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„Evaluation of protein, carotenoids and iron in popular dishes given to preschool children from Kiboga district in Uganda“

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List of Abbreviations

AAS	Atomic Absorption Spectrometry
BHT	butylhydroxytoluol
ESTD	External standard
FAO	Food and Agricultural Organisation
G.nuts	grounded peanuts
GHI	Global Hunger Index
HPLC	High Phase Liquid Chromatography
ID	iron deficiency
IDA	Iron deficiency anaemia
IOM	Institute of Medicine
ISTD	Internal standard
IU	International Unit
NRC	National Research Council
PEM	Protein-energy malnutrition
pVAC	provitamin A carotenoids
RAE	Retinol activity equivalent
RDA	Recommended Daily Allowances
RE	Retinol equivalent
rpm	rounds per minute
t-AC	<i>trans</i> - α -carotene
t-BC	<i>trans</i> - β -carotene
TEA	Triethylamine
UDHS	Ugandan Demographic Health Survey
VAD	Vitamin A deficiency
WHO	World Health Organization

1 Introduction

Uganda is a landlocked country in East Africa and is surrounded by Kenya in the East, Tanzania in the South, Rwanda in the Southwest, the Democratic Republic of Congo in the West and the South Sudan in the North. The country is administratively divided into 112 districts. The estimated population is 34.5 millions. The population growth rate of 3.2 % is one of the highest in the world [UBOS, 2012]. The proportion of people living in urban areas was about 18 % in 2010. Uganda is still one of the least urbanized countries in East Africa but urbanization increases and it is expected that the urban population will be tripled until 2025 [MUKWAYA et al., 2010].

In general, fertile soils, rainfalls and moderate temperatures during the whole year provide a basis for the production of many different food crops. In Central and Western Uganda soils are generally fertile but fertility decreases as one moves further to the Eastern and Northern regions. Except the North there are two main rainy seasons providing major harvest in July and December [FANTA-2, 2010]. Most likely, due to the global warming, the rainfall patterns and therefore harvest became more unpredictable [FAO, 2010].

Around 82 % of the Ugandan population is employed in the agricultural sector. Due to geographic and climate difference between the Northern and Southern regions there is a variety of foods being grown. For example, *matooke* (green cooking bananas) which is the traditional staple food in Central Uganda does not grow in the North of the country because of insufficient rainfalls. In Western Uganda millet is the most common staple food, in the Northern region it is posho (a maize flour dish) and sweet potatoes are most common in the Eastern regions [BADIRWANG et al., 2011].

The district of interest for this work, Kiboga, is located in Central Uganda and consists of 83 parishes and more than 500 villages. The total population was estimated 279 000 in 2009, with a growing rate of 4.2 %.

Like other districts in Uganda, Kiboga district is entirely reliant on agriculture. Farming (about 80 % of the total labour force) and livestock farming are the

major sources of employment and income. The main crops grown are bananas, cassava, corn and horticulture [FHRI, 2009].

Malnutrition (referred to undernutrition) is still a big problem in Uganda, especially in the rural parts. With a Global Hunger Index (GHI) score of 18.2, Uganda was ranked at place 56 out of 78 countries in 2013, describing the situation as a serious problem. The GHI of a country is calculated using the average of the percentage of the population being undernourished, the percentage of children younger than five years being underweight and the percentage of children dying before their second birthday [VON GREBMER et al., 2013].

Although the country possesses adequate food supply, a large number of children are still malnourished. Various parameters are contributing to the risk of malnutrition in children in developing countries such as dietary factors, maternal factors and socioeconomic factors [KIKAFUNDA et al., 1998].

Most time infants and small children eat together with their mothers and get the common family meal which is often mashed and diluted for the children. Nationally, about 40% of the children do not have an adequate variety in their diet [FANTA-2, 2010]. In 2006 the prevalence of stunting (chronic malnutrition) among children under the age of five years was 38 %, about 2 million preschool children were affected. Between 2006 and 2011 the prevalence declined but is still high at 33 % what is above the acceptable WHO threshold of 20 % (**Table 1**) [UBOS, 2012].

Especially the deficiency of vitamin A and iron as well as protein-energy malnutrition are severe health problems among children in Uganda [BACHOU, 2002]. An adequate intake of those nutrients is desirable. Studies have shown that food-based approaches are the most sustainable way of alleviating malnutrition among small-holder households in developing countries, therefore increasing the production and the intake of vitamin A and iron-rich foods are necessary [FAO, 2010].

For the nutritional evaluation of foods not only the amount of certain nutrients but also (or especially) their bioavailability or bioaccessibility have to be considered [WERNER and BÖHM, 2011]. Of all nutrients in food only certain amounts will be actually used by the organism for metabolic functions and storage. Bioavailability describes the fraction of an ingested nutrient which reaches the systemic circulation and is available for use in physiological functions. The bioavailable part of a food or diet is the proportion that the body can effectively use [FERNÁNDEZ-GARCÍA et al., 2009].

Bioaccessibility, on the other hand, is defined as the fraction of a nutrient that is available for intestinal absorption after its release from the food matrix. It includes all steps taking place during digestion and describes the transformation of foodstuffs into potentially bioaccessible material that can be assimilated by the enterocytes [EKESA et al., 2012], [FERNÁNDEZ-GARCÍA et al., 2009]. *In vitro* methods simulating gastrointestinal digestion are widely used at present and represent a rapid and safe alternative to costly and time-consuming *in vivo* methods [WERNER and BÖHM, 2011].

1.1 Objectives

The focus of this work is to assess the eating patterns of rural banana-dependent smallholder households to enable an evaluation of the nutrient value of their dishes. The data used for the experimental work results from a household survey which was carried out at rural Kiboga district in Central Uganda.

The specific objectives of the master thesis are as followed:

- To establish the most popular dishes fed to children aged 12-59 months from Kiboga district in Uganda.
- To master the procedures to prepare these meals (including all ingredients, amounts of ingredients, cooking method and cooking time) and prepare samples.
- To evaluate the content of micronutrients (provitamin A carotenoids and iron) and protein in the popular dishes.

- To evaluate the *in vitro* bioaccessibility of provitamin A carotenoids and iron in the popular dishes.
- To assess if children aged 12-59 months from Kiboga district meet their nutritional needs.

Based on the results of the master thesis, other scientists will be able to develop appropriate preparation and combination methods with focus on protein and micronutrient-rich diets for children aged 12-59 months to decrease nutrient deficiencies.

2 Nutrition Situation in Uganda

All in all there isn't a lack of food in Uganda. About 73 % of the households are categorized as food secure [SHIVELY and HAO, 2012]. In 1996 food security was defined as existing "*when all people at all times have access to sufficient, safe, nutritious food to maintain a healthy and active life*" [WHO]. Although the country produces sufficient food to meet the needs of its population, many Ugandans do not have access to the recommended amount of calories because of the uneven distribution of food. Some parameters for a limited access are regional and seasonal factors (e.g. declining soil fertility, climatic conditions and unreliable rainfall patterns), poverty and diseases as well as prolonged civil insecurity in some regions [FANTA-2, 2010], [FAO, 2010].

Uganda is affected by high levels of macronutrient (especially protein) and micronutrient deficiencies with infants, young children, adolescents, pregnant and lactating women representing the most vulnerable groups. In many parts of the country undernutrition is endemic [HARVEY et al., 2010].

Generally the Ugandan diet is quite monotonous. The composition of dishes is depending on foods being grown and the availability and accessibility on local markets. This lack of diversity leads to an insufficient intake of micronutrients. In addition the prices for vegetables, fruits and animal products high in protein increased, what resulted in a decreased consumption of these products [SHIVELY and HAO, 2012].

The Ugandan diet is mainly based on foods of vegetable origin. Only 11-13 % of the total energy intake derives from foods of animal origin [HARVEY et al., 2010]. Plantain (green cooking bananas which are locally known as '*matooke*'), starchy roots like cassava and sweet potato and cereals like corn and millet are the main staple foods in the Ugandan diet. There are regional differences in the consumption; cassava, corn and millet are mainly used in the Northern and Eastern regions, cooking bananas, sweet potatoes, corn and rice are the most important staple foods in the central and western regions. Commonly these dishes are served together with a sauce. Grounded peanuts, beans, peas or green leafy vegetables are widely used ingredients for sauces. In rural families it is common to have two main meals a day (without breakfast).

Overall, in 2005 the two major food groups for human consumption were fruits/vegetables (mainly plantain, which is falling under that category according to FAOSTAT) and starchy roots (mainly cassava and sweet potato) followed by cereals with a lower supply (just one-third compared to the supply with fruits/vegetables). Corn and millet are the major compounds of this food group. From the nutritional point of view, cereals contain more health-promoting micronutrients than plantains and starchy roots [FAO, 2010].

In central Uganda, where Kiboga district is located, the main staples include cassava and potatoes followed by *matooke*, beans and corn [FANTA-2, 2010].

With 0.70 kg/person/day Ugandans have the highest per capita consumption of banana in the world; on average 30 % of their energy, 10 % of protein and 5 % of their fat intake is obtained by bananas [SHIVELY and HAO, 2012].

3 Malnutrition among children

Malnutrition, which consists of protein-energy malnutrition and micronutrient deficiencies, is a major health burden in many developing countries. As malnutrition increases the susceptibility to illness, it is a major contributor to death from diseases [MÜLLER and KRAWINKEL, 2005]. Around 50 % of deaths among children under the age of five years are associated with malnutrition [BRYCE et al., 2005].

Causes for child malnutrition are multifaceted and categorized in different levels (**Figure 1**). Basic causes appear at the social level which includes resources and environment, economic and policy factors as well as institutions. The three main causes at the household/family level are access to food, maternal and childcare practices and the quality of health services. These three causes are influenced by several factors like, among other things, agriculture, poverty and food prices, education and women's empowerment. Food/nutrient intake and general health are the major causes at the individual level which are influenced by many factors such as breastfeeding, dietary diversity, frequency and severity of illnesses, hygiene and food/water safety.

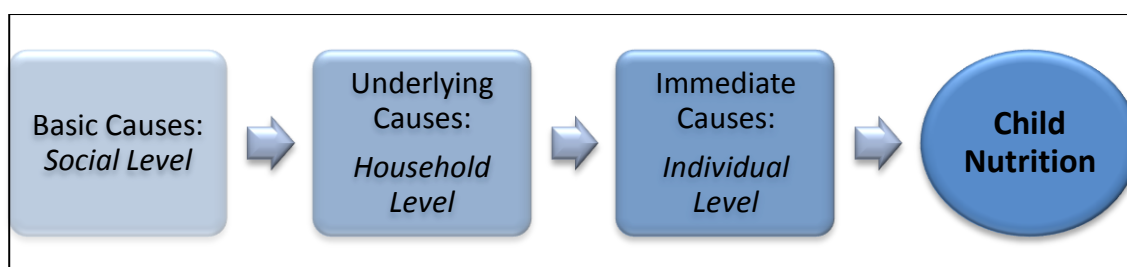


Figure 1. Causes of malnutrition [FANTA-2, 2010]

In Uganda malnutrition is kind of a “hidden problem” because most children who are affected are moderately malnourished or have micronutrient deficiencies that are not frequently assessed. In addition, Uganda has to fight the “double burden” of malnutrition: high levels of undernutrition coexisting with an increasing prevalence of overweight and obesity [FANTA-2, 2010].

3.1 Protein-energy malnutrition

An insufficient supply of macronutrients (protein, carbohydrates and fat) resulting in an inadequate intake of calories and severe diseases – especially those producing diarrhea – are the major causes of protein-energy malnutrition. Severe forms of PEM appear as Marasmus, Kwashiorkor or a mixed picture of both [MÜLLER and KRAWINKEL, 2005]. Moreover malnutrition is associated with structural as well as functional pathology of the brain and chronic PEM

during childhood can affect the development of higher cognitive processes. Long lasting cognitive impairments may be the result [KAR et al., 2008].

Due to their increased metabolic requirements, infants and children are the most vulnerable groups. The prevalence of Marasmus and Kwashiorkor in developing countries adds up to 5 % but 30-70 % of children aged five years or younger suffer from subclinical forms of PEM [ELMADFA and LEITZMANN, 2004]. It is estimated that one third of all children under 5 years are stunted (178 millions) and around 112 millions are underweight, which makes malnutrition the most common “disease” among children [AHMED et al., 2012].

3.1.1 Measurement of nutritional status

The nutritional status of children can be measured with the anthropometric data on weight and height. Stunting as well as wasting and underweight are classified using Z-scores (or standard deviation (SD) scores). The Z-score is defined as the deviation of an individual's value from the median value of a reference group, divided by the standard deviation of the reference group. If the Z-score of an individual is below -2 standard deviations from the median of the reference population, malnutrition is referred to as moderate, if the Z-score is below -3 standard deviations malnutrition is referred to as severe [WHO, 2006].

The *height-for-age-index*, which means that children are very short for their age, gives information about chronic malnutrition (stunting). This is an indicator for inadequate nutrition intake over a long period of time and is hardly influenced by short-term changes in the dietary intake [UBOS, 2012]. Besides a short adult age childhood stunting could also lead to a delayed development of cognitive functions as well as permanent cognitive impairments [KAR et al., 2008].

The *weight-for-height-index* describes the current nutritional status. It provides data for both actually malnourished (wasted) and also overweight children. Wasting is linked to an inadequate nutritional intake or an illness causing weight

loss in the recent past and means that a child is too thin for its size. Wasting occurs if children fail to gain the expected weight compared to healthy children at the same age [FANTA-2, 2010].

The *weight-for-age-index* describes underweight in general and is considered as a combination of both chronic and current malnutrition. Weight-for-age is used as an overall indicator for the nutritional health situation of a population [UBOS, 2012].

3.1.2 Marasmus

Marasmus is characterized by a quantitative nutrient and energy deficiency. It is defined as severe wasting, which means that children are too thin for their height [MÜLLER and KRAWINKEL, 2005] [UBOS, 2012]. Infants and children under the age of five years are most vulnerable to suffer from Marasmus because of their increased requirement of calories as well as their higher susceptibility to diseases. A low birth weight, short breast feeding periods and short intervals between childbirth may contribute to the development of Marasmus [ELMADFA and LEITZMANN, 2004]. The depletion of subcutaneous fat stores and the wasting of muscles are results of the body's adaption to starving [GROVER and EE, 2009]. Weight loss up to 50 % of the normal weight is not uncommon [ELMADFA and LEITZMANN, 2004].

3.1.3 Kwashiorkor

Kwashiorkor is characteristic for children whose diet ensures an adequate caloric intake but is insufficient in protein. The diseases name derives from the Ga language and means "the sickness the older baby gets when the new baby comes". Abrupt weaning of an infant after the birth of another child typically leads to the development of Kwashiorkor [ELMADFA and LEITZMANN, 2004]. Most of the affected children gain normal weight for their age but symptoms are oedemas, dermatoses and distended abdomen [GROVER and EE, 2009].

3.1.4 Situation in Uganda

In Uganda 33 % of children aged five years or younger are stunted and 14 % are severely stunted, with substantial regional variations. Therefore children living in rural areas are more likely to be chronically malnourished (e.g. 45 % in Karamoja) than those in urban areas (e.g. 14 % in Kampala).

With increased age the prevalence of being stunted also increases. The highest rates of stunting were found in children aged 24-35 months (43 %) and the lowest in children aged 6-8 months (12 %).

Another factor contributing to the development of stunting is size at birth. The likelihood for chronic malnutrition is more common among children of small birth size (43%) compared to those children with an average or larger size at birth [UBOS, 2012].

5 % of Ugandan children under the age of five are wasted. In contrast to chronic malnutrition, acute malnutrition has the highest rates in children aged 8-10 months (14 %) and the lowest in children aged 24-59 months (2 %). Infants with a very small size at birth (12 %) are more susceptible to be wasted than those with an average or larger size (4 %). The prevalence of wasting is also increased among children of thin mothers (13 %), among children living in Karamoja (7 %) and among those whose mothers have no education (7 %) [UBOS, 2012].

14 % of Ugandan children are underweight and 3 % are severely underweight. The highest rates can be found in children aged 6-8 months (19 %). In addition, children of thin mothers (BMI < 18.5) and children with a small birth size are three times as likely to be underweight as those who are born to overweight/obese mothers or having an average size at birth. Children in urban areas are less likely to be underweight (7 %) compared to their rural counterparts (15 %) [UBOS, 2012]. Other factors that contribute to underweight are regional variations, a poor health status, a low education level of the mother, the use of unprotected water supplies and also an insufficient consumption of milk [KIKAFUNDA et al., 1998], [UBOS, 2012].

According to WHO standards Uganda's situation for stunting is classified as serious and poor for both wasting and underweight (**Table 1**).

Table 1. WHO classification for assessing severity of malnutrition [FANTA-2, 2010]

	Acceptable	Poor	Serious	Critical
Stunting	<20%	20-30%	30-40%	>40%
Wasting	<5%	5-10%	10-15%	>15%
Underweight	<10%	10-20%	20-30%	>30%

Note: levels in Uganda are highlighted.

These high rates of malnutrition are related to an insufficient access to food, inadequate feeding practices and poor health, sanitation and hygiene practice. The deficiency of micronutrients contributes to childhood morbidity and mortality. The child mortality rate due to malnutrition is assumed to be up to 60 %, making malnutrition one of the most significant causes for childhood mortality in the country as malnourished children are much more susceptible for diseases and infections [FANTA-2, 2010]. Over the past years the proportion of wasted Ugandan children has not changed. However, prevalence of stunting and underweight decreased over the last years. The reduction of stunting among children shows an improvement in chronic malnutrition (**Figure 2**) [UBOS, 2012]. Under these positive circumstances Uganda is still on track to meet the first Millennium Goal (halve the number of people suffering from hunger between 1995 and 2015). The hunger-reduction target will be fulfilled if the prevalence of underweight in preschool children declines to 10 % by 2015. The Uganda Nutrition Action Plan 2011-2016 was established to support reaching this target. The improvement of maternal nutrition and care, exclusively breastfeeding during the first 6 months of life, appropriate complementary feeding and a sufficient intake of micronutrients for children aged 6 to 24 months, as well as fortification of staples are the main courses of action [UNDP, 2013].

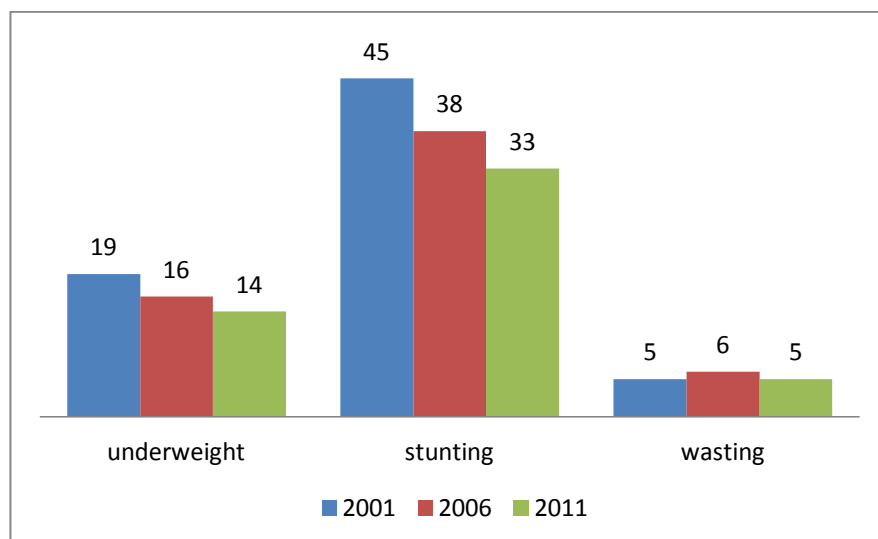


Figure 2. Trends in malnutrition among children under 5 years (in percent) in Uganda [UBOS, 2006 and 2011]

3.1.5 Recommended protein intake

To ensure normal body growth and development a sufficient intake of protein is necessary. In addition to the PEM symptoms mentioned before (see chapter 3.1.2 and 3.1.3) an inadequate intake of protein may cause delayed and irreversible cognitive development [KAR et al., 2008].

Based on 1.5 g/kg/day for infants, 1.1 g/kg/day for children 1-3 years and 0.95 g/kg/day for children 4-13 years, the Institute of Medicine gives the following recommendations (**Table 2**) [IOM, 2002].

Table 2: Dietary Reference Intakes: Protein [IOM, 2002]

Age of children	RDA (g/day)
0-6 months	9.1
7-12 months	11
1-3 years	13
4-8 years	19

RDA = recommended daily allowances

It has to be considered that proteins from plant origin (nuts, seeds, legumes, grains, vegetables ...) lack in one or more essential amino acids. The combination of different plant origin products and the addition of animal products may avoid deficits of amino acids, which are important especially during growth [IOM, 2002].

3.2 Vitamin A deficiency (VAD)

A chronic deficit of vitamin A leads to low body stores and consequently to a failure to meet physiological needs and an increased susceptibility for infections and diseases [UBOS, 2012] [WHO, 2009]. There are two ways to define VAD. One is to assess eye signs and the other one is measuring the serum retinol concentrations. Values below 0.70 $\mu\text{mol/l}$ represent VAD and values below 0.35 $\mu\text{mol/l}$ imply severe stages of VAD.

Xerophthalmia is the most specific disorder caused by VAD and reason for preventable blindness of children all over the world. The term xerophthalmia comprises the whole spectrum of eye diseases caused by VAD – from milder forms like night blindness to potentially blinding stages [WHO, 2009]. In addition there are some unspecific symptoms like increased morbidity and mortality, a higher risk of anaemia as well as slowed growth and development [FAO, 2004]. VAD appears most frequently in populations consuming mostly provitamin carotenoids to cover their vitamin A needs and have just small amount of fat in their diet [FAO, 2004].

WHO estimated that between 1995 and 2005, in 45 countries VAD in children aged 5 years and younger is a public health concern based on the prevalence of night blindness and in 122 countries on the prevalence of low serum retinol concentrations ($<0.70 \mu\text{mol/l}$). Described in absolute numbers it is estimated that 5.2 million preschool children are affected by xerophthalmia and 190 million children are affected by low serum retinol [WHO, 2009].

3.2.1 Vitamin A and carotenoids

Vitamin A (all-trans-retinol) is an essential micronutrient that plays an important role in the human metabolism. It has two main tasks: firstly, vitamin A maintains

growth and functions of cells in body tissues – it is required during growth and development and for the function of the immune system. Secondly, it functions in the retina of the eye where it regulates the visual cycle [FAO, 2004].

Foods rich in retinol, which have a high bioavailability, come from animal origin like meat, liver, eggs and dairy products. Provitamin A carotenoids are synthesized by plants and can be converted to retinol in the human body. Compared to retinol provitamin A carotenoids are less well absorbed and therefore have a lower bioavailability. They can be found in plant origin foods like dark green leafy vegetables, carrots, pumpkins, mangoes, etc. [ELMADFA and LEITZMANN, 2004] [FAO, 2010]. Among the provitamins β -carotene is the most potent one, followed by α -carotene and β -cryptoxanthin [RODRIGUEZ-AMAYA and KIMURA, 2004].

3.2.1.1 Metabolism

In animal foods preformed vitamin A occurs as retinyl ester, in foods of plant origin provitamin A carotenoids are found [FAO, 2004]. During digestion the retinyl esters are hydrolysed; the formed retinol and freed carotenoids are incorporated into lipids containing, water-miscible micelles. As Vitamin A is fat-soluble, an adequate intake of dietary fat (at least 10 g per day) is required to ensure an efficient solubilisation of retinol and the carotenoids in the aqueous milieu of the intestine [FAO, 2004]. The absorption rate of retinol is quite high (> 80 %) whereas the absorption rate of the carotenoids comes up to just 1/3 compared to retinol. In the small intestine the enzyme 15,15'-dioxygenase converts the carotenoids into retinol. One-half of the β -carotene molecules are transformed to retinol (**Table 3**) [ELMADFA and LEITZMANN, 2004]. After the absorption in the small intestine retinol is re-esterified and, together with transformed carotenoids and lipids, incorporated into chylomicrons. They are delivered to the blood and taken up by the liver where the retinyl esters are stored in the hepatocytes. Carotenoids are also stored in the skin, fat tissue, testes and the macula of the eye [ELMADFA, 2004]. When needed, the retinyl esters are hydrolysed, attached to retinol-binding protein and delivered to the tissues [FAO, 2004].

Table 3. Usability of provitamin A carotenoids [ELMADFA, 2004]

	Compared to retinol
β-carotene absorption rate	1/3
β-carotene transformation rate	1/2
β-carotene activity	1/6
α-Carotene and β-cryptoxanthin activity	1/12

3.2.1.2 Recommended intake

The mean requirements are set as the minimum daily intake of vitamin A to prevent children from any clinical or subclinical signs of VAD and allow normal growth [FAO, 2004]. To ensure that children meet their nutritional needs and to avoid the appearance of deficiency-related symptoms like xerophthalmia, an intake as described in **(Table 4)** is recommended [IOM, 2001]. The requirements for infants (0-6 months) are derived from the vitamin A content in human milk. Exclusive breastfeeding during the first 6 months of life provides enough vitamin A to enable adequate growth.

The safe level of intake is set as the daily amount of vitamin A that allows normal growth and other vitamin A dependent functions as well as an acceptable body storage [FAO, 2004].

As the liver can store vitamin A in adequate amounts for about four to six months, periodic administration of supplements is an opportunity for children whose diet contains insufficient amounts of the vitamin [UBOS, 2012].

Table 4. Dietary Reference Intakes: vitamin A (on basis of RE) [FAO, 2004]

Age of children	mean requirement (µg RE/day)	safe level of intake (µg RE /day)
0-6 months	180	375
7-12 months	190	400
1-3 years	200	400
4-6 years	200	450

To describe the vitamin A activity of carotenoids, the concept of retinol equivalents (RE) was established by a Joint FAO/WHO Expert Group. It should be considered that these conversion factors refer to a mixed diet and may be

higher or lower depending on the source of the carotenoids, in combination with other nutrients and preparation methods [FAO, 2004].

1 µg retinol = 1 µg retinol equivalent (RE)

1 µg RE = 6 µg β-carotene

1 µg RE = 12 µg other provitamin A carotenoids [NRC, 1989].

Another approach is to use retinol activity equivalents (RAE). Recent data shows that the absorption of provitamin A carotenoids is just one-sixth, rather than one-third. Therefore larger amounts of foods rich in carotenoids are needed to meet vitamin A requirements [IOM, 2001].

1 µg retinol = 1 µg retinol activity equivalent (RAE)

1 µg RAE = 12 µg β-carotene

1 µg RAE = 24 µg other provitamin A carotenoids [IOM, 2001].

The application of RAE is useful if the total amount of Vitamin A in mixed foods is calculated [IOM, 2001].

Based on this approach the following recommendations (**Table 5**) were set to prevent children from any forms of VAD:

Table 5. Dietary Reference Intakes: vitamin A (on basis of RAE) [IOM, 2001]

Age of children	RDA (µg RAE/day)
0-6 months	400
7-12 months	500
1-3 years	300
4-6 years	400

3.2.2 Situation in Uganda

Based on the prevalence of low serum retinol concentrations (<0.70 µmol/l) 28 % of Ugandan preschool children (around 1 600 000 individuals) between 6 and 59 months suffer from vitamin A deficiency which describes a severe public health problem [FIEDLER and AFIDRA, 2012] [WHO, 2009]. Using the prevalence of night blindness to assess VAD it is estimated that 1.5 % of

preschool-aged children (around 87 000 individuals) are affected, which is still defined as a moderate public health problem [WHO, 2009].

Especially in rural areas the intake of foods from animal origin is low. To cover their nutritional needs people rely heavily on foods which provide provitamin A carotenoids [WHO, 2009]. It is estimated that in Africa more than 80 % of the dietary vitamin A comes from provitamin A carotenoids [RUEL and LEVIN, 2000]. Boiled or steamed green leafy vegetables are the main source of vitamin A [BACHOU, 2002]. Moreover the content of lipids in the diet is relatively low (in 2005 it was just 16 % of the dietary energy supply). For an effective utilization of vitamin A adequate amounts of lipids are required [FAO, 2010].

Through twice-yearly high-dose supplements VAD could be prevented [LUTTER, 2008]. The high-potency supplements are containing 100 000 IU (1 µg RE = 3.3 IU) of vitamin A for children aged 7-12 months and 200 000 IU for children younger than five years [WHO, 2009]. In 2011, the UDHS (Ugandan Demographic Health Survey) evaluated data concerning vitamin A supplementation. 57 % of children aged 6-59 months received supplements six months prior the survey [UBOS, 2012] which is still far away from the national goal of vitamin A supplementation coverage of 80% [FANTA-2, 2012]. Another and the most important approach to control VAD includes the improvement of both availability and intake of foods rich in vitamin A. Nutrition education is the required key to change dietary habits, improve dietary diversification and encourage home growing of vitamin A-rich vegetables and fruits. The third approach is the fortification of staple foods, as it is already a common practice in high income countries [WHO, 2009].

3.3 Iron deficiency

Iron deficiency is among the leading risk factors for death worldwide and it is estimated that 2 billion people are affected [ZIMMERMANN and HURRELL, 2007]. It is the result of a negative iron balance over a long period which leads to an empty body storage and inhibits a normal iron turnover in the body [PETTIT et al., 2011]. If iron intake is insufficient firstly body stores are exhausted, then the formation of erythrocytes is diminished and finally the activity of iron-dependent enzymes is reduced. An inadequate intake of iron is the most contributing factor in the development of iron deficiency. Other contributors are impaired absorption or hepatic diseases [CLARK, 2008]. Early symptoms of iron deficiency include fatigue, dizziness, headache and alteration of the mucous membrane of the mouth and oesophagus. A more severe form of iron deficiency is anaemia with disturbed thermoregulation and malfunctions of the immune and nervous system [ELMADFA, 2004].

The onset of iron deficiency is hard to establish so it is difficult to estimate the exact prevalence. As iron deficiency is the most significant contributor to anaemia, iron deficiency anaemia (IDA) is often used as a proxy [PETTIT et al., 2011]. Nevertheless it has to be considered that there exist other factors than iron deficiency which can cause anaemia like hereditary defects in haemoglobin synthesis, deficits in other nutrients like Vitamin A or folic acid, blood loss because of hookworm infestation for instance [WHO, 2001].

To define anaemia haemoglobin thresholds are used. For children aged 6 months to 5 years the threshold is set at 110 g/l. Below this cut off anaemia is present [DE BENOIST et al, 2008], [PETTIT et al., 2011].

Global data for the prevalence of iron deficiency is not available; therefore anaemia is used as an indirect indicator. All over the world 1.6 billion people are affected by anaemia which corresponds to almost 25 % of the population. The highest prevalence can be found in preschool children and is 47.4 %. In Africa 67.7 % of preschool children are affected [DE BENOIST et al, 2008]. Using haemoglobin alone to estimate the prevalence of IDA may lead to overestimations because other causes of anaemia are not taken into account [ZIMMERMANN and HURRELL, 2007].

3.3.1 Iron

Iron is required for the oxygen transport in humans and is an important factor in the energy metabolism [ELMADFA, 2004]. It is part of enzymes and necessary for cognitive development and the immune system [UBOS, 2012]. In early life iron is essential for an adequate neurogenesis and the differentiation of brain cells. A deficit of the element during infancy is often related to poorer motor, cognitive and socio-emotional function [PETTIT et al., 2011].

Foods of animal origin like meat, poultry and fish are rich in heme iron (Fe^{2+}) which has a high bioavailability. Non-heme iron (Fe^{3+}) can be found in plant origin foods like cereals and pulses [ELMADFA, 2004] as well as in dairy products and eggs [RUEL and LEVIN, 2000]. As people in Uganda often do not have access to meat, they mainly consume foods of vegetable origin with a lower bioavailability of iron [FAO, 2004]. In low income countries about 50 % of the dietary iron comes from cereals [RUEL and LEVIN, 2000]. The presence of ascorbic acid (e.g. certain fruit juices, fruits, vegetables and potatoes) as well as meat or fish in the diet helps to enhance the absorption of iron, whereas phytates (e.g. in grains, seeds, nuts, vegetables, fruits) and some iron-binding polyphenols (e.g. in coffee, cocoa, some spices) inhibit absorption. Phytates inhibit iron uptake in a dose-dependent fashion but already small amounts have an effect. If food which contains heme iron is cooked too long at a high temperature a transformation to non-heme iron can occur [FAO, 2004]. The contribution of plant origin sources to control and in the best case decrease iron deficiency in developing countries is questionable [RUEL and LEVIN, 2000].

3.3.1.1 Metabolism

The absorption rate of heme-iron is approximately 10-25 %, whereas just 3-8 % of non-heme iron is taken up [BIESALSKI and GRIMM, 2007]. Low iron stores trigger an increased absorption by up- and down regulation of various proteins [FAO, 2004].

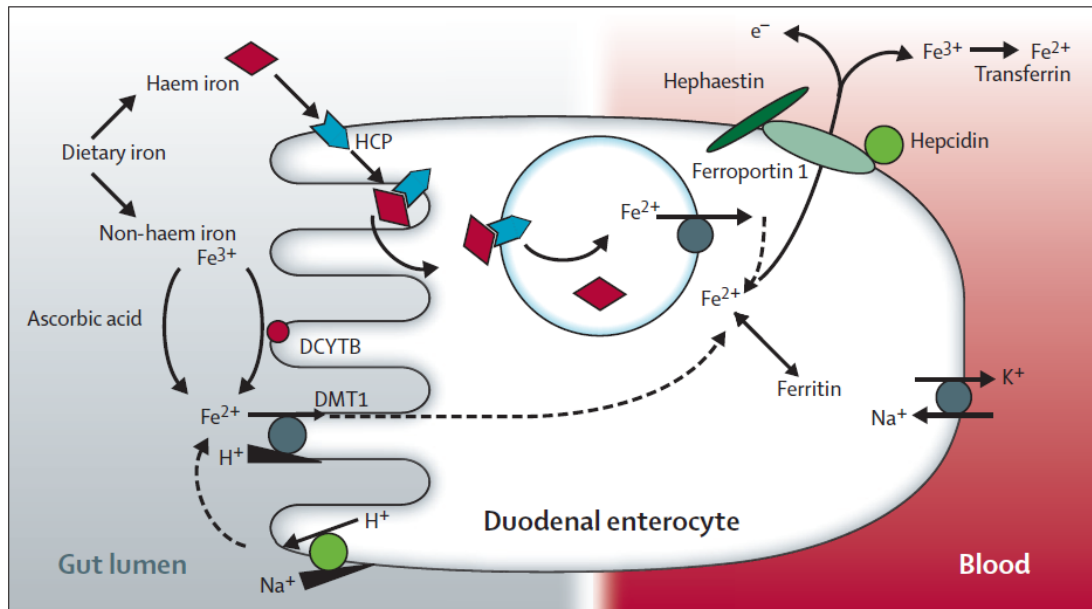


Figure 3. Regulation of iron uptake in the small intestine [ZIMMERMANN and HURRELL, 2007]

Dietary iron is absorbed by the small intestine (**Figure 3**). Heme-iron is taken up into the enterocyte by an intestinal heme iron transport (HCP). Non-heme iron in the diet has to be reduced from the ferric (Fe³⁺) to the ferrous (Fe²⁺) form before its uptake into the enterocyte by the divalent metal ion transport 1 (DMT1). As mentioned above, ascorbic acid may enhance iron absorption, as well as an enzyme on the brush border (duodenal cytochrome b, DCYTB). On the other hand absorption of non-heme iron can be decreased by some food components like calcium, phytates or phenolic compounds [PETTIT et al., 2011], [ZIMMERMANN and HURRELL, 2007].

The protein ferroportin 1 is responsible for the transport of iron across the basolateral membrane into the blood. Ferroportin, as well as DMT1 and DCYTB expression is stimulated if iron deficiency is present and thereby absorption is increased. Hepcidin, on the other hand, is a regulatory hormone which inhibits absorption as well as release of iron from other cells by binding to ferroportin 1 and causing its degradation. Expression of hepcidin is decreased in iron deficiency which leads again to a maximum absorption. The membrane protein hephaestin oxidises the iron from the divalent to its trivalent form which then can bind to transferrin [ZIMMERMANN and HURRELL, 2007].

Transferrin, a plasma protein which is synthesized in the liver, is responsible for the transport of iron from the mucosa cell to erythrocytes precursors in the bone marrow or other body cells [PETTIT et al., 2011]. Transferrin loaded with iron binds to specific transferring receptors (TfRs) and receptor-mediated endocytosis leads to the uptake into body cells or erythroid precursors. Inside the cell iron is released from transferrin by a pH reduction and is finally available [ZIMMERMANN and HURRELL, 2007]. Iron is stored mostly in the liver as either ferritin or haemosiderin [ELMADFA, 2004].

3.3.1.2 Recommended Intake

During their first year of life, children's demand for iron is increased because of rapid growth [PETTIT et al., 2011]. At birth an infant's iron store consists of 250 mg iron. These stores are exhausted during breastfeeding, because breast milk provides only 0.15 mg of absorbed iron per day, whereas 0.55 mg are required [ZIMMERMANN and HURRELL, 2007]. Children's requirements of absorbed iron are very high in relation to their body size and total energy requirements [FAO, 2004].

The recommended daily allowances (RDA) are set to meet the needs of almost all members in a group (**Table 6**) [IOM, 2001]. The following recommendations refer to a low bioavailability of iron because of a diet poor in animal protein.

Table 6. Dietary Reference Intakes: Iron [IOM, 2001]

Age of children	RDA (mg/day)
7-12 months	11
1-3 years	7
4-8 years	10

The absorption of non-heme iron is lower for individuals consuming just vegetarian diets compared to those eating a mixed diet. Therefore, iron requirement for those consuming a vegetarian diet is greater (approximately 2-fold) [IOM, 2001]. Blood loss (as a reason of hookworm infection, for instance) also increases iron demands [ELMADFA and LEITZMANN, 2004].

3.3.2 Situation in Uganda

On the basis of haemoglobin levels below 110 g/l the WHO estimated in 2006 that 64 % of Ugandan preschool children were affected by anaemia which represents a severe public health problem [DE BENOIST et al, 2008].

In 2011 haemoglobin was measured in 2,120 children from different regions. Almost 50 % of Ugandan children aged 6-59 months suffered from any anaemia with the highest rates in children aged 9-11 months because of the increased iron requirements during growth. There is a higher prevalence of anaemia in rural areas (51 %) compared to urban areas (38 %). The intake of iron-rich foods is higher in rural than in urban areas [UBOS, 2012]. A lower fibre intake in urban areas (e.g. Kampala) may also lead to a better bioavailability of iron [HARVEY, 2010]. Besides insufficient iron intake malaria, chronic diseases like HIV/AIDS, some parasites like hookworm or other infections can lead to anaemia [FAO, 2010].

Between 2006 and 2011, the prevalence of anaemia decreased from 73 to 46 %, mainly because of the decline in the prevalence of moderate anaemia (**Figure 4**) [UBOS, 2012].

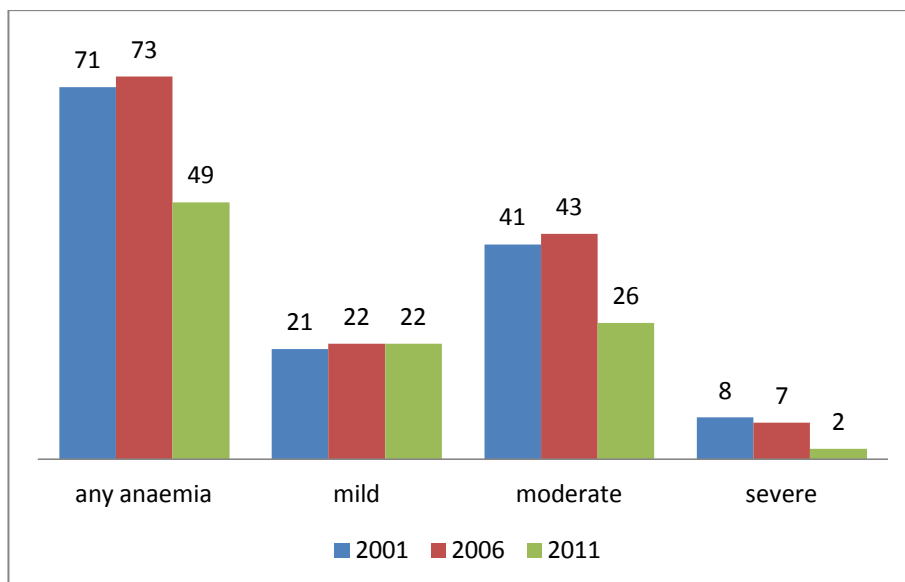


Figure 4. Trends in anaemia among children under 5 years (in percent) [UBOS, 2006 and 2011]

Strategies to prevent iron deficiency are widely discussed. Interventions need to start in early pregnancy by improving the mother's iron status. Supplementation as well as deworming and promoting the intake of iron-rich foods (from animal-source or iron-fortified, if available) are recommended practices. At birth early initiation of breast-feeding should be promoted. Although breast milk contains just little amounts of iron, it is in a high bioavailable form. Exclusively breast-feeding is recommended until the age of 6 months; other liquids or solid foods may reduce the absorption of iron present in breast milk and should be avoided. Between 6 and 24 months of age requirements are quite high whereas children consume comparatively small amounts of food. During that period meeting their iron needs through food alone is nearly impossible and supplementation is recommended. To prevent and treat ID, periodic supplementation (daily or weekly) is necessary, which requires attention to numerous factors (a working delivery system, motivated staff, creating consumer demand through education...) [LUTTER, 2008].

In Uganda supplementation coverage is generally low. In 2011, the UDHS (Ugandan Demographic Health Survey) showed that only 7 % of children between 6 and 59 months received iron supplements in the 7 days prior the survey [UBOS, 2012].

4 Methodology

4.1 Study design

The first aim of this study was to evaluate the most common dishes fed to children aged 12-59 months from Kiboga district in Uganda and hence the content of provitamin A carotenoids, iron and protein. The second objective was to establish the bioaccessibility of provitamin A carotenoids and iron in these dishes to find out to what extent these diets meet the nutritional needs of preschool children.

This is an experimental study which bases its sample type selection on findings from a cross-sectional survey to establish popular dishes given to preschool children from Kiboga district. This work is part of *Bioversity International's* project on "Developing agrobiodiversity-based strategies for the alleviation of micronutrient and protein deficiencies among smallholder households in banana growing regions of East Africa".

4.2 Sample population

The study was carried out in rural Kiboga district which is located in Central Uganda. Kiboga district is subdivided into 13 sub-counties of which Lwamata is one of them. Kisweeka and Ssinde, two parishes located in Lwamata, were purposively selected because of existing high poverty levels and high dependency on agriculture. Kisweeka has 7 villages, Ssinde has 5 villages.

In Lwamata the proportion of children below five years is 17.2 % of the total population.

Three and two villages were randomly selected from Kisweeka and Ssinde parishes, respectively. Based on the population size and proportion of preschool children in Lwamata (17.2 %), the exact sample size for the preliminary survey (questionnaires) was established and set to be 219 households with preschool children. The distribution of the 219 households with preschool children within the villages was proportional to the number of households in the villages. Once the number of households being samples in each village was determined, the village heads listed all households with

preschool children and systematic random sampling was used to select the 219 households.

4.3 Analysis of diagnostic data from household survey

Structured questionnaires were used for data collection and the respondents of the 219 households included preschool children's mothers, or any adult within the household responsible for feeding the respective pre-schooler. Information collected included:

- The common name of the meal.
- The ingredients both major and minor used to prepare the dish.
- The detailed cooking procedure including time when each ingredient should be added and total cooking time.

The analysis of the diagnostic data was carried out using SPSS Version 15 for Windows. The frequency in consumption of each meal was identified and out of these meals the three most popular dishes and the most common combination of ingredients used to prepare them were established.

4.4 Determination of preparation methods of dishes by caregivers

Three families from Kisweeka/Ssinde parish in Lwamata were picked randomly, facilitated to prepare the common dishes which were established at the statistical analysis and visits were made to these households to observe and also take part in the preparation of these common dishes. The information collected included all ingredients, amount of ingredients, total duration of cooking and also at which point ingredients were added and materials used for cooking. As expected, the preparation methods varied slightly from family to family. Nevertheless, the differences were non-serious, so the aim was to draw up one recipe for each dish that combines all information. Kitchen scales were not available, so the amounts of the used ingredients were measured using local cups or spoons and finally equated to known measures in grams/kilograms/litres.

4.5 Follow-up questionnaire

To affirm the findings from the preliminary household survey and the information given from the caregivers preparing the dishes, another questionnaire was developed to collect more detailed data of the most common dishes from 11 randomly selected households from Kisweeka and Ssinde and focus group discussions carried out.

4.6 Establishment of recipes of the most common dishes

The data from the household survey, the information of the caregivers who prepared the dishes and the follow-up questionnaire were combined and one recipe for each dish was established. These dishes were used for further laboratory analysis of nutrients.

4.7 Sample preparation

Fresh green cooking bananas as well as banana leaves were purchased on a local market in Kampala. Grounded peanuts and maize flour were purchased in a supermarket in Kampala and finally all samples were transported to Vienna (Austria, Universität für Bodenkultur BOKU, Institute of Food Science). Tomatoes, onions, salt and sugar were purchased in an Austrian supermarket. At the laboratory the dishes were prepared according to the established recipes (**Table 13**) although care was taken to ensure that cooked samples of each individual ingredient were collected before the dish (containing all necessary ingredients) was made. Both the single ingredients and the dishes were then stored at -18°C to await analysis.

A portion of each sample (20-40 g) was weighed in Petri plates, frozen at -24°C for 6 hours and then freeze-dried for 24 hours (Freeze Dryer Modulyo, Edwards). Dry matter was determined, samples were homogenized (Osterizer, Pulseomatic 10) and stored at -24°C until analysis.

4.8 Vitamin A/carotenoid analysis

As most of the dishes were just on plant-based or contained very small amounts of animal origin compounds analysis was focused on provitamin A carotenoids (pVAC).

Carotenoids are very sensitive to light and oxygen. Exposure to light leads to trans-cis isomerisation and destruction of pVAC. Therefore carotenoid analysis was carried out as fast as possible under subdued light and flasks wrapped with aluminum foil [RODRIGUEZ-AMAYA and KIMURA, 2004].

4.8.1 Instrumentation

- High Phase Liquid Chromatography (HPLC): AccelaTM (Autosampler, 600 Pump, PDA detector (80 Hz)), C18 column (ACE 5, 250x4 mm), Thermo Fisher Scientific
- BÜCHI Rotavapor R-134, BÜCHI Waterbath B-480, BÜCHI Vacuum Pump V-700, BÜCHI Vacuum Controller V-850.
- Shaking apparatus: Unimax 1010 + Inkubator 1000, Heidolph Instruments.
- pH 213 Microprocessor pH meter, HANNA instruments
- HERAEUS MULTIFUGE X3 FR centrifuge, Thermo Scientific
- SuperVario-N centrifuge, FUNKE GERBER
- WhatmanTM Filter 0.2 µm, diameter 47 mm, GE Health care life Sciences
- One-way syringe Luer Solo, inject 10 ml, BBRAUN
- One-way hypodermic-needle Sterican, size 12, 0.70x30 mm BL/LB, 226x1¼", BBRAUN

4.8.2 Reagents and Chemicals

- NaHCO₃, KCl, CaCl₂.H₂O, MERCK
- acetonitrile, acetone, petroleum ether, hexane, ethanol, NaCl > 99.8 %, ROTH
- methanol, ethyl acetate, K₂HPO₄ > 99.3 %, VWR

All following standards and enzymes were purchased from Sigma-Aldrich (product numbers are shown in parentheses)

- Trans- β -apo 8' $\geq$ 96% (UV), (10810)
- β -carotene $\geq$ 97% (UV), (22040)
- mucin from porcine stomach (M1778, Type III)
- α -amylase from porcine pancreas (A3176-500 KU, Type VI-B, $\geq$ 10 units/mg solid; 16.7 G solid, 30 units/mg solid)
- pepsin from porcine gastric mucosa (P7000, $\geq$ 250 units/mg solid)
- pancreatin from porcine pancreas (P1750, 4xUSP specifications)
- bile extracts porcine (B8631)

4.8.3 Analysis of carotenoid content

Extraction

Analysis was carried out in triplicate. To 1 g of each freeze-dried sample (0.5 g of samples 'plain cooking banana' and 'matooke', containing just plantain) 1 ml of trans- β -apo-8'-carotenal (0.5 mg/L acetone) as internal standard and 15 ml of petroleum ether (0.1 % butylhydroxytoluol, BHT) were added. After shaking, the sample was centrifuged for 5 min and 1100 rpm (rounds per minute). The supernatant was filtered through a funnel stuffed with glass wool and collected in a flask. 10 ml of petroleum ether, followed by 5 ml petroleum ether were used for the following extraction steps until the supernatant was clear.

A rotary evaporator was used to vaporize the petroleum ether in which the carotenoids were solved. To eliminate existing lipids the remaining extract was dissolved in 1 ml acetone and frozen at -24°C for 3-4 hours. 1 ml of frozen acetone was then added and the liquid sample was filtered through a funnel stuffed with glass wool to separate fat from the carotenoids [RODRIGUEZ-AMAYA and KIMURA, 2004]. The filtered sample was filled in a vial and stored at -24°C under argon awaiting analysis.

HPLC analysis

A linear gradient elution as pictured in **Figure 5** was used for analysis. The mobile phase consisted of acetonitrile (containing 0.05 % triethylamine (TEA), 0.1 % BHT) as eluent A and methanol:ethyl acetate (1:1, v/v, containing 0.05 % TEA, 0.1 % BHT) as eluent B. Flow rate was set at 1000 μ l/min and the injection

volume at 25 μ l. TEA was added to ensure a slightly improved peak resolution [DAVEY et al., 2006].

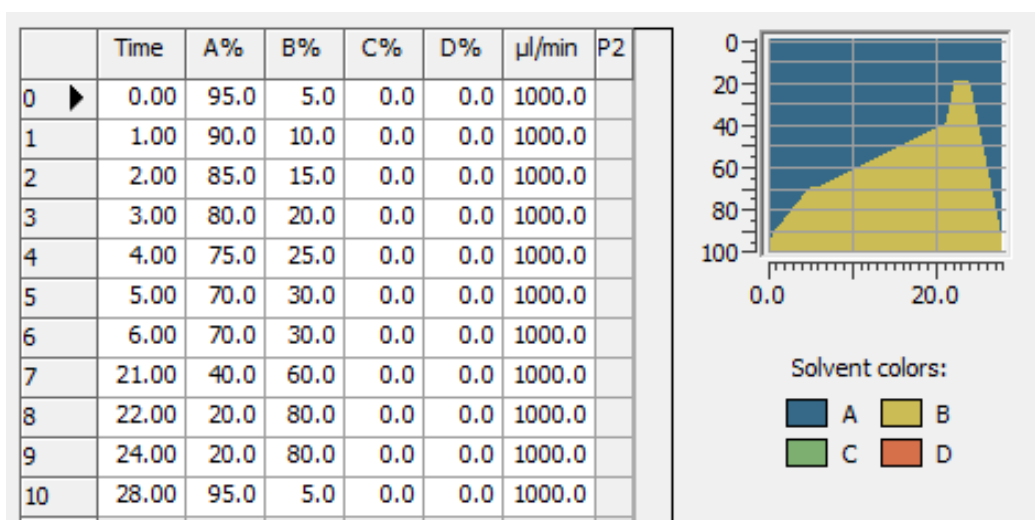


Figure 5. Linear gradient elution for HPLC analysis of carotenoids

A = acetonitrile, B = methanol/ethyl acetate (1:1)

For quantification of the carotenoids a calibration curve was established. Therefore 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 (**Table 7**).

For the preparation of the internal standard 5 mg of trans- β -apo-8'-carotenal were weighed into a 100 ml flask and filled up to the mark with acetone (0.1 % BHT). 5 ml of this solution were collected, transferred to a 50 ml flask and diluted with acetone (0.1 % BHT).

For the preparation of the external standard 2.5 mg of β -carotene were weighed into a 50 ml flask and filled up to the mark with acetone (0.1 % BHT). 4 ml of this solution were collected, transferred to a 25 ml flask and diluted with acetone (0.1 % BHT)

Table 7. Pipetting scheme: serial dilution for calibration curve

	ml of diluted ESTD	ml of diluted ISTD (c=0.5 mg/L)
Standard 1	1	4
Standard 2	2	4
Standard 3	3	4
Standard 4	4	4
Standard 5	5	4

According to their characteristic absorption spectra and retention times (compared to the added standards) carotenoids of each sample were identified and quantified [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.

4.8.4 *In vitro* digestion of carotenoids

***In vitro* digestion**

Analysis was carried out in triplicate. The *in vitro* digestion model was based on a previous study [EKESA et al., 2012] but slight modifications during the centrifugation step were made.

5 g of each fresh sample was weighed in a flask. 6 ml of a saliva solution (containing NaHCO₃, NaCl, KCl, CaCl₂.H₂O, K₂HPO₄, mucin, α -amylase and ultrapure water) was added. If necessary, pH was adjusted to 7 $\pm$ 0.2 by adding 1 M NaOH or 1 M HCl. The mixture was incubated in a shaking apparatus for 10 min at 37°C and 200 rpm.

15 ml of a saline solution (0.9 % NaCl) was added to the sample and again incubated for 10 min at 37°C and 200 rpm.

To simulate the gastric digestion of an infant pH was adjusted to 4 ± 0.2 by adding 1 M HCl. To ensure activity of enzymes, it was necessary to acidify the sample for the next step [ETCHEVERRY et al., 2012].

2 ml of porcine pepsin (40 mg/ml in 0.1 M 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 was again incubated at a shaking apparatus for 30 min at 37°C and 200 rpm.

To simulate the intestinal digestion pH was first raised to 6 ± 0.2 by adding 0.45 M sodium bicarbonate. 9 ml of a mixture containing porcine pancreatin and bile extracts (2 mg/ml porcine pancreatin, 12 mg/ml bile extracts in 0.1 M trisodium citrate) and 4 ml of bile extracts (0.1 g/ml in 0.1 M trisodium citrate) were added [EKESA et al., 2012]. Bile extracts acted as emulsifiers [ETCHEVERRY et al., 2012]. The mixture was again incubated at a shaking apparatus for 30 min at 37°C and 200 rpm to complete the digestion process.

Flasks were rinsed with ultrapure water twice and the digested samples were transferred to centrifugation tubes. In recent years, ultracentrifugation (up to 167000 g) was frequently replaced by low-speed centrifugation which is more applicable in practice and decreases the loss of instable micelles [WERNER and BÖHM, 2011]. Micelles were separated by centrifugation which lasted 1 hour at 10°C and 11 000 rpm. This speed was sufficient to clarify the suspension and enable microfiltration.

Aliquots (10 ml) of the resulting aqueous fraction were collected [EKESA et al., 2012] with a syringe and filtered through a 0.2 µm filter. For the filtration step a vacuum flask was used. The purpose of the microfiltration was to separate crystals, vesicles and oil droplets [WERNER and BÖHM, 2011].

Extraction

1 ml of a trans-β-apo-8'-carotenal (50 µg/L) was added to the filtered aliquot (10 ml) of the micellar aqueous fraction as an internal standard. 15 ml of hexane/ethanol (2:1, v/v) were used for the extraction. The digested sample and the solvent was shaken, centrifuged 3 min at 1100 rpm and the supernatant

was pipetted into a flask. This step was repeated three times. The collected solution was vaporized using a rotary evaporator, the remaining carotenoids were dissolved in acetone, filtered through a pipette stuffed with glass wool and sodium sulphate, filled in vials and stored at -24°C until analysis [EKESA et al., 2012].

HPLC analysis

Samples were injected, analysed and calculated as described above. After digestion the amount of carotenoids in the samples (bioaccessible fraction) was expected to be lower than in undigested samples, therefore standards for the calibration curve were diluted 1:10.

Bioaccessibility was calculated using the following formula [GAUTAM et al., 2010]:

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

4.9 Iron analysis

For the determination of the samples' iron content a microwave digestion was carried out followed by a flame atomic absorption spectroscopy (FAAS). To analyze the iron concentration in the AAS the sample solution was transformed into aerosols and transported to the flame which vaporizes and atomizes the sample [WELZ and SPERLING, 1999, S.149]. This step leads to a reduced intensity of the light (by absorption of a defined quantity of energy), coming from a hollow-cathode lamp. A following detector measures how much of the incoming light was absorbed. The difference between the radiation without sample and including sample (absorbance) was used to calculate the iron concentration.

Freeze-dried samples of all dishes were used for the analysis.

4.9.1 Instrumentation

- Microwave system: MLS-ETHOS plus microwave; Terminal 320 color touch, MLS GmbH; Leutkirch, Germany
- Flame Atomic Absorption Spectrometer (FAAS): PerkinElmer; Massachusetts, USA
- Centrifuge 5810 R, Eppendorf

4.9.2 Reagents and Chemicals

- Iron standard (1000 mg/L): Sigma Aldrich, St. Louis, USA
- Nitric acid (HNO₃) 69 % SUPRA-QUALITY ROTIPURAN® Supra: Carl Roth, Karlsruhe, Germany
- Hydrogen peroxide (H₂O₂) 30 % ROTIPURAN® p.a., ISO: Carl Roth, Karlsruhe, Germany
- 5 % Calcium chloride (CaCl₂)

All following enzymes were purchased from Sigma-Aldrich (product numbers are shown in parentheses)

- mucin from porcine stomach (M1778, Type III)
- α-amylase from porcine pancreas (A3176-500 KU, Type VI-B, ≥ 10 units/mg solid; 16.7 G solid, 30 units/mg solid)
- pepsin from porcine gastric mucosa (P7000, ≥ 250 units/mg solid)
- pancreatin from porcine pancreas (P1750, 4xUSP specifications)
- dialysis tubing ZelluTrans ROTH (E.666.1), Carl Roth, Karlsruhe, Germany

4.9.3 Analysis of iron content

Digestion

0.5 g of each sample were weighed in a Teflon vessel. 5 ml of 69 % HNO₃ and 1 ml of 30 % H₂O₂ were added. The vessels were closed, surrounded by high pressure safety coats, put into rotor segments and placed inside the microwave. To control heating, a temperature sensor was assigned to one of the vessels. The following program was applied: 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.

After the time has elapsed, the Teflon vessels were removed from the microwave and opened to let the nitrogen oxides volatilize.

The digested liquid samples were transferred to 50 ml volumetric flasks. The vessels and breeches were rinsed with bi-distilled water three times. The flasks were filled up until the mark, shaken well and filtered through a paper filter (MN 619 eh, Macherey-Nagel).

Measurement

For the calibration curve the iron standard was diluted to four different concentrations: 0,04 mg/L; 0,1 mg/L; 0,5 mg/L; 1 mg/L.

A blank value, consisting of 3% HNO₃ was necessary for the measurement as well.

250 µl of a 5 % CaCl₂ buffer was added to 10 ml of each sample, standard and the blank value. The tubes were closed and mixed on a Vortexer.

The AAS measurement started with the analysis of the blank value, followed by the standards and the samples.

On basis of 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.

4.9.4 *In vitro* digestion of iron

Analysis was carried out in triplicate. The *in vitro* digestion model was based on [LUTEN et al., 1989] but slight modifications were made. 5 g of each freeze dried sample were weighed into a flask and 80 ml of ultrapure water were added.

To simulate gastric digestion pH was adjusted to 2 with 6 M HCl, controlled after 15 min and readjusted if necessary. 3 ml of a pepsin solution (16 g/100 ml 0.1 M HCl) were added and the volume was made up to 100 ml with ultrapure water. The mixture was mixed and incubated at a shaking apparatus for 2 hours at 37°C and 200 rpm [LUTEN et al., 1996].

Before continuing digestion it was necessary to determine the titratable acidity. Samples were cooled to stop enzyme activity. 20 ml of the gastric digest were removed and taken to another flask. 5 ml of a pancreatin mixture (4 g pancreatin and 25 g bile extracts/1000 ml 0.1 M NaHCO₃) were added. The pH was measured and adjusted to 7.5 with 0.5 M NaOH. The pH was checked after 15 min and readjusted if necessary. Titratable acidity was defined as the amount of NaOH used to achieve a pH of 7.5 [LUTEN et al., 1996].

To stimulate intestinal digestion 20 ml 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; Ø dry: 20.4 mm) 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 25 ml ultrapure water was added to the digest (**Figure 6**) and incubated for 30 min in the shaking apparatus at 37°C and 200 rpm. The length of the tubing was set at approximately 20 cm 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]. 5 ml of the pancreatin/bile extracts mixture 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 which represents the bioaccessible fraction of the element. The dialysates were transferred into plastic tubes. To 15 ml of the samples 800 µl of 69% HNO₃ were added, the mixture was centrifuged for 10 min at 10°C and 11000 rpm. The supernatant was filtered through a paper filter. To determine the bioaccessible fraction of iron, filtered samples were measured using AAS as described above.

Bioaccessibility was calculated as follows [GAUTAM et al., 2010]:

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

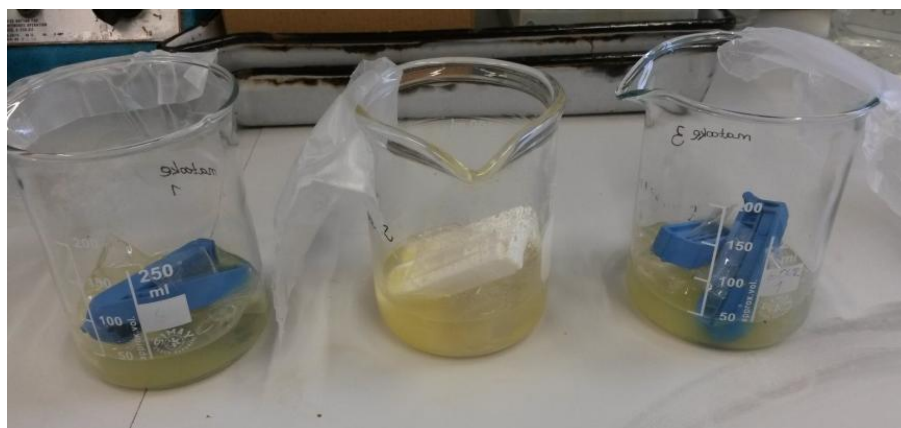


Figure 6. Dialysis tubings soaked in digests

4.10 Protein analysis

For the determination of the protein content of the dishes Kjeldahl method was applied. Freeze-dried samples were used for analysis.

4.10.1 Instrumentation

- DK 205 Heating Digester: VELP Scientific, Italy
- UDK 142 Automatic Distillation Unit, VELP Scientific, Italy
- Titro Line easy: VELP Scientific, Italy

4.10.2 Reagents and Chemicals

All following chemicals were purchased from Carl Roth GmbH, Germany

- Concentrated sulphuric acid (H_2SO_4) 98 %
- Catalytic mixture: 500 g potassium sulphate + 2 g copper-(II)-sulphate
- Boric acid 99.8 %
- Sodium hydroxide ≥ 99 %
- Hydrochloric acid (4 M standard solution)

4.10.3 Analysis of protein content

For the chemical decomposition 7.5 g of a catalytic mixture, small glass balls to avoid boiling retardation and the sample were weighed into flasks. The original sample weight was dependent on the expected protein content. 15 ml of

concentrated sulfuric acid were added, and then the freeze dried food sample was digested by heating. After decomposition nitrogen was available as ammonium sulfate and the flasks were connected to a VELP Automatic Distillation unit.

During the distillation step ammonium sulfate is 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 the ammonium ion and boric acid to the borate ion, respectively. Nitrogen content was established by titrating the formed ammonium borate with 0.1 M hydrochloric acid. The amount of hydrochloric acid used for the titration was used for the calculation of the nitrogen content and following the total protein content [MCCLEMENTS, 2003].

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

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

TN	total nitrogen (%)
V (HCl)	usage of hydrochloric acid (ml)
n (HCl)	molarity of hydrochloric acid (mol/L)
M (N)	molecular weight of nitrogen (14.008 mol/L)
IW	initial weight (mg)
F	factor 6.25

5 Results and discussion

5.1 Preliminary household survey

Caregivers were asked to mention the three dishes they prepare most often for their preschool children. As not every household named three dishes, the data of 482 dishes was analyzed. **Table 8** shows the most common meals given to preschool children in Kiboga district. Those dishes among the 482 which were stated with a frequency below 5 % are not mentioned. Just the three most common, Porridge, Matooke and Katogo, were of further interest.

Table 8. Most common dishes in general

Name of the dish	Frequency	%
Porridge	104	21.6
Matooke	80	16.6
Katogo	77	16.0
Rice	51	10.6
Sweet Potatoes	36	7.5
Posho	32	6.6
Irish Potatoes	31	6.4

Porridge

Porridge is the most common dish fed to preschool children in Kiboga district. Amongst all different kinds of flour used for the preparation, maize flour was the most common, followed by millet flour, cassava flour and soya flour (**Table 9**). White maize flour is sold most frequently in local stores in Lwamata sub-county. Different combinations of ingredients were used for the preparation of the maize flour. The most common combination was maize flour with milk and sugar and water, followed by maize flour with sugar and water, just maize flour with water and finally maize flour with milk and water (**Table 10**).

Table 9. Types of flour used for preparation of porridge

Type of flour	Frequency	%
Maize flour	79	76.0
Millet flour	15	14.4
Cassava flour	7	6.7
Soya flour	2	1.9
Millet flour + Soya flour	1	1

Table 10. Combination of ingredients for preparation of porridge

Ingredients	Frequency	%
maize flour + milk + sugar + water	33	41.8
maize flour + sugar + water	24	30.4
maize flour + water	15	19.0
maize flour + milk + water	7	8.9

Matooke

Steamed and mashed cooking bananas, locally known as *matooke*, are the second most common dish fed to preschool children from Kiboga district. Matooke is served together with gravy made of peanuts or vegetables, beans, peas or whatever ingredient is available. Lots of different combinations used to prepare the dish. The most common dish was matooke served with a sauce made of grounded peanuts/groundnuts (G.nuts) (18.8 %) followed by matooke together with the sauce of grounded peanuts and small silver fish, locally known as mukene (12.5 %) (**Table 11**).

Amongst the 80 caregivers who prepared matooke for their children, 39 combined it with grounded peanuts sauce or the peanuts sauce and some other ingredients (like mukene, tomatoes, onions, etc.). 25 caregivers used beans together with some other ingredients (like cooking oil, tomatoes or onions). A few others used fish, meat or different kinds of vegetables. Combinations with a frequency below 5 % are not mentioned.

Table 11. Combination of main ingredients for preparation of Matooke

Ingredients	Frequency	%
Cooking banana (matooke) + G.nuts sauce	15	18.8
Cooking banana (matooke) + G.nuts sauce + mukene	10	12.5
Cooking banana (matooke) + beans + cooking oil	7	8.8
Cooking banana (matooke) + G.nuts sauce + tomatoes	7	8.8
Cooking banana (matooke) + beans	6	7.5

Katogo

A dish where a staple is cooked in the same pot together with some sauce is referred to as Katogo, which is the third most common meal given to children aged 12-59 months from Kiboga district.

Staples used for the preparation were cooking bananas, followed by cassava and irish potatoes (**Table 12**). Having a closer look at the Cooking Banana Katogo, there is also a variety in the combination of the ingredients. 22 caregivers, which is about two-thirds of all who made Cooking Banana Katogo, cooked the bananas in a peanuts sauce and added some onions, tomatoes and salt (62.9 %). A few others added mukene, beans or some green leafy vegetables.

Table 12. Staples used for preparation of Katogo

Staple	Frequency	%
Cooking banana (matooke)	35	45.5
Cassava	23	29.9
Irish Potatoes	13	24.7

5.2 Preparation procedures of dishes by caregivers

5.2.1 Porridge

The families were provided with 1 kg of maize flour, 0.5 l of milk and 0.5 kg of sugar. They were asked to follow their usual procedure to prepare the dishes. In following paragraph the three different preparation methods are described.

Preparation procedure 1

Total duration of cooking: 45 minutes

Ingredients:

- 2.5 l of water
- 0.5 l of milk
- 250 g of maize flour
- 200 g of sugar

Preparation:

- Put 2 l of water in a sauce pan (without cover) and put it on fire. Boil for 20 min.
- Mix 0.5 l of cold water with 250 g of maize flour and mingle with a spoon.
- Put the mixture into the boiling water, mingle it with a stick and boil on medium fire for 10 min.
- Add 0.5 l of milk and boil it for 15 min.
- Add 200 g of sugar.

Preparation procedure 2

Total duration of cooking: 1 hour

Ingredients:

- 3 l of water
- 0.5-1.0 l of milk
- 400 g of maize flour
- 200 g of sugar

Preparation:

- Put 2.5 l of water in a sauce pan (without cover) and put it on fire. Boil for 30 min.
- Mix 0.5 l of cold water with 400 g of maize flour and mingle it.
- Put the mixture into the boiling water, mingle it with a stem of banana leaves and boil it for 15 more minutes with a high intensity of fire **(Figure 7)**.
- Add 0.5 l of milk (depending on availability 0.5-1 liter of milk is used) and boil it for 15 minutes.
- Add 2 heaped spoons of sugar to each cup (approx. 200 g of sugar all together)



Figure 7. Preparation of porridge

Preparation procedure 3

Total duration of cooking: 40 minutes

Ingredients:

- 4 l of water
- 0.75-1.0 l of milk
- 500 g of maize flour
- 250 g of sugar

Preparation:

- Put 3 l of water in a sauce pan (without cover) and put it on fire. Boil for 10 min.
- Mix 1 liter of cold water with 500 g of maize flour and mingle it.

- Put mixture into the boiling water, mingle and boil it for 10 more min.
- Add 0.75-1.0 l of milk (depending on availability).
- Add 250 g (measured with a local cup) of sugar directly to the sauce pan and boil for 20 more minutes.

5.2.2 Matooke with G.nuts sauce (and mukene)

The families were provided with 1 kg of G.nuts and 100 g of mukene. Matooke with a sauce made of G.nuts was established to be the second most popular dish given to preschool children. Women were asked again to follow their usual procedure to prepare the dish. It came out that it is not common to use just G.nuts when preparing a sauce. Women add at least some onions and also tomatoes if available to improve the taste.

In the following the three different preparation methods are described.

Preparation procedure 1

Total duration of cooking: Matooke - 1 hour, Sauce - 30 minutes

Ingredients:

- 200 small banana fingers (matooke)
- 4 l of water (matooke)
- 750 g of G.nuts (sauce)
- 3 l of water (sauce)
- 1 onion (sauce)
- 4 tomatoes (sauce)
- 50 g mukene (sauce)

Preparation of matooke:

- Put stems of banana leaves (or some wood) on the ground of the sauce pan to avoid that the bananas get soaked by the water during steaming (**Figure 8**). Put 4 l of water into the sauce pan and cover it with banana leaves.

- Peel 200 banana fingers with a knife, put the fingers into the sauce pan, cover them with more banana leaves and put the pan on the fire for 1 hour **(Figure 9)**.
- Mash the bananas not until the sauce is finished, otherwise matooke gets hard.



Figure 8. Stems of banana leaves to avoid soaking of bananas



Figure 9. Peeled bananas covered with banana leaves.

Preparation of sauce:

- Mix 3 l of water with 750 g of G.nuts and mingle until the mixture is smooth.
- Put sauce pan on fire (without a cover) and boil the sauce for 20 min.
- Cut 4 tomatoes and 1 onion and add them to the sauce. Boil for 5-10 more minutes and add 2 spoons of salt.

Preparation of sauce with mukene:

- 50 g of mukene are fried (without cooking oil) for 1 minute and added to the sauce together with onions and tomatoes.

When G.nuts sauce is prepared with mukene it is not necessary to add tomatoes and onions because the fish has a very intensive taste.

Preparation procedure 2

Total duration of cooking: Matooke - 1 hour, Sauce - 20 minutes

Ingredients:

- 70 medium/large banana fingers (matooke)
- 3.5 l of water (matooke)
- 500 g of G.nuts (sauce)
- 2 l of water (sauce)
- 3 onions (sauce)
- 50 g mukene (sauce)

Preparation of matooke:

- Put stems of banana leaves (or some wood) on the ground of the sauce pan to avoid that the bananas get soaked by the water during steaming. Put 3.5 l of water into the sauce pan.
- Peel 70 banana fingers with a knife, cover the fingers in banana leaves and fix it with some fibers, put it into the sauce pan, cover it with more banana leaves and put it on the fire for 1 hour (that is the traditional way of making matooke) (**Figure 10**).
- Mash the bananas not until the sauce is finished, otherwise matooke gets hard.



Figure 10. Traditional preparation of Matooke in banana leaves.

Preparation of sauce:

- Mix 1.5 l of water with 500 g of G.nuts and mingle it with a spoon until the mixture is smooth.
- Put the sauce pan on fire (without a cover), add 0.5 liter of water and boil the sauce for 15 minutes.
- Cut 3 onions, add them to the sauce and boil it for 5 more minutes and add 2 spoons of salt.

Preparation of sauce with mukene:

- Wash 50 g of mukene with cold water, fry (without cooking oil) for 1 minute and add fish to the sauce together with onions.

When G.nuts sauce is prepared with mukene it is not required to add tomatoes and onions because the fish has a very intensive taste.

Preparation procedure 3

Total duration of cooking: Matooke - 1 hour, Sauce - 15 minutes

Ingredients:

- 80 small/medium banana fingers (matooke)
- 1.5 l of water (matooke)
- 400 g of G.nuts (sauce)
- 2 l of water (sauce)

- 2 onions (sauce)
- 3 tomatoes (sauce)
- 50 g mukene (sauce)

Preparation of matooke:

- Put stems of banana leaves (or some wood) on the ground of the sauce pan to avoid that the bananas get soaked by the water during steaming. Put 1.5 l of water into the sauce pan and cover it with banana leaves.
- Peel 80 banana fingers with a knife, put the fingers into a sauce pan, cover them with more banana leaves and put the pan on the fire for 1 hour.
- Mash the bananas not until the sauce is finished, otherwise matooke gets hard.

Preparation of sauce:

- Mix 2 l of water with 400 g of G.nuts and mingle until the mixture is smooth.
- Put the sauce pan on fire (without a cover).
- Cut 2 onions and 3 tomatoes, add them to the sauce and boil for 15 min.
- Add 2 spoons of salt.

Preparation of sauce with mukene:

- Wash 50 g of mukene with cold water, fry (without cooking oil) for 1 minute and add fish to the sauce together with onions and tomatoes.

When G.nuts sauce is prepared with mukene it is not necessary to add tomatoes and onions because the fish has a very intensive taste.

5.2.3 Banana Katogo

The families were provided with 1 kg of G.nuts. They were asked to follow their usual procedure to prepare the dish. Again it turned out that it is not common to use just G.nuts for the preparation of the Katogo. To improve the taste women usually add small amounts of onions and tomatoes (if available). In the following paragraph the three different preparation methods are described.

Preparation procedure 1

Total duration of cooking: 1 hour

Ingredients:

- 62 medium banana fingers
- 2 l of water
- 250 g of G.nuts
- 4 small onions
- 3 teaspoons of salt

Preparation:

- Peel around 60 medium sized banana fingers with a knife and put them in the sauce pan.
- Add 1.5 l of water to the bananas and cover them with banana leaves.
- Put sauce pan on the heat and boil for 40 minutes until the bananas are soft.
- Mix 0.5 l of water with 250 g of G.nuts and mingle for 10 minutes.
- Add mixture to the bananas, cover it with leaves and boil again for 10 minutes.
- Cut 4 small onions into pieces and add it to the banana/G.nuts mixture.
- Add 3 teaspoons of salt and remain saucepan on the heat for 10 more minutes, then the dish is ready to serve **(Figure 11)**.



Figure 11. Freshly prepared Banana Katogo (banana in groundnut sauce)

Preparation procedure 2

Total duration of cooking: 50 minutes

Ingredients:

- 25 large banana fingers
- 1.5 l of water

- 300 g of G.nuts
- 1 spoon of salt

Preparation:

- Peel around 25 large banana fingers with a knife and put them in the sauce pan.
- Add 1 l of water to the bananas and cover them with banana leaves.
- Put sauce pan on the heat and boil for 40 min until the bananas are soft.
- Mix 0.5 l of water with 300 g of G.nuts and mingle it.
- Add mixture and one spoon of salt to the bananas and cover it.
- Put the Katogo on fire and boil for 10 more minutes.

Preparation procedure 3

Total duration of cooking: 25 minutes

Ingredients:

- 50 small/medium banana fingers
- 1.5 l of water
- 200 g of G.nuts
- 2 onions
- 2 spoon of salt

Preparation:

- Peel around 50 small to medium banana fingers with a knife and put them in the sauce pan.
- Cut 2 onions and add them to the bananas.
- Put 1 l of water to the bananas and the onions into the sauce pan and cover it with banana leaves.
- Put the sauce pan on the heat and boil for 15 minutes.
- Mix 0.5 l of water with 200 g of G.nuts and mingle it, then add the mixture to the bananas and cover it again.
- Boil for 10 more minutes and then add 2 spoons of salt.

5.3 Follow-up questionnaire and focus group discussion

Porridge: When caregivers were asked about the use of milk 9 out of 11 indicated to use milk 1-2 days/week for the preparation of porridge. 2 out of 11 did not have any access to milk at all. At the focus group discussion (**Figure 12**) it came out, that only 2 persons (out of 20) can afford milk daily. It was concluded, that milk is not a common ingredient and will therefore not be included in the recipe of porridge.

Information regarding the type and amount of flour (maize flour, 420 g on average), as well as the added quantity of water (3.1 l on average) and sugar (200-250 g on average) was very similar to the recipes of the caregivers mentioned above.

Matooke with groundnuts/peanut sauce (and mukene): The preparation of the G.nuts sauce was of particular interest. On average, 450 g of raw, pounded G.nuts and 1.8 l of water are used to prepare the sauce. 8 out of 11 caregivers indicated to use always tomatoes and onions when cooking G.nuts sauce. During the focus group discussion it came out, that these two ingredients are used regularly for the preparation of the sauce. Two tomatoes and three onions are used on average. Although those ingredients have not been among the most common ingredients at the household survey it seems that they are widely-used and so they will be included at the recipe.

In addition, 2 out of 11 caregivers use always mukene , 7 out of 11 use mukene at least 2 times/week for the preparation of the G.nuts sauce. These findings confirm the results from the household survey. The first recipe of the sauce will include tomatoes and onions, the second recipe of the G.nuts sauce will further include the usage of mukene.

Banana Katogo: On average, 50 medium sized banana fingers, mixed with 2.8 l of water and 400 g of raw, pounded G.nuts are used for the preparation of the Katogo. In addition to those ingredients which were established at the household survey it came out, that tomatoes and onions are commonly used as well. 7 out of 11 caregivers indicated to add tomatoes (two on average) and

onions (three on average) every time they prepare Banana Katogo, so these ingredients will also be included in the final recipe.



Figure 12. Farmers at the focus group discussion in Kiboga district

5.4 Recipes of the most common dishes

After summarizing all information (household questionnaire, information of the caregivers on-site, follow-up questionnaire) it was possible to establish one recipe for each dish.

Table 13. Recipes of the most common dishes

Dish	Ingredients	Preparation
Porridge	3 l of water 400 g of maize flour 200 g of sugar	- Put 2.5 liter of water in a sauce pan (without cover) and put it on fire. Boil for 20 min. - Mix 0.5 liter of cold water with 400 g of maize flour and mingle it. - Put the mixture into the boiling water, mingle it and boil it for 15 more minutes with a medium intensity of fire. When you smell a good scent and the color on top turns yellowish porridge is ready. - Add 200 g of sugar and serve.

Matooke with G.nuts sauce	70 medium bananas 3 l of water 500 g of G.nuts 2 l of water 3 onions 2 tomatoes	- Put stems of banana leaves (or some wood) on the ground of the sauce pan. Put around 3 liters of water into the sauce pan and cover it with banana leaves. - Peel 70 banana fingers with a knife, put the fingers into the sauce pan, cover them with more banana leaves and put the pan on the fire for 1 hour and then mash the bananas. - For the sauce mix 2 liters of water with 500 g of G.nuts and mingle it until the mixture is smooth. - Put the sauce pan on medium fire (without a cover). - Cut 2 onions and 3 tomatoes, add them to the sauce and boil for 15 minutes, then add 2 spoons of salt. When the sauce is ready the color changes and you find a cream on the surface.
Matooke with G.nuts sauce and mukene	70 medium bananas 3 l of water 500 g of G.nuts 2 l of water 3 onions 2 tomatoes 50 g mukene	- Put stems of banana leaves (or some wood) on the ground of the sauce pan. Put around 3 liters of water into the sauce pan and cover it with banana leaves. - Peel 70 banana fingers with a knife, put the fingers into the sauce pan, cover them with more banana leaves and put the pan on the fire for 1 hour and then mash the bananas. - For the sauce mix 2 liters of water with 500 g of G.nuts and mingle it until the mixture is smooth. - Put the sauce pan on medium fire (without a cover). - Cut 2 onions and 3 tomatoes, wash 50 g of mukene, fry for 1 minute (without cooking oil) and add all ingredients to the sauce. - Add 2 spoons of salt. When the sauce is ready the color changes and you find a cream on the surface.

Banana Katogo	50 medium bananas 2 l of water 300 g of G.nuts 3 onions 2 tomatoes	- Peel around 50 medium sized banana fingers with a knife and put them in the sauce pan. - Add 1.5 liters of water and cover the pan with banana leaves. - Put the sauce pan on the heat and boil for 30 min. on medium fire until the bananas are soft. - Mix 0.5 liters of water with 300 g of G.nuts and mingle it. - Add the mixture to the bananas, cover it with leaves and boil for 10 min. again. - Cut 2 small onions and 2 tomatoes and add it to the banana/G.nuts mixture. - Add 3 teaspoons of salt and remain saucepan on the heat for 10 more minutes. When you smell a good scent and see a cream on the surface the Katogo is ready to serve.
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5.5 Carotenoid content of popular dishes

Following the methodology described in chapter 4.8, provitamin A carotenoids were obtained in three of the four popular dishes. Matooke with G.nuts sauce, Matooke with G.nuts sauce and mukene as well as Katogo contained proper amounts of pVAC. Only porridge did not contain any carotenoids (**Table 14**). The banana derived dishes also contained lutein. Lutein, a xanthophyll which is responsible for the bananas yellow colour, does not have provitamin A activity and therefore it was not of further interest for this study.

All-*trans* β -carotene (t-BC), all-*trans* α -carotene (t-AC) and 13-*cis* β -carotene were found in the dishes. In all dishes the proportion of t-BC (around 48 %) was higher than that of t-AC (around 40 %). With around 12 %, 13-*cis* β -carotene had the smallest proportion of pVAC in all dishes. Katogo and Matooke with G.nuts sauce, tomato and onion contained exactly the same ingredients, just in a different ratio; the higher total pVAC content of Katogo (506 $\mu\text{g}/100$ g fresh weight) compared to the Matooke dish (399 $\mu\text{g}/100$ g fresh weight and 377 $\mu\text{g}/100$ g fresh weight, with and without mukene, respectively) can be explained by the proportionally larger amount of cooking bananas in Katogo.

As it was just possible to measure provitamin A carotenoids, the total vitamin A content of the Matooke dish containing mukene is expected to be slightly higher. It was reported that mukene is a rich source of Vitamin A and expected that 10 g are sufficient to cover a child's demand of the vitamin [ABILA, 2000]. Low value fish products like mukene are nutrient dense and can contribute to nutrition security in low income populations who rely on these products as their main animal food source. Because of the reported high values of iron and protein it is expected that mukene is also a good source of other nutrients normally present in fish products (such as vitamin A). Given the fact that both mukene stocks and catches have increased in Lake Victoria the usage of these small fish should be promoted [KABAHENDA et al., 2011].

However, the proportion of the dried silver fish in the meal was very small as it was just added to improve the taste of the dish.

Table 14. pVAC^a content of most common dishes fed to preschool children.

Dish	Content in µg/100 g fresh weight				RAE ^b
	β-carotene	α-carotene	cis β-carotene	total pVAC	
Porridge	n.d.	n.d.	n.d.		
Matooke, G.nuts sauce, tomato, onion	196±7.73	154±5.79	48.6±2.18	399	24.8
Matooke, G.nuts sauce, mukene , tomato, onion	180±1.52	153±2.13	44.5±1.26	377	23.2
Banana Katogo	240±5.69	202±6.56	63.2±1.86	506	31.1

^a The values are means of three determinations ± standard deviation.

^b conversion factors for calculation of RAE: trans- β-carotene/12, trans- α-carotene/24, cis β-carotene/24

Having a closer look at the single ingredients and components of the dishes also gives important information (**Table 15**). Lower t-BC and t-AC content of plain matooke (299 µg/100 g fresh weight, 273 µg/100 g fresh weight, respectively) compared to boiled cooking bananas (310 µg/100 g fresh weight, 361 µg/100 g fresh weight, respectively) may be explained by the processing procedure. Longer processing time as well as pureeing and mashing of food leads to a decrease in carotenoid content. Heat treatment also causes isomerization of the *trans*-carotenoids to its *cis*-isomers [RODRIGUEZ-AMAYA

and KIMURA, 2004]. Actually, the content of c-BC in plain matooke (70.5 µg/100 g fresh weight) is higher than in boiled cooking bananas (58.2 µg/100 g fresh weight).

Plain G.nuts sauce prepared of grounded peanuts, water and salt did not contain any carotenoids. In contrast, the addition of tomatoes and onions led to the detection of t-BC and c-BC. Responsible for the detection are the tomatoes which also contain reasonable amounts of lycopin. Despite its presumably health promoting impact lycopin does not have provitamin A activity and on that account will not be further discussed.

Table 15. pVAC^a content of the single components of the dishes.

Component	Content in µg/100 g fresh weight				RAE ^b
	β-carotene	α-carotene	cis β-carotene	total pVAC	
Boiled cooking banana	310±7.47	361±9.81	58.2±3.08	729	43.3
Plain matooke	299±12.3	273±8.16	70.5±1.75	643	39.2
G.nuts sauce	n.d.	n.d.	n.d.		
G.nuts sauce, tomato, onion	47.2±3.97	n.d.	7.83±0.84	55	4.26
G.nuts sauce, mukene, tomato, onion	47.2±1.86	n.d.	10±0.45	57.2	4.35

^a The values are means of three determinations ± standard deviation.

^b conversion factors for calculation of RAE: trans- β-carotene/12, trans- α-carotene/24, cis β-carotene/24

All in all it can be said that cooking bananas are mainly responsible for the carotenoids in the dishes. Nevertheless, tomatoes can contribute a slight increase in the amount of provitamin A carotenoids and therefore use of these vegetables should be maintained and further promoted.

5.5.1 Bioaccessibility of carotenoids

The amount of carotenoids released from the food matrix and transferred into mixed micelles is referred to as bioaccessibility. In other words it describes the efficiency of micellarization and the maximum amount that is available for absorption by the enterocytes [EKESA et al., 2012], [WERNER and BÖHM,

2011]. Therefore total pVAC after *in vitro* digestion will be explained using RE instead of RAE.

Table 16. Bioaccessibility of pVAC in the most common dishes.

Dish	% Bioaccessibility			RE ^b in µg/100g fw
	β-carotene	α-carotene	cis β-carotene	
Matooke, G.nuts sauce, tomato, onion	26.2±1.20	27.0±1.28	33.3±1.83	13.3
Matooke, G.nuts sauce, mukene , tomato, onion	27.3±2.59	28.0±2.84	35.1±2.14	13.1
Katogo	20.8±1.86	20.7±1.32	22.7±1.60	13

^b conversion factors for calculation of RE: trans- β-carotene/6, trans- α-carotene/12, cis β-carotene/12.

To detect the bioaccessible carotenoid fraction of the dishes analysis was carried out as described in chapter 4.8.4. The bioaccessibility of the Matooke dishes was quite similar and slightly higher than in Katogo. The percentages of bioaccessibility of the different provitamin A carotenoids are shown in **(Table 16)**. The Matooke dishes showed a more efficient micellarization of carotenoids. This may be explained by the higher amount of peanuts (and therefore higher amount of fat, which can be close to 50 % [EJIGUI et al., 2005]) in the dish. Compared to t-BC and t-AC (between 26.2 % and 28.0 % in the Matooke dishes and 20.7 % in Katogo), bioaccessibility of *cis* β-carotene was higher in all samples (between 33.3 and 35.1 % in the Matooke dishes and 22.7 % in Katogo). Similar findings were reported by other authors [EKESA et al., 2012]. The Matooke dishes with a lower initial pVAC content showed a higher bioaccessibility whereas Banana Katogo contained higher amounts of pVAC before digestion but had a lower bioaccessibility (because of the smaller amount of peanuts and therefore fat, compared to the Matooke dishes). Expressed in RE all three dishes almost had the same amount of provitamin A carotenoids after digestion.

The *in vitro* digestion of the single components of the dishes resulted in a big variation and was not very meaningful. Especially in the G.nuts sauces just little

amounts of carotenoids were left after digestion. Analysis of those traces was difficult and therefore results were not reliable.

Different studies showed that different pH conditions during gastric and intestinal digestion lead to different results in bioaccessibility. At a gastric pH at 2, for example, carotenoids were less bioaccessible than at pH 4 (which was used in this study to simulate gastric pH of an infant). The more acid media increases the loss of instable carotenoids. Though a lower pH may enhance the transfer of carotenoids from the food matrix to the lipid phase, the following transfer to mixed micelles is reduced. To make different *in vitro* studies comparable, equal pH settings have to be applied [WERNER and BÖHM, 2011].

5.5.2 Potential contribution of banana-derived dishes to vitamin A RDA

An average portion size for children aged 1 to 5 years was estimated to be 200 g [EKESA et al., 2011]. With these consumption levels children are not able to meet even a quarter of their nutritional needs of vitamin A by consuming any of the tested dishes. Enormous amounts of the dishes would have to be consumed to cover the requirements. Using the concept of RAE and a daily RDA of 400 µg, preschool children would meet 11.6 % and 12.4 % of the vitamin A RDA while consuming Matooke and G.nuts sauce without and with mukene, respectively (however, contribution of Matooke with G.nuts and mukene to the daily allowances is expected to be higher because of the vitamin A in the fish which was not analyzed). The consumption of Katogo would contribute 15.6 % to the daily demand. Porridge, which is the most common dish of all and consumed most frequently does not contain any provitamin A carotenoids at all.

Expressed in RE the contribution to the daily vitamin A recommendations is even less. By consuming the banana-based dishes children would meet around 6.5 % of their vitamin A RDA (**Table 17**).

Table 17. Contribution of banana-derived dishes to vitamin A recommendations

Dish	µg RE/ 200 g fw	% RDA (400 µg RE/day)	µg RAE/ 200 g fw	% RDA (400 µg RAE/day)
Matooke, G.nuts sauce, tomato, onion	26.6	6.7	49.6	12.4
Matooke, G.nuts sauce, mukene , tomato, onion	26.2	6.6	46.4	11.6
Katogo	23	6.5	62.2	15.6

g fw = gram fresh weight

Big differences in the vitamin A activity of the dishes were observed depending on whether RAE of the undigested samples or RE of the bioaccessible fraction was used for the calculation (**Figure 13**). Using the classical estimates of retinol activity equivalents may lead to an overestimation of the actual activity of provitamin A carotenoids in foods. Bioaccessibility of pVAC should therefore be taken into account to provide meaningful recommendations of the amount of a food needed to meet nutritional demands. Overestimations of the vitamin A content of a dish may lead to insufficient consumption levels of foods and dishes to meet nutritional needs.

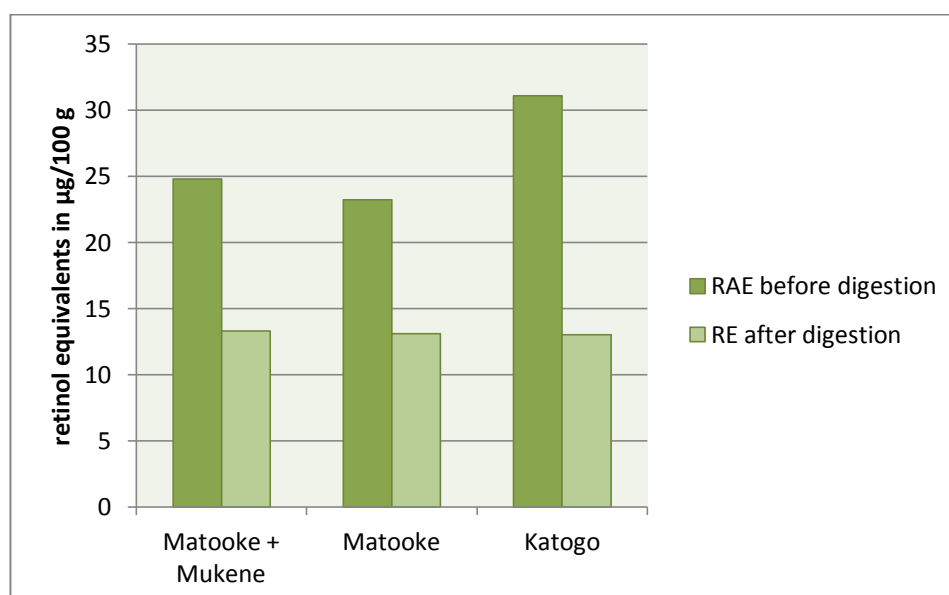


Figure 13. Comparison of retinol equivalents expressed in RAE and RE.

Type and intensity of food processing influences the food matrix and bioaccessibility of carotenoids. In general, the absorption of uncooked food is low. Mild cooking enhances the absorbability of carotenoids by destruction of the food matrix and thus facilitates absorption. Although bioaccessibility can be increased by cooking, a loss of total pVAC can be observed. Long time gaps between preparation and cooking, as well as long cooking times attribute to higher carotene losses in traditional cooking methods [VEDA et al., 2010].

When preparing dishes containing bananas the cooking procedure should immediately start after peeling the bananas. To avoid too much exposure of the food to atmospheric oxidation they should be cooked with the lid on. If possible handling with bananas should be carried out under subdued light to reduce *trans-cis* isomerisation of carotenoids. Rapid processing at high temperatures helps to preserve carotenoids [RODRIGUEZ-AMAYA and KIMURA, 2004].

Matooke with G.nuts sauce (and mukene) as well as Banana Katogo are among the four most often consumed dishes from Kiboga district in Uganda. However, it should be kept in mind that there are also other foods that may provide provitamin A carotenoids. In the period after harvest mangoes and papayas are common fruits which are often consumed and contain considerable amounts of pVACs, as well as green leafy vegetables like amaranth and pumpkin leaves which are sometimes added when preparing Katogo.

Higher amounts of peanuts, therefore higher amounts of fat, showed an increased bioaccessibility of carotenoids in the dishes. Similar results were reported in another study where the addition of rapeseed oil to carrot juice significantly enhanced bioaccessibility [COURRAUD et al., 2013]. The addition of lipids to dishes containing pVAC should be promoted.

5.6 Iron content of popular dishes

As the absorption rate of iron depends on the iron storage in the body the indication of the iron content of foods to meet physiological needs is not very meaningful [ELMADFA and LEITZMANN, 2004]. A high mineral content is not always related to a high accessibility and availability for the body [SAHUQUILLO et al., 2003].

Table 18. Iron content of most common dishes fed to preschool children.

Dish	µg/100 g fresh weight
Porridge	n.d.
Matooke, G.nuts sauce, tomato, onion	423±3.54
Matooke, G.nuts sauce, mukene , tomato, onion	460±50.2
Katogo	311±17.7

Data is represented in means of at least two determinations ± standard deviation

Table 19. Iron content of the single components of the dishes.

Component	µg/100 g fresh weight
Boiled cooking bananas	207±31.1
Plain matooke	202±21.9
G.nuts sauce	927±12.7
G.nuts sauce, tomato, onion	946±39.6
G.nuts sauce, mukene , tomato, onion	1077±2.12
mukene	6325±147*

Data is represented in means of at least 2 determinations ± standard deviation

* µg/100 g dry weight

Following the methodology described in 4.9 the amount of iron in the dishes was analysed. The total iron content of the most popular dishes fed to preschool children is given in **(Table 18)**. Besides provitamin A carotenoids, porridge also did not contain any iron. Katogo contained 311 µg iron/100 g fresh sample and Matooke with G.nuts sauce 423 µg/100 g fresh sample. As expected, Matooke with G.nuts sauce and mukene showed the highest total iron content (460 µg/100 g fresh weight). This can be explained having a closer look at the single components of the dishes **(Table 19)** representing mukene as a good source of iron (6325 µg/100g dried fish). Boiled cooking bananas and plain Matooke almost contained the same amount of iron, which is quite low compared to the average literature value for iron in bananas (325 µg/100 g fresh weight) [SOUCI et al., 2008]. However, it should be kept in mind that there is a great variety of bananas. The nutrient content of foods may vary widely due to different cultivars, the stage of ripening, geographic site of production, handling during

harvesting and storage [VAN JAARSVELD et al., 2014]. Plain G.nuts sauce and a G.nuts sauce with tomatoes and onions also showed similar amounts of the element. The addition of mukene to the sauce showed an increase in total iron content. A study on low-value fish products carried out in East Africa emphasizes the results of our study. It also showed high iron contents of mukene, between 8.2 and 10.7 mg/100 g dried fish, depending on the processing method [KABAHENDE et al., 2011].

5.6.1 Bioaccessibility of iron

After *in vitro* digestion just small amounts of iron were found in the bioaccessible fraction. Bioaccessibility (in %) of the three dishes containing iron is listed below in **(Table 20)**. All three dishes showed a low bioaccessibility. Similar results were reported by other authors [HEMALATHA et al., 2007], [LUTEN et al., 1996]. 3.02 % of iron present in Katogo was bioaccessible. The Matooke dish with G.nuts sauce showed similar results but had a very high standard deviation (values between 0.29 and 7.49 % were detected). An insufficient homogenization of the sample and hence an uneven distribution of the single components may be the reason for the high standard deviation. The Matooke dish containing mukene surprisingly showed the lowest iron content after *in vitro* digestion (1.84 %). Low values can be explained by small amounts of the fish added to the dish. Because of technical limitations of the homogenizer (Osterizer, Pulsematic 10) the sample was eventually not homogenized completely and hence the fish was not evenly distributed.

The presence of oxalates, phytates, dietary fibre or polyphenoles is likely to decrease absorption of non-heme iron in the present dishes. The bioavailability of iron in food of plant origin is known to be low [VAN JAARSVELD et al., 2014] and can vary 10-fold in dishes with a similar content of iron depending on the content of protein, fat and other compounds [FAO, 2004]. Plain Matooke and plain cooking bananas showed a bioaccessibility of 10.2 % and 14.2 ± 3.88 %, respectively. On the other hand, the bioaccessibility of the G.nuts sauce was, if at all detectable, below 1 %. Peanuts are known to contain antinutritional compounds like phytates, leading to a limited usage and nutritional value of iron

and other elements [EJIGUI et al., 2005]. Those results can explain the low bioaccessibility of the dishes as all of them are containing peanuts.

The *in vitro* method consisted of various steps which may have an effect on the final result. An increased initial sample weight, for example, may hence increase the amount of bioaccessible iron. To compare values of bioaccessibility it is important to use standardized conditions of the *in vitro* digestion model [SAHUQUILLO et al., 2003]. Another critical step was the pH adjustment of the samples. Each dish was behaving different because of the various components. Uneven distributions of the components in the homogenized dishes lead to different pH values among the triplicate samples. A deficit of the *in vitro* digestion model is that heme iron is not distinguished from non-heme iron and absorption enhancing and inhibiting compounds are not further investigated.

Table 20. Bioaccessibility of iron in the most common dishes

Dish	% Bioaccessibility
Matooke, G.nuts sauce, tomato, onion	3.48±3.67
Matooke, G.nuts sauce, mukene , tomato, onion	1.84±0.24
Katogo	3.02±0.46

Data is represented in means ± standard deviation

5.6.2 Strategies to improve iron absorption

Heat processing of food generally alters bioavailability of nutrients. Both digestibility and hence absorption of iron is believed to be enhanced because of heat treatment. The alteration of factors inhibiting mineral absorption like phytates and fibre, as well as softening of the food matrix and hence release of protein-bound iron result in an improved bioaccessibility of the micronutrient [HEMALATHA et al., 2007].

To reduce the most potent inhibitor of iron absorption in food, phytic acid, both enzymatic and non-enzymatic methods can be applied. Germination (soaking in water to promote sprouting), fermentation and simple soaking lead to the activation of phytase enzymes which promote the degradation of phytic acid.

Milling can lead to the reduction of phytic acid if the compound is localized in the germ or aleurone layer. This method, however, also results in a loss of iron which is found as well in the germ or aleurone layer [RUEL and LEVIN, 2000].

A study carried out in 2005 reported the effect of processing methods on iron and phytates in peanuts. Roasting and dehulling lead to a significant decrease of the iron content compared to raw peanuts, germination did not significantly affect the element. Processing had a positive impact on phytate content. Germination, roasting, as well as the combination of both methods showed a significant decrease in phytate [EJIGUI et al., 2005].

Vitamin C is considered to be the most potent enhancer of ferric (non-heme) iron absorption. Each meal, especially the ones containing high amounts of non-heme iron (like the sauce made of peanuts) should be accompanied with at least 25 mg of ascorbic acid. Hence iron absorption may be doubled or even tripled. The addition of certain fruits and vegetables to meals containing iron should be recommended to increase the nutritional value [FAO, 2004].

However, it has to be questioned if plant foods with rather low iron contents can contribute to minimize the prevalence of iron deficiency in developing countries [VAN JAARSVELD et al., 2014].

5.6.3 Potential contribution of the most common dishes to iron RDA

To meet their nutritional needs children aged 7-12 months require 11 mg iron/day, children between 1 and 3 years require 7 mg/day and children aged 4-8 years 10 mg/day [IOM, 2001]. An average portion size was expected to be 200 grams. As shown in **(Table 21)** this uptake is not sufficient for children to meet their recommendations. 200 g of the different dishes contribute between 5.65 % and 13.1 % to the daily recommendations, depending on the meal and age group. The dietary references mentioned above refer to a low bioavailability because of a mainly vegetarian diet. Iron contents before *in vitro* digestion were used for the determination of the contribution to the RDA.

Table 21. Contribution of popular dishes to iron recommendations

Dish	µg/200 g fresh weight	% RDA 7-12 months	% RDA 1-3 years	% RDA 4-8 years
Matooke, G.nuts sauce, tomato, onion	846	7.69	12.1	8.46
Matooke, G.nuts sauce, mukene , tomato, onion	920	8.36	13.1	9.2
Katogo	622	5.65	8.89	6.22

5.7 Protein content of popular dishes

Following the methodology described in 4.10 showed that all of the common dishes fed to preschool children contained detectable amounts of protein (**Table 22**). The lowest protein content was found in porridge (0.85 g/100 g). Higher amounts were obtained in dishes containing cooking bananas and G.nuts, with the highest amount in the Matooke dish with additional mukene (3.31 g/100 g), followed by the Matooke dish without mukene (2.94 g/100 g) and Banana Katogo (2.14 g/100 g). The Matooke dishes contained proportionally more of the G.nuts sauce than bananas compared to Katogo explaining the higher protein content.

Table 22. Protein content of most common dishes fed to preschool children.

Dish	g/100 g fresh weight	%
Porridge	0.85±0.01	4.84±0.04
Matooke, G.nuts sauce, tomato, onion	2.94±0.01	10.8±0.02
Matooke, G.nuts sauce, mukene , tomato, onion	3.31±0.11	12.7±0.42
Katogo	2.14±0.06	11.0±0.31

Data is represented in means of at least two determinations ± standard deviation

In **Table 23** the protein contents of the single components are shown. Boiled cooking bananas as well as plain matooke had low protein contents (0.76 and 0.85 g/100 g fresh weight, respectively). The sauce prepared of grounded peanuts on the other hand represented a good source of protein. If the sauce

was prepared with mukene just small amounts of the small fish were used, therefore, no high increase in protein content was observed.

The high protein content in mukene was already discovered by other researchers. There still may occur protein loss along the processing chain. Fish products are often not dried enough to inhibit enzyme activity, lipid oxidation and proteolytic processes. Poor handling practices are likely to cause spoilage [KABAHENDE et al., 2011]. However, mukene is a good source of protein and can be purchased on local markets at low prices. Adding the small fish to dishes is an affordable solution of increasing the protein content of meals. Because of the strong taste traditionally just small amounts (around 5 g) are added to the food. By explaining the benefit of an increased intake of mukene, consumption may be encouraged.

Table 23. Protein content of the single components of the dishes.

Component	g/100 g fresh weight	%
Boiled cooking bananas	0.76±0.06	3.26±0.27
Plain matooke	0.85±0.06	3.18±0.24
G.nuts sauce	8.69±0.08	33.6±0.33
G.nuts sauce, tomato, onion	8.42±0.03	31.9±0.12
G.nuts sauce, mukene , tomato, onion	9.61±0.14	35.6±0.51
Mukene	65.6±0.72*	65.6±0.72

Data is represented in means of at least two determinations ± standard deviation

* dry weight

5.7.1 Potential contribution of the most common dishes to protein RDA

To meet nutritional needs 11 g protein/day are recommended for children aged 7-12 months, 13 g/day for children between 1 and 3 years and 19 g/day for children between 4 and 8 years. With an average portion size of 200 g [EKESA et al., 2012] the dishes, especially the ones containing peanuts, can contribute to meet the daily protein RDAs (**Table 24**). The consumption of Matooke with G.nuts sauce and mukene is most susceptible to reach the recommended daily amount of proteins. Eating a portion of 200 g could provide 50 % of the RDA for a child between 1 and 3 years. Porridge can contribute between 9 and 15.5 %

to children's protein RDA depending on age. Using wholegrain maize flour would slightly increase the total protein content of porridge and hence make the dish a better source to meet dietary protein needs.

Table 24. Contribution of popular dishes to protein recommendations

Dish	g/200 g fresh weight	% RDA 7-12 months	% RDA 1-3 years	% RDA 4-8 years
Porridge	1.7	15.5	13.1	8.95
Matooke, G.nuts sauce, tomato, onion	5.88	53.5	45.2	30.9
Matooke, G.nuts sauce, mukene , tomato, onion	6.62	60.1	50.9	34.8
Katogo	4.28	38.9	32.9	22.5

Although not amongst the four most popular dishes, beans are often used when preparing dishes. Beans are known to have a high protein content and are rich in the amino acid lysine. The combination of beans and maize which is cultivated in Kiboga district and contains considerable amounts of methionin, would be a good nutritional practice because of a high biological value [ELMADFA, 2004].

6 Conclusion

The evaluation of a questionnaire, interviews with caregivers as well as a focus group discussion lead to the result, that porridge, Matooke with a sauce of grounded peanuts, tomatoes, onions and once with mukene, once without mukene, as well as Banana Katogo are the four most common dishes fed to preschool children in Kiboga district in Uganda.

Matooke and G.nuts sauce with and without mukene as well as Banana Katogo contained between 23.3 µg and 31.1 µg REA/100 g. Bioaccessibility of the pVAC for all three dishes was between 20.8 % and 27.3 % of *trans*-β-carotene and α-carotene and between 22.7 % and 35.1 % of *cis*-β-carotene. Porridge did not contain any provitamin A carotenoids.

The analysis of Matooke and G.nuts with and without mukene as well as Banana Katogo showed that the dishes contained iron between 311 µg and

460 µg/100 g. Just porridge did not contain any iron. Bioaccessibility of iron was quite low and ranged between 1.84 % and 3.42 %.

Protein was detected in all four popular dishes with the lowest amount found in porridge (0.85 g/100 g) and the highest in Matooke with G.nuts sauce and mukene (3.31 g/100 g).

At present state, the common dishes fed to preschool children from Kiboga district can contribute between 5.65 % and 13.1 % to the RDAs for iron, between 11.6 % and 15.6 % to the vitamin A RDAs and between 8.95 % and 60.1 % of the protein RDAs. However, the amount of those critical nutrients contained in the dishes is not sufficient for children to meet their nutritional needs. To avoid deficiencies the combination with other, more bioavailable nutrients as well as nutrient sparing cooking methods have to be applied. Suggestions include the encouragement of households to use wholegrain flour and milk for the preparation of porridge. Dishes containing iron should be accompanied by vitamin C rich foods to increase the elements absorption. The addition of vitamin A rich local palm oil, which is and affordable for most of the families, should be promoted. To achieve high levels of carotenoids the usage of already ripe bananas and avoiding of direct sunlight during processing is recommended. A combination of these and other actions may have the potential to increase the nutritional value of the popular dishes fed to preschool children.

7 Summary

Malnutrition, vitamin A deficiency as well as iron deficiency anaemia are severe public health problems in developing countries. Reasons therefore are an insufficient uptake of essential nutrients as well as diseases that lead to an impaired metabolism. The aim of the study was to figure out the most common dishes fed to preschool children in Kiboga district, as well as the protein, vitamin A and iron content and bioaccessibility of vitamin A and iron of those dishes. Based on the results of the laboratory work it was assessed if preschool children meet their nutrition needs when consuming their common diet.

To figure out the popular dishes a questionnaire, interviews with caregivers as well as a focus group discussion were applied. Porridge, Matooke with a sauce of grounded peanuts, tomatoes, onions and once prepared without mukene, once with mukene, as well as Banana Katogo were established to be the four most common dishes consumed by preschool children in rural Kiboga district in Uganda. Vitamin A, especially in the form of provitamin A carotenoids was analysed using HPLC and was available in the three banana-derived dishes but not in porridge. Using the concept of retinol activity equivalents (RAE) a daily consumption of 200 g of each banana-derived dish may contribute 11.6 % - 15.6 % to a child's vitamin A RDA. Bioaccessibility of the meals ranged from 20.8 % to 27.3 % of trans- β -carotene, 20.7 % to 28 % of α -carotene and 22.7 % to 35.1 % of cis- β -carotene. Carotenoids are susceptible to be destroyed by heat treatment especially in the presence of light and oxygen. Cooking leads to a decrease of total carotenoid concentrations due to molecule destruction as well as isomerisation of the all-*trans* to the *cis*-form of β -carotene. The proportional increase of the *cis*-isomers decreases its biological vitamin A activity [VEDA et al., 2010]. On the other hand, cooking increases the release of carotenoids from the food matrix and therefore bioaccessibility [ETCHEVERRY et al., 2012]. To ensure maximum carotenoid concentrations in the banana-derived meals together with a high bioaccessibility a happy medium in food processing has to be found. Further studies on different processing methods (lower temperature and longer cooking time, higher temperature and shorter cooking time) should be carried out.

Iron was detected in the banana-based dishes but not in porridge. 200 g of the banana-based dishes could contribute between 5.65 % and 13.1 % to the iron RDA of children. The sauce made of grounded peanuts was used both for the preparation of Katogo and was served together with Matooke. It was established that the sauce itself was a reasonable source of iron. But iron bioaccessibility of all dishes containing peanuts was rather low (< 3.42 %), therefore processing methods and combination with vitamin C rich foods to improve the absorption of iron should be considered.

Protein was found in all four of the most common dishes. Porridge contained smaller amounts and could contribute 8.95 % - 15.5 % to the protein recommendations, depending on the age of the child. Banana-derived dishes served with G.nuts provide between 22.5 % and 60.1 % of the protein RDA.

Although consumed most frequently, porridge contained the fewest nutrients among all dishes. To improve its nutritional value and increase both protein and iron content it should be suggested to use wholegrain flour instead of milled white flour.

To sum it up, the consumption of the four most common dishes can contribute to the requirements of the investigated nutrients. However, amounts of iron and provitamin A are not likely to be sufficient to alleviate the prevalence of micronutrient deficiencies like VDA and IDA. For this reason, further research on appropriate preparation and processing techniques to optimize retention and bioaccessibility of the dishes is necessary.

8 Zusammenfassung

Fehlernährung, Vitamin A sowie Eisenmangel sind in Entwicklungsländern weit verbreitet und tragen zu einer hohen Morbidität und Mortalität in der Bevölkerung bei. Gründe für Fehl- und Mangelernährung sind zum Einen eine unzureichende Aufnahme essentieller Nährstoffe und zum Anderen Krankheiten, die den Stoffwechsel und somit die Verwertung der Nahrung beeinträchtigt. Säuglinge und Kleinkinder stellen eine Risikogruppe dar und sind am häufigsten betroffen. In Uganda lag die Prävalenz für chronische Mangelernährung bei Kindern unter fünf Jahren 2011 bei 33 %, was weit über dem von der WHO akzeptiertem Grenzwert von 20 % liegt [UBOS, 2012].

Kiboga Distrikt in Zentraluganda war Untersuchungsort dieser Studie. Ziel war es jene Mahlzeiten zu eruieren, welche von Kindern unter fünf Jahren am häufigsten verzehrt werden. Infolge dessen wurden Protein-, Provitamin A und Eisengehalte sowie die Provitamin A und Eisen Bioaccessibility (jener Teil der Nahrung, der nach Herauslösen aus der Lebensmittelmatrix für die intestinale

Absorption zur Verfügung steht [FERNÁNDEZ-GARCÍA et al., 2009]) der Mahlzeiten ermittelt.

Porridge, Matooke (zerdrückte Kochbananen) mit einer Soße aus Erdnüssen, Tomaten, Zwiebeln mit oder ohne Mukene (kleine Silberfischchen) sowie Bananen Katogo (Kochbananen in einer Erdnusssoße) wurden als die vier am häufigsten konsumierten Gerichte identifiziert.

Provitamin A Gehalte wurden mittels HPLC ermittelt und in den drei Gerichten auf Bananenbasis, allerdings nicht in Porridge, detektiert. Durch jeweils 200 g der unterschiedlichen Gerichte können zwischen 11,6 % und 15,6 % der empfohlenen Tagesdosis von Vitamin A gedeckt werden. Die Provitamin A Bioaccessibility der Mahlzeiten, welche Anhand einer *in vitro* Verdauungsmethode ermittelt wurde, lag zwischen 20,8 % und 27,3 % für trans- β -Carotin, zwischen 20,7 % und 28 % für α -Carotin und zwischen 22,7 % und 35,1 % für cis- β -Carotin. Hitzebehandlung, speziell unter der Anwesenheit von Licht und Sauerstoff führt zur Zerstörung von Carotinoiden. Kochen führt somit zu einer Reduzierung der gesamt Carotinoidkonzentration sowohl durch Zerstörung der Moleküle als auch durch Isomerisierung von der *trans* in die *cis* Form, was wiederum zu einer verminderten biologischen Vitamin A Aktivität führt [VEDA et al., 2010]. Andererseits wird durch Kochen das Herauslösen der Carotinoide aus der Lebensmittelmatrix begünstigt und somit die Bioaccessibility verbessert [ETCHEVERRY et al., 2012]. Um einen möglichst hohen Gehalt an Carotinoiden gemeinsam mit einer hohen Bioaccessibility zu gewährleisten, muss ein goldener Mittelweg bei der Zubereitung gefunden werden. Weitere Studien zu unterschiedlichen Zubereitungsmethoden (niedrigere Temperaturen bei längerer Kochdauer, hohe Temperaturen bei kurzer Kochdauer) sind dafür nötig.

Der Eisengehalt der Gerichte wurde mittels FAAS ermittelt. Eisen wurde in den Gerichten auf Bananenbasis, allerdings nicht in Porridge, detektiert. Jeweils 200 g der jeweiligen Gerichte können zwischen 5,65 % und 13,1 % zur empfohlenen Tagesdosis von Eisen für Kinder beitragen. Die aus gemahlenden Erdnüssen hergestellte Soße wurde für die Zubereitung aller drei Mahlzeiten auf Bananenbasis verwendet. Der Eisengehalt dieser Soße erwies sich als

beträchtlich, allerdings war die Bioaccessibility der Bananengerichte mit Erdnusssoße gering (< 3,42 %). Alternative Zubereitungsmethoden sowie die Zugabe von Vitamin C reichen Lebensmittel zu den Mahlzeiten sollten in Betracht gezogen werden, um die Absorption des vorhandenen Eisens zu verbessern.

Mittels der Kjeldahl Methode konnte Protein in allen vier Gerichten detektiert werden. 200 g des Gerichts können, je nach Alter des Kindes, zwischen 8,95 % und 15,5 % zur empfohlenen Tagesdosis von Protein beitragen. Die drei Mahlzeiten auf Bananenbasis zeigten höhere Gehalte auf. Durch 200 g der jeweiligen Gerichte können zwischen 22,5 % und 60,1 % der täglich empfohlenen Proteinaufnahme gedeckt werden.

Porridge wird von Kindern unter fünf Jahren im ländlichen Kiboga Distrikt am häufigsten verzehrt, weist allerdings die geringsten Nährstoffgehalte auf. Die Verwendung von Vollkornmehl wäre eine Möglichkeit, um den Nährwert und den Gehalt an Eisen und Protein dieser Mahlzeit zu erhöhen.

Zusammenfassend kann gesagt werden, dass die vier gängigen Gerichte einen Beitrag zur Bedarfsdeckung der untersuchten Nährstoffe leisten können. Allerdings sind die Gehalte von Provitamin A und Eisen in den Mahlzeiten nicht ausreichend, um das Auftreten von VAD und IDA zu verhindern. Aus diesem Grund sind weitere Studien zu geeigneten Verarbeitung- und Zubereitungsmethoden, sowie Kombinationen von unterschiedlichen Lebensmitteln nötig, um den Erhalt und die Bioaccessibility der Nährstoffe zu optimieren.

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