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Full Length Research Paper

Evaluation of micronutrients in seeds of Pumpkin varieties grown by smallholder farmers in the Lake Victoria Basin

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Abstract

This study shows micronutrients malnutrition and its devastating effects taking toll of the world, affecting over two billion of its population. Measures have been put in place that includes supplementation, fortification and bio fortification among others. Utilization of indigenous crops are known to be nutritious and are acceptable among the communities mainly, developing countries favours the later method. The micronutrient levels in pumpkin seeds of six varieties, cultivated in four districts within the Lake Victoria Basin, East Africa were determined. This was with a view to establish if any significant differences existed between the varieties as well as set the background upon which the better varieties should be improved. The results showed levels of moisture (11.62-26.23 mg /100 g), β-carotene (0.02-0.42 mg /100 g), thiamine (0.19-0.54 mg /100 g), niacin (0.43-1.03 mg /100 g) and pyridoxine (0.13-0.26 mg /100 g), zinc (3.73-9.70 mg /100 g) and selenium (5.43-11.07 µg /100 g) significantly differed ($p < 0.05$). The varieties evaluated did not show significant differences in vitamin C, α-tocopherol and iron. The carnival squash (*C. maxima*) seeds, generally exhibited better levels of most micronutrients among the varieties sampled. Specifically however, bottle gourd (*Lagenaria siceraria*) samples showed higher levels of selenium, while butternut (*C. moschata*) seeds had high levels of zinc (9.70 mg /100 g) and green kabacha (*Cucurbita pepo*) the B complex vitamins. With sufficient nutritional education and purposefull improvement of the better varieties through breeding, pumpkin seeds can go along way as an alternative path for bio fortification as a method of fighting micronutrients malnutrition.

Keywords: Pumpkin seeds, malnutrition, micronutrients, Lake Victoria basin, biofortification, pumpkin varieties, β-carotene, B complex vitamins.

INTRODUCTION

The major challenge to food security in Africa, is it's under developed agricultural sector that is characterized by over reliance on primary agriculture, low fertility soils, minimal use of external farm inputs, environmental degradation, and significant food crops loss both pre and post harvest, inadequate food storage and preservation that result in significant commodity price fluctuation (Mwaniki, 2005). Ninety five percent of the food in sub-Saharan Africa is grown under rain fed agriculture (Inter-Academy Council, 2004). The food production is therefore vulnerable to adverse weather conditions. Food insecure populations are vulnerable to rampant cases of malnutrition. Micronutrients malnutrition affects approximately 2 billion people worldwide, with the adverse deficiency ranging from pre-mature death, poor health, blindness, growth stunting, mental retardation, learning disabilities to work capacity (Christopher *et al*., 1998). The deficiencies increases the general risk of infections, illness and of dying from diarrhea, measles, malaria and pneumonia. These conditions are among the leading causes of diseases in the world (WHO, 2001).There is therefore an urgent need for developing countries to increase their investment in long term interventions such as dietary diversification, food sufficiency and bio fortification. These have lower maintenance costs, a higher probability of reaching the poor who are vulnerable to food security and produce sustainable results.

The deficiencies particularly due to vitamin A, iodine, iron and zinc are a characteristic of victims who live in low income countries and are typically deficient in more than one micronutrient (Bruno *et al*., 2006). The use of indigenous vegetables, fruits and seeds like those of pumpkins would be to a large extent, one of the remedies to counter the deficiencies. Such crops are rich in nutrients. The levels of some of the micronutrients like β-carotene, increases on storage (Kahn *et al*., 1992) in contrast to the milled cereals. In addition, they keep fresh for as long as six months if stored whole or years if canned (Dolson, 2008). Pumpkin fruits are consumed as vegetables or dessert (pie) while pumpkin seeds, also known as pepitas are small flat, green, edible seeds. They are popular snack when roasted and to a lesser extent, as cooking oil (Lazos, 1992). They have many health benefits, some of which include good source of protein, zinc and are even said to lower cholesterol (Mateljan, 2008). They are a good source of magnesium, manganese, phosphorous and phytosterols which can benefit the liver and can increase immune response (Levin *et al*., 2008). Though pumpkin varieties have been grown in the Lake Victoria Basin (Ondigi *et al., 2008),* little has been done on this crop. Farmer-based research demonstrates wealth of traditional knowledge and beliefs on the health, sensory and culinary properties of local varieties (FAO, 2001). Research studies carried out in Tanzania on pumpkins, have only been limited to surveys for seed collection and provision of scanty information on nutritional values, appropriate agronomic practices and pest management methods (Hamishy, 2002). No deliberate efforts have been made even in Kenya and Uganda's lake basin to know the nutritional level of the seed varieties. Indeed, pumpkins have no established place in the production system as compared to elite food crops like rice, wheat, maize and barley (FAO, 1998; 2002).

The purpose of this investigation was to determine levels of micronutrients of pumpkin seeds of the varieties grown in the Lake Victoria basin, with the aim of establishing if there are any variations, thereby laying the foundation for indentifying better sources of specific micronutrients.

MATERIALS AND METHODS

Freshly harvested mature pumpkin fruits were purposively collected from pumpkin growing households randomly chosen from the lists provided by agricultural extension officers in four districts in the Lake Victoria Basin, Tarime ($1^{0}21^{1}0^{11}$S, $34^{0}22^{1}0^{11}$E, altitude range 900-1800 m above sea level), Jinja ($0^{0}25^{1}28^{11}$N, $33^{0}12^{1}15^{11}$E, altitude 1167 m), Gucha ($0^{0}25^{1}27^{11}$N, $33^{0}12^{1}15^{11}$E, altitude range 1000-2000 m) and Busia ($0^{0}1^{1}36^{11}$S, $34^{0}54^{1}32^{11}$E, altitude range 1130-1375 m). They were purposely selected to represent the key landscape features within the Lake Victoria Basin. The samples collected were kept for four weeks before they were cut and seeds removed. Husks were removed and weighed before crushing them in a motor using a pestle. The extractions were done. Standards were run at the same time as extracts. Calibration curve equations were derived and used to determine concentrations in each case.

β- Carotene and α- tocopherol

The Nyambaka and Ryle, (2001) procedure was adopted in the extraction though instead of petroleum ether, hexane was used. This protocol agrees with AOAC (1989) method. In each extraction about 2.00 g of the mashed samples were used. The extracts were purified and diluted to a standard volume of 25 ml in the mobile phase constituted as methanol; dichloromethane; water (79:18:3). Three extractions were done in duplicate for each fruit's seeds. The extracts were filtered using a 0.45 micro millipore filter before 30 µl were injected in a HPLC (Hitachi, model L4000H), pump (L6000), RP C_{18} column (25 cm x 4.5 mm)) rate set at 1 ml / min with a UV/Visible detector at 450 nm for β-carotene and 470 nm α-tocopherol.

Vitamin C

About 2.00 g of the mashed samples were mixed with 1% metaphosphoric acid, shaken with a mechanical shaker for ten minutes. The mixture was centrifuged and filtered. The residue was re
extracted two more times before topping up to 25 ml. The extracts were titrated with 2, 6-dichlorophenol indophenol (DCPIP) standardized with L (+) ascorbic acid.

The B complex Vitamins

The method of Vinas *et al*., (2003) was adopted in the extraction and determination, though there was use of methanol instead of acetonitrile in the mobile phase. About 2.00 g of the mashed sample was homogenized with 25 ml 0.1M HCl for ten minutes before heating in a

Table 1. Levels of micronutrients in seeds of different pumpkins varieties

Nutrient	Variety					
	Crown Prince	Green Kabacha	Carnival squash	Bottle gourd	Butternut squash	Banana squash
% moisture	21.29± 2.00	22.92 ±3.25	19.87 ±5.61	11.62 ±0.21	13.85 ±4.21	26.23 ±2.36
Vitamin C (mg)	2.09± 1.26	2.36 ±1.40	2.32 ±0.92	1.52 ±0.02	2.94 ±1.98	1.00 ±0.75
β-carotene (mg)	0.23 ±0.19	0.21 ±0.05	0.42 ±0.18	0.02 ±0.02	0.27 ±0.02	0.23 ±0.31
Thiamine(B_1) (mg)	0.22 ±0.03	0.54 ±0.11	0.32 ±0.11	0.18 ±0.03	0.22 ±0.06	0.31 ±0.23
Riboflavin(B_2) (mg)	0.59 ±0.09	0.49 ±0.13	0.68 ±0.17	0.39 ±0.09	0.35 ±0.01	0.79 ±0.68
Niacin(B_3) (mg)	1.04 ±0.02	2.33 ±1.15	0.99 ±0.35	0.43 ±0.06	0.82 ±0.04	0.59 ±0.41
Pyridoxine(B_6) (mg)	0.11 ±0.02	0.26 ±0.06	0.17 ±0.02	0.14 ±0.01	0.14 ±0.09	0.14 ±0.05
α–tocopherol mg	2.35 ±3.28	4.22 ±3.04	3.99 ±3.06	2.39 ±0.64	6.04 ±2.70	7.56 ±2.45
Iron (mg)	12.57 ±4.19	11.89 ±0.97	11.52 ±3.79	13.15 ±0.77	9.70 ±4.86	11.13 ±5.30
Zinc (mg)	7.21 ±0.27	7.12 ±0.92	7.19 ±2.34	4.25 ±0.71	9.70 ±4.86	5.92 ±2.22
Selenium (µg)	6.19 ±0.54	7.06 ±2.35	5.47 ±1.62	10.32 ±1.21	6.46 ±1.90	6.29 ±0.16

water bath set at 80^0 C for a similar duration. Sodium acetate was added for pH adjustment before 1.00 g of taka diastase was added and the mixture incubated at 50^0 C for two hours. Acetonitrile was added, shaken before the mixture was heated to 90^0 C in a water bath. The mixture was filtered, topped up to 25 ml and about 30 µl injected into the HPLC with UV/Visible detector set at 234 nm, 266 nm, 324 nm, 261 nm for thiamine, riboflavin, pyridoxine and niacin respectively. The concentrations were calculated from the integrated peak areas of the sample and corresponding standards. In each case, the mobile phase was 0.1 mM KH_2PO_4 (pH =6):CH_3OH (90:10) and flow rate fixed at 1 ml / 1 min with column operated at 25 ^{0}C.

Iron, Zinc and Selenium

Wet ashing using 10 ml of 69 % concentrated nitric acid on about 2.50 g of the dry sample was done. The digest was cooled, 2 ml perchloric acid was added before further heating. Filtration was done, topped up to 50 ml with distilled water. The standards and the sample were run in an AAS (Buck, model 210 VGP) machine at 247.93 nm and 324.8 nm for iron and zinc respectively. The hydride generation atomic absorption spectrophotometer, at wavelength 196.4 nm, was used for selenium.

Data Analysis

The SPSS version 11.5 program was run to give the mean levels of micronutrients; analysis of variance was done to check variations in micronutrients between the varieties from the same location and different locations. Duncan tests were performed to establish varieties which were extremely high in the tested micronutrients. Correlation coefficients were calculated to seek associations between the different micronutrients.

RESULTS AND DISCUSSION

Levels of micronutrients in seeds of different pumpkin varieties

The micronutrients in seeds of six pumpkin varieties were found to establish if any differences existed between varieties (Table 1).

A preliminary examination of the trends in the micronutrients shows variation in the levels between the varieties.

Moisture

Moisture varied widely ranging from 11.62 mg / 100 g in bottle gourd to 26.23 mg / 100 g in banana squash seeds. The moisture content in bottle gourd seeds has also been found in other studies. Hanaa and Hala (2010) in a separate study found it to be 8.38±0.24 % while Emuejeuoke and Marcella (2009) found it to be 10.50±2.00 %. Mohammed (2004) found similar values.

Vitamin C

The vitamin C in the seeds varied from 1.52 mg / 100 g in the bottle gourd to 2.94 mg / 100 g in butternut. There

Table 2. The F and P values obtained in the one way ANOVA of micronutrients in seeds of different pumpkin varieties

Nutrient	F- value	P- value
% moisture	6.142	0.002
Vitamin C	1.281	0.315
β-Carotene	6.529	0.001
Thiamine	4.341	0.012
Riboflavin (B_2)	1.351	0.288
Niacin (B_3)	5.291	0.004
Pyridoxine (B_6)	5.251	0.005
α–tocopherol	1.697	0.186
Iron	0.296	0.909
Zinc	3.163	0.038
Selenium	7.033	0.001

was a tendency to cluster implying limited variation of this micronutrient in the pumpkin seed varieties. Emuejeuoke and Marcella (2009) in a separate study found it to be 4.164 mg / 100 g.

β-carotene

The β-carotene varied widely from 0.02 mg / 100 g in bottle gourd to 0.42 mg / 100 g in carnival squash. The range covers average value of β-carotene in pumpkin seeds established at 0.24 mg / 100 g in other studies like Mateljian (2008), in which varieties of pumpkins derived from the three species i.e. *Cucurbita pepo*, *C. maxima* and *C. moschata* were analysed. A cross breed of *C. maxima* and *C. moschata* was also tested and the content of the *β*-carotene ranged from 0.06 to 7.4 mg / 100 g (Murkovic *et al.*, 2002).

The B-complex vitamins

Thiamine level in the seeds of the pumpkin varieties varied widely from 0.19 mg / 100 g in bottle gourd to 0.54 mg / 100 g in green kabacha. The riboflavin levels varied though there was a tendency of clustering around 0.5 mg / 100 g. The concentrations varied from 0.35 mg / 100 g in butternut to 0.70 mg / 100 g in crown prince. The niacin levels on the other hand varied widely from 0.43 mg / 100 g in bottle gourd to 1.03 mg / 100 g in crown prince. Pyridoxine levels ranged from 0.13 mg / 100 g in crown prince to 0.26 mg / 100 g in green kabacha. The levels of the B–vitamins established in this study are within the same range as found by Grubben, (2004) in a separate study.

α-tocopherol

The levels of α-tocopherol varied from 2.39 mg / 100 g in bottle gourd to 6.04 mg / 100 g in butternut though the levels tended to cluster around 4.0 mg / 100 g. In a similar study of the varieties of *cucurbita pepo*, Murkovic *et al.*, (1996), found a wide range of 41-620 mg / 100 g in gamma tocopherol but no significant difference in alpha tocopherol between the varieties conforming to the findings in this study.

The trace elements

The levels of iron in the pumpkin seed varieties were higher than any other micronutrient analysed in this study. It varied from 9.70 mg / 100 g in butternut to 13.15 mg / 100 g in bottle gourd with a tendency to cluster. The zinc levels however were evenly distributed ranging from 3.73 mg / 100 g in bottle gourd to 9.70 mg / 100 g in butternut. The selenium levels varied widely ranging from 5.47 µg / 100 g in carnival squash to 11.07 µg / 100 g in bottle gourd. Lazos (1986) and El Adawy and Taha (2001) in separate studies established the levels of iron in pumpkin varieties to average 13.66±1.60 mg / 100 g while zinc was 1.09±0.06 mg / 100 g. Emuejeuoke and Marcella (2009) reported much higher values of iron (36.99 mg / 100 g) and zinc (32.43 mg / 100 g) in bottle gourd they studied. The difference in levels in this case can be explained. Elemental composition of plants is affected by the plant nutritional status (Mcdonald, 1988), geographical area, climate, day length, soil type, incidence of pests and disease and cultivation practices (Garrow *et al.*, 1998; Woolfe, 1992).

The analysis of variance was then done on the levels of the micronutrients in the pumpkin seeds to support the preliminary examination. This was with a view to establish any significant differences between the varieties. Table 2 shows the results.

There were significant differences noted in moisture, β-Carotene, thiamine, niacin, pyridoxine and selenium ($p < 0.05$). Since varieties were the main factor that was changing during the analysis, it implies that the noted variation was because levels of micronutrients in the varieties differed significantly. These significant variations in micronutrients between the varieties are supported by earlier studies.

Achu, (2004) evaluated five species of *cucurbita* family and showed that moisture ranged between 4.33 and 7.25 per cent among the varieties. In the evaluation of the fluted pumpkin *Telfairia occidentalis* hook seeds, a

Table 3. The average levels of moisture and micronutrients obtained from the post hoc (Duncan's rank test).

Content	Subset α=0.5	Variety Crown prince	Butternut squash	Carnival squash	Green k.	Banana squash	Bottle Gourd
Moisture	1		13.86				11.7
	2		13.86	19.87	22.72	26.23	
	3	21.29		19.87	22.72	26.23	
β-Carotene	1						0.02
	2	0.23	0.27	0.42	0.21	0.24	
Thiamine	1	0.23	0.22	0.31		0.31	0.19
	2			0.31	0.54	0.31	
Niacin	1	1.03	0.82	0.99		0.57	0.43
	2				2.33		
Selenium	1	6.19	8.11	5.47	7.08	6.29	
	2						11.07
Zinc	1						3.73
	2	7.21	9.70	7.2	7.43	5.92	

Table 4. The varieties that had the lowest and highest of each micronutrient analyzed

content	Variety with lowest concentration	Variety with the highest concentration
Moisture	White gourd	Banana
β-Carotene	White gourd	Carnival squash
Thiamine	White gourd	Green Kabacha
Niacin	White gourd	Green Kabacha
Pyridoxine	Crown Prince	Green Kabacha
α-Tocopheral	White gourd	Banana
Zinc	White gourd	Butternut squash
Selenium	Carnival Squash	White gourd

within species study to compare the contents of selenium and iodine(Christian, 2007) showed moisture to be 10.93 per cent, iron to be 69 µg / g, vitamin A as 890 IU and vitamin C to be 0.7 µg / g as the nutritional status of this variety of pumpkin. In the evaluation of micronutrients (minerals) in pumpkins, Akiwaowo *et al.,* (2000) established the levels of iron as 9.82 mg / 100 g in the seeds and 6.8 mg / 100 g of zinc in old leaves. In an evaluation of the variation and identification of micronutrients' content in seeds , Einkom wheat Ozkan *et al.*, (2007) found that the contents of iron and zinc among the 54 accessions varied from 0.21 to 2.16 µg per seed (zinc) and from 0.54 to 3.09 µg per seed (iron). In another analysis of nutrient constituents from eight lines of naked seed squash (*cucurbita pepo* L.), Indouraine, (1996) reported significant variation in protein (37.1 ± 0.45% to 44.4 ± 0.45 %), oil (34.5 ± 0.42 % to 43.6 ± 0.06 %) and in all minerals except magnesium and manganese. It is therefore important to know the varieties that are high in the various nutrients in order to be used in alleviation of malnutrition. A post hoc analysis of the mean values to establish the varieties that actually differed in the micronutrient values was therefore done. Table 3 shows the results obtained.

Different varieties of pumpkin seeds differed in various micronutrients as in the ANOVA table 2.These differences were however between some of the varieties, for example, the differences in moisture of seeds were caused by the differences in butternut, bottle gourd which had low amounts as compared to banana, crown prince seeds which had higher amounts comparatively. Crown prince and bottle gourd seeds' low levels of β-carotene were significantly different from the levels in butternut, banana, and carnival squash seeds that had high levels. All the varieties had lower levels of niacin as compared to the levels in green kabacha. Bottle gourd seeds had higher amounts of selenium as compared to other varieties. Some of the varieties had comparable levels of some micronutrients; for example the level of β-carotene in green kabacha, carnival squash and banana variety seeds did not differ significantly. Table 4 shows varieties that exhibited the lowest and highest of each micronutrient analyzed.

The bottle gourd a *Lagenaria siceraria* seed exhibited lower micronutrients except in selenium than regular pumpkin varieties sampled. Green kabacha seeds had higher values of the B- complex vitamins while the butternut squash seeds are better in the zinc levels

Table 5. Levels of micronutrients in assorted pumpkin seeds from different districts (mg / 100 g DM)

Nutrient	Gucha district	Tarime district	Jinja District	Busia district
% Moisture	21.44±6.20	21.46±0.53	20.14±5.22	16.49±6.95
Vitamin C	2.04±1.38	2.29±1.37	2.62±1.12	2.05±0.48
β-Carotene	0.26±0.15	0.32±0.22	0.19±0.09	0.39±0.05
Thiamin	0.39±0.19	0.25±0.04	0.36±0.16	0.22±0.04
Riboflavin	0.61±0.39	0.69±0.21	0.47±0.09	0.55±0.18
Niacin	1.09±0.86	1.16±0.31	1.81±1.37	0.74±0.09
Pyridoxine	0.17±0.07	0.12±0.03	0.20±0.06	0.17±0.03
α-Tocopherol	4.54±4.04	4.62±3.63	3.62±3.62	5.65±2.48
Iron	10.24±3.63	14.59±3.94	10.94±1.70	11.67±1.34
Zinc	7.16±1.66	8.36±1.69	5.64±1.08	7.54±1.57
Selenium	6.43±2.03	6.51±0.59	6.44±2.08	6.55±3.41

Table 6. The F and P values of micronutrients in seeds from the districts

Nutrient	F-value	P-value
Moisture	0.315	0.814
β-Carotene	3.183	0.697
Vitamin C	0.485	0.048
Thiamine	0.588	0.630
Riboflavin	0.502	0.685
Niacin	0.231	0.874
Pyridoxine	1.479	0.252
α-Tocopherol	0.732	0.546
Iron	1.959	0.156
Zinc	3.301	0.043
Selenium	0.055	0.983

while niacin levels were comparatively high in the crown prince varieties.

The seed samples collected in a given district were grouped together and analyzed. This was disregarding the variety in order to compare whether micronutrients differ significantly between the four study sites. The results are shown in table 5.

The four locations had different mean values for the various micronutrients. The β-carotene ranged from 0.26±0.15 mg / 100 in Gucha, 0.32±0.22 mg / 100 g in Tarime, 0.19±0.09 mg / 100 g in Jinja, 0.39±0.05 mg / 100 g in Busia. Iron content ranged from 10.24±3.63 mg / 100 g in Gucha, 14.59±3.94 mg / 100 g in Tarime, 10.94±1.70 mg / 100 g in Jinja while Busia varieties had 11.67±1.34 mg / 100 g. Dry matter content can vary widely depending on the cultivar, geographical area, climate, soil type, incidence of pests and diseases (Garrow *et al*., 1998; woolf, 1992). Seeds as biological material are subject to random variation in the mineral content (Greenfield and southgate, 1992; Torelm and Damubon, 1998). In the study of natural variation and identification of micronutrients content in seeds of Einkom wheat in four locations in Germany, Turkey and Italy Ozkan *et al*., (2007) reported different mean values varying from 1.09 to 2.16 µg per seed zinc and from 0.83 to 1.97 µg per seed iron, 1.43 to 1.97 µg per seed manganese and 0.14 to 0.24 µg per seed copper.

An ANOVA of the micronutrients between districts in the study area was done to establish if there were significant differences. Table 6 shows the results.

Vitamin C and zinc varied between the districts while all other nutrients in the assorted pumpkins did not show significant variations in the nutrients. The correlation coefficients of micronutrients in seeds were calculated and table 7 shows the results.

At $p = 0.05$ significance level, some of the micronutrients had negative correlations. Both selenium and β-carotene are negatively correlated with moisture. The β-Carotene, the B-complex, iron and selenium were all negatively associated with vitamin C. The trace elements were also negatively associated with thiamine (B_1). These negative correlation coefficients means that high level of one micronutrient influences the level of the other micronutrients negatively. This can be explained using the physicochemical properties of some of the micronutrients. The oxidizing property of vitamin C must affect the levels of Fe^{2+} which easily turn to Fe^{+3}. Enzymes such as ascorbic acid oxidase, phenolase can oxidize ascorbic acid. In a non enzymatic oxidation process, copper and iron salts catalyses the oxidation (Milindi, 2008), thereby justifying the vitamin C and iron correlation of $r = -0.33$ established in this study. Similarly, since β-carotene is easily oxidized it should not co-exist positively with vitamin C.

Table 7. Correlation coefficients of micronutrient in the seeds**.**

Nutrient	Moistue	Vit. C	β-Car.	B_1	B_2	B_3	Fe	Zn	Se
% Moisture	I								
Vitamin C	0.04	I							
β- carotene	-0.09	-0.19	I						
B_1	0.34	-0.07	0.01	I					
B_2	0.22	-0.34	0.30	0.35	I				
B_3	0.31	0.04	0.03	0.75**	0.01	I			
Fe	0.08	-0.33	0.41	-0.16	-0.01	0.07	I		
Zn	-0.14	0.08	0.57**	-0.04	-0.07	0.11	0.53*	I	
Se	-0.46*	-0.24	-0.13	-0.01	-0.05	-0.09	0.30	0.21	I

* correlation is significant at 0.05 level (2 tailed) , ** correlation is significant at 0.01 level (2 tailed)

The β-carotene, iron and zinc were positively correlated conforming to a separate study in which there was a positive correlation in the sweet potato germplasm between beta-carotene, iron, and zinc (January 2011). Both zinc and iron levels were positively correlated ($r = 0.53$). This implies that a high level of one nutrient influences positively the level of the other nutrients. The association between zinc and iron has been reported in the study of natural variation and identification of micronutrients contents in the seeds of wheat (Ozkan, 2007). Muhamba and Msola (2010) did a study on the diversity of common bean genotypes in iron and zinc. Results showed a positive and significant correlation ($r = 0.416$; $P < 0.001$) between iron and zinc, suggesting that genetic factors for increasing iron and zinc are co-segregating with genetic factors for increasing zinc. This is in conformity with this study.

CONCLUSION AND RECOMMENDATIONS

The carnival squash pumpkin seeds on average, represents the basis of reducing and controlling nutritional deficiencies related to lack of enough intake of micronutrients β-carotene, the B-complex vitamins, iron and zinc (table 3). Specifically however, the green kabacha seeds are high in the B-complex vitamins, the bottle gourds are high in selenium and butternut squash seeds have higher levels of zinc. Backed with nutritional education, pumpkin seeds could compliment existing strategies to combat micronutrient malnourishment within the Lake Victoria basin, disaster management sites in most developing countries. They also form the basis for recombination technology to increase these micronutrients content through bio fortification to levels which are higher than the current ones. Through cycles of recurrent selection, pumpkin varieties with superior concentrations of the micronutrients can be bred by plant breeders. The high levels of the anti oxidants and zinc in pumpkin seeds offers them as one of the food choices for people living with HIV and AIDS.

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