# RETENTION AND BIOACCESSIBILITY OF PROVITAMIN A CAROTENOIDS IN POPULAR *MUSA* FRUIT AND THEIR DERIVED PRODUCTS CONSUMED IN EASTERN DEMOCRATIC REPUBLIC OF CONGO

**BY**

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**H87/11175/2008**

**A RESEARCH THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD) IN THE SCHOOL OF APPLIED HUMAN SCIENCES OF KENYATTA UNIVERSITY**

**MAY 2012**

## DECLARATION

This Thesis is my Original Work and has not been presented for a Degree in any other University

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## DEDICATION

I dedicate this thesis to my son Gabriel Miruka and husband David Onyango for the joy they continue to grant me and their patience during my pursuit of this course.

## ACKNOWLEDGEMENT

I acknowledge the Consortium for Improving Agriculture-based Livelihoods in Central Africa (CIALCA) and HarvestPlus through Bioversity International for providing the necessary funding to carry out this research. Sincere gratitude and appreciation goes to the team of persons who facilitated the data collection from the study areas and ensured that samples arrived in Kampala in good condition. These include Mr. Charles Lwanga and Professor Ndung'o Vigheri of Beni territory, North Kivu; Mr. Jules Ntamwira and Mr. Charles Bisimwa of Bukavu territory, South Kivu.

IITA and CIP personnel at Namulonge laboratory (Kampala, Uganda) are thanked for their help during sample preparation and processing. Ms. Ifigeneia Mellidou of the Laboratory of Fruit Breeding and Biotechnology, Katholieke University of Leuven (Belgium) and Ms. Marie Poulaert of CIRAD (France) are thanked for their guidance during the laboratory studies.

I also thank, Professor Judith Kimiywe, Dr. Mark Davey and Dr. Claudie Dhuique-Mayer for their tireless contribution in seeing that I realise my research objectives and writing of this thesis. My colleagues in Bioversity International and especially Dr. Guy Blomme, Dr. Eldad Karamura and Dr. Inge Van den Bergh are thanked for making sure that necessary structures were in place to facilitate the research activities.

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## ABBREVIATIONS AND ACRONYMS

**ACC/SCN** - Administrative Committee on Coordination/Standing Committee on Nutrition

**ANOVA** - Analysis of Variance

**BCE** - Beta Carotene Equivalent

**BCX/βCX** - Beta Cryptoxanthin

**BHT** - Butylated Hydroxytoluene

**$CaCI_2H_2O$** - Calcium Chloride and Water

**CIALCA** - Consortium of Improving Agricultural-based livelihoods in Central Africa

**CIP** - Centro Internacional de la Papa (International Potato Center)

**CIRAD** - Centre for International Research in Agricultural Development

**DALYS** - Disability Adjusted Life Years

**DDS** - Dietary Diversity Score

**DMEM** - Dulbecco's Modified Eagle's Medium

**DRC** - Democratic Republic of Congo

**EAHB** - East African Highland Banana

**EDTA** - Ethylenediaminetetraacetic acid

**FAO** - Food and Agricultural Organization

**FBS** - Fetal Bovine Serum

**FGDs** - Focus Group Discussion

**FVS** - Food Variety Score

**GOK** - Government of Kenya

**HDDS** - Household Dietary Diversity Score

**HCL** - Hydrochloric Acid

**HHs** - Households

**HPLC** - High Performance Liquid Chromatography

**IITA** - International Institute of Tropical Agriculture

**IU** - International Unit

**KCI** - Potasium Chloride

**$K_2HPO_4$** - Potasium Hydrogen Phosphate

**KUL** - Katholiek University of Leuven

**MeOH** - Methanol

**MTBE** - Methyl-tert-butyl-ether

**NaCL** - Sodium Chloride

**$NaHCO_3$** - Sodium Hydrogen Cabonate.

**NaOH** - Sodium Hydroxide

**NEAA** - Non-Essential Amino Acid

**NK** - North Kivu

**OFSP** -Orange Fleshed Sweet Potato

**PVPP** - Polyvinylpolypyrolidine

**PVAC** - Provitamin A Carotenoid

**RAE** - Retinal Activity Equivalent

**RE** -Retinal Equivalent

**RDA** - Recommended Dietary Allowances

**SCN** -Sub-Committee on Nutrition

**SK** -South Kivu

**SPSS** - Statistical Package for Social Sciences

**t-AC** - *trans* Alpha Carotene

**t-BC** - *trans* Beta Carotene

**t-BME** - *tert*-butyl methyl ether

**THF** - Tetrahydrofuran

**UNICEF** - United Nations Children's Fund

**U5MR** - Under Five Mortality Rate

**VAD** - Vitamin A Deficiency

**WFP** -World Food Programme

**WHO** - World Health Organization

## ABSTRACT

In the Democratic Republic of Congo (DRC), bananas and plantains (*Musa* spp.) production is predominant in the Eastern region where they are also a major part of the diet. Recent micronutrient analyses on raw bananas from The Philippines, Uganda and Hawaii indicate that certain cultivars can contribute substantially to the daily vitamin A requirements. The objective of this study was to establish the retention and bioaccessibility of provitamin A carotenoids (pVACs) in the most popular *Musa* fruit and their locally derived products consumed in Eastern DRC. The study sites included Beni territory (North Kivu-NK) and Bukavu territory (South Kivu-SK). The localities, villages and specific households were established through multistage sampling. Sample size was calculated using Fisher's formula. A total of 14 focus group discussions were carried out and 371 households visited and mothers/caregivers interviewed. The most popular *Musa* cultivars identified were sub-sampled at ripening stages 1, 3, 5 and 7 based on peel color and another sub-sample processed into most common products. All the samples were then frozen at $-20^0$c and lyophilised. Using HPLC, these samples were subsequently analysed for fruit pulp pVACs contents. For bioacessibility studies, *Musa* samples and ingredients were processed into products following local procedures and bioaccessibility was estimated using an in vitro digestion method, content of pVACs in the products and dishes was verified using HPLC. Findings showed that the preferred cooking banana varieties included yellow-pulped AAA-East African Highland bananas [AAA-EAHBs] 'Nshikazi' (SK) and 'Vulambya' (NK). Preferred plantains (AAB genome), were orange-pulped and included 'Musheba' (SK) and 'Musilongo' (NK). The most common cooking method was simply boiling of bananas/plantains and the main accompaniments included beans and amaranth leaves. The predominant pVACs in both raw and processed fruit pulp were all-*trans* β- and all-*trans* α-carotene, together constituting about 90% of total pVACs. The proportion of β-carotene was twice that of α-carotene in the plantains varieties, while in the EAHBs tested, the proportion was almost equal. Provitamin A carotenoids observed in the fruit pulp of the tested *Musa* cultivars were retained and a significant increase observed during ripening. The highest levels of the pVACs were observed at ripening stage 3 in all four cultivars. Values were as high as 1081µg/100gFM in 'Vulambya' and 1820µg/100gFM in 'Musilongo'. Although boiling AAA-EAHB cultivars led to substantial losses (40%-60%) in total pVACs contents, boiling and deep frying of the plantains led to retention and an apparent increase. After *in-vitro* digestion, the percentage of micellarized t-BC was higher in 'Vulambya' (29 %) than in 'Musilongo' (16.6 %). In the two *Musa* cultivars, the incorporation into micelles was similar for t-BC and t-AC, but significantly higher for 13-*cis* isomer. The best performing *Musa*-based dishes made from 'Musilongo' and 'Vulambyo' provided about 22% and 28% of the daily vitamin A Recommended Dietary Allowance (RDA) for a child under 5 years old. These results can guide consumer consumption patterns to maximize vitamin A intake for improved health in these regions and also direct researchers in the selection of *Musa* cultivars to be fast-tracked in the fight against VAD.

# CHAPTER ONE: INTRODUCTION

## 1.1 Background of the study

The highlands of the Great Lakes region (Eastern Democratic Republic of Congo-DRC, Rwanda, Burundi, and Western Uganda) contain the most intensely cultivated agricultural regions of Africa. In DRC, 67% of the population depends on agricultural production for both food and income and the principal food crops include; cassava, banana and sweet potatoes (Ndungo, Fiaboe, & Mwangi, 2008). Banana and plantain production occurs predominantly in forest regions of the equator in North and South Kivu. The most dominant banana types in DRC are the plantains and cooking bananas accounting for 27% of the global production estimated to be 282,520 tonnes and 1,071,900 tonnes respectively (Ndungo et al., 2008).

Studies and reports (Karamura, Karamura, & Gold, 1996; Karamura, 1998; Lescot, 1999) indicate that the majority of bananas and plantains grown within this region are consumed locally in various forms. Even though the consumption rates/patterns for the various forms of banana-based foods are not known, the different forms in which they are prepared and consumed include; cooked green, cooked ripe, cooked in the peel, steamed, prepared as juice, ripened for desert, roasted, chipped and fried or dried and floured to make a host of confectionaries. In other words, there is an affordable banana dish for virtually every income category of consumers in the region (HarvestPlus, 2007). In addition, apart from being intercropped with other nutritious ground covering food crops such as legumes (Ddungu, 1987) thus promoting agricultural biodiversity, bananas

can be served with a variety of accompaniments that would ensure diversity in diet and thus nutrient adequacy.

Data on the fruit pulp micronutrients content of fresh fruits of bananas grown in the Eastern African region is not well documented. However, recent analysis of the micronutrient content of bananas suggests that banana and plantain may be one of the few foods that can provide very high amounts of dietary micronutrients, in particular vitamin A (Davey, Van den Bergh, Markham, Swennen, & Keulemans, 2009). However, this needs to be elucidated further. Most of the studies already carried out are on raw, mature bananas and the few studies carried out on the effect of off-plant development and post-harvest treatment on the pVACs content in *Musa* fruit are from other regions of the world (Ngoh-Newilah, Lusty, Van den Bergh, Akyeampong, Davey, & Tomekpe, 2008; Englberger, Schierle, Marks, & Fitzgerald, 2003a, 2003b), no *Musa* fruit samples from either Eastern or Central Africa have been tested.

Following consumption of food, the release of carotenoids during digestion is determined by the extent to which the cell wall is degraded during processing (Tumuhimbise, Namutebi, & Muyonga, 2009). This is influenced by factors, such as the matrix, processing/cooking method and fat, fiber or other additional components (Failla, Thakkar, & Kim, 2009). The amount of food carotenoids released from the food matrix is commonly referred to as bioaccessibility and constitutes the maximum amount available for absorption by the enterocytes (Failla, et al., 2009). Data on the bioaccessibility of pVACs is a critical step in determining the vitamin A activity of a particular food product (Bengtsson, Brackmann, Enejder, Alminger, & Svanberg, 2010). Previous studies have

examined the effect of cooking on carotenoids bioaccessibility from fruits and vegetables, including numerous studies on cooked/processed orange-fleshed sweet potatoes (Ryan, O'Connell, O'Sullivan, Aherne, & O'Brien, 2008; Failla, Thakkar & Kim, 2009; Bengtsson, Larsson-Alminger & Svanberg, 2009; Bengtsson, Brackmann, Enejder, Alminger, & Svanberg, 2010). However, no research has looked at the effect of cooking on the carotenoids bioaccessibility of *Musa* fruit.

**1.2 Problem Statement**

Apart from the high banana and plantain production, DRC is endowed with a wealth of natural resources and a substantial agricultural potential capable of feeding the whole of Africa. Unfortunately the exploitation of these resources has so far not been utilized to promote a decent life for the Congolese population (WFP, 2009). Out of the 154 territories in the whole of DRC, more than 117 are moderately food insecure and 28 others are in a state of acute food insecurity and livelihood crisis. Most of the food insecure households are concentrated in the Eastern part of DRC (WFP, 2009). Seventy four percent of the total population is undernourished and the prevalence of acute malnutrition (wasting) is 9.6 %. This rate is near the wasting threshold of the WHO (10 %). The levels of severe global malnutrition (Underweight) affect 10 % of Congolese children. This is more marked in rural areas (11.9 %) than in urban areas (5.8 %) (Luntala, Mbile, Jean Claude, & Okulo, 2000). Although there is no documentation on the prevalence of VAD in either South Kivu or North Kivu, based on WHO estimates, 61.1% of preschool children in the whole of DRC have VAD. According to the classification provided by the International vitamin A Consultative Group (known as the

Annecy Accords), a prevalence of over 30% of the population with less than 20 μg/dl serum retinol defines a situation of severe VAD (SCN, 2010). Therefore in DRC, VAD is clearly a major public health problem requiring immediate attention.

For children, VAD causes severe visual impairment and blindness, and significantly increases the risk of severe illness, and even death, from such common childhood infections as diarrhoeal disease and measles (WHO, 2011). For women VAD occurs especially during pregnancy and in the last trimester when demand by both the unborn child and the mother is highest. The mother's deficiency is demonstrated by the high prevalence of night blindness during this period and may increase the risk of maternal mortality (WHO, 2011).

Based on the fact that banana and plantain form a major part of the diets of people in Eastern DRC and following findings indicating that some raw banana and plantain cultivars could be good sources of provitamin A carotenoids (pVACs) (Davey, Keulemans, & Swennen, 2006; Englberger, Schierle, Marks, & Fitzgerald, 2003a, 2003b), if sustainably exploited, bananas and plantains could be part of a strategy to reduce the levels of VAD in these regions. Although it is known that there is variation in pVACs content in banana and plantain grown in the region, none of the samples in the above mentioned studies were from DRC. In addition, although there are certainly very few studies that have evaluated the effect of ripening on pVACs of some banana cultivars, the results obtained so far are not conclusive (Davey, Keulemans, & Swennen; 2006, Ngoh-Newilah, Lusty, Van den Bergh, Akyeampong, Davey, & Tomekpe, 2008). In addition, although the amount of food carotenoids released from the food matrix

(bioaccessible) constitutes the maximum amount available for absorption by the enterocytes, there is no knowledge on the percentage of the pVACs that are eventually miceralised and available for absorption by the body. This study therefore aspires to fill in these knowledge gaps and make major steps towards understanding the actual cause of high VAD in DRC.

### 1.3 Justification

Banana is a highly important crop in DRC. In Eastern DRC, it forms a major part of the staple diet, complementing other sources of food (Ndungo, Fiaboe, & Mwangi, 2008). Bananas are grown by small holder farmers and the diverse cultivars can be cooked, roasted, eaten as dessert, made into flour or brewed. Reports have indicated that effective, culturally appropriate food-based strategies are essential for sustainable solutions to alleviating VAD (Ayewole-Olusola & Asagbra, 2003). These strategies empower individuals and households, leading to family food production, wise food selection and preparation methods, the provision of multiple nutrients simultaneously and an enhancement of cultural pride and identity (Englberger, Schierle, Marks, & Fitzgerald, 2003a).

Since studies have suggested that some banana varieties have among the highest carotene levels in the world capable of providing up to half of the total daily vitamin A requirements in a single fruit (Davey, Van den Bergh, Markham, Swennen, & Keulemans, 2009; Englberger, Schierle, Marks, & Fitzgerald, 2003a, 2003b), if sustainably exploited, bananas and plantains could go a long way in reducing the levels of VAD among populations dependent on them. In addition, the actual consumption

patterns of bananas, retention level of micronutrients such as pVACs during both storage and processing/cooking and its bioaccesibility from commonly consumed banana products/dishes have barely been studied and documented.

### 1.4 Purpose of study

This study aspired to fill these literature gaps and lead to a better understanding of the caused of VAD in Eastern DRC. It will also lead to recommendations for better post-harvest handling techniques that will enhance retention of nutrients especially pVACs - in *Musa* products and promote bioaccessibility for better nutrition and health status of populations in these regions. The results will also guide researchers on potential existing *Musa* cultivars that can be fast tracked within communities for enhanced nutrition and health.

### 1.5 Main objective

To establish the retention of pVACs in popular *Musa* fruit cultivars during ripening and local processing/cooking and its bioccessibility from *Musa* post-harvest products consumed by small holder households in Eastern DRC.

#### 1.5.1. Specific objectives

1. To obtain and validate information on banana varietal popularity, common banana processing techniques, dietary patterns and nutrition status among banana growing and consuming regions of Eastern DRC.

2. To determine the cultivar-dependent changes in pVACs contents related to developmental stage (ripening) of *Musa* fruit post-harvest.
3. To determine the cultivar-dependent changes in pVACs content related to different post-harvest treatment methods practiced by community members in Eastern DRC.
4. To determine pVACs bioaccessibility from two *Musa* products consumed in Eastern DRC using an *in vitro* digestion model.

### 1.6 Hypotheses

1. The banana cultivars grown and consumed by small holder households in Eastern DR Congo do not have substantial levels of pVACs

2. The level of pVACs in *Musa* fruits is not significantly affected by post-harvest development (ripening) and processing/cooking.

3. The amount of Beta-carotene released from the food matrix and available for absorption is not significantly different from the amount in the consumed banana product/dish.

### 1.7 Significance and anticipated output

Banana and plantain fruits typically are processed and consumed in various forms by households; in some instances, they are also kept for some time before being processed into various products. It was therefore important to find out what changes in the micronutrient content take place during both storage and processing and whether the

post-harvest handling and processing procedures affect the retention of important micronutrients such as pVACs. It was also important to establish the proportion of pVACs in the banana product that are available for absorption and utilisation by the body after consumption. Through documentation of the findings, this study will contribute to the existing knowledge on the effect of ripening and post-harvest processing of *Musa* fruit on the retention of pVACs and more insight on the bioaccessibility of pVACs from different post-harvest *Musa* products. The anticipated outcomes include the adoption of post-harvest handling and processing techniques that enhance the retention of pVACs in *Musa* fruit and products. The beneficiaries include community members, non-governmental organisations, government organisations and other stakeholders working towards the alleviation of micronutrient deficiencies.

### 1.8 Limitations

Although more than 50 different banana cultivars are grown in Eastern DRC (Ndungo, Fiaboe, & Mwangi, 2008), through focus group discussions (FGDs),only two most popular banana cultivars from each of the two sites were selected and samples drawn from them. In addition not all the banana products and dishes used by the community were sampled; through the FGDs only two of the most popular products for each selected cultivar were selected for sampling.

**1.9 Conceptual Framework**

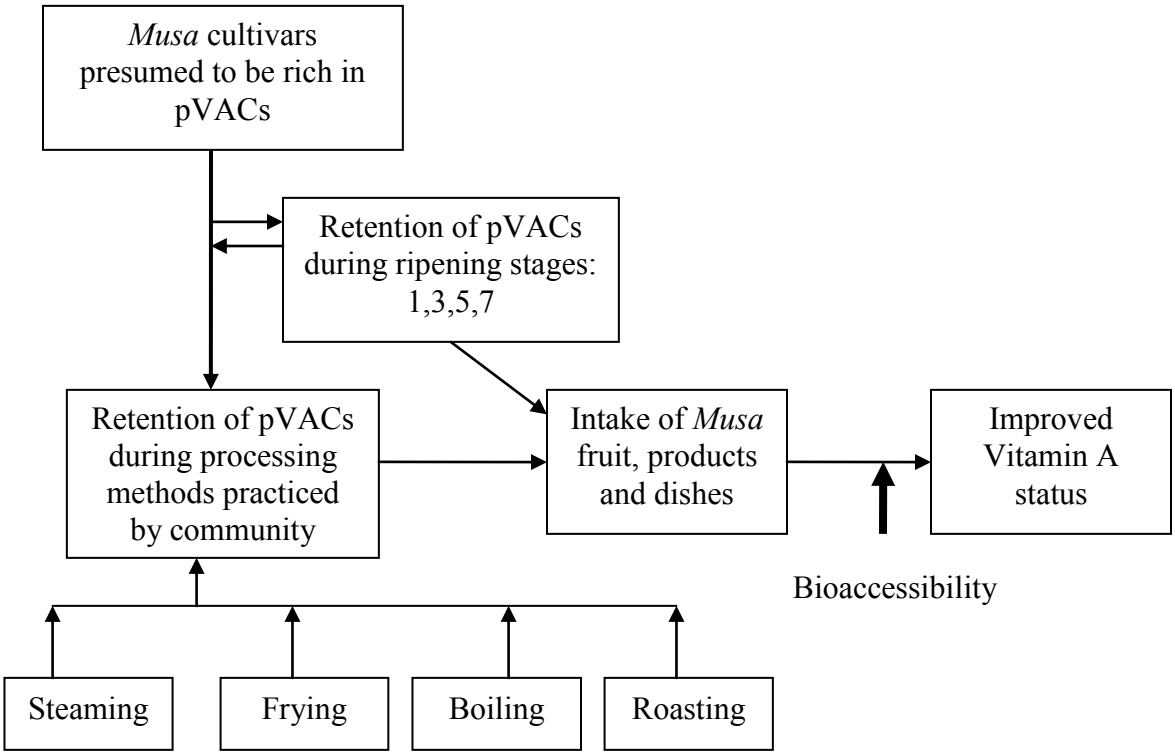


**Figure 1.1.** Interaction between the study variables and the expected outcomes (Source: adopted from the UNICEF conceptual framework as modified by Black *et al*, Lancet, 2008)

The conceptual framework (Figure 1.1), shows that by establishing the effect of ripening local processing on retain of pVACs and establishing the derived dishes/products with the highest content and bioaccesisbility of pVACs,the communities dependent on bananas will be able to apply the best practices and enhance their nutrition with regards to Vitamin A status. This is because the *Musa* fruit will be utilised at the optimal development stage, appropriate post-harvest processing/cooking techniques and dietary

combinations that optimise on retention and bioaccessibility of pVACs will be put into practice.

### 1.10 Standard and operational definition of key terms

**Agricultural Biodiversity** - Variety and variability of plants and animals necessary to sustain key functions for, and in support of, food production, availability and access.

**Banana –** Refers to dessert and cooking types, including plantain, of *Musa* app. fruit (AAA, AAB and ABB groups)

**Banana dependent households**- Households that rely on banana production for both food and income

**Bioaccessibility of *trans* beta-carotene (t-BC)** - The amount of t-BC that is released from the food matrix and available for absorption.

**Bioavailability of *trans* beta-carotene (t-BC)** - The fraction of the ingested t-BC that is absorbed and available for utilisation in normal physiological functions and for storage.

**Bioconversion of *trans* beta-carotene (t-BC)** – The fraction of the absorbed t-BC that is converted to retinol.

**Carotene-** The precursor of active vitamin A, mostly yellow-orange pigments found only in foods of plant origin where they are closely associated with chlorophyll.

**Dietary diversity** - The number of different foods consumed over a period of seven days.

**Family food security-** A situation that exists when a family has sufficient safe and nutritious food throughout the year for all members to meet their dietary needs and food preferences.

**Food Security –** When all people at all times have both physical, social and economic access to sufficient safe and nutritious food to meet their dietary needs and food preferences for a productive and healthy life.

**Macronutrients-** Nutrients (such as carbohydrates, fats and proteins) required by the body in large amounts.

**Malnutrition-** An abnormal physiological condition caused by deficiencies, excesses or imbalance of energy and nutrients.

**Micronutrients-** Nutrients (like vitamins and minerals) required by the body in very small amounts.

**Nutrient-** Part of the food that is absorbed and used by the body for energy, growth, repair, and protection from disease.

**Nutrition** The study of foods, diets and food-related behaviors, and how nutrients are used in the body. People also use the term to describe the food intake of a person.

**Nutrient security** – Ability to meet the recommended nutrient requirements of the body at all times.

**Nutritional status -** the state of the body, as expressed according to scientifically tested parameters: weight for height, height for age and weight for age.

**Preschool Children** – Children aged between two years and five years.

**Provitamin A Carotenoids** - a precursor of vitamin A; a substance found in mostly, green, red or orange plant foods and when it is consumed it can be converted into an active form of vitamin A when needed by the body.

**Retinol** - the form of vitamin A absorbed from eating animal food sources, it is a yellow, fat-soluble substance. Since the pure alcohol form is unstable, the vitamin is found in tissues in a form of retinyl ester.

**Retinol Equivalent (RE)** - a unit used for quantifying the vitamin A value of sources of vitamin A, including both preformed retinoids in animal foods and precursor carotenoids (pVACs) in plant foods.

**Stunting -** Shortness due to a deficit in growth in height that has failed to reach genetic potential. Low height-for-age and defined as <-2standard deviations (SD) of the height-for-age median value of a reference healthy population.

**Underweight –** Low weight-for-age. A composite of stunting and wasting. Defined as <-2standard deviations (SD) of the weight-for-age median value of a reference healthy population.

**Vitamin A -** Vitamin A includes several bioactive compounds known as retinoids as well as precursor forms of the vitamin (provitamin A) known as carotenoids. In foods from

animal origin: Preformed vitamin A: absorbed in the form of retinol / In foods from plant origin: Provitamin A carotenoids (pVACs): made into retinol in the body.

**Vitamin A deficiency (VAD) -** All the physiological disturbances caused by lack of vitamin A, including clinical signs and symptoms.

**Wasting –** Low weight-for-height. A condition that results from the loss of both tissue and fat in a body that usually reflects severely inadequate food intake or infectious processes happening at present. Defined as <-2standard deviations (SD) of the weight-for-height median value of a reference healthy population.

# CHAPTER TWO: LITERATURE REVIEW

## 2.1. Introduction

This chapter reviews literature related to the variables in this study. The causes and magnitude of VAD and dietary sources of both preformed vitamin A and pVACs, the importance of banana to the smallholder households in Eastern DRC have been looked at. Other studies on the effect of post-maturity development and processing on pVACs of different fruits and methods of estimating bioaccessibility of pVACs have also been reviewed. Lastly existing literature gaps have been identified.

## 2.2. Vitamin A, its importance and dietary sources

### 2.2.1 Vitamin A

Vitamin A can be considered the most important vitamin in supporting human life; it is made up of a family of compounds called the retinoids (William, 1994). The retinoid designation resulted from finding that vitamin A had the biological activity of retinol (Williams, 1994). Vitamin A is a fat-soluble nutrient that occurs in nature in two forms: retinol or preformed vitamin A and provitamin A or precursor vitamin A (Kirschmann & Kirschmann, 1996). While retinol, occurs only in foods of animal origin, fruits and vegetables that contain certain carotenoids also provide vitamin A activity (Kirschmann & Krschmann, 1996). Carotenoids are plant pigments, responsible for the red, orange, and yellow color of fruits and vegetables. The body can convert certain members of the carotenoid family, including beta-carotene, alpha-carotene, and gamma-carotene, into

vitamin A. These are the carotenoids referred to as "provitamin A carotenoids' (pVACs) (Kirschmann & Kirschmann, 1996).

In plants, carotenoids are synthesized from geranylgeranyl diphosphate (Figure 2.1) and accumulate in leaf chloroplasts and in the chromoplasts of fruits, seeds and flowers (Giuliano, Aquiliani, & Dharmapuri, 2000). Animals cannot synthesise these components and they rely on the diet as the source of these compounds (Fraser & Bramley, 2004). In the intestines, pVACs can be transformed into retinal, a precursor of vitamin A (Figure 2.1). Retinal will later be reduced to retinol (vitamin A). This process will only take place if the body has a need for vitamin A (Yeum & Russel, 2002).

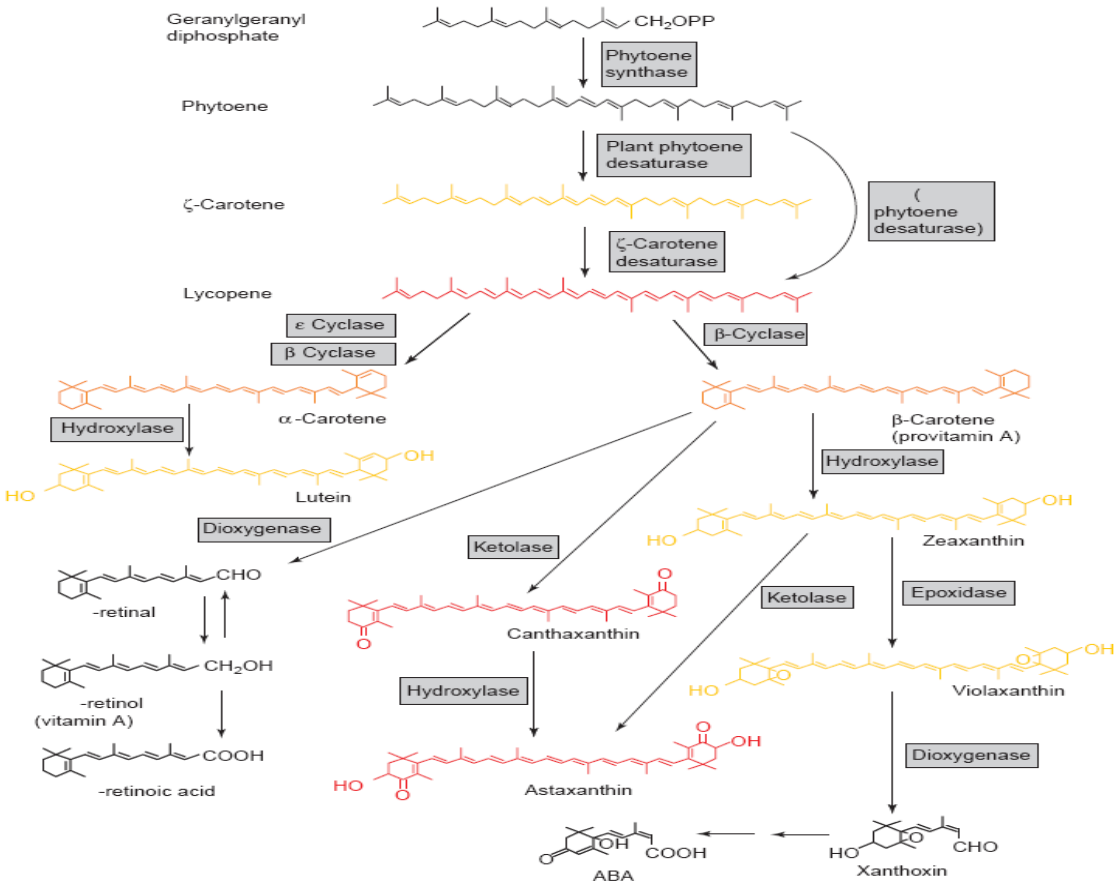


**Figure 2.1.** An overview of carotenoid biosynthesis. pVACs, Source: Giuliano et al., 2000

### 2.2.1.1. Carotenoids commonly found in *Musa* fruit

#### β- carotene (BC)

β-carotene (Figure 2.2) is the most abundant carotenoid in nature. It is mostly found in orange colored fruits and vegetables like carrots, pumpkin, mango etc., but also in dark green leafy vegetables like spinach. β-carotene is the carotenoid with the largest vitamin A activity but it also has an important function as an antioxidant (Schieber & Carle, 2005; Johnson, 2002).

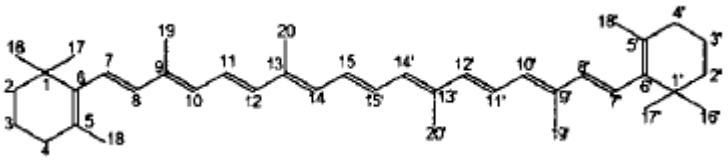

**Figure 2.2**. Chemical structure of all *trans* BC

Source: Rodriguez-Amaya, 2001

#### α-carotene (AC)

This carotenoid usually is present in much lower concentrations than β-carotene. It can be found in carrots, green vegetables, etc. The most important function of α-carotene is its pro-vitamin A activity. In structure it only differs from β-carotene in the positioning of a double bond in the second ring (Figure 2.3) (Britton et al., 2004).

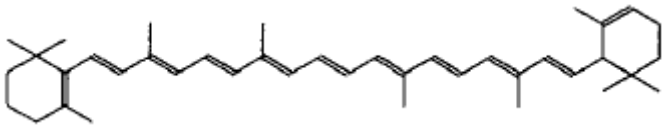

**Figure 2.3:** Chemical structure of all *trans* AC

Source: Rodriguez-Amaya, 2001

**Lutein**

Lutein is a xanthophyl (Figure 2.4). It is mostly found in cereals such as maize, in leafy vegetables like spinach, salad etc. and in other green vegetables like broccoli (Schieber & Carle, 2005). In the human body, lutein is mostly found in the eye. The highest concentration is found in the yellow spot in the eye and in the lens. Here it functions as an antioxidant and protects the eyes from UV-light (Fraser & Bramley, 2004; Johnson, 2002).

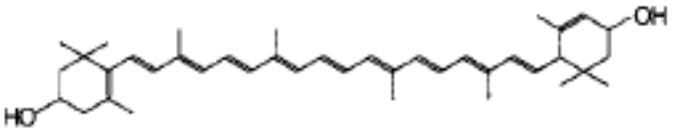


**Figure 2.4.** Chemical structure of lutein

Source: Rodriguez-Amaya, 2001

**2.2.2 Role of vitamin A in the human body**

While vitamin A is best known for its vital role in vision, this vitamin also participates in physiological activities related to the immune system, maintenance of epithelial and mucosal tissues, growth, reproduction, and bone development (Kirschmann & Kirschmann, 1996).

Vision- The human retina contains four kinds of photopigments that store vitamin A compounds. One of these pigments, called rhodopsin, is located in the rod cells of the retina. Rhodopsin allows the rod cells to detect small amounts of light, and, thus, plays a fundamental role in the adaptation of the eye to low-light conditions and night vision. Retinal, the aldehyde form of the vitamin, participates in the synthesis of rhodopsin, and

in the series of chemical reactions that causes visual excitation, which is triggered by light striking the rod cells. The remaining three pigments, collectively known as iodopsins, are found in the cone cells of the retina and are responsible for day vision (Kirschmann & Kirschmann, 1996).

Immune function- Vitamin A stimulates several immune system activities, possibly by promoting the growth, and preventing the stress-induced shrinkage, of the thymus gland (Kirschmann & Kirschmann, 1996). Vitamin A is known to enhance the function of white blood cells, increase the response of antibodies to antigens, and to have anti-viral activity. In addition, retinoic acid is needed to maintain the normal structure and function of epithelial and mucosal tissues, which are found in the lungs, trachea, skin, oral cavity, and gastrointestinal tract. These tissues, when healthy and intact, serve as the first line of defense for the immune system, providing a protective barrier that disease-causing microorganism cannot penetrate (Kirschmann & Kirschmann, 1996).

Cell growth - Vitamin A is also necessary for normal cell growth and development. Although the mechanisms by which vitamin A promotes cell growth and development are not yet fully understood, it is known that retinoic acid is necessary for the synthesis of many glycoproteins, which control cellular adhesion (the ability of cells to attach to one another), cell growth and cell differentiation (Kirschmann & Kirschmann, 1996).

Other roles for vitamin A - It is also known that vitamin A is essential for reproductive processes in both males and females and plays a role in normal bone metabolism (Kirschmann & Kirschmann, 1996).

### 2.2.3 Dietary sources of preformed and provitamin A carotenoids and its daily recommended dietary allowances

The dietary sources of vitamin A are of two categories: vitamin A or retinol, also known as preformed vitamin A; and pVACs, which are the carotenoids that are biologically active as retinol following consumption (Kirschmann & Kirschmann, 1996). Of approximately 600 carotenoids found in nature, only three are important precursors of vitamin A in humans: t-BC, t-AC and t-BCX. Of these three, all *t*-BC (t-BC) is the major pVAC component of most foods containing carotenoids and has the highest vitamin A activity (i.e. is converted into retinol more efficiently than other pVACs) (Kirschmann & Kirschmann, 1996; Williams, 1994).

Foods rich in preformed vitamin A include milk and milk products, eggs, fish and associated oils, shellfish, liver, organ meats and chicken. Preformed vitamin A occurs in food in the form of retinyl esters. People in developing countries, where the most serious problems of vitamin A deficiency are prevalent, derive most of their vitamin A carotenoids from plant sources, because foods containing preformed vitamin A are usually expensive or can rarely be obtained (Mulokozi, 2003). Main sources of pVACs include the following: red palm oil, dark green leafy vegetables, red and yellow vegetables and roots/tubers, and red and yellow/orange fruits (Williams, 1994). **C**rude red palm oil has been documented to be the richest natural source of carotenoids with 10 to 15 times more carotenes than carrots and 50 times more than tomatoes and between 200 to 700 μg provitamin A carotenoids per gram (Nestel & Nalubola, 2003; Monde, Michel, Carbonneau, Tiahou, & Vernet, 2009).

The recommended dietary allowances (RDA) for vitamin A for children as formulated by FAO/WHO (2002) (Table 2.1.).

**Table 2.1.** Reccomended Dietary Allowances (RDAs) of vitamin A for children and women.

| Life Stage | Age | RDA µg retinol/day |
|---|---|---|
| Infants | 0 - 6 months | 400 |
| | 7 - 12months | 500 |
| Children | 1 - 3 yrs | 300 |
| | 4 - 8 yrs | 400 |
| Female | 9 - 13 yrs | 600 |
| | 14 - 18 yrs | 700 |
| | 19 - 30 yrs | 700 |
| | 31 - 50 yrs | 700 |

Although RDAs are given in ug of retinol equivalents (RE), the vitamin A content of foods appears on the label in International Units (IU). The conversion of IUs to retinol equivalents is made using conversion factors that depend on the food source. One Retinol Equivalent (RE) of vitamin A (in µg) = 6 International Units (IU) from BC

10 IU from other carotene-rich plant foods

4.10 IU from milk and yogurt

3.33 IU from animal sources and fortified foods

### 2.2.4 Metabolist of Vitamin A in the human body

Seventy to ninety percent of vitamin A from the diet is absorbed in the intestine. The efficiency of absorption for vitamin A continues to be high (60-80%) as intake continues to increase. Greater than 90% of the retinol store within the body enters as retinyl esters that are subsequently found within the lipid portion of the chylomicron (Ross, 1999). Absorption of vitamin A is very rapid, with maximum absorption occurring two to six hours after digestion (Ross, 1999). Within the intestinal lumen the vitamin is incorporated into a micelle and absorbed across the brush border into the enterocytes. Within the enterocyte, precursors of vitamin A (carotenoids) are converted to active forms of the vitamin. The newly formed products and additional precursors are then packaged into chylomicrons and readied for transport throughout the body (Groff, Gropper, & Hunt, 1995).

Transport- After leaving the enterocytes chylomicrons, which carry retinyl esters, carotenoids, and unesterfired retinol along with triglycerides, are circulated first through the lymphatic system and then through the general circulation. Upon arriving at extra-hepatic cells chylomicrons release triglycerides, however vitamin A remains within the chylomicron. The vitamin A is then incorporated into a chylomicron remnant (Groff, Gropper, & Hunt, 1995). The chylomicron remnant then travels back to the liver where it is taken up and further metabolized or stored. When needed retinol is mobilized from the liver and requires the use of a carrier for transport through the blood. As shown in Figure 2.5, retinol-binding protein (RBP) is the specific carrier used to transport all-trans retinol in the plasma. The all-trans isoform accounts for more than 90% of all plasma vitamin A (Ross, 1999). This specific carrier is manufactured and secreted by the parenchymal cells

of the liver (Groff, Gropper, & Hunt, 1995; Ross, 1999). Each mole of retinol released binds equivocally with RBP to form holo-RBP. This compound then binds with a molecule of transthyretin (TTR), formerly known as prealbumin. This newly formed retinol-RBP-TTR complex is not filtered by the glomerulus, but instead freely circulates throughout the plasma. Tissues are then able to take the retinol up as needed via cellular retinoid-binding protein (Ross, 1999). Retinoic acid is believed to be manufactured by the cells as needed. Therefore, transport of retinoic acid is likely not substantial. Instead, the cell possesses intra-cellular proteins that regulate the amount of retinoic acid produced. The proteins also help to determine the intra-cellular usage of retinoic acid (Groff, Gropper, & Hunt, 1995).

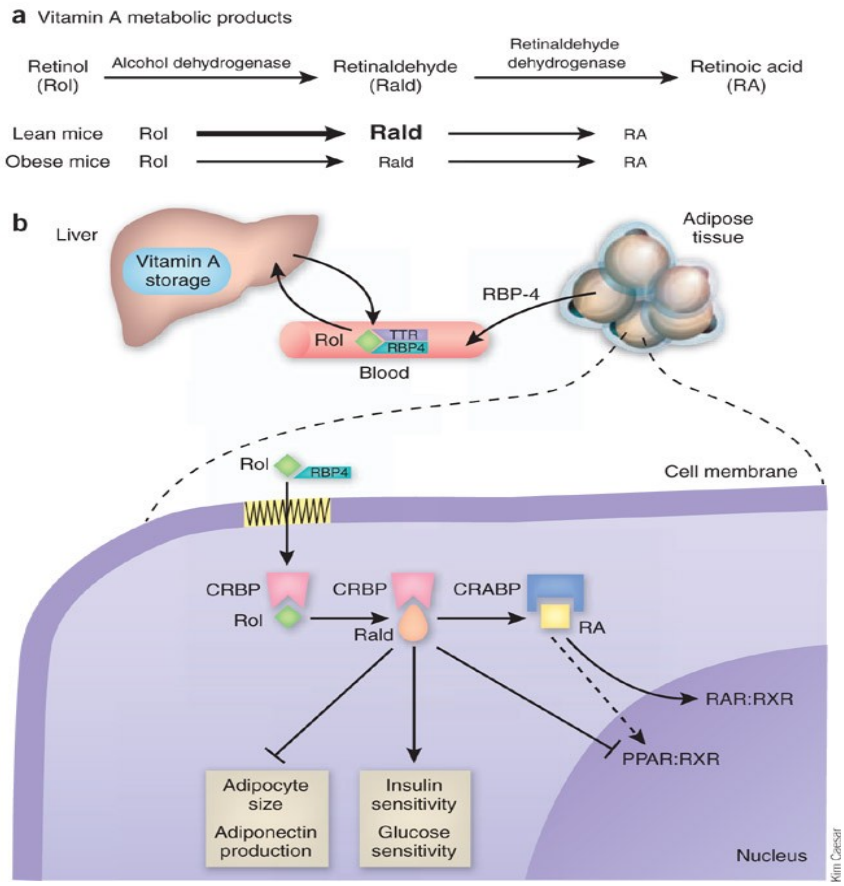


**Figure 2.5.** Vitamin A metabolism; Source Desvergne, 2007

Storage- Approximately 50 to 85% of the total body retinol are stored in the liver when vitamin A status is adequate (Ross, 1999). Retinol returning to the liver is re-esterfied before storage. Because of this, over 90% of the retinol is stored in the form of retinyl esters. The retinol is stored in hepatic stellate (star-shaped) cells along with droplets of lipid (Groff, Gropper, & Hunt, 1995). Thus constitutes the fat-soluble property of vitamin A. The size of stellate cells increase linearly with increasing retinol levels. Once hepatic stellate cells are saturated with all the retinol they can hold, hypervitaminosis can result. (Ross, 1999). The precursor to vitamin A, beta-carotene, can be stored in adipose cells of fat depots throughout the body and to date the only side effect of excess beta-carotene supplementation appears to be yellowing of the skin. Serum levels of beta-carotene are an indicator of recent intake and not body stores (Groff, Gropper, & Hunt, 1995).

Excretion- The kidneys are the main paths of RBP and retinol excretion from the body. This is achieved manly via renal catabolism and glomerular filtration (Ross, 1999). Those persons suffering from renal disease often experience elevated serum levels of RBP and retinol and therefore must be more aware of vitamin A toxicity (Rodrigues, Goncalves, & Bairos, 2004).

**2.3. Vitamin A Deficiency (VAD) and its prevalence in DRC**

Vitamin A Deficiency (VAD) occurs in endemic proportions in developing countries and the most obvious result is progressive damage to the eyes, eventually leading to blindness. The only biochemical parameter validated and found practical for routine survey of VAD use is serum retinol concentration (Sommer & Davidson, 2002). One agreed cut-off point is 20 µg/dl or 0.7µmol/l, and the criterion for establishing a public

health problem is >15% prevalence of VAD (SCN, 2010). The term ‘sub-clinical’ has been generally dropped, and low serum retinol can be refereed to as VAD, meaning the state of inadequate vitamin A nutrition (SCN, 2010).

An estimated 250 million preschool children are vitamin A deficient and it is likely that in vitamin A deficient areas a substantial proportion of pregnant women is vitamin A deficient. An estimated 250 000 to 500 000 vitamin A-deficient children become blind every year, half of them dying within 12 months of losing their sight (WHO, 2009).

In all developing countries, an estimated 163 million children are vitamin A deficient (by low serum retinol) with a prevalence of about 30% (SCN, 2010). Young children are at high risk of developing VAD due to their increased requirements during growth and their vulnerability to infections (Mulokozi, 2003). For children, VAD causes severe visual impairment and blindness, and significantly increases the risk of severe illness, and even death, from common childhood infections such as diarrhoeal disease and measles (FAO/WHO, 2002). For pregnant women, VAD occurs especially during the last trimester when demand by both the unborn child and the mother is highest, the mother’s deficiency is demonstrated by the high prevalence of night blindness during this period (FAO/WHO, 2002).

In Central Africa just as in other parts of the developing world, foods of animal origin that contain vitamin A are rarely eaten on a sustained basis because the majority of people cannot afford them. Thus pVACs from plants (green leafy vegetables, yellow/orange fruits, tubers and vegetables) constitute the main source of vitamin A (Mulokozi, 2003). Although, data on malnutrition levels among banana growing regions

in Central Africa is scanty, according to Sinkiyajako (2006), the majority of the population in Central Africa depends on agriculture for food and income and bananas are among the three most important staples in the region. Despite this, a large number of people are still poor and the level of malnutrition among children below five years is still a problem of public health concern (Sinkiyajako, 2006).

In DRC, malnutrition continues to be a leading cause of morbidity and mortality among children below five years especially in Eastern parts of the country (Luntala, Mbile, Jean Claude, & Okulo, 2000). In 2005 UNICEF reported that DRC had one of the highest under-five mortality rates in the world with more than 200 deaths in every 1000 live births, (UNICEF, 2003). A study by WFP indicated that in DRC acute malnutrition affects 11% of children less than five years old, chronic malnutrition affects about 40% and 30% of the children are underweight. Some of the causes of malnutrition are poor food consumption and inadequate breastfeeding practices (WFP, 2008). According to the $6^{th}$ report on the world nutrition situation, the prevalence of VAD in DRC was 61.1% based on WHO estimates. According to the classification provided by the International vitamin A Consultative Group (known as the Annecy Accords), a prevalence of over 30% of the population with less than 20 µg/dl serum retinol defines a situation of severe VAD (SCN, 2010). Therefore in DRC, VAD is clearly a major public health problem requiring immediate attention.

Reports have indicated that effective, culturally appropriate food-based strategies are essential for sustainable solutions to alleviating VAD (Ayewole-Olusola & Asagbra, 2003). Eastern DRC (north and south Kivu) is the most predominant banana production

region in DRC and banana is one of the main dietary starchy sources, and the most frequently consumed weaning dish. Despite this, food insecurity and under five malnutrition levels are concentrated in this region (WFP, 2008).

### 2.4. Importance of bananas to the people of Africa and Eastern DRC

Bananas (cooking, plantain and dessert), constitute the fourth most important global food commodity (after rice, wheat and maize) (Frisson & Sharrock, 1999). Bananas are grown in more than 100 countries over a harvested area of approximately 10 million hectares, with an annual production of 88 million tonnes (Frisson & Sharrock, 1999). The all year round fruiting habit of bananas puts this crop in a superior position in bridging the 'hunger gap' between harvests. It therefore contributes significantly to food and income security of the people involved in its producing and trade especially in developing countries (IITA, 1998).

The great lakes region covering parts of Uganda, Rwanda, Burundi, Tanzania, Kenya and DRC, is the largest producer and consumer of bananas in Africa (Smale, 2006) where per capita consumption had been estimated at more than 250kg; the highest in the worlds (FAO, ). In DRC bananas production is predominant in the eastern reagion (Luntala, Mbile, Jean Claude, & Okulo, 2000) and they are the second most important source of calories after cassava ((Luntala et al., 2000; Dowiya, Rweyemamu & Maerere, 2009). The production level is as high as 75 000-80 000 tonnes a year and the consumption rate ranges from 136.9 to 173.9kg/person/year (Dowiya et al., 2009). The majority of cultivated bananas and plantains are triploid varieties belonging to the Eumusa section of

the genus *Musa*, family *Musaceae* (Tenkouano, Vuylsteke, & Swennen, 2007). These varieties evolved from intra- and inter-specific crosses involving two diploid ancestor species, *M. acuminata* Colla (genome AA) and *M. balbisiana* Colla (genome BB), which originated from Malaysia and India, respectively (Stover & Simmonds, 1991).

The banana crop is mainly used for food and as a cash crop, banana plantations also contribute to environmental concervation because it grows through out the year (Dowiya, Rweyemamu, & Maerere, 2009). A variety of banana cultivars are grown by households in South Kivu and North Kivu and the selection criteria of preferred cultivars includes flavour/taste, juice production capacity, bunch size and resistance to diseases (Dowiya et al., 2009). Table 2.2 gives a list of some of the banana cultivars values for their high production capacity interms of bunch yield/size.

Over 90% of the bananas produced in the region are consumed locally in many different forms (Frison, Sharrock, Karamura, & Karamura, 1998). Dessert bananas are consumed raw as snacks and desserts. Plantains are fried in various ways and eaten as side dishes and fast foods. East African highland bananas are pounded into thick porridges (“fufu” and “matooke”) (Luntala, Mbile, Jean Claude, & Okulo, 2000).

**Table 2.2.** The most productive and best testing banana cultivars in North Kivu and South Kivu

| Trait | South Kivu | North Kivu |
|---|---|---|
| Most Productive cultivars in order of priority | Nshikazi[1] | Nguma[4] |
| | Barhebesha[2] | Vulambya[1] |
| | Kameramasenge[3] | Tuntu (Tundu)[1] |
| | Kisamunyu[2] | Kitika Sukari kiri3 |
| | Ngoss Michel[3] | Mukingiro[1] |
| | Musheba[4] | Kitoke/Mathoke[2] |
| | Malaya (Kitika)[3] | Kisubi Mangango[1] |
| | Chidege[3] | Kisubi Musa[1] |
| | Yangambi Km 5[1] | Mudjuva[2] |
| | Chisukari/Green red[3] | KaloleII/Kamela[3] |
| Best tasting banana cultivars in order of priority | Kameramasenge[3] | Vulambya[1] |
| | Barhebesha[2] | Pakuma[2] |
| | Nshikazi[1] | Nyaghenge[2] |
| | Kisamunyu[2] | KaloleII/Kamela[3] |
| | Ngoss Michel[3] | Musilongo[4] |
| | Musheba[4] | Nguma[4] |
| | Malaya (Kitika)[3] | Kitika Sukari kiri[3] |
| | Ndundu[1] | Nsirabahima co[2] |
| | Chisukari/Green red[3] | Mudjuva[2] |
| | Sukumba[1] | Kitoke/Mathoke[2] |

1 Beer type, 2 Cooking type-AAA-EA, 3 Dessert banana, 4 Plantain type

## 2.5 General nutrient and provitamin A carotene content in bananas

The nutritional composition of bananas has been elucidated, starch being the predominant carbohydrate in green fruit (Adeniji, Tenkouano, Ezurike, Ariyo, & Vroh-Bi, 2010). According to Rieger (2009), one large banana, is about 9 inches in length, has about 140 calories and 36gms carbohydrates. *Musa* fruits provides some essential minerals and

contribute substantial amount of vitamin C and carotene (pro-vitamin A), which are among the six vitamins included in the daily Recommended Dietary Allowances of the Food and Nutrition Board of the National Research Council (Ogazi, 1996). Bananas are easy to digest and, since they are similar in chemical composition to the mucus of the stomach lining, they have a soothing effect in the treatment of gastric ulcers and diarrhea (Adeniji et al., 2010). The major sugars in bananas are glucose and fructose. Glucose is the most easily digestible sugar, which gets into the bloodstream rapidly and can be utilized for a quick release of energy, while fructose is absorbed more slowly, and thus it provides a more lasting fuel release (Adeniji et al., 2010)

Bananas are famous as a good source of potassium, a mineral involved in proper muscle contraction (Adeniji, Tenkouano, Ezurike, Ariyo, & Vroh-Bi, 2010). An average sized banana contains 475mg of potassium, providing almost 24% of the current recommended dietary allowance (RDA) and 16% of the proposed recommendation of 3000mg (Rieger, 2009).

A few banana cultivars from Hawaii-10, Philippines-9, Cambodia-2 and Uganda-11 were assessed for levels of various micronutrients including; pVACs, iron (Fe) and zinc (Zn). The results revealed that the levels of Zn and Fe were not significant but the levels of total carotenoids and pVACs varied significantly with some varieties having very high contents (Davey, Van den Berge, Markham, Swennen, & Johan, 2009). The mean fruit pVAC levels between the different *Musa* genotypes, were determined by HPLC, and they varied with more than 200-fold from 0.23 - 59.56 μg/gdw (Davey, Van den Berge, Markham, Swennen, & Johan, 2009). The highest levels were found in the plantains and

lower levels in the other cooking bananas and commercial dessert banana types and the nutritional value varied according to the relative proportions of the individual pVACs. The proportion of t-BC varied between 15 and 79% of the total pVAC content. Varieties in which the proportion of t-BC is highest are likely to be the most interesting because t-BC has twice the vitamin A activity content of all-trans α-carotene (t-AC) (Davey, Van den Berge, Markham, Swennen, & Johan, 2009).

However, nutritional assessment of processed banana (banana products) and banana based dishes will give a clear picture of how much of the pVACs is present in the food after processing and how much an individual acquires after consumption of the banana food. This is because storage time and food processing/cooking significantly affects the nutritional quality of foods in addition not all the nutrient present in the food is finally available for use by the body.

### 2.6 Effect of post-maturity development (ripening) on Carotenoid retention and stability

Fruit ripening is a complex process that involves changes in various physiological and biochemical events. These events include chlorophyll breakdown, increased starch degradation and sugar synthesis, and fruit softening (Eng-Chung, Sumana, & Pei, 2003). According to Dadzie and Orchard (1997), the *Musa* fruit undergoes 7 major ripening stages; stage one where banana is full, hard and green skin, at stage two the fruit skin is green but with traces of yellow, at stage three, there is more of yellow than green on the skin indicating that the ripening has begun and the fruit is increasing its natural sugar content. At the forth stage the fruit is yellow some green hints while at stage five the fruit

skin is yellow with green tips and crowns, if it is a dessert variety it can be eaten at this stage. As the fruit gets to stage six, it is completely yellow no traces of green and the fruit starts to soften as it ages heading towards its top sugar content. When the fruit reaches stage seven, it is yellow but with brown/dark sugar spots (Dadzie & Orchard, 1997).

Several studies have confirmed that the nutritional composition of *Musa* pulp is diversely affected by natural ripening (Ahenkora, Kyei, Marfo, & Banful, 1997). In a study on the nutrition changes in '*Apantu pa'* a plantain variety during ripening, it was established that moisture levels increased, protein levels did not change, ash content increased, carbohydrate levels decreased, sugar levels increased and starch content decreased gradually as the fruit advanced in ripening (Adeyemi & Oladigi, 2009). Although carotenoids were not among the micronutrients tested, significant increase in mineral content due to ripening were observed for iron, copper and zinc although the increase were not consistent with the stages of ripening (Adeyemi & Oladigi, 2009). In another study, small increases in potassium, iron, calcium and sodium were found during ripening of plantain while phosphorus levels decreased as ripening progressed and the concentration of copper and magnesium ions remained fairly constant (Welford-Abbey & Omuani, 2006).

Studies on carotenoids retention and changes in *Musa* cultivars indicated that during postharvest ripening, pVACs contents may remain essentially unaltered, increase or even slightly decrease, but this appears to depend on the cultivar in question (Davey, Keulemans, & Swennen, 2006; Ngoh-Newilah, Dhuique-Mayer, Rojas-Gonzalez,

Tomekpe, Fokou, & Etoa, 2009). In 'Popoulou' a cooking banana, grown and consumed in Cameroun, the levels of carotenoids increased during the initial ripening stages with highest levels recorded at ripening stage 3 but there was no further significant change as the fruit progressed from ripe to fully ripe. A different trend was observed in 'Batard' a plantain (also grown and consumed in Cameroon), where the level of carotenoids decreased significantly as the fruit progressed from ripening stage 1 to stage 7 (Ngoh-Newilah, Lusty, Van den Bergh, Akyeampong, Davey, & Tomekpe, 2008).

**2.7 Carotene stability and effects of processing**

Although carotenoids are relatively stable in comparison with other vitamins, because they have a series of conjugated double bond, they are highly susceptible to oxidation which is accelerated by oxygen, light, acids, heavy metals and high temperature (Rodriguez-Amaya, 2001). They are also susceptible to *cis*/*trans* isomerisation catalysed by acid, light and heat (Mulokozi, 2003). In nature, carotenoids exist mostly in the more stable all-*trans* configuration, although *cis*-isomers are known to exist naturally in some fruits and vegetables ((Mulokozi, 2003).

During food processing, the vitamin A activity of the carotenoids may decrease and the colour change due to *cis*/*trans* isomerisation (Sweeney & Marsh, 1971). There is also a risk of oxidation of carotenoids during processing and the storage of the food, which occurs through free radical catalysed processes. Studies conducted to assess the effect of sun drying on total carotenes of green leafy vegetables have reported losses ranging from 58 to 98%. Generally, drying techniques involving heat, light and open air systems can be damaging to carotenoids as a result of oxidation, isomerisation and /or free radical

formation. Thermal processing of green leafy vegetables has been reported in some studies to cause substantial losses of pVACs content. A study by Khachik, Beecher, Goli and Lusby (1992) reported that the levels of α and β-carotenes in green vegetables under mild cooking conditions remain unchanged. However an increase of pVACs content has been reported by Mulokozi (2003). The reported increased content of carotenoids was explained by the heat induced tissue breakdown with a concomitant increase in the accessibility of the carotenes to the extracting solvent. Increased extractability by heat treatment may also be associated with an increased bioavailability of carotenoids from the vegetable matrix (Mulokozi, 2003). A study by Hedren, Diaz and Svanberg, (2002), established that 3% of the total BC content was released from raw carrots in pieces, but when homogenized (pulped) 21% was released. Cooking the pulp increased the accessibility to 27%. Addition of cooking oil to the cooked pulp further increased the released amount to 39%. The trends for AC were similar to those for BC.

The few studies carried out to investigate changes and retention of pVACs during processing of bananas indicate no consistent impact on carotenoid contents attributable to cooking (Englberger, Schierle, Marks, & Fitzgerald, 2003a; Lusty, Akyeampong, Davey, Ngoh-Newilah, & Markham, 2006). On the other hand, cooking, especially under high heat and for a long time, destroys carotenoids, and converts *trans* isomers into *cis* isomers, which have lower vitamin A activity (Booth, Johns, & Kuhnlein, 1992). When six sets of Micronesian banana samples were prepared as raw and cooked samples, five samples were boiled or steamed for 10 min, whereas one sample was baked for 60 min. For four sets, the cooked sample had higher carotenoid content, but for two, the raw sample had higher carotenoid content (Englberger et al., 2003a). For ‘uht en yap’ (the

sample that was baked), the raw sample carotenoid content was more than twice that of the cooked, which may be explained by the longer cooking time.

In a further analysis of the Micronesian banana cutivars, five sets of banana cultivars were tested raw and cooked, a higher content of carotenoid was generally found in the cooked samples (Englberger, Aalbersberg, Ravi, Bonnin, Marks, Fitzgerald & Elymore, 2003b). According to Englberger, et al (2003b), this might be explained by the greater ease with which carotenoids are extracted from cooked samples. However, the water content of the raw and cooked samples was not analyzed and thus exact comparison on a dry-weight basis is not possible. Reports have indicated that steaming has little effect on moisture content of starchy staples while boiling increases moisture. It is therefore likely that the increased carotenoid values observed were not due to concentration effects but rather due to moisture loss during cooking (Englberger, et al., 2003b). Although it is also documented that deep-frying, baking and pickling results in substantial loss of pVACs (Rodriguez-Amaya, 1997), another report suggests that a large percentage of carotenoids are retained in frying plantain (Rojaz-Gonzalez, Avallone, Brat, Trystram, & Bohuon, 2006).

**2.8 Bioaccessibility of Provitamin A Carotenoids**

Bioaccessibility is defined as the fraction of carotenoid transferred during digestion from the food matrix to mixed micelles and thus made accessible for intestinal absorption (Brown, Ferruzi, Nguyen, Cooper, Eldridge, Schwarts, & White, 2004). It is a critical concept in the assessment of the role of vitamin A in human health. Populations at risk of vitamin A deficiency usually rely on dietary pVACs to meet vitamin A needs; this makes

its bioaccessibility a critical concept (Cheryl, Jennifer, Curt, Mack, Shirley, & Schwartz, 1998). A number of factors have been identified that may affect the bioaccessibility of carotenoids from foods, e.g. the matrix in which the carotenoids are incorporated, the content of dietary fat and fiber, the particle size, and the food processing method applied (Hedren, Diaz, & Svanberg, 2002)

Cooking may enhance the carotenoid release by softening or breaking down the cell walls, another way of reducing matrix effects however is by homogenization or particle size reduction. Pureeing vegetables results in smaller particle size and mechanically disrupts plant cells, so that the carotenoids are presumably more available in the intestinal lumen for absorption. Dietary fat, as well, has been reported to have a positive effect on bioccessibility of carotenoids although the amount of fat required to optimize carotene bioaccessibility has not been clearly identified (Brown, Ferruzi, Nguyen, Cooper, Eldridge, Schwarts, & White, 2004). Dietary pectin, which is present in vegetables (such as carrots) along with the carotenoids, has been previously shown to adversely influence carotene absorption. Pectin increases the viscosity of the gastrointestinal contents, which disrupts mixing of these contents and proper micelle formation and thus interferes with carotenoid uptake (Brown, Ferruzi, Nguyen, Cooper, Eldridge, Schwarts, & White, 2004).

Studies on the effect of heat treatment on pVACs bioaccessibility have however been inconsistent. Some studies found a small improvement in t-BC bioaccessibility associated with mild heat treatment of carrot slurries in pre-ruminant calves while others did not observe a difference in the tissue uptake of t-BC and t-AC from heated and non-heated

carrot juice in ferrets. In addition, it was established that heat treatment promotes isomerization of the carotenoids in foods, from *trans* to *cis* isomeric forms, and the degree of isomerization is directly correlated with the intensity and duration of heat processing. In addition, fresh sweet potatoes, carrots, and tomatoes contain negligible quantities of *cis*--carotene, but when canned, the proportion in these vegetables is ~25, 27 and 47%, respectively (Cheryl, Jennifer, Curt, Mack, Shirley, & Schwartz, 1998).

**2.9 Methods to estimate carotenoid bioaccessibility and bioavailability**

Existing methods to estimate carotenoid bioavailability include both short- and long-term studies in humans as well as in appropriate animal models, such as ferret and preruminant calves. The most commonly used method involves measuring increases in plasma concentrations of carotenoids or retinol in humans following administration of an acute or chronic dose of an isolated carotenoid or a carotenoid-containing food. This however is a relative measure, as the plasma is highly dynamic and individual responses have been reported to be highly variable. To obtain a quantitative measure of carotenoid bioavailability, the oral faecal balance method is a better approach (Hedren, Diaz, & Svanberg, 2002). However, studies based on humans or animals are tedious and costly and are not suitable for the screening of a large number of food samples. There is therefore a need for an *in vitro* model that simulates the human digestion tract. Such a method should quantitatively assess the amount of carotenoids that becomes accessible both from different food sources and from foods subjected to different food treatments (Garett, Failla, & Sarama, 1999).

This *in vitro* digestion method allows a rapid estimation of carotene accessibility and bioavailability which may reliably predict *in vivo* behaviour. Recent reports have also documented that bioaccessibility as determined by *in vitro* digestion is highly correlated with data derived by sampling small intestinal luminal contents from human subjects fed carotene rich vegetables and bioavailability data from published human studies ( Failla, Thakkar, & Kim, 2009).

**2.10 Summary/literature gaps**

The total banana production in the Great Lakes region is adequate to feed the regional population that is dependent on it as a staple. Previous analysis has shown that bananas are not only sources of carbohydrates but could be good sources of vitamin A (Davey, Van den Bergh, Markham, Swennen, & Johan, 2009). Despite this, childhood malnutrition, in particular vitamin A deficiencies remain a significant public health problem in banana-dependent regions of Central Africa, especially DRC. In addition, although most of the bananas consumed are either boiled, steamed, roasted, fried or made into flour, the actual processing procedures are barely documented and the effect of post-maturing development/ripening and processing/cooking on the nutrient retention of micronutrients like pVACs in these products is not known. Populations at risk of VAD usually rely on dietary pVACs to meet their vitamin A needs, yet bioaccessibility of these compounds especially in products/dishes derived from banana has not been extensively researched and documented. This research therefore aspired to address these knowledge gaps.

# CHAPTER THREE: METHODOLOGY

## 3.1. Introduction

This chapter describes the design of the study, the variables (independent and dependent), locations at which the study was carried out, the sampling procedures, sample size, and sample preparation. A description of the research instruments, ethical considerations and analysis of the data obtained during the preliminary survey, HPLC analysis and *in vitro* digestion has been given.

## 3.2. Research design

This study adopted a cross-sectional and experimental research design and incorporated both quantitative and qualitative analysis.

## 3.3. Variables

Table 3.1. Independent and dependent variables of the study

| Independent variables | Dependent variables |
|---|---|
| Level/content of fruit pVACs | *Musa* cultivar variation |
| | Post-harvest development (ripening stage) |
| | Procesing/cooking method |
| Bioaccessibility of pVACs | *Musa* cultivar variation |
| | Processing/cooking method |
| | Food combination |

### 3.4. Location of study

The study was carried out in the DRC. Although there are 26 provinces, basing on secondary data on banana production, food insecurity and high malnutrition levels only two provinces North Kivu and South Kivu which both fall on the Eastern part of DRC were selected because of high levels of food insecurity and their high dependency on bananas and plantains (WFP, 2008). From each of these provinces, two territorial districts Beni and Bukavu were randomly selected for inclusion in the survey and from each of the territories three localities randomly selected: Beni (Mabuku, Kisungu Rwakhwa) and Bukavu (Kajeje, Murhesa, Miti) ( map: appendix 3).

### 3.5. Target Population

This study included banana dependent small holder households in North and South Kivu provinces of Eastern DRC. The population of North and South Kivu is 5,100,000 and 4,422,000 respectively, and more than 60% of the households in these provinces depend on banana production for both food and income. Food insecurity in Eastern DRC is characterised by low dietary diversity and low food intake frequency and affects more than 30% of the people (WFP, 2008).

### 3.6. Sampling procedures, sample size and sample preparation

**Objective 1-** Multistage sampling was used to select one territory from each of the provinces and three localities from each of the two territories. Two territories/districts Beni and Bukavu were randomly sampled from North Kivu and South Kivu, respectively. In Beni territory Mabuku, Kisungu and Rwakhwa localities were randomly sampled and

in Bukavu territory, the localities randomly sampled were Kajeje, Murhesa and Miti. A listing of all households with preschool children in each locality was compiled and systematic random sampling was used to select the specific households to be interviewed. Sample size was calculated using Fisher's formula:

$$n = \frac{t^2 \times p(1-p)}{m^2}$$

where n = required sample size, t = confidence level at 95% (standard value of 1.96) p = estimated proportion of households with preschool children with regards to total households, m = margin of error at 5% (standard value of 0.05) (Magnani, 1997).

In South Kivu, the population of the three sampled localities was 41,176, the population of preschool children was 4941 (12%) (Unpublished reports). In North kivu the population of the three samples localities was 48 612 while that of preschool children was 7877 (16.2%) (Unpublished reports), thus the sample size was:

$$N = \frac{1.962\ (0.12 \times 0.88)}{0.052}$$ N=163 Households with preschool children

$$N = \frac{1.962\ (0.162 \times 0.838)}{0.052}$$ N= 208 Households with preschool children

Focus group discussions were carried out at the locality level, to obtain information on the most popular and preferred *Musa* cultivars and the general processing (e.g., cooking)

methods. Through the support of local agronomists and administration leaders, participants who included local farmers and women with children below five years were drawn from the randomly samples localities and called to a common ground (local primary schools).

**Objective 2** – Following the preliminary survey, the four most popular *Musa* cultivars in Eastern DRC were identified using the botanical characteristics described in Table 3.2, Mature disease-free plants of these *Musa* cultivars were then identified in farmer's fields and marked by qualified agronomists from Beni (NK) and Bukavu (SK) territories. When the fruits of the bunch were mature (deep green skin, full and rounded fingers) according to Dadzie and Orchard (1997), the bunch was harvested and two middle hands ($2^{nd}$ and $3^{rd}$ hand) each containing between 12-16 fingers, were sampled.

Table. 3.2. Description and botanic characteristics used in identification of the popular cultivars from Beni territory (North Kivu) and Bukavu territory (South Kivu), Eastern DRC.

| Local name | Origin | Synonyms | Genomic group | Sub-group | Botanic characteristic used to identify the cultivar | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Pseudostem | leaves | Bunch | Fruit | Male bud |
| Vulambya | Beni (DRC) | Malambya[2], Bulambya[2], Nyalambya[2], Nyalambi[2] (Rukonjo (Uganda) [3] | AAA-EA[2,] | Lujugira-Mutika[2] | ≥ 3metres light green with black blotches[2] | Dirty green[2] | Hangs vertically, Compact, cylindrical, maximum 11 hands[2] | Straight, Medium (15cm-20cm long), pointed tips, maximum 18 fruits in 3rd hand, naked tips[2] | Ovoid shaped, at harvest about 23cm width and 36cm length, white male flower[2] |
| Musilongo | Beni (DRC) | Kasilongo[2], Munzabo[2] Kibeda | AAB[2, 4] | Plantain (French horn) [2] | ≥ 3 metres Red-purple[2] | Medium green[2], | Hangs vertically, Lax, assymetrical, maximum number of hands 9[2] | Sharp curved long fruit, pointed tip about 14 fruits in 3rd hand, persistent floral remains on fruit tips[2] | Lanceolate shaped, yellow male flower[2] |
| Nguma | Beni (DRC) | Mbaguma[2] Apakumo[2] Asongbe[2] | AAB[24,] | Plantain (French horn) [2] | ≥ 3 metres Light Green almost yellowish[2] | Medium green[2] | Hangs vertically, lax, slightly truncated cone shape | Fruits almost straight and a bit inflated. Slightly pointed tips with persistent old style[2] | Ovoid , large, red purple and persistent, yellow male flower[2] |
| Kiware | Beni (DRC) | Ndabaware[2], Maware[2] Ndyabawali[2] Ngagara (Ug)[3] Nandigobe (Ug) [3] | AAA-EA[2,] | Lujugira-Mutika[2] | ≥ 3 metres light green with black blotches[2] | Dirty green[2] | Hanging vertically, cylindrical, lax. Rachis with semi persistent floral parts, 10 hands | Fruits slender and angular slightly above 20cm, with persitent style on the fruit tip, 18 fruits in 3rd hand[2] | Intermediate Purple and pointed, yellow make flower[2] |
| Nshikazi | Bukavu (DRC) | 'Magizi'= "bitter" [3] Ishika'(Rwanda), [3] Ensika',(Ug) [3] 'Emburansika' Omuburasika'(Ug[3] | AAA-EA[4] | Lujugira-Mutika | ≥ 3 metres light green with black blotches[2] | Dirty green[2] | Oblique and compact cylindrical bunch[2] | Fruits medium and pulp astringent[2] | Ovoid shaped, at harvest about 23cm width and 36cm length, white male flower[2] |
| Musheba | Bukavu (DRC) | Busheba[3], misheba[3], | AAB[4] | Plantain (French) | ≥ 3 metres Light green, almost yellowish[2] | Medium green[2] | Hangs vertically, lax, slightly truncated cone shape[2] | Fruits almost straight and a bit inflated. Slightly pointed tips with persistent old style[2] | Male bud large, red purple and persistent, yellow male flower[2] |
| Barhebesha | Bukavu (DRC) | Njakara, Incakara, Barabesha[3], Mutant of mudjuva | AAA-EA[4] | Lujugira-Mutika[2] | ≥ 3 metres Green with black blotches[2] | Dirty green[2] | Hangs vertically, lax, almost cylindrical[2], | Fruits > 25cm, almost curved towards the rachis, bottle necked tips[2] | Intermediate, Purple, pointed, white male flower[2] |
| Kamera | Bukavu (DRC) | Kamaramasenge, Kilore[2], | AAB[2,4] | Kamaram asenge[2] | Green yellow with bron rusty pigmentation | Medium green[2] | Asymmetrical-bunch axis nearly straight, compact,, with bare rachis[2] | Short 15cm, almost perpendicular to rachis[2]. | Purplish blue, imbricated and pointed[2] |

[1]=Dowiya, Rweyemamu & Maerere, (2009); [2]= Universite Catholique de Graben (UCG) Butembo colection; [3]=Unpublished reports/personal communication; [4]=Simmonds & Shepherd, (1995).

The samples from Beni territory (North Kivu) were packed in an aerated carton box and transported as hand luggage by air to Kampala the morning of the harvest, while the samples from Bukavu were also packed in an aerated carton box and transported as hand luggage by an overnight bus to Kampala. Samples from both sites arrived at the laboratory in Kampala within 48 hours of harvest. The ripening stages were estimated based on the peel colour as described by Dadzie and Orchard (1997). These were as follows: 1= completely green: 2= green with hints of yellow: 3= more green than yellow: 4= more yellow than green: 5= only green tips remaining: 6= all yellow; 7=yellow flecked with brown (Dadzie & Orchard, 1997). Triplicate samples of each of the *Musa* cultivar were picked at ripening stages 1, 3, 5 and 7. Giving at least 12 samples from each cultivar (Figure 3.1).

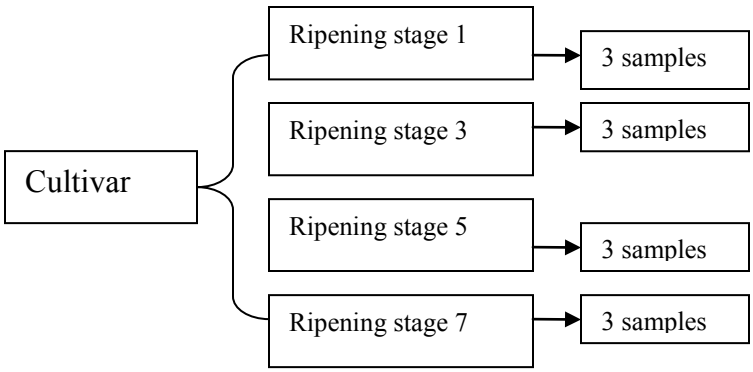


Figure 3.1. Samples for retention during ripening of *Musa* fruit

The samples were lyophilised (freeze dried) for 72 hours, repackaged in their labelled zip-closure plastic bags, air removed and all the samples put in a dark coloured carton box, transported for pVACs analysis to the Laboratory of Fruit Breeding and

Biotechnology, Department of Biosystems at the Catholic University of Leuven, Belgium.

**Objective 3**. Following the preliminary survey and after identification of the four most popular Musa cultivars, the *Musa* fruits were transported to the laboratory in Kampala. After obtaining necessary ingredients from local markets, the most common *Musa* products/dishes were processed/cooked following procedures described in Table 3.3**.** The cooking bananas and plantains were processed at ripening stages 3 and 5 respectively. They were then cooled to under room temperature and about 100g placed in labelled zip-closure plastic bags, the air removed manually and all bags stored in a freezer at $-20^0$c until all the products were made (within 2 days). The samples were then lyophilised for 72 hours, repackaged in their labelled zip-closure plastic bags, air removed manually and all the samples put in a dark coloured carton box and sentto the Laboratory of Fruit Breeding and Biotechnology, Department of Biosystems at the Catholic University of Leuven, Belgium for pVACs analysis.

Table 3. 3. Ingredients used and procedures followed in preparation and processing/cooking of the *Musa* products

| | Musa product | Ingredients | Cooking procedure | Comments |
|---|---|---|---|---|
| 1. | Boiled Musilongo/ Musheba in-peel | 3 fingers of plantain (Musilongo / Musheba with peel) 480g 300ml water | Musilongo/Musheba fingers were cleaned, weighed and places in an aluminium sauce pan, about 300ml water was added to submerge the plantain fingers. These were boiled covered at medium heat for about 20 minutes. The remaining water was drained; the food was cooled with the lid on. The boiled fruits were then hand peeled, weighed and placed in labelled zip-locked plastic bags with air removed manually and stored at -20$^0$c. | The two varieties were cooked in separate saucepans |
| 2. | Boiled Musilongo/ Musheba without peel | 3 fingers of hand peeled plantain (Musilongo/Musheba) 340g 300ml water | Musilongo/Musheba fingers were cleaned, hand peeled, weighed and places in an aluminium sauce pan, and about 300 ml water was added to just submerge the fruits. These were boiled covered at medium heat for about 15 minutes. When cooked the remaining water was drained, the food cooled with the lid on. The fingers were then re-weighed, placed in labelled zip-lock plastic bags with air removed manually and stored at -20$^0$c. | The two varieties were cooked in separate saucepans |
| 3. | Musilongo flour | Unripe Musilongo fingers | Mature unripe Musilongo fingers were peeled using a knife, put in polythene bags which were closed tightly and left to ferment for 3 days. The mould was scrapped off the finger using a knife and the bananas were put under the sun for 1-2 days to dry. Once dry, they were crushed into small pieces and ground into flour using a local grinding mill. | |
| 4. | Musheba flour | Unripe Musheba fingers | Mature unripe Musheba fingers were peeled using a knife, chopped into uneven pieces about 3 inches and put onto of a laid out polythene bag under the sun to dry (about 2 days). The dry pieces were crashed further and ground into flour using a local grinding mill. | |
| 5. | Boiled Vulambya/Nshikazi in-peel | 3 fingers of AAA-EAHB (Vulambya/Nshikazi) with peel 360g 300ml water | The Vulambya/Nshikazi fingers were cleaned weighed and put in an aluminium sauce pan, about 300ml water was added to just submerge the bananas and covered with a tight lid and let to boil for 15 minutes under medium heat. When cooked, the remaining water was drained, the bananas were let to cool with the lead still on, after cooling, they were weighed hand peeled, re-weighed and put in labelled zip-lock plastic bags with air removed manually and stored in dark room at 20$^0$c. | Each of the AAA-EAHB cultivars was boiled in a separate sauce pan |
| 6. | Boiled Vulambya/Nshikazi without peel | 3 fingers of AAA-EAHB (peeled Vulambya) 200g 200ml water, Pinch of salt | The Vulambya/Nshikazi fingers were carefully peeled with a knife, cleaned, weighed and put in an aluminium sauce pan, 200ml water was added, a pinch of salt added and covered with a tight lid and let to boil for 15 minutes under medium heat. Excess water was drained, the bananas let to cool with the lead still on, re-weighed and put in labelled zip-lock plastic bags with air removed manually and stored at 20$^0$c. | Each of the AAA-EAHB cultivars was boiled in a separate sauce pan |
| 7.. | Musilongo/Musheba deep fried in local palm oil & Musilongo/Musheba deep fried in cooking oil (Golden fry) | 3 fingers of each plantains cultivar (stage 5) Local palm oil/cooking oil (Golden fry) Salt | The Plantains (Musilongo/Musheba) were cut off at both ends and hand peeled. They were then sliced into ¼ - ½ inch rounds, and weighed. In a large, heavy saucepan, the local palm oil/cooking oil was heated over medium heat keeping a close watch on it. Once the oil sizzled when a slice of plantain was added, several plantain slices were added and browned for about 2 minutes on each side. They were then transferred into a bowl to drain the excess oil and cool. After cooling, they were re-weighed and put in labelled zip-lock plastic bags with air removed manually and stored at 20$^0$c. | Each variety of the plantain was deep fried separately and new oil was used. Weighing is not normally done by community members |

**Objective 4.** A sub-sample of the most popular *Musa* cultivars in Eastern DRC, which included only samples from North Kivu were selected. Mature disease-free plants of the selected cultivars 'Musilongo' (Plantain-AAB) and 'Vulambya (AAA-EAHB) were identified at farmer fields and marked by qualified agronomists from Beni territory (NK). When the fruits of the bunch were mature: deep green, full and rounded (Dadzie & Orchard, 1997), the bunch was harvested and two middle hands ($2^{nd}$ and $3^{rd}$ hand) each with between 12-16 fingers sampled. The *Musa* fruit samples were packed and transported just as described in objective 2 and 3. After obtaining other ingredients, all ingredients were taken to the CIRAD-laboratory in Montpellier. At the laboratory, the four common products/dishes from the AAA-EAHB cultivar 'Vulambya' were prepared/cooked when the fruit was at ripening stage 3 (green skin with yellow highlights). The 2 popular products from the plantain were prepared at ripening stage 5 (fully ripe). All the products and dishes were prepared following the procedures described by community members and documented in Table 3.4a and 3.4b. The products were then refrigerated at $-20^{0}$C before HPLC analysis and *in vitro* digestion.

Table 3.4a. Ingredients and procedures used in preparation of the Musa dishes from 'Musilongo' for bioaccessibility studies

| | ***Musa* Product** | **Ingredients** | **Cooking procedure** |
|---|---|---|---|
| T1 | Musilongo boiled in peel | 3 fingers of Musilongo, unpeeled 480 g<br><br>300 ml water | The fingers were cleaned, weighed and placed in an aluminum sauce pan. About 300 ml water was added to just submerge the fruits. These were boiled, covered, at medium heat for about 15 minutes. The remaining water was drained, and the fruits were cooled with the lid on. The boiled fruits were then hand peeled, weighed and placed in labeled zip-locked plastic bags with air removed manually and stored in a dark room at -20$^0$C. |
| T2 | Musilongo boiled without peel | 3 fingers of Musilongo, hand peeled 340 g<br><br>300 ml water | Idem[1] as T1, but fruits were hand-peeled after cleaning and before weighing. |
| T3 | Musilongo boiled without peel with olive oil | 3 fingers of Musilongo, hand peeled 340 g<br><br>300 ml water, 1 table spoon (10 g = 2.9%) of olive oil | Idem as T2, but 1 table spoon of olive oil was added during boiling. |
| T4 | Musilongo boiled without peel with palm oil | 3 fingers of Musilongo, hand peeled 340 g<br><br>300 ml water, 1 table spoon (10 g = 2.9%) of palm oil | Idem as T2, but 1 table spoon of palm oil was added during boiling. |
| T5 | Musilongo flour | Unripe Musilongo fingers | Mature unripe Musilongo fingers were peeled using a knife, put in polythene bags which were closed tightly and left to ferment for 3 days. The mould was scrapped off the finger using a knife and the bananas were put under the sun for 1-2 days to dry. Once dry, they were crushed into small pieces and ground into flour using a local grinding mill.<br><br>Note: This is the flour used in making the porridge (T6) |

Idem= the same as

Table 3.4b. Ingredients and procedures used in preparation of the *Musa* dishes from 'Vulambya' for bioaccessibility studies

| | ***Musa* Product** | **Ingredients** | **Cooking procedure** |
|---|---|---|---|
| T7 | Vulambya boiled in peel | 3 fingers of Vulambya, unpeeled 360 g<br>300 ml water | Idem as T1 (table3.3a) |
| T8 | Vulambya boiled without peel | 3 fingers Vulambya, peeled 200 g<br>200 ml water, Pinch of salt | Idem as T1 (table 3.3a), but fruits were carefully peeled with a knife after cleaning and before weighing. A pinch of salt was added to the water. |
| T9 | Vulambya boiled with beans | 3 fingers of Vulambya, hand peeled 200g<br>100 g boiled fresh beans, Pinch of salt<br>500 ml water | The fresh beans were sorted and put to boil. When they were soft (cooked), peeled and cleaned banana fingers were added to the beans, and 500 ml of water added and salt to taste. The mixture was covered and let to cook for about 15 minutes under medium heat until the bananas were cooked. After cooking, the dish was let to cool and put in labeled zip-lock bags with air removed manually and stored in a dark rook -$20^0$c awaiting manipulation. |
| T10 | Vulambya boiled with beans and olive oil | 3 fingers of Vulambya, hand peeled 200g<br>100 g boiled fresh beans, Pinch of salt<br>500 ml water, 1 table spoon (10 g = 3.3%) of olive oil | Idem as T9, but 1 table spoon of olive oil was added to the mixture when both the bananas and beans were cooked; the mixture was stirred a little and left to cook for about 2 more minutes. |
| T11 | Vulambya boiled with beans and palm oil | 3 fingers of Vulambya, hand peeled 200g<br>100 g boiled fresh beans, Pinch of salt<br>500 ml water, 1 table spoon (10 g = 3.3%) of palm oil | Idem as T9, but 1 table spoon of palm oil was added to the mixture when both the bananas and beans were cooked; the mixture was stirred a little and left to cook for about 2 more minutes. |
| T12 | Vulambya boiled with beans and green vegetables | 3 fingers of Vulambya, hand peeled 200 g<br>100 g fresh beans, 60 g sorted and cleaned amaranth leaves, 600 ml water | The fresh beans were sorted and put to boil, when they were soft (cooked), peeled and cleaned banana fingers were added in the beans, and 500ml of water added and salt to taste. The mixture was covered and let to cook for about 12 minutes under medium heat. Amaranth leave were added after the 12 minutes, the mixture was mixed a little bit and let to cook for 5 more minutes before being left to cool with lid on. The dish was put in labeled zip-lock bags with air removed manually and stored in a dark rook -$20^0$c awaiting manipulation |
| T13 | Vulambya boiled with beans and green vegetables and olive oil | 3 fingers of Vulambya, hand peeled 200 g<br>100 g fresh beans, 60 g sorted and cleaned amaranth leaves, 600 ml water<br>2 table spoons (20 g = 2.8%) of olive oil | Idem[2] as T12, but 2 table spoons of olive oil were added were added to the bananas, beans and amaranths, and mixed in well just 2 minutes before the dish was removed from the heat. |

Idem= the same as

### 3.7. Research instruments and procedures

**Focus Group Discussions (FGDs) checklist;** this is a tool with general questions addressing general, agricultural nutrition and health issues affecting the community. It was used to collect information on cultivar preference, popularity, common banana products and also obtain a community food list that was used during household interviews. The participants included local farmers and caregivers (women with children below five). Through community leaders the participants were called to a common ground and divided according to gender with each group having between 10-15 participants and the FGDs conducted in the local language with the guide of a research assistant.

**Household structured questionnaires;** this tool contained questions targeting; household food production, food processing/preparation/cooking and dietary patterns. In addition nutrition status (weight and height taken using standardised tools and procedures) and health status of preschool children in the sampled households was assessed. The respondents were caregivers of the child or any other person responsible for food provision and preparation at household level. The enumerator filled-in the questionnaire during the interview

**Information sheet for retention and HPLC machine;** A data sheet for each sample was prepared to facilitate the recording of all information on the sample and the results obtained for both the retention and the HPLC machine used to establish the levels of pVACs in the samples

**Information sheet for *in vitro* bioaccessibility**; The information sheet was used to fill in the results obtained after in vitro digestion and after HPLC analysis of the undigested and digested sample to be able to determine bioaccessibility of pVACs.

### 3.8 Pilot study

The household questionnaire Appendix II was administered to about 10% of the sample group. The households included in the pre-test were from 2 villages from Beni territory and Bukavu territories respectively that had all the qualities necessary for inclusion in the survey but were not sampled. The purpose of this was to test the reliability and validity of the tools as well as test response rate. Complete data was obtained from 87% of the pilot study sample. The tool was then modified accordingly.

For the pVACs HPLC analysis and *in vitro* bioaccessibility, triplicate samples of each of the samples were obtained and independently tested to measure the validity and reliability of the equipment used.

### 3.9 Ethical Considerations

Permission to carry out the research was sought from the administration office within the regions, the local administration and leaders were informed and briefed on the objectives, procedures and the requirements of the research. The identified household- heads were briefed on the research procedures and assured of confidentiality and verbal consent obtained.

For retention and bioaccessibility sample collection, permission was obtained to collect the relevant *Musa* samples from the farmers' fields. All legal procedures to enable inter-country and inter-continental transportation of samples were adhered to.

### 3.10. Data analysis and sample analysis

#### 3.10.1 Data analysis

Data obtained from the focus group discussions was first reviewed, summarised according to set codes, interpreted and results presented in tables. Data from household interviews was analysed using the statistical package for social sciences (SPSS) version 17. The quantitative data such as, most popular *Musa* cultivars, most popular *Musa* products, number of food groups, variety of food items consumed and prevalence of childhood infections was organised, described and summarised using frequency tables, charts and graphs. Dietary diversity data was further analysed in accordance with the guidelines set by FAO (2007).

Epi Info programme was used to compute nutrition indices weight, height and age into Z-scores and the results classified according to WHO (2006). Regression analysis with an $r^2$ threshhold of 0.045 was further carried out to establish the the relationship between dietary diversity, nutrition status and prevalence of infections.

### 3.10.2 Extraction and pVACs analysis for retention during ripening and local processing (KU Leuven laboratory- Belgium)

#### 3.10.2.1. Buffers and solvents

HPLC-grade tetrahydrofuran (THF) and *tert*-butyl methyl ether (t-BME) were obtained from Sigma Aldrich, (Bornem, Belgium), HPLC-grade methanol (MeOH) from LabScan (Dublin, Ireland). Lutein, all-*trans* ß-carotene (t-BC), all-*trans* ß-8-apocarotenal (apocarotenal), butylated hydroxytoluene (BHT), triethylamine and insoluble polyvinylpolypyrolidine (PVPP) were all obtained from Sigma-Aldrich.

#### 3.10.2.2. Extraction method

All extractions were carried out in triplicate according to procedures specifically developed for the analysis of *Musa* tissues (Davey, Van den Berge, Markham, Swennen, & Johan, 2009; Davey, Keulemans, & Swennen, 2006). In brief, around 100 mg aliquots of powdered lyophilised *Musa* fruit pulp were homogenised for 30 seconds at maximum speed with 3-5 glass beads in a 'FastPrep' reciprocal shaker in 400µl of ice-cooled extraction buffer. The extraction buffer consists of THF:MeOH, 1:1 (v/v), containing 0.25% BHT and 2% insoluble PVPP Following centrifugation (14,000 rpm for 20 min at 4 ˚C), the pellet was re-extracted twice more with 400µl of THF:MeOH, 1:1 (v/v), containing 0.25% BHT without PVPP. After each extraction step, the supernatant was collected and combined. Retention times relative to known standards were available (Davey et al., 2006; Azevedo-Meleiro & Rodriguez-Amaya, 2004; Howe &

Tanymihardjo, 2006). Apocarotenal at a final concentration of 0.002 µg/ml was added as an internal standard to correct for differences in extraction volumes.

### 3.10.2.3. Carotenoid analysis

The combined supernatants were directly analysed by RP-HPLC using a Waters Alliance, 2690 Separations System fitted with an autosampler, thermostated at 8˚C, a pulse dampener and a 996 UV-Vis photodiode array detector (Waters, Massachusetts, USA) (Davey, Van den Berge, Markham, Swennen, & Johan, 2009; Davey, Keulemans, & Swennen, 2006). The entire system was controlled and the data were collected and integrated using the Millennium 4.0 software package. Extracts were resolved on a 150 x 4.6 mm, YMC $C_{30}$ 3-µm particle size HPLC column (Achrom, Zulte, Belgium), using a 24 minute linear gradient of 2-50 % t-BME in MeOH at 1.0 ml/min and regenerated with 95% t-BME in MeOH. All buffers contained 0.05 % BHT and 0.05% triethylamine. Peaks were quantified at 450 nm using a freshly-prepared standard curve of t-BC in extraction buffer and identified on the basis of their characteristic absorption spectra. 8-*Apo β*-carotenal at a final concentration of 0.002µg/ml was added as an internal standard to correct for differences in extraction volumes.

These results were based on lyophilised dry matter. Therefore, to obtain the equivalent fresh-matter approximate values in the raw samples, the formulae [fresh matter value= value of dry matter/ (100/100-moisture %)] was used. The same formula was used for the processed samples although moisture content took into account the water absorbed during

boiling and the moisture lost during deep frying. Results of the flours were presented per unit dry matter.

### 3.10.2.4. Assessment of carotenoids contents and impact on vitamin A requirements

The relative vitamin-A activity of 13 *cis*- β-carotene (c-BC) has been estimated to be 53% of t-BC (Schieber & Carle, 2005), while that of trans ά-carotene (t-AC) is only 50% of the activity of t-BC (Fraser and Bramley 2004; Trumbo, Yates, Schlicker-Renfro, & Suitor, 2003). Using the formula total -BC equivalent = 0.5 t-AC + t-BC + 0.53 c-BC', the mean total pVAC values derived from results of three independent HPLC analyses at each of the four ripening stages presented in nmol/gdw were converted into all *trans* β-carotene equivalents (t-BCE). These values were then converted into 'Retinal Activity Equivalents' (RAE) assuming that $1/12^{th}$ of the total t-BCEs ingested are taken up into the body (Yeum & Russell, 2002). The RAE in µg/100gFM values were compared to the daily recommended dietary allowances (RDAs) for vitamin A in population groups particularly vulnerable to vitamin A deficiency (VAD) who according to McLaren and Frigg (2001) include children between 1-5years and non-pregnant, non-lactating women whose RDAs are 400µg/day and 700µg/day respectively (FAO/WHO, 2002). This was to establish the potential contribution of the *Musa* fruit to the Vitamin A RDAs within normal consumption patterns.

### 3.10.3. *In vitro* pVACs bioaccessibility study (CIRAD- Montpellier France)

#### 3.10.3.1. Buffers, solvents and other chemicals

Extraction solvents were RPE-grade hexane, ethanol and dichloromethane from Carlo-Erba (Val de Reuil, France). Analytical solvents were HPLC-grade methanol, acetonitrile and tetrahydrofuran (THF) also from Carlo-Erba (Val de Reuil, France), and methyl-tert-butyl-ether (MTBE) from Sigma-ALDRICH (Steinheim, Germany). Carotenoid standards (98% pure) used for HPLC analysis were purchased from Extrasynthese (Genay, France): b-carotene, alpha+beta-carotene mixture, lutein and b-apo-8-carotenal. Dulbecco's modified Eagle's medium (DMEM) containing 4.5g/l glucose and trypsin-EDTA (500 and 200mg/l, respectively) was purchased from Bio Whittaker (Fontennay-sous-Bois, France), Fetal bovine serum (FBS) was purchased from Biomedia (Lssy-les-Moulineaus, France), and NEAA and penicillin/streptomycin were from Gibco BRL (Cergy-Pentoise, France). Pepsin, porcine pancreatin, porcine bile extract and pyrogallol were purchased from Sigma-Aldrich (St Quentin Fallavier, France).

#### 3.10.3.2. Carotenoids extraction and HPLC analysis of *Musa*-based products and dishes

Carotenoid extraction was carried out as according to a previous study (Dhuique-Mayer, Tbatou, Carail, Caris-Veyrat, Dornier, & Amiot, 2007a). Samples of boiled bananas, dishes, or bananas flour (0.5-2g) were extracted with ethanol/hexane (4:3, v/v). $\beta$-Apo-8'-carotenal was added as an internal standard. Carotenoid extracts were dissolved in 500 µl of dichloromethane and 500 µl of an 80:20 (v/v) mixture of MTBE and methanol before

HPLC analysis. Carotenoids were analyzed by reverse-phase HPLC using an Agilent 1100 system (Massy, France) Carotenoids were separated using a $C_{30}$ column (250 x 4.6 mm i.d., 5 µm YMC (EUROP GMBH, Germany), mobiles phases were $H_2O$ as eluent A, methanol as eluent B, and MTBE as eluent C. Flow rate was fixed at 1mL/min$^{-1}$, column temperature was set at 25 °C, and injection volume was 20 $\mu$L. The following gradient program was performed: initial condition was: 0-5 min, 40% A, 60% B; 5-10 min, 20% A, 80% B; 10-60 min, 4% A, 81% B, 15% C; 60-71 min, 4% A, 11% B, 85% C; 71-72 min 100% B, and back to the initial condition for reequilibration. Absorbance was followed using an Agilent 1100 photodiode array detector. Quantification of carotenoids was achieved using calibration curves with β-carotene at 450nm. Quantification of carotenoids was achieved using calibration curves with β-carotene, lutein with five concentration levels. Correlation coefficients ranged from 0.994 to 0.998.

**3.10.3.3. *In vitro* digestion**

The *in vitro* digestion model was based on previous studies of Reboul, Richelle, Perrot Desmoulins-Malezet, Pirisi and Borel, (2006) and Dhuique-Mayer, Borel, Reboul, Caporiccio, Besancon and Amiot, (2007b) with modifications (an "oral" phase for *Musa* samples was added in order to be closer to physiological digestion). Triplicate samples (5g) of boiled *Musa* were subjected to simulated oral, gastric and small intestinal phases of digestion. Briefly, samples were mixed with a saliva solution (6 ml) prepared by dissolving in 100 ml of ultrapure water 0.5208 g of $NaHCO_3$ (99.5%), 0.0878 g of NaCl (99.5%), 0.0478 g of KCl (99.5%), 0.044 g of $CaCl_2.H_2O$ (97%), 0.1044 g of $K_2HPO_4$

mucin (0.216g) and porcin α-amylase (200 units/ml) that was closed to physiologic conditions the pH was adjusted to 7.0 and the mixture was incubated for 10 min at 37°C in a shaking water bath.. Then, samples (5g for boiled bananas and dishes/products and 10g for porridge) were mixed in saline solution (NaCl 0.9 %) and homogenized for 10 min at 37°C in a shaking water bath.

To mimic the gastric step, the pH was adjusted to 4.00 ± 0.02 with 1 M NaOH, then 2 ml porcine pepsin (40 mg/ml in 0.1 M HCl) were added. The homogenate was incubated at 37°C in a shaking water bath for 30 min. To mimic the intestinal step, the pH of the partially digested mixture was raised to 6.00 ± 0.02 by adding around 20 ml of 0.45 M sodium bicarbonate pH 6.0. Then a mixture of porcine bile extract and pancreatin (9 ml containing 2 mg/ml pancreatin and 12 mg/ml bile extract in 100 mmol/l tri sodium citrate, pH 6.0) and 4 ml bile extract at 0.1g/ml were added. Samples were incubated in a shaking water bath at 37°C for 30 min to complete the digestion process. Micelles were separated by centrifugation (20,000 rpm for 4 h at 10°C using a Beckman JA 21 rotor). The aqueous fraction was collected and then filtered through a 0.22 $\mu$m filter (Millipore). Aliquots were stored at –20°C under nitrogen until analysis.

**3.10.3.4. Carotenoid Extraction from digested samples**

Carotenoid extraction from the final digested samples was carried out as described previously (Dhuique-Mayer, Borel, Reboul, Caporiccio, Besancon, & Amiot, 2007b). An aliquot of micellar aqueous fraction from digested sample (10 ml) was extracted 3 times with 10 ml of hexane and 5 ml of ethanol containing 100 µl of beta-apo-8'-carotenal as

recovery standard. The pooled hexane extracts were evaporated and redissolved in 500 µl of the HPLC mobile phase (250 µL of dichloromethane and 250 µL of an 80:20 (v/v) mixture methyl-*tert*-butyl-ether (MTBE) and methanol). Samples were then injected according to analytical conditions as described above.

### 3.11. Statistical analysis of all the results

Differences in mean values of provitamin A carotenoids observed following the different ripening stages, processing methods and *in vitro* bioaccessibility were tested using analysis of variance (ANOVA) and determination of the significance of difference among samples using *p*-values obtained by post hoc tests of homogenouse subsets, Duncan's Multiple Range Test (DMRT). Results were summarized using tables and graphs.

# CHAPTER FOUR: RESULTS AND DISCUSSION

## 4.1 Introduction

Bananas (*Musa* spp.) serve as important food crops in much of Africa. Together they provide over 25% of the carbohydrate needs and 10% of the daily calorie requirements for over 70 million people on the African continent (HarvestPlus, 2007). Production of the bananas is concentrated in Eastern DRC and ranges between 75,000 and 80,000 tones/year and they rank second in importance after cassava in the Democratic Republic of Congo (DRC) (Luntala, Mbile, Jean Claude, & Okulo, 2000). More than 80% of the population in Eastern DRC consume bananas with a frequency of more than 10 times in a week (Ouma et al., In Press).

Apart from being the main dietary starch sources (Luntala, Mbile, Jean Claude, & Okulo, 2000), studies have suggested that some banana cultivars have high pro-vitamin A carotenoids (pVACs) levels and are capable of providing up to half of daily recommended dietary allowances (RDAs) of vitamin A in a single fruit (Davey, Keulemans, & Swennen, 2006; Davey, Van den Berge, Markham, Swennen, & Johan, 2009). Despite this the 6$^{th}$ report on the world nutrition situation indicates that an estimated 163 million children in developing countries are vitamin A deficient (as defined by a serum retinol content of < 20 µg/dl serum retinol), and based on WHO estimates over the period 1995-2005, the prevalence of VAD in DR Congo was 61.1% while according to the United Nations Sub-Committee on Nutrition (UNSCN) estimates of 2007, the prevalence was 42.2%. According to the classification provided by the

International vitamin A Consultative Group/Micronutrient Forum (known as the Annecy Accords), a >30% prevalence of people with < 20 μg/dl serum retinol defines a situation of severe VAD (SCN, 2010). Therefore in DRC, VAD is clearly a major public health problem requiring attention.

Despite the major role that bananas play in Eastern DRC, there has been limited research and hardly any documentation on the preferences for the various banana cultivars grown, their processing/cooking techniques and consumption patterns among small-holder farmer households. In addition, reports have indicated that effective, culturally appropriate food-based strategies are essential for sustainable solutions to alleviating vitamin A deficiency (VAD) (Ayewolu-Olusola & Asagbra, 2003). Most *Musa* cultivars are stored for some time before processing or cooking and processed into various forms before consumption. The changes in pVACs content during fruit ripening appear to be cultivar-specific, and pVACs contents may remain essentially unaltered, increase or even slightly decrease depending on the genotype (Davey, Keulemans, & Swennen, 2006; Ngoh-Newalah, Dhuique-Mayer, Rojas-Gonzalez, Tomekpe, Fokou, & Etoa, 2009). Studies from several other fruits and starchy staples have confirmed that heat processing has an effect on carotenoids and that even after consumption, the release of carotenoids from food matrix during digestion (bioaccessibility) is determined by the extent to which the cell wall is degraded during processing (Tumuhimbise, Namutebi, & Muyonga, 2009) and it is a critical step in the process of establishing the vitamin A activity of a particular food product.

This chapter first gives an overview of the social cultural/economic factors of the small-holder households dependent on banana in Eastern DRC. The results of the study are then presented in accordance to set objectives.

## 4.2. Social-cultural/economic factors of the *Musa*-dependent communities of Eastern DRC

Social-cultural/economic factors thought to influence the consumption and utilization of *Musa* fruit and products in this region included; age of respondents, marital status, education level, household size, number of children below five years in the households, prevalence of under five mortality, household main source of income and household average monthly income.

### 4.2.1. Gender, marital status and age distribution of respondents

A total of 371 small holder households with preschool children from Beni territory (163) and Bukavu territory (208) were visited and interviewed. FAO studies confirm that rural women make a tremendous contribution to food and agricultural production. They also play a crucial role in determining and guaranteeing food security and well-being for the entire household (FAO, 2003). It was therefore necessary that questions requiring details on household consumption patterns were addressed to women (caregivers). This explains the high percentage of women respondents (91.7%) observed. The majority of the women interviewed were between 25 - 35 years old, with 20% of them between the ages of 15 and 24 years (Figure 4.1), indicating that the women get married or become caregivers at

a very early age. In DRC, the legal minimum age for marriage is 15 for women and 18 for men and 74% of women between 15 and 19 years of age are already married (FIDH, 2010).

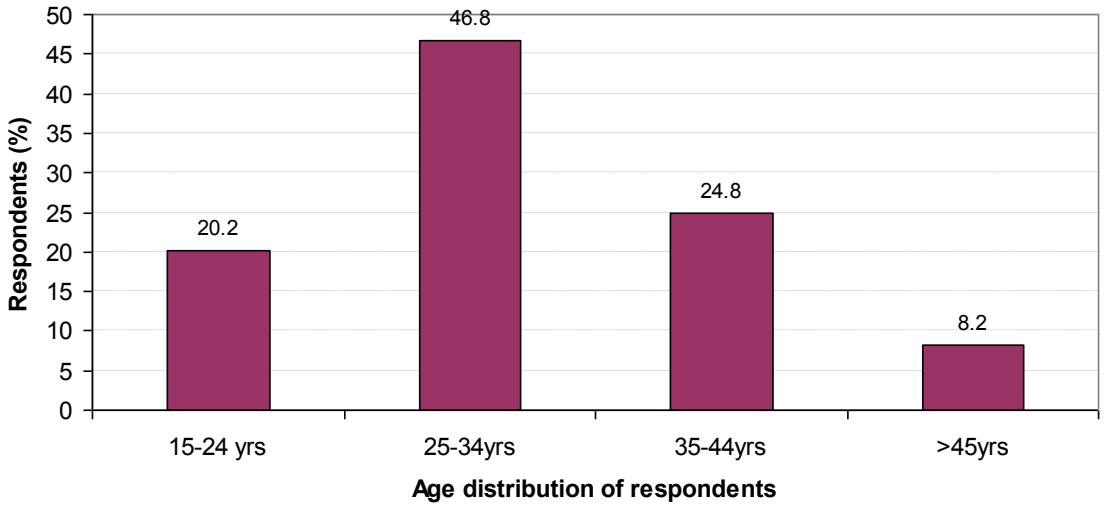


**Figure 4.1.** Age distribution of the respondents in both Beni and Bukavu territory N= 367

One of the most common outcomes of marriage at an early age is the withdrawal of the girl from formal education, or no school enrolment at all. This could explain the low level of education observed in this study, which puts these women at a great disadvantage since most educated women are well empowered to influence decisions affecting food choices, availability and accessibility (ACC/SCN, 2004). In addition, the low level of education reduces the ability of these women to process information, acquire skills and

model positive care-giving practices for better health, nutrition and education outcomes for their children (ACC/SCN, 2004). The low literacy level could further be explained by the irregularity and inadequacy of teachers' salary payments and inadequate infrastructure (negatively affecting teaching morale) (Luntala, Mbile, Jean Claude, & Okulo, 2000). The absence of a primary education fee waiver (Luntala et al., 2000) and the observed low monthly average income (<US$30), could also be a contributor to high illiteracy. The girls may be forced to drop out of school due to a lack of money for tuition fees. This low income level was also observed by FAO (2003), more than 80% of Congolese were reported to be living on less than US $1 per day.

### 4.2.2. Household size, age distribution of members

The majority of the households (60.9%) had between 5 to 8 household members and the average household size was 6.37 in North Kivu and 7.08 in South Kivu. All the households interviewed had to have at least one preschool child, and findings showed that slightly more than half of the households (51.8%) had two children below five years, 32.4% had 1 child below five years, 15% had 3 children below five years old and there were 3 households (0.8%) that had 4 children below five years old.

### 4.2.3. Education level of respondents

Education level and especially that of the mother significantly affects the dietary patterns of the household members. According to ACC/SCN (2004), most educated women are well empowered to influence decisions affecting food choices, availability and

accessibility (ACC/SCN, 2004). Despite all the above, the majority of the women in Eastern DRC are illiterate, 44.7% of the respondents had not had any form of education, 32% had attended but not completed some primary education, 10.8% had completed primary education, while ony 0.5% had got to vocational college and none of the respondent had any college or university diploma/degree. The observed low level of education could be explained by the fact that the education sector in DRC has serious problems. There is an inbalane between supply and demand, and the government is not able to take care of this sector; as illustrated by irregularity and insufficient salary payment, inadequate infrastructures, and the fact that most pupils have to abandon school because of no payment of tuition due to the parent's low income (Luntala, Mbile, Jean Claude, & Okulo, 2000).

### 4.2.4. Main source of household income and average monthly income

The majority of households (76.3%) interviewed depended on agricultural production as their main source of income. Fourteen percent were involved in casual labour while 5.3%, and 4.3% were involved in small scale business and formal employment respectively. The findings of this study also indicated that the mean monthly household income was between 20 and 50 US$ with more than 80% of the households had a monthly average income of less than US$ 30. This agrees with reports that have indicated that more than 80% of Congolese people live on less than 1US$ per day and the poverty level is at 70% (Fund for peace, 2006).

### 4.2.5. Under-five mortality among the respondents

Although the findings showed that the mean number of children below five years in the households was two, there were 17.3% and 11.7% households in South Kivu and North Kivu respectively that had 3 children below five years old. Of the 371 women of child bearing age that had been interviewed, 38.5% of them had lost at least one child. These findings can be supported by the very high mortality rate of children below five years old - 150 to 200 deaths per 1000 live births reported in the Democratic Republic of Congo (Ministry of Health-DRC, 2005).

## 4.3 Nutrition status, dietary diversity, most popular banana cultivars and common processing techniques of *Musa*-dependent communities of Eastern DRC

### 4.3.1 Nutrition status of preschool children from Beni territory (NK) and Bukavu territory (SK) eastern DRC

Malnutrition remains one of the main factors associated with the high child morbidity and mortality rates in the DRC. Hundreds of thousands of children have died due to malnutrition in the DRC over the past 12 years (UNICEF, 2011). Anthropometric measurements of 141 and 201 preschool children (12-59months) from Beni territory (NK) and Bukavu territory (SK) respectively were taken and compared to their age in months and z-scores classified according to WHO (2006).

#### 4.3.1.1 Underweight (weight-for-age z-scores)

Under-weight reflects achieved body mass to chronological age and is the most commonly-used nutritional indicator in defining malnutrition (El Sayed, Ashry, Leila, Ahmed, & Hamdy, 2001). One official Millennium Development Goal indicator of progress towards the poverty and hunger goal is the rate of low weight for-age (underweight) of children 0-5 years (GOK, 2004). Findings of this study showed that the proportion of children who were underweight was significantly higher among preschool children from south Kivu (50.5%) as compared to those from north Kivu (18.0%). In addition more cases of severe underweight (24.5%) were observed among preschool children from South Kivu (Table 4.1).

**Table 4.1.** Prevalence of underweight based on weight-for-age z-score and by sex

| | North Kivu | | | South Kivu | | |
|---|---|---|---|---|---|---|
| Underweight | All n = 139 | Boys n = 74 | Girls n = 65 | All n = 196 | Boys n = 116 | Girls n = 80 |
| Underweight[1] | 18.0 % (13.3 - 23.8) | 20.3 % (11.5 - 33.1) | 15.4 % (6.7 - 31.6 ) | 50.5 % (46.1 - 54.9) | 52.6 % (47.9 - 57.2) | 47.5 % (40.2 - 54.9) |
| Moderate underweight[2] | 12.2 % (10.2 - 14.6) | 14.9 % (9.4 - 22.8) | 9.2 % (2.7 - 26.9) | 26.0 % (21.4 - 31.2) | 25.0 % (21.7 - 28.6) | 27.5 % (19.4 - 37.5) |
| Severe underweight[3] | 5.8 % (3.4 - 9.7) | 5.4 % (0.9 - 25.8) | 6.2 % (2.0 - 17.4) | 24.5 % (17.7 - 32.8) | 27.6 % (22.3 - 33.5) | 20.0 % (10.3 - 35.2) |

Values are frequency in percentage and confidence intervals (C.I) at 95%
[1]<-2 z-score; [2]<-2 and >=-3 z-score; [3]<-3 z-score.

The prevalence of underweight observed in both South Kivu (SK) and North Kivu (NK) were higher than that of 11.9% observed among rural households from DRC by Luntale et al (2000). Just as observed by Luntala et al (2000), there was a significant correlation

between weight for age and household average monthly income ($r^2$= 0.113-NK and 0.091-SK), indicating that between 9% and 11% of the changes in underweight are influenced by social economic status. Other studies have shown that dietary diversity is significantly associated with nutritional status indicators especially among preschool children (Ruel, 2002; Onyango, Koski & Tucker, 1998). In this study, $r^2$ values of 0.062-NK and 0.074-SK were observed between weight for age and dietary diversity, indicating that about 6% of the changes in weight for age could be attributed to the diversity of diets consumed by the preschool children. Although the number of boys that were underweight was slightly higher compared to the number of girls in both NK and SK, the difference was not statistically significant. (Table 4.1).

#### 4.3.1.2. Stunting (height-for-age z-score)

The value 'stunting' measures the cumulative growth deficiency associated with long-term factors, including insufficient dietary intake, frequent infections, and poor feeding practices over a sustained period, and low socioeconomic status of households (El Sayed, Ashry, Leila, Ahmed, & Hamdy, 2001). Apart from the physical effects, stunting (growth retardation) is also associated with impaired cognitive functioning. Taken as a whole, growth retardation can leave an individual physically and cognitively less able to contribute to the workforce, a significant factor that may influence productivity and overall development (Hoffman & Soo- Kyung, 2005).

In this study, stunting was the most prevalent form of malnutrition in both north Kivu and south Kivu, with levels as high as 79.1% and 77.4% respectively (Table 4.2). These

levels are almost similar to those of 78.9% observed by Ekesa, Garming and Blomme (2011) when preschoolers from Butembo territory, also in eastern DRC were assessed. The correlation observed between dietary diversity and height for age in both NK and SK was below the threshold of 0.045, this indicates that the population in eastern DRC has gone through prolonged periods of inadequate food. There is also a high possibility that food inadequacy begins even before birth since fetal growth restriction is mostly caused by maternal malnutrition which reduces neonatal survival and has a permanent stunting effect on postnatal growth (Wu, Bazer, Wallace & Spencer, 2009; Ricci et al., 2006).

**Table 4.2.** Prevalence of stunting based on height-for-age z-score and by sex

| | North Kivu | | | South Kivu | | |
|---|---|---|---|---|---|---|
| | All n = 129 | Boys n = 68 | Girls n = 61 | All n = 186 | Boys n = 112 | Girls n = 74 |
| Stunting[1] | 79.1 % (69.7 - 86.1) | 80.9 % (67.8 - 89.5) | 77.0 % (66.0 - 85.3) | 77.4% (70.7 - 82.9) | 80.4 % (71.8 - 86.8) | 73.0 % (65.6 - 79.2) |
| Moderate stunting[2] | 36.4 % (26.0 - 48.3) | 35.3 % (20.7 - 53.2) | 37.7 % (29.1 - 47.1) | 29.6 % (22.4 - 37.9) | 27.7 % (21.8 - 34.4) | 32.4 % (13.2 - 60.1) |
| Severe stunting[3] | 42.6 % (32.9 - 52.9) | 45.6 % (35.2 - 56.4) | 39.3 % (26.2 - 54.2) | 47.8 % (35.5 - 60.5) | 52.7 % (49.3 - 56.0) | 40.5 % (16.9 - 69.6) |

Values are frequency in percentage and confidence intervals (C.I) at 95%
[1]<-2 z-score; [2]<-2 and >=-3 z-score; [3]<-3 z-score.

In addition, although the levels observed in this study are significantly higher (by 20%) than those of UNICEF statistical reports of 2006, these results are supported by documentation that levels of stunting can even double in rural communities. Although food production on farms is mainly in rural areas, this does not mean that rural children are better nourished. Safe water, adequate sanitation, access to health services and information needed by mothers and other care givers are equally important to provide

children with effective care; these services are less accessible in rural areas (Deean, Richard, Malegapuru, Eduard, Florence, Karen & Khama, 2006). The compromised overall health in this population can also be backed up by the very high under five mortality rates that have been documented in the region (DRC,> 200 deaths/1000 live births) (UNICEF DRC, 2006; Arthur et al., 2009).

#### 4.3.1.3 Acute malnutrition/wasting (weight-for-height z-score)

The findings here show that acute malnutrition was the least prevalent form of malnutrition in both Beni and Bukavu territories (Table 4.3.). Although there were no cases of wasting in Beni territory (NK), previous studies in Pinga Health zone also in North Kivu have documented higher levels of wasting (17.1%), surpassing the emergency global acute malnutrition threshold of 10% (UNICEF, 2008). The difference observed could be due to the difference in sample size where by in this study only a few villages were sampled while in the UNICEF study all the villages in Pinga health Zone were represented. In Bukavu territory (SK), a prevalence rate of 9.1% was observed, although this value is slightly below the WHO threshold, it shows that if sustainable interventions are not put in place the levels are likely to rise.

**Table 4.3.** Prevalence of acute malnutrition based on weight-for-height z-score and by sex

| | North Kivu | | | South Kivu | | |
|---|---|---|---|---|---|---|
| | All n = 147 | Boys n = 79 | Girls n = 68 | All n = 197 | Boys n = 117 | Girls n = 80 |
| Global malnutrition[1] | 0.0 % (0.0 - 0.0) | 0.0 % (0.0 - 0.0) | 0.0 % (0.0 - 0.0) | 9.1 % (5.6 - 14.7) | 10.3 % (6.2 - 16.5) | 7.5 % (3.5 - 15.5) |
| Moderate malnutrition[2] | 0.0 % (0.0 - 0.0) | 0.0 % (0.0 - 0.0) | 0.0 % (0.0 - 0.0) | 6.1 % (1.7 - 19.5) | 6.0 % (1.2 - 24.4) | 6.3 % (1.9 - 18.5) |
| Severe malnutrition[3] | 0.0 % (0.0 - 0.0) | 0.0 % (0.0 - 0.0) | 0.0 % (0.0 - 0.0) | 3.0 % (1.0 - 8.9) | 4.3 % (1.6 - 11.2) | 1.3 % (0.2 - 7.3) \] |

Values are frequency in percentage and confidence intervals (C.I) at 95%
[1]<-2 z-score; [2]<-2 and >=-3 z-score; [3]<-3 z-score.

Taking into consideration all the indices of malnutrition (stunting, wasting, underweight), results here indicate that the prevalence of malnutrition among preschool children from south Kivu is significantly higher compared to that in children from North Kivu. This could be supported by the earlier observation in this study where more children from SK were consuming diets low in diversity as compared to those from NK. In addition, other reports from SK have indicated that majority of households are not able to access the desired food in terms of quantity and quality and that there is higher food insufficiency in SK as compared to other regions of DRC such as bas-Congo and NK (Ouma et al., In press).

### 4.3.2 Dietary Diversity and consumption of banana in relation to other staples

#### 4.3.2.1 Consumption patterns across food groups

With reference to the FAO guidelines of measuring household dietary diversity (FAO, 2007), 14 food groups were taken into consideration excluding the sweets, spices, condiments and beverages groups. The results on consumption frequencies with regards to these food groups are summarized in Table 4.4. The group consisting of white roots, tubers and bananas reported the highest consumption rate of more than 95% from both north Kivu and south Kivu.

**Table 4.4.** Consumption patterns of households Beni territory (NK) and Bukavu territory (SK), eastern DRC across food groups

| Food Groups | North Kivu (N=163) | | South Kivu (N=208) | |
|---|---|---|---|---|
| | %HH | SD | %HH | SD |
| Cereals & grains | 17.50 | (0.38) | 24.00 | (0.43) |
| White roots/tubers/bananas | 96.80 | (0.18) | 97.10 | (0.17) |
| Vitamin A rich vegetables | 9.30 | (0.29) | 1.40 | (0.12) |
| Dark green leafy vegetables | 79.10 | (0.40) | 49.00 | (0.50) |
| Other vegetables | 10.50 | (0.30) | 16.30 | (0.37) |
| Vitamin A rich fruits | 3.30 | (0.17) | 1.00 | (0.10) |
| Other fruits | 8.60 | (0.28) | 3.80 | (0.19) |
| Organ meats | 0.70 | (0.08) | 0.00 | (0.00) |
| Flesh meats | 10.50 | (0.31) | 3.40 | (0.18) |
| Fish | 22.40 | (0.42) | 53.80 | (0.50) |
| Eggs | 0.00 | (0.00) | 0.00 | (0.00) |
| Legumes and pulses | 52.00 | (0.50) | 54.80 | (0.50) |
| Milk & products | 0.00 | (0.00) | 0.00 | (0.00) |
| Fats and oils | 74.30 | (0.44) | 96.20 | (0.00) |

The other popular group was that of dark green leafy vegetables. The community could access a wide range of dark green leafy vegetables, (amaranth leaves, bean leaves,

pumpkin leaves, cassava leaves and spinach), but in both South and North Kivu the most popular dark green leafy vegetables was cassava leaves with 18.3% and 58.5% of the households from South and North Kivu respectively consuming this food item. As mentioned earlier, this popular green leafy vegetable 'cassava leaves', is a major relish locally known as 'sombe' and usually accompanies a hard paste made from cassava flour locally called 'ugali'. Despite the high consumption of vegetables and especially the 'cassava leaves' it is likely that the nutrients in the vegetables are lost due to the cooking methods employed. The leaves are boiled for prolonged periods, the water drained and the leaves then pounded in a mortar. The main aim of this is to break-down and get rid of the cyanide in the cassava leaves (J.Ntamwira, personal communication, August 26, 2009).

The food groups with reported poor consumption rates included the group with vitamin A rich vegetables. In addition, there was virtually 0.00% consumption of preformed vitamin A (eggs, milk and milk products and organ meats groups) among households from both South and North Kivu in the 24 hours preceding the survey. Despite this, the consumption of Oils and fats was relatively high; a total of 96.2% and 74.3% of the households from SK and NK respectively had consumed food cooked with local red palm oil in the last 24 hours. This could be explained by the high production of palm oil in the region (Carrere, 2010). In 2005, total palm oil production in DRC was estimated at 225,000 tonnes, of which 25,000 tonnes came from the agribusiness sector and 200,000 from the village plantation sector. Of this, approximately one quarter represented

commercial oil sold on the consumer market, while the rest went to self-consumption by the producers and their family circles, in the broad sense of the term (Carrere, 2010).

#### 4.3.2.2 Household dietary diversity score (HDDS)

The food groups were further reduced to 12 by having all the vegetables in one group, all the fruits in another group and all the meats together, this was in order to come up with the household dietary diversity score (HDDS) as according to FAO (2006). Findings showed that more than 50% of the households had consumed less than 3 food groups in the last 24 hours preceding the survey (Figure 4.2), this means that the diversity of diets consumed in eastern DRC is very low.

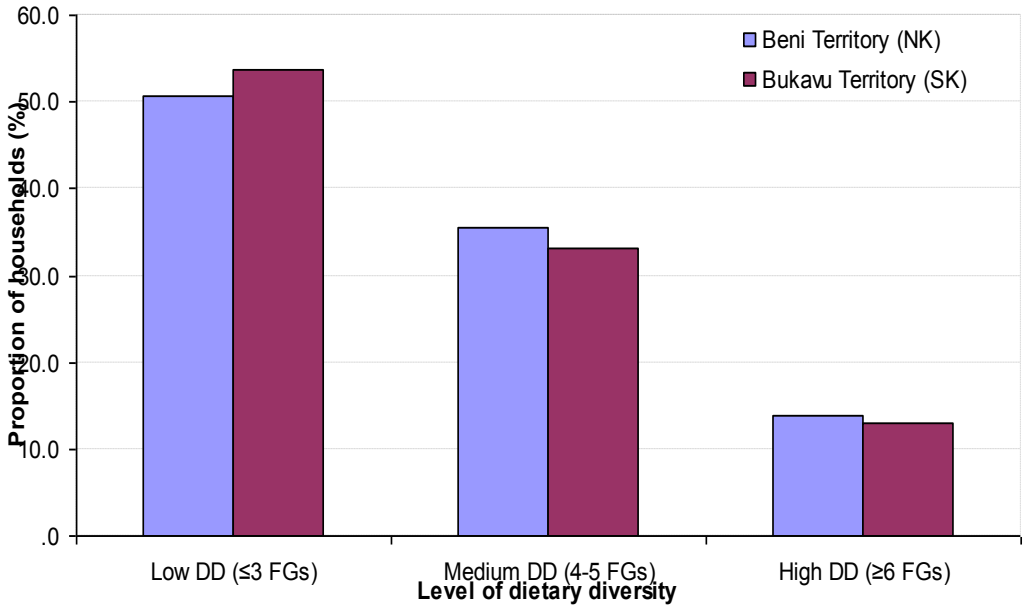


**Figure 4.2.** Diversity of diets consumed by households from South Kivu and North Kivu, Eastern DRC.

The fraction of households consuming highly diversified diets (>6FGs) was less than 10% in both Beni territory (NK) and Bukavu territory (SK). Although the SK had a slightly higher proportion of households consuming diets low in diversity, the difference in HDDS between NK and SK was not statistically significant (p=0.320). A similar observation was made when Ekesa, Bloome and Garming (2011) assessed the diversity of diets consumed by households in Butembo territory (North Kivu, eastern DRC), and found that the diets of the general household was not different from the diets of preschool children within the same households. Therefore, although moving from a monotonous diet to one containing a more diverse range of foods has been shown to increase intake of energy as well as micronutrients in developing countries (Gina, Maria, Chiara, Guy, & Inge, 2007), following the findings of this study the diets of most households in Eastern DRC and more specifically those of preschool children are starchy based and monotonous. This puts them at a higher risk of malnutrition and especially micronutrient deficiencies.

The poor relationship observed between dietary diversity and stunting/underweight/wasting indicates that although dietary diversity has an effect on the nutrition status, in DRC, there is more to malnutrition than just diet. Therefore to tackle the problem of malnutrition, it is important that comprehensive interventions that address the basic and underlying causes of malnutrition which range from presence of appropriate economic, political and educaton structures to acces to enough and nutritious food, access to good health services and appropriate maternal care (UNICEF, 2009). Having these appropriate structures and keeping them operational has been a great challange in the

DRC due to conflict over basic resources such as water, minerals and land and the fight for political power. The result has been prolonged civil war and political instability (Anup, 2012).

#### 4.3.2.3 Consumption of banana fruit and other starchy staples accessible to the community

Of all the staples considered in this study, cassava root was the most highly consumed. This finding is supported by the high cassava production of 74% observed by Luntala, Mbile, Jean Claude and Okulo (2000). These authors also indicated that DRC has the highest annual cassava consumption in the world, with an estimated 390 kg fresh root (equal to 1,100 kcal) per person per day. In addition, cassava is cultivated in around 50% of the arable land in DRC and provides to 70% of Congolese population about 60% of food energy intake. Just as reported by Luntala et al (2000), in our study, EAHB were the second most consumed starchy staple recording a consumption rate of ≥70% among households in both SK and NK (Table 4.5 and Figure 4.3), the difference in consumption levels between SK and NK was not statistically significant ($p=<0.05$).

**Table 4.5.** Consumption of cooking bananas, plantains and other starchy staples available to communities in Eastern Democratic Republic of Congo.

| | South Kivu N= 208 | | North Kivu N=163 | |
|---|---|---|---|---|
| Starchy staples | Frequency (%) | S.E.Mean | Frequency (%) | S.E.Mean |
| Millet | 0.50 | (±0.005) | 0.00 | (±0.000) |
| Sorghum | 5.80 | (±0.016) | 0.60 | (±0.006) |
| Maize | 17.80 | (±0.027) | 14.20 | (±0.028) |
| Wheat & products | 0.00 | (±0.000) | 3.10 | (±0.014) |
| Rice | 1.00 | (±0.007) | 1.80 | (±0.011) |
| Irish potatoes | 0.50 | (±0.005) | 0.60 | (±0.006) |
| Sweet potatoes | 0.50 | (±0.005) | 0.00 | (±0.000) |
| Cassava & products | 86.60 | (±0.026) | 99.40 | (±0.006) |
| Yam/Taro | 0.00 | (±0.000) | 1.20 | (±0.009) |
| Cooking banana | 66.50 | (±0.034) | 64.00 | (±0.038) |
| Plantain | 4.80 | (±0.015) | 10.00 | (±0.021) |

These findings support results by Jagwe, Ouma and Van Asten (2009), who reported EAHB consumption rates of almost 80% in DRC. Although Jagwe et al (2009) reported that consumption of plantain bananas was about 36% in SK and that plantains have traditionally been a starchy staple food of rural populations in the humid lowlands of DRC, the low level of plantain consumption observed (4.8% SK and 10% NK) could be explained by the fact that due to their high market value, farmers are increasingly selling plantains as a cash crop to urban consumers. With rapid urbanization and the growing prosperity of city dwellers, demand is outstripping supply, thereby leaving the rural poor households to depend on either cooking varieties or other starchy staples. The consumption of dessert bananas was even lower with consumption rates of 0% and 2% in SK and NK, respectively (Figure 4.3), because dessert bananas are mostly consumed as snacks and their production is also very low in Eastern DRC.

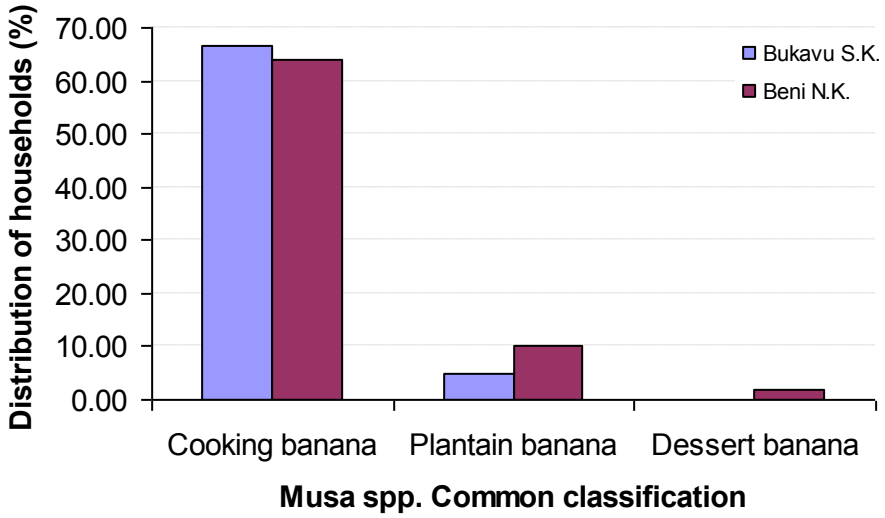


**Figure 4.3.** Consumption rates of cooking, plantain and dessert bananas among households in Eastern Democratic Republic of Congo (SK-South Kivu: N=208, NK-North Kivu: N=163)

Bananas are consumed in various forms, mostly boiled, roasted, as dessert, beer, and juice. This study showed that in both NK and SK, bananas are mostly boiled (Table 4.6). Although some households in SK (47%) indicated having consumed roasted banana, the consumption of banana beer was reported in both NK and SK. The high consumption of boiled banana and banana beer is explained by the high production area under the two popular cultivars in both regions ('Nshikazi' and 'Vulambya') whose main form of utilisation is either cooking (boiling) or production of beer (Dowiya, Rweyemamu, & Maerere, 2009).

**Table 4.6.** Consumption of local banana products among households in South Kivu (N=208), North Kivu (N=163), Eastern Democratic Republic of Congo**.**

| Region/ Site | Banana product | Consumption freq (%) | S.E.Mean | Cultivars commonly used | Genomic group | Ripening stage at use |
|---|---|---|---|---|---|---|
| SK | Steamed banana | 1.20 | (±0.00) | Nshikazi, Barhebesha | AAA | 1-3(unripe) |
| | Boiled banana | 75.00 | (±0.030) | Nshikazi, Barhebesha | AAA | 1-3(unripe) |
| | Roasted banana | 47.00 | (±0.035) | Nshikazi, Barhebesha | AAA | 1-3(unripe) |
| | Banana chips/crisps | 0.00 | (±0.000) | Musheba | AAB | 5(ripe) |
| | Banana beer/wine | 6.00 | (±0.000) | Nshikazi, | AAA | 6(ripe) |
| | Banana juice | 13.00 | (±0.000) | Nshikazi, | AAA | 6(ripe) |
| | Banana porridge | 4.00 | (±0.014) | Musheba | AAB | 5(ripe) |
| NK | Steamed banana | 0.00 | (±0.000) | - | - | - |
| | Boiled banana | 69.00 | (±0.036) | Vulambya, | AAA | 1-3(unripe) |
| | Roasted banana | 0.00 | (±0.000) | - | - | - |
| | Banana chips/crisps | 0.00 | (±0.0000 | Musilongo | AAB | 5(ripe) |
| | Banana beer/wine | 6.00 | (±0.019) | Kisubi, | AB | 6(ripe) |
| | Banana juice | 1.00 | (±0.006) | Banane | | 6(ripe) |
| | Banana porridge | 1.00 | (±0.006) | Musilongo | AAB | 5(ripe) |

Although a significant amount of roasted banana was consumed in SK (47 %), none of the households in NK consumed the *Musa* fruits in this form. Other products were banana juice, beer and porridge in SK, and banana beer in NK. The beer banana cultivars were the AAA-EAHB 'Nshikazi' cultivar in SK and the AB 'Kisubi' cultivar in NK, which were kept up to ripening stage 6 (all yellow) before use (Table 4.6).

A large proportion of the household members (>55%) from both NK and SK had consumed *Musa* fruit (bananas and plantains) between 2 to 4 times in the 7 days before the interview and there were some households that had consumed *Musa* fruit every day of the last 7 days preceding the survey (Figure 4.4).

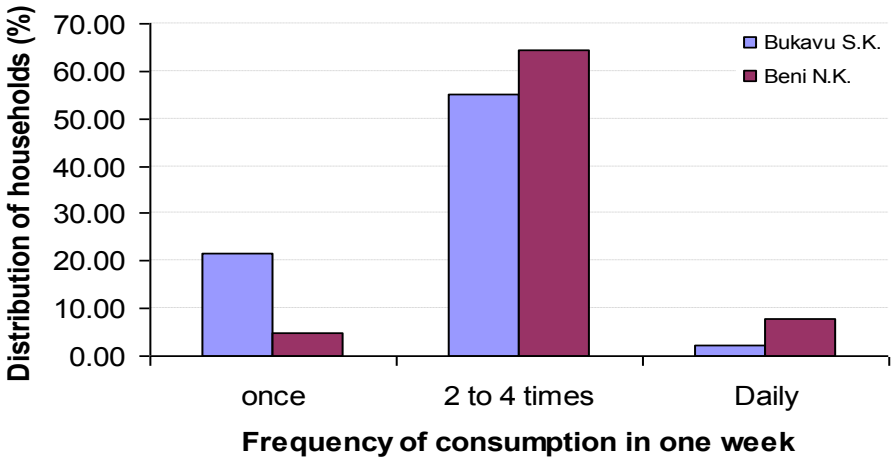


**Figure 4.4.** Consumption frequency of bananas and banana products among households in Eastern Democratic Republic of Congo (SK-South Kivu: N=208, NK-North Kivu: N=163)

Although there were several geographic sources of food for the community, more than 90% of the households from NK and SK indicated having obtained the bananas and plantains from their farms (Figure 4.5).

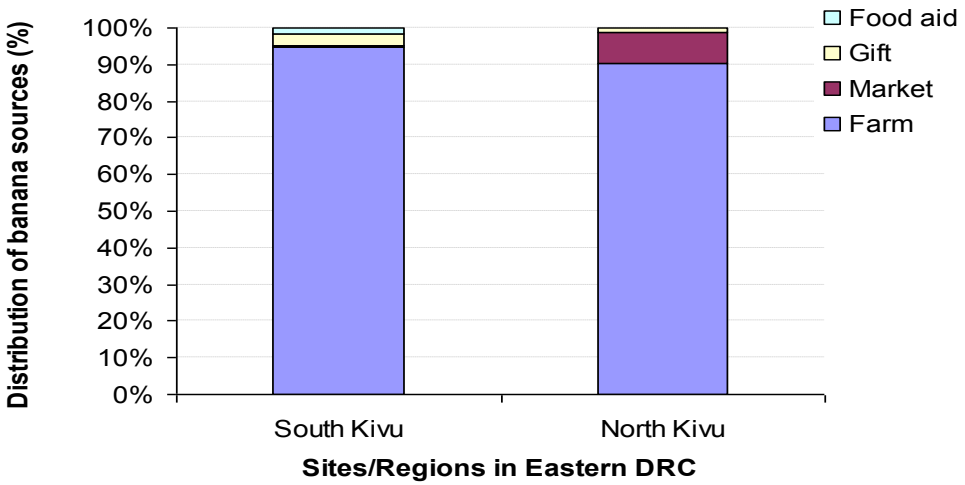


**Figure 4.5.** Sources of bananas consumed by households in Eastern Democratic Republic of Congo (SK-South Kivu: N=208, NK-North Kivu: N=163)

This is higher than values reported by Jagwe, Ouma, Van Asten and Abele (2009), who reported that only 64% of households in SK obtained their bananas from own production, whereas 24% relied on purchase. This difference could be because in the study carried out by Jagwe et al (2009), the respondents included both rural and urban traders in *Musa* while in this study the respondents were exclusively small-holder farmers.

The relationship between consumption of bananas especially the cooking banana and dietary diversity was a positive and significant one ($r^2$=0.537), indicating that more than 50% of the children/households that had consumed diversified diets had atleast consumed the cooking bananas. This was expected following the very high rate (>60%) of banana consumption reported in both NK and SK. Despite this, the correlation between banana consumption and any of the three indices of malnutrition (stunting, wasting,

underweight), was negligible, this means that although bananas form a major part of the dieta and it could provide a good avenue for accessing not only the carbohydrates but also micronutrients, it is not possible for only one food item to make a substancial contribution to health and thus the strong advocacy for dietary diversity.

### 4.3.3 Most Popular and Prefered banana cultivars and the common processing methods

#### 4.3.3.1 Most popular and preferred banana cultivars

Through 14 focus group discussions (FGDs) in SK (8) and NK (6), a list of all *Musa* cultivars grown within the community was compiled. A total of 16 and 19 *Musa* cultivars were listed in SK and NK, respectively. This number was lower than that indicated by Dowiya, Rweyemamu and Maerere (2009), where a total of 29 and 32 *Musa* cultivars were reported as being cultivated in SK and NK respectively. Both results show, however, that the diversity of *Musa* cultivars is slightly higher in NK as compared to SK, the higher number reported by Dowiya et al (2009) could be due to the survey methodology used, where direct observation (transect walks) were carried out to establish the varied cultivars. In addition, Dowiya et al (2009) included more territories in his study as opposed to this study, where information was only collected through the FGDs and household interviews and only one territory/district from each of the two provinces (South Kivu and North Kivu) was included. In addition, not all villages were visited from the selected territories.

The participants then arranged the named cultivars in order of priority and as shown earlier in Table 3.1, in SK, Bukavu territory, the four most popular cultivars consisted of three East African Highland cooking banana (AAA-EAHB) cultivars and one plantain (AAB) cultivar while in NK, Beni territory the four included two East African Highland cooking banana (AAA-EAHB) cultivars and two plantain (AAB) cultivars.

The cultivars were then scored on a scale of 1 to 5 by FGD participants for cooking quality, market price, bunch size (yield) and plant disease resistance in order to identify the two most popular and preferred from each of the two sites. In SK, the EAHB 'Nshikazi' also called 'Magizi' had the best overall score, with 4.65 for cooking quality, 3.93 for market price, 4.02 for bunch size and 2.39 for disease resistance (Table 4.7). The second highest score was obtained by the plantain 'Musheba', with 3.98 for cooking, 4.60 for market price, 3.43 for bunch size (yield) and 2.08 for disease resistance. These findings confirm findings by Dowiya, Rweyemamu and Maerere (2009), carried out in the same province (SK) although at different districts which also cited 'Nshikazi' as the most preferred *Musa* cultivar. According to Dowiya et al (2009), Nshikazi' was occupying 52% of the land under banana and apart from having the traits already mentioned (high yield, high market price and good cooking quality), 'Nshikazi' tastes better after cooking, and made good banana juice and banana beer. 'Nshikazi' is also grown in Rwanda where it is known as 'Ishika' and Uganda where it has different names depending on the regions i.e. 'Ensika', 'Emburansika' 'Omuburasika' or 'Nsika' (Table 3.1).

As indicated earlier and shown in table 4.1, ‘Musheba’ was the most popular plantain cultivar in SK. According to Dowiya, Rweyemamu and Maerere (2009), apart from the reasons given by the community (high market price, good yield, and good cooking quality), ‘Musheba’ is a popular cultivar because it matures faster and its taste is very acceptable (good). In addition, ‘Musheba’ has social-cultural importance in times of marriage and the ‘Mashi’ tribe of SK had obtained it from Uganda as a form of dowry (Dowiya et al., 2009). In NK, the EAHB ‘Vulambya’ received the highest score, followed by the plantain ‘Musilongo’ , the traits that made ‘Vulambya’ and ‘Musilongo’ popular were similar to those observed in ‘Nshikazi’ and ‘Musheba’ respectively from SK. The principal weakness of the popular cultivars was their low disease resistance (Table 4.6). These popular AAA-EAHB cultivars in NK were also among the six most preferred *Musa* cultivars identified by Dowiya et al (2009) in the same region (NK). Although this study indicates that the plantains (‘Musilongo’ and ‘Nguma’) are popular (Tables 4.6 & 4.7) and that ‘Musilongo’ produces a bigger bunch and matures faster, according to Dowiya et al (2009), ‘Nguma’ was the most productive plantain *Musa* cultivar in NK and ‘Musilongo’ was not among the ten most popular cultivars.

**Table 4.7.** Most popular and preferred banana cultivars grown and consumed by households in North Kivu-NK (N=163) and South Kivu-SK (N=208), Eastern Democratic Republic of Congo.

| Region | Banana Cultivar | Trait/Attribute (1=lowest score and 5= highest score) and standard deviation (S.D) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Name (genome group) | Yield[1] | S.D | Market price[1] | S.D | Cooking quality[1] | S.D | Disease resistance[1] | S.D | Total Score |
| SK | Nshikazi (AAA) | 4.02 | 0.68 | 3.93 | 0.67 | 4.65 | 0.49 | 2.39 | 0.57 | 15.00 |
| | Barhebesha (AAA) | 3.46 | 0.77 | 2.44 | 0.58 | 3.83 | 0.69 | 2.26 | 0.59 | 11.99 |
| | Kamera (AAA) | 2.2 | 0.69 | 2.24 | 0.73 | 1.4 | 0.58 | 1.39 | 0.55 | 7.25 |
| | Musheba (AAB) | 3.43 | 0.81 | 4.6 | 0.53 | 3.98 | 0.75 | 2.08 | 0.91 | 4.08 |
| NK | Vulambya (AAA) | 4.02 | 0.68 | 4.03 | 0.71 | 4.66 | 0.49 | 2.41 | 0.57 | 15.12 |
| | Kiware (AAA) | 3.35 | 0.74 | 2.71 | 0.69 | 4.52 | 0.71 | 2.21 | 0.65 | 12.79 |
| | Nguma (AAB) | 2.86 | 1.02 | 3.9 | 0.75 | 2.32 | 0.79 | 2.57 | 0.69 | 11.65 |
| | Musilongo (AAB) | 3.39 | 0.93 | 4.63 | 0.52 | 3.86 | 0.73 | 2.12 | 0.91 | 14.00 |

[1]Values of each trait are means of the scores given by the participants of the focus group discussions

A verification of the results on the preferred cultivars obtained through FGDs was carried out during the individual household interviews. Respondents confirmed that 'Nshikazi' and 'Musheba' in South Kivu and 'Vulambya' and 'Musilongo' in North Kivu were the most preferred *Musa* cultivars (Table 4.8). In SK more than half of the households (69%) indicated 'Nshikazi' as the most preferred cultivar. The only popular and preferred plantain was 'Musheba'. In NK, 'Vulambya' was the most preferred cultivar overall (36%). The two popular plantain cultivars were; 'Nguma' and 'Musilongo' although more households preferred 'Musilongo' to 'Nguma' (Table 4.8). Table 4.8 also shows

that the most preferred cultivars had pulp colour ranging from ivory and yellow (EAHB cooking varieties) to orange (plantain varieties).

**Table 4.8.** *Musa* cultivars preferred by household members in North Kivu (N=163) and South Kivu (N=208), Eastern Democratic Republic of Congo.

| Region | Cultivar local Name (genome) | Pulp color At use | Harvest+ colou Strip-carotene | Number of household indicating choice 1st Choice | 2nd Choice | 3rd Choice |
|---|---|---|---|---|---|---|
| South Kivu | Nshikazi (AAA) | yellow | 7/2 121U | 143(68.8%) | 9(4.3%) | 1(0.5%) |
| | Barhebesha (AAA) | yellow | ½ 101 | 6(2.9%) | 36(17.3%) | 6(2.9%) |
| | Kamera (AAA) | Ivory | 3/3 1205U | 8(3.8%) | 11(5.3%) | 22(10.6%) |
| | Musheba (AAB) | Orange | 9/2 1355U | 27(13.0%) | 9(4.3%) | 9(4.3%) |
| North Kivu | Vulambya (AAA) | Yellow | 7/2 121U | 59(36.4%) | 17(10.5%) | 9(5.6%) |
| | Kiware (AAA) | Yellow | - | 20(12.3%) | 29(17.9%) | 6(3.7%) |
| | Nguma (AAB) | Orange | 9/2 1355U | 5(3.1%) | 2(1.2%) | 9(5.6%) |
| | Musilongo (AAB) | Orange | 9/3 7507U | 19(11.7%) | 14(8.6%) | 11(6.8%) |

Although a report by Ndungo, Bakelana, Eden-Green and Blomme (2005) cited the beer banana 'Pisang Awak' (ABB), known locally as 'Kayinja', as the most popular cultivar occupying nearly ¾ of the land cropped with banana, in our study 'Kayinja' was neither mentioned among the first four popular cultivars in SK and it did not even emerge as one of the preferred cultivars. This could be because the participants of the FGDs were mostly local farmers and women of child-bearing age and they seemed to prefer cultivars that could be used to make food.

The fruit pulp colour of the above preferred *Musa* cultivars grown in Eastern DRC was pre-screened using the HarvestPlus carotenoid colour strip. Findings showed that of the four most popular *Musa* cultivars (two AAB-plantains and two AAA-EAHBs), the plantains varieties from both South Kivu and North Kivu had a dark yellow/orange pulp (RHS[1] 9/2 1355U), while the East African Highland banana (EAHB) cultivars varieties had a yellow pulp (RHS 3/1 107U) (Figure 4.6).


**Figure 4.6.** Longitudinal cross-section of 'Musheba'-plantains (left) and 'Nshikazi'-AAA-right).

These results are similar to those observed after pre-screening of cultivars from Uganda, Cambodia, Philippines and Hawaii, which revealed that more than 80% of the plantain cultivars (AAB) screened had an orange pulp while more than 80% of the cooking banana cultivars (AAA) had pulp colour ranging from ivory to white (HarvestPlus, 2007). Although the pulp colour is not a definite indication of levels of carotenoids, it helps in filtering cultivars that could be further analyzed for enhanced carotenoids content.

[1] Royal Horticultural Society

#### 4.3.3.2 Popular cooking/processing methods of the preferred banana cultivars

As indicated earlier, in Eastern DRC bananas are a major part of the staple diet, complementing other sources of food. The methods of processing and cooking the *Musa* fruit range from simple boiling to fermentation, drying and grinding to make flour; The fruit can also be eaten as a dessert, or processed into juice, beer and wine (Ndungo, Fiaboe, & Mwangi, 2008). Findings of this study showed that the processing techniques applied to the banana cultivars in North Kivu were similar to those practiced in South Kivu (Table 4.9). The two most popular EAHB cultivars ('Nshikazi' and 'Vulambya') were mostly utilized between stages 1 and 3 of ripening, i.e., before the fruit was ripe. According to community members, the EAHB banana fruits were generally peeled and boiled with only the addition of salt. Occasionally locally processed palm oil was added during the boiling process. The cooked banana was eaten either alone or with boiled beans or peas. In some instances the banana would be boiled together with beans and some amaranth leaves, 'lengalenga'.

**Table 4.9.** Local cooking/processing methods applied to common banana cultivars in Eastern Democratic Republic of Congo.

| Banana cultivar | Cooking method | Cooking ingredients | Common accompaniments |
|---|---|---|---|
| Nshika(AAA) | Boiling ('kitika') | Salt, water | Beans, peas, amaranths, |
| | Steaming | Salt, water, | Beans, meat[2], Fish[2] |
| Vulambya(AAA) | Boiling ('kisamunyu') | Salt and water | Beans, peas, amaranths, |
| Musheba(AAB) | Boiling ('ndizi') | Water | Peas, meat[2], chicken[2] |
| | Deep frying[3] | Palm oil | Meat[2], chicken[2] |
| | Roasting (snack) | - | - |
| | Porridge (flour) | Water, cassava flour[1] | - |
| Musilongo(AAB) | Boiling ('ndizi') | Water | Peas, meat[2], chicken[2] |
| | Deep frying[3] | Palm oil | Meat[2], chicken[2] |
| | Porridge (flour) | Water, cassava flour[1] | - |

[1] = Optional, [2] = Used occasionally, [3] = Prepared occasionally

The plantain cultivars (‘Musheba’ and ‘Musilongo’) were also boiled but generally no salt was added because of the cultivars’ natural sweetness and were eaten either alone or served with boiled green peas. ‘Musheba’ and ‘Musilongo’ were also used to make ‘Nchimba’ banana flour. To make ‘Nchimba’, the fruit was harvested at maturity, kept for about 1 week to reach stage 5 of ripening (all yellow with green tips), peeled, chopped into pieces (in some cases put under warm conditions and left to ferment), sun-dried on the ground and either pounded or milled into flour. This flour (Nchimba) was then used to make porridge, a weaning food. The plantain flour was also boiled in water to make a thick paste locally called ‘ugali’, although the ‘ugali’ could be accompanied by any relish, the most common one was reported to be boiled and pounded cassava leaves, locally called ‘sombe’. In some cases, cassava flour was added to the plantain flour at a ratio of 2:3. Occasionally, the plantain cultivars were deep fried in the local palm oil and eaten as ‘banana fries’.

Boiling *Musa* fruit (banana and plantain) is also a common practice in Uganda, Cameroon, Burundi and West Indies. In Uganda, EAHB cultivars are typically boiled with beans and ghee then mixed with pepper, salt and onions to make a dish called 'akatogo'. In Cameroon, the green banana is boiled and served in a sauce of palm oil with fish, cooked meat, green beans, beans and seasoning, whereas in the West Indies boiled green banana is served with salted fish or meat (FAO, 1990). In Gabon and Cameroon, the mature green form of banana is dried and stored and may be used for cooking after grinding into flour, the difference is that in these two countries (Gabon and Cameroon) this product was mainly used as a famine reserve and not as an everyday meal (FAO, 1990). Although steaming and roasting are not popular cooking methods in Eastern DRC, they are very popular banana cooking methods in Uganda; Generally, the Ugandan EAHB cultivars are mostly steamed in banana leaves and either pounded or eaten whole (a dish called 'matoke'), while the plantains are roasted and sold as street food.

Findings of this study have shown that the nutritions status of the preschool children from Eastern DRC is poor with most of then stunted and that majority of them consume diets low in diversity (<3FGs/day). In addition, bananas form a major part of the diets of households in Eastern DRC and the most popular cultivars include 2 AAA-EAHBs ('Vulambya' and 'Nshikazi') and 2 plantains ('Musilongo' and 'Musheba'). Despite these, the levels of VAD remain high in DRC (SCN, 2010), it was therefore necessary that more evaluations to establish the content of pVACs in these popular cultivars, the retention of these pVACs during ripening and percentage of pVACs miceralised and ready for absorption following ingestion of the Musa product/dish. The objective of using

bananas in the fight against VAD follows findings that some banana cultivars could be very good sources of pVACs (Davey, Van den Berge, Markham, Swennen, & Johan, 2009; Englberger, Schierle, Marks, & Fitzgerald, 2003a; Englberger et al., 2003b). In addition, research has proven that food-based strategies offer sustainable solutions in alleviation of VAD (Ayewole-Olusola & Asagbra, 2003). And the other benefit is that there is simultaneous provision of multiple nutrients (Englberger et al*.,* 2003a).

## 4.4 Cultivar-dependent changes in pVACs contents related to post harvest development (ripening) and local post-harvest processing of *Musa* cultivars popular in Eastern DRC

### 4.4.1 Principal carotenoids observed

Figures 4.7 and 4.8 shows the chromatograms of 'Nshikazi' and 'Musheba', the peaks were identified by comparing the retention times with these of the standards of apocarotene, β-carotene and lutein. As previously observed by other researchers, the principal carotenoids identified in both the raw *Musa* fruits and the processed *Musa* fruits were (t-BC), all-*trans* α-carotene (t-AC), with minor amounts of lutein and the 13-*cis* isomers of AC and BC, (Davey, Van den Berge, Markham, Swennen, & Johan, 2009; Englberger, Schierle, Marks, & Fitzgerald, 2003a; Englberger et al., 2003b; Englberger, Ron, Wills, Dufficy, Daniells, & Coyne, 2006a; Ngoh-Newilah, Dhuique-Mayer, Rojas-Gonzalez, Tomekpe, Fokou, & Etoa, 2009; Ngoh-Newilah, Lusty, Van den bergh, Akyeampong, Davey, & Tomekpe, 2008).

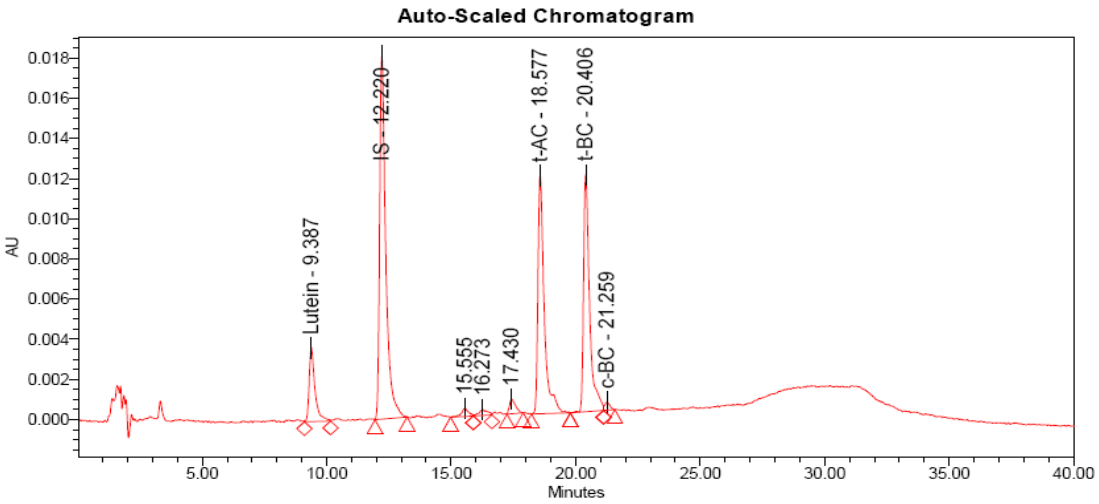


**Figure 4.7.** A chromatogram of 'Nshikazi' an AAA-EAHB cultivar from South Kivu at ripening stage 3 showing the separation of different carotenoids at different retention times.

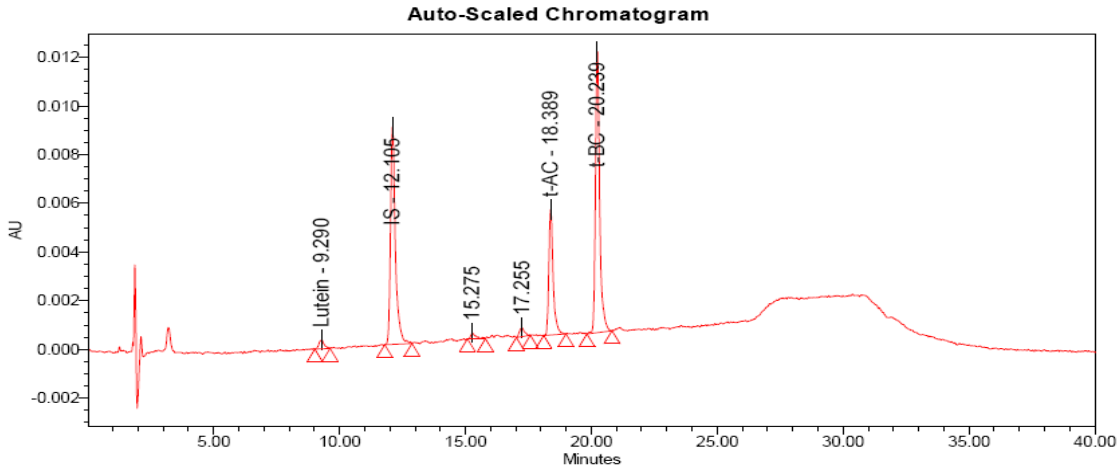


**Figure 4.8.** A chromatogram of 'Musheba' an AAB-French plantain cultivar from South Kivu at ripening stage 3 showing the separation of different carotenoids at different retention times (x-axis).

### 4.4.2. Changes in the pVACs during ripening of *Musa* fruit

#### 4.4.2.1. Total and individual pVACs in the four *Musa* cultivars

The means of individual carotenoids, total carotenoid and total pVACs (as determined by HPLC) are summarized in Table 4.10, and they were based on triplicate extractions per cultivar, per plant and per ripening stage.

Total pVACs were calculated as the sum of the concentrations of t-AC, t-BC and *cis*-carotenoids (13-*cis* BC and 13-*cis*-AC). The total pVACs content ranged from 7.07 to 33.88nmol/gFM (379.66 to 1819.36 µg/100 gFM) representing a 5-fold variation. These values are comparable to those of between 232-2000 µg/100 gFM observed in 9 *Musa* cultivars from Cameroon (Ngoh-Newilah, Lusty, Van den Bergh, Akyeampong, Davey, & Tomekpe, 2007).

The levels of t-BC in the four tested *Musa* cultivars ranged from 3.6-22.6nmol/gFM (193.3- 1212.0 µg/100gFM) while that of t-AC ranged from 2.36-11.2nmol/gFM (126.7-597.7 µg/100gFM). The content of 13 *cis*-carotenoids observed was too low to have any significant contribution to nutrition. In addition, the AAA-EAHB (creamish/yellow pulp color) had significantly lower levels of both individual and total pVACs as compared to the AAB plantains (light orange pulp color) at all the four ripening stages ($p=<0.05$). This confirms the previously reported positive correlations between the fruit pulp colour and the total pVACs content (Ngoh-Newilah, Dhuique-Mayer, Rojas-Gonzalez, Tomekpe, Fokou, & Etoa, 2008).

**Table 4.10.** The content of specific and total carotenoids/provitamin A carotenoids (pVACs) observed after HPLC analysis of four popular *Musa* cultivars from Eastern Democratic Republic of Congo

| | | | | Content of carotenoids in nmol/gFM during ripening | | | | | Total | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Origin Country | Local name | Genomic group | Ripening stage | t-AC(SD) | t-BC(SD) | cis-AC(SD) | cis-BC(SD) | Lutein(SD) | Carotene nmol/gFM | pVACs nmol/gFM | BCE nmol/gFM | BCE µg/100gFM |
| Bukavu | Nshikazi | AAA-EA | Stage 1 | 5.28(1.10) | 4.31(1.07) | 0.00(0.00) | 0.08(0.03) | 0.45(0.21) | 10.70 | 9.67 | 7.00 | 375.66 |
| South Kivu | | | Stage 3 | 6.47(0.02) | 6.83(0.61) | 0.23(1.08) | 0.18(0.01) | 0.53(0.36) | 15.39 | 13.70 | 10.28 | 551.92 |
| DRC | | | Stage 5 | 3.77(0.21) | 3.60(0.92) | 0.18(0.21) | 0.22(0.38) | 0.45(0.03) | 9.03 | 7.77 | 5.70 | 305.95 |
| | | | Stage 7 | 6.36(1.02) | 7.32(1.21) | 0.05(0.12) | 0.43(0.03) | 0.40(0.26) | 14.79 | 14.16 | 10.76 | 577.74 |
| | Musheba | AAB | Stage 1 | 2.36(0.42) | 4.71(0.82) | 0.00(0.00) | 0.00(0.00) | 0.11(0.04) | 7.25 | 7.07 | 5.89 | 316.05 |
| | | | Stage 3 | 6.74(1.06) | 15.51(1.05) | 0.00(0.00) | 0.30(0.07) | 0.18(0.00) | 23.64 | 22.55 | 19.04 | 1022.37 |
| | | | Stage 5 | 5.63(0.04) | 11.94(0.03) | 0.00(0.00) | 0.24(0.10) | 0.23(1.07) | 19.01 | 17.80 | 14.88 | 799.12 |
| | | | Stage 7 | 4.39(0.11) | 7.09(1.22) | 0.00(0.00) | 0.05(1.02) | 0.36(0.38) | 12.70 | 11.53 | 9.31 | 500.13 |
| Beni | Musilongo | AAB | Stage 2 | 9.38(1.05) | 17.76(0.37) | 0.00(0.00) | 0.16(0.53) | 0.13(0.08) | 28.18 | 27.31 | 22.54 | 1210.37 |
| North Kivu | | | Stage 3 | 11.13(2.10) | 22.57(0.01) | 0.00(0.00) | 0.18(0.36) | 0.21(0.67) | 34.91 | 33.88 | 28.23 | 1515.87 |
| DRC | | | Stage 5 | 8.17(1.20) | 15.44(0.29) | 0.00(0.00) | 0.11(0.57) | 0.16(0.32) | 24.29 | 23.73 | 19.59 | 1051.96 |
| | | | Stage 7 | 9.00(0.03) | 17.04(1.07) | 0.00(0.00) | 0.18(0.05) | 0.18(0.24) | 26.40 | 26.22 | 21.64 | 1161.86 |
| | Vulambya | AAA-EA | Stage 1 | 5.50(0.09) | 4.71(0.06) | 0.00(0.00) | 0.09(0.00) | 0.24(0.05) | 10.55 | 10.31 | 7.51 | 403.52 |
| | | | Stage 3 | 10.48(0.68) | 9.39(1.03) | 0.00(0.00) | 0.26(0.07) | 0.33(0.03) | 20.85 | 20.13 | 14.77 | 793.10 |
| | | | Stage 5 | 9.58(0.38) | 7.81(1.28) | 0.00(0.00) | 0.22(1.05) | 0.33(0.10) | 18.44 | 17.61 | 12.72 | 682.91 |
| | | | Stage 7 | 8.86(0.08) | 7.50(0.10) | 0.00(0.00) | 0.26(1.23) | 0.25(0.10) | 17.28 | 16.63 | 12.07 | 648.27 |

Values are means of three independentanalyses. DRC= Democratic Republic of Congo, t/cis-AC/BC= trans/cis-Alpha carotene/beta carotene, SD- Standard Deviation.

The findings showed that total pVACs content of the *Musa* fruit was affected by the stages of ripening. Just as observed by Ngoh-Newila et al (2008), the difference in the level of individual and total pVACs observed at each of the ripening stages was significant (<0.05) for each of the four tested cultivars. In 'Musheba', 'Musilongo', and 'Vulambya' the highest levels of t-BC, t-AC and total pVACs were obserbed at ripening stage 3, and the difference observed as the fruit progressed in ripening was significant (Tables 4.11a,b,c). In 'Nshikazi' the highest levels were observed at ripening stages 3 and 7 (Table 4.11d).

**Table 4.11.** Differences in the content of t-BC, T-AC and total pVACs in the four *Musa* cultivars tested in nmol/gFM

Table 4.11a. Musilongo AAB plantain

| Ripening stage | t-AC | t-BC | Total pVACs |
|---|---|---|---|
| 2 | 9.38[c] | 17.76[c] | 27.31[c] |
| 3 | 11.13[d] | 22.57[d] | 33.88[d] |
| 5 | 8.17[a] | 15.44[a] | 23.73[a] |
| 7 | 9.00[b] | 17.04[b] | 26.22[b] |
| p- Value | <0.001 | <0.001 | <0.001 |

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

Table 4.11b. Musheba AAB

| Ripening stage | t-AC | t-BC | Total pVACs |
|---|---|---|---|
| 1 | 2.36[a] | 4.71[a] | 7.07[a] |
| 3 | 6.74[d] | 15.51[d] | 22.55[d] |
| 5 | 5.63[c] | 11.94[c] | 17.80[c] |
| 7 | 4.39[b] | 7.09[b] | 11.53[b] |
| p-Value | <0.001 | <0.001 | <0.001 |

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

Table 4.11c. Vulambya AAA-EAHB

| Ripening stage | t-AC | t-BC | Total pVACs |
|---|---|---|---|
| 1 | 5.50 [a] | 4.71 [a] | 10.31 [a] |
| 3 | 10.48 [d] | 9.39 [d] | 20.13 [d] |
| 5 | 9.58 [c] | 7.81 [c] | 17.61 [c] |
| 7 | 8.86 [b] | 7.50 [b] | 16.63 [b] |
| p-Value | <0.001 | <0.001 | <0.001 |

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

Table 4.11d. Nshikazi AAA-EAHB

| Ripening stage | t-AC | t-BC | Total pVACs |
|---|---|---|---|
| 1 | 5.28 [b] | 4.31 [b] | 9.67 [b] |
| 3 | 6.47 [c] | 7.32 [c] | 13.70 [c] |
| 5 | 3.77 [a] | 3.60 [a] | 7.77 [a] |
| 7 | 6.36 [c] | 6.83 [c] | 14.16 [c] |
| p-Value | <0.001 | <0.001 | <0.001 |

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

A similar observation was made when 'Popoulou' a cooking banana, grown and consumed in Cameroun, was tested, the levels of carotenoids in 'Popoulou' increased during the initial ripening stages with highest levels recorded at ripening stage 3 (Ngoh-Newilah, Lusty, Van den Bergh, Akyeampong, Davey, & Tomekpe, 2008). In this study, as the fruits progressed from ripe to fully-ripe and then to over-ripe (ripening stages 3 to 7), each of the four cultivars showed a different trend and each of this changes was significant at a p value of 0.05. These findings are different from those observed in 'Popoulou' where after ripening stage 3 the changes in total pVACs were not significant (Ngoh-Newilah et al., 2008). An evaluation of 'Batard' a plantain cultivar also grown in Cameroun, showed a totally different trend with the level of total pVACs decreasing considerably as the fruit progressed from ripening stage 1 to stage 7 (Ngoh-Newilah et al., 2008).

#### 4.4.2.2. *Musa* fruit pVACs profiles during ripening

In all the four cultivars tested more than 90% of the total pVACs consisted of t-AC and t-BC alone, the remaining less than 10% comprised of their respective 13 *cis*-isomers (Figure 4.9).

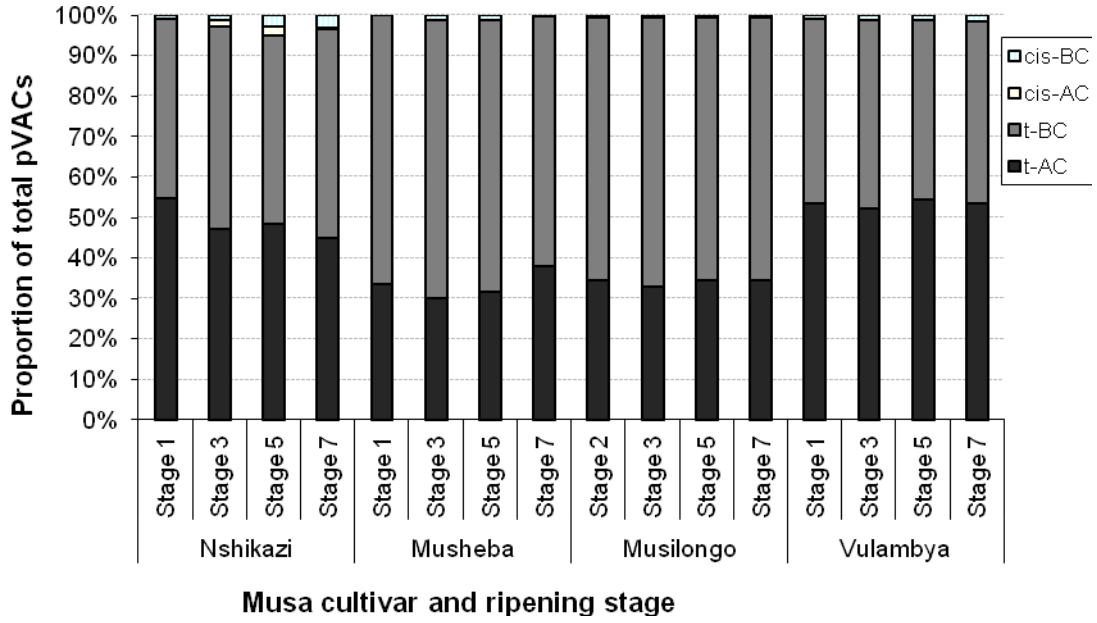


**Figure 4.9.** Overview of the variation in proportion of individual pVACs at different ripening stages within individual fruit of four *Musa* cultivars from Eastern DRC

The pVACs profile observed in this study was quite different from the profile reported for other crops such as maize and wheat where the pVACs always represent between 10-20% of the total carotenoid content. Never the less, it was similar to observations made by Davey, Van den Berge, Markham, Swennen and Johan (2009), where in the 171 *Musa* cultivars from Cameroon analysed over 90% of the total pVACs invariably consisted of t-AC and t-BC alone.

The vitamin A nutritional value of banana and plantain fruits depends not only on the concentration of pVACs but also on the relative proportion of the individual pVACs present. This is because, from a nutritional viewpoint, fruit/foods with higher t-BC contents are preferred as t-BC is the pVAC with the highest vitamin A nutrition value. T-AC has only 50% of the vitamin A activity of t-BC, thus the relative proportions of t-AC and t-BC affect the overall vitamin A nutritional values of the fruit (Fraser & Bramley, 2004; Trumbo, Yates, Schlicker-Renfro, & Suitor., 2003).

In the two plantains tested ('Musheba' and 'Musilongo') the proportion of t-AC ranged from 30% to 38% while that of t-BC ranged from 61% to 69% indicating that the proportion of t-BC is almost double that of t-AC. Among the East African Highland (EAHB) cultivars ('Vulambya' and 'Nshikazi'), the proportion of t-AC was slightly higher than that of t-BC with that of t-AC being between 44% to 54% and that of t-BC being between 44% to 51%, but these differences were not statistically significant (Figure 4.9). This therefore makes the two plantains tested ('Musilongo' and 'Musheba') preferred sources of carotenoids as compared to the AAA-EAHB cultivars.

### 4.4.3. Effect of processing on levels of pVACs in four *Musa* cultivars

Provitamin A carotenoids are known to be easily destroyed during processing, exposure to light and heat treatment. Although the net retention rate of pVACs from different *Musa* fruit processing techniques has not been adversely documented, it is clear that prolonged exposure to heat treatments such as deep frying, boiling and a combination of several processing techniques will result in substantial losses (Ruel & Bouis, 2004). However

data are somehow conflicting and often difficult to interpret (Ngoh-Newilah, Dhuique-Mayer, Rojas-Gonzalez, Tomekpe, Fokou, & Etoa, 2008).

#### 4.4.3.1. Changes and retention of pVACs during boiling

All the four cultivars were boiled in-peel and without peel (Table 3.2). The content of total pVACs in the boiled AAB-plantains and AAA-EAHB cultivars ranged from 6.7-47.9nmol/gFM (357.6-2570.1 μg/100gFM) edible portion. Among the AAA-EAHB cultivars ('Nshikazi' and 'Vulambya'), findings showed a statistically higher content of total pVACs in the raw fruit at ripening stage 3 as compared to when the fruit was boiled in peel or without peel at the same stage of ripening (Table 4.12 & Table 4.13a,b). This therefore confirms findings reporting that boiling results into breakdown of carotenoids thus causing relative loss. In 'Nshikazi', there was 50.1% retention of total pVACs when the cultivar was boiled in-peel and 39.74% retention when the cultivar was boiled without peel. 'Vulambya' retained 89.60% when boiled in-peel and 65.40% when boiled without peel. Boiling of the two plantains led a significantly higher retention of pVACs, a loss of less than 5% was observed in 'Musheba' while in 'Musilongo there was not only retention but also more carotenoids were observed following boiling of either in peel or without peel (Table 4.12). The difference in the content of individual and total pVACs observed in the raw AAB plantains at ripening stage 5 and that observed following boiling at the same ripening stages was statistically significant (Table 4.13c,d).

Other studies have reported different levels of retention. When a Cameroonian plantains 'Batard' was boiled for 30minutes (peeled) and 40minutes (un-peeled), retention levels of 72% and 70% recorded in-peel and without peel respectively but the difference was not

statistically significant (Ngoh-Newilah, Lusty, Van den Bergh, Akyeampong, Davey, & Tomekpe, 2007). In another study, there were higher levels of retention (78%) of all t-BC following boiling of orange-fleshed sweet potato (OFSP) for 20minutes (Bengtsson, Alminger, & Svanberg, 2009).

**Table 4.12.** The content of specific and total carotenoids/provitamin A carotenoids (pVACs) observed after HPLC analysis of processed Musa cultivars from Eastern Democratic Republic of Congo

| Site of origin | Local name | Genomic group | Banana/plantain product | Content of carotenoids in raw and processed Musa fruit (nmol/gFM) | | | | | Total Carotene nmol/gFM | Total pVACs nmol/gFM (% R) | Total BCE nmol/gFM | BCE µg/100g ep |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | t-AC(SD) | t-BC (SD) | c-AC(SD) | c-BC(SD) | Lutein(SD) | | | | |
| Bukavu territory South Kivu DRC | Nshikazi' | AAA-EA | Raw (stage 3) [a] | 7.75(1.67) | 9.01(0.54) | 0.00(0.00) | 0.00(0.00) | 4.47(0.12) | 21.23 | 16.76 | 12.88 | 691.84 |
| | | | Boiled with peel | 4.07(1.07) | 4.31(0.85) | 0.00(0.00) | 0.00(0.00) | 3.74(0.72) | 12.12 | 8.39(50.06) | 6.35 | 340.94 |
| | | | Boiled no peel | 3.41(0.10) | 3.25(0.26) | 0.00(0.00) | 0.00(0.00) | 3.49(0.22) | 10.14 | 6.66(39.74) | 4.95 | 265.92 |
| | Musheba' | AAB | Raw (Stage 5)[a] | 4.62(1.31) | 10.07(3.24) | 0.00(0.00) | 0.00(0.00) | 1.36(0.15) | 16.04 | 14.68 | 12.37 | 664.45 |
| | | | Boiled with peel | 6.38(0.59) | 7.62(0.94) | 0.00(0.00) | 0.00(0.00) | 1.62(0.23) | 15.62 | 14.00(95.37) | 10.81 | 580.43 |
| | | | Boiled no peel | 6.18(1.47) | 7.72(1.87) | 0.00(0.00) | 0.00(0.00) | 1.84(0.42) | 15.73 | 13.89(94.62) | 10.80 | 580.16 |
| | | | Deep fried-palm oil | 42.93(2.03) | 84.42(3.66) | 0.00(0.00) | 22.14(0.90) | 8.12(0.57) | 157.62 | 149.50(1018.4) | 117.63 | 6316.51 |
| | | | Deep fried- veget oil | 20.02(0.20) | 31.24(0.15) | 0.66(0.31) | 2.24(0.04) | 15.68(0.20) | 69.85 | 54.16(368.9) | 42.97 | 2297.77 |
| | | | Musheba flour[b] | 11.64(0.48) | 14.43(0.01) | 0.00(0.00) | 0.00(0.00) | 0.83(0.16) | 26.90 | 26.07(177.55) | 20.25 | 1087.59 |
| Beni territory North Kivu DRC | Vulambya' | AAA-EA | Raw (stage 3) [a] | 7.90(3.99) | 7.35(3.95) | 0.00(0.00) | 0.00(0.00) | 0.12(0.06) | 15.36 | 15.25 | 11.30 | 606.69 |
| | | | Boiled with peel | 8.09(1.40) | 5.58(0.57) | 0.00(0.00) | 0.00(0.00) | 0.39(0.10) | 14.06 | 13.67(89.60) | 9.63 | 516.94 |
| | | | Boiled no peel | 5.65(2.43) | 4.33(1.83) | 0.00(0.00) | 0.00(0.00) | 0.30(0.03) | 10.27 | 9.97(65.40) | 7.15 | 383.91 |
| | Musilongo' | AAB | Raw (stage 5) [a] | 7.20(2.37) | 13.01(4.17) | 0.00(0.00) | 0.00(0.00) | 0.08(0.02) | 20.28 | 20.20 | 16.60 | 891.67 |
| | | | Boiled with peel | 16,67(0.45) | 22.87(0.88) | 0.00(0.00) | 0.00(0.00) | 0.45(0.03) | 39.98 | 39.54(195.74) | 31.20 | 1675.57 |
| | | | Boiled no peel | 18.10(2.67) | 29.76(5.66) | 0.00(0.00) | 0.00(0.00) | 0.52(0.14) | 48.38 | 47.86(239.50) | 38.81 | 2083.90 |
| | | | Deep fried-palm oil | 47.75(0.82) | 82.79(1.95) | 0.00(0.00) | 0.00(0.00) | 0.95(0.02) | 131.48 | 130.53(646.2) | 106.66 | 5727.64 |
| | | | Deep fried- veget oil | 26.75(0.84) | 43.57(1.28) | 0.00(0.00) | 0.00(0.00) | 0.80(0.03) | 71.12 | 70.32(348.1) | 56.95 | 3058.14 |
| | | | Musilongo flour[b] | 1.27(0.16) | 1.93(0.83) | 0.00(0.00) | 0.00(0.00) | 0.00(0.00) | 3.20 | 3.20(16.83) | 2.57 | 137.86 |

Values are means of three independent analyses. [a]= Reference samples for establishing retention, [b]= Results expressed in terms of dry matter, (% R) = Percentage retention, ep=edible portion, veget oil=pure vegetable oil

**Table 4.13.** Statistical difference between the content of t-BC, t-AC and total pVACs in the raw and processed Musa cultivars

4.13a. Vulambya AAA-EAHB

| | Provitamin A carotenoids in nmols/gfm | | |
|---|---|---|---|
| Processing method | t-AC | t-BC | Total pVACs |
| Raw | 10.48[c] | 9.39[c] | 20.13[c] |
| Boiled with peel | 8.09[b] | 5.58[b] | 13.67[b] |
| Boiled no peel | 5.65[a] | 4.33[c] | 9.98[a] |
| p- Values | <0.001 | <0.001 | <0.001 |

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

4.13b. Nshikazi AAA-EAHB

| | Provitamin A carotenoids in nmols/gfm | | |
|---|---|---|---|
| Processing method | t-AC | t-BC | Total pVACs |
| Raw | 6.47[c] | 6.83[c] | 13.69[c] |
| Boiled with peel | 4.07[b] | 4.31[b] | 8.38[b] |
| Boiled no peel | 3.41[a] | 3.25[a] | 6.67[a] |
| p- Values | <0.001 | <0.001 | <0.001 |

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

4.13c. Musilongo AAB plantain

| | Provitamin A carotenoids in nmols/gfm | | |
|---|---|---|---|
| Processing method | t-AC | t-BC | Total pVACs |
| Raw | 8.17[b] | 15.44[b] | 23.73[b] |
| Boiled with peel | 16.67[c] | 22.87[c] | 39.54[c] |
| Boiled no peel | 18.10[d] | 29.76[d] | 47.86[d] |
| Deep fried vegeable oil | 26.75[e] | 43.57[e] | 70.32[e] |
| Deep fried palm oil | 47.75[f] | 82.79[f] | 130.54[f] |
| Musilongo flour | 4.07[a] | 6.18[a] | 10.26[a] |
| p-Values | <0.001 | <0.001 | <0.001 |

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

4.13d. Musheba AAB plantain

| | Provitamin A carotenoids in nmols/gfm | | |
|---|---|---|---|
| Processing method | t-AC | t-BC | Total pVACs |
| Raw | 5.63[a] | 11.94[b] | 17.80[b] |
| Boiled with peel | 6.38[c] | 7.62[a] | 13.89[a] |
| Boiled no peel | 6.18[b] | 7.71[a] | 14.00[a] |
| Deep fried vegeable oil | 20.02[d] | 31.24[c] | 54.16[c] |
| Deep fried palm oil | 42.93[f] | 84.42[e] | 149.50[e] |
| Musheba flour | 37.24[e] | 46.19[d] | 83.42[d] |
| p-Values | <0.001 | <0.001 | <0.001 |

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

#### 4.4.3.2. Changes and retention of pVACs during deep frying

In the two plantains ('Musilongo' and 'Musheba'), the content of total pVACs observed following deep frying in pure vegetable cooking oil 'Golden fry' for 2 minutes was significantly higher than that observed in the raw fruits (4.12 & 4.13c,d). Although deep frying and especially for prolonged periods has been documented to result into substantial loss in pVACs (Rodriguez-Amaya, 1997). In this study, the reported higher levels of pVACs observed after deep fryingcan be supported by Rojaz-Gonzalez, Avallone, Brat, Trystram and Bohuon (2006). In addition the deep frying for only 2 minutes, can explain the difference when these findings are comparaed to those of studies on orange fleshed sweet potato (OFSP) where lower levels of retention 76-80% were reported following deep frying for 10 minutes (Bengtsson, Alminger, & Svanberg, 2008). Another reason could be that, in our study we used pure vegetable oil fortified with 2.06mg/100g of retinal, in the OFSP study, sunflower oil containing insignificant carotenoids was used (Bengtsson, Alminger, & Svanberg, 2008). Therefore, although studies have indicated loss of pVACs following deep frying (Rodriguez-Amaya, 1997; Azizah, Wee, Azizah, & Azizah, 2009), more pVACs can be retained following reduction of frying time to 2 minutes or less.

#### 4.4.3.3. Changes and retention of pVACs during processing plantain into flour

Through comparison of the total pVACs in the raw *Musa* cultivars and the two plantain flours; 'Musilongo' and 'Musheba', a loss of about 98.3% of pVACs was recorded. This means that by the time this flour is being used in porridge or the local hard paste 'Ugali' almost all the carotenoids have been destroyed. As mentioned earlier, prolonged storage,

exposure to sunlight and chopping into small pieces (and grinding into flour) leads to substantial loss in pVACs. As described in Table 3.2, processing 'Musilongo' into flour, involved peeling, chopping the bananas into small pieces fermenting for 2-3 days, direct sun drying and grinding into flour. This process that takes at least a week and it explains the very high loss of carotenoids observed. Contrary to the observations above, processing of 'Musheba' the plantain from South Kivu resulted into a loss of only 23% of the total pVACs (Table 4.11), this could be because when processing 'Musheba' into flour, some of the stages such as keeping in a warm place and allowing to ferment for 2-3 days did not take place and thus the process was shorter and less rigorous (Table 3.2).

### 4.4.3.2. Profile of pVACs in processed *Musa* fruit

Figures. 4.10 and 4.11 show the profiles of the carotenoids observed after heat processing of 'Vulambya', 'Nshikazi', 'Musheba' and 'Musilongo'.

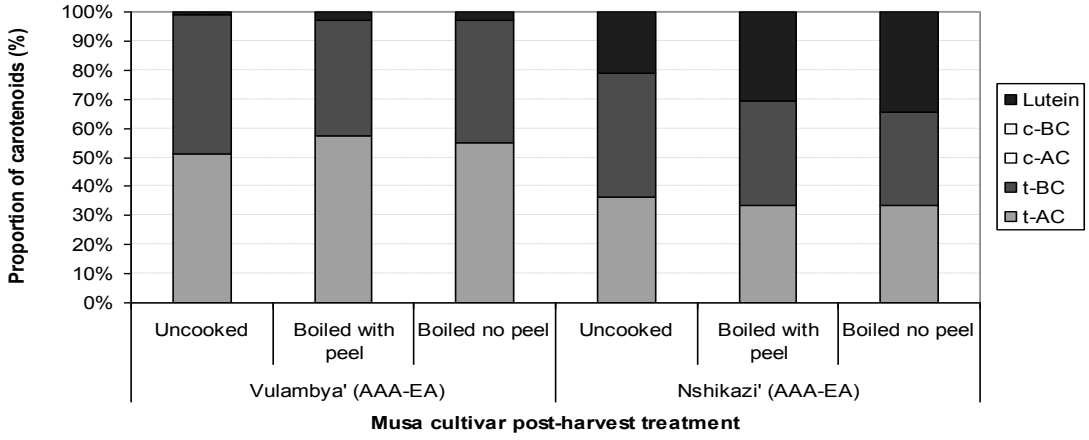


**Figure 4.10.** Overview of the variation in proportion of individual pVACs in boiled AAA-EAHB cultivars popular in Eastern Democratic Republic of Congo

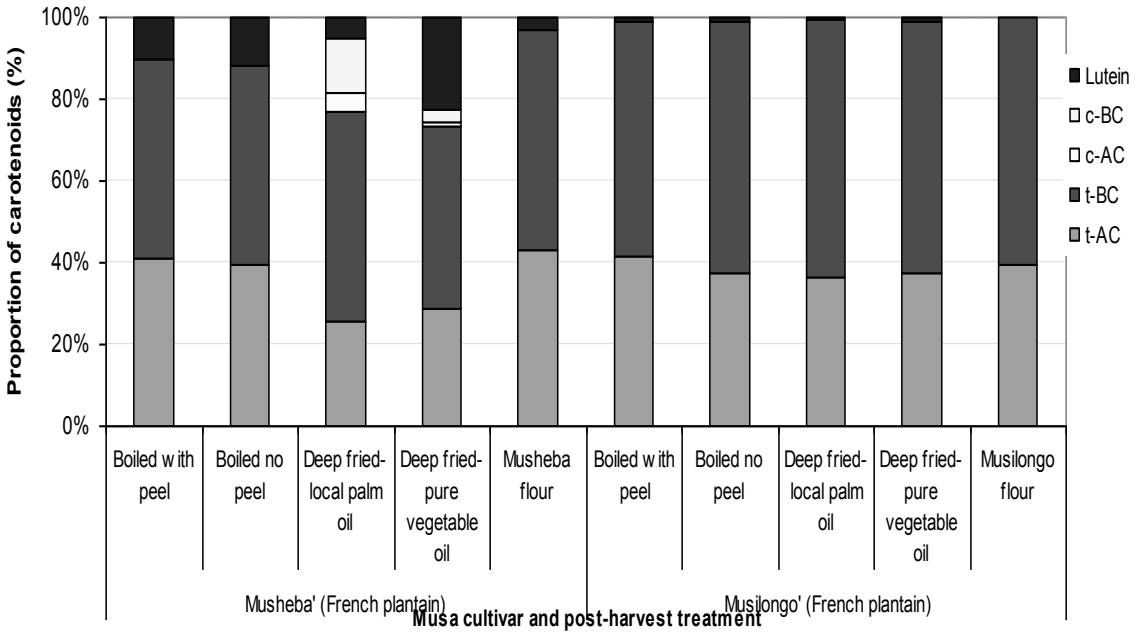


**Figure 4.11.** Overview of the variation in proportion of individual pVACs in heat processed (boiled and fried) plantain cultivars popular in Eastern Democratic Republic of Congo

Just as observed in the raw samples of the same four cultivars, the main carotenoids observed were t-AC and t-BC. These principal carotenoids constituted between 82% and 99% of the total carotenoids in 'Vulambya' and between 65% and 78% of total carotenoids in Nshikazi, the difference in proportion of the sum of main carotenoids in the two EAHBs following boiling in water was statistically significant ($p=0.015$). In the two EAHB products the proportion of t-AC to t-BC was not statistically different. The proportion of Lutein was significantly higher in 'Nshikazi' as compared to 'Vulambya' ($p=0.01$). No 13 cis-carotenoids were observed in the boiled EAHB cultivars. The proportion of t-AC to t-BC in 'Musheba' and 'Musilongo' was statistically difference ($p=<0.001$) and ($p=<0.001$) respectively, with that of t-BC almost double that of t-AC.

The deep fried Musheba had some 13 *cis*-carotenoids indicating that cooking especially under high heat destroys carotenoids, and converts transe isomers into cis isomers, which have lower vitamin A activity (Booth, Johns, & Kuhunlein, 1992).

### 4.4.4. Impact of consuming raw and processed *Musa* fruit on Vitamin A requirement of children <5yrs and women between 15-45yrs

Table 4.14 shows that at the normal ripening stages of consumption (AAA-EAHB-stage 3, plantains-stage 5), 'Nshikazi', and 'Vulambya', had RAE values ranging from 45.99 µgRAE/100gFM to 66.09 µgRAE/100gFM while 'Musheba' and 'Musilongo' had RAE values ranging from 66.59µgRAE/100gFM to 87.7µgRAE/100gFM. In all the four cultivars the highest RAE values were reported during ripening stage 3 (Table 4.14). In the boiled *Musa* fruits the RAE values ranged from 22.26 µgRAE/100gFM to 173.66µgRAE/100gFM, while the RAE values in the plantains deep fried in local palm oil was as high as 526.38 µgRAE/100gFM. Following the consumption levels of *Musa* fruit recommended by Englberger et al (2006a), the two plantains 'Musilongo' and 'Musheba' are good sources of vitamin A while the two AAA-EAHB cultivars 'Nshikazi' and 'Vulambya' also have considerable values of vitamin A.

According to FAO/WHO (2002), a child aged 1-5years and a woman of reproductive age[2] needs 400µg and 700 µg of vitamin A per day respectively. Following the findings of the focus group discussions, the cultivars tested in this study are normally processed into various forms ranging from boiling, frying, roasting, to grinding into flour before consumption.

[2] Woman of reproductive age is referring to a non-pregnant, non-lactating woman aged 15-45years

**Table 4.14.** Beta carotene equivalent (BCE), retinal activity equivalent (RAE) and % contribution to recommended daily allowances (RDAs) of vitamin A for a child 1-5years consuming 200g (at least 2 fingers) of fruit and an adult woman consuming 500g (at least 4 fingers) of fruit.

| Origin | Local Name | Ripening Stage | Total BCE µg/100gFM | RAE in µg/100gFM | % RDA child <5yrs 100g ep | 250g ep | % RDAs woman 100g ep | 500g ep |
|---|---|---|---|---|---|---|---|---|
| Bukavu South Kivu DRC | Nshikazi | Raw stage 1 | 375.66 | 31.30 | 7.83 | 19.57 | 4.47 | 22.36 |
| | | Raw stage 3 | 551.92 | 45.99 | 11.50 | 28.75 | 6.57 | 32.85 |
| | | Raw stage 5 | 305.95 | 25.50 | 6.37 | 15.93 | 3.64 | 18.21 |
| | | Raw stage 7 | 577.74 | 48.15 | 12.04 | 30.09 | 6.88 | 34.39 |
| | | Boiled no peel | 265.92 | 22.26 | 5.54 | 13.83 | 3.17 | 15.83 |
| | | Boiled in-peel | 340.94 | 28.41 | 7.10 | 17.76 | 4.06 | 20.29 |
| | Musheba | Raw stage 1 | 316.05 | 26.34 | 6.58 | 16.46 | 3.76 | 18.81 |
| | | Raw stage 3 | 1022.37 | 85.20 | 21.30 | 53.25 | 12.17 | 60.86 |
| | | Raw stage 5 | 799.12 | 66.59 | 16.65 | 41.62 | 9.51 | 47.57 |
| | | Raw stage 7 | 500.13 | 41.68 | 10.42 | 26.05 | 5.95 | 29.77 |
| | | Boiled no peel | 580.16 | 48.35 | 12.09 | 30.22 | 6.91 | 34.53 |
| | | Boiled in-peel | 580.43 | 48.37 | 12.09 | 30.23 | 6.91 | 34.55 |
| | | Deep fried palm oil | 6316.51 | 526.38 | 131.59 | 328.98 | 78.20 | 375.98 |
| | | Deep fried refined oil | 2297.77 | 191.48 | 47.87 | 119.68 | 27.35 | 136.77 |
| | | Musheba flour | 1087.59 | 90.63 | 22.66 | 56.65 | 12.95 | 64.74 |
| Beni North Kivu DRC | Musilongo | Raw stage 2 | 1210.37 | 100.86 | 25.22 | 63.04 | 14.41 | 72.05 |
| | | Raw stage 3 | 1515.87 | 126.32 | 31.58 | 78.95 | 18.05 | 90.23 |
| | | Raw stage 5 | 1051.96 | 87.66 | 21.92 | 54.79 | 12.52 | 62.62 |
| | | Raw stage 7 | 1161.86 | 96.82 | 24.21 | 60.51 | 13.83 | 69.16 |
| | | Boiled no peel | 2083.90 | 173.66 | 43.41 | 108.54 | 24.81 | 124.04 |
| | | Boiled in-peel | 1675.57 | 139.63 | 34.91 | 87.27 | 19.95 | 99.74 |
| | | Deep fried in palm oil | 5727.64 | 477.30 | 119.33 | 298.31 | 68.19 | 340.93 |
| | | Deep fried refined oil | 3058.14 | 254.84 | 63.71 | 159.28 | 36.41 | 182.03 |
| | | Musilongo flour | 137.86 | 11.49 | 2.87 | 7.18 | 1.64 | 8.21 |
| | Vulambya | Raw stage 1 | 403.52 | 33.63 | 8.41 | 21.02 | 4.80 | 24.02 |
| | | Raw stage 3 | 793.10 | 66.09 | 16.52 | 41.31 | 9.44 | 47.21 |
| | | Raw stage 5 | 682.91 | 56.91 | 14.23 | 35.57 | 8.13 | 40.65 |
| | | Raw stage 7 | 648.27 | 54.02 | 13.51 | 33.76 | 7.72 | 38.59 |
| | | Boiled no peel | 383.91 | 31.99 | 8.00 | 20.00 | 4.15 | 30.77 |
| | | Boiled in-peel | 516.94 | 43.08 | 10.77 | 26.92 | 6.15 | 36.11 |

Values are means of three independent analysess; DRC= Democratic Republic of Congo; ep=Edible portion

In addition, consumption of 250g (approximately 2 fingers) of cooked *Musa* fruit by a child below five years and 500g (approximately 3 fingers) by a woman of reproductive age is considered to be within normal consumption levels (Rodriguez-Amaya, 1997).

Nutrients compete with other nutrients for absorption and some nutrients will enhance/reduce the amounts of other nutrients being absorbed by the body (Chetyrkin, 2000). In addition, the health status of an individual will definitely affect the amount of nutrient eventually available for utilisation by the body (Chetyrkin, 2000). Despite these factors the the contribution of the tested *Musa* products to the daily vitamin A RDAs was calculated with the assumption that bioaccessibility and bioavailability of these pVACs is optimal. Therefore within the stated normal consumption levels, the boiled AAA-EAHB cultivars tested ('Vulambya' and 'Nshikazi') would meet between 14% and 27% of the daily vitamin A recommended dietary allowances (RDAs) of a child 12 – 59 months and between 16% and 36% of the daily Vitamin A RDAs of a woman of reproductive age. The boiled plantains would meet at least 30% of the daily vitamin A RDAs of the same child and woman. Just 100g of the plantains whether deep fried in local palm oil or ordinary cooking oil would meet more than 100% of daily vitamin A RDAs for a child 12-59months and a woman of reproductive age (Table 4.14). Therefore within normal consumption levels both a preschool child and a non-pregnant non-lactating woman, would receive significant amounts of vitamin A from consuming any of the four *Musa* cultivars tested. These findings confirm that there already exist *Musa* cultivars with sufficiently high pVACs contents to immediately have a noticeable impact on population at risk of VAD at modest and realistic consumption levels (Englberger et al., 2006b).

## 4.5. Bioaccessibility of provitamin A carotenoids (pVACs) from local dishes made from cooking banana and plantain (*Musa* spp.) consumed in the Democratic Republic of Congo

### 4.5.1. Content of Provitamin A carotenoids (pVACs) in freshly prepared Musa products and *Musa*-based dishes

The carotenoids content in the dishes derived from 'Musilongo' and 'Vulambya' were represented by three *main* pVACs: (t-BC), (t-AC) and 13-*cis*- β-carotene. Lutein was also detected but in traces.The difference in carotenoids content observed between *Musa* cultivars boiled with or without peel were not statistically significant (p=0.05). However, there were significant differences between the carotenoids profiles from 'Musilongo' and 'Vulambya', with the plantain 'Musilongo' having higher levels of all three pVACs than the EAHB 'Vulambya'. In 'Musilongo', the proportion of t-BC (49%) was significantly higher than the t-AC (37%); in contrast, in 'Vulambya', the proportion of t-BC represented 36% compared to 56% t-AC (Table 4.15).

Among the *Musa*-based dishes, 'Musilongo' porridge had the lowest content of both total specific pVACs (7µg/100gdw) and specific pVACs. This low content can be explained by the double processing, first into flour involving fermentation, sun-drying and grinding, and then into porridge (Table 3.3b). In addition, during the making of porridge the ratio of flour to water was 1:11.5, i.e. 30g of flour was put into 320ml of water, thus the flour constituted only about 8%. Just as observed in 4.4.3.3., the flour had a total pVACs content of 333µg/100gdw compared to 7593 µg/100gdw observed in raw 'Musilongo', indicating that the pVACs present in the flour are very low, even before preparation of the porridge.

**Table 4.15.** Content of pVACs in freshly processed *Musa* products and *Musa*-based dishes consumed in Eastern Democratic Republic of Congo

| *Mush* product/Dish | Content of pVACs[1] (µg/gFM) in freshly prepared Products and *Musa* dishes | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | All-*trans*-β-carotene | | α-carotene | | 13 *cis*-β-carotene | | Total | RAE in | RDA child | RDA-FA |
| | Mean (ratio) | SD | Mean(ratio) | SD | Mean(ratio) | SD | pVAC | µg/100g | 200g/day | 500g/day |
| Musilongo boiled in peel | 5.75(49.4%) | 0.09 | 4.27(36.7%) | 0.44 | 1.62(13.9%) | 0.05 | 11.64 | 72.9 | 36.50% | 52.07% |
| Musilongo boiled without peel | 5.93(51.3%) | 0.06 | 4.03(34.9%) | 0.12 | 1.59(13.8%) | 0.03 | 11.55 | 73.4 | 36.70% | 52.42% |
| Vulambya boiled in peel | 1.39 (35.6%) | 0.14 | 2.19 (56.2%) | 0.07 | 0.32 (8.2%) | 0.02 | 3.9 | 22 | 11.00% | 15.71% |
| Vulambya boiled without peel | 1.19 (35.8%) | 0.01 | 1.85 (55.7%) | 0.06 | 0.28 (8.4%) | 0.01 | 3.32 | 19.2 | 9.60% | 13.70% |
| Musilongo flour | 1.76 (52%) | 0.08 | 1.42 (46%) | 0.07 | 0.15(4.5 %) | 0.04 | 3.33 | - | - | - |
| Musilongo porridge | 0.07 (53.8%) | 0.01 | 0.06 (46.2%) | 0.01 | 0.00 (00.0%) | 0 | 0.13 | 0.83 | 0.41% | 0.60% |
| Musilongo boiled olive oil | 4.51 (48.7%) | 0.21 | 3.36 (36.3%) | 0.16 | 1.39 (15.0%) | 0.05 | 9.26 | 57.6 | 28.30% | 41.14% |
| Musilongo boiled palm oil | 11.5 (52%) | 0.3 | 7.22 (32.6%) | 0.15 | 3.36 (15.2%) | 0.03 | 22.08 | 139 | 69 .5 % | 99.00% |
| Vulambya, Beans | 0.80 (33.5%) | 0.05 | 1.41 (59.0%) | 0.16 | 0.18 (07.5%) | 0.02 | 2.39 | 13.4 | 6.70% | 9.57% |
| Vulambya, beans, olive oil | 0.71(33.2%) | 0.01 | 1.10 (51.4%) | 0 | 0.33 (15.4%) | 0.04 | 2.14 | 11.9 | 5.95% | 8.57% |
| Vulambya, beans, palm oil | 7.45 (55.6 %) | 0.13 | 4.40 (32.8 %) | 0.07 | 1.54 (11.5) | 0.05 | 13.39 | 86.82 | 43.41 | 62.01% |
| Vulambya, beans, amaranth | 6.68 (62.4%) | 0.15 | 2.26 (21.1%) | 0.35 | 1.77 (16.5%) | 0.04 | 10.71 | 72.8 | 36.40% | 52.00% |
| Vulambya, beans, amaranth, olive oil | 8.62 (68.4%) | 0.09 | 2.47 (19.6%) | 0.07 | 1.51(12.0%) | 0.03 | 12.6 | 88.69 | 44.35% | 63.35% |

Values are means of three independent determinations (percentage proportion of the specific carotenoids in relation to total provitamin A carotenoids), SD=standard deviation. RAE= Retinal Activity Equivalent= µg *trans* β-carotene /12 and 13 *cis*- β-carotene /24. FA=Female Adult. (p=0.05)

Other ingredients used in the dishes included fresh beans, amaranth leaves and the occasional addition of olive or palm oil during the boiling process. The addition of olive oil to the boiled banana did not lead to any significant change in the content of total carotenoids, this could be because according to the bottle label the olive oil contained no carotenoids or vitamin A. Addition of beans did not also change the carotenoids content, this was expected as testing of the beans as a single ingredient showed that the beans did not contain any carotenoids.

In the contrast, addition of amaranth leaves led to an increase in the content of total pVACs and especially t-BC in the dish [80µg/100gFM of t-BC for Vulambya with beans compared with 668 µg/100gFM in Vulambya with beans plus amaranth]. This is due to the reported high level of t-BC in amaranth leaves as reported by Grubben and Denton, (2004). Similar trends were observed when palm oil was added during boiling [451 µg/100gFM of t-BC for boiled 'Musilongo' as compared to 1150 µg/100gFM of t-BC when palm oil was added to the 'Musilongo' during boiling; 80 µg/100gFM of t-BC for 'Vulambya' with beans as compared to 745 µg/100gFM observed when palm oil was added to 'Vulambya' during boiling]. As extensively documented, palm oil is well known for its high level of provitamin A carotenoids (Nestel & Nalubola, 2003; Monde, Michel, Carbonneau, Tiahou, & Vernet, 2009). Therefore although the addition of beans and/or olive oil does not significantly enhance the content of pVACs, the addition of amaranths and/or palm oil to boiled *Musa* fruit leads to significant increase (>200%) in content of pVACs especially t-BC.

### 4.5.2. Contribution of the *Musa* products and *Musa*-based dishes to Recommended Daily allowances of vitamin A

The estimated RAE for the tested *Musa*-based dishes and their potential to contribute to the nutritional requirements of children below five years and women of reproductive age are reported in Table 4.13. Boiled 'Vulambya' and 'Musilongo', had RAE levels ranging from 19.2 - 22.0 µg/100gFM and from 72.9 - 73.4 µg/100gFM respectively, while the 'Musilongo' porridge had negligible RAE values. Consumption of 200g and 500g of boiled *Musa* fruit by a child below 5 years and a woman of reproductive age respectively is within normal consumption levels (Rodriguez-Amaya, 1997).

With these consumptions levels, boiled 'Musilongo' would meet between 36% and 52% of the daily vitamin A RDAs of a child 1-5 years old and a woman of reproductive age, respectively. Consumption of the same quantities of boiled 'Vulambya' would meet only 10-11% and 14-16% of the daily vitamin A RDAs needed by the same child and the same woman, respectively. Boiling the *Musa* fruit together with beans did not have any effect on the RDAs, but addition of amaranth leaves and/or palm oil to the *Musa* fruit during boiling led to a significant increase in the contribution of the dish to daily vitamin A RDAs. Within the earlier stated normal consumption levels, boiling of 'Vulambya' in fresh beans, amaranth leaves and olive oil would meet 44.35% and 63.35% of the daily vitamin A RDAs of a child of 1-5 years and a woman of reproductive age respectively, while consumption of 'Musilongo' boiled with or without olive oil would meet 28.8-36.7% and 41.1-52.4 % of the vitamin A RDA of the same child and the same woman, respectively (Table 4.15).

### 4.5.3. Bioaccessibility of pVACs from freshly prepared *Musa* products and *Musa*-based dishes

The percentage micellarization of t-BC, t-AC, and 13-*cis*-BC are reported in Table 4.16. The level of bioaccessible t-BC was similar to that of t-AC, while more pVACs were micellarized (bioaccessible) from boiled 'Vulambya' (28.9%) as compared to boiled 'Musilongo' (16.61%). Bioaccessibility of 13-*cis* BC was significantly higher (33% and 21% for boiled 'Vulambya' and 'Musilongo' respectively). These observations were previously reported by several authors with different types of food (Failla, Thakkar, & Kim, 2009; Bengtsson, Alminger, & Svanberg, 2009; Reboul, Richelle, Perrot, Desmoulins-Malezet, Pirisi, & Borel, 2006). Addition of an oral phase with a concentration of artificial salivary optimal in regard of physiological conditions had no effect on the bioaccessibility of pVACs from boiled *Musa* cultivars in this *in vitro* digestion model ($p=<0.05$).

Although the t-BC and t-AC levels in 'Musilongo' porridge were very low, they were significantly more bioaccessible as compared to those from boiled 'Musilongo'. The difference in bioaccessibility could be explained by the fact that in porridge, micellarization of carotenoids was facilitated by the dissentegrated food matrix (liquid form). However, despite the relatively high bioaccessibility, the very low initial levels of pVACs in 'Musilongo' porridge make that this food insufficient in provision of vitamin A.

**Table 4.16.** Bioaccessibility of pVACs in freshly processed *Musa* products and dishes consumed in Beni territory, Eastern Democratic Republic of Congo

| Musa based products and dishes | BC % Bioac (SD) | AC % Bioac (SD) | 13 cis-BC % Bioac (SD) | µg RE* /200g after in vitro dig.. | %RDA child < 5 years |
|---|---|---|---|---|---|
| Musilongo no oral phase | 16.6 (2.7)[a] | 15.5 (1.8)[a] | 21.5 (1.3)[a] | 48.88 | 12.2 |
| Musilongo with oral phase | 14.1 (0.4)[a] | 15.2 (1.0)[a] | 23 (1)[a] | - | - |
| Vulambya no oral phase | 28.9 (1.0)[b] | 30.5 (0.8)[be] | 33 (2)[b] | 22.38 | 7 |
| Vulambya with oral phase | 28.7 (1.8)[b] | 28.6 (1.2)[be] | 33.5 (1.2)[b] | - | - |
| Musilongo porridge | 31 (1)[bd] | 41 (1)[c] | Nd | - | - |
| Musilongo olive oil | 14.6 (2.1)[a] | 14 (1.8)[a] | 23.7 (2.0)[a] | 35.2 | 8.8 |
| Musilongo palm oil | 15.3 (1.8)[a] | 13.8 (1.6)[a] | 20 (2)[a] | 86.44 | 21.6 |
| Vulambya and Beans | 24 (2)[c] | 23.5 (2.5)[d] | 16.2 (2.2)[c] | 12.4 | 3.1 |
| Vulambya, beans, olive oil | 27.5 (3.3)[b c] | 26(3.6)[e] | Nd | 5.63 | 1.4 |
| Vulambya, beans, palm oil | 32 (3)[d] | 31.1(3.3)[b] | 34.4 (4.2)[b] | 110.08 | 27.75 |
| Vulambya, beans, Amaranth | 10.2 (0.2)[e] | 22.7 (1.6)[d] | 21.6 (1.3)[a] | 38.96 | 9.74 |
| Vulambya, beans,amaranth,olive oil | 15.8 (1.9)[a] | 23.7 (2.7)[d] | 22 (2.1)[a] | 60.66 | 15 |

BC= β-carotene, AC= ά-carotene, * RE= retinol equivalent = µg trans β-carotene /6 and 13 cis- β-carotene /12; n≥3 independent experiments presented as means (SD); nd, not detected; Significant differences in the same column are shown by different letters ($p<0.05$)

The addition of olive oil, when boiling 'Musilongo', did not significantly increase the bioaccessibility of pVACs. This could be explained by the preparation method, where oil was added to the water during the boiling process and the banana fruits were subsequently removed from the oil and water mixture. As such, the oil can be assumed to have been discarded with the cooking water and was hence not incorporated into the food. Similar results with respect to bioaccessibility were observed when palm oil was added to boiled 'Musilongo' (no increase in bioaccessibility), with the difference that the initial pVACs content was greatly increased, resulting in a Retinol Equivalent of 86 µg providing 21.6% of the RDA for children.

In the 'Vulambya'-based dishes, the pVACs bioaccessibility increased slightly in the presence of olive and palm oil. This could be explained by the difference in the food

matrix and texture of boiled ‘Musilongo’ and boiled ‘vulambya’, The boiled ‘vulambya’ was soft and the particles seemed to dis-intergrate when pressed between the thumb and the fore finger, while the boiled ‘Musilongo’ was firm and seemed to keep its form and it was even harder to grind and homogenise. This made the oil was better incorporated into the ‘Vulambya’ dish probably improving micellarization of carotenoids. When amaranth leaves were added to the ‘Vulambya’ and beans dish the bioaccessibility particularly for β-carotene decreased from 24% to 10.2%. This was explained by the fact that the majority of t-BC, came from amaranth leaves, 6.7 µg/g compared with 0.8 µg/g for the dish ‘Vulambya’ and beans (Table 14.16). It has also been documented that the bioavailability of β-carotene is higher for *Musa* fruits as compared to green leafy vegetables (Van Het Hof, West, Weststrate, & Hautvast, 2000; Dee Pee, West, Permeisih, Martuti, & Hautvast, 1998) due to the localization of carotenoids in the food matrix. Carotenoids from vegetables are found in chloroplasts bound to protein and fiber and the release from the food matrix is difficult which can explain the differences compared to bioaccessibility of fruits, where carotenoids are in chromoplasts dissolved in oil droplets (O’Connell, Ryan, & O’Brien, 2007). In the dish ‘Vulambya’-bean-amaranth dish, the main β-carotene from amaranth leaves resulted in a lower bioaccessibility of carotenoids. The addition of olive oil slightly improved the bioaccessibility of β-carotene.

Amongst the different *Musa*-based dishes analyzed, the two dishes with palm oil (plantain ‘Musilongo’ boiled without the fruit peel and palm oil, and the AAA-EA ‘Vulambya’ boiled with beans and palm oil) were the most interesting in terms of their potential to contribute to meeting the daily vitamin A RDAs of target populations. These

were followed by 'Vulambya' boiled with beans, amaranth leaves and olive oil, and boiled 'Musilongo'.

Estimation of the vitamin A activity in *Musa*-based dishes calculated from an undigested food using a classical estimate (RAE) or calculated from a digested food (RE) taking into account bioaccessibility, leads to different results (Figure 4.12).

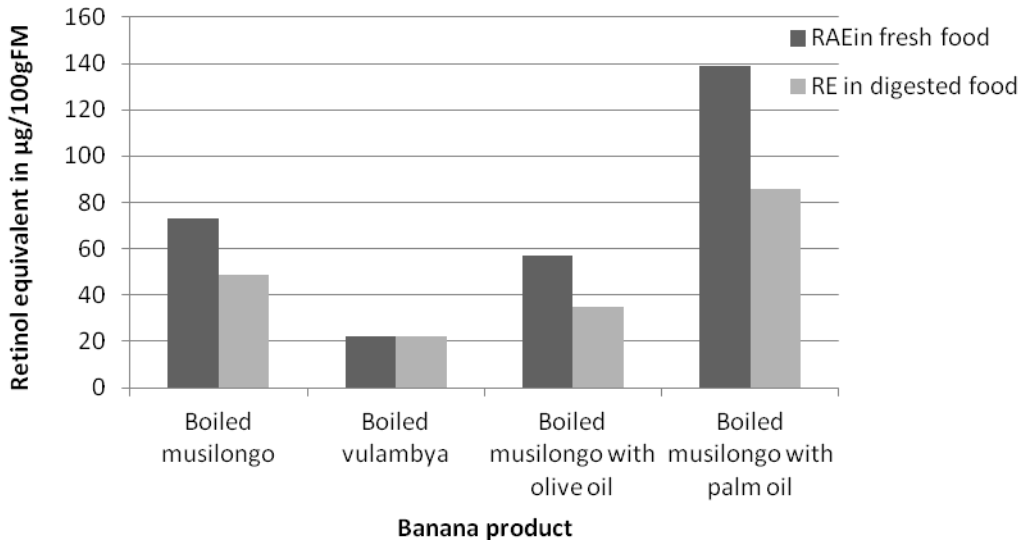


**Figure 4.12.** Retinal equivalent expressed in RAE1 or RE2 per 100g in the freshly prepared and digested *Musa* products. 1 RAE: retinol activity equivalent= µg trans β-carotene /12 and 13 cis- β-carotene /24; 2 RE : retinol equivalent = µg trans β-carotene /6 and 13 cis- β-carotene /12

Classical estimates from undigested food overestimate RAE compared with estimates from digested food. These last results suggest that it is very important to take into account the t-BC bioaccessibility of *Musa*-based dishes in carotenoids absorption and consequently in estimations of the vitamin A activity to meet nutritional requirements.

# CHAPTER V: SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

## 5.1. Summary of the findings

The purpose of this study was to describe the dietary patterns/diversity with regards to *Musa* fruit consumption, establish nutrition and health status and assess the retention and bioaccessibility of pVACs in *Musa* fruit and post-harvest products consumed by households in Eastern DRC. This research project included both cross-sectional surveys and experimental studies. Cross-sectional survey was to establish the most common banana cultivars, banana products, dietary patterns and nutrition status. Experimental studies involved; a) HPLC analysis to determine the retention of pVACs in *Musa* fruit during ripening and after processing using local procedures and b) *In vitro* bioaccesisbility studies to determine the amount of pVACs that are micelarised and available for absorption from popular *Musa*-based dishes.

Study sites included Beni territory (North Kivu) and Bukavu territory (South Kivu) in Eastern Democratic Republic of Congo (DRC). Households with preschool children were used to give a general indication of the population. The localities, villages and specific households that were included in the preliminary survey were established through multistage sampling techniques. In Beni territory, Mabuku, Kisungu and Rwakhwa localities were randomly sampled and in Bukavu territory, the localities randomly sampled were Kajeje, Murhesa and Miti. A listing of all households with preschool children in each locality was compiled and systematic random sampling was used to select the specific households to be interviewed. Sample size was calculated using Fisher's formula. A total of 14 focus group discussions were carried out (8-SK and 6-

NK) and 371 households (208-SK and 163-NK) with preschool children visited and mothers/care-givers interviewed. Through local scientists, samples of the identified most popular *Musa* cultivars were obtained from farmer fields and transported within 48hours of harvest at room temperature to IITA laboratory at Namulonge-Kampala. A sub-sample of the bananas was collected at ripening stage 1, 3, 5 and 7 based on peel color and another sub-sample processed into two most common banana products for each cultivar. All the samples were then frozen at $-20^0$c and lyophilised. Lyophilised samples were subsequently transported to the Laboratory of Fruit Breeding and Biotechnology at Katholiek University of Leuven (Belgium) for pVAC analysis using standardised procedures developed for banana.

For bioacessibility studies, banana samples were obtained from farmer fields in Eastern DRC, together with the ingredients needed to prepare the identified common dishes. Samples were then transported as hand luggare by air to the CIRAD laboratory in Montpellier France. The products were prepared following procedures given by community members, and the pVACs content in the products and dishes verified using HPLC. Bioaccessibility was estimated using an *in vitro* digestion method that simulates conditions in the human gastrointestinal tract.

Findings showed that the preferred cooking banana varieties included yellow-pulped AAA-East African Highland bananas [EAHB] ‘Nshikazi’ (SK) and ‘Vulambya’ (NK), valued for their cooking qualities, large bunches and suitability for production of banana beer. Preferred plantains were orange-pulped and included ‘Musheba’ (SK) and ‘Musilongo’ (NK) reasons for preference were short maturation period, large bunches

and higher prices. Over 60% of households had consumed EAHB within the last 24 hours, whereas <10% had consumed plantains. The common cooking method was simple boiling of bananas/plantains and main accompaniments include beans and amaranth leaves. Most of the households (90%) obtain banana/plantains from their farms, >55% of households from SK and NK consume banana products between 2-4 times/week.

The predominant pVACs were all-*trans* β- and all-*trans* α-carotene, together constituting about 90% of total pVACs in both raw and processed fruit pulp. The proportion of β-carotene was around twice that of α-carotene in the plantains varieties 'Musilongo' and 'Musheba', while in the East African Highland Bananas ('Nshikazi' and 'Vulambya'), the proportion was almost equal. Total pulp pVACs were found to increase significantly during ripening and highest levels were observed at ripening stage 3 in all four cultivars. Values were as high as 1080.98µg/100gFM in 'Vulambya' and 1819.38µg/100gFM in 'Musilongo'. Although boiling of AAA-EAHB cultivars led to substantial losses (40%-60%) in total pVACs, boiling and deep frying of the plantains led to retention and an apparent increase.

After *in-vitro* digestion, the percentage of micellarized t-BC was higher in 'Vulambya' (29 %) than in 'Musilongo' (16.6 %). But because 'Musilongo' had a higher initial content of pVACs than 'Vulambya', the final retinol equivalent was higher in 'Musilongo' (44.9 µg/200gFM) than in 'Vulambya' (22.4 µg/200gFM). In the two *Musa* cultivars, the incorporation into micelles was similar for t-BC and t-AC but significantly higher for 13-*cis* isomer. Addition of other ingredients such as palm oil, amaranths and beans to the boiled *Musa* fruit affected both bioaccessibility of pVACs and the retinol

equivalent. The best performing *Musa*-based dishes made from 'Musilongo' and 'Vulambyo' provided an estimated 22% and 28% of the daily vitamin A Recommended Dietary Allowance (RDA) for a child under 5 years old. Although it is necessary to carry out retention and bioaccessibility studies on a large number of *Musa* cultivars basing on the existing diversity of the cultivars, the findings from this study indicate that with appropriate post-harvest practices (storage, cooking, dietary combination), the tested cultivars can substantially contribute to vitamin A requirements of vulnerable groups.

### 5.2. Implications of the findings

These findings have shown that although there is a significant increase in the level of provitamin A carotenoids as the *Musa* fruit moves from unripe to ripe, as the fruit progresses from ripe to over-ripe there is a decrease in the total pVACs. This therefore implies that to maximize pVACs intake from *Musa* foods, communities should be encouraged and supported in developing best-practices regarding post-harvest handling, processing/cooking and diversification of diets based on bananas and plantains.

Despite the negligible levels of pVACs observed in the 'Musilongo' porridge, the very positive level of bioaccesibility from this dish implies that with appropriate flour processing techniques, that should probably involve drying under a shade and eliminating the fermentation stage, this plantain porridge would be able to substantially contribute to the daily vitamin A RDAs of vulnerable groups.

## 5.3. Conclusions

Bananas (cooking banana and plantains) are the second most popular starchy staples consumed by small holder households in SK and NK of Eastern DRC. 'Nshikazi' (AAA-EAHB) and 'Musheba' (AAB-French plantain) are the most popular and preferred *Musa* cultivars in South Kivu while 'Vulambya' (AAA-EAHB) and 'Musilongo' (AAB-French plantain) are the most popular and preferred *Musa* cultivars in North Kivu. The AAA-EAHB cultivars ('Vulambya' and 'Nshikazi') are preferred because they produce big bunches, taste better when cooked and they make good banana beer. The plantain cultivars ('Musheba' and 'Musilongo') are preferred because they mature faster, produce big bunches and have high market prices.

Of the three common *Musa* fruit classifications (cooking banana, plantain banana and desert banana); cooking bananas are the most consumed, having a consumption rate of more than 65% in both North Kivu and South Kivu.

Although the *Musa* fruit is normally processed/cooked using various methods such as boiling (with/without peel), steaming, roasting, drying and milling into flour, the most popular cooking method applied to the *Musa* cultivars consumed in Eastern DRC is simply boiling without peel. More than 50% of the households consume boiled bananas between two and four times per week, with 20% of the households consuming it on daily basis and 90% of the bananas are obtained from the household's farm.

The principal pVACs in popular bananas and plantains consumed in Eastern DRC are trance β-carotene (t-BC), trance α-carotene (t-AC) and 13-*cis*- β-carotene, with the

plantains ‘Musilongo’ and ‘Musheba’ having higher levels of all three pVACs than the EAHB ‘Vulambya’ and ‘Nshikazi’. In Plantains the proportion of trance β-carotene (t-BC) is almost double that of trance α-carotene (t-AC), while in the EAHB cultivars the proportion of trance β-carotene (t-BC), trance α-carotene (t-AC) is almost equal. Therefore following the difference in RAE between t-BC and t-AC, plantains are better sources of carotenoids.

During post-harvest maturation (ripening) of ‘Musheba’, ‘Musilongo’, Vulambya’ and ‘Nshikazi’, there is not only retention of the pVACs in the fruit at the time of harvest but also a significant increase with the highest levels observed when the fruit is beginning to ripen (stage 3). The popular *Musa* cultivars in Eastern DRC have between 46 RAE µg/100gFM and 87.7 RAE µg/100gFM when raw.

While boiling of plantains leads to retention of almost all the pVACs, as observed in Musheba (95%), and even an increase in the pVACs as observed in ‘Musilongo’, boiling of ‘Vulambya’ and ‘Nshikazi’ for 15 minutes leads to a substantial loss in the pVACs. Deep frying of the plantains leads to not only maximum retention but also increase in the pVACs.

Bioaccessibility (micellarization) of t-BC is significantly more efficient in the boiled AAA-EAHB ‘Vulambya’ (28.9%) than in the plantain ‘Musilongo’ (16.61%). However, taking into account the lower initial levels of pVACs, observed in ‘Vulambya’ final Retinol Equivalent (22.4µg.200g$^{-1}$) as compared to ‘Musilongo’ (48.9 µg.200g$^{-1}$), plantains remain better sources of vitamin A. With respect to the different carotenoids species, the bioaccessibility of t-BC and t-AC are similar (29 and 17% for t-BC, and 31

and 16% for t-AC, respectively in boiled 'Vulambya' and 'Musilongo') while that of 13-*cis* BC are higher (33 and 21% respectively).

Within normal consumption levels described by Rodriguez-Amaya, (1997), 'Vulambya' and 'Nshikazi' would meet between 14% and 40% of the daily Vitamin A RDAs of a child 12-59months and a woman of reproductive age. Boiled 'Musilongo' and 'Musheba' would meet between 30% and 108% of the daily vitamin A RDAs of the same child and woman. Just 100g of deep fried 'Musilongo'/'Musheba' would meet more than 100% of daily vitamin A RDAs for a child 12-59months, and a non-pregnant or non-lactating woman.

### 5.4. Recommendations for policy

Poor policies have greatly affected the food security, nutrition status and general livelihood status of populations in Africa. A need to direct policies towards agricultural research especially on how to sustainably exploit the much diversified *Musa* species with an objective of increasing food security, and elevating the incomes of small holder households should be realized. Emphasis should be on policies that exploit synergies for better nutrition between increased agricultural production and an improved nutrition and health status and at the same time allow the two related ministries (agriculture and health) to act as independently as possible. Integrated interventions linking health, nutrition and food security components should be the future focus in DRC for providing the most sensible approach to address the needs of the affected population.

## 5.5. Recommendations for further research

The findings of this study should be disseminated back to stakeholders in the target community. These stakeholders include local NGOs, government representatives, community change agents and more importantly the community members. The findings should be backed up with awareness creatioin on practices that promote carotenoids retention and bioaccessibility.

There is need to explore this research further by 'establishing the optimal processing/cooking precudures that would lead to maximum retention, increase and bioaccessibility of provitamin A carotenoids in popular *Musa* cultivars.' This calls for testing the same processing/cooking methods with different variations in liquids used (water/oil) and cooking times i.e. boiling for 10 minutes, 15 minutes and 20 minutes; steaming for 30 minutes, 1 hour, 11/2hours, 2 hours; deep frying for 1 minute, 2 minutes, 4 minutes; stir frying for 2 minutes, 4 minutes, 6 minutes. This will help in further passing on the best practises with regards to cooking/processing for better health and nutrition outcomes.

This study took into consideration only the two popular plantains and two popular cooking bananas (AAA-EAHB), and only evaluated provitamin A carotenoids. There are over 20 *Musa* cultivars in Eastern DRC (Dowiya et al., 2009) and research has indicated that there is always nutrient-nutrient interaction i.e. an increase in dietary fat content increases carotene absorption and protein deficiency decreases intestinal absorption of vitamin A (Caballero, 1988). There is a need to therefore carry out an

evaluation of more cultivars and take into consideration the nutrition profile of both macronutrients and micronutrients.

More investigations are still needed to evaluate carotenoid bioaccessibility from different local *Musa*-based dishes prepared from a wider range of cultivars currently consumed in *Musa*-dependent regions within east and central Africa.

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# APPENDICES

## 1. STUDY AREA MAP

## 2. FOCUS GROUP DISCUSSION (FGD) CHECKLIST

**Objective; To obtain information on food crops grown by the community, most popular banana cultivars within the community, the most common processing/cooking procedures and to generate a community food list**

## Instructions:

**The participants of the focus group discussions (FGDs) was local farmers, community leaders and women of child bearing age. Through the contact persons in each of the sites, the participants was invited to a common ground i.e. Health facility or local administrative office. Each group discussion will have between 10-20 participants and they was divided according to gender and if necessary age. Each site should have minimum 2 groups and maximum 4 groups.(The participants was welcomed to the exercise and thanked for their participation, they was informed that no answer is right or wrong, and probing was done to get more details. All information was recorded on flip charts by the field assistant)**

### A. GENERAL INFORMATION

**1.Country……. 2. District/territory/zone…….....**

**3. Sub-county/Collectivity/Commune…… 4. Parish/locality/colline…….**

**5. Date of FGD…… 6. Number of participants……. Men……Women……..**

### B. FOOD PRODUCTION IN THE COMMUNITY

**1. What are the major sources of income of the community members in your region? (Get information on the type of income generation activities and their order of important from the most important)**

**……**
**……**
**……**
**……**
**……**
**……**

**2. What are the main food crops grown and animals reared by households in your region**

**(List according to level of importance)**

**a) Food crops grown**
**……**
**……**
**……**
**b) Animals reared**
**……**
**……**

**3. What is the annual trend of food availability in your community? (Tick where appropriate at each month depending on the level of food availability)**

| | **Annual trend in food availability** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Food availability** | **Jan** | **Feb** | **March** | **April** | **May** | **June** | **July** | **Aug** | **Sept** | **Oct** | **Nov** | **Dec** |
| Very available | | | | | | | | | | | | |
| Available | | | | | | | | | | | | |
| Some shortage | | | | | | | | | | | | |
| Severe shortage | | | | | | | | | | | | |

**B. BANANA AND PLANTAIN UTILISATION IN THE COMMUNITY**

**4.a) (If banana is listed in 2 above) What are the banana cultivars grown by members of your community and how are they utilized i.e. cooking, beer, roasting juice, etc?**

| | **Local name of banana cultivar** | **Genomic name (can be filled later)** | **Form of Utilization** |
|---|---|---|---|
| **1.** | | | |
| **2.** | | | |
| **3.** | | | |
| **4.** | | | |
| **5.** | | | |
| **6.** | | | |
| **7.** | | | |
| **8.** | | | |
| **9.** | | | |

**b) Of the banana cultivars mentioned above which five of them are most popular and what are the reasons behind the popularity?**

| | **Local name of banana cultivar** | **Genomic name/s** | **Pulp color of the cultivar (H+ color chart)** | **Reasons for popularity** |
|---|---|---|---|---|
| **1.** | | | | **1.** |
| | | | | **2.** |
| | | | | **3.** |
| **2.** | | | | **1.** |
| | | | | **2.** |
| | | | | **3.** |
| **3.** | | | | **1.** |
| | | | | **2.** |
| | | | | **3.** |
| **4.** | | | | **1.** |
| | | | | **2.** |
| | | | | **3.** |

**c) What are the banana post-harvest products used by the community and how are they processed? (Please describe the steps followed in processing, ingredients used. In addition find out the level of popularity of the products),**

| | **Banana post-harvest product** | **Method of processing** | **Procedure followed in processing/cooking** | **Cooking duration** | **Popularity level** |
|---|---|---|---|---|---|
| **1.** | | | | | |
| **2.** | | | | | |
| **3.** | | | | | |
| **4.** | | | | | |

**d) Give four most popular banana-based dishes (2 consumed by children 12-59months and 2 consumed by other members of community)**

| | **Name of banana-based dish** | **Local name of banana cultivar used** | **Genomic name of cultivar used** | **Other Ingredients used** | **Method of preparation** | **Cooking duration (in minutes)** |
|---|---|---|---|---|---|---|
| **1.** | | | | | | |
| **2.** | | | | | | |
| **3.** | | | | | | |
| **4.** | | | | | | |

**C. COMMUNITY FOOD LIST (Necessary for measurement of dietary diversity during household survey)**

**5. Give a list of all food items whether grown, gathered, hunted, purchased, given as gifts or given as food aid that are used by the community members in your region ( The interviewer to allow the community members freely list all the food items, and later assist them to put them in their respective food groups. Objective is to come up with a community FOOD LIST)**

| | Food group | Food items |
|---|---|---|
| 1. | Cereals | |
| 2. | Vitamin A rich vegetables and tubers | |
| 3. | White roots and tubers | |
| 4. | Dark green leafy vegetables | |
| 5. | Other vegetables | |
| 6. | Vitamin A rich fruits | |
| 7. | Other fruits | |
| 8. | Organ meat | |
| 9. | Other meats | |
| 10. | Eggs | |
| 11. | Fish | |
| 12. | Legumes, nuts and seeds | |
| 13. | Milk and Milk products | |
| 14. | Oils and fats | |
| 15. | Sweets | |
| 16. | Spices, condiments & beverages | |

Adopted from FAO version, 2007

## 3 HOUSEHOLD STRUCTURED QUESTIONNAIRE

**Dietary Patterns, and Nutrition & Health Status of Preschool Children from Small-holder Households in Banana Growing and Consuming Regions of East and Central Africa**

**Household Questionnaire**

Questionnaire Id: ..... Household id/code……..
Date of interview..... Enumerator's name……..
Supervisor name……
Is respondent household head (HHH)? 1=Yes 2=No
If N**o**, What is he/she in the HH……..

### Household Location

| | **Name** |
|---|---|
| 1.Country and Province | |
| 2.District/Territory/Zone | |
| 3.Sub-county/Collectivity/Commune | |
| 4.Parish/Locality/Colline | |
| 5.Village | |

## SECTION I

### A: GENERAL INFORMATION

| **Variable** | **Respons e** | **Codes** |
|---|---|---|
| Demographic data | | |
| 1. Gender of respondent | | 1=Female, 2=Male |
| 2. Age of respondent in years | | |
| 3. Marital status of respondent | | 1=Single, 2=Monogamously married, 3=Polygamous married, 4= Widowed, 5=Separated/Divorced, 6=Other (Specify) |
| 4. If married, age of spouse | | |
| 5 Education level of respondent | | 0= none, 1= Informal Education 2= some primary 3=completed primary 4=some secondary 5=completed secondary 6= vocational training 7=college 8=University |
| 6 Education level of Spouse | | |
| 7 Main source of income for the HHH | | 1=farming 2= casual labor 3=business 4=employment 5=Other specify)…… |
| 8. How many other household members are working | | |
| 9. What is the approximate total monthly income of the household | | 1= <20$, 2= 20-50$, 3= 50-100$, 4=100-200$, 5=>200$ |
| 10. Household size | | All members of household that normally share the same pot |
| 11. Number of times the woman has given birth | | |
| 12. How many children does the woman have | | Indicate the number of children alive |
| 13. How many children are below five years old | | |
| 14. Alternative source of income of the mother /female caretaker of the index child | | 1= farming 2= casual labour 3=business 4=employment 5=none 6=Other (specify)_____ |
| | | |

## SECTION II

### FOOD INTAKE, NUTRITION AND HEALTH STATUS

### A. Household and Individual child Dietary Diversity

**Introduction**: The following questions examine the food consumption pattern of your (household) and your child (age 12 - 59 months) in the last 24 hours. Please describe the foods (meals and snacks) that your household and the child under five years ate yesterday during the day and night, whether at home or outside the home. Start with the first food eaten in the morning. [Household level: consider foods eaten by any member of the household, and exclude foods purchased and eaten outside of the home]. Also get information on main source of the food (Farm=1, market=2 Gift=3 food aid=4 Gathering/hunting=5) method of preparation (Raw=1, boiling=2, roasting=3, frying/stewing=4, other……) For quantity of food consumed this will only apply to the child and two sets of models (cup and plate) of three sizes was used: small (<150g/ml), medium (150-300g/ml), large (300-500g.ml) Small=1, medium=2, large=3

| | **Household level consumption** | | | | | **Preschool child (12-59months)consumption** | | | |
|---|---|---|---|---|---|---|---|---|---|
| **CODE** | **Groups & varieties consumed** | | **Yes=1 No=2** | **Main source** | **Preparation method** | **Yes=1 No=2** | **Main source** | **Preparation method** | **Quantity consumed** |
| **1.0** | **Cereals/grains and products** | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| **2.0** | **White roots, tubers and bananas** | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| **3.0** | **Vitamin A rich vegetables, tubers and bananas** | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| **4.0** | **Dark green Leary vegetables** | | | | | | | | |
| | | | | | | | | | |

| | Household level consumption | | | | | Preschool child (12-59months)consumption | | | |
|---|---|---|---|---|---|---|---|---|---|
| CODE | Groups & varieties consumed | | Yes=1 No=2 | Main source | Preparation method | Yes=1 No=2 | Main source | Preparation method | Quantity consumed |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| 5.0 | Other vegetables | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| 6.0 | Vitamin A rich fruits | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| 7.0 | Other fruits even wild | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| 8.0 | Organ meats (iron rich) | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| 9.0 | Flesh meats | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| 10.0 | Eggs | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| 11.0 | Fish | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| 12.0 | Legumes, nuts & oil seeds | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| 13.0 | Milk and milk products | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |

| | Household level consumption | | | | | Preschool child (12-59months)consumption | | | |
|---|---|---|---|---|---|---|---|---|---|
| CODE | Groups & varieties consumed | | Yes=1 No=2 | Main source | Preparation method | Yes=1 No=2 | Main source | Preparation method | Quantity consumed |
| 14.0 | Oils and fats | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| 15.0 | Sweets | | | | | | | | |
| | | | | | | | | | |
| 16.0 | Spices, condiments & beverages | | | | | | | | |

**(To be worked out by the interviewer immediately after the interview)**

2i. How many food groups were consumed in the last 24 hours excluding group 15 and16 by

(a) Household……. (b) Index child…….

2ii. How many food varieties were consumed in the last 24 hours excluding groups 15 and16 by

(a) Household……. (b) Index child…….

3. Did any household member consume any food items listed below in the last 24hours?

| Food items commonly fortified with Vitamin A | Consumed 1=Yes 0=No | If yes, who consumed All members=1 preschool child=2 |
|---|---|---|
| a. Margarine | | |
| b. Cooking oil fortified with Vit A | | |
| c. Fortified flour | | |
| d. Fortified sugar | | |

4a. Is (child's name) currently being breastfed? 1=yes 2=No ☐

4b If **YES**, how many times is the child breastfed in a day? _____ times/day

**B. Production of bananas and Consumption of Post-Harvest Banana Products**

1. If banana was mentioned in section A 2.0 and A3.0, how often did the household members and the preschool child consume post-harvest banana products in the last one week? (Daily=3, 2-4 times/wk=2, once/wk=1, not consumed=0)

| **Post-harvest banana products** | **Yes=1 No=0** | **Local name of cultivar** | **Genomic name of cultivar** | **Post maturity stage at usage (1=unripe, 2=ripe)** | **Pulp color at use** | **Consumption frequency** | |
|---|---|---|---|---|---|---|---|
| | | | | | | **HH** | **Child** |
| a.Steamed banana/plantain | | | | | | | |
| b. Boiled banana/plantain | | | | | | | |
| c. Roasted banana | | | | | | | |
| d. Banana pancake/bread | | | | | | | |
| e. Banana crisps/chips | | | | | | | |
| f. Banana beer/wine | | | | | | | |
| g. Banana juice | | | | | | | |
| h. Banana porridge (Flour) | | | | | | | |
| i. Dessert banana | | | | | | | |
| j. Other specify …… | | | | | | | |

2a. How much land does your household [ ]e under food production?

<1/4 acre=1, ½ -1 acre=2, >1acre=3

2b.What proportion of the land under food production is under banana/plantain?

1/4=1, ½=2, >1/2= 3

3. What are the banana cultivars grown, what proportion of the land do they occupy and how are they utilized? (cooking=1, roasting=2, dessert=3, beer/juice=4, flour=5, other=6)

| | **Local name** | **Genomic name** | **Proportion of land** | **Pulp color (H+ color Chart)** | **Form of utilization** |
|---|---|---|---|---|---|
| **a** | | | | | |
| **b** | | | | | |
| **c** | | | | | |
| **d** | | | | | |
| **e** | | | | | |
| **f.** | | | | | |

**SECTION III**

**A. Health Status of Preschool child**

1. What is the immunization and vitamin A supplementation status of your child

1= Not immunized and received no vitamin A Supplementation

2= Immunized only

3= Received vitamin A supplement only

4= Immunized and received vitamin A supplement

(if the answer is 1-3, interviewer to **probe further for evidence)**

2. If child is **not immunized and/or not supplemented**, what is the reason?

1=Health facility too far

2= Not aware of its importance

3=has negative effect and may harm my child

4= had no time to take the child

5 other (specify)_____

3. Within the last two weeks did (**name of the index child**) experience the following symptoms

| **Symptoms** | **Last 2 weeks** | **Did you take the child to hospital** |
|---|---|---|
| | **No=0 Yes=1** | **No=0 Yes=1** |
| a) Fever | | |
| b) Cough | | |
| c) Diarrhoea | | |
| d) vomiting | | |
| e) Flu | | |
| f) Asthma | | |

4. Does (child's name) have any problem seeing in day time? 1=yes 2=No

5. Does (child's name) have any problem seeing at night? 1= yes 2=No

**B. Nutrition status of the preschool child**

Nutritional status of index child (12-59months) for age please refers to child health card/birth certificate).

1. Name of child..... 2.Sex: Boy=1 Girl=2 ☐

3. Age of child (**months**)..... 4. Date of birth.....

| **Anthropometric measurements** | **1st reading** | **2nd reading** | **Average** | **Computed z- score (to be filled later)** |
|---|---|---|---|---|
| 5. Height (in centimeters) Cm | | | | |
| 6. Weight (in Kilograms) Kg | | | | |
| 7. Mid upper arm circumference (MUAC) | | | | |

**Interview finished at …… Time taken ……(Minutes)**

**Name of interviewer…… Signature……**

**END**

## 4 *MUSA* FRUIT RIPENING STAGES