No.1. The clear solution was subjected to atomic absorption spectrophotometer (AAS) (Shmadzu UNICAM 919 - Cambridge, UK) using the hollow cathode lamps set at 248.3 and 213.9 nm, respectively. The AAS was calibrated using standard solutions specific for each mineral element.

Statistical analysis

The data obtained was analysed using Statistical Product and Service Solutions (SPSS 16.0) software (Version 20). Analysis of variance (ANOVA) was computed at 5% level of significance to determine any significant difference in the levels of nutrients between the vegetable varieties and the three maturity stages (21, 28 and 35 days).

RESULTS

Effect of maturity stage on vitamin C contents

The results of vitamin C content are presented in Table 1. At the first harvest stage, the highest amount of vitamin C

was observed in Madiira AM 38, which was 69.2 ± 4.6 mg/100 g. Madiira EX Zim had highest vitamin C content (113.5 ± 3.6 mg/100 g) at the second stage and Spider plant had highest amount of vitamin C content (163.4 ± 2.3 mg/100 g) at maturity stage III. The lowest vitamin C scores were observed in sample Olevolosi SS 49 (27.7 ± 3.9 mg/100 g and 41.5 ± 1.3 mg/100 g) at maturity stage I and II, respectively and in Madiira AM 38 (102.0 ± 3.4 mg/100 g) and in Olevolosi SS 49 (104.7 ± 3.2 mg/100 g) at maturity stage III. Similar vitamin C content was observed in Nduruma BG 16 at stages I and II and there was a significant decrease observed in Madiira AM 38. In addition, there was a significant difference in vitamin C content among the two cultivars of *S. villosum* and *A. cruentus* at all maturity stages. The highest amount of vitamin C was observed at stage III of harvesting for all the studied vegetables.

Effect of maturity stage on iron content

Iron content of the five cultivars from three types of vegetables is presented in Table 2. Sample Madiira EX Zim scored the highest iron concentrations (73.3 ± 0.3, 84.6 ± 0.6 and 99.9.0 ± 3.7 mg/100 g), at all three maturity stages I, II and III, respectively while samples Olevolosi SS 49, Madiira AM 38 and Spider plant had the lowest iron concentrations (23.1 ± 1.5 mg/100 g, 31.3 ± 0.8 mg/100 g and 48.6 ± 3.3 mg/100 g) at maturity stages I, II and III, respectively. In all five vegetable, varieties iron content increased continuously from stage I to stage III. Generally, the increase from maturity stage I to stage II was slightly lower compared to that of stage II to stage III. The two cultivars of *A. cruentus*, Madiira EX Zim had higher iron content compared to Madiira AM 38. Likewise, there was a difference in iron concentration in the two cultivars of *S. villosum* (Nduruma BG 16 and Olevolosi SS 49) but they had same concentration at the third stage of maturity.

Effect of maturity stage on zinc content

The contents of zinc across different stages decreased

Table 2. Iron contents (mg/100 g DM) in different stages of maturity of different vegetables.

Vegetable	Stage of Maturation		
	Stage I (21 Days)	Stage II (28 Days)	Stage III (35 Days)
<i>Solanum villosum</i> (Nduruma BG 16)	27.4±0.8 ^b	35.8±0.5 ^d	85.3±0.8 ⁱ
<i>Solanum villosum</i> (Olevolosi SS 49)	23.1±1.5 ^a	42.4±0.4 ^e	85.1. ±0.6 ⁱ
<i>Cleome gynandra</i> (Spider Plant)	36.3±3.3 ^d	41.1±3.6 ^e	48.6±3.3 ^f
<i>Amaranthus cruentus</i> (Madiira Ex Zim)	73.3±0.3 ^h	84.6±0.6 ⁱ	99.9±3.7 ^j
<i>Amaranthus cruentus</i> (Madiira AM 38)	29.2±0.6 ^{bc}	31.3±0.8 ^c	52.6±1.3 ^g

Data presented as arithmetic means ± SD (n = 3). Means within the vegetable variety in row/column with different superscript letters are significantly different at p<0.05.

Table 3. Zinc content in different vegetable varieties at different maturity stages.

Vegetable	Stage of Maturation		
	Stage I (21 Days)	Stage II (28 Days)	Stage III (35 Days)
<i>Solanum villosum</i> (Nduruma BG 16)	7.2±1.8 ^f	5.4±0.0 ^c	4.4±0.9 ^b
<i>Solanum villosum</i> (Olevolosi SS 49)	5.8±0.9 ^d	5.4±0.4 ^c	4.5±0.9 ^b
<i>Cleome gynandra</i> (Spider Plant)	7.8±0.4 ^g	5.2±0.9 ^c	3.9±0.0 ^a
<i>Amaranthus cruentus</i> (Madiira Ex Zim)	4.6±0.9 ^b	4.6±0.8 ^b	3.7±0.0 ^a
<i>Amaranthus cruentus</i> (Madiira AM 38)	6.2±0.9 ^e	5.8±0.9 ^d	4.0±0.9 ^a

Data presented as arithmetic means ± SD (n = 3). Means within the vegetable variety in row/column with different superscript letters are significantly different at p<0.05.

with plant maturity (Table 3). The highest zinc contents were 7.8 ± 0.4 mg/100 g in sample *C. gynandra* at maturity stages I, 5.8 ± 0.9 mg/100 g in Madiira AM 38 at maturity stage II and 4.5 ± 0.9 mg/100 g in Olevolosi SS 49 at maturity stage III. The lowest zinc contents were in sample Madiira EX Zim at all three maturity stages. In all five varieties, zinc content decreased with plant age with the lowest zinc content at stage III of maturity. Significant differences in zinc content were also observed among the two varieties of *S. villosum*, which is Olevolosi SS 49 and Nduruma BG, 16 only in the first maturity stage but no difference was observed in the second and third maturity stages. There were also differences in the two varieties of amaranthus Madiira EX Zim and Madiira AM 38 at the first and second stages of maturity but zinc content was not significantly different at stage III of maturity.

DISCUSSION

In this study, variation in vitamin C, iron and zinc contents in vegetables harvested at different stages of maturity was investigated. Variation was observed in vitamin C content across the cultivars. Vitamin C content was similar with what was reported by other researchers for vegetables grown in Africa (Ayua et al., 2016). There was

relatively higher vitamin C content at the third stage of maturity in all studied vegetables. *C. gynandra* had the highest vitamin C content at 35 days. These results are in agreement with the work of Amanabo et al. (2011) who found that vitamin C content increased from market maturity (94.60 ± 5.60 mg/100 g) to reproductive stage (160.50 ± 7.10 mg/100 g) in *A. cruentus* (Amanabo et al., 2011). Some researchers reported that vitamin C concentration is highest in mature leaves with fully developed chloroplasts (Khan et al., 2011) and regardless of fertilizers used, vitamin C content increased with maturity in spider plant and black nightshade grown in Kenya (Ayua et al., 2016). This suggest that harvesting green leafy vegetables at advanced age potentially provides the greatest concentrations of vitamin C. Apart from maturity stage and variety type, other factors that affect vitamin C concentration in vegetables include postharvest handling conditions (time lapse between harvest to consumption and extent of physical damage), processing methods such as blanching, cooking, drying and canning (Kader and Seung, 2000). This implies that even when vegetables are harvested at the maturity stages when vitamin C content is highest, care should be taken during handling, processing and cooking to ensure maximum intake of this important vitamin.

There was variation in iron content across the varieties

and across the cultivars. *Amaranthus cruentus* (Madiira Ex Zim) had the highest iron content. Iron content in the studied vegetables was higher than other indigenous African vegetables as reported by other researchers (Kamga et al., 2013; Lyimo et al., 2003). In a recent study, variation in nutrient content was reported, specifically zinc and iron contents in vegetables grown from different districts in Tanzania (Amuri et al., 2017). An increase in iron content was observed in all varieties of the studied vegetables across the harvesting stages. The increasing trend of iron suggests that the mineral may be indissociable ion and accumulates as age increase. These findings were similar to those of Flyman and Afolayan who reported increase in iron content at each stage in *Vigna unguiculata* as the plant matured from 21 to 57 days after sowing the seeds (Flyman and Afolayan, 2008). Modi and colleagues found that iron concentration in *A. cruentus* increased significantly ($p < 0.05$) from 40 ± 2.5 mg/100 g at 20 days after sowing to 58 ± 2.1 mg/100 g at 40 days after sowing (Modi et al., 2006). It was reported in other studies that iron concentration decreased in green leafy vegetables as they mature towards fruiting (Khader and Rama, 2003). The possible reason for the increasing trend observed in this study is that the 35 days that were set as maximum for harvesting the leaves was still early for the vegetables to start flowering. Further studies to investigate later harvesting stages on nutrients may be necessary.

Zinc content was significantly higher in *C. gynandra* at the first stage of maturity. The amount was higher than what was reported in other commonly consumed vegetables like sweet potato leaves, lettuce, jute mallow, and kales (Kamga et al., 2013). The observed variations could be attributed by harvesting stage or nature of the soil where the vegetables were grown. The differences in zinc content between them may be attributed to differences in absorption efficiencies between the varieties. Different plant varieties have got different root system and hence different efficiency in exploiting and taking up nutrients from the soil. There was a slight but not significant decrease in zinc concentration at all stages of maturity. The decreasing zinc content may be attributed to diversion of this mineral towards plant development. During fruit initiation and development, some metabolites for cellular synthesis and growth substances are translocated from the leaves, stems, and roots to the developing fruits (Khader and Rama, 2003). Moreover, Lanyasunya et al. (2007) observed that the rapid uptake of mineral by plants during early growth and the gradual dilution that occurs as plant matures would have been responsible for the decrease in some of the mineral content during fruiting. Zinc content decreased with increasing maturity stages in *Amaranthus* and *Spinaces* species (Khader and Rama, 1998).

Flyman and Afolayan (2008) found that zinc content was higher at 21 days (389.08 ± 5.08 mg/100 kg) and decreased continuously to 161.53 ± 3.59 mg/100 kg at 28

days and decreased further to 85.83 ± 1.30 mg/100 g, at 35 days after sowing. Amanabo et al. (2011) observed that zinc content in *A. cruentus* decreased from 0.08 ± 0.01 mg/100 g dry weight at market maturity (vegetative) stage to 0.05 ± 0.01 mg/100 g dry weight at heading (reproductive) stage. Generally, the variation in nutrient content across the cultivars and across different vegetables can be explained by their genetic variations (Marles, 2017).

Associations of soil nutrient and concentration in vegetables

Nutrient concentrations in foods depend largely on the quality of soil in which the crops are grown. Soil fertility management practices were reported to affect zinc and iron concentration in vegetables and there was a direct association between soil chemical properties and vegetable mineral concentrations (Amuri et al., 2017). Some studies noted that soil pH may affect zinc content in vegetables and in some study sites zinc in the soil was negatively correlated with zinc content in vegetables (Amuri et al., 2017; Harter, 1983). The authors noted that levels of zinc in vegetables varied due to soil management and not necessarily zinc concentration in the soil (Amuri et al., 2017). A study in Bangladesh found that elemental concentrations vegetables varied in different samples and which reflect the difference in uptake capabilities and their further translocation to the edible portion of the plants (Jolly et al., 2013). They also found variation according to location which could be related to soil properties and soil nutrient management. Other studies found that increased zinc content in the soil increased its concentration in *Amaranthus* leaves (Ondo et al., 2012). Although soil nature and other environmental factors contribute to the mineral deposition in plants, it was not the case in this study since all vegetables were grown in similar soil type.

Conclusions

There were marked differences in nutrient content among the various varieties and cultivars involved in this investigation. There were variations in vitamin C content in all vegetables investigated where the highest amount was in Madiira AM 38 in stage I, in Madiira Ex Zim in stage II and in *C. gynandra* in stage III. Madiira EX Zim was the best in iron content at all maturity stages. *C. gynandra* contained highest levels of zinc at stage I which decreased to about half at stage III. Iron and vitamin C concentrations increased with plant age implying that the vegetables should be harvested at advanced plant age. On the contrary, levels of zinc decreased with plant maturity implying that vegetables should be harvested at early maturity stages preferably the third week after

sowing. This suggests that consuming different vegetables varieties in combination would be the best approach to get all the essential nutrients in adequate amounts needed to maintain normal body functions. It would be interesting to investigate the consumer acceptance in consuming vegetables harvested at different stages of maturity. While considering nutrient content as an important factor, palatability and other sensory attributes and hence consumer acceptance should not be ignored.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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