CAREGIVER AND CHILD ACCEPTABILITY OF A PROVITAMIN A CAROTENOID, IRON AND ZINC RICH COMPLEMENTARY FOOD PREPARED FROM COMMON BEAN AND PUMPKIN IN UGANDA

BY

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PREFACE

The work described in this thesis was carried out in the School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, from July 2018 to December 2020, under the supervision of Dr Kirthee Pillay and Professor Muthulisi Siwela.

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As the candidate's supervisors, we agree to the submission of this thesis.

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Professor Muthulisi Siwela (Co-Supervisor)

DECLARATION

I, Edward Buzigi declare that:

1. The research reported in this thesis, except where otherwise stated, is my original research.
2. This thesis or any part of it has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons writing, data, pictures, graphs, or other information unless specifically acknowledged as being sourced from them. Where other written sources have been quoted, then:
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ABSTRACT

Vitamin A deficiency (VAD), iron deficiency (ID), and zinc deficiency (ZnD) are the three leading micronutrient deficiencies causing morbidity and mortality among children under five years in developing countries, including Uganda. A high prevalence of VAD, ID and ZnD among children in developing countries begins during the period of complementary feeding, which is between the ages of six to 24 months. This is the period when children are fed complementary foods (CFs) prepared from vitamin A, iron, and zinc deficient staple tubers and cereals. To combat VAD, ID, and ZnD, the World Health Organization (WHO) recommends that CFs be diversified with vitamin A, iron and zinc rich food sources, such as animal source foods (ASFs), food supplements and commercially fortified foods. However, ASFs, commercially fortified foods and food supplements are either unaffordable or inaccessible to rural Ugandan caregivers. Therefore, the aim of this study was to prepare a complementary food (CF) rich in provitamin A carotenoids (PVACs), iron and zinc using locally available common bean and pumpkin and to test the acceptability of the CF among caregivers and their children in rural Uganda. The study objectives were to: (i) select one common bean landrace superior in iron and zinc, and one pumpkin landrace superior in PVACs from a variety of local landraces available in the local market; (ii) evaluate the effect of home cooking methods on provitamin A carotenoid (PVAC) retention in the selected pumpkin (superior in PVACs), and iron and zinc retention in the selected common bean (superior in iron and zinc); (iii) assess child acceptability of an innovative CF- a common bean pumpkin blend (BPB) prepared with common bean (superior in iron and zinc) and pumpkin (superior in PVACs); (iv) assess caregiver perceptions and acceptability of the innovative CF (BPB). The study was conducted in rural Kyankwanzi district, Uganda, East Africa. Cross-sectional and randomised control trial designs were used in this study for the consumer acceptability investigations; and a controlled laboratory experiment for the nutrient retention investigation. Three pumpkin landraces on the local market of the study area were screened for PVACs, whilst five common bean landraces also on the local market were screened for iron and zinc content. Iron and zinc content were determined by flame atomic absorption spectrometry (FAAS), whilst PVAC content was determined by high performance liquid chromatography (HPLC). True retention of iron, zinc and PVAC was determined after expert caregivers cooked pumpkin by either boiling or steaming, whilst the common bean was cooked by either boiling with or without prior soaking. Caregivers prepared the test CF and the control according to the consistency (thickness or

thinness) fit for child consumption based on the child's age and stage of development. The test CF (BPB) was prepared by mixing and blending two parts of cooked pumpkin and one part of cooked common bean, whilst the control CF, pumpkin puree (PP) was prepared by mashing one part of cooked pumpkin. Seventy children, aged 6 to 24 months participated in the child acceptability randomised crossover study. In the current study, the CFs test food (BPB) and control (PP) were considered acceptable if the child consumed at least 50 g and more of the 100 g of the CF offered. Mean duration for intake of the CFs and vitamin A, iron and zinc intake were calculated. A paired t-test was used to determine whether there were significant differences in the amount, duration, and micronutrient intake between the BPB and PP. Further, 70 caregivers (whose children participated in the child acceptability study) participated in the caregiver acceptability study. A cross-sectional sensory evaluation study design was used to assess caregiver perceptions and acceptability of the study CFs. Sensory attributes (taste, colour, aroma, texture and general acceptability) of the BPB and PP were rated using a five-point facial hedonic scale (1=very bad, 2=bad, 3=neutral, 4=good, 5=very good). Focus group discussions (FGDs) were also conducted to assess caregiver perceptions about using the BPB as a CF. A chi-square (X^2) test was used to detect the proportionate difference for each sensory attribute between BPB and PP, whilst focus group discussions (FGDs) data was analysed by thematic analysis. A *p* value of 0.05 was considered statistically significant. For objective one (first investigation), β-carotene content in *Sweet cream* (1 704 µg/100 g) was significantly higher compared to *Dulu* (1 333 µg/100 g) and *Sun fish* (1041 µg/100 g) (p<0.0001). The α-carotene content of *Sweet cream* was significantly lower (46 µg/100 g, p<0.0001) compared to *Dulu* (77.3 µg/100 g) and *Sun fish* (79.3 µg/100 g). However, the total retinol activity equivalent (RAE) was highest in *Sweet cream* (143.9 µg/100 g), compared to *Dulu* (115.4 µg/100 g) and *Sun fish* (90.1 µg/100 g). Iron content was highest in *Obwelu* (7.75 mg/100g), compared to *Masavu* (6.95 mg/100 g), *Nambale* (6.55 mg/100g), *Kanyebwa* (7.15 mg/100 g) and *Obwayelo* (6.5 mg/100 g). *Obwelu* had significantly higher iron concentrations than *Obwayelo* (p<0.05). Zinc content was highest in *Obwelu* (3.05 mg/100 g), but was not significantly different (p >0.05) compared to the other common bean landraces of *Masavu* (2.95 mg/100 g), *Nambale* (2.35 mg/100 g), *Kanyebwa* (2.9 mg/100 g) and *Obwayelo* (3.0 mg/100 g). The findings of the first investigation suggested that *Sweet cream* was superior in PVAC content compared to the other pumpkin landraces, whilst *Obwelu* was superior in iron and zinc content compared to the other common bean landraces. Therefore, *Sweet cream* and *Obwelu* were selected for use in the preparation of a CF rich in PVACs, iron and zinc. For objective two (second investigation), β-carotene, α-carotene, and total provitamin A content in raw

pumpkin was 1704 µg/100 g, 46 µg/100 g and 1437 µg/100 g, respectively. Either boiling or steaming pumpkin resulted in over 100% retention of PVACs and total provitamin A. Iron and zinc retention in soaked boiled common bean was 92.2% and 91.3%, respectively. Boiling common bean without soaking resulted in 88.4% and 75.6% retention of iron and zinc, respectively. The findings of the second investigation suggested that there was a high retention of PVACs in pumpkin, *Sweet cream* after boiling or steaming, and a high retention of iron and zinc in common bean, *Obwelu* after boiling with prior soaking. For objective three (third investigation), the mean amount of BPB (53.9 g) and the control (PP) (54.4 g) consumed by children was high, but not significantly different from each other ($p>0.05$). The mean duration for child consumption of BPB was 20.6 minutes and 20.3 minutes for the control and the durations for child consumption were not significantly different from each other ($p<0.05$). The mean child intake of vitamin A was significantly higher ($p<0.05$) from the control (PP) (152.5 µgRAE) compared to the test food (BPB) (100.9 µgRAE). The mean iron intake was significantly higher ($p<0.05$) from BPB (1.1 mg) compared to the control (0.3 mg). Furthermore, zinc intake was significantly higher ($p<0.00001$) from the (0.58 mg), compared to control (0.13 mg). For objective four (fourth investigation), between 64% and 96% of the caregivers rated both BPB and PP as acceptable (good to very good) for all the sensory attributes. There was no significant difference ($p>0.05$) in caregiver acceptability for all sensory attributes between BPB and PP ($p>0.05$). Caregivers had positive perceptions about the taste, texture, aroma, and colour of the BPB. Caregivers were keen to know the specific varieties of common bean and pumpkin used to formulate the PVAC, iron and zinc rich BPB. Findings from this study suggest that a complementary food, BPB, rich in PVACs, iron and zinc prepared from locally available common bean, *Obwelu* and pumpkin, *Sweet cream* was acceptable to caregivers and their children who were in the age range of complementary feeding in Uganda. To contribute towards combating child VAD, ID and ZnD, policy makers in Uganda, such as the district nutrition coordination teams should support and promote the cultivation and utilisation of common bean, *Obwelu* and pumpkin, *Sweet cream* as major ingredients of CFs. The use of BPB as a CF should not replace other existing nutrition interventions such as micronutrient supplementation, commercial fortification, biofortification programmes and the use of ASFs that aim to combat micronutrient deficiencies during the period of complementary feeding. However, the use of BPB as a CF should be a complementary strategy to these existing nutrition interventions. Future studies should investigate the effect of BPB intake on the vitamin A, iron and zinc status of children.

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DEDICATIONS

This PhD is dedicated to my mother, Ms. Justine Nattu. I am deeply grateful for your support and care since childhood to present.

I also dedicate this PhD thesis to my late father, Mr. Joshua Ssemugenyi Munoga. You persistently told me that the only offer you would give me in this world was education. Dad, you are hugely missed but never forgotten.

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LIST OFABBREVIATIONS

AI	Adequate Intake
ANOVA	Analysis of Variance
ASFs	Animal Source Foods
BPB	Common Bean Pumpkin Blend
CF(s)	Complementary Food(s)
CDC	Centers for Disease Control and Prevention
DRIs	Dietary Reference Intakes
EAR	Estimated Average Requirement
FAO	Food and Agriculture Organization of the United Nations
FAAS	Flame Atomic Absorption Spectroscopy
FAOSTAT	Food and Agriculture Organization of the United Nations Statistics
FGD(s)	Focus Group Discussion(s)
GAM	Global Acute Malnutrition
HAZ	Height for Age Z score
Hb	Haemoglobin
HHs	Household(s)
HICs	High Income Countries
HPLC	High Performance Liquid Chromatography
HIV/AIDS	Human Immunodeficiency Virus/Acquired Immune deficiency Syndrome
ID	Iron Deficiency
IDA	Iron Deficiency Anaemia
IU	International Units
IYC	Infant and Young Children
LAZ	Length for Age Z score
LMICs	Low and Middle Income Countries
MAM	Moderate Acute Malnutrition
MND	Micronutrient Deficiencies
MOH	Ministry of Health
NB	Fat-Based Nutri-Butter
NCDs	Non-Communicable Diseases
ND	Nutrient Density
NT	Crushable Nutri-Tablets

OFSP	Orange-Fleshed Sweet Potato
PP	Pumpkin Puree
PSF(s)	Plant Source Food(s)
PVAC (s)	Provitamin A Carotenoid(s)
RBP	Retinol Binding Protein
RCTs	Randomised Control Trial(s)
RDA	Recommended Dietary Allowance
SAM	Severe Acute Malnutrition
SD	Standard Deviation
SP	Sprinkles Powder
SSA	Sub-Saharan Africa
STATA	Statistics and Data
UDHS	Uganda Demographic Health Survey
UNICEF	United Nations Children’s Fund
VAD	Vitamin A Deficiency
VAS	Vitamin A Supplementation
VHT(s)	Village Health Team member(s)
WAZ	Weight for Age Z score
WFSP	White-Fleshed Sweet Potato
WHO	World Health Organization
WHZ	Weight for Height Z score
YCC	Young Child Clinic
ZnD	Zinc Deficiency

CHAPTER ONE

INTRODUCTION, THE PROBLEM AND ITS SETTING

1.1 Importance of the study

Micronutrients are essential vitamins and minerals required from the diet to sustain almost all normal biochemical functions of life (Shergill-Bonner, 2017). Micronutrient deficiencies (MNDs) are common and affect more than two billion people worldwide (Bailey, West and Black, 2015). However, children are among the most vulnerable to suffer from MNDs (Bailey, West and Black, 2015). The three most common MNDs that affect more than 50% of the world's children under the age of two years are vitamin A deficiency (VAD), iron deficiency (ID) and zinc deficiency (ZnD) (Shergill-Bonner, 2017). However, the highest burden of VAD, ID and ZnD is seen in children residing in the developing countries of Africa and Asia (Bailey, West and Black, 2015). This high burden of MNDs is a result of consuming foods that are low in micronutrients such as iron, zinc and vitramin A (FAO *et al.,* 2019; 2020). The consequences of VAD, ID and ZnD are devastating, because they lead to high child morbidity, mortality, and cognitive impairment in the developing world (Stevens *et al.*, 2015; Millward, 2017). It is worth noting that VAD, ID and ZnD usually coexist with other forms of child malnutrition such as wasting (low weight for height) and stunting (low height for age) (Millward, 2017).

The highest prevalence of VAD, ID, and ZnD in the developing world is observed among children between six and 24 months of age, which is also the period of complementary feeding (Millward, 2017; WHO, 2019). The World Health Organization (WHO) recommends exclusive breastfeeding for the first six months of an infant's life, because breast milk alone is adequate to meet the vitamin A, iron and zinc requirements for health, growth and development during this time (WHO, 2019). However, from six months of age, breast milk alone is no longer sufficient to meet the vitamin A, iron and zinc requirements of the infant (Qian *et al.*, 2010). Therefore, breast milk should be complemented with complementary foods (CFs) rich in vitamin A, iron and zinc and other essential nutrients (WHO, 2019).

Unfortunately, most CFs fed to infants and young children (IYC) in countries in sub-Saharan Africa (SSA) are low in micronutrients, because they are predominantly prepared from staple cereals and tubers (Gibson *et al.*, 2010; Amaral, Herrin and Gulere, 2018). Although cereals and tubers are rich in energy (FAO, 2017), they are low in vitamin A, iron and zinc, which increases the risk of VAD, ID and ZnD when they dominate the diets of children (Gegios *et al.*, 2010; Harika *et al*., 2017) . Moreover, cereals are rich in anti-nutritional factors, such as phytic acid and polyphenols, which reduce the bioavailability of iron and zinc in the body (Gibson *et al.*, 2010).

Uganda is a low-income country in SSA, located in East Africa (Uganda Bureau of Statistics, 2017; Uganda Bureau of Statistics and Inner City Fund, 2018), with a high prevalence of child VAD, ID and ZnD (Wessells and Brown, 2012; Wirth, *et al*., 2017; Gardner and Kassebaum, 2020). Caregivers in rural areas of Uganda feed their children CFs formulated from staple cereals and tubers as the main ingredients and these CFs are generally deficient in vitamin A, iron and zinc (Amaral, Herrin and Gulere, 2018; Ekesa, Nabuuma and Kennedy, 2019). Consumption of such vitamin A, iron and zinc deficient CFs puts Ugandan children at risk of VAD, ID, and ZnD (Gegios *et al.*, 2010). To combat MNDs such as VAD, ID and ZnD, it is recommended that caregivers feed their children CFs prepared from commercially fortified foods, food supplements and animal source foods (ASFs) (WHO, 2019; Darapheak *et al.*, 2013; Muslimatun and Wiradnyani, 2016; Zhang, Goldsmith and Winter-Nelson, 2016). This recommendation is plausible because fortified CFs, food supplements and ASFs are rich sources of vitamin A, iron, and zinc (Sazawal *et al.*, 2010; Krebs *et al.*, 2011, 2012; WHO, 2016). However, fortified foods, food supplements and ASFs are not affordable to caregivers of low socio-economic status such as rural caregivers (FAO, 2017). Therefore, this study aimed to identify and use locally available vitamin A, iron, and zinc rich foods to prepare an innovative CF rich in vitamin A, iron, and zinc for future use to combat VAD, ID and ZnD among rural Ugandan children in the age range of complementary feeding (six to 24 months old), as recommended by the WHO (2019).

This study developed an innovative CF blend rich in provitamin A carotenoids (PVACs), iron and zinc using locally available pumpkin and common bean in Uganda (Kiwuka *et al.*, 2012; Nakazibwe *et al.*, 2019), and tested its acceptability among caregivers and their children. Common bean and pumpkin were used in the preparation of the innovative CF because the former is a rich source of iron and zinc (Carvalho *et al.*, 2012; Ferreira *et al.*, 2014; Haas *et al.*, 2016; Moloto *et al.*, 2018), while the latter is rich in PVACs (Koh and Loh, 2018). It is worth noting that after ingestion, PVACs in pumpkin are bioconverted to retinol, an active form of vitamin A used by the body (Van Loo-Bouwman, Naber and Schaafsma, 2014).

Although the high prevalence of consuming CFs low in vitamin A, iron and zinc is well known among Ugandan children (Amaral, Herrin and Gulere, 2018; Ekesa, Nabuuma and Kennedy, 2019), there are no previous published studies conducted in Uganda that have sought to formulate a multiple micronutrient rich (PVACs, vitamin A, iron, and zinc) CF made from locally available ingredients such as pumpkin and common bean. Therefore, this study aimed to prepare a homemade PVAC, iron and zinc rich CF blend from common bean and pumpkin to support Uganda's food and nutrition policy agenda of combating all forms of malnutrition, including MNDs (The Republic of Uganda, 2003).

Uganda has several landraces of common bean and pumpkin (Kiwuka *et al.*, 2012; Nakazibwe *et al.*, 2019). However, their micronutrient composition is unknown (Hotz, Lubowa and Sison, 2010). To this end, it was necessary to analyse the nutritional composition of the two food sources and select one common bean landrace superior in iron and zinc, and one pumpkin landrace superior in PVACs. Home preparation/cooking methods are known to affect iron and zinc retention in common bean (Carvalho *et al.*, 2012) and provitamin A carotenoid (PVAC) retention in pumpkin Thus, it was also important to assess the iron and zinc retention in common bean and PVAC retention in the pumpkin used to prepare the innovative CF in Uganda.

Assessing caregiver and child acceptability of the innovative CF was also necessary because the caregiver is the gatekeeper of the consumption of CFs and as such, makes decisions regarding which CFs to prepare and feed to the child whist, the child is the target for receiving the CFs (FAO, 2017). It is worth noting that several previous studies have concentrated on testing the acceptability of CFs among caregivers only, while neglecting to assess child acceptability of CFs because it is argued that children are too young to make a rational and valid sensory judgment of CFs (Martin, Laswai and Kulwa, 2010; Govender *et al.*, 2014). However, caregiver acceptability of CFs does not guarantee child acceptability of the same CF (Paul *et al.*, 2008). Moreover, testing child acceptability is possible by using non-sensory evaluation methods (Ahmed *et al.*, 2014). To this end, this study aimed to prepare a PVAC, iron and zinc rich CF using locally available common bean and pumpkin from the local market and to test its acceptability among caregivers and the children in their care in Uganda. A PVAC rich pumpkin puree (PP) was used as a control as pumpkin is commonly used as a single CF in Uganda (Uganda Bureau of Statistics and Inner City Fund, 2018).

1.2 Summary of research focus

This study determined the PVAC composition of pumpkin landraces and, iron and zinc composition of common bean landraces found on the local market in rural Uganda to select the landraces superior in these micronutrients. Further, the study assessed the effect of home cooking on PVAC, iron and zinc retention in the common bean and pumpkin landraces selected for use in the preparation of the PVAC, iron and zinc rich CF. Furthermore, the acceptability of the PVAC, iron and zinc rich CF prepared from common bean and pumpkin by home cooking methods was evaluated among caregivers and the children in their care from a low socio-economic rural community in Uganda.

1.3 Aim of the study

To assess the potential of using a CF blend prepared from locally available PVAC rich pumpkin and iron and zinc rich common bean for use in combating VAD, ID and ZnD prevalent among children aged six to 24 months in the rural areas of Uganda.

1.4 Study hypotheses

The following hypotheses were tested in the study:

1.4.1 The PVAC content of various pumpkin landraces available on the rural Ugandan market differ.

1.4.2 The iron and zinc content of various common bean landraces available on the rural Ugandan market differ.

1.4.3 Home cooking methods have an impact on PVAC retention in pumpkin purchased from the Ugandan local market.

1.4.4 Home cooking methods have an impact on iron and zinc retention in common bean purchased from the Ugandan local market.

1.4.5 The BPB is more acceptable to Ugandan caregivers and their children compared to the control PVAC rich PP.

1.5 Study objectives

The specific objectives were to:

1.5.1 Select one common bean landrace superior in iron and zinc, and one pumpkin landrace superior in PVACs from a variety of local landraces available on the local market.

1.5.2 Evaluate the effect of home cooking methods on PVAC retention in the selected pumpkin (superior in PVACs), and iron and zinc retention in the selected common bean (superior in iron and zinc).

1.5.3 Assess child acceptability of a CF, BPB prepared with common bean (superior in iron and zinc) and pumpkin (superior in PVACs).

1.5.4 Assess caregiver perceptions and acceptability of the CF, BPB.

1.6 Study parameters and general assumptions

This study was conducted in rural Kyankwanzi district located in central Uganda. Central Uganda was chosen because IYC from this region are at a higher risk of VAD, ID and ZnD as they are fed CFs low in vitamin A, iron, and zinc (Amaral, Herrin and Gulere, 2018; Ekesa, Nabuuma and Kennedy, 2019). Moreover, the study district is rural, and most caregivers are of a low socio-economic status. Therefore, it was assumed that the caregivers in rural Kyankwanzi district would not afford to purchase or physically access vitamin A, iron and zinc supplements, ASFs and commercially fortified foods to feed their children during complementary feeding. However, they could access the locally cultivated pumpkin and common bean landraces in rural central Uganda (Kiwuka *et al.*, 2012; Nakazibwe *et al.*, 2019).

Since common bean is a rich source of iron and zinc (Ferreira *et al.*, 2014), and pumpkin is rich in PVACs (Koh and Loh, 2018), this study assumed that a CF blend formulated from common bean and pumpkin would be a potential rich source of PVACs, iron and zinc. The CF developed in this study was prepared by peer caregivers using local preparation methods used to cook common bean and pumpkin for immediate consumption by household members in the study community. Children aged six to 24 months old and their caregivers were used to test the acceptability of the innovative CF. These study participants were used because children six to 24 months old are the target population for CFs, whilst caregivers decide whether or not they will offer the innovative CF to their children.

1.7 Definitions of key terms

Acceptance tests: Type of sensory evaluation tests used to determine if panellists like a product, and/or if they intend to use a product (Penfield and Campbell, 1990).

Caregivers: People who care for children and provide nutrition care and support (FAO, 2017).

Complementary feeding: The process that starts when breast milk alone is no longer enough to meet the nutritional requirements of infants and other foods and liquids are fed, along with breast milk. The age range for complementary feeding is generally six to 24 months (FAO, 2017).

Complementary foods: Foods that are manufactured or locally prepared, suitable to complement breast milk or infant formula, when either become insufficient to meet the nutritional requirements of the infant. At this age, the infant is developmentally ready to be introduced to other foods (FAO, 2017).

Home cooking methods: Methods used to prepare and cook food in the household, ready for human consumption (Carvalho *et al.,* 2012).

Landrace: A domesticated, locally adapted, traditional variety of a species of animal or plant that has developed over time, through adaptation to its natural and cultural environment of agriculture, and due to isolation from other populations of the species (Tadesse *et al.*, 2019).

Micronutrient deficiency/malnutrition: A term to describe dietary inadequacy of vitamins and/or minerals (Bailey, West and Black, 2015).

Mortality: Mortality is the term used for the number of people who have died within a population (Wunsch and Gourbin, 2018).

Morbidity: Morbidity refers to the state of being diseased or unhealthy within a population (Wunsch and Gourbin, 2018).

Nutrient retention: The ratio of the nutrient content in the cooked food to the nutrient content in the raw food (Li *et al.*, 2007).

Provitamin A carotenoids: An inactive form of vitamin A, predominantly found in plant food sources that is bioconverted by the human body to retinol, an active form of vitamin A used by the body (Van Loo-Bouwman, Naber and Schaafsma, 2014).

Sensory evaluation/analysis: A scientific discipline used to invoke, measure, analyse, and interpret reactions to characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch and hearing (Penfield and Campbell, 1990).

1.8 Outline of the thesis

Chapter 1 presents the importance of the thesis, summary of research focus, aim of the study, study hypotheses, study objectives, study parameters, general assumptions, and definitions of key terms. It also presents the outline and referencing styles used in the thesis. A review of the related literature on the prevalence and risk factors of the different forms of child manlnutrition at global and Ugandan level, link between complementary feeding and child malnutrition, guiding principles for complementary feeding and existing interventions to combat child malnutrition is presented in Chapter 2. Listed in the footnote of the introduction page of chapter 2 is one publication based on this chapter. Chapter 3 presents the description of the study site, philosophical approach guiding the study, study design, and ethical approvals. Chapter 4 presents the screening of common bean varieties to select one variety superior in iron and zinc; and the screening of pumpkin varieties to select one variety superior in PVACs. Chapter 5 evaluates the effect of cooking of selected common bean, *Obwelu* on iron and zinc retention; and the effect of cooking selected pumpkin, *Sweet cream* on PVAC retention. Also listed in the footnote of the introduction page of chapter 4 is one publication based on this chapter. Chapter 6 assesses child acceptability of common bean pumpkin blend (BPB), a PVAC, iron and zinc rich CF prepared from common bean (*Obwelu*) and pumpkin (*Sweet cream*). Listed in the footnote of the introduction page of chapter 6 is one publication based on this chapter. Chapter 7 assesses caregiver perceptions and acceptability of BPB. Listed in the footnote of the introduction page of chapter 7 is one publication based on this chapter. Chapter 8 discussess key findings from chapters 4, 5, 6 and 7 and their implications for current and future approaches to alleviate VAD, ID and ZnD. Chapter 8 concludes with recommendations for policy and research. The appendices contain questionnaires and ethical aprovals.

1.9 Referencing style used in the thesis

The Harvard referencing style was used in this thesis.

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CHAPTER TWO

[1]LITERATURE REVIEW

2.1 Introduction

This chapter reviews the prevalence of malnutrition among children under five years of age mainly in low and middle-income countries (LMICs) including Uganda, with emphasis on chronic malnutrition (stunting), acute malnutrition and underweight [World Health Organization (WHO), 2006]. The three most prevalent micronutrient deficiencies of public health importance which include VAD, ID and ZnD, are also reviewed (Wessells and Brown, 2012; Wirth *et al*., 2017; Gardner and Kassebaum, 2020). The association between micronutrient deficiencies (VAD, ID and ZnD) and other nutritional deficiencies such as stunting and acute malnutrition is also reviewed. This chapter also presents an overview of the causes of child malnutrition, with emphasis on how the complementary feeding period is a risk factor for child malnutrition. The chapter also reviews existing nutrition interventions such as supplementation, commercial fortification, dietary diversity, biofortification, home gardening and nutrition education, which are used to combat child malnutrition during the period of complementary feeding (Ruel and Alderman, 2013; Vir, 2016). Furthermore, the potential of a complementary food (CF) formulated from pumpkin and common bean to meet the dietary references intakes (DRIs) for vitamin A, iron, and zinc for infants and young children (IYC) in the age range of complementary feeding (six to 24 months old), is also reviewed.

2.2 Chronic malnutrition, acute malnutrition, underweight and micronutrient deficiencies

2.2.1 Chronic malnutrition (stunting)

Chronic malnutrition, also known as child stunting or linear growth retardation is defined as a low length/height for age in children or a length/height for age Z score (HAZ) below -2, i.e. more than two standard deviations (SD) below the population median (WHO and UNICEF, 2019). Stunting is the most prevalent form of undernutrition globally among children below five years old, compared to wasting and underweight (UNICEF/WHO/World Bank Group, 2017). It is estimated that in 2019, 144 million children under 5 were stunted worldwide (FAO

[1]Publication based on this chapter:
Buzigi E, Pillay K, & Muthulisi Siwela (2021): Potential of pumpkin to combat vitamin A deficiency during complementary feeding in low and middle income countries: Variety, provitamin A carotenoid content and retention, and dietary reference intakes, *Critical Reviews in Food Science and Nutrition*, doi: 10.1080/10408398.2021.1896472

et al., 2020). Out of the 144 million stunted children in 2019, over 90% reside in Asia and Africa (FAO *et al.*, 2020). This confirms a study by De Onis, Blössner and Borghi (2012), which predicted that the majority of the world's stunted children will be in Asia and Africa by the year 2020. Globally, the prevalence of stunting has decreased from 22.2% in 2017 (FAO *et al.*, 2018) to 21.3% in 2019 (FAO *et al.*, 2018). However, the rate of decrease is too slow to achieve the world health assembly target of a 40% reduction in the number of stunted children by 2025, as well as the 2030 sustainable development goal (SDG) target of a 50% reduction (FAO *et al.*, 2019). Figure 2.1 shows the trends of stunting among children below five years globally, in Africa and Asia.

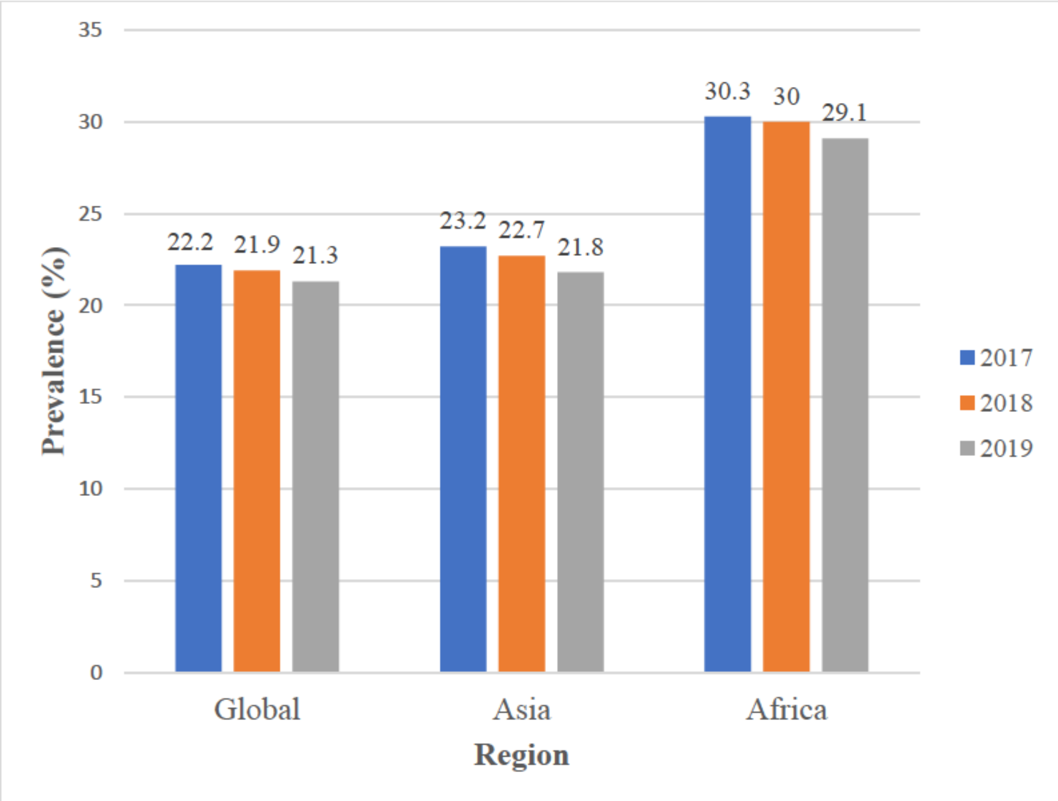


Figure 2.1: Trends of child stunting between 2017 and 2019 globally, in Asia and Africa (FAO *et al.*, 2018; FAO *et al.*, 2019; FAO *et al.*, 2020)

Based on Fgure 2.1, the prevalence of child stunting has persistently remained higher in Africa compared to Asia and the globe betwen 2017 and 2019. Moreover, stunting is reducing at a lower rate in Africa compared to that in Asia and worldwide (FAO *et al.*, 2019).

Stunting is diagnosed when children aged 0 to 59 months are more than two SD below the median height for age using the WHO child growth standards (WHO and UNICEF, 2019). Stunting is a recurrent process because women who were themselves stunted in childhood tend to have stunted offspring, creating an intergenerational cycle of malnutrition that is difficult to break (Martorell and Zongrone, 2012). Stunting occurs during the first 1000 days of life, which is from conception until the child is two years old (Prentice, *et al*., 2013). The first 1000 days of life can be divided into periods of pregnancy (270 days), and two years (730 days) of exclusive breastfeeding (0 to 6 months) and complementary feeding (six to 24 months) (Prentice *et al*., 2013). Therefore, to address child stunting, adequate nutrition and health interventions should be promoted and supported during the periods of pregnancy, exclusive breastfeeding and complementary feeding (Dewey, 2016).

The consequences of stunting are devastating because it is associated with increased risk of morbidity and mortality, and impacts negatively on physical growth and cognitive, and economic development (Hoddinott *et al*., 2013; Leonard *et al*., 2014). In Bolivia, children who were stunted at two years had poor muscle growth at 10 years (Leonard *et al.*, 2014). In Guatemala, being stunted at two years was associated with less schooling, a lower test performance, a lower household per capita expenditure, and an increased risk of living in poverty during adulthood (Hoddinott *et al.*, 2013). A more recent study conducted in Nepal, revealed that children who were stunted before celebrating their fifth birthday were 1.64 times more at risk of having hearing loss, compared to those non-stunted at 16 to 23 years (Emmett *et al.*, 2018).

The Uganda national prevalence of stunting among children below five years old has remained static at 29% between 2016 (Buzigi, 2018) and 2019 (FAO *et al.*, 2020). This indicates that stunting is more prevalent in Uganda (29%) compared to the globe (21.3%) (FAO *et al*., 2020). At this slow rate in the reduction of stunting, it is unlikey that Uganda will achieve the sustainable development goal (SDG) target of a 50% reduction in stunting by 2030 (FAO *et al.*, 2020). According to Buzigi (2018), the prevalence of stunting in Uganda is reducing at a very low rate. The annual reduction in the prevalence of stunting between 1995 and 2016 was 0.45%, and unless something is done to address this, Uganda may not achieve the 40% reduction rate in stunting between 2010 and 2025, as agreed on at the 65th World Health Assembly (WHA) (Buzigi, 2018). Figure 2.2 shows the trends of child stunting, wasting and underweight from 1995 to 2016 in Uganda. However, the trends in Figure 2.2 should be interpreted with caution because the periods covered straddle both the MDGs (2000-2015) and SDGs (2015 to present), when different global efforts were at play.

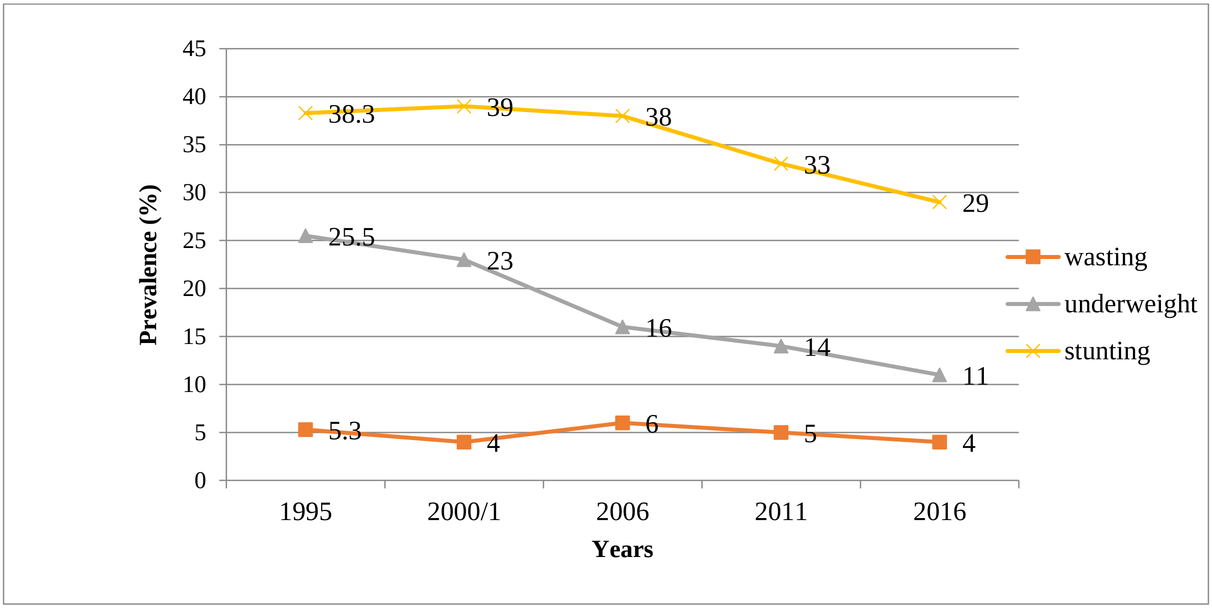


Figure 2.2: National trends of stunting, wasting and underweight in Uganda between 1995 and 2016 (Buzigi, 2018; Uganda Bureau of Statistics and Inner-City Fund, 2018).

There are inequalities in the prevalence of child stunting among regions within Uganda. Some regions like Karamoja, Acholi, Kigezi, Westnile, Bunyoro and Tooro registered unacceptably high rates of stunting compared to the national prevalence in 2016, ranging from 30.8% in Kigezi to 40.6% in the Tooro region (Buzigi, 2018) (Figure 2.3).

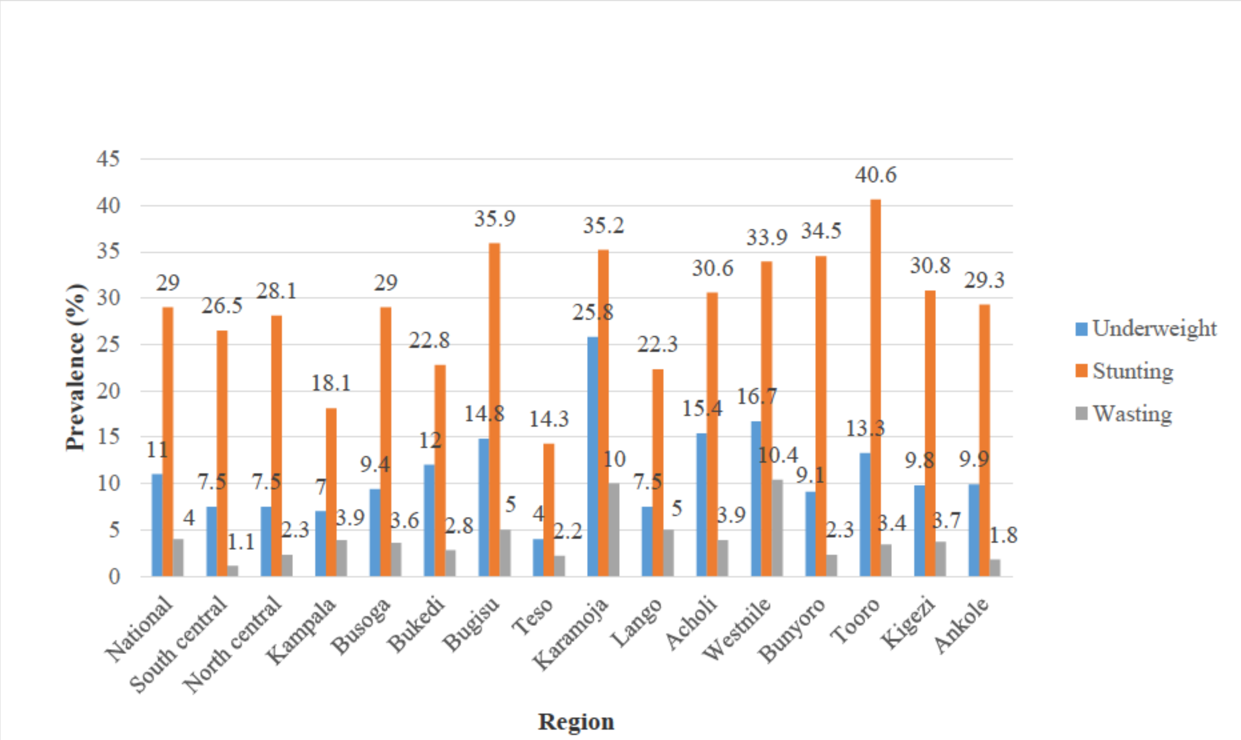


Figure 2.3: National prevalence versus regional prevalence of stunting, wasting and underweight in Uganda by the year 2016 (Buzigi, 2018)

2.2.2 Acute malnutrition

According to the 2019 report on the State of the World's Food Security and Nutrition Status by the Food and Agriculture Organization of the United Nations (FAO) and other partners, the global prevalence of acute malnutrition was 7.3% (49.5 million) among children under five years of age (FAO *et al.,* 2019. It is worth noting that more than 75% of the 49.5 million global children with acute malnutrition reside in sub-Saharan Africa (SSA) and Asia (FAO *et al.,* 2019). Acute malnutrition can present as either oedematous (nutritional pitting oedema) or non-oedematous (wasting) (Ghosh-Jerath *et al.,* 2017; Ubesie *et al.,* 2012). Nutritional pitting oedema is usually bilateral starting from the lower limbs, which can spread all over the other parts of the body including the abdomen and face (WHO, 2013b). In contrast to oedematous acute malnutrition, acute malnutrition due to wasting is characterised by visible body wasting (WHO, 2013b).

Generally, acute malnutrition is classified into global acute malnutrition (GAM), moderate acute malnutrition (MAM) and severe acute malnutrition (SAM) (WHO, 2013b; The Sphere Hand Book, 2018). It is worth noting that GAM is the total sum of MAM and SAM (The Sphere

Hand Book, 2018). Table 2.1 shows the classification of acute malnutrition for children six to 59 months old based on GAM, MAM, and SAM.

Table 2.1: Classification of acute malnutrition for children six to 59 months old (WHO, 2013b)

Global acute malnutrition	Moderate acute malnutrition	Severe acute malnutrition
• WFH <-2 SD on Z score chart and/or • MUAC <12.5 cm and/or • Nutritional oedema	• WFH -3 to -2 SD on Z score chart and/or • MUAC 11.5-12.5 cm	• WFH <-3 SD on Z score chart and/or • MUAC <11.5 cm and/or • Nutritional oedema

WFH: Weight for height; MUAC: Mid-upper arm circumference; SD: Standard deviation.
Z score charts are based on the World Health Organization standard charts for weight and height Z scores (WHO, 2013b).

The consequences of MAM and SAM are of concern because both are associated with child morbidity and mortality (Mahgoub and Adam, 2012; Munthali *et al.,* 2015; Pravana *et al.,* 2017). Several studies conducted in various African countries including Uganda revealed that child mortality due to SAM was high at over 9% (Kerac *et al.*, 2014; Attia *et al.*, 2016; Rytter *et al.*, 2016; Nabukeera-Barungi *et al.*, 2018). Moreover, morbidities such as diarrhoea, high intestinal inflammation, and high systemic inflammation are significantly associated with mortality in SAM (Attia *et al.*, 2016). In a hospital-based study of 1097 Sudanese children with SAM below five years old, 71.1% and 10.2% of children had diarrhoea and malaria co-morbidities, respectively (Mahgoub and Adam, 2012). Furthermore, 5.5% of these children died (Mahgoub and Adam, 2012). It is worth noting that despite following the WHO guidelines for the treatment of SAM, child mortality due to SAM remains unacceptably high in the developing countries of Africa (Irena, Mwambazi and Mulenga, 2011; Talbert *et al.*, 2012; Kerac *et al.*, 2014; Rytter *et al.*, 2016). For example, in Malawi, Kerac *et al.* (2014) showed that 42% of children with SAM below two years of age died during or after the recommended SAM treatment. Of the 42% deaths, 25% occurred after normal programme discharge or three months after admission (Kerac *et al.*, 2014).

It is worth noting that the mortality rate may be higher among children with SAM who also present with other childhood co-morbidities such as human immunodeficiency virus/ acquired immune deficiency syndrome (HIV/AIDS), diarrhoea, malaria, and respiratory tract infections.

Severe respiratory tract infections and SAM are both highly prevalent and strongly associated with death among hospitalised children who are HIV infected or exposed (Preidis *et al.*, 2011; Kerac *et al.*, 2014). A systematic review and meta-analysis of 17 studies conducted in SSA, showed that children with SAM and HIV co-morbidities were more likely to die (30.4%) than children with SAM, uninfected with HIV (8.4%) (Fergusson and Tomkins, 2009). Two studies conducted in Zambia and Kenya showed that diarrhoea increased the risk of death among children with SAM (Irena, Mwambazi and Mulenga, 2011; Talbert *et al.*, 2012).

In Uganda, 9.8% of children under five years of age with SAM died during hospitalisation (Nabukeera-Barungi *et al.*, 2018). Predictors of mortality included diarrhoea on admission, suspected sepsis, skin ulcers, respiratory tract infections, and confirmed HIV infection (Nabukeera-Barungi *et al.*, 2018). In Zambia, similar findings were reported among children with SAM, infected with HIV (Munthali *et al.*, 2015). Another study conducted in Uganda showed a higher mortality rate of 14% among children receiving in-patient management of SAM (Rytter *et al.*, 2016). Infection such as oral thrush was significantly associated with SAM deaths (Rytter *et al.*, 2016). Similar findings have been observed in Zambia, where overall mortality was higher at 46% among children with non-oedematous SAM or severe wasting (Munthali *et al.*, 2015). Furthermore, SAM among children in SSA is associated with a higher risk of developing NCDs in later life. A Malawian study showed that children with SAM who survived death in childhood had a higher risk of developing NCDs such as cardiovascular and metabolic diseases (Lelijveld *et al.*, 2016). This is a very important finding because NCDs will be the leading cause of premature deaths by the year 2030 in LMICs, including countries in SSA (Mathers and Loncar, 2006).

Globally, the number of wasted children below five years old increased from 50 million in 2015 (UNICEF/WHO/World Bank Group, 2016) to 52 million in 2017 (UNICEF/WHO/World Bank Group, 2017). However, the 2018 global nutrition report showed that the number of children below five years old with wasting increased from 50 million in 2017 (UNICEF/WHO/World Bank Group, 2017), to 50.5 million in 2018 (Development Initiatives, 2018). Based on the most current 2019 global food security and nutrition status by the FAO *et al.* (2019), the prevalence of child wasting was 7.3%, indicating that the global prevalence of wasting reduced by 0.2% between 2018 and 2019.

It is noteworthy that the highest prevalence burden of global child wasting has consistently been observed in Asia and Africa (UNICEF/WHO/World Bank Group, 2016; UNICEF/WHO/World Bank Group, 2017; Development Initiatives, 2018; FAO *et al.*, 2019). In 2015, more than 75% of all wasted children under five years old lived in Asia (68%) and more than 28% lived in Africa (UNICEF/WHO/World Bank Group, 2016). Similar findings were observed in 2016, where more than two thirds (69%) of all wasted children below five years old lived in Asia and more than one quarter (27%) lived in Africa (UNICEF/WHO/World Bank Group, 2017). Furthermore, more than 75% of wasted children below five years old were found in Asia and Africa by the year 2018 (FAO *et al.*, 2019) and 2019 (FAO *et al*., 2020).

In Uganda, the national prevalence of wasting (weight for height Z score < -2 SD) reduced from 5.3% in 1995, to 4.0% in 2016, as shown in Figure 2.2 (Buzigi, 2018). However, by the year 2016, the prevalence of wasting was disproportionately higher in some rural regions of Uganda than the national prevalence, as shown in figure 2.3 (Buzigi, 2018). For example, Figure 2.3 shows that by the year 2016, the prevalence of wasting in rural Karamoja and Westnile regions was at 10% and 10.4%, respectively, compared to the national prevalence of 4% (Buzigi, 2018).

2.2.3 Underweight

The United Nation's Children Fund (UNICEF) estimates that one in every four children below five years old from developing countries in Asia and Africa are underweight (UNICEF, 2006). Underweight in children is defined as a low weight for age (WHO and UNICEF, 2019). According to the WHO child growth standards, underweight is defined as a weight for age *Z* score less than two SD below the global median standards (WHO and UNICEF, 2019). It has been argued that underweight is not an objective measure of child malnutrition because it is used as a composite of stunting and wasting and does not distinguish between the two (WHO and UNICEF, 2019). However, measuring underweight is recommended in growth monitoring programmes because weight is easy to measure and plot on growth monitoring charts (WHO and UNICEF, 2019). Based on convincing evidence from a meta-analysis of prospective studies, underweight is associated with child mortality in LMICs (McDonald *et al.*, 2013).

More than 75% of children below five years with underweight reside in developing countries of Asia and Africa (Akombi *et al.*, 2017; Kumar *et al*., 2019). Based on the 1995 and 2016 Uganda Demographic and Health Surveys, the prevalence of child underweight decreased from

25.5% in 1995 (Statistics Department-Uganda and Macro International Inc, 1996) to 11% in 2016 (Uganda Bureau of Statistics and Inner City Fund, 2018). However, by the year 2016, some Ugandan regions such as rural Bukedi, Bugisu, Karamoja, Acholi, and Westnile had a higher prevalence of underweight in children, ranging from 12% to 26% (Uganda Bureau of Statistics and Inner City Fund, 2018). The most recent data compiled by the Mundu Index (2019), revealed that in the year 2019, the prevalence of underweight among children below five years old in Uganda was lower (10.2%), compared to other countries of SSA such as Kenya (11%), Tanzania (13.7%), Burundi (29.3%), Democratic Republic of Congo (23.4%) and Ethiopia (23.6%) (Mundu Index, 2019).

2.2.4 Micronutrient deficiencies

Micronutrient deficiency is also known as hidden hunger because a person may suffer from a micronutrient deficiency without showing any immediate visible signs (UNICEF 2018) Micronutrients include both vitamins and minerals and there are many forms of micronutrient deficiencies. However, this study reviews VAD, ID and ZnD because these are the three most prevalent micronutrient deficiencies of public health importance, especially in LMICs, including Uganda (Ahmed, Hossain and Sanin, 2012; Wirth *et al.*, 2017; UNICEF, 2018). Table 2.2 shows the global prevalence of VAD, ID and ZnD.

Table 2.2: Global prevalence of child VAD, ID and ZnD (Wessells and Brown, 2012; Stevens *et al.*, 2015; Wirth *et al.*, 2017; Gardner and Kassebaum, 2020)

Region	Vitamin A deficiency (%)	Iron deficiency (%)	Zinc deficiency (%)
Low and middle income countries	29	56	17.3
Sub-Saharan Africa	48	72	>25
Asia	44	67	15-25
North America and Europe	< 5	<6	<15

2.2.4.1 Vitamin A and prevalence of vitamin A deficiency

Vitamin A (retinol) is a fat-soluble vitamin (Ross *et al.*, 2014). The two sources of vitamin A include the pre-formed and pro-formed sources. The former is found in ASFs such as liver, eggs and organ meats, while the latter is found in plant source foods (PSFs), such as carrots, spinach and pumpkin, and provitamin A-biofortified food crops such as orange-fleshed sweet potato (OFSP), yellow or orange maize and yellow cassava (Ross *et al.*, 2014). Provitamin A is the main source of vitamin A for people in LMICs because they have a high and low consumption rate of PSFs and ASFs, respectively (Sanghvi, Ross and Heymann, 2015). Pre-formed vitamin A is also called retinol, an active form of vitamin A in the body (Tanumihardjo, Palacios and Pixley, 2010). In contrast, pro-formed vitamin A is in the form of provitamin A carotenoids (PVACs), the pigments that give the yellow and orange colour to the edible parts of plants rich in pro-formed vitamin A (Saini, Nile and Park, 2015). When provitamin A-rich foods are consumed, their PVACs are metabolised into retinol in the human body (Tanumihardjo, Palacios and Pixley, 2010; Green *et al.*, 2016).

Vitamin A has several functions in the body including a well defined function in vision (Ross *et al.,* 2014). When retinal tissue is deprived of vitamin A, eye function is impaired resulting in blindness because vitamin A is also required for the integrity of epithelial cells, cell differentiation and growth (Ross *et al.,* 2014). To this end, vitamin A is important for child growth. A review of several epidemiological studies confirmed that vitamin A deficient children have reduced linear growth (Marasinghe *et al.,* 2015; Akhtar *et al.*, 2013). Furthermore, vitamin A plays a role in immune function by fighting common childhood illnesses such as measles and diarrhoea (Mayo-Wilson et al., 2011; Kartasurya *et al*., 2020).

Total serum retinol is the most used indicator of vitamin A status in population studies (Samba *et al.*, 2010; Martínez-Torres, Meneses-Echavéz and Ramírez-Vélez, 2014; Stevens *et al.*, 2015). Vitamin A deficiency is diagnosed when serum retinol is less than 20 µg/dL or 0.7 µmol/L (Stevens *et al.*, 2015). However, concentrations of retinol binding protein (RBP), a specific vitamin A transport protein, has been used to diagnose VAD in population field surveys (Baingana, Matovu and Garrett, 2008), because the ratio of circulating serum retinol to RBP is 1:1(Tanumihardjo *et al.,* 2016). In addition to using biochemical biomarkers, clinical problems of the eye such as xerophthalmia (inflammation and dryness of the conjunctiva and cornea), have been used to diagnose VAD (Tanumihardjo, 2011). Xerophthalmia is a population indicator of VAD, and a minimum prevalence of 0.5% in preschool children signifies a public health problem (Sherwin *et al.,* 2012).

The problem of VAD is prevalent among children worldwide (Ahmed, Hossain and Sanin, 2012; Wirth *et al*., 2017). Globally, one third of all children under five years of age suffer from VAD, which causes blindness or death when associated with severe bouts of diarrhoea or measles (Stevens *et al.*, 2015). The prevalence of VAD is higher in LMICs compared to HICs

(Wirth et *al*., 2017). The prevalence of VAD in LMICs among children under five years old reduced from 39% in 1991 to 29% in 2013 (Stevens *et al.*, 2015). However, the prevalence is estimated to be decreasing very slowly (Mason *et al.*, 2015). The rate at which VAD in children is reducing in LMICs is about 0.3 percentage points per year, and at this rate, it will take another 100 years to eliminate the problem of VAD in LMICs (Mason *et al.*, 2015).

By the year 2013, the prevalence of VAD among children under five years of age in LMICs was 29%, of which SSA and eastern Asia contributed 48% and 44%, respectively (Stevens *et al.*, 2015). A review study conducted in South Asian countries showed that India had the highest prevalence of clinical and subclinical VAD among South Asian countries, with 62% of children under five years old reported to be deficient in vitamin A (Akhtar *et al.*, 2013).

In a Latin American country, Columbia, Martínez-Torres *et al.* (2014) showed that the prevalence of VAD (serum levels less than 20µg/dL) among children 12 to 59 months was 24.3%. The prevalence of child VAD is unacceptably high in several SSA countries (Wirth *et al.,* 2017). In Congo Brazzaville, a national representative assessment survey of vitamin A status showed that 50% of children between six months and six years had mean retinol levels of 14.2µg/dL (Samba *et al.*, 2010), a higher VAD prevalence than that reported in LMICs and the SSA region (Stevens *et al.*, 2015). In the Democratic Republic of Congo, a VAD prevalence of 49% (serum retinol less than 20µg/dL) was reported among children between six to 59 months old (Samba *et al.*, 2006).

To contribute towards combating VAD, the government of Uganda has been running bi-annual (six monthly) high dose [200,000 International Units (IU) equivalent to 60, 000µg of retinol] vitamin A supplementation (VAS) for more than a decade (Baingana, Matovu and Garrett, 2008; Wirth *et al.,* 2017). However, the prevalence of VAD among Ugandan children under five years of age increased from 20.4% in 2006 (Baingana, Matovu and Garrett, 2008) to 32.6% in 2016 (Wirth *et al.,* 2017). The likely explanation for the increase in the prevalence of VAD in Ugandan children is that VAS programmes may not combat VAD, when implemented as a single strategy (Mason *et al.,* 2015). This is because the liver is unable to store the high dose of 200,000 IU (nearly 100 times the daily allowance for children below five years), and therefore, excess vitamin A is broken down by the liver and excreted (D'Ambrosio, Clugston and Blaner, 2011). To this end, the rise in serum retinol resulting from six monthly VAS is small, short-lived, and lasts for only one to three months (Pedro *et al.*, 2004). Therefore, VAS programmes are necessary but not sufficient to combat VAD (Mason *et al.*, 2015).

Furthermore, the increase in the prevalence of child VAD in Uganda is linked to the fact that caregivers feed their children CFs prepared from cereals such as white maize and tubers such as yams, white cassava and white sweet potato (Amaral, Herrin and Gulere, 2018; Ekesa, Nabuuma and Kennedy, 2019). It is worth noting that such cereals and tubers consumed by Ugandan children are deficient in vitamin A (Ekesa, Nabuuma and Kennedy, 2019), and their regular consumption is associated with child VAD (Gegios *et al.*, 2010). To this end, it is important to identify locally available foods rich in vitamin A or PVACs and use them to prepare vitamin A rich CFs. Consumption of such CFs prepared from locally available PVAC rich foods can be a complementary strategy to VAS programmes towards combating child VAD in Uganda, as shown in other African countries (Faber, *et al.*, 2002; Faber & van Jaarsveld, 2007; Gershon, 2017).

2.2.4.2 Zinc and prevalence of zinc deficiency

Zinc is an essential micronutrient because the human body cannot produce zinc and does not have an adequate mechanism for storing or releasing it (Kambe *et al.*, 2015). Therefore, regular dietary intake is necessary (Kambe *et al*., 2015). Zinc serves as a cofactor for many enzymes and is important for the maintenance of biochemical functions throughout the human life cycle (Kambe *et al.*, 2015). ZnD is associated with a variety of biochemical and physiological abnormalities, such as retarded growth in children and impaired immune function leading to childhood illnesses (Millward, 2017). Moreover, ZnD may result from dietary inadequacy, increased requirements during rapid growth periods such as complementary feeding, malabsorption, increased losses, and impaired utilisation of nutrients (Sheng *et al.*, 2009; Krebs *et al.*, 2012).

The quality of dietary zinc sources is determined by the amount and bioavailability of zinc from food sources (Hambidge *et al.*, 2004; Sheng *et al.*, 2009). Animal and insect source foods are the richest food sources of zinc, and they do not contain phytate, an anti-nutrient that reduces the absorption of zinc (Ross *et al.*, 2014). PSFs such as cereals and legumes contain a modest amount of zinc; however, their high phytate content reduces the amount of zinc available (Gibson *et al.*, 2010). Serum or plasma zinc concentration is the most frequently used biochemical indicator of zinc status in populations (Hess *et al.*, 2009; Lowe, Fekete and Decsi, 2009; Wieringa *et al.*, 2015). Serum/plasma zinc concentration of less than 65 µg/dL (9.9 µmol/l) in children less than 10 years old indicates ZnD (De Benoist *et al.*, 2007). A ZnD prevalence of higher than 20% is a public health problem, requiring an intervention to improve population zinc status (De Benoist *et al.*, 2007; Hess *et al.*, 2009). Alternatively, the risk of ZnD may be estimated based on dietary intake (Hotz, 2007) and childhood stunting (Hotz, 2001; Engle-Stone *et al.*, 2014). The three common recommended indicators for risk assessment of population ZnD are stunting rates among children aged less than five years, prevalence of inadequate dietary zinc intake, and the prevalence of low plasma zinc (De Benoist *et al.*, 2007). Therefore, where biochemical assessment of zinc status is not possible, ZnD can be estimated based on the prevalence of inadequate zinc intake of >25% and a prevalence of stunting of >20% (De Benoist *et al.*, 2007).

By 2012, an estimated 17.3% of the world's population was having ZnD (Wessells and Brown, 2012). In Latin America and the Caribbean, the national data for serum zinc from Mexico, Colombia, Ecuador, and Guatemala showed that the prevalence of ZnD ranged from 19.1% to 56.3% among children below six years old (Cediel *et al.*, 2015). Moreover, the countries with the highest risk of ZnD (estimated prevalence of inadequate zinc intake >25% plus prevalence of stunting >20%), were Belize, Bolivia, El Salvador, Guatemala, Haiti, Honduras, Nicaragua, Saint Vincent and the Grenadines (Cediel *et al.*, 2015).

Africa and Asia have the highest burden of ZnD among children under five years of age. The prevalence of inadequate zinc intake ranges from 15% to ≥ 25%, compared to Europe and North America with less than 15% (Wessells and Brown, 2012). A study conducted in India showed that 73.3% of preschool children were zinc-deficient (plasma zinc <70 µg/dL), of which 33.8% had plasma zinc levels below 60 µg/dL (Dhingra *et al.*, 2009). In urban Sri Lanka, deficiencies of zinc and vitamin A occurred among 67% and 38% of children below five years old, respectively (Marasinghe *et al.*, 2015). A higher prevalence of ZnD is also common in countries in SSA (Wessells and Brown, 2012). A study conducted in Cameroon showed that the prevalence of ZnD (serum zinc <65 mg/dL) among children under five years old was 83% (Engle-Stone *et al.*, 2014). Furthermore, in Benin, the prevalence of inadequate intakes of zinc among children below five years ranged from 11% to 80%, when compared to the estimated average requirement (EAR) (Galetti *et al.*, 2016).

The prevalence of inadequate zinc intake in Uganda ranged from 15% to 25% between 2003 and 2007 (Wessells and Brown, 2012). Between October 1999 and January 2000, Bitarakwate and colleagues showed that the serum zinc levels in Ugandan children aged six to 36 months with persistent diarrhoea, was significantly lower than that of children without diarrhoea (Bitarakwate, Mworozi and Kekitiinwa, 2003). The prevalence of ZnD in children with persistent diarrhoea was 47.9% (Bitarakwate, Mworozi and Kekitiinwa, 2003). Moreover, of the children with persistent diarrhoea, 66.7% were stunted, wasted or both (Bitarakwate, Mworozi and Kekitiinwa, 2003). It is worth noting that the dietary intake of zinc has been shown to prevent both acute and chronic diarrhoea in children (Lukacik, Thomas and Aranda, 2008). Therefore, it is important to identify locally available zinc rich CFs to reduce the incidence and prevalence of child diarrhoea in Uganda.

Furthermore, a multi-clinic study by Ndeezi *et al.* (2010) conducted between 2005 and 2008, revealed that the prevalence of ZnD among children between 12 and 59 months of age living with HIV/AIDS in Uganda was 54.3% (serum zinc less 10 μmol/L). The study by Ndeezi *et al.* (2010) suggests that special groups of children such as those living with HIV/AIDS, may need specific considerations while addressing the problem of ZnD in Uganda. Ugandan staple foods such as cereals and tubers are used to prepare CFs (Amaral, Herrin and Gulere, 2018). However, tubers are low in zinc (Tidemann-Andersen *et al.*, 2011), while cereals contain phytic acid which reduces the bioavailability of zinc. Moreover, consumption of tubers is linked to ZnD among children (Gegios *et al.*, 2010). Since Ugandan children are predominantly fed CFs low in zinc (Amaral, Herrin and Gulere, 2018; Ekesa, Nabuuma and Kennedy, 2019), there is a need to identify locally available and affordable foods rich in zinc such as common bean (Beebe, Gonzalez and Rengifo, 2000; Kiwuka *et al.*, 2012), and use them to prepare zinc rich CFs for caregivers to feed their children during the period of complementary feeding.

2.2.4.3 Iron and prevalence of iron deficiency

The two dietary classifications of iron are haem and non-haem iron. The former is predominantly from ASFs, while the latter is from PSFs (Hurrell and Egli, 2010). Haem iron is an important dietary source of iron because it is more effectively absorbed than non-haem iron (Anderson and Frazer, 2017). The levels of stored iron in the body influence the extent of its absorption. In cases of low iron status, dietary absorption of iron is promoted, whereas with high iron levels, iron absorption is reduced (Andrews, 2005; Abbaspour, Hurrell and Kelishadi, 2014). Several other dietary and endogenous factors can influence iron absorption, for example, ascorbic acid (vitamin C) reduces ferric iron to the more bioavailable form of ferrous iron (Anderson and Frazer, 2017), while polyphenols and phytic acid can interfere with iron absorption (Hurrell and Egli, 2010).

Anaemia is diagnosed when there is a decrease in the total amount of red blood cells (RBC) or haemoglobin (Hb) in the blood based on age, sex and race (Hercberg, Preziosi and Galan, 2001). The highest proportion of anaemia in LMICs is due to defects in Hb synthesis or iron deficiency anaemia (IDA) (Kassebaum *et al.*, 2014). To this end, most LMICs use low Hb levels as a proxy measure of ID and IDA (Petry *et al.*, 2016). However, other RBC defects cause low Hb or anaemia including production defect (aplastic anaemia), maturation defect (megaloblastic anaemia), genetic defects of haemoglobin maturation (thalassaemia) or due to the synthesis of abnormal haemoglobin (haemoglobinopathies, sickle cell anaemia and thalassaemia), and physical loss of RBCs (haemolytic anaemias) (Schneider *et al.*, 2005; White, 2005). In addition to ID, other nutrient deficiencies such as vitamin C and infectious diseases such as malaria and intestinal worm infestations common in LMICs, are associated with IDA (Stevens *et al.*, 2013).

In contrast to LMICs, HICs use specific and more sensitive tests for individual assessment of depleted iron stores, such as serum ferritin and transferrin saturation (Hercberg, Preziosi and Galan, 2001). Plasma ferritin is a secretory component of intracellular ferritin synthesis, and reflects the extent of iron stores in normal individuals. A decrease in plasma ferritin to less than 12 µg/L and 30 µg/L among children less than five years old without infection and with infection respectively, indicates ID (Zimmermann and Hurrell, 2007). In contrast, serum transferrin is an iron transport protein produced by the liver (Lynch *et al.*, 2018). When the human body's stores of iron run low, the human body produces more transferrin to get more iron into the blood (Hercberg, Preziosi and Galan, 2001). Therefore, a high serum transferrin (total iron binding capacity) is an indicator of ID because it means that the body is producing more of the transport protein to bind and use all the iron available in the body (Lynch *et al.*, 2018). A soluble transferrin receptor level greater than 8.3 mg/L is considered as ID among children 6 to 24 months old (Serdula *et al.*, 2013). Many of the clinical symptoms of ID are a consequence of IDA (low Hb and ferritin levels based on age, sex, and race), and a decrease of iron in different tissues or a combination of both processes (Abbaspour, Hurrell and Kelishadi, 2014). Childhood consequences of ID include IDA, reduced work capacity, poor mental and motor functioning in later life (Lynch *et al*., 2018).

More than 50% of the world's population has IDA, with the highest burden in LMICs (WHO/UNICEF/UNU, 2001; McLean *et al.*, 2008). Children less than five years old, pregnant women, and women of childbearing age from low socio-economic status such as those from LMICs, have a higher risk of developing IDA because they have an inadequate dietary iron intake with an increased iron demand (WHO/CDC, 2008). The causes of anaemia are multi-factorial (Balarajan *et al.*, 2011; Kassebaum *et al.*, 2014). However, 30% to 50% of anaemia in LMICs is due to IDA (Kassebaum *et al.*, 2014). Other causes of anaemia in LMICs include infections such as malaria and intestinal worms (Stevens *et al.*, 2013).

The risk for ID and IDA among children is particularly high during the second six months of life, when prenatal stores of iron are depleted and breast milk alone no longer provides the iron that is required to meet the physiological needs for children in the age range of complementary feeding (Black *et al.*, 2011). A cohort study conducted in Bolivia showed that low iron status was common among infants between six to eight months of age (Burke *et al.*, 2018). Moreover, 56% of these infants were iron deficient, 76% were anaemic, and 46% had IDA (Burke *et al.*, 2018). In contrast to LMICs, the prevalence of ID and IDA among children during the period of complementary feeding in HICs of Europe was low (Hercberg, Preziosi and Galan, 2001). Hercberg and colleagues showed that the prevalence of ID (measured by serum ferritin) among children six to 24 months was 2%, 24.6%, 39.8%, and 29.2% in Denmark, Italy, Spain and France, respectively (Hercberg, Preziosi and Galan, 2001). The prevalence of IDA in France was low at 4.2%; however, there was no IDA data available for Italy, Denmark and Spain (Hercberg, Preziosi and Galan, 2001).

In Uganda, Totin *et al.*, (2002) conducted a study between January 1995 and June 1998, and established that the prevalence of IDA was 44.3% and 45.4%, among HIV positive and HIV negative Ugandan children in the age range of complementary feeding, respectively. According to the 2000-2001 Uganda Demographic Health Survey (UDHS), the prevalence of anaemia in Uganda was 64% among children less than five years of age (Uganda Bureau of Statistics and ORC Macro., 2001). A subsequent 2006 UDHS noted that the prevalence of anaemia had increased to 72% in Uganda (Uganda Bureau of Statistics and Macro International Inc., 2007). The most recent UDHS conducted in 2016, showed that the prevalence of anaemia in children six to 24 months old, the age range of complementary feeding, was 33.2%, compared to an anaemia prevalence of 2% to 11.7% among children 12-59 months old (Uganda Bureau of Statistics and Inner-City Fund 2018). Moreover, the prevalence of child anaemia in rural districts of Uganda such as Kyankwanzi district was more than twice (6.8%) than that for urban districts (3.3%) (Uganda Bureau of Statistics and Inner-City Fund 2018). In addition to malaria infection, the high prevalence of anaemia among Ugandan children is due to IDA (Menon and Yoon, 2015). Iron deficiency anaemia among rural Ugandan children is linked to low quality CFs deficient in iron (Kikafunda, Lukwago and Turyashemererwa, 2009). To this end, identifying and promoting the preparation and consumption of iron rich CFs using locally available iron rich foods such as common bean (Beebe, Gonzalez and Rengifo, 2000; Kiwuka *et al.*, 2012), is necessary to contribute towards combating IDA.

2.2.5 Micronutrient deficiencies as risk factors for stunting and acute malnutrition

A child may be underweight because it is either stunted (low height for age) or acutely malnourished/wasted (low weight for height) (WHO and UNICEF, 2019) Therefore, the link between micronutrient deficiencies and stunting or wasting are reviewed. Several cross-sectional studies conducted in LMICs have revealed that individual micronutrient deficiencies including VAD, ID and ZnD are positively associated with stunting among children (Galetti *et al.*, 2016; Ssentongo *et al.*, 2020). For example, in Uganda, Ssentongo *et al.* (2020), showed that VAD was positively associated with stunting among children aged six to 59 months old. In Benin, Galetti *et al.* (2016) and in Cameroon, Engle-Stone *et al.* (2014), demonstrated that ZnD was prevalent in stunted children. Another study in Pakistan, similarly reported that IDA was common among stunted children (Rahman, Razak and Hassan, 2010). Stunting is an indicator of poor linear growth (WHO and UNICEF, 2019). A systematic review of 36 intervention studies assessed the effect of zinc supplementation on linear growth in children < 5 years from developing countries (Imdad and Bhutta, 2011). In eleven of these studies, zinc was supplemented in combination with other micronutrients, including iron and vitamin A. Imdad and Bhutta (2011) established that multiple micronutrient supplementation improved linear growth. In addition, Imdad and Bhutta (2011) showed that a zinc dose of 10 mg/day for a duration of 24 weeks led to a net linear gain of 0.37 cm in the zinc supplemented group, compared to the control. Another systematic review of multiple micronutrient supplementation including iron, zinc and vitamin showed little effect on linear growth (Mayo-Wilson *et al.*, 2014). Findings from Imdad and Bhutta (2011) and (Mayo-Wilson *et al.*, 2014) may suggest that micronutrient intake has a positive effect on linear growth.

In Bangladesh, acute malnutrition was positively associated with ZnD (Mahfuz *et al*., 2019), while Kangas *et al.* (2020) reported VAD, ID and IDA among children with SAM in Burkina Faso. Since micronutrient deficiencies are common in acute malnutrition, therapeutic foods such as formula 75 (F75), formula 100 (F100) and plumpy nut, a ready to use therapeutic food (RUTF), which are used in the treatment of SAM, are designed to contain micronutrients including zinc, vitamin A and iron (WHO, 2013b; Kangas *et al.*, 2020). Moreover, VAS and iron supplementation are recommended in the management of acute malnutrition (WHO, 2013b). The role of iron and zinc in therapeutic foods for the treatment of acute malnutrition have been demonstrated (Kangas *et al*., 2020). For example, iron, and zinc status of children with acute malnutrition improved following WHO protocols for the therapeutic management of acute malnutrition (Kangas *et al*., 2020).

2.3 Complementary feeding and child malnutrition

Complementary feeding refers to the provision of liquids and solid foods in addition to breastfeeding, which should be practiced from six to 24 months of age, after exclusive breastfeeding for the first six months of life, and should be continued after 24 months (FAO/WHO, 2013). From the age of six months, an infant's energy and nutrient requirements starts to exceed that which is provided by breast milk alone, and complementary feeding becomes necessary to fill the energy and nutrient gap. For example, vitamin A, iron and zinc are some of the most problematic nutrients during the period of complementary feeding (Vossenaar and Solomons, 2012), largely because their concentrations in human milk are low relative to needs.

Breast milk zinc concentrations decrease sharply during the first three months of lactation, followed by a gradual decline throughout lactation (Qian *et al.*, 2010). It is estimated that the mean daily zinc transfer to the infant via breast milk is 4 mg in colostrum at birth, 1.75 mg at one month old and 0.7 mg at six months old (Brown *et al.*, 2009). The 0.7 mg of zinc transferred at six months is too low to meet the recommended dietary allowance (RDA)/adequate intake (AI) for zinc of 3mg for IYC, six to 24 months old (Institute of Medicine, 2001). To this end, CFs rich in zinc are necessary for IYC in the age group of complementary feeding. Furthermore, infants are particularly susceptible to the consequences of ID due to rapid growth and brain development (Eussen *et al.*, 2015). Iron requirements for the full-term infant during the first six months of life are met through the utilisation of hepatic reserves accumulated mainly during the final trimester of gestation (Cao and O'Brien, 2013). However, by six months, the iron concentration in breast milk is insufficient to meet the RDA/AI for iron for IYC, six to 24 months old Moreover, maternal dietary intake of iron rich foods and iron supplementation does not improve breast milk iron concentrations (Kelleher and Lönnerdal, 2005; Mahdavi, Nikniaz and Gayemmagami, 2010). Therefore, IYC require iron rich CFs during complementary feeding (FAO/WHO, 2013).

2.3.1 Causes of child malnutrition during the period of complementary feeding

Infant growth faltering may result if CFs are not introduced timeously at around six months of age or if they are inadequate in quality and quantity (Chang, He and Chen, 2008). In many LMICs, the highest incidence of growth faltering, and micronutrient deficiencies occur during the period of complementary feeding (Victora *et al.*, 2010). Figure 2.4 shows the UNICEF (1990) conceptual framework on the causes of child malnutrition.

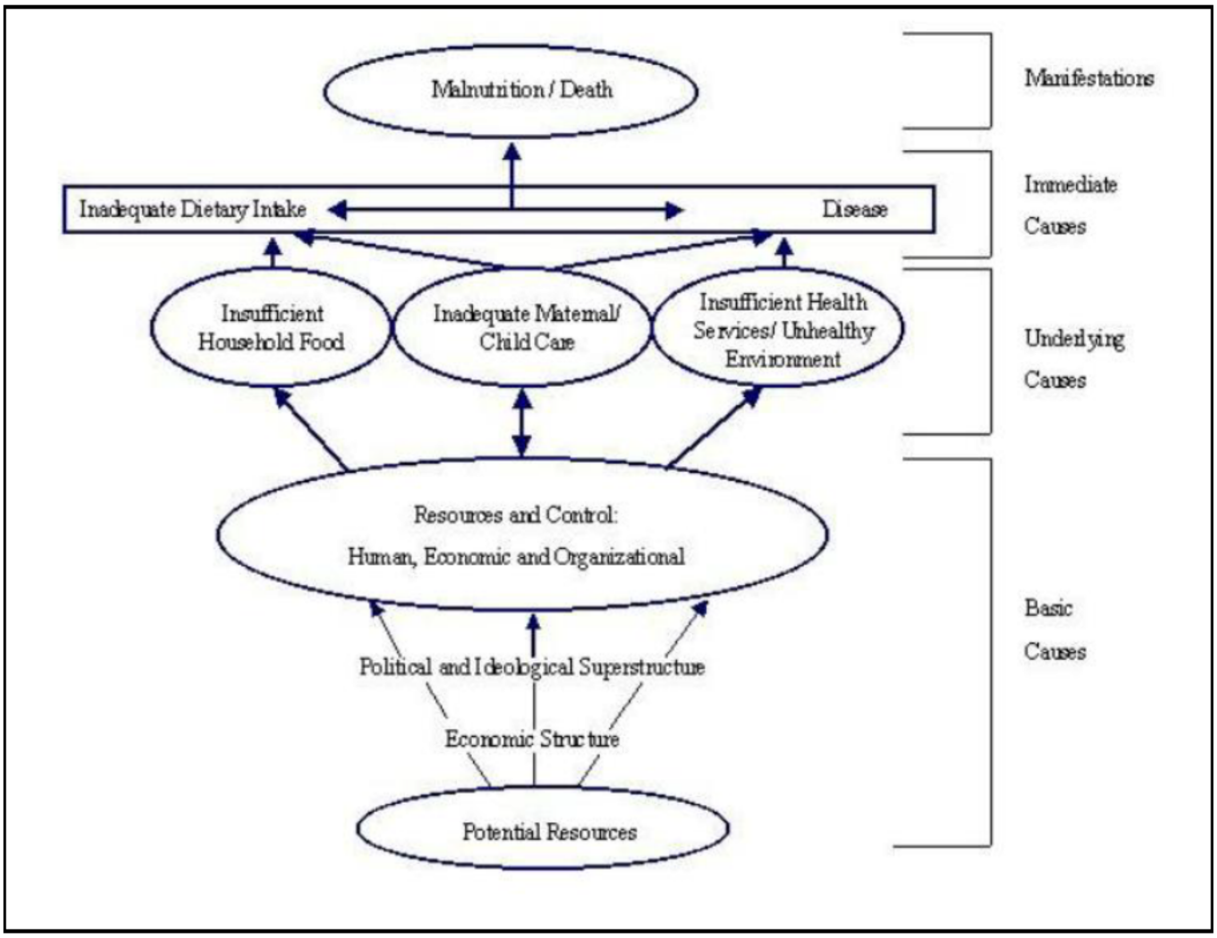


Figure 2.4: United Nations Children's Fund conceptual framework on the causes of child malnutrition (UNICEF, 1990)

Acoording to the UNICEF (1990) the causes of child malnutrition are multi-factorial and multi-level, grouped into basic, underlying and immediate causes. Inadequate dietary intake including consuming foods deficient in micronutrients during the period of complementary feeding is an immediate cause of malnutrition/death (UNICEF, 1990). Studies conducted in LMICs show that child malnutrition is common among IYC, six to 24 months old who consume CFs with low energy and micronutrient density (Kimmons *et al.*, 2005; Gibson *et al.*, 2009). Childhood infections such as diarrhoeal diseases, malaria, respiratory tract infections and intestinal parasites are other immediate causes of child malnutrition (UNICEF, 1990). This is common among complementary fed IYC from LMICs, as CFs are often prepared under unhygienic conditions (Muhoozi *et al.*, 2016). Several studies conducted in LMICs have shown that childhood infections are associated with child malnutrition (Buzigi 2015; Jones and Berkley 2014; Schlaudecker, Steinhoff, and Moore 2011). Infections cause child malnutrition by reducing appetite and nutrient absorption, and increasing nutrient catabolism (Millward, 2017). Moreover, infections are both a risk factor and consequence of malnutrition (Millward, 2017).

The causes of child malnutrition in Uganda are in line with the UNICEF (1990) conceptual framework on the causes of child malnutrition. The first stratum of immediate causes includes infections and inadequate food intake (Kikafunda, Agaba and Bambona, 2014; Rytter *et al.*, 2015; Mokori, Schonfeldt and Hendriks, 2017; Nabukeera-Barungi *et al.*, 2018). The second stratum are the underlying causes that manifest at the household level and impact on quality of care such as inadequate child supervision, a heavy workload for caregivers (usually mothers), and maternal attitude and behaviours; and inadequate access to resources such as income and food (Ssewanyana and Kasirye, 2010; Kikafunda, Agaba and Bambona, 2014; Muhoozi *et al.*, 2016). The third stratum, which are the basic causes, includes poor contextual factors (political, economic and cultural) and inadequate potential resources (Ssewanyana and Kasirye, 2010; Uganda Bureau of Statistics and Inner City Fund International Inc., 2012; World Food Program, 2016; Uganda Bureau of Statistics and Inner City Fund, 2018).

2.3.2 Guiding principles for complementary feeding

Poor feeding practices lead to child malnutrition and growth faltering during the period of complementary feeding because the nutritional demands for growth increase during this essential period of life (Victora *et al.*, 2010). Therefore, combating child malnutrition and promoting sustainable child growth and development through sufficient infant and young child feeding practices is a well-recognised strategy for the survival and wellbeing of infants and young children (FAO/WHO, 2013). The guiding principles for complementary feeding were established by the WHO as shown in Table 2.3 (FAO/WHO 2013).

<u>Table 2.3:</u> Guiding principles for complementary feeding (FAO/WHO 2013)

• Practise exclusive breastfeeding from birth to 6 months of age and introduce complementary foods at 6 months of age (180 days), while continuing to breastfeed. • Continue frequent, on-demand breastfeeding until two years of age or beyond. • Practise responsive feeding, applying the principles of psychosocial care. • Practise good hygiene and proper food handling. • Start at six months of age with small amounts of food and increase the quantity as the child gets older, while maintaining frequent breastfeeding. • Gradually increase food consistency and variety as the infant gets older, adapting to the infant's requirements and abilities. • Increase the number of times that the child is fed complementary foods as he/she gets older. • Feed a variety of foods to ensure that nutrient needs are met. • Use fortified complementary foods or vitamin-mineral supplements for the infant, as needed. • Increase fluid intake during illness, including more frequent breastfeeding, and encourage the child to eat soft, varied, appetising, favourite foods. After illness, give food more often than usual and encourage the child to eat more.

2.4 Existing interventions to combat child malnutrition

The commonly recommended strategies or interventions to combat malnutrition in IYC during complementary feeding are food supplementation, fortification, and dietary diversification (DD). However, their acceptability to both child caregivers and IYC is very important because caregivers make decisions on the kinds of CFs that are prepared and fed to the children in their care (Govender *et al.*, 2014; Amod *et al.*, 2016), and children are the target population for receiving the CFs (FAO/WHO, 2013). Moreover, caregiver acceptability of CFs may not guarantee their acceptability to the children in their care (Paul *et al.*, 2008). To this end, the guiding principles and effectiveness of food supplementation, fortification and dietary diversification for IYC are reviewed.

2.4.1 Supplementation

Supplementation is a term used to describe the provision of relatively large doses of micronutrients, usually in the form of pills, capsules, or syrups (WHO and FAO, 2006). In LMICs, supplementation programmes have been widely adopted to provide iron and folic acid to pregnant women, and vitamin A and zinc to IYC under five years of age (Department of Health South Africa, 2012; Preedy, 2013). In settings where VAD is a public health problem, VAS is recommended for IYC six to 59 months of age, as a public health intervention to reduce child morbidity and mortality (WHO, 2013a). A suggested VAS scheme for infants and children six to 59 months of age is presented in Table 2.4 (WHO, 2013a).

Table 2.4: Vitamin A supplementation scheme for children six to 59 months old (WHO, 2013a)

Target group	Infants 6-11 months old	Children 12-59 months old
Dose	100 000 IU (30mgRAE) of vitamin A	200 000 IU (60 mgRAE) of vitamin A
Frequency	Once	Every 4 to 6 months
Route of administration	Oral liquid, oil-based preparation of retinyl palmitate or retinyl acetate[a]	
Settings	Populations where the prevalence of night blindness is 1% or higher in children 24-59 months of age or where the prevalence of VAD (serum retinol 0.70µmol/L or lower) is 20% or higher in infants and children 6-59 months of age	

IU= international units; RAE= retinol activity equivalent; VAD= Vitamin A deficiency

[a]An oil-based vitamin A solution that can be delivered using soft gelatin capsules, as a single-dose dispenser or a graduated spoon (WHO, 2013a).

Several studies suggest that providing VAS to children six to 59 months of age from LMICs is associated with a reduced risk of mortality and diarrhoea (Awasthi *et al.*, 2013; Mason *et al.*, 2015; Imdad *et al.*, 2017). A possible explanation for the reduced diarrhoea morbidity observed with the use of VAS is that the vitamin A may improve gut integrity and therefore decrease the severity of some cases of diarrhoea (Mayo-Wilson *et al.*, 2011). The WHO (2013a) recommends that universal vitamin A distribution should involve periodic administration of supplemental doses to all preschool-age children or regions at highest risk of VAD, xerophthalmia, and night blindness. The WHO (2013a) recognises VAS as an essential nutrition action for IYC, six to 59 months of age bi-annually in LMICs during health system contacts.

Furthermore, children from areas where VAD is known to occur or where measles case fatality is likely to be more than 1%, should receive two doses of vitamin A supplements, given 24 hours apart, to help prevent eye damage and blindness (WHO, 2013a). VAS has been shown to reduce the number of deaths from measles by 50% (Mayo-Wilson *et al.*, 2011). The recommended age-specific doses are 50 000 IU for infants aged less than 6 months, 100 000 IU for infants aged six to 11 months and 200 000 IU for children ≥ 12 months (WHO, 2013a). If the child has clinical signs of VAD (such as Bitot's spots), a third dose should be given 4-6 weeks later (WHO, 2013a). Iron supplementation is proposed for IYC, six to 24 months old as they have higher iron requirements in comparison to other age groups, due to rapid growth (Grammatikaki and Huybrechts, 2016). Where the CFs do not include fortified foods, or if the prevalence of anaemia in children at approximately one year of age is severe (above 40%), supplements of iron at a dosage of 2 mg/kg of body weight per day should be given to all children between six to 24 months of age, as shown in Table 2.5 (WHO, 2013a). Moreover, a systematic review and meta-analysis of randomised control trials by Pasricha *et al.* (2013), showed that daily iron supplementation effectively reduced anaemia among children in the age range of complementary feeding.

Table 2.5: Guidelines for iron supplementation for children six to 24 months old (WHO, 2013a)

Age group	**Indications for supplementation**	**Dosage schedule**	**Duration**
Children from six to 24 months of age.	Where the diet does not include foods fortified with iron or where anaemia prevalence is above 40%.	2mg/kg/body weight/day.	From six to 23 months of age, for three months duration.

Zinc supplementation in LMICs aims to treat childhood diarrhoea, a leading cause of childhood morbidity, mortality, and malnutrition in LMICs (Checkley *et al.*, 2008; UNICEF/WHO, 2009; Moore *et al.*, 2010). A community-based, prospective, randomised controlled trial conducted in Bangladesh, showed that zinc supplementation combined with oral rehydration solution (ORS) significantly treated diarrhoea and prevented future episodes of diarrhoea, compared to ORS alone among children (Baqui *et al.*, 2002). Moreover, zinc plus ORS does not affect overall ORS use and decreases antibiotic/antidiarrheal use with good adherence without side-effects (INCLEN Childnet Zinc Effectiveness for Diarrhoea Group, 2006).

Convincing evidence from systematic reviews and meta-analyses of randomised control trials conducted in LMICs, showed that zinc supplementation reduced the duration and severity of acute and persistent diarrhoea among pre-school children (Bhutta *et al.*, 2000; Lukacik, Thomas and Aranda, 2008). Furthermore, zinc supplementation is an effective therapy for diarrhoea and reduces diarrhoea morbidity and mortality when introduced in LMICs (Walker and Black, 2010; Mayo-Wilson *et al.*, 2014). Zinc supplementation benefits children with diarrhoea because it is a vital micronutrient essential for protein synthesis, cell growth and differentiation, immune function and intestinal transport of water and electrolytes (Nriagu, 2011). Besides treating diarrhoea, zinc supplementation improves child growth (Imdad and Bhutta, 2011). A systematic rewiew of zinc supplementation interevention studies in developing countries revealed that zinc with or without combination with other micronutrients such as iron and zinc significantly increased linear child growth compared to placebo (Imdad and Bhutta, 2011). This is plausible because zinc may improve the secretion of insulin like growth factor-(IGF-1), a hormone that promotes linear growth in the first two years of life (Devesa, Almengló and Devesa, 2016). It is worth noting that zinc supplementation does not cause any serious adverse reactions (Gulani and Sachdev, 2014).

Furthermore, combined micronutrient supplementation improves micronutrient status and child linear growth. A combined vitamin A and zinc supplement significantly increased serum retinol, serum zinc, IGF-1 and SD scores for stunting among children below five years old, compared to the non-supplementation control group (Adriani and Wirjatmadi, 2014). These results suggest that a combination of micronutrients for supplementation programmes may be very important in improving child linear growth, since a meta-analysis and systematic review of randomised control trials of only zinc supplementation, had little or no significant effect on improving child growth (Mayo-Wilson *et al.*, 2014). Moreover, combined zinc and VAS significantly reduced the prevalence of persistent diarrhoea (Rahman *et al.*, 2001), compared to a stand-alone zinc supplementation (Siregar *et al.*, 2011).

To contribute towards combating child VAD in Uganda, bi-annual (six monthly) VAS programmes for children six to 59 months have been running for over a decade with over 65% coverage in Uganda (Wirth, 2017). However, the prevalence of child VAD among Ugandan children six to 59 months old has increased from 20.4% in 2006 (Baingana, Matovu and Garrett, 2008) to 32.6% in 2016 (Wirth et *al*., 2017). This may suggest that stand-alone VAS may not be sufficient to combat VAD (Mason *et al.*, 2015). Therefore, to contribute towards fighting VAD, VAS programmes may need to be complemented with other nutrition specific interventions such as fortification and dietary diversification or nutrition sensitive interventions such as provitamin A biofortification (Ruel and Alderman, 2013). In Uganda, zinc supplementation has been promoted and recommended in the treatment of child diarrhoea (Hategeka *et al.*, 2019). For example, the 2016 Uganda Demographic Health Survey showed that 47%-56% of children six to 24 months of age who had diarrhoea in the two weeks preceding the survey received zinc supplementation (Uganda Bureau of Statistics and Inner-City Fund, 2018). However, child morbidity and mortality due to ZnD in Uganda remains unacceptably high (Walker, Ezzati and Black, 2008), and therefore it is important to promote and support the intake of zinc rich foods among Ugandan children.

2.4.2 Food fortification

Fortification is defined as the addition of micronutrients to foods (WHO, 2016a). Three types of food fortification have evolved, and these include industrial or commercial fortification, fortification of homemade foods, and biofortification. Industrial or commercial fortification (ICF) refers to the addition of micronutrients to processed foods (WHO and FAO, 2006). ICF of staple CFs such as maize and rice with micronutrients of public health importance including vitamin A, iron and zinc, is widely recognised and supported by the WHO (WHO, 2016b; WHO 2018).

Consumption of commercially fortified CFs with iron rich foods improves Hb levels or prevents anaemia among infants in the age group of complementary feeding. Owino *et al.* (2007) randomly assigned Zambian infants at six months of age to receive a fortified blend of maize, common beans, bambara nuts and groundnuts or a similar blend with α-amylase for three months. At nine months, Hb levels were significantly higher in both intervention groups, than in the control group (Owino *et al.*, 2007). Moreover, the commercially fortified foods did not replace breastfeeding during the period of complementary feeding (Owino *et al.*, 2007). In comparison to unfortified maize flour, industrial fortification of maize flour products with iron and other micronutrients reduced the risk of ID in children (Garcia-Casal *et al.*, 2018). Moreover, maize fortification with iron was not harmful to children (Garcia-Casal *et al.*, 2018). Sazawal *et al.* (2010) randomly allocated children, including those in the age group of complementary feeding to receive either zinc, iron, and vitamin A-fortified milk or control milk (milk without micronutrient fortification). Intervention with micronutrient-fortified milk provided an additional 7.8mg of zinc, 9.6mg of iron, and 156mg of vitamin A per day (from three servings) for one year, compared to control milk (Sazawal *et al.*, 2010). Compared to children consuming control milk, children consuming fortified milk had a significantly lower risk of IDA and showed significant improvement in weight and height gain (Sazawal *et al.*, 2010). Improvement in height gain suggests that micronutrient fortification may prevent childhood stunting. Similar findings were reported in Chile, where a zinc-fortified formula significantly improved the height of children with SAM (Schlesinger *et al.*, 1992). Furthermore, in Malawi, Phuka *et al.* (2008) showed that consumption of CFs formulated from micronutrient-fortified maize soy flour for over one year improved the linear growth of infants, six to 12 months old.

Home fortification of CFs is another form of fortification. Home fortification, also referred to as point of use fortification, includes several products in powder or paste form that are mixed into CFs just before consumption (Adu-Afarwuah *et al.*, 2007; Jefferds *et al.*, 2013). These products are available as combinations of micronutrients alone (multiple micronutrient powders) or with other essential nutrients, such as protein, essential fats, amino acids, or enzymes that are missing or not available in adequate amounts in the usual diet (Jefferds *et al.*, 2013). Home fortification programmes have shown to improve child micronutrient status, growth and development during the period of complementary feeding (Siekmans et al., 2017). A randomised control trial by Adu-Afarwuah *et al.* (2007) assigned Ghanaian infants six to 12 months old to receive home-fortified food products including sprinkles powder (SP), crushable Nutri-tablets (NT), or energy-dense (108kcal/day), fat-based Nutri-butter (NB), containing 6, 16, and 19 vitamins and minerals, respectively, daily for 12 months. All the three home-fortified food products had positive effects on motor development by 12 months, compared with no intervention, while only NB affected growth (Adu-Afarwuah *et al.*, 2007). Moreover, all three home-fortified food products used to fortify CFs were well accepted by infants (six to 12 months old), because they consumed 87% of the SP, NT and NB given to them (Adu-Afarwuah *et al.*, 2008). Furthermore, all three intervention groups (SP, NT and NB) had significantly higher iron stores (ferritin), compared to the non-intervention control group. This finding indicates that home-fortified foods have the potential to combat ID (Adu-Afarwuah *et al.*, 2008). In addition to improving iron status, Adu-Afarwuah *et al.* (2008) revealed that the mean Hb was significantly higher in the NT (112 g/L) and NB (114 g/L) groups, but not in the SP (110 g/L) group, compared to non-intervention infants (106 g/L). More so, the prevalence of IDA was 31% in the non-intervention control group compared with 10% in the intervention groups (Adu-Afarwuah *et al.*, 2008). Compared to non-fortified food, iron multi-micronutrient fortification increased Hb levels by 0.87 g/dL and reduced the risk of anaemia by 57% (Eichler *et al.*, 2012). Moreover, compared to non-fortified food, fortification increased serum levels of vitamin A (Eichler *et al.*, 2012). Multiple micronutrient powders (MNP) are single-dose packets containing several vitamins and minerals in powder form that can be sprinkled onto any semi-solid food. The WHO (2011) recommends the use of MNP for home fortification of CFs as a strategy for improving micronutrient intake in children, six to 24 months old (WHO, 2011). A suggested scheme for home fortification with MNP of foods consumed by infants and children aged six to 24 months is presented in Table 2.6 (WHO, 2016a).

Table 2.6: Suggested scheme for home fortification with multiple micronutrient powders of foods consumed by infants and young children six to 24 months old (WHO, 2016a)

Composition per sachet[a]	Iron: 12.5 mg of elemental iron, preferably as encapsulated ferrous fumarate[b] Vitamin A: 300 μg of retinol, Zinc: 5 mg of elemental zinc, preferably as zinc gluconate.
Frequency	One sachet per day.
Duration and time interval between periods of intervention	At minimum, for a period of two months, followed by a period of 3 to 4 months of supplementation, so that use of micronutrient powders is started every six months.
Target group	Infants and children six to 24 months of age, starting at the same time as when complementary foods are introduced into the diet.
Settings	Populations where the prevalence of anaemia in children under two years or under five years of age is 20% or higher.

[a]The recommendation for the composition of the powder is based on the doses and nutrients included. In addition to iron, vitamin A and zinc, multiple micronutrient powders may contain other vitamins and minerals at current dietary reference intake doses for the target population. [b]12.5mg of elemental iron equals 37.5mg of ferrous fumarate, 62.5 mg of ferrous sulfate heptahydrate or 105mg of ferrous gluconate.

Home fortification of foods with MNP containing at least iron, vitamin A and zinc, is recommended to improve iron status and reduce anaemia among IYC in the age range of complementary feeding (WHO, 2011). In poor rural China, a soy-based MNP containing iron, and other micronutrients including vitamin A, zinc, calcium, vitamin A, folic acid, vitamin B_{12}, and vitamin B_2, significantly improved Hb status and prevented anaemia among children in the age group of complementary feeding (Huo *et al.*, 2015). Use of MNP containing zinc and iron reduces IDA in young children (Soofi *et al.*, 2013). However, the excess burden of diarrhoea and respiratory morbidities associated with MNP use and their very small effect on growth recorded, suggest that a careful assessment of risks and benefits is required in populations with malnourished children and high diarrhoea burdens (Soofi *et al.*, 2013). There is convincing evidence that MNP is acceptable to IYC and their caregivers. A randomised control study conducted in India showed that MNP was acceptable to both IYC, six to 24 months old and their mothers (Young *et al.*, 2018).

Biofortification is another form of fortification that involves the breeding of staple food crops to increase their nutritional value, including increased amounts of micronutrients or their precursors (Saltzman *et al.*, 2013; Bouis and Saltzman, 2017; Schnurr, Addison and Mujabi-Mujuzi, 2018). Biofortification is a nutrition sensitive agriculture intervention that can be achieved through three methods, including conventional or traditional plant breeding, application of target nutrient rich fertilizers to the soil or leaves, and genetic engineering, such as genetic modification and transgenesis (Garcia-Casal, *et al.*, 2016). Biofortification aims to increase the nutrient density of edible parts of staple crops during plant growth rather than during processing of the crops into foods (Saltzman *et al.*, 2016). Table 2.7 shows some LMICs which have adopted biofortification of staple food crops, together with their target micronutrients.

Table 2.7: Biofortification of staple food crops and their target micronutrients in low and middle-income countries (Meenakshi *et al.*, 2010; Bouis and Saltzman, 2017)

Country	Staple food crop	Target micronutrients
Uganda	Sweet potato	Vitamin A
Philippines	Rice	Zinc and iron
Pakistan	Wheat	Iron and zinc
Nigeria	Cassava	Vitamin A
Nicaragua	Common beans	Iron and zinc
Kenya	Maize	Vitamin A
India	Rice, pearl millet and wheat	Iron and zinc
Honduras	Common bean	Iron and zinc
Ethiopia	Maize	Vitamin A
Democratic Republic of Congo	Cassava	Vitamin A
Brazil (North west)	Cassava	Vitamin A
Bangladesh	Rice	Iron and zinc
South Africa	Sweet potato	Vitamin A
Rwanda	Common bean	Iron
Zambia	Maize	Vitamin A
Mozambique	Maize	Vitamin A

Several studies conducted in Uganda and other LMICs in Africa have evaluated the sensory properties (taste, texture, colour, and aroma) of CFs formulated from biofortified staple food crops (Ssebuliba, Muyonga and Ekere, 2006; Govender *et al.*, 2014; Amod *et al.*, 2016; Pillay, Khanyile and Siwela, 2018). In South Africa, Govender *et al.* (2014) found no significant difference in sensory acceptability between complementary porridges prepared from provitamin A biofortified maize and white maize. Child caregivers rated taste, texture, aroma, colour and overall acceptability of both porridges as “good”, with an average score of four on a five-point hedonic scale (Govender *et al.*, 2014). Moreover, during focus group discussions, caregivers expressed a willingness to give their infants porridge made with provitamin A-biofortified maize if it was more affordable, readily available, and beneficial to health (Govender *et al.*, 2014).

Another South African study by Amod *et al.* (2016), used maize meal of two provitamin A-biofortified maize varieties, separately, and a white variety (control) as the major ingredient in the composite CFs containing chicken stew. Overall caregiver acceptability was rated as “very good” for all the three CFs (Amod *et al.*, 2016). Moreover, caregivers had positive perceptions about the taste, texture, aroma and colour of the composite CF prepared with the two varieties of biofortified maize (Amod *et al.*, 2016). Furthermore, Pillay and colleagues assessed the acceptance of CFs made from orange-fleshed sweet potato (OFSP) and white-fleshed sweet potato (WFSP) among infant caregivers (Pillay, Khanyile and Siwela, 2018). The CFs prepared from OFSP were well accepted, especially its colour and soft texture (Pillay, Khanyile and Siwela, 2018). Moreover, during focus group discussions (FGDs), caregivers expressed a willingness to buy OFSP if it was available and cheaper than the WFSP (Pillay, Khanyile and Siwela, 2018). Ssebuliba and colleagues tested the acceptability of four and two varieties of OFSP and WFSP, respectively, among Ugandan children (Ssebuliba, Muyonga and Ekere, 2006). The acceptability scores of both test sweet potato varieties ranged from 3.5 to 5.0, which represented fair to very good ratings (Ssebuliba, Muyonga and Ekere, 2006).

Foods formulated from biofortified food crops have been shown to improve the nutritional status of children and women. For example, a randomised control trial conducted in Zambia, established that β-carotene from biofortified maize significantly improved total body reserves of vitamin A in rural Zambian children (Gannon *et al.*, 2014). In Kenya, the vitamin A status of children fed provitamin A-biofortified cassava improved significantly, compared to children fed non-biofortified cassava (Talsma *et al.*, 2016). Furthermore, in Rwanda, Hb and total body iron significantly increased among iron-depleted university females who consumed biofortified common beans, compared to the control group (Haas *et al.*, 2016). Furthermore, studies conducted by Hotz *et al.* (2012a) and Hotz *et al.* (2012b) in Mozambique and Uganda, respectively, showed that vitamin A status significantly improved among women and children from households which cultivated and consumed OFSP.

It is worth noting that the success of biofortified programmes depends on whether biofortified foods are accepted by target consumers for consumption (Birol *et al.*, 2015). However, acceptability remains a huge challenge to implement and sustain biofortified programmes on a large scale at a national level in developing countries, because there is a global campaign mobilising people from the developing world to resist biofortification (GRAIN, 2019a). For example, on 13th February 2019, a rally in Bangladesh protested against the release of biofortified rice (golden rice) as they do not want genetically modified foods in their country (GRAIN, 2019b). In Uganda, genetically modified provitamin A biofortified banana is expected to be ready for commercial release by 2021 (Schnurr, Addison and Mujabi-Mujuzi, 2018). However, the president of Uganda, for the second time refused to sign the genetically modified organisms (GMO) bill (New Vision, 2019), a situation which threatens the release and acceptability of provitamin A biofortified banana in Uganda. To this end, there is a global campaign to reject biofortified foods and encourage dietary diversification by using locally available foods to combat micronutrient deficiencies (GRAIN, 2019a).

2.4.3 Dietary diversification

Dietary diversity (DD) involves adding a variety of foods from various food groups to the diet such as fruit and vegetables, legumes, starch and ASFs (FAO, 2010). DD is important because it can improve the micronutrient density of poor-quality cereals and tubers predominantly fed to children during complementary feeding in the developing world. The WHO (2010) recognises DD as a key indicator for assessing IYC feeding practices during the period of complementary feeding. Minimum dietary diversity (MDD) which refers to the consumption of four or more food groups from the seven food groups (grains, roots and tubers; legumes and nuts; dairy products such as milk cheese, and yoghurt; flesh foods such as meat, poultry, fish and liver/organ meats; eggs; vitamin A rich fruits and vegetables; and other fruits and vegetables), is an acceptable measurement of DD (WHO, 2010).

Dietary diversification programmes that promote and support diversified food production and DD in LMICs have improved the nutritional status of children (Ruel, Quisumbing and Balagamwala, 2018). These programmes are nutrition sensitive and include ASF production, vegetable home gardening and biofortification (Ruel, Quisumbing and Balagamwala, 2018). In Cambodia, consumption of ASFs significantly reduced stunting and underweight by 69% and 74%, respectively among children six months to five years old, after adjusting for socio-economic and geographic factors (Darapheak *et al.*, 2013). In Indonesia, higher DD scores reduced child stunting by 89% (Mahmudiono, Sumarmi and Rosenkranz, 2017). In Bangladesh, a diverse diet formulated according to the proportion of a balanced diet, was shown to treat MAM among children below two years (Alam *et al.*, 2013). In Ethiopia, low DD (consumption of less than four food groups) was significantly associated with stunting among children in the age range of complementary feeding (Getachew and Argaw, 2017), while low household DD was associated with oedematous SAM in Uganda (Rytter *et al.*, 2015). Home gardening and consumption of provitamin A carotenoid rich foods have consistently been shown to improve the vitamin A status of children in rural Africa (Faber, Venter and Benade, 2002; Faber *et al.*, 2017). Furthermore, biofortification programmes in Africa (Van Jaarsveld *et al.*, 2005; Low *et al.*, 2007; Hotz *et al.*, 2012a; Chomba *et al.*, 2015) and Asia (Kodkany *et al.*, 2013; Finkelstein *et al.*, 2015), have been shown to improve vitamin A, iron and zinc status in children by diversifying their foods with vitamin A, iron and zinc biofortified food crops.

In addition to food supplementation, fortification and dietary diversification, other community-based strategies such as nutrition education of caregivers and home gardening have been shown to improve the nutritional status of IYC. In Malawi, nutrition education of child caregivers significantly improved DD of IYC in the age range of complementary feeding (Kuchenbecker *et al.*, 2017). In rural South Africa, home gardening of provitamin A rich vegetables, followed by nutrition education to encourage caregivers to feed these vegetables to their children, improved the vitamin A status of children (Faber, Venter and Benade, 2002). In Lesotho, the prevalence of underweight, stunting and wasting was significantly lower in households with home gardens compared to households without home gardens (Makhotla and Hendriks, 2004). It is worth noting that there is limited use of food supplements and commercially fortified foods and poor diversification of CFs with ASFs by the rural poor from LMICs including Uganda, due to unaffordability (Faber, 2001; Wamani *et al.*, 2005). Therefore, there is a need for Uganda to follow the Food and Agriculture Organization of the United Nations (FAO) recommendation that encourages the use of locally available foods from the community with superior nutrient composition to prepare nutritious CFs (FAO, 2017).

2.5 Potential of pumpkin and common bean to formulate a complementary food

This section reviews pumpkin as a source of provitamin A carotenoids (PVACs) and its potential to contribute towards meeting the RDA/AI for vitamin A for IYC, six to 24 months old. Furthermore, this section also reviews the potential of common bean towards meeting the RDA/AI for iron and zinc for IYC, six to 24 months old.

2.5.1 Pumpkin consumption to combat vitamin A deficiency during complementary feeding

Several pumpkin species are potentially rich sources of PVACs (Carvalho *et al.*, 2012). However, pumpkin is underutilised as a CF to combat VAD (Sharma and Ramana Rao, 2013; Ndegwa, 2016), yet when humans consume foods rich in PVACs, the PVACs are bioconverted into the active form of vitamin A called retinol (Tang, 2010). Three common varieties of pumpkin are cultivated or marketed in LMICs i.e. *C. pepo, C. moschata* and *C. maxima* (Table 2.8). However, in Brazil, Azevedo-Meleiro and Rodriguez-Amaya (2007) introduced another variety *"Tetsukabuto"* (*C. maxima× C. moschata hybrid)* after a cross breed between *C.*

maxima and C. moschata. Table 2.8 shows the PVAC content of pumpkin varieties cultivated in some LMICs.

<u>Table 2.8:</u> Provitamin A carotenoid content of pumpkin varieties in different LMICs

Study	**Country**	**Method**	**Variety**	**Provitamin A carotenoid mean content expressed as µg/100 g of edible portion**		
				β-carotene	**α carotene**	**β cryptoxanthin**
(Kim *et al.*, 2012)	Korea	HPLC	*C. pepo*	148	NT	ND
			C. moschata	570	NT	ND
			C. maxima	1704	NT	65
(Carvalho *et al.*, 2012)	Brazil	HPLC	*C. moschata-A*	24422	6706	NT
			C. moschata-B	14195	7299	
(Azevedo-Meleiro and Rodriguez-Amaya, 2007)	Brazil	HPLC	*C. moschata* "Menina Brasileira".	6670	2680	NT
			C. moschata "Goianinha"	5670	2380	NT
			C. maxima	1540	ND	NT
			C. maxima× C. moschata hybrid Tetsukabuto	3050	TR	NT
			C. pepo	540	ND	
(Pandey *et al.*, 2003)	India	HPLC	*C. moschata*	234-1485	NT	NT
(Provesi, Dias and Amante, 2011)	Brazil	HPLC	*C. moschata*	1945	1260	NT
			C. maxima	1338	43	
(Carvalho *et al.*, 2014)	Brazil	HPLC	*C. moschata*	17220	3995	NT
(Azizah *et al.*, 2009)	Malaysia	HPLC	*C. moschata*	1980	NT	NT
(Koh and Loh, 2018)	Malaysia	HPLC	*C. maxima*	4340	NT	NT
(Tee and Lim, 1991)	Malaysia	HPLC	*C. maxima*	578-1170	75.6	NT
(Pepping, Vencken and West, 1988)	Tanzania	HPLC	*C. moschata*	1170	1100	NT
(Norshazila *et al.*, 2014)	Malaysia	HPLC	*C. moschata*	2916 - 154760	1260- 10200	NT
(Usha, Lakshmi and Ranjani, 2010)	India	HPLC	*C. moschata*	4857.6 -1079.6	NT	NT

NT= Not tested, ND= Not detected, HPLC= High Performance Liquid Chromatography

Crossbreeding findings by Azevedo-Meleiro and Rodriguez-Amaya (2007) and Murkovic *et al.* (2002), suggest that pumpkin is a potential food crop for provitamin A biofortification through conventional plant breeding. Biofortification involves breeding food crops to increase the nutrient density of their edible parts (Meenakshi *et al.*, 2010; Prasad *et al.*, 2015). Moreover, other studies have also argued that it is possible to biofortify pumpkin for higher β-carotene content (Paris, 2011; Prasad *et al.*, 2015). The three known PVACs are β-carotene, α-carotene and β-cryptoxanthin (Rodriguez-Amaya, 2001; Rodriguez-Amaya and Kimura, 2004). Figure 2.5 shows the structure of PVACs.

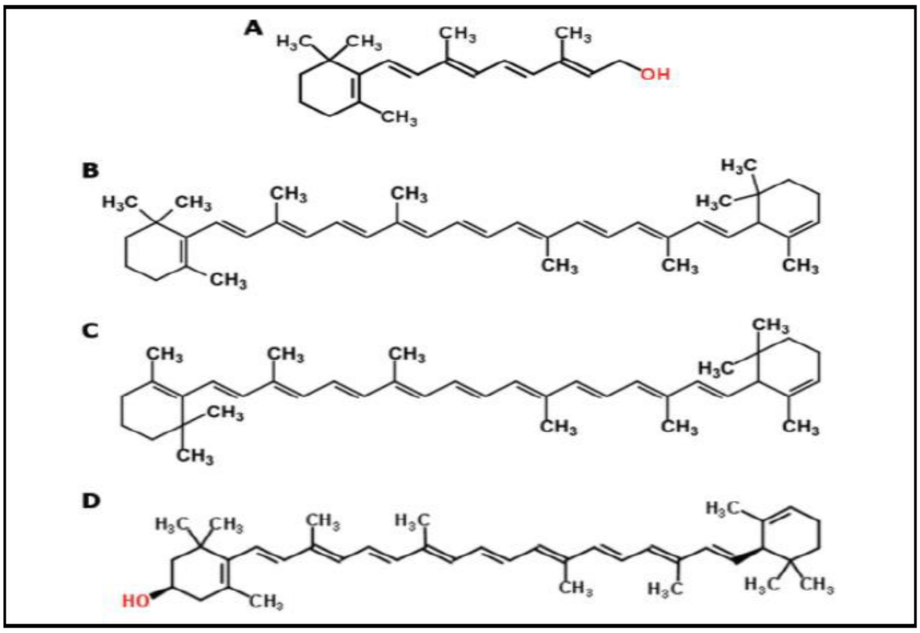
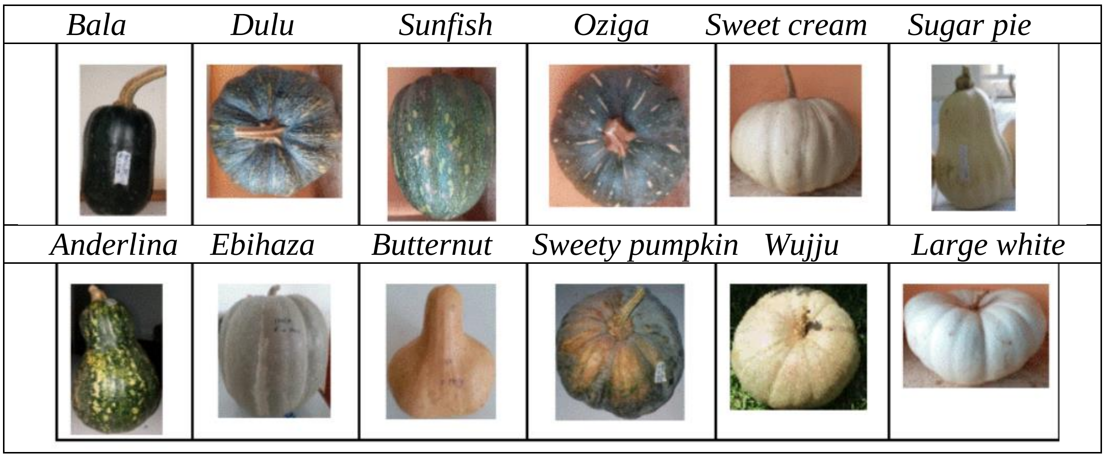


Figure 2.5: Structural formula of retinol (A) (Ross *et al.*, 2014), β-carotene (B), α-carotene (C) and β-cryptoxanthin (D) (Rodriguez-Amaya and Kimura, 2004).

Pumpkin is a potential source of all PVACs (Carvalho *et al.*, 2012; Kim *et al.*, 2012). However, most studies consistently tested for β-carotene in pumpkin as shown in Table 2.8. Furthermore, there are variations in PVAC content among pumpkin varieties cultivated within or across countries. The β-carotene content in *C. moschata* varies across countries at 9680 µg/100 g, 24422 µg/100 g, 1980 µg/100g and 1170 µg/100g in India (Muzzaffar *et al.*, 2016), Brazil (Carvalho *et al.*, 2012), and Malaysia (Azizah *et al.*, 2009), respectively. A similar variation was observed in *C. maxima* with values of 4340 µg/100 g, 5670 µg/100 g and 1704 µg/100 g in Malaysia (Koh and Loh, 2018), Brazil (Azevedo-Meleiro and Rodriguez-Amaya, 2007) and Korea (Kim *et al.*, 2012), respectively. Variations in PVAC content of pumpkin cultivated in LMICs are consistent with studies from HICs (Murkovic, Mulleder and Neunteufl, 2002; Itle and Kabelka, 2009; Bergantin *et al.*, 2018). Such variations may inform that cultivation of pumpkin in different localities may affect its PVAC content.

The observed variation in PVAC content across countries may be due to differences in the cultivation environments such as soil fertility, climate change, and water stress, that influence the productivity of pumpkin (Ferrandino and Smith, 2007; Yavuz *et al.*, 2015; Safahani Langeroodi *et al.*, 2019) and its PVAC content (Norshazila *et al.*, 2014). Such findings are significant in food and nutrition security programmes as they can encourage food and nutrition security policy makers, agencies and agricultural officers to identify and choose a variety of pumpkin with superior PVAC composition. The cultivation (home gardening) and consumption of this pumpkin should be supported and promoted at the community or household level, to alleviate VAD (Ruel and Alderman, 2013). When such programmes are implemented in the community through home gardening of PVAC rich foods, they improve the vitamin A status of children (Faber, *et al.*, 2002; Faber & van Jaarsveld, 2007; Faber & Laurie, 2011; Gershon, 2017). Differences in PVAC content in pumpkin could also be due to genetic variation as some varieties of pumpkin with variations in genetic make-up have been shown to have varying PVAC content (Carvalho *et al.*, 2015; Ribeiro *et al.*, 2015). These findings suggest that pumpkin can also be biofortified by genetic modification. Moreover, the PVAC content in provitamin A biofortified pumpkin by genetic modification (Carvalho *et al.*, 2015; Ribeiro *et al.*, 2015), was significantly higher than those biofortified by conventional breeding (Murkovic, Mulleder and Neunteufl, 2002; Azevedo-Meleiro and Rodriguez-Amaya, 2007). These findings are important when expanding provitamin A pumpkin biofortification programmes in LMICs, with the aim of improving provitamin A nutrition sensitive agriculture (Ruel and Alderman, 2013). There are several varieties of pumpkin cultivated in Uganda with unknown PVAC content (Nakazibwe *et al.*, 2019). Figure 2.6 shows several varieties of pumpkin cultivated in Uganda.



<u>Figure 2.6:</u> Pumpkin varieties cultivated in Uganda (Nakazibwe *et al.*, 2019).

Nutrient retention or the ratio of nutrient content in cooked food to raw food during food preparation and cooking, is paramount in achieving adequate nutrient status (Bechoff *et al.*, 2017). To this end, pumpkin cannot be consumed raw, so it must be cooked to make it ready and palatable for human consumption. At household level, raw pumpkin can be cooked by boiling and steaming. Carvalho and colleagues showed that PVAC retention in pumpkin was over 100% after boiling or steaming (Carvalho *et al.*, 2014), which is consistent with findings from Azizah *et al.* (2009) and Carvalho *et al.* (2015). A possible explanation for the high retention of β-carotene content in pumpkin may be due to the increase in *cis*-β-carotene isomers when PVAC rich foods are heated (Bengtsson *et al.*, 2008; Azizah *et al.*, 2009; De Moura, Miloff and Boy, 2013; Mugode *et al.*, 2014; Bechoff *et al.*, 2017). In addition, there is convincing evidence that maceration (softening) due to heat processing improves β-carotene bioaccessibility from PVAC rich foods, which is probably due to the rupture of the microstructure of plant tissue and subsequent release of PVACs from the complex food matrix (Tumuhimbise, Namutebi and Muyonga, 2009; Provesi and Amante, 2014).

Table 2.9 shows the RDA/AI for vitamin A for IYC aged six to 24 months. The RDA is the intake level that meets the nutrient needs of almost all (97% to 98%) individuals in a group (Institute of Medicine, 2001). On the other hand, the AI is the observed average or experimentally determined intake by a defined population or subgroup that appears to sustain a defined nutritional status, such as growth rate, normal circulating nutrient values or other functional indicators of health (Institute of Medicine, 2001).

Table 2.9: RDA/AI for vitamin A for IYC aged six to 24 months old (Institute of Medicine, 2001)

Age group (months)	RDA/AI (µgRAE/day)
6	400
7-12	500
13-24	300

RDA=Recommended dietary allowance; AI= Adequate intake

PVACs in PSFs can be bioconverted to active vitamin A (retinol) (Tanumihardjo, Palacios and Pixley, 2010). The Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) of 1967 (FAO/WHO, 1967), and the United States Institute of Medicine of 2001 (Institute of Medicine, 2001) established recommendations for the bioconversion of PVACs to retinol. The former recommends that 6 µg of β-carotene is equivalent to 1µg of retinol, whereas 12 µg of all other PVACs each, are equivalent to 1µg of retinol (FAO/WHO, 1967). However, the FAO/WHO (1967) recommendation was questioned because it is argued that it overestimated the bioconversion rates of PVACs to retinol (Institute of Medicine, 2001). Therefore, in 2001 the Institute of Medicine (2001) recommended that 12 µg of β-carotene be equivalent to 1 µg of retinol, whilst 24 µg of other PVACs each, be equivalent to 1µg retinol. The bioconversion of PVACs to vitamin A using the Institute of Medicine (2001) and FAO/WHO (1967) is shown in Table 2.10.

Table 2.10: Bioconversion rates of provitamin A carotenoids to vitamin A

Provitamin A carotenoid (µg)	1µgRAE[a]	1µgRAE[b]
β-carotene	6	12
α-carotene	12	24
β-cryptoxanthin	12	24

[a] Based on the FAO/WHO (1967) bioconversion rates of PVACs to retinol (FAO/WHO, 1967).
[b] Based on the United States of America, Institute of Medicine (2001) bioconversion rates of PVACs to retinol (Institute of Medicine, 2001).
1µg RAE = 1 µg Retinol

Carvalho *et al*. (2014) determined the PVAC content of boiled and steamed non-biofortified pumpkin. The mean content of β-carotene and α-carotene in boiled pumpkin was 18 480 and 4 397 µg/100 g, respectively, while the mean content of β-carotene and α-carotene in steamed pumpkin was 20 200 µg/100 g and 4 709 µg/100 g, respectively (Carvalho *et al.*, 2014). Using the Institute of Medicine (2001) bioconversion rates of PVACs to retinol, boiled pumpkin would contribute 1 540 µg/100 g and 183 µg/100 g of RAE from β-carotene and α-carotene, respectively, giving a total of 1 723 µg of RAE per 100 g of non-biofortified pumpkin consumed. Therefore, only 17.5 g to 29 g of non-biofortified boiled pumpkin (Carvalho *et al.*, 2014), would be required to meet the RDA/AI for vitamin A for IYC aged 13 to 24, and six to 12 months, respectively. Furthermore, steamed pumpkin would contribute 1 683 µg and 196 µg of RAE from β-carotene and α-carotene, respectively, giving a total of 1879 µg of RAE per 100 g of non-biofortified pumpkin consumed. To this end, only 16 g to 27 g of non-biofortified Brazilian steamed pumpkin by Carvalho *et al.* (2014), would be required to meet the RDA/AI for vitamin A for IYC aged 13 to 24 and six to 12 months old, respectively.

The average recommended portion per serving of a CF for an IYC, six to 24 months old during complementary feeding is 50 g (FAO/WHO, 2013). However, based on the Institute of Medicine (2001) bioconversion rates of β-carotene to vitamin A, less than 50g per day of either boiled or steamed non-biofortified pumpkin would meet the RDA/AI for vitamin A for an IYC, six to 24 months old. In addition to non-biofortified pumpkin, Carvalho *et al.* (2015) showed that the β-carotene content of boiled and steamed provitamin A biofortified pumpkin was 10 696 µg/100 g to 65 554 µg/100 g and 117 55 µg/100 g to 65 054 µg/100 g, respectively. Using the Institute of Medicine (2001), bioconversion rates of β-carotene to retinol, 100g of provitamin A biofortified boiled and steamed pumpkin would provide 891 µg to 5 463 µg and 980 µg to 5 421 µg of retinol, respectively. Therefore, only 5.5g to 34 g and 6 g to 31 g of boiled and steamed provitamin A biofortified pumpkin, respectively, would be required to meet the RDA/AI for vitamin A for a 13 to 24-month-old child (Carvalho *et al.*, 2015). Furthermore, only 7.5 g to 45 g and 7.5 g to 41 g of boiled and steamed provitamin A biofortified pumpkin, respectively, would be required to meet the RDA/AI for vitamin A for a six-month-old infant. In conclusion, pumpkin has the potential to combat VAD because several varieties are widely cultivated in LMICs with high β-carotene content. It is also important to note that pumpkin is a provitamin A biofortifiable food crop, with over 100% retention of PVACs when processed using home cooking methods (boiling and steaming) (Carvalho *et al.*, 2015). However, provitamin A biofortified pumpkin varieties are not available in Uganda (Nakazibwe *et al.*, 2019). Nevertheless, consuming less than 50 g of either provitamin A biofortified or non-biofortified cooked pumpkin per day has the potential to meet 100% of the RDA/AI for vitamin A for children six to 24 months old (Carvalho *et al.*, 2015).

2.5.2 Common bean as a potential source of iron and zinc

Common bean (*Phaseolus vulgaris*) is consumed worldwide, in both HICs and LMICs (Mitchell *et al.*, 2009; McDermott and Wyatt, 2017). However, it is a staple food crop cultivated and marketed in several LMICs including Uganda (Akibode and Maredia, 2011). More than 50% of households in Uganda cultivate common beans (Akibode and Maredia, 2011). Common bean harvest in Uganda averages at 164 kg per household per season (Larochelle *et al.*, 2015). Based, on the Food and Agriculture Organization of the United Nations Statistics (FAOSTAT), in the year 2013, 941 000 tonnes of common bean were produced in Uganda, of which 28 tonnes were exported and none was imported (FAOSTAT, 2013). In 2013, the estimated amount of common bean consumed by Ugandans was 22.7 kg/capita/year or contributing to 210 kCal/capita/day (FAOSTAT, 2013). These findings show that common bean is important in Uganda in terms of contributing towards food and nutrition security in the country (FAOSTAT, 2013; Larochelle *et al.*, 2015). Table 2.11 shows common bean varieties cultivated in rural and peri-urban Uganda, including their local names, seed colour, size and shape.

Table 2.11: Common beans grown in rural and peri-urban Uganda, and their local names, size, colour and shape (Kiwuka *et al.*, 2012).

Local name	Seed colour	Seed size	Seed shape
Nakyewogola	Maroon with white speckles	Large	Kidney
Obumyufu	Red	Small	Round
Obuddugavu	Small	Small	Oval
Yellow-Omumpi	Yellow	Medium	Oval
Kanyebwa (A)	Pink with maroon speckles	Medium	Oval
Nkola nkubalile	Orange	Medium	Oval
Khaki//MP	Cream	Small	Small
Obwelu (A)	White	Small	Kidney
Carolina	Red	Small	Round
Kifudu	Brown with white stripes	Medium	Oval
Kanyeebwa (B)	Cream with red stripes	Medium	Oval
Nambale Omudugavu	Purple with white stripes	Large	Kidney
Obumyufu (B)	Red	Medium	Round
Namunye (L)	Cream with maroon stripes	Large	Kidney
Obote	Black with white speckles	Large	Kidney
Mutikke omumyufu	Red	Large	Kidney
Obumyufu (C)	Red	Medium	Kidney
Nambaale-omumpi	Calima	Medium	Oval
Nambaale-omuwanvu	Calima	Large	Kidney
Yellow-omuwanvu	Yellow	Large	Kidney
Obwelu (B)	White	Medium	Round
Mutiike-Purple	Purple with white speckles	Large	Kidney
Namunye (M)	Brown with maroon stripes	Medium	Round
Congo	White	Large	Kidney

Large = 1.6 -<2 cm; Medium = 1-1.5cm; Small = 0.5-0.9 cm

The maximum iron and zinc content in cultivated common bean is 8.4 mg/100g and 5.4 mg/100g, respectively (Beebe, Gonzalez and Rengifo, 2000). The RDA/AI for iron for IYC aged 7 to 11 and 12 to 24 months old is 11 mg/day and 7 mg/day, respectively (Institute of Medicine, 2001). To this end, 100 g/day of common bean by Beebe, Gonzalez and Rengifo (2000), would meet 76% of the RDA/AI for iron in infants aged seven to 11 months old. In contrast, only 84g/day of common bean by Beebe, Gonzalez and Rengifo (2000), would meet 100% of the RDA/AI for iron in children 12 to 24 months old. The RDA/AI for zinc for IYC, six to 24 months old is 3 mg/day (Institute of Medicine, 2001). Therefore, only 56g of common bean by Beebe, Gonzalez and Rengifo (2000), would be needed to meet the RDA/AI for zinc in IYC, six to 24 months old during the period of complementary feeding. Furthermore, a study that evaluated the quantity of minerals in four varieties of common beans cultivated in Burundi, established that the mean concentrations of iron and zinc were 7.63 mg/100g and 7.33 mg/100g,

In contrast to Beebe, Gonzalez and Rengifo (2000), lower concentrations of iron and zinc were reported in common bean (red kidney bean) cultivated in Poland at 3.5 mg/100 g and 1.9 mg/100g, respectively (Suliburska and Krejpcio, 2014). This may echo that the cultivation environment and variety of common bean may play an important role in influencing the iron and zinc concentrations of the legume. Although concentrations of iron and zinc may be lower in some common bean varieties (Suliburska and Krejpcio, 2014), there is some convincing evidence that the nutrient content of iron and zinc of common bean may be increased through biofortification (Blair *et al.*, 2010; Hoppler *et al.*, 2014; Petry *et al.*, 2015; Moloto *et al.*, 2018). It is worth noting that legumes such as common bean are sources of phytic acid (Gibson *et al.*, 2010), which reduces the absorption of zinc and iron (Feil, 2001). To this end, it is necessary to consider feasible home-based food preparation methods such as soaking and boiling that could reduce the concentration of phytic acid in legumes (Lestienne *et al.*, 2005; Feitosa *et al.*, 2018).

2.6 Conclusions

Child malnutrition during the period of complementary feeding remains a major problem of public health importance in the developing world. Moreover, it is associated with CFs of low nutritional quality and low DD. The prevalence of child stunting in Uganda is unacceptably high, compared to wasting and stunting. In addition, the national prevalence of stunting in Uganda is higher than the worldwide prevalence. In Uganda, child stunting is associated with the consumption of vitamin A, iron and zinc deficient CFs, predominantly formulated from root tubers and cereals. Strategies including food fortification, supplementation and advising caregivers to diversify CFs with ASFs have been in existence in Uganda, and other LMICs to combat malnutrition during complementary feeding. However, these strategies are not sustainable to the rural poor because fortified foods, food supplements and ASFs are not affordable to them. Feasible and sustainable initiatives such as identifying and diversifying local nutritious foods cultivated by the rural population is paramount. This study aimed to identify local common bean and pumpkin for use in the preparation of an innovative CF rich in PVACs, iron and zinc in Uganda. This would be possible because common bean is a staple legume in rural Uganda, and is a potential source of iron and zinc. Furthermore, pumpkin is locally cultivated and available in Uganda and is a potential source of PVACs. Therefore, preparation of both common bean and pumpkin using home-based cooking methods and blending them together has the potential to form a nutritious CF, rich in PVACs, iron and zinc. To this end, when children eat the CF, it could contribute towards combating deficiencies of vitamin A, iron and zinc, and stunting in the long-term. However, to predict its future use as a CF, it is important to assess its acceptability among caregivers and target children.

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CHAPTER THREE

BACKGROUND ON THE STUDY SITE, PHILOSOPHICAL APPROACH GUIDING THE STUDY, STUDY DESIGN, AND ETHICAL APPROVALS

This chapter presents the study site, philosophical approach guiding the study, study design, and ethical approvals.

3.1 Background information on the study site

This study was conducted in rural Kyankwanzi district, central Uganda. Uganda's latitude and longitude is 1° 00' N and 32° 00' E, respectively. Uganda is a low income landlocked country located in East Africa. It is bordered to the east by Kenya, to the north by South Sudan, to the west by the Democratic Republic of the Congo, to the south-west by Rwanda, and to the south by Tanzania. The total land area is 199 810 km^2 (Worldometer, 2020). The current population of Uganda is 46 070 113 as of Sunday, September 27, 2020, based on Worldometer elaboration of the latest United Nations data (Worldometer, 2020). Nearly 74% of Uganda’s population reside in rural settings (Worldometer, 2020). Kyankwanzi is among the rural districts with a low socio-economic status located in central Uganda (Uganda Bureau of Statistics and Inner City Fund, 2017). The total population of Kyankwanzi district is 214 693, of which 48% are females, 16% are child mothers aged 12-19 years, 19% are children 0 to 4 years old, 34% are illiterate, 12% are children aged six to 15 years not going to school and 25% have not completed ordinary level education (Uganda Bureau of Statistics, 2017). The district has 22 210 households (HHs) of which 6% to 28% have one meal per day, 81% are dependent on subsistence farming as a main source of livelihood and 40% are 5 km or more from the nearest public health facility (Uganda Bureau of Statistics, 2017). Children in central Uganda are fed CFs deficient in vitamin A, iron and zinc (Amaral, Herrin and Gulere, 2018; Ekesa, Nabuuma and Kennedy, 2019). Caregiver and child acceptability studies were conducted at Ntwetwe Health Centre IV, the only referral health sub-district hospital in Kyankwanzi district. Figure 3.1 shows the map of Uganda and location of Kyankwanzi district. Figure 3.2 shows the map of Kyankwanzi district showing Ntwetwe Sub-county, the location of Ntwetwe Health Center IV.

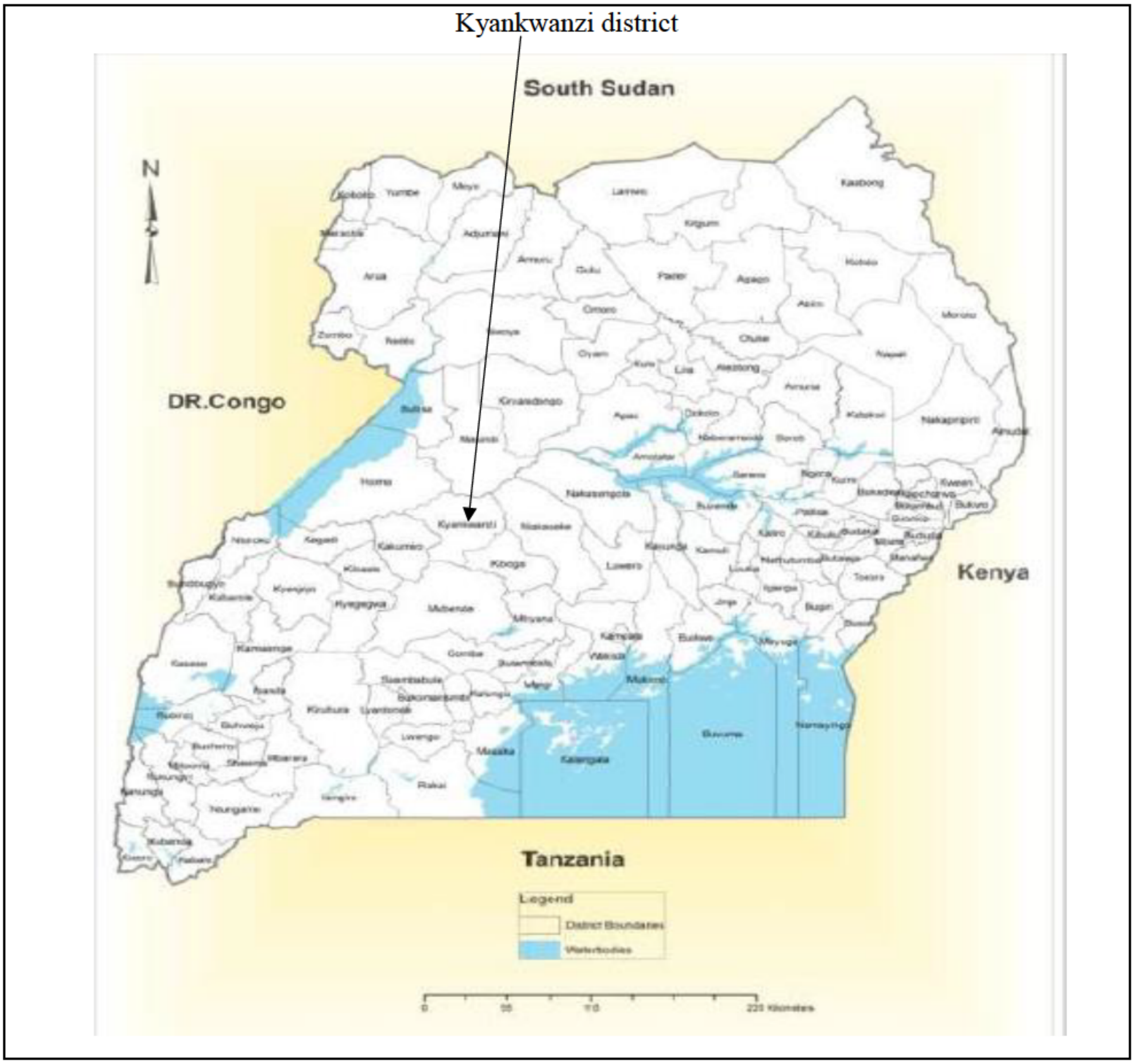


Figure 3.1: Map of Uganda showing the location of Kyankwanzi district (Uganda Bureau of Statistics, 2017).

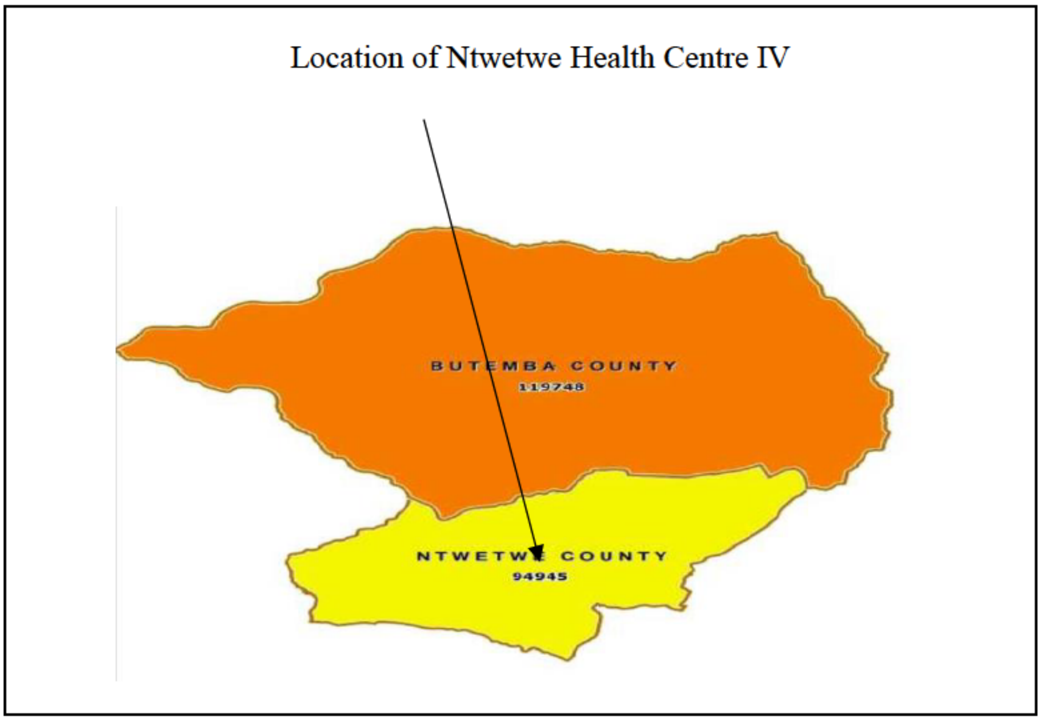


Figure 3.2: Map of Kwankwanzi district showing the county where Ntwetwe Health Centre IV is located (Uganda Bureau of Statistics, 2017).

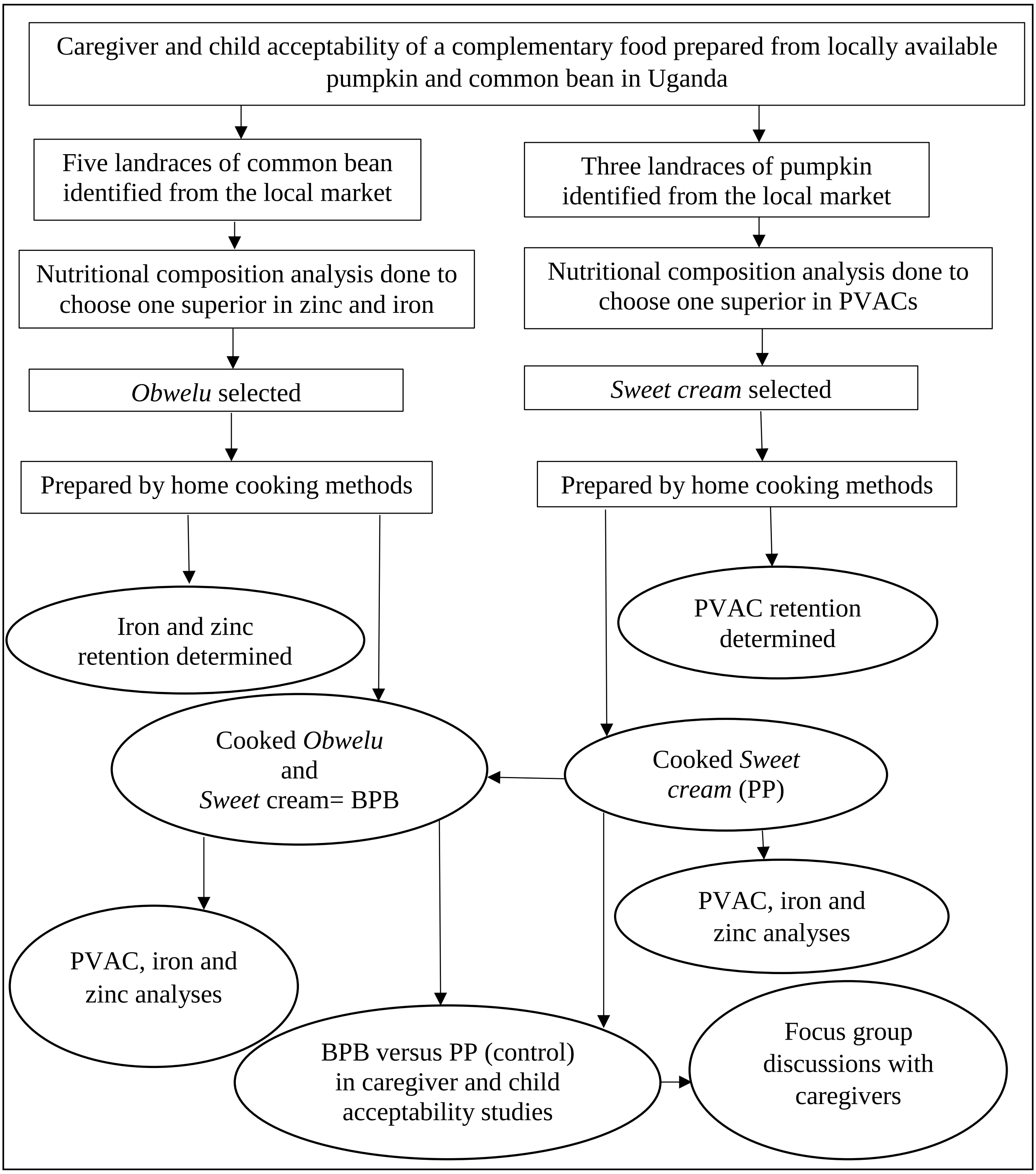
3.2 Philosophical approach guiding the study

This study was guided by the food-based approach (FBA) strategy (Thompson and Amoroso, 2014). The FBA encourages the use of locally available or indigenous nutritious foods that are accessible and affordable to the community members (Thompson and Amoroso, 2014). Dietary diversification, which is the consumption of foods from different food groups is among the recommended FBA strategies to tackle micronutrient deficiencies (MNDs) such as vitamin A deficiency (VAD), iron deficiency (ID) and zinc deficiency (ZnD) in low and middle income countries (Gopalan and Tamber, 2003). Guided by the FBA, this study prepared a provitamin A carotenoid, iron and zinc rich complementary food (CF), common bean pumpkin blend (BPB) from common bean and pumpkin because: (i) the former is rich in iron and zinc (Ferreira *et al.*, 2014), whilst the latter is rich in PVACs (Carvalho *et al.*, 2014); (ii) there is a sustainable access of indigenous common bean and pumpkin in Uganda because they are locally cultivated season after season, and readily available in the local markets at an affordable price (Kiwuka, *et al.* 2012; Nakazibwe, Olet and Kagoro-Rugunda, 2020); (iii) indigenous common bean and pumpkin are staple foods in rural Uganda, hence acceptable for human consumption (Kiwuka, *et al.* 2012; Nakazibwe, Olet and Kagoro-Rugunda, 2020).

3.3 Study design

This study used an experimental study design to assess the association between independent (exposure) and dependent (outcome) variables. Child acceptablity (chapter 6) and caregiver acceptability (chapter 7) studies used a randomised experimental design. The distinguishing feature of the randomised experimental design is that the various treatments being contrasted (including no treatment at all) are randomly assigned to experimental groups (Cash, Stankovic and Štorga, 2016). Randomisation is done so that every research participant has an equal chance of being selected to participate in the study (Woodward, 2014). Screening for iron and zinc content in common bean, and PVACs in pumpkin (chapter 4) and evaluating of cooking on micronutrient retention (chapter 5) were conducted using a quasi experimental design (Cash, Stankovic and Štorga, 2016). The difference between the randomised experimental and quasi experimental designs is that the latter lacks random assignment of study participants /materials (Cash, Stankovic and Štorga, 2016).

The conceptual framework of the study flow involved the following. First, common bean and pumpkin landraces in Uganda were screened for micronutrient composition. Iron and zinc analyses were conducted in the former and PVAC analysis in the latter. One common bean landrace rich in iron and zinc, and one pumpkin landrace rich in provitamin A carotenoids (PVACs) were selected for use in the preparation of a CF used in the acceptability studies. The selected common bean and pumpkin were cooked before blending them to make a suitable CF. The suitability of the CF was based on consistency (thinness or thickness) of the CF in relation to the child's age and ability to swallow as recommended by the Food and Agriculture Organization of the United Nations (FAO) (FAO, 2017). The retention of PVACs, zinc and iron after cooking pumpkin and common bean was determined. After cooking, the selected common bean and pumpkin were mixed and mashed to form a CF, BPB. Pumpkin alone was mashed to form pumpkin puree (PP) which served as the control. The BPB and PP (control) were analysed for PVACs, iron and zinc before using them in child and caregiver acceptability studies. Focus group discussions (FGDs) were also conducted with caregivers to elicit their perceptions of and willingness to use BPB as a CF. The conceptual framework of the study flow is shown in Figure 3.3.



PVAC= Provitamin A carotenoid (s), form of preformed vitamin A, found in plants; BPB=Common bean pumpkin blend, mixuture of cooked common bean and pumpkin; PP= Pumpkin puree, mashed cooked pumpkin

Figure 3.3: Conceptual framework of the study flow

3.4 Ethical approvals

Ethical approval was obtained from the Biomedical Research Ethics Committee, University of KwaZulu-Natal, South Africa (Reference number: BE438/19) (Appendix A). Permission to conduct the study was received from the District Health Office Kyankwanzi district, Uganda (Appendix B). In Uganda, the AIDS Support Organisation Research Ethical Committee (Reference number TASO REC/066/19-UG-REC-009) granted ethical approval (Appendix C). The child acceptability randomised control trial was registered by Pan African Clinical Trials Registry (www.pactr.org) as PACTR202002576768667 (Appendix D). Informed consent for child participation in the child acceptability study was obtained from caregivers (Appendix Ei in English and Eii in Luganda). Informed and signed consent were obtained individually from caregivers to participate in focus group discussions (Appendix Fi in English and Fii in Luganda) and sensory evaluation (Appendix Gi in English and Gii in Luganda). All data were coded to remove identifying information and secure confidentiality.

The next chapter screens common bean and pumpkin landraces identified from the local market for iron/zinc and PVACs, respectively to select one landrace of common bean superior in iron and zinc, and one landrace of pumpkin superior in PVAC for use in the preparation of a CF rich in PVAC, iron and zinc.

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CHAPTER FOUR

SELECTING MICRONUTRIENT-RICH COMMON BEAN AND PUMPKIN LANDRACES FOR PREPARING AN INNOVATIVE COMPLEMENTARY FOOD RICH IN PROVITAMIN A CAROTENOIDS, IRON AND ZINC IN UGANDA

Abstract

Pumpkin is a rich source of provitamin A carotenoids (PVAC), whilst common bean is rich in iron and zinc. There are several locally available landraces of pumpkin and common bean in Uganda. However, the PVAC composition for the former, and iron/zinc composition of the latter is unknown because Uganda lacks reliable food composition data bases. The objective of this study was to select one landrace of common bean (superior in iron and zinc) and one landrace of pumpkin (superior in PVAC), for use in the future preparation of a CF rich in PVAC, iron and zinc in Uganda. Three landraces of pumpkin, *Sweet cream*, *Dulu* and *Sunfish*; and five common bean landraces, *Obwelu*, *Nambale*, *Obwayelo*, *Kanyebwa* and *Masavu* were identified from the local market. High performance liquid chromatography (HPLC) was used to determine the PVAC composition in pumpkin, whilst flame atomic absorption spectroscopy (FAAS) was used to determine iron and zinc composition in common bean. One-way analysis of variance was used to detect mean differences in micronutrient composition between landraces at $p=0.05$. β-carotene composition in pumpkin, *Sweet cream* (1704 µg/100 g) was significantly higher compared to *Dulu* (1333 µg/100 g) and *Sun fish* (1041 µg/100 g) at $p<0.0001$. The α-carotene composition in *Sweet cream* was significantly lower (46 µg/100 g, $p<0.0001$) compared to *Dulu* (77.3 µg/100 g) and *Sun fish* (79.3 µg/100 g). However, the total retinol activity equivalent (RAE) was highest in *Sweet cream* (143.9 µg/100 g), compared to *Dulu* (115.4 µg/100 g) and *Sun fish* (90.1 µg/100 g). Iron composition was highest in common bean, *Obwelu* (7.75 mg/100 g) compared to *Masavu* (6.95 mg/100 g), *Nambale* (6.55 mg/100 g), *Kanyebwa* (7.15 mg/100 g) and *Obwayelo* (6.5 mg/100 g). *Obwelu* had significantly higher iron content than *Obwayelo* ($p<0.05$). Zinc content was highest in *Obwelu* (3.05 mg/100 g), but was not significantly different ($p>0.05$) from all other common bean landraces of *Masavu* (2.95 mg/100 g), *Nambale* (2.35 mg/100 g), *Kanyebwa* (2.90 mg/100 g) and *Obwayelo* (3.0 mg/100 g). Pumpkin, *Sweet cream* was superior in PVAC, whilst common bean, *Obwelu* was superior in iron and zinc. Therefore, *Sweet cream* and *Obwelu* were selected for use in the future preparation of a CF rich in PVAC, iron and zinc.

Keywords: Vitamin A; Provitamin A carotenoids (PVACs); Iron; Zinc; Common bean; Pumpkin

4.1 Introduction

Micronutrient deficiencies of vitamin A, iron and zinc are prevalent among children below five years old worldwide and are considered to be nutritional problems of public health importance (Bailey, West and Black, 2015; WHO, 2019). However, more than 50% of children below five years old with vitamin A deficiency (VAD), iron deficiency (ID) and zinc deficiency (ZnD) reside in the developing countries of Africa and Asia (Shergill-Bonner, 2017). Deficiencies of vitamin A, iron and zinc are underlying causes of child morbidity and mortality (Black, 2003). Vitamin A deficiency is a leading cause of preventable childhood night blindness and diarrhoea (Stevens *et al.*, 2015). Iron deficiency is a leading cause of iron deficiency anaemia in the developing world (Regine *et al.*, 2016), while ZnD is a key risk factor for childhood diarrhoea, morbidity, and mortality (Sanghvi, Ross and Heymann, 2007). Moreover, VAD, ID and ZnD are recognised risk factors for stunting (low height for age) in children (Golden, 1996; Millward, 2017).

The highest prevalence of child VAD, ID and ZnD in the developing world begins during complementary feeding, usually between six to 24 months of age, when homemade CFs are introduced (Shergill-Bonner, 2017). In the presence of increased demands for vitamin A, iron and zinc during complementary feeding (Dorea, 2000; Mahdavi, Nikniaz and Gayemmagami, 2010; Souza *et al.*, 2015), caregivers in the developing regions feed their children complementary foods (CFs) predominantly formulated from staple cereals and tubers (Gibson *et al.*, 2010). These staple cereals and tubers are either deficient in vitamin A, iron and zinc or contain anti-nutrients such as phytic acid that reduce the bioavailability of these micronutrients (Gegios *et al.*, 2010; Gibson *et al.*, 2010). This partly explains why most micronutrient deficiencies in the developing world begin during the complementary feeding stage (Gibson *et al.*, 2010). Children living in rural Uganda are vunerable to VAD, ID, and ZnD because they are predominantly fed cereal and tuber-based CFs (Amaral, Herrin and Gulere, 2018; Ekesa, Nabuuma and Kennedy, 2019). To combat these micronutrient deficiencies, strategies such as diversifying CFs with animal source foods (ASFs), supplementation, fortification and biofortification have been widely recommended (WHO, 2009; De Moura *et al.*, 2014). However, ASFs, food supplements, and fortified foods are not affordable or accessible to the rural poor in Africa, including Uganda (Faber, 2005; Wamani *et al.*, 2005), and are hence also unsustainable. Furthermore, the acceptability of biofortified foods is very low in Uganda (Birol *et al.*, 2015; New Vision, 2019).

A potentially feasible strategy to contribute towards combating VAD, ID and ZnD is to improve dietary diversity of CFs with locally available foods, which are rich in provitamin A, iron, and zinc, but are also affordable, accessible, and acceptable to the rural communities, who are generally of low socio-economic status. Thus, this study aimed to identify locally available common bean rich in iron and zinc (Carvalho *et al.*, 2012a; Nakitto, Muyonga and Nakimbugwe, 2015; Petry *et al.*, 2015) and pumpkin rich in PVACs (Carvalho *et al.*, 2014; Koh and Loh, 2018). PVACs are precursors of vitamin A, found mainly in plant source foods (PSFs) such as pumpkin (Rodriguez-Amaya and Kimura, 2004). After consumption, PVACs in pumpkin can be bioconverted by the body into retinol, the active form of vitamin A (Fernández-García *et al.*, 2012; La Frano *et al.*, 2014).

It is worth noting that a wide range of common bean and pumpkin landraces are cultivated, available and accessible for human consumption in Uganda (Kiwuka *et al.*, 2012; Nakitto, Muyonga and Nakimbugwe, 2015; Nakazibwe *et al.*, 2019). Pumpkin puree (PP) is usually given as a single CF in Uganda (Uganda Bureau of Statistics and Inner City Fund, 2018). However, a CF blend rich in PVACs, iron and zinc prepared from locally available common bean and pumpkin has not been developed for use to combat VAD, ID and ZnD during the period of complementary feeding in Uganda (Uganda Bureau of Statistics and Inner City Fund, 2018). Therefore, this investigation aimed to select one landrace of common bean, superior in zinc and iron, and one landrace of pumpkin, superior in PVACs from Uganda and then use the selected food materials as the main ingredients in the preparation of an innovative complementary food rich in PVAC, iron and zinc, common bean pumpkin blend (BPB).

Ideally, to select micronutrient-rich common bean and pumpkin, food composition databases would be used (Elmadfa and Meyer, 2010). However, Uganda lacks food composition databases (Baingana, 2004). Although there is one available for central and Eastern Uganda, it was developed by extrapolating food composition databases from other countries such as the United States of America and South Africa, and may not be appropriate for use in Uganda (Hotz, Lubowa and Sison, 2010). Therefore, it would be more accurate to screen for micronutrients in the pumpkin and common bean landraces using laboratory food analysis methods based on the standard methods of the Association of Official Analytical Chemists (AOAC) (AOAC International, 2005; Elmadfa and Meyer, 2010). The aim of the current investigation was to screen for iron and zinc content in common bean and PVACs in pumpkin landraces in Uganda, using laboratory food analysis methods in order to prepare a multiple micronutrient rich CF, BPB.

4.2 Materials and methods

4.2.1 Raw pumpkin and common bean materials

Five landraces of common bean (*Phaseolus vulgaris*), with local names of *Masavu, Nambale, Obwelu, Kanyebwa, and Obwayelo* (Kiwuka *et al.*, 2012), were identified and purchased from the local market in rural Kyankwanzi district, Uganda. For each common bean landrace, five samples of 250 g each were purchased from the five vendors found in the market. Figure 4.1 shows the five common bean landraces identified from the local market and their local names, whilst figure 4.2 shows the pumpkin landraces with their local names.

Masavu	*Obwelu*	*Nambale*	*Kanyebwa*	*Obwayelo*

Figure 4.1: Common bean landraces and their local names used in the study

Further, three pumpkin landraces (*Cucurbita moschata*), with local names of *Sweet cream, Dulu* and *Sunfish* (Nakazibwe *et al.*, 2019) were also identified and purchased from the local market for use in the study.

Sweet cream	*Dulu*	*Sun fish*

Figure 4.2: Pumpkin landraces and their local names used in the study

For each pumpkin landrace, one sample of fresh pumpkin was purchased from each of the six vendors found in the market. However, one pumpkin sample was picked by simple random sampling from each landrace for PVAC analysis. It is worth noting that the study food crop samples were purchased from Ntwetwe market. Ntwetwe market was selected because it serves as the central market in Kyankwanzi districtrct, where food crops are collected before they are distributed to the peripheral markets in the district.

4.2.2 Preparation of pumpkin and common bean samples

Sampling and preparation of pumpkin for PVAC analysis was done following procedures recommended by Carvalho *et al.* (2012b). Five samples from each of the purchased pumpkin landraces were peeled, and each one divided into four parts by two longitudinal cuts from one end to the other. Of these four sections, two sections (opposite from each other) were discarded. The remaining two were chopped into smaller pieces, freeze-dried and blended using the Biochef high performance blender (TM800) to obtain a homogenous powdered sample ready for analysis (Rodriguez-Amaya and Kimura, 2004). For common bean preparation, around 300 g of each landrace of common bean was dried in an oven at 80°C for eight hours. The samples were then milled separately by a willey mill (40 mesh) to form a fine flour and kept in an airtight polythene bag ready for analysis.

4.2.3 Analysis of provitamin A carotenoids and retinol activity equivalents in pumpkin

PVAC analysis was done at METLAB East Africa limited laboratory, Kampala, Uganda. The PVAC content in pumpkin was determined by high performance liquid chromatography (HPLC) as described in the HarvestPlus handbook for carotenoid analysis (Rodriguez-Amaya and Kimura, 2004). Carotenoids are very sensitive to light and oxygen, which may lead to *trans-cis* isomerization and destruction of PVACs (Saini, Nile and Park, 2015). To this end, carotenoid analysis was carried out as fast as possible under subdued light and flasks wrapped with aluminium foil (Rodriguez-Amaya and Kimura, 2004). Carotenoids were extracted from the pumpkin prior to PVAC analysis (identification and quantification) by HPLC.

When extracting carotenoids from biological samples such as foods, which contain large amounts of water (e.g. pumpkin), a water-miscible organic solvent (e.g. acetone, methanol, ethanol, or mixtures thereof) should be used to allow better solvent penetration (Rodriguez-Amaya and Kimura, 2004). For carotenoid extraction, 25 mL of acetone was successively added to 1 g of powdered freeze-dried pumpkin until a paste was obtained (Rodriguez-Amaya and Kimura, 2004). The paste was transferred to a sintered funnel (5 µm) coupled to a 250 mL Buchner flask and filtered. This procedure was repeated at least three times until the sample was colourless. The obtained extract was transferred to a 500 mL separatory funnel containing 40 mL of petroleum ether. The acetone was removed through the slow addition of ultrapure water (Milli-Q-Millipore) to prevent the formation of an emulsion. The aqueous phase was discarded, and this procedure was repeated four times until no residual solvent remained.

For the identification and quantification of α-carotene and β-carotene, 2mL were removed from the carotenoid extract and dried in an amber flask under flowing nitrogen. The sample was diluted in 100 µL of acetone while mixing in a vortex mixer and then transferred to a 2mL amber flask for HPLC analysis. All the solvents and chemicals including α-carotene and β-carotene standards were purchased from valid sources. Calibration curves were used to calculate the concentration of the respective carotenoids. For HPLC analyses, 10 mL of the extracts were injected on to a reversed phase column (201 TP54, Vydac, Hesperia, CA, USA., 250 mm, 4.6 mm, 5 mm) with a pre-column (ODS1, metal free, 4.6 mm, 5 mm) from the same producer. The HPLC equipment used was a HP1100 liquid chromatograph (Hewlett Packard, Waldbronn, Germany) with a variable wavelength detector (1050, Hewlett Packard, Waldbronn, Germany). The columns were eluted with a mobile phase of acetonitrile/methanol/ dichloromethane (76/20/4.5) with 0.1% BHT as antioxidant and triethylamine as mobile phase modifier. The flow rate was 1.0 mL/min and the absorption of the effluent was monitored at a wavelength of 450 nm for the determination of the PVACs. The analysis temperature was 30 °C with a total analysis time of 60 minutes (Rodriguez-Amaya & Kimura, 2004).

The content of α-carotene and β-carotene was calculated according to the formula:

$$C(\mu/g) = \frac{A_X \times C_s(\mu/g) \times V(mL)}{A_S \times P(g)}$$

Where, C is the PVACs; Ax = Carotenoid peak area; Cs = Standard concentration; As=Standard area; V=Total extract volume and P=Sample weight.

It is worth noting that PVACs are bioconverted to retinol, the active form of vitamin A used by the human body (De Pee *et al.*, 1998; Van Loo-Bouwman, Naber and Schaafsma, 2014). To this end, the Institute of Medicine (2001) bioconversion rates of PVACs to retinol (retinol activity equivalents) were used, i.e. 12 µg of β-carotene is equivalent to 1µg of retinol, whilst 24 µg of α-carotene is equivalent to 1 µg retinol.

4.2.4 Analysis of iron and zinc in common bean

Iron and zinc content of common bean was conducted at the Cedara Agriculture Laboratory, KwaZulu-Natal, South Africa. Iron and zinc were determined by FAAS, as described by Santelli *et al.* (2006) and Ekesa, Nabuuma and Kennedy (2019). The FAAS generally requires the oxidation of organic matter of biological samples to render a solution ready for analysis of mineral elements (Hill and Fisher, 2017). To this end, microwave digestion was used to dissolve iron and zinc, and oxidise the organic molecules in common bean prior to analysis by FAAS. Digestion was done by closed vessel microwave oven following the procedure by Santelli *et al.* (2006). Accurately, 0.5 g of each sample was carefully weighed into perfluoroalkoxy (PFA) liners of the oven and 8 ml of concentrated nitric acid was added. After standing overnight in contact with nitric acid, 2 mL of 30% m/m hydrogen peroxide was added and the liners were closed, placed into the microwave oven cavity and two irradiation cycles of 25 minutes (1000W maximum power) were run. The equipment was adjusted to avoid internal pressures higher than 180 psig and temperatures higher than 190^{o}C and between the first and second cycle, the liners were opened to release gases formed during digestion. Applying this procedure, perfectly clear and colourless solutions were obtained. Then, this solution was transferred to the volumetric flask and the volume was made up to 25 mL. This final solution was used in the iron and zinc determination by FAAS. The sample solution was then transformed into aerosols and transported to the flame, to vaporize and atomize the sample. This step leads to a reduced intensity of the light (by absorption of a defined quantity of energy) coming from a hollow-cathode lamp. The attached detector quantified the absorbed incoming light. The difference between the radiation without sample and including sample (absorbance) was used to calculate the iron and zinc concentration. The standard concentration for iron and zinc calibration curve (1000 ppm) were prepared as documented in the data book for the atomic absorption spectrophotometer (The Perkin-Elmer Corporation, 1996). The wavelengths (nm) for iron and zinc were 248.3 nm and 213.9 nm, respectively (The Perkin-Elmer Corporation, 1996).

4.2.5 Data analysis

Analysis of each of the five common bean and three pumpkin landraces was conducted four times to increase precision, the degree to which repeated measurements from the same food sample of the same variable give the same value. All data were reported as the mean (mg/100g) of dry weight ± standard deviations (SD) of four determinations of common bean and mean (µg/100g) freeze-dried weight ± SD of four determinations of pumpkin samples. One-way analysis of variance (ANOVA) was used to detect the individual micronutrient composition mean differences between landraces. The level of significant difference was set at a probability value of 5% (p =0.05). Where the one-way ANOVA revealed a significant mean difference, a Bonferroni test was conducted to identify where the specific micronutrient composition mean difference existed between landraces. Statistical analysis was conducted using Statistics and Data (STATA), version 13.1.

4.3 Results

The results of the PVAC composition in three pumpkin landraces and the iron and zinc composition in five common bean landraces purchased from the local market in rural Uganda are presented.

4.3.1 Provitamin A carotenoid content in pumpkin and retinol activity equivalents

The mean β-carotene content in *Sweet cream*, *Dulu* and *Sun fish* was 1704µg/100 g, 1333 µg/100g, and 1041µg/100g of pumpkin, respectively. *Sweet cream* had a significantly higher β-carotene content than *Dulu* (p<0.0001) and *Sun fish* (p<0.0001). Furthermore, *Dulu* had a significantly higher β-carotene content than *Sunfish* (p<0.0001). Table 4.1 shows the PVAC content and RAE of each pumpkin landrace.

Table 4.1: Provitamin A carotenoid content and retinol activity equivalents in pumpkin landraces

Local name	β-carotene Mean (µg/100g ± SD)	α-carotene Mean (µg/100g)	RAE µg retinol/100g of pumpkin
Sweet cream	1704 ± 5.0^{a}	46.0 ± 3.0^{a}	143.9
Dulu	1333 ± 4.7^{b}	77.3 ± 1.5^{b}	114.4
Sun fish	1041 ± 38.1^{c}	79.3 ± 1.5^{b}	90.1

Values in the same column with different superscript letters are significantly different (p <0.05) according to the Bonferroni test.
RAE=Retinol Activity Equivalent (retinol).
RAE= β-carotene (µg/100g)/12+ α-carotene (µg/100g)/24 (Institute of Medicine, 2001).

The mean α-carotene content in *Sweet cream*, *Dulu* and *Sun fish* was 46 µg/100 g, 77.3 µg/100 g and 79.3 µg/100 g, respectively. *Sweet cream* had significantly lower mean α-carotene content than *Dulu* (p<0.0001) and *Sun fish* (p<0.0001). However, there was no significant difference in α-carotene content between *Dulu* and *Sun fish* (p =0.884). The RAE in *Sweet cream*, *Dulu* and *Sunfish* was 143.9 µg/100 g, 114.4 µg/100 g and 90.1 µg/100 g, respectively.

4.3.2 Iron and zinc content of common bean

Table 4.2 shows the iron and zinc content of the five common bean landraces.

Table 4.2: Iron and zinc content in the five common bean landraces

Local name	Iron Mean (mg/100g ± SD)	Zinc Mean (mg/100 g ± SD)
Masavu	6.95 ± 0.49[a]	2.95 ± 0.7
Obwelu	7.75 ± 0.07[a]	3.05 ± 0.01
Nambale	6.55 ± 0.21[a]	2.55 ± 0.21
Kanyebwa	7.15 ± 0.21[a]	2.9 ± 0.50
Obwayelo	6.5 ± 0[b]	3.0 ± 0.5

Values in the same column with different superscript letters are significantly different (p <0.05) according to the Bonferroni test.

The mean iron content in *Masavu, Obwelu*, *Nambale*, *Kanyebwa* and *Obwayelo* was 6.95 mg/100g, 7.75 mg/100 g, 6.55 mg/100 g, 7.15 mg/100 g and 6.5 mg/100 g dry weight of common bean, respectively. *Obwelu* had significantly higher iron concentrations than *Obwayelo* (p=0.049). However, the iron content in *Obwelu* was not significantly different from *Masavu* (p=0.279), *Nambale* (p=0.058) and *Kanyebwa* (p=0.697). The mean zinc content in *Masavu, Obwelu*, *Nambale*, *Kanyebwa* and *Obwayelo* was 2.95 mg/100 g, 3.05 mg/100 g, 2.55 mg/100 g, 2.9 mg/100 g and 3.0 mg/100 g dry weight of common bean, respectively. *Obwelu* had the highest zinc content, but it was not significantly different from the other common bean landraces (p>0.05).

4.4 Discussion

The aim of this study was to select a common bean landrace superior in iron and zinc; and pumpkin landrace superior in PVACs and then use them in the preparation of a CF, BPB, which could be suitable for use in combating VAD, ID and ZnD prevalent among children in rural areas of Uganda.

4.4.1 Provitamin A carotenoids and retinol activity equivalents in pumpkin landraces

The concentration of β-carotene was higher than that of α-carotene in all the pumpkin landraces. This finding has implications for vitamin A bioavailability because the bioconversion rate of β-carotene to retinol in the human body is twice that of α-carotene (Institute of Medicine, 2001). Therefore, the significantly higher concentrations of β-carotene in *Sweet cream* compared to *Dulu* (p<0.0001) and *Sunfish* (p<0.0001), would justify the selection of *Sweet cream* for the preparation of the BPB. In contrast, the mean α-carotene content in *Sweet cream* (46 µg/100 g) was significantly lower ($p<0.0001$) compared to *Dulu* (77.3 µg/100 g) and *Sunfish* (79.3 µg/100 g). Nevertheless, *Sweet cream* was chosen as the pumpkin landrace for preparation of the BPB because it provided the highest amounts of RAE compared to *Dulu* and *Sun fish.*

The highest RAE for *Sweet cream* is attributed to the significantly higher concentrations of β-carotene compared to the other landraces, and lower concentrations of α-carotene compared to β-carotene across all the three pumpkin landraces. It is worth noting that according to the Institute of Medicine (2001), 12 µg of β-carotene in PVAC rich foods is equivalent to 1 µg of retinol (Institute of Medicine, 2001). Moreover, a review of vitamin A equivalency of β-carotene in various food matrices for human consumption confirms that 12 µg of β-carotene in pumpkin is equivalent to 1 µg of retinol (Van Loo-Bouwman, Naber and Schaafsma, 2014). In contrast, 24 µg of α-carotene is equivalent to 1µg of retinol (Institute of Medicine, 2001). To this end, bioconversion calculations of PVACs to retinol measured in RAE (Table 4.1), showed that concentrations of β and α-carotene in *Sweet cream* provided the highest concentrations of retinol, compared to the other two study pumpkin landraces. The higher β-carotene and lower α-carotene content observed in *Sweet cream* is almost similar to varieties of pumpkin in Korea, Brazil and India (Pandey *et al.*, 2003; Azevedo-Meleiro and Rodriguez-Amaya, 2007; Provesi, Dias and Amante, 2011; Kim *et al.*, 2012). In contrast, the PVAC content in *Sweet cream* was lower compared to the provitamin A biofortified pumpkin (Azevedo-Meleiro and Rodriguez-Amaya, 2007; Carvalho *et al.*, 2015; Ribeiro *et al.*, 2015). This is plausible because biofortification, the breeding of pumpkin with PVACs increases its PVAC content (Azevedo-Meleiro and Rodriguez-Amaya, 2007).

The recommended dietary allowance (RDA)/adequate intake (AI) for vitamin A for a child in the complementary feeding age range of a child, 13 to 24 months old is 300 µg of retinol/day (Institute of Medicine, 2001). It is worth noting that the average recommended intake per serving of a CF for a child 12 to 24 months old during complementary feeding is 50 g (FAO/WHO, 2013). If a child 12 to 24 months old is served 50 g of pumpkin twice a day, then the 100 g of *Sweet cream* served would contribute nearly 50% of the RDA/AI for vitamin A for a child aged 13-24 months old.

To make pumpkin suitable for child consumption as a CF, it should be prepared using the same cooking methods that are used at the household level such as boiling and steaming. Therefore, the RAE in *Sweet cream* is expected to be higher after cooking because the retention of PVACs in pumpkin is over 100% after home cooking methods (Azizah *et al.*, 2009; Carvalho *et al.*, 2014). The proposed high retention of β-carotene content in pumpkin may be explained by β-carotene isomerism, characterised by the increase of *cis*-β-carotene isomers on heating PVAC rich foods (Bengtsson *et al.*, 2008; Azizah *et al.*, 2009; De Moura, Miloff and Boy, 2013; Mugode *et al.*, 2014; Bechoff *et al.*, 2017). Besides, maceration (softening) due to heat processing improves β-carotene bioaccessibility from PVAC rich foods by rupturing the microstructure of plant tissue and subsequently releasing more PVACs from the complex food matrix (Tumuhimbise, Namutebi and Muyonga, 2009; Provesi and Amante, 2014).

4.4.2 Iron and zinc content in common bean landraces

The mean iron content of common bean landraces was 6.5 mg/100 g, 6.55 mg/100 g, 6.95 mg/100 g, 7.15 mg/100 g and 7.75 mg/100 g in *Obwayelo*, *Nambale*, *Masavu*, *Kanyebwa* and *Obwelu*, respectively. There was no significant mean difference in the iron content of *Obwayelo*, *Nambale*, *Masavu*, and *Kanyebwa* ($p>0.05$). However, the mean iron content in *Obwelu* was significantly higher than that in *Obwayelo* ($p=0.048$). *Obwelu* was superior in iron content compared to other landraces, and was therefore selected for preparing the CF. There was no significant difference in the mean zinc content across all common bean landraces under study. However, *Obwelu* had the highest zinc content at 3.05 mg/100 g compared to the other four common bean landraces that had a zinc content ranging from 2.55 mg/100 g to 3 mg/100 g. To this end, *Obwelu* was selected because of its superior zinc content.

It is worth noting that the mean iron content of 7.75 mg/100 g observed in *Obwelu* was higher than that of common bean varieties cultivated in neighbouring countries to Uganda such as Burundi (7.3 mg/100 g) (Barampama and Simard, 1993) and Tanzania (ranging from 3.1 to 6.2 mg/100 g) (Tryphone and Nchimbi-Msolla, 2010). In contrast, the Burundian common bean had higher zinc concentrations (7.33 mg/100 g) (Barampama and Simard, 1993), than *Obwelu*. However, the iron and zinc content in *Obwelu* was higher than that of the common bean in Poland at 3.5 mg/100 g and 1.9 mg/100 g, respectively (Suliburska and Krejpcio, 2014).

Further, the mean iron and zinc content in *Obwelu* was higher than that observed in some of the biofortified common bean varieties cultivated in Columbia, Guatemala and Costa Rica, which ranged from 4.9 mg/100 g to 6.0 mg/100 g for iron and 2.3 mg/100 g to 2.8 mg/100 g for zinc (Blair *et al.*, 2010). In contrast, some other varieties of biofortified common beans cultivated in the same countries contained higher concentrations of iron and zinc ranging from 7.6 mg/100 g to 10.1 mg/100 g and 3.1 mg/100 g to 3.4 mg/100 g, respectively, compared to *Obwelu* (Blair *et al.*, 2010). These variations in zinc and iron concentrations in common bean could be explained by factors such as variety, biofortification strategies and different cultivation environments across countries (Barampama and Simard, 1993; Blair *et al.*, 2010; Suliburska and Krejpcio, 2014). It is worth noting that iron-zinc biofortified common bean varities were released on the Ugandan market for human consumption (Glahn, Wiesinger and Lung'aho, 2020). However, the locally available *Obwelu* (7.8 mg/100 g) is superior in iron composition compared to the iron biofortified common bean varities such as NAROBEAN 1(6.7 mg/100 g), NAROBEAN 3 (6.4 mg/100 g), and NAROBEAN 5 (7.2 mg/100 g) (Glahn, Wiesinger and Lung'aho, 2020).

Obwelu would also be prepared by home cooking methods to make it suitable as a CF for child consumption. Assuming that iron and zinc retention of *Obwelu* is 90% as reported for other common bean landraces by Carvalho *et al.* (2012a), cooked *Obwelu* would yield 7.0 mg/100g and 2.7mg/100g of iron and zinc, respectively. The RDA/AI for iron for a child in the age range of complementary feeding of 12 to 24 months old is 7 mg/day (Institute of Medicine, 2001). To this end, 100 g/day of cooked *Obwelu* would meet 100% of the RDA/AI for iron in a child 12 to 24 months old. Furthermore, the RDA/AI for zinc in a child 12 to 24 months old is 3mg (Institute of Medicine, 2001). Therefore, 100 g/day of cooked *Obwelu* would meet 90% of the RDA for zinc in a child 12 to 24 months old.

4.4.3 Study strengths and limitations

Uganda lacks food composition databases for its locally available foods (Baingana, 2004; Hotz, Lubowa and Sison, 2010). The main strength of this study is that it revealed the iron and zinc content of the study common bean landraces and PVAC content with vitamin A (RAE) for the study pumpkin landraces, which will be useful in future development of the Ugandan food composition data bases. Furthermore, this study analysed PVAC composition using HPLC, whilst iron and zinc composition were analysed by FAAS. The use of HPLC was neccssary because it is considered a gold standard method for PVAC analysis, probably because it has a significantly greater power of resolution, reproducibility, and high speed of separation of these carotenoids (Melendez-Martinez, Vicario, and Heredia 2007).

Some limitations are also inherent in this study. For example, the three known PVACs are β-carotene, α-carotene and β-cryptoxanthin (Institute of Medicine, 2001). However, due to financial constraints and failure to find the standard for β-cryptoxanthin in Uganda, this study only analysed β-carotene and α-carotene in pumpkin. However, it is worth noting that failure to analyse β-cryptoxanthin may not have significantly affected the PVAC content of pumpkin because evidence from previous studies that analysed β-cryptoxanthin in pumpkin, established that β-cryptoxanthin was either not detected or negligible (Kim *et al.*, 2012). It is also worth noting that the study common bean and pumpkin were sampled at one point in time. Therefore, the study samples may not be representative in terms of environmental effects on the study food crops grown in the different seasons of the year or different years.

4.5 Conclusions

Based on the superior zinc and iron content in *Obwelu*, compared to the other common bean landraces, and the superior PVAC content in *Sweet cream*, compared to other pumpkin landraces, this study selected *Obwelu* and S*weet cream* for use in the preparation of a complementary food, BPB, rich in PVACs, iron and zinc. Future studies should assess the impact of different cooking methods on iron and zinc retention in *Obwelu*, and PVAC retention in *Sweet cream*.

Therefore, chapter 5 will evaluate micronutrient retention in common bean *Obwelu* and pumpkin *Sweet cream* after cooking.

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CHAPTER FIVE

[2]EFFECT OF COOKING LOCALLY AVAILABLE COMMON BEAN (*OBWELU*) ON IRON AND ZINC RETENTION, AND PUMPKIN (*SWEET CREAM*) ON PROVITAMIN A CAROTENOID RETENTION IN RURAL UGANDA

Abstract

Pumpkin is a potential rich source of vitamin A precursors called provitamin A carotenoids (PVACs), whilst common bean is a potential rich source of iron and zinc. This study evaluated the effect of cooking locally available pumpkin, *Sweet cream* (*Cucurbita moschata*) on provitamin A carotenoid (PVAC) retention in Uganda. Furthermore, the effect of cooking locally available common bean, *Obwelu* (*Phaseolus vulgaris*) on iron and zinc retention was evaluated. Expert caregivers from the local community cooked pumpkin by either boiling or steaming, whilst common bean was cooked by either boiling with prior soaking or boiling without prior soaking. The PVACs in raw and cooked pumpkin were analysed by high performance liquid chromatography (HPLC), whilst iron and zinc in raw and cooked common bean were analysed by flame atomic absorption spectroscopy (FAAS). Conversion of PVACs into vitamin A retinol activity equivalents (RAE) was calculated using the Institute of Medicine (2001) recommendations for the bioconversion of PVACs into vitamin A (retinol). Micronutrient retention was measured using true retention. β-carotene, α-carotene, and vitamin A content in raw pumpkin was 1704 µg/100 g, 46 µg/100 g and 1437 µgRAE/100 g, respectively. Either boiling or steaming pumpkin resulted in over 100% retention of PVACs and vitamin A. Iron and zinc retention in boiled common bean with prior soaking was 92.2% and 91.3%, respectively. Boiling common bean without prior soaking resulted in 88.4% and 75.6% retention of iron and zinc, respectively. In conclusion, to retain a high proportion of PVACs, caregivers should be advised to cook *Sweet cream* by either boiling or steaming, while to retain a high proportion of iron and zinc, *Obwelu* should be prepared by boiling with prior soaking.

Keywords: Retention; Common bean; Pumpkin; Provitamin A carotenoids; Zinc; Iron; cooking

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[2] Publication based on this chapter:
Buzigi, E., Pillay, K. and Siwela, M. (2020) 'Effect of cooking locally available common bean (*Obwelu*) on iron and zinc retention, and pumpkin (*Sweet cream*) on provitamin A carotenoid retention in rural Uganda', *Food Science and Nutrition*, 00, pp. 1–10. doi: 10.1002/fsn3.1873.

5.1 Introduction

The consequences of vitamin A deficiency (VAD), iron deficiency (ID) and zinc deficiency (ZnD) among children are debilitating because they are associated with childhood mortality and morbidities such as night blindness, diarrhoea, iron deficiency anaemia, and stunting (Millward, 2017). In rural Uganda, children aged six to 24 months are at a higher risk of developing VAD, ID and ZnD because they are fed complementary foods (CFs) predominantly formulated from staple cereals such as white maize and tubers such as white cassava, white sweet potato and yams (Amaral, Herrin and Gulere, 2018; Ekesa, Nabuuma and Kennedy, 2019). These staples are deficient in vitamin A, iron, and zinc or contain anti-nutrients that reduce the bioavailability of zinc and iron (Gibson *et al.,* 2010). Hence, their predominance in the diets of children increases the risk for VAD, ID and ZnD (Gegios *et al.,* 2010).

To combat child VAD, ID and ZnD, the WHO (2009) recommends strategies such as micronutrient supplements, commercially fortified foods, and animal source foods (ASFs) to prepare CFs for children in the age range of complementary feeding. This is plausible because micronutrient fortified foods, supplements and ASFs are rich sources of vitamin A, iron, and zinc. However, rural caregivers in Uganda cannot afford food supplements, commercially fortified foods and ASFs (Wamani, 2005). Therefore, rural caregivers resort to using locally available vitamin A, iron and zinc deficient CFs prepared from vitamin A, iron and zinc deficient staples cereals and tubers (Ekesa, Nabuuma and Kennedy, 2019), which increases child vulnerability to VAD, ID and ZnD. Locally available pumpkin species in Uganda such as *Cucurbita moschata* and *Cucurbita maxima* (Ondigi *et al.,* 2008; Nakazibwe *et al.,* 2019), are potential sources of PVACs such as β-carotene, α-carotene, and β-cryptoxanthin (Kim *et al.,* 2012; Saini, Nile and Park, 2015; Koh and Loh, 2018). Moreover, when PVAC rich foods are consumed, the human body bioconverts these PVACs into retinol, the active form of vitamin A used by the body (Van Loo-Bouwman, Naber and Schaafsma, 2014). Furthermore, common bean (*Phaseolus vulgaris*) is locally available in Uganda, and is a potential rich source of iron and zinc (Beebe, Gonzalez and Rengifo, 2000; Carvalho *et al.,* 2012; Kiwuka *et al.,* 2012).

To contribute towards improving the vitamin A, iron and zinc density of CFs in Uganda (Ekesa, Nabuuma and Kennedy, 2019), locally available PVAC rich pumpkin (*Sweet cream*), and iron and zinc rich common bean (*Obwelu*) were identified from the local community to prepare a PVAC, iron and zinc rich CF in rural Uganda (chapter 4, sections 4.3.1 and 4.3.2).

It is worth noting that raw foods should be prepared by cooking to make them palatable and soft for children to consume as CFs (FAO, 2017). However, nutrient retention, defined as the ratio of nutrient content in the cooked food to the nutrient content in the raw food is affected by different cooking methods (Li *et al.*, 2007). Several cooking methods impact differently on PVAC retention in PVAC rich foods, including different pumpkin varieties (Bengtsson *et al.*, 2008; Azizah *et al.*, 2009; De Moura, Miloff and Boy, 2013; Carvalho *et al.*, 2014, 2015; Mugode *et al.*, 2014; Bechoff *et al.*, 2017). Similarly, cooking impacts differently on iron and zinc retention in common bean varieties (Carvalho *et al.*, 2012; Ferreira *et al.*, 2014). However, the effect of cooking pumpkin, *Sweet cream* on PVAC retention and the effect of cooking common bean, *Obwelu* on iron and zinc retention is unknown. To this end, this study evaluated the effect of household cooking methods used in rural Uganda on the retention of PVACs in pumpkin (*Sweet cream*) and iron and zinc retention in common bean (*Obwelu*). The cooking methods found to retain a high proportion of micronutrients in the current study will be recommended for use in the preparation of pumpkin and common bean in Uganda.

5.2 Materials and methods

5.2.1 Pumpkin and common bean materials

Pumpkin (*Sweet cream*) and common bean (*Obwelu*) were purchased from the local market in rural Kyankwanzi district, central Uganda.

5.2.2 Selection of home cooking methods for common bean and pumpkin

Pumpkin and common bean were prepared using local household-level cooking methods as recommended by the 2017 Food and Agriculture Organization of the United Nations (FAO) guide to conducting participatory cooking demonstrations to improve complementary feeding practices (FAO, 2017). To this end, this study worked with Village Health Team members (VHTs) and peer mothers from the study community. The Ugandan Ministry of Health (MOH) recognises VHTs as community health workers at the village level with the roles of promoting, supporting, and protecting community health programmes such as nutrition and health in their respective villages (MOH, 2016). By using simple random sampling, the head of VHTs identified one expert peer mother from each of the 10 villages located in Ntwetwe sub county, Kyankwanzi district, Uganda, to participate in the cooking of pumpkin and common bean, using household-level cooking methods for common bean and pumpkin as used in the study community.

Expert peer mothers, were asked one by one to mention the cooking methods commonly used at household level to cook common bean and pumpkin in their community. Of the 10 expert peer mothers, 9 and 10 mentioned overnight soaking followed by boiling and boiling without soaking, respectively as the commonly used household-level cooking methods for preparing common bean in their community. Furthermore, of the 10 expert peer mothers, 9 and 8 mentioned that pumpkin was commonly cooked by boiling and traditional steaming, respectively. To this end, PVAC retention in pumpkin was evaluated either by boiling and steaming pumpkin, while iron and zinc retention in common bean was evaluated by boiling with or without overnight soaking. Figure 5.1 shows the flow chart with methods used to cook pumpkin and common bean.

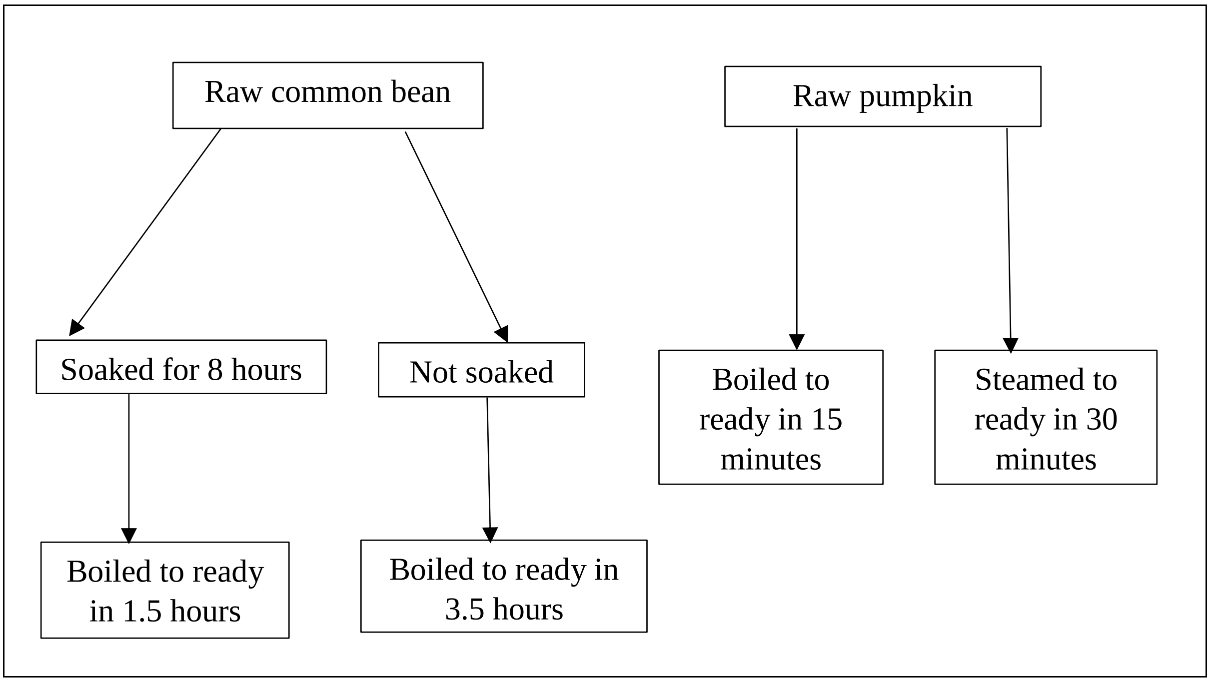

Figure 5.1: Flow chart of methods used to cook common bean and pumpkin

5.2.3 Preparation and cooking of pumpkin

Expert peer mothers participated in the preparation and cooking of the study pumpkin and common bean. Pumpkin pulp was washed, cut into three relatively equal sections, and seeds discarded. One raw section was peeled and blended for about one minute using a blender to obtain a homogenous sample for PVAC analysis. The other sections were cooked according to one of the two following procedures commonly used by households in the community, as indicated by expert peer mothers. The first section was peeled and further divided into four relatively equal parts. The four parts were then put into a cooking pot and tap water was added to the pumpkin level. The pot with a lid was transferred to a hot charcoal stove and boiled for about 15 minutes. The second section was also cut into four relatively equal parts, peeled, and made ready for a local method of steaming.

The local method of steaming involved putting 1 L of tap water into the cooking pot, followed by banana stalks, then banana leaves, in which the pumpkin was wrapped before being put onto the hot charcoal stove for steaming. The role of the banana stalk was to create an elevation and separation between the water in the cooking pot and the banana leaves, where the pumpkin was wrapped. To this end, when water in the pot boils, it releases steam that vaporises through the banana stalk spaces to heat the banana leaves in which the pumpkin is wrapped. Pumpkin was steamed for about 30 minutes. Figure 5.2 shows raw and cooked pumpkin.

Raw peeled pumpkin	Boiled pumpkin	Steamed pumpkin

<u>Figure 5.2:</u> Raw and cooked pumpkin

The durations of boiling and steaming were sufficient to soften the plant tissue as assessed by the penetration of the tip of a knife without resistance (Carvalho *et al.*, 2014). Boiled and steamed pumpkin were blended separately in a blender for approximately 1 minute to form separate homogenous samples of boiled and steamed pumpkin, ready for PVAC analysis. Water was not added during blending to prepare pumpkin for analysis.

5.2.4 Preparation and cooking of common bean

A mass of 1000 g of common bean was divided into two portions i.e. 200 g (raw) and 800 g (for cooking). Raw common bean was dried in an oven at 80°C for 8 hours. Thereafter, it was milled to form a fine powder and kept in an airtight polythene bag ready for analysis. The remaining 800g of common bean was further divided equally into two portions of 400 g each. The first portion was put into a cooking pot and soaked in cold tap water overnight (8 hours). The water was discarded in the morning, and the soaked common bean was transferred to the regular cooking pot. The second portion was washed in cold tap water and transferred to the regular cooking pot. The cooking pots were filled with tap water to cover just above the level of the common bean in the pot. A lid was placed on each pot prior to putting it on the hot charcoal stove ready for boiling. The expert peer mothers regularly checked on the common bean to confirm whether it was soft and ready for consumption. When it was established that it was ready, common bean was taken off the charcoal stove. Figure 5.3 shows raw and cooked common bean.

Raw, unsoaked	Raw, soaked	Boiled without prior soaking	Boiled with prior soaking

Figure 5.3: Raw and cooked common bean

The cooking time for boiled common bean with and without soaking was 1.5 and 3.5 hours, respectively. Thereafter, the water used to boil the common bean was drained, and the common beans were put in a blender and blended for around 1 minute ready for analysis. Water was not added during blending to prepare common bean for analysis.

5.2.5 Analysis of provitamin A carotenoids and retinol activity equivalents in pumpkin

The PVAC content was analysed by high performance liquid chromatography (HPLC) as described in the HarvestPlus handbook for carotenoid analysis (Rodriguez-Amaya and Kimura, 2004). Carotenoids were extracted from the pumpkin prior to PVAC identification and quantification by HPLC. For the carotenoid extraction, 25mL of acetone was successively added to 1 g of blended freeze-dried pumpkin (separate for raw, boiled and steamed), until a paste was obtained. The paste was transferred to a sintered funnel (5 µm) coupled to a 250 mL Buchner flask and filtered. This procedure was repeated at least three times until the sample was colourless. The obtained extract was transferred to a 500 mL separatory funnel containing 40 mL of petroleum ether. The acetone was removed through the slow addition of ultrapure water (Milli-Q-Millipore) to prevent the formation of an emulsion. The lower aqueous phase was discarded and this procedure was repeated four times.The upper organic layer was transferred to a 25 mL measuring flask having 4.5 mL acetone.

For the identification and quantification of PVACs, 2 mL was removed from the carotenoid extract for raw, boiled and steamed pumpkin and dried in an amber flask under flowing nitrogen. Nitrogen flow was used to evaporate 2 mL of the extract stored in petroleum ether and re-suspended in acetone. 20 to 30 mL of filtered extracts from 0.45-mm filter unit were injected into the chromatographic column for HPLC analysis. All the solvents and chemicals including α-carotene and β-carotene standards were purchased from valid sources. Calibration curves were used to calculate the concentration of the respective carotenoids. The samples were analysed at 450 nm. The content of α-carotene and β-carotene was calculated according to the formula:

$$C\ (\mu g/g) = \frac{A_X \times C_S\ (\mu/g) \times V\ (mL)}{A_S \times P\ (g)}$$

Where: A_X = Carotenoid peak area; Cs = Standard concentration; A_S=Standard area; V=Total extract volume and P=Sample weight.

5.2.6 Conversion of provitamin A carotenoids in pumpkin to vitamin A (retinol activity equivalents)

The Institute of Medicine (2001) bioconversion rates of PVACs to retinol (retinol activity equivalents) were used, i.e. 12 µg of β-carotene is equivalent to 1µg of retinol, whilst 24 µg of α-carotene is equivalent to 1 µg retinol (Institute of Medicine, 2001).

5.2.7 Analysis of iron and zinc in common bean

Iron and zinc concentrations in both raw and boiled common bean were determined by FAAS, as described previously (Santelli *et al.*, 2006; Ekesa, Nabuuma and Kennedy, 2019). Iron and zinc determinations were performed using a Perkin–Elmer Model 3110 flame atomic absorption spectrometer. Microwave digestion was used to dissolve iron and zinc to oxidise the organic molecules in common bean prior to analysis by FAAS. Microwave digestion was done by closed vessel microwave oven following the procedure by Santelli and colleagues (Santelli *et al.*, 2006). 0.5g of each sample was carefully weighed into perfluoroalkoxy (PFA) liners of the oven and 8mL of concentrated nitric acid was added. After standing overnight in contact with nitric acid, 2mL of 30% m/m hydrogen peroxide was added and the liners were closed, placed into the microwave oven cavity and two irradiation cycles of 25 minutes (1000W maximum power) were run. The program of the equipment was adjusted to avoid internal pressures higher than 180psig and temperatures higher than 190^{o}C and between the first and second cycle, the liners were opened to release gases formed during digestion. Applying this procedure, perfectly clear and colourless solutions were obtained. This solution was transferred to the volumetric flask and the volume was made up to 25mL. This final solution was used in the iron and zinc determination by FAAS. The sample solution was then transformed into aerosols and transported to the flame to vaporise and atomise the sample. This step led to a reduced intensity of the light (by absorption of a defined quantity of energy) coming from a hollow-cathode lamp. The attached detector quantified the absorbed incoming light. The difference between the radiation without sample and including sample (absorbance) was used to calculate the iron and zinc concentration.

5.2.8 Retention of provitamin A carotenoids, iron and zinc

Micronutrient retention in processed foods may be calculated using either apparent retention (AR) or true retention (TR) (Murphy *et al.*, 1975; Li *et al.*, 2007). Apparent retention assumes that the amount of solids lost during processing are negligible and is expressed on a dry weight basis (Murphy *et al.*, 1975; Li *et al.*, 2007). In contrast, true retention is expressed on a fresh weight basis (Li *et al.*, 2007). It is worth noting that TR considers the loss of physical mass (i.e. soluble solid losses) more especially in food processing methods such as cooking, which cause significant soluble solid losses (Bechoff *et al.*, 2017). Therefore, TR is more accurate than AR (Bechoff *et al.*, 2017). To this end, micronutrient retention of PVACs, iron and zinc was calculated by TR using the following equation (Bechoff *et al.*, 2017).

$$TR\ (\%) = M_2 \times C_2 \div M_1\ C_2 \times 100$$

Where TR is true retention, M_2 is mass of cooked food in grams, C_2 is micronutrient content per gram of cooked food, M_1 is mass of raw food in grams and C_1 is micronutrient content per gram of raw food.

5.2.9 Data analysis

Iron and zinc content were reported as mean (mg/100g) of dry weight ± standard deviation (SD) of three determinations for raw and boiled common bean with or without prior soaking. The PVACs were reported as (µg/100g) fresh weight ± SD of three determinations for raw, boiled, and steamed pumpkin. Vitamin A was reported as retinol activity requirements (RAE) measured in µg/100g for raw, boiled, and steamed pumpkin. Data was analysed using Statistics and Data (STATA), version 13.1.

5.3 Results

The aim of the study was to determine the effect of household cooking methods on PVAC retention in locally available pumpkin (*Sweet cream*) in Uganda. Furthermore, it evaluated the effect of household cooking methods on zinc and iron retention in common bean (*Obwelu*) in Uganda.

5.3.1 Effect of cooking pumpkin on provitamin A carotenoid retention

Pumpkin was cooked by either boiling or steaming. Boiling and steaming pumpkin took 15 and 30 minutes, respectively. The mean β-carotene content was higher in boiled (3326.5 µg/100 g) and steamed (3466.2 µg/100 g) pumpkin, than in raw pumpkin (1704 µg/100 g). Furthermore, mean β-carotene content was higher in steamed (3466.2µg/100g) compared to boiled pumpkin (3326.5 µg/100 g). The TR for β-carotene in boiled and steamed pumpkin was 195% and 203%, respectively. Mean α-carotene was higher in boiled (75.1 µg/100 g) and steamed pumpkin (88 µg/100 g), compared to raw pumpkin (46 µg/100 g). It is worth noting that the mean α-carotene content was higher in steamed pumpkin compared to boiled pumpkin. Table 5.1 shows the PVAC content and retention after boiling and steaming pumpkin.

Table 5.1: Provitamin A carotenoid content and retention in boiled and steamed pumpkin, *Sweet cream* in Uganda

PVACs	Raw	Boiled	Steamed	True retention (%)	
				Boiled	Steamed
β-carotene µg/100g (±SD)	1704 (±5.0)	3326.5 (± 0.7)	3466.2 (± 3.9)	195	203
α-carotene µg/100g (±SD)	46 (± 3.0)	75.1 (± 0.3)	88.0 (± 0.6)	161	194
Vitamin A, (RAE) µg/100g (±SD)	143.9 (±4)	280.3 (± 0.8)	292.5 (± 2)	194.8	203.3

PVACs = Provitamin A carotenoids; SD = Standard deviation; RAE = Retinol activity equivalents, Vitamin A (retinol). Observations of PVACs and retention are given as a mean of three determinations. RAE = β-carotene (µg/100g)/12+ α-carotene (µg/100g)/24 (Institute of Medicine, 2001).

The TR for α-carotene in boiled and steamed pumpkin was 163% and 191%, respectively. The RAE was calculated using the 2001 Institute of Medicine bioconversion rates for PVACs to retinol (Institute of Medicine, 2001). The calculated mean RAE was higher in boiled pumpkin (280.3 µg/100 g) and steamed pumpkin (292.5 µg/100 g) than in raw pumpkin (143.9µg/100 g). The TR for RAE in boiled and steamed pumpkin was 194.5% and 203.3%, respectively.

5.3.2 Effect of cooking common bean on iron and zinc retention

Common bean was cooked either by soaking followed by boiling or boiling without prior soaking. Common bean was cooked until they were soft and ready for human consumption. The cooking time for soaked common bean was 1.5 hours, whilst unsoaked common bean took 3.5 hours to cook. Table 5.2 shows iron and zinc retention in boiled common bean in rural Kyankwanzi district, Uganda.

Table 5.2: Iron and zinc retention in cooked common bean, *Obwelu* in Uganda

	True retention (%)	
Cooking method	Iron	Zinc
Boiling with prior soaking	92.2	91.3
Boiling without soaking	88.4	75.6

The mean iron content reduced from 7.75 mg/100 g in raw common bean to 7.13 mg/100 g in boiled common bean with prior soaking, giving an iron retention of 92.2%. Likewise, mean zinc content reduced from 3 mg/100 g in raw common bean to 2.73 mg/100 g in boiled common bean with prior soaking, giving a zinc retention of 91.3%. Furthermore, mean iron content reduced from 7.75 mg/100 g in raw common bean to 6.84 mg/100 g in boiled common bean without prior soaking, giving an iron retention of 88.4%. Furthermore, mean zinc content reduced from 3 mg/100 g in raw common bean to 2.26 mg/100 g in boiled common bean without prior soaking, giving a zinc retention of 75.6%.

5.4 Discussion

The two methods that can be used to calculate nutrient retention are AR and TR (Murphy *et al.*, 1975; Li *et al.*, 2007). However, the current study used TR because it is more accurate than AR since AR tends to overestimate nutrient retention (Murphy *et al.*, 1975; Bechoff *et al.*, 2017; Hummel *et al.,* 2020).

5.4.1 Provitamin A carotenoid retention in pumpkin, *Sweet cream*

The higher concentration of PVACs observed in boiled and steamed pumpkin, compared to raw pumpkin, shows that there was over 100% retention of PVACs with cooking. The higher PVAC retention observed in this current study is consistent with other studies conducted in other PVAC rich foods such as provitamin A biofortified maize, provitamin A biofortified cassava and orange-fleshed sweet potato, which showed that PVAC retention ranged from 90% to over 100% with either boiling or steaming (Thakkar *et al.*, 2009; Pillay *et al.*, 2011; Vimala, Nambisan and Hariprakash, 2011). Furthermore, these findings agree with previous PVAC retention studies that used boiled or steamed pumpkin (Carvalho *et al.*, 2014, 2015). Carvalho and colleagues demonstrated that the retention of β-and α-carotene in both provitamin A biofortified and non-biofortified pumpkin was over 100% after boiling or steaming (Carvalho *et al.*, 2014, 2015).

The greater than 100% PVAC retention observed in the current study is plausible probably because of the PVAC isomerism characterised by extraction and release of *cis*-β-carotene isomers during heating of PVAC rich foods (Bengtsson *et al.*, 2008; Azizah *et al.*, 2009; Mugode *et al.*, 2014; Bechoff *et al.*, 2017). Furthermore, maceration (softening) and heating increases the PVAC concentration by rupturing the microstructure of PVAC rich plant tissue and then releasing more PVACs from the complex food matrix (Tumuhimbise, Namutebi and Muyonga, 2009; Provesi and Amante, 2015).

It is worth noting that the higher concentrations of PVACs observed in steamed compared to boiled pumpkin, could be attributed to the cooking method. It is likely that in the boiling method, PVACs released by rupturing the microstructure of the plant tissue are drained into the boiling water used to cook the PVAC rich food (Tumuhimbise, Namutebi and Muyonga, 2009; Provesi and Amante, 2015). Moreover, the current study did not analyse the PVAC content of the water in which the pumpkin was boiled because the water was discarded. The greater than 100% retention of PVACs (β-carotene and α-carotene) observed after cooking pumpkin also led to the increased RAE in boiled and steamed pumpkin. This is because the RAE calculation is based on the quantity of PVACs (Institute of Medicine, 2001). To this end, an increase in PVAC retention increases RAE. Such an increase in RAE has implications in meeting the recommended dietary allowance (RDA)/adequate intake (AI) for vitamin A for a target population group such as Ugandan children, six to 24 months old, an age group vulnerable to VAD (Amaral, Herrin and Gulere, 2018; Ekesa, Nabuuma and Kennedy, 2019). The RDA is the intake of a nutrient that meets the needs of almost all (97% to 98%) individuals in a group (Institute of Medicine, 2001). In contrast, the AI is a value based on observed or experimentally determined approximations of daily nutrient intake by a group (or groups) of healthy people (Institute of Medicine, 2001). The AI is used when an RDA cannot be determined (Institute of Medicine, 2001).

5.4.2 Zinc and iron retention in common bean

Iron and zinc content in cooked common bean were lower than in raw common bean, indicating that boiling with or without prior soaking of common bean reduces its iron and zinc retention. These findings are consistent with previous studies by Carvalho *et al.* (2012), Ferreira *et al.* (2014) and Hummel *et al.* (2020), who demonstrated that the zinc and iron retention in boiled common bean with or without soaking was below 100%. However, the current study showed that iron retention was higher in boiled common bean with prior soaking (92.2%), compared to common bean without soaking (88.4%).

Furthermore, zinc retention was higher in boiled common bean with prior soaking (88.4%), compared to common bean without prior soaking (75.6%). Findings from the current study are consistent with other studies, which demonstrated that iron and zinc retention in boiled common bean with prior soaking was higher than boiled common bean without prior soaking (Carvalho *et al.*, 2012; Hummel *et al.*, 2020). For example, in Brazil, Carvalho *et al.* (2012) showed that iron retention was 99% and 94% for boiled common bean (*BRS* *marfim*) with prior soaking and boiled common bean (*BRS marfim*) without prior soaking, respectively. Furthermore, Carvalho *et al.* (2012) demonstrated that zinc retention was higher (84%) in boiled common bean (*BRS Radiante*) with prior soaking compared to boiled common bean (*BRS Radiante*) without prior soaking (78%). Hummel *et al*. (2020) reported consistent findings from South American and Eastern African cultivated common bean varieties by demonstrating that the mean true retention for iron in boiled common bean with prior soaking (87%), was higher compared to boiled common bean without prior soaking (82%) among iron biofortified common bean varieties. In contrast to iron, Hummel *et al*. (2020) showed that mean zinc retention was higher in boiled zinc biofortified common bean varieties without prior soaking (81%), compared to boiled with prior soaking (78%). However, there was no statistically significant difference in zinc retention between boiled zinc biofortified common bean varieties with or without prior soaking (Hummel *et al*., 2020).

Previous studies have shown that it is not unanimous that boiled common beans with prior soaking have a higher retention of iron and zinc than those boiled without prior soaking (Fernandes, Nishida and Da-Costa Proença, 2010; Carvalho *et al.*, 2012; Hummel *et al.*, 2020). Several other common bean varieties from Eastern African countries and South American countries such as Brazil, and Columbia, boiled with or without prior soaking, had a varied retention of zinc and iron, ranging from 90% to over 100% (Carvalho *et al.*, 2012; Ferreira *et al.*, 2014; Hummel *et al.*, 2020). These findings were not unanimous, since zinc and iron content and retention was higher in some boiled common bean varieties without prior soaking, compared to those boiled with prior soaking (Carvalho *et al.*, 2012; Ferreira *et al.*, 2014; Hummel *et al.*, 2020). This may indicate that the variety of common bean and the environment in which they are cultivated are possible determinants of iron and zinc content and retention. For example, iron and zinc biofortified varieties of common bean cultivated in different countries of South America and East Africa had different iron and zinc content and retention, irrespective of either boiling with or without soaking, compared to the non-iron/zinc biofortified common bean varieties (Ferreira *et al.*, 2014; Hummel *et al.*, 2020).

The mean iron content in raw non-biofortified common bean was lower at 7.8 mg/100 g, compared to the iron biofortified common bean varieties with a mean iron content of 8.9 mg/100 g, as reported by Hummel *et al.* (2020). However, the average iron content of non-biofortified common bean varieties used by Hummel *et al.* (2020), was lower at 5.7 g/100 g, compared to that observed in common bean of 7.8 mg/100 g. Similarly, the zinc biofortified common bean varieties used by Hummel *et al.* (2020) had a higher zinc content at 3.9 mg/100 g, compared to 3mg/100g observed in common bean. In contrast, the mean zinc content in common bean observed in this current study is almost similar to 3.1 mg/100 g of the non-biofortified varieties used by Hummel *et al.* (2020). It is not surprising that biofortified common bean varieties are richer in the micronutrients under study because biofortification is a process that increases the nutritional value of food crops by increasing the vitamin and mineral density of the crop through conventional plant breeding, agronomic practices or biotechnology (HarvestPlus, 2007, 2012).

Hummel *et al.* (2020) boiled common bean with prior soaking, which is similar to the cooking method used in the current study. However, the TR of iron (92%) observed in boiling common bean, *Obwelu* prior to soaking was slightly higher, compared to that of 87% and 88% in iron biofortified and non-biofortified varieties of common bean, respectively, as reported by Hummel *et al.* (2020). Furthermore, the TR for zinc (91%) observed in soaked boiled *Obwelu* was higher than that in zinc biofortified (80%) and non-zinc-biofortified (75%) varieties of common bean used by Hummel *et al.* (2020), respectively. It is worth noting that a retention of over 90% of iron and zinc observed in boiled common bean with prior soaking in the current study is considered to be high (Carvalho *et al.*, 2012; Ferreira *et al.*, 2014). The higher retention of iron and zinc in boiled common bean with prior soaking compared to boiled common bean without prior soaking, observed in the current study could be explained by soaking. This is because soaking is more effective in increasing the extractability of iron and zinc from the food matrix, compared to not soaking (Sripriya, Antony and Chandra, 1997; Perlas and Gibson, 2002; Fernandes, Nishida and Da-Costa Proença, 2010).

Furthermore, the water used to soak *Obwelu* was discarded prior to boiling. This is plausible because discarding water after soaking is necessary to reduce phytic acid, an anti-nutrient that reduces the bioavailability of iron and zinc (Fernandes, Nishida and Da-Costa Proença, 2010). In contrast to increasing iron and zinc extractability (Fernandes, Nishida and Da-Costa Proença, 2010), soaking may lead to a loss of negligible amounts of iron and zinc, because iron and zinc may be lost in the discarded water used for soaking and boiling common bean (Fernandes, Nishida and Da Costa Proença, 2010). This may explain why the retention of iron and zinc was not 100% in soaked, boiled *Obwelu*.

In addition to a higher nutrient retention, soaked common bean cooked faster (1.5 hours) compared to un-soaked common bean (3.5 hours). This finding indicates that boiling with prior soaking of common bean was more fuel saving compared to boiling without prior soaking. This is consistent with a review of previous studies, which confirms that boiling common bean with prior soaking results in a shorter cooking time compared to common bean boiled without prior soaking (Reyes-Moreno, Paredes-López and Gonzalenz, 1993). A long cooking time for common bean increases fuel costs as indicated by the higher quantity of charcoal used to cook common bean in the current study (Mendum and Njenga, 2018). It is worth noting that caregivers prefer cooking methods that save time and subsequently save fuel (Reyes-Moreno, Paredes-López and Gonzalenz, 1993). To this end, caregivers should choose a cooking method such as boiling with prior soaking of common beans because it cooks faster, and consequently saves fuel, compared to boiling without prior soaking, as demonstrated in the current study.

5.4.3 Study strengths and limitations

Some strengths are inherent in the current study. For example, caregivers from the local community prepared pumpkin and common bean by using the locally acceptable home cooking methods. This was important because the locally acceptable cooking methods, which led to a higher retention of the micronutrients under study, should be promoted and supported through community nutrition education programmes (FAO, 2017). However, some limitations also exist. For example, boiling and steaming time impacts differently on PVAC retention in pumpkin (Ribeiro, *et al.*, 2015). However, in the current study, the boiling and steaming times for pumpkin were limited to 15 and 30 minutes, respectively.

Furthermore, iron and zinc retention in common bean are affected differently by different periods of soaking and cooking time (Fernandes, Nishida and Da-Costa Proença, 2010). However, the current study used only overnight soaking (8 hours), prior to boiling common bean. It is important to note that it was difficult to compare micronutrient retention in relation to cooking time for both common bean and pumpkin in the current study, since it was not feasible to set a constant cooking temperature when using local cooking equipment such as a charcoal stove used in the current study. It is worth noting that local cooking equipment such as charcoal and firewood stoves commonly used to cook food in developing countries, lack temperature regulators, compared to electric cookers commonly used in developed countries (Maes and Verbist, 2012). Furthermore, although there are three forms of PVACs i.e. β-carotene, α-carotene, and β-cryptoxanthin (Institute of Medicine, 2001), the current study did not analyse for β-cryptoxanthin. Nevertheless, failure to analyse β-cryptoxanthin may not have significantly affected the PVAC content of pumpkin because evidence from previous studies that analysed β-cryptoxanthin in pumpkin, established that its content was either not detected or negligible (Kim *et al.*, 2012). It is also likely that the study food samples were not representative of the environmental variation in micronutrients analyzed.

5.5 Conclusions

To retain a high proportion of PVACs, caregivers should be advised to cook pumpkin, *Sweet cream* by either boiling or steaming, while to retain a high proportion of iron and zinc, common bean, *Obwelu* should be prepared by boiling with prior soaking.

The next chapter prepares an innovative PVAC, iron and zinc rich CF by boiling pumpkin, *Sweet cream* and boiling with prior soaking common bean, *Obwelu*. Thereafter, the prepared PVAC, iron and zinc rich CF is fed to children with the aim of determining whether it is acceptable to them or not.

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CHAPTER SIX

[3]CHILD ACCEPTABILITY OF A PROVITAMIN A CAROTENOID, IRON AND ZINC RICH COMPLEMENTARY FOOD BLEND PREPARED FROM PUMPKIN AND COMMON BEAN IN UGANDA: A RANDOMISED CONTROL TRIAL

Abstract

A common bean pumpkin blend (BPB) complementary food (CF), rich in provitamin A carotenoids (PVACs), iron and zinc made from pumpkin (*Sweet cream*) and common bean (*Obwelu*) and a provitamin A carotenoid (PVAC) rich pumpkin puree (PP) CF made from *Sweet cream*, were prepared by peer mothers. This study compared child acceptability of the BPB and PP (control). The crossover acceptability study randomly assigned Ugandan children six to 24 months old to either receive 100 g of BPB (n=35) or 100 g of PP (n=35) on day one. After a washout period of one day, children crossed over to receive either BPB (n=35) or PP (n=35). The amount of CF consumed, duration of consumption, and micronutrient intake were assessed. The CF was acceptable if children consumed ≥ 50g (50%) of the served CF (100 g). A paired t-test was used to determine the mean differences within participants between BPB and PP. The level of statistically significant difference was set at a probability value of 5% (p=0.05). The mean amount of BPB and PP consumed was 53.9 g and 54.4 g, respectively. The mean duration for consumption of BPB and PP was 20.6 and 20.3 minutes, respectively. There was no significant difference in the amounts consumed, and duration of consumption in BPB and PP (p>0.05). The mean intake of vitamin A was significantly higher (p<0.00001) from PP (152.5 µgRAE) compared to BPB (100.9 µgRAE). The mean iron intake was significantly higher from BPB (1.1 mg) (p<0.00001) compared to PP (0.3 mg). Furthermore, zinc intake was significantly higher (p<0.00001) from BPB (0.58 mg) compared to PP (0.13 mg). In conclusion, a homemade complementary food, BPB, made from locally available common bean and pumpkin rich in PVACs, iron and zinc is acceptable to children in the age range of complementary feeding in Uganda.

Key words: Child acceptability; Complementary food; Common bean pumpkin blend; Pumpkin puree; Provitamin A carotenoids; Iron; Zinc; Vitamin A; Uganda

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[3] Publication based on this chapter:
Buzigi, E., Pillay, K. and Siwela, M. (2020) 'Child acceptability of a novel provitamin A carotenoid, iron and zinc-rich complementary food blend prepared from pumpkin and common bean in Uganda : A randomised control trial', *BMC Pediatrics*, 20 (412). doi:10.1186/s12887-020-02326-z.

6.1 Introduction

The burden of micronutrient deficiencies resulting from inadequate intakes of key micronutrients, particularly iron, zinc, and vitamin A contributes to reductions in linear growth, vulnerability to infection, reduced cognitive function, and significant child morbidity and mortality in the developing world (Millward, 2017). In developing countries, vulnerability to vitamin A deficiency (VAD), iron deficiency (ID) and zinc deficiency (ZnD) begins during the period of complementary feeding, when children are fed CFs deficient in vitamin A, iron and zinc (Gegios *et al.*, 2010; Gibson *et al.*, 2010). According to the World Health Organization (WHO), the period of complementary feeding begins at six to 24 months or beyond, when nutritious homemade foods are supposed to be given alongside breast milk to meet the increased nutritional demands for a child's growth and development (WHO, 2009). The nutritional demands for vitamin A, iron and zinc increase during the age range of complementary feeding (Dorea, 2000; Sakurai *et al.*, 2005; Qian *et al.*, 2010), indicating that vitamin A, iron and zinc rich complementary foods (CFs) are necessary at this critical period of child growth and development (WHO, 2009). However, Ugandan children are fed CFs predominantly prepared from staple cereals and tubers such as white maize, cassava, sweet potato and yams (Amaral, Herrin and Gulere, 2018). Such staples are deficient in vitamin A, iron and zinc, and their consumption has been linked to VAD, ID and ZnD (Gegios *et al.*, 2010). A recent study conducted in central rural Uganda analysed the micronutrient content of CFs, and established that they were deficient in iron and vitamin A (Ekesa, Nabuuma and Kennedy, 2019).

The WHO (2009) guidelines recommend that in order to combat micronutrient deficiencies such as vitamin A, iron and zinc, child caregivers should feed their children CFs formulated from animal source foods (ASFs), fortified foods and food supplements. This is plausible because ASFs, fortified foods and food supplements are rich sources of iron, zinc and vitamin A (Bhutta *et al.*, 2000; Dary and Mora, 2002; Lukacik, Thomas and Aranda, 2008; Dror and Allen, 2011; Shah *et al.*, 2016). However, the rural poor, including child caregivers in Uganda are unable to physically access or afford ASFs, fortified foods and food supplements (Faber, 2001; Wamani *et al.*, 2005). Moreover, despite a high child vitamin A supplementation (VAS) coverage of over 65% in Uganda (Wirth *et al.*, 2017), the prevalence of VAD among Ugandan children less than five years old has unacceptably increased from 20.4% (Baingana, Matovu and Garrett, 2008) to 32.6% (Wirth *et al.*, 2017) in the last decade. To this end, it is necessary to identify locally available and affordable food ingredients rich in vitamin A, iron and zinc and use them to prepare CFs rich in vitamin A, iron and zinc to supplement nutrition-specific interventions such as supplementation and fortification. Therefore, this study selected common bean, *Obwelu* and pumpkin, *Sweet cream*, locally cultivated in rural Uganda (Kiwuka *et al.*, 2012; Nakazibwe *et al.*, 2019), for use in the home preparation of an innovative PVAC, iron and zinc rich complementary food, BPB. *Obwelu* was selected because it was superior in iron and zinc compared to other locally available common bean landraces (chapter 4, section 4.3.2), whilst *Sweet cream* was superior in PVACs compared to other locally available pumpkin landraces (chapter 4, section 4.3.1). PVACs are an inactive form of vitamin A predominantly found in plant food sources (Codjia, 2001). When PVAC rich foods such as pumpkin are consumed, the PVACs are bioconverted into retinol, the active form of vitamin A used by the body (Van Loo-Bouwman, Naber and Schaafsma, 2014).

Testing caregiver and child acceptability of innovative CFs is necessary because it is a measure that can inform whether caregivers will feed the CF to their children and whether children will accept to ingest the CF, respectively (Skinner *et al.*, 2002). However, several studies have used child caregivers to test the acceptability of CFs (Martin, Laswai and Kulwa, 2010; Govender *et al.*, 2014; Amod *et al.*, 2016; Pillay, Khanyile and Siwela, 2018), hence neglecting the target group for CFs, who are children six to 24 months old. Such studies argue that caregivers were used instead of children because children are too young to provide a rational judgement on sensory attributes such as taste, aroma, colour and texture, usually used to test for acceptability of foods (Martin, Laswai and Kulwa, 2010). However, caregiver acceptability of a CF does not guarantee child acceptability (Paul *et al.*, 2008). It is worth noting that child acceptability can be assessed by feeding the innovative CF to the child, followed by measuring the amount of CF consumed, and the duration taken to complete eating the CF (Guinard, 2001; Adu-Afarwuah *et al.*, 2008; Aaron *et al.*, 2011; Ahmed *et al.*, 2014). Children aged six to 24 months are the target consumers for CFs (FAO/WHO, 2013). Therefore, this study assessed the acceptability of an innovative PVAC, iron and zinc rich homemade complementary food, BPB, among Ugandan children in the age range of complementary feeding (six to 24 months old).

6.2 Methods

6.2.1 Study setting

This study was conducted in rural Kyankwanzi district, central Uganda. Uganda's latitude and longitude is 1° 00' N and 32° 00' E, respectively. The total population of Kyankwanzi district is 214 693, of which 34% are illiterate, 48% are females, 16% are child mothers 12-19 years old and 19% are children 0-4 years old (Uganda Bureau of Statistics, 2017). Children in this study area are fed CFs deficient in vitamin A, iron and zinc (Amaral, Herrin and Gulere, 2018; Ekesa, Nabuuma and Kennedy, 2019). The preparation of the CFs and child acceptability study were conducted at Ntwetwe Health Centre IV, Kyankwanzi district.

6.2.2 Description of the intervention

6.2.2.1 Ingredients for preparation of the complementary foods used in the intervention

This study formulated two homemade complementary foods, BPB and PP. The former was formulated from boiled common bean (*Obwelu*) with prior soaking and boiled pumpkin (*Sweet cream*), whilst the latter (control CF) from boiled pumpkin, *Sweet cream* as described in chapter 5, section 5.2.3 and 5.2.4. The PP was selected as a control because pumpkin is commonly used as a single CF in Uganda (Uganda Bureau of Statistics and Inner City Fund, 2018). Common bean, *Obwelu* and pumpkin, *Sweet cream* were used as ingredients because the former is superior in iron and zinc compared to other common bean landraces, whilst the latter is superior in PVACs as established in chapter 4, section 4.3.1 and 4.3.2. Moreover, these ingredients are cultivated in rural Uganda and available on the local markets (Kiwuka *et al.*, 2012; Nakazibwe *et al.*, 2019). Figure 6.1 shows raw common bean (*Obwelu*) and pumpkin (*Sweet cream*), PP and BPB.

Raw common bean (*Obwelu*)	Raw pumpkin (*Sweet cream*)	Pumpkin puree (PP)	Common bean pumpkin blend (BPB)

Figure 6.1: Study complementary foods and the ingredients used to prepare them

6.2.2.2 Preparation of common bean pumpkin blend and pumpkin puree

Community health workers randomly identified 10 expert peer mothers from 10 villages and invited them to Ntwetwe Health Centre IV to participate in the preparation of CFs used in the acceptability study. Expert peer mothers were encouraged to prepare CFs using the locally acceptable home-based methods used in the community to prepare common bean and pumpkin for child consumption. Common bean (*Obwelu*) and pumpkin (*Sweet cream*) were purchased from the local market with assistance from expert peer mothers. Expert peer mothers prepared *Sweet cream* by peeling and discarding seeds followed by boiling the pulp. For *Obwelu*, expert peer mothers used overnight soaking (for about eight hours), followed by boiling (for about 1.5 hours). After cooking, expert mothers indicated that they prepare homemade CFs in their community based on consistency (thinness or thickness of food) suitable for the child's stage of development. To this end, after cooking by expert peers mothers, research assistants mixed the ingredients to form CFs based on the consistency as suggested by caregivers and the 2017 Food and Agriculture Organization of the United Nations (FAO) recommended guidelines for conducting participatory cooking demonstrations to improve complementary feeding practices (FAO, 2017). Research assistants prepared three different formulations of BPB by mashing and mixing *Sweet cream* and *Obwelu* together. Table 6.1 shows the ratio of *Sweet cream* and *Obwelu* that was used to prepare the three formulations of BPB.

Table 6.1: Ratio of mixing *Sweet cream* and *Obwelu* to formulate BPB

BPB formulations	**BPB -1**	**BPB-2**	**BPB-3**
Sweet cream : *Obwelu*	1:1	1:2	2:1

BPB=Common bean pumpkin blend

After preparation, the three varieties of BPB were put on a table in three different serving dishes and presented to expert peer mothers. Based on consistency, expert mothers, one by one entered the room and were asked to choose one formulation of BPB that they would choose to feed their children, six to 24 months old (FAO, 2017; Bekele and Turyashemererwa, 2019). All the 10 expert mothers unanimously selected BPB-3, prepared by mixing two parts of *Sweet cream* and one part of *Obwelu*. Mashed cooked pumpkin in Uganda is usually given as a single CF (Uganda Bureau of Statistics and Inner City Fund, 2018). Therefore, PP prepared from *Sweet cream*, served as the control. Triplicate samples of prepared BPB (test food) and PP (control) were transported to METLAB East Africa limited laboratory, Kampala, Uganda for PVAC, iron and zinc analyses.

6.2.2.3 Vitamin A, iron and zinc analysis of BPB and PP

The PVAC content was analysed by high performance liquid chromatography (HPLC) as described in the HarvestPlus handbook for carotenoid analysis (Rodriguez-Amaya and Kimura, 2004) and section 5.2.5. To analyse the vitamin A content, the Institute of Medicine (2001) bioconversion rates of PVAC to vitamin A, retinol (retinol activity equivalents) were used, i.e. 12µg of β-carotene is equivalent to 1µg of retinol, whilst 24µg of α-carotene is equivalent to 1µg retinol (Institute of Medicine, 2001). Iron and zinc concentrations in the CFs were determined by flame atomic absorption spectroscopy (FAAS) as described by Santelli *et al.* (2006), Ekesa, Nabuuma and Kennedy (2019) and section 5.2.5. Triplicate analysis for BPB and PP were done separately to get the mean content of PVACs, iron and zinc in both CFs.

6.2.2.4 Provitamin A carotenoid, iron and zinc content of BPB and PP

The mean content of PVACs, iron and zinc were determined per 100g of edible portion of BPB and PP (Table 6.2).

Table 6.2: Provitamin A carotenoid, iron and zinc content of edible portion of BPB and PP

Micronutrient	BPB/100 g	PP/100 g
Iron (mg)	1.99	0.57
Zinc (mg)	1.08	0.23
β-carotene (µg)	2219	3326.5
α-carotene (µg)	50.5	75.1
Vitamin A (µgRAE)	187	280.3

BPB=Common bean pumpkin blend; PP=Pumpkin puree
RAE=Retinol activity equivalent (retinol)
RAE= β-carotene (µg/100 g)/12+ α-carotene (µg/100 g)/24 (Institute of Medicine, 2001)

6.2.3 Study participants, enrolment, inclusion and exclusion criteria

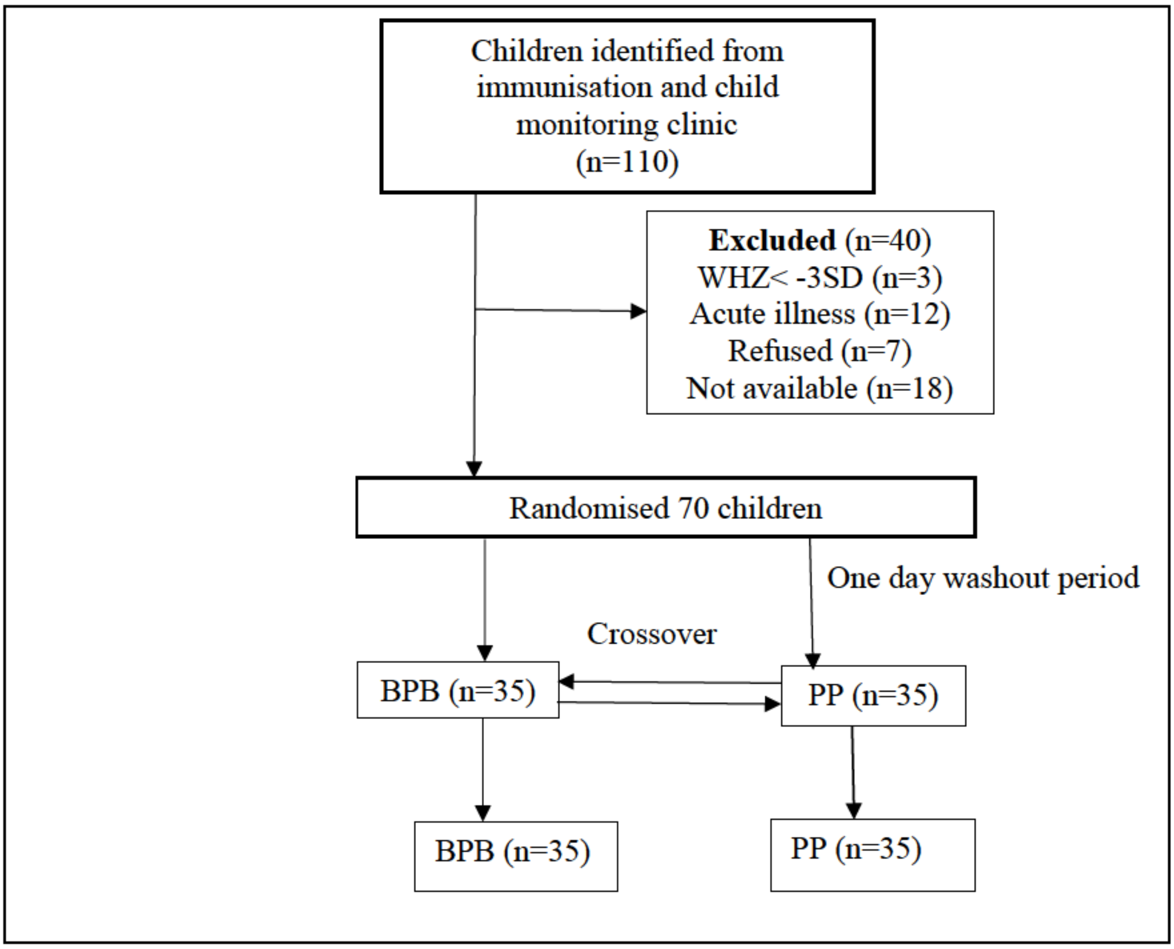
All children (aged 6 to 24 months old) coming for growth monitoring and immunisation at Ntwetwe Health Centre IV, Kyankwanzi district Uganda were screened for nutritional status and presence of any illness. Upon fulfilling the enrolment criteria (age 6 to 24 months, on complementary feeding) and obtaining consent for participation from the caregivers, the children were randomly allocated to two different study groups (BPB and PP) and children were enrolled. Children did not meet the enrolment criteria if their weight for age or weight for height Z scores were below -3 standard deviations (SD) of the WHO reference (WHO and UNICEF, 2019), indicating severe underweight or severe wasting, respectively. Furthermore, children were excluded from the study if they had any childhood acute illness or features suggestive of any chronic disease such as tuberculosis or congenital abnormalities such as cleft lip or palate.

6.2.4 Sample size determination

A maximum of 50 g of CF per serving is considered adequate for children in the age range of complementary feeding (Lutter and Dewey, 2003; FAO/WHO, 2013). Therefore, the sample size was determined to test the hypothesis that the mean consumption of CF during the acceptability test would be at least 50 g (50%) of the amount offered (100 g). Assuming a mean difference of 5 g between test CF and control, and a SD of 10 g in a normally distributed population of children 6 to 24 months old, a sample size of 63 for each CF would allow for rejection of the null hypothesis with 80% power. However, this was a crossover study in which participants consumed both the test and control CFs. In order to cater for loss to follow-up, an additional seven participants were added to the 63 to give a total of 70 participants. To this end, the same 70 participants were enrolled in each group of BPB and PP.

6.2.5 Study design

This was a randomised crossover acceptability study. A total of 110 children from the growth monitoring and immunisation clinic at Ntwetwe Health Centre IV, Kyankwanzi district Uganda were identified for randomisation (Figure 6.2). Out of the 110 children, 70 were eligible and assigned to BPB (test) and PP (control) using simple random sampling according to computer-generated random numbers. Computer-generated numbers were given to participants by a research assistant who was located off site. On the first day, 35 children were assigned to each group of BPB and PP. A washout period of one day was granted, and on the third day participants crossed over to the opposite CF group. Figure 6.2 shows the study design.



BPB: Common bean pumpkin blend; PP: Pumpkin puree; SD: Standard deviation; WHZ: Weight for height Z score

Figure 6.2: Child acceptability study design

6.2.6 Measurement of study outcomes

Child acceptability was assessed by feeding the study CFs to the child, followed by measuring the amount of CFs consumed, and the time taken to consume the CFs (Guinard, 2001; Adu-Afarwuah *et al.*, 2008; Aaron *et al.*, 2011; Ahmed *et al.*, 2014). The primary outcome of the study was to measure the amount of CF consumed by children. The two secondary outcomes were to measure the time taken by the child to consume the served CF; and to analyse the PVAC, iron and zinc intake of each child based on the amount of CF consumed. Caregivers were also requested to report any discomfort or adverse effects experienced by the children after being fed the study CFs.

6.2.6.1 Amount of complementary food consumed by children

This study ensured that children were offered the assigned CF (BPB or PP) at least one hour after they were last fed. A portion of 100 g of CF was offered to the child in a serving dish by the caregiver. The CF was considered acceptable if the child ingested at least 50g of the offered food (FAO/WHO, 2013). The amount of food ingested was calculated by subtracting the left-over from the offered amount. Pre-weighed napkins were provided; any food that was regurgitated, vomited or spilled was swabbed, the napkin weighed and subtracted from the amount of the food offered.

6.2.6.2 Duration of feeding

Duration of feeding was measured as described by Ahmed *et al.* (2014). Caregivers were asked to spoon feed their children the assigned CF until the child refused to eat. After a two-minute pause, the same food was offered a second time until the child refused again. After a second two-minute pause, the food was offered a third time until refused again. After the third refusal, the feeding episode was considered terminated. The duration of feeding (excluding the intervening 'pause periods') was recorded by stopwatch, and the total duration of the feeding was noted. The feeding episode took place under the direct supervision of a trained research assistant to ensure that feeding was not forced. Children were considered as refusing intake if they moved their head away from the food, cried, clamped their mouth shut or clenched their teeth, or became agitated, spat out the food or refused to swallow as described by Ahmed *et al.* (2014).

6.2.6.3 Micronutrient intake measurement

The micronutrient intake (MNI) for each child was calculated using the formula, MNI= A(g)×B/100, where A was the amount of CF (BPB or PP) consumed by the child and B was the nutrient composition in 100g of CF served to the child. For example, if the child consumed 50g of 100g of BPB served, then MNI for iron, zinc and vitamin A (retinol) would be 50×1.99/100 (0.995 mg), 50×1.08/100 (0.54 mg) and 50×187/100 (93.5 µgRAE), respectively.

6.2.7 Determination of background characteristics and anthropometric measurements

Data on background characteristics such as age, gender and nutritional status of study participants were collected. Age was calculated in months based on the difference between the date of visit and date of birth. If the exact date of a child's birth was unknown, the month and year of birth were estimated using a local events calendar. In such cases, age was calculated after inputing the day of birth as the 15th of the month, as recommended by the 2019 WHO guidelines (WHO and UNICEF, 2019). Date of birth was extracted from the child's immunisation and growth monitoring chart. Nutritional status was determined by using anthropometry, and diagnosed by Z scores based on the 2019 WHO recommendations for data collection, analysis and reporting on anthropometric variables such as weight and height in children under five years old (WHO and UNICEF, 2019). Anthropometric measurements were taken by trained Nutritionists with prior experience in conducting child anthropometric measurements. The weight of each child wearing minimal clothing was measured to the nearest 100 g using a hanging Salter weighing scale (Model 235 6S, England), with a measuring capacity of 25k g. The scale was adjusted to read zero before starting the measurements. The average of the three measurements of weight was recorded. Length was measured to the nearest centimetre using a stadiometer (wooden length board). The child was laid down with back against the board, with heels, buttocks and shoulders straight. The child's legs, knees and ankles were placed together. The sliding headpiece was brought down onto the upper most point on the head and the length was recorded to the nearest 0.1cm at the examiner's eye level. The average of the three measurements of length was recorded. A child was stunted, wasted and underweight if the length for age Z score (LAZ), weight for length Z score (WLZ) or weight for age Z score (WAZ) was below -2 SD of the WHO reference, respectively (WHO and UNICEF, 2019).

6.2.8 Data analysis

Statistical data analysis was done using Statistics and Data (STATA) version 13.1. Background characteristics of the participants were evaluated using descriptive statistics. The mean ± SD of the amount of the CF consumed, duration of consumption, and MNI were calculated. The paired t-test was used to detect the mean differences of outcome variables within participants between BPB and PP. The level of significant difference was set at a probability value of 5% ($p = 0.05$).

6.2.9 Ethical considerations

Permission to conduct the study was granted by the District Health Office, Kyankwanzi district, Uganda. In South Africa, ethical approval was obtained from the Biomedical Research Ethical Committee, University of KwaZulu-Natal (Reference number: BE438/19). In Uganda, ethical approval was granted by the AIDS Support Organisation Research Ethical Committee

(Reference number: TASO-REC/066/19-UG-REC-009). This randomised control trial was registered by the Pan African Clinical Trials Registry (www.pactr.org) as PACT R202002576768667. Informed and signed consent were obtained individually from caregivers of child participants in the study, and all data were coded to remove identifying information and ensure confidentiality.

6.3 Results

6.3.1 Description of study participants

On day one, 35 children were either fed BPB or PP, while day two was a washout period. On day three, 35 participants crossed over to receive either BPB or PP. A total of 70 eligible children were enrolled and completed the acceptability test. They included 37 girls (52.9%) and 33 boys (47.1%), and their mean age ± SD was 12.3 ± 3.9 months. The proportion of wasting, underweight and stunting among study participants was 7%, 17% and 29.3%, respectively. Caregivers did not observe any discomforts or adverse effects in their children after they tasted the BPB or PP. The mean age and SD of child caregivers was 23.6 ± 6.1 years. Of the 70 caregivers, 63 (90%) and 7 (10%) caregivers were female and male, respectively. Table 6.3 shows the demographic characteristics and nutritional status of child participants.

Table 6.3: Demographic characteristics and nutritional status of child participants in the acceptability study

Characteristics	Participants (n=70)
Demographic	
Age (months), mean ± SD	12.3 ± 3.9
Gender	
Female, n (%)	38 (54.3)
Male, n (%)	32 (45.7)
Nutritional status	
Wasted (WHZ < -2SD)	
Yes, n (%)	5 (7.1)
No, n (%)	65 (92.9)
Underweight (WAZ < -2SD)	
Yes, n (%)	8 (11.4)
No, n (%)	62 (88.6)
Stunting (HAZ < -2SD)	
Yes n (%)	27 (38.6)
No n (%)	43 (61.4)

SD = Standard deviation
WHZ= Weight for height Z score
WAZ= Weight for age Z score
HAZ= Height for age Z score

6.3.2 Child acceptability test

The aim of this study was to assess child acceptability of a homemade CF (BPB), rich in PVACs, iron and zinc, compared to a PVAC rich PP (control). Acceptability was measured by the amount of CF consumed by the child and duration of consumption. Table 6.4 shows results from the child acceptability test.

Table 6.4: Child acceptability and micronutrient intake from BPB and PP

Variable	**BPB (n=70)**	**PP (control) (n=70)**	***p* value**
Amount consumed (g) (mean ±SD)	53.9 ± 2.97	54.4 ± 3.51	0.440
Feeding duration (minutes) (mean ±SD)	20.6 ± 1.4	20.3 ± 1.6	0.140
Iron received from consumed food (mg) (mean ±SD)	1.1 ± 0.59	0.3 ± 0.02	<0.00001
Vitamin A received from consumed food (µgRAE) (mean ±SD)	100.9 ± 0.7	152.5 ± 1.2	<0.00001
Zinc received from consumed food (mg) (mean ±SD)	0.58 ± 0.04	0.13 ± 0.01	<0.00001

BPB=Common bean pumpkin blend; PP=Pumpkin puree; SD=Standard deviation
µgRAE=Microgram Retinol Activity Equivalent (Retinol, active form of vitamin A)

6.3.3 Amount of complementary food consumed

Children consumed an average of 54.2 ± 3.3 g of served CF. The mean consumption of BPB and PP was 53.9 g and 54.4 g, respectively. There was no significant difference in the amount consumed between BPB and control PP (p=0.440). The CF was acceptable if the child ate 50 g (50% and above) of the 100 g of CF offered. To this end, both BPB and PP were 100% acceptable to the study children.

6.3.4 Duration of consumption of BPB and PP

The mean duration for consumption of BPB was slightly longer (20.6 minutes) compared to PP (20.3 minutes). However, there was no significant difference in mean duration of consumption for BPB and PP (p=0.14).

6.3.5 Vitamin A, iron and zinc intake from consumed BPB and PP

The mean vitamin A (RAE) intake was 100.9 µgRAE and 152.5 µgRAE from BPB and PP, respectively. The mean intake of vitamin A was significantly higher from PP compared to BPB (p<0.00001). The mean iron intake was significantly higher (p<0.00001) from BPB (1.1 mg) compared to PP (0.3 mg). Furthermore, zinc intake was significantly higher from BPB (0.58 mg), compared to PP (0.13 mg).

6.4 Discussion

The BPB was superior in iron and zinc compared to PP because the common bean was blended with pumpkin to form BPB (Table 6.2). This is plausible because common bean is a rich source of iron and zinc (Carvalho *et al.*, 2012; Ferreira *et al.*, 2014; Haas *et al.*, 2016; Moloto *et al.*, 2018). Besides, PP was superior in vitamin A because 100% of PP was prepared from pumpkin, a rich source of PVACs (Azevedo-Meleiro and Rodriguez-Amaya, 2007; Azizah *et al.*, 2009; Koh and Loh, 2018). The average recommended amount of CF per serving for a child, 6 to 24 months old during complementary feeding is 50 g (Lutter and Dewey, 2003). It was hypothesised that the CFs would be acceptable if children consumed 50g or more of the offered CF. The mean amount consumed of both CFs was above 50 g, indicating that both CFs were 100% acceptable. Moreover, there was no significant difference in the mean amount consumed between the BPB and PP. Preparation and acceptability of micronutrient rich CFs from locally available food ingredients has been reported previously (Konyole *et al.*, 2012; Govender *et al.*, 2014; Bauserman *et al.*, 2015; Amod *et al.*, 2016). Based on the amount consumed, Bauserman *et al.* (2015) established that a micronutrient rich CF prepared from caterpillar, corn and palm oil was acceptable to children in the age range of complementary feeding, in the Democratic Republic of Congo. The CF was developed in accordance with international standards on the formulation of foods intended for infants and children up to two years of age as outlined in Codex Alimentarius (FAO/WHO, 2013; Bauserman *et al.*, 2015). However, the current study developed CFs based on consistency as determined by child caregivers and as recommended for the preparation of homemade CFs (FAO, 2017). Preparation in accordance with the international standards on the formulation of foods intended for infants and children up to two years of age outlined in the Codex Alimentarius is a more objective indicator of the nutrient content of CFs, compared to the subjective method of using consistency. This may explain why the iron and zinc content in the study by Bauserman *et al.* (2015) was higher than that in BPB. The vitamin A (in the form of RAE), iron and zinc content of BPB was reported in the current study. However, Bauserman and colleagues only reported zinc and iron content of their CF, despite the use of PVAC rich palm oil as one of the ingredients in the formulation of the CF (Codjia, 2001; Bauserman *et al.*, 2015). Therefore, it is difficult to conclude the vitamin A content in the CF developed by Bauserman and colleagues (Bauserman *et al.*, 2015).

In Kenya, the highest proportion of children aged 6 to 24 months old consumed over 75% of the CF developed from locally available termites and small fish, which was regarded as acceptable (Konyole *et al.*, 2012). However, the Kenyan study also reported the estimated content of iron and zinc, but not vitamin A. In South Africa, CFs were developed from provitamin A-biofortified foods (Govender *et al.*, 2014; Amod *et al.*, 2016). However, these studies did not test the acceptability of these CFs in the target age group of complementary feeding (children 6to 24 months old) (Govender *et al.*, 2014; Amod *et al.*, 2016). In contrast to other previous studies (Konyole *et al.*, 2012; Govender *et al.*, 2014; Bauserman *et al.*, 2015; Amod *et al.*, 2016), the current study developed a CF rich in vitamin A, iron and zinc, and tested its acceptability among children in the age range of complementary feeding. It is worth noting that vitamin A, iron and zinc are the three leading micronutrients of public health importance in the developing world needed to prevent child mortality and common childhood morbidities such as diarrhoea, respiratory tract infections, night blindness, and iron deficiency anaemia (Millward, 2017).

The role of CFs in meeting the dietary reference intakes (DRIs) such as the recommended dietary allowance (RDA)/adequate intake (AI) for children in the age range of complementary feeding is well recognised (Dewey and Brown, 2003). The RDA is the intake that meets the nutrient need of almost all (97% to 98%) individuals in a group, while the adequate intake (AI) is the recommended average daily nutrient intake level, based on observed or experimentally determined estimates of the average nutrient intake by a healthy population (Institute of Medicine, 2001). The RDA/AI for retinol (vitamin A), iron and zinc for a child 13 to 24 months old is 300 µgRAE/day, 7 mg/day and 3 mg/day, respectively (Institute of Medicine, 2001). This study showed that the average intake of vitamin A, iron and zinc from one serving of BPB (53.9 g) was 109.5 mg, 1.1 mg and 0.58 mg, respectively. This suggests that one serving of BPB would contribute 37%, 16% and 19% towards meeting the RDA/AI for vitamin A, iron and zinc, respectively in children 13 to 24 months old. If BPB was served to children twice daily, it would contribute 74%, 32% and 38% towards meeting the RDA/AI for vitamin A, iron and zinc, respectively in the children under study. It is worth noting that provitamin A-biofortified food crops such as maize and orange-fleshed sweet potato are bred to provide 50% of the mean daily vitamin A dietary requirement through normal consumption habits (HarvestPlus, 2007, 2012). However, if BPB is served twice daily it would provide over 50% of the RDA/AI for vitamin A for a child 13 to 24 months old. Iron and zinc concentration in the BPB were low, compared to other studies that developed and tested child acceptability of iron and zinc rich CFs (Konyole *et al.*, 2012; Bauserman *et al.*, 2015). However, there is convincing evidence from a systematic review study that low dose daily iron and zinc intake has a positive effect on iron and zinc status of children, six to 24 months old (Petry *et al.*, 2016).

It is worth noting that the rural poor in Uganda feed their children CFs such as staple cereals and tubers, which are deficient in vitamin A (Amaral, Herrin and Gulere, 2018; Ekesa, Nabuuma and Kennedy, 2019), because they lack physical and economic access to vitamin A rich foods such as ASFs and vitamin A-fortified foods (Wamani *et al.*, 2005). To contribute towards combating VAD in Ugandan children, bi-annual (six monthly) high dose (200,000 IU or 60,000 µgRAE) VAS programmes have been running for more than a decade (UNICEF, 2007; Wirth *et al.*, 2017; Factfish, 2019). However, the prevalence of child VAD in Uganda has increased from 20.4% (Baingana, Matovu and Garrett, 2008) to 32.6% (Wirth *et al.*, 2017) in the last decade. This is plausible because standalone VAS programmes may not combat VAD (Mason *et al.*, 2015). This is because the liver is unable to store the high dose of 60 000 µg of retinol (200 times the RDA for a child 12 to 24 months old), and therefore, the excess vitamin A is destroyed by the liver and excreted (Blomhoff, Green and Norum, 1992). Moreover, the rise in serum retinol resulting from six-monthly VAS is small, short-lived, and lasts only for one to three months (Pedro *et al.*, 2004). To this end, a PVAC, iron and zinc rich BPB or a PVAC rich PP may be necessary to complement VAS programmes in the fight against child VAD in Uganda.

6.4.1 Study strengths and limitations

Bioavailability is defined as the proportion of the ingested nutrients that are absorbed in the small intestine, enter the circulation, and become available for utilisation or storage in organs (Saini, Nile and Park, 2015). Common bean, an ingredient of BPB is a potential source of phytic acid, an anti-nutrient that reduces the bioavailability of iron and zinc in the body (Gibson *et al.*, 2010; Kumar *et al.*, 2010). However, this study did not analyse for anti-nutrient compounds such as phytic acid in BPB. It is worth noting that common home cooking methods such as soaking and boiling used in this study are known to reduce the amounts of phytic acid in common bean (Fernandes, Nishida and Da Costa Proença, 2010; Miller, Hambidge and Krebs, 2015). Because PVACs are fat soluble (Saini, Nile and Park, 2015), incorporating fat during preparation of BPB or PP could have improved the bioavailability of the PVACs contained in them. However, the widely accepted 2001 Institute of Medicine bioconversion recommendations of PVACs to retinol used in this study, are independent of the use of fat as an ingredient in the preparation of PVAC rich foods (Institute of Medicine, 2001).

Furthermore, the current study considered that the offered CF was acceptable to children, six to 24 months old, if they ingested at least 50 g of the CF offered. This is in line with the 2013 FAO and WHO guidelines on formulated CFs for older infants and young children, which considers 50g of offered CF as a reasonable maximum quantity which children in the age range of complementary feeding can ingest per feeding (FAO/WHO, 2013). However, child acceptability findings from the current study with regard to the amount of CF ingested in a specified duration should be interpreted with caution. This is because a wide range of 10g to 50g of a formulated CF is considered a reasonable quantity that an older infant or a young child in the complementary feeding stage can ingest easily in one feeding session for a specified duration, depending on age (FAO/WHO, 2013). Therefore, older infants (six to 12 months old) may ingest less CF in a specified duration than young children (13 to 24 months old).

6.5 Conclusions

Child caregivers prepared a PVAC, iron, and zinc rich complementary food, BPB from locally available pumpkin and common bean using home preparation methods. The newly developed, multiple micronutrient rich BPB was acceptable to children in the age range of complementary feeding, and has the potential to contribute towards combating VAD, ID and ZnD among children aged six to 24 months old. The study implies that children are likely to ingest BPB, when it is continuously given as a CF. Future studies should evaluate the effect of BPB intake on child vitamin A, iron, and zinc status.

The next chapter evaluates the acceptablity of BPB among caregivers. It is neccessary to to evaluate caregiver acceptablity of innovative CFs because caregivers are the gatekeepers of CFs, ie they decide whether they will prepare and give the innovative CFs to their children.

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CHAPTER SEVEN

[4]CAREGIVER PERCEPTIONS AND ACCEPTABILITY OF A PROVITAMIN A CAROTENOID, IRON AND ZINC RICH COMPLEMENTARY FOOD BLEND PREPARED FROM COMMON BEAN AND PUMPKIN IN RURAL UGANDA

Abstract

Caregivers decide whether or not to feed innovative complementry foods to their children. This study developed an innovative provitamin A carotenoid (PVAC), iron and zinc rich common bean pumpkin blend (BPB) complementary food (CF) from locally available pumpkin and common bean in Uganda and aimed to determine its acceptance, compared to a control, pumpkin puree (PP). Seventy caregivers participated in the study. The sensory attributes (taste, colour, aroma, texture and general acceptability) of BPB and PP were rated using a five-point facial hedonic scale (1=very bad, 2=bad, 3=neutral, 4=good, 5=very good). Focus group discussions (FGDs) were conducted to assess the perceptions of caregivers about the BPB. A chi-square test was used to detect the proportion difference for each sensory attribute between BPB and PP, whilst focus group discussion (FGD) data was analysed by thematic analysis. A proportion of 64 to 96% of the caregivers rated both BPB and PP as acceptable (good to very good) for all the sensory attributes. There was no significant difference in caregiver acceptability for all attributes between BPB and PP (p>0.05). Caregivers had positive perceptions about the taste, texture, aroma and colour of the BPB. Caregivers were keen to know the specific varieties of common bean and pumpkin used to formulate the PVAC, iron and zinc rich BPB. In conclusion, BPB was acceptable to caregivers, and they were interested to know how to prepare and use it as a CF.

Keywords: Complementary foods; Common bean pumpkin blend; Pumpkin puree; Provitamin A carotenoids; Iron; Zinc; Caregiver acceptability; Uganda

[4] Publication based on this chapter:
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7.1 Introduction

The World Health Organization (WHO), and the Food and Agriculture Organization of the United Nation (FAO) define complementary feeding as a critical period of child growth and development (six to 24 months of age), when breast milk should be supplemented with nutrient-rich CFs (WHO, 2009; FAO, 2017). Feeding low quality CFs during this critical period of growth and development increases the vulnerability to child morbidity, mortality and growth faltering (Victora *et al.*, 2010; Millward, 2017). Micronutrients such as vitamin A, iron and zinc are necessary for child growth, and preventing childhood illnesses such as night blindness, diarrhoea, respiratory tract infections and iron deficiency anaemia (Millward, 2017). From the age of six months, breast milk alone is no longer sufficient to meet the child's nutritional requirements for vitamin A, iron and zinc (Dorea, 2000; Sakurai *et al.*, 2005; Qian *et al.*, 2010). Therefore, children need to be fed vitamin A, iron and zinc rich CFs (WHO, 2009). However, caregivers from developing countries including Uganda, feed their children low quality homemade CFs predominantly formulated from staple cereals and tubers such as white maize, sweet potatoes, cassava and yams (Gegios *et al.*, 2010; Gibson *et al.*, 2010; Amaral, Herrin and Gulere, 2018). These staple cereals and tubers are rich energy sources; however, they are low in vitamin A, iron and zinc (Ekesa, Nabuuma and Kennedy, 2019). Consumption of such staples during the period of complementary feeding is associated with child vitamin A deficiency (VAD), iron deficiency (ID) and zinc deficiency (ZnD) (Gegios *et al.*, 2010).

To combat VAD, ID and ZnD during complementary feeding, it is recommended that children should be fed micronutrient fortified CFs, food supplements and animal source foods (ASFs) (WHO, 2009; FAO, 2017). This recommendation is plausible because fortified CFs, food supplements and ASFs are rich sources of vitamin A, iron and zinc (Sazawal *et al.*, 2010; Krebs *et al.*, 2011, 2012; WHO, 2016). However, rural caregivers from developing countries including Uganda lack both physical and economic access to fortified foods, food supplements and ASFs (Faber, 2001; Wamani *et al.*, 2005). One potential and sustainable recommended strategy to combat micronutrient deficiencies such as VAD, ID and ZnD is to feed children CFs formulated from intrinsically vitamin A, iron and zinc rich foods that are locally available, acceptable, and affordable to caregivers (FAO, 2017).

In Uganda, common bean and pumpkin are widely cultivated and affordable to the rural poor (Kiwuka *et al.*, 2012; Nakazibwe *et al.*, 2019). Pumpkin is a rich source of provitamin A carotenoids (PVACs), a proformed form of vitamin A (Azevedo-Meleiro and Rodriguez-

Amaya, 2007; Azizah *et al.*, 2009; Koh and Loh, 2018). When pumpkin is consumed by humans, the PVACs are bioconverted into retinol, measured as retinol activity equivalents (RAE), a form of vitamin A used by the human body (Van Loo-Bouwman, Naber and Schaafsma, 2014). Furthermore, common bean is rich in iron and zinc (Carvalho *et al.*, 2012; Ferreira *et al.*, 2014; Haas *et al.*, 2016; Moloto *et al.*, 2018). Besides, cooked pumpkin has over 100% PVAC retention (Carvalho *et al.*, 2014, 2015), whilst common bean has over 90% retention for either iron or zinc (Carvalho *et al.*, 2012; Ferreira *et al.*, 2014). To this end, by using home cooking methods, this study prepared an innovative PVAC, iron and zinc rich CF, common bean pumpkin blend (BPB) from locally available common bean (*Obwelu*) and pumpkin (*Sweet cream*). Caregivers decide whether or not to offer CFs to their children based on sensory acceptability of the CF (Skinner *et al.*, 2002; Martin, Laswai and Kulwa, 2010). Therefore, this study aimed to assess caregiver perceptions and acceptability of the test CF, BPB compared to the control CF, PP in rural Uganda.

7.2 Materials and methods

7.2.1 Preparation of BPB and PP

The materials (common bean and pumpkin) used to prepare BPB (test food) and PP (control food) were described in chapter 6, sections 6.2.2.1 and 6.2.2.2. These ingredients were chosen because common bean is rich in iron and zinc (Carvalho *et al.*, 2012; Ferreira *et al.*, 2014; Haas *et al.*, 2016; Moloto *et al.*, 2018), and pumpkin is rich in PVACs (Azevedo-Meleiro and Rodriguez-Amaya, 2007; Azizah *et al.*, 2009; Koh and Loh, 2018). Pumpkin and common bean are cultivated in rural Uganda and available on the local markets (Kiwuka *et al.*, 2012; Nakazibwe *et al.*, 2019). Mashed cooked pumpkin is usually given to children as a single CF in Uganda (Uganda Bureau of Statistics and Inner City Fund, 2018). Therefore, PP prepared from *Sweet cream* was used as a control in this study.

The FAO (2017) recommends that caregivers should prepare homemade CFs based on consistency (thinness and thickness) of the food in relation to the child's age and ability to swallow. To this end, CFs were prepared by expert peer mothers in accordance with food consistency as recommended by the 2017 FAO guide to conducting participatory cooking demonstrations to improve complementary feeding practices (FAO, 2017). Iron and zinc concentrations in the study CFs were determined by flame atomic absorption spectroscopy (FAAS), as described by Santelli *et al.* (2006) and Ekesa, Nabuuma and Kennedy (2019). The PVAC content was analysed by high performance liquid chromatography (HPLC) as described by Rodriguez-Amaya and Kimura, (2004) in the HarvestPlus handbook for carotenoid analysis and the Institute of Medicine (2001) bioconversion of PVACs to retinol as described in chapter 6, section 6.2.2.4. Figure 6.1 in chapter 6, section 6.2.2.1 shows study CFs (BPB and PP) and the ingredients used to prepare them. Table 6.2 in chapter 6, section 6.2.2.4 shows the PVAC, iron and zinc content of BPB and PP.

7.2.2 Study area and study participants

This study was conducted in rural Kyankwanzi district, central Uganda, an area with a high prevalence of illiteracy and young child caregivers (Uganda Bureau of Statistics, 2017). Children from this socio-economically disadvantaged area are fed CFs low in vitamin A, iron and zinc (Amaral, Herrin and Gulere, 2018; Ekesa, Nabuuma and Kennedy, 2019). Caregivers, are the gatekeepers for CFs (FAO/WHO, 2013; FAO, 2017; Bekele and Turyashemererwa, 2019). Therefore, all the 70 caregivers of children who participated in the child acceptability study of the innovative complementary food, BPB (Chapter 6, Section 6.2.5) were eligible to participate in the caregiver acceptability study.

7.2.3 Selection of caregiver participants and measurement of caregiver acceptability

Caregiver acceptability was measured by sensory evaluation and focus group discussions (FGDs) as done in previous studies (Martin, Laswai and Kulwa, 2010; Govender *et al*., 2014; Amod, *et al.*, 2016; Pillay, Khanyile and Siwela, 2018). All the 70 caregivers whose children participated in the child acceptablity study (chapter 6, section 6.2.5), were selected to participate in the sensory evaluation and FGDs.

7.2.4 Pilot study for sensory evaluation and focus group discussions

The aim of this pilot study was to test the sensory evaluation questionnaire, procedures for conducting the sensory evaluation and the focus group discussions (FGDs). A pilot study of the sensory evaluation and the FGDs was conducted two days prior to the main study. Ten caregivers with children aged six to 24 months old attending the young child clinic (YCC) for child immunisation and growth monitoring at Ntwetwe Health Centre IV, Kyankwanzi district, participated in a sensory evaluation pilot study. A short while later, all the caregivers who had participated in the sensory evaluation, participated in a pilot FGD. The pilot study was conducted on a different day to the main study in order to prevent the pilot study participants from participating in the main study. As the pilot study venue was too far away from the YCC, a closer alternative venue was used for the main study. No changes were made to the sensory evaluation and FGD questionnaire after the pilot study.

7.2.5 Procedure for sensory evaluation

A sample size of more than 50 caregivers is adequate for a valid sensory evaluation acceptability study (Stone and Sidel, 2004). To this end, 70 caregivers, who cared for children aged six to 24 months and participated in the child acceptability study (chapter 6, section 6.2.5), were recruited to participate in the sensory evaluation study. Sensory evaluation was conducted in a designated private room, close to the YCC. The caregivers were seated a distance away from each other and were asked not to communicate with each other during the sensory evaluation session. Samples were labelled with random numbers so that panellists would not form judgments based on labels, but rather on their sensory experiences (Lawless and Heymann, 2010). To this end, the samples were randomly labelled, using a unique three-digit code obtained from a table of random numbers, and were served in a random order (Lawless and Heymann, 2010).

The CF samples were warmed in a microwave oven for 10 seconds, at a medium heat, before serving. Each caregiver received 100g samples of BPB and PP in separate polystyrene cups. The caregivers were provided with a spoon and a cup of water to rinse their palates between samples. A facial hedonic scale has been found to be appropriate for use by semi-illiterate and illiterate panellists (Stone and Sidel, 2004). Therefore, caregivers rated the taste, texture, aroma, colour and overall acceptability of both BPB and PP (control) samples using a five-point facial hedonic scale (1=very bad, 2=bad, 3=neutral, 4=good and 5= very good), based on the sensory evaluation questionnaire developed in the local language (*Luganda*). Research assistants trained in conducting FGDs explained the questionnaire to the caregiver panellists and assisted them during the evaluation when necessary.

7.2.6 Procedure for focus group discussions

The FGDs were conducted to assess caregivers' acceptability or willingness to use the BPB as a CF. The FGDs were conducted about 30 minutes after the sensory evaluation study was completed. All the 70 caregivers who participated in the sensory evaluation agreed to participate in the FGDs. The acceptable sample size for each FGD is between seven and 12 participants (Harris *et al.*, 2009). To this end, participants were divided into focus groups, each containing 10 participants. The study sample had seven and 63 male and female caregivers, respectively. Therefore, at least one male caregiver was allocated to each of the seven FGDs to ensure male representation in each of the focus groups.

Through established community relationships, four facilitators, experienced in conducting FGDs in the local language (*Luganda*) were recruited for a one-day training session in focus group discussion moderation, in relation to the objectives of this study, following guidelines explained by Amico *et al.* (2011). A trained facilitator directed the discussions, using a structured FGD guide. The FGD guide consisted of a brief explanation of the samples that were tasted during the sensory evaluation as well as a set of questions for initiating and facilitating the discussion. Guidelines for conducting FGDs with a structured set of open-ended questions were followed, as recommended by Krueger and Casey (2015). The question guide included questions that captured factors from themes that influence the general acceptability or willingness to use CFs by caregivers. These themes included sensory attributes (taste, aroma, texture, and colour), physical access, affordability, cultural acceptability and feasibility of preparing a CF (Govender *et al.*, 2014; FAO, 2017; Pillay, Khanyile and Siwela, 2018). Table 7.1 shows the questions that were included in the FGD guide.

Table 7.1: Focus group discussion questions

Focus group discussion questions
1. How would you grade the taste, colour, odour, and texture of the BPB compared to the PP?
2. To what extent is common bean and pumpkin accessible and affordable in your community?
3. How feasible would the preparation of common bean and pumpkin be during the development of BPB?
4. What cultural factors in your community or households may prevent you from using the BPB as a complementary food?

BPB= Common bean pumpkin blend
PB= Pumpkin puree

The FGDs were facilitated in the local language, *Luganda* by trained FGD facilitators. A digital voice recorder was used to record the FGDs after participants consented to the use of the voice recorder. The recordings were later translated from *Luganda* into English by the three FGD facilitators. The translated recordings were cross-checked by a *Luganda* professional teacher against the English translation for accuracy.

7.2.7 Data analysis

A sensory attribute was considered acceptable if it was rated as good to very good by caregiver panellists. The proportion of caregivers was calculated according to their sensory attribute ratings for the BPB and PP. A chi-square test was conducted to test for significant differences in the sensory attributes (taste, colour, odour, texture and general acceptability) between BPB and PP at a *p* value of 0.05. Statistical and data analysis was done using Statistics and Data (STATA), version 13.1.

Data generated from the FGDs were analysed using deductive thematic analysis. Thematic analysis is a method for identifying and analysing patterns (themes) of meaning in a dataset (Braun and Clarke, 2006). This study used a deductive thematic analysis because themes were predetermined before FGDs were conducted (Fereday and Muir-Cochrane, 2006). After conducting FGDs, themes were first summarised for each focus group, and then compared across all the seven FGDs to explore the most prominent themes, triangulate caregiver perspectives, and subsequently explore any potential context-specific variations associated with the predetermined themes of sensory attributes (taste, aroma, texture, and colour), physical access, affordability, cultural acceptability and feasibility of preparing a CF.

7.3 Results

7.3.1 Demographic characteristics of caregivers

A total of 70 eligible caregivers completed both the sensory evaluation study and FGDs. They included 63 (90%) and 7 (10%) females and males, respectively. The mean age of caregivers was 23.6 years. Only 24% of the caregivers had at least completed a primary level education. The mean age of the children being cared for by the caregivers who participated in the study was 12.3 months.

7.3.2 Sensory evaluation findings

Over 64% of the 70 caregivers rated both study CFs as good to very good. Out of the 70 caregivers, 61(87%), 48(67%), 52(74%), 62 (89%) and 53(6%) scored taste, texture, aroma, colour and overall acceptability for BPB as good to very good, respectively. Furthermore, out of the 70 caregivers, 67(96%), 45(64%), 57(81%), 66(94%) and 58(83%) scored taste, texture, aroma, colour and overall acceptability for PP as good to very good, respectively. Table 7.2 shows the sensory acceptability ratings for the BPB and PP, according to the different sensory attributes and the number and percentage of caregivers who gave the different ratings for each sensory attribute of BPB and PP.

Table 7.2: The number and percentage of caregivers who gave the different ratings for the sensory attributes evaluated for BPB and PP (n=70)

CFS	Attributes	Very bad n (%)	Bad n (%)	Neutral n (%)	Good n (%)	Very good n (%)
BPB	Taste	1(1.4)	2(2.7)	6(8.6)	38(54.3)	23(32.9)
	Texture	2 (2.9)	4(5.7)	16(22.9)	16(22.9)	32(45.7)
	Aroma	3(4.3)	3(4.3)	12(17.1)	28(40.0)	24(34.3)
	Colour	1(1.4)	2(1.4)	6(8.8)	35(50.0)	27(38.6)
	Overall acceptability	1(1.4)	4(5.7)	12(17.1)	29(41.4)	24(34.3)
PP	Taste	1(1.4)	0(0)	2(2.9)	47(67.1)	20(28.6)
	Texture	2(2.9)	2(2.9)	13(18.6)	25(35.7)	20(28.6)
	Aroma	2(2.9)	2(2.9)	9(12.9)	38(54.3)	19(27.1)
	Colour	1(1.4)	1(1.4)	2(2.9)	36(51.4)	30(42.9)
	Overall acceptability	0(0)	2(2.9)	10(14.3)	38(54.3)	20(28.6)

CFS= Complementary food sample; BPB = Common bean pumpkin blend; PP =Pumpkin puree

7.3.3 Association of sensory acceptability between BPB and PP

A binary outcome of sensory acceptability (yes or no) was created for each sensory attribute of BPB and PP. The CF was regarded as unacceptable if caregivers scored the sensory attribute as very bad to neutral. In contrast, the CF was regarded as acceptable if caregivers scored the sensory attributes as good to very good. Findings showed that 64% to 96% of the caregivers rated the sensory attributes of both BPB and PP as acceptable (good to very good). Table 7.3 shows the association between the study CFs and sensory acceptability.

Table 7.3: Association of sensory acceptability between BPB and PP

Sensory attribute	Acceptable (n= 70 across rows)		X^2	*p* value
	Yes, n (%)	No, n (%)		
Taste			3.28	0.07
BPB	61(87)	9(13)		
PP	67(96)	3(4)		
Texture			0.29	0.59
BPB	48(69)	22(31)		
PP	45(64)	25(34)		
Aroma			1.11	0.31
BPB	52(74)	18(26)		
PP	57(81)	13(19)		
Colour			1.46	0.23
BPB	62(89)	8(11)		
PP	66(94)	4(6)		
Overall			1.09	0.30
acceptability	53(76)	17(24)		
BPB	58(83)	12(28)		
PP				

BPB: Common bean pumpkin blend; PP: Pumpkin puree
X^2: Chi-square test

Out of the 70 caregivers, 61(87%), 48(69%), 52(74%), 62(89%), and 53 (76%) scored taste, texture, aroma, colour and overall acceptability of BPB as acceptable (good to very good), respectively. Furthermore, out of the 70 caregivers, 67(96%), 45(64%), 57(81%), 66(94%), and 58(83%) scored taste, texture, aroma, colour and overall acceptability of PP as acceptable (good to very good), respectively. A chi-square test revealed that there was no significant difference (p>0.05) in caregiver acceptability for all attributes between BPB and PP.

7.3.4 Focus group discussions findings

7.3.4.1 Taste

All participants in the FGDs indicated that they had tasted PP before, but not BPB. They further noted that the taste of PP and BPB were similar.

> '*I thought this was pumpkin alone (BPB). If you had not said that this is a mixture of pumpkin and common bean, I wouldn't have realised that it was a mixture of the two food ingredients.*' (Female caregiver)

Caregivers wondered why BPB and PP would taste the same, yet they had different ingredients. Therefore, most of the caregivers were interested to know the specific varieties used in the preparation of the BPB.

> '*… how come that PP and BPB taste almost the same? We have several varieties of common bean such as Obwayelo, Nambale, Obote, Kanyebwa and several varieties of pumpkin such as Bala, Sweet cream, Dulu, Ozinga, Wujju among others. Could you please let us know the specific amounts of ingredients of pumpkin and common bean varieties used to prepare BPB?*' (Female caregiver)

7.3.4.2 Texture and colour

The softness and colour of the BPB appealed to caregivers. They indicated that children would also accept it. Caregivers were interested to know the ratio of pumpkin and common bean used to prepare the BPB.

> '*The colour of BPB is not any different to the usual mashed pumpkin we give our children. This yellow colour is good for children since it is bright, infants and young children like colourful things.*' (Female caregiver)

> '*In addition to the bright yellow colour, BPB was soft. Please what ratios of pumpkin and common bean did you use to prepare BPB?*' (Female caregiver).

> '*As a matter of fact, we could not differentiate the softness between BPB and PP. It is important we know the ratios you used to mix pumpkin and common bean. This will help us to use these ratios when we are preparing BPB while at home.*' (Female caregiver)

7.3.4.3 Aroma

Caregivers indicated that the aroma of BPB was not any different from the PP.

> '*Recognising the difference in smell between BPB and PP was difficult. These two CFs smell the same.*' (Male caregiver)
>
> '*For me I thought both foods were the same because they were almost similar in smell and colour.*' (Female caregiver)

7.3.4.4 Cultural acceptability

Caregivers agreed that the ingredients for BPB, i.e. pumpkin (*Sweet cream*) and common bean (*Obwelu*) are culturally acceptable for human consumption in their community.

> '*…on many occasions, our in-laws and husbands dictate on the new foods we have feed to our children. I remember, two years back, they introduced to us a nutritious maize porridge fortified with termites. However, my in-law and husband refused me to feed my twins with this porridge, because eating termites is not acceptable in their clan. BPB seems new to us. However, the common bean and pumpkin used to make BPB are culturally acceptable for consumption in our community. I am pretty sure that we shall not get any resistance from our in-laws and husbands to use them in the preparation of BPB.*' (Female caregiver)

> '*Pumpkin and common bean are widely acceptable for consumption in Uganda and our community. As a matter of fact, common bean is frequently consumed in our households. If you need to confirm, just ask them, whether there is anyone who did not prepare common beans at their home in last two days….*' (Male caregiver)

7.3.4.5 Access and affordability of pumpkin and common bean

Caregivers revealed that pumpkin and common bean are easily accessible from their gardens or local market and affordable to them. However, they wanted to know the variety of pumpkin and common bean that was used to prepare the BPB so that they could cultivate or buy them for use in the preparation of BPB.

> '*Accessing common bean is never a problem to us. Almost every household cultivates common bean every season, and the surplus is sold off to traders.*' (Male caregiver)

> '*For those who do not cultivate, common bean is affordable because on average, 1 kilogram of common bean costs 1000 Uganda shillings.*' (Male caregiver). [1000 Uganda shillings is equivalent to 0.2 United States Dollars (USD)].

> '*Not very many households cultivate pumpkin as it is done with beans. However, the price of pumpkin is affordable at the local market. For example, on average a small to medium sized pumpkin costs 500 Uganda shillings.*' (Male caregiver) (500 Uganda shillings is equivalent to 0.1 USD).

‘*What specific variety of common bean and pumpkin did you use to prepare BPB? Then we would cultivate them, because our main source of common bean is from our household gardens.*’ (Female caregiver)

7.3.4.6 Feasibility to prepare common bean and pumpkin

Several caregivers emphasised that they would not frequently prepare common bean because it takes long to cook, hence consuming a lot of fuel. However, other caregivers advised their peers on how to reduce the cooking time of common bean. On the other hand, caregivers noted that pumpkin is easy to prepare because it cooks fast.

‘*…cooking common beans takes quite a longer time, minimum of three hours. Ideally it consumes a lot of firewood or charcoal.*’ (Female caregiver)

‘*Now days firewood is very scarce, whilst charcoal is too expensive. As a matter of fact, to cook common bean, one must fill the charcoal stove three times before common beans are ready. This is unacceptably expensive to us.*’ (Female caregiver)

‘*Pumpkin is among the easiest foods to cook, once it starts boiling, it will be ready in a few minutes. However, common bean can take a couple of hours…*’ (Female caregiver)

‘*…reducing cooking time for common bean is possible by soaking them over night. Soaking makes common bean soft, and quick to get ready after boiling.*’ (Female caregiver)

‘*In addition, there is this type of salt called Ekisula, which also softens common bean when cooking, hence reducing cooking time.*’ (Female caregiver)

7.4 Discussion

Ugandan children are predominantly fed CFs formulated from staple cereals and tubers, devoid of vitamin A, iron and zinc (Amaral, Herrin and Gulere, 2018; Ekesa, Nabuuma and Kennedy, 2019). This increases child vulnerability to VAD, ID and ZnD. Therefore, the preparation of a BPB rich in PVACs, iron and zinc was necessary. Testing for caregiver acceptability of BPB was plausible because CFs disliked by caregivers are unlikely to be fed to the children in their care (Skinner *et al.*, 2002). The results of this study indicate that the sensory attributes of BPB and PP were equally acceptable (rated as good to very good) to child caregivers who evaluated them. These findings are consistent with other studies conducted in Uganda and South Africa, which revealed that micronutrient rich CFs prepared by home cooking methods were acceptable to child caregivers (Ssebuliba, Muyonga and Ekere, 2006; Govender *et al.*, 2014; Amod *et al.*, 2016; Pillay, Khanyile and Siwela, 2018). However, these studies formulated CFs similar to PP because they were predominantly rich in PVACs, as they used provitamin A biofortified foods such as OFSP (Ssebuliba, Muyonga and Ekere, 2006; Pillay, Khanyile and Siwela, 2018), and provitamin A biofortified maize (Govender *et al.*, 2014; Amod *et al.*, 2016). It is worth noting that the current study formulated a PVAC, iron and zinc rich CF from locally available common bean (*Obwelu*) and pumpkin (*Sweet cream*) (chapter 6, section 6.2.2.1, Figure 6.1).

Based on Table 6.2 (Chapter 6, Section 6.2.2.4), adding common bean to PP increased iron and zinc content from 0.57 mg and 0.23 mg in PP to 1.99 mg and 1.8 mg in BPB, respectively. In contrast, vitamin A reduced from 280.3 µgRAE in PP to 187 µgRAE in BPB. The 2013 WHO/FAO guidelines on formulated CFs for older infants and young children recommends that a CF should contribute at least 50% of the RDA for all nutrients (FAO/WHO, 2013). The RDA/AI for iron, zinc and vitamin A for children 12 to 24 months old is 7 mg, 3 mg, and 300 µgRAE, respectively (Institute of Medicine, 2001). Therefore the 50% of the RDA/AI recommendation of a CF for a child 12 to 24 months would be 150 µgRAE, 3.5 mg and 1.5 mg for vitamin A, iron and zinc, respectively. Hence, PP would contribute 16%, 15% and 186% of iron, zinc and vitamin A, respectively of the 50% requirement from a CF for a child, 12 to 24 months old. In contrast, BPB would contribute 57%, 73% and 125% of iron, zinc and vitamin A of the 50% requirement of a CF for a child, 12 to 24 months old. To this end, BPB significantly contributed a higher proportion of iron and zinc to meet the RDA/AI for children. It is worth noting that both BPB and PP contributed over 100% of the 50% RDA/AI for vitamin A for a CF for a child 12 to 24 months old. Therefore, BPB would be preferred to PP because it is a multiple micronutrient rich CF compared toPP. One may be concerned about vitamin A toxicity or hypervitaminosis which may occur after consumption of either BPB or PP. However, vitamin A toxicity is not associated with PVAC intake because the conversion of PVACs to vitamin A (retinol) is regulated according to the body's requirements for vitamin A

(Allen and Haskell, 2002). Therefore, the PVACs are only converted to vitamin A when needed by the body (Allen and Haskell, 2002).

Furthermore, during FGDs, caregivers had positive attitudes towards the taste, colour, texture and aroma of the BPB. They revealed that the taste, colour and aroma of BPB was the same as that of the regular PP they feed their children. This suggests that caregivers accepted the colour, aroma and taste of BPB because they were similar to that of the PP (control), hence the willingness to feed their children the BPB. Furthermore, caregivers noticed that the BPB was soft enough to prevent choking. This suggests that caregivers perceived the consistency/texture of the BPB to be suitable for their children in the age range of complementary feeding, and therefore was in accordance with the recommendations on consistency of homemade CFs (FAO, 2017).

Affordability and physical access to innovative CFs are key factors that may influence its potential to be used in complementary feeding (Govender *et al.*, 2014; FAO, 2017; Pillay, Khanyile and Siwela, 2018). During FGDs, caregivers noted that they had adequate access to common bean and pumpkin from their own gardens. Moreover, those who could not access them from their gardens noted that the common bean and pumpkin from the local market was affordable to them. These findings confirm that pumpkin and common bean, the ingredients used to prepare the PVAC, iron and zinc rich BPB are locally available and affordable in Uganda (Kiwuka *et al.*, 2012; Nakazibwe *et al.*, 2019; Selina Wamucii, 2019).

Throughout the FGDs, caregivers were interested to know the specific varieties of pumpkin and common bean that were used in the preparation of the BPB. Furthermore, they wanted to know the ratio of common bean and pumpkin used to prepare the BPB. These findings suggest that caregivers were interested to know more in order to prepare the BPB in the future. Such caregiver interest indicates that there is a need for caregiver sensitisation to promote the BPB by providing adequate information, education and communication on its preparation. Moreover, conducting such sensitisations are highly recommended to improve complementary feeding practices (FAO, 2017). Furthermore, participation of caregivers in CF formulation and acceptability testing encourages them to gain and share nutrition knowledge and have positive perceptions towards good feeding practices (Pelto, Levitt and Thairu, 2003).

A long cooking time for common bean and the associated high cost of fuel were the main challenges that would prevent caregivers from preparing the BPB for children. However, peer caregivers indicated that soaking the common beans before cooking softens it, thus reducing the cooking time and use of fuel. The information shared by caregivers is consistent with a review of studies that demonstrated that common bean soaked before cooking, cooked faster compared to the non-soaked common bean (Reyes-Moreno and Paredes-López, 1993). It is worth noting that in the current study, the common bean was soaked before cooking during the preparation of the BPB. Sharing such nutrition-related knowledge among expert and novice peer caregivers is necessary and recommended in promoting and supporting positive infant and young child feeding practices in the community (FAO, 2017).

Compared to other CF acceptability studies that recruited only female caregivers (Ssebuliba, Muyonga and Ekere, 2006; Paul *et al.*, 2008; Govender *et al.*, 2014), this study recruited both male and female caregivers. It is worth noting that male involvement and participation in such complementary feeding studies is very important, particularly in Africa, where men/fathers are decision makers regarding how money is spent on food (Mukuria *et al.*, 2016). Moreover, male involvement is necessary in the promotion and support of adequate complementary feeding practices (Moyo and Schaay, 2019).

Furthermore, caregivers showed interest in cultivating the pumpkin and common bean varieties used in the preparation of BPB. However, yield and productivity of cultivated common bean and pumpkin in sub-Saharan Africa (SSA) is influenced by agronomic factors such as soil fertility (Jansa *et al.,* 2011; Musa and Ogbadoyi, 2012). To this end, the Food and Nutrition Technical Assistance (FANTA) encourages the district nutrition coordination committee, which is comprised of several food and nutrition security experts including agricultural extension workers, to support caregivers on how to improve soil fertility during cultivation of food crops, including common bean and pumpkin (FANTA, 2017).

7.4.1 Study strengths and limitations

This study analysed for the micronutrients of public health importance in the formulated CFs (Millward, 2017). However, it did not analyse for the percentage moisture and calorie (energy) contents of the formulated CFs. It is worth noting that the calorie (energy) content is needed when calculating the nutrient density (ND) of a given food, the ratio of nutrients to calories (energy). Since this study did not analyse the calorie (energy) content of the formulated CFs, it is difficult to calculate the ND of BPB and PP and compare them to other CF blends. Furthermore, PVACs are fat soluble, so incorporating fat during the preparation of PVAC rich foods can increase PVAC bioavailability (Saini, Nile and Park, 2015). However, fat was not used during the preparation of BPB or PP. Although, the widely acceptable 2001 Institute of Medicine bioconversion recommendations of PVACs to retinol used in this study are independent of the use of fat as an ingredient in the preparation of PVAC rich foods (Institute of Medicine, 2001), it is important that caregivers are advised to add some fat such as cooking oil or margarine when preparing or eating PVAC rich foods, to increase vitamin A bioavailability (Saini, Nile and Park, 2015).

7.5 Conclusions

The PVAC, iron and zinc rich complementary food, BPB, prepared from locally available pumpkin and common bean was acceptable to the child caregivers who tasted it. Caregivers were interested to know how to prepare and use it as a CF. These findings suggest that the BPB has the potential to be used by caregivers in complementary feeding to improve vitamin A, iron and zinc intake among children in the age range of complementary feeding, a group that is vulnerable to VAD, ID and ZnD.

The next chapter discussess key findings from chapters 4, 5, 6 and 7 and their implications for current and future approaches to alleviate micronutrient deficiencies especially VAD, ID and ZnD.

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CHAPTER EIGHT

GENERAL DISCUSSION

Vitamin A deficiency (VAD), iron deficiency (IDA) and zinc deficiency (ZnD) are the three most prevalent micronutrient deficiencies (MND) affecting children in developing countries, including Uganda (Wessells and Brown, 2012; Ruel-Bergeron *et al.*, 2015; Shergill-Bonner, 2017). The highest prevalence of VAD, ID and ZnD is seen in children 6 to 24 months old, the age range of complementary feeding. Children in the age range of complementary feeding experience the highest prevalence of VAD, ID and ZnD because of the increased nutritional demands for vitamin A, iron and zinc during complementary feeding (Biesalski and Tinz, 2018). Children in Uganda and other developing countries are fed complementary foods (CFs) deficient in vitamin A, iron and zinc (Gibson *et al.*, 2010; Amaral, Herrin and Gulere, 2018; Ekesa, Nabuuma and Kennedy, 2019).

To combat MND, including VAD, IDA and ZnD during the period of complementary feeding, the World Health Organization (WHO) recommends that caregivers should use commercially fortified foods, food supplements and animal source foods (ASFs) as part of CFs (WHO, 2003). However, Ugandan rural caregivers either cannot afford commercially fortified foods, food supplements and ASFs or fortified and food supplements are inaccessible in Ugandan rural markets (Wamani *et al.*, 2005). To this end, it was necessary to identify locally available foods for use in the preparation of a vitamin A, iron and zinc rich complementary food (CF). This study identified locally available pumpkin and common bean in rural Uganda for the preparation of a provitamin A carotenoid (PVAC), iron and zinc rich CF, common bean pumpkin blend (BPB). Common bean and pumpkin were selected for use in the preparation of BPB because the former is a rich source of iron and zinc (Carvalho *et al.*, 2012), while the latter is a rich source of provitamin A carotenoids (PVACs) (Bergantin *et al.*, 2018). Moreover, when PVAC rich foods are consumed by humans, the human body bioconverts the PVACs to retinol, the active form of vitamin A (Van-Loo-Bouwman, Naber and Schaafsma, 2014). The current study assessed the acceptability of the innovative BPB among children, six to 24 months old and their caregivers because the former is the target group of CFs (WHO, 2003), whilst the latter decides whether or not to prepare and give innovative CFs to their children (Skinner, 2002). In Uganda, there are several landraces of common bean (Kiwuka *et al.*, 2012) and pumpkin (Nakazibwe *et al.*, 2019). However, their nutritional composition is unknown since Uganda lacks food composition data bases (Baingana, 2004). To this end, the first objective of this study was to select one common bean landrace superior in iron and zinc, and one pumpkin landrace superior in PVACs from a variety of local landraces available on the local market.

Findings from objective one revealed that common bean, *Obwelu* was superior in iron and zinc compared to other common bean landraces *Obwayelo*, *Masavu*, *Nambale* and *Kanyebwa* found on the local market. Furthermore, pumpkin, *Sweet cream* was superior in PVACs, compared to other pumpkin landraces, *Dulu,* and *Sun fish* found on the local market. Therefore, pumpkin *Sweet cream* and common bean, *Obwelu* were selected for use in the preparation a PVAC, iron and zinc rich CF, BPB. This current study is similar to the previous studies that screened common bean varieties to identify those superior in iron or zinc (Beebe, Gonzalez and Rengifo, 2000; Glahn, Wiesinger and Lung'aho, 2020), and pumpkin to identify those superior in PVACs (Azevedo-Meleiro and Rodriguez-Amaya, 2007; Norshazila, 2014). It is worth noting that Uganda lacks food composition data bases for its locally available foods (Baingana, 2004).

Therefore, findings from objective one can provide the micronutrient compostion of common bean and pumpkin landraces in Uganda, which can be used in the future development of Uganda's food composition data bases. One limitation in this current study is that the three known PVACs are β-carotene, α-carotene and β-cryptoxanthin (Rodriguez-Amaya and Kimura, 2004). However, due to financial constraints, this study only analysed β-carotene and α-carotene in pumpkin, which may have led to the underestimation of PVACs in study pumpkin landraces and study CFs. However, other studies that analysed PVACs of pumpkin either did not detect β-cryptoxanthin or found trace amounts of β-cryptoxanthin (Azevedo-Meleiro and Rodriguez-Amaya, 2007; Tee and Lim, 1991), suggesting that the content of β-cryptoxanthin in pumpkin may be negligable.

At household level, common bean and pumpkin must be cooked to make them soft and palatable for human consumption. However, Ugandan caregivers use different methods to cook common bean and pumpkin. Therefore, the second objective evaluated the effect of home cooking methods on PVAC retention in the selected pumpkin (superior in PVACs), and iron and zinc retention in the selected common bean (superior in iron and zinc). Findings from objective two showed that boiling *Obwelu* with prior soaking retained a higher proportion of iron and zinc compared to boiling, without prior soaking. These findings are consistent with previous studies that showed that cooking common bean by boiling with prior soaking retains a higher proportion of iron and zinc (Carvalho *et al*., 2012; Ferreira *et al*., 2014). One strength in this study is that boiling with prior soaking of common bean degrades phytic acid, an anti-nutrient in common bean that inhibits the absorption of iron and zinc in the human body (Fernandes, Nishida and Da Costa Proença, 2010). To this end, the current study concluded that in order to retain a higher proportion of iron and zinc, caregivers should first soak common bean *Obwelu* overnight for eight hours, followed by boiling. Furthermore, boiling or steaming pumpkin, *Sweet cream* retained a higher proportion of PVACs. These findings are consistent with other studies which showed that boiling or steaming PVAC rich foods such as orange-fleshed sweet potato and PVAC fortified cassava retained over 100% of PVACs (Chavez *et al.*, 2007; Pillay *et al.*, 2011; De-Moura, Alexander Millff and Boy, 2013; Bechoff *et al.*, 2017). The higher PVAC retention observed in either boiling or steaming pumpkin could be explained by the PVAC isomerism characterised by extraction and release of *cis*-β-carotene isomers during the heating of PVAC rich foods (Azizah *et al.*, 2009; Bechoff *et al.*, 2017). Furthermore, maceration (softening) due to cooking increases the PVAC concentration by rupturing the microstructure of PVAC rich plant tissue and then releasing more PVACs from the complex food matrix (Bengtsson *et al.*, 2008; Tumuhimbise, Namutebi and Muyonga, 2009). This suggests that it may be beneficial to consume PVAC rich foods in a cooked form, rather than in a raw form.

Objective three of the current study was to assess child acceptability of a CF prepared with common bean (superior in iron and zinc) and pumpkin (superior in PVACs) blend (BPB). Based on objective one, common bean, *Obwelu* was superior in iron and zinc compared to other common bean landraces. In addition, pumpkin, *Sweet cream* was superior in PVACs compared to other pumpkin landraces on the local market. Therefore, caregivers used common bean, *Obwelu* and pumpkin, *Sweet cream* to prepare BPB. Caregivers cooked *Obwelu* and *Sweet cream* by boiling with prior overnight soaking (about eight hours) and boiling, respectively. The selected common bean and pumpkin were cooked by caregivers before making a suitable CF. The suitability of the CF was based on consistency (thinness or thickness) of the CF in relation to the child's age and ability to swallow as recommended by the Food and Agriculture Organization of the United Nations (FAO) (FAO, 2017). Preparation of BPB (test CF) and pumpkin puree (PP) (control CF) based on consistency was practically plausible because homemade CFs prepared from locally available foods in Uganda are prepared based on consistency at the household level (Uganda Bureau of Statistics and Inner City Fund, 2018). It was also necessary to use PP as a control because PP is usually given as a single CF in Uganda (Uganda Bureau of Statistics and Inner City Fund, 2018).

The primary outcome variable for assessing child acceptability was the amount of CF consumed by the child. The secondary outcome was the duration taken to consume BPB and PP. An additional secondary outcome was the amount of vitamin A, iron and zinc consumed by child participants in each of the BPB and PP groups. The CF was acceptable if the child consumed 50g or more of the offered study CFs (100g). The study considered 50g as the reference amount of food for child acceptability because 50g is the minimum recommended amount of CF intake per serving for children six to 24 months on complementary feeding (FAO/WHO, 2013). Findings showed that the amount of BPB and PP consumed by child participants was above the minimum recommended amount of CF intake per serving for children six to 24 months old, and not significantly different from CF group. Moreover, the duration taken by child participants to consume BPB and PP was not significantly different from each other. These findings agree with Ahmed *et al.* (2014), Muhimbula, Issa-Zacharia and Kinabo (2011), Konyole *et al.* (2012) and Abebe *et al.* (2006), who demonstrated that micronutrient dense CFs prepared from locally available foods are acceptable to children. It was necessary to use children to assess the acceptability of an innovative PVAC, iron and zinc rich CF, BPB because children aged six to 24 months are the target consumers for CFs (WHO, 2003). In addition, children in the study area are fed CFs deficient in vitamin A, iron and zinc (Amaral, Herrin and Gulere, 2018; Ekesa, Nabuuma and Kennedy, 2019).

The PP was higher in PVACs compared to BPB. This may explain why the intake of vitamin A (retinol activity equivalent) by child participants in the PP group was significantly higher than in the BPB group. Child iron intake was significantly higher from BPB compared to PP, and zinc intake was significantly higher from BPB, compared to PP. This is likely because BPB was superior in iron and zinc compared to PP. The prevalence of child stunting reported in the current study was unacceptably higher than Uganda's national prevalence of child stunting (Uganda Bureau of Statistics and Inner City Fund, 2018). It is worth noting that MND such as VAD, ID and ZnD are risk factors for child stunting (Golden, 1996; Millward, 2017). Moreover, multiple micronutrient interventions and not single micronutrient interventions alone, improve child stunting (Ramakrishnan *et al.*, 2004; Ramakrishnan, Nguyen and Martorell, 2009). Therefore, the preparation and use of a PVAC, iron and zinc rich CF, BPB among study children may improve their vitamin A, iron and zinc status, and consequently increase their linear growth, hence preventing stunting (Ramakrishnan *et al.*, 2004; Ramakrishnan, Nguyen and Martorell, 2009).

One strength of this child acceptability study is that it recruited child participants, six to 24 months old, who are the target population of CFs (WHO, 2003). However, the amount of CF consumed by infants (children below 12 months) and young children (children above 12 months in a specified duration may be lower and higher, respectively. Therefore, a limitation is that it may be difficult to generalise the child acceptability findings based on the wide age range of study participants (six to 24 months old). Ahmed *et al.* (2014) tested child acceptability of foods using a non-crossover randomised control trial, which does not allow study participants to test both the test and control foods, hence making it difficult to compare acceptability within participants. However, another strength of this child acceptability study is that it employed a randomised crossover control trial study design. The crossover design ensured that each child participant had a chance to consume both the test (BPB) and control (PP) CFs, hence allowing a comparison of acceptability within participants (Ding *et al.*, 2015).

The fourth objective assessed caregiver perceptions and acceptability of the CF, BPB. It was important to assess caregiver acceptability of BPB because caregivers are the gatekeepers of innovative CFs and decide whether or not to prepare and give the CFs to their children (Skinner *et al.*, 2002; Wansink, 2006). Study findings revealed that the highest proportion of caregivers rated both BPB and PP as acceptable (good to very good) for all the sensory attributes of taste, smell, texture, colour and general acceptability. Moreover, there was no significant difference in caregiver acceptability for all attributes between BPB and PP. These findings agree with studies that evaluated caregiver acceptability of PVAC rich CFs prepared from provitamin A biofortified foods in Uganda (Ssebuliba, Muyonga and Ekere, 2006) and South Africa (Pillay, Khanyile and Siwela, 2018).

During focus group discussions (FGDs), caregivers displayed positive perceptions about the taste, texture, aroma and colour of the BPB. Such positive perceptions may inform that caregivers are willing to use innovative CFs such as BPB during the period of complementary feeding (Wansink, 2006; Pillay, Khanyile and Siwela, 2018). Furthermore, caregivers were keen to know the specific varieties of common bean and pumpkin that were used to formulate the PVAC, iron and zinc rich BPB. This finding may suggest that caregivers were keen to use the locally available study pumpkin and common bean landraces to prepare BPB for feeding their children at home (FAO, 2017). In addition to quantitative methods (sensory evaluation), one strength of the caregiver acceptability study is that it also used qualitative methods (FGDs) during data collection. The FGDs explored caregivers' perceptions and intentions to use a PVAC, iron and zinc rich CF, BPB.

This study adopted a food-based approach (FBA) to prepare a PVAC, iron and zinc rich CF using locally available iron/zinc rich common bean and PVAC rich pumpkin. The FBA is a sustainable approach that encourages the use of nutritious foods that are locally available, affordable, accessible and acceptable to the local communities to combat malnutrition, including micronutrient deficiencies of public health importance such as VAD, ID and ZnD. The innovative PVAC, iron, zinc rich CF, BPB was acceptable to children and their caregivers. However, these findings should be interpreted with caution. First, one should not conclude that pumpkin and common bean are the only locally available foods rich in PVAC or vitamin A and iron/zinc, respectively in Uganda. Other alternatives do exist. For example, iron-zinc biofortified common bean (Centre for Agriculture and Bioscience International, 2020; Glahn, Wiesinger and Lung'aho, 2020) and provitamin A biofortified orange-fleshed sweet potato (OFSP) (Hotz *et al.*, 2012) were introduced in Uganda to combat the high prevalence of ID, ZnD and VAD, respectively. Biofortification is the breeding of staple food crops to increase their micronutrient density of their edible parts and bioavailability to prevent micronutrient deficiencies (MNDs) in developing countries (Bouis and Welch, 2010).

It is worth noting that compared to the other conventional strategies to combat MNDs, such as supplementation and commercial fortification, biofortification is sustainable similar to the growing of indigenous food crops. This is because biofortification needs a single investment of breeding, thereafter the food crops can be grown season after season for human consumption (Bouis and Welch, 2010). A food blend, "Mugoyo", prepared from non-biofortified common bean and non-biofortified white-fleshed sweet potato (WFSP) is commonly given as a CF in Uganda (Uganda Bureau of Statistics and Inner City Fund, 2018). However, "Omugoyo" is likely to be low in PVACs, iron, and zinc because it is prepared from non-biofortified common bean and WFSP. Therefore, future studies should try to prepare an innovative PVAC, iron and zinc rich CF using OFSP and iron-zinc biofortified common bean as an alternative to BPB, and test its acceptability among caregivers and their children.

Furthermore, this study informs that while adopting the FBA in combating MNDs among children, the concept of food systems should not be neglected. A food system gathers all the elements such as environment, people, inputs, processes, infrastructures, institutions and activities that relate to the production, processing, distribution, preparation and consumption of food (Meybeck and Gitz, 2017). This study utilised several elements and activities involved in the food system. For example, it used inputs such as locally available common bean and pumpkin to prepare BPB; people, including caregivers, were used to prepare and test acceptability of the BPB, and children to test acceptability of the BPB. The study also considered several activities including processing/preparation and consumption. For example, the effect of cooking common bean on iron and zinc retention, the effect of cooking pumpkin on PVAC retention, and consumption of the CF by the target group of complementary feeding. However, the study also appreciates an inter-sectoral collobaration from different departments such as agriculture and health among others, to promote and support the production, distribution and consumption of nutritious foods, including the PVAC, iron and zinc rich CFs in the wider community.

In general, the PVAC, iron and zinc rich CF, BPB prepared from locally available pumpkin and common bean was acceptable to both caregivers and their children, suggesting that there is a possibility of Ugandan caregivers using BPB as a CF. Future studies should assess the effect of feeding BPB on the vitamin A, iron and zinc status of target children. Furthermore, future studies should also consider identifying other food alternatives rich in PVACs or vitamin A, iron and zinc from the local community, and using them to prepare PVAC and iron rich CFs, and testing their acceptability among the target consumers.

The next chapter concludes with recommendations for policy and research.

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CHAPTER NINE

CONCLUSIONS, IMPLICATIONS OF FINDINGS, RECOMMENDATIONS AND LIMITATIONS

Micronutrient deficiencies of vitamin A, iron and zinc continue to be a problem of public health importance that affects children below five years old residing in developing countries. In Africa, including Uganda, the highest proportion of vitamin A deficiency (VAD), iron deficiency (ID) and zinc deficiency (ZnD) among children below five years old begins during the period of complementary feeding, when children are fed vitamin A, iron and zinc deficient complementary foods (CFs), predominantly prepared from staple cereals and tubers. To increase the micronutrient density of complementary foods (CFs), the World Health Organization (WHO) recommends that staple cereal and tuber-based CFs should be given along with ASFs, food supplements and fortified foods. However, caregivers from rural Uganda, either lack physical or economic access to animal source foods (ASFs), food supplements or fortified foods. Therefore, a more sustainable strategy is to identify locally available micronutrient rich foods and use them to prepare innovative CFs. To this end, this study aimed to evaluate caregiver and child acceptability of an innovative common bean pumpkin blend (BPB) prepared from a local common bean landrace rich in iron and zinc and a local pumpkin landrace, rich in provitamin A carotenoids (PVACs), in rural Uganda. Both caregiver and child acceptability were assessed because the former is the gatekeeper of CFs who decides which CF will be fed to a child, whilst the latter is the target population for CFs. This study (i) selected one common bean landrace superior in iron and zinc, and one pumpkin superior in PVAC for use in the preparation of a PVAC, iron and zinc rich CF, BPB; (ii) evaluated iron and zinc retention after cooking common bean, and PVAC retention after cooking pumpkin; (iii) assessed child acceptability of BPB; (iv) assessed caregiver acceptability of BPB.

9.1 Conclusions

9.1.1 Selecting one common bean landrace superior in iron and zinc and one pumpkin landrace superior in provitamin A carotenoids

This study hypothesised that iron and zinc content of common bean varieties found on the Ugandan market would differ. Five landraces of common beans were found on the local market and screened for iron and zinc content. It was established that the iron and zinc content of common bean landraces differed. Out of the five common bean landraces, *Obwelu* was found to be superior in iron and zinc content, compared to the other common bean landraces. It was also hypothesised that the PVAC content of pumpkin landraces on the Ugandan local market would differ. The study established that there was a difference in the PVAC content of pumpkin varieties. Out of the three pumpkin landraces, *Sweet cream* was superior in PVACs. Therefore, common bean, *Obwelu* and pumpkin, *Sweet cream* were selected for use in the preparation of a PVAC, iron and zinc rich CF, BPB.

9.1.2 Effect of cooking common bean, *Obwelu* on iron and zinc retention and pumpkin, *Sweet cream* on provitamin A carotenoid retention

This study hypothesised that home cooking methods would have an impact on PVAC retention in the pumpkin superior in PVACs. The study established that cooking had an impact on PVAC retention. Study findings revealed that either boiling or steaming pumpkin, *Sweet cream* led to higher PVAC retention. Furthermore, it was hypothesised that cooking methods would have an impact on iron and zinc retention in common bean superior in iron and zinc. Findings from this study established that iron and zinc retention were higher in boiled common bean with prior soaking, compared to boiled common bean without prior soaking. To maintain a higher retention of PVACs, caregivers should be encouraged to cook *Sweet cream* by either boiling or steaming. To maintain a higher retention of iron and zinc, caregivers should be encouraged to cook *Obwelu* by boiling (for about one and a half hours) with prior soaking (for about eight hours).

9.1.3 Child and caregiver acceptability of a provitamin A carotenoid, iron and zinc rich complementary food prepared from pumpkin (*Sweet cream*) and common bean (*Obwelu*)

The recommended average amount of CF intake by children six to 24 months old in the age range of complementary feeding is at least 50 g per serving. Caregivers prepared BPB (test CF) and pumpkin puree (PP) (control CF) based on consistency. This study also hypothesised that BPB would be more acceptable to children and their caregivers than PP. The mean amount of BPB and PP consumed by study children was higher than the recommended 50 g. These findings revealed that the BPB was acceptable to children in the age range of complementary feeding. Furthermore, a high proportion of caregivers rated both the BPB and PP as acceptable (good to very good) for all the sensory attributes. There was no significant difference in caregiver acceptability for all sensory attributes between BPB and PP. In general, there was no difference in caregiver and child acceptability of BPB and PP.

Moreover, caregivers had positive perceptions about the taste, texture, aroma, and colour of the BPB, and were willing to use the BPB as a CF. These findings suggest that a complementary food, BPB, rich in PVACs, iron and zinc prepared from locally available common bean, *Obwelu* and pumpkin, *Sweet cream*, was acceptable to caregivers and their children in the age range of complementary feeding, in rural Uganda.

9.2 Implications of findings and recommendations

9.2.1 Uganda lacks food composition databases for its locally available foods including pumpkin and common bean. This study established the iron and zinc content of locally available common bean landraces and PVAC content of locally available pumpkin in Uganda. Study findings revealed that pumpkin, *Sweet cream* was superior in PVACs, compared to other locally available pumpkin landraces, while common bean, *Obwelu*, was superior in iron and zinc, compared to other locally available common bean landraces. To this end, PVAC rich pumpkin, *Sweet cream* and iron and zinc rich common bean, *Obwelu*, should be selected for use in the preparation of a PVAC, iron and zinc rich BPB. Furthermore, the iron and zinc content of the study common bean landraces, and the PVAC content of the study pumpkin landraces established from this study will be useful during future development of the Ugandan food composition databases. The developed food composition databases will be useful in designing diets for vulnerable populations such as children in the age range of complementary feeding and in dietary intake assessment studies.

9.2.2 The commonly used local home-based cooking methods of common bean in Uganda is boiling, either with prior soaking or without prior soaking. However, this study revealed that boiling with prior soaking of iron and zinc rich common bean, *Obwelu* retained higher amounts of zinc and iron, compared to boiling without prior soaking. Therefore, Ugandan caregivers should be advised to cook common bean, *Obwelu* by boiling with prior soaking. Furthermore, pumpkin in Uganda is commonly cooked by either boiling or steaming. However, this study established that either boiling or steaming PVAC rich pumpkin *Sweet cream* retained a higher proportion of PVACs. Therefore, Ugandan caregivers should cook pumpkin, *Sweet cream* by either boiling or steaming.

9.2.3 This study revealed that a PVAC, iron and zinc rich complementary food, BPB prepared from locally available pumpkin (*Sweet cream*) and common bean (*Obwelu*) in Uganda, was acceptable to both caregivers and their children in the age range of complementary feeding (six to 24 months old). To contribute towards combating child VAD, ID and ZnD in Uganda, the use of BPB as a CF should be promoted and supported at the community level through nutrition education campaigns, by encouraging caregivers to prepare BPB to feed their children. At the health facility level, health workers at young child clinics should conduct nutrition education. Community health workers (known as village health team members in Uganda) through home visits, and facility health workers, who attend community outreach programmes, such as child immunisation programmes, can conduct nutrition education. It is worth noting that the use of a BPB as a CF towards combating VAD, ID and ZnD does not replace the other existing nutrition interventions such as micronutrient supplementation, commercial fortification and biofortification programmes and the use of ASFs, which aim to combat micronutrient deficiencies during the period of complementary feeding. However, the use of BPB as a CF should be a supplementary strategy to the above-mentioned existing nutrition interventions.

9.2.4 This study was conducted in a rural Ugandan setting, where the main economic activity is subsistence farming including the cultivating and selling of different landraces of common bean and pumpkin. To this end, the government of Uganda should support and promote the cultivation and marketing of PVAC rich pumpkin, *Sweet cream*, and iron and zinc rich common bean, *Obwelu.* This can be done through existing structures in Uganda such as the District Nutrition Coordination Committee (DNCC), a nutrition cluster represented by sectors such as agriculture production, nutrition, and health, among others. The main aim of the DNCC is to combat all forms of malnutrition at the district level in Uganda, and it is responsible for coordinating the implementation of all activities that promote and support food security and nutrition activities in the district.

9.2.5 This study showed that the innovative PVAC, iron and zinc rich BPB is acceptable to children in the age range of complementary feeding. However, the effect of consuming BPB on child vitamin A, iron and zinc status is unknown. Therefore, future studies should consider investigating the effect of BPB consumption on child vitamin A, iron and zinc status. This can be done through randomised control trials, a gold standard study design, to establish a cause and effect relationship.

9.3 Study limitations

9.3.1 The three known PVACs are β-carotene, α-carotene and β-cryptoxanthin. However, due to financial constraints, this study only analysed β-carotene and α-carotene in pumpkin, PP and BPB. This may have led to an underestimation of PVACs in study pumpkin landraces, PP and BPB.

9.3.2 Common bean is a potential source of anti-nutrients such as polyphenols and phytic acid, which may reduce the bioavailability of iron and zinc in common bean and BPB. However, anti-nutrients were not analysed in this study. Analysing these anti-nutrients could give better insight into the anti-nutritional value of common bean and BPB.

9.3.3 It is likely that the variability in micronutrient content observed in the study food crops could be attributed to the different environmental conditions under which they were grown. However, it was difficult for the current study to establish the environmental conditions under which the food crops were grown because they were purchased from food traders in the local market and not farmers. Food traders have limited knowledge on the growing conditions of food crops.

APPENDICES

APPENDIX A: ETHICS APPROVAL FROM THE BIOMEDICAL RESEARCH ETHICS COMMITTEE, UNIVERSITY OF KWAZULU-NATAL

UNIVERSITY OF KWAZULU-NATAL™
INYUVESI YAKWAZULU-NATALI

22 October 2019

Mr E Buzigi (218087161)
School of Agricultural, Earth and Environmental Sciences
College of Agriculture, Engineering and Science
buzigie@live.com

Dear Mr Buzigi

Protocol: Nutritional and sensory properties of a complementary food prepared from pumpkin and common bean in Uganda
Degree: PhD **BREC Ref No: BE438/19**

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 24 May 2019.

The study was provisionally approved pending appropriate responses to queries raised. Your response received on 19 September 2019 to BREC letter dated 17 September 2019 has been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have been met and the study is given **full ethics approval** and may begin as from 22 October 2019. **Please ensure that outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.**

This approval is valid for one year from **22 October 2019.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be noted by a full Committee at its next meeting taking place on **12 November 2019.**

[redacted]

Prof V Rambiritch
Chair: Biomedical Research Ethics Committee

cc: Postgrad Admin: manjoom@ukzn.ac.za Supervisor: pillayk@ukzn.ac.za siwelam@ukzn.ac.za

Biomedical Research Ethics Committee
Professor V Rambiritch (Chair)
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Telephone: +27 (0) 31 260 2486 **Facsimile:** +27 (0) 31 260 4609 **Email:** brec@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

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APPENDIX B: PERMISSION FROM DISTRICT HEALTH OFFICE, KYANKWANZI DISTRICT TO CONDUCT RESEARCH

THE REPUBLIC OF UGANDA
KYANKWANZI DISTRICT LOCAL GOVERNMENT
DISTRICT HEALTH OFFICE
P.O. BOX 90 KIBOGA

March 20th, 2019
Edward Buzigi
Department of Dietetics and Human Nutrition
University of KwaZulu Natal, South Africa

Dear Mr Edward Buzigi

Letter of Authorization to Conduct Research at Ntwetwe Health Center IV, Kyankwanzi District, Uganda

I am very pleased, on behalf of the District Health Team (DHT), Kyankwanzi district that you have been authorized to conduct the research project entitled "Nutritional and sensory properties of a complementary food prepared from pumpkin and dry (common) bean" to be conducted in Kyankwanzi district, Uganda at Ntwetwe Health center IV, Kyankwanzi district.

The DHT acknowledges that it has reviewed the protocol presented by the researcher, as well as the associated risks to the district. The district accepts the protocol and the associated risks to the facility, and authorizes the research project to proceed. The research project should be implemented at the facility upon approval from the relevant Institutional Review Board or Research Ethical Committee of the University of KwaZulu Natal. .

This research project is very relevant to our community, and we shall provide you with the necessary support during data collection. If you have any concerns or require additional information please contact Dr Joel Sserwadda at sserwaddajoel@gmail.com or call +256771478477

Yours Sincerely,

[redacted]

Dr Joel Sserwadda MD, MPH
District Health Officer

DISTRICT HEALTH OFFICE
20 MAR 2019
KYANKWANZI DISTRICT

APPENDIX C: ETHICS APPROVAL FROM THE AIDS SUPPORT ORGANISATION, UGANDA

The AIDS Support Organisation (TASO) Uganda Ltd.

TASO Headquarters
Mulago Hospital Complex
P.O. Box 10443, Kampala-Uganda
Tel: +256 414 532 580/1
Fax: +256 414 541 288
Email: mail@tasouganda.org
Website: www.tasouganda.org

30th September, 2019

Our Ref: TASOREC/066/19-UG-REC-009

Edward Buzigi,
Principal Investigator
buzigie@live.com

Dear Edward,

RE: RESEARCH APPROVAL "NUTRITION AND SENSORY PROPERTIES OF A COMPLEMENTARY FOOD PREPARED FROM PUMPKIN AND COMMON BEAN IN UGANDA."

Thank you for submitting an initial ethics review application of the above-referenced protocol.

I am pleased to inform you that your correspondence dated **23rd September, 2019** with responses to initial review comments of **20th September 2019**, met the requirements for approval.

TASO REC, at its secretariat meeting gave annual approval of the study, effective **30th September 2019**, valid until **29th September 2020**.

Documents reviewed and approved:

Document Type	Date	Version
1. The Study Protocol.	23/09/2019	2.0
2. Informed Consent Forms with Translations	23/09/2019	2.0
3. Data Collection Tools with Translations	23/09/2019	2.0
4. TASO REC Research Review Application and DOC of Interest.	27/07/2019	1.0
5. Introductory Letter, University of KwaZulu-Natal	24/05/2018	
6. Letter of Permission, Kyankwanzi District Local Government	29/07/2019	

Amendments: All proposed amendments to the study (including personnel, procedures, or documents) must be approved by the REC in advance before implementation.
Adverse Events/Unanticipated Problems: Please keep in mind that it is your responsibility to inform the REC of any adverse consequences to participants that occur in the course of the study.
Site Monitoring Visits: shall be undertaken to verify that only approved procedures are being implemented, to ensure that the rights and welfare of participants are being protected.
Study Reports: It is a requirement by the REC that you submit timely progress reports.
Renewal of the study approval. This should be through submission of the Annual Report and a Continuing Review Application, at least 60 days prior to expiration date.
Protocol documents which contain the REC-stamp (if applicable), must be utilized during recruitment of participants, obtaining informed consent and data collection processes.

We recommend that you proceed with the registration and final clearance of your study by the Uganda National Council of Science and Technology (UNCST) before commencement.

Yours sincerely,

[redacted] 29 SEP 2020

Dr. Kaguma David,
Vice Chairperson, TASO RESEARCH ETHICS COMMITTEE (REC)
CC: Executive Director, TASO (U) Limited
CC: Uganda National Council for Science & Technology (UNCST

APPENDIX D: CLINICAL TRIAL FOR CHILD ACCEPTABILITY STUDY REGISTRATION BY PAN AFRICAN CLINICAL TRIALS REGISTRY

saMRC advancinglife

Cochrane South Africa

25 February 2020

To Whom It May Concern:

RE: Nutrition and sensory properties of a complementary food prepared from pumpkin and common bean in Uganda

As project manager for the Pan African Clinical Trial Registry (www.pactr.org) database, it is my pleasure to inform you that your application to our registry has been accepted. Your unique identification number for the registry is **PACTR202002576768667**.

Please be advised that you are responsible for updating your trial, or for informing us of changes to your trial.

Please note that it is now a WHO requirement to include, at a minimum, summary results or a link to summary results within the trial registration record. This should be done within 12 months of the study completion date.

Additionally, please provide us with copies of your ethical clearance letters as we must have these on file (via email or post or by uploading online) at your earliest convenience if you have not already done so.

Please do not hesitate to contact us at +27 21 938 0835 or email epienaar@mrc.ac.za should you have any questions.

Yours faithfully,

Elizabeth D Pienaar
www.pactr.org Project Manager
+27 021 938 0835

PACTR
PAN AFRICAN CLINICAL TRIALS REGISTRY

The South African Medical Research Council
Cochrane South Africa | PO Box 19070, Tygerberg, 7505
Tel: +27 (0)21 938 0438 | Email: cochrane@mrc.co.za | Web: www.southafrica.cochrane.org

APPENDIX E (i): CONSENT TO PARTICIPATE IN CHILD ACCEPTABILITY STUDY (ENGLISH)

Good morning/afternoon, Mr/Mrs/Ms…………………………………………………………. We are from [Kyankwanzi District Health Office and Department of Dietetics and Human Nutrition, UKZN). My name is Edward Buzigi, a PhD student from the Department of Dietetics and Human Nutrition, UKZN, South Africa. I am a Ugandan, and my Ugandan phone number is +256772867437, email is buzigie@live.com

We are working on a project to determine the child acceptability of complementary foods formulated from common bean and pumpkin in Uganda. We would like to invite your child to participate in the study. Now, the project is just starting, and we are completing a survey among participants to know more about child acceptability of complementary foods prepared from common bean and pumpkin. All the information we obtain will remain strictly confidential and your answers and name will never be revealed. The child acceptability test will take 45 to 60 minutes.

The objective of this study is to assess child acceptability of foods prepared from locally available common bean and pumpkin. This is not to evaluate or criticize you or your child, so please do not feel pressured to give a specific response and do not feel shy if you do not know the answer to a question. Participation in this research is voluntary (and you may withdraw participation at any point), and that in the event of refusal/withdrawal of participation you or your child will not incur penalty or loss of treatment or other benefit to which you have come for or entitled to get.

You will be required to feed your child the offered study complementary foods. We will record the amount of food your child will ingest and the duration of ingesting it. These study complementary foods have been prepared by your fellow child caregivers using the traditional methods of cooking you use at home.

In the event of any problems or concerns/questions you may contact the researcher at (+256772867437/+256772321871, email: buzigie@live.com

Do you agree to participate in this interview?

Yes ___ No ___ *If yes, sign the consent form; if no, stop the interview*.

CONSENT

I (Name…………………………………………………………………….)

Sign………………………………………………………………………………

I have been informed about the study entitled “child acceptability of a complementary food prepared from common bean and pumpkin s in Kyankwanzi district, Uganda by (Edward Buzigi/principal investigator…………………………/Sign…………………………………

APPENDIX E (ii): CONSENT TO PARTICIPATE IN CHILD ACCEPTABILITY STUDY (LUGANDA)

Olupapula olukkiririzibwako abalabirira abaana okwetaba mu kunoonyereza ku ngeri abaana gye bakkirizaamu emmere ey'ennyongerezaa (emmere ey'okulozaako)

Osiibye otya nnyabo/ssebo, ……………………………………………………… Tuvudde mu ofiisi ya disitulikiti ya Kyankwanzi ey'eby'obulamu wamu ne'ekitongole ky'eby'endya n'endiisa mu bantu (Kyankwanzi District Health Office ne Department ya Dietetics and Human Nutrition, UKZN). Amannya gange nze Edward Buzigi, omuyizi asoma okufuuka pulofeesa, okuva mu Department ya Dietetics ne Human Nutrition, UKZN, South Africa. Ndi munna Uganda era ennamba yange ey'essimu nga ndi mu Uganda eri +256772867437, n'omukutu/email eri buzigie@live.com.

Tukola okubuuza ebibuuzo (eddakiika nga ana mu ttano ku nkaaga) okwekenneenya mu abo abaneetaba mu kunoonyereza okusobola okumanya ebisingawo ku ngeri abaana gye bakkirizaamu emmere ey'ennyongereza ekoleddwa okuva mu bijanjaalo ebya bulijjo n'ensujju. Byonna bye tunaafuna tujja ku bikuuma nga bya kyaama era okuddamu kwo n'erinnya tebiriyasanguzibwa. Nnja kukusaba oliise omwana wo emmere ey'ennyongereza ekoleddwa okuva mu bijanjaalo ebya bulijjo n'ensujju ekuweereddwa, nga bwe weetegereza enneyisa y'omwana/bwe yeeyisizza eri emmere ng'omuliisa. Emmere eno etegekeddwa abalala abalabirira abaana nga bakozesa enkola ey'okufumba ey'ekinnansi gy'okozesa [redacted] Kino tekikolebwa kukupimaapima oba kukunenya, kale nno towulira kuwalirizibwa kuwa kuddamu okumu era towulira nsonyi singa oba tomanyi kya kuddamu ku kibuuzo. Okwetaba mu kunoonyereza kuno kwa kyeyagalire (era osobola okukuvaamu ekiseera kyonna), era singa ogaana/ova mu kunoonyereza, tojja kubonerezebwa oba kufiirwa bujjanjabi oba okuganyulwa okulala kwonna kwojjiridde oba kw'oteekeddwa okufuna. Okunoonyereza kuno kukakasiddwa era ne kukkirizibwa akakiiko ka UKZN Biomedical Research Ethics Committee (ennamba y'okukkiriza…………………………………………………)

Singa wabaawo obuzibu bwonna oba ebibuuzo, osobola okutuukirira omunoonyereza ku ssimu (+256772867437, email: buzigie@live.com oba akakiiko ka UKZN Biomedical Research Ethics Committee, ng'oyita ku ndagiriro zino wammanga: Biomedical Research Ethics Administration Research Office, Westville Campus; Govan Mbeki Building**;** Private Bag X

54001, Durban 4000, KwaZulu-Natal, South Africa, Essimu: 27 31 2604769 - Fax: 27 31 2604609, Email: BREC@ukzn.ac.za.

Okkiriza okwetaba mu kubuuzibwa ebibuuzo kuno?

Yee ___ Nedda ___ Oba Yee, teeka omukono ku foomu ekkiririzibwako okwetaba mu kunoonyereza*; oba nedda, yimiriza okubuuzibwa ebibuuzo.*

OKUKKIRIZA

Nze (Amannya……………………………………………………………………….)

Omukono………………………………………………/Ennaku z'omwezi…………………………

Ntegezeddwa ku kunoonyereza okuyitibwa Enkola z'okuliisa ebiriisa by'emmere ebigere n'enkolagana zaakwo n'embeera y'endiisa y'abaana mu disitulikiti ya Kyankwanzi mu Uganda (kukolebwa Edward Buzigi/akola mu byalo..)

APPENDIX F (i): CONSENT FOR CAREGIVERS TO PARTICIPATE IN FOCUS GROUP DISCUSSION AND SENSORY EVALUATION STUDY (ENGLISH)

Good morning/afternoon, Mr/Mrs/Ms……………………………………………………….
We are from [Kyankwanzi District Health Office and Department of Dietetics and Human Nutrition, UKZN). My name is Edward Buzigi, a PhD student from the Department of Dietetics and Human Nutrition, UKZN, South Africa. I am a Ugandan, and my Ugandan phone number is +256772867437, email is buzigie@live.com

We are working on a project to determine the acceptability of complementary foods formulated from common bean and pumpkin. We would like to invite you to participate in the study. Now, the project is just starting, and we are completing a survey among participants to know more about child caregiver acceptability of complementary foods prepared from common bean and pumpkin. All the information we obtain will remain strictly confidential and your answers and name will never be revealed. The sensory evaluation will take 45 to 60 minutes for either sensory evaluation or focus group discussion.

The objective of this study is to assess caregiver perceptions and acceptability of common bean pumpkin blend prepared from locally available common bean and pumpkin. This is not to evaluate or criticize you, so please do not feel pressured to give a specific response and do not feel shy if you do not know the answer to a question. Participation in this research is voluntary (and you may withdraw participation at any point), and that in the event of refusal/withdrawal of participation you will not incur penalty or loss of treatment or other benefit to which you have come for or entitled to get.

I am not expecting you to give a specific answer; I would like you to answer the questions honestly, telling me about how you feel (very bad, good, neutral, bad, very bad) of the taste, smell, colour, texture, and overall acceptability of the study foods. These foods have been prepared by your fellow child caregivers using the traditional methods of cooking you use at home. I will request you to allow me tape record the focus group discussion to facilitate its recollection.

In the event of any problems or concerns/questions you may contact the researcher at (+256772867437, email: buzigie@live.com).

Do you agree to participate in this interview?

Yes ___ No ___ *If yes, sign the consent form; if no, stop the interview*.

CONSENT

I (Name…………………………………………………………………….)

Sign………………………………………………………………………………

I have been informed about the study entitled “caregiver perceptions and acceptability of a complementary food prepared from common bean and pumpkin s in Kyankwanzi district, Uganda by (Edward Buzigi/principal investigator…………………………/Sign…………………………………

APPENDIX F (ii): CONSENT FOR CAREGIVERS TO PARTICIPATE IN FOCUS GROUP DISCUSSION AND SENSORY EVALUATION STUDY (LUGANDA)

Olupapula lw'okukkiriza okwetaba mu kunoonyereza n'okubuuzibwa ebibuuzo mu kyaama

Engeri abalabirira abaana gye basalawo ku mmere ey'ennyongereza nga bakozesa engeri abantu ze bawulirizaamu omuli okulaba, okuwunyiriza, okuloza, okuwulira n'empulikika mu kukwata ku mmere ekolebwa mu bijanjaalo ebya bulijjo n'ensujju.

Osiibye otya nnyabo/ssebo, ……………………………………………………. Tuvudde mu ofiisi ya disitulikiti ya Kyankwanzi ey'eby'obulamu wamu ne'ekitongole ky'eby'endya n'endiisa mu bantu (Kyankwanzi District Health Office and Department of Dietetics and Human Nutrition, UKZN). Amannya gange nze Edward Buzigi, omuyizi asoma okufuuka pulofeesa, okuva mu Department of Dietetics and Human Nutrition, UKZN, South Africa. Ndi munna Uganda era ennamba yange ey'essimu nga ndi mu Uganda eri +256772867437/+256772321871, n'omukutu/email eri buzigie@live.com.

Tukola mu puloojekiti okusobola okusalawo engeri emmere ey'ennyongereza ekoleddwa mu bijanjaalo ebya bulijjo n'ensujju gy'ekkirizibwamu. Twandiyagadde okukwaniriza okwetaba mu kunoonyereza. Ekitandikirwako, tumaliriza okwekenneenya mu abo abaneetaba mu kunoonyereza okusobola okumanya ebisingawo ku ngeri abalabirira abaana gye bakkirizaamu emmere ey'ennyongereza (emmere ekoleddwa awaka eweebwa abaana abayonka abali wakati w'emyezi omukaaga n'emyaka ebiri) ekoleddwa okuva mu bijanjaalo ebya bulijjo n'ensujju. Byonna bye tunaafuna tujja ku bikuuma nga bya kyaama (okuddamu kwo n'erinnya lyo tebirimanyisibwa eri muntu yenna) okupima kw'okukozesa empulira, endaba, empooma, empunya n'okukwata ko kujja kutwala eddakiika eziri wakati w'ana mu ttaano n'enkaaga. Kino tekikolebwa kukupimaapima oba kukunenya,kale nno towulira kuwalirizibwa kuwa kuddamu okumu era towulira nsonyi singa oba tomanyi kya kuddamu ku kibuuzo. Okwetaba mu kunoonyereza kuno kwa kyeyagalire (era osobola okukuvaamu ekiseera kyonna), era singa ogaana/ova mu kunoonyereza, tojja kubonerezebwa oba kufiirwa bujjanjabi oba okuganyulwa okulala kwonna kwojjiridde oba kw'oteekeddwa okufuna.

Tusuubira nti okunoonyereza kujja kuteekawo emigaso gino: Ng'abalabirira abaana, mujja kufuna amagezi n'obukugu ku ngeri y'okuteekateeka emmere ey'ennyongereza ejjudde ekiriisa okuva mu bijanjaalo ebya bulijjo n'ensujju nga mukozesa enfumba z'awaka. Okwongereza ku ekyo, mujja kumanya engeri abaana bammwe gyebayimiriddemu mu by’endiisa, era n’abo abanaasangibwa nga bakoozimbye, bajja kuweerezebwa awalabirirwa abaana mu by’endiisa bayambibwe n’okuzzibwamu amaanyi okusinziira ku mateeka ga Uganda bwe galagira. Ssikusuubira kuwa kuddamu kulambikiddwa; okumbuulira engeri gy’owuliramu (bubi nnyo, bulungi, ssi bulungi ssi bubi, bubi, bubi nnyo) ku mpooma, empunya, langi, obukwafu n’engeri emmere etegekeddwa okuva mu bijanjaalo ebya bulijjo n’ensujju gy’ekkirizibwamu yonna okutwaliza awamu. Emmere eno etegekeddwa abalala abalabirira abaana nga bakozesa enkola ey’okufumba ey’ekinnansi gy’okozesa awaka. Okunoonyereza kuno kukakasiddwa era ne kukkirizibwa akakiiko ka UKZN Biomedical research Ethics Committee (ennamba y’okukkiriza………………………………………………)

Singa wabaawo obuzibu bwonna oba ebibuuzo, osobola okutuukirira omunoonyereza ku ssimu (+256772867437, email: buzigie@live.com oba akakiiko ka UKZN Biomedical Research Ethics Committee, ng’oyita ku ndagiriro zino wammanga: Biomedical Research Ethics Administration Research Office, Westville Campus; Govan Mbeki Building**;** Private Bag X 54001, Durban 4000, KwaZulu-Natal, South Africa, Essimu: 27 31 2604769 - Fax: 27 31 2604609, Email: BREC@ukzn.ac.za.

Okkiriza okwetaba mu kubuuzibwa ebibuuzo kuno?

Yee ___ Nedda ___ Oba Yee, teeka omukono ku foomu ekkiririzibwako okwetaba mu kunoonyereza*; oba nedda, yimiriza okubuuzibwa ebibuuzo.*

OKUKKIRIZA

Nze (Amannya…………………………………………………………………….)

Omukono…………………………………………………/Ennaku z’omwezi…………………………

Ntegezeddwa ku kunoonyereza okuyitibwa Enkola z’okuliisa ebiriisa by’emmere ebigere n’enkolagana zaakwo n’embeera y’endiisa y’abaana mu disitulikiti ya Kyankwanzi mu Uganda (kukolebwa Edward Buzigi/akola mu byalo..

APPENDIX G (i): SURVEY QUESTIONNAIRE TO DETERMINE CHILD ACCEPTABILITY OF COMMON BEAN PUMPKIN BLEND (ENGLISH)

Age of caregiver………… Gender of caregiver ……………………

A. Sociodemographic information and anthropometric measurements of children

Infant/young children		
1. Child number	Give a child a number e.g. 001	----------
2. Gender of child	Is (*the name of the child*) male or female?	Male ☐ Female ☐
3.Weight	Please, allow me take three weight measurements of your chid	1…………. 2………….. 3…………… Average …………..(kg)
4.Height	Please allow me take three height measurements of your child	1……………. 2……………. 3…………….. Average …………… (Cm)
Mid Upper Arm Circumference (MUAC)	Please, allow me take three MUAC measurements of your child	1………… 2………….. 3…………… Average ………………(Cm)
5. Child's age	When is your child's birthday? *Probe if necessary*: On what day and in which month and year was (*name of the child*) born? Does he/she have a health/vaccination card with the birth date recorded? *If yes, record the date of birth as documented in the card*	_ _ _ _/ _ _/ _ _ year month day
	How old was (*name of the child*) at his/her last birthday? *Record age in completed years and/or months*	Age in completed years _ _ Age in completed months _ _

B. Ingestion of prepared complementary foods common bean pumpkin blend (BPB) and pumpkin puree (PP)

A research assistant should ensure that a child is offered the assigned complementary food (BPB or PP) at least 1 hour after was last fed. A portion of 100g of complementary food was offered to the child in a serving dish by the caregiver. Caregivers are asked to spoon feed their children the assigned complementary food until the child refuses to eat. After a two-minute pause, the same food should be offered a second time until s/he refuses again. After a second two-minute pause, the food should be offered a third time until the child refuses again. After this third refusal, the feeding episode should be terminated.

Amount of study complementary foods ingested

Common bean pumpkin blend (BPB)			**Pumpkin puree (PP)**		
Amount offered (A)	Amount of leftover (B)	Amount* ingested (A-B)	Amount offered (C)	Amount of leftover (D)	Amount ingested (C-D)

*The amount of food ingested was calculated by subtracting the left-over from the offered amount. Pre-weighed napkins were provided; any food that was regurgitated, vomited or spilled was swabbed, the napkin weighed and subtracted from the weight of the amount offered.

C. Duration of feeding

The duration of feeding (excluding the intervening 'pause periods') was recorded by stopwatch, and the total duration of the feeding was noted. The feeding episode took place under the direct supervision of a trained research assistant to ensure that feeding was not forced. Children were considered as refusing intake if they moved their head away from the food, cried, clamped the mouth shut or clenched the teeth, or became agitated, spat out the food or refused to swallow.

Duration of feeding study complementary foods

Common Bean Pumpkin Blend			**Pumpkin puree**		
Time food is offered (P)	Time feeding is terminated (Q)	Duration of feeding (Q-P)	Time food is offered (S)	Time feeding is terminated (T)	Duration of feeding (S-T)

APPENDIX G (ii): SURVEY QUESTIONNAIRE TO DETERMINE CHILD ACCEPTABILITY OF COMMON BEAN PUMPKIN BLEND (LUGANDA)

Emyaka gyalabilila……………………….. Ekikula kyomulabilizi

A. Ebikwata kumwanawo

Infant/young children		
1. Erinya	Wa omwana enamba geza 001	----------
2. Ekikula kyomwana	Muwala oba mulenzi	Mulenzi ☐ Muwala ☐
3.Obuzito	Omwana mupime emirundi esatu	1…………. 2………….. 3…………… Gata obuzito bwona ogabizemu emirundi 3 …………..(kg)
4.Obuwanvu	Omwana mupime emirundi esatu	1……………. 2……………. 3…………….. Gata obuzito bwona ogabizemu emirundi 3 …………… (Cm)
5.Obunene bwekitundu kyomukono ogwawagulu	Omwana mupime emirundi esatu	1……….. 2………….. 3…………… Gata obuzito bwona ogabizemu emirundi 3 ………………(Cm)
6.Emyako gyomwanawo	Omwana wamuzala/bamuzala ddi? *Buuza oba kyetagisa*: Omwana wamuzala ddi? Buza enaku zomewezi nomyaka Omwan a alina ekipalati kyokugema *Bwaba alina wnadika nga bwekili ku kipalati kye*	_ _ _ _/ _ _/ _ _ Omwaka/ omwezi/olunaku
	Omwana wo yalina emyaka emmeka lwewasemba okukuza amazalibwa ge *Wandiika mu mwaka ob emyezi*	Obukulu bwomwana mu myaka _ _ Obukulu bwomwana mu myezi _ _

B. **Okulya emmere ey'ennyongereza etegekeddwa mu bijanjaalo ebya bulijjo n'ensujju ebisotteddwa awamu (BPB) n'ensujju ensotte yokka (PP)**

Omunoonyereza alina okukakasa nti omwana aweebwa emmere ey'ennyongereza emugerekeddwa (BPB or PP) waakiri essaawa emu okuva lwe yasembyeyo okulya. Alabirira omwana yamuwadde ekitundu ky'emmere ey'ennyongereza kya ggulaamu kikumi (100g) ku ssowaani/ebbakuli.
Abalabirira abaana basabibwa okuliisa abaana baabwe emmere ey'ennyongereza ebagerekeddwa okutuusa ng'omwana agaanye okweyongera okulya. Oluvannyuma lw'akabanga ka ddaakika bbiri ak'okuwummulamu, emmere y'emu eweebwa omwana okutuusa lw'agaana okweyongera okulya nate. Oluvannyuma lw'okuwummulamu okw'eddakiika ebbiri okw'okubiri, emmere erina okuweebwa omwana omulundi ogw'okusatu okutuusa era omwana lw'agaana okweyongera okulya. Oluvannyuma lw'okugaana omulundi ogw'okusatu, ekiseera ky'okulya ekyo kirina okukomekkerezebwa.

Obunji bw'emmere ey'ennyongereza obuliiriddwa

Ebijanjaalo ebya bulijjo ebisotteddwa wamu n'e nsujju (BPB)			**Ensujju esotteddwa yokka (PP)**		
Obunji obuweeredd wa (A)	Obunji bw'esigadde wo (B)	Obunji bw'eriiridd wa * (A-B)	Obunji obuweeredd wa (C)	Obunji bw'esigadde wo (D)	Obunji bw'eriiridd wa (C-D)

*Obunji bw'emere eriiriddwa bwaafuniddwa nga twawula obunji bw'esigaddewo okuva ku bunji bw'eweereddwa; emmere yonna eyaboogeddwa, eyasesemeddwa oba eyayiise, yasiimuddwa n'akagoye akagoye nekateekebwa ku minzaani era obuzito obuvaamu ne bwawulibwa okuva ku bunji bw'emmere eyaweereddwa.

C. **Obuwanvu bw'ekiseera ekiriirwamu**

Obuwanvu bw'ekiseera ekiriirwamu (ng'ojjeeko ebiseera byonna eby'okuwummulamu mu kulya) bwaakwatiddwa ku ssaawa ebala obutikitiki, era obuwanvu bw'ekiseera ekiriirwamu bwonna awamu ne buwandiikibwa. Ekiseera ky'okulya kyonna kyabaddewo nga omunoonyereza omutendeke alaba okukakasa nti teewabaddewo kukabibwa kulya. Abaana baatwaaliddwa nti baganye okulya singa omwana yatambuzza omutwe okuva awali emmere, yakaabye, yagasse emmimwa oba okuluma amannyo, oba yeekyaye, yawandudde emmere oba yagaanye okumira.

Obuwanvu bw'ekiseera ekiriirwamu emmere y'ennyongereza mu kunoonyereza

Ebijanjaalo ebya bulijjo ebisotteddwa wamu n'e nsujju			**Ensujju esotteddwa yokka**		
Essaawa emmere lw'egabibwa (P)	Essaawa okulya wekuyimiriziddwa (Q)	Obuwanvu bw'ekiseera ky'okulya (Q-P)	Essaawa emmere lw'egabibwa (S)	Essaawa okulya wekuyimiriziddwa (T)	Obuwanvu bw'ekiseera ky'okulya (S-T)

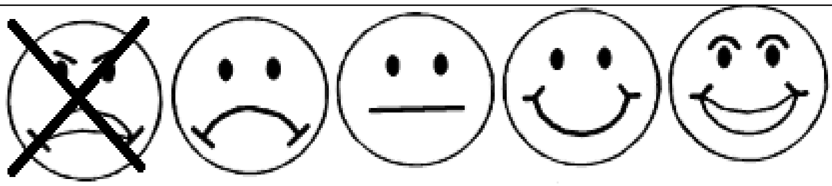
APPENDIX H (i): SENSORY EVALUATION QUESTIONNAIRE TO ASSESS CAREGIVER ACCEPTABILITY OF COMMON BEAN PUMPKIN BLEND

Participant number: Sample number: Date of birth (dd/mm/yy):

Instructions:

- Please rinse your mouth before and after tasting each sample.
- Please taste the samples in the order given i.e. from left to right.
- Please rate the taste, texture, aroma, colour and overall acceptability of the samples by putting a cross on the picture that best describes that sample.
- You may re-taste the sample if needed.

Example:

Colour	 Very bad Bad Neutral Good Very good

FIVE-POINT FACIAL HEDONIC SCALE IN ENGLISH

Taste

Very bad Bad Neutral Good Very good

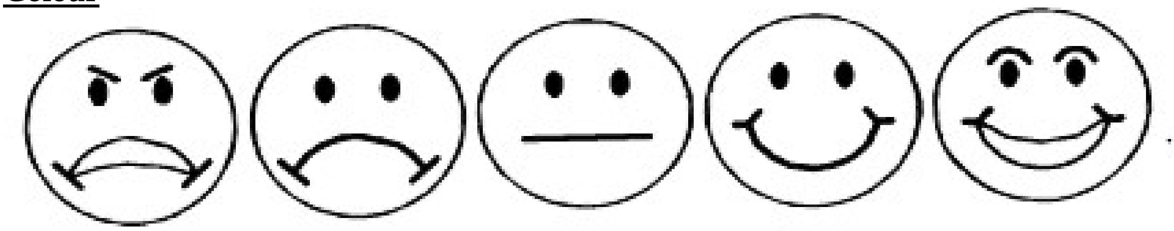
Texture

Very bad Bad Neutral Good Very good

Aroma

Very bad Bad Neutral Good Very good

Colour



Very bad Bad Neutral Good Very good

Overall acceptability

Very bad Bad Neutral Good Very good

Thank you for participating in the study!

APPENDIX H (ii): SENSORY EVALUATION QUESTIONNAIRE TO ASSESS CAREGIVER ACCEPTABILITY OF COMMON BEAN PUMPKIN BLEND (LUGANDA)

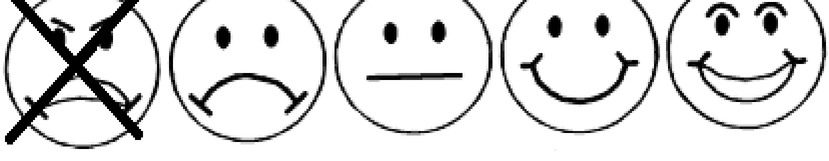
OKUKOZESA ENGERI ABANTU GYE BAWULIRAMU OMULI OKULABA, OKUWUNYIRIZA, OKULOZA, OKUWULIRA N'EMPULIKIKA MU KUKWATA MU KUPIMA EMMERE EY'ENNYONGEREZA EKOLEDDWA MU BIJANJAALO EBYA BULIJJO N'ENSUJJU

Ennamba y'eyeetabye mu kunoonyereza: ……………………………………………
Ennamba y'emmere erozebwako: ……………………………………………….
Amazaalibwa (olunaku/omwezi/omwaka): …………………………………….

Ebiragiro:

- Nkusaba onyumunguze mu kamwa nga tonnaloza ne ng'omaze okuloza ku buli mmere.
- Nkusaba oloze ku buli mmere ekuweereddwa nga bw'etegekeddwa buli emu mu kifo kyaayo kwe kugamba, okuva ku kkono ng'odda ku ddyo.
- Nkusaba ogabe obubonero ku mpooma, obukwafu, akawoowo, langi, n'okukkirizibwa kwonna okwa buli mmere ekuweereddwako ng'osaza emirundi ebiri (X) mu kifaananyi ekisinga okunnyonnyola obulungi emmere eyo erozeddwako.
- Osobola okuddamu okuloza ku mmere emu ssinga kiba kyetaagisa.

Eky'okulabirako:

Langi	 Mbi nnyo Mbi Ssi nnungi ssi mbi Nnungi Nnungi nnyo

OBUBONERO OBUTAANO OBW'EMITWE GY'ABANTU OBUKOZESEBWA MU LULIMI OLUGANDA

Empooma

Mbi nnyo | Mbi | ssi nnungi ssi mbi | nnungi | nnungi nnyo

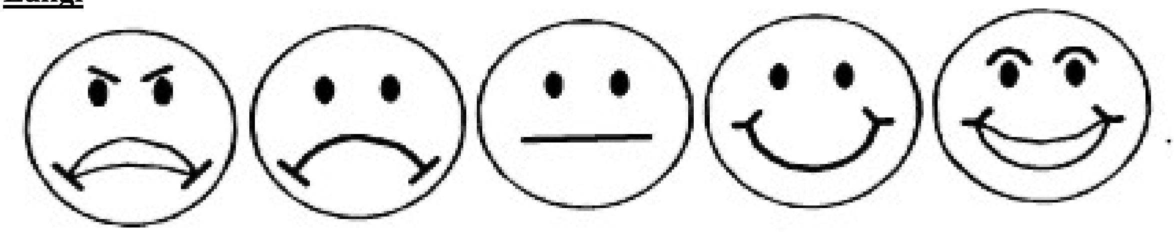
Obukwafu (empulikika mu kamwa)

Mbi nnyo | Mbi | ssi nnungi ssi mbi | Nnungi | Nnungi nnyo

Akawoowo

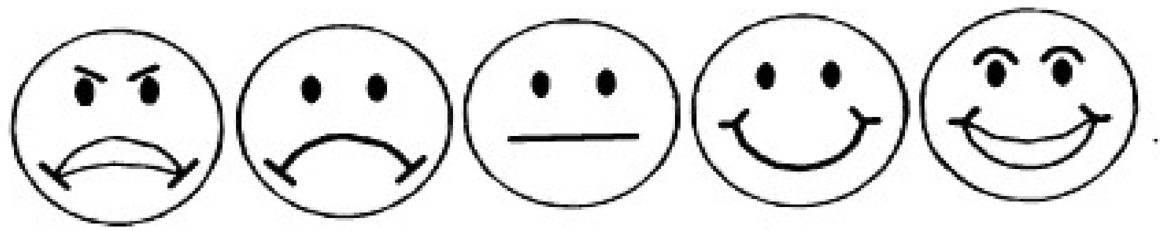
Kabi nnyo | Kabi | ssi kalungi ssi kabi | Kalungi | Kalungi nnyo

Langi



Mbi nnyo | Mbi | Ssi nnungi ssi mbi | Nnungi | Nnungi nnyo

Okukkirizibwa kwonna wamu



Mbi nnyo | Mbi | Ssi nnungi ssi mbi | Nnungi | Nnungi nnyo

Weebale kwetaba mu kunoonyereza!

APPENDIX I (i): FOCUS GROUP DISCUSSION QUESTIONNAIRE TO ASSESS CAREGIVER PERCEPTIONS OF COMMON BEAN PUMPKIN BLEND (ENGLISH)

Focus group discussion data collection questions
1. Have you ever cooked common bean and pumpkin before? Please share your experience
2. How would you grade the taste, colour, odour (smell), and texture of the BPB compared to the PP?
3. To what extent is common bean and pumpkin accessible and affordable in your community?
4. How feasible would the preparation of common bean and pumpkin be during the development of BPB?
5. As a caregiver, would you feed your child a complementary food made from common bean and pumpkin? Explain your reasons
6. What cultural factors in your community or households may prevent you from using the BPB as a complementary food?

BPB: Common Bean Pumpkin Blend

PP: Pumpkin puree

APPENDIX I (ii): FOCUS GROUP DISCUSSION QUESTIONNAIRE TO ASSESS CAREGIVER PERCEPTIONS OF COMMON BEAN PUMPKIN BLEND (LUGANDA)

EKITUNDU AWAWEEBWA OBUBAKA OBULALA: OLUKALALA LW'EBIBUUZO OLUKOZESEBWA MU KWE KENNEENYA ENDOWOOZA Z'ABALABIRIRA ABAANA ERI EBIJANJAALO EBYA BULIJJO EBISOTTEDDWA N'ENSUJJU

Ebibuuzo ebikozesebwa mu kukuŋŋanya ebikubaganyizibwako ebirowoozo mu kibinja
1. Wali ofumbyeko ku bijanjaalo ebya bulijjo n'ensujju? Nkusaba ogabaneko naffe nga bwekyaali. 2. Waandigabye otya obubonero ku mpooma, langi, akawoowo (empunya), n'obukwafu bw'ebijanjaalo ebya bulijjo ebisotteddwa wamu n'ensujju (BPB) ng'ogerageeranya ku nsujju esotteddwa yokka (PP)? 3. Kyanguwa kwenkanawa okusobola okufuna ebijanjaalo ebya bulijjo n'ensujju mu kitundu kyo? 4. Bwangu bwenkanaki obuli mu kutegeka ebijanjalo ebya bulijjo n'ensujju mu kukola ebijanjaalo bya bulijjo ebisotteddwa wamu n'ensujju (BPB) 5. Nga ggwe alabirira omwana, waandimuwadde okulya emmere ey'ennyongereza ekoleddwa mu bijanjaalo ebya bulijjo n'ensujju? Nyonnyola ensongazo. 6. Nsonga ki ez'obuwangwa mu kitundu oba mu maka eziyinza okukuziyiza okukozesa emmere ey'ennyongereza ekoleddwa mu bijanjaalo ebya bulijjo ebisotteddwa wamu n'ensujju (BPB)?

BPB: Ebijanjaalo ebya bulijjo ebisotteddwa wamu n'ensujju

PP: Ensujju ensotte yokka