FORMULATION AND CONSUMER ACCEPTABILITY OF BIOFORTIFIED MAIZE (*Zea mays*) AND SUGAR BEAN (*Phaseolus vulgaris*) BLEND AS SUPPLEMENTARY FOOD FOR CHILDREN AGED 6-59 MONTHS

A dissertation submitted in partial fulfilment of the requirements for the Master of Science Degree in Food Security and Sustainable Agriculture
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DECLARATION

I hereby declare that the research project entitled “FORMULATION AND CONSUMER ACCEPTABILITY OF BIOFORTIFIED MAIZE (*Zea mays L.*) AND SUGAR BEAN (*Phaseolus vulgaris L.*) BLEND AS SUPPLEMENTARY FOOD FOR CHILDREN AGED 6-59 MONTHS” submitted to Bindura University of Science Education, Department of Agricultural Economics, Education and Extension is a record of an original work done by me under the guidance and supervision of Dr. B. Masamha and this work is submitted in partial fulfilment of the requirements for the award of a Master of Science Degree in Food Security and Sustainable Agriculture. The results embodied in this thesis have not been submitted to any University or Institute for the award of any degree of diploma.

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DEDICATION

This thesis is dedicated to the following special people in my life:

- My beloved parents Mr Sebastian & Mrs Elizabeth Mahuni (née Masaiti). My siblings Solomon, David and Ropafadzo. Thank you for being always there for me and sacrificing a lot for the sake of my future.
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ABSTRACT

Globally, about 2 billion people experience micronutrient deficiency due to the consumption of poor quality foods that lack diversity and essential micronutrients. Biofortified crops offer a potential route for tacking micronutrient malnutrition in at-risk population subgroups particularly children. This study aimed to formulate biofortified maize-sugar bean blendsand evaluate their consumer acceptability as supplementary food for children aged 6-59 months. The grains of locally available provitamin A-biofortified maize, iron-biofortified sugar beans and white maize (control) were analysed for carotenoids, iron and zinc using standard or referenced methods. The analysis results showed that biofortified maize had significantly high total provitamin A carotenoid concentration (9.958 µg/g DW) compared to both iron biofortified sugar beans (0.071 µg/g DW) and white maize (0.013 µg/g DW) which served as a control. The biofortified sugar bean had significantly higher iron and zinc concentrations (32.680 µg/g and 77.203 µg/g DW respectively) compared to both biofortified maize (0.045 µg/g and 0.261 µg/g DW respectively) and white maize (0.070 µg/g and 0.040 µg/g DW respectively). These findings indicate that biofortified maize is a superior source of provitamin compared to biofortified sugar beans and white maize. The findings also indicate that biofortified sugar beans is a superior source of iron and zinc compared to biofortified maize and white maize.

The biofortified maize and sugar bean were then combined in different proportions (50:50 and 67:33) to formulate meal blends meeting at least 20% of the recommended daily intakes of vitamin, iron and zinc for children aged 5 years and below. A cross-sectional survey was conducted to evaluate the consumer acceptability of the blends as supplementary food in the form of thin porridge. Thin porridges prepared from sole white maize and sole biofortified maize meal were used as controls. The thin porridges were evaluated for appearance, taste, aroma, texture and overall acceptability using a 5 point hedonic scale by 30 untrained adult women caregivers in Mbare, Harare. Thin porridge prepared using biofortified maize:sugar bean blend (ratio 50:50) had the highest mean score for overall acceptability (3.59) among all the thin porridges. Interestingly, both thin porridges prepared using biofortified maize:sugar bean blends were more accepted by the caregivers than thin porridges prepared using sole white maize meal and sole biofortified maize. Such blends of biofortified maize and sugar bean had never been evaluated before and hence their high acceptability among women caregivers is a new and important finding. The high acceptability of blends of biofortified maize and sugar beans suggests they can potentially be used to deliver provitamin A, iron and zinc to vulnerable individuals particularly children under the age of 5. Findings from this study also suggest that there is a low level awareness on biofortified crops among urban consumers. There is need for nutrition awareness and education programmes on biofortification targeting urban areas as current efforts are mainly focused on rural areas.

The more accepted blend (50:50) was then analysed for proximate composition. The blend had an acceptable crude fibre content of 4.5 g/100g as per the Codex Alimentarius guidelines on formulated complementary foods for children. The blend also had a low moisture content of 7.7% indicating that ithas good keeping qualities. The proximate analysis results indicate that biofortified maize-sugar bean blends are good sources of energy, protein and carbohydrates and should be used in tackling protein energy malnutrition (PEM).

Key words: biofortified, supplementary food, sensory evaluation

LIST OF ACRONYMS AND ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of Variance
CGIAR	Consultative Group on International Agricultural Research
CIMMYT	International Maize and Wheat Improvement Center
DR&SS	Department of Research and Specialist Services
DW	Dry Weight
GoZ	Government of Zimbabwe
HPLC	High-Performance Liquid Chromatography
FAO	Food and Agriculture Organisation
NNS	National Nutrition Survey
RLA	Rural Livelihoods Assessment
SPSS	Statistical Package for Social Sciences
UNICEF	United Nations International Children's Emergency Fund
UZ	University of Zimbabwe
USAID	United States Agency for International Development
VAD	Vitamin A Deficiency
WFP	World Food Programme
WHO	World Health Organisation
ZDHS	Zimbabwe Demographic and Health Survey
ZimVAC	Zimbabwe Vulnerability Assessment Committee

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CHAPTER 1: INTRODUCTION

1.1 Background of the study

Globally, about 2 billion people experience micronutrient deficiency due to the consumption of poor quality foods that lack diversity and essential micronutrients (Govender et al., 2017). This was corroborated by findings from a systematic analysis of health effects of dietary risks in 195 countries for the period 1990-2017 conducted by Afshin et al., (2019) which revealed that poor diet is the leading cause of mortality and morbidity worldwide. At the global scale, deficiencies in Vitamin A, iron and zinc continue to be ranked amongst the main manifestations of micronutrient malnutrition and are associated with retarded growth, higher morbidity and mortality, with high rates among young children. Iron, zinc and vitamin A deficiencies are ranked 9th, 11th and 13th among the 26 major risk factors of the global burden of disease (Ezzati et al., 2012). An estimated 10.8 million child deaths occur annually due to Fe, Zn and vitamin A deficiencies (World Health Organisation (WHO), 2002).

Children under the age of 5 years from low- and middle-income countries are highly vulnerable to micronutrient deficiencies. Consequently, children are considered a high priority population subgroup due to relatively high nutritional needs for rapid growth and development (Black et al., 2013) accompanied with inadequate micronutrient intakes, lower energy, higher infection burden (Ijarotimi, 2013) and the need to end the cycle of intergenerational micronutrient malnutrition. This is of importance to Zimbabwe where 25% of the children have Vitamin A deficiency (VAD), 72% are living with iron deficiency and 33% have iron deficiency anemia (National Nutrition Micronutrient Survey, 2015; United Nations International Children's Emergency Fund (UNICEF), n.d.).

Data on zinc deficiency are limited, but an estimated 48.4%of the Zimbabwean population including children is at risk of inadequate zinc intake (Wessells and Brown, 2012). Unfortunately, most people affected by micronutrient deficiencies neither show observable clinical symptoms nor are they themselves aware that they have the deficiencies, a phenomenon referred to as “hidden hunger” (World Food Program (WFP), 2006). This gives rise to the need for an accelerated and sustained national action to end malnutrition in all its forms including micronutrient deficiencies, leaving no one behind. This entails an inclusive approach that takes into consideration children from poor rural and urban households.

The missing link to ending malnutrition lies in provision of improved food from household based value addition initiatives by vulnerable and affected groups such as women, children and the elderly. Biofortified crops conventionally bred for enhanced micronutrient concentrations (Nestel et al., 2006) can act as low cost vehicles for delivering target vitamins and minerals to consumers help eliminate micronutrient deficiencies in vulnerable populations in developing countries (Banerji et al., 2013). However, the use of single nutrient approaches in alleviating micronutrient malnutrition has proved ineffective thus far. This is mainly because almost half of children with micronutrient deficiencies are suffering from multiple deficiencies (UNICEF, 2019).

Low income households from both urban and rural settings usually rely on low cost but often poorly formulated and therefore nutritionally imbalanced maize based complementary foods for feeding their children due to prohibitive cost of commercially fortified complementary foods and nutritionally dense foods of animal origin. This might explain the persistent trident malnutrition problem of vitamin A, iron and zinc deficiency in children under the age of 5 in Zimbabwe where white maize (*Zea mays* L.) is a major part of the diet. White maize is devoid of provitamin A carotenoids (Nuss and Tanumihardjo, 2010) which are required to prevent VAD in children. This is where locally produced provitamin A-biofortified maize also known as orange maize conventionally bred for increased levels of provitamin A carotenoids, which are precursors of vitamin A in the body, can succeed as an alternative cereal. Given that children commonly consume cereal based supplementary food mostly in the form of porridge referred to as *bota* in Shona throughout early childhood, utilizing orange maize in such foods can go a long way in complementing efforts aimed tackling VAD in children. This is an attractive and sustainable complementary food based approach given that VAD remains a public health challenge despite the implementation of multiple strategies including mandatory food fortification of selected commercial food products and oral vitamin A supplementation to address it.

From a nutritional point of view, orange maize just like white maize is a relatively poor source of iron and zinc (Ortiz-Monasterio et al., 2007; Oikehet al., 2004). Maize based foods therefore need to be fortified with locally available iron and zinc rich sources in a way that can be easily implemented at household level. In view of this, home based food-to-food fortification of orange maize based food with biofortified sugar bean (*Phaseolus vulgaris* L.) rich in iron and zinc (Bouis and Welch, 2010) is an interesting and plausible approach for producing high quality supplementary food for children. Provitamin A-biofortified maize and iron-biofortified sugar bean, which are grown and consumed by about 250000 rural farmers in Zimbabwe, can fulfill a significant portion of Estimated Average Requirement (EAR) of iron (up to 35%) and vitamin A (> 50%) when consumed as staples (Bouis and Welch, 2010; HarvestPlus, 2018), without the need for industrial fortification. Though promising, the use of biofortified legumes in enriching biofortified cereal based supplementary food for instance porridge for children is uncommon and remains largely unexplored in Zimbabwe and beyond based on the lack of documented research work. Most of these biofortified cereals and legumes have not been considered for local based value addition and food fortification to combat malnutrition.

Despite the health benefits associated with the consumption of biofortified crops especially orange maize, the success of biofortification particularly in Southern Africa will likely depend on its acceptance and consumption by target populations who at present largely consume and prefer white maize over yellow/orange maize for historical, cultural and sensory reasons (De Groote et al., 2010; Rubey and Lupi, 1997; Muzhingi et al., 2008). The addition of sugar bean to maize depending on the formulation can impact the sensory properties of the supplementary food and hence its acceptance by consumers. At present, the consumer acceptability of biofortified maize-sugar bean blends is unknown though such findings could be useful in determining (i) whether or not such blends can be used as vehicles to deliver provitamin A, iron and zinc to at-risk children (ii) identify the traits that breeders need to target in order to improve the undesirable sensory attributes of the blends (if any). This study therefore seeks to formulate supplementary food from a blend of locally produced biofortified maize and sugar bean for children under 5 years of age. The study also seeks to establish the consumer acceptability of the supplementary food.

1.2 Problem statement

There is a paucity of empirical evidence on the proper combination and formulation of provitamin A biofortified maize and biofortified sugar bean blend rich in iron and zinc to yield supplementary food containing a good balance of vitamin A, iron and zinc at household level targeting children. The wide use of the white maize devoid of provitamin A carotenoids as a basic ingredient of traditional complementary foods highly consumed by children especially from poverty stricken rural and urban households is contributing to the high prevalence of micronutrient deficiencies in Zimbabwe. This is supported by Oikeh et al., (2004) who postulates that the iron and zinc concentrations of white maize range from 1.69-2.07 mg/100 g and 1.85-2.04 mg/100 g respectively. These Fe and Zn concentrations of white maize are too low to meet the recommended daily intake of 12.1 mg/day and 8.95 mg/day (from diets poor in zinc) of both Fe and Zn for children aged 5 years and below (WHO/Food and Agriculture Organization (FAO), 2004). This can potentially be addressed by replacing white maize with provitamin A-rich biofortified maize in the popularly consumed foods. Biofortified maize can provide as much as 50% of the recommended daily intake for vitamin A (Bouis and Welch, 2010).

The concentration ranges of both iron (1.1-3.9 mg/100 g) and zinc (1.5-4.7 mg/100 g) in provitamin A-biofortified maize (Ortiz-Monasterio et al., 2007) are low for the dietary needs of children hence the need for their supplementation. This can be potentially achieved by blending biofortified orange maize with zinc rich and iron-biofortified sugar bean in defined ratios using simple processing methods that can be adopted at household level. The proper blending should be guided by food composition data of both the biofortified maize and sugar bean which is currently unavailable in Zimbabwe. The plausible approach of food-to-food fortification using nutritionally enhanced biofortified crops to produce nutritious supplementary foods remains unexplored. There is also a paucity of data on the consumer acceptance of such supplementary foods and therefore it is not known whether it could be successfully used as a vehicle to deliver provitamin A, iron and zinc to vulnerable children particularly those in the age category of 6-59 months. This study therefore seeks to determine the nutritional composition of biofortified maize and sugar bean, use that data to formulate supplementary food for children aged 6-59 and evaluate its acceptability by consumers.

1.3 Objectives

1.3.1 Main objective

To formulate biofortified maize-sugar bean blend and assess its consumer acceptability as supplementary food to enhance vitamin A, iron and zinc status for children aged 6-59 months.

Specific objectives

1. To determine the provitamin A, iron and zinc content of white maize, biofortified maize and biofortified sugar beans.

   Hypothesis: i. Biofortified maize contains significantly higher levels of provitamin A compared to both white maize and biofortified sugar beans.

   ii. Biofortified sugar beans contain significantly higher levels of iron and zinc compared to both white maize and biofortified maize.
2. To formulate the biofortified maize-sugar bean supplementary food blend by combining biofortified maize and sugar bean meal at different levels.

   Hypothesis: Supplementary food prepared from biofortified maize- sugar bean blend can provide at least 25% (one quarter) of the recommended daily intakes for vitamin A, iron and zinc.
3. To assess the proximate composition of the more acceptable supplementary food.

   Hypothesis: Biofortified maize- sugar bean blend can meet the recommended minimum levels for macronutrients in complementary foods for children aged 6-59 months
4. To evaluate the acceptability of biofortified maize-sugar bean blends using organoleptic test of supplementary food among urban mothers/caregivers of children aged 6-59 months.

   Hypothesis: Supplementary food prepared from biofortified maize- sugar bean blends will be more acceptable compared to the sole white maize and sugar beans consumed as sole diet.

1.4 Justification

This study focused particularly on children from low resource settings that have long been identified as highly vulnerable to micronutrient malnutrition partly due to inadequate dietary intake of vitamins and minerals that has persisted despite the availability and access to nutritionally superior biofortified agricultural commodities. This can be attributed to on-going reliance on poorly combined and formulated diets. This study can make a timely contribution in providing food composition data that can be used to guide the proper combination of locally produced biofortified ingredients and optimal formulation that can be adopted at household level to produce nutritionally balanced supplementary food containing a good balance of iron, zinc and vitamin A for child feeding purposes. Such a supplementary food may offer poor households a sustainable and affordable means of maintaining or improving the vitamin A, iron and zinc status of at risk or affected children aged 6-59 months.

Furthermore, findings from this study can be used by private, public and non-governmental organisations to guide the design of integrated nutrition interventions including but not limited to community based nutrition education and awareness on the optimal use of locally produced biofortified crops for deriving improved nutritional benefits and thus possibly promote their consumption at household level. Such knowledge can also encourage women, who are also responsible for the care of children and family nutrition in general, to develop positive attitudes towards dietary improvements derived from proper use of biofortified food resources. The lack of knowledge on how to use the available food resources has been cited as a contributing factor to child malnutrition (FAO, International Fund for Agricultural Development (IFAD), UNICEF, WFP and WHO, 2017) and in the interest of public health this area requires urgent attention targeting vulnerable children in Zimbabwe.

The generation of data on the consumer acceptability of the resultant supplementary food could help breeders in identifying the traits that they need to focus on, in order to make such food more acceptable. Currently, such data is lacking and hence a knowledge gap exists. This needs to be investigated. Findings from this study can be rich and exciting in their contributions to policy and programming appropriate nutritional interventions hence help in curbing vitamin A, iron and zinc deficiency in children. If uncontrolled through innovative sustainable and targeted food based interventions that can be realistically implemented at household and community level, malnutrition is likely to lead to increased loss of productivity, child morbidity and mortality in Zimbabwe.

1.5 Scope/delimitations and limitations of study

The study focused on formulating biofortified maize-sugar bean blend and assessing its consumer acceptability as supplementary food (thin porridge) to enhance vitamin A, iron and zinc for children aged 6-59 months. The study used varieties of biofortified maize (ZS242A), sugar beans (NUA45) and a composite of non-biofortified maize grown and consumed in Zimbabwe. Data on the nutritional composition of these specific biofortified varieties and the most accepted supplementary food blend established through a sensory evaluation study were obtained through laboratory tests conducted at the University of Zimbabwe (UZ) and

Standards Association of Zimbabwe (SAZ). The data can therefore be used for comparative purposes and as a baseline for future studies. However, the study did not analyse the anti-nutritional factors present in any of the grain samples (raw materials) used nor evaluate the shelf life of biofortified food mixes as these were beyond the scope of the work. The study only used two formulations of biofortified maize:sugar beans (50:50 and 67:33) as it targeted to produce blends that could potentially meet at least 20% (one fifth) of the RDI of vitamin A, iron and zinc for children aged 6-59 months respectively when consumed as supplementary food in the form of thin porridge.

A cross sectional survey on sensory acceptability of the thin porridge was done in the low income, high density suburb of Mbare located in Harare by 30 consenting adult women caregivers of at least a single child aged 6-59 months. Both 100% white maize and 100% biofortified maize were used as controls for the sensory acceptability study. The small sample size of panellists due to budgetary constraints means that the findings of this study cannot be generalized though the work lays a foundation for future studies. The lack of baseline data on the acceptability of the test meals in the study area effectively limits the ability to do a comparative analysis. The study did not investigate the willingness of urban consumers to buy biofortified maize and/or sugar beans and their biofortified food mixes.

1.6 Outline of Thesis

This thesis comprises of the following six chapters:

Chapter 1: Introduction

This introductory chapter encompasses the background to the study covering the problem of malnutrition at global level and in Zimbabwe with a particular focus on the prevalence of VAD, iron and zinc deficiency in Zimbabwean children under 5 years. The potential of biofortified maize and sugar beans in complementing existing public health strategies for combating malnutrition is discussed. The problem statement, study objectives and their corresponding hypothesis, the justification for conducting the study and the scope/delimitations and limitations of the study are also covered in this introductory section.

Chapter 2: Literature review.

This chapter reviews and critically analyses the existing literature relevant to this study. The importance of vitamin A, iron and zinc in human health and the prevalence of their associated deficiencies in Zimbabwean children are reviewed. The nutritional inadequacies of popularly consumed home based complementary foods are discussed and gaps identified. The nutritional compositions of both biofortified and non-biofortified maize and sugar beans are reviewed. Published findings from previous on consumer acceptability of yellow/orange maize based foods are also reviewed. The conceptual framework for this study is presented in this chapter.

Chapter 3: Methodology

This chapter gives the background of the study site, presents the research design (experimental and cross-sectional survey), procedures of sampling, data collection and data analysis employed in the study. The completely randomized design was used in food nutritional analysis. The cross-sectional survey was used to evaluate the sensory acceptability of supplementary food prepared from blends of biofortified maize and sugar beans. The chapter also presents the ethical considerations of the research and finally a summary of the methodology.

Chapter 4: Manuscript 1 "Formulation and nutritional evaluation of supplementary food prepared using biofortified orange maize-sugar bean blends for children aged 6-59 months".

This chapter presents the first manuscript of the original study. An abstract of the work is presented first followed by an introduction of the problem of malnutrition particularly VAD, iron and zinc deficiencies and their manifestations. The potential of biofortification in tackling micronutrient malnutrition is also introduced with biofortified maize and sugar bean as the crops of focus. The methodology including the materials, methods, sampling procedure, data collection procedure and data analysis procedure used in the study are described. Challenges encountered during data collection are highlighted. The steps followed in formulating the food blends by combining biofortified maize:sugar beans in different ratios (50:50 and 67:33) are described. The results of the study on the nutritional composition of the raw materials and the supplementary food are presented and discussed in comparison to findings of. previous studies reported in literature. The recommendations from this study are also presented. A conclusion based on the main findings of the study is given.

Chapter 5:Manuscript 2 "Consumer acceptability of supplementary food prepared using blended biofortified orange maize and sugar beans in Mbare, Harare"

This chapter presents the second manuscript entitled of the original study beginning with an abstract of the work. It also gives an introduction relevant to the study. The methodology including the materials, methods, sampling procedure, data collection procedure and data analysis procedure used in the study are described. Challenges encountered during data collection are highlighted. The main findings from the cross-sectional survey on the sensory acceptability of supplementary food prepared from blends of biofortified maize and sugar beans are presented and discussed in comparison to findings of previous studies reported in literature. The recommendations from this study are also presented. A conclusion based on the main findings of the study is given.

Chapter 6: Summary, conclusions and recommendations

This section presents a summary of main research findings, conclusion drawn from the study, the policy implications of the findings, recommendations and proposed areas for further studies are covered. The various sources used in this study are acknowledged under the references section. Appendices are available at the very end of the chapter.

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CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

According to Neuman (2011), the aim of reviewing literature is to demonstrate familiarity with existing knowledge and establish credibility in the process. Reviewing literature is important as it helps to place a research study in a context, thereby demonstrating its relevance. For the purposes of this study, this chapter reviews the role of vitamin A, iron and zinc in human health and nutrition, recommended dietary intakes of the 3 aforementioned micronutrients for children as well as the prevalence of VAD, iron (Fe) and zinc (Zn) deficiency in Zimbabwean children. Documented findings on the nutritional quality of popular cereal based complementary foods are also reviewed. A review of biofortification together with its pros and cons is provided. The nutritional profiles of both biofortified and non-biofortified maize and sugar bean are reviewed. Published data and information on the consumer acceptability of yellow/orange maize based foods are also reviewed. Gaps in the previous studies that this study seeks to address are highlighted throughout the chapter.

2.2 Literature subsections

2.2.1 The role of vitamin A human metabolic processes

Vitamin A is an essential micronutrient for metabolic functions in humans though it is required in small quantities by the body as any other micronutrient. The term vitamin A includes provitamin A carotenoids, which are dietary precursors of physiologically active forms of vitamin A (WHO, 2009). Carotenoids are natural pigments produced by plants only. As such, mammals including humans rely solely on plant-derived foods as sources of carotenoids. According to the Institute of Medicine (2000) vitamin A activity is the only known function of carotenoids in humans. It has long been established that vitamin A plays an important role in the normal functioning of the visual system (Sommer and West, 1996). Severe vitamin A deficiency (VAD) can cause irreversible eye damage resulting in impaired vision or irreversible blindness. VAD is the leading cause of preventable childhood blindness. Xerophthalmia is the collective term for all ocular manifestations of VAD (WHO, 2009).

Vitamin A is essential for cell division, bone growth and production of red blood cells (Institute of Medicine, 2001). Various reports indicate that Vitamin A promotes absorption of non-haeme iron from plant based foods by increasing Fe bioavailability and hence can contribute to improved iron status in humans (Welch, 2002; Garcia-Casal et al., 2000).

Vitamin A is important to all people and continues to be a micronutrient of focus particularly in developing countries characterised by high prevalence of VAD. Children have increased vitamin A requirements for supporting their rapid growth. It is therefore important to provide children with a balanced diet that meets the recommended daily intake (RDI) for vitamin A for their age group as indicated in Table 2.1 below.

Table 2.1 Recommended Dietary Intakes for vitamin A for children aged 1-6 years

Nutrient	**1-3 years**	**4-6 years**
Vitamin A (µg RE)†	200	200

†Calculated estimated average requirements. RE, retinol equivalents. 1RE = 1µg retinol = 12µg β-carotene or 24µg other provitamin A carotenoids or 6 µg synthetic b-carotene

Source: WHO/FAO (2004).

2.2.2 The role of iron in human metabolic processes

Iron plays a key role in various human metabolic processes such as electron transport and synthesis of deoxyribonucleic acid (Abbaspour et al., 2014). As a component of the erythrocyte proteins haemoglobin and myoglobin, iron serves as a transporter of oxygen in the body (McDowell, 2003). Iron deficiency limits the production of haemoglobin in the body resulting in anaemia. Anaemia can lead to increased morbidity from infectious diseases, impaired cognitive development, loss of energy and stunting in affected individuals (Lozo et al., 2008; Bailey et al., 2015). Children have been identified as one of the population that is vulnerable to iron deficiency. They need iron for rapid growth and combat infections. According to WHO/FAO (2004), the RDI of iron for children varies with age group. The RDI for iron also depends on its bioavailability in the food source as shown in Table 2.2 below.

Table 2.2 Recommended Dietary Intakes for iron for children aged 1-6 years

Nutrient	**1-3 years**	**4-6 years**
Iron (mg)§ 15% bioavailability	3.9	4.2
10% bioavailability	5.8	6.3
5% bioavailability	11.6	12.6

§15% bioavailability from a diet rich in vitamin C and animal protein, 10% for diets rich in cereals plus vitamin C, 5% bioavailability for diets low in vitamin C and animal protein. Based on average bodyweights.

Source: WHO/FAO (2004).

2.2.3 The role of zinc in human metabolic processes

According to Shankar and Prasad (1998), zinc is an indispensable micronutrient that plays a central role in the maintenance of cell and organ integrity given that it stabilizes the molecular structure of multiple cellular components and membranes as well as protects cells from oxidative damage (Rostan et al., 2000; Prasad et al., 2004). Zinc improves wound healing through its involvement in membrane signaling systems that influence the growth and proliferation of cells (MacDonald, 2000). Clinical features of zinc deficiency in humans depending on severity include retarded growth, weak immune system, delayed bone maturation, poor appetite, diarrhoea, skin lesions and loss of hair (Hambridge et al., 1987;Roohani et al., 2013). Adequate dietary intake of zinc that meets the needs of the consumer is of importance in preventing and managing zinc deficiency. Zinc intake in children can be guided by the WHO/FAO (2004) RDIs (which can also be referred to as recommended daily allowance (RDA)) which takes into account the age group and level of zinc bioavailability in the food source as shown in Table 2.3 below.

Table 2.3 Recommended Dietary Intakes for zinc for children aged 1-6 years

Nutrient	**1-3 years**	**4-6 years**
Zinc (mg) High bioavailability	2.4	2.9
Moderate bioavailability	4.1	4.8
Low bioavailability	8.3	9.6

Levels of bioavailability: High - from diets rich in animal protein. Moderate - from diets rich in pulses or including yeast fermented cereals (e.g. leavened breads). Low from diets poor in animal protein or zinc-rich plant foods. Based on average bodyweights.

Source: WHO/FAO (2004).

Information on the RDIs of target nutrients including vitamin A, iron and zinc should therefore guide selection, combination, formulation and consumption of nutritious foods that meet both the nutritional needs of children. Biofortified foods are no exception. This arguably has not been the case at household level due to a number of factors including lack of knowledge for instance pertaining to the nutritional composition of such food ingredients, defined formulations for meeting a specific proportion of the recommended dietary intake for particular nutrients at household level. The optimal use of available food is therefore not being achieved most likely leading to consumption of nutritionally imbalanced foods and exacerbating childhood malnutrition.

2.3 Micronutrient malnutrition in Zimbabwean children

2.3.1 Prevalence of VAD in children

According to UNICEF (2019), 25% of the children in Zimbabwe have VAD. Data on the trends in vitamin A status of children in Zimbabwe is scarce. The prevalence of childhood VAD gives an indication that the problem is widespread and it may be mainly attributed to low dietary intake of the vitamin A which is insufficient to meet physiological needs. Low dietary intake of vitamin A by children can be an indication of poor child feeding practices even in situations where food is available and accessible. Optimal use of available and accessible food resources aimed at meeting the Vitamin A requirements of children is therefore an important but arguably overlooked aspect of food and nutrition security especially at household level.

2.3.2 Prevalence of iron deficiency in children

The prevalence of iron deficiency in children age 6-59 months has recently been documented in the Zimbabwe Demographic and Health Survey (ZDHS) 2015 report. According to the report, 37% of children aged 6-59 months (37%) are anaemic. Of these, 22% of the children are mildly anaemic, 15% are moderately anaemic, and less than 1% are severely anaemic. The prevalence of anaemia in Zimbabwean children is generally unacceptable and varies by province, from a low of 29% in Masvingo to a high of 42% in Harare. This shows that urban areas are also becoming iron deficiency hotspots and deserve attention. On average, the prevalence of anaemia issimilar for children in urbanand rural areas (38% and37%, respectively). Although the prevalence of anaemia in children has been on a downward trend over the years, it remains unacceptably high as shown in Figure 2.1 below.

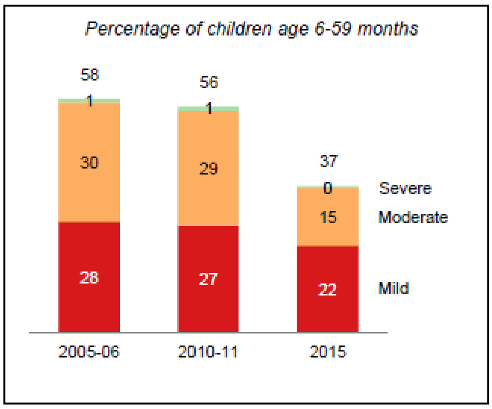

Figure 2.1 Trends in anaemia status among children

Source: ZDHS (2015).

The high prevalence of anaemia in children calls for innovative approaches to complement the existing strategies for tackling the problem. Since iron deficiency is largely attributed to low dietary intake of iron, ensuring that children consume iron rich foods such as iron-biofortified beans can potentially go a long in reducing the risk of the deficiency.

2.3.3 Prevalence of zinc deficiency in children

Data on the prevalence of zinc deficiency in Zimbabwean children aged 6-59 months is unavailable. Wessells and Brown (2012) estimated that 48.4% of the Zimbabwean population including children is at risk of inadequate zinc intake. Increasing dietary intake of zinc can go a long way in reducing the risk to zinc deficiency. Food based interventions can be used to complement other strategies aimed at controlling zinc deficiency in Zimbabwe. The identification and optimal use of locally available and accessible zinc rich food resources such as beans can be a sustainable means of reducing the vulnerability of consumers especially children to zinc deficiency.

2.4 Home-based complementary foods

Due to their wide consumption, home based complementary foods have become a subject of interest. The nutritional adequacy of popular cereal based complementary foods has been investigated. Dewey (2005) reported that home-based foods often contain insufficient amounts of iron and zinc which according to WHO (2006) can lead to Fe and Zn deficiencies respectively. Gibbs et al., (2011) evaluated multiple fortified plant-based complementary foods available in developing countries and concluded that only 96 and 98% of the porridges failed to meet the recommended daily intake for iron and zinc respectively as jointly set by WHO and FAO. This can be attributed in part to low content and/or low availability of iron and zinc in the cereal based ingredients. According to Oikehet al., (2004), the iron and zinc concentrations of white maize range from 1.69-2.07mg/100g and 1.85-2.04mg/100g respectively. The Fe and Zn concentration of white maize is too low to meet the recommended daily intake of 10mg/day of both Fe and Zn for children (WHO/FAO, 2004).

White maize based complementary foods are a poor source of vitamin A unless if vitamin A containing ingredients are added to such foods. Findings from previous studies indicate that white maize is devoid of provitamin A carotenoids (Nuss and Tanumihardjo, 2010; Menkiret al., 2008; Li et al., 2007). Consumers of unfortified complementary foods prepared from white maize who lack access to vitamin A rich foods and/or supplements are therefore at an increased risk of vitamin A deficiency. The possibility of substituting staple ingredients particularly white maize with nutritionally enhanced orange maize (biofortified) should be explored. Furthermore, combining orange maize with iron-biofortified beans can improve the nutritional quality of commonly consumed maize based foods.

Reports by many researchers on the nutritive potentials of non-biofortitified cereal-legume food mixes for children have been promising (Badamosi et al., 1995; Owolabi, 1996; FAO, 1997; Okoh, 1998; Ladeji et al., 2000). Surprisingly, the nutritive potentials of biofortitified cereal-legume food mixes as supplementary food for tackling micronutrient deficiencies in children have not been reported in literature even though biofortified crops appear to be ideal candidates for such food given that they are specially bred for enhanced levels of vitamin A, iron and zinc (Bouis and Welch, 2010). As such, the prospect of combining locally produced provitamin A-biofortified maize with the biofortified sugar bean containing high levels of iron and zinc to produce a multi-nutrient rich home-based supplementary food for children is appealing and therefore needs to be explored. Children from resource poor settings are often affected by multiple micronutrient deficiencies (Villegas et al., 2008) hence single nutrient approaches alone are not the key to solving such nutrition problems and preventing chronic diseases. However, biofortified cereal-legume food mixes should follow proper combination and formulation guided by nutritional composition data of the food constituents. In Zimbabwe, there is paucity of data on the nutritional profiles of the biofortified crops released in the country to date. This needs to be investigated.

2.5 Biofortification

The goal of biofortification is to contribute to reducing the high prevalence of selected micronutrient deficiencies particularly of vitamin A, iron and zinc that commonly occur in low income populations. This is to be achieved by improving the micronutrient density of staple food crops that are produced and consumed by target populations. When consumed regularly, biofortified staple foods should lead via increased intakes of these micronutrients to improved health outcomes. More than 290 varieties of 12 biofortified staple food crops are in testing or have been released in 60 countries around the world including Zimbabwe (FAO, 2018). Over 20 million people worldwide are currently consuming biofortified crops (Bouis and Saltzman, 2017).

Zimbabwe has adopted biofortification as a complementary strategy for fighting micronutrient malnutrition particularly in resource poor settings. To date, provitamin A-biofortified maize also referred to as orange maize due to its orange colour and iron-biofortified sugar beans have been released in Zimbabwe. The development and release of biofortified crops in Zimbabwe is spearheaded by Consultative Group on International Agricultural Research (CGIAR) HarvestPlus and International Maize and Wheat Improvement Center (CIMMYT) together with Zimbabwe’s Department of Research and Specialist Services (DR&SS) and other partners. HarvestPlus is part of the CGIAR Research Program on Agriculture for Nutrition and Health (A4NH). All biofortified crop varieties being promoted and released in Zimbabwe are conventionally bred CIMMYT (2016) although other pathways such as genetic engineering and agronomic fortification are being explored in other countries (Laurie et al., 2012). The micronutrients currently being targeted by the HarvestPlus Biofortification Challenge Program are provitamin A, iron and zinc (Bouis and Saltzman, 2017).

The advantages of biofortification as a complementary strategy to alleviate micronutrient deficiencies include the following:

i. It is a sustainable strategy. This is because after the initial investment of developing the nutritionally enhanced biofortified crops, the crops can be shared internationally thus keeping recurrent costs low (Nestelet al., 2006).
ii. No behaviour modification on the part of the consumer is needed in most cases (Bouis, 2003; Bouis, 1999) as biofortification targets staples commonly consumed in large quantities by the poor consumers at risk of micronutrient deficiencies. The case

of orange maize is unique as some of its sensory characteristics such as colour and aroma differ from those of the commonly consumed white maize.

iii. It is cost-effective as there are no annual costs associated with the purchasing and addition of fortificants to the food supply (Bouis, 2002; Asare-Marfo et al., 2014) unlike in the cases of commercial fortification and supplementation.

iv. The strategy offers poor populations at risk of poor nutritional status living in remote and rural areas that may not have access to commercial fortified foods the opportunity to access and grow their own micronutrient rich foods (Nestel et al., 2006; Bouis, 2003).

v. There is low risk of vitamin A toxicity from consuming provitamin A biofortified foods. This is because such biofortified foods provide the body with carotenoids that the body converts to vitamin A as needed and hence the conversion process is controlled and regulated by the body (Penniston and Tanumihardjo, 2006).

However, biofortification is not without its shortcomings including its inability to provide as high mineral and/or vitamin content compared to supplementation or fortification strategies alone. Biofortification is therefore not expected to treat micronutrient deficiencies or eliminate them but contribute to increased micronutrient intake (Bouis et al., 2017).

2.6 Nutritional composition of biofortified and non-biofortified maize and sugar beans

Researchers from around the world have over the years investigated the chemical composition of various crops including biofortified and non-biofortified maize and bean to evaluate their nutritive value. Notably, fewer studies have investigated the nutritional composition of biofortified crops compared to the wide literature on the nutritional composition of non-biofortified crops. This might be because biofortification is a relatively a new food based strategy for tackling micronutrient malnutrition. This section presents the reported chemical compositions of both biofortified and non-biofortified maize and bean from previous studies. The review is mostly limited to vitamin A, iron and zinc and proximate parameters (protein, fat, fibre, ash, moisture and carbohydrates as they are the nutrients of interest in this study).

2.6.1 Nutritional composition of provitamin A-biofortified

i. Micronutrient composition of biofortified maize

More than 40 provitamin A rich biofortified maize genotypes have been released in different African countries (Andersson et al., 2017) to curb vitamin A deficiency. Several studies have investigated the nutritional composition including micronutrients contained by various provitamin A maize genotypes. In South Africa, Pillay et al., (2011) investigated the carotenoid content of 13 lines of provitamin A maize and reported that the ranges varied from 7.3-8.3ug/g against the current breeding target of 17 ug/g on dry weight basis (DW) for biofortified maize set by HarvestPlus (Bouis and Welch, 2010). A survey on of carotenoid variation in maize out by Islam (2004) reported a total provitamin A concentration range of 7.58-14.05 for 6 inbred lines.Ortiz (2019) analysed the carotenoid profile of 5 biofortified maize genotypes. The total carotenoid concentrations of the biofortified maize genotypes ranged from 32.7–61.0 µg/g DW. The amounts of provitamin A carotenoids in β-carotene equivalents in the varied by genotype and were found to range from 4.3–9.3 µg/g DW. The variation in carotenoid content of yellow maize due to genetic factors has been reported by (Muzhingi et al., 2008) and hence it is important to generate variety specific nutritional data including carotenoid content. Provitamin A-biofortified is a good source of vitamin A as it contains high amounts of provitamin A carotenoids particularly β-carotene found to have twice vitamin A activity than other provitamin A carotenoids (α-carotene and β-cryptoxanthin) (Gregory, 1996).

Data on the iron and zinc contents in biofortified maize is scarce. According to Ortiz-Monasterio et al., (2007)provitamin A maize genotypes have varying contents of iron (1.1-3.9 mg/100 g) and zinc (1.5-4.7 mg/100g). Pillay et al., (2011) also investigated the iron and zinc contents in 13 lines biofortified maize. The iron content of the lines ranged from 1.90-5.77 mg/100 g (mean = 3.23 mg/100 g). The zinc content of the lines ranged from 1.75-2.90 mg/100 g (mean = 2.19 mg/100 g). The ranges indicate that there is variation in the contents of iron and zinc depending on the maize variety or line. Data on nutritional composition of biofortified maize produced in Zimbabwe is still very limited in spite of its expanding cultivation and consumption. This needs to be investigated.

The proximate composition of biofortified maize has been previously investigated. A study by Pillay et al., (2011) investigated the macronutrient composition of 34 lines of provitamin A-biofortified maize. The results of this investigation are presented in Table 2.4 below.

Table 2.4 Mean macronutrient composition of 34 biofortified maize lines

Macronutrient	**Average % of the kernels of 34 lines**
Starch	71.3
Protein	8.7
Fat	4.1
Fiber	3.0
Sugars	11.4

Source: Pillay et al., (2011)

2.6.2 Nutritional composition of non-biofortified maize

The kernels of normal maize contains multiple macronutrients as shown in Table 2.5 below. Maize is an important dietary source of nutrients for humans and animals. The carbohydrate and phosphorus are the most abundant macronutrient and micronutrient found in maize kernels respectively. Trace minerals are found in low amounts in maize (FAO, 1992). The proximate composition of normal dent maize reported by (Johnson, 2000) indicates that starch is the major component of normal dent maize. It accounts for 71.3% of the kernel composition, followed by sugars (11.4%) and protein which constitutes 8.7% of the maize used in this study. Fat and fibre account for 4.1% and 3.0% of the maize of the kernel composition. Other studies reported protein levels of between 8.92-10.52% in white maize (Machida et al., 2010). There is variability in the amount of a given macronutrient in different maize types even in earlier studies as shown in Table 2.6 below.

Table 2.5 Composition per 100 g of edible portion of maize

Nutrient	**Mean quantity in a kernel**
Carbohydrate	71.88 g
Protein	8.84 g
Fat	4.57 g
Fibre	2.15 g
Ash	2.33 g
Iron	2.3 mg

Source: Shah et al., (2015); Gopalan et al., (2007).

Table 2.6 Proximate composition of 5 different maize types

Maize type	**Moisture**	**Ash**	**Protein**	**Crude fibre**
Salpor	12.2	1.2	5.8	0.8
Crystalline	10.5	1.7	10.3	2.2
Floury	9.6	1.7	10.7	2.2
Starchy	11.2	2.9	9.1	1.8
Sweet	9 5	1 5	12.9	2.9

Source: Cortez and Wild-Altamirano, (1972).

2.6.3 Nutritional composition of biofortified sugar beans

From the limited literature available, the concentrations of nutrients in biofortified beans varies with the cultivar. Brigide et al., (2014) determined the nutritional composition of 5 different biofortified bean cultivars. The results of that study are presented in Table 2.7 below. From the results, the biofortified bean cultivars are rich in protein, iron and zinc and thus have potential to improve dietary intakes of consumers when consumed as staples.

Table 2.7 Macronutrient composition of 5 biofortified bean cultivars

Bean variety	**Carbohydrate (%)**	**Protein (%)**	**Fat**	**Ash (%)**	**Crude fibre (%)**	**Moisture (%)**	**Iron** mg/kg	**Zinc** mg/kg
Perola	22.69	23.38	1.94	4.57	34.01	13.4	61.2	22.29
Porto Real	18.52	25.81	2.13	4.10	34.21	15.24	73.93	31.11
Brasil	16.18	31.59	1.94	4.34	30.32	15.62	80.82	37.68
Pirata	20.30	27.34	1.66	4.54	32.27	13.89	72.23	30.08
Supremo	19.1	28.03	1.73	4.55	32.27	14.04	78.95	31.06

Source: Brigide et al., (2014).

The variation in nutritional composition of biofortified beans indicates that there is need for variety specific data. Currently, data on the nutritional composition of a variety of iron biofortified sugar beans (NUA45) released in 2015 is lacking in Zimbabwe. Such data can be used for optimal use of beans in food products.

2.6.4 Nutritional composition of non-biofortified common beans

The nutritional composition of normal common beans has been reported in literature. The nutritional profile of beans varies with the variety as shown in Table 2,8 below. Carbohydrate is the major component of bean seed and it varies more than other nutrients. From the table below protein constitutes about 8% of the bean seed. However, beans is a low in fat therefore it can be a good source of low fat protein.

Table 2.8 Nutritional composition of normal beans

Bean variety	**Carbohydrate (%)**	**Protein (%)**	**Fat (%)**	**Crude fibre (%)**	**Iron mg/g**
Black	21	8	0.5	8	2
Cranberry	22	8	0.4	9	2
Great northern	19	7	0.4	6	2
Navy	24	8	0.6	9	2
Pink	24	8	0.4	5	2
Pinto	22	8	0.5	8	2
Small red	20	8	0.2	6	3

Source: United States Department of Agriculture (USDA, 2016)

2.7 Consumer acceptability of yellow/orange maize based foods

The success of biofortified maize-based foods will partly depend upon whether Zimbabwean consumers prefer provitamin A maize over white maize or not since both types of maize cost the same on the market. Studies have established that white maize is generally preferred over yellow/orange maize in African countries and as such change in dietary preferences for provitamin A biofortified is a daunting task (Pillary et al., 2011; Stevens and Winter-Nelson, 2008). The poor acceptance of yellow maize in Africa can be attributed to come from prejudice and negative associations such as food aid and animal feed, lack of nutrition education and awareness as well as undesirable sensory characteristics that affect overall acceptability.

Muzhingi et al., (2008) investigated consumer acceptability of yellow maize products in both urban and rural Zimbabwe more than 5 years prior to the introduction of biofortified crops in

Zimbabwe. The study showed a definite preference for white maize over yellow maize. However, 94% of households registered their willingness to consume yellow maize if they knew it was more nutritious than white maize. A paltry 2% of households had some knowledge about the nutritional value of yellow maize. Nutrition education therefore has potential to improve consumer acceptability of yellow maize and arguably orange maize too. Generation of variety specific compositional data of yellow/orange maize could be a good starting point and hence requires further investigation. Also, findings from studies limited to yellow maize are likely to be inaccurate if they are used to determine the consumer acceptance for biofortified orange maize. The chemical composition for instance carotenoid content of yellow maize differs from that of orange maize and compositional differences may result in incomparable organoleptic properties and consumer acceptance.

An earlier consumer acceptability study conducted by Tschirley and Santos (1995) in Mozambique indicated that white maize was preferred over yellow maize although poorer consumers were more willing to purchase yellow maize if it was offered at a discounted price. A more recent market survey conducted by Stevens and Winter-Nelson (2008) found that many Mozambican participants had a favourable response to the orange maize contrary to the earlier findings by Tschirley and Santos (1995). This could be attributed to inherent genetic differences between yellow and orange maize, an indication that yellow and orange maize may need to be assessed separately. The consumer acceptability of yellow maize in Zimbabwe has been previously evaluated by Muzhingi et al., (2008) although that of orange maize and its products is poorly understood in Zimbabwe hence this study seeks to fulfil this knowledge gap.

Pillary et al., (2011) evaluated the acceptability of popularly consumed maize food products (*phuthu,* thin porridge and samp) prepared from biofortified yellow maize using 212 subjects aged 3-55 years in rural KwaZulu-Natal. The researchers found that adult and secondary school panelists preferred products made with white maize compared to those made with biofortified maize. On the contrary preschool children favoured the food products made from biofortified maize compared to those made with white maize. This showed that biofortified maize based products could be accepted by preschool children and hence be used for targeted feeding of children particularly from VAD hotspots. This study focuses on children aged 6-59 months in Zimbabwe where 33% of children under the age of 5 have VAD (UNICEF, n.d; National Nutrition Micronutrient Survey, 2015).

Although limited studies aimed at establishing consumer acceptance of orange maize have been carried out, similar studies focusing on blended orange maize-biofortified sugar bean for use as potentially micronutrient rich supplementary food seem fewer or non-existent and this requires further investigation. Work on such blends can provide important data on the acceptability and possible consumption of biofortified cereal-legume food mixes and also inform breeding decisions for improving the sensory quality of biofortified staples. Provitamin A-biofortified maize can only reach its full potential if it is bred with features that are more acceptable to consumers (Stevens and Winter-Nelson 2008).

Interventions such as biofortification primarily target rural communities as they are considered to be vulnerable to malnutrition (De Groote and Kimenju, 2008). However, poor and food insecure urban households are also vulnerable to malnutrition hence need to be targeted too. It is therefore plausible to extend the consumer acceptability studies to the urban poor. It is equally important to target young children's mothers when conducting sensory evaluations as they are in most cases responsible for making dietary decisions pertaining to child feeding at household level and hence can influence the quantity and quality of supplementary food to be consumed. The participation of young children's mothers in consumer acceptability studies of supplementary food encourages them to gain nutrition knowledge and positive attitudes towards dietary improvements (Pelto et al., 2003).

2.3 Conceptual/Theoretical framework

The design of the study was guided by an adapted UNICEF (1990) conceptual framework on causes of malnutrition. The underlying causes of childhood malnutrition are household food insecurity and poor child feeding practices which are influenced by the nutrition knowledge of the caregiver. These underlying causes lead to inadequate nutrient intake which is an immediate cause of malnutrition. Therefore level of knowledge on proper child feeding practices is a prerequisite to prevent malnutrition in children. This knowledge influences the choice, combination, formulation and preparation of food especially from staples at household level. Knowledge on optimal use of locally available and accessible food resources can be considered as a factor that is important in child care. It partly influences whether food availability and access translates into intake of nutritionally balanced food that promotes child health and well-being. This study is premised on the idea that women caregivers lack adequate knowledge on the proper selection, combination and formulation of locally available food resources for feeding children. The lack of food composition data limits the ability of women caregivers to make informed decisions that result in the consumption of nutritionally balanced foods is likely contributing to the poor nutritional quality of home based complementary foods.

The study is also premised on the idea that staples are widely consumed food resources and as such are a key determinant of the nutritional status of a significant portion of consumers. Food-to-food fortification plays a key role in improving the quality and diversity of food. Therefore this study utilises biofortified maize and beans to formulate nutritious blends. The study is also premised on the idea that sensory characteristics of food are key determinants of whether individuals accept the food or not. The study will try to use the framework to try and relate how food-to-food fortification can lead to achieving improved supplementary foods of an acceptable sensory quality as part of child feeding practices.

2.4Summary of literature Review

Vitamin A, iron and zinc deficiency remain unacceptably high in Zimbabwean children. This can be attributed in part to poor nutritional quality of home based complementary foods based on white maize containing very low levels of iron, zinc and vitamin to meet the recommended daily intakes of these micronutrient for children. This constitutes poor infant and child feeding practices. Biofortified maize an sugar beans contain enhanced levels of vitamin A and iron and zinc respectively and can potentially be combined to produce micronutrient rich supplementary foods for children. The formulations must be guided by food compositional data and the RDIs for vitamin A, iron and zinc. The acceptability of biofortified foods largely depends on consumer acceptability which is a difficult task. The limited studies carried out on the acceptability of biofortified maize based foods indicate that, generally, white maize is more acceptable and preferred compared to yellow/orange maize. The acceptability of biofortified maize-sugar has not been reported in literature.

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CHAPTER 3: METHODOLOGY

3.1 Introduction

This chapter describes the background of the study site, presents the research design, procedures of sampling, data collection and data analysis employed in the study. The chapter also presents the ethics of the research and finally a summary of the methodology.

3.2 Description of study site

3.2.1 Sourcing of raw materials

A total of 3 raw materials comprising of (i) composite white maize (control), (ii) provitamin A-biofortified maize (variety ZS242A) and (iii) iron-biofortified sugar beans (variety NUA45) were sourced and used in this study..

i. White maize

A total of 3 different samples of white maize popularly sold and consumed in the study area of Mbare were purchased from traders at Mbare Musika Agricultural Produce Market which is Zimbabwe’s biggest agricultural market. The market is located in Mbare, a high density area located in Harare with geographical coordinates of 17° 51^{II} 29^{II} S in latitude and 31° 2^{II} 13^{II} E in longitude (Sengwe and Musemwa, 2016). The market was purposively selected for this study as it supplies hundreds of Harare residents including those from Mbare with tonnes of agricultural produce and commodities including locally produced dried common beans and white maize grain throughout the year. The location of Mbare Musika Agricultural Produce Market is shown in Figure 3.1.

Major supplies of maize and sugar beans are delivered and subsequently sold at the market throughout the year from a number of areas including Mashonaland West, Mashonaland Central and Manicaland due to high demand and better prices on offer compared to other markets. The maize and common beans is usually delivered by farmers and usually bought by traders and informal middlemen locally referred to as *makoronyera* for resell at a higher price. On average white maize grain and sugar beans costs 0.30 and 1.00 United States Dollars (USD) per kilogramme (kg) respectively at Mbare Musika Agricultural Produce Market. However, biofortified sugar beans is usually in short supply at the market although it can fetch as much as 2.50 USD per kg. At present, the market does not supply provitamin A-biofortified maize.

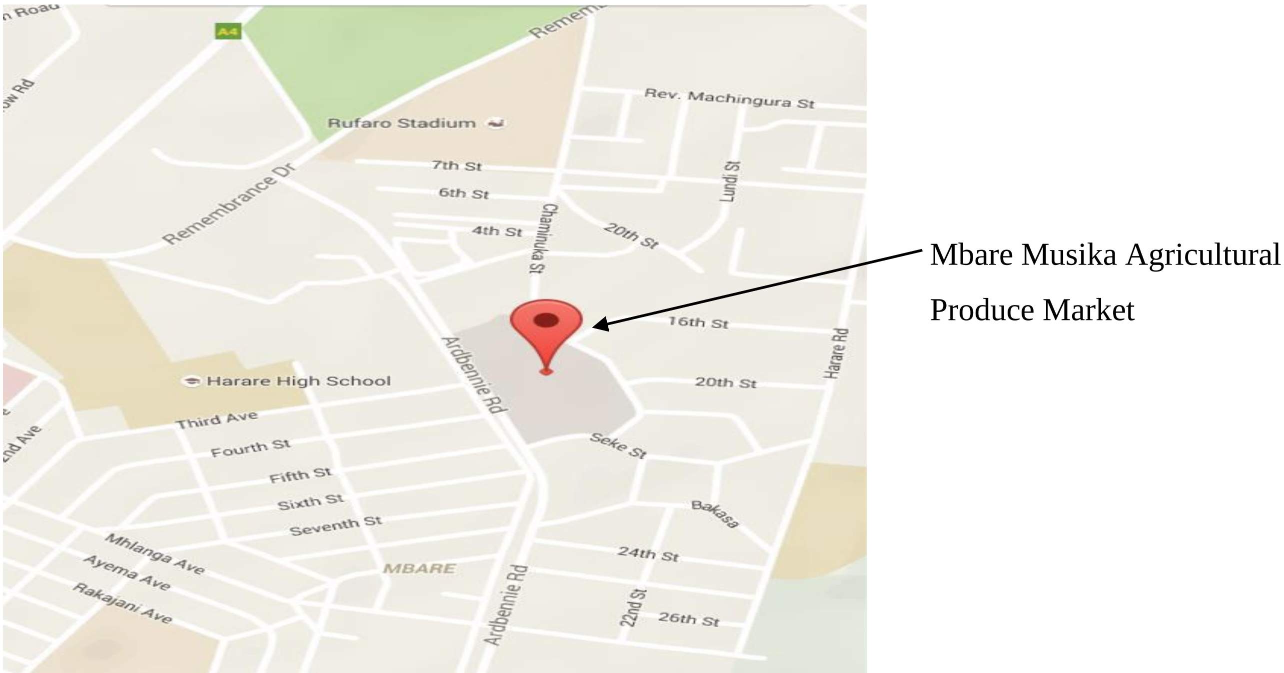

Figure 3.1 Map of Mbare showing the location of Mbare Musika Agricultural Produce Market

Source: Sengwe and Musemwa (2016).

ii. Sourcing of Provitamin A-biofortified maize and iron-biofortified sugar beans

Samples of provitamin A biofortified maize (variety ZS242A) and iron-biofortified sugar beans (variety NUA45) weighing 5kg each were sourced from HarvestPlus. The biofortified maize and sugar beans were purposively selected as raw materials for this study due to their availability locally, staple status and nutritional quality. ZS242 contains high levels of provitamin A whilst NUA45 is rich in iron and zinc compared to their non-biofortified counterparts.

3.2.2 Nutritional analysis and formulation of food blends

The vitamin A, iron and zinc analysis of both the raw materials and supplementary food were carried out at the University of Zimbabwe and Standards Association of Zimbabwe located in using the same standard methods. Both of the institutions are located in Harare and have well equipped laboratories.

3.2.3 Consumer acceptability study)

The study was carried out in Mbare, one of the oldest high-density suburbs located in the southern part of Harare, the capital city of Zimbabwe. Reports indicate that Mbare was the first high-density suburb established in 1907. It is located about 5 kilometres from the central business district of Harare. The suburb is divided into ward 3 and ward 4 with a combined population of 37 213 comprising of 19 283 males constituting 52% of the total population and 17 930 females constituting 48% of the total population. The constituency has 9 653 households (ZimStat, 2012). Mbare is predominantly a low income suburb dominated by the informal activities notably illegal vending. Mbare ward 3 has a population of 22 243. Some of Mbare's poorest residents reside in overcrowded housing units particularly in the dilapidated flats such as Mbare Hostels under Mbare East ward 3. A significant number of such poor inhabitants and their family members including children are likely to food insecure, undernourished and either suffering or at high risk of micro and/or micronutrient deficiencies.

Mbare Hostels comprise of 9 blocks having either 2 or 3 floors as shown in Figure 3.3 (a) and 3.3 (b) below. Each floor comprises of 35 rooms with each room housing at least one family. Some rooms house as many as 3 to 4 families. This is usually the case with the poorest families that either cannot afford to rent a full room or rent out part of the room to earn some money. The hostels are less than a kilometre from the largest farm produce market Zimbabwe popularly known as Mbare Musika Agricultural Produce Market. A number of vendors mostly female from Mbare Hostels buy vegetables and fruits from Mbare Musika Agricultural Produce Market for resale within Mbare Hostels at both legal and illegal vending spaces.

For the purposes of the sensory evaluation, Mbare Hostels was identified as a convenient sample due to its close proximity to the researcher's residential area (less than 5 km), its high number of accessible and closely located households forming a significant part of Mbare's population, its low income status and hence reflects the resource poor settings targeted in the study. It is highly likely that a significant number of children from Mbare Hostels commonly consume white maize based complementary food and suffer or are at high risk of VAD, iron and zinc deficiencies. The consumption of the proposed supplementary food is uncommon in this area and therefore its acceptability is largely unknown. A map showing the location of Mbare Hostels in Mbare is shown in Figure 3.2 below.

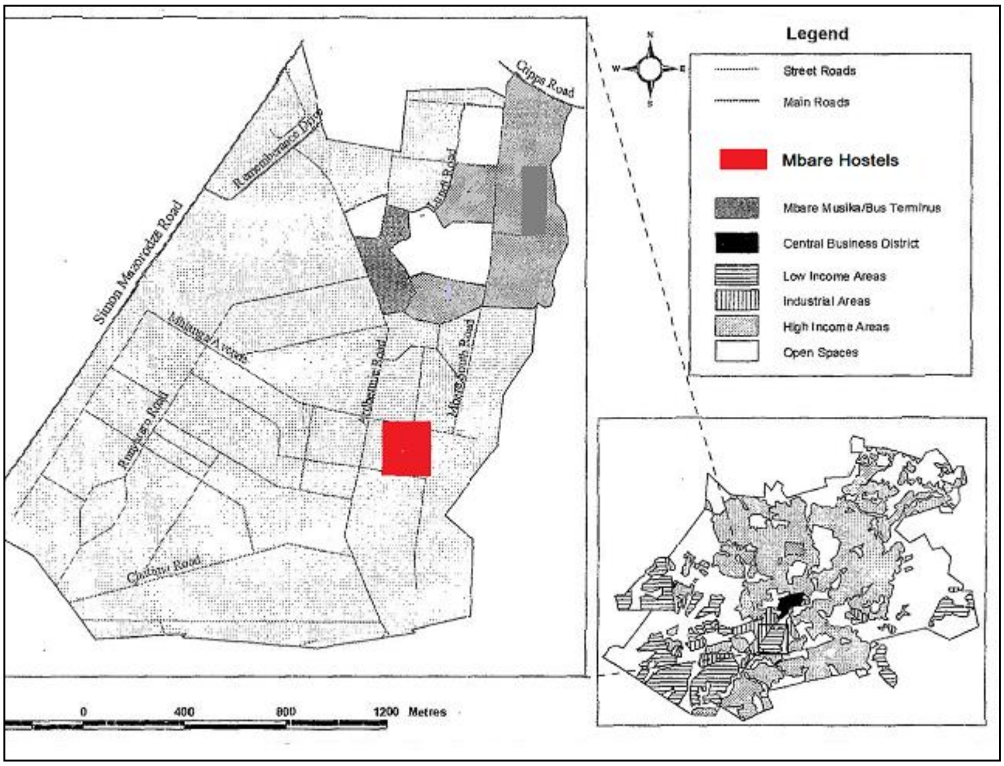

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Figure 3.2 Location of the study area (Mbare Hostels) within Mbare

Source: Tevera and Chikanda (2000).

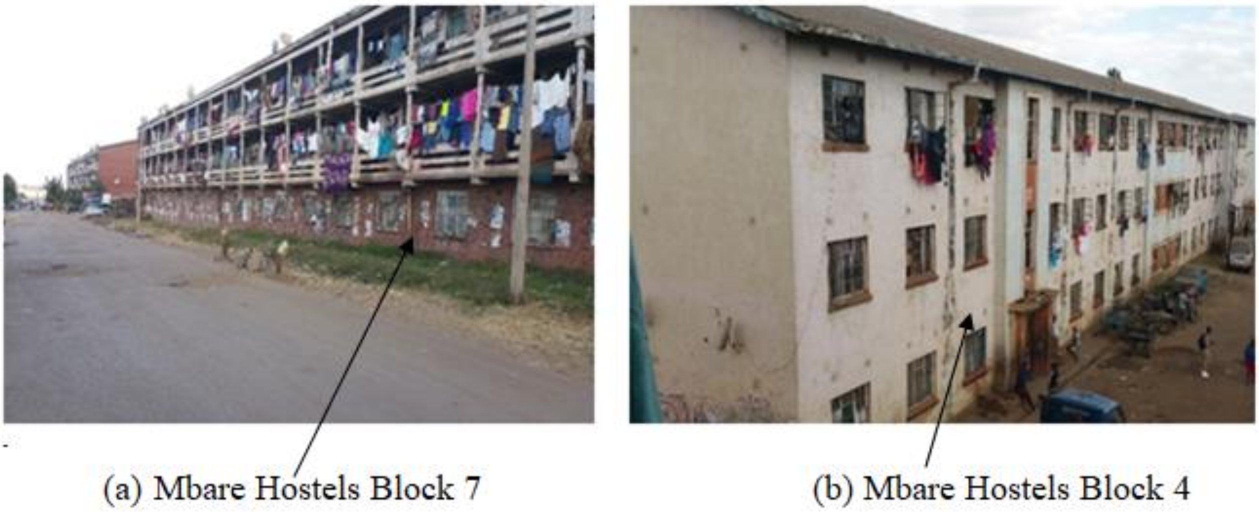

Figure 3.3 Mbare Hostels

Source: Author

3.3 Population and sampling

Sampling was conducted on the study populations from two sites (i) Mbare Musika Agricultural Produce Market and (ii) Mbare Hostels for purposes of procuring raw materials and conducting the cross-sectional study on consumer acceptability of the supplementary food blends.

3.3.1 Procurement of white maize at Mbare Musika Agricultural Produce Market

A total of 30 traders selling white maize grain at Mbare Musika Agricultural Produce Market were purposively selected from a population of over 300 traders selling varying agricultural produce and/or commodities at the market. The final sample of 3 traders from which a total of 60kg of white maize was purchased from were randomly selected from the pool of 30 due to limited funds. The minimum quantity of white maize grain that one can buy from traders at the market is 20kg at a cost of USD6.00. The hat-and-draw method was used for random sampling purposes due to its simplicity and low cost. The method was found to be suitable as the traders were selling the maize at designated trading spaces and hence could be easily assigned unique numbers.

A total of 30 small pieces equal dimensions and made from the same material were each labelled with a unique number from 1 to 30. The numbered pieces of paper were then put in a hat in a random order then thoroughly but carefully mixed up by hand to avoid dropping any of the pieces of paper. After this, 3 numbered pieces of paper where picked by hand one at a time without looking into the hat. The picking was done without replacement. The traders who had been assigned numbers corresponding to those on the 3 numbered pieces of paper that were drawn from the hat were then used to form the sampling subset. A total of 3 samples of white maize each weighing 20 kg were then purchased from the selected 3 traders. The traders provided the white maize most preferred by consumers based on their experience and trading volumes. Representative samples of white maize grain (1.67 kg) were then drawn from each of the 3 samples using a compartmented hand probe, weighed using a digital scale and mixed to obtain a 5 kg composite sample according to the National Biotechnology Authority sampling guidelines for bagged grain (unpublished). The composite sample was then stored in a clean and airtight plastic container for further use.

3.3.2 Consumer acceptability study

The study employed multistage sampling to select a sample of participants for the sensory acceptability study in Mbare Hostels which comprises of 9 individual blocks (block 1 to 9). Mbare Hostels was purposively selected as the study area due to urban location, its high density and low income status as targeted by the study. The 9 blocks of Mbare Hostels were treated as clusters and from these a total of 5 clusters were then chosen using simple random sampling. This was accomplished using the hat-and-draw method. A total of 9 small pieces equal dimensions and made from the same material were each labelled with a unique number from 1 to 9. The numbered pieces of paper were then put in a hat in a random order then thoroughly but carefully mixed up by hand to avoid dropping any of the pieces of paper. After this, 5 numbered pieces of paper where picked by hand one at a time without looking into the hat. The picking was done without replacement.

Snowball sampling was then used to identify all women caregivers (n=104) of at least a single child aged 6-59 months from all the 5 blocks. A total of 83 women caregivers who met the set inclusion criteria for the study (listed in the next paragraph) and were willing to take part in the study were purposively sampled. From these, random sampling using the hat-and-draw method was then employed to select a subset of 6 respondents from each of the 5 blocks thus the final sample size for the study was 30. This was done in a similar manner already described in the random sampling of blocks. From the remaining 29 unselected women caregivers, a further 5 women (one from each of the 5 blocks) were also randomly selected to take part in a pilot study only.

The following inclusion and exclusion criteria was used in determining the eligibility of women caregivers sensory acceptability. To be eligible to take part in the sensory evaluation, each participant had to meet all of the following requirements:

i. Be a woman aged 18 years and above who has at least a single child aged 6 to 59 months in their care at the time of the study.
ii. Be from a household that uses maize-based porridge to feed its child or children of the aforestated age group at least 3 days a week.
iii. Be a current resident of Mbare Hostels and staying in one of the selected blocks.
iv. Personally consent to take part in the evaluation.
v. Have no known history of being allergic to all the ingredients of the test meals.
vi. Have no aversions to the test meals.

3.4 Research design

The study employed a mixed method design as it combined the experimental design and survey (cross-sectional study design). The experimental design was used for formulating the supplementary foods. A single factor Completely Random Design (CRD) was used in the laboratory analysis of vitamin A, iron and zinc contents of the raw materials. The crop was used as a factor with white maize, biofortified maize and biofortified sugar beans as independent variables and vitamin A, iron and zinc as dependent variables. Quantitative on the nutritional composition of the raw materials and the supplementary food were collected.

The cross sectional survey was used to gain a quick understanding of the magnitude of variation in acceptance of supplementary food blends among consumers with varying demographic characteristics at a specific point in time. This information is difficult to measure using observational techniques. Also, a cross-sectional study design was employed due to its simplicity involving only one contact with the study population and therefore comparatively cheap to undertake and easy to analyse. The study had 2 control groups (100% white maize and 100% orange maize) and 2 experimental groups (blended orange maize-sugar bean blends in ratios 50:50 and 67:33.The survey used pre-coded questionnaires to collect quantitative data on the acceptability of supplementary food blends by consumers.

Both the experimental design and cross-sectional study design provided a rationalised and justified procedural plan for operationalizing variables so they can be measured, selecting the study sample, collecting quantitative data for testing the study hypothesis and analysing the results. The choice to collect quantitative data was on the premise that it can be easily analysed by statistical methods in order to accept or refute alternative knowledge claims. The research process flow is presented in Figure 3.4 below.

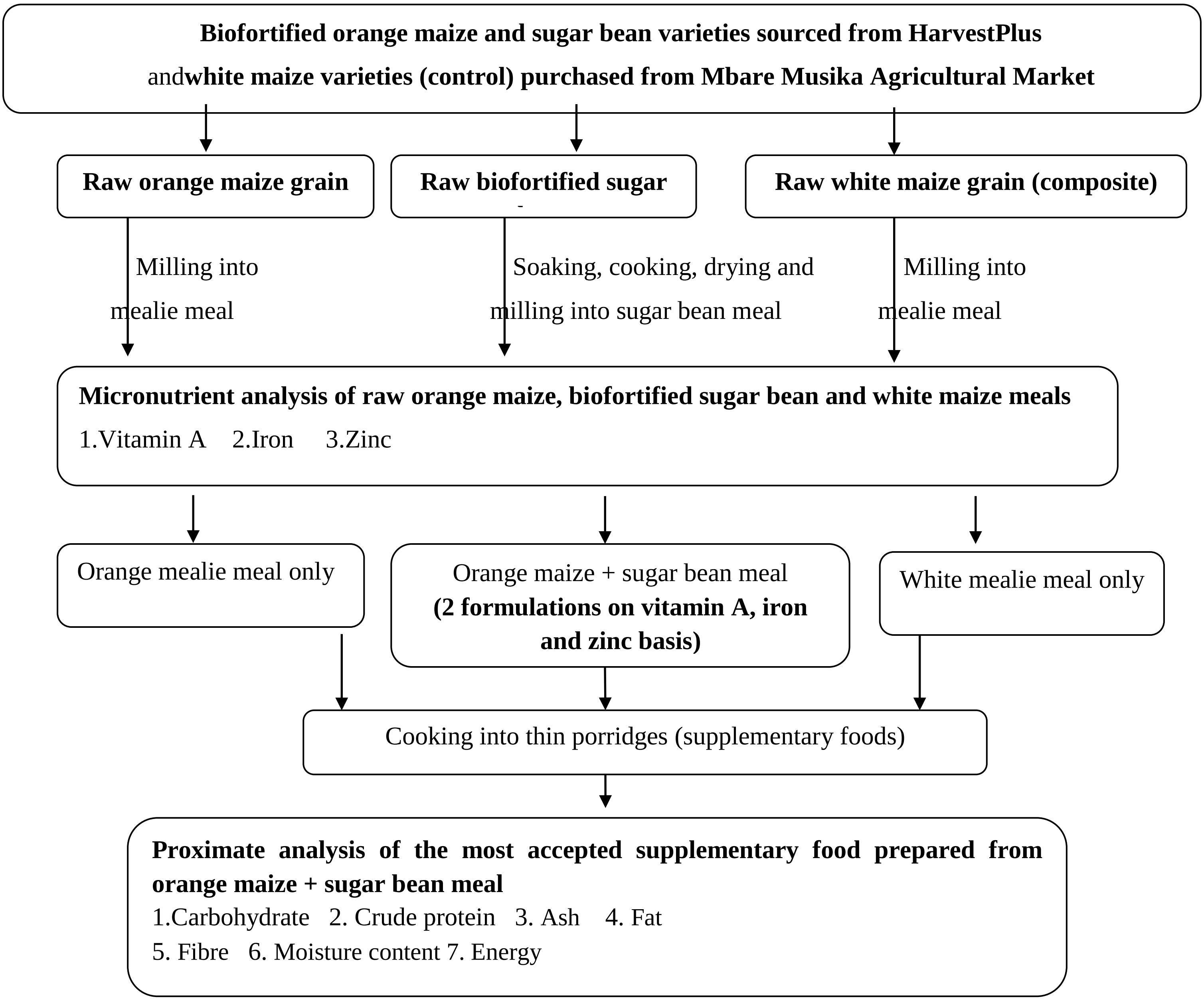

Figure 3.4 Research process flow diagram**.**

Source: Author

3.4.1 Experimental food formulation

All work on the food formulation was carried out at National Biotechnology Authority GMO Testing Laboratory.

3.4.1.1 Preparation of ingredients

i. Cleaning and sorting of raw materials

The raw food commodities (5 kg each; n=3) shown in Figure 3.5 (a – c) below were thoroughly cleaned from dust, extraneous materials and admixture of other food grains by winnowing prior to use. Sorting of the grain was done using an aperture sieve. Visual inspection was used to check for damaged and immature grains which were then removed by hand.

Figure 3.5 (a) White maize grain **Figure 3.5 (b)** Orange maize **Figure 3.5 (c)** Biofortified sugar beans

Figure3.5 Raw white maize, orange maize and biofortified sugar beans

Source: Author

ii. Preparation of maize meal

The cleaned samples of white maize and orange maize were then ground until fine and homogenous meals were obtained using a clean stainless steel electric blender to pass a 1.0 mm screen. Samples of the fine maize meals were then packed in labelled, dark, air tight polythene bags and stored in a refridgerator at 4°C for further use.

iii. Preparation of sugar bean meal

The sugar beans was soaked overnight (12 hours) and then cooked by boiling for 90 minutes on an electric plate. Boiling reduces the content of anti-nutritional factors present in pulses and legumes. The cooked sugar beans were then oven dried at 50°C for 4 hours to improve its keeping qualities and also to allow for dry milling which is common in Zimbabwe. The dried sugar beans were then roasted using an electric plate on high heat for 25 minutes. Roasting enhances the taste, flavour and digestibility of food. The roasted beans were then allowed to cool down to room temperature. The roasted beans were ground using a clean stainless steel electric blender to produce fine and homogenous sugar bean meal to pass a 1.0 mm screen. Samples of the fine bean meal were then packed in labelled, dark, air tight polythene bags and stored in a refridgerator at 4°C for further use. The meals of all the 3 raw materials are shown in Figure 3.6 (a – c) below.

Figure 3.6(a) White maize meal Figure 3.6(b) Sugar bean meal Figure 3.6(c) Orange maize meal

Figure 3.6 Milled white maize, orange maize and biofortified sugar beans.

Source: Author

3.4.1.2 Carotenoid, iron and zinc analysis of the meals

The total iron and zinc contents of white maize, orange maize and sugar bean meal were determined by Inductively Coupled Plasma-Optic Emission Spectrometry (ICP-OES) using a (Model ICAP 6000, Thermo-Fischer Scientific). For each sample, the meal was ground into fine powder and transferred to 100ml beakers. A closed-tube digestion method was then used for digesting samples (Wheal et al., 2011). Working standards for the iron and zinc were prepared by serial dilution of the standard solutions. Certified Standard Reference Material provided by Institute for Reference Materials and Measurements of the European Joint Research Centre were used for method and results validation. The tests were done in triplicate for each sample meal.

The β-carotene, β-cryptoxanthin and α-carotene content of the meals were determined by High-Performance Liquid Chromatography (HPLC) method described by Pillay et al., (2011) and de Carvahlo et al., (2012) respectively. Total provitamin A concentration was expressed as β-carotene equivalents, calculated using the formula:

Total provitamin A concentration =β-carotene+(β-cryptoxanthin+α-carotene)/2.

The sum of β-cryptoxanthin+α-carotene was divided by 2 because the vitamin A activity of each of these 2 carotenes is half (50%) that of β-carotene. All tests were done in triplicate.

3.4.1.3 Blend formulation

A total of 2 supplementary food blends were formulated by combining meals of biofortified maize and biofortified sugar bean in different ratios indicated in Table 3.1 below. The treatments for the blends were determined to provide predetermined percentages of the WHO/FAO, (2004) average RDI for vitamin A, iron and zinc per 100g on dry weight (DW) of the food blend for children aged 6 to 59 months as indicated in table 3.1 below. The calculations were done using the results of provitamin A, iron and zinc analysis for white maize, biofortified maize and biofortified sugar bean as shown in Table 4.1 and Table 4.2 (Chapter 4 section 4.3 Results and Discussion). From the results, biofortified maize contained statistically significant higher mean concentration of provitamin A carotenoids expressed in β-carotene equivalents (9.958 µg/g DW) compared to both white maize and biofortified sugar bean ($p<0.01$). Biofortified sugar bean contained the statistically significant higher concentrations of iron and zinc (32.68 µg/g DW and 77.20 µg/g DW µg/g DW respectively) compared to both biofortified maize and white maize ($p<0.01$). As a result, biofortified maize and biofortified sugar bean were selected for formulating the food blends. Since maize is the dominant staple food and energy source among the study's food commodities, it was used as the principal ingredient in all the blends.

The 2 controls for the study consisted of 100% white maize meal designated treatment 1 (T1) and 100% biofortified maize meal designated treatment (T2) as shown in Table 4.1. Treatment 3 and treatment 4 consisted of blended biofortified maize and sugar bean meal combined in the ratios 50:50 and 67:33 respectively. The experiments followed a completely randomized design with 3 replications per treatment.

The determination of the formulations (blending ratios) for T3 and T4 were computed in stages (i-iv) as follows:

i. Calculation of the average RDI for each of the 3 micronutrients (vitamin A, iron and zinc) using the WHO/FAO (2004) RDI guidelines presented in Chapter 3 (sections 2.2.1–2.2.3) as follows for the calculations:

   Average RDI = (RDI for the 0-3 age group + RDI for the 4-6 age group) divided by the number of age groups. The number of age groups for all micronutrients was 2.

- Average RDI for vitamin A = (200 + 200) RE ÷ 2

= <u>200 RE</u>

RAE, retinol activity equivalents. 1RE = 1µg retinol

= 12 µg β-carotene (WHO/FAO 2006).

Therefore 200 RAE = 12 x 200 µg β-carotene = <u>2400 µg β-carotene</u>

- Average RDI for iron from food with 10% bioavailability = (5.8 + 6.3) mg ÷ 2

= <u>6.05 mg</u>

Classification of study's food blends as foods with a 10% iron bioavailability was done using the WHO/FAO (2004) guidelines.

- Average RDI for zinc from food of moderate bioavailability = (4.1 + 4.8) mg ÷ 2

= <u>4.45 mg</u>

Classification of study's food blends as foods of moderate zinc bioavailability was done using the WHO/FAO (2004) guidelines.

ii. Estimation of the final vitamin A content in biofortified maize (as the major source of vitamin A in the blends) and iron and zinc in biofortified sugar beans (as the major source of iron and zinc in the blends) guided by the reported percentage (%) retention for the micronutrient in the food constituent after processing including cooking from literature. Calculations were done using the formula:

Final micronutrient content = (average micronutrient content in the major source of the

= micronutrient in the blended food/100g x % retention)

For maize, the β-carotene equivalents (BEC) retention of 100% was assumed as previous studies involving a combined 40 genotypes of yellow/orange maize reported that cooking by boiling for 20 to 30 minutes increases the carotenoid content (Mugode et al., 2014, Muzhingi et al., 2008). The β-carotene equivalents retention for sugar beans were not estimated as the total provitamin A content in this food constituent were confirmed to be significantly low and therefore considered to be negligible from laboratory analysis results presented in Table 4.1 (Chapter 4 section 4.3 Results and Discussion).

The sugar beans used in this study were first soaked and the boiled thus the average true retentions of 92,2% and 85.6% for iron and zinc respectively after soaking and boiling of beans reported in a recent study by Hummel et al., 2020) involving 3 biofortified bean varieties were adopted in this study for estimating the final iron and zinc content of the sugar beans after processing as follows:

Final iron content of processed sugar beans = 92.20 x (77.20 ÷ 1000) mg/100g
= <u>7.12 mg/100g (2 d.p)</u>

Final zinc content of processed sugar beans = 85.6 x (32.68 ÷ 1000) mg/100g
= <u>2.78 mg/100g</u>

The iron and zinc retention after processing for maize were not estimated as the iron and zinc contents in this food constituent were confirmed to be significantly low and therefore considered to be negligible from laboratory analysis results presented in Table 4.2 (Chapter 4 section 4.3 Results and Discussion).

iii. Set the minimum target percentages of the average RDIs for vitamin A, iron and zinc per 100g of the 2 food blends guided by the recommended RDIs children aged 1-6 years by WHO/FAO (2004) and the micronutrient contents of the individual food constituents as presented in presented in Table 4.1 and 4.2 (Chapter 4 section 4.3 Results and Discussion). The formulations were targeting to produce at least one blend of biofortified maize and sugar that could meet at least 20% (one fifth) of the recommended daily intakes for vitamin A, iron and zinc respectively. The formulations were also guided by the prevalence rates of VAD (25%), iron deficiency (72%) in Zimbabwean children under the age of 5 (National Nutrition Micronutrient Survey, 2015; UNICEF, n.d) and the estimated percentage of the Zimbabwean population (48%) including children that is at risk of zinc deficiency (Wessells and Brown, 2012). Iron deficiency is therefore the most prevalent deficiency in Zimbabwean children and this was considered in the formulating the blends.

iv. Calculation of the required blending ratio to meet the target percentages of the average RDIs for vitamin A, iron and zinc per 100g of the food blend for children aged 6-59 months was done as follows:

The amount of the biofortified maize required to meet the target percentage for example 38% of the average RDI (200µg) for vitamin A retinol activity equivalents in supplementary food T3 (as indicated in Table 8 below) was computed as shown below.

$$\text{Target \% of the RDI} \quad = \frac{amount\ of\ provitamin\ A\ in\ maize\ in\ RAE}{Average\ RDA\ in\ RAE} \text{ x } 100$$

(25 x 200 RAE µg) ÷ 100 = amount of provitamin A in maize
Therefore the amount of provitamin A in maize = 50 RAE µg
Converting RAE to provitamin A in beta carotene equivalents µg using a factor of 12.
Therefore the amount of provitamin A in maize = 50 x 12 µg

= 600 µg

Using results from the laboratory provitamin A analysis presented in Table 4.1 (Chapter 4 section 4.3 Results and Discussion), 100 g of biofortified maize contains 995.8 µg of provitamin A and therefore 912 µg of provitamin A is supplied by 60g of biofortified maize. Since the blends are per 100g the amount of sugar beans to be added will be calculated as 100g – 60g = 40g. Therefore the blending ratio of biofortified maize:biofortified sugar bean for treatment T3 was 60:40 as shown in Table 1 below.

The percentages of iron that can be provided by the 8g of sugar beans were then calculated as a proportion of 100g using the final iron content of sugar beans after processing.

Final iron content of processed sugar beans = 7.12 mg/100g

Therefore iron content of 8g of sugar beans = (40 ÷ 100) x 7.12 mg

= 2..85 mg

$$\text{\% of the RD1 for iron} = \frac{iron\ content\ in\ a\ given\ amount\ of\ sugar\ bean}{Average\ RDA\ for\ iron\ for\ children\ aged\ 1-6\ years} \text{ x } 100$$

= (2.85 mg ÷ 6.05 mg) x 100

= 47% (0 d.p)

Final zinc content of processed sugar beans = 2.78 mg/100g

Therefore zinc content of 4g of sugar beans = (40 ÷ 100) x 2.78 mg

= 1.11 mg

$$\% \text{ of the RD1 for zinc} = \frac{zinc\ content\ in\ a\ given\ amount\ of\ sugar\ bean}{Average\ RDA\ for\ zinc\ for\ children\ aged\ 1-6\ years} \times 100$$

= (1.11 mg ÷ 4.45 mg) x 100

= 25% (0 d.p)

Table 3.1 Treatments for food constituents and the estimated % of the average RDIs for vitamin A, iron and zinc that they can meet per 100g DW

Ingredients	**Treatments**			
	1 (T1)	**2 (T2)**	**3 (T3)**	**4 (T4)**
White maize	100 (control 1)	0	0	0
Orange maize	0	100 (control 2)	50	67
Sugar beans	0	0	50	33
Minimum target % of the average RDI for vitamin A, iron and zinc per 100g to be met by the treatment	-	-	1. 20% (vitamin A) 2. 20% (iron) 3. 20% (zinc)	
Estimated % of the average RDI for vitamin A, iron and zinc per 100g that can be met by the treatment	<1.2 % for all 3	1. 41.5 % (vitamin A) 2. <1% for both iron and zinc	1. 25% (vitamin A) 2. 47% (iron) 3. 25% (zinc)	1. 28% (vitamin A) 2. 39% (iron) 3. 21% (zinc)

The 2 formulated food blends T3 and T4 (Figure 7.7(a) and 7.7(b)) were then used to prepare thin porridges for the consumer acceptability study with the 100% white maize meal and 100% biofortified maize being used as controls.

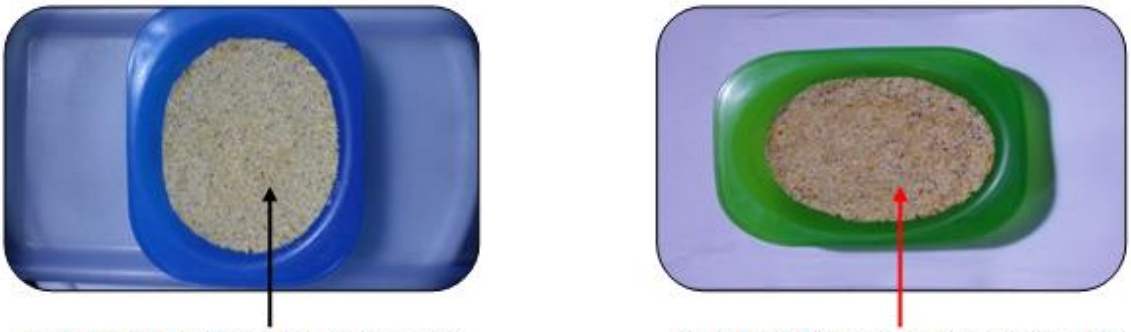

Figure 3.7(a) Blend formulation T3

Figure 3.7(b) Blend formulation T4

Figure 3.7 Blend formulations T3 and T4
Source: Author

3.4.2 Cross-sectional survey for sensory acceptability of supplementary food

The survey was conducted in Mbare, Harare as described in the following subsections.

3.4.2.1 Preparation of the supplementary porridges

Two women caregivers residing in Mbare Hostels with appropriate experience in cooking maize based porridge for children were recruited to cook the thin supplementary porridges for both the pilot and main study. Both cooks were not included as participants in the sensory evaluation of the porridges. For the pilot study, the thin porridge was prepared from orange maize only. For the main study, the thin porridges were prepared from each of the 4 meals prepared separately using 100% white maize, 100% orange maize, orange maize:sugar beans ratio 50:50 and orange maize:sugar beans ratio 67:33) on the day that the sensory evaluation was conducted.

Separate but similar pots and utensils were used for cooking each of the meals to avoid any carry-over. All the thin porridges regardless of their base ingredients were prepared using a standardized recipe. For each cooking run, clean tap water (1 500 ml) was brought to boil in a stainless steel pot using a hot plate on high heat (plate control setting 6). Two cups (200 g) of any given meal were added to 380 ml of cold water in a bowl and stirred for 2 minutes using a wooden spoon to obtain a homogenous paste. Soon after, the paste was added to the boiling water, stirred until smooth and left to boil with occasional stirring. The mixture was cooked on high heat for 20 minutes with the pot lid on and only temporarily lifted to allow for stirring of the contents as shown in Figure 3.8 below. Salt (1g) was added to the porridge during cooking. Sugar (40g) was immediately added to the porridge once it was cooked. The thin porridge was then allowed to cool down to the serving temperature of 40°C. The porridge was kept in a separate insulated box in order to maintain its temperature until served. For the main sensory study, >3 500 ml of thin porridge was prepared for each of the study's 4 meals to allow for 50 g servings in duplicate to each of the 30 panellists.

Figure 3.8 Cooking of one of the thin porridges in progress

Source: Author

3.4.2.2 Pilot study

A pilot study of the sensory evaluation was conducted in Mbare using, Shona-speaking African women caregivers (n=5) from Mbare Hostels with the help of 2 trained research assistants. The researcher with the help of the assistants gathered the untrained panelists in a single room and briefly explained the purpose and procedure of the study to all of them. Furthermore, the researcher informed the caregivers of the study's ethical considerations including the right of participants to withdraw from the study even after having initially consented to take part in the study.

Each of the participants were then handed a consent form either in Shona or English depending on the participant's preference and given time to go through it, ask the researcher questions (if any) and only append their signatures upon making an individual decision to participate in the study. Consenting participants (seated one metre apart) were then each given the study's pre-coded questionnaire, a new pen for use in filling the questionnaire and a clean plastic spoon for use in eating the porridge. Each of the 5 caregivers was then served with 50 ml of white maize-based thin porridge with a temperature of 40°C in a small, clean 100 ml disposable plastic cup by the research assistants. The porridge was one of the test meals for the main study and was prepared on the day of the pilot study using the recipe described in section 3.4.2.1 which was later used for all the test meals of the main study. The participants were asked to taste the thin porridge and indicate their rating for 5 sensory attributes on the questionnaire using a 5 point hedonic scale. The participants were asked to express any comments on the questionnaire and the organisation of the study whether positive or negative.

The main findings of the pilot study were as follows:

1. The study to be conducted during the period 9 am to 12 am to allow the participants enough time to first finish their morning chores before presenting themselves for the study and also prepare afternoon meals for their families.
2. The participants indicated that they preferred to initially be grouped into batches and be given a starting time for their evaluation session. This would allow the batch members to report for their session at the allocated times rather than wait for their turn outside the study room.
3. The participants were satisfied with the structure, length and simplicity of the questionnaire.
4. It took an average of 2.5 minutes for a panellist to complete the evaluation of a single test meal including filling in the questionnaire.

The pilot study served to pre-test the research instruments (questionnaires) for face validity and determine how they would work in practice. The main findings of the pilot study were used to adapt the study and its instruments accordingly.

3.4.2.3 Sensory evaluation (main study)

Sensory evaluation of the thin porridges prepared from each of the food constituents (n=4) presented in Table 5 was conducted in Mbare Hostels by a total of 30 women caregivers (untrained panellists). All thin porridge samples were prepared on the day of the study using the recipe described in section 3.4.2.1 by the same experienced cooks who had prepared thin porridge for the pilot study.

The following precautions were taken before the study commenced. These were mandatory to everyone including the researcher. The body temperatures of each of the participants, cooks and research team were checked using an infra-red thermometer and anyone found to have a temperature above 37.2 °C as per WHO guidelines for COVID was not permitted to take part in the study. Any individual with any flu like symptoms including headache, coughing, sneezing and running nose was not allowed to take part in the study. It was also mandatory to put on a face mask throughout the study unless when tasting the food samples. One litre of commercial hand sanitizer was provided for mandatory cleaning of hands upon entering the study room as well as upon leaving the study room. The surface of the dining table used by the panellists was thoroughly sanitized before each batch of panellists were allowed to use it. Participants were not allowed to bring any accompanying person(s), pet, electronic gadget or anything into the room.

The study commenced in the morning. The panellists were grouped into batches of 3 as they arrived at the study room and were immediately allocated a specific time to return for their evaluation session. Upon returning, the researcher informed each of the 10 batches of panellists of the study's purpose, procedure and the rights of the participants including but not limited to confidentiality and withdrawal from the study even after having initially consented to take part in the study. Consent was obtained from all the panellists who participated in the study using the same procedure followed during the pilot study described in section 3.4.2.2. The research team also sought verbal consent from the participants for the purpose of taking photographs and use in any publications on this study. Sensory evaluation was carried out in small groups of 3 panellists using the same well lit room at room temperature (25°C) for all the groups. The panellists were seated one metre from each other to maintain social distancing and were not allowed to have any discussion with each other during the evaluation to reduce chances of influencing each other's decisions and as a precautionary measure against the spread of Coronavirus-19 (COVID-19). Participants who wanted assistance or anything were instructed to simply their hands and encouraged to communicate their message in writing whenever possible. Only a single research assistant was allowed to be in attendance for any given batch of panellists to ensure a minimum number of people in the room.

Each panellist was given the study's pre-coded questionnaire, a new pen straight from the box for use in filling the questionnaire and a clean plastic spoon for use in eating the porridge. All the panellists were served with 50 ml of each of the 4 thin porridges with a temperature of 40°C in clean, disposable 100 ml plastic cups by the research assistants. The servings were done in duplicate for each test meal. The participants were allowed to request for more of any of the thin porridges for evaluation purposes. The serving was done in an order such that the 3 participants were given a different sample from that of the rest of the batch for each round of tasting and were informed about this so as to limit chances of bias among participants. Furthermore, the polyestrene cups were labelled with three digit random codes that were generated using an online random number generator (www.calculatorsoup.com). Blind presentation was meant to reduce bias and allow the panelists to focus only on the sensory characteristics of the test meals. Panelists were neither shown the packaging and no given information deemed to possibly cause biased evaluations.

All panellists were asked to try the samples and rate the degree to which they liked the taste, appearance, aroma, texture and overall acceptability of each sample on a 5-point hedonic scale (1 = dislike very much, 2=dislike like moderately, 3=neither like nor dislike 4=like moderately and 5 like very much). All panellists were provided with water in clean 100ml disposable cups to rinse their mouths in-between testing of the samples to avoid carry over effect. The supplementary food blend with a higher overall acceptability between T3 and T4 was then subjected to proximate analysis

3.4.3 Proximate analysis of supplementary food

The carbohydrate content, crude protein, crude fat, crude fibre, ash and moisture contents of the supplementary food were determined according to standard methods described by (AOAC, 2020). The analysis were done using analytical grade reagents and standard stock solutions and double distilled water. Clean glassware soaked in 10% nitric acid overnight and washed with distilled water and dried in an oven were used. Energy value of the supplementary food was calculated using the following from fat, carbohydrate and protein contents using Atwater’s conversion factors using the formula:

Energy in (Kcal) = 4 x protein value+ 4x carbohydrate value + 9 x fat value

Determination of crude protein content

The crude protein content will be determined using the standard method described by the Association of Official Analytical Chemists (AOAC) (2000).Kjeldahl catalyst was used. A protein nitrogen factor of 6.5 was used for calculation of crude protein content.

Determination of crude fat

The fat content of the supplementary food triplicate samples was determined according to the Soxhlet procedure, using a Soxhlet Fat extractor according to the method described by AOAC (2000). Petroleum ether was used for extraction.

Determination of ash (total mineral) content

The ash content was determined using a standard method described by AOAC. The hunter process was utilised in the analysis of the individual minerals (AOAC, 2000).

Determination of moisture content

The moisture content was determined using a standard method described by AOAC (2000) in which the samples of known weight were put in evaporating dishes and dried in an oven set at 60^0C for 8 hours until a constant as achieved. The moisture content of the food products was determined by weight difference formula as given in the method.

3.5.1 Calorimetric determination of available carbohydrates by DNS method

The carbohydrate content was determined using the AOAC standard DNS method (AOAC, 2000).

Crude fibre content

The crude fibre content will be determined using a standard method described by AOAC, (2000). In which petroleum free from fat was used for sample extraction on a Soxhlet apparatus.

Gross Energy determination

This was calculated using the formula:

Energy in (Kcal) = 4 x protein value+ 4x carbohydrate value + 9 x fat value

3.5 Data collection procedure

3.5.1 Nutritional analysis

Quantitative data on the nutritional composition of raw materials and supplementary food blend were collected through laboratory analysis using standard methods. Each determination was carried out in triplicate and results reported as mean value ± standard error. Data was captured into SPSS version 20.

3.5.2 Consumer acceptability study

A survey on the sensory acceptability of four different thin porridges was conducted in Mbare Hostels with an untrained panel of 30 women caregivers. Quantitative data on the sensory acceptability of the thin porridges were collected using group administration of pretested close ended questionnaires. The data collected included demographic characteristics of 30 consenting adult participants, the degree to which individual panellists liked or disliked the liked the taste, appearance, aroma, texture and overall acceptability on a 5 point hedonic scale for all the thin porridge samples. Data from the acceptability survey were cleaned and captured into SPSS version 20.

3.5.3 Validity and reliability

The questionnaire for the acceptability survey was pretested for face validity using women caregivers from Mbare Hostels. These households were not considered in the main study. Based on this study the data collection instrument (questionnaire) was revised to make sure that it was clear, understandable and gathered the intended data. All the nutritional analysis runs were done using calibrated equipment, analytical grade reagents and thoroughly cleaned glassware to avoid contamination. Runs were conducted in triplicate to check for consistency of results and reliability of data.

3.6 Data analysis procedure

3.6.1 Nutritional analysis

The data obtained from the nutritional analysis of study samples were statistically analysed in complete randomized design for analysis of variance (ANOVA) for comparing means of the samples using SPSS version 20.0. Post-hoc Tukey HSD test was used to separate the means. Significant differences were determined at the 0.01 level of significance. The results were presented in tabular form.

3.6.2 Consumer acceptability study

All collected data were entered into and analysed using SPSS version 20. At the data entry stage, responses from the 5–point hedonic scale ratings of the 4 study meals by panellists were coded as follows for all the 5 attributes (1=Dislike extremely, 2=Dislike moderately, 3 = Neither like nor dislike, 4=Like moderately, 5= Like extremely). Non-parametric data from acceptability analysis were assessed using the non-parametric ANOVA, which is the Kruskal-Wallis test. The Post-hoc Tukey HSD test was used to separate the means. The results were presented in tabular form. Significant differences were determined at the 0.05 level of significance

3.7 Ethical considerations

Ethics are a key consideration in research. Research ethics dictates that participants should be provided with sufficient and accessible information about the research so that they make informed and independent decisions as to whether to be involved or not. As such, the researcher explained to the assessors the purpose of the study, without exaggerating or understating the benefits or risks. Great effort was made to ensure that sampling of participants and traders was done as fairly as possible by strictly following the set inclusion and exclusion criteria and sampling plan for the study. No study participant was coerced into participating in the study against their will. Panellists were allowed to withdraw from the panel at any time, without any penalty being levied on them. The researcher strived to preserve confidentiality of all sensitive information gathered from the participants and no personal information that could lead to the identification of the participant was collected using the survey questionnaire. Each questionnaire had a slot for respondent number as a way of protecting the identity of the respondent. Furthermore, responses from this evaluation were presented in aggregate form only.

In light of the COVID-19 pandemic, the safety of the study participants was highly prioritised. Necessary precautions were taken and national and WHO guidelines were followed in the highest level of strictness and diligence. The researcher was well aware that study work must not cause any harm or injury to the participants and there safety must be prioritised at all costs. To avoid plagiarism, sources of text and graphics used in this research work were acknowledged. The researcher did not use any pirated content of any form in this study. Collected data from the study was analysed without any devious alteration or falsification meant to alter the true findings of the study.

3.8 Summary

This chapter provided a description of the 2 study sites for purchase of raw materials (Mbare Musika Agricultural Produce Market) and sensory evaluation (Mbare Hostels) both located in

Mbare, Harare. The research employed a mixed study design (experimental and cross-sectional survey) in formulating the food blends and evaluating the sensory acceptability of supplementary food blends respectively. The blending experiments followed a completely randomized design with 100% white maize and 100% biofortified maize as controls.The biofortified maize and sugar beans were combined in the ratios (50:50 and 67:33) to produce 2 food blends. The formulations were guided by the vitamin A, iron and zinc contents of the raw ingredients established through laboratory nutritional analysis and the RDIs for children aged 6–59 months. The cross-sectional survey was conducted using 30 consenting women caregivers following a pilot study for pre-testing the research questionnaire (data collection instrument). Thin porridges prepared using the 2 formulated blends of biofortified maize-sugar bean were subjected to sensory evaluation with thin porridges prepared solely on white maize and biofortified maize as controls. The chapter also described the ethical considerations of the research.

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CHAPTER 4

Formulation and nutritional evaluation of biofortified maize (*Zea mays*) and sugar bean (*Phaseolusvulgaris*) blend as supplementary food for children aged 6-59 months

Abstract

The high rates of micronutrient malnutrition particularly at global level has been attributed to continued consumption of poor diets that lack diversity and essential micronutrients. The consumption of micronutrient rich biofortified cereals and legumes bred for increased levels of vitamin A, iron and zinc can complement existing strategies aimed at tackling malnutrition. This study aimed to formulate biofortified maize-sugar bean blend and evaluate its nutritional quality as supplementary food for children aged 6-59 months. The grains of locally available provitamin A-biofortified maize, iron-biofortified sugar beans and white maize (control) were analysed for carotenoids, iron and zinc using standard and referenced methods. The analysis results showed that biofortified maize had significantly high total provitamin A carotenoid concentration (9.958 µg/g DW) compared with biofortified maize and white maize (control). The biofortified sugar bean had significantly high iron and zinc concentrations (32.680 µg/g and 77.203 µg/g DW respectively) compared to both biofortified maize and white maize. The biofortified maize and sugar bean were then combined in different proportions (50:50 and 67:33) to formulate meal blends on vitamin A, iron and zinc basis. The blends were formulated to meet at least 20% of the recommended daily intakes of vitamin, iron and zinc for children aged 6-59 months.

The blends were subjected to sensory evaluation for acceptability. The biofortified maize and sugar bean blend (50:50) was the more accepted. The more accepted blend (50:50) was then analysed for proximate composition using standard methods. The blend had an acceptable crude fibre content of 4.5 g/100g as per the Codex Alimentarius guidelines on formulated complementary foods for children. The blend also had a low moisture content of 7.7% indicating that it has good keeping qualities. The proximate analysis results indicate that biofortified maize-sugar bean blends are good sources of energy, protein and carbohydrates and should be used in tackling protein energy malnutrition (PEM).

- 5 keywords: **orange maize, biofortified, supplementary food**

4.1 Introduction

The high rates of micronutrient malnutrition particularly at global level has been attributed to continued consumption of poor diets that lack diversity and essential micronutrients (Govender et al., 2017 with children being the most affected. An estimated 10.8 million child deaths occur annually due to Fe, Zn and vitamin A deficiency WHO (2002). Children are a high priority group due to high nutritional demands for growth and the need to end the cycle of intergenerational micronutrient malnutrition. This is of importance to Zimbabwe where 25% of the children have Vitamin A deficiency (VAD), 72% are living with iron deficiency and 33% have iron deficiency anaemia (National Nutrition Micronutrient Survey, 2015; United Nations International Children's Emergency Fund (UNICEF), n.d.). About 48.4%of the Zimbabwean population including children is at risk of inadequate zinc intake (Wessells and Brown, 2012).

Low income households from both urban and rural settings usually rely on low cost but often poorly formulated and therefore nutritionally imbalanced maize based complementary foods for feeding their children due to prohibitive cost of commercially fortified complementary foods and nutritionally dense foods of animal origin. This might explain the persistent trident malnutrition problem of vitamin A, iron and zinc deficiency in children under the age of 5 in Zimbabwe where white maize (Zea mays) is a major part of the diet. White maize (*Zea mays*) is devoid of provitamin A carotenoids (Nuss and Tanumihardjo, 2010) which are required to prevent VAD in children. This is where locally produced biofortified maize also known as orange maize conventionally bred for increased levels of provitamin A carotenoids, which are precursors of vitamin A in the body, can succeed as an alternative type of maize.

Given that children commonly consume cereal based supplementary food mostly in the form of porridge referred to as *bota*in Shona throughout early childhood, utilizing orange maize in such foods can go a long way in complementing efforts aimed tackling VAD in children. However, iron and zinc also deserve attention and hence there is need to combine orange maize with iron and zinc rich sugar beans. Home based food-to-food fortification of orange maize based food with biofortified sugar bean (*Phaseolus vulgaris* L.) rich in iron and zinc (Bouis and Welch, 2010) is an interesting and plausible approach for producing high quality supplementary food containing significant levels of vitamin A, iron and zinc. However, this should be guided by the nutritional composition of the constituent raw materials and the recommended dietary intakes for the target consumers.

Data on the nutritional composition of locally available biofortified crops in Zimbabwe is lacking. This is despite that biofortified crops are micronutrient rich and can fulfil a significant portion of Estimated Average Requirement (EAR) of iron (up to 35%) and vitamin A (> 50%) when consumed as staples (Bouis and Welch, 2010; HarvestPlus, 2018. Though promising, the use of biofortified legumes in enriching biofortified cereal based supplementary food for instance porridge for children is uncommon and remains largely unexplored in Zimbabwe. This study aimed to formulate biofortified maize-sugar bean blend and evaluate its nutritional quality as supplementary food for children aged 6-59 months.

4.2 Material and Methods

4.2.1 Description of study area (Refer to Chapter 3 Subsection 3.2.1 and 3.2.2)

4.2.2 Sampling procedure (Refer to Chapter 3 Section 3.3.1)

4.2.3 Research Design (Refer to Chapter 3 Section 3.4.1)

4.2.4 Data collection procedure (Refer to Chapter 3 Section 3.6.1)

4.2.5 Data analysis procedure (Refer to Chapter 3 Section 3.7.1)

4.2.6 Challenges encountered during data collection

1. The lockdown restrictions imposed by the Government of Zimbabwe, though necessary, delayed the sample collection and analysis. This meant working extra hours to compensate for the lost time.
2. This study was self-funded using local currency. Avoiding a budget overrun was difficult as prices of materials and services used in this study were always increasing due to the instability of the local currency. More resources had to be channelled towards the study.
3. The high costs of sample analysis limited the number of samples and nutritional parameters that could be collected and analysed. For instance it cost 35USD to analyse a single sample for vitamin A.

4.3 Results and Discussion

4.3.1 Micronutrient analysis of raw white maize, biofortified maize and sugar beans

4.3.1.1 Carotenoid composition of raw materials

Results showing the total β-carotene and total provitamin A carotenoid contents in the whole meals of white maize (composite), provitamin A-biofortified maize (variety ZS242A) and iron-biofortified sugar beans (variety NUA45) are presented in Table 4.1 below.

Table 4.1 Carotenoid composition of white maize, ZS242A and NUA45

Raw material	**Total β-carotene** (µg/g dry weight (DW))	**Total Provitamin A carotenoids** (µg/g DW)
1. ZS242A	5.690±0.006[a]	9.958±0.010[a]
2. NUA45	0.047±0.001[b]	0.071±0.001[b]
3. White maize	0.008±0.000[c]	0.013±0.001[c]

Values within the same column with different letters are significantly different according to Tukey's HSD test at the 0.01 level ($p < 0.01$). Carotenoid data are expressed as mean±standard error of mean (SEM); n = 3. Each mean is for triplicate determinations. Provitamin A content is expressed in β-carotene equivalents.

The results showed that there were significant differences ($p<0.01$) in total β-carotene and total provitamin A carotenoid content for all the three raw materials. Provitamin A-biofortified maize had a significantly higher mean content of total provitamin A carotenoids in β-carotene equivalents (9.958 µg/g DW) compared to both white maize (0.013 µg/g DW) and iron-biofortified sugar beans (0.071 µg/g DW). The total provitamin A carotenoids of the biofortified maize (9.958 µg/g DW) used in this study was slightly higher than the mean values of 9.45 µg/g DW, 9.3 µg/g DW and 8.8 µg/g DW for ranges reported by (Mugode et al., 2014), Ortiz et al., (2019) and Pillay et al., (2011) respectively. The total provitamin A carotenoid content of ZS242A was at least 3 times higher compared to the highest concentration of 2.5µg/g DW generally reported for non-biofortified yellow maize (Nuss and Tanumihardjo, 2010). Both findings from this study are encouraging finding given that the consumption of maize with increased provitamin A density can significantly reduce the risk of VAD among vulnerable population subgroups in maize consuming areas. The human body cannot synthesize vitamin A on its own and hence relies on dietary sources for this essential micronutrient.

The slight differences between the provitamin A contents of the biofortified maize used in this study and those reported in earlier studies by Ortiz et al., (2019) and Pillay et al., (2011) can be attributed mainly to genotypic factors since all the studies were conducted using different varieties. A study by Menkir and Maziya-Dixon (2004) found that β-carotene, which accounted for a significant portion (57%) of the total provitamin A carotenoid content of orange maize used in this study as also reported by Li et al., (2007), is strongly influenced by the crop's genotype. According to Pfeiffer and McClafferty (2007), there is genetic variability in the expression of provitamin A carotenoids in plants. The provitamin A content of ZS242A was 59% of the breeding target of 17 µg/100g DW set by HarvestPlus (Bouis and Welch, 2010). This can potentially be attributed to provitamin A losses at the postharvest stage. Carotenoids are known to be sensitive to light, heat and oxygen because of their structure, which contains a conjugated double bond. Exposure to these abiotic agents can reduce the carotenoid content of a given food (Peace and Dolfini, 1991). It is important to ensure proper postharvest handling and storage of orange maize along the entire value chain to minimize nutrient losses particularly provitamin A carotenoids. The results may also be an indication that further research is required to improve the concentration of provitamin A carotenoids in biofortified maize possibly through extensive screening and recombination of superior lines. This may go a long way in ensuring that the orange maize provides consumers with the target concentration of provitamin A carotenoids upon consumption.

The results show that white maize used in this study had a total provitamin A carotenoids of 0.013 µg/g DW, indicating a lack of carotenoids and low vitamin A activity. This confirms that white maize is a poor source of vitamin A as previously reported by (Nuss and Tanumihardjo, 2010). The results also showed that biofortified sugar bean contained a significantly higher content of total provitamin A carotenoids (0.071 µg/g DW) compared to white maize. However, the aforestated value was significantly lower (0.7%) compared to that for biofortified maize (9.958 µg/g DW). These results indicate that provitamin A-biofortified maize is a much better source of provitamin A carotenoids compared than both white maize and biofortified sugar bean and can therefore be used in formulating foods for consumers at risk of VAD.

Results presented in Table 4 show that provitamin A-biofortified maize had a significantly higher mean content of total β-carotene content (5.690 µg/g DW) compared to both white maize (0.008µg/g DW) and iron-biofortified sugar beans (0.047 µg/g DW). The total β-carotene content of the biofortified maize used in this study was higher than the total β-carotene concentrations of maize lines used in a study by Pillay et al., 2011 and Kurilich and

Juvick (1999). This shows that ZS242A can be a good candidate for the production and consumption of β-carotene rich maize based foods. β-carotene is the most important provitamin A carotenoid as it has twice provitamin A activity compared to all the other provitamin A carotenoids and hence can be efficiently converted into retinol by the body (Gregory, 1996). From the results, provitamin A-biofortified maize is a better sole source of vitamin A than white maize and sugar bean combined as had a higher β-carotene content. The high total β-carotene content in ZS242A makes it ideal for use in feeding children who have a small stomach capacity and cannot consume large quantities of food but have a nutritional demand due to rapid growth.

4.3.2.1 Iron and zinc composition of raw materials

Results showing the iron and zinc contents in the whole meals of composite white maize grain, ZS242A and NUA45 are presented in Table 4.2 below.

Table 4.2 Iron and zinc concentrations of white maize grain, ZS242A and NUA45

Raw material	**Zinc** (µg/g DW)	**Iron** (µg/g DW)
1. NUA45	32.68±0.0120^a	77.203±0.015^a
2. White maize	0.040±0.000^b	0.070±0.000^b
3. ZS242A	0.261±0.001^b	0.045±0.001^b

Values within the same column with different letters are significantly different according to Tukey's HSD test at the 0.01 level ($p < 0.01$). All iron and zinc data are expressed as mean±standard error of mean (SEM); n = 3. Each mean is for triplicate determinations.

From the results, white maize had a zinc and iron content of 0.04 µg/g DW and 0.07 µg/g DW respectively. Provitamin A-Biofortified maize (ZS242A) had a zinc and iron content of 0.26 µg/g DW and 0.045 µg/g DW respectively. Iron-biofortified sugar beans (NUA45) had a zinc and iron content of 32.68 µg/g DW and 77.20 µg/g DW respectively. The results showed that there were significant differences ($p<0.01$) in both iron and zinc content for all the three raw materials. Biofortified sugar beans had a higher iron and zinc content compared to both biofortified orange maize and non-biofortified white maize. This indicates that biofortified sugar beans is a better source of iron and zinc compared to both biofortified maize and white maize.

The concentrations of iron (77.20 µg/g DW) and zinc (36.68 µg/g DW) of the biofortified sugar beans used in this study were within the ranges of 30–110 µg/g for iron (and 25–60 µg/g for zinc reported in literature (Pfeiffer and McClafferty, 2007; Islam et al., 2002). The iron and zinc content of the biofortified sugar beans used in this study were lower than the breeding targets for iron and zinc in common beans of 107 µg/g and 56 µg/g DW set by HarvestPlus (Bouis and Welch, 2010). The iron and zinc contents of the sugar beans were 66% and 72% of the final target content for iron and zinc. The results may also be an indication that further research is required to improve the concentration of iron and zinc in biofortified sugar beans possibly through extensive screening and recombination of superior lines.

The results also showed that biofortified maize is an inferior source of iron and zinc compared to compared to both biofortified sugar beans and white maize. However, the iron and zinc content of white maize were a paltry 0.09% and 0.12% of the iron and zinc concentrations of biofortified sugar beans. However, biofortified maize is richer in provitamin A compared to both white and sugar beans. Therefore, combining the otherwise provitamin A rich biofortified maize with iron and zinc rich sugar beans using the concept of complementary micronutrients can yield food products that are more nutritionally balanced compared to products made solely from either of constituents.

4.3.2 Proximate composition of maize:sugar bean

The macronutrient composition of the biofortified maize:sugar bean blend (ratio 50:50) used to prepare the supplementary food (thin porridge) which had the highest overall acceptability according to the sensory evaluation results is presented in Table 4.3 below.

Table 4.3 Proximate composition of biofortified maize:sugar bean blend (T3)

Blend (T3)						
Energy (Kcal/100g)	**Carbohydrate** (g/100g)	**Protein** (g/100g)	**Fat** (g/100g)	**Crude fibre** (g/100g)	**Total ash** (g/100g)	**Moisture content** (%)
344.70±0.00	58.41±01	23.31±0.01	1.98±0.01	4.50±0.25	3.24±0.01	7.75±0.01

Kilocalories = Kcal. All data are for dry weight.

The blend had the following average contents of the following proximate parameters: Energy (344 Kcal per 100 g), Carbohydrate (58.41 g/100 g), Protein (23.31 g/100 g), Fat (1.98g/

100g), Crude fibre (4.50), Total ash (3.24 g/100 g). The average moisture of the blend was 7.7%. The energy content of the blend of 3.44 cal/g is slightly lower than the recommended minimum energy density of 4 cal/g for formulated complementary food on dry weight basis by Codex Alimentarius (2017). The energy density of the blend can be increased by incorporating energy containing raw materials with digestible carbohydrates and or fats and oils. The mean dietary fibre of the blend (4.5 g/100 g) is within the limit of 5 g per 100 g on a dry weight basis (Munasinghe et al., 2013; Codex Alimentarius, 2017). High levels of dietary fibre in foods for children can cause may flatulence and loss of appetite which is undesirable (Codex Alimentarius (2017).

The moisture content of the blend of 7.7% was comparable to the 7.06% that Tufa et al., 2016 reported for their soybean-cereal supplementary food diet 6. According to Amankwah et al., (2009), the moisture content prepared cereals should have 3-8% moisture content. Therefore the moisture content can be considered to be acceptable which is important as it has long been established that low moisture content limits food spoilage (Temple et al., 1996). The blend had a higher protein content compared to that of complementary food diets soybean and various cereals by Tufa et al., 2016. This bulk of the protein is likely to have been from the sugar beans as legumes are known to be rich sources of protein. This probably shows the importance of combining cereals and legumes to produce nutritious food mixes. Foods high in protein can be useful in fighting protein energy malnutrition which is a serious public health challenge in sub-Saharan Africa (De Onis and Blössner, 2003).

The fat content of the blend was lower than the minimum recommended level in foods for children. Fats play an important role in the development of the neurological and immunological systems in children. Fat can also serve as an important source of energy (Garrow et al., 1999). The total ash content of the blend is below limit of 5 g/100 g for complementary and is therefore acceptable according to Munasinghe et al., (2013). The blend contains a high content of carbohydrates which are important sources of energy in the body . (Garrow et al., 1999).

4.4 Conclusion

Biofortified maize a superior source of dietary provitamin A compared to both biofortified sugar beans and white maize due to the significant amounts of total β-carotene and total provitamin A that it contains in its grains. However, the maize has low levels of iron and zinc similarly to white maize. On the contrary, biofortified sugar beans contains significant amounts of iron and zinc making good candidate for blending with biofortified to produce nutritionally enhanced food blends. The resultant food blends can potentially meet at least 20% of the RDI of vitamin A, iron and zinc for children under the age of 5. The food blend contains acceptable levels of energy, protein, total ash , carbohydrates and has low moisture content which is an important keeping quality indicator.

4.5 Recommendations

1. Mothers or caregivers should replace white maize with biofortified maize:sugar bean blends (ratio 50:50 or 67:33 respectively) for preparing supplementary thin porridge for feeding infants and children aged 6 – 59 months.
2. There is need for sustained breeding efforts to further develop biofortified maize and sugar beans with higher levels of provitamin A, iron and zinc than the currently available varieties.
3. The nutritional composition of other available maize varieties being grown and consumed in Zimbabwe should be determined for comparison and crop breeding purposes.

4.6 References

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CHAPTER 5

Consumer acceptability of biofortified maize (*Zea mays*) and sugar bean (*Phaseolusvulgaris*) blend as supplementary food for children aged 6-59 months

Abstract

The prevalence of vitamin A, iron and zinc deficiencies in children is partly due to consumption of nutritionally poor foods. Biofortified crops offer a potential route for tacking micronutrient malnutrition in at-risk population subgroups. However, the majority of Southern African consumers largely consume and prefer white maize over yellow/orange maize for various including undesirable sensory characteristics. This study evaluated the consumer acceptability of supplementary food in the form of thin porridges prepared using blends of biofortified orange maize and sugar beans. The sensory attributes (appearance, aroma, taste, texture and overall acceptability) of the thin porridges were evaluated using a 5 point hedonic scale by 30 untrained adult women caregivers in Mbare, Harare. Thin porridge preparing biofortified maize:sugar bean meal (blend ratio 50:50) had the highest mean score for overall acceptability, aroma and taste among all the porridges. Both thin porridges prepared using biofortified maize:sugar bean blends were more accepted by the caregivers than thin porridges prepared using sole white maize meal or biofortified maize. Such blends of biofortified maize and sugar bean had never been evaluated before and hence their high acceptability among women caregivers is a new and important finding. This suggests that blends of biofortified maize and sugar beans can potentially succeed as vehicles for tackling multiple micronutrient deficiencies particularly in children. Findings from this study also showedthat there is a low level awareness on biofortified crops among urban consumers. There is need for nutrition awareness and education programmes on biofortification targeting urban areas.

Keywords biofortified, supplementary food, consumer acceptability

5.1 Introduction

Globally, about 2 billion people experience micronutrient deficiency due to the consumption of poor quality foods that lack diversity and essential micronutrients (Govender et al., 2017). Notably, iron, zinc and vitamin A deficiencies are ranked 9th, 11th and 13th among the 26 major risk factors of the global burden of disease(Ezzatiet al., 2012). Children in particular are vulnerable to vitamin and mineral deficiencies due to various factors including higher requirements of micronutrients needed for supporting their rapid growth, low micronutrient intake and increased exposure to infections. An estimated 10.8 million child deaths occur annually due to Fe, Zn and vitamin A deficiencies with no end in sight (WHO, 2002) and Zimbabwe is no exception.

Zimbabwe has a serious problem of childhood malnutrition as 25% of the children have Vitamin A deficiency (VAD), 72% are living with iron deficiency and 33% have iron deficiency anaemia ((National Nutrition Micronutrient Survey, 2015; United Nations International Children's Emergency Fund (UNICEF), n.d.). About 48.4%of the Zimbabwean population including children is at risk of inadequate zinc intake (Wessells and Brown, 2012). Consequently, provitamin A rich-biofortified maize and iron and zinc rich-biofortified sugar bean were introduced in the country in 2015 by HarvestPlus working in partnership with the Government of Zimbabwe. Biofortification was adopted as a complementary strategy for tackling multiple micronutrient deficiencies in the country due its huge potential to deliver vitamin and mineral rich foods at a limited cost to poor and vulnerable consumers (Meenakshi et al., 2010).

Despite the health benefits associated with the consumption of biofortified crops, the acceptance of biofortified commodities especially orange coloured provitamin A-maize by Southern African consumers is not without its challenges. The biofortification process can result in a change of the intrinsic and/or extrinsic properties of food as is the case with provitamin A-maize which has a yellow or orange colour due to chemical nature of carotenoids present in its kernels (Muzhingi et al., 2008). The yellow/orange colour is markedly different from that of white maize and together with other sensory attributes such as taste and aroma influence the consumer acceptability of a given type of maize. Previous consumer acceptability studies revealed that the majority of Southern African consumers largely consume and prefer white maize over yellow/orange maize for historical, cultural and organoleptic reasons (De Groote et al., 2010; Rubey et al., 1993; Muzhingi et al., 2008). Contrary to this, a consumer acceptability study carried out in Mozambique reported a more favourable response to orange maize (Stevens and Winter-Nelson 2008). In another study conducted in South Africa, children preferred orange maize-based products whereas adults preferred products prepared using white maize Pillay et al., (2011). The findings of these studies indicate that the sensory acceptability of biofortified maize and most likely other biofortified agricultural commodities vary by age, type of food product and geographical location. It is therefore difficult to generalize findings beyond the geographical location where the study was conducted. There is a need to carry out acceptability studies in previously untargeted areas.

In Zimbabwe, there is a paucity of published data on the acceptability of sole provitamin-A biofortified maize and iron biofortified sugar bean diets. Furthermore, studies on the consumer acceptability of innovative supplementary foods specially formulated for at-risk population subgroups using biofortified maize-sugar bean blends are non-existent although this is interesting prospect. As such, the aim of this study seeks to evaluate the acceptability of supplementary thin porridge prepared using blends of provitamin A-biofortified maize and iron-biofortified sugar bean by consumers in Mbare, a low income high density suburb located in Harare, Zimbabwe. The thin porridge was selected as the food of choice in this study considering that it is popularly consumed by children, a population group that is highly vulnerable to micronutrient malnutrition.

5.2 Material and Methods

5.2.1 Description of study area (Refer to Chapter 3 Section 3.2)

5.2.2 Sampling procedure (Refer to Chapter 3 Section 3.3.2)

5.2.3 Research Design (Refer to Chapter 3 Section 3.4.2)

5.2.4 Data collection procedure (Refer to Chapter 3 Section 3.6.2)

5.2.5 Data analysis procedure (Refer to Chapter 3 Section 3.7.2)

5.2.6 Challenges encountered during data collection

1. The researcher could not travel from Harare where he is based to Shamva for the purposes of carrying out the consumer acceptability study due to total lockdown restrictions that lasted for more than a month. As a result the researcher resorted to carry out the consumer acceptability study in Mbare, Harare when the restrictions were relaxed as time was running out.
2. The lockdown restrictions on the number of people per gathering negatively impacted the sample size of respondents. The study using a lesser number of panelists than initially targeted.

3. The use of additional resources for the consumer acceptability study such as infra-red thermometer and hand sanitizers which were initially unbudgeted for presented a financial challenge for the researcher.

5.3 Results and Discussion

A total of 30 adult female caregivers participated in the study. The majority of the respondents (46.7%) were in the age group 18-25 years age group. Women caregivers in the 18-25 years age group accounted for 33.3% of the respondents with those in the 34-41 age group and over 41 accounting for 13.3% and 6.7% of the respondents respectively. The highest level of education attained by participants was primary (10%) , secondary (83.3%) and college level (6.7%).

The results of the sensory evaluation of thin porridges prepared using 100% white maize meal as a control (sample code 203), 100% biofortified maize meal (sample code 319), biofortified maize:sugar bean meal (blend ratio 50:50) (sample code 872) and biofortified maize:sugar bean meal (blend ratio 94:8) (sample code 132) are presented in Table 5.1 below.

Table 5.1 Mean scores of sensory attributes of thin porridges

	Sensory attributes				
Sample code	**Appearance**	**Aroma**	**Taste**	**Texture**	**Overall acceptability**
203 (control)	2.57±0.568^{a}	2.07±1.112^{a}	2.40±1.037^{a}	2.60±4.98^{a}	2.60±0.770^{a}
319	4.03±0.658^{b}	3.58±0.502^{b}	2.87±0.499^{a,c}	2.42±8.07^{a}	2.61±0.882^{a}
872	3.80±0.407^{b,d}	4.00±0.643^{b}	3.40±0.669^{c}	2.40±0.643^{a}	3.59±0.791^{b}
136	3.60±1.037^{d}	3.80±0.814^{b}	3.03±0.814^{c}	2.58±0.498^{a}	3.17±0.733^{b}

Values within the same column with different letters are significantly different according to Tukey's HSD test at the 0.05 level ($p < 0.05$). Data for all sensory attributes are expressed as mean±standard error of mean (SEM);

The mean scores for overall acceptability of the thin porridges were 2.60 for control sample 203 (100% white maize), 2.61 for sample 319 (100% biofortified maize), sample 872 (biofortified maize:sugar bean meal (blend ratio 50:50) and (biofortified maize:sugar bean meal (blend ratio 94:6). According to the Tukey's HSD test results, there was a significant difference in the overall acceptability of the thin porridges prepared using biofortified maize-sugar bean meal compared to those prepared using 100% white maize and 100%. Thin porridge preparing biofortified maize:sugar bean meal (blend ratio 50:50) had the highest mean score for overall acceptability, aroma and taste among all the porridges. Both thin porridges prepared using biofortified maize:sugar bean blends were more accepted by the caregivers than thin porridges prepared using sole white maize meal or biofortified maize. This finding corroborates the results from a consumer acceptability study reported by Stevens and Winter-Nelson (2008). The finding suggests that the addition of sugar bean meal to biofortified maize meal can improve its acceptability by consumers. This might also explain the differences in findings from this study compared to those from previous studies by (Tschirley and Santos, 1995; De Groote et al., 2010; Muzhingi et al., 2008).

The high acceptability of the blends used in this study among consumers is encouraging. This is because from a nutritional point of view, blending micronutrient biofortified maize and sugar bean meal can be used for the preparation of food containing significant amounts of vitamin A, iron and zinc especially for young infants. Such food may prove to be useful tackling multiple deficiencies particularly VAD, iron and zinc which are highly prevalent in children under the age of 5.

From the mean sensory attribute scores shown in Table 5.1, thin porridge prepared from sole biofortified maize had the highest mean scores for the appearance with that prepared from sole white maize having the lowest score. This suggests that the yellow colour of thin porridge prepared using biofortified maize might be appealing to consumers. This might be a reflection that young mothers in the 18-25 age group that constituted the majority of the respondents (46.7%) might have little or less preconceptions about yellow/maize than older people that went through the period in the early 1990s when yellow maize was popularly distributed in Zimbabwe as food aid due to serious food shortages. That period saw yellow maize being labelled as food for the poor and therefore not liked by many from that point onwards. The poor quality of some of the grain imported into the country also affected its acceptance by consumers (Muzhingi et al., 2008). Thin porridge prepared using sole white maize had the highest score for texture of all the porridges although this was not statistically significant ($p<0.05$). The texture of the porridge was generally not liked by the panellists. This might be because adults prefer fine flour to meals which are coarser. This can be easily addressing through milling to the required fine particle size.

Only one and none of the 30 participants had heard of biofortified maize and sugar bean before respectively. This might indicates a seriously low level of awareness and education on biofortified crops among urban consumers. This represents an opportunity to target urban women caregivers who are in most cases responsible for making dietary decisions pertaining to child feeding at household level and hence can influence the quantity and quality of supplementary food to be consumed. The participation of young children's mothers in nutrition programmes may encourage them to gain nutrition knowledge and positive attitudes towards dietary improvements.

5.4 Conclusion

Supplementary food in the form of thin porridges prepared using blended biofortified maize:sugar beans (using formulation ratios 50:50 and 67:33) was more acceptable by urban women caregivers compared to supplementary food based on sole white maize or biofortified maize. Thin porridge preparing biofortified maize:sugar bean meal (blend ratio 50:50) had the highest mean score for overall acceptability, aroma and taste among all the porridges. The addition of sugar bean meal to biofortified maize meal can potentially improve its acceptability.

5.5 Recommendations

1. Nutrition programmes promoting biofortified crops need to be extended to urban areas especially those considered to be highly vulnerable to malnutrition.
2. Mothers or caregivers should use biofortified maize:sugar bean blend (ratio 50:50 or 67:33 respectively) for preparing supplementary thin porridge for feeding infants and children aged 6 – 59 months.
3. The consumption of biofortified food blends such as the one used in this study must be promoted in both urban and rural areas.
4. Further consumer acceptability studies should be carried out using larger sample sizes of participants from other areas in Zimbabwe for comparison and decision making purposes. The sensory evaluation should be combined with laboratory analysis of volatile compounds that affect sensory characteristics of biofortified food. Furthermore, the use of trained panellists in sensory evaluations of biofortified foods should be undertaken. This could give more objective results.

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CHAPTER 6: SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Introduction

In this chapter the main conclusions and recommendations of this thesis will be discussed. A summary of the research will be presented. The aim of the study was to formulate biofortified maize-sugar bean blends and assess their consumer acceptability as supplementary food to enhance vitamin A, iron and zinc status for children aged 6-59 months. The objectives of this research work were:

1. To determine the provitamin A, iron and zinc content of white maize, biofortified maize and biofortified sugar beans.
2. To formulate the biofortified maize-sugar bean supplementary food blend by combining biofortified maize and sugar bean meal at different levels on vitamin A, iron and zinc basis
3. To evaluate the acceptability of biofortified maize-sugar bean blends using sensory test of supplementary food among urban mothers/caregivers of children aged 6-59 months.
4. To assess the proximate composition of the more acceptable supplementary food.

6.2 Research summary

This work investigated the nutritional composition of locally produced and consumed provitamin A-biofortified maize and iron-biofortified sugar beans and as a result has provided important baseline, variety specific nutritional data that were either lacking or scarce. The nutritional composition of a composite of white maize sourced locally has been determined in this study.

The total provitamin A carotenoid concentration (9.958 µg/g) on dry weight basis in the provitamin A-biofortified maize (variety ZS242A) used in this study was higher than the levels in the other study raw materials (white maize and biofortified sugar bean). The provitamin A carotenoid concentration in the biofortified maize was generally higher than the levels reported for yellow/orange maize varieties reported in literature. However, the concentration was lower than the current breeding target (17 µg/g) for biofortified maize as set by HarvestPlus. This indicates that further breeding efforts are required to develop varieties with the targeted provitamin A level. The findings of this study also indicate that in terms of iron and zinc, biofortified sugar beans is a superior source compared with both provitamin A-biofortified maize and white maize. However, the iron and zinc concentration of the biofortified sugar beans used in this study is still lower than the current breeding target of 107 µg/g for iron and 56µg/g for zinc on dry weight basis for biofortified beans as set by HarvestPlus. This affirms that further breeding efforts are required to develop varieties with the targeted provitamin A level.

The study findings show that biofortified maize and sugar beans can be combined to produce blends that can meet a significant portion of the average RDA of vitamin A, iron and zinc for children under the age of 5 after processing into supplementary food in the form of thin porridge. Thin porridges prepared using such blends are more acceptable by women caregivers compared to those prepared using sole white maize or biofortified maize. This finding indicate that incorporating sugar beans into maize based supplementary food can improve its acceptability. Proximate analysis of one of the blends selected on the basis of having the highest score of overall acceptability from the sensory evaluation showed that such a food mix can contain acceptable levels of energy, carbohydrate, fat, crude fibre, total ash according to Codex Alimentarius guidelines for complementary foods.

6.3 Conclusions

Biofortified maize (ZS242A) contains significant amounts of total β-carotene and total provitamin A carotenoids making it a superior source of dietary provitamin A compared to both biofortified sugar beans and white maize. However, biofortified maize similarly to white maize contains low levels of iron and zinc meet their recommended daily intakes. Biofortified sugar beans contains significant amounts of iron and zinc making good candidate for blending with biofortified to produce a nutritionally enhanced food mix for use in preparing supplementary food for children. Blends of biofortified maize:sugar beans can be formulated to potentially meet at least 20% of the RDI of vitamin A, iron and zinc for children under the age of 5. Supplementary food in the form of thin porridges prepared using blended biofortified maize:sugar beans is more acceptable by urban women caregivers compared to supplementary food based on sole white maize or biofortified maize and hence can potentially be used to deliver vitamin A, iron and zinc to consumers. The proximate parameters of the blend: energy, carbohydrate, crude protein, fat need do not meet the Codex Alimentarius minimum recommended levels and hence need to be increased. The crude fibre and total ash content of the blend are within the recommended limits. The blend has moisture content and should have good shelf life.

6.4 Policy implication and recommendations

1. The findings of the study draw attention to the need to review food and nutrition policies to promote the development of national food composition databases for locally produced nutrient dense food resources including provitamin A–biofortified maize and iron biofortified sugar beans to guide proper food combination, formulation of healthier recipes and food products for all.
2. Food-to-food fortification using locally available nutritionally improved crops such as biofortified maize and sugar beans needs to be prioritised and supported through policy.
3. Agricultural policy needs to consider biofortified crops under national agricultural programmes to boost their production and local supply.
4. Mothers or caregivers should use biofortified maize:sugar bean blend (ratio 50:50 or 67:33 respectively) for preparing supplementary thin porridge for feeding infants and children aged 6 – 59 months.

6.5 Areas for further research

1. Future research should investigate the effect of household level food processing methods such as cooking, roasting and fermentation and on the levels of antinutritional factors and retention of nutrients in the blends of provitamin A maize and biofortified sugar bean.
2. Future studies should evaluate the shelf life of biofortified cereal-legume food mixes.
3. There is need to carry out bioavalaibility studies on the biofortified food blends.

6.6 Appendices

APPENDIX A: STANDARD RECIPE FOR PREPARATION OF THIN PORRIDGE

INGREDIENTS
6 cups (1500 ml) water
2 cups (200 g) mealie meal
2 cups (400 ml) water
1 g salt
40 g sugar

METHOD

1. Bring 6 cups (1500 ml) of water to the boil in a pot with a holding capacity of >2500 ml on a hot plate on high heat (plate control setting 6 for 4 or 3 plate stoves and setting 3 for 2 or 1 plate stove).

2. Combine 1 cup (200 g) of meal with 2 cups (380 ml) of cold water and mix by stirring to make a homogenous smooth paste.

3. Add the paste to the boiling water and stir until smooth.

4. Cook at high heat for 25 minutes with the pot lid on and occasional stirring.

4. Add 1 g of salt while cooking.

5. Add 40 g of sugar once the porridge is cooked.

APPENDIX B: CONSENT FORM FOR WOMEN CAREGIVERS (IN ENGLISH)

CONSENT FORM FOR FEMALE PANELLISTS

This confirmation of consent relates to a research project titled "Consumer acceptability of supplementary food prepared using biofortified maize-sugar bean blends", which is being conducted by Cyprian Mahuni, a final year student pursuing an MSc in Food Security and Sustainable Agriculture degree at Bindura University of Science Education, registration number B1852483. I understand that participation is entirely voluntary and I can withdraw my consent at any time in which case I have authority to have the results of the participation returned to me, removed from the study records, or destroyed. A total of 30 consumers will participate in this evaluation which will take about 10-15 minutes to complete. The following points have been explained to me:

1. The aim of the study is to evaluate the sensory acceptability of supplementary food prepared using biofortified maize-sugar bean blend among women caregivers of children aged 6-59 months in Mbare, Harare. The only benefit that I may expect from this evaluation is a satisfaction that I have contributed to the knowledge that will be generated from this study.
2. In any case, it is my responsibility to report prior to participation to the investigator any allergies I may have.
3. The procedure is as follows: Four coded samples prepared using 1) white maize, 2) biofortified maize, 3) biofortified maize-sugar bean (ratio 1) and 4) biofortified maize-sugar bean (ratio 2) will be placed in front of me and I will evaluate them using my senses and indicate my rating for each of the 5 attributes on a score sheet using a 5 point hedonic scale. I will not be allowed to talk to or influence the ratings of other participants when the evaluation is in progress. Participation entails minimal risks. The consumption of beans can potentially cause flatulence.
4. The data collected in this evaluation will be treated in strict confidence, used for statistical purposes, presented in aggregate form only and will not be released in any individual identifiable form without my prior consent unless required by law.

The study has been discussed with me and all my questions have been answered. I understand that additional questions regarding the study should be directed to the study supervisor Dr. B. Masamha on (+263)778467297 or via the email address blemasamha@gmail.com. I agree with the terms above and acknowledge that l have been given a copy of the consent form.

I, ________________________Signature______________agree to participate in this study.

Signature of Investigator_________________Witness______________Date_____________

APPENDIX C: QUESTIONNAIRE FOR SENSORY EVALUATION (IN ENGLISH)

QUESTIONNAIRE GUIDE

My name is Cyprian Mahuni, a final year student pursuing a Master of Science in Food Security and Sustainable Agriculture degree at Bindura University of Science Education located in Bindura, Zimbabwe. My student registration number is B1852483. I am carrying out a study titled "**Consumer acceptability of supplementary food prepared using biofortified maize-sugar bean blends**" in partial fulfilment of the aforementioned degree programme.

The main aim of the study is to gather information on consumer acceptance of supplementary food prepared using blended biofortified maize-sugar bean among women caregivers of children aged 6-59 months in Mbare, Harare.

You were selected for the study because you meet the eligibility criteria and your contribution will be relevant to my study. Your participation in this study is **voluntary**, you can decide to **withdraw at any time**. The questionnaire comprises of sections A, B and C. It will approximately take about 10 to 15 minutes to complete the evaluation.

Please complete all the questions. Responses from this evaluation will be kept confidential and presented in aggregate form only to pursue academic fulfilment and for publication. The responses will not be released in any individual identifiable form without your prior consent unless required by law.

For the investigator

Date of completion……………...............Respondent No..............

SECTION A: Personal information

1. Tick one box to indicate your gender

☐— Male ☐—Female ☐—Prefer not to answer

2. Tick one box to indicate your age group

☐— Under 18 ☐—18-25 ☐—26-33 ☐—34-41 ☐—Over 41

3. Tick one box to indicate highest level of education attained

☐— No education ☐—Primary ☐—Secondary ☐—College

SECTION B: Sensory rating of food samples

Instructions:

1. Please taste all the food samples that are in front of you in any order of your choice.
2. After tasting each sample, please check its sample code on the serving cup and in table 1 below indicate how you feel about its taste, appearance, aroma and texture as well as the overall acceptability by placing a tick inside the circle that best corresponds to your rating of choice.

Table 1. Sensory evaluation using 5 point hedonic scale

Sample code	**Attribute**	**Dislike extremely**	**Dislike**	**Neither Dislike nor like**	**Like**	**Like extremely**
1. **203**	Taste	○	○	○	○	○
	Appearance	○	○	○	○	○
	Aroma	○	○	○	○	○
	Texture	○	○	○	○	○
	Overall acceptability	○	○	○	○	○

Table 1. Continued....

Sample code	**Attribute**	**Dislike extremely**	**Dislike**	**Neither Dislike nor like**	**Like**	**Like extremely**
2. **319**	Taste	○	○	○	○	○
	Appearance	○	○	○	○	○
	Aroma	○	○	○	○	○
	Texture	○	○	○	○	○
	Overall acceptability	○	○	○	○	○
3. **872**	Taste	○	○	○	○	○
	Appearance	○	○	○	○	○
	Aroma	○	○	○	○	○
	Texture	○	○	○	○	○
	Overall acceptability	○	○	○	○	○
4. **136**	Taste	○	○	○	○	○
	Appearance	○	○	○	○	○
	Aroma	○	○	○	○	○
	Texture	○	○	○	○	○
	Overall acceptability	○	○	○	○	○

SECTION C: Biofortified crops

1. Have you ever heard of biofortified maize that is being grown and consumed in Zimbabwe?

Yes ☐—
No ☐—

2. Have you ever heard of biofortified beans that is being grown and consumed in Zimbabwe?

Yes ☐—
No ☐—

End of questionnaire

THANK YOU FOR PARTICIPATING IN THIS EVALUATION

APPENDIX D: SAMPLE PREPARATION AND SENSORY EVALUATION STUDY IN PICTURES

Figure 6.1 Processing of maize and sugar bean samples.

Figure 6.2(a) Set-up for sensory evaluation

Figure 6.2(b) Sensory testing in progress

Figure 6.2 Set-up for sensory evaluation and sensory testing in progress