Provitamin A carotenoids content and bioaccessibility in the modified local diet for children aged 6-23 months in Bukoba, Tanzania

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ABSTRACT

Dietary diversification is one of the strategies to address nutrient deficiencies. The study modified eight diets for children aged 6-23 months in order to improve diversify; vitamin A content and its bio-accessibility. All samples were analysed in triplicates. Three carotenes; all-trans α-carotene, 13-cis-β-carotene and all-trans β-carotene were determined by High Performance Liquid Chromatography. Bio-accessibility was assessed using *invitro* bio-accessibility model in three phases; simulated gastrointestinal system oral, gastric and intestinal. The analysed provitamin A Carotenoids (pVACs) were converted into 'Retinol Activity Equivalents' (RAE), and determined total RAE of the modified diets ranged from 8.8 to 137.4 µg/100g, and after *in-vitro* digestion ranged from 0.87 to 13.3µg/100g. The bio-accessibility of pVACs ranged from 12.2% to 33.6%. In cooked food, pumpkin fruit contributed high amount of provitamin A followed by palm oil, '*Bira'* and amaranths. '*Bira*' banana variety contributed high pVACs than local '*Nshakala'* banana variety. pVACs from pumpkins leaves were more accessible than those from amaranths and red palm oil fruit. Our results suggests that when carrying out interventions to improve diets, it is very important to take into account the estimation of dietary source of vitamin A and pVCAs and their bio-accessibility to meet nutritional requirements for vitamin A.

Keywords: Diet modification, recipes, children, vitamin A, bioaccessibility

INTRODUCTION

Dietary modification is one of the strategies to address nutrient deficiencies by increasing nutrient adequacy intake. Vitamin A is an essential nutrient though needed in small amounts by humans body, it plays a vital role on the normal functioning of the visual system; growth and development; and maintenance of epithelial cellular integrity, immune function, and reproduction. The dietary needs for vitamin A are normally provided as preformed retinol from animal source and provitamin A carotenoids (pVACs) from plant sources. Provitamin A carotenoids are usually converted to the active forms (retinol) to be used by the body (Bailey *et al.,* 2015; FAO, 2004). Apart from preventing vitamin A deficiency, carotenoids rich foods protects human bodies against chronic diseases including cancers, cardiovascular disease, diabetes, cataracts, some inflammatory diseases, and age-related muscular degeneration due to their antioxidant properties (Englberger et al., 2003; Englberger *et al.,* 2004;

Table 1. Food recipes and their ingredients used in the study

Recipe name	Recipe code	Food Ingredients in the Recipe
Banana purée '*Nshakala*' dry bean	1N	EAHB '*Nshakala*' variety, dry red kidney beans, amaranths, palm oil, salt, onion, tomatoes, bitter tomatoes
Banana purée '*Nshakala' fresh bean*	2N	EAHB '*Nshakala*' variety, fresh red kidney beans, pumpkin leaves, sunflower oil, salt, onion, tomatoes, bitter tomatoes
Banana purée '*Bira*' dry bean	2B	AAB '*Bira*' variety, dry red kidney beans, amaranths, palm oil, salt, onion, tomatoes, bitter tomatoes
Banana purée '*Bira*' fresh bean	4B	AAB '*Bira*' variety, fresh red kidney beans, pumpkin leaves, sunflower oil, salt, onion, tomatoes, bitter tomatoes
'Katogo'/'matoke' *katogo'or 'matoke'* is a local recipe prepared from banana, beans and other ingredients purée	5N	EAHB '*Nshakala*' variety, pumpkin fruit, groundnuts flour, salt, onion, tomatoes, bitter tomatoes
Orange-fleshed sweet potato porridge	6OFSP is orange fleshed sweet potato rich in vitamin A; kabode variety	Fermented maize flour, orange fleshed sweet potatoes, groundnut flour, sugar
'*Bira'* porridge	7B	Fermented maize, AAB '*Bira*' variety, groundnut flour, sugar
Egg porridge	8E	Fermented maize flour, egg, red kidney beans, sugar

Etcheverry et al., 2012; Nagao, 2009). The most common six provitamin A carotenoids include β-carotene (the carotenoid with the most provitamin A activity), α-carotene and β-cryptoxanthin (McLaren and Frigg, 2001). Other three carotenoids which cannot be converted to retinol are lutein, zeaxanthin, and lycopene (Mezzomo and Ferreira, 2016). The absorption of pVACs by the body depends on its availability. Bioavailability is the fraction of ingested nutrients that is available for utilization in normal physiological functions and/or for storage, while Bio-accessibility is the amount of nutrient released from the food matrix in the gastrointestinal tract and accessible for absorption (Etcheverry *et al.,* 2012).

Vitamin A deficiency (VAD) during infancy can cause long-term health problems that cannot be reversed even with adequate intake later in life (Ekesa *et al.,* 2011; Grantham-McGregor *et al.,* 2007). These deficiency increases the severity of infections such as measles and diarrhea disease in children (NBS, 2016). VAD affects over two billion people worldwide (Bailey *et al.,* 2015; Detzel and Wieser, 2015). In Tanzania, about one third of children below five years of age are vitamin A deficient (NBS, 2016). Particularly in Kagera region 47% of children below five years of age are vitamin A deficient (ICF-Macro, 2011; NBS, 2016). Interventions to address VAD include high-dose vitamin A capsule distributed to children (NBS, 2011) and, to a lesser extent, vitamin A fortified-foods such as, vegetable oil and fats, and flour (Hotz, 2012). Complementary foods are expected to bridge the gaps in energy and nutrients between daily requirements for children and the amount consumed. The most reliable and sustainable source of vitamin A in children is through dietary intake. However, many children are always fed from monotonous starch based diet with limited vitamin A rich foods. Godson (2014) conducted a control study, in Bukoba district in Kagera region and observed that local diets prepared with banana and bean had low vitamin A content (44µg RAE/100g) which is far below the recommended daily allowance (RDA) for children below five years of age for vitamin A of 400 µgRAE/day (recommended safe intake level) (Dewey, 2003; FAO, 2004). The amount is also below the Estimated Average Requirement (EAR) of 210µgRAE/day (Dewey, 2003). This clearly showed the importance of complementary food with micronutrients source to contribute to the nutrient requirements of children. This study used participatory dietary modification strategy to modify eight food recipes by diversifying the food ingredients per recipe to increase level and intake of vitamin A. The availability of Provitamin A and its bio-accessibility from the eight modified food recipes from plant sources was determined.

MATERIALS AND METHODS

Eight food recipes including five banana-based from EAHB '*Nshakala' Nshakala*' is East African Hybrid Banana (EAHB) local variety and AAB '*Bira' 'Bira'* is triploid hybrid of *Musa acuminata* and *Musa balbisiana*

(AAB) varieties, and three maize-based porridges were developed in combination with other ingredients (Table 1), to improve diversity of food items in order to enhance intake of vitamin A were used in this study.

Study Area

Dietary modification was conducted in Izimbya ward of Bukoba district in Kagera region of Tanzania. The region is located in the north-western Tanzania, west of Lake Victoria. The main farming system in the study area is banana farming system integrated with coffee and other annual crops.

Sample Preparation and Processing

The food ingredients were purchased from the local market at Izimbya ward, Tanzania. They were properly packaged in an aerated carton and air-freight to the department Food Science and Biotechnology at BOKU University in Vienna, Austria for laboratory analysis. Triploid hybrid of *Musa acuminata* and *Musa balbisiana* (AAB) '*Bira*' variety were obtained from neighboring country, Burundi, because no mature fruit bunches were available in Tanzania at the time sample collection. The banana varieties were transported on the morning of the harvest from Burundi to Kampala, Uganda where they were air-freight to Vienna, Austria within 48hrs of harvest.

On arrival at BOKU laboratory, all food samples were stored in a cold room maintained at 4ºC for 24 hours. The recipes were prepared and cooked according defined procedures (Mbela *et al.,* 2017 –In press). Sunflower cooking oil was added during cooking to increase bio-accessibility of provitamin A. All food samples and the single raw ingredients were initially freeze-dried (lyophilized) and then homogenized to a fine powder by grinding in mortar and pestle, and later stored in sealed tubes in the dark at -24ºC until analysis. *Invitro* digestion was carried out as described in Ekesa *et al.,* (2013).

Analysis of the Improved Recipes for Children of Age 6-23 Months

Pro-vitamin A Carotenoids Extraction

Carotenoids extraction was done in five banana and two porridge recipes; 0.5g of each freeze-dried sample was weighed into a flask and added with 2ml of trans-β-apo-8'-carotenal (internal standard) and 15ml of petroleum ether (0.1% butylated hydroxytoluene; BHT). The flask was then shaken for 60 seconds and centrifuged for 5 min at 1100 rpm (revolutions per minute). The supernatant was filtered through a funnel stuffed with glass wool and collected in a flask and added with 10ml of petroleum, then was added with 5ml petroleum ether for the following extraction steps until the supernatant was clear. A rotary evaporator was used to vaporize the petroleum ether.

To eliminate existing lipids the remaining extract was dissolved in 1ml ethyl-acetate and frozen at -24°C for 3-4 hours, then added with 1ml of frozen acetone and the liquid sample was filtered through a funnel stuffed with glass wool to separate fat from the carotenoids (Gleize et al., 2012; Rodriguez-Amaya and Kimura, 2004). The filtered sample was filled into a vial and stored at -24°C under argon before the analysis; storage did not exceed 48 hours.

Pro-vitamin A Carotenoid Analysis

The whole process leading to pVACs analysis was carried out as fast as possible using alluminium foiled covered following Rodriguez-Amaya Kimura (2004) protocol. This is because carotenoids are very sensitive to light and oxygen and exposure to light leads to trans-cis isomerisation and destruction of pVAC.

High-performance Liquid Chromatography (HPLC)

A linear gradient elution was used for HPLC analysis. The mobile phase consisted of eluent A; acetonitrile (containing 0.05% triethylamine (TEA), and eluent B; 0.1% BHT) and methanol: ethyl acetate (1:1, v/v, containing 0.05% TEA, 0.1% BHT). Flow rate was set at 1000µl/min and the injection volume was 25µl. TEA was added to ensure a slightly improved peak resolution (Davey et al., 2006). For quantification of the carotenoids a calibration curve was established. Five standard solutions with different concentrations containing trans-β-apo-8'-carotenal as internal standard (IStd) and β-carotene as external standard (EStd) were prepared.

Preparation of standards

Internal standard (IStd) was prepared by weighing 5mg of trans-β-apo-8'-carotenal into a 100ml flask and filled up to volume with acetone (0.1% BHT). Then 5ml of IStd solution was transferred to a 50ml flask and diluted with acetone (0.1% BHT) to the volume. For the preparation of the external standard (EStd), 2.5mg of β-carotene were weighed into a 50ml flask and filled up to volume with acetone (0.1% BHT). Then 4 ml of EStd solution were collected, transferred to a 25ml flask and diluted with acetone (0.1% BHT) to volume.

Identification of carotenoids was based on the characteristics in absorption spectrum and retention time (compared to the added standards) of different carotenoids (Davey *et al*., 2006). Chromquest 5.0 Software was used for the interpretation of the chromatograms. Values of peak areas were used to calculate the carotenoid contents. The areas under the curve ratios between trans-β-apo-8'-carotenal as internal standard and the compounds were used for the determination of concentrations (Courraud *et al.,* 2013). Calculations were carried out using Microsoft Office Excel 2007. The analysis was done in triplicates.

Carotenoid Contents and Daily Vitamin A Recommended Dietary Allowance (RDAs) for Children

Different pVACs are converted in the body to vitamin A (retinol) with different efficiencies. Thus, to determine the relative vitamin A nutritional content of samples, total pVACs contents were first converted to all-trans β-carotene equivalents (t-BCEs) using the formula t-BCE=0.5 t-AC + t-BC + 0.53 c-BC', where c-BC is the sum of 13-cis β-carotene and 13-cis ɑ-carotene. These values were then converted into 'Retinol Activity Equivalents' (RAE) assuming that 1/12th of the total t-BCEs ingested are taken up into the body (Yeum and Russell, 2002). The RAE in µg/100 gfw were compared to the vitamin A RDIs (Ekesa et al,. 2013).

Determination of Provitamin A Carotenoids bio-accessibility of the improved recipes

Invitro digestion

The invitro bio-accessibility model was based on previous studies (Ekesa et al., 2012; Patted, 2010) with slightly modifications from this study. To mimic the human digestion, the model was divided into three phases; of oral, gastric and intestinal phase. Triplicate food samples were subjected to simulated oral, gastric and intestinal phase of digestion. For each freeze-dried food sample 1.3g was weighed in a flask and filled up with water to 5g and mixed with 6ml of saliva solution (containing 0.521g $NaHCO_3$ (99.5%), 0.087g NaCl (99.5%), 0.048g KCl (99.5%), 0.044g $CaCl_2.H_2O$ (97%), 0.104g K_2HPO_4, 0.216g mucin and 200 units/ml of porcine ɑ-amylase in 100ml of ultrapure water). The pH of the saliva solution was adjusted to 7 ± 0.2 by adding 1M NaOH. The mixture of saliva and food sample was incubated by shaking at 200 rpm for 10 min at 37°C. The food samples were t hen mixed with 15ml saline solution (0.9% NaCl) and again incubated for 10 min at 37°C while shaking at 200 r pm. To simulate the gastric digestion of an infant, pH of the food sample was adjusted to 4 ± 0.2 by adding 1M HCl. After which 2 ml of porcine pepsin (40 mg/ml in 0.1M HCl) were added to acidify the food sample. For the next step to ensure activity of enzymes, 2ml of porcine pepsin (40 mg/ml in 0.1M HCl) were added (Ekesa et al., 2012). Pepsin, which is responsible for the digestion of proteins, needs an acidic milieu and loses its activity at a pH=>5 (Etcheverry et al., 2012). The mixture (homogenate) was again incubated on a shaking apparatus for 30min at 37°C and 200 rpm. To simulate the intestinal digest ion step, the pH of the partially digested mixture was first raised to 6 ± 0.2 by adding 0.45M sodium bicarbonate. Subsequently, 9ml of a mixture containing porcine pancreatin and bile extracts (2mg/ml porcine pancreatin, 12mg/ml bile extracts in 0.1M trisodium citrate) and 4ml of bile extracts (0.1g/ml in 0.1M trisodium citrate) were added (Ekesa et al., 2012). Bile extracts act as emulsifiers (Etcheverry et al., 2012). The food samples mixture was again incubated on a shaking apparatus for 30min at 37°C and 200 rpm to complete the digestion process. The digested samples were transferred to centrifugation tubes, and micelles were separated by centrifugation at 11000 rpm for 1hour at 10°C. This speed was sufficient to clarify the suspension and enable micro filtration. The resulting aqueous fraction was filtered through a 0.2µm filter using a vacuum flask. Aliquots (10ml) of the resulting aqueous fraction were collected (pipette) into a pyrex tube. Aliquots were stored at -24°C under nitrogen prior to analysis (Etcheverry et al., 2012; Gautam et al., 2010).

Extraction and HPLC Analysis
Extraction

Three (3)ml of a trans-β-apo-8'-carotenal (50 µg/L) was added to the aliquot (10ml) and 15ml of hexane/ethanol (2:1) were used for the extraction. The digested sample and the solvent was briefly centrifuged for 3min at 11000 rpm and the supernatant transferred into a flask (Ekesa et al., 2012). This step was repeated three times. The collected solution was vaporized using a rotary evaporator, the remaining carotenoids were dissolved in 1ml ethylacetate, filtered through a pipette stuffed with glass wool and sodium sulphate, filled in vials and stored at -24°C until analysis.

Bio-accessibility of Pro Vitamin A analysis

For bio-accessibility analysis, samples were injected, analysed by HPLC and calculated as described above. After digestion the amount of carotenoids in the samples (bioaccessible fraction) was expected to be lower than in

Table 2. Provitamin A Carotenoids (pVACs) in Raw Food Ingredients µ/100g

Food item	α carotene µ/100g (SD)	All trans β µ/100 g Carotene (SD)	13 cis β µ/100g carotene (SD)	Total pVAC µ/100g	Total RAE in µg/100g fw
OFSP	6743 (904)	118(5.9)	0.0(0.0)	6861	290
Pumpkin fruit	1377(28.6)	2066(69.1)	131(5.0)	3574	235
Egg	0.0 (0.0)	17.2(3.8)	0.0(0.0)	17.2	1.4
Amaranths	7.1(0.0)	377(13.3)	42.5(0.9)	427	33.5
Pumpkin leaves	8.4(0.6)	228(4.3)	27.1(1.2)	263	20.4
Fresh red kidney bean	0.0(0.0)	6.6(0.5)	0.0(0.0)	6.6	0.6
Bitter tomato (*'entongo'*)	3.7(0.7)	49.7(3.2)	0.0(0.0)	53.4	4.3
Palm oil fruit	4986(334)	4205(288)	502(30.2)	9693	579
EAHB '*Nshakala*'	840(7.0)	426(17.8)	26.9(0.4)	1282	71.2
AAB *'Bira'*	1716(1.0)	1930(54.9)	96.6(1.2)	3712	234
Tomato	7.8(0.1)	504(2.0)	23.3(0.3)	535	43.3
p≤0.05					

SD=Standard deviation

undigested samples, therefore standards for the calibration curve were diluted 1:10. Bioaccessibility was calculated using the following formula:

$$bioaccessibility\,(\%) = \frac{content\ of\ bioaccessible\ fraction\ (\mu g/100\ g)}{total\ content\ of\ nutrient\ (\mu g/100\ g)} * 100$$

Statistical Analysis

Statistical analyses were performed using GenStat 14th Edition software. Means separation was done by Turkey mean separation test using Least Significant Differences (LSD) at p≤0.05. Differences in mean content of total and individual provitamin A carotenoids observed following analysis were tested using one way analysis of variance (ANOVA). Microsoft Excel 2007 was used to compute data.

RESULTS

Provitamin A Carotenoids (pVACs) Content in single raw ingredients

Provitamin A carotenoid contents (pVACs) were derived from all the recipes and presented by three pVACs: all-trans ɑ-carotene (t-AC), all-trans β-carotene (t-BC) and 13-cis-β-carotene. Of all the recipes, palm oil had high amount of provitamin A carotenoids of 579RAE/100g followed by orange fleshed sweet potato (291RAE/100 g), pumpkin fruit (235RAE/100g) and the least was AAB '*Bira'* variety (234RAE/100 g) (Table 1). Triploid hybrid banana *Musa acuminata* and *Musa Balbisiana* (AAB), '*Bira*' variety had high amount of pVAC (234 RAE/100g) than EAHB '*Nshakala*' (71.2 RAE/100g) local variety. For the analysed green leaves, amaranths showed higher amount of provitamin A carotenoids of 33.5RAE/100g compared to pumpkin leaves with 20.4RAE/100g (Table 2).

Table 3. pVACs Content (in 100g) in the Optimized (modified) Diets for Children Aged 6 -23 months in Bukoba Rural

Recipes	α carotene µ/100g (SD)	All trans β carotene µ/100g (SD)	13 cis β carotene µ/100g (SD)	Total pVAC µ/100g	Total RAE in µg/100gfw
Banana purée '*Nshakala*' dry bean (1N): EAHB '*Nshakala' variety*, dry red kidney beans, amaranths, palm oil, salt, onion, tomatoes, bitter tomatoes	472 (3.6)	451(39.4)	19.0(3.7)	942	58.1
Banana purée '*Nshakala' fresh bean* (2N): EAHB *'Nshakala' variety*, fresh red kidney beans, pumpkin leaves, sunflower oil, salt, onion, tomatoes, bitter tomatoes	359(6.6)	350(9.9)	8.4(1.4)	718	44.5
Banana purée '*Bira*' dry bean(3B): AAB *'Bira' variety*, dry red kidney beans, amaranths, palm oil, salt, onion, tomatoes, bitter tomatoes	581(33.2)	793(75.6)	29.9(22.2)	1404	91.6
Banana purée '*Bira*' dry bean(3B): AAB *'Bira' variety*, fresh red kidney beans, pumpkin leaves, sunflower oil, salt, onion, tomatoes, bitter tomatoes	86.1(2.2)	197(20.8)	3.7(0.4)	286	20.1
Katogo purée (5N): EAHB 'Nshakala' variety, pumpkin fruit, groundnuts flour, salt, onion, tomatoes, bitter tomatoes	870(30.7)	1096(222)	234(15.1)	2200	137
Orange-fleshed sweet potato porridge (6OFSP): Fermented maize flour, orange fleshed sweet potatoes, groundnut flour , sugar	55.8(7.6)	219(27.3)	0.0(0.0)	275	20.6
***Bira'* porridge (7B):** Fermented maize, AAB *'Bira' variety*, groundnut flour, sugar	49.7(2.7)	67.1(4.3)	27.8(4.3)	145	8.8
Egg porridge (8E): Fermented maize flour, eggs red kidney beans, sugar	0.0(0.0)	13.1(1.3)	0.0(0.0)	13.1	1.1*
p≤0.05					

*100 g of egg has 227.0REµg which meet 56.8%RDA vitamin A (Miranda et al., 2015); SD=Standard deviation

Contribution of Modified Diets to Carotenoids Recommend Daily Allowances (RDAs) of Children Aged 6-23 Months

The potential of the modified diets to contribute to nutritional requirements of children aged 6-23 months is shown in Table 2. Recipe 5N had the highest content of pVACs with 137.4RAE/100g followed by recipe 3B and 1N with 91.6 and 58.1 RAE/100g, respectively. Recipe 5N had pumpkin fruit, EAHB 'Nshakala' variety as the main source of vitamin A while recipe 3B had red palm oil, AAB 'Bira' variety and amaranths as the main source of vitamin A. Red palm oil, EAHB 'Nshakala' variety and amaranths were also the main source of vitamin A in recipe 1N. Recipe 5N had high beta (1096µg/100g) and alpha (870µg/100g) carotene content followed by recipe 3B with bet

Table 4. Means of carotenoid (in the form of beta, alpha and 13-cis) content in the 8 modified recipes

Pro-vitamin A carotenoids (pVACs) µg/100g		**Pro-vitamin A % bio-accessibility**	
Recipe	**Mean**	**Recipe**	**Mean**
5N beta	1096.3[a]	4B alpha	20.1[a]
5N alpha	870.0[b]	6OFSP beta	18.9[a]
3B beta	793.0[b]	6OFSP alpha	18.3[ab]
3B alpha	581.3[c]	3B alpha	16.4[abc]
1N alpha	472.2[cd]	3B beta	14.2[bcd]
1N beta	451.1[cd]	4B beta	13.6[cde]
2N alpha	359.0[de]	2N beta	12.4[cdef]
2N beta	350.3[def]	7B beta	11.5[def]
5N13-cis	234.1[efg]	7B alpha	11.2[def]
6OFSP beta	219.2[efgh]	1N alpha	9.64[efg]
4B beta	196.5[fghi]	5N beta	8.27[fg]
4B alpha	86.2[ghij]	1N beta	6.75[g]
7B beta	67.1[hij]	2N alpha	6.01[g]
6 OFSP13-cis	55.8[ij]	5N alpha	5.93[g]
7B alpha	49.6[ij]	4B 13-cis	0.00[h]
3B 13-cis	29.9[j]	3B 13-cis	0.00[h]
7B13-cis	27.8[j]	2N 13-cis	0.00[h]
1N 13-cis	19.0[j]	1N 13-cis	0.00[h]
8E beta	13.1[j]	5N 13-cis	0.00[h]
2N13-cis	13.1[j]	6OFSP 13-cis	0.00[h]
2N13-cis	8.4[j]	7B 13-cis	0.00[h]
4B13-cis	3.7[j]	8E 13-cis	0.00[h]
6OFSPalpha	0.0[j]	8E alpha	0.00[h]
8E13-cis	0.0[j]	8E beta	0.00[h]
8E alpha	0.0[j]		
p≤0.05			

Means in the same column with the same letter are not significantly different

(793µg/100g) and alpha (58 µg/100g) carotene content and 1N with beta carotene (451/100g) and alpha carotene (472µg/100g). Recipe 2N and 4B, which had 'Nshakala'/pumpkin leaves and 'Bira'/pumpkin leaves (Table 3), respectively as source of vitamin A were lower in pVACs compared to other banana recipes. Conversely, recipe 4B had slightly higher vitamin A compared to recipe 2N due to AAB 'Bira' variety (234RAE/100g). For porridge recipes, recipe 7B had low pVACs content (145RAE/100g The mean content of vitamin A ranged from 13.1 to 1,096 µg/100g beta, 0 to 870 µg/100g alpha and 0 to 234 µg/100g-cis carotenes with grand mean of 249µg/100g. The mean of t-BC was significantly higher 1096µg/100g at (p≤0.05) with the overall mean of 249µg/100g. There was a significant different between mean provitamin A carotenoids contents in recipes at p≤0.05 (Table 4).The percentage recommended daily allowance (RDA) for vitamin A in 100g for all recipes ranged from 2.2 to 34.1% (Figure 1). Except recipe 8E which had egg, thus, 100g of egg has 227REµg which meet 56.8%RDA vitamin A (Miranda et al., 2015).

Recommended daily intake for children aged 6-23 months to meet daily vitamin A requirements

The RDA for vitamin A from the modified diets in 250g and 500g consumption size ranged from 5.5%-85.6% RDA and 11.0%-171% RDA vitamin A, respectively (Table 5). Of the seven recipes, 5N showed high amount of vitamin A in both intakes on 250 and 500g/day with 85.6% and 171% RDA vitamin, respectively, it corresponds to the daily requirements. It was followed by recipe 8E and 3B, which will meet 75%-150% RDA vitamin A and 57.2-115 RDA vitamin A, respectively for children.

Provitamin A Carotenoids (pVACs) After *in-vitro* Digestion in 100g of the Modified/improved Diets

The provitamin A carotenoids (pVACs) contents of the modified recipes after *invitro* digestion are presented in Table 6, as all-trans ɑ-carotene (t-AC), all-trans β-carotene (t-BC) and 13-cis-β-carotene. The Amount of t-AC ranged from 5.6 to 94.7 µg/100g and 7.7 to 112 µg/100g in t-BC after *invitro* digestion. There was no 13-cis-β-caroten in all recipes after *invitro* digestion. After *invitro* digestion recipe 3B showed high amount of pVAC (207µg/100g) and total RAE of 13.4µg/100g, followed by recipe 5N (114µg/100g) and 1N (75.9µg/100g) with total RAE of 7.4 µg/100g and 4.4 µg/100g, respectively.

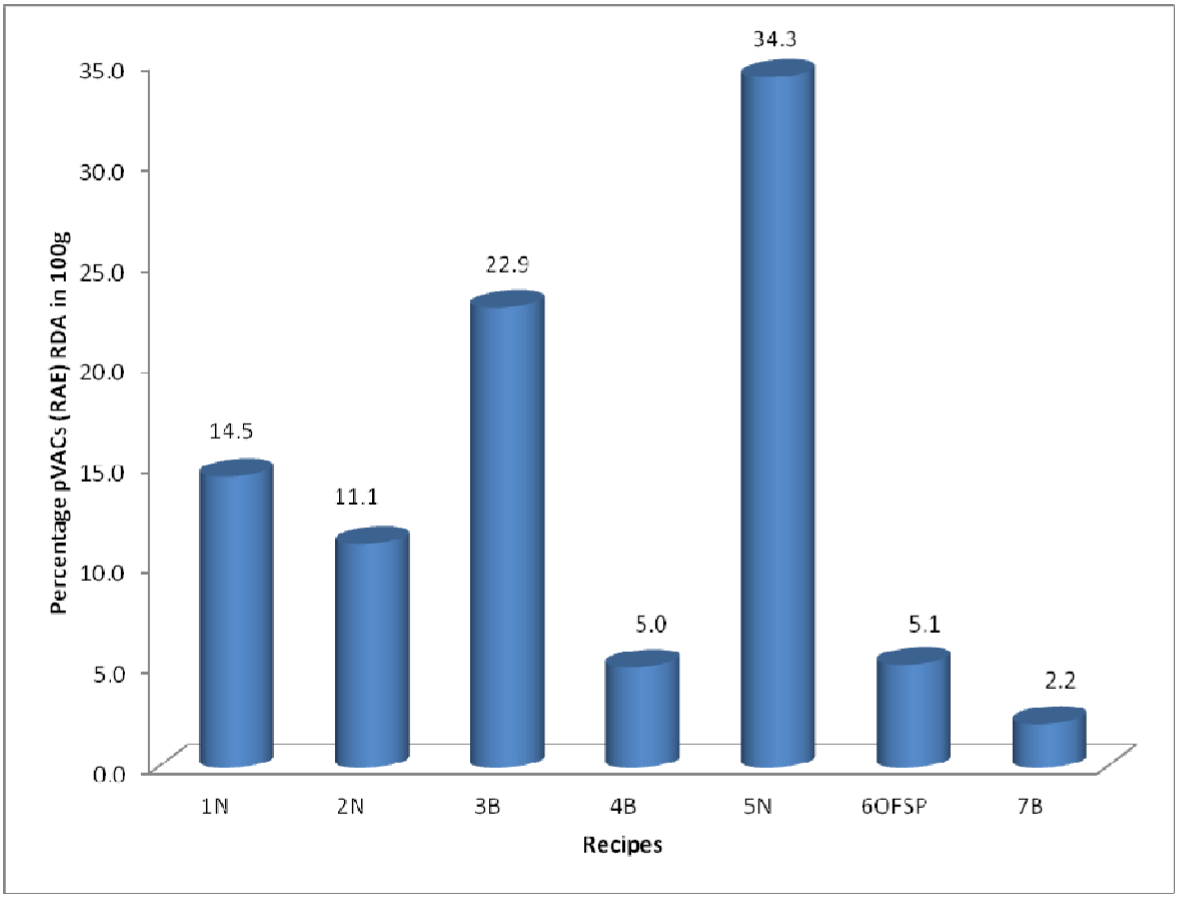

Figure 1 Percentage RDA for pVACs in 100g for children aged 6-23months in different recipes analysed in this study

However, recipe 2N had slightly high amount of RAE after invitro digestion of 4.5µg/100g despite having total pVAC of 64.7µg/100g. Total RAE after invitro digestion ranged from 0.87-13.3µg/100g.

Provitamin A Bioaccessibility

Table 7 shows that the percentage bioavailability of t-AC (20.1%) and t-BC (16.4%) were significantly high at $p \leq 0.05$ in recipe 4B and 3B, respectively. The bioaccessibility of pVACs ranged from 12.2% to 33.6% in the modified local diets. Recipe 4B had high bioaccessibility followed by recipe 3B and 6OFSP with 33.6%, 30.5% and 25.9 %, respectively. Unlike the high pVACs content in recipe 5N, this recipe had the least bioaccessibility followed by recipe 1N, which had 'Nshakala'/pumpkin fruits and 'Nshakala'/amaranths/palm oil as source of vitamin A respectively. There was significant difference between recipes in the mean percentage of pVACs bio-accessibility at $p \leq 0.05$ with grand mean of 7.2 and least significant differences (LSD) of 2.3.

DISCUSSION

Provitamin A Contents in the Modified Diets

Pro-vitamin A content varied with type of raw food in following order: palm oil, orange fleshed sweet potato (OFSP), pumpkin fruit and AAB 'Bira' variety. Of all the recipes recipe 5N cooked with 'Nshakala', pumpkin fruit and groundnut had higher amounts of provitamin A, presumably from pumpkin fruit. Similarly, palm oil, AAB 'Bira' variety and amaranths contributed high amounts of provitamin A in recipe 3B ('Bira'+ amaranths + palm oil + dry red kidney beans). This indicates that diversification of food items in a recipe can increase micronutrients content and hence intake. While pumpkin fruit was the third in pVACs contents in raw food ingredients, in

Table 5. Percentage Retinol Equivalent (RAE) RDA if 250g and 500g Consumed Per Day by a Child Aged Between 6 to 12 months and 12 to 23 months, Respectively.

Recipes	RAE %RDA if 250g consumed /day	RAE %RDA if 500g consumed /day
Banana purée '*Nshakala*' dry bean (1N): EAHB '*Nshakala' variety*, dry red kidney beans, amaranths, palm oil, salt, onion, tomatoes, bitter tomatoes	36.3	72.6
Banana purée '*Nshakala' fresh bean* (2N): EAHB *'Nshakala' variety*, fresh red kidney beans, pumpkin leaves, sunflower oil, salt, onion, tomatoes, bitter tomatoes	27.8	55.6
Banana purée '*Bira*' dry bean(3B): AAB *'Bira' variety*, dry red kidney beans, amaranths, palm oil, salt, onion, tomatoes, bitter tomatoes	57.2	114
Banana purée '*Bira*' dry bean(3B): AAB *'Bira' variety*, fresh red kidney beans, pumpkin leaves, sunflower oil, salt, onion, tomatoes, bitter tomatoes	12.6	25.1
Katogo purée (5N): EAHB 'Nshakala' variety, pumpkin fruit, groundnuts flour, salt, onion, tomatoes, bitter tomatoes	85.6	171
Orange-fleshed sweet potato porridge (6OFSP): Fermented maize flour, orange fleshed sweet potatoes, groundnut flour, sugar	12.9	25.8
***Bira'* porridge (7B):** Fermented maize, AAB *'Bira' variety*, groundnut flour, sugar	5.5	11.0
Egg porridge (8E): Fermented maize flour, egg, red kidney beans, sugar	75.0	150

cooked recipes showed to be a good source of pVACs than other ingredients. The difference in provitamin A carotenoids content was due to the retention effect after cooking. There are varieties of pumpkins, which cannot lose carotenoids while others can lose up to 22% (Ribeiro et al., 2015). Another reason could be due to high content of β-carotene in recipe 5N, which is the carotenoid with the most provitamin A activity (McLaren, and Frigg, 2001). AAB 'Bira' variety showed to contribute high pVACs than local EAHB 'Nshakala' variety. Therefore, production and consumption of AAB 'Bira' variety should be promoted to increase provitamin A intake by the groups vulnerable to vitamin A deficiency.

Contribution of modified diets to Daily Recommended Dietary Allowances (RDA) of Vitamin A for Children Aged 6-23 Months

The daily requirement of the amount of complementary food for children aged 6-23 months is 500g per day and 600ml of breast milk consumed (Arbeit and Kouevi, 2013; Dewey et al., 2006). Other studies have documented that child aged 6 to 23 months require 600 to 674ml of breast milk intake per day and decreases with age (Dewey et al., 2006). Moreover, breastfed children aged 6-9 months and 9-24 months are recommended to be fed 2-3 and 3-4 meals, respectively, and all non-breastfed children 6-23 months are recommended to be fed 4-5 times a day (Berti et al., 2014; WHO, 2010). In the present study the assumption was that a child will be able to consume 250 to 500g of the modified diets per day. The RDA for provitamin A (retinol equivalent, RE) from the modified diets of 250g ranged from 5.5% to 85.6% and that of 500g ranged from 11.0% to 171%. The Estimated Average Requirements (EAR) for children aged 1-3 years is 210µg/d (Dewey, 2003). In this regard the percentage intake from the amounts of 250 and 500g will range from 10 to 163% and 21 to 326% of RDA, respectively. The

Table 6.Amount of RAE after in-vitro Digestion in 100g of Optimized (modified) Diets for Children Aged 6-23 months in Bukoba Rural

Recipes	α carotene µg/100g (SD)	All trans β carotene µg /100g (SD)	13-cis-β carotene µg/100g	Total pVACs µg/100g	Total RAE in µg/100g
Banana purée 'Nshakala' dry bean (1N): EAHB 'Nshakala' variety, dry red kidney beans, amaranths, palm oil, salt, onion, tomatoes, bitter tomatoes	45.5(0.7)	30.3(0.6)	0.0	75.9	4.4
Banana purée 'Nshakala' fresh bean (2N): EAHB 'Nshakala' variety, fresh red kidney beans, pumpkin leaves, sunflower oil, salt, onion, tomatoes, bitter tomatoes	21.5(3.6)	43.2(5.0)	0.0	64.7	4.5
Banana purée 'Bira' dry bean(3B): AAB 'Bira' variety, dry red kidney beans, amaranths, palm oil, salt, onion, tomatoes, bitter tomatoes	94.7(9.8)	112(11.1)	0.0	207	13.3
Banana purée '*Bira*' dry bean(3B): AAB '*Bira*' variety, fresh red kidney beans, pumpkin leaves, sunflower oil, salt, onion, tomatoes, bitter tomatoes	17.4(3.1)	26.7(4.7)	0.0	44.0	2.9
Katogo purée (5N): EAHB '*Nshakala*' variety, pumpkin fruit, groundnuts flour, salt, onion, tomatoes, bitter tomatoes	51.1(19.6)	62.9(25.8)	0.0	114.1	7.4
Orange-fleshed sweet potato porridge (6OFSP): Fermented maize flour, orange fleshed sweet potatoes, groundnut flour , sugar	24.3(11.9)	7.7(1.6)	0.0	31.9	1.7
Bira' porridge (7B): Fermented maize, AAB 'Bira' variety, groundnut flour, sugar	5.6(1.4)	7.7(2.3)	0.0	13.3	0.7

SD=Standard deviation

Table 7: Bio-acessibility of pVAC of Optimized (modified) Diets for Children Aged 6-23 months in Bukoba Rural

Recipes	α carotene (%) (SD)	All trans β carotene (%) (SD)	13-cis-β carotene (%) (SD)	Total bioaccessibility %/100g
Banana purée '*Nshakala*' dry bean (1N): EAHB '*Nshakala' variety*, dry red kidney beans, amaranths, palm oil, salt, onion, tomatoes, bitter tomatoes	9.6(0.1)	6.7(0.5)	0.0(0.0)	16.4
Banana purée '*Nshakala' fresh bean* (2N): EAHB *'Nshakala' variety*, fresh red kidney beans, pumpkin leaves, sunflower oil, salt, onion, tomatoes, bitter tomatoes	6.0(1.0)	12.4(1.8)	0.0(0.0)	18.4
Banana purée '*Bira*' dry bean(3B): AAB *'Bira' variety*, dry red kidney beans, amaranths, palm oil, salt, onion, tomatoes, bitter tomatoes	16.4(2.6)	14.2(0.9)	0.0(0.0)	30.5
Banana purée '*Bira*' dry bean(3B): AAB *'Bira' variety*, fresh red kidney beans, pumpkin leaves, sunflower oil, salt, onion, tomatoes, bitter tomatoes	20.1(3.1)	13.6(1.0)	0.0(0.0)	33.6
Katogo purée (5N): EAHB 'Nshakala' variety, pumpkin fruit, groundnuts flour, salt, onion, tomatoes, bitter tomatoes	5.9(2.5)	6.2(3.8)	0.0(0.0)	12.2
Orange-fleshed sweet potato porridge (6OFSP): Fermented maize flour, orange fleshed sweet potatoes, groundnut flour, sugar	11.7(7.4)	14.2(5.0)	0.0(0.0)	25.9
***Bira'* porridge (7B):** Fermented maize, AAB *'Bira' variety*, groundnut flour, sugar	11.2(2.5)	11.5(3.4)	0.0(0.0)	22.7

$p \leq 0.05$

SD=Standard deviation

mean requirement for vitamin A for children aged between 7-12 and 12-36 months is 190 and 200µgRE/day (FAO, 2004). Therefore, if a child of 7-12months consumes 250g and of 12-36months 500g of modified diets the intake will range from 11-180% and 22-342%, respectively.

Breast milk provides a significant portion of daily vitamin A requirement for children. Assuming an average breast-milk intake, the amount of vitamin A required from complementary food is 63RE, 92RE and 400RE for 6-8.9 months, 9-11.9 months and 12-23.9 months children, respectively (Dewey et al., 2006). Thus, all the modified diets will meet the RDA for vitamin A in children aged 6 to 23 months old and above. As reported (Carvalho et al., 2012) that food can be labeled as the 'source' of a nutrient when 100g of the product provides more than 15% of the dietary reference intake for the desired nutrient. Recipe 7B fall below recommended RDA (2.2% RDA vitamin A for recommended safe intake) as it has low percentage RDA in 100g and low amount of 8.8µRE/100g.

Bioaccessibility of pVACs in the Modified/improved Diets

The study shows that provitamin A carotenoids (pVACs) from pumpkin leaves in 4B recipe were more bio-accessible than those from amaranths and red palm oil fruit in recipe 3B chronologically. This indicates clearly that pumpkin leaves contribute to high bio-accessibility of pVACs content, because both recipe 4B and 3B had AAB '*Bira*' variety. Red palm oil showed higher pVACs in raw food ingredients as it contains high concentrations of β-carotene (t-BC) and ɑ-carotene (t-AC). This also confirmed by You *et al.,* (2002) that red palm oil contain high vitamin A value. However, the low bio-accessibility of red palm oil was demonstrated in this study when red palm oil was added in recipe 3B and recipe 1N, it could not significantly increase the bioaccessibility of pVACs, hence confirmation for low bioaccessibility of red palm oil. Thus, provitamin A carotenoids bioaccessibility is not determined by pVACs contents rather is determined by food variety. Furthermore, all the recipes had oil to improve micellarization of carotenoids. For porridge recipe, recipe 6OFSP with orange fleshed sweet potato the total pVACs were more bioaccessible than AAB '*Bira*' variety in recipe 7B. Recipe 5N with pumpkin fruit and '*Nshakala*' as source of pVACs showed to have high amount of RAE but its bioaccessibility was very low compared to other recipes. This indicates that pVACs in pumpkin fruit had low bioaccessibility compared to pVACs in pumpkin leaves and AAB '*Bira*' variety. Thus, production and consumption of pumpkin leaves should be encouraged to enhance intake of vitamin A. To translate vitamin A requirements into recommendations for daily dietary intakes requires an estimate of vitamin A bioaccessibility. Therefore, nutrient bioaccessibility must be considered because when RDA is high does not mean the nutrient will be 100% bio-accessible.

CONCLUSION

Chronologically palm oil fruit and orange fleshed sweet potato showed high amount of provitamin A carotenoids (pVAC's). Fresh red kidney bean and bitter tomatoes showed the least pVAC's. Recipe 5N had the highest content of pVACs whereby pumpkin fruit was the main source of vitamin A. Recipe 7B had low pVACs, whereby AAB *'Bira' variety* was the main source of pVACs. Recipe 4B had high bioaccessibility. The percentage RDA for vitamin A in 100g for all recipes ranged from 2.2 to 34.1% for plant source while animal source porridge with egg, in 100g meets 56.8% RDA vitamin A. The bioaccessibility of pVACs ranged from 12.2% to 33.6% in the modified local diets. The modified diets are good source of vitamin A for children as well as adults combating Vitamin A defficiency. Our results suggests that it is very important to take into account the estimation of dietary source of vitamin A and their pVACs bioaccessibility to meet nutritional requirements of children aged 6-23 months.

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