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Bioavailability Studies of Vitamin A and E in Indigenous Vegetables and their Potential Use in the Management of HIV and AIDS

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Authors' contributions

This work was carried out in collaboration among all authors. The author have read and approved the final draft.

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ABSTRACT

Levels of vitamins in selected indigenous vegetables in Butula, western Kenya were determined and foods containing high levels of vitamin A and E were used to make food formulation. The bioavailability of these vitamins in food formulation was estimated using algorithm procedure.
Determination of β-carotene and α-tocopherol content was done using HPLC procedure. Fresh blanched vegetables contained high levels of β-carotene; 4000 − 9700 µg/100g and α-tocopherol levels; 3000 − 7350 µg/100g. Solar dried vegetables contained β-carotene levels ranging from 572 − 854 µg/g and α-tocopherol levels ranging from 281 to 673 µg/g dry weights. Solar dried vegetables contained significantly lower ($P<0.05$) amounts of β-carotene and α-tocopherol as compared with fresh vegetables. The mean serum retinol α-tocopherol and β-carotene levels were 0.937, 0.144 and 17.787µmol/l respectively. Bioavailability estimated using algorithm indicated a +2.17 change in serum β-carotene and +7.776 changes in serum α-tocopherol, a positive indication that consumption of indigenous vegetables can meet the recommended dietary allowances of vitamins A (750 µg retinol equivalent/day) and E (8 mg/day). The bioavailable vitamins are capable of boosting the immune system and therefore delay early use of ARV'S.

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Keywords: Indigenous vegetables; bioavailability; β-carotene; α-tocopherol; algorithm.

1. INTRODUCTION

Bioavailability relates to the proportion of ingested nutrient that is absorbed, retained and metabolized through normal pathways to exert normal physiological function [1].The chemical analysis of such vitamins will yield measures of the total vitamin contents which will be overestimates of the biologically relevant amounts. Nutrients bioavailability in food may be estimated by *in-vivo* or *in-vitro* methods. *In-vivo* methods use metabolic balance technique in animals or small scale human clinical trials, whereas *in-vitro* methods use simulated gastrointestinal digestion of food and determination of nutrients solubility [2] or dialysis through artificial membrane [3] or uptake by caco-2 cells using radio labeled nutrients or ferritin levels in the cell [4,5]. Although *in vivo* methods determine bioavailability of nutrients in natural physiological conditions, where regulatory mechanism and transit time are not altered [6], they suffer from large inter individual variations and their use is limited due to high cost and ethical issues involved [7]. On contrary, *in-vitro* methods are cheap, flexible and rapid [7]. *In-vitro* methods have been used to develop better iron supplement and screen cultivars for bioavailable nutrients, because the results of *in-vitro* methods correlate to nutrients bioavailability determined by *in-vivo* methods [5,8] thus providing a useful bioavailability index that allows ranking of foods. Some algorithms have been developed for quick assessment of bioavailability of a few nutrients ([9], Miller et al., 2007), but they generally have poor predictions, because they do not factor degradation of inhibitors and enhancers resulting from storage and cooking, and the food composition data used with the algorithms are often incomplete and may not be accurate [4,5]. However, algorithms provide a cheaper and faster way of assessing bioavailability of nutrients from different diets, therefore are widely used in the dietary surveys. Algorithms are also used to screen foods for unknown dietary factors by comparing their predictions with the more accurate *in-vivo* or *in-vivo* methods [9].

Bioavailability algorithms or mathematical models used to estimate nutrient bioavailability from different diets have great appeal because of the potential to apply general principles to a complex dietary matrix, the increase in predictability without direct measurement of absorption and retention and ability to facilitate dietary assessements and recommendations [10]. The procedure is useful especially in populations with low nutrient status [1].

The role of nutrition in the management of HIV/AIDS has assumed increasing importance over the past decade because nutritional status affects the progression of HIV to AIDS and survival of HIV individuals (FANTA, 2004; Piwoz and Preble, 2004). Some of the factors contributing to the high HIV/AIDS prevalence include poverty, adoption of foreign lifestyles, socio-cultural practices such as wife inheritance, cross border movements, stigma and discrimination (NACC, 2005). The immune system in HIV patients is compromised, resulting in prolonged illness, reduced appetite and interference with the body's absorption of nutrients (Baum, 1994; Piwoz and Ellen, 2000). HIV infection is characterized by a high preference of micronutrients deficiencies and wasting that vary considerably among different HIV infected population (Baum and others 1995). Micronutrients deficiencies are a common occurrence due to malabsorption or altered metabolism and habitual consumption of poor diets (Baum and others 1995; Semba and others 1995). High intake of foods with α-tocopherol and β-carotene has been associated with reduced progression of HIV to AIDS and improved survival (Semba and others 1995).

PLWHA in some regions like in Busia County in Kenya may be accessible to indigenous vegetables rich in immune boosting micronutrients but do not take these vegetables regularly and in right combination. The aim of the study was to determine the bioavailability of vitamin A and E in the indigenous foods with a view of preparing locally acceptable food formulation that will improve availability of the vitamins in PLWHA.

The level of vitamins in foods does not necessarily translate to similar levels biologically available in the body after consumption because their bioavailability is affected by such factors as differences in biopotencies, significant losses during storage, processing and/or cooking (Alan, 1995), affect the absorption of some vitamins by affecting intestinal transit time or/ the enteric formation of mixed micelles.

2. MATERIALS AND METHODS

2.1 Sampling and Food Formulation

Fresh raw vegetables samples were collected from Butula market and surrounding household gardens. The fresh vegetable samples were thoroughly washed under tap water and destalked to remove all inedible parts before shredding according to household practice and blanched for 1-3 minutes in boiling water and solar dried using an indirect solar dryer. The solar dried samples were stored under nitrogen flashed polythene bags, and then sealed tightly to prevent any oxygen getting in.

2.2 Apparatus and Reagents

All the glassware used was cleaned with chromic acid followed by a washing detergent. They were then rinsed with distilled water. Before the glassware was used in the analysis of fat soluble vitamins they were rinsed with methanol. Standard reagents (α-tocopherol and β-carotene) and all other chemicals and reagents were analytical grade from Sigma Aldrich (Steinhelm, Germany).

2.3 Instrumentation

The Hitachi HPLC chromatograph was used (model L-6000, Tokyo, Japan) for β-carotene and α–tocopherol analysis. The detection wavelength was 297nm for both β-carotene and α–tocopherol in the vegetables.

2.4 Procedures for Analysis

2.4.1 Extraction of β–carotene and α–tocopherol in vegetables

Twenty five grams of vegetable samples were blended for 5 minutes with 0.3 g ascorbic acid into a puree. Five grams of the pretreated sample was weighed and transferred into 150 ml round bottomed flask. A mixture of hexane and dichloromethane (30 ml) in the ratio of 3:2 was added to the flask, shaken for 2 minutes and the hexane layer decanted into a 250 ml separating flask after allowing for separation. The residue was similarly re-extracted with 50 ml of n-hexane three times before washing the combined hexane layer with 50 ml of saturated potassium hydroxide in methanol followed by portions of 50ml distilled water until there was no colouration on phenolphthalein indicator. The hexane layer was then dried over anhydrous sodium sulphate and evaporated to dryness under a stream of nitrogen. The residue was immediately dissolved in 10ml of methanol. An aliquot of the solution was filtered with 0.45 µm millipore filter and injected into HPLC column.

2.5 Method Validation

The methods for β-carotene and α-tocopherol analysis were validated by regression and recovery studies. Regression analysis for β-carotene gave the equation y = 6459.2 x + 2066.9 and the correlation coefficient ($R^{2)}$ of 0.9975, while α-tocopherol gave the equation, y = 1429.8 x and r^2 = 0.9973. The correlation coefficient, comparable to other studies indicates that over 99.7% of the HPLC response relates linearly to the concentration of standard solutions [11-15]. The calibration curves were used to determine the concentrations in the vegetable samples. The coefficients of variation ranged from 2.0 % to 3.1 % for β-carotene and 2.3 % to 6.3 % for α-tocopherol, while mean recoveries of 95.5% and 93.6% respectively (Table 1). The results indicate that the extraction process was satisfactory and no significant losses occurred during the extraction and analysis process.

2.6 Levels of β-carotene and α-tocopherol in the vegetables

Table 2 shows the β-carotene and α-tocopherol levels in dry and fresh vegetables. The β-carotene content in the dry vegetable samples ranged from 548.00 µg/g to 854.00 µg/g dry matters (DM) while in the fresh vegetables it ranged from 7000 µg/100g to 9700 µg/100g. The α-tocopherol content ranged from 281.60 µg/g to 693.55 µg/g DM in the dry vegetable samples and from 2800 µg/100g to 7500 µg/100g in the fresh vegetables. The concentration of β-carotene and α-tocopherol varied with the individual vegetables, with the frying spider having the highest β-carotene level and nightshade had the highest α-tocopherol level. While slender leaf had the lowest (Table 2).

Table 1. Percentage recovery and reproducibility results of β-carotene and α-tocopherol in some vegetables

Material	β-carotene				α-tocopherol			
	Recovery	CV	Regression equation	R^2	Recovery	CV	Regression equation	R^2
Standards			Y = 6459.2x + 2066.9	0.9975			Y= 1429.8x	0.9973
Flying spider		2.0				6.3		
Amaranthus	97.6	3.1		48.8	90.5	3.1		54.3
Nightshade	98.4	-		49.2	97.5	-		78.0
Pumpkin leaves	94.8	3.1		47.4	95.3	2.3		76.2
Cowpea leaves	91.2	2.9		54.7	90.8	3.1		54.3
Serum								

Table 2. β-carotene and α-tocopherol Mean content in µg/g (± Standard deviation) of some selected vegetables (dry matter) and µg/100g wet weight

Vegetable	β-carotene dry matter	α-tocopherol dry matter	β-carotene wet weight	α-tocopherol wet weight
Cow pea leaves	680.00 ± 4.35	513.60 ± 13.95	7437 ± 391.38	6400 ± 582.54
Pumpkin leaves	548.00 ± 54.68	693.55 ± 66.53	8000 ± 604.18	7350 ± 941.60
Amaranthus LL	650.00 ± 9.065	653.63 ± 48.67	7400 ± 337.40	6750 ± 714.83
Slender leaf	572.60 ± 43.68	281.60 ± 117.70	7000 ± 226.21	2800 ± 778.13
Frying spider	854.00 ± 82.17	445.75 ± 44.29	9700 ± 1246.72	3000 ± 702.54
Night shade	717.00 ± 20.90	680.60 ± 60.74	7625 ± 462.44	7500 ± 998.30

Table 3. Bioavailability of vitamins in indigenous vegetables obtained using algorithms

Vegetable	β-carotene(µg/g) dry matter	α-tocopherol (µg/g) dry matter	Serum β-carotene change (µmol/l)	Serum α-tocopherol change (µmol/l)
Cowpea leaves (CL)	580	514	0.025	4.873
Pumpkin leaves	548	694	0.004	4.903
Amaranthus	650	654	0.019	4.897
Slender leaf	572	281	0.020	4.840
Frying spider (FS)	680	446	0.091	4.862
Nightshade	717	681	0.115	4.901
CL+FS	1260	960	0.472	4.947

The values for β-carotene and α-tocopherol obtained in this study are generally within the range of values reported although some values obtained cannot be directly compared. The variation could be due to the fact that β-carotene and α-tocopherol content is dependent on sample varieties, stage of maturity, soil fertility, climate or geographical site of production, harvesting and post harvesting handling, processing, storage conditions and different methods of analysis [14,16].

2.7 Bioavailability Studies

The algorithm equations were developed from literature studies on β-carotene and α-tocopherol intervention using vegetables and carrying out regression analysis of data on absorption and retention. Two mathematical models were developed using β-carotene and α-tocopherol nutrition intervention data obtained from similar studies in the internet. Data collected was subjected to multiple linear regression analysis. β-Carotene and α-tocopherol absorption were each separately regressed against dietary, pre-study serum and duration of intake of the diet. Multiple regression analysis was used to develop the algorithms for determining the bioavailability of β-carotene and α-tocopherol in indigenous foods. The data on β-carotene used are: absorption ranged from 0.01 to 7.8435 µmol/l, the duration of intake ranged from 30hours to 4 years, the number of subjects ranged from 2 to 29,133, while the amount of β-carotene administered ranged from 1.32 to 25 mg ([17,18]; Cheryl and others 1992; [19]; Ribaya and others 2007; Matti and others 1997; Francois and others 1999; Sabrina and others 2002).

3. RESULTS AND DISCUSSION

3.1 Bioavailability of Vitamins in Indigenous Vegetables by use of Algorithms

The bioavailabilty of vitamins in indigenous vegetables was estimated by use of algorithms developed and the results are shown in Table 3. β-Carotene absorbed was regressed against the duration of intake of supplement (days), the dietary level of β-carotene and the pre-study serum level of β-carotene (µmol/l) and results presented in Table 4. The dietary level of β-carotene had (B=0.657; p<0.000), pre-study serum level (B=12.806; P<0.000) while duration of study (B=0.009; P<0.002). Pre-study serum level was the most influential positive significant predictor. The three predictors accounted for over half of the variance in absorption (r^2=0.877) which was highly significant. Therefore the regression equation 1 is given as follows.

$$Y= -3.010 +12.806X+0.657Z+0.009W \quad (1)$$

The B's (-3.010, 12.806, 0.657 and 0.009) are the regression coefficients representing the amount the dependent variable Y changes when the corresponding independent changes by one unit. In the equation pre-study serum β-carotene level is represented by X, dietary β-carotene levels is Z, duration of study (days) was W. The equation shows that the change in serum β-carotene level is partly dependent on dietary β-carotene levels, pre study serum and duration of intervention. -3.010 is the constant, where the regression line intercepts the y axis, representing the amount the dependent (Y) will be when all the independents variables are zero. The negative constant (-3.010) may be due to influence of absorption efficiency by other food related factors such as food matrix effect, cooking technique, dietary fat, dietary interactions and host related factors such as clinical status, age, metabolic rate and physiological changes [1].

The model validity, goodness of fit, satisfaction of regression assumptions and quality of parameter estimates were evaluated using standard statistical criteria. The goodness of fit as reflected by r^2=0.877 is supportive of the model indicating that 87.7% of the variance is explained by the model. Plots of residual squares (Fig. 1 and 2) obtained from regression analysis of β - carotene absorption show that, assumptions about linearity, normal distribution and equal variances were met.

The data for α-tocopherol used are: absorption ranged from 0.17 to 27.2 µmol/l, the duration of intake ranged from7days to 3 years, the number of subjects ranged from 5 to 29,133, while the amount of α-tocopherol administered ranged from12.7 to 800 mg ([18,19]; Matti and others 1997; [20]; Richard and others 2006; [17]).

The dietary level of α-tocopherol (B=0.166; P<0.000) and duration of study (B=0.005; P< 0.000) were the most influential positive significant predictors as shown in Table 5. Pre-study serum had a negative coefficient (B= -0.702; P<0.008) that was insignificant. The predictors accounted for over a half of the variance (0.731) in absorption which was highly

significant at P<0.000. Therefore the regression equation 2 was as follows.

$$Y=16.824 – 0.702X +0.166Z+0.005W \quad (2)$$

The B's (16.824, –0.702, 0.166 and 0.005) are the regression coefficients representing the amount the dependent variable Y changes while the corresponding independent changes one unit. Pre-study serum α-tocopherol level is represented by X, dietary α-tocopherol level is Z and duration of study (days) is W, where Y is change in serum α-tocopherol level. The equation shows that the change in serum α-tocopherol level is partly dependent on dietary α-tocopherol levels, duration of intervention and partly dependent on pre study serum. The positive constant (+16.824) may be due to influence of absorption efficiency by several foods related factors such as dietary fat and host related factors [1].

The model validity, goodness of fit, satisfaction of regression assumptions and quality of parameter estimates were evaluated using standard statistical criteria. The goodness of fit as reflected by r^2=0.731 is supportive of the model indicating that 73.1% of the variance is explained by the model. Plots of residual squares (Figs. 3 and 4) obtained from regression analysis of α-tocopherol absorption show that, assumptions about linearity, normal distribution and equal variances were met.

3.2 Bioavailability of the Vitamins

The regression analyses were used to estimate the bioavailability of the vitamins in cowpea leaves, pumpkin leaves, amaranthus, slender leaf, frying spider and nightshade and results are presented in Table 5. There was a positive change in the serum β-carotene and α-tocopherol levels determined by use of algorithms.The serum β-carotene change ranged from +0.020 to +0.115. Nightshade which had a high β-carotene level (717 µg/g) produced the highest change in serum β-carotene (+0.115) while pumpkin leaves which had the least β-carotene level (548 µg/g) produced the lowest change in serum β-carotene (+0.020). The serum α-tocopherol change ranged from +4.840 to +4.903. Pumpkin leaves which had a high α-tocopherol level (694 µg/g) produced the highest change in serum β-carotene (+4.903) while slender leaf which had the least α-tocopherol level (281 µg/g) produced the lowest change in serum β-carotene (+4.840).

The results are comparable to a study carried out by Wachira 2008 [21] who reported an increase in β-carotene and α-tocopherol levels after a 3 months intervention study with PLWHA using a food supplement consisting of cowpea leaves, pumpkins and carrots. At the onset of the three months intervention study the mean β-carotene level was 77.6 ± 58.93 µg/dL while at the end term the mean β-carotene level was 82.1 µg/dL ± 61.82 µg/dL. The mean α-tocopherol at the on-set of intervention period was 648.44 ± 186.83 µg/dL while at the end term the α-tocopherol mean level was 711.0 ± 216.15 µg/dL. It was therefore hypothesized that the bioavailabilty of β-carotene and α-tocopherol in blood serum increases with consumption of green leafy vegetables.

The low relative bioavailabilty of β-carotene from vegetables is in line with reports from other studies. De Pee and others 1995 [22] reported only 7% relative bioavailabilty of β-carotene from green leafy vegetables. Later De Pee and others 1998 [23] reported a relative bioavailabilty of 23% for β-carotene from green leafy vegetables and carrots. The presence of carrots in the diet may have resulted to have higher bioavailability. Miller,1988;Nawiri,2008;Nderitu,2006;Nyambaka, 1988).

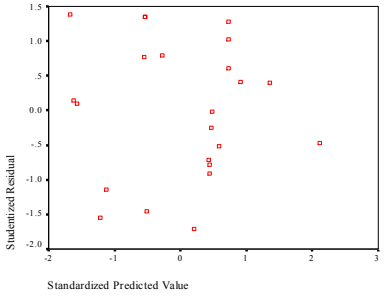

Fig. 1. Regression residual plot for β-carotene

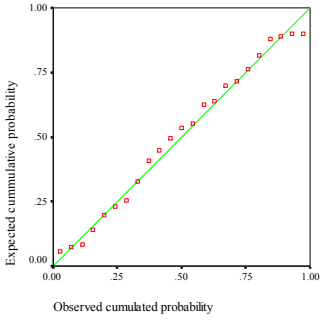

Fig. 2. Normal probabilty plot of regression residuals β-carotene

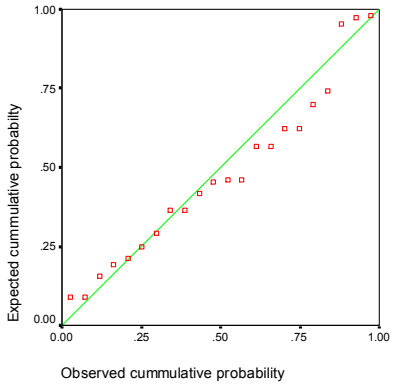

Fig. 3. Normal probability plot for α-tocopherol

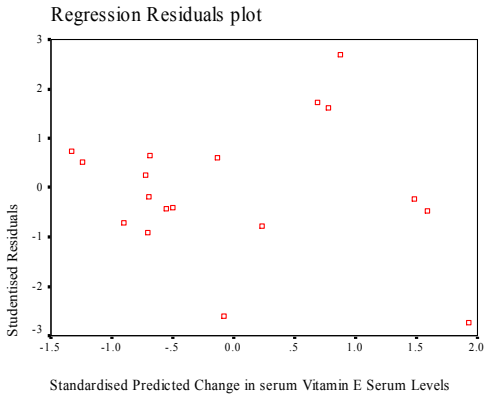

Fig. 4. Regression residuals plot for α-tocopherol

Table 4. Vitamins status of PLWHA in Butula division

Vitamin	Mean serum level (µmol/l)	Serum level range (µmol/l)	Number of male PLWHA		No of female PLWHA	
			deficient	normal	deficient	Normal
β-carotene	0.144	0.080-4.029	6	10	20	21
α-tocopherol	17.787	1.602-28.051	5	11	17	24
Retinol	0.937	0.022-0.835	5	11	18	23

Table 5. Regression analysis for β–carotene and α-tocopherol

Vitamin	Predictor variables	Unstandardized coefficients (B)	P=value
β – Carotene			
	Constant	-3.010	0.000
	Pre- study serum level(µmol/L)	12.806	0.000
	β-carotene levels in food (mg)	0.657	0.000
	Duration of study(days)	0.009	0.002
α-Tocopherol			
	Constant	16.824	0.006
	Pre- study serum level(µmol/L)	- 0.702	0.008
	α-Tocopherol levels in food (mg)	0.166	0.000
	Duration of study(days)	0.005	0.000

Dependent variable: change in serum vitamin µmol/l
Adjusted R^2 = 0.877 for β–carotene and R^2 = 0.731 for α-tocopherol

Table 6. Preparation of indigenous food mixture

Vegetables	β-carotene µg/g (dry weight)	α-tocopherol µg/g dry weight
Cowpea leaves (CL)	580	514
Frying spider (FS)	680	446
Total	1260	960
RDA	750 µg RE/day	8 mg/day
Serum β-carotene change	+2.170	
Serum α-tocopherol change	+7.776	

Cowpeas leaves(CL) and frying spider(FS) were used to prepare food supplement because they contained high levels of β-carotene and α-tocopherol and are grown in large quantities. The food supplement consists of cowpea leaves and frying spider in the ratio of 1:1. Table 6 shows nutrients composition used to prepare the food supplement. The vitamin A value is calculated by dividing β-carotene by 6 as approved by WHO/FAO (FAO/WHO 1988). One gram of cowpea leaves produces 580 µg while one gram of frying spider produces 680 µg making up a total of 1260 µg in 2 grams. Therefore 2 g of CL+FS mixture provide 1260 µg β-carotene which is equivalent to 210 µg RE vitamin value obtained by dividing by 1260 µg by 6. The contribution to the RDA is based on the assumption of 100% bioconversion that is set at 750 µg β-carotene and 8 mg α-tocopherol for adults. Required mixture to meet RDA is 7.14g of CL and FS mixture in the ratio of 1:1.α-tocopherol RDA is set at 8 mg/day for adults. Two grams of CL+FS mixture provide 960 µg/g with cowpea leaves contributing 514 µg/g while frying spider contributing 446 µg/g to the mixture. Required mixture to meet the RDA is 16.67 g CL+FS mixture in the ratio of 1:1. If 18 g of the CL+FS mixture was to be taken by each of the PLWHA on daily basis the mixture would provide 2.5xRDA β- carotene and 1xRDA α-tocopherol. The food supplement should be taken together with other foods in order to provide all the nutrients required by the body. Algorithms for β-carotene and α-tocopherol were used to determine the effect the food supplement would have on the serum levels. There was a positive change in serum β-carotene and α-tocopherol levels by +2.17 and +7.776 respectively thus indicating an improvement in the bioavailabilty of these nutrients in the blood.

4. CONCLUSION

Bioavailability studies using algorithms show that all the selected indigenous vegetables produce a positive change in serum levels with β-carotene showing a positive change of range between +0.019 to +0.472 while α-tocopherol shows a a positive change of range +4.862 to +4.947. The positive change in serum level would indicate an improvement in bioavailability of the two nutrients in the blood, hence strengthening the immune system of PLWHA and delay in the progression of HIV and early use of ARV's.

ETHICAL APPROVAL

Ethical apptoval was taken to carry out the study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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