Protein and iron contents and bio accessibility in local modified diets for children aged 6 to 23 months in Bukoba, Tanzania

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ABSTRACT

Micronutrient deficiencies often occur as part of a cycle of malnutrition and may be coupled with protein energy malnutrition. One of the measure to fight malnutrition is through participatory dietary modification was used. Five recipes of banana-based porridges from two EAHB '*nshakala'* and AAB '*bira' banana* varieties and three maize-based porridges were formulated in combination with other ingredients. The contents of Iron and protein in separate ingredients and in modified recipes were analysed using flame atomic absorption spectrophotometry and Kjeldahl´s method, respectively, and bio-accessibility of iron was estimated using *invitro* simulating gastrointestinal digestion method. Beans were a good source of both iron and protein while groundnuts flour was a good source of protein. Contribution of iron and protein in recommended daily allowance (RDA) ranged from 75.3 to 458% and 106 to 146% in a portion of 500g respectively. Iron bioaccessibility in all recipes ranged from 7.4% to 31.1%. Iron in porridge recipes (6OFSP, 7B) was more bio-accessible. The developed and improved recipes showed great potential of contributing substantially to iron and protein needs of children 6-23 months. The importance of food diversification for intake of iron and protein is discussed.

Key words: Diet modification, children below five years, protein, iron bioaccessibility

INTRODUCTION

Micronutrient and protein deficiencies are a global public health concern (Detzel and Wieser, 2015; NBS, 2016). Globally, pregnant women and their children below five years are at the highest risk of micronutrient deficiencies (Bailey *et al*., 2015). Iron is an essential micronutrient for the immune system and critical for optimal growth and cognitive function (Bailey *et al*., 2015; NBS, 2016).

Bioavailability is the fraction of an ingested nutrient that is available for utilization in normal physiological functions and/or for storage (Etcheverry et al., 2012). Of the two forms of iron: heme from animal sources and non-heme from plant source. Neither form is highly bioavailable by the body. The bioavailability heme-iron is estimated to be 12 to 25% while the non-heme iron is 5% (Bailey *et al.,* 2015; Trumbo *et al.,* 2001). On the other hand, bio accessibility is the amount of nutrient released from the food matrix and accessible for absorption (i.e. the transfer into micelles) (Etcheverry et al., 2012; van Lieshout *et al.,* 2001).

Iron deficiency anaemia (IDA) is mainly due to iron deficiency. IDA affects over two billion people worldwide and 190 million preschool-age children (Bailey *et al.*, 2015; Detzel and Wieser, 2015). Iron deficiency affects 60% of children below five years In Tanzania and 58% of

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

Recipe name	Recipe code	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	3B	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	5N	
purée		EAHB *'nshakala' variety*, pumpkin fruit, groundnuts flour, salt, onion, tomatoes, bitter tomatoes
Orange-fleshed sweet potato porridge	6OFSP	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

them are anaemic (NBS, 2016). In Kagera 25% of children suffered iron deficiency and 49% of them are anaemic; (NBS, 2011). Protein energy malnutrition (PEM) occurs when the body requirements for protein or energy or both cannot be met through diets. Similarly, micronutrient deficiencies often occur as part of a cycle of malnutrition and may be coupled with protein or energy malnutrition (Bailey *et al.,* 2015). Prolonged PEM can cause irreversible changes in organ function and growth; delayed and irreversible cognitive development (Klein, 2012). Children are at increased risk for protein and iron deficiencies when introduced to complementary foods, which are usually made of starchy-staple such as banana, cereal or root and tuber crops with little or no fruit and vegetable or animal source of protein and iron (Christides *et al.,* 2015; Kennedy *et al.,* 2010;). These starchy-staple foods are often poor dietary sources of iron as they contain high levels of iron absorption inhibitors such as phytates (Amagloh and Coad, 2014; Saunders, *et al.,* 2012; Soetan and Oyewole, 2009). One of the methods to prevent micronutrient deficiencies is dietary modification. These deficiencies can be addressed by consumption of animal and plant based foods, but animal sources are expensive compared to plant sources (Bailey *et al.,* 2015). Godson, 2014 reported that in Bukoba district, local diets for children prepared with banana and beans were low in iron content (1.18mg/100g). According to Kennedy *et al,.* (2010) a modified diet is any diet altered by nutritionist or dietician to include or exclude certain components, such as vitamins, minerals, protein, calories and fat, mainly using the available local types of foods. Information on the nutrient content of foods commonly consumed in rural communities of Tanzania is limited (Kinabo et al., 2006). The present study analysed iron and protein contents and iron bio-accessibility in order to enhance their intake to children aged 6-23 months using modified local diets mostly from plant sources that are affordable to smallholder farmers.

MATERIALS AND METHODS

Eight food recipes were modified to enhance intake of iron and protein to children below five years (Table 1). Five recipes were banana-based from '*nshakala'*[1] *Nshakala*' is East African Hybrid Banana (EAHB) local variety and AAB '*bira'*[1] *'Bira'* is triploid hybrid of *Musa acuminata* and *Musa balbisiana* (AAB) *banana* varieties, and three maize-based porridges. To improve nutrient and spontaneous fermentation (using the microorganisms that are naturally present in food) was done to ferment maize flour for 24 hours fermentation to reduce phytic acid in cereals and legumes to enhance the content of micronutrients and improve micronutrient bio accessibility (Berhanu et al., 2014; Gibson and

Study Area

The study was conducted in Izimbya ward of Bukoba district in Kagera region 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 such as beans, maize, sweetpotato and yams.

Sample Preparation and Processing

Food ingredients (Table 1) were purchased from the local market at Izimbya ward in Bukoba district. 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 neighbouring country, Burundi, because no mature fruit bunches were available in Tanzania. 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 the department, all food samples were stored in a room maintained at 4oC until analysis. Recipes were prepared and cooked at the department laboratory according to the already defined procedures (Mbela et al., 2017–In press). All food samples were freeze-dried (lyophilized). Lyophilized foods were homogenized to a fine powder by grinding in mortar and pestle and later stored in sealed tubes in the dark at-24ºC until analysis and in vitro digestion were carried out. All the food samples were analysed in triplicates for protein and iron contents and iron bio accessibility.

Determination of Protein Contents of the Modified Recipes for Children Aged 6-23 months using Kjeldahl´s method

Analysis of Protein Content

Catalyst mixture of 7.5g containing (4g of copper sulphate per 1kg potassium sulphate) and some glass beads were filled in a digestion tube to avoid boiling retardation. The original sample weight was dependant on the expected protein content. Food samples were weighed into a digestion tube and 15ml sulphuric acid (H2SO4) was added. Digestion was done at different temperature regimes starting at 150°C for 15 min, 2 50°C for 15 min, 380°C for 60 min and 420°C for 90 min. Then the digestion tubes were water cooled for about half an hour then 60ml ultra high quality water was added. The digestion tubes were added with 20ml boric acid (2%) and 50ml NaOH (32%) and distilled for 3 min at steam distilling unit. After distillation, titration was done with 0.1M HCl until the end point of pH 4.7 (McClements, 2003). After decomposition nitrogen was available as ammonium sulfate and the flasks were connected to a VELP Automatic Distillation unit.

During distillation step ammonium sulfate was converted to ammonia by the addition of sodium hydroxide (NaOH). The volatile ammonia gas moves to another flask containing boric acid (2%). A chemical reaction leads to the conversion of ammonia to ammonium ion and boric acid to the borate ion, respectively. Nitrogen content was established by titrating the formed ammonium borate with 0.1M hydrochloric acid. The amount of hydrochloric acid used for the titration was used for the calculation of the nitrogen content and following total protein content (McClements, 2003).

Protein calculation

$$TN(\%) = \frac{V(HCl) * n(HCl) * M(N) * 100}{SW}$$

Protein content (%) = TN (%) * F

TN	total nitrogen
V(HCl)	volume of hydrochloric acid (ml) needed for titration
n(HCl)	morality of hydrochloric acid (mol/L)
M(N)	molecular weight of nitrogen
SW	sample weight (mg)
F	factor 6.25

Determination of Iron (Fe) Contents of the Modified Recipes for Children Aged 6-23 months

Iron Content Analysis

Determination of iron content of all freeze-dried samples was achieved through microwave digestion followed by a flame atomic absorption spectroscopy (FAAS). To analyze the iron concentration in the FAAS, sample solutions were transformed into aerosols and passed through the flame, which vaporizes and atomizes the sample. This step leads to a reduced intensity of the light (by absorption of a defined quantity of energy), coming from a hollow-cathode lamp. The detector measured how much of the incoming light was absorbed. Iron concentration is calculated from the difference between the radiation without sample and with sample (absorbance).

Digestion of Freeze-dried food samples

For each freeze-dried sample, 0.5g of was weighed in a

Table 2. Mean Iron and Protein Content in 100g in Sample Weight of Raw Ingredients

Raw ingredients	Iron mg/100gfw (SD)	Protein g/100g (SD)
Orange fleshed sweet potatoes	2.1(0.0)	2.6(0.0)
Pumpkin fruit	2.3(0.0)	1.4(0.0)
Amaranths	3.6(0.0)	5.7(0.0)
Pumpkin leaves	3.3(0.0)	6.3(0.6)
Fresh red kidney beans	6.9(0.0)	11.9(0.4)
Dry red kidney beans,	6.5(0.1)	22.8(0.4)
Bitter tomato (*'entongo'*)	3.2(0.2)	1.3(0.0)
EAHB ('*nshakala*' variety)	0.9(0.0)	1.1(0.0)
AAB (*'bira'* variety)	0.7(0.0)	0.9(0.0)
Tomatoes	3.7(0.0)	1.0(0.5)
Groundnuts flour	1.9(0.1)	27.5(0.2)
Fermented maize flour	2.1(0.0)	8.2(0.0)
Egg	9.0(0.0)	13.3(0.1)
Onions	4.9(0.2)	1.5(0.0)

*fw=Fresh weight; SD=Standard deviation

Teflon vessel and added with 5ml of 69 % HNO3 and 1ml of 30 % H2O2. Vessels were closed, surrounded by high pressure safety coats (red or fluorescent dyes to aid in visual leak detection), put into rotor segments inside microwave and heated using the following program: 34 min heating (10 min/1000 W/160°C, 4 min/1000 W/190°C, 20 min/700 W/190°C) followed by 30 min cooling. Teflon vessels were removed from the microwave and opened to let the nitrogen oxides volatilize. The digested liquid samples were transferred into 50ml volumetric flasks. The vessels and breeches were rinsed with bi-distilled water three times. The flasks were filled up with water until the mark (50mls), shaken well several time and filtered through a paper filter (MN 619 eh, Macherey-Nagel).

Measurement of Iron content

Preparation of calibration curve was prepared by diluting iron standard to 4 different concentrations: 0.04 mg/L; 0.1mg/L; 0.5mg/L; 1 mg/L Fe. A blank value, consisting of 3% HNO3 was prepared. To avoid masking of iron, 250µl of a 5% CaCl2 buffer was added to 10ml of each food sample, standard and the blank value. The tubes were closed, mixed and briefly vortexed. The AAS measurement started with the analysis of the blank value, followed by the standards and the samples. Basing on the calibration curve and the specific absorption of the different samples, the AAS calculates the iron concentration of each sample. Concentration values were used for the calculation of the total iron content. By measuring the amount of absorption, the concentration of the analyte were calculated using the Beer-Lambert law; where A is the absorbance, ε is the wavelength-dependent molar absorptivity coefficient and b is the path length. The sample concentration was calculated as follows:

Iron Bio accessibility of the modified diets for children aged 6-23 months

In vitro digestion of iron

The invitro digestion model was based on (Luten et al., 1989) with slight modifications in this study. In a 150ml flask, 5g of each freeze-dried food samples were weighed and 80ml ultrapure water was added. To simulate gastric digestion pH of gastric digest was adjusted to pH 2.0 with 6M HCl, controlled after 15min and readjusted if necessary. Then 3ml of a pepsin solution (16g/100 ml 0.1 M HCl) were added to the gastric digest and made to volume of 100ml with ultrapure water. The mixture was mixed and incubated for 2 hours at 37°C at a shaking apparatus at 200 rpm (Luten et al., 1989). The gastric digests were cooled to stop enzyme activity. Subsequently, 20ml of the gastric digest were removed and taken to another flask. Then, 5ml of a pancreatin mixture (4g pancreatin and 25g bile extracts/1000ml 0.1M NaHCO3) were added. The pH was measured and adjusted to pH 7.5 with 0.5M NaOH and it was checked after 15 min and readjusted if necessary. (Luten et al., 1989). To stimulate intestinal digestion 20ml of the gastric digests were put into flasks. Dialysis tubings (molecular weight cut-off of 6000-8000 Da; flat width: 32 mm; wall thickness: 30 µm; Φ dry: 20.4mm) were soaked in water for at least 15 min prior to use. Dialysis tubing containing NaHCO3 (in moles equivalent to the NaOH used to identify titratable acidity) diluted in 25ml ultrapure water was added to the digest and incubated for 30 min in the shaking apparatus at 37°C and 200 rpm. The length of the tubing was set at approximately 20cm from clamp to clamp (Luten et al., 1996). The sodium bicarbonate buffer diffuses slowly out of the tubing and neutralizes the digest (Etcheverry et al., 2012). Next, 5ml of the pancreatin/bile extracts mixture

Table 3. Mean Iron and Protein Contents and iron bioaccessibility (in 100g of edible portion) in the Modified foods for Children Aged 6-23 months in Bukoba Rural

Recipe code	Modified Food Recipes	Protein g/100g(SD)	Iron mg/100gfw (SD)	Iron Bio accessibility %/100g(SD)
1N	**Banana puree**: '*Nshakala'*, dry red kidney beans, amaranths, palm oil, salt, onion, tomatoes, bitter tomatoes (1N)	3.4(0.1)	9.1(0.1)	7.9(0.2)
2N	**Banana puree**: *'Nshakala'*, fresh red kidney beans, pumpkin leaves, sunflower oil, salt, onion, tomatoes, bitter tomatoes (2N)	2.8(1.0)	6.1(0.3)	12.2(0.9)
3B	**Banana puree**: *'Bira'*, dry red kidney beans, amaranths, palm oil, salt, onion, tomatoes, bitter tomatoes (3B)	3.3(0.1)	9.0(0.1)	7.4(0.2)
4B	**Banana puree**: *'Bira'*, fresh red kidney beans, pumpkin leaves, sunflower oil, salt, onion, tomatoes, bitter tomatoes (4B)	2.8(0.9)	5.8(0.3)	11.1(1.5)
5N	**Katogo:** *'Nshakala'*, pumpkin, groundnuts flour, salt, onion, tomatoes, bitter tomatoes (5N)	3.8(0.2)	1.9(0.1)	26.7(1.4)
6OFSP	**Porridge**: Fermented maize flour, orange fleshed sweet potatoes, groundnut flour, sugar (6 OFSP)	3.7(0.1)	1.6(0.2)	31.1(2.3)
7B	**Porridge:** Fermented maize, *'bira'*, groundnut flour, sugar (7B)	3.5(0.4)	1.5(0.2)	25.6(1.1)
8E	**Porridge:** Fermented maize flour, egg, red kidney beans, sugar (8E)	3.0(0.4)	3.5(0.1)	12.7(0.5)
	p≤0.05			

SD=Standard deviation

were added and the digests were incubated again for 2 hours (Luten et al., 1996). At the end of simulated gastrointestinal digestion the liquid inside the tubing contains iron represents the bioaccessible fraction of the element. The dialysates were transferred into plastic tubes. Then 15ml from the dialysates were transferred into centrifugation tubes and added with 800µl of 69% HNO3 followed by centrifugation for 10 min at 10°C and 11,000 rpm. The supernatant was filtered through a paper filter. To determine the bioaccessible fraction of iron, filtered supernatant were measured using AAS and bioaccessibility was calculated as follows:
(Gautam et al., 2010).

Statistical analysis

Statistical analyses were performed using GenStat 14th Edition software. Mean were separated by Turkey mean separation test using Least Significant Differences (LSD) at p≤0.05. Determination of the significant difference between food samples using p-values was obtained by homogenous sets. Differences in mean content of total and individual protein and iron were observed following analysis and were tested using One Way analysis of Variance (ANOVA).

RESULTS

Iron and Protein Content in Raw Food ingredients used in Modified food recipes

Egg had more iron content with 9.0mg/100gfw followed by fresh red kidney beans with 6.9mg/100gfw and red kidney beans with 6.5mg/100gfw. Of all recipes groundnuts flour showed higher source of protein (27.5g/100g) than dry red kidney bean (22.8g/100g) and egg (13.3g/100g) (Table 2).

Iron and Protein Content in the Modified Diet and Recommended Daily Allowances (RDA) of Children Aged 6-23 months

The mean iron content in the modified recipes range from 1.5 to 9.1mg/100g, with recipe 1N showing the highest mean iron content of 9.1mg/100g and recipe 7B having the least mean Iron content. These mean represent 6-fold variation. Mean protein contents ranged from 2.8g/100g to 3.8g/100g, with recipe 5N having the highest protein content of 3.8g/100g followed by porridge recipe 6 OFSP (3.7g/100g) and the least was 2N (2.8g/100g) (Table 3).

Bio accessibility of Iron in the Modified Recipes for Children Aged 6 to 23 months

The bio accessibility of iron in all recipes ranged from 7.4% to 31.1%. Iron in porridge recipes were more bio accessible than in banana recipes. Recipe 6OFSP had high bio accessibility percent followed by recipe 5N and 7B with bioaccessibility of 31.1%, 26.7% and 25.6%, respectively (Table 3). There was significant difference between

Table 4. Means of protein and iron content in the 8 modified recipes at p≤0.05

Protein mean (g)*		Iron mean (mg/100g)*		Iron mean bio accessibility*	
5N	3.75 [a]	1N	9.15 [a]	6OFSP	31.1 [a]
6OFSP	3.70 [a]	3B	9.07 [a]	5N	26.7 [b]
7B	3.48 [a]	2N	6.06 [b]	7B	25.6 [b]
1N	3.44 [a]	4B	5.84 [b]	8E	12.3 [c]
3B	3.34 [a]	8E	3.53 [c]	2N	12.2 [c]
8E	3.01 [a]	5N	1.86 [d]	4B	11.1[cd]
4B	2.79 [a]	6OFSP	1.57 [d]	1N	7.92 [de]
2N	2.77 [a]	7B	1.51 [d]	3B	7.42 [e]
p≤0.05					

*Means in the same column with the same superscripts are not significantly different

mean percentage of iron bio-accessibility at p≤0.05 with grand mean of 16.8% and LSD of 2.158.

Table 4 show that mean the protein content between modified food recipes was not significant different at p<0.05. The mean iron bioaccessibility ranged from 7.42 to 31.1% and there was significant different within their mean at p≤0.05. Recipe 6OFSP had high mean bioaccessibility of 31.1% followed by recipe 5N (26.7%) and 7B (25.6%).

Iron and Protein Contents in the Consumption Size of 250g and 500g for Children Aged 6-23 months

In this assessment the assumption was that a child will be able to consume 250g to 500g of the modified recipes per day. The intake of iron from the modified foods ranged from 37.8 to 229% RDA iron and 75.3 to 458% RDA iron at consumption levels of 250g/day and 500g/day, respectively. The percent of protein intake with consumption size of 250g ranged from 53.1 to 72.8 RDA protein and 106–46% RDA protein with consumption size of 500g/day. Dry red kidney beans, onion, tomatoes, amaranths and bitter tomatoes, contributed significant iron content, respectively in recipe 1N at p≤0.05. For iron recipe 1N, 2N, 3B, 4B and 8E meets the RDA by more than 100% by consuming 500 g/day, while by consuming 250g/day only recipe 1N, 2N, 3B and 4B will meet the RDA for iron. However, for breastfed child all recipes will still meet the RDA fro iron and protein (Table 5).

Recommended Daily Allowances (RDA) for iron and protein in 100g ranged from 15.1-91.5% and 21.2-29.1%, respectively (Figure1).

DISCUSSION

The study analysed for raw food ingredients and the modified recipes. This helped to understand, which food is a good source of iron and/or protein. From the raw ingredients, fresh red kidney beans showed to have high amount of iron when compared to the dry ones. This result does not differ much with the study conducted in Tanzania by Mamiro et al., 2012 they found that the levels of iron in fresh bean grains were significantly higher than the average amount found in dry bean grains (Fe 5.6 – 8.0 mg/100g) (Mamiro et al., 2012). The higher iron content in fresh bean grain might be due to the presence of ferritin in fresh seeds which are also loaded in leaves (Ayala-Vela et al., 2008). Therefore consumption of fresh red kidney bean is recommended to enhance iron intake.

The results show that red kidney beans, pumpkin leaves and amaranths had high content of iron and contributed significantly iron content in recipes. It is therefore these ingredients are recommended for preparation of complementary foods to enhance iron intake. Onion, tomatoes and bitter tomatoes are regarded as spice only and not as nutrient source, in this study they contributed significant iron content in recipes. Therefore consumption of these spices sholud be encouranged for iron source. Only recipe 8E had egg as animal source of protein and iron. Raw egg showed high content of iron although in the recipe contributed less in recipe 8E. Groundnuts were a good source of protein. Similar results were reported in a study conducted in Nigeria which recommended that groundnuts are good source of protein for human diet, animal feeds, and an antidote for children suffering from malnutrition (Ayoola et al., 2012). Red kidney beans were also good source of iron and protein. This study agrees with other study conducted in India which showed that red kidney beans are a good source of iron and protein, they analysed for raw and blanched (processed) red kidney beans (Chaudhary and Sharma, 2013). Also in Nigeria they analysed for roasted and boiled red kidney beans and found the same results (Olanipekun et al., 2015).

Recipe 1N and 3B are recommended for iron and protein intake, since they showed high content in both iron and protein. Both recipes had dry red kidney beans, amaranths, palm oil, salt, onion, tomatoes, and bitter tomatoes as ingredients except the first had ‘nshakala’ and the later had ‘bira’ banana variety. However, recipe 5N had slightly high protein content compared to recipe 1N and 3B.

Fermentation improves the bioavailability of minerals such as iron and zinc as a result of phytic acid hydrolysis (Temesgen, 2013). Fermentation has positive effect on

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

Modified food recipe	Iron		Protein	
	%RDA in 250g	%RDA in 500g	%RDA if 250g eaten/day	% RDA if 500g eaten/day
Banana puree: (1N)	228.7	457.5	66.2	132.3
Banana puree: (2N)	151.5	302.9	53.1	106.2
Banana puree: (3B)	226.8	453.6	64.3	128.6
Banana puree: (4B)	145.9	291.9	53.8	107.6
Katogo: (5N)	46.4	92.9	72.8	145.6
Porridge: (6 OFSP)	39.3	78.7	71.2	142.4
Porridge: (7B)	37.7	75.3	66.9	133.7
Porridge: (8E)	88.3	176.6	58.0	115.9

SD=Standard deviation

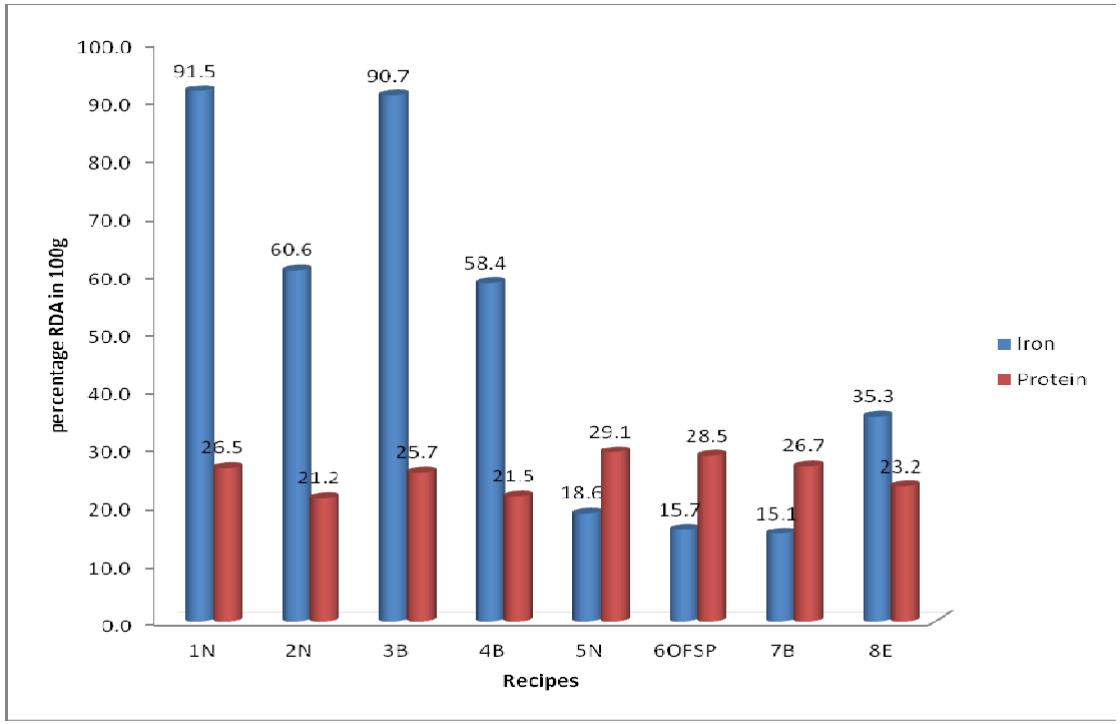

Figure1. Percentage RDA for Iron and Protein in 100g for Children Aged 6-23 months (ingredients per recipes are given in Table 1)

protein content (Temesgen, 2013). Fermented food recipes showed higher bioaccessibility of iron compared to unfermented one for example banana purée recipe (1N) had 9.1mg/100g with bioaccessibility of 7.9% while fermented porridge recipe (6OFSP) had 1.6mg/100g with bioaccessibility of 31.1%. The high bioaccessibility percentage in fermented porridge recipe (6OFSP) might be due to; orange-fleshed sweet potato which is rich in vitamin C (ascorbic acid) and beta-carotene, vitamin C is a known enhancer of iron uptake and other studies indicates that ß-carotene improve iron bioavailability (Christides and Sharp, 2013; García-Casal, 2006; Hurrell and Egli, 2010). Also vitamin C concentrations are positively associated with the ferritin (iron) values (Andre et al., 2015). Furthermore, fermentation increases the amount and bioavailability of iron and protein in cereals. It has also positive effect on protein content (Temesgen, 2013). The assumption is that iron bioaccessibility in fermented porridge (6OFSP) might have been contributed by vitamin C, beta-carotene and fermentation process. Therefore, sweet potato-based porridge is recomended for complementary foods, since has better iron bioavailability.

It is concluded that porridge recipe with fermented maize flour, orange fleshed sweet potatoes, and groundnut flour is recommended for high iron intake since has better iron (31.1%) followed by katogo with 'nshakala', pumpkin, groundnut flour, onion, tomatoes, bitter tomatoes (26.7%) and porridge with fermented maize, 'bira' and groundnut flour (25.6%). In the study area fermentation of maize flour for porridge is not common, therefore it should be promoted.

Bio accessibility of iron was not associated with iron content in the modified recipes, for example; recipe 6OFSP had moderate iron content but with high bioaccessibility compared to recipe 1N with high iron content. This means food with high iron content does not mean will be 100% bio accessible. It is therefore to translate iron requirements into recommendations for daily iron dietary intakes requires an estimation of iron bioavailability.

With the average breast milk intake, the daily requirement of complementary food for children aged 6-23 months is 500g per day and 600 to 674 ml of breast milk consumed in a day (Arbeit and Kouevi, 2013; Dewey et al., 2006). Moreover, a breastfed child aged 6-9 months and 9-24 months is recommended to eat 2-3 and 3-4 meals per day respectively, while all non-breastfed children 6-23 months are advised to eat 4-5 times a day (Berti et al., 2014; WHO, 2010). The amount of protein required to satisfy a child daily nutritional requirement is 9.1 g/day for 6–8 months, 9.6g/day for 9–11 months, and 10.9g/day for 12–23 months (Dewey et al., 2006). Breast milk provides a significant portion of daily protein requirement for children. When average breast-milk intake is assumed, the amount of protein needed from complementary foods is 1.9g/day at 6–8 months (21%), 4.0g/day at 9–11 months (42%), and 6.2g/day (57%) at 12–23 months (Dewey, 2001; Dewey et al., 2006). Thus if the child will consume 250g or 500g of the modified recipes per day; protein intake will be 6.92 to 13.9g/day or 9.47 to18.9g/day, respectively per child. From this point of view the child will consume RDA protein from 63 to 207% in the modified recipes which is more than what is needed from the complementary food. However, this does not consider bioaccessibility.

As stated earlier, the child will get some nutrients from breast milk making the complementary food to contribute significant amount of nutrients. However, other studies have reported that a food can be labelled as the 'source' of a nutrient when 100g of the product presents more than 15% of the dietary reference intake (DRI) for the desired nutrient (Carvalho et al., 2012). The modified diet had more than 15% RDA for iron and protein, therefore the modified diets are recommended for iron and protein intake to meet RDA for children aged 6 to 23 months.

CONCLUSION

Egg is a good source of iron followed by fresh and dry red kidney bean. Groundnut and dry red kidney bean showed to be good source of protein. Banana had the lowest iron and protein content. Dry red kidney beans, was the main source of iron in recipe 1N which had higher iron content than other diets. Recipe 5N had higher protein content with groundnut as the main source of protein in the recipe. Fermented porridge recipes had more bioaccessible iron than in banana recipes. Thus cereal fermentation and food diversity (mixture orange-fleshed sweet potato, fermented maize flour, groundnut flour, sugar) would contribute significant to the bio accessibiliy of iron in recipes. Therefore to enhance intake of iron and protein in the diet; consumption of eggs, red kidney beans, groundnut and food diversity should be given priority. The developed and improved recipes have great potential of contributing substantially to iron and protein needs of children 6-23 months.

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