Vol. 12(11) pp. 316-322, November 2018
DOI: 10.5897/AJFS2018.1735
Article Number: 4573A3C58862
ISSN: 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

Full Length Research Paper

Oxalate, phytate and nitrate content in African nightshade, spider plant and amaranths at different stages of maturity

Mwanri A. W.[1*], Mamboleo T. F.[2], Msuya J. M.[1] and Gowele V. F.[1]

[1]Department of Food Technology, Nutrition and Consumer Sciences, P.O Box 3006, Morogoro. Tanzania.
[2]Tanzania Food and Nutrition Centre, Dar es Salaam. Tanzania.

Received 23 June, 2018; Accepted 3 September 2018

African indigenous green leafy vegetables play important role in income generation and subsistence; they are the cheapest and most readily available sources of important minerals and vitamins. On the other hand, they contain anti-nutritional factors that reduce availability of vital nutrients. This study was conducted to determine oxalates, phytates and nitrates content in commonly consumed *Amaranths cruentus*, Spider plant (*Cleome gynandra)* and African night shade (*Solanum villosum)* at 21, 28 and 35 days age of the plant harvest. Vegetables were planted on plots and harvested at 21, 28 and 35 days. At each stage, about 600±2 g of the edible part was harvested and standard chemical analyses procedures were followed to determine oxalate, phytate and nitrate contents. Using Statistical Product and Service Solutions (SPSS 20) data were analysed and results presented as simple means, ranges and standard deviations. Analysis of variance (ANOVA) with 5% level of significance was done to determine differences in the levels of nutrients between the vegetable varieties and three maturity stages. African nightshade Nduruma BG 16 had lowest oxalate concentrations (28.7 ± 0.1 mg/100 g) at maturity stage I while African nightshade Olevolosi SS 49 had the highest value (60.9 ± 0.9 mg/100 g) at maturity stage III. There was no particular trend for phytates in all maturity stages and in all varieties but amaranths Madiira AM 38 had exceptionally the highest values (0.7 ± 0.0 mg/100 g) at stage III of maturity. The highest nitrate content was 85.6 ± 1.8 mg/100 g in Olevolosi SS 49 at maturity stage I, whereas the lowest value was 45.3 ± 1.3 mg/100 g in amaranths Madiira AM 38 at stage III. There was a slight variation in antinutrients composition of the studied vegetables and the composition was generally very low.

Key words: Antinutrients, indigenous vegetables, maturity stage, Tanzania.

INTRODUCTION

African indigenous green leafy vegetables (GLV) play important role in income generation and subsistence; they are the cheapest and most readily available sources of important minerals and vitamins (Schippers, 2000).

*Corresponding author. E-mail: akwmwanri@sua.ac.tz or akwmwanri@hotmail.com. Tel: +255 754 336650.

They are important commodities for poor households because their prices are relatively affordable when compared with other food items. In Tropical Africa where the daily diet is dominated by starchy staples, indigenous GLVs are the cheapest and most readily available sources of important minerals and vitamins (Dzomeku et al., 2011). In addition, indigenous vegetables have the added advantage of possessing other desirable traits such as aroma and flavor which make them quite acceptable in the local communities. These vegetables are often easier to grow, resistant to pests and diseases and do not require intensive management (Moyo et al., 2013). Furthermore, they are a rich source of vitamins and other components that contribute to antioxidant activity in the diet and contain non-nutrient bioactive phytochemicals that have been linked to protection against cardiovascular and other degenerative diseases (Moyo et al., 2013). On the other hand, green leafy vegetables contain antinutrients that compromise digestion and absorption of vital nutrients (Uusiku et al., 2010). These components occur in combinations and may act synergistically or may have contraindicating effects with each other. Understanding the role and contents of these components is crucial for managing micronutrient deficiencies and chronic diseases of lifestyle.

Green leafy vegetables are harvested by rural communities from crop fields at different stages of plant growth (Modi et al., 2006). For most of green leafy vegetables, there is a preferred stage of plant development when flavor and palatability are favorable for human consumption. The maturity stage of a conventional vegetable is universally defined, and a crop is normally harvested and consumed at a known stage of plant development, irrespective of environmental conditions for plant growth. Unlike conventional crops such as staple cereals, information about the stage of plant development to define harvest maturity for indigenous green leafy vegetables is scarce. Moreover, studies have indicated that levels of nutrients and toxic substances in vegetables are influenced by stages of plant development (Khader and Rama, 2003).

Anti-nutritional factor is known to interfere with metabolic processes such that growth and bioavailability of nutrients are negatively influenced (Agbaire and Emoyan, 2012). Phytate and oxalates have the ability to form chelates with metallic ions such as Cd, Mg, Zn and Fe to form poorly soluble compounds that are not readily absorbed from the gastrointestinal tract thus decreasing their bioavailability. Ladji et al. (2004) reported that oxalates causes irritation and swelling in the mouth and throat. They further noted that phytate inhibits the functions of some digestive enzymes. Oxalate binds to calcium to form calcium oxalate crystals; these prevent the absorption and utilization of calcium by the body and aiding the formation of kidney stones. Addition of a source of calcium to vegetables containing high levels of soluble oxalate has shown to reduce intestinal available oxalate content in such foods (Radek and Savage, 2008). Phytate is the major phosphorus storage compound in African leafy vegetables (Schlemmer et al., 2009). Although it is an antioxidant, it has been shown to inhibit absorption of minerals. Phytate chelates multivalent metal ions such as calcium, iron and zinc, thus it is a strong inhibitor of iron mediated free radical generation hence the diet high in phytate content reduces bioavailability of zinc and iron. A number of studies indicate that green leafy vegetables contain various amounts of phytic acid (Agbaire, 2012; Agbaire and Emoyan, 2012; Nkafamiya et al., 2010). While some studies reveal that domestic thermal processing methods can significantly reduce phytate content in vegetables (Imaobong et al., 2013; Yada and Sehgal, 2003); other studies report that phytate content increases or remains unchanged when heat processed (Embaby, 2010). Nitrates form part of the essential chemistry of soils and plants. Thus plant roots are able to absorb nitrate directly from the soil. Nitrate contamination in vegetables occurs when crops absorb more than they require for their optimal growth. Nitrate content of vegetables may range from 1 to 10,000 mg/kg (Renseigné et al., 2007). The nitrate content varies with plant species, cultivars of the same species, and even genotypes with different ploidy (Grzebelus and Baranski, 2001; Sunaga et al., 2005). Thus, selection of genotypes that accumulate fewer nitrates may contribute significantly to reduction in the nitrate consumption by humans through vegetables and the subsequent risk of nitrate poisoning. The main concern for the public health is the link between nitrates and stomach cancer due to the fact that nitrates may lead to formation of carcinogenic nitrosamines (Renseigné et al., 2007; Walker, 1990).

Consumption of GLV is recommended as a strategy of preventing and alleviating minerals and vitamin deficiencies in both rural and urban societies, hence it is important that vegetables are harvested and consumed when the levels of micronutrients is at maximum; and when the quantities of anti-nutritional factors is at the minimum. However, there is limited information about the stage of plant development at which antinutrients are at minimum levels with balanced flavor, palatability and other nutrients. The need for exploration of anti-nutritional information in traditional leaf vegetables is significant in overcoming micronutrient deficiencies in order to contribute to health and nutritional security in Africa. It is in this view that the research is designed to determine oxalates, phytates and nitrates content in commonly consumed *Amaranths cruentus*, Spider plant (*Cleome gynandra)* and African night shade (*Solanum villosum)* at 21, 28 and 35 days age of the plant harvest.

MATERIALS AND METHODS

This study was conducted at Sokoine University of Agriculture

(SUA) situated in Morogoro urban district. The district is characterized with temperatures ranging from 16 to 33°C and average annual rainfall ranging from 821 to 1505 mm (URT, 2016). The field sites were the crop experimental plots located at SUA.

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* or African night shade (Nduruma BG 16 and Olevolosi SS49) and Spider plant (C*. gynandra)* were used in the study. Seeds of these vegetables were purchased from Horticulture Training Institute of Tengeru (HORTI – Tengeru) Arusha and from the horticulture unit of Sokoine University of Agriculture (SUA), Morogoro.

Planting, management and collection of samples

The seeds were sown at the crop experimental plots at SUA, Morogoro Tanzania. Before sowing the seed, the land was cleared, ploughed, harrowed and fertilized using poultry manure. It is a common practice for farmers to use poultry manure for vegetable growing in Morogoro urban because it is of low cost and results to relatively more yield. Seeds of the five vegetables varieties were sown on a plot 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.

The three maturity stages were 21, 28 and 35 days from sowing the seeds. At each stage, about 600 ± 2 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 running tap water, drained to remove excess water, cut into small pieces (about 2 mm) using domestic sharp knife and cutting board and homogenized. Samples were oven dried at 60°C until constant weight was obtained.

The dry samples were removed from the oven and immediately ground into a fine powder using motor and pestle. The powdered samples were placed in transparent polythene bags, labeled and stored ready for oxalate, phytate and nitrate analyses.

Determination of oxalate content

The titration method as described by Baker (1952) was followed. 2.0 g of powdered sample was heated in 50 ml distilled water. 0.3 M HCl was then added to the heated sample. The cold filtrate was treated with 3 drops of methyl red indicator and NH_4OH solution before heating the mixture to 100°C. The mixture was left to cool. After cooling, the filtrate was heated further before the addition of 10 cm^3 of 10% $CaCl_2$ solution and allowed to stand overnight. The mixture was filtered by using Whatman paper No. 1. After filtration, the precipitate formed was washed to remove traces of Ca^{2+} before dissolving in H_2SO_4 solution. The solution formed was brought to boil by heating before warm titrating with 0.05 M $KMnO_4$ solution until a faint pink colour persisted for at least 30 s. The oxalate content was then be calculated by taking 1 ml of 0.05 m $KMnO_4$ as equivalent to 2.2 mg oxalate using the following formula:

$$O = T_s \times M_d \times M_o \times 100 / W_s$$

where O = Oxalate concentration in mg/100 g, T_s = Volume of potassium permanganate used for sample, M_d = number of moles of potassium permanganate reacted, M_o = number of moles of oxalate reacted, and W_s = sample weight.

Determination of phytate content

Phytic acid contents of the vegetable samples were determined by method as described by Wheeler and Ferrel (1971). About 0.2 g of each powdered sample was weighed into a 125 ml Erlenmeyer flask. The phytic acid was extracted using 50 ml 3% Trichloroacetic acid (TCA) for 30 min with occasional swirling by hand for 45 min. The suspension was centrifuged and 10 ml aliquot of the supernatant was transferred to a 50 ml conical flask. Four milliliters of $FeCl_3$ solution was added to the aliquot by lowing rapidly from the pipette. The content was heated in a boiling water bath for 45 min. After 30 min, two drops of 3% sodium sulphate were added in 3% TCA extract and continued to be heated. The supernatant was centrifuged for 15 min and decanted. The precipitate was washed twice by dispensing well in 20 to 25 ml of 3% TCA, heated in boiling water bath for 10 min and centrifuged. Washing with water was repeated. The precipitate was dispersed in 27 ml of water and 3 ml of 1.5 N NaOH with mixing. The volume was brought to approximately 30 ml with water and heated in boiling water bath for 30 min. The precipitate was filtered through a moderately retentive paper Whatmann No. 2. The precipitate was washed with 70 ml hot water and the filtrate was discarded. The precipitate was dissolved from the paper with 40 ml 3.2 N HNO_3 into a 100 ml volumetric flask. The filter paper was washed with several portions of water and the washed papers were collected in the same flask taking care not to exceed the 100 ml volume. The flask was cooled at room temperature and diluted to volume with water. A 5 ml aliquot was transferred to another 100 ml volumetric flask and diluted to approximately 70 ml. 20 ml of 1.5 M KSCN was added and diluted to volume and color was read immediately within 1 min at 480 nm. A reagent blank was run with each set of samples.

Phytate content in sample was calculated using the following formula:

$$\text{Phytate content in } \mu g/100 \text{ g sample} = (C \times E / S \times Av) \times 100$$

where C = phytate concentration from standard graph, E = total extraction volume, S = analytical sample taken, and Av = analytical volume

Determination of nitrate content

Determination of nitrate contents was done by using spectrophotometric method as outlined by Gaya and Alimi (2006) where 10 g of each sample was taken into a 250 cm^3 beaker and 2.5 ml of 4% NaOH was added. The content of the beaker was warmed at 80°C for 25 min with occasional shaking. The resulting solution was filtered through a fluted filter paper into 100 cm^3 volumetric flask and made up to the mark. An aliquot of 4 cm^3 was taken into a test tube cooled in ice. 1 cm^3 of 5% Ag_2SO_4 solution was added followed by subsequent addition of 7 cm^3 of 98% H_2SO_4 and 0.1 cm^3 of 5% phenol 25 solutions. The solution was allowed to stand for 20 min while shaking occasionally. The resulting mixture was extracted in 50 cm^3 separating funnel by adding toluene and shaking for 5 to 10 min. The lower aqueous layer was discarded. The organic phase was washed twice with 10 ml of distilled water by shaking for 2 min and each time discarding the aqueous phase. The organic phase was extracted again by shaking for 1 min with 10 cm^3 of 10% Na_2CO_3 solution and collected in a test tube. Absorbance was read at 407 nm. Since 4 cm^3 of the 100 cm^3 filtrate was used for analysis.

The amount of nitrate (mg/g) in the vegetable was calculated by

Table 1. Oxalate content (mg/100 DM) in different vegetables at different stages of maturity.

Vegetable	Stage of Maturation		
	Stage I (21 Days)	Stage II (28 Days)	Stage III (35 Days)
African nightshade (*Solanum villosum)*(Nduruma BG 16)	28.7±0.1^{a}	38.2±1.5^{c}	59.6±0.3^{f}
African nightshade (*Solanum villosum)* (Olevolosi SS 49)	42.9±0.2^{d}	55.9±1.2^{e}	60.9±0.9^{f}
Spider plant *(Cleome gynandra)*	38.4±1.7^{c}	48.3±1.5^{d}	30.5±0.5^{a}
Amaranths cruentus (Madiira Ex Zim)	39.9±0.8^{c}	54.9±0.5^{e}	60.3±2.0^{g}
Amaranths cruentus (Madiira AM 38)	34.6±1.3^{b}	38.1±0.3^{c}	31.1±1.2^{c}

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 2. Phytate content (mg/100 DM) in different vegetables at different stages of maturity.

Vegetable	Stage of Maturation		
	Stage I (21 Days)	Stage II (28 Days)	Stage III (35 Days)
African night shade *(Solanum villosum)* (Nduruma BG 16)	0.04±0.0^{ab}	0.2±0.0^{ab}	0.3±0.1^{cd}
African night shade *(Solanum villosum)* (Olevolosi SS 49)	0.2±0.0^{bc}	0.3±0.0^{cd}	0.4±0.0^{cd}
Spider plant *(Cleome gynandra)*	0.02±0.0^{a}	0.3±0.0^{bcd}	0.3±0.0^{bcd}
Amaranths (Madiira Ex Zim)	0.2±0.0^{bcd}	0.4±0.0^{cd}	0.5±0.0^{d}
Amaranths (Madiira AM 38)	0.02±0.0^{ab}	0.2±0.0^{abc}	0.7±0.0^{e}

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.

the formula:

$$\text{Nitrate} = \frac{C \times 100}{Ws \times 4}$$

where C = concentration of nitrate in the sample as from calibration graph (mg cm^{-3}) and Ws = weight of the sample used (g).

Statistical analysis

The data obtained was analysed using Statistical Product and Service Solutions software (Version 20). Results were presented as simple means, ranges and standard deviations. Analysis of variance (ANOVA) with 5% level of significance was done to determine any significant differences in the levels of nutrients between the vegetable varieties and the three maturity stages (21, 28 and 35 days).

RESULTS

Oxalate content

Table 1 shows the oxalate concentration in the study vegetables at three stages of maturity. Oxalate content increased significantly (p<0.05) with plant age in *S. villosum* (Nduruma BG 16), *S. villosum* (Olevolosi SS 49) and Amaranthus (Madiira Ex Zim). In Spider Plant *(C. gynandra*) and Amaranthus (Madiira AM 38), oxalate content increased from stages I to II and then decreased at stage III. In both cultivars of *S. villosum* (Nduruma BG 16 and Olevolosi SS 49), the changing pattern of oxalate was the same, that is, it increased significantly with plant age and similar oxalate levels at stage III. However, this was not the case in the two cultivars of *A. cruentus* (Madiira AM 38 and Madiira EX Zim) where oxalate content increased continuously with age in Madiira EX Zim but in Madiira AM 38 it increased from stages I to II and then decreased at stage III.

Phytate content

The phytate concentration in all vegetables varieties increased from stages I to III as shown in Table 2; the increase being very high in Amaranths (Madiira AM 38) from stages II to III of maturity. The increase in phytate content in Spider plant (*C. gynandra*) from stages II to III was not significantly different. All varieties had similar phytate contents at the first harvesting stage that is at 21 days.

Nitrate content

In all five varieties, the nitrate content decreased with maturity but the differences were not always statistically different (p<0.05) except in in *S. villosum* (Olevolosi 49). The highest level of nitrate concentration at any maturity stage was observed in *S. villosum* (Olevolosi 49) while

Table 3. Nitrate content (mg/100 DM) in different vegetables at different stages of maturity.

Vegetable	Stage of maturation		
	Stage I (21 Days)	Stage II (28 Days)	Stage III (35 Days)
African night shade *(Solanum villosum)* (Nduruma BG 16)	66.8±1.4^{bc}	64.5±0.8^{b}	63.0±1.4^{b}
African night shade *(Solanum villosum)* (Olevolosi SS 49)	85.6±1.8^{g}	79.9±0.3^{ef}	75.4±1.8^{de}
Spider plant *(Cleome gynandra)*	81.2±1.6^{fg}	78.6±0.8^{f}	64.3±3.7^{b}
Amaranths cruentus (Madiira Ex Zim)	81.4±1.1^{fg}	66.2±1.7^{bc}	63.0±1.7^{b}
Amaranths cruentus (Madiira AM 38)	71.2±0.1^{cd}	66.4±0.6^{cd}	45.3±1.3^{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.

the lowest levels were observed in *A. cruentus* (Madiira AM 38). The nitrate content in *C. gynandra* was significantly different between stages II and III only. Nitrate content was slightly lower in Nduruma BG 16 as compared to Olevolosi SS 49, the difference being significant at all maturity stages (Table 3).

DISCUSSION

This study aimed to determine variation in oxalate, phytates and nitrates in five varieties of indigenous vegetables harvested after 21, 28 and 35 days. Generally, oxalate levels increased with maturity stage but in Spider Plant *(C. gynandra*) and Amaranthus (Madiira AM 38) oxalate content increased from stages I to II and then decreased at stage III. These results resembled the findings reported by Kitchen and Burns (2006) who worked on dark green bloomsdale spinach and found that total oxalate content was maximum at 32 days after planting and decreased subsequently as the plants developed towards the vegetative phase. Musa et al. (2011) also reported an increase in total oxalate content in *A. cruentus* grown in Nigeria from 4.40 ± 0.19 mg/100 g at market maturity stage (vegetative stage) to 5.27 ± 0.24 mg/100 g at heading (reproductive stage). This suggests that harvesting the vegetables after 35 days could have reduced oxalate concentration. Variation was observed in oxalate content across the cultivars. Oxalate content was much lower than reported in other vegetables like sweet potato leaves and amaranths consumed in Tanzania, Kenya and in Nigeria (Musa and Ogbadoyi, 2014; Mwanri et al., 2011; Mziray et al., 2001). Studies show that chemical composition of plants is affected among other things the variety and even the cultivar (Singh et al., 2001). Therefore, the differences observed in oxalate concentration in the vegetables in this study might be attributed to varietal differences as well as the part of plant consumed.

Processing methods such as pressure cooking and open pan cooking can help to reduce oxalate levels significantly. A study by Virginia et al. (2012) on effects of cooking and processing on oxalate content in green leafy vegetables and pulses revealed that blanching, pressure cooking for 10 min and open pan cooking of green leafy vegetables reduced their oxalate concentration from 88.8 mg/100 g fresh samples to 48.4, 57.2 and 60.13 mg/100 g, respectively. Other studies reported reduction of oxalates in boiling and drying of vegetables (Essack et al., 2017; Ilelaboye et al., 2013; Mwanri et al., 2011); which means the amount of oxalate present in studied vegetables may not be harmful for human consumption since the contents were relatively low and these vegetables are usually boiled before consumption.

There was variation in phytate content in different varieties but no significant difference was observed at 21 days. Phytate content were lower than reported for other vegetables commonly consumed in Africa. For example, studies by Gupta et al. (2005) found 1.95 mg/100 g in Amaranthus tricolor at market maturity stage. In addition, higher levels of phytic acid were observed in Amaranths (4.12 mg/100) and Solanum (7.39 mg/1000) species at market maturity and in several leafy vegetables consumed in Nigeria (Agbaire, 2012; Agbaire and Emoyan, 2012). Significant variation of phytic acid content among different varieties were also reported by Hossain and Becker (2001) who found that three varieties of Sesbania seeds (*S. aculeate, S. rostrata* and *S. sesban*) had 0.28, 0.16 and 0.39 g/100 g phytic acid content, respectively. Phytic acid varies not only among varieties but also among different cultivars of the same variety (Purvika et al., 2012). Phytate content in green leafy vegetables can be reduced by various processing methods including blanching and cooking. A study by Ilelaboye et al. (2013) reported that phytate content in *Amaranthus hybridus* was reduced from 191 mg/100 in raw vegetables to 81.65 mg/100 by blanching and 56.67 mg/100 g by cooking.

Generally, nitrate content decreased with maturity stage. The decreasing trend in nitrate content in vegetables with maturity was also reported by Musa et al. (2011) who found that nitrate content of *A. cruentus* decreased from 17.71±2.42 g/kg at market maturity to 7.62±1.00 g/kg at vegetative stage. The amount of nitrate in plants is determined mainly from its genetically based metabolism, the age of the plant, environmental factors

and the amount of available nitrate in the soil (Masclaux-Daubresse et al., 2010; Rachmilevitch et al., 2004). Nitrate content was higher in *S. villosum* (Olevolasi SS 49) and lowest in Nduruma BG 16 variety. The variation of nitrate according to varieties were reported by other researchers who found higher levels in arugula (4354.9 mg/kg) as compared to kale (603.0 mg/kg), but all were below the maximum recommended levels for human consumption (Brkić et al., 2017). Strategies to reduce amount of nitrate in diets include selecting varieties with low nitrate, avoiding excessive nitrate fertilizers, avoid application of nitrogen fertilizer shortly before harvesting the vegetables and harvesting vegetables in afternoons and sunny period (Renseigné et al., 2007).

It is important to note that poultry manure used in this study was not analysed so its chemical composition is not known. However, it is known that nitrogen composition in organic manure increase nitrogen in the soil which ultimately may affect nitrate concentration in vegetables since excess nitrogen tends to accumulate as nitrate. Compared to dairy and goat manure, poultry manure was reported to contribute relatively more soil nitrogen (Maerere et al., 2001). Therefore, nitrate concentration observed could be overestimated due to nitrogen content of the poultry manure used. But for the sake of comparisons made in this study, it is important to bear in mind that all the experimental plants were treated the same.

Conclusions

Given the findings of this study, it is therefore not possible to generalize the 'best' stage to harvest and consume these three African indigenous green leafy vegetables based on the plant contents of three known anti-nutrient factors (oxalate, phytate and nitrate). Mixing of the plant parts harvested at varying stages when preparing these vegetables for consumption, may offer a good opportunity of minimizing intake of such compounds and for that case, any danger associated with them. Variation of antinutritional compounds across the varieties confirms the importance of diversification for improved nutrient intake. In addition, the three antinutrients investigated were generally in low levels so consumption of vegetables to increase micronutrient intake is encouraged.

ACKNOWLEDGEMENT

The authors would like to acknowledge financial support from USAID funded Horticulture CRSP project titled 'sustainable African Indigenous Vegetable Production and Market Chai Development for Improved Health and Nutrition and Income Generation by Small Holder Farmers in Kenya, Tanzania and Zambia.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Agbaire PO (2012). Levels of anti-nutritional factors in some common leafy edible vegetables of southern Nigeria. African Journal of Food Science and Technology 3(4):99-101.

Agbaire PO, Emoyan OO (2012). Nutritional and anti-nutritional levels of some local vegetables from Delta State, Nigeria. African Journal of Food Science and Technology 6(1):8-11.

Baker CJL (1952). Determination of oxalates in fresh plant material. Analyst 77(77):340-344.

Brkić D, Bošnir J, Bevardi M, Bošković AG, Miloš S, Lasić D, Trstenjak, NU (2017). Nitrate in leafy green vegetables and estimated intake. African Journal of Traditional, Complementary and Alternative Medicines 14(3):31-41.

Dzomeku BM, Kwenin WKJ, Wolli M (2011). Assessing the nutritional value of some African indigenous green leafy vegetables in Ghana. Journal of Animal and Plant Sciences 10(2):1300-1305.

Embaby HE (2010). Effect of soaking, dehulling and cooking methods on certain antinutrients and in vitro protein digestibility of bitter and sweet lupin seeds. Food Science And Biotechnology 19:1055-1062.

Essack H, Odhav B, Mellem JJ (2017). Screening of traditional South African leafy vegetables for specific anti-nutritional factors before and after processing. Journal of Food Science and Technology 37:462-471.

Gaya UI, Alimi S (2006). Spectrophotometric determination of nitrate in vegetables using phenol. Journal of Applied Sciences and Environmental Management 10(1):79-82.

Grzebelus D, Baranski R (2001). Identification of accessions showing low nitrate accumulation in a germplasm collection of garden beet. Acta Horticulturae 563:253-257.

Gupta S, Jyothilakshmi A, Manjunath M, Prakash J (2005). Analysis of nutrient and antinutrient content of underutilized green leafy vegetables. LWT-Food Science and Technology 38:339-345.

Hossain MA, Becker K (2001). Nutritive value and antinutritional factors in different varieties of Sesbania seeds and their morphological fractions. Journal of Food Science and Technology 73:421-431.

Ilelaboye NOA, Amoo IA, Pikuda OO (2013). Effect of cooking methods on mineral and anti nutrient composition of some green leafy vegetables. Archives of Applied Science Research 5(3):254-260.

Imaobong IU, Roland U, Efiok JU (2013). Effect of thermal processing on antinutrients in common edible green leafy vegetables grown in Ikot Abasi, Nigeria. Pakistan Journal of Nutrition12(2):162-167.

Khader V, Rama S (2003). Effect of maturity on macromineral content of selected leafy vegetables. Asia Pacific Journal of Clinical Nutrition 12(1):45-49.

Kitchen JW, Burns EE (2006). The effect of maturity on the oxalate content of spinach (*Spinacia oleraceae L.*). Journal of Food Science 30(4):589–593.

Ladji O, Akin CU, Umaru HA (2004). Level of antinutritional Factors in vegetables commonly eaten in Nigeria. Journal of Natural Sciences Research 7:71-73.

Maerere AP, Kimbi GG, Nonga DLM (2001). Comparative effectiveness of animal manures on soil chemical properties, yield and root growth of amaranthus (amaranthus cruentus l.) African Journal of Science and Technology 1(4):14-21.

Masclaux-Daubresse C, Daniel-Vedele F, Dechorgnat J, Chardon F, Gaufichon L, Suzuki A (2010). Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. Annals of Botany 105(7):1141-1157.

Modi M, Modi AT, Hendriks S (2006). Potential Role for Wild Vegetables in Household Food Security: A Preliminary Case Study in Kwazulu-Natal, South Africa. African Journal of Food, Agriculture, Nutrition and Development 6(1):1-13.

Moyo M, Amoo SO, Ncube B, Ndhlala AR, Finnie JF, van Staden J (2013). Phytochemical and antioxidant properties of unconventional leafy vegetables consumed in southern Africa. South African Journal

of Botany 84:65-71.
Musa A, Ogbadoyi EO (2014). Determination of Anti-nutrients and Toxic Substances of Selected Fresh Leafy Vegetables Obtained from Minna Town, Nigeria. Nigerian Journal of Basic and Applied Sciences 22(3 & 4):79-83.
Musa A, Oladiran JA, Ezenwa MIS, Akanya HO, Ogbadoyi EO (2011). Effect of heading on some micronutrients, anti-nutrients and toxic substances in Amaranthus cruentus grown in Minna, Niger State, Nigeria. American Journal of Food and Nutrition 1(4):147-154.
Mwanri AW, Kogi-Makau W, Laswai HS (2011). Nutrients and Anti-nutrient composition of raw, cooked and sun dried sweet potato leaves. African Journal of Food, Agriculture, Nutrition and Development 11(5):5142-5156.
Mziray RS, Imungi JK, Karuri EG (2001). Nutrient and antinutrient contents of raw and cooked Amaranthus hybridus. Ecology of Food and Nutrition (40):53-65.
Nkafamiya II, Osemeahon SA, Modibbo UU, Aminu A (2010). Nutritional status of non-conventional leafy vegetables, Ficus asperifolia and Ficus sycomoru. African Journal of Food Science 4(3):104-108.
Purvika S, Rajni M, Monika S, Chuhan PK (2012). Anti-nutrient profile of different chenopodium cultivars leaves. Annals. Food Science and Technology 13(1):66-74.
Rachmilevitch S, Cousins AB, Bloom AJ (2004). Nitrate assimilation in plant shoots depends on photorespiration. Proceedings of the National Academy of Sciences of the United States of America 101(31):11506-11510.
Radek M, Savage GP (2008). Oxalates in some Indian green leafy vegetables. International Journal of Food Sciences and Nutrition 59(3):246-260.
Renseigné N, Shahid U, Iqbal M (2007). Nitrate accumulation in plants, factors affecting the process, and human health implications. A review. Agronomy for Sustainable Development. Springer Verlag/EDP Sciences/INRA 27(1):45-57.
Schippers RR (2000). African Indigenous Vegetables: An overview of the cultivated species. Chatham, UK. Natural Resources Institute, London UK.
Schlemmer U, Frolich W, Prieto RM, Grases F (2009). Phytate in foods and significance for humans: food sources, intake, processing, bioavailability, protective role and analysis. Molecular Nutrition & Food Research 53(Suppl 2):S330-375.
Singh G, Kawatra A, Sehgal S (2001). Nutritional composition of selected green leafy vegetables, herbs and carrots. Plant Foods for Human Nutrition 56(4):359-364.
Sunaga Y, Harada H, Hatanaka T (2005). Varietal differences in nitrate nitrogen concentration of Sudangrass (Sorghum sudanense (Piper) stapf). Grassland Science 51(2):169-177.
United Republic of Tanzania(URT) (2016). Morogoro Region Investment Guide. President's Office, Regional Administration and Local Government, Morogoro, Morogoro Urban District 87 p.
Uusiku NP, Oelofse A, Duodu KG, Bester MJ, Faber M (2010). Nutritional value of leafy vegetables of sub-Saharan Africa and their potential contribution to human health: A review. Journal of Food Composition and Analysis 23(6):499-509.
Virginia P, Swati VS, Ajit P (2012). Effect of cooking and processing methods on oxalate content of green leafy vegetables and pulses. Asian Journal of Food and Agro-Industry 5(4):311-314.
Walker R (1990). Nitrates, nitrites and N-nitrosocompounds: A review of the occurrence in food and diet and the toxicological implications. Food Additives and Contaminants 7(6):717-768.
Wheeler EL, Ferrel RE (1971) A method for phytic acid determination in wheat and wheat fractions. American Association of Cereal Chemists 48:312-320.
Yada S, Sehgal S (2003). Effect of domestic processing and cooking on selected antinutrient contents of some green leafy vegetables. Plant Foods for Human Nutrition 58(1):1-11.