NUTRITIONAL COMPOSITION AND ACCEPTANCE OF A COMPLEMENTARY FOOD MADE WITH PROVITAMIN A-BIOFORTIFIED MAIZE

BY

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ABSTRACT

Introduction: Micronutrient malnutrition has been identified as a serious health problem globally and is on the rise in South Africa. This is evident from the escalating burden of vitamin A deficiency (VAD) in South Africa. Rural infants are the most affected, as their diets often lack micronutrients. Food fortification, vitamin A supplementation and dietary diversity are the strategies that have been employed in South Africa to alleviate VAD. However, these strategies have not been effective, for various reasons. Biofortification is the production of micronutrient dense staple crops to alleviate micronutrient deficiencies. This strategy could complement existing strategies in the alleviation of VAD in South Africa and in other countries, especially in sub-Saharan Africa (SSA), where VAD is prevalent.

Aim: The aim of this study was to investigate the nutritional composition and acceptance of a complementary food (soft porridge) made with provitamin A-biofortified maize by female infant caregivers from the rural areas of Umgungundlovu District of KwaZulu-Natal, South Africa.

Objectives: (i) To evaluate the nutritional composition of soft porridge made with provitamin A-biofortified maize compared to non-biofortified white maize porridge; (ii) To assess the sensory acceptability of soft porridge made with the biofortified maize by black African female infant caregivers of varying age; and (iii) To determine the perceptions of the black African infant caregivers about the biofortified maize relative to the non-biofortified white maize.

Methods: A cross-sectional study was conducted. Grains of two provitamin A-biofortified maize varieties and one white variety (control) were used. Grain and soft porridge of each variety of maize were analysed for their nutritional composition. The sensory acceptability of the porridges were evaluated by black African female infant caregivers, using a five-point facial hedonic scale. Focus group discussions were conducted, using some of the study subjects, to determine their perceptions about the provitamin A-biofortified maize.

Results: The results showed that the grains of the provitamin A-biofortified maize varieties and their soft porridges were more nutritious than the control white variety in terms of energy, fibre, fat, protein, iron, zinc and phosphorus content. The results of the sensory evaluation indicated that there was no significant difference in the sensory acceptability of the biofortified soft porridges and the white maize soft porridge, irrespective of the age of the

sensory evaluation panellists. The female caregivers perceived the biofortified maize as nutritious and health-beneficial and thought that infants would like its unique yellow colour and taste. However, the black African female caregivers perceived the provitamin A-biofortified maize as an animal feed or food for the poor. Nevertheless, the female caregivers expressed a willingness to give their infants porridge made with provitamin A-biofortified maize if it was cheap, readily available and health-beneficial.

Conclusion: This study suggests that provitamin A-biofortified maize has the potential to be used as a complementary food item. Biofortification of maize with provitamin A could be used as a possible complementary strategy to assist in the alleviation of VAD in SSA. Furthermore, the relatively higher energy, fibre, fat, protein, iron, zinc and phosphorus content of the biofortified maize could contribute to the alleviation of protein-energy malnutrition and mineral deficiencies, respectively, which are prevalent in children of SSA. Although the findings of this study, like other previous studies, indicate that there are some negative perceptions about the provitamin A-biofortified maize, this study shows that provitamin A-biofortified maize soft porridge is as acceptable as white maize soft porridge to female infant caregivers from the rural areas of Umgungundlovu District of KwaZulu-Natal, South Africa. The female caregivers are thus likely to accept the biofortified maize for use as an infant complementary food in the form of soft porridge. Further research is recommended to expand the study area and consumer sample size in order to increase the confidence of inferring these results for large rural populations.

PREFACE

The work described in this dissertation was carried out in the School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, from March 2012 to January 2014, under the supervision of Dr Kirthee Pillay, Dr Muthulisi Siwela and Professor John Derera.

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As supervisors of the candidate we agree to the submission of this dissertation.

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Dr Muthulisi Siwela (Co-supervisor)

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Professor John Derera (Co-supervisor)

DECLARATION

I, Laurencia Govender, declare that:

1. The entirety of the work contained in this dissertation is my original work, except where otherwise stated.

2. This dissertation, or any part of it, has not been submitted for any degree or examination at any other university.

3. Where other sources have been used they have not been copied and have been properly acknowledged.

4. This dissertation does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the relevant reference section.

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CHAPTER 1: INTRODUCTION, THE PROBLEM AND ITS SETTING

1.1 Importance of the study

The prevalence of childhood malnutrition is escalating worldwide, especially in developing countries (World Health Organization (WHO) 2011; United Nations Children's Fund (UNICEF) 2012). Malnutrition is the leading cause of death during childhood, resulting in more than 33% of child deaths worldwide (WHO 2013a). In Africa, 25% of children under the age of five years were shown to be underweight and 10% were wasted (UNICEF 2013a). In SSA, the number of children with malnutrition has risen by 6% between 1990 and 2010 (Garcia 2012). In South Africa, a 1.8% increase in the number of underweight children was observed between 1990 and 2010 (Lutter, Daelmans, de Onis, Kothari, Ruel, Arimond, Deitchler, Dewey, Blössher & Borghi 2011). According to the District Health Information System (DHIS) (2011), in South Africa, the KwaZulu-Natal province has the highest incidence of malnutrition in children under five years of age. The Umgungundlovu District in Pietermaritzburg is one of the areas worst affected by malnutrition in this age group (DHIS 2011).

Childhood malnutrition can manifest in two ways, namely, protein-energy malnutrition and micronutrient malnutrition (Department of Health (DOH) 2013a, p70). Protein-energy malnutrition is the most commonly diagnosed form of malnutrition and can present as severe acute malnutrition, moderate acute malnutrition, wasting, underweight and stunting, depending on the macronutrient deficiency. Severe acute malnutrition can present as marasmus, kwashiorkor or marasmic kwashiorkor (DOH 2012a; Mahan, Escott-Stump & Raymond 2012, p55; WHO 2013a). This type of malnutrition results from a deficiency of protein and energy from the diet (DOH 2013a, p70; Manary & Sandige 2008). Micronutrient deficiencies are highly prevalent in developing countries. The most common micronutrient deficiencies are vitamin A, iodine, iron, zinc and folic acid (Ramakrishnan 2002). Vitamin A deficiency (VAD) has been identified as the leading micronutrient deficiency in South Africa (DOH 2013a, p70; WHO 2013b). According to the WHO (2009), VAD is a serious public health problem in South Africa. The South African Vitamin A Consultative Group (SAVACG) study of 1994 reported that one in three children had VAD (Labadarios & Van Middelkoop 1995). The National Food Consumption Survey (NFCS) of 1999 indicated that almost half of the children living in South Africa had VAD (Labadarios, Steyn, Maunder, Macintyre, Swart, Gericke, Huskisson, Dannhauser, Vorster & Nesamvuni 2000). The

National Food Consumption Survey-Fortification Baseline (NFCS-FB) (2005) reported that two in three South African children were vitamin A deficient (Labadarios, Moodie & van Rensburg 2007). The VAD situation is South Africa has worsened (Labadarios *et al* 2007; Labadarios & Van Middelkoop 1995). Vitamin A plays a vital role in the health of human beings. It plays several physiological roles, including being required in vision, ocular health and immune function (Loveday & Singh 2008). Since vitamin A is derived mainly from food (American Academy of Pediatrics Committee on Nutrition 2009, p457), the low accessibility of vitamin A-rich foods, especially in low income communities, can be identified as the major cause of VAD (Faber & Wenhold 2007; Faber 2005a). Rural South African communities, similar to those in many other developing countries, are generally of low socio-economic status and have limited access to expensive animal products and commercial plant foods that are fortified with vitamin A or rich in vitamin A. A majority of these lower socio-economic communities generally consume a diet that consists of predominately starchy staples and only a few individuals are able to consume vegetable or animal products rich in vitamin A (Faber & Wenhold 2007; Faber 2005a; Labadarios *et al* 2000).

In South Africa, the strategies in place to address VAD include food fortification of certain foods during commercial processing and vitamin A supplementation and promotion of dietary diversity (DOH 2012a; Swart, Sanders & McLachlan 2008, p133; DOH & UNICEF 2007, p8). However, fortified foods are not always accessible to individuals who are at risk of malnutrition (Loveday & Singh 2008; Ortiz-Monasterio, Palacios-Rojas, Meng, Pixley, Thethowan & Pena 2007). The South African Department of Health recommends that all children aged six to 59 months receive an oral dose of vitamin A in the form of an oil-based drop every six months (DOH 2012b). The challenge with vitamin A supplementation is that it does not reach all infants, especially those living in rural areas, as there may be limited access to clinics or children are not taken for regular check-ups (WHO 2011; UNICEF 2007). The dietary diversity strategy aims to alleviate nutrient deficiency, by promoting the consumption of a variety of foods. However, this strategy appears to be failing due to the fact that poor communities have limited access to a diversified diet, as stated earlier. Thus, despite the implementation of these strategies, the problem of VAD has worsened in South Africa (Swart *et al* 2008, p133).

Biofortification is an emerging strategy aimed at alleviating micronutrient deficiencies (Meenakshi, Johnson, Manyong, De Groote, Javelosa, Yanggen, Naher, Garcia & Meng 2010a). This process involves modifying the micronutrient content of popular staple crops (Khush, Lee, Cho & Jeon 2012). HarvestPlus has identified maize as one of the six staple crops suitable for biofortification (HarvestPlus 2013). Biofortification is regarded as a complementary strategy that should be used in conjunction with other existing strategies to address micronutrient malnutrition (Meenakshi *et al* 2010a). However, the biofortification process may cause sensory changes to the staple crops which may lead to poor consumer acceptance of these crops (Nuss & Tanumihardjo 2010). Provitamin A-biofortified maize is yellow or orange in colour as a result of the carotenoids present in the maize (Chapman 2012; Nuss & Tanumihardjo 2010). The colour and aroma of the maize are both altered by carotenoid biofortification, which may render the end-product unacceptable to consumers (Meenakshi *et al* 2010a; Nestel, Bouis, Meenakshi & Pfeiffer 2006; Stevens & Winter-Nelson 2008).

Appropriate nutrition in the early stages of an infant's life is important for optimal growth and development. Breast milk is sufficient to meet an infant's dietary requirements up to six months of age. However, six months after birth, breastfeeding alone cannot meet the infant's nutritional requirements and complementary foods are required (UNICEF 2012; WHO 2011). It has been reported that communities of a poor socio-economic background have diets that are high in starch, such as maize, and low in animal food sources (Mayer, Pfeiffer & Beyer 2008; Stevens & Winter-Nelson 2008). Infants from these communities are generally given soft maize porridge as the first, and in most cases, the only complementary food (WHO 2000). Unfortified white maize porridge is devoid of vitamin A and the use of soft white maize porridge as the main complementary food increases the risk of VAD in infants. Using provitamin A-biofortified maize, instead of the unfortified white maize as a complementary food, could improve vitamin A intake in vulnerable children (Faber & Benadé 2007; Faber, Kvalsvig, Lombard & Benadé 2005b).

Although previous studies have indicated a low acceptance of provitamin A-biofortified maize by consumers, due to its undesirable sensory attributes (Pillay, Derera, Siwela & Veldman 2011a; Stevens & Nelson-Winter 2008; Muzhingi, Langyintuo, Malaba & Banziger 2008), it has been found that mothers would be willing to try new foods if they thought it would be beneficial to their infants and if it was culturally acceptable (UNICEF 2012). A

study conducted in KwaZulu-Natal by Pillay *et al* (2011a) reported that the acceptance of provitamin A-biofortified maize varied with consumer age and maize food type. The authors found that the variation in the acceptance of the provitamin A-biofortified maize with food type highlighted the need to identify maize food types in which the biofortified maize was most acceptable to consumers (Pillay *et al* 2011a). Studies have also reported that sensory attributes, especially texture, are a major determinant of the acceptance of provitamin A-biofortified maize by consumers (Khumalo, Schönfeldt & Vermeulen 2011; Stevens & Winter-Nelson 2008). Soft porridge made with provitamin A-biofortified maize in place of white maize may thus be acceptable to female caregivers for use as a complementary food. However, it appears that the acceptance of soft porridge made with provitamin A-biofortified maize and its nutritional composition have not been investigated.

1.2 Aim of the study

The aim of this study was to investigate the nutritional composition and acceptance of a complementary food (soft porridge) made with provitamin A-biofortified maize.[1]

1.3 Research objectives

The objectives of this study were:

1.3.1 To evaluate the nutritional composition of soft porridge made with provitamin A-biofortified maize compared to white maize[2].

1.3.2 To assess the sensory acceptability of soft porridge made with biofortified maize by black African female infant caregivers of varying age.

1.3.3 To determine the perceptions of black African female infant caregivers about provitamin A-biofortified maize relative to white maize.

1.4 Hypotheses

The following hypotheses were tested in the study:

1.4.1 The nutritional composition of the provitamin A-biofortified maize porridge is superior to that of white maize porridge, which is not biofortified.

1.4.2 The soft maize porridge made with provitamin A-biofortified maize is less acceptable relative to white soft maize porridge, due to the undesirable sensory properties.

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[1] The provitamin A-biofortified maize used in this study was yellow in colour.

[2] Non-biofortified white maize was used as a control in this study.

1.4.3 Black African female caregivers perceive provitamin A-biofortified maize to be unfit for human consumption in comparison to white maize, due to the undesirable sensory properties and negative social beliefs.

1.5 Study parameters

The parameters for the study were as follows:

1.5.1 The study was limited to two varieties of provitamin A-biofortified maize (PVA pool A and PVA pool B) and one variety of white maize (control).

1.5.2 The study was restricted to black African female caregivers residing in the Umgungundlovu District and attending the paediatric out-patient department and Khanyisa clinic at Edendale hospital. This hospital was selected for this study as it is the referral hospital for many rural clinics, namely: Caluza, Elandskop, Embo Community Health Clinic, Enkumane, Gomane, Imbalenhle Community Health Clinic, Indaleni, Mbuthisweni, Mpumuza, Ntembeni, Nxamalala, Pata, Richmond, Sondelani, Songonzima and Taylors Halt.

1.5.3 The provitamin A-biofortified maize and the white maize grains were analysed for the following nutrients only: gross energy, protein, fat, ash (total mineral content), fibre, provitamin A, calcium, phosphorus, iron and zinc.

1.6 Assumptions

The following assumptions were made:

- It was assumed that the study participants consumed maize on a regular basis.
- The food items used in this research project were safe for human consumption.
- The subjects answered all questions honestly and without bias.
- All the participants understood isiZulu, the language in which the questionnaire was presented, and completed the questionnaire honestly. Similarly, it was assumed that the focus group discussion participants gave honest responses about their perceptions of provitamin A-biofortified maize.

1.7 Definition of terms

Biofortification – The process of breeding staple crops that are micronutrient dense by classical plant breeding methods or modern technology (Johns & Eyzaguirre 2007).

Caregiver – A caregiver is a person who takes care of the basic needs of a person who does not have the capacity to take care of themselves (Hermanns & Mastel-Smith 2012).

Complementary foods – These are foods that are given to infants above six months of age to supplement breastfeeding (WHO 2011).

Food fortification – The addition of micronutrients to accessible and affordable foods that are regularly consumed by a significant proportion of the population at risk of micronutrient deficiencies (Gillespie & Mason 1994, p23).

Infant – An infant is a young baby aged between six months and one year (Rudolf, Lee & Levene 2011, p4).

Maize porridge – Porridge made by cooking refined maize meal with water and salt added. It is a traditional African dish that is served for breakfast (Spearing, Kolahdooz, Lukasewich, Mathe, Khamis & Sharmas 2012).

Provitamin A carotenoids – These are the carotenoids, β-cryptoxanthin, β-carotene and α-carotene. These compounds can be converted to vitamin A in the body (Preedy 2012, pp143-157).

1.8 Abbreviations

AOAC	: Association of Official Analytical Chemists
DOH	: Department of Health
INP	: Integrated Nutrition Programme
NDF	: Neutral Detergent Fibre
NFCS	: National Food Consumption Survey
NFCS-FB	: National Food Consumption Survey: Fortification Baseline
MAM	: Moderate Acute Malnutrition
SAM	: Severe Acute Malnutrition
SAVACG	: South African Vitamin A Consultative Group
SSA	: sub-Saharan Africa
VAD	: Vitamin A deficiency
WHO	: World Health Organization

1.9 Summary

Malnutrition is a major contributing factor to childhood deaths. Micronutrient malnutrition has been identified as a serious health problem, globally, and is on the rise in South Africa. This is evident from the escalating burden of VAD in South Africa. Rural infants are the most affected, as their diets often lack micronutrients. Food fortification, vitamin A supplementation and dietary diversity are the strategies that have been employed in South Africa to alleviate VAD. These strategies have not been effective, for various reasons. Biofortification is the production of micronutrient dense staple crops to alleviate micronutrient deficiencies. This strategy could complement existing strategies in the alleviation of VAD in South Africa and in other countries, especially in SSA, where VAD is prevalent. Appropriate introduction of complementary foods after six months of age is vital for normal health, growth and development in infants. Many rural infants' diets are made up predominately of a bulky starch, maize which, if unfortified, is devoid of vitamin A. Provitamin A-biofortified maize contains a significant amount of vitamin A. Although earlier studies have shown that the sensory properties could affect the acceptability of provitamin A-biofortified maize, there is evidence that if a product is beneficial to the health of infants, it may be accepted by female caregivers. It has been shown that population age, maize type and sensory attributes are crucial determinants of the acceptance of products made with provitamin A-biofortified maize. Therefore, substituting white maize for provitamin A-biofortified maize to produce a soft porridge could possibly be accepted by caregivers and children. There is a need to investigate the sensory acceptability and nutritional composition of provitamin A-biofortified maize porridge as a complementary food.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

The literature review covers the extent of malnutrition in South Africa and focuses on vitamin A deficiency. The strategies employed to combat VAD in South Africa are reviewed. The proposal to use provitamin A-biofortified maize as a complementary food to combat VAD is evaluated.

2.2 Malnutrition in children, with special focus on South Africa

According to the WHO (2013b), it was estimated that more than 33% of child deaths worldwide were caused by malnutrition (WHO 2013b). In SSA there was an increase in the percentage of underweight children from 11.7% to 13.5% between 1990 and 2010 (Lutter *et al* 2011). High rates of stunting were noted in SSA (de Onis, Frongillo & Blössner 2000). In 2011, 40% of children under five years of age living in SSA were stunted (UNICEF 2013b). South Africa, like many other developing countries in SSA, is a country that is faced with the double burden of malnutrition, which includes both under and over-nutrition. The reason for the double burden is the transition from rural to urban areas (Lutter *et al* 2011; Schönfeldt, Gibson & Vermeulen 2010). Traditional diets, which are high in carbohydrate and fibre, are being abandoned for high fat foods, resulting in over-nutrition. South African women are the most affected by over-nutrition, whereas infants are at high risk of under-nutrition (Bourne, Lambert & Steyn 2002). The rural-urban transition results in stunted children becoming overweight in adulthood (Kimani-Murage, Kahn, Pettifor, Tollman, Dunger, Gómez-Olivé & Norris 2010). Common trends observed in South Africa are over-weight amongst individuals with higher education and under-nutrition among impoverished individuals (Steyn, Labadarios, Maunder, Nel, Lambard & Directors of the National Food Consumption Survey 2005). A study that was conducted on the mortality rate in South Africa in 2007 showed that 14.6% of infants younger than one year died as a result of malnutrition (Statistics South Africa 2009). Malnutrition can lead to childhood mortality due to diarrhoea, pneumonia, severe infections, malaria and measles (Caulfield, de Onis, Blössner & Black 2004). The next section reviews malnutrition in childhood.

2.2.1 General malnutrition in childhood and its causes

The major form of malnutrition observed in children in developing countries is protein-energy malnutrition. Protein-energy malnutrition results from a lack of one or more macronutrients that are required by body tissue in order to sustain optimal function of the human body (Manary & Sandige 2008). It is caused largely by deficiencies in protein and glycaemic carbohydrates (Mahan *et al* 2012, p55). Malnutrition can manifest in different ways depending on the symptoms presented. Malnutrition can be classified as severe acute malnutrition (SAM) or moderate acute malnutrition (MAM) (DOH 2012a). SAM is characterised by the presence of severe wasting or pitting oedema and can clinically present as marasmus, kwashiorkor or marasmic kwashiorkor (DOH 2012a). If a child is deficient in both energy and protein and presents with no oedema, it is known as marasmus. If a child is consuming a predominately high carbohydrate diet and is deficient in protein and has oedema, it is known as kwashiorkor (Bain, Awah, Geraldine, Kindong, Sigal, Bernard & Tanjeko 2013; Mahan *et al* 2012, p55). Marasmic kwashiorkor is the presence of both wasting and bilateral pitting oedema (DOH 2012b). MAM is classified as the presence of moderate wasting with no oedema (DOH 2012b). Malnutrition can be classified using anthropometric measurements (Table 2.1).

Table 2.1 The classification of malnutrition using anthropometric measurements (DOH 2013a, p72; DOH 2012b; Berry, Hall & Hendricks 2010; WHO & UNICEF 2009, p2)

Anthropometric Measurement	Measurement	Classification
Weight for height	Below -3SD	SAM
	Above -3SD , Below -2SD	MAM
Mid upper arm circumference (MUAC) (Children aged 6-60 months)	Less than 11.5cm	SAM
	Above 11.5cm, Below 12.5cm	MAM
Weight for age	Below -2 SD	Indicates weight loss, malnutrition
Height for age	Below -2SD	Indicates chronic malnutrition

SD=standard deviation

When a child is malnourished, the body is in a state of starvation (Mahan *et al* 2012, p55). The human body requires glucose as an energy source from the food consumed. In the absence of glucose, free fatty acids are utilised as an energy source. Certain organs, such as the kidneys, brain, eyes and red blood cells, are unable to use free fatty acids as a source of

fuel and require glucose as an energy source. During starvation, the main aim of the body is to provide the brain with a constant energy supply (Mahan *et al* 2012, p55). The gluconeogenic activity increases in order to increase the basal metabolic rate (Mahan *et al* 2012, p51). Glycogen is required to maintain glucose levels in the human body, but in states of starvation the glycogen levels are depleted and not replenished (Mahan *et al* 2012, p55). Starvation affects the gastrointestinal tract, cardiac muscle, liver, kidneys and the immune system (Manary & Sandige 2008). During starvation, the gastrointestinal tract cannot absorb nutrients needed by the body as a result of damage caused by muscle atrophy. The cardiac muscles do not function optimally and are unable to tolerate excess fluid. There is inadequate production of glucose, due to the damaged liver being unable to support gluconeogenesis. The liver is not able to metabolise protein. Sodium and excess fluid cannot be excreted by the kidneys during starvation. Appropriate nutrition is required to correct electrolyte imbalance, prevent further organ damage and provide the correct amounts of macronutrients for sustainability (Mahan *et al* 2012, pp54-56).

The causes of malnutrition are best illustrated in the United Nations' UNICEF conceptual framework (Figure 2.1). There are many factors that are responsible for the vicious cycle of malnutrition, such as poverty, food insecurity, inadequate infrastructure and access to healthcare facilities, lack of education and inadequate food intake (Bain *et al* 2013; Kimani-Murage *et al* 2010; Chopra Daviaud, Pattinson, Fonn & Lawn 2009; Manary & Sandige 2008; Smuts, Faber, Schoeman, Laubscher, Oelofse, Benadé & Dhansay 2008).

Basic causes at societal level

The basic causes at societal level are limited access to resources and environmental technology (UNICEF 1998). The majority of the South African population consists of more than 75% black Africans, with a high percentage of this population living below the poverty line (Argent, Finn, Leibbrandt & Woolard 2009). It was estimated that poverty affects 43% of South African households (Charlton & Rose 2002). Many rural individuals rely on social grants as the main source of income (Schoeman, Faber, Adams, Smuts, Ford-Ngomane, Laubscher & Dhansay 2010; Smuts *et al* 2008). These communities do not have enough money to purchase adequate food, resulting in an increased susceptibility to infection and disease. In comparison to other provinces in South Africa, KwaZulu-Natal has the highest proportion and number of impoverished individuals (Argent *et al* 2009).

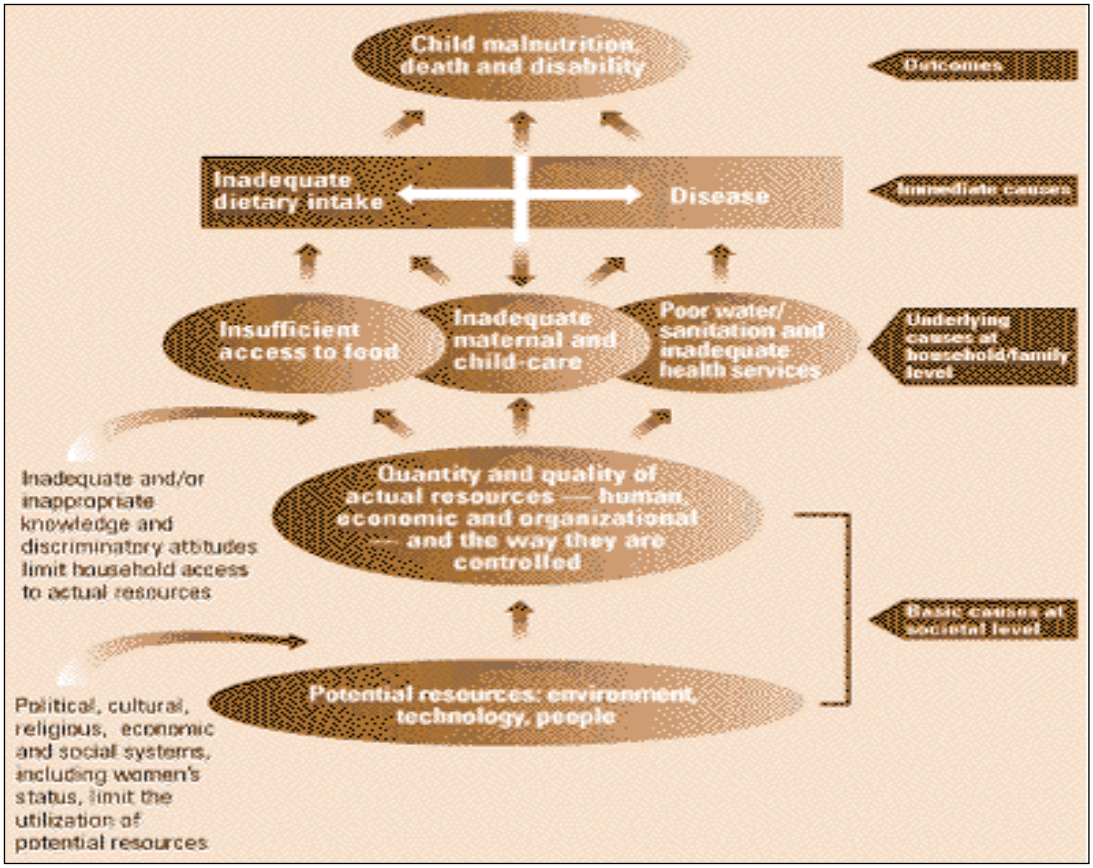

Figure 2.1: The UNICEF conceptual framework (UNICEF 1998).

Underlying causes at household/family level

The underlying causes at household/family level are due to insufficient access to food, inadequate maternal and child-care and poor water/sanitation and inadequate health services (UNICEF 1998). Poverty results in food insecurity (Kimani-Murage *et al* 2010). The NFCS (2005) showed that one in five households in South Africa were food insecure (Labadarios *et al* 2007). Access to food still remains a problem, despite the fact that there is adequate supply of food worldwide (Schönfeldt *et al* 2010). The high rate of unemployment in rural areas is the main cause of the inadequate access to foods. Rural diets lack variety and tend to lack fruit and vegetables, which are high in micronutrients (WHO 2013b). The rise in food prices directly affects the amount and variety of food consumed by the low-income South African population (Kruger, Schönfeldt & Owen 2008). The inadequate intake of nutritious food leads to nutrient deficiencies, reduced immunity and child and maternal undernutrition (WHO 2013b; Magadi 2011).

Some rural communities lack adequate infrastructure (Chopra *et al* 2009). In South Africa, approximately eight individuals reside in one household (Schoeman *et al* 2010). This

household consists of a single room, with no electricity or clean, safe water. This has a negative impact on the health of its occupants (Chopra *et al* 2009; Department of Health (DOH), Medical Research Council (MRC) & OrcMacro 2007). Poor sanitation and infrastructure can exacerbate the disease process and result in malnutrition (Figure 2.1).

Education is a major factor that could result in the reduction of malnutrition. Many of the individuals living in areas of low socio-economic status have little or no form of education. Maternal education on family planning and the utilisation of available resources efficiently could reduce the degree of malnourished children and overpopulation (Bain *et al* 2013). A lack of infrastructure and sanitation, adequate food, adequate healthcare services and education results in malnutrition and could lead to mortality (Bain *et al* 2013; Chopra *et al* 2009; Smuts *et al* 2008).

Immediate causes

A major cause of malnutrition is inadequate food intake (Manary & Sandige 2008). In infants, under-nutrition results in a high risk of becoming stunted, wasted or underweight. The high rates of malnutrition in these infants may be attributed to the mothers being malnourished and HIV infected (Magadi 2011). Malnutrition is a vicious cycle that has long-term effects in adulthood. Infants who are malnourished have a slower rate of development and do not reach their full potential in adulthood (Bain *et al* 2013). This results in a vicious cycle of poverty and malnutrition and could lead to death (Faber & Wenhold 2007). Although macronutrient malnutrition is a major concern, micronutrient deficiency is mostly overlooked. The next section discusses micronutrient deficiencies.

2.2.2 Micronutrient malnutrition, particularly vitamin A deficiency

Micronutrient deficiencies affect as many as two billion people, worldwide. It is caused by individuals consuming a poor quality diet with a lack of diversity. The most common micronutrient deficiencies experienced in developing countries are iron, iodine, zinc and vitamin A (Bain *et al* 2013; Smuts, Dhansay, Faber, Van Stuijvenberg, Swanevelder, Gross & Benadé 2005b). Micronutrient deficiencies result in several health conditions, including growth retardation and delayed development (Bain *et al* 2013). The NFCS (1999) showed that children in South Africa were deficient in the following micronutrients: vitamin A, calcium, iron, zinc, folate, vitamin B6, niacin, riboflavin, vitamin C and vitamin E. This study further indicated that the highest numbers of micronutrient deficiencies were seen in the

rural communities of South Africa (Labadarios *et al* 2000). Children who are infected with HIV/AIDS are at a higher risk of micronutrient malnutrition. HIV infection worsens the disease process and results in developmental delays and growth retardation (Anabwani & Navario 2005).

Vitamin A is a fat-soluble vitamin that is important for vision, protein synthesis, cell differentiation, immunity, growth and development and plays a role as an anti-oxidant (Whitney, De Bruyne, Pinna & Rolfes 2011, pp203-204; Loveday & Singh 2008). One of the most common attributes associated with VAD is diarrhoeal disease (Nannan, Norman, Hendricks, Dhansay, Bradshaw & South African Comparative Risk Assessment Collaborating Group 2007). Vitamin A supplementation, together with other micronutrients, may decrease the prevalence of stunting and diarrhoea in children (Chhagan, Van den Broeck, Luabeya, Mpontshane, Tomkins & Bennish 2010). Poor communities have diets that are high in starch such as maize, and poor in animal foods. These diets lack dietary diversity, are nutritionally insufficient and may lead to VAD (Mayer *et al* 2008; Stevens & Winter-Nelson 2008).

Vitamin A-rich animal foods contain retinyl esters. Plant sources of vitamin A contain provitamin A carotenoids, from which vitamin A activity is derived (Harrison 2012). The liver and intestine are important sites for β-carotene conversion to vitamin A in human beings (Harrison 2012). Animal sources contain the active form of vitamin A (Mahan *et al* 2012, p57). Retinoids are formed from a group of compounds known as carotenoids, which are found in vitamin A-rich animal and plant foods (Mahan *et al* 2012, p57). Good plant sources of vitamin A are papaya, ripe mango, butternut, pumpkin, carrot, orange sweet potato, spinach and indigenous green leafy vegetables (Faber, Laurie, Ball & Andrade 2013a, p51). Xanthophylls and carotenes are the two forms of carotenoids that are produced by plants and cannot be made by animals. There are many forms of carotenoids that possess vitamin A activity namely alpha, β, gamma and β-cryptoxanthin (Chapman 2012). β-carotene is the most common type of carotenoid and is present in fruit and vegetables. The darker the colour of the fruit and vegetables the greater the amount of β-carotene present (Chapman 2012). The most bioavailable source of vitamin A comes from animal products such as egg yolks, liver and dairy products. These forms of vitamin A are not accessible to many rural individuals, as these foods are expensive (Faber & Wenhold 2007). The addition of fats to vitamin A rich foods increases the absorption of Vitamin A (Chapman 2012).

Individuals from low-income communities do not have sufficient money to purchase foods rich in micronutrients. This results in micronutrient deficiencies such as vitamin A deficiency (West & Mehra 2010). Children living in rural areas rely on plant sources for vitamin A (Sommer 2008). Not only is nutritional intake a problem in the rural areas, there is also poor utilisation of healthcare services, resulting in many individuals not obtaining treatment for various conditions (Smuts *et al* 2008). Mothers are not utilising healthcare facilities and their infants are not receiving adequate vitamin A supplementation. Young children and pregnant women are mostly affected by micronutrient deficiencies such as VAD (WHO 2013c). VAD can lead to xerophthalmia, keratomalacia, irreversible damage to the cornea, night blindness, vomiting, hair loss, anorexia, muscle weakness, poor immune function, increased susceptibility to infection, pneumonia and pigmentary retinopathy (Chapman 2012; Whitney *et al* 2011, p205; American Academy of Pediatrics 2009, p461). Inadequate dietary intake leads to infectious diseases and, if untreated, could result in either malnutrition or death (Faber *et al* 2013a, p18)

The SAVACG (1995) study showed that 33% of children between the ages of six and 71 months suffered from marginal vitamin A deficiency in South Africa (Labadarios & Van Middelkoop 1995). The NFCS (1999) indicated that 88.9% of children had an inadequate vitamin A status. This study showed that KwaZulu-Natal had the highest rate of vitamin A deficiency, with 44% of children between one and nine years being vitamin A deficient (Labadarios *et al* 2000). A study conducted in two disadvantaged communities in the Western Cape, South Africa, found that 23% of black infants had marginal vitamin A deficiency (Oelofse, Van Raaij, Benadé, Dhansay, Tolboom & Hautvast 2002). The NFCS (2005) reported that the number of children with VAD in South Africa had increased to 64% (Labadarios *et al* 2007).

The millennium development goals have been put into place to assist with improving the nutritional status of individuals by 2015. The first millennium developmental goal (MDG) is to eradicate hunger and poverty and the fourth is to reduce child mortality (United Nations Systems Standing Committee on Nutrition 2004, pp5-6). These goals are far from being achieved in South Africa. To reach these goals, the nutritional status of children must be improved by trying to prevent and treat both micronutrient and macronutrient deficiencies; but there is slow progress towards achieving these goals (Lutter *et al* 2011). Even though the South African government has implemented various strategies to alleviate VAD, it is still a major health problem. The next section will discuss these strategies.

2.3 Strategies employed to combat VAD in South Africa and their shortcomings

As mentioned earlier, the VAD situation in South Africa is worsening. The South African government have put various strategies in place to assist in the alleviation of VAD. These include food fortification, vitamin A supplementation and dietary diversity (DOH 2012a; Swart *et al* 2008; DOH & UNICEF 2007, p8).

2.3.1 Food fortification

Food fortification is a process whereby micronutrients are added to foods that are commonly consumed by a population that is at risk of micronutrient deficiencies (Pretorius & Schönfeldt 2012; Gillespie & Mason 1994). This may be seen as a cost-effective way of increasing the micronutrient intake of individuals living in developing countries. Many products are already being fortified with vitamin A in South Africa, namely maize meal, wheat flour and, less often, margarine (Roelf 2011; DOH 2002). The findings of the NFCS of 1999 resulted in the South African Department of Health fortifying maize and wheat flour with vitamin A, iron, zinc, folic acid, thiamin, niacin, vitamin B6 and riboflavin, as of October 2003, and are part of South African legislation (DOH & UNICEF 2007, p8). This was decided, as the study found that bread and maize meal were the most commonly consumed staples by the rural population (Labadarios *et al* 2000). Food fortification was implemented as an approach to help alleviate malnutrition (Pretorius & Schönfeldt 2012). Even though this is one of the long-term strategies employed to alleviate VAD it is not always accessible by communities residing in remote areas (Horton 2006; Dary & Mora 2002; Latham, Ash, Ndossi, Mehansho & Tatala 2001). Food fortification is only an effective strategy if the target population is able to purchase and utilise fortified foods (Steyn, Nel & Labadarios 2008). As with many low-income households, there is a low consumption of commercially processed foods (Dary & Mora 2002). The low consumption of fortified foods by individuals living in rural areas may be due to the high reliance on social grants and the poor affordability of fortified foods (Loveday & Singh 2008; Ortiz-Monasterio *et al* 2007). The other challenges with food fortification are keeping the micronutrients stable in the fortification mixture, the fortification mixture not being mixed properly, milling companies having inadequate quality control and the fact that sunlight exposure may result in losses of vitamin A, riboflavin and folic acid (Pretorius & Schönfeldt 2012; DOH & UNICEF 2007, p8).

2.3.2 Vitamin A supplementation

Vitamin A supplementation is another strategy that was implemented by the South African Department of Health (DOH) in order to alleviate VAD. According to the DOH regulations, all infants and children are to be supplemented with routine and therapeutic doses of vitamin A. Previously, all infants under six months of age received a dose of vitamin A. However, since 17 September 2013, only infants over six months and under 59 months of age are to receive a routine dose of vitamin A supplementation (DOH 2012a). Therapeutic doses of vitamin A are given to infants who present with clinical signs of vitamin A deficiency or who are severely malnourished (DOH 2012a).

Although vitamin A supplementation is available at clinics and hospitals, many infants do not receive it. There has been poor utilisation of health care facilities by individuals living in KwaZulu-Natal. The poor utilisation of healthcare facilities was confirmed by the high percentage of home deliveries recorded in KwaZulu-Natal. Twenty-five percent of mothers residing in rural areas of KwaZulu-Natal had no access to healthcare facilities (Smuts *et al* 2008). Many poor rural communities are unable to bring their children to clinics and hospitals for them to get their routine doses of vitamin A supplementation. Many of these infants only receive a therapeutic dose of vitamin A supplementation due to a poor diet and lack of appropriate health care treatment (DOH 2012a).

A high percentage of individuals from the Umkhanyakude and Zululand districts in KwaZulu-Natal showed poor vitamin A coverage. Twenty-three percent of children from the Umkhanyakude and 33% of children from Zululand did not receive vitamin A supplementation (Schoeman *et al* 2010). Education may be a contributing factor to the poor coverage, as many caregivers from these communities were unaware of the importance of vitamin A (Faber & Benadé 2007).

Other issues with vitamin A supplementation include the possibility of overdosing on vitamin A supplementation, as well as the vitamin A supplements being ineffective due to incorrect storage (DOH 2012a). Unopened bottles of vitamin A capsules can be stored for a maximum of two years in a cold, dark place. However, if they are stored in direct sunlight or frozen, these capsules lose their nutritional value (DOH 2012a; Loveday & Singh 2008).

2.3.3 Dietary diversification

Dietary diversity is viewed as a long-term strategy to combat VAD in South Africa (Latham *et al* 2001). A diversified diet consists of a variety of food types, including fruit and vegetables, legumes, starch and animal products. Unfortunately, vegetables, fruit and animal products are not often consumed by impoverished communities living in developing countries, due to the high cost of these foods (Faber *et al* 2002). Food gardens are becoming popular in the KwaZulu-Natal province of South Africa. This trend is as a result of community initiatives and/or the initiatives and support of local and provincial government institutions and other organisations (Smuts *et al* 2008; Faber *et al* 2002).

A cross-sectional study conducted by Smuts *et al* (2008) indicated that as many as 49% of households in KwaZulu-Natal had their own food gardens. However, β-carotene-rich foods and vegetables were only eaten by a few individuals. The vitamin A status could be improved if a greater quantity of these vegetables were eaten (Faber 2005a).

Similarly, in the Eastern Cape Province of South Africa, The Medical Research Council/Agricultural Research Council (MRC/ARC) has given support to individuals to plant vitamin A-rich crops, together with their normally grown crops (Faber, Witten & Drimie 2011). There were two projects involving in home gardens, namely the Ndunakazi project and the Lusikisiki project (Faber *et al* 2011). The first project involved demonstration gardens being conducted at training sites in the local community. Individuals from the community were taught the most effective ways to cook vitamin A-rich foods, in order to achieve the greatest availability of provitamin A carotenoids (Faber *et al* 2011). The second project aimed to modify existing gardens to optimise vitamin A intake. Demonstrations and training were conducted on existing gardens (Faber *et al* 2011). The "Mdantsane for Vitamin A" project was another project implemented to combat vitamin A deficiency. This project targeted children between the ages of six and 59 months. The main aim was to improve vitamin A supplementation, as well as to promote the gardening of vitamin A-rich foods (Faber *et al* 2011).

Despite the use of food gardens to alleviate VAD, there are many problems that limit their effectiveness. These include lack of knowledge, insect pests, plant diseases, animals destroying produce, no fencing or protection, lack of seeds, gardening supplies and money (Faber *et al* 2013a, p13; Faber, Laubscher & Laurie 2013b). Dietary diversification can be viewed as a long-term goal to alleviate VAD. However, this strategy requires significant

economic resources and is therefore not appropriate for the low-income communities of South Africa. This is the same in almost all other countries in SSA (Faber, Phungula, Venter, Dhansay & Benadé 2002).

2.3.4 Integrated Nutrition Programme (INP)

In 1995, the Integrated Nutrition Programme (INP) was introduced by the DOH in order to help improve the nutritional status of South Africans. The three main components of the INP are: health facility-based nutrition programmes and strategies; community-based nutrition programmes and strategies; nutrition and HIV and AIDS support programmes and strategies (Swart *et al* 2008). The INP programme was aimed at three target groups, namely: malnourished children and their households; pregnant mothers and their families; families and households at nutritional risk (Health Systems Trust 2013). There are six key focus areas of this programme, which includes: disease-specific nutrition support, treatment and counselling; growth monitoring and promotion; control of micronutrient deficiencies; promotion, protection and support of breastfeeding; contribution to household food security and social assistance (Hendricks, Eley & Bourne 2006). Each focus area has a programme or programmes targeted at specific groups (Hendricks *et al* 2006).

Even though these strategies are in place, there are many factors that lower the effectiveness of the INP (Figure 2.2).

Fortification supplementation and dietary diversification are ways to alleviate VAD, but are not always effective. It is vital to explore other complementary strategies to assist in the alleviation of VAD. One of these strategies is biofortification, which will be discussed in the next section.

2.4 Biofortification as a new strategy to combat vitamin A deficiency

2.4.1 What is biofortification?

It is evident that some communities do not have access to supplementation and are unable to purchase fortified foods. A more effective and sustainable way of alleviating the burden of VAD could be through biofortification (Saltzman, Birol, Bouis, Boy, De Moura, Islam & Pfeiffer 2013).

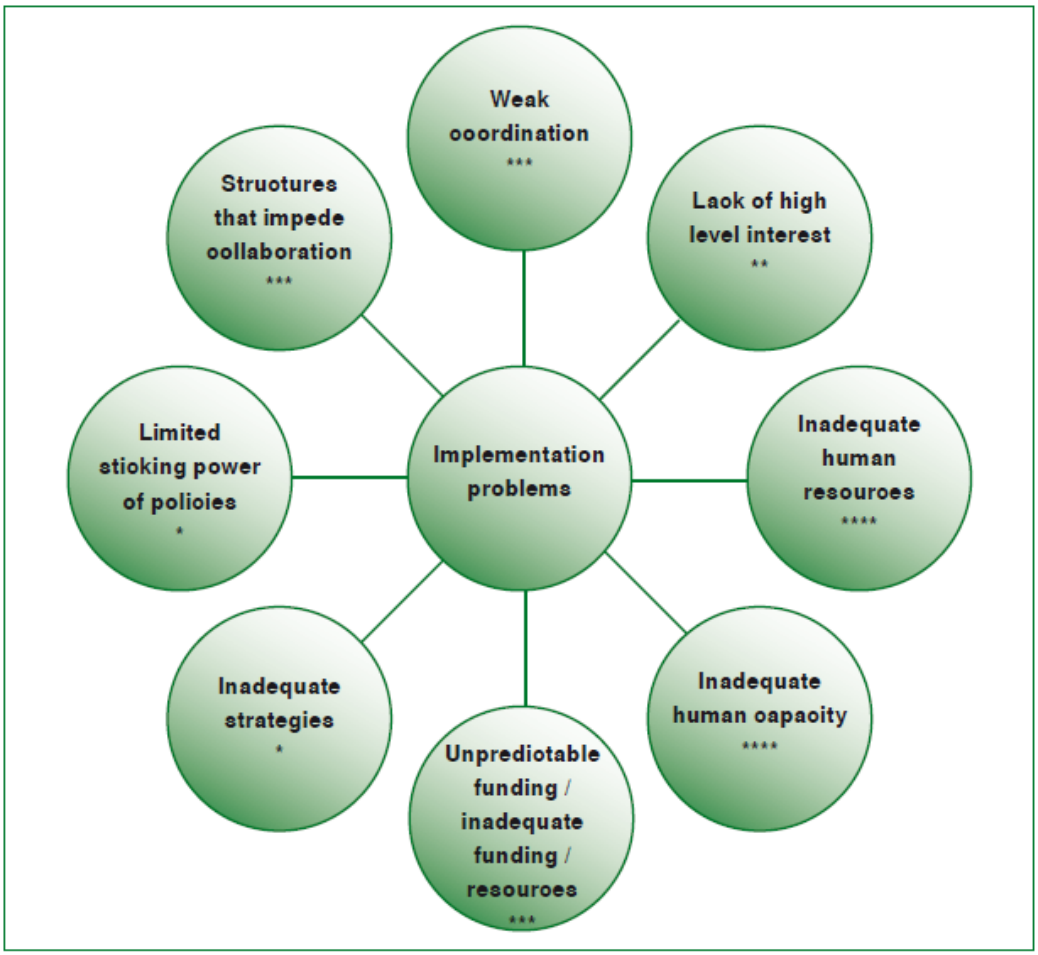

Figure 2.2 Core problems reducing the effectiveness of the INP (Swart *et al* 2008, citing Morris *et al* 2008)

Biofortification is a complementary strategy that involves enhancing popular staple crops with vitamins and minerals through different biofortification methods (Khush *et al* 2012; Meenakshi *et al* 2010a). Biofortification can be achieved through conventional breeding or genetic modification (Saltzman *et al* 2012; Stevens & Nelson-Winter 2008). Currently, HarvestPlus has a biofortification programme in place that makes use of conventional breeding. The main focus of this programme is to use iron, zinc and provitamin A for biofortification (HarvestPlus 2009a). Seven staple crops have been identified for the biofortification process. These are beans, cassava, maize, pearl millet, rice, sweet potato and wheat (HarvestPlus 2009a). These crops were chosen as they are the most commonly eaten staple foods in the specific areas targeted by the HarvestPlus programme. Biofortication is an ideal medium to ensure that individuals living in specific areas at risk of micronutrient

deficiencies receive these micronutrients through the popular staple foods (HarvestPlus 2009a).

2.4.2 Possible advantages of biofortification

Biofortification is a cost-effective and sustainable way of providing micronutrients to disadvantaged communities. It is more cost-effective than other strategies that are currently in place. Biofortification is a single investment to produce crops that can yield micronutrient dense crops that can be used for many years (Nestel *et al* 2006). There is no need to add any fortificants to the staple crop, as it has already been genetically modified or conventionally bred (Bouis 2003). Biofortification is particularly targeted at low-income, malnourished communities. Children are not receiving vitamin A supplementation for various reasons and many households are unable to purchase vitamin A-rich foods. Thus biofortification with provitamin A could be adopted to help alleviate VAD and provide infants with more nutritious foods, as well as access to the crops. Many of these rural individuals live in remote areas and may be able to benefit from biofortified crops, as these crops are more accessible to them (Nestle *et al* 2006; Bouis 2003). Biofortified crops may also enhance the nutritional composition of breastmilk. Breastfed infants over six months of age may benefit from maternal consumption of biofortified crops, thus decreasing the risk of micronutrient deficiencies (HarvestPlus 2010). There is a risk of toxicity from the overconsumption of vitamin A-rich foods, as well as high doses of vitamin A supplementation, which could cause undesirable side-effects. With biofortification there is no risk of toxicity (Stevens & Winter-Nelson 2008). However, consumers have to accept the biofortified crops, in order for it to have health benefits (Johns & Eyzaguirre 2007).

2.4.3 Provitamin A-biofortified maize

The HarvestPlus challenge programme was officially launched in 2004, making it a global leader in biofortification. HarvestPlus is managed within the Consultative Group on International Agricultural Research (CGIAR) programmes (HarvestPlus 2009a). This initiative is being co-ordinated by the International Centre for Tropical Agriculture (CIAT) and the International Food Policy Research Institute (IFPRI), both of which are part of the CGIAR institutions (HarvestPlus 2009a). HarvestPlus targets over 25 countries that are faced with micronutrient deficiencies and provides nutrient dense crops to the target population at risk of micronutrient deficiencies. HarvestPlus is most active in Africa, Asia and Latin America (HarvestPlus 2009a). Zambia has been identified as a target country for the

biofortification of maize, due to the high levels of VAD and maize consumption. According to HarvestPlus (2009b), most individuals are accustomed to white maize while provitamin A-biofortified maize exhibits an orange-yellow colour, thus affecting its acceptability. Fifty-three percent of Zambian children have VAD (UNICEF 2008). It was suggested that the orange maize taste would be well accepted by the target population. Orange maize has a distinct taste separating it from yellow maize which is not as well accepted as other maize varieties, thus improving the acceptance of orange maize in the target population (HarvestPlus 2009b). The provitamin A content of HarvestPlus staple crops is 15µg/g (HarvestPlus 2009b). Some maize varieties contain between 0.5 and 1.5 µg of β-carotene (Harjes, Rocheford, Bai, Brutnell, Kandianis, Sowinski, Stapleton, Vallabhaneni, Williams, Wurtzel, Yan & Buckler 2008). This is below the target level for fortification, which is 15 µg of β-carotene (Nuss, Arscott, Bresnahan, Pixley, Rocheford, Hotz, Siamusantu, Chileshe & Tanumihardjo 2012).

Provitamin A biofortified maize is not readily available on the South African market (Khumalo *et al* 2011). Not many farmers in African countries such as Zimbabwe currently grow yellow biofortified maize on their farms (Muzhingi *et al* 2008). However, many farmers are willing to grow provitamin A-biofortified maize due to a number of attractive attributes (Muzhingi *et al* 2008). It is drought-resistant and pest-resistant and yellow maize matures at a faster rate than white maize. Provitamin A-biofortified maize may be viewed as a potential complementary strategy for combating VAD. It is also cost effective (Muzhingi *et al* 2008). Similar to Zambia and other countries in SSA, South Africa would be an appropriate target for the biofortification of maize with provitamin A, due to the high consumption of white maize in South Africa. This is reviewed in the next section.

2.4.3.1 Utilisation of maize as a staple crop in South Africa

Maize (*Zea mays*), also known as corn, is the most popular staple food, consumed worldwide, especially in developing countries (Tumuhimbise, Namutebi, Turyashemererwa & Muyonga 2013; Nuss & Tanumihardjo 2010; Johnson 2000, p31). Globally, 85% of yellow maize and between 10 and 12 % of white maize are grown annually (Ram & Mishra 2010, p285). Maize is the second most produced crop in the world and is a major staple food in SSA (IITA 2010; Johnson 2000, p31). Maize was first introduced to East Africa by the Portuguese during the 16th century (Hassan 1998, p4). Maize was not seen in South Africa by Jan van Riebeeck in 1652, although other food sources were identified in the Cape (McCann 1999, p251). It was

recommended by Jan van Riebeeck that maize seeds should be shipped into South Africa to be grown among local crops (McCann 1999, p251). Maize has become a major cereal grain in Africa over the past two decades. The International Centre for Maize and Wheat Improvement (CIMMYT) has developed 441 maize breeding lines for South Africa (McCann 1999, pp248-266). In South Africa about eight thousand tonnes of maize grain is produced annually and about 50% of the maize grain grown is for human consumption (Du Plessis 2003).

Maize is a tall plant with an extensive fibrous root system. This plant belongs to the grass family, Poacaeae [Serna-Saldivar 2010, p9; Ram & Mishra 2010, p284; Food and Agriculture Organization of the United Nations (FAO) 1992]. The maize plant contains both male and female parts. The male parts are borne in the tassel and the female parts are located in the ear or pistillate area (Serna-Saldivar 2010, p12). The grain is produced in the ear of the plant (FAO 1992). Maize grains are one of the largest grains produced. The kernel of the maize is hard, and on average, weighs 350 mg (Delcour & Hoseney 2010, pp9-11; Ram & Mishra 2010, p287). The maize plant grows optimally at 40^{o}C. It is highly susceptible to low temperatures and frosting. Maize is usually grown during spring and summer seasons, as there is an optimum temperature for growth (Serna-Saldivar 2010, pp10-12). There are six types of maize kernels that vary in quantity, quality, shape of the endosperm, composition and colour (Johnson 2000, p34). There are seven types of maize kernels, which vary from yellow to blue in colour (Johnson 2000, p34). In South Africa, similar to other sub-Saharan countries, maize is processed into a variety of foods, including porridges, breads and beverages (Ortiz-Monasterio *et al* 2007).

2.4.3.2 Maize kernel structure and chemical composition

Kernel structure

Figure 2.3 shows the four major structures of the maize grain, namely pericarp, endosperm, germ and tip cap. The maize kernel contains 5-6% pericarp, approximately 83% endosperm, 10-14% germ and the remainder is the tip cap. The endosperm contains a protein matrix, which holds together polygonal starch molecules (Delcour & Hoseney 2010, p9; Ram & Mishra 2010, p287; FAO 1992). The maize kernel contains an opaque area situated at the centre of the kernel and a translucent area close to the aleurone. The yellow kernel colour of some maize varieties is attributed to the presence of carotenoids in the maize kernel (Hallauer

2001, p4). White and yellow maize varieties contain more vitreous endosperm than floury endosperm (Delcour & Hoseney 2010, p9; Ram & Mishra 2010, p287).

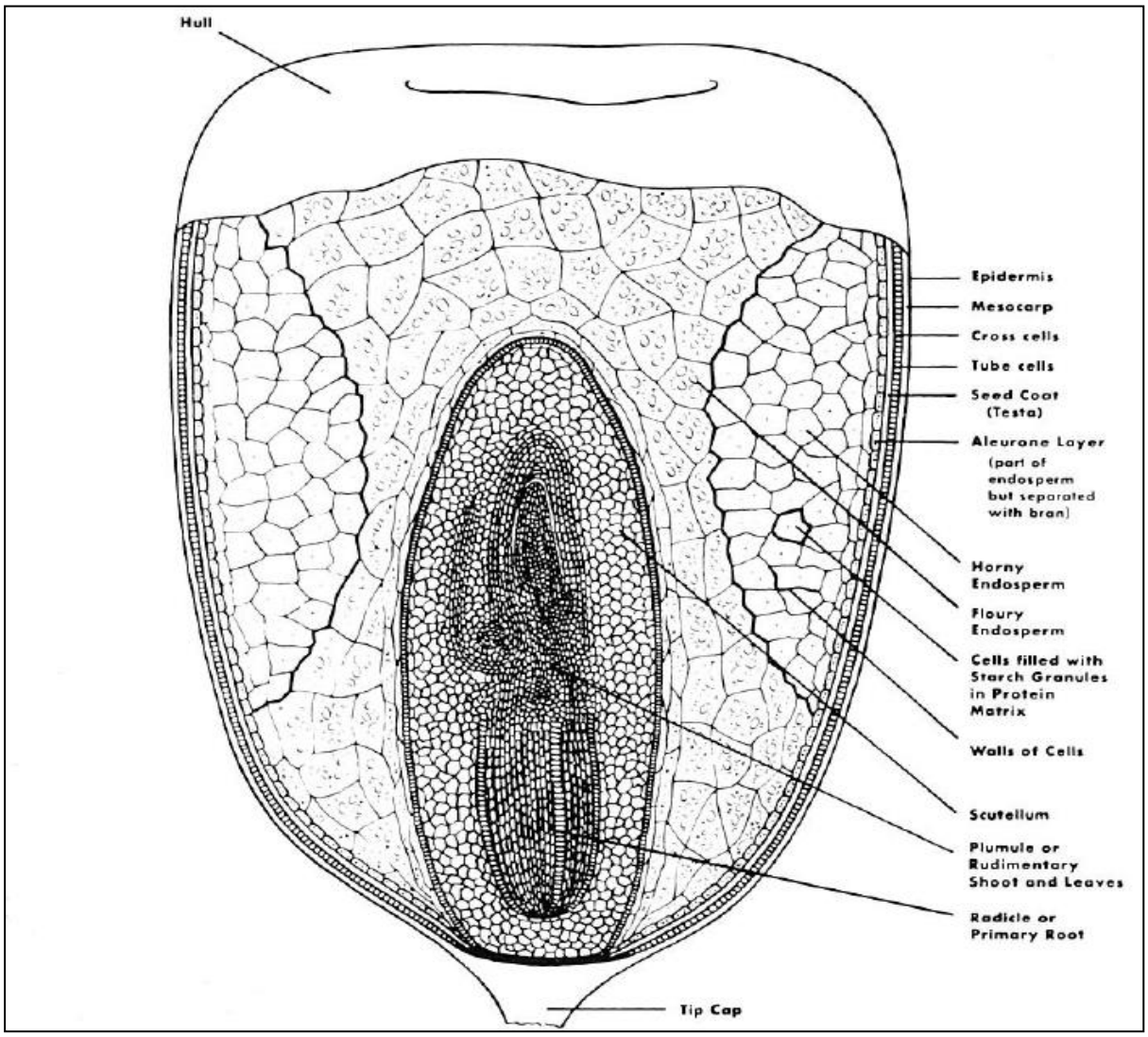

Figure 2.3 Structure of the maize kernel (Delcour & Hoseney 2010, p10)

Nutritional composition

The maize kernel consists of 71.3% starch. The starch is located in large amounts in the endosperm layer (Johnson 2000, p38; Hallauer 2001, p9). The starch granule contains 70% amylase and 100% amylopectin components (Delcour & Hoseney 2010, p29; Hallauer 2001, p10). Starch is a vital component, as it is a significant source of energy (Robinson 1987, pp62-66). Although white maize contains high quantities of starch it is limited in other nutrients. As with other cereal grains, maize has a limited protein content and the protein quality is low. The normal dent maize contains an average of 8.7% protein (Table 2.2). Zein is a type of prolamin protein and is the largest constituent of protein found in the maize kernel. The endosperm contains 44% zein proteins, 28% glutelines and 5% albumin and

globulins (Delcour & Hoseney 2010, p64). With the maturation of the kernel there is a decrease in the quality of protein, as there are low levels of lysine, methionine and tryptophan (FAO 1992). Normal dent maize contains about 4.1% fat (Table 2.2). The germ of the maize is used for the production of oil (Delcour & Hoseney 2010, pp 80-81). The oil extracted from the maize kernel is low in saturated fatty acids and contains only small amounts of linoleic and arachidonic acids (FAO 1992).

About 1.3% of the kernel contains micronutrients (FAO 1992). Of that, 78% contains minerals, with phosphorus found in high amounts (FAO 1992; Watson & Ramstad 1987, p73). White maize contains B-vitamins and the fat soluble vitamin, vitamin E (Watson & Ramstad 1987, p73). White maize is devoid of provitamin vitamin A and the mature kernel does not contain vitamin B12 (FAO 1992). The aleurone contains proteins and some minerals and vitamins. However, with processing, most of these nutrients are decreased (Oritz-Monasterio *et al* 2007). Yellow or orange maize contains two fat soluble vitamins, namely vitamin A and E, which are located in the kernel (FAO 1992). There is limited data on the nutritional consumption of yellow and orange maize. However, as stated earlier, non-biofortified white maize is devoid of vitamin A, while some varieties of yellow and orange maize contain significant levels of provitamin A. As reviewed earlier (section 2.4.3) provitamin A-biofortified maize varieties have been found to contain 0.5 to 15 µg/g β-carotene (Harjes *et al* 2008).

White maize naturally contains phytates, which hinder the absorption of minerals such as iron, calcium and zinc (Hotz & Gibson 2001). However, yellow and orange maize have lower levels of phytic acid present (Raboy 2007). Pillay, Siwela, Derera & Veldman (2013) found higher levels of starch, fat and protein in provitamin A-biofortified maize varieties, relative to a white maize variety. The provitamin A-biofortified maize was found to have higher quantities of essential amino acids relative to white maize, except for histidine and lysine. The low levels of these essential amino acids can be overcome by making use of complementary proteins (Mahan *et al* 2012, p52). Complementary proteins are consumed to help compensate for inadequate amino acids from a certain food item. Two protein-rich foods that contain different types of amino acids are combined to yield a complementary protein. In the case of provitamin A-biofortified maize, another protein containing food that is high in the essential amino acids histidine and lysine could be consumed together with the biofortified maize, to form complementary proteins (Whitney *et al* 2012, p133).

Table 2.2 Composition of normal dent maize (Johnson 2000, p38)

Component	Normal Dent
Starch (%)	71.3
Protein, Nx6.25 (%)	8.7
Fat (%)	4.1
Fibre (%)	3.0
Sugars[a] (%)	11.4
Ash (%)	1.5
Amylose (g/100g starch)	24[b]
Amylopectin (g/100g starch)	76[b]
Lysine (g/100g protein)	2.7[c]

[a] Calculated by difference after subtracting starch, protein, fibre and ash
[b] Kazarian & Hall (1965)
[c] Keener *et al* (1985)

Carotenoid retention

The length of storage of carotenoid containing maize can affect the amount of carotenoids present, as observed by Burt, Grainger, Young, Shelp & Lee (2010). The study assessed the degradation of carotenoids in two genotypes (HiC-7 & HiC-26). The samples were analysed in triplicate at 0, 3, 6 and 18 months. It was found that in the first three months the carotenoid levels remained constant, however at six months the levels decreased significantly between 35% and 40% and thereafter remained constant for a year (Burt *et al* 2010). This study further indicated that the lutein and total zeaxanthin ratio did not change in either sample implying that the carotenoid losses were similar between the samples (Burt *et al* 2010). Another retention study focused on carotenoid retention using the following processes: soaking and wet milling, fermentation of wet flour and cooking (Li, Tayie, Young, Rocheford & White 2007). These authors found statistically significant losses of provitamin A carotenoids during soaking and wet milling of kernels. Further carotenoid losses were observed in the fermentation step. Oxidation, degradation and isomerization are the causes of carotenoid losses during food processing. With this said, the authors found a higher carotenoid loss in unfermented, cooked porridge in comparison to cooked, fermented porridge (Li *et al* 2007). The milling process directly affects the provitamin A content, as seen in the study by Li *et al* (2007). In a study by Pillay, Siwela, Derera & Veldman (2011b), which

assessed the retention of provitamin A carotenoids in biofortified maize during processing and preparation, it was found that the lowest retention of provitamin A carotenoids was seen in cooked thin maize porridge after the milling process, whereas *phutu* and samp had the highest provitamin A carotenoid retention (Pillay *et al* 2011b). Fat can be added to provitamin A-biofortified maize to increase the absorption of vitamin A. The poor retention can be compensated for by combining fat-containing foods, together with provitamin A-biofortified maize to improve the absorption of vitamin A (Morgan & Dickerson 2003).

Processing maize into food products

There are a number of methods used to process maize into food products. According to Ram & Mishra (2010, p291), milling is a procedure used to make maize cereal grains more palatable. Maize kernels are milled using three different processes. The method used depends on the use of the maize kernel. The three different types of milling are dry milling, wet milling and nixatamalisation. The dry milling process separates the maize into the outer layer fraction (bran), the germ and the endosperm (Ram & Mishra 2010, p291; Serna-Saldivar 2010, p27). The dry milling products are processed, using different methods, into various food products, including porridges, snacks and breakfast cereals (Johnson 2000, p50). The endosperm is ground into a meal. Wet milling is different from dry milling in that the maize kernel is further broken up to obtain its chemical components (Delcour & Hoseney 2010, p139; Ram & Mishra 2010, p292). The main products of wet milling are starch, protein, fibre and an oil-rich germ. These products have both industrial and food applications (Johnson 2000, pp50-56). Nixatamalisation involves soaking the grain to loosen the outer layers, which are then removed. After treatment, the grain is usually ground into a meal for producing food products such as tortillas and *roti*. Nixatamalisation is not used in Africa but is commonly used in South America. One major advantage of nixatamalisation is that it increases the availability of nutrients (Serna-Saldivar 2010, pp239-242). The next section reviews at the consumer acceptability of provitamin A-biofortified maize.

2.4.3.4 Consumer acceptance of provitamin A-biofortified maize

Maize is a well-consumed staple, worldwide, and an ideal medium for biofortification. Table 2.3 summarises several consumer acceptance studies conducted in Africa.

Table 2.3 A summary of studies on the consumer acceptance of yellow or orange maize (including provitamin A-biofortified maize)

Author/s	Study type and participants	Location of the study	Foods tested	Findings, implications and recommendations
De Groote & Kimenju (2008)	Open-ended questions were used to determine the willingness to purchase biofortified maize	Fifteen supermarkets in Nairobi, Kenya	Biofortified maize	- White maize is preferred over yellow maize. - A discount is needed for consumers to purchase yellow maize. - Consumers that had a higher education preferred white maize. Higher income was associated with an increased interest in biofortified maize. - Nutritional education and awareness programmes should be conducted on biofortified maize, targeting the urban population. - Further studies should be conducted in rural areas where yellow maize is popular.
Stevens & Winter-Nelson (2008)	Framed field experiment, using 201 participants of mixed gender, aged 14-70 years, selected from two market places on different days	Maputo, Mozambique	Maize meal porridge (*Xhima*)	- Meat, eggs and fish were reported to be consumed less than twice a week thus contributing to VAD. - Acceptance of orange maize is more likely when there are children in a household. - The appearance of orange maize was rated higher by women than men. - Aroma and appearance of orange maize was well accepted. - If orange maize was sold at a cheaper price it would be purchased. - Texture of the orange maize is not well accepted; if the texture was improved through breeding, the orange maize would be better accepted. - Orange maize could be used in areas where maize is widely eaten, as this study indicated that frequency of porridge consumption did not affect acceptance. - Nutrition bioavailability studies need to be conducted. The amount of orange maize an individual should consume should be determined. - Overall, yellow maize was well accepted by the participants.
Muzhingi *et al* (2008)	Structured interview & food frequencies, 10 households from 10 villages in the districts and 30 households from the urban area	Mutasa, Mudzi & Nkayi districts and Harare & Bulawayo urban centres, Zimbabwe	Yellow maize	- Only 6% of farmers grow yellow maize, but more than 90% are willing to grow yellow maize as they are more resilient than white maize varieties. - Maize is consumed in many ways, but the most common are porridge, roasted or boiled maize and maize bread. - Yellow maize bread was more preferred in comparison to white maize bread. - Over 50% of the consumers preferred the taste of yellow maize. Almost 33% of consumers disliked the smell. - The female consumers who were heads of households were indifferent in their willingness to purchase yellow or white maize. Males would purchase yellow maize if it was cheaper than white maize.

				- Households that were of low socio-economic status were willing to substitute yellow maize for white maize if they were made aware of the health benefits.
De Groote, Tomlins, Haleegoah, Awool, Frimpong, Banerji, Chowdury & Meenakshi (2010)	Sensory evaluation & individual auctions or Becker De Groot-Marschak procedure;. 703 consumers were interviewed	Ashanti, Central & Eastern region, Ghana	*Kenkey* made with yellow & orange maize	- The results from all three areas were different. - The Ashanti consumers preferred white maize over yellow maize and lastly orange maize. These consumers were willing to spend more on white maize than yellow maize and a lower amount for orange maize than yellow maize. - The central consumers preferred yellow maize over orange or white maize. The central consumers were willing to pay more for yellow maize over white and orange maize. - The eastern consumers preferred both yellow and orange maize over white maize. These consumers were willing to purchase yellow and orange maize over white maize. - It is evident from this study that the acceptance of biofortified maize depends on the geographical location. It will be well accepted in areas where biofortified maize is well known. - Nutrition education via different modes of media could influence consumers' acceptance of biofortified maize.
Meenakshi, Banerji, Manyong, Tomlins, Hamukwala, Zulu & Mungoma (2010b)	Sensory evaluation, 10 semi-trained panellists. Central location testing and home use testing	National Institute for Scientific and Industrial Research in Lusaka, Zambia	Yellow and orange maize cooked into a paste	- Orange maize may possibly be accepted by Zambian consumers. - Orange maize would be purchased without needing any discount, whereas one would be needed for yellow maize. - Nutrition education on biofortified maize could possibly improve the acceptance and willingness to purchase orange maize.
De Groote, Kimenju & Morawetz (2011)	Experimental auction, 100 heads of households, spouse or the person responsible for household purchases was selected	Two districts, Siaya & Vihiga in Western Kenya	Yellow maize was tested	- There is a high prevalence of yellow maize use in Western Kenya. This is why these two districts were selected for the study. - Fortified white maize was the most preferred in Western Kenya. Yellow maize was preferred over white maize in the Siaya district, however. - Consumers were only willing to purchase yellow maize at discounted prices, but will pay a premium for fortified white maize. - The more familiar the consumers were with yellow maize the lower was the aversion to it. - This study indicated that it would be easier to introduce provitamin A-biofortified maize to areas in which yellow maize is usually grown.

Khumalo, *et al* (2011)	Quantitative sensory evaluation tests, using 48 females older than 18 years and residing in one of two villages in Giyani	Giyani district municipality, in the northwest of Limpopo province, South Africa	Maize meal porridge	- The finer the texture of the porridge the higher the acceptance of the porridge. - The study found that price was an important factor to determine if the maize would be purchased. One village was willing to purchase yellow maize for the nutritional benefit, rather than the cost, and the other village would purchase it if it was cheaper than white maize. - There would be better exposure to yellow maize if it was readily available, rather than only during drought periods.
Pillay *et al* (2011a)	Cross sectional study, using primary school and high school children and adults as a convenience sample	Mkhambathini Municipality, KwaZulu-Natal, South Africa	*Phutu*, maize meal porridge and samp	- The acceptance of maize varied with the age of the participants and the type of food consumed. - The sensory attributes of colour, flavour and aroma were not favoured in the study. - The participants in this study would purchase yellow maize if it was available at a cheaper price. - There is a need for education to promote the acceptance of yellow maize among adult consumers. - There is a need to identify the type of provitamin A-biofortified foods that would be highly acceptable to consumers.
Meenakshi, Banerji, Manyong, Tomlins, Mittal & Hamukwala (2012)	Central location and home-use testing, randomised sample of 10 households	Central and southern provinces of Zambia	Maize meal porridge	- Participants of this study were willing to purchase orange maize without any nutritional intervention. - Orange maize and white maize were scored similarly for all sensory attributes (appearance, taste, aroma, texture and overall acceptability) by a trained panel. - Orange maize scored better in comparison to yellow maize. - A discount is needed, so that yellow maize would be purchased, however, participants would be willing to purchase orange maize for a higher price.
Nuss *et al* (2012)	27-35 children between 3 and 5 years participated in the trial	Nyimba District, Eastern Province of Zambia	Maize meal porridge (*Nshima*)	- Yellow maize is negatively associated with food aid, as it is used when there is a shortage of white maize. - Within the first week of the study the children accepted orange maize dishes. - There is potential to use provitamin A-biofortified maize in communities with micronutrient deficiencies.

The sensory attributes of provitamin A-biofortified maize is an important factor in the acceptance of the maize. The research findings summarised in Table 2.3 indicate that, generally, white maize is more acceptable to consumers than the yellow or orange maize (including the provitamin A-biofortifid maize). Biofortification leads to a final product that consumers are unfamiliar with (Stevens & Nelson-Winter 2008). The characteristic aroma, flavour and colour of yellow/orange maize were found to contribute to the lower sensory acceptability of yellow/orange maize relative to white maize (Pillay *et al* 2011a; Stevens & Winter-Nelson 2008). These atypical sensory attributes of the biofortified maize are due to carotenoid pigments that are present in yellow or orange maize and absent in white maize (Chapman 2012; Nuss & Tanumihardjo 2010).

Negative perceptions about yellow maize contribute to its poor acceptance. Many rural communities have the perception that yellow maize is not suitable for human beings and should only be used as animal feed (Nuss *et al* 2012; Khumalo *et al* 2011). In some African countries, such as Zimbabwe, yellow maize is used as food aid (Muzhingi *et al* 2008). The majority of the maize grown in rural communities is white maize and only a small fraction of farmers grow yellow maize (Muzhingi *et al* 2008).

The cost of maize is a major factor in determining if consumers are willing to buy yellow maize. It was evident from a number of studies that if yellow maize was cheaper than white maize it would be purchased (Khumalo *et al* 2011: Stevens & Winter-Nelson 2008; Muzhingi *et al* 2008; De Groote & Kimenju 2008). Consumers are also willing to purchase yellow maize if it is more nutritionally superior to white maize (Muzhingi *et al* 2008). Paradoxically, it was reported that the higher a person's education the more likely they were to choose white maize over yellow maize (De Groote & Kimenju 2008). This may be due to the common perception that yellow maize is eaten by poor people (Muzhingi *et al* 2008).

Although many studies have indicated that provitamin A-biofortified maize is not acceptable to some consumers, with frequent exposure to yellow maize it can become as acceptable as white maize (Nuss *et al* 2012). It was noted by UNICEF (2012) that mothers would give their infants a product if it had health-promoting properties, which is the case with provitamin A-biofortified maize (UNICEF 2012). Pillay *et al* (2011a) have suggested that nutrition education on provitamin A-biofortified maize should be given to adult caregivers, especially females, which could possibly improve the acceptance of biofortified maize (Pillay *et al* 2011a). There is a need to identify provitamin A-biofortified foods that are highly acceptable

to consumers, so that these foods may be promoted. Complementary foods made from provitamin A-biofortified maize could be an acceptable food. This is reviewed in the next section.

2.5. Complementary feeding

Malnutrition is directly linked to the nutritional intake of an infant. It is therefore important to provide nutritious foods to a growing infant. Exclusive breastfeeding is the ideal way of feeding an infant less than six months of age. However, at six months breastfeeding alone or other forms of replacement feeds alone are insufficient to meet the nutritional requirements of the growing infant. A variety of nutritious foods should be supplemented to all infants over six months of age, in addition to breast milk (WHO 2012; Krebs & Hambidge 2007). The first two years of an infant's life is regarded as a critical period, as this is the time when the infant is prone to growth faltering, micronutrient deficiencies and infectious diseases (Aggarwal, Verma, Faridi & Dayachand 2008; WHO 2005, p6; Dewey 2001). This vulnerable stage is the time to provide nutritious, energy-dense complementary foods to prevent macronutrient and micronutrient deficiencies and to promote optimal growth and cognitive development (Kuriyan & Kurpad 2012).

At six months of age the infant's body undergoes changes allowing the body to tolerate solid foods (Whitney *et al* 2012, p302). By this age the infant is able to swallow semi-solid foods (Whitney *et al* 2012, pp302-303). Small amounts of food should be offered at a time. A single food item should be offered when it is a new food (American Academy of Pediatrics 2009, p131). This food should be given to the infant for four to five days to check for allergies. Rice cereals are first offered to infants as they are less likely to cause allergies (Whitney *et al* 2012, p303; American Academy of Pediatrics 2009, p131). If tolerated, other cereal grains are introduced into the infant's diet. Wheat is the last cereal grain that is introduced, as it is the most allergenic in comparison to other cereal grains (Whitney *et al* 2012, pp303-304). This grain is cooked into a semi-solid porridge and offered to an infant. Foods that are initially given to infants should be pureed, mashed or should be semi-solid (WHO 2005, p9). New foods can be accepted by an infant in the early stages, with frequent exposure. A longitudinal study conducted over two years in the Netherlands found that, with frequent exposure of vegetables in a child's diet, there was an increase in the vegetable intake and acceptance of vegetables (Barends, de Vries, Mojet & de Graaf 2013). Another study, conducted by Lange, Visalli, Jacob, Chabanet, Schlich & Nicklaus (2013), indicated that

mothers have a direct impact on the acceptance of new foods. It was also deduced that if vegetables were introduced earlier during the complementary feeding process, infants would accept a wider range of vegetables (Lange *et al* 2013).

During the initial phase of complementary feeding, foods may lack energy and be low in protein and micronutrients (Chang, He & Chen 2008; Faber 2005a). Many infants are given complementary foods that are inappropriate for their age group (Lutter *et al* 2011). Malnutrition in infants may be caused by the incorrect duration of breastfeeding or giving infants foods that lack nutrients (WHO 2013b). Formula is sometimes given to infants as a complementary food. Infant formula generally contains both vitamin A and vitamin E (Chávez-Servín, Castellote & López-Sabater 2008). Chávez-Servín *et al* (2008) showed that once the formula package was opened, or even during storage, the vitamin A content decreased slightly (Chávez-Servín *et al* 2008). Because the early introduction of complementary foods may predispose the infant to the development of allergies, the correct timing of complementary foods is vital. A study conducted by Nwaru, Takkinen, Niemelä, Kaila, Erkkola, Ahonen, Haapala, Kenward, Pekkanen, Lahesmaa, Kere, Simell, Veijola, Llonen, Hyoty, Knip & Virtanen (2013) found that, when infants were given complementary foods as early as three months of age, it resulted in various allergies, depending on the type of complementary food that was introduced (Nwaru *et al* 2013).

Evidence from various studies have shown a direct link between traditional and cultural beliefs and the types of food and feeding practices followed by caregivers (Nuss *et al* 2012; WHO 2012; Shi & Zhang 2011). Socio-economic status plays a major role in the compliance levels of mothers to education or guidelines given to mothers (Broilo, Louzada, de Lourdes Drachler, Stenzel & Vitolo 2013). Complementary feeding in a household depends on the practices of the caregiver and the foods available (WHO 2002). Many infants are given plant based staples as the main source of food. This leads to an unbalanced diet (WHO 2005, p15). The unfortified plant-based staples lack iron, zinc, calcium and vitamin B12 (WHO 2005, p13). Cereal grains tend to be low in the essential amino acid lysine (WHO 2005, p15). Fat is an important macronutrient needed by infants. It improves the palatability of foods, increases the energy density and provides free fatty acids that assist with the absorption of fat-soluble vitamins such as vitamin A (WHO 2005, p16).

Although infants need optimal nutrition from six months of age, Lutter *et al* (2011) reported that less than 33% of children between the ages of six and 23 months met minimum

nutritional requirements, as the diet lacked variety. The poor nutritional practices may be attributed to poor socio-economic status, lack of knowledge and inadequate access to a variety of foods (Lutter *et al* 2011). Inappropriate feeding practices are the cause of the high mortality rate seen in developing countries (WHO 2000). South African practices with regards to complementary feeding are reviewed in the next section.

2.5.1 South African practices

A study conducted in the Ndunakazi community, KwaZulu-Natal, indicated that maize meal porridge was the first complementary food offered to infants (Faber & Benadé 1999). The diets of these infants were low in fruit and vegetables and animal products, but high in carbohydrates. Low ferritin and retinol levels were noted in these infants (Faber & Benadé 1999). The results were similar to several other studies conducted in South Africa. In the Moretele district, Northwest Province, South Africa, mothers started weaning their infants as early as two to three months of age. The majority of these infants were given maize meal porridge as a weaning food, which was boiled for a long period of time in water to give it a soft consistency. This soft porridge was overcooked and over-diluted (Kruger & Gericke 2003). The mothers added formula powder and margarine to the cooked soft maize porridge. Over-diluted formula was also commonly given to infants in addition to complementary foods (Kruger & Gericke 2003).

A study conducted in two disadvantaged communities in the Western Cape found that infants were given complementary foods before the age of four months. The black infants in the study received soft maize porridge, which was prepared with fortified maize meal as their main source of complementary foods. The fortified maize meal was micronutrient dense and high in carbohydrates. This showed that mothers diluted this porridge, thus not supplying the infants with appropriate nutritional needs (Oelofse *et al* 2002). A cross-sectional study conducted by Faber (2005a) showed similar results to the previous studies reviewed. More than half the participants gave their infants soft porridge made with maize meal. A small percentage (4%) added margarine, peanut butter, sugar, formula powder and eggs to the soft porridge. This study found that, although some mothers increased the energy density of the soft porridge, the diets lacked calcium, iron and zinc (Faber 2005a).

In a study conducted in rural KwaZulu-Natal it was reported that mothers initiated complementary feeding earlier than four months after the birth of their babies. Of these mothers, 55% gave their infants soft maize porridge as their first complementary food. The

infants in this study had a poor intake of fruit and vegetables and animal food sources. The same study indicated that 20% of the infants had VAD (Faber & Benadé 2007). A more recent study, conducted in four African countries, including South Africa, found that there was early introduction of complementary foods, because mothers lacked adequate knowledge. Complementary foods such as porridge and formula were introduced as mothers thought that they did not have enough breastmilk (Nor, Ahlberg, Doherty, Zembe, Jackson & Ekström 2012). The nutritional quality of the South African diet will be discussed in the next section.

2.5.2 Nutritional quality of South African complementary foods

From the discussion in the previous section it appears that white maize and water are the main complementary foods used. Unfortified maize meal is a bulky food that contains limited quantities of micronutrients (Faber & Benadé 2007). Complementary foods made with these ingredients are sufficient in energy, fibre and B-vitamins, but are deficient in the essential amino acids lysine and tryptophan, some minerals and, most notably, vitamin A. A diet that consists predominantly of white maize tends to be low in the amino acids phenylalanine, tyrosine, tryptophan, threonine, methionine, cystine, leucine, valine, isoleucine and histidine. The most limiting amino acid found in maize-based diets is lysine (Duvenage & Schönfeldt 2007).

From the literature it is evident that white maize is devoid of vitamin A and contains phytates. If white maize is eaten in large quantities, which is the case in South Africa, it could hinder the absorption of iron, zinc and calcium (Hotz & Gibson 2001). In rural areas of South Africa, where unfortified white maize is consumed in large quantities, there is a concern, as unfortified white maize is deficient in vitamins and minerals. Maize meal porridge is usually overcooked and over-diluted. Although some studies indicate that margarine, peanut butter, eggs, sugar and formula powder were added to the porridge, the porridge may still lack nutrients due to the loss of nutrients from over-dilution. These infants were deficient in calcium, iron and zinc (Faber 2005a; Faber & Benadé 1999). Over-diluted formula is sometimes added to the porridge, making it nutritionally inadequate. Over-dilution is a sign of incorrect preparation. It increases the risk of bacterial contamination, thus negatively impacting on the health and nutritional status of these infants (Andresen, Rollins, Sturm, Conana & Greiner 2007).

In low-income South African communities, unfortified white maize is the main ingredient used to prepare complementary infant foods. Unfortunately, unfortified white maize is devoid

of vitamin A and infants consuming this maize are at high risk of developing VAD. The next section reviews the potential use of provitamin A-biofortified maize as a complementary food.

2.5.3 Proposal to use provitamin A-biofortified maize in complementary foods

Provitamin A-biofortified maize should be suitable for use as a complementary food since maize is the main ingredient in most South African complementary foods, including those used in KwaZulu-Natal. The use of provitamin A-biofortified maize would be critical to address the risk of VAD associated with the use of complementary foods made with white maize. Other nutritional problems may also be addressed, especially protein-energy malnutrition. Fat and protein, which were found in higher quantities in provitamin A-biofortified maize relative to white maize, may assist in improving protein-energy malnutrition in sub-Saharan African children (Pillay *et al* 2013). Although provitamin A-biofortified maize could be used as a complementary food to positively impact on the nutritional status of infants, consumer acceptance of the biofortified maize could be an obstacle, as has been found to be the case with other biofortified maize foods. The acceptance of the biofortified maize complementary foods would largely depend on the mothers and caregivers as they usually decide what the infants should eat. Nevertheless, mothers have been found to be willing to try new foods for their children especially if it is of nutritional benefit (UNICEF 2012). They may thus be willing to try complementary foods made with biofortified maize. Since the consumer acceptance of provitamin A-biofortified maize was found to vary with maize food type, the biofortified maize complementary foods could be acceptable to the mothers and caregivers, as suggested earlier. Therefore there is a need to evaluate the acceptance of complementary foods made with provitamin A-biofortified maize.

2.6 Conclusion

Biofortification is an emerging, cost-effective complementary strategy that could be used to help alleviate VAD, which is prevalent in sub-Saharan countries, including South Africa. Maize is the most popular staple food eaten in South Africa and would be an ideal medium for biofortification with provitamin A. Provitamin A-biofortified maize contains lower levels of minerals and higher levels of starch, fat and protein in comparison to white maize. Consumer acceptance of provitamin A-biofortified maize has been found to vary with maize food types, which seems to be an opportunity to identify maize food types in which the biofortified maize is well accepted. The acceptance of provitamin A-biofortified maize has been reported to vary with the age of the consumer. It was more acceptable to younger children than to older

children and adults. Infants could therefore easily be given biofortified maize foods, but it would also depend on the acceptance of the food by the caregivers.

Although maize biofortified with provitamin A may cause sensory changes to the final maize food product, mothers are willing to give their infants a food item if they know that it has possible health-promoting properties. Many rural infants receive soft, white maize porridge as a complementary food that is deficient in vitamin A. Provitamin A-biofortified maize could be used in complementary foods with the aim of contributing to the alleviation of VAD. There is a lack of data on the use of provitamin A-biofortified maize in complementary foods and the nutritional composition of these foods. It would therefore be highly beneficial to investigate the acceptance of provitamin A-biofortified maize as a complementary food by black African rural female caregivers and also the nutritional composition of these foods.

CHAPTER 3: METHODOLOGY

3.1 Introduction

Chapter 3 describes the methods used in this study. It covers the background information on the study site, study design, materials and methods, the pilot study, data quality control and reduction of bias the statistical analysis and ethical considerations.

3.2 Background information on the study site

The study was conducted at the Khanyisa clinic at Edendale Hospital (Figure 3.1) in the Umgungundlovu District (Figure 3.2) of KwaZulu-Natal province, South Africa. The paediatric out-patient department provides medical care for infants and children less than 12 years of age. The Khanyisa clinic provides medical treatment specifically to infants and children less than 12 years old who are infected with the Human Immunodeficiency Virus (HIV). Patients with side-effects caused by Anti-Retroviral Drugs (ARVs) that cannot be treated at clinic level are referred to Khanyisa clinic. The paediatric out-patient department is the first point of paediatric admission into the hospital and is situated in the main building of the hospital.

Figure 3.1 Entrance to Edendale Hospital (Department of Health 2013b)

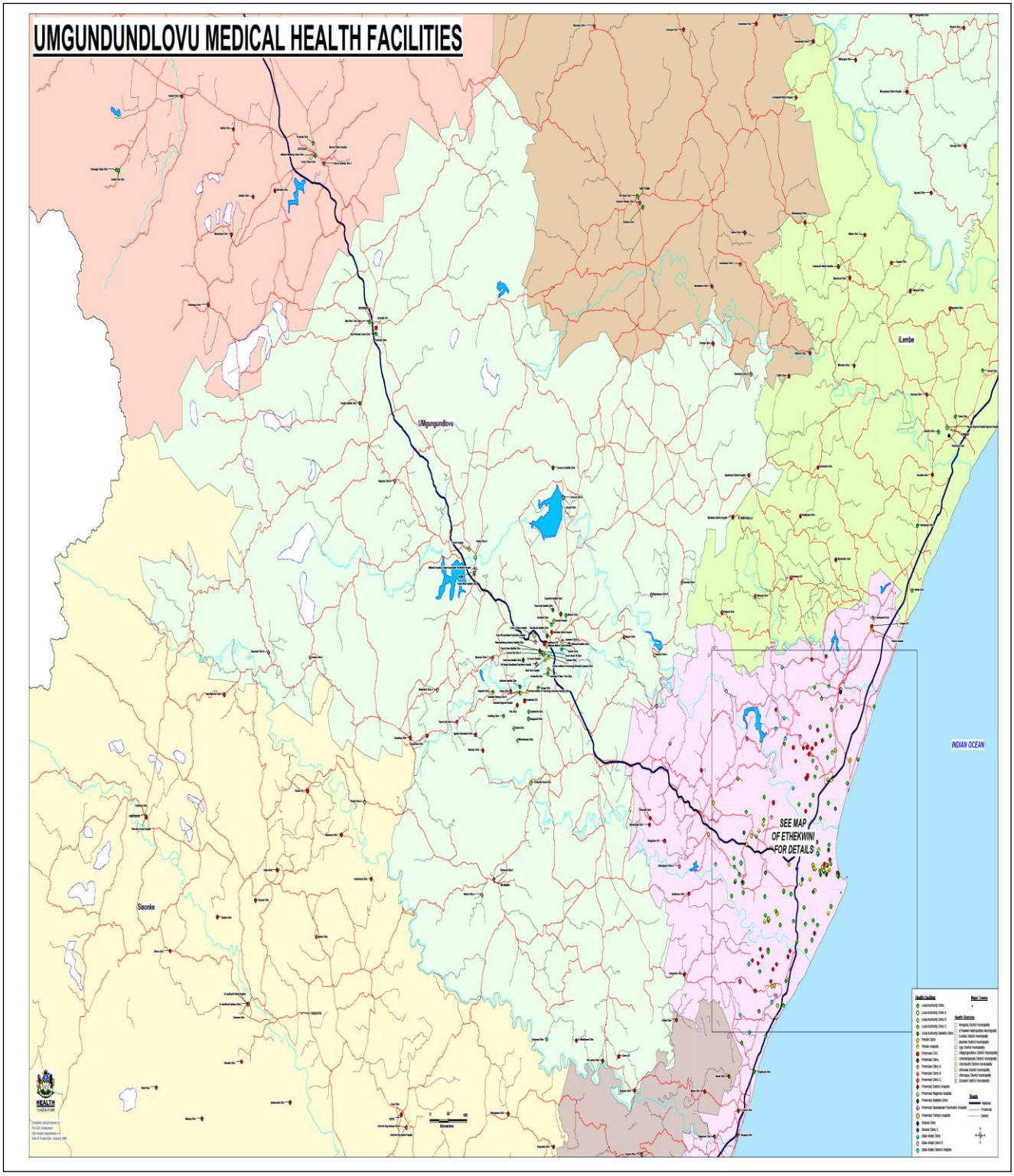

Figure 3.2 Map of the Umgungundlovu District area (Department of Health 2013b)

3.3 Study design

With respect to the consumer acceptance investigations, a cross-sectional study was conducted. Grains of two provitamin A-biofortified maize varieties and one white maize variety (control) were used. Each variety of maize grain was analysed for its nutritional composition. The soft porridge was made using the maize meal of each of the maize varieties. The porridges were evaluated for consumer acceptability through a sensory evaluation by black African female caregivers. Thereafter, focus group discussions were conducted, using some of the subjects, to determine the perceptions concerning the porridges. The study design is shown in Figure 3.3.

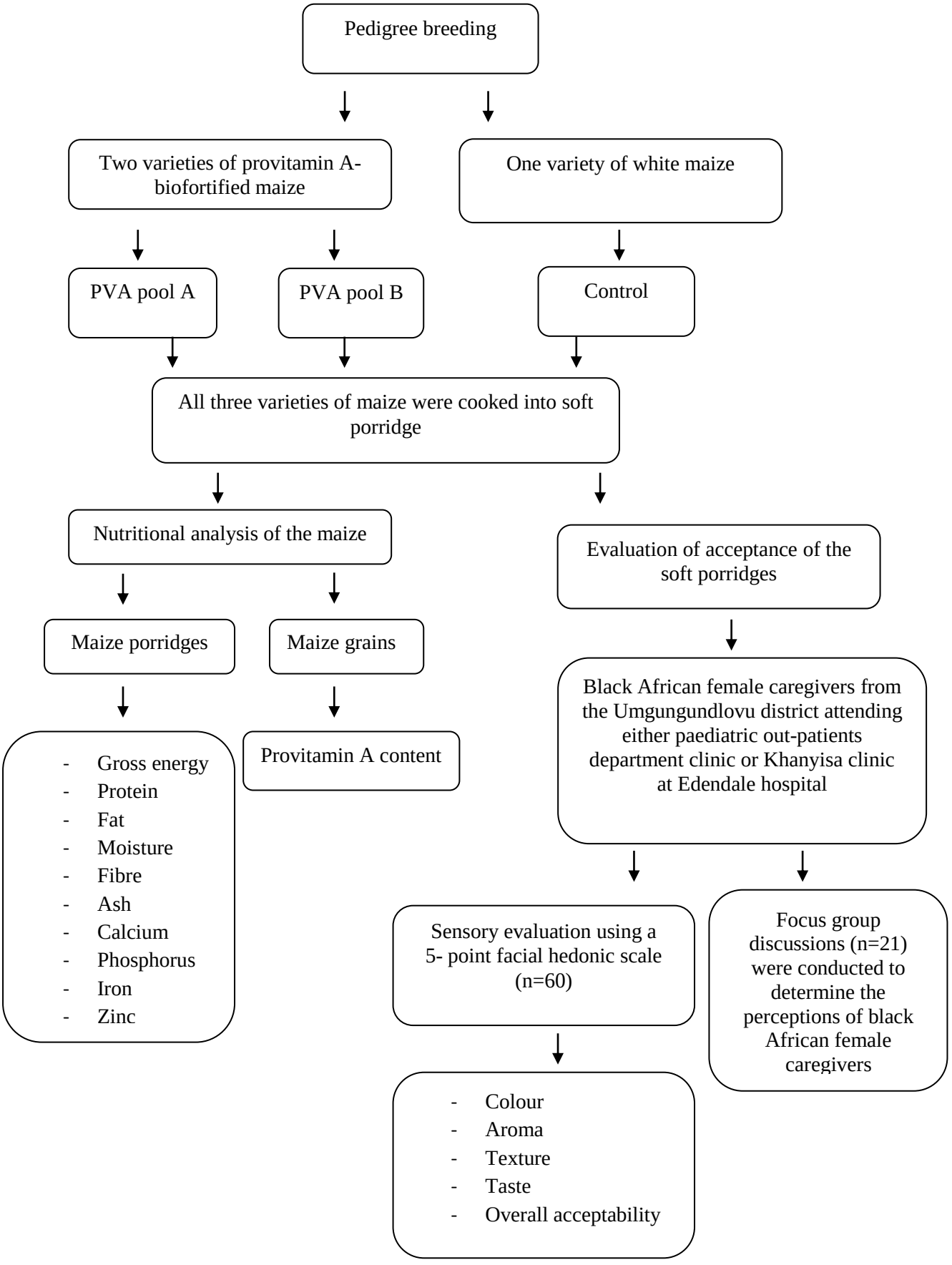

Figure 3.3 Study design

3.4 Study materials and methods

3.4.1 Maize grain samples

Dried (about 10% moisture) grain of three maize varieties: two provitamin A-biofortified varieties (PVA pool A and PVA pool B) and one white variety (control) were used in this study (Figure 3.4). The white maize variety was not biofortified and therefore lacked vitamin A. For this reason it was adopted as a control for the study. The maize grains were produced by breeders at the University of KwaZulu-Natal, as described in section 3.4.2.

3.4.2 Breeding of provitamin A-biofortified maize

Orange maize inbred lines were developed through pedigree breeding from 2008 to 2011. During phenotypic selection, emphasis was placed on grain colour intensity, such that lines exhibiting deep orange colour were advanced to the next generation. The deep orange grain colour is positively correlated with total carotenoids content in maize grain. Experimental F1 maize hybrids were then developed by cross-pollination of the inbred lines. The hybrids were divided into three groups, depending on the colour intensity, as follows: Group A deep orange, B medium and C light orange. Each group was used to make a synthetic population by mixing the grain of the hybrids and allowing them to mate randomly. For convenience of the study, the three synthetic populations were designated Pool A, B and C. These were grown at Cedara Research Station, near Pietermaritzburg, South Africa, to bulk the grain. Standard cultural practices for maize production were followed. The maize was harvested manually and left to dry under ambient conditions (±25 °C) for 21 days at Ukulinga Research Farm of the University of KwaZulu-Natal on the Pietermaritzburg campus. The maize was then threshed by hand and the grain was stored in a cold room (approx. ±5°C) at Ukulinga Research Farm until it was required for the research.

Figure 3.4 Grains of three maize varieties (from left to right: PVA pool A, PVA pool B and the control)

3.4.3 Maize milling

A grain cleaner (R.G Garvie and Sons, Agricultural Engineers, Aberdeen, Scotland, UK) was used to clean the maize grains. After cleaning the grains, moisture was adjusted to 15% (w/v). A pilot roller mill (Model MK 150, Roff Industries, Kroonstad, South Africa) was used to mill the maize grains. This type of miller has a three-break system and yields super meal, maize grits and fine meal. Super meal was collected from the last two break systems for the purpose of the study. This maize meal passed through a 459 µm aperture screen. Figure 3.5 shows the different maize meal varieties.

Figure 3.5 Maize meal of the maize varieties used in this study (from left to right; PVA pool A, PVA pool B and the control)

3.4.4 Nutritional Analysis

The nutritional composition of the three maize grains and their porridges was determined using standard methods.

Gross energy

The energy content of the samples was determined using a LECO AC500 automatic bomb calorimeter (LECO Corporation, St Joseph, Michigan, USA). The samples were dried and weighed. Thereafter they were placed in a thick-walled chamber, where the samples were completely combusted. The samples were combusted by igniting them in oxygen under high pressure.

Protein

The protein content of the samples was measured with a LECO Truspec Nitrogen Analyser (LECO Corporation, St Joseph, Michigan, USA) using the AOAC official method 990.03 (AOAC 2003). Controls and samples were measured in duplicate and placed into a combustion chamber at 950^{o}C with an autoloader. The following equation was used to calculate the percentage of protein:

% crude protein = % N x 6.25

Fat

The fat content of the samples was determined according to the Soxhlet procedure, using a Büchi 810 Soxhlet Fat extractor (Büchi, Flawil, Switzerland) according to the AOAC Official Method 920.39 (AOAC 2003). Petroleum ether was used for extraction. Percentage crude fat was calculated using the following equation.

$$\% \text{ crude fat} = \frac{\text{Beaker + fat} - \text{Beaker}}{\text{Sample mass}} \times 100$$

Moisture

The moisture content of the samples was measured according to the AOAC Official Method 934.01 (AOAC 2003). The samples were dried at 95 ºC for 72 hours in an air-circulated oven. The weight loss of the samples was used to calculate the moisture content. The following equation was used to calculate the moisture content:

$$\% \text{ moisture} = \frac{\text{(mass of the sample + dish)} - \text{(mass of sample + dish after drying)}}{\text{(mass of the sample + dish)} - \text{(mass of petri dish without the lid)}} \times 100$$

Ash (total mineral content)

The total mineral content of the samples was determined as ash according to the AOAC Official method 942.05 (AOAC 2003). The samples were weighed and placed in a furnace at 550ºC overnight. The minerals remained as a residue of ash in the crucibles after the volatilisation of the organic matter from the samples. The following equation was used to determine the percentage of ash that was found in the sample:

$$\% \text{ ash} = \frac{(\text{mass of sample + crucible after ashing}) - (\text{mass of pre-dried crucible})}{(\text{mass of sample + crucible}) - (\text{mass of pre-dried crucible})} \times 100$$

Fibre

Fibre was determined as neutral detergent fibre (NDF). The samples were dried and the fat content of the samples measured. If the samples had a fat content of > 10%, the samples had to be defatted before the analysis could be conducted. Samples were weighed and Neutral Detergent Reagent and the enzyme amylase were added. The samples were boiled for one hour and samples were then filtered, using a low vacuum. The contents were dried overnight at 105^0C. These samples were allowed to cool and their weights recorded. The samples were ashed for three hours at 550^0C and weighed after being cooled. The following equation was used to calculate NDF (van Soest, Robertson & Lewis 1991):

$$\% \text{ NDF} = \frac{(\text{crucible + dry residue}) - (\text{crucible + ash})}{\text{sample mass}} \times 100$$

Calcium, phosphorus, iron and zinc

Calcium, phosphorus, iron and zinc were analysed using the Agricultural Laboratory Association of Southern Africa (ALASA) Method 6.5.1. The first step of this process was to freeze dry the samples in a freeze drier (Edwards, High Vacuum International, Sussex, England). Samples were ashed overnight at 550^oC, in a furnace. The samples were dissolved in HCl, thereafter HNO_3 was added. The samples were analysed using an atomic absorption spectrophotometer. Calcium and phosphorus were determined using the Analytik Jena Spekol 1300 spectrophotometer (Analytik Jena AG, Achtung, Germany). Iron was determined with the Varian SpectrAA atomic absorption spectrophotometer (Varian Australia Pty Ltd, Mulgrave, Victoria) and the zinc with the GBC 905AA spectrophotometer (GBC Scientific Equipment Pty Ltd, Dandenong, Victoria, Australia).

Provitamin A

Provitamin A was determined by high performance liquid chromatography (HPLC). The procedures followed in this analysis were described by Lacker, Strohschein & Albert (1999). The provitamin A-biofortified maize grains were analysed for the provitamin A content. The PVA pool A and PVA pool B varieties contained 5.70µg/g and 4.88µg/g, respectively.

3.4.5 Evaluation of the acceptance of the maize porridges

The acceptance of the porridges made with provitamin A-biofortified maize was evaluated by sensory evaluation and focus group discussions.

Pilot study

A pilot study of the sensory evaluation and focus group discussions was conducted prior to the main study. The purpose of the pilot study was to detect and correct any methodological problems related to the preparation of the porridges, the sensory evaluation and the focus group discussion. Ten black African female caregivers participated in the pilot study. These participants were recruited from the paediatric out-patient department and Khanyisa clinic at Edendale hospital, which was the chosen site for the main study. The research assistant went to both clinics to recruit volunteers to participate in the pilot study. Five subjects from each of the two clinics were recruited to participate in the pilot study. Subjects who participated in the pilot study were not allowed to participate in the main study. To ensure this, the researcher selected a different day and week to conduct the main study, as most patients have monthly follow-up visits close to the same date each month. Focus group discussions were conducted after the sensory evaluation, using some of the sensory evaluation panellists.

The outcomes of the pilot study were: (1) a standardised porridge recipe was established after several cooking trials (Appendix A); (2) the sensory evaluation questionnaires in English (Appendix B) and in isiZulu (Appendix C) were modified; and (3) the process to be followed during the focus group discussions was finalised.

Several changes were made to the main study to improve it. The recipe was modified by adjusting the quantities of certain ingredients to ensure that it was acceptable to the sample group. The main study was conducted at an earlier time than the pilot study, so that sufficient participants could be recruited to participate in the study. The porridge samples were warmed for eight seconds in a microwave oven, to reach an ideal temperature for the sensory

evaluation. All panellists were allocated a number, to assist with identification. The consent form and the sensory evaluation attributes were explained, in detail, to ensure that all the participants understood what was expected of them.

Sensory evaluation

Panellists

Sixty black African female caregivers residing in the Umgungundlovu area and attending either the paediatric out-patients department clinic or the Khanyisa clinic at Edendale Hospital participated in the study. Research assistants explained the study to the patients attending both clinics and recruited volunteers. Thereafter research assistants randomly selected panellists from patients who volunteered to participate in the study. The volunteers were asked if they had participated in the pilot study the previous month. Those volunteers that participated in the pilot study were not permitted to participate in the main study.

Preparation of porridges

The maize meal of each of the three maize varieties was used to prepare a soft porridge, according to the recipe in Appendix A. The porridges were prepared by a black African woman living in a rural area in the Umgungundlovu District with experience in cooking soft porridge. This ensured that the porridges were culturally acceptable to the participants. The porridges were prepared on the morning of the day of data collection in the Food Science Laboratory, situated at the Human Nutrition and Dietetics Department at the University of KwaZulu-Natal, Pietermaritzburg. Thereafter the porridges were transported to the research site in air-tight containers. The porridges were not prepared at the research site as there were no cooking facilities available. Figure 3.6 shows the preparation of the soft maize meal porridge and Figures 3.7a, 3.7b and 3.7c show the three different varieties of cooked porridges; PVA pool A, PVA pool B and the control, respectively.

Figure 3.6 Preparation of the maize meal porridge

Figure 3.7a Maize meal porridge made from PVA pool A

Figure 3.7b Maize meal porridge made from PVA pool B

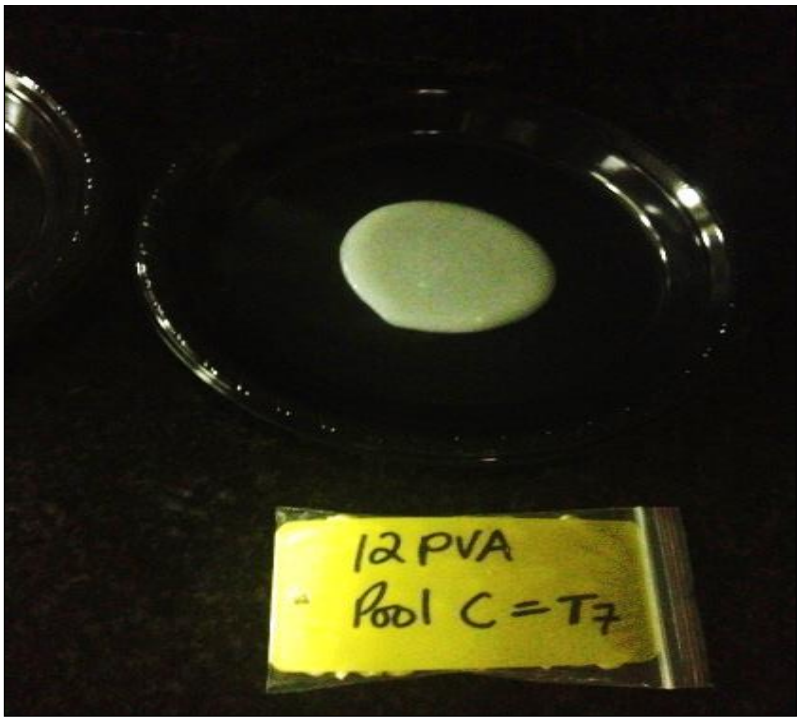

Figure 3.7c Maize meal porridge made from the control

Sample coding, serving order and sensory evaluation set-up

The panellists were seated some distance from each other and were asked not to communicate during the sensory evaluation session. This was done to prevent the panellists from

influencing each other's responses. Each of the three varieties of maize porridge was assigned a unique three-digit code obtained from a Table of Random Numbers (Heymann 1995). The three digit codes were known to the researcher, but not to the panellist and research assistants, to prevent bias. The serving order of the porridge samples was determined by a Table of Random Permutations of Nine (Heymann 1995). Each participant received 12.5 ml (one heaped tablespoon) of each sample in a polystyrene cup. Each sample was warmed for eight seconds before being given to the participant. The ideal temperature was determined during the pilot study. Each panellist was provided with a pencil, a cup of water to rinse the palate between samples and the sensory evaluation questionnaire in isiZulu (Appendix C), the local language in KwaZulu-Natal. The English version of the sensory evaluation questionnaire (Appendix B) was translated into isiZulu by a translator proficient in both languages. The questionnaire made use of a five-point facial hedonic scale (1=very bad; 5=very good), so that illiterate participants could record their responses. The hedonic scale is a popular sensory evaluation tool that indicates the degree of likes and dislikes (Bergara-Almeida, Aparecida & da Silva 2002; Gacula & Singh 1984, p30).

The participants were required to fill in a consent form before starting the evaluation. A research assistant explained the consent form in detail in isiZulu to all the participants. The participants were asked if they understood everything that was on the consent form before they signed the forms. If anything was unclear it was explained before the participant signed. The consent form was formulated in English (Appendix D) and was also translated into isiZulu (Appendix E). After the consent forms were signed, the research assistant explained the questionnaire in isiZulu to the participants. All the sensory attributes were explained to the participants. If the explanation for a sensory attribute was unclear the research assistant repeated the explanation for that sensory attribute. Illiterate participants were further helped by research assistants if they required more assistance to fill out the questionnaire.

3.4.6 Focus Group Discussions

Focus group discussions were conducted to determine the perceptions of black African female caregivers about provitamin A-biofortified maize. Focus group participants were recruited on a voluntary basis from the sensory evaluation panel. Twenty-one participants were randomly selected from the caregivers who had volunteered to participate in the sensory evaluation. The participants were divided into three groups of seven. According to Greenbaum (2000, p2)

and Merton, Fiske & Kendall (1990, p137), the ideal size of a focus group is between seven and twelve individuals.

The focus group discussion was facilitated by an individual who was fluent in isiZulu. All questions asked in the focus group discussions were formulated in advance and checked by an expert in the field. The focus group discussion questions were first formulated in English (Appendix F) and then translated into isiZulu (Appendix G) by two isiZulu-speaking individuals. Focus group discussions were recorded using a digital voice recorder. The recordings were later translated into English by the focus group discussion facilitator. The recordings were cross-checked by an isiZulu speaking person against the English translation for accuracy in translation.

3.5 Data quality control and reduction of bias

Nutritional analysis was conducted in triplicate, using standard methods. During the preparation of the soft maize meal porridge various steps were taken to ensure quality control. These included calibrating the balance that was used to weigh the dry ingredients, using the same brands of salt and sugar to prepare the different varieties of soft porridges and using the same measuring cup, measuring jug and measuring spoons, to measure the ingredients.

Pots of the same type and size were used in the preparation of the soft porridges. The porridges were transported in air-tight containers to the study site. The same amount of porridge (12.5 ml) was served to all the participants. All the porridges were heated just before testing so that they would be palatable to the participants. Sample labelling and serving order were randomised and panellists were not allowed to communicate during the sensory evaluation session. Data from the nutritional analysis and sensory evaluation was captured on a spread-sheet and cross-checked to ensure that all the data was entered correctly. The focus group discussions that were recorded using a digital voice recorder were translated and cross-checked by an isiZulu-speaking person, for accuracy.

3.6 Statistical analysis

Data was captured onto Excel spread-sheets and transferred to a statistical package, SPSS (Statistical Package for Social Science version 15.0 SPSS Inc, Chicago III USA). The data was analysed using appropriate statistical techniques, including ANOVA, Tukey and Dunnet tests. A p-value of < 0.05 was regarded as being statistically significant.

3.7 Ethical considerations

Ethical approval was obtained from the University of KwaZulu-Natal, Humanities and Social Science Ethics Committee (HSS/0180/012M) (Appendix H). The Edendale Hospital Ethics Committee issued a supporting letter for the research to be conducted (Appendix I). The Department of Health granted permission for the study to be conducted at Edendale Hospital (HRKM95/12) (Appendix J). Each panellist was required to sign a consent form before participating in the sensory evaluation (Appendix D). The consent form was read to the participants in isiZulu so that illiterate participants could understand the content of the consent form. They were shown where to sign or initial on the consent form if they understood what was explained. If they did not understand anything it was re-explained and when everything was clear to them they signed the consent form.

CHAPTER 4: RESULTS

Chapter 4 presents and describes the results of the study, in which two types of provitamin A-biofortified maize and a control variety were tested for their acceptance and nutritional composition.

4.1 Nutritional composition of provitamin A-biofortified maize meal and their porridges

Table 4.1 shows the nutritional composition of two varieties of provitamin A-biofortified maize meal and maize porridges, compared with the meal and porridge of the white maize (the control).

Table 4.1 Nutritional composition of provitamin A-biofortified maize meal and porridge compared to white maize meal and porridge [dry basis (db)]

	Moisture g/100g	Energy KJ/100g	NDF g/100g	Fat g/100g	Protein g/100g	Ash[c] g/100g	Iron mg/100g	Zinc mg/100g	Phosphorus mg/100g	Calcium mg/100g
Maize meal										
PVA pool A	**13.65[a](0.00)[b]**	**17455.83 (2.43)**	**5.28 (0.00)**	**2.15 (0.00)**	**10.44 (0.00)**	**1.23 (0.00)**	**2.70 (0.00)**	**3.50 (0.00)**	**75.30 (0.03)**	**13.83 (0.02)**
PVA pool B	**14.65 (0.00)**	**17471.10 (135.0)**	**5.60 (0.00)**	1.90 (0.00)	**9.86 (0.00)**	**1.15 (0.00)**	**5.20 (0.00)**	**2.51 (0.00)**	**91.27 (0.02)**	**26.40 (0.04)**
Control	13.43 (0.00)	17345.30 (33.03)	2.93 (0.00)	2.13 (0.00)	7.81 (0.00)	0.97 (0.00)	0.07 (0.00)	0.07 (0.00)	1.18 (0.00)	0.19 (0.00)
Porridge										
PVA pool A	86.70 (0.00)	**17187.10 (108.65)**	**2.50 (0.00)**	**0.94 (0.00)**	**7.77 (0.00)**	**1.53 (0.00)**	**6.87 (0.00)**	**6.68 (0.00)**	**47.15 (0.07)**	34.67 (0.04)
PVA pool B	87.40 (0.01)	**17318.00 (43.57)**	**2.53 (0.00)**	**0.89 (0.00)**	**7.94 (0.00)**	**1.40 (0.00)**	**6.10 (0.00)**	**10.0 (0.00)**	**126.5 (0.04)**	62.33 (0.02)
Control	86.50 (0.00)	17081.40 (53.05)	2.40 (0.00)	0.66 (0.00)	6.13 (0.00)	1.16 (0.00)	4.74 (0.00)	3.49 (0.00)	47.01 (0.06)	146.5 (0.07)

NDF= Neutral detergent fibre

[a] Mean of at least two determinations

[b] Standard deviation

[c] Total minerals

Values in bold are significantly different from the control (white maize) for that nutrient (Dunnet test, $p < 0.05$)

Table 4.1 shows that the fat content of one variety of provitamin-A biofortified maize meal (PVA pool A) was higher than the white maize meal (the control). The provitamin-A biofortified maize meal had a significantly higher energy, fibre, protein, iron, zinc, phosphorus and calcium content relative to the control maize meal.

Table 4.1 shows that the energy, fibre, fat, protein, iron, zinc and phosphorus levels of the provitamin A-biofortified porridges were higher than those of the control porridge. The calcium content of the control porridge was significantly higher than those of the porridges made with the provitamin A-biofortified maize (PVA pool A and PVA pool B).

4.2 Sensory evaluation of porridges made with provitamin A-biofortified maize varieties

4.2.1 Sample characteristics of the sensory evaluation panel

The number and percentage of the black African female caregivers in specific age groups is presented in Table 4.2.

Table 4.2 Number of sensory evaluation participants in each age group

Age group	n (%)*
16-25	21(35)
26-35	16 (27)
36-45	08 (13)
46-55	10 (16.7)
56-65	05 (8.3)

* total sample (n=60)

The results of the sensory evaluation of the provitamin A-biofortified maize porridges are presented in Table 4.3 and Figure 4.1.

Table 4.3 Sensory evaluation of provitamin A-biofortified maize porridges compared with the control white maize porridge

	Sensory attributes				
Porridge type	**Taste**	**Texture**	**Aroma**	**Colour**	**OA**
PVA pool A	4.0[a] (1.0)[b]	4.1 (0.9)	4.0 (0.8)	4.2 (0.7)	4.2 (0.8)
PVA pool B	3.7 (1.0)	4.0 (0.8)	3.9 (0.9)	4.2 (0.7)	4.0 (0.8)
Control	3.9 (1.0)	4.1 (0.8)	3.9 (0.9)	4.0 (0.9)	4.3 (0.8)

OA = Overall acceptability
[a] Mean
[b] Standard deviation

The Dunnet test indicated that there was no significant difference between the different sensory attributes and the two types of biofortified maize, in comparison to the control variety (Table 4.3).

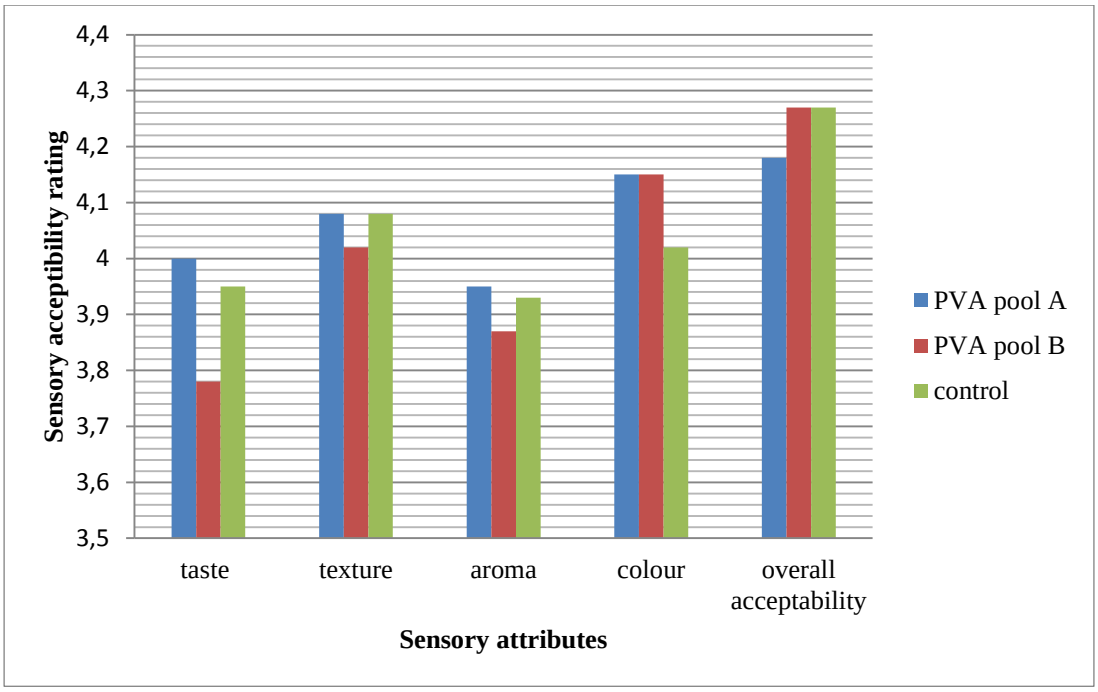

Figure 4.1 Sensory acceptability of soft maize porridge made with the maize varieties PVA pool A, PVA pool B and the control

The Tukey test indicated that there was no significant difference in the acceptability of all the maize porridges evaluated ($p<0.05$). The panellists rated the sensory attributes (including overall acceptability) of all the porridges as "good" (the scores on the facial hedonic scale were approximately 4). Table 4.4 and Figures 4.2, 4.3 and 4.4 show the percentages of panellists who gave the different ratings for the sensory attributes evaluated.

Table 4.4 Percentages of panellists who gave the different ratings for the sensory attributes evaluated (n = 60)

Porridge Type	Rating	Taste	Texture	Aroma	Colour	Overall acceptability
PVA pool A	Very bad	1[a] (1.7)[b]	1 (1.7)	0 (0)	0 (0)	2 (3.3)
	Bad	4 (6.7)	4 (6.7)	2 (3.3)	2 (3.3)	0 (0)
	Average	7 (11.7)	3 (5)	15 (25)	5 (8.3)	8 (13.3)
	Good	31 (51.7)	33 (55)	27 (45)	35 (58.3)	27 (45)
	Very good	17 (28.3)	19 (31.7)	16 (26.7)	18 (30)	23 (38.3)
PVA pool B	Very bad	3 (5)	1 (1.7)	1 (1.7)	1 (1.7)	2 (3.3)
	Bad	5 (8.3)	1 (1.7)	2 (3.3)	1 (1.7)	2 (3.3)
	Average	9 (15)	8 (13.3)	15 (25)	3 (5)	8 (13.3)
	Good	28 (46.7)	36 (60)	28 (46.7)	38 (63.3)	29 (48.3)
	Very good	15 (25)	14 (23.3)	14 (23.3)	17 (28.3)	19 (31.7)
Control	Very bad	1 (1.7)	1 (1.7)	0 (0)	2 (3.3)	0 (0)
	Bad	3 (5)	04 (6.7)	4 (6.7)	1 (1.7)	1 (1.7)
	Average	12 (20)	3 (5)	12 (20)	9 (15)	8 (13.3)
	Good	25 (41.7)	33 (55)	25 (41.7)	30 (50)	25 (41.7)
	Very good	19 (31.7)	19 (31.7)	19 (31.7)	18 (30)	26 (43.3)

[a] Number of subjects

[b] Percentage of total number of participants

Acceptability rating 1-5: 1= very bad; 2= bad; 3= average; 4=good; 5= very good

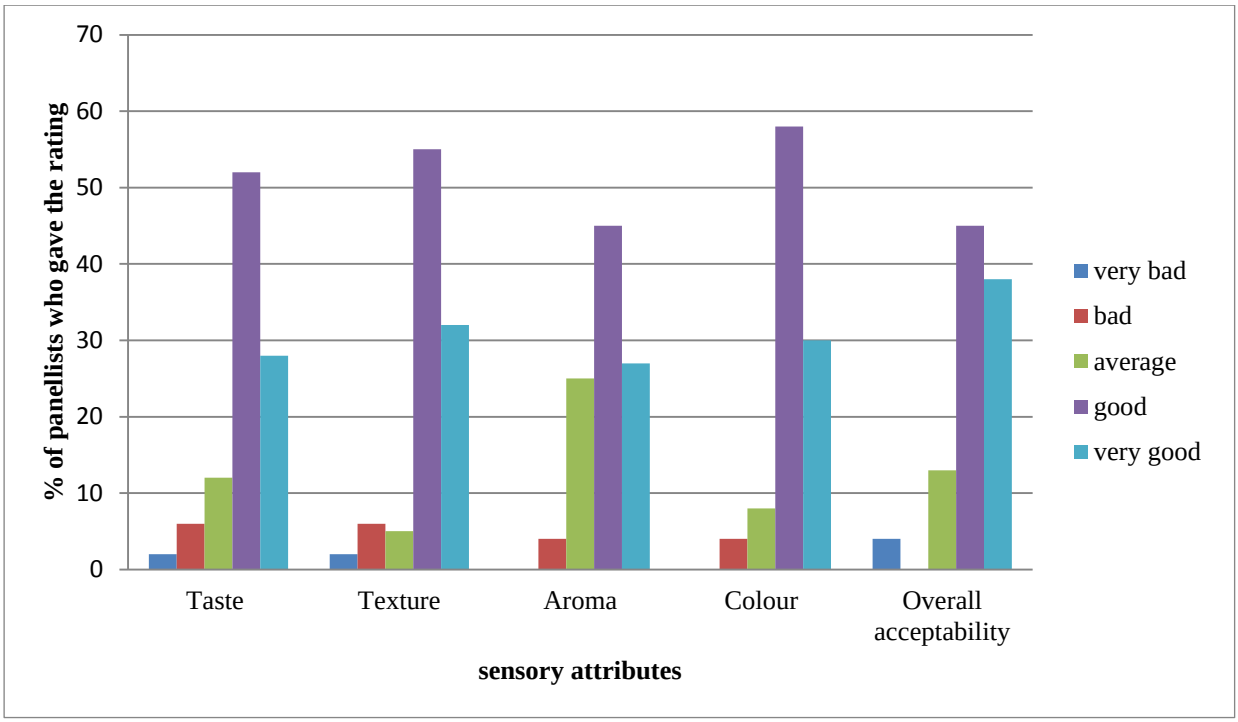

Figure 4.2 Ratings of sensory attributes of porridge made from PVA pool A

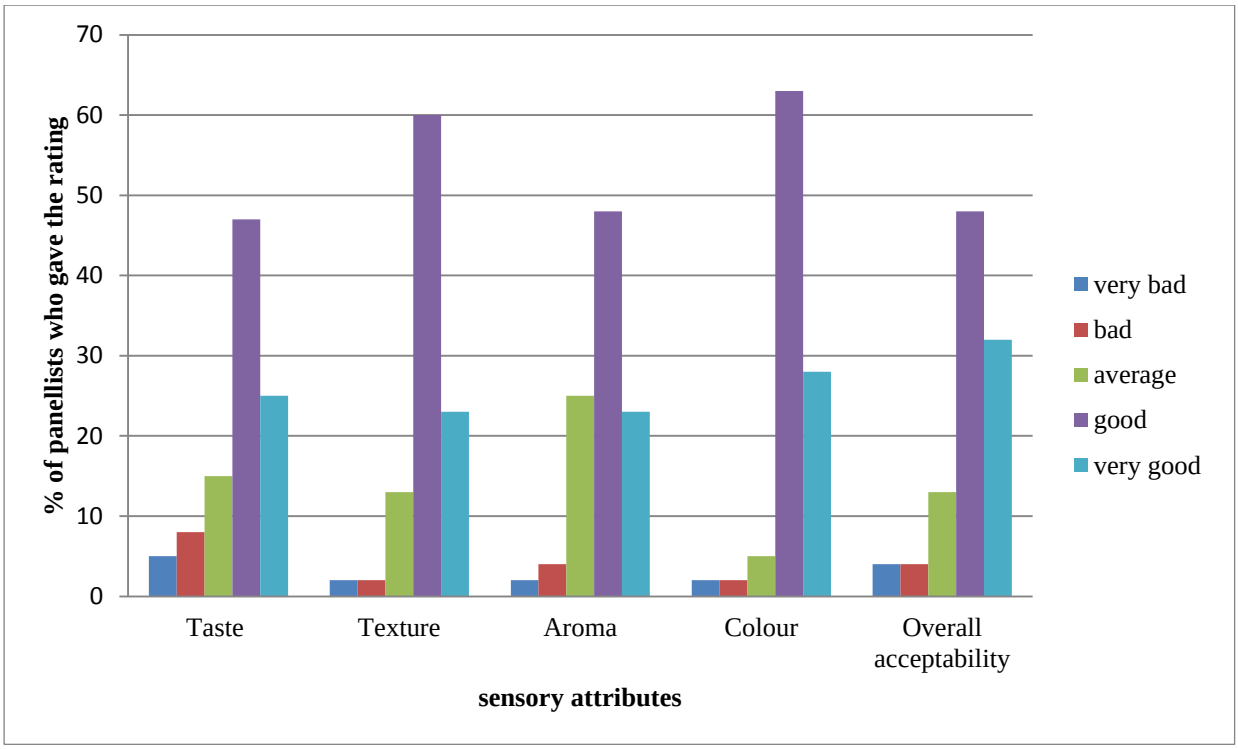

Figure 4.3 Ratings of sensory attributes of porridge made from PVA pool B

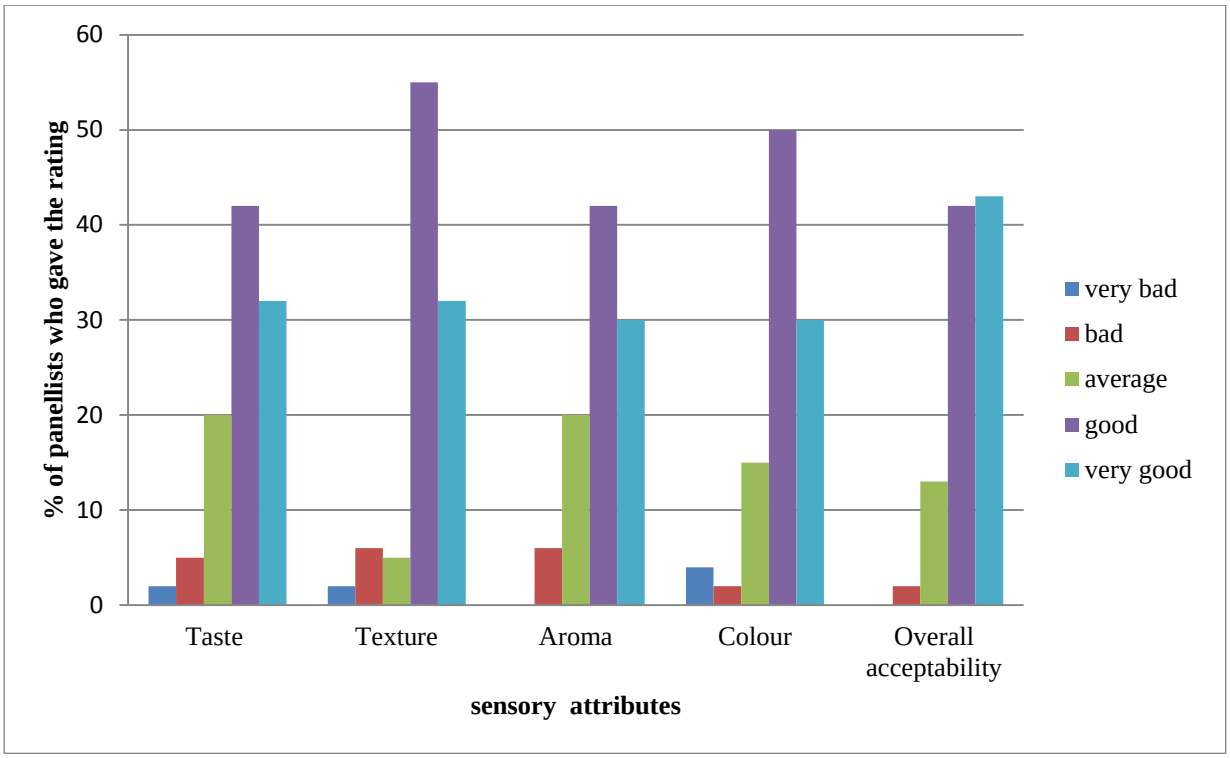

Figure 4.4 Ratings of sensory attributes of porridge made from the control

There was no statistically significant difference in the acceptability of the porridges across age groups (Figure 4.5). The Chi-square test indicated that there was no relationship between the age of the caregivers and the overall acceptability of the porridges made with the different maize varieties ($p>0.05$).

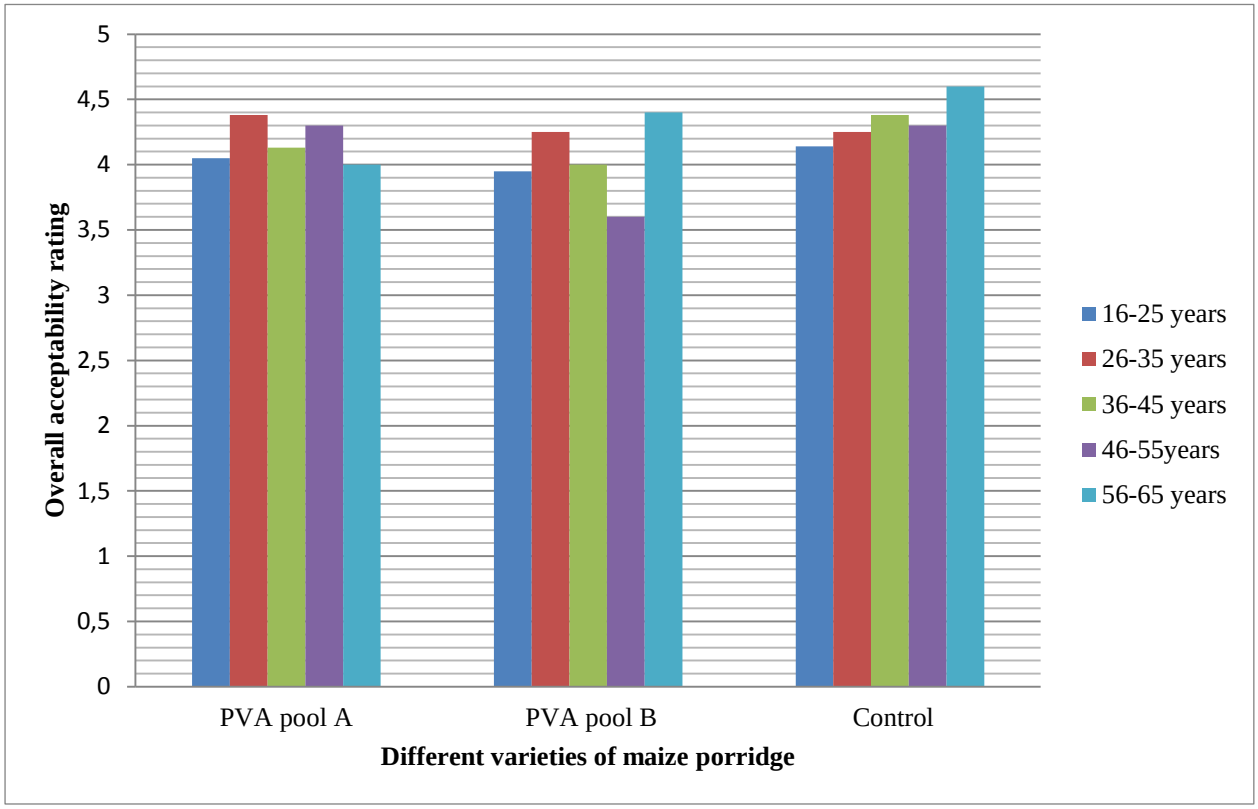

Figure 4.5 The effect of age on the overall acceptability of the maize porridges

4.3 Focus group discussions

Overall, the black African female caregivers had positive perceptions about the colour, aroma and texture of provitamin A-biofortified maize. However, the taste of the provitamin A-biofortified maize was not readily accepted. The black African female caregivers expressed a willingness to give their infants provitamin A-biofortified maize if it was cheaper than white maize, readily available and had a health benefit. The results are presented and discussed in detail in Chapter 5 (Table 5.1).

CHAPTER 5: DISCUSSION

This section interprets the findings of this study.

5.1 Nutritional composition of provitamin A-biofortified maize

The findings of the study indicated that the energy, fibre, fat, protein, iron, zinc and phosphorus levels of the biofortified maize porridges were higher than those of the control, white maize porridge. These findings were similar to those for fat and protein in a study conducted by Pillay *et al* (2013). Although the present study found the fat content of the biofortified maize porridges, PVA pool A (0.94/100g) and PVA pool B (0.89g/100g), were higher than that of the control porridge, these findings were lower than those found in the literature for white maize, which was 4.1g/100g (Johnson 2000, p38). Findings of this study indicated that the protein levels of the biofortified maize porridges, PVA pool A (7.77g/100g) and PVA pool B (7.94g/100), were lower than those found in the literature for the maize kernel, which was 8.7g/100g (Johnson 2000, p38). Pillay *et al* (2013) found higher amounts of starch in biofortified maize grain varieties than in the white maize grain variety. Starch was not evaluated in this study.

Pillay *et al* (2013) found lower iron levels in the provitamin A-biofortified maize grain varieties. The levels of zinc in their biofortified maize grain varieties were similar to those in their control white maize grain variety (Pillay *et al* 2013). A possible reason for the difference in iron and zinc levels in comparison to the present study could be genetic and environmental factors. Pillay *et al* (2013) found that the nutritional composition of provitamin A-biofortified maize varied with the different varieties of biofortified maize, which suggested that it was influenced by genetic factors. The iron and zinc levels for the biofortified maize porridges were between 6.10 and 6.87mg/100g and 6.68 and 10.0mg/100, respectively. They were higher than the levels obtained by the CIMMYT for provitamin A-biofortified maize, which was 1.1 to 3.9mg/100g and 1.5 to 4.7mg/100g for iron and zinc, respectively (Oritz-Monasterio *et al* 2007). Furthermore, this study found higher iron and zinc levels than another study. Oikeh, Menkir, Maziya-Dixon, Welch, Glahn & Gauch (2004) found yellow maize had higher concentrations of iron (2.05mg/100g) and zinc (2.12mg/100g) than most of the white varieties used in their study (Oikeh *et al* 2004). The results of the present study showed a higher concentration of iron and zinc than the study conducted by Oikeh *et al* (2004). The literature concerning the nutritional composition of biofortified maize is limited. The increase in the mineral content of all the maize varieties (including the

white variety) upon being processed into porridges (Table 4.1) was most probably due to the contribution of other ingredients, including water, to the total mineral content of the porridges.

The high energy content found in biofortified maize would be beneficial to infants who have high energy and fat requirement (Rolfes, Pinna & Whitney 2012, p465). Infants need approximately 100 kilocalories (kcal)/kg/day or 420 kilojoules (kJ)/kg/day of energy. Of that energy, between 30 and 40% should be from fat (Mahan *et al* 2012, p391). Fat is required by the body to assist with the absorption, digestion and transportation of fat-soluble vitamins (Mahan *et al* 2012, p40). Biofortified maize contains two fat-soluble vitamins namely vitamin A and E (FAO 1992). There fat content of the biofortified porridges were higher than that of the white maize porridge (control). Lipids could be added to biofortified maize to further increase the fat content (Morgan & Dickerson 2003). This could improve to the transportation of fat soluble vitamins in the body, impacting positively on the vitamin A status of infants at risk of VAD. The protein content of white maize is generally low (Johnson 2000, p38). The results of the protein content of the provitamin A-biofortified maize used in this study are encouraging, as they indicate that the biofortified maize had a relatively higher protein content than the control white maize used. In SSA, protein-energy malnutrition is a major problem and is responsible for a high percentage of deaths. The vicious cycle of malnutrition is endemic, due to poverty, food insecurity and diseases. The higher protein content of the biofortified maize, relative to white maize, found in this study suggests that provitamin A-biofortified maize could be used to improve the nutritional status of infants in SSA. Apart from the protein content of the maize, however, it is important to assess its protein quality. White maize is known to have low levels of the essential amino acids lysine, methionine and tryptophan (FAO 1992). A study conducted by Scott, Edwards, Bell, Schussler & Smith (2006) found that lower density maize plants contain limiting amounts of the essential amino acids lysine and tryptophan. Pillay *et al* (2013) found that provitamin A-biofortified maize varieties had lower levels of the essential amino acids histidine and lysine, although the amino acid content was higher than that of white maize (Pillay *et al* 2013). In the present study, the amino acid profiles of the biofortified maize and white maize was not determined and the protein quality of the two maize types and their porridges is thus not known.

The present study revealed a relatively higher iron, zinc and phosphorus content of the biofortified maize grain and porridges relative to white maize grain and porridge (Table 4.1). These results suggest that provitamin A-biofortified maize is nutritionally superior to white maize with respect to mineral content. Iron and zinc are among the prevalent micronutrient deficiencies affecting infants in developing countries (Bain *et al* 2013; Smuts *et al* 2005). This means that the deployment of biofortified maize varieties could be useful to complement strategies for minimising malnutrition. However, there is a need to determine the bioavailability of the minerals in the biofortified maize, as this may be negatively affected by other components of the grain. According to Johnson (2000), white maize is high in phosphorus, a proportion of which, as in other cereal grains, is in the form of phytate. Phytate inhibits the absorption of calcium, iron and zinc (FAO 1992; Watson & Ramstad 1987, p73). In the present study, although the phosphorus levels of the two biofortified maize varieties was higher than that of the white maize variety, the proportion of phosphorus stored in the form of phytate was not measured. The seemingly high potential of provitamin A-biofortified maize as a source of minerals would actually be lower if a large proportion of the phosphorus in the biofortified maize occurred in the form of phytate. It would be crucial therefore, to introduce genes for low phytic acid content in the biofortified maize varieties to enhance the availability of minerals.

The two varieties of biofortified grains, PVA pool A and PVA pool B, contained lower amounts of provitamin A (5.70µg/g and 4.88 µg/g, for PVA pool A and PVA pool B, respectively) than the amount recommended by HarvestPlus (15µg/g) (HarvestPlus 2009a). However, these provitamin A-biofortified grains had higher provitamin A than other maize varieties which have been found containing 0.5-1.5µg/g of provitamin A (Harjes *et al* 2008). There is thus a significant improvement already, which can be used for studies while further breeding is underway. The provitamin A-biofortified maize porridges were not analysed for their provitamin A content, due to financial constraints. There are many factors that affect the provitamin A content of biofortified maize. With long periods of storage the carotenoid content can decrease by 30-40% within a year (Burt *et al* 2010). Various processing operations, such as soaking and wet milling, fermentation of wet flour and cooking, that can reduce the carotenoid content of the maize food (Li *et al* 2007; Oritz-Monasterio *et al* 2007). Pillay *et al* (2011b) found the lowest retention of carotenoids in thin maize porridge. Although the carotenoid content may decrease during preparation, it still contains provitamin A, unlike white maize, which is devoid of vitamin A (FAO 1992). To compensate for the

losses of vitamin A during processing, fat containing foods such as margarine could be added to the prepared foods to enhance the absorption of vitamin A (Morgan & Dickerson 2003). Provitamin A-biofortified maize may have the potential to impact the vitamin A status of infants together, with other existing strategies that are in place to alleviate VAD. This is especially important in South Africa, as the number of children with VAD has doubled from 1994 to 2005 (Labadarios *et al* 2007; Labadarios *et al* 2000; Labadarios *et al* 1995). Therefore provitamin A-biofortified maize could be considered as a potential complementary strategy to improve the vitamin A status of South African infants.

The present study indicated that provitamin A-biofortified maize porridge was nutritionally superior to that of white maize porridge, as it was higher in energy, fibre, fat, protein, iron, zinc and phosphorus. With further research there is a possibility that provitamin A-biofortified maize could be used to assist with both macronutrient and micronutrient deficiencies, together with other strategies. This is especially important in developing countries, where there has been an increase in macronutrient and micronutrient deficiencies in infants. However, there is still a need to improve the biofortified maize varieties to obtain the desired levels of micronutrients.

5.2 Sensory acceptability of provitamin A-biofortified maize

Provitamin A-biofortified maize exhibits an orange-yellow colour, which consumers are not accustomed to (HarvestPlus 2009b). The maize undergoes chemical changes during the biofortification process that could result in the final product being unacceptable to consumers (Tumuhimbise *et al* 2013). In the present study the yellow colour and chemical changes seem not to have affected the sensory acceptability of the biofortified maize to the female caregivers. The results indicated that there were no significant differences in the sensory acceptability of the biofortified maize porridges and the white maize porridge. A study conducted by Stevens & Winter-Nelson (2008) indicated that women ranked the appearance of biofortified maize higher than men ranked it. This study had only female participants and there were no significant differences in their colour acceptance of the maize porridges. This is similar to the results obtained by Meenakshi *et al* (2012), working with yellow and orange provitamin A-biofortified maize and a semi-trained panel. The participants rated the taste, aroma, texture and overall acceptability of the biofortified maize as being similar to white

maize (Meenakshi *et al* 2012). The yellow maize was rated slightly lower than the orange maize (Meenakshi *et al* 2012).

Conversely, Pillay *et al* (2011a) found that there was poor acceptance of the colour, flavour and aroma of yellow provitamin A-biofortified maize. The acceptability of these sensory attributes varied with food types, namely samp, thin porridge and *phutu* (Pillay *et al* 2011a). This study only evaluated soft maize porridge. The texture of provitamin A-biofortified maize is another sensory attribute that has been found to be poorly accepted (Stevens & Winter-Nelson 2008). Khumalo *et al* (2011) reported that a finer texture was better accepted by consumers. Texture acceptability was not a problem in the present study. The differences in the results of sensory acceptability of biofortified maize among the studies may be attributed to the geographical location of the consumers, consumer demographic profiles, the colour of the biofortified maize (yellow or orange), maize food type evaluated and consumer familiarity with biofortified maize.

Although many researchers have found that white maize is sensorially preferred over the yellow/orange provitamin A-biofortified maize, the results of this study present a totally different picture. There was no significant difference in the acceptability of white maize and provitamin A-biofortified maize. It is generally accepted that if a food is accepted by mothers and other caregivers, they would give it to the children that they care for. The sensory acceptability findings of this study suggest that soft porridge made with provitamin A-biofortified maize could potentially be used as a complementary food by rural caregivers in the Umgungundlovu District of KwaZulu-Natal province of South Africa. More research needs to be conducted to determine whether or not the biofortified maize soft porridge would be acceptable for use as a complementary food to a much larger sample of caregivers in rural areas of SSA. Other types of complementary foods containing provitamin A-biofortified maize should be tested for their sensory acceptability to caregivers.

5.3 Black African female caregivers' perceptions about provitamin A-biofortified maize

The results of the focus group discussions are presented in Table 5.1. The female caregivers of this study perceived the yellow biofortified maize as nutritious. They compared the colour of the yellow maize to that of egg yolk. Egg yolk is usually used as a weaning item for infants and is a good source of iron and vitamin A (Mahan *et al* 2012, pp63-69; Makrides, Hawkes, Neumann & Gibson 2002). These results indicate that the participants in this study

believe that yellow maize was suitable for infants, as they perceived it to be nutritious. Indeed, in the focus group discussions, some of the participants indicated their willingness to use provitamin A-biofortified maize soft porridge as a complementary food (Table 5.1). Appropriate nutrition education on the health benefits of yellow maize could enhance this positive perception of yellow maize. This could increase its acceptance for use as a complementary food item for infants, especially in developing countries, where other options are limited or very expensive for the poor rural population.

The participants in this study expressed some negative perceptions about yellow maize (Table 5.1). These perceptions were similar to those reported in the literature. According to Nuss *et al* (2012); Khumalo *et al* (2011) and Muzhingi *et al* (2008), there are notable negative perceptions about yellow maize. It has been found that yellow maize (including provitamin A-biofortified maize), is perceived as animal feed and food aid. The fact that yellow maize (including the biofortified maize) is not readily available in the African market exacerbates this stigma. These perceptions were detected in the present study. However, these negative perceptions seem to be associated with yellow maize only. Orange biofortified maize has been found more acceptable to consumers compared to yellow maize (Meenakshi *et al* 2012; Meenakshi *et al* 2010b; Stevens & Nelson-Winter 2008). The stigma concerning yellow maize (including the provitamin A-biofortified maize) could be reduced by educating consumers on the health properties of biofortified maize. This could be done using several forms of media and through health workers. The consumer perceptions about yellow maize could be further improved by making it readily available in supermarkets, as it would then be perceived as being fit for human consumption. Because there is less stigma associated with orange maize, the colour of the biofortified maize could be developed to an orange colour through breeding techniques, thereby improving its acceptance. Orange biofortified maize was not tested in this study, but could possibly be used in further research.

Price was a limiting factor to whether or not provitamin A-biofortified maize would be purchased (Table 5.1). Several studies indicated that biofortified maize would be purchased if it was sold at a lower price (De Groote *et al* 2011; Pillay *et al* 2011a; De Groote & Kimenju 2008; Stevens & Winter-Nelson 2008). This was also a finding in this study. Other authors have indicated that orange maize did not require a discount for it to be purchased. Instead, some consumers were willing to pay a higher amount for orange maize, whereas a discount was needed for yellow maize (Meenakshi *et al* 2012; Meenakashi *et al* 2010). In the present

study it was found that some consumers were willing to purchase biofortified maize if it was more nutritious than white maize and had health benefits (Khumalo *et al* 2011; Muzhingi *et al* 2008). According to Stevens & Winter-Nelson (2008), there is better acceptance of biofortified maize when there are children living in the household. Similarly, in this study, the participants reported that the colour of the maize would be attractive to the children, who would like the taste of the biofortified maize because it was different to that of the usual white maize. This study clearly indicates that biofortified maize would be purchased if it was cheaper, contained a health-promoting property and was readily available on the market. The implications of this study are that provitamin A-biofortified maize could possibly be used as a complementary food item in SSA if it was reasonably priced, readily available and positively impacted on the health of infants. Research on the use of provitamin A-biofortified maize as a complementary food item is still inadequate and more research needs to be conducted on the use of biofortified maize in other complementary food items across SSA. Because maize foods other than porridge are used as complementary foods, these other maize foods should be investigated.

Table 5.1 Black African female caregivers' perceptions towards the consumption of soft porridge made with provitamin A-biofortified maize

Question	Theme	Concept	Quotes	Discussion
Consumer familiarity with yellow maize	Consumer perceptions of yellow maize	• Familiar food consumed in bygone times • White maize regarded as a modern food • Stigmatisation	*'You can never find yellow maize in any supermarket or spaza shop.'* *'Bokide was used to feed the animals.'* *'In the old days you could get it ,now you cannot find it.'* *'People feed the animals with it.'* *'Yellow maize is for poor people and used to feed animals.'*	Modernisation and market availability influences the preferences, consumption and utilisation of yellow maize. The fact that it is unavailable in supermarkets suggests that the white maize is of better quality, subsequently overshadowing yellow maize. Nutrition education on the health benefits of provitamin A-biofortified maize compared to white maize should be conducted to create a positive image and change consumer perceptions. Proper marketing of provitamin A-biofortified maize could increase the acceptance by consumers. The study subjects perceived yellow maize as being for poor people, because of the way in which it was introduced during times of drought. It was given as food aid. It is perceived as animal food because it is only available in animal stores and not in supermarkets. The stigmatisation of yellow maize jeopardises its acceptability.
Perceived description of the appearance of yellow maize	Physical attributes of yellow maize which are appreciated Physical attributes of yellow maize which are not appreciated	• Described as egg yolk colour • Rough and coarse texture • Smell of yellow maize was not different from that of white maize • Unusual taste	'*The colour of the yellow maize was beautiful and bright.'* *'The yellow maize had a nice colour.'* *'It tasted different from the white maize.'* *'It tastes okay.'* *'It was fine and I could get used to the taste.'* *'Children would not have a problem with it.'*	The colour, texture and appearance of the maize were satisfactory to the consumers. It should be noted that the yellow colour was very attractive to children. However, the taste of the provitamin A-biofortified maize was less acceptable, though it was perceived to be nutritious compared to the yellow maize meal from 'bygone times'. The caregivers mentioned that,although the yellow maize had an unusual taste it was something that they could get used to. Provitamin A-biofortified yellow maize has a rich yellow colour. This colour is similar to that of egg yolk and could have stimulated and influenced the belief that provitamin A-biofortified yellow maize was nutritious.

Question	Theme	Concept	Quotes	Discussion
Willingness to use yellow maize porridge as a complementary food	Willingness	• Colour • Perceived nutrition • Affordability	*'If it is good for my baby's health I would buy it.'* *'I would buy it if it was cheap.'* '*I can use it to make porridge, pap, sgwaqane and phutu.'*	The colour was attractive enough to entice children into liking it. Mothers believed that the change in taste indicated that the yellow maize was nutritious. Price was the main determinant of whether they would buy it or not.
	Food preparation methods	• Baby foods such as soft porridge, *phutu*, pap and beans		No distinct difference in the way in which yellow maize would be used, compared to white maize, with regards to food for babies. However, mothers indicated that if yellow maize had a specific primary benefit, this could possibly stimulate their willingness to purchase and consume yellow maize.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

In Chapter 6 conclusions are drawn from the main findings of the study and recommendations are made. The aim of this study was to investigate the nutritional composition and acceptance of a complementary food made with provitamin A-biofortified maize. The objectives were; (i) to evaluate the nutritional composition of soft porridge made with provitamin A-biofortified maize compared to white maize; (ii) to assess the sensory acceptability of soft porridge made with provitamin A-biofortified maize by black African female infant caregivers; and (iii) to determine the perceptions of black African female infant caregivers about provitamin A-biofortified maize relative to white maize.

6.1 Conclusions

The findings of this study indicated that provitamin A-biofortified maize varieties were nutritionally superior to white maize with respect to energy, fibre, fat, protein, iron, zinc and phosphorus content. The same trends were observed in the soft porridges made with these maize varieties. These findings are generally similar to those reported previously. The findings of this study suggest that complementary soft porridges made with provitamin A-biofortified maize could, in addition to contributing to the alleviation of vitamin A deficiency (VAD), improve the nutritional status of infants with protein-energy and mineral deficiencies. Further research needs to be conducted in order to make firm conclusions about these findings; among the investigations needed are the protein quality and mineral bioavailability of the provitamin A-biofortified maize.

The sensory evaluation results indicated that yellow, provitamin A-biofortified maize soft porridges were as acceptable as white maize soft porridge to infant caregivers from the rural areas of the Umgungundlovu District of KwaZulu-Natal province. Unlike in several previous consumer acceptance studies, the unique colour, flavour and aroma of the yellow maize were not objectionable to the consumers used in this study. Although consumers of various demographic profiles were found, in other studies, to generally prefer white maize over yellow maize (including the biofortified type), it appears that the sensory acceptability of yellow maize (including the biofortified type) soft porridge had not been evaluated before the current study. In this study, it was found that age did not affect the sensory acceptability of provitamin A-biofortified maize, which was different from the findings of similar studies reported in the literature. From these findings it can be inferred that infant caregivers from

rural areas are likely to accept yellow, provitamin A-biofortified maize for use as an infant complementary food in the form of soft porridge. These findings should be treated with caution, due to the limited sample size of the caregivers and study area.

The focus group discussions indicated that the infant caregivers had both positive and negative perceptions about the yellow provitamin A-biofortified maize. The caregivers perceived the biofortified maize as nutritious and health-promoting. They thought that children would find the unique yellow colour and taste of the biofortified maize attractive, yet the caregivers had some negative perceptions about the yellow biofortified maize. The negative perceptions were similar to those reported in previous studies. These included the perception that yellow maize is for animal feed, or is food for poor people in need of food aid. The caregivers said that the fact that the yellow maize was not readily available in the market supported their perceptions that it was inferior to white maize, which was abundant on the market. This study indicated that the infant female caregivers were willing to give infants porridge made with provitamin A-biofortified maize if it was cheap, readily available and had a health benefit for their infants. It can be deduced from this study that, with nutritional education and proper marketing strategies, the negative stigma attached to yellow maize could be minimised.

Overall, it appears that provitamin A-biofortified maize has the potential to be used as a complementary food item. It could be used as a complementary strategy to help alleviate VAD in South Africa and other SSA countries.

6.2 Recommendations

6.2.1 Study limitations

- Due to financial constraints, the amino acid profile, starch composition and the provitamin A content of the porridges were not analysed.
- This study was conducted at two clinics, only, in the Umgungundlovu District, KwaZulu-Natal, which limits inferring the study findings for rural populations of wider geographical regions.

6.2.2 Recommendations for improvement of the study

The following recommendations are made:

- Further nutritional quality assessments of the provitamin A-biofortified maize soft porridge, including starch, provitamin A, amino acid profile and mineral bioavailability, should be conducted.
- The cost of the porridge was not calculated for this study. This should be done in order to determine if provitamin A-biofortified maize is economical for individuals from poor socio-economic backgrounds.
- The sensory evaluation should be conducted using more black African female caregivers at each of the clinics in the Umgungundlovu District and other similar rural areas.

6.2.3 Implications for further research

The study has implications:

- Similar studies should be conducted in other provinces in order to assess the acceptability of using provitamin A-biofortified maize as a complementary food. The complementary food chosen should be one that is commonly given to infants in that province.
- Caregivers should be provided with appropriate education prior to the study, to determine if education has an impact on the choices that the caregivers make and the perceptions that they have about provitamin A-biofortified maize.
- Studies could be conducted to determine if marketing has an impact on whether or not provitamin A-biofortified maize would be grown, purchased and used.

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APPENDIX A: SOFT MAIZE PORRIDGE RECIPE

SOFT MAIZE PORRIDGE RECIPE

Ingredients

- 8 cups (2000ml) water
- 2 cups (268 g) maize meal
- 1.5 cups (375 ml) water
- 1 ml salt
- 80 ml sugar

Method

1. Bring 8 cups (2000 ml) of water to the boil in a heavy duty pot on a defy thermofan stove (plate control setting 6).
2. Combine the maize meal and water to make a smooth paste.
3. Add the paste to the boiling water and stir until smooth.
4. Add 1 ml of salt while cooking.
5. Add 80 ml sugar once the porridge is cooked.

APPENDIX B: SENSORY EVALUATION QUESTIONNAIRE IN ENGLISH

Sensory evaluation of complementary maize porridge

Instructions:

- Please rinse your mouth with water before starting.
- Please rinse your mouth with water after tasting each sample.
- Please taste the samples of porridge in the order presented, from left to right.
- Please rate the taste, texture, aroma, colour and overall acceptability of the samples by putting a cross on the picture that best describes that sample.
- You may re-taste the sample if you wish.

Example :

Aroma	Very bad Bad Average Good Very good

Participant number : Sample number :

Sensory Evaluation of complementary infant food

Date of birth (dd/mm/yy): _____

Taste

Very bad Bad Average Good Very good

Texture

Very bad Bad Average Good Very good

Aroma

Colour

Very bad Bad Average Good Very good

Overall acceptability

Very bad Bad Average Good Very good

Thank you

APPENDIX C: SENSORY EVALUATION QUESTIONNAIRE IN ISIZULU

Ukuhlolwa kwephalishi lemputhu lezingane usebenzisa imizwa yomzimba

Iziyalo

- Uyacelwa ukuthi uyakaze umlomo ngamanzi ngaphambi kokuba uqale
- Uyacelwa ukuthi uyakaze umlomo ngamanzi emva kokuzwa isampulo ngayinye
- Uyacelwa ukuthi amasampulo ephalishi uwenzwe ngalandlela abekwe ngakhona, kusukela ngasesandleni sokunxele kuya kwesokudla.
- Uyacelwa ukuthi ukale ukunambitheka, iphunga, umbala kanye nokwamkeleka kwesampula ngayinye ngokuthi ufaka isiphambano phezu kwesithombe esichaza isampulo ngayinye.
- Uvumelekile ukuthi uphinde uyizwe isampulo uma ifisa.

Isibonelo:

Iphunga	 Kubi kakhulu Kubi Kuntukuntuko Kumnadi Kumnandi kakhulu

INOMBOLO ONIKEZWE YONA: INAMBOLO YESAMPULO:

Ukuhlolwa kwephalishi lemputhu lezingane usebenzisa imizwa yomzimba

Usuku lokuzalwa: (dd/mm/yy) _____

Ukuzwa ngomlomo

Kubi kakhulu Kubi Kuntukuntuko Kumnadi Kumnandi kakhulu

Ukuzwa ngezandla

Kubi kakhulu Kubi Kuntukuntuko Kumnadi Kumnandi kakhulu

Iphunga

Kubi kakhulu Kubi Kuntukuntuko Kumnadi Kumnandi kakhulu

Umbala

Kubi kakhulu Kubi Kuntukuntuko Kumnadi Kumnandi kakhulu

Ukwamkeleka

Kubi kakhulu Kubi Kuntukuntuko Kumnadi Kumnandi kakhulu

Ngiyabonga

APPENDIX D: CONSENT FORM FOR FEMALE CAREGIVERS IN ENGLISH

Consent form for mothers and caregivers

I am currently a part-time student from the University of KwaZulu-Natal, doing my Masters in Dietetics. The aim of my research is to assess the potential of using provitamin A-biofortified maize as a complementary food. I would like to find out if mothers or caregivers would find yellow/orange maize acceptable to use as a complementary food. The participants will be required to taste four samples of porridge and rate the samples using a simple picture scale. There will no discomforts or hazards to participants who agree to participate in this study.

- The researcher's name is LaurenciaGovender (BSc Dietetics, PgDip Dietetics), who is from the Discipline of Dietetics and Human Nutrition at the University of KwaZulu-Natal. Contact details for the researcher are as follows 033-3954993 or 206511208@stu.ukzn.ac.za.
- For further information regarding the study, you may contact Dr Kirthee Pillay, who is the project supervisor. Contact details: 033-2605674 or pillayk@ukzn.ac.za.
- All the data collected from this study will remain confidential and will only be used for the purpose of this research project. All participants will remain anonymous.
- Participation in this study is completely voluntary. All participants may leave the study at any time they wish, without any negative consequences.
- There are no potential benefits from participating in this study. No participants will receive any payments or financial reimbursements for participating in this research project.
- Audio recordings from the focus group discussions will be used for the purpose of this study and will be stored appropriately.
- All data will be destroyed when it is no longer needed.

Declaration:

I _____ (full name and surname) hereby confirm that the questionnaire has been clearly explained to me and I understand the purpose of this research project and how the information will be collected. I consent to participating in the research project.

I understand that participation is voluntary and I can leave the study if I desire.

_____ _____

Signature Date

APPENDIX E: CONSENT FORM FOR FEMALE CAREGIVERS IN ISIZULU

Ifomu lemvume lomama kanye nabanakekela amantwana

Ngingumfundi wase University of Kwazulu Natal, oqhuba izifundo zakhe ze masters kwi dietetics. Ngenza ucwaningo lokuhlola ukwamkeleka kokusebenzisa i provitamin A Bioforifiedmaize ukwena ukudla kwezingane ezingaphezu kwezinyanga eziyisithupha. Ngifisa ukuzwa uvo lomama noma lwabanakekeli bezingane lokuthi impuphu ephuzi yamkelekile yini ukuthi ingasetshenziswa ekudleni kwezingane ezinezinyanga eziyisithupha nagaphezulu. Abantu abayingxenye yalolocwaningo bazocelwa ukuthi bezwe amasampulo amane ephalishi bese besebenzisa isikali esinezithombe ezinhlanu ukusho ukuthi lamaphalishi ezwakale enjani. Kulolucwaningo akukho ukungaphatheki kahle nobungozi obukhona.

- Igama lomcwaningi uLaurencia Govender (BSC Dietetics, Pgdip Dietetics) ukumkhakha wakwa dietetics and human nutrition e University of KwaZulu Natal. Utholakala ku 033 3954993 noma 20651120@stu.ukzn.ac.za.
- Uma udinga eminye imniningwane ngalolucwaningo ungathintana no Kirthee Pillay ongumphathi walolucwaningo. Utholakala ku 033 2605674 noma pillay@ukzn.ac.za.
- Imniningwane ezotholakala ngeke isetshenziselwe okunye okuseceleni, izosebenzizwa kulolucwaningo kuphela. Imininingwane yabantu abazobe beyingxenye yalolucwaningo izogodlwa.
- Abantu baba ingxenye yalolucwaningo ngokuvolontiya. Abantu abayingxenye yalolucwaningo bavumelekile ukuthi bashiye phakathi kwalo uma befisa, Akukho lutho olubi oluyokwenziwa kubona,
- Ayikho imikomelo ezotholwa abantu abayingxenye yalolucwaningo. Ayikho imali eyotholwa abantu abayinxenye yalolucwaningo.
- Okuzo rekhodwa ngenkathi kuxoxiswana nezixuku zabantu kuzosetshenziselwa lolucwaningo kuphela futhi kuzogcinwa endaweni ephephile.
- Yonke imininingwane yalolucwaningo izolahlwa uma ingasa dingakali.

Izwi lobufakazi:

Mina_____ (Amagama aphelele nesibongo) Ngiyaqiniseka ukuthi ngichazelekile kahle ngalembuzo engizobuzwa yona futhi ngiyasiqonda isizathu salolucwaningo nokuthi yonke imiciningwane etholakele izohlolwaNgiyavuma ukuba ingxenye yalolucwaningo.

Ngiyaqonda ukuthi kuyavolontiywa ukuba ingxenye yalolucwaningo nanokuthi ngingashiya phakathi uma ngifisa.

_____ _____

(Sayina) (Usuku)

APPENDIX F: FOCUS GROUP DISCUSSION QUESTIONS IN ENGLISH

Focus Group Questions

1. Have you ever cooked yellow/orange maize before? Please share your experience
2. Is it the first time you have tasted porridge made with yellow/orange maize? Please describe how you feel about its physical attributes
    - Taste
    - Smell
    - Texture
    - Colour
3. Is the yellow maize different from any other maize you ever used? Elaborate on the difference if there is or if not what are the similarities if there any.
4. As a mother would you feed your baby porridge made with yellow/orange maize? Explain your reasons
5. What kind of infant foods would you prepare with yellow/orange maize?
6. Can you access yellow/orange maize in any way in the area, if yes explain how and where and if no explain why do you think is like that.
7. Would you buy yellow/orange maize if it was cheaper than white maize?
8. Would you buy yellow/orange maize if it has positive effects on your baby's health?

APPENDIX G: FOCUS GROUP DISCUSSION QUESTIONS IN ISIZULU

Imibuzo ezobuzwa isixuku sabantu

1. Wake wapheka ngemputhu ephuzu noma eqanda phambilini? Uyacelwa ukuthi uchaze kafushane ukuthi kwaba njani ukuyisebenzisa?

2. Uyaqala ukulizwa iphalishi lempuphu eyellow noma ephuzu emlonyeni? Uyacelwa ukuthi uchaze ukuthi ulizwe linjani
    - Emlonyeni
    - Iphunga
    - Ezandleni
    - Umbala

3. Kungabe impuphu ephuzu ihlukile kwezinye izinhlobo zezimpuphu owake wazisebenzisa phambilini? Uyacelwa ukuthi uchaze kabanzi ngomehluko owuzwile uma ube khona. Uma ungekho umehluko uyacelwa ukuthi uchaze ngokuthole kufana nokwezinye izinhlobo zempuphu.

4. Njengoba uwumama ungamupha umntwana wakho iphalishi eliphekwe ngempuphu ephuzu noma eqanda? Uyacelwa ukuthi ubeke izizathu zempendulo yakho

5. Iziphi izinhlobo zokudla zabantwana ongazipheka ngempuphu ephuzu noma eqanda?

6. Iyatholakala impuphu ephuzu noma eqanda endaweni ohlala kuyo? Uma itholakala itholakala kanjani futhi laphi? Uma ingatholakali ubona ukuthi kwenziwa yini lokho?

7. Ungayithenga impuphu eyenziwe ngommbila ophuzu noma oqanda uma ibiza kangconywana kunempuphu emhlophe?

8. Ungayithenga impuphu ephuzu noma eqanda uma izoba nemphumela emihle empilweni yomntwana wakho?

APPENDIX H: ETHICS APPROVAL LETTER FROM UKZN

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

Research Office (Govan Mbeki Centre)
Westville Campus
Private Bag X54001
Durban, 4001
Telephone: 031 260 3587
EMAIL: ximbap@ukzn.ac.za

23 May 2012

Ms Laurencia Govender 206511208
School of Agricultural, Earth & Environmental Sciences

Dear Ms Govender

PROTOCOL REFERENCE NUMBER: HSS/0180/012M
PROJECT TITLE: An assessment of the potential of provitamin A-biofortified maize as a complementary infant food

PROVISIONAL APPROVAL

This letter serves to notify you that your application in connection with the above has been approved, subject to necessary gatekeeper permissions being obtained.

This approval is granted provisionally and the final approval for this project will be given once the above condition has been met. In case you have further queries/correspondence, please quote the above reference number.

Kindly submit your response to the Chair: Prof. S Collings Research Office as soon as possible

Yours faithfully

Professor Steven Collings (Chair)
Humanities & Social Sciences Research Ethics Committee

POST GRADUATE OFFICE
COLLEGE OF AGRICULTURE,
ENGINEERING AND SCIENCE
2012 -05- 28
PIETERMARITZBURG CAMPUS

cc Supervisor Dr Kirthee Pillay
cc Dr Mtulisi Siwela
cc Professor John Derera
cc Ms M Balawanth/Ms Sibingile Ntuli

APPENDIX I: APPROVAL LETTER FROM EDENDALE HOSPITAL

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

Edendale Hospital
Private Bag X 509, Plessislaer, 3216
Tel: 033 395 4040, Fax: 033 395 4087
email: thandiwe.ndlovu@kznhealth.gov.za
www.kznhealth.gov.za

OFFICE OF THE CHIEF EXECUTIVE OFFICER

Reference No.30/5/1
Enquiries: Mrs. TJ Ndlovu
Tel: 033-3954040

Date: 29 June 2012

Ms. L Govender
Dietetics Department
Edendale Hospital

Dear Ms. Govender

RE- REQUEST PERMISSION TO CONDUCT RESEARCH STUDY ON AN ASSESMENT OF THE POTENTIAL OF PROVITAMIN A-BIOFORTIFIED MAIZE AS A COMPLEMENTARY INFANT FOOD.

Your letter dated 13 June 2012 refers,

Your request to conduct the above-mentioned surveillance is supported by Edendale Hospital Management, subject to approval by Department of Health Research Committee.

Yours Sincerely,

DR OG OJO
ACTING CHIEF EXECUTIVE OFFICER
EDENDALE HOSPITAL

APPENDIX J: APPROVAL LETTER FROM THE DEPARTMENT OF HEALTH

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

Health Research & Knowledge Management sub-component
10 – 103 Natalia Building, 330 Langalibalele Street
Private Bag x9051
Pietermaritzburg
3200
Tel.: 033 – 3953189
Fax.: 033 – 394 3782
Email.: hrkm@kznhealth.gov.za
www.kznhealth.gov.za

Reference : HRKM95 /12
Enquiries: Mrs G Khumalo
Telephone: 033 – 395 2805

30 July 2012

Dear Ms L Govender

Subject: Approval of a Research Proposal

1. The research proposal titled **'An assessment of the potential of provitamin A-biofortified maize as a complementary infant food'** was reviewed by the KwaZulu-Natal Department of Health.

The proposal is hereby **approved** for research to be undertaken at Edendale Hospital.

2. You are requested to take note of the following:
   a. Make the necessary arrangement with the identified facility before commencing with your research project.
   b. Provide an interim progress report and final report (electronic and hard copies) when your research is complete.

3. Your final report must be posted to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hrkm@kznhealth.gov.za

For any additional information please contact Mrs G Khumalo on 033-395 3189.

Yours Sincerely

Dr E Lutge
Chairperson, Health Research Committee
KwaZulu-Natal Department of Health
Date: 01/08/2012