NUTRITIONAL QUALITY AND CONSUMER ACCEPTABILITY OF PROVITAMIN A-BIOFORTIFIED MAIZE

BY

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PREFACE

The work described in this thesis was carried out in the School of Agricultural Sciences and Agribusiness, University of KwaZulu-Natal from August 2009 to June 2011, under the supervision of Dr Muthulisi Siwela, Professor John Derera and Professor Frederick J Veldman.

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DECLARATION

I, Kirthee Pillay declare that:

1. The research reported in this thesis, except where otherwise stated, is my original research.

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

3. This thesis does not contain any other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from those persons.

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ABSTRACT

Vitamin A deficiency (VAD) is a major public health problem in developing countries, including South Africa. The potential of provitamin A-biofortified maize for use as a complementary strategy to alleviate vitamin A deficiency in developing countries, where maize is the dominant staple food, is currently a subject of research. Although the nutritional composition of white maize is thought to be similar to that of biofortified maize, apart from the differences in provitamin A carotenoid content, the comparative nutritional composition of the two maize types seems not to have been subjected to a comprehensive scientific study. When setting the target level of provitamin A in the provitamin A-biofortified maize, it is important to consider the potential effect of processing on the final provitamin A carotenoid content of the biofortified food products, as the provitamin A carotenoids levels may decrease on processing. Furthermore, the yellow/orange provitamin A-biofortified maize may not be widely accepted by African consumers who are vulnerable to VAD, and are traditional consumers of white maize.

This study firstly aimed to evaluate the nutritional composition, including provitamin A composition, and grain quality of provitamin A-biofortified maize varieties, compared to white maize. The second aim was to assess the effect of processing (milling and cooking) on the retention of provitamin A carotenoids and other nutrients in popular South African maize food products prepared with provitamin A-biofortified maize. Thirdly, the study aimed to assess the acceptability of maize food products prepared with provitamin A-biofortified maize by consumers of different age and gender in rural KwaZulu-Natal, South Africa.

The grains of the provitamin A-biofortified maize varieties and grain of a white maize variety (control) were analysed for their nutritional composition using standard or referenced methods. The carotenoid content of the grains was analysed by High-Performance Liquid Chromatography (HPLC) and mass spectroscopy. The provitamin A carotenoids β-cryptoxanthin, and *trans* and *cis* isomers of β-carotene, and other unidentified *cis* isomers of β-carotene were detected in varying levels in the provitamin A-biofortified maize varieties. The total provitamin A content in the biofortified maize varieties ranged from 7.3-8.3 μg/g dry weight (DW), with total β-carotene ranging from 3.5-3.6 μg/g DW, and β-cryptoxanthin from 3.7-4.8 μg/g DW, whilst no carotenoids were detected in the white maize variety. Results of the evaluation of the content of other nutrients showed that, when compared with the white maize variety, the provitamin A-biofortified maize varieties had higher levels of starch, fat and protein but were lower in iron.

The zinc and phosphorus levels in the white maize and the biofortified maize were comparable. The biofortified maize varieties were better sources of most of the essential amino acids relative to the white maize, but, similar to the white maize, they were deficient in histidine and lysine, indicating that further improvement is required. Selected quality attributes (grain density, susceptibility of kernels to cracking, milling quality and resistance of the kernels to fungal infection) of grains of 32 provitamin A-biofortified maize varieties and a white variety (control) were assessed. Overall, the quality of the grains of the provitamin A-biofortified maize varieties were found to be superior to that of the white maize grain, although the biofortified maize grains showed less resistance to fungi, including mycotoxin-producing types. This indicates that the trait of grain resistance to infection by fungi should also be incorporated in the provitamin A-biofortified maize varieties during breeding.

To assess the retention of provitamin A carotenoids and other nutrients in maize food products, three selected provitamin A-biofortified maize varieties and the control (white maize variety) were milled into mealie meal and samp. The milled products were cooked into three products: *phutu* and thin porridge (from the mealie meal) and cooked samp. Nutrient retention during processing was determined. Milling resulted in either an increase or slight decrease in the provitamin A carotenoid levels, but there was no major decrease in the total provitamin A level. Most of the other nutrients were well retained during milling, but there were substantial losses of fibre, fat and minerals. Provitamin A carotenoid levels decreased on cooking. In *phutu* 96.6 ± 20.3% β-cryptoxanthin and 95.5 ± 13.6% of the β-carotene was retained after cooking. In thin porridge 65.8 ± 4.6% β-cryptoxanthin and 74.7 ± 3.0% β-carotene; and in samp 91.9 ± 12.0% β-cryptoxanthin and 100.1 ± 8.8% of the β-carotene was retained after cooking, respectively. Provitamin A retention seemed to be influenced by both maize variety and food form, indicating that suitable varieties and food forms should be found. There was generally a high retention of the other nutrients in all the three cooked products, except for the substantial losses of fat in thin porridge and iron and phosphorus in cooked samp. These findings indicate that an optimal delivery of provitamin A to the consumer can be achieved by processing provitamin A-biofortified maize into foods that have a good retention of provitamin A carotenoids, such as *phutu* and samp. These food products would be recommended in areas where VAD is prevalent.

In order to assess consumer acceptability of provitamin A-biofortified maize, a total of 212 subjects aged 3-55 years from Mkhambathini Municipality, in KwaZulu-Natal province, South Africa, participated in the sensory evaluation of *phutu*, thin porridge and cooked samp prepared

with provitamin A-biofortified maize varieties and a white variety (control). Preference for yellow maize food products was negatively associated with an increase in the age of the subjects. Overall, preschool children preferred yellow maize to white maize food products: *phutu* (81% vs. 19%), thin porridge (75% vs. 25%) and samp (73% vs. 27%). In contrast, primary school children preferred white maize to yellow maize food products: *phutu* (55% vs. 45%), thin porridge (63% vs. 38%) and samp (52% vs. 48%). Similarly, secondary school children and adults also displayed a similar preference for white maize food products. There was no association between gender and preference for maize variety. Focus group discussions revealed that participants had a negative attitude towards biofortified maize due to its colour, taste, smell and texture. However, the participants expressed a willingness to consume biofortified maize if it was cheaper than white maize and was readily available in local grocery stores. These findings indicate that there is a potential to promote the consumption of provitamin A-biofortified maize and its food products in this part of South Africa, thereby contributing to a reduction in the incidence of VAD.

This study has shown that provitamin A-biofortified maize has a good potential to be used as an additional strategy to alleviate VAD in poor communities of South Africa, including similar environments in sub-Saharan Africa. However, the study has revealed that there are still challenges to be overcome in order to achieve the target provitamin A content of 15 μg/g in provitamin A-biofortified maize, set by HarvestPlus, an international challenge program. This may also explain why provitamin A-biofortified maize varieties with this level of provitamin A have been scarcely reported in the literature. Thus, more research is required to achieve the target provitamin A level in maize by conventional breeding. The results of this study indicate that besides provitamin A, the biofortified maize is also a good source of other nutrients including starch, fat, protein and zinc. However, improving the consumer acceptability of the provitamin A-biofortified maize remains a challenge, due to the negative attitudes towards the yellow/orange maize by African consumers. On the other hand, the results of this study indicate that there is an opportunity to promote the consumption of provitamin A-biofortified maize food products by preschool children, a finding which has not been previously reported in the literature. Nutrition education on the benefits of provitamin A-biofortified maize, as well as improved marketing are recommended, in this part of South Africa and also in similar environments in other sub-Saharan countries.

ACKNOWLEDGEMENTS

DEDICATION

This thesis is dedicated to my Mum, Kanthie Pillay (née Totaram) 1945-1983.

Thank you for the precious gifts of life and education.

I wish that you could have shared this experience with me.

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ABBREVIATIONS

AACC	American Association of Cereal Chemists
AOAC	Association of Official Analytical Chemists International
CGIAR	Consultative Group on International Agricultural Research
CIMMYT	International Maize and Wheat Improvement Center
DoH	Department of Health
DW	Dry Weight
EAR	Estimated Average Requirement
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agriculture Organization of the United Nations
HPLC	High-Performance Liquid Chromatography
IITA	International Institute of Tropical Agriculture
INP	Integrated Nutrition Programme
MSA	Malt Salt Agar
NDF	Neutral Detergent Fibre
NIT	Near-Infrared Transmittance
NFCS	National Food Consumption Survey
NFCS-FB	National Food Consumption Survey-Fortification Baseline
PAW	Predictive Analytics SoftWare
PCNB	Pentachlorobenzene Agar
PDA	Potato Dextrose Agar
PEM	Protein Energy Malnutrition
QPM	Quality Protein Maize
RAE	Retinol Activity Equivalents
RE	Retinol Equivalents
SAGL	South African Grain Laboratory
SAVACG	South African Vitamin A Consultative Group
SPSS	Statistical Package for Social Sciences
SSA	Sub-Saharan Africa
THF	Tetrahydrofuran
VAD	Vitamin A Deficiency
WHO	World Health Organization

CHAPTER 1

INTRODUCTION, THE PROBLEM AND ITS SETTING[1]

1.1 Importance of the study

Maize (*Zea mays*) belongs to the grass family *Poceae* (formerly *Gramineae*) and is a cross pollinating species with the female (silk) and male (tassel) flowers located separately on the plant [Food and Agriculture Organization (FAO) 1992]. Maize is also known as corn and is one of the three most important cereal crops worldwide. In sub-Saharan Africa (SSA), approximately 50% of the population relies on maize as a staple food [International Institute of Tropical Agriculture (IITA) 2010a]. According to the World Health Organization (WHO) (2003), the average maize consumption in the African diet is 106.2 g/person/day and is far greater than the calculated values from other maize-consuming regions in the world (WHO 2003). In South Africa, maize is a commonly consumed food item and contributes about 40% of total energy intake (McCann 2005, p9; Labadarios *et al* 2000). In Africa, the sub-Saharan Africa region is a leader in maize consumption (IITA 2010a). However, the nutritional composition of maize is unbalanced, especially due to the lack of provitamin A carotenoids (Nuss & Tanumihardjo 2010). This could partly explain why malnutrition still exists in SSA, where maize is a dietary staple (Nuss & Tanumihardjo 2010)

Vitamin A deficiency (VAD) is a major public health problem in lower income countries, especially in Africa and South-East Asia (WHO 2009). Globally, approximately 250 million preschool children have VAD and an estimated 250 000 to 500 000 of these children become blind every year as a result of VAD (WHO 2010). Population groups that are vulnerable to VAD include children under the age of five; children with infection and severe protein energy malnutrition (PEM); children from poor socioeconomic backgrounds; non-breastfed infants; and pregnant and lactating women (Ahmed & Darnton-Hill 2004). VAD is caused by low dietary intake of vitamin A, which is insufficient to meet physiological needs and leads to impaired tissue function (WHO 2009). VAD results in loss of appetite, poor growth in children and an impaired immune response (Gibson 2005, p908). Xerophthalmia, which starts with night blindness and can progress to irreversible blindness, can also result from VAD (Sommer & West 1996; Sommer 1995). Xerophthalmia is the leading preventable cause of blindness in children worldwide (Sommer & West 1996; Sommer 1995). Children with VAD

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[1] A concept note based on this chapter has been published as an abstract: South African Journal of Clinical Nutrition. Pillay K, Siwela M, Derera J, Veldman FJ (2010). Retention of provitamin A carotenoids during processing and consumer acceptability of provitamin A-biofortified maize food products. SAJCN 23(3): S36.

are more likely to die than children with a good nutritional status and approximately 1-2.5 million preschool children die annually as a result of VAD (ACC/SCN 2000, p121). As mentioned earlier, the problem of VAD is particularly serious in Africa, where maize is a major part of the diet.

According to the accepted criteria of the WHO, South Africa has a serious public health problem of poor vitamin A status (WHO 2009). The South African Vitamin A Consultative Group (SAVACG) study of 1994 reported that approximately one in three children was found to have VAD (serum retinol < 20 µg/dl), with the highest prevalence found in KwaZulu-Natal (38%) and Limpopo (43.5%) provinces (Labadarios & Van Middelkoop 1995). These findings were supported by the National Food Consumption Survey (NFCS) of 1999, which found that one of two children had a vitamin A intake of less than half the recommended level (Labadarios *et al* 2000). In 2000, approximately 3069 childhood deaths (0-4 years) and 222 maternal deaths were attributed to VAD in South Africa (Nojilana *et al* 2007). The 2005 National Food Consumption Survey-Fortification Baseline (NFCS-FB) revealed that the proportion of children with marginal vitamin A status had increased to 64%. This indicates that there has been a significant deterioration in the vitamin A status of South African children since 1994 (Labadarios *et al* 2007). This deterioration in the vitamin A status of South African children has occurred despite strategies implemented to overcome VAD (Swart *et al* 2008, p133). Therefore other sustainable and complementary strategies should be found.

The most bioavailable dietary source of vitamin A is preformed vitamin A, which is found in foods of animal origin such as milk, cheese, liver and egg yolk. These expensive sources of preformed vitamin A are inaccessible to many poor households in SSA. In plant foods, vitamin A is in the form of provitamin A carotenoids, which must be converted to retinol in the body, in order to achieve vitamin A activity (Institute of Medicine 2000a, p326). The bioavailability and bioconversion of provitamin A carotenoids can be influenced by various factors such as molecular linkage, amount of carotenoids consumed in a meal, matrix in which the carotenoid is incorporated, intake of dietary fat, fibre and alcohol, nutritional status of the individual as well as genetic and host-related factors (Castenmiller & West 1998). Many of the poor households rely on plant sources of vitamin A, such as yellow/orange-fleshed vegetables, dark-green leafy vegetables and yellow/orange-fleshed non-citrus fruit (Faber & Wenhold 2007). In the African diet, more than 80% of dietary intakes of vitamin A are from plant sources, which have a lower bioavailability than animal sources (WHO 1995),

hence the level of provitamin A should be improved in plant foods that are accessible and available to both the rural and urban poor populations.

The Integrated Nutrition Programme (INP), which was initiated by the South African Department of Health in 1995, has the elimination of micronutrient deficiencies as one of the key objectives within the focus area of micronutrient malnutrition control. The INP uses a combination of strategies to address micronutrient deficiencies in the population including: supplementation, food fortification, promotion of dietary diversification as well as other related public health measures (Labadarios *et al* 2005). Although dietary diversification may be a desirable way of preventing micronutrient malnutrition, poverty and limited access to a variety of foods prevents it from being successful. Despite legislation for the fortification of maize meal and wheat flour with vitamin A among other nutrients, since October 2003 (Labadarios *et al* 2005), the accessibility of these commercially fortified foods to poor people in rural areas is arguably a chronic problem (Faber & Wenhold 2007; Nestel *et al* 2006). This is where biofortification could succeed as a new and alternative strategy of dealing with micronutrient malnutrition (De Groote & Kimenju 2008; Mayer *et al* 2008).

Biofortification involves breeding staple crops for increased vitamin and mineral content using the best traditional breeding practices and modern biotechnology (De Groote & Kimenju 2008; Nestel *et al* 2006). Efforts in this regard are being led by the HarvestPlus Challenge Program, a biofortification initiative within the Consultative Group on International Agricultural Research (CGIAR) that aims to reduce micronutrient malnutrition among less privileged populations in Africa, Asia and Latin America (Ortiz-Monasterio *et al* 2007). Comprehensive plant breeding programmes with completed feasibility studies are currently in place for rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), cassava (*Manihot esculenta* Crantz), orange-fleshed sweet potato [*Ipomoea batatas* (L.) Lam.] and common beans (*Phaseolus vulgaris* L.) (HarvestPlus Brief 2006). Since maize is the preferred staple in Africa, including South Africa, provitamin A-biofortified maize[2] has the greater potential to succeed as a new strategy to combat VAD in Africa, than the other above-mentioned crops (Tothova & Meyers 2006; WHO 2003).

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[2] For the purpose of this thesis provitamin A-biofortified maize may also be referred to as yellow maize, orange maize, yellow/orange maize or biofortified maize.

Although breeding maize with a high provitamin A content has the potential to reduce VAD, there are some challenges that need to be addressed. Despite the fact that yellow/orange varieties[3] of maize have a superior provitamin A content compared to white maize, which contains nutritionally insignificant provitamin A carotenoids, the provitamin A carotenoid content of yellow/orange maize is known to vary, due to genetic factors and the levels are generally low (Menkir *et al* 2008; Li *et al* 2007; Ortiz-Monasterio *et al* 2007). Yellow maize varieties contain between 0.25 and 2.5 μg/g dry weight (DW) of provitamin A, while deep yellow or orange varieties may contain 15 μg/g DW of provitamin A (Nuss & Tanumihardjo 2010), but such varieties are not currently available to farmers. However, grain colour is not necessarily correlated with provitamin A content. This is due to the fact that among the yellow/orange maize varieties there are variable levels of provitamin A carotenoids in the seed coat, endosperm and germ of the maize kernels (Harjes *et al* 2008; Grogan & Blessin 1968) and there are also many other carotenoids and non-carotenoid pigments with no vitamin A activity (De Groote & Kimenju 2008). The current breeding target for maize as set by HarvestPlus is 15 μg/g DW of provitamin A (Ortiz-Monasterio *et al* 2007), and thus there are ongoing breeding efforts to achieve this target in provitamin A-biofortified maize varieties. Although the nutritional composition of white maize is thought to be similar to yellow/orange maize, apart from the differences in provitamin A carotenoid content, it has not been well reported. The nutritional composition of yellow/orange maize varieties may be significantly different from that of white maize with respect to nutrients other than provitamin A, similar to what was found in biofortified low-phytic acid white maize (Raboy *et al* 1989). It is therefore necessary to determine the nutritional composition, including provitamin A content of maize varieties bred for high provitamin A content.

Another challenge with biofortification is that provitamin A carotenoids can be destroyed by exposure to light, oxygen and processing (Rodriquez-Amaya 1997). Although the data are limited, environmental and processing conditions have been found to result in losses of 24.5% and 24.8% of β-carotene in the preparation of fermented and unfermented maize porridges, respectively (Li *et al* 2007). The average retention of provitamin A carotenoids in provitamin A-biofortified maize following *nixtamilization*[4] and frying, which are common processing

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[3] For the purpose of this thesis the word/s, variety/varieties is used interchangeably with hybrid/hybrids.

[4] N*ixtamilization* is the traditional form of processing maize in Mexico and Central America. It involves the cooking and steeping of maize kernels in an aqueous suspension of calcium hydroxide and is a key step in the conversion of maize grain to dough and eventually to corn flour, snacks and tortillas (Katz *et al* 1974).

methods used to prepare Mexican maize food products, was found to be only 64% (Lozano-Alejo *et al* 2007). Muzhingi *et al* (2008a) investigated the effect of cooking on the carotenoid content of raw maize flour and observed an increase in carotenoid levels in all cooking methods, except baking. The findings of the limited studies on the retention of β-carotene during processing of provitamin A-biofortified maize (Muzhingi *et al* 2008a; Li *et al* 2007; Lozano-Alejo *et al* 2007) suggest that provitamin A carotenoid retention is influenced by storage and processing conditions. The methods used to process maize into food products vary due to several factors, including culture, economics and available technology. In many parts of Africa, including South Africa, maize is milled into different products, such as mealie rice, samp and flour which are then processed, usually by cooking, into a wide variety of food products. Wet-cooking is one of the most predominant processing methods. It is therefore important to investigate the retention of provitamin A carotenoids and other nutrients during the processing of provitamin A-biofortified maize into different food products.

Increasing the provitamin A content of maize through breeding changes its sensory properties significantly when compared to the sensory properties of white maize, which may impact negatively on its consumer acceptability. The consumer's reactions to these changes may act as a deterrent to consumer acceptance of provitamin A-biofortified maize and related food products (Stevens & Winter-Nelson 2008). In particular, the characteristic yellow/orange colour of provitamin A-biofortified maize may pose a challenge with regard to consumer acceptance, especially in areas such as eastern and southern Africa where most of the maize produced and consumed is white (De Groote & Kimenju 2008; Stevens & Winter-Nelson 2008).

Although there is a lack of published South African data on consumer acceptability of provitamin A-biofortified maize, there are results from other African countries such as Kenya, Zambia, Mozambique and Zimbabwe. In Kenya, yellow maize is regarded as inferior and is associated with food aid and animal feed (De Groote *et al* 2010; De Groote & Kimenju 2008). In Zimbabwe, yellow maize is known to all but few people are aware of its nutritional benefits and few consume it. Zimbabweans view yellow maize as inferior to white maize mainly because of the unacceptable organoleptic properties that result from chemical changes as a result of poor handling during importation (Muzhingi *et al* 2008b). Studies in Mozambique found that consumers preferred white maize to yellow maize, but were willing to switch over

to yellow maize if given a price discount (Stevens & Winter-Nelson 2008; Tschirley & Santos 1995).

The limited studies on consumer acceptance of provitamin A-biofortified maize in Africa have been conducted on the adult group, living in urban areas. However, the urban consumer is not the main target of provitamin A-biofortified maize and more studies on rural consumers have been recommended (De Groote & Kimenju 2008). Furthermore, preschool children[5] are at increased risk for VAD, partly due to a lack of dietary diversity (Allen 2006; Ahmed & Darnton-Hill 2004). Consumer acceptability of provitamin A-biofortified maize may vary with age and gender and this may influence the decision to purchase and consume the maize. Data on the acceptability of provitamin A-biofortified maize varieties and their food products by consumers of different age and gender would be useful. It could help to identify the traits that the breeders need to focus on, in order to improve the acceptability of the maize across consumer age and gender groups.

1.2   Summary of research focus

This study will provide important data on the nutritional composition and other quality attributes of provitamin A-biofortified maize grain varieties. Retention of provitamin A carotenoids and other nutrients during milling and cooking of the maize varieties into popular South African maize food products will also be investigated. This study will also provide useful data on the consumer acceptability of provitamin A-biofortifed maize food products among Black South African children (in preschool[5], primary[6] and secondary[7] school) and adults of poor socio economic status, living in KwaZulu-Natal, who are highly likely to be at risk of VAD.

1.3   Purpose of the study

The purpose of this study was to assess the nutritional composition, retention of provitamin A during milling and cooking and consumer acceptability of provitamin A-biofortified maize, which is proposed to contribute to the alleviation of VAD.

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[5] Preschool children are under the age of 5 years and are not yet attending Primary School.
[6] Primary school children are generally between Grade 1 (at least 6 years) and Grade 7 (at least 12 years).
[7] Secondary school children are generally between Grade 8 (at least 13 years) and Grade 12 (at least 17 years).

1.4   Study objectives

The objectives of this study were:

1.4.1   To evaluate the nutritional composition and other quality attributes of provitamin A-biofortified maize grain varieties compared to white maize grain.

1.4.2   To determine the effect of milling and cooking on the provitamin A carotenoid content of different provitamin A-biofortified maize varieties.

1.4.3   To assess the acceptability of popular South African maize food products made with provitamin A-biofortified maize among Black African children and adults from a rural KwaZulu-Natal population.

1.5   Hypotheses

The hypotheses of this study were as follows:

1.5.1   Provitamin A carotenoid content varies among the provitamin A-biofortified maize varieties due to genetic factors.

1.5.2   The nutritional composition of the provitamin A-biofortified maize varieties is significantly different from that of white maize; in particular the biofortified maize has significantly higher levels of provitamin A carotenoids.

1.5.3   The other quality attributes of provitamin A-biofortified maize grain varieties is different from that of the white maize variety (control) due to genetic factors.

1.5.4   Milling decreases the provitamin A carotenoid content of the provitamin A-biofortified maize.

1.5.5   Cooking reduces the provitamin A carotenoid content of the provitamin A-biofortified maize food products but the provitamin A carotenoid content will still be nutritionally significant.

1.5.6   Food products made from provitamin A-biofortified maize are less acceptable relative to the corresponding white maize food products due to the undesirable sensory properties of the biofortified maize, which includes, colour, taste and aroma, across all consumer age and gender groups.

1.6   Study parameters and general assumptions

The consumer acceptability studies on the provitamin A-biofortified maize food products were carried out in the Mkhambathini Municipality, KwaZulu-Natal, South Africa. It was assumed that these subjects were of poor socio economic status and were representative of a poor, rural population in KwaZulu-Natal. Assumptions relevant to specific sub problems are

discussed in the relevant sections of this thesis. The study was limited to yellow/orange maize varieties which were obtained from conventional breeding performed at agricultural stations in KwaZulu-Natal province, South Africa.

1.7 Outline of the thesis

The thesis is laid out as follows:

Chapter 1:   Introduction, the problem and its setting.

Chapter 2:   Literature review.

Chapter 3:   Background to study design and the consumer acceptability study site.

Chapter 4:   Nutritional composition and other quality attributes of provitamin A-biofortified maize grain.

Chapter 5:   Retention of provitamin A carotenoids and other nutrients during processing of provitamin A-biofortified maize into popular South African maize food products.

Chapter 6:   Consumer acceptability of yellow, provitamin A-biofortified maize in KwaZulu-Natal, South Africa.

Chapter 7:   Conclusions and recommendations.

The referencing style used in this thesis is according to the guidelines used in the Discipline of Dietetics and Human Nutrition, University of KwaZulu-Natal, Pietermaritzburg.

References

ACC/SCN (2000). **4th Report On the World Nutrition Situation. Nutrition Throughout the Life Cycle**. United Nations Administrative Committee on Coordination, Sub-committee on Nutrition in collaboration with International Food Policy Research Institute.

Ahmed F, Darnton-Hill I (2004). Vitamin A deficiency, 1st ed. In: Gibney MJ, Margetts BM, Kearney JM, Arab L, eds. **Public Health Nutrition**. Oxford: Blackwell Science.

Allen LH (2006). Causes of nutrition-related public health problems of preschool children: available diet. **Journal of Pediatric Gastroenterology and Nutrition** 43: 8S-12S.

Castenmiller JJM, West CE (1998). Bioavailability and bioconversion of carotenoids. **Annual Review of Nutrition** 18: 19-38.

De Groote H, Kimenju SC (2008). Comparing consumer preferences for color and nutritional quality in maize: Application of a semi-double bound logistic model on urban consumers in Kenya. **Food Policy** 33: 362-370.

De Groote H, Kimenju SC, Morawetz UB (2010). Estimating consumer willingness to pay for food quality with experimental auctions: the case of yellow versus fortified maize meal in Kenya. **Agricultural Economics** 41: 1-16.

Faber M, Wenhold F (2007). Nutrition in contemporary South Africa. **Water SA** 33: 393-400.

Food and Agriculture Organization of the United Nations (FAO) (1992). **Maize in Human Nutrition**. Rome, Italy.

Gibson RS (2005). **Principles of Nutritional Assessment**, 2nd ed. New York: Oxford University Press.

Grogan CO, Blessin CW (1968). Characterization of major carotenoids in yellow maize lines of differing pigment concentrations. **Crop Science** 8: 730-732.

Harjes CE, Rocheford TR, Bai L, Brutnell TP, Kandianis CB, Sowinski SG, Stapleton AE, Vallabhaneni R, Williams M, Wurtzel ET, Yan J, Buckler ES (2008). Natural genetic variation in lycopene epsilon cyclase tapped for maize biofortification. **Science** 319: 330-333.

HarvestPlus Brief (2006). **HarvestPlus: Breeding Crops For Better Nutrition**. Washington DC, USA.

Institute of Medicine (2000a). **Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids.** Food and Nutrition Board. Washington DC: National Academy Press.

International Institute of Tropical Agriculture (IITA) (2010a). **New varieties to boost maize output in West and Central Africa.** http://www.iita.org/maize/-/asset_publisher/jeR0/content/new-varieties-to-boost-maize (date accessed 22/11/2010).

Katz SH, Hediger M, Valleroy L (1974). Traditional maize processing techniques in the New world. **Science** 184: 765-773.

Labadarios D, Moodie IM, Van Rensburg A (2007). Vitamin A status. In: Labadarios D. **National Food Consumption Survey: Fortification Baseline**, Chapter 9B: South Africa 2005. Stellenbosch: Department of Health.

Labadarios D, Steyn N, Maunder E, Macintyre U, Swart R, Gericke G, Huskisson J, Dannhauser A, Vorster HH, Nesamvuni EA (2000). **The National Food Consumption Survey (NFCS): children aged 1-9 years, South Africa**, **1999**. Department of Health: Directorate of Nutrition, Pretoria.

Labadarios D, Steyn NP, Mgijima C, Dladla N (2005). Review of the South African nutrition policy 1994-2002 and targets for 2007: achievements and challenges. **Nutrition** 21: 100-108.

Labadarios D, Van Middelkoop AM (1995). **The South African Vitamin A Consultative Group. Children aged 6-71 months in South Africa, 1994: their anthropometric, vitamin A, iron and immunisation coverage status**. Isando, South Africa.

Li S, Tayie FAK, Young MF, Rocheford T, White WS (2007). Retention of provitamin A carotenoids in high β-carotene maize (Zea mays) during traditional African household processing. **Journal of Agricultural and Food Chemistry** 55: 10744-10750.

Lozano-Alejo N, Carrillo GV, Pixley K, Palacios-Rojas N (2007). Physical properties and carotenoid content of maize kernels and its nixtamalized snacks. **Innovative Food Science and Emerging Technologies** 8: 385-389.

Mayer JE, Pfeiffer WH, Beyer P (2008). Biofortified crops to alleviate micronutrient malnutrition. **Current Opinion in Plant Biology** 11: 166-170.

McCann JC (2005). **Maize and Grace: Africa's encounter with a New World Crop 1500-2000**, 1st ed. Cambridge: Harvard University Press.

Menkir A, Liu W, White WS, Maziya-Dixon B, Rocheford T (2008). Carotenoid diversity in tropical-adapted yellow maize inbred lines. **Food Chemistry** 109: 521-529.

Muzhingi T, Yeum KJ, Russell RM, Johnson EJ, Qin J, Tang G (2008a). Determination of carotenoids in yellow maize, the effects of saponification and food preparations.

Muzhingi T, Langyintuo AS, Malaba LC, Banziger M (2008b). Consumer acceptability of yellow maize products in Zimbabwe. **Food Policy** 33: 352-361. **International Journal for Vitamin and Nutrition Research** 78: 112-120.

Nestel P, Bouis HE, Meenakshi JV, Pfeiffer W (2006). Biofortification of staple food crops. **The Journal of Nutrition** 136: 1064-1067.

Nojilana B, Norman R, Bradshaw D, van Stuijvenberg ME, Dhansay MA, Labadarios D and the South African Comparative Risk Assessment Collaborating Group (2007). Estimating the burden of disease attributable to vitamin A deficiency in South Africa in 2000. **South African Medical Journal** 97: 748-753.

Nuss ET, Tanumihardjo SA (2010). Maize: a paramount staple crop in the context of global nutrition. **Comprehensive Reviews in Food Science and Food Safety** 9: 417-436.

Ortiz-Monasterio JI, Palacios-Rojas N, Meng E, Pixley K, Trethowan R, Pena RJ (2007). Enhancing the mineral and vitamin content of wheat and maize through plant breeding. **Journal of Cereal Science** 46: 293-307.

Raboy V, Below FE, Dickison DB (1989). Alteration of maize kernel phytic acid levels by recurrent selection for protein and oil. **Journal of Heredity** 80: 311-315.

Rodriguez-Amaya DB (1997). **Carotenoids and Food Preparation: The retention of Provitamin A carotenoids in prepared, processed, and stored foods**. Washington DC: OMNI/USAID.

Sommer A (1995). **Vitamin A deficiency and its consequences: a field guide to detection and control**, 3rd ed. Geneva, World Health Organization.

Sommer A, West KP (1996). **Vitamin A deficiency: health, survival and vision**. New York University Press.

Stevens R, Winter-Nelson A (2008). Consumer acceptance of provitamin A-biofortified maize in Maputo, Mozambique. **Food Policy** 33: 341-351.

Swart R, Sanders D, McLachlan M (2008). Nutrition: a primary health perspective. In: **South African Health Review**, Chapter 9: 129-147.

Tothova M, Meyers WH (2006). **Predicting the acceptance for high beta-carotene maize: an ex-ante estimation method**. American Agricultural Economics Association Annual Meeting, July 23-26, Long Beach, California.

Tschirley DL, Santos AP (1995). **Who eats yellow maize? Preliminary results of a survey of consumer maize preferences in Maputo, Mozambique**. MSU International Department of Agricultural Economics Development, Department of Economics Working Paper No 53, Michigan State University, East Lansing, Michigan

World Health Organization (WHO) (1995). **Global prevalence of vitamin A deficiency.** Micronutrient Deficiency Information System Working Paper 2. Geneva, World Health Organization.

World Health Organization (WHO) (2003). **GEMS/Food Regional Diets: regional per capita consumption of raw and semi-processed agricultural commodities, food safety department**. WHO Document Production Services, Geneva, Switzerland.

World Health Organization (WHO) (2009). **Global prevalence of vitamin A deficiency in populations at risk 1995-2005: WHO Global Database on Vitamin A Deficiency**. http://whqlibdoc.who.int/publications/2009/9789241598019_eng.pdf (date accessed 23/08/2010).

World Health Organization (WHO) (2010). **Micronutrient deficiencies: Vitamin A deficiency**. http://www.who.int/nutrition/topics/vad/en/index.html (date accessed 14/06/2010).

CHAPTER 2
LITERATURE REVIEW

This chapter reviews the chemistry of vitamin A, its food sources and role in health and human nutrition as well as vitamin A deficiency in South Africa. Biofortification and the potential of maize as a candidate crop for provitamin A biofortification, to alleviate vitamin A deficiency, are also reviewed. Published data and information on the effects of processing on the retention of provitamin A carotenoids in provitamin A-biofortified maize and the consumer acceptability of provitamin A-biofortified maize food products are also reviewed.

2.1   The chemistry of vitamin A, its food sources and role in health and human nutrition

2.1.1   Vitamin A chemistry

Vitamin A is classified as a fat soluble vitamin. The term vitamin A includes provitamin A carotenoids, which are dietary precursors of retinol (Figure 2.1) and retinoids, including its active metabolites (Institute of Medicine 2001, p83). Retinaldehyde and retinoic acid are the main physiologically active forms of vitamin A and are both derived from retinol (Bender 2003, p30). Free retinol is not chemically stable and is present in food in small amounts as a variety of esters, mainly retinyl palmitate (Bender 2003, p31). Retinoic acid has a lower potency than retinol or retinyl esters and cannot be reduced to retinaldehyde or retinol (Bender 2003, p33).
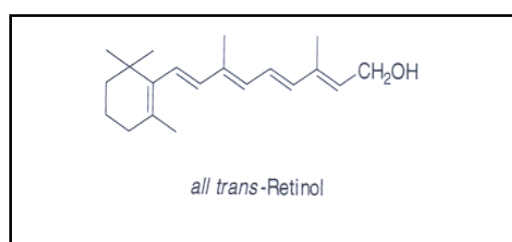
Figure 2.1   Structure of retinol

(Institute of Medicine 2001, p84)

Of the 600 known carotenoids, approximately 50 have vitamin A activity, but food composition data are available for only three of these carotenoids (α-carotene, β-carotene and β-cryptoxanthin) (Figure 2.2) (Institute of Medicine 2001, p83; Gregory 1996, p545). Alpha-carotene, β-carotene and β-cryptoxanthin are also called provitamin A carotenoids as they are

precursors of vitamin A that can be converted into retinol by the body (Institute of Medicine 2000a, p326).
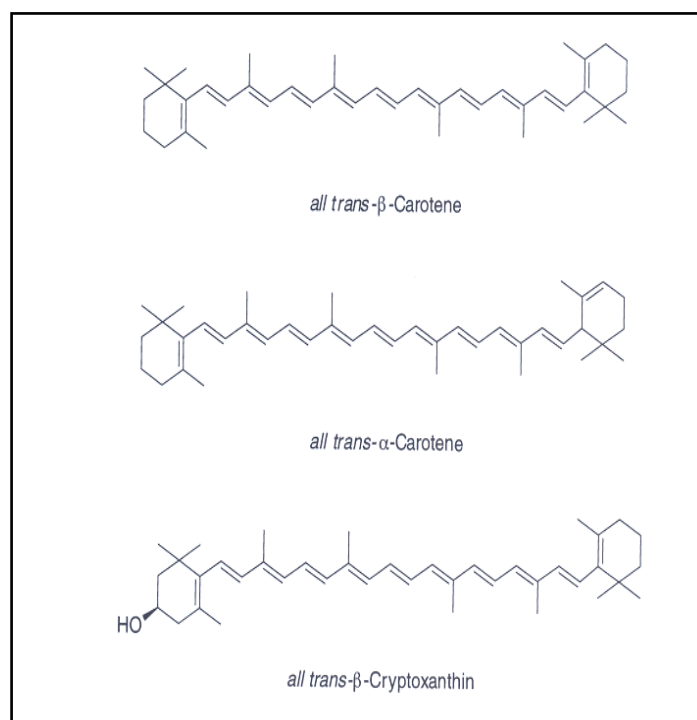
Figure 2.2   Structure of the provitamin A carotenoids
(Institute of Medicine 2001, p84)

Although many *cis* isomeric forms of each carotenoid exist, the all-*trans* isomers are the most common and stable (Institute of Medicine 2000a, p83). The all-*trans* isomers have the greatest vitamin A activity and are also the main forms of retinoids and carotenoids found naturally in foods (Gregory 1996, p546). Beta-carotene exhibits the greatest vitamin A activity of all the carotenoids. Two molecules of vitamin A can be produced from each molecule of dietary β-carotene (Gregory 1996, p546). The relative vitamin A activities of stereoisomeric forms of carotenes are shown in Table 2.1.

Table 2.1   Relative vitamin A activity of stereoisomeric

forms of carotenes (Gregory 1996, p548)

Compound and isomer	Relative vitamin A activity (%)
β-Carotene	
All-*trans*	100
9-*cis* (neo-U)	38
13-*cis* (neo-B)	53
α-Carotene	
All-*trans*	53
9-*cis* (neo-U)	13
13-*cis* (neo-B)	16

Through High-Performance Liquid Chromatography (HPLC), it has been shown that many foods contain a mixture of all-*trans* and *cis* isomers of retinoids and carotenoids. β-carotene and β-cryptoxanthin have been found to be the most abundant provitamin A carotenoids in provitamin A-biofortified maize, while α-carotene is present in much smaller quantities (Ortiz-Monasterio *et al* 2007).

2.1.2 Stability of provitamin A carotenoids

Provitamin A carotenoids can be easily destroyed by exposure to light, oxygen and prolonged processing (Rodriguez-Amaya 1997; Gregory 1996, pp545-546). The degradation of vitamin A (retinoids and provitamin A carotenoids) in food is similar to the oxidative degradation of unsaturated lipids. Vitamin A is degraded by the same factors that promote the oxidation of unsaturated lipids. This may occur by direct oxidation or indirect effects of free radicals. Carotenoid molecules are known to remain chemically intact during thermal processing but do undergo some isomerization. The conversion of all-*trans* forms of carotenoids to various *cis* isomers can result from thermal isomerization, exposure to light, acid, chlorinated solvents and dilute iodine (Gregory 1996, pp545-546).

2.1.3 Food sources of vitamin A

Vitamin A from foods of animal origin is present in the form of preformed vitamin A, mainly in the form of retinyl esters. Foods rich in vitamin A include: beef liver (10 503 µg Retinol Equivalents (RE)/100 g), butter (754 µgRE/100 g), cheddar cheese (390 µgRE/100 g), egg yolk (255 µgRE/100 g), full cream/whole milk (47 µgRE/100 g) and fish such as mackerel (130 µgRE/100 g), sardines (70 µgRE/100 g) and tuna (23 µgRE/100 g)

(Wolmarans *et al* 2010; Brody 1999, p554). However, liver oils of shark, halibut and polar bear are by far the richest sources of vitamin A (Brody 1999, p554). In addition, the preformed vitamin A found in foods of animal origin is the most bioavailable dietary source of vitamin A (Faber & Wenhold 2007). Provitamin A carotenoids are found in green, orange and yellow plant tissues (Combs 1998, p109). The richest sources of provitamin A carotenoids are dark green leafy vegetables such as spinach (819 μgRE/100 g), carrots (3250 μgRE/100 g), orange-fleshed sweet potatoes (2182 μgRE/100 g), mature squashes (396 μgRE/100 g) and pumpkin (213 μgRE/100 g) (Wolmarans *et al* 2010; Brody 1999, p554). Dark green leafy vegetables are regarded as the most common rich sources of provitamin A carotenoids among many developing countries while root crops such as carrot and yellow/orange sweet potato have the benefit of being available worldwide. Squashes and pumpkins are also regarded as being potentially important sources of provitamin A, given their long shelf-life and the fact that they are relatively easy to produce. However, the colour of fruit and vegetables is not a reliable indicator of the provitamin A carotenoid content (Ahmed & Darnton-Hill 2004, p193).

Although fruits have a lower provitamin A content than other plant sources, they have the benefit of being better accepted by both children and adults (Rodriguez-Amaya 1997). The richest plant source of provitamin A is crude red palm oil, an extract of the mesocarp of the oil palm *Elaeis guineensis* (Choo 1994; Rukmini 1994). A disadvantage of the red palm oil is that the carotenoids are destroyed when the oil is refined (Rodriguez-Amaya 1997). The conversion of provitamin A carotenoids to vitamin A is greater for ripe, coloured fruits and cooked, yellow tubers than equivalent amounts of dark, green leafy vegetables (Institute of Medicine 2001, pp82-83). Cereal grains are a poor source of provitamin A carotenoids, especially following the milling process (Ahmed & Darnton-Hill 2004, p193).

2.1.4 The role of vitamin A in health and human nutrition
Vitamin A is an essential component of human metabolism. This micronutrient is required for the normal functioning of the visual system, maintenance of cell function for growth, epithelial integrity, red blood cell production, immunity and reproduction. A deficiency of vitamin A can impact negatively on many body functions and overall health (WHO 2009). The 11-*cis*-retinaldehyde (retinal) form of vitamin A is needed for the visual cycle (Institute of Medicine 2001, p84). Retinoic acid has an important role to play in regulating the expression of various genes that encode for structural proteins, enzymes, extracellular matrix

proteins and retinol binding proteins and receptors. Retinoic acid is also essential for embryonic development and the maintenance of immune function (Institute of Medicine 2001, p85). Vitamin A activity is the only known function of carotenoids in humans (Institute of Medicine 2000a, p326). Although there is evidence that β-carotene and the other carotenoids have *in vitro* antioxidant activity, there is not enough evidence to suggest that they have *in vivo* antioxidant activity. As a result, β-carotene does not meet the criteria of a dietary antioxidant (Institute of Medicine 2000a, p43-44).

Vitamin A deficiency (VAD) is caused by a chronic inadequate dietary intake of vitamin A, which is insufficient to meet physiological needs, and leads to impaired tissue function (WHO 2009). In poorer households, the diet usually lacks the preformed vitamin A food sources which are expensive and inaccessible and is made up mostly of cheaper plant foods, with a reduced vitamin A bioavailability (Faber & Wenhold 2007; Ahmed & Darnton-Hill 2004, p201). In Africa, more than 80% of dietary intakes of vitamin A are from plant sources (WHO 1995). Populations in which the diets are of low quality due to lack of animal foods are more likely to have sub-clinical deficiency of vitamin A (serum retinol < 0.7 μmol/l). Inadequate breastfeeding, poor quality complementary feeding and a poor diet in childhood can also contribute to VAD in children (Ahmed & Darnton-Hill 2004, p201). The risk of vitamin A deficiency disorders is greatly increased with low intake of vitamin A during periods such as infancy, childhood, pregnancy and lactation, when requirement for the vitamin increases (WHO 2009).

Signs of VAD include xerophthalmia, anaemia and reduced immune function which can increase the severity of infection (WHO 2009). Xerophthalmia, which is the leading cause of preventable childhood blindness, is a collective term for all ocular manifestations of VAD. The earliest sign is night blindness, which can progress to structural eye damage resulting in impaired vision or irreversible blindness (WHO 2009; E-Siong 1995). The reduced immune system function and physiological changes that take place with VAD lead to an increase in morbidity and mortality throughout the life cycle (Ahmed & Darnton-Hill 2004, p196).

2.2 Vitamin A deficiency in South Africa

2.2.1 Vitamin A deficiency trends

The South African Vitamin A Consultative Group (SAVACG) study of 1994 was the first ever, truly representative national nutrition survey conducted in children under the age of six

years. About 3% of the sampled children had VAD (serum retinol < 10 μg/dL), while 39% of the children were found to have a marginal vitamin A status (serum retinol < 20 μg/dL), which was higher than the global average estimate of 21% for this age group. The following groups of children were found to be most affected: children in the age group of 36-47 months; children living in rural areas; children whose mothers were poorly educated. Some of the key recommendations of the SAVACG study (1994) were: a national high dose vitamin A capsule distribution programme for all children aged 6-71 months; lactating mothers receive a single high dose vitamin A supplement within the first month postpartum; investigate the feasibility of fortifying foods consumed in adequate amounts by children at risk of VAD (Labadarios & Van Middelkoop 1995). The findings of the National Food Consumption Survey (NFCS) (1999), which was conducted on South African children aged 1-9 years, supported the SAVACG (1995) study findings. The NFCS (1999) found that one of two children had a vitamin A intake of less than half the recommended level (Labadarios *et al* 2000).

The vitamin A status of children in South Africa was assessed again in the 2005 National Food Consumption Survey-Fortification Baseline (NFCS-FB). The NFCS-FB (2005) revealed that the vitamin A status of children has deteriorated significantly since 1994. Only 38% of children were found to have an adequate vitamin A status (serum retinol > 20 μg/dL). Approximately 15% of children were found to have severe VAD (serum retinol < 10 μg/dL). Inadequate vitamin A status was found to be similar across all age groups (1-9 years) with all provinces showing a ≥ 20% prevalence, making this a problem of severe public health significance (Labadarios *et al* 2007). The deterioration in the vitamin A status of children in South Africa as observed for the period 1994 to 2005 is of concern and has occurred despite the legislated fortification of bread flour and maize meal since 2003 and the national high-dose vitamin A supplementation programme, which was implemented in the majority of provinces since 2001 (Swart *et al* 2008, p133; Department of Health (DoH) 2003; DoH 2001). In 2000, approximately 3069 deaths in children aged 0-4 years (3.2% of all deaths in this age group) were attributed to vitamin A deficiency (Nojilana *et al* 2007).

Although national data exists on the vitamin A status of South African children, there are no available national data on the vitamin A status of South African adults (Steyn *et al* 2006). Local studies conducted in South Africa have shown high prevalence of VAD among Human Immunodeficiency Virus (HIV)-infected adults. Kennedy-Oji *et al* (2001) reported

rates of VAD and severe VAD of 66% and 37% respectively, among a cohort of HIV-infected women. Visser *et al* (2003) showed an overall prevalence of low plasma retinol concentrations in 52% of HIV-infected adults. In 2000, VAD accounted for approximately 11% of all maternal mortality (222 maternal deaths) in South Africa (Nojilana *et al* 2007).

2.2.2 Vulnerable groups

Children of preschool age are vulnerable to VAD because of the higher requirements for vitamin A needed for growth, low intake of vitamin A and increased exposure to infections. Children with measles, acute or prolonged diarrhoea, acute lower respiratory infection, severe protein energy malnutrition (PEM), from poor communities and non-breastfed infants are also more vulnerable to VAD (Ahmed & Darnton-Hill 2004, pp192-215). Pregnant and lactating women from poor socioeconomic backgrounds are also at risk for VAD as they may be unable to meet the increased requirements for vitamin A (Ahmed & Darnton-Hill 2004, pp192-215). In South Africa children between the ages of 6-59 months and from rural areas are the most at risk of VAD and experience more severe effects than any other age group (DoH 2001; Labadarios & Van Middelkoop 1995).

2.2.3 Strategies to address vitamin A deficiency in South Africa

The Integrated Nutrition Programme (INP) was initiated by the South African Department of Health in 1995. The aim of the INP is to ensure optimum nutrition for all South Africans by preventing and managing malnutrition (DoH 1999). The elimination of micronutrient deficiencies is the key objective within the focus area of micronutrient malnutrition control. The combination of strategies to address micronutrient deficiencies in the population include: supplementation, food fortification, promotion of dietary diversification as well as other related public health measures (Labadarios *et al* 2005). These strategies are briefly discussed in the next section.

2.2.3.1 Supplementation

The South African DoH implemented a vitamin A supplementation programme in 2002 as a primary prevention strategy which forms part of the routine immunization program, maternal health and the integrated management of childhood illnesses (Labadarios *et al* 2005). The programme involves providing vitamin A supplementation to children aged 6-59 months and women in the post partum period (DoH 2001). Various studies have evaluated the vitamin A supplementation programme in South Africa. Du Plessis *et al* (2007)

evaluated the programme in the Western Cape and reported many missed opportunities, poor recording of the dispensed doses on the Road-to-Health cards and a poor awareness of the programme among mothers. Hendricks *et al* (2007) also reported similar observations such as missed opportunities for supplementation, lack of awareness by mothers and a need for training of nurses (Hendricks *et al* 2007). The NFCS-FB (2005) concluded that the high-dose vitamin A supplementation programme had not been successful as it had been unable to reach the most vulnerable target groups (Swart *et al* 2007). It was recommended that the programme should be optimized in order to achieve maximum impact and that the efficiency, effectiveness and safety of the programme be evaluated by the relevant stakeholders (Dhansay 2007).

2.2.3.2 Food fortification

One of the recommendations from the NFCS (1999) was that maize be used as a food vehicle for fortification as it was found to be the most commonly consumed food item amongst children aged 1-9 years (Labadarios *et al* 2000). The fortification of two staple foods, namely maize meal and wheat flour with vitamin A, thiamin, niacin, pyridoxine, folate, riboflavin, iron and zinc, was legislated in South Africa in October 2003 (Labadarios *et al* 2005). Although the fortification of maize meal and wheat flour was implemented to address micronutrient deficiencies in vulnerable groups, the accessibility of these commercially fortified foods to poor people in rural areas is questionable (Faber & Wenhold 2007; Nestel *et al* 2006). According to Steyn *et al* (2006), it is unlikely that food fortification will make up for an inadequate dietary intake especially in children who are unable to consume large portions of fortified staple foods at a time. The success of the food fortification programme is also questionable given the deterioration in the vitamin A status of children from 1994 to 2005 (Swart *et al* 2008).

2.2.3.3 Dietary diversification/modification

Dietary diversification/modification refers to a variety of strategies that aim to increase the production, availability and access to foods rich in micronutrients. It also aims to increase the consumption of foods rich in micronutrients and/or the bioavailability of micronutrients from the diet. Dietary modification is a long-term strategy that can be achieved through horticultural approaches such as home gardens, behavioural change and improved methods of food preparation and preservation that minimizes the loss of micronutrients (Ruel 2001). Diversification of crops, introduction of new crops and the promotion of indigenous foods

are also strategies that can be used to address micronutrient malnutrition (Faber & Wenhold 2007). Although dietary diversification may be a desirable way of preventing micronutrient malnutrition, poverty and a lack of access to a variety of foods prevents it from being successful (Mayer *et al* 2008).

From the literature reviewed it is clear that the existing strategies that have been implemented to combat VAD in South Africa have not been successful. New strategies together with existing strategies from the National Department of Health may improve the vitamin A status of vulnerable population groups in South Africa. Biofortification could succeed as a new and alternative way of dealing with the problem of VAD (De Groote & Kimenju 2008; Mayer *et al* 2008).

2.3   Biofortification as a strategy to combat micronutrient deficiency

2.3.1 Current biofortification initiatives

Biofortification involves breeding staple crops for increased vitamin and mineral content using the best traditional breeding practices and modern biotechnology (De Groote & Kimenju 2008; Nestel *et al* 2006). Currently, breeding of crops with better nutrition is being led by the HarvestPlus Challenge Program. HarvestPlus is the biofortification initiative within the Consultative Group on International Agricultural Research (CGIAR). HarvestPlus has put together a scientific team from around the world to breed and disseminate crops for better nutrition (HarvestPlus Brief 2006). HarvestPlus aims to improve food security and enhance the quality of life by reducing micronutrient malnutrition among less privileged populations in Africa, Asia and Latin America. Although other micronutrients may be added in the future, HarvestPlus currently focusses on iron, zinc and provitamin A, which are three micronutrients that have been identified as limiting by the WHO (Ortiz-Monasterio *et al* 2007). Comprehensive plant breeding programmes are currently in place for six staple crops, for which feasibility studies have already been completed. These include rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), cassava (*Manihot esculenta* Crantz), orange-fleshed sweet potato [*Ipomoea batatas* (L.) Lam.] and common beans (*Phaseolus vulgaris* L.) (HarvestPlus Brief 2006).

2.3.2 Advantages of biofortification as an alternative strategy to alleviate vitamin A deficiency

One of the most important advantages of biofortification is that it is cost-effective. After the plants have been developed and grown by farmers there are no annual costs associated with the purchasing and addition of fortificants to the food supply (Bouis 2002). A second advantage of biofortified crops are their sustainability. After the initial investment of developing and disseminating the nutritionally improved crops, the recurrent costs are low and the germplasm can be shared worldwide (Nestel *et al* 2006). A third advantage of biofortification is that it takes advantage of the fact that staple crops are a predominant part of the diets of poor populations who have VAD or are at risk of VAD. There is also a consistent and large amount of staple foods consumed by all family members in poor households at risk of VAD (Bouis 2003). As a result this approach relies on existing consumer behaviour and no behaviour modification on the part of the consumer is needed (Bouis 2003; Bouis 1999). Fourth, biofortified crops may be more accessible to poor populations at risk of poor nutritional status living in remote and rural areas. Biofortification can deliver naturally fortified foods to people who may not have access to commercial fortified foods that are more readily available in urban areas (Nestel *et al* 2006; Bouis 2003). Biofortification and commercial fortification can therefore be regarded as complementary strategies to address micronutrient malnutrition (Bouis 2003). A fifth advantage of biofortification is that there is less risk of vitamin A toxicity from biofortification as compared to excessive consumption of fortified foods and massive doses of vitamin A supplements, as the conversion of carotenoids into vitamin A in the body is controlled and regulated (Penniston & Tanumihardjo 2006). The potential of maize as a candidate for biofortifiaction with provitamin A to alleviate VAD in Africa, including South Africa is evaluated in the next section.

2.3.3 The potential of maize as a candidate for biofortification with provitamin A to alleviate vitamin A deficiency in Africa, including South Africa

Maize (*Zea mays*) is the most widely grown cereal worldwide and is cultivated across a range of latitudes, altitudes, moisture conditions, slopes and soil types, using the simplest to the most sophisticated technologies (Smale & Jayne 2003). Maize makes a substantial contribution to the total worldwide cereal grain production and has an important place in the world economy and trade as a food, feed and an industrial grain crop (Vasal 2001). The six major types of corn kernels include: dent (indentation on the top of the kernel), flint, flour,

sweet, popcorn and pod corns. Differences between the types are based on quality, quantity and pattern of endosperm composition (Johnson 2000, p34). The dent, white maize is widely used for human consumption [Food and Agriculture Organization of the United Nations (FAO) 1992].

2.3.3.1History of maize in Africa and its importance as a staple crop

The historical evidence for the arrival of maize in Africa is vague. Initially maize was not a major staple crop in Africa, but provided a supporting role alongside older staple crops or new crops such as cassava, potatoes, beans, sweet potatoes, tobacco and squash (McCann 2005, pp95-96). Jan Van Riebeeck did not report seeing maize when he arrived in the Cape in 1652 but by 1658 he had recommended the sowing of maize brought from West Africa's Guinea Coast to the first generation of Dutch farmers.

The African continent depends on white maize as a food source more than any other continent (McCann 2005, p1). South Africa is one of the top 20 producers of maize worldwide and is the only country to have an explicit policy objective to export white maize (FAO 2008; FAO/CIMMYT 1997, p9). According to the WHO (2003), the average total cereal consumption in the African diet is 291.7 g/person/day with an average maize consumption of 106.2 g/person/day. This average per capita maize consumption is far greater than the calculated values from other maize-consuming regions such as Europe, Far East, Latin America or Middle East (WHO 2003). In Africa, the sub-Saharan Africa region is a leader in maize consumption (IITA 2010a). In South Africa, maize contributes about 40% of total energy intake (McCann 2005, p9). African maize consumption as a percentage of national diet is shown in Figure 2.3. The NFCS (1999) reported that maize was one of the most commonly consumed food items and it also recommended that maize be used as a food vehicle for micronutrient fortification (Labadarios *et al* 2000). In South Africa yellow maize is not readily consumed by humans, except when there is a severe shortage of white maize (Tothova & Meyers 2006).

Maize is consumed in several food forms. Some of the traditional foods made from maize are breads, porridges, steamed and roasted products, beverages and snacks. These foods are prepared using whole kernels, kernels without pericarp, milled germ and endosperm (Ortiz-Monasterio *et al* 2007). Fresh or fermented maize porridges are widely consumed in Africa

(Ortiz-Monasterio *et al* 2007). The processing of maize into various food forms is reviewed in section 2.3.4.1.
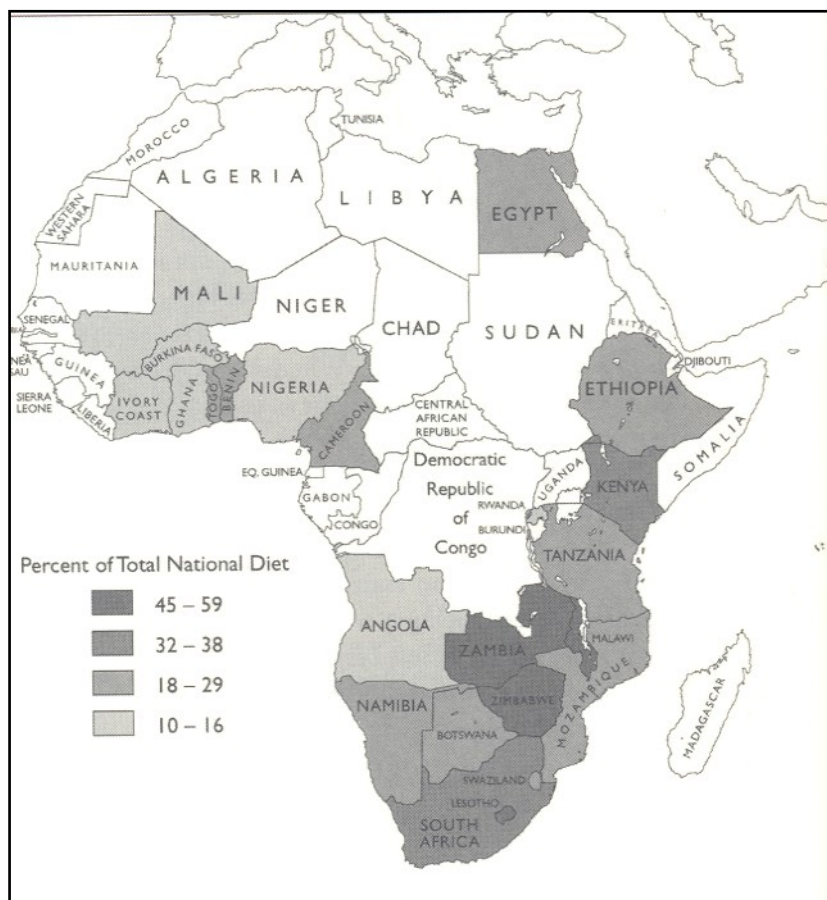
Figure 2.3   African maize consumption as a percentage of national diet, 2002 (McCann 2000, p10)

2.3.3.2 Anatomical structure, chemical composition and nutritional value of maize

Maize is a cross pollinating species with the female (silk) and male (tassel) flowers located separately on the plant. Maize kernels develop in the ear and each ear may hold between 300-1000 single kernels weighing between 19 to 40 g per 100 kernels. Figure 2.4 shows the maize kernel which is made up of the following major anatomical structures: the pericarp (hull or bran) (6% of kernel weight); the germ or embryo (11% of kernel weight); the endosperm (83% of kernel weight) (Johnson 2000, p34; FAO 1992).
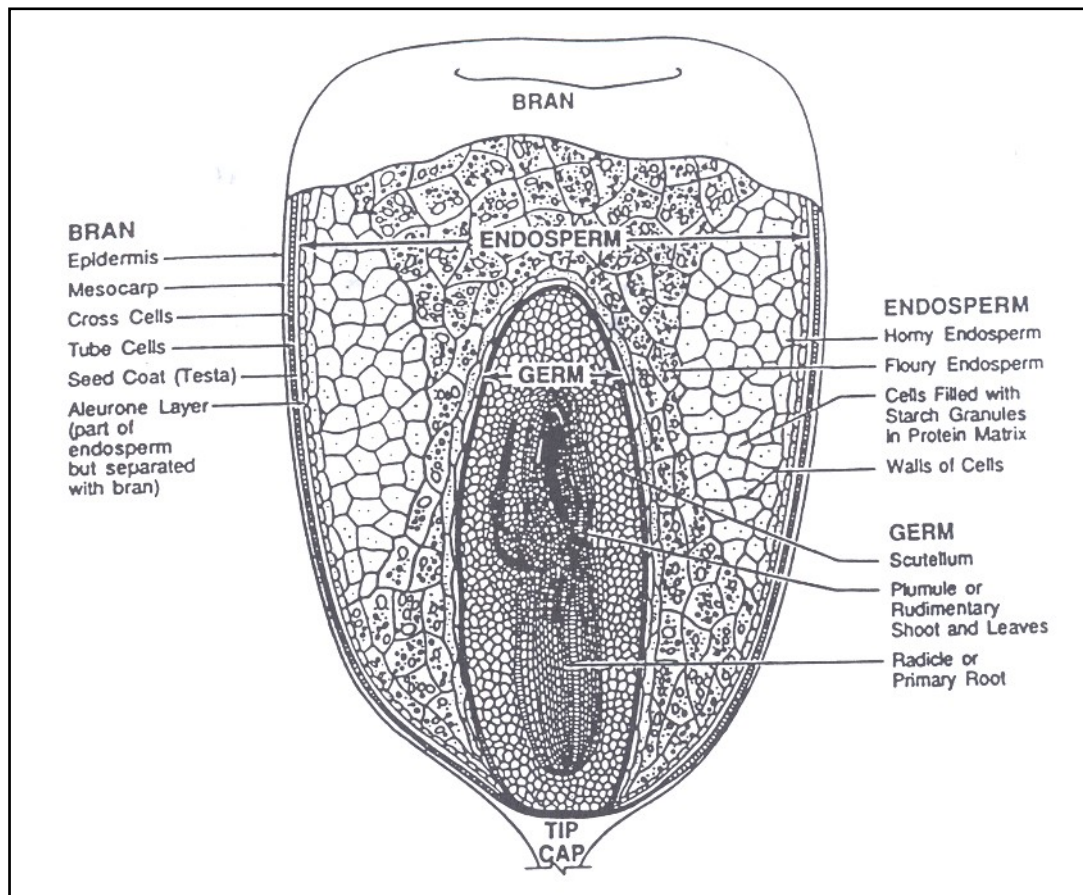
Figure 2.4   Structure of the maize kernel (Johnson 2000, p34)

The chemical composition of maize is known to vary greatly due to genetic make-up, environmental factors and agronomic practices, which may also influence the weight distribution and individual chemical composition of the endosperm, germ and bran of the kernel (FAO 1992). Table 2.2 shows the chemical composition of normal (dent) white maize.

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

Component	Normal Dent
Starch (%)	71.3
Protein, N x 6.25 (%)	8.7
Fat (%)	4.1
Fiber (%)	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, fat, fiber and ash
[b] Kazarian & Hall (1965)
[c] Keener *et al* (1985)

Starch is the major chemical component of the maize kernel and is concentrated in the endosperm. Both types of starch: amylose, a linear glucose polymer and amylopectin, a branched glucose polymer, are both present in maize. Other carbohydrates present include simple sugars such as glucose, sucrose and fructose that vary from 1-3% of the kernel. Protein is the next largest chemical component of the kernel and is mostly found in the endosperm of the kernel. Recent studies have reported protein levels of between 8.92-10.52% in white maize (Machida *et al* 2010). The normal white maize lacks the amino acids lysine and tryptophan but has a high leucine content. The oil content of maize also varies depending on the variety and comes mainly from the germ. Maize oil has a low level of saturated fatty acids and a relatively high level of polyunsaturated fatty acids, mainly linoleic acid. Dietary fibre is the chemical component found in greatest amounts in maize after carbohydrates, proteins and fats. Insoluble fibre makes a greater contribution to the total dietary fibre content as compared to soluble fibre. Phosphorus is the most abundant mineral found in maize while calcium and trace minerals are found in low amounts (FAO 1992).

The B-vitamins are the main water-soluble vitamins in the maize kernel. Thiamin, riboflavin and niacin are found in varying amounts depending on variety, while choline, folic acid and pantothenic acid are found in low amounts. Maize does not contain vitamin B_{12} and contains only small amounts of ascorbic acid in the mature kernel (FAO 1992). Fibre, minerals and B-vitamins are concentrated in the outer layers, mainly the pericarp and aleurone layer, of the maize kernel. Provitamin A carotenoids and vitamin E are the two fat-soluble vitamins found in the maize kernel. Provitamin A carotenoids are found in varying amounts in yellow/orange maize, while white maize has little or no carotenoids. The hard endosperm of the kernel contains most of the carotenoids, while small amounts can be found in the germ (FAO 1992).

While the chemical composition of normal (white) maize is widely documented, that of yellow/orange maize is lacking. Apart from the reported higher provitamin A levels in the provitamin A-biofortified maize varieties, data on other nutrients are scarce (Ortiz-Monasterio *et al* 2007; Oikeh *et al* 2004). Oikeh *et al* (2004) reported the iron and zinc concentrations of 19 white maize varieties and one yellow maize variety, with beta-carotene, which were all grown in the same environment. The results showed that the yellow maize variety had higher concentrations of both iron (2.05 mg/100 g) and zinc (2.12 mg/100 g) than almost all the white maize varieties (iron and zinc concentrations ranged from 1.69-2.07 mg/100 g and 1.85-2.04 mg/100 g, respectively). In a review paper, Ortiz-Monasterio *et al* (2007) reported the iron and zinc concentrations in provitamin A-biofortified maize samples from the International Maize and Wheat Improvement Center (CIMMYT). According to these authors, the iron and zinc content of the provitamin A-biofortified maize samples ranged from 1.1-3.9 mg/100 g and 1.5-4.7 mg/100 g, respectively. However, the authors did not include iron and zinc values of white maize varieties grown under the same conditions as the biofortified maize varieties to serve as controls. The provitamin A-biofortified maize may have a different nutritional composition, other than a higher provitamin A content, when compared with white maize due to genetic factors. Differences in nutritional composition have already been reported in low phytic acid maize varieties (Raboy *et al* 1989). The nutritional composition of provitamin A-biofortified maize compared to white maize needs to be subjected to a rigorous scientific study.

The review of the chemical composition of maize shows that, in terms of nutritional value, white maize is an important source of dietary energy. Although maize is an important source of protein in the African diet, the quality of the maize protein is poor due to deficiencies in

some essential amino acids, such as lysine and tryptophan. Maize is a significant source of fibre and micronutrients, particularly B-vitamins and some minerals, such as phosphorus. Maize is a poor source of dietary minerals such as zinc and iron due to the fact that these minerals are bound by antinutritional factors such as phytic acid and phenolic compounds (Hotz & Brown 2004; Gibson 1994). Furthermore, the nutritional value of white maize is limited by its lack of vitamin A.

Because maize is widely consumed as a staple in developing countries, including most of the African countries, and is known to have limited nutritional value, many attempts are being made to improve its nutritional value. These efforts include genetic manipulation, processing and fortification (FAO 1992). Provitamin A-biofortified varieties are being produced using traditional plant breeding techniques. These varieties have a yellow to orange colour which indicates the presence of carotenoid pigments, not necessarily provitamin A. The provitamin A content of the yellow provitamin A-biofortified maize varieties have been found to range from 0.25 to 2.5 µg/g dry weight (DW) while deep yellow or orange varieties contain 15 µg/g DW of provitamin A (Nuss & Tanumihardjo 2010). The provitamin A content of the biofortified maize varieties was found to be influenced by genetic factors, although published data are limited. The current breeding target for maize as set by HarvestPlus is 15 µg/g DW of provitamin A (Ortiz-Monasterio *et al* 2007). There may be a need to review this target as more published data on provitamin A losses during processing of maize into food products become available. Although there are no published data on efficacy trials using provitamin A-biofortified maize in humans, animal studies have shown that provitamin-biofortified maize has the potential to positively alter or maintain vitamin A status (Howe & Tanumihardjo 2006a).

HarvestPlus has identified maize as the target crop for biofortification to alleviate VAD in Zambia. It is hoped that if the maize biofortification programme is successful in Zambia, it will be implemented in other African countries, including South Africa. On the other hand, the orange fleshed sweet potato (OFSP) is a biofortified crop that has been promoted in various provinces of South Africa to address VAD. The Agricultural Research Council (ARC)-Vegetable and Ornamental Plant Institute (VOPI) of South Africa have established both short-term and long-term vegetable garden projects using OFSP in various parts of the country based on the approach that was used in the Ndunakazi project in KwaZulu-Natal, South Africa (Laurie 2007). A randomized controlled trial showed that feeding 125 g of the

cooked Resisto variety of OFSP to South African primary school children improved liver stores of vitamin A after a period of 53 school days compared to the control group, which consumed the white variety of sweet potato (Van Jaarsveld *et al* 2005). However, maize is the predominant staple crop for the majority of South Africans, including the poor communities who are affected by or at risk of VAD. Thus it seems appropriate that attention be shifted to biofortification of maize with provitamin A to alleviate VAD in South Africa. However, the possible challenges and/or disadvantages associated with biofortification of crops needs to be assessed and addressed; these are reviewed in the sections that follow.

2.3.4   Possible disadvantages or challenges of biofortification as an alternative strategy   to alleviate vitamin A deficiency

2.3.4.1 Possible effects of processing on the retention of provitamin A carotenoids in provitamin A-biofortified maize

Maize kernels are processed, prepared and consumed in many different ways worldwide (Nuss & Tanumihardjo 2010; Johnson 2000, pp40-45). The three major processing methods that are used to produce food products from maize are dry milling, wet milling and the distilling process for beverage alcohol. During these processes 65-70% of the maize is converted to primary end products (Wright 1987, pp456-457). Dry milling is a common maize processing technology and is used worldwide. Dry milling is carried out to separate the kernel into its anatomical parts (endosperm, bran and germ). Degerming and nondegerming are the two different systems used to dry mill maize. The nondegerming system grinds maize into meal with little or no separation of germ while the degerming system involves separation of the germ and hull from the rest of the kernel (Johnson 2000, p48). Because some nutrients are located in the germ and outer layers of the maize kernel, it is likely that the process of dry milling maize will result in a loss of certain nutrients, including provitamin A carotenoids. This is an important aspect to investigate further as the loss of nutrients that take place with dry milling can impact the overall nutritional value of the provitamin A-biofortified maize. The main products derived from dry milling are maize grits, maize meals and maize flours (Alexander 1987, pp353-355). In Southern Africa, including South Africa, the common products of dry milling of maize are maize flour (mealie meal) of different particle size and refinement; maize grits; samp and mealie rice. These milled products are processed using different methods into several food products, including cooked samp and mealie rice; fermented and non-fermented porridges (of different consistency), gruels and beverages. Wet cooking is a common thermal processing method used to prepare maize food products. In

KwaZulu-Natal province, South Africa, the popular maize foods among the Black African population are stiff porridge (*phutu*), thin porridge (*iphalishi*) and samp (*isitampu*) (Faber & Kruger 2005; Faber 2004; Faber *et al* 2001; Faber *et al* 1999). In terms of home processing, it is known that provitamin A carotenoid losses increase as a result of microwaving, steaming, boiling and sautéing. Substantial losses occur from deep-frying, prolonged cooking, combination of several preparation/processing methods, baking and pickling (Rodriguez-Amaya 1997). Although limited, published data indicate that significant amounts of provitamin A may be lost during processing of provitamin A-biofortified crops, including maize, which is reviewed in the next section.

2.3.4.2 Studies conducted to investigate the retention of provitamin A carotenoids in provitamin A-biofortified crops

Van Jaarsveld *et al* (2006) determined the retention of β-carotene in boiled, mashed orange-fleshed sweet potato (OFSP) under home-cooking and institutional-cooking conditions. Although retention of trans-β-carotene in OFSP varied with cooking conditions, overall, the trans-β-carotene content of boiled, mashed sweet potato was still substantial with retention ranging from 83% to 92%. Li *et al* (2007) investigated the retention of the major provitamin A carotenoids, α-carotene, β-carotene and β-cryptoxanthin, in high β-carotene maize during traditional processing of a fermented African porridge. The major provitamin A carotenoid found in the maize was all-*trans* β-carotene with the two prominent *cis* isomers of β-carotene being 9-*cis* and 13-*cis*. The cumulative losses of β-carotene in the final, cooked products were 24.5% and 24.8% for the fermented and unfermented porridges, respectively. This suggests that traditional fermentation does not adversely affect the retention of provitamin A carotenoids in high β-carotene maize porridges. Higher losses during the cooking of the unfermented porridge were observed for all carotenoids (Li *et al* 2007). Muzhingi *et al* (2008a) investigated the effect of cooking on the carotenoid content of raw, uncooked yellow maize flour. The cooking of *Sadza* (dumpling), porridge and *Mangai* (snack) resulted in an increase in the carotenoid levels, while muffin preparation resulted in a decrease in carotenoid levels (Muzhingi *et al* 2008a). No studies on the retention of provitamin A carotenoids during the milling of provitamin A-biofortified maize could be found. The retention of provitamin A carotenoids in provitamin A-biofortified maize may vary with the milled product. Lozano-Alejo *et al* (2007) investigated the losses of carotenoids following *nixtamilization* and frying, which are common processes used in Mexico to prepare maize food products. The average loss of provitamin A following *nixtamilization* and subsequent snack preparation by deep-

frying was found to be 36%, with the resulting snacks brighter in colour compared to the original kernels (Lozano-Alejo *et al* 2007).

The foregoing review indicates that provitamin A losses may occur when maize is processed using different methods. It is crucial to consider and quantify the losses of provitamin A carotenoids, due to processing and food preparation. These losses must be taken into account by biofortification programmes when setting breeding targets, in order to further support the feasibility of using provitamin A-biofortified maize as a means to alleviate VAD (Li *et al* 2007; Lozano-Alejo *et al* 2007). Because the levels of provitamin A losses seem to vary according to the processing and preparation methods used, the provitamin A losses during the processing of maize into popular maize products for a distinct population and culture should be taken into account. There is also a paucity of data on the effect of maize variety on the retention of provitamin A carotenoids (Muzhingi *et al* 2008a), and this also requires investigation. Research results may indicate the need to screen for provitamin A-biofortified maize varieties with good provitamin A retention during processing.

2.3.4.3 Consumer acceptability of provitamin A-biofortified maize food products

Background

In Africa, white maize is produced and sold by farmers and this is also preferred by consumers. The preference for white maize by African consumers as their staple crop can be traced back to the 1920s and 1930s. Yellow maize is preferred in live-stock feeding as the yellow maize gives egg yolks, poultry meat and animal fat a yellowish tinge which is preferred by consumers of these products in many cultures (McCann 2005, pp111-113). As mentioned earlier, increasing the provitamin A content of maize through breeding changes the colour of maize to yellow/orange and can also change other characteristics of the maize, such as flavour and aroma. The consumer's reactions to these traits may act as a deterrent to consumer acceptability of provitamin A-biofortified maize and related food products (Stevens & Winter-Nelson 2008). Although no in-depth studies on consumer acceptability of provitamin A-biofortified maize have been conducted in South Africa, studies have already been conducted in other African countries such as Zimbabwe, Kenya, Zambia and Mozambique.

Studies on consumer acceptability of provitamin A – biofortified maize products

In Zimbabwe yellow maize is thought to have little or no human consumption demand (Rubey 1993). Previously, production was limited to large-scale commercial farmers for stock feed and was only considered for human consumption when domestic production of white maize was inadequate or could not be procured on the international markets (Byerlee & Eicher 2001). Although the Zimbabwean consumer's preference for yellow maize is unknown there is potential for stimulating its production and consumption as maize is a staple food crop (Muzhingi *et al* 2008b). In Zimbabwe the main source of supply of yellow maize is through imported food aid which has two negative associations for consumers. Firstly, it is considered a "poor man's" grain and inferior to white maize. Secondly yellow maize undergoes chemical changes resulting in unacceptable organoleptic properties, if poorly handled during importation (Muzhingi *et al* 2008b). A study conducted by Muzhingi *et al* (2008b) on consumer acceptability of yellow maize in urban and rural Zimbabwe found that more than 94% of households were willing to consume yellow maize if they knew it was more nutritious than white maize. However, only 2% of households had some knowledge about the nutritional qualities of yellow maize. Although more than 50% of respondents liked the taste of the yellow maize, almost a third disliked the smell. The overall preference for white maize was based on its visual appeal. Results also suggested that male-headed households were more likely to consume yellow maize products compared to female-headed households in order to save money on food expenditure. Economic factors may influence the preference of yellow maize over white in Zimbabwe, as yellow maize is cheaper than white maize and is likely to be readily available through government intervention, if domestic production is inadequate (Muzhingi *et al* 2008b).

In Kenya, most of the maize that is consumed is white (FAO/CIMMYT 1997). Studies conducted on urban consumers have confirmed that white maize is preferred by Kenyans, but there is a preference for yellow maize in some parts of Kenya (De Groote *et al* 2010; De Groote & Kimenju 2008). Consumers with a higher education seem to prefer white maize while ethnic background also plays a role in preference (De Groote & Kimenju 2008). De Groote *et al* (2010) concluded that Kenyans were more interested in commercially fortified maize and would buy yellow maize only at a discount of 11%. Poor acceptance of yellow maize in Kenya seems to come from prejudice and negative associations, such as food aid and animal feed, rather than from sensory characteristics such as taste. Although the Kenyan studies have been conducted in urban areas, it is recommended that these types of studies be

extended to rural areas where large numbers of poor maize consumers reside (De Groote *et al* 2010; De Groote & Kimenju 2008).

White maize is also the most common staple crop produced and consumed in Mozambique (Stevens & Winter-Nelson 2008). Earlier studies in Mozambique indicated that white maize was preferred over yellow maize; however, poorer consumers were more willing to purchase yellow maize if it was offered at a discounted price (Tschirley & Santos 1995). The recent success with introducing the OFSP in Mozambique does suggest that consumers may be willing to change their eating habits in terms of food appearance, with appropriate education initiatives (Low *et al* 2007). The market survey conducted by Stevens & Winter-Nelson (2008) found that many participants had a favourable response to the orange maize. The appearance of the orange maize was rated lower by men compared to women. Although participants preferred the appearance of the white maize over the yellow maize, participants preferred the aroma of the orange maize over the white maize, which may increase the chances of acceptance. Results from Mozambique suggests that provitamin A-biofortified maize may be a self-targeting nutritional intervention, as those who are most vulnerable to VAD were the most likely to accept the orange maize (Stevens & Winter-Nelson 2008).

Although the consumer acceptability studies in Mozambique showed a favourable response to yellow maize, studies in Zimbabwe and Kenya have showed a definite preference for white maize over yellow maize (De Groote & Kimenju 2008; Muzhingi *et al* 2008b; Stevens & Winter-Nelson 2008). Both Muzhingi *et al* (2008b) and De Groote & Kimenju (2008) concluded that substantial efforts would be needed to make provitamin A-biofortified maize products more acceptable to urban consumers in Zimbabwe and Kenya. These efforts would include nutrition education and awareness programmes on the nutritional value of yellow maize as well as a reduction in price to make it more affordable and appealing to the poorer consumers (De Groote & Kimenju 2008; Muzhingi *et al* 2008b). It is clear from this review that the biofortification process may produce changes in the sensory properties of yellow maize, making it less acceptable compared to white maize. Provitamin A-biofortified maize can only reach its full potential if it is bred with features that are more acceptable to consumers (Stevens & Winter-Nelson 2008). These features should be clearly identified to improve the sensory quality of provitamin A-biofortified maize varieties for the target consumers.

Other possible factors affecting consumer acceptance of provitamin A-biofortified maize

Consumer acceptability studies on provitamin A-biofortified maize in Africa have thus far only been conducted on adult consumers, mostly living in urban areas. However, urban consumers are not the primary target of biofortified maize, so it is important to extend the consumer acceptability studies to rural areas (De Groote & Kimenju 2008). Given the fact that food preferences may change with age and gender (Cooke & Wardle 2005), it may be useful to conduct consumer acceptability studies on consumers of both genders and across a range of age groups.

2.4   Conclusions

Vitamin A deficiency (VAD) which is caused by a chronic inadequate dietary intake of vitamin A is a major public health problem in many developing countries. In South Africa, VAD continues to be a serious public health problem despite implemented strategies to alleviate VAD. Provitamin A-biofortified maize, which has been developed through plant breeding, has the potential to act as an additional strategy to eliminate VAD. Although provitamin A-biofortified maize is known to have a higher provitamin A content compared to white maize, the nutritional composition of provitamin A-biofortified maize compared to that of white maize, with regards to nutrients other than provitamin A, has not been thoroughly investigated. The influence of genetic factors (maize variety) on the nutritional composition of the provitamin A-biofortified maize in terms of nutrients other than provitamin A is not known. The limited research findings indicate that provitamin A carotenoids are lost during the processing of biofortified crops. The findings suggest that the extent of the loss of provitamin A carotenoids is influenced by the method of food processing. More research needs to be conducted to quantify the loss of provitamin A carotenoids that occurs during processing of provitamin A-biofortified maize, including milling and cooking. Findings from these studies should be communicated to and considered by maize biofortification projects, when setting breeding targets. Currently, there are no published data on provitamin A losses during the processing of provitamin A-biofortified maize into popular South African foods.

The feasibility of using provitamin A-biofortified maize to alleviate VAD is critically dependent on consumer acceptance of the provitamin A-biofortified maize. The yellow/orange provitamin A-biofortified maize needs to be widely accepted by African consumers who are vulnerable to VAD, and are traditionally consumers of white maize. The limited studies on consumer acceptability of provitamin A-biofortified maize carried out in

Africa indicate that, generally, yellow/orange maize is less acceptable and preferred relative to white maize. However, these studies have not investigated whether there are differences in consumer acceptability amongst consumers of different age and gender. Food acceptance and preference may vary with gender and age group. Data on consumer acceptability of provitamin A-biofortified maize varieties and their food products from consumers of different age and gender would be useful. It could help to identify the traits that the breeders need to focus on, in order to make the biofortified maize more acceptable. There is a lack of data on the consumer acceptability of provitamin A-biofortified maize in South Africa and this requires further investigation.

References

Ahmed F, Darnton-Hill I (2004). Vitamin A deficiency, 1st ed. In: Gibney MJ, Margetts BM, Kearney JM, Arab L, eds. **Public Health Nutrition**. Oxford: Blackwell Science.

Alexander RJ (1987). Corn Dry Milling: Processes, Products and Application, 1st ed. In: Watson SA, Ramstad PE, eds. **Corn: chemistry and technology**. Minnesota: St. Paul.

Bender DA (2003). **Nutritional Biochemistry of the Vitamins**, 2nd ed. United Kingdom: Cambridge University Press.

Bouis HE (1999). Economics of enhanced micronutrient density in food staples. **Field Crops Research** 60: 165-173.

Bouis HE (2003). Micronutrient fortification of plants through plant breeding: can it improve nutrition in man at low cost? **Proceedings of the Nutrition Society** 62: 403-411.

Bouis HE (2002). Plant breeding: a new tool for fighting micronutrient malnutrition. **The Journal of Nutrition** 132: 491S-494S.

Brody T (1999). **Nutritional Biochemistry**, 2nd ed. California: Academic Press.

Byerlee D, Eicher CK (2001). **Africa's Emerging Maize Revolution**. United Kingdom: Lynne Publishing.

Choo YM (1994). Palm oil carotenoids. **Food and Nutrition Bulletin** 15: 130-137.

Combs GF (1998). **The Vitamins: Fundamental Aspects in Nutrition and Health**, 2nd ed. London: Academic Press.

Cooke LJ, Wardle J (2005). Age and gender differences in children's food preference. **British Journal of Nutrition** 93: 741-746.

De Groote H, Kimenju SC (2008). Comparing consumer preferences for color and nutritional quality in maize: Application of a semi-double bound logistic model on urban consumers in Kenya. **Food Policy** 33: 362-370.

De Groote H, Kimenju SC, Morawetz UB (2010). Estimating consumer willingness to pay for food quality with experimental auctions: the case of yellow versus fortified maize meal in Kenya. **Agricultural Economics** 41: 1-16.

Department of Health (2003). **Foodstuffs, Cosmetics and Disinfectants Act (Act 54 of 1972)**. **Regulations relating to the fortification of certain foodstuffs**. **Government notice no.R2003**. Pretoria: Department of Health.
http://www.doh.gov.za (date accessed 01/12/2009).

Department of Health (2001). **Immunisation Awareness Campaign**. **Directorate Nutrition Fact Sheet: Vitamin A**. Pretoria: Department of Health.
http://www.doh.gov.za/docs/facts-f.html (date accessed 01/12/2009).

Department of Health (1999). **Policy Summary – The Integrated Nutrition Programme**. Pretoria.

Dhansay MA (2007). Vitamin A supplementation in South Africa: time for appraisal. **South African Journal of Clinical Nutrition** 20: 123-124.

Du Plessis LM, Najaar B, Koornhof HE, Labadarios D, Petersen L, Hendricks M, Kidd M (2007). Evaluation of the implementation of the vitamin A supplementation programme in the Boland/Overberg region of the Western Cape Province. **South African Journal of Clinical Nutrition** 20: 126-132.

E-Siong T (1995). The medical importance of vitamin A and carotenoids (with particular reference to developing countries) and their determination. **Malaysian Journal of Nutrition** 1: 179-230.

Faber M (2004). Complementary foods consumed by 6-12 month old rural infants in South Africa are inadequate in micronutrients. **Public Health Nutrition** 8 (4): 373-381.

Faber M, Jogessar VB, Benadé AJS (2001). Nutritional status and dietary intakes of children aged 2-5 years and their caregivers in a rural South African community. **International Journal of Food Sciences and Nutrition** 52: 401-411.

Faber M, Kruger S (2005). Dietary intake, perceptions regarding body weight, and attitudes toward weight control of normal weight, overweight, and obese Black females in a rural village in South Africa. **Ethnicity & Disease** 15: 238-245.

Faber M, Smuts CM, Benadé AJS (1999). Dietary intake of primary school children in relation to food production in a rural area in KwaZulu-Natal, South Africa. **International Journal of Food Sciences and Nutrition** 50: 57-64.

Faber M, Wenhold F (2007). Nutrition in contemporary South Africa. **Water SA** 33: 393-400.

Food and Agriculture Organization of the United Nations (FAO) and International Maize and Wheat Improvement Center (CIMMYT) (1997). **White Maize: A Traditional Food Grain in Developing Countries**. Rome, Italy.

Food and Agriculture Organization of the United Nations (FAO) (1992). **Maize in Human Nutrition**. Rome, Italy.

Food and Agriculture Organization of the United Nations (FAO) (2008). **The state of food insecurity in the world 2008: high food prices and food security – threats and opportunities**. Rome, Italy.
http://www.fao.org/docrep/011/i0291e/i0291e00.htm (date accessed 16/09/2010).

Gibson RS (1994). Content and bioavailability of trace elements in vegetarian diets. **American Journal of Clinical Nutrition** 59: 1223S-1232S.

Gregory JF (1996). Vitamins, 3rd ed. In: Fennema OR, ed. **Food Chemistry**. New York: Marcel Dekker Inc.

HarvestPlus Brief (2006). **HarvestPlus: Breeding Crops For Better Nutrition**. Washington DC, USA.

Hendricks M, Beardsley J, Bourne L, Mzamao B, Golden B (2007). Are opportunities for vitamin A supplementation being utilized at primary health-care clinics in the Western Cape Province of South Africa? **Public Health Nutrition** 10: 1082-1088.

Hotz C, Brown KH (eds) (2004). International Zinc Nutrition Consultative Group (IZiNCG). Technical Document No. 1: Assessment of the risk of zinc deficiency in populations and options for its control. **Food and Nutrition Bulletin** 25 (Supp 2): S94-S204.

Howe JA, Tanumihardjo SA (2006a). Carotenoid-biofortified maize maintains adequate vitamin A status in Mongolian gerbils. **The Journal of Nutrition** 136: 2562-2567.

Institute of Medicine (2001). **Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc.** Food and Nutrition Board. Washington DC: National Academy Press.

Institute of Medicine (2000a). **Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids.** Food and Nutrition Board. Washington DC: National Academy Press.

International Institute of Tropical Agriculture (IITA) (2010a). **New varieties to boost maize output in West and Central Africa.** http://www.iita.org/maize/-/asset_publisher/jeR0/content/new-varieties-to-boost-maize (date accessed 22/11/2010).

Johnson LA (2000). Corn: The Major Cereals of the Americas, 2nd ed. In: Kulp K, Ponte JG, eds. **Handbook of Cereal Science and Technology**. New York: Marcel Dekker.

Kazarian EA, Hall CW (1965). **Transactions of ASAE** 8: 33.

Keener HM, Henery JE, Schonauer SL, Anderson RJ (1985). **Burning Shelled Corn as an Alternative Fuel**. Ohio Report, July-August 1985.

Kennedy-Oji C, Coutsoudis A, Kuhn L, Pillay K, Mburu A, Stein Z, Coovadia H (2001). Effects of vitamin A supplementation during pregnancy and early lactation on body weight of South African HIV-infected women. **Journal of Health Population and Nutrition** 19: 167-176.

Labadarios D, Moodie IM, Van Rensburg A (2007). Vitamin A status. In: Labadarios D. **National Food Consumption Survey: Fortification Baseline**, Chapter 9B: South Africa 2005. Stellenbosch: Department of Health.

Labadarios D, Steyn N, Maunder E, Macintyre U, Swart R, Gericke G, Huskisson J, Dannhauser A, Vorster HH, Nesamvuni EA (2000). **The National Food Consumption Survey (NFCS): Children aged 1-9 years, South Africa, 1999**. Department of Health: Directorate of Nutrition, Pretoria.

Labadarios D, Steyn NP, Mgijima C, Dladla N (2005). Review of the South African nutrition policy 1994-2002 and targets for 2007: achievements and challenges. **Nutrition** 21: 100-108.

Labadarios D, Van Middelkoop AM (1995). **The South African Vitamin A Consultative Group. Children aged 6-71 months in South Africa, 1994: their anthropometric, vitamin A, iron and immunisation coverage status**. Isando, South Africa.

Laurie S (2007). **Experiences with orange-fleshed sweet potato in vegetable garden projects**. Proceedings of the orange-fleshed sweet potato symposium. Pretoria. 3 October 2007.

Li S, Tayie FAK, Young MF, Rocheford T, White WS (2007). Retention of provitamin A carotenoids in high β-carotene maize (Zea mays) during traditional African household processing. **Journal of Agricultural and Food Chemistry** 55: 10744-10750.

Low JW, Arimond M, Osman N, Cunguara B, Zano F, Tschirley D (2007). A food-based approach introducing orange-fleshed sweet potatoes increased vitamin A intake and serum retinol concentrations in young children in rural Mozambique. **The Journal of Nutrition** 137: 1320-1327.

Lozano-Alejo N, Carrillo GV, Pixley K, Palacios-Rojas N (2007). Physical properties and carotenoid content of maize kernels and its nixtamalized snacks. **Innovative Food Science and Emerging Technologies** 8: 385-389.

Machida L, Derera J, Tongoona P, MacRobert J (2010). Combining ability and reciprocal cross effects of elite quality protein maize inbred lines in subtropical environments. **Crop Science** 50: 1708-1717.

Mayer JE, Pfeiffer WH, Beyer P (2008). Biofortified crops to alleviate micronutrient malnutrition. **Current Opinion in Plant Biology** 11: 166-170.

McCann JC (2005). **Maize and Grace: Africa's encounter with a New World Crop 1500-2000**, 1st ed. Cambridge: Harvard University Press.

Muzhingi T, Yeum KJ, Russell RM, Johnson EJ, Qin J, Tang G (2008a). Determination of carotenoids in yellow maize, the effects of saponification and food preparations

Muzhingi T, Langyintuo AS, Malaba LC, Banziger M (2008b). Consumer acceptability of yellow maize products in Zimbabwe. **Food Policy** 33: 352-361.
International Journal for Vitamin and Nutrition Research 78: 112-120.

Nestel P, Bouis HE, Meenakshi JV, Pfeiffer W (2006). Biofortification of staple food crops. **The Journal of Nutrition** 136: 1064-1067.

Nojilana B, Norman R, Bradshaw D, van Stuijvenberg ME, Dhansay MA, Labadarios D and the South African Comparative Risk Assessment Collaborating Group (2007). Estimating the burden of disease attributable to vitamin A deficiency in South Africa in 2000. **South African Medical Journal** 97: 748-753.

Nuss ET, Tanumihardjo SA (2010). Maize: a paramount staple crop in the context of global nutrition. **Comprehensive Reviews in Food Science and Food Safety** 9: 417-436.

Oikeh SO, Menkir A, Maziya-Dixon B, Welch R, Glahn RP, Gauch G (2004). Environmental stability of iron and zinc concentrations in grain of elite early-maturing tropical maize genotypes grown under field conditions. **Journal of Agricultural Sciences** 142: 543-551.

Ortiz-Monasterio JI, Palacios-Rojas N, Meng E, Pixley K, Trethowan R, Pena RJ (2007). Enhancing the mineral and vitamin content of wheat and maize through plant breeding. **Journal of Cereal Science** 46: 293-307.

Penniston KL, Tanumihardjo SA (2006). The acute and chronic toxic levels of vitamin A. **American Journal of Clinical Nutrition** 83: 191-201.

Raboy V, Below FE, Dickison DB (1989). Alteration of maize kernel phytic acid levels by recurrent selection for protein and oil. **Journal of Heredity** 80: 311-315.

Rodriguez-Amaya DB (1997). **Carotenoids and Food Preparation: The retention of Provitamin A Carotenoids in Prepared, Processed, and Stored Foods**. Washington DC: OMNI/USAID.

Rubey L (1993). **Consumer Maize Meal Preferences in Zimbabwe: Survey Results and Policy Implications**. Report Prepared for The Ministry of Lands Agriculture and Water Development. Harare, Zimbabwe.

Ruel M (2001). **Can Food-Based Strategies Help Reduce Vitamin A and Iron Deficiencies?** A Review of Recent Evidence. International Food Policy Research Institute. Washington DC.

Rukmini C (1994). Red palm oil to combat vitamin A deficiency in developing countries. **Food and Nutrition Bulletin** 15: 126-129.

Smale M, Jayne T (2003). **Maize is Eastern and Southern Africa: "Seeds" of success in retrospect**. Environment and Production Technology Division Discussion Paper No. 97, International Food Policy Research Institute. Washington DC, USA.

Stevens R, Winter-Nelson A (2008). Consumer acceptance of provitamin A-biofortified maize in Maputo, Mozambique. **Food Policy** 33: 341-351.

Steyn NP, Bradshaw D, Norman R, Joubert JD, Schneider M, Steyn K (2006). **Dietary changes and the Health Transition in South Africa: Implications for Health Policy**. Cape Town: South African Medical Research Council.

Swart R, Labadarios D, Kruger S, Nel JH, Schloss I (2007). General and socio-demographic factors impacting on nutritional status. In: Labadarios D. **National Food Consumption Survey: Fortification Baseline**, Chapter 3: South Africa 2005. Stellenbosch: Department of Health.

Swart R, Sanders D, McLachlan M (2008). Nutrition: A Primary Health Perspective. In: **South African Health Review**, Chapter 9: 129-147.

Tothova M, Meyers WH (2006). **Predicting the acceptance for high beta-carotene maize: an ex-ante estimation method**. American Agricultural Economics Association Annual Meeting, July 23-26, Long Beach, California.

Tschirley DL, Santos AP (1995). **Who eats yellow maize? Preliminary results of a survey of consumer maize preferences in Maputo, Mozambique**. MSU International Department of Agricultural Economics Development, Department of Economics Working Paper No 53, Michigan State University, East Lansing, Michigan.

Van Jaarsveld PJ, Faber M, Tanumihardjo SA, Nestel P, Lombard CJ, Benade AJS (2005). β-carotene-rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modified-relative-dose-response test. **American Journal of Clinical Nutrition** 81: 1080-1087.

Van Jaarsveld PJ, Marais DW, Harmse E, Nestel P, Rodriguez-Amaya DB (2006). Retention of β-carotene in boiled, mashed orange-fleshed sweet potato. **Journal of Food Composition and Analysis** 19: 321-329.

Vasal SK (2001). **Keynote Address: Quality Protein Maize Development**. Seventh Eastern and Southern Africa Regional Maize Conference, 11th-15th February 2001: 3-6.

Visser ME, Maartens G, Kossew G, Hussey GD (2003). Plasma vitamin A and zinc levels in HIV-infected adults in Cape Town, South Africa. **British Journal of Nutrition** 89: 475-482.

Wolmarans P, Danster N, Dalton A, Rossouw K, Schonfeldt H (2010) (eds). **Condensed Food Composition Tables for South Africa**. Medical Research Council, Parow Valley, Cape Town.

World Health Organization (WHO) (1995). **Global prevalence of vitamin A deficiency**. Micronutrient Deficiency Information System Working Paper 2. World Health Organization, Geneva.

World Health Organization (WHO) (2003). **GEMS/Food Regional Diets: Regional Per Capita Consumption of Raw and Semi-processed Agricultural Commodities, Food Safety Department**. WHO Document Production Services, Geneva, Switzerland.

World Health Organization (WHO) (2009). **Global prevalence of vitamin A deficiency in populations at risk 1995-2005: WHO Global Database on Vitamin A Deficiency**. http://whqlibdoc.who.int/publications/2009/9789241598019_eng.pdf (date accessed 23/08/2010).

Wright KN (1987). Nutritional Properties and Feeding Values of Corn and Its By-Products, 1st ed. In: Watson SA, Ramstad PE, eds. **Corn: chemistry and technology**. Minnesota: St. Paul.

CHAPTER 3

BACKGROUND TO STUDY DESIGN AND THE CONSUMER ACCEPTABILITY STUDY SITE

This chapter gives the background to the experimental design, breeding of the provitamin A-biofortified maize varieties and the site at which the consumer acceptability studies were carried out.

3.1 Study design

The overall design of the study is shown in Figure 3.1.

Hybridization of provitamin A maize germplasm and production of bulk hybrid grain

Provitamin A-biofortified maize grain varieties
and
White maize grain variety (control)

Nutritional composition analysis

- Provitamin A carotenoids
- Moisture
- Starch
- Fat
- Protein
- Amino acids
- Iron
- Zinc
- Phosphorus

Analysis of other quality attributes

- 1000 kernel weight
- Hectolitre mass
- Stress cracks
- Milling index
- Grain fungal infection levels

Milling into mealie meal and samp

Assessment of retention of provitamin A and other nutrients

Cooking into phutu, thin porridge and samp

Assessment of retention of provitamin A and other nutrients

Assessment of consumer acceptability of *phutu*, thin porridge and samp

Figure 3.1   Study design

3.2   Background to breeding of provitamin A-biofortified maize

Breeding and production of bulk grain were done at three research stations in KwaZulu-Natal province, South Africa. To initiate the breeding process, the best 21 inbred maize line sources of provitamin A were obtained from the maize breeding programmes at the International Maize and Wheat Improvement Centre (CIMMYT) in Mexico (11 lines), and the International Institute of Tropical Agriculture (IITA) at Ibadan in Nigeria (10 lines), in 2007. Recombinant inbred lines (RILs) were derived from F_2 bi-parental populations among these elite lines following a pedigree selection process. This entailed selection of the best progenies between and within rows. Best progenies (based on agronomic and plant traits) were advanced to the next generation by self-pollination. Detailed description of the pedigree breeding process has been presented by Sleper & Poehlman (2006).

During the 2007/2008 summer season F_1 crosses were generated among elite lines at Ukulinga Research Farm at the University of KwaZulu-Natal, Pietermaritzburg, South Africa. These were then advanced to F_2 (S_1) generation at Makhathini Research Station in KwaZulu-Natal, South Africa during the winter of 2008. The best selections were then advanced to F_3 (S_2) at Ukulinga Research Farm during the 2008/2009 summer and to F_4 (S_3) during the 2009 winter seasons. In each breeding cycle, lines displaying a deep orange grain colour were selected for advancement. Single cross hybrids were created among 12 S_3 inbred lines at Makhathini Research Station during September 2009 to January 2010. The 10 F_1 hybrids with adequate seeds for planting in trials were planted in 20 rows of 5 m each at Makhathini Research Station during the winter season (May to September 2010). F_2 grain, which represents the generation of seed that is actually used to make maize grain products for consumption, were then generated by full-sib mating of F_1 plants within each row of 17 plants. At harvest all ears from the 20 rows of each hybrid were bulked and a sample of 5 kg was drawn for processing and to make food products. Figure 3.2 depicts the pedigree breeding and hybridization process and Table 3.1 shows a list of the names and grain colour of the maize varieties used in this study.

	November 2007		

Parents   **A**   **X**   **B** ⟶

Complementary breeding germplasm lines obtained from CIMMYT (Mexico) and IITA (Nigeria) were crossed to recombine desired traits

Pure lines genotypes   **AA**   **BB**

Genetically uniform with deep orange

Cross pollination

Homogenous but heterozygous

AB (F_1 hybrid)   2007/8

Self pollination

Heterogeneous segregating population

¼ AA   ½ AB   ¼ BB
F_2 (S1)

Self pollination

F_3 (S2)

Self pollination

Continued selection of lines with deep orange colour, and the trait was fixed by self-pollination

F_4 (S3)

F_4 lines X Testers crossed to produce new F_1 hybrids

Cross pollination

F_1 hybrids

Full sib-mating

F_2 grain (edible grain)

Bulked

Figure 3.2   Outline of breeding process (pedigree breeding)

Table 3.1   Experimental hybrids used in the study

Entry	Variety name	Grain colour
1	CC-37[ψ]	White
2	SC-701[†]	White
3	10 MAK 7-10[*]	Yellow/Orange
4	10 MAK 7-1	Orange
5	10 MAK 7-2	Orange
6	10 MAK 7-3	Orange
7	10 MAK 7-5	Orange
8	10 MAK 7-7	Orange
9	10 MAK 7-8	Orange
10	10 MAK 7-9	Orange
11	KPPVAH-1	Orange
12	KPPVAH-2	Orange
13	KPPVAH-3	Orange
14	KPPVAH-4	Orange
15	KPPVAH-5	Orange
16	KPPVAH-6	Orange
17	KPPVAH-7	Orange
18	KPPVAH-8	Orange
19	KPPVAH-9	Orange
20	KPPVAH-10	Orange
21	KPPVAH-11	Orange
22	KPPVAH-12	Orange
23	KPPVAH-13	Orange
24	KPPVAH-14	Orange
25	KPPVAH-15	Orange
26	KPPVAH-16	Orange
27	KPPVAH-17	Orange
28	KPPVAH-18	Orange
29	KPPVAH-19	Orange
30	KPPVAH-20	Orange
31	KPPVAH-21	Orange
32	KPPVAH-22	Orange
33	KPPVAH-23	Orange
34	KPPVAH-25	Orange
35	KPPVAH-26	Orange
36	KP-76	Yellow
37	KP-77[#]	Yellow
38	KP-78[#]	Yellow
39	KP-79[#]	Yellow
40	KP-80	Yellow

[ψ] White maize control used in Chapter 4 and Chapter 5
[†] White maize control used in Chapter 6
[*] Reference yellow/orange maize variety used in Chapter 4 and Chapter 5
[#] Yellow maize varieties used in consumer acceptability study (Chapter 6)

3.3 Colour of provitamin A-biofortified maize grain

Provitamin A-biofortified maize grain may range in colour from light yellow to dark orange (Ortiz-Monasterio *et al* 2007; HarvestPlus Brief 2006). In this study, the maize used to assess the nutritional composition of the maize grain (Chapter 4) and to assess the retention of provitamin A carotenoids and other nutrients after milling and cooking (Chapter 5) were orange in colour, as indicated by the higher Hunter *a* and *b* values [Hunter *a* values: 16.51-25.68; Hunter *b* values: 29.25-37.46]. The maize used to assess the consumer acceptability of provitamin A-biofortified maize (Chapter 6) was yellow in colour as indicated by the lower Hunter *a* and *b* values [Hunter *a* values: 11.38-13.78; Hunter *b* values: 21.25-22.02]. The Hunter *L*, *a*, *b* system, uses *L* as a measure of lightness (0 = black to 100 = white), *a* as a measure of redness and (+*a* = redness; -*a* = greenness), and *b* as a measure of yellowness (+*b* = yellowness; -*b* = blueness) (DeMan 1999, p237).

3.4 Background on Mkhambathini Municipality (site of consumer acceptability studies)

It was decided that the consumer acceptability studies would be carried out on Black African subjects from a rural area, as these subjects would be more vulnerable to vitamin A deficiency compared to similar subjects from an urban area. The Mkhambathini Municipality was chosen for the consumer acceptability studies as it has a large Black African population (constitutes approximately 93% of the total population in this area) and can be regarded as a low income area due to the high unemployment rate (44% in 2001) and low average annual household income (R5742.00 [approximately US$838.00] in 2004) (Mkhambathini Local Municipality 2007). The Mkhambathini Municipality lies about 20 km east of Pietermaritzburg, the capital city of KwaZulu-Natal province, in South Africa. The Mkhambathini Municipality is the second smallest municipality within Umgungundlovu District Municipality and is situated along the Southern-Eastern border of the Umgungundlovu District Municipality. It adjoins Richmond and Msunduzi local municipalities to the West, uMshwati Local Municipality to the North and Durban/eThekwini Metropolitan area to the East. It covers an area of approximately 917 km^2 (Mkhambathini Local Municipality 2010). Figure 3.3 shows the location of KwaZulu-Natal province in South Africa and Figure 3.4 shows a map of the Mkhambathini Municipality, in KwaZulu-Natal province.

Figure 3.3   Map of South Africa showing
KwaZulu-Natal Province
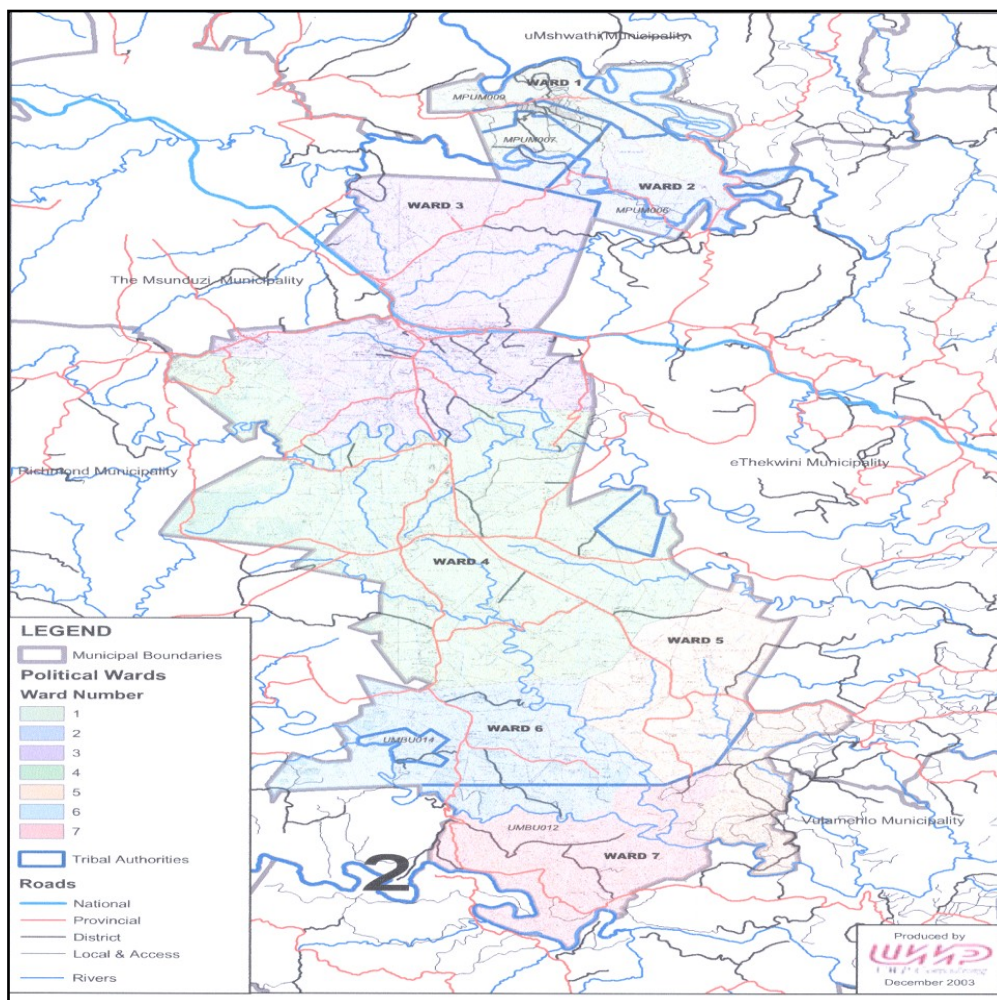
Figure 3.4   Map of Mkhambathini Municipality in KwaZulu-Natal showing
wards and Municipal boundaries (UWP Consulting 2003)

The total population of the Mkhambathini Municipality is approximately 45 569 with the following racial distribution: Black - 43 467 (93.3%), White - 2048 (4.4%), Indian/Asian - 1048 (2.3%) and Coloured - 6 (0.01%) (Mkhambathini Local Municipality 2008/2009). The predominant language spoken by the Black population is *isiZulu*. The area had an unemployment rate of 44% in 2001 as opposed to 30% in 1996. Cumulative individual income for the Mkhambathini Municipality increased from about R174 million [approximately US$25 million] per annum in 1996 to R324 million [approximately US$47 million] per annum in 2001 and to R409 million [approximately US$60 million] per annum in 2004. Annual household income shows the same trend. Income per capita increased from R3783.00 [approximately US$552.00] per annum in 1996 to R5478.00 [approximately US$800.00] per annum in 2001 and to R5742.00 [approximately US$838.00] per annum in 2004 and is indicative of a low income economy. The dominant employment industry is the agriculture, forestry and fishing sectors. The number of people employed in the agricultural sector shows that there is a continued dependence of residents in the Mkhambathini Municipality on employment opportunities in the primary sector of the economy (Mkhambathini Local Municipality 2007).

3.4.1   Schools used in study

The school-going subjects that were used to assess consumer acceptability of the provitamin A-biofortified maize food products were drawn from the following schools within the Mkhambathini Municipality: Cosmoore Primary School (Figure 3.5), Fairleigh Primary School (Figure 3.6) and Mabomvini Combined School (Figure 3.7). The adult subjects were drawn from parents and guardians attending the Parents Meetings held at Mabomvini Combined School and Cosmoore Primary School.

Preschool subjects used in the study were drawn from Cosmoore Primary School, Fairleigh Primary and Mabomvini Combined School. Primary School subjects and Secondary School subjects were drawn from Cosmoore Primary School and Mabomvini Combined School respectively. According to the South African Department of Education Resource Targeting List (2010), Fairleigh Primary School and Mabomvini Combined School both fall into quintile 1 of the National Quintile for Public Schools, while Cosmoore Primary School falls into quintile 2. According to the National Quintile for Public Schools, all South African public schools fall into one of five groups where the grouping is according to the poverty of the community around the school. Quintile 1 is the poorest and quintile 5 is the least poor.

Schools that fall into Quintiles 1-3 have been declared as no fee schools for 2010 (South African Schools Act 1996). This shows that there was a high prevalence of poverty in the schools used in this study and the community around the schools.

Figure 3.5   Cosmoore Primary School   **Figure 3.6**   Fairleigh Primary School

Figure 3.7   Mabomvini Combined School

3.5   Approvals to carry out study

Ethical approval to carry out this study was obtained from the University of KwaZulu-Natal, Humanities and Social Sciences Ethics Committee (Approval number HSS/0591/09D) (Appendix A, p171). Approval to perform sensory evaluation using subjects from the Mkhambathini Municipality was obtained from the Mkhambathini Municipal Manager (Appendix B, p172). Approval to use learners from the schools in the Mkhambathini Municipality was obtained from the Department of Education (Appendix C, p173).

References

DeMan JM (1999). **Principles of Food Chemistry**, 3rd ed. Maryland: Aspen Publishers.

HarvestPlus Brief (2006). **HarvestPlus: Breeding Crops for Better Nutrition**. Washington DC, USA.

Mkhamabthini Local Municipality (2010). **Mkhambathini Municipality**. http://www.mkhambathini.gov.za (date accessed 17/08/2010).

Mkhamabthini Local Municipality (2008/2009). **Municipality Annual Report (2008/2009)**. http://www.mkhambathini.gov.za (date accessed 17/08/2010).

Mkhambathini Local Municipality (2007). **The Final Local Economic Development (LED) Strategic Plan.** UMgungundlovu District Municipality, Pietermaritzburg.

Ortiz-Monasterio JI, Palacios-Rojas N, Meng E, Pixley K, Trethowan R, Pena RJ (2007). Enhancing the mineral and vitamin content of wheat and maize through plant breeding. **Journal of Cereal Science** 46: 293-307.

Sleper DA, Poehlman JM (2006). **Breeding field crops**, 5th ed. Iowa: Blackwell Publishing.

South African Department of Education Resource Targeting List (2010). South African Department of Education, Pretoria, South Africa.

South African Schools Act (1996) (Act No 84 of 1996). **Amended National Norms and Standards for School Funding**. South African Department of Education, Pretoria, South Africa.

UWP Consulting (2003). **Mkhambathini Municipality Map 1.** http://www.uwp.co.za (date accessed 18/08/2010).

CHAPTER 4

NUTRITIONAL COMPOSITION AND OTHER QUALITY ATTRIBUTES OF PROVITAMIN A-BIOFORTIFIED MAIZE GRAIN

Abstract

The potential of provitamin A-biofortified maize for use as a complementary strategy to alleviate vitamin A deficiency in developing countries is currently a subject of research. Apart from the difference in the provitamin A carotenoid content, it may be assumed that the nutritional composition of provitamin A-biofortified maize is similar to that of white maize. However, a difference in genetic factors between the two types of maize may also result in a difference in nutritional composition. Similarly, other quality attributes, e.g. storage and food processing properties of the two types of maize may be different. However, published data in this area are scarce. This study therefore aimed to evaluate the nutritional composition and other quality attributes of provitamin A-biofortified maize grain varieties compared to white maize grain. The total provitamin A content in the biofortified varieties ranged from 7.3-8.3 μg/g dry weight (DW) with total β-carotene ranging from 3.5-3.6 μg/g DW, and β-cryptoxanthin from 3.7-4.8 μg/g DW, whilst no carotenoids were detected in the white maize variety. The results showed that, when compared with the white maize variety, the biofortified maize varieties had higher levels of starch, fat and protein, but were lower in iron. Zinc and phosphorus levels compared well in the biofortified and white maize varieties. The biofortified maize varieties were better sources of most of the essential amino acids relative to the white maize, but similar to the white maize, they were deficient in histidine and lysine. The results of this study indicate that provitamin A carotenoids occur in varying levels in provitamin A-biofortified maize grain, but further improvement is required to increase the provitamin A levels to at least 15 μg/g, which is the HarvestPlus target. The results of the study indicate that in terms of the other nutrients, the biofortified maize grain is generally superior to white maize grain, except for minerals. The provitamin A-biofortified maize grain varieties have the potential to make a significant contribution towards meeting the nutritional requirements of poor communities who use maize as a staple food. Overall, the quality of the grain of the provitamin A-biofortified maize varieties was superior to that of the white maize grain, although the grain of the biofortified varieties showed less resistance to fungal infection.

4.1   Introduction

Maize (*Zea mays*), also known as corn, is one of the leading cereal grains worldwide with production exceeding 750 million metric tonnes in 2008 (USDA/FAS 2008). Maize is used as a food and feed worldwide (Nuss & Tanumihardjo 2010). The African continent is dependent on maize as a food source more than any other continent in the world (McCann 2005, p1). According to the World Health Organization (WHO), the average maize consumption in Africa is 106.2 g/person/day which is far greater than other maize-consuming regions such as Europe, the Far East, Latin America or the Middle East (WHO 2003). In South Africa, maize contributes about 40% of total energy intake (McCann 2005, p9). Maize is processed into a wide variety of both traditional and modern food products. The traditional foods made from maize include breads, porridges, steamed and roasted products, beverages and snacks. In Africa, fresh or fermented maize porridges are widely consumed (Ortiz-Monasterio *et al* 2007), whereas in developed countries maize is mainly used as a feed for livestocks.

The chemical composition of maize is known to vary due to genetic make-up, environmental factors and agronomic practices (FAO 1992). Although maize is an important source of energy and protein, the amounts of some macronutrients and micronutrients are unbalanced and inadequate for consumers that depend on maize as a major food source (Nuss & Tanumihardjo 2010). Carbohydrate (largely starch) is the major chemical constituent of the white maize kernel. The protein quality of maize is regarded as inferior as it is limiting in the essential amino acids, lysine and tryptophan. Maize oil has a low level of saturated fatty acids and a relatively high level of polyunsaturated fatty acids, mainly linoleic acid. White maize is a good source of thiamin, pyridoxine and phosphorus and a fair source of riboflavin, niacin, folate, biotin, iron and zinc. Micronutrients present in small amounts include vitamin E and calcium (FAO 1992). Provitamin A carotenoids in typical white maize grain include α-carotene, β-carotene and β-cryptoxanthin; however, concentrations are low (Kurilich & Juvik 1999) and hence nutritionally insignificant.

The unbalanced nutritional composition of white maize, especially the lack of provitamin A carotenoids could partly explain why malnutrition still exists in sub-Saharan Africa, where maize is a dietary staple (Nuss & Tanumihardjo 2010). In Africa, an estimated 33 million preschool-age children have vitamin A deficiency (VAD) (West 2002), which predisposes them to diseases such as anaemia, diarrhoea, malaria and respiratory infections (Villamor &

Fawzi 2000; West 2000; Shankar *et al* 1999; Sommer & West 1996). Approximately 20-24% of child mortality from diarrhoea, measles and malaria and 3% mortality from infectious diseases can be attributed to VAD (Rice *et al* 2004).

Maize is one of the six staple crops that has been targeted for biofortification with provitamin A carotenoids as part of an international effort to combat VAD (Tanumihardjo 2008; HarvestPlus Brief 2006). Biofortification of maize with provitamin A carotenoids, through conventional plant breeding has the potential to contribute to the alleviation of VAD by complementing existing strategies aimed at improving vitamin A status (Ortiz-Monasterio *et al* 2007). The provitamin A carotenoid content of provitamin A-biofortified maize is known to vary due to genetic factors (Menkir *et al* 2008; Li *et al* 2007; Ortiz-Monasterio *et al* 2007). Yellow maize varieties contain between 0.25 and 2.5 μg/g DW of provitamin A while deep yellow or orange varieties may contain 15 μg/g DW (Nuss & Tanumihardjo 2010). Current breeding targets for maize as set by HarvestPlus is 15 μg/g DW of provitamin A (Ortiz-Monasterio *et al* 2007), but currently this target provitamin A level has not been achieved in any maize variety that can be released for agricultural production. Thus, there is ongoing breeding efforts to achieve this target in provitamin A-biofortified maize varieties.

Although the nutritional composition of white maize is widely reported (Johnson 2000, p38; FAO 1992), published data on the nutritional composition of provitamin A-biofortified maize are scarce. It may be assumed that the nutritional composition of provitamin A-biofortified maize is similar to that of white maize, apart from the differences in provitamin A carotenoid content. However, breeding and selection of maize genotypes to produce provitamin A-biofortified maize may result in a significant change in the nutritional composition of the maize, similar to what has been reported in the mutation breeding of white maize genotypes to produce low phytic acid maize (Raboy *et al* 1989). The protein content of the low phytic acid maize hybrids were found to be significantly lower when compared to their normal high phytic acid counterparts (Raboy *et al* 1989). Because the processing of maize grain into food products usually involves milling it is important to assess its milling quality attributes. It is also important to assess levels of fungal infection as this may impact on its potential to be used as a safe and affordable food source. The aim of this study was to evaluate the nutritional composition including carotenoid composition and other quality attributes of provitamin A-biofortified maize grain varieties, compared to white maize grain.

4.2   Materials and Methods

4.2.1   Maize grain varieties

The grain varieties used in this research chapter were orange. They were planted and harvested in the 2009/2010 season. These experimental maize varieties were produced as described in Chapter 3, section 3.2. A total of 32 orange maize varieties were produced and analysed for their nutritional composition. A control white variety, CC-37 (Figure 4.1a) and a 10 MAK 7-10 (Figure 4.1b), a reference yellow/orange maize variety (commercial provitamin A variety), obtained from Seed Co Ltd. (Zimbabwe), produced under the same conditions as the experimental varieties, were included. Of the experimental varieties, only seven were produced in bulk (±5 kg). Grain colour of the seven varieties was measured in terms of the Hunter *L*, *a*, *b* system (Table 4.1), using a Colorflex instrument (Hunter Associates Laboratory, Inc., Reston, Virginia, USA). This system uses *L* as a measure of lightness (0 = black to 100 = white), *a* as a measure of redness and (+*a* = redness; -*a* = greenness), and *b* as a measure of yellowness (+*b* = yellowness; -*b* = blueness) (DeMan 1999, p237). Based on the grain colour results in Table 4.1, 10 MAK 7-5 (Figure 4.2a) was chosen as the lightest orange variety, 10 MAK 7-7 (Figure 4.2b) as the medium orange variety and 10 MAK 7-8 (Figure 4.2c) as the deepest orange variety. These three varieties were also used to prepare food products (Chapter 5).

Figure 4.1a   White maize (control) (CC-37) maize

Figure 4.1b   Reference yellow/orange maize (10 MAK 7-10)

Table 4.1 Colour of the maize varieties

Maize variety	Hunter *L*, *a*, *b* colour values		
	L[a]	*a*[b]	*b*[c]
CC-37[ψ]	63.90[d](0.14)[e]	3.05 (0.04)	18.43 (0.54)
10 MAK 7-1	52.35 (0.02)	25.68 (0.03)	37.46 (0.02)
10 MAK 7-2	58.20 (0.02)	17.59 (0.01)	29.25 (0.09)
10 MAK 7-3	54.57 (0.03)	23.22 (0.01)	34.91 (0.14)
10 MAK 7-5	60.29 (0.02)	16.51 (0.01)	29.98 (0.04)
10 MAK 7-7	57.39 (0.03)	20.34 (0.01)	32.35 (0.04)
10 MAK 7-8	48.76 (0.02)	24.05 (0.01)	36.68 (0.17)
10 MAK 7-9	57.34 (0.01)	17.80 (0.03)	31.89 (0.08)
10 MAK 7-10[*]	53.14 (1.63)	12.84 (1.36)	20.38 (0.24)

[a] Measure of lightness (0 = black to 100 = white)
[b] Measure of redness and (+*a* = redness; -*a* = greenness)
[c] Measure of yellowness (+*b* = yellowness; -*b* = blueness)
[d] Mean
[e] Standard deviation
[ψ] White maize variety (control)
[*] Reference yellow/orange maize variety

Figure 4.2a 10 MAK 7-5 (lightest orange)

(Hunter L = 60.29; a = 16.51; b = 29.98)

Figure 4.2b 10 MAK 7-7 (medium orange)

(Hunter L = 57.39; a = 20.34; b = 32.35)

Figure 4.2c   10 MAK 7-8 (deepest orange)

(Hunter L = 48.76; a = 24.05; b = 36.68)

The experimental design used to assess the nutritional composition of the maize grain, milled products and cooked products (Chapter 4 and Chapter 5) is shown in Figure 4.3.

PEDIGREE BREEDING (See Figure 3.2 in Chapter 3)

```
┌─────────────────┐      ┌─────────────────┐      ┌─────────────────┐      Nutritional
│ Reference       │      │ 32 Provitamin   │      │ White maize     │      composition
│ yellow/orange   │- - ->│ A-biofortified  │- - ->│ (CC-37)         │- - ->  analysis
│ maize variety   │      │ varieties       │      │ (control)(grain)│      - Carotenoids#
│ (10 MAK 7-10)   │      │ (grain)         │      │                 │      - Moisture
│ (grain)         │      │                 │      │                 │      - Starch
└─────────────────┘      └─────────────────┘      └─────────────────┘      - Fat
                                                                           - Protein
                                                                           - Iron
                                                                           - Zinc
                                                                           - Phosphorus
┌─────────────────┐      ┌─────────────────┐      ┌─────────────────┐      Assessment of
│ Reference       │      │ Seven           │      │ White maize     │      grain colour
│ yellow/orange   │- - ->│ varieties       │- - ->│ (CC-37)         │- - ->
│ maize variety   │      │ (grain)         │      │ (control)       │      Amino acid
│ (10 MAK 7-10)   │      │ (BULKED)        │      │ (grain)         │      composition
│ (grain)         │      │                 │      │                 │
└─────────────────┘      └─────────────────┘      └─────────────────┘      Assessment of
                                                                           grain quality

                         ┌──────────────────────────┐
                         │ Three provitamin A-bio-  │
                         │ fortified maize grain    │
                         │ varieties selected based │
                         │ on colour (light, medium,│
                         │ deep orange)             │
                         └──────────────────────────┘

                              ┌──────────────┐
                              │ Dry milling  │
                              └──────────────┘

┌──────────────┐                          ┌──────────────┐      Nutritional
│ Mealie meal  │- - - - - - - - - - - - ->│ Samp         │- - ->composition
└──────────────┘                          └──────────────┘      analysis
                                                                - Carotenoids*
                         ┌────────────────────────┐            - Moisture
                         │ Cooking of food        │            - Energy
                         │ products               │            - Starch
                         └────────────────────────┘            - NDF
                                                                - Fat
                         ┌────────────────────────┐            - Protein
                         │ Phutu, thin porridge,  │- - - - - ->- Minerals (ash)
                         │ samp                   │            - Iron
                         └────────────────────────┘            - Zinc
                                                                - Phosphorus
```

\# Maize grain of the following varieties were analysed for carotenoid composition: CC-37(white control), 10 MAK 7-5, 10 MAK 7-7 and 10 MAK 7-8

* Milled and cooked maize products of the following varieties were analysed for carotenoid composition: 10 MAK 7-5, 10 MAK 7-7 and 10 MAK 7-8

NDF = Neutral Detergent Fibre

Figure 4.3   Experimental design for the nutritional composition of maize grain, milled and cooked products

4.2.2   Nutritional composition of maize grain

The whole maize grains were analysed for their nutritional composition. Referenced methods were used in the analysis. Analytical reagents were used and analysis for nutrient content in a sample was repeated at least twice. Due to the high costs of maize carotenoid analysis in South Africa [R4000.00 (US$584.00) per sample], only three varieties of the provitamin A-biofortified maize (10 MAK 7-5, 10 MAK 7-7 and 10 MAK 7-8), which were selected based on grain colour (section 4.2.1), were analysed for carotenoid composition in this study. Only the seven bulked varieties were analysed for amino acid composition due to cost constraints [R820.00 (US$120.00) per sample].

Sample preparation

General sample preparation involved size reduction, as appropriate (e.g. milling of whole grain). Other sample preparation procedures were as per specific analysis method and are described specifically.

4.2.2.1 Carotenoids

Handling of samples for analysis

Raw whole maize grain samples were stored in amber glass bottles, which were flushed with nitrogen gas and then immediately placed into a cooler box. Ice packs were placed over the bottles and multi-layered newspaper sheets were used to insulate the cooler box. The cooler box was transported to the Council for Scientific and Industrial Research (CSIR), Pretoria, with an overnight courier service. Upon receipt, the samples were transferred to a freezer and stored at -20 °C. Thawed raw maize grain samples were milled using a 0.5 mm rotor mill (ultra centrifugal mill ZM 200, Retsch, Haan, Germany). The prepared samples were stored under nitrogen at -20 °C until they were analysed by High-Performance Liquid Chromatography (HPLC).

Chemicals and standards

All solvents used in the analysis were HPLC grade. The following solvents were used: methanol and tetrahydrofuran (THF) (Labscan, Gliwice, Poland), ethanol (Merck, Darmstadt, Germany) and *tert*-butyl methyl ether (Sigma-Aldrich, St. Louis, MO, USA). Analytical standards of β-carotene, β-cryptoxanthin (Sigma-Aldrich, St. Louis, MO, USA) and zeaxanthin (ChromaDex, Irvine, CA, USA) were used to calibrate and quantify the carotenoids.

Sample extraction

Samples were analysed in duplicate using the method described by Muzhingi *et al* (2008a). Extraction was performed by incubating 1 g of sample with 5 mL of methanol for 2 hours at room temperature (±25 °C). During that time, the sample mixture was vortexed for 30 seconds at 30 minute intervals. The sample mixture was flushed with nitrogen gas and stored overnight (16-18 hours) in the dark at room temperature. The mixture was centrifuged at 1409 x g for 5 minutes. The methanol layer was transferred into a 25 mL volumetric flask and the sample was extracted using 5 mL of tetrahydrofuran (THF), incorporating the vortexing and centrifugation steps. Extraction was repeated three times using 5 mL of THF each time. The THF layers were combined with the methanol layer. The combined methanol and THF extract was then filtered and concentrated to a volume < 3 mL using a rotary evaporator/concentrator. Thereafter, the concentrated extract was dried completely under nitrogen gas and re-suspended in a 1 mL mixture of ethanol and THF (1:1, v/v). The extract was filtered through a 0.45 μm PTFE/Teflon syringe filter before analysis by HPLC.

Instrumentation and Chromatography

Carotenoid analysis was carried out using a Hewlett-Packard 1100 HPLC (Agilent Technologies Incorporated, Loveland, CO, USA) consisting of a binary pump, autosampler, column thermostat, diode array detector and ChemStation software (Revision B.03 02, Agilent Technologies Incorporated, Loveland, CO, USA). The carotenoids were separated on a C_{30} column with polymeric bonding chemistry (250 x 2.0 mm, 5 μm, TMC Co., Ltd, Kyoto, Japan) with the corresponding guard column (20 x 2.0 mm, 5 μm) operated at a flow rate of 0.4 mL/minute. Two mobile phases were employed and they were both mixtures of methanol, *tert*-butyl methyl ether and water [Mobile Phase A: 83/15/2 (v/v/v) and Mobile Phase B: 8/90/2 (v/v/v)]. The gradient elution programme was set as follows: i) 0 to 1 min with 100% A, ii) 1 to 8 minutes with a linear gradient to 70% A, iii) 8 to 13 minutes held at 70% A, iv) 13 to 22 minutes with a linear gradient to 45% A, v) 22 to 24 minutes with a linear gradient to 5% A, vi) 24 to 25 minutes held at 5% A, vii) 25 to 27 minutes with a linear gradient to 100% A, and viii) 27 to 30 minutes held at 100% A. Carotenoids were monitored at 450 nm and the injection volume was 20 μL. All-*trans* β-carotene, β-cryptoxanthin and zeaxanthin in the samples were identified by comparing their peak retention times and characteristic UV/visible absorption spectra with that of their standards. Quantification of these carotenoids was accomplished using multilevel response curves with carotenoid standards at concentration ranges of 0.03-3 μg/mL for zeaxanthin, 0.02-4 μg/mL for β-

cryptoxanthin and 0.04-2 µg/mL for β-carotene. The concentration of each standard was calculated using the specific absorption coefficient ($A^{1\%}$) for each carotenoid: 2480 for zeaxanthin in ethanol at 450 nm, 2356 for β-cryptoxanthin in ethanol at 452 nm and 2620 for all-*trans* β-carotene in ethanol at 450 nm (Rodriguez-Amaya & Kimura 2004; Thomas *et al* 2001). The *cis* isomers of β-carotene were quantified using the standard response curve for all-*trans* β-carotene.

Isomer identification

Mass spectral investigation of carotenoid isomers was performed on a Waters SYNAPT QTOF HDMS G1 mass spectrometer (Waters, Milford, USA) equipped with an atmospheric pressure ionisation source and lock spray interface for continuous mass accuracy during the analysis process. Chromatographic separation was achieved using an Acquity UPLC system (Waters, Milford, USA) under reversed phase conditions and utilising a C18 stationary phase. To enable the detection of carotenoids like β-carotene, β-cryptoxanthin and zeaxanthin, standards were infused into the source and the ionisation conditions optimised. Optimised mass spectral conditions were used to detect the selected carotenoids after chromatographic separation.

4.2.2.2 Moisture

The moisture content of the samples was measured according to the Association of Official Analytical Chemists International (AOAC) Official Method 934.01 (AOAC 2002) in which the samples of known weight were dried in a forced-air oven set at 95 ºC for 72 hours. The moisture content of the food products was determined by weight difference after freeze drying in a freeze drier (Edwards High Vacuum International, Sussex, England).

4.2.2.3 Starch

Starch content was determined according to the AOAC Method 979.10 (Glucoamylase Method) (AOAC 2002). This method entails the gelatinization of starch in the sample by autoclaving followed by the enzymatic hydrolysis of the starch to glucose and then the determination of glucose content by the glucose oxidase method. Sugars present in the samples were removed by refluxing the samples in 80% ethanol before determining the starch. Amyloglucosidase (glucoamylase), in acetate buffer, was used to hydrolyse the gelatinized starch to glucose. An enzyme-buffer-chromogen mixture (enzymes: glucose oxidase and peroxidase, and o-Dianisidine.2HCl, in a phosphate buffer) was reacted with the

glucose, absorbance measured at 540 nm and then glucose content read off from a glucose concentration vs. absorbance standard curve.

4.2.2.4 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 2002). Petroleum ether was used for extraction.

4.2.2.5 Protein

The crude protein content of the samples was measured with a LECO Truspec Nitrogen Analyser (LECO Corporation, St Joseph, Michigan, USA) using the Dumas Combustion method (AOAC Official Method 968.06) (AOAC 2002).

4.2.2.6 Amino acids

The amino acid profile of the samples was analysed by the Pico-Tag method (Millipore Corporation 1986, 1987) using a Waters Breeze High-Performance Liquid Chromatography (HPLC) with Empower software (Waters, Millipore Corp., Milford, MA). Samples (400 mg) were hydrolysed with 6 N HCl for 24 hours and then derivatized with phenylisothiocyanate (PITC) to produce phenyltiocarbamyl (PTC) amino acids, which were analysed by reverse phase HPLC.

4.2.2.7 Minerals

Iron and zinc

The iron and zinc contents of the samples were determined by the atomic absorption spectroscopy methods described by Giron (1973). The Varian SpectrAA atomic absorption spectrophotometer (Varian Australia Pty Ltd, Mulgrave, Victoria) was used to analyse iron and the GBC 905AA spectrophotometer (GBC Scientific Equipment Pty Ltd, Dandenong, Victoria, Australia) was used to determine the zinc content.

Phosphorus

Phosphorus content was determined according to the AOAC Official Method 968.08 (AOAC 2002). Absorbances were measured with the Analytik Jena Spekol 1300 spectrophotometer (Analytik Jena AG, Achtung, Germany).

4.2.3   Maize grain quality

Grain of each of the experimental maize varieties as well as the white variety (CC-37) (control) and the reference yellow/orange variety (10 MAK 7-10) was analysed for selected grain quality attributes, *viz.* 1000 kernel weight, hectolitre mass, stress cracks, milling index and fungal infection.

4.2.3.1 1000 kernel weight

1000 kernel weight is the mass of 1000 grains and is a measure of grain density. Grain density is routinely used to indicate grain milling quality, where a higher grain density indicates a better milling quality (Taylor & Duodu 2009, p200). 1000 kernel weight was determined by counting 1000 kernels with a numigral 180900 series Chopin seed counter (Chopin SA, Villeneuve-La-Garenne, France) and thereafter measuring the mass of the kernels.

4.2.3.2 Hectolitre mass

Hectolitre mass is the mass of a hectolitre (100 L) of grain and is a measure of grain density. As mentioned earlier, grain density is used to indicate grain milling quality, where a higher grain density indicates a better milling quality. Grain defects such as insect infestation and mouldiness reduce grain density (Taylor & Duodu 2009, pp200-201). The hectolitre mass of grain was measured using an apparatus that consisted of a hopper and a 0.5 L receiver (cup) following the American Association of Cereal Chemists (AACC) Method 55-10 Test Weight per Bushel (AACC 2000). The hopper was filled with grain which was then emptied into the receiver until it overflowed. The grain in the receiver was levelled off and weighed, and the net mass of the grain used to calculate hectolitre mass.

4.2.3.3 Stress cracks

Maize kernels can crack during artificial drying from stress caused by the uneven contraction of different parts of the endosperm (Taylor & Duodu 2009, p201). Hard grain is more susceptible to cracking than soft grain. Grain cracking can have negative effects on the grain such as grain losses due to breakage of cracked kernels during grain handling and processing and a reduction in milling quality (Taylor & Duodu 2009, p201). The stress cracks of the maize grain samples were analysed according to the Southern African Grain Laboratory (SAGL) Industry Accepted Method for Stress Crack Analysis of Maize Kernels (SAGL 2001). A total of 100 sound kernels from each maize sample were selected and placed on a

light board. The kernels were then visually inspected for cracks. The cracks in the kernel were seen as dark lines when light was transmitted through the grains. A kernel was reported as positive for stress cracking if one or more cracks were seen on it.

4.2.3.4 Milling index

Milling Index is an indicator of grain hardness and hence milling quality (Taylor & Duodu 2009, p 201). In this study, the Milling Index of the maize grain was determined according to the SAGL Industry Accepted Method for estimating Milling Index using Near-Infrared Transmittance (NIT) (SAGL 2007). The basis of the method is that the light transmittance of light in the near-infrared wavelength through the grain is directly related to grain hardness and hence milling quality (Taylor & Duodu 2009, pp202-203). The method is calibrated against data obtained from pilot-scale roller milling trials, with Milling Index representing extraction rate (% meal obtained from milling the grain) (Taylor & Duodu 2009, p201). The Milling indices of the maize grain samples of this study were measured with the INFRATEC 1241 Grain Analyzer (Foss Tecator AB, Höganäs, Sweden) NIT machine, which had been calibrated as described above. Approximately 500 g of a maize grain sample was loaded into the machine.

4.2.3.5 Grain fungal infection levels

Fungi infecting the maize grains were enumerated, isolated and identified by the direct plating method described by Rabie & Lübben (1984) and Rabie *et al* (1997). According to this method, sample grains are evenly spread over three different growth media to ensure mycelia growth of all possible species present. The fungi, which use the growth media as nutrients, grow out of the test sample onto the medium. Potato Dextrose Agar (PDA) was used as a non-selective medium; Malt Salt Agar (MSA) for the selective growth of *Aspergillus*, *Eurotium* and *Penicillum* spp.; and Pentachlorobenzene Agar (PCNB) for the selective growth of *Fusarium* spp. Kernels of each grain type were surface-disinfected by shaking them in a flask of 76% (v/v) ethanol and then rinsing them three times with sterile distilled water. Five kernels were placed on plates (10 each) of PDA, MSA and PCNB incubated at 25 ºC for 2 to 14 days. Kernels with mycelia growth of any fungal type were counted and expressed as a percentage of the total kernels plated. The fungal colonies were isolated and purified on fresh PDA plates and then identified based on morphological features of their fruiting bodies by referring to Dugan (2006).

4.2.4   Statistical analyses

Predictive Analytics SoftWare (PASW) Statistics version 18.0 (IBM Corporation, New York) was used to analyse the data. Mean and standard deviations were calculated from the duplicate nutrient values. Univariate analysis of variance (UNIANOVA) and Tukey post-hoc multiple comparisons of means was used to analyse for differences in the nutrient content according to maize variety. The Dunnett test (post-hoc multiple comparisons) was used to compare the nutrient content of the CC-37 (white maize control) variety with the nutrient content of the provitamin A-biofortified maize varieties. A p value of < 0.05 was considered to be significant.

4.3   Results and Discussion

4.3.1   Nutritional composition of provitamin A-biofortified maize grain varieties

Carotenoids

The quantitative distribution of carotenoids in white maize grain (CC-37) and three varieties of provitamin A-biofortified maize grain (10 MAK 7-5, 10 MAK 7-7 and 10 MAK 7-8) is shown in Table 4.2.

Table 4.2   Carotenoid composition of white maize grain compared to provitamin A-biofortified maize grain varieties

			μg/g dry weight						
			β-carotene isomers						
Maize variety	Zeaxanthin[a]	β-cryptoxanthin[a]	All-*trans*[a]	9-*cis*[a]	Other cis[ab]	Total cis[ac]	Total β-carotene[ad]	Total provitamin A carotenoids[ae]	Total carotenoids[af]
CC-37 (white)	< 0.3[g] (0.0)[h]a	0.1 (0.0)a	ND[i]	ND	ND	ND	ND	0.5 (0.0)a	0.4 (0.2)a
10 MAK 7-5	15.7 (2.12)b	3.7 (0.5)b	1.7 (0.2)b	0.6 (0.1)b	1.3 (0.2)b	1.9 (0.5)b	3.6 (0.5)b	7.3 (1.3)b	23.0 (6.3)b
10 MAK 7-7	18.7 (1.8)b	4.3 (0.4)b	1.5 (0.1)b	0.5 (0.0)b	1.5 (0.1)b	2.0 (0.7)b	3.5 (0.6)b	7.8 (1.6)b	26.5 (7.6)b
10 MAK 7-8	14.1 (0.8)b	4.8 (0.2)b	1.5 (0.1)b	0.5 (0.0)b	1.5 (0.0)b	2.0 (0.7)b	3.5 (0.6)b	8.3 (1.9)b	22.4 (5.6)b

[a] Values within the same column with different letters are significantly different at $p<0.05$ (Tukey test)
[b] Concentration of unidentified *cis* isomers of β-carotene
[c] Sum of 9-*cis* and other *cis* isomers
[d] Sum of all-*trans*, 9-*cis* and other *cis*
[e] Sum of β-cryptoxanthin and total β-carotene
[f] Sum of Zeaxanthin, β-cryptoxanthin and total β-carotene
[g] Mean of duplicate values
[h] Standard deviation
[i] ND = Not detected; detection limit for β-carotene was < 0.1 μg/100 g

Figure 4.4 shows the chromatogram of the white maize grain (CC-37). As expected, β-carotene was not detected in the white maize grain, while negligible amounts of zeaxanthin and β-cryptoxanthin were found (Table 4.2 and Figure 4.4).
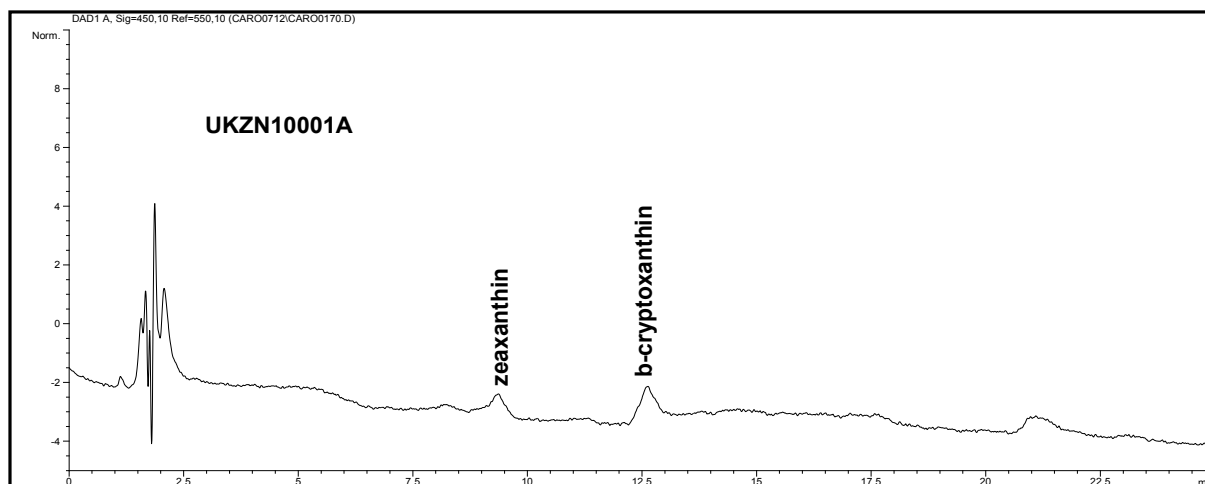
UKZN10001A = White maize (CC-37)

Figure 4.4   HPLC chromatogram of the carotenoids in an extract of white maize grain (CC-37)

Figures 4.5a, 4.5b and 4.5c show the chromatograms of the carotenoids present in the three varieties of provitamin A-biofortified maize grain.
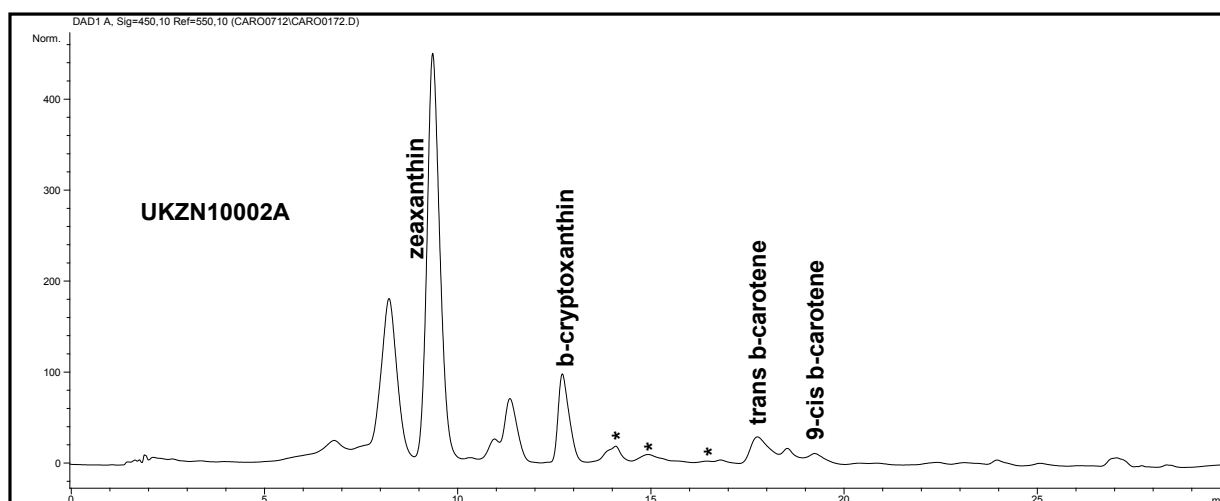
UKZN10002A = 10 MAK 7-5 (lightest orange grain)

Figure 4.5a   HPLC chromatogram of the carotenoids present in an extract of provitamin A-biofortified maize grain (10 MAK 7-5)
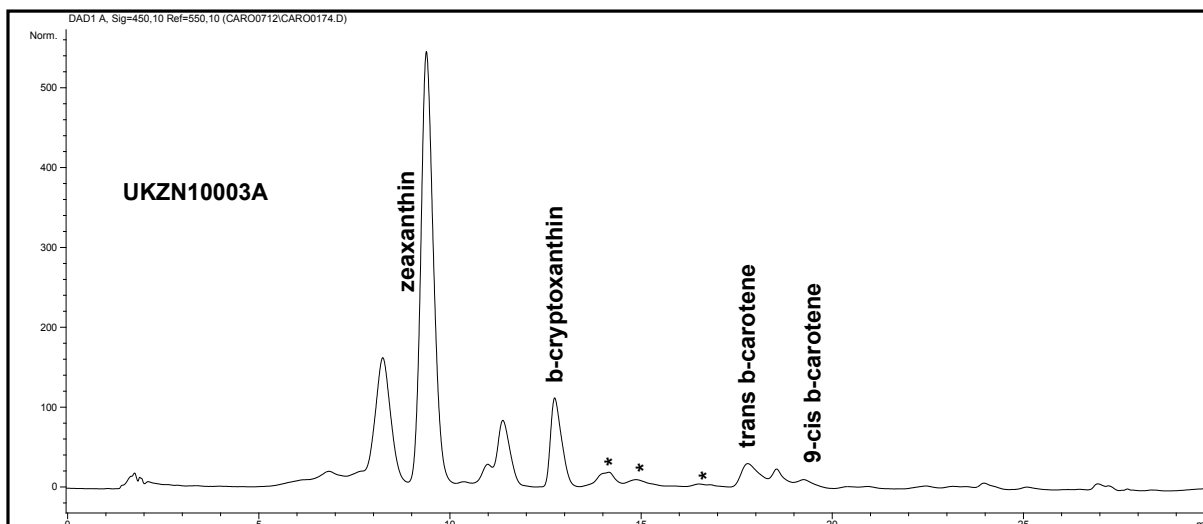
UKZN10003A = 10 MAK 7-7 (medium orange grain)

Figure 4.5b HPLC chromatogram of the carotenoids present in an extract of provitamin A-biofortified maize grain (10 MAK 7-7)
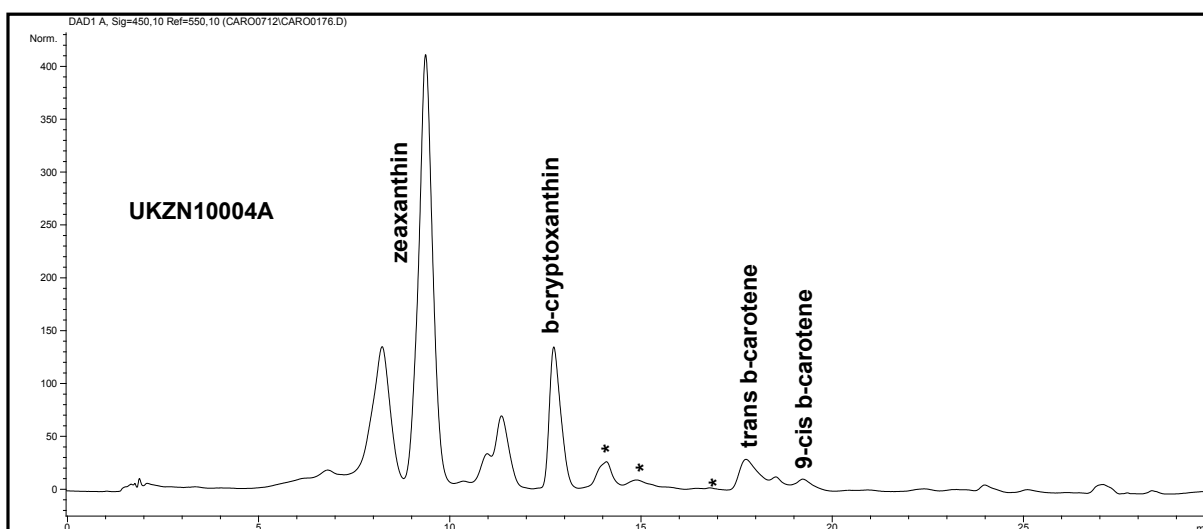
UKZN10004A = 10 MAK 7-8 (deepest orange grain)

Figure 4.5c HPLC chromatogram of the carotenoids present in an extract of provitamin A-biofortified maize grain (10 MAK 7-8)

The first peak eluted is likely to be lutein because it is one of the major carotenoids in maize. Lutein was not determined in the present study in order to limit expenditure on non-provitamin A carotenoids. All of the three maize forms had much higher concentrations of zeaxanthin, when compared to the concentration of the other carotenoids detected during the analysis. Apart from β-carotene, zeaxanthin also contributes to the orange colour of the

maize, but has no vitamin A activity. β-cryptoxanthin and β-carotene are both precursors of vitamin A.

Mass spectral analysis of the carotenoid extracts revealed four different β-carotene isomers: 9-*cis* β-carotene; 13-*cis* β-carotene; 9, 13-*dicis* β-carotene, and 13, 15-*di-cis* β-carotene. The 15-*cis* isomer was not detected with the specific experimental conditions applied in this study. The 9-*cis* isomer was tentatively identified using the mass spectrum isomer profile, the UV spectra and methods described by Muzhingi *et al* (2008a) and Lacker *et al* (1999). An authentic standard is required to confirm the 9-*cis* isomer peak. Although three remaining β-carotene isomers (indicated by * in Figures 4.5a, 4.5b and 4.5c) were identified in the extracts, individual isomer identification and quantification was not possible and hence a total concentration of the unidentified isomers is reported.

The total provitamin A carotenoid concentration in the provitamin A-biofortified maize grain ranged from 7.30-8.30 μg/g DW, which was higher when compared to the concentration (0.25-2.5μg/g DW) generally reported for typical yellow maize varieties (Nuss & Tanumihardjo 2010), but still lower when compared to the current breeding target for biofortified maize as set by HarvestPlus (15 μg/g DW of provitamin A) (Ortiz-Monasterio *et al* 2007). These results indicate that additional research is required to improve the concentration of carotenoids in maize hybrids, in order to reach the concentration set by HarvestPlus. This can be achieved by further recombination of the lines used in these hybrids.

β-cryptoxanthin (3.7-4.8 μg/g DW) was the most abundant provitamin A carotenoid present in the provitamin A-biofortified maize grain samples. Similar results were also reported by Lozano-Alejo *et al* (2007). However, the range of β-cryptoxanthin values reported in the present study is significantly higher. In contrast, a study by Li *et al* (2007) showed considerably less β-cryptoxanthin concentration, when compared to that of β-carotene, in high β-carotene containing maize. Yet, it has to be emphasised that although β-cryptoxanthin is present in higher concentrations than β-carotene in many varieties or breeding lines of provitamin A-biofortified maize, it has only one-half of the provitamin A activity of β-carotene (Kimura *et al* 2007). The total β-carotene content in the maize grain samples ranged from 3.4-3.6 μg/g DW, which was 5 times higher than the mean concentration reported in a survey of maize lines by Kurilich & Juvick (1999). Overall results indicate that our research programme has made some significant progress in developing recombinant inbred maize

lines with significant concentrations of carotenoids through conventional selection on the basis of grain colour intensity. The most predominant β-carotene isomer in the raw grain samples was the all-*trans* isomer (1.5-1.7 µg/g DW), which was higher in concentration when compared to the concentration reported in the study by Lozano-Alejo *et al* (2007). Further breeding progress that approximates the concentrations as envisaged by HarvestPlus could be achieved by combining molecular breeding tools with conventional processes in the future.

Nutrients other than provitamin A carotenoids

Table 4.3 shows the content of nutrients other than provitamin A in provitamin A-biofortified maize grain varieties.

Table 4.3 Content of nutrients other than provitamin A in provitamin A-biofortified maize grain varieties

Number	Variety	Moisture (%)[a]	Starch (g/100g)[a]	Fat (g/100g)[a]	Protein (g/100g)[a]	Iron (mg/100g)[a]	Zinc (mg/100g)[a]	Phosphorus (mg/100g)[a]
1	CC-37[ψ]	17.1[b](0.1)[c]l	59.4 (0.3)b	3.0 (0.0)a	10.7 (0.1)ab	5.90 (0.00)l	2.12 (0.08)a-f	393.97 (17.17)fg
2	10 MAK 7-1	12.1(0.1)de	**56.2** (0.2)ab	**3.9** (0.1)bc	**13.6** (0.5)lo	**4.63** (0.04)jk	2.24 (0.08)c-g	378.18 (12.87)c-g
3	10 MAK 7-2	11.3 (0.4)c	**54.3** (0.1)a	**4.7** (0.1)h-k	**13.4** (0.1)j-m	**4.91** (0.05)jk	2.27 (0.16)e-g	380.22 (1.29)c-g
4	10 MAK 7-3	11.9 (0.1)d	**55.6** (0.9)a	**5.2** (0.1)m-p	**11.9** (0.1)c-f	**4.67** (0.03)jk	1.83 (0.00)a-c	355.25 (1.34)b-h
5	10 MAK 7-5	10.5 (0.1)bc	**54.3** (0.0)a	**4.3** (0.1)c-g	**12.4** (0.1)f-h	**5.03** (0.15)k	1.97 (0.08)a-e	360.68 (0.26)b-g
6	10 MAK 7-7	9.4 (0.3)a	**55.0** (0.3)a	**4.3** (0.0)j-m	**13.5** (0.1)j-m	**4.88** (0.15)jk	2.23 (0.08)c-g	396.66 (2.05)fg
7	10 MAK 7-8	11.3 (0.1)c	**55.4** (0.7)a	**4.2** (0.0)b-f	**13.2** (0.1)h-k	**4.96** (0.07)jk	**2.90** (0.08)i	409.57 (11.08)g
8	10 MAK 7-9	14.5 (0.4)k	57.0 (0.0)ab	**4.0** (0.1)b-d	**11.2** (0.0)bc	4.96 (0.07)l	**2.71** (0.00)hi	396.52 (13.24)fg
9	10 MAK 7-10[*]	14.1 (0.1)k	57.2 (0.3)ab	**3.9** (0.0)a	**12.1** (0.1)ef	**4.52** (0.15)j	2.25 (0.00)c-g	**329.42** (7.01)a-c
10	KPPVAH-1	13.4(0.0)ij	**70.3** (1.7)d-i	**4.7** (0.1)i-k	**14.2** (0.0)no	**2.63** (0.08)c-g	2.27 (0.00)d-g	357.12 (9.63)b-g
11	KPPVAH-2	12.8 (0.0)f-h	**70.7** (0.3)e-i	**4.5** (0.1)f-i	**15.3** (0.0)p	**2.11** (0.01)ab	2.21 (0.08)b-g	389.31 (2.42)d-g
12	KPPVAH-3	12.9 (0.1)f-i	**72.0** (0.3)g-i	**5.0** (0.1)k-n	**13.5** (0.2)j-m	**2.98** (0.11)gh	2.21 (0.08)b-g	**338.08** (3.17)a-e
13	KPPVAH-4	12.6 (0.0)e-g	**70.8** (1.3)e-i	**5.0** (0.0)k-n	**12.9** (0.6)g-j	**2.63** (0.11)c-g	2.43 (0.08)f-h	365.60 (1.41)c-g
14	KPPVAH-5	13.1 (0.0)g-j	**69.1** (0.3)c-h	**4.4** (0.0)e-h	**13.5** (0.2)j-m	**2.63** (0.18)c-g	2.21 (0.08)b-g	366.17 (3.01)c-g
15	KPPVAH-6	13.3 (0.0)h-j	**71.9** (1.0)g-i	**4.2** (0.2)b-f	**12.5** (0.0)f-i	**2.72** (0.07)e-g	2.16 (0.16)a-g	363.98 (1.58)c-g
16	KPPVAH-7	13.1 (0.0)g-j	**73.9** (1.3)i	**4.9** (0.1)h-k	10.3 (0.1)a	**3.48** (0.00)i	2.04 (0.00)a-f	**339.84** (47.06)a-f
17	KPPVAH-8	13.0 (0.1)g-j	**72.3** (0.7)hi	**4.9** (0.1)i-k	**12.3** (0.1)k-m	**2.61** (0.00)c-g	2.03 (0.33)a-f	359.96 (11.98)b-g
18	KPPVAH-9	13.5 (0.0)j	**71.5** (1.3)f-i	**4.5** (0.1)g-j	**14.0** (0.1)m-o	5.77 (0.04)l	2.28 (0.17)e-g	390.55 (4.08)e-g
19	KPPVAH-10	12.7 (0.0)f-h	**70.6** (0.8)e-i	**5.0** (0.0)i-l	**12.9** (0.1)k-n	**2.78** (0.12)fg	2.09 (0.08)a-f	366.11 (33.93)c-g
20	KPPVAH-11	12.9 (0.0)f-j	**70.2** (0.7)d-i	**4.1** (0.0)b-e	**13.5** (0.1)j-m	**3.51** (0.13)i	2.03 (0.00)a-f	389.15 (8.80)d-g
21	KPPVAH-12	13.2 (0.0)g-j	**68.7** (1.1)c-h	**4.5** (0.1)g-j	**14.3** (0.1)o	**3.34** (0.15)hi	2.33 (0.08)e-h	**336.55** (7.68)a-d
22	KPPVAH-13	12.6 (0.0)e-g	**66.9** (0.9)cd	**5.1** (0.2)l-p	**14.2** (0.1)no	**3.28** (0.07)hi	2.20 (0.08)b-g	**343.95** (6.58)a-g
23	KPPVAH-14	12.9 (0.0)f-i	**67.6** (0.0)c-e	**5.7** (0.0)r	10.3 (0.1)a	**2.33** (0.01)a-f	**1.75** (0.08)a	**308.51** (12.03)ab
24	KPPVAH-15	12.7 (0.0)f-h	**68.0** (0.9)c-f	**5.4** (0.0)p-r	**12.5** (0.0)f-h	**2.64** (0.06)d-g	2.09 (0.08)a-f	367.66 (6.07)c-g
25	KPPVAH-16	12.9 (0.0)f-i	**65.7** (2.7)c	**5.0** (0.1)k-o	**11.4** (0.0)b-d	**1.98** (0.11)a	**1.75** (0.08)a	**346.21** (4.98)a-h
26	KPPVAH-17	12.5 (0.0)ef	**70.0** (1.1)d-h	**5.3** (0.0)o-q	**11.5** (0.0)c-e	**2.11** (0.05)ab	1.97 (0.08)a-e	368.01 (5.20)c-g
27	KPPVAH-18	12.9 (0.0)f-h	**70.0** (0.9)d-h	**5.6** (0.0)qr	**12.0** (0.0)d-f	**2.14** (0.01)a-c	1.86 (0.08)a-d	**350.06** (12.92)b-h
28	KPPVAH-19	12.7 (0.1)f-h	**68.1** (0.1)c-f	**4.8** (0.0)j-m	**13.1** (0.1)h-l	**2.58** (0.13)b-g	**2.55** (0.08)g-i	393.54 (1.20)fg
29	KPPVAH-20	12.7 (0.1)f-h	**68.4** (0.4)c-g	**5.3** (0.2)n-q	**12.9** (0.0)g-j	**2.60** (0.14)b-g	**2.55** (0.08)g-i	382.06 (6.67)c-g
30	KPPVAH-21	12.8 (0.0)f-h	**67.5** (0.1)c-e	**4.8** (0.0)i-l	**13.0** (0.0)h-l	**2.69** (0.13)e-g	2.32 (0.08)e-h	377.89 (0.16)c-g
31	KPPVAH-22	12.8 (0.0)f-h	**69.8** (1.0)d-h	**4.9** (0.1)i-k	**12.5** (0.0)k-m	**1.90** (0.04)a	2.26 (0.00)d-g	356.96 (5.72)b-g
32	KPPVAH-23	13.1 (0.1)g-j	**71.1** (0.8)e-i	**4.3** (0.0)d-g	**12.0** (0.2)d-f	**2.28** (0.09)a-e	1.81 (0.00)ab	**296.44** (12.67)a
33	KPPVAH-25	12.7 (0.1)f-h	**70.9** (0.6)e-i	**4.0** (0.0)b-e	**13.6** (0.2)k-m	**2.16** (0.11)a-d	2.15 (0.16)a-g	357.82 (15.03)b-g
34	KPPVAH-26	13.3 (0.0)h-j	**70.0** (0.4)d-h	**4.7** (0.0)i-k	**11.5** (0.0)c-e	**2.07** (0.11)a	2.27 (0.0)d-g	**336.50** (6.78)a-d
Grand mean		12.6[d] (0.9)[e]	**66.4** (6.4)	**4.7** (0.5)	**12.8** (1.1)	**3.23** (1.14)	2.19 (0.26)	363.28 (26.56)

[a] Values within the same column with different letters are significantly different at p<0.05 (Tukey test); [b] Mean; [c] Standard deviation; [d] Mean of provitamin A-biofortified varieties; [e] Standard error; Figures in bold are significantly different from CC-37 (white maize) for that nutrient (Dunnett Test, p significant at < 0.05); [ψ] White maize variety (control); [*] Reference yellow/orange maize variety

The starch content of the provitamin A-biofortified maize varied widely across the biofortified varieties; it ranged from 54.3-73.9 g/100 g (mean = 66.4 g/100g). The fat and protein content of the provitamin A-biofortified varieties also varied across variety, but within a narrower range, 3.9-5.7 g/100 g (mean = 4.7 g/100g) and 10.3-15.3 g/100 g (mean = 12.8 g/100g), respectively. No reports of the contents of the above-mentioned macronutrients in provitamin A-biofortified maize could be found in the literature.

Table 4.3 shows that the level of iron in the CC-37 (white maize) variety (5.90 mg/100 g) was significantly higher than the mean iron level in the provitamin A-biofortified maize grain varieties (3.23 mg/100 g) ($p < 0.05$). On the other hand, the levels of starch, fat and protein were significantly lower in the CC-37 variety compared to the provitamin A-biofortified maize grain varieties ($p < 0.05$). The mean starch (66.4 g/100 g) and fat (4.7 g/100 g) values of the provitamin A-biofortified maize from this study are similar to those of normal maize, 71.3 g/100 g and 4.1 g/100 g, respectively (section 2.3.3.2) (Johnson 2000, p38). On the other hand, the mean protein values (12.8 g/100 g) in the biofortified varieties of this study were much higher than the values reported in normal white maize of 8.92-10.52 g/100 g by Machida *et al* (2010) and 8.7 g/100 g by Johnson (2000, p38).

The fact that the provitamin A-biofortified maize varieties had higher protein and fat content than the white maize is encouraging. It indicates that in addition to provitamin A, the biofortified maize varieties could also contribute towards protein, fat and overall energy intake which could also potentially help to alleviate Protein Energy Malnutrition (PEM). PEM is also a major nutritional and health problem in sub-Saharan Africa (De Onis & Blössner 2003). The higher protein, fat and energy content of the provitamin A-biofortified maize varieties may also help to improve the overall nutritional intake in sub-Saharan Africa, where low protein and energy staples (including cereal grains) form the basis of dietary intake.

Iron content varied widely across the biofortified maize, 1.90-5.77 mg/100 g (mean = 3.23 mg/100 g), whilst zinc content varied within a narrower range, 1.75-2.90 mg/100 g (mean = 2.19 mg/100 g). As reviewed earlier (section 2.3.3.2), Ortiz-Monasterio *et al* (2007) stated that the International Maize and Wheat Improvement Center (CIMMYT) had analysed maize samples that were biofortified with provitamin A. The authors stated that the iron and zinc content of the provitamin A-biofortified maize samples ranged from 1.1-3.9 mg/100 g and

1.5-4.7 mg/100 g, respectively, and the average iron and zinc contents were 2.0 mg/100 g and 2.5 mg/100 g, respectively. Thus, the provitamin A-biofortified maize varieties of this study had higher iron content than the CIMMYT samples, whilst their average zinc content was similar to that of the CIMMYT samples. However, the iron and zinc values of the biofortified maize varieties of this study and those of the CIMMYT maize samples are still lower than the HarvestPlus target values of > 6.0 mg/100 g for both iron and zinc in biofortified maize (Ortiz-Monasterio *et al* 2007). The phosphorus levels in the biofortified maize varied widely across varieties (range: 296.44 - 409.57 mg/100 g; mean = 363.28 mg/100 g) and these values were comparable with that of the control white maize variety (CC-37) (393.97 mg/100 g). The phosphorus levels of the biofortified maize varieties were much higher than the levels of iron and zinc in the same maize varieties.

The iron values of the provitamin A-biofortified maize varieties of this study were lower than that of the white maize variety (CC-37) (control). On the other hand, the zinc values of the biofortified maize varieties compared well with that of the control variety. Although lower than that of the control (white maize), the mean values of iron in the provitamin A-biofortified maize varieties reported in this study are higher than the values reported by Šimić *et al* (2009), Oikeh *et al* (2004), Oikeh *et al* (2003a) and Oikeh *et al* (2003b) in normal white maize varieties. The mean zinc values reported in this study are also higher than the mean zinc values reported in studies by Šimić *et al* (2009), Oikeh *et al* (2004), Oikeh *et al* (2003a) and Oikeh *et al* (2003b) in normal white maize varieties. The fact that phosphorus was found in much higher levels compared to iron and zinc in this study is in line with the fact that phosphorus is the most abundant mineral found in maize (FAO 1992).

Approximately 1.5% and 1.4% of deaths worldwide are attributed to iron and zinc deficiency, respectively (WHO 2002b). In Africa, iron and zinc deficiency are also important contributors to the burden of disease (WHO 2002b). This is compounded by the fact that maize, which is an important staple food in Africa, contains low levels of iron and zinc (Šimić *et al* 2009; McCann 2005, p1). Furthermore, the absorption of iron and zinc in the human gastrointestinal tract is limited by certain antinutrients such as phytate and tannins found in plants (White & Broadley 2005; Welch & Graham 2004; Mendoza 2002). Phosphorus values are higher than the mean values reported by Bressani *et al* (1989) (299.6 ± 57.8 mg/100 g) in normal white maize varieties. It is noted that the high total phosphorus content of the provitamin A-biofortified maize varieties could also indicate high phytate

content since 65-80% of the phosphorus in maize is in the form of phytate (Raboy 1997, p445). That would imply a low bioavailability of the phosphorus, but this was not investigated in this study.

Overall, Table 4.3 shows that the provitamin A-biofortified maize varieties are superior sources of starch, fat and protein, compared to white maize. In order to account for the shortcomings in the nutritional composition of the provitamin A-biofortified maize varieties, food products made from this maize should be combined and complemented with foods that are good sources of the lacking nutrients.

Univariate analysis of variance (Table 4.4) showed that the maize variety had an effect on the starch, fat, protein, iron, zinc and phosphorus content of the provitamin A-biofortified maize varieties ($p = 0.000$).

Table 4.4 Effect of variety on the content of nutrients other than provitamin A in provitamin A-biofortified maize grain varieties

Nutrient	df	F	P value[a]
Starch	32	102.43	0.000
Fat	32	70.06	0.000
Protein	32	91.28	0.000
Iron	32	188.55	0.000
Zinc	32	13.10	0.000
Phosphorus	32	8.130	0.000

[a] Univariate analysis of variance, p significant at < 0.05
F = The mean square for each main effect or interaction divided by the residual mean square
df = Degrees of freedom

Levels of nutrients in the maize grain, including protein and minerals (e.g. iron and zinc) are affected by many complex factors such as genotype, soil properties, environment conditions and nutrient interactions (House 1999). Large variations in iron and zinc concentrations in maize kernels have been reported in normal white maize (Bänziger & Long 2000). Šimić *et al* (2009) found that mineral concentrations were affected by environmental conditions, while

Oikeh *et al* (2004) found that genotype x environmental interactions (GxE), varietal main effects and environment main effects contributed to variation in mineral content of maize grain. Although the influence of soil properties and environmental conditions on mineral content were not determined in this study, univariate analysis of variance found that maize variety did influence iron and zinc content of the provitamin A-biofortified maize grain varieties ($p<0.005$) (Table 4.4).   The results of this study suggest that the nutritional composition of provitamin A-biofortified maize can be partly manipulated by hybridization during conventional breeding.

Amino acids

Because of cost limitations, only seven of the 32 provitamin A-biofortified maize varieties were analysed for amino acid composition (Table 4.5a).

Table 4.5a   Amino acid composition of maize grain (g/100g, DW)

Varieties	Protein (g/100g)	Essential amino acids							Non-essential amino acids							
		His	Thr	Val	Isoleu	Leu	Phe	Lys	Asp	Glu	Ser	Gly	Arg	Ala	Pro	Tyr
CC -37[ψ]	10.7[a]	0.44	0.44	0.56	0.40	1.41	0.60	0.39	0.67	2.18	0.65	0.49	0.61	0.87	1.30	0.40
	(0.1)[b]	(0.02)	(0.01)	(0.02)	(0.00)	(1.11)	(0.00)	(0.00)	(0.06)	(0.16)	(0.04)	(0.05)	(0.00)	(0.01)	(0.19)	(0.01)
10 MAK 7-1	13.6	0.43	**0.52**	0.66	**0.49**	**1.79**	**0.73**	**0.29**	0.77	**2.68**	**0.75**	0.47	0.54	**1.15**	1.40	**0.48**
	(0.5)	(0.01)	(0.01)	(0.02)	(0.00)	(1.14)	(0.02)	(0.03)	(0.06)	(0.18)	(0.02)	(0.04)	(0.01)	(0.10)	(0.03)	(0.04)
10 MAK 7-2	13.4	0.42	**0.54**	**0.68**	**0.48**	**1.80**	**0.70**	**0.28**	0.77	**2.69**	**0.74**	0.51	0.59	**1.16**	1.38	0.45
	(0.1)	(0.04)	(0.00)	(0.00)	(0.01)	(0.04)	(0.02)	(0.00)	(0.04)	(0.08)	(0.04)	(0.06)	(0.01)	(0.11)	(0.12)	(0.02)
10 MAK 7-3	11.9	0.38	0.45	0.62	**0.45**	1.61	**0.66**	**0.30**	0.75	2.45	0.67	0.43	0.55	**1.04**	1.22	0.36
	(0.1)	(0.00)	(0.00)	(0.07)	(0.02)	(0.10)	(0.04)	(0.01)	(0.06)	(0.22)	(0.00)	(0.02)	(0.01)	(0.01)	(0.03)	(0.00)
10 MAK 7-5	12.4	0.39	**0.50**	0.64	**0.47**	**1.67**	**0.70**	**0.30**	0.78	2.52	0.70	0.44	0.57	**1.04**	1.25	0.41
	(0.1)	(0.00)	(0.00)	(0.05)	(0.01)	(0.06)	(0.02)	(0.02)	(0.02)	(0.18)	(0.01)	(0.02)	(0.01)	(0.04)	(0.06)	(0.05)
10 MAK 7-7	13.5	0.42	**0.54**	**0.69**	**0.51**	**1.96**	**0.75**	**0.32**	**0.87**	**3.03**	**0.74**	0.46	0.61	**1.15**	1.40	0.46
	(0.1)	(0.03)	(0.02)	(0.01)	(0.01)	(0.01)	(0.00)	(0.00)	(0.00)	(0.03)	(0.00)	(0.00)	(0.02)	(0.01)	(0.02)	(0.00)
10 MAK 7-8	13.2	0.40	**0.52**	0.63	**0.51**	**2.01**	**0.79**	**0.29**	**0.86**	**2.95**	**0.75**	0.43	**0.53**	**1.14**	1.31	**0.51**
	(0.1)	(0.00)	(0.01)	(0.02)	(0.00)	(0.03)	(0.01)	(0.01)	(0.00)	(0.04)	(0.00)	(0.00)	(0.00)	(0.01)	(0.01)	(0.03)
10 MAK 7-9	11.2	**0.38**	0.46	0.57	**0.44**	**1.68**	**0.66**	**0.32**	0.77	2.60	0.67	0.44	0.57	1.00	1.19	0.42
	(0.0)	(0.00)	(0.01)	(0.03)	(0.02)	(0.02)	(0.01)	(0.00)	(0.02)	(0.00)	(0.00)	(0.00)	(0.02)	(0.02)	(0.03)	(0.01)
10 MAK 7-10[*]	12.1	0.46	0.47	0.56	0.42	1.41	0.59	**0.28**	0.67	2.32	0.65	0.45	0.67	0.94	1.28	0.37
	(0.1)	(0.01)	(0.02)	(0.01)	(0.01)	(0.01)	(0.01)	(0.02)	(0.02)	(0.09)	(0.03)	(0.02)	(0.05)	(0.02)	(1.00)	(0.00)

[a] Mean; [b] Standard deviation; Figures in bold are significantly different from CC-37 (white maize) for that amino acid (Dunnett test, p significant at < 0.05); [ψ] White maize variety (control); [*] Reference yellow/orange maize variety

Table 4.5a shows that the provitamin A-biofortified maize varieties had a higher concentration of most of the essential amino acids (threonine, valine, isoleucine, leucine and phenylalanine) compared to the white maize (CC-37). However, the levels of histidine and lysine were generally lower in the provitamin A-biofortified maize varieties compared to the white maize (CC-37). With the non-essential amino acids, the levels of aspartic acid, glutamic acid, serine and alanine were generally higher in the provitamin A-biofortified maize varieties, while the levels of glycine and arginine were higher in the white maize (CC-37). The normal white maize is known to be nutritionally deficient in lysine, while its leucine content is high (FAO 1992). However, the results of this study show that the lysine levels were higher in the white maize (CC-37) compared to the provitamin A-biofortified maize varieties while the leucine levels were lower in the white maize (CC-37) compared to the provitamin A-biofortified maize varieties. Table 4.5b shows the comparison of essential amino acid concentration with the pattern of essential amino acid requirements. The concentrations of all the essential amino acids, except lysine, in all the provitamin A-biofortified maize varieties and the white variety were generally higher than the pattern of amino acid requirements for all age groups. The provitamin A-biofortified maize varieties were generally more nutritionally sufficient in the essential amino acids than the white maize variety. The lysine concentration in all maize varieties was consistently lower than the pattern of amino acid requirements in all age groups.

These results are in agreement with the fact that maize is a poor source of lysine (FAO 1992). In order to overcome the lysine deficiency in provitamin A-biofortified maize food products, the food products should be consumed with food sources that are rich in lysine, using the concept of complementary proteins. With complementary proteins the amino acid profiles complement each other such that the essential amino acids missing from one food source are supplied by other food sources (Whitney & Rolfes 2011, p188). Examples of inexpensive, good food sources of lysine that can be eaten with provitamin A-biofortified maize in order to improve overall lysine intake include legumes and eggs (Whitney & Rolfes 2011, p188; King & Burgess 1993, pp24-25). To overcome lysine and tryptophan deficiency in normal maize, researchers at CIMMYT have produced Quality Protein Maize (QPM) (Prasanna *et al* 2001). The QPM has been shown to contain 55% more tryptophan, 30% more lysine, 38% less leucine and has a higher biological value (80%) than that of normal maize (Bressani 1995; Paes & Bicudo 1995; Graham *et al* 1980).

Machida *et al* (2010) reported protein levels of 9.19-11.15 g/100 g in QPM, compared to 8.92-10.52 g/100 g in normal white maize. QPM varieties have already been released in many countries in Latin America, Africa and Asia with the aim of improving the amino acid balance of maize consumers (Prasanna *et al* 2001). Therefore there is an opportunity for breeding to stack genes for QPM with those of provitamin A carotenoids to enhance maize nutrition.

Table 4.5b Essential amino acid composition of maize grain (g/100g, DW) and comparison of essential amino acid concentration with the pattern of essential amino acid requirements

Varieties	Protein (g/100g)	Essential amino acids						
		His	Thr	Val	Isoleu	Leu	Phe	Lys
CC 37[ψ]	10.7[a]	0.44[b]	0.44	0.56	0.40	1.41	0.60	0.39
		41[c]	41	52	37	132	56	36
10 MAK 7-1	13.6	0.43	0.52	0.66	0.49	1.79	0.73	0.29
		32	38	49	36	132	54	21
10 MAK 7-2	13.4	0.42	0.54	0.68	0.48	1.80	0.70	0.28
		31	40	51	36	134	52	21
10 MAK 7-3	11.9	0.38	0.45	0.62	0.45	1.61	0.66	0.30
		32	38	52	38	135	55	25
10 MAK 7-5	12.4	0.39	0.50	0.64	0.47	1.67	0.70	0.30
		31	40	52	38	135	56	24
10 MAK 7-7	13.5	0.42	0.54	0.69	0.51	1.96	0.75	0.32
		31	40	51	38	145	56	24
10 MAK 7-8	13.2	0.40	0.52	0.63	0.51	2.01	0.79	0.29
		30	39	48	39	152	60	22
10 MAK 7-9	11.2	0.38	0.46	0.57	0.44	1.68	0.66	0.32
		34	41	51	39	150	59	29
10 MAK 7-10[*]	12.1	0.46	0.47	0.56	0.42	1.41	0.59	0.28
		38	39	46	35	117	49	23
Pattern of amino acid requirements (mg/g protein requirement)[d]								
0.5 years		20	31	43	32	66	N/A	57
1-2 years		18	27	42	31	63	N/A	52
3-10 years		16	25	40	31	61	N/A	48
11-14 years		16	25	40	30	60	N/A	48
15-18 years		16	24	40	30	60	N/A	47
> 18 years		15	23	39	30	59	N/A	45

[a] g/100g, dry weight; [b] Amino acid content (g/100g, db); [c] Amino acid concentration (mg/g protein; rounded off to a whole number; [d] WHO (2002a); N/A =Not available; [ψ] White maize variety (control); [*] Reference yellow/orange maize variety

4.3.2   Maize grain quality

Results of maize grain quality are shown in Table 4.6. The provitamin A-biofortified varieties had higher hectolitre mass and milling index values compared to the white maize (CC-37) and the reference yellow/orange maize (10 MAK 7-10). This indicates that the provitamin A-biofortified varieties have a better milling quality compared to the white maize variety (CC-37) and the reference yellow/orange maize variety (10 MAK 7-10). The maize varieties CC-37 (control) and 10 MAK 7-10 (reference) had a higher percentage of kernels with stress cracks compared to the provitamin A-biofortified maize varieties, which indicates that their grains were of inferior quality.

Table 4.6 shows that the maize grain varieties were infected with the following fungi: *Penicillium* spp., *Fusarium* spp., and the specific species of *Fusarium*, *Fusarium oxysporum*. The provitamin A-biofortified maize varieties 10 MAK 7-7 (50-72% infected), 10 MAK 7-9 (56-88% infected) and 10 MAK 7-10 (58-90% infected) had higher fungal infection levels than the white variety CC-37 (control) (28-56% infected). *Penicillium* spp. was the most predominant fungus infecting the maize grain (40-90% grains infected), followed by *Fusarium oxysporum* (8-80% grains infected) and *Fusarium* spp. (6-60% grains infected). Although it is expected to observe greater resistance to fungal infection in cereal grains with greater grain hardness (higher milling index) (Audilakshmi *et al* 1999; Jambunathan *et al* 1992) and higher protein content (Bueso *et al* 2000; Rodríguez-Herrera *et al* 1999; Kumari *et al* 1994; Kumari *et al* 1992), that was not the case in this study. Other factors could have caused the lower resistance of the biofortified maize varieties to fungal infection. The biofortified maize varieties had higher grain fat content than the white variety. The fat content of the biofortified maize varieties could have contributed to their lower resistance to fungi similar to findings of Ratnavathi & Sashidhar (2003) working with sorghum grain.

Table 4.6   Maize grain quality attributes

Varieties	1000 kernel weight (g)	Hectolitre mass (kg/hl)	Stress cracks (%)	Milling index	Fungal infection (% maize grains infected)		
					Penicillium spp.	*Fusarium* spp.	*Fusarium oxysporum*
CC-37	507.4	88.2[a] (0.5)[b]	23	77.7 (0.9)	48	28	56
10 MAK 7-1	429.1	93.6 (0.1)	0	96.6 (1.3)	66	14	24
10 MAK 7-2	410.8	93.5 (0.3)	0	101.9 (1.3)	40	32	30
10 MAK 7-3	355.5	91.7 (0.2)	21	104.6 (1.9)	68	18	18
10 MAK 7-5	328.8	94.6 (0.0)	0	104.0 (3.5)	48	6	8
10 MAK 7-7	444.1	93.1 (0.2)	1	101.8 (3.1)	72	50	56
10 MAK 7-8	376.7	94.9 (0.3)	1	100.9 (2.1)	62	20	22
10 MAK 7-9	345.1	96.3 (0.2)	13	98.6 (1.6)	88	60	56
10 MAK 7-10	677.9	88.2 (0.6)	17	70.5 (4.7)	90	58	80

[a] Mean
[b] Standard deviation

Fusarium spp. is the main pathogenic fungal genus causing spoilage of maize in the ear while *Penicillium* spp. can be found invading maize preharvest (Pitt & Hocking 1999, pp483-484). A study by Montes *et al* (2009) also reported that the major fungal genera found in maize hybrids were *Fusarium* spp. and *Penicillium* spp. Overall, the white maize hybrids were found to be less susceptible to pathogens and had a higher percentage of healthy grain compared to the yellow maize hybrids (Montes *et al* 2009). Roigé *et al* (2009) also found that *Penicillium* spp. and *Fusarium* spp. were the most common fungi isolated from maize. *Penicillium* spp. is often classified as storage fungi while *Fusarium* spp. is regarded as field fungi (Logrieco *et al* 2003). The field and storage fungi may cause grain discolouration, reduced germinability and overall grain deterioration as well as heating, mustiness, shriveling and rotting (Agarwal & Sinclair 1987, pp4-12; Christensen & Kaufmann 1974). This reduces the nutritional value of the maize and makes it unfit for human consumption (Fandohan *et al* 2003). Both *Fusarium* and *Penicillium* genera contain species that produce mycotoxins; some of which can be carcinogenic, mutagenic or teratogenic (Bennet & Klich 2003; Bauduret 1990; Abramson *et al* 1983). Mycotoxins can also cause large economic losses for many commercial sectors including crop producers, animal breeders and food and animal feed processors (Jestoi *et al* 2004; Miller 1999, pp1698-1706). Therefore, biofortified maize varieties whose grain has low resistance to fungi should be improved because fungal infection impacts negatively on grain quality and safety.

4.4   Conclusions

This study shows that various carotenoids, including zeaxanthin, β-cryptoxanthin and β-carotene isomers occur in provitamin A-biofortified maize grain varieties. Thus, it seems feasible to improve maize varieties for significant levels of provitamin A in a breeding programme. However, only a few varieties can be screened due to prohibitive costs and the long duration of the process. Priority should be given to developing a rapid and cheap assay for provitamin A analysis. Methods such as these could especially assist researchers in developing countries, where the biofortified maize technology is needed most. The quality of the grain of the provitamin A-biofortified maize varieties was superior to that of the white maize, although the biofortified varieties showed higher levels of fungal infection. Compared to the white maize grain, the provitamin A-biofortified grain varieties contained higher levels of starch, fat and protein and were superior sources of all essential amino acids, except for histidine and lysine. The provitamin A-biofortified maize varieties were lower in iron compared to the white maize, whilst the zinc and phosphorus levels compared well in the

biofortified and white maize varieties. In this study, variety was found to influence the nutritional composition of the provitamin A-biofortified maize grain varieties, including carotenoid composition. Thus, in terms of grain quality and nutritional composition, provitamin A-biofortified maize varieties would be, overall, a better food source than the normal white maize. Further breeding is required to increase the provitamin A carotenoid levels reported in this study, in order to reach the HarvestPlus Challenge Program target level of 15 μg/g DW of total provitamin A.

References

Abramson D, Mills JT, Boycott BR (1983). Mycotoxins and mycoflora in animal feedstuffs in western Canada. **Canadian Journal of Comparative Medicine** 47: 23-26.

Agarwal VK, Sinclair JB (1987). **Principles of Seed Pathology**, Vol 1. Boca Raton: CRC Press.

American Association of Cereal Chemists (AACC) (2000). **Approved Methods**, 10th ed. AACC Method 55-10 Test Weight per Bushel. St Paul: AACC International.

Association of Official Analytical Chemists (AOAC) International (2002). **Official Methods of Analysis of AOAC International**, 17th ed. Volume I and II. Maryland: AOAC International.

Audilakshmi S, Stenhouse JW, Reddy TP, Prasad MVR (1999). Grain mould resistance and associated characters of sorghum genotypes. **Euphytica** 107: 91-103.

Bänziger M, Long J (2000). The potential for increasing the iron and zinc density of maize through plant breeding. **Food and Nutrition Bulletin** 20: 397-400.

Bauduret P (1990). A mycological and bacteriological survey on feed ingredients and mixed poultry feeds in Reunion Island. **Mycopathologia** 109: 157-164.

Bennett JW, Klich M (2003). Mycotoxins. **Clinical Microbiology Reviews** 16: 497-516.

Bressani R (1995). In: **Proceedings of the International Symposium on Quality Protein Maize**, EMBRAPA/CNPMS, Sete Lagaos, Brazil.

Bressani R, Breuner M, Ortiz MA (1989). Contenidodefibraacidoy neutron-detergente y de minerals menores en maiz y su tortilla. **Archivos Latinoamericanos de Nutrición** 39: 382-391.

Bueso FJ, Waniska RD, Rooney LW, Bejosano FP (2000). Activity of antifungal proteins against mold in sorghum caryopses in the field. **Journal of Agricultural and Food Chemistry** 48: 810-816.

Christensen CM, Kaufmann HH (1974). Microflora. In: Christensen CM, ed. **Storage of Cereal Grains and their products**. St. Paul: AACC International.

De Onis M, Blössner M (2003). The World Health Organization global database on child growth and malnutrition: methodology and application. **International Journal of Epidemiology** 32: 518-526.

DeMan JM (1999). **Principles of Food Chemistry**, 3rd ed. Maryland: Aspen Publishers.

Dugan FM (2006). **The Identification of Fungi – an illustrated introduction with keys, glossary, and guide to literature**. St Paul: American Phytopathological Society.

Fandohan P, Hell K, Marasas WFO, Wingfield MJ (2003). Infection of maize by *Fusarium* species and contamination with fumonisin in Africa. **African Journal of Biotechnology** 2: 570-579.

Food and Agriculture Organization of the United Nations (FAO) (1992). **Maize in Human Nutrition**. Rome, Italy.

Giron HC (1973). Atomic Absorption Newsletter 12, 28. Perkin Elmer Atomic Spectrophotometer.

Graham GG, Placko RP, Maclean WC (1980). Nutritional value of normal, opaque-2 and sugary-2 opaque-2 maize hybrids for infants and children. 2. Plasma Free Amino Acids. **Journal of Nutrition** 110: 1070-1075.

HarvestPlus Brief (2006). **HarvestPlus: Breeding Crops for Better Nutrition**. Washington DC, USA.

House W (1999). Trace element bioavailability as exemplified by iron and zinc. **Field Crops Research** 60: 115-141.

International Institute of Tropical Agriculture (IITA) (2010b). **Giving tropical maize a vitamin-A boost**. IITA Annual Report. http://www.annualreport.iita.org (date accessed 24/4/2011).

Jambunathan R, Kherdekar MS, Stenhouse JW (1992). Sorghum grain hardness and its relationship to mold susceptibility and mold resistance. **Journal of Agricultural and Food Chemistry** 40: 1403-1408.

Jestoi M. Ritieni A, Rizzo A (2004). Analysis of the Fusarium mycotoxins fusaproliferin and trichothecenes in grains using gas chromatography mass spectrometry. **Journal of Agricultural and Food Chemistry** 52: 1464-1469.

Johnson LA (2000). Corn: The Major Cereals of the Americas, 2nd ed. In: Kulp K, Ponte JG, eds. **Handbook of Cereal Science and Technology**. New York: Marcel Dekker.

Kimura M, Kobori CN, Rodriguez-Amaya DB, Nestel P (2007). Screening and HPLC methods for carotenoids in sweetpotato, cassava and maize for plant breeding trials. **Food Chemistry** 100: 1734-1746.

King FS, Burgess A (1993). **Nutrition for developing countries**, 2nd ed. New York: Oxford University Press.

Kumari RS, Chandrashekar A, Shetty HS (1994). Antifungal proteins from sorghum endosperm and their effects on fungal mycelium. **Journal of the Science of Food and Agriculture** 66: 121-127.

Kumari RS, Chandrashekar A, Shetty HS (1992). Proteins in developing sorghum endosperm that may be involved in resistance to grain moulds. **Journal of the Science of Food and Agriculture** 60: 275-282.

Kurilich AC, Juvik JA (1999). Quantification of carotenoids and tocopherol antioxidants in *Zea mays*. **Journal of Agricultural and Food Chemistry** 47: 1948-1955.

Lacker T, Strohschein S, Albert K (1999). Separation and identification of various carotenoids by C_{30} reversed-phase High-Performance Liquid Chromatography coupled to UV and atmospheric pressure chemical ionization mass spectrometric detection. **Journal of Chromatography A** 854: 37-44.

Li S, Tayie FAK, Young MF, Rocheford T, White WS (2007). Retention of provitamin A carotenoids in high β-carotene maize (Zea mays) during traditional African household processing. **Journal of Agricultural and Food Chemistry** 55: 10744-10750.

Logrieco A, Bottalico A, Mulé G, Moretti A, Perrone G (2003). Epidemiology of toxigenic fungi and their associated mycotoxins for some Mediterranean crops. **European Journal of Plant Pathology** 109: 645-667.

Lozano-Alejo N, Carrillo GV, Pixley K, Palacios-Rojas N (2007). Physical properties and carotenoid content of maize kernels and its nixtamalized snacks. **Innovative Food Science and Emerging Technologies** 8: 385-389.

Machida L, Derera J, Tongoona P, MacRobert J (2010). Combining ability and reciprocal cross effects of elite quality protein maize inbred lines in subtropical environments. **Crop Science** 50: 1708-1717.

McCann JC (2005). **Maize and Grace: Africa's encounter with a New World Crop 1500-2000**, 1st ed. Cambridge: Harvard University Press.

Mendoza C (2002). Effect of genetically modified low phytic acid plants on mineral absorption. **International Journal of Food Science & Technology** 37: 759-767.

Menkir A, Liu W, White WS, Maziya-Dixon B, Rocheford T (2008). Carotenoid diversity in tropical-adapted yellow maize inbred lines. **Food Chemistry** 109: 521-529.

Miller JD (1999). Mycotoxins. In: Francis FJ, ed. **Encyclopedia of Food Science and Technology**. New York: John Wiley & Sons.

Montes GN, Reyes MCA, Montes RN, Cantu AMA (2009). Incidence of potentially toxigenic fungi in maize (*Zea mays* L.) grain used as food and animal feed. **CyTA-Journal of Food** 7: 119-125.

Muzhingi T, Yeum KJ, Russell RM, Johnson EJ, Qin J, Tang G (2008a). Determination of carotenoids in yellow maize, the effects of saponification and food preparations. **International Journal for Vitamin and Nutrition Research** 78: 112-120.

Nuss ET, Tanumihardjo SA (2010). Maize: a paramount staple crop in the context of global nutrition. **Comprehensive Reviews in Food Science and Food Safety** 9: 417-436.

Oikeh SO, Menkir A, Maziya-Dixon B, Welch R, Glahn RP (2003a). Assessment of concentrations of iron and zinc and bioavailable irons in grains of early-maturing tropical maize varieties. **Journal of Agricultural and Food Chemistry** 51: 3688-3694.

Oikeh SO, Menkir A, Maziya-Dixon B, Welch R, Glahn RP (2003b). Genotypic differences in concentration and bioavailability of kernel-iron in tropical maize varieties grown under field conditions. **Journal of Plant Nutrition** 26: 2307-2319.

Oikeh SO, Menkir A, Maziya-Dixon B, Welch R, Glahn RP, Gauch G (2004). Environmental stability of iron and zinc concentrations in grain of elite early-maturing tropical maize genotypes grown under field conditions. **Journal of Agricultural Sciences** 142: 543-551.

Ortiz-Monasterio JI, Palacios-Rojas N, Meng E, Pixley K, Trethowan R, Pena RJ (2007). Enhancing the mineral and vitamin content of wheat and maize through plant breeding. **Journal of Cereal Science** 46: 293-307.

Paes MCD, Bicudo MH (1995). In: **Proceedings of the International Symposium on Quality Protein Maize**, EMBRAPA/CNPMS, Sete Lagaos, Brazil.

Pitt JI, Hocking AD (1999). **Fungi and Food Spoilage**, 2nd ed. Maryland: Aspen Publishers.

Prasanna BM, Vasal SK, Kassahun B, Singh NN (2001). Quality Protein Maize. **Current Science** 81: 1308-1319.

Rabie CJ, Lübben A (1984). The mycoflora of sorghum salt. **South African Journal of Botany** 4: 251-255.

Rabie CJ, Lübben A, Marais GJ, Van Vuuren (1997). Enumeration of fungi in barley. **International Journal of Food Microbiology** 35: 117-127.

Raboy V (1997). Accumulation and Storage of Phosphate and Minerals. In: Larkins BA, Vasil IK, eds. **Cellular and Molecular Biology of Plant Seed Development**. Dordrecht: Kluwer Academic Publishers.

Raboy V, Below FE, Dickison DB (1989). Alteration of maize kernel phytic acid levels by recurrent selection for protein and oil. **Journal of Heredity** 80: 311-315.

Ratnavathi CV, Sashidhar RB (2003). Substrate suitability of different genotypes of sorghum in relation to Aspergillus infection and aflatoxin production. **Journal of Agricultural and Food Chemistry** 51: 3482-3492.

Rice AI, West KP, Black RE (2004). Vitamin A deficiency. In: **Comparative quantification of health risks**. http://www.who.int/publications/cra/chapters/volume1/0211-0256.pdf (accessed 25/10/2010).

Rodriguez-Amaya DB, Kimura M (2004). **HarvestPlus Handbook for Carotenoid Analysis**. Technical Monograph Series 2.

Rodríguez-Herrera R, Waniska RD, Rooney WL (1999). Antifungal proteins and grain mold resistance in sorghum with nonpigmented testa. **Journal of Agricultural and Food Chemistry** 47: 4802-4806.

Roigé MB, Aranguren SM, Riccio MB, Pereyra S, Soraci AL, Tapia MO (2009). Mycobiota and mycotoxins in fermented feed, wheat grains and corn grains in Southeastern Buenos Aires Province, Argentina. **Revista Iberoamericana de Micología**.

Shankar AH, Genton B, Semba RD (1999). Effect of vitamin A supplementation on morbidity due to *Plasmodium falciparum* in young children in Papua New Guinea: A randomized trial. **Lancet** 354: 203-209.

Šimić D, Sudar R, Ledenćan T, Jambrović A, Zdunić Z, Brkić I, Kovačević V (2009). Genetic variation of bioavailable iron and zinc in grain of a maize population. **Journal of Cereal Science** 50: 392-397.

Sommer A, West KP (1996). **Vitamin A deficiency: Health survival and vision**. New York: Oxford University Press.

Southern African Grain Laboratory (SAGL) (2007). **Industry Accepted Method for estimating Milling Index using NIT**. Pretoria, South Africa.

Southern African Grain Laboratory (SAGL) (2001). **Industry Accepted Method for Stress Crack Analysis of Maize Kernels**. Pretoria, South Africa.

Tanumihardjo SA (2008). Food-based approaches for ensuring adequate vitamin A nutrition. **Comprehensive Reviews in Food Science and Food Safety** 7: 373-381.

Taylor JRN, Duodu KG (2009) Applications for non-wheat testing methods. In: Cauvain SP, Young LS, eds. **The ICC Handbook of Cereals, Flour, Dough and Product Testing: Methods and Applications**. Pennsylvania: DEStech Publications.

Thomas JB, Kline MC, Gill LM, Yen JH, Duewer DL, Sniegoski LT, Sharpless KE (2001). Preparation and value assignment of standard reference material 968c fat-soluble vitamins, carotenoids, and cholesterol in human serum. **Clinica Chimica Acta** 305: 141-155.

United States Department of Agriculture and Foreign Agricultural Service (USDA/FAS) (2008). **Corn 2008: production, supply, demand database**.
http://www.pecad.fas.usda.gov/cropexplorer/ (date accessed 14/10/2010).

Villamor E, Fawzi WW (2000). Vitamin A supplementation: Implication for morbidity and mortality in children. **Journal of Infectious Diseases** 182: 122-133.

Welch RM, Graham RD (2004). Breeding for micronutrients in staple food crops from a human nutrition perspective. **Journal of Experimental Botany** 55: 353-364.

West CE (2000). Vitamin A and measles. **Nutrition Reviews** 58: 546-554.

West KP, Jr (2002). Extent of vitamin A deficiency among preschool children and women of reproductive age. **The Journal of Nutrition** 132: 2857-2866.

White PJ, Broadley MR (2005). Biofortifying crops with essential mineral elements. **Trends in Plant Science** 10: 586-593.

Whitney E, Rolfes SR (2011). **Understanding Nutrition**, 12th ed. Belmont: Wadsworth, Cengage Learning.

World Health Organization (WHO) (2002a). **Joint FAO/WHO/UNU Expert Consultation on Protein and Amino Acid requirements in Human Nutrition.** Technical Report Series No 935. Geneva, Switzerland.

World Health Organization (WHO) (2002b). **The World Health Report 2002: Reducing Risks, Promoting Healthy Life**. Geneva, Switzerland.

World Health Organization (WHO) (2003). **GEMS/Food Regional Diets: Regional Per Capita Consumption of Raw and Semi-processed Agricultural Commodities, Food Safety Department**. WHO Document Production Services, Geneva, Switzerland.

CHAPTER 5

RETENTION OF PROVITAMIN A CAROTENOIDS AND OTHER NUTRIENTS DURING PROCESSING OF PROVITAMIN A-BIOFORTIFIED MAIZE INTO POPULAR SOUTH AFRICAN MAIZE FOOD PRODUCTS[8]

Abstract

Provitamin A-biofortified maize may contribute to alleviating vitamin A deficiency (VAD), in developing countries. However, processing the maize into food products may result in significant reduction of its provitamin A content and other nutrients. The aims of this study were to assess the retention of carotenoids and other nutrients during processing of popular maize food products consumed in KwaZulu-Natal, South Africa. Milling provitamin A-biofortified maize into mealie meal resulted in a higher retention of carotenoids compared to milling into samp. Most of the other nutrients were well retained on milling, but there were substantial losses of fibre (75 ± 2.8%), fat (41.5 ± 5.8%), iron (77.2 ± 3.7%), zinc (32.9 ± 3.3%) and phosphorus (45.7 ± 3.5%) on milling into mealie meal and iron (62.8 ± 4.3%), zinc (25.5 ± 2.1%) and phosphorus (38.2 ± 2.3%) with milling into samp. The highest retention of provitamin A carotenoids was observed in cooked *phutu* and cooked samp, whilst cooking into thin porridge resulted in the lowest retention of provitamin A carotenoids. In *phutu*, 96.6 ± 20.3% β-cryptoxanthin and 95.5 ± 13.6% of the β-carotene were retained after cooking. In samp, 91.9 ± 12.0% β-cryptoxanthin and 100.1 ± 8.8% β-carotene; and in thin porridge, 65.8 ± 4.6% β-cryptoxanthin and 74.7 ± 3.0% β-carotene were retained after cooking. The retention of provitamin A in the maize food products seemed to be affected by both maize variety and maize food form ($p < 0.05$). Other nutrients were generally well retained on cooking of the three maize food products, except for substantial losses of fat (47.5 ± 3.1%) on cooking into thin porridge and iron (33.4 ± 9.7%) and phosphorus (36.2 ± 10.5%) on cooking into samp. This study demonstrates that provitamin A retention in maize is affected by the cooking method (and hence cooked food form) and therefore cooking methods that result in a good retention of provitamin A need to be identified and recommended. The results showed that a daily consumption of the usual portion size of each of the three maize food types (*phutu*, samp and thin porridge) made with provitamin A-biofortified maize has the potential

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to make a significant contribution towards meeting the Estimated Average Requirement (EAR)[9] for vitamin A, in all age and gender groups studied.

5.1   Introduction

Maize (*Zea mays*) is a staple food for more than 1.2 billion people in sub-Saharan Africa (SSA) and Latin America and is regarded as a vital crop in the perspective of global nutrition (IITA 2010a; Nuss & Tanumihardjo 2010). In sub-Saharan Africa, maize is a predominant staple. However, most of the maize that is produced and consumed is white and devoid of provitamin A carotenoids (Menkir *et al* 2008; Li *et al* 2007). This may partly explain why vitamin A deficiency (VAD) is a major public health problem in SSA (Nuss & Tanumihardjo 2010). VAD affects approximately 33 million preschool-age children in Africa (West 2002) and is responsible for an estimated 20-24% of child mortality from diarrhoea, measles and malaria and 3% of mortality from infectious diseases (Rice *et al* 2004). In South Africa, the number of children with VAD increased from 33% in 1994 to 64% in 2005 (Labadarios *et al* 2007; Labadarios & van Middelkoop 1995).

In an international effort to combat VAD, maize is one of the six staple crops that have been targeted for biofortification with provitamin A carotenoids by the HarvestPlus Challenge Program (Tanumihardjo 2008; HarvestPlus Brief 2006). Biofortification of maize varieties with provitamin A by conventional breeding is viewed as a potential long-term sustainable strategy to alleviate VAD in target groups (Howe & Tanumihardjo 2006a; Howe & Tanumihardjo 2006b; Nestel *et al* 2006). The current breeding target for maize as set by HarvestPlus is 15 µg/g dry weight (DW) of provitamin A (Ortiz-Monasterio *et al* 2007). An additional important aspect to consider when looking at the effectiveness of any biofortification strategy is the potential effect of food processing on the final provitamin A carotenoid content of the biofortified food products. Provitamin A carotenoids are sensitive and can be destroyed by environmental factors, such as heat, oxygen, light, and acidic conditions (Rodriguez-Amaya 1997; Gregory 1996, pp545-546). It is important to quantify the losses of provitamin A carotenoids during processing of provitamin A-biofortified maize. These losses should then be taken into account when setting targets for the provitamin A

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[9] The Estimated Average Requirement is one of four reference values that make up the Dietary Reference Intakes (DRIs). It is defined as the average daily nutrient intake level estimated to meet the requirement of half the healthy individuals in a particular life stage and gender group (Institute of Medicine 2000a, p3).

content of the maize. In addition, it is likely that other nutrients may also be lost due to processing and these losses should be quantified.

Although limited, data are available on the retention of provitamin A carotenoids during processing of biofortified maize. Li *et al* (2007) reported that only modest losses of provitamin A carotenoids in high β-carotene maize could be directly attributed to household processing steps in the preparation of African fermented maize porridges. The retention of β-carotene in the final, cooked products was 75.5 % for the fermented and 75.2% for the unfermented porridges, respectively. Muzhingi *et al* (2008a) investigated the effect of cooking on the carotenoid content of raw maize flour and observed an increase in carotenoid levels in all cooking methods, except baking. In contrast, the average retention of provitamin A carotenoids in provitamin A-biofortified maize following *nixtamilization* and frying, which are common processing methods used to prepare Mexican maize food products, was found to be only 64% (Lozano-Alejo *et al* 2007). These studies suggest that cooking methods have a significant effect on provitamin A retention in provitamin A-biofortified maize.

In South Africa, like in most countries in sub-Saharan Africa, maize is processed in several ways into a wide variety of food products. Usually, the processing steps involve milling of the maize grain into products of different particle size, followed by cooking of the milled products. Currently, there is a lack of data on the retention of provitamin A carotenoids and other nutrients when provitamin A-biofortified maize is processed into popular South African food products. This study therefore aimed to assess the retention of provitamin A carotenoids and other nutrients in provitamin A-biofortified maize during the preparation of popular maize foods consumed in KwaZulu-Natal, South Africa. The food products studied were *phutu* (a stiff porridge made from maize meal), thin porridge (porridge made from maize meal with a dry matter content of approximately 14%) and samp (broken maize grain), which are the most popular maize foods consumed by the rural African population in KwaZulu-Natal (Faber & Kruger 2005; Faber 2004; Faber *et al* 2001; Faber *et al* 1999).

5.2   Materials and Methods

5.2.1   Maize breeding and variety selection

The experimental maize varieties used in this study were produced as described in Chapter 3, section 3.2. The three maize hybrids that were used for the retention experiments in this chapter were 10 MAK 7-5 (lightest orange variety), 10 MAK 7-7 (medium orange variety)

and 10 MAK 7-8 (deepest orange variety). The three varieties were selected on the basis of grain colour, measured in terms of the Hunter *L*, *a*, *b* system as described in section 4.2.1.

5.2.2   Maize grain milling

Grain of the three provitamin A-biofortified maize varieties and the white maize variety (control) was milled into mealie meal (maize flour) (Figure 5.1a) and samp (Figure 5.1b). The milled products were used to prepare popular South African food products: *phutu* (a stiff porridge made from maize meal), thin porridge (porridge made from maize meal with a dry matter content of approximately 14%) and samp (broken maize grain). The maize grain was first cleaned using a grain cleaner (R.G Garvie and Sons, Agricultural Engineers, Aberdeen, Scotland, UK). The functional parts of the grain cleaner comprised a vibrating sieve and an aspirator. Grain moisture was adjusted to 15% (w/v) before milling into maize meal and samp. Samp was produced by milling the maize grain with a degerminator mill (Dayton Electric Manufacturing Company, Inc., Reston, Virginia, USA). The whole mill product coming out of the degerminator was collected. A pilot plant roller mill (Model MK 150, Roff Industries, Kroonstad, South Africa) with a three break system was used to mill the maize grain into super meal, maize grits, bran and fine meal. The three break system consisted of a set of three roller mills of decreasing roller gap size, which progressively broke up maize grain into smaller particles. Each roller mill had a set of sieves for separating the maize particles into mill fractions.   The mill fractions of larger particle size were manually transferred to the next roller mill for further size reduction. The super meal was the mill fraction which passed through a 495 µm aperture screen; it was collected from the last two roller mills.

Figure 5.1a   Raw mealie meal

Figure 5.1b   Raw samp

5.2.3   Preparation of maize food products

Three Black African women from a rural area in KwaZulu-Natal with experience in cooking the popular maize food products, *phutu*, thin porridge and samp were recruited to prepare these products for the study. The recipes and cooking procedures for all the three food products were standardised after several cooking trials. *Phutu* (Figure 5.2a) was prepared by bringing 280 mL of tap water to the boil. Two cups (268 g) of maize meal were added to the water and stirred as soon as the mixture reached boiling point. The *phutu* was allowed to stand on low heat for approximately 75 minutes with the lid on and occasional stirring. The thin porridge (Figure 5.2b) was prepared by bringing 8 cups (2,000 mL) of tap water to the boil. Two cups (268 g) of maize meal were added to two cups (500 ml) of cold water to make a paste, which was then added to the boiling water and stirred until it was smooth. The porridge was cooked on medium heat for 25 minutes with the lid on and occasional stirring. Two cups of samp (369 g) were soaked overnight in four cups (1,000 mL) of cold water. Four cups (1,000 mL) of boiling water were then added to the pre-soaked samp and boiled for an additional 135 minutes, with the lid on. An additional two cups (500 mL) of water were added to the samp (Figure 5.2c) during the cooking period. The temperature and pH of each of the three food products were recorded at the end of their cooking periods. Although sugar and salt are included in the standardised recipes [Appendix D (p174), Appendix E (p175) and Appendix F (p176)], no sugar or salt was included during the preparation of the food products in this research chapter, so as not to influence the nutritional composition.

Figure 5.2a   *Phutu*

Figure 5.2b   Thin porridge

Figure 5.2c   Cooked samp

5.2.4   Nutritional composition of milled and cooked maize products

The raw, milled and cooked maize products were analysed for their nutritional composition. Referenced methods were used in the analysis. Analytical reagents were used and analysis for nutrient content in a sample was repeated at least twice.

Sample preparation

General sample preparation involved freeze-drying of food products. Other sample preparation procedures were as per specific analysis method and are described specifically.

5.2.4.1 Carotenoids

Handling of samples for analysis

Sample handling was the same as that of whole maize grain samples described earlier (section 4.2.2.1). Raw milled and cooked maize samples were stored in amber glass bottles, which were flushed with nitrogen gas and then immediately placed into a cooler box. Ice packs were placed over the bottles and multi-layered newspaper sheets were used to insulate the cooler box. The cooler box was transported to the Council for Scientific and Industrial Research (CSIR), Pretoria, with an overnight courier service. Upon receipt, the raw milled and cooked maize samples were transferred to a freezer and stored at -20 °C. Cooked maize samples were first freeze-dried, then milled into a fine powder using a coffee grinder (Braun, Frankfurt,

Germany). The prepared samples were stored under nitrogen at -20 °C until they were analysed by High-Performance Liquid Chromatography (HPLC).

The rest of the procedures for carotenoid analyses were as described in section 4.2.2.1 (Chapter 4).

5.2.4.2 Energy

The energy content of the samples was determined with a LECO AC500 automatic bomb calorimeter (LECO Corporation, St Joseph, Michigan, USA) following the manufacturer's instructions.

5.2.4.3 Neutral detergent fibre

Fibre content was measured as Neutral Detergent Fibre (NDF). Neutral detergent (ND) solution and heat-stable α-amylase are used to dissolve easily digested proteins, lipids, sugars, starches and pectins in feeds. This leaves a fibrous residue that is primarily cell wall components in plant materials (cellulose, hemicellulose, and lignin) and indigestible nitrogenous matter in animal products. Sodium lauryl sulfate, an anionic detergent, and sodium sulfite are used to solubilise nitrogenous matter; Ethylenediaminetetraacetic acid (EDTA) is used to chelate calcium and enhance removal of pectins at boiling temperatures; triethylene glycol helps to remove some nonfibrous matter from concentrated feeds; disodium phosphate and sodium borate are used as buffers to maintain a neutral pH. The neutral detergent fibre (NDF) of the samples was analysed with a Dosi-Fibre machine (JP Selecta, Abrera, Barcelona, Spain) according to the AOAC Official Method 2002.04 (AOAC 2002).

5.2.4.4 Total minerals

The total mineral content of the samples was determined as ash by combusting the samples in a furnace set at 550 ºC for 4 hours (AOAC Official Method 942.05) (AOAC 2002).

5.2.4.5 Other nutrients

Referenced methods were used to analyse the raw milled and cooked maize samples for the other nutrients: moisture, starch, fat, protein, iron, zinc and phosphorus. These methods have been described previously (Chapter 4, section 4.2.2).

5.2.5   Retention of nutrients

Retention of nutrients was calculated as apparent retention, which is defined as the ratio of the nutrient content in the cooked food to the nutrient content in the raw food, expressed on a dry weight basis (Murphy *et al* 1975). Other researchers (e.g. Muzhingi *et al* 2008a; Li *et al* 2007) have found apparent retention to be straightforward to work with, due to the fact that analysis is on a dry matter basis. Apparent retention was therefore calculated, using the equation described by Murphy *et al* (1975), which is as follows:

% Apparent retention = <u>Nutrient content per g of cooked food (dry basis)</u> x 100
                       Nutrient content per g of raw food (dry basis)

5.2.6   Determination of usual portion sizes

As part of the consumer acceptability study (reported in Chapter 6), the usual portion sizes of the popular South African foods (*phutu*, thin porridge and samp) consumed, were obtained from 210 subjects with an age ranging from 4.3 to 55.5 years. Subjects were provided with cooked food samples and a serving spoon and were asked to plate out the usual portion size of each of the three food products they would consume into a bowl or plate (Figure 5.3a and Figure 5.3b). The food portion was then weighed with an electronic balance (Soehnle, Leifheit AG 56377, Nassau, Germany). The mean usual portion size was then calculated for each of the different age and gender groups (Table 5.1). These values were used to determine the percentage contribution of the three provitamin A-biofortified maize food products (*phutu*, thin porridge and samp) to the Estimated Average Requirement (EAR) for vitamin A if their usual portions are consumed by the different age and gender groups.

Figure 5.3a   Grade R subjects plating usual portions of cooked maize products

Figure 5.3b   Adult female subjects plating usual portions of cooked maize products

Table 5.1   Mean usual portion sizes of popular South African maize food products obtained from subjects aged 4.3-55.5 years (n=210)

Life stage group and age (years)	N	Mean age (years)	Phutu		Thin Porridge		Samp	
			Mean weight of usual portion (g)	Weight range (g)	Mean weight of usual portion (g)	Weight range (g)	Mean weight of usual portion (g)	Weight range (g)
Children								
4-8	70	6.04[a] (1.08)[b]	177.00[c] (68.83)[d]	50.0-420.0	240.37 (99.54)	62.0-524.0	332.03 (153.45)	66.0-896.0
Boys								
9 - 13	14	12.00 (1.49)	223.14 (39.09)	154.0-278.0	241.86 (66.55)	156.0-374.0	414.71 (128.22)	304.0-808.0
14 - 18	20	16.50 (1.23)	277.80 (88.05)	148.0-530.0	349.30 (129.15)	146.0-622.0	525.60 (209.87)	222.0-1096.0
Girls								
9 - 13	14	11.44 (1.48)	183.69 (60.34)	110.0-274.0	210.15 (30.84)	156.0-270.0	336.62 (87.13)	160.0-460.0
14 -18	27	16.46 (1.46)	170.89 (77.18)	62.0-480.0	287.04 (122.56)	136.0-716.0	357.63 (147.56)	166.0-712.0
Males								
19 - 70	31	35.26 (12.51)	257.23 (80.30)	126.0-442.0	376.39 (117.44)	120.0-730.0	454.00 (125.36)	172.0-738.0
Females								
19 - 70	34	37.54 (10.68)	186.18 (56.47)	58.0-318.0	335.94 (101.31)	150.0-580.0	351.06 (113.04)	124.0-550.0

[a] Mean of subject age (years)
[b] Standard deviation of subject age
[c] Mean of usual meal portion weight (g)
[d] Usual meal portion weight standard deviation

5.2.7   Statistical analyses

Predictive Analytics SoftWare (PASW) Statistics version 18.0 (IBM Corporation, New York) was used to analyse the data. Standard descriptive statistics (means and standard deviations) were used to express the duplicate nutrient measurements. Univariate analysis of variance (UNIANOVA) and Tukey post-hoc multiple comparisons of means were used to evaluate the influence of maize variety and maize food form on nutrient content and retention.

5.3   Results and Discussion

5.3.1   Carotenoid composition of processed provitamin A-biofortified maize products

Table 5.2 shows the carotenoid content of the processed provitamin A-biofortified maize products. The carotenoid composition of the maize grain varieties has been reported in Table 4.2 and discussed in section 4.3.1 (Chapter 4).

Table 5.2   Carotenoid composition of provitamin A-biofortified maize grain and its processed products (µg/g DW)

Maize variety	Maize form	Zeaxanthin[a]	β-cryptoxanthin[a]	β-carotene isomers			Total β-carotene[ac]	Total provitamin A carotenoids[ad]	Total carotenoids[ae]
				All-*trans*[a]	9-*cis*[a]	Other *cis*[ab]			
	RAW GRAIN[f]								
CC-37 (white)		< 0.3[g](0.0)[h]a	0.1 (0.0)a	ND[i]	ND	ND	ND	0.1 (0.0)a	0.4 (0.2)a
10 MAK 7-5		15.7 (2.1)b-f	3.7 (0.5)b-d	1.7[c](0.2)c-f	0.6 (0.1)b-d	1.3 (0.2)c-f	3.6 (0.6)b-e	7.3 (1.0)b-d	23.0 (6.3)b-e
10 MAK 7-7		18.7 (1.8)fg	4.3 (0.4)c-h	1.5 (0.1)b-e	0.5 (0.0)b	1.5 (0.1)c-h	3.5 (0.3)b-d	7.8 (0.7)c-f	26.5 (7.6)d-f
10 MAK 7-8		14.1 (0.8)b-e	4.8 (0.2)e-i	1.5 (0.1)b-e	0.5 (0.0)b	1.5 (0.0)c-h	3.5 (0.1)b-d	8.3 (0.3)c-g	22.4 (5.6)b-e
	MILLED								
	Raw mealie meal								
10 MAK 7-5		18.2 (2.2)e-g	4.4 (0.5)d-h	1.8 (0.2)d-g	0.8 (0.1)c-g	1.3 (0.2)c-f	3.9 (0.4)c-e	8.3 (0.9)c-g	26.5 (7.3)d-f
10 MAK 7-7		25.5 (1.6)i	5.9 (0.4)jk	2.2 (0.1)g	0.9 (0.1)e-h	1.7 (0.1)f-h	4.8 (0.3)f	10.7 (0.7)h	36.2 (10.4)g
10 MAK 7-8		16.8 (0.4)c-g	6.2 (0.1)k	1.9 (0.0)e-g	0.7 (0.0)c-f	1.5 (0.1)c-h	4.1 (0.1)c-f	10.3 (0.2)h	27.1 (6.7)d-f
	Raw samp								
10 MAK 7-5		17.8 (0.4)d-g	4.2 (0.2)c-g	1.9 (0.1)e-g	0.8 (0.0)c-g	1.5 (0.1)c-h	4.2 (0.1)d-f	8.4 (0.3)c-g	26.2 (7.1)c-e
10 MAK 7-7		17.0 (1.3)d-g	4.1 (0.3)c-f	1.7 (0.1)c-f	0.7 (0.0)c-f	1.7 (0.1)f-h	4.1 (0.1)c-f	8.2 (0.5)c-g	25.2 (6.8)c-e
10 MAK 7-8		13.4 (0.6)b-d	5.0 (0.3)f-j	1.7 (0.1)c-f	0.6 (0.0)b-d	1.7 (0.1)f-h	4.0 (0.2)c-f	9.0 (0.5)d-h	22.4 (5.2)b-e
	COOKED								
	Phutu								
10 MAK 7-5		23.4 (2.1)hi	5.2 (0.4)h-j	1.6 (0.1)c-f	0.7 (0.0)c-f	1.7 (0.1)f-h	4.0 (0.3)c-f	9.2 (0.7)e-h	32.6 (9.6)fg
10 MAK 7-7		20.3 (0.2)gh	4.6 (0.0)d-h	1.7 (0.0)c-f	0.7 (0.0)c-f	1.4 (0.0)c-g	3.8 (0.1)c-e	8.4 (0.1)c-g	28.7 (8.3)ef
10 MAK 7-8		16.7 (0.2)b-g	5.8 (0.1)i-k	1.7 (0.0)c-f	0.9 (0.0)e-h	1.4 (0.0)c-g	4.0 (0.1)c-f	9.8 (0.2)gh	26.5 (6.7)d-f
	Thin porridge								
10 MAK 7-5		12.2 (0.0)b	2.8 (0.1)b	1.2 (0.0)b	0.8 (0.0)c-g	0.8 (0.0)b	2.8 (0.0)b	5.6 (0.1)b	17.8 (4.9)b
10 MAK 7-7		16.0 (0.6)b-g	3.7 (0.1)b-d	1.4 (0.0)b-d	0.9 (0.0)e-h	1.1 (0.0)b-d	3.4 (0.1)b-d	7.1 (0.1)bc	23.1 (6.4)b-e
10 MAK 7-8		12.4 (0.5)bc	4.4 (0.2)d-h	1.4 (0.0)b-d	0.7 (0.0)c-f	1.1 (0.0)b-d	3.2 (0.1)bc	7.6 (0.2)c-e	20.0 (4.9)bc
	Samp								
10 MAK 7-5		16.0 (0.5)b-g	3.3 (0.1)bc	1.6 (0.0)c-f	1.1 (0.1)h	1.2 (0.1)c-e	3.9 (0.1)c-f	7.2 (0.3)b-d	23.2 (6.4)b-e
10 MAK 7-7		19.3 (0.6)f-h	3.9 (0.2)c-e	1.6 (0.0)c-f	0.8 (0.0)c-g	1.5 (0.0)c-h	3.9 (0.0)c-f	7.8 (0.2)c-f	27.1(7.8)d-f
10 MAK 7-8		16.3 (0.1)b-g	5.1 (0.0)g-j	1.9 (0.0)e-g	0.9 (0.0)e-h	1.6 (0.0)e-h	4.4 (0.0)e-f	9.5 (0.1)f-h	25.8 (6.4)c-e

[a] Values within the same column with different letters are significantly different at p < 0.05 (Tukey test); [b] Total concentration of unidentified *cis* isomers of β-carotene; [c] Sum of all-*trans*, 9-*cis* and other *cis*; [d] Sum of β-cryptoxanthin and total β-carotene; [e] Sum of zeaxanthin, β-cryptoxanthin and total β-carotene; [f] Values from Table 4.2 (Chapter 4); [g] Mean of duplicate values; [h] Standard deviation; [i] ND = Not detected, detection limit for β-carotene was < 0.1 µg/100 g

Figures 5.4a, 5.4b and 5.4c show the representative chromatograms of the carotenoids present in the three varieties of raw mealie meal. The chromatograms of the three varieties of mealie meal are similar.
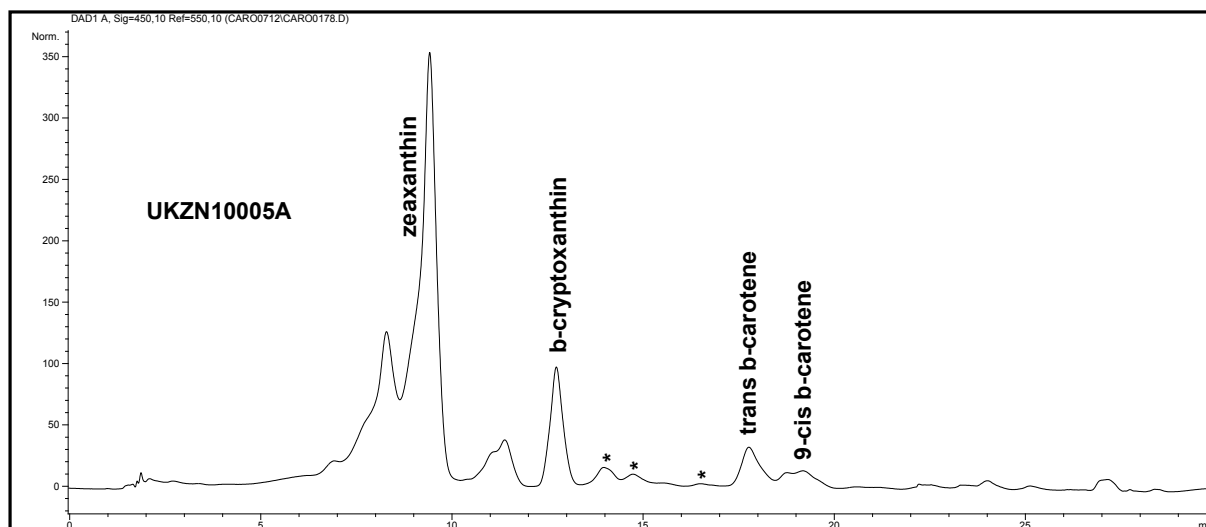
UKZN10005A = 10 MAK 7-5- raw mealie meal

Figure 5.4a   HPLC chromatogram of the carotenoids present in an extract of raw mealie meal (10 MAK 7-5)
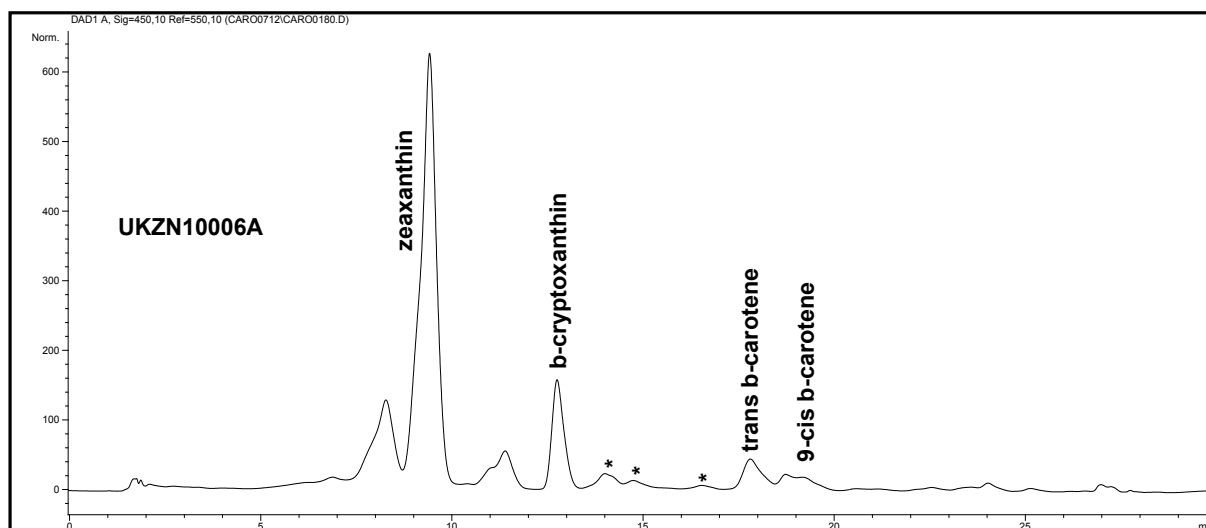
UKZN10006A = 10 MAK 7-7- raw mealie meal

Figure 5.4b   HPLC chromatogram of the carotenoids present in an extract of raw mealie meal (10 MAK 7-7)
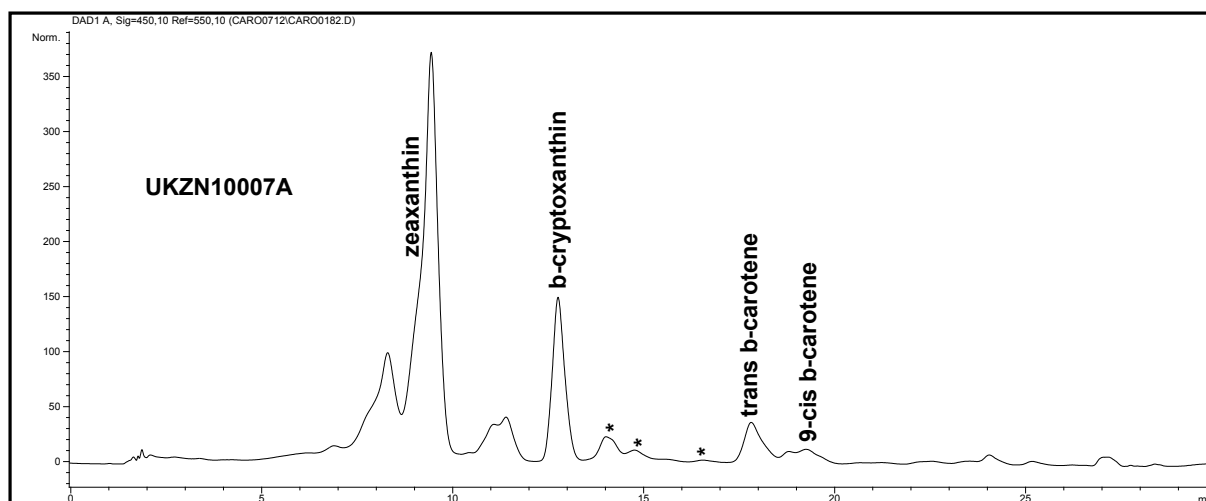
UKZN10007A = 10 MAK 7-8- raw mealie meal

Figure 5.4c   HPLC chromatogram of the carotenoids present in an extract of raw mealie meal (10 MAK 7-8)

The raw mealie meal of the variety 10 MAK 7-7 contained the highest amount of total β-carotene (4.8 ± 0.3 µg/g) and total provitamin A carotenoids (10.7 ± 0.7 µg/g). The highest amount of β-cryptoxanthin was found in the variety 10 MAK 7-8 (6.2 ± 0.1 µg/g).

Figures 5.5a, 5.5b and 5.5c show the representative chromatograms of the carotenoids present in the three varieties of raw samp.
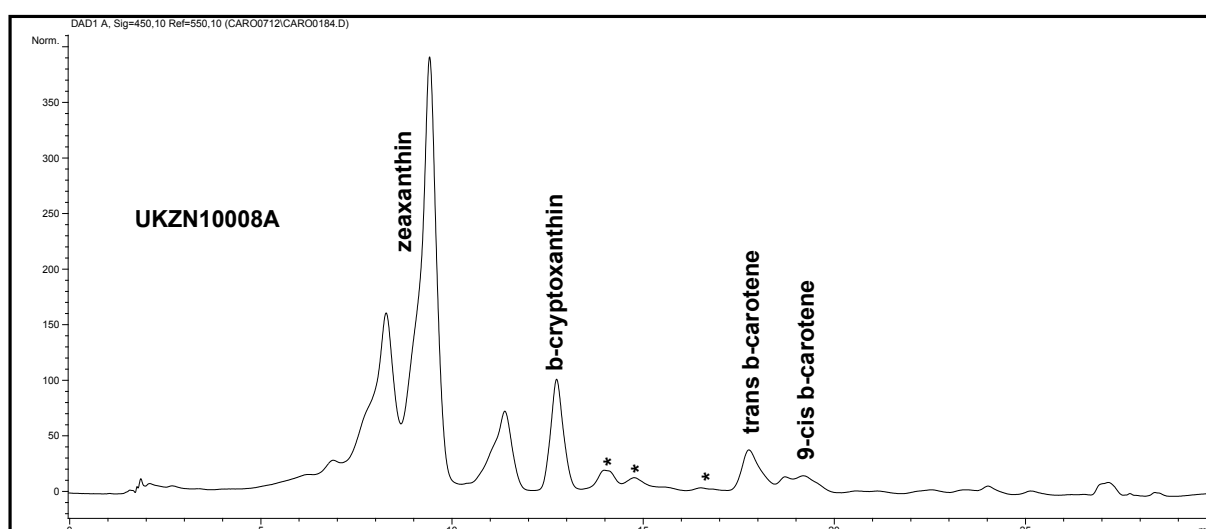
UKZN10008A = 10 MAK 7-5 – raw samp

Figure 5.5a   HPLC chromatogram of the carotenoids present in an extract of raw samp (10 MAK 7-5)
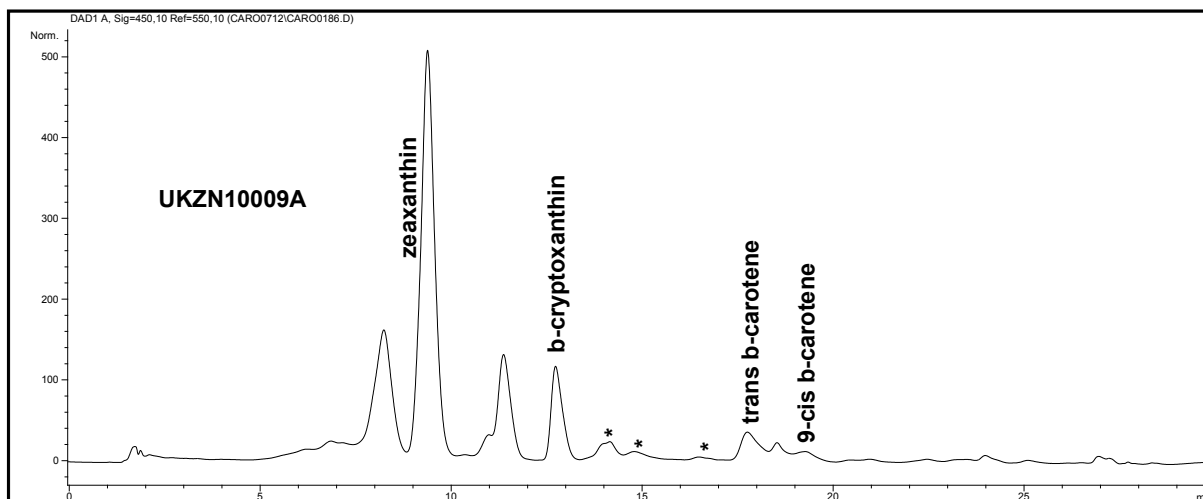
UKZN10009A = 10 MAK 7-7 – raw samp

Figure 5.5b   HPLC chromatogram of the carotenoids present in an extract of raw samp
(10 MAK 7-7)

UKZN10010A = 10 MAK 7-8 – raw samp

Figure 5.5c   HPLC chromatogram of the carotenoids present in an extract of raw samp
(10 MAK 7-8)

The raw samp of the variety 10 MAK 7-8 contained the highest amount of β-cryptoxanthin
(5.0 ± 0.3 µg/g) and total provitamin A carotenoids (9.0 ± 0.5 µg/g), whilst the variety 10
MAK 7-5 contained the highest amount of total β-carotene (4.2 ± 0.1 µg/g).

Overall, the raw mealie meal contained a higher amount of total provitamin A carotenoids
(9.8 ± 1.3 µg/g), total β-carotene (4.3 ± 0.5 µg/g) and β-cryptoxanthin (5.5 ± 1.0 µg/g)

compared to the raw samp. This shows that the raw mealie meal is a better source of provitamin A carotenoids than the raw samp.

Figures 5.6a, 5.6b and 5.6c show the representative chromatograms of the carotenoids present in the three varieties of cooked *phutu*.
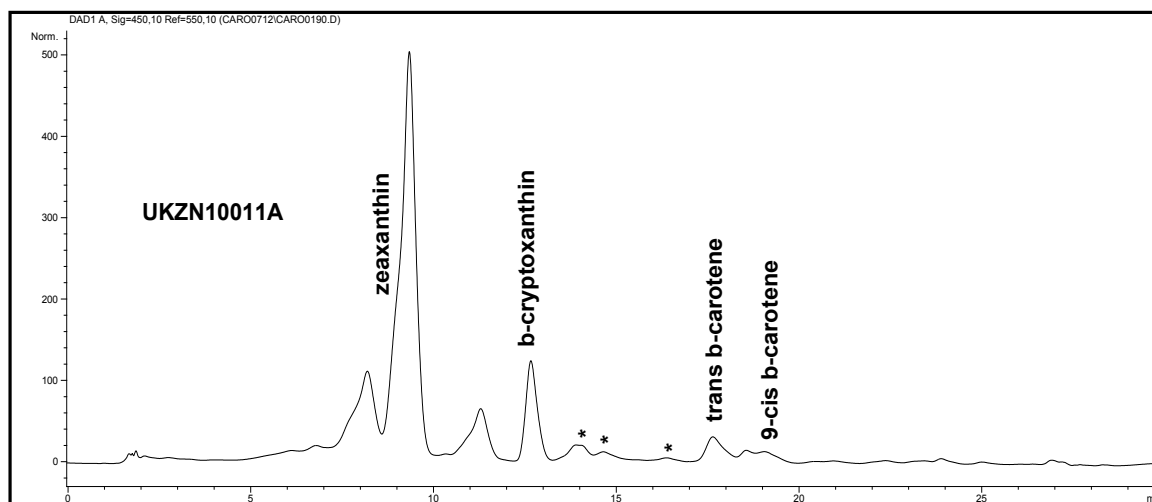
UKZN 10011A = 10 MAK 7-5- *Phutu*

Figure 5.6a   HPLC chromatogram of the carotenoids present in an extract of *phutu* (10 MAK 7-5)
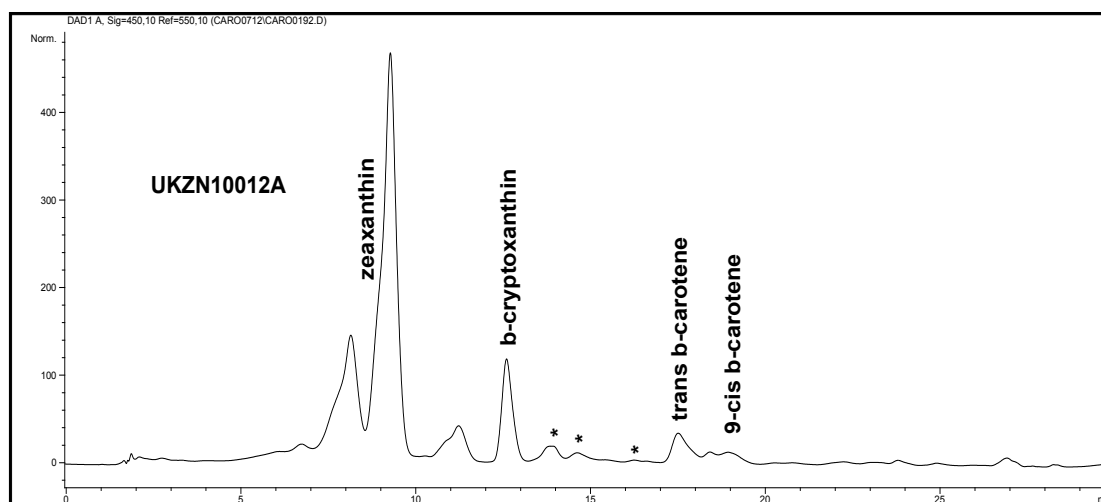
UKZN10012A = 10 MAK 7-7 – *Phutu*

Figure 5.6b   HPLC chromatogram of the carotenoids present in an extract of *phutu* (10 MAK 7-7)
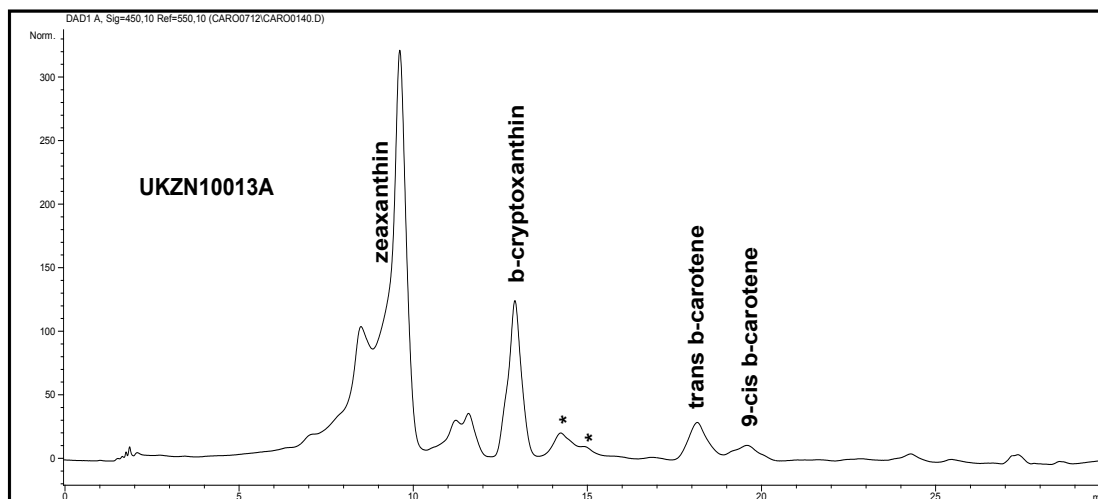
UKZN10013A = 10 MAK 7-8 - *Phutu*

Figure 5.6c   HPLC chromatogram of the carotenoids present in an extract of *phutu*

                (10 MAK 7-8)

The cooked *phutu* of the variety 10 MAK 7-8 contained the highest amount of β-cryptoxanthin (5.8 ± 0.1 μg/g), total β-carotene (4.0 ± 0.1 μg/g) and total provitamin A carotenoids (9.8 ± 0.2 μg/g).

Figures 5.7a, 5.7b and 5.7c show the representative chromatograms of the carotenoids present in the three varieties of cooked thin porridge.
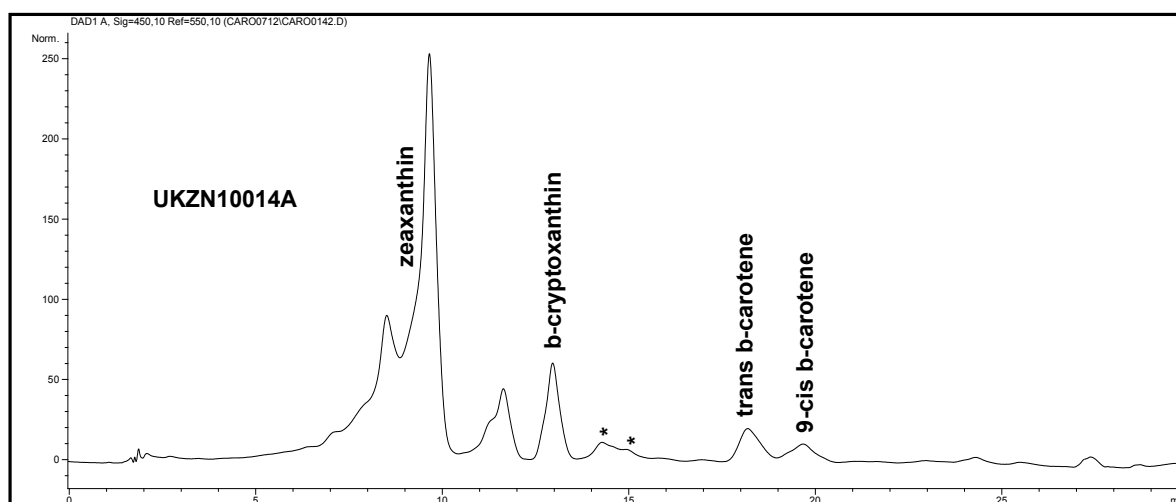
UKZN10014A = 10 MAK 7-5-thin porridge

Figure 5.7a   HPLC chromatogram of the carotenoids present in an extract of thin porridge
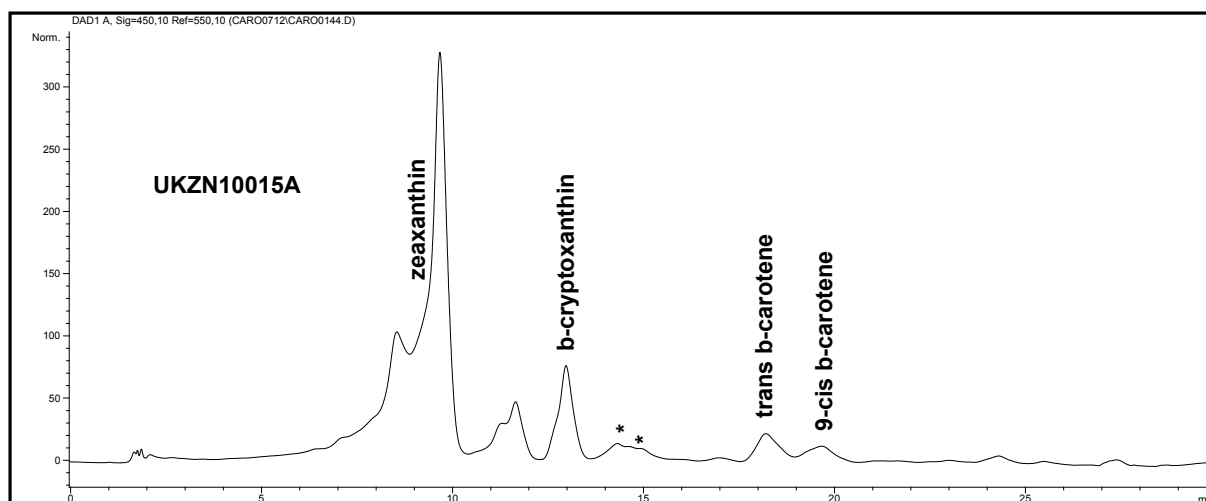
                (10 MAK 7-5)

UKZN10015A = 10 MAK 7-7-thin porridge

Figure 5.7b   HPLC chromatogram of the carotenoids present in an extract of thin porridge (10 MAK 7-7)
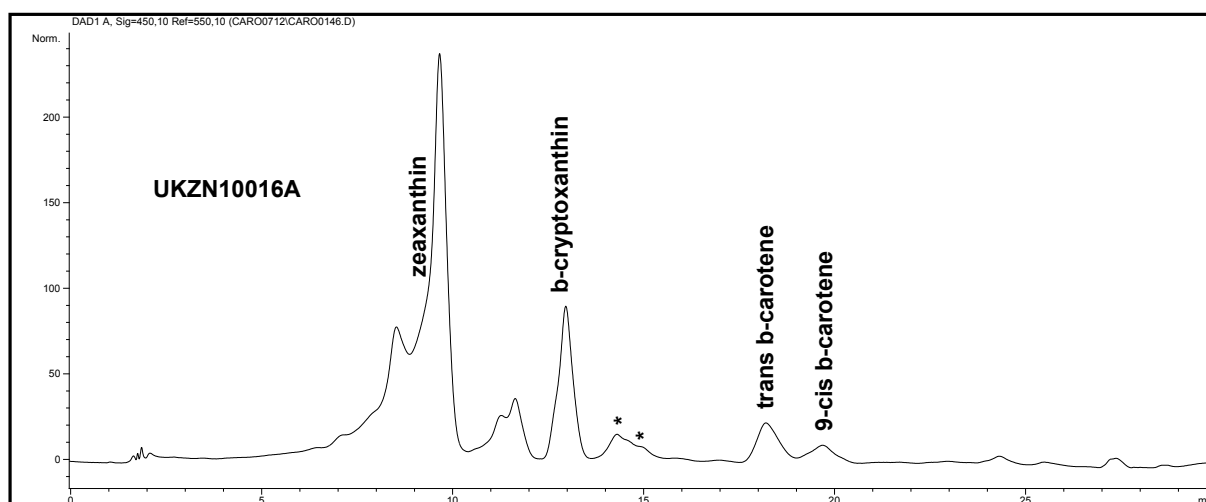
UKZN10016A = 10 MAK 7-8-thin porridge

Figure 5.7c   HPLC chromatogram of the carotenoids present in an extract of thin porridge (10-MAK 7-8)

The cooked thin porridge of the variety 10 MAK 7-8 contained the highest amount of β-cryptoxanthin (4.4 ± 0.2 μg/g) and total provitamin A carotenoids (7.6 ± 0.2 μg/g), whilst the variety 10 MAK 7-7 contained the highest amount of total β-carotene (3.4 ± 0.1 μg/g).

Figures 5.8a, 5.8b and 5.8c show the representative chromatograms of the carotenoids present in the three varieties of cooked samp.
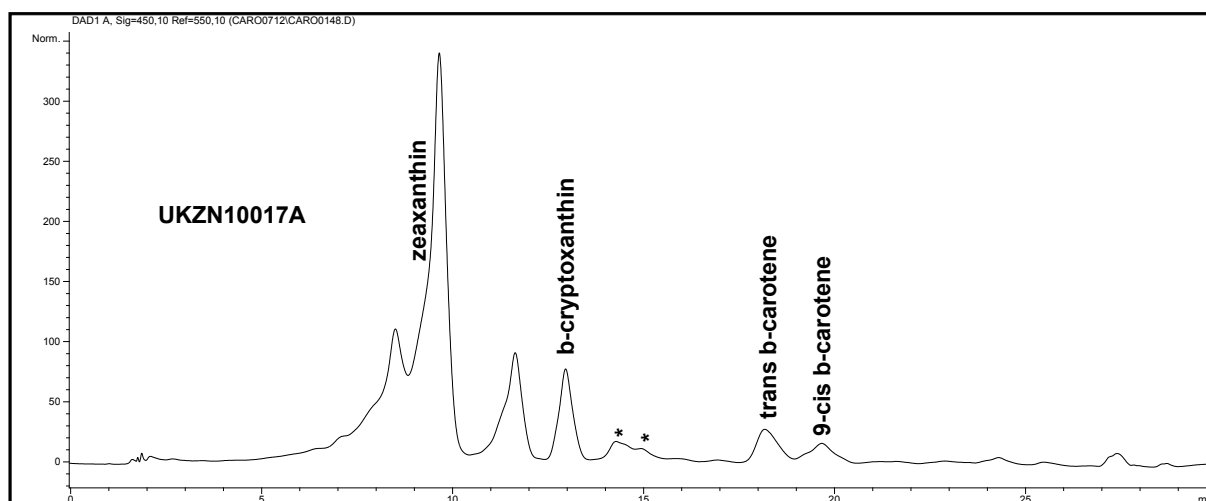
UKZN10017A = 10 MAK 7-5-samp

Figure 5.8a   HPLC chromatogram of the carotenoids present in an extract of cooked samp (10 MAK 7-5)
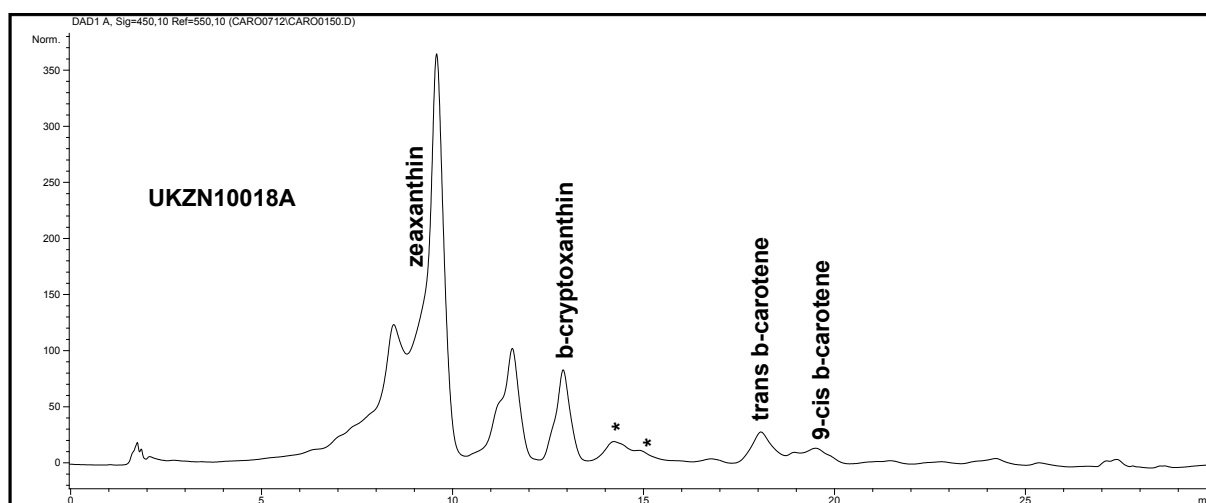
UKZN10018A = 10 MAK 7-7-samp

Figure 5.8b   HPLC chromatogram of the carotenoids present in an extract of cooked samp (10 MAK 7-7)
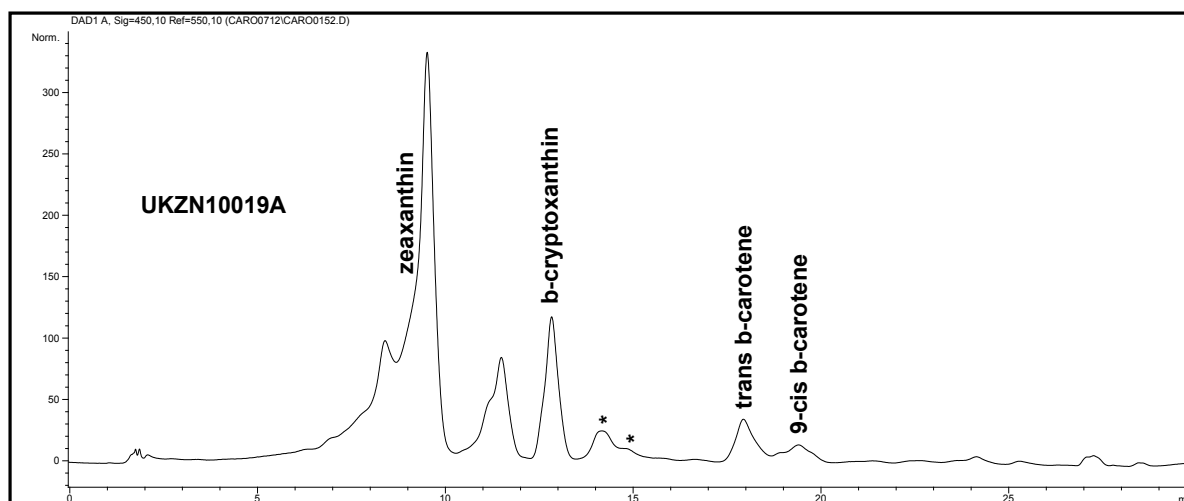
UKZN10019A = 10 MAK 7-8-samp

Figure 5.8c   HPLC chromatogram of the carotenoids present in an extract of cooked samp
(10 MAK 7-8)

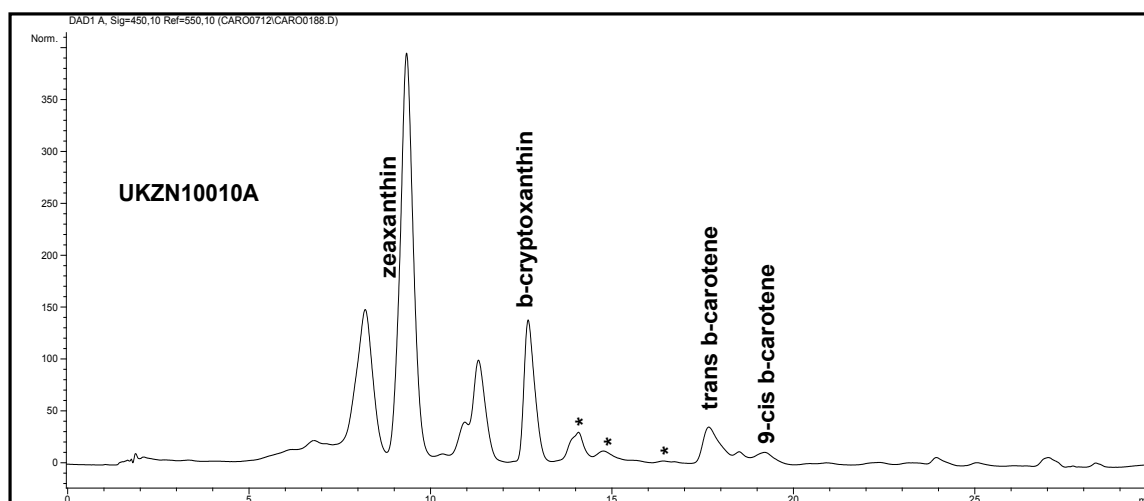The highest amount of β-cryptoxanthin (5.1 ± 0.0 μg/g), total β-carotene (4.4 ± 0.0 μg/g) and total provitamin A carotenoids (9.5 ± 0.1 μg/g) was found in the variety 10 MAK 7-8 of cooked samp.

The variety 10 MAK 7-8, has consistently shown the highest levels of total provitamin A carotenoids in all the three cooked foods. *Phutu* contained the highest amount of total provitamin A carotenoids (9.1 ± 0.7 μg/g), followed by the samp (8.2 ± 1.2 μg/g) and the thin porridge (6.8 ± 1.0 μg/g). *Phutu* had the highest β-cryptoxanthin content (5.2 ± 0.6 μg/g) whilst samp had the highest mean total β-carotene content (4.1 ± 0.3 μg/g). The highest amount of all-*trans* β-carotene isomers was found in the samp (1.7 ± 0.2 μg/g). This suggests that *phutu* and samp may be better choices of foods to prepare using provitamin A-biofortified maize, because of the superior provitamin A carotenoid content. These findings also suggest that the variety 10 MAK 7-8 yields the highest concentration of total provitamin A carotenoids when processed into cooked foods.

Distribution of β-carotene isomers in maize products
The effects of processing on the quantitative distribution of β-carotene isomers are shown in Table 5.3. In this study, milling provitamin A-biofortified maize grain into mealie meal resulted in an increase in the % *trans* isomers of β-carotene, relative to the total β-carotene content of the biofortified maize varieties. However, milling the maize varieties into samp

resulted in an overall decrease in the % *trans* isomers of the β-carotene. The opposite effect was observed on the % *cis* isomers, relative to the total β-carotene content. Milling the biofortified maize varieties into mealie meal resulted in a decrease in the % *cis* isomers of the β-carotene, whilst milling the maize varieties into samp resulted in an increase in the % *cis* isomers of the β-carotene. Cooking maize into *phutu*, thin porridge and samp resulted in an overall decrease in the % *trans* isomers of the β-carotene, whilst there was a corresponding increase in the % *cis* isomers. Compared to *trans* isomers, *cis* isomers are less stable and as stated earlier, they have lower vitamin A activity (Institute of Medicine 2000a, p83; Gregory 1996, p546). However, it is unlikely that the small percentage increase in the *cis* isomers during processing would significantly change the provitamin A activity of the biofortified maize food products.

Table 5.3   Quantitative distribution of β-carotene isomers in provitamin A-biofortified maize grain and its processed products (µg/g DW)

Maize variety	Maize form	β-carotene isomers			Total cis[ac]	Total β-carotene[ad]	% trans[e]	% cis[f]
		All-*trans*[a]	9-*cis*[a]	Other *cis*[ab]				
	RAW GRAIN							
CC-37 (white)		ND[g]	ND	ND	ND	ND	ND	ND
10 MAK 7-5		1.7[h](0.2)[i]	0.6 (0.1)	1.3 (0.2)	1.9 (0.5)	3.6 (0.6)	46.0 (0.7)	54.0 (0.7)
10 MAK 7-7		1.5 (0.1)	0.5 (0.0)	1.5 (0.1)	1.9 (0.7)	3.5 (0.3)	42.2 (2.8)	55.9 (0.2)
10 MAK 7-8		1.5 (0.1)	0.5 (0.0)	1.5 (0.0)	2.0 (0.7)	3.4 (0.1)	42.8 (0.1)	57.2 (0.1)
	MILLED							
	Raw mealie meal							
10 MAK 7-5		1.8 (0.2)	0.8 (0.1)	1.3 (0.2)	2.1 (0.3)	3.8 (0.4)	46.2 (0.4)	53.8 (0.4)
10 MAK 7-7		2.2 (0.1)	0.9 (0.1)	1.7 (0.1)	2.6 (0.6)	4.7 (0.3)	45.7 (0.6)	54.3 (0.6)
10 MAK 7-8		1.9 (0.0)	0.7 (0.0)	1.5 (0.1)	2.2 (0.6)	4.1 (0.1)	45.8 (0.4)	54.3 (0.4)
	Raw samp							
10 MAK 7-5		1.9 (0.1)	0.8 (0.0)	1.5 (0.1)	2.3 (0.5)	4.2 (0.1)	45.2 (0.3)	54.8 (0.3)
10 MAK 7-7		1.7 (0.1)	0.7 (0.0)	1.7 (0.1)	2.3 (0.7)	4.0 (0.1)	41.9 (0.1)	58.1 (0.1)
10 MAK 7-8		1.7 (0.1)	0.6 (0.0)	1.7 (0.1)	2.3 (0.7)	4.0 (0.2)	42.8 (0.0)	57.2 (0.0)
	COOKED							
	Phutu							
10 MAK 7-5		1.6 (0.1)	0.7 (0.0)	1.7 (0.1)	2.5 (0.7)	4.1 (0.3)	39.9 (0.2)	60.1 (0.2)
10 MAK 7-7		1.7 (0.0)	0.7 (0.0)	1.4 (0.0)	2.1 (0.4)	3.8 (0.1)	44.7 (0.4)	55.3 (0.4)
10 MAK 7-8		1.7 (0.0)	0.9 (0.0)	1.4 (0.0)	2.3 (0.3)	4.0 (0.1)	43.3 (0.3)	56.7 (0.3)
	Thin porridge							
10 MAK 7-5		1.2 (0.0)	0.8 (0.0)	0.8 (0.0)	1.6 (0.1)	2.8 (0.0)	43.3 (0.8)	56.7 (0.8)
10 MAK 7-7		1.4 (0.0)	0.9 (0.0)	1.1 (0.0)	2.0 (0.1)	3.4 (0.1)	40.0 (0.3)	60.0 (0.3)
10 MAK 7-8		1.4 (0.0)	0.7 (0.0)	1.1 (0.0)	1.8 (0.3)	3.2 (0.1)	43.8 (0.1)	56.2 (0.1)
	Samp							
10 MAK 7-5		1.6 (0.0)	1.1 (0.1)	1.2 (0.1)	2.3 (0.1)	3.9 (0.1)	41.3 (0.9)	58.7 (0.9)
10 MAK 7-7		1.6 (0.0)	0.8 (0.0)	1.5 (0.0)	2.3 (0.5)	3.9 (0.0)	40.9 (0.1)	59.1 (0.1)
10 MAK 7-8		1.9 (0.0)	0.9 (0.0)	1.6 (0.0)	2.5 (0.5)	4.4 (0.0)	42.8 (0.3)	57.2 (0.3)

[a] Measured in µg/g
[b] Concentration of unidentified *cis* isomers of β-carotene
[c] Sum of 9-*cis* and other *cis*
[d] Sum of all-*trans*, 9-*cis* and other *cis*
[e] Calculated from duplicate values for % trans
[f] Calculated from duplicate values for % cis
[g] ND = Not detected, detection limit for β-carotene was < 0.1 µg/100g
[h] Mean of duplicate values
[i] Standard deviation

5.3.2   Retention of carotenoids

Milling

Table 5.4 shows the carotenoid retention (%) during milling of maize grain into mealie meal and samp followed by cooking the milled products into *phutu*, thin porridge and samp. Milling maize grain into mealie meal resulted in a higher retention of zeaxanthin (115.9%-136.4%), β-cryptoxanthin (118.9%-137.2%) and β-carotene (105.6%-134.3%), compared to milling into samp. With milling into samp there was a lower retention of zeaxanthin (90.9%-113.4%) and β-cryptoxanthin (95.3%-113.5%).

Cooking

Cooking maize mealie meal into *phutu* resulted in the highest retention of zeaxanthin (128.6%), β-cryptoxanthin (118.2%) and β-carotene (107.9%) in the 10 MAK 7-5 variety (Table 5.4), with the lowest retention of zeaxanthin (79.6%), β-cryptoxanthin (78.0%) and β-carotene (80.9%) found in the 10 MAK 7-7 variety. With cooked samp, the highest retention of zeaxanthin (121.6%), β-cryptoxanthin (102.0%) and β-carotene (110.0%) was found in the 10 MAK 7-8 variety, whilst there was lower retention of carotenoids when the other two biofortified varieties were used. Overall, cooking into *phutu* and samp resulted in the highest retention of carotenoids, whilst the lowest retention of carotenoids was found for cooked thin porridge, irrespective of the maize variety used. The retention results of the present study should be interpreted with caution as there is a tendency of overestimating nutrient retention when apparent retention is used, as mentioned earlier.

Increases in carotenoid retention as a result of cooking have been reported previously. As stated earlier, Muzhingi *et al* (2008a) observed an increase in the carotenoid concentration in all cooked yellow maize products, except for baked muffins. Khachik *et al* (1992a) found that conventional blanching and cooking significantly increased the carotenoid concentration in several green vegetables. Granado *et al* (1992) also reported that the boiling of vegetables resulted in an increase in the amounts of carotenoids. The increase in carotenoid concentration is suspected to be due to the increased chemical extractability of carotenoids as a result of the breakdown of the food matrix (Khachik *et al* 1992b). Although the cooked foods had different moisture contents (*phutu*, 34.9 ± 3.8%; thin porridge, 91.2 ± 0.4%; and samp, 73.9 ± 4.1%), moisture content of the cooked food was found not to have an effect on carotenoid retention in the foods (p=0.620). Differences in carotenoid retention among the cooked maize foods could be attributed to the cooking temperature. Although the thin porridge was cooked for the

shortest time (25 minutes), it reached the highest cooking temperature of 96ºC, compared to 86ºC and 84ºC, for *phutu* and samp, respectively. Exposure to extreme heat is known to destroy provitamin A carotenoids (Rodriguez-Amaya 1997). It can be derived from the study that further investigations on cooking temperature and time for the various food products is required to find optimum cooking conditions that retain significant concentrations of provitamin A carotenoids in maize foods.

Table 5.4 Retention of carotenoids in processed maize products of three provitamin A-biofortified maize varieties

Maize variety	Maize form	Retention (%)		
			Provitamin A carotenoids	
		Zeaxanthin	β-cryptoxanthin	β-carotene
	MILLED			
	Raw mealie meal			
10 MAK 7-5		115.9	118.9	105.6
10 MAK 7-7		136.4	137.2	134.3
10 MAK 7-8		119.1	129.2	120.6
	Raw samp			
10 MAK 7-5		113.4	113.5	116.7
10 MAK 7-7		90.9	95.3	114.3
10 MAK 7-8		95.0	104.2	117.6
	COOKED			
	Phutu			
10 MAK 7-5		128.6[a]	118.2	107.9
		(149.0)[b]	(140.5)	(113.9)
10 MAK 7-7		79.6	78.0	80.9
		(108.6)	(107.0)	(108.6)
10 MAK 7-8		99.4	93.5	97.6
		(118.4)	(120.8)	(117.6)
	Thin porridge			
10 MAK 7-5		67.0	63.6	73.7
		(77.7)	(75.7)	(77.8)
10 MAK 7-7		62.7	62.7	72.3
		(85.6)	(86.0)	(97.1)
10 MAK 7-8		73.8	71.0	78.0
		(87.9)	(91.7)	(94.1)
	Samp			
10 MAK 7-5		89.9	78.6	92.9
		(101.9)	(89.2)	(108.3)
10 MAK 7-7		113.5	95.1	97.5
		(103.2)	(90.7)	(111.4)
10 MAK 7-8		121.6	102.0	110.0
		(115.6)	(106.3)	(129.4)

[a] Retention at a processing step
[b] Overall retention in parentheses; calculated as proportion of provitamin A carotenoids in cooked product compared with the provitamin A carotenoid content in whole grain

Table 5.5   Effect of maize variety and maize form on carotenoid content

Source of variation	Zeaxanthin			β-cryptoxanthin			Total β-carotene			Total provitamin A carotenoids		
	F	df	p value[a]	F	df	p value[a]	F	df	p value[a]	F	df	p value[a]
Maize variety	134.591	3	0.000	200.721	3	0.000	126.771	3	0.000	157.285	3	0.000
Maize form	32.239	5	0.000	49.876	5	0.000	21.220	5	0.000	30.724	5	0.000
Maize variety x maize form	6.939	10	0.000	5.359	10	0.001	3.293	10	0.012	4.139	10	0.004

[a] Univariate analysis of variance, p is significant at < 0.005
F = The mean square for each main effect or interaction divided by the residual mean square
df = Degrees of freedom

Both maize variety and maize food form significantly (p<0.05) influenced the carotenoid concentrations in the maize products (Table 5.5). Even though only a few biofortified maize varieties were studied, the results of the present study confirm that carotenoid concentration depends on the maize variety and that there is genetic variation for this trait (Muzhingi *et al* 2008). The results suggest that a combination of selective breeding for high-provitamin A maize varieties and the selective processing of the provitamin A-biofortified maize varieties into maize food forms that have good provitamin A retention, such as *phutu* and samp, would enable the delivery of significant concentrations of provitamin A to the consumer. The results suggest that selection for high-provitamin A maize varieties can be emphasised in a maize breeding programme. Although, maize grain colour is generally not correlated with provitamin A concentration, deep orange maize varieties have often been found to contain substantial concentrations of provitamin A. Due to the high costs of HPLC analysis, it is suggested that maize grain colour be used for the intial screening for the presence of provitamin A in maize varieties. HPLC analysis would then be used to determine provitamin A carotenoid concentrations in the promising varieties.

5.3.3 Contribution of provitamin A-biofortified maize products to the Estimated Average Requirement (EAR) for vitamin A

Table 5.6 shows the percentage of the EAR met for vitamin A for the different age and gender groups when usual portions of provitamin A-biofortified maize food products are consumed. The usual portions used are based on values from Table 5.1. The mean usual portions for boys and adult males were higher than the mean usual portion sizes for females of the corresponding age group. This is expected as males generally consume larger portions of food compared to females. Overall the highest percentage of the EAR would be met by consuming samp (36-61%), while *phutu* would make the lowest contribution to the EAR (19-35%). Although Table 5.6 shows the vitamin A value of the different food products using retinol activity equivalents (RAE), it does not take into account the bioavailability of vitamin A. Bioavailability is defined as the amount of the carotenoid from a food item or meal that is absorbed and available to be used by the body for normal physiological functions or for storage (White & Broadley 2005; Tanumihardjo 2002). There are a number of factors that have been identified which affect the bioavailability of carotenoids. These factors include: *cis* or *trans* configuration of the carotenoid, esterification, amount of carotenoid in the meal, matrix properties of the plant, effectors of absorption and bioconversion, nutrient status of the

individual, genetic factors, host-related factors and interactions between factors (Tanumihardjo 2002).

The consumption of usual portions of a single provitamin A-biofortified maize food product per day makes a reasonable contribution to the EAR for vitamin A in all age and gender groups. However, a more significant percentage of the EAR could be met if the provitamin A-biofortified maize products were eaten three times a day, i.e., thin porridge for breakfast, with *phutu* or samp eaten during the lunch or evening meals. In South Africa, thin porridge is often eaten as part of breakfast, while *phutu* and samp are eaten during the day or evening meal. In order to provide the optimal EAR of vitamin A to groups that are vulnerable to or have VAD, usual portions of all three provitamin A-biofortified maize products should be consumed on a daily basis. This can only be achieved with adequate nutrition education on the nutritional benefits of daily consumption of provitamin A-biofortified maize food products.

Table 5.6   Percentage of the Estimated Average Requirement met for vitamin A for the different age and gender groups when usual portions of provitamin A-biofortified maize food products are consumed

Life stage group	Age (y)	EAR[a] (µg/day)	PHUTU			THIN PORRIDGE			SAMP		
			Mean usual portion (g)	Vitamin A value (µg/RAE)[b]	% of EAR met	Mean usual portion (g)	Vitamin A value (µg/RAE)[b]	% of EAR met	Mean usual portion (g)	Vitamin A value (µg/RAE)[b]	% of EAR met
Children	4 - 8	275	177[c] (69)[d]	97	35	240 (100)	99	36	332 (153)	169	61
Boys	9 - 13	445	223 (39)	122	27	242 (67)	100	22	415 (128)	212	48
	14 - 18	630	278 (88)	152	24	349 (129)	144	23	526 (210)	268	43
Girls	9 - 13	420	184 (60)	101	24	210 (31)	87	21	337 (87)	172	41
	14 -18	485	171 (77)	93	19	287 (123)	119	25	358 (148)	183	38
Males	19 - 70	625	257 (80)	140	22	376 (117)	155	25	454 (125)	232	37
Females	19 - 70	500	186 (56)	102	20	336 (101)	139	28	351 (113)	179	36

[a] Institute of Medicine (2001).
[b] RAE (Retinol activity equivalents): 12µg β-carotene = 1µg RAE; 24 µg β-cryptoxanthin = 1µg RAE) (Trumbo *et al* 2001)
  Sum of β-carotene and β-cryptoxanthin (mean calculated from three varieties) using values presented in Table 5.2.
[c] Mean
[d] Standard deviation

5.3.4   Nutritional composition of processed provitamin A-biofortified maize products

Milled products

The nutritional composition of white maize milled products compared to provitamin A-biofortified maize milled products is presented in Table 5.7. The mealie meal of the provitamin A-biofortified maize contained significantly higher levels of energy, fat and protein compared to that of white maize mealie meal ($p<0.05$). However, the iron levels were lower in the biofortified mealie meal compared to the white mealie meal. Similar to the mealie meal, the biofortified maize samp contained significantly higher levels of fat and protein. This was clearly due to the fact that the provitamin A-biofortified maize grain contained higher levels of starch, fat and protein as reported in Chapter 4, Table 4.3. Interestingly, the iron levels of the samp of the provitamin A-biofortified maize varieties were higher than that of the white maize variety. Although the zinc levels in the mealie meal of the white maize variety and the biofortified maize varieties were comparable, the zinc levels in the samp of the biofortified maize varieties were significantly lower than that of the white maize samp. These results do not correspond with the nutritional composition of the whole white maize grain and provitamin A-biofortified maize grain, as reported in Chapter 4, Table 4.3. These results of iron and zinc levels in the milled products, which do not corroborate with those of whole grain, may not indicate trends in the retention of these minerals in the milled products, but could be due to a random variation in the nutritional composition of the milled products. Generally, the starch, total minerals and phosphorus levels of the provitamin A-biofortified maize varieties were comparable with those of the white maize variety. Overall, mealie meal and samp of the provitamin A-biofortified maize are superior sources of fat and protein when compared with the corresponding white maize products.

Table 5.7   Nutritional composition of white maize mealie meal and samp compared to provitamin A-biofortified maize mealie meal and samp of three varieties

Milled products and maize varieties	Moisture (%)	Energy[a] (kJ/100g)	Starch[a] (g/100g)	NDF[a,b] (g/100g)	Fat[a] (g/100g)	Protein[a] (g/100g)	Total Minerals[a] (mg/100g)	Iron[a] (mg/100g)	Zinc[a] (mg/100g)	Phosphorus[a] (mg/100g)
Mealie meal										
CC-37	14.0[c] (0.0)[d]	1737.5 (9.9)	79.4 (2.4)	3.1 (0.1)	2.0 (0.0)	10.3 (0.1)	975.0 (7.1)	1.41 (0.12)	1.70 (0.08)	205.83 (9.67)
10 MAK 7-5	13.9 (0.1)	**1776.0** (1.5)	77.5 (1.4)	3.3 (0.1)	**2.8** (0.0)	**11.9** (0.1)	**1185.0** (49.5)	**0.95** (0.01)	**2.09** (0.00)	200.02 (0.39)
10 MAK 7-7	**16.0** (0.3)	**1786.1** (1.4)	79.9 (0.2)	2.7 (0.1)	**2.4** (0.0)	**12.3** (0.0)	1015.0 (21.2)	**1.13** (0.04)	1.55 (0.16)	199.94 (2.60)
10 MAK 7-8	**16.7** (0.1)	**1790.6** (7.2)	78.1 (1.1)	2.9 (0.2)	**2.3** (0.1)	**12.1** (0.1)	**1205.0** (35.4)	1.30 (0.00)	1.88 (0.08)	**233.78** (1.58)
Samp										
CC-37	11.2 (0.0)	1764.4 (10.9)	74.5 (0.2)	8.8 (0.2)	2.3 (0.1)	11.3 (0.0)	1015.0 (35.4)	1.34 (0.02)	2.17 (0.01)	220.35 (14.17)
10 MAK 7-5	11.4 (0.0)	1776.9 (0.6)	73.5 (0.4)	8.4 (0.2)	**2.6** (0.0)	**12.4** (0.2)	1020.0 (42.4)	**1.71** (0.02)	**1.42** (0.07)	213.67 (3.95)
10 MAK 7-7	**14.0** (0.0)	1793.8 (1.7)	**69.2** (0.6)	**12.1** (0.1)	**3.4** (0.0)	**13.5** (0.1)	**1560.0** (155.6)	**2.05** (0.07)	**1.70** (0.09)	250.50 (9.46)
10 MAK 7-8	**14.1** (0.1)	1781.9 (13.2)	**69.5** (0.4)	**13.6** (0.2)	**3.6** (0.0)	**12.8** (0.1)	**1360.0** (28.3)	**1.76** (0.06)	2.18 (0.09)	**257.83** (1.84)

[a] Dry weight basis
[b] NDF = Neutral Detergent Fibre
[c] Mean
[d] Standard deviation
Values in bold are significantly different from CC-37 (white maize) for that nutrient (Dunnett test, p significant at < 0.05)

Cooked maize products

The nutritional composition of cooked white maize food products compared to cooked provitamin A-biofortified maize food products is presented in Table 5.8a. The energy, starch, fat, protein and phosphorus content of the provitamin A-biofortified maize *phutu* were significantly higher than that of the white maize *phutu* ($p < 0.05$). The provitamin A-biofortified maize thin porridge contained significantly higher levels of fibre, fat and protein compared to the white maize thin porridge ($p < 0.05$). The fat, protein and mineral content of the biofortified maize samp were significantly higher than the white maize samp ($p < 0.05$). The iron levels in the biofortified maize *phutu* and the samp were significantly lower than the corresponding white maize products, but the iron levels were generally higher in the biofortified maize thin porridge. The low levels of iron in the cooked products are in line with the results of the nutritional composition of the whole maize grains (Table 4.3, Chapter 4) which show that the iron levels in the biofortified whole maize grain were lower compared to the white maize grain. The zinc levels in all the cooked products of the biofortified maize varieties were lower than that in the corresponding white maize products. This is in contrast to the results in Table 4.3 (Chapter 4), which shows that the zinc levels in the biofortified maize and white maize grains were comparable. This suggests that more zinc was lost during the processing (milling and cooking) of the provitamin A-biofortified maize relative to the white maize. The results may be attributed to differences in the properties of the two types of maize, including their composition and milling properties.

The lower levels of iron and zinc in the cooked biofortified maize products could be addressed by combining these staple foods with other foods that are good sources of iron and zinc. However, in low-income communities where biofortified maize is likely to be used, high iron foods such as liver, kidney, mussels and red meat as well as medium iron foods such as chicken, fish and processed meat (Gibson 2005, p445) are unlikely to be available due to high cost. Therefore, the cooked biofortified maize products should be combined with foods such as dried beans, lentils and green leafy vegetables, which are relatively good, inexpensive sources of iron (Sizer & Whitney 2011, p304). A similar recommendation may also apply to zinc; the food sources of readily absorbable zinc include meat, liver, fish and shellfish, but these sources would be expensive for poor communities. Thus the plant food zinc sources, such as cereal grains, nuts and legumes would be recommended (Gibson 2005, p714). It is noted, however, that in plant foods, the bioavailability of nutrients, especially the divalent metal irons (including zinc and iron) is significantly limited by their interaction with

antinutritional factors, notably phytic acid, oxalic acid, trypsin inhibitors and tannins (Hotz & Brown 2004; Gibson 1994). The phosphorus levels were generally higher in the cooked biofortified maize products, compared to the white maize products. This could be because the phosphorus values in the milled products of the three varieties of the provitamin A-biofortified maize were in some cases higher than the phosphorus values in the corresponding white maize milled products. Overall, Table 5.8a shows that the cooked provitamin A-biofortified maize products are better sources of energy, fat, protein and phosphorus compared to the cooked white maize products. This is in line with the nutritional composition of the provitamin A-biofortified maize grain (Chapter 4, Table 4.3), which was found to be higher in starch, fat, protein, and therefore energy, compared to the white maize grain.

Table 5.8a Nutritional composition of cooked provitamin A-biofortified maize food products compared to the corresponding white maize food products

Food products and maize varieties	Moisture (%)	Energy[a] (kJ/100g)	Starch[a] (g/100g)	NDF[ab] (g/100g)	Fat[a] (g/100g)	Protein[a] (g/100g)	Total minerals[a] (mg/100g)	Iron[a] (mg/100g)	Zinc[a] (mg/100g)	Phosphorus[a] (mg/100g)
Phutu										
CC-37	33.4[c] (1.0)[d]	1727.8 (2.2)	76.6 (0.3)	8.9 (0.3)	1.6 (0.0)	10.4 (0.1)	1130.00 (14.14)	1.57 (0.01)	1.87 (0.08)	195.93 (2.01)
10 MAK 7-5	30.4 (1.0)	**1799.7** (5.6)	**74.8** (0.6)	**11.4** (0.3)	**1.9** (0.1)	**12.4** (0.0)	1125.00 (49.50)	**1.36** (0.03)	1.52 (0.16)	**262.03** (0.48)
10 MAK 7-7	**38.3** (1.2)	**1813.1** (0.1)	**73.9** (0.5)	**5.5** (0.1)	**2.2** (0.0)	**11.7** (0.0)	1200.00 (42.43)	**1.16** (0.02)	**1.34** (0.07)	**228.97** (2.48)
10 MAK 7-8	36.0 (0.8)	**1813.3** (2.9)	**74.0** (0.2)	8.1 (0.3)	**2.3** (0.0)	**12.1** (0.1)	**1720.00** (113.14)	**1.33** (0.05)	1.79 (0.07)	**237.15** (4.89)
Thin porridge										
CC-37	90.8 (0.1)	1726.0 (0.1)	74.9 (0.0)	6.7 (0.2)	1.0 (0.0)	10.7 (0.0)	960.00 (28.28)	1.53 (0.04)	2.00 (0.01)	196.30 (2.70)
10 MAK 7-5	**91.6** (0.1)	**1759.4** (9.3)	73.9 (0.7)	**5.5** (0.1)	**1.4** (0.1)	**11.9** (0.0)	1095.00 (35.35)	**1.35** (0.04)	**1.48** (0.07)	**220.80** (1.14)
10 MAK 7-7	90.8 (0.2)	**1771.8** (4.1)	74.6 (0.4)	**4.8** (0.1)	**1.2** (0.0)	**12.8** (0.2)	1085.00 (49.50)	1.57 (0.01)	**1.39** (0.08)	202.36 (4.91)
10 MAK 7-8	**91.2** (0.0)	1716.2 (1.7)	73.9 (1.3)	**5.5** (0.0)	**2.0** (0.1)	11.9 (0.1)	**1480.00** (28.28)	**1.77** (0.01)	1.95 (0.06)	**220.45** (8.61)
Samp										
CC-37	77.0 (0.1)	1788.0 (5.1)	69.3 (0.2)	18.4 (0.6)	2.3 (0.0)	11.2 (0.1)	955.00 (7.07)	1.45 (0.01)	2.26 (0.01)	155.35 (0.98)
10 MAK 7-5	78.6 (0.2)	1803.1 (5.0)	70.4 (0.6)	18.1 (0.6)	**2.6** (0.0)	**12.3** (0.0)	**895.00** (7.07)	**0.98** (0.04)	**1.45** (0.080	**121.40** (6.92)
10 MAK 7-7	73.1 (1.4)	**1839.7** (7.6)	**66.9** (0.0)	18.3 (0.8)	**3.9** (0.0)	**13.6** (0.1)	**1335.00** (7.07)	**1.35** (0.04)	1.94 (0.00)	**190.00** (3.33)
10 MAK 7-8	**70.2** (3.3)	**1839.0** (0.8)	**67.3** (0.7)	**22.2** (0.1)	**3.4** (0.0)	**13.0** (0.0)	**1280.00** (28.28)	**1.35** (0.01)	2.10 (0.09)	151.48 (2.68)

[a] Dry weight basis
[b] NDF = Neutral Detergent Fibre
[c] Mean
[d] Standard deviation
Values in bold are significantly different from CC-37 (white maize) for that nutrient according to the Dunnett test, p significant at < 0.05

5.3.5   Retention of nutrients, other than provitamin A carotenoids

Milling

Table 5.8b shows the retention of other nutrients in maize products after processing. After milling into mealie meal, starch, energy and protein were well retained in all the three maize varieties. The greatest nutrient loss after milling into mealie meal was for fibre (21.8-26.9%). Although both iron and zinc were lost after milling into mealie meal, zinc losses were lower compared to iron. The 106.1% increase in the zinc content in the biofortified variety 10 MAK 7-5 on milling into mealie meal could be due to a laboratory error in the analysis for zinc or contamination. Starch, energy and protein were well retained in all three maize varieties with milling into samp.

Cooking

Cooking into *phutu* resulted in an overall increase in the levels of energy, fibre, total minerals and iron with a decrease in starch, fat, protein and zinc. Cooking into thin porridge resulted in an overall increase in the levels of fibre, total minerals and iron levels, whilst there was a decrease in the levels of energy, starch, fat, protein and zinc. With cooking into samp, there was an increase in the levels of energy, fibre, fat, protein and zinc, whilst there was a loss of starch, total minerals and iron. Only one effect of cooking on nutrient retention was common to all the three food products - the fibre levels increased on cooking. The nutrients, starch, protein, iron and zinc are generally stable at cooking temperatures below 100 ºC. In this study, the food products were cooked in conditions of moisture, moderate heat (96 ºC, 86 ºC and 84 ºC, for thin porridge, *phutu* and samp, respectively) and very slightly acidic pH (6.4, 6.0 and 5.8 for thin porridge, *phutu* and samp, respectively). Most of the nutrients, including starch, minerals and proteins are generally stable in these conditions (Miller 1996, p639; Potter & Hotchkiss 1995, pp63-64). The decreases in the contents of these nutrients are likely to have been due to the interaction of the nutrients among themselves and with other cell components, resulting in them being less assayable. It is noteworthy that apart from decreasing their assayability, the suggested interactions of these nutrients would likely reduce their bioavailability.

The results of the retention of other nutrients in provitamin A-biofortified maize during processing, i.e. milling and cooking obtained from this study, are new and have not been previously reported.

Table 5.8b Retention of nutrients other than provitamin A during processing of provitamin A-biofortified maize into food products

Maize variety	Maize form	Energy	Starch	NDF	Fat	Protein	Total minerals	Iron	Zinc	Phosphorus
							Retention (%)			
	MILLED									
	Raw mealie meal									
10 MAK 7-5		98.4	142.7	26.9	65.1	95.7	79.3	18.9	106.1	55.5
10 MAK 7-7		97.1	145.3	26.3	56.0	91.1	64.0	23.2	69.5	50.4
10 MAK 7-8		98.1	140.9	21.8	54.3	91.7	69.5	26.2	64.8	57.1
	Raw samp									
10 MAK 7-5		98.5	135.4	69.3	59.3	99.5	68.2	34.0	72.1	59.2
10 MAK 7-7		97.5	125.8	117.3	79.8	100.1	98.4	42.0	76.2	63.2
10 MAK 7-8		97.6	125.3	102.6	85.9	97.1	78.4	35.5	75.2	63.0
	COOKED									
	Phutu									
10 MAK 7-5		101.3[a]	96.5	346.0	67.5	104.3	94.9	143.2	72.7	131.0
		(99.8)[b]	(137.8)	(93.2)	(44.0)	(99.8)	(75.3)	(27.0)	(77.2)	(72.7)
10 MAK 7-7		101.5	92.5	202.6	91.3	95.4	118.2	74.8	118.6	114.5
		((98.6)	(134.4)	(53.2)	(51.2)	(86.8)	(75.7)	(23.8)	(60.1)	(57.7)
10 MAK 7-8		101.8	106.5	59.8	64.1	94.3	126.5	102.3	95.2	101.4
		(99.4)	(133.4)	(61.4)	(55.0)	(91.6)	(99.1)	(26.8)	(61.7)	(57.9)
	Thin porridge									
10 MAK 7-5		99.1	95.3	168.0	50.0	100.2	92.4	142.1	70.8	110.4
		(97.5)	(136.1)	(45.2)	(32.6)	(95.9)	(73.2)	(26.8)	(75.1)	(61.2)
10 MAK 7-7		99.2	93.4	177.5	51.5	103.8	106.9	101.3	123.0	101.2
		(96.3)	(135.7)	(46.6)	(28.8)	(94.5)	(68.5)	(32.2)	(62.3)	(51.0)
10 MAK 7-8		96.3	106.4	40.8	56.0	93.2	108.8	136.2	103.7	94.3
		(94.0)	(133.4)	(41.8)	(48.1)	(90.5)	(85.3)	(35.7)	(67.2)	(53.8)
	Samp									
10 MAK 7-5		101.5	95.8	214.6	102.4	99.8	87.7	57.3	102.1	56.8
		(99.9)	(129.7)	(148.7)	(60.7)	(99.3)	(59.9)	(19.5)	(73.6)	(33.7)
10 MAK 7-7		102.6	96.7	150.9	112.8	100.4	85.6	65.9	114.1	75.9
		(100.0)	(121.7)	(177.1)	(90.0)	(100.4)	(84.2)	(27.7)	(87.0)	(47.9)
10 MAK 7-8		103.2	96.9	163.8	93.6	101.8	94.1	76.7	96.3	58.8
		(100.8)	(121.4)	(168.0)	(80.4)	(98.9)	(73.8)	(27.2)	(72.4)	(37.0)

[a] Retention at a processing step; [b] Overall retention in parentheses; calculated as proportion of nutrient in cooked product compared with the nutrient content in whole grain; NDF = Neutral Detergent Fibre

5.4   Conclusions

The present study shows that different carotenoids, including vitamin A precursors (provitamin A), are present in biofortified maize varieties, and also that the carotenoid composition seems to be influenced by maize variety. Thus, it seems feasible to screen maize varieties for significant concentrations of provitamin A in a breeding programme. Results of the present study indicate that milling provitamin A-biofortified maize into mealie meal result in a higher retention of carotenoids compared to milling into samp. Milling the maize grain into both mealie meal and samp resulted in substantial losses of iron, zinc and phosphorus, and there was also a substantial reduction of fibre on milling into mealie meal. The highest retention of provitamin A carotenoids was observed in cooked *phutu* and cooked samp, whilst the lowest retention of provitamin A carotenoids was observed in cooked thin porridge. The present study highlights the need to identify and recommend maize food forms in which there is a high retention of provitamin A carotenoids occur during processing, to ensure optimal delivery of the carotenoids to the consumer. In addition, the study also suggests that many varieties of maize should be tested for both value for cultivation and use, because of the association between maize variety and provitamin A concentration. This study is the first to report the retention of other nutrients in provitamin A-biofortified maize during processing, i.e., milling and cooking. The consumption of a single usual portion of a cooked provitamin A-biofortified maize product on a daily basis has the potential to make a reasonable contribution towards meeting the EAR for vitamin A, in all gender and age groups. However, a more substantial contribution could be achieved if the usual portions of the provitamin A-biofortified maize products were consumed three times a day. The implementation of intensive nutrition education on the nutritional benefits of consuming provitamin A-biofortified maize food products is essential, in order to achieve a substantial level of intake of provitamin A-biofortified maize food products that would result in a substantial intake of provitamin A.

References

Association of Official Analytical Chemists (AOAC) International (2002). **Official Methods of Analysis of AOAC International**, 17th ed. Volume I and II. Maryland: AOAC International.

Faber M (2004). Complementary foods consumed by 6-12 month old rural infants in South Africa are inadequate in micronutrients. **Public Health Nutrition** 8: 373-381.

Faber M, Jogessar VB, Benadé AJS (2001). Nutritional status and dietary intakes of children aged 2-5 years and their caregivers in a rural South African community. **International Journal of Food Sciences and Nutrition** 52: 401-411.

Faber M, Kruger S (2005). Dietary intake, perceptions regarding body weight, and attitudes toward weight control of normal weight, overweight, and obese Black females in a rural village in South Africa. **Ethnicity & Disease** 15: 238-245.

Faber M, Smuts CM, Benadé AJS (1999). Dietary intake of primary school children in relation to food production in a rural area in KwaZulu-Natal, South Africa. **International Journal of Food Sciences and Nutrition** 50: 57-64.

Food and Agriculture Organization of the United Nations (FAO) (1992). **Maize in Human Nutrition**. Rome, Italy.

Gibson RS (1994). Content and bioavailability of trace elements in vegetarian diets. **American Journal of Clinical Nutrition** 59: 1223S-1232S.

Gibson RS (2005). **Principles of Nutritional Assessment**, 2nd ed. New York: Oxford University Press.

Granado F, Olmedilla B, Blanco I, Rojas-Hidalgo E (1992). Carotenoid composition in raw and cooked Spanish vegetables. **Journal of Agricultural and Food Chemistry** 40: 2135-2140.

Gregory JF (1996). Vitamins, 3rd ed. In: Fennema OR, ed. **Food Chemistry**. New York: Marcel Dekker Inc.

HarvestPlus Brief (2006). **HarvestPlus: Breeding Crops for Better Nutrition**. Washington DC, USA.

Hotz C, Brown KH (eds) (2004). International Zinc Nutrition Consultative Group (IZiNCG). Technical Document No. 1: Assessment of the risk of zinc deficiency in populations and options for its control. **Food and Nutrition Bulletin** 25 (Supp 2): S94-S204.

Howe JA, Tanumihardjo SA (2006a). Carotenoid-biofortified maize maintains adequate vitamin A status in Mongolian gerbils. **The Journal of Nutrition** 136: 2562-2567.

Howe JA, Tanumihardjo SA (2006b). Evaluation of analytical methods for carotenoids extraction from biofortified maize (*Zea mays* sp.). **Journal of Agricultural and Food Chemistry** 54: 7992-7997.

Institute of Medicine (2000a). **Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids.** Food and Nutrition Board. Washington DC: National Academy Press.

Institute of Medicine (2000b). **Dietary Reference Intakes: applications in dietary assessment**. Food and Nutrition Board. Washington DC: National Academy Press.

Institute of Medicine (2001). **Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc.** Food and Nutrition Board. Washington DC: National Academy Press.

International Institute of Tropical Agriculture (IITA) (2010a). **New varieties to boost maize output in West and Central Africa.**
http://www.iita.org/maize/-/asset_publisher/jeR0/content/new-varieties-to-boost-maize (date accessed 22/11/2010).

Johnson LA (2000). Corn: The Major Cereals of the Americas, 2nd ed. In: Kulp K, Ponte JG, eds. **Handbook of Cereal Science and Technology**. New York: Marcel Dekker.

Khachik F, Beecher GR, Goli MB, Lusby WR (1992a). Separation and quantification of carotenoids in foods. **Methods in Enzymology** 213: 347-359.

Khachik F, Goli MB, Beecher GR, Holden J, Lusby WR, Tenorio MD, Barrera MR (1992b). Effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomatoes and several green vegetables. **Journal of Agricultural and Food Chemistry** 40: 390-398.

Labadarios D, Moodie IM, Van Rensburg A (2007). Vitamin A status. In: Labadarios D. **National Food Consumption Survey: Fortification Baseline**, Chapter 9B: South Africa 2005. Stellenbosch: Department of Health.

Labadarios D, Van Middelkoop AM (1995). **The South African Vitamin A Consultative Group. Children aged 6-71 months in South Africa, 1994: their anthropometric, vitamin A, iron and immunisation coverage status**. Isando, South Africa.

Li S, Tayie FAK, Young MF, Rocheford T, White WS (2007). Retention of provitamin A carotenoids in high β-carotene maize (Zea mays) during traditional African household processing. **Journal of Agricultural and Food Chemistry** 55: 10744-10750.

Lozano-Alejo N, Carrillo GV, Pixley K, Palacios-Rojas N (2007). Physical properties and carotenoid content of maize kernels and its nixtamalized snacks. **Innovative Food Science and Emerging Technologies** 8: 385-389.

Menkir A, Liu W, White WS, Maziya-Dixon B, Rocheford T (2008). Carotenoid diversity in tropical-adapted yellow maize inbred lines. **Food Chemistry** 109: 521-529.

Miller DD (1996). Minerals, 3rd ed. In: Fennema OR, ed. **Food Chemistry**. New York: Marcel Dekker Inc.

Murphy EW, Criner PE, Gray BC (1975). Comparisons of methods for calculating retentions of nutrients in cooked foods. **Journal of Agricultural and Food Chemistry** 23: 1153-1157.

Muzhingi T, Yeum KJ, Russell RM, Johnson EJ, Qin J, Tang G (2008a). Determination of carotenoids in yellow maize, the effects of saponification and food preparations. **International Journal for Vitamin and Nutrition Research** 78: 112-120.

Nestel P, Bouis HE, Meenakshi JV, Pfeiffer W (2006). Biofortification of staple food crops. **The Journal of Nutrition** 136: 1064-1067.

Nuss ET, Tanumihardjo SA (2010). Maize: a paramount staple crop in the context of global nutrition. **Comprehensive Reviews in Food Science and Food Safety** 9: 417-436.

Ortiz-Monasterio JI, Palacios-Rojas N, Meng E, Pixley K, Trethowan R, Pena RJ (2007). Enhancing the mineral and vitamin content of wheat and maize through plant breeding. **Journal of Cereal Science** 46: 293-307.

Potter NN, Hotchkiss JH (1995). **Food Science**, 5th ed. Chapman & Hall: New York.

Rice AI, West KP, Black RE (2004). Vitamin A deficiency. In: **Comparative quantification of health risks**. http://www.who.int/publications/cra/chapters/volume1/0211-0256.pdf (date accessed 25/10/2010).

Rodriguez-Amaya DB (1997). **Carotenoids and Food Preparation: the retention of Provitamin A Carotenoids in Prepared, Processed, and Stored Foods**. Washington DC: OMNI/USAID.

Sizer F, Whitney E (2011). **Nutrition Concepts and Controversies**, 12th ed. USA: Wadsworth Cengage Learning.

Tanumihardjo SA (2002). Factors influencing the conversion of carotenoids to retinol: bioavailability to bioconversion to bioefficacy. **International Journal of Vitamin and Nutrition Research** 72: 40-45.

Tanumihardjo SA (2008). Food-based approaches for ensuring adequate vitamin A nutrition. **Comprehensive Reviews in Food Science and Food Safety** 7: 373-381.

Trumbo P, Yates AA, Schlicker S, Poos M (2001). Dietary Reference Intakes: Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. **Journal of the American Dietetic Association** 101: 294-301.

West KP, Jr (2002). Extent of vitamin A deficiency among preschool children and women of reproductive age. **The Journal of Nutrition** 132: 2857-2866.

White PJ, Broadley MR (2005). Biofortifying crops with essential mineral elements. **Trends in Plant Science** 10: 586-593.

CHAPTER 6

CONSUMER ACCEPTABILITY OF YELLOW, PROVITAMIN A-BIOFORTIFIED MAIZE IN KWAZULU-NATAL, SOUTH AFRICA[10]

Abstract

Biofortification of maize with provitamin A through conventional breeding may contribute to alleviating vitamin A deficiency (VAD), especially in sub-Saharan Africa. However, the biofortified maize is yellow and may be less acceptable to consumers relative to the traditional white maize. In this study, consumer sensory tests were used to assess the consumer acceptability of popular maize food products (*phutu*, thin porridge and samp), prepared with three varieties of yellow, provitamin A-biofortified maize as well as a control white maize variety. A total of 212 subjects aged 3-55 years drawn from rural KwaZulu-Natal, South Africa, where white maize grain is the dominant staple food, participated in the study. Preference for yellow maize food products was negatively associated with an increase in the age of the subjects. Preschool children overall preferred yellow maize to white maize food products: *phutu* (81% vs. 19%), thin porridge (75% vs. 25%) and samp (73% vs. 27%). In sharp contrast, primary school children preferred white maize to yellow maize food products: *phutu* (55% vs. 45%), thin porridge (63% vs. 38%) and samp (52% vs. 48%). Similarly, secondary school children and adults also displayed a similar preference for white maize food products. Focus group discussions confirmed the preference for white maize to yellow maize by adults. The results of this study confirm previous study findings that adults prefer white maize to yellow maize. However, the preference for yellow maize to white maize among preschool children is a new and important finding. This suggests that yellow maize has the potential to succeed as a strategy to alleviate VAD in children of preschool age who are also the most vulnerable to VAD. It is also derived from the results that if children of preschool age are exposed to yellow maize, it could influence their choice as adults. In addition, intensive nutrition education programmes on the nutritional benefits of yellow maize, may improve its overall acceptability among older consumers.

6.1   Introduction

Globally, vitamin A deficiency (VAD) affects approximately 190 million children under the age of five (WHO 2009). According to the accepted criteria of the World Health Organization

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[10] Publication based on this research chapter:
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(WHO), South Africa has a serious public health problem of poor vitamin A status (WHO 2009). The South African Vitamin A Consultative Group (SAVACG) study of 1994 reported that approximately 33% of children had VAD (Labadarios & Van Middelkoop 1995). This was supported by the findings of the National Food Consumption Survey (NFCS 1999) which indicated that one out of two children in South Africa had a vitamin A intake of less than half the recommended level (Labadarios *et al* 2000). In 2000, approximately 3069 deaths in children aged 0-4 years (3.2% of all deaths in this age group) were attributed to VAD (Nojilana *et al* 2007). The 2005 National Food Consumption Survey-Fortification Baseline (NFCS-FB) revealed that the vitamin A status of South African children had deteriorated significantly since 1994, as 64% of South African children aged 1-9 years were found to have VAD (Labadarios *et al* 2007).

The South African Department of Health is currently addressing micronutrient deficiencies through supplementation, food fortification and promotion of dietary diversification (Labadarios *et al* 2005). However, these strategies have not resulted in an improvement in the vitamin A status of South African children since 1994 (Swart *et al* 2008). Despite South African legislation of the fortification of maize meal and wheat flour since 2003, the accessibility of these commercially fortified foods to poor people living in remote, rural areas remains questionable (Faber & Wenhold 2007; Nestel *et al* 2006; Labadarios *et al* 2005). Biofortification of maize varieties with provitamin A by conventional breeding has emerged recently as a potential long-term sustainable strategy to improve vitamin A status in humans (Howe & Tanumihardjo 2006a; Howe & Tanumihardjo 2006b; Nestel *et al* 2006). Biofortification by conventional breeding is an attractive approach as it can deliver naturally fortified foods to people who may not have access to commercially-biofortified food products that are more readily available to consumers in urban areas and in developed countries (Nestel *et al* 2006).

Biofortification of maize with provitamin A carotenoids changes the grain colour from white to yellow/orange as well as the aroma and flavour of the maize. In many parts of Africa, including Eastern and Southern Africa, there is a cultural preference for white maize, and thus changes in colour and other sensory properties associated with provitamin A-biofortification of maize may result in negative sensory perceptions (Stevens & Winter-Nelson 2008; HarvestPlus Brief 2006). Rigorous studies on consumer acceptance of yellow/orange maize have been conducted in some African countries, such as Zimbabwe (Muzhingi *et al* 2008b),

Kenya (De Groote *et al* 2010; De Groote & Kimenju 2008), and Mozambique (Tschirley & Santos 1995), where white maize is the predominant staple. A study in Kenya showed a general strong preference for white maize, whilst yellow maize was preferred only in some areas. The dislike of yellow maize in Kenya seems to come from prejudice and negative associations, such as food aid and animal feed rather than from sensory characteristics such as taste (De Groote *et al* 2010; De Groote & Kimenju 2008). In Mozambique, consumers indicated a preference for white maize to yellow maize; although poorer consumers indicated a willingness to purchase yellow maize if offered at a discounted price (Tschirley & Santos 1995). A more recent study in Mozambique showed a more favourable response to orange maize, particularly the aroma (Stevens & Winter-Nelson 2008). There is a lack of published data on the acceptability of yellow maize by South African consumers.

Studies on consumer acceptability of yellow/orange maize in Africa have thus far only been conducted on urban adult consumers. These consumers are not the primary target of provitamin A-biofortified maize. Rural consumers (including children) are more at risk of VAD (De Groote & Kimenju 2008; Allen 2006; Ahmed & Darnton-Hill 2004). The aim of this study was to assess the acceptability of maize food products prepared with yellow, provitamin A-biofortified maize by consumers in rural KwaZulu-Natal, South Africa. The food products studied wer: *phutu* (a stiff porridge made from maize meal), thin porridge (porridge made from maize meal with a dry matter content of approximately 14%) and samp (broken maize grain). These food products were chosen as they have been found to be the most popular to rural African communities in KwaZulu-Natal (Faber & Kruger 2005; Faber 2004; Faber *et al* 2001; Faber *et al* 1999).

6.2   Materials and Methods

6.2.1   Maize breeding

The experimental maize varieties used in this study were produced as described in Chapter 3, section 3.2. SC-701, which is a popular white maize grain variety in Southern Africa, was included as the control and was grown under the same conditions as the yellow maize hybrids. The maize was harvested manually and left to dry under ambient conditions (± 25 ℃) for 21 days. The maize was then threshed mechanically and the grain was stored in a cold room (± 4 ℃) before milling. A grain sample of 5 kg was then drawn for food processing.

6.2.2   Colour measurements and maize variety selection

Five varieties of yellow maize ranging in colour from light yellow to darker yellow were selected using visual assessment. Yellow grain colour intensity was then determined using a Colorflex instrument (Hunter Associates Laboratory, Inc., Reston, Virginia, USA), in terms of the Hunter *L, a, b,* system which uses *L* as a measure of lightness (0 = black to 100 = white), *a* as a measure of redness and (+*a* = redness; -*a* = greenness), and *b* is a measure of yellowness (+*b* = yellowness; -*b* = blueness) (DeMan 1999, p237). The measurements were done in duplicate and mean values were calculated (Table 6.1).

Table 6.1   Hunter *L, a, b* values for the five yellow maize varieties

Maize variety	**Grain characteristics**		
	L^a	a^b	b^c
KP-80	$56.81^d (0.33)^e$	11.66 (1.20)	21.62 (1.05)
KP-79	56.66 (2.14)	11.38 (1.61)	21.25 (0.64)
KP-78	57.00 (0.70)	11.48 (0.78)	21.30 (1.77)
KP-77	54.84 (0.39)	13.78 (0.04)	22.02 (1.30)
KP-76	53.57 (0.78)	13.66 (0.35)	21.30 (1.62)

[a] *L* - measure of lightness (0 = black to 100 = white)
[b] *a* - measure of redness and (+*a* = redness; -*a* = greenness)
[c] *b* - measure of yellowness (+*b* = yellowness; -*b* = blueness)
[d] Mean
[e] Standard deviation

Based on the data in Table 6.1, the hybrid KP-77 was chosen as the deepest yellow variety, KP-79 as the medium yellow variety and KP-78 as the lightest yellow variety.

6.2.3   Maize milling

Grain of the three selected yellow maize varieties and the white variety was milled to obtain samp and maize meal according to the procedures described in Chapter 5, section 5.2.2.

6.2.4   Preparation of maize food products

Three women from rural KwaZulu-Natal with appropriate cooking experience were recruited to cook the popular maize products, *phutu,* thin porridge and samp. The food products assessed by the consumers in this study were prepared fresh on a daily basis in the Food Processing Laboratory at the University of KwaZulu-Natal (Figure 6.1). The procedure for the preparation of each of the products is described in Chapter 5, section 5.2.3. Sugar and salt were included during preparation of the food products in this research chapter as it involved

sensory evaluation. Standardised recipes for the food products can be found in Appendix D (p174), Appendix E (p175) and Appendix F (p176). The food samples were transported to the study site in insulating plastic containers closed with tight-fitting lids.

Figure 6.1   Preparation of yellow maize food products
in the Food Processing Laboratory

6.2.5   Consumer sensory evaluation

Black African male and female subjects who were regular consumers of *phutu*, thin porridge and samp were recruited from the Mkhambathini Municipality in KwaZulu-Natal. The Mkhambathini Municipality was chosen as a site for the study as it has a large Black African population (approximately 93% of the total population) and can be regarded as a low income area due to the high unemployment rate (44% in 2001) and low average annual household income (R5742 in 2004) [approximately US$838.00] (Mkhambathini Local Municipality 2007). The schools that participated in the study were selected on the basis that they fall into quintile 1 and quintile 2 of the South African National Quintile for Public Schools, which indicates that the school is located in an area with a high general prevalence of poverty (South African Department of Education Resource Targeting List 2010). Preschool (n=52), primary (n=56) and secondary school (n=54) subjects were selected from two primary schools and one secondary school in the area. The schools were a convenience sample and were selected based on their accessibility and close proximity to each other. The learners were randomly selected using their class registers which listed the learners numerically in alphabetical order of their surnames. The numbers that listed the learner were each written on a piece of paper and

mixed together. The subjects for sensory evaluation were then drawn randomly. The adult subjects (n=50) were a convenience sample drawn from the School Parents Meetings held at the schools. The sample size for each age group was 50 or more subjects, which was in accordance with the accepted sample sizes for consumer acceptance and preference tests (Stone & Sidel 2004, pp 247-277).

Sensory evaluation was carried out in small groups of between five to eight panellists. Preschool and primary school subjects completed a paired preference test (Figure 6.2a; Figure 6.2b) [Appendix G (p177) - English version and Appendix H (p178) - Zulu version]. A paired preference test can be performed reliably by children over the age of two years (Guinard 2001; Kimmel *et al* 1994). The test involved tasting a sample and the control of each of the three food products (*phutu*, thin porridge and samp) prepared with KP-79 (medium yellow maize variety) and SC-701(white maize variety), separately. Fieldworkers assisted the pre- and primary school children to record their responses. The secondary school subjects and adults tasted each of the three food products (*phutu*, thin porridge and samp) prepared with four maize varieties (SC-701; KP-78; KP-79; KP-77), separately (Figure 6.3a; Figure 6.3b). Prior to each session, the sensory attributes aroma, texture and flavour were explained to the secondary school and adult subjects in *Zulu*. The panellists seemed to understand the sensory attribute concepts, although the researchers could not ascertain that all subjects were able to distinguish the different sensory attributes.The food products were evaluated using a five-point facial hedonic scale (1=very bad; 5=very good) [Appendix I (p179) - English version and Appendix J (p181) - Zulu version] and a preference ranking test (1=most preferred; 4=least preferred) [Appendix K (p184) - English version and Appendix L (p185) - Zulu version. Secondary school subjects were able to record their own responses whilst some adult subjects were assisted by the fieldworkers. The food samples were warmed to about ±45ºC with a microwave oven in small batches just before serving. The samples (±30ml) were served in 125 ml polystyrene cups. The samples were blind labelled with 3-digit codes obtained from a Table of Random Numbers (Heymann 1995) and were served in a random order, which was determined using a Table of Random Permutations of Nine (Heymann 1995). Each panellist was provided with a spoon and a polystyrene cup of water to cleanse the palate between samples.

Figure 6.2a   Preschool subjects completing paired preference tests

Figure 6.2b   Primary school subjects completing paired preference tests
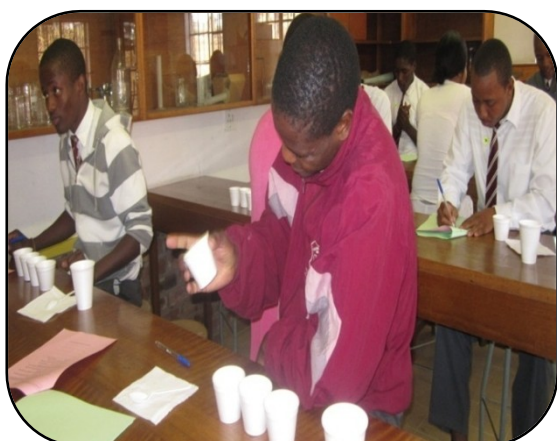
Figure 6.3a   Secondary school subjects completing five-point facial hedonic and preference ranking tests

Figure 6.3b   Adult subjects completing five-point facial hedonic and preference ranking tests

6.3   Focus group discussions

Nine men and nine women were randomly selected and participated as separate gender groups in the focus group discussions, which were conducted after the sensory evaluation sessions (Figure 6.4a; Figure 6.4b). The accepted sample size for focus group discussions is 8-12 subjects (Merton *et al* 1990, p137; Mullings 1985, pp5-6). Although several focus group discussions using different participants would have been desirable, only two adult focus group discussions were conducted due to time constraints. Themes on consumer perceptions of and attitudes towards yellow maize were developed and corresponding focus group discussion

questions generated. The focus group discussions were conducted by a trained research assistant in *Zulu*, which is the local language in the KwaZulu-Natal province. Both sessions were recorded using a digital voice recorder and the recordings were translated into English by a *Zulu*-speaking person. The English translations were then compared to the *Zulu* recordings and checked for accuracy of translation by another *Zulu*-speaking person. See Appendix M (p186) for Focus group questions in English and Appendix N (p187) for *Zulu*.

Figure 6.4a   Adult males participating in the focus group discussion

Figure 6.4b   Adult females participating in the focus group discussion

6.4   Ethical approval

Ethical approval to carry out this study was obtained from the University of KwaZulu-Natal, Humanities and Social Sciences Ethics Committee (Approval number HSS/0591/09D). Approval to carry out the study in the Mkhambathini Municipality was obtained from the Municipal Manager and approval to use the schools in the area was obtained from the KwaZulu-Natal Department of Education. Written consent was obtained from the Parents or Guardians of the learners [see Appendix O (p189) for consent document in English and Appendix P (p190) for consent document in Zulu] and the adult subjects [see Appendix Q (p191) for consent document in English and Appendix R (p192) for consent document in Zulu] prior to participation in the study.

6.5   Statistical analysis

SPSS (Statistical Package for Social Sciences) version 15.0 (SPSS Inc., Chicago, III, USA) was used to analyse the data. The *Z*-test was used to compare the proportions of subjects who preferred each of the two varieties in the paired preference test in the preschool and primary school groups. Logistic regression and simple linear regression analyses were used to

determine the effect of age on maize variety preference all groups. Chi-square analysis was used to determine the relationship between gender and maize variety preference in the secondary school and adult groups. Multiple linear regression analysis was used to determine the sensory attributes that had significant influence on the overall acceptance of a sample. One way analysis of variance (ANOVA) and Tukey post-hoc multiple comparison of means were used to analyse for differences in the acceptance of the sensory attributes evaluated. The level of significance was $p<0.05$.

6.6 Results and Discussion

6.6.1 Consumer sensory evaluation

A total of 212 subjects with an age range from 3.1 to 55.5 years participated in the study (Table 6.2).

Table 6.2 Consumer panel demographics

Group	Total number of subjects (n)	Number of males (%)*	Number of females (%)*	Age range (years)**	Mean age (years) (SD)
Preschool	52	22 (42)	30 (58)	3.1 - 6.5	5.4 (0.7)
Primary school	56	28 (50)	28 (50)	6.1-16.0	10.7 (3.0)
Secondary school	54	26 (48)	28 (52)	13.2-21.3	17.6 (1.9)
Adults	50	21 (42)	29 (58)	20.8-55.5	41.4 (8.1)

* Percentage (%) of total sample within each age group
** Age of subjects was obtained from the class register
SD = standard deviation

The results of the paired preference test for preschool and primary school subjects are shown in Table 6.3.

Table 6.3   Paired preference test results for preschool and primary school subjects

Group	*Phutu*		Thin porridge		Samp	
	SC-701	**KP-79**	**SC-701**	**KP-79**	**SC-701**	**KP-79**
PRESCHOOL						
No. of males (%)*	3 (14)	19 (86)	4 (18)	18 (82)	5 (23)	17 (77)
No. of females (%)*	7 (23)	23 (77)	9 (30)	21 (70)	9 (30)	21 (70)
Total no. of subjects (%)*	**10 (19)**	**42 (81)**	**13 (25)**	**39 (75)**	**14 (27)**	**38 (73)**
p value**	$p < 0.001$		$p < 0.001$		$p < 0.001$	
PRIMARY SCHOOL						
No. of males (%)*	14 (50)	14 (50)	16 (57)	12 (43)	15 (54)	13 (46)
No. of females (%)*	17 (61)	11 (39)	19 (68)	9 (32)	14 (50)	14 (50)
Total no. of subjects (%)*	**31 (55)**	**25 (45)**	**35 (63)**	**21 (38)**	**29 (52)**	**27 (48)**
p value**	$p = 0.478$		$p = 0.065$		$p = 0.779$	

SC-701: white maize (control)
KP-79 : medium yellow maize variety
* Percentage (%) of sample within gender group
** *Z*-test to compare proportions of subjects; p value is given for the total age group

The number of preschool children who preferred yellow maize food products was statistically significantly higher than that of children who preferred white maize food products (Table 6.3). In the primary school group, there was a tendency to prefer white thin porridge to yellow thin porridge, although this was not statistically significant ($p>0.05$).

The higher preference for white maize food products among the older children (primary and secondary school children) and adults compared with the younger children (preschool) could be due to the fact that older consumers had become more accustomed to white maize as they had been consuming it for a longer time than the younger children. The results suggest that provitamin A-biofortified maize may have the potential to solve the problem of VAD in children of preschool age, but this is dependent on the bioavailability of the β-carotene from the maize and the consumption of adequate amounts of the maize (Muzhingi, Gadaga, Siwela, Grusak, Russell & Tang 2011). However, it is generally the adult caregivers, particularly women, who purchase and prepare meals in the home. It is unlikely that the adult caregiver would prepare yellow maize food products separately for preschool children and white maize

for the rest of the household. This implies that education on the nutritional benefits of consuming yellow maize should be aimed at the adult caregivers, particularly women. The alternative would be to include yellow maize food products into preschool feeding schemes

The mean sensory attribute scores for all three food products from the secondary school and adult groups are shown in Table 6.4. In the secondary school group, the white variety had the highest scores for the acceptability of the sensory attributes of *phutu* and thin porridge, compared to the yellow varieties. However, in samp, the yellow variety KP-79 had the highest scores for appearance and aroma acceptability, whilst KP-77, another yellow variety, had the highest mean score for texture, flavour and overall acceptability. The white maize food products were generally more acceptable to adults relative to the yellow maize food products.

Table 6.4 Five-point facial hedonic rating and preference ranking of yellow maize food products by the secondary school and adult subjects in KwaZulu-Natal, South Africa.

	Phutu				Thin porridge				Samp			
	SC 701	KP-78	KP-79	KP-77	SC 701	KP-78	KP-79	KP-77	SC701	KP-78	KP-79	KP-77
Secondary school												
Appearance	3.93[a] (1.03)[b]	**3.41** (1.14)	**3.09** (1.14)	**3.31** (1.13)	4.19 (0.91)	**3.39** (0.92)	**3.52** (1.04)	**3.43** (0.98)	2.80 (1.23)	3.06 (1.11)	3.26 (1.07)	3.17 (1.11)
Aroma	3.91 (0.83)	**3.37** (1.09)	**3.33** (0.99)	3.52 (0.82)	3.59 (1.00)	3.20 (0.98)	3.39 (0.88)	3.35 (0.89)	3.13 (1.07)	3.20 (0.96)	3.37 (1.02)	3.24 (0.93)
Texture	3.81 (1.18)	3.52 (1.09)	**3.22** (1.19)	3.61 (0.92)	3.94 (0.88)	3.61 (1.12)	3.39 (1.28)	3.43 (1.28)	2.91 (1.14)	2.83 (1.04)	3.09 (1.03)	3.13 (1.05)
Flavour	4.02 (0.98)	3.69 (1.08)	**3.20** (1.12)	**3.43** (1.09)	3.96 (1.10)	3.78 (0.93)	3.52 (0.99)	3.63 (1.02)	3.17 (1.04)	3.04 (1.08)	3.04 (1.01)	3.19 (1.03)
Overall acceptability	4.24 (0.99)	3.85 (1.14)	**3.26** (1.17)	3.87 (1.12)	4.19 (0.97)	3.87 (0.91)	**3.69** (1.04)	3.85 (1.02)	3.20 (1.25)	3.04 (1.06)	3.17 (1.02)	3.35 (1.14)
Preference ranking	2.00 (1.26)	2.44 (0.98)	**2.85** (1.02)	**2.72** (1.04)	2.22 (1.30)	2.57 (0.92)	2.54 (1.18)	2.74 (1.05)	2.30 (1.31)	2.52 (1.06)	2.67 (1.01)	2.52 (1.08)
Adults												
Appearance	4.30 (0.91)	**3.08** (1.29)	**3.04** (1.09)	**3.16** (1.25)	4.36 (0.75)	**3.38** (1.31)	**3.40** (1.21)	**3.60** (1.03)	3.64 (1.23)	3.20 (1.11)	3.20 (1.18)	3.28 (1.14)
Aroma	4.14 (0.99)	**3.28** (1.18)	**3.04** (1.16)	**3.04** (1.21)	4.06 (0.79)	**3.34** (1.15)	**3.40** (1.13)	**3.52** (0.95)	3.52 (1.02)	3.16 (1.10)	3.22 (1.08)	3.24 (1.00)
Texture	4.32 (0.91)	**3.52** (1.34)	**3.10** (1.33)	**3.08** (1.31)	4.50 (0.74)	**3.38** (1.24)	**3.30** (1.22)	**3.72** (1.16)	3.78 (1.13)	**3.04** (1.18)	3.40 (1.05)	3.38 (1.19)
Flavour	4.48 (0.79)	**3.14** (1.20)	**3.14** (1.25)	**3.18** (1.40)	4.46 (0.58)	**3.28** (1.26)	**3.48** (1.19)	**3.42** (1.14)	3.70 (1.06)	**3.08** (1.09)	3.22 (1.04)	3.18 (1.14)
Overall acceptability	4.46 (0.97)	**3.30** (1.34)	**3.04** (1.31)	**3.14** (1.29)	4.54 (0.73)	**3.42** (1.31)	**3.34** (1.38)	**3.70** (1.18)	3.64 (1.23)	3.12 (1.15)	3.24 (1.14)	3.28 (1.18)
Preference ranking	1.32 (0.79)	**2.86** (0.99)	**2.80** (0.88)	**3.04** (0.90)	1.44 (0.86)	**3.00** (1.01)	**2.90** (0.97)	**2.66** (0.90)	1.96 (1.21)	**2.94** (1.08)	**2.58** (1.01)	**2.52** (0.97)

[a] Mean
[b] Standard deviation
Mean acceptability scores in bold were significantly different from those of the control maize (SC-701) according to the Tukey test (p<0.05)
SC-701: white maize variety (control); KP-78: light yellow maize variety; KP-79: medium yellow maize variety; KP-77: deep yellow maize variety
Five-point facial hedonic ranking ranged from 1 to 5 (1=very bad; 5=very good)
Preference ranking ranged from 1 to 4 (1=most preferred; 4=least preferred)

Table 6.5　　Preference ranking of maize food products by the secondary school and adult subjects in KwaZulu-Natal, South Africa

Food Products	*Phutu*				Thin porridge				Samp			
Maize varieties	**SC 701**	**KP-78**	**KP-79**	**KP-77**	**SC 701**	**KP-78**	**KP-79**	**KP-77**	**SC 701**	**KP-78**	**KP-79**	**KP-77**
Secondary school **(n=54)**												
Most preferred	31[a] (57)[b]	8 (15)	6 (11)	9 (17)	24 (44)	7 (13)	13 (24)	9 (17)	23 (43)	11 (20)	9 (17)	11 (20)
Second preferred	3 (6)	25 (46)	14 (26)	11 (20)	10 (19)	18 (33)	16 (30)	10 (19)	9 (17)	16 (30)	12 (22)	17 (32)
Third preferred	9 (17)	10 (19)	16 (30)	20 (37)	4 (7)	20 (37)	8 (15)	22 (41)	5 (9)	15 (28)	21 (39)	13 (24)
Least preferred	11 (20)	11 (20)	18 (33)	14 (26)	16 (30)	9 (17)	17 (32)	12 (22)	17 (32)	12 (22)	12 (22)	13 (24)
Adults **(n=50)**												
Most preferred	41 (82)	5 (10)	3 (6)	1 (2)	38 (76)	5 (10)	4 (8)	3 (6)	27 (54)	7 (14)	8 (16)	8 (16)
Second preferred	5 (10)	13 (26)	16 (32)	16 (32)	4 (8)	10 (20)	14 (28)	22 (44)	8 (16)	9 (18)	16 (32)	17 (34)
Third preferred	1 (2)	16 (32)	19 (38)	13 (26)	6 (12)	15 (30)	15 (30)	14 (28)	5 (10)	14 (28)	15 (30)	16 (32)
Least preferred	14 (14)	16 (32)	12 (24)	20 (40)	2 (4)	20 (40)	17 (34)	11 (22)	10 (20)	20 (40)	11 (22)	9 (18)

Preference ranking ranged from 1 to 4 (1=most preferred; 4=least preferred)
[a] Number of subjects; [b] % of total number of subjects

Preference ranking of maize food products revealed that all three white maize food products were the most preferred compared to the yellow maize food products by both the secondary school and adult groups (Table 6.5).

Logistical regression analysis showed that, in females, the likelihood of accepting yellow maize food products decreased significantly as age increased (r^2=-0.275; p=0.003). Although the same tendency was observed in males, it was not statistically significant (r^2=-0.132; p=0.128). In the secondary school and adult groups, Chi-square analysis showed that there was no statistically significant relationship between gender and maize variety preference (p<0.05). Simple linear regression analysis of the effect of age on maize variety preference showed that for *phutu* and thin porridge, preference for white maize increased with age (p<0.05). However, there was no association between preference for a maize variety and age, for *phutu*, thin porridge and samp made from KP-79 and KP-77.

Multiple linear regression analysis showed that in the secondary school group, texture had a significant influence on the overall acceptability of *phutu* and thin porridge, whilst flavour, texture and aroma had a significant influence on the overall acceptability of samp (Table 6.6). In the adult group, flavour had a significant influence on the overall acceptability of *phutu* and samp and texture influenced the overall acceptability of thin porridge. Overall, in both the secondary school and adult groups, texture and flavour had the greatest influence on overall acceptability of all the three maize food products.

Table 6.6   Multiple linear regression coefficients (r^2) showing the influence of the sensory attributes on the overall acceptability of the maize food products by the secondary school and adult groups

Group	Food product and variety	Appearance	Aroma	Texture	Flavour
Secondary school	*Phutu*				
	SC-701	0.080	0.145	0.524*	0.185
	KP-78	0.049	0.049	0.465*	0.529*
	KP-79	0.265	0.486*	0.389*	0.018
	KP-77	-0.049	0.399*	0.431*	0.346*
	Thin porridge				
	SC-701	0.235	0.061	0.561*	0.122
	KP-78	-0.142	0.216*	0.462*	0.262*
	KP-79	0.069	0.097	0.560*	0.184
	KP-77	0.141	0.071	0.372*	0.144
	Samp				
	SC-701	-0.090	0.176	0.029	0.763*
	KP-78	-0.003	0.239*	0.375*	0.413*
	KP-79	0.064	0.207	0.141	0.470*
	KP-77	0.266	0.189	0.548*	0.071
Adults	*Phutu*				
	SC-701	0.142	-0.460	0.558*	0.549*
	KP-78	0.141	0.102	0.295	0.574*
	KP-79	-0.014	0.071	0.418*	0.495*
	KP-77	-0.132	0.062	0.471*	0.416*
	Thin porridge				
	SC-701	0.107	-0.114	0.793*	0.208
	KP-78	0.068	0.021	0.321*	0.614*
	KP-79	-0.012	-0.018	0.698*	0.405*
	KP-77	-0.077	0.140	0.433*	0.593*
	Samp				
	SC-701	-0.233	0.112	0.618*	0.529*
	KP-78	0.116	0.048	0.344*	0.534*
	KP-79	0.119	0.091	0.371	0.459*
	KP-77	0.107	0.026	0.290*	0.602*

*Multiple linear regression analysis, significant at $p < 0.05$

The strong preference for white maize food products by older consumers shown in this study is in agreement with findings of other studies (De Groote *et al* 2010; De Groote & Kimenju 2008; Muzhingi *et al* 2008b; Tschirley & Santos 1995). Since the acceptability of the yellow maize food products was influenced by flavour and texture, breeding yellow maize with suitable flavour and texture traits may improve consumer acceptability. However it may be impossible to change the flavour of the yellow maize as it dependent on the oil and amino acid content, storage time and conditions, as well as processing (Schroeder 1997). Storing yellow maize under suitable conditions so as to prevent the development of unacceptable

sensory properties is also an important factor in ensuring its overall sensory acceptability. Furthermore, varying product formulation and processing methods, may also contribute to increased acceptance of yellow maize as was suggested by the variation in the acceptance of the different yellow maize food forms by the secondary school group.

6.6.2   Focus group discussions

The results indicate that the participants disliked the colour, flavour, aroma and texture of the yellow maize (Table 6.7). However, the participants were willing to consume yellow maize if it was cheaper than white maize and was readily available in local grocery stores.

Table 6.7 Perceptions towards the consumption of yellow maize food products by adults from KwaZulu-Natal, South Africa.

THEMES	CONCEPTS	DISCUSSION	QUOTES
Concerns towards consuming yellow maize food products	Expectations of sensory quality • Smell • Colour • Taste • Texture	Sensory properties such as smell, colour, taste and texture affected the likelihood to accept and consume yellow maize food products. Both genders shared the same concerns towards the consumption of yellow maize food products.	*'I cannot stand the colour'* *'The colour is unusual'* *'One will have to get used to the colour'* *'I hate its smell'* *'It is tasty but the smell'*
	Effects of preparation methods		*'It tastes like it is uncooked'* *'It tasted a bit salty'*
	Consumption with other food items (e.g. sour milk)		*'I cannot eat it with sour milk'*
Likelihood of acceptability of yellow maize food products	Unfavourable attitude towards taste by women	Female participants showed an unfavourable attitude towards the taste of all the yellow maize food products. Their main concern was for children as they thought the taste would be unacceptable to them. Therefore, such concern as care givers and food preparers might have been a key attitude influencing factor. On the contrary, male participants showed eagerness in accepting yellow maize food products and perceived them as 'nutritious', 'filling' and 'healthy'.	*'I do not like it"* *"It is tasteless'*
	Favourable attitude towards acceptance by men		*'I think it's got more nutrients than the white porridge it is good for the body'* *'.... It is making me healthy'* *'After eating you can feel that you had something'*
Likelihood to purchase yellow maize for consumption	Price factor and household economy	The majority of participants stated that they would buy yellow maize for human consumption. Price factors and availability in grocery stores were identified as two determinants for likelihood to purchase yellow maize. The female group was willing to buy the yellow maize if it were cheaper and would divert the money saved to other household needs. This indicates that domestic economic factors should be used to determine the possibility of purchase and consumption of yellow maize. The majority mentioned that the maize was not commonly found in the local grocery stores. Therefore the availability and supply of yellow maize to local grocery stores could influence the buying decisions.	*'I would try to get used to it if it is cheaper'* *'I would buy it if it is cheaper to save money'*
	Supply and availability at grocery stores		*'Yes in shops selling animal food like Agricol but not in shops selling food'* *'I used to see it in shops long ago, these days I don't see it'*
Psychological factors	Perceptions and experience	Both gender groups showed an unfavourable attitude towards the yellow colour of the maize, which seemed influenced by their past experiences. Both gender groups mentioned that they preferred white maize for human consumption and that yellow maize was used to feed chickens. Their perception of yellow maize as chicken feed was caused by the fact that yellow maize is mostly found in feed stores.	*'Yellow maize is good for feeding chickens'* *'I would not buy the maize because I am not used to it'*
Socio-cultural factors	Preferences towards other yellow maize food products	The participants suggested a higher acceptance of yellow maize if served in maize food forms other than the forms presented to them in this study. The other food forms suggested by the participants were maize bread, mealies with bean soup, grilled mealies, sour porridge and African beer. With regard to the food forms served in this study, both gender groups chose thin porridge as the best food form that could be made from yellow maize.	*'I can use it to make maize bread and cook dry mealies and beans'* *'I can also make African beer'* *'...I can drink sour porridge'*

Focus group discussions showed that adult subjects had a negative attitude towards the colour, flavour and aroma of yellow maize food products, which concurred with the sensory evaluation results that consumers preferred white maize to yellow maize. Although sensory evaluation results showed that gender had no effect on preference for yellow maize, the focus group discussions indicated that male subjects had a more positive view of yellow maize compared to female subjects. This suggests that education initiatives on the nutritional benefits of yellow maize should be directed at both men and women. The willingness to purchase yellow maize if it were sold at a lower price than white maize and the association of yellow maize with animal feed by participants is consistent with other studies (De Groote *et al* 2010; De Groote & Kimenju 2008; Tschirley & Santos 1995).

6.7   Conclusions

The study findings suggest that yellow, provitamin A-biofortified maize has the potential for use as a new strategy for dealing with the serious problem of VAD, especially among children of preschool age. The inclusion of yellow maize food products in preschool feeding programmes may be a strategy to promote its consumption in this group. However, in older groups, the use of yellow maize to alleviate VAD is unlikely to be successful unless intensive nutrition education programmes that highlight the nutritional benefit of this maize are developed. Other strategies should target the market price at which yellow maize is sold, in addition to increasing its availability in local grocery stores and improving its sensory properties through breeding.

References

Ahmed F, Darnton-Hill I (2004). Vitamin A deficiency, 1st ed. In: Gibney MJ, Margetts BM, Kearney JM, Arab L, eds. **Public Health Nutrition**. Oxford: Blackwell Science.

Allen LH (2006). Causes of nutrition-related public health problems of preschool children: available diet. **Journal of Pediatric Gastroenterology and Nutrition** 43: 8S-12S.

Cooke LJ, Wardle J (2005). Age and gender differences in children's food preference. **British Journal of Nutrition** 93: 741-746.

De Groote H, Kimenju SC (2008). Comparing consumer preferences for color and nutritional quality in maize: Application of a semi-double bound logistic model on urban consumers in Kenya. **Food Policy** 33: 362-370.

De Groote H, Kimenju SC, Morawetz UB (2010). Estimating consumer willingness to pay for food quality with experimental auctions: the case of yellow versus fortified maize meal in Kenya. **Agricultural Economics** 41: 1-16.

DeMan JM (1999). **Principles of Food Chemistry**, 3rd ed. Maryland: Aspen Publishers.

Faber M (2004). Complementary foods consumed by 6-12 month old rural infants in South Africa are inadequate in micronutrients. **Public Health Nutrition** 8: 373-381.

Faber M, Jogessar VB, Benadé AJS (2001). Nutritional status and dietary intakes of children aged 2-5 years and their caregivers in a rural South African community. **International Journal of Food Sciences and Nutrition** 52: 401-411.

Faber M, Kruger S (2005). Dietary intake, perceptions regarding body weight, and attitudes toward weight control of normal weight, overweight, and obese Black females in a rural village in South Africa. **Ethnicity & Disease** 15: 238-245.

Faber M, Smuts CM, Benadé AJS (1999). Dietary intake of primary school children in relation to food production in a rural area in KwaZulu-Natal, South Africa. **International Journal of Food Sciences and Nutrition** 50: 57-64.

Faber M, Wenhold F (2007). Nutrition in contemporary South Africa. **Water SA** 33(3): 393-400.

Guinard J-X (2001). Sensory and consumer testing with children. **Trends in Food Science & Technology** 11: 273-283.

HarvestPlus Brief (2006). **HarvestPlus: Breeding Crops for Better Nutrition**. Washington DC, USA.

Heymann H (1995). **Three-day Advanced Sensory Analysis Workshop**. Agricultural Research Council (ARC), Irene, Pretoria, South Africa, 19-21 April 1995.

Howe JA, Tanumihardjo SA (2006a). Carotenoid-biofortified maize maintains adequate vitamin A status in Mongolian gerbils. **The Journal of Nutrition** 136: 2562-2567.

Howe JA, Tanumihardjo SA (2006b). Evaluation of analytical methods for carotenoids extraction from biofortified maize (*Zea mays* sp.). **Journal of Agricultural and Food Chemistry** 54: 7992-7997.

Kimmel S, Sigman-Grant MJ, Guinard J-X (1994). Sensory testing with young children. **Food Technology** 48: 92-99.

Labadarios D, Steyn N, Maunder E, Macintyre U, Swart R, Gericke G, Huskisson J, Dannhauser A, Vorster HH, Nesamvuni EA (2000). **The National Food Consumption Survey (NFCS): Children aged 1-9 years, South Africa, 1999**. Department of Health: Directorate of Nutrition, Pretoria.

Labadarios D, Steyn NP, Mgijima C, Dladla N (2005). Review of the South African nutrition policy 1994-2002 and targets for 2007: achievements and challenges. **Nutrition** 21:100-108.

Labadarios D, Moodie IM, Van Rensburg A (2007). Vitamin A status. In: Labadarios D. **National Food Consumption Survey: Fortification Baseline**, Chapter 9B: South Africa 2005. Stellenbosch: Department of Health.

Labadarios D, Van Middelkoop AM (1995). **The South African Vitamin A Consultative Group. Children aged 6-71 months in South Africa, 1994: their anthropometric, vitamin A, iron and immunisation coverage status**. Isando, South Africa.

Merton RK, Fiske M, Kendall PL (1990). **The Focused Interview: A Manual of Problems and Procedures**, 2nd ed. New York: Free Press.

Mkhambathini Local Municipality (2007). **The Final Local Economic Development (LED) Strategic Plan**. UMgungundlovu District Municipality, Pietermaritzburg.

Mullings C (1985). Group Interviewing. In: **Crus Guides**. Sheffield: British Library Board.

Muzhingi T, Langyintuo AS, Malaba LC, Banziger M (2008b). Consumer acceptability of yellow maize products in Zimbabwe. **Food Policy** 33: 352-361.

Muzhingi T, Gadaga T, Siwela AH, Grusak MA, Russell RM, Tang G (2011). Yellow maize with high β-carotene is an effective source of vitamin A in healthy Zimbabwean men. **American Journal of Clinical Nutrition** 94: 510-519.

Nestel P, Bouis HE, Meenakshi JV, Pfeiffer W (2006). Biofortification of staple food crops. **The Journal of Nutrition** 136: 1064-1067.

Nojilana B, Norman R, Bradshaw D, van Stuijvenberg ME, Dhansay MA, Labadarios D and the South African Comparative Risk Assessment Collaborating Group (2007). Estimating the burden of disease attributable to vitamin A deficiency in South Africa in 2000. **South African Medical Journal** 97: 748-753.

Schroeder JW (1997). **Corn Gluten Feed: Composition, storage, handling, feeding and value**.
http://www.ag.ndsu.edu/pubs/ansci/dairy/as1127w.htm (date accessed 03/09/2010)

South African Department of Education Resource Targeting List (2010). South African Department of Education, Pretoria, South Africa.

Stevens R, Winter-Nelson A (2008). Consumer acceptance of provitamin A-biofortified maize in Maputo, Mozambique. **Food Policy** 33: 341-351.

Stone H, Sidel JL (2004). **Sensory Evaluation Practices**, 3rd ed. California: Elsevier Academic Press.

Swart R, Sanders D, McLachlan M (2008). Nutrition: A Primary Health Perspective. In: **South African Health Review**, Chapter 9: 129-147.

Tschirley DL, Santos AP (1995). **Who eats yellow maize? Preliminary results of a survey of consumer maize preferences in Maputo, Mozambique**. MSU International Department of Agricultural Economics Development, Department of Economics Working Paper No 53, Michigan State University, East Lansing, Michigan.

World Health Organization (WHO) (2009). **Global prevalence of vitamin A deficiency in populations   at risk 1995-2005: WHO Global Database on Vitamin A Deficiency**. http://whqlibdoc.who.int/publications/2009/9789241598019_eng.pdf (date accessed 23/08/2010).

CHAPTER 7
CONCLUSIONS AND RECOMMENDATIONS

In this chapter the main conclusions and recommendations of this thesis will be discussed. The aim was to assess the nutritional quality and consumer acceptability of provitamin A-biofortified maize. The objectives of this work were to: (i) to assess the nutritional composition of provitamin A-biofortified maize grain varieties compared to white maize grain (ii) to determine the effect of milling and cooking on the provitamin A carotenoid content of different provitamin A-biofortified maize varieties (iii) to assess the acceptance of popular South African maize food products made with provitamin A-biofortified maize among Black African children and adults from a rural KwaZulu-Natal population.

7.1　Summary of study findings

This work has provided useful and important baseline data on the nutritional quality and consumer acceptability of provitamin A-biofortified maize, an area in which data were either lacking or scarce.

The results indicate that provitamin A-biofortified maize contains various carotenoids, including the provitamin A carotenoids, β-cryptoxanthin, and the isomers of β-carotene, and the non-provitamin A carotenoid zeaxanthin. The results suggest that the carotenoid composition of provitamin A-biofortified maize varies with variety and is influenced by genetic factors, highlighting the need to breed for this trait in this maize. Although the total provitamin A carotenoid concentration in the provitamin A-biofortified maize grain was higher than the levels generally reported for typical yellow maize varieties, it was still lower than the current breeding target for biofortified maize as set by HarvestPlus. Thus, further breeding work is required to produce varieties with the desired provitamin A level. This study has made significant progress towards that goal, but the fact that no maize variety has been released with this trait reflects the real challenges of meeting the target set by HarvestPlus. Both the International Institute of Tropical Agriculture (IITA) and the the International Maize and Wheat Improvement Center (CIMMYT), which are the leading breeding groups within the HarvestPlus Challenge Program of the Consultative Group on International Agricultural Research (CGIAR), have not reported any varieties with the target level of 15 ug/g DW of provitamin A carotenoids in provitamin A-biofortified maize. According to the IITA Annual Report (2010b), they have realised a range of 2.5 to 10.5 ug/g DW total provitamin A concentration in the 300 adapted inbred lines.

The results show that the nutritional quality of provitamin A-biofortified maize is superior to that of white maize. The provitamin A-biofortified maize was found to contain higher levels of starch, fat and protein compared to white maize. The study findings indicate that the provitamin A-biofortified maize has a better amino acid composition than white maize; although not corrected for protein digestibility; the essential amino acids would be fairly nutritionally adequate for age groups > 0.5 years. However, similar to white maize, provitamin A-biofortified maize would not be adequate in lysine and histidine for this age group. These results emphasise the importance of combining cereal grains with food sources that have a better essential amino acid profile such as legumes and eggs.

The findings of this work indicate that when compared with white maize grain, the provitamin A-biofortified, yellow/orange maize grain is of superior quality. The provitamin A-biofortified maize varieties were found to have higher grain density, their kernels had a lower tendency to crack and they had better milling properties than the white maize variety (control). These attractive quality attributes of the provitamin A-biofortified maize highlight the potential of this maize type to be an alternative to white maize as a food source. The good milling quality would contribute to quality processed food products and a reduction in nutritional and economic losses due to low milling yields. However, the relatively lower storage quality (lower resistance to fungal infection) found in the provitamin A-biofortified maize varieties indicate that provitamin A-biofortified maize seeds and food grain would tend to be lost through fungal deterioration, which would impact negatively on food security and economy. Moreover, there would be a higher risk of contamination of the biofortified maize by mycotoxins, which would pose a health hazard. Therefore, during breeding, it would be important to improve the resistance of provitamin A-biofortified maize varieties to fungi.

The results of this study indicate that provitamin A retention in milled provitamin A-biofortified maize products varies with the type of milled product; there was a higher retention of provitamin A in mealie meal, compared to samp. It would therefore be recommended to mill the biofortified maize into products that tend to retain the provitamin A carotenoids, such as mealie meal. Another approach could be to either add back some of the bran to enrich the product that tends to lose the provitamin A carotenoids or to manipulate the milling process such that there is a limited loss of provitamin A. The basis of manipulating the milling process is that the carotenoid pigments are largely located in the endosperm of the maize grain and therefore limiting loss of the endosperm material during milling would limit

provitamin A loss. Wet cooking provitamin A-biofortified maize results in the loss of provitamin A carotenoids and reduction varies with the final form of cooking. *Phutu* and samp tend to lose less carotenoids than thin porridge, which is partly due to its higher cooking temperature. The maize food products were cooked under different conditions, which account for the differences in provitamin A retention. These findings highlight the need for optimising cooking conditions to reduce the loss of provitamin A carotenoids during wet cooking. Maize variety seems to also influence the retention of provitamin A in the cooked maize, which could be due to a variation in the chemical composition of the maize varieties. This presents breeders with the opportunity to select varieties for provitamin A retention and these varieties can be marketed based on this trait. It is noted, however, that although provitamin A losses were observed in this study, they were not significant.

The study findings indicate that preference for yellow maize food products decreases as the age of the consumer increases. The results showed that preschool children overall preferred yellow maize to white maize food products, whilst primary and secondary school children and adults preferred white maize to yellow maize food products. Focus group discussions similarly revealed that overall adult participants preferred white maize to yellow maize, although they may consume the yellow maize if it was readily available on the local markets and in various food forms. However, the adult males seemed to be more positive about the yellow maize than the females. These findings suggest that preference for white maize to yellow maize is significantly influenced by culture, which obviously becomes more entrenched with an increase in age. The consumers grow in a cultural environment where white maize is accepted as the traditional food. However, the findings of this study suggest that there is an opportunity to change the cultural mindset of preference for white maize, by promoting the consumption of yellow maize by children. The children could be educated about the nutritional benefits of yellow maize and the yellow maize food products could be made readily available to them, for example through school and community feeding programmes. A combination of intervention strategies, including nutrition education, improved marketing and economic incentives for utilisation of yellow maize could also increase the acceptance of this maize by adults.

Overall, it appears that provitamin A-biofortified maize has a good potential for application as an additional strategy to alleviate vitamin A deficiency amongst the poor communities of sub-Saharan Africa, including South Africa. Apart from it being a source of provitamin A, the

provitamin A-biofortified maize is a better source of starch, fat and protein than white maize. The biggest challenge is to improve consumer acceptability of the provitamin A-biofortified maize.

7.2   Implications of findings and recommendations

Although the total provitamin A carotenoid concentration in the provitamin A-biofortified maize grain was higher than the levels generally reported for typical yellow maize varieties, it was still lower than the current breeding target for biofortified maize as set by HarvestPlus. Future research should look at further recombination of the best lines used in these hybrids which is followed by selection, to achieve a higher level of provitamin A carotenoid concentration.

The research programme, of which this study is a part, has made some significant progress in developing recombinant inbred maize lines with significant levels of carotenoids through conventional selection on the basis of grain colour intensity. Approximating the levels as envisaged by HarvestPlus could be achieved by combining molecular breeding tools with conventional processes in the future.

Carotenoid levels as well as grain colour should be measured in more biofortified maize varieties, in order to perform correlation analysis of grain colour and carotenoid levels. If a positive correlation between grain colour and provitamin A content was found in the provitamin A-biofortified maize, its grain colour could be used to predict provitamin A content in the maize. This is because measuring grain colour is relatively much cheaper and more rapid than measuring provitamin A content by HPLC. The method of predicting provitamin A content using grain colour would enable the rapid screening of provitamin A-biofortified maize varieties for high provitamin A content.

The levels of the essential amino acids, histidine and lysine in the biofortified maize grain varieties were lower than that of the white maize. In order to overcome the lack of histidine and lysine in the biofortified maize, the maize should be eaten in combination with foods that are good sources of histidine and lysine, using a similar concept to that of complementary proteins. The iron levels were lower in the provitamin A-biofortified maize grain varieties compared to the white maize. Again, in order to account for this, the biofortified maize products should be eaten in combination with foods that are good sources of the nutrients that

are lacking. It is recommended that future studies should involve more in-depth nutritional analysis, including other micronutrients.

The effect of storage conditions, agricultural practices and environmental factors on the nutritional composition of provitamin A-biofortified maize should be determined in future studies, as they may influence nutritional composition.

Cooked *phutu* and samp contained higher levels of provitamin A carotenoids compared to the cooked thin porridge. This suggests that *phutu* and samp may be better choices of foods to prepare using provitamin A-biofortified maize. Future research should investigate more maize food forms that retain provitamin A better and can be used as carriers of provitamin A. Variety was found to influence the nutritional composition of the biofortified varieties. Biofortified maize varieties with superior nutritional composition should be developed and produced for the purpose of human consumption to improve nutritional status.

The effect of dry cooking on the retention of provitamin A carotenoids in provitamin A-biofortified maize food products should be carried out in future studies, as this may have a different effect on nutrient retention compared to wet cooking.

As stated earlier, nutrition education initiatives on the nutritional benefits of provitamin A-biofortified maize should start with children of preschool-age, as this study has shown that they have a preference for the provitamin A-biofortified maize products. Adults should also be educated on the nutritional benefits of the provitamin A-biofortified maize. Although females are traditionally the target of nutrition education, both females and males should be targeted as this study has shown that males had a positive attitude towards the biofortified maize. If nutrition education programmes are successful, the maize colour change could be beneficial as it may help to identify those maize varieties that have a superior nutritional quality. Future research could also investigate the effect of nutrition education on the consumer acceptability of the provitamin A-biofortified maize.

Elderly consumers were not included in this study as sensory evaluation data from individuals over 55 years is regarded as being unreliable due to a decrease in sensory sensitivity with aging. However, this life stage group is also vulnerable to vitamin A deficiency and should be included in future studies on consumer acceptability of provitamin A-biofortified maize.

Further consumer acceptability studies should be carried out using subjects from other provinces in South Africa and should include the popular maize foods eaten in those provinces. This will allow for more generalised conclusions on the consumer acceptability of provitamin A-biofortified maize in South Africa. Other popular South African maize food products that could be used in future studies on consumer acceptability and nutrient retention include: fresh, boiled or roasted mealies (maize on the cob), stiff porridge, fermented and unfermented porridges and fermented beverages.

Descriptive sensory analysis should be carried out using a trained panel to characterise the sensory properties of the provitamin A-biofortified maize products and the results thereof should be compared to the sensory evaluation results from the consumer panel. Further work should be done to improve the sensory characteristics of the provitamin A-biofortified maize through breeding and food product development.

The utilization of provitamin A-biofortified maize could be improved by reducing the market price of its seeds and grain in comparison to the white maize and increasing its availability in local grocery stores. This could be achieved by developing supportive policies by governments.

7.3 Study critique

- Besides the provitamin A carotenoids, only a limited number of other micronutrients were analysed in the maize samples, due to high cost constraints. Analysing more micronutrients, including some B-vitamins, could give a more in-depth view of the nutritional composition of provitamin A-biofortified maize.

- Carotenoid, amino acid and grain quality analysis was carried out on a limited number of provitamin A-biofortified maize varieties due to high cost constraints. The costs of carotenoid and amino acid analyses were approximately R4000.00 (US$584.00) and R820.00 (US$120.00) per sample, respectively. Analysing for the carotenoid and amino acid composition of a bigger sample of the maize varieties would have allowed for the determination of other factors influencing nutritional composition. The relationships between phenotypic characteristics and nutritional composition, such as provitamin A content and grain colour, could also have been determined.

- The consumer acceptability studies were carried out only in KwaZulu-Natal province, on *phutu*, thin porridge and samp, which are popular maize food products in this province. Although these foods are consumed in other provinces in South Africa, they are not the most popular. Therefore, the consumer acceptability findings can be generalised only for the KwaZulu-Natal province, with a limited inference for other provinces in South Africa.

- Urban commercial maize products were not included as this study focused on foods consumed by rural consumers. In practice, the biofortified maize would also be processed into commercial products and hence their acceptability should also be assessed.

- The foods used in this study were limited in that they were cooked using only the wet cooking method. Therefore the retention values obtained only apply to wet cooking. Dry cooking methods are also applied on maize in sub-Saharan Africa, including South Africa. The dry cooking methods may have different effects on provitamin A retention in maize foods.

- Retention of provitamin A carotenoids and other nutrients during storage of the maize was not assessed in this study, yet it may have an effect on the retention. In practice, maize grain is normally stored for varying periods before use and this may have an effect on its nutritional composition.

- The potential influence of agricultural practices and environmental factors on the nutritional composition of the biofortified maize grain varieties was not determined, even though these are known to influence the nutritional composition of crops.

REFERENCES

Abramson D, Mills JT, Boycott BR (1983). Mycotoxins and mycoflora in animal feedstuffs in western Canada. **Canadian Journal of Comparative Medicine** 47: 23-26.

ACC/SCN (2000). **4th Report on the World Nutrition Situation**. **Nutrition throughout the Life Cycle**. United Nations Administrative Committee on Coordination, Sub-committee on Nutrition in collaboration with International Food Policy Research Institute.

Agarwal VK, Sinclair JB (1987). **Principles of Seed Pathology**, Vol 1. Boca Raton: CRC Press.

Ahmed F, Darnton-Hill I (2004). Vitamin A deficiency, 1st ed. In: Gibney MJ, Margetts BM, Kearney JM, Arab L, eds. **Public Health Nutrition**. Oxford: Blackwell Science.

Alexander RJ (1987). Corn Dry Milling: Processes, Products and Application, 1st ed. In: Watson SA, Ramstad PE, eds. **Corn: Chemistry and Technology**. Minnesota: St. Paul.

Allen LH (2006). Causes of nutrition-related public health problems of preschool children: available diet. **Journal of Pediatric Gastroenterology and Nutrition** 43: 8S-12S.

American Association of Cereal Chemists (AACC) (2000). **Approved Methods**, 10th ed. AACC Method 55-10 Test Weight per Bushel. St Paul: AACC International.

Association of Official Analytical Chemists (AOAC) International (2002). **Official Methods of Analysis of AOAC International**, 17th ed. Volume I and II. Maryland: AOAC International.

Audilakshmi S, Stenhouse JW, Reddy TP, Prasad MVR (1999). Grain mould resistance and associated characters of sorghum genotypes. **Euphytica** 107: 91-103.

Bänziger M, Long J (2000). The potential for increasing the iron and zinc density of maize through plant breeding. **Food and Nutrition Bulletin** 20: 397-400.

Bauduret P (1990). A mycological and bacteriological survey on feed ingredients and mixed poultry feeds in Reunion Island. **Mycopathologia** 109: 157-164.

Bender DA (2003). **Nutritional Biochemistry of the Vitamins**, 2nd ed. United Kingdom: Cambridge University Press.

Bennett JW, Klich M (2003). Mycotoxins. **Clinical Microbiology Reviews** 16: 497-516.

Bouis HE (1999). Economics of enhanced micronutrient density in food staples. **Field Crops Research** 60: 165-173.

Bouis HE (2003). Micronutrient fortification of plants through plant breeding: can it improve nutrition in man at low cost? **Proceedings of the Nutrition Society** 62: 403-411.

Bouis HE (2002). Plant breeding: a new tool for fighting micronutrient malnutrition. **The Journal of Nutrition** 132: 491S-494S.

Bressani R (1995). In: **Proceedings of the International Symposium on Quality Protein Maize**, EMBRAPA/CNPMS, Sete Lagaos, Brazil.

Bressani R, Breuner M, Ortiz MA (1989). Contenidodefibraacidoy neutron-detergente y de minerals menores en maiz y su tortilla. **Archivos Latinoamericanos de Nutrición** 39: 382-391.

Brody T (1999). **Nutritional Biochemistry**, 2nd ed. California: Academic Press.

Bueso FJ, Waniska RD, Rooney LW, Bejosano FP (2000). Activity of antifungal proteins against mold in sorghum caryopses in the field. **Journal of Agricultural and Food Chemistry** 48: 810-816.

Byerlee D, Eicher CK (2001). **Africa's Emerging Maize Revolution**. United Kingdom: Lynne Publishing.

Castenmiller JJM, West CE (1998). Bioavailability and bioconversion of carotenoids. **Annual Review of Nutrition** 18: 19-38.

Choo YM (1994). Palm oil carotenoids. **Food and Nutrition Bulletin** 15: 130-137.

Christensen CM, Kaufmann HH (1974). Microflora. In: Christensen CM, ed. **Storage of Cereal Grains and their products**. St. Paul: AACC International.

Combs GF (1998). **The Vitamins: Fundamental Aspects in Nutrition and Health**, 2nd ed. London: Academic Press.

Cooke LJ, Wardle J (2005). Age and gender differences in children's food preference. **British Journal of Nutrition** 93: 741-746.

De Groote H, Kimenju SC (2008). Comparing consumer preferences for color and nutritional quality in maize: Application of a semi-double bound logistic model on urban consumers in Kenya. **Food Policy** 33: 362-370.

De Groote H, Kimenju SC, Morawetz UB (2010). Estimating consumer willingness to pay for food quality with experimental auctions: the case of yellow versus fortified maize meal in Kenya. **Agricultural Economics** 41: 1-16.

De Onis M, Blössner M (2003). The World Health Organization Global Database on Child Growth and Malnutrition: methodology and application. **International Journal of Epidemiology** 32: 518-526.

DeMan JM (1999). **Principles of Food Chemistry**, 3rd ed. Maryland: Aspen Publishers.

Department of Education and Culture Administration: House of Assembly (1991). **Foods and Cookery**. Pretoria, South Africa.

Department of Health (1999). **Policy Summary – The Integrated Nutrition Programme**. Pretoria.

Department of Health (2001). **Immunisation Awareness Campaign**. **Directorate Nutrition Fact Sheet: Vitamin A**. Pretoria: Department of Health. http://www.doh.gov.za/docs/facts-f.html (date accessed 01/12/2009).

Department of Health (2003). **Foodstuffs, Cosmetics and Disinfectants Act (Act 54 of 1972)**. **Regulations relating to the fortification of certain foodstuffs**. **Government notice no.R2003**. Pretoria: Department of Health. http://www.doh.gov.za (date accessed 01/12/2009).

Dhansay MA (2007). Vitamin A supplementation in South Africa: time for appraisal. **South African Journal of Clinical Nutrition** 20: 123-124.

Dugan FM (2006). **The Identification of Fungi – an illustrated introduction with keys, glossary, and guide to literature**. St Paul: American Phytopathological Society.

Du Plessis LM, Najaar B, Koornhof HE, Labadarios D, Petersen L, Hendricks M, Kidd M (2007). Evaluation of the implementation of the vitamin A supplementation programme in the Boland/Overberg region of the Western Cape Province. **South African Journal of Clinical Nutrition** 20: 126-132.

E-Siong T (1995). The medical importance of vitamin A and carotenoids (with particular reference to developing countries) and their determination. **Malaysian Journal of Nutrition** 1: 179-230.

Faber M (2004). Complementary foods consumed by 6-12 month old rural infants in South Africa are inadequate in micronutrients. **Public Health Nutrition** 8: 373-381.

Faber M, Jogessar VB, Benadé AJS (2001). Nutritional status and dietary intakes of children aged 2-5 years and their caregivers in a rural South African community. **International Journal of Food Sciences and Nutrition** 52: 401-411.

Faber M, Kruger S (2005). Dietary intake, perceptions regarding body weight, and attitudes toward weight control of normal weight, overweight, and obese Black females in a rural village in South Africa. **Ethnicity & Disease** 15: 238-245.

Faber M, Smuts CM, Benadé AJS (1999). Dietary intake of primary school children in relation to food production in a rural area in KwaZulu-Natal, South Africa. **International Journal of Food Sciences and Nutrition** 50: 57-64.

Faber M, Wenhold F (2007). Nutrition in contemporary South Africa. **Water SA** 33: 393-400.

Fandohan P, Hell K, Marasas WFO, Wingfield MJ (2003). Infection of maize by *Fusarium* species and contamination with fumonisin in Africa. **African Journal of Biotechnology** 2: 570-579.

Food and Agriculture Organization of the United Nations (FAO) (1992). **Maize in Human Nutrition**. Rome, Italy.

Food and Agriculture Organization of the United Nations (FAO) (2008). **The state of food insecurity in the world 2008: high food prices and food security – threats and opportunities**. Rome, Italy.
http://www.fao.org/docrep/011/i0291e/i0291e00.htm (date accessed 16/09/2010).

Food and Agriculture Organization of the United Nations (FAO) and International Maize and Wheat Improvement Center (CIMMYT) (1997). **White Maize: a Traditional Food Grain in Developing Countries**. Rome, Italy.

Gibson RS (1994). Content and bioavailability of trace elements in vegetarian diets. **American Journal of Clinical Nutrition** 59: 1223S-1232S.

Gibson RS (2005). **Principles of Nutritional Assessment**, 2nd ed. New York: Oxford University Press.

Giron HC (1973). Atomic Absorption Newsletter 12, 28. Perkin Elmer Atomic Spectrophotometer.

Graham GG, Placko RP, Maclean WC (1980). Nutritional value of normal, opaque-2 and sugary-2 opaque-2 maize hybrids for infants and children. 2. Plasma Free Amino Acids. **The Journal of Nutrition** 110: 1070-1075.

Granado F, Olmedilla B, Blanco I, Rojas-Hidalgo E (1992). Carotenoid composition in raw and cooked Spanish vegetables. **Journal of Agricultural and Food Chemistry** 40: 2135-2140.

Gregory JF (1996). Vitamins, 3rd ed. In: Fennema OR, ed. **Food Chemistry**. New York: Marcel Dekker Inc.

Grogan CO, Blessin CW (1968). Characterization of major carotenoids in yellow maize lines of differing pigment concentrations. **Crop Science** 8: 730-732.

Guinard J-X (2001). Sensory and consumer testing with children. **Trends in Food Science & Technology** 11: 273-283.

Harjes CE, Rocheford TR, Bai L, Brutnell TP, Kandianis CB, Sowinski SG, Stapleton AE, Vallabhaneni R, Williams M, Wurtzel ET, Yan J, Buckler ES (2008). Natural genetic variation in lycopene epsilon cyclase tapped for maize biofortification. **Science** 319: 330-333.

HarvestPlus Brief (2006). **HarvestPlus: Breeding Crops for Better Nutrition**. Washington DC, USA.

Hendricks M, Beardsley J, Bourne L, Mzamao B, Golden B (2007). Are opportunities for vitamin A supplementation being utilized at primary health-care clinics in the Western Cape Province of South Africa? **Public Health Nutrition** 10: 1082-1088.

Heymann H (1995). **Three-day Advanced Sensory Analysis Workshop.** Agricultural Research Council (ARC), Irene, Pretoria, South Africa, 19-21 April 1995.

Hotz C, Brown KH (eds) (2004). International Zinc Nutrition Consultative Group (IZiNCG). Technical Document No. 1: Assessment of the risk of zinc deficiency in populations and options for its control. **Food and Nutrition Bulletin** 25 (Supp 2): S94-S204.

House W (1999). Trace element bioavailability as exemplified by iron and zinc. **Field Crops Research** 60: 115-141.

Howe JA, Tanumihardjo SA (2006a). Carotenoid-biofortified maize maintains adequate vitamin A status in Mongolian gerbils. **The Journal of Nutrition** 136: 2562-2567.

Howe JA, Tanumihardjo SA (2006b). Evaluation of analytical methods for carotenoids extraction from biofortified maize (*Zea mays* sp.). **Journal of Agricultural and Food Chemistry** 54: 7992-7997.

Institute of Medicine (2000a). **Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids**. Food and Nutrition Board. Washington DC: National Academy Press.

Institute of Medicine (2000b). **Dietary Reference Intakes: applications in dietary assessment**. Food and Nutrition Board. Washington DC: National Academy Press.

Institute of Medicine (2001). **Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc.** Food and Nutrition Board. Washington DC: National Academy Press.

International Institute of Tropical Agriculture (IITA) (2010a). **New varieties to boost maize output in West and Central Africa.** http://www.iita.org/maize/-/asset_publisher/jeR0/content/new-varieties-to-boost-maize (date accessed 22/11/2010).

International Institute of Tropical Agriculture (IITA) (2010b). **Giving tropical maize a vitamin-A boost**. IITA Annual Report. http://www.annualreport.iita.org (date accessed 24/4/2011).

Jambunathan R, Kherdekar MS, Stenhouse JW (1992). Sorghum grain hardness and its relationship to mold susceptibility and mold resistance. **Journal of Agricultural and Food Chemistry** 40: 1403-1408.

Jestoi M. Ritieni A, Rizzo A (2004). Analysis of the Fusarium mycotoxins fusaproliferin and trichothecenes in grains using gas chromatography mass spectrometry. **Journal of Agricultural and Food Chemistry** 52: 1464-1469.

Johnson LA (2000). Corn: The Major Cereals of the Americas, 2nd ed. In: Kulp K, Ponte JG, eds. **Handbook of Cereal Science and Technology**. New York: Marcel Dekker.

Katz SH, Hediger M, Valleroy L (1974). Traditional maize processing techniques in the New world. **Science** 184: 765-773.

Kazarian EA, Hall CW (1965). **Transactions of ASAE** 8: 33.

Khachik F, Beecher GR, Goli MB, Lusby WR (1992a). Separation and quantification of carotenoids in foods. **Methods in Enzymology** 213: 347-359.

Khachik F, Goli MB, Beecher GR, Holden J, Lusby WR, Tenorio MD, Barrera MR (1992b). Effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomatoes and several green vegetables. **Journal of Agricultural and Food Chemistry** 40: 390-398.

Keener HM, Henery JE, Schonauer SL, Anderson RJ (1985). **Burning Shelled Corn as an Alternative Fuel**. Ohio Report, July-August 1985.

Kennedy-Oji C, Coutsoudis A, Kuhn L, Pillay K, Mburu A, Stein Z, Coovadia H (2001). Effects of vitamin A supplementation during pregnancy and early lactation on body weight of South African HIV-infected women. **Journal of Health Population and Nutrition** 19: 167-176.

Kimmel S, Sigman-Grant MJ, Guinard J-X (1994). Sensory Testing with Young Children. **Food Technology** 48: 92-99.

Kimura M, Kobori CN, Rodriguez-Amaya DB, Nestel P (2007). Screening and HPLC methods for carotenoids in sweetpotato, cassava and maize for plant breeding trials. **Food Chemistry** 100: 1734-1746.

King FS, Burgess A (1993). **Nutrition for developing countries**, 2nd ed. New York: Oxford University Press.

Kumari RS, Chandrashekar A, Shetty HS (1994). Antifungal proteins from sorghum endosperm and their effects on fungal mycelium. **Journal of the Science of Food and Agriculture** 66: 121-127.

Kumari RS, Chandrashekar A, Shetty HS (1992). Proteins in developing sorghum endosperm that may be involved in resistance to grain moulds. **Journal of the Science of Food and Agriculture** 60: 275-282.

Kurilich AC, Juvik JA (1999). Quantification of carotenoids and tocopherol antioxidants in *Zea mays*. **Journal of Agricultural and Food Chemistry** 47: 1948-1955.

Labadarios D, Moodie IM, Van Rensburg A (2007). Vitamin A status. In: Labadarios D. **National Food Consumption Survey: Fortification Baseline**, Chapter 9B: South Africa 2005. Stellenbosch: Department of Health.

Labadarios D, Steyn N, Maunder E, Macintyre U, Swart R, Gericke G, Huskisson J, Dannhauser A, Vorster HH, Nesamvuni EA (2000). **The National Food Consumption Survey (NFCS): Children aged 1-9 years, South Africa, 1999**. Department of Health: Directorate of Nutrition, Pretoria.

Labadarios D, Steyn NP, Mgijima C, Dladla N (2005). Review of the South African nutrition policy 1994-2002 and targets for 2007: achievements and challenges. **Nutrition** 21: 100-108.

Labadarios D, Van Middelkoop AM (1995). **The South African Vitamin A Consultative Group. Children aged 6-71 months in South Africa, 1994: their anthropometric, vitamin A, iron and immunisation coverage status**. Isando, South Africa.

Lacker T, Strohschein S, Albert K (1999). Separation and identification of various carotenoids by C_{30} reversed-phase High-Performance Liquid Chromatography coupled to UV and atmospheric pressure chemical ionization mass spectrometric detection. **Journal of Chromatography A** 854: 37-44.

Laurie S (2007). **Experiences with orange-fleshed sweet potato in vegetable garden projects**. Proceedings of the orange-fleshed sweet potato symposium. Pretoria. 3 October 2007.

Li S, Tayie FAK, Young MF, Rocheford T, White WS (2007). Retention of provitamin A carotenoids in high β-carotene maize (Zea mays) during traditional African household processing. **Journal of Agricultural and Food Chemistry** 55: 10744-10750.

Logrieco A, Bottalico A, Mulé G, Moretti A, Perrone G (2003). Epidemiology of toxigenic fungi and their associated mycotoxins for some Mediterranean crops. **European Journal of Plant Pathology** 109: 645-667.

Low JW, Arimond M, Osman N, Cunguara B, Zano F, Tschirley D (2007). A food-based approach introducing orange-fleshed sweet potatoes increased vitamin A intake and serum retinol concentrations in young children in rural Mozambique. **The Journal of Nutrition** 137: 1320-1327.

Lozano-Alejo N, Carrillo GV, Pixley K, Palacios-Rojas N (2007). Physical properties and carotenoid content of maize kernels and its nixtamalized snacks. **Innovative Food Science and Emerging Technologies** 8: 385-389.

Machida L, Derera J, Tongoona P, MacRobert J (2010). Combining ability and reciprocal cross effects of elite quality protein maize inbred lines in subtropical environments. **Crop Science** 50: 1708-1717.

Mayer JE, Pfeiffer WH, Beyer P (2008). Biofortified crops to alleviate micronutrient malnutrition. **Current Opinion in Plant Biology** 11: 166-170.

McCann JC (2005). **Maize and Grace: Africa's encounter with a New World Crop 1500-2000**, 1st ed. Cambridge: Harvard University Press.

Mendoza C (2002). Effect of genetically modified low phytic acid plants on mineral absorption. **International Journal of Food Science & Technology** 37: 759-767.

Menkir A, Liu W, White WS, Maziya-Dixon B, Rocheford T (2008). Carotenoid diversity in tropical-adapted yellow maize inbred lines. **Food Chemistry** 109: 521-529.

Merton RK, Fiske M, Kendall PL (1990). **The Focused Interview: A Manual of Problems and Procedures,** 2nd ed. New York: Free Press.

Miller DD (1996). Minerals, 3rd ed. In: Fennema OR, ed. **Food Chemistry**. New York: Marcel Dekker Inc.

Miller JD (1999). Mycotoxins. In: Francis FJ, ed. **Encyclopedia of Food Science and Technology**. New York: John Wiley & Sons.

Mkhamabthini Local Municipality (2010). **Mkhambathini Municipality**.
http://www.mkhambathini.gov.za (date accessed 17/08/2010).

Mkhamabthini Local Municipality (2008/2009). **Municipality Annual Report (2008/2009)**.
http://www.mkhambathini.gov.za (date accessed 17/08/2010).

Mkhambathini Local Municipality (2007). **The Final Local Economic Development (LED) Strategic Plan.** UMgungundlovu District Municipality, Pietermaritzburg.

Montes GN, Reyes MCA, Montes RN, Cantu AMA (2009). Incidence of potentially toxigenic fungi in maize (*Zea mays* L.) grain used as food and animal feed. **CyTA-Journal of Food** 7: 119-125.

Mullings C (1985). Group Interviewing. In: **Crus Guides**. Sheffield: British Library Board.

Murphy EW, Criner PE, Gray BC (1975). Comparisons of methods for calculating retentions of nutrients in cooked foods. **Journal of Agricultural and Food Chemistry** 23: 1153-1157.

Muzhingi T, Yeum KJ, Russell RM, Johnson EJ, Qin J, Tang G (2008a). Determination of carotenoids in yellow maize, the effects of saponification and food preparations. **International Journal for Vitamin and Nutrition Research** 78: 112-120.

Muzhingi T, Langyintuo AS, Malaba LC, Banziger M (2008b). Consumer acceptability of yellow maize products in Zimbabwe. **Food Policy** 33: 352-361.

Muzhingi T, Gadaga T, Siwela AH, Grusak MA, Russell RM, Tang G (2011). Yellow maize with high β-carotene is an effective source of vitamin A in healthy Zimbabwean men. **American Journal of Clinical Nutrition** 94: 510-519.

Nestel P, Bouis HE, Meenakshi JV, Pfeiffer W (2006). Biofortification of staple food crops. **The Journal of Nutrition** 136: 1064-1067.

Nojilana B, Norman R, Bradshaw D, van Stuijvenberg ME, Dhansay MA, Labadarios D and the South African Comparative Risk Assessment Collaborating Group (2007). Estimating the burden of disease attributable to vitamin A deficiency in South Africa in 2000. **South African Medical Journal** 97: 748-753.

Nuss ET, Tanumihardjo SA (2010). Maize: a paramount staple crop in the context of global nutrition. **Comprehensive Reviews in Food Science and Food Safety** 9: 417-436.

Oikeh SO, Menkir A, Maziya-Dixon B, Welch R, Glahn RP (2003a). Assessment of concentrations of iron and zinc and bioavailable irons in grains of early-maturing tropical maize varieties. **Journal of Agricultural and Food Chemistry** 51: 3688-3694.

Oikeh SO, Menkir A, Maziya-Dixon B, Welch R, Glahn RP (2003b). Genotypic differences in concentration and bioavailability of kernel-iron in tropical maize varieties grown under field conditions. **Journal of Plant Nutrition** 26: 2307-2319.

Oikeh SO, Menkir A, Maziya-Dixon B, Welch R, Glahn RP, Gauch G (2004). Environmental stability of iron and zinc concentrations in grain of elite early-maturing tropical maize genotypes grown under field conditions. **Journal of Agricultural Sciences** 142: 543-551.

Ortiz-Monasterio JI, Palacios-Rojas N, Meng E, Pixley K, Trethowan R, Pena RJ (2007). Enhancing the mineral and vitamin content of wheat and maize through plant breeding. **Journal of Cereal Science** 46: 293-307.

Paes MCD, Bicudo MH (1995). In: **Proceedings of the International Symposium on Quality Protein Maize**, EMBRAPA/CNPMS, Sete Lagaos, Brazil.

Penniston KL, Tanumihardjo SA (2006). The acute and chronic toxic levels of vitamin A. **American Journal of Clinical Nutrition** 83: 191-201.

Pitt JI, Hocking AD (1999). **Fungi and Food Spoilage**, 2nd ed. Maryland: Aspen Publishers.

Potter NN, Hotchkiss JH (1995). **Food Science**, 5th ed. Chapman & Hall: New York.

Prasanna BM, Vasal SK, Kassahun B, Singh NN (2001). Quality Protein Maize. **Current Science** 81: 1308-1319.

Rabie CJ, Lübben A (1984). The mycoflora of sorghum salt. **South African Journal of Botany** 4: 251-255.

Rabie CJ, Lübben A, Marais GJ, Van Vuuren (1997). Enumeration of fungi in barley. **International Journal of Food Microbiology** 35: 117-127.

Raboy V (1997). Accumulation and Storage of Phosphate and Minerals. In: Larkins BA, Vasil IK, eds. **Cellular and Molecular Biology of Plant Seed Development**. Dordrecht: Kluwer Academic Publishers.

Raboy V, Below FE, Dickison DB (1989). Alteration of maize kernel phytic acid levels by recurrent selection for protein and oil. **Journal of Heredity** 80: 311-315.

Ratnavathi CV, Sashidhar RB (2003). Substrate suitability of different genotypes of sorghum in relation to Aspergillus infection and aflatoxin production. **Journal of Agricultural and Food Chemistry** 51: 3482-3492.

Rice AI, West KP, Black RE (2004). Vitamin A deficiency. In: **Comparative quantification of health risks**. http://www.who.int/publications/cra/chapters/volume1/0211-0256.pdf (date accessed 25/10/2010).

Rodriguez-Amaya DB (1997). **Carotenoids and Food Preparation: The retention of Provitamin A Carotenoids in Prepared, Processed, and Stored Foods**. Washington DC: OMNI/USAID.

Rodriguez-Amaya DB, Kimura M (2004). **HarvestPlus Handbook for Carotenoid Analysis**. Technical Monograph Series 2.

Rodríguez-Herrera R, Waniska RD, Rooney WL (1999). Antifungal proteins and grain mold resistance in sorghum with nonpigmented testa. **Journal of Agricultural and Food Chemistry** 47: 4802-4806.

Roigé MB, Aranguren SM, Riccio MB, Pereyra S, Soraci AL, Tapia MO (2009). Mycobiota and mycotoxins in fermented feed, wheat grains and corn grains in Southeastern Buenos Aires Province, Argentina. **Revista Iberoamericana de Micología**.

Rubey L (1993). **Consumer Maize Meal Preferences In Zimbabwe: Survey Results And Policy Implications**. Report Prepared for The Ministry of Lands Agriculture and Water Development. Harare, Zimbabwe.

Ruel M (2001). **Can Food-Based Strategies Help Reduce Vitamin A and Iron Deficiencies? A Review of Recent Evidence**. International Food Policy Research Institute. Washington DC.

Rukmini C (1994). Red palm oil to combat vitamin A deficiency in developing countries. **Food and Nutrition Bulletin** 15: 126-129.

Schroeder JW (1997). **Corn Gluten Feed: Composition, storage, handling, feeding and value**.
http://www.ag.ndsu.edu/pubs/ansci/dairy/as1127w.htm (date accessed 03/09/2010).

Shankar AH, Genton B, Semba RD (1999). Effect of vitamin A supplementation on morbidity due to *Plasmodium falciparum* in young children in Papua New Guinea: A randomized trial. **Lancet** 354: 203-209.

Šimić D, Sudar R, Ledenćan T, Jambrović A, Zdunić Z, Brkić I, Kovačević V (2009). Genetic variation of bioavailable iron and zinc in grain of a maize population. **Journal of Cereal Science** 50: 392-397.

Sizer F, Whitney E (2011). **Nutrition Concepts and Controversies**, 12th ed. USA: Wadsworth Cengage Learning.

Sleper DA, Poehlman JM (2006). **Breeding field crops**, 5th ed. Blackwell Publishing.

Smale M, Jayne T (2003). **Maize is Eastern and Southern Africa: "Seeds" of success in retrospect**. Environment and Production Technology Division Discussion Paper No. 97, International Food Policy Research Institute. Washington DC, USA.

Sommer A (1995). **Vitamin A deficiency and its consequences: a field guide to detection and control**, 3rd ed. Geneva, World Health Organization.

Sommer A, West KP (1996). **Vitamin A deficiency: Health, survival and vision**. New York University Press.

South African Department of Education Resource Targeting List (2010). South African Department of Education, Pretoria, South Africa.

Southern African Grain Laboratory (SAGL) (2007). **Industry Accepted Method for estimating Milling Index using NIT**. Pretoria, South Africa.

Southern African Grain Laboratory (SAGL) (2001). **Industry Accepted Method for Stress Crack Analysis of Maize Kernels**. Pretoria, South Africa.

South African Schools Act (1996) (Act No 84 of 1996). **Amended National Norms and Standards for School Funding**. South African Department of Education, Pretoria, South Africa.

Stevens R, Winter-Nelson A (2008). Consumer acceptance of provitamin A-biofortified maize in Maputo, Mozambique. **Food Policy** 33: 341-351.

Steyn NP, Bradshaw D, Norman R, Joubert JD, Schneider M, Steyn K (2006). **Dietary changes and the Health Transition in South Africa: Implications for Health Policy**. Cape Town: South African Medical Research Council.

Swart R, Labadarios D, Kruger S, Nel JH, Schloss I (2007). General and socio-demographic factors impacting on nutritional status. In: Labadarios D. **National Food Consumption Survey: Fortification Baseline**, Chapter 3: South Africa 2005. Stellenbosch: Department of Health.

Swart R, Sanders D, McLachlan M (2008). Nutrition: A Primary Health Perspective. In: **South African Health Review**, Chapter 9: 129-147.

Tanumihardjo SA (2002). Factors influencing the conversion of carotenoids to retinol: bioavailability to bioconversion to bioefficacy. **International Journal of Vitamin and Nutrition Research** 72: 40-45.

Tanumihardjo SA (2008). Food-based approaches for ensuring adequate vitamin A nutrition. **Comprehensive Reviews in Food Science and Food Safety** 7: 373-381.

Taylor JRN, Duodu KG (2009) Applications for non-wheat testing methods. In: Cauvain SP, Young LS, eds. **The ICC Handbook of Cereals, Flour, Dough and Product Testing: Methods and Applications**. Pennsylvania: DEStech Publications.

Thomas JB, Kline MC, Gill LM, Yen JH, Duewer DL, Sniegoski LT, Sharpless KE (2001). Preparation and value assignment of standard reference material 968c fat- soluble vitamins, carotenoids, and cholesterol in human serum. **Clinica Chimica Acta** 305: 141-155.

Tothova M, Meyers WH (2006). **Predicting the acceptance for high beta-carotene maize: an ex-ante estimation method**. American Agricultural Economics Association Annual Meeting, July 23-26, Long Beach, California.

Trumbo P, Yates AA, Schlicker S, Poos M (2001). Dietary Reference Intakes: Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. **Journal of the American Dietetic Association** 101: 294-301.

Tschirley DL, Santos AP (1995). **Who eats yellow maize? Preliminary results of a survey of consumer maize preferences in Maputo, Mozambique**. MSU International Department of Agricultural Economics Development, Department of Economics Working Paper No 53, Michigan State University, East Lansing, Michigan.

United States Department of Agriculture and Foreign Agricultural Service (USDA/FAS) (2008). **Corn 2008: production, supply, demand database**. http://www.pecad.fas.usda.gov/cropexplorer/ (date accessed 14/10/2010).

UWP Consulting (2003). **Mkhambathini Municipality Map 1.** http://www.uwp.co.za (date accessed 18/08/2010).

Van Jaarsveld PJ, Marais DW, Harmse E, Nestel P, Rodriguez-Amaya DB (2006). Retention of β-carotene in boiled, mashed orange-fleshed sweet potato. **Journal of Food Composition and Analysis** 19: 321-329.

Van Jaarsveld PJ, Faber M, Tanumihardjo SA, Nestel P, Lombard CJ, Benade AJS (2005). β-Carotene-rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modified-relative-dose-response test. **American Journal of Clinical Nutrition** 81: 1080-1087.

Vasal SK (2001). **Keynote Address: Quality Protein Maize Development**. Seventh Eastern and Southern Africa Regional Maize Conference, 11th-15th February 2001: 3-6.

Villamor E, Fawzi WW (2000). Vitamin A supplementation: implication for morbidity and mortality in children. **Journal of Infectious Diseases** 182: 122-133.

Visser ME, Maartens G, Kossew G, Hussey GD (2003). Plasma vitamin A and zinc levels in HIV-infected adults in Cape Town, South Africa. **British Journal of Nutrition** 89: 475-482.

Welch RM, Graham RD (2004). Breeding for micronutrients in staple food crops from a human nutrition perspective. **Journal of Experimental Botany** 55: 353-364.

West CE (2000). Vitamin A and measles. **Nutrition Reviews** 58: 546-554.

West KP, Jr (2002). Extent of vitamin A deficiency among preschool children and women of reproductive age. **The Journal of Nutrition** 132: 2857-2866.

White PJ, Broadley MR (2005). Biofortifying crops with essential mineral elements. **Trends in Plant Science** 10: 586-593.

Whitney E, Rolfes SR (2011). **Understanding Nutrition**, 12th ed. Belmont: Wadsworth, Cengage Learning.

Wolmarans P, Danster N, Dalton A, Rossouw K, Schonfeldt H (2010) (eds). **Condensed Food Composition Tables for South Africa**. Medical Research Council, Parow Valley, Cape Town.

World Health Organization (WHO) (1995). **Global prevalence of vitamin A deficiency**. Micronutrient Deficiency Information System Working Paper 2. World Health Organization, Geneva.

World Health Organization (WHO) (2002a). **Joint FAO/WHO/UNU Expert Consultation on Protein and Amino Acid requirements in Human Nutrition**. Technical Report Series No 935. Geneva, Switzerland.

World Health Organization (WHO) (2002b). **The World Health Report 2002: Reducing Risks, Promoting Healthy Life**. Geneva, Switzerland.

World Health Organization (WHO) (2003). **GEMS/Food Regional Diets: Regional Per Capita Consumption of Raw and Semi-processed Agricultural Commodities, Food Safety Department**. WHO Document Production Services, Geneva, Switzerland.

World Health Organization (WHO) (2009). **Global prevalence of vitamin A deficiency in populations at risk 1995-2005: WHO Global Database on Vitamin A Deficiency**. http://whqlibdoc.who.int/publications/2009/9789241598019_eng.pdf (date accessed 23/08/2010).

World Health Organization (WHO) (2010). **Micronutrient deficiencies: Vitamin A deficiency**. http://www.who.int/nutrition/topics/vad/en/index.html (date accessed 14/06/2010).

Wright KN (1987). Nutritional Properties and Feeding Values of Corn and Its By-Products, 1st ed. In: Watson SA, Ramstad PE, eds. **Corn: Chemistry and Technology**. Minnesota: St. Paul.

APPENDICES

APPENDIX A: NOTIFICATION OF ETHICS APPROVAL FROM THE HUMANITIES AND SOCIAL SCIENCES ETHICS COMMITTEE, UNIVERSITY OF KWAZULU-NATAL

UNIVERSITY OF KWAZULU-NATAL

RESEARCH OFFICE (GOVAN MBEKI CENTRE)
WESTVILLE CAMPUS
TELEPHONE NO.: 031 – 2603587
EMAIL : ximbap@ukzn.ac.za

30 SEPTEMBER 2009

MRS. K PILLAY (942402804)
AGRICULTURAL SCIENCES AND AGRIBUSINESS

Dear Mrs. Pillay

ETHICAL CLEARANCE APPROVAL NUMBER: HSS/0591/09D

I wish to inform you that your application for ethical clearance has been granted full approval for the following project:

"Assessment of the nutritional quality and consumer preference of provitamin A – biofortified maize varieties and their food products"

PLEASE NOTE: Research data should be securely stored in the school/department for a period of 5 years

I take this opportunity of wishing you everything of the best with your study.

Yours faithfully

PROFESSOR STEVEN COLLINGS (CHAIR)
HUMANITIES & SOCIAL SCIENCES ETHICS COMMITTEE

cc. Supervisor (Dr. J Derera)
cc. Ms. M Francis

APPENDIX B: LETTER OF APPROVAL TO CONDUCT RESEARCH IN THE MKHAMBATHINI MUNICIPALITY

Private Bag X04
Camperdown
3720

Mkhambathini Municipality
For the Community

Tel no : (031) 785 1668/1184
Fax no: (031) 785 1463/ 2977

Website: www.mkhambathini.org.za
E-mail: mkhambamune@telkomsa.net

OFFICE OF THE MUNICIPAL MANAGER

03 November 2009

Mrs Kirthee Pillay
Lecturer and Student (Student number: 942402804)
Discipline of Dietetics and Human Nutrition
University of KwaZulu-Natal
Private Bag X01
Scottsville
3209

RE: REQUEST TO CONDUCT RESEARCH ON THE CONSUMER ACCEPTABLILITY OF PRO-VITAMIN A-BIOFORTIFIED YELLOW/ORANGE MAIZE VARIETIES AND THEIR FOOD PRODUCTS WITHIN THE MKHAMBATHINI MUNICIPALITY

Please be advised that you have been granted permission to conduct a research project on the consumer acceptability of pro-vitamin A -biofortified yellow/orange maize varieties and their food products within the Mkhambathini Municipality. We understand that you will be conducting the research on the following groups: Pre-School Children, Primary School Learners, High-School Learners, Women of Childbearing age (19-35 years), Adult males (up to 55 years) and Adult Females (35-55 years) and will use between 55-60 participants from each group.

We wish you well in your research

Yours sincerely

Mr D. A Pillay
Municipal Manager

APPENDIX C: LETTER OF APPROVAL TO CONDUCT RESEARCH FROM THE KWAZULU-NATAL DEPARTMENT OF EDUCATION

kzn education

Department:
Education
KWAZULU-NATAL

MRS K PILLAY
PRIVATE BAG X01
SCOTTSVILLE
PIETERMARITZBURG
3209

Enquiries:	Sibusiso Alwar
Date:	08/03/2010
Reference:	0025/2010

RESEARCH PROPOSAL: AN ASSESSMENT OF THE RETENTION OF PROVITAMIN A CAROTENOIDS IN THERMALLY PROCESSED PROVITAMIN A-BIOFORTIFIED MAIZE PRODUCTS AND THE CUSTOMER ACCEPTABILITY OF THESE FOOD PRODUCTS

Your application to conduct the above-mentioned research in schools in the attached list has been approved subject to the following conditions:

1. Principals, educators and learners are under no obligation to assist you in your investigation.
2. Principals, educators, learners and schools should not be identifiable in any way from the results of the investigation.
3. You make all the arrangements concerning your investigation.
4. Educator programmes are not to be interrupted.
5. The investigation is to be conducted from 08 March 2010 to 08 March 2011.
6. Should you wish to extend the period of your survey at the school(s) please contact Mr Sibusiso Alwar at the contact numbers above.
7. A photocopy of this letter is submitted to the principal of the school where the intended research is to be conducted.
8. Your research will be limited to the schools submitted.
9. A brief summary of the content, findings and recommendations is provided to the Director: Resource Planning.
10. The Department receives a copy of the completed report/dissertation/thesis addressed to:

The Director: Resource Planning
Private Bag X9137
Pietermaritzburg
3200

We wish you success in your research.

Kind regards

R. Cassius Lubisi (PhD)
Superintendent-General

...dedicated to service and performance
beyond the call of duty.

APPENDIX D: **STANDARDISED RECIPE FOR THE PREPARATION OF**
PHUTU

INGREDIENTS

280 mL water

1 mL salt

2 cups (268 g) mealie meal

METHOD

1. Bring 280 mL of water to the boil[11] in a heavy-bottom pot on a Defy Thermofan Stove (Model 731 MF) on high heat (plate control setting 6).

2. Add 1 mL of salt to the water.

3. Add 2 cups of mealie meal (268 g) to the boiling water and stir as soon as the mixture reaches boiling point.

4. Allow the _phutu_ to stand on low heat (plate control setting 1) for approximately 75 minutes, with the pot lid on and occasional stirring until cooked.

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[11] Boiling is a cooking method that involves the cooking of food in direct contact with the liquid at the boiling point of the liquid (Department of Education and Culture Administration: House of Assembly 1991).

APPENDIX E: **STANDARDISED RECIPE FOR THE PREPARATION OF THIN PORRIDGE**

INGREDIENTS

8 cups (2000 mL) water

2 cups (268 g) mealie meal ⎤
                          ⎬ To make a paste
2 cups (500 mL) water ⎦

1 mL salt

50 mL sugar

METHOD

1. Bring 8 cups (2000 mL) of water to the boil in a heavy-bottom pot on a Defy Thermofan Stove (Model 731 MF) on high heat (plate control setting 6).

2. Combine 2 cups (268 g) of mealie meal with 2 cups (500 mL) water to make a smooth paste.

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

4. Drop to medium heat (plate control setting 3) and cook for 25 minutes with the pot lid on and occasional stirring.

4. Add 1 mL of salt while cooking.

5. Add 50 mL of sugar once the porridge is cooked.

APPENDIX F: STANDARDISED RECIPE FOR THE PREPARATION OF SAMP

INGREDIENTS

2 cups (369 g) samp

4 cups (1000 mL) water   Soaked overnight

6 cups (1500 mL) water

5 mL salt

METHOD

1.   Soak 2 cups (369 g) of samp in 4 cups (1000 mL) of cold water in a heavy-bottom pot, overnight.

2.   Add 4 cups (1000 mL) of water to the samp and bring to the boil on high heat (plate control setting 6) using a Defy Thermofan Stove (Model 731 MF).

3.   Boil the samp with the pot lid on for 135 minutes, until cooked.

4.   Add 5 mL of salt to the samp while cooking.

5.   Add a further 2 cups (500 mL) of water while cooking.

APPENDIX G: PAIRED PREFERENCE TEST IN ENGLISH

Please rinse your mouth with water before starting.

Please taste the two food samples in the order given, from left to right.

Please circle the number of the sample that you prefer.

_____   _____

Thank you for taking part in this study

APPENDIX H: PAIRED PREFERENCE TEST IN ZULU

Sicela uxubhe umlomo wakho ngamanzi ngaphambi kukoqala.

Sicela uzwe lezinhlobo ezimbili zokudla ngendlela ezihlelwe ngayo, kusukela kwesokunxele kuyakwesokudla.

Sicela ubeke uphawu enambeni yesampula oyikhethayo.

_____   _____

Siyabonga ngokubamba iqhaza kululuncwaningo

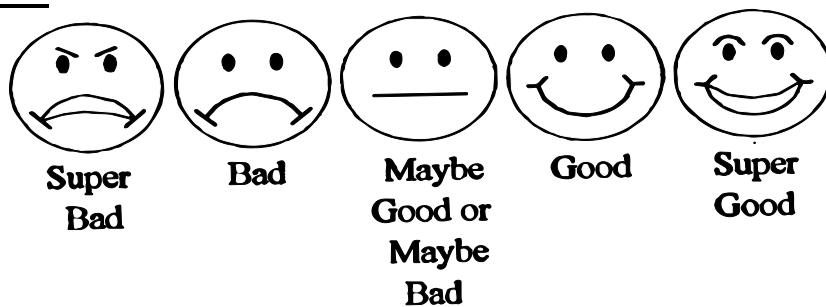**APPENDIX I:**   **FIVE-POINT FACIAL HEDONIC SCALE IN ENGLISH**

Please taste the food sample that is in front of you.
After you have tasted it please indicate how you feel about the appearance, aroma, texture and flavor as well as the overall acceptability by placing crosses over the relevant faces below.
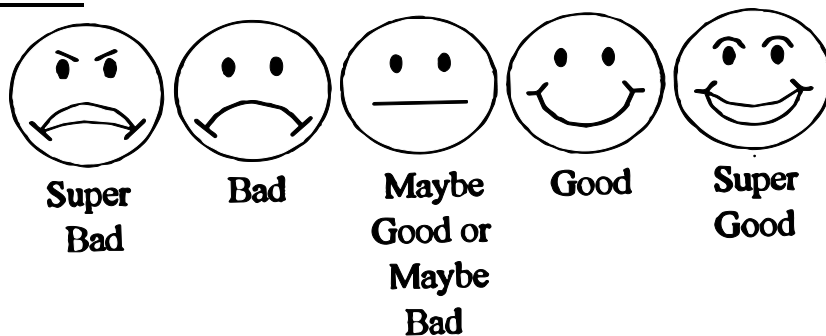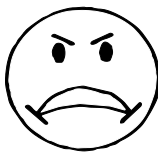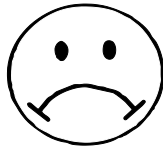
APPEARANCE

| Super Bad | Bad | Maybe Good or Maybe Bad | Good | Super Good |

AROMA

| Super Bad | Bad | Maybe Good or Maybe Bad | Good | Super Good |

TEXTURE

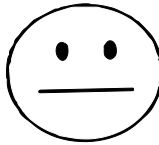| Super Bad | Bad | Maybe Good or Maybe Bad | Good | Super Good |
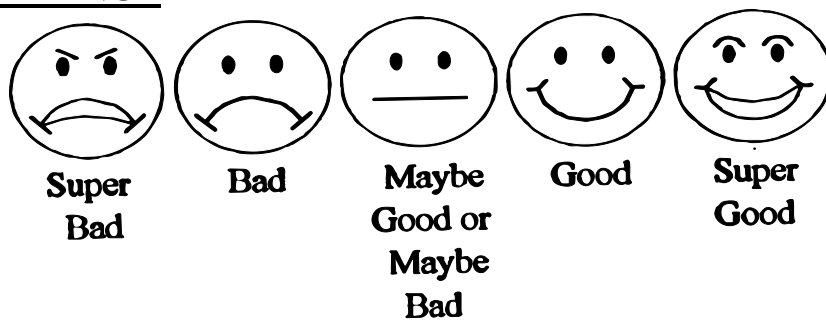
FLAVOUR
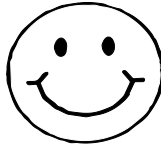
| Super Bad | Bad | Maybe Good or Maybe Bad | Good | Super Good |

OVERALL ACCEPTABILITY

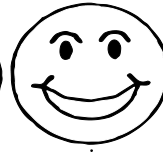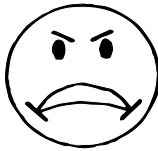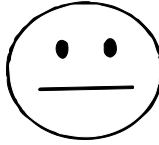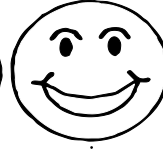| Super Bad | Bad | Maybe Good or Maybe Bad | Good | Super Good |

APPENDIX J: FIVE-POINT FACIAL HEDONIC SCALE IN ZULU

Besicela uzwe ukunambitheka kwalokhu kudla okuphambi kwakho.

Emuva kokuzwa besicela utshengise ngalezizimpawu ezilandelayo ukuthi kubukeka kanjani, iphunga, ukunambitheka, nohlobo kanye nesinqumo jikelele ngokubeka uphawu ezithombeni ezilandelayo.

<u>UKUBUKEKA</u>

| Kubi impela | Kubi | Mhlawumbe Kumnandi Noma Mhlawumbe kubi | Kumnandi | Kumnandi impela |

IPHUNGA

| Kubi impela | Kubi | Mhlawumbe Kumnandi Noma Mhlawumbe kubi | Kumnandi | Kumnandi impela |

UKUNAMBITHEKA

| Kubi impela | Kubi | Mhlawumbe Kumnandi Noma Mhlawumbe kubi | Kumnandi | Kumnandi impela |

UHLOBO

| Kubi impela | Kubi | Mhlawumbe Kumnandi Noma Mhlawumbe kubi | Kumnandi | Kumnandi impela |

ISINQUMO JIKELELE

| Kubi impela | Kubi | Mhlawumbe Kumnandi Noma Mhlawumbe kubi | Kumnandi | Kumnandi impela |

APPENDIX K: **PREFERENCE RANKING TEST IN ENGLISH**

Please rinse your mouth with water before starting and between tasting samples.

You may also rinse your mouth again at any time during the session if you need to.

Please taste the four samples in the order presented, from left to right.

You may re-taste the samples once you have tried all of them.

Rank the samples from most preferred to least preferred using the following numbers.

1 = most preferred, 4 = least preferred

Please do not use the same numbers more than once

If you have any questions please ask the assistants now.

SAMPLE	RANKING

Thank you for taking part in this study

APPENDIX L: PREFERENCE RANKING TEST IN ZULU

Uyacelwa ukuba uxubhe umlomo wakho ngamanzi ngaphambi kokuqala ukuzwa izinhlobo

zamasampulo, ungaphinda uxubhe umlomo usaqhubeka nokuzwa.

Ungaphinda uhlanze umlomo wakho futhi noma inini ngesikhathi sokuzwa uma uthanda.

Sicela uzwe amasampula amane ngohla olubekiwe kusukela kwesokunxele kuya kwesokudla

Uma usuwezwe wonke amasampula, ungaphinda uwezwa uma ufisa.

Linganisa kumasampula ukuthi ikuphi okuthandayo impela noma okuthandayo nje usebenzisa

izinombolo

1 = okuthandayo impela, 4 = okuthandayo nje

Sicela ungasebenzisi izinamba ezifanayo kaningi

Uma unombuzo cela usizo manje

ISAMPULA	UKULINGANISA

Siyabonga ngokubamba iqhaza kuloluphando

APPENDIX M: FOCUS GROUP QUESTIONS IN ENGLISH

PHUTU

1. Have you eaten phutu made from yellow/orange maize before?
2. What did you like about the yellow/orange phutu that you tasted today?
3. What did you not like about the yellow/orange phutu that you tasted today?
4. What did you think about the taste of the yellow/orange phutu that you tasted today?
5. What did you think about the colour of the yellow/orange phutu that you tasted today?

THIN PORRIDGE

1. Have you eaten thin porridge made from yellow/orange maize before?
2. What did you like about the yellow/orange thin porridge that you tasted today?
3. What did you not like about the yellow/orange thin porridge that you tasted today?
4. What did you think about the taste of the yellow/orange thin porridge that you tasted today?
5. What did you think about the colour of the yellow/orange thin porridge that you tasted today?

SAMP

1. Have you eaten samp made from yellow/orange maize before?
2. What did you like about the yellow/orange samp that you tasted today?
3. What did you not like about the yellow/orange samp that you tasted today?
4. What did you think about the taste of the yellow/orange samp that you tasted today?
5. What did you think about the colour of the yellow/orange samp that you tasted today?

GENERAL QUESTIONS

1. If the yellow/orange maize was available in the shops for you to buy to feed your family, would you buy it and why?
2. If the yellow/orange maize was cheaper than the white maize, which one would you buy and why?
3. Have you seen yellow/orange maize being sold anywhere? If yes, where?
4. If the yellow/orange maize was available for you to grow in your garden, would you grow it and why?
5. What other foods would you make using yellow/orange maize besides samp, phutu and thin porridge?

APPENDIX N: FOCUS GROUP QUESTIONS IN ZULU

UPHUTHU

1. Usuke waludla uphuthu olwenziwe ngempuphu yombila ophuzi/noma o olentshi ngaphambili?
2. Yini oyithandile ngophuthu olwenziwe ngempuphu yombila ophuzi/noma o olentshi oyizwile namhlanje?
3. Yini ongayithandanga ngophuthu olweziwe ngempuphu yombila ophuzi/noma o olentshi oluzwile namhlanje?
4. Ucabanga ukuthi lunambitheka kanjani uphuthu olwenziwe ngempuphu yombila ophuzi/noma o olentshi oluzwile namhlanje?
5. Ucabangani ngombala wophuthu lwe mpuphu yombila ophuzi/noma o olentshi ?

IPHALISHI ELIMANZI

1. Usuke walidla iphalishi elenziwe ngempuphu yombila ophuzi/noma o olentshi ngaphambili?

2. Yini oyithandile ngephalishi elenziwe ngempuphu yombila ophuzi/noma o olentshi olizwile namhlanje?

3. Yini ongayithandanga ngephalishi elenziwe ngempuphu yombila ophuzi/noma o olentshi oloizwile namhlanje?

4. Ucabangani ngokunambitheka kwephalishi elenziwe ngempuphu yombila ophuzi/noma o olentshi?

5. Ucabangani ngombala wephalishi lempuphu yombila ophuzi/noma o olentshi?

ISITAMBU

1. Usuke wasidla isitambu sombila ophuzi noma o olentshi ngaphambili na?
2. Yini oyithandile ngesitambu sombila ophuzi/noma o olentshi owuzwile namhlanje?
3. Yini ongayithandanga ngesitambu sombila ophuzi/noma o olentshi owuzwile namhlanje na?
4. Ucabanga ukuthi sinambitheka kanjani isitambu sombila ophuzi/noma o olentshi owuzwile?
5. Ucabangani ngombala wesistambu sombila ophuzi/noma o olentshi na?

IMIBUZO EJWAYELEKILE

1. Uma kukhona umbila ophuzi/noma o olentshi ezitolo, ungawuthenga ukupha umndeni wakho. Ungawuthenga na ngobani?

2. Uma umbila ophuzi/noma o olentshi ushibhile kunomhlophe,imuphi ongawuthenga ngobani?

3. Uke wawubona umbila ophuzi/noma o olentshi udayiswa noma kuphi. Uma impendulo kuwu yebo, wawubona udayiswa kuphi?

4. Uma umbila ophuzi/noma o olentshi ukhona ukuthi ungawutshala engadini yakho, ungawutshala na, ngobani?

5. Wuluphi olunye uhlobo lokudla ongakwenza ngombila ophuzi/olentshi ngaphandle kwesitambu, uphuthu, nephalishi elimanzi?

APPENDIX O: CONSENT DOCUMENT FOR LEARNERS IN ENGLISH

UNIVERSITY OF
KWAZULU-NATAL

School of Agricultural Sciences & Agribusiness
Dietetics & Human Nutrition
Private bag X01
3290 Scottsville
Tel +27 (0) 33 2605428
Fax +27 (0) 33 2606270

Dear Parent/Guardian
I am a Lecturer and Researcher from the Discipline of Dietetics and Human Nutrition at the University of KwaZulu-Natal. I am doing a study on yellow/orange maize food products. Your child/ward _____ has been selected to take part in this study. Your child/ward will be required to taste yellow/orange maize food products and tell us how they like them compared to the same white maize food products. I will visit the school on the _____. Your child's identity and all information from them will be kept confidential. If you agree for your child/ward to take part in this study please fill out and sign this form at the bottom.
Please do not hesitate to contact me if you need any further information.
Yours faithfully
Mrs Kirthee Pillay
033-2605674 (work)
0837853072 (cell)

_____

I, _____ Parent/Guardian of

_____
Hereby give consent for my child/ward to take part in the study on yellow/orange maize food products. I understand that the information obtained will be kept confidential and will be used for research purposes only.
NAME OF PARENT/GUARDIAN:

        _____
SIGNATURE: _____   DATE:
_____

APPENDIX P: CONSENT DOCUMENT FOR LEARNERS IN ZULU

<div align="right">

UNIVERSITY OF
KWAZULU-NATAL
School of Agricultural Sciences & Agribusiness
Dietetics & Human Nutrition
Private bag X01
3290 Scottsville
Tel +27 (0) 33 2605428
Fax +27 (0) 33 2606270

</div>

Sawubona Mzali/Mphathi

Mina ngiwu Thisha/no mcwaningi (Researcher) osebenza emnyangeni ophathelene nezokudla nendlela yokudla kwabantu eNyuvesi yakwaZulu-Natal.

Ngenza ucwaningo ngezinhlobo zokudla ezenziwe ngombila ophuzi no olentshi. Ingane yakho

ikhethiwe ukuba ibambe iqhaza kulolucwaningo. Umntwana uzocelwa ukuzwa ukunambitheka kokudla okwenziwe ngombila ophuzi no olentshi bese eqhathanisa nokwenziwe ngombila omhlophe.

Ngizovakashela isikole ngomhlaka…………………………………

Imininingwane yomntwana nemibona yakhe izogcinwa iyimfihlo.

Uma uvuma ukuthi umntwana abambe iqhaza kulolucwaningo, besicela ugcwalise

imininingwane kulifomu bese uyasayina.

Unelungelo lokungithinta uma ufisa ukubuza okuthile.

Yimina Nkosikaza Kirthee Pillay
Inombolo yomsebenzi (033) 2605674
Inombolo kamakhala ekhukhwini 083 785 3072

_____

Mina _____Mzali/Mphathi ka_____
Ngiyavuma ukuthi umntwana abambe iqhaza kulolucwaningo olumayelana nokudla okwenziwe ongombilo ophuzi no olintshi. Ngiyaqonda ukuthi yonke imininigwane izogcinwa iyimfihlo, futhi izosebenziswa kulolucwaningo kuphela.
IGAMA LOMZALI/MPHATHI: _____
Sayina: _____ USUKU:_____

APPENDIX Q: CONSENT DOCUMENT FOR ADULT SUBJECTS IN ENGLISH

UNIVERSITY OF
KWAZULU-NATAL
School of Agricultural Sciences & Agribusiness
Dietetics and Human Nutrition
Private bag X01
Scottsville, 3209
Tel +27 (0) 33 2605428
Fax +27 (0) 33 2606270

STUDY TITLE: ASSESSMENT OF THE CONSUMER ACCEPTABILITY OF YELLOW/ORANGE MAIZE FOOD PRODUCTS.

I am a student and Lecturer from the Discipline of Dietetics and Human Nutrition at the University of KwaZulu-Natal. As part of my studies I want to find out whether or not people like yellow/orange maize food products compared to white maize products.

- The person carrying out the study is Kirthee Pillay-[M.Sc Diet, RD (SA)] who is from the Discipline of Dietetics and Human Nutrition, University of KwaZulu-Natal. Contact – 033-2605674 or Pillayk@ukzn.ac.za

- For further information you may contact Dr Muthulisi Siwela (PhD) who is from the University of KwaZulu-Natal and is the project supervisor. Contact – 072 415 9652 or siwelam@ukzn.ac.za

- Subjects identified for the project are people who regularly consume maize products.

- The people who agree to take part in this study will be given different yellow/orange maize food products to taste and will then choose the products which they like the best. There will not be any possible discomforts or hazards involved with taking part in the study. The estimated time for completion of the tasting session is 30 minutes.

- There are no potential benefits from participating in the study however, results from the study will be made available to the participants.

- There are no payments or reimbursement of financial expenses for participating in the study.

- Audio recordings of focus group discussions will be made and these will be kept confidential and used only for the purpose of this study.

- Information gathered from this study will be kept confidential and all participants will remain anonymous.

- Information gathered from this study will be kept by the Discipline of Dietetics and Human Nutrition and will be destroyed when no longer required.

- If subjects decide not to participate in the study they will not be disadvantaged in any way.

- Participation in is voluntary and subjects are free to withdraw from the study at any stage and for any reason.

DECLARATION

I ………………………………………………… (Full names of participant) hereby confirm that I understand the contents of this document and the nature of the research project, and I consent to participating in the research project.

I understand that I am free to withdraw from the study at any time should I so desire.

…………………………………………   ……………………….
SIGNATURE OF PARTICIPANT   DATE

APPENDIX R: CONSENT DOCUMENT FOR ADULT SUBJECTS IN ZULU

UNIVERSITY OF
KWAZULU-NATAL
School of Agricultural Sciences & Agribusiness
Dietetics and Human Nutrition
Private bag X01
Scottsville, 3209
Tel +27 (0) 33 2605428
Fax +27 (0) 33 2606270

ISIHLOKO CWANINGO: UKUTHOLA UKUTHI YILUPHI UHLOBO LOMBILA OLUTHANDWA ABATHENGI PHAKATHI KOMBILA OMBALA UPHUZI NO OLENTSHI

Ngiwumfundi weziqu zobuDokotela kwezolimo kanti futhi ngingumsebenzi emnyangweni ophathelene nezokudla nendlela yokudla kwabantu(Dietetics & Human Nutrition) enyuvesi yakwaZulu-Natal. Njengenxenye yocwaningo lwezifundo zami, ngizama ukuthola ukuthi abantu/abathengi bathanda umbila ophuzi/noma o-olentshi noma abawuthandi, uma kuqhathaniswa nombila omhlophe.

- Umuntu owenza lolucwaningo unkosikazi Kirthee Pillay oneziqu zeMasters kwi Diet, RD (SA), osebenza emnyangweni ophathelene nezokudla nendlela yokudla kwabantu(Dietetics & Human Nutrition), enyuvesi yakwaZulu-Natal. Otholakala kunombolo yocingo ewu (033) 2605674
- Uma-ufisa ukuthola imininingwane ebanzi, ungaxhumana nomphathi walolucwaningo uDokotela Muthulisi Siwela enyuvesi yaKwaZulu-Natal. Inombolo yocingo u0724159652 noma siwelam@ukzn.ac.za
- Abantu abahlelelwe ukabamba iqhaza ngabantu abajwayele ukudla lezizinhlobo zombila
- bawuzwe ukunambitheka kwawo bese bekhetha ukuthi umuphi omnandi. Angeke kubekhona ukungazizwa kahle/nabungozi ngokumba iqhaza kulolucwaningo. Kuzothatha imizuzu engamashuni amathathu ukuzwa ukunambitheka.
- Abantu abazobamba iqhaza bayokwaziswa imiphumela yocwaningo, kodwa –ke akunanzuzo.
- Cha akunankokhelo yemali ngokubamba iqhaza.
  Amazwi qoshiwei abantu abazobamba iqhaza azogcinwa eyimfihlo, ezosetshenziswa kulolucwaningo kuphela.
- Imininingwane etholakele kulolucwaningo izogcinwa iyimfihlo, amagama abantu ababambe iqhaza azogcinwa eyimfihlo
- Imininingwane etholakele kulolucwaningo izogcinwa emnyangweni ophathelene nezokudla nendlela yokudla kwabantu(Dietetics & Human Nutrition), futhi iyoshatshalaliswa uma ingasadingeki.
- Uma ufisa ukushiya kulolucwaningo angeke ujeziswe ngalokho.
- Ukubamba iqhaza kuyigunya lakho, awuphoqiwe, futhi ungashiya noma inini nomangasiphi isizathu.

ISIVUNGO

Mina …………………………………………………… (Amagama aphelele ombambe iqhaza) ngiyavunga

ukuthi, ngiyaqonda konke okuqukethwe kulombalo nohlobo locwaningo , ngiyavuma ukubamba iqhaza

kulolucwaningo.Ngiyaqonda ukuthi nginelungelo lokuphuma kulolucwaningo noma inini umangifisa.

……………………………………………   ……………………………
ISIGINESHA YOBAMBE IQHAZA   USUKU