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OXALATE LEVELS IN SELECTED AFRICAN INDIGENOUS VEGETABLE RECIPES FROM THE LAKE VICTORIA BASIN, KENYA.

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ABSTRACT

African indigenous vegetables (AIVs) in Lake Victoria Basin that could provide micronutrients to fight malnutrition contain oxalates that reduce bioavailability. These can be reduced through appropriate traditional food processing techniques adopted by households. This study determined oxalate levels in formulated AIV recipes. Eleven selected AIVs and five AIV mixtures were each divided into two lots. One lot was boiled and fermented for 48 hours and other lot unfermented. The unfermented were subjected to three treatments; cooked by boiling in water, cooked by boiling with cow's milk and lye and cooked by sautéing. Oxalate levels in recipes were determined using HPLC. Independent t-test was used to compare the mean oxalate levels between fermented and unfermented recipes. One-way ANOVA was used to compare mean oxalate levels between different methods of cooking. Oxalate levels in unfermented recipes ranged from 2.62-10.17 mg/100g FW and in fermented, 1.54-20.36 mg/100g FW. The mean levels in some fermented recipes were significantly lower than unfermented ($p<0.05$). Cooking methods differently affected oxalate levels. Cooking methods and fermentation do not have a uniform effect on oxalate level reduction in all AIV recipes but could still be employed as household procedures in reducing oxalate levels in a number of AIV recipes.

Keywords: African indigenous vegetables, oxalate levels, fermentation, cooking methods

INTRODUCTION

The African Indigenous vegetables (AIVs) in Vihiga County in Lake Victoria Basin (LVB) include spider plant, African nightshade, pumpkin, cowpea, amaranths, jute mallow, and slender leaf (Abukutsa-Onyango 2007). The potential of these AIVs as sources of micronutrients is limited by the presence of anti- nutrients like phytate, oxalate, tannic acid, Ethylene diamine tetra acetic acid (EDTA) and sapon in which bind to some micronutrients in the vegetables hence limiting the micronutrient bioavailability (Makokha and Ombwara, 2005).Currently there is a lot of research on bioavailability of micronutrients in AIVsand AIV anti-nutrient content and the effects of these anti-nutrients on human health. It is only very recently that there has been a significant interest toward Africa's indigenous vegetables grown in home or backyard gardens (Abukutsa-Onyango, 2010) otherwise AIVs normally face stiff competition with exotic vegetables like cabbage, spinach, and lettuce among others (Maundu *et al*., 1999). The introduction of exotic vegetables in the African continent had some negative impact on the consumption and cultivation of indigenous vegetables. During the colonial time, a deliberate suppression of the indigenous vegetables was done and a lot of efforts were made to promote the exotic vegetables such as cabbage (Abukutsa-Onyango, 2010).The net effect of such suppression flowed into the post independent era where the governments perpetuated the agricultural policies developed by the colonial rulers. Changed food habits in favor of introduced temperate vegetables lowered the demand of indigenous vegetables, due to the fact that the former fetched higher prices in local markets (Abukutsa-Onyango, 2010). Indigenous vegetables were considered out of fashion, poor man's food that could only be used as a last resort. Thus they enjoy less social prestige, being associated with the low-income group. As the poor sought to imitate the eating habits of the affluent and were exposed to more fashionable exotic species, the indigenous species became neglected (Abukutsa-Onyango, 2010). The neglect and stigmatization was perpetuated by stakeholders like the policy makers, agricultural training institutions, researchers, consumers and traders (Mnzava,1997). Having been branded and denoted by the agriculturalists and researchers as weeds, the tendency was to eradicate them and not conserve them as it were. However, the potential of AIVs for use in the eradication of malnutrition in poor households has attracted a lot of research because of the numerous advantages these vegetables possess over exotic ones.

AIVs adapt easily to harsh or difficult environments and require less input to grow as compared to other crops. Furthermore AIVs are highly resistant to pathogens and require less attention (Abukutsa-Onyango *et al*., 2006). This makes them appropriate for the alleviation of

malnutrition in people living in areas with high population density in LVB. Their mineral and vitamin content exceed levels found in exotic vegetables like cabbage (Abukutsa-Onyango, 2010). They also contain ascorbic acid which has been known to enhance iron absorption. Populations who consume AIVs are less likely to suffer cardiovascular diseases, diabetes and other diseases and this property is attributed to the presence of non-nutrient bioactive phytochemicals (Smith and Eyzaguire, 2007). Some studies have also shown that AIVs contain health promoting compounds such as anti-cancer factors, minerals, vitamins and anti-oxidants(Abukutsa-Onyango, 2003). This boosts the body's immune system if consumed. Apart from providing important nutrients, AIVs can also play an important role in improving income and subsistence to people (Adebooye and Parody, 2004). However, the potential of AIVs in their use to fight malnutrition is limited by the anti-nutrients they contain. Anti-nutrients in vegetables which include phytate, oxalate, tannic acids and hydrocyanic acids are associated with less bioavailability of zinc, calcium, iron and magnesium in vegetables (Broadhurst and Jones, 1978;Akindahunsi, 2005). Anti-nutrients are organic in nature and chelate with mineral elements to form insoluble complexes which interfere with absorption and assimilation of these mineral elements in the human body (Munro and Bassir, 1969). AIVs also contain polyphenols such as phenolic acids, flavonoids and their polymerization products. They form insoluble complexes with iron and inhibit iron absorption. Tannin is a phenolic compound (Brown *et al*., 1990). Anti-nutrient levels increase with age in stems, roots and seeds (Ekpedema *et al*., 2000; Weinberger and Msuya, 2004).One anti-nutrient that has attracted study is the oxalate whose ionposes two main problems: it reduces the bioavailability of essential elements in the human body such as calcium, iron and zinc and its crystals block the kidney as kidney stones and also cause gout, rheumatoid arthritis and vulvodynia (Franceschi and Nakata, 2005). Oxalate is distributed in plants and this levels range in 3-15 % w/w of their dry weight (Franceschi and Nakata, 2005). Some plants like rice accumulate oxalate to detoxify aluminium, lead, strontium, copper and cadmium (Yang *et al*., 2000; Choi *et al*., 2001).Oxalate is actually a compound of oxalic acid (ethanedioic acid); oxalic acid is a colourless and toxic organic compound that belongs to the family of dicarboxylic acids whose formula is $(COOH)_2.2H_2O$. It is soluble in water, alcohol and ether. It occurs as oxalate in plants and more so in green leafy foods.

Lack of knowledge on the correct choice of food, dietary diversity and anti-nutrient levels in AIVs has led to underutilization of AIVs (Abukutsa-Onyango, 2003; Waudo *et al*., 2006; Kimiywe *et al*., 2007). Diets in households within the LVB are primarily composed of cereals and legumes that are high in anti-nutrients that inhibit micronutrient absorption. This can be reduced through appropriate traditional food processing techniques adopted in households (Walingo, 2009). The mineral and anti-nutrient content of local foods within LVB needs further research to identify suitable sources of absorbable minerals and possible suitable dietary combinations that can contribute towards the reduction of mineral deficiency (Walingo, 2009).

Traditional food processing methods and diet combinations usually reduce the levels of mineral anti-nutrients in the plant diets thus increasing mineral bioavailability. Household food processing methods that promote nutrient content and bioavailability for improved health and nutrient situation of rural populations whose diets are basically plant based with high anti-nutrient content should be identified (Walingo, 2009). Some of these processes include thermal processing, germination, milling/household pounding, microbial fermentation, and soaking.

Cooking has been shown as one of the factors that affect anti-nutrient and nutrient contents of vegetables. The main methods of cooking in the study area involve boiling in unspecified amounts of water contributing to nutrient loss and using additives like bicarbonate of soda, lye (traditional salt),milk, sesame and groundnut paste whose effects are unknown. Cooking in study area households is done by use of pots rather than pans as pots retain heat and give better simmering effects (Abukutsa-Onyango, 2010). Also, the covering of the cooking pot is preceded by sealing it completely with banana leaves and this helps to retain steam, which escapes with some volatile nutrients and the aroma (Musotsi *et al*., 2005). Results also show that the recipes in study area households are based on a mixture of different vegetables (Musotsi *et al*., 2005). There is some evidence that boiling vegetables induce losses of 5%-15% of phytate and that thermal processing can also enhance bioavailability of vitamins and carotenoids by releasing them from entrapment in the plant matrix (Sandberg, 1991). Microbial fermentation is also one of the food processing methods employed in the study area. Fermentation can induce phytate hydrolysis via action of microbial phytase enzymes, which hydrolyze phytate to lower inositolphosphates that do not affect mineral absorption (Sandberg, 1991). Microbial phytases originate from micro flora on the surface of cooked food (Sandberg, 1991). Studies also reveal that the enzyme phytase is affected by anti-nutrients like tannin hence interfere with hydrolysis of phytate (Sandberg, 1991). Employing both cooking and fermentation of AIVs may contribute to the increased bioavailability of micronutrients (Gibson *et al*., 2010). Kimiywe and Waudo (2007) documented preparation and cooking procedures that could lead to a decrease in the nutritive value of cooked food. These includes chopping before washing which leads to loss of vitamin C and vitamin B complex since they are water soluble vitamins, repeated boiling and frying destroys vitamin C and addition of sodium bicarbonate leads to loss of vitamin B complex i.e. B1, B2 and niacin.There is a delicate balance between loss of nutrients and reduction of anti-nutrients by traditional food processing methods adopted in LVB households.

Oke and Bolarinwa (2011) studied the effect of fermentation on oxalate content of cocoyam flour. It was demonstrated that 48 hour fermentation reduced calcium oxalate significantly by approximately 58%-65% and that the longer the fermentation period the higher the microbial population and the higher the reduction of oxalate in the cocoyam. Iwuoha and Kalu (1995) reported 82.1% and 61.9% oxalate reduction in cocoyam flour produced from boiled and roasted

cocoyam respectively. The study concluded that high temperatures during cooking significantly reduced the levels of oxalate in vegetables. Muchoki *et al* (2010) also demonstrated that high temperatures reduced oxalate levels in *Vigna unguiculata* leafy vegetable. The effects of cooking and microbial fermentation on levels of anti-nutrients needed a study. This study investigated the oxalate levels in selected African indigenous vegetable recipes from the Lake Victoria Basin, Kenya.

MATERIALS AND METHODS

Study site

The study was carried out in Vihiga County of Western part of Kenya in the LV Band is located at longitude 34º 30" East and latitude 00º 11" and 00º 15" North and occupies an area of 563 km^2 (CBS, 2001). It has four sub-counties: Emuhaya, Vihiga, Hamisi and Sabatia. It lies between 1300 m and 1500 m above sea level with an equatorial climate and a forest cover of 4 percent and an annual precipitation of 1900 mm (District Strategic Plan, 2005). Land is arable and supports a variety of crops (CBS, 1997). It is the third most densely populated County in Kenya with a population of 595,180 people as per 2005 census. Population density is 975 persons per sq. km (CBS, 2001). The County is dependent on food relief and its high population growth rate cannot be sustained by its infrastructure and productivity. Adverse poverty indicators hinder attainment of food security, as demand grows every year. With an average land size of 0.4 hectares per household, the county can no longer produce enough food. Malnutrition is a common feature here (Akelola *et al*., 2007). Land is scarce and 60 percent of the population lives below the poverty line. The main economic activity by residents is farming of maize, millet, tea, cassava, sweet potatoes, beans and a variety of vegetables and fruits. Dairy farming is practiced on small scale, as many people have been restricted to keeping one or two animals because of inadequate pasture. Uneconomical land use and HIV/AIDS contributes to poverty, and malnutrition is high due to wide spread poverty, poor feeding habits and over reliance on starchy foods. Nearly 133 children per 1000 children die before their fifth birthday due to maternal malnutrition (CBS, 2002). Despite having favorable climate and soils the area is not sufficient in food production.

SOURCE OF THE AFRICAN INDIGENOUS VEGETABLES

The eleven AIVs selected for use in recipe formulations were spider plant *(Cleome gynandra),* pumpkin leaves *(Curcubita moschata),* cowpea *(Vigna unguiculata),* green amaranth *(Amaranthusblitum*), jute mallow *(Corchorus olitorius),* sweet potatoes leaves (*Ipomea batatas),* African nightshade (*Solanum nigrum),* and cassava leaves (*Manihot esculenta*), slender leaf (*Crotolaria ochroleuca*), vine spinach *(Basella alba)* and African kales (*Brassica carinata).*

These vegetables were identified and about 1 kg of each purchased from markets within the study area: Chavakali, Shisejeri, Shamakhokho, Gambogi, Kiboswa, Wemilabi, Luanda and Majengo. Leaves of sweet potatoes (*Ipomea batatas),* cassava (*Manihot esculenta*) and nderema *(Basella alba)* were identified and purchased from small vegetable farms in homes within the study area. The purchased vegetables were de-stalked and the leaves washed with distilled water to remove dirt. Vegetables of the same species from different markets were mixed to get a representative sample for each species and transported to Kenyatta University in cool boxes for recipe preparation. Exactly 1kg of each vegetable sample was weighed and blanched in one litre of boiling water for two minutes to inactivate enzymes responsible for vitamin degradation (Mosha *et al*., 1997 and Nyambaka and Ryley, 1996) and immersed in ice cold water for two minutes to minimize premature cooking process.

PREPARATION OF LYE

Lye was prepared from pods of beans (Habwe *et al*., 2009). Pods of green beans were dried after removing the mature seeds. The dry pods were then burnt over a hot dry pan and the ash collected after complete burning. The ash was put in a container whose bottom had small holes and distilled water poured through the ash into another container underneath and filtrate (lye) collected.

PREPARATION OF RECIPES

There were five vegetable mixtures formulated, each containing 40g of the unmixedAIV as follows: First mixture (*S. nigrum* + *A. blitum*), second mixture (*C. ochroleuca* + *C. olitorius*), and third mixture (*C. ochroleuca* + *C. olitorius* + *V. unguiculata*), fourth mixture (*A. blitum* + *C. gynandra* + *S. nigrum*) and fifth mixture (*C. gynandra* + *C. moschata*).Each of these blanched five vegetable mixtures and each of the eleven unmixed selected AIVS were divided into two lots. One lot was fermented and the other lot unfermented. The lot to be fermented was boiled, cooled and left in the open to allow microbial fermentation to occur for 48 hours. The unfermented lot was divided into three portions for cooking. One portion was cooked by boiling in water, another cooked by boiling with lye and milk and the other cooked by sautéing according to the household cooking procedures commonly employed in the study area. The unfermented group was refrigerated at – 4^0C. This generated 16 AIV recipes for study.

Vegetable cooked by boiling in water

Exactly 40 g of the vegetable was boiled in 100 ml of distilled water for 10 minutes at 40ºC and recipe obtained was cooled to room temperature and sealed in black polythene bags to keep out light and refrigerated at - 4ºC, awaiting extraction to obtain the sample for laboratory analysis.

Vegetable cooked by boiling with lye and milk

Exactly 40 g of the blanched AIV to which 30 ml of traditional salt (lye) had been added was boiled for 7 minutes in 40 ml of distilled water at a moderate temperature of 40ºC. Exactly 30 ml of fresh milk was added and mixture simmered for three more minutes. The boiled mixture was left to cool to room temperature, sealed in a black polythene bag and refrigerated at - 4ºC awaiting extraction to obtain the sample for laboratory analysis.

Vegetable cooked by sautéing

Exactly 20 ml of vegetable cooking oil was transferred by means of clean plastic syringe to a clean cooking pan and placed on electric cooker plate set at a temperature of 40ºC to heat. One onion bulb was peeled to remove dry outer skin, washed, sliced and was sautéed in the oil till golden brown and a pinch of common salt added. Two tomatoes with intact skin were washed with distilled water, chopped and added to the mixture in the pan. Exactly 40 g blanched AIV was then added, stirred and mixture heated for 10 minutes. The sautéed AIV was left to cool to room temperature, sealed in a black polythene bag and refrigerated at - 4ºC awaiting extraction to obtain the sample for laboratory analysis.

Fermented recipe

Exactly 40 g of the blanched single AIV or AIV mixture was boiled for 10 minutes in 100 ml of distilled water and left to cool to room temperature, sealed in a black polythene bag to keep out light and kept in open air for 48 hours to allow microbial fermentation. After the fermentation period the fermented recipe was refrigerated at - 4ºC awaiting extraction to obtain the sample for laboratory analysis.

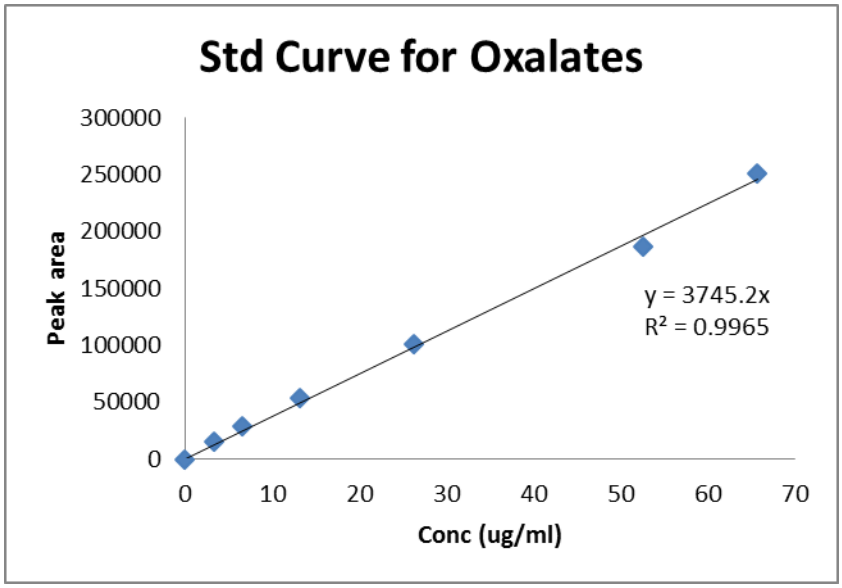
SAMPLE PREPARATION FOR OXALATE ANALYSIS

Glassware was initially washed with a detergent, chromic acid and further washed in 1:1 nitric acid before rinsing in distilled water. The glassware was dried overnight at 50 ^{0} C. Plastic containers were washed in 1:1 nitric acid and also rinsed in distilled water before drying them in an oven at 30 ^{0} C. Standards of oxalic acid were of analar grade and were sourced from Aldrich Chemicals. Exactly 0.2 g of vegetable sample was homogenized in 1 ml of 0.5 N HCl. The homogenate was heated at 80 ° C for 10 min with intermittent shaking. To the homogenate 5 ml of distilled water was added. About 2 ml of the solution was withdrawn and centrifuged at 12 000 g for 10 min. 1 ml of supernatant was passed through a filter (0.45 µm) before HPLC analysis. Standards were prepared at varying concentrations for quantification.

STANDARDS AND CALIBRATION CURVES

A stock solution of the standard containing 10 mg/ml of oxalic acid was prepared for calibration. The peak area was determined and used to obtain the concentration levels of oxalate in the samples. The regression coefficient (R) was obtained and from it the coefficient of determination (R^2) was worked out.

Figure 1: Calibration curve for oxalate



METHOD VALIDATION

The following performance parameters were evaluated for method validation: linearity domain of the concentration: limit of detection (LOD), precision (reproducibility), and accuracy (by recovery tests):

Linearity test of concentration: limit of detection

The linearity of the calibration curve is given by the equation y=mx-c, where the calculated blank sample absorbance is c and the method sensitivity (the slope) is m and the correlation coefficient is R. Limit of detection (LOD) was calculated using equation (i) (Eurachem guide, 1998). Absorbance values for 10 replicates of the blank solution were determined and transformed into concentration values in order to be compared with the data obtained from the calibration curve. The results are displayed in table 1.

$LOD = \bar{X}_{blank} + 3S_{blank}$..... (1)

$\bar{X}_{blank}$ is the mean absorbance obtained with the blank solutions:

S_{blank} is the standard deviations of the blank:

X_i are the values of the blank solutions while n is the number of replicates i.e. n=10.

Table 1: Limit of detection, equation of calibration curve, coefficient of determination (R^2) and regression coefficient (R)

Parameter	LOD(µg/ml)	Equation	R^2	R
Oxalate	0.002	Y=3684x+2881	0.997	0.99849

The R^2 values hence R values indicate that the established calibration curves are linear over the respective range of concentrations as R tends to unity. The method detection limits at 3 standard deviations for all the parameters were < 1 µg/ml which clearly indicates that the method was reliable for the determination of the levels of oxalates.

Precision

Reproducibility of results was calculated for 10 measurements. Precision was evaluated by Relative Standard Deviation (%RSD), according to the equation 2 (Eurachem guide, 1998). The results are given in table 2.

$RSD = \frac{s}{\bar{x}} \times 100$..... (2)

Table 2: Method precision

Parameter	mean	s	%RSD
Oxalate	5.34	0.02	0.37

The obtained results show good precision for the parameter under determination.

Accuracy

Accuracy was evaluated by recovery test, according to equation3 (Eurachem guide, 1998). The results are presented in table 3.

% Recovery = $\frac{C_F - C_U}{C_A}$ x 100..... (3)

Where C_U is the concentration in unfortified sample; C_A is the concentration of Fortification (added solution); C_F is the concentration determined in fortified sample.

Table 3: Accuracy by Recovery test

Parameter	C_U (mg/g)	C_A (mg/g)	C_F (mg/g)	%Recovery
Oxalate	0.20	0.70	0.89	98.40

The percentage recovery lies within the range (98.40−102.10). This confirms that the method is accurate and fit for analysis of the parameter.

ANALYSIS OF OXALATE

Analysis was done at Jomo Kenyatta University of Agriculture and Technology's Home economics laboratory by reversed-phase HPLC using Hypsil C-18 SUPELCO column (5 µM, 4.6 mmx250 mm) equipped Waters 550 (Waters, MA, USA) as the static phase and the mobile phase was a solution containing 0.5 % KH_2PO_4 and 0.5 mM TBA (tetrabutylammonium hydrogen sulphate) buffered at pH 2.0 with orthophosphoric acid. Flow rate was 1 ml min^{-1} (Libert, 1981; Yu *et al*., 2002) and detection wavelength was at 220nm.

DATA ANALYSIS

The data obtained was analyzed by SPSS software (version 17) where it was subjected to independent t-test to compare whether there was any significant difference in the mean levels of oxalates between fermented and non-fermented AIVs and one-way ANOVA to compare the mean values between different AIVs and cooking methods. Where one- way ANOVA showed significant difference it was followed by multiple range test (Student Newman Keul Test). All the significance tests were performed at 95% confidence level.

RESULTS AND DISCUSSION

The mean concentration levels of oxalate in fermented and unfermented AIV recipes

The mean levels of oxalate between fermented and unfermented AIV recipes were compared (Table 4). Unfermented *Amaranthus blitum* and *C. gynandra+C. moschata* recipe formulation recorded significantly higher mean levels of oxalate than any other unfermented recipes ($p<0.001$). Fermented *Amaranthus blitum* recorded significantly higher mean levels of oxalate than any other fermented recipes ($p=0.107$). The mean levels of oxalate in fermented *Ipomea batatas*, *Solanum nigrum*,*Manihot esculenta*, *Cleome gynandra* and *Basella alba* were significantly lower than in the unfermented ones ($p<0.05$). The mean levels of oxalate in the five recipe mixtures that were fermented were significantly lower than in the unfermented ones ($p<0.001$). There was no significant difference in the mean oxalate levels between fermented and unfermented *Vigna unguiculata* ($p=0.569$) and *Amaranthus blitum* ($p=0.107$) (Table 4). Apart from 2 AIV recipes (*Vigna unguiculata* and *Amaranthus blitum)* all the fermented AIV recipes in the study had significantly lower mean levels of oxalate than the unfermented ones. However, when the mean value of all unfermented vegetables was compared with the mean value of all fermented AIVs (table 5), there was no significant difference ($p=0.280$, t-test).It was observed that fermentation reduced oxalate levels in someunmixedAIV recipes and some recipe mixtures. The observations inthose recipes in which there was a significant reduction in mean levels of oxalates agreed withfindings of Oke and Bolarinwa (2011) who studied the effect of fermentation on oxalate content of cocoyam flour. The study showed that 48 hour fermentation reduced calcium oxalate significantly by approximately 58%-65% and that longer fermentation period resulted in higher microbial population leading to higher reduction of oxalate concentration levels.

Table 4: Mean concentration levels of oxalate in fermented and unfermented AIVs

	Oxalate		
AIVs	**Unfermented (Mean±SE)mg/100g (n=9)**	**Fermented (Mean±SE)mg/100g (n=9)**	**P value**
Curcubita moschata	9.20±1.61[a]	6.55±1.53[b]	0.030
Vigna unguiculata	2.62±.43[a]	2.24±.01[a]	0.569
Amaranthus blitum	15.42±1.56[b]	20.36±.01[c]	0.107
Corchorus olitorius	10.17±3.73[a]	3.04±.01[a]	0.001
Ipomea batatas	3.45±.53[a]	1.56±.08[a]	0.038
Solanum nigrum	6.72±.41[a]	2.69±.02[a]	<0.001

Manihot esculenta	3.07±.25[a]	1.94±.08[a]	0.006
Crotolaria ochroleuca	4.37±.58[a]	3.01±.00[a]	<0.001
Brassica carinata	6.10±1.65[a]	3.18±.06[a]	0.003
Cleome gynandra	5.12±.09[a]	1.54±.01[a]	<0.001
Basella alba	5.24±.52[a]	2.06±.45[a]	0.001
S. nigrum+A. blitum	3.04±.00[a]	2.06±.01[a]	<0.001
C. ochroleuca+ C. olitorius	2.53±.02[a]	2.04±.01[a]	<0.001
C. ochroleuca +C. olitorius+V. unguiculata	3.44±.01[a]	1.70±.03[a]	<0.001
A. blitum+C. gynandra+S. nigrum	4.14±.01[a]	2.30±.01[a]	<0.001
C. gynandra+C. moschata	5.88±.086[b]	3.02±.08[a]	<0.001
p-value	<0.001	<0.001	

Mean ± SE values within the same column followed by the same superscripts are not significantly different at $\alpha=0.05$, while values within the same row with $p<0.05$ are significantly different, 95% confidence level, independent t-test.

Table 5: oxalate levels in all non-fermented and all fermented recipes

Microbial Fermentation	Mean Oxalate level ±SE
All Non-fermented AIVs	5.48±0.87
All Fermented AIVs	3.88±1.16
p-value	0.280

Independent t test

The mean concentration levels of oxalate in AIVs by different cooking methods

Boiled *Curcubita moschata* recorded significantly higher mean levels of oxalate than in *Amaranthus blitum*, *Ipomea batatas*, *Brassica carinata*, *Solanum nigrum*, *Crotolaria ochroleuca* and *Cleome gynandra* (Table 6). There was no significant difference in mean oxalate levels in boiled *Curcubita moschata* compared to boiled *Vigna unguiculata*, *Corchorus olitorius* and *Basella alba* ($p>0.05$). *Curcubita moschata* boiled with lye and milk recorded significantly higher mean oxalate levels than in *Corchorus olitorius*, *Ipomea batatas*, *Brassica carinata* and *Amaranthus blitum* cooked the same way ($p<0.05$). However, there was no significant difference in mean oxalate levels in *Curcubita moschata* boiled with lye and milk when compared with *Vigna unguiculata*, *Solanum nigrum*, *Manihot esculenta*, *Cleome gynandra* and *Basella alba* cooked the same way ($p>0.05$). Sautéed*Corchorus olitorius* recorded significantly higher mean oxalate levels than sautéed, *Ipomea batatas*, *Solanum nigrum*, *Manihot esculenta*, *Crotolaria ochroleuca*, *Brassica carinata*, Cleome *gynandra* and *Basella alba*. The mean oxalate levels in sautéed *Amaranthus blitum* were not significantly different from sautéed *Vigna unguiculata*, *Curcubita moschata* and *Basella Alba* ($p>0.05$).

Table 6: The mean concentration levels of oxalate in AIVs by different cooking methods

OXALATE			
AIVs	Boiled (Mean±SE) mg/100g (n=3)	Lye+milk (Mean±SE) mg/100g (n=3)	Sautéed(Mean±SE) mg/100g (n=3)
Curcubita moschata	7.13±.01[a]	17.26±.46[c]	9.86±.18[b]
Vigna unguiculata	1.66 ±.01[a]	3.57±.01[c]	2.15±.06[b]
Amaranthus blitum	19.78±.01[c]	9.37±.02[a]	17.11±.06[b]
Corchorus olitorius	1.82±.00[a]	3.66±.01[b]	25.03±.32[c]
Ipomea batatas	7.14±.01[c]	3.53±.01[b]	1.40±.04[a]
Solanum nigrum	6.77±.09[b]	8.09±.02[c]	5.29±.02 [a]
Manihot esculenta	2.61± .03[b]	3.60±.08[c]	2.00±.02[a]
Crotolaria ochroleuca	4.04±.02[b]	6.52±.01[c]	2.55±.02[a]
Brassica carinata	15.18±.01[c]	5.14±.01[b]	3.22±.03[a]
Cleome gynandra	5.04±.01[b]	5.47±.06[c]	4.86±.01[a]
Basella alba	4.65±.03[a]	7.91±.06[c]	5.28±.01[b]

Mean±SE values within the same row followed by the same superscripts are not significantly different at $\alpha=0.05$, $p<0.001$, SNK test.

Sautéed recipes had significantly lower oxalate levels than those boiled with lye and milk except *Amaranthus blitum* and *Corchorus olitorius* ($p<0.05$) (Table 6).AIVs boiled with water had significantly lower mean oxalate levels than those boiled with lye and milk except boiled *Brassica carinata* which recorded significantly higher mean concentration levels of oxalate than *Brassica carinata* boiled with lye and milk (Table 6). The higher levels of oxalate in sautéed vegetables and in vegetables boiled with lye and milk compared to boiled ones could be attributed to addition of oxalate to the recipes present in lye, milk, tomatoes and onions during cooking. The results also showed that high temperatures during boiling and sautéing reduce doxalate levels in some recipes and not others. The observations in those recipes in which there was a significant reduction in mean levels of oxalates agreed with findings of Muchoki *et al* (2010) that high temperatures reduced oxalate levels in *Vigna unguiculata* leafy vegetable. Iwuoha and Kalu (1995) also reported 82.1% and 61.9% oxalate reduction in cocoyam flour produced from boiled and roasted cocoyam respectively. The mean levels of all boiled AIVs, all AIVs boiled with lye and milk and all sautéed AIVs were compared (table 7).The results show that there was no significant difference in the mean values of oxalates by different methods of cooking($p=0.986$, one-way ANOVA).

Table 7: mean levels of oxalate in all AIVs by different cooking methods

Cooking Method	Mean oxalate level ± SE
All AIVs Boiled	6.89±1.71
All AIVs boiled with Lye andMilk	6.74±1.22
All AIVs Sauteed	7.16±2.26
p-vlaue	0.986

One-way ANOVA

CONCLUSION

Fermentation of AIVs differently affected oxalate levels. In some recipes a decrease was observed while in others there was no significant change, suggesting that reduction of oxalate levels in AIVs may depend on other factors within the recipes other than microbial fermentation. Cooking methods also differently affected oxalate levels. In some recipes a decrease was observed while in others there was no significant change, suggesting that the degree of oxalate degradation may depend on other factors within the recipes apart from heat during cooking. Cooking methods and fermentation do not have a uniform effect on oxalate level reduction in all AIV recipes but could still be employed as household procedures in reducing oxalate levels in a number of AIV recipes.

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REFERENCES

1. Abukutsa-Onyango, M. O. (2003). Unexploited potential of Traditional African vegetables in Western Kenya. *Maseno Journal of Education, Arts and Sciences*, **4**: 103-122.

2. Abukutsa-Onyango, M. O., Tushaboomwe, K., Onyango, J. C and Macha, S. E. (2006). Improved community Land use for sustainable production and utilization of African indigenous vegetables in the Lake Victoria region. In: proceedings of the Fifth Workshop on Sustainable Horticultural Production in the Tropics, 23rd-26th November, 2005, ARC, Egerton University.
3. Abukutsa-Onyango, M.O. (2007). The diversity of cultivated African leafy vegetables in three communities in western Kenya. *African Journal of Food Agriculture, Nutrition and Development* (AJFAND) **7** (3) online journal www.ajfand.net.7-179. Njoro: Egerton University.
4. Abukutsa-Onyango, M.O. (2010). African Indigenous Vegetables in Kenya: Strategic Repositioning in the Horticultural Sector. Inaugural lecture, April 30th, 2010, Assembly Hall, Main Campus, JKUAT-Juja,
5. Adebooye, O. C and Parody, J. T. (2004). Status of conservation of the traditional leaf vegetables and fruits of Africa. *African Journal of Biotechnology*, **3:** 103-122.
6. Akelola, R., Karuri, N. M. and Mwaniki, D. 2007. The effect of otange fleshed sweet potato on vitamin A nutritional status of pre-school children in Nambale Division, Busia District, Proceedings of the 13th ISTRC Symposium 13:721-727.
7. Akindahunsi, S. (2005). Phytochemical screening and nutrient-anti-nutrient composition of selected tropical green leafy vegetables. *African Journal of Biotechnology*, **4:** 497-501.
8. Broadhurst, R. and Jones, W. T. (1979). Analysis of condensed tannins using acidified vanillin. *Journal of the Science Food and Agriculture*, **29**: 783-798.
9. Brown, R. C., Klein, A.K., Simons, W. K and Hurrel, K. F. (1990). The influence of Jamaican herbal teas and other polyphenols containing beverages on iron absorption in the rat. *Nutrition Research Reviews*, **10**: 343-353.
10. Central Bureau of Statistics (1997). Vihiga District Plan 1997-2001. Government Printers, Nairobi: 114-119.
11. Central Bureau of Statistics and Ministry of Planning and National Development (2001). Population distribution by administrative areas and urban centers, Kenya, 1999 Population and Housing census vol. **1**, 13-25.
12. Central Bureau of Statistics and Ministry of Planning and National Development (2002). Kenya 1999 Population and Housing Census. Analytical report on mortality vol. **V**, 115-122.
13. Choi, Y. E., Harada, E., Wada, M., Tsuboi, H., Morita, Y., Kuusano, T and Sano, H. (2001). Detoxification of Cadmium in tobacco plants: formation of active excretion of crystal containing cadmium and calcium through trichomes. *Journal of Plant physiology*, **213**: 45-50.

14. District Strategic Plan (2005). Implementation of the national population policy for sustainable development. National coordinating Agency for population and development, Nairobi, Kenya.
15. Ekpedema, U., Akwaowo, Ndun, A and Ekaete, U. (2000). Minerals and anti-nutrients in fluted *Curcubita moschata* (*Telfairia occidentalis Hook f.*) Analytical, Nutritional and Clinical Methods Section. *Food Chemistry Journal*, **70**: 235-240.
16. Eurachem (1998). The Fitness of Purpose of Analytical methods, A Laboratory Guide to Method Validation and Related Topics, Internet edition: 46-49.
17. Franceschi, V. R and Nakata, P. A. (2005). Calcium oxalate in plants: formation and function. *Annual Review of Plant Biology*, **56**:41-71.
18. Gibson, R. S., Bailey, K. B., Gibbs, M and Ferguson, E. L. (2010). A review of Phytate, iron, zinc and calcium concentrations in plant based complementary foods used in low-income countries and implications for bioavailability. *Food and Nutrition Bulletin*, **31**: S134-46.
19. Habwe, F. O., Walingo, M. K., Abukutsa-Onyango, M. O and Oluoch, M. O. (2009). Iron content of the formulated East African Traditional AIV recipes. *African Journal of. Food, Agriculture, Nutrition and Development*, **3**: 393-397.
20. Iwuoha, C. I and Kalu, F. A. (1995). Calcium oxalate and physicochemical properties of cocoyam tuber flours as affected by processing. *Food Chemistry Journal*, **54**: 61-66.
21. Kimiywe, J., Waudo, J., Mbithe, D and Maundu, P. (2007). Utilization and medical value of traditional leafy vegetables consumed in Urban and peri-urban Nairobi. *African Journal of. Food, Agriculture, Nutrition and Development.* ISSN, **7**: 1684-5374.
22. Libert, B and Franceschi, V. R. (1987). Oxalate in crop plants. *Journal of Agriculture and Food Chemistry,* **35**:926-938.
23. Libert, B. (1981). Rapid determination of oxalic acid by reversed-phase high performance liquid chromatography. *Journal of chromatography*, **210**:540-543.
24. Makokha, A.O. and F. Ombwara, (2005). Potential for increased use of indigenous Kenyan Vegetables as functional foods. In: **In**: *Proceedings of the third HorticultureWorkshop on* Sustainable Horticultural Production in the Tropics, 26th -29th November 2003. Maseno University MSU, Maseno, Kenya. ISBN: 9966-758-11-9. pp. 102-116.
25. Maundu, P.M, G.W. Ngugi and C.H.S. Kabuye, 1999.Traditional Food Plants of Kenya. KENRIK National Museums of Kenya, Nairobi, Kenya. 270 pages.
26. Mnzava, N.A. (1997). Vegetable crop diversification and the place of traditional species. In: Guarino Editor 1997, Traditional African vegetables. Promoting the conservation and use of Underutilized and neglected crops. 16. Proceedings of the IPGRI International Workshop on Genetic Resources of Traditional Vegetables in Africa. Conservation and

use, 29-31 August 1995, ICRAF-HQ, Nairobi, Kenya. Institute of Plant Genetic and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome. Italy. Pages 1-15.

27. Mosha, T. C., Pace, R. D., Adeyeye, S., Laswai, H. S and Mtebe, K. (1997). Plant Foods for Human Nutrition. , *Journal of Agriculture and Food Chemistry*, **50**: 189-201.
28. Muchoki, C. N., Lamuka, P. O and Imungi. (2010). Reduction of nitrates oxalate and phenols in fermented solar- dried store Vigna unguiculata (*Vigna unguiculata*) leaf vegetables. *African Journal of. Food, Agriculture, Nutrition and Development*, **10**: 4398-4412.
29. Munro, A and Bassir, O. (1969). Oxalate in Nigerian vegetables. *West African Journal of Biological and Applied Chemistry*, **12**: 14-18.
30. Musotsi, A.A., Sigot, A. and M.O.A. Onyango (2005). African Indigenous Vegetables Recipe Documentation and their role in food security. In: Proceedings of the third Horticulture Workshop on Sustainable Horticultural Production in the Tropics, 26th -29th November 2003. Maseno University MSU, Maseno, Kenya. ISBN: 9966-758-11-9. pp. 105-111.
31. Nyambaka, H and Ryley. (1996). an isocratic reversed-phase HPLC separation of stereo isomers of the provitamin A carotids (α and β carotene) in dark green vegetables, *Journal of Food Chemistry*, **55**: 63-72.
32. Oke, M. O and Bolarinwa, I. F. (2012). Effect of fermentation on physicochemical properties and oxalate content of cocoyam (Colocasia esculenta) flour.
33. Sandberg, A. S (1991). Phytate hydrolysis by phytase in cereals; effects on in vitro estimation of iron availability. *Journal of Food Science*, **56**: 1330-1333.
34. Smith, I. F and Eyzaguire, P. (2007). African heavy vegetables; their role in the World Health Organization Global Fruit and Vegetable Initiative. *African Journal of. Food, Agriculture, Nutrition and Development*, **7**: 1674-1684.
35. Walingo, M. K. (2009). Traditional food processing methods that improve zinc absorption and bioavailability of plant diets consumed by the Kenyan population. *African Journal of. Food, Agriculture, Nutrition and Development*, **9**: 523-535.
36. Waudo, J., Tuitoek, P., Msuga, J and Kikafunda, J. (2006). Food consumption patterns and nutrient intakes by women and children under five in the wetlands of Lake Victoria Basin. Research week, Egerton University, July 2006.
37. Weinberger, K and Msuya. (2004). Indigenous vegetables in Tanzania-Significance and Prospects. Shanhua, Taiwan: AVRDC-The world vegetable centre. Technical Bulletin no. 31, AVRDC publication 04-600. p. 70.

38. Yang Y. Y., Jung, J. Y., Song, W. Y., Suh, H. S and Lee, Y. (2000). Identification of rice varieties with high tolerance or sensitivity to lead and characterization of the mechanism of tolerance. *Plant Physiology Journal*, **124**: 1019-1026.

39. Yu, L., Peng, X. X., Yang, C., Liu, Y. H and Fan, Y. P. (2002). Determination of oxalic acid in plant tissue and root exudates by reversed phase high performance liquid chromatography. *Chinese Journal of Analytical Chemistry*, **30**: 1119-1122.